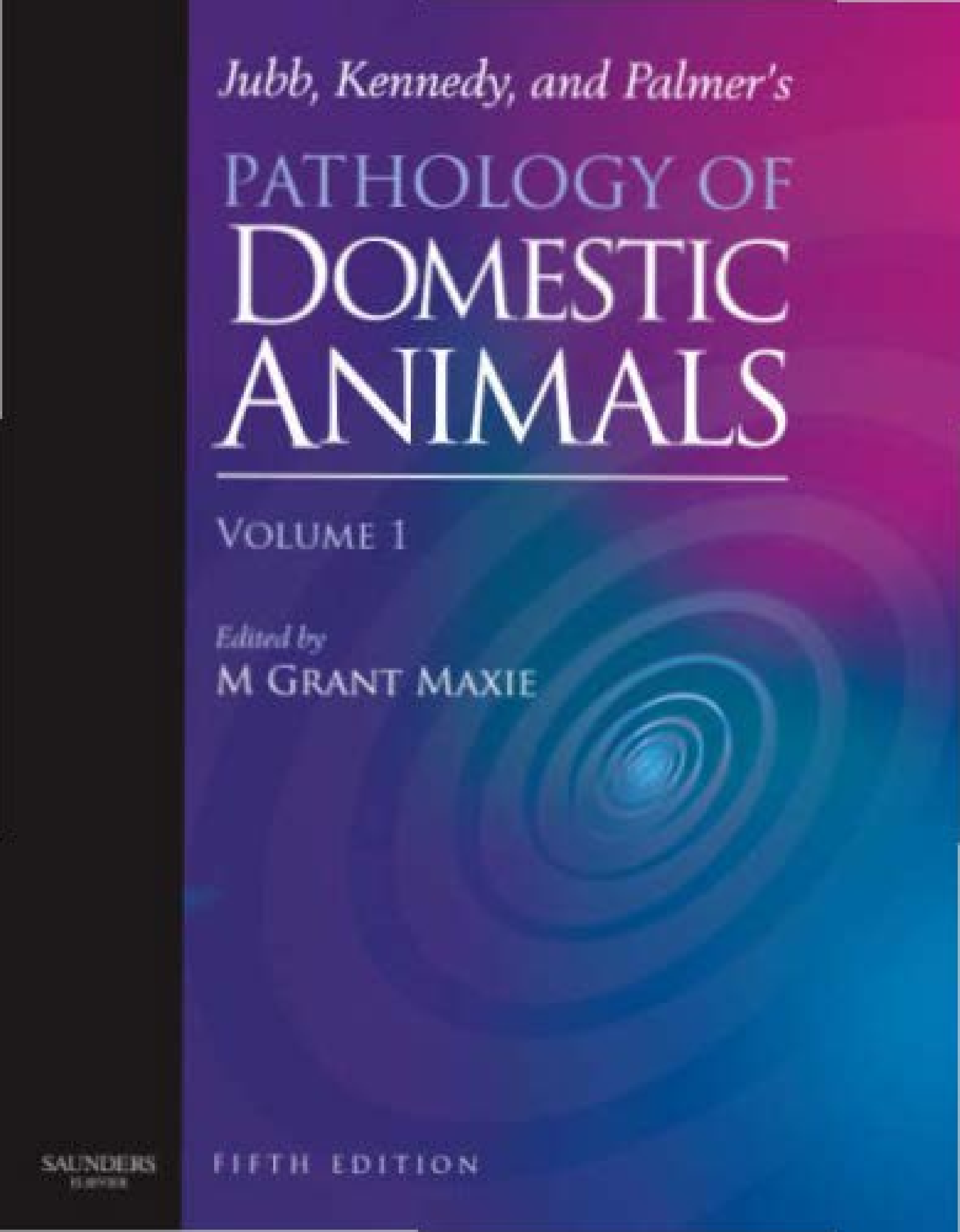

Jubb, Kennedy, and Palmer's

PATHOLOGY OF DOMESTIC ANIMALS

VOLUME 1

Edited by

M GRANT MAXIE

FIFTH EDITION

SAUNDERS
ELSEVIER

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Bones and joints

Keith Thompson

Diseases of bones

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Diseases of bones

GENERAL CONSIDERATIONS

Bone is a highly specialized connective tissue, its properties depending largely on the unique nature of its extracellular matrix. In addition to providing mechanical support and protecting key organ systems from traumatic injury, bone is intimately involved in the homeostasis of calcium, an essential cation in a wide range of bodily functions. In spite of their apparent inertia, *bones are dynamic organs, undergoing constant remodeling throughout life.* Even in mature individuals, bone tissue is continually undergoing localized resorption and replacement in response to the demands of mineral homeostasis and alterations in mechanical forces. The dynamic nature of bones is well illustrated by their impressive powers of repair following injury.

Because of the difficulties associated with processing mineralized tissue, the study of bones, both by researchers and diagnosticians, has lagged behind that of most other organ systems. The skeleton is seldom examined in detail during routine necropsy and it is highly likely that many disorders go undiagnosed. Even in cases where a bone disease is suspected, many veterinary pathologists do not feel confident in their approach to making a diagnosis. *Familiarity with the gross and microscopic anatomy of bones, factors regulating bone formation and resorption, and an understanding of the responses of bone to injury are key to an appreciation of the pathogenesis and pathology of bone diseases.* The initial sections of this chapter will therefore focus on these aspects and outline an approach to examining the skeleton at necropsy.

STRUCTURE AND FUNCTION OF BONE TISSUE

Cellular elements

Bone tissue consists of four cell types: *osteoblasts, osteocytes, lining cells, and osteoclasts.* The first three are derived from primitive

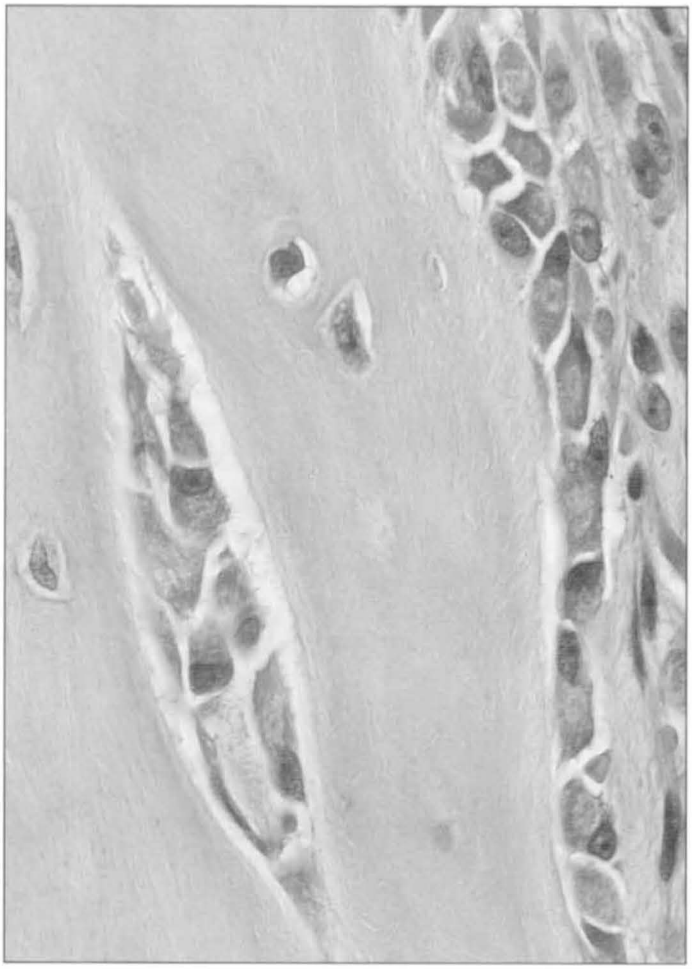

Figure 1.1 Active osteoblasts at a site of rapid bone formation in a newborn kitten. Note the eccentric nuclei, basophilic cytoplasm and prominent Golgi zone in many of the cells. Some osteoblasts have surrounded themselves with osteoid to become osteocytes.

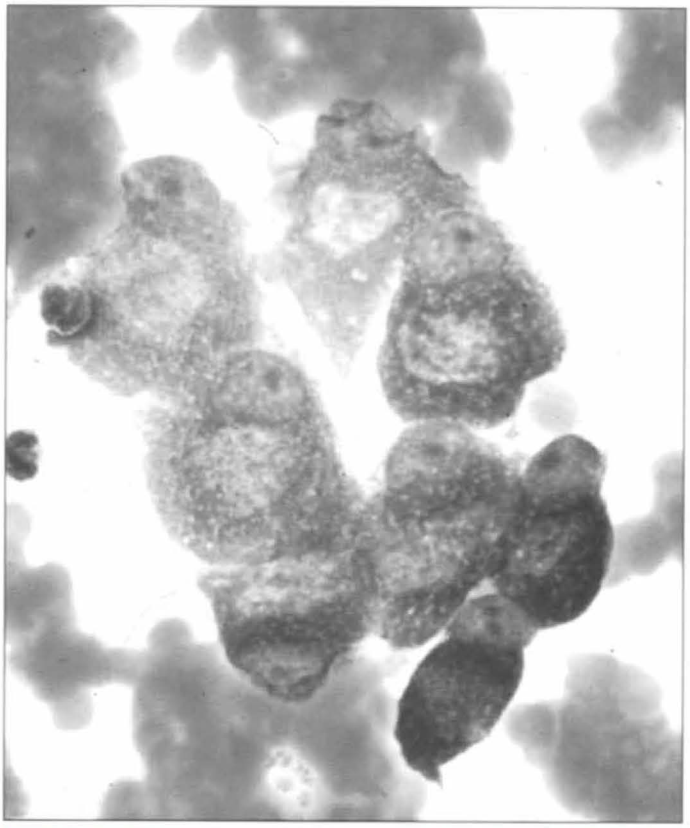

Figure 1.2 Active osteoblasts in a cytological preparation showing the characteristic eccentric nucleus, basophilic cytoplasm and prominent Golgi zone.

osteoprogenitor cells of mesenchymal origin, which are present in bone as well as other tissues, and which can be induced to differentiate into osteoblasts under the influence of appropriate paracrine and autocrine stimuli. Osteoclasts are derived from hematopoietic stem cells, probably of the monocyte series.

Osteoblasts *are responsible for manufacturing osteoid*, the organic component of bone matrix. Active osteoblasts, which line surfaces where bone formation is occurring, have abundant rough endoplasmic reticulum and a prominent Golgi apparatus, reflecting their role in protein synthesis. Histologically they appear as plump, cuboidal cells with basophilic cytoplasm, their nuclei sometimes being polarized away from the adjacent bone surface (Fig. 1.1). The typical morphologic features of osteoblasts are often more easily appreciated in cytologic preparations (Fig. 1.2). Not only do osteoblasts produce the osteoid, *they play a role in initiating its mineralization*, although the mechanism is not fully understood. The membranes of osteoblasts are rich in alkaline phosphatase, which appears to be involved in mineralization of both osteoid and cartilage. The high level of alkaline phosphatase activity measured normally in the serum of rapidly growing young animals presumably reflects the intense osteoblastic activity that is occurring in the developing skeleton. There is mounting evidence that the osteoblast is the central cell through which bone resorption and formation are mediated. In addition to osteoid, they produce an array of *regulatory factors* that are deposited in bone matrix and which play a critical role in bone remodeling.

Inactive osteoblasts, or **bone-lining cells**, are flattened cells with few organelles that cover endosteal bone surfaces that are undergoing neither formation nor resorption. Although barely visible in histological sections, these are the most abundant cells on the endosteal surface of the adult skeleton and link with each other to *form a functional barrier* between the extracellular fluid compartment of bone tissue and that of surrounding tissues. Bone-lining cells are believed to be involved in calcium homeostasis by regulating the exchange of calcium and other ions, such as sodium and magnesium, between bone fluid and extracellular fluid, under the influence of parathyroid hormone. It is likely that bone-lining cells can produce osteoid and contribute to bone deposition should the demand arise at a site of previous inactivity. Endosteal osteoprogenitor cells may be residents of this population of bone-lining cells.

During active bone formation, about 10–20% of osteoblasts at regular intervals along a bone-forming surface surround themselves with osteoid and become **osteocytes** (see Fig. 1.1). *These are the most abundant cells of bone tissue, residing in small spaces (lacunae) within the mineralized matrix.* Newly formed osteocytes retain some morphological and functional characteristics of osteoblasts, but as they mature and become embedded deeper in the mineralized matrix, the amount of rough endoplasmic reticulum in their cytoplasm is reduced considerably and they develop features more typical of phagocytic cells. Osteocytes maintain contact with adjacent osteocytes, and with bone-lining cells or osteoblasts on the surface, by a network of branching cytoplasmic processes extending through *canaliculi*. In routinely stained histological sections, the canaliculi are not visible and only the nuclei of osteocytes are usually apparent.

Osteocytes are thought to be capable of producing and resorbing bone in their immediate vicinity, thereby decreasing or increasing the size of their lacunae. This process is known as *osteocytic osteolysis*. The perilacunar bone matrix is less heavily mineralized and more labile than that in other areas. The action of osteocytes on this perilacunar bone is believed to be important in regulating the concentration of calcium and other minerals in the bone fluid compartment. Because of the large surface area of mineralized bone exposed to either osteocytes or their canaliculi, significant quantities of calcium can be mobilized from this source very rapidly in response to the demands of calcium homeostasis. Although osteocytes can enlarge their lacunae by resorbing perilacunar bone matrix, this process of osteocytic osteolysis does not play a significant role in structural modifications of bones, or in the development of bone lesions associated with disease processes.

Osteocytes probably survive for several years in the mature skeleton but eventually die, leaving empty lacunae. The gradual loss of osteocytes is presumably a normal phenomenon, compensated by the construction of new bone as part of the remodeling process that occurs throughout life. In disease states in which bone necrosis is a feature, there is no immediate loss in structural integrity of the dead bone tissue, but efforts to remove the remaining mineralized matrix and replace it with new bone suggest that its function is impaired.

Osteoclasts *are primarily responsible for resorption of bone tissue*. They are probably related to monocytes and macrophages, which are capable of resorbing bone in vitro, but are sufficiently different histochemically to suggest that they may possess a distinct stem cell. Osteoclasts are rich in acid phosphatase and a range of other acid hydrolases, packaged in primary lysosomes. The acid phosphatase isoenzyme present in osteoclasts is tartrate-resistant, unlike the tartrate-sensitive acid phosphatase found in monocytes and macrophages. Osteoclasts are easily recognizable histologically as large, multinucleated cells with eosinophilic cytoplasm, typically situated on bone surfaces and often within shallow pits called **Howship's lacunae**. The presence of Howship's lacunae on a bone surface is convincing evidence of previous resorption at that site, even if no osteoclasts are present at the time of observation. Although not always apparent histologically, osteoclasts involved in active bone resorption have a highly specialized "ruffled" or brush border contiguous with the bone surface (Fig. 1.3). A clear zone adjacent to the brush border is free of organelles but contains actin-like filaments, which may assist in anchoring the cell to the bone matrix. This attachment of active osteoclasts to the bone surface is an essential requirement for resorption to occur, as is the activity of a specific intracellular, membrane-bound tyrosine kinase. Deletion of the gene coding for this enzyme in mice has been shown to induce osteopetrosis, a disease characterized by defective osteoclastic activity.

During **osteoclastic bone resorption**, an acid environment is created in the narrow space between the cell and the bone surface. Hydrogen and bicarbonate ions are generated from carbon dioxide and water by the action of carbonic anhydrase II on the brush border membrane of osteoclasts. An ATP-mediated proton pump, also located on the brush border membrane, actively transfers hydrogen ions into the extracellular space. The acidity of the local environment not only induces demineralization of the bone, it enhances the activity of the acid hydrolases released from osteoclasts when primary lysosomes fuse with the cell membrane of the brush border. Fragments of degraded matrix are endocytosed by osteoclasts and further digested within secondary lysosomes.

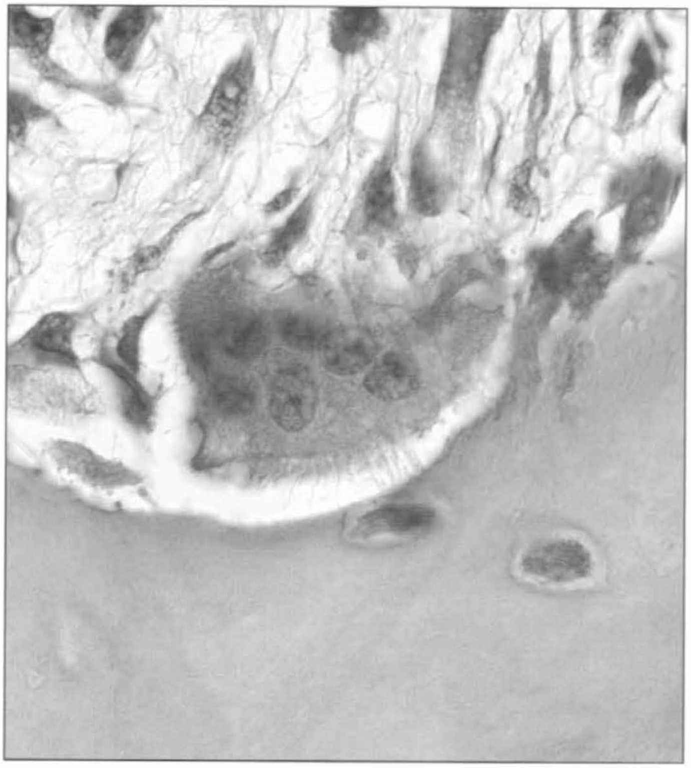

Figure 1.3 Multinucleated osteoclast in a shallow pit (Howship's lacuna) on a bone surface undergoing resorption. Note the ruffled border of the osteoclast adjacent to the bone.

The potential rate of removal of bone by osteoclasts is much greater than the rate of formation by osteoblasts. An individual osteoclast can erode approximately 400 μm^3 of bone, and travel 100 μm across a bone surface, per hour. As a result, localized or generalized removal of bone during the normal physiological processes of modeling and remodeling, or in disease states, can occur very rapidly.

Once osteoclasts have completed their required phase of resorption, they most likely undergo apoptosis and disappear from resorption sites. This is characterized by condensation of nuclear chromatin, loss of the ruffled border and detachment from the bone surface. Drugs that inhibit bone resorption have been shown to induce osteoclast apoptosis both in vitro and in vivo. Importantly, parathyroid hormone promotes the survival of osteoclasts and in cases of either nutritional or renal hyperparathyroidism, surviving osteoclasts are found in medullary spaces mixed with fibroblastic elements. The abnormal persistence of osteoclasts in these sites is an important aid to the diagnosis of these conditions.

Bone matrix

Bone matrix consists of an *organic component, called* **osteoid**, *and an inorganic component comprised predominantly of hydroxyapatite crystals*. The main constituent of osteoid (approximately 90%) is **type I collagen**, which is also the predominant form of collagen in tendons, ligaments, dentine, and the ocular sclera. Each collagen molecule consists of three polypeptide chains assembled into a triple helix, which is a highly stable configuration, resistant to proteolytic degradation, and which forms the basic unit of all collagenous structures. The strength of bone and other collagenous structures is due in part to the manner in which individual collagen molecules are aggregated into fibrils, with each fibril overlapping its neighbor by about one quarter of its length. This creates a characteristic banding pattern, clearly evident on transmission electron microscopy. The tensile strength of collagenous structures is further enhanced by intermolecular cross-links, which form by the oxidative deamination of either lysyl or hydroxylysyl residues under the influence of the copper-dependent enzyme lysyl oxidase. The number of these cross-links in bone collagen is greater than that of the collagen types found in soft tissues. Interference with the formation of cross-links, as occurs in copper deficiency or certain toxicity diseases (see below), may significantly alter the mechanical properties of bone and other connective tissues.

Several *noncollagenous proteins* are also produced by osteoblasts and form part of the organic matrix of bone. The most abundant of these is **osteonectin**, a phosphoprotein that interacts with both type I collagen and hydroxyapatite, and has been shown to facilitate the mineralization of type I collagen in vitro. Osteonectin concentration is highest in mature bone, especially in areas with the highest degree of mineralization. **Osteocalcin**, also referred to as *bone Gla protein* because of its γ-carboxyglutamic acid (Gla) residues, is also abundant in bone, accounting for up to 10% of total noncollagenous proteins. Its synthesis by osteoblasts is vitamin K-dependent and is stimulated by 1,25 dihydroxyvitamin D. Osteocalcin is deposited in osteoid shortly before mineralization and binds strongly to calcium ions and to hydroxyapatite, suggesting that it is important in the mineralization process. There is also evidence to suggest that it may be involved in the recruitment of osteoclasts to sites of bone resorption or remodeling. Interestingly, depletion of osteocalcin concentration to less than 1% of normal in rats fed warfarin is not accompanied by a reduction in the mechanical strength of bone. Osteocalcin is also found in plasma, where it serves as a marker for osteoblastic activity. A second Gla-containing protein, *matrix Gla protein*, occurs in bone as well as several other tissues. Its function is not known but it appears early in skeletal development when osteocalcin levels are still low, suggesting a possible role in bone development.

The **proteoglycans** of bone matrix are considerably smaller and less abundant than those found in cartilage matrix, possessing a relatively small protein core and only one or two glycosaminoglycan (chondroitin sulfate) side-chains. The bone proteoglycans are concentrated near the mineralization front where they are believed to play a *key role in the organization and mineralization of the matrix*. During this process, the protein core is degraded, leaving the chondroitin sulfate side-chains, which persist in the mineralized matrix of bone.

Several other noncollagenous proteins have been detected in bone matrix, including *osteopontin*, a sialoprotein that binds strongly to hydroxyapatite, and many other glycoproteins and phosphoproteins whose functions are unknown.

Bone matrix also contains a *variety of growth factors* that are capable of inducing mitogenic responses in a range of cell types, including bone cells. These factors, which probably play an important role in bone development, modeling and remodeling, especially at the local level, include: bone morphogenetic proteins, fibroblast growth factors, platelet-derived growth factors, insulin-like growth factors, and transforming growth factors β.

The **inorganic (mineral) component** of bone matrix is known to consist largely of **hydroxyapatite** [$Ca_{10}(PO_4)_6(OH)_2$], but its structure and properties are poorly understood. In addition to calcium and phosphate, bone mineral contains considerable quantities of *carbonate, magnesium, sodium and zinc*, not all of which are available for exchange. *Fluoride* is also present in small amounts in bone matrix. Ultrastructurally, hydroxyapatite is present in bone matrix either as thin, needle-like crystals oriented in the same direction as collagen fibrils, or as an amorphous, granular phase, depending on the type of bone.

Matrix mineralization

The mineralization of skeletal tissues is a highly complex process, and is only partly understood. In organ systems throughout the body, extracellular tissue fluids in equilibrium with plasma are supersaturated with respect to hydroxyapatite. Many also contain type I collagen similar to that in bone, but mineralization does not normally occur. This is most likely due to the presence of potent inhibitors, which must be enzymatically degraded before mineralization can be initiated. In bone, the selective and localized degradation of such inhibitors, and the synthesis by osteoblasts of unique molecules that promote mineralization, could account for the orderly manner in which mineral deposition occurs in this tissue. However, the presence of substrates that promote nucleation at humoral solute concentrations is also required.

There is no doubt that **matrix vesicles**, *tiny extracellular organelles originating as cytoplasmic blebs from osteoblasts, chondrocytes and odontoblasts*, play an important role in initiating the mineralization process, particularly in cartilage undergoing endochondral ossification. These vesicles are rich in calcium-binding phospholipids and proteins, alkaline phosphatase, pyrophosphatases, phospholipase A_2 and in metalloproteinases that degrade proteoglycans, potential inhibitors of mineralization. Although the mechanism is still uncertain, the initial nucleation of hydroxyapatite crystals occurs on the inner surface of matrix vesicle membranes, at least in mineralizing cartilage matrix. Phospholipids in vesicles are believed to sequester calcium, while pyrophosphatases and metalloproteinases inactivate local inhibitors and alkaline phosphatase generates phosphates, allowing mineralization to proceed.

Although mineral deposition in some tissues such as growth plate cartilage appears to be mediated almost exclusively by matrix vesicles, this is not the case for bone matrix. Unlike cartilage, where mineralization of collagen fibrils does not occur, hydroxyapatite crystals are deposited in the type I collagen fibrils of bone. Evidence suggests however that collagen fibrils alone are not capable of initiating primary nucleation. The adsorption to collagen of bone-specific noncollagenous proteins such as osteonectin and osteocalcin, both of which are strong binders of Ca^{2+} ions, may create appropriate sites for nucleation. Once initiated, the mineral spreads in an orderly manner throughout collagen fibers until the entire aqueous space of the fiber is filled with hydroxyapatite crystals. The mineralization of individual fibers occurs rapidly, as evidenced by the sharp division between highly and sparsely mineralized matrix at the junction between mineralized bone and osteoid seams.

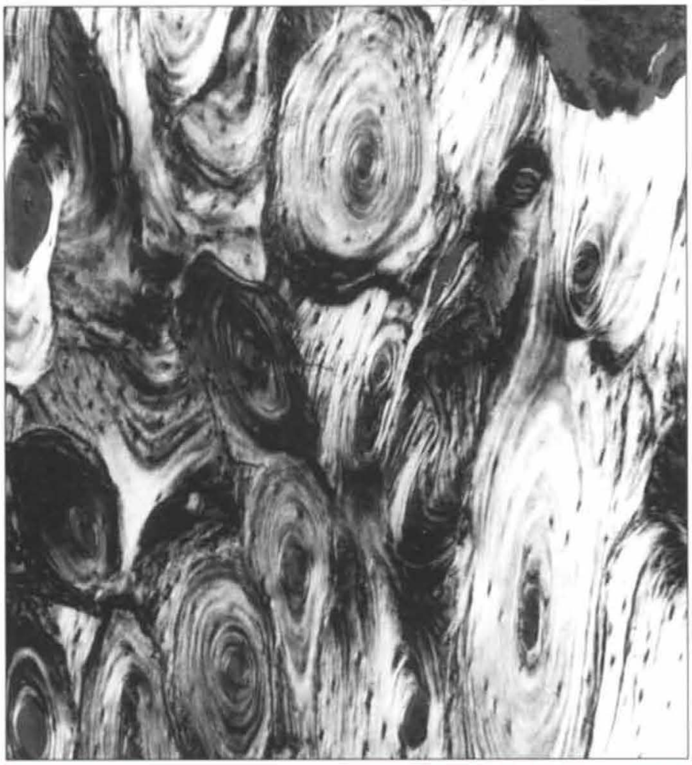

Figure 1.4 Transverse (slightly oblique) section of cortical bone viewed under polarized light to show the **osteons or Haversian systems**, which consist of concentric lamellae of bone surrounding a central vascular canal.

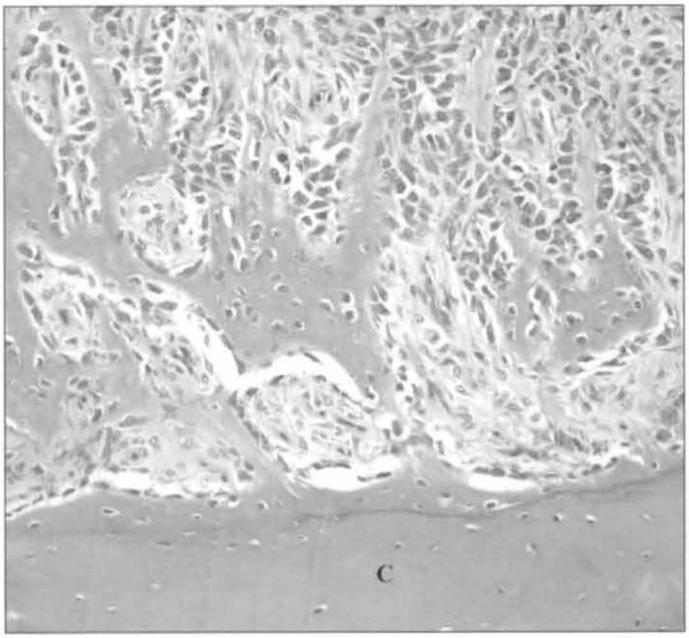

Figure 1.5 Trabeculae of **woven bone** emerging from the cortex (C) beneath an elevated periosteum. The osteocytes in the woven bone are more numerous than in the mature lamellar bone of the cortex and are irregularly distributed. The matrix of the woven bone is slightly more basophilic than that of the mature bone.

Osteoid does not become mineralized for 5–10 days after deposition. As a result, *a thin layer of unmineralized osteoid*, the **osteoid seam**, covers the surfaces where bone is being formed. Although not always apparent histologically in decalcified tissue sections, the osteoid seam is usually more eosinophilic than previously mineralized bone tissue and, in lamellar bone, separated from it by *a basophilic line*, the **mineralization front**. The osteoid seam may be 5–15 μm in depth, depending on its rate of formation. Once mineralization of osteoid begins, it occurs very rapidly, with over 60% of the matrix becoming mineralized almost immediately. However, the remaining deposition of mineral is a slow cumulative process that can take weeks to complete.

Structural organization of bone tissue

Although the cellular elements of bone tissue, and the basic composition of the matrix, are relatively constant, there is variation in the organization of these components both at the macroscopic and microscopic level. The adult skeleton consists predominantly of mature **lamellar bone**, where the collagen fibers of the bone matrix are oriented in parallel layers. This pattern is clearly apparent in histological sections viewed under polarized light (Fig. 1.4). Osteocytes are present in small slit-like lacunae between layers in a regular pattern, their distribution reflecting the orderly manner in which osteoblasts manufacture lamellar bone. In dense cortical bone, the lamellae are organized into **osteons** or **Haversian systems**, consisting of concentric lamellae surrounding a central vascular canal (Fig. 1.4). *Osteons run longitudinally through the cortex and are cemented together by interstitial lamellae.* The trabecular or spongy bone of medullary cavities consists of variable numbers of lamellae arranged parallel to the surface rather than organized into osteons.

The alternating pattern of birefringent and nonbirefringent layers in lamellar bone has traditionally been interpreted as reflecting a 90° switch in orientation of collagen fibers between successive layers, creating a structure with physical strength similar to plywood. This model has remained unquestioned since the early 20th century, but has been challenged by recent studies using scanning electron microscopy. An alternative model proposes that lamellar bone consists of alternating layers of collagen-rich (dense) and collagen-poor (loose) lamellae, only the former showing birefringence.

A variant of lamellar bone is often seen on the weight-bearing aspects of long bones of rapidly growing animals, especially young ruminants. In these areas, the outer cortex is often arranged in laminar arrays rather than conventional Haversian systems, and is known as **laminar bone**.

In the developing fetus, and at sites of rapid bone formation during postnatal life, the *collagen fibers in bone matrix are arranged in a haphazard, interwoven fashion*. This immature form of bone tissue is referred to as **woven bone**, or coarse-bundle bone. Its matrix is more basophilic than that of lamellar bone and the osteocytes are larger, more numerous, and are irregularly arranged (Fig. 1.5). During skeletal maturation and remodeling, woven bone is resorbed and replaced with lamellar bone, which has greater strength, but it is seen in adults at sites where bone is produced rapidly in response to injury, inflammation or neoplasia. Fracture calluses invariably contain this form of bone tissue, as do bone-forming tumors.

A third type of bone, **chondroid bone**, arises directly from fibrocartilaginous origins and is found in ossifying tendon sheaths, of bone derived from neural crest origins and probably from some mixed tumors.

Formation of cranial bones

The pathology of the cranial bones often differs from bones elsewhere in the body, and some of the cranial bones may be spared in disorders that affect other bones. This is probably due to the complex origins of the cranial bones. The base of the cranium develops from a hyaline cartilage model that mineralizes and subsequently undergoes endochondral ossification. The remainder of the cranial bones and also the facial bones arise from mesenchyme directly by the process of intramembranous bone formation. Experimental studies using chimeric chick embryos and transgenic mice indicate that neural crest cells populate the sutures of membranous cranial bones, contribute to tooth morphogenesis and the formation of odontoblasts, dentine and cementum of teeth, and in the formation of the mandible and its cartilaginous articulation with the temporal bones. Chondroid bone that develops from neural crest cells populates the cranial bone sutures, and also forms as an intermediate step in the formation of intramembranous skull bones. Sutures remain as active sites of intramembranous bone production, and are probably the site of origin for the distinctive tumor that arises in the skull, the multilobular tumor of bone.

The embryology and development of the vertebral column is also complex, and in most vertebrae there are eight separate ossification centers. However, the atlas and axis differ in their structure and development.

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Regulation of bone formation and resorption

The regulation of bone cell function is accomplished by the action of a variety of systemic hormones and the local action of cytokines, which not only influence the recruitment and action of differentiated osteoblasts and osteoclasts, but also may stimulate the proliferation of their precursors. A list of systemic and local factors involved in bone remodeling is presented in Table 1.1.

Systemic hormones known to have either direct or indirect effects on the skeleton include: parathyroid hormone, 1,25-dihydroxyvitamin D$_3$, calcitonin, glucocorticoids, growth hormone, insulin, sex steroids, and thyroid hormones. **Parathyroid hormone** (PTH) is a potent activator of osteoclastic bone resorption, but its action appears to be mediated through osteoblasts or bone-lining cells as PTH receptors have been identified on osteoblasts but not osteoclasts. Furthermore, in vitro studies have demonstrated that the activation of osteoclasts by PTH depends on the presence of osteoblasts. The TNF-family molecule RANK-L (receptor activator of NF-kappaB ligand) has recently been identified as an essential signal in osteoclast development, activation, and survival. RANK-L is highly expressed in areas of bone remodeling and at sites of pathological bone loss in a variety of disease states in man and animals.

Osteoclasts are poorly able to resorb unmineralized bone matrix and since a thin layer of osteoid covers all bone surfaces, except those undergoing resorption, this layer must be removed before resorption can be initiated. Osteoblasts also may be involved in this process through activation of a latent form of collagenase, the synthesis and secretion of which are enhanced by PTH. In addition to its effect on bone resorption, PTH has been shown experimentally both to inhibit and stimulate bone formation, depending on the duration of administration and the dose rate. High doses cause increased bone resorption leading to fibrous osteodystrophy. In contrast, low and intermittent doses of PTH that are too small to affect serum calcium concentration have been shown to promote bone formation and increase bone mineral density. This anabolic effect of PTH is mediated through inhibition of apoptosis of osteoblasts and osteocytes, thereby increasing their lifespan.

Table 1.1 Factors controlling bone remodeling

Systemic factors	Local factors
Polypeptide hormones - Parathyroid hormone - Calcitonin - Insulin - Growth hormone **Steroid hormones** - Vitamin D (1,25-dihydroxyvitamin D$_3$ - Sex hormones - Corticosteroids **Thyroid hormones**	**Cytokines** - Interleukins (IL-1, IL-6, IL-11) - Insulin-like growth factors (IGF-I, IGF-II) - Transforming growth factors (TGF-β) - Bone morphogenetic proteins - Fibroblast growth factors - Platelet-derived growth factor - Tumor necrosis factors - Interferons - Colony stimulating factors (M-CSF, GM-CSF) **Prostaglandins** - Prostaglandin E2 **Nitric oxide**

Like PTH, **1,25-dihydroxyvitamin D$_3$** (1,25(OH)$_2$D$_3$), *the active form of vitamin D$_3$, is a potent stimulator of osteoclastic bone resorption.* It promotes the differentiation and fusion of osteoclast progenitors and activates mature osteoclasts, probably by a mechanism similar to, but independent of, PTH. Receptors for 1,25(OH)$_2$D$_3$ do not occur on osteoclasts but are present on osteoblasts, supporting the involvement of osteoblasts in mediating osteoclastic resorption triggered by 1,25(OH)$_2$D$_3$. The effect of 1,25(OH)$_2$D$_3$ on bone formation is complex, but it appears to be required for normal bone growth and mineralization. This effect on mineralization is partly related to maintaining adequate serum concentrations of calcium and phosphorus, but evidence for a direct effect of 1,25(OH)$_2$D$_3$ on mineralization has been provided by experiments in mice and rats. The synthesis of osteocalcin by osteoblasts is stimulated by 1,25(OH)$_2$D$_3$, but there is confusion over the exact role of this peptide hormone and other metabolites of vitamin D$_3$ on bone formation.

Calcitonin *has a direct, but transient, inhibitory effect on osteoclastic bone resorption* but does not appear to influence bone formation. Following exposure to calcitonin, active osteoclasts rapidly lose their ruffled border and become physically separated from the underlying bone surface. There is also an inhibitory effect on osteoclast formation through reduced proliferation of progenitor cells and reduced differentiation of committed precursors. Calcitonin is capable of inhibiting PTH-induced bone resorption in vitro, but the mechanism and significance of this action is unclear.

Insulin does not regulate bone resorption but *plays an important role in the synthesis of bone matrix, and in cartilage formation.* As such, it has a major influence on normal skeletal growth. Rather than influence bone cell replication, insulin stimulates bone matrix synthesis by differentiated osteoblasts. **Growth hormone** promotes longitudinal growth of bones in immature individuals and appositional bone growth in adults, but its action is indirect, being mediated through insulin-like growth factors produced by the liver.

Glucocorticoids *have a significant catabolic effect on the skeleton* through actions on both osteoblasts and osteoclasts. There is direct inhibition of osteoblastic activity, thereby reducing bone formation, and an indirect enhancement of osteoclastic resorption. The latter is probably due to inhibition of enteric calcium absorption and impaired renal tubular calcium reabsorption, leading to reduced serum ionized calcium concentration and secondary hyperparathyroidism. Reduction in bone mass is a recognized sequel to long-term corticosteroid therapy both in human and animal patients.

Estrogens and **androgens** appear to be *important regulators of skeletal growth and maturation.* Androgen receptors are present on osteoblasts, but at low densities. During puberty, androgens stimulate bone growth, while in male adults they are involved in the maintenance of the skeleton. Androgens do not affect osteoclasts directly, but may inhibit bone resorption indirectly by inhibiting the recruitment of osteoclast precursors from bone marrow. A significant effect of estrogens appears to be to inhibit bone resorption. Immediately after menopause, a decline in circulating estrogen levels in some women is accompanied by a rapid acceleration in bone loss, leading to osteoporosis. Treatment of postmenopausal women with estrogen has been shown to significantly reduce the risk of osteoporosis-related fractures. Estrogen may inhibit bone resorption by reducing the synthesis of cytokines such as interleukin-6, although the exact mechanism is unknown.

Thyroid hormones are important for normal skeletal development, primarily through *stimulation of cartilage growth,* but they also stimulate bone resorption. In human patients, accelerated bone resorption in hyperthyroidism may result in hypercalcemia and increased risk of osteoporosis. Hypothyroidism during fetal development has been linked to congenital abnormalities of the skeleton in animals. Reduced longitudinal bone growth, delayed ossification and impaired bone resorption were reported in lambs following fetal thyroidectomy, while delayed ossification of carpal bones in newborn foals has been described in association with hyperplastic goiter.

In addition to the range of systemic hormones capable of influencing bone formation and resorption, several locally produced **growth regulatory factors** or **cytokines** with paracrine, autocrine, or juxtacrine functions have been identified. In fact, these factors are likely to be more important than the systemic hormones in initiating physiologic bone resorption and remodeling, which occurs in discrete localities throughout the skeleton. Many of these growth factors become stored in the matrix and their release in active form during future episodes of bone resorption may stimulate the differentiation and proliferation of osteoblast precursors. They can be grouped into distinct families based on their action and target cell, but there is considerable overlap and redundancy in their activities.

The **insulin-like growth factors** (IGF-I and IGF-II) are growth hormone-dependent polypeptides produced by several tissues, including bone. *They are known to have powerful systemic and local effects on bone formation and maintenance of bone mass.* IGF-I, which is more potent than IGF-II, stimulates the proliferation of osteoblast precursors and enhances matrix synthesis by differentiated osteoblasts. It also inhibits the degradation of bone collagen, most likely by inhibiting the expression of interstitial collagenase by osteoblasts. The synthesis of IGF-I is stimulated by PTH and other agents that stimulate cAMP in bone cells, but is inhibited by glucocorticoids. The reduced bone mass that occurs in association with glucocorticoid excess may be due in part to this inhibition of IGF-I.

Transforming growth factor-β (TGF-β), another family of polypeptide hormones produced both in bone and other tissues, *has powerful effects on both osteoclasts and osteoblasts, and probably plays a key role in bone remodeling.* During bone resorption, TGF-βs are released from the bone matrix in active form. They inhibit the proliferation and differentiation of osteoclast precursors, as well as the activity of mature osteoclasts. TGF-βs also stimulate the replication of osteoblast progenitors, increase collagen synthesis by differentiated osteoblasts and induce osteoblast chemotaxis in vitro.

Bone morphogenetic proteins (BMPs) are a *large subgroup of signaling molecules* within the TGF-β superfamily and have been the subject of intense research in recent years. Approximately 30 different BMPs with overlapping expression patterns have been identified. BMP-specific antagonists, such as noggin and chordin, have also been identified. BMPs appear early in embryogenesis and are involved in the induction of bone and cartilage development during organogenesis. They stimulate osteoblast differentiation and have a unique property of inducing heterotopic bone formation in vivo. This has led to considerable interest in the possible therapeutic use of BMPs in disease conditions where enhanced bone formation is desirable.

Acidic and basic **fibroblast growth factors** (FGFs) also have been shown to *stimulate bone formation* but, unlike TGF-βs, they do not influence bone resorption. FGFs probably generate additional osteoblast precursors, leading to increased numbers of differentiated osteoblasts, and are effective in stimulating new bone formation to restore bone mass. Abnormalities in FGF receptors have been identified in human patients with certain inherited skeletal disorders, including achondroplasia. **Platelet-derived growth factor** (PDGF) is a *potent stimulator of new bone formation, but also promotes bone resorption.*

Estrogen depletion induced by ovariectomy in a rat model markedly increases the synthesis of **interleukin-6** (IL-6) and **interleukin-11** (IL-11) by osteoblasts or their precursors in the bone marrow stroma. *These cytokines appear to play a crucial role in the recruitment, proliferation, and differentiation of osteoclast progenitors that eventually lead to reduced bone mass in estrogen deficiency.* Further support is derived from studies in mice following deletion of the IL-6 gene. Unlike normal controls, mice lacking the IL-6 gene do not show any reduction in bone mass after ovariectomy. **Interleukin-1** (IL-1) and **tumor necrosis factor-α** (TNF-α) are related cytokines that act synergistically on bone and also have been implicated in the pathogenesis of postmenopausal osteoporosis. Both are potent stimulators of osteoclastic activity in vitro and in vivo and may be involved in mediating focal bone resorption in certain inflammatory disorders. Unlike other cytokines, **γ-interferon** does not stimulate bone resorption. In fact, it selectively inhibits the resorption stimulated by IL-1 and TNF, probably through inhibition of prostaglandin synthesis. **Osteoprotegerin** (OPG), a newly described receptor-like protein, is a member of the TNF receptor family, and acts as a decoy receptor for RANK-L. Its major role appears to be in regulating bone remodeling through a negative effect on the maturation and activation of osteoclasts.

Several **colony-stimulating factors** (CSFs) influence bone resorption by regulating the proliferation and differentiation of osteoclast precursors. Their importance is highlighted by studies in mice with the *op/op* variant of osteopetrosis, where there is impaired production of macrophage colony-stimulating factor (M-CSF or CSF-1). A decrease in osteoclast formation, leading to reduced bone resorption and osteopetrosis, can be reversed transiently by the administration of M-CSF. Granulocyte macrophage colony-stimulating factor (GM-CSF) can also increase osteoclast differentiation from their precursors, thereby promoting bone resorption. Osteoblasts are capable of secreting GM-CSF following exposure to bacterial endotoxin or PTH.

Prostaglandins (PGs), in particular those of the E series, are another group of *important mediators of local bone resorption.* They are characterized by restricted, local activity before being rapidly degraded, making studies of their in vivo action extremely difficult. Consequently, their precise role has not been determined. PGE_2 appears to directly stimulate osteoclastic activity and may be a mediator of bone resorption regulated by osteoblasts. Local production of PGE_2 in response to inflammation, mechanical trauma, or neoplasia is likely to contribute to the bone resorption that is often associated with such conditions. PGE_2 has also been linked to the bone resorption and hypercalcemia associated with certain malignancies.

Nitric oxide, generated by the nitric oxide synthase group of enzymes, may be an important mediator of bone cell function. In vitro studies have revealed a biphasic effect on bone resorption. At low concentrations, nitric oxide potentiates IL-1-induced osteoclastic bone resorption, suggesting that small amounts of nitric oxide may be required for normal osteoclast activity. In contrast, high concentrations of nitric oxide strongly inhibit bone resorption both in organ cultures and in cultures of isolated osteoclasts. This inhibitory effect appears to be due to apoptosis of osteoclast progenitors, induced by the action of nitric oxide derived from osteoblasts. The effect of nitric oxide on osteoblast function is less clear, although estrogen has been shown to stimulate nitric oxide synthase activity in both endothelial cells and osteoblasts, suggesting a possible role of nitric oxide in mediating the protective effects of estrogen on bone. At high concentrations, nitric oxide inhibits osteoblast growth and differentiation. This provides a possible explanation for the reduced bone formation and osteoporosis that occurs in association with certain inflammatory conditions. The role of nitric oxide in vivo has yet to be established, but there is increasing evidence to support its role as an important regulator of bone turnover.

Remodeling of bone tissue

Throughout postnatal life, old or defective bone is constantly being replaced with new bone tissue by a process of **remodeling**. This occurs at a local level and involves the coordinated activities of osteoclasts and osteoblasts in so-called **bone remodeling units**, presumably mediated by a complex interaction between systemic hormones and locally acting cytokines. The remodeling sequence starts with activation of osteoblasts (probably bone-lining cells) by external signals such as hormones, cytokines, or growth factors to induce accelerated formation of osteoclasts from their precursors. The osteoclasts attach to the bone surface, seal it off from the extracellular space, and resorb bone matrix and mineral. Then follows a reversal phase during which resorption ceases, the osteoclasts detach from the bone and disappear, probably by apoptosis. Osteoblasts are attracted by an unknown mechanism to the resorption site where they deposit new bone.

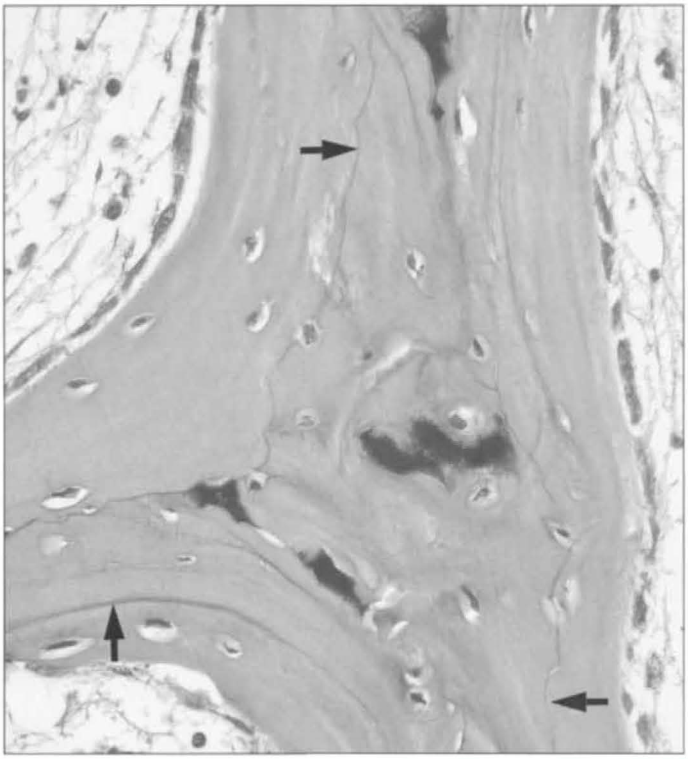

Figure 1.6 Cementing lines in a segment of trabecular bone. The smoothly contoured lines (vertical arrow) are referred to as **resting lines** and indicate sites at which bone formation had ceased for a period then recommenced. The scalloped lines (horizontal arrows) are **reversal lines** and reflect previous resorption, followed by deposition of new bone.

In the cortex, activated osteoclasts form a "cutting cone" which bores longitudinally through the dense, primary bone, creating a *resorption canal.* As the canal advances, it becomes lined by osteoblasts, which fill the space with concentric layers of new lamellar bone, creating a *secondary osteon or Haversian system.* This process provides a mechanism for on-going internal replacement of cortical bone without altering its gross form or function. Remodeling of trabecular bone follows a similar sequence, but from the trabecular surface, without the formation of resorption canals.

In histological sections, the separate units of secondary bone that form during remodeling can be distinguished from each other, and from adjacent primary bone, by the presence of deeply basophilic **cementing lines** (Fig. 1.6). These lines are created by the deposition of a thin layer of highly mineralized, collagen-free matrix at sites where bone resorption or formation ceases. Two types of cementing lines are recognized. Those with a scalloped appearance are termed **reversal lines**, and indicate a site where previous bone resorption had occurred, then new bone deposited in its place. Smoothly contoured cementing lines, or **resting lines**, mark sites where bone formation ceased for a period, then re-commenced. The number and pattern of cementing lines may provide useful information on the recent history of an area of bone, in particular regarding the rate of turnover.

Markers of bone remodeling

Although not used routinely in veterinary medicine, various **markers of bone remodeling** or turnover can be measured in serum or urine and may add support to a clinical diagnosis, or be of value in research. *Serum alkaline phosphatase activity is a well-recognized indicator of osteoblastic activity*, increased levels occurring in diseases characterized by increased bone formation such as hyperparathyroidism. Its diagnostic value is limited however by the fact that high levels are also detected normally in rapidly growing young animals. Furthermore, other isoforms of alkaline phosphatase are commonly used as indicators of cholestatic liver disease in several species and hyperadrenocorticism in dogs. Another potentially useful indicator of osteoblastic activity is *serum osteocalcin*. Approximately 10–25% of the osteocalcin synthesized by osteoblasts escapes into the circulation and serum concentrations are proportional to the rate of osteoid synthesis.

Bone resorption, associated with increased osteoclastic activity, is reflected by increased serum activity of *tartrate-resistant acid phosphatase*, an enzyme released by osteoclasts during the degradation of bone matrix. Increased urinary concentration of *hydroxyproline* has long been considered a marker of bone resorption, but this amino acid is present in all types of collagen, not just the type I collagen of bone, and cannot therefore be considered specific. Recent interest has focused on measurement of urinary concentrations of the *pyridinium cross-links* that bind the nontriple helical portions of one collagen molecule to another. Of these molecules, hydroxylysyl pyridinoline is the most abundant, but is less specific for type I collagen than deoxypyridinoline. Both can be detected in urine as either free or peptide-bound forms using commercially available kits.

In dogs, horses, rats and humans, considerable diurnal variations in the serum and urinary concentrations of bone markers have been demonstrated. This may reflect circadian rhythms in the rates of bone formation and resorption. There has been increasing interest in such markers of bone resorption in human medicine as indicators of post-menopausal bone loss or monitoring the effectiveness of antiresorptive therapy, but their application in animals is still largely confined to research.

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Ectopic mineralization and ossification

Extraskeletal deposition of either amorphous calcium phosphate or hydroxyapatite crystals, referred to as **ectopic** or **heterotopic mineralization** (calcification), occurs in a range of disease conditions, and must be differentiated from **ectopic ossification**, where the mineral is deposited in the form of bone tissue.

Three main forms of ectopic mineralization are recognized in animals: metastatic, dystrophic, and idiopathic. **Metastatic mineralization** is a frequent complication of disease conditions associated with hypercalcemia and/or hyperphosphatemia, such as vitamin D toxicity, hypercalcemia of malignancy, primary hyperparathyroidism and uremia. In dogs, direct precipitation of mineral occurs when the *calcium-phosphate solubility product*, expressed in mg/dL, persistently exceeds 60. The mineral deposited in metastatic mineralization typically occurs in the outer medullary region of the kidney, the fundus of the stomach, and the lungs, all of which are involved in acid secretion and possess a local alkaline environment favoring mineral precipitation. Other favored sites include the media of large arteries and the elastic tissue of the endocardium, but the reason for such predilection is not clear.

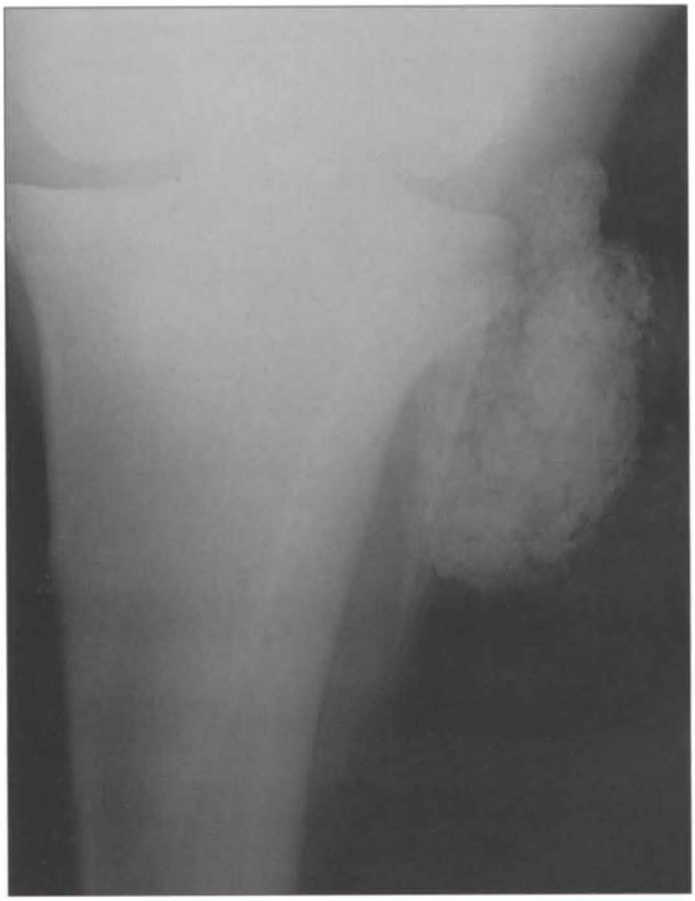

Figure 1.7 Tumoral calcinosis in a horse. A large, discrete mass lateral to the femoro-tibial joint contains multiple radiodense deposits.

Dystrophic mineralization occurs in association with *damaged tissue* rather than an elevated calcium-phosphate solubility product, probably due to the release or establishment of cellular components with nucleation properties following injury. Examples include mineral deposition in the caseous lesions of bovine tuberculosis and caprine paratuberculosis, and the mineralization of muscle fibers in animals with nutritional myodegeneration.

Idiopathic forms of ectopic mineralization occur in a variety of unrelated disease syndromes, including **calcinosis cutis**, which is characterized by widespread mineralization of dermal elastic fibers in association with hyperadrenocorticism, and **tumoral calcinosis** (*calcinosis circumscripta*), where tumor-like nodules of ectopic mineralization develop in soft tissues, often close to joints. Tumoral calcinosis in horses usually occurs in animals about 2–4 years of age, and 90% of lesions develop on the lateral aspect of the stifle (Fig. 1.7). The lesions may be single or multiple and are sometimes attached to the joint capsule, but not to the overlying skin. They usually appear as hard, well-circumscribed, nonpainful, subcutaneous swellings ranging in size from about 3–12 cm in diameter. Individual lesions consist of a tough, outer, fibrous capsule with collagenous trabeculae dividing the interior into numerous variable-sized locules consisting of finely granular, chalky white accumulations of calcium salts, surrounded by a rim of granulomatous inflammation cells. The lesions usually do not cause lameness and do not recur after surgical removal. Tumoral calcinosis in humans is also characterized by deposition of mineral in locular masses adjacent to major joints, particularly the shoulder and hip. Autosomal recessive inheritance is suggested to account for approximately one third of human cases, and hyperphosphatemia is often present, but the pathogenesis is unknown.

In dogs, the manifestations of calcinosis circumscripta are variable, and show breed preferences. In German Shepherds, the lesions are generally solitary, occurring most often in the skin near pressure points, but multiple lesions occur in some dogs, often attached to tendons, joint capsules, or the periosteum. Involvement of the vertebral processes of C-4 and C-5 was reported in three 5–6-month-old Great Dane puppies from the same litter, suggesting the possibility of a familial etiology. The lesions are usually nonpainful and nonprogressive, but may cause clinical signs if they interfere with the function of adjacent structures. For example, spinal cord compression caused by paravertebral calcinosis circumscripta occurred in two young dogs of different breeds. Although the pathogenesis is not known, repetitive trauma has been suggested as a predisposing factor, at least in some cases with skin involvement. Trauma is less likely to be responsible for lesions associated with vertebrae or involving the tongue, as has been reported.

A syndrome characterized by calcium hydroxyapatite deposition in the spinal canal of young Great Dane puppies has been reported to cause paraplegia and incoordination. Mineral deposition may also be present in joints and soft tissues of affected pups. In one report, the condition occurred in two litters of related Great Danes and was considered familial.

Ectopic ossification refers to the *formation of nonneoplastic trabecular bone in extraosseous sites*, presumably following the induction of pluripotential stem cells by appropriate growth factors. In most cases, ectopic bone is detected as an incidental finding at necropsy without an obvious predisposing cause. Typical sites include the pulmonary connective tissue of dogs and cattle, and the cervical and lumbosacral dura mater of aged dogs (ossifying pachymeningitis). Ectopic ossification also occurs in the supporting connective tissue of certain tumors in dogs, particularly mammary carcinomas, where it is very common, and to a lesser extent in thyroid and salivary carcinomas.

Specific disease entities associated with widespread ossification of soft tissues have been described both in human patients and animals and may involve overexpression or dysregulation of bone morphogenetic proteins. The temptation with such diseases in animals is to classify them according to the human syndrome that they most closely resemble. Such comparisons are usually subjective and may not be appropriate in all cases. A syndrome resembling human **fibrodysplasia ossificans progressiva** has been described in several cats and occasionally in the dog, although some of these cases more closely resemble myositis ossificans. In cats, the disease is characterized by *progressive, symmetrical, hyperplasia and ossification of connective tissue in the subcutis and epimysium of the neck, dorsum and limbs.* Affected cats have ranged in age from 5 months to 6 years. A feature of the disease in humans, and in some reported cases in animals, is the formation of bone in soft tissue sites by the process of endochondral ossification. In the 5-month-old cat referred to above, there was massive thickening of joint capsules and synovial membranes of several limb joints with disorganized hyaline cartilage (Fig. 1.8A, B). There was bilateral involvement of carpal and stifle joints in addition to several metatarsophalangeal and interphalangeal joints. Many foci of endochondral ossification were scattered throughout the hyperplastic

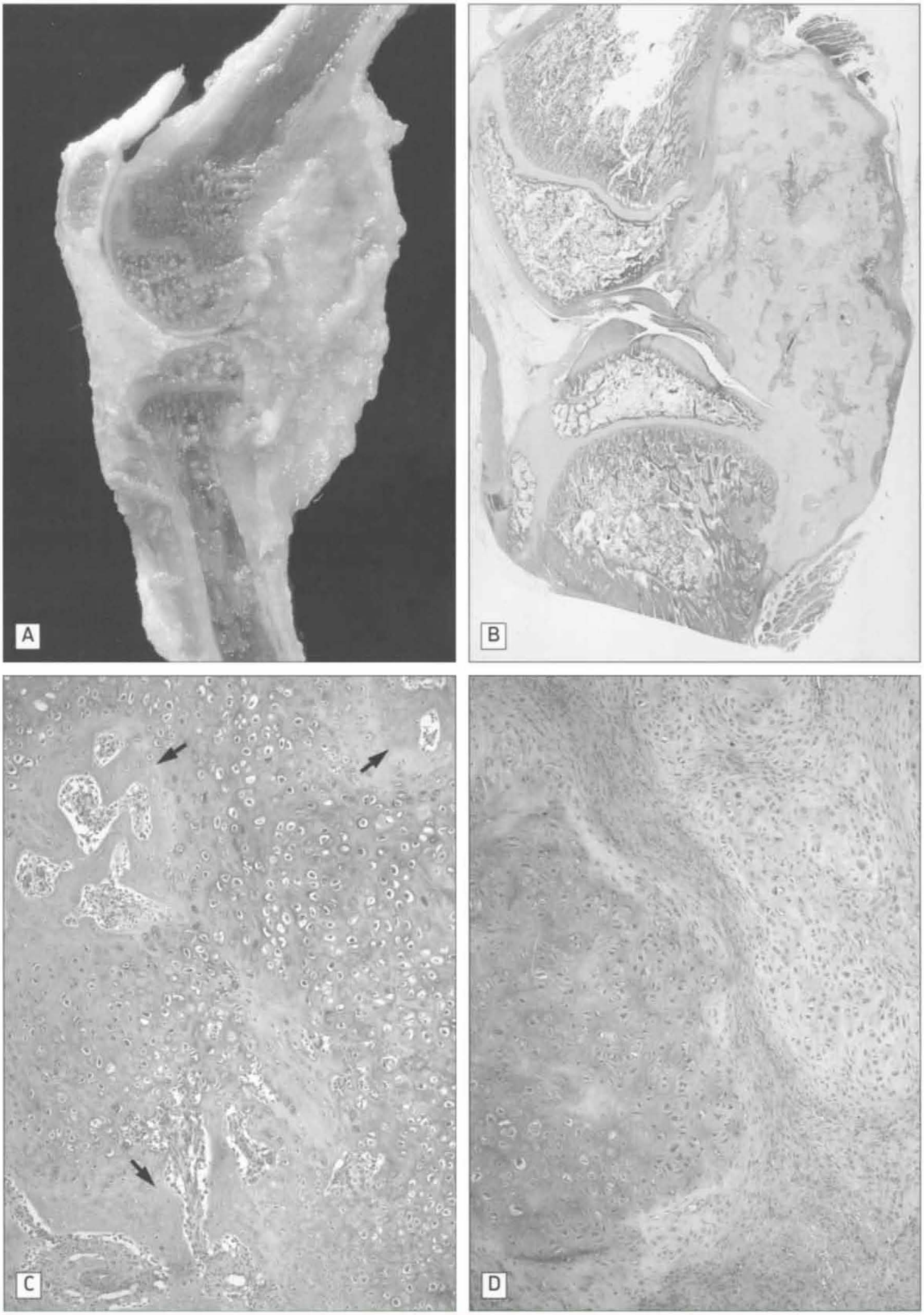

Figure 1.8 Fibrodysplasia ossificans progressiva in a 5-month-old kitten. **A.** Left stifle joint showing massive thickening of the joint capsule on the caudal aspect of the joint with tissue resembling cartilage. Similar lesions involved the right stifle, and several other limb joints. **B.** Subgross preparation of the same lesion, showing a cartilaginous mass bridging the stifle joint and attaching to the cortex of the adjacent femur and tibia in the region of the capsular insertions. **C.** Microscopically, the lesion consists of disorganized hyaline cartilage containing many plump chondrocytes and undergoing multifocal endochondral ossification (arrows). **D.** Biopsy of an early lesion from the same kitten, showing proliferating nodules of disorganized hyaline cartilage with features suggestive of chondrosarcoma.

cartilage, which, in some areas, had microscopic features suggestive of chondrosarcoma (Fig. 1.8C, D). The disease in man is associated with overexpression of at least one of the BMPs and is inherited as an autosomal dominant trait. Although there is no evidence of a genetic etiology in cats, there have been too few reported cases for this to be established.

Myositis ossificans has also been reported in cats and in a dog, but differs from fibrodysplasia ossificans in being *localized and asymmetric*. The lesions characteristically possess a peripheral zone of orderly maturation from fibrous tissue to mineralized osteoid, which is gradually replaced by lamellar bone. The prognosis following surgical removal is very good. Trauma is considered the initiating factor in most human cases of myositis ossificans, and the same probably applies to animals. Ossification of a hematoma may be the mechanism in some cases.

Generalized forms of myositis ossificans have been reported in cats and pigs but may have been more appropriately classified as fibrodysplasia ossificans. The disease in pigs was familial, occurring at 2–6 months of age in 34 of 115 offspring of an affected boar, and was characterized by widespread ossification of soft tissues, especially around vertebrae. The boar had developed similar lesions at 9 months of age.

Ectopic bone may form in lesions that have been mineralized for an extended period, possibly due to metaplasia of cells involved in the initial process of mineralization. This may account for the disseminated foci of metaplastic bone that form occasionally in the skin of dogs with hyperadrenocorticism and calcinosis cutis. Alternatively, the cutaneous osseous metaplasia in such cases may be due to local production of specific growth factors, including TGF-β, FGFs, and BMPs, by inflammatory cells in the dermis. TGF-β has been found in large quantities in inflammatory disorders of the skin of human patients with osteoma cutis, a condition similar to that described in dogs, and has been associated with the differentiation of fibroblastic cells into osteoblasts.

Where ectopic bone forms in close association with or becomes attached to a bone, differentiation of the lesion from a fracture callus or a parosteal osteosarcoma may prove difficult. Depending on its stage of maturation, a fracture callus is likely to contain remnants of cartilage or chondro-osseous bone, while *ectopic bone is composed exclusively of trabecular bone*. In parosteal osteosarcoma, the spaces between trabeculae will be populated with mesenchymal cells showing features of malignancy, rather than a single layer of osteoblasts lined up along the surface of bone trabeculae.

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BONES AS ORGANS

Development and anatomy

There are two distinct processes by which bone formation occurs in the developing fetus. *Flat bones of the skull develop by* **intramembranous ossification**. Mesenchymal progenitor cells migrate from the neural crest to form condensations at specific, highly vascular, sites in the head region and some other flat bones, where they differentiate directly into osteoblasts and produce anastomosing trabeculae of woven bone. These centers of ossification expand by on-going osteoblastic differentiation of mesenchymal cells at the periphery and apposition of new bone on the surface of trabeculae, to form a plate. A fibrous layer, *the periosteum*, separates the developing membrane bone from adjacent tissues and controls its shape. Individual bones of the developing skull are separated by connective tissue **sutures** until growth ceases, at which time a bony union forms. With maturity, the woven bone is remodeled and replaced by lamellar bone. Intramembranous bone formation also occurs at the periosteal surfaces of all bones during growth.

Most bones of the skeleton, including those of the limbs, vertebral column, pelvis, and base of the skull, develop by **endochondral ossification**. In this process, condensations of primitive mesenchymal cells differentiate into chondrocytes and produce crude cartilage models of the adult bone destined to form at that site. An avascular fibrous layer, the perichondrium, surrounds each cartilage model. As expansion of the model continues by interstitial growth, chondrocytes near the center become hypertrophic, and the matrix undergoes mineralization. Meanwhile, the perichondrium becomes invaded with capillaries, converting it into a periosteum, and a narrow cuff of bone forms by intramembranous ossification around the mid-shaft region of the developing bone. Capillaries and osteoclasts then invade the hypertrophic cartilage from the periosteum and establish a vascular network. Preosteoblasts also enter with the invading capillaries and differentiate into osteoblasts, which deposit osteoid on remnants of the mineralized cartilage, creating a **primary ossification center**. This process of endochondral ossification continues as the chondrocytes at either end of the developing bone continue to proliferate and the model expands in length and width. Once the bone reaches a certain stage of development, **secondary ossification centers** appear at one or both ends (depending on the bone), and expand by endochondral ossification to form the **epiphyses** of long bones. As the epiphyses expand, they remain separated from the primary ossification center, now occupying the **diaphysis** and **metaphysis** of the developing bone, by the **physis** or **growth plate**. Limited growth in size of the epiphysis continues by endochondral ossification beneath

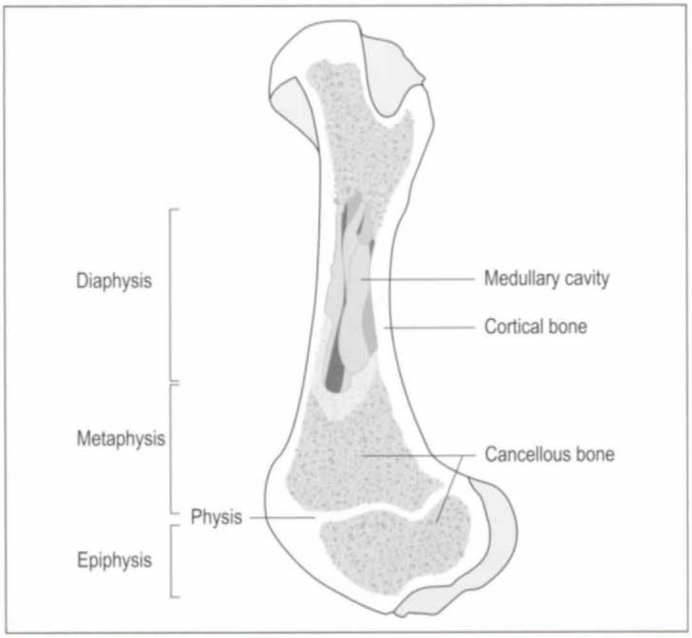

Figure 1.9 Drawing of the femur from a newborn calf, illustrating the **gross anatomy and terminology** of the different regions.

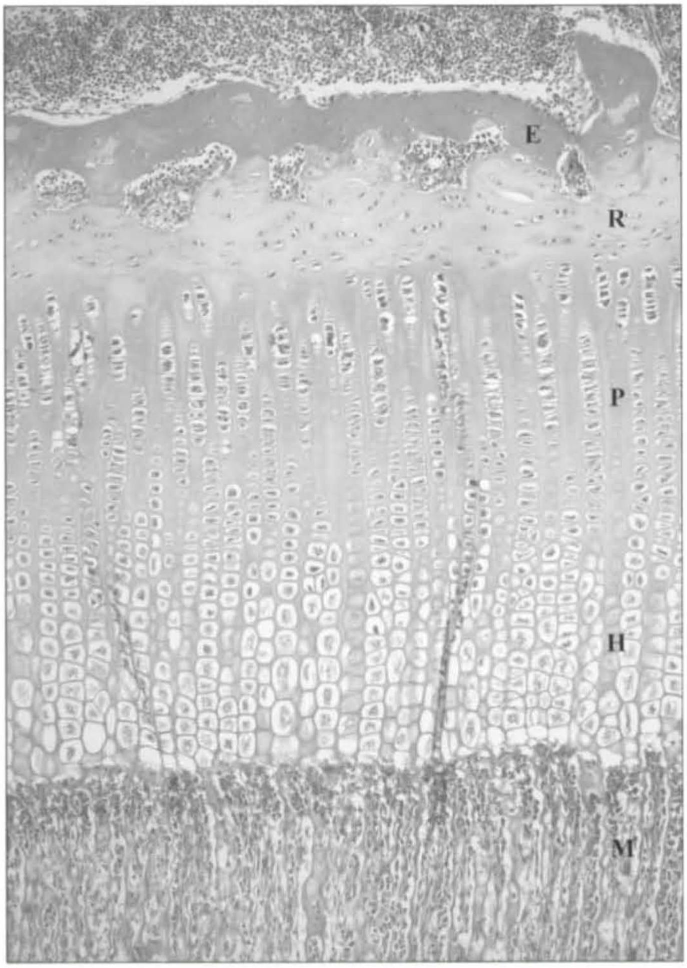

Figure 1.10 Physis or growth plate of a young animal showing the reserve (R), proliferative (P), and hypertrophic (H) zones. The reserve zone is anchored to trabecular bone of the epiphysis (E). Also note the abrupt transition from the hypertrophic zone of the physis to the metaphysis (M).

the articular cartilage at the articular–epiphyseal cartilage complex. The epiphyseal side of the growth plate soon becomes capped by a layer of trabecular bone, preventing further growth from that side, but proliferation of chondrocytes in the growth plate and endochondral ossification on the metaphyseal side continues until maturity. The gross anatomy and terminology of a developing long bone, in this case the femur of a newborn calf, is illustrated in Figure 1.9.

During active bone growth, *the hyaline cartilage of the growth plate is organized into three easily recognizable zones* (Fig. 1.10). A **reserve**, or **resting zone**, with irregularly dispersed chondrocytes and pale-staining matrix, is anchored to the trabecular bone of the epiphysis. The chondrocytes in this zone have the lowest concentration of intracellular ionized calcium, but the matrix has the highest concentration of type-II collagen. In the **proliferative zone**, the chondrocytes are tightly packed into longitudinal columns, the cell at the top being the progenitor cell for longitudinal growth of each column. The chondrocytes in this zone are actively dividing, accumulating glycogen, and synthesizing matrix proteoglycans. The columns of chondrocytes are separated by deeply basophilic cartilage matrix rich in aggregated proteoglycans, which inhibit mineralization in spite of the presence of matrix vesicles. Within columns, only thin matrix septa separate individual chondrocytes. The chondrocytes of the **hypertrophic zone** become enlarged, but remain metabolically active and are responsible for preparing the matrix for mineralization. They rely on anaerobic glycolysis for energy production because of the distance from epiphyseal blood vessels, which terminate at the top of the proliferative zone, and the inability of oxygen to diffuse from the metaphysis through the mineralized matrix of the lower hypertrophic zone. The energy is used primarily in the accumulation, storage, then release of calcium as part of the mineralization process. The lower region of the hypertrophic zone is commonly referred to as the *zone of degeneration*, as the chondrocytes appear to have separated from the pericellular matrix and become degenerate in sections prepared for histology and electron microscopy by routine methods. Following the development of more sophisticated techniques for preparing sections of cartilage for transmission electron microscopy, this concept has been challenged. Rather than being degenerate, these chondrocytes appear to be highly differentiated cells capable of synthesizing type-X collagen, chondrocalcin and other macromolecules that, together with matrix vesicles, are likely to be involved in initiating matrix mineralization. Mineralization of the cartilage matrix occurs in the deepest layer of the hypertrophic zone and is an essential event in the process of endochondral ossification. This mineralized layer is not evident in histological sections prepared after demineralization. The transition from growth plate to metaphysis is abrupt, and is designated by the last intact layer of chondrocytes.

Around the perimeter of the growth plate there is a wedge-shaped groove of cells, termed the **ossification groove of Ranvier**. The cells in this groove proliferate and are responsible for increasing the diameter of the physis during growth. A dense layer of fibrous tissue, the **perichondrial ring of LaCroix**, surrounds the groove of Ranvier and is continuous with the fibrous layer of the periosteum. As such, it provides strong mechanical support at the bone–cartilage junction of the growth plate, an area that is prone to injury in fast-growing young animals.

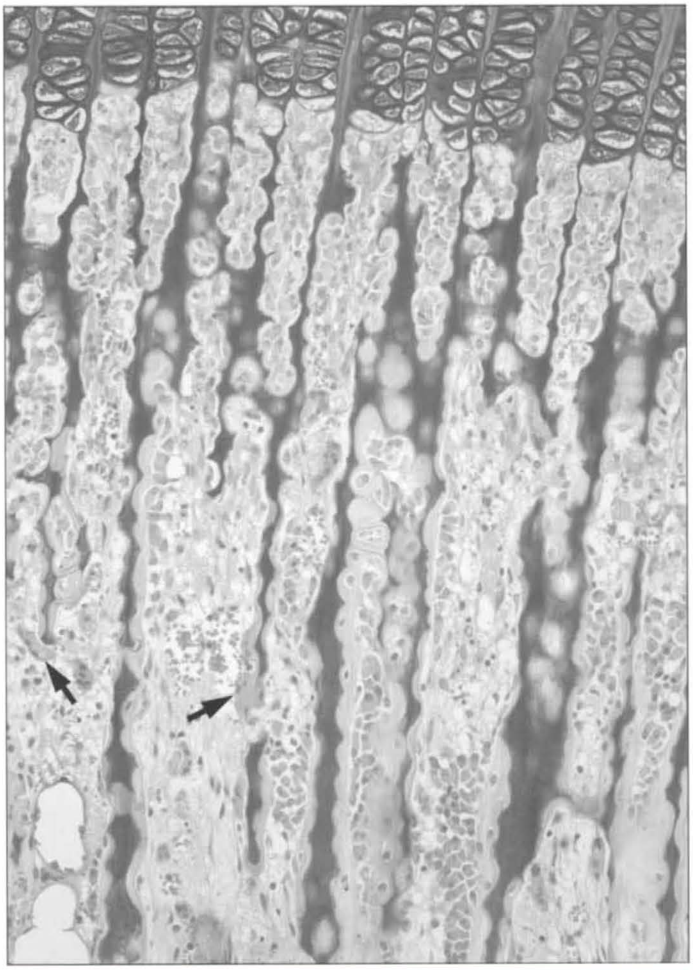

Figure 1.11 Primary spongiosa in a rapidly growing young animal. Basophilic spicules of calcified cartilage matrix extending into the metaphysis at right angles to the growth plate, form a lattice on which osteoblasts are lining up and depositing osteoid. Osteoclasts (arrows) are resorbing some trabeculae from the medullary end.

From the metaphyseal side of the growth plate, osteoclasts attack the mineralized cartilage matrix and rapidly remove the delicate transverse septa between individual chondrocytes within columns, allowing vascular invasion. The thicker longitudinal septa of mineralized cartilage matrix between columns of chondrocytes are not resorbed at this stage. Instead, they provide a framework on which newly differentiated osteoblasts line up and deposit a layer of woven bone (Fig. 1.11). *This lattice of trabeculae, with a basophilic core of mineralized cartilage covered by a thin, eosinophilic layer of bone is termed the* **primary spongiosa.** Trabeculae of the primary spongiosa extend at right angles to the direction of the growth plate, but deeper in the metaphysis, the trabeculae are remodeled by the coordinated action of osteoclasts and osteoblasts and are realigned in directions most suited to withstanding the mechanical forces acting on the bone. During this process, the cartilage cores and woven bone of the primary spongiosa are largely removed and replaced by thicker trabeculae of lamellar bone, which form the **secondary spongiosa.** While growth in length of a bone is continuing from the growth plate, osteoclastic resorption of trabeculae occurs at the metaphyseal–diaphyseal junction in order to *create the medullary cavity.*

The thickness of a growth plate is relatively constant across the width of the bone and is proportional to its rate of growth. So too is the distance to which trabeculae of the primary spongiosa extend into the metaphysis before they are remodeled. As growth slows, the different layers within the growth plate become narrow, and a transverse layer of trabecular bone forms on the metaphyseal side. The cartilage of the growth plate is then replaced with a bony scar, which is gradually remodeled into trabecular bone, blurring the margin between the epiphysis and metaphysis. The timing of growth plate closure varies both between and within bones and is controlled to a large degree by androgens and estrogens, but it is likely that nutritional factors can also play a role. In the radius, the distal growth plate remains open longer, and contributes significantly more to the length of the bone, than the proximal growth plate. In the humerus, femur, and tibia, the opposite is true. *The fastest-growing growth plates are the ones that are most likely to suffer damage due to trauma or nutritional imbalances*, and are therefore worthwhile sites to examine at necropsy, and to sample for histopathology.

The growth in width of the diaphysis in young animals occurs by intramembranous ossification beneath the **periosteum**, *which covers the surface of bones except at their articular ends and at insertion points of muscles and tendons.* The periosteum has a tough outer fibrous layer and a more cellular inner layer, *the cambium*, which contributes preosteoblasts for new bone formation (Fig. 1.12). Where muscle fibers and tendons insert onto bones, dense collagen fibers termed **Sharpey's fibers** become embedded in the bone matrix. The periosteum has a rich supply of nerve endings and blood vessels. *The inner bone surface is lined by a thin layer of osteogenic cells called the* **endosteum.**

Regulation of physeal growth

Many of the systemic hormones and local growth factors that regulate the formation and resorption of bone tissue also influence growth plate function. Their effect may be on a particular zone of the growth plate, and may vary with the age of the animal. **Growth hormone** and its mediators, the **insulin-like growth factors** (IGF-I and IGF-II), act throughout all zones of the growth plate, but **IGF** receptors are most abundant in the proliferative zone, implying a strong influence on cellular proliferation and hence longitudinal growth. **Thyroid hormones** are essential for cartilage growth and the maturation of chondrocytes, and appear to act synergistically with IGF-I. Deficiency of thyroid hormones leads to growth retardation and cretinism. **PTH** acts primarily on the proliferative and upper hypertrophic zones of the growth plate, and has a direct mitogenic effect on chondrocytes, as well as stimulating proteoglycan synthesis. It enhances the mitogenic effect of local growth factors. **Calcitonin** has been shown to accelerate chondrocyte maturation and matrix mineralization. The primary influence of **glucocorticoids** on the growth plate is to inhibit chondrocyte differentiation and proliferation. In young animals, excessive glucocorticoids from either endogenous or exogenous sources result in growth retardation. At physiological concentrations, **androgens** are anabolic factors, stimulating proteoglycan synthesis by chondrocytes in young animals, but they also stimulate mineralization in the growth plate. High doses of androgens actually depress growth and accelerate growth plate closure. **Estrogens**, in general, exhibit an inhibitory effect on longitudinal bone growth, excessive levels leading to premature closure of growth plates.

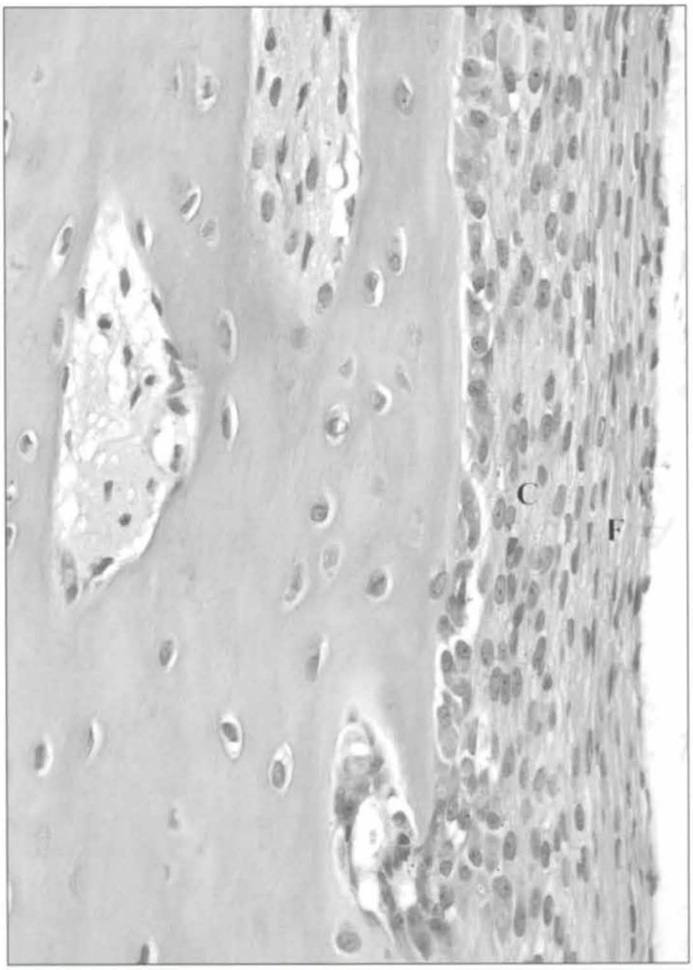

Figure 1.12 Periosteum in an actively growing young animal. Note the outer fibrous layer (F) and the cambium layer (C) containing primitive mesenchymal cells. A single layer of active osteoblasts lines the bone surface.

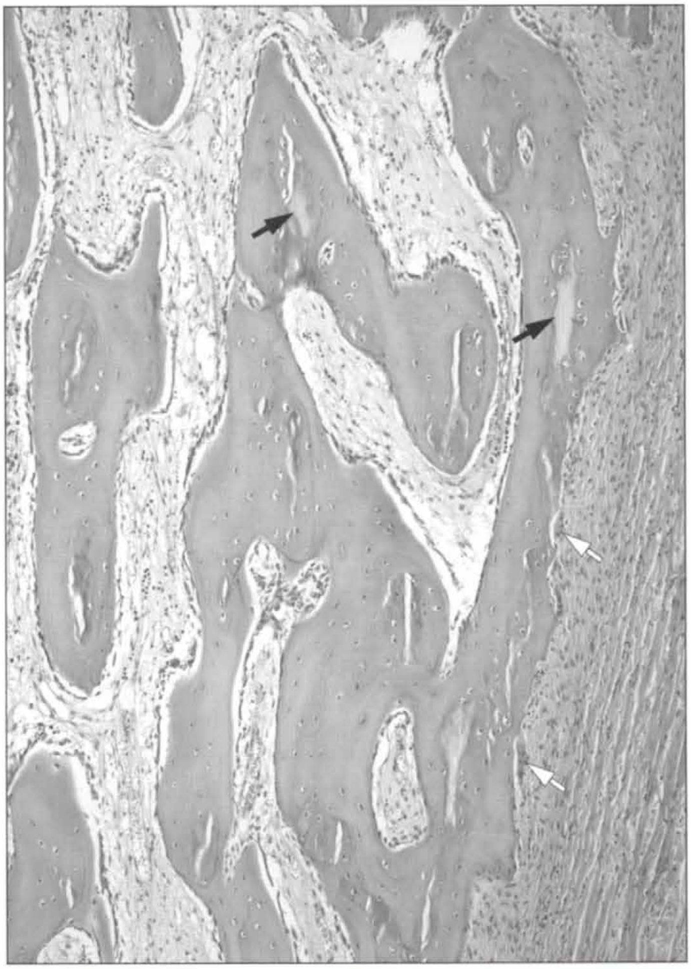

Figure 1.13 Architectural modeling in the metaphysis of a long bone during growth in length. In order to maintain the flare in the metaphyseal region, osteoclasts (white arrows) resorb bone at the periosteal surface, while osteoblasts actively deposit bone along trabeculae of the primary and secondary spongiosa. Trabecular bone of endochondral origin is thus converted into dense cortical bone. The spicules of mineralized cartilage (black arrows) derived from the growth plate persist until the cortex is remodeled.

Replication of chondrocytes is stimulated by several local growth factors, including **TGF-β**, **FGF**, and **epidermal growth factor** (EGF). TGF-β, in addition to its role as a regulator of bone formation, is also involved in the formation of cartilage, and has been shown to be a potent inhibitor of interleukin-1, which induces degradation of growth plate chondrocytes. **Prostaglandins** are present at low levels in the growth plate and although they have been shown to inhibit alkaline phosphatase activity and collagen synthesis while stimulating proteoglycan synthesis, their overall effect on growth plate function in unclear.

Modeling

In order to establish the unique shape of a long bone, extensive architectural **modeling** *occurs throughout the growth phase.* As the bone increases in size, the diameter of the diaphysis increases by deposition of new bone beneath the periosteum and resorption from the endosteal surface. However, growth in length is more complex and involves the coordinated actions of osteoclasts and osteoblasts operating on different bone surfaces. The diameter of most long bones is greatest at the level of the growth plate, then tapers through the metaphyseal region to its narrowest region in the diaphysis. This basic funnel shape is maintained during growth in length by continual osteoclastic resorption beneath the periosteum around the circumference of the metaphysis, thereby reducing its diameter. Meanwhile, osteoblasts rapidly deposit new bone within tunnels between the peripheral trabeculae of the primary and secondary spongiosa, converting it into dense cortical bone (Fig. 1.13). During this process, spicules of mineralized cartilage originating from the growth plate become incorporated into the cortex and will remain there until they are removed by remodeling.

The peripheral metaphysis of a growing long bone is therefore an area of intense osteoclastic and osteoblastic activity. The cortex is relatively porous, consisting of trabecular bone undergoing compaction, and there is extensive peritrabecular fibrosis. This must be borne in mind when examining histological sections from such areas in young animals with suspected metabolic bone diseases, particularly fibrous osteodystrophy.

The normal curvature present in some bones is produced during growth by a modeling process referred to as **osseous drift**, whereby the shaft of a bone moves on its long axis. This is accomplished by successive waves of osteoblastic and osteoclastic activity beneath appropriate periosteal and endosteal surfaces of the diaphyseal cortex,

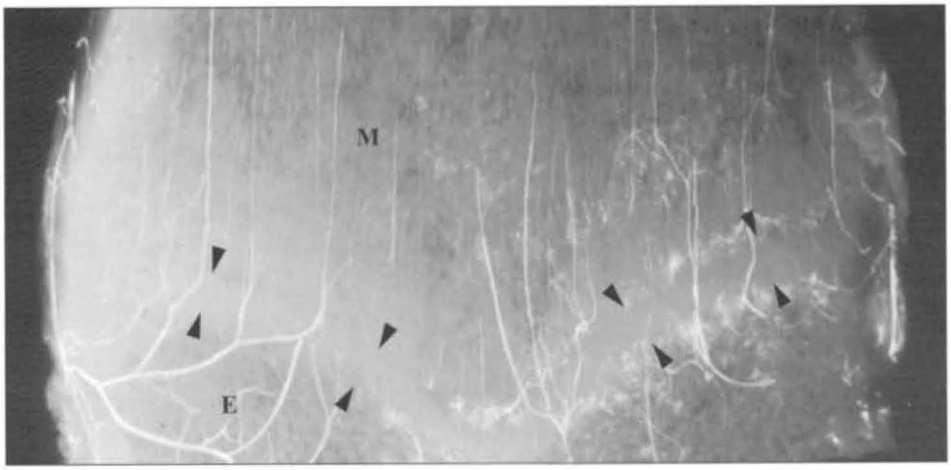

Figure 1.14 Transphyseal blood vessels. Cabinet radioangiogram of a 2 mm thick slice of decalcified distal 3rd metacarpal bone from a 13-day-old foal. The physis is between the arrowheads. The arterial blood supply to this area had been injected with radio-opaque dye immediately after death. Numerous transphyseal arteries can be seen crossing from the epiphysis (E) to the metaphysis (M). (Courtesy of EC Firth.)

presumably under the influence of both genetic and mechanical forces, leading to the formation of laminar bone deposits. The same process is involved in efforts to correct shape abnormalities in long bones resulting from malunited fractures, or other acquired defects altering the mechanical forces acting on a bone.

Bones respond to increased usage during the growth phase by an increase in bone mass, particularly in the density and thickness of the cortex. In adults, increased mechanical usage does not increase bone mass, but can decrease remodeling and conserve the amount of bone already present.

Blood supply

The blood supply to bones is derived from arteries entering the medullary cavity through foramina in the cortices of the diaphysis, metaphysis, and epiphysis, as well as periosteal arteries. In young growing animals, **nutrient arteries** supply the diaphyseal marrow and most of the central area of the metaphysis, while **metaphyseal arteries** supply the peripheral regions. Terminal branches from these vessels pass vertically towards the metaphyseal surface of the growth plate, where they end in fenestrated capillary loops immediately below the last intact transverse septum of the mineralized cartilage matrix. At this point they turn back sharply into wide-bore venules characterized by low flow rate. Some terminal branches of the nutrient and metaphyseal arterial systems anastomose with each other but they do not penetrate the growth plate.

Epiphyseal arteries supply the epiphyses or secondary centers of ossification and small branches pass through narrow cartilage canals in the reserve zone of the growth plate to terminate at the start of the proliferative zone. This is the only source of oxygen and nutrients to the growth plate as no blood vessels terminate in the hypertrophic zone. Further branches of the epiphyseal artery pass to the undersurface of the overlying articular cartilage, where they form vascular loops similar to those on the metaphyseal side of growth plates, and participate in endochondral ossification.

Transphyseal blood vessels have been identified in newborn animals of several species (Fig. 1.14), but their function remains obscure. Most evidence suggests that the direction of arterial flow in these vessels is from the epiphysis to the metaphysis, but that venous flow occurs in the opposite direction. Transphyseal blood vessels do not provide nutrients to the growth plate but may enhance the blood supply to the metaphysis during the rapid growth phase in very young animals. At sites where transphyseal vessels enter the metaphysis, they are surrounded by cartilage projections, which might be expected to strengthen the union between the epiphysis and metaphysis at a time when the growth plate is highly susceptible to shear forces. These vessels may also be involved in certain diseases of bones, such as osteomyelitis, where they provide a possible route for spread of infection across the growth plate. The periphery of the growth plate is supplied by perichondrial arteries to the perichondrial ring of LaCroix, and by metaphyseal arteries.

The blood supply to the bone cortex in young animals is predominantly derived from the endosteal surface by way of nutrient arteries, and the flow of blood within the cortex is centrifugal. Arterial blood enters Haversian systems of the cortex through capillaries communicating with medullary sinusoids, but venous drainage occurs through the periosteal surface. With age, the cortex becomes increasingly dependent on **periosteal arteries** for its blood supply.

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POSTMORTEM EXAMINATION OF THE SKELETON

Of all organ systems, the skeleton is perhaps the most neglected during postmortem examination, even by experienced pathologists. Most organs are examined as part of the routine necropsy technique, but examination of the skeleton is more often confined to those occasions where the clinical history clearly indicates a skeletal problem. As a result, many skeletal disorders are likely to be missed. Furthermore, lack of familiarity with the normal appearance of skeletal structures commonly leads to misinterpretation in cases where a skeletal disease is suspected and a detailed examination of the skeleton is performed.

Gross examination

Complete examination of the skeleton is both impractical and unnecessary. A standard procedure for examining the skeleton should include an assessment of the shape, flexibility and breaking strength of readily accessible bones, such as ribs, cranium and key limb bones during routine necropsy. *No skeletal examination is complete without sectioning one or two representative long bones longitudinally* to reveal the growth plates, the thickness of the cortex, and the amount and density of trabecular bone in metaphyseal and epiphyseal regions. When the clinical history suggests the possibility of a skeletal disorder, a more detailed assessment is required. Antemortem radiographs are a valuable component of the gross examination in such cases and may highlight areas requiring special attention. The pathologist should insist on viewing them before commencing the necropsy. Radiographs of lesions identified during necropsy, either in the form of whole bones or sawn slabs, can also provide valuable information on the extent and severity of bone lysis or demineralization, but is an insensitive indicator of diffuse bone loss, as occurs in osteoporosis.

The manifestations of generalized skeletal diseases are likely to be most severe in certain bones. Even within bones, some regions may be affected more severely than others. For example, lesions associated with metabolic bone diseases, such as rickets and fibrous osteodystrophy, will be most marked at sites of rapid bone formation. The growth plates of the distal radius, proximal humerus, distal femur, and proximal tibia should therefore be targeted for gross and histological examination. Costochondral junctions of the largest ribs are also useful sites to examine in such cases. In osteoporosis, the depletion of trabecular bone is more rapid than that of cortical bone, presumably due to the greater surface area available for resorption in trabecular bone tissue.

Histological techniques and stains

Bone specimens for histological processing should be sawn at approximately 5 mm thickness and immersed in neutral buffered formalin. Other than in a few specialist laboratories equipped to prepare undemineralized bone sections, the specimens must then be demineralized prior to sectioning. In most laboratories, this involves the use of *commercial decalcifying agents*, usually consisting of strong acid solutions, which induce decalcification within 24–48 h. In the interests of section quality, the specimen should not be left in the decalcifying fluid any longer than necessary. It is important that bone slabs are no thicker than 5 mm, in order to minimize the time they spend in the fluid. The endpoint for decalcification can be judged by probing the tissue with a needle or by radiography. The decalcified tissue should be immersed in flowing tap water for 2–4 h to remove the acid, which would otherwise interfere with staining procedures. Although strong decalcifying solutions will allow the rapid preparation of sections for diagnostic purposes, they will also cause more tissue damage and may therefore impair interpretation. *Slower decalcification* in a chelating solution such as ethylene-diamine tetra-acetic acid (EDTA), will take approximately 7 days, but enables the preparation of higher-quality histological sections.

The *preparation of undecalcified sections* requires the use of plastic embedding media, such as methyl methacrylate, and a heavy-duty sledge microtome. Several useful staining methods for undemineralized bone sections are available, including hematoxylin and eosin, Von Kossa, and Villanueva's bone stain.

Hematoxylin and eosin is also a good general-purpose stain for routine histological examination of demineralized bone sections, allowing clear differentiation of bone and cartilage matrices, and providing adequate cellular detail. However, it does not reliably allow assessment of the thickness of osteoid seams, which is of diagnostic significance in diseases such as rickets or osteomalacia. These seams generally appear pale orange/pink, in contrast to the slightly more basophilic bone that was previously mineralized, but the distinction is often too subtle or variable to allow confident interpretation. The Masson's trichrome method is another useful general-purpose stain for bone sections, but has similar limitations with regard to identifying osteoid seams. Staining methods that allow identification of unmineralized osteoid in demineralized sections have been published (see Ralis and Ralis, 1975 and Tripp and MacKay, 1972 in bibliography) and, although not used routinely, can be easily performed in laboratories that are unable to cut undemineralized sections.

Preparation artifacts in histological sections

Because of the difficulty in preparing histological sections from bones, artifactual changes are often present and could be misinterpreted as lesions. During the process of sawing bones prior to demineralization, multiple small, irregular-sized fragments of *bone "sawdust"* and soft-tissue debris often become embedded in spaces between bone trabeculae (Fig. 1.15). Such fragments are commonly misinterpreted as necrotic bone. Rinsing the cut surface of the bone under running water, and gently brushing it before fixation, can minimize this artifact. Since the fragments will be most abundant near sawn surfaces, further trimming of the face to be sectioned, after demineralization, will further reduce them. In a section where "sawdust" is a problem, slicing deeper into the paraffin block is likely to yield cleaner sections for examination.

The heat generated by a band saw, or power drill in the case of bone biopsies, may create coagulative changes, resembling early ischemic necrosis, along the edges of the specimen. Overexposure to strong acid solutions during decalcification inhibits the staining of nucleic acids by hematoxylin, and of collagen by eosin, resulting in poor cellular detail and difficulties in interpretation.

Another common histological artifact is the presence of empty clefts between bone surfaces and the soft tissues of the marrow cavity. This reflects the much greater shrinkage of the soft tissues, when compared to bone, during

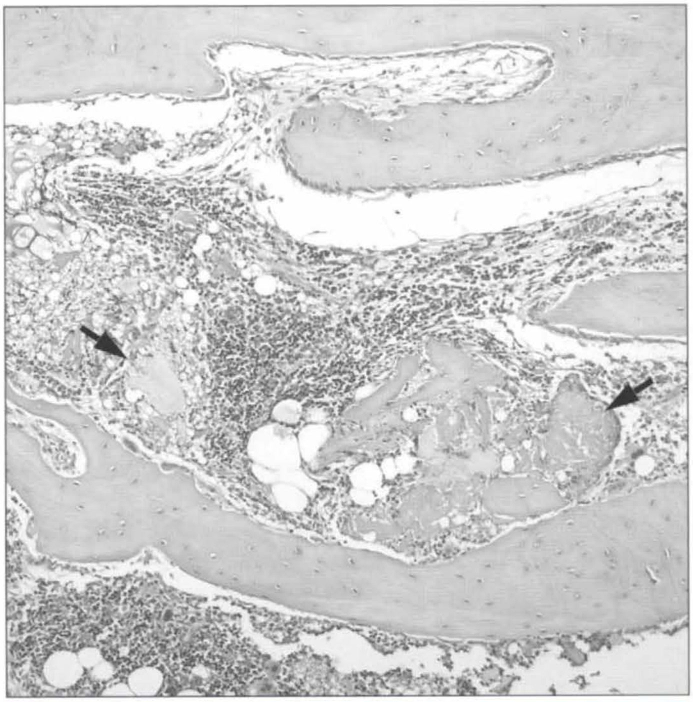

Figure 1.15 The multiple small fragments of bone and cartilage (arrows) embedded in the marrow spaces between trabeculae are **artifacts of sawing** ("sawdust") and should not be misinterpreted as lesions. Such artifacts are commonly present if sections are prepared too close to a sawn surface.

fixation in formalin. Consequently, osteoblasts or osteoclasts lining the bone surface may become separated from their site of activity. However, where bone resorption has been occurring, the surface of the bone will have a characteristic scalloped appearance. Also, bone does not adhere to microscope slides as well as soft tissues of the marrow spaces and may become dislodged, leaving large spaces lined by osteoblasts. These could be misinterpreted as vascular spaces.

Other laboratory techniques

The use of technetium labeling to identify areas of metabolically active bone, detected by scintigraphy, has dramatically improved the ability to detect bone abnormalities in the live animal. This technique greatly assists in locating multifocal lesions, such as the spread of metastatic disease within the skeleton, and although widely used in human medicine, has a relatively limited use for this purpose in animals.

A variety of other techniques may be used in the study of bones, but most are confined to the research laboratory. **Bone ash** measurements have historically been performed in animals with suspected metabolic bone diseases, but are of limited value for routine diagnosis because of the variability between individual bones and the lack of reliable reference ranges for animals of different age groups. More sophisticated and accurate methods for determining **bone density**, such as dual energy X-ray absorptiometry (DEXA scanning), have been developed for assessing bone mineral density in human patients, but are not readily available to most veterinary pathologists.

Microradiography of thick sections provides an indication of the pattern and degree of mineralization within the bone. Sections of bone, 60–100 μm thick, are placed in close contact with X-ray film and exposed. This creates an image of the bone section, which can be examined microscopically in association with histological sections prepared from the same slab.

The periodic administration to growing animals of **fluorescent markers**, which are deposited at sites of active mineralization, allows objective measurement of the rate of bone formation in physiological and disease states. The most commonly used marker is the antibiotic **tetracycline**, which fluoresces bright yellow when examined in undemineralized sections under UV light. Since the marker is only deposited at sites of active mineralization, a thin fluorescent line results from each dose. The distance is measured between lines representing sequential periods of exposure to the marker, and the rate of bone formation estimated.

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RESPONSE TO MECHANICAL FORCES AND INJURY

The cells of bone tissue are capable of the same basic cellular responses as most other tissues, including *atrophy, hypertrophy, hyperplasia, metaplasia, neoplasia, degeneration, and necrosis*. Bones have an excellent capacity for repair or modification in response to a wide range of injurious stimuli or changes in mechanical demand. Depending on the stimulus, the response may be localized or generalized but, in general, the magnitude of skeletal response is greater in young growing animals than in adults. If the response is generalized, it is likely to be most prominent at sites of rapid bone growth or modeling.

Mechanical forces

Bone adapts or remodels in response to the mechanical demands placed upon it. According to **Wolff's law**, *it is deposited at sites where it is required and resorbed where it is not*. For example, trabeculae in the epiphyseal and metaphyseal regions of long bones are aligned in directions which best reflect the compressive forces associated with weight bearing, and the tension associated with muscle insertions. In young individuals, increased mechanical stress on the skeleton increases the density of metaphyseal trabecular bone and the thickness of cortices. Increased mechanical usage in adults does not lead to an increase in bone mass, but reduces remodeling activity and conserves the amount of bone already present. Decreased activity accelerates bone loss by removing the inhibition of remodeling, and reduces formation, leading to a net reduction in bone mass.

Reduced mechanical stress on bones due to partial or complete immobilization, as occurs during fracture repair, leads to increased resorption, resulting in decreased bone strength and stiffness. If an implant, such as a metal plate, remains attached to a bone after a fracture has repaired, it will share the mechanical load with the bone. The bone will then atrophy in proportion to the decreased load and its strength will be greatly reduced. For this reason, *rigid implants should be removed soon after a fracture has healed*. Such implants may also trigger the development of an osteosarcoma at the site (see below), providing further reason for their removal.

Prolonged weightlessness associated with space travel has also been shown to result in decreased bone mass in weight-bearing bones. In contrast, increased mechanical stress associated with strenuous exercise has been associated with increased bone density.

Growth plate damage

In young growing animals, the growth plate is the weakest structure in the ends of long bones and is prone to traumatic injury resulting from shearing forces, compressive forces, or, in the case of traction epiphyses (e.g., lesser trochanter of the femur), excessive tension. In general, *the fastest growing growth plates are the most susceptible to injury*, the distal radial physis being the most commonly affected. Undulations in the growth plates of some bones increase their resistance to separation in response to shearing forces.

The consequences of growth plate injury depend on several factors, including the nature of the lesion, its location, the age of the animal and the status of the blood supply. Growth plates subjected primarily to traction consist at least partly of fibrocartilage, which imparts increased resistance to tensile forces. Such growth plates are sometimes referred to as **apophyses**.

Complete separation through the growth plate, referred to as **epiphyseolysis** (or "slipped epiphysis"), is a relatively common sequel to severe trauma or horizontal shear forces acting in the region of the bone–growth plate interface. The separation almost invariably occurs through the hypertrophic zone, where the cell volume is greatest and the matrix, which provides strength to the physis, is relatively sparse. Providing the epiphyseal vasculature has not been disrupted, the prognosis for this type of fracture is very good as the proliferative zone of the growth plate, and its blood supply, are likely to remain intact. However, epiphyseolysis of the capital femoral epiphysis, which may be associated with birth trauma in calves and occurs with some frequency in growing foals and puppies, may result in avascular necrosis of the femoral head. This reflects the greater risk of vascular damage as the nutrient vessels to the proximal femoral epiphysis travel along the neck of the femur and traverse the rim of the growth plate. The vessels supplying most other long bone epiphyses enter the bone at some distance from the growth plate and are protected by the periosteum or the fibrous layer of the joint capsule. A syndrome characterized by physeal dysplasia and slipped capital femoral epiphysis has been described recently in cats (4.5–24 months of age), most of which were male and obese. The Siamese breed was over-represented. In affected cats, the physeal cartilage was abnormally thick and chondrocytes were in disorganized clusters surrounded by abundant matrix. A similar syndrome is recognized in adolescent, overweight boys. Epiphyseolysis of the femoral head also occurs in pigs and deer as a manifestation of osteochondrosis, where there is likely to be an underlying weakness in the growth plate. Slipped capital femoral epiphysis must be distinguished from Legg–Calvé–Perthes disease and fractures through the femoral neck.

The *most common type of physeal fracture* reported in dogs, cats, horses, and humans, is characterized by extension of the fracture along the growth plate for a variable distance, then out through the metaphysis, leaving a triangular fragment of metaphyseal bone still attached to the growth plate. As with complete epiphyseolysis, the prognosis for further growth is very good. In contrast, fractures that cross the growth plate, with displacement of the fragments, will lead to the formation of a bony bridge between the metaphysis and epiphysis, precluding further growth in length at that site.

It is relatively common for epiphyseal separations, similar to those described above, to be induced during postmortem examination of young animals when limb joints are disarticulated forcefully. Such "fractures" are not accompanied by hemorrhage and are therefore easily distinguished from antemortem epiphyseolysis.

Growth plates of major limb bones, particularly the distal radius and ulna, are also susceptible to crushing injuries caused by compressive forces transmitted through the epiphysis. Such injuries, if severe enough, damage the epiphyseal blood supply as well as chondrocytes in the proliferating zone, leading to cessation of growth. *When the lesion is confined to one side of the growth plate, as it often is, continued growth on the other side leads to* **angular limb deformity**.

In dogs, *premature closure of the distal ulnar growth plate* is a common cause of limb deformity. Shearing forces acting on this growth plate result in crushing injury rather than epiphyseolysis, because of its conical shape, and are therefore more likely to result in retarded growth. If the growth plate of the adjacent radius escapes injury, the required synchrony between the two bones during development will be disturbed. Shortening of the limb will be accompanied by cranial bowing of the radius, valgus deformity, and outward rotation of the carpal and metacarpal bones.

Angular limb deformities ("bent leg") have been associated with a range of disease syndromes in several species, and are not always due to growth plate lesions. These will be discussed separately in a later section of this chapter.

Detachment of the ischial tuberosity from the pelvis is a well-recognized entity in young breeding sows, resulting in acute lameness. The separation, which may be bilateral, usually occurs between 8 and 14 months of age, prior to the closure of the apophyseal growth plate between the tuber ischiadicum and the rest of the ischium. The tuber ischiadicum serves as the origin for the semitendinosus and semimembranosus muscles, and as an attachment for the sacrotuberous ligament. As such, it is subject to considerable traction force and any weakness in the growth plate, as may occur in osteochondrosis, predisposes it to fracture.

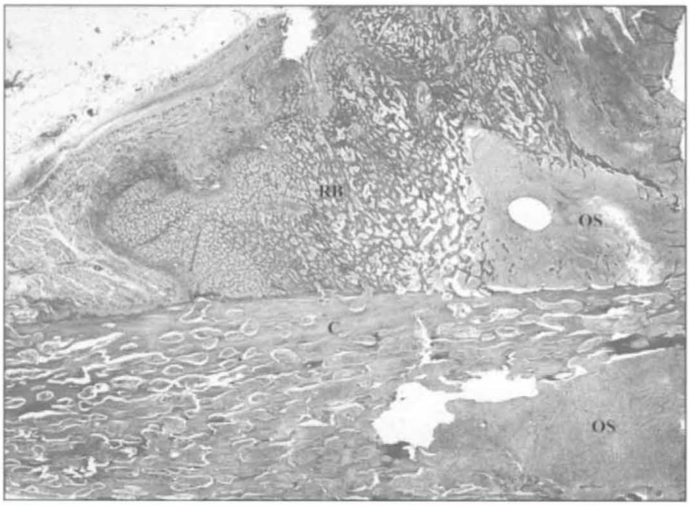

Figure 1.16 Reactive bone (RB) forming a so-called **"Codman's triangle"** beneath an elevated periosteum in a dog with osteosarcoma. The cortex (C) is porous due to tumor invasion. Sheets of tumor cells (OS) fill the medullary cavity and are replacing the sub-periosteal new bone in some areas.

Periosteal damage

Periosteal damage due to trauma stimulates rapid formation of new or **reactive bone** following activation and proliferation of osteoblast progenitors in the cambium layer. Trabeculae of woven bone extend from the underlying bone surface at acute angles, and can be readily distinguished histologically from the mature lamellar bone of the cortex (see Fig. 1.5). Separation of the periosteum from the bone surface by hemorrhage, inflammatory exudate, or neoplasia, or following surgical intervention, is also followed by *sub-periosteal new bone formation.* A pyramid-shaped region of new bone, referred to as **Codman's triangle**, may form beneath the periosteum in association with osteosarcoma (Fig. 1.16), but can also occur in association with other bone lesions, such as osteomyelitis. The mechanism of periosteal new bone formation is not clear, but it often precedes actual involvement of the periosteum by an underlying osteosarcoma or inflammatory process, suggesting that it may involve either local circulatory disturbances or the release of growth factors in response to bone resorption.

Localized outgrowths of new bone beneath the periosteum are referred to as **exostoses**. Depending on their size, and the inciting cause, they may either persist or gradually be removed by remodeling.

Fracture repair

Bone fractures are very common in animals and occur either when a bone is subjected to a mechanical force beyond that to which it is designed to withstand, or when there is an *underlying disease process that has reduced its normal breaking strength.* The latter is referred to as a **pathological fracture** and unless the predisposing disorder is corrected then the repair process is unlikely to be successful. The possibility of a localized bone disease (e.g., neoplasia or osteomyelitis) or a generalized disorder (e.g., fibrous osteodystrophy or osteoporosis) should always be considered if bone fracture has occurred without evidence of trauma.

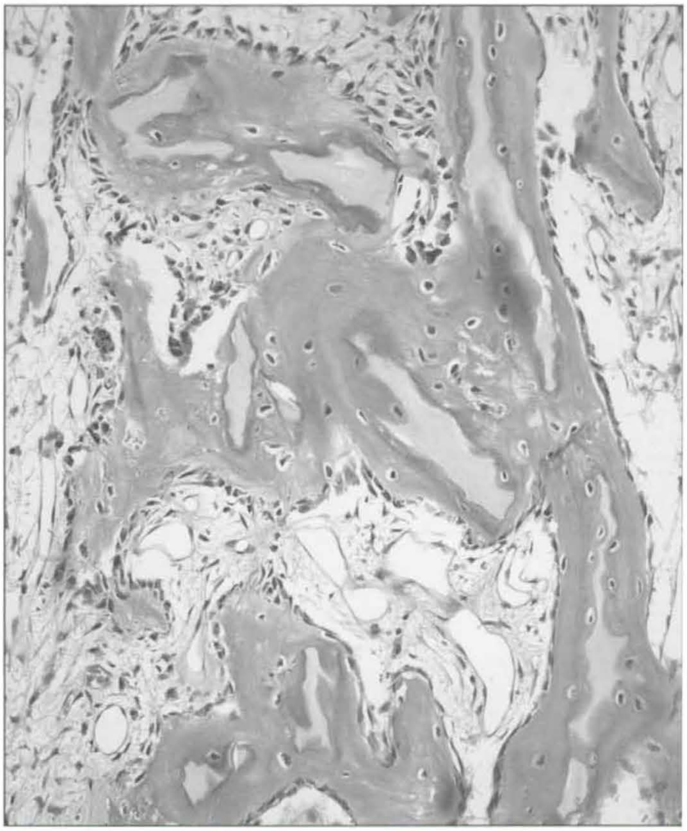

Figure 1.17 Trabecular microfractures in a calf with **osteogenesis imperfecta**. Note the abnormal alignment of cartilage cores in adjacent trabeculae that have been incorporated in a microcallus.

Types of fractures

Fractures are classified as **simple,** if there is a clean break separating the bone into two parts, or **comminuted**, if several fragments of bone exist at the fracture site. When one segment of bone is driven into another the fracture is referred to as an **impacted** fracture, and when there is a break in the overlying skin, usually due to penetration by a sharp fragment of bone, the fracture is referred to as **compound**. If there has been minimal separation between the fractured bone ends, and the periosteum remains intact, the lesion is classified as a **greenstick** fracture. An **avulsion** fracture occurs when there is excessive trauma at sites of ligamentous or tendinous insertions and a fragment of bone is torn away.

Microscopic fractures of individual trabeculae, or localized segments of cortical bone, also occur and are referred to as **microfractures**. Trabecular microfractures can sometimes be detected in histological sections by the abnormal alignment of their cartilage cores, which are normally situated at right angles to the growth plate, and parallel to the cartilage cores of adjacent trabeculae (Fig. 1.17). Such microfractures must however be differentiated from artifactual alterations in trabecular alignment that may occur when a bone is being sawn during processing. Once trabeculae have lost their cartilage core through remodeling this does not apply, and since the direction of remodeled trabeculae is less predictable, detection of microfractures is more difficult. *Multiple microfractures involving several adjacent trabeculae without gross displacement of the bone ends are referred to as* **infractions.** These are sometimes seen

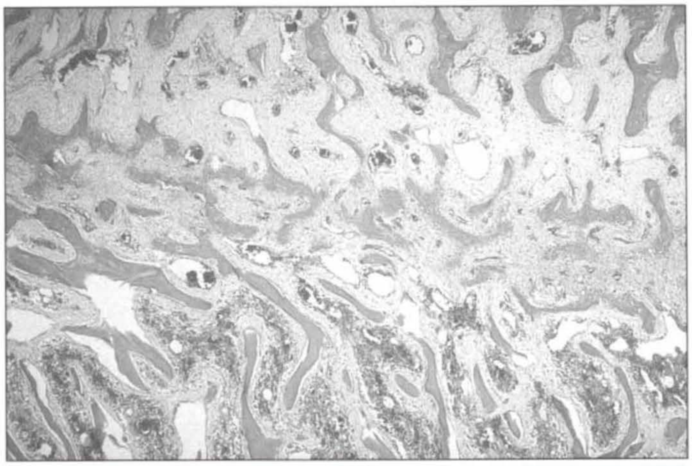

Figure 1.18 Infraction line in the metaphysis of a young pig with fibrous osteodystrophy. The abnormal alignment of trabeculae across the center of the field represents a band of healed trabecular microfractures.

in association with weight bearing on bones weakened by an underlying disease process, such as fibrous osteodystrophy (Fig. 1.18). Repeated bone trauma associated with strenuous exercise may lead to a **stress** fracture in the cortex of a limb bone. This represents the accumulation of several cortical microfractures, rather than a single traumatic event, and is typically seen in the dorsal or dorsomedial cortex of metacarpal III in young racehorses when they first enter training (see below).

Process of fracture repair

Unlike most other tissues, bone is capable of repair by regeneration rather than scar formation and successful repair of a fracture can return the bone both to its original shape and strength. The process of fracture repair follows a consistent pattern, but can be modified by methods of stabilization and by interfering factors, such as infection or the presence of an underlying bone disease.

The initial event in uncomplicated fracture repair is the *formation of a hematoma* between the bone ends. With disruption of the blood supply, *ischemic necrosis of bone* and other tissues in the vicinity of the fracture is inevitable. An *acute inflammatory response* is triggered by mediators released from the hematoma and from necrotic tissues. Inhibition of this inflammatory phase by anti-inflammatory drugs has been shown to interfere with the natural process of fracture repair. Although neutrophils and macrophages are the first cells to arrive at the fracture site, mesenchymal cells from the medullary cavity, endosteum, and cambium layer of the periosteum rapidly proliferate in and around the hematoma, forming a **callus** consisting initially of loose connective tissue. Subperiosteal new bone formation commences on the bone surface adjacent to the bone ends while primitive mesenchymal cells in the fracture gap differentiate into chondroblasts and replace the loose connective tissue with chondroid matrix. Meanwhile, osteoclasts appear and start to remove the dead bone. Osteoblasts producing new bone have been identified ultrastructurally in the medullary callus as early as 24 hours after fracture. Evidence suggests that at least some of these osteoblasts may be derived from transformed endothelial cells from capillaries and small venules in the vicinity of the fracture.

The early callus, consisting predominantly of hyaline cartilage, forms very rapidly and serves to anchor the fractured bone ends, allowing limited function while the repair process continues. As revascularization of the fracture site occurs, endochondral ossification within the callus leads to progressive replacement of the cartilage by trabeculae of woven bone. The *development of a bony callus* further stabilizes the fracture and allows a return to normal function, although the repair process continues. The *final phase* may take several months, or even years, and *involves the replacement of woven bone in the callus with mature lamellar bone*, and modeling of the callus to eventually restore the bone to its original shape. Once this process is completed, the strength of the bone will also be returned to its previous state. Modeling of the callus is more rapid in young animals than in adults and is more likely to result in complete resolution. In adults, residual changes such as persistence of medullary trabeculae and thickening of the periosteal bone surface are likely to persist at the fracture site.

The size of a fracture callus is proportional to the amount of movement between the fractured bone ends. Where there is considerable movement, callus formation will be exuberant, and may create diagnostic problems for the pathologist, especially in cases where the clinical history is incomplete and radiographs of the lesion are not available. In the early stages of callus formation the abundance of pleomorphic spindle cells, sometimes exhibiting primitive osteoid formation, can easily be misinterpreted as an osteosarcoma in biopsy specimens (Fig. 1.19). As the callus matures, a more organized pattern develops, with osteoblasts lining up along trabeculae of woven bone and the cells between trabeculae appearing less primitive. It must be remembered that an underlying osteosarcoma could have predisposed the bone to fracture and the two processes may in fact be present.

In fractures in which the separated ends have been perfectly aligned, and rigidly immobilized by metal plates or other methods of fixation, callus formation is minimal, or even nonexistent. The repair process in this situation is more protracted as it relies on the process of internal remodeling, whereby resorption canals form across the fracture line in the apposed cortices and new osteons are created. This is referred to as **primary cortical healing**.

There seems little doubt that local and systemic regulators of bone growth influence fracture repair, but their precise role has yet to be determined. Platelet-derived growth factor, transforming growth factor-β, fibroblast growth factor and bone morphogenetic proteins are released from the hematoma and necrotic bone soon after a bone fractures. These factors are important in regulating normal bone formation and resorption (see above) and are believed to play an important role in the modulation and coordination of callus formation during fracture repair. The possible therapeutic use of such growth factors in enhancing fracture repair is an exciting area of current research.

Complications of fracture repair

The repair process does not always proceed smoothly. In comminuted fractures, *fragments of necrotic bone that are too large for removal by osteoclastic resorption may persist at the site and interfere with the healing process*. Such bone fragments are referred to as **sequestrae**. The repair of compound fractures may be delayed by the development of bacterial osteomyelitis following contamination of the fracture site through the open skin wound. Failure to control the

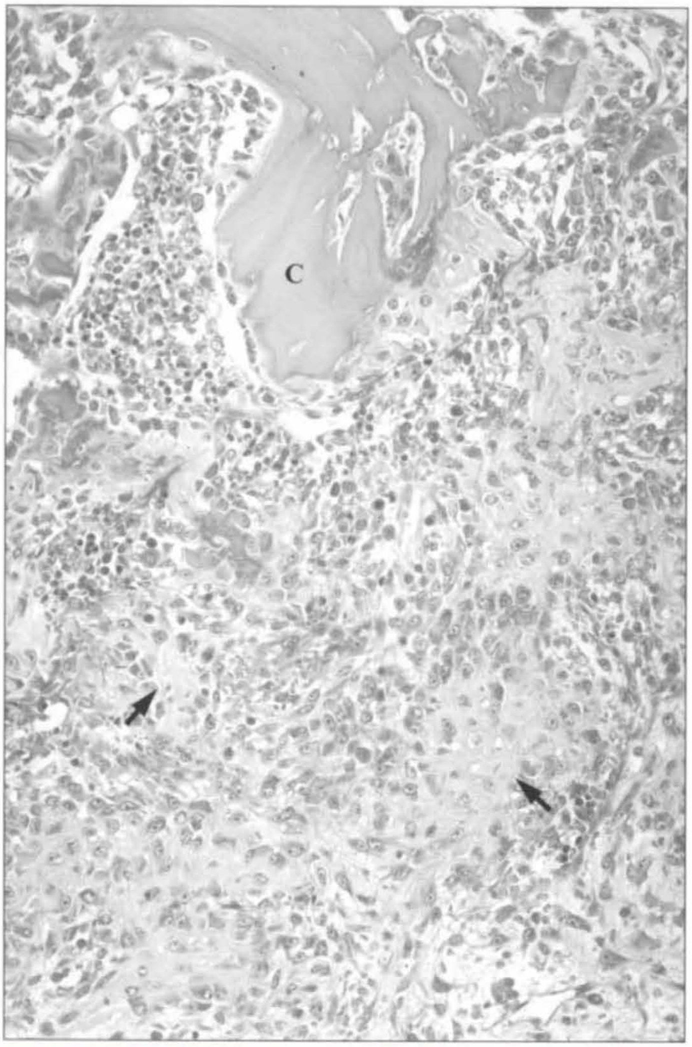

Figure 1.19 Repairing fracture at 1 week Disorganized condensations of plump mesenchymal cells are producing osteoid (arrows) in a manner similar to that which occurs in some osteosarcomas. A fragment of pre-existing cortical bone (C) is still present and serves as an attachment site for some of the newly formed bone spicules.

infection in the early stages will interfere with new bone formation and the resorption of dead bone. If the infection becomes chronic, new bone may form at the margin of healthy and diseased tissue in an attempt to wall off the infected area. The result may be the development of a large callus containing pockets of infection or fistulae surrounded by granulation tissue and trabecular bone. Any large fragments of bone engulfed by the inflammatory process are likely to persist as sequestrae, causing irritation, delayed healing, or nonhealing.

Excessive movement between bone ends during the repair process may inhibit the formation of a bony callus by continually disrupting attempts at revascularization. This favors the formation of cartilage and fibrous tissue and may lead to the development of a false joint or **pseudoarthrosis** at the fracture site. A pseudoarthrosis may also develop if soft tissues separate the fractured bone ends or if persistent infection inhibits callus formation.

Generalized skeletal disorders or deficiencies of nutrients crucial to bone formation will inevitably delay or prevent fracture repair, and must be corrected if normal healing is to proceed. Certain drugs, for example corticosteroids, can also interfere with bone healing.

Stress-related lesions in horses

A spectrum of stress-related lesions, including **bucked shins** and **incomplete cortical fractures**, *commonly affect the dorsal cortex of the third metacarpal bone in young sport horses*, particularly Thoroughbreds or Quarter Horses, undergoing intensive training for their first season of racing. Estimations of prevalence for bucked shins vary from 30–90% of all young horses in training. *Incomplete cortical fractures (so-called saucer fractures)* usually occur several months after the initial signs of bucked shins. Both lesions affect the left third metacarpal bone more often than the right, but they may be bilateral.

Bucked shins is characterized by the formation of smoothly contoured foci of periosteal new bone on the dorsal aspect of the metacarpal bone, accompanied by pain and swelling. For many years, bucked shins was considered to be the result of fatigue injuries leading to microfractures in the dorsal cortex of the metacarpal bone, with subsequent callus formation. This seems unlikely, as the extent of new bone formation is in excess of what would be expected as a response to such microfractures with negligible bone displacement and instability. Recent studies have suggested an alternative hypothesis. According to this hypothesis, repeated high-strain fatigue during training or racing decreases bone stiffness. This *then induces the formation of reactive bone on the periosteal surface in an effort to increase the inertial properties of the bone and increase its resistance to bending*, as would be predicted by Wolff's law.

Incomplete cortical fractures (stress or saucer fractures) seldom occur in horses that have not previously had bucked shins, suggesting that the conditions are related, but only about 12% of horses with bucked shins eventually develop such fractures. They usually occur 6–12 months after the onset of bucked shins and involve the periosteal new bone that forms on the dorsal or dorsolateral aspect of the third metacarpal. Until this bone is remodeled and strengthened, it is susceptible to fatigue injury during the high-strain cyclic loading associated with training or racing. Failure of the bone may be in the form of many small saucer-shaped stress fractures, extending part way into the cortex before returning to the surface.

Stress-related lesions are often found incidentally in the **distal condyles** of the third **metacarpal** and **metatarsal** bones of Thoroughbred racehorses. Linear defects in the articular cartilage adjacent to the sagittal ridge are closely related to increased density of the subchondral bone and to intense focal remodeling of the bone immediately adjacent to the articular defects. These changes are presumably a response to increased strain associated with training and may predispose to condylar fractures.

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SKELETAL DYSPLASIAS

A variety of genetic abnormalities primarily affecting bone formation or remodeling have been reported in humans and domestic animals. These are collectively known as skeletal dysplasias and are *usually associated with short stature, abnormally shaped bones, and/or increased bone fragility*. Not surprisingly, human skeletal dysplasias have been subjected to much greater scientific scrutiny than those in animals, leading to the development of detailed and sometimes confusing systems of classification, based on their radiographic appearance, the bones involved, age of onset, or pathogenesis. A comprehensive international classification system for skeletal dysplasias, proposed in 1991, includes well over 100 entities. Advances in molecular biology will inevitably lead to the development of a more precise classification system based on the actual genetic defect. Already, the mutations underlying several human skeletal dysplasias have been identified and it is now apparent that some disorders previously thought to be separate entities, are merely examples of variable expression of the same genetic defect. Skeletal dysplasias of animals are seldom investigated in as much detail as their human counterparts and classification is often imprecise, but it is clear that a similar range of conditions exist, creating opportunities for the development of potentially useful animal models. Studies in animals, particularly laboratory mice, are already proving valuable in helping to identify the molecular basis of inherited disorders of the skeleton and other body systems. Such studies may also generate new information on the role of specific proteins in bone function and physiology.

The diversity of skeletal dysplasias reflects the complexity of the processes involved in bone formation and remodeling, and the large number of genes required for normal development. In some dysplasias the entire skeleton is involved, while in others the defect is confined to individual bones or regions within bones. The terminology generally reflects either the distribution of lesions or the nature of the defect. Some examples of commonly used terms are listed in Table 1.2.

Most skeletal dysplasias in animals are usually either lethal or semi-lethal, but the gene frequency of some disorders in some breeds, has reached surprisingly high levels. This most likely reflects either inbreeding, or excessive use of a sire carrying a defective gene. The latter has clearly been responsible for the very high prevalence reached by certain genetic diseases in cattle, where artificial breeding is widely practiced.

Table 1.2 Terminology of some congenital abnormalities in skeletal development

Term	Nature of the defect
Generalized	
• Achondroplasia	— absence of cartilage development
• Chondrodysplasia	— Disordered cartilage development
• Osteogenesis imperfecta	— Genetic defect in type-I collagen formation characterized by osteopenia and excessive bone fragility
• Osteopetrosis	— Persistence of primary and/or secondary spongiosa due to defect in osteoclastic bone resorption
• Skeletal dysplasia	— Disordered skeletal development
Head	
• Brachycephalic	— Shortening of the head
• Brachygnathia	— Abnormally short jaw (inferior or superior)
• Campylognathia	— Harelip
• Palatoschisis	— Cleft palate
• Prognathia	— Abnormal projection of the jaw
Spine	
• Kyphosis	— Abnormal dorsal curvature of the spinal column
• Lordosis	— Abnormal ventral curvature of the spinal column
• Scoliosis	— Lateral deviation in the spinal column
Limbs	
• Amelia	— Absence of one or more limbs
• Hemimelia	— Absence of the distal part of a limb
• Micromelia	— Presence of abnormally small limbs
• Notomelia	— Accessory limb attached to the back
• Peromelia	— Congenital deformity of the limbs
• Phocomelia	— Absence of the proximal portion of one or more limbs
Digits	
• Adactyly	— Absence of a digit
• Dactylomegaly	— Abnormally large digits
• Ectrodactyly	— Partial or complete absence of a digit
• Polydactyly	— Presence of supernumerary digits
• Polypodia	— Presence of supernumerary feet
• Syndactyly	— Fusion of digits

Not all skeletal abnormalities are caused by genetic defects. Exposure of developing fetuses to certain toxic principles, mineral deficiencies, or infectious agents at appropriate stages of gestation can create skeletal lesions virtually indistinguishable from those with a genetic etiology. For the veterinary pathologist, it is as important to accurately determine the cause of the problem as it is to characterize the lesions. Otherwise valuable stud animals may be slaughtered unnecessarily or teratogenic agents may go undetected. In this section, discussion will focus on those skeletal dysplasias of domestic animals that are known, or strongly believed, to be genetic in origin. Acquired skeletal abnormalities will be discussed in a later section.

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Generalized skeletal dysplasias

Several important generalized disorders of bones are recognized in animals, all of them having analogous human counterparts. The underlying defect may lie in the formation of cartilage, thus affecting all bones that form by endochondral ossification. Such disorders are referred to as **chondrodysplasias** and affected individuals, no matter what the species, usually *show various degrees of dwarfism.* The term **achondroplasia** is often used in place of chondrodysplasia to describe diseases characterized by *disproportionate dwarfism,* especially in human medicine, and although it is less accurate, the term is entrenched in the literature. Alternatively, the defect may involve the synthesis of a specific component of bone matrix, e.g., type I collagen, as occurs in **osteogenesis imperfecta**. A defect in bone remodeling is the mechanism involved in another group of skeletal dysplasias, the **osteopetroses**. Only those disorders reported in domestic animals are included in this section but from time to time, the veterinary pathologist will be confronted with bone deformities that do not resemble any of the syndromes currently recognized in animals. In such cases, reference to the substantial human literature on skeletal dysplasias may be useful.

Chondrodysplasias

The *dwarfism of the chondrodysplasias is disproportionate,* in contrast to the proportionate or primordial dwarfism associated with somatotropin deficiency. Primordial dwarfism is usually inherited as a recessive trait and is well recognized in horses and dogs, where it forms the basis of several distinct breeds, e.g., Miniature Poodle. The chondrodystrophic dog breeds, e.g., Dachshund, Pekingese, and Basset Hound, are actually variant forms of disproportionate dwarfs. These forms of chondrodysplasia are inherited as polygenic traits associated with defects in hormone receptors, matrix components, or chondrocyte regulatory factors, and differ from the less "desirable" chondrodysplasias discussed below, which are believed to be caused by single gene defects.

Cartilage grows by both interstitial proliferation and surface apposition. Longitudinal growth of the cartilage model in the fetus, and at growth plates in young animals, relies primarily on interstitial proliferation of chondrocytes, while transverse growth occurs by interstitial growth and apposition. In some chondrodysplasias, appositional growth is normal but interstitial growth of cartilage is defective, resulting in premature closure of growth plates and reduced length of long bones. The intersphenoid, spheno-occipital, and interoccipital synchondroses at the base of the skull also develop by interstitial proliferation of chondrocytes and close prematurely in such disorders. In some chondrodysplastic calves, bony ridges project into the cranial cavity, possibly due to early fusion of the spheno-occipital synchondrosis and altered growth in this region of the skull. Although there is hypoplasia of basocranial bones and impaired development of the ethmoids and turbinates, the mandible, which enlarges by appositional growth, develops normally, leading to prognathia inferior. As the brain continues to grow, the cranium becomes domed and hydrocephalus may develop. The spinal column is shorter than normal due to reduced length of individual vertebrae, as are the ribs, but costochondral junctions may be enlarged since the cartilage expands by appositional growth.

The underlying molecular defects in most chondrodysplasias of animals have yet to be determined but their identification will eventually provide a more precise system of classification than is currently possible. In human achondroplasia, the most common form of heritable disproportionate dwarfism in people, the cartilage lacks a receptor for a growth factor as a result of a mutation in the gene encoding fibroblast growth factor receptor 3 (FGFR3). Such discoveries provide clues for investigating the mechanisms of chondrodysplasias in animals and may lead to the development of valuable animal models for assessing treatment options in human patients.

Chondrodysplasias of cattle

Several phenotypic forms of inherited chondrodysplasia occur in different breeds of cattle, and are broadly classified on the basis of their morphological characteristics into *"bulldog," Telemark, "snorter" (brachycephalic), and long-headed (dolichocephalic) types.* Because of the range of syndromes, it is likely that more than one gene locus is involved, or at least that there are different modifying alleles influencing the expression of a major gene.

The most severe form of bovine chondrodysplasia is the lethal **bulldog type**, which occurs in the Dexter and Holstein breeds, and possibly in Charolais and Jersey. Dexter cattle are considered short-legged derivatives of the Kerry breed, originating from Ireland, and are gaining in popularity in many countries because of the desirability of small, easily managed cattle for "lifestyle" farms. Some short-legged Dexters are heterozygous for an *incompletely dominant gene* which, when homozygous, gives rise to a bulldog calf. Bulldog calves may be carried to full term but are usually aborted before the seventh month of gestation and may not be detected by the owner. In all cases, they possess severe, relatively consistent, skeletal abnormalities. As well as being *much smaller than normal for the stage of pregnancy, they have extremely short limbs, which are usually rotated, a domed head with retruded muzzle and protruding mandible, and a large ventral abdominal hernia* (Fig. 1.20A). The tongue is of normal size so protrudes markedly, and the hard palate is absent. The shortened limb bones consist of mushroom-shaped, cartilaginous epiphyses separated by a short, central segment of diaphyseal bone. Histologically, the long bones lack distinct growth plates. Instead, the physeal cartilage consists of densely packed chondrocytes showing no orderly arrangement into columns (Fig. 1.20B), and a fibrillar intercellular matrix surrounding large, vascular, cartilage canals. Due to defective endochondral ossification at growth plates there is little growth in the length of long bones and no distinct primary or secondary spongiosa (Fig. 1.20B). In contrast, intramembranous ossification beneath the periosteum appears to proceed normally and contributes disproportionately to the bone volume (Fig. 1.20C). Holstein bulldog calves are similar to those of the Dexter breed, *but in Holsteins, the defect is characterized by autosomal recessive inheritance* rather than as an incomplete dominant. The defective gene coding for chondrodysplasia in Dexter cattle has recently been identified, and a test for carriers is now commercially available.

The **Telemark lethal** form of bovine chondrodysplasia is inherited as an *autosomal recessive trait* and, as such, the heterozygous parents are phenotypically normal. Affected calves are born alive but cannot stand, and die of suffocation shortly after birth. The head and facial abnormalities are similar to those of bulldog calves, and the limbs, although not quite as short as in bulldog calves, are much shorter than normal and rotated to varying degrees. A similar

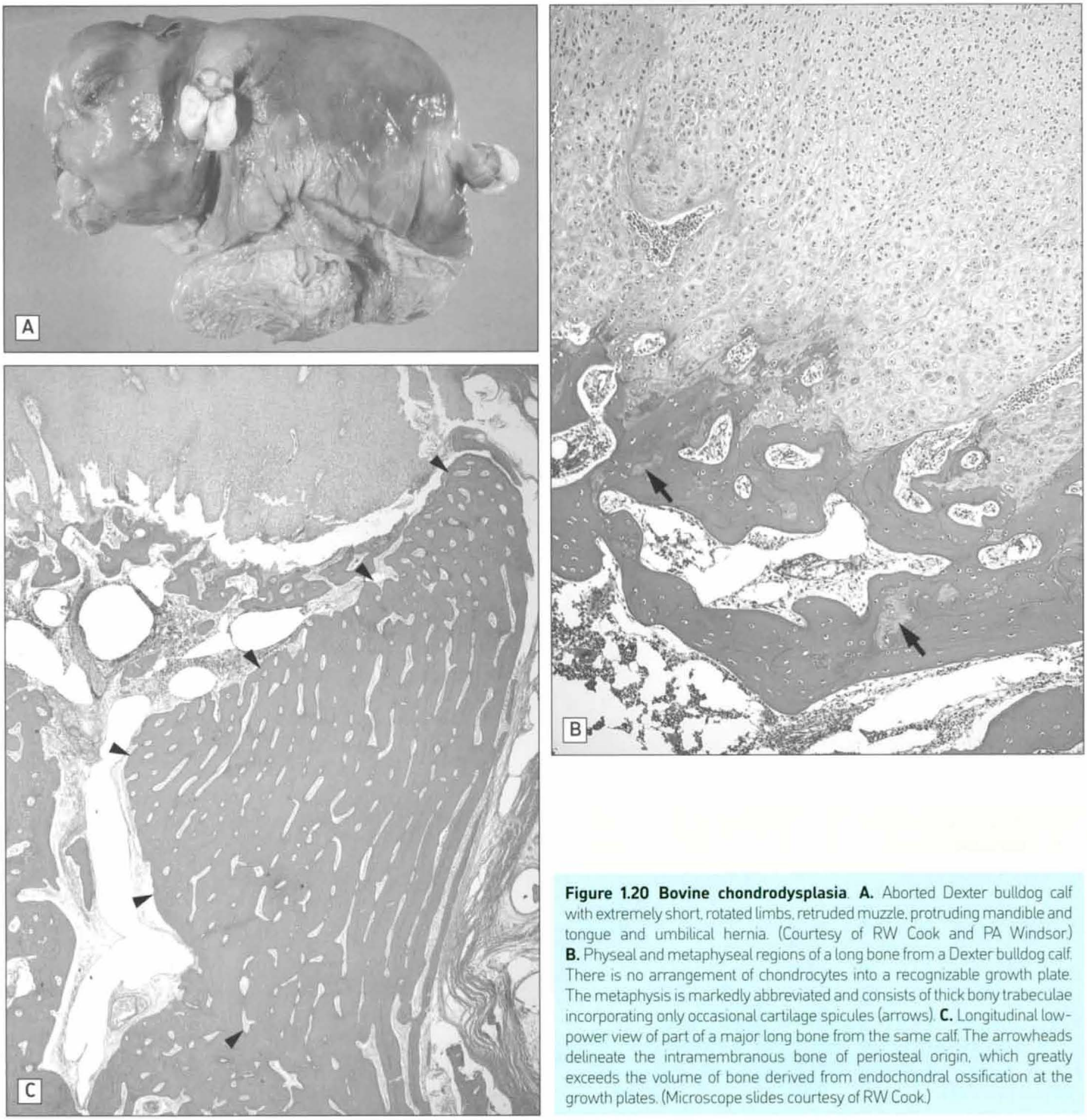

Figure 1.20 Bovine chondrodysplasia. A. Aborted Dexter bulldog calf with extremely short, rotated limbs, retruded muzzle, protruding mandible and tongue and umbilical hernia. (Courtesy of RW Cook and PA Windsor.) **B.** Physeal and metaphyseal regions of a long bone from a Dexter bulldog calf. There is no arrangement of chondrocytes into a recognizable growth plate. The metaphysis is markedly abbreviated and consists of thick bony trabeculae incorporating only occasional cartilage spicules (arrows). **C.** Longitudinal low-power view of part of a major long bone from the same calf. The arrowheads delineate the intramembranous bone of periosteal origin, which greatly exceeds the volume of bone derived from endochondral ossification at the growth plates. (Microscope slides courtesy of RW Cook.)

disorder, characterized by autosomal recessive inheritance, occurs in Jersey cattle, but shows much greater phenotypic variability. The lesions in Jerseys may include a short, broad head, deformed mandible, cleft palate, and short, spiraled limbs, but many calves have mild lesions and are viable.

Brachycephalic ("snorter") type dwarfism was common in the Hereford breed in North America and New Zealand during the late 1940s and early 1950s, but also occurred in other beef breeds, especially the Angus. Although inherited as an *autosomal recessive trait*, the defect appears to be partially expressed in heterozygotes, which are slightly smaller and more compact than normal. Selection for this phenotype most likely facilitated the spread of the chondrodysplasia gene in these traditional beef breeds and accounted for the high gene frequency. With a change in emphasis towards a larger frame score in beef cattle, and the introduction of programs to eliminate the defective gene, *snorter dwarfism is now rare.*

Snorter dwarfism is a much milder form of chondrodysplasia than the bulldog and Telemark types, but *affected calves have a short, broad head with bulging forehead, retruded upper jaw and a slightly protruding mandible* (Fig. 1.21). The eyes are prominent and laterally displaced. The vertebral column is shortened and the ventral borders of individual vertebrae are flattened, a useful diagnostic feature

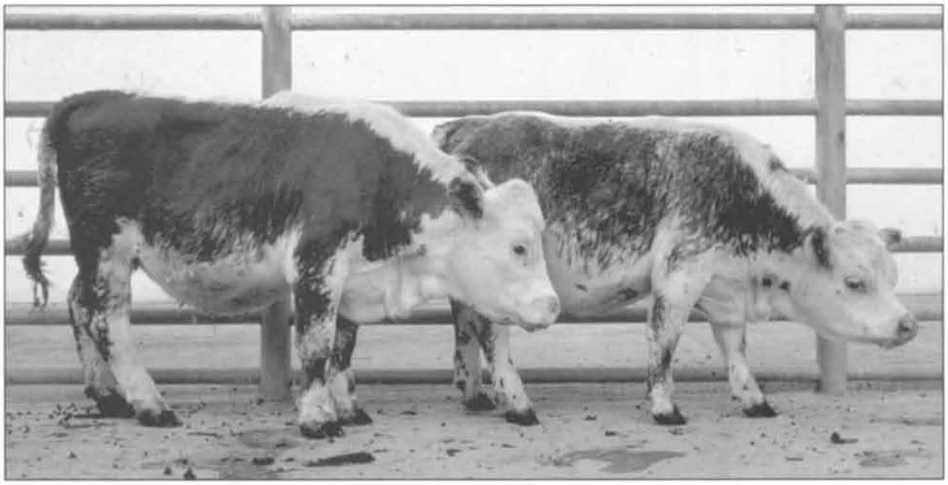

Figure 1.21 Bovine chondrodysplasia. Two brachycephalic ("snorter") dwarf Hereford calves with characteristic short stature, domed forehead, retruded upper jaw and distended abdomen. (Courtesy of RD Jolly.)

visible radiographically in young calves. Chronic ruminal tympany is a feature of snorter dwarfs, possibly as a result of reduced intra-abdominal space and impaired eructation. There is premature closure of basocranial synchondroses leading to compression of the cerebellum and shortening of the brain stem. The distal limb bones are proportionately shorter than proximal bones, the metacarpi showing the greatest degree of shortening. Interestingly, the width of long bones from brachycephalic dwarfs is comparable to, or greater than, that of normal calves. *The ratio of total metacarpal length to diaphyseal diameter is therefore a useful diagnostic indicator of this form of dwarfism.* In snorter dwarfs the ratio is usually 4.0 or less, but in normal animals it is greater than 4.5. Histological changes in the growth plates of snorter dwarfs are relatively mild and are not of diagnostic value. The microscopic organization of growth plates into their different zones is normal, but the columns of palisading chondrocytes are shorter, less hypertrophied and more irregular.

Long-headed or **dolichocephalic** dwarfs are slightly larger than snorter dwarfs, but the main difference is that *the head is proportionately much longer and tapers to a narrow muzzle.* Affected animals may have crooked limbs, slow growth rate and are unthrifty. Another form of chondrodysplasia, considered to be *rhizomelic* in type, occurs in Japanese Brown cattle. Lesions are confined to the long bones of the limbs and are associated with degenerative changes in growth plates and disordered endochondral ossification.

Proportionate dwarfism has been reported in an estimated 21 of 63 (33%) progeny from a clinically normal purebred Charolais bull. The miniature calves were derived both from Charolais cows and crossbred cows of diverse breeding, over several years. Affected calves weighed from 5–16 kg at birth and were born 2 weeks or more premature, with a high proportion dead at birth. Other than their small size, there were no anatomical differences between the miniature and normal calves sired by the bull. The condition was considered to be inherited as an *autosomal dominant trait*, probably resulting from a new mutation in the bull's primordial germ cells. This would explain the fact that the bull was clinically normal, and that less than 50% of its progeny were affected.

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Chondrodysplasias of sheep

The most common, and potentially important, form of hereditary chondrodysplasia in sheep is **spider lamb syndrome**, *a semi-lethal condition of Suffolk and Hampshire sheep.* Spider lambs were first recognized in North America in the late 1970s and the defect has since been introduced to Australia and New Zealand with imported Suffolk genetic material. The trait is characterized by *autosomal recessive inheritance with complete penetrance*, but with variation in expressivity between individuals. The prevalence of spider lamb syndrome in North America has reached a much higher level than would normally be expected for a semi-lethal genetic disorder, possibly due to selection for long-legged animals heterozygous for the "spider" gene.

Lambs with spider syndrome may be aborted or stillborn, but most are born alive showing skeletal deformities of variable severity. Some appear clinically normal at birth but develop typical signs of the disease within their first month of life, including *disproportionately long limbs and neck, shallow body, scoliosis and/or kyphosis* (Fig. 1.22A), *sternal deformity, and valgus deformity of the forelimbs below the carpus, creating a "knock-kneed" appearance.* Hindlimb deformities may also be present, but are less severe than those involving the forelimbs. Facial deformities, including Roman nose (Fig. 1.22A), deviated nasal septum and shortening of the maxilla are common, but not consistent. The deformities of the limbs and spinal column become progressively more severe with age. *Diagnosis is best confirmed by demonstrating characteristic radiographic changes in the elbow, sternum, and shoulder.* Multiple, irregular islands of ossification are present consistently at these sites and there is malalignment and displacement of sternebrae.

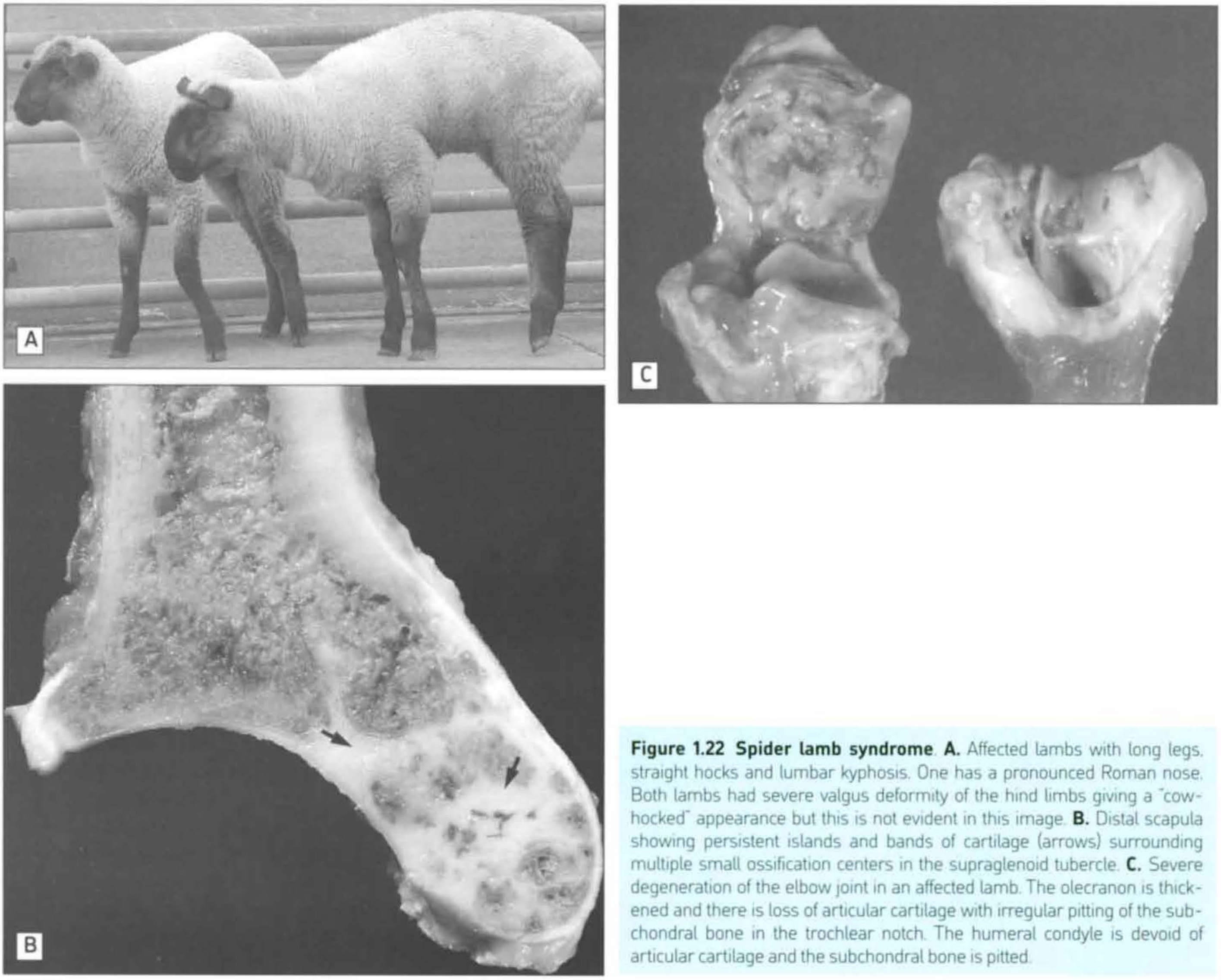

Figure 1.22 Spider lamb syndrome. **A.** Affected lambs with long legs, straight hocks and lumbar kyphosis. One has a pronounced Roman nose. Both lambs had severe valgus deformity of the hind limbs giving a "cow-hocked" appearance but this is not evident in this image. **B.** Distal scapula showing persistent islands and bands of cartilage (arrows) surrounding multiple small ossification centers in the supraglenoid tubercle. **C.** Severe degeneration of the elbow joint in an affected lamb. The olecranon is thickened and there is loss of articular cartilage with irregular pitting of the subchondral bone in the trochlear notch. The humeral condyle is devoid of articular cartilage and the subchondral bone is pitted.

In addition to the changes described above, other gross lesions may be detected at necropsy. In particular, there is *elongation of occipital condyles in a craniocaudal direction*, sometimes with erosion of the articular cartilage and dorsal deviation of the sternum between the second and sixth sternebrae. The caudal sternebrae often fail to fuse across the midline. Cervical and thoracic vertebral bodies contain excessive quantities of cartilage with disorganized arrangement, often accompanied by abnormalities in shape and symmetry. The olecranon and distal scapula also contain an excess of cartilage surrounding the multiple, irregular-shaped islands of ossification (Fig. 1.22B). Severe degenerative arthropathy, particularly involving the atlanto-occipital, elbow (Fig. 1.22C), and carpal joints, is present in lambs older than 3 months.

Histologically, the changes reflect abnormal development of ossification centers in bones developing by endochondral ossification. Multiple small ossification centers develop in nodules of hypertrophic cartilage but they fail to coalesce and expand in normal fashion towards articular surfaces (Fig. 1.23). The lack of subchondral bone predisposes to degenerative arthropathy. The proliferative and hypertrophic zones of growth plates in vertebrae and long bones are irregularly thickened and tongues of cartilage extend into the metaphysis or epiphysis. Chondrocyte columns in growth plates are often irregular. An interesting feature of the disease is that the ossification centers most severely affected are those that develop around the time of birth. Those that complete their development in utero, where they are not subjected to the mechanical forces of weight bearing and locomotion, appear normal. The lesions associated with spider lamb syndrome therefore may reflect the influence of mechanical stress on a defective cartilage model. This could help to explain the variable expressivity that is such a feature of the disease.

A *point mutation in ovine FGFR3* has been identified as the underlying defect in spider lamb syndrome. The gene encoding this receptor has largely been cloned and sequenced, and an accurate test for detecting heterozygous individuals is now available.

Chondrodysplastic syndromes characterized by **disproportionate dwarfism** do occur in sheep, but are rare. The best known of these is the *Ancon mutant*, which at one time gained popularity as a breed for its inability to jump walls. Although the breed is now extinct, the defect has reappeared on odd occasions, presumably due to new mutations. The Ancon abnormality is a *mesomelic, short-limbed dwarfism* caused by premature closure of certain growth plates.

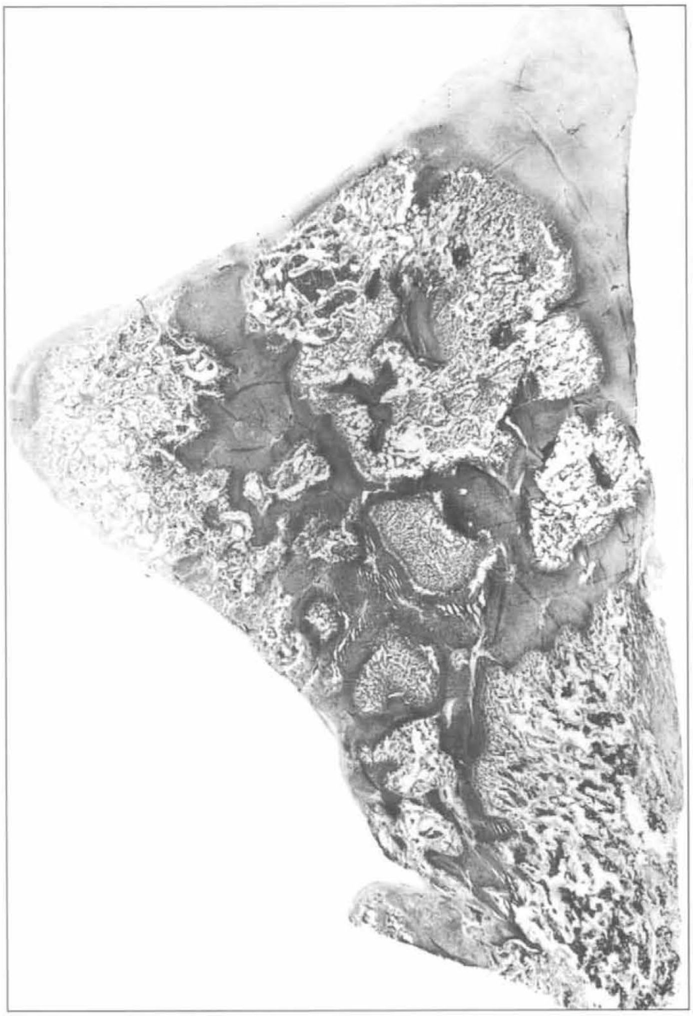

Figure 1.23 Subgross preparation of the olecranon from a **spider lamb** showing multiple ossification centers separated by irregular bands of cartilage.

Another form of chondrodysplasia reported in South Country Cheviot sheep in Scotland, was characterized by ectrodactyly, domed forehead and protruding eyes, together with shortened ears and tail. The syndrome was believed to be of genetic origin with recessive inheritance, but this was not proven.

A severe form of dwarfism affecting 27 of 110 lambs born to 70 mixed-breed ewes occurred in the United Kingdom. Most affected lambs were alive at birth but died within a few minutes. The abnormalities included a domed head, shortened nose, short, paddle-like limbs, narrow thorax, and swollen abdomen, and were said to resemble bulldog calves. Recessive inheritance could be eliminated due to the high incidence of affected lambs and the genetic diversity of the ewe flock. It is possible that a new, dominant mutation occurred in primordial germ cells of a ram in the flock.

A chondrodysplasia characterized by *dwarfism and varus deformity of the forelimbs* has recently been recognized in **Texel sheep** in New Zealand. The syndrome is inherited as an *autosomal recessive trait, but with variable expression.* Affected lambs appear normal at birth, but by 2–4 weeks of age show evidence of reduced growth rate, shortened neck and legs, varus forelimb deformities and a wide-based stance. Severely affected lambs show progressive reluctance to walk and often die within the first 4 months of life. In such cases, the articular cartilage on major weight-bearing surfaces of the hip and shoulder joints may be completely eroded, exposing the subchondral bone. The trachea is flaccid, sometimes kinked, and tracheal rings are partially flattened. *Histologically, there is disorganization of chondrocytes in articular and physeal cartilage and multiple foci of chondrolysis,* which sometimes coalesce to form large clefts. The matrix surrounding chondrocytes has an abnormal fibrillar and granular appearance. Similar microscopic changes are present in tracheal cartilage, confirming the generalized nature of the chondrodysplasia.

Chondrodysplasias of pigs

Disproportionate dwarfism was reported in three litters of Danish Landrace pigs sired by the same boar. The abnormalities were confined to the long bones of the limbs, and were most severe in the forelimbs. The bones of the skull and axial skeleton were unaffected. Signs of the disease were first apparent at 1–2 weeks of age and became more apparent with time. By weaning, affected pigs had an abnormal gait due to loose attachment of the limbs to the body and excessive mobility of joints. Few of them reached breeding age. Histologically, the growth plates had reduced thickness of the proliferative zone and irregularity of the hypertrophic zone, but the chondrocytes within each zone appeared normal. The disorder was inherited as an autosomal recessive trait.

In another report of dwarfism in pigs, almost 100 of 800 juvenile pigs developed gait abnormalities and marked shortening of limb bones, especially those of the hindlimb. The reduced length of limbs was due to premature closure of growth plates. The skull and axial skeleton were unaffected. In spite of the very high incidence, autosomal recessive inheritance was suspected.

Dwarfism also occurs in pig with vitamin A toxicosis (see later) but the lesions are more widespread than in this heritable form of chondrodysplasia, affecting vertebral bodies as well as limb bones.

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Chondrodysplasias of dogs

A variety of different forms of inherited chondrodysplasia are recognized in dogs of different breeds. Some are clinically apparent at birth

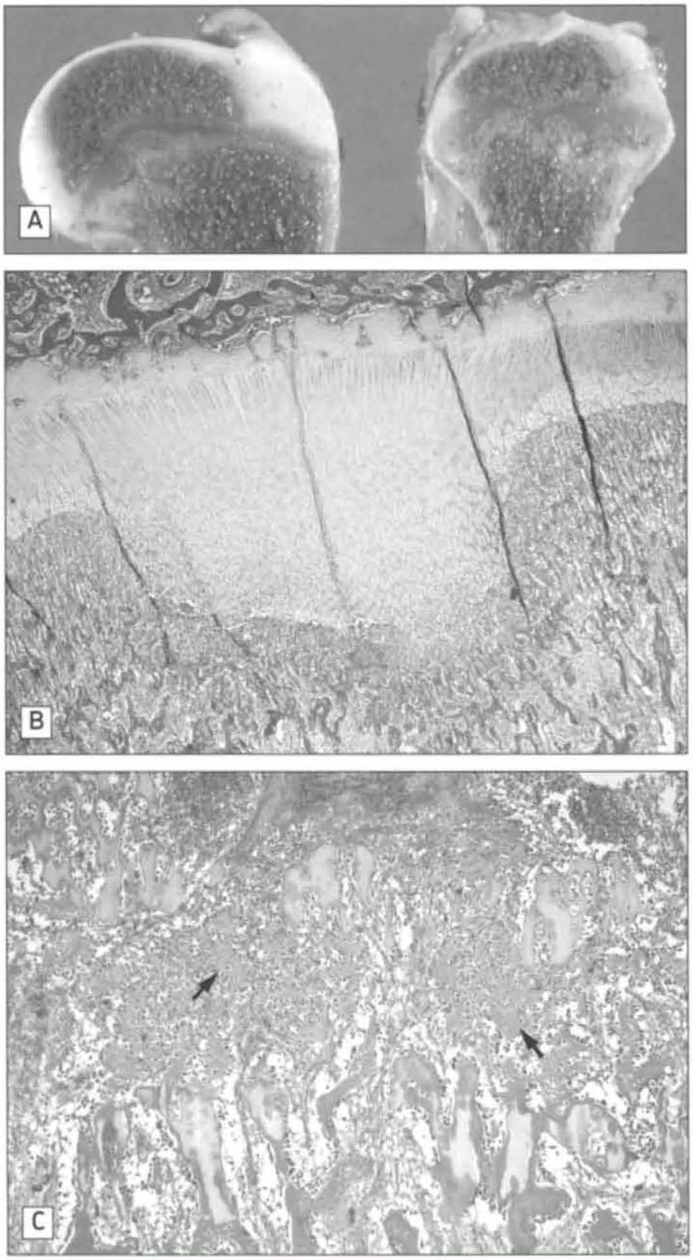

Figure 1.24 Alaskan Malamute chondrodysplasia. A. Proximal humerus (left) and distal radius of an affected pup showing irregular thickening of the physes. **B.** Marked, segmental thickening of the hypertrophic zone in the physis of an Alaskan Malamute pup with chondrodysplasia. **C.** Primary spongiosa immediately beneath a thickened segment of physis in the same Malamute pup. Note the disruption of the normal trabecular architecture and replacement by proliferating osteoblasts producing disorganized spicules of osteoid (arrows). An area of hemorrhage is present closer to the physis.

but others are not manifest for several weeks. **Chondrodysplasia in the Alaskan Malamute** is characterized by *disproportionate, short-legged dwarfism*, and *autosomal recessive inheritance* with complete penetrance and variable expression. At birth, the growth plates of affected puppies appear normal radiographically. Radiographic changes are apparent as early as 7–10 days of age but are more pronounced after 3 weeks. Similarly, there is little difference in growth plate and metaphyseal histology between dwarf and normal Malamutes at birth. During growth, the changes become marked, suggesting that mechanical force is required in order to create the clinical and pathological changes. Irregular thickening of growth plates in the limb bones is a feature of the disease (Fig. 1.24A). Broad tongues of hypertrophic cartilage extend into the metaphysis in close proximity to healing trabecular microfractures (Fig. 1.24B, C). Disruption of the metaphyseal blood supply, leading to impaired vascular invasion of the developing growth plate, is considered responsible for the physeal thickenings. *The lesions bear a remarkable resemblance to rickets*, both grossly and in demineralized bone sections, but the mineralization of cartilage is normal. Abnormal endochondral ossification occurs throughout the body, but *the most striking lesions occur in the distal ulna and radius*, resulting in enlarged carpal regions and valgus deformity of the forelimbs. This most likely reflects the proportionately greater weight-bearing responsibility of the forelimbs compared to the hindlimbs, and the greater susceptibility to injury. A reduction in the hydroxyproline content of cartilage, and in the stability of type II collagen cross-links, has been demonstrated in dwarf Alaskan Malamutes, the collagen defect possibly increasing the susceptibility of trabeculae in the primary spongiosa to fractures. Hemolytic anemia characterized by stomatocytosis, macrocytosis, and increased concentration of sodium within erythrocytes, accompanies the chondrodysplasia in this breed. Heterozygotes have intermediate hemoglobin concentrations, suggesting that this manifestation of the syndrome is inherited as an incompletely dominant trait.

Chondrodysplasia in the Norwegian Elkhound is a *disproportionate, short-legged dwarfism* similar to the disease in Malamutes but with significant morphologic differences. In Elkhounds, the vertebral bodies are also shortened and there is a marked reduction in the width of the proliferative zone in growth plates. *A highly distinctive feature is the presence of one or more large, intracytoplasmic inclusions in the chondrocytes of all zones* (Fig. 1.25). The inclusions stain deep blue by the Alcian Blue-periodic acid-Schiff method at pH 1.0 and 2.6. Inclusions that escape from degenerate chondrocytes persist and lie free in lacunae. The cell columns in the zone of hypertrophy and degeneration are generally disorganized and separated by wide matrix bars. Trabeculae in the primary spongiosa are coarse and shortened, with many horizontal bridges and thick osteoid seams. No inclusions are present in osteoblasts or osteocytes.

Chondrodysplasia in the English Pointer has been reported in the United Kingdom and Australia in related dogs. Inheritance appears to be *autosomal recessive*. Affected puppies are smaller than their normal littermates and develop locomotory abnormalities, including a bunny-hopping gait. Some also have inferior prognathism. Growth plates are irregularly thickened due to increased width of the hypertrophic zone, but the lesion varies in severity between different bones. Increased mineralization of laryngeal and tracheal cartilage occurs in some affected animals. At around 10 weeks of age, articular cartilages appear normal but by 16–18 weeks, there are abnormalities in the cartilage of all major limb joint, including wrinkles, projections and sometimes fibrillation. In the epiphyseal cartilage beneath these lesions there are irregular cavities containing degenerate chondrocytes and strands of collagen. Severe degenerative arthropathy develops in some joints.

Chondrodysplasia in the Great Pyrenees also appears to be inherited as *an autosomal recessive trait*. Affected pups are normal at birth but by 2 weeks they are shorter and lighter than their littermates. Mature dwarfs are less than half of normal size. Radiographic abnormalities are restricted to the metaphyses of long bones and vertebrae and are characterized by *delayed ossification*. Histologically, chondrocyte columns in growth plates are disorganized and many chondrocytes contain cytoplasmic vacuoles that consist of dilated cisternae of rough endoplasmic reticulum. Metaphyseal trabeculae are thicker than normal and are often joined by lateral bridges.

Pseudoachondroplastic dysplasia, a disease of **Miniature Poodles**, was originally named epiphyseal dysplasia. Inheritance of the trait is *probably autosomal recessive*. The disease is not apparent at birth but by 2–3 weeks of age affected pups are noticeably smaller than their normal littermates and have difficulty standing and walking. The skull is usually normal but mild inferior prognathism may be present. Vertebral bodies are short and show delayed ossification, costal cartilages are longer than normal and costochondral junctions are enlarged (Fig. 1.26). Severely affected pups have dorsoventral flattening of the rib cage, presumably due to persistent recumbency. The limb bones are also short, particularly those of the forelimbs, and possess enlarged epiphyses which are sometimes flared over the metaphyses (Fig. 1.27). Histologically, the cartilage matrix is sparse and lacks basophilia. Chondrocytes vary in size and are sometimes clumped together in enlarged lacunae. Chondrocyte

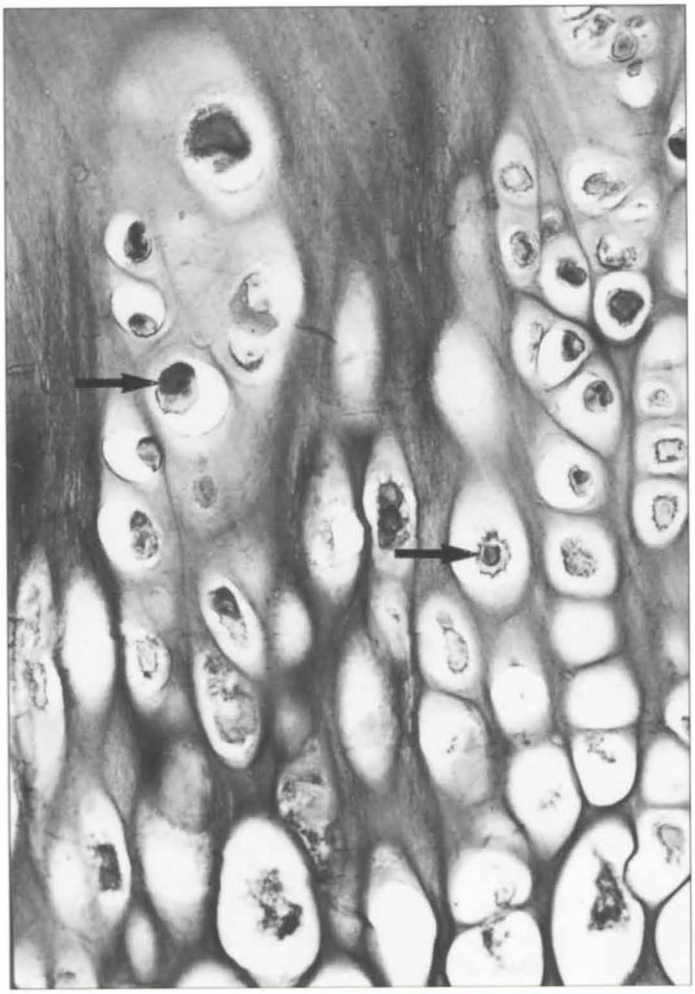

Figure 1.25 Norwegian Elkhound chondrodysplasia. Inclusion bodies in chondrocytes (arrows). (Courtesy of SA Bingel.)

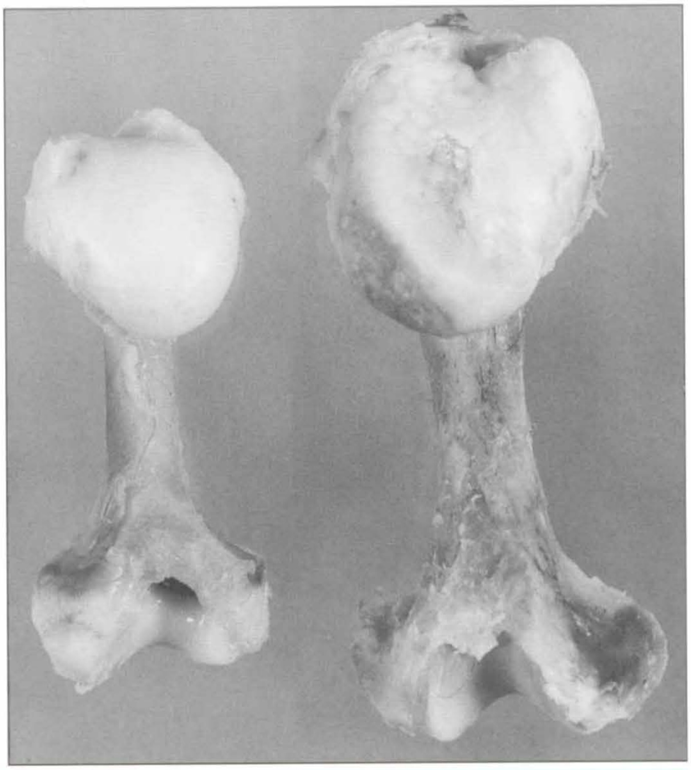

Figure 1.27 Pseudoachondroplastic dysplasia in Miniature Poodle. Humerus of immature and mature dogs. The articular cartilage of the humeral head in the adult dog is degenerate.

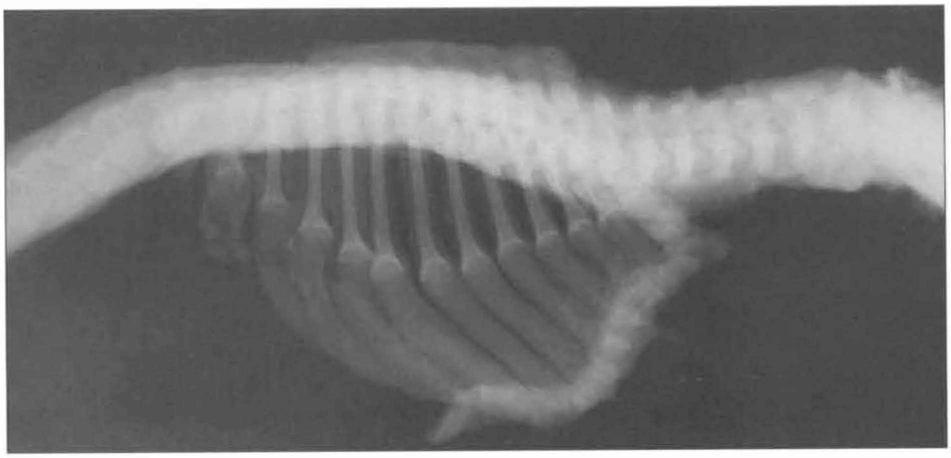

Figure 1.26 Pseudoachondroplastic dysplasia in Miniature Poodle. Thoracic radiograph showing enlarged costochondral junctions, long costal cartilages, and shortened vertebrae.

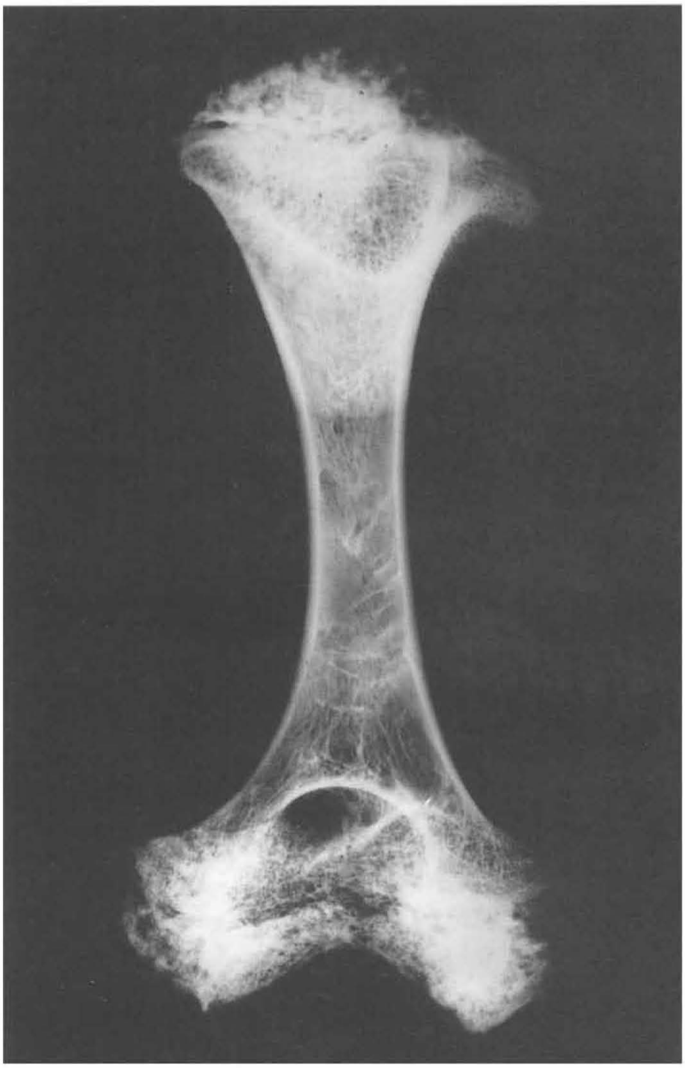

Figure 1.28 Pseudoachondroplastic dysplasia in Miniature Poodle. Radiograph of humerus showing irregular, multifocal development of ossification centers, creating a stippled appearance.

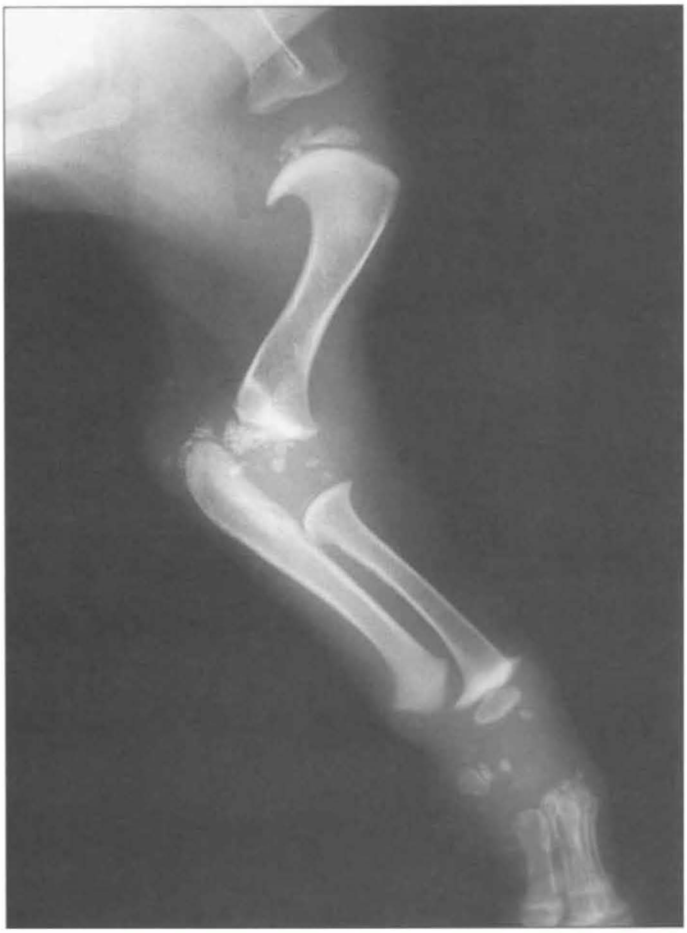

Figure 1.29 Multiple epiphyseal dysplasia in a Beagle dog. Forelimb radiograph showing stippled epiphyses in shoulder, elbow and carpal regions. (Courtesy of GP Rasmussen.)

columns in growth plates are irregular and the proliferative zone is abnormal. Ossification centers develop later than normal and are multifocal, creating a stippled appearance radiographically in affected pups (Fig. 1.28). By 2 years of age, ossification is complete and the long bones are only half to two-thirds normal length. Interestingly, the lumbar vertebrae are longer than in normal dogs of the same age. Degenerative arthropathy and spondylosis may develop in pups less than a year of age. *The underlying defect in cartilage matrix formation not only causes defective endochondral ossification, but also results in abnormal formation of the trachea and nasal septum.* Collagen synthesis is normal but the defect appears to involve decreased sulfation of glycosaminoglycans.

Multiple epiphyseal dysplasia is a rare condition of **Beagle dogs**, probably inherited as an *autosomal recessive trait.* Affected puppies walk with a swaying gait but, in adults, lameness is only apparent after exercise. The earliest radiographic signs are not visible until 3 weeks of age and consist of *stippled epiphyseal mineralization* (Fig. 1.29), especially in the tarsal and carpal bones, the femoral, humeral, metacarpal and metatarsal epiphyses, and in the bodies of the sixth and seventh lumbar vertebrae. By 5 months the stippling is no longer apparent, having been incorporated into the normal ossification centers. The abnormal foci of epiphyseal mineralization develop in a specific subarticular zone of the epiphyses. The initial lesion is a floccular accumulation of chondroitin sulfate and glycoprotein in chondrocyte lacunae. As adjacent lacunae coalesce and liquefy to form cysts, their contents mineralize. The stippled radiographic appearance of epiphyses resembles that of pseudoachondroplastic dysplasia of Miniature Poodles but the histologic changes are distinct.

Osteochondrodysplasia in the Scottish Deerhound becomes apparent at around 4–5 weeks of age, with affected puppies showing exercise intolerance and retarded growth. These pups go on to develop a bunny-hopping gait, kyphosis, marked bowing of the limbs, and joint laxity. Mature dogs have shortened, porotic long bones, shortened vertebrae, distorted carpal and tarsal bones, incongruencies of hip and elbow joints, and enlargement of muscle attachment sites. Growth plates are irregular in width and physeal–metaphyseal junctions are uneven. Histologically, there are islands of basophilic, vacuolated chondrocytes in epiphyses, but physeal and metaphyseal lesions are intermittent. Physes contain some areas that are virtually normal, in addition to areas that are narrow and hypocellular, or lack proliferative or hypertrophic zones. A characteristic feature is the *presence of periodic acid-Schiff-positive, diastase-resistant cytoplasmic inclusions in proliferative and hypertrophic chondrocytes.* The metaphyses may consist of normal primary and secondary spongiosa in some areas, while elsewhere the trabeculae are thin, sparse and largely replaced by fibrovascular connective tissue. The irregularity of the physeal and metaphyseal lesions may reflect localized trauma. The condition is probably inherited as an *autosomal recessive trait.*

A syndrome with combined **ocular and skeletal dysplasia** occurs in the **Labrador Retriever** and **Samoyed** breeds. The bone lesions are confined to the appendicular skeleton and include *shortening of the limb bones, valgus deformity of the carpi, and abnormally shaped radii and ulnas.* Affected dogs have a *characteristic "downhill" conformation* due to more severe involvement of the forelimbs. In some dogs, the coronoid processes are ununited and there is hypoplasia of both the coronoid and anconeal processes, but these changes may be incidental as they also occur as separate defects in these breeds. Retained cartilage cores may be present in the distal ulna. Although clinical evidence of dwarfism may not be evident until about 8 weeks of age, *a radiographic image-classification system allows identification of affected puppies at birth.* The ocular lesions in both breeds consist of *cataracts and retinal detachment.* In Retrievers, there is also retinal dysplasia. The ocular and skeletal lesions appear to be inherited together, but the defective gene has recessive effects on the skeleton and incomplete dominance on the eye. Heterozygotes therefore possess mild eye lesions but a clinically normal skeleton.

Chondrodysplasias of cats

There are *few reports* of chondrodysplasias in cats. One report describes a severe form in a stillborn kitten *in association with homozygous Pelger–Huët anomaly.* The kitten was small and had a dome-shaped skull, flattened face, and protruding tongue. Vertebrae were shortened, as were ribs and long bones of the limbs. The limbs were thickened and had broad, mushroom-shaped epiphyses. Growth plates were incompletely formed with partial or irregular ossification at the center of the physis, and more normal peripheral regions. A live littermate, which was heterozygous for the Pelger–Huët anomaly, also had skeletal abnormalities, including pectus excavatum, lordosis, shortened vertebral bodies, and flared distal radial metaphyses. The skeletal lesions in the stillborn kitten were very similar to those seen in rabbits with homozygous Pelger–Huët anomaly.

A *possible metaphyseal chondrodysplasia* was also diagnosed in two unrelated kittens with shortened limbs, bowed forelimbs, marked widening of the growth plates and flaring of the metaphyses. The most severe growth plate lesions were present in the distal radius and proximal humerus. The radiographic changes in growth plates initially led to a diagnosis of rickets, but neither kitten responded to treatment and there was no evidence of defective mineralization. One kitten had two siblings with similar limb deformities and its probable sire was also known to have short, deformed forelimbs, suggesting a genetic etiology.

An osteochondrodysplasia characterized by a short, thick, inflexible tail and misshapen distal limbs with extensive new bone formation on the plantar aspect of the tarsi and metatarsi, is reported in **Scottish Fold** cats. Other abnormalities in affected cats include irregularity in the size and shape of tarsal, carpal, metatarsal, and metacarpal bones, phalanges, and caudal vertebrae, narrowing of joint spaces, progressive new bone formation around distal limb joints and diffuse osteopenia of adjacent bones. Histologically, there is evidence of defective endochondral ossification in physes and beneath articular cartilages. Cartilage columns in physes are disorganized and there is irregular thickening of physeal cartilage. Islands of cartilage may also extend from articular cartilage into epiphyses. Lesions can be detected in kittens as young as 7 weeks of age, but the bony changes are slowly progressive and may not be detected for several years. Originally, it was thought that affected cats were homozygous for the Fd (fold-eared) gene and resulted only from matings between fold-eared cats. Recent evidence suggests that the disease can also develop in heterozygous Scottish Folds and that significant lesions may be present in such animals as early as 6 months of age.

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Osteogenesis imperfecta

Osteogenesis imperfecta is one of the most frequently observed inherited connective tissue disorders of humans, but *occurs rarely in domestic animals.* The disease is characterized by *excessive bone fragility,* which in severe cases may result in multiple intrauterine fractures, marked skeletal deformity, and either stillbirth or perinatal death. Milder forms may be inapparent at birth but lead to an increased incidence of postnatal fractures and bowing of the limbs. Other abnormalities may include fragile, opalescent teeth (dentinogenesis imperfecta), joint laxity, and blue sclerae. Based on clinical features and mode of inheritance, *at least four distinct forms of osteogenesis imperfecta are recognized in human patients.* Most human cases of osteogenesis imperfecta are inherited as autosomal dominant traits and represent new mutations, although rare recessive forms also exist. A quantitative or qualitative defect in type I collagen is the molecular basis of the disease and may be due to one of many mutations in either of the two genes, *COL1A1* or *COL1A2,* that code for the $\alpha1$ and $\alpha2$ chains, respectively. Type I collagen is the

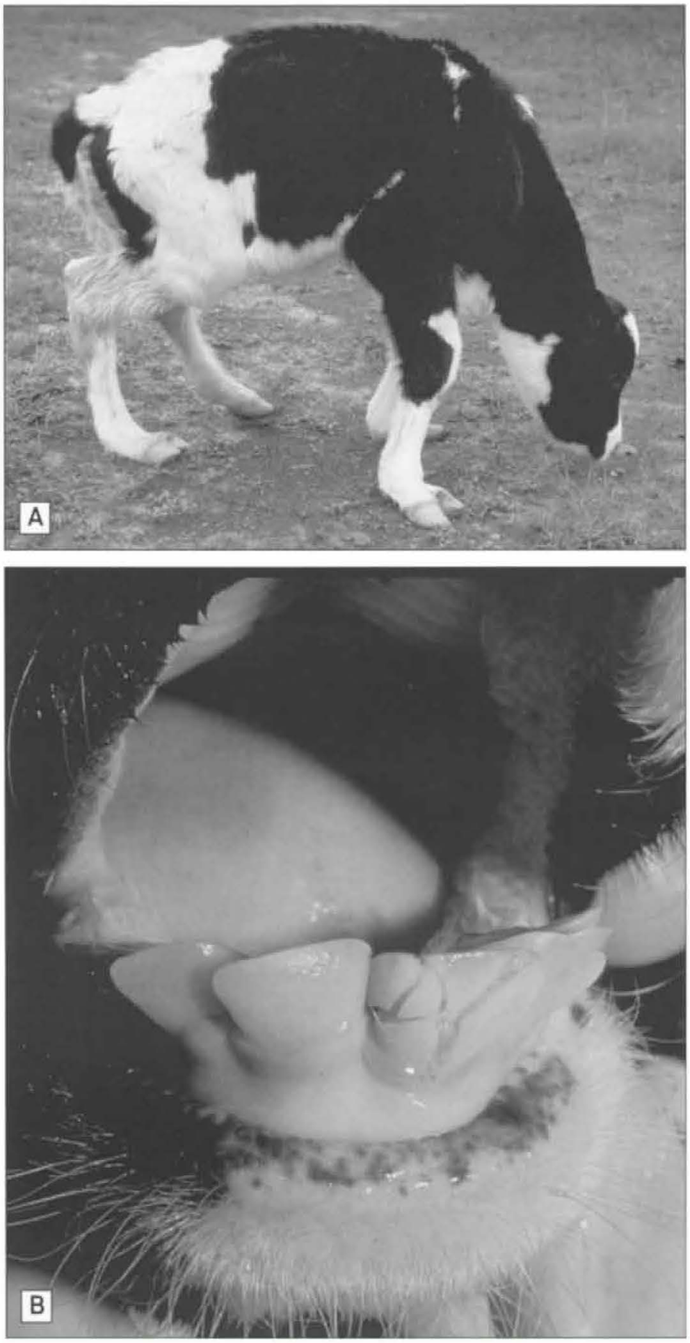

Figure 1.30 Bovine osteogenesis imperfecta **A.** Affected Holstein calf, 2 weeks old. Because of excessive joint laxity, the calf has a crouched stance and its pasterns almost touch the ground. **B.** Fractured incisor tooth due to dentinal dysplasia in the same calf.

predominant collagen type in bone, dentin, ligaments, tendons, and in the ocular sclera, thus accounting for the characteristic distribution of lesions.

Most reports of osteogenesis imperfecta in animals are in calves and lambs and appear analogous to the most severe form of the disease in humans. In **cattle**, the disease has occurred in Australia, the US, and Denmark in the offspring of three clinically normal, unrelated **Holstein-Friesian** bulls, and in **Charolais** calves in Denmark. A single crossbred **Hereford** calf with osteogenesis imperfecta has been identified in New Zealand. There is convincing evidence to support an *autosomal dominant* mode of inheritance, at least in Holstein-Friesian cattle, although the percentage of calves affected from the three Holstein-Friesian bulls varied from approximately 9% to 44%. This presumably reflects different degrees of gonadal mosaicism following mutations in the germ cell lines of each bull. The manifestations of the disease in each report in calves have been similar, but minor variations exist, suggesting that different mutations may be involved. Affected calves are usually born alive and are of normal size, but most are unable to stand due to marked hypermobility of the joints and, in some cases, the presence of limb fractures. Some affected Holstein-Friesian calves are able to stand and can walk with difficulty, but have a characteristic crouched stance with pasterns almost touching the ground (Fig. 1.30A). As in some forms of the human disease, calves with osteogenesis imperfecta have dentinal dysplasia characterized by small, translucent pink-gray teeth, which are barely erupted at birth and may fracture in calves that survive for a few weeks (Fig. 1.30B). In some calves the sclerae are distinctly blue, but this is more evident during postmortem examination following enucleation of the eye. Skin fragility is not a feature of the disease in calves.

The main findings during *postmortem examination* relate to the skeleton. *Although the bones are essentially normal in shape, they are extremely brittle* and can usually be broken with little effort. Acute fractures are common in the mandibles and major limb bones; the latter probably occurring during attempts to stand. Severely affected calves may have multiple, well-developed calluses on their ribs (Fig. 1.31A), indicating intrauterine fractures. In spite of their fragility, the cortices of long bones are similar in thickness to those of normal calves but the volume of trabecular bone in metaphyses and epiphyses is reduced and shows a finer, denser pattern. In calves that have stood and walked, infraction lines may be present in the epiphysis and metaphysis of major limb bones (Fig. 1.31B). These represent bands of healing trabecular microfractures presumably resulting from trauma during weight bearing. Tendons are thinner than normal and discolored pink.

Histologically, osteoblastic activity appears normal but calcified cartilage spicules in the primary spongiosa are lined by only a thin layer of basophilic bone matrix and there is little osteoclastic resorption or realignment of trabeculae. Infractions and trabecular microfractures are common (see Fig. 1.17). Bone cortices are porous due to incomplete compaction of primary osteons and consist predominantly of deeply basophilic woven bone with few lamellae (Fig. 1.32A, B). The dentin in affected calves is basophilic, coarsely laminated and approximately one fifth as thick as in control calves. Dentinal tubules are irregularly oriented and reduced in number, and there is an undulating margin with the pulp cavity. The sclera is approximately one half of normal thickness. Ultrastructurally, collagen fibrils in the tendons, dentin, sclera, and skin of affected calves are significantly narrower than normal.

Chemical analysis of bone from Holstein-Friesian calves with osteogenesis imperfecta has provided evidence that *different mutations are involved*. In bone tissue from both American and Australian calves there is reduced size of apatite crystals and a decrease in the amount of type I collagen and proteoglycans. The *main difference is in the concentration of osteonectin*, which is normal in the Australian calves with osteogenesis imperfecta, but only 10% of normal in the affected American calves. Phosphophoryn, a dentin-specific protein, and an osteonectin-like protein of dentin are also markedly deficient in these calves.

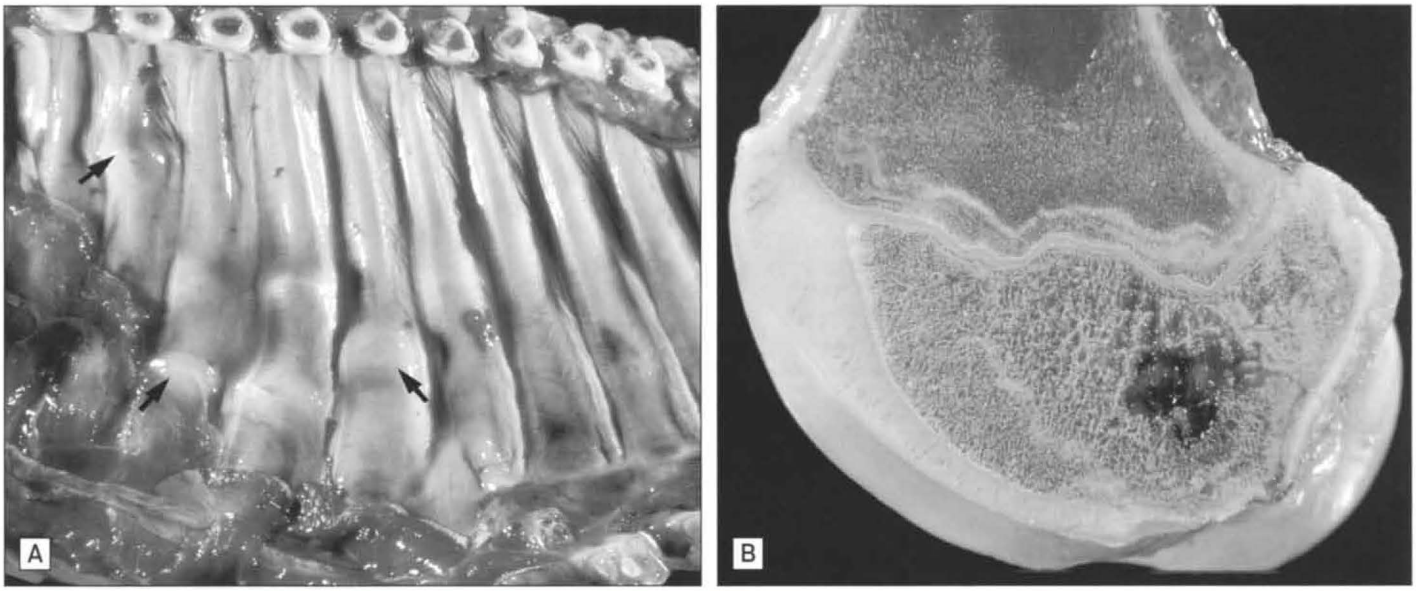

Figure 1.31 Bovine osteogenesis imperfecta. **A.** Several mature calluses (arrows) on the ribs of a newborn calf, indicating intrauterine fractures due to extreme bone fragility. **B.** Distal femur from a calf that had survived for a couple of weeks and had walked. Multiple infraction lines are present in the epiphysis and metaphysis. The cancellous bone is more porous than normal.

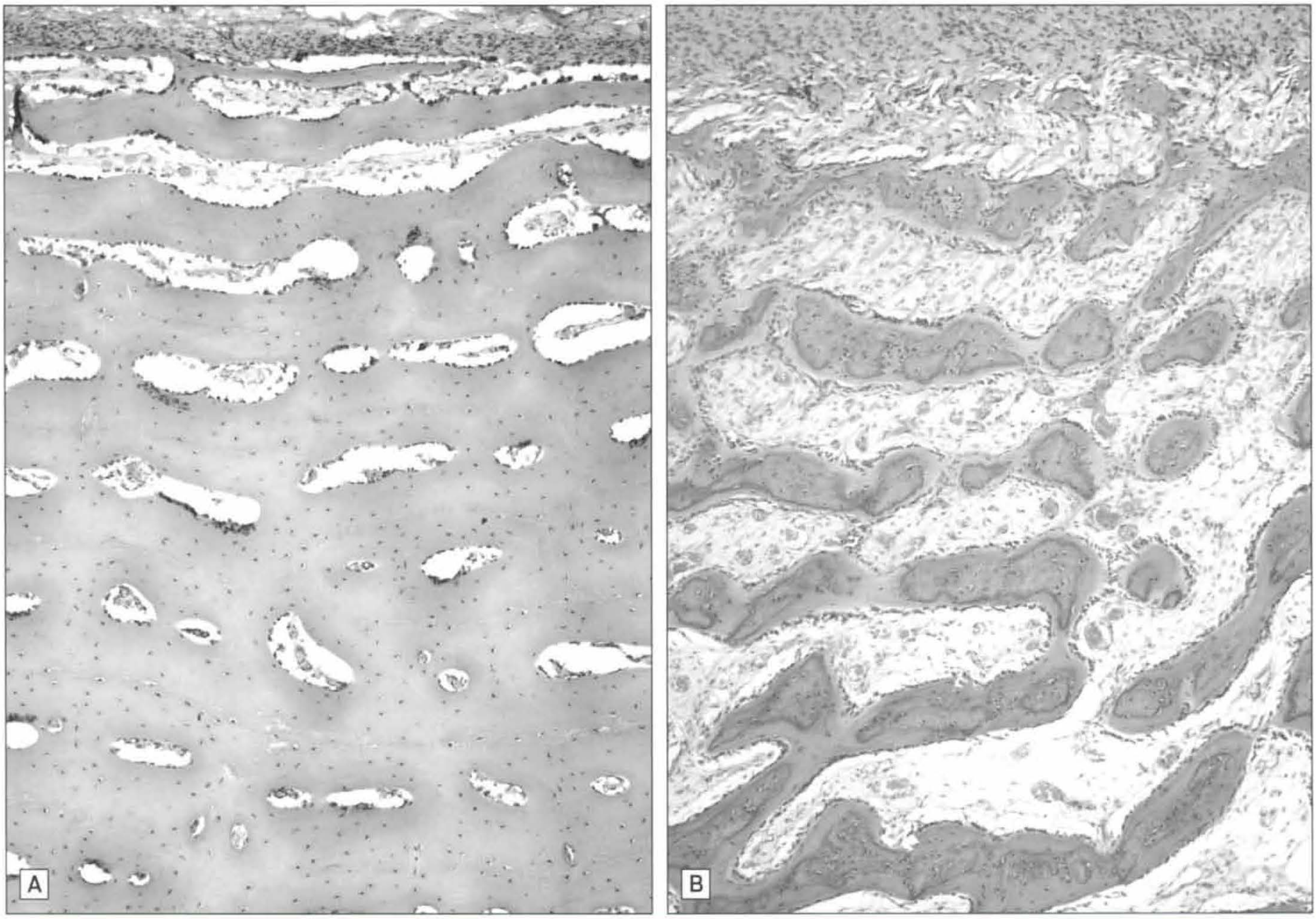

Figure 1.32 Bovine osteogenesis imperfecta. **A.** Microscopic view of periosteum and developing cortical bone in a **normal newborn calf**. **B.** Similar view of the developing cortex in an **affected calf** showing basophilic spicules of woven bone and minimal compaction by the addition of lamellar bone.

Skin fragility is not reported in calves with osteogenesis imperfecta, but Belgian calves with dermatosparaxis do have skeletal lesions. These calves are deficient in the activity of procollagen *N*-peptidase, an enzyme involved in the conversion of procollagen to collagen. Although the bones are grossly normal and not prone to spontaneous fractures, they are abnormally brittle when processed. Histologically, the cortical bone of periosteal origin is disorganized and hypermineralized, while that derived from compaction of the chondro–osseous complex is normal. **Arachnomelia**, a lethal defect in German Simmental and Brown Swiss-Braunvieh cattle, is also accompanied by increased bone fragility but affected calves also have arthrogryposis, distortion of the vertebral column and brachygnathia inferior, in addition to cardiovascular anomalies. The limbs are long and distorted, and the bones have very narrow diaphyses.

In **sheep**, severe forms of osteogenesis imperfecta have occurred in two flocks in New Zealand and one in the United Kingdom. In both New Zealand reports, one of which involved the **Romney** breed, affected lambs died either during parturition or soon after. The Romney lambs had a domed head (Fig. 1.33A) with brachygnathia inferior, dark blue sclerae, fragile, pink teeth, and marked joint laxity. A feature of the disease in Romneys, which involved about 50 lambs, was the presence of marked skin fragility. This has not been described in any of the other reports of osteogenesis imperfecta in humans or animals. Using DNA fingerprinting, the defect was traced to one of five clinically normal Romney rams being used in the flock and a suspicion of *dominant inheritance* was confirmed by test-matings with unrelated ewes of a different breed. In affected lambs, the bones could be easily bent or cut with a knife and were often abnormally shaped with thickened diaphyseal regions (Fig. 1.33B). Most lambs had recent fractures surrounded by hemorrhage and older (in utero) fractures with poorly formed calluses, especially involving the ribs. Microscopic changes are similar to those described in calves with osteogenesis imperfecta, but more severe. Persistent trabeculae of calcified cartilage, lined by a thin layer of basophilic bone, extend from growth plates deep into the diaphyses of long bones, filling the marrow cavities (Fig. 1.34A, B). Osteoclastic resorption of the primary spongiosa is impaired, and there is no formation of a recognizable secondary spongiosa. The cortices are extremely porous, but thicker than normal, and consist of narrow, basophilic trabeculae separated by loose connective tissue. There is no evidence of compaction into normal cortical bone.

Similar gross and microscopic bone lesions occurred in lambs from the other New Zealand flock, together with tooth abnormalities and skin fragility. The breed of these sheep was not disclosed and the mode of inheritance not determined, but circumstantial evidence favors a dominant trait. The United Kingdom outbreak occurred in lambs of the **Clun Forest** breed. These lambs often survived parturition and were bright and alert, but usually could not stand. Blue sclerae were reported but there was no mention of tooth abnormalities. Multiple recent and intrauterine bone fractures were present in most lambs, and the histological lesions in bone closely resembled those seen in the New Zealand lambs.

A milder form of osteogenesis imperfecta is described in **Barbados Blackbelly** sheep. Although fractures occur in utero, affected lambs are born alive and can stand, in spite of joint laxity. The teeth of these lambs are normal. Autosomal recessive inheritance is suspected in this breed.

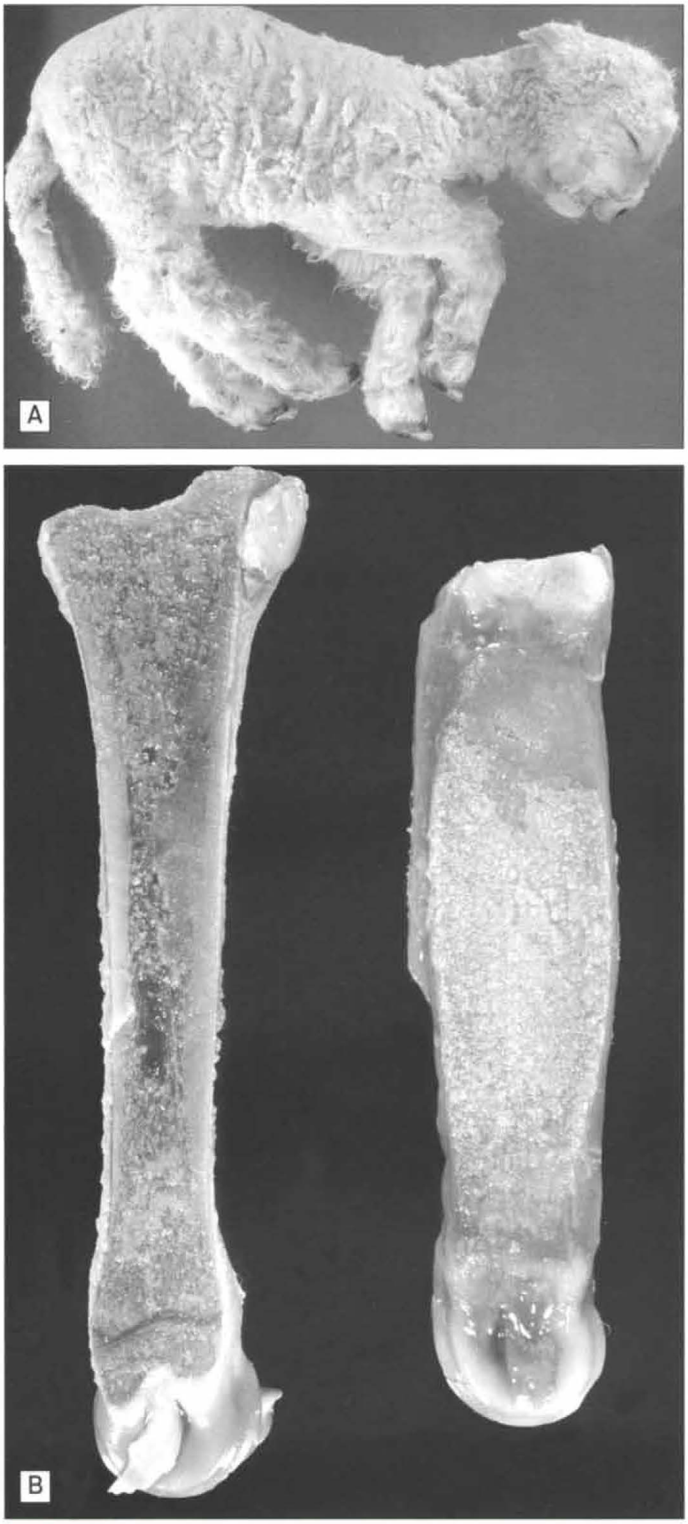

Figure 1.33 Ovine osteogenesis imperfecta in Romney lamb. **A.** Note the domed forehead and brachygnathia inferior. **B.** Sagittal sections of metatarsal bones from a **normal newborn lamb** (left) and a **lamb with osteogenesis imperfecta**. The bone from the affected lamb is shorter, has a thickened diaphysis and no medullary cavity.

Osteogenesis imperfecta has been described in **puppies** and **kittens**, although it appears likely that early reports of the disease in these species were misdiagnoses of fibrous osteodystrophy caused by nutritional secondary hyperparathyroidism. More recently,

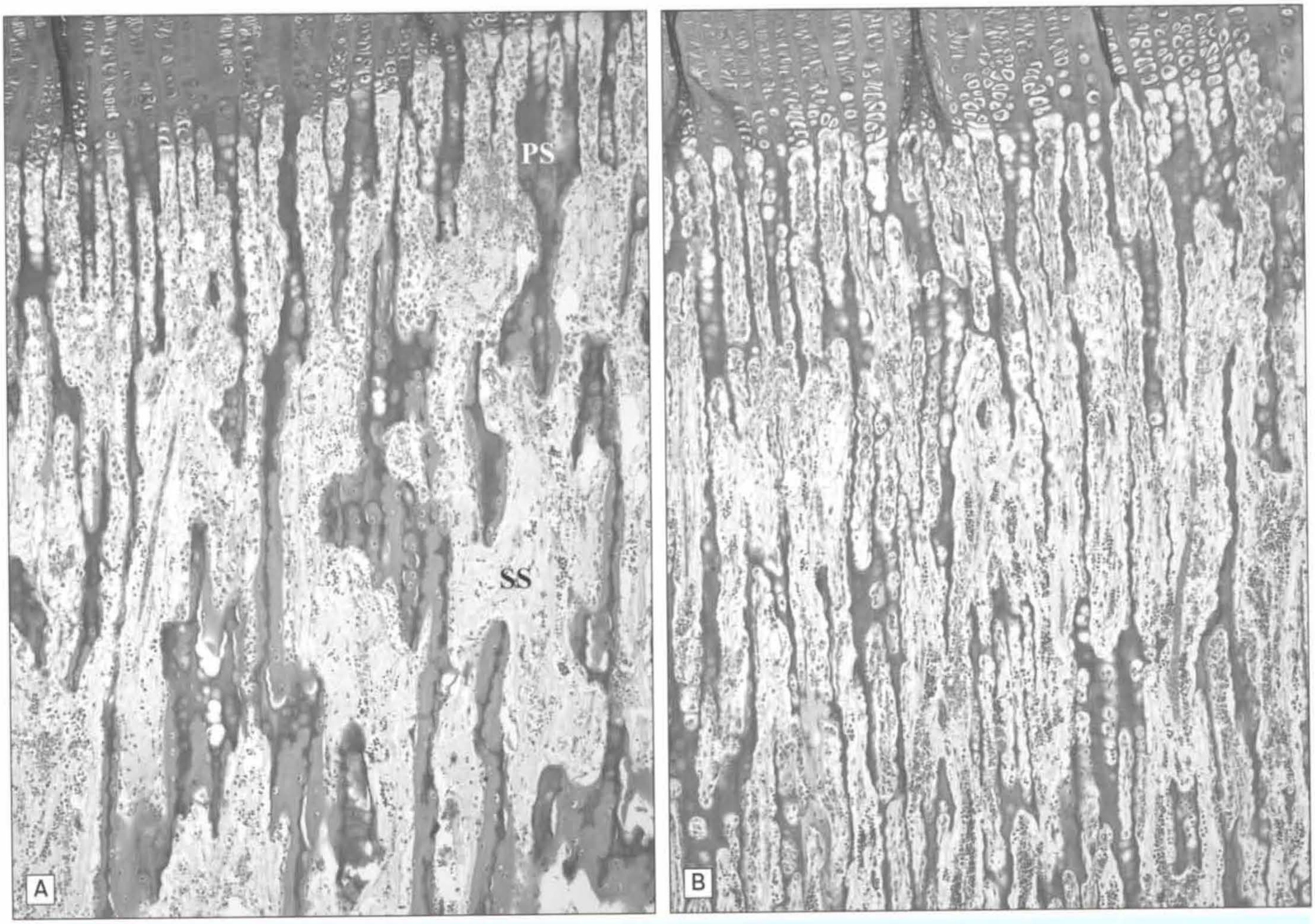

Figure 1.34 Ovine osteogenesis imperfecta. **A.** Metaphysis from a long bone of a **normal newborn lamb** showing primary spongiosa (PS) and secondary spongiosa (SS). **B.** Similar microscopic field from a **newborn lamb with osteogenesis imperfecta.** The primary spongiosa persists deep into the metaphysis and consists of delicate trabeculae lined by a thin layer of basophilic bone.

convincing cases have been recognized in both species. Multiple bone and tooth fractures, together with joint laxity, were reported in 2/4 and 3/6 pups from two litters of **Dachshunds** sired by the same dog. The disease was apparent from 3–4 weeks of age and histologic lesions in bone and dentine resembled those described in calves and lambs with osteogenesis imperfecta. Isolated cases of osteogenesis imperfecta have also occurred in **Golden Retriever, Beagle**, and **Standard Poodle** pups. Defective type I collagen formation was demonstrated in these three breeds using cultured skin fibroblasts. The disease was also diagnosed in a 12-week-old **domestic longhair** kitten that developed multiple spontaneous fractures of long bones. The kitten also had fractured teeth. Histologic examination of bones revealed a marked reduction in the quantity of cortical bone, most of which was woven bone, and an absence of well-developed osteons. These cases of osteogenesis imperfecta in the puppies and kitten appear analogous to the milder forms of the disease in humans.

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Osteopetrosis

Osteopetrosis, or "*marble bone disease*," is a group of rare disorders occurring in humans, as well as in laboratory and domestic animals, characterized by *defective osteoclastic bone resorption and the accumulation of primary spongiosa in marrow cavities.* Two main forms of the disease are recognized in humans – a severe, recessively inherited, *lethal (malignant) form* with lesions present at birth, and a *dominant (benign) form* which becomes manifest later in life. Intermediate forms also exist. *In animals, most descriptions of osteopetrosis appear analogous to the malignant form and autosomal recessive inheritance is suspected in most cases.* Because of the complexity of osteoclast differentiation and function there are many points at which bone resorption can be interrupted, thus accounting for the clinical and morphologic variability associated with the osteopetroses. In some forms, osteoclasts are present in abundance but are incapable of resorbing bone, while in others osteoclasts are either absent or markedly reduced in number. For example, in the *op/op* osteopetrotic mouse, a deficiency in macrophage specific growth factor (M-CSF or CSF-1) results in an absence of osteoclasts. The disorder in these mice can be successfully treated with M-CSF. Deficiency of carbonic anhydrase II causes osteopetrosis in humans by interfering with the intracellular generation of protons by osteoclasts. Affected individuals have abundant osteoclasts, but acidification of the microenvironment at the cell–bone interface is not possible and resorption cannot occur. Patients with this form of osteopetrosis also have renal tubular acidosis and cerebral mineralization, reflecting a systemic deficiency of the enzyme.

In **cattle**, osteopetrosis is best studied in the **Angus** breed, where it is inherited as an *autosomal recessive trait.* Affected calves are small, premature (250–275 days of gestation) and usually stillborn. Clinically, they show brachygnathia inferior, impacted molar teeth and protruding tongue (Fig. 1.35A, B). The long bones are shorter than normal and easily fractured. Radiographically, the medullary cavities are dense, without clear differentiation between the cortex and medulla. Vertebrae are shortened, frontal and parietal bones are thick, and the bones of the cranial base are thick and dense. On cut surface, *the metaphyses and diaphyses of long bones, are filled with dense, unresorbed cones of primary spongiosa extending from the metaphysis to the center of the diaphysis* (Fig. 1.36A). In spite of their increased density, the bones are more fragile than normal and fractures are sometimes detected at necropsy. The fragility is much less marked than in osteogenesis imperfecta. Although the skeletal lesions of osteopetrosis are generalized, they vary in expression throughout the body. Nutrient foramina are either absent or hypoplastic. As a result of the abnormalities of the skull, the cerebral hemispheres are rectangular with flattened dorsal surfaces, the cerebellum is partially herniated through the foramen magnum, and optic nerves are hypoplastic.

Histologically, growth plates are essentially normal but metaphyses are relatively avascular. Dense chondro-osseous tissue, consisting of cartilage matrix lined by a thin layer of woven bone, occupies the medulla (Fig. 1.36B). Osteoclasts are rare and when present, appear to be inactive. *Failure to replace the primary spongiosa and its associated woven bone with thicker trabeculae of mature lamellar bone, presumably accounts for the increased fragility of osteopetrotic bones.* Cortical bone is apparently normal. In the brain, mineralized vessel walls and/or neurons may be found in the thalamus, cerebellum, meninges, choroid plexus, and around the aqueduct of Sylvius.

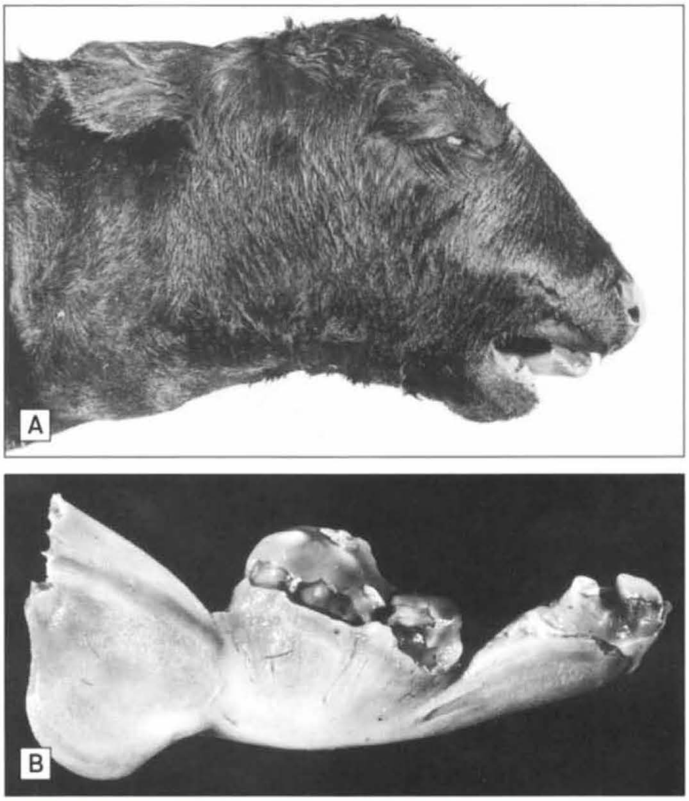

Figure 1.35 Bovine osteopetrosis. A. Stillborn Angus calf with inferior brachygnathia. **B.** Impacted molars in deformed, fragile mandible from the same calf.

Osteopetrosis of **Hereford** and **Simmental** breeds resembles that of Angus calves in most respects, including mode of inheritance, but in affected Herefords the frontal bones are markedly thickened and filled with cystic spaces. The domed forehead of these calves could be misinterpreted as hydrocephalus unless the skull is sectioned. An outbreak of an osteopetrosis-like disorder involving five of 16 Angus and Angus-Charolais cross fetuses has been reported but was associated with multiple skeletal deformities, including arthrogryposis and kyphoscoliosis in addition to frontal bone lesions similar to those of osteopetrosis in Hereford calves. Although a genetic etiology was suspected, the more likely possibility of a teratogenic agent was not excluded. In particular, infection of fetuses or young calves with ***Bovine viral diarrhea virus*** is capable of causing zonal osteopetrosis-like lesions, most likely secondary to transitory virus-induced osteoclast depletion. Acquired osteopetrosis-like lesions with varying degrees of metaphyseal sclerosis may also occur in association with ***Canine distemper virus*** infection in pups and in **lead poisoning** (see below).

A syndrome characterized by *persistence of the secondary spongiosa, perhaps analogous to metaphyseal dysplasia,* a rare human osteosclerotic disorder, was reported in three aborted or neonatal Hereford, Holstein, and Japanese Black calves. The macroscopic and histologic changes in these calves differed from those of osteopetrosis and

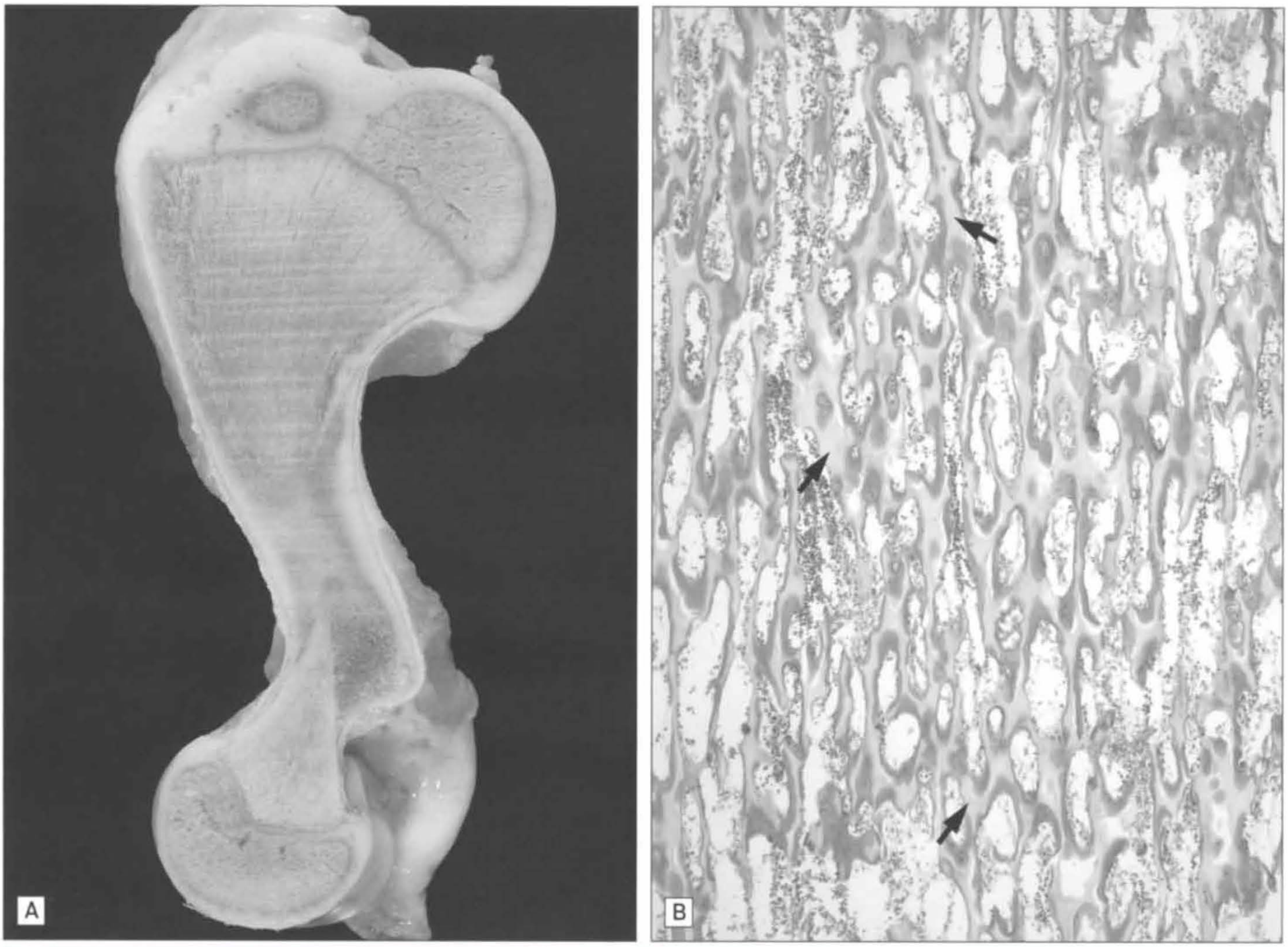

Figure 1.36 Bovine osteopetrosis. A. Sagittal section of humerus showing unresorbed cones of primary spongiosa extending from the proximal and distal growth plates into the diaphysis. No medullary cavity is evident. (Courtesy of RA Fairley.) **B.** Section of metaphysis showing a dense lattice of delicate interconnected trabeculae consisting of cartilage matrix (arrows) covered by a thin layer of bone.

their significance is questionable. In two of the calves, the shape of the bones was essentially normal and there was no mention of fragility. Osteoclast numbers were not reduced but those present were thin and inactive. Such subtle skeletal abnormalities could be much more common than is currently recognized, as without radiography or longitudinal sectioning of long bones they would remain undetected.

Osteopetrosis in **horses** with clinical, radiographic and pathologic features similar to the severe lethal form in Angus calves has been reported in four **Peruvian Paso foals** and one **Appaloosa**. Affected foals are of normal size at birth and are born alive but unable to stand. Brachygnathia inferior is a consistent finding and there is malpositioning and impaction of the teeth, reflecting the requirement of osteoclastic activity for normal tooth eruption. Long bones and vertebrae contain medullary cones of primary spongiosa extending from the physis to the center of the diaphysis, filling the medullary cavity (Fig. 1.37). The fragility of the bones is illustrated by the presence of multiple rib fractures, some of which probably occur in utero. Histologic changes are similar to those described for Angus calves except for the presence of normal to increased numbers of osteoclasts in affected foals. The osteoclasts appear larger than normal but no ruffled border is evident ultrastructurally, *suggesting a functional defect in osteoclasts as the basis of the disease in foals*. Although the total number of cases is small, it is likely that the disease in Peruvian Paso horses is inherited as an autosomal recessive trait.

Osteopetrosis has also been reported in an inbred captive herd of **white-tailed deer**. In addition to marked brachygnathia inferior, impacted teeth and protruding tongue, affected fawns had characteristic radiodense bones and calluses on several ribs, indicating in utero fractures.

In **dogs**, osteopetrosis is reported in three neonatal **Dachshund** puppies from the same litter. Their bones were of normal size and shape but had increased fragility. Isolated cases in an **Australian Shepherd** and a **Pekingese** were diagnosed at 12 months and 30 months of age respectively. Both dogs developed severe myelophthisic anemia.

In **cats**, osteopetrosis has been associated with chronic vitamin D toxicosis and subsequent to infection with *Feline leukemia virus*. Increased density of vertebrae and thickening of the cortices of long bones characterized an osteopetrosis-like disorder in two adult

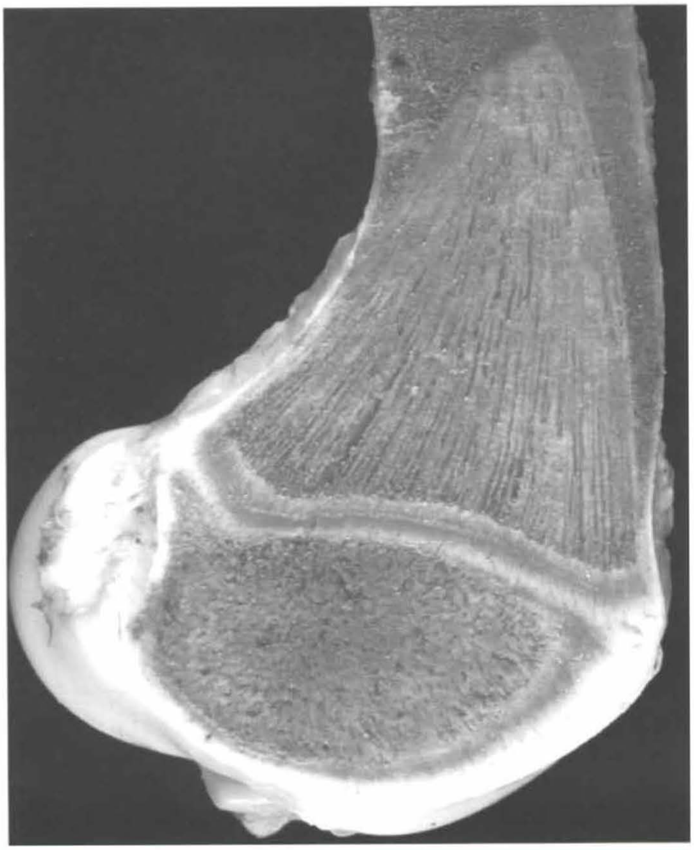

Figure 1.37 Equine osteopetrosis. Distal femur of an affected foal. Note the cone of unresorbed primary spongiosa extending into the metaphysis from the physis. (Courtesy of MW Leach.)

cats. The shape and size of the bones were normal. This syndrome, and that in the Australian Shepherd and Pekingese dogs, may be analogous to one of the benign forms of human osteopetrosis, or could be acquired lesions of undetermined pathogenesis.

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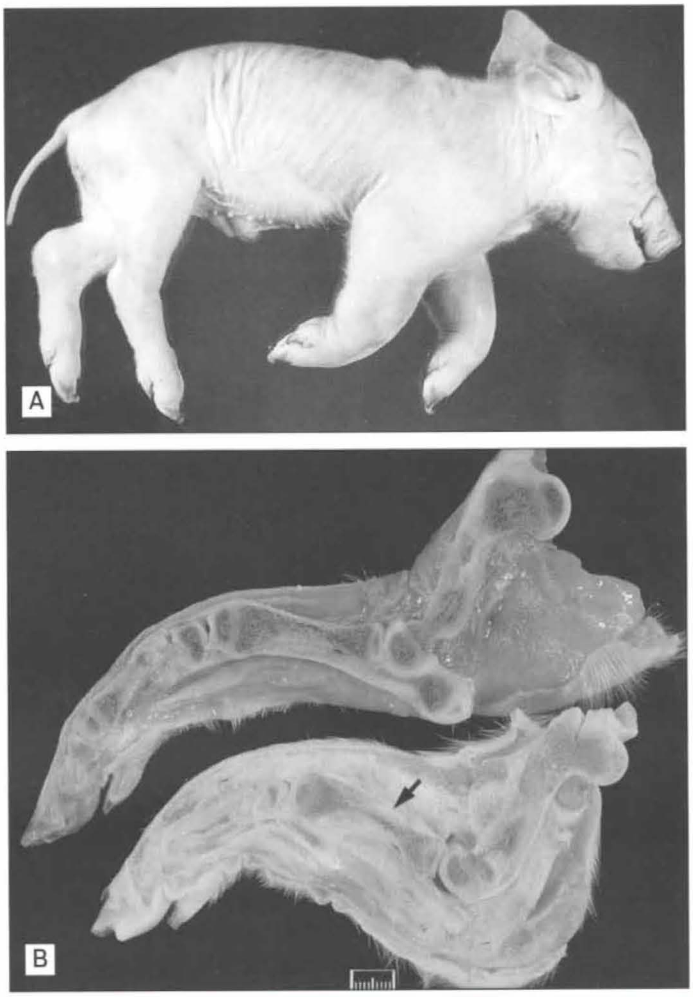

Figure 1.38 Porcine congenital hyperostosis. A. Marked swelling of both forelegs and less severe involvement of the left hindleg. **B.** Comparison of forelegs from a normal (above) and affected piglet. The radius of the affected limb is thickened due to increased periosteal bone (arrow). There is extensive edema and swelling of soft tissues throughout the entire limb. (Courtesy of RA Fairley.)

Congenital hyperostosis

Congenital hyperostosis (*cortical hyperostosis, diaphyseal dysplasia*) is a rare disease of newborn **pigs**. Affected piglets are either stillborn or die within the first few days of life, and show various degrees of thickening of one or more forelimbs (Fig. 1.38A), and sometimes the hindlimbs as well. The thickened limbs are hard and may be up to twice their normal size. The radius and ulna are the most severely affected and in some cases are the only bones involved. Grossly, *a thick layer of extracortical bone extends along the diaphyses* (Fig. 1.38B). Marked edema of the surrounding soft tissues contributes to the thickness of the limb. Histologically, radiating trabeculae of woven bone extend from the surface of apparently normal cortical bone beneath a thickened periosteum (Fig. 1.39A) containing multiple layers of active osteoblasts. The periosteum merges with edematous, poorly vascular connective tissue containing scattered, stellate fibroblasts, which infiltrate adjacent muscle bundles and the overlying dermis (Fig. 1.39B). No lesions are found in bones of the axial skeleton.

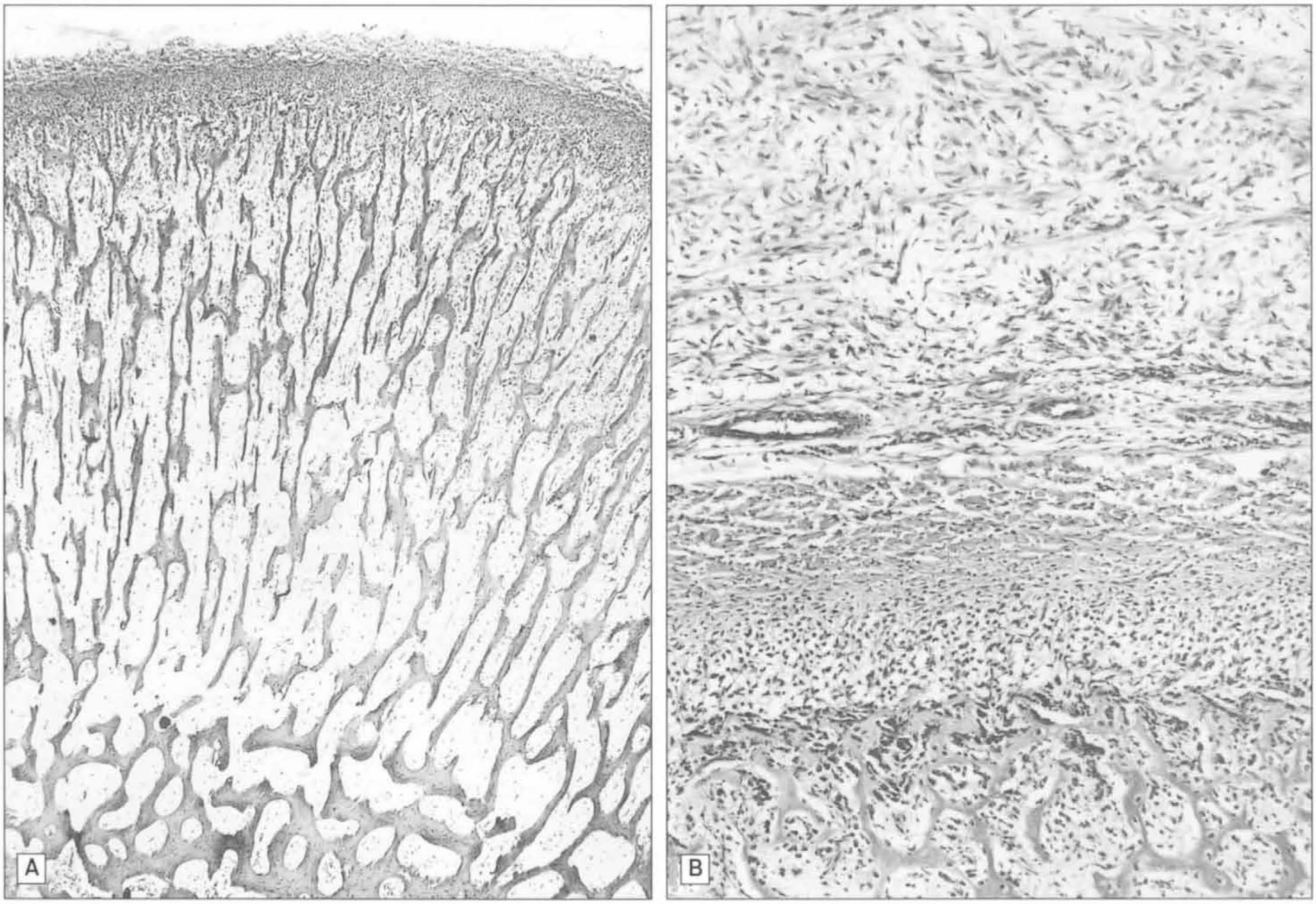

Figure 1.39 Porcine congenital hyperostosis **A.** Transverse section of bone showing radiating trabeculae of woven bone extending out from the cortex beneath a thickened periosteum. **B.** Closer view of reactive bone forming at the periosteal surface. The surrounding muscle is edematous, myofibers are atrophic and there are many stellate fibroblasts throughout.

Congenital hyperostosis of pigs is believed to be inherited as an autosomal recessive trait, but this has not been proven. In fact, the disease may not be inherited at all. The pathogenesis is not known, but the periosteal reaction is consistent with prolonged edema caused by a local circulatory disturbance. Arteriosclerotic lesions in small arteries and arterioles have been identified in vessels supplying the radioulnar region in affected piglets, supporting the possibility that the mechanism may involve localized hypertensive changes, perhaps due to abnormal intrauterine positioning of the fetus.

Infantile cortical hyperostosis, or Caffey's disease, is a disease of children, which resembles congenital hyperostosis of pigs. The disease may be congenital but more often develops in infancy and is characterized by extensive periosteal new bone formation at multiple sites, most commonly the mandible, clavicle, and long bones, including those of the forearm. However, the human syndrome is self-limiting and regresses over several years. A hyperostotic disease similar to infantile cortical hyperostosis was reported in a **West Highland White Terrier** dog that had periosteal new bone formation involving the pelvis, scapulae, humeri, ulnae, femora, radii, and tibiae when it was destroyed at 22 months of age.

Osteochondromatosis

Osteochondromatosis, also known as *multiple cartilaginous exostosis*, is a skeletal dysplasia characterized by the formation of single or multiple tumor-like exostoses. In humans, dogs, and horses, the disease is inherited as an *autosomal dominant trait*. In dogs, the lesions usually are not present at birth but arise in young animals on the surface of endochondral bones adjacent to physes or subarticular growth cartilages, and continue to enlarge until the skeleton matures. In affected horses, the lesions are often present at birth and tend to be bilaterally symmetrical. Because of their tumor-like appearance, these lesions are discussed in more detail in the section on cartilage-forming tumors (Tumors and tumor-like lesions of bones, p. 119).

Idiopathic multifocal osteopathy

This syndrome, characterized by *multifocal absence of bone in the skull, cervical vertebrae, and proximal regions of the radius, ulna and femur*, has recently been reported in four **Scottish Terriers**. Three of the four dogs were related, suggesting a genetic etiology. It was not clear whether the disorder was acquired or congenital as the abnormalities were not detected until the dogs were young adults, by which time the lesions were well developed. The localized areas of absent bone were replaced with fibrous tissue but whether this reflects abnormal development of the bone, or lysis of bone and replacement fibrosis, is not clear. Histological evidence of osteolysis was present in one case, supporting the latter option. A similar condition characterized by multicentric bone lysis and replacement fibrosis occurs in humans where it is referred to as *Winchester syndrome or "vanishing bone disease."*

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Localized skeletal dysplasias

Skeletal dysplasias characterized by localized anomalies of the appendicular or axial skeleton are included in this section, together with discussion of the vertebral abnormalities associated with wobbler syndrome in dogs and horses.

Limb dysplasias

The most frequent malformations of the limbs are the localized chondrodysplasias seen in chondrodystrophoid breeds of dog. These are inherited as polygenic traits and are mediated by variations in receptor activities for hormones and other factors that regulate the proliferation of physeal chondrocytes. The defect is usually confined to the distal bones, producing the sort of dwarfism seen in such breeds as the Basset Hound and Dachshund. In some breeds, such as the Pekingese, chondrocytes of neural crest origin are also involved and there are basocranial abnormalities. In other breeds, such as the Boston Terrier and Boxer, only the basocranium is affected, the limbs being of normal length. The cartilage abnormality may also involve the intervertebral disks, the central or perinuclear portions of which develop as immature hyaline cartilage with only a gradual transition at the periphery towards the normal laminated fibrocartilage of the annulus fibrosus. Such disks are predisposed to degeneration of the hyaline cartilage followed by herniation of the disks (see disk degeneration and spondylosis, p. 154).

A wide range of defects of the limbs and/or digits has been reported in domestic animals, often as isolated cases of unknown etiology. Rather than attempt to present a comprehensive list, discussion in this section will focus on those conditions known or suspected to be genetic in origin.

Syndactyly, a defect characterized by *partial or complete fusion of functional digits*, occurs in several breeds of cattle, including Holstein-Friesian, Angus, Chianina, Hereford, Simmental, German Red Pied, Indian Hariana, and Japanese native cattle. Inheritance in cattle is *autosomal recessive with incomplete penetrance and variable expression.* The disorder became very common in the Holstein-Friesian breed following extensive use of a heterozygous bull by artificial breeding, but is rare in other breeds. In Holstein-Friesians, the disorder seldom affects all four digits. The right forelimb is most frequently affected, followed by the left forelimb then the right hindlimb. In contrast, the defect in Angus cattle usually affects all four digits. *The lesions of syndactyly may vary from complete horizontal fusion of paired phalanges to fusion of only one or none of the phalanges, but fusion of interdigital soft tissues.* Vertical fusion of phalanges is reported in some affected Angus cattle. The production of affected calves from Angus-Holstein matings suggests that the same gene locus is involved, at least in these breeds, but differences in the manifestations of syndactyly between affected Angus and Holstein-Friesian calves suggests the existence of two recessive alleles. In the Holstein-Friesian breed, syndactyly is linked to increased susceptibility to hyperthermia and affected cattle often die of heat stress. Syndactyly has been reported in Shorthorn cattle in association with **dactylomegaly**, a form of club foot (talipes). The condition was characterized by various degrees of dew claw enlargement. Syndactyly is also reported in pigs, where it is presumed to be caused by a simple dominant gene, and occasionally in sheep and dogs.

Incomplete fusion of the paw is reported in dogs and cats and referred to as **ectrodactyly**. In cats, a *dominant gene* with variable expressivity is responsible and the defect is bilateral. In dogs, dominant inheritance is likely and the defect is usually unilateral. The cleft in the paw extends to the level of the metacarpals, and occasionally reaches the carpus. Various other limb defects may accompany the ectrodactyly, including aplasia or hypoplasia of carpal and metacarpal bones, duplication of digits, fusion of metacarpals, and elbow joint luxation. Syndromes characterized by total or partial absence of phalanges have been reported as ectrodactyly in calves and lambs. These defects differ from the disease in cats and dogs and may be better termed **adactyly**, a condition characterized by the *absence of phalanges* and reported as a possible inherited defect in Southdown lambs.

Polydactyly *is an increase in the number of digits.* The anomaly is observed in all species but is perhaps best known in *cats, dogs, horses, and cattle.* Polydactyly is an inherited trait in various bovine breeds but the inheritance pattern is poorly understood. In most cases, it is the medial digit (digit 2) that is duplicated and although all four feet may be affected, the anomaly is more frequently confined to the forelimbs. A polygenic mode of inheritance requiring a dominant gene at one locus and two recessive genes at another is postulated in Simmental cattle. In horses, two forms of polydactyly occur. The *common, atavistic type* features an extra medial digit, usually involving a forelimb, which articulates with a second metacarpal. *The rare, teratogenic form* is characterized by duplication of bones distal to the fetlock joint, producing a cloven hoof. In goats, a Shami buck with two extra digits on both hindlegs sired a doe kid with a similar defect, suggesting a genetic etiology. An inherited syndrome of multiple skeletal defects including polydactyly and syndactyly together with cleft palate, shortened tibia, brachygnathism, and scoliosis occurred in a family of Australian Shepherd Dogs. An X-linked lethal gene was suspected. Polydactyly is also reported in pigs where it is usually associated with cleft palate and is believed, but not proven, to be inherited.

Hemimelia *refers to the partial absence of one or more limbs* and occurs as a recessively inherited lethal defect in Galloway calves. Affected calves have bilateral agenesis of the tibial bones and patellae. Other bones of the hindlimbs are apparently normal, highlighting the localized nature of the defect. A similar syndrome characterized by tibial hemimelia, meningocele, retained testicles and abdominal hernia is reported in Shorthorn calves and is also believed to be transmitted as an autosomal recessive trait. Bilateral tibial hypoplasia has been reported in a Simmental calf. A recessively inherited form of hemimelia involving distal forelimbs is reported in Chihuahua dogs. The abnormality may be either transverse, in which case the entire forearm is absent, or paraxial, where there is aplasia of only some metacarpals and digits. Paraxial hemimelia with bilateral agenesis of radial bones is reported in goat kids. In lambs, hemimelia characterized by incomplete development of distal limbs has been reported but in one "outbreak" was most likely due to ingestion of lupin alkaloids by pregnant ewes, rather than a genetic defect.

Peromelia, a term sometimes used synonymously with hemimelia, has been used to describe a syndrome characterized by *agenesis of distal parts of the limbs in Angora goats*. Affected kids lack the phalanges and parts of the metacarpus or metatarsus on one or more limbs. Autosomal recessive inheritance is suspected. In isolated cases where the distal limb is absent, it is important to exclude the possibility that the missing component was accidentally ingested by the dam when she was eating the placenta. This is most likely to occur in goats. *Congenital absence of one or more limbs*, **amelia**, has been reported in a foal and a calf.

Skull

Brachygnathia inferior, *shortening of the mandibles*, and **brachygnathia superior**, *shortening of the maxillae*, may occur alone or in combination with other skeletal defects. Brachygnathia inferior is commonly encountered in otherwise normal domestic animals and, while not life-threatening, is considered an undesirable characteristic. Genetic and teratogenic etiologies have been suggested and both are likely to occur. Brachygnathia superior occurs in association with degenerative joint disease as a lethal genetic defect in Angus calves. In addition to brachygnathia superior, affected calves have other skull changes similar to those of brachycephalic dogs and degenerative changes are present in articular cartilage throughout the body. The nature of the underlying defect is not known, but histologically there is degeneration of cartilage matrix and necrosis of chondrocytes in articular cartilage.

Sternum

Lateral curvature of the sternum occurs in association with vertebral scoliosis, especially when the latter shows simultaneous torsion. *Retraction of the caudal sternebrae and xiphoid* is seen in lambs and calves, and is apparently due to shortness of the tendinous portions of the diaphragm. *Clefts of the sternum* may occur as isolated defects, but are usually accompanied by ectopia cordis or form part of the defect known as *schistosomus reflexus*. In this syndrome, there is lordosis, dorsal reflection of the ribs with more-or-less total eventration, nonunion of the pelvic symphysis and dorsal reflection of the pelvic bones. *Sternal deformity* also occurs in "spider lamb" chondrodysplasia.

Ribs

Costal abnormalities are usually secondary to malformation of the vertebral column or sternum. Absent or fused ribs correspond to absent or fused vertebrae and may accompany severe scoliosis.

Pelvis

Lesions of the lumbosacral region, arising from abnormalities of the notochord failing to give rise to appropriate dermatomes, occur in humans and lead to various abnormalities often collectively termed the **caudal regression syndrome**. Abnormalities of this derivation result in lumbosacral agenesis, as seen in English bulldogs, but often are accompanied by anorectal abnormalities, urogenital anomalies, various anomalies of the spinal cord, and in humans the development of a variety of tumors and cysts. These include yolk sac cysts, cysts of urogenital origin, lipomas, teratomas, and nephroblastomas. The sacrum may be absent, or it may be hypoplastic or deviated in association with absence of the coccygeal vertebrae. Hypoplastic chondrodysplasia of the coccygeal vertebrae is a characteristic of French bulldogs and occurs occasionally, with kinking of the remnants, in cattle and cats. Malformations of the sacrum accompany other severe spinal defects. The pubic bones are separated and may be absent, in association with ectropion of the bladder.

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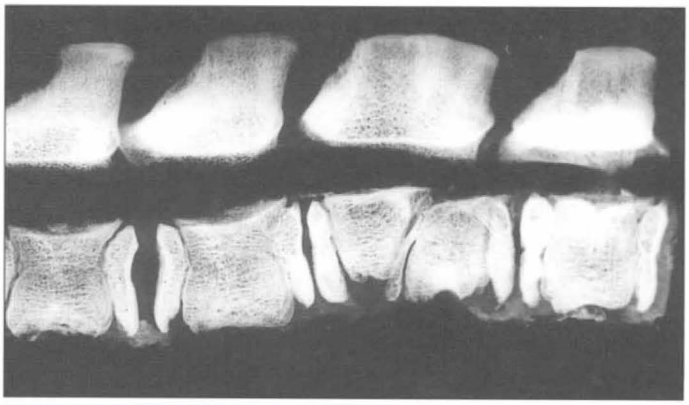

Figure 1.40 Block vertebra. Two vertebral bodies are incompletely separated and their dorsal processes are fused.

Vertebrae

The development of the vertebrae and intervertebral disks is a complex process involving interactions between ectodermal and mesodermal elements. Defects can arise from errors at many stages of development and, because of the proximity of the spinal cord, the consequences are often serious. **Spina bifida** *results from defective closure of dorsal vertebral laminae in a segment of the vertebral column.* It has been reported in several different breeds of cattle and there is some evidence to support autosomal recessive inheritance. Teratogenic agents acting early in pregnancy can no doubt produce similar lesions. The lesions vary markedly in severity and are often accompanied by defects in the overlying skin, meningocele, and sometimes myelodysplasia. The defect may occur at virtually any level of the vertebral column but appears to favor the lumbar and sacral regions. Various nonskeletal defects have been described in association with spina bifida, including arthrogryposis, due to spinal damage and defective innervation of some muscle groups, fusion of the kidneys, unilateral aplasia of one uterine horn, atresia ani, and kyphoscoliosis.

Perosomus elumbus, a rare congenital defect characterized by *agenesis of the lumbosacral spinal cord and vertebrae*, is reported in cattle, sheep, and pigs. In two affected Hereford calves, the spinal column terminated at the last thoracic vertebra. Both calves had arthrogryposis of the hindlimbs and one also had other skeletal defects, including absence of some ribs, unilateral agenesis of the scapula and humerus, and polydactyly involving one leg. The absence of the lumbar spinal cord in affected cattle is associated with extreme muscle atrophy and replacement with adipose tissue. In an affected stillborn Holstein heifer calf, the skeletal abnormalities were accompanied by anomalies in the urogenital system and distal colon/rectum. It has been suggested, but not proven, that perosomus elumbus in Holstein cattle is inherited.

Block vertebra *results from improper segmentation of the somites in the embryo, resulting in complete or partial fusion of adjacent vertebrae* (Fig. 1.40). The fused structure may be equal to or shorter than the pair of vertebrae it replaces. There are usually no clinical signs. **Butterfly vertebrae** are so called because of the *appearance of the indented vertebral end plates on ventrodorsal radiographs.* The cause is *persistence of notochord*, or a sagittal cleft of notochord, that leads to a dorsoventral, sagittal cleft in the vertebral body. The halves may spread laterally but the condition is usually an incidental radiographic finding in screw-tailed dogs such as Bulldogs, Pugs, and Boston Terriers. **Hemivertebrae** may arise as a result of displacement and inappropriate fusion of somites. In this case, *one member of a pair of somites fuses diagonally with a somite cranial or caudal to it*, forming a vertebra but leaving the other members of the pairs to persist as hemivertebrae.

Complex vertebral malformation is a lethal congenital defect of Holstein calves characterized by shortening of the cervical and thoracic vertebral column due to multiple hemivertebrae, fused and misshapen vertebrae, and scoliosis. Affected calves are smaller than normal, sometimes born premature, and consistently show symmetrical arthrogryposis of the forelimbs. The hindlimbs may also show mild arthrogryposis. Approximately 50% have heart malformations, including ventricular septal defects and dextraposition of the aorta. A genetic etiology is suspected. Two common ancestral sires were identified in the pedigrees of 18 affected calves in Denmark and it is suggested that one is a carrier. Both are former elite sires of US origin and it is possible therefore that the defect is present in several countries. There is one recent report of the syndrome in the USA.

Subluxations, fusions, and other anomalies of cervical vertebrae are described with developmental disturbances of joints.

Cervical vertebral stenotic myelopathy (wobbler syndrome)

Cervical vertebral stenotic myelopathy (cervical vertebral malformation, cervicospinal arthropathy, wobbler syndrome) is a neurological disease characterized by slow, progressive, ataxia of the hindlimbs, and occasionally the forelimbs, due to compression or stretching of the spinal cord as a result of a primary abnormality in the vertebral column. The syndrome occurs most commonly in horses and large breeds of dog, particularly Great Danes and Doberman Pinschers. In both species, male animals are affected much more often than females, and rapid growth is an apparent predisposing factor. The vertebral lesions reported in horses and dogs with the disease are similar, suggesting a common pathogenesis. In both species there are two pathologic syndromes, *cervical vertebral instability* and *cervical static stenosis*, the former damaging the cord only when the neck is extended or flexed, the latter resulting in cord compression no matter what position the neck is in. Usually the onset is insidious, but vague signs are often exacerbated following trauma or strenuous activity. The disease in

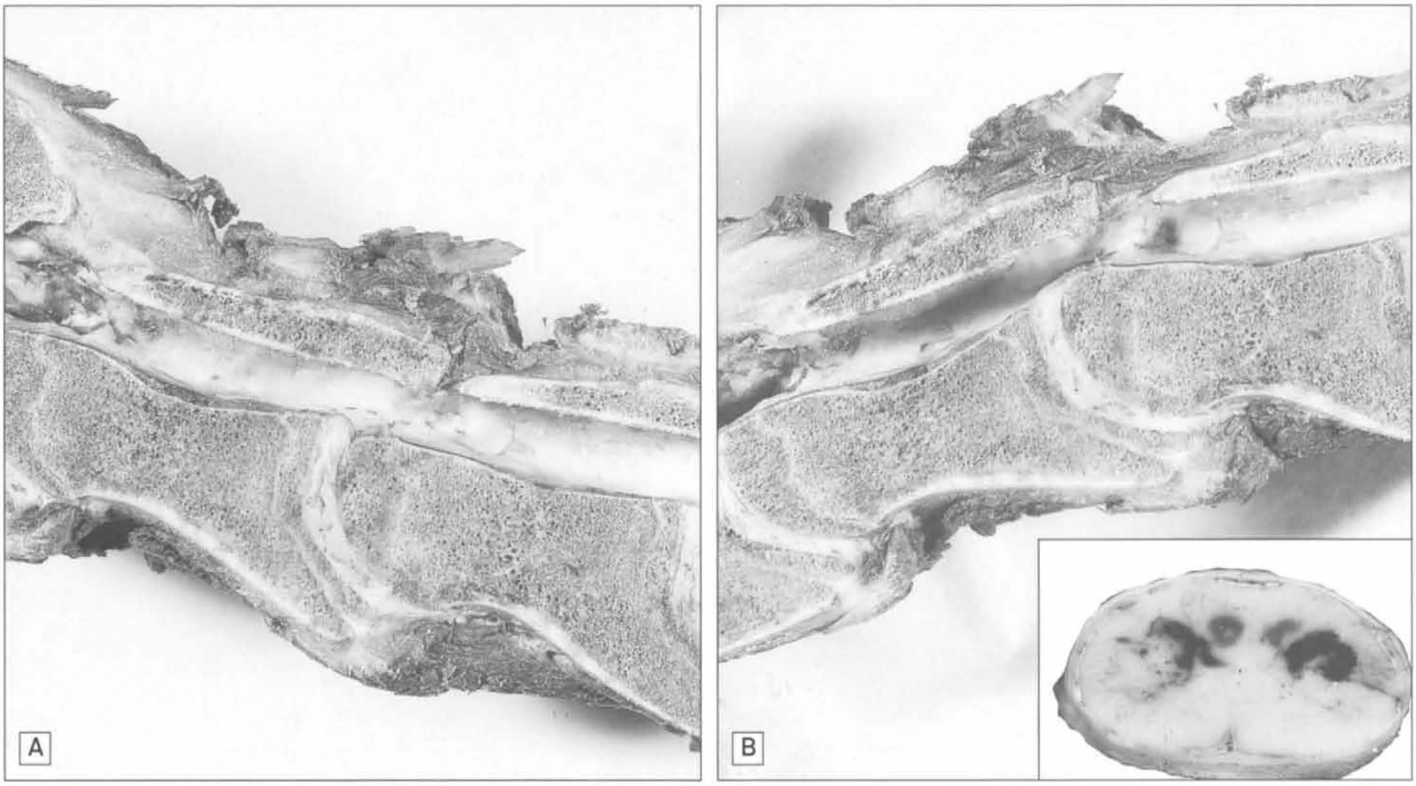

Figure 1.41 Cervical vertebral stenotic myelopathy. C-3 and C-4 from a "wobbler" horse. **A.** Neck extended. **B.** Neck ventroflexed to show stenosis of the spinal canal. Malacia of cervical cord is illustrated in the inset.

horses is not progressive, but if the signs are severe, recovery does not occur.

In **horses**, cervical vertebral stenotic myelopathy occurs most often in *Thoroughbreds* and *Quarter Horses*. **Cervical vertebral instability** usually affects fast growing young horses 8–18 months of age and is associated with lesions in the region of C-3 to C-5. The lesions vary and are not always easy to detect at necropsy, especially since the problem may only be apparent when the neck is ventroflexed (Fig. 1.41A, B). In a few cases, a lip may be palpated on the dorsal border of the vertebral body, apparently due to expansion of the cranial epiphysis. More often, a distinct lip is not palpable, but a mid-sagittal section of affected vertebrae shows that the floor of the spinal canal angles upwards and the roof angles downwards to produce a funnel-shaped canal with cranial stenosis. Occasionally, the cranial articular processes of the vertebral bodies project in a ventro-medial direction and impinge upon the spinal canal, compressing the dorsolateral parts of the cord. In a small proportion of cases, increased mobility of adjacent cervical vertebrae, which allows subluxation is apparently responsible for the cord compression. Although the lesions in cervical vertebral instability occur most often at C-3 to C-5, other vertebrae may also be affected, and more than one lesion may be present in a single animal.

Articular facets of vertebrae in horses with cervical vertebral instability often contain lesions typical of the degenerative arthropathy that follows osteochondrosis (see below). These lesions may be accompanied by osteophytic outgrowths at the articular margin, altering the size and shape of the facet and occasionally encroaching on the spinal canal. Similar changes can however be found in many clinically normal horses and their presence in a wobbler must be interpreted with caution. Asymmetry of articular facets is also present in many affected horses (Fig. 1.42), but this too is of questionable significance, since many clinically normal horses have similar changes. In some affected horses, there is also evidence for osteochondrosis affecting vertebral growth plates in the cervical

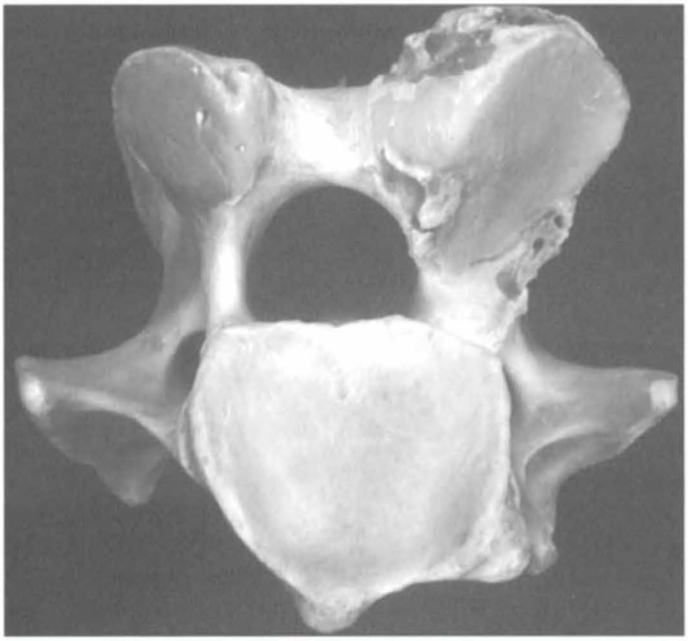

Figure 1.42 Asymmetry of articular facets in a "wobbler" horse. (Courtesy of JD Baird.)

region. Nonetheless, in some horses the spinal cord injury is likely to be due to vertebral malalignment created by asymmetry of articular facets.

The fundamental cause of cervical vertebral instability in horses is not clear. There is growing evidence that the multiple genetic factors that permit rapid growth, and the dietary excesses that encourage it, are responsible. In particular, the *ad libitum feeding of high-protein, high-energy rations to weanlings to produce maximum-sized yearlings seems to be the major influence.* Such feeding practices are commonly associated with osteochondrosis and many affected horses have lesions of this disease, but the significance of this to the overall incidence of cervical vertebral instability is presently undetermined.

Cervical static stenosis, the second, less common form of cervical vertebral stenotic myelopathy, usually occurs in large male horses between 1 and 4 years of age and is caused primarily by *thickening of the ligamentum flavum and the dorsal lamina of the vertebral arches.* The spinal cord is compressed by the encroachment of these structures on the spinal canal. Histologically, there is increased fibrocartilage at the attachment of the ligamentum flavum to the dorsal lamina, and deposition of fibrovascular tissue both in the ligamentum and the contiguous joint capsule. The thickening of the dorsal lamina is due to osteosclerosis, with the dense trabeculae being formed by compaction of the fibrocartilage of the ligamentum flavum. Degenerative arthropathy of articular facets is usually present and, although its severity is not correlated with the severity of signs, it may be the result of a primary vertebral instability that also subjects the ligaments and laminae to abnormal mechanical forces. The osteosclerosis and development of fibrocartilage are consistent with increased forces and joint movement. Compression of the spinal cord by synovial cysts has also been reported.

In the final analysis, *interpretation of osseous lesions depends on their linkage with spinal cord degeneration.* There is variation in the suddenness and the severity of cord injury, and in the immediate pathogenesis of injury, which may occur directly by pressure on the nervous tissues, or indirectly if local blood vessels are compromised. Thus, there may be one or more primary foci of softening in the cervical cord with Wallerian degeneration of the ascending and descending tracts. Careful examination of the cord, with the dura mater reflected, sometimes reveals a slight depression in the contour of the nervous tissue at the affected site. Sometimes, the primary foci of malacia are visible after fixation as brown areas in the gray matter, usually adjacent to an articulation (Fig. 1.41B). The fixed cord should be sliced at intervals of no more than 2 mm, otherwise a lesion may be missed. Histologic examination is necessary to confirm and demonstrate lesions.

Microscopically, there is more-or-less extensive demyelination in the cord. In severe cases with gross lesions, hemorrhagic and necrotic foci can be found in the gray matter in either the ventral or lateral horns, and may be either unilateral or bilateral. Even when lesions are absent from the gray matter, the primary focus or foci are visible in short segments of the white matter, which shows the moth-eaten appearance typical of demyelination. In the primary foci there are ragged microcavitations, which represent areas of liquefactive necrosis. Many adjacent degenerate fibers are swollen and basophilic. About the primary focus, demyelination may be present in all tracts while *cranial to the focus, the lesions can be seen in ascending fibers, most of which are in the dorsolateral funiculi. Caudal to the focus the demyelination is primarily in the ventral and ventrolateral funiculi*, symmetrical on either side of the ventromedian fissure, and extending as far as the lumbar region in some animals. As a consequence of localized edema, often there is striking astrocytosis in chronic lesions and cells are increased both in number and size, becoming gemistocytic in type.

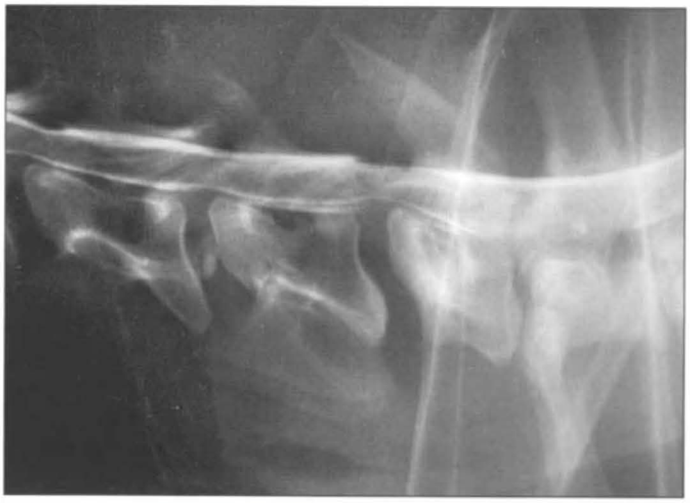

Figure 1.43 Caudal cervical spondylomyelopathy in a Doberman Pinscher. Myelogram showing compression of the spinal cord at the cranial opening of C-7. The body of this vertebra is malformed. (Courtesy of HM Burbidge.)

There are many similarities between cervical vertebral stenotic myelopathy in **dogs** and the disease in horses. The *age of onset varies from a few weeks to several years* although, in general, Great Danes develop signs at an earlier age than Dobermans. There is mounting evidence for an *inherited predisposition*, although overnutrition and abnormal biomechanical forces involving cervical vertebrae have also been suggested as contributing factors. *A variety of anatomical or functional abnormalities have been described in affected dogs, usually in the region of C-6 to C-7.* Malformation of vertebrae, including stenosis of the cranial opening (Fig. 1.43), degeneration and asymmetry of articular facets, and misshapen vertebral bodies caused by early closure of the dorsal part of the caudal physis with elongation of the ventral part of the body, are detected in some affected dogs. Instability of cervical vertebrae, particularly of those that are misshapen, is also reported but is difficult to assess objectively. There is no doubt that some cases of wobbler syndrome in dogs are caused by alterations in the supporting ligaments rather than the vertebrae. In one study, the most common cause of spinal cord compression in dogs was considered to be hyperplasia of the ligamentum flavum with protrusion into the spinal canal. Compression of the cord by the bulging ligament is greatest when the neck is extended and is relieved when the neck undergoes traction or full flexion.

Stenosis of the cervical vertebral canal also occurs in **sheep** and although little is known about the syndrome in this species, it is probably linked to heavy feeding and perhaps the trauma associated with fighting.

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GENETIC DISEASES INDIRECTLY AFFECTING THE SKELETON

Lysosomal storage diseases

The lysosomal storage diseases are a large group of inherited or acquired deficiencies in the activity of specific lysosomal enzymes, culminating in the accumulation of otherwise digestible substrates in the lysosomal system of various cell types. Although many such diseases have been reported in domestic animals, only the mucopolysaccharidoses and the gangliosidoses affect the skeleton. In both groups of diseases there are lesions of greater significance in other organ systems, particularly the central nervous system (see Vol. 1, Nervous system).

The **mucopolysaccharidoses** (MPS) are characterized by the *accumulation of partially catabolized glycosaminoglycans in lysosomes* and, in human medicine, are classified on the basis of the specific enzyme defect and clinical phenotype as MPS I through MPS VII (with several subgroups):

- **MPS I** (Hurler's syndrome), caused by a deficiency of α-L-iduronidase,
- **MPS VI** (Maroteaux-Lamy syndrome), caused by arylsulfatase-B deficiency, and
- **MPS VII**, caused by β-glucuronidase deficiency.

All three have all been associated with skeletal deformities in **cats**.

MPS VI occurs in Siamese cats where it is inherited as an autosomal recessive trait. By 2 months of age the typical features of the syndrome, including broad flattened face, small ears, diffuse corneal clouding, large forepaws, and pectus excavatum are evident. Radiographic lesions are present in the axial and distal skeleton by 6 months of age and are progressive. Most affected cats have symmetrical epiphyseal dysplasia, short stature, and develop degenerative joint disease. Thoracic vertebral bodies are short and there are fusions of cervical and lumbar vertebrae, absence or dysplasia of cervical and thoracic vertebral spinous processes, and increased width of intervertebral spaces. The ribs broaden at the costochondral junctions. In older animals, the epiphyses and metaphyses of long bones are distorted by bony proliferation, and articular surfaces are irregular. Some animals develop posterior ataxia and paresis at less than a year of age due to compression of the spinal cord by osteophytes, which project into the vertebral canal between T-12 and L-2. Affected kittens can be recognized at 1 week of age by their excessive concentrations of urinary dermatan sulfate. Metachromatic granules are present in the cytoplasm of circulating neutrophils. *Microscopic abnormalities in the skeleton are centered on articular and physeal cartilage.* Growth plates have poorly organized proliferative zones, lack of column formation in hypertrophic zones, disorganized zones of mineralization, and uneven chondro–osseous junctions. Chondrocytes are swollen with abundant, finely vacuolated cytoplasm. Osteoclast numbers in the primary spongiosa are reduced. Membrane-bound cytoplasmic inclusions are present in hepatocytes, bone marrow granulocytes, vascular smooth muscle cells and fibroblasts of the skin, heart valves, and cornea. This disease of cats closely resembles MPS VI of human patients.

Feline MPS I is reported in domestic shorthaired cats and most likely has an autosomal recessive mode of inheritance. The bony lesions in this disease are milder than those of MPS VI and are not accompanied by epiphyseal dysplasia of long bones. Nor are there metachromatic granules in circulating neutrophils. Although the number of cases is small, cats with MPS I appear to have a high incidence of meningiomas. Only *isolated cases of MPS VII* are reported in domestic shorthaired cats but the skeletal lesions are severe and resemble those of MPS VI.

MPS I also occurs in **Plott Hounds**, probably as an autosomal recessive trait. Affected dogs are stunted and develop progressive lameness in addition to diffuse, bilateral corneal opacity. A feature of the canine disease is the development of *severe degenerative joint disease* with extensive periarticular bone proliferation. Cytologic evaluation of synovial fluid may reveal mononuclear and multinucleated cells containing abundant basophilic granules (on Wright-Giemsa stain). Allogeneic bone marrow transplantation of irradiated dogs reduces the neurovisceral lesions of MPS I but the effect is minimal in chondrocytes, except those near the articular surface.

GM$_1$ gangliosidosis *occurs in cats, calves, sheep, and dogs, and is due to a deficiency of beta-galactosidase.* In sheep, there is a concomitant deficiency of α-neuraminidase. In dogs, skeletal lesions are present in **English Springer Spaniels** but in the Beagle, only the neurovisceral changes are present. Also, the stored undegraded oligosaccharides differ between the two breeds. In both breeds, the trait is autosomal recessive. Affected Springers develop neurologic signs at 4–6 months. Skeletal lesions may include proportional dwarfism with frontal bossing and hypertelorism, irregular intervertebral spaces, and degenerative changes in the femoral and tibial articular cartilages.

A storage-like disease with visceral and skeletal (but not neural) involvement occurred in a **Rhodesian Ridgeback dog**. The dog was lame from an early age and showed multiple osteolytic lesions in radiographs of long bones and vertebrae. Osteoarthritic changes, especially in the elbow, hip, and stifle, were accompanied by marked enlargement of acetabular and olecranon fossae. The liver, lymph nodes, and synovial villi were enlarged due to accumulation of cells with abundant vacuolated cytoplasm. Similar cells were seen in endocrine glands and vertebrae. The stored material, which appeared to be confined to the monocyte–macrophage system and synovial lining cells, was not identified but was variably oil red O-positive.

Congenital erythropoietic porphyria

Red-brown discoloration of the teeth and bones, caused by a recessively inherited defect in porphyrin metabolism, is reported in several breeds of cattle, including Hereford, Holstein, Ayrshire, Shorthorn, and Jamaica Red and Black. A deficiency of uroporphyrin III cosynthetase leads to the accumulation of uroporphyrin I and coproporphyrin I in blood, bone, and a variety of other tissues. The urine may also be red-brown or turn red on exposure to sunlight. The action of sunlight on porphyrins accumulated in the skin results in photodynamic dermatitis. The teeth, bones, and urine of affected animals show bright cherry-red fluorescence on exposure to ultraviolet light in a darkened room. Congenital erythropoietic protoporphyria in Limousin cattle, caused by a deficiency of the mitochondrial enzyme ferrochelatase, is associated with photosensitivity but no discoloration of the bones or teeth.

Inherited forms of porphyria associated with discoloration of the bones and teeth have been reported in cats and Duroc pigs, but the enzyme defect is unknown.

Acquired porphyria with pink discoloration of bones was recognized at slaughter in approximately 300 of 390 *crossbred lambs in Australia*. Similar "outbreaks" have also occurred in lambs and young deer in New Zealand. The discoloration was most prominent in the cortex of long bones and fluoresced on exposure to ultraviolet light, but teeth were not affected. On cut surfaces of long bones in a sample of affected lambs and deer, the pink discoloration was confined to areas of bone that had formed in the weeks prior to death, in particular the outer cortex. This reflects the fact that *porphyrins are only deposited at sites of active mineralization*. In congenital porphyria, the constant availability of porphyrins during dental and skeletal mineralization results in diffuse discoloration of all mineralized tissues. The extraction of coproporphyrin and protoporphyrin from the bones of lambs in the Australian outbreak suggested an enzyme block towards the end of the heme synthetic pathway, most likely induced by a toxin. Although lead toxicity can induce porphyrin accumulation, no lead was detected in affected lambs. Toxicity caused by some chlorinated hydrocarbons can also result in impaired heme synthesis and the detection of 1,2,4-trichlorobenzene in fat samples supported this possibility in the lambs. This compound is a *major metabolite of lindane*, an organochlorine insecticide that was widely used in parts of Australia and New Zealand before being banned. The occurrence of acquired porphyria in grazing animals may therefore reflect environmental contamination due to leakage from chemical dumpsites.

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ACQUIRED ABNORMALITIES IN SKELETAL GROWTH, DEVELOPMENT, AND REMODELING

Bone growth and maturation is a complex process, requiring an interaction between genetic factors, local and systemic hormones, dietary nutrients, and mechanical forces. Anything that interferes with the synthesis of proteoglycans or collagen by chondroblasts or osteoblasts, the differentiation of precursor cells, or the resorption of bone by osteoclasts can result in a skeletal abnormality. The expression of an abnormality depends on many factors, including the phase of skeletal development that is altered, the severity of the defect, the age of the animal at the time of the insult, and how long it persists. As a result, the range of possible skeletal defects is huge, and a single etiology may vary considerably in its manifestations.

An accurate etiological diagnosis is not always possible in an animal with a skeletal defect, particularly since the inciting cause is often no longer present at the time of examination. In many cases the pathologist is presented with lesions reflecting an insult that occurred several weeks or months earlier and can only speculate as to its cause. It may not even be possible to determine with confidence whether a defect is genetic or acquired. Such decisions should not be taken lightly as they can greatly influence the actions of the owner, sometimes leading to unnecessary culling of related animals, resulting in considerable wastage of valuable breeding stock. *Many teratogenic agents have been shown to mimic genetic diseases of the skeleton and other organ systems*, and unless there is clear evidence that the problem is inherited, or it resembles an established genetic disorder, any temptation to attribute a genetic etiology should be resisted.

The term "*developmental orthopedic disease*" has been used to encompass a complex and important group of skeletal problems in horses, including osteochondrosis, subchondral cystic lesions, physeal dysplasia, wobbler syndrome, acquired angular limb deformities, flexural deformities, and cuboidal bone deformities. These disorders are associated with abnormalities in endochondral ossification at either the articular/epiphyseal cartilage complex or the growth plate of developing bones, but their etiology and pathogenesis are obscure. Although a genetic component is suspected, at least in some of these conditions, most appear to have a multifactorial etiology that includes nutritional and biomechanical factors. Rather than discuss the developmental orthopedic diseases as a group they will be included as separate entities in this chapter, reflecting the fact that although some of them may be different manifestations of the same underlying problem, their clinical and pathological presentation varies. Although osteochondrosis is a disorder of endochondral ossification, its clinical and pathological

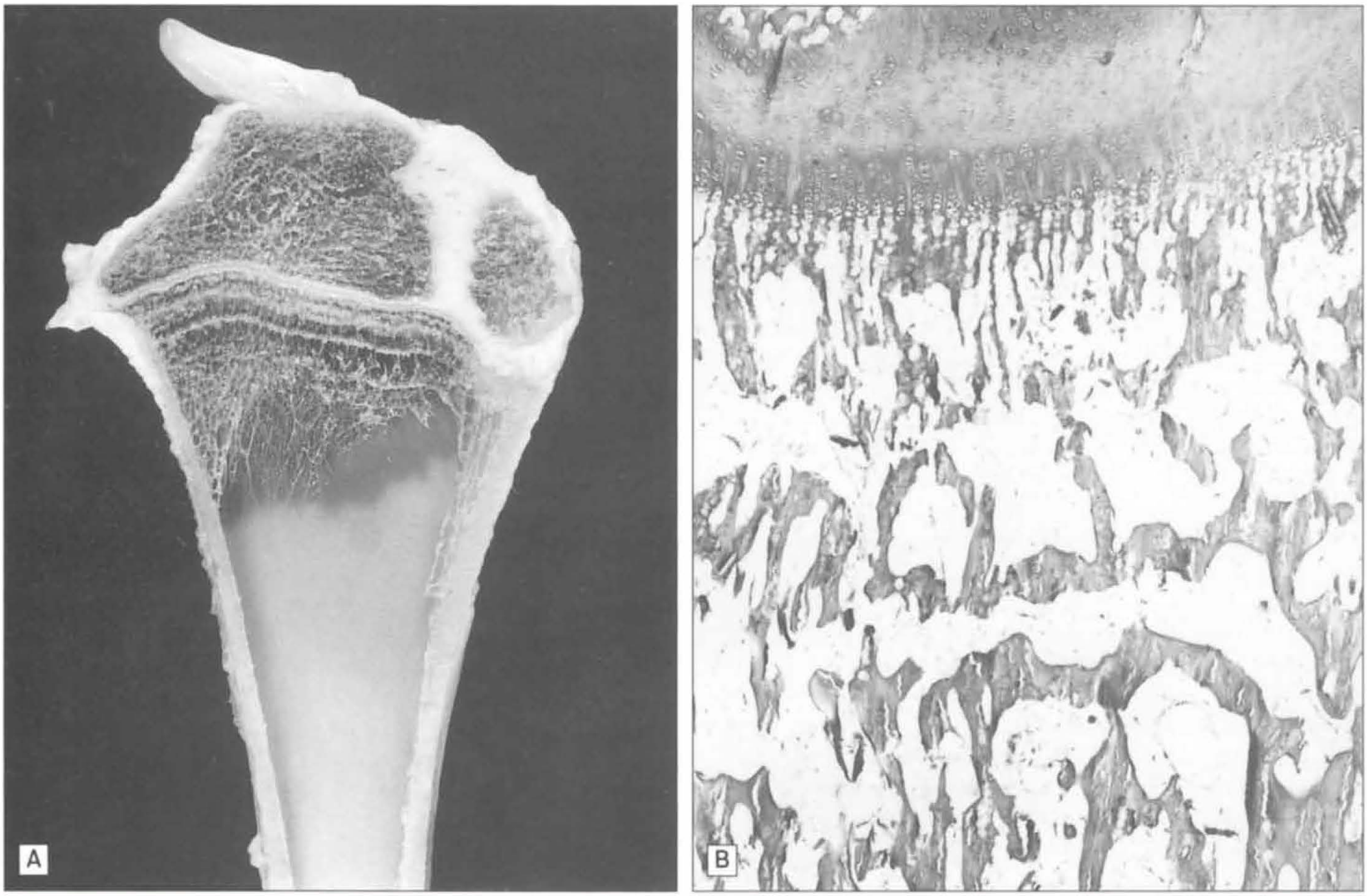

Figure 1.44 Growth arrest lines A. Proximal tibia from a lamb with osteoporosis. Several growth arrest lines are parallel to the physis. **B.** Microscopic section showing the **transverse trabeculae** that form during periods of growth arrest. These are left behind in the metaphysis once growth at the physis

manifestations in most species relate principally to its effect on joints, and is therefore included in a later section (Developmental diseases of joints).

Nutritional imbalances and toxins affecting skeletal growth

Bone development by endochondral ossification is sensitive to quantitative and qualitative alterations in nutrition, both in fetal and postnatal life. *Malnutrition or starvation*, if severe enough, will retard longitudinal bone growth, resulting in narrow growth plates that may become sealed by a horizontal plate of bone on the metaphyseal side. If the nutritional abnormality is corrected, physeal growth will resume and the plate of bone will be displaced into the metaphysis, persisting for some time as a radiographically and grossly visible **growth arrest line**, parallel to the growth plate. The presence of several parallel lines of transverse trabeculae (Fig. 1.44A, B) indicates recurrent episodes of retarded growth. Growth arrest lines are often accompanied by osteoporosis due to reduced bone formation and increased resorption in response to starvation, and the contents of the marrow cavity may be gelatinous due to serous atrophy of medullary adipose tissue. *Growth arrest lines may be detected in fetuses*, in which case they imply either defective maternal nutrition or retarded fetal development due to some other cause, such as intrauterine infection. Similar, but less well-defined lines in the metaphysis also occur in fetuses and in postnatal animals in association with certain infectious or toxic agents that interfere with osteoclastic resorption of the primary or secondary spongiosa and must be differentiated from growth arrest lines. These bands of metaphyseal sclerosis are discussed later in this section.

Overnutrition, in particular chronic excess of dietary calcium, in rapidly growing young dogs of large breeds, has been linked to a range of skeletal disorders, including osteochondrosis, metaphyseal osteopathy, and hip dysplasia. It is proposed that hypercalcitoninism, secondary to persistent stimulation by gastrointestinal hormones and post-prandial elevations in plasma calcium concentration, causes impaired osteoclastic activity and defective bone remodeling. The possibility that this may impair the development of bony foramina and predispose to conditions such as wobbler syndrome and eosinophilic panosteitis has been suggested, but remains to be proven.

Deficiencies or excesses of specific nutrients, including minerals and vitamins, can have a major impact on the developing skeleton. So too can a variety of toxic principles, primarily of plant origin, that an animal is exposed to during skeletal development. These will be discussed in some detail in this section, drawing comparisons between different animal species where appropriate. The so-called metabolic bone diseases, rickets, osteomalacia, fibrous osteodystrophy, and osteoporosis, caused by deficiencies or imbalances of vitamin D, calcium, or phosphorus, are discussed in detail in a later section of this chapter.

Mineral imbalances

Manganese is an essential trace element in animals, being required for the activation of xylosyltransferase, the first enzyme in the biosynthetic pathway of sulfated glycosaminoglycans. *A deficiency of manganese causes decreased production and increased degradation of cartilage glycosaminoglycans* and therefore potentially affects all bones that develop by endochondral ossification.

Manganese deficiency has been incriminated as the cause of skeletal deformities in newborn calves and other farmed livestock in several countries. The deficiency does not appear to affect adult animals but calves born to cows fed on manganese-deficient rations during pregnancy may show various degrees of **skeletal deformity**. The syndrome resembles that described as "*crooked calf disease*," which is discussed in more detail below under "Plant toxicities." In one study, pregnant cows fed 183 mg of manganese per day produced normal calves whereas those fed 115–123 mg manganese per day produced calves with deformities of the limbs, including reduced length and breaking strength of humeri, enlarged joints, and twisted legs. Similar skeletal defects occur in lambs and kids. The reduced breaking strength of bones in this syndrome may reflect the fact that manganese is necessary for the glycosylation of collagen as well as in the synthesis of cartilage glycosaminoglycans.

In New Zealand, manganese deficiency was incriminated in a naturally occurring "outbreak" of **chondrodystrophy** involving 32 purebred Charolais calves to various degrees. Because of severe drought, the pregnant cows had been fed a diet of apple pulp and corn silage, both of which are low in manganese. The calves had short limbs with enlarged joints, and collapsed tracheas with thick cartilage rings. The limb bones were short and had large mushroom-shaped epiphyses (Fig. 1.45). Microscopic lesions included irregular alignment and degeneration of physeal chondrocytes, absence of hypertrophic cells, and degeneration of matrix with unmasking of collagen fibers. Calves with mild lesions that were able to walk gradually recovered, but their legs remained bent and shortened for several months.

Manganese deficiency is also suggested as a possible cause of unusual skeletal deformities in calves from several properties in western Canada. **Congenital spinal stenosis** and myelomalacia, together with focal premature closure of growth plates, malformations of the cranial base and shortening of long bones characterize the syndrome. The spinal stenosis appears to be the result of flaring of the ends of vertebral bodies and protrusion of vertebral articular processes. Focal closure of growth plates is found in the offspring of manganese-deficient rats.

Congenital skeletal malformations in 47 Holstein calves on a property in South Africa were also suggested as being due to manganese deficiency, but the evidence was not convincing. Affected calves were small and showed variable joint laxity, doming of the forehead, brachygnathia superior, and shortening of their long bones. These outbreaks of skeletal deformities highlight the difficulty in establishing a definitive etiologic diagnosis in a disease that is not discovered until several months after the initial damage has occurred.

There is no doubt that **copper deficiency** influences skeletal development, but full appreciation of the range of manifestations and the mechanisms involved is lacking. The role of copper deficiency in **osteoporosis** and its association with **osteochondrosis** in some species are discussed further elsewhere in this chapter. *As a component of the enzyme lysyl oxidase, copper is required for cross-linkage of collagen molecules.* This is an important step in strengthening the matrix elements of bone tissue, cartilage, and other connective tissues that rely on collagen for support. It is not surprising therefore that increased fragility of bone, and possibly cartilage, is a feature of copper deficiency. Studies in dogs and swine have demonstrated reduced osteoblastic activity in animals with copper deficiency, leading to narrow cortices of long bones and reduced deposition of bone on persistent spicules of mineralized cartilage in the primary spongiosa. These metaphyseal changes resemble those of vitamin C deficiency, which is not surprising since both deficiencies interfere with collagen synthesis and cross-linkage.

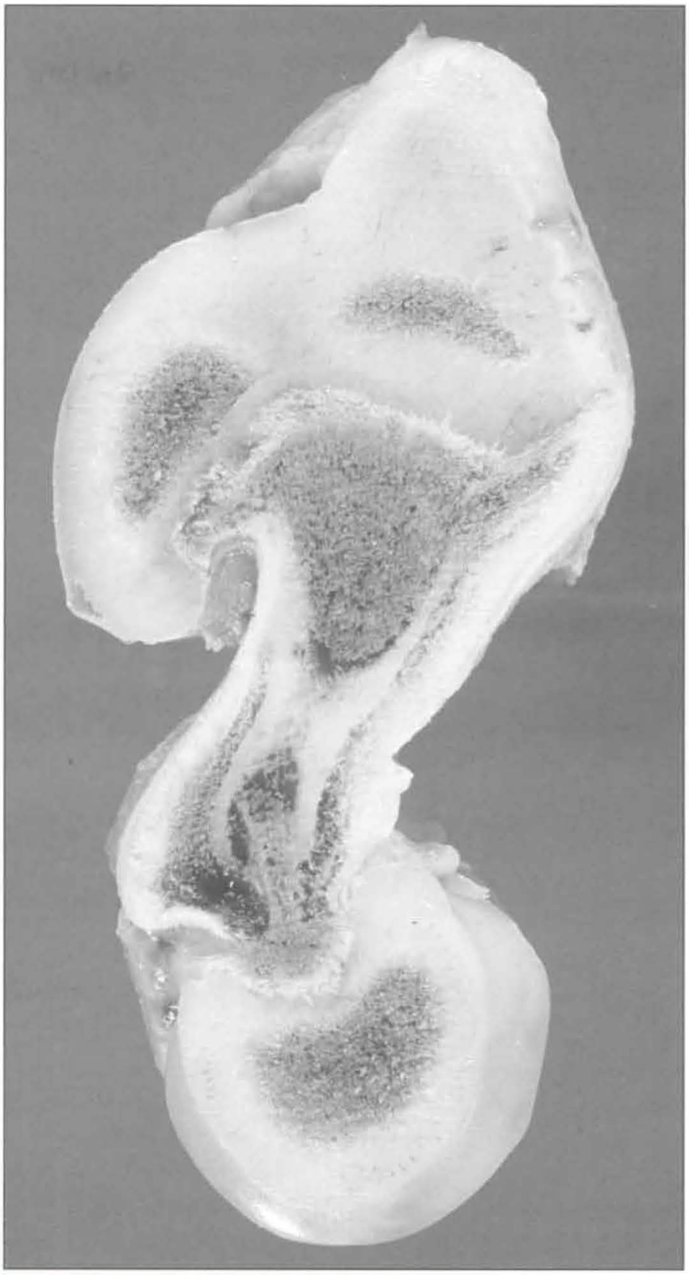

Figure 1.45 Chondrodystrophy in a newborn calf caused by intrauterine **manganese deficiency**. The humerus is considerably shorter than normal and the epiphyses are excessively large in comparison.

In calves, foals, pigs, and dogs, copper deficiency has been associated with grossly visible focal thickenings in rapidly growing growth plates. These foci consist of *retention of hypertrophic zone chondrocytes*, which protrude into the metaphysis, but the mechanism for this lesion in copper deficiency is not clear. It is possible that the stress of weight bearing causes microfractures of fragile trabeculae in the primary spongiosa with local disruption of the metaphyseal blood supply and impaired invasion of the mineralized cartilage. A similar mechanism has been proposed for the focal thickening of growth plates observed in chondrodystrophic Alaskan Malamutes and for the physeal thickening seen in some cases of osteochondrosis. In support of this hypothesis, trabecular microfractures, adjacent to foci of physeal thickening, are described in copper-deficient swine, calves, and foals, but it is seldom possible to accurately assign cause and effect in natural cases as the lesions are frequently too advanced to provide clues as to their origin. Whatever the mechanism, *these growth plate lesions bear a strong resemblance, grossly and histologically, to those of rickets and osteochondrosis*. Differentiation from rickets may be further complicated by the possibility of spontaneous long-bone fractures in copper-deficient animals due to osteoporosis and increased bone fragility.

Physeal changes are not a feature of copper deficiency in lambs. In one report, lambs with experimentally induced copper deficiency developed osteoporosis, but no lesions were detected in growth plates. Nor are there any descriptions of growth plate thickening in sheep with natural copper deficiency, suggesting either a species difference, or the fact that lambs, because of their lower bodyweight, are less likely to develop trabecular microfractures than foals, calves, or pigs.

Copper deficiency may be either *primary*, due to inadequate dietary copper, or *secondary* to increased dietary levels of copper antagonists, but the mechanisms vary between species. Absorption of copper is reduced by the presence of divalent cations such as zinc, iron, and cadmium that compete with copper for a common transport mechanism. Zinc may also induce the formation of metallothionine, a protein that binds copper in the intestine, liver, and kidney and reduces its availability. Phytates in the carbohydrate diets may form insoluble ligands with copper and prevent its absorption. Skeletal lesions consistent with osteochondrosis are described in pigs and foals fed experimental diets high in zinc, and in foals grazing pastures contaminated with zinc close to industrial plants. The possibility of a direct toxic effect of zinc on developing bone or cartilage must be considered, but a secondary effect through induction of copper deficiency is favored.

Calves and lambs grazing pastures containing high levels of *molybdenum* have developed skeletal lesions consistent with copper deficiency. This is believed to be due to the formation of thiomolybdates in the sulfide-rich environment of the rumen and *induction of secondary copper deficiency*. Thiomolybdates do not form in the digestive tract of nonruminants, providing a possible explanation for the higher tolerance of monogastric species to dietary molybdenum. However, although dietary molybdenum has little effect on copper uptake in horses, skeletal lesions resembling those of copper deficiency have been reproduced in rats and rabbits fed on rations with high levels of molybdenum.

Molybdenosis in sheep is associated with *epiphysiolysis of the greater trochanter and sub-periosteal hemorrhages of long bones*, leading to formation of bony excrescences. Sub-periosteal new bone formation at sites of muscle and tendon insertion has also been described in rats fed a low-copper diet supplemented with tetrathiomolybdate. In feedlot cattle, molybdenum poisoning caused weight loss and animals died over many months despite a short exposure to toxic levels, with both severe nephrosis and hepatotoxicity found in fatal cases. Although it has been suggested that bone lesions, at least in lambs, may be due to molybdenum toxicity rather than copper deficiency, the latter seems to be a better option. In ruminants, molybdenum may also interfere with the metabolism of sulfur, and affect ruminal microflora. Weakening of connective tissues with tearing of insertion sites due to reduced strength of tendons and ligaments would not be surprising in copper-deficient animals with reduced cross-linkage of collagen. The formation of reactive bone would be an expected response to periosteal elevation induced by hemorrhage at such sites. In affected animals, the other commonly associated lesions of copper deficiency are absent, so the exact pathogenesis of bone lesions in molybdenosis remains uncertain.

Fluorine is an essential trace element but, when present in chronic excess, is capable of inducing *characteristic dental and/or bony changes*. **Fluorosis** *is the term used to denote chronic fluoride toxicity*. All species are susceptible but because of the manner in which chronic poisoning occurs, *fluorosis is most common in herbivorous animals*. Discussion here is based on fluorosis in cattle, which are more susceptible than sheep and horses and for which the abnormalities are better described.

Toxic levels may be obtained from subsurface waters, especially where *rock phosphate* is plentiful. Rock phosphates vary considerably in their fluoride content, and chronic poisoning has been observed in cattle and sheep given rock phosphates as "licks." Contamination of pastures adjacent to *mineral ore refineries* may also cause toxicity, either directly or by uptake of fluorine by plants, since fluorine is volatile at high temperatures and is part of the gaseous and particulate effluent of many industrial processes. *Dust from volcanic eruptions* also contains abundant fluorides. Because of the widespread distribution of fluorine in nature, many animals obtain small nontoxic doses, partly from drinking water and partly from wind-blown dust, which settles on pasture.

Fluoride is removed rapidly from the blood, by renal excretion and deposition in bones and teeth. A small amount is deposited in soft tissues. Some fluorine crosses the placental barrier and although plasma levels are lower in fetal than maternal circulation, under certain circumstances fetal fluorosis may develop. Evidence regarding fluorine levels in milk is conflicting. The deposition of fluoride in bone may be functionally comparable to that of other elements, such as lead and strontium, and *may represent a detoxification mechanism*. Unless the intake of fluoride is very low, there is a steady accumulation of the element since it is deposited as calcium fluoride or fluorapatite, which are of low solubility. Many normal cattle have 600–900 μg/g in their bones but fetal lesions apparently occur at concentrations as low as 100–200 μg/g. Evidence of fluorosis does not develop in older cattle until the concentration reaches 2500–3000 μg/g. A level of 40–60 mg/kg (dry matter) in feed will produce such concentrations in the bone of cattle after 2–3 years. In general, the toxicity of fluorine depends on the aqueous solubility of the compound fed; sodium compounds are more soluble and more toxic than calcium compounds.

Fluoride toxicity is enhanced by poor nutrition, and alleviated somewhat by high dietary intakes of calcium and aluminum. While ingesting toxic levels of fluorine, urine of cattle usually contains

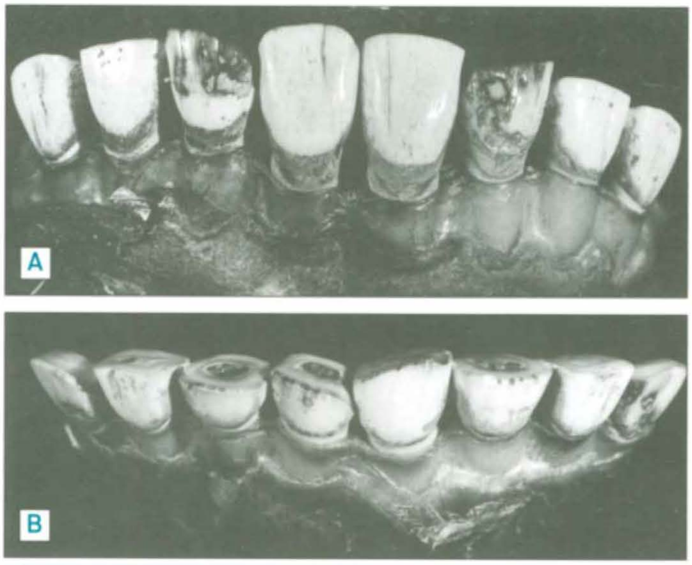

Figure 1.46 Fluorosis. (Courtesy of JL Shupe.) **A.** Dark brown discoloration and hypoplasia of enamel in the incisors of a cow. The variable involvement of the teeth reflects variation in levels of the toxin during enamel formation. **B.** Accelerated wear of incisor teeth in a cow with fluorosis.

10 μg/g or more although high urinary concentrations may persist for some time after excess fluorine is removed from the diet. Plasma fluorine is also elevated during periods of exposure but declines a few days after fluorine is removed from the diet.

The characteristic changes of severe fluorosis occur in teeth and bones and are accompanied by shifting lameness, loss of production, and a variety of nonspecific signs of debility. **Dental lesions** develop only if intoxication occurs while teeth are in the developmental stages and enamel is forming. Ameloblasts and odontoblasts are extremely sensitive to fluorine, which causes them to produce a matrix that mineralizes abnormally and is reduced in quality and quantity. In particular, *the outer layer of enamel is hypomineralized.* The incremental lines in the enamel are disrupted and the normal subsurface pigment band of bovine incisors is distorted.

The mildest macroscopic evidence of dental fluorosis is the *presence of small foci with a dry, chalky appearance compared to the normal glistening surface of enamel.* These mottled areas are readily visible as opacities when the tooth is transilluminated. In more severe cases, *all the enamel in affected teeth may be chalky, opaque and show various degrees of yellow, dark brown, or black discoloration* (Fig. 1.46A), *which is virtually pathognomonic for fluorosis.* Affected teeth show accelerated wear (Fig. 1.46B) and may develop chip fractures. In chronic cases, they may be worn to the gum line. The pigment is present in the enamel layer and possibly in the dentin, and may reflect oxidation of the organic matrices of the teeth. Unlike the pigment of food stains and tartar, it is not limited to the surface and cannot be removed by scraping. Hypoplasia of enamel may occur in severe fluorosis and is evident as punctate pits or as horizontal grooves, usually most prominent on the lateral aspect of the tooth. The horizontal disposition of the grooves and pits is attributable to periodic interference with mineralization of enamel during odontogenesis.

Although the fetus does not accumulate high levels of fluorine, under some circumstances lesions may develop in the deciduous teeth of calves exposed during gestation. Microscopically the odontoblasts are disorganized and have vacuolated cytoplasm. There is excessive predentin and formation of globular dentin. Fibrosis of the pulp cavity with ectopic bone formation is also seen. Lesions in permanent teeth, especially those that are last to erupt, are much more common, those that erupt first showing little or no damage. In cattle, the permanent teeth are sensitive to fluorosis from about 6–36 months of age. The most severe effects occur when exposure coincides with initiation of crown formation. In areas of endemic fluorosis, where ingestion of the element is more-or-less continuous, the lateral incisors of cattle show the most obvious lesions and, together with the second premolars and second and third molars, the most severe lesions. Lesions of similar severity should be present in teeth that develop simultaneously. These associations include the first incisor with the second molar, second incisor with the third molar, and the third incisor with the second premolar. Lesions in the second incisor must be severe before lesions are prominent in the third molar, and in general, incisor abrasion develops prior to molar abrasion. Lesions may develop in the fourth incisor in the absence of changes in other teeth.

The bone lesions of fluorine toxicity, **osteofluorosis**, are generalized but not uniform and, in severe cases, are characterized grossly by the formation of *periosteal hyperostoses*, which give the macerated bones a chalky roughened appearance (Fig. 1.47A, B, C). Lesions occur first on the medial surface of the proximal third of the metatarsal and later on the mandible, metacarpals, and ribs. The pelvis, vertebrae, and other bones of the distal limbs are also affected. In chronically affected cattle, fracture of the digital bones in the medial claw is common, leading to lameness and a preference for affected animals to stand cross-legged. A similar pattern of development occurs in horses. Although exostoses often develop at sites of tendinous or fascial insertions, this is not the rule and the reasons for their occurrence and distribution are unknown. Endostoses seldom occur in farm animals. Articular surfaces are normal, and lameness is due to involvement of the periosteum, encroachment of osteophytes on tendons and ligaments and, in some cases, to mineralization and even ossification of the latter structures. Fluorine is incorporated into bone matrix during mineralization and will therefore be most abundant in young animals at sites of active formation but is also deposited in older animals during remodeling. Exposure of cattle after 3 years of age may therefore produce osteofluorosis but not dental fluorosis.

Depending on the level of exposure to fluoride, and its concentration in bone, the bones may appear grossly and radiographically normal. A characteristic microscopic feature that may be detected in ground bone sections in such cases is the presence of brown discoloration of osteons, similar to that in enamel. The discoloration is apparently due to the effect of fluoride on osteoblasts, and its extent depends on the rates of bone growth or remodeling. Both endosteal and periosteal osteoblasts are affected and produce an abnormal matrix, which mineralizes abnormally. Sections of cortex may have a mottled appearance with some lamellae in some osteons showing discoloration and others appearing normal, depending on whether they were exposed to toxic levels of fluorine during formation. In fetuses, brown mottling of lamellar bone occurs at much lower concentrations of fluorine than in older cattle.

At *highly toxic levels*, the gross lesions of osteofluorosis occur rapidly. Not only is any new bone abnormal, preformed bone is apparently

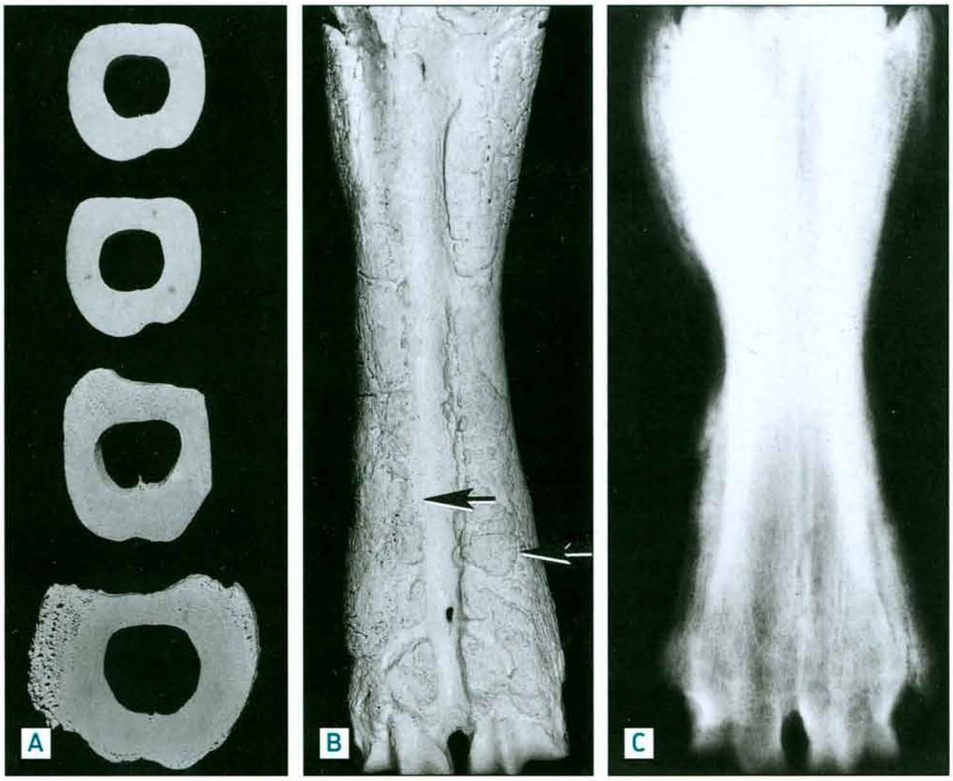

Figure 1.47 Chronic osteofluorosis in a cow. (Courtesy of JL Shupe.) **A.** Transverse sections of the diaphysis of metatarsals from cattle with increasing severity of osteofluorosis (top to bottom). Note the increased thickness of the cortex due to periosteal new bone formation. **B. Extensive periosteal new bone formation** (arrows) on the metatarsal bone. **C.** Radiograph of a metatarsal from an affected animal illustrating the extent of the periosteal hyperostosis.

altered in its mechanical properties and has a reduced life span. The rate of remodeling is correspondingly increased both for fluoridated normal bone and for bone formed under the influence of fluoride. The medullary cavity is enlarged and resorption spreads progressively outward through the cortex, and may involve the laminar periosteal bone. Resorption cavities may be excavated much more rapidly than they can be refilled, and the cortex becomes porotic. The impaired mechanical properties induce periosteal reinforcement with laminar bone or, if the need is greater and the deposition of new bone more rapid, by coarse woven cancellous bone. When fluoride levels are very high, the new matrices produced are abnormal and remain unmineralized, as in osteomalacia.

In young, growing dogs and pigs, and presumably in other species, fluorine intoxication produces lesions, which, in many respects, resemble rickets. This is presumably due to inhibition of mineralization when fluorine is present in high doses. The ends of long bones and the costochondral junctions are enlarged while physes are usually increased in depth, softer than normal, and yield to the pressure of weight bearing. The change at osteochondral junctions appears to be due to continued proliferation of chondrocytes, which fail to mature and align themselves. Associated with immaturity, there is a reduced amount of cartilaginous matrix and, although this appears to mineralize normally, albeit intermittently, the mineralized spicules are thin and fragile. The wide seams of osteoid that are deposited are poorly and irregularly mineralized.

Lead toxicity is better recognized for its effects on the nervous system, but also causes bone lesions. *The characteristic lesion is a band of sclerosis, referred to as a* **"lead line,"** visible radiographically and grossly in the metaphysis of developing bones. This is a relatively early morphological lesion in children with lead poisoning, and also occurs in animals (Fig. 1.48A). The sclerosis is due to persistence of mineralized cartilage trabeculae in the metaphysis (Fig. 1.48B) because of impaired osteoclastic resorption. Microscopically, many osteoclasts are present but they may be separated from the surface of bone trabeculae (Fig. 1.48C) and appear poorly able to degrade mineralized matrix. Some osteoclasts may contain *acid-fast intranuclear inclusions.* Ultrastructurally, a large amount of mineralized cartilage matrix is present in the cytoplasm of osteoclasts suggesting a defect in intracellular processing of the matrix. The metaphyseal sclerosis associated with lead toxicity radiographically and grossly resembles that seen in association with some cases of canine distemper viral infection and intrauterine *Bovine viral diarrhea virus* infection, both of which may interfere with osteoclast function or number.

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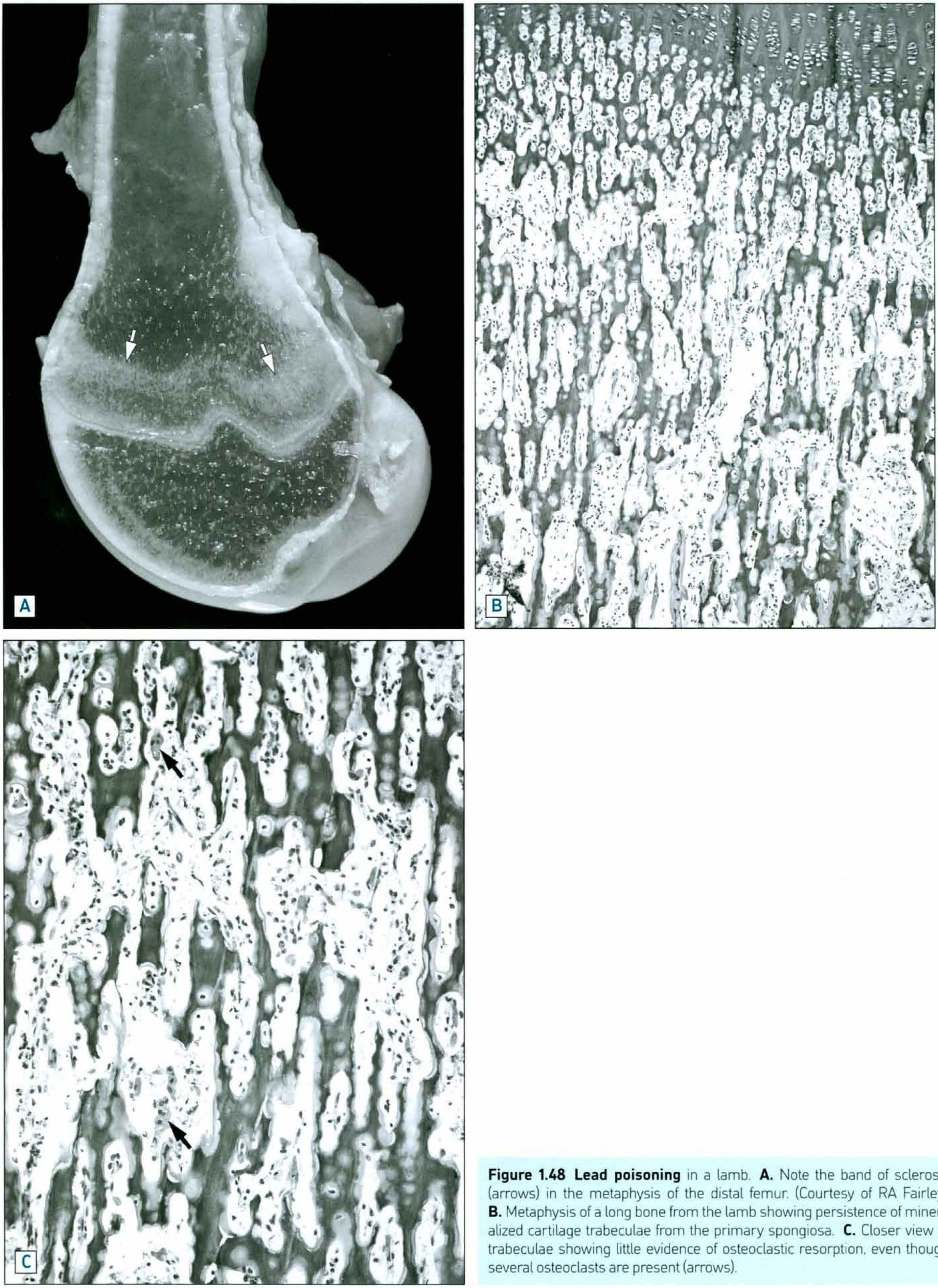

Figure 1.48 Lead poisoning in a lamb. **A.** Note the band of sclerosis (arrows) in the metaphysis of the distal femur. (Courtesy of RA Fairley.) **B.** Metaphysis of a long bone from the lamb showing persistence of mineralized cartilage trabeculae from the primary spongiosa. **C.** Closer view of trabeculae showing little evidence of osteoclastic resorption, even though several osteoclasts are present (arrows).

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Vitamin imbalances

Deficiencies and excesses of vitamins A and D can significantly influence skeletal development and remodeling, as can deficiency of vitamin C. The skeletal manifestations of imbalances in these vitamins will be discussed here, except for vitamin D deficiency, which will be included with the metabolic bone diseases because of its role in the etiology of rickets and osteomalacia.

Vitamin A in the diet originates from both plant and animal sources. Retinyl esters of animal origin are hydrolyzed in the gut to retinol, which is bound to lipid and absorbed, then re-esterified and transported to the liver. Retinol is transported from the liver to target tissues bound to a specific transport protein, retinol-binding protein, an α1-globulin. Provitamins such as carotenes and carotenoids of plant origin are absorbed in the small intestine, where a molecule of β-carotene, for example, can be converted enzymatically in epithelial cells to two molecules of retinaldehyde (retinal). Most of this is reduced to the alcohol form, retinol. A small proportion of retinaldehyde is oxidized to retinoic acid, which is not converted to retinol in the body and is not stored in the liver. Instead, it is excreted in bile and urine after being transported in plasma bound to albumin. Retinol is essentially synonymous with vitamin A since it, or its derivatives, can supply all the requirements for reproduction, vision, and growth supplied by the vitamin. Retinoic acid, and its natural and synthetic derivatives, are classified as retinoids. These compounds do not support the visual functions of vitamin A and only some of its reproductive functions.

The functions of vitamin A, other than that of retinal in relation to vision (see Vol. 1, Eye and ear), are incompletely understood. *Retinol and retinoic acid stimulate the activity of osteoclasts*, causing them to increase their acid phosphatase content and resorb bone. *Development and differentiation of epithelial structures and bone are sustained by retinol, retinyl esters, retinal, and retinoic acid*, but the mechanisms are unclear. It is postulated that retinol and retinoids may either act as carriers of sugar for glycoprotein biosynthesis or function through a specific receptor protein. The metabolically active form is also unknown – the model of 11-*cis*-retinal in the rhodopsin cycle does not apply to other functions. Retinyl phosphate, retinoic acid, or some other metabolite, may be the active compound.

Vitamin A deficiency *occurs in cattle and pigs fed unsupplemented rations of grain and/or old hay.* Yellow corn, new hay, and fresh silage are adequate sources of carotene but potency decreases with storage. Animals grazing green pasture or crops are unlikely to suffer from vitamin A deficiency, but dry summer pastures may be deficient. Although deficiency of vitamin A affects many tissues, the emphasis here is on the skeletal manifestations and their sequelae. *The underlying skeletal abnormality in vitamin A deficiency involves defective remodeling of membranous bone, presumably due to the stimulatory effect of vitamin A on osteoclastic activity.* Consequently, there is asynchrony between the developing central nervous system and the bones of the skull and spinal column. Failure of the cranial cavity and spinal canal to enlarge sufficiently to accommodate the brain, cranial nerves, and spinal cord results in secondary changes in the central nervous system and a variety of nervous signs. Membrane bones in other locations, including the periosteal surface of long bones, are also affected and may develop a coarse profile, but these changes are not significant clinically.

In the cranium, the defect is particularly severe in the bones of the caudal fossa, and *the cerebellum may herniate into the foramen magnum.* In puppies with vitamin A deficiency, deafness is a prominent sign because of changes in the internal auditory meatus, while affected *calves and pigs develop blindness due to narrowing of the optic foramina and compression of optic nerves.* The basis for these variations between species is not clear but lesions are modified according to the severity of the deficiency and the stage of skeletal growth. Spinal nerve roots, especially those in the cervical cord, may herniate into the intervertebral foramina, although the lesions are not always bilaterally symmetrical.

The pathogenesis of the asynchrony in development between the nervous system and skeleton is complex and is related to altered patterns of drift in bones that are growing during the period of deficiency. Normally osteoclasts are responsive to vitamin A, and in the cranium of deficient animals, there is inadequate resorption of endosteal bone. Often, bone is produced at sites where resorption should be occurring thus exacerbating the retarded expansion of the cranial cavity and the various foramina. Endochondral bone does not appear to be directly influenced by vitamin A deficiency.

The nervous signs in growing animals with vitamin A deficiency may be related to cranial and spinal nerve degeneration and to hydrocephalus. The hydrocephalus probably is due mainly to decreased absorption of cerebrospinal fluid into the blood, a process that occurs in the arachnoid granulations and villi. The granulations are located in the tentorium cerebelli, which is severely affected by the thickening that occurs in the dura mater in vitamin A deficiency. Hypersecretion of cerebrospinal fluid with vitamin A deficiency also contributes to the development of hydrocephalus.

Clinical signs of deficiency in growing cattle may include edema of the brisket and limbs, irreversible blindness, night blindness, and the neurologic effects of increased intracranial pressure. Growing pigs may show incoordination and posterior paresis. Pigs less than a week old may be affected if maternal supplies are low but signs usually are seen in older animals. Gross neurologic lesions include osseous thickening of the tentorium cerebelli, cerebral edema and coning of the cerebellum. Microscopically, there is Wallerian degeneration in the spinal cord and brain (see Vol. 1, Nervous system).

The most characteristic extraskeletal lesion of vitamin A deficiency is squamous metaplasia of various glandular or ductular epithelia. *In cattle, squamous metaplasia of the parotid salivary duct is virtually pathognomonic for vitamin A deficiency*, but this resolves within a few weeks following correction of the deficiency. In vitamin A-deficient pigs, focal squamous metaplasia in the urinary bladder may be grossly visible as 1–2 mm light-yellow nodules on the mucosa.

The effect of vitamin A deficiency on dental development is well studied in the continuously growing incisors of rats and guinea pigs, but not in teeth of other species. Inadequate differentiation and spatial organization of odontoblasts lead to irregular formation of poor-quality dentin. Ameloblastic differentiation is also suboptimal and results in enamel hypoplasia. In addition, undifferentiated ameloblasts invade the dental pulp where they induce the odontoblasts to form concretions.

Vitamin A deficiency, like excess, is **teratogenic**, and swine and large Felidae appear to be very susceptible. Abortions, stillbirths, and a variety of lesions, including subcutaneous edema, microphthalmia, retinal dysplasia, hypotrichosis, supernumerary ears, polydactyly, arthrogryposis, cleft palate, pulmonary hypoplasia, diaphragmatic hernia, hepatic cysts, and cardiac, renal, and gonadal malformations may occur in the offspring of vitamin A-deficient swine. Hydrocephalus, and protrusions of spinal cord through intervertebral foramina, may also occur. The frontal and parietal bones may be thin and the basioccipital bone thicker than normal. The latter bone is also thickened in neonatal calves from vitamin A-deficient dams, as are the squamous occipital, basisphenoid and presphenoid bones. Stillbirths, and congenital blindness, incoordination and thickened carpal joints are also seen in calves as well as hydrocephalus, with herniation of cerebellar vermis through the foramen magnum, constriction and degeneration of optic nerves, and retinal dysplasia.

Vitamin A toxicity is well recognized as a cause of skeletal disease in man and animals. Natural cases of toxicity usually follow an accidental overdose with vitamin A concentrate or excessive feeding of diets containing high concentrations of the vitamin, such as liver. Depending on the age and species of animal, and the duration and level of exposure to excess vitamin A, *the manifestations of toxicity may include physeal damage, osteoporosis, or the development of exostoses (osteophytes).* High concentrations of carotenes in green feed oats may predispose to rickets in lambs through inhibition of vitamin D activity.

The **physeal lesions** of vitamin A toxicity are characterized by reduced chondrocyte proliferation and reduced size of hypertrophic chondrocytes, resulting in narrowing of growth plates. Loss of proteoglycans and unmasking of collagen fibers occur, and sometimes with high doses there is complete destruction of segments of growth plates. Rapidly growing physes, especially those subjected to the greatest compressive forces are most likely to show changes, resulting in reduced length of certain long bones. In organ culture, vitamin A inhibits chondrocyte proliferation and reduces RNA and protein synthesis. Lysis of matrix may be the result of destabilization of lysosomal membranes with the release of acid proteases that attack matrix proteins.

The **osteoporosis** of vitamin A toxicity is associated with decreased numbers of osteoblasts and fewer, thinner osteoid seams than normal, and is most severe in cortical bone and some of the membranous bones of the skull. Periosteal bone formation is more severely affected than endosteal and intracortical formation. In long bones, this results in thin cortices, reduced diaphyseal diameter and an exaggerated metaphyseal flare. Metaphyseal trabeculae tend to be reduced in number but thicker than normal. Hypervitaminosis A interferes with cell differentiation in the embryonic skeleton and may have similar effects on osteoblasts in postnatal bone. The decrease in osteoid production also suggests a direct toxic effect on osteoblasts. The osteoporosis may be exacerbated by continued bone resorption, osteoclasts apparently being less sensitive than osteoblasts to the effects of increased vitamin A. Fractures are not a feature of vitamin A toxicity in farm animals and pets as they are in some laboratory species. Osteoporosis is reversible in animals removed from diets high in vitamin A but the physeal damage is permanent and the consequences emphasized with time.

The *formation of exostoses* in association with vitamin A toxicity is often extensive. The pathogenesis of this lesion is not clear but may be associated with fragility of periosteal attachments, and provoked by tensions and minor trauma.

Toxicity associated with *single, large doses of vitamin A* has been recognized in pigs and calves. In baby **pigs** given excess vitamin A orally as part of disease prevention programs, a characteristic syndrome develops. Grossly there is shortening of long bones and prolongation of the traction epiphyses of the tibial tuberosity, the greater trochanter of the femur, and the humeral tuberosity. Rotation of epiphyses, particularly those of the distal femur and proximal and distal humerus, is also described, and tarsal and carpal bones may be distorted. The medial and lateral metatarsal bones, and to a lesser extent the metacarpal bones, may be of different lengths. Pigs treated shortly after birth show an abnormal gait and possess noticeably short legs by 6–8 weeks of age. The condition occurs with as little as twice the recommended dose of vitamin A. *The basis for the gross lesions is destruction and focal closure of growth plates.* Continued growth of the intact parts of the physis, and superimposed adaptational deformities, account for the characteristic appearance of the bones.

Similar lesions occur in **kittens** given daily oral doses of 40–100 μg/g body weight of vitamin A for 4 to 5 weeks, followed by a 6–15 week recovery period. The metacarpal lesions tend to be more severe than in piglets, the tibial lesions are similar, while those in the distal femur and other sites are less severe.

In 7-week-old **pigs** given about 20 μg/g body weight for 5 weeks then killed, osteoporosis is prominent. Lesions are especially severe in the squamous occipital bone, which may be so thin that the cerebellum can be crushed by manual pressure to the overlying skin. The long bones are short and fragile with thin cortices, flared metaphyses, and typical physeal lesions, but the latter are less severe than at higher dose rates and gross deformities do not occur. Exostoses may be present, especially near the insertion of the brachialis muscle on the proximal radius.

Vitamin A toxicity has been incriminated as the cause of **"hyena disease"** in **calves**. This unusual form of dwarfism is characterized

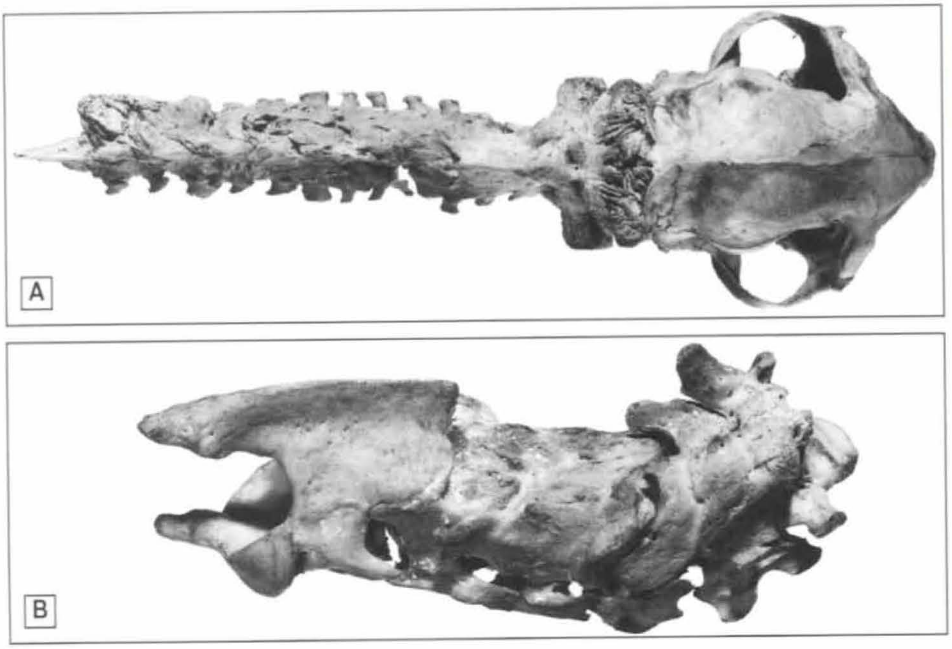

Figure 1.49 Vitamin A toxicity in a cat. **A, B.** Dorsal and lateral views of cervical vertebrae showing extensive exostoses leading to ankylosis. (Courtesy of AA Seawright.)

by *relative underdevelopment of the caudal body structures* due to premature closure of growth plates, particularly in the pelvic limbs, and has been associated with the injection of newborn calves with vitamins A and D. Affected calves have a characteristic "bunny-hopping" gait and a sloped back with small hindquarters, resembling a hyena. Although the lesions are most severe in the bones of the pelvic limbs and lumbosacral vertebrae, the pectoral limbs are also affected. Physes are narrow, but many focal triangular projections of physeal cartilage may protrude into the metaphysis. Administration of excess vitamin A to calves has been shown to induce premature mineralization of physeal cartilage leading to early closure of growth plates and impaired longitudinal bone growth, supporting vitamin A toxicity as the cause of hyena disease. An autoimmune mechanism associated with *Bovine viral diarrhea virus* has also been suggested but is not supported by convincing evidence.

Osteophyte formation is the hallmark of prolonged exposure to excess vitamin A in many species and is the outstanding feature of *chronic vitamin A poisoning in* **cats**, producing the syndrome referred to as **deforming cervical spondylosis.** The syndrome results from prolonged feeding of bovine livers to adult cats, which ordinarily contain large amounts of vitamin A when derived from grazing animals. The disease was common in Australia and Uruguay where beef livers are plentiful and relatively cheap, but also occurs sporadically in other countries when domestic cats are given unconventional diets. In young growing cats, this same diet would be expected to result in fibrous osteodystrophy because of the imbalance in calcium and phosphorus.

Deforming cervical spondylosis is typically *seen in cats after 2 years of age* and is characterized by postural changes, cervical ankylosis and forelimb lameness, sometimes accompanied by cutaneous hyperesthesia or anesthesia. The vertebral lesions are similar to those of mucopolysaccharidosis VI but the age of the cat should prevent confusion. *Extensive confluent exostoses develop, especially on the dorsal and lateral aspects of the cervical vertebrae and sometimes on the occipital bone.* In general, the exostoses do not encroach on the neural canal and only occasionally involve ventral aspects of the vertebrae (Fig. 1.49A, B). The intervertebral foramina are considerably altered in shape and reduced in size, causing compression and degeneration of nerves leading to denervation atrophy of muscles. Exostoses occasionally occur on the cranial thoracic vertebrae and may be accompanied by similar outgrowths on the sternum and fixation of the ribs. Periarticular osteophytes also occur about the proximal joints of one or both forelimbs and, if extensive, may cause fixation of the shoulder and elbow joints, usually in the flexed position. Lumbar vertebrae and pelvic limbs are seldom affected. The osteophytes consist initially of either osteocartilaginous tissue that overgrows joints and causes ankylosis, or of woven bone that develops at the site of muscle insertions and extends into the perimysium. Later, the woven bone and cartilage are replaced by lamellar bone. The stress on cervical intervertebral joints associated with regular grooming probably accounts for the concentration of exostoses in the cervical region of cats. In animals with chronic lesions, patches of ungroomed fur may develop in inaccessible sites. Interestingly, cats fed bovine liver tend to have higher and more persistently elevated plasma vitamin A concentration than cats supplemented with the vitamin, even at a higher equivalent dosage.

Oral and dental lesions, including hypermobility and loss of incisor teeth, may also occur in older cats in association with vitamin A toxicity and cervical spondylosis, but it is difficult to determine whether the changes are primary or secondary. Liver has a low calcium content, which could lead to resorption of alveolar bone, but excess vitamin A causes dental lesions even when dietary calcium is normal.

Several *other lesions* are associated with vitamin A toxicity. In some species, including humans, rats, rabbits, and dogs, it causes *ectopic mineralization* in internal organs. In cattle, pigs, and dogs there is decreased cerebrospinal fluid pressure and, in calves at least, this is associated with *thinning of the fibrous cap of arachnoid granulations.* Vitamin A is stored in the liver in *Ito cells*, which increase in size with dietary excess. Peliosis-like changes in the liver, in addition to hemorrhages around joints and hair loss, are reported in people with hypervitaminosis A. *Alopecia and dermatitis* are features of toxicity in horses.

Like vitamin A deficiency, *excess vitamin A is* **teratogenic**, the effects depending to some extent on dose, stage of gestation, and the compound administered. Teratogenicity is increased by protein or protein-energy malnutrition. Vitamin A is used extensively to treat skin disease in people and to a lesser extent in dogs. The half-life of some retinoids is many months, and anomalies in children exposed in utero are well recognized, but are not reported in puppies. *Experimental hypervitaminosis A during pregnancy produces defects in many systems in a variety of animals.* Vitamin A toxicity was incriminated as the cause of continued high prevalence of cleft palate and lip, pulmonary hypoplasia, and abortions near term in a swine herd. The problem in this herd ceased following reduction of vitamin A supplementation to normal levels. The mechanism of teratogenesis is unknown, but excess vitamin A in the embryo inhibits chondrogenesis and osteogenesis, possibly by modifying the differentiation of mesenchyme.

Vitamin D is essential for normal bone development, in particular mineralization of cartilage during endochondral ossification and of newly formed osteoid, but in excess, it is highly toxic. **Vitamin D toxicity** may result from accidental over-supplementation of young animals, ingestion of plants that contain the active form of the vitamin, or accidental ingestion of rodenticide containing cholecalciferol (vitamin D_3). The potency of the latter toxin is illustrated by the fact that cats and dogs may be poisoned by ingesting the carcasses of poisoned rats. Cats appear to be more sensitive to vitamin D toxicity than dogs. When administered as one or two massive doses shortly before parturition, vitamin D appears to prevent postparturient hypocalcemia ("milk fever") in cows but mineralization of soft tissues may result, particularly in pregnant Jersey cows, and in this breed its use is contraindicated. Vitamin D is stored in muscle and fat, and meat from vitamin D-treated "downer cows" is a potential hazard to dogs and cats. Significant amounts of vitamin D may be secreted in milk, and soft tissue mineralization in suckling puppies has been attributed to high dietary levels in the bitch.

Several plants, including *Solanum malacoxylon* (syn. *glaucum, glaucophyllum*), *Cestrum diurnum* and *Trisetum flavescens* cause diseases similar to hypervitaminosis D in grazing animals in various parts of the world (see Enteque seco and Manchester wasting disease, in Vol. 3, Endocrine glands). Traditionally, vitamin D_2 (ergocalciferol) is regarded as the plant form of vitamin D, but *the toxic principle in these plants is 1,25-dihydroxycholecalciferol-glycoside.* Lesions in poisoned animals are indistinguishable from those produced by excess vitamin D. Other calcinogenic plants in which the active principle is less well defined include *Solanum torvum, S. verbascifolium*, possibly *S. esuriale*, and *Dactylis glomerata*. Alfalfa (*Medicago sativa*) contains vitamin D_3 in addition to vitamin D_2.

The *mechanism of vitamin D toxicity* is related primarily to its effect on increasing calcium absorption from the intestine, mobilizing it from bone and reducing its excretion by the kidney. The end result is *hypercalcemia* together with *hyperphosphatemia*, which, if persistent, will lead to widespread mineralization of soft tissues, in particular the kidneys, gastric mucosa, lungs, endocardium, and arterial walls. Death from renal failure is likely to occur before noticeable changes develop in the skeleton. In fact, there are few reports of the skeletal lesions of vitamin D toxicity in animals.

The skeletal changes in hypervitaminosis D may be characterized by either sclerosis or rarefaction, depending on the level of dietary calcium and the pattern of exposure. An early response in bone is widespread, intense osteoclastic activity, which may remove most of the primary spongiosa and cause active resorption in other sites. With continued administration, the matrix produced by osteoblasts accumulates, sometimes in large amounts and in a distinctive pattern. It often has a tangled fibrillary arrangement and appears somewhat mucoid, floccular, and intensely basophilic (Fig. 1.50). Osteoblasts are abundant. Mineralization of this basophilic matrix is delayed but it is gradually converted to a relatively homogeneous eosinophilic substance resembling osteoid. Initially, the maturation of the matrix is local and irregular in distribution, and is unrelated to normal patterns of osteogenesis. If toxicity is prolonged, the abnormal matrices continue to accumulate and virtually obliterate the marrow spaces. This produces a mosaic of basophilic and acidophilic matrix, and newly formed woven bone. *The presence of abundant basophilic matrix is virtually pathognomonic for vitamin D toxicity* and is valuable diagnostically when plasma levels of the vitamin are not known.

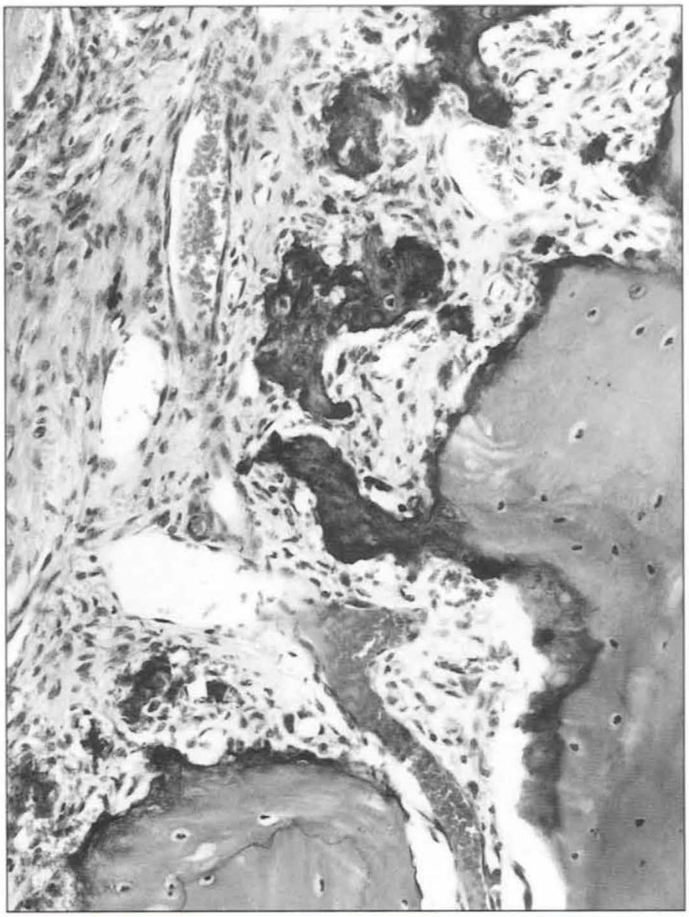

Figure 1.50 Vitamin D toxicity. Intensely basophilic bone matrix deposited on surface of trabeculae and in marrow spaces.

Vitamin D poisoning is usually associated with intermittent toxic doses of the vitamin. This results in surges of osteoblastic activity during periods of withdrawal with rapid production of large amounts of abnormal matrix. This matrix may slowly mature and mineralize, but the process is hastened by further administration of the vitamin. With continued intermittent administration of vitamin D, there are further cycles of matrix deposition, maturation, and mineralization. *Broad, basophilic, resting lines separate the layers of* *bone produced in each cycle.* Necrosis of osteocytes occurs with high doses of vitamin D and groups of empty lacunae are often present in cortical bone and in the center of trabeculae.

Virtually all bones are affected to some extent in vitamin D toxicity, but *the outstanding changes occur in long bones, especially in the ends where growth is most rapid.* The epiphyses are usually normal and appear to escape even the resorptive changes. The growth plates are also normal. The lesions involve the metaphyseal spongiosa and extend well into the diaphysis to fill the medullary cavity. Little active bone marrow remains and is gradually replaced by dilated veins and loose fibrous tissue, which is sparsely populated with hematopoietic cells. Areas of sclerosis may alternate with transverse bands of resorption, indicating intermittent toxicity. Active resorption of primary spongiosa may be accompanied by an intense neutrophilic reaction. Both the periosteum and endosteum are involved, and new periosteal bone contributes to the thickness of the metaphysis. Usually, the perichondrium is not affected, but sometimes it produces new cartilage and bone. In addition, there is rapid metaplastic bone formation in the thickened fibrous layer of the periosteum, producing a collar of new bone around the metaphysis.

Vitamin D deficiency is discussed in the section Metabolic bone diseases (Rickets and osteomalacia).

Vitamin C (ascorbic acid) is a co-factor for the enzymes prolyl and lysyl hydroxylase, which are required for the hydroxylation of proline and lysine during collagen synthesis, and is also an important antioxidant. In **vitamin C deficiency**, *there is reduction or failure in the secretion and deposition of collagen.* Furthermore, the deficiency of hydroxylysine results in impaired formation of intermolecular crosslinks and any collagen that is produced is of reduced strength. Vitamin C also promotes the differentiation of osteoblasts from their progenitors and the hypertrophy of chondrocytes, both of which are critical to the process of endochondral ossification. Most mammals synthesize ascorbic acid from glucose via glucuronic acid and gulonic acid. Some species, including humans, certain nonhuman primates, and guinea pigs, lack the hepatic microsomal enzyme L-gulonolactone oxidase and, in the absence of a dietary source of ascorbic acid, develop **scurvy**. The disease normally does not occur in other species except as a result of genetic defects in vitamin C synthesis. *A spontaneous mutation involving L-gulonolactone oxidase is recorded in pigs.* The defect in pigs is inherited as an autosomal recessive trait and homozygous recessive piglets develop classic scorbutic lesions shortly after weaning. Maternal milk is a rich source of vitamin C and prevents signs of deficiency prior to weaning. A similar defect is also recognized in ODS rats.

The lesions of scurvy in guinea pigs with dietary vitamin C deficiency and in pigs with an inherited deficiency of L-gulonolactone oxidase are similar, although the lesions described in pigs are more severe. This probably reflects the complete absence of vitamin C in affected pigs, and the effect of greater body mass on weakened connective tissues. In guinea pigs, a dietary deficiency of vitamin C may be partial rather than absolute, or may be sporadic, and the bone lesions therefore vary in severity. Pigs with inherited L-gulonolactone oxidase deficiency are normal at birth but lose condition, become reluctant to stand or move, and develop swellings around their joints. Gross lesions are dominated by *sub-periosteal accumulations of clotted blood* around the shafts of the long bones, the scapulas, the bones of the head, especially the mandible, and on the ribs. The metaphyses are fragile, discolored by hemorrhage and separate easily from the adjacent physes. Similar hemorrhages occur around major joints, along costochondral junctions and on mandibles of affected guinea pigs. The bones are osteopenic and fragile. Subclinical scurvy in guinea pigs is accompanied by such nonspecific signs as diarrhea, weight loss, and dehydration, and may be more common than is generally appreciated as the early bone lesions may not be detected clinically or on gross postmortem examination.

The most characteristic microscopic lesion of scurvy is in the metaphysis. *Naked spicules of calcified cartilage, derived from the zone of provisional calcification in the growth plate, persist as a "scorbutic lattice"* (Fig. 1.51A, B). The layer of bone that is normally deposited on this cartilage framework by active osteoblasts is absent or deficient. Osteoblasts are sparse and appear to have lost their polarity. The marrow cavity may be filled with a population of poorly differentiated mesenchymal cells showing little if any collagen formation (Fig. 1.51B). In some cases, apparently normal bone trabeculae are present in the secondary spongiosa, representing bone formed during a period when dietary vitamin C was adequate. The cartilage spicules of the scorbutic lattice do not have the strength of normal trabeculae. Microfractures or infractions associated with hemorrhage and fibrin, but with little evidence of normal repair (Fig. 1.51A, B), are often present. Mechanical forces no doubt influence the development of such lesions.

Physes are generally thin and contain a reduced number of chondrocytes, poorly organized into columns. Cortices of long bones are thin and the periosteum may be elevated with hemorrhage or thin, irregular spicules of basophilic, woven bone separated by loose connective tissue.

The cartilage spicules of the scorbutic lattice have a high mineral content and may be visible radiographically as a radiodense band in the metaphysis.

Early suggestions that vitamin C deficiency may be a cause of metaphyseal osteopathy (hypertrophic osteodystrophy) in young dogs have been refuted. Dogs are capable of manufacturing their own vitamin C and although there are radiographic similarities between scurvy and metaphyseal osteopathy, the histologic lesions are quite different.

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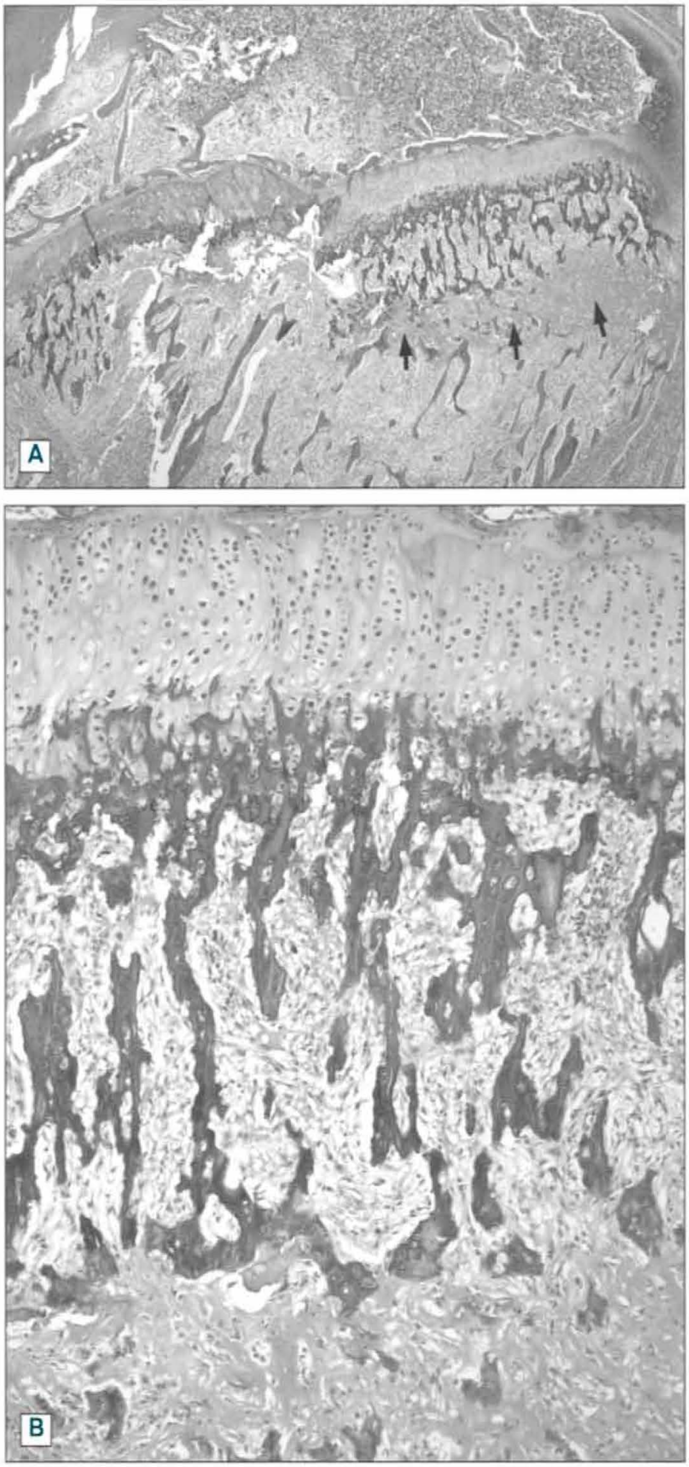

Figure 1.51 Scurvy. **A.** Physis and metaphysis from an affected guinea pig. Note the basophilic spicules of cartilage matrix without osteoid deposition in the area of the primary spongiosa. This is referred to as a "scorbutic lattice." Trabeculae in the secondary spongiosa do possess an osteoid layer, indicating that the deficiency of vitamin C is relatively recent. An infraction line (arrows) between the primary and secondary spongiosa reflects the fragility of bone trabeculae. **B.** Closer view of physis and **"scorbutic lattice"** in the same guinea pig. The calcified cartilage spicules are devoid of an osteoid layer and no osteoblasts are apparent. The spaces between trabeculae are filled with poorly differentiated mesenchymal cells. Trabecular fragments surrounded by fibrin at the bottom of the illustration form part of the infraction shown in (**A**). There is no evidence of repair.

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Plant toxicities

Many plant species are known, or suspected, to cause skeletal abnormalities, either by inducing a teratogenic effect on the developing fetus, or influencing skeletal remodeling in young growing animals. Those with a teratogenic action typically exert their influence early in pregnancy and require ingestion by the dam during a specific stage of fetal development. The classic example is ***Veratrum californicum*** (false hellebore, wild corn, or skunk cabbage), which causes **cyclopia** (Fig. 1.52) in the progeny of ewes that consume the plant on day 14 of gestation. Similar anomalies also occur in calves and kids whose dams are fed the plant at this stage of gestation, when the neural groove has formed and the first cranial somites are developing. Ingestion of *V. californicum* by pregnant ewes near day 29 produces marked shortening of the metacarpals, metatarsals, and tibiae, and sometimes the radii, while tracheal and laryngeal stenosis occurs in the offspring of ewes ingesting the plant on or about day 31 of gestation. There is variation in the degree of susceptibility, but lesions are usually bilaterally symmetrical and twins tend to be affected equally. Fusion of metacarpals may result in postnatal bowing of the legs and fusion or deformities of joints may cause **arthrogryposis**. Obviously not all ewes will graze the plant at precisely the same time of gestation and in a natural outbreak the changes detected in affected lambs would be expected to include a range of abnormalities. Prolonged gestation is also a feature of malformed fetal lambs and embryonic losses may be substantial. Death of about 17% of embryos occurs with maternal ingestion of *V. californicum* on days 19–21 of gestation, and up to 75% of embryos when the plant is ingested on day 14.

Ingestion of *V. californicum* by *pregnant cows* between days 12 and 30 of gestation may induce a range of anomalies, including cleft palate, harelip, brachygnathia, hypermobility of hock joints, and

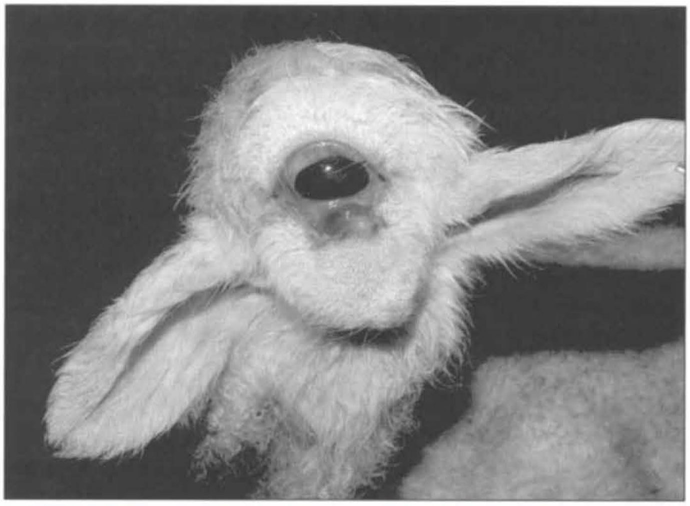

Figure 1.52 Cyclopia in a lamb. This deformity has been associated with ingestion of *Veratrum californicum* by pregnant ewes at around day 14 of gestation.

syndactyly and reduced number of coccygeal vertebrae. The length and diameter of all limb bones may be reduced, or the changes may be restricted to the metacarpals and metatarsals. Between days 30–36 of gestation, the teratogens selectively inhibit growth in length of the metacarpal and metatarsal bones.

V. californicum contains several steroidal alkaloids, the most active being cyclopamine, cycloposine, and jervine. *Cyclopamine is most abundant in the plant and is responsible for the craniofacial defects*, but the cause of the other anomalies is unknown.

A syndrome referred to as **crooked-calf disease** occurs when pregnant cows ingest certain **wild lupins**, including *Lupinus caudatus, L. sericeus,* and *L. formosus*, especially between days 40 and 80 of gestation. Malformation of the limbs, notably the forelimbs, is the most common alteration, but the axial skeleton may also be involved. *The limb abnormalities consist of flexion contracture and arthrogryposis associated with disordered growth of joints*, in addition to shortening and variable rotation of the bones. *Torticollis, and either scoliosis or kyphosis are common*, involvement of the thoracic spine being associated with costal deformities. There are various subtle and often asymmetric changes in the skull, where *the most definable malformations are cleft palate, or brachygnathia superior.* Affected calves are usually born alive and may survive, depending on the nature and severity of their malformations, but growth is retarded and the malformations persist, often becoming more severe with age. Neuromuscular structure and function are regarded as normal, or at least not primarily responsible for the skeletal deformity. No remarkable or consistent histological changes are reported in crooked calves.

Lupins contain several alkaloids, some of which are teratogenic. The quinolizidine alkaloid *anagyrine* is present in *L. caudatus* and *L. sericeus* and is almost certainly a teratogen. In *L. formosus* the piperidine alkaloid *ammodendrine* may be responsible. Inhibition of fetal movement caused by a sedative or anesthetic effect of the alkaloids may be the basis for the skeletal deformities. Alternatively, secondary effects of direct toxicity to the dam may be involved; sustained uterine contractions producing deformities in the fetus have been suggested. *Lupinus caudatus* (tailcup lupine) does not produce lesions in fetal lambs or kids, suggesting differences between cattle and other species in the metabolism of anagyrine.

Lupins cause two other distinct diseases in animals; a nervous disorder associated with the alkaloids in bitter lupins (see Vol. 1, Nervous system) and a mycotoxicosis caused by *Phomopsis leptostromiformis* (see Vol. 2, Liver and biliary system).

Conium maculatum (poison hemlock, European hemlock, spotted hemlock) is toxic to various species and causes a syndrome characterized by excitation and subsequent depression. There are five piperidine alkaloids in *C. maculatum*, the major ones being *coniine and* γ*-coniceine*. Both are thought to be toxic, and the latter teratogenic. Cattle are most susceptible, and there is decreasing susceptibility through sheep, horses, and pigs. *Conium maculatum* is also teratogenic, especially for cattle, with pigs being less susceptible and sheep relatively resistant. The plant causes *arthrogryposis and spinal deformities in the offspring of sows and cows* when fed between days 43–53 and 55–75 of gestation, respectively. *Cleft palate* occurs in the offspring of pigs exposed to the plant between days 30–45 and goats exposed between days 30 and 60 of gestation. *Carpal flexure* occurred in lambs and kids when *Conium* was fed to pregnant ewes and goats from days 30–60 of gestation, but resolved spontaneously as they grew.

Nicotiana tabacum (burley tobacco) causes arthrogryposis and sometimes brachygnathia and kyphosis in piglets exposed to the plant in utero. ***Nicotiana glauca*** (wild tree tobacco) causes similar lesions and also produces arthrogryposis and spinal deformities in calves exposed between days 45–75 of gestation. The teratogen is an α-substituted piperidine alkaloid, anabasine, which is present also in *N. tabacum*. It produces the lesions when given between days 43 and 53 of gestation and causes cleft palate when administered between days 30 and 37.

Jimsonweed (*Datura stramonium*) and **wild black cherry** (*Prunus serotina*) are suspected teratogens in sows, both being linked to *arthrogryposis*.

Various etiologies, including plant toxicities, have been incriminated in a limb deformity syndrome of lambs referred to as **"bent leg."** In Australia, the syndrome has been associated with ingestion of *Trachymene ochracea* (white parsnip), *T. cyanantha* and *T. glaucifolia* (wild parsnip) by ewes, which preferentially graze the inflorescence of *Trachymene* sp. The toxic principle is unknown, but the deformity may either be congenital, due to exposure of lambs in utero, or may develop during postnatal growth, probably following exposure of lambs through the milk. Gross deformities are usually most prominent in the forelimbs, and include outward bowing, flexion, and lateral rotation of the carpal joints and medial or lateral rotation of the fetlocks. Similar syndromes are described in New Zealand (where it is called **"bowie"**) and South Africa, but plant toxicities are not suspected. In both of these conditions, calcium deficiency and osteoporotic bone disease play a major role in the weakening and deformity that develop. Further discussion of these syndromes is included later in this chapter under the heading "Angular limb deformity."

Outbreaks of congenital limb deformity and abortion have been reported in cattle, sheep, and horses in association with ingestion of **locoweed** (species of *Astragalus* and *Oxytropis*) by pregnant animals. The abnormalities include *brachygnathia, contracture or over-extension of joints, limb rotations, osteoporosis, and bone fragility.* The toxic agent responsible for the skeletal defects is not known but is most likely unrelated to that responsible for the neurological manifestations of locoweed toxicity (see Vol. 1, Nervous system), which inhibits the lysosomal enzyme α-mannosidase, inducing a lysosomal storage disease.

The leguminous plant ***Lathyrus odoratus*** (sweet pea) contains the amino acid β-aminopropionitrile, which inhibits lysyl oxidase and interferes with the formation of intermolecular cross-links in collagen and elastin. Skeletal deformities have been produced experimentally when the plant was fed to young rats, but natural disease associated with ingestion by grazing animals is unlikely.

Fetal ankylosis and abortion are recorded in foals whose dams graze **hybrid sorghum and Sudan grass** (*Sorghum sudanense*) during days 20–50 of gestation, and a similar association has been made in cattle. The lesions in foals involve many joints but are not well described. It is not clear whether the foals have ankylosis or arthrogryposis.

Other toxicities

Overdosing of ewes with the anthelmintic **parbendazole** between days 12–24 of gestation may produce skeletal abnormalities in a high proportion of lambs. Neurologic lesions may also be present (see Vol. 1, Nervous system). The skeletal malformations include compression and/or fusion of vertebral bodies, fusion of proximal ribs, curvature of long bones, hypoplasia of articular surfaces and absence of various bones such as ulna, humerus, and metacarpals. Lesions are reliably reproduced by a double dose of the drug and may even occur with less than twice the recommended dose.

A range of fetal abnormalities, including twisted limbs, brachygnathia inferior, and arthrogryposis is reported in several sheep flocks in New Zealand where ewes had been affected by **nitrate toxicity** during early pregnancy. The ewes had been fed during the winter on crops containing high levels of nitrate and although the evidence to incriminate nitrate toxicity as the cause of fetal abnormalities was circumstantial, the possible involvement of agents such as pestivirus and *Akabane virus* was excluded. The problem occurred on seven properties and the incidence of abnormal lambs varied from 1 to 11%.

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Skeletal anomalies of unknown etiology

A syndrome of neonatal foals, characterized by **thyroid hyperplasia** *accompanied by* **musculoskeletal deformities,** has been recognized in western Canada for several years. The skeletal abnormalities include mandibular prognathia, flexural deformities of the forelimbs, ruptured common and deep digital extensor tendons, and delayed ossification of carpal and tarsal bones. In some affected foals, abnormal development of carpal bones, especially the third carpal, is considered to cause angular limb deformity. Lesions typical of osteochondrosis are sometimes present on articular surfaces of carpal bones, perhaps secondary to delayed ossification and increased susceptibility to injury during weight bearing. Hyperplasia of the thyroid gland is a consistent finding in affected foals, and similar abnormalities have been reproduced in partially thyroidectomized foals, but the etiology is not known. Unlike iodine deficiency goiter, the thyroid glands in foals with this syndrome usually are only slightly enlarged, if at all, and could easily be overlooked if not examined microscopically. The syndrome is not genetic and it is considered that *some factor in the feed, perhaps a mycotoxin or plant toxin*, is responsible for interfering with thyroid function, leading to skeletal and other changes normally associated with hypothyroidism. Exposure to cruciferous plants may be responsible.

A syndrome characterized by the birth of deformed calves with a range of abnormalities including joint laxity, disproportionate dwarfism, and sometimes varus or valgus forelimb deformities is well recognized in the western United States, Canada, and Australia. Severely affected calves walk on the palmar and plantar surfaces of their phalanges and some have brachygnathia superior. Originally called **"acorn calves"** because the defect was attributed to ingestion of acorns by pregnant cows, this etiology has now been dispelled, but the name persists in the literature. In Canada, the disease has been referred to as **congenital joint laxity and dwarfism.** Although the actual cause is not known, nutritional deficiency or possibly ingestion of a toxin by the dam, probably between 3 and 6 months of gestation, is suspected. Up to 10% of calves may be affected in herds grazing native pastures of foothills in the western United States. Similar environmental conditions occur in Australia, but the disease incidence tends to be lower, and in Canada where 2–40% of calves in a herd may be affected. The calves usually survive but do not prosper.

In Canada the disease is produced by feeding grass/legume silage during gestation and prevented by adding hay and grain to the silage ration. Timothy grass, fed as silage, produces the disease while timothy from the same fields fed as hay does not. It is suspected that fermentation of silage leading to production of mycotoxins, in particular those from *Fusarium*, is responsible.

Other, isolated examples of generalized abnormalities do occur sporadically. For example, forelimb abnormalities, resembling human "thalidomide babies," affected up to 29% of beef calves in two herds in Australia. There was an axial malformation in one calf and congenital blindness in another, but otherwise the lesions were restricted to the forelimbs. The scapulae were present and usually completely cartilaginous, but distal portions of the limbs were either vestigial or absent. A teratogen was suspected but health and management records provided no clues.

A disproportionate dwarfism in two dairy Shorthorn siblings also occurred in Australia, and was characterized by marked shortening of the pelvic limbs, mild shortening of pectoral limbs, and a normal axial skeleton. Retardation of growth in the femurs, tibias, and metatarsals was prominent, and there was deformity of the femorotibial joints. The calves were affected at birth, suggesting that this syndrome may be *different from hyena disease* (see above), which may be a manifestation of vitamin A toxicity. In hyena disease, the abnormality is usually not apparent until 3–4 months of age.

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Angular limb deformity

Angular limb deformities are relatively common in young animals of several domestic species, particularly in fast-growing breeds. In many cases, the deformity can be traced to an asymmetric lesion involving an active growth plate, such as the distal radius, but growth plate damage is not always the underlying cause, and even when it is, the etiology is often not apparent. Abnormal development of carpal or tarsal bones is also reported as causing limb deformities, especially in horses, as has joint instability due to laxity of supporting structures. *Lateral deviation* of the limb distal to the affected growth plate or joint is referred to as a **valgus deformity** and *medial deviation* as a **varus deformity**.

In **horses**, angular limb deformities occur primarily in young foals and are included with a group of disorders referred to as *developmental orthopedic diseases*. A variety of congenital and acquired lesions have been identified in foals with limb deformities and in many cases it is not possible to determine which changes are primary and which are secondary. The abnormality is often centered on the carpal, tarsal, or fetlock joints. Many foals are born with weakness of the supporting periarticular tissues of these joints and have minor structural abnormalities in the osteochondral junctions of developing joint surfaces or the physes of adjacent bones. These minor defects are often clinically manifest as angular limb deformities at birth but most resolve spontaneously within the first week or two of life. During this period, the periarticular attachments strengthen and any regions of physis or developing joint surface that are subjected to extra strain or compressive force during locomotion or weight bearing compensate by an increase in the rate of endochondral ossification. This allows correction of most minor structural abnormalities. If the deformity is more severe, the compensatory mechanisms are exceeded and the deformity will persist without intervention.

Congenital **defects in carpal and tarsal bones** are reported as a *cause of angular limb deformity in newborn foals*. The bones may be abnormal in size or contour, or may possess osteochondrosis-like lesions, both of which can alter the alignment of bones within the joint and therefore the angle of the lower limb. As described above, these lesions may occur in association with other musculoskeletal abnormalities and thyroid hyperplasia. Delayed ossification of affected bones due to hypothyroidism is suggested as a possible mechanism. Even in foals with no evidence of hypothyroidism, the bones of the carpus and tarsus vary considerably in their degree of ossification at birth. In premature foals, or foals with inadequate mineralization of these bones, any minor conformational defects associated with joint laxity may become permanent due to abnormal compression and plastic deformation of the thick cartilaginous layer.

Physeal lesions are occasionally present at birth but are more often the cause of acquired angular limb deformities during the first 6 months of life. *Physes are the weakest structure in the developing ends of long bones and are very susceptible to injury.* In rapidly growing young foals, physeal lesions may also occur as a manifestation of osteochondrosis. The physes most at risk of both trauma and osteochondrosis are those that grow most rapidly, such as the distal radius. Other common sites are the distal physes of the metacarpals and metatarsals, the distal physis of the tibia and the proximal physis of the first phalanx. Such lesions, if detected radiographically as thickened foci in the growth plate, are *often termed "physitis" or "epiphysitis,"* both of which create the misleading impression that they are primarily inflammatory. *These are essentially clinical terms and should not be used by pathologists.* Although many lesions of this type resolve spontaneously without causing clinical disease, some will predispose to angular limb deformity. Microscopically, the lesions of "physitis" reflect focal disruption of endochondral ossification and resemble those associated with osteochondrosis. Septic infection of bone in young foals may also involve physes, causing destruction of the growth cartilage (Fig. 1.53) (see osteomyelitis, in Inflammatory diseases of bones). Foals that survive such infections may develop angular limb deformity.

In **dogs**, angular limb deformity is most commonly associated with *premature closure of the distal ulnar physis*, which is particularly susceptible to trauma, presumably because of its conical shape. If the physis of the adjacent radius continues to grow, the resultant asynchrony will induce valgus deformity. The syndrome occurs most often in large breeds but is described as an autosomal recessive trait in the Skye Terrier. Less frequently, angular limb deformity in young pups results from a lesion in either the proximal or distal physis of the radius, the latter being the most common. Such lesions may be due to a fracture through the hypertrophic zone of the

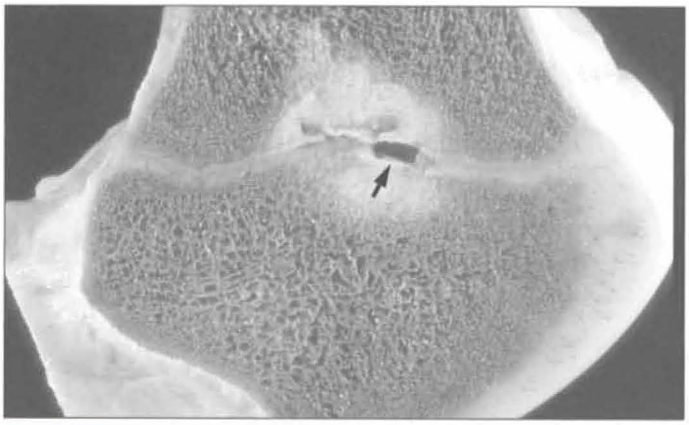

Figure 1.53 Septic physitis and osteomyelitis causing disruption of the distal femoral growth plate in a young foal. Had the foal survived, there would have been fusion between the epiphysis and metaphysis in the area of physeal disruption (arrow) possibly leading to angular limb deformity.

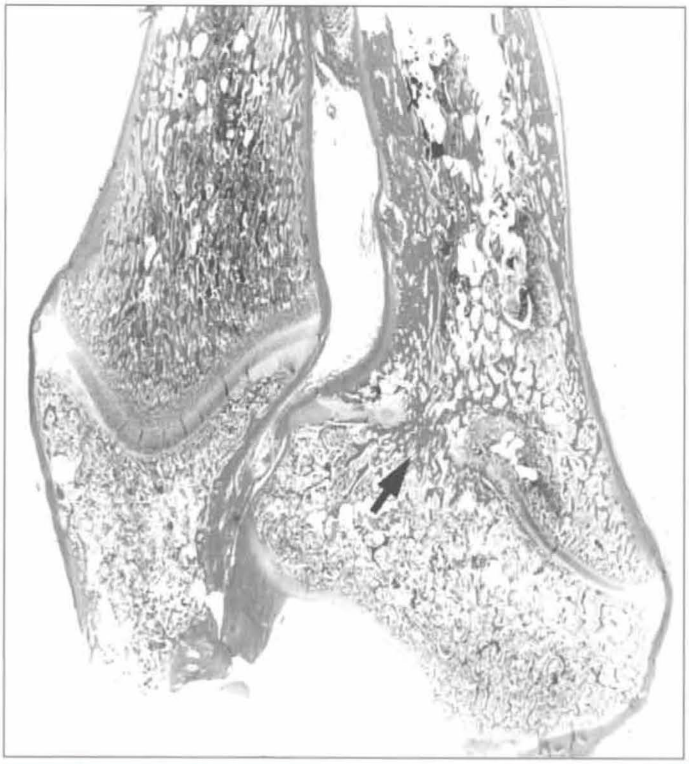

Figure 1.54 Distal radius and ulna from a pup with **angular limb deformity** secondary to traumatic damage to the radial physis. One side of the physis has been destroyed and is penetrated by thick bony trabeculae (arrow). Fusion of the epiphysis and metaphysis on this side has prevented further growth, while growth has continued from the undamaged segment of physis, resulting in angular deformity. (Courtesy of DH Read.)

growth plate, or to a crushing force transmitted through the epiphyseal bone. If the injury affects just one side of the growth plate, and growth continues on the other side, angular deformity will result (Fig. 1.54). Premature closure of the distal tibial physis, resulting in curvature of the tibia/fibula, also occurs occasionally in pups.

In large dogs, especially Great Danes, *closure of the distal ulnar growth plate can be confused with retarded endochondral ossification* (retained endochondral cartilage core). Although the clinical manifestations are often similar, the underlying lesion and its pathogenesis are different. The lesion is bilateral, often clinically silent, and may heal uneventfully. Large retained cores are more likely to interfere with longitudinal growth of the ulna and cause angular deformity. The lesion consists of a cone-shaped mass of unmineralized hypertrophic cartilage with its base at the center of the ulnar growth plate and its apex projecting into the metaphysis (Fig. 1.55A, B). The periphery of the plate is normal. The cause of the abnormality is not known but it could be a manifestation of osteochondrosis, or perhaps due to a temporary interruption or insufficiency in the metaphyseal blood supply. Retained cartilage in the distal ulna occurred in nine of nine pups in two successive litters of captive Mackenzie Valley timber wolves and was associated with limb deformities in all animals.

Angular limb deformity is also described in young, rapidly growing **sheep**, in different parts of the world and is often referred to as **"bent leg"** or **"bowie."** It is relatively common in ram lambs in feedlots, but also occurs in pasture-fed lambs. The forelimbs are affected more frequently than the hindlimbs and the deformity be either unilateral or bilateral, and either valgus or varus (Fig. 1.56A). The etiology is not known, but there is a *strong association with high-energy rations and rapid growth*, suggesting a nutritional component. Dietary imbalances of calcium and phosphorus have been implicated but not proven. One outbreak involved approximately 100 of 160 lambs, 6–9 weeks of age, some of which had been implanted with an anabolic hormone to increase growth rate. Bowie was common in sheep grazing unimproved hill country in New Zealand during the 1950s, involving up to 40% of lambs on some properties, but is now rare. The etiology was never identified but superphosphate topdressing was partially effective in controlling the disease.

Most reports of angular limb deformity in sheep describe gross and microscopic lesions in the *fast-growing physis of the distal radius*, and to a lesser extent in the *distal metacarpals or metatarsals*. The lesions typically consist of *focal or segmental thickening of the physis* (Fig. 1.56B) due to expansion of the hypertrophic zone with extension into the proximal metaphysis. These lesions closely resemble the physeal manifestations of osteochondrosis in foals and pigs, a syndrome that also occurs in rapidly growing young animals, predominantly males (see later in this chapter), and it is likely that the etiology and pathogenesis are similar. Although rickets may also produce similar lesions, there is no evidence of impaired provisional mineralization of cartilage in animals with bent leg, or of reduced bone strength, as would be expected in rickets.

Angular limb deformity is also described in young **goats**, especially those of *fast-growing dairy breeds*, when fed concentrate rations. Males are affected more often than females. The signs of deformity usually are first noticed around the time of weaning at 2–3 months of age, but occasionally in kids only a few days old. Many affected kids recover spontaneously by the time they are 5 months old. *Focal thickening of the physes of the distal radius or distal metacarpal is the characteristic gross lesion* and microscopically these consist of persistent hypertrophic chondrocytes. The syndrome is probably analogous to that in lambs and may be a manifestation of osteochondrosis.

In contrast, limb deformities reported in **llamas** and **fallow deer** have been in animals that are in poor condition and small for their age, suggesting a different mechanism. In llamas, the deformity is almost invariably valgus and centered on the distal radial physis. Radiographic evidence suggests that the deformities in this species

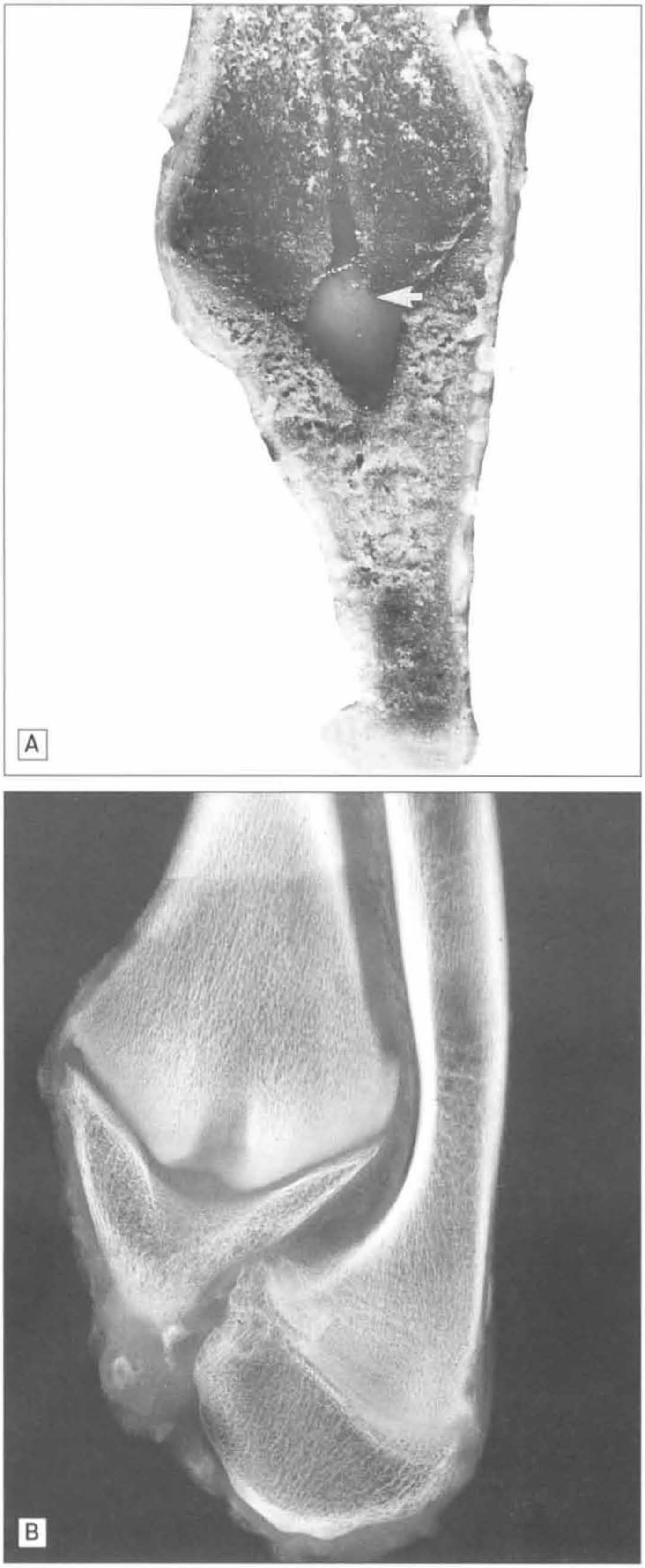

Figure 1.55 Retained cartilage core in the distal ulna of a dog. **A.** A mass of unresorbed physeal cartilage (arrow) projects into the metaphysis. **B.** Slab radiograph showing retained cartilage core in distal ulna of a dog with angular limb deformity secondary to reduced growth from the ulna physis.

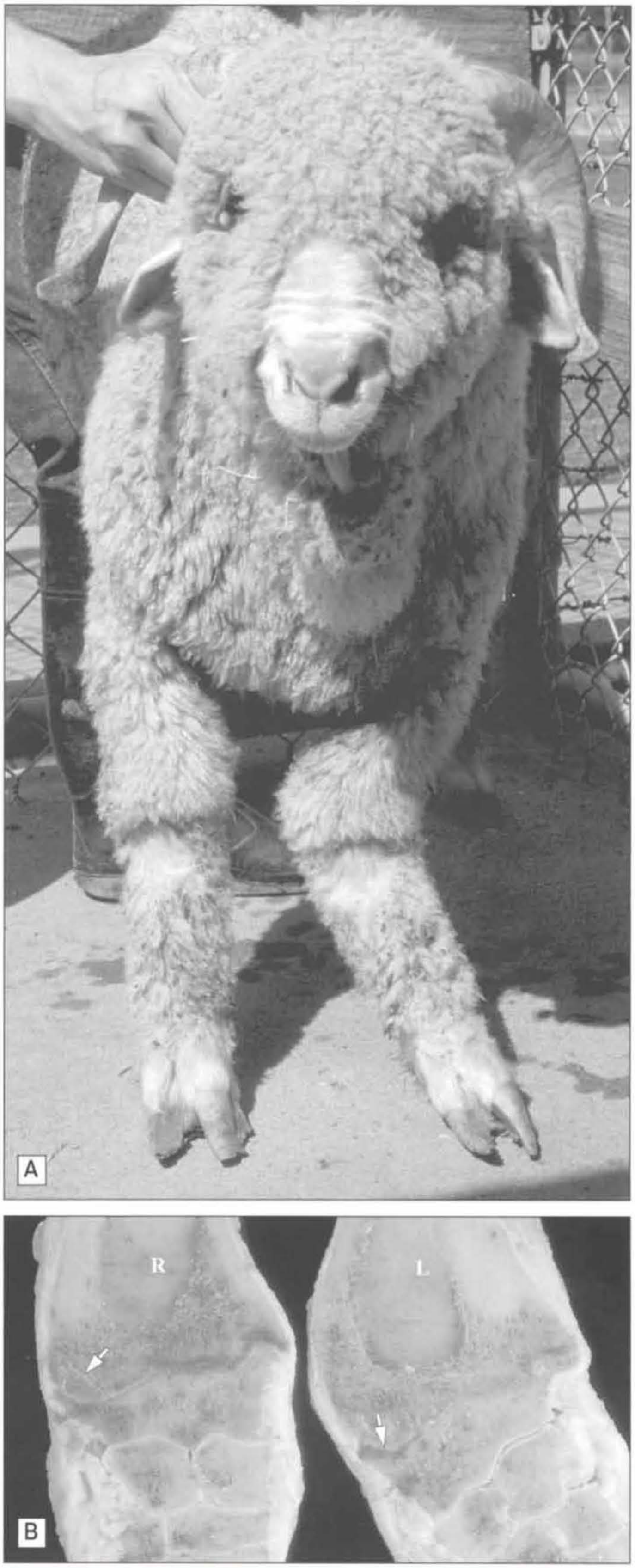

Figure 1.56 Angular limb deformity in a sheep. **A.** Varus deformity of the right foreleg and valgus deformity of the left in a young Rambouillet ram. **B.** Distal radial physes from the same ram. Note the focal duplication of the physeal cartilage (arrow) in the right leg (R) and focal thickening of the physis (arrow) in the left leg (L). In both legs, the physeal abnormalities are on the side that has grown more.

are due to premature closure of the distal ulnar physis, as occurs in dogs. In one report describing several cases of limb deformity in an inbred group of llamas, a genetic etiology was suggested, but not proven. A high incidence of limb deformity was recorded in fallow deer from a large deer park in the United Kingdom. In these animals, the lesion was usually centered on the distal metacarpal or metatarsal physes. Thickening of the physes was seen radiographically and grossly only in deer less than 1 year of age, while deformities were detected only in older deer whose growth plates had fused. In addition to the physeal lesions, some affected deer had widened metaphyses. Also, young deer in the park had previously been found with spontaneous fractures, suggesting the possibility of a metabolic bone disease, such as rickets. *The physeal lesions of rickets could predispose to angular limb deformity* and may have been involved on this property. Such physeal lesions could also be involved in angular limb deformities in llamas as camelids appear to be more susceptible to rickets than other grazing ruminants, especially when farmed at low altitudes (see p. 76).

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Effect of infectious agents on skeletal development and remodeling

Various infectious agents, in particular viruses, have been shown to infect bone cells and induce abnormalities in the developing skeleton. Infectious agents causing inflammatory changes in bones are discussed in a later section (see Inflammatory diseases of bones). Discussion here is confined to those infectious agents that influence skeletal development *by directly influencing the activity of bone cells.*

The ***Bovine viral diarrhea virus*** (BVDV) has been associated with the formation of zones of metaphyseal sclerosis parallel to the growth plate, referred to as **growth retardation lattices**, in aborted calves following maternal infection (Fig. 1.57A, B). These lattices consist of zones of persistent primary spongiosa with increased cross-connections between cartilage spicules (Fig. 1.57C), presumably reflecting transient periods of impaired osteoclastic activity. Similar zonal **osteopetrosis-like lesions** have also been linked to type 2 BVDV infection in older calves, up to 4 months of age. In these calves, a dense lattice of persistent primary spongiosa adjacent to growth plates contrasted sharply with the apparently normal trabecular arrangement deeper in the metaphysis. The paucity of osteoclasts detected histologically in affected calves, and reduced cellularity of marrow spaces, suggests that *viral destruction of osteoclasts*, or osteoclast precursors, is a likely mechanism for the lesion. It is possible that some cases of osteopetrosis in calves where there is widespread persistence of the secondary spongiosa might be caused by intrauterine viral infection but no specific agent has been incriminated.

Classical swine fever virus, which is closely related to BVDV, causes similar lesions in pigs that survive the acute phase of the disease.

Growth retardation lattices similar to those described in calves have been observed in association with natural and experimental infection by ***Canine distemper virus*** (Fig. 1.58), and in cats infected with ***Feline herpesvirus***. In experimental infections in weanling dogs, viral antigen was demonstrated by immunocytochemistry in osteoclasts, osteoblasts, and less often in osteocytes, from about day 5 to day 41 after infection. Viral antigen was most abundant in bone cells of the primary spongiosa and was strongly correlated with osseous changes, which were most prominent between days 26 and 32 post-infection. Impaired osteoclastic resorption of the primary spongiosa was considered the likely pathogenesis and was supported by the detection of necrotic osteoclasts. The lesions were transitory and resolved once viral antigen disappeared.

The term "growth retardation lattice" has become widely used for lesions of the type described here and in association with lead poisoning, but it creates a misleading impression of the nature of the lesion and *could easily be confused with "growth arrest lines."* The lattice of persistent primary or secondary spongiosa that comprises the former lesion is the result of reduced osteoclastic activity, not reduced growth of the bone. Physeal cartilage is not necessarily affected and growth in length of the bones may be continuing normally. In contrast, growth arrest lines, as discussed earlier in this section, occur when physeal growth ceases then commences again later. *The two lesions differ both in pathogenesis and in microscopic appearance, and should not be confused.*

Toxigenic strains of ***Pasteurella multocida*** cause osteoblast necrosis and are associated with the development of atrophic rhinitis in pigs.

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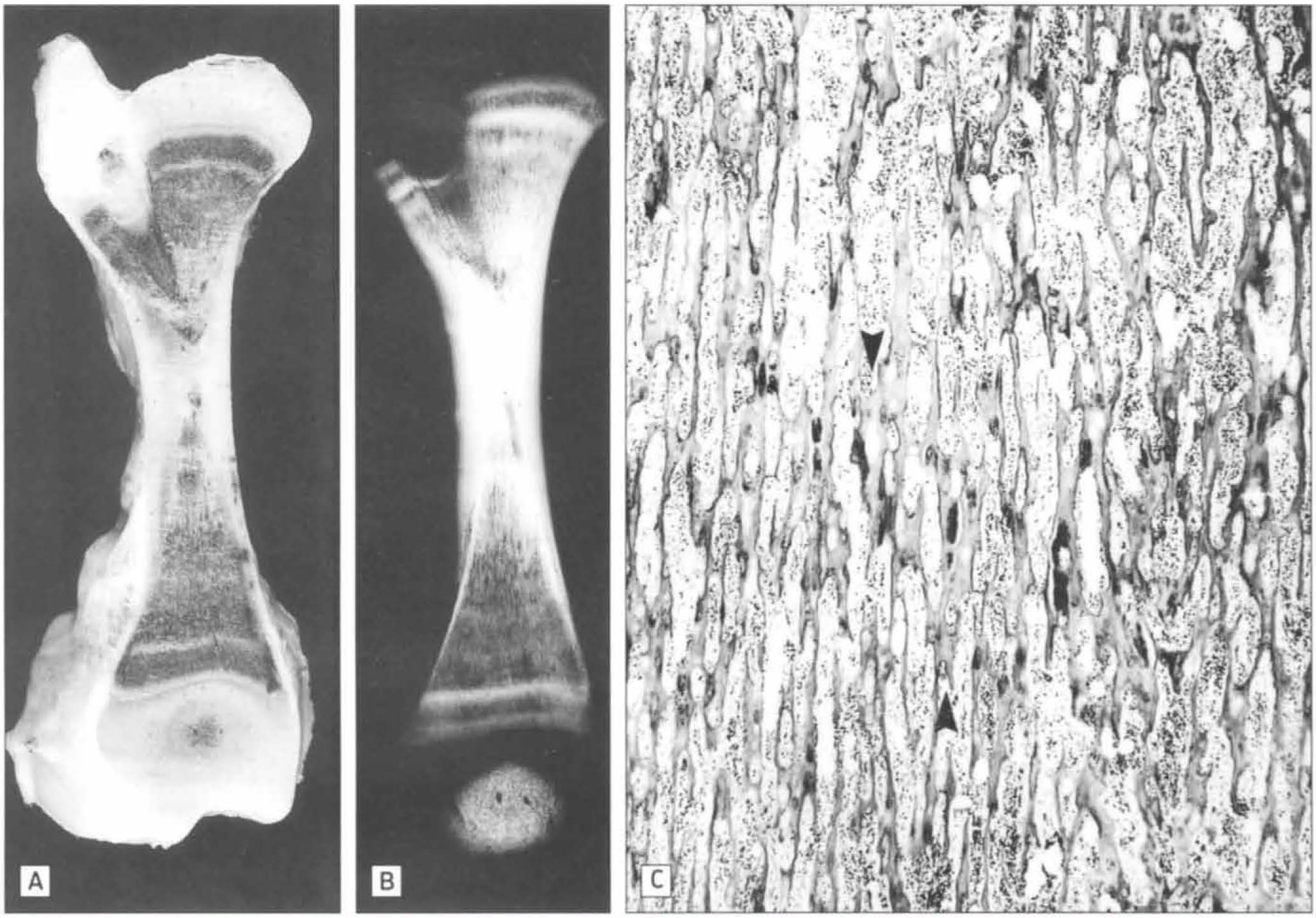

Figure 1.57 Bovine fetus. Intrauterine ***Bovine viral diarrhea virus* infection**. **A.** Narrow sclerotic bands in the metaphyses and relative persistence of medullary bone. **B.** Radiograph of (**A**) showing increased radiographic density of the metaphyseal bands and diaphysis. **C.** Histological preparation of the metaphysis, showing persistence of the primary spongiosa and a poorly defined zone (between arrowheads) where there are increased numbers of cross-connections between cartilage spicules. This corresponds to the sclerotic band seen grossly and radiographically.

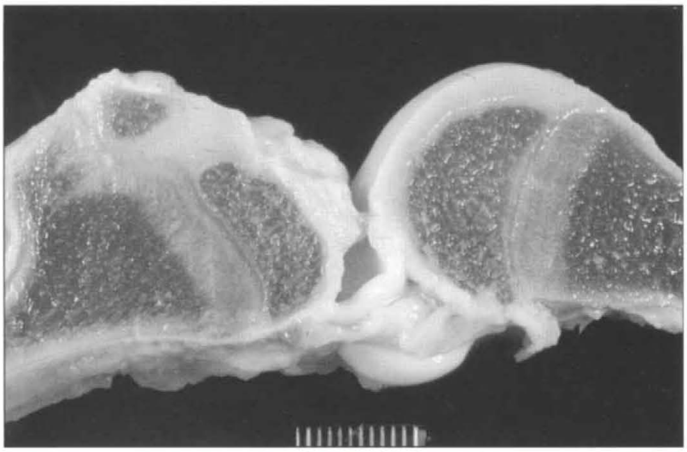

Figure 1.58 ***Canine distemper virus* infection** in a pup. Note the thick bands of metaphyseal sclerosis adjacent to the physes in the distal femur and proximal tibia. (Courtesy of TM Monticello.)

METABOLIC BONE DISEASES

Metabolic bone diseases, also referred to as **osteodystrophies**, *are the result of disturbed bone growth, modeling, or remodeling due to either nutritional or hormonal imbalances.* Because of the complexity of the processes involved in bone development, errors may occur at many stages, the consequences varying with the nature and severity of the imbalance. Genetic defects involving specific enzymes or receptors critical to the activity of hormones or cells participating in bone formation are also reported as causing metabolic bone disease in man and animals, but these are rare. The manifestations of metabolic bone diseases are generally most severe in young animals where the skeleton is undergoing rapid turnover, but lesions also occur in adults due to an effect on the quality or quantity of bone formed during remodeling. In addition to variations with age, there are variations between species in the manifestations of dietary mineral imbalances.

Metabolic bone diseases are traditionally classified as **rickets, osteomalacia, fibrous osteodystrophy**, or **osteoporosis**. Although these are distinct morphological entities with characteristic pathogenesis and lesions, *they can occur in combination in the same individual*. This may create confusion diagnostically. Furthermore, *their etiology can vary between species*. For example, calcium deficiency in sheep is likely to result in osteoporosis, but in a rapidly growing pig, fibrous osteodystrophy is a more likely result. Also, each of these disorders can be caused by more than one dietary or endocrine imbalance. The distinguishing features of each entity, together with their etiology, pathogenesis, and occurrence in different species are discussed in detail below. The term **osteopenia** is often used to describe syndromes associated with increased radiolucency of bone, but makes no inference as to its quality. As such, it includes osteoporosis, where only the quantity of bone is abnormal, as well as osteomalacia, fibrous osteodystrophy, and osteogenesis imperfecta, where the bone is not only reduced, it is abnormal in quality.

Since bone matrix is composed largely of calcium and phosphate ions in the form of hydroxyapatite crystals, any dietary or physiological factors affecting the metabolism of calcium and/or phosphorus can interfere with the formation of bone tissue and cause an osteodystrophy. Because of the importance of these two elements in bone formation, and hence in the etiology of metabolic bone diseases, a brief review of their homeostasis is presented below. Vitamin D and parathyroid hormone are integral components of homeostatic control mechanisms for calcium and phosphorus and their role also is discussed.

Metabolic bone diseases can usually be treated successfully, providing the problem is recognized early enough and there are no lasting effects caused by pathologic fractures or disruption of growth plates in young, growing animals. However, in cases where severe generalized osteoporosis has developed, insufficient bone surfaces may remain to allow appositional bone deposition to proceed and affected animals remain susceptible to hypocalcemic crises and the deleterious effects of chronically weakened bones. In production animals, an early and accurate diagnosis is important if significant economic loss is to be avoided. This requires a good history, recognition of species differences and an ability to accurately interpret radiographic and/or pathologic alterations. Whenever possible, more than one affected animal should be examined as the lesions may vary in severity between animals even if they are being fed the same ration. *It is important to examine and sample bones from several sites, particularly those that are showing obvious abnormalities and those from sites of rapid growth or movement. This includes the physes of the distal radius, proximal humerus, proximal tibia, distal femur, and costochondral junctions of the largest ribs.*

In reality, metabolic bone diseases seldom result from a deficiency of a single dietary nutrient. *More often, there is either a deficiency of several nutrients or a dietary imbalance in the ratio of calcium and phosphorus.* Diseases of other organ systems, especially gastrointestinal disorders that cause malabsorption, can affect the skeleton by reducing the absorption of essential minerals or vitamins. Metabolic bone disease also occurs in humans and animals in association with renal failure, due to interference with mineral metabolism and reduced synthesis of 1,25-dihydroxyvitamin D. This will be discussed in more detail below. Hepatobiliary and pancreatic disorders can, at least potentially, predispose to osteodystrophy through impaired absorption of vitamin D and other nutrients, but this is seldom recognized in domestic animals.

Calcium and phosphorus homeostasis

Calcium and phosphorus are required for a variety of essential bodily functions in addition to those involving skeletal development. *Approximately 99% of the calcium in the body is present in the skeleton as hydroxyapatite, the remaining 1% being contained in extracellular fluids and soft tissues.* The concentration of calcium in extracellular fluids and in the cytosol must be kept within narrow limits in order to maintain critical functions such as muscle contraction, neuromuscular irritability, blood coagulation, and mineralization of bone matrix. A small fraction of the calcium in bone (about 1%) is freely exchangeable with the extracellular fluid compartment. The skeleton is therefore an important storage site for calcium, providing a readily available source should dietary levels become temporarily deficient. Phosphorus is also a component of hydroxyapatite in bone matrix but, like calcium, has other essential functions; in particular the generation and transfer of cellular energy. *Approximately 85% of the phosphorus in the body is included in the skeleton.*

There are *three distinct fractions of calcium* in extracellular fluids (including plasma); an ionized fraction (about 50%), a protein-bound fraction (about 40%), and calcium complexed to other ions such as citrate and phosphate (about 10%). The *ionized fraction of calcium* (Ca^{2+}) *is the physiologically active form* and is tightly controlled by the actions of parathyroid hormone, 1,25-dihydroxyvitamin D and calcitonin on the intestinal tract, skeleton and, to a lesser extent, the kidney. Most of the protein-bound calcium is bound to albumin and plasma concentrations vary with the concentrations of plasma albumin. The binding of calcium to albumin is highly pH-dependent. Acute acidosis decreases binding and increases ionized calcium concentrations, while acute alkalosis increases binding and reduces the concentration of ionized calcium in plasma and extracellular fluids. Such alterations in ionized calcium are seldom reflected in the total serum calcium concentrations routinely measured by diagnostic laboratories.

Inorganic phosphate also exists in ionized, protein-bound, and complexed forms in plasma. The protein-bound fraction is relatively small (about 10%), but 35% of plasma phosphate is complexed to sodium, calcium, and magnesium, the remaining 55% being ionized. The predominant form of ionized phosphate in plasma is the divalent anion HPO_4^{2+}. Plasma phosphorus concentration is controlled by the action of parathyroid hormone and 1,25-dihydroxyvitamin D on the kidney and intestinal tract, but the control mechanisms are not as precise as for calcium. Consequently, plasma phosphorus concentration varies more widely than calcium, and is more easily influenced by diet, age, sex, pH, and various hormones. An inverse relationship exists between plasma ionized phosphate and plasma ionized calcium concentrations, *an increase in phosphate resulting in a reduction in ionized calcium.*

Parathyroid hormone (PTH) is intimately involved in the regulation of ionized calcium and phosphate concentrations in plasma through its action on specific cells in bone and kidneys, and indirectly on the intestine:

- stimulates the release of calcium and phosphate from bone by osteoclastic resorption,
- stimulates reabsorption of calcium and inhibits reabsorption of phosphate from renal glomerular filtrate,
- stimulates renal synthesis of 1,25-dihydroxyvitamin D from 25-dihydroxyvitamin D, thereby increasing intestinal absorption of calcium and phosphate.

The parathyroid gland responds to any decrease in the concentration of plasma-ionized calcium by releasing PTH. *The net effect of PTH is to increase plasma-ionized calcium and reduce plasma phosphate concentrations.* Once the ionized calcium has been restored to an acceptable level, PTH secretion is halted. Although PTH releases phosphate from bone, together with calcium, the increased loss of phosphate in the urine under the influence of PTH results in a net decrease in plasma phosphate. Osteoclasts do not possess receptors for PTH but are stimulated indirectly through increased expression of RANK-L by osteoblasts under the influence of PTH. This functional linkage between osteoblasts and osteoclasts may account for the concurrent increase in osteoblastic and osteoclastic activity in the bones of animals with hyperparathyroidism. PTH also reduces expression of osteoprotegerin, an inhibitor of osteoclast maturation and activation, and is closely involved in regulating the formation of the active form of vitamin D through its action on renal 1α-hydroxylase. Interestingly, intermittent, low doses of PTH have been shown to have a powerful anabolic effect on bone, creating new opportunities for treating osteoporosis in human patients.

Vitamin D is biologically inert, but undergoes two successive hydroxylations, in the liver and kidney, to form the *active hormone 1,25-dihydroxyvitamin D*, which acts in concert with PTH in maintaining plasma ionized calcium concentrations. 1,25-dihydroxyvitamin D has various actions on target organs:

- enhances absorption of calcium and phosphate from the small intestine,
- stimulates release of calcium and phosphate from bone by osteoclastic resorption,
- enhances phosphate reabsorption from glomerular filtrate.

In the intestine, 1,25-dihydroxyvitamin D increases the efficiency of calcium absorption from about 10% to 70%, probably by stimulating the local production of calcium-binding protein, although several mechanisms may be involved. Phosphorus absorption in the intestine is also increased by 1,25-dihydroxyvitamin D. *The principal action of 1,25-dihydroxyvitamin D on bone is to enhance calcium mobilization when the dietary levels are inadequate to maintain normal plasma-ionized calcium concentrations.* This requires the presence of both 1,25-dihydroxyvitamin D and PTH and is achieved by inducing the differentiation of osteoclasts from monocytic stem cells in the bone marrow as well as stimulating osteocytic osteolysis. In the absence of vitamin D metabolites, osteoid is poorly mineralized and calcium cannot be efficiently mobilized by osteoclasts. Although vitamin D is considered essential for normal mineralization of bone matrix, there is uncertainty whether this is a direct effect or mediated through maintenance of adequate extracellular concentrations of calcium and phosphorus. In addition to its effects on bone, 1,25-dihydroxyvitamin D has other biological effects, including inhibiting the synthesis of PTH and interleukin 2, and enhancing the secretion of insulin and thyroid-stimulating hormone. In vitro, 1,25-dihydroxyvitamin D has been shown to induce monocytes to differentiate into osteoclast-like multinucleated giant cells.

Vitamin D exists in two forms, *vitamin D_2 and vitamin D_3*, both of which have similar potency in humans and domestic animals. Some species however, such as New World primates (Cebus monkeys, tamarins (*Saguinus* spp.)), can utilize only vitamin D_3, while in chickens vitamin D_3 has about ten times greater potency than vitamin D_2. *Vitamin D_2 (ergocalciferol)* is of dietary origin, being present predominantly in yeasts and plants. *Vitamin D_3 (cholecalciferol)* may also be derived from dietary sources, especially fatty fish and cod liver oil, but most is formed in the skin following photochemical conversion of 7-dehydrocholesterol by energy derived from solar ultraviolet light with wavelengths from 290–315 nm (ultraviolet B). The previtamin D_3 formed by this process is then transformed into vitamin D_3 over a period of a few hours by heat-induced isomerization. Latitude, time of day, and season can all have a dramatic effect on vitamin D_3 production in the skin due to lessening of the intensity of ultraviolet light when the sun is at lower angles. At higher latitudes, sunlight is incapable of stimulating vitamin D_3 synthesis in the skin during the winter months. Furthermore, dark-skinned people require longer exposure to sunlight than people with light skin to manufacture the same amount of vitamin D_3. The same most likely applies to animals, and a dense hair coat is likely to reduce cutaneous vitamin D_3 synthesis even further. In fact, there is doubt that vitamin D is synthesized at all in the skin of dogs and cats. The need for carnivores to manufacture their own vitamin D is perhaps obviated by the ample supply of this fat-soluble vitamin in their usual diet.

Once formed in the skin, vitamin D_3 is transported to the circulation where it is bound to an α-1-globulin. Vitamin D_2 or D_3 absorbed from the intestine is transported in chylomicrons, reflecting its lipid solubility. Vitamin D from either source is transported to the liver where it is converted to 25-hydroxyvitamin D by the addition of a hydroxyl group. This is the major form of vitamin D in circulation. Since the reaction of the hepatic enzyme vitamin D-25-hydroxylase is not closely regulated, the concentration of 25-hydroxyvitamin D in circulation reflects the level of cutaneous vitamin D_3 formation and/or dietary levels of vitamin D. Measurements of 25-hydroxyvitamin D in plasma are therefore used to determine whether an individual has adequate or deficient vitamin D status.

The most active form of vitamin D is 1,25-dihydroxyvitamin D, which is formed by the addition of a second hydroxyl group to 25-hydroxyvitamin D under the direction of 25-hydroxyvitamin D-1α-hydroxylase. This reaction occurs predominantly in mitochondria of renal proximal tubular epithelial cells and is closely regulated by circulating concentrations of PTH and inorganic phosphorus. In pregnant animals, 1,25-dihydroxyvitamin D is also formed in the placenta, from where it may become involved in fetal bone and mineral metabolism. Synthesis of 1,25-dihydroxyvitamin D is stimulated directly by high PTH and low phosphorus, and indirectly by low ionized calcium, which acts through PTH. Once formed, 1,25-dihydroxyvitamin D also induces negative feedback on its own synthesis. When plasma phosphorus and ionized calcium concentrations are adequate, and PTH levels are low, 25-hydroxyvitamin D is converted to either 24,25-dihydroxyvitamin D or 1,24,25-trihydroxyvitamin D. These biologically inert metabolites are considered to represent the initial steps in biodegradation pathways.

Calcitonin also plays an important role in calcium and phosphorus homeostasis, and in bone metabolism. Produced by thyroidal C-cells, and secreted in response to increased concentrations of plasma-ionized calcium, *the main biological function of calcitonin is to inhibit osteoclastic bone resorption.* Within minutes of calcitonin administration, osteoclasts shrink and reduce their bone-resorbing activity. As a result, the release of calcium from bone is inhibited and plasma calcium returns to normal.

Osteoporosis

Osteoporosis is easily the most common of the metabolic bone diseases, both in man and animals. Rather than being a specific disease, *osteoporosis is a lesion characterized by a reduction in the quantity of bone, the quality of which is normal.* In effect, osteoporosis represents an imbalance between bone formation and resorption in favor of the latter, resulting in bone that is structurally normal but with reduced breaking strength. Strictly speaking, all osteoporoses should be

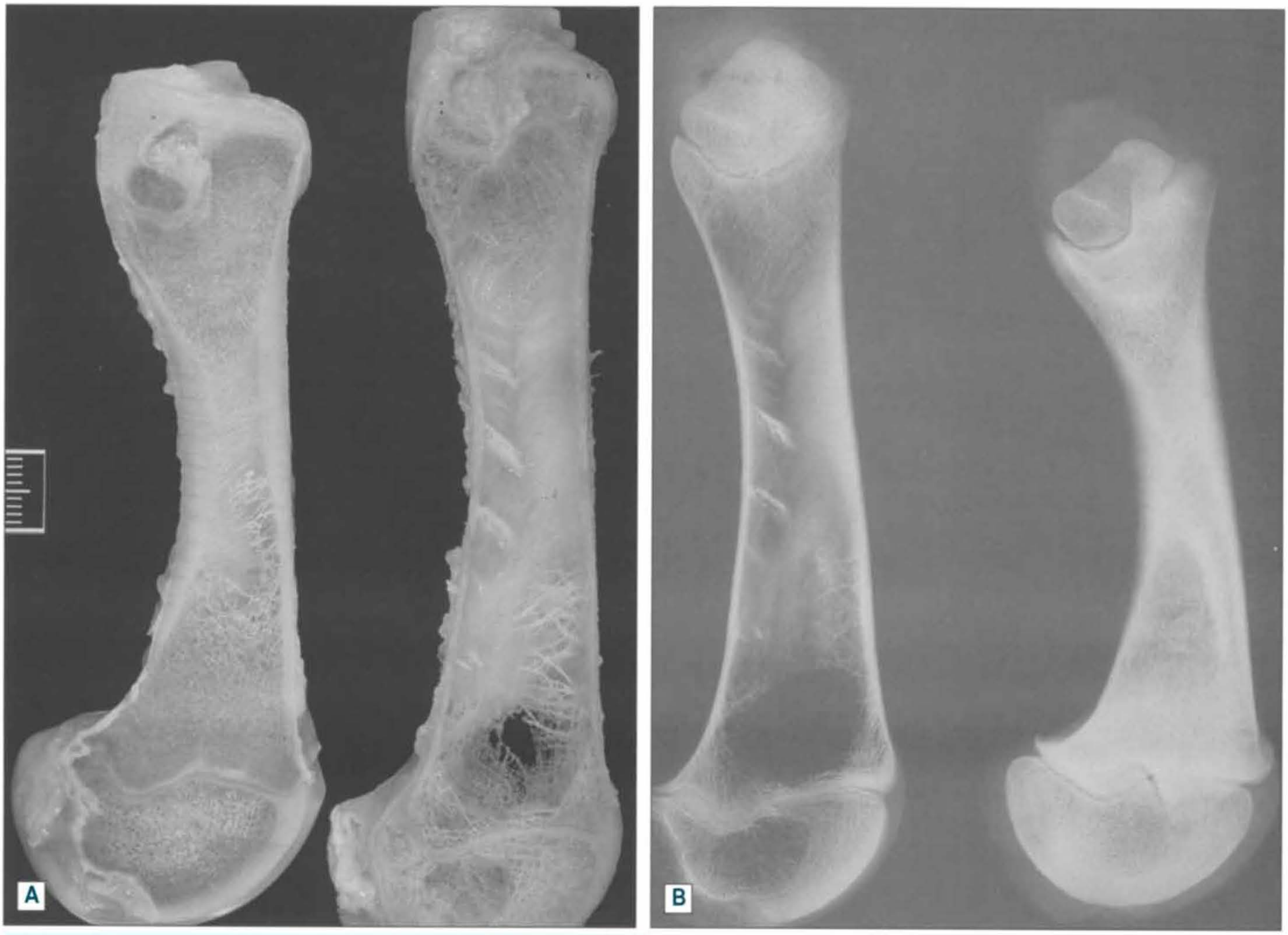

Figure 1.59 Osteoporosis in a pig. (Courtesy of RA Fairley.) **A.** Femur from a pig with severe osteoporosis (right) compared to that of a normal pig. Note the marked depletion of cancellous bone in the metaphyses and epiphyses and the very thin cortices in the affected pig. **B.** Radiograph of the same two bones (positions reversed) showing marked differences in the quantity of cancellous and cortical bone.

called osteopenias pending qualitative assessment of the bone tissue but, for practical purposes, this seldom occurs. Some diseases, for example osteogenesis imperfecta, are characterized by a reduction in bone quality as well as an alteration in bone mass and should not therefore be referred to as osteoporosis, even though the skeleton may appear porotic radiographically and show marked fragility.

The development of a negative balance between bone formation and resorption is recognized as a *normal part of the aging process* in man and animals. Osteoporosis could therefore be considered a natural phenomenon, but there are several physiological situations and disease states that either accelerate bone loss or increase bone resorption, leading to premature depletion of the skeleton. Those of most importance in animals are discussed in detail below.

Many mild cases of osteoporosis in animals remain undetected, even at postmortem examination, as the shape of individual bones is not altered and unless there have been pathological fractures, lameness is not likely to have been observed clinically. The *occurrence of a bone fracture without evidence of excessive trauma may be the first indication that an animal, or human, is suffering from osteoporosis.* In farmed livestock, there may be an unusually high incidence of fractures in the herd or flock, suggesting increased bone fragility.

Confirmation of osteoporosis can be difficult, especially when the degree of bone depletion is relatively mild. Routine radiographic procedures are insensitive in the early stages of osteoporosis as approximately 30–50% of skeletal calcium must be lost before the change can be reliably detected by this method. For this reason, and because of the importance of osteoporosis in human patients, *new diagnostic techniques* have been developed for its early recognition and quantitation. These techniques, which include photon absorptiometry, dual-energy X-ray absorptiometry, ultrasonography, magnetic resonance imaging, and computed tomography, are not widely used for the diagnosis of osteoporosis in animals. *Subjective assessment of the cut surface of bones at necropsy will allow diagnosis of advanced cases of osteoporosis,* but knowledge of what is normal for an animal of the age and species in question is essential, especially when the changes are equivocal. Radiography of isolated bones can detect much smaller alterations in bone density than whole-body radiographs, but comparison with an age-matched control may still be necessary before osteoporosis can be diagnosed with confidence. When it is important to be sure of the diagnosis, objective measurement of radiographs, or use of one of the more precise imaging techniques mentioned above, is recommended. Objective measurement of histological parameters such as bone volume, trabecular thickness, osteoid volume, osteoblastic and osteoclastic activity and bone mineral apposition rate is commonly used by medical pathologists (see review by Bullough et al.), but *histomorphometry* remains largely a research tool in veterinary pathology. *Bone ash measurements* have been traditionally used for the diagnosis of osteoporosis in animals and although they may provide information on the matrix–mineral relationship, reliable reference ranges are seldom available and interpretation of results is difficult. Because the ratio of calcium to phosphorus is fixed in deposits of hydroxyapatite within bone, bone ash samples do not identify low calcium or low phosphorus as causes for osteopenic bone disease, merely that the matrix to mineral ratio is abnormal.

Osteoporosis in human subjects is defined as bone mass 2.5 standard deviations below the young adult mean. In animals, such precise data are not available and the cut-off point between a normal and osteoporotic skeleton is somewhat arbitrary. For this reason, the diagnosis tends to be restricted to cases where there is an obvious reduction in bone mass.

Gross lesions of osteoporosis are generally most marked in bones, or areas of bones, which consist predominantly of *cancellous bone*, presumably because trabecular bone has a greater surface area to volume ratio than cortical bone and is resorbed more rapidly. Vertebral bodies in particular are affected early in the disease in human patients and animals with osteoporosis and may contain pathological fractures. Flat bones of the skull, scapula, ilium, and the ribs may also be severely affected and the breaking strength of the ribs, as assessed during necropsy, may be noticeably reduced. Cancellous bone in the metaphyses and epiphyses of long bones may be reduced in amount and more porous than normal (Fig. 1.59A, B). Trabeculae that are most concerned with transmission of weight-bearing stress are relatively spared and may become more prominent. Thickened trabeculae extending partially or completely across the medullary cavity may be present in the metaphysis or diaphysis of human patients and animals with chronic osteoporosis (Fig. 1.60). The pathogenesis of these *so-called reinforcement lines or bone bars* is uncertain but they may represent an attempt to reinforce weakened bones at locations of particular biomechanical stress. In most forms of osteoporosis, an exception being disuse osteoporosis (see below), cortical bone is resorbed most rapidly from the endosteal surface and along vascular channels. In advanced stages, the medullary cavities of affected long bones are enlarged and the cortices thin (Fig. 1.59A, B). Osteoporotic bones are usually light and fragile, but an accompanying reduction in soft tissue mass and muscular power in many cases tends to preserve the skeleton against stress fractures.

Histological examination of severely osteoporotic bones will not necessarily yield information of diagnostic value, as the depletion of bone tissue is likely to have been clearly established on the basis of either radiography or gross examination. However, it may allow assessment of bone quality, thus allowing differentiation from other metabolic bone diseases and certain other forms of osteopenia. Furthermore, *although it is not likely to allow an etiologic diagnosis, histology may provide an indication of the pathogenesis of bone loss*. For example, there is evidence that osteoporosis evolving from increased resorption is characterized by a decline in trabecular numbers, while in osteoporosis caused by decreased formation there are normal numbers of thin trabeculae. Quantitative histomorphometry may be of value in the diagnosis of osteoporosis in equivocal cases, but this is essentially a research tool rather than one to be used in routine diagnosis.

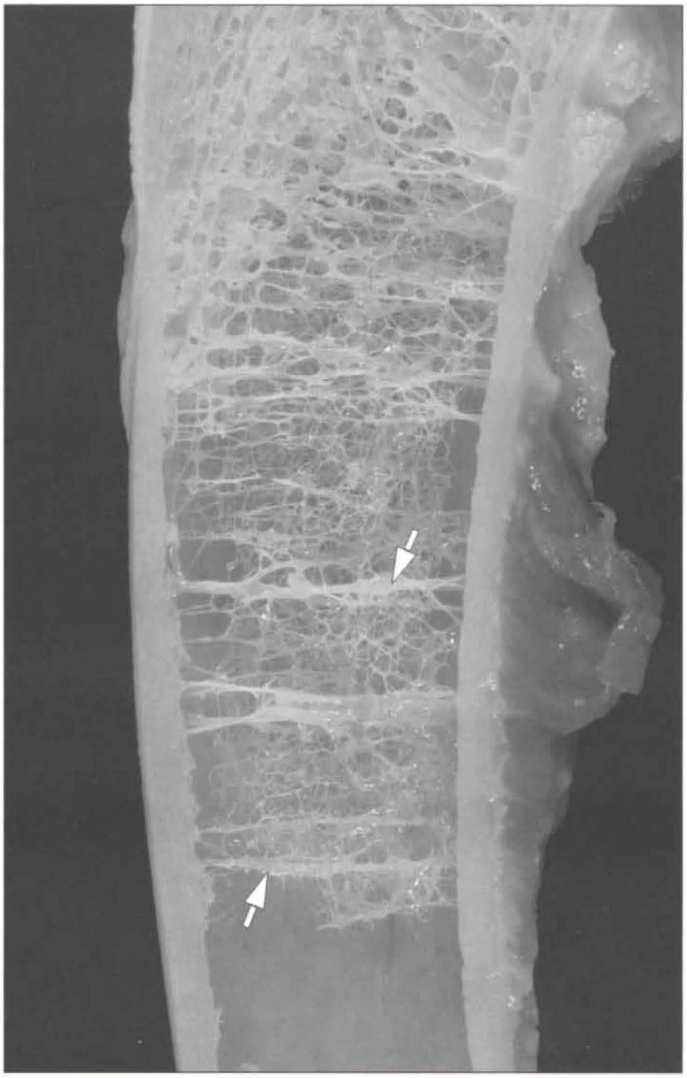

Figure 1.60 Osteoporosis in a dog. Narrow cortices, depletion of cancellous bone and formation of transverse reinforcement trabeculae (arrows), in a dog with chronic osteoporosis. (Courtesy of RA Fairley.)

In severely osteoporotic young animals, the hypertrophic zone of the growth cartilages may be narrowed or absent and when growth ceases, the physis is sealed by a plate of bone (Fig. 1.61A, B). Microscopically, trabeculae are reduced in number and/or size and often are fractured. Depending on the cause of the osteoporosis, evidence of abnormal activity of osteoblasts and/or osteoclasts is present. Intracortical resorption in long bones occurs along vascular channels and is produced by teams of osteoclasts working parallel to the long axes of the bones, the so-called *cutting cones*. Intracortical resorption is functionally important because a small loss of compact bone has a greater effect on bone stiffness than a comparable loss of cancellous bone.

Postmenopausal osteoporosis is the most important form of osteoporosis in human patients and, although the etiology is multifactorial, estrogen deficiency around the time of menopause is believed to play a significant role. Estrogen indirectly inhibits osteoclastic bone resorption through inhibition of interleukin-1 synthesis by peripheral blood monocytes. After estrogen withdrawal, interleukin-1 enhances the production of interleukin-6, a potent stimulator of

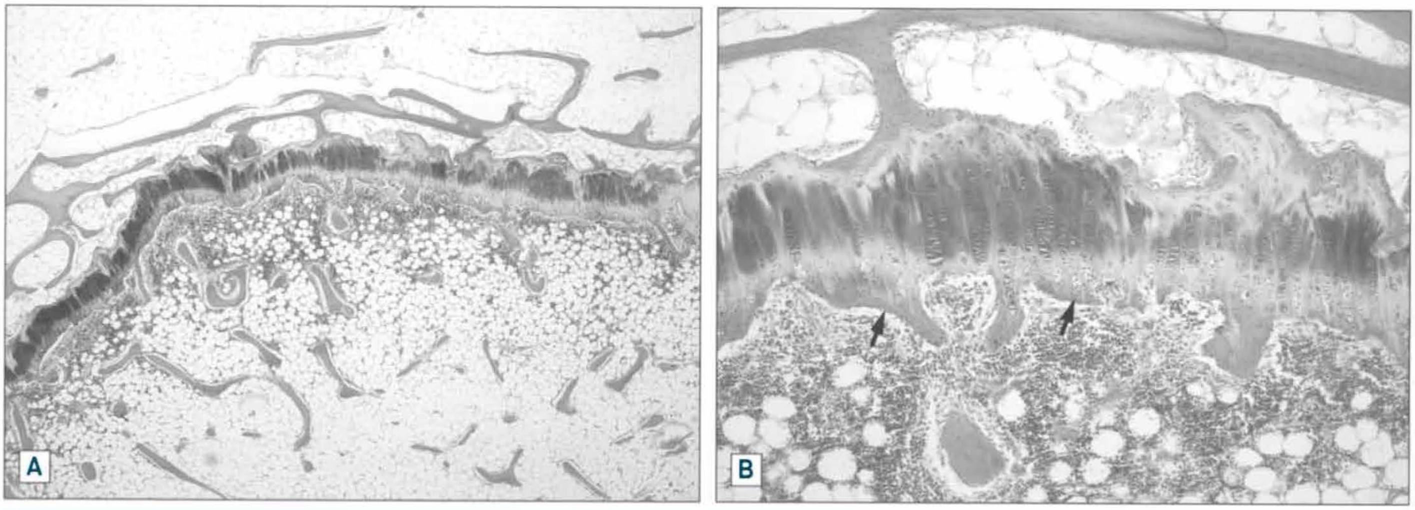

Figure 1.61 Osteoporosis: physeal closure. **A.** Narrowing of the physis and marked deficiency of trabecular bone in the epiphysis and metaphysis of a young pig with severe osteoporosis. **B.** Closer view of the physis in the same animal, showing almost complete absence of hypertrophic chondrocytes and sealing of the metaphyseal side of the physis with narrow plates of bone (arrows). Occasional plump trabeculae extend for a sort distance into the metaphysis.

osteoclastic activity. The inhibitory effect of estrogen on bone resorption may also involve promotion of osteoclast apoptosis by this hormone. This has been demonstrated both in vitro and in vivo and appears to be mediated by TGF-β. An equivalent form of osteoporosis is not currently recognized in animals but there are several other possible causes, many of which are discussed below.

Senile osteoporosis is common in humans and occurs in other animals, but *seldom appears as a clinical problem in veterinary medicine.* This may reflect the considerably shorter lifespan of most domestic animals when compared with humans, although in reality, it is often difficult for the veterinary pathologist to distinguish between the effects of age and other factors that might contribute to reduced skeletal mass in old animals. Beagle dogs aged 7–8 years have approximately 8% less bone tissue in their forelimbs than do young adults, but this reduction is not clinically significant.

Most cases of osteoporosis in animals, especially farm animals, are **nutritional** in origin and may be due to deficiency of a specific nutrient, such as calcium, phosphorus, or copper, or to **starvation**, where there is restricted intake of an otherwise balanced ration. Starvation is relatively common in grazing animals in areas prone to drought or due to overstocking in seasons when pasture growth is below expectations. Poor-quality milk replacers fed to young calves or other young animals may also result in starvation due to inadequate digestibility and absorption of nutrients. In starved animals, the mechanism of osteoporosis is complex, but the lack of dietary protein and energy most likely contribute, either directly or indirectly.

The *effects of starvation* on the skeleton are greater in young growing animals than in adults. Growth of the skeleton is retarded and may cease during periods of starvation due to reduced production of bone matrix. The physes are narrower than normal and the chondrocytes are small, with a relative increase in the amount of cartilage matrix. Thin, widely spaced bone trabeculae are present on the epiphyseal side of the physis, and the metaphyseal side may be sealed by a narrow, horizontal plate of bone (Fig. 1.61B), indicating cessation of growth. As a result, the epiphyseal and metaphyseal sides of the growth plate may appear similar. The cortical and trabecular bone of the diaphyses is reduced in amount. In some cases, characteristic transverse trabeculae may be evident grossly and microscopically in the metaphysis (see Fig. 1.44A, B). These **growth arrest lines**, which differ from the reinforcement lines referred to above, result from intermittent cessation and reactivation of physeal growth and represent a succession of sealing layers from the physis. Transverse radiodense striations also develop in limb bones of fetuses from ewes on a low plane of nutrition. In these lambs, there may be considerable variation in the number of arrest lines between littermates, the number of lines being correlated positively with retardation of fetal size and skeletal maturity. Serous atrophy of medullary adipose tissue is a common feature of starvation-induced osteoporosis in animals (Fig. 1.62).

The *pathogenesis of starvation osteoporosis* in terms of remodeling imbalance is not completely defined. The histologic appearance of inactive osteoblasts, and *a priori* reasoning, both *suggest that deficient bone formation is responsible.* Support for this hypothesis is derived from findings in dogs with malabsorption syndrome, in which tetracycline-labeled sites of bone formation are sparse or absent. The level of bone resorption during starvation is not known.

Severe undernutrition also has a profound effect on tooth development. Delayed formation and eruption of the whole dentition, with a relatively greater delay in the growth of the jaws, leads to overcrowding of the teeth, especially the permanent molars. This causes malocclusion and malalignment of teeth, and there is partial or complete elimination of the diastemata between incisors and canines, and between canines and deciduous molars. Nutritional rehabilitation allows rapid growth of jaws and teeth but malocclusion of permanent dentition persists and often there is abnormal development of parts of some teeth.

Uncomplicated **calcium deficiency**, in the presence of optimum dietary levels of phosphorus and vitamin D, causes osteoporosis experimentally in mature and immature animals, but rarely if ever occurs as a natural disease. In gilts, **lactational osteoporosis** occurs when rations marginally deficient in calcium, and with normal or excess phosphorus, are fed over extended periods during gestation and/or lactation. Generalized osteoporosis, and a tendency to fracture vertebrae, femurs, and phalanges, characterizes the condition.

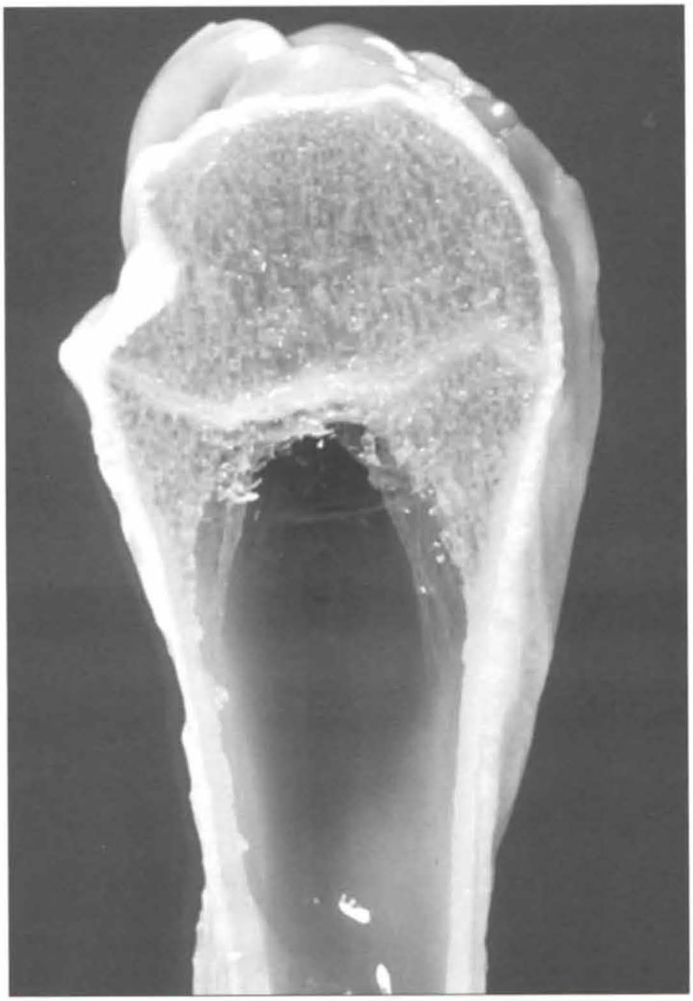

Figure 1.62 Starvation-induced osteoporosis in a lamb. Marked depletion of metaphyseal cancellous bone, thin cortices, and serous atrophy of medullary adipose tissue.

Significant losses associated with lactational osteoporosis occur in some swine herds. The condition may be complicated by disuse osteoporosis caused by confinement in farrowing crates, or by other dietary deficiencies and affected animals may have microscopic evidence of concurrent fibrous osteodystrophy.

The *osteoporosis induced by calcium deficiency is due to excess bone resorption* as a result of increased activity of parathyroid hormone following a reduction in plasma-ionized calcium concentration. In adult animals, the bone loss probably results from an increase in the activation of new remodeling sites. Cortical bone is lost through increased endosteal resorption and, in severe calcium deficiency, intracortical resorption as well. In both adult and growing animals, there is severe loss of cancellous bone, especially in bones with a high trabecular component such as vertebrae. In immature metaphyses, the primary spongiosa may be removed completely, so that there is no framework remaining for the production of secondary spongiosa.

Unless the deficiency is extremely severe, physeal growth and mineralization of cartilage are normal. Osteoid seams are of normal width and frequency, and mineralization occurs normally. *Rickets is not a feature of uncomplicated calcium deficiency* because calcium resorption from bone ensures that this mineral is not a limiting factor at sites of mineralization. In mature animals, calcium deficiency is usually asymptomatic, but in the young, trabecular microfractures, infractions, and complete fractures of bones may occur in both the axial and appendicular skeleton, and animals may die suddenly from hypocalcemic crises when stressed. Parathyroid glands are slightly enlarged due to hyperplasia, and fibrous osteodystrophy sometimes develops, but is less florid than in the osteodystrophy of calcium deficiency induced by phosphorus excess or vitamin D deficiency.

Prolonged calcium deficiency causes dental abnormalities in sheep, including delayed eruption, and increased susceptibility to wear due to mild enamel hypoplasia. When adequate calcium becomes available, superior brachygnathia develops because of inadequate repair of the upper skull compared to the mandible.

Classically, **phosphorus deficiency** *produces osteomalacia in adults and rickets in growing animals, both under natural and experimental conditions. However, under some circumstances it causes osteoporosis.* The reasons for this are not clear, but it may be related to the anorexia that often accompanies phosphorus deficiency, the age and growth rate of the animals, and the severity and duration of phosphorus deficiency. It is not clear whether osteoporosis and osteomalacia are interconvertible or whether osteoporosis is a stage in the development of osteomalacia.

Osteoporosis is recognized as a feature of naturally occurring and experimental **copper deficiency** in lambs, calves, foals, pigs, and dogs. As a component of the enzyme lysyl oxidase, *copper is required for the cross-linkage of collagen and elastin.* Deficiency of this enzyme is probably responsible for the reduced osteoblastic activity observed in swine and dogs with copper deficiency, while the impaired cross linkage of collagen in bone matrix most likely accounts for the increased bone fragility in copper-deficient animals when compared to animals with osteoporosis of other causes.

The bone lesions associated with primary and secondary copper deficiency are discussed earlier in this chapter in the section Mineral imbalances and will not be repeated here. It should be mentioned however that *the gross and microscopic bone lesions of copper deficiency may resemble the lesions of rickets and osteochondrosis*. In fact, there is increasing evidence, at least in some species, that copper deficiency is a cause of, or predisposing factor in, osteochondrosis.

Osteoporosis is often present in animals with severe **gastrointestinal parasitism**, most likely *secondary to malabsorption*. However, there is convincing evidence that subclinical parasitism may also cause osteoporosis, probably by a different mechanism. In one study, continuous dosing of lambs with *Trichostrongylus colubriformis* between 4 and 7 months of age produced a mineral osteoporosis characterized by a reduction in cortical and trabecular bone. This was accompanied by a reduction in the length and volume of bones, and in their degree of mineralization. Some animals showed widened osteoid seams on trabeculae and physeal changes suggestive of concurrent rickets. The lambs had low plasma phosphorus concentrations and normal plasma calcium, suggesting that the bone lesions were due to an induced phosphorus deficiency, although the possible effect of increased corticosteroid production on bone formation was also considered. In goats infused with a subclinical dose of *Trichostrongylus colubriformis* in another study, concurrent subclinical coccidial infection was shown to have an additive effect. Recently, trichostrongyle infections have been shown to lower the concentration of phosphorus carrier proteins in the intestinal mucosa, leading to phosphorus malabsorption in parasitized animals.

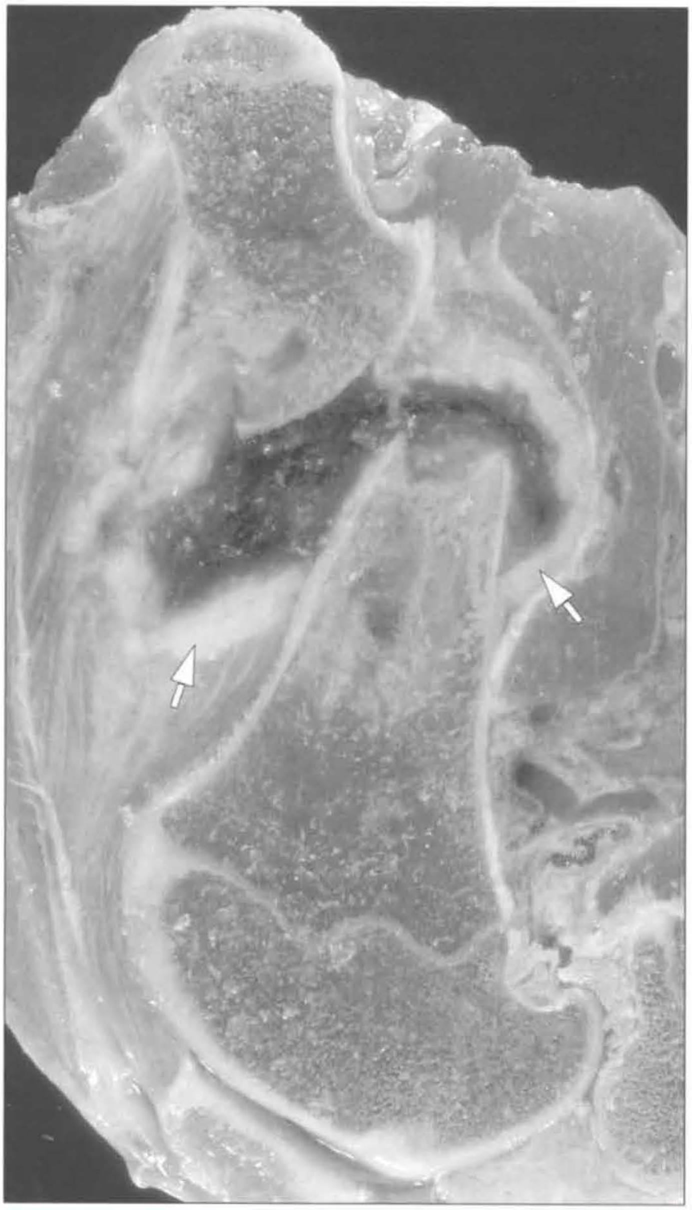

Figure 1.63 Spontaneous femoral fracture (approximately 1 week duration) in a heavily parasitized young sheep with **severe osteoporosis**. Note the thin cortices and porous cancellous bone. There is hemorrhage and early callus formation (arrows) between the malaligned bone ends.

Subclinical infection of lambs with *Ostertagia circumcincta* has also been shown to produce osteoporosis. This was a *matrix osteoporosis*, mediated apparently through restrictions in the availability of protein and energy for matrix deposition. Reduced bone volume was a consistent finding, but there was no effect on growth plates and the length of bones was normal. In calves infected experimentally with *Ostertagia* spp., the utilization of phosphorus is adversely affected.

The *importance of parasite-induced osteoporosis in grazing animals is unknown* but it is undoubtedly much more common than is recognized and may be responsible for some of the unexplained cases of spontaneous bone fractures encountered in cattle and sheep (Fig. 1.63).

Osteoporosis is a complication of **inflammatory bowel disease** in humans and has been described in dogs with **malabsorption syndrome**, but the mechanism is unknown. Reduced intake and/or absorption of vitamin D and calcium have been suggested as a possible mechanism for the osteoporosis in human patients with ulcerative colitis or Crohn's disease. So too has corticosteroid therapy, which is often used in such patients and is well known as a cause of osteoporosis, but recent human studies have failed to demonstrate a link between corticosteroid therapy and inflammatory bowel disease. The generation of proinflammatory cytokines such as interleukin-1 and interleukin-6, both of which induce bone resorption and inhibit its formation, may play an important role in the osteoporosis associated with inflammatory bowel diseases of humans, and may also be important in various parasitic and other enteric diseases of animals. Recent evidence that activated T lymphocytes may play a role in local or systemic bone loss through the production of RANK-L has provided an alternative hypothesis for the osteoporosis of inflammatory bowel disease.

Corticosteroid-induced osteoporosis is common in humans and its occurrence in animals may be underestimated. Moderate doses of corticosteroids inhibit both the synthesis of collagen by pre-existing osteoblasts and the differentiation of osteoblasts from their precursor cells, resulting in *decreased apposition rate*. In addition to their effect on bone formation, corticosteroids have been shown to *stimulate osteoclastic bone resorption*, resulting in a generalized loss of skeletal mass. Indirect effects of corticosteroids on the skeleton are mediated through reduced calcium absorption in the intestine and reduced calcium reabsorption in renal tubules, both of which would be expected to enhance bone resorption through secondary hyperparathyroidism. Interference with vitamin D metabolism has also been implicated in the development of corticosteroid-induced osteoporosis but the evidence from various studies is conflicting.

The clinical importance of osteoporosis in dogs and other animal species with *spontaneous and iatrogenic hyperadrenocorticism* is not clear as few studies have been reported, but hyperadrenocorticism is considered one of the most common causes of generalized osteopenia observed radiographically in dogs. In one study, a 25% reduction in mean trabecular bone volume was detected in Beagle dogs with clinical hyperadrenocorticism. In addition, a significant reduction in osteoblast number and the amount of osteoid lining trabecular surfaces was also demonstrated in Beagles with subclinical hyperadrenocorticism. Reduced bone formation in these dogs did not appear to be accompanied by increased osteoclastic activity.

Other causes of *generalized osteoporosis* in humans and domestic animals include **vitamin A toxicity**, **hyperthyroidism,** and chronic **metabolic acidosis**. The immunosuppressive agent **cyclosporin** and certain **anticonvulsant drugs** (e.g., phenobarbital, diphenylhydantoin, and carbamazepine) have also been associated with osteoporosis in human patients.

Disuse osteoporosis is a loss of bone mass due to muscular inactivity and reduced weight bearing. It may be localized, following paralysis or fracture of a limb, or more generalized in association with prolonged recumbency or inactivity. *Bones that normally carry the greatest loads suffer the greatest proportional loss in mass from disuse.* Measurable loss of mineral occurs in horses that have a limb cast for prolonged periods. The mechanism of disuse osteoporosis is not clear but it does occur in immobilized parathyroidectomized animals, indicating that it is *not PTH-dependent,* and is seen in human fetuses with neuromuscular disease.

In experimental long-term disuse osteoporosis of the forelimb in young adult Beagle dogs, the lesion develops in several stages. The first is marked by rapid loss of tissue, which reduces the bone mass by about 16% in 6 weeks. This results from imbalanced resorption on endosteal, periosteal, and intracortical surfaces, and may be due to nonspecific activation of numerous remodeling sites. This is followed by an increase in bone mass from 8–12 weeks after immobilization, presumably as a result of net bone formation at activated remodeling sites, and the bone mass approaches normal. In the period between 8–12 and 24–32 weeks following immobilization, there is slower bone loss, 80–90% of which is subperiosteal bone. The reduction in bone mass may reach 30–50% of normal and be maintained at this level. The distal bones of the limb lose more bone than the proximal, but in all cases the subperiosteal bone is diminished.

The effects of remobilization on disuse osteoporosis are not clearly defined. Recovery from relatively short periods of immobilization does occur but the age of the patient influences the response. The effect of weightlessness associated with prolonged space travel is well documented and the loss of bone strength remains for a prolonged period after returning to normal gravity.

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Rickets and osteomalacia

It is convenient to consider these two diseases together as they have *a similar etiology and pathogenesis, differing only in the age at which they occur.* **Rickets** is a disease of the developing skeleton in young animals and is accompanied *by abnormal endochondral ossification at growth plates, in addition to defective bone formation.* Its expression is greatly influenced by the level of physical activity undertaken; sedentary animals are less likely to suffer from fractures, infractions, and joint collapse. **Osteomalacia** occurs only in adults and although there are no lesions associated with growth cartilages, *the bone changes are the same as those that occur in rickets.* Both diseases occur in all domestic animal species and wildlife, but there are differences between species in the circumstances under which they occur, and in the most likely cause.

The pathogenesis of both rickets and osteomalacia involves **defective mineralization.** In young animals with rickets, this includes cartilage matrix at sites of endochondral ossification, as well as newly formed osteoid. In adults, where skeletal growth by endochondral ossification is no longer occurring, the defective mineralization affects only the osteoid formed during skeletal remodeling. The nature of the lesions varies, depending on the etiology, the age and growth rate of the animal, and the duration of the disease process. Furthermore, the dietary abnormality may involve a deficiency of more than one nutrient. As a result, the macroscopic and microscopic changes are not always classical, and the lesions may overlap with those of the other metabolic bone diseases. Accurate diagnosis on the basis of morphology alone may therefore be difficult.

Anything that interferes with the mineralization of cartilage or bone matrix may cause rickets or osteomalacia, but *most cases in animals result from dietary deficiencies of either vitamin D or phosphorus.* It is still uncertain whether the protective effect of 1,25-dihydroxyvitamin D against rickets is mediated by a direct effect on matrix mineralization or whether it is based on maintenance of adequate extracellular ionized calcium concentrations. The role of 1,25-dihydroxyvitamin D in matrix mineralization may involve its action in stimulating the synthesis of noncollagenous proteins such as osteocalcin, which is thought to be required for normal mineralization.

Vitamin D deficiency may occur in grazing animals where the combination of relatively high latitudes and temperate climates allows them to be pastured for much of the year. Such conditions occur in parts of the United Kingdom, South America, New Zealand, and southern Australia. Photobiosynthesis is a more important source of vitamin D than diet in grazing animals, but may be inadequate during the winter months in some regions. When the winter sun is at an angle of less than 30 degrees to the horizontal, the short-wavelength ultraviolet rays required for the activation of 7-dehydrocholesterol in the skin are reflected by the atmosphere and dermal synthesis of previtamin D_3 is impaired. Mature grass and sun-cured hay are relatively good alternative sources of vitamin

D_2, but the levels present in immature pasture are likely to be inadequate. This may be further compounded by the anti-vitamin D activity of carotenes present in lush pasture and green cereal crops. The extra demands of pregnancy and lactation during winter and early spring may also contribute significantly to the development of clinical osteomalacia. *It is likely that many grazing animals are vitamin D deficient for a period during the winter*, but clinical rickets or osteomalacia are only likely to develop if the deficiency is marked or persists for longer than usual. Problems may also occur if vitamin D deficiency is combined with deficiencies of other essential nutrients, such as phosphorus or copper.

Sheep appear to be more susceptible to vitamin D deficiency than **cattle**, possibly because a dense fleece covers much of their skin. Not surprisingly, the concentration of vitamin D in blood increases following shearing. **Alpacas** and **llamas** *may be even more susceptible to vitamin D deficiency than sheep*. Rickets has been diagnosed in young camelids in New Zealand, South Australia, and northern regions of the USA. The disease occurs during winter months and is accompanied by low blood concentrations of both 25-hydroxyvitamin D and phosphorus. In the New Zealand report, lambs grazing the same pasture had normal serum phosphorus concentrations and the phosphorus content of the pasture was normal. Vitamin D deficiency was considered the most likely etiology, the low serum phosphorus concentrations being secondary to reduced absorption of phosphorus from the intestine. The natural environment for alpacas and llamas is at high altitude near the equator, where solar irradiation is intense. Their dense fiber and pigmented skin may have evolved as a protective mechanism to prevent excessive solar irradiation reaching the skin. This could prove to be a disadvantage in animals moved to lower altitudes, especially at latitudes with limited solar irradiation during the winter.

Dietary deficiency of vitamin D also occurs in housed animals, particularly **piglets** and **calves** (at higher latitudes), due to lack of sunlight and errors in the formulation of rations. Piglets grow rapidly and are weaned early. If exposed to rachitogenic diets while the cartilages are still growing rapidly, the lesions tend to be florid. Vitamin D deficiency rickets occurs only rarely in **pups** and **kittens**, even though neither species seems capable of manufacturing vitamin D in their skin. The widespread availability of balanced commercial diets has reduced the likelihood of deficient rations being fed to dogs and cats, although inadequate rations are occasionally incriminated in the etiology of osteodystrophies in pups, as indicated in a report of rickets in a litter of Greyhounds. In the presence of adequate dietary levels of calcium and phosphorus, the vitamin D requirements of dogs appear to be very low. The vitamin D concentration of canine milk is very low, but deficiency in neonatal pups is unlikely, providing sufficient vitamin D has been stored in the liver of the pups during pregnancy.

Phosphorus deficiency *is well established as a cause of rickets and osteomalacia*, although the exact mechanism is uncertain. Presumably, the delayed mineralization in phosphorus-deficient animals reflects inadequate extracellular concentrations of the phosphate ions essential for the formation of hydroxyapatite. *Rickets and osteomalacia due to phosphorus deficiency are uncommon, but do occur in animals grazing pastures low in phosphorus*. There are many areas of the world, including South America, South Africa, northern Australia, and New Zealand, where soil phosphorus levels are very low and successful livestock production requires application of phosphorus either to the soil or the animals. Supplementation of individual animals is often not practical, but the problem can be prevented by regular applications of phosphorus-containing fertilizers. Reduced fertilizer use in an effort to cut production costs has led to an increased incidence of rickets, osteomalacia, and other diseases associated with phosphorus deficiency in some countries. **Cattle** appear to be more susceptible than **sheep** to phosphorus deficiency, while **horses** seem to be remarkably resistant. Rickets has also been diagnosed in farmed red **deer** grazing phosphorus deficient pastures in New Zealand. Phosphorus-deficiency rickets is not recognized in piglets but hypophosphatemia may be a complication of vitamin D deficiency.

Signs of phosphorus deficiency develop slowly, especially in the mature skeleton, and many animals with subclinical osteomalacia no doubt remain undiagnosed. Clinical disease is most likely to occur in cows where the deficiency is exacerbated by the extra demands of pregnancy or lactation. Such animals lose condition, develop transient, shifting lameness, and show an increased susceptibility to fractures. They may crave phosphorus-rich materials, and *osteophagia and pica are characteristic signs of the deficiency*. Fertility can be severely reduced and estrum may be irregular, inapparent, or absent. Newborn calves are of normal size and development (at the expense of their dams) but while suckling, their growth is subnormal, and this is accentuated after weaning. Growth is also aberrant in that maturity is delayed and deficient animals appear long and lean, and have a rough hair coat. Hypophosphatemia develops early but also returns to normal rapidly if the animals are supplemented. Serum calcium concentrations are usually normal or increased. The presence of normal serum phosphorus concentration in an animal with osteodystrophy does not therefore exclude phosphorus deficiency as the cause. The poorly mineralized bone is removed only slowly and will persist until well after the underlying dietary deficiency has been corrected.

Dietary phosphorus deficiency is virtually impossible in carnivores because of the high levels of phosphorus normally present in their rations. In fact, excess dietary phosphorus resulting in nutritional secondary hyperparathyroidism and fibrous osteodystrophy is relatively common in pups and kittens.

Calcium is also required for mineralization but there is doubt that uncomplicated calcium deficiency is capable of inducing rickets. Since any decrease in extracellular concentrations of ionized calcium is rapidly corrected by the actions of PTH and 1,25-dihydroxyvitamin D on bone and small intestine, *calcium is unlikely to be a limiting factor at sites of mineralization. Persistent dietary deficiency or unavailability of calcium is more likely to lead to fibrous osteodystrophy or osteoporosis* (depending on age and species) due to hyperparathyroidism and excessive bone resorption. Hypocalcemia does not occur until the advanced stages of calcium deficiency, by which time skeletal growth will be retarded and any effect on growth plates reduced. It is important to recognize however, that vitamin D deficiency will be accompanied by hypocalcemia due to impaired resorption of calcium from bone and absorption from the small intestine. The skeletal lesions of vitamin D deficiency in young animals would therefore be expected to include changes characteristic of both rickets and fibrous osteodystrophy. In contrast, lesions attributable to hyperparathyroidism should not complicate phosphorus-deficiency rickets or osteomalacia. More likely, chronic phosphorus deficiency will be accompanied by anorexia, and the skeletal changes may therefore reflect a combination of osteomalacia and osteoporosis.

Interestingly, calcium deficiency has been shown to induce a deficiency of vitamin D in rats. The mechanism is not certain, but evidence suggests that increased secretion of PTH in calcium deficiency stimulates increased production of 1,25-dihydroxyvitamin D, which acts directly or indirectly to enhance inactivation of 25-hydroxyvitamin D in the liver. This may explain the concurrent development of vitamin D deficiency in certain human disorders characterized by hyperparathyroidism, including gastrointestinal disease, chronic liver disease, and anticonvulsant therapy.

Several other, less common, causes of rickets and osteomalacia are recognized in man and animals. These include two types of **vitamin D-dependent rickets** (types I and II) caused by inborn errors in vitamin D metabolism. **Type I** is caused by a defect in the renal enzyme 1α-hydroxylase, which converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D in proximal tubules. The disease is inherited as an *autosomal recessive trait* in people and pigs, and sporadic cases have been suspected in dogs and cats. The disease is characterized by high levels of serum parathyroid hormone and 25-hydroxyvitamin D but low or undetectable levels of 1,25-dihydroxyvitamin D. Affected pigs are clinically normal until 4–6 weeks of age, but then plasma calcium and phosphate levels fall and alkaline phosphatase activity rises. Florid rickets develops over the next 3–4 weeks and the pigs die unless treated. **Type II** vitamin D-dependent rickets, which is also more appropriately called **hereditary vitamin D-resistant rickets**, is due to a defect in 1,25-dihydroxyvitamin D receptor-effector systems in the cells of target organs. The disease occurs in people, and possibly in the common marmoset, *Callithrix jacchus*. These animals, like other New World monkeys, cannot utilize vitamin D_2 but, in addition, require unusually large amounts of vitamin D_3 and have very high levels of plasma 1,25-dihydroxyvitamin D without hypercalcemia. Despite the elevated 1,25-dihydroxyvitamin D, some marmosets develop a bone disease with features of rickets.

Hypophosphatemic vitamin D-resistant rickets (renal hypophosphatemic rickets) is characterized by hypophosphatemia, normocalcemia, decreased renal tubular reabsorption of phosphate, and skeletal deformities. Plasma 1,25-dihydroxyvitamin D levels are inappropriately low. Hypophosphatemia results from impaired renal reabsorption and intestinal absorption of phosphorus. Combined treatment with 1,25-dihydroxyvitamin D to depress parathyroid hormone and thus phosphaturia, and phosphate has beneficial effects on bone lesions. The disease is inherited as an X-linked dominant trait in children and in mice. Hypophosphatemic vitamin D-resistant rickets and osteomalacia have also been associated with various neoplasms in human patients. The mechanism is unknown but is thought to involve production by the tumor of a humoral substance that interferes with phosphate absorption in renal proximal tubules. This syndrome has yet to be reported in animals but could easily be missed both clinically and at necropsy.

Rickets and osteomalacia are recognized in human patients with **gastrointestinal malabsorption** resulting from chronic disorders of the small bowel, hepatobiliary system, or pancreas. Since vitamin D is fat soluble, any interference with the digestion or absorption of fat would be expected to reduce dietary vitamin D absorption and predispose to deficiency. Reduced absorption of calcium in malabsorption syndromes may enhance the efficiency of vitamin D, as outlined above. Although the same would be expected to occur in animals, there are no reports. This may reflect less complete radiographic and pathologic evaluation of the skeleton in animals with such diseases or the fact that affected animals do not live long enough for the skeletal lesions to become manifest clinically.

Rickets has been reproduced experimentally in rats by the addition of **iron** to a normal, nonrachitogenic diet. *Iron interferes with the absorption of phosphorus* and high dietary levels may induce phosphorus deficiency. High dietary levels of other phosphorus antagonists, such as **calcium** and **aluminum**, might also be expected to induce phosphorus-deficiency rickets. Lesions resembling osteomalacia have been observed in cattle ingesting high concentrations of **fluoride**, which *interferes with mineralization* when incorporated into bone matrix.

Animals with *rickets* are generally stiff or lame, and in severe cases are reluctant to stand. The limbs, especially the forelimbs, may be bowed. Swelling of the carpals and other joints, due to enlarged ends of long bones, may lead to an initial suspicion of arthritis. Varus or valgus deformity may be present, reflecting asymmetric involvement of growth plates. Affected pigs may stand with a hunched back, walk on the tips of their toes, or adopt a dog-sitting position. Knuckling of the carpus is also described in pigs with rickets. In many animals however, the clinical signs are mild and nonspecific.

Macroscopic lesions of rickets are most prominent at sites of rapid growth, including metaphyseal and epiphyseal regions of long bones and costochondral junctions of the large middle ribs. Enlargement of costochondral junctions is a classic feature of the human disease and is referred to as the "*rachitic rosary*." Costochondral junctions are normally prominent in fast-growing young animals and any enlargement must be interpreted with caution. In rickets, the metaphysis of the rib is often wider than the cartilaginous portion and on sagittal section, the chondro–osseous junction is irregular, with tongues of unresorbed cartilage extending into the metaphysis (Fig. 1.64A). The normal architecture of the metaphysis is replaced by a mixture of disorganized trabeculae, irregular tongues and islands of cartilage, fibrous tissue, and sometimes hemorrhage (Fig. 1.64B). Loss of the normal trabecular arrangement in the metaphysis is best appreciated radiographically, at least in severe cases (Fig. 1.65). Similar changes may be evident in sagittal sections of long bones (Figs 1.66A, B; 1.67A, B), especially at sites of most rapid growth, such as distal radius, proximal humerus, distal femur, and both ends of the tibia. Metacarpal and metatarsal physes are also likely to show gross changes (Fig. 1.68). The severity of lesions between different physes may vary considerably, even within the same animal, and although the disease is systemic, the physeal lesions are often multifocal rather than diffuse. This emphasizes the importance of examining sagittal sections of several bones during postmortem examination. The enlargement of the ends of long bones is due partly to flaring of the metaphysis and partly to compression caused by weight bearing on metaphyseal bone of reduced strength. Also, there is accumulation of poorly mineralized osteoid, which is not removed efficiently by osteoclasts.

Focal thickening of physeal cartilage, similar to that observed in rickets is also described in osteochondrosis in various species, and in the inherited dwarfism of Alaskan Malamute dogs. In rickets however, the physeal lesions are generally accompanied by evidence of bone fragility, including trabecular disruption, hemorrhage, and infractions. Pathological fractures may be present in the limbs, ribs, or vertebrae in severe cases. When the deficiency is relatively recent but severe, a distinct zone where abnormal bone formation commenced may be present in the metaphysis (Fig. 1.64A). This reflects a change from adequate to inadequate dietary phosphorus or vitamin D and

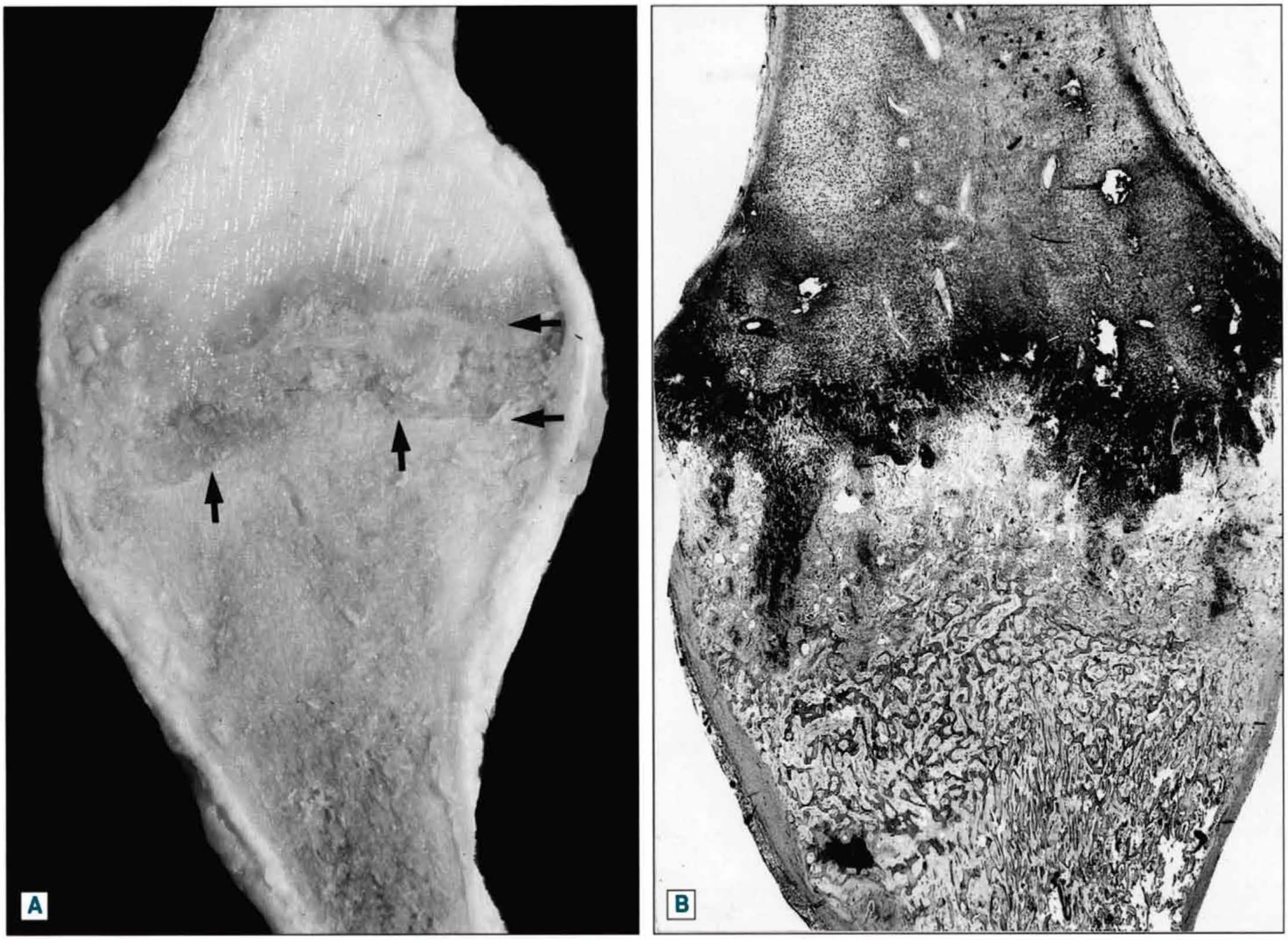

Figure 1.64 Rickets, bovine. **A.** Phosphorus deficiency rickets in a 1-year-old steer. Swelling of metaphysis at the costochondral junction and disruption of normal architecture in the zone between the horizontal arrows. Broad tongues of cartilage extend into the metaphysis from the physeal cartilage. The stage at which defective bone formation commenced is indicated by the vertical arrows. **B.** Subgross preparation of costochondral junction from a calf with rickets showing irregular tongues of cartilage remaining in the metaphysis and disorganization of metaphyseal bone.

may be of value in relating the disease to a particular change in diet or environment.

Endochondral ossification occurring beneath articular cartilage is also abnormal in animals with rickets, but since the rate of epiphyseal bone formation is slower than that of the metaphysis, the lesions are milder. The articular cartilage may be irregularly thickened and in severe cases, there may be infolding and irregularity of the articular surface due to collapse of weakened subchondral bone. These articular lesions of rickets occur most often in pigs, but also occur in association with fibrous osteodystrophy, and tend to involve large, weight-bearing joints such as the shoulder and hip. Similar macroscopic lesions occur in the articular cartilage of pigs with osteochondrosis, but since this disease is not associated with defective bone formation, the subchondral bone does not collapse.

In mild rickets, the lesions may not be apparent grossly or radiographically. Furthermore, reduced growth rate in animals with phosphorus-deficiency rickets may reduce the severity of lesions. In such cases, histology is essential for confirmation.

The microscopic lesions of rickets reflect the failure of mineralization of cartilage at sites of endochondral ossification, and of osteoid deposited during bone growth and remodeling. Mineralization of cartilage matrix is a critical event in the process of endochondral ossification at physes and appears to be functionally coupled with vascular invasion from the metaphyseal side. As the physeal cartilage becomes mineralized, the hypertrophic chondrocytes degenerate, the narrow septa between adjacent cells are broken down and capillary sprouts derived from metaphyseal vessels invade their vacant lacunar spaces. These invading capillaries are accompanied by osteoprogenitor cells, which differentiate into osteoblasts and deposit osteoid on the spicules of mineralized cartilage, which previously separated the columns of chondrocytes. Experiments in vitamin D-deficient rachitic rats demonstrated that metaphyseal angiogenesis and invasion of physeal cartilage is halted or impaired. Within 96 hours of vitamin D repletion, the metaphyseal vasculature returns to normal and capillary sprouts recommence invasion of the physeal cartilage.

Persistence of hypertrophic chondrocytes at sites of endochondral ossification, both at physes and beneath articular cartilage, is the hallmark of rickets in histological preparations. The lesions are often irregular and are usually accompanied by disruption of the underlying trabecular bone. As the cartilage continues to accumulate, its growth rate slows, possibly

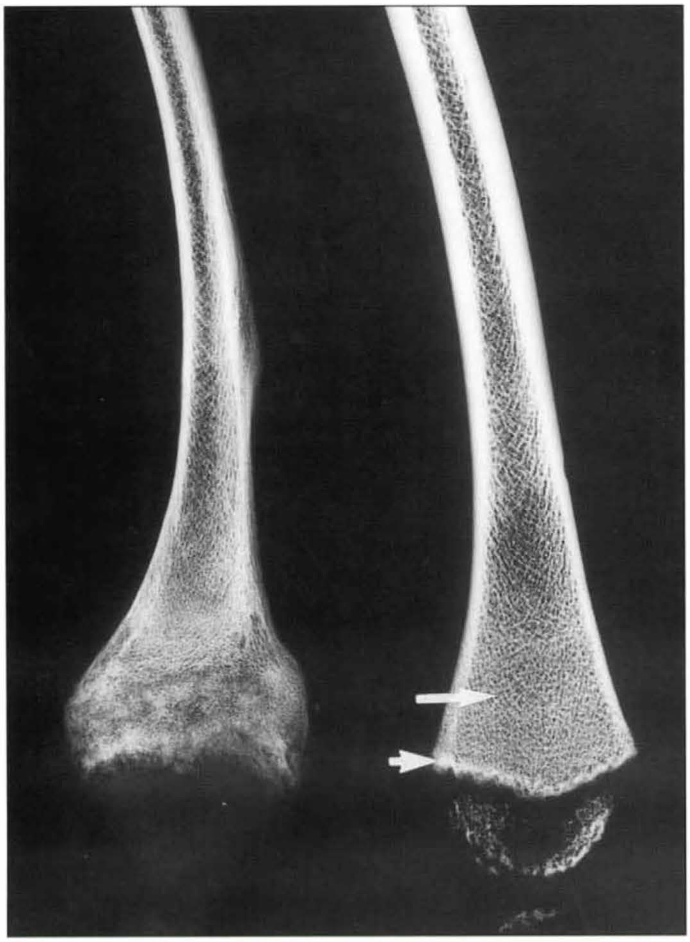

Figure 1.65 Rickets, calf. Normal rib on right. There is metaphyseal swelling and disorganization of trabecular bone in the rachitic bone. Note the regular trabecular pattern in the primary (short arrow) and secondary spongiosa (long arrow) in the normal rib.

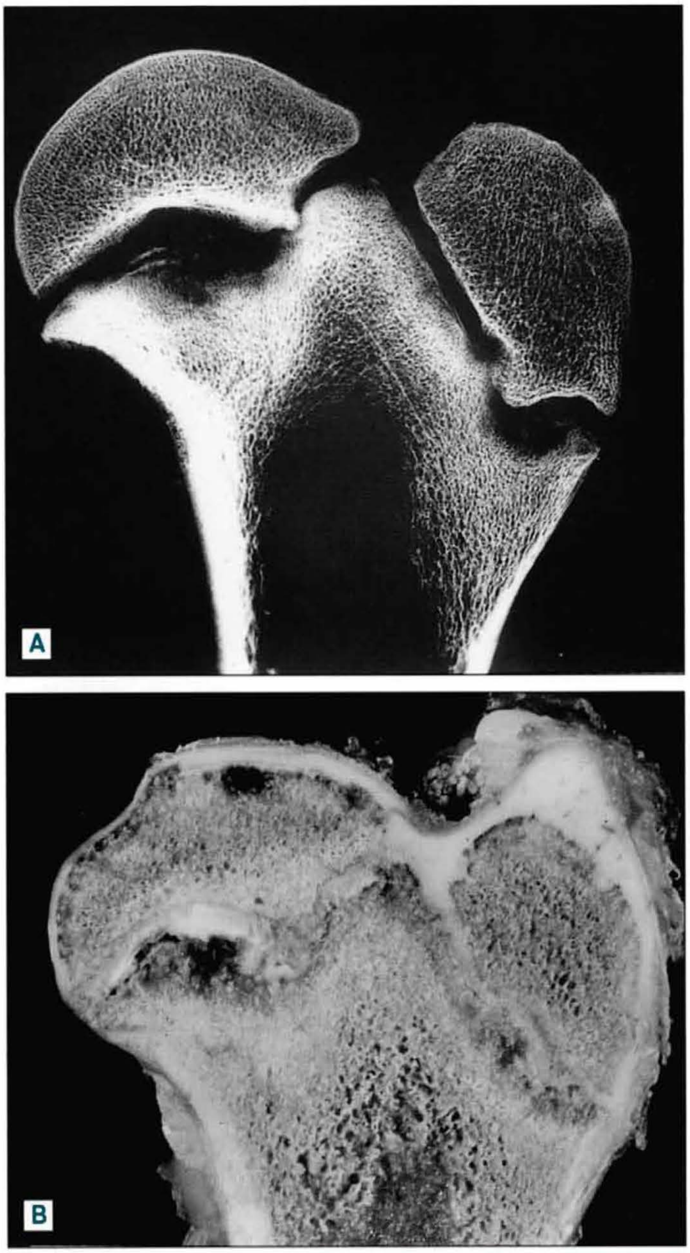

Figure 1.66 Rickets, pig. **A.** Slab radiograph of proximal femur showing increased depth of physeal cartilage. **B.** Proximal femur showing irregular thickening of the physes in addition to hemorrhage and disruption of trabeculae in metaphyses. Also note the collapse of the articular cartilage of the femoral head and abnormalities in the subchondral bone. (Courtesy of PA Taylor.)

because of inadequate diffusible nutrient, and the hypertrophic cells form irregular clumps instead of the characteristic columns. Some of these clumps are bypassed by the irregular ingrowth of vascular and osteogenic tissue and ultimately come to lie in the metaphysis as unmineralized clusters of hypertrophic chondrocytes surrounded by osteoid (Fig. 1.69A, B). Occasionally, the persistent tongues of cartilage degenerate and are replaced by connective tissue, in which metaplastic osteoid forms. Degenerate cartilage, which loses its basophilia and stains like osteoid, is sometimes referred to as pseudo- or cartilaginous osteoid. If the dietary deficiency is corrected, plates of rachitic cartilage may become undermined by invading vessels and stranded in the metaphysis, forming a grossly visible, translucent band parallel to the growth plate.

Metaphyseal trabeculae are thicker than normal, irregular in shape and may be partly covered by seams of unmineralized osteoid (Fig. 1.70A). Trabecular microfractures are common. Infractions (incomplete fractures) in various stages of repair are also relatively common and reflect the fragility of the trabeculae (Fig. 1.70B). *In vitamin D deficiency, the histologic lesions may have features of fibrous osteodystrophy in addition to rickets*, because of the hypocalcemia and secondary hyperparathyroidism. In such cases, osteoblastic and osteoclastic activity are prominent and trabeculae are separated by loose fibrous connective tissue, even in areas distant from repairing infractions. A combination of rickets and fibrous osteodystrophy can, at least in theory, also occur in association with chronic renal disease (see Fibrous osteodystrophy section below) due to renal secondary hyperparathyroidism and impaired renal synthesis of 1,25-dihydroxyvitamin D. However, in animals with renal disease the lesions of fibrous osteodystrophy generally predominate.

Cortical bone is also affected in rickets, but the lesions are generally overshadowed by those involving growth plates. Grossly, the diaphyses of long bones may appear shorter and thicker than normal with a narrow medullary cavity. In spite of their increased thickness, the cortices are more susceptible to weight-bearing trauma and, in severe cases may be soft, bent, or contain pathological fractures.

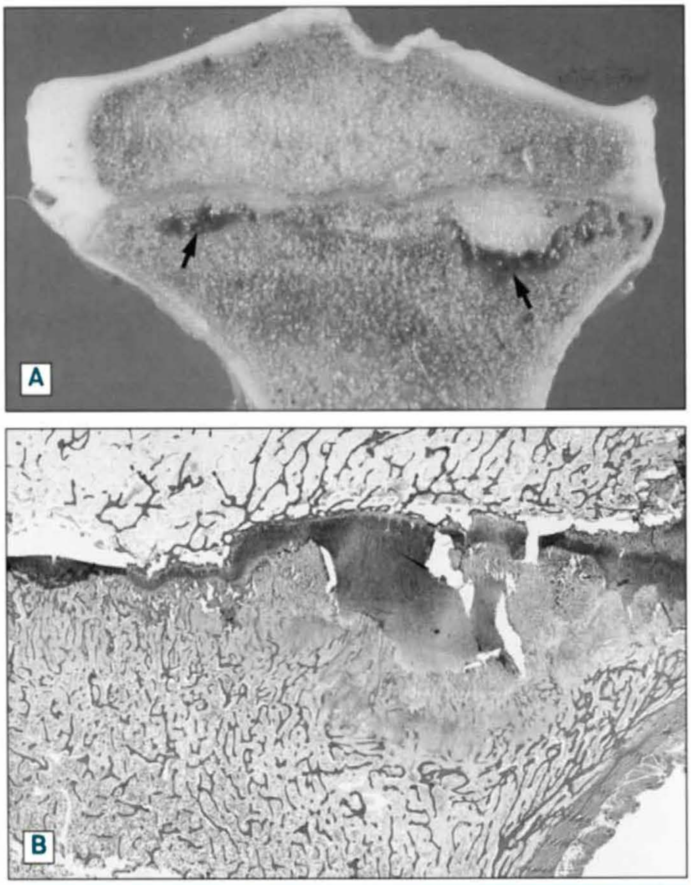

Figure 1.67 Rickets, pig. **A.** Massive, focal thickening of the physis in the proximal tibia. The dark areas, which appear gelatinous grossly (arrows), indicate trabecular disruption and replacement with highly vascular fibrous connective tissue. **B.** Subgross photograph of the same specimen showing the thickened physeal cartilage and replacement of trabecular bone in the underlying metaphysis with repair tissue.

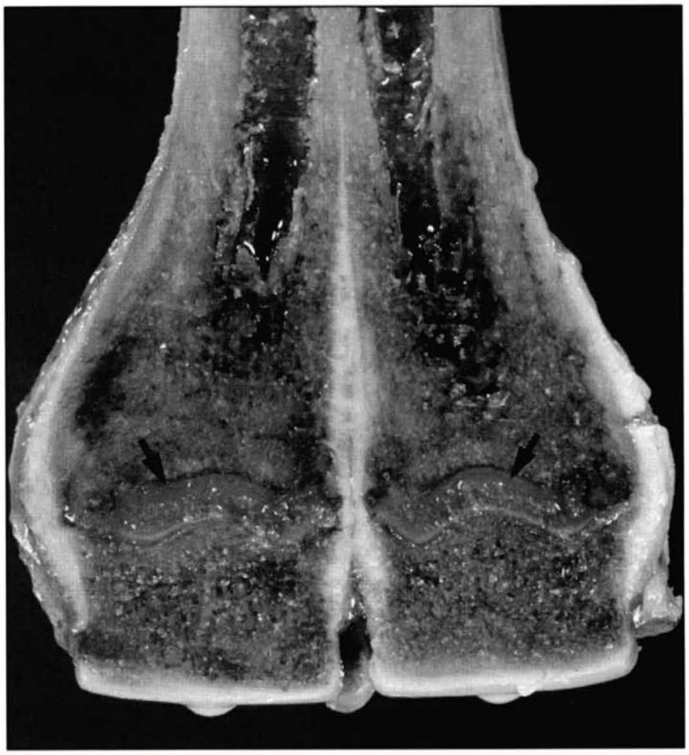

Figure 1.68 Rickets, alpaca. The physes of the distal metacarpal bone (arrows) are diffusely thickened and bulging from the cut surface.

Histologically, a layer of poorly mineralized osteoid may accumulate within partly filled Haversian systems and on the periosteal surface.

Osteoid seams are best illustrated in undemineralized sections, but can be appreciated in demineralized sections stained with hematoxylin and eosin as pale, eosinophilic matrix against the darker staining bone tissue that was previously mineralized (Fig. 1.70A). Special stains for demonstrating unmineralized osteoid in demineralized sections (see page 18) can be of value but are not used routinely and sections may be difficult to interpret in the absence of controls. *It is important to recognize that osteoid seams are not pathognomonic for rickets and osteomalacia.* Nor are they invariably present. They also may occur in other syndromes at sites of rapid bone formation rather than due to delayed mineralization.

The **lesions of osteomalacia** *are similar to those of rickets but since they occur in adult animals, growth plates are not involved.* Osteomalacic bones have reduced resistance to pressure and tension, and increased susceptibility to the stress and strain of ordinary activity. As a result, there is *excessive deposition of matrix where mechanical stimuli are strongest*, such as at insertions of tendons and fascia, places of angulation and curvature, and on stress-oriented epiphyseal trabeculae. When the disease is advanced, the *bones fracture readily*, the marrow cavity is expanded and may extend into the epiphysis, and the cortex is thin, spongy and soft. In affected cattle, fractures are most common in the ribs, pelvis, and long bones, while in pigs they occur most often in the vertebral column. *Deformities* are often present, including kyphosis or lordosis, medial displacement of the acetabula with compression of pubic bones, twisting of the ilia, and narrowing of the pelvis. The ribs and transverse processes of the lumbar vertebrae droop so that the thorax is narrowed and flattened and the sternum prominent. Collapse of articular surfaces and degeneration of cartilages is sometimes observed and tendons may separate from their attachments. In long-standing cases of osteomalacia, cachexia and anemia are often present.

The *histological changes of osteomalacia reflect the inadequate mineralization of osteoid formed during remodeling*, the lesions resembling those in the cortex of animals with rickets. In many cases, particularly those caused by phosphorus deficiency, osteoporosis associated with concurrent deficiency of protein and other nutrients is superimposed. The lesions develop slowly but in advanced cases, trabeculae are reduced in size and number, and the cortices are thin and porous. Osteoid covers the trabeculae and lines the expanded Haversian canals. Localized accumulations of osteoid occur at sites of mechanical stress.

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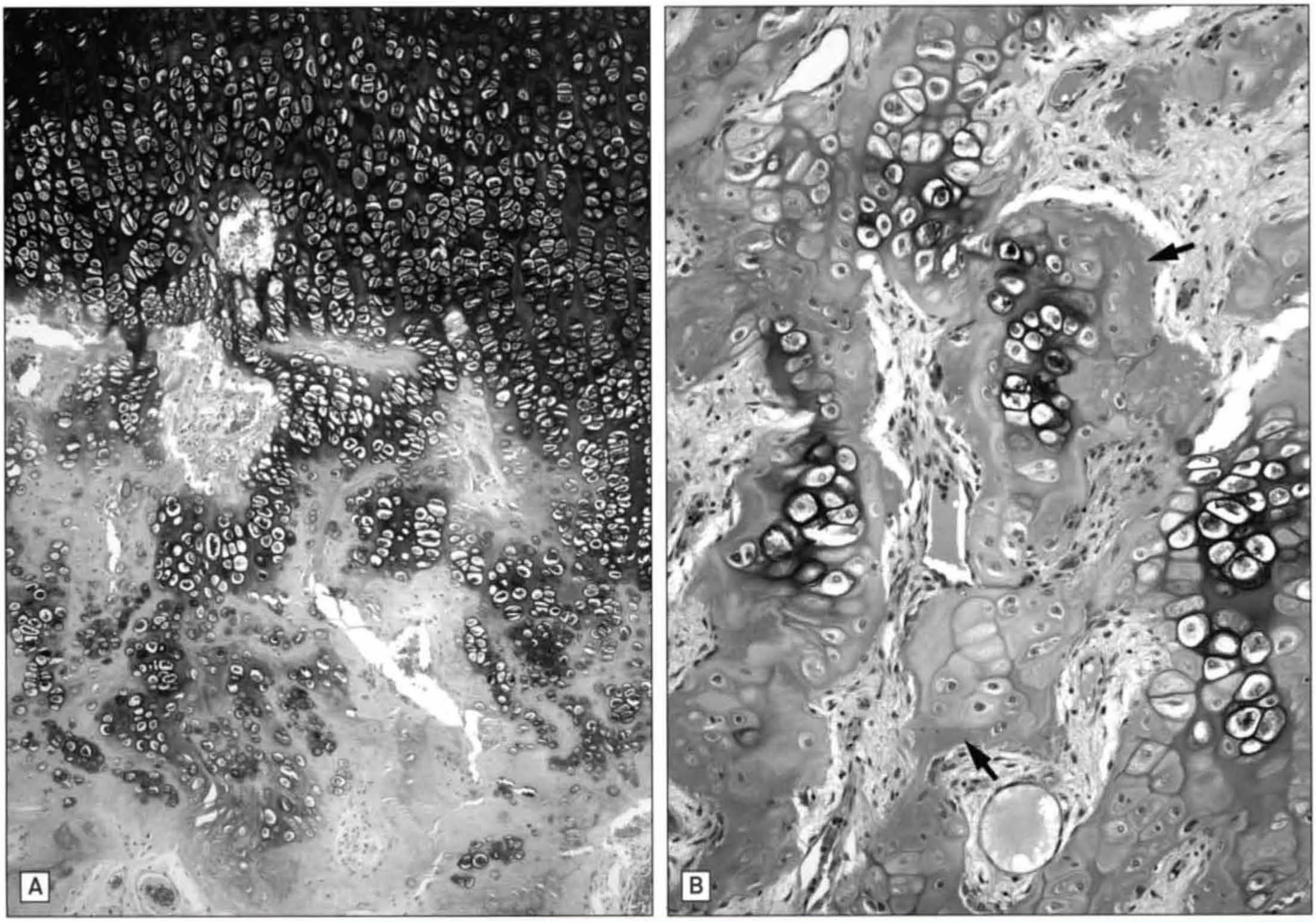

Figure 1.69 Rickets, pig. **A.** Thickening and disorganization of the hypertrophic zone in the physis. Metaphysis consists of persistent tongues and islands of hypertrophic chondrocytes surrounded by a thick layer of osteoid. **B.** High-power view of metaphysis showing clusters of hypertrophic chondrocytes, some of which are degenerate, surrounded in some areas by seams of unmineralized osteoid (arrows).

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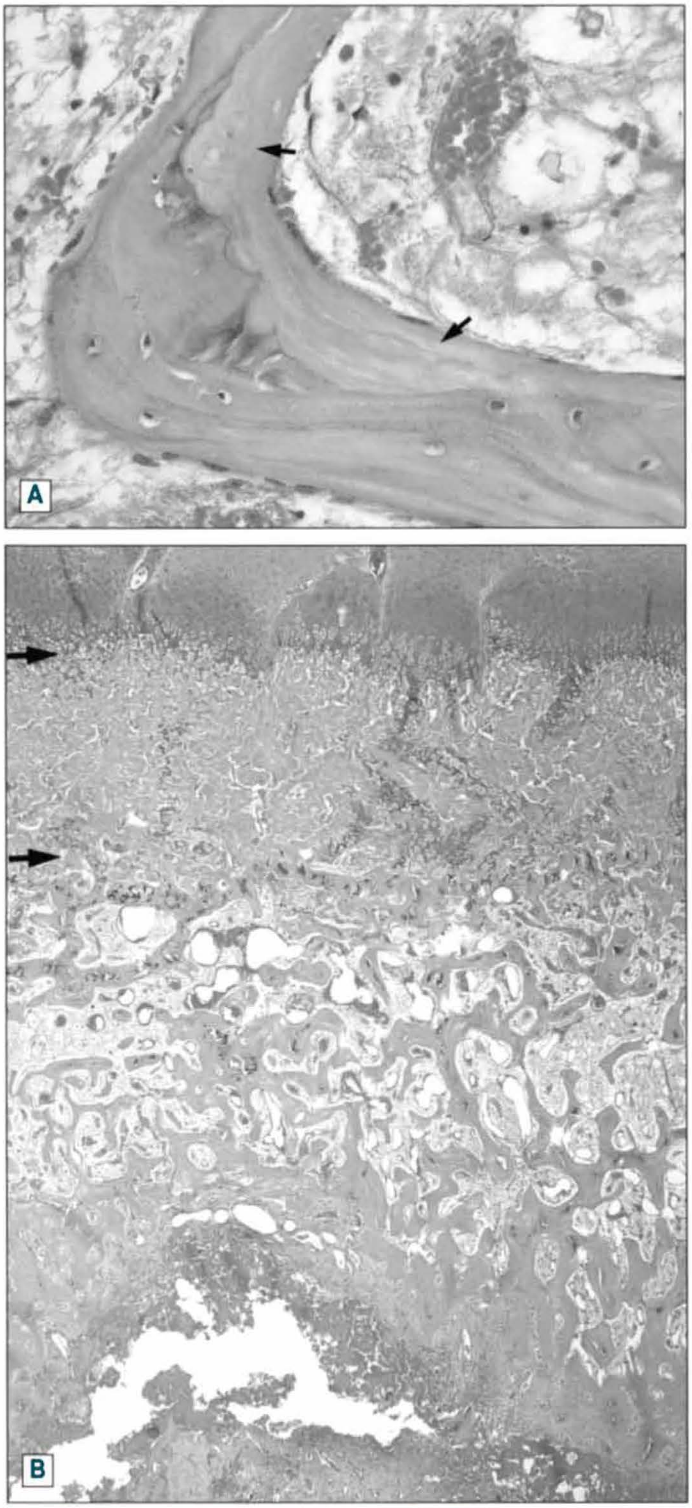

Figure 1.70 Rickets, due to phosphorus deficiency, in a farmed red deer. **A.** Pale-staining osteoid seam (arrows) on the surface of a metaphyseal trabecula. Note the scalloped reversal line separating the osteoid seam from the underlying mineralized bone, indicating previous osteoclastic resorption at the site. **B.** Low-power view of costochondral junction from the same deer showing an irregular line of recent trabecular fractures deep in the metaphysis. Note the disruption of trabecular architecture and persistent islands of cartilage in the primary and secondary spongiosa (between arrows).

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Fibrous osteodystrophy

Fibrous osteodystrophy (osteodystrophia fibrosa, osteitis fibrosa cystica) is a relatively common metabolic bone disease characterized by extensive bone resorption accompanied by proliferation of fibrous tissue and poorly mineralized, immature bone. The pathogenesis involves persistent elevation of plasma PTH and the lesion can be considered to represent the skeletal manifestation of primary or secondary hyperparathyroidism. Different animal species vary in their susceptibility to fibrous osteodystrophy and, to some degree, in the distribution of lesions. Horses, pigs, dogs, cats, ferrets, and goats are often affected, as are reptiles and New World nonhuman primates, but the disease is rare in sheep and cattle.

Primary hyperparathyroidism *is usually the result of a functional parathyroid gland adenoma* (see Vol. 3, Endocrine glands). This occurs rarely in adult dogs and there are isolated reports in horses and cattle. Hereditary primary hyperparathyroidism associated with diffuse parathyroid hyperplasia was also reported in a litter of German Shepherd puppies with fibrous osteodystrophy. *In primary hyperparathyroidism, autonomous secretion of PTH results in persistent hypercalcemia and hypophosphatemia*, the latter reflecting increased urinary clearance of phosphate. This differs from secondary hyperparathyroidism where plasma total calcium concentrations are usually either normal or slightly decreased and, depending on the cause, plasma phosphorus concentrations are either normal or increased. The persistent hypercalcemia in primary hyperparathyroidism is generally accompanied by *polydipsia/polyuria, muscular weakness, and widespread mineralization of soft tissues*. Affected animals may succumb to the effects of *nephrocalcinosis* before the skeletal changes are severe enough to become clinically apparent. The skeletal changes in the German Shepherd pups with primary hyperparathyroidism (discussed below) were more severe than those described in adult dogs with functional parathyroid adenomas. This probably reflects the more rapid turnover of bone in young animals. Generalized bone resorption also occurs in **pseudohyperparathyroidism** or **hypercalcemia of malignancy** caused by the production of parathyroid-related hormone by tumors such as malignant lymphoma and apocrine gland adenocarcinoma of the anal sac. However, although increased osteoclastic activity and reduced trabecular bone volume have been demonstrated by histomorphometric techniques, the bone lesions are usually mild and are not apparent clinically or even radiographically.

Secondary hyperparathyroidism is a much more common cause of fibrous osteodystrophy in animals than primary hyperparathyroidism and *may be due to either chronic renal disease or a dietary imbalance of calcium and phosphorus*. PTH secretion is stimulated by a reduction in plasma-ionized calcium, whatever the cause, and if the stimulus persists then generalized bone resorption results. **Renal secondary hyperparathyroidism** occurs most often in dogs, and occasionally in cats, as a complication of chronic renal failure. Impaired glomerular filtration in animals with renal failure leads to progressive hyperphosphatemia due to reduced renal clearance of phosphate. Hypocalcemia develops as a result of the inverse relationship between plasma-ionized phosphate and calcium concentrations, and stimulates the release of PTH. In addition, chronic renal disease may result in impaired formation of 1,25-dihydroxyvitamin D, thereby reducing calcium absorption from the intestine and contributing further to the hypocalcemia. This combination of secondary hyperparathyroidism and deficient synthesis of 1,25-dihydroxyvitamin D in animals and humans with renal failure may give rise to a bone disease with features of both fibrous osteodystrophy and osteomalacia. For this reason, the disorder is often referred to by the more general term, **renal osteodystrophy**.

Nutritional secondary hyperparathyroidism *may be due to a simple dietary deficiency of calcium, excess dietary phosphorus, or to a deficiency of vitamin D.* As discussed previously in this chapter, vitamin D deficiency alone is also a cause of rickets or osteomalacia, but reduced calcium absorption from the intestine in animals with vitamin D deficiency often results in concurrent fibrous osteodystrophy. Excess dietary phosphorus may cause fibrous osteodystrophy even in animals receiving adequate dietary calcium. Increased plasma phosphate concentration, resulting from increased intestinal absorption of phosphorus, depresses plasma-ionized calcium and indirectly stimulates the release of PTH.

In practice, *nutritional secondary hyperparathyroidism is most often caused by diets containing low calcium and a relatively high concentration of phosphorus* and, with the exception of horses, affects young, rapidly growing animals. Horses seem to be remarkably sensitive to the effects of high-phosphorus diets and relatively resistant to the effects of rations low in phosphorus. In all species, there are several factors that influence the development and the severity of lesions in secondary hyperparathyroidism. These include the degree to which dietary calcium is deficient and, perhaps more importantly, the degree to which dietary phosphorus is in excess. Although the efficiency of calcium absorption decreases markedly at high intakes, that of phosphorus seems to be unchanged. Furthermore, over a wide range of intakes, plasma calcium concentration is more sensitive to dietary phosphorus than to dietary calcium.

Differences between species in the susceptibility, likely etiology, and distribution of lesions in fibrous osteodystrophy are discussed below in some detail.

In the **horse**, the disease occurs at any time after weaning but the prevalence, and possibly the susceptibility, declines after about the seventh year. Horses require a calcium:phosphorus ratio of approximately 1:1. Diets in which the calcium:phosphorus ratio is 1:3 or wider, can result in osteodystrophia fibrosa depending to some extent on individual and familial susceptibility, and on alternative sources of calcium, such as drinking water. The condition usually occurs after maintenance for some months on diets consisting largely of grain, corn, and grain by-products such as bran, hence the term "bran-disease." Fibrous osteodystrophy also occurs in horses grazing tropical grasses high in oxalate, even though dietary calcium and phosphorus are normal. Prevalences of 10–15% occur in Sri Lanka and the Philippines. The problem is also reported in northern Australia and southern states of the USA. Lush pastures are most hazardous, some containing as much as 7.8% oxalate on a dry weight basis. Those with total oxalate over 0.5% and calcium:oxalate ratios below 0.5 are potentially dangerous because oxalate binds the calcium and makes it unavailable for absorption. Several grasses, including *Setaria sphacelata*, *Cenchrus ciliaris* (buffel grass), *Brachiaria mutica* (para grass), *Digitaria decumbens* (pangola grass), and *Panicum* spp., contain sufficient oxalate to produce clinical disease. Renal secondary hyperparathyroidism is not reported in horses as renal failure in this species typically results in hypercalcemia rather than hypocalcemia.

The *clinical signs and gross lesions* of fibrous osteodystrophy generally develop more rapidly in young, growing horses due to their increased rate of bone formation and remodeling. Early signs consist of minor changes in gait, stiffness, transient and shifting lameness, and lassitude. Loss of appetite with progressive cachexia and anemia develop later. The anemia may be due to depression of erythropoiesis by parathyroid hormone or its metabolites. *The most characteristic feature is bilateral swelling of the bones of the skull* (Fig. 1.71A), including both the maxillae and mandibles, hence the term "*bighead*." The bony swelling begins along the alveolar margins of the mandibles, producing cylindrical thickenings and reducing the intermandibular space. The molar margins of the maxillae then begin to swell and the enlargement spreads to involve the palate, the remainder of the maxillae, and the lacrimal and zygomatic bones. In severe cases (Fig. 1.71B), the nasal and frontal bones are also swollen. Initially, the swellings are soft but later they harden. Involvement of the palate reduces the nasal passages and may cause dyspnea. Palatine and mandibular thickening causes reduction of the buccal cavity and mastication is impaired. The teeth loosen and are partially buried or may exfoliate, and the softened bone yields to pressure, further impairing prehension and mastication. The food may be swallowed unchewed or allowed to drop out of the mouth. The face may be continually wet from occlusion of the lacrimal canals. In advanced cases, the enlargement of the head may be extreme and although the swelling is invariably bilateral, it is not always symmetrical.

Gross lesions in the remainder of the skeleton indicate advanced disease. In such cases, the scapulae may be thickened and curved so that the shoulder joint is displaced forward and the trunk droops in the pectoral girdle, giving undue prominence to the sternum. The vertebral column curves downwards, sometimes upwards, and the arch of the ribs is reduced. There is an increased susceptibility to *fractures* and to avulsion of ligaments. *Detachment of ligaments and tendons* occurs chiefly in the lower limbs. Some animals escape the fractures and ligamentous detachments and pass into *cachectic recumbency* but multiple fractures may occur if such animals are forced to rise. Radiography of affected bones may show generalized osteopenia, but not until the relatively advanced stages of the disease. Macerated bones from severely affected animals are finely cancellous or pumice-like and may be brittle and crumbly. The weight of the macerated bones may be less than 50% of normal.

Severe cases of fibrous osteodystrophy in horses are now rare and animals with facial swelling due to nutritional secondary hyperparathyroidism are seldom seen. Milder forms of the disease, however, may still be a cause of poorly defined lameness and could perhaps predispose to spontaneous fractures of sesamoid bones or phalanges in racehorses in training, which are fed rations high in grain.

Serum concentrations of calcium and phosphate are of limited value in the diagnosis of nutritional secondary hyperparathyroidism in horses, although a slight increase in serum phosphate is reported as a common finding in affected horses. Serum concentrations of PTH are likely to be of more diagnostic value and the test has been validated for use in horses. Increased urinary fractional clearance of phosphorus is also considered a sensitive indicator of secondary hyperparathyroidism. In one survey, abnormally high phosphorus clearance ratios were detected in 87.5% of racehorses in Hong Kong, suggesting that subclinical hyperparathyroidism is relatively common.

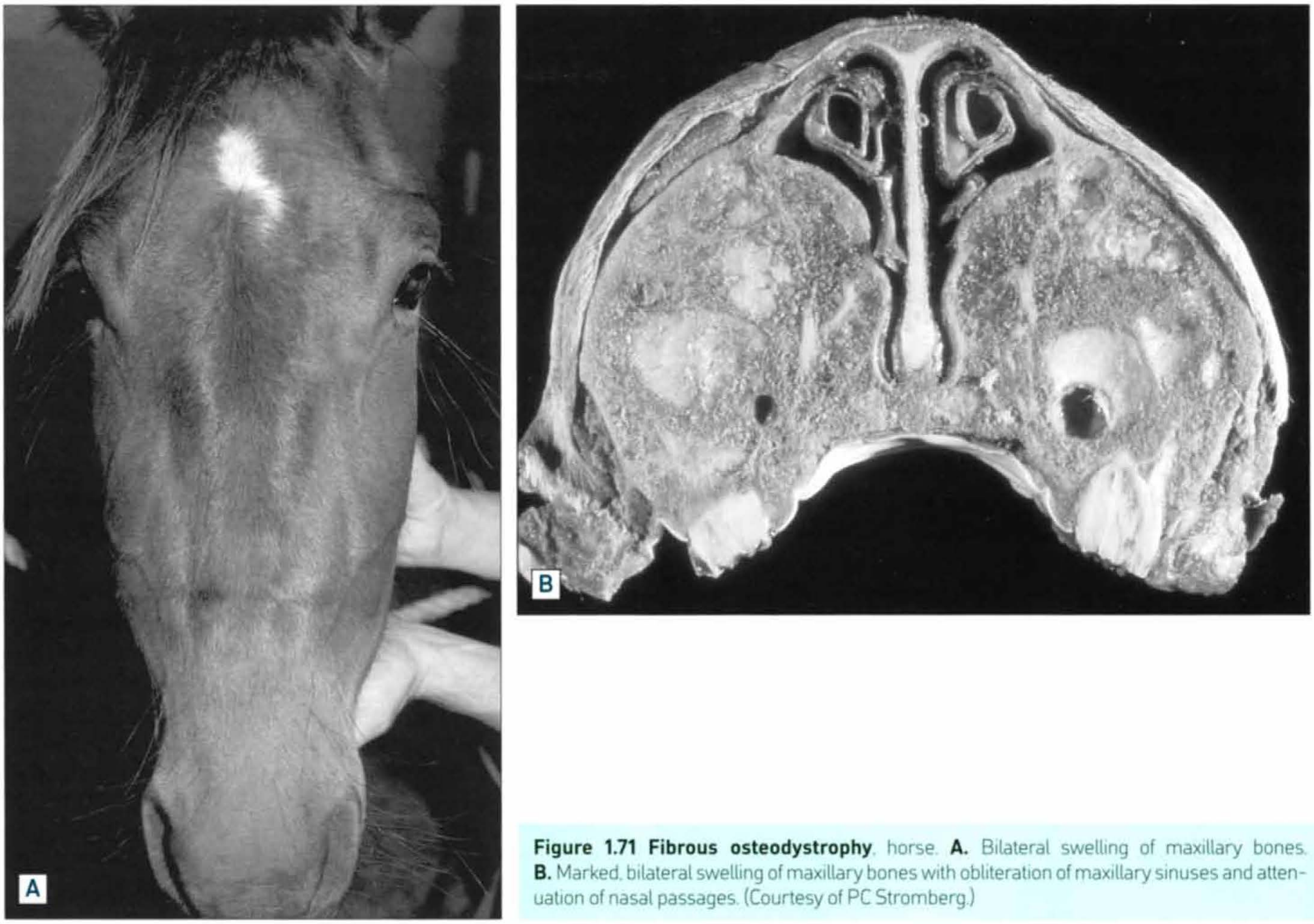

Figure 1.71 Fibrous osteodystrophy, horse. **A.** Bilateral swelling of maxillary bones. **B.** Marked, bilateral swelling of maxillary bones with obliteration of maxillary sinuses and attenuation of nasal passages. (Courtesy of PC Stromberg.)

In **swine**, *fibrous osteodystrophy usually occurs in young growing animals fed unsupplemented grain rations.* The disease also accompanies rickets in association with vitamin D deficiency. Clinical signs include stiffness, reluctance to rise and, in severe cases, lameness. Some affected animals may develop enlargement of the skull similar to that seen in horses. The mandibles may be affected first, and sometimes are the only site of gross lesions, but in other cases, the basocranium is also enlarged. Sometimes, the mouth cannot be closed and the tongue protrudes because of the swelling of the jaws and the shortness of the mandibular rami. The teeth are mobile and often deeply and obliquely embedded in the fibro-osseous connective tissue. Radiographically, there is a generalized reduction in bone density.

At necropsy, the ribs may bend or snap with little effort. Recent or healing pathological fractures are often present. Articular surfaces of major joints may be irregular and focally depressed due to collapse of subchondral bone (Fig. 1.72). Growth plates are of normal thickness, except in cases caused by vitamin D deficiency where rickets and fibrous osteodystrophy occur concurrently.

Severe fibrous osteodystrophy is relatively common in **goats** fed rations high in concentrates, but there are no convincing reports of the disease in either **sheep** or **cattle**, suggesting species variation in susceptibility. In goats, fibro-osseous enlargement of the mandibles and maxillae is a characteristic clinical feature (Fig. 1.73). Respiratory distress may be present due to encroachment by swollen maxillary bones on the nasal cavity. The relative severity of lesions in bones of the skull in goats and certain other species may be related to the mechanical stimulus associated with chewing. The bones of the limbs are also affected in goats with fibrous osteodystrophy and may be markedly bowed.

In **dogs** and **cats**, nutritional secondary hyperparathyroidism is usually caused by *diets consisting largely or entirely of meat or offal.* The calcium content of such diets is low and the calcium:phosphorus ratio very wide. For example, the calcium content of cardiac and skeletal muscle is approximately 10 mg/100 g and the ratio of calcium to phosphorus approximately 1:20. The calcium:phosphorus ratio of liver is approximately 1:50. The ideal ratio is about 1:1, and skeletal abnormalities develop when ratios are 1:2 or greater. Supplementation of all-meat diets with appropriate levels of calcium corrects the imbalance and prevents fibrous osteodystrophy.

Clinical signs of nutritional secondary hyperparathyroidism usually begin a few weeks after weaning. Kittens fed exclusively on beef heart develop signs of fibrous osteodystrophy within 4 weeks. In both kittens and puppies, signs of the disease are progressive and include reluctance to move, hindlimb lameness and incoordination. Enlargement of facial bones is uncommon. Affected animals become depressed and may develop sudden lameness due to infractions of long bones or vertebrae, the latter resulting in paralysis. Callus formation at fracture sites does occur but consists primarily of fibrous tissue with little evidence of mineralization. Radiographically there is generalized, often marked, osteopenia (Fig. 1.74). Fibrous osteodystrophy in cats has been inappropriately referred to as osteogenesis imperfecta in the early veterinary literature because of the osteopenia and

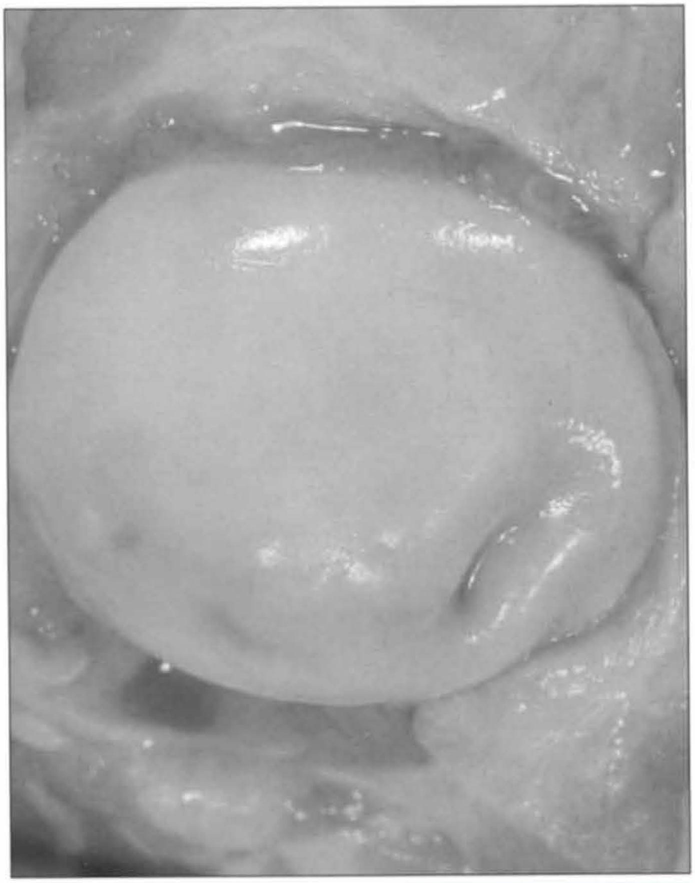

Figure 1.72 Fibrous osteodystrophy, pig. Irregular articular surface of humeral head due to collapse of subchondral bone. (Courtesy of RA Fairley.)

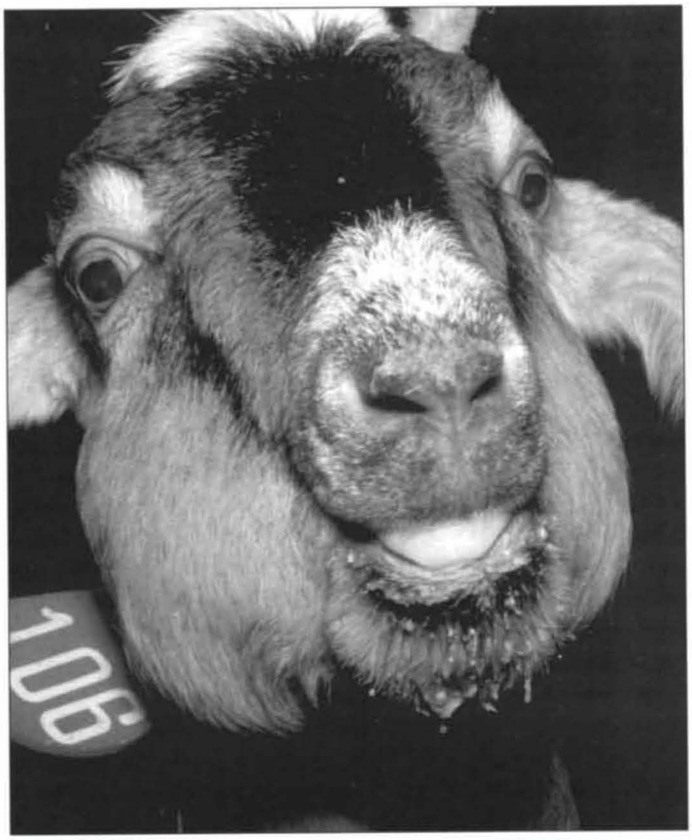

Figure 1.73 Fibrous osteodystrophy, goat. Bilateral swelling of maxillary bones and mandibles.

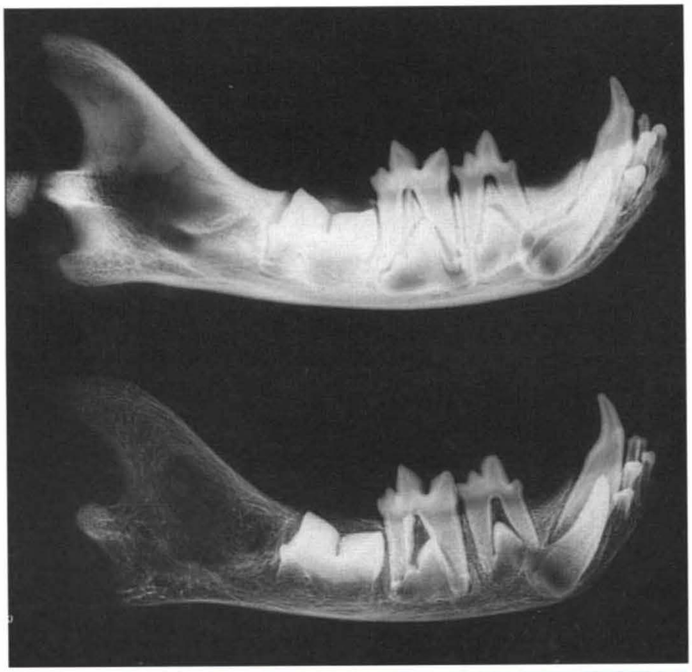

Figure 1.74 Fibrous osteodystrophy, kitten. Mandible from a normal kitten (top) and a kitten with fibrous osteodystrophy. Note the marked, diffuse rarefaction in the affected kitten and the loss of alveolar bone.

bone fragility. Osteogenesis imperfecta has however been diagnosed in the cat and remains a differential for fibrous osteodystrophy.

Renal secondary hyperparathyroidism may occur in adult dogs with renal failure, but the skeletal lesions are usually of secondary importance to the manifestations of uremia. Resorption of alveolar socket bone and loss of lamina dura dentes occurs early in the disease and may be evident radiographically. As the disease progresses, there is accelerated resorption of cancellous bones of the maxilla and mandibles, occasionally resulting in soft, pliable mandibles (so-called "*rubber jaw*"). Skull bones may have a moth-eaten appearance due to localized areas of accentuated resorption. Facial hyperostosis is a feature of some dogs with renal secondary hyperparathyroidism, but usually occurs in dogs with familial renal disease that develop slowly progressive renal failure from a young age. In such dogs, there is symmetrical enlargement of the head due to marked swelling of the maxillae and mandibles. The bones are firm rather than hard and can usually be cut with a knife. Focal red-brown areas of recent or old hemorrhage may be evident on cut surface. Young dogs with renal osteodystrophy may also have enlarged costochondral junctions and irregularly thickened physes, reflecting concurrent rickets due to impaired formation of 1,25-dihydroxyvitamin D.

In the German Shepherd pups with *hereditary primary hyperparathyroidism*, clinical signs were apparent by 2 weeks of age and included stunted growth, muscular weakness, and polydipsia/polyuria. Diffuse reduction in bone density was detected radiographically. At necropsy, the long bones were fragile and had narrow cortices. Facial bones were not enlarged but firm, pale yellow, swollen regions in the

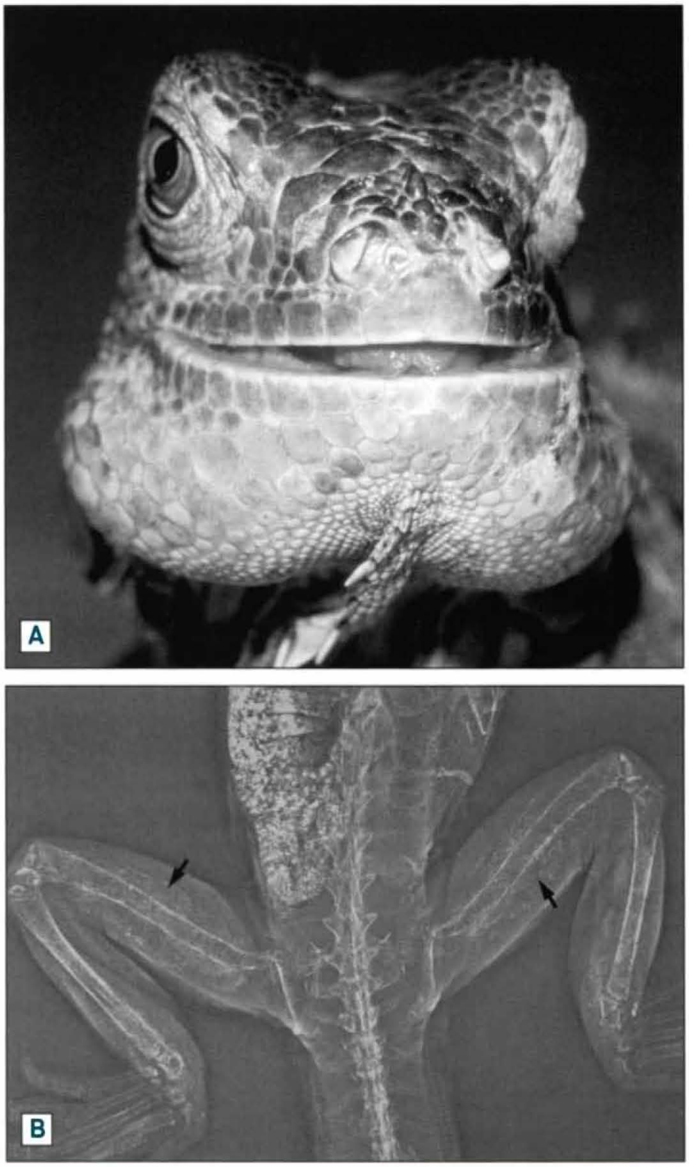

Figure 1.75 Fibrous osteodystrophy, iguana. **A.** Bilateral mandibular swelling. **B.** Xeroradiograph of the hindlegs and spine of the same iguana. The cortices of long bones are very thin and there is extensive periosteal new bone formation, particularly along the femurs (arrows).

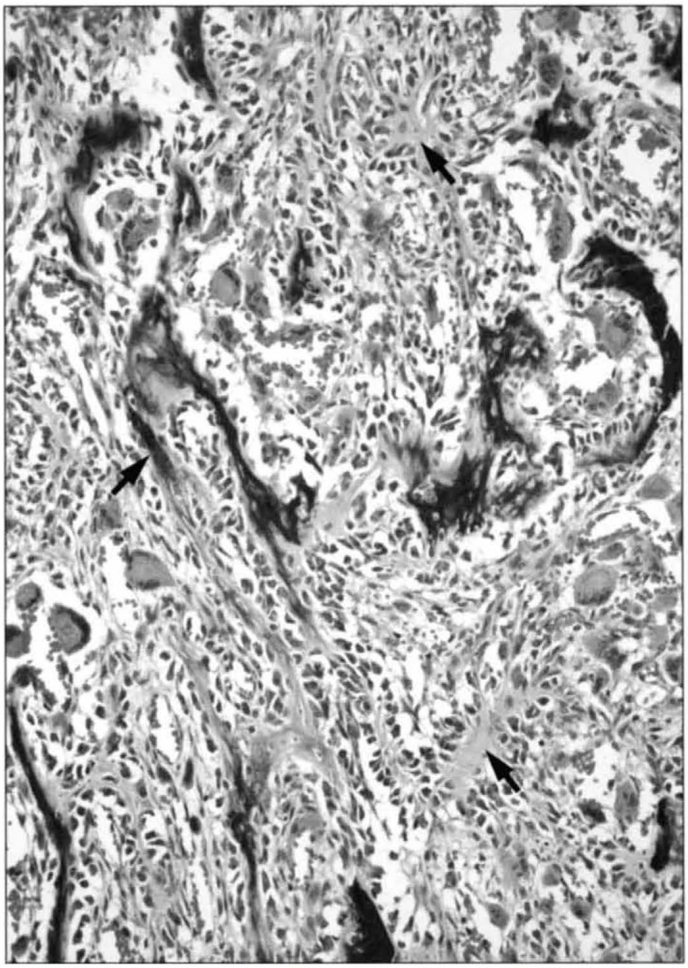

Figure 1.76 Fibrous osteodystrophy, ferret. Undemineralized section stained by the Von Kossa method and counterstained with hematoxylin and eosin. Highly reactive osteoblasts are forming delicate spicules of poorly mineralized or unmineralized bone (arrows). Many large osteoclasts are also present.

metaphyses of the ribs extended 2–3 cm along the shaft from close to costochondral junctions. Similar swollen segments were also present in the diaphyses of some ribs.

Fibrous osteodystrophy was reported in a strain of German Shepherd pups in Japan and although the possibility of a familial increase in susceptibility was suggested, the syndrome was apparently due to nutritional secondary hyperparathyroidism and was not analogous to the hereditary primary hyperparathyroidism referred to above.

Nutritional secondary hyperparathyroidism is relatively common in **reptiles** fed on diets that are either high in phosphorus (e.g., meat) or low in calcium (e.g., fruits, vegetables). Typically, there is thickening and softening of the bones of the skull and limbs, and a reduction in radiographic bone density (Fig. 1.75A, B).

The **microscopic features** *of fibrous osteodystrophy are similar in all domestic animal species and are characterized by increased osteoclastic bone resorption, marked fibroplasia, and increased osteoblastic activity with formation of immature woven bone.* These are also features of normal bone during development, and can be found in other disease processes. For example, longitudinal growth of long bones in rapidly growing young animals is accompanied by prominent osteoclastic and osteoblastic activity and intertrabecular fibrosis in the peripheral metaphyses as part of the normal modeling process. Such areas must be avoided when examining bone for histological evidence of fibrous osteodystrophy. Repairing fractures at certain stages of maturity, and diseases associated with hyperostosis (discussed elsewhere in this chapter), may also be confused with fibrous osteodystrophy if histological sections are examined without an awareness of their exact location and an adequate clinical and/or radiographic knowledge of the case.

The lesions of fibrous osteodystrophy vary between different bones, depending on their rate of turnover, and with the stage of the disease process. It is important therefore to examine several sections from different parts of the skeleton, particularly those areas that are growing most rapidly. In early lesions, the increase in bone resorption is reflected by increased numbers of plump, active osteoclasts, often within Howship's lacunae along the surface of bone trabeculae. Osteoblastic activity is also prominent (Fig. 1.76) and it is not unusual to find

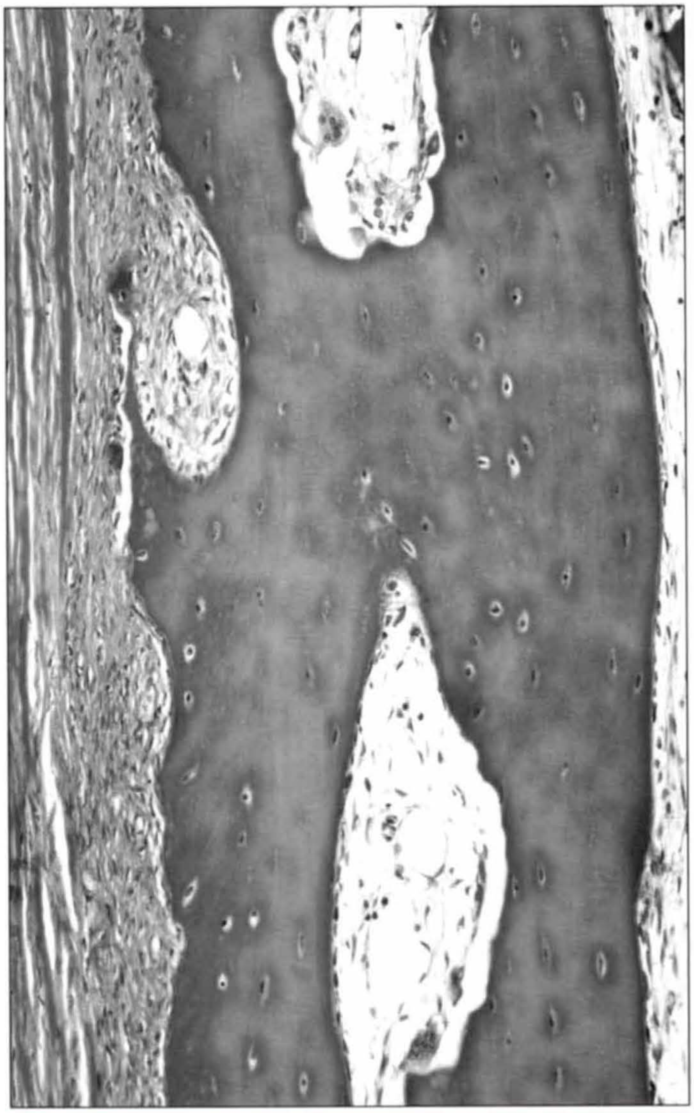

Figure 1.77 Fibrous osteodystrophy, kitten. Large resorption cavities in the cortex. Osteoclasts and Howship's lacunae indicate active resorption.

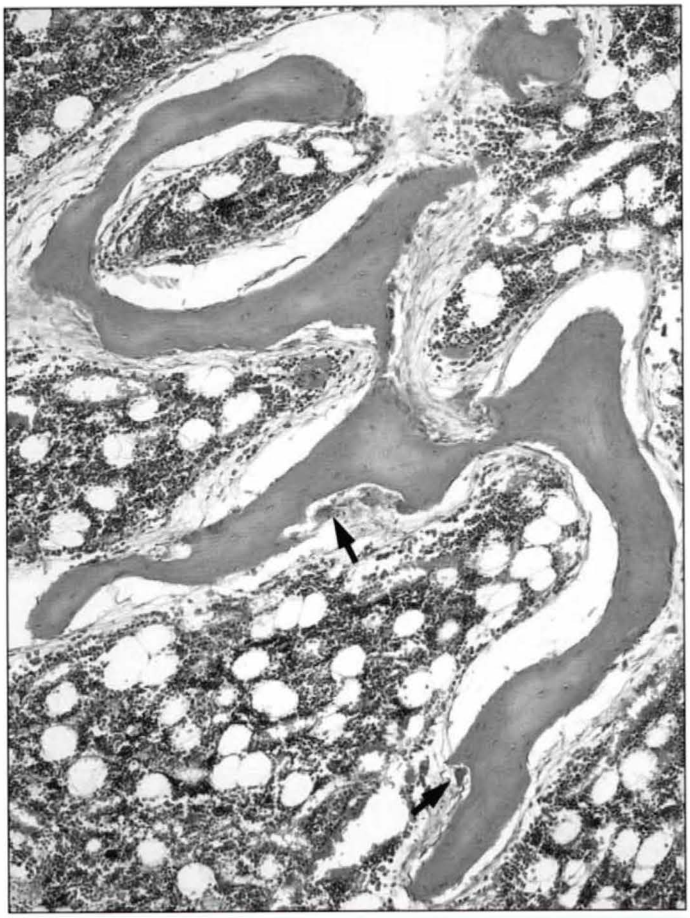

Figure 1.78 Fibrous osteodystrophy, pig. Early lesion with peritrabecular fibrosis and osteoclastic resorption (arrows).

bone trabeculae being resorbed on one side while new bone is being added to the other. Wide osteoid seams may be present at sites of rapid bone formation, especially in renal osteodystrophy where there may be concurrent rickets or osteomalacia due to deficient renal synthesis of 1,25-dihydroxyvitamin D. Resorption cavities lined by osteoclasts may be present in cortical bone (Fig. 1.77), and even within trabeculae, providing convincing evidence for exaggerated bone resorption. Lightly fibrillar connective tissue initially surrounds trabeculae undergoing resorption (Fig. 1.78), but later progresses to fill the intertrabecular spaces. As the disease progresses, *mature trabecular and cortical bone are extensively replaced by a combination of loose fibrous connective tissue and irregular trabeculae of poorly mineralized or unmineralized woven bone*, which may encroach on the medullary cavity and expand peripherally to elevate the periosteum (Fig. 1.79). The compact bone of the cortex is resorbed from endosteal and periosteal surfaces, and from within. In advanced lesions, only porous remnants of the original cortex may remain (Fig. 1.79). The soft, swollen mandibles and maxillae in animals with facial hyperostosis consist of highly cellular fibrous connective tissue surrounding delicate trabeculae of poorly mineralized woven bone (Fig. 1.80A, B). Osteoclastic activity may be largely absent in such advanced lesions, or remain in isolated pockets where remnants of the original bone are still being resorbed. In the absence of an adequate history or gross description, such lesions could be confused with benign or malignant bone tumors, or perhaps a fracture callus. Cyst-like cavities are sometimes present, possibly secondary to localized hemorrhage, and there may be sufficient hemosiderin in some areas to cause brown discoloration grossly.

Growth plates are histologically normal in fibrous osteodystrophy unless there is concurrent rickets or interference with vascular invasion due to trabecular fractures in the adjacent metaphysis. Premature resorption of the primary spongiosa is a feature of fibrous osteodystrophy in young, growing animals. The primary spongiosa normally consists of delicate trabeculae extending into the metaphysis at right angles to the growth plate and containing a central core of mineralized cartilage. In fibrous osteodystrophy, the newly formed trabeculae are resorbed almost as rapidly as they form and are replaced by disorganized spicules of woven bone lacking the cartilage core (Fig. 1.81). The spicules are often lined by osteoclasts and/or active osteoblasts, two to three cells deep, and are separated by a moderate amount of fibrous connective tissue.

In severe cases, articular cartilage of weight-bearing joints is often herniated into the epiphysis, causing an irregular articular surface. This is the result of osteoclastic resorption of the calcified zone of

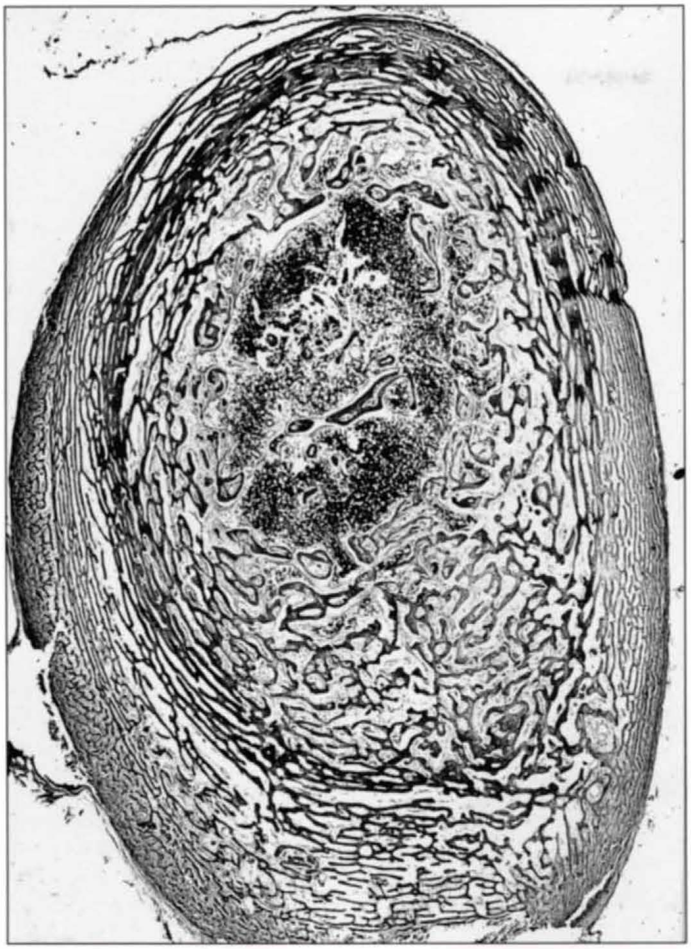

Figure 1.79 Fibrous osteodystrophy, pig. Transverse, subgross section of humeral diaphysis in an advanced lesion. Rarefaction of original and proliferation of new bone on medullary and periosteal surfaces.

articular cartilage, as well as resorption and collapse of subchondral epiphyseal bone.

In humans with renal failure, histologic lesions of osteomalacia are a prominent feature, but in dogs with renal secondary hyperparathyroidism, fibrous osteodystrophy predominates. An inherited disease known as Albright's syndrome appears as fibrous osteodystrophy in children, and in some children a phosphorus-losing nephropathy has been identified. Even in relatively young pups, histologic evidence of rickets is unlikely because the characteristic physeal lesions of rickets require relatively rapid growth and pups with renal failure generally grow poorly. *The lesions of renal osteodystrophy in dogs are usually most severe in the bones of the skull.* Histologic evidence of bone resorption by numerous large osteoclasts is prominent, particularly around teeth where the alveolar bone may be removed completely (Fig. 1.80A). Although facial hyperostosis occurs in some cases, osteopenia is more common in dogs with renal failure. Increased bone resorption on endosteal and periosteal surfaces is not balanced by formation, resulting in reduced cortical thickness.

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OSTEONECROSIS

Like any living tissue, *bone will die when deprived of its blood supply.* This is referred to as **osteonecrosis**, or the synonymous term **osteosis**. In animals, bone ischemia is most often associated with trauma, particularly fractures, where vascular disruption to part of the fractured bone is inevitable. Osteonecrosis also occurs in many acute inflammatory diseases of bones, where the periosteal or myeloid vascular supplies may be disrupted by exudate accumulating either beneath the periosteum or between trabecular bone in marrow spaces. In such cases, the presence of a *large fragment of necrotic bone,* or **sequestrum**, often proves to be a major hindrance to successful repair. This is discussed in more detail in the following section Inflammatory diseases of bones. Other causes of ischemic necrosis of bone, or **bone infarction**, include infiltrating neoplasms, thromboembolism and peripheral vasoconstriction in association with ergotism, fescue foot, or chronic anemia. Severely dehydrated animals in cold climates may also develop peripheral vasoconstriction and necrosis of bone, together with ischemic necrosis of other tissues in peripheral regions. As a rule, regions of bone that are served by end-arteries and have poor collateral circulation, such as the femoral head, are most prone to nonseptic osteonecrosis.

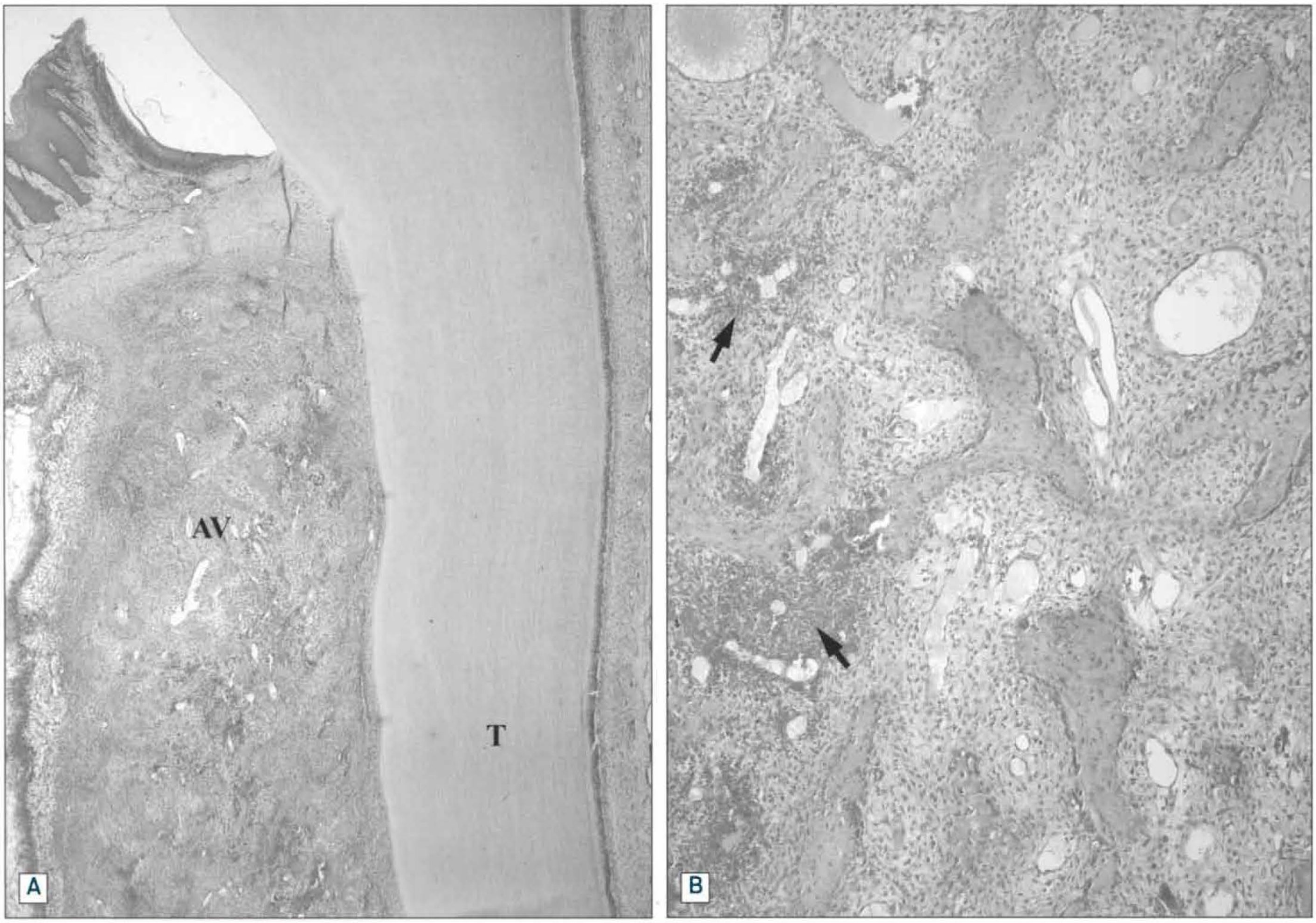

Figure 1.80 Fibrous osteodystrophy, dog. **A.** Section of tooth (T) and alveolar bone (AV) from a young dog with renal secondary hyperparathyroidism. The normally dense alveolar bone has been completely replaced by disorganized trabecular bone and fibrous connective tissue. **B.** Higher-power view showing the irregular trabeculae of woven bone surrounded by highly cellular fibrous connective tissue. The trabeculae are surrounded by active osteoblasts and occasional osteoclasts. Foci of hemorrhage (arrows) are also present.

In humans, bone infarction occurs in association with steroid therapy, alcoholism, hyperviscosity, hemoglobinopathies, and in divers or tunnel workers who work in dysbaric conditions. **Steroid-induced bone necrosis** is believed to be secondary to fat embolism of small blood vessels and although it can occur in many locations, lesions are found most often in the femoral head, distal femur, and proximal humerus. In animals, bone necrosis has been induced experimentally in rabbits and horses, but its prevalence in association with routine steroid therapy is unknown. The availability of sensitive imaging tools such as MRI now provides an opportunity to investigate the occurrence of bone infarcts in animals on long-term steroid therapy. Fat embolism is also believed to be involved in the pathogenesis of alcohol-induced osteonecrosis in humans. Although alcoholism is not a problem in animals, other causes of impaired lipid metabolism that do occur in animals might be expected to predispose to bone infarction. Dysbaric osteonecrosis is caused by the lodgment of nitrogen gas emboli in subarticular bone and medullary cavities following rapid decompression.

Necrosis of bone and bone marrow is described in calves with the juvenile form of sporadic lymphosarcoma. Infarcts of various sizes are found both in vertebral bodies and long bones (see Fig. 1.124A). The pathogenesis of the lesion is not known but may involve vascular obstruction due to increased intra-osseous pressure associated with neoplastic infiltration of the bone marrow.

A possible sequel to bone infarction is the *development of osteosarcoma.* Multiple bone infarcts have been associated with the formation of osteosarcoma in breeds of dog not usually susceptible to this tumor (e.g., Miniature Schnauzer). The reparative process triggered by the infarcts may be responsible for initiating the malignancy, similar to the proposed mechanism of tumor induction at sites of fracture repair and osteomyelitis in dogs and cats (see below).

Morphology and fate of necrotic bone

In the early stages, necrotic bone is often impossible to recognize on gross examination. Furthermore, its mineral composition will be unaltered and its radiographic appearance will therefore resemble that of normal healthy bone. *The earliest recognizable alteration is usually a change in the periosteum to a dull, dry, parchment-like sheath, which can be detached easily.* This contrasts with the normal periosteum and cortical surface, which is smooth, white, glistening, and firmly adherent, except where muscles and fascia are inserted. The sharp contrast in

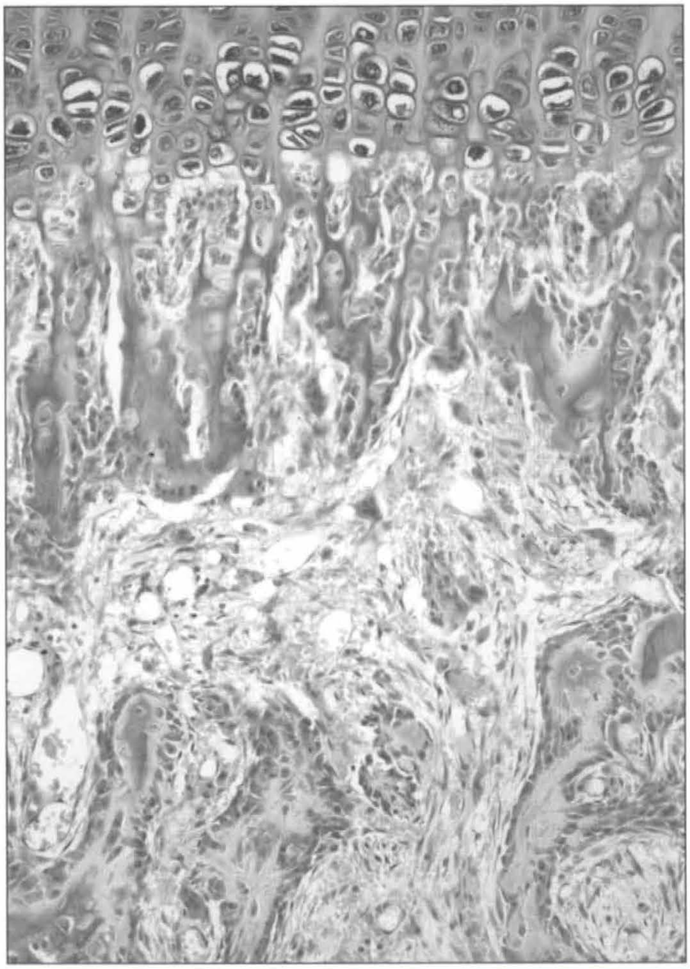

Figure 1.81 Fibrous osteodystrophy, kitten. Physis is normal, but there is premature osteoclastic resorption of trabeculae in the primary spongiosa and replacement with disorganized spicules of woven bone.

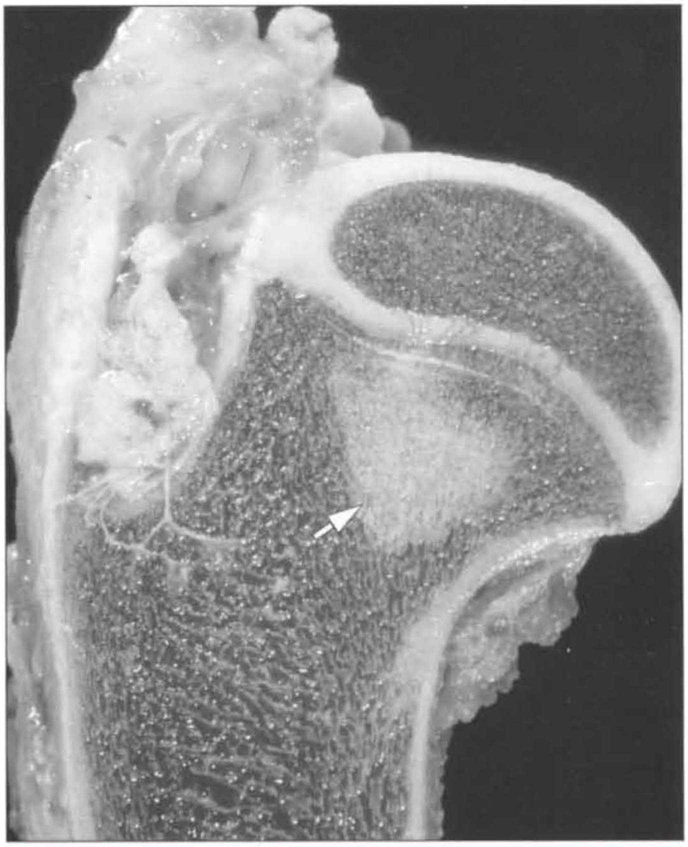

Figure 1.82 Necrosis of bone in the metaphysis of a young foal with **acute osteomyelitis**. The necrotic bone (arrow) is much paler than the surrounding hematopoietic bone marrow.

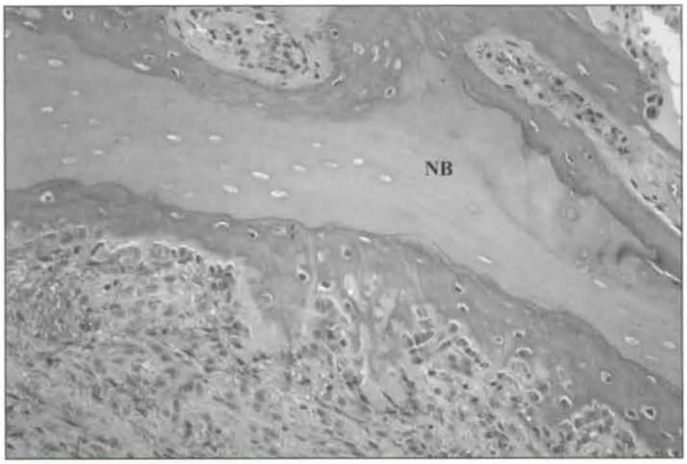

Figure 1.83 Necrotic bone surrounded by new, woven bone at a **fracture site** in a cat. Note the empty lacunae in the necrotic bone (NB) and the more basophilic matrix of the new bone. The necrotic bone has a scalloped margin, reflecting a period of osteoclastic resorption before the new bone was deposited on its surface.

color between a pale tan area of ischemia and the adjacent areas of normal marrow is an early indication of necrosis during gross examination, especially in young animals where the marrow is red due to active hematopoiesis (Fig. 1.82). Necrotic bone is slowly but progressively resorbed by osteoclasts, but these cells can only gain access to areas where the blood supply is intact and oxygen tension is maintained. Within a few weeks, evidence of the resorptive process will be apparent grossly as a margin of fibrous connective tissue separates the necrotic bone from adjacent viable bone. The necrotic bone may remain chalky white or become light brown.

Microscopically, zones of empty lacunae due to loss of osteocytes characterize necrotic bone (Fig. 1.83), but pyknotic nuclei from dead osteocytes may take anywhere from 2 days to 4 weeks to disappear. Hematopoietic tissue in the marrow cavity will show evidence of necrosis within 2 to 3 days after ischemia and can therefore provide an early indication, but adipose tissue is more resistant to ischemia and will not show evidence of necrosis for about 5 days. If an ischemic event is only transient, then there may be death of hematopoietic cells and perhaps osteocytes, but not adipocytes. Gas gangrene occasionally develops in the necrotic bones of livestock, but is rare in companion animals. The discovery of scattered empty lacunae in a section of bone does not justify a diagnosis of osteonecrosis as this may reflect normal turnover or even be an artifact of preparation. Prolonged immersion in acidic demineralizing solutions can also mimic osteocyte death. In osteonecrosis, all the lacunae in an area of bone should be empty, and the contents of the marrow cavity should be necrotic. Sometimes, focal dystrophic calcification occurs in the necrotic medullary fat.

The fate of necrotic bone depends on several factors, the most important being its volume, whether it is accompanied by sepsis, and whether it is in contact with viable tissue. In pyogenic infections of bone, sequestration is a common problem and usually has serious implications. The prognosis is more favorable if the necrotic bone is at a site that is both sterile and has good collateral circulation, and the volume of necrotic bone is small. In such cases, a zone of granulation tissue develops at the interface between the necrotic and viable tissue. This encroaches on the dead tissue and over a period of several weeks, the necrotic fat is replaced with collagenous connective tissue. *Osteoblasts derived from local progenitor cells begin to deposit seams of new woven bone on the remnants of necrotic bone* (Fig. 1.83). This new bone is sharply demarcated from the necrotic bone, which will usually be lamellar, and can be easily distinguished by its increased basophilia and the presence of viable osteocytes. Meanwhile, osteoclasts are recruited to the site and commence removal of the necrotic bone that is being used as a framework for deposition of new bone. This repair process, sometimes referred to as "*creeping substitution*," therefore involves the gradual resorption of dead bone at the same time that it is being replaced by new bone. Eventually, the new woven bone will be replaced with lamellar bone, but until such remodeling is complete, the combination of woven bone and partly resorbed dead bone may not be strong enough to withstand the forces of weight bearing, and the fragile, repairing trabeculae may collapse.

When the volume of necrotic bone is small, and is not in an area predisposed to further damage during the repair process, resolution is likely to be uncomplicated and complete. In fact, many such events probably occur throughout life but do not become clinically apparent. Repetitive injury at the same site may lead to an exaggerated response with formation of local exostoses.

The healing process is more complex when the necrotic bone is separated from viable connective tissue, as occurs frequently with necrosis or detachment of the periosteum. In such lesions, resorption of necrotic bone may still be complete if its volume is small, but if a relatively large volume of cortical bone is involved, sequestration may occur. Even when infection is not present, the sequestrum may interfere with the healing process by acting as a foreign body. *Attempts to wall off the sequestrum will lead to the formation of a layer of granulation tissue and reactive bone, the latter being referred to as an* **involucrum** (Fig. 1.84).

The efficacy of the collateral circulation is another important factor in the likely outcome of osteonecrosis. When the nutrient artery is occluded, large areas of the bone marrow become necrotic, along with the adjacent cancellous and compact bone. If the damage is restricted to the diaphyseal extremities, the prognosis is more favorable as this region has a dual blood supply of osseous origin – from the nutrient vessels on the diaphyseal side, and from the vessels of the joint capsule and ligaments on the epiphyseal side – as well as that from adjacent soft tissues.

As a rule, *the collateral circulation in cortical bone is inefficient* because, in spite of abundant anastomoses, the vessels are small and because they are confined within narrow canals, they are incapable of effective compensatory dilation. The resting and proliferative cartilage of the major growth plates depends on the epiphyseal circulation, while the sinusoidal circulation of the primary spongiosa is derived from metaphyseal vessels and is vulnerable to trauma. In contrast, the articular cartilages are relatively insensitive to regional ischemia since their nutrient supply is obtained by diffusion from the synovial fluid.

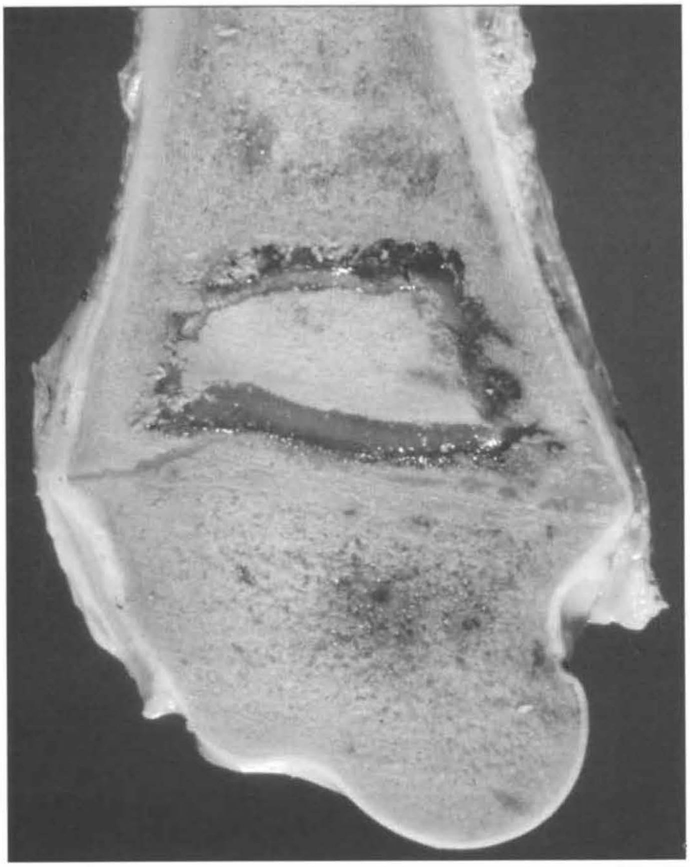

Figure 1.84 Large sequestrum surrounded by an involucrum of granulation tissue in the distal radius of a foal with salmonellosis.

Osseous sequestration is a relatively common sequel to distal limb wounds in horses, the most common sites being the proximal half of the third metatarsal bone and the third metacarpal bone. Although the initial event may be traumatic, it is likely that the introduction of infection to the site predisposes to sequestration, rather than reduced peripheral cortical circulation alone. Sequestra may be detected at about 14 days after injury, when there is some separation from adjacent viable bone and early formation of an involucrum.

Legg–Calvé–Perthes disease

This disease, characterized by *avascular necrosis of the femoral head*, is a well-recognized entity in children but also occurs with some frequency in dogs, especially smaller breeds. Miniature Poodles are particularly susceptible, as are West Highland white and Yorkshire Terriers, and there is evidence to suggest that the disease is inherited as an *autosomal recessive trait.* Avascular necrosis of the femoral head may also occur in other breeds of dog, and in other species, as a result of fractures of the femoral head, but such cases should not be confused with Legg–Calvé–Perthes disease which has a different pathogenesis. Clinically, the disease has an insidious onset, usually between 4 and 8 months of age, and is bilateral in approximately 15% of cases. There is no obvious sex or leg preference in dogs, unlike children where boys are affected five times more frequently than girls.

Anatomical and experimental evidence supports the theory that *the osteonecrosis in Legg–Calvé–Perthes disease is initiated by one or more episodes of ischemia.* As maturation takes place, the developing blood

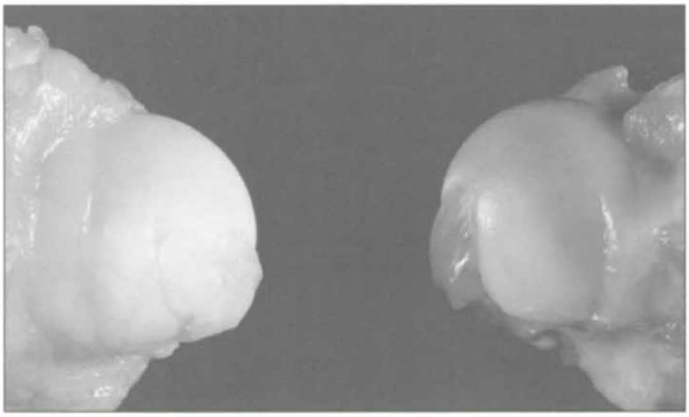

Figure 1.85 Legg–Calvé–Perthes disease in a dog. The articular surface of the femoral head on the left is irregular due to collapse of necrotic subchondral bone. The unaffected femoral head from the same dog is included for comparison. (Courtesy of MW Leach.)

vessels supplying the femoral head are progressively incorporated into fibro-osseous canals, which offer protection as they travel along the femoral neck. In highly susceptible Miniature Poodles, the incorporation of vessels into these canals is delayed, or is incomplete, in contrast to mongrels, where the vessels run mainly intra-osseously. In experimental studies, even a relatively slight degree of intracapsular tamponade produces lesions similar to those in the naturally occurring disease, probably by occluding the veins that drain the femoral head. A transient increase in intra-articular pressure caused by an effusion associated with synovitis or trauma would therefore be expected to interfere with venous drainage from the femoral head and produce the natural disease.

In the early stages of the disease, the shape of the femoral head, and the outlines of the articular cartilage and physis appear grossly and radiographically normal, even though the subchondral bone may be necrotic. If the area of necrosis is small, and the vascular supply is quickly re-established, healing may occur uneventfully. When the subchondral infarct is more extensive, *continued weight-bearing leads to fracture and collapse of the necrotic trabecular bone and flattening of the femoral head* (Fig. 1.85), predisposing to degenerative arthropathy. The physis of the femoral head is also disrupted in some cases, presumably due to interruption of its epiphyseal blood supply at the time of the initial insult.

The repair process involves initial revascularization and proliferation of mesenchymal cells from the margin of the necrotic area, followed by deposition of woven bone on the remnants of necrotic trabeculae. Gradually, the dead bone is removed by osteoclasts and eventually the woven bone is replaced by lamellar bone.

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INFLAMMATORY DISEASES OF BONES

Inflammation of bones inevitably originates in vascular areas of either the medullary cavity or the periosteum and is referred to as either **osteomyelitis** or **periostitis** respectively. **Osteitis** is a more general term for inflammation of bones, but is used less frequently. Most inflammatory diseases of bones are caused by bacterial infections, although mycotic and viral agents can also infect bones. *Bacterial osteomyelitis is particularly common and important in domestic animals* and will be discussed in some detail below, as will other forms of infectious osteitis.

Noninfectious osteitis also occurs, usually in response to local periosteal injury, and typically results in the formation of **exostoses**. This may be due to a single insult if there is damage to the periosteum or to repeated minor trauma. Tearing of ligamentous insertions will often induce local periostitis and the development of exostoses or osteophytes. Small exostoses may be completely resorbed if the stimulus is removed, but larger ones may be converted from woven to lamellar bone and persist indefinitely. These often remain clinically inapparent but may interfere with the function of adjacent structures, such as tendons or ligaments, as occurs in some cases of "splints" on the second and fourth metacarpal bones of horses. There is evidence to suggest that traumatic injury to the periosteum may predispose to bacterial osteitis at the site, even if the overlying skin is intact. The mechanism is uncertain but damage to adjacent tissues may increase their susceptibility to infection. More extensive periostitis, particularly involving vertebrae, is reported in dogs in association with parasitic myositis caused by *Hepatozoon americanum* infection. Presumably, the periosteum is reacting to the release of inflammatory mediators or other factors in response to the presence of parasitic stages in muscle fibers.

Bacterial infections of bones

Bacterial infections of bones are very common in animals, especially young horses and ruminants. Since the route of infection is *usually hematogenous*, most are centered on the medullary cavity and are referred to as osteomyelitis. There is little doubt that bacterial osteomyelitis is far more common than is diagnosed, as affected animals often die of septicemia before the bone lesions become evident and the skeleton is generally not closely examined at necropsy unless clinical signs have suggested a skeletal disorder.

During bacteremia or septicemia, bacteria can become localized in many organs and at many sites. In bones, there is a strong predilection for sites of active endochondral ossification within the metaphyses and epiphyses of long bones and vertebral bodies. This reflects the unique nature of the vascular architecture at the physis, and at the equivalent site in expanding epiphyses. Capillaries invading the mineralized cartilage make sharp loops before opening into wider sinusoidal vessels that communicate with the medullary veins. The capillaries are fenestrated, thus permitting ready escape of bacteria into the bone marrow. Furthermore, sluggish circulation in the sinusoidal system, and the relative inefficiency of the phagocytic cells lining it, also tend to favor the development and persistence of infection. Experiments in rabbits have suggested that trauma, or some other factor that alters the metaphyseal environment, enhances the establishment of bone infection in animals with bacteremia. Thus, *a combination of physeal, metaphyseal, or epiphyseal injury and concurrent bacteremia or septicemia may be involved in the pathogenesis of hematogenous osteomyelitis.* Localization of bacteria in the vessels of cartilage canals is also common in hematogenous infections of young animals. As the skeleton matures, the vascular morphology at chondro–osseous junctions alters to make it less suitable for bacterial localization. In fact, there is probably only a narrow window during which bacteria, be they derived from umbilical infections in colostrum-deprived animals, or from infections in the respiratory or alimentary tracts, are able to establish in bones. The clinical manifestations of osteomyelitis may not develop until several months later when the bone lesion becomes extensive enough to cause pain, disfigurement of the bone, or perhaps result in a pathological fracture.

Some bacteria appear to have a predilection for localization within bone. This probably reflects the fact that *certain bacteria* (e.g., *Staphylococcus aureus*) *possess receptors to bone surface proteins,* such as sialoproteins and collagen. It is possible that trauma makes binding sites on these proteins more accessible to bacterial receptors. This might account for the frequent establishment of bone infection at sites of blunt trauma, even when the skin surface has remained intact. Once bound to bone proteins, *S. aureus* bacteria surround themselves with a thick mucopolysaccharide glycocalyx, which allows tighter adhesion to the bone surface and favors persistence of the infection by protecting the organisms from host defense mechanisms and inhibiting the penetration of antibiotics. Other bacteria that commonly establish in bones (e.g., *Salmonella* spp.) may possess similar mechanisms of survival.

At sites of bacterial infection, cytokines such as tumor necrosis factor, interleukin-1, and prostaglandin E_2 released from inflammatory cells activate *bone resorption by osteoclasts.* There is also evidence that inflammatory cells themselves may be able to resorb bone.

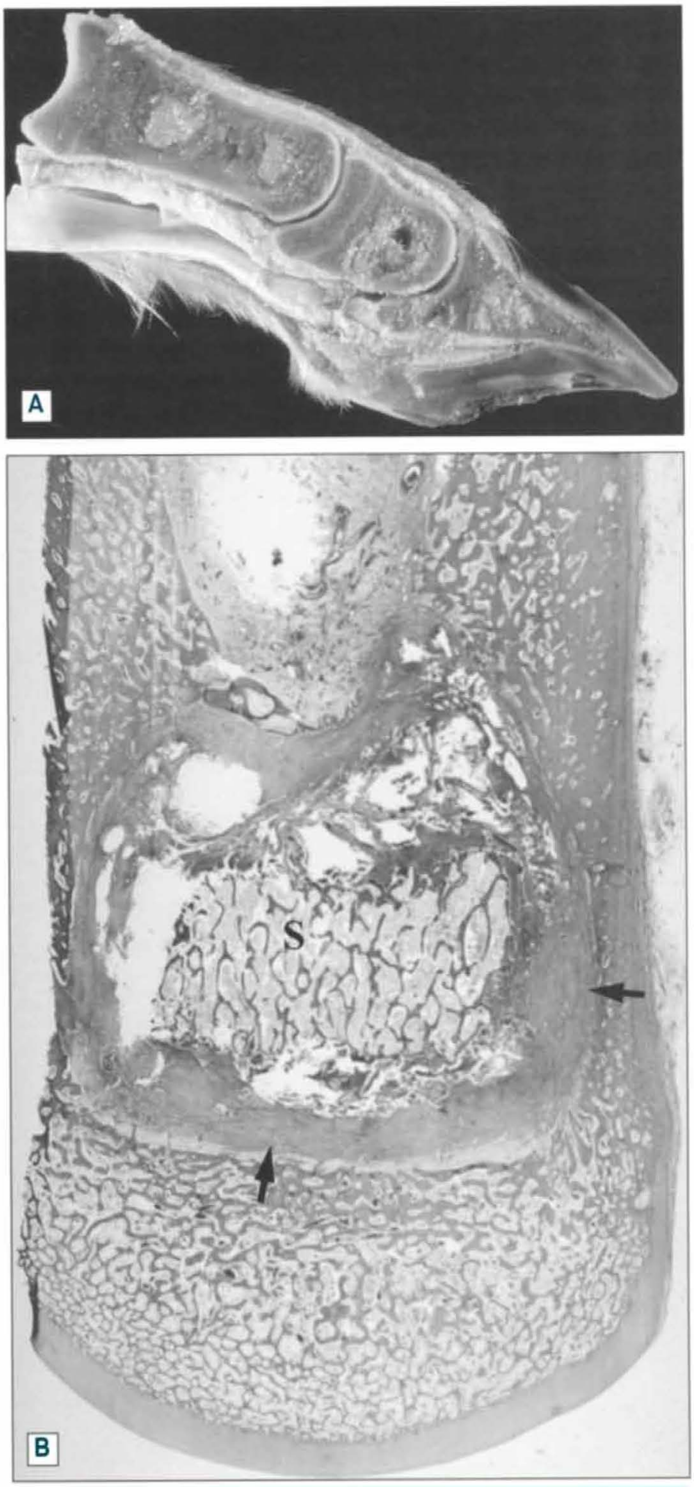

Figure 1.86 Osteomyelitis in a foal with salmonellosis. **A.** All three digits contain large sequestra. **B.** Sequestrum in the distal metaphysis of P-1 from the same foal. Note the thick involucrum of granulation tissue (arrows) surrounding the sequestrum of necrotic cancellous bone (S).

There are several possible sequelae to hematogenous bacterial infection of bone. The initial response is characterized by *edema and acute purulent inflammation.* Many infections are probably eliminated spontaneously by host defenses at this early stage, perhaps assisted by prompt and vigorous treatment with specific antibacterial agents. If the infection is not eliminated it may become segregated by fibrous inflammatory tissue and woven bone, with development of a *metaphyseal or epiphyseal abscess* (*Brodie's abscess*). Alternatively, the exudate may percolate through the adjacent marrow cavity causing necrosis of soft tissues and trabecular bone. These early lesions appear grossly as discrete areas of pallor, sharply demarcated from the normal red bone marrow (Fig. 1.82). *Resorption of necrotic trabecular bone* from the edge of the lesion, and *proliferation of granulation tissue*, results in separation of the necrotic, infected bone from adjacent viable tissues (Fig. 1.84). It is not unusual to find several foci of osteomyelitis in different bones within the same animal (Fig. 1.86A), or even within the same bone. Osteoclasts or inflammatory cells

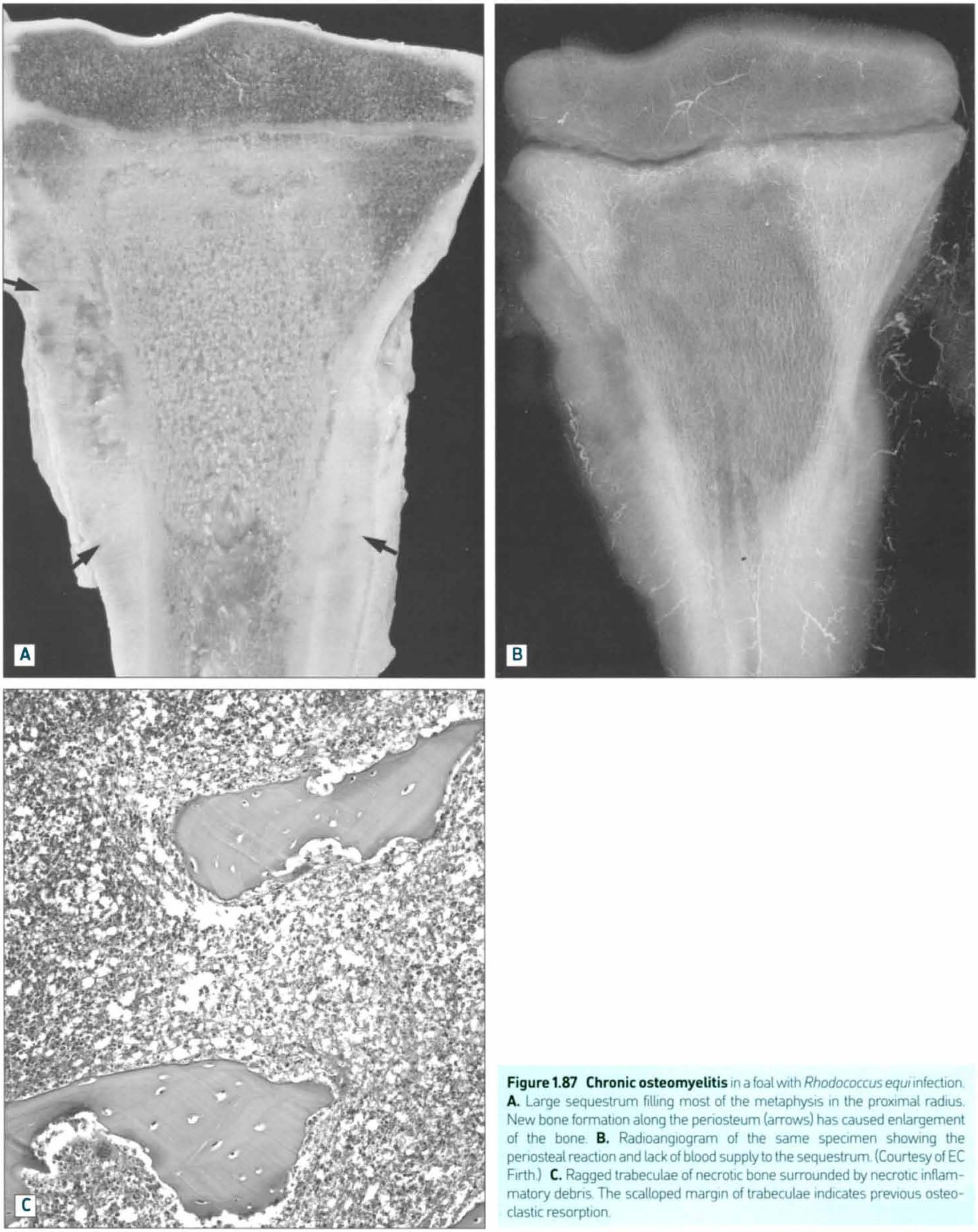

Figure 1.87 Chronic osteomyelitis in a foal with *Rhodococcus equi* infection. **A.** Large sequestrum filling most of the metaphysis in the proximal radius. New bone formation along the periosteum (arrows) has caused enlargement of the bone. **B.** Radioangiogram of the same specimen showing the periosteal reaction and lack of blood supply to the sequestrum. (Courtesy of EC Firth.) **C.** Ragged trabeculae of necrotic bone surrounded by necrotic inflammatory debris. The scalloped margin of trabeculae indicates previous osteoclastic resorption.

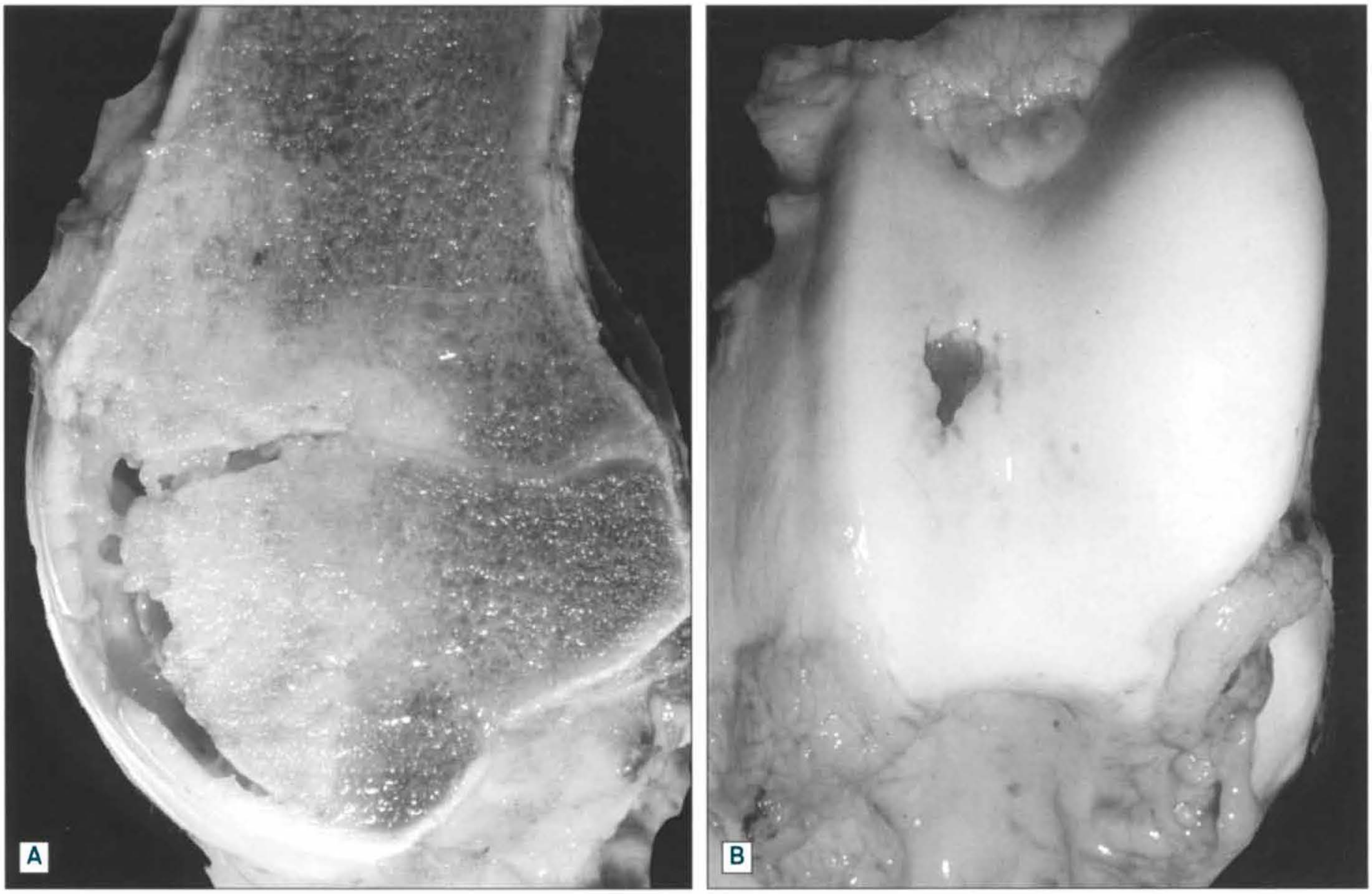

Figure 1.88 Osteomyelitis, foal. *Salmonella typhimurium* infection. **A.** Extensive destruction of subchondral bone in the distal femur. The infection has spread to involve much of the epiphysis, as well as the physis and part of the metaphysis. **B.** Same lesion prior to sectioning, showing communication with the stifle joint through a focal defect in the trochlear groove.

may resorb small foci of necrotic bone, but larger foci, which become separated from a viable vascular supply, persist as **sequestra**, harboring bacteria and interfering with the repair process. *Granulation tissue or reactive bone, which often forms a layer around sequestra* (Figs 1.84; 1.86A, B) is referred to as an **involucrum**.

The physis usually prevents the spread of infection from the metaphysis to the epiphysis, but transphyseal blood vessels in very young animals may transmit infection from one side to the other. In such cases, the physis is usually involved in the septic inflammatory process and is destroyed locally (see Fig. 1.53). The cortex is relatively porous in young animals and offers little resistance to the spread of infection, which penetrates through vascular canals to the periosteum. If cortical penetration occurs within the attachment of a joint capsule, then *septic arthritis* may result but, more commonly, a *subperiosteal abscess* develops. Disruption or thrombosis of the blood supply from the metaphysis to the inner portion of the cortex, together with periosteal elevation and interference with periosteal blood vessels, causes *segmental cortical necrosis and formation of sequestra*. In severe cases of bacterial osteomyelitis, there may be locally extensive necrosis and sequestration of metaphyseal and cortical bone, with an exuberant involucrum formed beneath an elevated periosteum, resulting in swelling and disfigurement of the bone (Fig. 1.87A, B).

The *predilection sites* for long bone lesions of osteomyelitis vary in distribution between species. There is also some variation in the bacteria involved and in the age distribution of affected animals. In **foals**, osteomyelitis seldom occurs after 4 months of age and the lesions occur more often in the secondary ossification centers of the epiphyses than the metaphysis. *Most infections establish immediately beneath the thickest part of the articular cartilage*, particularly in the caudal aspect of the lateral and medial femoral condyles, dorsal to the weight-bearing articular surface. Other common sites for epiphyseal localization of infection in foals include the distal intermediate ridge of the tibia, medial styloid process of the radius, and the proximal humerus, but many other sites also may be involved. The vascular arrangement at sites of thickened cartilage is characterized by an increased arterial supply and greater sinusoidal filling than in areas where the articular cartilage is thinner. If the infection becomes established, there may be extensive destruction of subchondral bone with underrunning of the articular cartilage (Fig. 1.88A, B). The inflammation may progress to involve much of the epiphysis and may communicate with the joint space through defects in the overlying cartilage.

Metaphyseal lesions in foals are most common at sites where the physis deviates greatest from a horizontal plane. Approximately 70% of foals with bacterial osteomyelitis, including virtually all of those with epiphyseal lesions, also have septic arthritis. This may reflect either concurrent establishment of infection in the synovium during bacteremia, or direct spread from bone. The bacteria most commonly involved in foals with osteomyelitis are *Escherichia coli*,

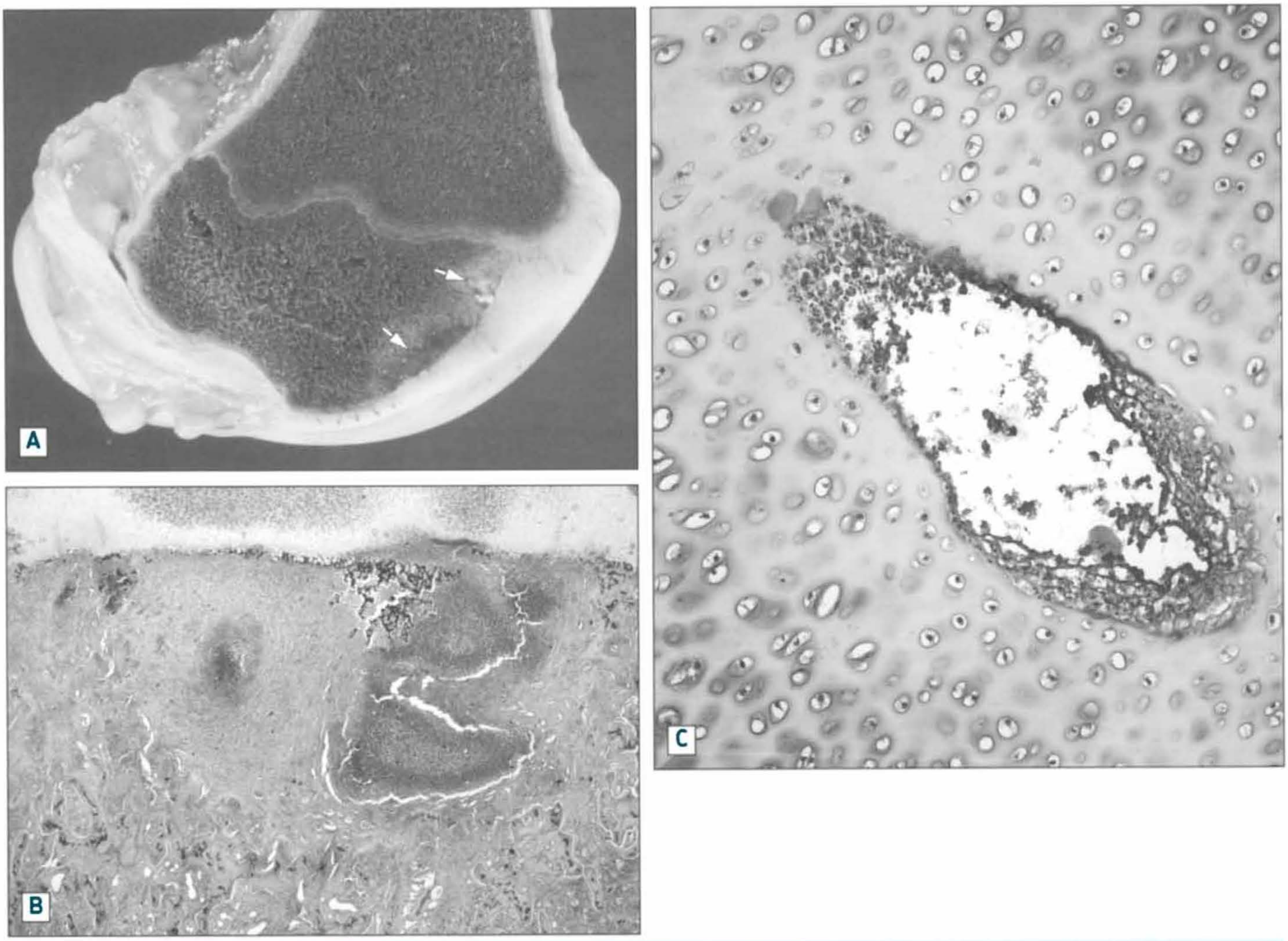

Figure 1.89 Subacute, suppurative osteomyelitis in the epiphysis of the distal femur in a calf. **A.** Foci of suppuration and trabecular destruction (arrows) immediately beneath the articular surface of the trochlear groove. The calf also had polyarthritis (see Fig. 1.154A, B) **B.** Low-power microscopic view showing foci of intense, suppurative inflammation and effacement of epiphyseal trabecular bone immediately beneath the articular cartilage. The pale areas of cartilage are degenerate. **C.** Cartilage canal in the same calf, showing destruction of vascular structures and replacement with necrotic debris.

Streptococcus spp., *Salmonella* spp., *Klebsiella* spp., and *Rhodococcus equi*. Hematogenous osteomyelitis of the tarsal bones, usually in association with infectious arthritis, is reported as an entity in young foals less than a month of age.

In **cattle**, hematogenous osteomyelitis is not confined to young animals, as it is in foals. In one study, the age range in cattle was 2 weeks to 5 years. *Metaphyseal osteomyelitis* in this study occurred at many sites in long bones, but particularly the distal metacarpus, metatarsus, radius, and tibia. *Epiphyseal osteomyelitis* occurred most often in the distal femoral condyle, distal radius and in the patella, and was usually associated with infection by *Salmonella* spp. In contrast, metaphyseal infections were most often caused by *Arcanobacterium pyogenes*. The *Salmonella* spp. infections were almost invariably in calves less than 3 months of age, while *Arcanobacterium pyogenes* usually affected cattle older than 6 months. As in foals, septic osteomyelitis generally accompanies bacterial arthritis in calves (Fig. 1.89A). Hematogenous osteomyelitis and arthritis also frequently occur together in young **lambs**, **goats**, and **deer**, and are associated with a range of organisms.

Histological changes in bacterial osteomyelitis are predictable, but depend on the causative agent and the stage of infection. *Acute osteomyelitis* in young animals may be distinguished from active myelopoiesis by the presence of fibrin, and a dense population of neutrophils and necrotic cells in the primary spongiosa, where hematopoietic tissue is usually absent. Inappropriate or excessive osteoclastic resorption of bone trabeculae is another characteristic microscopic feature and there may be necrosis of physeal or articular cartilage. In the epiphysis, inflammatory foci typically start immediately beneath the articular cartilage resulting in local destruction of subchondral trabecular bone (Fig. 1.89B). Degeneration or necrosis of the overlying articular cartilage is common in young animals with hematogenous bacterial osteomyelitis due to septic embolism or thrombosis of blood vessels in cartilage canals (Fig. 1.89C). In *chronic osteomyelitis*, marrow spaces between necrotic, partly resorbed bone trabeculae may be filled with necrotic debris (Fig. 1.87C), fibrous connective tissue, or a mixed population of inflammatory cells. A thick layer of granulation tissue infiltrated with neutrophils may surround larger areas of necrotic bone, with prominent osteoclastic activity at the margins. Proliferation of connective tissue and woven bone in chronic lesions may create difficulty in distinguishing the reaction from neoplasia, especially in biopsy specimens where the sample is small and perhaps not representative. The presence of neutrophils and plasma cells amongst the reactive bone and connective tissue supports a diagnosis of chronic osteomyelitis. Osteomyelitis secondary to a compound fracture may present a considerable diagnostic challenge, as the inflammatory changes will be superimposed on the proliferative components of fracture repair. In such cases, the history and radiographic changes are indispensable.

Hematogenous bacterial osteomyelitis is rare in **dogs** but may occur in pups that survive canine parvovirus infection, or other diseases associated with short-term or persistent neutropenia. A myelokathexis-like syndrome in Border Collie pups, characterized by persistent neutropenia, is typically accompanied by hematogenous osteomyelitis involving the metaphyses of several long bones. More often, bacterial osteomyelitis in dogs and **cats** is a *complication of compound fractures, wound contamination during open fracture repair, bite wounds, or gunshot injury*. Staphylococci, especially *Staphylococcus intermedius*, and streptococci are the most common organisms involved, although mixed infections including gram-negative bacteria, such as *Escherichia coli* and *Proteus* spp., also occur. Because of the association with fractures, osteomyelitis in dogs is most common in long bones of the appendicular skeleton, particularly the femur. Foreign material implanted traumatically beneath the skin, or surgical implants, may act as a nidus for infection. The surfaces of such implants are soon coated with matrix and serum proteins, fibronectin, and carbohydrates. Cell membrane receptors on staphylococci and some other gram-positive bacteria bind with fibronectin, while anaerobes and gram-negative bacteria attach less firmly via pili and fimbriae. In addition, the mucopolysaccharide glycocalyx produced by staphylococci and other gram-positive aerobes protects them from phagocytosis, antibodies, and may even alter the action of some antibiotics. Even after apparent resolution, bacterial infections of bone may recur as a result of proliferation of bacteria that have survived within the glycocalyx or in fragments of necrotic bone.

Vertebral osteomyelitis is a relatively common manifestation of bacterial osteomyelitis in horses and farmed livestock. Most cases probably result from hematogenous spread of infection to the bones during the neonatal period in animals with inadequate passive immunity. Bacteria may enter via the umbilicus, respiratory tract, digestive tract, or perhaps via the placenta immediately before parturition. In piglets and lambs, tail biting and tail docking respectively are believed to provide a portal of entry for bacteria in at least some cases of vertebral osteomyelitis. Following localization in the epiphysis or metaphysis of a vertebral body, or adjacent to a growth plate in a developing vertebral arch, the infection causes progressive destruction and weakening of the affected vertebral bone. As in long bones, sequestration and abscessation may occur. The *most common sequela is pathological fracture and collapse of affected vertebrae* with dorsal displacement of pus and necrotic bone fragments into the spinal canal (Fig. 1.90). Such an event is accompanied by the sudden onset of neurological signs caused by compression of the spinal cord. The severity and nature of the clinical signs will depend on the segment of cord involved and the degree of damage. In cases where vertebral fracture does not occur, the suppurative process may permeate the cortex in any direction, spreading to involve the pleura or peritoneum, the spinal muscles, or protrude as an encapsulated abscess into the spinal canal and cause partial compression of the cord. If the infection is contained or does not result in cord compression it may go undetected, or be found incidentally at slaughter.

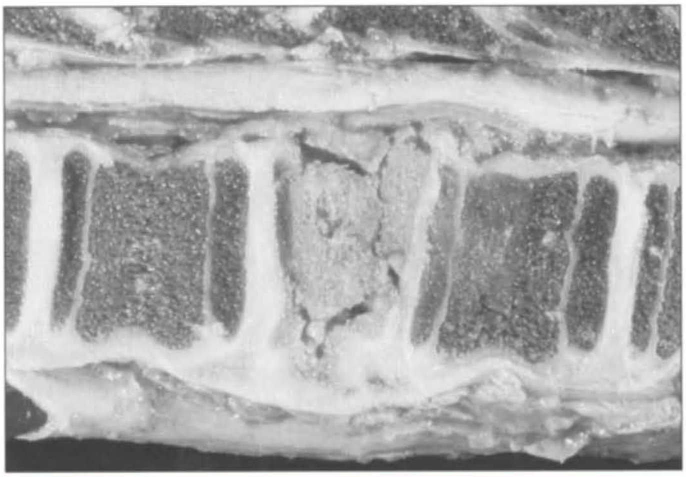

Figure 1.90 Vertebral osteomyelitis, calf. *Escherichia coli* infection. The vertebral body is completely necrotic and has collapsed. Several fragments have been forced into the spinal canal, compressing the cord. (Courtesy of PC Stromberg.)

Arcanobacterium pyogenes is the most common causative organism in vertebral osteomyelitis in most species, but a range of other bacteria may be involved. These include: *Escherichia coli, Salmonella typhimurium,* staphylococci, streptococci, and *R. equi* in foals; tuberculosis in older horses; *Fusobacterium necrophorum* in calves; *Mannheimia (Pasteurella) haemolytica, F. necrophorum,* and staphylococci in sheep; and *Erysipelothrix rhusiopathiae*, staphylococci, and streptococci in pigs. In one survey of cervico-thoracic vertebral osteomyelitis in calves, *Salmonella dublin* was isolated from eight of ten cases at postmortem. The lesions in this study all involved one or more of the vertebrae between C5 and T1. In regions where brucellosis is endemic in swine, *Brucella suis* may produce vertebral lesions as a sequel to diskospondylitis.

In dogs, bacterial osteomyelitis has been linked to penetration of grass seeds. Since the lesions are confined to the lumbar region, it is suggested that the grass seeds have most likely been ingested and penetrate the intestinal wall, perhaps at the caudal duodenal flexure, and ascend in the mesoduodenum to the lumbar vertebrae. Possible sequelae include sublumbar abscessation, osteomyelitis, and fistulation to the skin of the flank.

Localized bacterial periostitis is often associated with primary inflammatory conditions in adjacent tissues. Examples include infections of the feet of cattle with *F. necrophorum* ("footrot"), necrotic stomatitis of cattle caused by the same organism, infections of the paranasal sinuses as an extension from perforating injuries, bite wounds that produce cellulitis or abscesses, chronic paronychia and pressure sores over bony prominences. Penetrating wounds of the hoof in cattle or horses may also spread to involve the adjacent third phalanx.

Atrophic rhinitis of pigs, induced by exposure to toxins from certain strains of *Pasteurella multocida,* is associated with mixed bacterial infections and rhinitis. Absorption of the toxins leads to localized osteonecrosis of turbinate bones, and affected bones may lyse completely, leaving no substrate for repair (see Vol. 2, Respiratory system). A similar lytic condition may affect the turbinates of dogs.

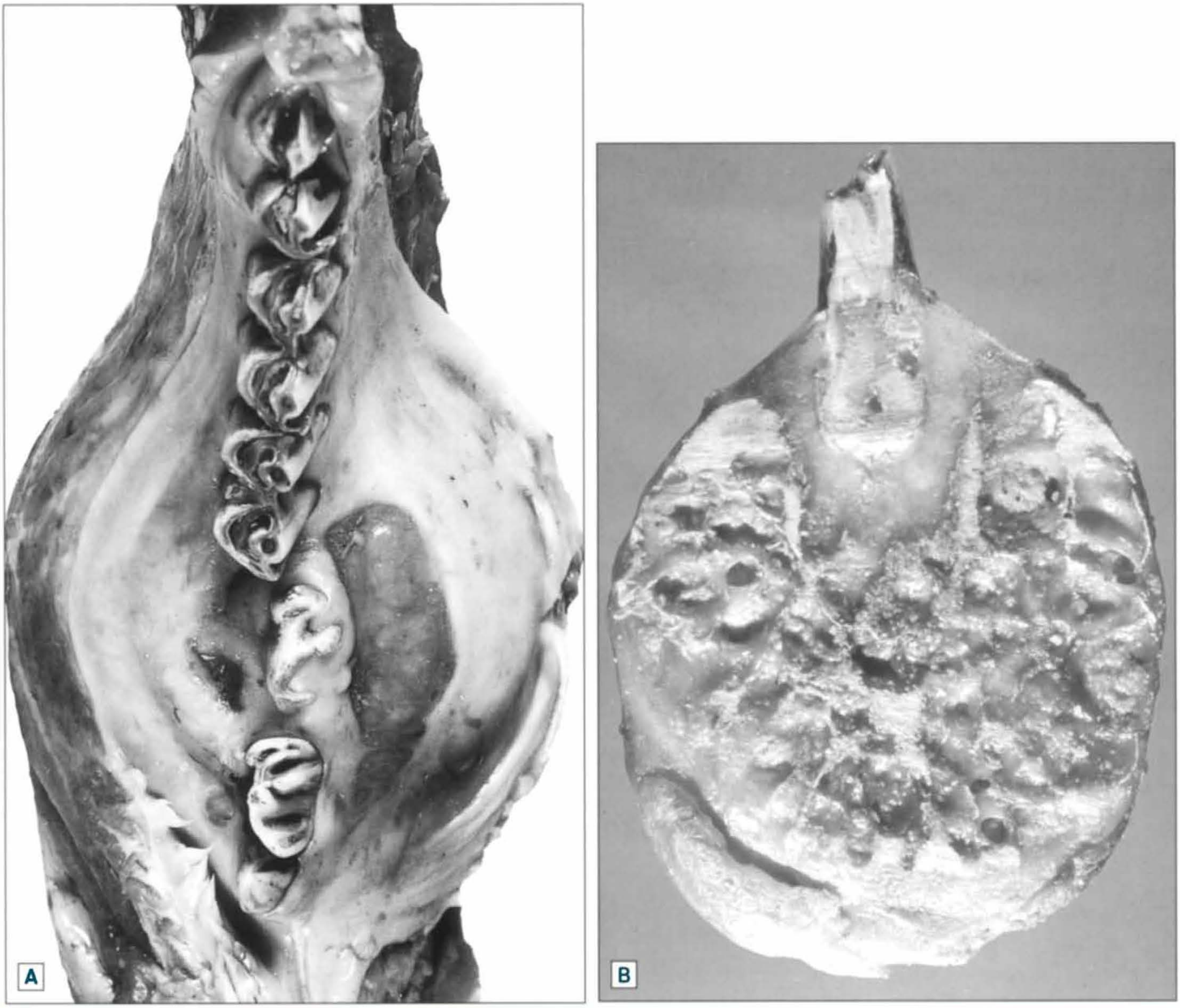

Figure 1.91 Mandibular osteomyelitis, cow. *Actinomyces bovis* infection. **A.** The mandible is markedly swollen. There is gingival ulceration and displacement of a tooth. **B.** Section through mandible showing "honeycomb" appearance caused by pockets of inflammatory tissue surrounded by reactive bone.

Mandibular osteomyelitis is primarily a disease of cattle caused by *Actinomyces bovis*, but occasionally occurs in horses, pigs, deer, sheep, and dogs. In cattle, the disease is known as **actinomycosis** or **"lumpy jaw,"** and *the classic lesion is confined to the mandible.* The maxilla is rarely involved and the organism rarely spreads even to regional lymph nodes, which, although large and indurated, are not infected. *A. bovis* is probably an obligate parasite of the oropharyngeal mucosa in a number of animal species, and most infections involve the buccal tissues. The organism is not particularly virulent, and in most, perhaps all cases, the surface tissues must be injured by some other agent or by a foreign body for invasion to occur. The osteomyelitis follows direct extension of the infection from the gums and periodontium. Extension to the periosteum causes actinomycotic periostitis, and the infection may not progress any further. Similar lesions may be produced by *A. pyogenes. Actinomyces bovis* may invade bone directly though the periosteum, but *osteomyelitis usually develops from periodontitis*, presumably via lymphatics, which drain into the mandibular bone. Once in the bone, *A. bovis* causes a chronic, pyogranulomatous inflammatory reaction. Suppurative tracts permeate the medullary spaces leading to multiple foci of bone resorption and proliferation. Large sequestra do not develop, even when the cortex is invaded, probably because of the slow, progressive nature of the disease. Fistulae often extend into the overlying soft tissue and may discharge through the skin or mucous membranes. Periosteal proliferation is excessive and the bone may become enormously enlarged (Fig. 1.91A), the normal architecture of the mandible being destroyed. The teeth in the affected portion of the jaw become loosened, lost, or buried in granulation tissue. On cut surface, the affected mandible has a "honeycomb" appearance with reactive bone surrounding pockets of inflammatory tissue (Fig. 1.91B). Fragments of necrotic trabecular bone accumulate in purulent exudate as "bone sand." The pus is also likely to contain many 1–2-mm diameter, soft,

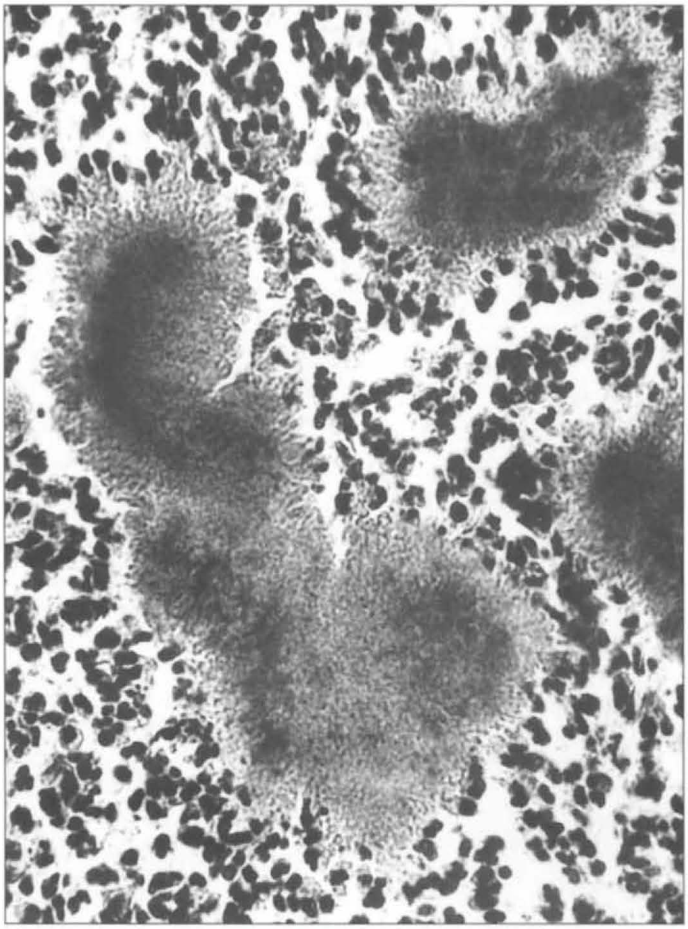

Figure 1.92 Colonies of *Actinomyces bovis* with characteristic club formation. The colonies are surrounded by neutrophils.

light yellow granules referred to as "sulfur granules." These consist of an internal mass of tangled, gram-positive filaments mixed with some bacillary and coccoid forms, and a periphery consisting of closely packed, club-shaped, gram-negative bodies (Fig. 1.92).

A similar tissue reaction, accompanied by club-colonies, occurs in association with some other bacteria, in particular *Actinobacillus lignieresi*, but the colonies in actinomycosis are much larger and the clubs are smaller and less discrete than in actinobacillosis. Furthermore, actinobacillosis is a disease of soft tissues rather than bone. *Fusobacterium necrophorum* and a variety of nonspecific bacteria may cause osteomyelitis by direct spread from periodontitis, but the lesions are usually more destructive and less proliferative than with *Actinomyces bovis*.

Mandibular osteomyelitis associated with loosening or loss of molar teeth (Fig. 1.93A) is sometimes detected in ill-thrifty **sheep** in the absence of other likely causes of weight loss, and may be a more important problem than is currently recognized in commercial sheep flocks. Grass awns or other plant matter may be wedged into pockets between teeth and the alveolar bone (Fig. 1.93B), providing a route of entry for opportunistic bacteria into the mandible.

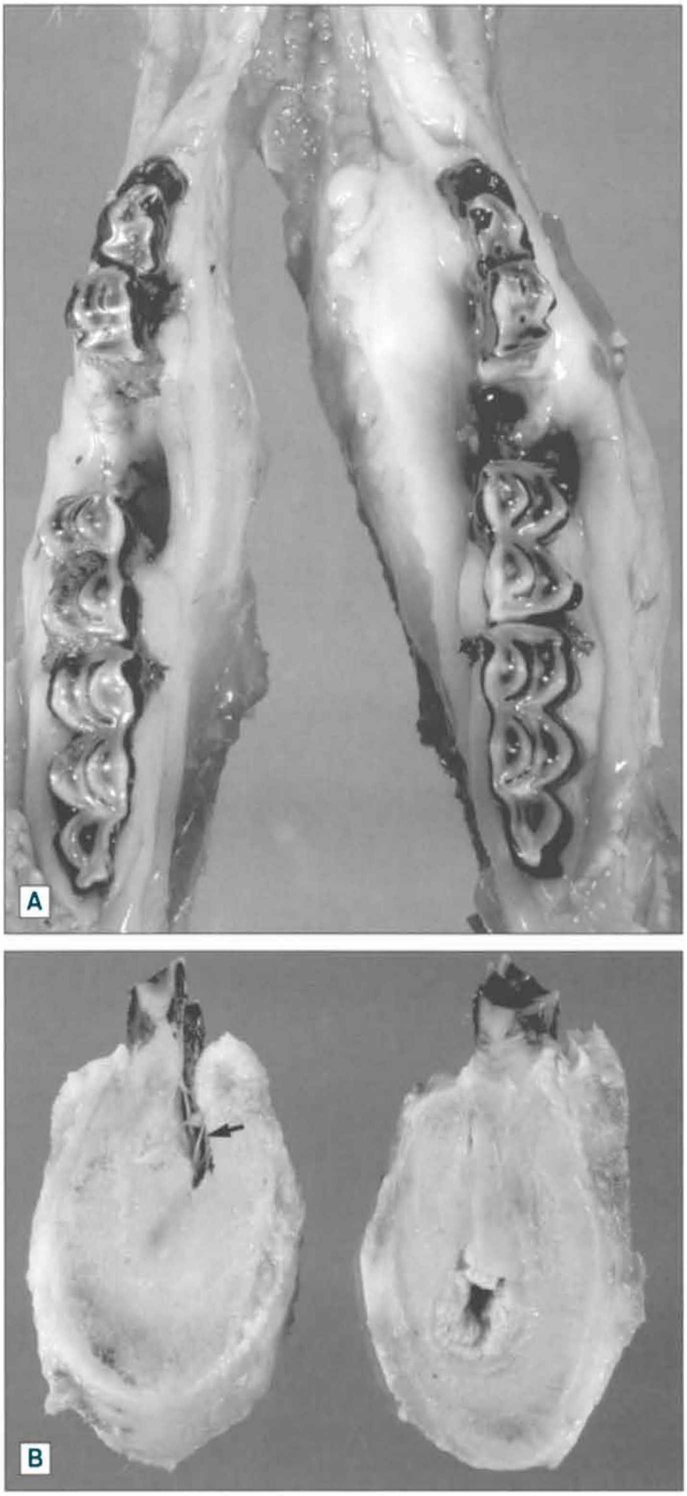

Figure 1.93 Mandibular osteomyelitis, sheep. **A.** Bilateral loss of individual molar teeth and swelling of the mandible on the right. **B.** Sections through swollen mandible shown in (**A**). Note the plant material (arrow) wedged between the tooth and adjacent bone in one section and the lytic focus filled with necrotic debris near the base of the tooth in the other.

Mycotic infections of bones

Mycotic infections of bone are less common than those caused by bacteria but certain pathogenic fungi, in particular the yeasts *Coccidioides immitis, Blastomyces dermatitidis, Cryptococcus neoformans*, and *Histoplasma capsulatum* may cause osteomyelitis following inhalation of spores and hematogenous dissemination. Multiple bones may be involved and the lesions may vary from lytic to highly proliferative, depending on the stage of the disease and the immune status of the host. Histologically, *the inflammatory response is pyogranulomatous*

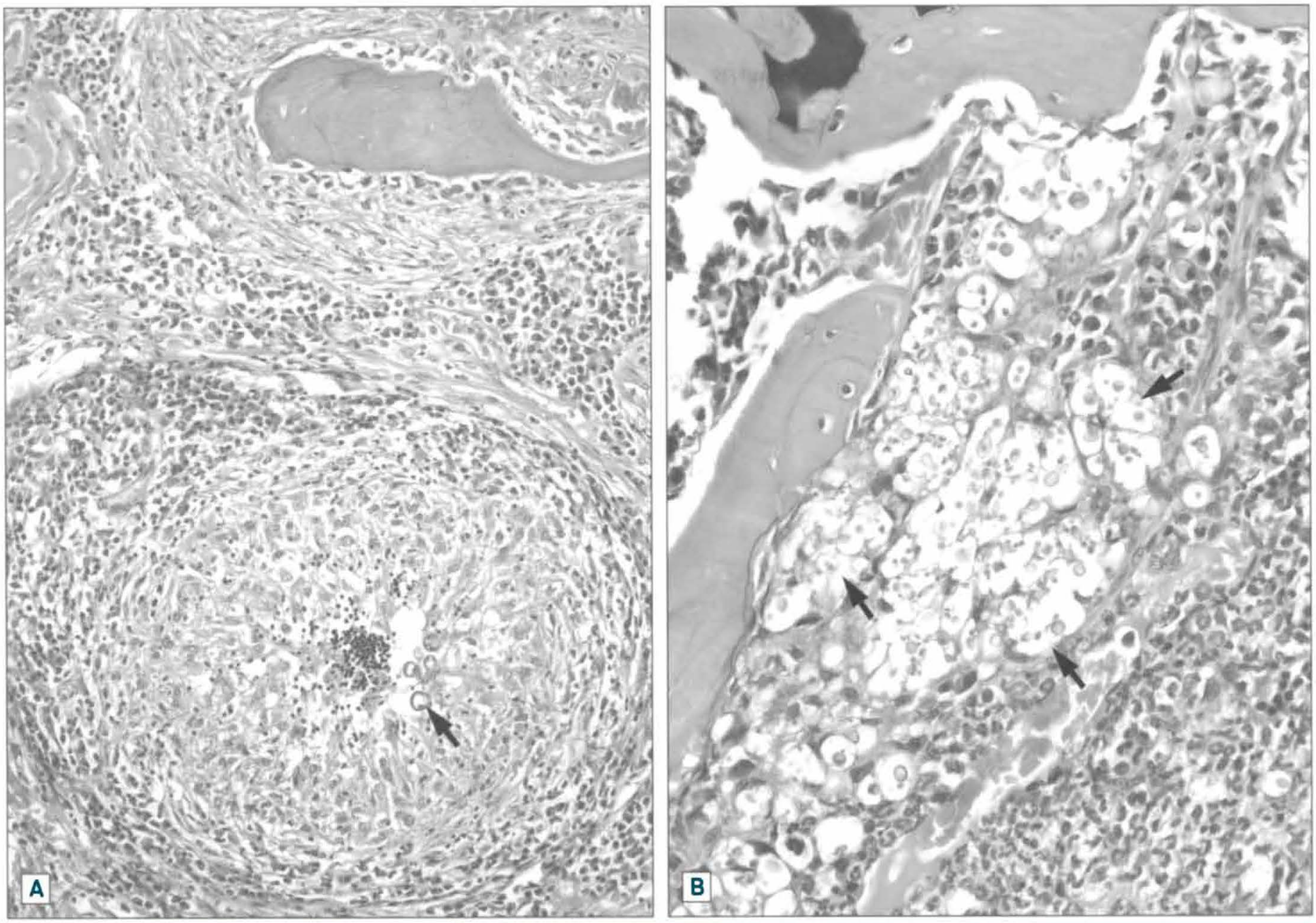

Figure 1.94 Mycotic osteomyelitis, dog. **A.** *Coccidioides immitis* infection. Focus of pyogranulomatous inflammation containing occasional organisms (arrow) and surrounded by a mantle of plasma cells. **B.** *Cryptococcus neoformans* infection. Numerous clusters of organisms with characteristic thick capsule (arrows) in marrow spaces of the rib.

(Fig. 1.94A) and is generally confined to the medullary cavity, at least in the early stages. The organisms can usually be detected readily, especially in cryptococcosis, where the host response may be minimal, perhaps reflecting impaired immunity (Fig. 1.94B). Lesions may also be present in a range of other tissues in animals with systemic mycoses and the presence of bone involvement may be overlooked.

Paecilomyces sp., a saprophyte in soil and decaying organic material, is generally considered nonpathogenic but is occasionally reported to cause osteomyelitis in dogs in association with systemic infection. The bone lesions may be multifocal and lytic, resembling plasma cell myeloma radiographically and grossly, especially when vertebral bodies are involved. Alternatively, they may be associated with extensive periosteal new bone formation leading to marked thickening of long bones (Fig. 1.95A, B). *Aspergillus* infections occasionally cause osteomyelitis in dogs, less so in other species. Other fungi, such as *Pithium insidiosum,* localize sporadically in bone and cause osteomyelitis.

Viral infections of bones

Several viruses are known to cause distinctive lesions in bones. Zones of metaphyseal sclerosis may be associated with ***Canine distemper virus*** infection and the **pestiviruses** of **bovine viral diarrhea** and ***Classical swine fever***, presumably due to destruction of osteoclasts or their precursors. This has been discussed in more detail earlier in this chapter.

***Canine adenovirus* type I** infection in pups may be accompanied by grossly visible metaphyseal hemorrhages at costochondral junctions due to virus-induced injury to endothelial cells, some of which contain characteristic intranuclear inclusion bodies. Necrosis of all cellular elements in the metaphysis also occurs, possibly due to ischemia, but there are no reports of residual lesions.

Feline herpesvirus causes necrosis in the turbinate bones of germ-free cats following intranasal inoculation, and produces necrosis in the metaphyses and periosteum of growing bones when administered intravenously. Sites of active osteogenesis are most susceptible. The virus infects osteoprogenitor cells, osteoblasts, osteoclasts, and endothelial cells, all of which may contain intranuclear inclusions.

Medullary sclerosis may occur in cats infected with ***Feline leukemia virus***, and is associated with nonregenerative anemia (see Vol. 3, Hematopoietic system). It is not clear whether the osteosclerosis is a direct result of the viral infection or is secondary to chronic anemia or hypoxia. Myelofibrosis may also be a feature of this infection.

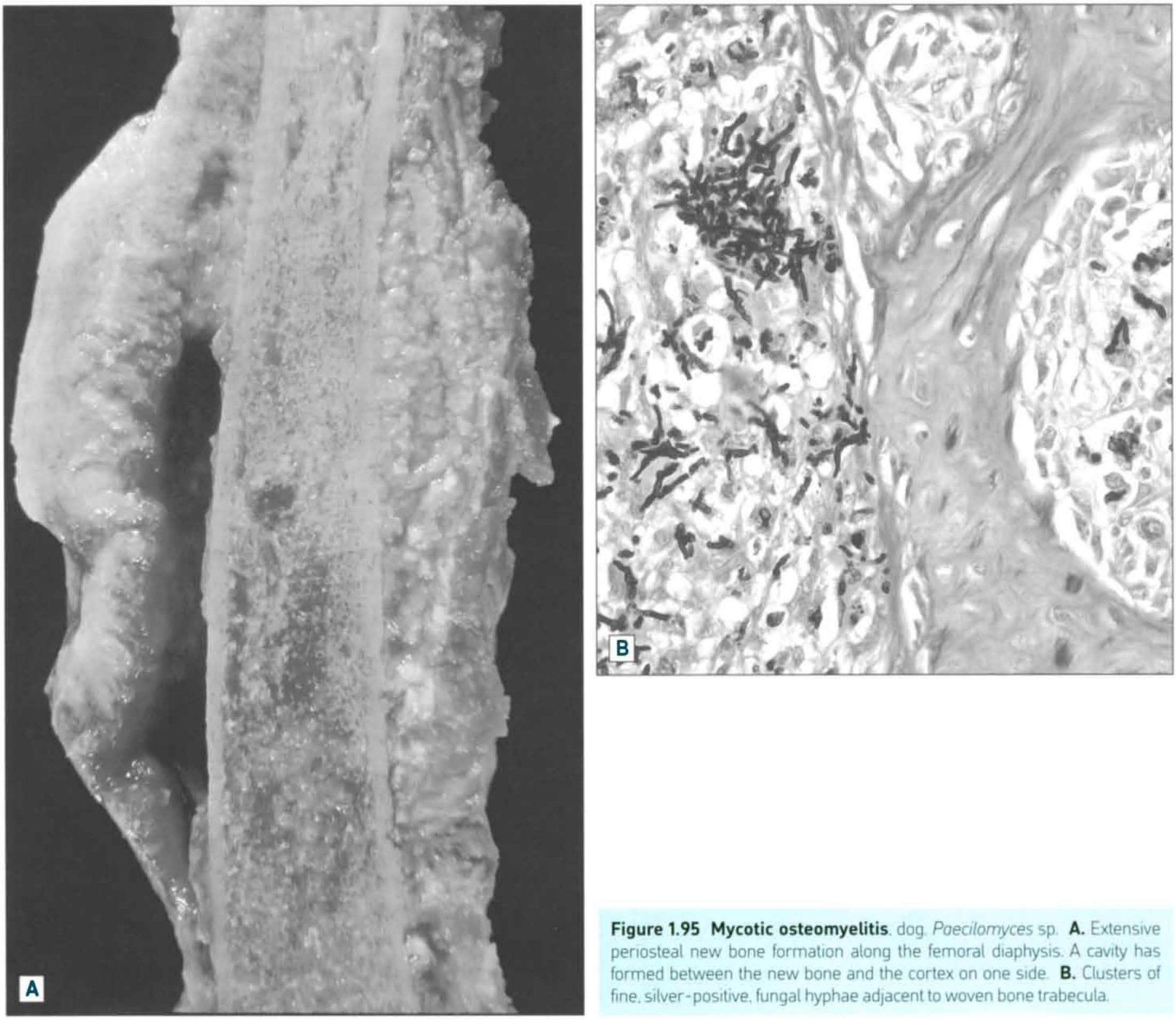

Figure 1.95 Mycotic osteomyelitis, dog, *Paecilomyces* sp. **A.** Extensive periosteal new bone formation along the femoral diaphysis. A cavity has formed between the new bone and the cortex on one side. **B.** Clusters of fine, silver-positive, fungal hyphae adjacent to woven bone trabecula.

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Metaphyseal osteopathy

Although *the cause of canine metaphyseal osteopathy is unknown*, and a variety of noninfectious etiologies have been proposed, the disease

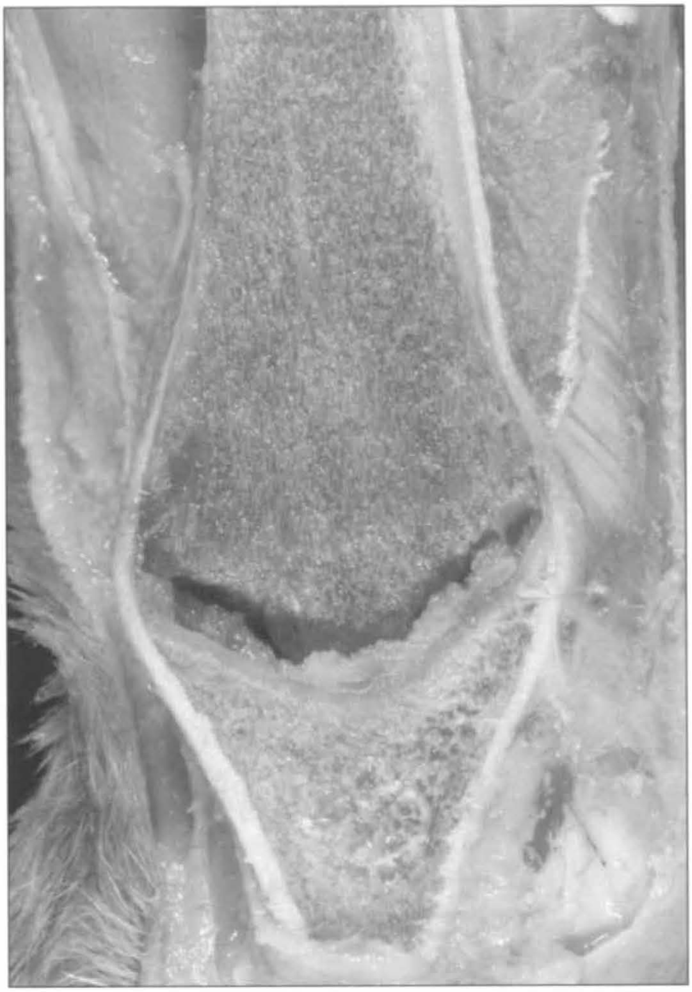

Figure 1.96 Metaphyseal osteopathy, dog. Acute lesion showing an infraction line through weakened trabeculae in the primary spongiosa of the distal ulna. (Courtesy of DJ Meuten.)

is included here amongst inflammatory diseases of bones because of the nature of the early lesions and increasing evidence that it is a manifestation of infectious osteomyelitis and periostitis. The disease is commonly referred to as "*hypertrophic osteodystrophy*" but *this term implies a metabolic rather than an inflammatory origin and should be discontinued*. Similarly, the term "skeletal scurvy" is purely historic and is inappropriate.

Metaphyseal osteopathy is a *disease of young growing dogs*, mainly those of large and giant breeds, although Weimaraners are over-represented in published reports. Affected dogs are usually 3–6 months of age at the time of initial presentation and typically show signs of fever, anorexia and severe lameness associated with swelling and pain of the metaphyseal regions of long bones. Some animals are reported to show diarrhea, or some other clinical abnormality, 7–10 days prior to the onset of fever. The *distal radius and ulna* are usually most severely affected but all fast-growing bones including ribs may be involved. Bones distal to the tarsus and carpus are usually spared. Radiographs taken early in the disease show alternating radiodense and radiolucent zones in the metaphysis parallel to the physis, and there is often lipping of the metaphyseal margins. Acute lesions are characterized grossly by a narrow band of pallor in the primary spongiosa adjacent to rapidly growing physes. Infractions (antemortem or postmortem) may be present through the primary spongiosa of some bones (Fig. 1.96) reflecting the fragility of the trabeculae in these areas of acute inflammation.

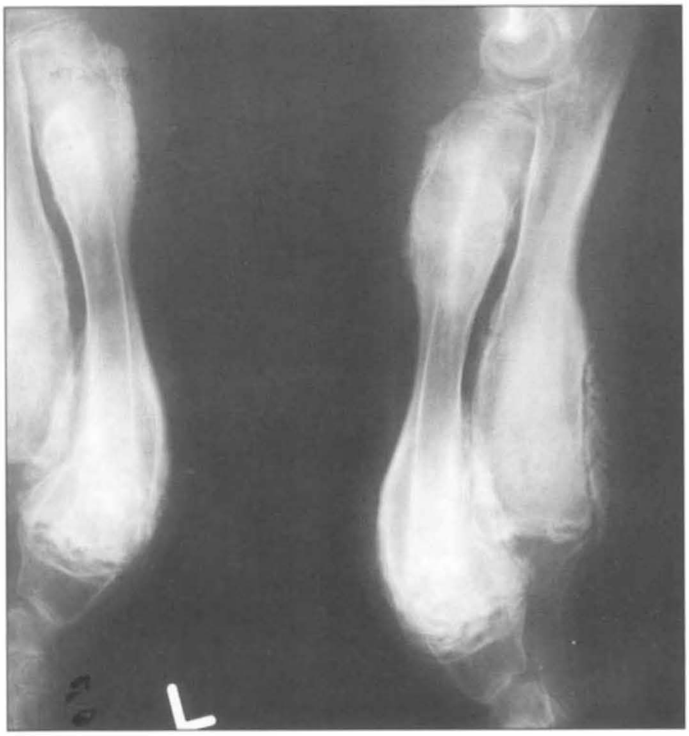

Figure 1.97 Metaphyseal osteopathy, dog. Radiograph of chronic lesion with extensive periosteal reaction involving the radius and ulna.

In most cases, the lesion does not progress but in others, periosteal and extraperiosteal ossification develops and the ends of the long bones become swollen and hard. Occasionally these swellings are extensive and involve almost two-thirds of the length of the bone, excluding only the mid-diaphysis and epiphyses (Fig. 1.97). The lesions are *usually bilaterally symmetrical*. Remissions and exacerbations may occur over a period of weeks to months and, rarely, a chronically affected dog dies or, more commonly, is killed for humane reasons. Most animals recover completely without, or in spite of, treatment and the excess metaphyseal bone is gradually removed.

The *histologic lesions* in acute cases are characteristic. The growth plates and primary spongiosa are structurally normal but there is *persistence of the calcified cartilage lattice of the primary spongiosa and intense intertrabecular, suppurative inflammation with necrosis and disappearance of osteoblasts* (Fig. 1.98A, B). Deposition of osteoid on the calcified cartilage spicules of the primary spongiosa is absent and the delicate trabeculae frequently fracture. Osteoclastic activity is prominent in some areas. The suppurative inflammation and necrosis of marrow contents may extend throughout much of the metaphysis and fibrin thrombi may be present in small blood vessels. Aggregates of segmented neutrophils are often present in the periosteum, associated with hemorrhages, as well as in cartilage canals of the epiphyses, and in bones such as vertebrae, mandibles, and turbinates. Enamel hypoplasia associated with inflammation of the dental crypt may also occur.

If the disease progresses, periosteal and extraperiosteal woven bone may develop in areas of previous hemorrhage and inflammation, eventually forming a collar of bone around metaphyses. At the same

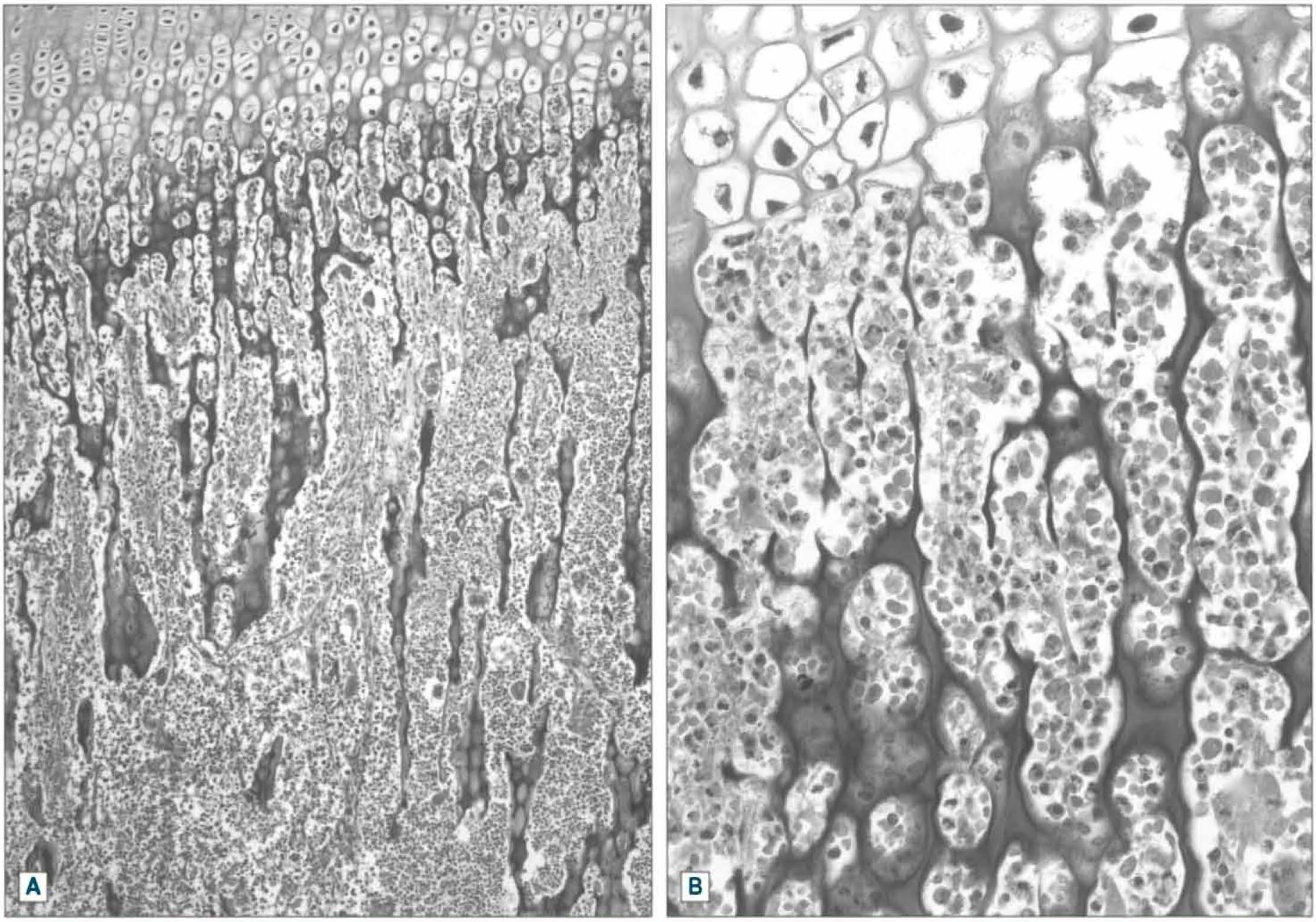

Figure 1.98 Metaphyseal osteopathy, dog. **A.** Acute disease showing normal physis and intense inflammatory reaction between trabeculae in the primary and secondary spongiosa. Trabeculae are delicate and consist largely of calcified cartilage with little, if any, osteoid. **B.** Higher-power view of primary spongiosa from the same animal. Calcified cartilage spicules devoid of an osteoid layer are separated by neutrophils and necrotic cellular debris. No osteoblasts are apparent.

time there is proliferation of mesenchymal tissue in the marrow spaces, replacing the suppurative exudate and providing precursor cells for the reconstruction of the damaged tissues. In those animals that have recurrences of the disease, acute inflammatory changes are superimposed on the osteogenic fibrous tissue in marrow spaces. Radiologically, the collar of periosteal bone may obscure metaphyseal lesions.

Although there have been numerous proposed causes for metaphyseal osteopathy, none has been proven. Initial reports suggested vitamin C deficiency as a likely cause because of the radiographic similarity between this disease and scurvy of children, but the histological changes are quite different and, in cases of metaphyseal osteopathy where liver levels of ascorbic acid were measured, they were normal. Hypervitaminosis D and "overnutrition" have also been incriminated, but neither of these, nor imbalances of other nutrients such as calcium, phosphorus, or vitamin A would be expected to produce the inflammatory lesions typical of metaphyseal osteopathy. Arguments in support of a nutritional cause are unconvincing. Based on clinical signs, the suppurative nature of the early lesions, and their predilection for osteochondral complexes of metaphyses and epiphyses, *an infectious etiology, probably bacterial, seems likely*. This possibility was suggested in an excellent description of metaphyseal osteopathy in a litter of Weimaraner pups, which also had suppurative lesions in soft tissues (see Woodard 1982). Although hematogenous osteomyelitis is considered uncommon in dogs, it is a consistent feature of Border Collie puppies with an inherited form of persistent neutropenia, and occurs in some puppies that survive canine parvovirus infection, probably following bacteremia established during a period of neutropenia. The lesions in affected animals are indistinguishable from those of metaphyseal osteopathy (Fig. 1.99). Further support for an infectious etiology in metaphyseal osteopathy is provided by the demonstration of *E. coli* bacteremia in a dog with characteristic radiographic lesions.

A possible link has been suggested between metaphyseal osteopathy and canine distemper vaccination, at least in Weimaraner puppies, but radiographic evidence has not been supported by histopathology. Canine distemper virus mRNA has been detected in osteoblasts and osteoclasts from metaphyseal bone of dogs with metaphyseal osteopathy, but viral mRNA can also be detected in the metaphysis of distemper-infected dogs without metaphyseal osteopathy. Canine distemper virus is known to induce metaphyseal sclerosis secondary to its action on osteoclasts (see above), but this change does not resemble the suppurative, inflammatory lesion of metaphyseal osteopathy.

Based on the clinical and histological evidence available, the concept of an infectious, probably bacterial, etiology for metaphyseal osteopathy is the most appealing. This does not however imply the involvement of a specific agent. Several bacteria are known to cause hematogenous osteomyelitis in other species, and a similar range of organisms may be involved in dogs. Proof of bacterial infection in metaphyseal osteopathy is difficult as most affected animals survive, and those that are subjected to postmortem examination are usually either at an advanced stage of the disease or have been treated with antibiotics prior to death or euthanasia. In reality, many cases of metaphyseal osteopathy caused by bone infection in dogs could remain undetected, especially in dogs that are able to re-establish an effective immune response and eliminate the infection. Perhaps clinical disease is confined to animals with defective immunity or those that receive an overwhelming infection during a period when their defense mechanisms are reduced by an immunosuppressive viral infection.

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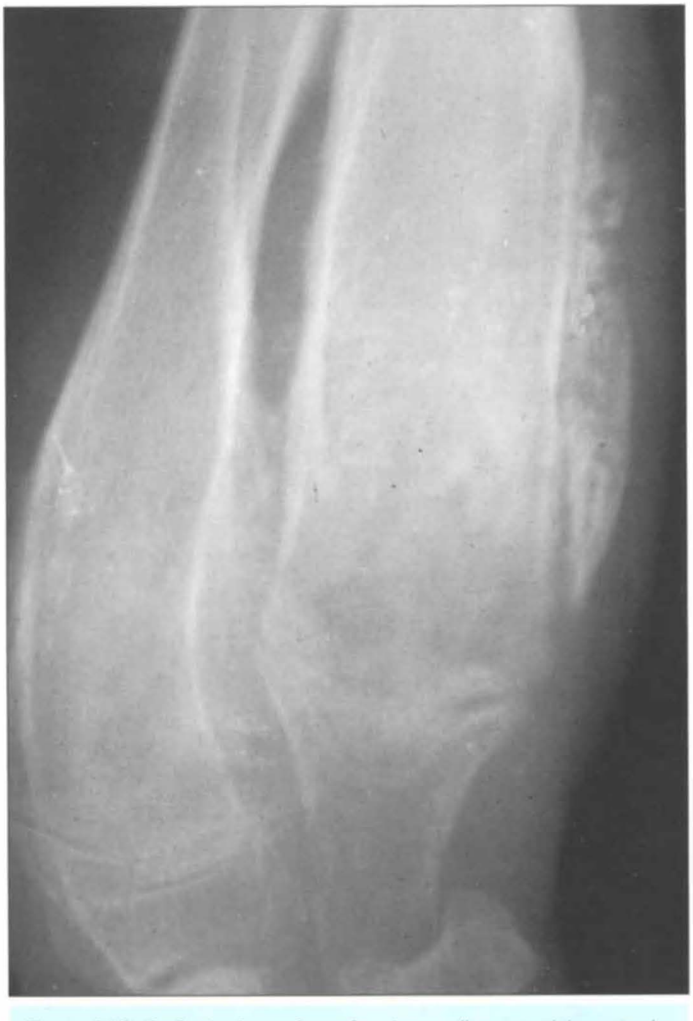

Figure 1.99 Periosteal new bone forming a collar around the metaphysis of the distal radius and ulna in a 5-month-old pup that had survived infection by *Canine parvovirus*. The lesions were bilateral and radiographically, were indistinguishable from chronic metaphyseal osteopathy.

Canine panosteitis

Canine panosteitis (panostosis, juvenile osteomyelitis, enostosis, eosinophilic panosteitis) is a disease of unknown cause that, like metaphyseal osteopathy, usually affects dogs of the large and giant breeds between 5–12 months of age. Occasionally, small dogs such as Miniature Schnauzer and Scottish Terrier are affected and the age range extends from 2 months

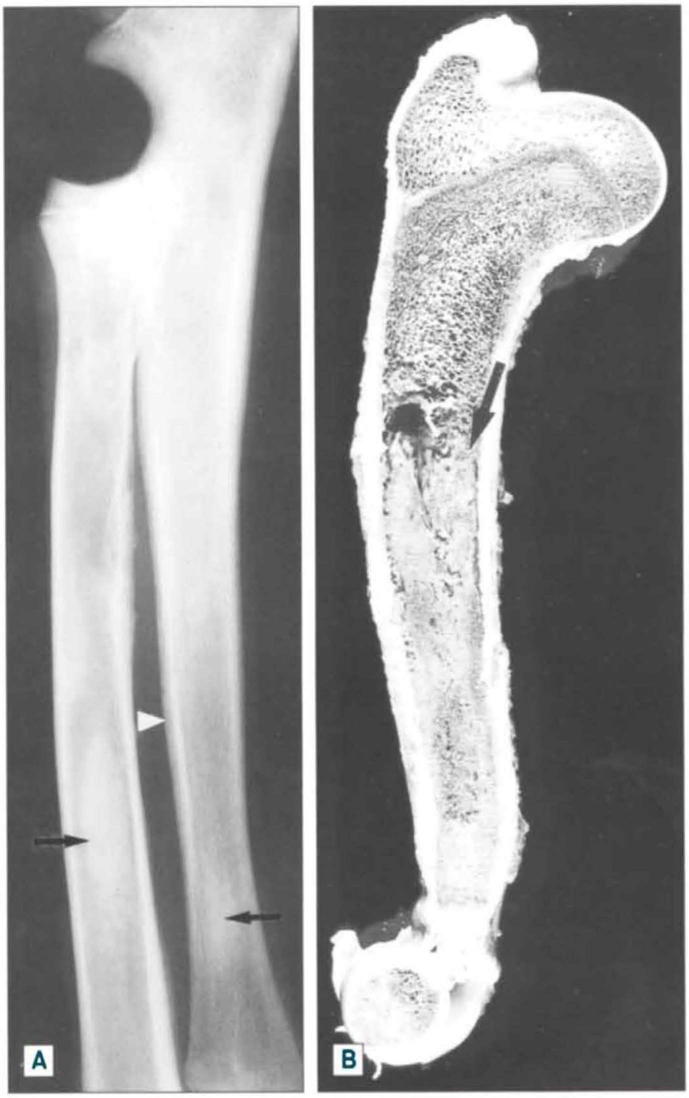

Figure 1.100 Panosteitis, dog. **A.** Radius and ulna showing focal and diffuse medullary densities (arrows) and periosteal reaction (arrowhead). (Courtesy of DC Van Sickle.) **B.** Humeral marrow distal to arrow is filled with woven bone.

to 5 years. The disease was first described as eosinophilic panosteitis of German Shepherds and 75% of reported cases involve this breed. More than two-thirds of affected dogs are males. Although an inflammatory origin for panosteitis is not convincing, the disease is included here for convenience.

Clinical signs vary from *mild to severe lameness*, which may shift from one bone to another. The disease is prone to remission and exacerbation, and is *usually self-limiting* after one to several months. The lameness is associated with abnormalities in the diaphysis of a long bone, usually in the foreleg. Multiple bone involvement occurs in about 50% of cases and rarely, other bones such as ilium and metatarsals are affected.

Because the disease is self-limiting and is well recognized by radiologists, pathologic studies are few and the lesions are defined in terms of their radiographic appearance. At the time when clinical signs begin, no lesions may be visible. About 10 days later, initial involvement of the long bone is seen as an increased density or densities of the medulla in the region of the nutrient foramen. The density may increase in size, sometimes filling the entire medullary cavity, or may remain localized (Fig. 1.100A, B). If the reaction extends to the cortex, marked cortical thickening may result due to periosteal proliferation. When the animal recovers, the increased density disappears over a period of weeks to months.

The initial increased radiodensity is due to expanding areas of fibrovascular tissue in the bone marrow, which are rapidly replaced by woven bone (Fig. 1.101A). This bone is subject to consecutive episodes of formation and resorption, thus reversal and resting lines are often present in the same trabeculae, and osteoclasts and osteoblasts are active in the same microscopic field. Cartilage is sometimes formed in the fibrovascular tissue. Periosteal woven bone formation may be stimulated and this, like the medullary bone, is replaced by lamellar bone before being replaced over the ensuing months (Fig. 1.101B). The older, more mature bone in the center of the medullary lesions is removed first.

In most dogs, there is no evidence of inflammatory infiltrates in the lesions, but sometimes plasma cells and histiocytes are present. Eosinophilia is an inconstant feature of the hemogram and serum chemistry is unremarkable. Abnormalities in clotting times are inconstant findings.

The cause of canine panosteitis is not known. Genetic and allergic factors, hyperestrogenism, filterable agents, and various bacteria have been proposed, but the evidence is inconclusive. A viral etiology, particularly *Canine distemper virus*, has also been suggested because of the pyrexia, tonsillitis, and leukocytosis that occur in some cases. In females, the onset of signs is sometimes associated with estrus and it is thought that other physiological stress may precipitate the disease. The occurrence of panosteitis in dogs with hemophilia A suggests that sometimes the disease might be a sequel to intraosseous hemorrhage.

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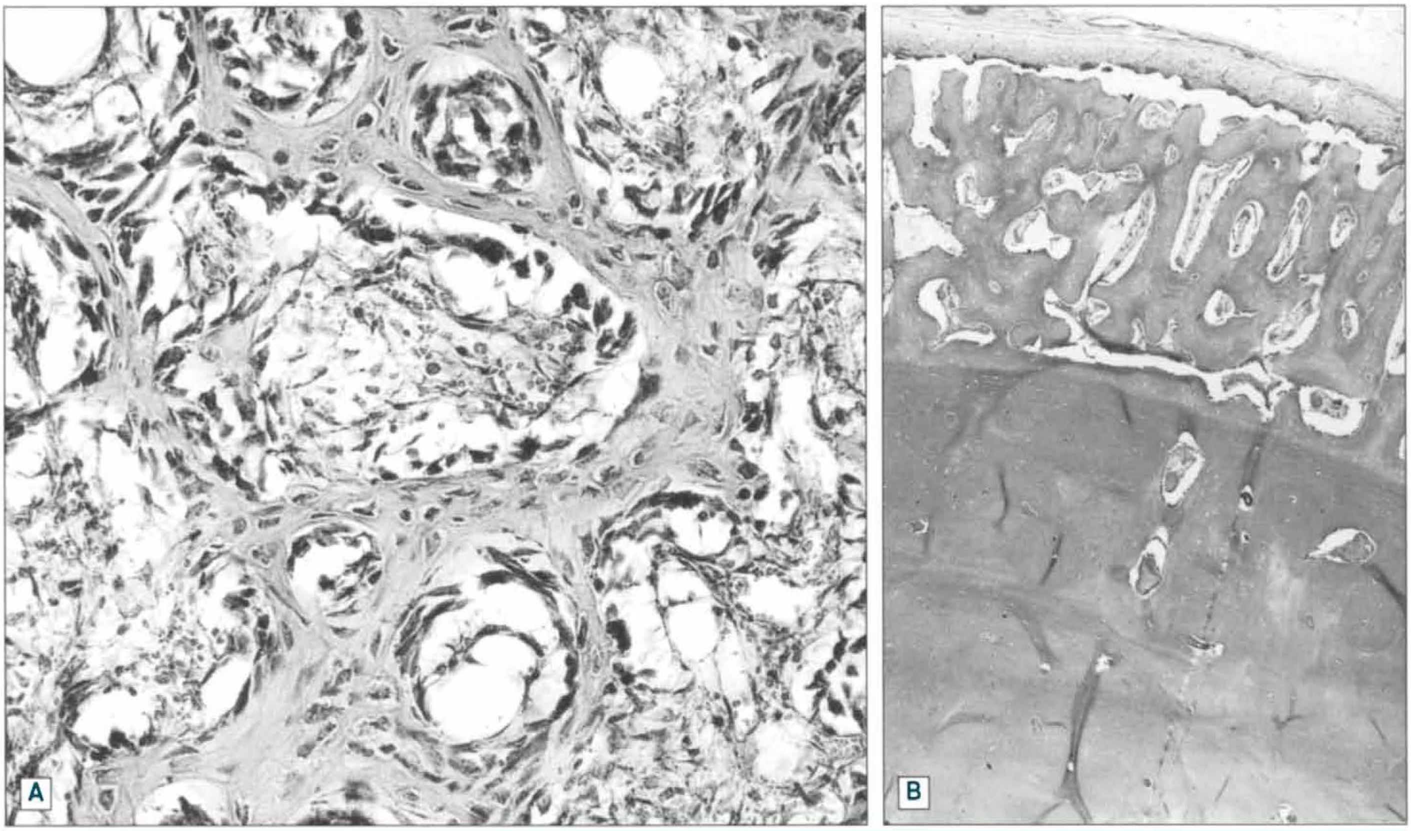

Figure 1.101 Panosteitis, dog. **A.** Rapidly developing trabeculae of woven bone in the marrow cavity. (Courtesy of DC Van Sickle.) **B.** New bone formation beneath the periosteum. (Courtesy of KA Johnson.)

HYPEROSTOTIC DISEASES

Excessive bone formation or hyperostosis occurs as a nonspecific response to various forms of bone injury, including trauma, infection, metabolic disturbances, mineral and vitamin imbalances, subperiosteal hemorrhage, and neoplasia. Many of these have been discussed already in this chapter. The periarticular exostoses or osteophytes that develop in association with degenerative joint disease further illustrate this predictable response of bone to injury or inflammation in adjacent tissues. This section includes a group of poorly understood diseases of humans and animals, characterized by marked hyperostosis in the absence of any apparent cause.

Craniomandibular osteopathy

Craniomandibular osteopathy, also known as *"lion jaw", is a proliferative disorder usually confined to the bones of the skull, in particular the mandibles, occipital, and temporal bones.* The tympanic bullae are often severely affected. Lesions are bilateral but irregular and may also involve the parietal, frontal, and maxillary bones. Occasionally, there is subperiosteal and extraperiosteal proliferation of new bone associated with long bones. The disease is most common in West Highland White Terriers, but is also described in several other breeds, including Scottish Terriers, Labrador Retrievers, Great Danes, Doberman Pinschers, Cairn Terriers, German Wirehaired Pointers, Pyrenean Mountain Dogs, and in a Boxer, a Boston Terrier and an English Bulldog.

The disease is usually recognized at approximately 4–7 months of age because of either discomfort while chewing or inability to open the mouth to eat. Proliferative changes may be palpable or grossly visible, and pain is elicited when attempting to open the mouth. Radiographic findings are characterized by more or less symmetrical enlargement of the mandibles and tympanic bullae, although the lesions may occur independently. Ossification of soft tissues, especially near the angular processes of the mandibles, may result in fusion of the processes to the bullae and ankylosis. The disease has an intermittent, progressive course lasting several weeks to a few months. It is often self-limiting and nonfatal, although some dogs are killed because they cannot eat. During clinical exacerbations, which tend to recur every 2–3 weeks, fever is present but laboratory data are normal.

At the height of the disease, atrophy of the muscles of mastication and increased fibrous and osseous tissue of the head are obvious. The full extent of the bone changes is best demonstrated in radiographs and macerated specimens (Fig. 1.102). In the mandible, new bone deposition is usually most prominent in the region of the angle, involving medial, ventral, and lateral aspects but tending to avoid the alveolar bone. The tympanic bullae are often enlarged two to three times and filled with new bone.

Histologic changes in the mandible involve the endosteum, periosteum, and trabecular bone and present a complex pattern of intermittent and concurrent formation and resorption. The mosaic of reversal and resting lines resulting from this cellular activity (Fig. 1.103) bears some resemblance to the changes seen in Paget's disease of man, but further comparison is unjustified. The significance of this mosaic pattern is easily overemphasized since the mandible of growing dogs is a site of intense bone modeling as teeth mature, erupt, and drift during normal development. Bone resorption in craniomandibular osteopathy appears to be random and disoriented, involving both pre-existing lamellar trabeculae and newly formed woven bone. Similarly, bone formation occurs simultaneously in many areas, evolving from fibrous tissue, which frequently fills the marrow

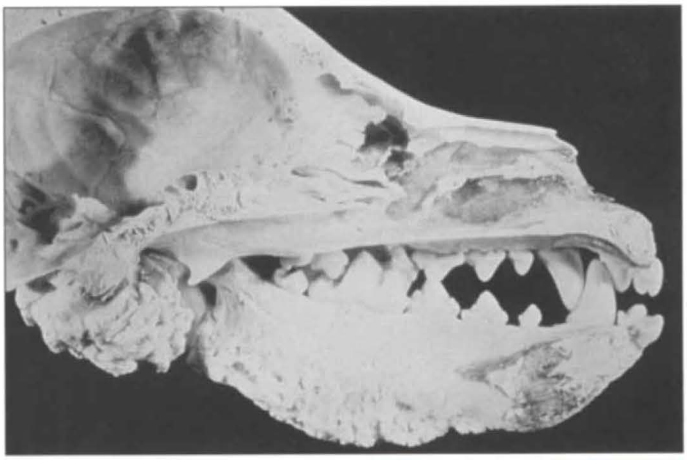

Figure 1.102 Craniomandibular osteopathy, dog. Macerated specimen showing extensive new bone formation along the mandible and around the tympanic bulla.

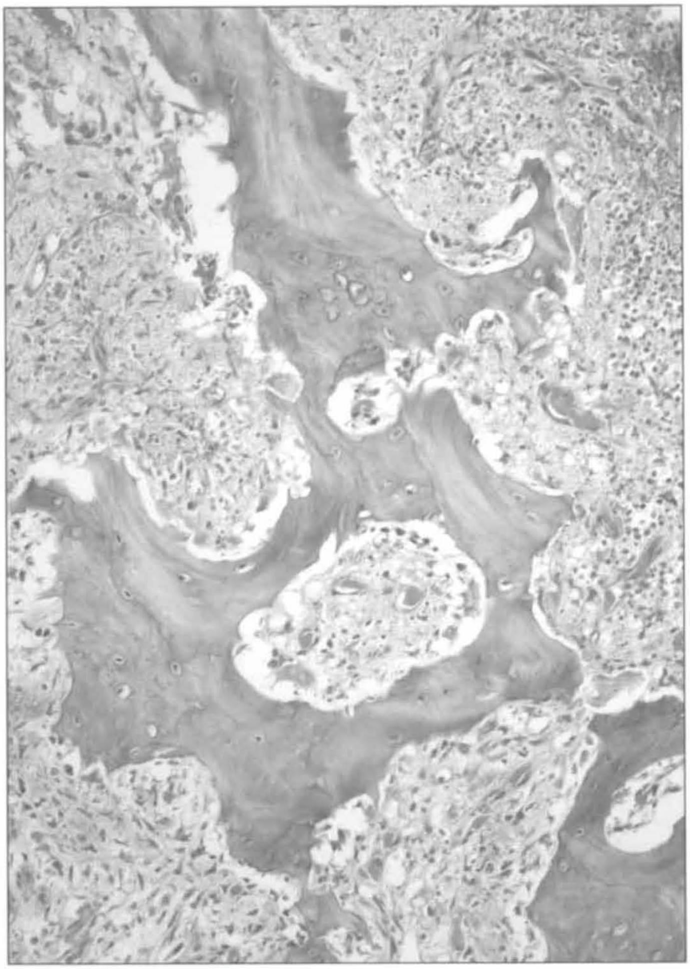

Figure 1.103 Craniomandibular osteopathy, dog. Ragged bone trabeculae with scalloped margin indicating active osteoclastic resorption. The trabeculae contain a mosaic of woven and mature lamellar bone demarcated by cementing lines.

spaces and thickens the periosteum. Lymphocytes, plasma cells, and neutrophils are sometimes present in areas of active osteogenesis.

As the disease subsides, formation of woven bone may be superseded by lamellar bone production, and existing woven trabeculae are replaced by lamellar bone, which is then gradually resorbed. In some cases, especially where fusion of mandibles to tympanic bullae is present, resorption is insufficient to allow restitution of normal function.

The cause of craniomandibular osteopathy is not known. The presence of inflammatory infiltrates raises the possibility of an infectious agent, but none has been demonstrated. In West Highland White and Scottish Terriers, there is evidence of a genetic etiology with autosomal recessive inheritance. However, the occurrence of the syndrome in diverse breeds suggests that there may be more than one cause of the condition. Alternatively, there may be an inherited predisposition in several breeds but the presence of an additional factor, perhaps an infectious agent, is required for the disease to be expressed. The etiology and pathogenesis of a similar condition in children, infantile cortical hyperostosis, are also unknown.

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Hypertrophic osteopathy

This syndrome, which is also known as "Marie's disease" or hypertrophic pulmonary osteopathy, is characterized by diffuse, periosteal new bone formation along the diaphyses and metaphyses of certain limb bones in association with a chronic inflammatory or neoplastic lesion, usually in the thoracic cavity. It occurs in humans, and is reported in several domestic animal species, most often dogs. Occasionally, hypertrophic osteopathy occurs in association with nonthoracic lesions, such as botryoid rhabdomyosarcoma of the urinary bladder in dogs and ovarian tumors in horses. Rarely, the disease occurs in animals with no detectable visceral lesions.

The thoracic lesions associated with hypertrophic osteopathy are diverse. In dogs, it occurs most often with primary or secondary pulmonary neoplasms, but also with granulomatous pleuritis, granulomatous lymphadenitis of bronchial or mediastinal nodes, chronic bronchitis, *Dirofilaria immitis* infection, esophageal granulomas, and tumors provoked by *Spirocerca sanguinolenta,* and with neoplastic disease of the thoracic wall. In horses, it is more often associated with granulomatous inflammatory diseases involving the thoracic cavity. Hypertrophic osteopathy is present only in a minority of animals with thoracic lesions, but the incidence is much higher in some types than in others. For example, dogs with pulmonary metastases from osteosarcoma are much more likely to develop the syndrome than are dogs with metastatic carcinoma. It is also possible that many mild cases of hypertrophic osteopathy are missed both clinically and through incomplete examination of the limb bones at necropsy.

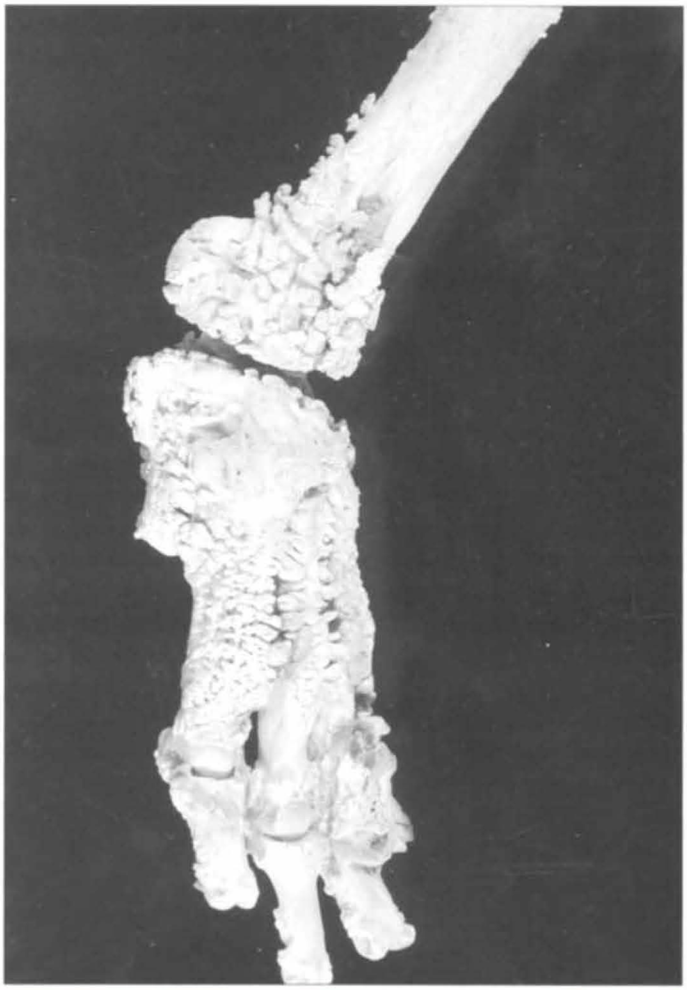

Figure 1.104 Hypertrophic osteopathy, dog. Periosteal new bone involving the metacarpals and radius-ulna, but with relative sparing of the phalanges.

The *periosteal new bone formation in hypertrophic osteopathy is usually confined to the limbs*, and in the dog, involves particularly the radius, ulna, tibia, metacarpals, and metatarsals, with relative sparing of the bones of the upper limbs and phalanges (Fig. 1.104). The more distal limb bones are generally affected first, the lesions then extending proximally. The extent of the osteophytes is best demonstrated in radiographs and macerated specimens where, in florid cases, they appear as wart or cauliflower-like accretions extending along the whole length of the bone (Fig. 1.105). Cross-sections of bone demonstrate the asymmetric development of these accretions, which tend to occur initially and most severely where the periosteum is free of tendinous insertions or adjacent bones. Occasionally, osteophytes occur on the vertebrae and skull, and minor endosteal lesions occur infrequently. *Articular surfaces are not involved in animals but are often affected in human cases*, hence the frequent use of the term hypertrophic osteoarthropathy for the human disease. Although the lesions of hypertrophic osteopathy are progressive, they regress if the primary thoracic lesion is removed.

Histologically, the earliest changes are hyperemia and edema, with proliferation of highly vascular connective tissue in the periosteum, in some cases leading to extensive hemorrhage. These changes are associated with a rapid increase in peripheral blood flow in the distal half of the limbs and may be accompanied by a light infiltrate of lymphocytes and plasma cells. Shortly thereafter, osteoblasts deposit osteoid on the existing cortical bone and then, if the disturbance is severe, begin to lay down new trabeculae perpendicular to the original cortex. The new bone may be deposited with extraordinary rapidity, and the width of the cortex may be doubled in a few weeks. In the early and active stages of the disease, the new bone is clearly distinguishable from the original cortex because the former is trabecular and the latter compact. Later, the distinction may be less distinct because the original cortex is converted to spongy bone by resorption from within the vascular canals and the endosteal surface.

The *pathogenesis of hypertrophic osteopathy is obscure* but increased blood flow to the limbs is a consistent early change. Several hypotheses have been proposed, including hypoxia, arteriovenous shunting, neurogenic and humoral mechanisms, but none consistently fits the clinical and experimental observations. According to the *neurogenic theory*, impulses originating in the thoracic lesion travel via the vagus nerve to the brainstem and initiate reflex vasodilation in the limbs, either by humoral or neural means. Limited support for this theory is provided by the regression of bone lesions in some, but not all, human and canine cases treated by vagotomy. The *humoral hypothesis* proposes either that the primary lesion produces a hormone or hormone-like substance or that arteriovenous anastomoses in the primary lesion prevent catabolism of such a substance in the lung. The hormone then produces the secondary skeletal lesions. Support for this hypothesis is derived from the production of typical skeletal lesions following experimental venoarterial shunts in dogs, but there is no evidence of such shunts in most spontaneous cases. In a very limited number of cases, Marie's disease has been restricted to one limb, and vascular abnormalities are generally found in those cases. It has also been suggested that reduced oxygenation of peripheral blood, resulting from congenital or acquired arteriovenous shunts, stimulates periosteal osteophyte production. More recently, it has been proposed that the primary lesion in hypertrophic osteopathy might cause inappropriate stimulation of an extra-renal volume receptor system controlled by innervation of blood vessels by branches of the vagus and glossopharyngeal nerves. It is suggested that stimulation of this system as a result of blood flow in anomalous vascular channels associated with the primary thoracic lesion, may activate the cerebral salt center leading to retention of extracellular sodium, fluid retention, and increased blood supply to the limbs. This does not however explain the occurrence of hypertrophic osteopathy in animals in association with tumors of the urinary bladder or ovary.

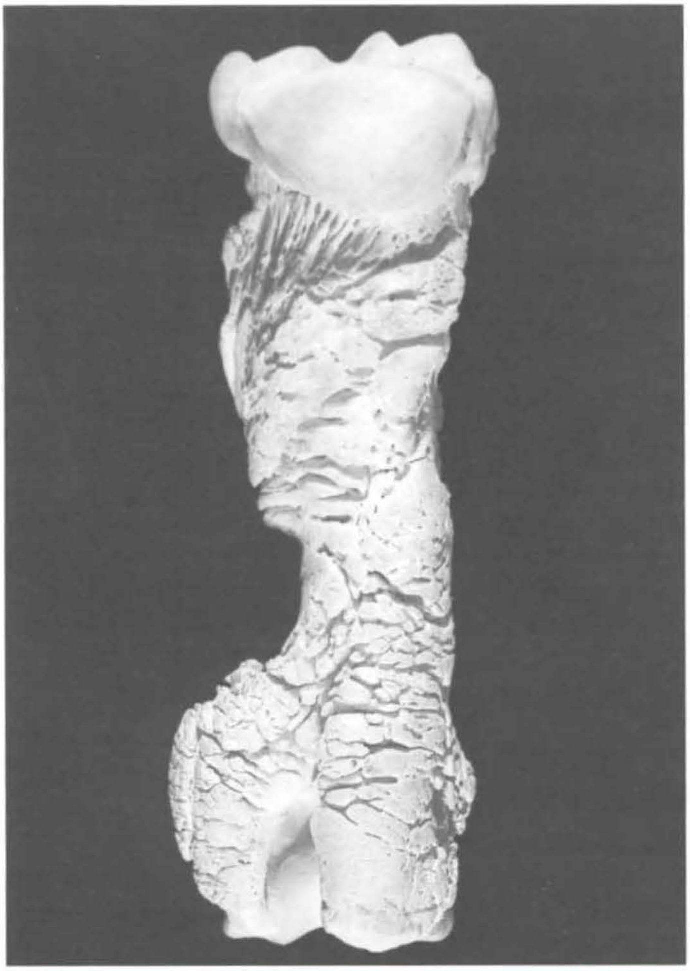

Figure 1.105 Hypertrophic osteopathy, horse. Florid periosteal reaction along the shaft of the humerus.

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Canine diffuse idiopathic skeletal hyperostosis

Diffuse idiopathic skeletal hyperostosis is a hyperostotic disorder of man that appears to have a counterpart in dogs, particularly large and giant breeds such as Labrador Retrievers and Great Danes. *The disease in dogs is characterized by progressive new bone formation along vertebral bodies, sometimes leading to fusion; progressive osseous fusion of the ilium, pubis, femur, and ischium; and development of para-articular bony lesions around certain joints of the appendicular skeleton.*

The vertebral exostoses tend to occur at ligamentous insertions (enthesiophytes) and appear to vary in their distribution between different regions of the spine. The lesions are more extensive than those of ankylosing spondylosis, involving the ventral and lateral aspects of three or more contiguous vertebral bodies, and are not accompanied by evidence of disk degeneration. Furthermore, the new bone formation also occurs on vertebral arches, dorsal spinous processes, and articular processes, where it is very unlikely to be associated with degenerative disk disease. The initial para-articular lesions in the appendicular skeleton are fibrocartilaginous, but undergo endochondral ossification to produce bony lesions, which may progress to result in joint fusion. Intra-articular structures are not affected.

There are several other hyperostotic diseases in dogs that must be differentiated from diffuse idiopathic skeletal hyperostosis. These include ankylosing spondylosis, spondylosis deformans, spinal osteoarthrosis, vitamin A toxicity, and hepatozoonosis, all of which are discussed elsewhere in this chapter. *Radiographic criteria* that might suggest a diagnosis of diffuse idiopathic skeletal hyperostosis in dogs include the following:

- flowing calcification and ossification along ventral and lateral aspects of three contiguous vertebral bodies leading to bony ankylosis;
- preservation of normal disk width in affected areas and absence of significant degenerative disk disease, such as end-plate sclerosis, nuclear calcification and localized spondylosis deformans;
- osteophytes around vertebral articular facets;

- formation of pseudoarthroses between the bases of spinous processes;
- periarticular osteophytes in addition to calcification and ossification of soft-tissue attachments (enthesiophytes) in the axial and appendicular skeleton;
- periarticular osteophytes, sclerosis and ankylosis of sacroiliac joints; and
- bony ankylosis of the symphysis pubis.

The presence of any four of the first five criteria is considered sufficient to support the diagnosis.

The *etiology of diffuse idiopathic skeletal hyperostosis is unknown*, but it appears to represent an exaggerated response to stimuli that would normally induce little, if any new bone formation. For this reason, *the disease has been referred to as an ossifying diathesis*. In human patients with diffuse idiopathic skeletal hyperostosis, excessive heterotopic ossification occurs after hip surgery, and in dogs, the formation of periarticular osteophytes is out of proportion to any degenerative changes in the associated joint. No predisposing cause can be found for most of the skeletal lesions in this syndrome but their close association with joints, and insertion sites for ligaments, tendons, and muscles cannot be ignored. Perhaps minor traumatic episodes at these sites result in excessive local production of specific growth factors, (e.g., TGF-β, FGFs, and BMPs) in susceptible individuals.

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Canine hepatozoonosis

Periosteal new bone formation, similar to that occurring in hypertrophic osteopathy, is a feature of canine hepatozoonosis caused by *Hepatozoon americanum* infection of dogs in the southern USA. The osseous lesions also consistently develop in experimentally infected dogs and coyotes. *The periosteal reaction occurs primarily on the diaphyseal regions of the more proximal limb bones* (Fig. 1.106A), but also may involve bones of the axial skeleton, including the ilium (Fig. 1.106B) and vertebrae, the lesions differing in distribution to those of hypertrophic osteopathy. In early reports of canine hepatozoonosis, it was suggested that the periosteal new bone formation was secondary to local irritation caused by the presence of *Hepatozoon americanum* cysts in adjacent muscle fibers. The absence of microscopic evidence to support this hypothesis, and the widespread, often symmetrical, distribution of lesions *suggests that humoral rather than local factors are involved in the pathogenesis of the bony lesions.*

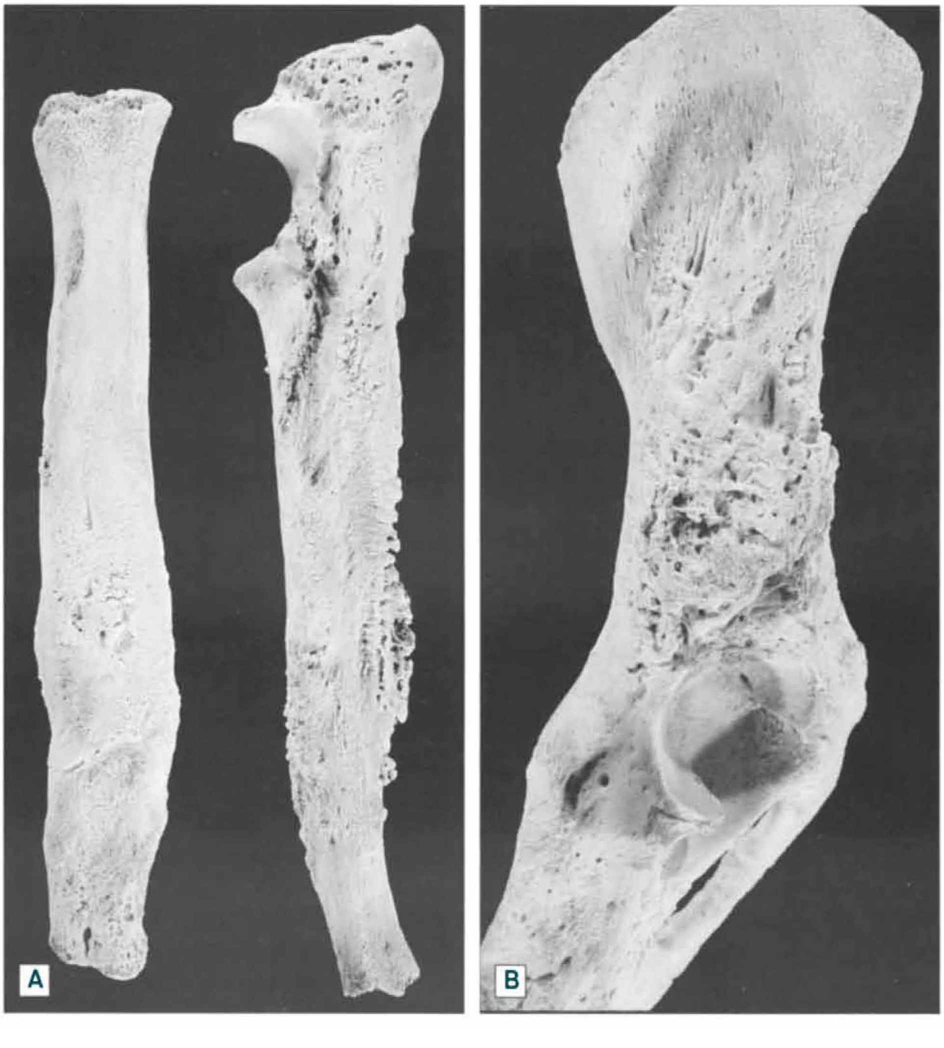

Figure 1.106 Canine hepatozoonosis. A. Periosteal reaction along the diaphyses of the radius and ulna of a 1-year-old dog that had been infected with *Hepatozoon americanum* for approximately 9 months. **B.** Ilium from the same dog, showing prominent periosteal reaction.

Clinical signs of canine hepatozoonosis include pyrexia, depression, weakness, muscle atrophy, and gait abnormalities ranging from stiffness to recumbency. Leukocytosis due to mature neutrophilia, and sometimes a left shift, is a consistent laboratory finding. The disease leads to *chronic debilitation and eventual death*. Different stages of this protozoon agent may be found in skeletal muscles throughout the body, often inducing a pyogranulomatous inflammatory response. The parasite is not present in bone, but may be detected in muscle fibers close to the periosteal surface. Except for a single report from Japan, periosteal new bone formation has not been described in association with *Hepatozoon canis* infection, which occurs in the Old World. The agent of canine hepatozoonosis in North America was initially considered a more virulent strain of *Hepatozoon canis*, but differences in the nature and distribution of lesions, in addition to morphologic differences in gamonts, led to its classification as a separate species.

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TUMORS AND TUMOR-LIKE LESIONS OF BONES

Primary tumors of bones are common in dogs and to a lesser extent in cats, but occur infrequently or rarely in other domestic animals. They may arise from any of the mesenchymal tissues present in bones, including precursors of bone, cartilage, fibrous tissue, adipose tissue, and vascular tissue, but *tumors of bone and cartilage-forming cell lines are the most common*. In humans, most primary tumors of bones are benign but this does not apply to all animal species. In dogs, most tumors of bones are malignant. Benign and malignant tumors of bones occur with approximately equal frequency in cats, but in horses, cattle, and other domestic animals, benign tumors of bones are much more common than malignant ones. Secondary tumors of bones, in particular metastatic carcinomas, are very common in humans but are seldom diagnosed in animals. The prevalence of secondary bone tumors in animals is no doubt underestimated as the skeleton is seldom examined in detail either radiographically or at necropsy, but it is almost certainly much lower than in humans. Several comprehensive surveys and reviews of bone tumors have been published and are included in the bibliography at the end of this section.

The classification of primary tumors and tumor-like lesions of bones in domestic animals is generally similar to that developed for humans, but there are too many differences in morphology and prognosis to allow direct extrapolation. The system used in this chapter is based on that proposed in the most recent World Health Organization fascicle on *Histological Classification of Bone and Joint Tumors of Domestic Animals* (Table 1.3). Rather than discuss benign and malignant tumors separately, as in the WHO system, the most important of these tumors will be grouped according to the nature of the predominant matrix that they produce. This reflects the manner in which a pathologist is likely to approach the diagnosis of a bone tumor.

Table 1.3 Histological classification of tumors and tumor-like lesions of bones*

Benign tumors
- Chondroma
- Feline osteochondromatosis
- Hemangioma
- Myxoma of the jaw
- Ossifying fibroma
- Osteoma
- Osteochondroma

Malignant tumors
- Central
    1. Chondrosarcoma
    2. Fibrosarcoma
    3. Giant cell tumor of bone
    4. Hemangiosarcoma
    5. Liposarcoma
    6. Multilobular tumor of bone
    7. Osteosarcoma
        a. poorly differentiated
        b. osteoblastic
            — nonproductive
            — productive
        c. chondroblastic
        d. fibroblastic
        e. telangiectatic
        f. giant cell type
- Peripheral
    1. Maxillary fibrosarcoma (dogs)
    2. Parosteal osteosarcoma
    3. Periosteal chondrosarcoma
    4. Periosteal fibrosarcoma
    5. Periosteal osteosarcoma
- Tumors of bone marrow
    1. Malignant lymphoma
    2. Plasma cell myeloma

Tumor-like lesions
- Aneurysmal bone cyst
- Epidermoid cyst of the phalanx
- Exuberant fracture callus
- Fibrous dysplasia
- Solitary (unicameral) bone cyst
- Subchondral (juxtacortical) bone cyst

**Based on World Health Organization International Histological Classification of Tumors of Domestic Animals.*

It is often difficult to classify a bone tumor into one category or another with confidence, as the predominant matrix may vary throughout the tumor and poorly differentiated sarcomas may reveal little evidence of their lineage. Since the prognosis of different bone tumors varies markedly, accurate classification by the pathologist will greatly assist the clinician or surgeon and should be attempted, but only in cases where sufficient tissue and information is provided. The pathologist must not feel pressured into making a definitive diagnosis in cases where either the specimen or the history is inadequate. There are many benign lesions of bones that may resemble malignant bone tumors and the consequences of an incorrect diagnosis can be substantial.

The clinical history and radiographic appearance are often crucial to an accurate diagnosis of a bone lesion and the pathologist should not rely on microscopic features alone. In addition to knowledge of the species, breed, and age of the animal, it is important to be aware of the location of the lesion and its duration. Certain bone tumors in man and animals occur with increased frequency at specific sites, hence the value of knowing the exact location of the lesion. It is also important to know if there has been a previous fracture or infection at the site, or whether the animal has recently suffered from a systemic disease or soft tissue tumor, which may have spread to the bone. Radiographs provide valuable additional information on the extent, nature, and behavior of a bone tumor. They can also be of considerable value to the surgeon or pathologist in establishing a list of likely differential diagnoses, and selecting sites for histological or cytological examination. Radiographs of a bone lesion at necropsy will often provide more information of diagnostic relevance than gross examination of the affected bone, but the radiographic appearance can seldom allow reliable differentiation of primary tumors, secondary tumors, and inflammatory lesions of bones.

Proliferation of new, woven bone is a common response to various forms of bone injury and should not be misinterpreted as osteosarcoma. This reactive bone may be deposited beneath an elevated periosteum and form the so-called *Codman's triangle* (see Fig. 1.16), which often occurs in osteosarcoma but also in other lesions where the periosteum is separated from the underlying bone. When the periosteum is breached, the radiating spicules of reactive bone expanding out from the original lesion may create a "sunburst" effect in radiographs. Reactive bone is not confined to the periosteal surface. Many primary and secondary tumors growing within the bone marrow stimulate endosteal new bone formation, as do many inflammatory lesions. In the early stages of formation, reactive bone consists of rapidly proliferating, immature mesenchymal cells producing spicules of unmineralized osteoid. These spicules usually merge with broader trabeculae of woven bone lined by a single layer of plump osteoblasts (Fig. 1.107), unlike osteosarcoma, where the spaces between spicules or islands of tumor bone are generally filled with malignant osteoblasts (see Fig. 1.109B). However, in small biopsy samples, differentiation of early reactive bone from osteosarcoma may be extremely difficult, if not impossible.

In addition to new bone formation, *osteolysis is also a feature of many bone tumors* and osteoclasts can often be seen resorbing mature bone as the advancing tumor permeates the marrow cavity or invades the cortex. The gross and radiographic appearance of a bone tumor reflects the balance between bone resorption and new bone formation. *Biopsy samples, or samples collected for histology or cytology from a suspected bone tumor during necropsy, should always include areas of lysis, as these areas are most likely to contain cells of diagnostic significance.* Too often, samples submitted to the pathologist for examination consist entirely of reactive bone and no diagnosis is possible. In such cases, it is important that the pathologist does not exclude the possibility of an underlying tumor and if the history and radiographic appearance suggest neoplasia, examination of further biopsies, including areas of lysis, is recommended.

Bone-forming tumors

Osteoma, ossifying fibroma, and fibrous dysplasia

These benign tumors are uncommon in domestic animals and there is considerable confusion over their classification and diagnosis. All three arise primarily from the bones of the skull, particularly those bones that develop by intramembranous ossification. Fibrous dysplasia is not a true neoplasm of bones but is included in this group because of the need to differentiate it from osteoma and ossifying fibroma. Furthermore, some authors have suggested that osteomas are hamartomas rather than true tumors, while others claim that they represent the sclerotic end stage of fibrous dysplasia.

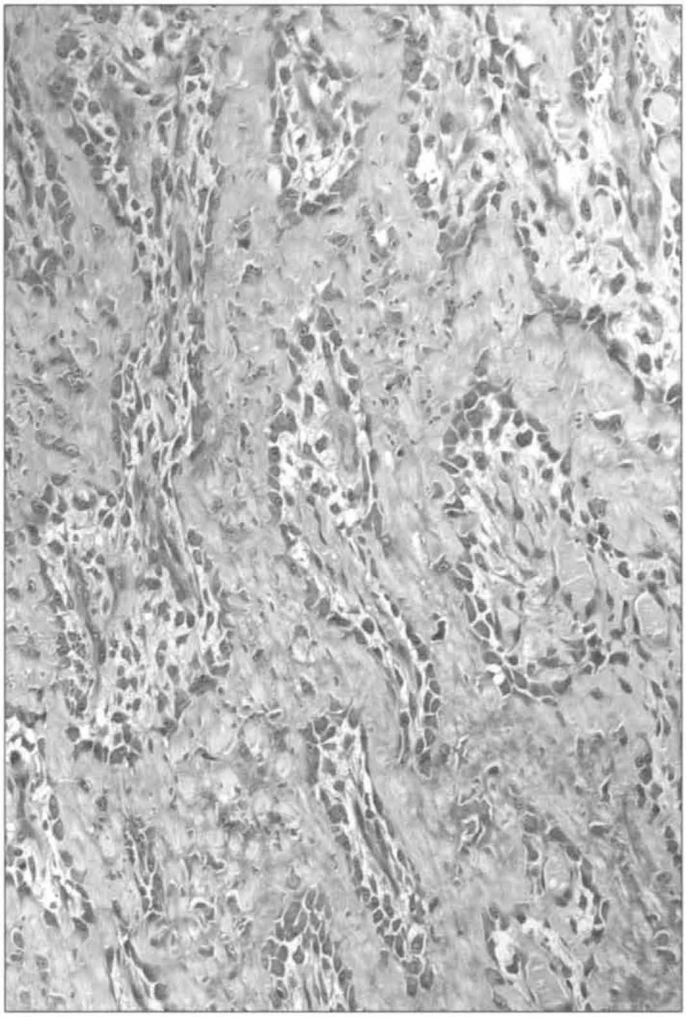

Figure 1.107 Trabeculae of **reactive, woven bone** lined by a single layer of plump osteoblasts.

An **osteoma** is a *dense, smoothly contoured, protruding mass of well-differentiated bone* originating from a periosteal surface and merging with the underlying bone. Exostoses induced by localized periosteal damage may closely resemble osteomas, but osteomas generally show *slow, progressive enlargement* and sometimes achieve a very large size. They are found most often in the mandible, maxilla, nasal sinuses, and bones of the face and cranium in horses and cattle, but also occur in other species. Osteomas occasionally arise from bones of the pelvis, or tubular bones of the limbs, and *must be differentiated from osteochondromas.* In osteochondroma, the bone forms by endochondral ossification beneath a cap of hyaline cartilage and there is continuity between the intertrabecular spaces of the tumor and the marrow cavity of the bone.

Osteomas are often extremely hard, especially if they have been growing for some time, and must be cut with a saw. Clinical signs, other than disfigurement, may be nonexistent or may relate to compression of adjacent structures.

Histologically, osteomas consist of trabecular bone formed by osteoblasts, remodeled by osteoclasts and ultimately converted almost entirely to *lamellar bone*. The trabeculae are gradually thickened by appositional growth so that ultimately the spaces between them are relatively inconspicuous, and the density of the tumor mass approaches that of mature cortex. Such tumors are virtually indistinguishable from exostoses; location and clinical history should be considered in differentiating the two. Actively expanding osteomas have a border of connective tissue resembling periosteum and the slender trabeculae forming beneath this layer are arranged approximately perpendicular to the surface. The superficial trabeculae of an osteoma may consist of woven bone while those in deeper regions are broader and consist largely or completely of lamellar bone.

Osteoid osteoma, a distinct form of osteoma in humans, has also been reported in a cat. This tumor is characterized by a discrete, intra-osseous nidus of active bone formation in which disorganized trabeculae are surrounded by a rim of sclerotic bone. The trabeculae of immature bone are lined by plump osteoblasts but show no evidence of malignancy.

Ossifying fibroma is a rare fibro-osseous tumor of the head, occurring most frequently in young horses less than a year of age, and primarily involving the rostral mandible. There are occasional reports of the tumor in cats, dogs, and sheep. Typically, ossifying fibroma is a sharply demarcated, expansile mass, which distorts the normal contours of the affected bone. The tumor *consists of spindle-shaped fibroblasts, which appear to transform into osteoblasts and form irregular spicules of woven bone*, which are lined by plump osteoblasts. Lamellar bone is extremely rare in ossifying fibroma, but may be formed where woven bone trabeculae are resorbed and replaced.

There are several lesions from which ossifying fibroma must be distinguished, including osteoma, fibrous dysplasia, osteosarcoma, and perhaps even fibrous osteodystrophy, especially in horses. In osteomas, the spaces between trabeculae may contain marrow rather than fibrous connective tissue, and the trabeculae are larger and denser with a much greater proportion of lamellar bone. The bone spicules of fibrous dysplasia are usually more uniform and are not rimmed by osteoblasts. The connective tissue cells of ossifying fibroma lack the pleomorphism and high mitotic index of osteosarcoma and the bone is formed in a more regular and uniform manner. Confusion with fibrous osteodystrophy is unlikely as long as an adequate history accompanies a small bone biopsy. The lesions of fibrous osteodystrophy are bilaterally symmetrical and large osteoclasts are often present amongst the loose connective tissue surrounding trabeculae of woven bone.

The biologic behavior of ossifying fibroma is obscure but *malignant transformation is not known to occur*. Bone is not an inert tissue and it seems reasonable that remodeling of woven trabeculae and replacement by lamellar bone within the tumor could occur. If so, then a maturing ossifying fibroma might eventually resemble an osteoma with hematopoietic cells colonizing spaces between trabeculae of lamellar bone.

Fibrous dysplasia is a rare, fibro-osseous lesion of bone that is thought to be a *developmental abnormality of bone-forming mesenchyme rather than a neoplasm*. It is characterized by smoothly contoured, expanding fibro-osseous lesions involving one or several bones. Although well documented in humans, there are few reliable reports of fibrous dysplasia in animals, but there seems little doubt that the condition does occur in several animal species, including horses, dogs, and domestic cats. As in humans, young animals are most commonly affected.

Some published reports of fibrous dysplasia in animals are questionable, the lesions in some cases appearing more consistent with ossifying fibroma. Furthermore, a description of familial canine polyostotic fibrous dysplasia in three Doberman Pinscher pups most likely represents a polyostotic form of unicameral bone cysts. It is also possible that some cases of fibrous dysplasia have been misdiagnosed as various tumors of bones.

Osteosarcoma

This malignant tumor is the most common primary neoplasm of the appendicular skeleton in dogs and cats. In general, it is a rapidly progressive tumor with early metastasis to the lungs leading to early mortality. Most osteosarcomas arise from within bones, particularly in the metaphyseal regions of long bones, and are referred to as **central osteosarcomas**. Less commonly, osteosarcomas arise in the periosteum or even in extraskeletal tissues. Two types of **peripheral osteosarcoma** may arise in the periosteum. One is referred to as **periosteal osteosarcoma** and may show similar biologic behavior to central osteosarcoma; the other is **parosteal (juxtacortical) osteosarcoma**, which shows greater differentiation, slower growth, and a much better prognosis than central osteosarcoma.

Osteosarcoma accounts for approximately 80% of primary bone tumors in dogs and 70% in cats. In **dogs**, the mean age of occurrence is around 7.5–8 years, but the range is broad and animals less than 2 years of age are sometimes affected. The tumor occurs predominantly in large and medium-sized breeds and has a strong site preference. The appendicular skeleton is affected three to four times as often as the axial skeleton and the forelimbs 1.6–1.8 times as often as the hindlimbs. Most osteosarcomas in the forelimbs involve the *metaphyses of either the distal radius or proximal humerus* (Fig. 1.108A). The *distal femur and proximal tibia* (Fig. 1.108B) are favored sites in the hindlimb. Approximately 50% of osteosarcomas of the axial skeleton occur in bones of the head and 50% in the ribs, vertebrae, and pelvis. Osteosarcomas of the appendicular skeleton occur more often in male dogs than in females, but this sex difference does not appear to apply to tumors of the axial skeleton. In addition to the sites mentioned above, osteosarcomas occasionally occur at sites of chronic irritation and repair, such as those associated with osteomyelitis, bone infarcts, or the presence of an internal fixation device. In such cases, the tumor may originate from the diaphyseal region of long bones, or other locations not normally considered predilection sites for osteosarcoma.

The mean age of osteosarcoma in **cats** is 10.5 years (range 3–18 years) and there is no apparent breed predisposition. Most studies of osteosarcoma in cats involve fewer cases than in dogs and information on predilection sites is less precise. Although the appendicular skeleton appears to be involved more frequently than the axial skeleton, the ratio is less than in dogs and there is no apparent predilection for the forelimbs. Unlike dogs, most osteosarcomas in cats arise in the diaphyses of long bones, rather than the metaphyses.

Osteosarcomas are rare in domestic animals other than dogs and cats, but are reported occasionally in horses, cattle and sheep. In these species, the tumor generally involves bones of the head, particularly the mandible.

The *gross and radiographic appearance of central osteosarcoma varies markedly*, depending on the behavior of the tumor cells and the matrix they produce. Some subtypes are predominantly lytic, some

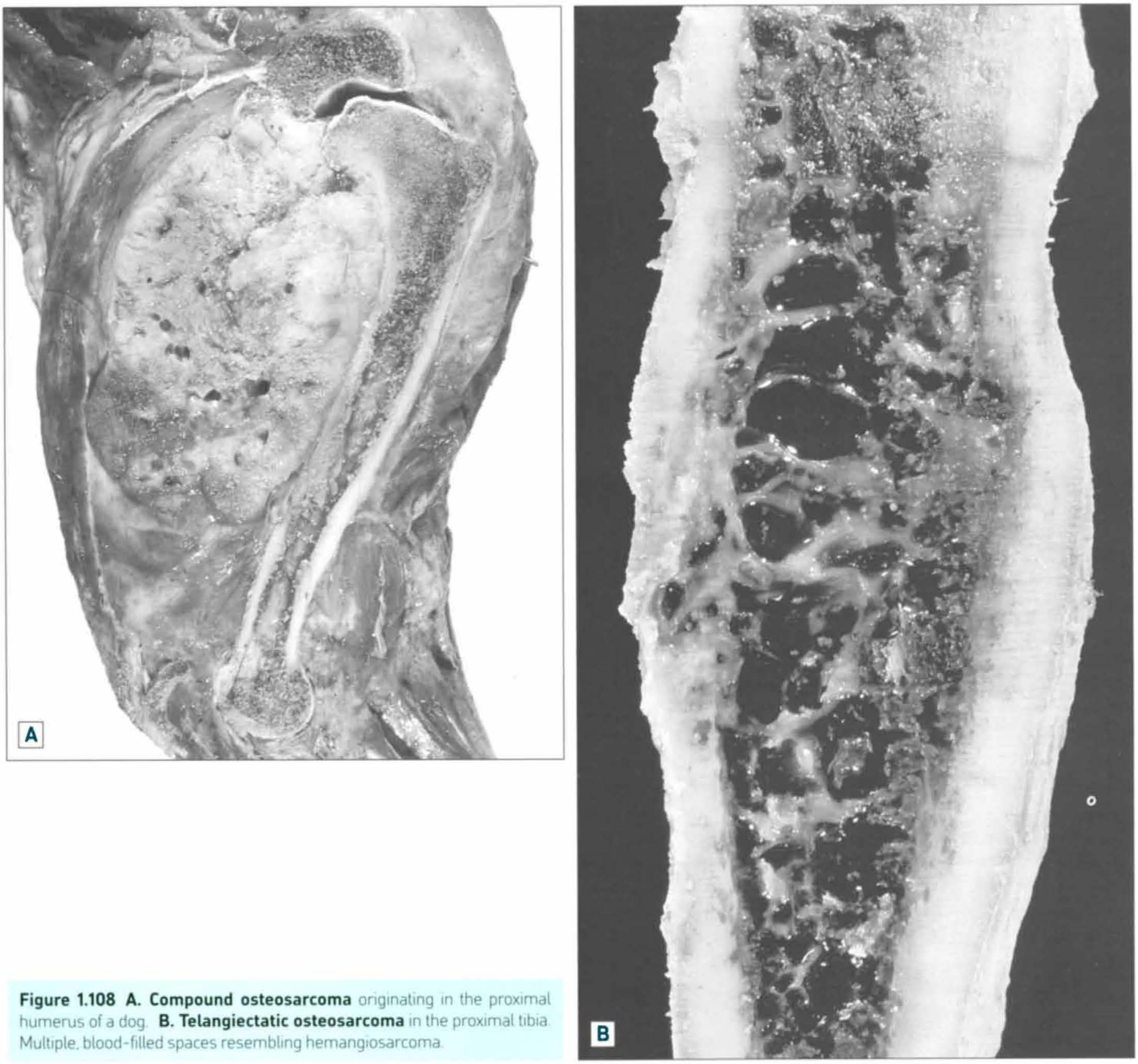

Figure 1.108 A. Compound osteosarcoma originating in the proximal humerus of a dog. **B. Telangiectatic osteosarcoma** in the proximal tibia. Multiple, blood-filled spaces resembling hemangiosarcoma.

are productive, while others comprise a mixture of both destructive and proliferative elements. Assessment of the gross features is greatly enhanced by radiographs of dissected and bisected specimens. Radiolucent or osteolytic tumors are usually hemorrhagic and soft, and often contain light yellow areas of necrosis. Pathological fractures may accompany erosion of cortical bone and osteolytic tumors tend to invade the adjacent soft tissues early in their course. Radiodense tumors are various shades of gray on gross examination and areas of tumor bone and tumor cartilage give a stippled appearance to radiographs and a gritty texture when cut. In compound osteosarcomas, tumor cartilage is sometimes recognizable by its texture and color, and tends to be located at the periphery of the neoplasm. Productive tumors frequently provoke a more marked periosteal reaction than the osteolytic tumors. Osteosarcomas of long bones commonly, perhaps invariably, erode cortical bone but although they tend to invade the epiphysis rather than the diaphysis, they rarely penetrate the adjacent articular cartilage. Telangiectatic osteosarcomas are soft and dark red, and closely resemble hemangiosarcomas grossly.

The *histologic appearance of osteosarcomas varies markedly,* but the production of osteoid and/or tumor bone by malignant osteoblasts is a common factor. The tumor matrix may also contain variable quantities of cartilage and collagen, but *if tumor osteoid is present then the tumor is classified as osteosarcoma.* This reflects the greater malignant potential of malignant osteoblasts than either chondroblasts or fibroblasts. Bone formation also occurs in some chondrosarcomas, but indirectly through endochondral ossification of tumor cartilage, and should not be misinterpreted as tumor bone. Similarly, osseous metaplasia of multipotential mesenchymal cells in tumors of nonosseous origin, such as mammary carcinomas, may create confusion.

Malignant osteoblasts may be spindle-shaped cells resembling fibroblasts or plump, oval or rounded cells with basophilic cytoplasm and eccentric, hyperchromatic nuclei, more closely resembling non-neoplastic osteoblasts. Mitotic figures are often very common. The nature and amount of osteoid is highly variable. In some cases, it consists of hyaline, eosinophilic material arranged in thin strands or narrow ribbons amongst the malignant cells, producing a lace-like pattern (Fig. 1.109A). In others, the osteoid is present as irregular islands or spicules separated by malignant osteoblasts (Fig. 1.109B). Thin strands of osteoid may closely resemble collagenous fibrous tissue and distinguishing the two forms of matrix may be impossible. Both are birefringent, reflecting their collagen content, and reliable differentiation is not possible using histochemical stains. Fibrin in areas of hemorrhage may also be mistaken for osteoid, but is not birefringent. In general, osteoid is less fibrillar than collagen, more amorphous, and often partly or completely surrounds the tumor cells, entrapping them in lacunar spaces.

Formation of endosteal and periosteal new bone is a feature of a variety of inflammatory and neoplastic bone lesions, not just osteosarcoma, and must be differentiated from tumor bone. This may be difficult in small biopsy samples. Distinguishing between osteosarcoma and an early fracture callus presents an even greater challenge. Rapidly proliferating, plump mesenchymal cells surrounding themselves with osteoid are a feature of fracture repair (see Fig. 1.19), especially during the first few days, and may easily be confused with osteosarcoma. Evidence of maturation within the callus is a useful differentiating feature, and the cell population would be expected to show less atypia than most osteosarcomas. Not surprisingly, the histologic appearance may be extremely difficult to interpret in cases where pathological fracture has occurred in association with an osteosarcoma. *Knowledge of the clinical history and radiographic findings are particularly important adjuncts to histology in such cases.*

Multinucleate giant cells with features of osteoclasts are often scattered throughout osteosarcomas, sometimes in sufficient numbers to suggest a diagnosis of giant cell tumor, but demonstration of osteoid production by the tumor cells allows exclusion of the latter option. In rapidly growing osteosarcomas, large areas of coagulation necrosis and hemorrhage are often present, probably due to localized ischemia.

As shown in Table 1.3, osteosarcomas may be subclassified according to the predominant histologic pattern. This scheme is an adaptation of a system developed for use in human medicine and is now well accepted by veterinary pathologists. *Subclassification of osteosarcomas can be justified in that it may lead to the identification of correlations of osteosarcoma subtype with prognosis, and susceptibility to therapy.* For example, fibroblastic osteosarcomas in dogs and human patients have been shown to possess a relatively favorable prognosis, while the prognosis for the telangiectatic form is very poor. However, the exercise is not always straightforward because of the inherent variability present within many osteosarcomas, and the fact that a subdominant pattern may appear to be the most malignant. *Classification into one of the six categories is determined by the predominant pattern in representative sections from the tumor*, but if no single pattern is dominant, the tumor is best categorized as a *combined type osteosarcoma*. In cases where only small fragments of tissue are received for examination, the main aim should be to determine whether the tumor is an osteosarcoma, rather than what subtype it belongs to.

- In **poorly differentiated osteosarcoma**, the tumor cells vary from small cells resembling those of bone marrow stroma, to large, pleomorphic cells of an undifferentiated sarcoma. The only clue that the tumor is an osteosarcoma is the presence of small quantities of *unequivocal tumor osteoid*. These tumors are generally highly aggressive, forming lytic bone lesions, and often are associated with pathological fractures.
- **Osteoblastic osteosarcomas** are recognizable by the presence *of cells with features of anaplastic osteoblasts* throughout much of the tumor (Fig. 1.109C). The tumor cells have hyperchromatic, often eccentric nuclei and variable quantities of basophilic cytoplasm. The cell borders are often angular and there may be a pale Golgi zone adjacent to the nucleus. Osteoblastic osteosarcomas may be *further subclassified into nonproductive and productive osteosarcomas* depending on the quantity of tumor bone produced. Moderately productive osteoblastic osteosarcoma (Fig. 1.109A, B) is the most common subtype of osteosarcoma in dogs. Radiographically, there is a mixed pattern of destruction and production. In some cases with abundant tumor bone formation, differentiation of tumor bone from reactive bone may be difficult or impossible.
- In **chondroblastic osteosarcomas**, *the malignant mesenchymal cells directly produce both osteoid and chondroid matrices.* Although the two components are usually intermixed, they remain separate in some tumors and small biopsy specimens may lead to an incorrect diagnosis of chondrosarcoma.
- **Fibroblastic osteosarcomas** consist of a population of *spindle cells* similar to those of fibrosarcoma, within which there is evidence of *osteoid or bone formation by tumor cells.* The spicules of bone may be sparse, especially in early lesions, and it is not uncommon for such tumors to initially be diagnosed as fibrosarcomas then later reclassified as osteosarcoma following examination of further biopsies. In one study, the prognosis for dogs with fibroblastic osteosarcoma was more favorable than for other subtypes.
- **Telangiectatic osteosarcoma** is an *aggressive, osteolytic tumor* consisting of a mixture of solid areas and blood-filled spaces, grossly and radiographically resembling hemangiosarcoma (Fig. 1.108B). Histologically, telangiectatic osteosarcoma can be *differentiated from hemangiosarcoma by the presence of occasional spicules of osteoid among pleomorphic, malignant mesenchymal cells* (Fig. 1.110), although a careful search is often required in order to detect osteoid. Furthermore, the blood-filled spaces present throughout the tumor are lined by tumor cells, not endothelium. Metastases of telangiectatic osteosarcomas generally resemble the primary tumor, containing many cystic spaces filled with blood. In human patients and dogs, *this subtype is associated with a less favorable prognosis than all other forms of osteosarcoma.*
- **Giant cell osteosarcomas** resemble nonproductive osteoblastic osteosarcoma histologically, but possess areas in which tumor giant cells predominate and must be differentiated from malignant giant cell tumors of bone.

Although cytology is usually less reliable than histology in the diagnosis of mesenchymal tumors, central osteosarcomas can often be diagnosed with confidence on examination of *fine-needle aspiration biopsies or imprints* prepared from tissue biopsy samples. Cytological preparations from osteosarcomas are usually more cellular than aspirates or imprints from soft tissue sarcomas, and the cells may have characteristic features of malignant osteoblasts. In many cases,

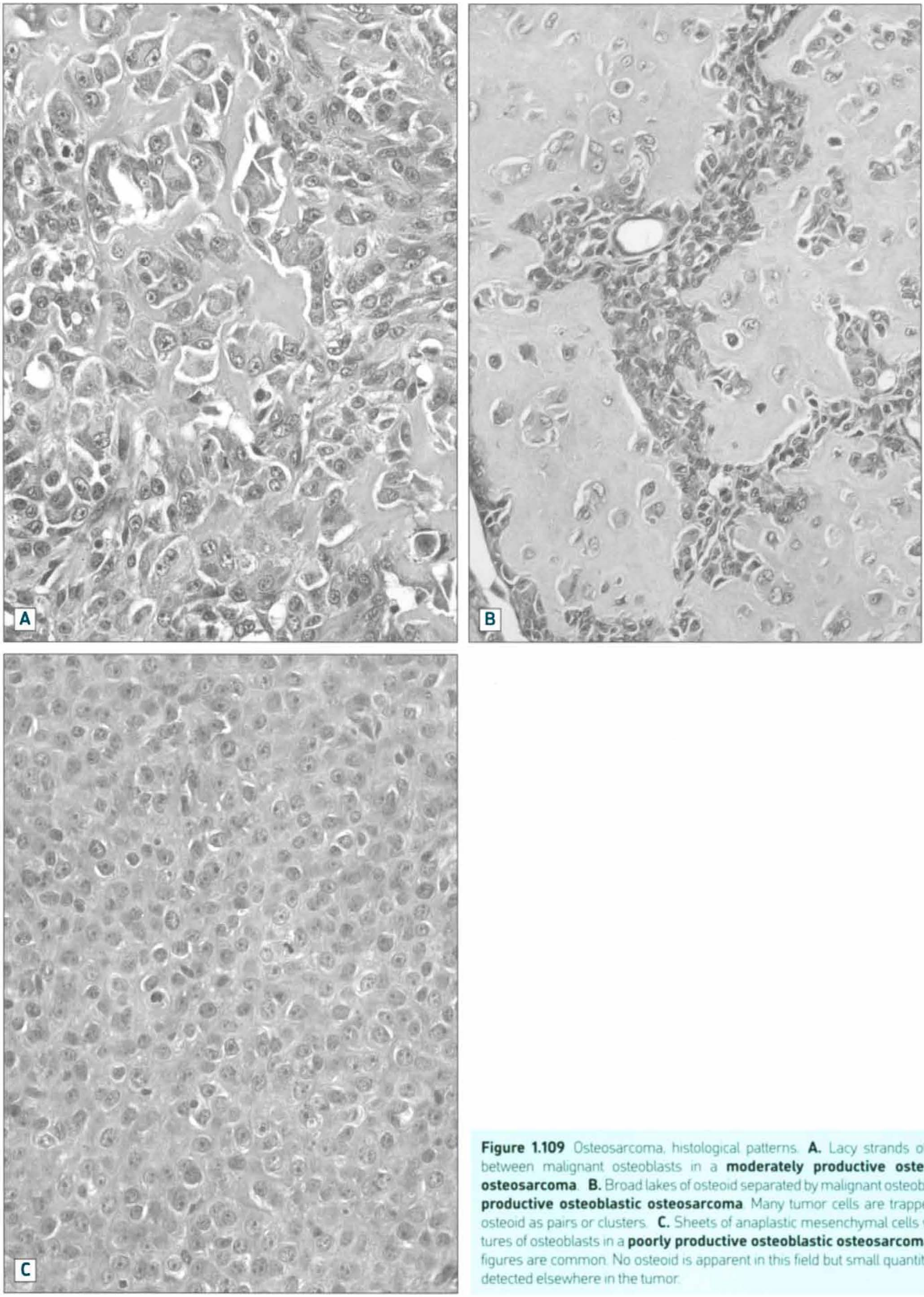

Figure 1.109 Osteosarcoma, histological patterns. **A.** Lacy strands of osteoid between malignant osteoblasts in a **moderately productive osteoblastic osteosarcoma**. **B.** Broad lakes of osteoid separated by malignant osteoblasts in a **productive osteoblastic osteosarcoma**. Many tumor cells are trapped in the osteoid as pairs or clusters. **C.** Sheets of anaplastic mesenchymal cells with features of osteoblasts in a **poorly productive osteoblastic osteosarcoma**. Mitotic figures are common. No osteoid is apparent in this field but small quantities were detected elsewhere in the tumor.

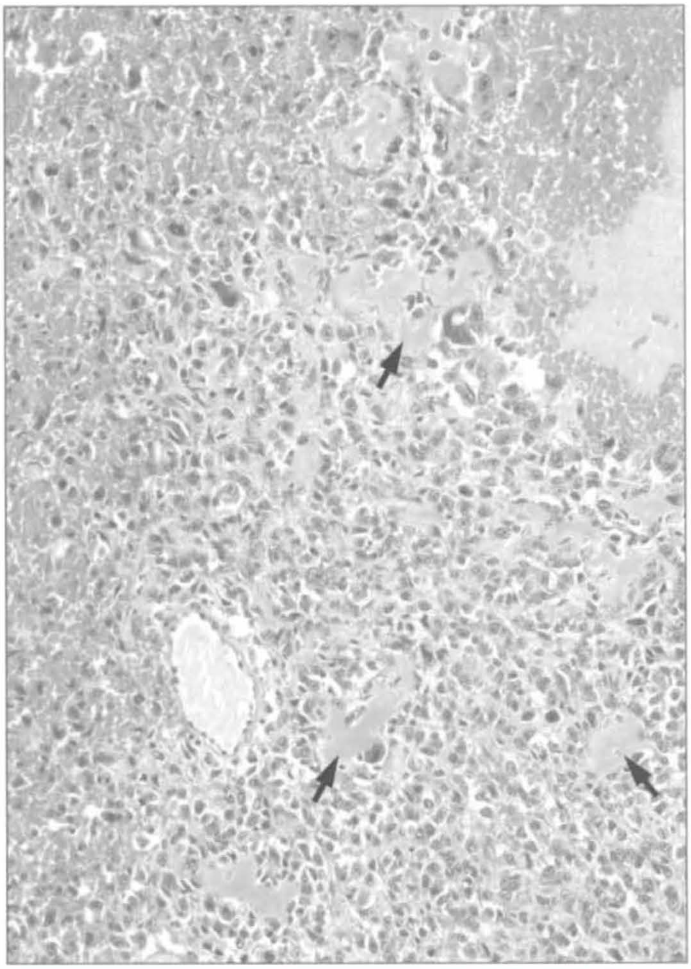

Figure 1.110 Telangiectatic osteosarcoma, dog. Malignant mesenchymal cells forming spicules of matrix resembling osteoid (arrows) and closely associated with large, blood-filled spaces.

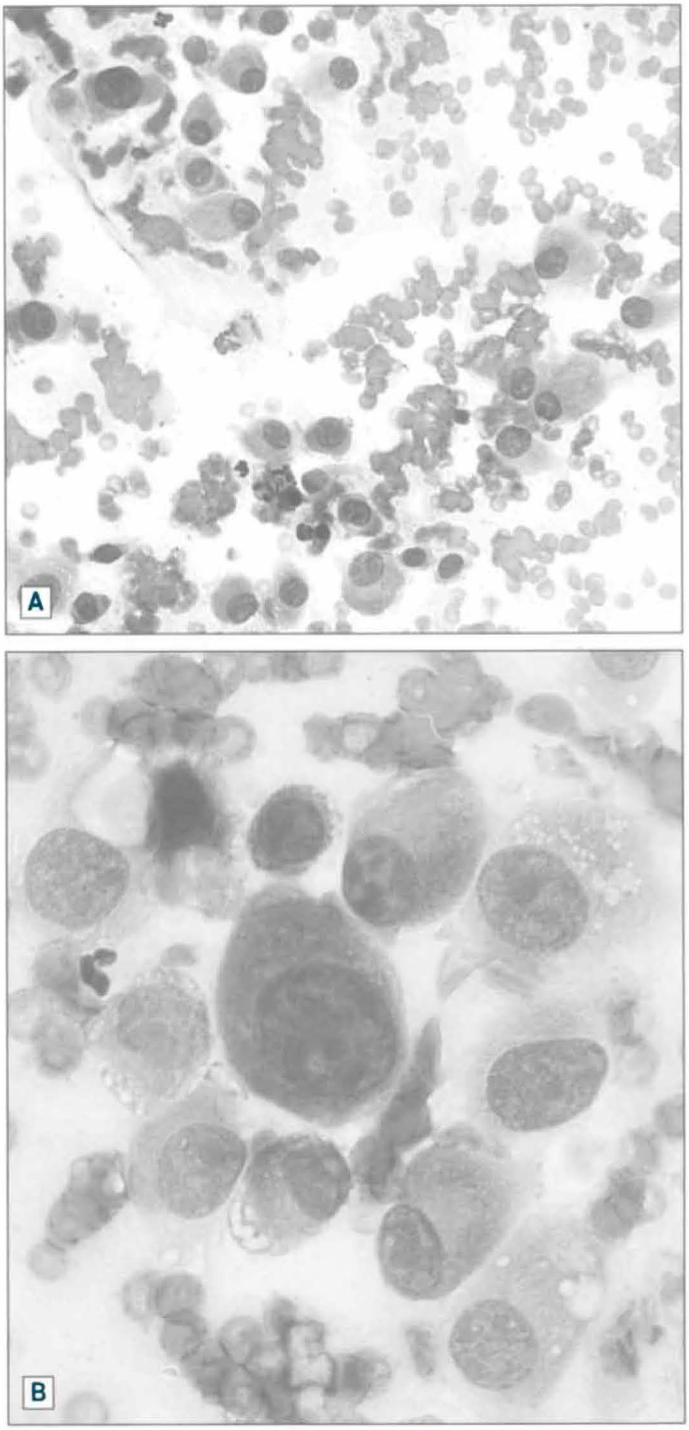

Figure 1.111 Cytological appearance of **osteosarcoma**. **A.** Moderate numbers of oval to pyriform-shaped cells with eccentric nuclei, basophilic cytoplasm and pale Golgi zone adjacent to the nucleus of some cells. There is moderate variation in nuclear size and N:C ratio. **B.** Higher-power view of malignant osteoblasts including one cell with a very large, hyperchromatic nucleus and deeply basophilic cytoplasm.

the cytological characteristics, together with clinical history and radiography, will be sufficient to allow a definitive diagnosis of osteosarcoma, but malignancy cannot be excluded on the basis of cytology as the sample may not be sufficiently representative of the lesion. Since most osteosarcomas originate from within the medullary cavity, shallow aspirates may be largely acellular or, at best, just contain a small number of reactive osteoblasts (see Fig. 1.2). Furthermore, some productive subtypes of osteosarcoma with extensive tumor bone formation may not yield significant numbers of tumor cells to cytological preparations and aspirates from telangiectatic osteosarcomas may be too heavily contaminated with blood to be of value. Even in cases where a cytological diagnosis of osteosarcoma can be made, classification of the tumor into one of the subtypes listed above is not possible.

Malignant osteoblasts may be present individually or in clusters in cytological preparations and are sometimes closely associated with brightly eosinophilic strands or islands of *osteoid*. They vary from round or oval to plump, fusiform cells, often with an eccentric nucleus and deeply basophilic cytoplasm (Fig. 1.111A, B). The cytoplasm may have a pale Golgi zone adjacent to the nucleus and, in some cases, there are variable numbers of *small, clear, intracytoplasmic vacuoles and/or fine pink granules*. Similar pink granules may also be present in tumor cells from chondrosarcomas or occasionally fibrosarcomas. In anaplastic osteosarcomas, there may be marked anisokaryosis, multiple large, irregular-shaped nucleoli and variable nuclear to cytoplasmic ratio. Mitotic figures are common and may be abnormal. In contrast, well-differentiated osteosarcomas may consist largely of relatively uniform-sized cells with many features of reactive

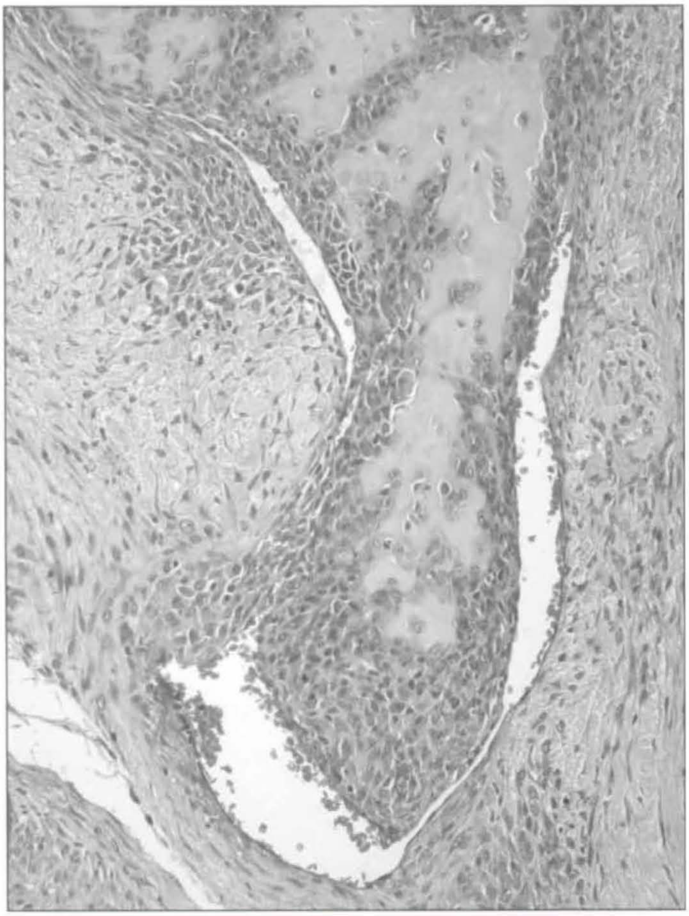

Figure 1.112 Tumor tissue containing osteoid invading a large vein at the margin of an **osteosarcoma**.

osteoblasts. A similar population of reactive osteoblasts associated with strands of osteoid may be harvested from an early fracture callus, highlighting the importance of clinical history and knowledge of the radiographic appearance of the lesion. *Unless the characteristics of malignancy are unequivocal, histological examination is recommended.*

Central osteosarcoma is perhaps the most malignant group of tumors of animals, at least in dogs. *The prognosis for all subtypes is very poor.* Hematogenous metastasis to the lungs commonly occurs early in the disease. Vascular invasion can sometimes be detected at the tumor margins (Fig. 1.112). Pulmonary metastases are detected radiographically in approximately 10% of canine appendicular and axial osteosarcomas at the time of initial diagnosis and it is likely that most dogs with osteosarcoma will eventually develop metastases if their lives are prolonged by surgery or other forms of treatment. In one study, dogs with telangiectatic osteosarcoma had a 100% metastatic rate. Hematogenous metastasis to other organs, including the skeleton, also occurs but osteosarcomas seldom spread to regional lymph nodes. The median survival time for dogs with appendicular skeletal osteosarcomas is only 14–19 weeks. Survival time is only marginally better (22 weeks) in dogs with osteosarcomas of the axial skeleton, although osteosarcomas of the mandible, and those with osteoblastic tumors of paranasal sinuses and calvaria generally have a better prognosis.

The incidence of *metastasis in cats* with central osteosarcoma is considerably less than in dogs and the prognosis is therefore more favorable. A median survival time of 49.2 months has been observed in cats with appendicular osteosarcoma following treatment by amputation, but the number of animals in most studies of feline osteosarcoma has been small.

Periosteal and parosteal (juxtacortical) osteosarcoma are referred to as **peripheral osteosarcomas** because of their origin on or near the surface of bones. Two types of **periosteal osteosarcoma** have been recognized in dogs. One is a highly aggressive tumor with similar histologic features and biologic behavior as central osteosarcoma, the other being more differentiated and with a more favorable prognosis. Too few cases of periosteal osteosarcomas have been reported in dogs and other domestic animals to provide reliable data on the age, sex, and site incidence. It is possible that the more aggressive forms of periosteal osteosarcoma in dogs are actually central osteosarcomas arising from the outer metaphyseal regions of a long bone and adopting an eccentric growth pattern.

Parosteal osteosarcomas also arise in the periosteal connective tissue but have a characteristic clinical behavior and histologic pattern. These tumors are reported in dogs, cats, and a horse and follow a longer clinical course than central osteosarcomas. Most cases are presented as a firm, slowly enlarging mass on the surface of a bone. Radiographically, the tumor has an evenly contoured margin and the underlying cortex is generally intact. This is in contrast to osteochondroma, where the marrow cavity of the tumor is continuous with that of the adjacent bone. In histological sections, parosteal osteosarcoma consists of *broad trabeculae of well-differentiated bone separated by moderately cellular fibrous connective tissue showing some pleomorphism, but no convincing evidence of malignancy* (Fig. 1.113). Differentiation from reactive bone may present a significant challenge. Metastasis of parosteal osteosarcoma to the lungs occurs late, if at all.

Osteosarcomas occasionally arise in soft tissues of dogs and cats, in the absence of a primary bone lesion. These **extraskeletal osteosarcomas** occur most often in the *mammary gland*, but may also arise in the gastrointestinal tract, subcutaneous tissues, spleen, urinary tract, liver, skin, muscle, eye, and thyroid gland. They also arise in the esophagus in association with *Spirocerca lupi* infestation. In general, extraskeletal osteosarcomas occur in older dogs than skeletal osteosarcomas (mean age 10.6–11.5 years) and show no apparent predilection for large breeds. Although distant metastases are common, the lungs are involved less often than in skeletal osteosarcomas, and death is usually due to either local recurrence or euthanasia at the time of diagnosis. The mean survival time is reported to be lower than for skeletal osteosarcomas, in part due to the later detection of intra-abdominal tumors and limited surgical options for tumors at some sites. Osteosarcoma of the mammary gland of bitches should not be confused with the *more common, nonneoplastic, osseous metaplasia* that occurs in association with mammary carcinomas.

Extraskeletal osteosarcoma appears to be less common in cats than in dogs, but has been reported in the eye following ocular trauma, and in the mammary area of a 12-year-old cat. In the latter case, the tumor recurred following surgical removal and widespread lung metastases were detected at necropsy.

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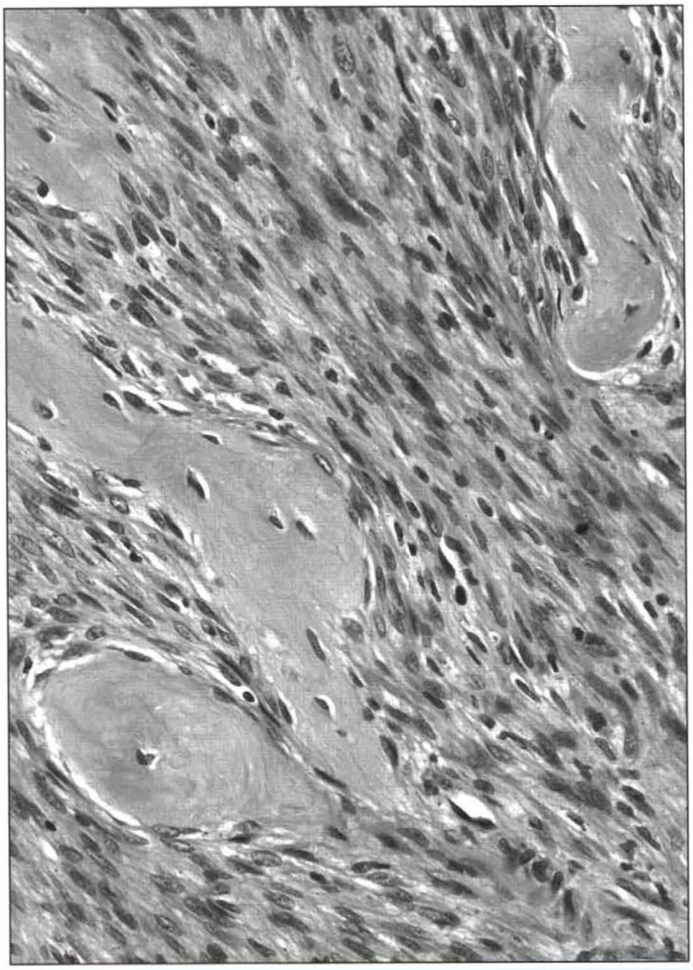

Figure 1.113 Parosteal osteosarcoma, dog. Broad, disoriented, bony trabeculae separated by uniformly cellular tissue resembling immature fibrous tissue.

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Cartilage-forming tumors

Tumors consisting entirely or predominantly of cartilage are *uncommon* in the skeleton of domestic animals. They are diagnosed most frequently in dogs, where approximately 10% of skeletal tumors are cartilaginous, and in sheep, where chondromas comprise a relatively high proportion of skeletal tumors. Cartilaginous tumors often contain areas of bone formed by endochondral ossification of tumor cartilage. This should not be mistaken for the tumor bone formed directly from neoplastic mesenchymal cells in osteosarcoma. Repairing fractures also contain cartilage and bone in various proportions at different stages of maturity and could be confused with cartilage- or bone-forming tumors.

Extra-skeletal tumors containing cartilage also occur, particularly those arising as a component of lipomatous soft tissue masses (chondrolipomas). Cartilaginous tissue is also present and may become dominant in mixed tumors of mammary and salivary gland origin.

Chondroma

A chondroma is a benign neoplasm of cartilage but, in veterinary medicine, the term has often been used loosely to include benign proliferations of cartilage in several extraskeletal tissues. Primary chondromas of bone are separable into *enchondromas*, which originate within the medullary cavity of a bone and *ecchondromas*, which arise from cartilage elsewhere in the skeleton. Both forms are rare in animals. Enchondromas are sometimes polyostotic, in which case the syndrome is referred to as *enchondromatosis*. It is likely that many tumors diagnosed as chondromas in animals are in fact osteochondromas or low-grade chondrosarcomas, especially in cases where the pathologist is not provided with an adequate history or access to radiographs of the lesion.

Chondromas occur rarely in dogs, cats, and cattle, and although considered to be more common than other skeletal tumors in sheep, they are infrequently diagnosed in this species. The tumors are typically firm to hard, smooth or nodular, roughly spherical masses with a relatively thin fibrous capsule. They involve flat bones and ribs more commonly than long bones. On cut surface, a lobular pattern may be evident due to dissection of the blue-white cartilage by fibrous septa. Areas of mineralization or ossification appear as chalk-white stippling. Myxomatous tissue, which occurs in some chondromas, has a gelatinous texture grossly and seems prone to hemorrhage and necrosis.

Histologically these benign tumors consist of irregular lobules of hyaline cartilage with cells that are, by definition, quite regular in size and appearance and typically chondrocytic. This is especially true of enchondromas. The matrix is usually more fibrous than that of normal hyaline cartilage. Foci of endochondral ossification and mineralization may be present, and lobules of myxomatous tissue sometimes develop. Differentiation between chondroma and low-grade chondrosarcoma is difficult on the basis of histopathology and may require the assessment of biological behavior in sequential radiographs.

Chondromas expand slowly, and a rapid change in size may indicate malignant transformation. Expanding tumors within the medullary cavity cause bone deformation and may predispose to fracture.

Osteochondroma

An osteochondroma is a benign, cartilage-capped tumor-like exostosis arising from the surface of an endochondral bone adjacent to a physis or subarticular growth cartilage. The tumor may be monostotic or polyostotic, in which case the terms **multiple cartilaginous exostosis** or **osteochondromatosis** are often used. In **humans, dogs**, and **horses**, the disease is inherited as an autosomal dominant trait and would be more appropriately classified as a *skeletal dysplasia* than a neoplasm, but is included here for convenience.

In dogs, the lesions usually are not present at birth but arise in young animals and continue to enlarge until the skeleton matures. In affected horses, the lesions are often present at birth and tend to be bilaterally symmetrical. During the phase of expansion, the outgrowths consist of a thin outer cap of hyaline cartilage resembling poorly organized growth plate, adjacent to the epiphyseal plate, which undergoes endochondral ossification from its deep surface (Fig. 1.114A). *Trabecular bone and bone marrow within the mass are continuous with the marrow cavity of the parent bone. This is a useful diagnostic feature in the differentiation of osteochondromas from some other hyperplastic or neoplastic bony masses.* Once bone growth ceases, the cartilage cap is replaced by bone. The trabecular bone in osteochondromas is not remodeled and the mineralized cartilage cores persist. *The nature of the genetic defect in osteochondroma is not known but is suspected to involve the perichondrial ring.* Peripheral nodules of physeal cartilage are pinched off and grow laterally, the lesions eventually finishing up in the metaphyseal region some distance from the original growth plate.

The lesions of osteochondroma are variable in their location but common sites in dogs and horses are the metaphyses of long bones, the pelvis, ribs, scapula, and vertebrae. Although many cases are merely of esthetic rather than clinical significance, lesions in some locations interfere with the action of ligaments or tendons and exostoses derived from vertebrae sometimes protrude into the spinal canal, resulting in spinal cord compression (Fig. 1.114B). Furthermore, *they may undergo malignant transformation into chondrosarcoma or osteosarcoma.* From 5 to 25% of human patients with the syndrome eventually develop chondrosarcoma, and similar cases have occurred in dogs and horses. In one series of eight cases of multiple cartilaginous exostoses in dogs, three developed chondrosarcoma, suggesting that the risk of malignant transformation in affected dogs is relatively high.

Osteochondromatosis in **cats** occurs in mature animals and tends to involve flat bones, including bones derived from intramembranous ossification. Long bones are seldom affected. The lesions in cats appear to arise as *multifocal areas of osteocartilaginous hyperplasia in the periosteum and undergo progressive enlargement.* Their behavior is therefore more consistent with that of a true tumor, unlike that of osteochondromas in dogs and horses. Furthermore, the lesions in cats are usually not continuous with the marrow cavity of the adjacent bone. It seems likely that this syndrome in cats is not analogous to that in the other species and a link with *Feline leukemia virus* has been made, and virus particles identified within lesions. Malignant transformation of feline osteochondroma is also reported.

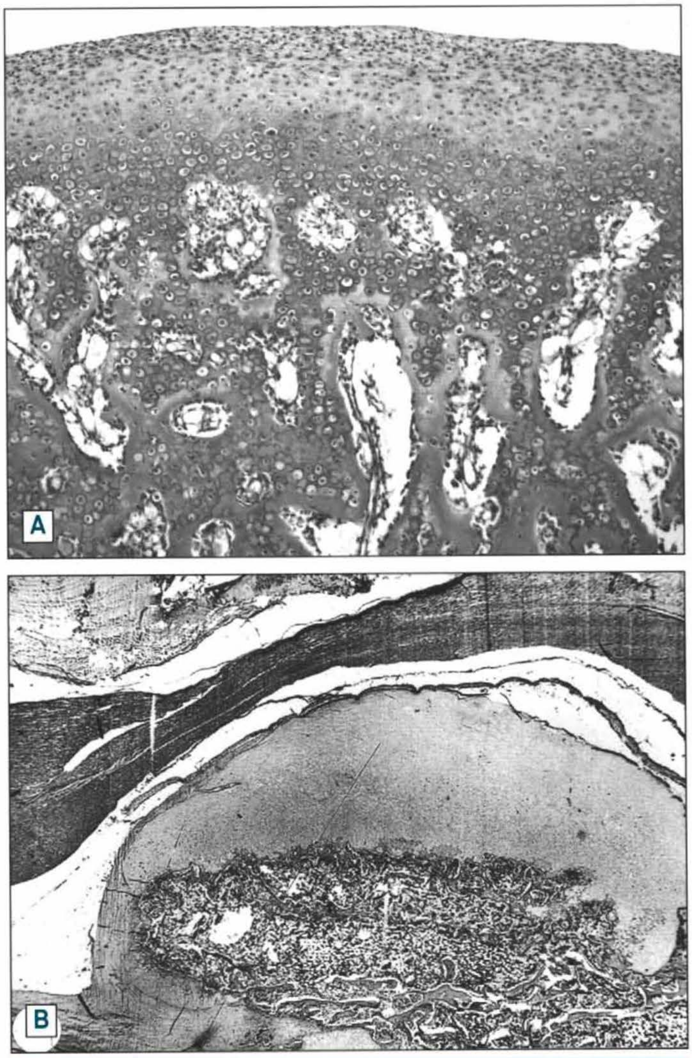

Figure 1.114 Osteochondroma, dog. **A.** Outer layer of proliferating chondrocytes differentiating into disorganized hyaline cartilage with randomly distributed hypertrophic chondrocytes. Endochondral ossification is occurring at the deep surface of the cartilage cap. **B.** Compression of spinal cord by osteochondroma originating from vertebra.

Multilobular tumor of bone

Multilobular tumor of bone is a slow-growing but locally aggressive and potentially malignant tumor, occurring most often in the skull of dogs, but occasionally in the cat and horse. Many alternative terms have been proposed for the tumor, including chondroma rodens, cartilage analogue of fibromatosis, calcifying aponeurotic fibroma, multilobular chondroma or osteoma, and multilobular osteochondrosarcoma. In dogs, it is primarily a disease of middle-aged to older animals, occurring most often in medium or large breeds, but rarely in giant breeds. In one study of 39 cases, the median age was 8 years (range 4–17 years) and the median weight was 29 kg, although four dogs weighed less than 25 kg. Three cases have been recorded in cats, varying in age from 9 months to 8 years, and there is one report in a 12-year-old Thoroughbred mare.

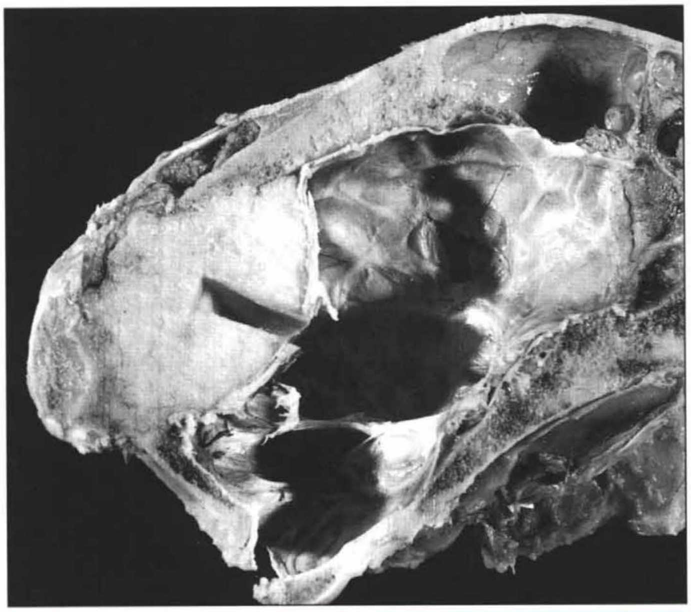

Figure 1.115 Multilobular tumor of bone impinging on the brain of a dog.

The tumor is usually present as a *firm, immovable mass on the surface of skull bones* and clinical signs relate to the compression and disturbance of function in adjacent structures. The swelling apparent from the skull surface may provide little indication of the degree to which the tumor impinges on adjacent organs, such as the brain (Fig. 1.115). Multiple small gritty foci are usually evident grossly on cut surface and a stippled appearance may be observed in radiographs of the tumor.

Histologically, the multilobular tumor of bone has a characteristic pattern consisting of multiple circular, oval or irregular-shaped nodules of cartilaginous, osseous, or osteocartilaginous tissue separated by narrow fibrovascular septa (Fig. 1.116A, B). In certain areas of some tumors, broad, ill-defined zones of mesenchymal tissue merge with the nodules. The cartilaginous nodules usually are surrounded by spindle-shaped septal cells, contiguous with the plump oval nuclei of the neoplastic chondrocytes in the center of each lobule. The tumor cells produce a pale, eosinophilic or faintly basophilic matrix, which mineralizes at the center of the lobule. Resorption of mineralized cartilage and endochondral bone formation sometimes occurs and occasionally, osteoclastic remodeling of bone develops. In these nodules, the matrix-producing cells are unequivocally chondrocytic in appearance. In bony nodules, the mesenchymal cells produce a matrix that

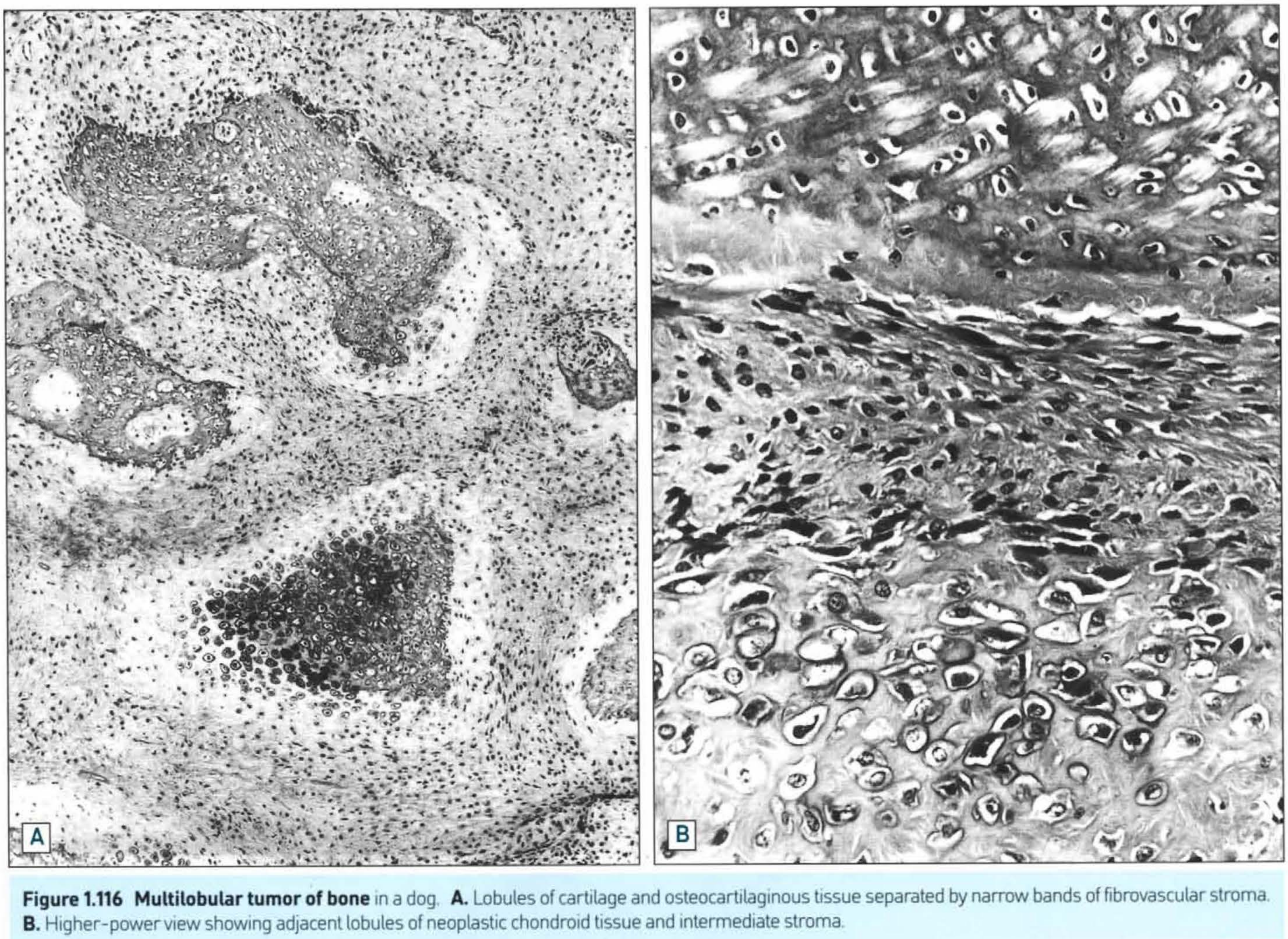

Figure 1.116 Multilobular tumor of bone in a dog. **A.** Lobules of cartilage and osteocartilaginous tissue separated by narrow bands of fibrovascular stroma. **B.** Higher-power view showing adjacent lobules of neoplastic chondroid tissue and intermediate stroma.

ossifies rather than mineralizes. In some tumors, the bone is produced by angular osteoblasts with abundant basophilic cytoplasm but in others, oval cells of indeterminate type produce a tissue reminiscent of chondroid bone. The mineralization process tends to progress centrifugally to involve the entire nodule, and the septal tissue then consists of dense collagen. In these areas, the tumor is extremely hard. Osseous and cartilaginous tissue may be present in the same nodule and some tumors contain both osseous and cartilaginous nodules. Although the nodular pattern predominates, resorption of the hard tissues does occur and nodules may be replaced by dense connective tissue containing areas of cartilaginous metaplasia.

Surgical removal is often difficult because of the location of these tumors and local recurrence occurs in about 50% of cases. Malignant transformation often occurs in tumors that are long-standing or recurrent. Indicators of malignancy include mitotic activity, loss of the orderly lobular architecture, necrosis, hemorrhage, and overgrowth of one of the mesenchymal elements. The characteristic appearance of multilobular tumors is retained in metastases.

Chondrosarcoma

Chondrosarcomas are malignant mesenchymal tumors in which the neoplastic cells produce variable quantities of cartilaginous or fibrillar matrix, but not osteoid. Although bone may be present in chondrosarcomas, it forms by endochondral ossification of tumor cartilage, rather than being produced directly by malignant mesenchymal cells.

Primary chondrosarcomas are those that arise from either within a bone (central or medullary chondrosarcoma) or from the periosteal surface (peripheral chondrosarcoma), the former being the most common in animals. The term *secondary chondrosarcoma* refers to those that develop by malignant transformation of cartilage in an osteochondroma.

Chondrosarcoma is *reported most frequently in the dog,* where it accounts for approximately 10% of primary tumors of bones and is second only to osteosarcoma in incidence. In most other domestic species, chondrosarcoma is a relatively rare tumor and too few cases are reported to provide reliable information on its clinicopathological features. In sheep, chondrosarcoma is considered to occur more frequently than osteosarcoma, but it remains a rare tumor in this species.

In dogs, chondrosarcoma occurs most often in medium to large breeds, particularly Boxers, German Shepherds, Golden Retrievers and various mixed breeds, but is rare in small and giant breeds. Although a broad age range is reported, the tumor is *most common in middle-aged to older dogs,* the mean age of affected animals varying from 5.9 to 8.7 years. No sex predilection is recognized. There are fewer data on the age incidence of chondrosarcoma in cats, but in one review, a mean age of 8.8 years was reported.

In all species, chondrosarcomas *involve flat bones more often than long bones.* The ribs, turbinates, and pelvis are common sites in dogs, although nasal chondrosarcomas are rare in Boxers. Chondrosarcomas may also occur in the appendicular skeleton of dogs, including, but not restricted to, sites of predilection for osteosarcomas. In cats, chondrosarcomas are reported in both flat bones (especially the scapula) and long bones, but in this species, the published data are inadequate to draw reliable conclusions regarding site predilection. In sheep, the cartilages of the sternocostal complex were the most common site for chondrosarcoma in one survey, followed by the scapula and tuber coxae. In cattle and horses, chondrosarcomas are found most often on flat bones, but long bones are occasionally affected.

Grossly, central chondrosarcomas are firm or hard and may consist of either several large lobules resembling hyaline cartilage or multiple small, contiguous nodules of translucent, blue-white or pink tissue. In some tumors, especially those in the nasal cavity, there are soft, mucoid areas, and in others, there are chalky white foci of mineralization or ossification. Chondrosarcomas *tend to grow expansively* and have a smooth surface and a capsule that merges with surrounding fibrous tissue. The more highly malignant tumors may contain areas of necrosis and hemorrhage. Nasal chondrosarcomas tend to destroy turbinates, fill the nasal cavity (Fig. 1.117A, B) and may either spread into adjacent sinuses or penetrate overlying bone and infiltrate adjacent soft tissues. When in long bones, chondrosarcomas may produce lytic lesions similar to osteosarcomas. Periosteal chondrosarcomas are less common in animals than central chondrosarcomas and usually occur as slow-growing, nodular masses on the surface of flat bones in older dogs and cats.

Histologically, well-differentiated chondrosarcomas show few indications of malignancy and closely resemble benign tumors of cartilage (Fig. 1.118A). Endochondral ossification of tumor cartilage may be present and should not be mistaken for the formation of tumor bone. *Knowledge of the clinical and radiographic features is often essential to accurate classification of cartilaginous tumors.* Fortunately for the veterinary pathologist, most chondrosarcomas in animals are well advanced before the owner seeks veterinary advice and biopsy samples are collected. In fact, the challenge may be to decide whether a malignant mesenchymal tumor involving bone is a chondrosarcoma, osteosarcoma, or some other sarcoma of bone. Such a decision is important, bearing in mind the differences in prognosis. In some chondrosarcomas, the matrix becomes fibrillar and hyalinized, resembling osteoid, and differentiation from osteosarcoma may be very difficult, especially in small biopsy samples. Chondrosarcomas in the sinonasal region of dogs are often characterized by nodules of variously differentiated chondroid tissue forming within poorly differentiated mesenchymal elements (Fig. 1.117C, D).

Microscopic features of malignancy in central chondrosarcomas include the presence of many tumor cells with plump nuclei and prominent nucleoli, binucleate tumor cells, and large chondrocytes with single or multiple nuclei (Fig. 1.118A, B). Mitotic figures are seldom present in well-differentiated chondrosarcomas and even a single mitotic figure strongly supports a diagnosis of malignancy in this tumor. In poorly differentiated, more malignant chondrosarcomas, mitotic figures may be relatively common (Fig. 1.119).

Fine-needle aspiration biopsies from chondrosarcomas usually contain fewer cells than osteosarcomas but, on low-power examination, *lakes of bright pink chondroid matrix* may be evident (Fig. 1.120). The tumor cells are similar to those from osteosarcomas, varying from round to fusiform, possessing large, hyperchromatic nuclei and basophilic cytoplasm, which sometimes contains fine pink granules. Anisokaryosis is generally a prominent feature and there may be multinucleate tumor cells. Although a presumptive diagnosis of chondrosarcoma may be possible in cytologic specimens, *confirmation requires histopathology.* Malignant chondroblasts and osteoblasts have too many features in common to allow reliable differentiation cytologically, and some osteosarcomas have extensive chondroid matrix.

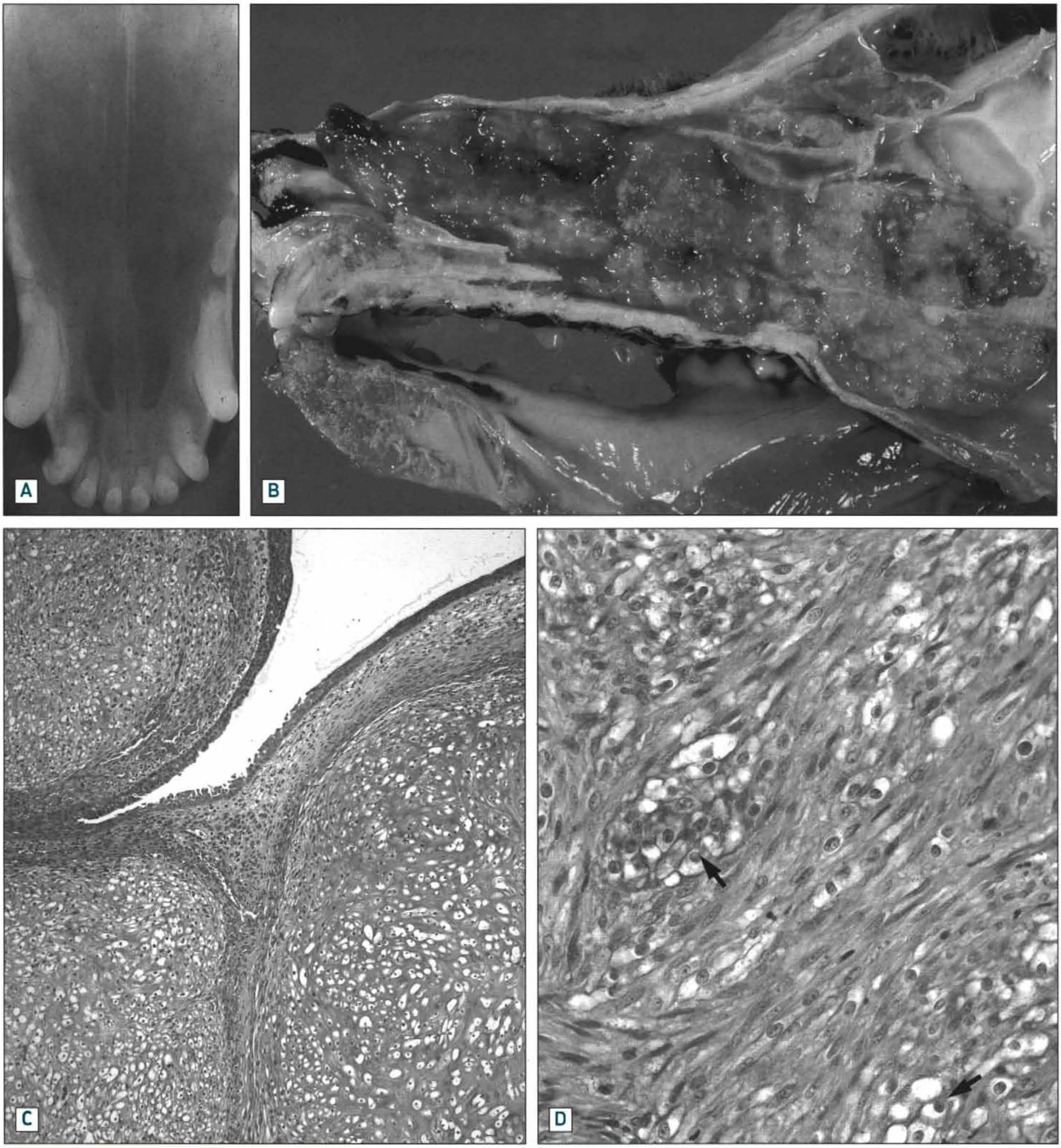

Figure 1.117 Nasal chondrosarcoma, dog. **A.** Radiograph showing loss of trabeculae and replacement by tumor tissue with radiodensity similar to soft tissue. **B.** Sagittal section through the skull of the same dog. Nodules of tumor tissue resembling hyaline cartilage fill the nasal cavity and extend into the nasopharynx. The tumor is eroding into the presphenoid bone, cribriform plate, and hard palate. **C.** Nodules of relatively well-differentiated hyaline cartilage beneath the nasal mucosa bulge into the attenuated lumen of the nasal cavity. **D.** Higher-power view of the same tumor, showing areas of chondroid differentiation (arrows) closely associated with poorly differentiated mesenchymal elements.

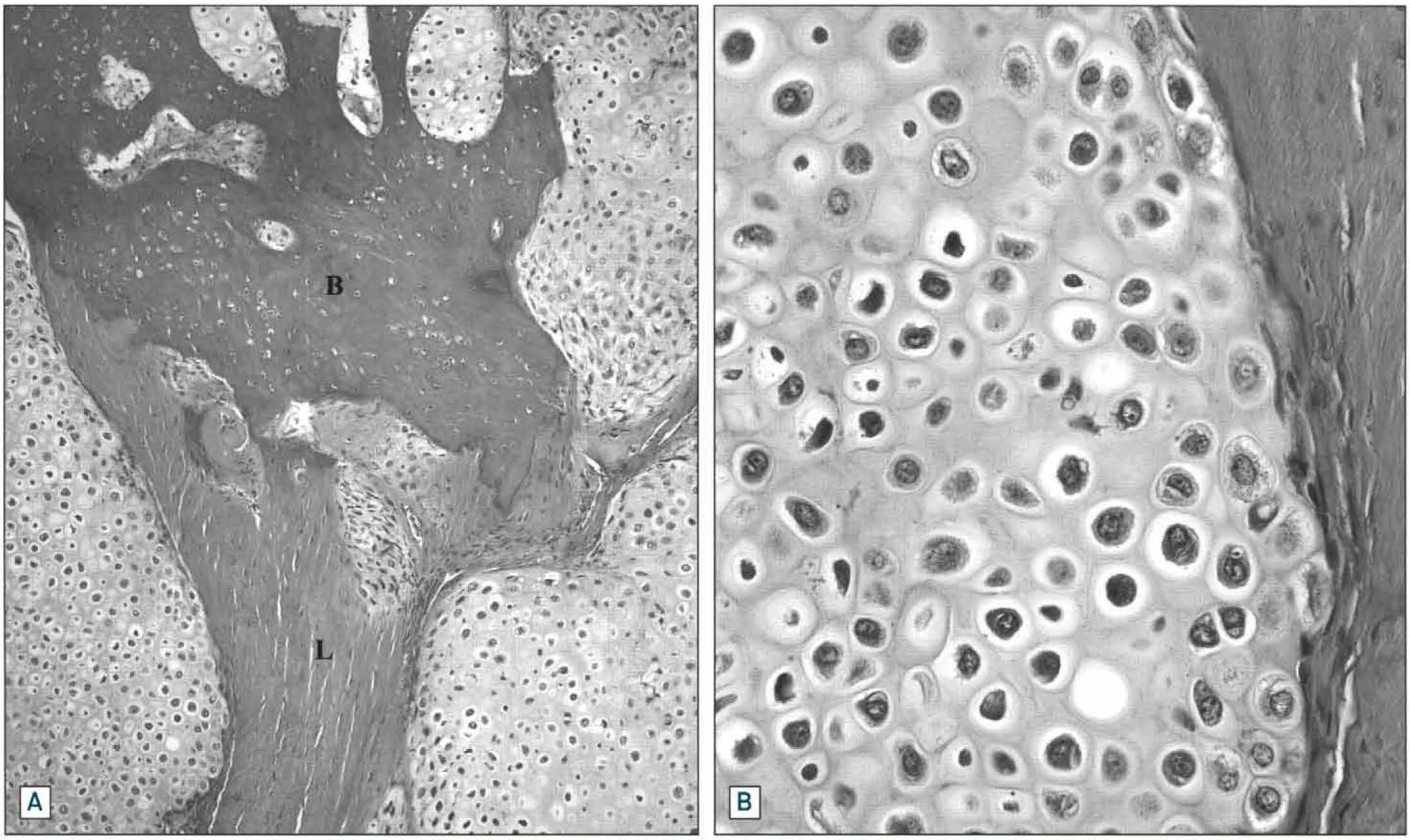

Figure 1.118 Chondrosarcoma, dog. Distal radius. **A.** Nodules of well-differentiated hyaline cartilage surrounding a ligament (L) at its insertion site and invading the adjacent bone (B). **B.** Higher-power view showing the plump, neoplastic chondrocytes compressing the ligament. Most of the tumor cells have large, variably sized nuclei and prominent nucleoli. Some lacunae contain more than one cell.

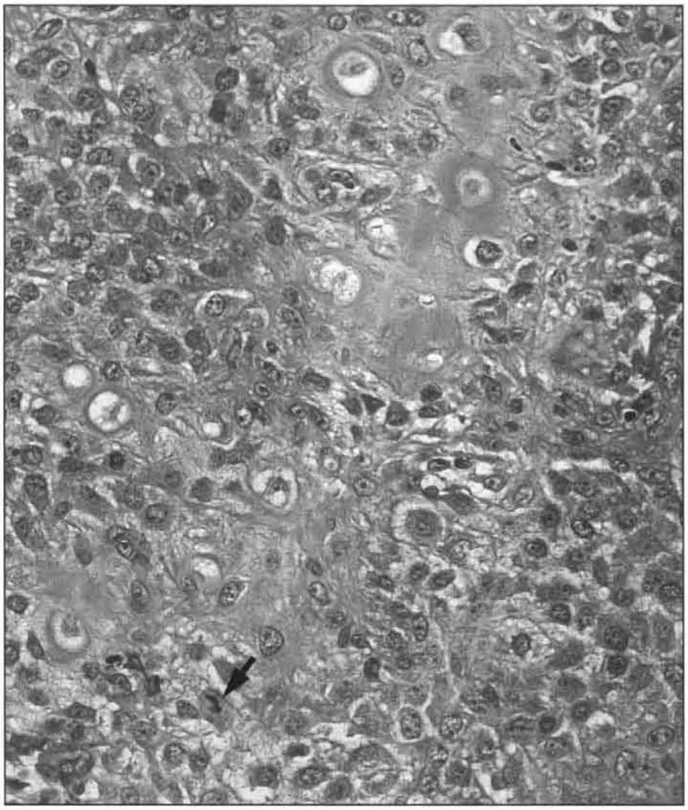

Figure 1.119 Poorly differentiated chondrosarcoma, dog. Anaplastic mesenchymal cells showing evidence of chondroid differentiation in some areas. Note the mitotic figure (arrow).

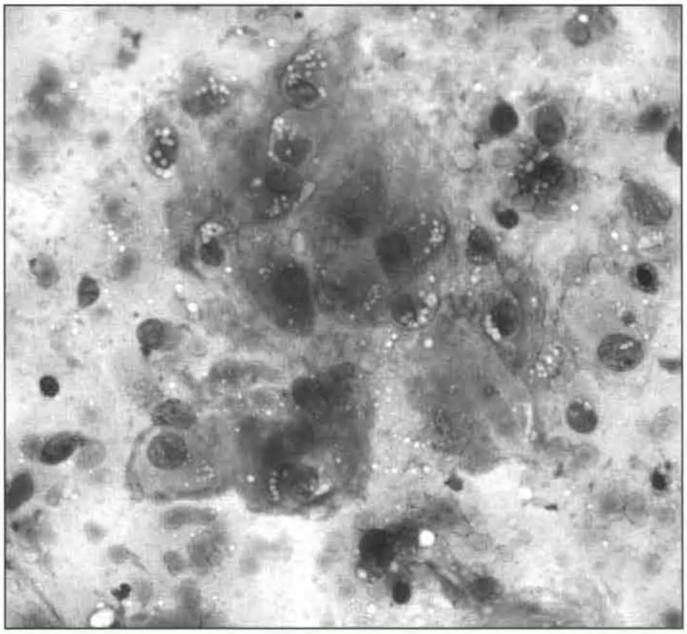

Figure 1.120 Cytological preparation from a **chondrosarcoma** in a dog. Several oval to fusiform cells, often with eccentric nuclei, prominent nucleoli, and basophilic cytoplasm, are closely associated with a background of brightly eosinophilic matrix.

Chondrosarcomas tend to grow more slowly and metastasize later than osteosarcomas, and typically follow a longer clinical course. Local invasion is common, as is recurrence following surgical removal, but the chance of successful removal from accessible sites is much greater than for osteosarcoma. Metastasis is usually to the lungs, but other visceral organs such as the kidney, liver, heart, and skeleton are sometimes involved.

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Fibrous tumors of bones

Fibrous tumors of bones are less common in domestic animals than tumors forming bone or cartilage. Ossifying fibroma and fibrous dysplasia are discussed above with osteoma, because of the similarities between these three benign tumors. Skeletal **fibromas** are extremely rare in animals, unlike humans where nonossifying fibromas are relatively common.

Skeletal **fibrosarcomas** arise from connective tissue stroma in either the medullary cavity or periosteum. Their histologic and gross appearances are essentially the same as that of fibrosarcomas elsewhere, although their innocuous microscopic appearance often belies their invasive tendencies. Central (medullary) fibrosarcomas are less common than those originating from the periosteum. They probably comprise about *5% of primary skeletal tumors in dogs* and occur predominantly in mature male dogs of large and medium breeds, but are rarely reported in other species.

In dogs, **central fibrosarcomas** arise most often in *metaphyses of long bones* and cause lytic lesions that must be differentiated from fibroblastic osteosarcomas. They are also reported in the mandible and vertebral column of dogs. It is likely however, that the prevalence of skeletal fibrosarcoma is overestimated. In one study, six of 11 tumors originally diagnosed as skeletal fibrosarcoma were reclassified as osteosarcoma following re-examination and identification of areas of osteoid production by tumor cells. Because of the poorer prognosis of osteosarcoma, an accurate diagnosis carries considerable clinical relevance. The pathologist should not be tempted to diagnose skeletal fibrosarcoma if the quantity of tissue available for examination is inadequate. Sequential radiographs of the lesion may be of diagnostic value because *fibrosarcomas of bone, although they are primarily destructive, usually progress more slowly than osteosarcomas or other highly malignant bone tumors.*

Periosteal fibrosarcomas occur in most species and *tend to involve flat bones* especially those of the head, but also occur on the scapula and occasionally on long bones. They enlarge slowly, causing disfigurement of the involved bone and are intimately attached to the bone surface. Erosion of the adjacent bone cortex may eventually predispose to pathological fracture.

Maxillary fibrosarcoma of the dog is technically a periosteal fibrosarcoma but it is classified separately from other tumors of this type because of its more frequent occurrence, and evidence that *it responds to combined radiation–hyperthermia therapy*. Maxillary fibrosarcomas occur primarily in middle-aged dogs, usually around 7 years of age, and appear to be most common in the Golden Retriever, Doberman Pinscher and German Shepherd breeds. In one large survey of canine bone tumors, ten of 31 fibrosarcomas involved the maxilla. Typically, the mass is broadly adherent to the periosteal surface of the maxilla (Fig. 1.121A, B).

Classification of periosteal fibrosarcomas as low-grade fibrosarcomas is based more on their invasive behavior than their histological appearance, which is often deceptively benign. They are less cellular than fibrosarcomas in other sites, lack the characteristic pattern of interwoven bundles and often contain a highly collagenous stroma with minimal pleomorphism of tumor cells (Fig. 1.121C). Biopsy samples from such tumors will often be misinterpreted as fibroma or fibrous repair tissue, especially if the pathologist is unaware of the clinical history or radiographic findings.

Although periosteal fibrosarcomas can often be dissected free from the underlying bone, *they often recur*. Metastasis to the lungs rarely occurs.

Vascular tumors of bones

Although tumors of vascular tissue are described with the circulatory system, *they can occur as primary bone tumors* and will therefore be mentioned briefly here. Intraosseous **hemangioma** is extremely rare in domestic animals. **Hemangiosarcoma** occurs occasionally as a primary bone tumor, especially in dogs, and is considered slightly less common than primary fibrosarcoma of bone. In one survey of 152 primary bone sarcomas in dogs, four were hemangiosarcomas. The tumor is found mainly in large and medium-sized breeds, with Boxers, German Shepherds and Great Danes overrepresented. The *proximal and distal ends of long bones are most often affected* but tumors are also reported in the pelvic bones, sternum, ribs, maxilla, and vertebrae.

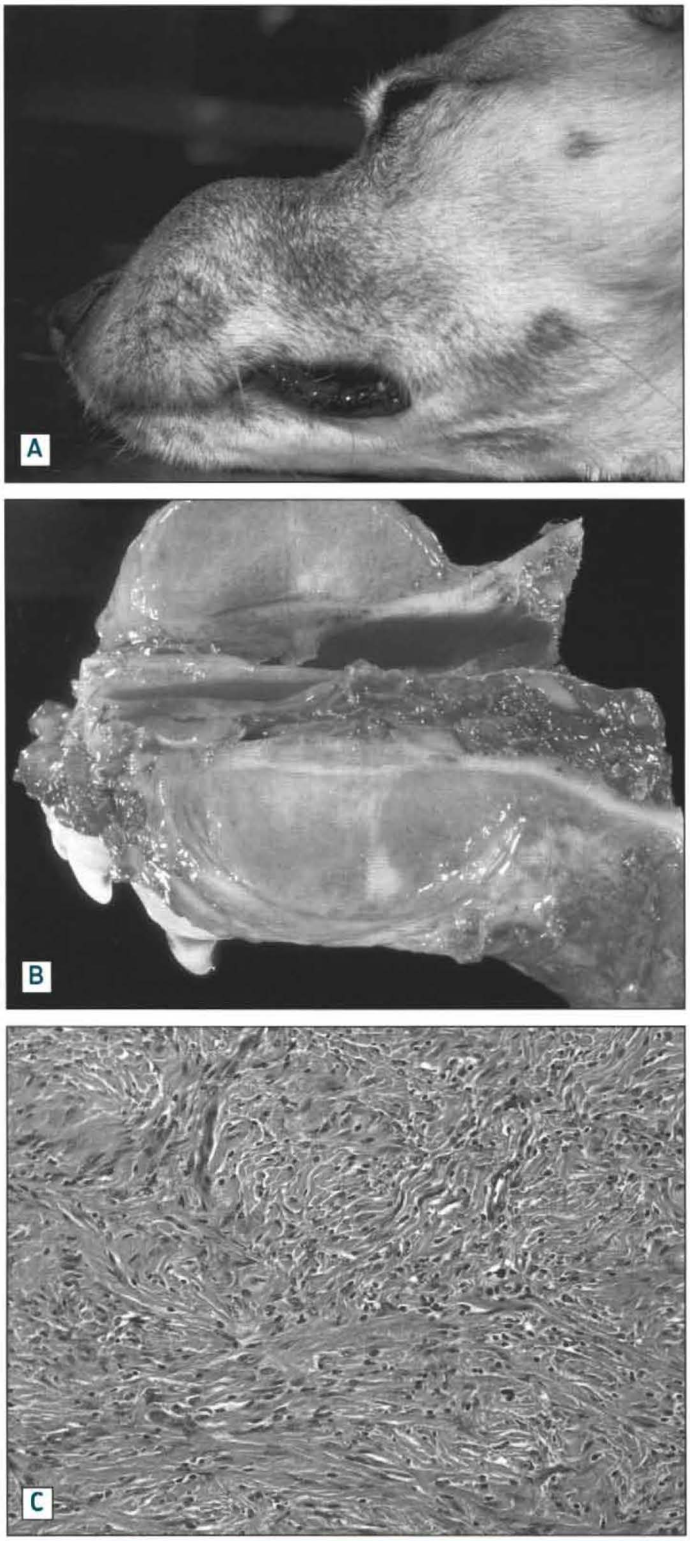

Figure 1.121 Maxillary fibrosarcoma, dog. **A.** Firm swelling over the rostral maxilla. **B.** Sagittal section through the nasal cavity of the same dog. The mass is firmly adherent to the outer surface of the maxillary bone and partly surrounding it. **C.** Interwoven bundles of well-differentiated fibrous tissue showing little microscopic evidence of malignancy.

Skeletal hemangiosarcoma is an aggressive tumor causing bone destruction and often predisposing to pathological fracture. The tumor tends to remain within the medullary cavity rather than invade adjacent soft tissues, but erodes and weakens the cortex. *Grossly*, the tumor resembles hemangiosarcomas of other organs, consisting of spongy, dark red tissue, and *cannot be reliably differentiated from telangiectatic osteosarcoma and aneurysmal bone cysts. Diagnosis is based on the demonstration in microscopic sections of malignant endothelial cells forming vascular channels*, at least in some areas of the tumor. The supporting stroma may become impregnated with plasma proteins, forming a hyaline material resembling osteoid and leading to an incorrect diagnosis of telangiectatic osteosarcoma. *In telangiectatic osteosarcoma, malignant osteoblasts rather than endothelial cells line the blood-filled spaces*, but the distinction is often difficult, especially in small biopsy samples or at sites of pathological fracture. Hemangiosarcomas occasionally metastasize to bones from other tissues and thorough postmortem examination of an affected animal is required before the tumor can be considered to be of bone origin.

Other primary tumors of bones

Giant cell tumor of bone is a recognized entity in humans but is rare in domestic animals with only isolated reports in **dogs** and **cats**. Some reported cases in animals are not convincing and may represent other tumors in which osteoclasts are prominent. Giant cell tumors of humans and animals *typically cause expansile osteolytic masses in the ends of long bones*, often involving much of the subchondral epiphyseal bone. Involvement of metacarpal bones and of the axial skeleton is also recorded in dogs and cats. As the mass expands, it destroys cortex but tends to remain at least partly circumscribed by a thin shell of bone. The osteolytic nature of the tumor combined with its bony shell creates a characteristic "soap-bubble" appearance in clinical radiographs.

The tumor is characterized *histologically* by the presence of *large numbers of multinucleated giant cells closely associated with neoplastic mononuclear cells*. The giant cells, which resemble osteoclasts, are often very large and are scattered uniformly throughout the tumor (Fig. 1.122). Their nuclei resemble those of the mononuclear cells. In some tumors there may be areas of collagen and/or osteoid formation, but this is not a prominent feature and the matrix may be produced by reactive fibroblasts and/or osteoblasts respectively, rather than by the tumor cells. The tumor is highly vascular and may contain cavernous spaces and areas of hemorrhage, leading to possible confusion with aneurysmal bone cyst, which also contains variable numbers of multinucleated giant cells. Osteosarcomas may also contain many osteoclasts in some areas and be mistakenly diagnosed as giant cell tumors.

In cytological preparations, the presence of a large percentage of multinucleated giant cells among many plump, spindle-shaped, or ovoid mesenchymal cells, suggests the possibility of giant cell tumor. Giant cells may also be present in aspirates from other bone lesions, particularly osteosarcoma, but the percentage in giant cell tumors is likely to be much higher. In one report of this tumor in a dog, 25% of all cells obtained by fine-needle aspiration biopsy were multinucleated giant cells. However, definitive diagnosis still requires histological examination of sections from representative areas of the mass. The histogenesis of giant cell tumor is uncertain, but immunochemical staining suggests that the mononuclear tumor cells are of histiocytic origin and that the giant cells arise from their fusion. C-type viral particles were found to be budding from tumor cells in one feline giant cell tumor but their significance is unknown.

Because there are so few documented cases of giant cell tumor in animals, there is inadequate information to provide an indication of their biological behavior. Benign and malignant forms are also reported in animals, the former being most common. In humans, most giant cell tumors are benign, but they commonly recur following surgery and approximately 5–10% are malignant, metastasizing predominantly to the lungs.

Because of the marked differences in prognosis between giant cell tumor and osteosarcoma, both of which may contain many multinucleated giant cells, *a diagnosis of giant cell tumor should never be made from small biopsy samples that are not representative of the lesion.*

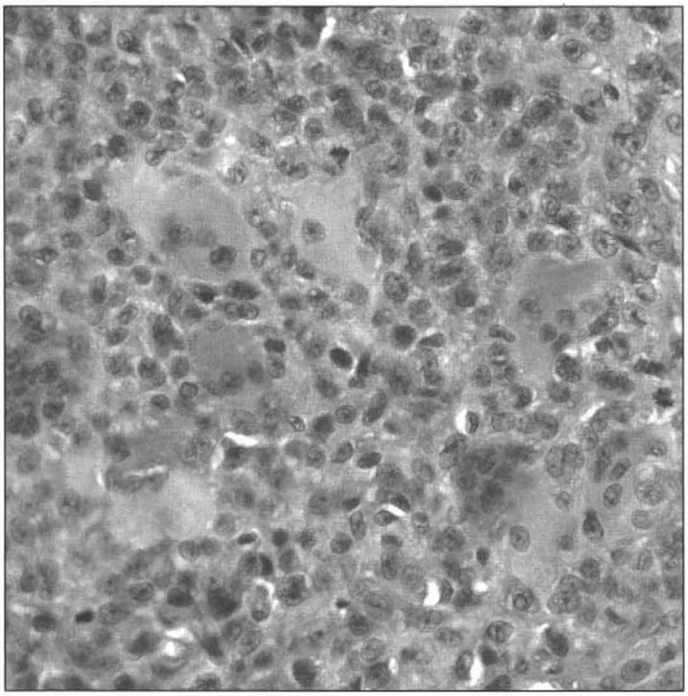

Figure 1.122 Giant cell tumor of bone, cat. Several large, sometimes abnormally shaped, multinucleated giant cells resembling osteoclasts scattered amongst a population of mononuclear cells.

Liposarcoma is a rare primary bone tumor in animals, resembling its soft-tissue counterpart but forming osteolytic bone lesions. The tumor cells typically contain variable numbers of intracytoplasmic vesicles, often with an eccentrically positioned nucleus but, in some areas, may consist of less well-differentiated spindle cells. **Osteoliposarcoma**, a form of **malignant mesenchymoma**, is a malignant neoplasm containing liposarcomatous and osteosarcomatous tissue and is recorded in the dog.

Plasma cell myeloma, a malignant tumor of plasma cells within the bone marrow, typically produces discrete, multicentric, lytic lesions in bones, especially those involved in active hematopoiesis. The disease occurs most often in **dogs** but is also reported occasionally in **cats** and **horses**. The mean age of affected dogs is approximately 9.5 years (range 2.5 to 16 years) and males are affected more frequently than females. In cats, like dogs, there is a higher incidence in males but no sex predilection is recognized in the small number of cases reported in horses.

A feature of plasma cell myeloma in all species is the *production of a homogeneous immunoglobulin or immunoglobulin fragment (paraprotein or M-component),* which appears as a monoclonal spike on serum protein electrophoresis. The disease may also be accompanied by Bence–Jones proteinuria, hyperviscosity syndrome, and hypercalcemia. Involvement of vertebral bodies may result in paraplegia due to protrusion of tumor masses into the spinal canal, or secondary to pathological fractures of vertebral bodies. Discrete, "punched-out" foci of osteolysis (Fig. 1.123A), of various sizes, and often involving multiple bones, are present in approximately two-thirds of dogs with plasma cell myeloma, but appear to be less common in affected horses. In dogs, the principal sites of bone involvement are vertebrae, especially in the thoraco-lumbar region, femur, pelvis, humerus, and ribs. Bones of the distal limb are seldom involved in dogs, unlike cats where they appear to be involved as often as proximal bones of the limb.

Grossly, the lytic foci consist of soft, fleshy or gelatinous, dark red nodules, replacing trabecular bone. The lesions are often multiple and may be associated with a pathological fracture. Histological or cytological examination reveals a relatively pure, dense population

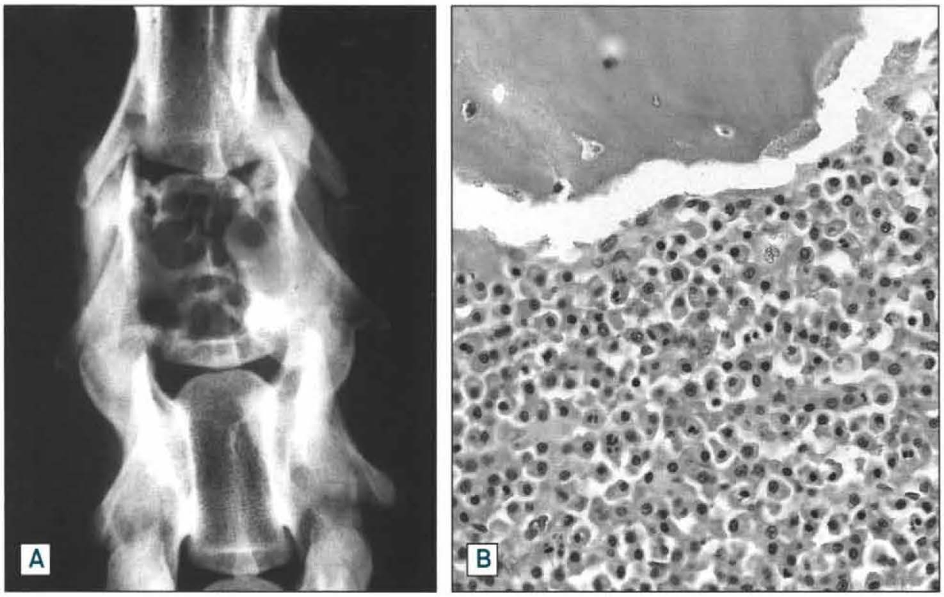

Figure 1.123 Plasma cell myeloma, dog. **A.** Discrete, "punched out" osteolytic foci in a cervical vertebra. **B.** Monomorphic population of plasma cells replacing normal marrow architecture.

of plasma cells (Fig. 1.123B), which may be either well-differentiated or large, anaplastic round cells with a high mitotic index.

Malignant lymphoma may also occur as a primary tumor of bone in humans and animals but, more frequently, bone involvement occurs in association with multicentric lymphoma. There are few reported cases of primary skeletal lymphoma in **dogs**, but most have occurred in animals less than 1 year of age. The lesions in dogs are typically lytic and multiple, and cannot be distinguished radiographically or grossly from plasma cell myeloma. Pathological fractures and hypercalcemia are reported, but hypercalcemia is not a consistent feature of the disease, even when the bone lesions are extensive. Microscopically, sheets of monomorphic lymphocytes replace bone trabeculae and the contents of the marrow cavity in areas of lysis.

Infiltration of bone marrow, together with *multifocal to locally extensive bone infarction*, is reported in **calves** with the juvenile, sporadic form of malignant lymphoma. Affected calves also have generalized lymph node enlargement and variable involvement of other organs, including liver, kidney, and spleen. The bone infarcts are readily visible grossly as discrete, pale areas (Fig. 1.124A), but are not detected unless the bones are sectioned during postmortem examination. The mechanism of the tumor-associated bone marrow necrosis is not clear, but ischemia secondary to infiltration of the marrow with malignant cells is the most likely explanation. Histologically, the infarcted areas may include either neoplastic or nonneoplastic tissue and are often bordered by a zone of edematous connective tissue (Fig. 1.124B).

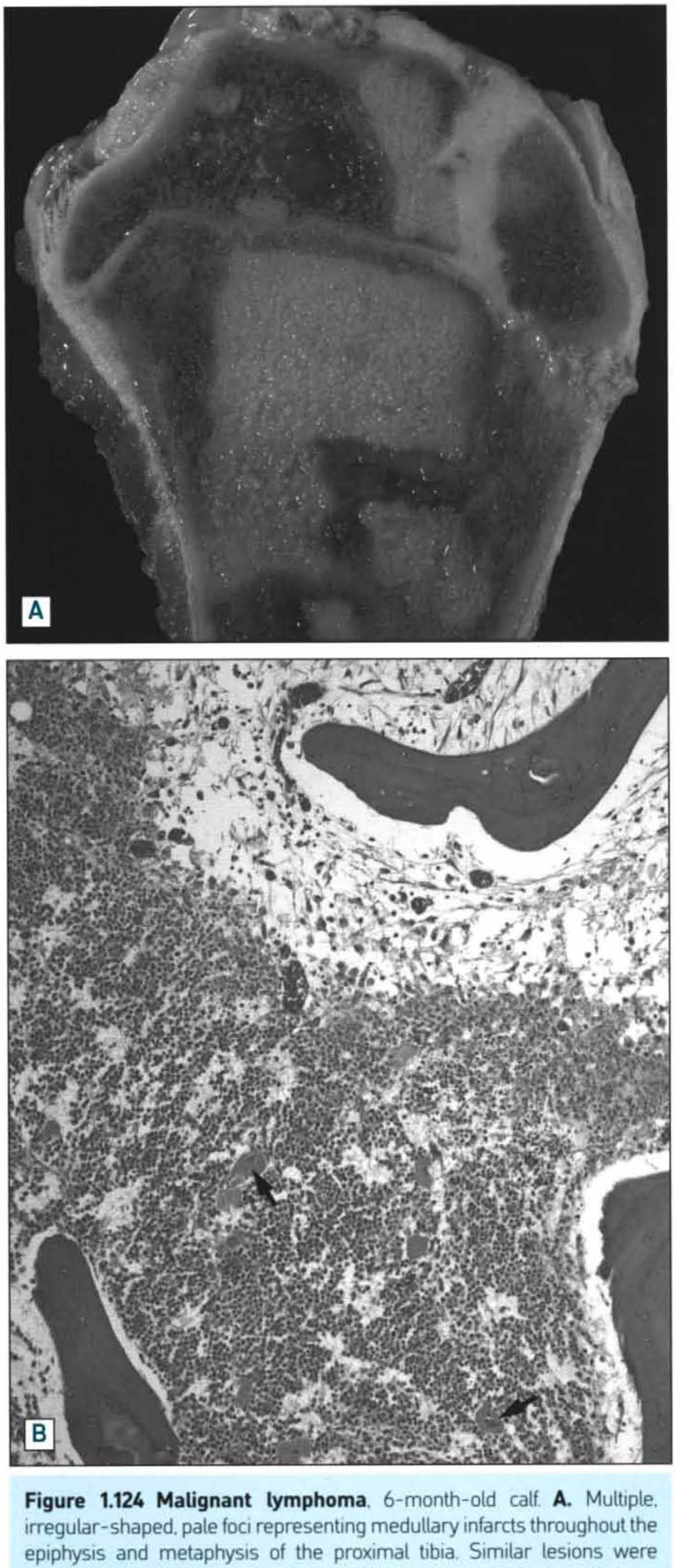

Figure 1.124 Malignant lymphoma. 6-month-old calf. **A.** Multiple, irregular-shaped, pale foci representing medullary infarcts throughout the epiphysis and metaphysis of the proximal tibia. Similar lesions were detected in most other long bones. **B.** Sheets of necrotic tumor cells in an infarcted area, bordered by a zone of edematous connective tissue. Several necrotic megakaryocytes (arrows) are scattered amongst the tumor cells.

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Secondary tumors of bones

Malignant neoplasms originating in soft tissues or in the skeleton may involve bone secondarily either by hematogenous metastasis or direct

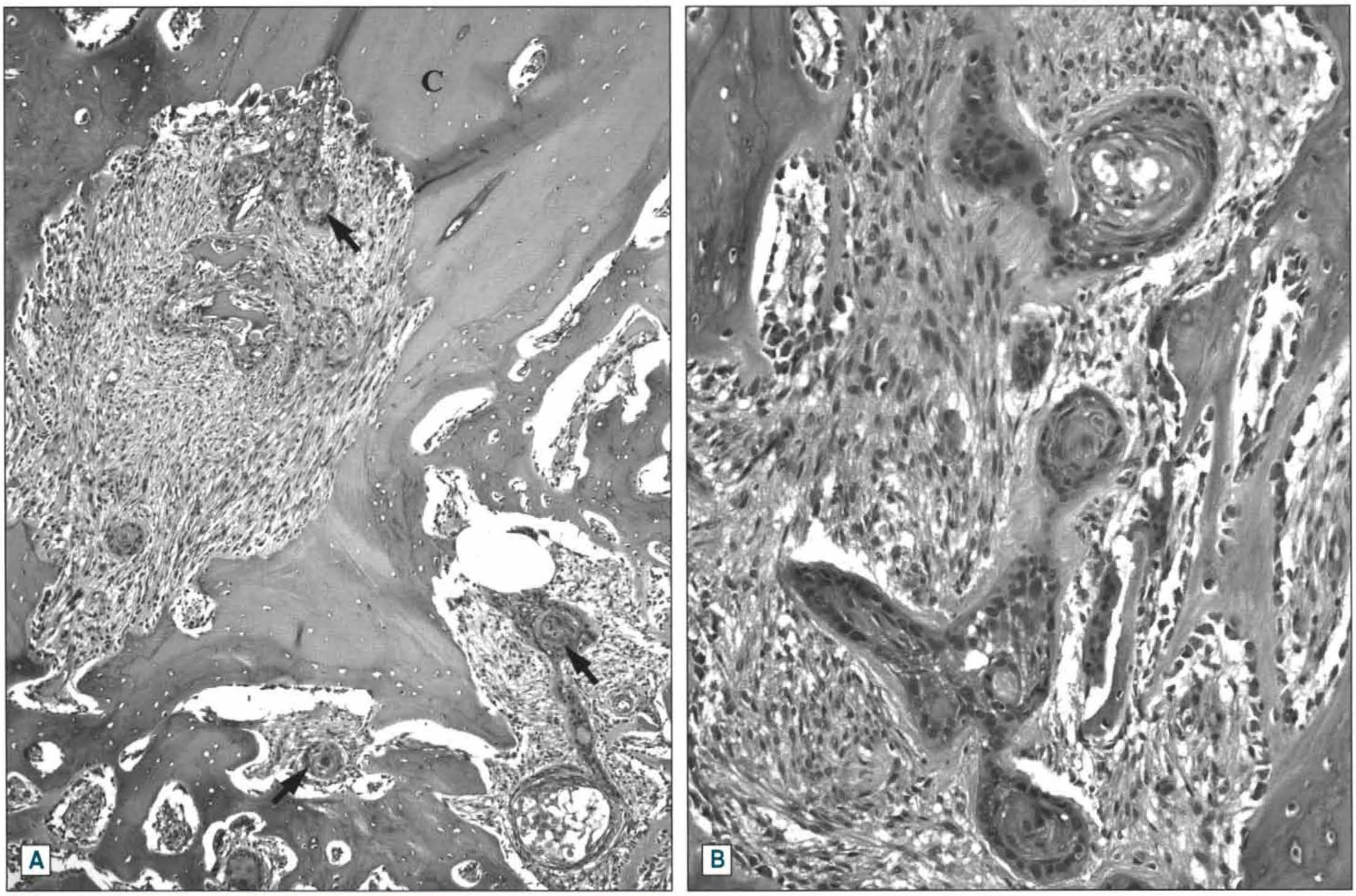

Figure 1.125 Squamous cell carcinoma, cat. Mandible. **A.** Cords of epithelial cells (arrows) surrounded by a marked fibroblastic response invading the dense cortical bone (C) and adjacent reactive bone. **B.** Closer view of tumor cells showing evidence of squamous differentiation.

extension. **Metastatic bone disease** is very common in humans but is *generally considered rare in domestic animals*. Several explanations for this discrepancy have been proposed. The life span of most farm animals is abbreviated by slaughter, and that of many pets by euthanasia, thus limiting the time for tumors to develop, metastasize and, perhaps most importantly, produce signs of metastatic disease.

While there appears to be a genuine difference between the frequency of skeletal metastases in humans and domestic animals, *metastatic bone disease in animals is likely to be much more common than is realized*. The skeleton of animals with malignancies at other sites is seldom examined in detail, either radiographically or during necropsy, and skeletal metastases could easily be missed. In one survey, skeletal metastases were identified in 5.8% of dogs with metastatic carcinoma, but since fewer than 20% of the dogs underwent bone scan, skeletal radiography, or necropsy, the true prevalence was probably much greater. In another study, 98 dogs with a variety of carcinomas were subjected to thorough examination of the skeleton at necropsy. The spine, pelvis, and long bones were sectioned longitudinally and the ribs inspected visually. Macroscopic metastases were detected in 21 (21.4%) of the 98 dogs. The prevalence of metastases would almost certainly have been even higher had the bones been scanned or radiographed, or if the skeleton had been subjected to detailed microscopic examination.

Carcinomas metastasize to the skeleton of dogs much more commonly than sarcomas and the most common tissues of origin are the mammary gland, thyroid, prostate, ovary, and lung. The ribs, vertebrae, and proximal long bones are the favored locations for skeletal metastases in dogs.

Information on the prevalence of skeletal metastases in cats is scarce, but there are occasional reports of pulmonary and mammary carcinomas metastasizing to the bones in this species. Interestingly, metastatic carcinomas in the skeleton of cats more frequently involve bones of the distal limb (acrometastases), unlike humans and dogs where skeletal metastases are uncommon in bones distal to the elbow or stifle.

Secondary bone tumors generally produce osteolytic lesions that are difficult, if not impossible, to distinguish radiographically from primary bone tumors. This may contribute to the underdiagnosis of secondary bone tumors in cases where the radiographic diagnosis is not confirmed by histological or cytological examination of biopsy specimens. For example, in a report of 20 metastatic bone tumors in dogs, the radiographic diagnosis was primary bone tumor in all but one case.

In general, dogs with skeletal metastases are older than dogs with primary tumors of bones (median age 8.5–10 years), and there is no obvious breed or size predilection. Unlike primary bone sarcomas, metastatic skeletal carcinomas are found most commonly in dogs weighing less than 25 kg and approximately 30% of such tumors are found in small dogs weighing less than 15 kg.

A definitive diagnosis of metastatic bone tumor is based on either cytological or histological examination of fine-needle aspirates or tissue biopsies respectively but, in most cases, the tumor cells provide few indications as to the tissue of origin. Even after thorough postmortem examination, the primary site of a metastatic skeletal carcinoma may not be apparent, although malignant epithelial cells within a skeletal lesion can only be derived from an extraskeletal carcinoma. Osteosarcomas sometimes metastasize from the bone of origin to other skeletal sites, in which case the secondary lesions may be in atypical locations and may appear to have been present for different lengths of time. For some sarcomas, such as hemangiosarcoma, which may originate in bone or other tissues, it may be impossible to determine whether skeletal involvement is primary or secondary but if the tumor is present in several skeletal sites then metastasis is more likely.

Malignant tumors in soft tissues adjacent to a bone may penetrate the bone by direct extension and can be referred to as **invasive tumors of bones**. *This is a feature of some types of tumor, in particular squamous cell carcinoma of the digits and oral cavity in dogs and cats.* These aggressive tumors frequently invade the periosteum of the underlying bone, erode the cortex, and penetrate the medullary cavity. In dogs, bone invasion is reported to occur in 77% of oral squamous cell carcinomas. Microscopically, cords of anaplastic epithelial cells, showing evidence of squamous differentiation, permeate the osseous and extra-osseous tissues, and are accompanied either by an osteoblastic reaction or a prominent fibroblastic response with osteoclastic resorption of adjacent bone (Fig. 1.125A, B). Squamous cell carcinomas of the canine digit not only invade bone but also metastasize to local lymph nodes, and eventually to the lungs.

Other tumors that appear to have an affinity for bone invasion are *malignant melanomas of the oral cavity in dogs* and *fibrosarcomas of the canine skull and long bones*. Tumors of dental origin, including acanthomatous epulis, ameloblastoma, and fibroameloblastoma, may also extend into the maxilla or mandible of dogs and cats.

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Tumor-like lesions of bones

Several nonneoplastic lesions of bones occur in humans and domestic animals and must be differentiated from primary and secondary bone tumors. Included amongst these are various forms of **bone cysts**, some of which are not true cysts as defined by pathologists but appear "cystic" radiographically.

Solitary or **unicameral bone cysts** are reported in the *metaphyses of long bones in children and young dogs*, a breed predisposition having been suggested in Doberman Pinschers. The lesions may be monostotic or polyostotic and are generally lytic and expansile, with erosion of the cortex and little or no periosteal new bone formation. In radiographs, shelves of bone often project from the cyst wall, but in most cases, these are incomplete and the cysts are unicameral. Pathological fracture may occur due to the localized bone destruction. *The cyst cavity is filled with clear or sanguineous fluid and lined by a connective tissue membrane of variable thickness, with scattered multinucleate giant cells and hemosiderin-containing macrophages.* The pathogenesis of unicameral bone cysts is uncertain but they may be the result of impaired venous drainage from sites of active endochondral ossification. Recurrence following surgical drainage and curettage is uncommon.

Aneurysmal bone cysts have been reported rarely in dogs, cats, horses, and cattle. Radiographically, they appear as *expansile, osteolytic lesions* (Fig. 1.126A) *contained by a thin, "ballooned" periosteum and with an internal "soap-bubble" appearance* and must be differentiated from osteosarcoma, hemangiosarcoma, fibrosarcoma, and plasma cell myeloma. Too few cases are reported in animals to establish age or site prevalence, although lesions are described in bones of both the axial and appendicular skeleton. The gross appearance of aneurysmal bone cysts closely resembles telangiectatic osteosarcoma and hemangiosarcoma, typically exuding blood from the cut surface, and may contain solid areas in addition to multiple blood-filled cysts. Pathological fracture may be present.

Fine-needle aspiration biopsies are unlikely to be useful in the diagnosis of either aneurysmal or unicameral bone cysts as the preparations are likely to be heavily contaminated with blood. Confirmation of the diagnosis requires histopathological examination of biopsy specimens. *The lesion consists of cavernous blood-filled spaces* (Fig. 1.126B) *separated by septa of loosely arranged spindle cells with scattered multinucleate giant cells and hemosiderin-containing macrophages*, similar to the lining of unicameral bone cysts. Osteoid or spicules of reactive bone may be present in the surrounding connective tissue (Fig. 1.126C) and should not be mistaken for tumor bone. The pathogenesis of these lesions is not known, but altered blood flow, perhaps secondary to trauma or some other bone disease, such as malignancy, is believed to play a role.

Subchondral (juxtacortical) bone cysts occur in young horses, pigs, and occasionally in other species, as a manifestation of osteochondrosis. The lesions are typically 5–10 mm in diameter and may be multilocular. In horses, subchondral cysts are found most frequently beneath the articular surfaces of phalanges, but also occur in femoral condyles and a range of other bones. *The cysts may develop either from residual nodules of epiphyseal cartilage or within foci of subarticular hemorrhage.* Histologically, the lesions are not always cystic, instead consisting of fibrous tissue with extensive myxoid matrix or, in some cases, degenerate cartilage. Cystic spaces lined by fibrous tissue and containing fluid, possibly of synovial origin, are present within some lesions. In humans, subchondral cysts occur in association with degenerative joint disease and are thought to be secondary to either synovial fluid intrusion or bony contusion.

Intraosseous epidermoid cysts *are reported rarely in dogs as a cause of lytic lesions in the distal phalanx.* At this site, they must be differentiated from nail-bed carcinomas, malignant melanomas, and osteomyelitis. One reported case involved the tenth thoracic vertebral body of a dog. Similar lesions occur in the distal phalanx and skull of humans. Radiographically, there may be a sclerotic reaction around one or more lytic foci and extensive periosteal new bone formation. Grossly, the lesion consists of multilocular cysts containing pale cream, crumbly material. Microscopically, *the cysts are lined by well-differentiated, stratified squamous, keratinizing epithelium and filled with layers of keratinized squames.* The squamous epithelium may show marked pseudoepitheliomatous hyperplasia and could be mistaken

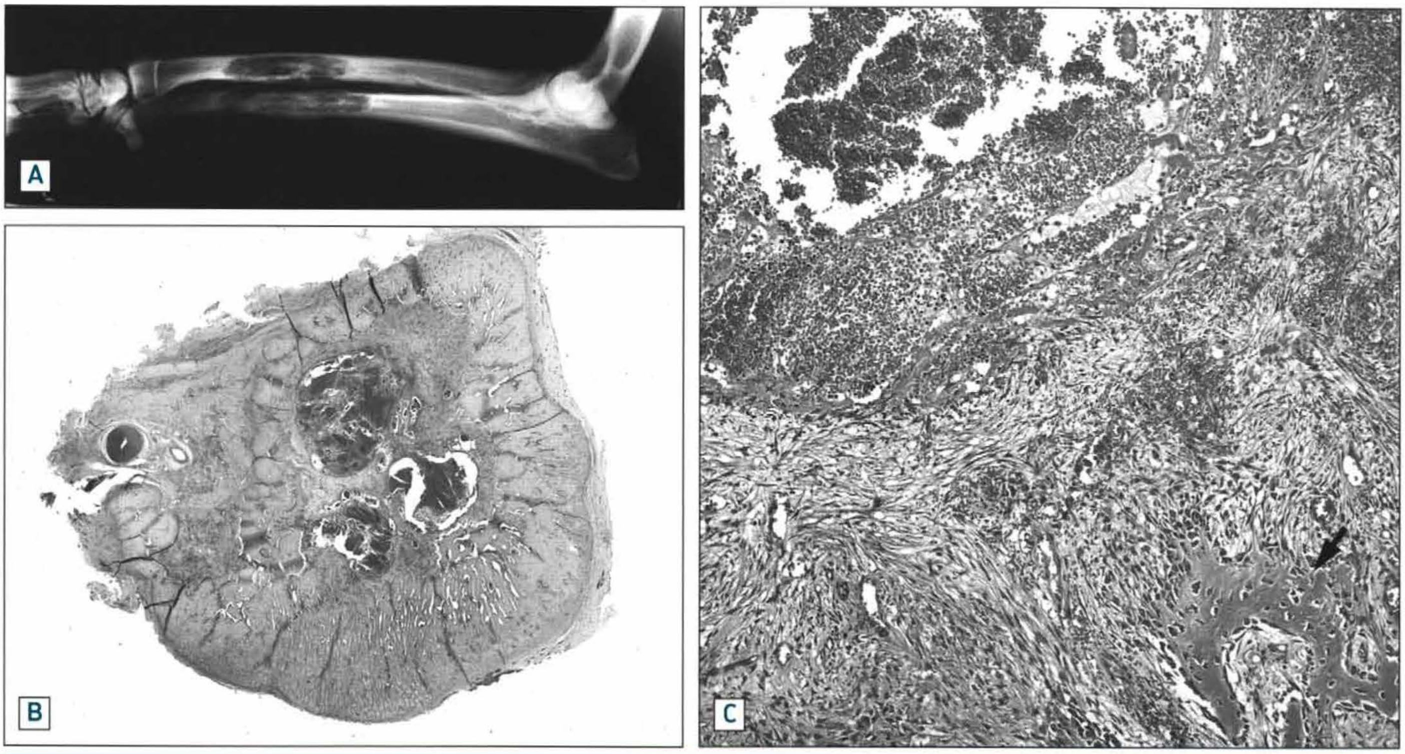

Figure 1.126 Aneurysmal bone cyst. 1-year-old dog. Radius and ulna. **A.** Radiograph showing expansile, osteolytic lesions in the distal diaphyseal regions of the radius and ulna. **B.** Subgross, transverse section of ulna showing cavernous, blood-filled spaces surrounded by loose connective tissue, reactive bone, and remnants of cortical bone. **C.** Reactive bone (arrow) forming in the connective tissue near a blood-filled space.

for squamous cell carcinoma, especially if a biopsy sample is inadequate. The cysts are generally supported by a dense fibrous stroma and surrounded by thickened bone trabeculae. The pathogenesis of intra-osseous epidermoid cysts is uncertain, but the digital lesions are believed to be *secondary to a penetrating wound*, with traumatic implantation of epidermal fragments into the underlying bone. Cysts involving the skull of humans, and vertebral body of a dog, may represent *rests of heterotopic ectoderm* that have become sequestered along lines of closure during embryonic development.

An **exuberant fracture callus** containing extensive areas of disorganized and primitive bone, cartilage, and fibrous tissue *may also resemble a primary bone tumor.* Even in cytological and histological preparations, differentiation of an early callus from osteosarcoma and chondrosarcoma can present a significant challenge, especially in cases where the clinical and radiographic history is inadequate.

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Diseases of joints

GENERAL CONSIDERATIONS

Three main types of joints or articulations unite adjacent bones and/or cartilaginous structures throughout the skeleton. These are classified on the basis of their morphology and tissue composition as *fibrous, cartilaginous, or synovial joints.* This method of classification has limitations as some joints contain a mixture of the different tissue types, while others change their composition during maturation. Furthermore, even within each category, there is considerable variation in the amount of movement between adjacent skeletal structures.

In spite of these limitations, the system is widely accepted in the medical and veterinary literature and is used throughout this chapter.

Fibrous joints

In these joints the *bones are united by fibrous tissue*, which allows little movement between them. Fibrous joints are *subdivided into sutures, syndesmoses, and gomphoses.*

Sutures are limited to the *skull*, where they allow continued growth of cranial bones by intramembranous ossification as the brain matures. Osteogenic cells form a cambial layer adjacent to the bone-forming surfaces and are separated by intervening layers of fibrous tissue, which vary in thickness depending on their location. Broader sheets of fibrous tissue often occur at the junctions of three adjacent skull bones and are referred to as **fontanelles**. A bony union or **synostosis** replaces the fibrous tissue of many sutures once growth ceases.

A **syndesmosis** is a fibrous joint in which *adjacent bones are united by an interosseous ligament or membrane*, such as occurs in some species between the shafts of the tibia and fibula and between the radius and ulna. Syndesmoses contain fibrous and elastic connective tissue in variable proportions. Consequently, minor movement may occur between the bones due to stretching of the ligament or membrane.

Gomphoses are specialized fibrous *joints between the teeth and either the mandible or maxilla.* The membrane between tooth and bone is termed the **periodontal ligament**, and although it contains no elastic fibers, it allows slight movement of the tooth.

Cartilaginous joints

These are joints in which the *union consists of either hyaline or fibrocartilage*, or a combination of the two. There are two types of cartilaginous joints, *synchondroses and symphyses.*

Synchondroses are *temporary joints* that exist only while the skeleton is growing and are replaced by bone once the skeleton matures. *Physeal growth plates*, which unite the separate centers of ossification in long bones, are synchondroses consisting of well-organized hyaline cartilage. Synchondroses also exist between bones forming by endochondral ossification in the basicranium.

Symphyses are located in the *mid-sagittal plane of the body and are permanent joints*, unlike synchondroses. The adjacent bones are capped by a thin layer of hyaline cartilage, which blends with fibrocartilage, forming a joint with great strength while still allowing a limited amount of movement. Examples are the *pubic symphysis and intervertebral disks.* Intervertebral disks unite each pair of vertebrae in the vertebral column, with the exception of the atlas and axis.

Diseases of **intervertebral disks** are very common in humans and certain domestic animals. For this reason, a brief discussion of the unique structure and function of these joints is appropriate. Each disk contains *a central core, the* **nucleus pulposus**, which is a remnant of the notochord. In young animals, the nucleus pulposus is gelatinous and translucent (Fig. 1.127). The matrix consists of glycosaminoglycans, particularly chondroitin-6-sulfate, keratan sulfate, and hyaluronan (hyaluronic acid), collagen (mainly type II) and a large amount of water. The cellular concentration is relatively sparse and consists predominantly of *chondrocytes*, which are often arranged in small clusters, and fibrocytes. The nucleus pulposus of immature animals may also contain clusters of notochordal or physaliferous cells, which have abundant finely vacuolated cytoplasm filled with glycogen. With aging, the glycosaminoglycan and water concentration of intervertebral disks declines and the number of fibrocytes increases. *The nucleus pulposus is surrounded by the* **annulus fibrosus**, which is broader ventrally than dorsally and consists of concentric layers of fibrocartilage. The direction of the collagen fibers alternates between each layer. The matrix of the annulus fibrosus consists predominantly of type I collagen, with lesser quantities of glycosaminoglycans. *Fibrocytes* are the predominant cell type. Fine nerve endings are present in the outer third of the annulus fibrosus. *The cranial and caudal boundaries of intervertebral disks are occupied by* **cartilaginous end plates**, which consist of hyaline cartilage and are in direct apposition to the vertebral bodies on either side of the joint. Collagen fibers from the annulus fibrosus merge with those of the cartilaginous end plates and become embedded in the bony trabeculae of the vertebral body, forming a strong, stable union.

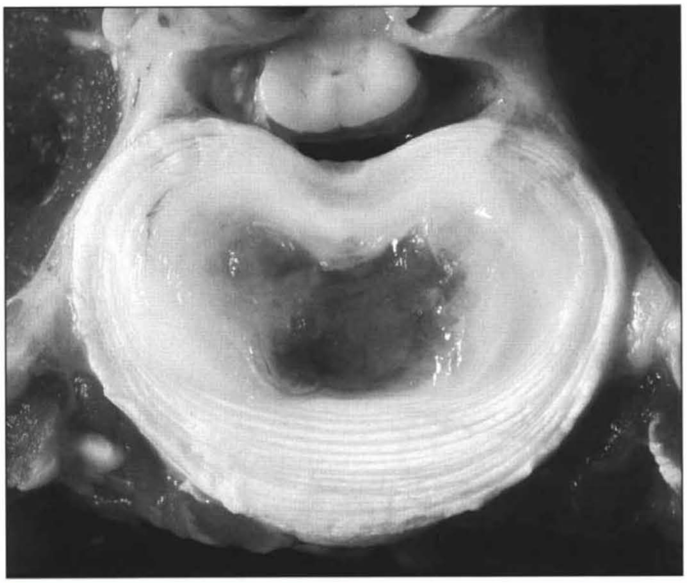

Figure 1.127 Intervertebral disk of a young dog. The gelatinous nucleus pulposus is surrounded by the annulus fibrosus, which consists of concentric layers of fibrocartilage.

Dorsally and ventrally, the intervertebral disks merge with the **dorsal and ventral longitudinal ligaments**, which run the length of the spinal column. The dorsal longitudinal ligament lies in the floor of the vertebral canal, merging with each disk as it passes, except between the second and tenth thoracic vertebrae. In this region, *conjugal ligaments* connecting the heads of the corresponding ribs cross the floor of the canal between the dorsal longitudinal ligament and the dorsal portion of the annulus fibrosus. The extra support provided by the conjugal ligaments is no doubt responsible for the low incidence of disk protrusions between the second and tenth thoracic vertebrae in dogs.

Intervertebral disks are designed to allow limited movement between adjacent vertebral bodies when the vertebral column is subjected to a wide variety of different loading conditions, including compression, tension, bending, shear forces, and torsion. Degenerative changes occurring in the nucleus pulposus and/or annulus fibrosus as part of the aging process or in disease states can markedly alter the ability of intervertebral disks to withstand such forces.

Synovial joints

Synovial or **diarthrodial** joints are found predominantly in the appendicular skeleton and allow considerable movement between adjacent bones. The bone ends in these specialized joints are covered by *hyaline articular cartilage* and an *articular capsule* surrounds a central cavity filled with *synovial fluid*. Some synovial joints are supported by ligaments, while others, such as the femorotibial joint, contain fibrocartilaginous menisci. Because of the importance of these joints, and the frequency with which they are involved in disease processes, the individual components of synovial joints will be discussed in more detail.

Articular cartilage is the key component of synovial joints, being required to withstand the compressive forces associated with weight bearing in addition to the shear forces that occur during motion. Grossly, it is smooth, bluish-white, and turgid in young animals but, with advancing age, it becomes yellow, opaque, and less elastic. The thickness of articular cartilage varies between and within joints, tending to be thickest at points of maximum weight bearing.

The matrix of articular cartilage is approximately 70–80% water. *The organic component consists largely of proteoglycans, type II collagen, and lesser quantities of types V, VI, IX, X, and XI.* Although not apparent in routine histologic preparations, the type II collagen fibrils are arranged in loops with either end firmly embedded in the calcified zone. Therefore, the fibrils located in the superficial zone are oriented parallel to the articular surface, while those in the radial zone are more vertical. This arrangement presumably enhances the ability of articular cartilage to withstand the range of shear and compressive forces to which it is exposed. In addition to covalent cross-links between chains of type II collagen in articular cartilage, there are intermolecular links between type II and type IX collagen. Type IX collagen is a small, nonfibrillar molecule, comprising only 1–2% of articular cartilage, but appears to be important in anchoring proteoglycan molecules to collagen and stabilizing the network of collagen fibers within the matrix.

The *proteoglycan subunits of articular cartilage* consist of a core protein with the glycosaminoglycan molecules chondroitin-6-sulfate, chondroitin-4-sulfate, and keratan sulfate attached. A small peptide referred to as *link protein* links the amino-terminal end of the core protein in proteoglycan subunits to hyaluronan (hyaluronic acid) filaments, thus forming massive, stable proteoglycan aggregates. The term *aggrecan* is applied to the proteoglycan monomers that link with hyaluronan, and the complex is referred to as an aggrecan aggregate. The glycosaminoglycan molecules in these aggregates are negatively charged because of the presence of many carboxyl and sulfate groups and therefore remain separated when attached to the core protein. Water molecules are trapped and immobilized by the negative charges and the meshwork of large, hydrophilic molecules, resulting in the *formation of a gel* supported by a delicate reticular arrangement of collagen fibrils. Water can move only slowly within this meshwork, thus allowing articular cartilage to maintain its turgidity when subjected to a compressive load. This flow of water during movement is important in promoting the transport of nutrients to chondrocytes within the articular cartilage and providing lubricant for the joint. Small quantities of nonaggregating proteoglycan monomers, including decorin and biglycan, are also found in articular cartilage. The role of these proteoglycans, which consist of chondroitin sulfate and dermatan sulfate, is not clear.

Histologically, the cellular concentration of articular cartilage is low, especially in adults, and the chondrocytes are not distributed randomly. In the *superficial or gliding zone*, the chondrocytes are relatively small and flattened, with their long axis parallel to the articular surface. Beneath this layer, is an *intermediate (transitional) zone* in which the chondrocytes are round or ovoid, then a *radial zone*, in which large, round chondrocytes line up vertically in short columns reminiscent of those in the physis. The proteoglycan content of the transitional and radial zones is greater than that of the superficial zone. The fourth recognizable zone in mature articular cartilage is the *calcified zone*, which is separated from the radial zone by an irregular basophilic line, referred to as the "*tidemark*." Although collagen fibrils do not cross from the calcified zone of articular cartilage into subchondral bone, the undulating nature of this osteochondral interface provides a strong attachment with considerable resistance to shearing forces. In immature animals, endochondral ossification beneath the articular cartilage contributes to the growth in size of the epiphysis. The metachromasia of cartilage matrix with stains such as toluidine blue is due to its glycosaminoglycan content. When proteoglycans are lost, metachromasia is reduced, the intercellular substance stains positively by the periodic acid-Schiff (PAS) method, and collagen fibers are more prominent.

Although the collagen component of articular cartilage is relatively stable, the *proteoglycan macromolecules are constantly being degraded and replaced*, perhaps as part of an internal remodeling system mediated by autodegradative enzymes. Various zinc-dependent metalloproteinases are secreted by the chondrocytes and cleave the core protein, link protein, and hyaluronan molecules at specific locations. The fragments diffuse into the synovial fluid then into the synovial membrane, where they are removed by lymphatics. Overactivity of these proteases may be responsible for cartilage degradation in degenerative joint disease and immune-mediated arthritis. Chondrocytes in articular cartilage must continually synthesize new matrix components in order to replace those that are degraded and lost. They respond to various soluble mediators, including synovial cytokines, such as tumor necrosis factor and interleukin-1, in addition to mechanical loading, changes in hydrostatic pressure and even to electric fields. A serine proteinase inhibitor and various collagenase inhibitors have been isolated from human and bovine articular cartilage. These are probably important in the regulation of extracellular matrix degradation.

Articular cartilage is devoid of blood vessels and nerves. The chondrocytes within articular cartilage therefore depend on diffusion of nutrients from synovial fluid through the extracellular matrix, except in immature animals where a proportion of the nutrients entering articular cartilage may diffuse through the relatively permeable subchondral bone. Because of the absence of nerve endings, damage to articular cartilage does not cause pain unless there is concomitant injury to the subchondral bone and/or joint capsule, both of which are well supplied with nerves. The surface of the cartilage appears to be porous, at least in macromolecular dimensions, and the diffusion of nutrients into cartilage is aided by the massaging effect of normal movement. It appears that the integrity of the cartilage is preserved only if joint movements are physiological in their range and pressure. *Sustained excesses of pressure and prolonged disuse accelerate degeneration.* The latter is of particular relevance in animals with limb fractures, where one or more joints may be immobilized by casting or external fixation. Such fixators should be left in place for the shortest time possible and, ideally, allow up to 10 degrees of joint motion.

The chemical composition of articular cartilage alters with advancing age. Although the collagen content remains relatively stable throughout life, the proteoglycan content slowly decreases from birth. The protein core and glycosaminoglycan chains are longer in immature animals than in adults and there is a rapid decline in the concentration of chondroitin-4 sulfate with aging. This is accompanied by a corresponding increase in the concentration of keratan sulfate.

The thin plate of **subchondral bone**, to which the articular cartilage is attached, is approximately *ten times more deformable than cortical bone.* This is important in allowing more even distribution of the load between the articular cartilage and the bone at times of peak loading. In chronic degenerative joint diseases, the subchondral bone may become denser. In such cases, the articular cartilage is required to bear an increased proportion of the burden and the degenerative process is accelerated. There is some debate as to whether thickening of the subchondral bone precedes degeneration of the articular cartilage in degenerative joint diseases, or occurs as a sequel, but it is likely that the two processes occur concurrently.

In some joints of cattle, horses, and pigs, nonarticulating depressions known as **synovial fossae** (Fig. 1.128) are present near the midline. These normal structures are bilaterally symmetrical and are acquired during the first months of postnatal life as a consequence of joint modeling. During skeletal growth, acquired incongruities in central areas of certain joints cause progressive loss of contact between apposing articular surfaces. While the superficial or gliding layer of the articular cartilage in these areas persists, chondrocytes in the deeper zones of the subarticular growth cartilage are not maintained and gradually disappear. Gradually, synovial fossae appear as central depressions having distinct borders and a smooth, blue to pink surface, reflecting the proximity of the subchondral capillary bed. A study in 50 swine found that synovial fossae were not present at birth, but were present commonly after 4–5 months of age on the articular surfaces of the scapula, distal humerus, proximal radius, distal radius, and distal surface of the intermediate carpal bone. *It is important that synovial fossae are not mistaken for lesions in the articular cartilage or as indicators of collapsed subchondral bone.* In general, they are of no significance, although in horses they may be structural points of weakness where infection can be passed between the joint cavity and the subchondral bone. In diseased joints, synovial fossae may become more prominent due to hyperemia of the synovium or the underlying epiphyseal bone.

An **articular capsule** surrounds each synovial joint and consists of an outer fibrous capsule and an inner synovial membrane. The **fibrous capsule** consists of parallel bundles of dense, fibrous connective tissue and merges with the periosteum of the bones on either side of the joint. This strong capsule restricts the range of movement possible between articulating bone ends and is supported in some areas by focal thickenings or **ligaments**. Intra-articular ligaments, such as the cruciate ligaments of the stifle, add further support in some joints. **Tendons** may also attach to the articular capsule, adding strength to areas that require it. Collagen fibers from ligaments and tendons may be attached to the fibrous capsule or may attach to bone at sites referred to as **entheses**. At these sites, collagen fibers from the tendon merge with zones of unmineralized then mineralized fibrocartilage, before becoming incorporated into bone as **Sharpey's fibers**. Entheses are well served by anastomosing blood vessels originating within the peritenon, perichondrium, and periosteum, and have a prominent nerve supply. Excessive tension on ligaments may lead to rupture of the ligament, or to an avulsion fracture, where a fragment of bone is detached with the enthesis intact. The latter is not uncommon in animals with rickets or fibrous osteodystrophy. The fibrous capsule is well supplied with blood vessels, lymphatics, proprioceptive nerves, and pain receptors. Thickening of the fibrous capsule in animals with chronic joint diseases leads to reduced motion or stiff joints but may be difficult to appreciate clinically or at necropsy.

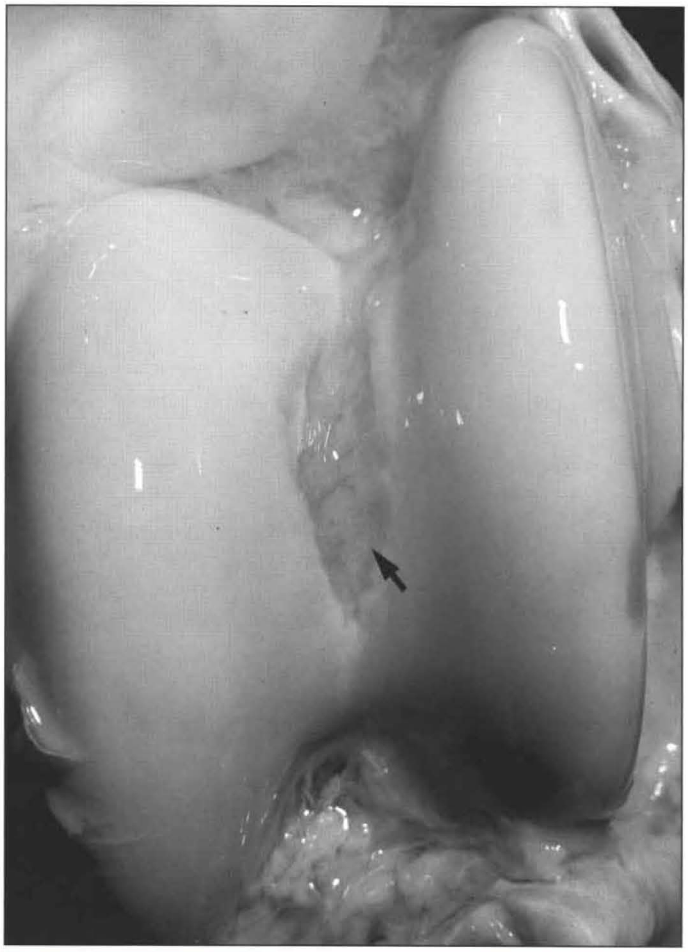

Figure 1.128 Synovial fossa (arrow) in the groove between articular condyles of the talus in a horse.

In the *femorotibial joints* of domestic animals, *semilunar fibrocartilaginous disks, or* **menisci**, provide additional stability. These structures are firmly attached to ligaments, or to the fibrous layer of the joint capsule, and extend into the joint space between the articulating bone surfaces. Menisci are not lined by a synovial membrane but are innervated and have a blood supply. Similar, but more circular or oval structures, referred to as **articular disks**, *are present in the temporomandibular joint.* Articular disks may possess a central perforation. Intrameniscal calcification and ossification in the stifle joint occurs uncommonly in humans and has been reported in cats. Such lesions must be differentiated from intra-articular avulsion fractures or loose bodies in clinical radiographs.

The **synovial membrane** is a smooth, glistening, highly vascular layer that lines the inner surface of the fibrous capsule. It also covers any intra-articular ligaments or tendons and is reflected on intra-articular bone where it merges with the periosteum or the perichondrium, as the case may be. In the transition zone, it merges with the articular margins and spreads for a short distance over the non-weight-bearing articular cartilage. In some areas, particularly in recesses of the joint, the synovial membrane has many *small, villus projections*, which presumably permit expansion of the synovial membrane in association with joint movement or changes in intra-articular pressure. These synovial villi are not easily discerned macroscopically in normal joints but may become enlarged, hyperemic, and more numerous in some chronic inflammatory or degenerative diseases of joints. In addition, thickened folds of synovial membrane, often containing adipose tissue, extend into the joint cavity. These *fat pads* generally occupy depressions on the articular surface and are displaced during motion. Synovial membranes lining tendon sheaths and bursae are structurally similar to those lining diarthrodial joints.

The *synovial membrane generally consists of two layers*, a thin, cellular **intima** on the inner surface and a **subintima**, which contains variable quantities of areolar, adipose, and fibrous tissue. The loose fibrous connective tissue of the subintima merges with the dense fibrous capsule. The subintima is richly vascular, and contains many lymphatics and nerves, together with a small number of histiocytes. Dendritic cells similar to the antigen-processing Langerhans cells of skin have been identified in the synovium and have been shown to interdigitate with T-lymphocytes. Specialized post-capillary venules with cuboidal endothelial cells, referred to as *high endothelial venules*, allow lymphocytes to migrate easily from the blood into the subintima of the synovial membrane in certain inflammatory conditions. In areas where the synovial membrane lines intra-articular ligaments or tendons, the subintimal layer is usually attenuated or inapparent.

The synovial intima consists of *synoviocytes* forming an ill-defined layer, one to three cells deep. The cells vary in shape from fusiform to polygonal. No basement membrane exists between the synovial intima and subintima and the *synoviocytes are of mesenchymal rather than epithelial origin*. In some areas, the synoviocytes are closely packed, while elsewhere they are relatively sparse and the subintimal layer may be directly apposed to the joint cavity. Two types of synoviocytes, referred to as type A (macrophage-like) and type B (fibroblast-like) cells, are recognized on the basis of their morphology, function, and immunochemical staining. *Type A synoviocytes originate from the bone marrow and have phagocytic and antigen-processing functions*. Ultrastructurally, they resemble tissue macrophages, possessing a dense, heterochromatin-rich nucleus, many cytoplasmic vacuoles and poorly developed rough endoplasmic reticulum. They are primarily responsible for removing and degrading particulate matter from the joint cavity and possess antigen-processing properties. *Type B synoviocytes are probably of fibroblastic origin*. They have a well-developed Golgi apparatus, prominent rough endoplasmic reticulum and are responsible for the synthesis of hyaluronan in addition to matrix components, including collagen. They are also equipped with various enzymes capable of degrading cartilage and bone. Both cell types produce cytokines and other mediators. Cells with ultrastructural characteristics intermediate between types A and B synoviocytes, so-called *intermediate or type C cells*, have also been described.

The synovial membrane is freely permeable in either direction to molecules of small dimension, which may be removed by the capillaries and lymphatics. Larger particles are phagocytosed by type A synoviocytes. The removal of particulate matter from the joint and its deposition in the subintimal layer is a continuous process. Because of the long life span of type A synoviocytes, estimated to be from 3–6 months, phagocytosed particulate matter may persist in the synovium for long periods. When the volume is large, as may occur in diseased joints, its presence in the synovial membrane stimulates fibrosis of the capsule, contributing to the swelling and fixation of diseased joints. *The synovial membrane proliferates markedly in certain disease states*, and has considerable powers of regeneration following injury or synoviectomy. *In chronic synovitis, lymphocytes and plasma cells diffusely infiltrate the synovial membrane, and may accumulate in hypertrophic synovial villi*. The lymphocytes are sometimes arranged in follicles.

The transitional zone of the synovial membrane is characterized by a gradual merging of the synovial membrane with the periosteum and cartilage margin. The intima is well vascularized where it extends onto the cartilage surface and it is in this area that erosion, or alternatively lipping and osteophyte formation, often occurs in chronic arthritis and degenerative arthropathy. The transitional zone of the synovial membrane is also the initial site of synovial proliferation and development of the *vascular granulation tissue or* **pannus,** which sometimes spreads across the articular cartilage in chronically inflamed joints. Pannus may also develop from the marrow spaces of subchondral bone and extend onto the articular surface through defects in the cartilage. *This layer of granulation tissue interferes with the normal nutrition of articular cartilage and is capable of eroding and destroying cartilage through the activity of cytokines and collagenases*. Collagenases may be synthesized by cells in diseased synovial membranes, by granulation tissue, and by neutrophils in the synovial fluid. The collagens of the synovial membrane itself are generally resistant to these collagenases because of their cross-linking. Adhesion of pannus to opposing articular surfaces *may cause fibrous ankylosis* and, if bone is formed in the connective tissue, bony ankylosis results. Few animals are permitted to survive to the stage of bony ankylosis, but fibrous ankylosis occurs occasionally in pigs with mycoplasma arthritis and in other chronic arthritides. Bony ankylosis sometimes involves the intercarpal joints of racing horses.

Synovial chondromatosis (osteochondromatosis) is a syndrome characterized by the presence of *many nodules of hyaline cartilage, some of which may undergo ossification, in the synovial membrane* (Fig. 1.129). It is widely believed that these nodules develop by metaplasia, or even benign neoplasia, of mesenchymal elements in the synovium. An alternative hypothesis is that, at least in some cases, they are derived from fragments of cartilage eroded from a damaged articular surface as part of a pre-existing joint disease. Such fragments are capable of surviving, and growing, in the synovial fluid, which provides a nutritious culture medium. Some may attach and become embedded in the synovial membrane, where survival and proliferation of chondrocytes within fragments would also be expected. Some of these bodies then become vascularized and undergo endochondral ossification. Some human patients and animals with synovial chondromatosis also have either few or multiple loose bodies free within the synovial fluid. These could represent nodules that have developed within the synovial membrane and become detached, but it seems more likely that they are of articular cartilage origin and have grown within the joint space, as occurs in some animals with osteochondrosis. However, synovial

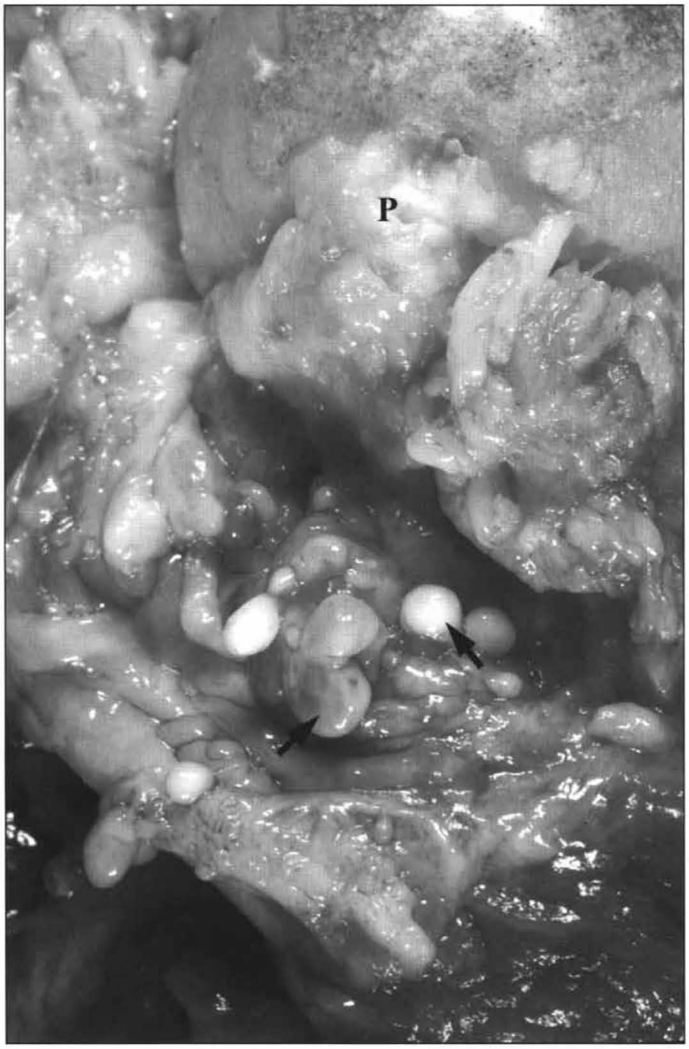

Figure 1.129 Synovial chondromatosis associated with degenerative joint disease in the hip of a dog. Several smooth, oval, cartilaginous nodules (arrows) are present within or attached to the synovium. Also note the villus hypertrophy, pannus formation (P), and eburnation of the femoral head. (Courtesy of RA Fairley.)

chondromatosis sometimes occurs in the absence of an underlying articular disease, or involves extra-articular structures such as bursae or tendon sheaths. Such cases, referred to as **primary synovial chondromatosis**, are more likely to be due to chondroid metaplasia of subsynovial fibroblasts, although the possibility that they are seeded by microscopic fragments of cartilage released from mildly damaged articular surfaces should be considered.

Synovial fluid is a viscous, clear, colorless or slightly yellow fluid, and is the main source of nutrients for articular cartilage. Essentially, *it is a dialysate of plasma, modified by the addition of hyaluronan, glycoprotein, and various other macromolecules by synoviocytes and other cells in the synovial membrane.* Electrolytes and small molecules such as glucose, lactate, and some small plasma proteins are able to move freely into and out of the synovial fluid through the synovial membrane, but large proteins such as fibrinogen are excluded. The viscous nature of synovial fluid reflects its high concentration of hyaluronan and varies between joints, as does the volume of fluid.

Hyaluronan is believed to function as a lubricant for the synovial membrane and periarticular tissues, but it probably plays little, if any part, in lubricating the motion between opposing cartilage surfaces. Lubrication of synovial joints under relatively light loading relies on the presence of the glycoprotein **lubricin**, which adheres to the surface of articular cartilage and binds a highly insoluble, **surface-active phospholipid** with strong lubricating properties. Recent studies have demonstrated that *it is the surface-active phospholipid, rather than lubricin, that actually lubricates the joint.* This is referred to as boundary lubrication. *Lubrication of articular surfaces under heavier loads depends on hydrostatic (weeping) lubrication*, which involves the seepage of water from the matrix when the cartilage is compressed. The water is squeezed onto the articular surface just ahead of the contact area and accumulates in the many minute depressions that are normally present. Once the compressive force is removed, the fixed charges within the cartilage attract the water and small solutes back into the matrix, and the cartilage returns to its original shape.

Normal diarthrodial joints contain only a very small volume of synovial fluid but the volume generally increases greatly in response to injury or inflammation. This is most likely due in part to increased vascular permeability in the synovial membrane following the release of inflammatory mediators such as prostaglandins and cytokines. The resulting increase in protein concentration of synovial fluid alters the normal oncotic balance and therefore fluid volume. In damaged joints, increased lymphatic drainage accelerates the clearance of proteins and cartilage breakdown products from synovial fluid.

Synovial fluid normally contains a small number of mononuclear cells and occasional free synoviocytes. Neutrophils and erythrocytes are uncommon unless the joint has been damaged, the synovial membrane is inflamed, or the sample has been contaminated with blood during collection. *The number of neutrophils increases markedly in both septic and nonseptic inflammatory diseases of the joint.* The synovial fluid may become turbid and less viscous depending on the number of neutrophils that are present.

Response of articular cartilage to injury

Because of its avascular nature and the absence of undifferentiated cells with the ability to respond to injury, *articular cartilage has only limited powers of regeneration.* The chondrocytes of mature articular cartilage show little if any sign of mitotic activity and have limited capacity for increasing matrix synthesis. Furthermore, their encasement in lacunae restricts their capacity to migrate to areas of damage. The regenerative potential of articular cartilage decreases even further with advancing age as the number of chondrocytes declines and the size of matrix proteoglycans decreases.

The response of articular cartilage to injury varies with the nature of the insult and with the depth of the lesion. *Superficial lacerations that do not penetrate the tidemark, and therefore fail to cause hemorrhage or inflammation, do not heal.* Chondrocytes adjacent to the lesion may proliferate, forming small clusters (*chondrones*), and may produce new matrix, but they do not migrate into the lesion. Within a few weeks of injury the chondrocyte response subsides, the lesion persisting for long periods but without progressing to chondromalacia or degenerative joint disease.

The repair of injuries that involve the full depth of the articular cartilage and penetrate subchondral bone differs markedly from the repair of superficial lesions. Hemorrhage occurs from blood vessels in the subchondral bone and the lesion becomes filled with a hematoma. Inflammatory cells and primitive mesenchymal cells invade the hematoma, probably under the influence of local growth factors, such as PDGF and TGF-β, derived from platelets and from the damaged bone. Within approximately 2 weeks of injury, some of the mesenchymal cells in the lesion have features of chondrocytes and begin to produce a matrix rich in proteoglycans, but also containing type II collagen. By 6–8 weeks, the defect is filled with fibrocartilage, which is firmly bonded to the adjacent hyaline articular cartilage. New bone formation occurs at the base of the lesion, restoring the subchondral bone plate, but the new bone does not extend into the area previously occupied by articular cartilage, remaining well below the articulating surface. The fibrocartilage repair tissue is analogous to the fibrous scar that repairs most other tissues and although it is an adequate replacement for articular cartilage at sites of deep injury, it does not perform as well when subjected to mechanical loading. In general, *most large defects in articular cartilage will eventually progress to degeneration after being filled with fibrocartilaginous repair tissue.* Early signs of degeneration may be present within a year of injury, although in some situations the repair tissue appears to function satisfactorily for a prolonged period and may become remodeled to more closely resemble normal articular cartilage. Interestingly, continuous passive motion of articular surfaces subjected to full-thickness injury has been shown to stimulate more rapid and successful healing of the articular cartilage than either complete immobilization or intermittent active motion.

Subchondral cystic lesions in horses are considered a manifestation of osteochondrosis, but in a recent study, similar lesions were induced experimentally by creating full-thickness defects in the articular cartilage and extending them 4 mm into the subchondral bone. Histologically, the lesions consisted of variable quantities of fibrous connective tissue, fibrocartilage, and bone. This study suggests that *trauma to weight-bearing surfaces, in addition to osteochondrosis, may predispose to subchondral cystic lesions in horses.*

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DEVELOPMENTAL DISEASES OF JOINTS

Osteochondrosis

Osteochondrosis is a common and important disease of pigs, horses, and large breeds of dog, but also occurs in cattle, sheep, and farmed deer. Young, fast-growing animals are most susceptible to osteochondrosis, especially breeds selected for rapid growth. The disease is most likely analogous to human osteochondrosis and to tibial dyschondroplasia in chickens and turkeys. It is characterized by multifocal abnormalities in endochondral ossification involving articular–epiphyseal cartilage complexes (the immature cartilage covering the ends of growing long bones) and growth plates. As such, osteochondrosis is not strictly a disease of joints, but since the clinical and pathological manifestations relate primarily to lesions in articular cartilage, it is included in this section. **Dyschondroplasia** is a more appropriate name for this disease as the initial lesion is in the growing cartilage, but the term osteochondrosis has become entrenched in the literature and a change in nomenclature is now unlikely to gain widespread acceptance. Other synonyms for the disease are **osteochondrosis dissecans** and **osteochondritis dissecans**. Such differences in terminology are due, at least in part, to the variation in lesions when examined at different stages. Severe degenerative joint disease is a common sequel to osteochondrosis and is one of the most common causes of lameness in domestic animals, especially in swine, horses, and certain large breeds of dog.

The *etiology and pathogenesis of osteochondrosis are poorly understood* and have been the subject of considerable recent debate, but valuable clues have emerged from studies in swine, horses, and dogs. The etiology is multifactorial, but most likely involves the effect of trauma or biomechanical factors on cartilage that has been weakened by nutritional or hormonal imbalances, vascular disruption, or genetic factors. Whatever the cause, the initial lesion in the articular–epiphyseal cartilage complex of each species where detailed studies have been performed are remarkably similar, *suggesting a common pathogenesis, most likely involving ischemic damage to the growing cartilage.*

In adult animals articular cartilage is avascular, relying on diffusion of nutrients from synovial fluid, but the articular–epiphyseal cartilage complex of immature animals depends on the presence of viable blood vessels within cartilage canals, at least for the first few months of life. These vessels, which appear to arise from the perichondrium, gradually disappear as the lumens of cartilage canals become filled with hyaline cartilage in a process referred to as *chondrification.* Investigation of the vascular supply to the femoral condyles of swine

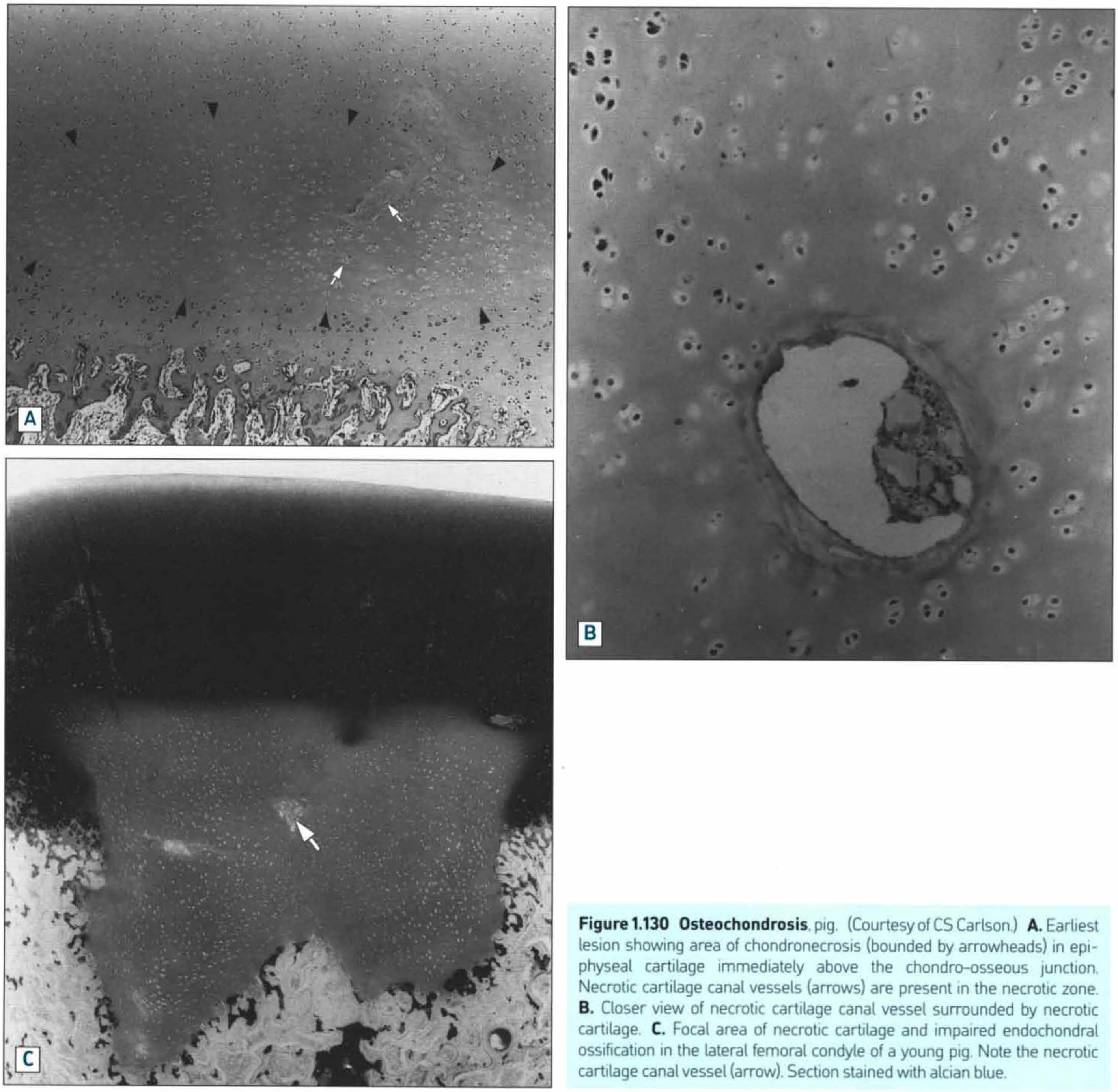

Figure 1.130 Osteochondrosis, pig. (Courtesy of CS Carlson.) **A.** Earliest lesion showing area of chondronecrosis (bounded by arrowheads) in epiphyseal cartilage immediately above the chondro-osseous junction. Necrotic cartilage canal vessels (arrows) are present in the necrotic zone. **B.** Closer view of necrotic cartilage canal vessel surrounded by necrotic cartilage. **C.** Focal area of necrotic cartilage and impaired endochondral ossification in the lateral femoral condyle of a young pig. Note the necrotic cartilage canal vessel (arrow). Section stained with alcian blue.

revealed that the cartilage of both condyles is fully vascularized at 2 weeks of age but that the blood supply recedes with maturity. By the time the pigs are 4–5 months of age the condyles are virtually avascular. In horses, cartilage canal blood vessels have disappeared from the articular–epiphyseal cartilage complex of the distal tibia by 3 months of age, and from the femoral condyles by 5 months.

Surgical interruption of the cartilage canal vascular supply in growing pigs at approximately 2 months of age results in necrosis of subarticular epiphyseal cartilage, but not of the overlying articular cartilage. This closely resembles the earliest lesion of osteochondrosis identified in the articular–epiphyseal cartilage complex of pigs and horses, wherein focal areas of cartilage necrosis and chondrolysis associated with necrotic cartilage canal blood vessels are confined to the epiphyseal cartilage and do not involve either the overlying articular cartilage or the subchondral bone (Fig. 1.130A, B). Similar early lesions have also been recognized in dogs and calves. These observations suggest that there is a risk period during which the epiphyseal cartilage of the articular–epiphyseal cartilage complex is dependent on the blood supply delivered by cartilage canal vessels. Disruption of the blood supply during this period in a young, rapidly growing animal may be the initial event in osteochondrosis. Local ischemia to this zone of developing cartilage would lead to necrosis, delayed endochondral ossification, and an extension of necrotic cartilage into the subchondral bone. Such lesions would not be expected to occur once the cartilage canals have closed and the cartilage is deriving its nutrients by diffusion from synovial fluid.

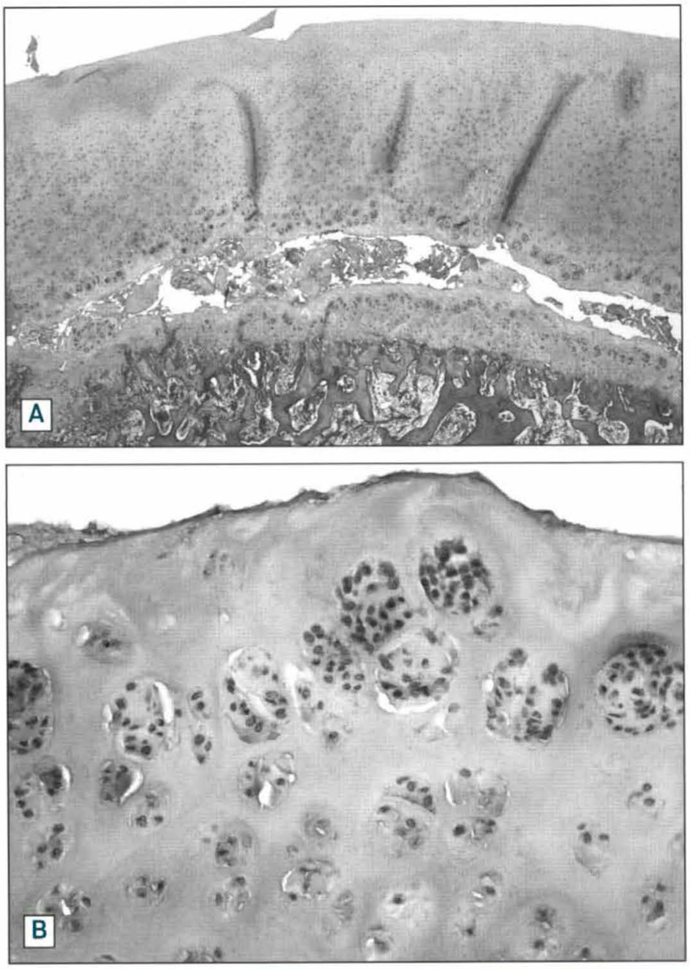

Figure 1.131 Osteochondrosis, foal. **A.** Horizontal cleft in deep layer of necrotic cartilage. **B.** Higher power of cartilage lining the cleft, showing acellular areas of chondronecrosis and many clusters of proliferating chondrocytes (chondrones) indicating a response to injury.

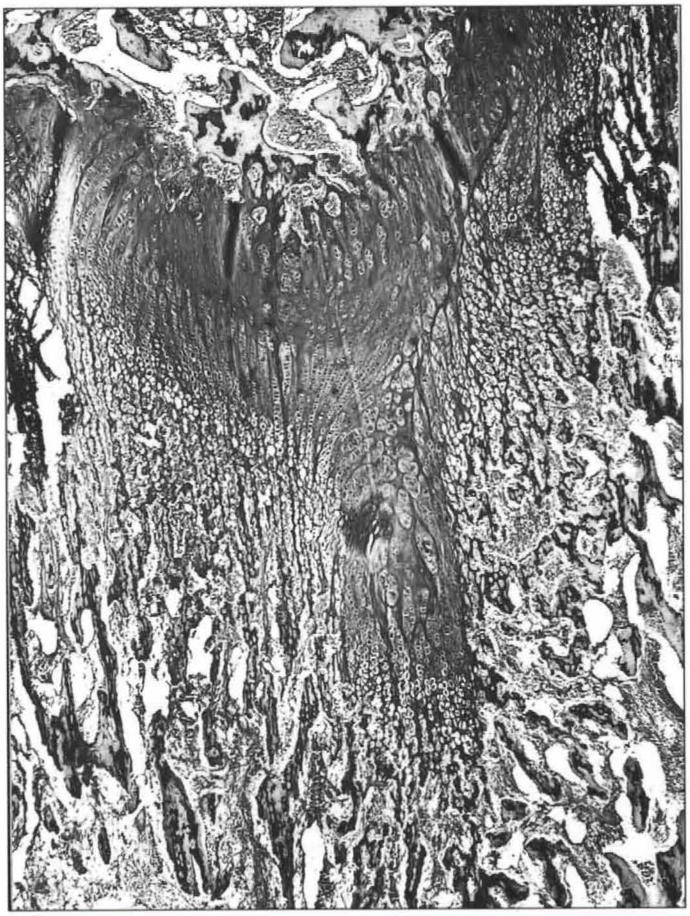

Figure 1.132 Focal retention of physeal cartilage (**metaphyseal dysplasia**) in osteochondrosis in a pig.

The presence of necrotic cartilage in the articular–epiphyseal cartilage complex induces *localized failure of endochondral ossification*. The necrotic cartilage does not mineralize or become invaded by blood vessels from the epiphyseal bone, and extends into the subchondral bone as ossification continues in adjacent, unaffected areas of cartilage (Fig. 1.130C). Small defects are unlikely to cause problems and no doubt heal spontaneously. Islands of necrotic cartilage left behind in the subchondral bone of the epiphysis are often found incidentally in various stages of repair. If the lesion is sufficiently large, clefts form in the deep layers of necrotic cartilage (Fig. 1.131A, B), presumably due to the trauma of weight bearing. The cleft may cause extensive underrunning of the articular cartilage and eventually join with the articular surface, creating a flap, which either remains attached or becomes separated, *forming a loose body or "joint mouse."* Loose bodies may survive and enlarge within the joint space, deriving their nutrients from the synovial fluid, and may even undergo endochondral ossification. Some become attached to the synovial membrane. At this advanced stage, the lesion is accompanied by inflammatory changes in the synovium and is often referred to as osteochondritis dissecans.

Another potential sequel of osteochondrosis involving the articular–epiphyseal cartilage complex is the *formation of subchondral cystic lesions* consisting initially of degenerate or necrotic cartilage, which is eventually replaced with fibrous tissue. This lesion, which appears to be most common in horses, will be discussed in more detail later in this section.

The physeal lesions of osteochondrosis differ morphologically from those involving the articular–epiphyseal cartilage complex, suggesting the possibility of a different pathogenesis. *Early lesions in the growth plate consist of cone-shaped foci of retained cartilage extending into the metaphysis* (Fig. 1.132), but rather than containing necrotic cartilage, these foci of metaphyseal dysplasia consist of viable hypertrophic chondrocytes. This does not exclude the possibility of an ischemic origin as experimental vascular disruption has been shown to induce similar changes in the growth plate of young pigs and lesions identical to tibial dyschondroplasia in chickens. It has also been suggested that these retained wedges of hypertrophic cartilage may be secondary to the development of trabecular microfractures in the primary spongiosa near the chondro–osseous interface. Such fractures would interfere with vascular invasion of the mineralized cartilage during endochondral ossification leading to persistence of hypertrophic zone chondrocytes. A similar mechanism has been proposed for the multifocal physeal thickening observed in Alaskan Malamute dogs with chondrodysplasia.

Although several possible causative factors have been incriminated in osteochondrosis, *there seems little doubt that the etiology is multifactorial*, and it is likely that differences between species exist. A *genetic predisposition* to osteochondrosis appears to exist in most species. This is at least partly related to the *predisposition for rapid growth*, as the prevalence is greatest in species and breeds where this attribute has been emphasized. Furthermore, in species other than pigs, the prevalence is higher in males than females, presumably due to their more rapid growth. The occurrence of many familial cases of osteochondrosis in humans, and the apparent increase in prevalence within certain genetic lines in animals, suggests that there is *also a direct genetic component to the disease*, at least in some cases. A heritability coefficient of 0.24–0.27 has been estimated from a study of Standardbred horses in Sweden.

Trauma almost certainly plays an important role, as supported by the increased prevalence of articular lesions at sites of greatest weight-bearing force. Furthermore, pigs housed on hard surfaces appear to have increased incidence and severity of the disease. Whether trauma alone is capable of causing osteochondrosis is questionable, but the forces associated with weight bearing and motion, when inflicted on large foci of degenerate or necrotic cartilage, would be expected to predispose to cleft formation and separation of cartilage flaps from subchondral bone. The role of trauma in the etiology of physeal lesions is difficult to determine, but compressive forces acting vertically on articular surfaces inevitably are transmitted through epiphyseal bones to the growth plate. This could damage the vascular supply to the physis, especially in young animals still dependent on transphyseal blood vessels within cartilage canals.

Rapid growth and optimal nutrition are considered necessary for the development of osteochondrosis in most animal species, but contradictory evidence has been obtained from experimental studies and *the exact role of nutritional factors in the etiology of osteochondrosis remains to be determined*. A comparison of weanling swine fed either 12% or 16% protein in their ration demonstrated a reduced growth rate in the former group but no reduction in the incidence of osteochondrosis. This does not however exclude the possibility that further reduction in growth rate may have been beneficial, as in this study all pigs in both groups developed lesions. In another study, swine fed a balanced ration at 70% of that of controls grew more slowly, and had less severe lesions of osteochondrosis than controls when slaughtered at the same weight (90 kg). However, a comparison of lesions in pigs from both groups when they were slaughtered at the same age (208 days) revealed that the lesion score was highest in pigs on the restricted ration. These data suggest that although restricted growth rate did not reduce the severity of osteochondrosis, there was some resolution of the lesions in pigs on the restricted ration by the time they reached slaughter weight.

Experimental prolonged excess of dietary calcium is reported to increase the prevalence and severity of osteochondrosis in susceptible breeds of dog but does not appear to influence the disease in pigs and its relevance to the naturally occurring disease is questionable. Increased dietary calcium is believed to result in hypercalcemia and hypergastrinemia, both of which would be expected to induce hypercalcitoninemia and impaired bone resorption, but suggestions that osteochondrosis results from this mechanism are speculative and unproven. Of more significance is the apparent link between primary or secondary copper deficiency and osteochondrosis in horses, pigs, and deer. *Low dietary copper or high dietary levels of copper antagonists, including zinc and molybdenum, have been associated with both experimental and natural osteochondrosis*. Calcium is also a copper antagonist thus providing a possible explanation for the reported effect of high dietary calcium on osteochondrosis in dogs. As a component of the enzyme lysyl oxidase, copper is involved in strengthening collagen molecules in articular cartilage, and other connective tissues, by the formation of intermolecular cross-links. Copper deficiency could therefore weaken articular cartilage and the walls of small blood vessels in cartilage canals, rendering them more susceptible to the forces of weight bearing or other trauma.

Various hormones have been implicated in the etiology of osteochondrosis. Growth hormone and somatomedins stimulate the activity of chondrocytes both in vitro and in vivo and have been shown experimentally to increase the severity of osteochondrosis lesions in the articular–epiphyseal cartilage complex of pigs. Furthermore, high levels of growth hormone are present in young dogs of large breeds at the stage where they are most susceptible to the development of osteochondrosis. Insulin and insulin-like growth factors I and II are also mitogenic for chondrocytes and may provide an explanation for the apparent link between osteochondrosis and the feeding of high-energy diets, which would be expected to induce hyperinsulinemia. Lesions resembling osteochondrosis with extensive underrunning of cartilage have occurred in horses following the administration of glucocorticoids. A possible link between copper and hormone levels has been suggested and warrants further investigation. Several hormones, including glucocorticoids, insulin, insulin-like growth factor-I, interleukin-1, estrogen, and progesterone have been shown to alter metallothionein synthesis and are therefore capable of altering copper metabolism. Furthermore, growth hormone has been shown to reduce copper retention in pigs. This may help to explain the increased incidence of osteochondrosis in rapidly growing young animals.

Thrombosis of small blood vessels within cartilage canals has been suggested as a possible cause of ischemia to the articular–epiphyseal cartilage complex and to physeal cartilage. Lipid emboli were observed in the lumen of these vessels in one study, but their origin and significance is not known. Fibrin thrombi occurring in association with bacteremia or septicemia could also cause thrombosis of cartilage canal vessels. It is unlikely however that the majority of cases of osteochondrosis occur by this mechanism.

Because of differences between species in the nature and distribution of lesions, and their sequelae, it is appropriate to consider each species separately.

1. **Swine** As in other species, *microscopic lesions of osteochondrosis in swine are far more common than clinical signs or gross examination at necropsy would suggest*. In some studies, up to 100% of rapidly growing young pigs had microscopic changes of osteochondrosis in their growth plates or articular–epiphyseal cartilage complexes. Clearly, the vast majority of such lesions heal uneventfully and only those that are sufficiently large, or subjected to excessive trauma, progress to become clinically significant. At this stage, gross lesions are likely to be present in one or several joints. *Predilection sites in pigs* are the joint surfaces of the medial humeral and femoral condyles, anconeal process, lumbar vertebrae, mediodistal part of the talus, humeral head, glenoid cavity of the scapula, distal ulna, and dorsal acetabulum. *Physeal lesions* occur most commonly in the distal ulna, distal femur, costochondral junctions, femoral and humeral heads, thoracolumbar vertebrae, and the ischial tuberosity.

   The articular lesions of osteochondrosis are often bilateral, and may be symmetrical. It is important to distinguish them from synovial fossae, which are present as normal structures in some joints. *Early articular lesions* appear as thickened, white or cream foci of articular cartilage, which are sharply demarcated from adjacent normal areas of cartilage. In some cases the articular cartilage, which is not directly affected in osteochondrosis, will be depressed or wrinkled due to collapse of necrotic cartilage in the underlying articular–epiphyseal cartilage complex. *In more advanced lesions, the affected cartilage usually shows evidence of separation and underrunning* (Fig. 1.133), and there may be nodules of cartilage reflecting attempts at repair. In other cases, *underrun segments of articular cartilage may be detached completely, leaving a deep ulcer with exposure of subchondral bone* (Fig. 1.134). Lesions of this type are referred to as **osteochondritis dissecans** and occur most frequently on weight-bearing surfaces of the medial condyles of the humerus and femur in pigs of 5–7 months of age. In such cases, large fragments of articular cartilage may be present within the joint space. Since these so-called loose bodies or joint mice obtain nutrients by diffusion from synovial fluid, they can survive and grow within the joint, and may be larger than the defect from which they originated. The dissecting lesion does not involve bone but sometimes flaps of cartilage contain osseous tissue. This probably develops by endochondral ossification following invasion of vessels and cells from the area of attachment. The defect in the articular surface is initially filled with vascular connective tissue, which eventually is converted to fibrocartilage. Even if a dissecting lesion does not result, hemorrhage and fibrosis may occur in the subchondral bone and the resulting localized radiolucent area may be the basis for one type of "bone cyst" in osteochondrosis. *Degenerative arthropathy is common in the late stages of the disease*, but evidence that osteochondrosis was involved may no longer be apparent grossly. Even histologically, the changes may be too altered by secondary changes to reflect the initial lesion. Synovial villi may be enlarged and mild lymphoplasmacytic inflammation with scant fibrinous exudate may be present in the synovium, but these changes are nonspecific.

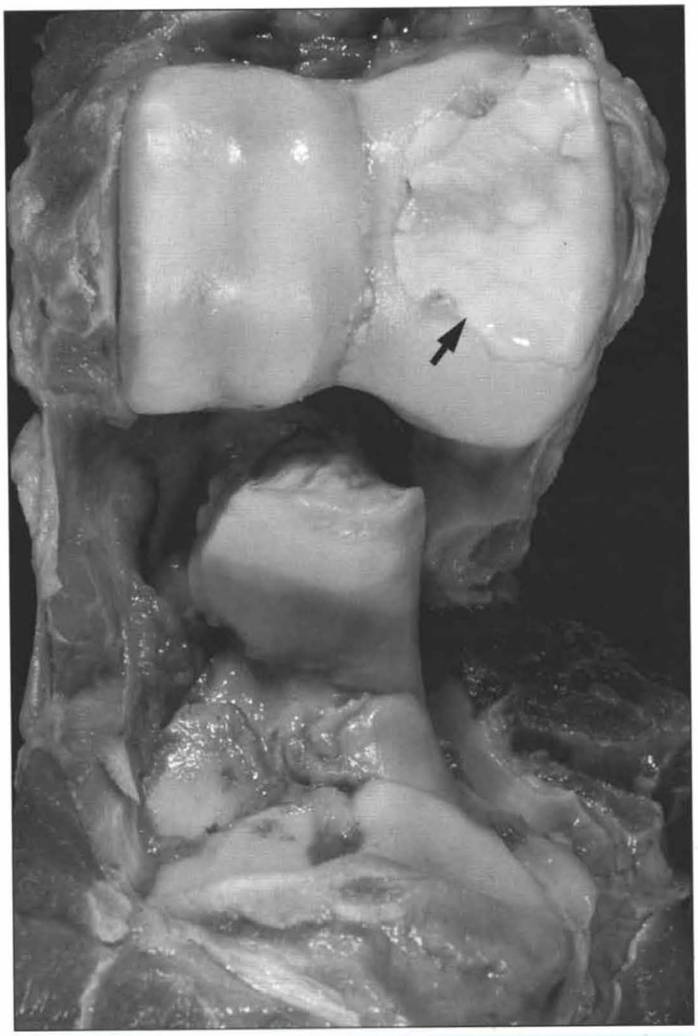

Figure 1.133 Osteochondrosis, pig. Elbow joint. Large area of thickened, underrun articular cartilage (arrow) on the medial humeral condyle, sharply demarcated from adjacent normal cartilage. (Courtesy of RA Fairley.)

Severe **growth plate lesions** associated with osteochondrosis may appear grossly as thickened foci. *Microscopically these foci, which are sometimes referred to as metaphyseal dysplasia, consist of persistent hypertrophic chondrocytes* (Fig. 1.132) and are associated with delayed mineralization. *Such lesions are similar to those of rickets* and may present a diagnostic challenge to the pathologist, especially since trabecular microfractures in the primary spongiosa may also occur in osteochondrosis and imply an underlying metabolic bone disease. In osteochondrosis, the mineralization failure is transient

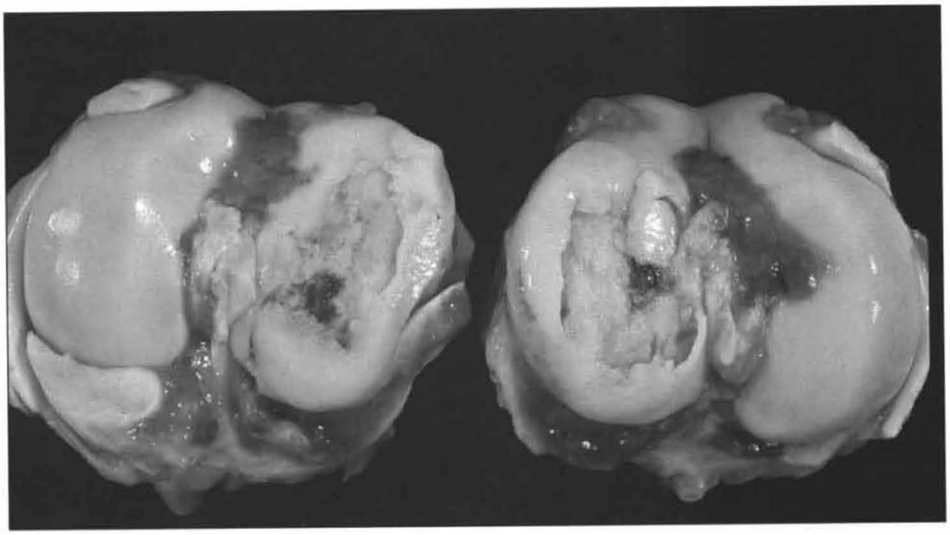

Figure 1.134 Osteochondrosis, pig. Bilateral detachment of articular cartilage on medial femoral condyles with exposure of subchondral bone. Lesions of this type are referred to as **osteochondritis dissecans**. (Courtesy of RA Fairley.)

and focal, and there is no evidence of delayed mineralization of osteoid, as occurs in rickets. The fate of these metaphyseal lesions is varied. Some undergo relatively normal ossification, others persist as nodules of cartilage in the metaphysis, while many degenerate and are replaced by fibrous tissue in which woven bone or fibrocartilage develops.

Irregular eosinophilic streaks, associated with areas of disorganized growth plate architecture, also occur in osteochondrosis (Fig. 1.135A). These may reflect either vestiges of cartilage canals or infraction lines occurring as a sequel to growth plate trauma. Similar eosinophilic streaks are normally present in the growth plates of young animals but are usually parallel to cartilage columns. In osteochondrosis they are often stellate and may subdivide the physeal cartilage into disorganized, sometimes degenerate, lobules. *Bony bridges*, uniting the epiphysis and metaphysis and effectively closing the growth plate at that location, may develop at sites of physeal degeneration (Fig. 1.135B). *Angular limb deformity* resulting from premature closure of a growth plate on one side and continued growth on the other is a potential sequel to this lesion but appears to be less common in pigs than in other species.

Another manifestation of osteochondrosis in swine is **epiphysiolysis**, *the separation of an epiphysis from metaphyseal bone*. This is a traumatic lesion, predisposed to by a defect in growth cartilage of the physis, and probably develops either from an extended eosinophilic streak or from areas of necrosis in the growth cartilage, rather than from foci of metaphyseal dysplasia. *The common sites for epiphysiolysis are femoral head* (Fig. 1.136*), ischiatic tuberosity of females, and lumbar vertebrae.* The distal epiphysis of the ulna and the anconeal process may also be involved, although strictly speaking the lesion involving the latter should not be called epiphysiolysis. In pigs, unlike dogs, the anconeal process does not develop from a separate ossification center, so **apophysiolysis** is a more appropriate term. Separation may be complete, as is often the case with the ischiatic tuberosity, or partial, as occurs in the head of the femur, which sometimes remains attached at its lateral margin. Separation probably occurs when the process of endochondral ossification reaches or approaches the cartilage defect. The resulting fracture may extend in a jagged crack through primary and secondary spongiosa.

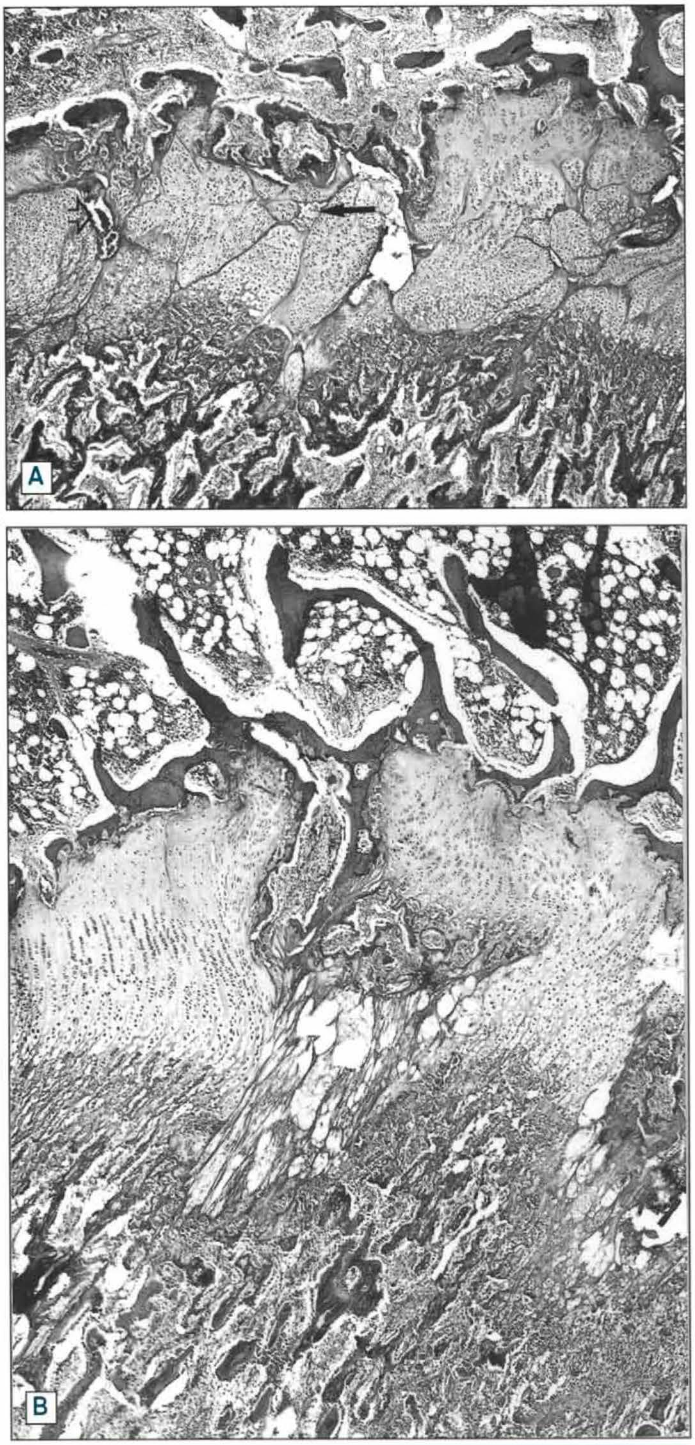

Figure 1.135 Osteochondrosis, pig. **A.** Eosinophilic streaks and focal cavitation (arrow) in a disorganized physis. **B.** Focal degeneration of the physis with formation of a bony bridge between the epiphysis and metaphysis.

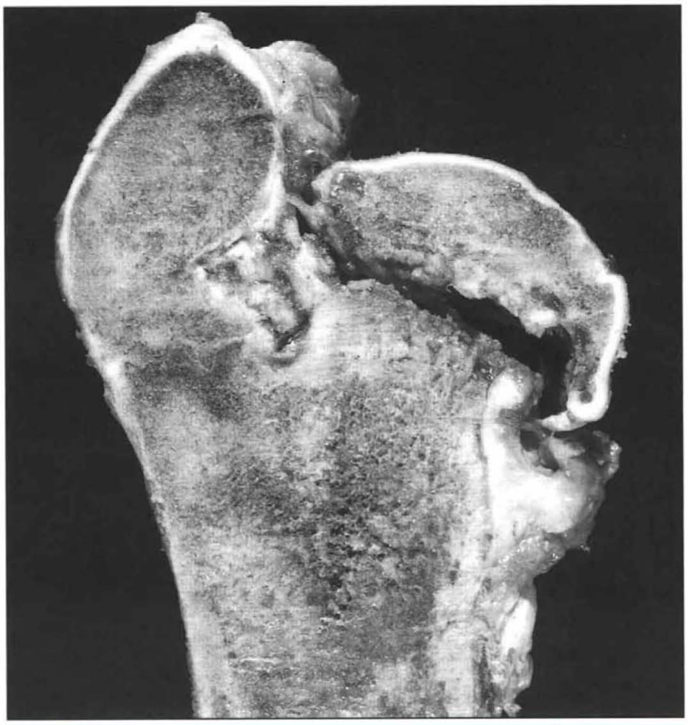

Figure 1.136 Osteochondrosis, pig. Epiphysiolysis of the femoral head.

2. **Dogs** *Osteochondrosis in dogs includes several orthopedic diseases, usually occurring in young fast-growing males of large and giant breeds.* Osteochondritis dissecans of the shoulder joint is the classic presentation of the disease, but similar lesions may also occur in other major joints. In addition, *ununited anconeal process and fragmented coronoid process* are believed to be manifestations of osteochondrosis in dogs.

The major predilection site for **osteochondritis dissecans** *in dogs is the caudal aspect of the humeral head.* In susceptible breeds, lesions are common between 4 and 8 months of age and are bilateral in approximately 70% of cases. Many lesions resolve spontaneously but others progress to cause severe lameness due to degenerative joint disease. As in pigs, *early lesions* are visible grossly as discrete gray/white or cream foci in the articular surface representing areas of thickened or underrun cartilage (Fig. 1.137A, B, C). Flaps of cartilage often detach from these sites and form loose bodies, leaving large ulcers that are gradually filled with fibrocartilage. Healing of such defects is never perfect and partly healed lesions of osteochondritis dissecans are often detected several months, or even years, later in the shoulder joints of dogs with chronic degenerative joint disease (Fig. 1.138). Such lesions may also be found incidentally, reflecting their high prevalence in certain breeds and the fact that they are not always symptomatic.

Osteochondritis dissecans also occurs in several other locations in dogs, including the medial aspect of the humeral condyle, the lateral, and less frequently the medial, condyle of the femur, and the medial trochlear ridge of the tibial tarsal bone. Lesions at these sites can be bilateral and may either cause lameness at a young age when the lesion are active, or predispose to degenerative joint disease.

Lesions in the humeral condyle form part of the **elbow dysplasia syndrome,** *which also includes ununited anconeal process and fragmented (ununited) medial coronoid process of the ulna.* The latter two conditions may also occur separately (see below), or in other combinations. Together the three lesions, which are often bilateral, cause foreleg lameness in young, usually large, growing dogs and often lead to arthropathy associated with periarticular osteophytes. The elbow dysplasia syndrome is characterized by incongruity of the joint caused by a step between the cranial margin of the proximal ulna and the caudal margin of the proximal radius. An increase in length of the proximal ulna is responsible for the incongruity and results in disruption of the normal continuous curve made by the profile of the trochlear notch of the ulna and the articular cartilage of the radius. Trauma from normal use most likely precipitates lesions.

Ununited anconeal process, which develops as an *epiphysiolysis,* is most common in the German Shepherd, but also occurs in other large and giant breeds. The ossification center of the anconeal process develops at about 11–13 weeks and expands for about 6 weeks until osseous union with the ulna is achieved by 4–5 months. If extensive fissures develop in the bipolar growth cartilage between the process and the ulnar diaphysis, normal plate closure does not occur and a fibrocartilaginous or fibrous union results.

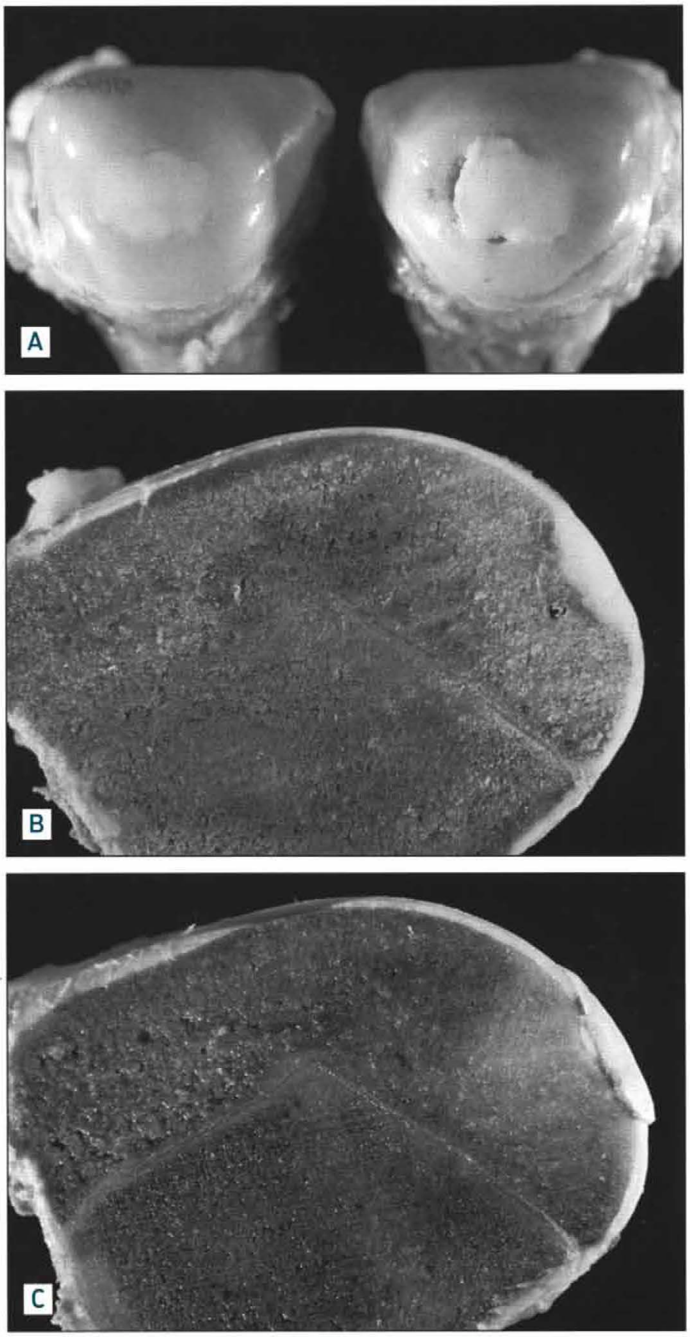

Figure 1.137 Osteochondrosis, dog. **A.** Early, bilateral lesions involving the caudal aspect of the humeral head in a 5-month-old Irish Wolfhound. **B, C.** Sagittal sections of the lesions shown in **(A)**. In **(B)** the articular cartilage is thickened, while in **(C)** the thickened cartilage has become detached from the subchondral bone.

Ununited (or fragmented) medial coronoid process of the ulna is less well known, but apparently more common, than ununited anconeal process. The coronoid process does not develop from a separate ossification center and is not easily seen in radiographs. Usually the first indications of disease are osteophytes around the anconeal process and the medial epicondyle of the humerus. Golden and Labrador Retrievers and Rottweilers are particularly prone to this condition. *In affected dogs, the medial coronoid process consists of one or several fragments, which may be in their normal position or completely separated from the articular surface of the proximal ulna as joint mice.* Lesions begin as vertical fissures in the proximal face of the articular cartilage and extend into the subchondral bone. They may reach the vertical joint surface or the area of insertion of the annular ligament.

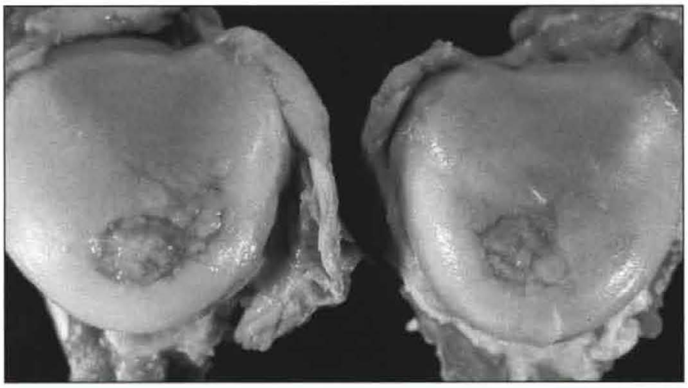

Figure 1.138 Partly healed lesions of **osteochondritis dissecans** in both humeral heads of a 4-year-old Great Dane with degenerative joint disease of both shoulder joints.

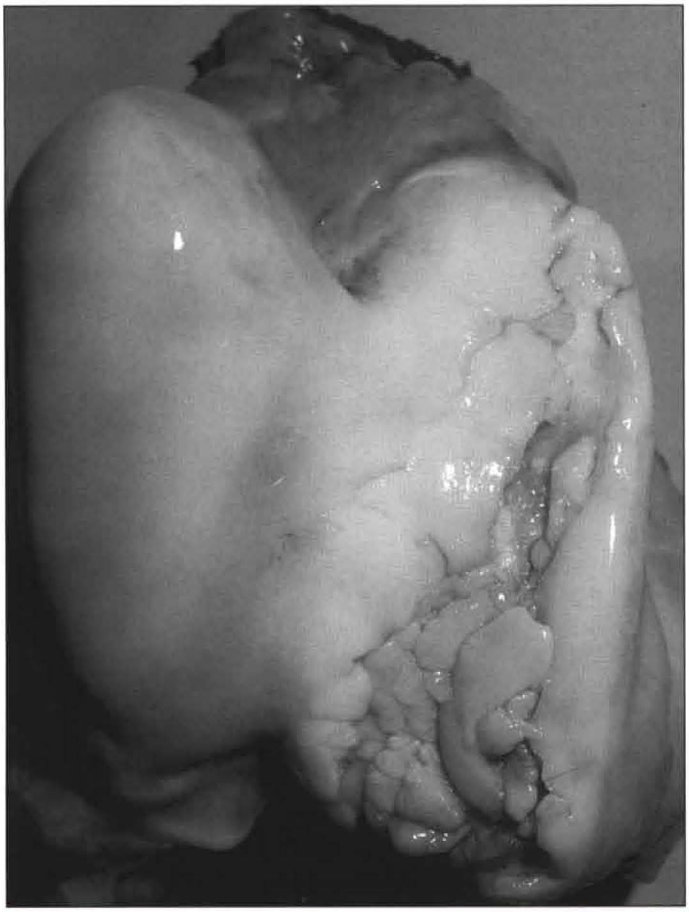

Figure 1.139 Osteochondrosis, horse. Extensive underrunning and displacement of articular cartilage on the lateral trochlear ridge of a 6-month-old foal. The many small cartilage nodules represent attempts at repair.

When complete separation of the fragment or fragments does not occur, the walls of the fissure become lined by fibrocartilage.

Other conditions in dogs, which are probably manifestations of osteochondrosis, include *retained cartilage core of the distal ulna* (Fig. 1.55A, B), and *slipped capital femoral epiphysis*. Retained cartilage cores also develop in the other long-bone metaphyses and may lead to growth deformities of the distal femur and proximal tibia. Canine hip dysplasia has been suggested as a possible manifestation of osteochondrosis but because of doubts over this classification, the disease is considered separately.

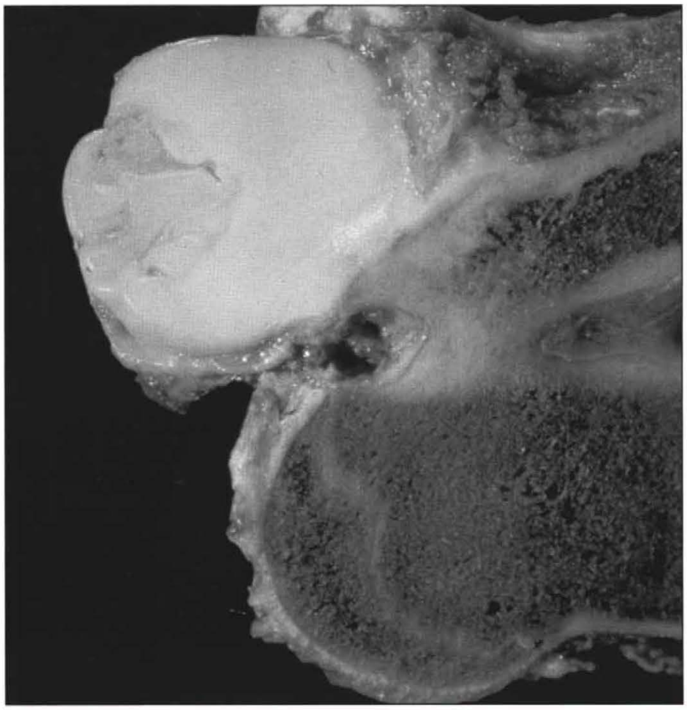

Figure 1.140 Osteochondrosis, horse. Flaps of detached cartilage on an articular facet of a cervical vertebra in a foal.

3. **Horses** Osteochondrosis is an important skeletal disease in horses of several breeds, and its incidence worldwide appears to be increasing. The term **developmental orthopedic disease** has been used to group osteochondrosis with several other skeletal diseases of young horses, some of which may occur as a sequel to, or as different manifestations of osteochondrosis. *These include osteochondritis dissecans, physitis, subchondral bone cysts, angular limb deformities, and wobbler syndrome.*

Although the lesions of osteochondrosis in horses may be widespread, there are several *predilection sites*, including the lateral trochlear ridge (Fig. 1.139) and medial condyle of the femur, the patella, the dorsal edge of the sagittal ridge of the distal tibia, and various sites in the tarsal and fetlock joints. In these locations, the pathogenesis of lesions is probably similar to that described earlier. Ossification of attached cartilage flaps seems to be more common in the horse than in other species. Lesions of osteochondrosis may also involve articular processes of cervical vertebrae (Fig. 1.140). Healing of such lesions may be characterized by formation of exostoses, which encroach on the spinal canal, or create asymmetry of articular facets, thus predisposing to wobbler syndrome.

Osteochondrosis in horses has been incriminated as a *predisposing lesion in the development of* **subchondral cystic lesions**, which are most common on the distal aspect of the medial femoral condyle (Fig. 1.141), distal aspect of the metacarpus, in addition to the carpus, elbow, and phalanges. It is suggested that subchondral cysts may develop from invaginations of articular cartilage, remnants of necrotic cartilage derived from the articular–epiphyseal cartilage complex, or in response to leakage of synovial fluid into subchondral bone through defects in articular cartilage. Such

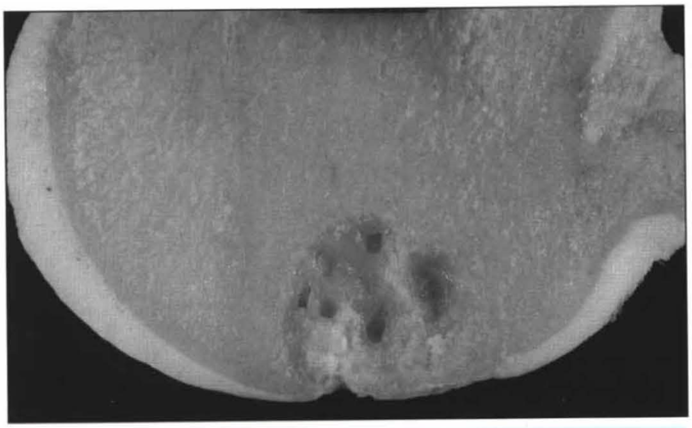

Figure 1.141 Osteochondrosis, horse. Subchondral cystic lesion in the medial femoral condyle. (Courtesy of NC State University.)

lesions have also been reproduced experimentally in young horses following traumatic damage to the articular cartilage and its supporting subchondral bone, suggesting that not all subchondral cysts are related to osteochondrosis. Histologically, the cysts usually consist of variable quantities of fibrous tissue and fibrocartilage, and are surrounded by sclerotic bone trabeculae.

Focal thickening of the growth plate, similar to metaphyseal dysplasia of pigs, is recognized as a manifestation of osteochondrosis in young foals. **"Physitis"** *or* **"epiphysitis"** *are commonly used clinical terms to describe these lesions, but both are misleading as the lesions are not inflammatory in origin*. The latter term is particularly inappropriate since the epiphysis is not involved. The lesions are thought to predispose to premature closure of growth plates and therefore *may induce angular limb deformity*. Epiphysiolysis is seldom reported in horses as a manifestation of osteochondrosis but has been described recently involving the physes of all cervical vertebrae from C3 to C7 in a 16-month-old filly.

Foals with congenital and neonatal angular limb deformities sometimes have concomitant hypoplasia of carpal bones and osteochondritis dissecans. These early lesions may both be manifestations of defective growth and maturation of cartilage. Lesions consistent with osteochondrosis were found at necropsy in the tarsal joints of 36 of 50 young horses with *spavin*, suggesting that osteochondrosis may be an important cause of this condition, at least in young animals.

Lesions of the palmar surface of the distal end of the metacarpal in adult racing Thoroughbreds are often discussed with the osteochondroses. These defects develop on the articular surface in contact with the proximal sesamoids. The *initial lesion* consists of fracture and necrosis of the subchondral bone, probably secondary to the trauma of hard running. With rest, *the fracture may heal* since articular cartilage above the fracture, nourished by synovial fluid, remains viable and probably holds the bone fragment in place. Collapse of subchondral tissues causes depression of the intact articular cartilage, but revascularization and healing may occur. On occasion, the complete fragment breaks free and this type of osteochondritis dissecans is analogous to some of the lesions described in man. The fact that horses so affected are adults, and are athletes in hard training, suggests that these lesions of the palmar surfaces of metacarpals *should be classified with stress fractures* rather than with osteochondrosis.

The relationship between copper deficiency and osteochondrosis in horses is compelling. Lesions indistinguishable from naturally occurring osteochondritis dissecans were reproduced experimentally in foals weaned from their dams at birth and fed on copper-deficient milk. Furthermore, there are several reports of similar lesions occurring in different parts of the world in foals grazing either copper-deficient pasture or pasture contaminated with the copper antagonists zinc and cadmium. Neonatal foals obtain copper primarily from milk, and yet mare's milk normally contains low copper concentrations and does not respond to supplementation. Foals born with inadequate hepatic copper reserves may therefore develop copper deficiency at an early age unless supplemented. Interestingly, supplementation of mares during late pregnancy and of their foals from 90 to 180 days of age appeared to reduce the prevalence and severity of osteochondrosis, even though unsupplemented control animals were fed at a level slightly above National Research Council recommended levels. There are also claims that copper supplementation has a palliative effect on established lesions of osteochondrosis. Possible mechanisms by which copper deficiency might predispose to osteochondrosis were discussed earlier in this section. Clearly, further work is needed in order to determine the precise role of copper in the etiology of osteochondrosis in horses and other species.

4. **Cattle** There are only occasional reports of osteochondrosis in cattle, but *the disease is likely to be much more common in this species than is currently recognized*. Due to financial constraints and difficulties in detailed radiologic examination, many lame cattle are sent for slaughter without definitive diagnosis. In a survey of middle-aged bulls slaughtered for nonmedical reasons, lesions of osteochondrosis were detected in the stifle joint of three of 25 animals in the absence of clinical lameness. Degenerative joint disease was detected in the stifle of 14 of the 25 bulls and although these lesion were too advanced to accurately determine their origin, the fact that the lesions identified as osteochondrosis, and those of degenerative joint disease both had a predilection for the lateral trochlear ridge, suggested that at least some cases of degenerative joint disease in this population of bulls were secondary to osteochondrosis. Other predilection sites for osteochondrosis in cattle are the humeral head, distal radius, elbow joint, and the tibial tarsal and occipital condyles.

Lesions characterized by focal thickening and chondrolysis are described in the growth cartilage of the articular–epiphyseal cartilage complex in young calves, *suggesting that the pathogenesis of the articular lesions in cattle is the same as in other species*. An association has been made between high-energy concentrate feeding and osteochondrosis in cattle, but the syndrome has also been reported in unsupplemented beef cattle grazing pasture. A possible genetic predisposition was suggested in the latter report.

Physeal lesions similar to those in pigs with osteochondrosis are described in the metacarpal and metatarsal bones of fast-growing, grain-fed, housed calves. In many cases, radiographic lesions characterized by focal thickening of the growth plate are accompanied by lameness and postmortem examination has revealed eosinophilic streaks and focal persistence of the hypertrophic zone. Disruption of the growth plate with bony bridging between the metaphysis and epiphysis is also recorded in affected calves. Hard floors apparently increase the number and severity of lesions, supporting the role of trauma in susceptible animals.

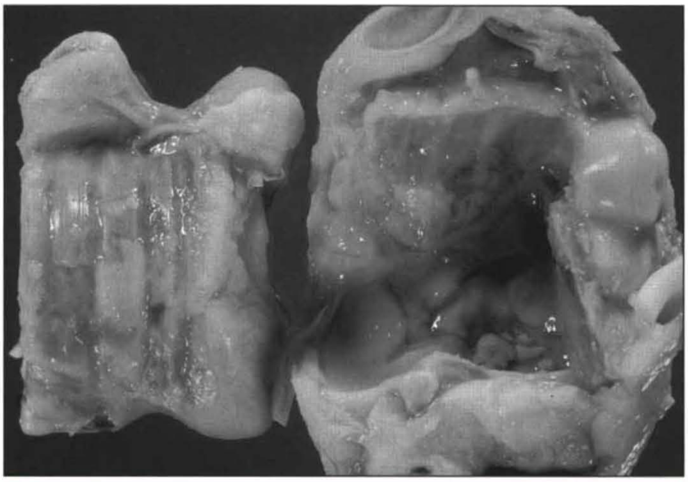

Figure 1.142 Osteochondrosis associated with copper deficiency in a farmed red deer. Extensive erosion of articular cartilage on the plantar surface of the talus and its opposing articular surface. Only small islands of cartilage remain and there are deep linear grooves in the exposed subchondral bone.

5. **Deer** *A strong association is recognized between osteochondrosis and copper deficiency in farmed red deer and wapiti x red deer hybrids in New Zealand.* On some farms, more than 30% of the fawns are affected. Lameness may be noticed as early as 1 month of age, but more commonly, the problem is not noticed until weaning at around 4 months of age. *Epiphysiolysis of the femoral head* is a common manifestation of the disease and may be bilateral. Animals with this lesion are severely lame and may adopt a "bunny-hopping" gait or "cow-hocked" stance. Other predilection sites are the carpal, hock (Fig. 1.142), and stifle joints. The lesions are usually bilateral. Affected deer invariably have low serum and/or liver copper concentrations and the disease can be prevented by copper supplementation of pregnant hinds.
6. **Sheep** Osteochondrosis appears to be *rare* in sheep, but has been reported as a cause of lameness in young, rapidly growing, Suffolk ram lambs. It is likely that the incidence of osteochondrosis in sheep will increase with continued selection for rapid growth. Microscopic lesions are common in growth plates of fast-growing lambs, but few of these progress to gross lesions. Valgus and varus limb deformities are common in ram lambs in feedlots and test stations in the USA and also occur in other countries. Grossly, some affected rams have thickening of the distal radial physis, similar to the growth plate lesions of osteochondrosis but joint changes are absent. Vascular and growth plate lesions mimicking osteochondrosis can be produced experimentally in lambs by procedures that increase weight bearing. Epiphysiolysis of the distal radial growth plate, associated with deformity of the forelimbs, is reported in 3-month-old lambs, supporting the concept that the physeal lesions of osteochondrosis may be one cause of angular limb deformity in sheep.

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Hip dysplasia

Hip dysplasia is the most common skeletal disease of large and giant breeds of dogs, but may occur in all dog breeds and is occasionally reported in cats, cattle, and horses. *The disease is characterized by a lack of conformity between the femoral head and acetabulum, resulting in subluxation and, invariably, degenerative joint disease.* A polygenic mode of inheritance is postulated in dogs, and estimates of heritability have varied from 0.2 to 0.6. Environmental effects are believed to play a significant role in the severity of lesions. In particular, growth rate during the first few weeks of life appears to be important; faster-growing dogs having more severe lesions. Overfeeding young pups of susceptible breeds has been shown to shorten the time to first appearance of hip dysplasia and increase its severity. In another study, limiting the food consumed by Labrador Retrievers between 6 weeks and 1 year of age reduced both the incidence and severity of the disease.

1. **Dogs** Increased joint laxity is a common feature in dogs with hip dysplasia and appears to have a hereditary basis. *Although the cause of joint laxity is not clear, it is well established that dogs with hip dysplasia generally have low pelvic muscle mass.* The hip joint, probably more than any other joint, depends on muscle action to maintain the relationships between articulating surfaces during weight bearing and motion. Breeds such as Greyhounds, which do not get hip dysplasia, have a much greater muscle mass than German Shepherd Dogs, in which the disease is common. Hypotrophy of pectineal muscles, due to a developmental neuropathy, has also been suggested as a predisposing cause of hip dysplasia in dogs. Spasm or shortening of the pectineal muscle would be expected to create an upward force by the femoral head on the dorsal rim of the acetabulum. Such a force during early postnatal development of the hip joint could result in abnormal modeling and a dysplastic joint.

   It is possible that the basic defect in hip dysplasia is intrinsic to the hip joint, rather than to its supporting structures. It has been suggested that hip dysplasia is a manifestation of osteochondrosis but this hypothesis does not appear to be widely supported.

   Radiographic evidence of hip dysplasia in affected pups is usually not apparent until about 6 months of age, but may be detected earlier in severe cases or much later in dogs with mild lesions. *A radiographic diagnosis of hip dysplasia is based on the presence of subluxation of the joint or evidence of degenerative joint disease, or both.*

   The *gross lesions* of canine hip dysplasia vary with the stage of the disease. In the early stages, acetabula appear shallow, there is subluxation of the femoral head, and the articular cartilage may be dull or roughened. *The lesions are most prominent in weight-bearing areas of the femoral head and the dorsal rim of the acetabulum.* As the disease progresses, the articular cartilage becomes yellow or gray and erosion leads to fibrillation, then eburnation, with sclerosis of the subchondral bone. Osteophytes may develop at chondro-osseous margins. The shape of the femoral head may be altered due to the abnormal forces associated with subluxation and the femoral neck may become thickened as enthesiophytes develop at the attachment of the joint capsule. The joint capsule may be thickened with fibrous connective tissue and granulation tissue developing within the synovial membrane may extend over part of the articular surface as a pannus. In advanced stages, evidence of hip dysplasia may be masked by the manifestations of severe, degenerative joint disease. In some cases, the formation of osteophytes around the acetabular rim may disguise its original shallowness. In dogs with severely dysplastic joints, advanced changes of degenerative joint disease may be present by one year of age.

   Microscopic changes in hip dysplasia are those of degenerative joint disease (see below) and contribute nothing specific to the diagnosis. Early lesions include edema and tearing of collagen fibers in the ligament of the head of the femur and hypertrophy and hyperplasia of synoviocytes in the synovial membrane. Later in the disease, synovial villi may be infiltrated with mononuclear cells, the joint capsule is thickened with fibrous tissue, and the eroded articular cartilage contains clusters of proliferating chondrocytes.

2. **Cats** *Hip dysplasia is much less common in cats than in dogs*, but its frequency in the general cat population is probably underestimated. Many cats with radiographic evidence of hip dysplasia are asymptomatic and *the diagnosis is often incidental.* In a survey of 648 cats representing 12 breeds, the overall frequency of hip dysplasia was about 6.6%. Persians and Himalayans had a higher prevalence rate (15.8% and 25.0%, respectively) but only small numbers of these purebreds were included in the study and the results may be misleading. Other studies have indicated a very high prevalence of hip dysplasia in the Maine Coon breed of cat, which, like the Persian and Himalayan breeds, has a larger body size and a relatively small gene pool. As in dogs, no sex predilection is apparent in cats.

   The role of joint laxity in the pathogenesis of feline hip dysplasia is uncertain and requires further investigation. A consistent observation in cats with hip dysplasia is an *abnormally shallow acetabulum*, rather than subluxation of the joint, which is a feature of the disease in dogs. The shallow acetabulum predisposes to degenerative joint disease but the distribution of lesions in cats differs from that in dogs. The most extensive remodeling and proliferative changes in affected cats involve the craniodorsal acetabular margin, whereas in dogs the dorsal rim of the acetabulum is most severely affected. A further difference is the lack of remodeling of the femoral neck in cats with hip dysplasia.

3. **Cattle** *Hip dysplasia in cattle is best known in Herefords* but also occurs in the Aberdeen Angus, Galloway, and Charolais breeds. An inherited component is suspected but too few cases are reported to allow confirmation or characterization of the mode of inheritance. The disease is largely confined to males. Although some calves may be affected at birth, clinical lameness usually commences from 3 months to 2 years of age. Since lame bulls are more often sent for slaughter than subjected to postmortem examination, the true prevalence of hip dysplasia in cattle may be much higher than is realized. The lesions are generally bilateral and are characterized by shallow acetabula and degenerative arthropathy involving both the femoral head and acetabulum. *Rapid growth and mineral deficiencies appear to exacerbate the condition*, and it is likely that at least some cases of hip dysplasia in cattle represent manifestations of osteochondrosis.

Luxations and subluxations

Congenital luxations are rare in animals but **atlanto-axial subluxations** are reported in dogs, goats, cattle, and horses. In dogs, miniature and toy breeds are usually affected. *The underlying lesion appears to be failure of fusion of the odontoid process to the body of the axis.* Clinical signs vary from neck pain to tetraplegia, with age of onset varying from a few months to several years. *Absence or hypoplasia of the odontoid process occurs in calves*, often in conjunction with atlanto-occipital fusion. Tetraplegia may be present at birth or develop at several months of age. In both dogs and calves, *fusion of the odontoid process with the axis* normally occurs in the early months of life and it is possible that in some cases postnatal influences are responsible for the condition.

Atlanto-axial subluxations occur in some *Arabian foals* with a familial, probably inherited, syndrome involving occipitalization of the atlas and atlantalization of the axis. Affected foals may be dead or tetraparetic at birth, or develop progressive ataxia within a few months. They have atlanto-occipital fusion and atlantal condyles that articulate with a malformed axis, thereby displacing the cranio-vertebral articulation caudally. *Congenital atlanto-occipital fusion with a wedge-shaped vertebral piece and cervical scoliosis* also occurs in horses as a sporadic defect unrelated to the Arabian condition. Subluxations of cervical vertebrae in horses and dogs are discussed with cervical vertebral stenotic myelopathy ("wobbler syndrome") earlier in this chapter.

Temporomandibular subluxation is reported in Basset Hounds and Irish Setters. Clinically, open-mouth locking of jaws occurs when the zygomatic arch interferes with the normal movement of the coronoid process of the mandible. Developmental abnormalities in the condyloid process of the mandible and the mandibular fossa of the temporal bone are responsible for subluxation but the *cause of the dysplasia is unknown.* The lesions are bilateral. A similar condition in American Cocker Spaniels may be bilateral or unilateral. In these animals, temporomandibular subluxation may be elicited by manipulation but is not associated with spontaneous clinical signs. Dysplasia of the mandibular fossa and hypoplasia or aplasia of the retroglenoid process are responsible for the excess joint mobility.

Subluxation of the carpus is reported as a sex-linked recessive trait in a colony of dogs in which hemophilia A also existed, although the two disorders do not occur in the same animals. Signs of carpal subluxation first appear when the animals begin to walk. Most carpal subluxations and luxations are secondary to trauma.

Patellar luxations and **subluxations** are common in dogs, less so in horses, and rare in other species. In **dogs**, most are associated with anatomical defects and are probably inherited as polygenic traits. Females are affected 1.5 times as often as males. The condition may be unilateral or bilateral and luxations of variable severity may occur either medially or laterally. Occasionally they occur in both directions. Medial luxations account for about three-quarters of the cases and are *most common in small dogs*, especially Pomeranians, Yorkshire Terriers, Chihuahuas, Miniature and Toy Poodles and Boston Terriers. Lateral luxations tend to occur in larger dogs including some giant breeds, such as Great Dane, St. Bernard, and Irish Wolfhound. Clinical signs commonly develop within the first few months of life, but may be present at birth. Various anatomical defects involving the femorotibial joint, and sometimes the entire limb, are present in affected dogs and may be adaptational deformities. Patellar luxation in **horses** almost always occurs in a lateral direction and is associated with hypoplasia of the lateral ridge of the femoral trochlea. It is sometimes congenital and bilateral in both foals and calves. The condition probably is inherited in ponies, and perhaps also in horses. Patellar luxation is rarely reported in **cats** and most cases appear to have a traumatic origin. A genetic predisposition has however been suggested in the Abyssinian and Devon Rex breeds.

The *consequences of luxations and subluxations* vary with the species of animal and the joint involved. In general, subluxations, whether they are genetic or traumatic in origin, *predispose to degenerative joint disease* due to instability of the joint. Luxations are more likely to damage periarticular soft tissues and, if the articular cartilage remains in persistent contact with soft tissues, it degenerates and is replaced by fibrous tissue and bone. If movement is minimal, a *fibrous or bony fixation* may develop, but continued movement may result in the formation of a new joint. Fibrous tissue about the dislocated bone ends sometimes becomes organized into an articular capsule with a synovial lining, but the articular surfaces are covered with fibrous tissue rather than articular cartilage.

Abnormal positioning of joints in terms of overextension or overflexion occurs in animals with **arthrogryposis**, but only in the most severe cases are the articular surfaces deformed. The primary lesion is in the central nervous system, where the congenital absence of motor neurons supplying selected muscles results in muscular atrophy and overextension or overflexion of the opposing unaffected muscle groups. **Congenital torticollis**, whether or not it is associated with a branchiocephalic fold, is associated with articular abnormalities of the cervical vertebrae in sympathy with the concertina-like compression that occurs on the concave side of the deviation.

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DEGENERATIVE DISEASES OF JOINTS

Synovial joints

Degenerative diseases involving the major weight-bearing joints of the limbs are very common in humans and domestic animals. In human medicine, the term **"osteoarthritis"** is preferred for this group of diseases although it incorrectly implies an inflammatory origin. **Degenerative joint disease** is a more appropriate term, based on the putative pathogenesis, and will be used in this chapter. Other commonly used *synonyms include osteoarthrosis and degenerative arthropathy*.

Degenerative joint disease is not a specific entity, but a common sequel to various forms of joint injury and involves an interaction between biologic and mechanical factors on the articular cartilage, subchondral bone, and synovium. It can be either *monoarticular or polyarticular* and may be classified as either *primary or secondary*. **Primary** *degenerative joint disease refers to those cases where there is no apparent predisposing cause and generally occurs in older animals*. Such cases may reflect an acceleration of the normal aging changes that occur in joints. Mild degenerative changes in weight-bearing articular surfaces, including yellowing and fibrillation of cartilage, are common incidental findings in adult dogs at necropsy. In the absence of clinical signs of lameness it is difficult to justify a diagnosis of degenerative joint disease in such cases, but it is likely that these represent the early manifestations of the disease and probably account for the mild lameness and stiffness seen in many old dogs.

Secondary *degenerative joint disease is associated with an underlying abnormality in the joint or its supporting structures, predisposing to premature degeneration of the cartilage. Any condition that causes direct damage to the articular cartilage, creates instability, or results in abnormal directional forces can predispose to degenerative joint disease.* For example, secondary degenerative joint disease is an inevitable consequence of the joint laxity in dogs with hip dysplasia, due to the effect of abnormal mechanical forces on articular cartilage. Traumatic injuries to ligaments may also create joint instability leading to degenerative joint disease. Incongruity of opposing articular surfaces, as occurs in many animals with osteochondrosis, is a common cause of degenerative joint disease involving the shoulder, elbow, stifle, and hock joints of dogs and other species. Other disorders that may predispose to secondary degenerative joint disease include malaligned limb fractures, angular limb deformities, aseptic necrosis, or metabolic bone diseases with collapse of subchondral bone, inherited defects in cartilage or collagen formation, septic arthritis, and hemarthrosis. Persistent hemarthrosis, as occurs in animals with hemophilia or other coagulopathies, may lead to chronic, proliferative synovitis with accumulation of hemosiderin-containing macrophages in the synovium. Invasive pannus may develop from the hyperplastic synovial tissue and spread out over the articular surface.

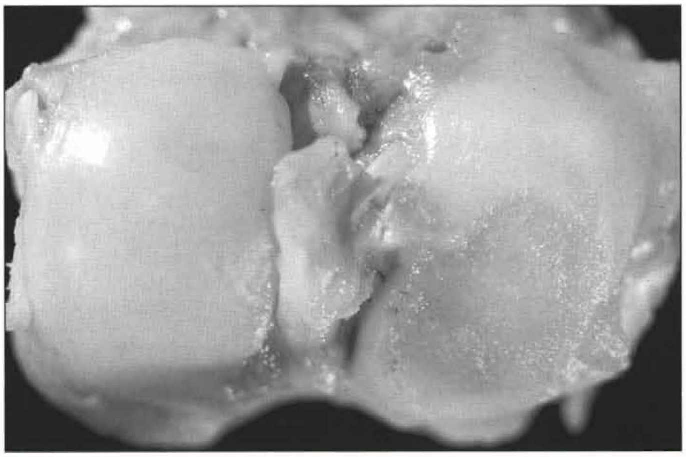

Figure 1.143 Degenerative joint disease, dog. Fibrillation of articular cartilage on the femoral condyle.

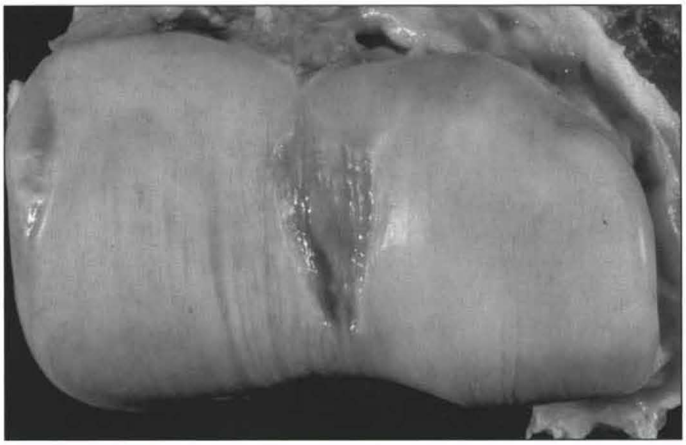

Figure 1.144 Degenerative joint disease, horse. Linear grooves (wear lines) in the articular cartilage of the distal humerus. The depressed area between the condyles is a developing synovial fossa. (Courtesy of SE Weisbrode.)

The *gross lesions* of degenerative joint disease are similar whether the disease is primary or secondary in origin, although the lesions of secondary degenerative joint disease are generally more severe by the time an affected animal comes to necropsy. *The earliest gross lesion is roughening of the articular cartilage in areas of weight bearing* (Fig. 1.143) due to loss of proteoglycans from the matrix and unmasking of the collagen fibrils. This is referred to as **fibrillation**. Initially, only the superficial layers are involved but, with continued abrasion of the degenerate cartilage, vertical fissures develop in deeper layers in the direction of collagen fibril alignment and may extend to the subchondral bone. In hinge-type joints, such as the hock, fetlock, and elbow, *linear grooves (wear lines)* may be present in the cartilage in the direction of joint movement (Fig. 1.144). These are found relatively commonly in the joints of adult horses. Their cause is uncertain and they are not necessarily associated with progressive lesions. Progressive erosion of fibrillated cartilage is accompanied by sclerosis of the subchondral bone. In advanced lesions, *the articular cartilage may be*

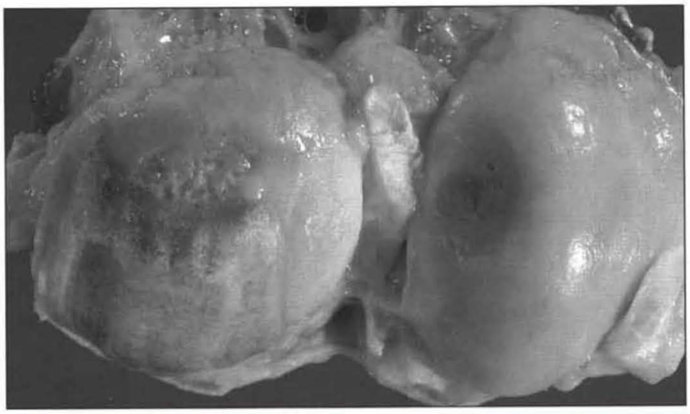

Figure 1.145 Chronic degenerative joint disease of the stifle joint in a cow. Loss of articular cartilage on the medial condyle with eburnation of the exposed subchondral bone. A smaller lesion is present on the lateral condyle.

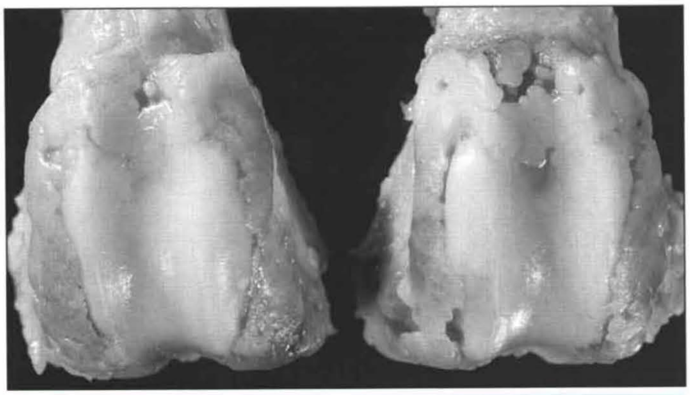

Figure 1.146 Degenerative joint disease, dog. Extensive osteophyte formation along the chondro-osseous margins of both stifles.

completely ulcerated in areas of maximum weight bearing and the exposed bone worn smooth, a process referred to as **eburnation** (Fig. 1.145). Increased stiffness of the sclerotic subchondral bone may accelerate the loss of the overlying cartilage due to reduced flexibility during weight bearing. In fact, some investigators have proposed that sclerosis of subchondral bone may be the initial lesion in degenerative joint disease. More likely, this is just one of a combination of factors involved in progression of the disease. Cystic lesions are often present beneath eburnated surfaces in human patients with degenerative joint disease but are seldom seen in domestic animals. *Another consistent gross feature of degenerative joint disease is the formation of* **osteophytes** *at the margin of articular cartilage and bone* (Fig. 1.146). These small, nodular, outgrowths of bone, covered by a thin layer of hyaline cartilage may surround the articular surface and either distort its shape or obscure the boundary between the articular surface and the supporting bone. Osteophytes develop rapidly following joint injury and can be detected as early as 7 days after a joint has been unstable experimentally. Changes also occur in the synovium and joint capsule of animals with degenerative joint disease. The joint capsule is thickened with fibrous connective tissue and there is likely to be hypertrophy of synovial villi.

A variety of *histologic changes* have been reported in the articular cartilage of animals and humans with degenerative joint disease. *Reduction in metachromatic staining of the superficial cartilage matrix*, presumably associated with the loss of proteoglycan, is a common early change and may be accompanied by necrosis of chondrocytes in the tangential layer. *Clusters of proliferative chondrocytes (brood capsules or chondrones) may be present*, especially adjacent to fissures in areas of fibrillation (Fig. 1.147A). Elsewhere, the density of chondrocytes is

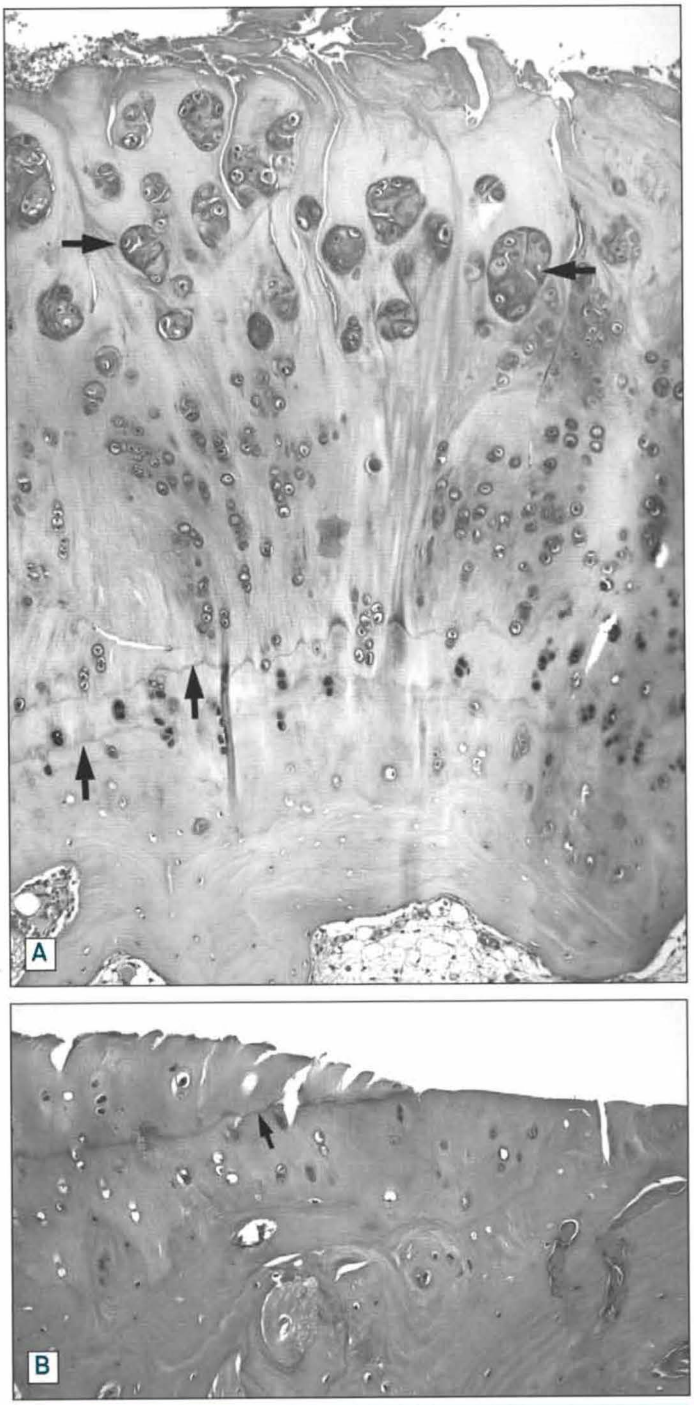

Figure 1.147 Degenerative joint disease, dog. **A.** Fibrillated articular cartilage with irregular surface, eosinophilic matrix and disorganization of chondrocytes. Several chondrones (horizontal arrows) are adjacent to clefts in superficial layers. The tidemark is duplicated in some areas (vertical arrows). **B.** Junction between areas of fibrillation and eburnation. The frayed cartilage becomes eroded to the level of the tidemark (arrow) at which stage it is replaced by an articular surface comprised of mineralized cartilage then sclerotic bone.

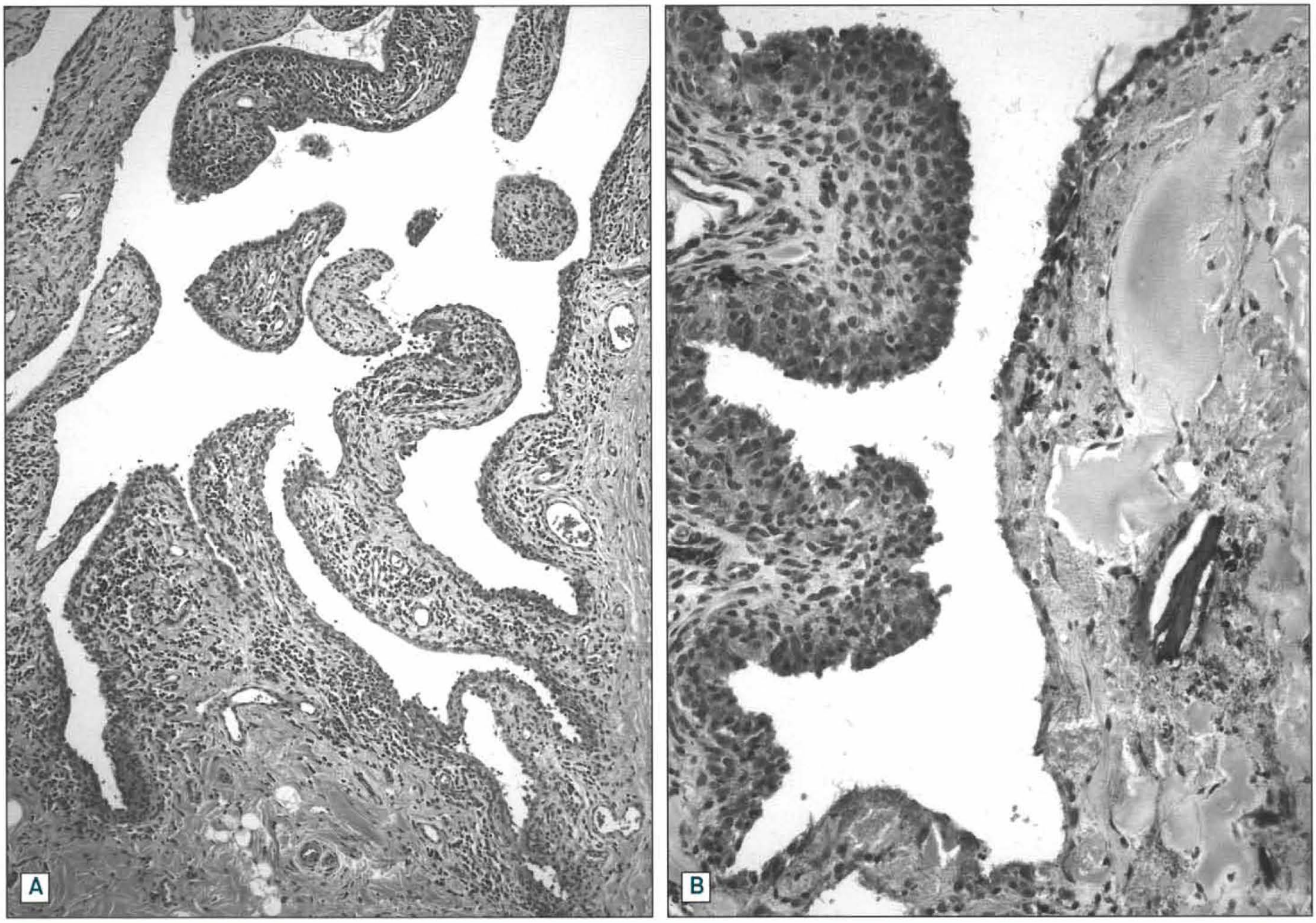

Figure 1.148 Chronic degenerative joint disease. A. Hypertrophic villi lined by plump synoviocytes and infiltrated with mononuclear inflammatory cells. **B.** Fragments of degenerate cartilage and occasional spicules of bone, most likely derived from the eroded articular surface, embedded in the synovium. The adjacent villi are lined by hyperplastic synoviocytes.

often reduced but remnants of degenerate cells may still be apparent. In advanced lesions, *the overall thickness of the degenerate cartilage may be markedly reduced*, the surface is irregular, and deep clefts may be present. Collagen fibrils within the matrix appear more prominent. The tidemark is often duplicated or disrupted, and may be penetrated by blood vessels. *Fibrillated cartilage merges with areas of eburnation* (Fig. 1.147B), where the articular cartilage is absent and the trabeculae of subchondral bone show variable degrees of thickening and/or remodeling. In chronic degenerative joint disease, *synovial villi are hypertrophic*, sometimes branching and are lined by plump, hyperplastic synoviocytes (Fig. 1.148A). Small to moderate numbers of *mononuclear inflammatory cells*, predominantly lymphocytes, in addition to hemosiderin-containing macrophages are often present. *Fragments of degenerate cartilage*, presumably derived from the eroded articular surface, may be embedded in the synovium or attached to its surface (Fig. 1.148B). Such fragments may enlarge through proliferation of surviving chondrocytes and may be an early stage of secondary osteochondromatosis (see p. 134).

The *pathogenesis of degenerative joint disease is complex* and continues to be debated in human medicine, even after many decades of research and observation. Although there are differing hypotheses on the exact sequence of events, most investigators conform to the view that the disease is *primarily degenerative in nature and that the accompanying inflammatory changes are secondary*. It must be recognized however, that lesions of degenerative joint disease will also develop as a sequel to chronic inflammation in many primary inflammatory conditions of man and domestic animals. There is convincing evidence that the *chondrocytes* of articular cartilage play an important role in the early stages of disease development. These post-mitotic cells normally survive throughout the life of the individual and maintain a balance between degradation and repair of the cartilage matrix under the influence of cytokines, growth factors, and direct physical stimuli. Disruption of this balance in favor of matrix catabolism occurs in the early stages of degenerative joint disease and is reflected by depletion of the proteoglycan aggregates, aggrecan, which are a major component of cartilage matrix. Continued reduction in the proteoglycan content of the matrix, together with damage to collagen fibrils, interferes with the viscoelastic properties of articular cartilage. As a result, progressive loss of cartilage occurs under the influence of normal biomechanical forces.

The early loss of matrix components in degenerative joint disease is mediated by degradative enzymes, including metalloproteinases, serine proteinases, cysteine proteinases, and aggrecanase. Many of these enzymes are derived from chondrocytes, but synoviocytes and inflammatory cells may also produce them. Natural inhibitors of these enzymes are normally present in articular cartilage but are deficient in degenerative joint disease. Several zinc-dependent metalloproteinases are recognized but the collagenases, stromelysins, and gelatinases appear to be the most important in cartilage degradation. Collagenase and stromelysin are synthesized as latent enzymes in cartilage and can be activated by plasmin and plasminogen activator or inhibited by plasminogen activator inhibitor-1. Collagenase breaks down the scaffolding of type-II collagen, while stromelysin cleaves aggrecan in addition to some types of collagen. Degradation products of aggrecan and type-II collagen may remain in the cartilage matrix or diffuse into the synovial fluid, where they can be detected. Proteoglycan fragments in synovial fluid may be either phagocytosed by synovial cells or enter lymphatics in the synovium.

Various cytokines and growth factors are also believed to be involved in the pathogenesis of degenerative joint disease, particularly in the generation of the inflammatory response. Most attention has focused on the role of the cytokines interleukin-1, interleukin-6, and tumor necrosis factor-α. Interleukin-1 and tumor necrosis factor-α have been shown to increase the synthesis of metalloproteinases and plasminogen activators, and to induce the resorption of cartilage both in vitro and in vivo. Interleukin-1 also stimulates fibroblasts to synthesize collagen type I and type II, and may therefore contribute to fibrous thickening of the joint capsule in degenerative joint disease. The role of interleukin-6 is less clear but it is released in vitro by chondrocytes from normal and degenerate cartilage and may be involved in autocrine stimulation of chondrocyte proliferation. It has also been detected in the synovial fluid of human patients with various arthropathies and may be an important intermediate signal for the activities of interleukin-1 and tumor necrosis factor-α. Growth factors, such as insulin-like growth factor-1 and transforming growth factor-β, have an anabolic effect on connective tissues and have been shown to stimulate proteoglycan and collagen synthesis. They can also inhibit or reverse some of the catabolic effects of interleukin-1. Similarly, the cytokine γ-interferon inhibits the action of interleukin-1 on metalloproteinase production and proteoglycan depletion from cartilage. These observations support the concept of a complex interaction between cytokines and growth factors in the pathogenesis of degenerative joint disease.

Inflammatory changes in the synovium of humans and animals with degenerative joint disease are most likely secondary to the stimulation of interleukin-1 and tumor necrosis factor-α by synoviocytes following the release of degraded collagen and proteoglycan fragments from degenerate cartilage. The neuropeptide, substance P, may also be involved. This peptide has been detected in the synovial membrane and fluid of human patients with degenerative joint disease and has been shown to activate both inflammatory cells and synoviocytes, in addition to stimulating the secretion and action of interleukin-1.

Primary and secondary degenerative joint diseases are common in most domestic animal species, but in dogs, horses, and cattle, certain types are either sufficiently common or important to warrant discussion as separate entities.

Degenerative joint diseases of horses

Although degenerative joint diseases may affect a wide range of synovial joints in horses, those involving the *interphalangeal, metacarpophalangeal, and hock joints* are of particular importance. *These joints are subjected to considerable biomechanical loading during motion*, especially in performance horses, and are therefore predisposed to traumatic injury to the articular cartilage, subchondral bone, or supporting structures. *In joints subjected to considerable motion, such as the metacarpophalangeal joints, the changes of degenerative joint disease are usually characterized by gradual erosion of articular cartilage, sclerosis of subchondral bone, chronic synovitis, and gradual stiffening of the joint due to fibrous thickening of the joint capsule.* In low-motion joints, such as the proximal and distal interphalangeal, distal intertarsal, and tarsometatarsal joints, maximum loading during motion is focused on a restricted area. In these joints, the lesion typically is characterized by full-thickness necrosis of the articular cartilage with limited wearing, focal damage to subchondral bone, and bony ankylosis. Degenerative changes involving the navicular bone and the adjacent deep digital flexor tendon, referred to as *navicular syndrome*, probably have a similar pathogenesis and will be discussed here, although they are more appropriately classified as a form of bursitis rather than with degenerative joint diseases.

Metacarpophalangeal (fetlock) joints

Traumatic and degenerative diseases of the metacarpophalangeal (fetlock) joints of the foreleg are *more common than similar lesions affecting any of the other limb joints.* This susceptibility to injury presumably reflects the relatively small surface area of this joint in comparison to most others and the considerable range of motion expressed by the joint. Furthermore, during racing, the entire weight of the animal is transmitted to the ground through this joint during one phase of the stride. *Poor conformation and excessive training of horses at a young age no doubt contribute to the development of lesions in this and other joints.* **Traumatic synovitis** of the dorsal joint capsule often results from repeated overextension of the fetlock joint in the racehorse. At the point of maximum extension, the *proximodorsal margin of the proximal phalanx traumatizes the fibrous synovial pad* covering the dorsal surface of the distal end of the cannon bone. In response to constant trauma, the fibrous pad becomes enlarged due to hyperemia, edema, and fibroplasia, and extends across the adjacent dorsal articular margin of the cannon bone. Degeneration of the underlying articular cartilage occurs as a result of impaired access to synovial fluid and release of inflammatory mediators from the inflamed synovium. In cases where the inflammation resolves and the synovial pad retracts, the degenerate, pitted articular surface remains as evidence. In other cases, hemorrhage from the traumatized synovial pad and the insertion line of the joint capsule results in the formation of an organizing hematoma. *The firm yellow/brown lesion on the dorsal surface of the distal cannon bone is referred to as* **villonodular synovitis**. The histological features of these lesions vary with their duration. In the subacute stages, they include a mixture of hemosiderin-containing macrophages and well-ordered granulation tissue. Chronic lesions consist of dense, poorly vascularized fibrous tissue infiltrated by mononuclear inflammatory cells and covered by a layer of synoviocytes.

Another common change involving the metacarpophalangeal joint in performance horses is the *formation of a periarticular lip along the dorsal articular margin of the proximal phalanx*. Initially, the lip consists of an overgrowth of articular cartilage, but its deeper layers undergo endochondral ossification, creating an *osteophyte* that can be detected radiographically. With repeated trauma, microscopic or macroscopic chip fractures may develop in the bony support of the lip. The repair of such fractures is often compromised by the continued trauma of racing or training, and loosened chip fractures, attached to the adjacent bone by granulation tissue, may cause synovitis and acute clinical signs.

Traumatic osteochondrosis

Small, ovoid, "bruised" defects about 2–4 mm in diameter are relatively common incidental findings in the palmar articular surface of the condyle of the cannon bone in racehorses at necropsy. More severe lesions of this type are symptomatic and are characterized by apparent collapse of the articular cartilage into a defect in the subchondral bone. These lesions are referred to as traumatic osteochondrosis. They typically occur in highly trained, mature racehorses and are believed to result from focal sclerosis of subchondral bone, leading to microfractures and bone necrosis. During the repair process, the lesion is susceptible to displacement or collapse, particularly if strenuous exercise persists during this period.

Transverse ridge arthrosis

This is a degenerative lesion involving the transverse ridge of the condyle of the cannon bone, particularly in the foreleg. The lesions vary from mild fibrillation to the development of deep ulcers extending into the underlying bone. The transverse ridge develops between the dorsal articular surface of the condyle, which articulates with the proximal phalanx, and the palmar surface of the condyle, which articulates with the proximal sesamoid bones. If the fetlock joint is overextended during racing, the base of the proximal sesamoid bone overrides the transverse ridge, exposing it to shearing forces and eventually leading to degeneration.

Instability of the metacarpophalangeal joint created by experimental transection of the medial collateral and the lateral collateral sesamoidean ligaments in horses has been used to induce lesions similar to those of naturally occurring degenerative joint disease. The lesions included scoring lines (linear erosions) in the articular cartilage and osteophyte formation along the lateral articular margin of the sesamoid bone and lateral aspect of the proximal phalanx.

Ringbone

Degenerative diseases of **interphalangeal joints** are commonly referred to as *ringbone*. *High* or *low* ringbone refers to involvement of the *proximal or distal interphalangeal joints* respectively. *The lesions are typically bilateral and primarily involve the forelimbs.* In young horses, subchondral cystic lesions associated with osteochondrosis are considered an important predisposing factor in high ringbone, although similar subchondral lesions can also be induced by trauma to the articular surface of young animals. In older horses, instability of the joint secondary to traumatic injuries to the joint capsule, ligaments, or tendons supporting the joint may be involved. Alternatively, repeated episodes of minor trauma from athletic activity, or mechanical stresses associated with faulty conformation, may place excessive strain on the insertion lines of the dorsal joint capsule. Degenerative disease of the proximal interphalangeal joint also occurs secondary to subluxation of the joint following fractures of the palmar or plantar eminences of the middle phalanx. High ringbone is found most often in adult horses used for western events or polo, where horses running at high speed make abrupt stops, turns, and twisting motions.

The severity of the articular and periarticular lesions of high ringbone varies markedly between individuals. In the *early stages of the disease*, affected joints may be partly immobilized by fibrous thickening of the dorsal joint capsule and there may be early cartilage degeneration of one or both condyles of the distal first phalanx and the apposing glenoid cavity of the proximal second phalanx. Joint fluid is normal and the synovium is not congested. The periarticular response, which includes fibrous thickening of the dorsal joint capsule and bone formation beginning in the joint capsule insertion line, is a much more prominent feature than the cartilaginous changes. *The periarticular bony response on the dorsal surface of the joint gives the lesion its name.* In *advanced lesions*, full-thickness necrosis of the articular cartilage, followed by erosions in the subchondral bony plate, may lead to *ankylosis*. In cases where there is residual joint motion, there is eburnation of the ulcerated articular surfaces, thickening of the subchondral bony plates and inhibition of ankylosis.

Degenerative disease of the distal interphalangeal (coffin) joint

This occurs most often in racehorses, probably as a result of repeated minor trauma. *The Quarter Horse breed appears predisposed to problems of this joint*, possibly because their relatively small hoof diameter, short, upright pasterns, and large body size place greater stress on the joint during racing at high speeds. Periarticular osteophytes and enthesiophytes involving the distal aspect of the middle phalanx or the extensor process of the distal phalanx, characteristic of low ringbone, are present in some, but not all cases of degenerative joint disease of the coffin joint. In other cases the disease is less obvious clinically and radiographically and may be confused with navicular syndrome.

Bone spavin

This is a degenerative disease of the tarsus of the horse and occasionally the ox. Structural changes in this disorder are essentially the same as those occurring in high ringbone and presumably have a similar pathogenesis, initiated by sustained loading and joint immobilization. The *major lesions develop on the medial side of the tarsus*, primarily involving the distal intertarsal joint and, less commonly, the tarsometatarsal and proximal intertarsal joints. Examination of early cases of bone spavin, centered in the distal intertarsal joint, indicates that periarticular fibrosis initially immobilizes the dorsomedial side of the joint. "Kissing lesions" subsequently develop in articular cartilage adjacent to the dorsomedial margins of the apposing articular surfaces of the central and third tarsal bones. *Early lesions* show full-thickness necrosis of the apposed cartilage surfaces and intense bone remodeling within the thickened subchondral bony plate. *Intermediate lesions* show penetration of the necrotic cartilage by granulation tissue from the areas of intense subchondral bone remodeling. As the *lesion progresses*, granulation tissue extends across the joint space through areas of necrotic cartilage and establishes a *fibrous ankylosis*, which later gives way to a more stable *bony ankylosis*.

Navicular syndrome (navicular disease)

This is a degenerative disorder involving the distal half of the flexor surface of the navicular bones of the forelegs of mature horses. Various theories for its etiology have been proposed, but a biomechanical origin, associated with the combined effect of abnormal forces and poor conformation, is currently favored. An alternative hypothesis, whereby thrombosis of arterioles supplying the distal region of the navicular bone leads to ischemic necrosis of the bone, has failed to gain support.

Horses having *abnormal foot conformation*, i.e., bone and joint alignments at either end of the spectrum for the breed, are predisposed to navicular syndrome. At one end of this spectrum are *racing Thoroughbreds with very long toes and low heels*. In horses with this conformation, the deep digital flexor tendon places sustained pressure against the distal border of the flexor surface of the navicular bone. At the other end of the spectrum are those American Quarter Horses with an *extremely upright foot conformation*. In such animals, the deep digital flexor tendon exerts repetitive concussive forces on the distal border of the flexor surface of the navicular bone during exercise. Either sustained pressure or repetitive concussion can activate the remodeling processes in the subchondral region of the navicular bone in an attempt to adapt the flexor cortex to these biomechanical stresses. If pressure or concussive forces are of low intensity and of limited duration, the subchondral bone and spongiosa thicken sufficiently to absorb, diffuse, and redistribute the load. In such horses, the navicular bones are sclerotic and the fibrocartilage of the flexor surface of these horses often shows degenerative changes, but there is no clinical lameness.

When the sustained load or concussive forces acting on the distal border of the flexor surface of the navicular bone exceeds the physiologic limit, an exaggerated bone remodeling response is initiated. This eventually creates *lytic lesions in the subchondral bone beneath the flexor surface of the navicular bone*. Granulation tissue arises within these sites of intense bone remodeling in the same manner as it does in high ringbone and bone spavin. The granulation tissue eventually penetrates the overlying layer of degenerate fibrocartilage and forms fibrous adhesions to the apposing dorsal surface of the deep digital flexor tendon.

Bone marrow fibrosis and distended veins can be demonstrated in the marrow spaces of the subchondral bone and spongiosa located in the distal halves of the navicular bones of horses with navicular syndrome. It appears that vessels participating in the intense bone remodeling response leak edema fluid into the marrow spaces of the subchondral spongiosa supporting the flexor surface of the bone. This fluid is organized by fibrous tissue, which forms at the drainage angle in the medullary cavity of the distal border of the navicular bone. Blood vessels in this area are entrapped in the fibrous tissue, which retards venous drainage, resulting in distended veins, venous hypertension, and pain.

Enlarged vascular channels can often be seen radiographically in horses with navicular syndrome. As the tiny nutrient arteries enter along the floor of the synovial fossae, which are located on the distal border of the navicular bone, each main artery sends off small branches to the synovial lining of each of the respective synovial fossae. As part of the hyperemia associated with the remodeling process, the synovial lining of the fossae also undergoes active hyperemia. Osteoclasts are attracted to the site and activated to resorb bone, thus enlarging the synovial fossae and eventually eroding into the navicular bone. The *resorptive process* follows the pathway of the nutrient artery into the medullary cavity of the distal border of the navicular bone, creating deep synovial invaginations that are recognized on radiographs as enlarged vascular channels.

Degenerative joint diseases of dogs

Primary and secondary degenerative joint diseases are relatively common in adult dogs, particularly medium and large breeds, and usually involving the major weight-bearing joints. Secondary degenerative joint disease in dogs most often occurs as a sequel to joint instability or incongruity following dysplastic diseases of the hip or elbow or to the various manifestations of osteochondrosis. These are discussed in more detail elsewhere in this chapter.

Primary degenerative joint disease is sufficiently common in aged dogs to be regarded by many as an *inevitable consequence of aging*. Gross lesions are often found incidentally at necropsy in animals that had shown no clinical evidence of lameness. In one study, 31 (21%) of 150 randomly selected dogs had degenerative lesions in the *stifle joint* at necropsy. In 23 of these dogs, the lesions were bilateral. Degenerative joint disease of the shoulder and hip joints are also common in middle-aged and old dogs. The lesions are usually bilateral and develop slowly, starting at around 5–6 years of age as areas of softening and yellow discoloration on regions of articular cartilage subjected to maximum weight-bearing stress, then progress to fibrillation (see Fig. 1.143) and sometimes eburnation. Osteophytes frequently line the chondro-osseous junction (see Fig. 1.146) and may encircle the articular surface. In severe cases, the joint capsule is thickened and synovial villi are hypertrophic.

Degenerative joint disease of the *shoulder joint* may be related to joint laxity. Like the hip joint, the shoulder relies on muscular strength for stability. Atrophy of muscles with aging could therefore permit excessive joint mobility and persistent mild trauma to the articular cartilage. The fact that the dog bears 65–70% of its body weight on its forelegs may account for the development of lesions in shoulders more frequently than in hips. The shoulder lesions of primary degenerative joint disease may be difficult to distinguish from the degenerative lesions that occur as a sequel to osteochondrosis, especially in the advanced stages. Both typically affect the caudal aspect of the humeral head, and both are generally bilateral. However, as described earlier in this chapter, osteochondrosis develops at a much earlier age than primary degenerative joint disease and although healed lesions of osteochondrosis may be found incidentally in older dogs, they represent the repair of a cartilage defect, rather than progressive degeneration of articular cartilage from its superficial layers.

Degenerative joint diseases of cattle

Degenerative joint disease of the *stifle joint* occurs in mature dairy cows and is reported as a *possible inherited trait in Holsteins and Jerseys*. Clinical signs include lameness and muscle atrophy. The lesions are bilateral, and appear to develop in the conventional manner. Cartilage degeneration, erosion, eburnation, and osteophyte formation occur on the distal femur and are most severe on the medial aspect (see Fig. 1.145). Complementary lesions are present in the proximal tibia and shredding of the medial meniscus is common (Fig. 1.149).

Stifle arthropathy also occurs in stud dairy and beef bulls and may be secondary to poor conformation, ligament damage, or ruptured meniscus during fighting, or as a consequence of osteochondrosis. The latter may be an important cause of wastage of well-grown beef bulls that have been fed for optimal growth prior to sale or showing.

Degenerative joint disease of the *hock joints (spavin)* has been described as a problem in *tied Swedish dairy cows*. In one study, 37%

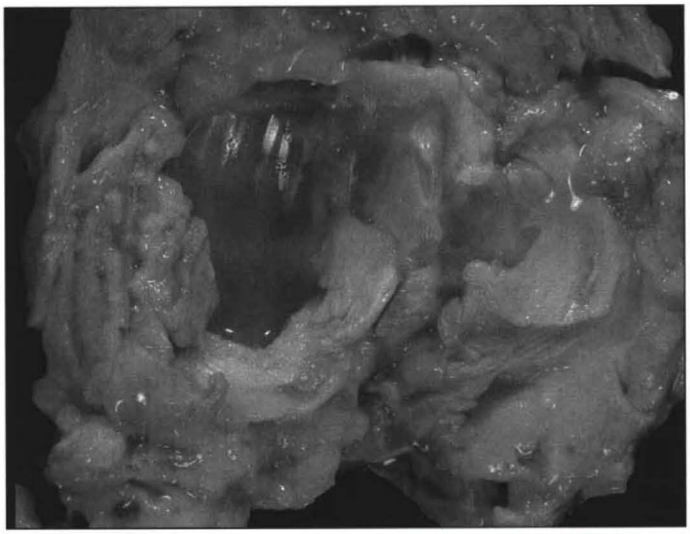

Figure 1.149 Degenerative joint disease, cow. Proximal tibia of same stifle joint as shown in Fig. 1.145. Shredding of medial meniscus, eburnation of medial articular surface (corresponding to medial condylar lesion), and marked fibrous thickening of joint capsule.

of animals were affected. The changes developed before the cows were 2 years old and increased in severity with age, sometimes progressing to ankylosis.

A lethal syndrome characterized by generalized degenerative joint disease and brachygnathia superior is reported in Angus calves. Affected calves have facial abnormalities, including reduced length of maxillary and palatine bones, together with abnormalities in all major joints. Macroscopic changes vary from reduced luster of articular cartilage to erosions and deep ulcers, eburnation of exposed subchondral bone, and thickening of joint capsules. Histologic lesions include degeneration of the articular cartilage matrix and necrosis of chondrocytes, in addition to fibrillation, chondrone formation, and chronic synovitis. Physeal abnormalities are also described. A genetic defect in the composition of cartilage matrix is suspected but the mode of inheritance is uncertain.

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Cartilaginous joints

The most important degenerative diseases of cartilaginous joints are those affecting *intervertebral disks*. Such diseases are of particular importance in humans and dogs, but are rare in other species, as are degenerative diseases of other cartilaginous joints. **Degeneration of intervertebral disks** occurs in all dog breeds as part of the aging process, but there are significant differences between chondrodystrophic and nonchondrodystrophic breeds in the nature of the degeneration, and the age at which it occurs.

In **chondrodystrophic breeds**, such as the Dachshund, Pekingese, Poodle, Beagle and Cocker Spaniel, *the nucleus pulposus starts to degenerate early in life* and by one year of age, it has become largely replaced by dry, gray/white or yellow, cartilaginous material (Fig. 1.150A). The initial microscopic change is thickening of the delicate fibrocartilaginous septa between cellular clusters, dividing the nucleus pulposus into lobules. Chondrocyte proliferation within the nucleus leads to *replacement of the original structure with chondroid tissue* within the first year of life. No lesions are apparent in the annulus fibrosus at this stage. The change in the nucleus pulposus in chondrodystrophic dogs *occurs in all disks* throughout the length of the vertebral column and is accompanied by a decline in the glycosaminoglycan and water content, and an increase in the collagen content of the matrix. Beginning at the periphery, *the chondroid tissue in the nucleus pulposus degenerates and mineralizes*, eventually becoming a crumbly mass (Fig. 1.150B). At this stage, there is *degeneration of the inner lamellae of the annulus fibrosus*, probably due to the altered viscoelastic properties of the degenerate nucleus pulposus. Individual lamellae may tear and allow degenerate nuclear material to escape into the annulus. Meanwhile, degeneration of the annulus fibrosus continues until the outer lamellae are also involved.

In **nonchondrodystrophic dogs**, *the normal mucoid nature of the nucleus pulposus persists, at least until middle age*, and although it may become dry and more fibrous with advancing age, it seldom mineralizes. Occasionally, fibrous metaplasia of the nucleus pulposus occurs in relatively young dogs of nonchondrodystrophic breeds, but such changes are believed to be secondary to *focal disruption of lamellae in the annulus fibrosus*, presumably as a result of trauma. In support of this belief is the observation that these lesions are *generally confined to a single disk*, unlike the generalized changes that occur in the nucleus pulposus of chondrodystrophic dogs. Traumatic damage to the annulus fibrosus would be expected to alter the biomechanical properties of

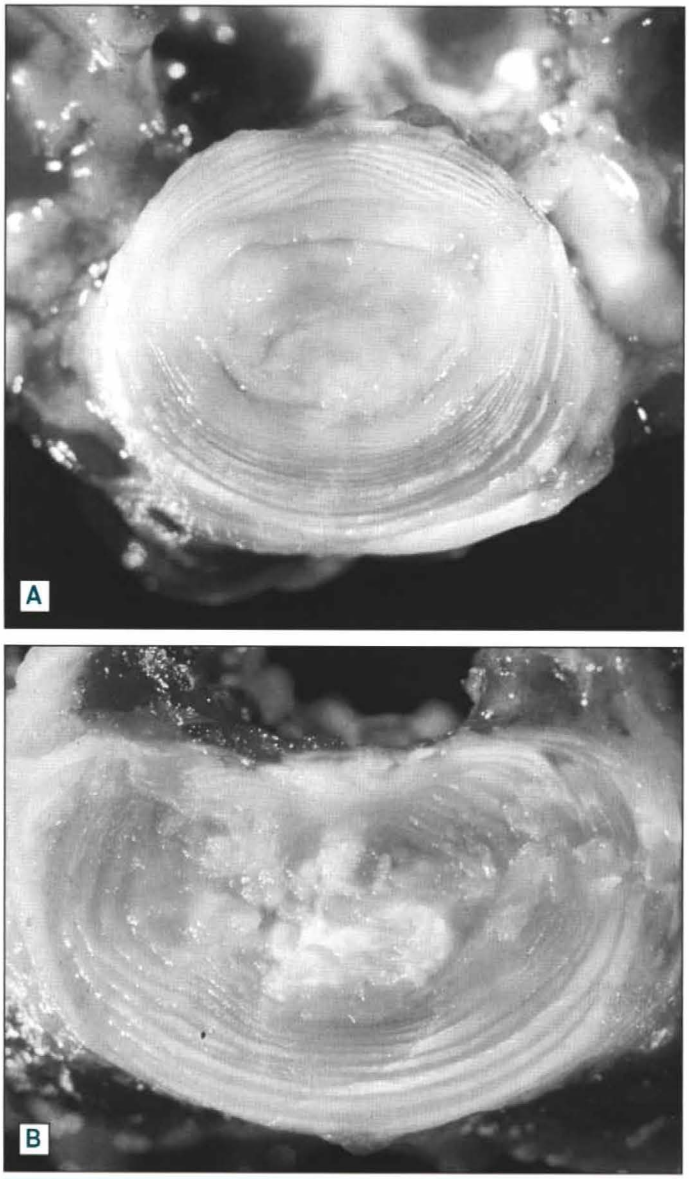

Figure 1.150 Intervertebral disk degeneration. **A.** Dry, gray/white nucleus pulposus (compare with Fig. 1.127) **B.** Chalky white foci of mineralization in the nucleus pulposus of a chondrodystrophic dog. Inner layers of annulus fibrosus are also degenerate.

the entire disk, leading to adaptive, and eventually degenerative, changes in the nucleus pulposus. Presumably, this same mechanism of intervertebral disk degeneration can occur in chondrodystrophic breeds, but the changes would be difficult to differentiate from the more severe, and more common, lesions outlined above.

Not surprisingly, *significant differences exist between chondrodystrophic and nonchondrodystrophic breeds of dog in the biochemical composition of their intervertebral disks.* At birth, the nucleus pulposus of chondrodystrophic disks contains up to 12 times more collagen than proteoglycan. The collagen composition increases rapidly with maturity, and by 11 months of age averages 25%. In nonchondrodystrophic breeds the collagen content of intervertebral disks remains below 5% for most of the animal's life. At all ages, the proteoglycan content of the intervertebral disk in chondrodystrophic breeds is significantly less than that of nonchondrodystrophic breeds.

The nucleus pulposus of *equine intervertebral disks* is more fibrous than that of dogs and its demarcation from the annulus fibrosus is less distinct. Although the nucleus pulposus of horses becomes less cellular with age, no chondroid or fibrous metaplasia occurs. In *cats*, macroscopic signs of intervertebral disk degeneration are not apparent until the animal is almost senile. Degenerative changes similar to those occurring in nonchondrodystrophic dogs are described in *adult sows and boars*, but dorsal herniation of the nucleus pulposus into the spinal canal has not been reported in this species. *Although disk protrusions are reported occasionally in cats and horses, discussion in the following section will concentrate on the syndrome in dogs*, for which there is a considerable volume of information.

Herniation of intervertebral disks

Because of their altered biomechanical properties, and their inability to withstand the normal range of forces associated with motion, *degenerate intervertebral disks are predisposed to complete or partial herniation or protrusion of nucleus pulposus material through the annulus fibrosus.* Many herniations are asymptomatic, but damage to adjacent structures, such as the spinal cord or peripheral nerve roots, is common and will be reflected by the clinical signs. Because of the eccentric location of the nucleus pulposus within the annulus fibrosus, most herniations occur through the narrower dorsal or dorsolateral regions of the annulus. In fact, *dorsal herniation of disk material into the spinal canal is considered the most common cause of paresis or paralysis in dogs.* Ventral herniation also occurs and may predispose to spondylosis (see below) and, on rare occasions, *nuclear material may herniate through the cranial or caudal cartilaginous end plate into a vertebral body, forming a so-called "Schmorl's node."*

Two types of dorsal herniation (types I and II) were recognized in classical descriptions of the disease in dogs by Hansen. **Type I herniations** *are characterized by a massive extrusion of degenerate nuclear material through the annulus fibrosus and dorsal longitudinal ligament into the spinal canal* (Fig. 1.151A). The sudden compression of the spinal cord, and/or peripheral nerve roots, typically causes *acute pain, paresis, or paralysis*, depending on the volume of extruded material and its location. Extradural hemorrhage may accompany laceration of longitudinal venous sinuses in the spinal canal. In some cases, severe damage to the spinal cord, or its vascular supply, results in *extensive hemorrhagic myelomalacia and ascending syndrome.* More commonly, the myelomalacia remains localized to a few cord segments. *The irritant nuclear material induces an inflammatory reaction* within the spinal canal and may become adhered to the dura.

Type II herniations generally develop slowly and are characterized by *partial herniation of the nucleus pulposus through ruptured annular fibers*, eventually resulting in bulging of outer lamellae and the intact dorsal longitudinal ligament into the spinal canal (Fig. 1.151B). Damage to the cord or peripheral nerve roots is much less than in type I herniations and clinical signs are generally mild.

Type I herniations of intervertebral disks are largely confined to chondrodystrophic dogs, particularly Dachshunds, and generally occur between 3 and 7 years of age. *Type II herniations are usually seen in nonchondrodystrophic dogs* between 6 and 8 years of age but can occur in any breed, as well as in humans, cats, and occasionally other species. Type I herniations are seldom reported in nonchondrodystrophic dogs.

Disk protrusions occur most frequently at sites of greatest vertebral mobility. In dogs, 70% of clinical cases of intervertebral disk disease are the

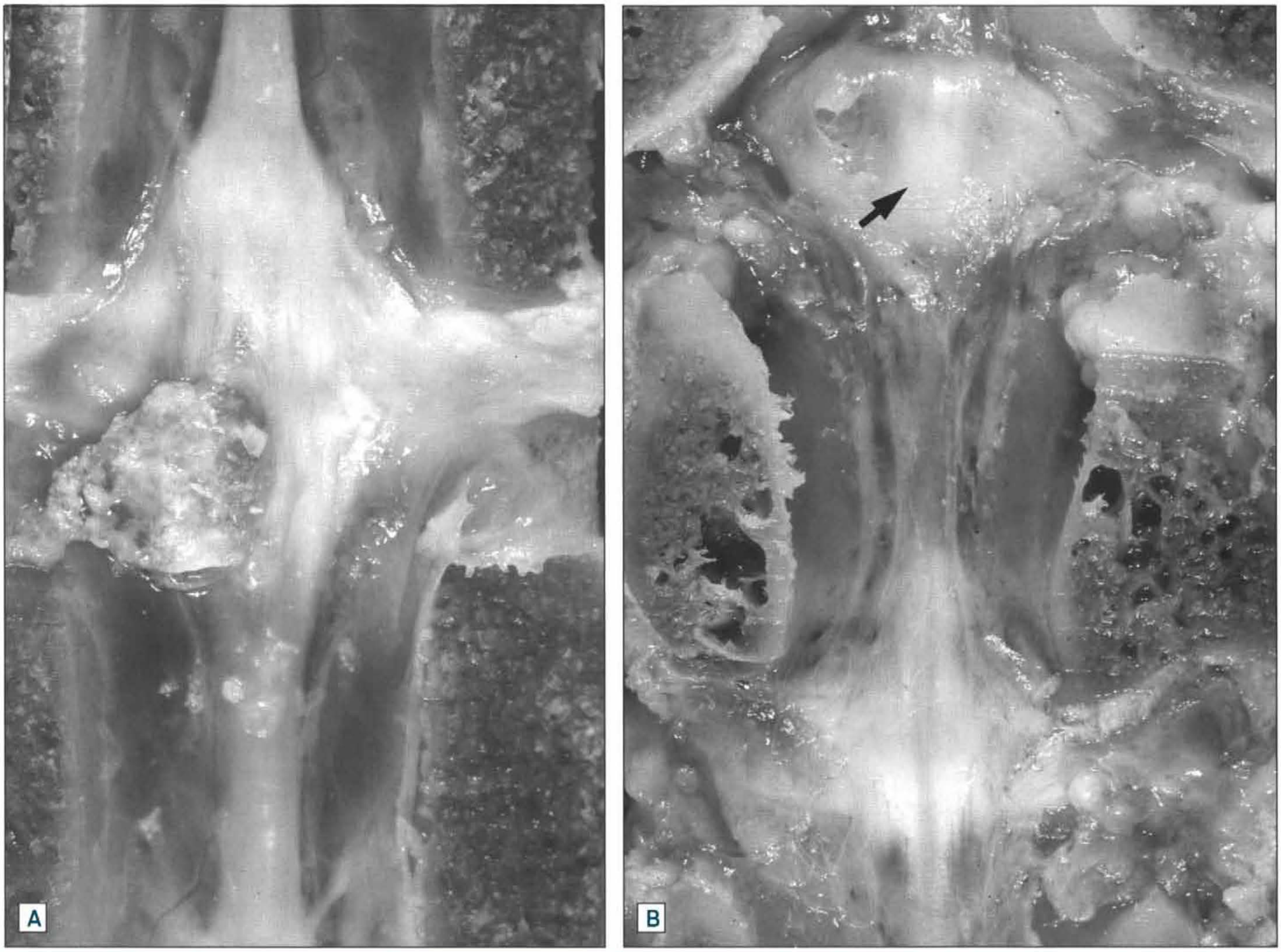

Figure 1.151 Degeneration and herniation of canine intervertebral disks. **A.** Herniation of a crumbly mass of degenerate disk material into the spinal canal through a tear in the dorsal longitudinal ligament (type I herniation). (Courtesy of RA Fairley.) **B.** Partial herniation of degenerate disk material (type II herniation) causing dorsal longitudinal ligament to bulge into the spinal canal (arrow). The adjacent disk is normal. (Courtesy of RA Fairley.)

result of herniations between the 12th thoracic and 2nd lumbar vertebrae. Approximately 15% of cases occur in the cervical region. Neurological signs associated with cervical disk protrusions are usually less severe than those involving the thoracolumbar region as the cervical cord occupies comparatively less space in the spinal canal, allowing more room for displacement before compressive damage occurs.

Herniations of intervertebral disks are *rare in horses* but occasionally are reported in the *cervical region*. Furthermore, in one study of cervical intervertebral disks in 17 clinically normal horses, partial extrusion of nuclear material through the annulus fibrosus, similar to type II lesions in dogs, was detected histologically in five horses. No intervertebral disk herniations have been reported in the thoracolumbar region of horses, probably because of the relative inflexibility of the equine spine in comparison to dogs and humans, and the strong longitudinal ligaments supporting the spine in this species. The mechanism of the cervical herniations in horses is not clear, but traumatic damage to the disk or adjacent vertebrae is a likely possibility, at least in some cases.

Ischemic myelopathy caused by **fibrocartilaginous embolism** *of spinal blood vessels is recognized as a possible sequel to degenerative disk disease in dogs.* Clinical signs relate to acute, severe, spinal cord damage similar to that associated with type I herniations of intervertebral disks, but the syndrome is *generally associated with type II herniations and is most common in nonchondrodystrophic dogs of large and giant breeds.* The spinal lesions may involve several vertebral segments and appear grossly as areas of swelling, malacia, and hemorrhage. Microscopically, fibrocartilaginous material, similar to that found in the degenerate nucleus pulposus, can usually be detected in the lumen of venules or arterioles (sometimes both) in areas of malacia. The mechanism by which embolic nuclear material enters the spinal vasculature is not clear. Venous emboli are probably caused by herniation of nuclear material directly into the overlying venous sinus, but arterial emboli are more difficult to explain. One suggestion is that they pass through arteriovenous communications from the venous circulation. Alternatively, they may directly penetrate arterioles in the degenerate annulus fibrosus and be extruded up to the radicular artery, from where they could gain access to the spinal cord. The possibility that such emboli develop following prolapse of the nucleus pulposus into the marrow cavity of an adjacent vertebral body has also been suggested, and is supported by experimental evidence. Demonstration of

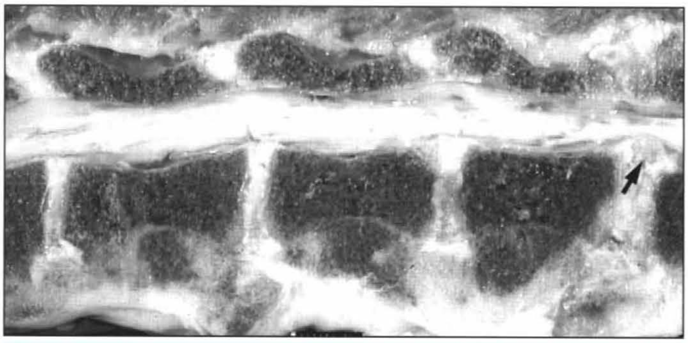

Figure 1.152 Ankylosing spondylosis, dog. Adjacent vertebral bodies united by thick, bony bridges along their ventral aspect. Also notice the type II disk herniation (arrow). (Courtesy of R. A. Fairley.)

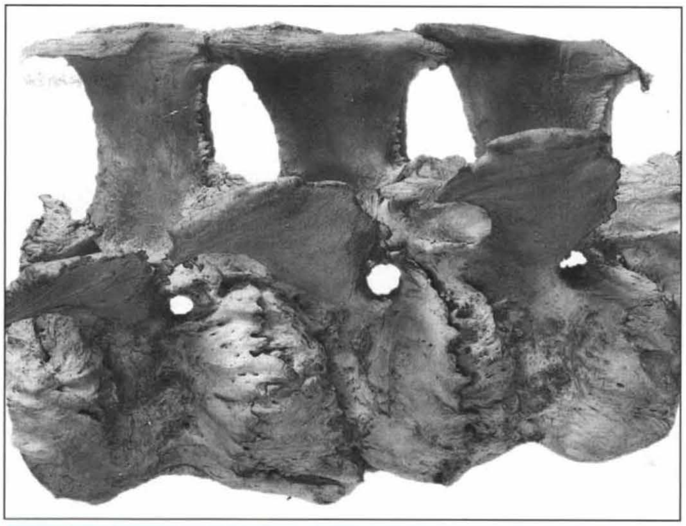

Figure 1.153 Ankylosing spondylosis, bull. Fusion of adjacent vertebral bodies by new bone deposited on ventral and ventrolateral surfaces.

Schmorl's nodes in vertebral bodies in the vicinity of spinal lesions would add further support to this hypothesis.

Fibrocartilaginous embolism has also been reported occasionally in pigs, cats, horses, and sheep. Of these species, pigs appear to be affected most frequently. The syndrome has occurred in weaner pigs as young as 45 days of age and, on one property, occurred in up to 25 of 1800 finishing pigs that had been sorted for slaughter at a weight of 126 kg. No degenerative changes in intervertebral disks, or other apparent predisposing factors, have been recognized in affected pigs. It is suggested that a *combination of heavy muscling and abrupt activity or excitement* may create increased pressure within intervertebral disks of young pigs and predispose to herniation of nuclear material into adjacent vertebrae or blood vessels. However, detailed dissection of disks and vertebrae in the vicinity of spinal lesions does not appear to have been performed.

Spondylosis

Spondylosis (spondylosis deformans, ankylosing spondylosis) is a common degenerative disease of the vertebral column characterized by the formation of osteophytes at the ventral and lateral margins of vertebral bodies adjacent to intervertebral spaces. The osteophytes may appear as spurs growing towards the adjacent vertebral body or as complete bony bridges with fusion of vertebrae (Fig. 1.152). Osteophytes also may be found dorsolaterally, projecting into the vertebral canal, but these are small and uncommon. In some cases, spondylosis is accompanied by degeneration of the synovial joints of the articular facets, and the reactive osteophytes may produce concurrent ankylosis of these articulations.

The *pathogenesis* of spondylosis is believed to involve an *initial degenerative change in the ventral annulus fibrosus*, probably secondary to trauma. Separation or tearing of the collagenous attachment of the annulus fibrosus from the adjacent vertebral body predisposes to mild ventral displacement of the annulus fibrosus and stretching of the ventral longitudinal ligament. This induces formation of bony outgrowths or spurs at the ventral margin of the intervertebral disk. As the disease progresses, further displacement of the annulus fibrosus and stretching of the ventral longitudinal ligament leads to more extensive osteophyte formation and eventually to bony bridging of the intervertebral space.

Spondylosis occurs most frequently in bulls, pigs, and dogs, and is seen less often in other domestic animal species. It is important in bulls kept in artificial breeding centers where it is presumably related to repeated traumatic damage to intervertebral disks during semen collection; *lesions are found in almost any bull past middle age.* Osteophytes develop mainly on the caudal end of thoracic vertebrae and the cranial end of lumbar vertebrae, and their incidence and size tends to decrease in either direction from the thoracolumbar junction. The greatest number and size of osteophytes is therefore in the area of greatest spinal curvature where maximum pressure on the disks during the thrust of service would be expected. The sequence of osteophyte development begins with severe degeneration of the annulus fibrosus, which may be present in bulls as young as 2 years of age. The osteophytes develop first in the outer annulus fibrosus and at its insertion to the rim of the vertebra. Growth is also in part by periosteal apposition and osseous metaplasia of ligaments. The trabecular bone of the osteophytes becomes continuous with that of the vertebral body and eventually is densely sclerotic. A thick layer of bone may be deposited along the ventral and ventrolateral aspects of the vertebral bodies (Fig. 1.153). In late stages, the heads of many ribs and the articular processes of the vertebrae bear large irregular osteophytes, which frequently cause ankylosis of the corresponding joint.

Although spondylosis is a *common incidental finding in breeding bulls, the disease is sometimes associated with mild or severe clinical signs.* Affected bulls may show caudal weakness and ataxia, or even paralysis, after dismounting from service. They may continue to be mildly ataxic or recover, only to be affected again later. The onset of signs is usually associated with fracture of the vertebral bodies and of the ankylosing new bone, which is dense, but tends to be brittle. The line of fracture tends to follow a large penetrating vessel to the intervertebral disk, which is frequently separated, and then to diverge across the dorsal corner of one or other vertebra. There is little displacement of the fractured ends in most cases, which accounts for the incomplete spinal syndrome. Trauma to the spinal cord is usually mild and paralysis is usually secondary to either hemorrhage or repeated trauma.

In *dogs*, the incidence of spondylosis increases with age after about the fifth year, and *vertebral osteophytes are a common incidental* *finding during radiography or at necropsy.* As in bulls, the primary morphologic change in dogs and cats with spondylosis is in the *annulus fibrosus*. In dogs, most lesions occur in the region of the first and second lumbar vertebrae, an area of relatively high mobility. The lumbosacral articulation is also often involved. The link between spondylosis and clinical signs in dogs is questionable, but it seems likely that severe lesions are responsible for spinal stiffness and pain, and reduced enthusiasm for physical activity.

Ankylosing spondylosis is a relatively common incidental finding in adult sows and boars. Although no specific predisposing factors have been identified, degenerate intervertebral disks or narrow disk spaces are often associated with the lesions. The osteophytes most commonly involve *lumbar vertebrae*, especially those in the lumbosacral region. Ankylosis, if present, may be confined to the ventral aspect of the vertebral bodies, but in some cases there is extensive new-bone formation in the vertebral arches, thus fusing the articular processes and encroaching on the spinal canal.

Spondylosis in *horses* is comparable to that in other species, but the evolution of the osteophytes has not been studied in detail. Vertebral ankylosis in *cats* with chronic vitamin A toxicity is discussed elsewhere.

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INFLAMMATORY DISEASES OF JOINTS

Inflammatory diseases of joints are generally referred to as either **arthritis** or **synovitis**. Although these terms are sometimes used interchangeably, they have slightly different meanings. *Synovitis refers to inflammation of a synovial membrane, whereas arthritis implies inflammation of other joint components in addition to the synovial membrane.* Inflammation of *tendon sheaths* often accompanies inflammation of an adjacent synovial joint and is referred to as **tenosynovitis** (synonyms: tendosynovitis or tendovaginitis). Secondary inflammatory changes are a feature of many chronic degenerative joint diseases, hence the commonly used term *osteoarthritis*, but in this section discussion will be confined to joint diseases that are primarily inflammatory in origin.

Arthritis may be either *infectious or noninfectious*, depending on the etiology. *Infectious arthritis occurs most frequently in farmed livestock and horses*, especially in young animals where it is a common sequel to neonatal bacteremia. *Most cases of noninfectious arthritis occur in dogs or cats and are immune-mediated.* Further classification is based on the duration of the lesion, the nature of the exudate, and the host response.

Fibrinous arthritis

This is typical of many *acute inflammatory diseases* of synovial joints, particularly those caused by *bacterial infections*. The presence of fibrin within synovial fluid indicates increased permeability of blood vessels in the synovial membrane, as fibrinogen and other large molecules are normally excluded. *Fibrin clots* may be floating free within the joint fluid, attached to the synovial membrane or lodged within recesses of the joint (Fig. 1.154A). In some cases, *sheets of yellow fibrin* partially or completely cover the synovial membrane, which is often edematous and hyperemic or may be studded with petechiae (Figs 1.154B, 1.155). *Synovial villi*, which are barely noticeable in normal joints, may become prominent macroscopically due to the edema and hyperemia. The synovial fluid is increased in volume and is usually slightly *turbid and mucinous*. When the inflammatory reaction is severe, there may be *gross edema of the periarticular tissues*. At this early stage, microscopic changes in the synovial membrane consist of edema and vascular engorgement, with few inflammatory cells. *Serous fluid or serofibrinous exudate* often infiltrates the fibrous layer of the articular capsule and the adjacent periarticular tissue.

In arthritis of longer duration, edema of synovial tissues is less apparent but the joint capsule and synovial membrane are thickened due *to proliferation of stromal cells and synoviocytes*, the latter often becoming several layers thick. *Sheets of fibrin containing variable numbers of neutrophils and fibroblasts may be attached to the surface* (Fig. 1.156). Villi continue to enlarge as a result of the cellular proliferation and may become extensively branched, with increasing numbers of lymphocytes and plasma cells, but few neutrophils (Fig. 1.157). Extravasated neutrophils pass quickly into the synovial fluid but seldom in sufficient numbers to give the fluid a purulent character. *Hypertrophy of villi* is greatest in the transition zone, and the proliferating fibrous stroma is joined by proliferating perichondrium to produce a fringe of granulation tissue, which spreads across the articular cartilage as a pannus and causes degeneration of the underlying cartilage.

Early resolution of the infection in animals with fibrinous arthritis is common, especially in smaller joints. However, the extensive deposits of fibrin in severe cases cannot be effectively removed by fibrinolytic mechanisms. Instead, the fibrin, which is deposited on the synovial membrane and within the layers of the articular capsule and periarticular tissue, is progressively invaded by fibrous tissue, leading to enlargement and restricted movement of affected joints.

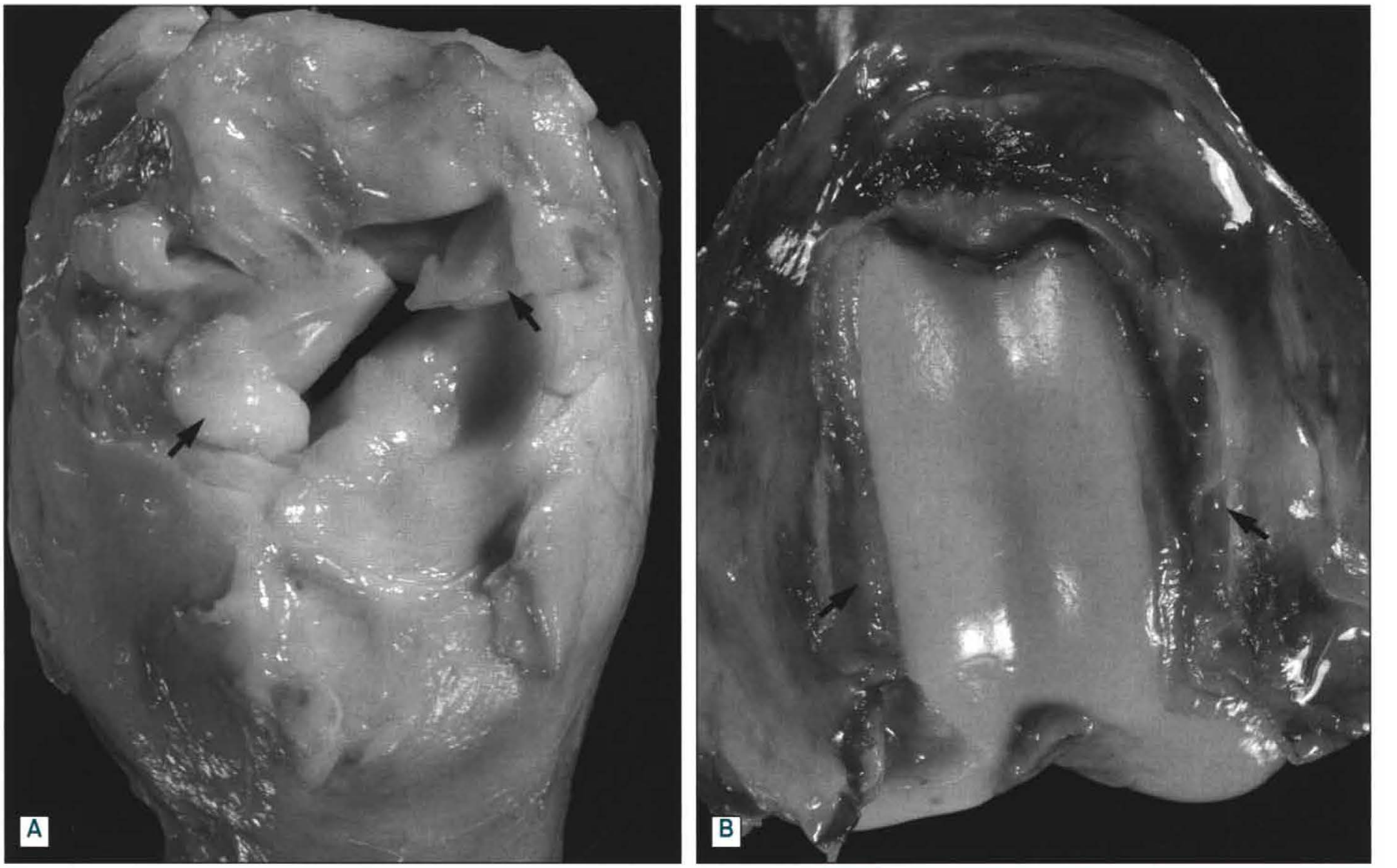

Figure 1.154 Fibrinous polyarthritis, calf. **A.** Large fibrin clots in the carpal joint (arrows). **B.** Stifle joint from the same calf showing an edematous, hyperemic synovial membrane covered by fibrin (arrows). The calf also had suppurative osteomyelitis (see Fig. 1.89A).

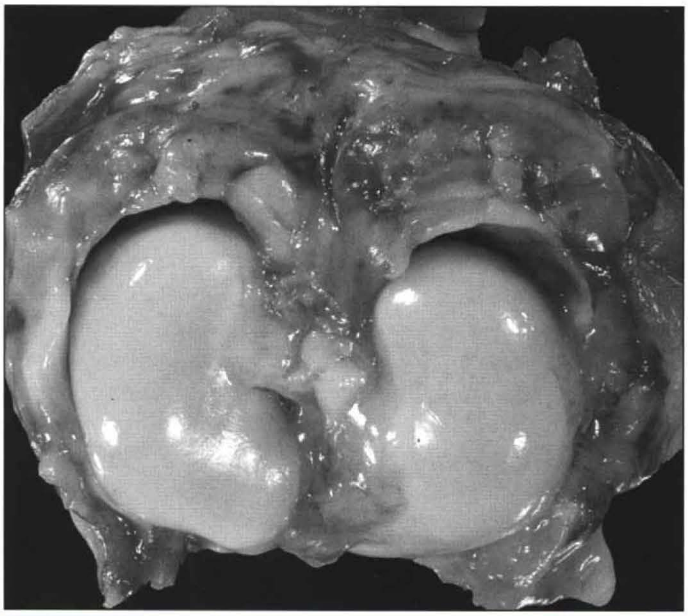

Figure 1.155 Fibrinous arthritis, pig. *Staphylococcus aureus* infection. The synovial membrane surrounding the femoral condyles is markedly thickened due to edema and an adherent layer of fibrin. Many petechiae are present in some areas.

The synovial lining is repaired by proliferation of synoviocytes. *Articular cartilage generally remains intact* in fibrinous arthritis, except in areas where it is covered by pannus. Pannus formation in joints with restricted movement may result in adhesions between apposed articular surfaces, leading to ankylosis.

Low-grade inflammation, with intense lymphocytosis, may persist in the synovial membrane, even in cases where the infection has apparently resolved. This may be due either to the persistence of an infectious agent that cannot be cultured or to ineffective removal by macrophages of the peptidoglycan components of microbial cell walls. For example, the cell wall of group A streptococci is relatively resistant to degradation by mammalian lysosomal enzymes, and is capable of provoking *persistent inflammation* in synovial tissues. Cell wall peptidoglycans from various other organisms, including *Erysipelothrix rhusiopathiae* also have this ability. All bacterial cell walls contain peptidoglycans, but there is considerable structural heterogeneity between bacterial species, and the types of side chain on the molecules probably determine their arthritogenic potential.

Purulent (suppurative) arthritis

This is characterized by the presence of *significant numbers of neutrophils in the synovial fluid, synovial membrane, and sometimes in adjacent structures*. When caused by bacterial infection, the neutrophils are usually abundant and may show *degenerative changes* in cytologic preparations of joint fluid. This is often referred to as **septic**

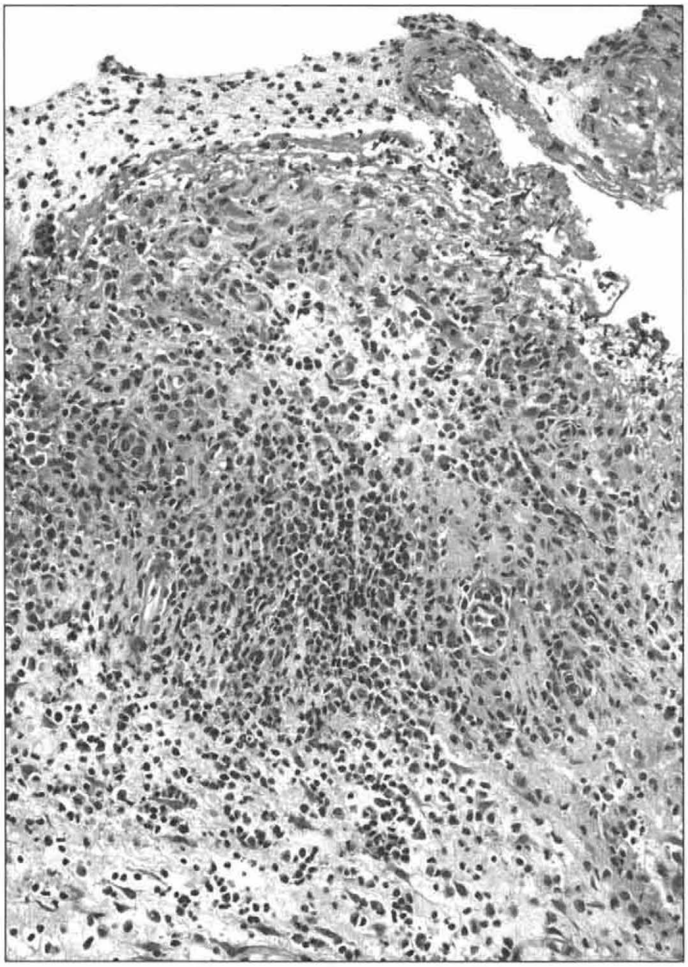

Figure 1.156 Subacute fibrinous arthritis, pig. *Streptococcus suis* type 1 infection. Edema, early proliferation of stromal cells and mixed cellular infiltration of synovial membrane. Fibrinous exudate containing moderate numbers of neutrophils is attached to the surface.

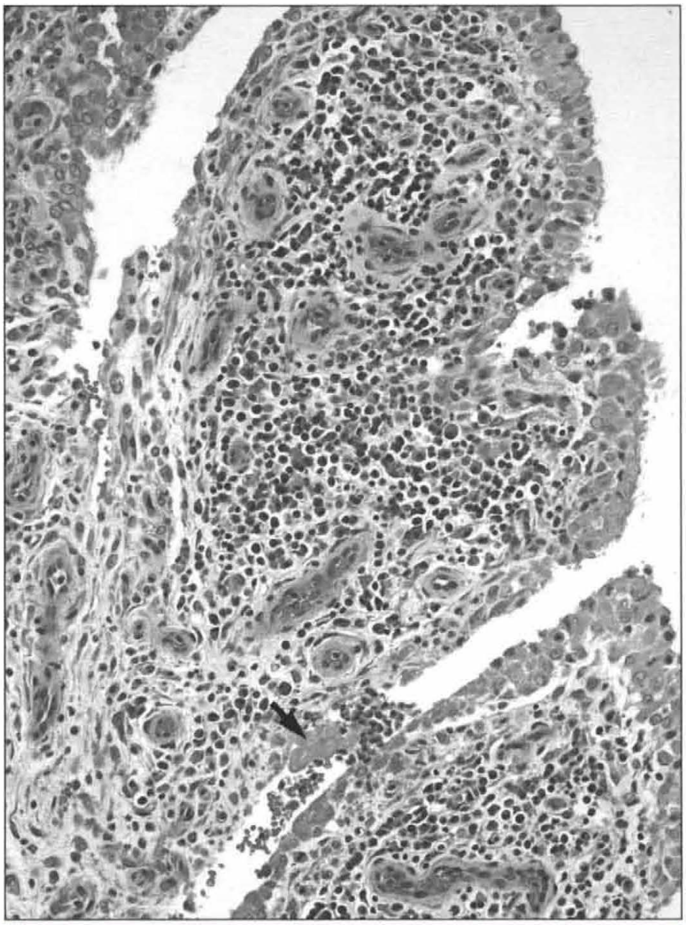

Figure 1.157 Chronic fibrinous arthritis, pig. *Streptococcus suis* type 2 infection. Hypertrophic villi infiltrated with mononuclear cells and lined by hypertrophic synoviocytes. A small fibrin tag and a cluster of neutrophils are trapped between two villi (arrow).

arthritis. Neutrophilic inflammation is a feature of arthritis caused by *Mycoplasma* spp., *Borrelia burgdorferi*, and certain viruses, but in these infections the neutrophils in synovial fluid are *nondegenerate*. Noninfectious, *immune-mediated arthritis* is also characterized by the presence of nondegenerate neutrophils in synovial fluid and differentiation from infectious arthritis is often difficult. *Bacteria are seldom observed in synovial fluid in animals with septic arthritis and false negatives on bacterial culture are common.*

Septic arthritis is often monoarticular and is *potentially a much more destructive process than fibrinous arthritis.* The synovial fluid is initially thin and cloudy but may resemble frank pus after a few days. *Destruction of articular cartilage* is much more likely to occur in septic arthritis than in fibrinous arthritis. *Lysosomal enzymes*, particularly collagenase, released from degenerating neutrophils probably play an important role in the cartilage destruction. Cytokines of macrophage origin, such as interleukin-1 and tumor necrosis factor-α, have also been shown to induce the resorption of cartilage both in vitro and in vivo and are most likely involved in chondrocyte-mediated cartilage degradation by stimulating the synthesis of metalloproteinases and plasminogen activators.

Complete resolution of septic arthritis is possible if the infection is eliminated spontaneously or by antibiotic therapy before erosion of cartilage occurs, but if the inflammatory process persists, the joint and adjacent structures will be severely altered. Cartilage degeneration occurs mainly at sites of weight bearing or at the articular margins, the latter in association with pannus formation. *Erosion of the degenerate cartilage may allow infection to enter the subchondral bone, resulting in purulent osteomyelitis* with extensive underrunning and separation of the articular cartilage. In such cases, it may be difficult to determine whether the arthritis preceded the osteomyelitis or vice versa, or whether the infectious agent gained access to both sites independently. Granulation tissue originating in the subchondral bone may grow out over the degenerate articular surface and predispose to ankylosis.

The suppurative process may extend to *involve adjacent tendon sheaths* and outwards from the synovial membrane of the articular capsule to *produce cellulitis in periarticular tissues.* The articular region is then greatly enlarged and the proliferation of fibrous tissue in response to inflammation, or during the healing process, results in permanent joint stiffness. In some cases, localization of the cellulitis into a periarticular abscess may be followed by *fistulation to the skin.* Fistulation to the skin surface may also result directly from empyema of the joint. Adhesions between tendons and tendon sheaths frequently occur in cases where tenosynovitis has developed in association with septic arthritis.

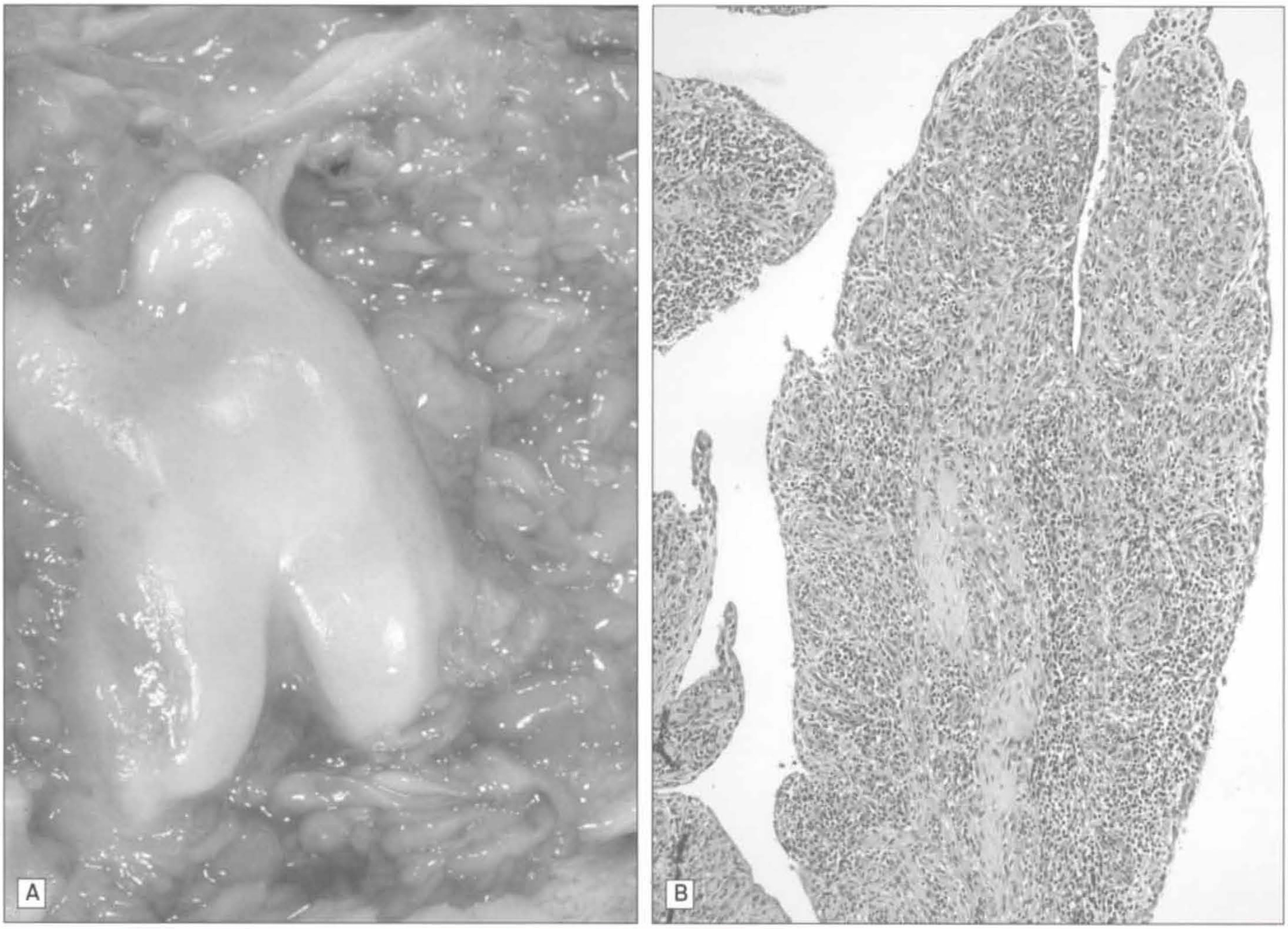

Figure 1.158 Proliferative arthritis, pig. **A.** Marked hypertrophy and hyperplasia of synovial villi in the stifle joint. (Courtesy of RA Fairley.) **B.** Hypertrophic villus in a pig with chronic *Erysipelothrix rhusiopathiae* infection. Note the stromal cell proliferation, mononuclear infiltration, and prominent synoviocyte layer.

Fibrinopurulent arthritis

Although it is convenient to classify inflammatory diseases of joints as fibrinous or purulent on the nature of the exudate, in reality, *many diseases would be better classified as* **fibrinopurulent arthritis**, *because the exudate consists of both fibrin and neutrophils.* In the chronic stages of many infectious and noninfectious forms of arthritis, lymphocytes and/or plasma cells are the major cell types infiltrating the synovial membrane (see Figs 1.148; 1.157). In such cases, **lymphocytic/plasmacytic synovitis** is a more appropriate morphological diagnosis. The term **proliferative arthritis** is often used to describe *chronic inflammatory diseases of joints where hypertrophy and hyperplasia of synovial villi is a prominent feature* (Fig. 1.158A, B).

An accurate etiologic diagnosis in animals with inflammatory diseases of joints may be crucial to a successful clinical outcome. In particular, failure to differentiate infectious and noninfectious causes of purulent arthritis can lead to inappropriate treatment and significantly alter the prognosis. *It is also important for septic arthritis to be recognized early and treated with an appropriate antibiotic in order to prevent degeneration of articular cartilage.* In addition to clinical history, analysis of synovial fluid often provides useful information but seldom allows a definitive diagnosis. As mentioned elsewhere in this section, many infectious and noninfectious forms of arthritis are characterized by increased numbers of neutrophils in synovial fluid. The neutrophils may show degenerative changes in bacterial infections but this is often difficult to appreciate in synovial fluid where the high concentration of hyaluronan prevents the cells from flattening out on the slide. Furthermore, not all bacteria induce such changes in neutrophils. Culture of bacteria or other agents from an inflamed joint allows a definitive diagnosis but false negatives are common. Culture of synovial membrane has been considered more sensitive but, in one study, *incubation of synovial fluid in a blood culture medium prior to culture yielded better results than either direct culture of the synovial fluid or culture of synovial membrane biopsies.* Microscopic examination of *synovial membrane biopsies* provides information on the nature of the inflammatory response and may be useful in differentiating chronic inflammatory and degenerative diseases of joints. Further information on diagnostic methods and details of laboratory results in specific diseases are discussed below.

Infectious arthritis

A variety of infectious agents, including *bacteria, viruses, and fungi*, are capable of infecting diarthrodial joints in humans and domestic animals. *In many, if not most situations, the arthritis is but one manifestation of a systemic infection*, with inflammatory lesions involving several tissues. In other cases, the infection may appear to be confined to one or more joints, suggesting either an affinity for synovial membranes or persistence of the infection in joints after being cleared from other sites. *Certain infectious agents may initiate a sterile synovitis in response to primary inflammatory lesions at sites distant from joints*. The mechanism of this form of arthritis is not clear, but it most likely involves an *immune-mediated reaction to nonviable bacterial components*, such as the bacterial wall antigen peptidoglycan, deposited in the synovial membrane, rather than direct infection of the joint by the agent. These forms of arthritis are discussed later under the heading Noninfectious (immune-mediated) arthritis.

A list of microorganisms that are commonly associated with infectious arthritis in domestic animals is presented in Table 1.4. Many agents other than those included in the list are capable of causing arthritis, but usually in isolated cases. Cats are not included in the list as infectious arthritis in this species is rare.

Some generalizations may be made about the prevalence of these infections relative to age. In *sheep*, with the exception of infection by *Mycoplasma* spp., infectious arthritis is primarily a disease of lambs. In *cattle*, streptococcal and coliform polyarthritis are neonatal, whereas infections due to *A. pyogenes* and *Salmonella* spp. may occur at any age. Streptococcal polyarthritis in *swine* is often a neonatal disease but the other infections are usually observed in weaned pigs. In *horses*, the organisms listed, other than *Salmonella* spp., generally cause intrauterine or neonatal infections.

Table 1.4 Common causes of infectious arthritis in domestic animals

Sheep
- *Chlamydophila pecorum*
- *Erysipelothrix rhusiopathiae*
- *Escherichia coli*
- *Histophilus somni*
- *Mycoplasma* spp.
- *Staphylococcus* spp.
- *Streptococcus* spp. (including types 1 & 2)

Goats
- *Mycoplasma* spp.
- Retrovirus

Swine
- *Actinobacillus suis*
- *Arcanobacterium pyogenes*
- *Brucella suis*
- *Erysipelothrix rhusiopathiae*
- *Escherichia coli*
- *Haemophilus parasuis*
- *Mycoplasma* spp.
- *Salmonella* spp.
- *Staphylococcus aureus*
- *Staphylococcus hyicus* ssp. *hyicus*
- *Streptococcus* spp. (including groups C & L)
- *Streptococcus suis* (types 1 & 2)

Cattle
- *Arcanobacterium pyogenes*
- *Escherichia coli*
- *Histophilus somni*
- *Mycoplasma bovis*
- *Salmonella* spp.
- *Streptococcus* spp.

Horses
- *Actinobacillus equuli*
- *Escherichia coli*
- *Klebsiella* spp.
- *Rhodococcus equi*
- *Salmonella* spp.
- *Streptococcus* (Group C)

Dogs
- *Blastomyces dermatitidis*
- *Borrelia burgdorferi*
- *Ehrlichia ewingii*
- *Escherichia coli*
- *Staphylococcus* spp.
- *Streptococcus* spp.

Bacteria

Bacterial arthritis is common in horses and food animals, usually as a sequel to neonatal bacteremia following omphalophlebitis ("navel ill"), or infections of the gastrointestinal tract or lungs. In many cases, the origin of the infection is not apparent either clinically or at necropsy, but inadequate transfer of colostral immunoglobulins is a common predisposing factor. The richly vascular synovial membrane appears to be a favored site for localization of bloodborne bacteria. Experimental studies have shown that viable bacteria injected intravenously lodge in the synovial membrane and gain access to synovial fluid more readily than to spinal fluid, aqueous humor, and urine.

Hematogenous bacterial infections in neonatal animals typically cause *polyarthritis*. Infected joints are generally hot, painful, and swollen due to the hyperemia and edema of the synovial membrane and joint capsule, and the increased quantity of synovial fluid. Although the infection may resolve in some joints, it often persists in others, particularly the large joints of the limbs, causing severe septic arthritis with destruction of articular cartilage. *Many, if not most young animals with septic arthritis of hematogenous origin also have osteomyelitis in the epiphyses or metaphyses of adjacent bones* (Figs 1.89A, 1.154A, B). This may be due to concurrent localization of the organism in the bone and synovial membrane, or may reflect the close vascular relationship between the epiphyseal bone and synovial membrane in young animals, with spread of infection from one site to the other. Foci of osteomyelitis originating at sites of endochondral ossification in the epiphysis, immediately beneath the articular cartilage, may underrun and penetrate the cartilage, spreading the infection directly into the synovial fluid. In joints where the capsule attaches distal to the physis, inflammatory foci in the metaphysis may contaminate the synovial fluid by penetrating the cortex near the physeal margin. This region is relatively porous in young animals because of the intense structural modeling that occurs in the metaphyseal cortex during rapid growth. The prevalence of concurrent bone involvement should always be considered in animals with bacterial arthritis as even if the arthritis can be successfully treated, the animal may eventually succumb to the effects of chronic osteomyelitis.

The reason why certain organisms are more likely to localize in the synovial membrane than others during bacteremia is not clear, but experimental evidence suggests that it is not purely due to chance. Studies in mice have indicated that *adherence* of *Staphylococcus aureus* to collagen is likely to be involved in the pathogenesis of septic arthritis and osteomyelitis. Other organisms causing arthritis have also been shown to possess *collagen-binding components*.

Spread of infection to joints from adjacent soft tissues is uncommon as the dense, fibrous layer of the joint capsule provides an effective barrier, but it may occur in necrotizing disorders such as necrobacillosis and footrot in cattle.

Direct implantation of bacteria into a synovial joint may occur as a sequel to a *penetrating wound* from the skin surface. This is the most common cause of bacterial arthritis in *dogs and cats*, where the disease occurs more often in adolescent and adult animals than in neonates. The arthritis is *monoarticular* and a mixed population of opportunistic bacteria is likely to be involved, often resulting in a highly destructive inflammatory response. *Arthroscopic surgery* and collection of synovial fluid by *fine-needle aspiration* may also introduce bacteria to a joint.

Although many different bacteria have been associated with arthritis in domestic animals, some specific types of bacterial arthritis are sufficiently important to warrant discussion in more detail.

Erysipelas

***Erysipelothrix rhusiopathiae**, the cause of porcine erysipelas and erysipeloid in man, is a gram-positive bacillus with a wide geographic distribution and host range.* It causes outbreaks of disease in *pigs, lambs, and birds*, and sporadic disease in the other domestic species. The organism is widespread in nature and is capable of survival, and perhaps growth, in decaying material of animal origin. It may be present in the soil of pig pens and in pit slurry, and survives for 2–3 weeks on pasture spread with slurry. It is resistant to many disinfectants and is capable of infecting many species, some of them in epidemic proportions. In spite of these epidemiological features, *pigs are probably the principal source of infection for other pigs. Erysipelothrix rhusiopathiae* can persist for many months in the lesions of diseased pigs, and it is often carried in the tonsils, intestine, bone marrow, and gallbladder of healthy swine.

Porcine erysipelas occurs in pigs of all ages, but the most susceptible are those from 2 months to 1 year of age, and pregnant sows. The latter may abort or give birth to stillborn young, from which the organism can be cultured. The disease can be produced by ingestion of the organism, contamination of cutaneous wounds, or as a result of bites of infected flies. The manifestations of erysipelas in pigs vary from an *acute septicemic form*, which is usually fatal, to *mild and chronic forms* characterized by necrosis of the skin, endocarditis, and polyarthritis. *In epidemics, the septicemic form predominates, whereas in endemic areas the disease tends to be sporadic, with cases of septicemia, polyarthritis, or endocarditis occurring in varying proportions.*

The **acute disease** is a *febrile septicemia*, which usually develops within 24 h of exposure to virulent organisms and produces *disseminated intravascular coagulation*. Endothelial cells of capillaries and venules throughout the body swell, monocytes adhere to them, and by 2–3 days microthrombi are widespread. By 4 days, accumulation of fibrin within and around vessels, bacterial invasion of endothelium and diapedesis of erythrocytes are prominent. Perivascular fibrin incites connective tissue proliferation in sites such as synovial membranes. *Grossly*, there is *purple discoloration of the skin* due to congestion and, in some cases, thrombosis of dermal capillaries and venules. Lesions may not be specific in pigs that die at this stage. *Petechiae or ecchymoses* may be present on serous membranes, the *spleen is almost always swollen and red,* and there may be *congestion and infarction in the gastric mucosa*. The latter lesion occurs in many acute infections in pigs. Subsidence of the acute disease, or a milder initial course, often leads to swelling of joints, lameness, and characteristic erythematous lesions in the skin.

The *cutaneous lesions* of porcine erysipelas are *roughly rhomboidal* and slightly raised. They are readily visible in light-skinned pigs and palpable when not visible in dark-skinned pigs. The skin within the rhomboid may be uniformly *bright red or purple*, but in some lesions only the margins and center are discolored. The latter lesions may progress to complete discoloration, or may return to normal within a few days. The uniform, bright red lesions may also resolve, leaving only an area of scurfiness as a residue. The dark red or purple lesions undergo dry necrosis and may eventually peel off or, if forcibly detached, expose a raw base. Occasionally, the skin lesions coalesce over large areas and lead to extensive cutaneous necrosis. The *tip of the tail and ears* may also become dark, shrunken, and leathery due to *ischemic necrosis*. Microscopically, there is neutrophilic infiltration of the walls of small dermal arterioles, many of which contain *cellular thrombi*. Suppurative inflammation of sweat glands is also a consistent microscopic finding, but otherwise the reaction is most severe at the junction of dermis and subcutis. *Similar cutaneous infarcts occasionally occur in other septicemic bacterial diseases* and the lesions should not therefore be considered pathognomonic for erysipelas. Confirmation requires culture of the organism from the undersurface of skin lesions or from other tissues.

By the time the cutaneous lesions of porcine erysipelas develop, lesions also may be found in *visceral organs*. In addition to *splenic enlargement*, numerous *petechiae may be visible in the renal cortices* and there is sometimes intense interstitial hemorrhage in the medulla. The renal cortical petechiae arise in part from small venules, but mainly from glomeruli. The *characteristic glomerular lesion of erysipelas is focal fibrinoid necrosis of the tufts, with intracapsular hemorrhage*. Less specific changes that might also be present include diffuse glomerulitis, characterized by swelling or proliferation of the glomerular endothelium, and segmental sludging of erythrocytes in some glomerular tufts. The latter lesion probably precedes fibrinoid necrosis and intratubular hemorrhage. Occasionally, gram-positive bacterial colonies are found in the necrotic tufts or in intertubular capillaries, where they are associated with tiny, intense foci of neutrophils.

The articular lesions of acute erysipelas are typically those of *fibrinous polyarthritis* (see above). The volume of synovial fluid is increased and the synovial membrane is hyperemic. In some cases, the synovial arterioles show necrotizing inflammation and extensive plugging by cellular thrombi.

Lesions in other tissues are not specific. There is leukocytosis with many mononuclear cells in hepatic sinusoids. The *lungs show typical lesions of bacteremia*, with organisms sequestered in the pulmonary capillaries, but without the focal localization of embolic pneumonia. The alveolar capillaries are intensely hyperemic and contain numerous sequestered leukocytes. Later, the alveolar walls tend to thicken as a result of cellular infiltration, mononuclear cells predominating, and this is accompanied by alveolar flooding and increased alveolar macrophages. The *skeletal musculature often shows extensive degeneration*, although it may be too recent to be visible grossly. Whether the muscular degeneration is an example of the classic change described as Zenker's degeneration or is mainly due to peripheral arteriolitis is difficult to determine. Of the remaining tissues, the *brain and eyes* appear to harbor lesions most consistently. The organisms localize in the choroid and the ciliary process and provoke an intense neutrophilic inflammatory response. In the brain, there is cerebral leukocytosis and degeneration of blood vessels in the cerebral white matter. Embolic bacterial colonies, together with a tiny surrounding zone of neutrophils and a few eosinophils, may be found in small vessels of the brain and spinal cord. Such foci of inflammation are quite common and are not always associated with visible organisms. The neural lesions do not produce neurological signs.

Chronic erysipelas in swine is characterized by *vegetative valvular endocarditis and/or arthritis*. Localization of the organism in heart valves and joints occurs as a sequel to either acute, nonfatal septicemia or mild, perhaps inapparent, systemic disease. The *mitral valves* are most often involved and pigs that die of endocarditis have congestive heart failure and embolism. *Large infarcts in the spleen and kidneys* are particularly common. The valvular lesions may be very large, consisting of layers of fibrin with variable numbers of trapped inflammatory cells. During the active stage, masses of gram-positive bacteria can be found near the surfaces of the thrombi. In older lesions, the bacterial colonies are buried more deeply in the thrombi and usually cannot be cultured.

Arthritis is a common expression of chronic erysipelas in pigs and may be unassociated with earlier acute or subacute signs of infection. It can be reproduced experimentally by injections of the organism, and also occurs in vaccinated swine. *Although vaccination seems useful in preventing the acute syndrome and mortality, it appears to enhance susceptibility to polyarthritis.* The number of joints affected following experimental inoculation of susceptible pigs depends on the number of organisms used and the strain of organism. In pigs with acute arthritis, organisms may be isolated from grossly normal as well as inflamed joints, but isolation from affected joints may be difficult in the chronic disease. Persistence of the inflammation in such cases may be due to the presence in synovial tissues of bacterial antigens, rather than intact organisms. Antigens persist for up to 18 months in arthritic joints and specific antibodies found in synovial fluid may be produced by plasma cells in the synovial membrane. Culture of the sediment from centrifuged synovial fluid however, usually yields a few organisms, even in very chronic erysipelas.

The *lesions in chronic erysipelas arthritis* vary in severity. In mild cases, there is excess synovial fluid and villus hypertrophy, but the articular capsule may appear normal. In severe cases, there is extensive villus hyperplasia and hypertrophy over much of the synovial membrane (Fig. 1.158A), together with pannus formation and cartilage degeneration. The hypertrophic villi are hyperemic and infiltrated with mononuclear cells, including plasma cells (Fig. 1.158B). *Diskospondylitis* is also a feature of chronic erysipelas.

Acute and chronic arthritis, similar to that caused by *Erysipelothrix rhusiopathiae* infection, also occur in pigs in association with certain other bacterial and mycoplasmal infections and *in many cases, the etiology remains undetermined.* The development of molecular biological techniques capable of demonstrating the presence of small quantities of persistent antigen may improve our diagnostic success with such cases in the future.

Erysipelas in sheep is usually the result of *percutaneous infection*, entry being gained through the *umbilicus, docking and castration wounds, shear wounds, and cuts or abrasions acquired during dipping.* The lesion in sheep may be confined to the skin and subcutis at the point of entry, or there may be bacteremia with localization in joints. Rarely, death may occur from septicemia.

Fibrinopurulent polyarthritis, and osteomyelitis, is the usual form of erysipelas seen in young lambs after docking or castration, and sometimes following umbilical infections. The arthritis is subacute or chronic and the morbidity may be as high as 50%. Mortality is low, and is a consequence of severe lameness rather than a direct result of the infection. The main limb joints are involved. In the early stages, there is synovitis with an increased volume of turbid synovial fluid. Later, the fibrinous exudate may coagulate into firm pads. Articular cartilage is initially unaffected, but foci of osteomyelitis in the subchondral epiphyseal bone may lead to collapse of the overlying articular surface and formation of irregular pits or ulcers with a base of granulation tissue. In the chronic stages, the joints are stiffened and deformed by periarticular fibrosis and by periosteal and perichondral osteophytes.

Cutaneous infections following dipping are associated with contamination of nonbactericidal dips with *E. rhusiopathiae.* Lesions occur most often about the *fetlocks* but invasion may occur wherever the skin is injured and contaminated. Post-dipping infections, which mimic cutaneous erysipelas, are occasionally caused by both *Arcanobacterium pyogenes* and *Corynebacterium pseudotuberculosis.* Cutaneous erysipelas may also occur when sheep are confined in wet, contaminated pens.

Lameness is severe and out of proportion to the gross changes in affected feet. The disease is febrile in some animals and associated with rapid wasting, but recovery occurs in 2–3 weeks. The affected pasterns are hot and painful, and there is regional lymphadenitis. The coronary band may be swollen and the swelling, which is always slight, may extend to the metacarpal or metatarsal regions. The affected areas are progressively depilated. Incision reveals moderate erythema and slight edema of the subcutis.

Histologically, there is acute inflammation very similar to that seen in the cutaneous lesions of porcine erysipelas. There are superficial pustules in the epidermis. In the outer layers of the dermis, there is perivascular edema, accumulation of neutrophils and cellular thrombi within vessels. The reaction is more severe in the deeper layers of the dermis and is characterized by suppurative hydradenitis, necrotizing vasculitis, and vascular thrombosis. Similar changes occur in the *sensory laminae of the foot* and are responsible for the severe lameness. In the course of 2–3 weeks, the inflammatory reaction resolves completely, except for residual lesions in the larger blood vessels.

Streptococcal septicemia and polyarthritis

Streptococci cause a variety of infections in domestic animals, including *septicemia, meningitis, polyarthritis, bronchopneumonia, and endocarditis.* In **swine**, there are well-defined syndromes of streptococcal septicemia, with localization in synovial structures, meninges, and elsewhere, caused by ***Streptococcus suis***. This organism shares cell-wall antigens with streptococci of Lancefield's group D but is not genetically related to them. Over 30 serotypes have now been recognized but those most frequently associated with arthritis are types 1, 2, 7, and 14. *In general,* Streptococcus suis *serotype 1 infects suckling pigs and is not pathogenic for other species, whereas serotype 2 tends to cause disease in weaner and feeder pigs and also cause meningitis and septicemia in man.* Serotype 7 may also have zoonotic significance. Interestingly, some serotype 2 strains cause only nonfatal pneumonia in gnotobiotic pigs, even following dexamethasone treatment. Increased virulence may depend on certain high-molecular-weight, cell-wall proteins that are released by muramidase.

***Streptococcus suis* type 1** may cause disease in piglets from 2 to 10 weeks of age, but most cases occur in the *2–3-week age group.* The disease may occur in one or all pigs in a litter, and a number of litters may succumb over a period of weeks. The infection is initially *bacteremic or septicemic* and death may occur at this stage with little evidence of localization in visceral organs. The spleen is slightly enlarged and purple, petechiae may be present in the renal cortex, and the lungs are hyperemic with small pleural hemorrhages. There may be a slight excess of fluid in serous cavities. When the course of the disease is slightly longer, there are signs of *arthritis and meningitis*. All joints may be affected but the lesions are most obvious in the large limb joints. The synovial fluid is increased in volume, slightly mucinous, and turbid due to the presence of neutrophils. Occasional gram-positive cocci may be seen in smears. The meningitis is fibrinopurulent and affects mainly the basal meninges. Clumps of fibrin may be found floating free in lateral ventricles or attached to choroid plexuses. The cerebral aqueduct and/or foramina of Luschka may be occluded by exudate or by ependymal reaction, leading to internal hydrocephalus.

Streptococcus suis type 1 is most likely *carried in the nasopharynx of sows* and transferred to their offspring shortly after birth. The portal of entry is the palatine tonsil. Piglets infected experimentally may have bacteremia for 2–3 weeks, after which time, the developing immunity eliminates infection from the blood and internal organs. The organism is relatively harmless unless it becomes established in joints or meninges.

Disease caused by ***Streptococcus suis* type 2** may be clinically and pathologically similar, but often the range of lesions is greater. The organism is *carried in the palatine tonsils of pigs*, and *infection is probably by the respiratory route*. In infected herds, it is isolated from up to 80% of clinically normal pigs and is commonly found in nasal turbinates and pneumonic lungs, where it is probably a secondary invader. Limited outbreaks do occur but *sporadic, isolated disease is more common*. The incubation period varies from 1 to 14 days and the clinical course from 4 to 48 h. Affected pigs are usually about 10–14 weeks of age and the most significant lesion is *purulent meningitis*, which, along with *polyserositis*, is visible grossly in about 50% of pigs. The bacteria probably enter the cerebrospinal fluid within monocytes via the choroid plexuses. *Purulent arthritis* occurs in a few animals, usually those at the lower end of the age range. *Fibrinopurulent pericarditis, endocarditis, or hemorrhagic, necrotizing myocarditis* occur in some pigs. Endocarditis most often affects the mitral valve. The myocarditis grossly may resemble mulberry heart disease but *the histologic lesions of necrotizing vasculitis and diffuse inflammation associated with bacteria are distinctive*.

Occasionally, *Streptococcus suis* type 2 causes *septicemia in newborn piglets*. Pigs dying acutely may show few lesions other than marked congestion of lungs and meninges with perhaps a few strands of fibrin adherent to the pleura, but purple discoloration of the skin and other signs of septicemia are usually present. *Streptococcus suis* is occasionally isolated from suppurative lesions in ruminants.

Streptococcus equisimilis also has been isolated from the joints of pigs with purulent arthritis. In experimental infections, other streptococci, such as *Streptococcus pyogenes* (Lancefield's group A) and group C streptococci produce arthritis and osteomyelitis, in swine.

Streptococci also cause *polyarthritis and meningitis* in **calves**. Metastatic iridocyclitis occurs in many bacterial infections in which polyarthritis occurs, but in none is it so consistent or prominent as in the *streptococcal disease of neonatal calves*. The disease is probably secondary to *umbilical infection* in most cases but, because ocular lesions may be visible grossly by 24 hours of life, there is a possibility that some infections are intrauterine. The clinical signs are of *hypopyon, corneal opacity and meningitis* and because of the strong affinity of the organisms for the meninges and eyes, the disease is without a chronic phase. The arthritis is acute and there is no obvious joint swelling.

In **lambs**, *Streptococcus* spp. are probably second only to *Erysipelothrix rhusiopathiae* as a cause of polyarthritis, although in a UK study, *Streptococcus dysgalactiae* was easily the most common isolate from the joints of arthritic lambs. The *umbilicus* is accepted as the likely route of entry in most cases and this is supported by the high prevalence of the disease in sucklings. There is some variation in the combination of lesions seen at necropsy, probably due to differences in the species and strains of organisms involved. Specific typing of *Streptococcus* spp. is not a routine procedure.

Localization of *Streptococcus* spp. in the joints and other organs of lambs is a sequel to bacteremia. Some lambs die acutely of septicemia and show few gross lesions. *Localization* in various tissues occurs in the course of 1–2 days and may involve any one or a combination of sites, including *the uvea, cerebrospinal meninges, valvular endocardium, myocardium, kidneys, and joints*. Meningeal localization seldom occurs in the absence of polyarthritis, but the latter may not be clinically evident in cases with a short clinical course. Polyarthritis may however occur alone. The infection may subside in many joints, persisting only in the larger limb joints and causing chronic lesions of purulent arthritis. In approximately 20% of lambs with subacute or chronic polyarthritis, there is coincident valvular endocarditis.

Coliform polyarthritis

Escherichia coli *often localizes in the joints or meninges (or both) in farm animals, but is a rare cause of arthritis in dogs.* In septicemic colibacillosis of neonatal **calves**, polyarthritis and tenosynovitis are common, but are easily overlooked or overshadowed by other manifestations of acute septicemia, including meningitis and polyserositis. The organism may enter the blood from the gut, oropharynx, or umbilicus. The lesions in calves are similar to those of streptococcal infection although iridocyclitis, with grossly visible hypopyon, occurs less frequently in association with coliform polyarthritis. In some cases, the polyarthritis is chronic, with lesions restricted to one or two of the larger limb joints and tending to be symmetrical. Chronic coliform arthritis in calves is often coincident with interstitial nephritis (white-spotted kidney), which may develop into descending pyelonephritis.

Polyarthritis caused by *Escherichia coli* also occurs commonly in **horses** and **pigs**, but does not appear to be a common isolate from the joints of lambs with polyarthritis.

Staphylococcal arthritis

Staphylococcus aureus *is a relatively common cause of polyarthritis in farm animals and monoarticular arthritis in dogs.* In lambs, *Staphylococcus aureus* may occur as a sporadic infection or as a complication of tick pyemia. In the UK, the latter syndrome occurs in lambs born onto tick-infested ground, particularly during the spring when tick activity is at a peak. The agent gains entry to the blood through bites of the blood-sucking nymphal stage of the tick *Ixodes ricinus*, then localizes in the synovial membrane and various internal organs. Vertebral osteomyelitis is a frequent complication of *Staphylococcus aureus* septicemia in affected lambs, often leading to ataxia or paralysis following collapse of necrotic bone into the spinal canal.

In one study, ***Staphylococcus hyicus*** subspecies *hyicus* was an important cause of fibrinopurulent arthritis in **pigs** up to 12 weeks of age. Lesions were most common in the *elbow and tarsal joints*. The palatine tonsil is considered an important site of entry for this organism in pigs. *Staphylococcus hyicus* subspecies *hyicus* produces *protein A*, which aids in attachment of the bacteria, and *exfoliative factors*, which may promote epithelial erosion and assist in the invasive process. The altered epithelial environment created by *Staphylococcus hyicus* subspecies *hyicus* in tonsillar crypts may also facilitate the entry of other bacteria to the blood or lymph of young pigs.

Staphylococcus spp. were the most common bacteria isolated from the synovial fluid in a study of 19 cases of *septic arthritis* in **dogs**. Interestingly, although all dogs included in the study had increased numbers of neutrophils in their synovial fluid, the *neutrophils were nondegenerate* in all but one case. This reinforces the point that detection of toxic neutrophils in synovial fluid is not a reliable means of differentiating septic arthritis from noninfectious, immune-mediated arthritis in dogs.

Haemophilus and *Histophilus* septicemia and arthritis

Glasser's disease *is a fibrinous meningitis, polyserositis, and/or polyarthritis of* ***pigs****, caused by* ***Haemophilus parasuis***. The organism is part of the normal flora of the upper respiratory tract of conventional pigs but is often isolated from pneumonic lungs and regarded as a secondary invader. It is acquired from the dam shortly after birth but colostral antibodies usually protect young piglets against disease. Active immunity develops by 7–8 weeks. *Haemophilus parasuis* occasionally produces severe outbreaks of disease in animals aged around 5–12 weeks, usually following transportation or other management stress. Cesarian-derived pigs are susceptible at any age and often develop Glasser's disease after introduction to a boar test-station or a conventional herd.

Glasser's disease is defined on an etiological basis, as there are other organisms, such as *Streptococcus suis*, which produce similar lesions and similar diseases. *Mycoplasma* spp. also produce serositis and arthritis in swine but the diseases are more chronic, and meningitis is either absent or lymphocytic, depending on the species of mycoplasma involved.

Glasser's disease is peracute, with high fever, lameness, and neurological disturbances, including paresis, stupor, and hyperesthesia. As in other septicemic diseases of pigs, the skin may show purple discoloration. The course is 1–2 days and without treatment, the mortality rate is very high. Usually, *serofibrinous meningitis, pericarditis, pleuritis, peritonitis, and synovitis of many joints* characterize the morbid picture. In individual cases, all these tissues, or any combination of them, may be inflamed. Occasionally the lesions occur in only one site and, in some pigs or some outbreaks, gross lesions are absent. *Predilection sites* for lesions of Glasser's disease are *meninges, joints, peritoneum, pleura, and pericardium in descending order*. Meningitis, which is more severe in the cranial than the spinal meninges, occurs in more than 80% of affected pigs. *Polyarthritis is most severe in the atlanto-occipital and large limb joints*. The synovial fluid is increased in volume and turbid due to increased numbers of neutrophils. The *synovitis* is characterized by the presence of gray-yellow fibrin, which covers the membrane or accumulates as a meniscus-like pad between articular surfaces. The *gastric fundic mucosa* is often intensely reddened due to venous infarction, a change that accompanies septicemia of several causes in pigs.

The *microscopic lesions* of *Haemophilus parasuis* infection in pigs are those of septicemia and fibrinous inflammation. Thrombosis of vessels in the skin, meninges, and renal glomeruli is often prominent. The organism can best be cultured from visceral pleura, providing the interval from death to postmortem examination is relatively short. It is seldom isolated from other sites.

Histophilus somni, previously known as ***Haemophilus agni***, ***Histophilus ovis***, or ***Haemophilus somnus***, produces an acute, fulminating, septicemic disease in **lambs** and a more chronic disease in older sheep. The disease appears to be associated with high levels of feeding but the reason for this association is unclear. Little is known of the biology of the organism, or the pathogenesis of the infection, but *H. somni* is a commensal of the genital tract of sheep and could be transmitted to newborn lambs during parturition.

Lambs with *H. somni* septicemia are usually *found dead*, the course of the disease being less than 12 h. Clinically, there is fever, depression, and extreme reluctance to move. Animals that survive for more than 24 h develop *fibrinopurulent arthritis, choroiditis, and basilar meningitis*. The disease tends to remain enzootic in a flock and losses may continue for several months. *The most constant postmortem findings are multiple hemorrhages throughout the carcass*. The hemorrhages vary in severity, but are usually most obvious in lambs dying acutely. The intermuscular connective tissue is wet and slightly stained with blood. The large blotchy hemorrhages of the serosa lack specificity, but *tiny streaks of hemorrhage that are quite diagnostic can usually be seen in the muscles*. They are best appreciated by viewing the intact muscle through the perimysium, and are usually most common near the tendinous attachments. In all cases except the most acute, small foci of necrosis, surrounded by a red halo, are present in the liver. The spleen is enlarged, the pulp soft and juicy. The *absence of pulmonary lesions* usually allows differentiation of this infection from *Mannheimia haemolytica* septicemia, which produces similar hepatic lesions. In *mature animals*, a more extended course is usual and lesions include myocarditis, embolic nephritis, and meningoencephalitis, similar to the lesions of *H. somni* infection in cattle.

Histologically, there is evidence of *overwhelming intravascular bacterial multiplication*. Blood vessels in many tissues are plugged with bacteria and some of the emboli give rise to *acute vasculitis and secondary infarctions*. All tissues share to some extent in this reaction, but there is preferential involvement of liver and muscle. In these organs, there is often severe inflammation in the parenchyma adjacent to the damaged vessels.

In spite of the fact that the tissues often teem with the organisms, bacteriological confirmation may not be obtained unless samples are taken very soon after death and cultured in media enriched with blood or tissue fragments, and preferably in an atmosphere of 10% carbon dioxide. Even so, previous antibiotic treatment significantly impairs isolation of this organism.

Acute serofibrinous arthritis involving one or two joints, usually including the atlanto-occipital joint, occurs regularly in **cattle** with meningoencephalitis caused by ***H. somni***. These animals die suddenly, usually have hemorrhagic necrosis in the brain, and yield *H. somni* on culture (see Vol. 1, Nervous system).

Borreliosis

Borrelia burgdorferi was first recognized in 1975 as a cause of a multisystemic disease (*Lyme disease, Lyme borreliosis*) in man. More recently, it has been associated with disease in domestic animals, including *dogs, horses, cattle, and cats*. In all species, *infection is far more common than disease*, but the host–bacterium interactions responsible are not well defined. *Arthritis is a common feature of disease in animals and human patients with borreliosis*. Other lesions include *myocarditis and nephritis in dogs, ocular disease and probably encephalitis in horses, and abortion in cattle.*

In North America, the three-host tick *Ixodes dammini* is the primary vector for *B. burgdorferi*. Tick eggs are deposited by engorged females in summer and hatch in about 1 month. Larvae engorge for a few days in the period May–September and about 2 months later molt to the nymphal stage. In the following spring–summer period they feed, drop off and molt to the adult stage. This 2-year cycle may be extended to 4 years since unfed larvae and nymphs may overwinter. Larvae and nymphs feed mainly on birds and small mammals in North America, *Peromyscus* spp. (white-footed mouse and deer mouse) are the main mammalian hosts. The adults attach mainly to large mammals, particularly *Odocoileus virginianus*, the white-tailed deer, but raccoons, dogs, horses, cattle, and people are among the potential victims. *Borrelia infection in the principal wildlife hosts is essentially asymptomatic.* Similar epidemiological patterns involving ticks of the *Ixodes ricinus* group and various animal hosts exist in other parts of the world. In North America, *B. burgdorferi* has been isolated from deer flies and horse flies, which are potentially effective mechanical vectors, and from mosquitoes, which are likely less important. Such vectors are significant only in areas where efficient tick vectors maintain infection among wildlife populations.

Both transovarial and trans-stadial transmission of *B. burgdorferi* occur, but only the latter is epizootiologically important. Intrastadial and interstadial transmission, which occur when several larvae and nymphs feed on an infected host, is of greatest significance in the spread of infection. *Transmission of infection to large mammals is usually by the bite of an adult tick and requires attachment for about 24 h.* The organism may be present in the urine of *Peromyscus* spp. and cattle, but the prevalence of infection by direct contact is not known.

The most common clinical signs in **dogs** with borreliosis are anorexia, lethargy, and lameness in association with fever and lymphadenopathy. Severe depression occurs in some cases and may be due to meningitis. The *arthritis is typically intermittent and involves one or more joints*, often including the carpus. Detailed pathologic descriptions are unavailable since antibiotic treatment appears to be successful and experimental reproduction of disease in dogs is not. In people, the arthritis may be recurrent, leading eventually to ulceration of cartilage. In experimentally infected Lewis rats, acute exudative arthritis, tendonitis and bursitis occur by 30 days postinfection, then regress, but exacerbations develop in some animals. Synovial, but not cartilaginous, ulceration occurs in rats, and there is villus hyperplasia with lymphoplasmacytic and macrophage infiltrates in the synovial membranes. Lymphoplasmacytic myocarditis also occurs in rats, and nonsuppurative myocarditis develops in a small proportion of affected dogs. Clinical evidence of renal disease is also seen in a few dogs and organisms can be demonstrated in the kidney, but the lesions are poorly defined.

In **horses**, signs of borreliosis include lethargy, low-grade fever, painful, swollen joints, and reluctance to move. Laminitis and panuveitis may also occur but are less common. The organism has been demonstrated in anterior chamber fluid and a probable case of encephalitis caused by *B. burgdorferi* is recorded in a horse. Few necropsies on affected horses are reported but it appears that arthritis is present and resembles that seen in dogs, with the addition of marked thickening and hyalinization of arterioles in synovial tissues.

Although there are few reports in **cattle,** it appears that the disease in this species is associated with *abortion*, and may develop into a chronic illness with relapses following treatment, eventually leading to emaciation. In other respects, the disease resembles that seen in dogs and horses. *Arthritis is common and there is limited evidence of myocarditis and nephritis.* In people, a characteristic skin lesion, *erythema chronicum migrans*, may develop at the site of the tick bite, and represents the first stage of Lyme disease. Similar areas of discoloration have been described on bovine mammary skin.

Borrelia burgdorferi has been isolated from blood, urine, colostrum, and synovial fluid of cows, and the blood of a newborn calf. Antibodies in the blood of an aborted fetus suggest that the organism may cross the placenta. Diagnosis of borreliosis is made to some extent by exclusion. *Borrelia burgdorferi* may be demonstrable by *dark-field or phase-contrast microscopy in urine or synovial fluid* and by *silver, immunoperoxidase or immunofluorescent stains in synovial membranes or other tissues*. Isolation of the organism may be possible by using special media. Often the diagnosis is based on typical signs in an animal with a high titer and potential or known exposure to the organism. There is serological cross-reactivity between *B. burgdorferi* and some other spirochetes, but apparently not with leptospires.

Rickettsial polyarthritis

Ehrlichiosis has occasionally been associated with polyarthritis in **dogs**. The syndrome is characterized by acute-onset fever with pain and swelling in one or more joints of the limbs. The synovial fluid has an increased protein concentration and a high cell count, of which 60–80% are neutrophils. *Morulae are occasionally observed within the cytoplasm of neutrophils, eosinophils, or monocytes in synovial fluid, and in circulation.* This differs from *Ehrlichia canis* infection, in which morulae typically are found in monocytes and lymphocytes. Although *Ehrlichia canis* titers are generally positive in affected dogs, the strain of the organism involved in *canine polyarthritis* has been tentatively named ***Ehrlichia ewingii***.

Chlamydial polyarthritis

The genera ***Chlamydia*** and ***Chlamydophila*** contain several species of obligate intracellular gram-negative bacteria characterized by the absence of cell-wall peptidoglycans. The former *Chlamydia psittaci* has been redefined as the *Chlamydophila* species: *C. abortus* (mammals), *C. psittaci* (birds), *C. felis* (cats), and *C. caviae* (guinea pigs). *Chlamydia pecorum* is renamed *Chlamydophila pecorum*. The former *Chlamydia trachomatis* has been subdivided to include the *Chlamydia* species: *C. trachomatis*, which infects humans, and *C. suis*, which infects swine.

- ***Chlamydophila abortus*** is an important cause of abortion in several species, including goats, cattle, and sheep, in which it is responsible for *"enzootic" abortion of ewes* in the United Kingdom.
- ***Chlamydophila psittaci*** is well recognized as an infectious agent of birds with zoonotic potential.
- ***Chlamydophila felis*** causes conjunctivitis and rhinitis in cats.
- Only ***Chlamydophila pecorum*** is currently recognized as a cause of arthritis in domestic animals.

Sporadic or epidemic C. pecorum polyarthritis occurs in calves and lambs, and occasionally in other animals. The natural habitat of chlamydiae is the intestinal tract, where the organism multiplies in the mucosal epithelium before entering the lamina propria. Nonvirulent strains rarely progress beyond the mesenteric nodes. Following oral exposure of calves to virulent strains, the chlamydiae spread to the liver and mesenteric nodes via blood vessels and lymphatics of the lower small intestine. Multiplication in these sites is followed by a primary, low-level chlamydemia with localization and multiplication in spleen, liver, lungs, and kidneys. The joints are infected during a secondary chlamydemia, and arthritogenic strains produce their most severe effects in synovial membranes, with clinical arthritis developing about 10 days after oral inoculation. Severe watery or bloody diarrhea often occurs a few days prior to the development of arthritis.

The disease in calves is severe, causing a high mortality rate both naturally and experimentally. Affected calves may be weak at birth, suggesting intrauterine infection. In these animals, fever, anorexia, reluctance to stand or move, and swelling of joints develop in 2–3 days and death occurs 2 days to 2 weeks after the onset of signs. *All or many joints are affected*, those of the limbs most severely. The subcutaneous and adjacent periarticular tissues are edematous with clear fluid, which also extends around tendon sheaths. Surrounding muscles are hyperemic and edematous with petechiae in the fascia. Joint cavities are distended with turbid yellow-gray fluid, and strands or wads of fibrin adhere to the synovium. Viscera may show changes attributable to systemic infection.

The ovine syndrome is less severe, being characterized by high morbidity but negligible mortality, even though the infection is systemic. Affected lambs show conjunctivitis, depression, anorexia, reluctance to move, joint stiffness that disappears with exercise, and loss of weight. Most lambs recover, but a few are permanently lame. *The articular lesions are similar to those in calves although modified by the milder reaction*, the longer course allowing fibrotic thickening of tendon sheaths and articular capsules and hyperplasia of synovial villi. In soft tissues, including the central nervous system, histologic traces of inflammation are present. The organism is sometimes demonstrable in the cytoplasm of synovial cells stained by the Giemsa, Gimenez, or modified Stamp methods.

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Mycoplasmal arthritis

Mycoplasmas are ubiquitous organisms, which are difficult to isolate, propagate, and identify, and often difficult to associate with specific diseases. Polyserositis and polyarthritis produced by these organisms are problems of considerable magnitude in farm animals, and often complicate mycoplasma ("enzootic") pneumonia or other disease syndromes. The diseases are seldom fatal, but produce a lingering debility from which animals never completely recover. Because of the difficulty in isolating mycoplasmas from routine diagnostic specimens, the prevalence of diseases caused by these agents is probably underestimated.

Swine

Three *Mycoplasma* species are pathogenic for swine:

- *Mycoplasma hyopneumoniae (suipneumoniae)* causes enzootic pneumonia;
- *Mycoplasma hyorhinis* causes polyserositis and *polyarthritis*, usually in animals about 3–10 weeks of age;
- *Mycoplasma hyosynoviae* also causes *polyarthritis*, but not polyserositis, and usually in pigs over 10 weeks of age.

Other mycoplasmas are sometimes associated with various diseases of swine but they are not primary pathogens.

Mycoplasma hyorhinis *is a commensal or parasite of the nasal passages in most pigs*, but often colonizes pneumonic lesions, especially those of mycoplasmal pneumonia. Although it does not noticeably alter the lung lesion, infected lungs might be the primary site for entry of the agent into the blood. The pathogenicity of *M. hyorhinis* is enhanced by intercurrent disease and various forms of stress. After an incubation period of 3–4 days, the infection produces an erratic febrile reaction, anorexia, depression, and loss of body weight. Growth rate remains retarded, although after a month or so there may be signs of recovery. The infection is more likely to be fatal in suckling piglets or those with intercurrent pneumonia.

Mycoplasma hyorhinis has a predilection for collagen-containing structures, including serous and synovial membranes and cartilage, but evidence of meningitis is either absent or mild. When present, the meningitis is lymphocytic in nature. The systemic infection persists for at least 2 months after experimental inoculation. *The serosal lesions may vary and any combination of epicarditis, pleuritis, and/or peritonitis is possible.* The serosal reaction is serofibrinous, and the cellular component is mononuclear with focal accumulations of neutrophils. The epicardium is uniformly affected, whereas pleuritis may be confined to the ventral margin of the lung and the pleura between lobes, and may extend to the outer surface of the pericardial sac. The peritoneal exudate is patchy and may be concentrated in the anterior abdomen, especially between the liver and diaphragm, but often involves loops of intestine and the coiled colon. Progressive organization of exudates produces tough adhesions, but even after many weeks there is still histologic evidence of active inflammation, which is consistent with the persistence of the organism.

While the effects of polyserositis predominate early in the disease, signs of *polyarthritis* become evident once villus hypertrophy, synovial thickening, and organization of fibrinous exudate replace the initial synovial edema and hyperemia. In the chronic disease, thickening and brown discoloration of joint capsules, erosion and discoloration of articular cartilages, and pannus formation are observed. Discoloration of joint structures is probably a sequel to synovial hemorrhage.

Early *microscopic changes* consist of hyperemia, diffuse lymphocyte and macrophage infiltration, and mild villus hypertrophy, followed by ulceration of synovial membranes. Fibrinopurulent exudate is consistently present in virtually all joints, and is most abundant in the recesses where the synovial membrane is reflected from the articular cartilage. Later, perivascular lymphoplasmacytic accumulations and more pronounced villus hypertrophy become evident and fibrinous exudate is less prominent. Early in experimental infections, mycoplasmas invade the lacunae of superficial chondrocytes, and degeneration of chondrocytes combined with the enzymatic activity of adherent neutrophils produces erosions of articular cartilage.

In chronic *Mycoplasma hyorhinis* synovitis, there is marked villus hypertrophy and proliferation of synovial cells. Perivascular cuffs are less prominent, but *lymphoid follicles*, often surrounded by lymphatics, occur frequently in the villi. Dense fibrosis of some villi is evident. Erosions, developing first at the periphery of the articular cartilages, later occur centrally, and *erosion of cartilage and subarticular bone by pannus* is not unusual in advanced cases. Joint contractures are associated with the chronic arthritis and thickened joint capsules. Chronic lesions remain active for many months, and the organism can often be cultured from surface exudate in these joints.

Mycoplasma hyosynoviae is carried persistently in the pharynx and tonsils of many sows and may be transmitted to their offspring. Infection of pigs less than 6 weeks of age is uncommon and separation of piglets from their dams by 5 weeks of age usually prevents transmission. Clinical disease often develops following management stress and is more frequent, and more severe, in pigs with poor conformation and gait.

Following experimental infection with *Mycoplasma hyosynoviae*, there is an incubation period of 5–10 days. Lameness, lasting 3–10 days, occurs in many pigs and may involve one joint or several. *Many pigs recover completely but lameness due to chronic arthritis persists in 5–15% of infected swine.* The early pathologic changes resemble those caused by *Mycoplasma hyorhinis*. Chronic lesions are not fully described but are also likely to be qualitatively similar to those of *Mycoplasma hyorhinis*.

Failure to isolate organisms from the joints of many pigs with *chronic mycoplasma arthritis* may be due to difficulties in culturing the organism. Alternatively, the continued inflammatory reaction may be due to an immune response to persisting mycoplasma antigen, rather than a viable agent. Support for this view is derived from the observation that rabbits sensitized to *Mycoplasma arthritidis*, either by infection or hyperimmunization, develop chronic arthritis when challenged with nonviable *Mycoplasma arthritidis* antigen. Antibody in the synovial fluid of sensitized animals could retain antigen in immune complexes. These complexes may become deposited in synovial tissues and articular cartilage and stimulate a cell-mediated immune response with generation of cytokines capable of inducing tissue damage. The nature of the putative antigens is undetermined. Since mycoplasmas lack a cell wall and cannot synthesize peptidoglycans, the antigen must differ from the antigens responsible for inducing chronicity in some bacterial arthritides. Whether this hypothesis is correct, or relevant to the natural disease, is undetermined.

Goats

Mycoplasma spp. cause several important disease syndromes in goats, including contagious caprine pleuropneumonia (see Vol. 2, Respiratory system) and contagious agalactia (see Vol. 3, Male genital system). A **septicemia-arthritis** syndrome caused by either ***Mycoplasma mycoides* subsp. *mycoides*** or ***Mycoplasma capricolum* subsp. *capricolum*** occurs in goats. *Mycoplasma mycoides* subsp. *mycoides* is separable into two groups on the basis of colony type (large and small). *Mycoplasma mycoides* subsp. *mycoides* (small colony) type is the etiologic agent of contagious bovine pleuropneumonia and should not be confused with *Mycoplasma mycoides* subsp. *mycoides* (large colony) type, which is a common infection of goats in several countries.

Mycoplasma putrifaciens occasionally causes polyarthritis and mastitis in goats. *Mycoplasma agalactiae*, the cause of contagious agalactia, also may be associated with arthritis following extension of infection from periarticular connective tissues.

The **septicemia-arthritis syndrome in goats** is acutely febrile and associated with a drop in milk yield due to *mastitis*. In the course of about 2 days, affected animals develop *acute polyarthritis*, but in suckling kids, the disease is peracute and localization in joints is less obvious. During the *systemic phase of the infection*, there is a generalized acute lymphadenitis, splenitis, histiocytic meningitis, glomerulitis, renal tubular degeneration, and focal coagulative necrosis in the liver. Fibrinous peritonitis and pleuritis are sometimes present. Capillaries in many organs may contain thrombi, probably reflecting disseminated intravascular coagulation. Diffuse interstitial pneumonia characterized by infiltration of alveolar walls with neutrophils or monocytes, and leakage of protein-rich fluid into alveoli, develops in kids. In adults, the pneumonia tends to be less diffuse and more chronic. *The articular lesion is a severe fibrinopurulent inflammation, usually involving many synovial structures.*

The epidemiology of the septicemia-arthritis syndrome is incompletely understood. Some do probably acquire *Mycoplasma capricolum* subsp. *capricolum* via the teat canal and infection can spread to the opposite gland. Some kids are probably infected via milk but horizontal transmission of both *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma mycoides* subsp. *mycoides* (large colony) type occurs among young kids housed together. An outbreak of septicemia and fibrinous polyarthritis caused by *Mycoplasma mycoides* subsp. *mycoides* (large colony type) occurred in New Zealand in 3–20 day old dairy goat kids fed pooled colostrum contaminated with organisms by a doe with mastitis. In total, 34 of 64 kids were affected and 3 died.

Sheep

Septicemia and arthritis caused by *Mycoplasma capricolum* subsp. *capricolum*, or a closely related organism, is reported in **lambs** exposed to goats' milk. The disease was similar to that described in kids.

Cattle

Mycoplasma mycoides subsp. *mycoides* (small colony) type, the agent of **contagious bovine pleuropneumonia**, causes *polyarthritis and endocarditis* in a proportion of **calves** in which it is used as a vaccine. Sporadic outbreaks of polyarthritis in cattle, not associated with *Mycoplasma mycoides* subsp. *mycoides* vaccination also occur in North America and Australia and are probably caused by *Mycoplasma bovis*. This organism also causes mastitis, with or without arthritis, and is often isolated from bovine fibrinous pneumonia where its significance is poorly understood. *Mycoplasma alkalescens, Mycoplasma californicum,* and *Mycoplasma canadense* have all been associated with arthritis and mastitis in cattle. Although *Mycoplasma mycoides* subsp. *mycoides* (large colony) type is not considered to be pathogenic for cattle, an outbreak of arthritis caused by this agent was recently reported in artificially reared dairy calves in New Zealand. This was the result of feeding contaminated goats' milk from a dairy goat farm where an outbreak of arthritis was also occurring in goat kids. No lateral spread of infection occurred amongst the calves.

Mycoplasma bovis probably enters the circulation *from the lungs* and localizes in synovial membranes. Oral infection of calves from the milk of a mastitic dam may also occur. Outbreaks of disease usually occur in *feedlots* and are often preceded by management stress. Usually 15% of animals show signs of lameness, followed within 1 or 2 days by severe swellings around joints. The *stifle, shoulder, hock, and elbow joints are most often affected*, but severe lesions usually develop in only one limb.

In *acute disease*, the joints are distended by opaque cream-colored fluid containing flakes of fibrin. Synovial membranes are red and swollen. Microscopic changes include light to diffuse infiltrations of lymphocytes, macrophages, and plasma cells in addition to hyperplasia of synovial cells and synovial villi. Focal perivascular necrosis and ulceration of the synovial membrane may be present and attract numerous neutrophils, which accumulate in joint spaces.

Most affected animals recover in 1–2 months but a few develop *chronic arthritis* and are culled. These animals have a *proliferative and erosive arthritis with well-vascularized pannus* extending from the thickened synovial membrane across the articular cartilage. Spread of inflammation to the subchondral bone with development of *osteomyelitis* occurs in some animals. The predominant cells in the chronic synovial lesions are lymphocytes and plasmacytes, with fewer neutrophils and macrophages. Isolation of mycoplasmas is relatively easy in the acute disease but is often more difficult in chronic stages.

Cats and dogs

Occasionally, mycoplasma arthritis occurs in cats and dogs. ***Mycoplasma gateae*** is reported as a cause of polyarthritis and tenosynovitis in cats, and ***Mycoplasma felis*** was recovered from the joint of a cat with suspected immune deficiency. ***Mycoplasma spumans*** was isolated from a Greyhound with polyarthritis and is considered a possible cause of the infectious arthritis seen in that breed in Australia.

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Other infectious agents

Viral arthritis

Few viral agents have been incriminated either directly or indirectly with arthritis in domestic animals.

Goats

The best-characterized example of viral arthritis in animals is **caprine arthritis-encephalitis**. This syndrome is caused by persistent infection with a *nononcogenic retrovirus (lentivirus)*. The neurologic, mammary, respiratory and systemic features are described in Vol. 1,

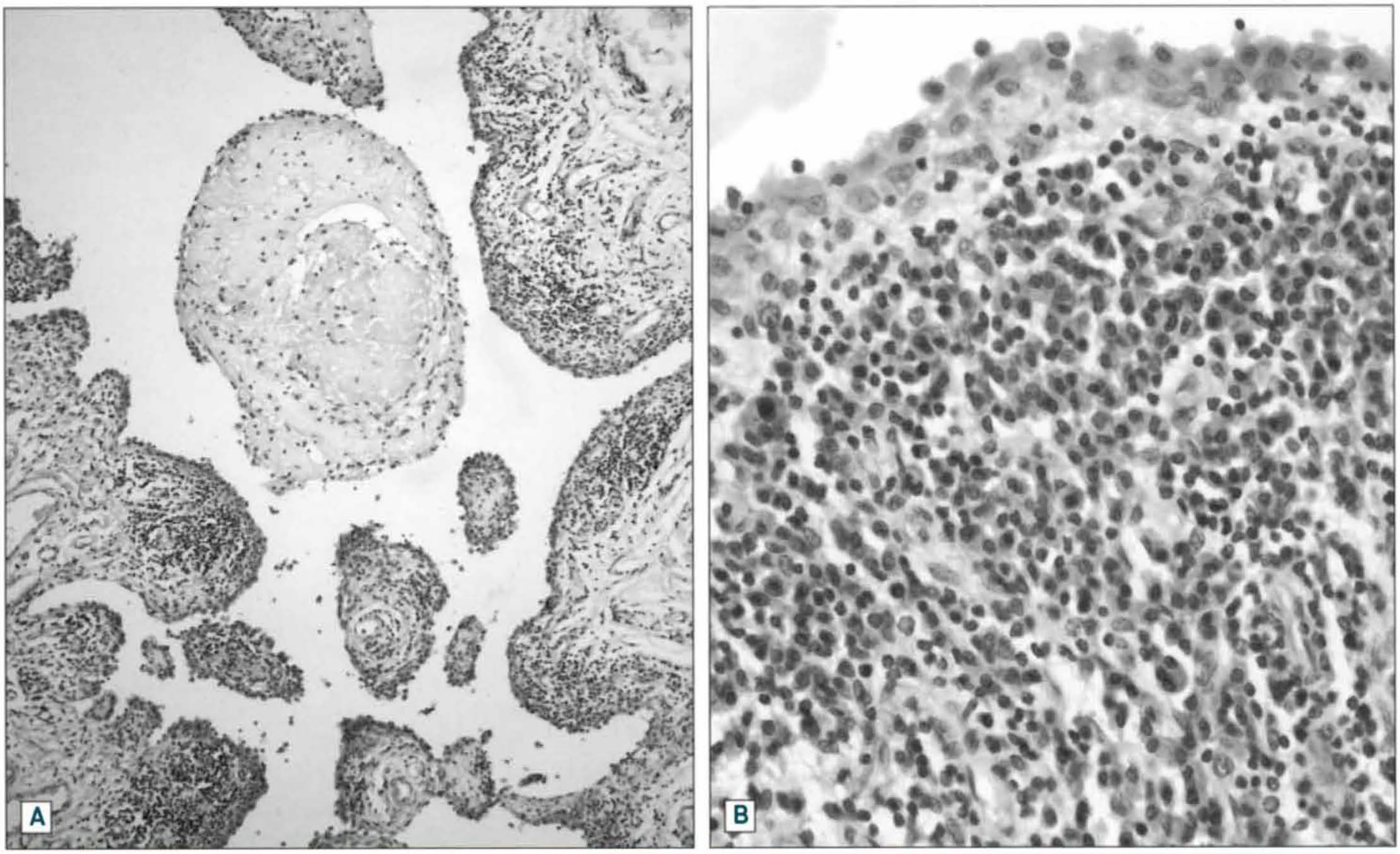

Figure 1.159 Caprine arthritis-encephalitis **A.** Hypertrophic, edematous synovial villi containing aggregates of mononuclear cells. **B.** Closer view showing intense plasmacytic infiltration of the synovium and hypertrophy of synoviocytes on the surface.

Nervous system. *In many herds, arthritis is the major or only clinical manifestation of infection*, with signs of lameness often associated with carpal hygromas, weight loss, and reduced milk production. The prevalence of infection in a herd may reach 100%, but expression of disease is variable and rarely exceeds 25–30%.

A high prevalence of unilateral or bilateral carpal hygromas is a characteristic clinical feature of caprine arthritis-encephalitis. The hygromas are chronic lesions that appear as flattened, cystic, subcutaneous distensions over the cranial carpus, and are filled with yellow or bloody fluid, often containing fibrinous or gelatinous masses. Usually, there is no communication with the carpal joint or tendon sheaths. The tendon sheaths and joint capsule are thickened by fibrous tissue, in which collagen degeneration and mineralization may occur. In many cases, the major joints, especially carpus and stifle, are distended with clear yellow fluid. Other gross changes, which are also seen most often in the carpus and stifle, include hypertrophy of synovial villi, fibrillation and erosion of cartilage, and destruction of joint structures by pannus. Lymphocytic/plasmacytic arthritis is demonstrable microscopically in many joints.

Caprine arthritis encephalitis virus is readily transmitted to kids in colostrum and milk, and horizontal transmission probably occurs. Although the colostrum may contain antibody this does not influence viral transmission. In blood, the virus is present in monocytes and is activated when they mature into macrophages. Following infection, there is vascular injury to synovial structures, with exudation of plasma proteins into synovial fluid. Synovial villi hypertrophy, become edematous and accumulate focal and diffuse infiltrations of plasma cells and lymphocytes (Fig. 1.159A, B) and scattered macrophages, which are sometimes multinucleated. Some villi may be fibrosed, and there is hyperplasia of synoviocytes on their surfaces. Hyalinized masses of fibrin also form villus-like structures in inflamed joints, and layers of fibrin may cover the synovial membrane in some areas.

Sheep

A related lentivirus of sheep, the ***Visna/maedi virus***, causes *chronic, nonsuppurative arthritis* in this species, and also produces arthritis in goat kids experimentally. Similarly, the *Caprine arthritis encephalitis virus* induces arthritis experimentally in lambs but natural cross infections are not known to occur.

In *lentivirus arthritis*, macrophages and lymphocytes are the predominant cells in synovial fluid. A small number of the macrophages are infected with the virus but there is a reduction in virus gene expression, possibly due to the presence of interferon in the synovial fluid. Plasma cells in the inflamed synovia produce immunoglobulin G_1, presumably in response to persistent infection, and the concentration in synovia is higher than that in serum. The relevance of this to lesion development is unclear since the predominant lymphocytes in the synovia are T cells and there is some suggestion that interaction between these cells and macrophages may be responsible for the chronic inflammatory response.

Cats

Lameness caused by acute arthritis is reported in cats in association with ***Feline calicivirus*** infection, or 5–7 days after live-virus vaccination. In one study, intra-articular inoculation or contact exposure of cats to a field strain of *Feline calicivirus* resulted in acute arthritis in addition to oral and nasal ulceration, conjunctivitis, and ocular discharge, which are more typical of the infection. Only mild lameness was induced by the vaccine strain. The gross and microscopic changes have not been well characterized, at least in natural infections, although viral antigen has been demonstrated in the supporting and lining cells of the synovial membrane by immunostaining.

Fungal arthritis

Arthritis involving one or more joints occurs in some dogs with systemic fungal infections. In some cases, the arthritis is probably *immune-mediated*, following the deposition of immune complexes in the synovial membrane, but *hematogenous localization* of fungal agents in joints, or *direct spread from osteomyelitic lesions* in adjacent bones also occurs. Fungi identified in arthritic joints of dogs have included *Blastomyces, Histoplasma, Coccidioides, Cryptococcus,* and *Sporothrix* species. Radiography may reveal a destructive process in a bone or bones adjacent to the joint and erosive changes in articular cartilage. The causative agent is sometimes evident on cytological examination of synovial fluid obtained by arthrocentesis. Histologically, the synovial inflammation is usually *pyogranulomatous*.

Protozoal arthritis

Proliferative synovitis may be associated with *visceral leishmaniasis* caused by the protozoal agent *Leishmania donovani*. Synovial villi in affected joints are hypertrophic and infiltrated with plasma cells and macrophages, some of which may contain the organism. The agent is occasionally demonstrated in macrophages collected from synovial fluid.

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Miscellaneous inflammatory lesions of joint structures

Bursitis

Bursitis often occurs as part of the synovial localization of *hematogenous* infections. In addition, there are *isolated forms* of bursitis, especially in horses, that appear to be initiated by *trauma* and are characterized by accumulation of excess serous fluid and variable degrees of synovial hypertrophy. They represent loci of lowered resistance and are predisposed to the localization of organisms, which may alter the nature of the inflammatory reaction while contributing to its progression. *The bursae that cover bony prominences are especially liable to traumatic injury*. Capped elbow and capped hock are examples of *serous bursitis*. Carpal bursitis is also known as *hygroma* and is not uncommon in cattle and sheep. The distended bursae are sometimes filled with fibrin concretions (Fig. 1.160). In cattle, large hygromas should be suspected of being secondarily infected with *Brucella abortus*. Carpal hygromas are also common in adult goats infected with *Caprine arthritis encephalitis virus*.

"Fistulous withers" and "poll evil" are the most serious forms of bursitis in horses and mules, affecting the supraspinous and atlantal bursae respectively. The affected bursae are those between the nuchal ligament and the second, or less often, the third, fourth, or fifth, thoracic spine in the case of "fistulous withers," and between the nuchal ligament and the dorsal arch of the atlas in the case of "poll evil." In both syndromes, the inflammation is *suppurative and granulomatous*, and there

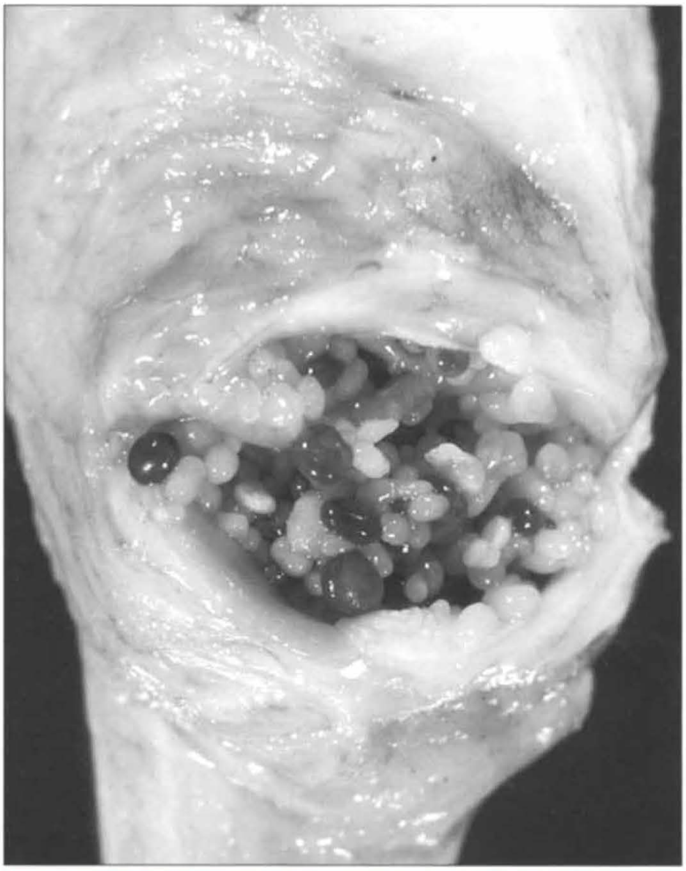

Figure 1.160 Chronic carpal bursitis in a sheep. Thickened fibrous wall surrounding numerous fibrin concretions.

is a tendency to fistulate to the skin surface. Less commonly, the inflammation extends into adjacent fibrous connective tissues as a cellulitis, or induces destructive osteitis of adjacent bones. Inflammation of the navicular bursa is discussed with navicular syndrome.

The primary lesions of "fistulous withers" and "poll evil" may be either *traumatic or inflammatory*. The trauma may be caused by a penetrating wound, with introduction of infection, or blunt injury, producing contusions in which organisms localize hematogenously. Inflammatory lesions are associated with a variety of organisms, including the nematode *Onchocerca cervicalis,* which localizes in the nuchal ligament and environs, provoking mineralized granulomas when it dies. However, in areas where the prevalence of *O. cervicalis* infection is high, the prevalence of bursitis is not, and *the etiologic role of the parasite is questionable*. In brucellosis-endemic areas, *Brucella abortus* is often isolated from closed lesions of bursitis, and vaccination is considered a useful method of treatment. *Actinomyces bovis*, a common component of the normal bovine oral flora, sometimes can be cultured from closed lesions in horses, and exposure to cattle is considered a predisposing factor. In North America, and other locations where the incidence of brucellosis and the exposure of horses to cattle are declining, isolation of *Brucella abortus* and *Actinomyces bovis* from equine bursitis has also waned. *Streptococcus zooepidemicus* is now the most common isolate from closed lesions but several other bacteria have also been implicated. The infections are *probably hematogenous*, but it is not clear whether a predisposing bursal lesion is required. *Actinomyces bovis,* in particular, is an unlikely primary pathogen, since in its own niche it is an uncommon opportunistic invader of traumatized oral mucosa. Furthermore, it does not establish itself in regional lymph nodes draining the lesions of osteomyelitis in which it flourishes.

Adventitious bursitis of the hock is common in finishing pigs, particularly those kept on slatted floors. A study conducted on 21 properties in south-west England revealed an overall prevalence of 51.0% with a range of 10.1 to 84.0%. The bursae are typically found immediately distal to the hock on the lateroplantar, plantar, and medial aspects of the joint. The lesions are thought to arise through traumatic injury to lymphatic vessels and capillaries, followed by the accumulation of fibrinous exudate within affected bursae.

Diskospondylitis

Diskospondylitis is inflammation of an intervertebral disk leading to osteomyelitis of contiguous vertebrae. It occurs occasionally in dogs and pigs, and less often in cats, horses, cattle, and sheep. Most cases are caused by bacteria, usually as a result of primary bacteremia, but also secondary to a chronic infection elsewhere in the body. Fungi, such as *Aspergillus terreus*, are occasionally involved in dogs.

In **dogs**, diskospondylitis occurs most often in *large males* and usually involves the *lumbosacral spine*, causing hyperesthesia, stilted gait, and/or pelvic limb lameness. *Staphylococcus aureus* is the most common cause, although several other bacteria have been incriminated and *Brucella canis* should be considered in countries or regions where it is known to occur. Often, there is concurrent disease, such as a urogenital infection. Diskospondylitis and osteomyelitis of lumbar vertebrae has been associated with migrating grass seeds in dogs. It is suggested that ingested seeds occasionally penetrate the intestinal wall at the caudal duodenal flexure and ascend in the mesoduodenum to the lumbar vertebrae, carrying bacteria with them.

In **pigs**, most lesions occur in the *upper thoracic and upper lumbar spine* following *hematogenous* localization of bacteria. *Erysipelothrix rhusiopathiae, Arcanobacterium pyogenes,* and *Staphylococcus aureus* are the most common agents involved but, in some geographic areas, *Brucella suis* may be an important cause of the disease. Diskospondylitis in **horses** most often involves the cervical vertebrae, inducing signs of neck pain. In some cases, there is a history of a penetrating wound to the neck. Medical or surgical therapy is often successful in dogs but seldom in horses.

Organisms causing diskospondylitis probably localize initially in the outer part of the annulus fibrosus, where they stimulate an inflammatory reaction. Alternatively, localization in the vertebral body with extension to the disk may occur. Early radiographic changes are reduction of the intervertebral disk space and loss of density in the vertebral endplates. Gross lesions appear as soft, gray areas of discoloration and disruption in the disk, often extending into adjacent vertebrae through disrupted end plates (Fig. 1.161). *Complete destruction of disks, with fibrous replacement and formation of vertebral osteophytes, occurs late in the disease.* Such lesions are easily confused with spondylosis due to primary degeneration of the annulus fibrosus. The inflammatory reaction may extend dorsally into the spinal canal causing meningomyelitis, or laterally, causing paravertebral abscessation. *Microscopic lesions* of diskospondylitis vary with the stage of the disease and the nature of the causative organism. Early bacterial infections are characterized by suppurative inflammation, hemorrhage, and necrosis of intradiskal structures and adjacent bones. In chronic lesions, vascular connective tissue, infiltrated with mononuclear or mixed inflammatory cells, predominates.

Calcium crystal-associated arthropathy (pseudogout)

A syndrome characterized by the deposition of *calcium pyrophosphate dihydrate* ($Ca_2P_2O_7{\cdot}2H_2O$) *crystals* in articular and para-articular tissues is well recognized in humans and is reported occasionally in *aged dogs*. The lesions may occur as single, tumor-like, periarticular masses (Fig. 1.162) developing over a period of several months or even years. The lateral and dorsal aspects of the metatarsophalangeal joint of the 5th digit were involved in a 13-year-old female Golden Retriever. A similar mass involving the carpal joint was diagnosed in a 15-year-old male English Pointer. Mild, progressive lameness was reported in

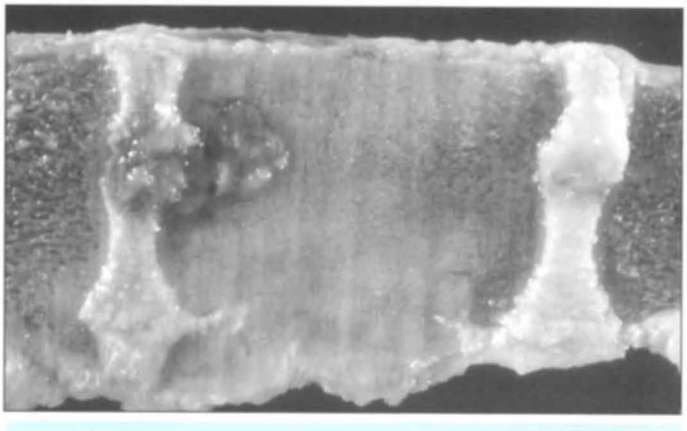

Figure 1.161 Diskospondylitis, dog. Inflammation of intervertebral disk and extension into adjacent vertebral body, causing local bone lysis. The remainder of the vertebral body is sclerotic.

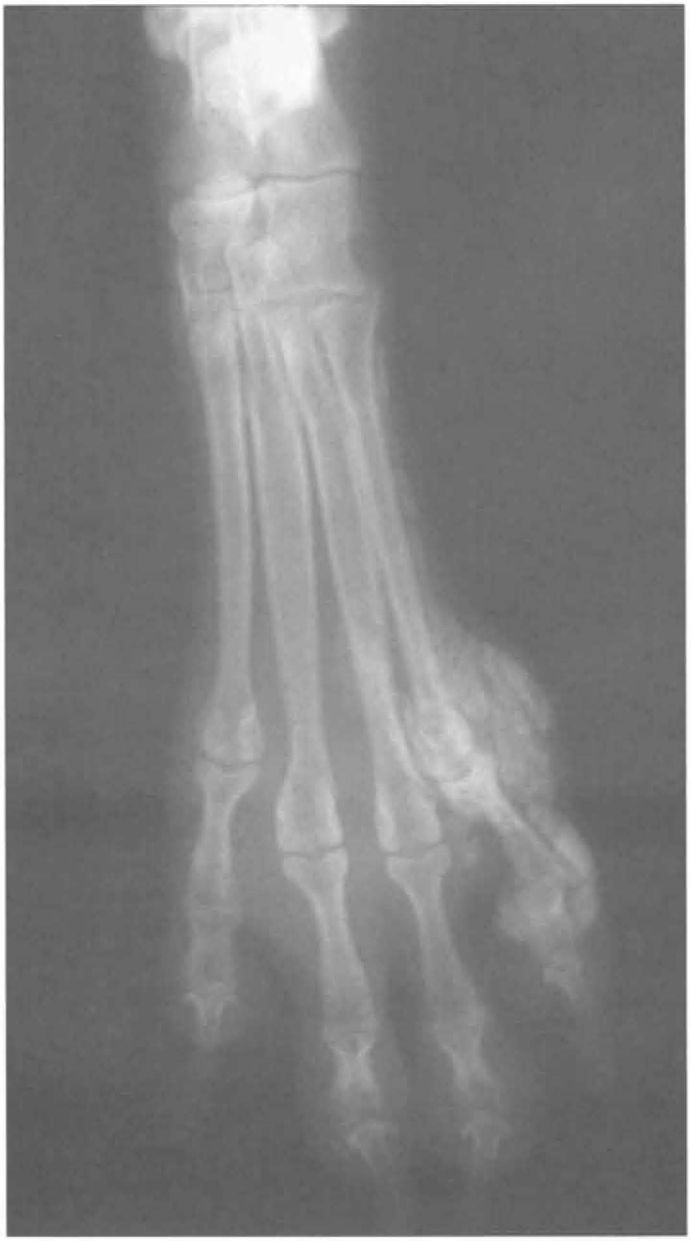

Figure 1.162 Calcium pyrophosphate dihydrate crystal deposition disease (**pseudogout**), dog. Radiodense mass adjacent to the metatarsophalangeal joint of the 5th digit and extending along the 1st phalanx.

the latter dog. In both cases, the lesions consisted of *multiple, hard, chalky white nodules separated by fibrous septa.* Although the masses were adherent to the joint capsule, they did not involve joint cavities or articular surfaces. Microscopically, the chalky nodules consisted of variable-sized deposits of pale brown crystalline material and were separated by bands or islands of fibrous or cartilaginous connective tissue. A *granulomatous inflammatory reaction*, which included many large, multinucleated giant cells, was closely associated with the crystalline deposits. The crystals were weakly birefringent, rhomboidal or rectangular, and stained positively with Alizarin red.

A more acute form of the disease characterized by nonweight-bearing lameness in both forelimbs and the left hindlimb was diagnosed in a 13-year-old Keeshond. Cytological examination of synovial fluid from both carpal joints revealed 60% mononuclear cells and 40% neutrophils. Weakly birefringent rectangular and rhomboid crystals, which stained positively with Alizarin red, were present extracellularly and within neutrophils, monocytes, and synovial lining cells. Clinically, *this acute form of pseudogout closely resembles septic arthritis.*

The pathogenesis of this syndrome, now referred to as calcium pyrophosphate dihydrate crystal deposition disease, is unknown, but acute episodes in human patients may follow surgery, trauma,or severe medical conditions, such as myocardial infarction.

True gout, which is associated with the deposition of urate crystals within joint structures occurs commonly in humans, but does not occur in mammalian species possessing the enzyme uricase. *There are no convincing reports of gout in either dogs or cats, and even Dalmatian dogs, with their high serum uric acid concentrations, do not appear to develop the disease.*

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Noninfectious (immune-mediated) arthritis

The term **immune-mediated arthritis** is used to classify a group of *inflammatory but noninfectious diseases of joints.* The inflammation is in response either to persistence of antigenic material in the synovium of affected joints, possibly as a sequel to previous infection, or to deposition in the synovium of immune complexes derived from inflammatory lesions elsewhere in the body. The *lesions may resemble those of infectious arthritis*, which is not surprising, as the mediators produced during the inflammatory process are quantitatively and qualitatively similar. Furthermore, *synovial fluid from both forms of arthritis is characterized by the presence of increased numbers of neutrophils. Demonstration of toxic change in the neutrophils is often mentioned as a means of differentiating infectious from immune-mediated arthritis, but this is unreliable.* First, not all bacteria induce significant toxic change in synovial neutrophils. Second, unless the viscosity of the synovial fluid is reduced by the lytic action of lysosomal enzymes on hyaluronan, the neutrophils may not "flatten out" sufficiently in direct smears for signs of nuclear degeneration to be apparent.

Immune-mediated arthritis is typically a *polyarthritis* and occurs most often in *dogs and cats*. Erosive and nonerosive forms of the disease are recognized.

- In the *erosive form*, the immunologic process is centered in the joint and stimulates pannus formation, which may result in erosion of the margins of articular cartilage, instability or luxation of joints, or fusion of low-motion joints. The erosive pattern of immune-mediated arthritis is a feature of *rheumatoid-like arthritis of dogs, polyarthritis of Greyhounds, and feline chronic progressive polyarthritis.*
- In the *nonerosive form*, the primary disease is located elsewhere in the body and the products of the immune process are transported to the capillary bed of the synovium of affected joints. Since the primary disease may be transient, cyclic,or responsive to treatment, and is not centered in the joint, products of the immune response that initiate the synovitis can be cleared periodically or permanently from the joint. Consequently, there is no chronic stimulation of pannus or destruction of articular surfaces. Nonerosive immune-mediated arthritis is seen in *idiopathic polyarthritis of dogs, drug-induced arthritis, polyarthritis/myositis syndrome of dogs, lymphocytic/plasmacytic arthritis, and systemic lupus erythematosus*. It is also seen in some animals with chronic enteropathies and other chronic infections.

Erosive athritis

Rheumatoid arthritis

This potentially deforming and crippling disorder is well described in humans, where it is defined by a combination of clinical, pathological, and laboratory criteria. The American Rheumatism Association lists 11 features of the disease, at least seven of which must be present to establish the diagnosis. Furthermore, certain diseases, such as bacterial arthritis, bacterial endocarditis, and systemic lupus erythematosus, must be excluded in order to permit the diagnosis. *The cause of rheumatoid arthritis in humans is not known*, although many aspects of its pathogenesis are understood.

A disease resembling human rheumatoid arthritis is recognized uncommonly in **dogs** and rarely in **cats**. In dogs, it typically affects small and toy breeds, but there is no sex or age predilection. Affected animals initially have episodes of anorexia, depression, and fever, with generalized or shifting lameness associated with swelling around one or more joints.The clinical course is progressive and in the advanced stages of the disease, multiple joints, particularly carpal, tarsal, and phalangeal joints, are persistently swollen, painful, and may be unstable due to weakening of periarticular soft tissues and destruction of articular cartilage and subchondral bone.

Radiographic changes in the early stages of rheumatoid arthritis may be confined to soft tissue swellings, with no apparent alterations to the joint space.As the disease progresses, there is loss of bone density around the joint, collapse of the joint space, and focal radiolucent areas may develop in the subchondral bone (Fig. 1.163A). Other changes may include periarticular osteophytosis, subluxation, or luxation of the joint and fibrous or bony ankylosis, especially in the advanced stages of the disease.

The prominent gross features of early rheumatoid arthritis are thickening and brown discoloration of the joint capsule, with hypertrophy and hyperplasia of synovial villi (Fig. 1.163B, C). Fibrin may be attached to the synovial membrane. Histologically, the enlarged villi are infiltrated with large numbers of lymphocytes and plasma cells and are lined by hypertrophic synoviocytes. Extravasated erythrocytes are often seen within the synovium, as are hemosiderin-laden macrophages, which account for the brown discoloration seen grossly. Degeneration of articular cartilage commences at the margins of the joint where *granulation tissue from the inflamed synovium either spreads across the articular cartilage as a pannus* (Fig. 1.164A) or invades the epiphysis, destroying subchondral bone, and undermining the articular cartilage (Fig. 1.164B). Erosion of central regions of articular cartilage (Fig. 1.163B, C) occurs later in the disease process, probably as a consequence of joint instability and the action of enzymes and mediators released as part of the chronic synovitis. Periosteal new bone sometimes develops at the attachment of thickened joint capsules, but productive osseous changes are always minimal.

Various infectious agents have been implicated as etiologic factors in rheumatoid arthritis of humans and animals. Although none has been shown to be directly involved, indirect involvement through the generation of immune complexes or induction of autoimmunity by molecular mimicry, are possible mechanisms. *Whether the disease is triggered by an infectious agent, or is a manifestation of autoimmunity, there seems little doubt that deposition of immune complexes in articular structures is central to the pathogenesis of rheumatoid arthritis*. Local activation of the complement cascade by these immune complexes leads to generation of proinflammatory peptides such as C3a and C5a, which are chemotactic for neutrophils and induce their degranulation. Type A synoviocytes are efficient antigen-presenting cells and are capable of secreting cytokines that modulate the immune response. Furthermore, high concentrations of granulocyte-monocyte colony stimulating factors in rheumatoid joints can induce monocyte precursors to differentiate into dendritic cells, which are also highly effective at antigen presentation. *Rheumatoid factors*, autoantibodies of the IgM, IgG, or IgA subclasses directed against altered host IgG, are found in synovial fluid, and sometimes in the serum, of dogs with rheumatoid arthritis. *Anti-type II collagen autoantibodies* have also been demonstrated in the synovial fluid of dogs with rheumatoid arthritis.About 25% of dogs with rheumatoid arthritis have rheumatoid factor of the IgM type in their serum.The IgG rheumatoid factor may be more important in dogs but its prevalence in the serum of affected dogs is not known. Rheumatoid factors also are found in a small proportion of dogs and people that do not have joint disease.

Canine distemper virus antigens have been demonstrated in immune complexes precipitated from synovial fluids of dogs with rheumatoid arthritis, suggesting the possibility of a causal role for this virus either following natural infection or vaccination. This would not be surprising since several viral agents have been implicated in human rheumatoid arthritis and the disease in humans often follows viral infection or vaccination.

The joint lesions of *chronic erysipelas and mycoplasmal infections in swine* have features of rheumatoid arthritis and the difficulty in isolating infectious organisms from such joints suggests that an immune mechanism may be involved. In fact, these diseases have been suggested as models for human rheumatoid arthritis.

Polyarthritis of Greyhounds

An *erosive polyarthritis*, which does not appear to have an immune basis, is described in young Greyhounds in Australia, the UK, and the

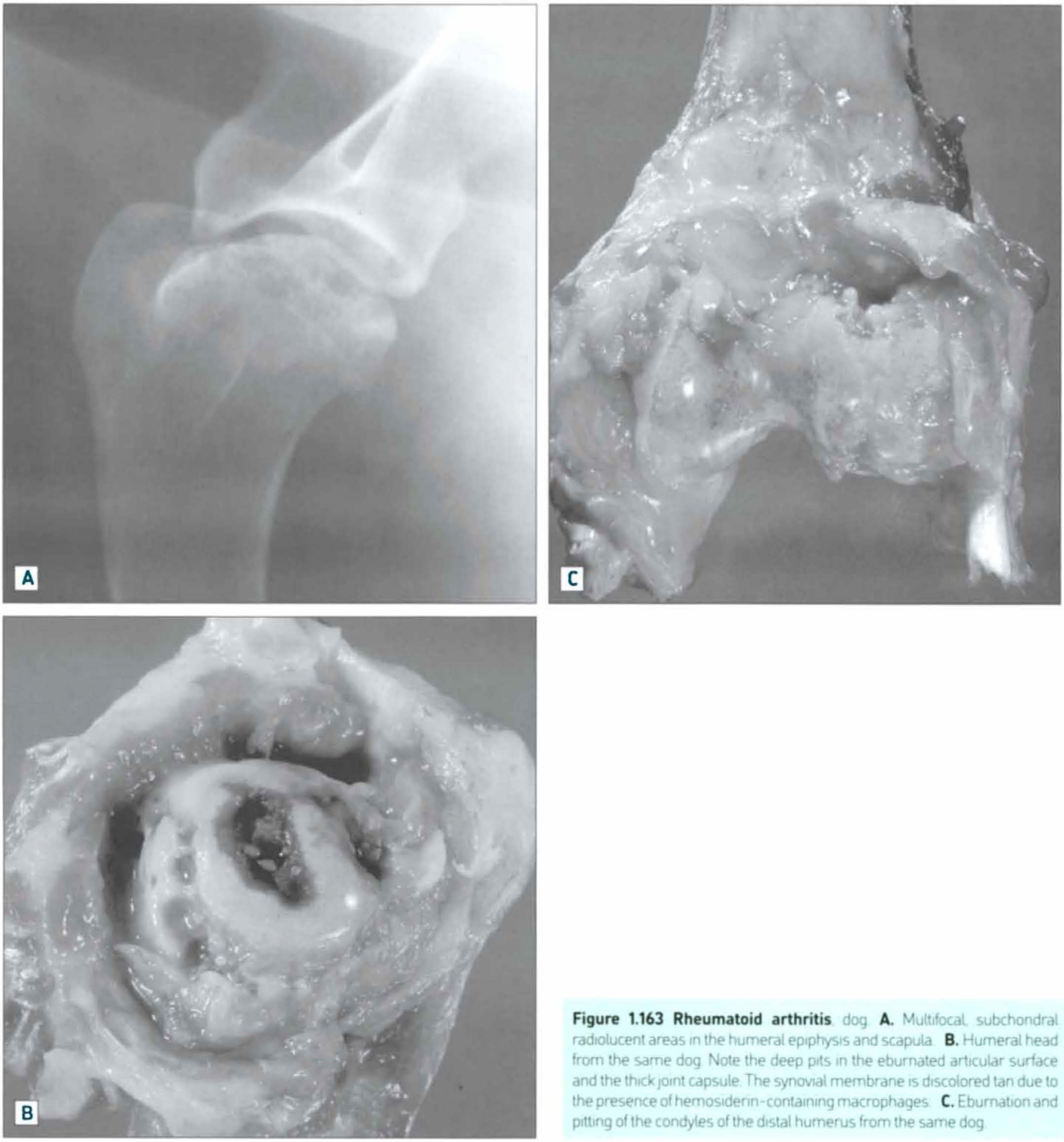

Figure 1.163 Rheumatoid arthritis, dog. **A.** Multifocal, subchondral radiolucent areas in the humeral epiphysis and scapula. **B.** Humeral head from the same dog. Note the deep pits in the eburnated articular surface and the thick joint capsule. The synovial membrane is discolored tan due to the presence of hemosiderin-containing macrophages. **C.** Eburnation and pitting of the condyles of the distal humerus from the same dog.

US. The disease affects dogs of both sexes and usually begins insidiously between 3 and 30 months of age. Mild to severe lameness and joint swellings, involving limb joints distal to, and including, elbows and stifles are observed clinically. Superficial lymph nodes are enlarged. Despite considerable effort, *no infectious agent has yet been identified and the syndrome does not appear to be related to athletic activity.*

Gross findings in affected joints include an excess of turbid synovial fluid, with fibrin clots in severe cases, yellow/brown discoloration, and thickening of synovial membranes, sometimes with adherent fibrin plaques, ecchymotic hemorrhages in the synovial membrane and erosions of articular cartilage. *Lesions are common in the first and second phalangeal joints, and in carpal, tarsal, elbow, and stifle joints.* Occasionally, shoulder, hip, and atlanto-occipital joints are involved.

Microscopic lesions include *necrosis of articular cartilage and proliferative synovitis.* The articular cartilage shows either full-thickness necrosis or necrosis of deep layers with relative sparing of superficial zones. Cartilage erosion sometimes extends to the subchondral bone. Typical changes in the synovium include villus hypertrophy, proliferation of synovial cells, and infiltration by lymphocytes, plasma cells, and neutrophils. In some areas, the lymphocytic infiltrates are

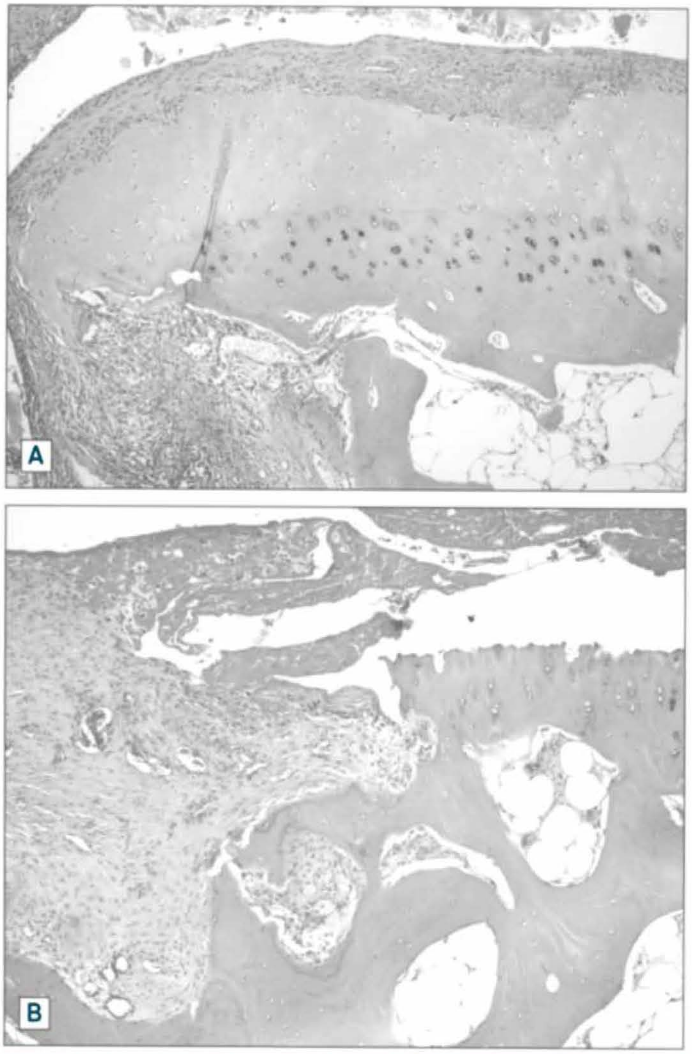

Figure 1.164 Rheumatoid arthritis, dog. **A.** Pannus spreading across articular cartilage from the joint margin. The cartilage beneath the pannus is degenerate. **B.** Granulation tissue invading the epiphysis and encroaching on the eroded articular surface. Note the osteoclasts resorbing bone in front of the advancing granulation tissue. Fibrin is adherent to the surface.

intense and may form follicles. There is no evidence of vasculitis. Pannus formation is often present, but never extensive, and changes in subchondral bone are usually minimal.

Feline chronic progressive polyarthritis

Two forms of this disease are recognized; an *erosive form*, which resembles rheumatoid arthritis of dogs and humans, and a more common form characterized by *periosteal proliferation*.

The **erosive form** occurs in older cats and can affect either sex. It has an *insidious onset, chronic progression,* and clinical signs are largely restricted to symmetrical deformities of the *carpus, tarsus, and digits* due to loss of joint margins, subluxation, collapse of joint space, and ankylosis. The synovial fluid contains low to moderate numbers of neutrophils and lymphocytes. Synovial membranes are heavily infiltrated with lymphocytes and plasma cells and enlarged synovial villi often contain prominent lymphoid follicles with germinal centers. Rheumatoid factor is absent. Infection with *Feline leukemia virus* is present in about 50% of affected cats.

The **periosteal proliferative form** occurs only in relatively *young, intact or neutered male cats,* usually between 18 months and 5 years of age. The disease is etiologically *linked to infection with Feline syncytia-forming virus and Feline leukemia virus.* Evidence of *Feline syncytia-forming virus* infection is present in all affected cats and 60% also have evidence of *Feline leukemia virus* infection, but the disease cannot be reproduced by experimental infection with either of the two agents, or with cell-free synovial tissue from affected cats. Because many cats are infected with both viruses without ever becoming ill, it is thought that the periosteal proliferative form of feline chronic progressive polyarthritis is an *unusual manifestation of Feline syncytia-forming virus infection in cats that are somehow predisposed.* The role of *Feline leukemia virus* is unclear, but it may potentiate the activity of the other viral agent. A similar syndrome has been reported in a 4-year-old cat that was seropositive for both *Feline syncytia-forming virus* and *Feline immunodeficiency virus.*

The disease is characterized by sudden onset of high fever, severe pain in joints and tendon sheaths, and lymphadenopathy associated with affected joints. Synovitis is initially characterized by the presence of *fibrinopurulent synovitis but later, plasma cells and lymphocytes predominate.* Rheumatoid factor is absent. Synovial lining cells are hyperplastic throughout the course of the disease, but villus hypertrophy and pannus formation are uncommon until late in the disease. Swollen lymph nodes biopsied early in the disease have exuberant lymphoid hyperplasia, including marked extracapsular extension, and could be mistaken for lymphosarcoma.

The skeletal lesions are bilateral and *most frequently involve the tarsometatarsal and carpometacarpal joints,* but the elbow and stifle joints, and the articular facets of the thoracic and lumbar spine, may also be affected. The periosteal new bone consists of trabeculae of woven bone and the intertrabecular spaces often are infiltrated with lymphocytes and plasma cells. Pannus formation usually occurs late in the disease and leads to periarticular erosions of joint margins, collapse of joint spaces, and fibrous ankylosis, but subluxation is not a feature. This pattern of disease is similar to Reiter's arthritis of humans.

Nonerosive arthritis

There are several diverse causes of noninfectious, nonerosive arthritis in dogs and cats, but the mechanism in each syndrome appears to be immune mediated and the presenting signs are similar. Clinically, the joint disease tends to be intermittent, and periods of remission may occur. One or several joints of the distal limbs may be involved, particularly the smaller joints of the manus and pes. There are essentially no radiographic changes except for joint distension and soft tissue swelling, although signs may have been present for months. Occasionally, slight narrowing of the joint space is evident, and distension of the joint cavity is demonstrable by contrast arthrography. Gross lesions in affected joints are minimal. Histologically, there is edematous thickening and inflammation of the synovial membrane. Neutrophils are concentrated at the intimal layer of the synovium and may be quite numerous, depending upon the stage of the disease. Sparse accumulations of plasma cells and lymphocytes are present in the deeper layers of the synovium and fibrous joint capsule. Villus hyperplasia and pannus are not seen.

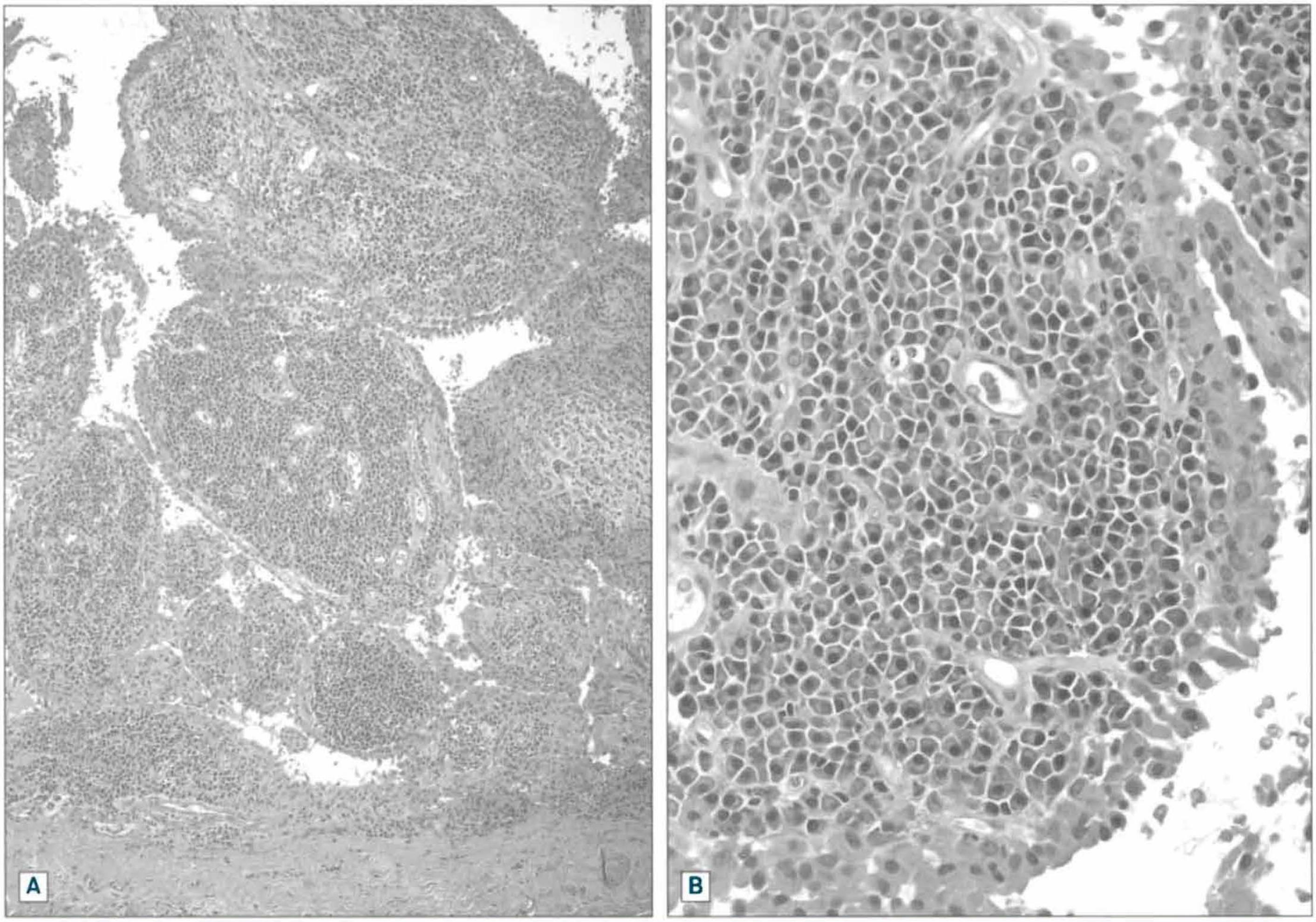

Figure 1.165 Lymphocytic/plasmacytic synovitis, dog. **A.** Hypertrophic villi heavily infiltrated with mononuclear cells in the stifle joint of a dog, 2 years after surgery for correction of patellar luxation. **B.** High-power view of a villus from the same dog. The villus is infiltrated with an almost pure population of plasma cells. Note the hypertrophy and hyperplasia of synovial cells.

Synovial fluid cytology is useful in the diagnosis of noninfectious, nonerosive arthritis, but is not specific and often does not allow differentiation from infectious arthritis or from erosive forms of noninfectious arthritis. The characteristic feature is a mild to moderate increase in cellularity, most cells of which are nondegenerate neutrophils.

Idiopathic polyarthritis is considered the most common form of nonerosive, immune-mediated arthritis in dogs, but is diagnosed only when other types of inflammatory joint disease have been excluded. Arthritis is the only manifestation, but pyrexia, inappetance, and lethargy generally accompany the lameness. The polyarthritis associated with **systemic lupus erythematosus** of dogs and cats is similar to that of idiopathic polyarthritis, but may be accompanied by evidence of disease in other organs systems, such as the skin, kidney, bone marrow, or neuromuscular system. *In many cases, only the presence of antinuclear antibody in serum distinguishes the two syndromes.* Neutrophils containing phagocytosed, partially degraded nuclear material, referred to as lupus erythematosus cells (LE cells), are rarely detected in the synovial fluid of animals with systemic lupus erythematosus. The phagocytosed nuclear material in LE cells is dense, diffusely pink or purple, and displaces the nucleus to the periphery of the cell. Leukophagocytic macrophages and neutrophils containing more granular nuclear remnants are common in the synovial fluid of acutely inflamed joints and should not be misinterpreted as LE cells.

Nonerosive polyarthritis has been recognized in animals and humans in association with a **variety of chronic diseases**, such as bacterial endocarditis, urinary tract infections, diskospondylitis, periodontitis, chronic otitis media or interna, pyometra, dirofilariasis, and fungal infections. **Inflammatory bowel disease** has also been associated with polyarthritis in canine and human patients. Although the possibility that infection may be involved in some of these nonerosive forms of polyarthritis is virtually impossible to exclude, the weight of evidence suggests that the synovitis is secondary to deposition of circulating immune complexes in the synovial capillary bed. In those cases associated with inflammatory processes at other sites, the inflammation presumably does not persist long enough, or is not severe enough, to initiate pannus formation or induce cartilage erosion. In the most common primary or idiopathic form, the origin and nature of the antigen responsible for initiating the disease is unknown.

Drug-induced polyarthritis, thought to be immune-mediated, develops in a small proportion of dogs, particularly Dobermans, treated with *sulfonamides*. Combination drugs containing sulfadiazine are usually responsible but sulfamethoxazole is also incriminated. The drug may act directly as *an antigen or as a hapten* in combination with host proteins. Signs of polyarthritis and fever, sometimes with lymphadenopathy, anemia, leukopenia, and thrombocytopenia, develop after 8–20 days of treatment. Clinical signs regress within 2–5 days of drug withdrawal and, in one report, recurred within 2 hours to 5 days in six of six dogs that were re-exposed.

A syndrome characterized by nonerosive **polyarthritis** and **polymyositis** has been reported in six young adult dogs, five of which were Spaniels. Clinical signs included stiffness, pain and swelling of multiple joints, muscle pain, muscle atrophy, and pyrexia. Affected dogs have no circulating antinuclear antibodies or rheumatoid factors but show chronic active inflammatory changes in muscle and synovial membrane biopsies.

Plasmacytic/lymphocytic synovitis is an insidious, nonerosive arthritis involving primarily the *stifle joint of small and medium-sized breeds of dog*. The syndrome may be accompanied by joint laxity and partial or complete rupture of the cranial cruciate ligament but it is seldom possible to determine which lesion came first. It is possible that the synovitis in these animals reflects an exaggerated immune response to mediators or antigens released following ligament damage, surgical correction, or as a result of an ensuing degenerative joint disease. Radiographic evidence of degenerative joint disease may be present in some cases. The synovial membrane is thickened, edematous, and red/yellow on gross examination. Microscopic examination reveals a *marked diffuse and/or nodular plasmacytic/lymphocytic infiltration of the synovium and hypertrophy of synovial cells* (Fig. 1.165A, B). Villus hypertrophy may also be a feature. Unlike other forms of immune-mediated arthritis, the increase in cellularity of the synovial fluid is due predominantly to small mononuclear cells rather than neutrophils, the latter comprising only 10–40% of the cell population.

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TUMORS AND TUMOR-LIKE LESIONS OF JOINTS

A range of neoplastic and non-neoplastic lesions occurs in domestic animals in the vicinity of joints, but most are rare and poorly characterized. The most recent World Health Bulletin on the histological classification of bone and joint tumors lists only synovial sarcoma and villonodular synovitis as tumors and tumor-like lesions of joints and related tissues. Although veterinary pathologists recognize many lesions other than these two, only the most common are included here. For further information, the reader is referred to the bibliography at the end of this section.

Tumors in the region of joints may be either *benign or malignant* and *arise from synovioblastic cells* in the synovial membrane *or from a range of supporting structures*, including fibroblastic, vascular, and nervous tissue. Because of the similarity between synovial tumors and other mesenchymal tumors, the potential for misdiagnosis is high, especially when these lesions occur so infrequently and diagnostic criteria are largely extrapolated from the human medical literature.

As with tumors of bones, the availability of a detailed clinical history and knowledge of the radiographic and gross findings prior to microscopic examination of the lesion are likely to enhance the accuracy of the diagnosis. In particular, radiography will usually indicate whether the lesion is centered on a joint or tendon sheath, the presence of mineralization, and any bone involvement. Cytological evaluation of fine-needle aspirates or scrapings prepared from tissue biopsies may provide useful information on the nature of the tumor cells, but in most cases, histologic examination is required.

Benign tumors and tumor-like lesions of joints

Although several benign proliferative lesions of the synovium have been described in man and animals, most are rare and, for many of them, there is debate over whether they are neoplastic or inflammatory.

Pigmented villonodular synovitis is recognized occasionally in the *major weight-bearing limb joints, particularly the stifle and hip, of* *adult dogs*. Grossly, the synovial membrane in affected joints is thickened with plump, tan or dark brown villi and articular surfaces show features of mild degenerative joint disease. Histologically, the thickened synovial membrane consists of *closely packed villi of various sizes and shapes, and slit-like spaces lined by synovial cells*. The spaces may represent tangential sections through gaps between adjacent villi. Hyperplastic synovial cells are present throughout much of the thickened synovium but do not invade the joint capsule and although binucleate forms are often present, they show no cytologic features of malignancy. Multinucleated giant cells are sometimes present, together with hemosiderin-containing macrophages. *The lesion may be difficult to differentiate from chronic infectious synovitis, or a chronic synovial response to either hemarthrosis or degenerative joint disease.*

Villonodular synovitis of the *equine metacarpophalangeal or metatarsophalangeal joints* is a chronic inflammatory condition characterized by the formation of a nodular mass involving the synovium near the dorsoproximal attachment of the joint capsule. As mentioned earlier in this chapter, this lesion is considered *secondary to persistent trauma* and has features of a resolving hematoma.

Localized nodular tenosynovitis is another benign lesion of questionable origin and with a variety of synonyms in the human literature, including benign synovioma; a term that is no longer advocated for use in either humans or animals. The lesion has been reported only occasionally in *dogs*, although nodular lesions of tendons previously diagnosed as benign synovioma probably constitute additional examples of localized nodular tenosynovitis. The most common sites for these firm, solitary lesions are the *tendons and tendon sheaths of the extensors and flexors of the feet, particularly in the region of the metacarpus and metatarsus*. The microscopic appearance varies between and within lesions, but usually includes cellular areas containing proliferating synoviocytes, hemosiderin-containing macrophages, multinucleated giant cells and mononuclear inflammatory cells. Cleft-like spaces lined by synoviocytes are often present in some areas. There are *similarities between this lesion and benign giant cell tumor of tendons and tendon sheaths*, and it is possible that these are variants of the same disease process.

Benign giant cell tumors of tendons and tendon sheaths appear to arise from synovioblastic cells surrounding tendons and tendon sheaths and are relatively common in animals. Microscopically, the lesion is characterized by the presence of *numerous multinucleated giant cells, which are thought to develop from proliferating synovioblasts*. Some lesions are predominantly solid while others contain many cleft-like spaces. The tumor cells show no convincing features of malignancy but, although they do not metastasize, recurrence following surgical removal is likely.

Benign vascular lesions referred to as either **synovial hemangiomas** or **vascular hamartomas** have been reported in the *carpal and digital sheaths of the forelegs in young horses*. The lesions may be apparent from an early age and enlarge slowly, or may become acutely apparent following rupture and formation of hematomas. Microscopically, the lesions consist of *multiple nodules of well-differentiated capillaries separated by septa of fibrous tissue*. Apparent invasion of adjacent structures, including tendons, occurs in some cases, but the endothelial cells lining capillaries show no features of anaplasia, and metastasis is not reported. Reports of hemangiosarcomas involving the tendon sheaths of young horses are most likely further examples of benign vascular tumors that would be more appropriately classified as vascular hamartomas.

Synovial chondromatosis (osteochondromatosis) is a tumor-like lesion characterized by the presence of *multiple nodules of hyaline cartilage, some of which may undergo ossification, in the synovial membrane*. Although synovial chondromas are widely considered to arise by metaplasia of synovial cells within the synovial membrane, an alternative hypothesis suggests that the cartilage nodules may in fact be derived from fragments of cartilage from the articular surface. Such fragments released into the joint space may continue to enlarge within the joint as they derive their nutrients from the synovial fluid. Some may become incorporated into the synovial membrane, where they could also survive and grow within the richly vascular synovial membrane and, in some cases, become ossified following vascular invasion. This mechanism is most likely in cases where there is an associated degenerative joint disease, but the syndrome sometimes occurs in the synovial membrane of apparently normal joints or bursae. In such cases, chondroid metaplasia of synovial mesenchymal cells is a more attractive pathogenesis.

Various other benign, tumor-like lesions occurring in the vicinity of joints, including **tumoral calcinosis** and **fibrodysplasia ossificans progressiva** are discussed earlier in this chapter under the heading Ectopic mineralization and ossification.

Malignant tumors of joints

Because of the infrequent occurrence of these tumors in animals, their classification has largely been based on extrapolation from the medical literature. This has not been straightforward, as the classification of certain malignant periarticular tumors of humans is confusing. For example, there is evidence that tumors classified as synovial sarcoma in human patients are not of synovial origin at all. Furthermore, there is debate over the use of such terms as malignant fibrous histiocytoma and malignant giant cell tumors of soft parts, both in animals and humans. The use of such categories in this section is more in recognition that they are well established in the veterinary literature than an endorsement of their validity. *A more precise and widely accepted classification system for malignancies of joints will necessitate the use of immunochemical or molecular techniques to better determine the histogenesis of the tumor cells.*

Synovial sarcoma in humans is characterized by a biphasic pattern, comprising both *mesenchymal and epithelial elements*. In some tumors, a "pseudoglandular" appearance is created by epithelial elements, which often form irregular cleft-like spaces surrounded by neoplastic mesenchymal cells. In other cases, the mesenchymal elements predominate and the tumor may be difficult to differentiate from other sarcomas, particularly fibrosarcoma. The neoplastic epithelial cells in these tumors possess tight junctions and are arranged on a basal lamina. In addition, they stain positively with the epithelial markers cytokeratin and epithelial membrane antigen. *Synovial cells are of mesenchymal, rather than epithelial, origin* and are not arranged on a basal lamina. The fact that these tumors in human patients frequently occur at sites distant from joints creates further doubt over their synovial origin and suggests that extrapolation to animals is inappropriate.

Synovial sarcoma is rare in animals, but has been reported in dogs and occasionally in cats and cattle. In **dogs**, synovial sarcoma is reported mainly *in large but not giant breeds* and although it is most common around middle age, the *age range is broad* (1–15 years). The tumor is found most often near one of the *major weight-bearing joints*, the *stifle* being the most common and followed in decreasing order by the elbow, shoulder, tarsus, carpus, and hip. Occasionally, joints of the axial skeleton are involved. Synovial sarcoma may also originate from the region of *tendons or tendon sheaths*, usually the flexor or extensor tendons of the limbs proximal to the carpus and tarsus. Grossly, *the tumor has indistinct borders* and may infiltrate along fascial planes leading away from the affected joint. Some synovial sarcomas contain cystic spaces filled with mucinous material. The synovial membrane may be largely replaced by tumor tissue and invasion of epiphyseal bone may occur at the insertion of the joint capsule.

Histologic descriptions of synovial sarcoma in animals frequently imply the presence of two distinct cell populations by reference to a synovioblastic or "epithelioid" cell type and a fibroblastic cell type. This no doubt reflects extrapolation from synovial sarcoma in humans, although *there is some doubt that these biphasic tumors actually occur in animals.* The tumor cells in synovial sarcomas of animals may vary in shape from polygonal to spindle-shaped, the relative proportion of each type varying between tumors, and even within the same tumor. *In cases with a predominance of spindle cells, the tumor may be virtually indistinguishable from fibrosarcoma, but other tumors may consist predominantly of sheets of synovioblastic cells more closely resembling normal synoviocytes.* Cavities or slit-like spaces containing *proteinaceous fluid or mucoid material* and lined by a poorly defined layer of malignant synovioblasts may form within sheets of tumor cells (Figs 1.166A, B, 1.167A). The malignant synovioblastic cells of synovial sarcoma often have eccentric circular to oval nuclei, which vary in size and may contain one or more prominent nucleoli. The mitotic rate is highly variable, as is the frequency of binucleate and multinucleate giant cells. Individual cells may be surrounded by an eosinophilic matrix resembling osteoid (Fig. 1.167B), sometimes leading to an incorrect diagnosis of osteosarcoma. In some canine synovial sarcomas, especially those involving tendons or tendon sheaths, *nodules of myxomatous tumor tissue* are scattered among areas that are more solid. The myxoid matrix may surround individual stellate tumor cells, clusters of tumor cells, or fill multiple spaces within the tumor. Synovial sarcomas with abundant myxoid change are difficult to differentiate from myxosarcoma or malignant peripheral nerve sheath tumors. The perivascular orientation of synovioblastic or fibroblastic tumor cells in some tumors might also cause confusion with hemangiopericytoma, especially in lesions where there is infiltration of the skin adjacent to the joint or tendon sheath.

In cytologic preparations, synovial sarcoma may yield a population of plump, malignant mesenchymal cells with eccentric nuclei and basophilic cytoplasm, together with variable numbers of spindle cells and multinucleated giant cells, but histopathology is required to reliably differentiate the tumor from osteosarcoma and various other sarcomas.

Canine synovial sarcomas *commonly recur following surgical excision* and, in one study, *20 of 37 cases (54%) metastasized.* Metastases were found most often in the lungs and regional lymph nodes. The clinical course may vary from less than a month to greater than a year.

Only isolated cases of synovial sarcoma are reported in species other than dogs, and the diagnosis is not always conclusive.

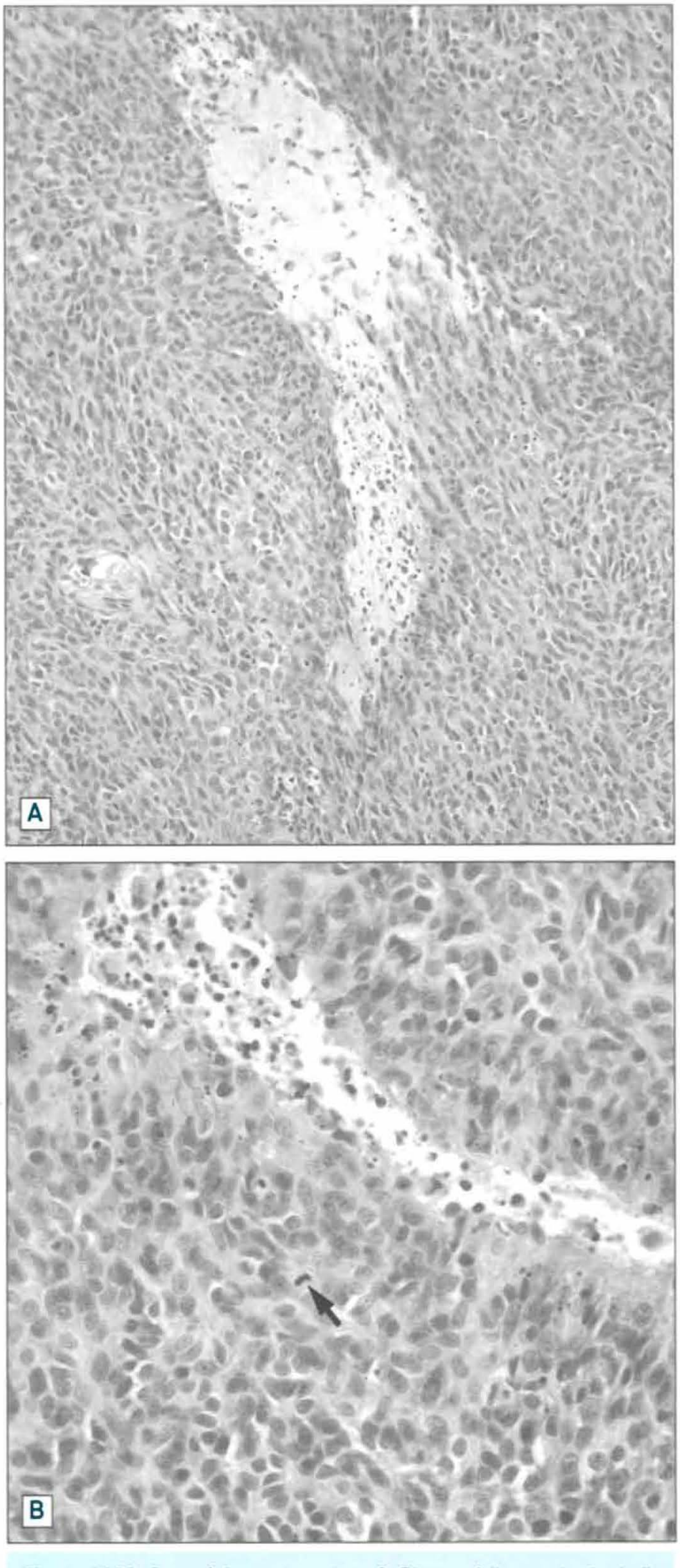

Figure 1.166 Synovial sarcoma, dog. **A.** Sheets of disorganized spindle cells surrounding and lining a poorly defined cavity. **B.** Higher-power view of the same tumor showing a narrow cleft surrounded by tumor cells. Occasional mitotic figures are present (arrow).

Histiocytic sarcoma is recognized as a *rapidly growing, locally aggressive tumor* often occurring in *close proximity to a joint in dogs*, particularly flat-coated Retrievers, Golden Retrievers, Labrador Retrievers, Bull Mastiffs and Rottweilers. The tumor has been recognized in dogs of both sexes from 4 to 13 years of age. Similar tumors also arise from the skin or subcutaneous tissue and infiltrate underlying tissues. It is likely that many histiocytic sarcomas originating in the joint capsule are incorrectly diagnosed as synovial sarcoma.

A recent retrospective study of 35 canine synovial tumors, using immunochemical staining for antibodies to cytokeratin and CD18, found that 51% of them were histiocytic sarcomas.

Dendritic (Langerhans) cells, which are known to occur in the synovial membrane, are considered the cell of origin of histiocytic sarcomas in the region of joints, although precise classification of the tumor requires the development of specific immunochemical stains.

Clinically, the tumor produces soft tissue swellings around or in the vicinity of major limb joints and may be accompanied by lytic lesions in the epiphyses or metaphyses of adjacent bones. On gross examination, the synovial membrane and joint capsule may show marked nodular or diffuse thickening with gray/pink, fleshy tumor tissue (Fig. 1.168A), which may extend into adjacent soft tissues. Histologically, *the synovial membrane and joint capsule are infiltrated with malignant histiocytic cells*, sometimes forming nodules protruding into the joint space (Fig. 1.168B) or causing marked thickening of synovial villi. The nuclei of tumor cells vary considerably in size and shape, and often contain large, irregularly shaped nucleoli (Fig. 1.168C). The cells have a moderate to large quantity of eosinophilic cytoplasm, which sometimes contains many small vacuoles. Multinucleated giant cells with variable-sized nuclei are common and the mitotic rate is usually high. Neutrophils, eosinophils, lymphocytes, and plasma cells may be present in variable numbers throughout the tumor. Similar tumor cells may be present between bone trabeculae in adjacent bones, presumably due to direct invasion from the attachment of the joint capsule or the adjacent muscle. *Cytological preparations* generally reveal a monomorphic population of large round cells with circular to oval or indented nuclei and abundant cytoplasm, which may contain many discrete vacuoles (Fig. 1.169A, B). Multinucleated giant cells containing nuclei of various sizes are common, as are abnormal mitotic figures.

Canine histiocytic sarcoma is not only locally aggressive, but *frequently metastasizes* to regional lymph nodes and occasionally to the lungs and other visceral organs. In the retrospective study referred to above, 91% of histiocytic sarcomas metastasized, and the average survival time was 5.3 months. The prognosis is therefore poor, and early diagnosis followed by amputation of the affected limb offers the best chance of survival.

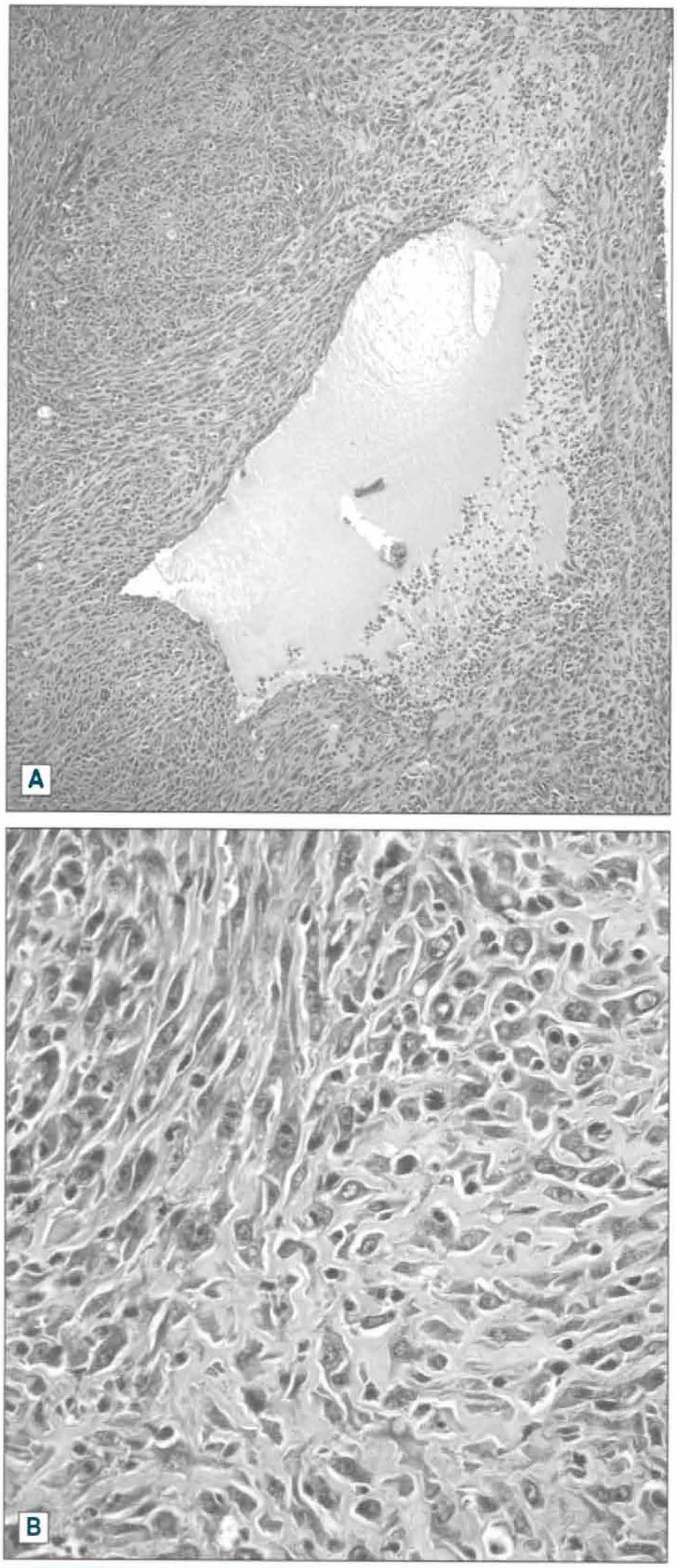

Figure 1.167 Synovial sarcoma, dog. **A.** Large cavity surrounded by malignant synovioblastic cells and containing proteinaceous fluid. **B.** Same tumor at higher power showing fusiform to polygonal tumor cells, many of which are surrounded by eosinophilic matrix similar to osteoid.

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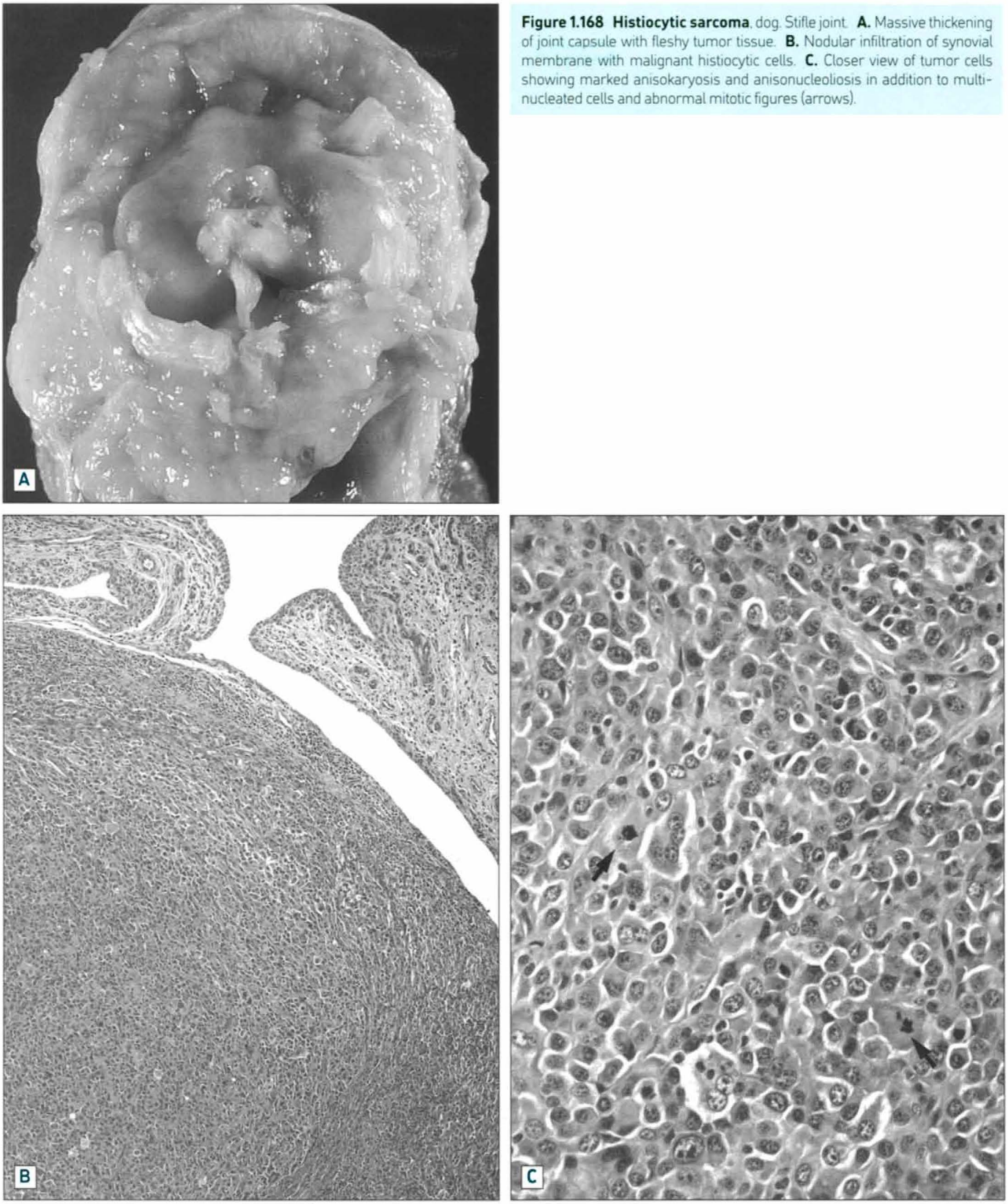

Figure 1.168 Histiocytic sarcoma, dog. Stifle joint. **A.** Massive thickening of joint capsule with fleshy tumor tissue. **B.** Nodular infiltration of synovial membrane with malignant histiocytic cells. **C.** Closer view of tumor cells showing marked anisokaryosis and anisonucleoliosis in addition to multinucleated cells and abnormal mitotic figures (arrows).

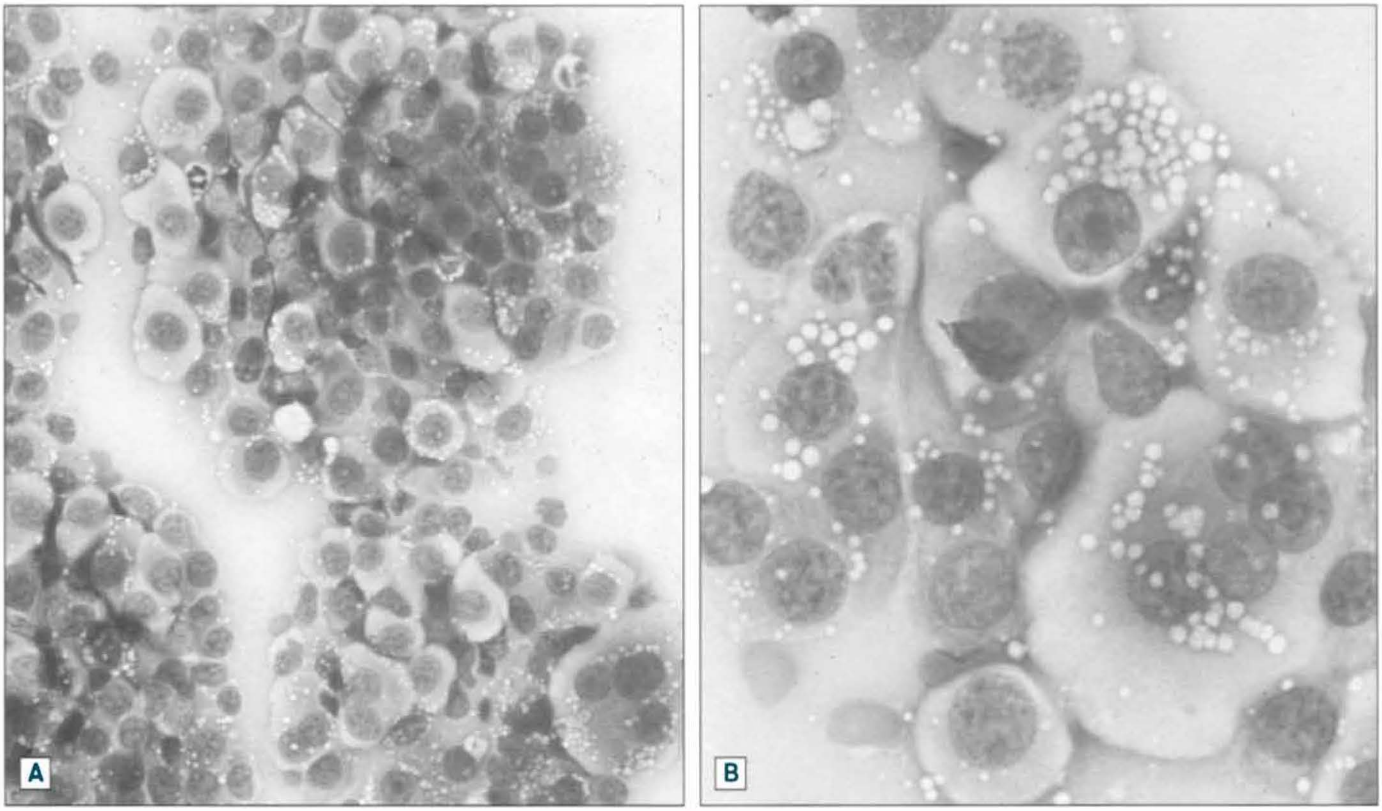

Figure 1.169 Histiocytic sarcoma, dog. **A.** Cytological preparation (scraping) of tumor involving the shoulder joint of a Retriever. The tumor cells are sometimes binucleate or multinucleate and many contain discrete intracytoplasmic vacuoles. **B.** Closer view showing large histiocytic cells, including a multinucleate form.

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2

Muscle and tendon

John F. Van Vleet and Beth A. Valentine

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Muscles

DEVELOPMENT AND STRUCTURE

Development

Development of striated muscle in the embryo is from *mesodermal somites,* which give rise to *myotomes.* Within each myotome, which corresponds roughly to a vertebral body segment, with its spinal nerve, the individual muscles develop by a process of aggregation and migration of presumptive myoblastic cells. It is very likely that the undifferentiated mesodermal cells will be committed to a muscle destiny some time before significant structural change is visible, and the earliest detectable modification to presumptive myoblasts is a cross-sectional rounding of the spindle-shaped cells. By this time as well, there may be subtle distinctions between those cells that will become myofibers and those that will become satellite cells.

The first clear sign of differentiation is the *migration of presumptive myoblasts,* destined to become myofibers, into the regions where future muscles will appear; this occurs before any nerve influence is exerted. The direct connection of the nerve to the myotome determines the subsequent route of innervation, but because migration has occurred, the muscle may receive nerve sprouts (as it has received myoblast group components) from more than one myotome.

The second phase of muscle development is incompletely separated from the first and subsequent phases. It begins with the *early development of sarcoplasmic components,* such as myofibrils, which identify the cells as muscle. The commitment to myogenesis is mutually exclusive with cell replication, and, with possible rare exceptions, committed myoblasts are post-mitotic. Myoblasts begin to fuse into elongated multinucleated cells about the time myogenesis begins.

Subsequent development of the myotube allows it to become a well-developed muscle fiber and consists of stepwise construction of *actin and myosin filaments,* the formation of the *Z band* into which the thin actin filaments insert, and the evolution of the *tubular systems.* The last of these steps, the invagination of the T tubular system from caveolae or other small, regular recesses on the outer sarcoplasmic membrane, provides an elaborate system of tubules that run parallel to the Z bands and make contact with all developing myofibrillar units.

The fourth phase of development is one in which the evolving fiber grows, increases the number of myofibrils and nuclei, and moves the latter to the subsarcolemmal position. During the final phase of development, the development of a basal lamina and an additional sheath of collagen, fibroblasts and capillaries invest each developing myotube as orientation of the fiber into its final position of tension takes place. Development of fibers up to this point, just after the end of the first trimester, is independent of any neural connection, but subsequent fiber enlargement and the considerable increase in the number of fibers that occurs during the immediate prenatal and postnatal periods, is dependent on a functional neural connection. The terminal differentiation of skeletal muscle is therefore largely determined by the characteristics of the neural connections although locally acting myogenic factors also contribute.

The genetic and molecular events in muscle development, and also in muscle regeneration, are rapidly becoming known. As might be expected, the organization and control of gene and protein expression during muscle cell specification and differentiation is complex. *Myogenic regulatory factors* (MRF) are centrally involved in transcription regulation in muscle and include MyoD, myogenin, Myf5, and MRF4. These muscle-specific proteins and a variety of growth factors influence gene and protein expression during the development of skeletal muscle.

The muscle fiber increase in late gestation in domestic animals is probably comparable in all species, but it has been particularly well studied in the pig and is more orderly in that species than in others. Within what will become a primary bundle of muscle fibers, *1–6 primary or template myofibers become the focal point of the proliferation* that, by early postnatal life, has produced one or more sublobular clusters of fibers within each primary bundle. It is not clear whether new fibers are produced next to the template fiber by a process of template fiber budding, or by incomplete or complete longitudinal division. Alternatively they may be derived from local myoblastic or satellite cells that begin development within the basal lamina of the primary fiber and then separate and develop their own completely investing basal lamina. What is clear is that they are produced in waves, with subsequent fibers pushing those formed earlier to the periphery of the sublobule. Template fibers are always histochemically type 1, but the newly formed fibers are type 2 until near the time of birth when a proportion of them (few or several depending on the muscle and the species) which lie closest to the template fiber will begin to modify to become type 1 fibers (Fig. 2.1A, B). The latter process may continue for 6 months or more after birth, and it may be that the process reverses again in early postnatal life in some muscles of the body.

During the period when templating is occurring, new fibers rapidly become innervated and, it seems, do not later change neural connections. In view of the described changes of fiber type, thought to be associated with exercise, it is difficult to reconcile other evidence that suggests that a single motor nerve axon can serve only one histochemical type of muscle fiber. The paradox may be resolved if it is accepted that some fibers have, or develop, different kinds of energy metabolism that they use selectively depending on work demands. Alternatively, a change in the activity of the nerve may induce biochemical changes in the muscle fiber clusters, and hence a change in fiber type identity. This explanation is consistent with the fact that the functional capacity of a muscle fiber is limited by its nerve connection and its cytoplasmic components and of these probably only the latter can be modified.

Gross anatomy

Muscle structure is arranged around *muscle fibers.* Muscle fibers are variable in size depending on age, exercise, nutritional status, position and function of the muscle in question, and on species, although the fibers of a mouse are only a little smaller than those of a horse. Muscle fibers in the extrinsic muscles of the eye are consistently small (10–30 μm in diameter) and round; those of the major limb muscles vary from an average least diameter of 40–65 μm and, although rounded in the fresh state, often appear polygonal when

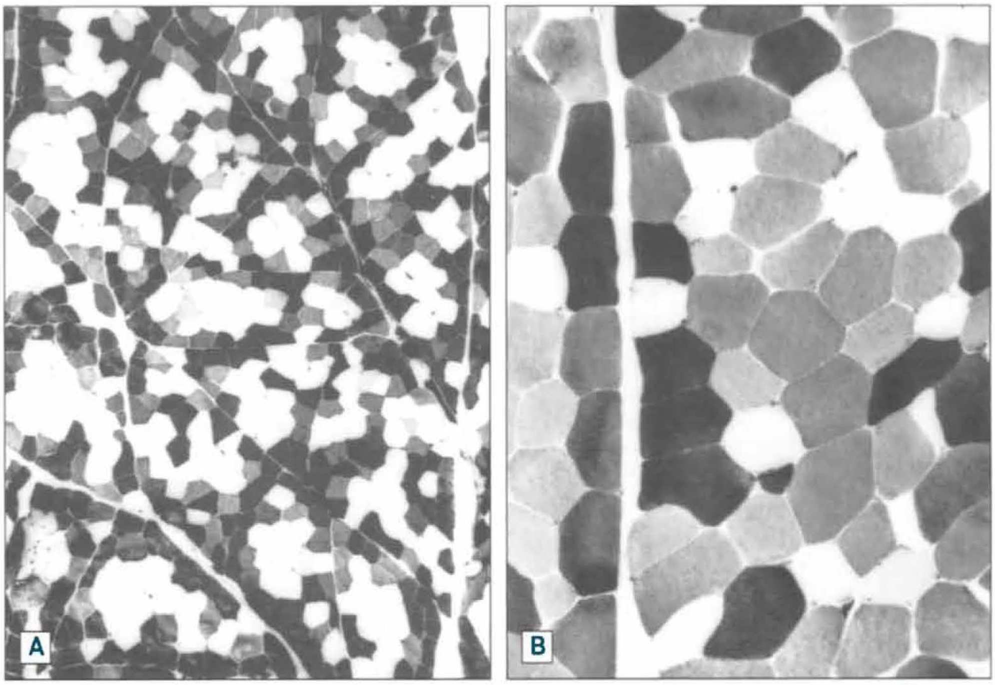

Figure 2.1 A. ATPase (alkaline) histochemical preparation of normal calf muscle to show light type 1 sublobular "**template clusters**." **B. Dark type 2 fibers** clustered along edges of primary bundles. (Courtesy of TJ Hulland.)

fixed. The size of fibers increases with age until puberty, at which time males have slightly larger fibers than females. In old age, with certainty, but perhaps beginning in early maturity, the fiber diameter slowly decreases. In those domestic animals that have been studied, the size distribution of muscle fibers conforms more or less to a biological distribution curve, although in most postural muscles the curve is skewed by a small population of large fibers extending upwards from the expected upper size limit. In most instances, these large fibers are from the type 1 fiber population, but in some muscles in some species they may be from the type 2 group. A distinctly bimodal curve readily develops in a muscle when disease, pregnancy, or nutritional status prompts withdrawal of muscle protein for general maintenance. This also increases the likelihood that large fibers will be round rather than polygonal in routine preparations.

Microscopic structure

The **structure of the muscle cell** or muscle fiber is quite well defined (Fig. 2.2). The outer component is a thin amorphous, but apparently quite tough, *basal lamina* consisting of three layers which, on most muscle cell surfaces, seems to be thrown into gentle folds. Atrophy of the fiber leads to much more obvious accordion-like folds of the basal lamina. *Within this basal lamina are two separate cell populations with very similar nuclei: the multinucleate myofiber, and the small, more numerous satellite cells,* which play an important role in fiber repair and regeneration (Fig. 2.3A, B). The nuclei of both cell types are oriented to the long axis of the muscle fiber and are distributed regularly in a spiral manner. In normal muscle, less than 3% of the nuclei of the multinucleate myofiber cell are displaced internally, but

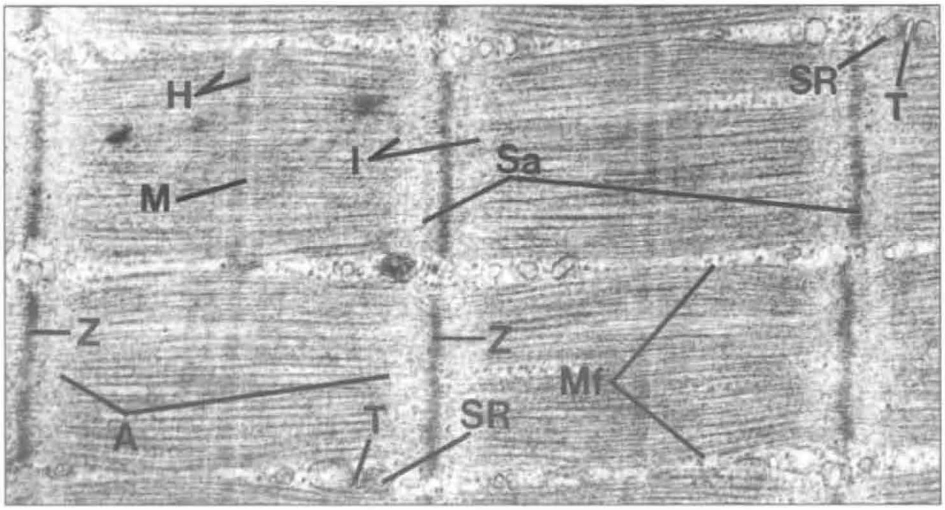

Figure 2.2 Normal horse muscle. Anisotropic band (A), H band (H), isotropic band (I), M line (M), myofibril (Mf), one sarcomere (Sa), sarcoplasmic reticulum (SR), T-tubule (T), Z band (Z). (Courtesy of TJ Hulland.)

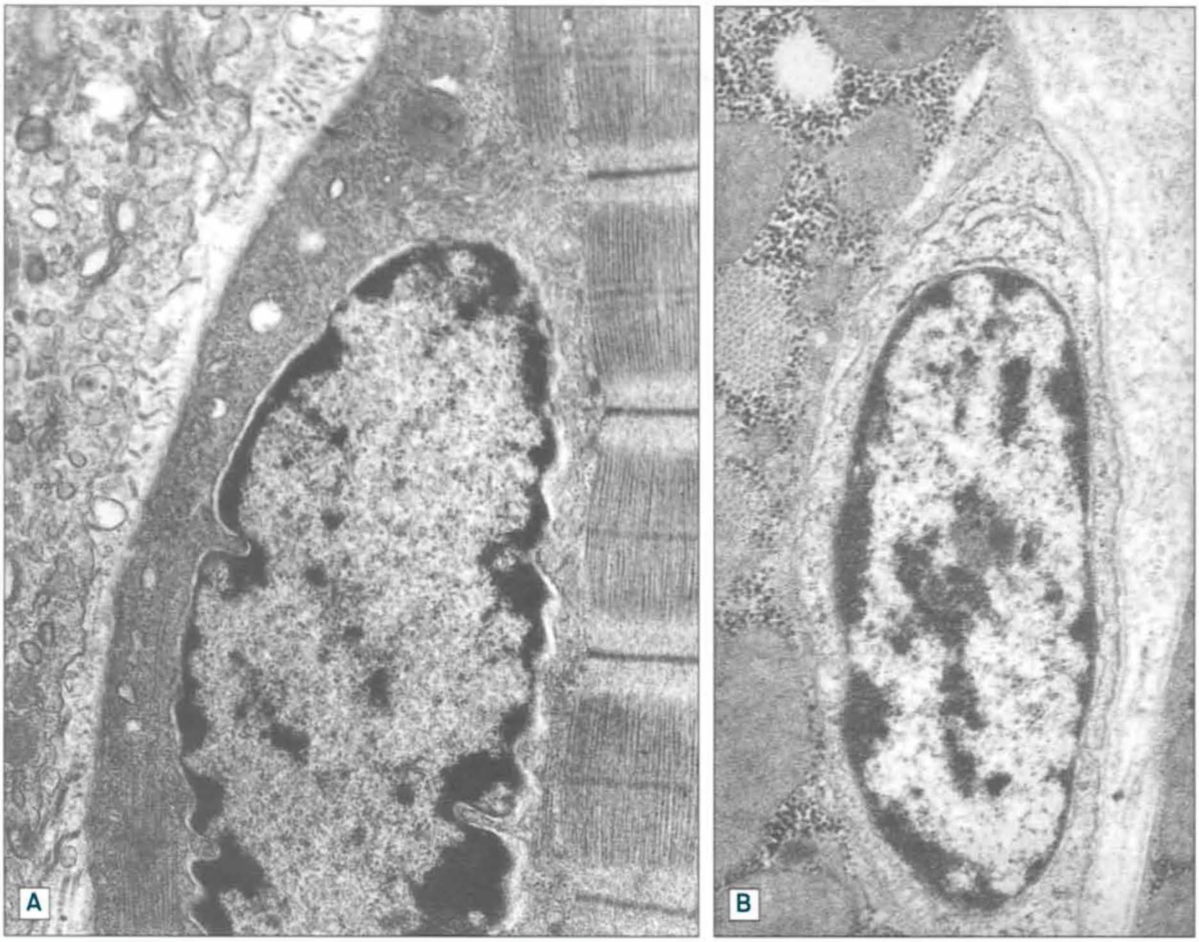

Figure 2.3 **A.** Myofiber nucleus and adjacent myofibril. **B.** Satellite cell nucleus. (Courtesy of TJ Hulland.)

the number tends to be higher adjacent to points where muscle and tendon interdigitate, or where muscle and bone meet through a short ligament or tendon. Nuclei are slender, oval, have evenly distributed chromatin and single, small nucleoli. The **satellite cells**, initially described by Mauro in 1961, consist of a simple cell membrane around a nucleus, a minimum of cytoplasm with mitochondria and a scant tubular system, all of which lie in a shallow indentation on the myofiber surface within the basal lamina. Satellite cells constitute 1–30% of the visible nuclei associated with a muscle fiber; the higher figure describes the neonatal state, the lower, old age. In mature muscle, 3–5% of the nuclei are satellite cells; a modest concentration of them occurs adjacent to motor end plates.

Satellite nuclei have been thought to be the only ones in muscle capable of mitotic division in postnatal life, and each time these cells divide in a growing muscle they contribute one myocytic nucleus and a minute amount of cytoplasm to the growing pool of nuclei in the enlarging multinucleate, myofiber cell. The other daughter cell remains as part of the satellite cell pool outside the myocyte and retains mitotic capability. It is clear that this mechanism will make the satellite cells a progressively smaller proportion of the total nuclear population within the basal lamina since their number remains constant from birth while the nonreplicating postmitotic myofiber nuclei increase in number cumulatively.

New concepts are emerging on the exclusive role of satellite cells in postnatal growth, repair and maintenance of skeletal muscle. Recent studies in mice have demonstrated that *hematopoietic stem cells from bone marrow may participate in regeneration of skeletal muscle.* However, the contribution of bone marrow-derived myogenic progenitor cells in the overall process of muscle cell regeneration, a process previously thought to exclusively involve satellite cells producing myogenic precursor cells, is still unclear. The potential may now exist for therapy by delivery of bone marrow-derived stem cells, via injection into the circulation, to damaged muscle cells to augment regeneration in diseases such as muscular dystrophy where the satellite cell population has become depleted and/or ineffective.

Ultrastructure

The most distinctive characteristic of skeletal muscle cells is the presence of **striated myofibrils**, approximately 0.5–1.0 μm in diameter, consisting, in cross-section, of thousands of regularly sized and regularly oriented **myofilaments** (Fig. 2.2). In longitudinal section, *contractile units or sarcomeres* about 20 μm long extend from one Z band to the next, and consist of bands and lines created by the stacking of thick and thin filaments with protein aggregates at certain places. The *thin filaments* (6 nm wide; actin, tropomyosin, and troponin) are fixed into the electron-dense *Z band* of noncontractile protein and incompletely overlap the *thick filaments* (16 nm wide; myosin). The zone of no overlap adjacent to the Z band is referred to as the *I band*. The central zone between the ends of the thin filaments in which only thick filaments are visible is the *H band*, and this is divided by a *thin dense line of M substance* on each filament, and collectively these create the *M line*. The *A band* is the wide central zone which extends from one end of the thick filaments to the other and alternates with the I band. *In sarcomere contraction, Z bands move closer together reducing I and H band widths.* In cross section at a central point where thick and thin filaments are present, one myosin filament is surrounded at regular intervals by six thin filaments, or 12 when the sarcomere is in strong contraction.

Myofibrils are surrounded by *sarcoplasm*, which makes up 30–40% of the fiber volume, and in it are elements of the T-tubular system, the sarcoplasmic reticulum, mitochondria, lysosomes, glycogen granules, and often, fat droplets. The endoplasmic tubular systems in the muscle fibers of all mammals regularly come together to surround myofibrils and meet in triads with the T tubule between two tubular segments of sarcoplasmic reticulum at frequent regular intervals. In many animals these triads can be found at each end of each sarcomere, an arrangement which ensures very extensive direct surface membrane contact between the cell surface, the sarcomeres and the endoplasmic reticulum. Sarcoplasm between the myofibrils and the outer cell membrane is often rich in mitochondria and glycogen granules.

Quite apart from their ability to shorten in active contraction, *myofibrils are capable of easy and rapid length adjustment accomplished by shedding or adding one or more sarcomeres.* Under ideal experimental circumstances, up to 25% of the sarcomeres may be lost in a 24-hour period, a statistic which emphasizes that, even in the healthy animal, there is potential for a considerable flux of muscle protein to allow for rapid dismantling and reconstruction of myofibrils. Muscle has a high requirement for arginine, glucogenic amino acids, and energy, reflected in part by the high turnover of glycogen in normal muscle. The breadth of myofibrils increases or decreases equally rapidly by building or discarding myofilaments. When new myofibrils are required, an existing large myofibril splits longitudinally, but when atrophy reduces the need for some myofibrils they are simply dismantled sarcomere by sarcomere or reduced in size by destruction of the most peripheral myofilaments.

Outside the muscle fibers and their satellite cells are *three magnitudes of connective tissue framework.* Intimately applied to the basement membrane of each muscle fiber is the net-like **endomysium,** which carries the capillary network with its longitudinal orientation. Around each primary bundle of 40–150 fibers is the thicker **perimysium** in which run larger vessels, nerve trunks, and sensory neuromuscular spindles. Around the outside of the muscle, or a major head of a muscle, lies the **epimysium,** which carries tendon organ sensory endings and sometimes prominent tendinous bands.

Histochemical fiber types

Types of muscle fibers may be distinguished by many histochemical and immunohistochemical techniques available for studying the enzyme components; each new technique demonstrates an ever-expanding heterogeneity of muscle fibers. Many of these techniques subdivide the muscle fiber population in the same ways, and in many instances the subdivisions seem to bear little relationship to the biological response created by disease. The two techniques that seem best to identify different biological activities are the *alkaline- and acid-resistant adenosine triphosphatase procedures* and the *nicotinic acid dehydrogenase tetrazolium reductase (NADH-TR) test* for oxidative enzyme activity. These allow the division of the muscle population into two major groups and several subgroups (Fig. 2.1A, B):

- **type 1 fibers** rich in oxidative enzymes and showing slow-twitch, red color characteristics (myoglobin); generally assumed to be fibers with a predominantly oxidative energy source appropriate for sustained activity;
- **type 2 fibers** rich in glycogen showing a fast-twitch response and paler color; generally assumed to be fibers with a predominantly glycolytic energy source appropriate for short-term, strong activity.

Both types of fiber contain a broad spectrum of enzymes, which makes them capable of a wide range of biochemical activity and, in certain circumstances, an interconversion of working types.

Some species, particularly the pig and the horse, have fibers apparently rich in both oxidative and glycolytic ingredients, and *many domestic animals have fibers that lack the clear-cut divisions that seem evident in the muscles of man and laboratory animals.* The type 2 fibers can be histochemically subdivided by ATPase response into subtypes but, for purposes of predicting biological activity and identifying diseases, the two main types seem to have the greatest relevance. A special subgroup of the glycolytic, type 2 group found in the masticatory muscles of the dog represent an exception to this because they display antigenic differences from other muscles and this makes them vulnerable to a unique disease process. Examination of a muscle in which about equal numbers of type 1 and type 2 fibers are present might suggest a random checkerboard distribution of the types; however, *type 2 fibers are disproportionately found on the periphery of primary bundles, and type 1 fibers are disproportionately at the center of nests of fibers within the primary bundle* (Fig. 2.1A, B). These observations are consistent with knowledge of the late fetal fiber evolution.

Muscles used repetitively but slowly and persistently should have high levels of type 1 fibers while those used for short bursts of activity should have high levels of type 2 fibers. This is often the case; for example the diaphragm of cattle often has 80–95% of type 1 fibers while most other skeletal muscles have 10–60% type 1 fibers. On the other end of the scale, the longissimus and semitendinosus muscles, the two muscles which are most enlarged in racing Greyhounds and Thoroughbred horses, have ~80–95% type 2 fiber content. Obligatory postural muscles, such as the supraspinatus, tend to have a 40–50% type 1 component, but so do muscles which are used relatively sparingly. Individual variation in fiber proportions is quite wide, and this is magnified by breed, muscle region, and species differences. The anatomic position and the function of a muscle in an animal can provide only a general indication of its fiber composition. In the horse, the proportion of type 1 fibers increases in deeper levels of large muscle masses, and the very deep postural muscles of the limbs, such as the medial head of the triceps and the vastus intermedius, are almost entirely type 1 fibers.

Specialized structures

Sensory muscle spindles *are found in all skeletal muscles in and anchored to the perimysial connective tissue and associated with a small nerve radicle, which contains 6–20 large sensory fibers.* They are more numerous in some muscles than others and are generally difficult to find in the larger muscles of mature animals. Spindles are about 0.5–3.0 mm long and 200–500 μm wide (Fig. 2.4). The intrafusal (central) space

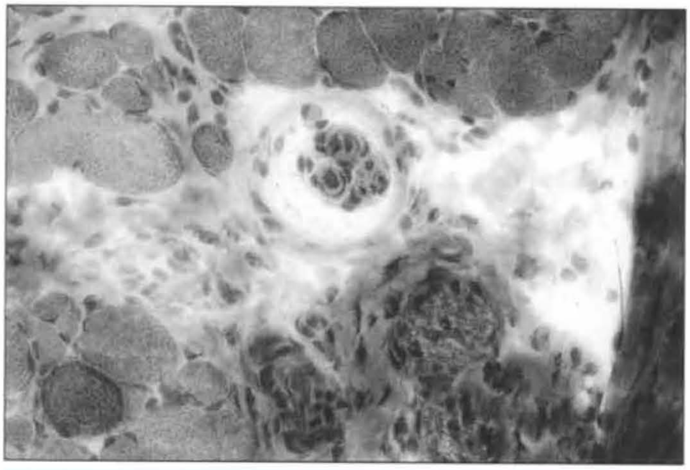

Figure 2.4 Sensory muscle spindle in cross-sectioned normal muscle. Frozen section, modified Gomori's trichrome.

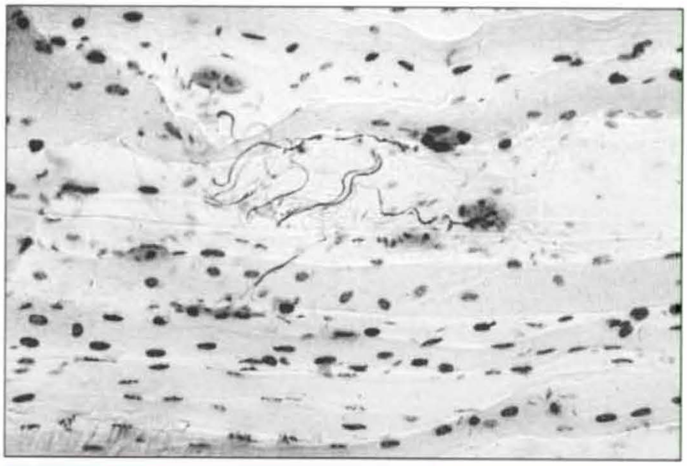

Figure 2.5 Motor end-plate in longitudinal section of canine muscle. Silver stain. (Courtesy of BJ Cooper.)

enclosed within the fibrous, multilayered outer sheath is continuous with lymphatic space and contains 2–20 specialized *small intrafusal muscle fibers*. The intrafusal fibers are histochemically different from the extrafusal fibers but share the same motor nerve. However, intrafusal fibers are innervated by a different, smaller group of motor neurons than those innervating extrafusal fibers. The intrafusal fibers have a high content of nuclei often contained in a central "nuclear bag" region, or linearly distributed up the middle of the small round fibers. Abundant sensory axons entering the spindle have "flower-spray," "grape-cluster," or spiral endings on the intrafusal fibers, and these structures collectively give rise to impulses, which record muscle stretch and contribute to proprioception. *Complex spindles* consisting of 2–10 spindles, incompletely overlapping end on end, are sometimes found in skeletal muscles and may be an expression of anomalous development. Intrafusal fibers generally do not participate in the pathologic processes, which involve extrafusal fibers, with the possible exception of nutritional myopathy. Intrafusal fibers are also resistant to atrophy.

Motor end-plates *are the sites of synaptic transmission of acetylcholine from nerve ends to muscle receptors at which sites a surface-membrane-conducted, polarizing impulse is initiated.* The interface takes the form of a complex, pretzel-shaped neural termination, which is pressed into a shallow but convoluted, cholinesterase-laden, synaptic gutter of matching shape on the myofiber surface. The end plate is contained within the endomysium, and extensions of the endomysial connective tissue ensheath the bare terminal axon for a short distance until its myelin sheath is reached (Fig. 2.5). A little more than one end-plate for each muscle fiber exists in skeletal muscle, and although supernumerary endings (subterminal sprouts) can develop, such structures are rarely found in normal muscle. One motor neuron gives rise to an extremely variable number of terminal axons with one end-plate each, the number being inversely proportional to the fineness of motor movement required of the muscle, and directly proportional to the relative diameter of the muscle fibers. Thus, in the extrinsic ocular muscle the axon:end-plate ratio may be as low as 1:10 on small round muscle fibers, whereas in the gastrocnemius or gluteus muscles the ratio may be 1:2000 on muscle fibers near the upper size limits. Terminal axons are given off at nodes of Ranvier in clusters of 5–30 per node to make muscle end-plate contact locally via a short, fine terminal axon. Although a low neural impulse level to skeletal muscle is translated into contraction of only a proportion of the muscle fibers, this is clearly not a form of muscle regulation that can lead to finely controlled movement and consequently fine movement must be controlled by a low neural ratio.

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DISTURBANCES OF GROWTH AND POSTMORTEM ALTERATIONS

As with many tissues, muscle has a fairly limited repertoire of changes. Many of the same histopathologic features are seen in different muscle disorders. Determination of possible causes for muscle changes often requires careful evaluation of the signalment, clinical history, and clinicopathologic findings as well as gross and histopathologic findings. *Muscle atrophy, hypertrophy*, and *postmortem changes* are commonly encountered in pathologic specimens and are the focus of this section. Degenerative and regenerative reactions are discussed in the next section.

Atrophy

Muscle atrophy *can refer to reduction in overall muscle mass.* Reduction in mass most often reflects *decreased myofiber diameter* that can involve all fibers uniformly or that can selectively involve muscle fiber types. Histochemical evaluation of fiber types is often necessary to clearly distinguish the type of atrophy present. In many circumstances, type 2 glycolytic fibers are more sensitive to atrophy than are type 1 oxidative fibers. Morphometric analysis of overall fiber diameter may be necessary to confirm a decrease in muscle mass due to mild overall reduction of myofiber diameter.

All fibers undergoing a reduction in size *lose myofilaments and myofibrils* by a process of more or less simultaneous disaggregation of actin and myosin filaments and disintegration of the protein of Z bands. Briefly, in the sequence, a slight sarcoplasmic space appears between the shrinking myofibrillar mass and the plasma membrane, but the membrane (and the basal lamina) soon begins to wrinkle and condense to accommodate the new slimmer lines. After a brief lag, mitochondria, glycogen granules, and lysosomes are reduced in number and size. After a slightly longer lag phase, the tubular systems are reduced in volume. Removal of cytoskeletal proteins, organelles, and glycogen is by autophagy. Atrophy does not involve sarcolemmal damage and therefore is not associated with increased serum activity of CK or AST. Provided that the time interval is relatively short, the influences inducing atrophy are removed, and the muscle environment is returned to normal, *the sequence can be reversed* and fibers can be restored to normal size.

Denervation atrophy

Myofibers that have lost connection with peripheral nerves due to neuropathy or neuronopathy undergo rapid and severe atrophy due to denervation. This form of atrophy has also been referred to in the somewhat contradictory terms "neurogenic atrophy." Denervation atrophy is characterized histologically by *severe fiber atrophy involving type 1 and type 2 fibers*. Mild denervation will result in scattered single or small contiguous groups (*small group atrophy*) of severely atrophied fibers compressed into angular shapes (*angular atrophy*) by adjacent innervated fibers (Fig. 2.6). More extensive denervation will result in large groups of small rounded fibers (*large group atrophy*), as there are no innervated fibers in the area to cause angular change in the denervated fibers (Fig. 2.7). Even in the absence of histochemical preparations, muscle containing extensive small and large group atrophy is most likely to be denervated, and a careful examination of intramuscular nerves (Fig. 2.8), peripheral nerve trunks, ventral nerve roots, and motor neurons is indicated. In addition to those features of atrophy described above, the rapid and severe atrophy due to denervation often results in clustering of myonuclei (nuclear clumping).

Denervation atrophy is relatively common in animals. It is part of many congenital dysplasias involving skeletal muscle that cause contracture or arthrogryposis. Denervation atrophy is always accompanied by muscle weakness or paralysis, but the clinical signs may be mild if the nerve is small or the damage is mild. Some of the best-known examples of denervation atrophy in animals are *laryngeal hemiplegia in horses* caused by axonal degeneration of the left recurrent laryngeal nerve, injury to the supraspinatus nerve by trauma or the pressure of a poorly fitting collar in a work horse ("*sweeney*"), symmetrical (such as due to equine motor neuron disease), or

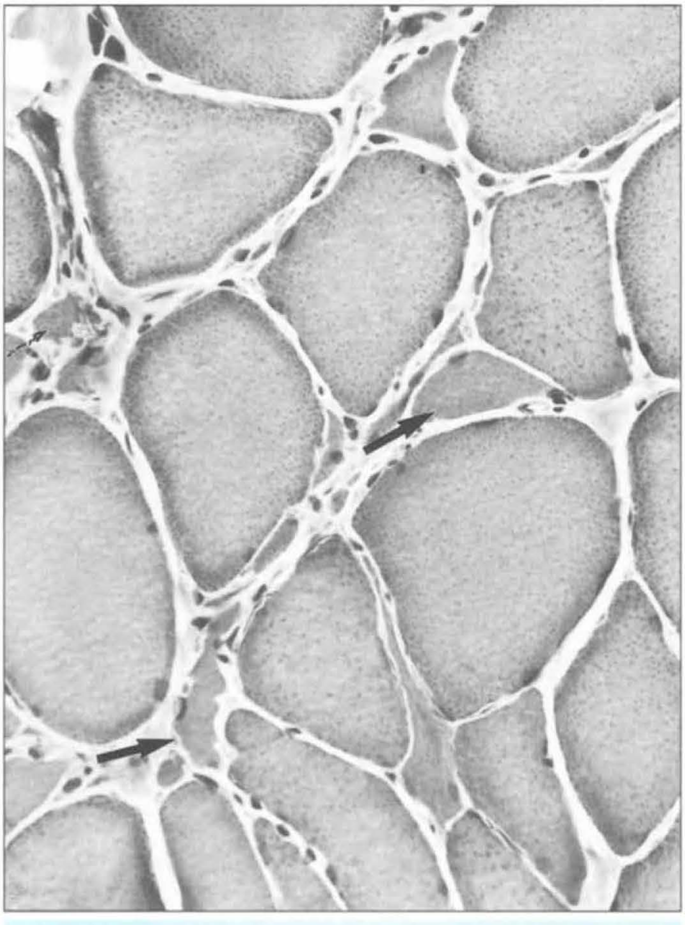

Figure 2.6 Severe angular atrophy (arrows) of small continguous groups of fibers (small group atrophy) due to denervation in a dog. (Courtesy of TJ Hulland.)

asymmetrical (the hallmark of equine protozoal myeloencephalitis) *gluteal atrophy in the horse*, and *radial or brachial paralysis in dogs and horses* due to trauma. Lesions involving the ventral gray matter of the spinal cord or the ventral roots emerging from the spinal canal, and inherited or acquired peripheral neuropathies, are also common causes of denervation atrophy.

Denervation atrophy is rapid and severe. It is accompanied by abnormal spontaneous activity (fibrillations, positive sharp waves, and sometimes myotonic bursts) detectable with concentric needle electromyography. *Within 2–3 weeks, two-thirds of the muscle mass can be lost.* This reduction in mass may be readily observed or may require careful palpation of muscle mass for detection. Given the variable muscling of different breeds of dogs and horses, a diagnosis of symmetric muscle atrophy can be difficult.

As stated above, *the hallmark of denervation atrophy is involvement of both type 1 and type 2 fibers.* Type 2 fibers can, however, be preferentially atrophied, especially early on in the denervation process. The denervation of equine motor neuron disease is somewhat unique, in that there is preferential atrophy of type 1 fibers, presumably due to oxidative injury to type 1 motor neurons due to vitamin E deficiency (Fig. 2.9). The fiber type of the muscle is determined by the electrical activity of the nerve fiber supplying that motor unit. If a denervated motor unit is reinnervated by terminal nerve sprouts from an adjacent intact nerve, the fiber type will be converted to that of the newly innervating nerve, and the reinnervated myofibers will rapidly regain normal diameter. Thus, alteration of the normal mosaic distribution of fiber types to one of clusters of type 1 and type 2 fibers (*fiber type grouping*), which can only be demonstrated on histochemical preparations, is characteristic of denervation followed by reinnervation (Fig. 2.10). If there is subsequent degeneration of the reinnervating nerve this will result in denervation atrophy of a group of fibers all of the same fiber type (*type-specific group atrophy*). This finding is indicative of on ongoing denervating process. Although such disorders occur, it is curious that type-specific group atrophy is rarely seen in animals.

Severe and chronic denervation will be accompanied by a variable degree of endomysial fibrosis. Admixed innervated fibers often undergo

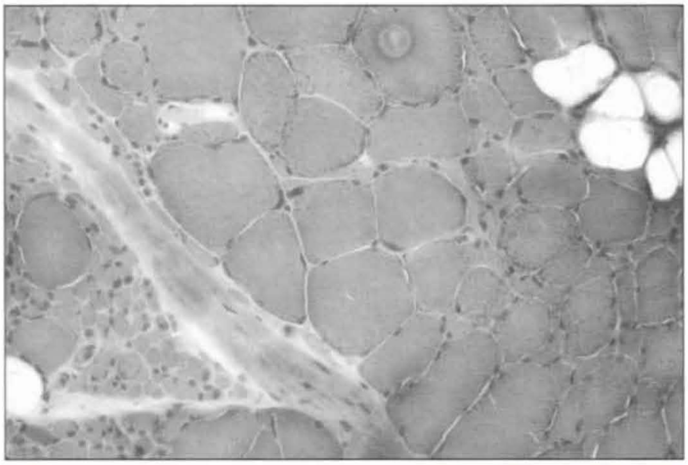

Figure 2.7 Small and large group atrophy in the vastus intermedius muscle of a horse with denervation due to equine motor neuron disease. Denervated fibers are admixed with moderately hypertrophied fibers. Frozen section, modified Gomori's trichrome. (From McGavin MD, Zachary JF. Pathologic Basis of Veterinary Disease, 2006, with permission of Elsevier Ltd.)

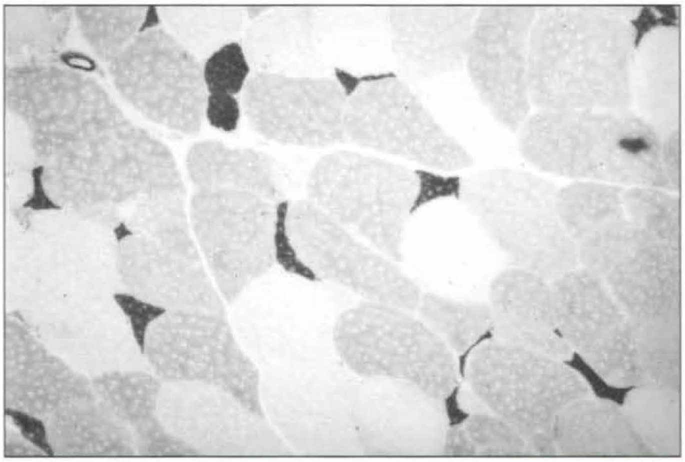

Figure 2.9 Selective angular atrophy of type 1 (dark staining) fibers in a horse with equine motor neuron disease. Frozen section, ATPase, acid preincubation.

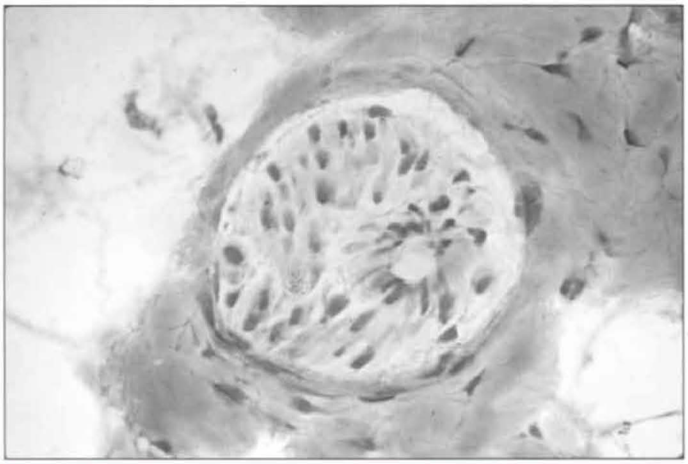

Figure 2.8 Intramuscular nerve from a horse with equine motor neuron disease. There is marked **loss of myelinated fibers** (only 1 remains), with endoneurial fibrosis. Frozen section, modified Gomori's trichrome.

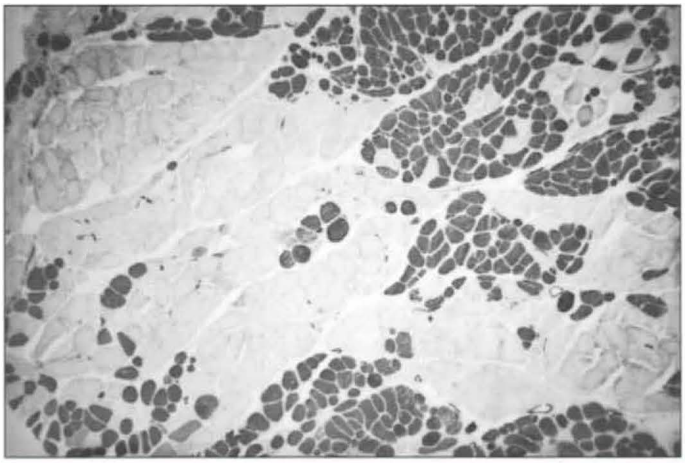

Figure 2.10 Fiber type grouping of type 1 (dark staining) and type 2 (light staining) fibers due to denervation and reinnervation in the laryngeal muscle of a horse. Frozen section, ATPase, acid preincubation.

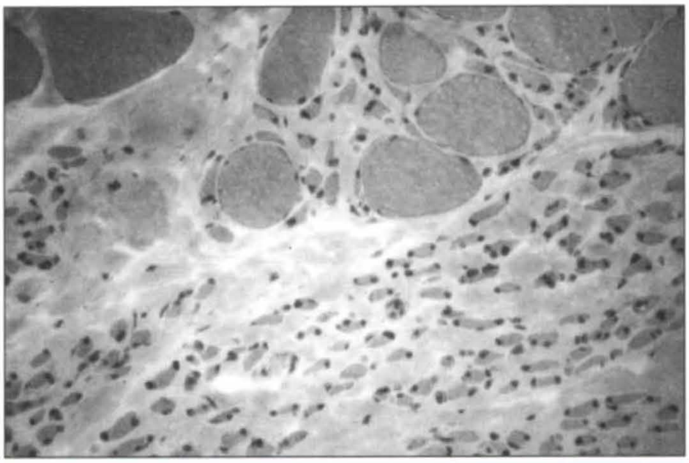

Figure 2.11 Severe large group atrophy with marked endomysial fibrosis due to chronic denervation in the medial head of the triceps of a horse with chronic equine motor neuron disease. There is severe compensatory hypertrophy of other fibers. Frozen section, modified Gomori's trichrome.

compensatory hypertrophy (see below; Figs 2.7, 2.11). Although both type 1 and type 2 fibers will undergo hypertrophy, type 1 muscle fibers appear to be somewhat resistant to denervation atrophy in many circumstances, and may be the predominant hypertrophied fiber type seen. Even without histochemical preparations to determine fiber types, *a pattern of large-diameter fibers admixed with severely atrophied fibers is very characteristic of severe and chronic denervation.* In very severe cases, there can be eventual loss of denervated myofibers, with replacement of muscle by fibrous connective tissue and often also by fat. Determining the cause of the severe atrophy in such "end-stage muscles" can be very difficult, as both severe chronic neuropathic processes and severe chronic myopathic processes are possible.

Disuse atrophy

Disuse atrophy *occurs due to decreased contractile activity of innervated muscle.* Decreased muscular activity due to painful lameness, bone fracture or disease, or limb immobilization are most common. Disuse atrophy in humans and experimental animals *classically involves predominantly type 2 fibers*, although this pattern is not seen in all muscles in people with disuse atrophy. Although a type 2 predominant atrophy is seen in some cases of disuse atrophy in domestic animals, in many cases there is overall atrophy of all fiber types with no clear preferential pattern. As no workload is imposed on muscle fibers undergoing disuse atrophy, there will be no compensatory hypertrophy of fibers.

Atrophy of cachexia

Atrophy of cachexia and malnutrition *occurs when an animal is unable to supply enough dietary nutrients to maintain muscle; muscle proteins become the source of nutrients for the rest of the body.* One to five per cent of the contractile muscle substance is dismantled each day, and in normal animals an equal or greater amount is reconstructed. In view of the large bulk of the body muscle, this represents a very large amount of protein that can be borrowed on a daily basis. Net loss of muscle protein probably starts hours after negative nitrogen balance has been reached. The muscle atrophy of cachexia associated with chronic illness and neoplasia is hastened by circulating cytokines such as *tumor necrosis factor* ("*cachectin*"), which act systemically to increase catabolism, including catabolism of myofibers. The atrophy of cachexia is gradual, and the process may take years if the net withdrawal of muscle protein is subject to fluctuations, or is irregular or slight.

In the dog, *atrophy of temporal muscles* is often prominent and can occur fairly rapidly in animals ill for any reason. The *back and thigh muscles* are also susceptible to severe atrophy due to cachexia. Histochemically, *type 2 fibers are depleted preferentially in cachexia* (Fig. 2.12A, B). Similar to the case in denervation atrophy and some cases of disuse atrophy, type 1 muscle fibers are resistant to atrophy due to cachexia. In most cases, the degree of atrophy achieved through cachexia is not as severe as that seen in denervation atrophy, and the history will provide clinical features and a time frame for the atrophy that should enable differentiation of cachexia from disuse and denervation atrophy.

Atrophy of endocrine disease

Neuromuscular weakness and muscle atrophy can accompany hypothyroidism and hypoadrenocorticism in the dog. In both disorders, *selective atrophy of type 2 fibers* is seen (Fig. 2.13). No compensatory type 1 hypertrophy occurs. The finding of contiguous groups of mildly angular atrophied fibers can suggest denervation atrophy, and histochemical preparations to reveal the selective type 2 involvement may be necessary. In general, the atrophy of type 2 fibers in endocrine myopathy is not as severe as the atrophy of denervation. *Selective type 2 fiber atrophy due to endocrine disease must be differentiated from that of cachexia or disuse.*

Myopathic atrophy

Atrophy of fibers commonly occurs in myopathic conditions, and contributes to the nonspecific myopathic change of increased fiber size variation. Selective type 1 fiber atrophy is seen in many congenital myopathies in humans, and also occurs in cats with nemaline myopathy (Fig. 2.14). In such cases, *the use of histochemical procedures is essential to distinguish the selective type 1 atrophy that is suggestive of primary myopathy*. Other myopathies result in mild rounded atrophy of both fiber types, usually occurring randomly throughout the sections. Chronic myopathic change often involves hypertrophy (see below) as well as atrophy. Hypertrophied fibers are prone to fiber splitting, and some of the rounded small-diameter fibers interpreted as atrophic in chronic myopathic conditions may represent fiber splitting.

Hypertrophy

As with atrophy, **hypertrophy** *can refer to the muscle as a whole or to increased diameter of myofibers.* Overall muscular hypertrophy occurs in cattle as an inherited defect in myostatin, resulting in an increased number of otherwise relatively normal-diameter fibers ("*double muscling*"). Overall muscular hypertrophy occurs due to physiologic increase in myofiber diameter due to *exercise conditioning*. The muscles of animals with myotonic myopathy often appear enlarged, which may be due to prolonged muscle contraction as well as to myofiber

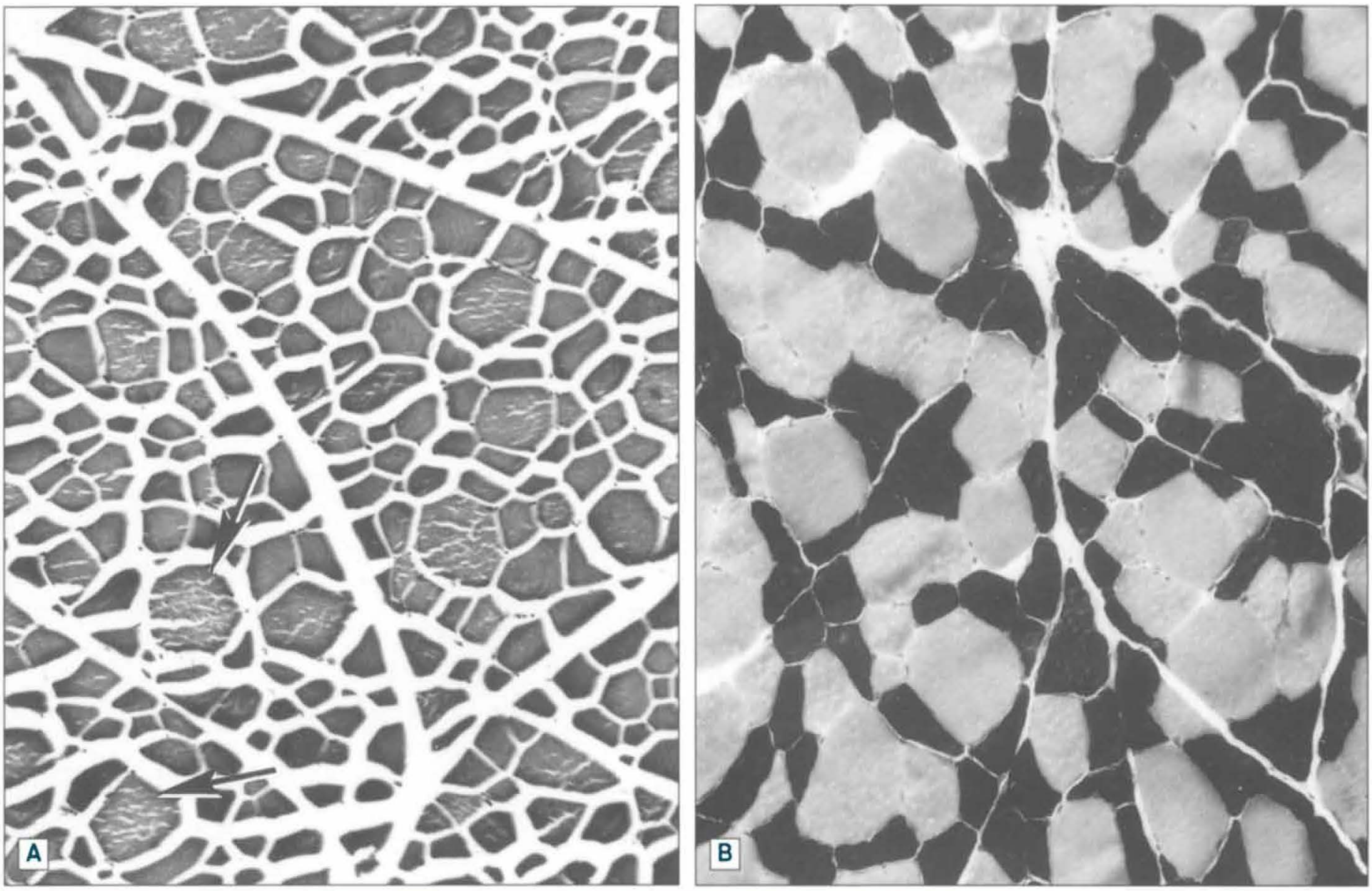

Figure 2.12 **Atrophy of cachexia**. **A.** Atrophy-resistant type 1 fibers are prominent (arrows) in formalin-fixed muscle from a calf; H&E stain. **B.** Preferential atrophy of type 2 (dark-staining) fibers in an emaciated sheep; frozen section, ATPase, alkaline preincubation. (Courtesy of TJ Hulland.)

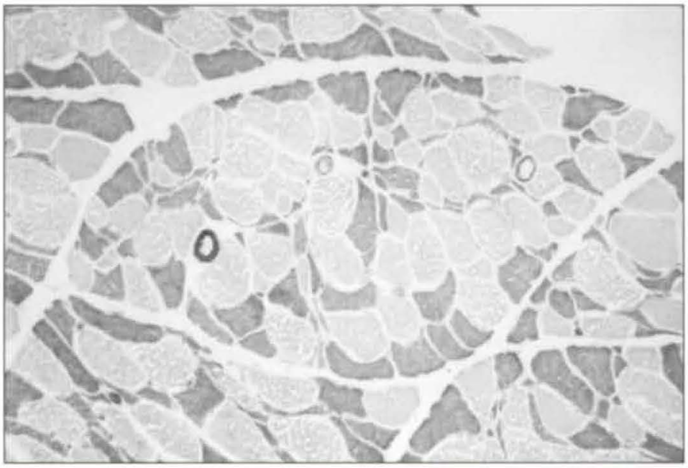

Figure 2.13 Preferential atrophy of type 2 (dark staining) fibers in a dog with **hypothyroid myopathy**. Frozen section, ATPase, alkaline preincubation.

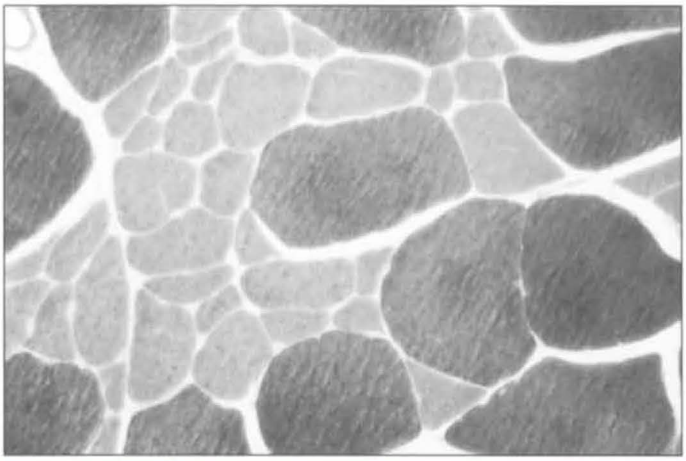

Figure 2.14 Selective atrophy of type 1 (light staining) fibers in a cat with congenital **nemaline myopathy**. Frozen section, ATPase, alkaline preincubation.

hypertrophy. Muscular hypertrophy is a characteristic feature of Duchenne-type X-linked muscular dystrophy in the cat, and occurs to a lesser degree in dystrophic dogs. Individual myofiber hypertrophy is often seen histologically in myopathic and neuropathic conditions in which there is overall reduction in muscle mass due to concurrent myofiber atrophy or loss. Lastly, muscle may appear enlarged due to pseudohypertrophy, as in chronically damaged muscles replaced by masses of fibrous tissue and/or fat, or due to fascial thickening in cats with fibrodysplasia ossificans progressiva.

Physiologic hypertrophy

Myofiber hypertrophy is considered physiologic when it occurs due to increased workload. This is a desirable process in athletes, and is accomplished through exercise conditioning. This process of physiologic hypertrophy of fibers is *accomplished by adding sarcomeres, by adding myofilaments to the periphery of myofibrils, and by adding new myofibrils to the existing ones by a process of longitudinal splitting.* There are sharp upper limits for this type of hypertrophy, and for animals it lies somewhere between 80 and 100 μm in diameter.

Compensatory hypertrophy

Compensatory myofiber hypertrophy occurs in muscle in which some myofibers are weak or dysfunctional due to myopathic or neuropathic disorders. In a sense, *this is a type of workload-increased hypertrophy* imposed on fibers less severely affected or unaffected by the myopathic or neuropathic condition. In such cases, fibers of 150–200 μm diameter can develop. Hypertrophied fibers in myopathic and neuropathic conditions are often abnormal, and may contain one or more internal nuclei, may undergo longitudinal splitting, or can develop bizarre cytoarchitectural disarray such as the formation of ring fibers and whorled fibers (Fig. 2.15). Longitudinal splitting allows one or more capillaries to be located near the center of the muscle fiber (Figs 2.16A, B, 2.17). When division is more or less complete, two or often more fibers become arranged in an "orange section" array (Fig. 2.16B) and appear as a cluster of the same fiber type that should not be mistaken for fiber type grouping.

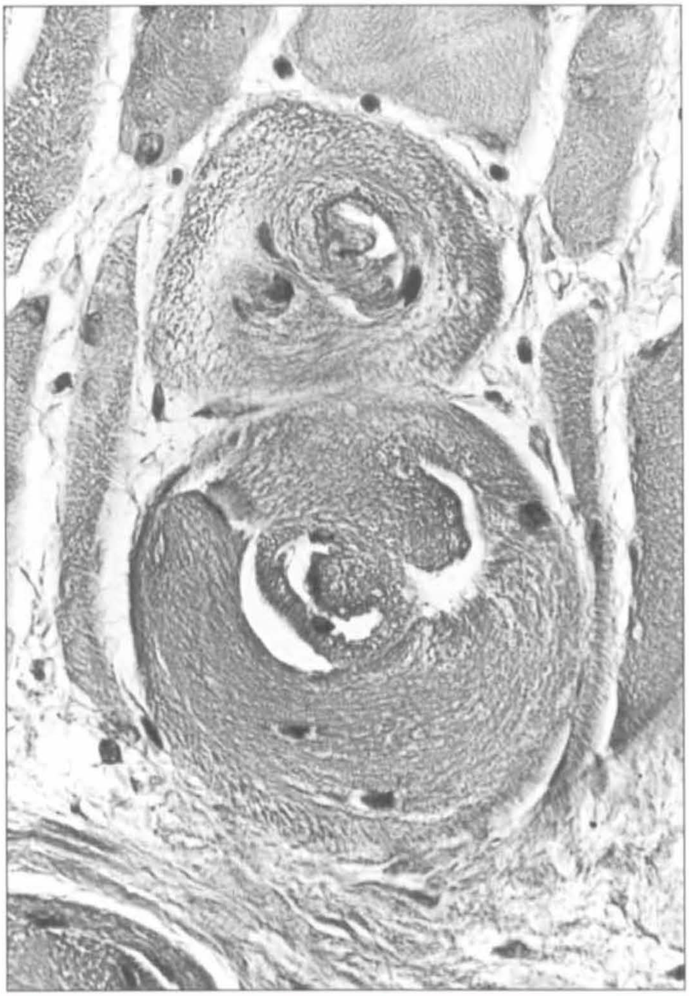

Figure 2.15 Bizarre cytoarchitectural alterations (clefting, whorling and internal nuclei) of **hypertrophied fibers in a denervated muscle**. (Courtesy of TJ Hulland.)

Postmortem changes

It is an unfortunate fact that veterinary pathologists are often involved in the postmortem examination of animals that have not recently expired. *Postmortem changes in muscle are common but variable,* making interpretation of the gross appearance of muscle difficult. In a well-fed animal that dies suddenly, the muscles can become very pale. Pallor may be caused by the accumulation of lactic acid in ischemic muscle. In other animals, particularly those whose glycogen stores have been depleted by chronic disease or malnutrition, the muscles become unusually dark after death. A wide range of differences in color and consistency of muscle normally exists among various species. Pallor of muscles also is present in animals that are anemic from copper or iron deficiency or blood loss, and is often normal in neonatal animals. Dark-red staining of muscle can be indicative of antemortem rhabdomyolysis, or can occur during putrefaction. Given the difficulty in interpretation of gross changes, it behooves the pathologist to take multiple muscle samples for histologic evaluation.

Rigor mortis *is contracture of the skeletal muscle that develops after death.* Rigor mortis is characterized by stiffening of the muscles and immobilization of the joints. It proceeds in orderly fashion from the muscles of the jaw to those of the trunk and then to those of the extremities, and it passes off in the same order. The time of onset, in average circumstances, is 2–4 h after death; maximum rigor is achieved in 24–48 h, after which it disappears. The intensity of rigor varies considerably as does the time of onset. *The factors influencing the time of onset and degree of rigor are the glycogen reserves, the pH of the muscles at the time of death, and the environmental temperature.* Rigor is slight or absent in cachectic or chronically debilitated animals. Rigor occurs with extraordinary rapidity in animals that die during or shortly after intense muscular activity, when muscle pH and glycogen stores are low. Onset of rigor can be delayed in well-rested, well-fed animals. It is hastened in onset and disappearance in a warm environment, and retarded in a cold environment. The chemical events in rigor are a modification of those occurring in normal contraction. Immediately after death, glycogen is converted to lactic acid by anaerobic glycolysis and creatine phosphate is broken down to produce creatine. These are both mechanisms for the resynthesis of adenosine triphosphate from adenosine diphosphate. Rigor will occur when the rate of adenosine triphosphate degradation exceeds its rate of synthesis. Muscle does not require energy to contract, but contraction is dependent on the presence of free calcium ions. Sequestration of calcium requires energy and is necessary for muscle relaxation. *Rigor develops because muscles deprived of energy are unable to maintain calcium in sequestered stores.* The eventual disappearance of rigor, and its failure to develop in cachectic animals, may be due to complete exhaustion of the chemical systems that produce energy and/or myofibrillar protein loss or breakdown.

Skeletal muscle is also prone to various *artifactual changes* that interfere with accurate histologic evaluation. Muscle collected from freshly dead animals, especially horses, is often still capable of vigorous

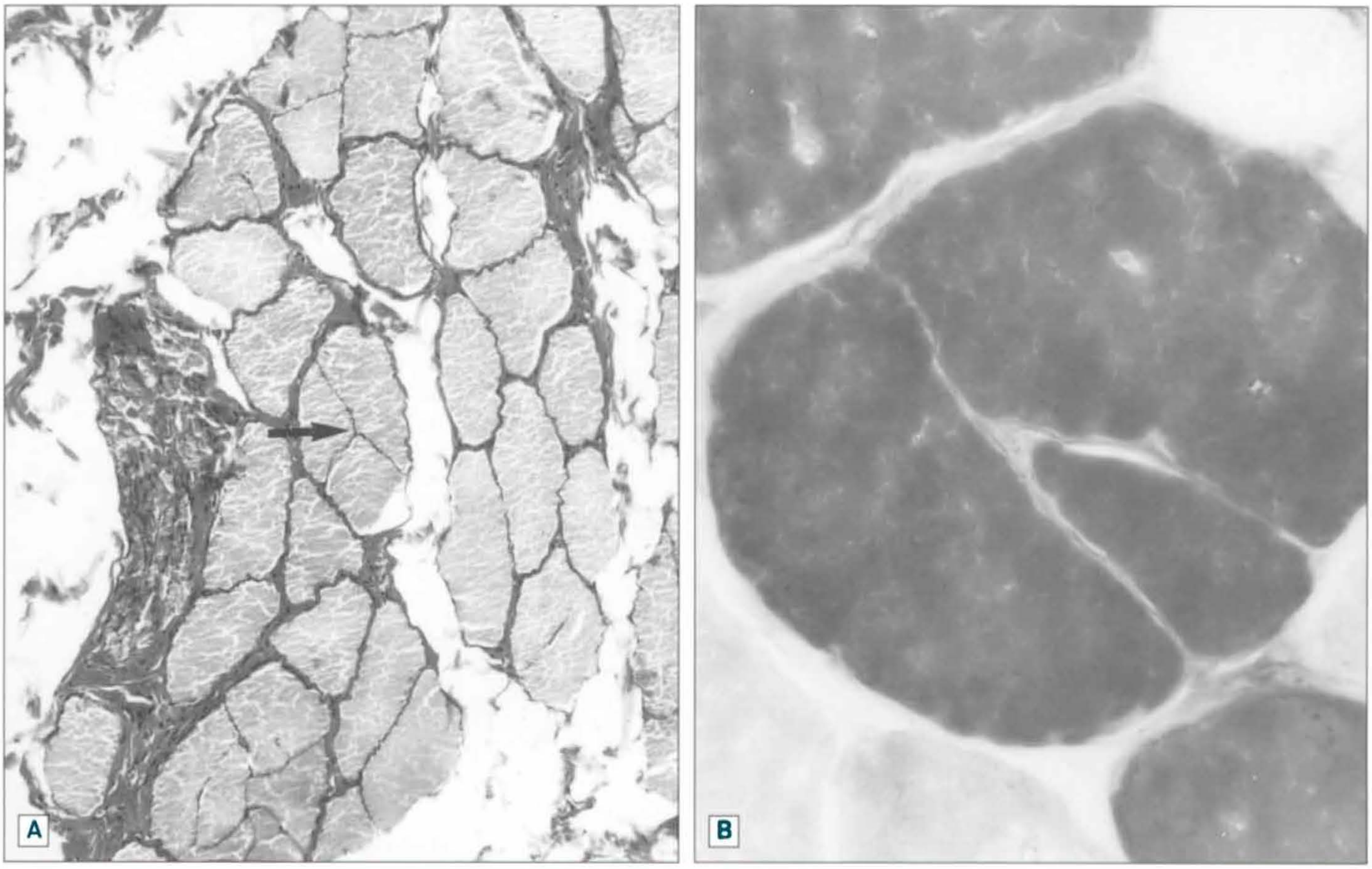

Figure 2.16 Fiber splitting. A. Fiber hypertrophy with fiber subdivisions (arrow). **B.** Hypertrophied fiber with early subdivision ("orange section" array). (Courtesy of TJ Hulland.)

contraction when myofiber ends are cut, allowing calcium-rich extracellular fluid to enter the myofiber and trigger the contractile apparatus. Exposure to formalin can also trigger contraction in fresh muscle samples. Various procedures have been advocated for elimination of the resultant contraction band artifact in muscle. It has been suggested that delayed sampling, following "curing" of the carcass, will result in better histologic preparations. Although the myofilamentous elements of myofibers are somewhat resistant to autolysis, and overall architecture of muscle will be better preserved postmortem than that of nervous tissue or gastrointestinal mucosa, this type of processing must still be considered less than ideal. Rapid postmortem loss of glycogen will often result in inability to diagnose a glycogen storage myopathy, and postmortem alterations in mitochondria and other organelles will interfere with ultrastructural evaluation. Histochemical procedures are also affected by postmortem autolysis, with resultant loss of fiber typing ability.

An extremely valuable procedure for collection of fresh muscle samples at necropsy, but also by biopsy, is *collection of muscle strips in a specially designed muscle clamp*. Samples are clamped in situ, following careful undermining of a strip of longitudinally arranged muscle fibers. The clamp ensures that the calcium influx occurring when fibers are cut transversely does not result in fiber contraction. It is recognized, however, that not all veterinary pathologists will have access to these muscle clamps. Utilization of a similar sampling technique, i.e., the isolation of a longitudinal strip of muscle approximately 2–3 cm long and 1 cm diameter and undermining the strip prior to cutting transversely across myofibers, will minimize artifact during sampling if followed by *pinning the strip to a rigid surface such as a piece of wooden tongue depressor*. This procedure will mimic the action of a muscle clamp and will provide good pathologic specimens for routine pathologic evaluation. Fixation in formalin cooled to refrigerator temperature can aid in preservation of glycogen.

When evaluating skeletal muscle samples, *it is essential to prepare both transverse and longitudinal sections*, and the sampling method described above will help to ensure that such sections are obtained. *Transverse sections* are needed in order to evaluate fiber diameter and presence of internal nuclei, as well as for detection of various cytoarchitectural changes. *Longitudinal sections* are often useful to confirm acute myonecrosis and regeneration as well as to determine the length of segment involved. Intramuscular nerves are often easier to find and to examine in longitudinal muscle sections. The use of *special staining techniques*, such as Masson trichrome stain, reticulin stain, phosphotungstic acid hematoxylin, and periodic acid-Schiff (PAS) stain for glycogen can be particularly useful when evaluating muscle samples fixed in formalin. Interpretation of early degenerative change and its distinction from artifactual or autolytic change sometimes requires all available information about time and circumstances of death as well as clinical signs and biochemical changes shown prior to death.

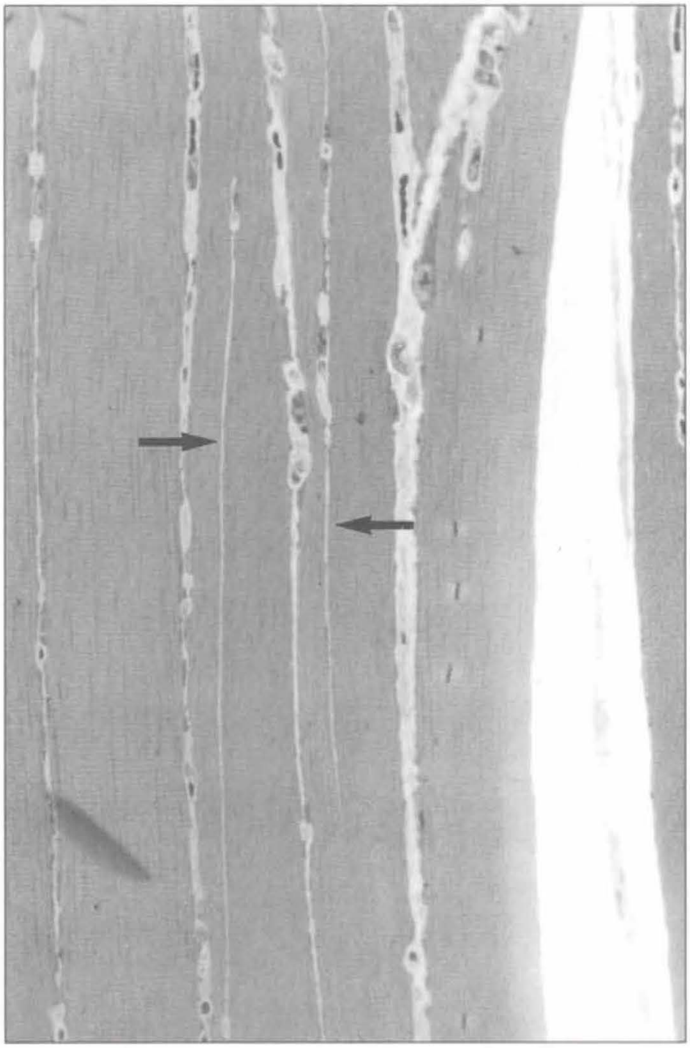

Figure 2.17 Longitudinal splitting in two adjacent fibers (arrows). (Courtesy of TJ Hulland.)

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DEGENERATION AND REPAIR OF MUSCLE

Degeneration may involve parts or all of the cellular structures and often occurs segmentally along the length of the myofibers. The myofibrils alone, or myofibrils and sarcoplasm may undergo degenerative change leaving the sarcolemmal basal lamina, myonuclei, and satellite cells viable and intact. The next level of segmental degeneration leaves only the satellite cell nuclei and the basal lamina in place, and the third level destroys the satellite cells. The fourth level of segmental destruction destroys endomysial connective tissue cells and capillaries as well. *Regenerative responses* differ at each of these levels. Because the myofibers are so long, it is quite possible for a segment or several segments of a fiber to be destroyed without all of the cells being adversely affected although subsequent reparative events sometimes fail to reconstruct the original fiber completely. On the other hand, it is distinctly possible for a fiber to suffer repeated segmental destruction throughout its life span, and to be restored each time to completeness.

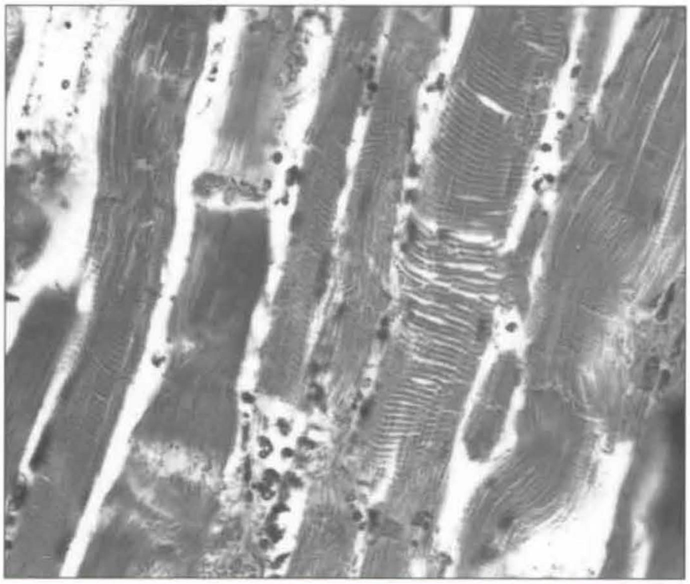

Figure 2.18 Hypercontracted fibers in a downer cow. (Courtesy of TJ Hulland.)

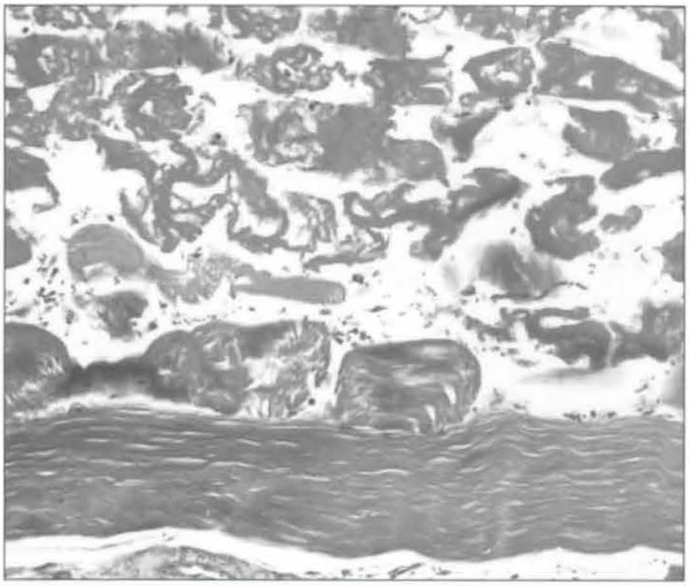

Figure 2.19 Hyaline degeneration in nutritional myopathy in a cow. (Courtesy of TJ Hulland.)

Thus, complete muscle cell death is an exceptional event even in extensive lesions, but destructive events usually have some lasting effect on the shape or number of fibers (see Regeneration and repair of muscle).

Histopathologic evidence of segmental degeneration of muscle fibers along with the events of regeneration and repair are the common expression of many muscle diseases in animals. Kakulas developed a classification for the degenerative lesions of muscle based on spatial distribution and temporal patterns that is often useful to identify broad etiologic categories of muscle disease. *Four categories of muscle injury reactions were established as follows: (1) focal monophasic, (2) multifocal monophasic, (3) focal polyphasic, and (4) multifocal polyphasic* (Kakulas, 1981).

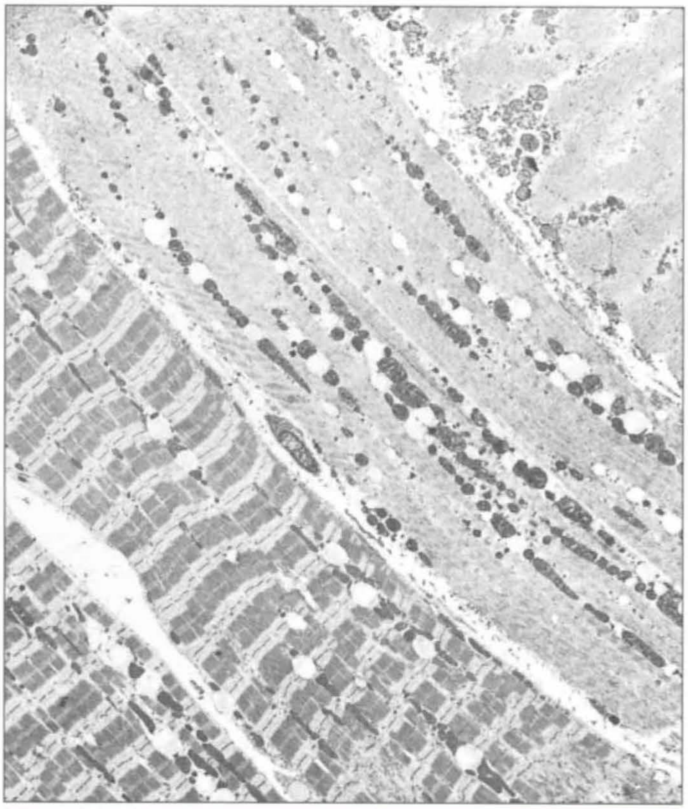

Figure 2.20 Coagulative necrosis (top) and areas of myofibrillar lysis (bottom) in a foal. (Courtesy of TJ Hulland.)

- *Focal monophasic reactions* result from an isolated single mechanical injury, such as external trauma or needle insertion.
- In *multifocal monophasic reactions*, a single insult – such as exposure to various myotoxic drugs, chemicals, or metabolic disorders – may initiate widespread muscle lesions, but all the alterations are in the same phase of injury.
- *Focal polyphasic reactions* result from repeated mechanical injury in the same site.
- *Multifocal polyphasic reactions* are frequent in muscular diseases of animals, and result from continued insults applied over a prolonged time, such as from nutritional deficiencies and genetic disorders (as in muscular dystrophies). The lesions are widespread in the musculature and various pathological reactions – including degeneration, leukocytic invasion during resolution, and regeneration – will occur concurrently.

Muscle fiber degeneration is frequently associated with alterations of cellular membranes, and membrane failure takes the form of segmental myofiber degeneration. Initiation of events that lead to contraction is by nerve-triggered activation of skeletal muscle ion channels resulting in a muscle action potential. Muscle contraction is initiated by passive release of calcium ions from the sarcoplasmic reticulum, but energy is required to recapture calcium ions to allow muscle fiber relaxation. Exhaustion of phosphate-bonded energy reserves often leaves the muscle fiber in a state of calcium-abundant contraction or hypercontraction which soon reduces the complex myofilaments to a coagulum of contractile proteins (Figs 2.18–2.21). *There appears to be a final common destructive pathway of* **mitochondrial calcium overload,** *which begins with many different causes of*

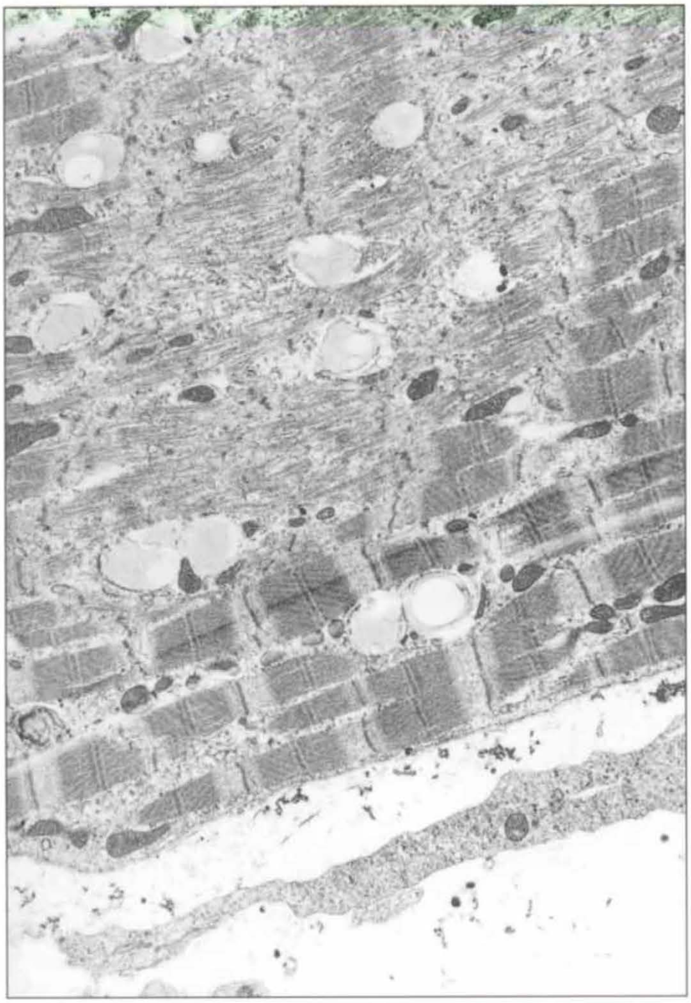

Figure 2.21 Myofibrillar lysis and lipid droplet accumulation in a foal. (Courtesy of TJ Hulland.)

membrane or energy failure and ends with hypercontraction and coagulation of the contractile proteins. Most animal myopathies seem to fit well into this sequence of events, particularly the nutritional myopathies. Grossly visible mineralization occurs several hours after the onset of protein coagulation during which striations are lost (*hyaline degeneration*). Calcium is released belatedly in degenerated myofibrils from calcium-saturated mitochondria as they disintegrate. Although the almost chalky mineralization seen grossly in many cases of nutritional myopathy is usually referred to as dystrophic, it is not at all certain that such accumulations can occur as a result of a purely passive collection of mineral from the blood on dead tissue protein. At the very least, it seems to require that mitochondria become overloaded with calcium early in the degenerative process.

Some cases of nutritional myopathy and toxic myopathy may have very similar microscopic degenerative changes not characterized by mineralization to any appreciable extent, even though the lesions are as extensive as those in typical "white muscle disease." Degenerate muscle may be very difficult to detect on gross examination when it is unmineralized, and consequently is likely to escape detection. The event that determines whether mineralization occurs in addition to the variable of the amount of blood calcium available may well be the period after hypercontraction, during which cellular membranes allow calcium into the cell and mitochondria continue to collect calcium from the cytosol. Eventually such activity will be ended by membrane disintegration and a release of calcium to the cytosol again, but calcium gathering could continue for a period of several hours.

The hypercontracted eosinophilic coagulum in segmental hyaline degeneration is readily removed by macrophages both in its earlier, premineralized state and in the later mineralized stage of granular degeneration. *The early pale, hyaline stage develops into typical* **Zenker's degeneration**, *which is a highly eosinophilic, homogeneous mass.* It sometimes retains faintly visible, tightly contracted striations, although these are eventually lost.

Regeneration and repair of muscle

The ability of muscle to repair itself is remarkable considering its high specialization and the great length and great vulnerability of individual fibers. The ability to rapidly repair the damaged segment of a fiber, without apparent complication to the rest of the fiber, is without parallel in other cells of the body. A muscle cell consists of a single large multinucleate myofiber adjacent to which is a large population of uninuclear satellite cells, outside the plasma membrane but within the tough basal lamina. *The major participants in the regenerative sequence are macrophages from the blood, the satellite cells, because only they, within the muscle, have retained the capability for mitotic division, and the basal lamina, which acts as a very efficient scaffold.* The integrity of the basal lamina determines from the very beginning how effective regeneration will be and whether the outcome will be regeneration of myofibers, fibrous replacement, or a mixture of the two. An intact basal lamina effectively keeps myonuclei, satellite nuclei, and myoblastic cells inside. It is equally efficient in keeping fibroblastic cells out, but allows phagocytic cells easy entry and exit.

If damage to the fiber is *segmental* and such that only the myofibrils and the sarcoplasm along with its subcellular components have been injured, the first visible event of regeneration is an early rounding-up of satellite cells as they prepare to undergo mitotic division. The proliferated satellite cells become apparent in hours as they leave their former sublaminal site. After about 12 hours, macrophages appear and they may or may not be accompanied by a short-lived wave of neutrophils and eosinophils. These inflammatory cells are rich sources of growth factors and cytokines such as insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF), and interleukin (IL)-6 that promote satellite cell and myoblast proliferation and differentiation. These cell types freely enter the mineralized damaged fiber. Macrophages dissolve and remove the debris (Fig. 2.22); neutrophils disappear unless infection complicates the process. Removal of sarcoplasmic debris leaves some space enclosed within the collapsing sarcolemma; and proliferating satellite cells are seen in the sarcolemmal tube. These cells are now termed myoblasts.

Myoblasts increase in number until a critical myoblastic mass is reached. Only then do myoblasts begin to fuse, and this triggers the next sequence of events. Five or six days after degeneration occurred, fusion of myoblasts has produced *unpolarized myotube giant cells* that begin to send out cytoplasmic processes as their nuclei divide (Fig. 2.23). Guided by the basal lamina, some processes make contact with remaining viable segments of the original fiber. They make effective union by dissolving cell membranes and uniting the cytosols into a single myofiber. The free pole of the myotube giant cell then

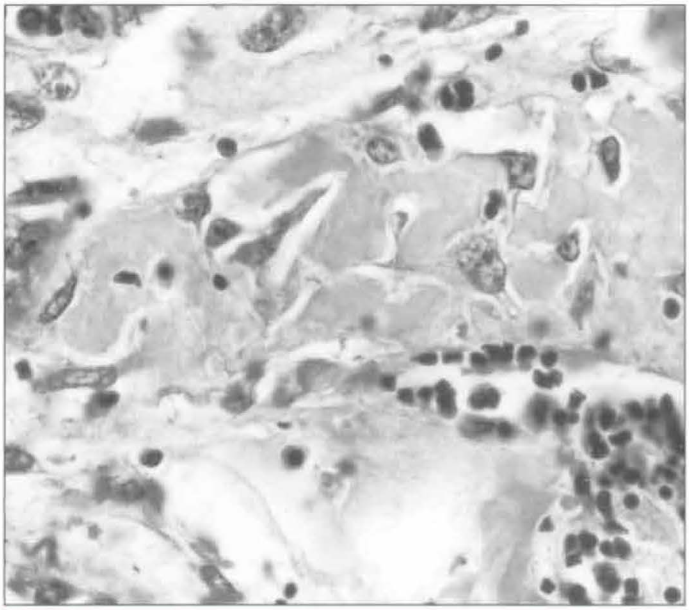

Figure 2.22 Necrotic myofiber with surviving satellite cells and invading macrophages. (Courtesy of TJ Hulland.)

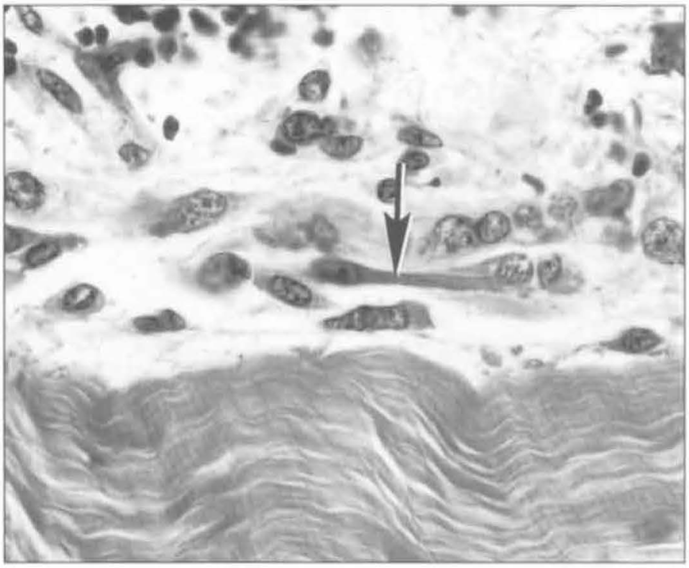

Figure 2.23 Regeneration, with elongating myoblasts (arrow). (Courtesy of TJ Hulland.)

becomes a *polarized regenerative probe,* which grows within the cleared space of the collapsed sarcolemmal sheath. Other myotube cells in the regenerating myofiber may not make contact with other myotubes for some time, and remain as unpolarized giant cells until an adjacent myotube's process contacts them. *A growing myotube characterized by its basophilic cytoplasm and central row of nuclei can grow for relatively long distances as long as the basal lamina is intact and no fibrous tissue intrudes and obstructs* (Fig. 2.24). If the basal lamina sheath is ruptured, the myotube protrudes from the ruptured sarcolemmal tube as a syncytial giant cell and can grow into the proliferating fibrous tissue for only 2–5 mm.

When a myotube has made contact with the next viable segment of the original fiber, at about 10–14 days, the next stage consists mostly of *production of new sarcomeres in the enlarging fiber.* The new fiber has to find growing space between existing fibers but seems able to do this efficiently. As myofibrils, sarcoplasm, and other organelles are added, the fiber becomes less basophilic, and from 7–21 days the nuclei migrate from their central or internal position to lie just under the new cell membrane – the normal position in a mature myofiber. Only in the most ideal of circumstances are all the parts restored to the predegeneration state by the efficient regeneration process described above. At the very least, two basal laminae, one inside the other, persist; later they may fuse. Any proliferating satellite cell/myoblasts, which do not enter the original cell, or any myotube processes which escape from it, may produce muscle giant cells that reflect abortive attempts at myofiber regeneration.

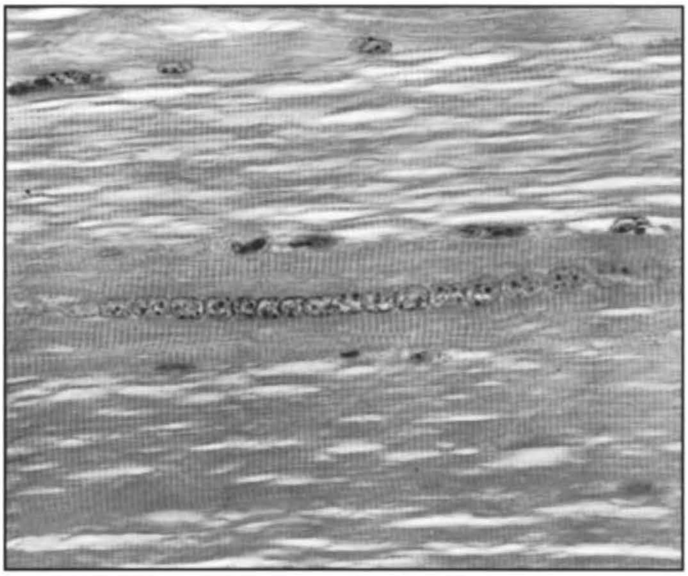

Figure 2.24 Regeneration, with a fiber with a row of central nuclei. (Courtesy of TJ Hulland.)

Considering the possibility of breaks in the sarcolemmal tubes, the potential for myoblast formation, which may be inside or outside the original myofiber and the potential for distortion of development by fibrous tissue invasion, it is easy to understand that the final result may not be perfect. *The longer and more numerous the gaps in sarcolemmal tubes are, the more potential there is for aberrations in repair.* The most important factors in preventing such aberrations seem to be the viability of nuclei and the wholeness of the basal lamina. Although sometimes extensively destructive, *nutritional, exertional and most toxic myopathies are likely to repair very well with minimal fibrosis. Primary dystrophies repair well but degenerate again* and eventually residual lesions of fibrosis will become prominent. *Ischemic damage repairs badly or not at all* because satellite nuclei and endomysial cells may have been killed. Fibrous replacement will be the end result. Ischemia with early reflow permits some regenerative repair, particularly around the outside of primary bundles where blood flow returns first but central fibrous replacement will occur. *Fibrosis may be the dominant feature in healed lesions of myositis and physical injury.*

The reduced regenerative capability that develops with maturity may be related to low numbers of potential myoblasts as the proportion of satellite cells to the total nuclear population in the fiber drops with age.

Other myofiber alterations

Vacuolar degeneration, an infrequent alteration in muscle fibers (Fig. 2.25) is associated with *electrolyte abnormalities,* such as hyperkalemia in horses with hyperkalemic periodic paralysis, and in the diaphragm of rodents given intraperitoneal injections of doxorubicin. **Fatty degeneration** with small fat vacuoles is a very common finding in *emaciated or anorectic animals* because muscle is able to use triglycerides directly as a source of energy. Rows of minute droplets are held in considerable quantity by type 1 (oxidative) fibers, but type 2 fibers often contain neutral fat also (see Fig. 2.21). The presence of these fat droplets may be more evident in those myofibrils remaining in advanced cachectic atrophy, but it apparently implies no pathologic change in muscle as even well-nourished animals carry some droplets in their muscles. Quite separate from these aggregations of minute fat droplets, larger fat droplets may be seen in muscle fibers in the dog and horse. Their cause is not known.

So-called **discoid degeneration** represents the phenomenon of *hypercontraction*. It develops when hypoxia plays a major part in the production of muscle lesions. The muscle striations, but not all of their fine features, are preserved and the fiber begins to separate at the Z bands (see Fig. 2.18).

Xanthomatosis (lipofuscinosis) is the term used to describe the *accumulation of abnormal amounts of yellow-brown to bronze pigment in some skeletal muscles and myocardium, and often also in adrenal cortex and distal convoluted tubules of the kidney*. Black hair coats may become brown, and white skin may become yellow, but the presence of the pigment appears not to be detrimental to the health of the animal. The build-up of "wear-and-tear pigments" in old animals, particularly high-producing dairy cows is indistinguishable morphologically from xanthomatosis but it is sometimes associated with myocardial failure. There appears to be a disproportionately high incidence of xanthomatosis in Ayrshire cows and among old cows of all breeds slaughtered for human consumption; in one study the incidence was about 0.1–0.5% overall compared to 10–25% for Ayrshires.

Skeletal muscles most frequently or most extensively involved are the *masseter muscles* and the *diaphragm*. Muscle fibers are of normal diameter or smaller, but may be dark brown or even black-brown. Pigment granules, some of which stain positively with lipid and PAS stains, tend to be located within lysosomes found perinuclearly under the sarcolemma or centrally in the fiber. Particles are irregular in size and shape and on electron microscopic examination show a wide variation in shape and organization. All are electron-dense and some are membrane-bound and it is presumed that they represent degraded lipid substances that have accumulated in phagolysosomes.

Other types of degeneration

A variety of inclusion-like structures or focally abundant or altered structural components occur in the myopathies of animals and are termed **cytoarchitectural changes** (Figs 2.26, 2.27). Similar alterations in the muscles of humans sometimes are associated with specific diseases but such relationships have not been established in most animal diseases. **Nemaline rods** have been found in a myopathy of cats and in occasional cases in dogs, with or without hypothyroidism. The rods represent accumulations of Z band material as confirmed by electron microscopy (see Congenital and inherited defects). **Targetoid fibers** (central zones of myofibrillar disruption)

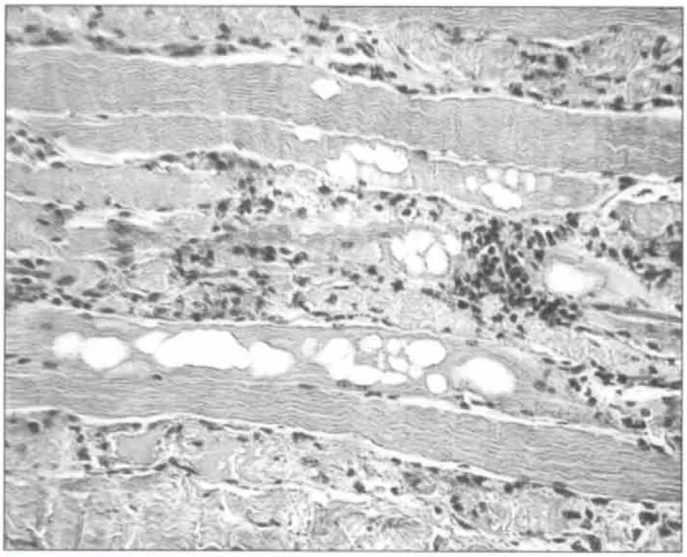

Figure 2.25 Vacuolar degeneration of muscle in a cow. (Courtesy of W Hadlow.)

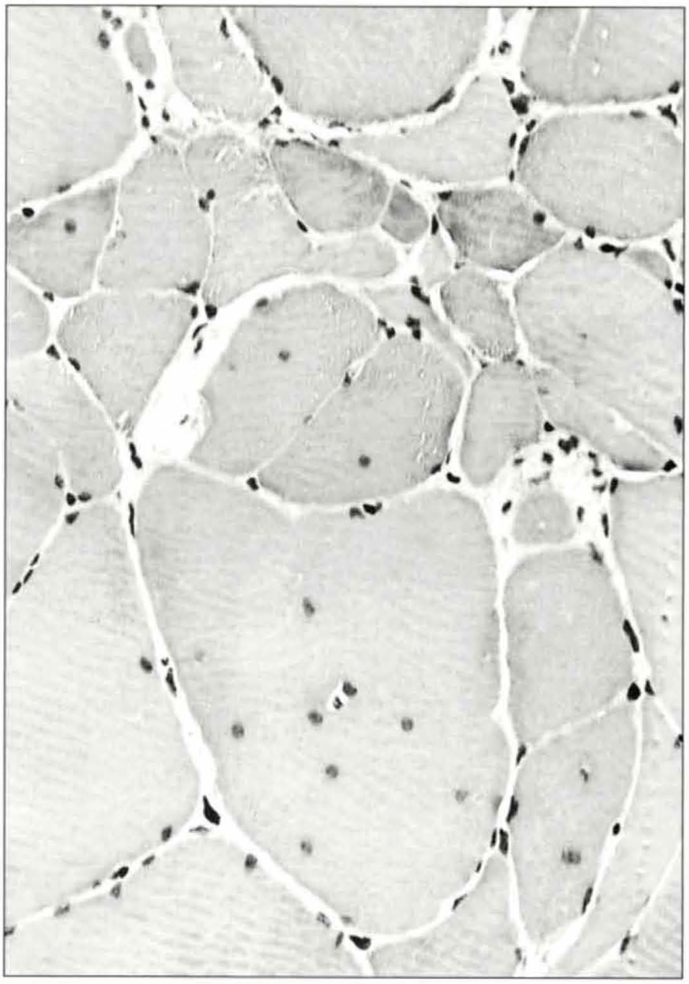

Figure 2.26 Myopathic alterations of internal nuclei and variation in fiber size in a horse. (Courtesy of TJ Hulland.)

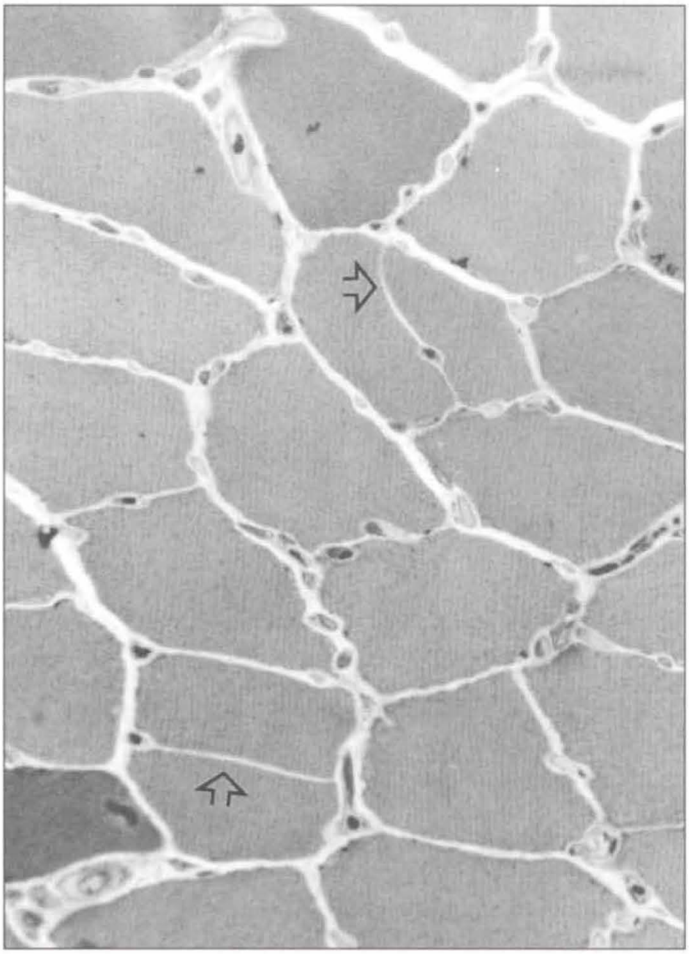

Figure 2.27 Myopathic alteration of **fiber splitting** (arrows) in a horse. (Courtesy of TJ Hulland.)

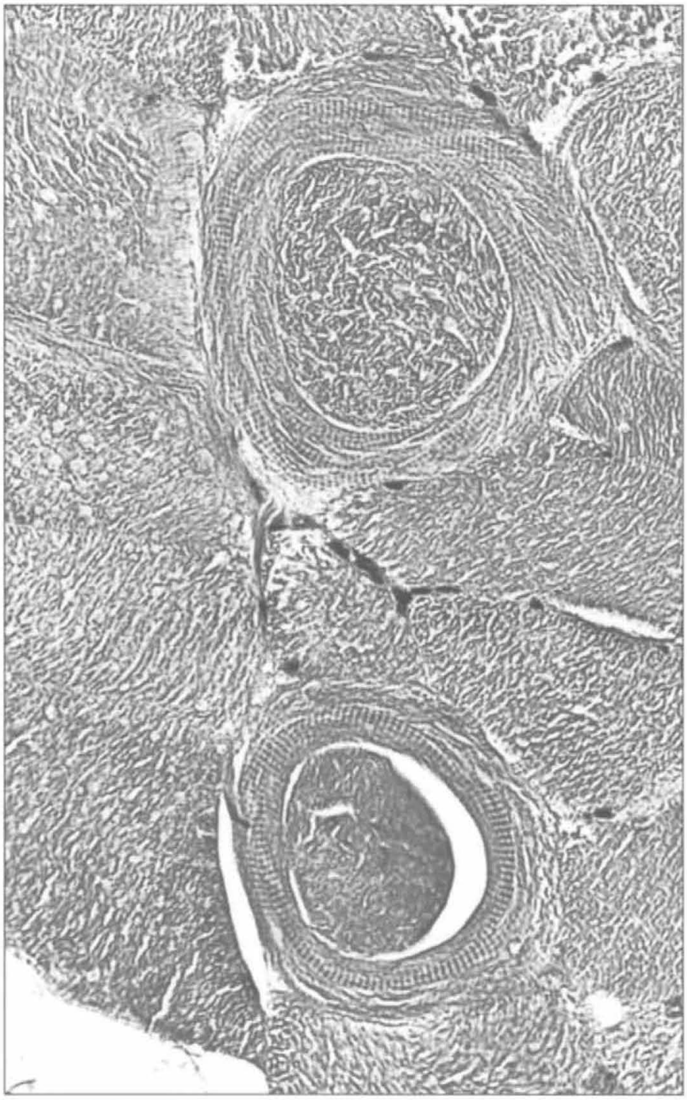

Figure 2.28 Two fibers with **ringbinden** in a horse. (Courtesy of TJ Hulland.)

and **ragged red fibers** (mitochondrial-laden) have been seen in myotonic muscle disease in dogs, and **peripheral and central sarcoplasmic masses** have been observed in the muscles of sheep and cattle in dystrophy. Abnormal **glycogen accumulations** may develop in the muscles of dogs, sheep, cattle, cats, and horses (see also Congenital and inherited defects). In all species, these are generally nonmembrane-bound collections. The deposits are PAS positive and digested by diastase and are usually just under the sarcolemma.

Ringbinden (ring fibers) is one of the unusual findings that may be left behind when denervation or tenotomy is combined with atrophy and regeneration. *It is a formation of disoriented peripheral myofibrils, which wrap themselves around a muscle fiber in a tight spiral fashion* (Fig. 2.28). Satellite cell nuclei can migrate and they sometimes orient their long axes transversely rather than longitudinally. Circular myofibrils accompanied by a mass of disrupted subsarcolemmal contractile elements presumably represent a true, but nonspecific, degeneration of myofibers. Ring fibers most often occur naturally in animals showing evidence of denervation atrophy, with or without evidence of ischemic muscle damage. Horses cast and anesthetized for several hours may also develop these lesions.

The role of **apoptosis** in skeletal muscle disease is still unclear. Although apoptosis represents an important activity in myoblasts during normal differentiation and development, its *role in postmitotic mature myofibers is uncertain*, but activated satellite cells may undergo apoptosis. Multinucleated skeletal muscle myotubes in cell culture exposed to staurosporine, a protein kinase inhibitor known to induce apoptosis in a variety of cell types, underwent the morphologic and biochemical features of apoptosis. In several models of muscle disease (X-linked muscular dystrophy in mouse, bupivacaine injury of immature rat muscle), apoptosis precedes the development of necrosis. Further studies are needed to establish the role and the importance of apoptosis in muscle diseases of animals and human beings.

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CONGENITAL AND INHERITED DEFECTS

There are numerous conditions affecting skeletal muscle development, integrity, and function in animals. Congenital myopathic and neuropathic conditions are apparent at birth or soon thereafter, usually within the first year of life, and may or may not be inherited. *Pedigree analysis and breeding studies often help to distinguish those congenital disorders that are also inherited.* Inherited disorders affecting skeletal muscle in animals in which the gene defect is known are listed in Table 2.1. Gene mutations leading to skeletal muscle dysfunction are most often *point mutations*, e.g., hyperkalemic periodic paralysis (HYPP) in horses, phosphofructokinase (PFK) deficiency in dogs, and myophosphorylase deficiency in cattle. Some disorders, e.g., canine X-linked muscular dystrophy, can occur due to either point mutations or deletions, and the genetically characterized cases of feline X-linked muscular dystrophy have been due to a gene deletion.

Following tradition, congenital and inherited defects of skeletal muscle are discussed separately from the congenital and inherited myopathies, although it is recognized that the distinction between these two groups is not always clear. Numerous disorders are difficult to classify using traditional classification schemes, and diseases or syndromes can change their status as a result of investigation and new information.

Abnormal development of neuroectoderm that involves the lower motor neuron portion of the nervous system will also affect skeletal muscle. Although the primary defect is in the nervous system, the profound effect these disorders can have on skeletal muscle development and function warrant a brief discussion here.

Primary CNS conditions

Neuroectodermal defects affecting the innervation of individual myotomes and the muscles that form from them can result in abnormal muscle development. Given the complex interaction of myogenic and neurogenic cells during development, it is often difficult to define the exact nature of the defect. Neuroectodermal defects can result in abnormal muscle development or structure due to lack of innervation, to loss of innervation, or to abnormal motor neuron activity.

For proper in utero development, muscles require normal innervation. Without it they atrophy or fail to develop normally (hypotrophy) and are more or less replaced by fibrous tissue and fat. Associated with each embryonic spinal cord segment is a myotome, a collection of cells with myoblastic potential, which migrate as the fetus develops and that ultimately form muscle. Committed myoblastic cells from one myotome may contribute to several muscles. Later, nerves from the same spinal segment follow the route of the myoblastic cells and innervate the same muscles. Most developing muscles are formed from myoblastic cells and nerves from two to six cord segments, but a few small muscles of the distal limbs receive contributions from only one. This explains why some muscles innervated from only one segment are vulnerable to denervation or lack of innervation resulting from a cord lesion involving a single segment, while a cord lesion of similar extent contributing innervation to muscles receiving cells and nerves from multiple segments can be associated with apparently normal muscle and nerve development. In the latter case, collateral innervation from nerves arising in normal cord segments can develop.

Developing skeletal muscle motor units are innervated by more than one nerve and, in some species such as the dog, this polyinnervation is still present at birth. With maturation of the neuromuscular unit the innervation pattern is simplified to one of one nerve fiber per motor unit. This pattern of polyinnervation can further complicate the pathologic findings in muscle from animals with neuroectodermal defects.

Arthrogryposis and dysraphism

Arthrogryposis *literally means crooked joint.* The terms *congenital articular rigidity* and *arthrogryposis multiplex congenita* are also used to describe this syndrome. Arthrogryposis can involve one or more limbs, depending on the underlying neuroectodermal defect. Severely affected animals may also have scoliosis, kyphosis, and torticollis, and the limbs or parts of limbs may be rotated, abducted, or curled backwards or forwards in grotesque positions (Fig. 2.29). Newborn animals afflicted with arthrogryposis are sometimes stillborn and may show autolysis indicative of in utero death 2–4 days earlier. Hydramnios is disproportionately associated with these, as is dystocia. Many, perhaps most, are of normal skeletal size although some are

Table 2.1 Animal myopathies in which the gene defect is known

Disorder	Gene affected	Type of disease	Species/breed affected
X-linked muscular dystrophy	Dystrophin	Muscular dystrophy	Various dog breeds. DSH cats
Double muscling	Myostatin	Muscular hyperplasia	Various beef breeds
Porcine stress syndrome	Ryanodine receptor	Malignant hyperthermia	Various pig breeds
Phosphofructokinase (PFK) deficiency	PFK, muscle isozyme	Carbohydrate metabolic defect	English Springer Spaniels, American Cocker Spaniels
Hyperkalemic periodic paralysis (HYPP)	Skeletal muscle sodium channel	Channelopathy leading to myotonia and paralysis	Impressive line of Quarter Horses
Glycogen storage disease	Myophosphorylase	Carbohydrate metabolic defect	Charolais cattle
Canine myotonia	Skeletal muscle chloride channel - ClC-1	Channelopathy leading to myotonia	Miniature Schnauzer

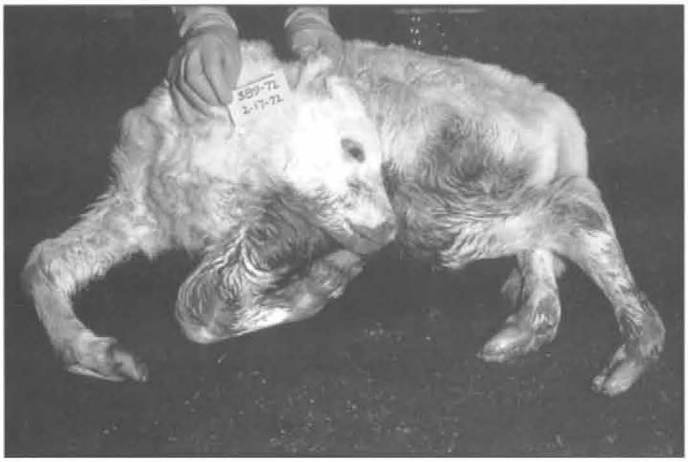

Figure 2.29 Newborn calf with **arthrogryposis**. (Courtesy of S Snyder.)

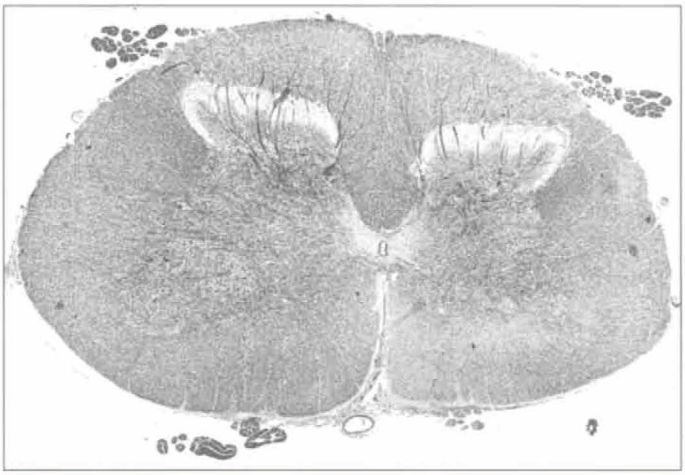

Figure 2.30 Failure of development of the right ventral horn in a calf with **myelodysplasia**. (Courtesy of TJ Hulland.)

considerably smaller than normal or smaller than littermates. In the rare cases in which the limb involvement is unilateral, the animal is likely to be born alive.

The causes of arthrogryposis are often not clear, although the underlying cause is generally agreed to be decreased limb movement in utero. Both myogenic and neurogenic disorders can result in decreased limb movement and arthrogryposis, but *denervation is by far the most common cause*. Arthrogrypotic fixation of the joints of lambs, calves, piglets, and foals, and less frequently of kittens and puppies, occurs with some regularity, and sometimes in epidemics. In cases of epidemic incidence, viral infections or toxins affecting the nervous system (see below) are most likely.

Unequivocal links exist between arthrogryposis and *the recognizable lesions caused by arrest or delay of neural tube closure* (**dysraphism**). These include, in the extreme, spina bifida and cord agenesis, but also anomalies of the dorsal septum, anomalies of the central canal such as hydromyelia and syringomyelia, anomalies of the dorsal, central or ventral gray matter (Fig. 2.30), and anomalies of the ventral median fissure (see Vol. 1, Nervous system). Dysraphism does not always lead to arthrogryposis; segmental lesions are sometimes found in clinically normal animals.

Obvious spinal cord and/or peripheral nerve abnormalities are present in a proportion, perhaps a majority, of arthrogrypotic domestic animals. In the rest, although an absence of primary myogenic or osteogenic lesions seems to point to a neurologic cause, the identity of the failed neural component is not obvious on routine investigation. Several careful studies of unilateral arthrogryposis in animals and children have indicated that the neural changes can be subtle and varied. The number of motor neurons in an apparently normal cord may be segmentally reduced, particularly in the caudal portion of the thoracic and lumbar eminences; or the number of motor neurons may be increased, or the neurons may be disoriented. Beyond this, conjecturally, there could be failure of neural direction, or connection, or of end-plate development.

Affected animals may have obvious reduction in mass of affected muscle due to atrophy or hypotrophy. Muscle mass can, however, appear superficially normal if there is replacement of the lost muscle mass by local masses of neonatal fat (Fig. 2.31). Dissection of major nerve trunks and muscle often is very difficult. Nerve trunks often appear to be normal on gross examination. Where comparisons are possible, peripheral nerve trunks in affected limbs may be demonstrably smaller and lacking some fascicles, although individual axons are normal.

Histologically, marked fiber size variation due to irregularity of muscle development may be evident. Groups of normal muscle can occur admixed with, or adjacent to, groups of small round muscle fibers (Fig. 2.32). Rarely, a typical denervation pattern of clusters of large

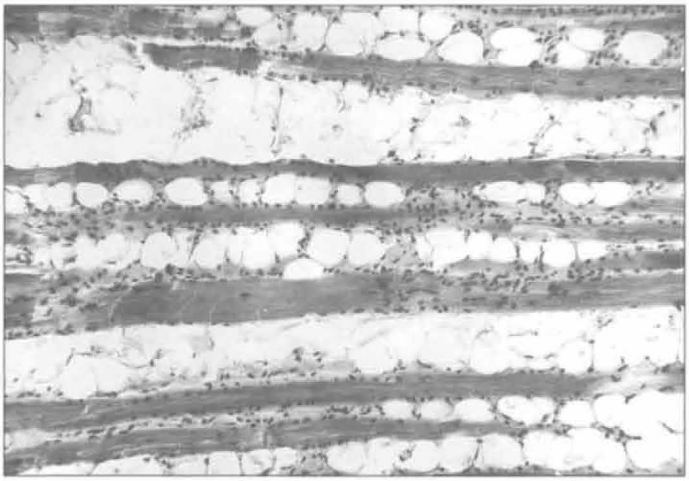

Figure 2.31 Muscle hypotrophy and fat replacement in a calf with arthrogryposis due to myelodysplasia. (Courtesy of TJ Hulland.)

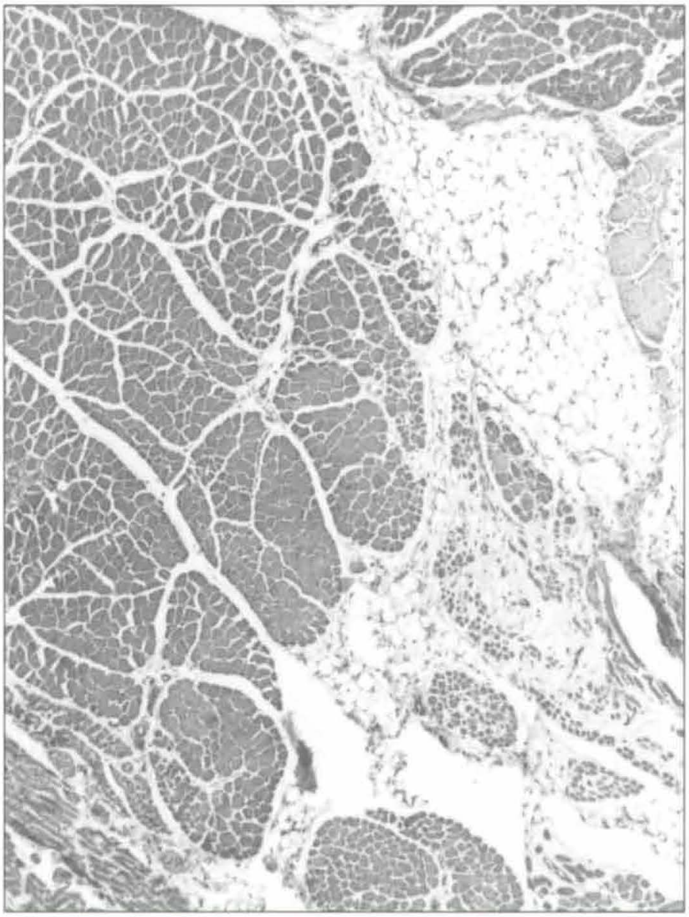

Figure 2.32 Admixed fascicles composed of myofibers of varied diameter, often with admixed fat infiltration, indicative of **asynchronous myofiber development** in an animal with arthrogryposis. (Courtesy of TJ Hulland.)

and clusters of small fibers can be seen. Abnormalities resulting in denervation and reinnervation will result in fiber type grouping. Other alterations in the fiber type pattern, such as type 1 fiber predominance (Fig. 2.33), are more difficult to explain but may reflect abnormal innervation or neural activity. Variable portions of muscle may be composed of adipose tissue.

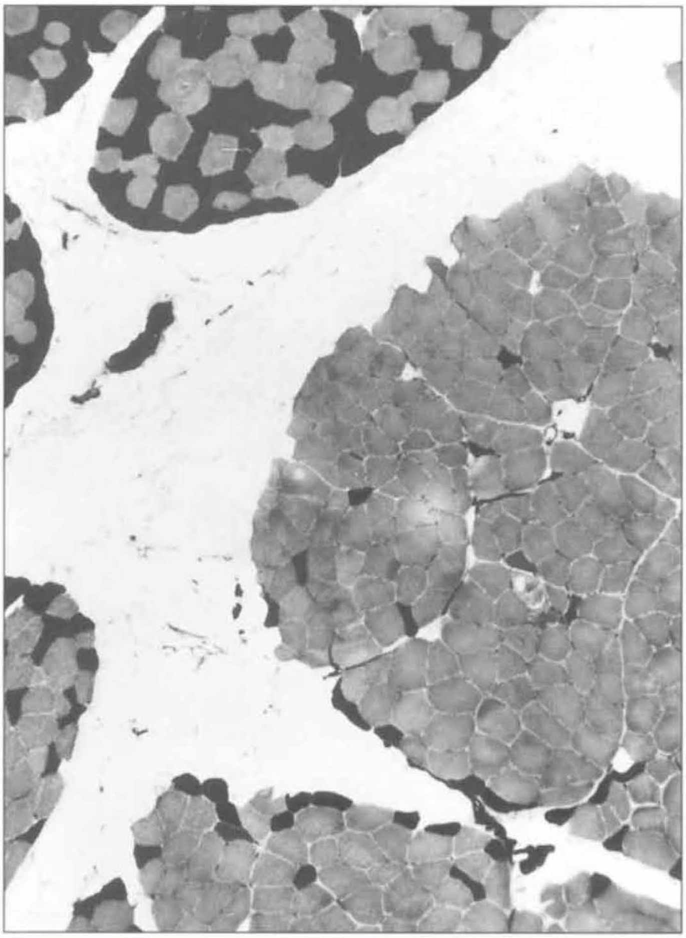

Figure 2.33 Fascicles exhibiting **type 1** (pale staining) **fiber predominance** in a lamb with arthrogryposis. Frozen section, ATPase, alkaline preincubation. (Courtesy of TJ Hulland.)

As noted above, it is often impossible to relate lesions to a specific cause. The critical event must occur early in pregnancy. *Genetic causes* are postulated in calves, sheep, and pigs, but the establishment of such a relationship does not require a different pathogenetic mechanism since the gene effect would apparently be directed at the neural component. Syndromes in the Charolais, Friesian, Swedish, and Red Danish breeds of cattle, sometimes associated with cleft palate, are consistent with that of a simple recessive or modified recessive characteristic. *Environmental toxins or viruses* may result in a similar disease pattern. The viruses of Akabane disease, Cache Valley fever, bluetongue, and border disease cause outbreaks of arthrogryposis in cattle and/or sheep. *Lupinus laxiflorus* (wild lupine), and other plant toxins, also cause deformities in fetuses when they are ingested by the dam during early pregnancy (see Vol. 1, Bones and joints).

Congenital flexures

Pastern contracture and immobility by itself can sometimes be part of arthrogryposis. If the flexure is combined with some degree of distal limb rotation, the pathogenesis is likely to be associated with neural and muscle changes as a minor expression of arthrogryposis. Many lambs, foals, and calves are born with an apparent inability to straighten the fetlock and sometimes other distal joints, yet deformities are minimal and muscle loss is not apparent. The problem in these cases seems to relate to the holding of the affected joint or joints in flexion without relief during a period of time in late gestation. The affected muscles appear to have reacted as they would following tenotomy by losing sarcomeres from the ends of myofibrils. This makes the joint initially incapable of extension, but under more or less constant tension the lost sarcomeres are quickly returned in hours or days. Such an explanation seems applicable because many affected foals and lambs recover completely within a few days to weeks. The original joint-fixing event can only be guessed at, but it would be easy to understand how it might happen in species in which the fetal size and limb length are high in relation to dam size.

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Muscular defects

Defects in the form and disposition of muscles associated with neuraxial deformities are quite common but there are four syndromes of importance affecting muscle that are not associated with lesions in nervous tissue.

"Splayleg" (myofibrillar hypoplasia) in piglets

Splayleg, *also called spraddle-leg, is a disease of neonatal piglets.* It occurs in all countries with a well-developed pig-rearing industry. The incidence of the disease fluctuates inexplicably but frequently appears as a farm or regional outbreak. The disease incidence within litters is variable and a sow may produce consecutive litters with the disease. Genetic predisposition and infectious or nutritional causes are postulated but not proven.

Piglets with splayleg assume a posture with the hindlegs or all four legs laterally extended (Fig. 2.34). They are apparently unable to adduct them or retrieve them from a forward or backward position. Mobility is reduced and affected pigs are susceptible to accidents or may starve due to an inability to compete to suckle. *The locomotor defect is transient and within a week, or at most two, survivors appear normal.*

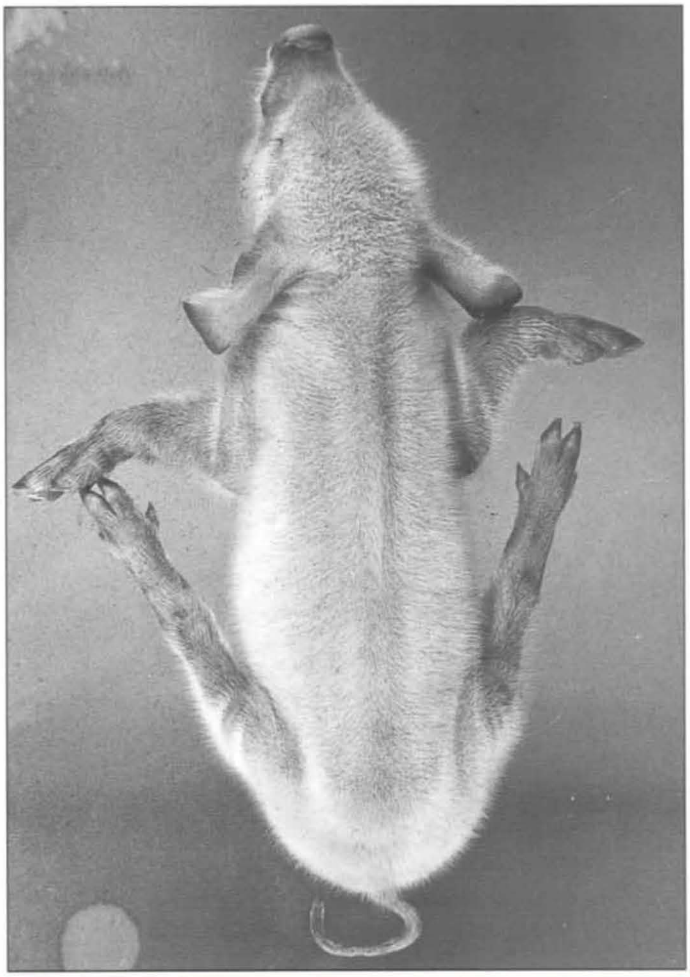

Figure 2.34 Piglet with **splayleg**. (Courtesy of TJ Hulland.)

The clinical signs of splayleg reflect muscle weakness. Not all piglets suffering from splayleg have primary muscle disease; other conditions cause similar signs, but much less frequently. Developmental abnormalities of spinal closure may be responsible (see Arthrogryposis and dysraphism) and myopathy induced by the injection of saccharated iron may produce a similar syndrome in one or more litters. The splayleg syndrome described here is unrelated to either of the above causes. The most common finding in skeletal muscle of affected pigs is *reduced diameter of myofibers with an abnormally small mass of myofibrils within individual muscle fibers* (Fig. 2.35A, B), and increased cytoplasmic glycogen filling the large extramyofibrillar spaces. It is also possible, however, to find the same histologic picture of apparent myofiber immaturity in clinically normal neonatal piglets. It may be that subtle differences in the degree of myofiber immaturity is the explanation for the variable clinical expression.

In the late intrauterine development of skeletal muscles in pigs, the muscle mass is increased by the *"stem line-template" method of new muscle fiber production.* A large type 1 muscle fiber centrally located within a sublobule (a segment of a primary bundle) of developing

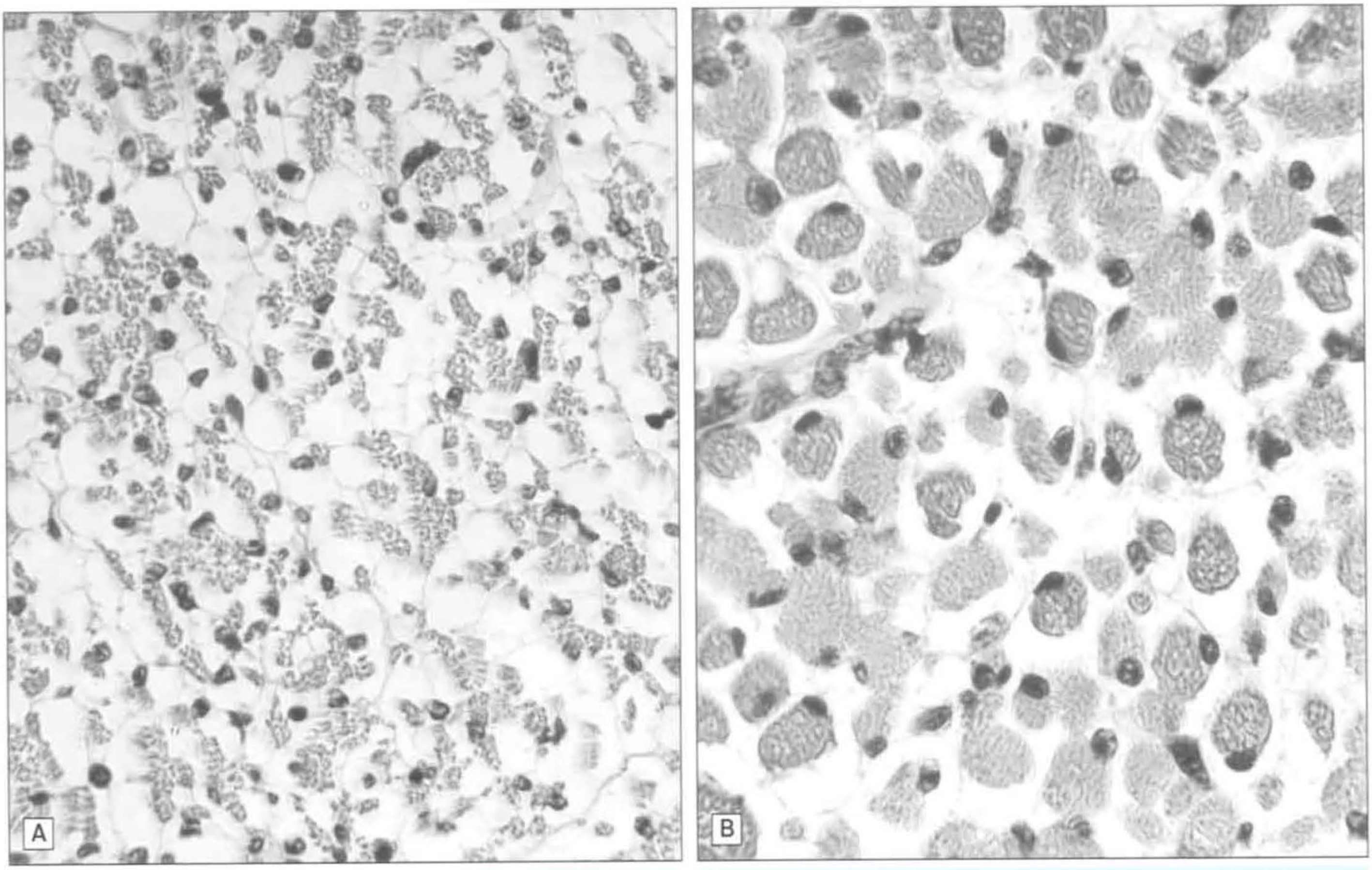

Figure 2.35 A. Reduced diameter of myofibers in a neonatal piglet with **splayleg**. (Courtesy of W Hadlow.) **B. Normal** neonatal piglet muscle. (Courtesy of W Hadlow.)

muscle apparently divides longitudinally to create a smaller daughter fiber, or acts as a template beside which a new fiber evolves from myoblasts. The new fiber has characteristics of a type 2 fiber. The central template fiber continues to spawn new fibers, gradually pushing those formed earlier to the periphery and thereby creating a sublobule of 10–20 fibers. Beginning at about the time of birth, a few of the most recently formed type 2 fibers adjacent to the primary or template fiber take on the characteristics of the central type 1 fiber. This early postnatal period also seems to mark the end of vigorous new fiber production, although some increase may continue in some circumstances. In the critical period just before and just after birth, splayleg pigs show an irregularity or retardation in the transition of type 2 fibers to type 1 fibers in the central sublobular region. *These findings in pigs with splayleg suggest delayed maturation of muscle in utero.* Examination of muscle from recovered piglets indicates that postnatal maturation continues, and the muscles from such pigs are indistinguishable from control piglets.

The characteristic lesions of myofibrillar hypoplasia are only partly revealed by light microscopy. Affected muscles, chiefly the longissimus, semitendinosus, and triceps, are incompletely or irregularly involved, and *the most obvious change is lightness of staining with eosin due to the low volume of myofibrils within outer cell membranes of normal volume.* The remainder of the fiber sheath appears to be empty but sometimes contains a nucleus or thin pink proteinaceous fluid. In longitudinal section, myofibrils appear to be diluted by watery fluid or clear strips of space. PAS staining reveals *marked accumulation of cytoplasmic glycogen.*

Electron microscopic examination of muscle from pigs with splayleg reveals segmental Z-line distortion with dispersal of the electron-dense material (Z-line streaming), loss of register and alignment of sarcomeres, reduced volume of myofilaments, and myofilament splitting and disarray. Glycogen granules are abundant, and there are increased numbers of ribosomes. Although degenerative changes, including increased phagolysosomes and lysis of myofilaments, have been described, this is not a consistent finding.

Myofibrillar hypoplasia has been reported as an isolated case in a *6-week-old calf.* Lesions very similar to those in splayleg pigs were seen. A clinical syndrome similar to splayleg occurs in puppies, and affected pups are known as "*swimmer pups*." Affected pups may have underlying myopathy or neuropathy, but more often this syndrome is seen in rapidly growing, overnourished pups. Dorsoventral flattening of the sternum often occurs. Affected pups often recover completely if measures such as use of a type of harness to help keep legs under the body and assisted exercise are employed.

"Double muscling" (muscular hyperplasia) in cattle

Economic pressure to produce beef cattle with increased muscling has undoubtedly led to perpetuation of multiple genetic defects involving muscle development. The best characterized of these is the defect known as double muscling. This disorder occurs within several beef breeds, including Charolais, Santa Gertrudis, South Devon, Angus, Belgian Blue, Belgian White, and Piedmontese cattle.

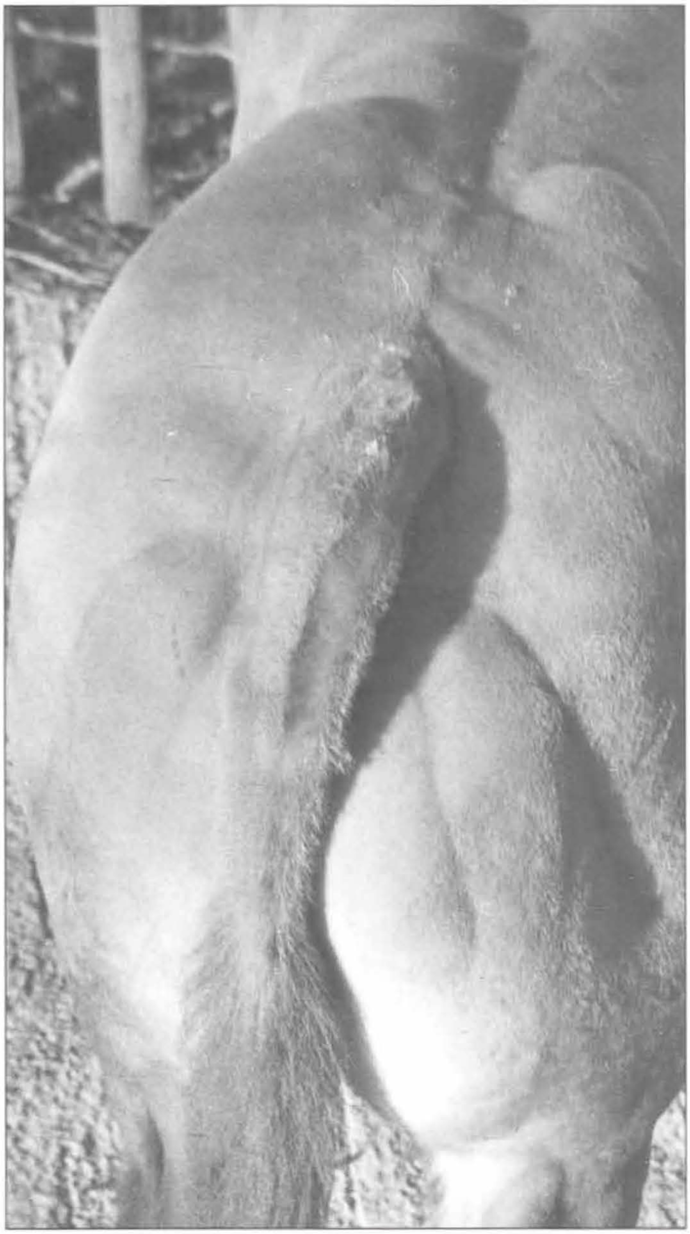

Figure 2.36 Congenital muscular hyperplasia in a calf. (Courtesy of JC MacKellar.)

It is characterized by an *increase in the number of fibers in affected muscles. The individual fibers are of normal size and structure.* This is in contrast to findings in other cattle with increased muscle bulk that are not double-muscled, in which a normal number of hypertrophied muscle fibers is found. In double-muscled homozygotes showing the full effect of the hyperplasia, the increase in gross muscle size is substantial, although not twice normal as the common name "double muscle" might suggest. In fact, the contours of the large muscles are regular and normal except for rather prominent topographical clefts between muscles which are associated with large individual muscle size, reduced body fat proportion and thin skin (Fig. 2.36). Bones may be lighter than normal and are often marginally shorter. Probably all striated muscles of the body are affected to some degree but *the muscles showing the most obvious change are those of the thighs, rump, loin, and shoulder* giving the calf a very athletic appearance and predisposing to dystocia. Some fiber increase is manifested by heterozygotes but the level of heritability seems to be low and subject to capricious variability of penetrance or expression. A higher proportion of type 2B glycolytic fibers and a decreased capillary density are found in muscle from doubled-muscled cattle.

This disorder has been found to be due to *genetic defects in the myostatin gene*, which normally functions to limit skeletal muscle growth. Other genes are likely to be involved in the development of double muscling, as the degree of double muscling in Belgian Blue cattle is significantly greater than in double-muscled Piedmontese cattle, despite the fact that both breeds have genetic defects that apparently result in inactivation of myostatin.

Muscular hyperplasia is also well documented in the *Callipyge phenotype in sheep* – the disease is only seen in heterozygote males and the molecular basis of the defect is associated with a mutation on chromosome 18. The condition is associated with hyperplasia of hindquarters and hindlimb musculature.

Muscular steatosis

Muscular steatosis *is a disease characterized by too much fat deposited within muscles.* It carries an inference that the fat is where muscle once was or ought to have been; effectively, *fat replacement of muscle fibers.* Fat infiltration of muscle is a *common nonspecific finding in chronic myopathic and neuropathic disorders that affect muscle.* The adipocytes that infiltrate muscle are derived from intramuscular mesenchymal cells. Fat infiltration may be accompanied by variable degrees of fibrosis. Steatosis of muscle is *best considered a reaction of chronically damaged or denervated muscle*, rather than a true developmental muscular defect.

The syndrome known as muscular steatosis in livestock is not typically associated with fibrosis. *It appears in clinically healthy animals, and it is usually a problem only in meat inspection.* Although previous damage due to neural lesions, nutritional myopathy, exertional myopathy, ischemia, or trauma are possible underlying causes, in most cases an exact cause is not determined. Studies on steatosis in normal market pig carcasses indicate that about 1–5% of pigs have small steatotic lesions and that a smaller proportion have extensive lesions of the anterior thigh or loin muscles (Fig. 2.37).

Sometimes the steatosis affects several muscles of one limb, but more often it affects only one or several muscles in one region. It may be bilaterally symmetrical or asymmetrical. Rarely does it affect all of one muscle and the dividing line between normal and fatty muscle is not sharp. Surviving muscle fibers in the marginal areas may be normal or smaller than normal but are often angular in fixed tissue as a result of adjacent pressure from turgid lipocytes.

Congenital clefts of the diaphragm

The embryologic development of the diaphragm is complex, with contributions from several different tissues. The septum transversum forms the central tendon, the dorsal aspect of the caudal mediastinum (the dorsal mesoesophagus) forms the diaphragm surrounding the esophagus and aorta, and the right and left pleuroperitoneal membranes fuse to close the pleuroperitoneal canals and form the final complete diaphragm.

Congenital diaphragmatic clefts affect all species either alone or as part of a more generalized malformation. *The most common defect involves the left dorsolateral and central portions of the diaphragm, indicative of failure of closure of the left pleuroperitoneal canal.* The clefts may permit

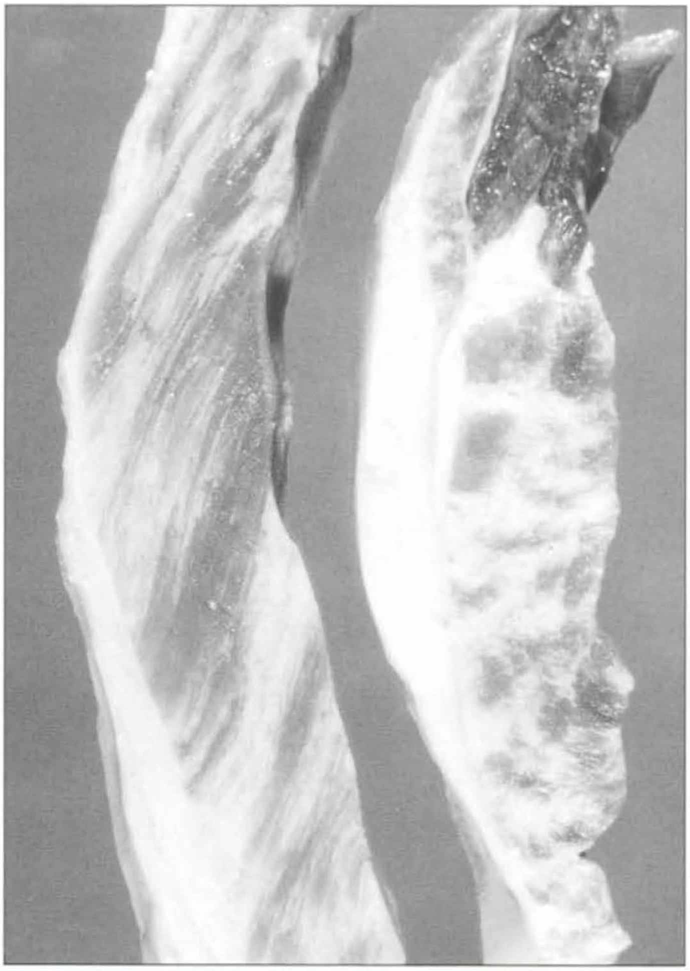

Figure 2.37 Steatosis of the loin muscle of a clinically normal pig. (Courtesy of TJ Hulland.)

the herniation of tissues ranging in size from a small button of liver to extensive displacement of viscera. In very large congenital clefts, the diaphragm may be represented by only a narrow rim of muscle that does little more than mark the diaphragmatic origin. Congenital clefts must be differentiated from acquired but healed lacerations of the diaphragm. The occurrence of congenital diaphragmatic hernia is best documented in the *dog* and in the *rabbit*. In the dog, incomplete closure of the left pleuroperitoneal canal is most common. In the rabbit, both defective closure of the left pleuroperitoneal membrane and absence of the major portion of the left diaphragm have been reported. Autosomal recessive inheritance has been proposed in both dogs and rabbits. The occurrence of congenital diaphragmatic hernias in five of 27 puppies from three father–daughter matings would support this form of inheritance, however further inbreeding of this particular colony of dogs failed to produce any subsequent pups with diaphragmatic hernias, suggesting that the genetic defect is likely to involve multiple genes.

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Muscular dystrophy

The muscular dystrophies of humans are defined as inherited progressive myopathies characterized histologically by ongoing muscle fiber degeneration and regeneration. Peripheral nerves and neuromuscular junctions are normal, although a recent study in humans and mice found cognitive disorders and increased susceptibility of the CNS to injury in individuals with Duchenne dystrophy. The advent of molecular genetics has begun to further designate these disorders according to their genetic basis and has aided in validating several animal disorders as true animal models of human disease. Given the high conservation of genes on the mammalian X chromosome, it was suspected that progressive degenerative myopathies inherited as an X-linked recessive trait in animals were likely to be homologs of the X-linked Duchenne and Becker muscular dystrophies of humans. Molecular genetic analysis has proven this to be the case. **True muscular dystrophy**, *homologous to Duchenne and Becker muscular dystrophy of humans, occurs in the dog, cat, and mouse.* Duchenne and Becker muscular dystrophy are X-linked recessive disorders of humans caused by defects in the gene coding for dystrophin, a sarcolemmal-associated cytoskeletal protein. Immunostaining of frozen sections of muscle and Western blot analysis for dystrophin in snap-frozen muscle samples are necessary for confirmation of dystrophin-deficient muscular dystrophy. It is interesting that dystrophin deficiency results in progressive muscle atrophy in most breeds of dogs, but causes marked muscular hypertrophy in the mouse, cat, and Rat Terrier dog. Muscle fiber hypertrophy occurs, especially in early stages of the disorder, but extensive fiber necrosis leads to overall muscle atrophy in most cases. At this time there is no explanation for this phenomenon, although it would appear that muscle hypertrophy is more apparent in animals of small stature.

In animals, a number of muscle disorders have been erroneously designated as "muscular dystrophy," most notably those degenerative myopathies occurring secondary to nutritional deficiency. An inherited progressive myopathy in sheep has been described as a muscular dystrophy and is included in this section, although this disorder might be better classified as a congenital progressive myopathy because cytoarchitectural alterations are the hallmarks of this disorder, and on-going fiber degeneration and regeneration are not typical features. Other inherited myopathies in dogs and cattle, many of which have been described as muscular dystrophies, are considered to be less likely candidates for true muscular dystrophy and are described in the Congenital myopathy section.

Canine X-linked muscular dystrophy

Sporadic reports from around the world of a severe progressive degenerative myopathy affecting young male dogs led to the establishment of a breeding colony of affected Golden Retriever dogs. Although a similar disorder has now been identified in many breeds, including Irish Terrier, Samoyed, Rottweiler, Dalmatian, Shetland Sheepdog, Labrador Retriever, German Shorthaired Pointer, Brittany Spaniel, Rat Terrier, Belgian Groenendael Shepherd, and Schnauzer, *the disease is best characterized in the Golden Retriever.*

Affected pups may be normal or slightly small at birth. Some affected pups may show severe progressive weakness leading to death within the first few weeks of life. This may be particularly true of affected pups in large litters that include normal and carrier pups, and may in part be due to inability to compete for food. Other pups show no signs of disease until approximately 8 weeks of age, when reduced jaw mobility, exercise intolerance, and a stiff-legged gait become apparent. Serum activities of creatine kinase (CK) and aspartate aminotransferase (AST) are, however, markedly increased, indicative of severe myodegeneration, even in neonates with inapparent disease, and levels of CK and AST continue to rise until peaking at about 6 months of age. Serum CK and AST levels in older dogs are always high, but tend to be decreased as compared to younger dystrophic dogs. Serum activities of CK and AST are highest if blood is drawn 4–6 hours after exercise. *Clinical signs of neuromuscular weakness are progressive until approximately 8–12 months of age, when the disease tends to stabilize.* The severity of the disorder is variable, with some dogs developing severe muscle atrophy and contractures by 6 months of age (Fig. 2.38) and others remaining stiff, muscle wasted, and exercise intolerant but without severe impairment of joint mobility. Dystrophic dogs have a characteristic stiff-legged shuffling gait and a thickening of the muscles of the base of the tongue and under the jaw, tend to drool excessively, and develop abdominal breathing and often deformation of the ribcage due to diaphragmatic contracture. Esophageal dysfunction is common, and dystrophic dogs may develop aspiration pneumonia due to regurgitation. The severity of the disease varies among breeds. Although muscle atrophy is more obvious than hypertrophy in the Golden Retriever and Rottweiler, in other breeds such as the Rat Terrier there is progressive development of muscular hypertrophy resulting in obvious increased bulk, particularly involving muscles of the thigh, neck, and shoulder girdle. Breed size may have some role in the type and severity of changes, with larger breeds being most severely affected. Female carriers are clinically normal, although higher than normal levels of serum CK and AST are apparent, particularly in carriers under 6 months of age.

Concentric needle electromyography reveals marked spontaneous activity in the muscles of dystrophic dogs. Although the complex repetitive activity generated by canine dystrophic muscle was initially interpreted as a myotonia, resulting in characterization of this disorder as a myotonic myopathy, careful analysis of the electrical activity indicates that these bursts do not wax and wane but rather start and stop abruptly and are *characteristic of pseudomyotonia.* Although the exact cause of this electrical activity is not known, membrane instability associated with clusters of degenerating and regenerating fibers is suspected.

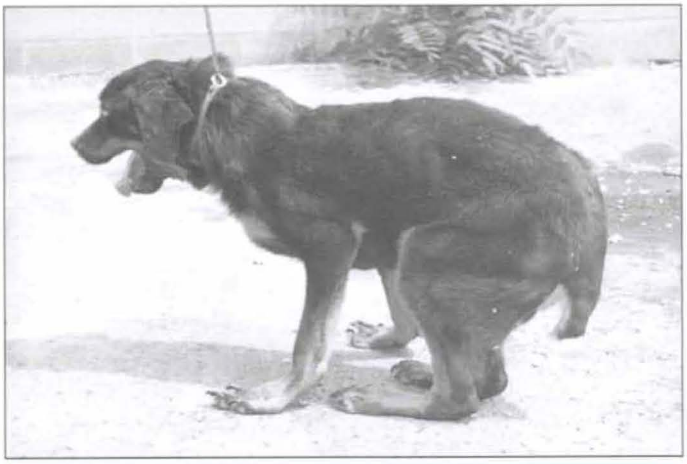

Figure 2.38 One-year-old male Rottweiler dog with severe **X-linked muscular dystrophy**.

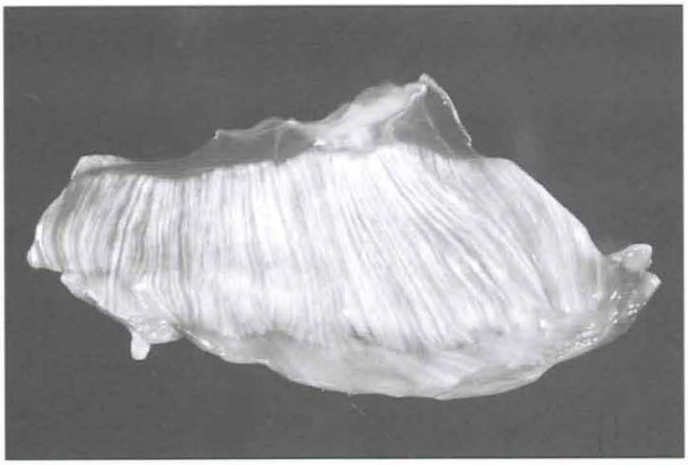

Figure 2.39 Pale streaking of diaphragm muscle due to **massive necrosis** in a neonatal pup with fulminant X-linked muscular dystrophy. (From McGavin MD, Zachary JF. Pathologic Basis of Veterinary Disease. 2006, with permission of Elsevier Ltd.)

Pups dying with fulminant neonatal disease have characteristic gross pathologic findings of severe degeneration of the diaphragm and strap muscles, including the trapezius and sartorius muscles of the limbs, characterized by pale white streaks within affected muscles (Fig. 2.39). Histologic evaluation reveals massive acute muscle fiber necrosis and mineralization, and similar findings are present in tongue muscle. This selective involvement of muscles in neonatal pups may reflect exercise-induced injury in muscles used for breathing, crawling, and suckling and may also involve the susceptibility to injury of more mature, larger-diameter fibers.

Gross pathologic examination of affected dogs that survive the neonatal period typically reveals pale musculature that may be slightly firm, especially true of the sartorius muscles of the hindlimbs. The diaphragm often exhibits the most striking changes, with thickening, contracture, and fibrosis evident (Fig. 2.40). On histologic examination, muscle samples exhibit the characteristic findings of muscular dystrophy. *Numerous swollen and dark staining fibers (so-called large dark*

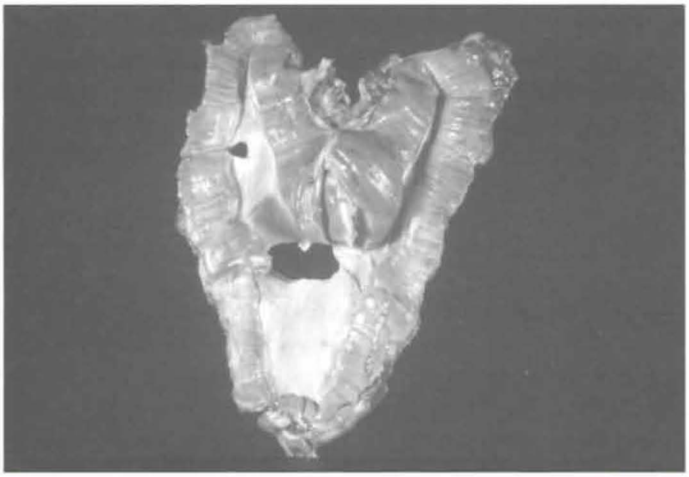

Figure 2.40 Severe diaphragmatic **muscular fibrosis and contraction** in a young adult dog with X-linked muscular dystrophy.

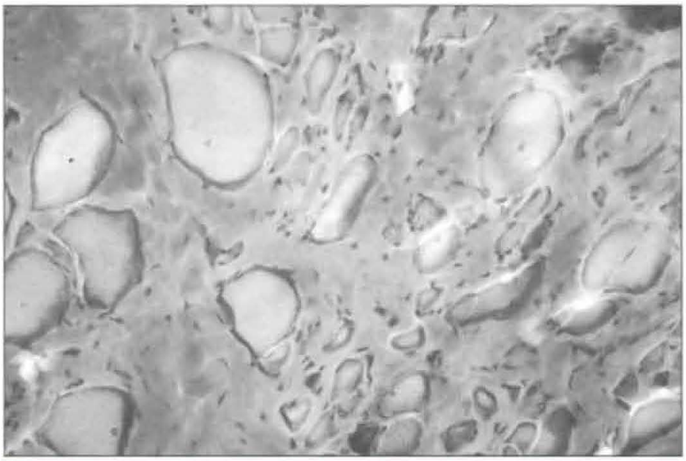

Figure 2.42 Marked endomysial fibrosis in the sartorius muscle of a dog with X-linked muscular dystrophy. Remaining fibers have variable diameter and frequently contain internal nuclei. Frozen section, modified Gomori's trichrome.

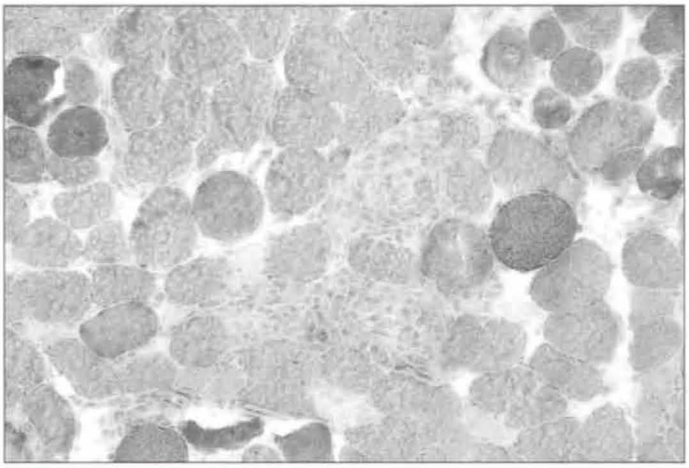

Figure 2.41 Characteristic **multifocal, polyphasic necrosis** in muscle from a dog with X-linked muscular dystrophy. There are characteristic large dark fibers as well as clusters of overtly necrotic fibers with macrophage infiltration. Frozen section, modified Gomori's trichrome. (Courtesy of BJ Cooper.)

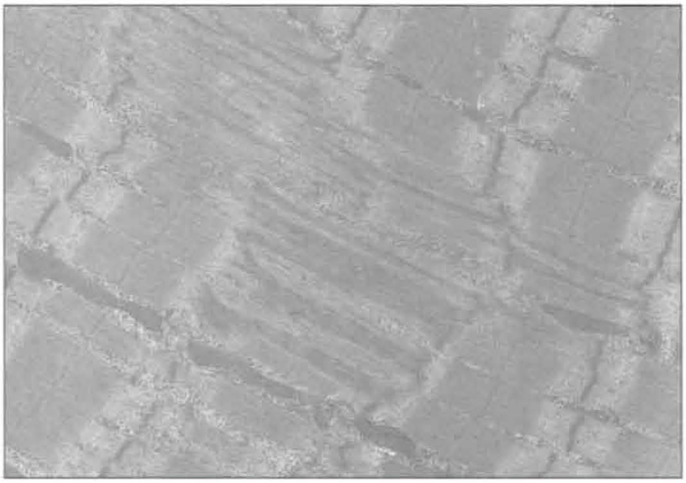

Figure 2.43 Streaming of Z bands in a pup with X-linked muscular dystrophy.

fibers) are seen throughout the sections and are considered to be the earliest stage of muscle fiber degeneration. Small clusters of overtly *necrotic fibers*, either in a coagulative state or macrophage-infiltrated, and of *regenerate fibers* are seen (multifocal, polyphasic necrosis; Fig. 2.41). Scattered *mineralized fibers* are common. The basal lamina of necrotic fiber segments is preserved, resulting in the presence of "empty sarcolemmal tubes" and in the ability to fully regenerate affected fiber segments. Muscle spindles are unaffected. With increasing age the degree of overt fiber necrosis and regeneration decreases. In *older dystrophic dogs*, marked fiber size variation, with internal nuclei, cytoarchitectural changes including nemaline rods, and a type 1 fiber predominance may be seen. Endomysial fibrosis and fat infiltration, the hallmark of advanced Duchenne dystrophy in boys, occurs in some muscles (Fig. 2.42), but is variable and typically less apparent in the dog.

In addition to evidence of fiber necrosis and regeneration, ultrastructural studies of muscle from dystrophic dogs have found small areas of apparent sarcolemmal defects in otherwise normal-appearing fibers. *Non-specific cytoarchitectural changes*, including increased mitochondrial content and Z-line streaming are also seen (Fig. 2.43). Alizarin red staining of frozen sections has confirmed that influx of calcium, probably through defects in the sarcolemma, is an early event leading to muscle fiber necrosis in dystrophic muscle (Fig. 2.44). Although considered to be an essential cytoskeletal protein and known to be part of a membrane-associated complex, *the exact function of dystrophin and the cause of muscle fiber necrosis in dystrophin-deficient muscle is still not clear.*

Cardiac muscle of dystrophic dogs 6 months or older exhibits myofiber necrosis, mineralization, and fibrosis of the myocardium in a subepicardial pattern. The left ventricular wall and right side of the interventricular septum are most severely affected (Fig. 2.45). The development of *progressive degenerative cardiomyopathy* is a hallmark of Duchenne and Becker muscular dystrophy in man, and often eventually results in congestive heart failure in older dystrophic dogs.

Immunostaining of frozen sections for dystrophin typically reveals complete absence of this sarcolemmal-associated protein (Fig. 2.46A, B), although partial expression may be present in Becker-type mutations or in so-called revertant fibers, which are fibers that have undergone genetic mutation allowing dystrophin expression. Cardiac muscle in dystrophic dogs completely lacks dystrophin. Although dystrophin expression has been absent or nearly absent in the dystrophic dogs studied to date, it is possible that animals expressing an abnormal truncated or elongated dystrophin will be found.

Muscle from young female carriers often contains numerous large dark fibers and scattered necrotic and regenerating fibers. The degree of overt necrosis and regeneration appears to be less than the proportion of large dark fibers seen, suggesting that membrane repair in some large dark fibers is possible. By about 6 months of age, evidence of degeneration or regeneration is rare. This reflects the mosaic pattern of dystrophin expression in carrier muscle. This mosaic pattern is most apparent in neonatal carriers, with loss of skeletal muscle mosaicism by about 6 months of age. This phenomenon is thought to reflect up-regulation or translocation of dystrophin throughout the mosaic muscle fiber. Cardiac muscle, composed of uninucleate cells incapable of regeneration, maintains a striking mosaicism for dystrophin for the life of the animal, and carrier females also develop cardiac muscle necrosis and progressive fibrosis in a pattern similar to that seen in dystrophic males. Curiously, although the fibrosis of cardiac muscle in older female carriers is quite striking, overt cardiac failure does not occur.

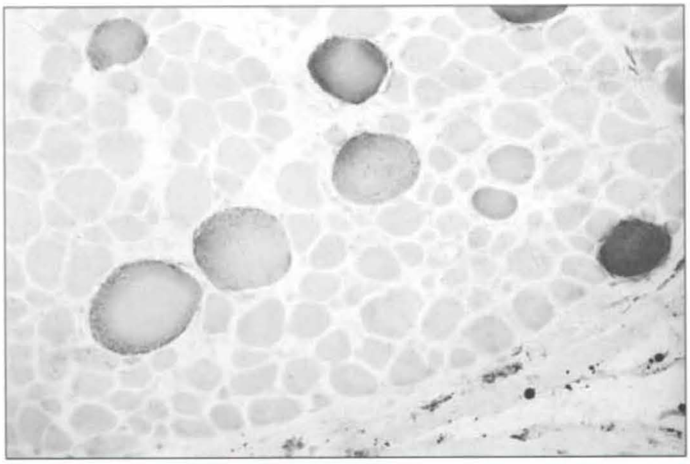

Figure 2.44 Numerous large apparently intact fibers with dark staining due to **calcium influx** indicative of sarcolemmal damage in a dog with X-linked muscular dystrophy. Frozen section, alizarin red S.

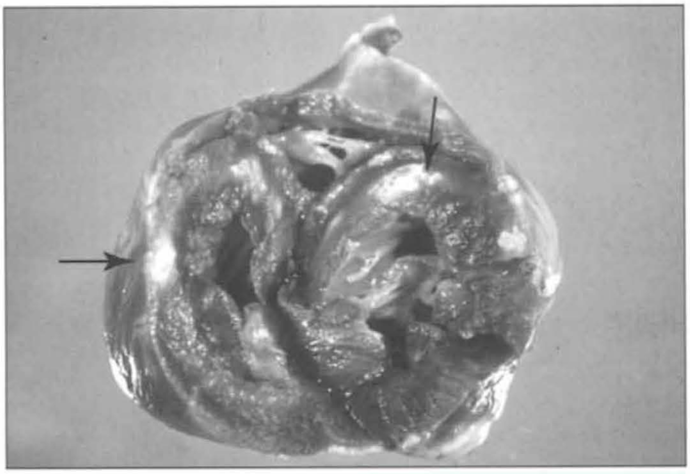

Figure 2.45 Multifocal pale zones of **myocardial necrosis and mineralization** (arrows) within the left ventricular wall and interventricular septum of a dog with X-linked muscular dystrophy. (Courtesy of BJ Cooper)

Feline X-linked muscular dystrophy

As in studies of canine muscular dystrophy, the recognition of a severe progressive degenerative myopathy in young male cats led to breeding studies of affected cats. To date, this disorder has only been reported in cats of mixed breeding. Its occurrence in Europe as well as North America suggests that, similar to canine X-linked muscular dystrophy, the feline dystrophin gene is prone to spontaneous mutation.

Affected cats develop marked muscle hypertrophy, stiff gait, "bunny-hopping" in the rear limbs, reduced activity and difficulty jumping up onto furniture and other objects. Clinical signs may be recognized as early as 5 months or as late as 2 years of age. Thickening of tongue muscle may result in difficulties prehending food and in drooling. Regurgitation due to esophageal dysfunction may also be evident. Given the fairly subtle signs, it is likely that affected cats may not be recognized by owners as being abnormal. Muscular hypertrophy is most evident in the muscles of the neck and proximal limbs (Fig. 2.47). Dystrophic cats are prone to development of a *malignant hyperthermia-like syndrome* associated with restraint or general anesthesia. Serum activities

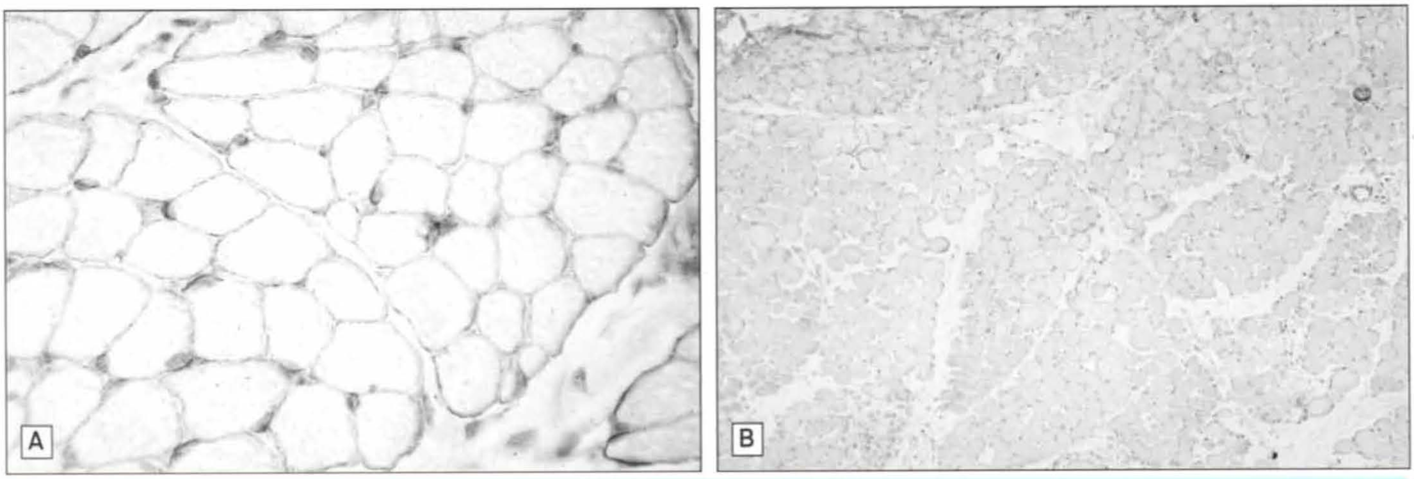

Figure 2.46 A. Dystrophin immunostaining of **normal** canine muscle, showing diffuse sarcolemmal staining. Frozen section, immunoperoxidase. (Courtesy of BJ Cooper) **B. Dystrophin is completely absent** in muscle from a dog with X-linked muscular dystrophy. Frozen section, immunoperoxidase. (Courtesy of BJ Cooper)

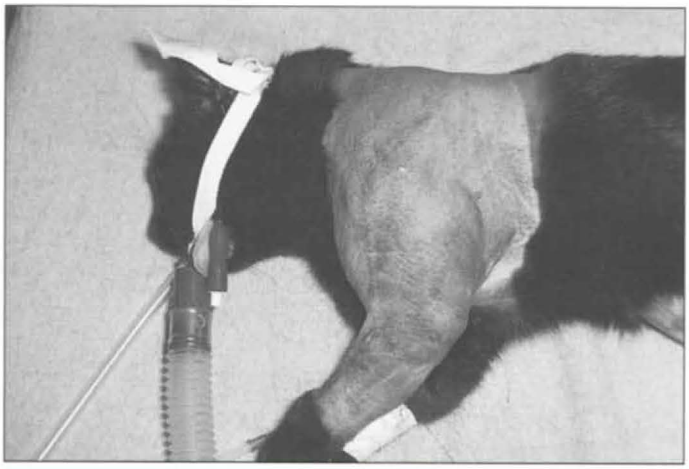

Figure 2.47 Marked hypertrophy of cervical and proximal forelimb muscles in a **cat with X-linked muscular dystrophy**. (Courtesy of BJ Cooper.)

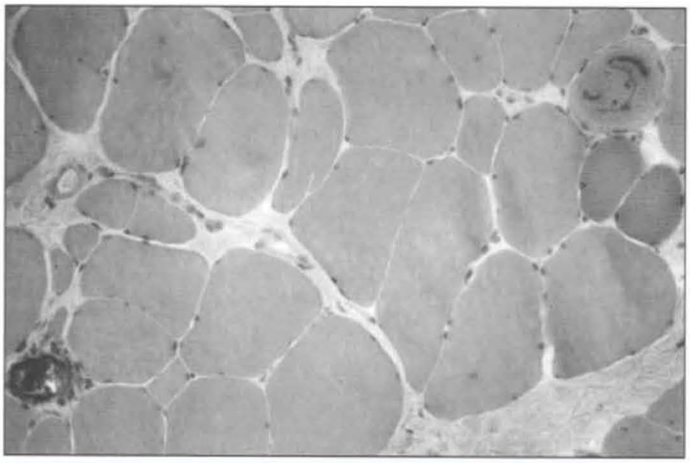

Figure 2.48 Excessive fiber size variation with both atrophy and hypertrophy, internal nuclei, and with scattered mineralized and bizarre fibers, in a cat with X-linked muscular dystrophy. Frozen section H&E. (Courtesy of BJ Cooper.)

of CK and AST are typically very high, and increases can be detected in kittens as young as 3 weeks of age. Electromyographic studies of affected cats reveal bizarre complex repetitive activity similar to that seen in the dystrophic dog.

At necropsy, affected cats have thickening of the muscular wall of the esophagus as well as marked thickening and contraction of the diaphragm. Skeletal muscle is often diffusely pale as well as displaying various degrees of hypertrophy. Pale streaks are often visible in the left ventricular wall and interventricular septum.

Histologic evaluation reveals *marked fiber size variation with numerous hypertrophied fibers often containing multiple internal nuclei.* Scattered clusters of necrotic and regenerating fibers with scattered mineralized myofibers are seen, and fibers with bizarre cytoarchitecture can occur (Fig. 2.48). The degree of myofiber degeneration and regeneration is often less than that seen in dystrophic dogs. Endomysial fibrosis occurs, but is minimal.

Foci of necrosis and mineralization of cardiac myofibers, followed by progressive fibrosis, can be seen in the left ventricular wall and interventricular septum of affected cats in a pattern similar to that seen in dystrophic dogs and in Duchenne and Becker muscular dystrophy. Cardiac lesions are not, however, a consistent finding in dystrophic cats, and clinical signs of cardiac insufficiency have not been reported, although experimental studies using inbred cats demonstrated left ventricular hypertrophy in all affected animals.

Muscular dystrophy, not X-linked, has also been described in cats associated with laminin deficiency. Affected cats developed progressive muscle weakness and atrophy, and also had peripheral neuropathies. In cases that survive for extended periods, fibrous replacement of atrophic muscle is severe.

Ovine muscular dystrophy

Muscular dystrophy of Merino sheep is an autosomal recessive disorder that occurs in widely separated flocks in Australia. This disorder affects about 1–2% of the progeny each year. The sexes are equally affected and *skeletal muscle of affected sheep expresses dystrophin in a normal manner, consistent with the non-X-linked nature of this disorder.* Although similarities to myotonic dystrophy of man have been proposed, there is no compelling evidence that this is the case.

Initial signs are *lack of normal growth and reduced flexion of the hindlimbs* that lead to aberrations of gait or, in mild cases, just stiffness. Subtle clinical signs may be detected in lambs at 3–4 weeks of age. The rate of progression of this disorder is extremely variable but the majority of cases have clear-cut signs of hindlimb gait abnormalities by 6–12 months of age and are severely affected by 2–3 years of age. Under field conditions, the animals often die of inanition at 6–18 months of age. With care, some animals can be maintained for up to 5 years, during which time the clinical disease is slowly progressive. Serum activity of CK is increased at rest in animals with unequivocal clinical signs of muscle dysfunction and is increased further following exercise. No electromyographic abnormalities are found.

On postmortem examination, apart from emaciation, the prominent changes are in the vastus intermedius, soleus, anconeus, and medial head of the triceps, where normal muscle may be replaced by mature adipose tissue. The muscles in advanced cases appear gray, and are hard and atonic. Involvement of other muscles, most often the extensors of the hip, stifle, and hock joints and flexors of the shoulder, carpus, and digits, is limited to microscopic changes only, although these muscles may exhibit some degree of pallor. Affected muscles have a high proportion of type 1 fibers, and it appears that this accounts for the distribution of lesions, as this disorder affects only type 1 fibers.

The initial stages of the dystrophic lesion are heralded by a general hypertrophy of type 1 fibers followed by loss of myofibrils and formation of characteristic sarcoplasmic masses at the periphery or in the central portion of the cell (Fig. 2.49A, B). This is followed by an irregular atrophy of fibers. Fiber proportions remain normal. Compensatory hypertrophy of fibers is often accompanied by fiber splitting, resulting in fiber nesting ("orange section" clusters). A few fibers develop peripheral annular fibrils ("ring fibers," or "ringbinden"). Many fibers acquire internally located nuclei. Hypercontracted fiber segments are observed only sporadically, and regenerative fibers are rare or nonexistent. Muscles undergo progressive loss of fibers which may reach 80–100% in animals over 2 years of age, and undergo progressive fat infiltration and/or endomysial fibrosis (Fig. 2.50A, B, C). In advanced muscle lesions, aggregates of lymphoid and histiocytic cells occur but these seem to be unimportant in the genesis of lesions.

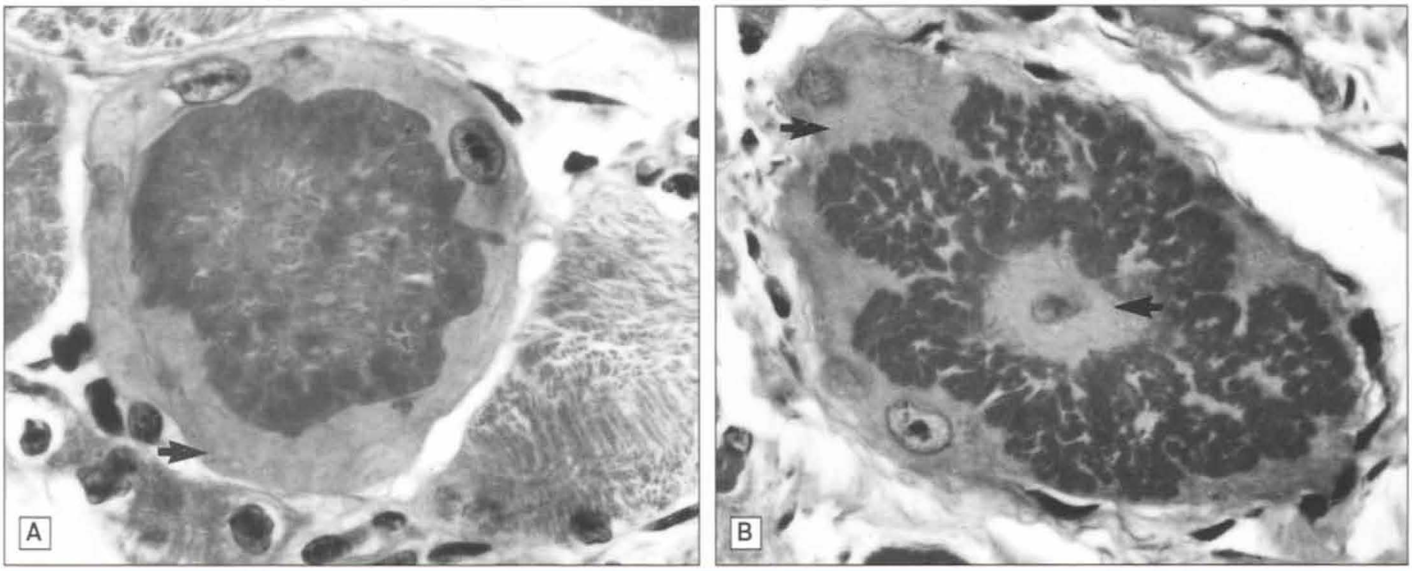

Figure 2.49 Myofibers in the vastus intermedius muscle of a **sheep with muscular dystrophy**. **A.** Myofiber with pale peripheral sarcoplasmic masses (arrow) and large vesicular nuclei. **B.** Myofiber with central and peripheral pale sarcoplasmic masses (arrows) and large vesicular nuclei. (Courtesy of MD McGavin.)

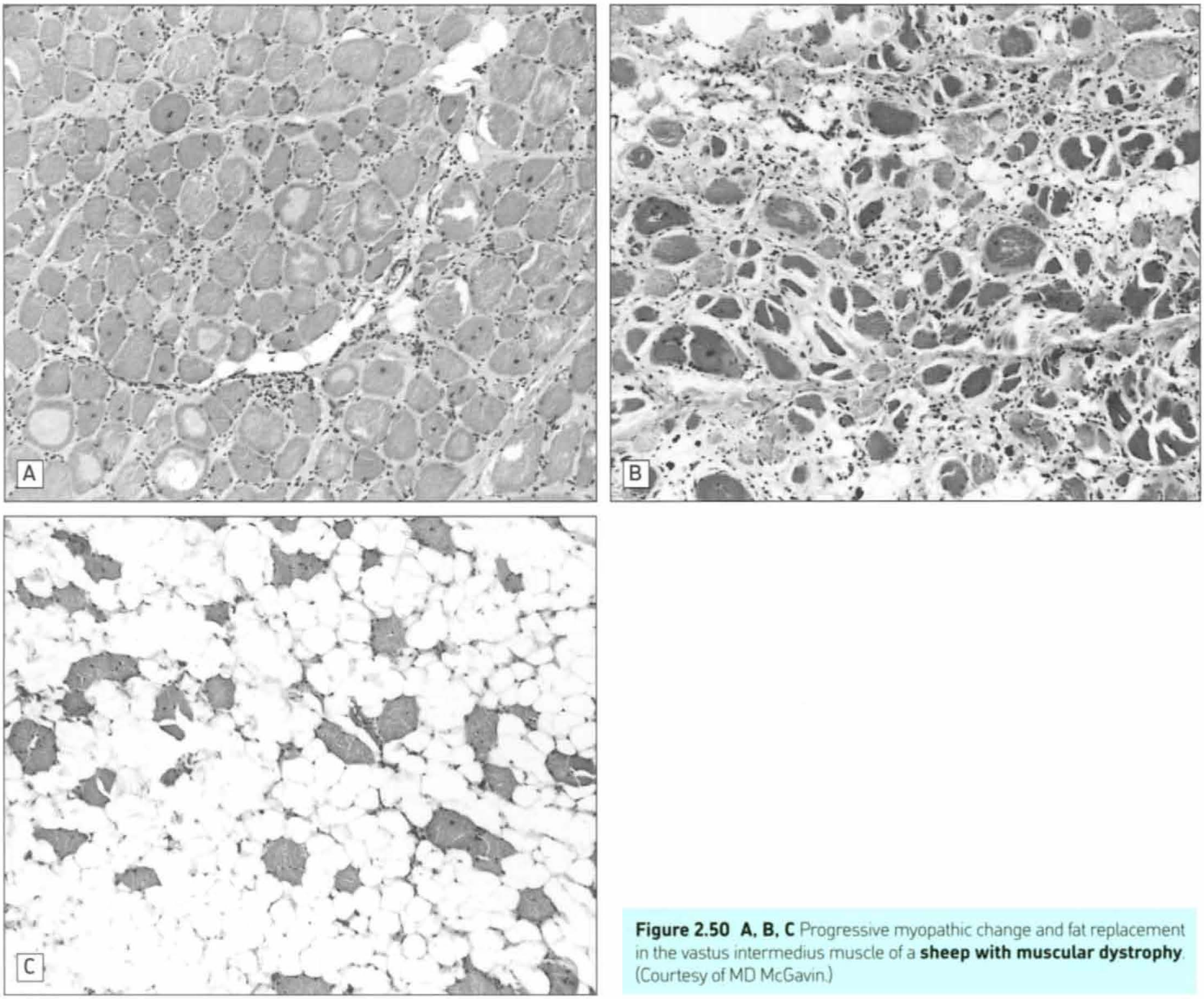

Figure 2.50 A, B, C Progressive myopathic change and fat replacement in the vastus intermedius muscle of a **sheep with muscular dystrophy**. (Courtesy of MD McGavin.)

Ultrastructural findings include *focal myofibril degeneration and Z-line abnormalities, including nemaline rods*. The distinctive sarcoplasmic masses are found to contain a mixture of normal organelles and tubular and fibrillar structures, including abnormal Z-line material. Immunocytochemical studies indicate profound alterations in cytoskeletal elements, including *loss of alpha actinin and proliferation of desmin*. Desmin forms the major constituent of the sarcoplasmic masses. Similar abnormal expression of desmin has been demonstrated in a growing number of myopathies affecting people and animals, and the term "myofibrillar myopathy" has been proposed for such disorders.

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Inherited and congenital myopathies

Many animal disorders have been compared to similar disorders in humans, but such comparisons are rarely validated. Several disorders are included in this section that have previously been designated as muscular dystrophies; the myopathy of sheep included in the muscular dystrophy section might be better classified as an inherited progressive myopathy. Inherited metabolic disorders are described in the Metabolic myopathies section. *The classification of myopathies in animals, similar to those in people, is subject to modification based on findings of on-going genetic and pathogenetic studies as well as on changes in disease definitions.*

Breed-associated myopathies in the dog

Several myopathic disorders are recognized in dogs that are breed-specific and it is likely that many more will be identified in the future. An inherited basis is suspected, if not confirmed, in most cases of breed-associated myopathies.

Myopathy of Labrador Retrievers

An inherited myopathy in Labrador Retrievers has been reported from Europe, North America, and Australia. The disorder occurs in working dog lines and not in show lines and is inherited as an *autosomal recessive disorder*. Both yellow and black Labrador Retrievers are affected.

Clinical severity is quite variable. Evidence of *stunting, reduced exercise tolerance, and a stiff "bunny hopping" gait* may be apparent soon after birth or may not occur until about 6 months of age. Affected dogs may always have a slightly stiff gait and low head carriage, or may be apparently normal at rest. Exercise in affected dogs leads to increasing signs of weakness and collapse. Clinical signs are exacerbated by cold ambient temperature. Muscle atrophy may or may not be apparent. *The temporalis muscles appear to be particularly prone to atrophy*. Decreased or absent patellar reflexes are a consistent finding in affected dogs. Megaesophagus leading to regurgitation may occur, but is uncommon. This disorder may progress, but often stabilizes by 6 months to 1 year of age, and affected dogs may show some degree of improvement with age. Electromyography reveals bizarre spontaneous, high-frequency activity. Serum activities of CK and AST are normal or only slightly increased.

Gross pathologic examination may indicate normal or slightly reduced muscling. On histological examination, *affected muscles exhibit a marked increase in fiber size variation that is most severe in older dogs*. Although initial reports indicated a type 2 fiber deficiency,

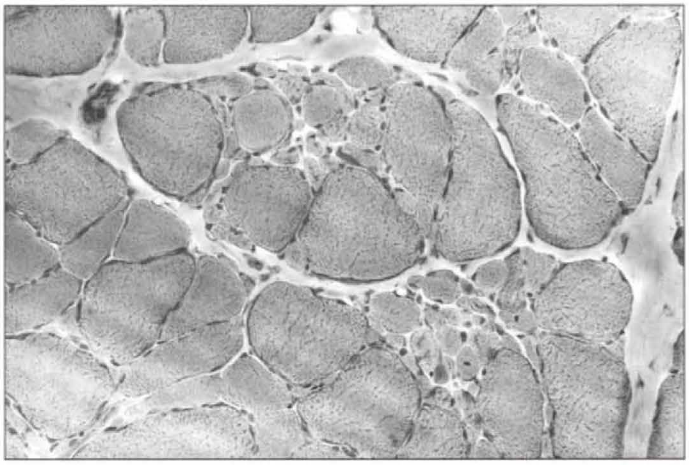

Figure 2.51 Increased variation in fiber diameter with groups of angular atrophied fibers and groups of fibers with rounded atrophy (mimicking denervation atrophy) in a Labrador Retriever with **congenital myopathy**. Frozen section, modified Gomori's trichrome.

subsequent studies indicate that, although alterations in fiber type are typical, atrophy or deficiency of type 2 fibers is variable. Scattered angular atrophied fibers may be seen. Fiber type grouping and small and large group atrophy are common (Fig. 2.51). Alterations in mitochondrial distribution are often striking, with peripheral mitochondrial aggregates and cytoplasmic zones devoid of mitochondria. Fibers with internal nuclei, whorled fibers, and split fibers are seen. There are scattered necrotic and regenerate fiber segments. Endomysial fibrosis is minimal. Despite the angular atrophy and fiber type grouping suggestive of denervation, motor nerve conduction velocities are normal and no abnormalities have been found in the peripheral or central nervous systems.

Myopathy of Bouvier des Flandres dogs

A *degenerative polymyopathy* occurs in the Bouvier des Flandres dog. Although the inheritance is not known, both sexes are affected. *Esophageal and swallowing muscles appear to be preferentially involved*, although generalized weakness occurs and histopathologic changes are seen in multiple muscles.

Affected dogs may not be recognized until 1–2 years of age when regurgitation due to megaesophagus becomes apparent. Clinical severity is variable, however, and affected pups may be slightly stunted and display locomotor and swallowing difficulties as early as 7 weeks of age. Generalized muscle atrophy, weakness, and a peculiar paddling gait are characteristic. Serum activities of CK and AST are typically moderately increased. Bizarre high-frequency activity is seen with concentric needle electromyography.

Gross pathologic findings are *generalized muscle atrophy and pallor and megaesophagus*. Histopathologic changes include moderate to severe increase in fiber size variation with rounded atrophy and hypertrophy of both fiber types. Severe cytoarchitectural changes are seen, including whorled fibers, split fibers, and fibers with internal nuclei. Multifocal myofiber necrosis and regeneration occurs, but is generally not striking. Endomysial and perimysial fibrosis occurs, but is relatively mild. Cardiac myofiber degeneration and fibrosis may also be present.

Canine dermatomyositis

A syndrome of crusting and alopecic skin lesions and associated myositis has been described in *Shetland Sheepdogs, Collies* and in a Pembroke Welsh Corgi dog. Canine dermatomyositis is thought to be inherited as an *autosomal dominant disorder* based on genetic studies in the Collie dog. This disorder has been likened to human dermatomyositis, which is thought to involve autoimmune mechanisms. In people, characteristic findings are a skin rash of the face, upper chest, and skin overlying joints, and muscle weakness with perifascicular atrophy and immune complex deposition and damage in skeletal muscle capillaries. Dogs with dermatomyositis develop distinctive skin lesions with basal cell degeneration and subepidermal cleft formation, but muscle lesions are not consistently found. Serum activities of CK and AST are normal. Nonsuppurative inflammation and atrophy of temporal and masseter muscles have been seen in affected dogs, however *electrodiagnostic and histologic studies of a group of affected Shetland Sheepdogs did not reveal convincing evidence of a primary myositis*. Muscle inflammation, when present, appeared to represent extension of inflammation from overlying ulcerated and inflamed skin lesions.

Other canine myopathies

A juvenile-onset distal myopathy occurs in Rottweiler dogs. Both males and females are affected, and clinical signs of muscle weakness and plantigrade and palmigrade stance are evident at about 2 months of age. The disorder is progressive, and is characterized histopathologically by *marked myofiber atrophy and fat infiltration with mild myonecrosis and endomysial fibrosis.* Serum activities of CK and AST are normal or only slightly increased. Electromyographic studies reveal rare fibrillations and positive sharp waves. The finding of decreased serum and muscle carnitine levels and improvement following carnitine supplementation suggest that *this may be a form of metabolic myopathy involving lipid metabolism*.

A *polymyopathy* with involvement of esophageal musculature occurs in association with dyserythropoiesis and cardiomegaly in *English Springer Spaniel dogs*. Findings in affected skeletal muscle include marked increase in fiber size variation with both rounded atrophy and hypertrophy and many fibers with internal nuclei. Central linear or irregular granular inclusions within myofibers that are visible on H&E and Gomori trichrome-stained frozen sections are characteristic. Inclusions are often associated with fiber splitting.

A myopathy with scattered degenerate and regenerate fibers and intramyofiber core-like structures has been seen in a young adult *Great Dane dog* with muscle weakness and atrophy. Ultrastructural evaluation revealed that the cores consist of disarrayed myofilaments and thickened Z-lines similar to nemaline rods.

Nemaline rods within myofibers occur as a nonspecific change in various canine myopathic conditions, and have been described in dogs with *X-linked muscular dystrophy*, hypothyroidism, and Cushing's syndrome. *Nemaline rods are formed by expanded Z-lines and are visible only in frozen sections stained with Gomori's trichrome stain or on ultrastructural evaluation.* Ultrastructurally, nemaline rods are electron-dense structures with periodicity similar to the Z-band material from which these structures arise. Congenital nemaline myopathy has been described in a 10-month-old Border Collie dog and adult-onset nemaline myopathy occurred in an 11-year-old Schipperke dog. Affected dogs displayed exercise intolerance and muscle weakness.

Endocrine myopathies are well recognized in humans, but in dogs are uncommon conditions sporadically associated with hypothyroidism, hyperadrenocorticism, and diabetes mellitus.

Inherited and congenital myopathies of cats

Primary congenital myopathies are less commonly described in cat than in dogs. Some are better characterized than others. It is possible that, given the relatively sedentary life of most house cats, a subtle neuromuscular dysfunction could be overlooked.

Nemaline myopathy of cats

An apparently inherited congenital myopathy characterized by intramyofiber nemaline rods occurs in mixed-breed cats. Both males and females are affected. This congenital myopathy is similar to congenital nemaline rod myopathy of people.

Affected cats exhibit signs of *progressive neuromuscular dysfunction* characterized by reluctance to move, a crouched "jerky" and hypermetric gait, muscle twitching, muscle atrophy, and hyporeflexia. Serum activities of CK and lactic dehydrogenase are mildly increased. No abnormalities are detected on electromyographic studies.

Muscle atrophy most severe in the proximal limb muscles and muscles of mastication is seen on gross examination. Characteristic histopathologic findings are seen with frozen section histochemistry, and include *atrophy of type 1 and type 2A fibers and presence of nemaline rods.* Nemaline rods are only visible with Gomori's trichrome stain (Fig. 2.52) and occur primarily within atrophied type 1 and 2A fibers and only rarely in type 2B fibers. Nemaline rods are also seen in toluidine blue-stained Epon-Araldite embedded sections and in sections examined by electron microscopy (Fig. 2.53). Fiber size variation and marked fiber splitting are also seen. Fiber typing is often indistinct in areas in which myofibers exhibit extensive fiber splitting. Fiber necrosis and regeneration are uncommon but may be seen, especially in younger cats.

Other feline myopathies

A syndrome of *feline hyperesthesia* has been recognized for many years. Detailed studies of frozen section muscle biopsies of epaxial muscle from affected cats have found numerous rimmed vacuoles. The contents of these vacuoles stain with monoclonal antibodies to paired helical filaments and beta amyloid, and this disorder has been likened to *human inclusion-body myositis.*

A myopathy causing spasticity has been reported in Devon Rex cats. Autosomal recessive inheritance is suspected. Affected cats exhibit neuromuscular dysfunction from an early age, typically from 3 to 23 weeks of age. Ventroflexion of the neck, exercise intolerance, megaesophagus, a hypermetric forelimb gait, muscle tremors, and collapse with exercise are characteristic. Serum activities of CK and AST are normal. Electromyographic studies indicate that abnormal spontaneous activity is rare and motor nerve conduction and repetitive nerve stimulation studies are normal. Sudden death due to laryngospasm is common. Histopathologic findings are consistent with a primary myopathy and are characterized by excessive fiber size variation due to rounded and angular atrophy and marked hypertrophy, internal nuclei, fiber splitting, and scattered necrotic and regenerate fibers. Endomysial fibrosis occurs in older affected cats.

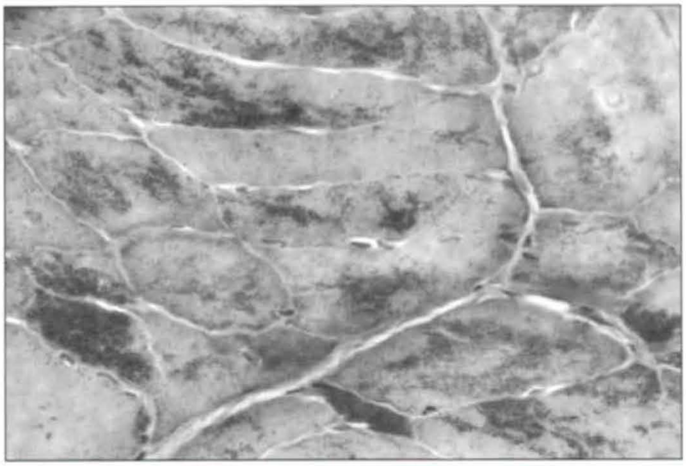

Figure 2.52 Numerous aggregates of dark staining nemaline rods in muscle from a cat with **congenital nemaline myopathy**. Frozen section, modified Gomori's trichrome.

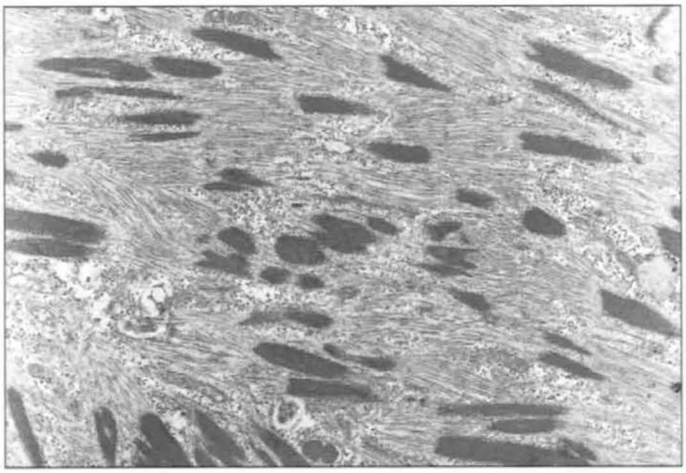

Figure 2.53 Dense staining elongate bodies composed of Z-disk material with associated myofilaments characteristic of nemaline rods in a cat with **congenital nemaline myopathy**. (Courtesy of BJ Cooper.)

Inherited and congenital myopathies of cattle

Congenital and inherited myopathies have been described in beef and dairy breeds. Double muscling in beef breeds, a genetic defect in the myostatin gene, is discussed under the heading Congenital and inherited defects. Myopathy due to glycogen storage disease is described in the Metabolic myopathy section.

Myopathy of the diaphragmatic muscle (diaphragmatic dystrophy)

An inherited myopathy affecting primarily diaphragmatic and intercostal muscles has been described in *Meuse-Rhine-Yssel cattle in The Netherlands and in Holstein-Friesian cattle in Japan.* The disorder in these two breeds appears to be identical. The disorder affects males and females, and is suspected to be transmitted as an autosomal recessive trait.

Clinical signs in affected cattle occur in adults from 2 to 10 years of age and include *loss of appetite and condition, decreased rumen activity, and recurrent bloat.* Serum activities of CK, AST, and LDH are normal.

Abnormal spontaneous activity was not seen in an electromyographic study of the diaphragm of affected Meuse-Rhine-Yssel cattle, although subtle changes in motor unit potentials were found.

The most severe gross pathologic changes are found in the *diaphragm, which is swollen, pale, and inflexible.* Similar changes are seen in intercostal muscles. Microscopic changes of myopathy occur in many other muscles and become more obvious as the disease progresses. Affected fibers exhibit marked cytoarchitectural alterations including vacuolar change, sarcoplasmic masses, atrophy and hypertrophy of fibers, numerous internal nuclei, marked fiber splitting, and central core-like lesions. *These core-like lesions are the most distinctive finding in this disorder.* Frozen section histochemistry studies demonstrate lack of mitochondrial enzyme staining within the cores, but strong staining at the periphery, similar to the cores and rimmed vacuoles described in several human myopathies. Scattered necrotic fiber segments are seen, often with macrophage infiltration. Endomysial fibrosis occurs and appears to be progressive. *Myocardial fibers contain distinctive centrally located inclusions* that are intensely acidophilic on H&E stain and dark green with Gomori's trichrome stain. On ultrastructural examination, the core-like lesions in skeletal muscle and the inclusions within cardiac myocytes consist of *disordered myofilaments with streaming of Z-line material.* Immunohistochemical studies of affected Holstein-Friesian cattle demonstrate that the core-like lesions in skeletal muscle contain actin and ubiquitin, but lack alpha actinin, desmin, and vimentin. Similar studies of myocardial fibers found variable ubiquitin reactivity of cardiac inclusions, and increased desmin reactivity on the periphery of the inclusions. It is proposed that this bovine disorder *may be a form of myofibrillar myopathy.*

Other bovine myopathies

Congenital myopathy occurs in *Braunvieh × Brown Swiss calves.* Both males and females are affected. These calves are abnormal at or soon after birth, with rapidly progressive muscle weakness and recumbency developing within 2 weeks of birth. Histological findings are marked fiber size variation due to fiber atrophy and hypertrophy, fiber splitting of hypertrophied fibers, disorganization of myofibrils, nemaline rods, internal nuclei, and peripheral eosinophilic inclusions. Fiber necrosis, regeneration, and endomysial fibrosis are not seen. Ultrastructural evaluation reveals that peripheral inclusions consist of tightly packed filamentous structures. Myofibrillar and mitochondrial disarray are also seen.

A congenital myopathy has been reported in a single Friesian calf that showed progressive weakness from birth. Muscle fibers were poorly developed and often exhibited lack of adequate myofibrils similar to myofibrillar hypoplasia of piglets. The most striking abnormality was the absence of Z-lines and presence of intracytoplasmic electron dense inclusions.

A degenerative myopathy characterized by necrotizing vasculopathy within skeletal muscle occurs in *young Gelbvieh cattle* (Fig. 2.54). The cause of this disorder is not known, although both immune-mediated and vitamin E deficiency-associated vasculopathy have been proposed.

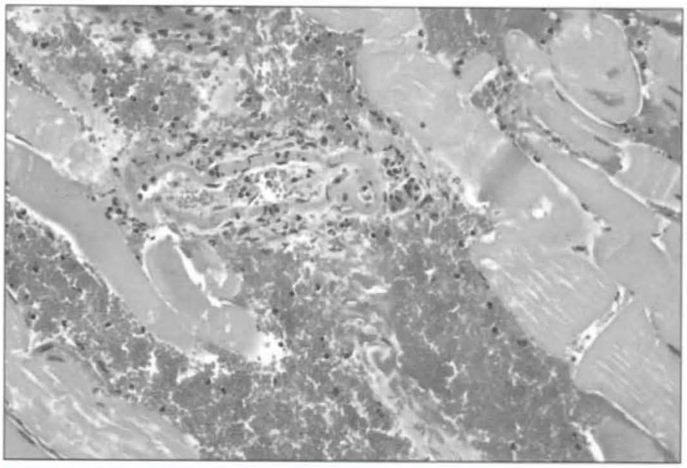

Figure 2.54 Necrotizing vasculitis and hemorrhagic necrosis of muscle in a Gelbvieh steer. (Courtesy of BJ Cooper.)

Inherited and congenital myopathies of sheep

The progressive inherited myopathy of *Merino sheep* in Australia has been described in the Muscular dystrophy section. A suspected autosomal recessive congenital myopathy has been described in *Border Leicester sheep.* At birth, affected lambs have clinical signs similar to lambs with cerebellar cortical atrophy ("daft lambs") but central nervous system lesions are absent. Clinical signs consist of difficulty or inability to rise and a peculiar arching of the neck with the head pressed back in an opisthotonos-like position. Pathologic findings in muscle are primarily within the cervical muscle and consist of abnormal fiber size variation due to fiber atrophy and hypertrophy. It has been proposed that small-diameter fibers represent delayed myofiber differentiation and maturation rather than atrophy. Ultrastructural abnormalities, including electron-dense bodies within axons of intramuscular nerves, suggest that myofiber lesions may reflect functional deficits of peripheral nerves rather than a primary myopathic process.

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Myotonic and spastic syndromes

Myotonia *is defined clinically as a temporary inability of skeletal muscle fibers to relax, resulting in transient uncontrollable muscle tension as a result of voluntary muscle contraction, and is manifested clinically as muscle stiffness.* Electromyographically, myotonia is characterized by waxing and waning spontaneous electrical activity ("dive bomber" sounds) evident following needle insertion or muscle percussion during concentric needle electromyography. In many, but not all, myotonic syndromes, prolonged focal muscle contraction may be elicited by muscle percussion, a phenomenon known as "*dimpling*" (Fig. 2.55). Myotonia may be inherited or acquired. Myotonia is most obvious on initiation of muscle contraction and often improves with continued exercise. Affected individuals may be normal between episodes or have persistent muscle stiffness resulting in a stiff gait. Myotonic syndromes reflect muscle membrane electrical abnormalities, most often associated with *ion channel defects*. Ion channel disorders are a diverse group of disorders, collectively termed the *channelopathies*, and many involve the abnormal regulation of chloride and sodium ions.

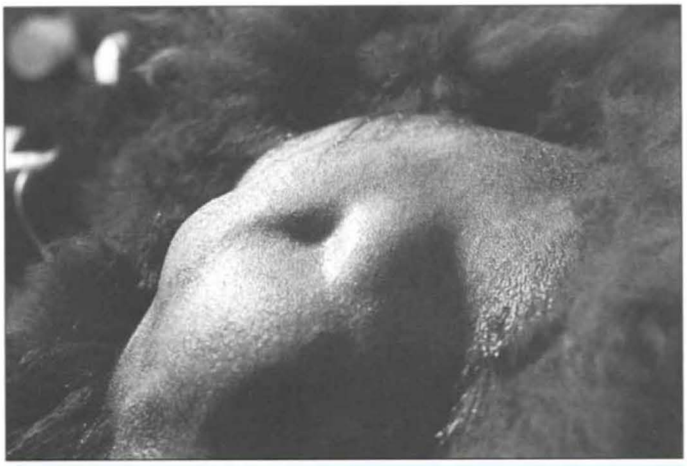
Figure 2.55 Dimpling following percussion in a Chow Chow dog with **congenital myotonia**. (Courtesy of L Fuhrer.)

In animals, confirmed or suspected inherited myotonias are most common. Drug- or toxin-induced myotonia occurs in humans, and a report of transient myotonia in a dog following ingestion of a phenoxy herbicide indicates that such induced forms of myotonia are possible in animals. Hypercortisolism due to endogenous or exogenous corticosteroids can induce a myotonia-like syndrome in dogs, although this disorder is best classified as a *pseudomyotonia*. Pseudomyotonic electrical discharges are also a feature of X-linked Duchenne-type muscular dystrophy in the dog and cat, and the canine disorder has been erroneously described as a myotonic myopathy.

Several syndromes involving *episodic spasticity of limb movements* are recognized in animals. The cause of these spastic syndromes is poorly understood, although an inherited basis is often suspected. Although primary myopathy is suspected in some cases, it is also possible for abnormal peripheral or central nervous system activity to result in episodic spasticity.

Myotonia in the dog

A *congenital myotonic syndrome* has been described in several breeds of dog, including Chow Chow, Staffordshire Terrier, and Miniature Schnauzer dogs. A suspected *adult-onset myotonic syndrome* has been described in the Rhodesian Ridgeback and Boxer dog. Clinical signs in myotonic dogs are often misinterpreted as a shifting leg lameness. Myotonia has been best characterized in the Chow Chow and Miniature Schnauzer dog.

Myotonia in the Chow Chow

This disorder occurs in Chow Chow dogs worldwide, including North America, Europe, Australia, and New Zealand. It may be that breeding for heavy muscling has concentrated the trait in this breed. Myotonia in Chow Chows affects both sexes, and is likely to be an *autosomal recessive trait*. Mildly affected animals, however, may go undetected. The specific muscle defect leading to myotonia in the Chow Chow is still not known.

Clinical signs of stiff gait and muscular spasm on initiation of movement may be apparent as early as 6 weeks of age. Electromyographic evidence of myotonia precedes obvious clinical signs and persists for the life of the animal. Laryngospasm is common, causing episodic collapse or cyanosis, and affected dogs often have a weak or hoarse bark. Muscular contraction occurring when affected dogs attempt to rise often results in transient splaying of the forelimbs followed by sufficient relaxation to allow the dog to stand and ambulate, although affected Chow Chows often have a persistent stiffness of gait. Affected dogs may have a stiff "bunny hopping" gait in the pelvic limbs, and muscular hypertrophy is evident in early stages. Percussion of limb or tongue musculature results in dimpling (Fig. 2.55). Serum activity of creatine kinase may be mildly increased. Muscle atrophy may develop in older animals.

In young myotonic dogs, the muscles may appear hypertrophied. In older affected dogs, muscle atrophy may be more obvious, and muscles may appear slightly pale. Histopathologic findings in affected muscle are minimal in early stages of the disorder. Only rare necrotic fibers are seen. With time, *progressive myopathic changes*, including excessive fiber size variation due to both hypertrophy and atrophy, internal nuclei, and mild endomysial fibrosis may be apparent. *Chronic myopathic changes, in particular numerous fibers with one or more internal nuclei, are the most consistent finding in muscle from older myotonic Chow Chows.*

Myotonia in the Miniature Schnauzer

Myotonia in the Miniature Schnauzer, reported as "myotonic myopathy," is also suspected to be due to *autosomal recessive inheritance* and appears to be clinically and histopathologically similar to myotonia in the Chow Chow. Abnormal muscle membrane chloride conductance in the Miniature Schnauzer is due to mutations in a skeletal muscle voltage-dependent chloride channel, CIC-1.

Myotonia in the cat

A suspected inherited congenital myotonia occurs in mixed breed cats. Clinical signs include a *crouched and stiff hindlimb gait and marked hypertrophy of proximal appendicular muscle evident at about 4 months of age*. These clinical signs of myotonia resemble those of male cats with muscular dystrophy, however serum activities of CK and AST in myotonic cats are normal. Vocalization is weak, facial muscle spasms may be seen, and transient laryngospasm may occur. Electromyography reveals characteristic waxing and waning myotonic activity. Muscle percussion induces "dimpling." *Increased variation in fiber size due to prominent fiber hypertrophy is the primary histopathologic finding.* Fibers with multiple tiny clear cytoplasmic vacuoles may be seen, especially in plastic-embedded sections. Ultrastructural evaluation reveals mild dilation of T-tubules and terminal cisternae of the sarcoplasmic reticulum (Fig. 2.56). Clinical signs are apparently not progressive. Both males and females are affected, and the underlying defect is as yet unknown.

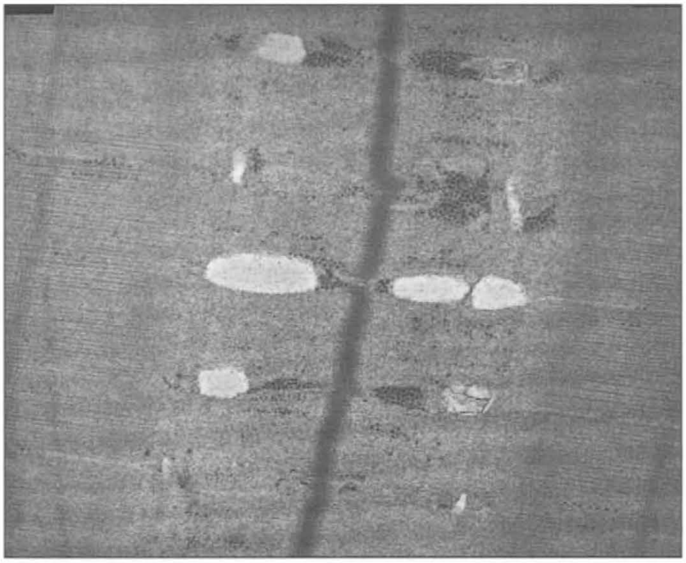

Figure 2.56 Dilated sarcotubular elements in muscle from a cat with **congenital myotonia**. (Courtesy of JF Cummings.)

Myotonia in the goat

Congenital myotonia in the goat has been recognized for many years. *The myotonic goat is said to be the oldest recognized animal model of an inherited human skeletal muscle disease.* In fact, these so-called "*fainting goats*" are prized by certain breeders. If nothing else, the advantages of keeping such goats include the difficulty myotonic goats have in jumping fences due to muscle spasms associated with initiation of muscle contraction.

Myotonia in goats is inherited as *an autosomal dominant trait, with probable incomplete penetrance*. This disorder has been shown to be due to mutation in a skeletal muscle voltage-dependent chloride channel, CIC-1, which causes decreased skeletal muscle chloride channel conductance. This condition is very similar to congenital myotonia (Thomsen's disease) in humans.

Affected goats show clinical signs of myotonia within 2 weeks of birth. Attacks are most often initiated by startling an affected goat. Affected goats assume a "sawhorse" stance and often fall over. Myotonic episodes are transient and affected goats are normal in between episodes. Percussion induces a prolonged muscle "dimpling," and concentric needle electromyography reveals characteristic waxing and waning myotonic potentials. Serum activities of CK and AST are normal.

There are no gross abnormalities in myotonic goats, and affected goats do not appear to be heavily muscled. Histopathologic changes in muscle examined by light microscopy are minimal, with only mild increase in fiber size variation due primarily to fiber hypertrophy and, in some cases, increased internal nuclei. On ultrastructural examination, dilated and proliferated sarcoplasmic reticulum and T-tubules are evident.

Periodic paralyses

The periodic paralyses are a group of inherited muscle disorders. In humans, hypokalemic, normokalemic, and hyperkalemic periodic paralyses are recognized. Although electrical myotonia and pseudomyotonia is a feature of many of the periodic paralyses, the transient attacks of paralysis are associated with muscle hypotonia rather than hypertonicity. These disorders are *sometimes referred to as paramyotonias* rather than true myotonic syndromes. The periodic paralyses are *due to ion channel defects that affect muscle sodium channel activity* and alter the muscle membrane potential. To date, only hyperkalemic periodic paralysis has been recognized in animals. The most common and the best-characterized syndrome occurs in horses. A similar disorder has been seen in a young American Pit Bull dog.

Hyperkalemic periodic paralysis in horses

Hyperkalemic periodic paralysis (HYPP) occurs in horses related to the Quarter Horse stallion "Impressive" and is inherited as an autosomal dominant trait. Homozygotes exhibit more severe signs of muscle dysfunction than do heterozygotes. The diagnosis of HYPP in horses can be confirmed by DNA testing.

Foals homozygous for HYPP often exhibit a peculiar respiratory noise due to *laryngospasm*, and may develop dysphagia and emaciation during the first 2 years of life. Laryngospasm and pharyngeal collapse associated with exercise is common. Such horses must be considered nonviable as performance animals. Heterozygotes may appear to be clinically normal, but are prone to transient attacks of paralysis. Initial signs include muscle fasciculations, flashing of the third eyelid, muscle spasm, and inspiratory stridor. In severe attacks, these signs will be followed by collapse to sternal or lateral recumbency associated with limb hypotonia. Attacks typically last from a few minutes to a few hours. Electromyography performed when animals are apparently normal reveals myotonic discharges and complex repetitive activity (pseudomyotonia).

Serum chemistry analysis often reveals higher than normal potassium levels in affected horses, particularly during attacks of weakness, but this finding is variable. Serum activities of CK and AST are normal or only very slightly increased. Clinical signs of weakness can be induced in HYPP horses with an oral potassium challenge test, high potassium feeds such as alfalfa or molasses-containing feeds, stress, or cold weather. *Sudden death* may occur in HYPP horses, perhaps due to effects of increased blood potassium on cardiac function. Attacks may also be precipitated by inhalant anesthetic agents, and may resemble malignant hyperthermia.

The underlying defect in HYPP in horses, as in man, is a mutation in the muscle sodium channel leading to increased open time. The increased influx of sodium through defective channels results in compensatory loss of muscle potassium, hence the hyperkalemia. Increased concentration of potassium ions outside the cell may further activate the abnormal sodium channels. The alteration in resting muscle membrane potential can lead to increased muscle action potentials and muscle fasciculations and spasms as well as to complete depolarization with inactivation of sodium channel activity and collapse.

There are no gross findings in horses with HYPP, other than frequent heavy and well-defined muscling. Although a vacuolar myopathy similar to humans with hyperkalemic periodic paralysis has been reported, in most cases of equine HYPP, *abnormalities are not found on light microscopy*. Ultrastructural findings of dilated sarcotubular elements are characteristic of HYPP, and are similar to those seen in myotonia in the cat and goat. DNA testing is now available on cells obtained from mane hairs rather than on blood, which is a boon for the pathologist who can now obtain the appropriate sample for testing in cases of otherwise unexplained death in Quarter-Horse-related breeds.

Myotonic dystrophy-like disorders in dogs and horses

Myotonic dystrophy is the most common inherited myotonic disorder in humans, affecting approximately 5 per 100 000 people, and is inherited as an autosomal dominant condition. Onset of obvious clinical signs is most often in late childhood, although this is quite variable, and a congenital form is also recognized. Myotonic dystrophy is a multisystem disorder associated with a constellation of signs, including cataracts and testicular atrophy. Muscle dysfunction is progressive, although the clinical signs of muscle dysfunction remain relatively mild. Findings in muscle biopsies include selective atrophy of type 1 fibers, hypertrophy of type 2 fibers, internal nuclei, ring fibers, and sarcoplasmic masses. However, these histopathologic findings are not specific for myotonic dystrophy. Attempts have been made to classify a group of animal disorders as animal models of myotonic dystrophy based on some similarities in clinical and pathologic findings, but the evidence for this is unconvincing. In particular, the involvement of other organ systems is generally lacking. The genetic defect in human myotonic dystrophy has been shown to be an abnormal expansion of a trinucleotide (CTG) repeat in a gene coding for a serine-threonine protein kinase. Similar molecular studies are lacking in animals. Myotonic dystrophy-like disease has been reported in a Rhodesian Ridgeback dog and in a Boxer dog. The best characterized of the myotonic dystrophy-like disorders occurs in the horse.

Myotonic dystrophy-like disease in the horse

A progressive early-onset myopathy associated with clinical and electrical myotonia and profound cytoarchitectural alterations in muscle occurs sporadically in horses. Breeds affected include Quarter Horse, Thoroughbred, and Standardbred, and both males and females appear to be equally represented. Although the early onset of clinical signs suggests that this disorder is congenital, to date there is no proof that it is inherited, and affected foals appear to occur only sporadically.

Affected foals may have difficulty standing at birth or, more commonly, develop a *progressive stiff gait with marked prominence of proximal limb muscles*, especially the gluteal muscle, evident at about 1 month of age. Percussion of muscle induces prolonged muscle dimpling. Affected foals become progressively weaker and have difficulty rising, with later development of muscle atrophy. Serum activities of CK and AST may be mildly increased. Concentric needle electromyography reveals characteristic myotonic and pseudomyotonic discharges.

At necropsy, affected muscles are often hypertrophied (Fig. 2.57) and may contain fibrous tissue and linear streaks due to fat

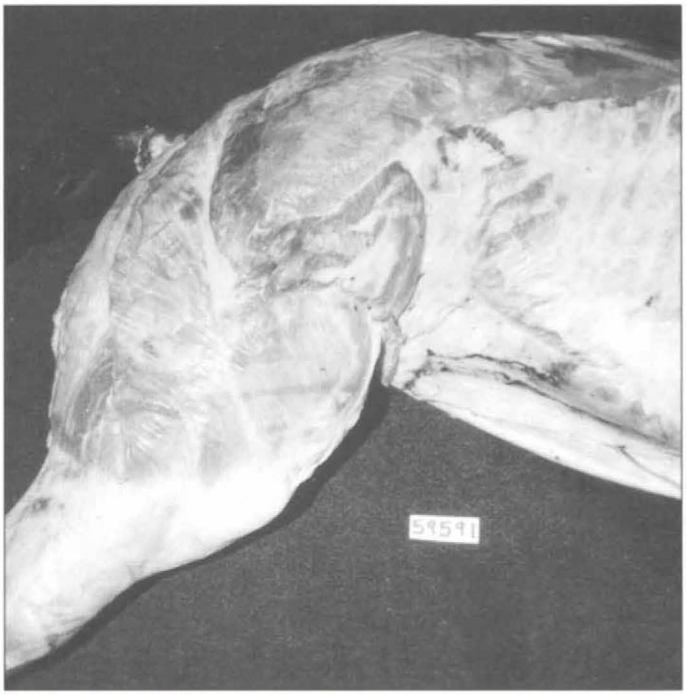

Figure 2.57 Muscular hypertrophy of the hindquarters in a foal with **myotonic dystrophy-like disease**. (Courtesy of TJ Hulland.)

infiltration. Severe lesions are pale and yellow-white. Atrophy of affected muscles may also be seen. There is a selective involvement of muscle, with *the most severe lesions found in the longissimus, iliopsoas, medial gluteal, biceps femoris, semitendinosus, semimembranosus, and sartorius muscles.* The triceps brachii and rectus femoris are less severely affected.

Histopathologic findings are a marked increase in fiber size variation with fiber hypertrophy as well as rounded atrophy and severe cytoarchitectural changes including pale-staining peripheral sarcoplasmic masses, internal nuclei, and small clear vacuoles (Fig. 2.58). Ring fibers and fiber splitting can also be seen. Only scattered necrotic and regenerate fibers are seen. Alterations in fiber type distribution in affected muscles include type 1 fiber predominance and fiber type grouping. Peripheral and intramuscular nerves, however, are histologically normal. Endomysial and perimysial fibrosis occurs, but is variable.

On ultrastructural examination, sarcoplasmic masses are composed of ribosomes, mitochondria, and disarrayed myofibrils.

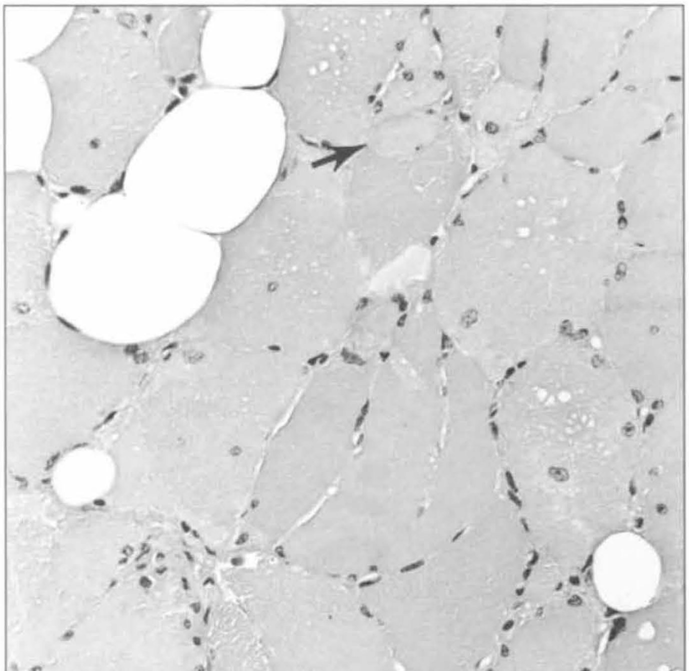

Figure 2.58 Excessive fiber size variation, pale peripheral sarcoplasmic masses (arrow), internal nuclei, fine cytoplasmic vacuoles, and fat infiltration in muscle from a **foal with myotonic dystrophy-like disease**.

Other myotonic-like disorders in the horse

A syndrome resembling *stiff-man syndrome* occurs in the horse. *Affected horses exhibit periodic muscle stiffness and spasms.* Muscle spasms are initiated by voluntary movement. Muscle hypertrophy may be evident. Electromyography reveals persistent motor unit activity, but no abnormal primary spontaneous activity of muscle is seen. Muscle biopsy samples are reported to be normal. A disorder involving *autoantibodies to glutamic acid decarboxylase*, resulting in a neuromyotonic syndrome similar to that occurring in humans, is proposed.

A syndrome of episodic painful muscle spasms associated with prolapse of the third eyelid has been described in horses with ear tick (Otobius megnini) infestation. Percussion of affected muscle groups results in muscle dimpling. Serum activities of CK and AST are often moderately to markedly increased. Electromyographic studies are normal unless muscle contracture occurs secondary to needle placement. No abnormalities have been reported in muscle biopsy samples. Clinical signs resolve following removal or insecticidal treatment of the ear ticks.

Spastic syndromes

"Scotty cramp" is an episodic motor disease, primarily of Scottish Terrier dogs. This disorder most often begins at 2–6 months of age, but adult onset is also possible. It is characterized by *hindlimb and pelvic muscle hypertonicity* along with upward arching of the back for periods of 1–30 minutes, and is precipitated by exercise or excitement. No consistent gross or histologic abnormalities are found in the central nervous system, peripheral nerves or skeletal muscles.

Muscle hypertonicity precipitated by exercise or excitement has been described in several *Cavalier King Charles Spaniels.* Vacuolation of scattered muscle fibers and several minor ultrastructural changes occur, but there is no clear indication of the cause.

"Dancing Doberman" disease has been described as a spastic disease of *one or both hocks* in Doberman Pinschers of any age. The animals are reluctant to bear weight continuously and exhibit a peculiar shifting hindleg action resembling dancing. This appears to be a nonspecific sign, since some of these dogs apparently have a localized myopathy and others have evidence of a neuropathy.

A congenital myopathy in *Devon Rex cats* results in spasticity and is described in the section on Inherited and congenital myopathies.

Spastic paresis of cattle affecting one or both hindlegs is usually seen in calves 3–7 months of age, although onset of signs can occur in adults. The disease appears first as an exaggerated straightness of the hock that increases to the point of *inability to flex the hock.* In the later stages, in animals with unilateral involvement, the affected limb may swing stiffly like a pendulum or be held out in an abnormal cranial or caudal position. The disease, also called *"contraction of the Achilles tendon," "straight hock," or "Elso heel"* (the latter derived from the name of the Friesian bull, some of whose progeny, had the disease), is worldwide in distribution. It affects primarily Holstein-Friesian cattle but has been found in several other dairy and beef breeds, and has a distinct familial pattern. Deafferentation (cutting of the sensory nerve root) of the gastrocnemius muscle relieves the effect, suggesting that the defect is an *unmodulated or uncontrollable sensory-motor reflex loop.*

A related syndrome, referred to as *"spastic syndrome," "crampy spasticity," or "stretches"* affects mature animals, usually *bulls*, and causes periodic and sudden extension and stiffening of both hindlegs. During episodes the back is arched and the neck is extended. Episodes last for a period of seconds to minutes. The disease is progressive and it is reportedly associated with straight hocks and a variety of other abnormalities of bone, joints, or spinal column.

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Metabolic myopathies

Abnormal skeletal muscle metabolism leading to neuromuscular dysfunction is most often due to inborn errors of metabolism, but can occur due to acquired metabolic defects. In some disorders, a metabolic defect is suspected based on clinical and pathologic findings but an exact cause remains elusive. Metabolic defects can affect multiple organ systems, with metabolically active organs such as skeletal muscle, cardiac muscle, nervous tissue, and liver being most severely compromised. Muscle dysfunction can, however, be the primary or the only clinical sign. In some such cases, the occurrence of tissue-specific isozymes of enzymes involved in metabolism can explain selective organ dysfunction, and in other cases the cause is not clear.

In *humans*, metabolic myopathies are broadly classified into four groups according to the type of metabolism affected:

- carbohydrate metabolic disorders,
- disorders of lipid metabolism,
- disorders of mitochondrial metabolism ("mitochondrial myopathies"),
- disorders of adenine nucleotide metabolism (myoadenylate deaminase deficiency).

Clinical signs of progressive muscle weakness, or of exercise-induced muscle cramping, contracture, and rhabdomyolysis are most common. Skeletal muscle dysfunction may occur due to inadequate energy production, to disruption of myofibrillar structure due to storage of carbohydrate or lipid, or to a combination of these factors. To date, the confirmed or suspected metabolic myopathies in animals that have been identified include disorders of carbohydrate metabolism, mitochondrial function, and lipid metabolism.

It should be noted that the terminology regarding *periodic acid-Schiff (PAS) positive, amylase-resistant inclusions* that occur within myofibers in some metabolic disorders is confusing. Terms such as *amylopectin, amylopectin-like, polyglucosan bodies, Lafora bodies, and complex polysaccharide have all been applied to this material.* Until detailed biochemical analysis of this type of stored material is available it is safe to say that *all these terms describe a similar type of material.*

Metabolic myopathies of the dog

Glycogen storage disease type II in Lapland dogs

A canine model of Pompe's disease due to acid maltase (acid α-glucosidase) deficiency has been recognized in the Lapland dog. Massive glycogen storage occurs in multiple organs, but skeletal, myocardial, and smooth muscle are most severely involved. The disorder is inherited as an autosomal recessive trait.

Clinical signs of progressive weakness and regurgitation due to megaesophagus are present from about 6 months of age. Myocardial dysfunction is also a feature. Affected dogs typically die at 10–18 months of age.

Overall muscle atrophy and megaesophagus are found on gross pathologic examination. Histologic examination reveals massive glycogen storage in multiple organs. Stored material is visible in routine preparations as clear vacuoles. This material stains positively with PAS stain and is digested by amylase (diastase), consistent with glycogen. Ultrastructural studies indicate that *the stored glycogen is membrane-bound within lysosomes.*

Phosphofructokinase deficiency (glycogenosis type VII) in English Springer Spaniels and American Cocker Spaniels

Phosphofructokinase (PFK) is a rate-controlling glycolytic enzyme. Quantitative and qualitative alterations in PFK activity can significantly alter energy metabolism in cells such as erythrocytes and skeletal muscle cells that rely heavily on glucose metabolism for energy. PFK is a tetramer composed of liver, muscle, and platelet type subunits in tissue-specific patterns. *The genetic mutation is a point mutation in the muscle isozyme of PFK, and a PCR-based test can detect affected and carrier animals.*

In English Springer Spaniels and American Cocker Spaniels, deficiency of the muscle isozyme of PFK results in *recurrent hemolytic episodes due to defective erythrocyte carbohydrate metabolism.* Variable hematocrit and persistent high reticulocyte counts are typical findings. Episodes are most often initiated by exercise or excitement, as PFK-deficient erythrocytes are particularly sensitive to lysis associated with a mildly increased pH of blood (alkalemia) resulting from hyperventilation. Skeletal muscle cells are apparently protected to some degree by up-regulation of the liver isozyme of PFK. Exercise intolerance, exercise-induced muscle cramps, and progressive muscular atrophy and weakness can occur, however, particularly in older PFK-deficient dogs. Erythrocyte PFK activity is markedly reduced in affected dogs, and moderately decreased in heterozygotes.

In humans with PFK deficiency, clinical signs are entirely due to muscular dysfunction, with exercise-induced cramps and myoglobinuria being most common. Late-onset progressive muscle weakness may also occur. The differences in clinical signs in affected dogs reflect the sensitivity of canine erythrocytes to alkalosis-induced lysis and possibly also to the high reliance of canine muscle on oxidative metabolism and less reliance on anaerobic glycolysis.

In older PFK-deficient dogs, examination of skeletal muscle may reveal *chronic myopathic changes including excessive fiber size variation and intramyofiber vacuoles and inclusions of material* that is stained lightly by hematoxylin in H&E-stained sections. This material is *intensely PAS-positive* and resists digestion by amylase (diastase), consistent with an amylopectin-like complex polysaccharide. Accumulation of this material appears to be a function of age. Ultrastructural studies indicate that this material is composed of nonmembrane-bound granular filamentous and amorphous material. Other findings at necropsy include marked extramedullary hematopoiesis and hemosiderosis due to recurrent hemolysis.

Other canine metabolic myopathies

- *Mitochondrial myopathy* causing episodic weakness and exercise-induced lactic acidosis has been described in a litter of Old English Sheepdogs.
- *Familial myoclonic epilepsy* with polyglucosan bodies in skeletal muscle occurs in miniature wire-haired Dachshunds.
- Neuromuscular weakness associated with *increased lipid accumulation and decreased carnitine activity* within skeletal muscle has been described as an acquired disorder in a variety of dog breeds.
- *Lipid storage myopathy* is thought to reflect abnormal lipid metabolism. It should be noted that increased intramyofiber lipid is normal in pups up to about 2 months of age and should not be interpreted as a lipid storage myopathy.

Metabolic myopathy of the cat

Metabolic myopathy in Norwegian Forest cats

A glycogen storage disease type IV due to deficiency of glycogen branching enzyme occurs as an autosomal recessive trait in Norwegian Forest cats. Although glycogen storage occurs in multiple organ systems in the cat, it is dysfunction of skeletal muscle, cardiac muscle, and nervous tissue that characterizes the feline disorder. In contrast, glycogen storage type IV of humans typically results in early-onset and progressive hepatic cirrhosis and liver failure. The molecular basis for this difference between cats and humans is as yet unknown.

Two clinical syndromes have been identified. The most common is the birth of fully developed kittens that are either stillborn or that die within a few hours of birth. Affected kittens that survive the neonatal period develop *progressive neuromuscular weakness, muscle atrophy, and cardiac dysfunction* beginning at about 5–7 months of age. Affected cats are often mildly febrile, exhibit generalized muscle tremors and a "bunny hopping" pelvic limb gait. *Progression to tetraplegia is rapid*, occurring within about 2 months following onset of clinical signs. Electromyography reveals abnormal spontaneous activity in multiple muscles. Cardiologic abnormalities include concentric left ventricular hypertrophy, left atrial dilation, decreased relaxation of the left ventricular wall during diastole, and focal hyperechoic areas in the subendocardial region of the left ventricular wall. Serum activities of CK and ALT are often increased. Glycogen branching enzyme activity in muscle, liver, and leukocytes is markedly reduced in affected cats, and moderately to severely reduced in clinically normal parents of affected cats.

Gross pathologic findings are absent in stillborn kittens or kittens dying soon after birth. In older affected animals, *severe generalized skeletal muscle atrophy* is evident. Fibrous replacement of muscle may be seen. *Left ventricular hypertrophy and left atrial dilation* are typical, and foci of fibrosis may be evident within the left ventricular myocardium.

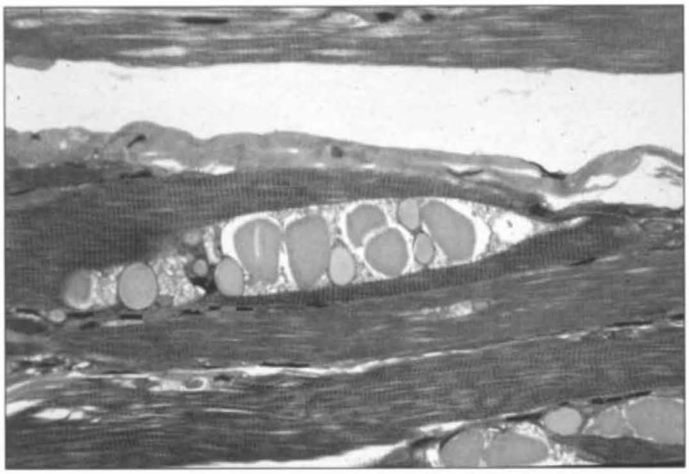

Figure 2.59 Multiple spherical to ovoid pale hyaline inclusions of complex polysaccharide in muscle from a Norwegian Forest cat with **glycogen branching enzyme deficiency** (glycogenosis type IV). (Courtesy of TJ Van Winkle.)

Characteristic histopathologic findings are nonmembrane-bound inclusions of *pale blue material within cells of many organs* (Fig. 2.59). This material is PAS-positive and amylase-resistant. Skeletal muscle, cardiac muscle, and central and peripheral nervous tissue are most severely affected and accumulation of stored material in these organs is accompanied by severe degenerative changes.

Metabolic myopathies of the horse

Equine polysaccharide storage myopathy

A glycogen storage myopathy occurs in many breeds of horses. This disorder has been recognized sporadically for many years. Only recently have studies begun to reveal the variety of clinical disorders associated with polysaccharide storage myopathy in horses as well as the wide range of breeds affected.

Equine polysaccharide storage myopathy is most common in draft, warmblood, and Quarter-Horse-related breeds. In draft-related breeds, a prevalence of over 50% has been reported. This disorder has also been recognized in virtually all other breeds, including ponies and miniature horses. Clinical signs range from recurrent exertional rhabdomyolysis, to progressive weakness that may or may not be accompanied by overt muscle atrophy, to mechanical lameness of the pelvic limbs. Subclinical cases are common. Serum activities of CK and AST may or may not be increased in affected horses. Although *an inherited basis is suspected*, the nature of the inheritance is still unclear.

Gross pathologic findings vary from no significant findings to obvious pale streaks within affected muscle. Muscle atrophy may or may not be evident. Red staining of muscle due to myoglobin release may be seen at postmortem examination of horses dying due to rhabdomyolysis. Careful evaluation of the diaphragm is indicated in animals dying with respiratory failure following clinical rhabdomyolysis, as *severe diaphragmatic necrosis can occur.*

Histopathologic findings are variable. In the most severe cases numerous round to irregularly shaped *blue-gray inclusions are seen within skeletal muscle fibers* on H&E-stained sections (Fig. 2.60). *Inclusions may be single or multiple and are segmental, replacing up to 90% or more of the cross-sectional area of affected fiber segments* (Fig. 2.61). These

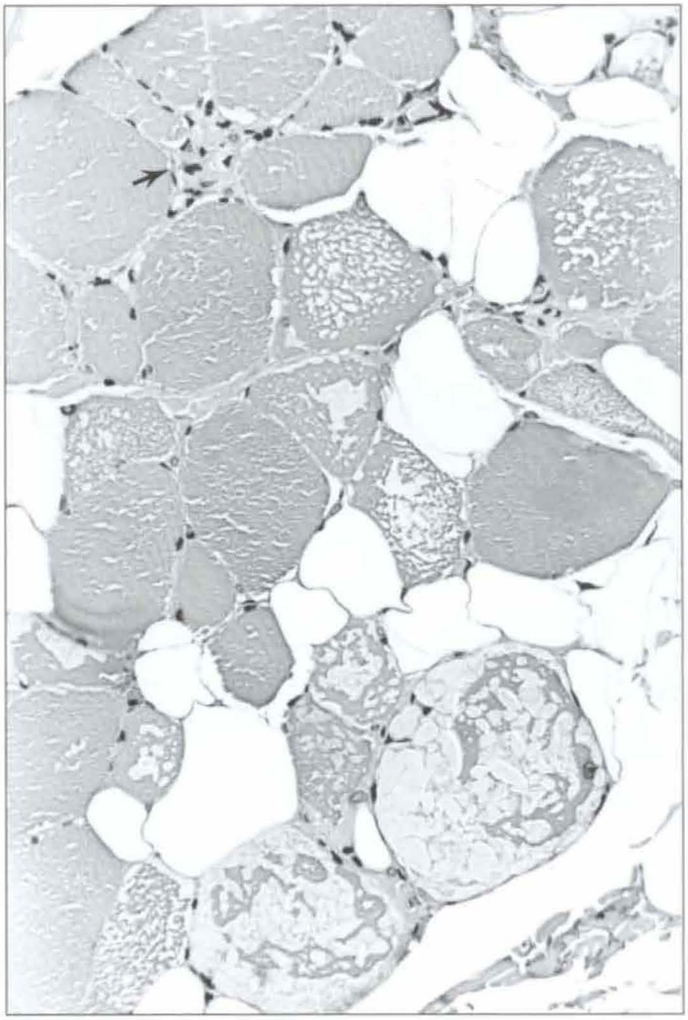

Figure 2.60 Marked fiber size variation with numerous fibers containing multiple vacuoles and inclusions of pale complex polysaccharide in a draft horse with severe chronic **polysaccharide storage myopathy**. A focus of interstitial macrophages (arrow) is indicative of previous segmental necrosis.

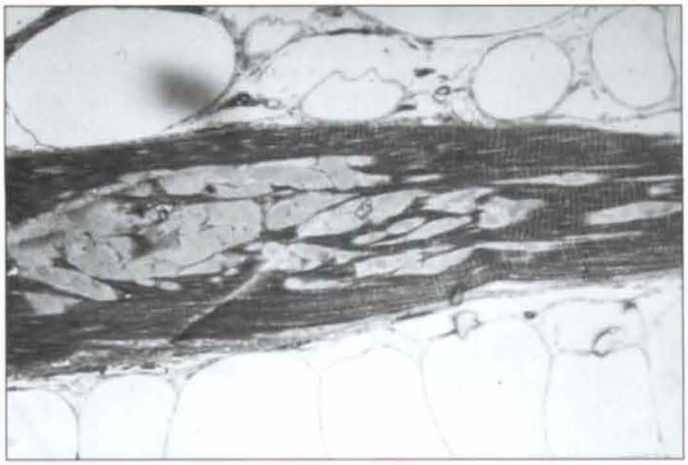

Figure 2.61 Multiple pale inclusions of complex polysaccharide replace large portions of fiber segments in a horse with **polysaccharide storage myopathy**. (Courtesy of JF Cummings.)

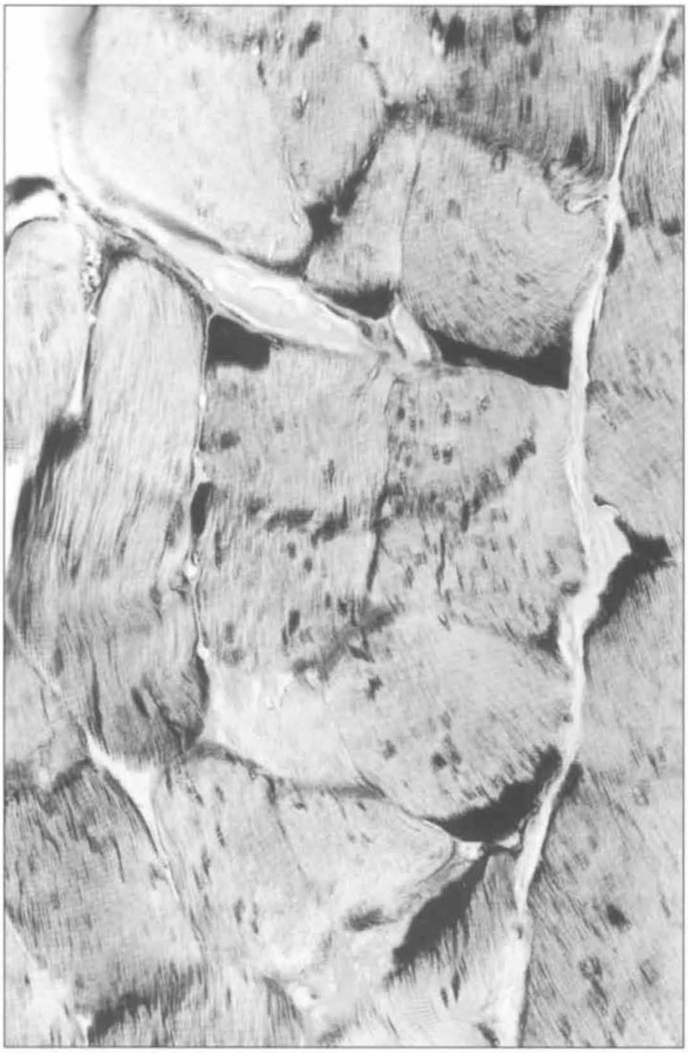

Figure 2.62 Multiple fibers with peripheral aggregates of densely stained glycogen in muscle from an Arabian horse with **polysaccharide storage myopathy**. Fibers also contain increased coarse granular glycogen. Periodic acid-Schiff.

inclusions are PAS-positive and amylase-resistant, consistent with a form of *complex polysaccharide. Inclusions occur only in type 2B glycolytic and 2A oxidative-glycolytic fibers.* Muscles that are strongly type 2 in composition, including semimembranosus, semitendinosus, gluteal, longissimus, and pectoral muscles, are most severely affected. In all cases in which inclusions of complex polysaccharide are seen, other fibers contain subsarcolemmal rounded vacuoles that are either clear or that contain pale pink hyaline material on H&E-stained sections. This material is PAS-positive and amylase-sensitive, consistent with *glycogen.* Fibers with complex polysaccharide or glycogen inclusions often occur in clusters, particularly at the periphery of fascicles. A more subtle pathologic change, involving only subsarcolemmal inclusions of glycogen, is seen in horses believed to have a form of the same disorder (Fig. 2.62). Numerous clear vacuoles within the fiber interior as well as in a subsarcolemmal location can also be seen. Aggregates of glycogen and of amylase-resistant material often contain ubiquitin. *Chronic myopathic changes* are often found, especially in older affected horses, and include excessive fiber size variation due to fiber atrophy and/or hypertrophy and increase in internal nuclei within myofibers. Scattered myofiber necrosis or regeneration may or may not be seen. Macrophages within necrotic fiber segments or found as small clusters within the interstitium often contain PAS-positive, amylase-resistant complex polysaccharide. In very severe cases, to date only documented in draft-related horses with apparent sudden onset of inability to rise, massive replacement of skeletal muscle by infiltrating adipose tissue can be seen. Other organs, with the exception of rare cases with inclusions of complex polysaccharide within cardiac myofibers, are unaffected.

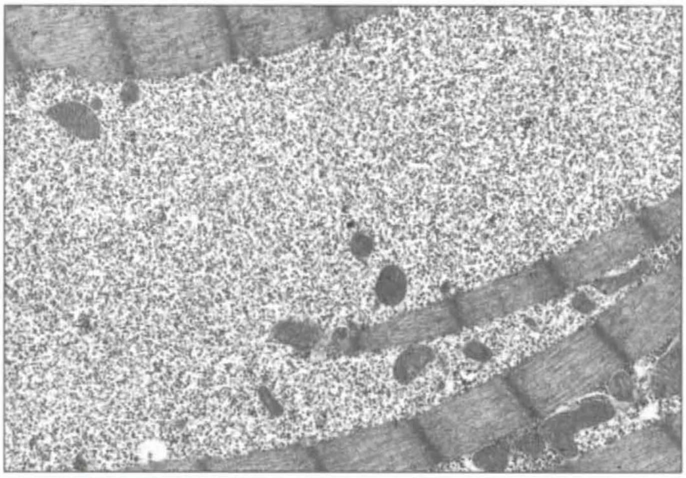

Figure 2.63 Aggregates of granular glycogen with attenuation of myofibrils in a horse with **polysaccharide storage myopathy**. (Courtesy of JF Cummings.)

Ultrastructural examination of affected muscle indicates that *glycogen storage is not intralysosomal but rather is dispersed within the cytoplasm.* Loss or attenuation of myofibrils occurs (Fig. 2.63). Organized inclusions of PAS-positive, amylase-resistant material are composed of tangled fibrillar material studded with glycogen particles.

Despite the clinical and pathologic similarities to myopathies due to carbohydrate metabolic defects in people, dogs, and cats, extensive studies have failed to document a defect in either glycolytic or glycogenolytic pathways in muscle from affected horses. However, diets designed to minimize starch and sugar intake and maximize fat intake have proven to be extremely successful in controlling clinical signs of skeletal muscle dysfunction, *suggesting that this equine disorder involves an as-yet unknown abnormality in carbohydrate metabolism.* Curiously, studies of a small number of horses indicate that pathologic findings are not obviously altered by dietary therapy.

Other metabolic myopathies of horses

A *mitochondrial myopathy* due to deficiency of complex 1 respiratory chain enzyme has been described in an Arabian. Profound exercise intolerance and exercise-induced lactic acidosis without evidence of exercise-induced muscle necrosis were seen.

Glycogen brancher enzyme deficiency occurs in Quarter Horse foals. Affected foals may be aborted or stillborn, or develop weakness and die of cardiac failure in the first few months of life. Characteristic spherical or ovoid inclusions of PAS-positive amylase-resistant amylopectin are seen within skeletal and myocardial fibers, particularly Purkinje fibers (Fig. 2.64). Similar material is seen to a lesser degree in many other organs.

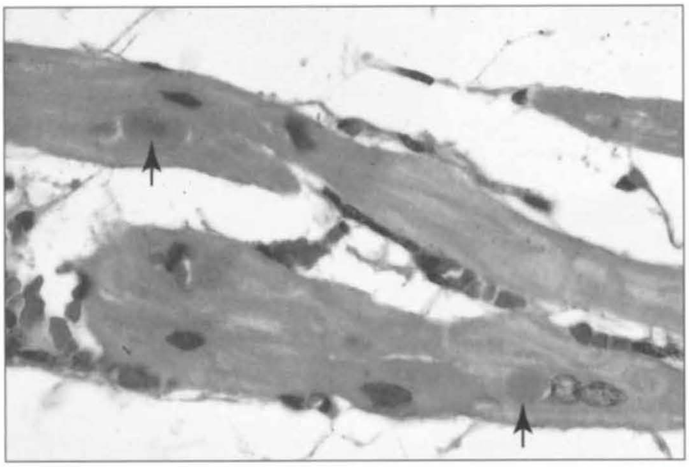

Figure 2.64 Spherical-to-ovoid bodies of complex polysaccharide within Purkinje fibers and cardiac myocytes in a Quarter Horse foal with **glycogen branching enzyme deficiency**. (Courtesy of BJ Cooper.)

Metabolic myopathies of cattle and sheep

Biochemical abnormalities leading to abnormal glycogen storage within skeletal muscle occur in sheep and cattle. In some cases, the disorder is generalized and in others it is confined to skeletal muscle. *Clinical signs of neuromuscular weakness in young sheep and cattle due to metabolic myopathy must be distinguished from the more common nutritional myopathies.*

Bovine metabolic myopathy due to myophosphorylase deficiency

Glycogen phosphorylase is an essential glycolytic enzyme. Tissue-specific isoforms exist, and the skeletal muscle isoform is known as myophosphorylase. *An autosomal recessive defect in the myophosphorylase gene similar to McArdle's disease in people occurs in Charolais cattle, and results in metabolic dysfunction confined to skeletal muscle.* Clinical signs of exercise intolerance and exercise-induced collapse can be seen in affected cattle as young as 7 weeks of age. Increased serum activities of CK and AST are characteristic. Subsarcolemmal vacuoles due to glycogen storage within myofibers are characteristic, and biochemical and histochemical studies reveal a *complete lack of myophosphorylase activity.* Testing of DNA from peripheral white blood cells will detect homozygotes and heterozygotes.

Glycogenosis type II (Pompe's disease) in cattle

Glycogen storage disease due to acid maltase (acid α-glucosidase) deficiency occurs as an autosomal recessive trait in Shorthorn and Brahman cattle. Two clinical syndromes are reported in Shorthorn cattle. An *early-onset form* results in intralysosomal glycogen storage within skeletal, cardiac, and smooth muscle, and in neurons of the central and autonomic nervous systems. Clinical signs of cardiac dysfunction predominate, leading to death due to cardiac failure. A *late-onset form* develops clinical signs predominantly of muscle weakness. Serum activity of CK is often increased, sometimes markedly. In Brahman cattle, glycogenosis type II results in early-onset signs of poor growth and neurologic dysfunction.

Glycogen storage diseases of sheep

A *generalized form* of glycogen storage disease occurs in sheep. Glycogen storage confined to skeletal muscle due to *myophosphorylase deficiency* also occurs, and is similar to the disease in cattle.

Metabolic myopathies of other species

Acid maltase deficiency (glycogenosis type II) resulting in generalized glycogen storage occurs in *Japanese quail.* These birds have been studied as an animal model of acid maltase deficiency of people. Clinical signs of muscle dysfunction predominate, and include inability to right themselves following placement in dorsal or lateral recumbency, and poor wing movements. Weakness is progressive. Abnormal accumulation of membrane-bound glycogen particles occurs as early as day 16 of embryonal development. Progressive glycogen storage resulting in *vacuolar myopathy*, with formation of autophagic vacuoles followed by fiber loss and fatty replacement, are characteristic. Abnormal glycogen storage also occurs in cardiac and smooth muscle as well as in neurons in the brain and spinal cord.

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Congenital myasthenia gravis

Two clearly defined types of myasthenia gravis occur in humans and animals; both congenital and acquired myasthenia gravis have been described in dogs and cats.

- The *congenital disease* is due to an inherent defect in acetylcholine end-plate receptors.
- The more common *acquired disease* is an immune-mediated disorder due to circulating anticholinesterase receptor antibodies and is discussed in the section on Immune-mediated conditions.

Canine congenital myasthenia

In the dog, congenital myasthenia gravis has been described in Jack Russell Terriers, Springer Spaniels, Smooth Fox Terriers, and the Gammel Dansk Hønsehund. The disorder is inherited as an *autosomal recessive trait*. Affected dogs are clinically normal at birth but develop clinical signs of *exercise-induced weakness and collapse* at an early age. Clinical signs are apparent at about 5–8 weeks of age in affected Springer Spaniels and Jack Russell Terriers, and at about 12–16 weeks of age in the Gammel Dansk Hønsehund. Clinical signs are progressive in growing Jack Russell Terriers and Springer Spaniels, often leading to recumbency. The disorder is not progressive in the Gammel Dansk Hønsehund. Esophageal dysfunction leading to megaesophagus may occur in the Springer Spaniel and Smooth Fox Terrier. A decremental response occurs with repetitive nerve stimulation studies. Circulating antibodies to acetylcholine receptors are not found, but affected dogs improve with anticholinesterase therapy.

No abnormalities are seen in the motor end plate by light microscopy. Ultrastructural evaluation reveals *decreased density of postsynaptic acetylcholine receptors and shallow secondary clefts in the end-plate synaptic gutters*. Studies in the Jack Russell Terrier have shown a decreased rate of insertion of acetylcholine receptors into the postsynaptic membrane. Young animals are apparently able to function normally, but with growth this reduced acetylcholine receptor density results in neuromuscular weakness.

Feline congenital myasthenia

Myasthenia gravis is less commonly described in cats. This disorder has been described in Siamese and in domestic shorthaired cats. Mode of inheritance is not known. Clinical signs of *episodic weakness* are first noted at about 4–5 months of age. Affected cats may have a weak voice and the disorder may progress to tetraplegia. Megaesophagus has not been reported. A decremental response is seen with repetitive nerve stimulation, and circulating antibodies to acetylcholine receptors are not found. Clinical improvement can be seen with oral or intravenous anticholinesterase therapy. Acetylcholine receptor density was found to be 66% of normal in one cat studied.

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Malignant hyperthermia

Malignant hyperthermia (MH) *is a condition that results in a sudden increase in myoplasmic calcium concentration leading to prolonged myofiber contraction and muscle rigidity, hypermetabolism, tachycardia, dyspnea, metabolic acidosis, and life-threatening hyperthermia. Severe acute rhabdomyolysis is the primary histopathologic finding.* Episodes are triggered by a variety of circumstances, including stress and pharmacologic agents such as halothane anesthesia. Malignant hyperthermia is an inherited disorder in humans, pigs, and some dogs. *The defect is in the ryanodine receptor*, a calcium-release channel of the sarcoplasmic reticulum that serves a critical role in triggering release of calcium from the sarcoplasmic reticulum during excitation–contraction coupling. Malignant hyperthermia in humans may be due to more than one genetic defect involving the ryanodine receptor. Other myopathic disorders leading to susceptibility to MH occur, and therefore *MH is best regarded as a syndrome rather than a single entity*. Susceptible individuals may have mildly increased serum CK activities and increased erythrocyte fragility. Testing for MH susceptibility classically involves in vitro exposure of muscle biopsy samples to various concentrations of caffeine and halothane. Muscle from MH-susceptible individuals exhibits contraction at relatively low concentrations of these pharmacologic agents as opposed to normal individuals.

Malignant hyperthermia-like episodes also occur in *anesthetized horses*, although an inherited basis has not been documented in the horse. Ryanodine receptor defects have been documented in a small number of cases. It may also be triggered in horses by injection of succinylcholine. Malignant hyperthermia-like episodes may also occur in people or animals with other underlying myopathies. This is certainly the case in cats with X-linked muscular dystrophy, in which anesthesia or the stress of restraint can trigger episodes of fatal hyperthermia. Underlying HYPP has been associated with malignant hyperthermia-like episodes associated with anesthesia in horses, and it is possible that other myopathic conditions may predispose horses to this disorder.

Malignant hyperthermia in pigs (porcine stress syndrome)

Malignant hyperthermia in pigs renders them susceptible to episodes associated with stresses such as handling, transportation, or fighting, and *may result in sudden death*. The meat of affected pigs is usually *pale, soft, and exudative (PSE pork)*. Porcine stress syndrome has been recognized in Europe and North America for a long time as "*herztod*" or *back muscle necrosis* of pigs. *Susceptible pigs exhibit intense, immobilizing limb and torso muscle rigidity, respiratory difficulty, tachycardia, acidosis and, often, rapid death.* Heavy-muscled pigs seem to be most susceptible to the clinical disease. Prior to identification of the ryanodine receptor gene defect, a large body of literature accumulated regarding various biochemical abnormalities detected in affected muscle and in affected animals. Recently, *a single point mutation in the skeletal muscle ryanodine receptor (ryr1) at locus HAL-1843 leading to increased channel open time has been shown to be the cause of MH* in domestic pigs, including Pietrain, Yorkshire, Poland China, Duroc, and Landrace breeds. DNA testing of peripheral blood for the HAL-1843 gene defect is available commercially. Genetic studies point to a single affected founder pig followed by widespread dissemination of the gene, and it has been suggested that these pigs have been selected for based on their heavy muscling and decreased body fat. Malignant hyperthermia occurs in all pork-producing countries of the world. It has been estimated that 2–30% of purebred breeding pigs are susceptible to malignant hyperthermia. A similar syndrome of MH appears to occur in *Vietnamese pot-bellied pigs*. In one case of a pot-bellied pig dying due to anesthesia-induced hyperthermia, a gene defect at HAL-1843 was detected. Testing of parents of another pot-bellied pig with suspect malignant hyperthermia, however, did not reveal HAL-1843 defects, suggesting that in pigs, as in humans, *more than one gene defect may lead to MH.*

Postmortem examination of the muscles of susceptible pigs that have not endured an episode of hyperthermia recently reveals normal muscles. *Pigs dying of hyperthermia have pale muscles that are wet and apparently swollen. Rigor mortis develops unusually rapidly.* In addition to lesions of skeletal muscle, *lesions of acute heart failure*, such as pulmonary edema and congestion, hydropericardium, hydrothorax, and hepatic congestion, often are present. The muscles most likely to be affected are those of the *back, loin, thigh, and shoulder.* Although both type 1 and type 2 myofibers undergo necrosis, muscles with a high proportion of type 2 fibers such as longissimus, psoas, and semitendinosus are most extensively and most frequently affected, and these should be examined histologically. Hemorrhages sometimes are present in muscles, and in the warm carcass a marked lowering of muscle pH to 5.8 or lower can be detected. On cooling, the pH rises rapidly towards neutrality. Myocardial pallor involving the ventricular muscles sometimes occurs but the clinical signs of tachycardia are probably related to acidosis.

Microscopic examination of malignant hyperthermia susceptible animals not recently affected by hyperthermic episodes reveals normal muscle fibers, or there may be a few degenerate fibers. *There is nothing distinctive about the appearance of the degenerate fibers.* In pigs dying acutely of malignant hyperthermia, *muscle fibers are separated by edema fluid.* This is evident in rapidly fixed specimens only and may be lost in processing. Changes in muscle fibers are widespread and are typically characterized as *multifocal monophasic injury*. It is possible, however, to find polyphasic injury in pigs with recent non-fatal episodes of MH, and underlying chronic myopathic changes may also be observed. Degenerative changes vary from segmental hypercontraction (Fig. 2.65) to overt coagulative necrosis. *Hypercontraction* is the most common lesion in skeletal muscle. Myocardial lesions include multifocal granular degeneration of myocytes, contraction band necrosis, and myocytolysis.

Malignant hyperthermia in dogs

There are sporadic reports of *MH-like episodes* in various breeds of dogs. *Exercise-induced hyperthermia* has been seen in English Springer Spaniels and in Labrador Retrievers. *Ingestion of hops* can trigger a MH-like episode in susceptible dogs, and this condition has been most commonly seen in Greyhounds. Studies of a breeding colony of mixed-breed dogs susceptible to anesthesia-induced MH determined that the disorder was inherited as a dominant trait with variable severity. A genetic defect has yet to be identified in MH-susceptible dogs and *it is possible that MH in dogs is a heterogeneous disorder.* Chronic myopathic changes including internal nuclei, increased fiber size variation, and fiber hypertrophy may be seen in muscle from MH-susceptible dogs. Histologic lesions in dogs dying due to

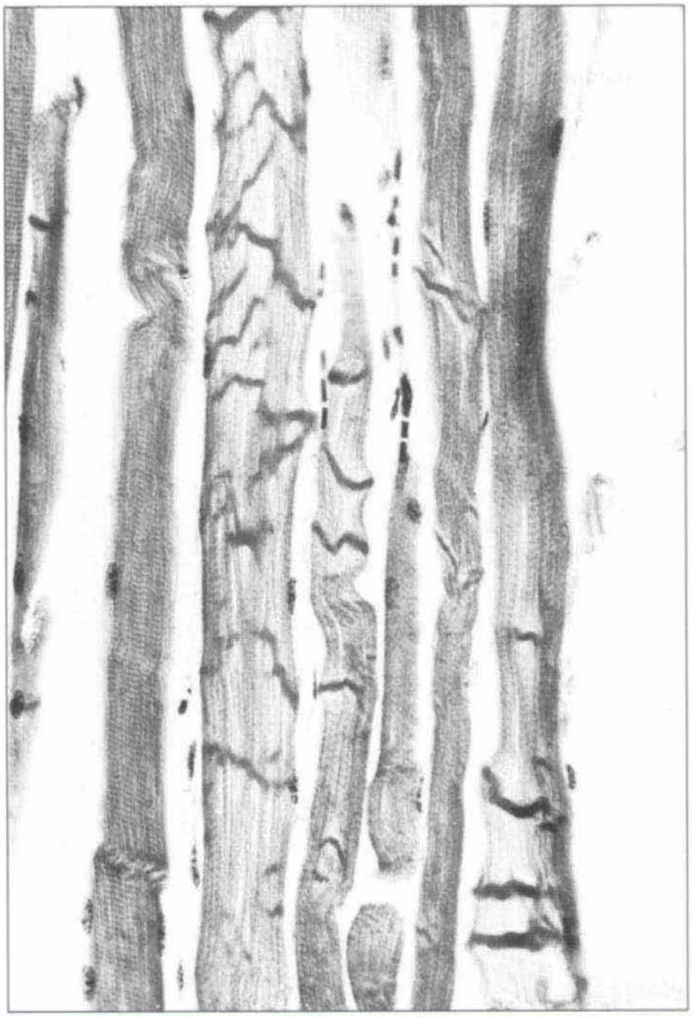

Figure 2.65 Acute myopathy in a pig with malignant hyperthermia (porcine stress syndrome) with characteristic **segmental hypercontraction**. (Courtesy of TJ Hulland.)

hyperthermia are similar to those seen in other MH-susceptible species.

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CIRCULATORY DISTURBANCES OF MUSCLE

Skeletal muscle is a highly vascular tissue with an abundant capillary bed that forms an extensive system of anastomoses. It is generally not possible to induce muscle fiber necrosis by ligation of, or damage to, intermuscular arteries, because most muscles receive small collateral arterioles from tendons, fascial sheaths, and major nerve trunks. Naturally occurring examples of ischemic muscle necrosis are most often due to vascular occlusion secondary to pressure. Occlusion of major arteries such as aortic-iliac thrombosis ("saddle thrombi") can also cause ischemic muscle necrosis.

Each muscle fiber is served, at any given level, by three to 12 capillaries that run mainly longitudinally in the endomysium. Type 1 fibers are served by slightly more capillaries than are type 2 fibers of comparable size. Muscles such as those of mastication are particularly well supplied; nearly twice as many capillaries serve each of these fibers than is the case for the major thigh or shoulder muscles.

Maximum myofiber diameter is limited to some extent by the distance from the capillary to the center of the fiber(s) supplied. When the distance becomes abnormally great, the fiber is likely to form a longitudinal cleft down one side (*fiber splitting*) into which a capillary slips, effectively serving the fiber interior (Figs 2.16, 2.17). Complete or incomplete longitudinal fiber division appears to be a mechanism primarily initiated to improve the capillary-to-fiber ratio.

Ischemic damage to muscle fibers, and the capacity to repair, will depend on the completeness and duration of oxygen and nutrient deprivation.

- Least damaging is *transient hypoxia* of a few hours duration, which causes coagulation of muscle contractile proteins but does little harm to the other cellular components or to the satellite cells. Muscles are ordinarily restored to normal function in about 16–20 days by myoblast activation, division and fusion to form myotubes (see Degeneration and repair of muscle, Fig. 2.22) that mature to myofibers.
- The next level of injury is induced by *episodes of ischemia lasting 6–24 hours*, which cause death of both myofiber and satellite cell nuclei and coagulative necrosis of long segments of myofibers. The capacity for regenerative muscle fiber repair is lost or greatly reduced and reaction is largely local proliferation of fibroblasts and endothelial cells within the endomysium. The repair process is quite rapid but leads to a randomly distributed mixture of regenerated or original fibers interspersed with fibrosis.
- The *third level of ischemic injury is that lasting for more than 18–24 hours*, which leads to death of all cells within an area of muscle. The tissue response in this case is confined to a slow peripheral sequestration followed by phagocytic removal of dead muscle and fibroblastic proliferation that can take months to complete. The end result is a mass of scar tissue that can include adipose tissue, with a peripheral narrow zone of distorted muscle fibers.

During the *acute phase of ischemic muscle destruction*, a relatively orderly sequence of functional and morphological changes can be expected. After as little as 1 h of ischemia, the ability of the muscle fiber to contract in response to any stimulus is lost. Ultrastructurally, changes in subcellular components begin after about 6–7 h of ischemia when the tubular systems become dilated and begin to disintegrate. Some mitochondria may show evidence of swelling and disarray at 8 h, but even after 24 h many mitochondria may be well preserved. Z bands begin to disintegrate at about 12 h. Other components of the sarcomere, including thick and thin myofilaments, remain intact for at least 48 h, and it is only after this time that an amorphous hyaline mass forms, free of cross or longitudinal striations. Degeneration of the fiber at the Z bands occurs after 48 h and may be an extension of the earlier Z band disintegration.

Regardless of the duration and extent of the ischemic change, *the muscle fiber plasma membrane becomes permeable to enzymes and myoglobin*. Serum creatine kinase activity is markedly increased but often returns to near normal in 4–5 days, even when destruction of muscle is extensive. Myoglobin is released from damaged muscle and the amount released will vary directly with the severity and extent of damage. When a large mass of muscle is physically injured or when ischemic degenerative changes are extensive, large amounts of myoglobin are released into the bloodstream. Much of this is excreted by the kidneys. Myoglobin has a direct toxic effect on convoluted tubules leading to nephrosis. Perhaps more importantly, renal ischemia due to shock will contribute to the potential for severe renal damage and renal shutdown. Hyperkalemia due to massive muscle fiber breakdown can result in acute heart failure.

Four syndromes of ischemic muscle necrosis are recognized in humans and animals, namely, compartment syndrome, downer syndrome, muscle crush syndrome, and vascular occlusive syndrome. The distinctions among these syndromes are not always clear. In veterinary medicine, *hypotensive myopathy in anesthetized horses constitutes a fifth syndrome.* In the compartment syndrome, the downer syndrome, and the muscle crush syndrome, ischemia is caused by increasing intramuscular pressure. The vascular occlusive syndrome results from physical obstruction of the blood supply to muscle, and hypotension leading to poor muscle perfusion is thought to be the cause of hypotensive myopathy in horses.

Compartment syndrome

Muscles that are surrounded by either a heavy aponeurotic sheath or by bone and sheath are vulnerable to ischemia when muscle fibers are subjected to moderately vigorous but not exhaustive contraction. This syndrome occurs in well-conditioned athletes, but nowhere is the syndrome more clearly primary and specific than in the infarction that occurs in the *supracoracoid muscles of some breeds of broiler chickens and in some breeds of turkeys*. In these birds, a brief, vigorous flapping of the wings increases intramuscular pressure of the supracoracoid muscle within the inelastic breastbone and the outer muscle sheath. Muscle in full contraction increases in volume up to 20% and this causes partial or transient collapse of the venous outflow. At the same time, muscle activity increases arterial blood flow to the muscle. Subsequent muscle contractions tend to build internal pressure until the intramuscular pressure exceeds first venous and then arterial blood pressure. Metabolites of the muscle fibers exerting an increased osmotic tension coupled with increased arteriolar blood pressure cause accumulation of interstitial water early in the process and this further increases intramuscular pressure. Once blood flow has stopped, ischemic changes of both muscle and vessels begin, and further water escapes from damaged endothelial cells. Pressure builds for 1–4 h after muscle exercise and the extent and severity of damage to muscle increases with time. In both humans and birds, early fasciotomy releases intramuscular pressure and restores the potential for complete regenerative repair.

The so-called *spontaneous rupture of the gastrocnemius muscle of Channel Island breeds of cattle* may represent an example of compartment syndrome leading to ischemia and subsequent rupture (Fig. 2.66). Similarly, the swelling of muscle that can occur in dogs and horses with *masticatory myopathy*, and in horses with *exertional rhabdomyolysis*, may occur at least partly due to compartment syndrome, with initial muscle damage leading to increased pressure against thick overlying fascia.

Downer syndrome

Humans and most of the domestic species share a muscle ischemia syndrome that is initiated by external pressure of objects or by pressure created by the weight of body, torso, or head on a limb tucked under the body for prolonged periods. This condition in humans is usually related to drug overdose, while in animals it is induced by prolonged anesthesia, muscle, joint, or bone damage causing prostration, or metabolic or neurologic disease causing paresis. Absolute size and body weight has some influence on the incidence of the disease. Animals in good condition are particularly susceptible, and thin animals seldom suffer from ischemic muscle necrosis. Rams and heavy ewes, boars and sows, and even large dogs are occasionally susceptible, but this disorder does not occur in cats. *Cows are the species most frequently affected*, partly because of their weight and their muscle bulk and

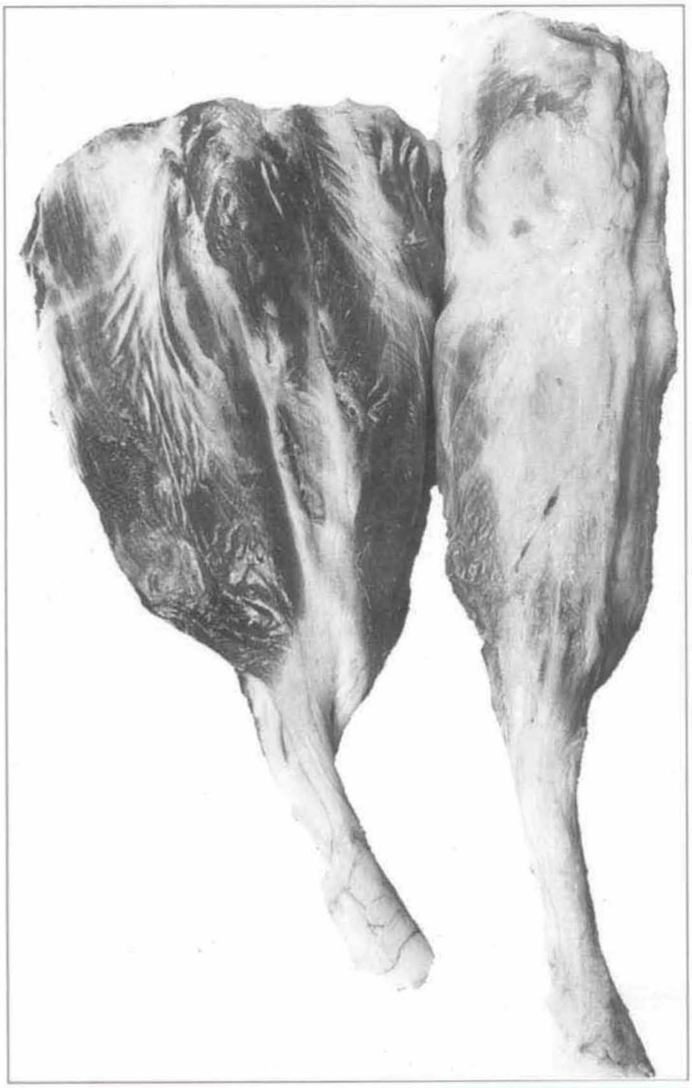

Figure 2.66 Rupture of the gastrocnemius muscle in a Channel Island cow, probably due to **compartment syndrome**. Affected muscle is on the right; normal muscle is on the left. (Courtesy of TJ Hulland.)

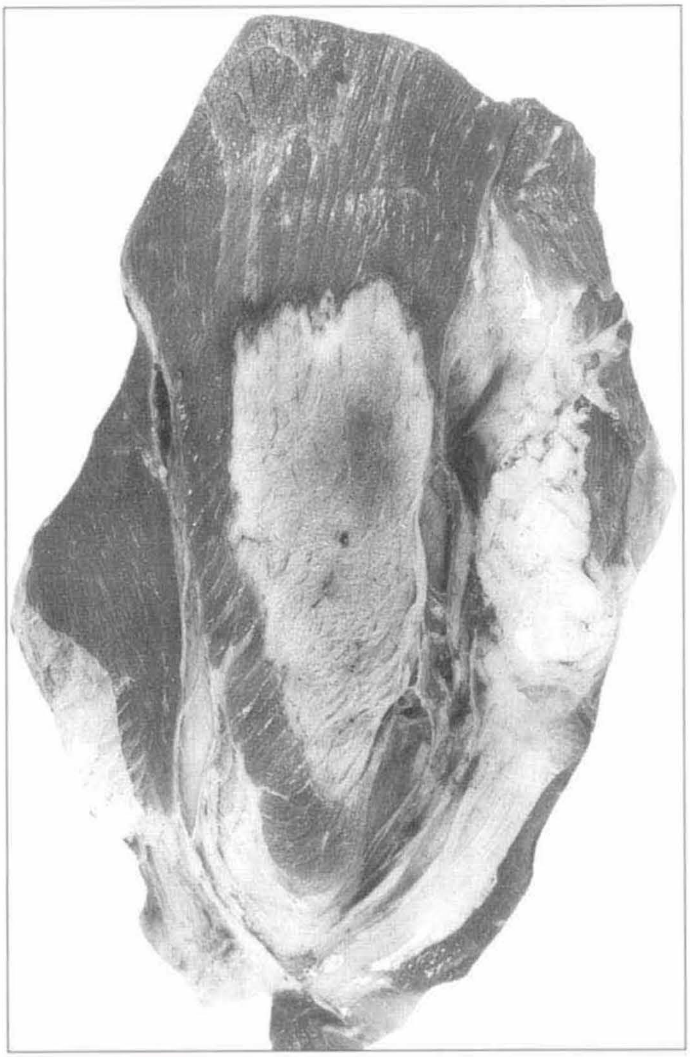

Figure 2.67 Locally extensive pale zone of infarcted thigh muscle due to ischemia in a **downer cow**. (Courtesy of TJ Hulland.)

partly because they are subject to diseases in which paresis is common (Fig. 2.67).

The pathogenesis of the downer syndrome depends upon the fact that the weight of the body can cause pressure within muscles to rise to levels considerably higher than both venous and arterial pressure. Muscles of limbs in a flexed or tucked position are particularly susceptible. The intramuscular pressure soon serves to collapse veins of the fascial sheaths and skin, causing congestion, and then collapses arteries. In cows and horses, extensive ischemic lesions are sometimes created by a period of inertia as short as 6 h, while some cows seem to be able to tolerate 12 or even more hours of immobility with minimal residual lesions. As time passes and as the pressure is removed, the affected limb continues to swell as edema fluid increases under returned arterial flow. *Reperfusion injury is likely to contribute to ischemic damage in the downer syndrome.* The extent of lesions within a muscle mass is quite variable but seldom involves more than half of the mass. The degree of damage will reflect the extent, duration, and severity of the ischemic episode as well as the extent and rapidity of reflow.

The clinical presentation of downer syndrome can be complicated by *pressure-induced peripheral nerve injury*. Even 6 h of anesthesia or comparable inactivity in horses and cows can cause sciatic or other nerve damage leading to *peroneal nerve paralysis* and a flexed rear fetlock, or a dropped shoulder and elbow due to *radial paralysis*. This neuropathy is, however, more often due to nerve conduction block than to structural nerve damage, allowing for the possibility of a relatively rapid recovery. If there is actual structural damage to nerves, effective recovery and mobilization may be delayed until nerves to muscles are regenerated, and complete recovery is not always possible.

Muscle crush syndrome

This form of muscle ischemia has characteristics in common with downer syndrome. *It is usually initiated by acute accidental trauma, often including bone fracture.* It occurs less frequently in animals than in humans, but has been seen in the dog and perhaps the cow. Initial events center on the *traumatic laceration of muscle*, which leads to a combination of high osmotic tension and hyperemia that result in accumulation of abundant edema fluid in the area. Edema causing increased pressure can exacerbate the muscle damage. If the damaged muscle and bone are still confined within a relatively firm sheath, conditions resembling those of compartment syndrome are set up. The limb swells and extends, and becomes turgid. Ischemia of variable extent ensues but, because of the great amount of myoglobin released, *renal dysfunction* may dominate the syndrome.

Vascular occlusive syndrome

When a *major vessel to a limb is occluded* the limb becomes cool, the arterial pulse is lost, skin over the limb loses its ability to sweat, and some limitation of movement may be apparent. When this occurs as a result of *aortic-iliac artery thrombosis in the horse* the effects are usually transient, apparently due to effective collateral circulation. Some muscle degeneration probably occurs but is repaired rapidly and completely. In the *cat with aortic-iliac thrombosis* associated with underlying cardiomyopathy, more of the aorta is likely to be occluded than is the case in the horse, and collateral circulation may be less effective at restoring circulation to hindlimb muscles. Muscle lesions vary from mild to severe (Fig. 2.68). The *anatomic pattern of degeneration* varies from one case to another and more distal muscles are not necessarily more vulnerable. Hindlimb muscle from cats with aortic-iliac thrombosis can also exhibit chronic myopathic changes indicative of previous bouts of subclinical ischemic myopathy.

An ischemic lesion of muscle seen in *sheep in advanced pregnancy* appears also to be caused by arterial occlusion. Ewes carrying twins or triplets sometimes suffer from ischemic necrosis of the internal abdominal oblique muscle without evidence of congestion or hemorrhage. This muscle does not have a confining sheath thus the necrosis is not part of a compartment syndrome. The arterial supply to the abdominal oblique is via a tortuous branch of the internal iliac artery which turns back on itself inside the iliac tuberosity, and it may be vulnerable to stretch and/or trauma. Ischemia of the muscle is followed by rupture, and subsequently the other abdominal muscles also rupture. In spite of all of this, the ewes sometimes lamb at term without difficulty.

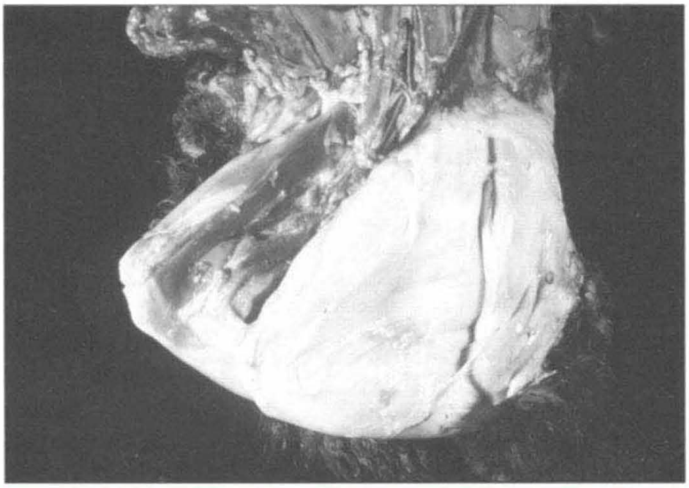

Figure 2.68 Pale thigh muscle due to infarction in a cat with **ischemic myopathy** due to aortic-iliac thrombosis.

Postanesthetic myopathy in horses

Postoperative weakness due to neuromuscular dysfunction occurs with some regularity in horses. A *variety of etiologies* are possible. Heavily muscled horses anesthetized and laid on poorly padded surfaces can develop *pressure-induced ischemic damage* to muscle similar to downer syndrome. *Compartment syndrome* affecting selected muscles can also occur. Cases of compartment syndrome in which a hemorrhagic infiltrate is prominent suggest predominantly venous occlusion. Pressure-induced neuropathy is a frequent complication. Horses with underlying myopathy, such as hyperkalemic periodic paralysis (HYPP) (see Myotonic and spastic syndromes) and polysaccharide storage myopathy (see Metabolic myopathies) are particularly prone to postanesthetic myopathy. A hyperthermia-like condition is also possible, especially in horses with HYPP.

A syndrome of ischemic myopathy due to hypotension and decreased muscle perfusion occurs in horses, most often associated with *halothane anesthesia*. This syndrome can be reproduced experimentally. Marked increases in serum CK and AST activities and lactate concentration occur. Swelling is most common in downside muscles, with increased intracompartmental pressure indicative of associated compartment syndrome.

Fibrotic myopathy

Fibrotic myopathies occur in a number of species including horses, dogs, cats, and humans, and most commonly involve the semitendinosus muscles. *Fibrotic myopathy is nonspecific and may be a consequence of intramuscular injections, primary myopathies, or peripheral neuropathies.* It may occur spontaneously in Quarter Horses and Greyhounds that rupture muscles upon explosive exercise, but is predisposed in conditions such as hemophilia where repeated hemorrhage into muscles leads to extensive fibrosis. *Affected muscles are replaced by contracted bands of dense scar tissue.*

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PHYSICAL INJURIES OF MUSCLE

Traumatic injuries of muscle (*laceration, contusion, tearing, penetrating wounds*) are common and may be the result of external trauma, or they may be a result of a muscle rupture or tear of the fascia as occurs occasionally in violent contraction, rarely in overextension. The effects of external trauma are very variable and depend on the qualities of the applied force, the presence or absence of concomitant fracture of the adjacent bones, and especially on the degree of hemorrhage, injury to blood vessels, and injury to motor nerves. The principles governing the outcome of these types of lesions have been discussed above.

Violent contraction of muscle may result in either hernia or rupture with hemorrhage. A hernia occurs when the belly of the muscle protrudes through a rent in the overlying fascia and epimysium. The hernia can be reduced by pressure when the muscle is relaxed and it hardens and bulges further when the muscle contracts, providing thereby a point of useful distinction between hernia and tumor of soft tissue.

Actual rupture of muscle tissue may also occur during violent exercise and is probably more common than rupture of the tendon. The muscle bulges at the end opposite to that which is torn. Such ruptures are not necessarily complete from the outset, but may become so later from additional strain or degeneration caused by infiltrating hemorrhage with pressure and ischemia. Regeneration in large defects is ineffective and the gap is filled in by scar tissue. The muscle most frequently ruptured in animals is the *diaphragm*. Trauma, with acute abdominal compression, is the usual excitant in the dog. In cattle, it tends to follow diaphragmatic myositis secondary to traumatic reticulitis. In horses it is a consequence (in foals) of abdominal compression at parturition, and diaphragmatic rupture occurs occasionally with acute gastric dilation. *Acquired ruptures must be differentiated from congenital and postmortem ruptures by attention to the edges of the cleft and the disposition of abdominal viscera.* Muscles subjected to continuous pressure undergo degeneration, necrosis, and fibrous replacement. It is possible that pressure can have a direct effect on the fibers by disorganizing their internal structure, but the principal effect is to interfere with circulation.

The histologic appearance of traumatic injury depends on the age of the lesions. Initially it includes sarcolemmal rupture with adjacent intact fibers showing hyaline and granular degeneration, accompanied by edema and hemorrhage and neutrophilic infiltration. Reparative changes are conventional, but if the endomysial tubes are extensively disrupted, regeneration will be by "budding" with accompanying fibrosis. Bleeding into muscle, whether caused by trauma or spontaneous hemorrhage, as in some hemorrhagic diatheses, is often sufficient in volume to result in hematoma, the fate of which will depend largely on its volume. Metaplastic bone may form in the capsule of the hematoma. Severe trauma-induced swelling in muscle will result in secondary ischemic injury, which causes muscle necrosis. Primary healing is by regeneration by budding and extensive fibrosis because endomysial tubes are transected.

Ossifying fibrodysplasia

Also termed *myositis ossificans or fibrodysplasia ossificans*, this infrequent condition of the dog, cat, pig, and horse represents heterotopic mineralization within the connective tissues of skeletal muscle. It occurs as either a localized or a generalized form. The generalized form is described under Fibromatous disorders of tendons and aponeuroses.

The *localized form* of myositis ossificans occurs in the dog and horse. In the dog, the lesion often, but not always, occurs secondary to trauma and results in firm swollen areas in the affected muscles generally in the caudal hip, shoulder, quadriceps, or neck. Microscopically, the lesion typically has *three zones* – a central area of actively proliferating undifferentiated connective tissue, a middle zone with osteoid and immature bone, and an outer zone of mature trabecular bone.

Strains/tears/ruptures/fibrotic myopathies/contractures

Strains are the result of *overstretching of muscles* that have disrupted muscle fibers, most commonly at the muscle–tendon junction. The severity of damage varies from mild localized disruption to complete rupture of muscle fibers. Hemorrhage and edema accompany the muscle damage and healing is by fibrosis.

Fibrotic myopathy of the semitendinosus, semimembranosus, and gracilis muscles occurs most often in Quarter Horses performing sliding halts. Physical tearing and hemorrhage from these abrupt maneuvers initiates the damage with fibrous replacement as an outcome. Additionally, some cases are the result of denervation injury.

Several syndromes of limb muscle dysfunction, possibly associated with trauma, are described in dogs. A variety of names have been applied including *infraspinatus contracture, quadriceps contracture, gracilis contracture, fibrotic myopathy of semitendinosus, and gracilis-semitendinosus myopathy*. The contractures are the result of functional shortening of affected muscles following injury and healing by fibrosis. German Shepherds, especially males, are involved with the gracilis/semitendinosus fibrotic myopathy. The affected muscle may contain a thin fibrous band.

A myopathy of the tail muscles in hunting dogs ("limber tail" or "frozen tail") appears to be associated with overwork and environmental stresses.

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NUTRITIONAL MYOPATHY

Nutritional myopathies (also named nutritional myodegeneration, nutritional muscular dystrophy, white muscle disease, stiff-lamb disease) are *principally diseases of calves, lambs, swine, and foals.* They infrequently affect carnivores. *The nutritional deficiencies are principally selenium and vitamin E.* Various environmental factors may, at times, also contribute to the muscle lesions historically associated with selenium/vitamin E deficiency. The clinical syndrome was first produced experimentally in 1928 in the suckling young of female rats fed a diet deficient in vitamin E but the skeletal muscle lesions were not identified until further studies in 1938 when the disease was incorrectly called a "dystrophy," a term appropriately applied to some inherited diseases of muscle. Selenium was established as an essential nutrient and implicated in nutritional myopathy in the late 1950s. Muscle fiber degeneration, as seen in nutritional myopathy, was discussed earlier (see Degeneration and repair of muscle); *it is a selective, segmental polyfocal and polyphasic degeneration of contractile components of the muscle cell* which leaves the ensheathing basal lamina and satellite cells intact, and therefore enables a rapid and efficient regenerative repair to take place. Myoglobinuria is usually absent in the enzootic disease of young animals but may occur in the sporadic cases in young adult animals, as those have a higher concentration of myoglobin in skeletal muscle. Frequently, skeletal muscle damage is concurrent with myocardial lesions.

Etiology and pathogenesis

Nutritional myopathy is a problem around the world but it occurs most often in those countries with intensive livestock agriculture operations. *Selenium moves through a soil–plant–animal cycle.* Sedimentary rocks provide most of the Se that becomes incorporated into soils. Alkaline and well-aerated soils provide much higher amounts of Se available to growing plants than acid, poorly aerated soils. This difference in availability from soils is related to the chemical form of Se and not to the Se concentration in the soil. *Soluble selenates* predominate in alkaline soils, and sparingly soluble *selenites* complexed with iron salts are in acid soils. As Se moves from soils into growing plants, it is largely incorporated into organic compounds, mainly in those selenoproteins with abundant selenomethionine. The Se content in the lush forage of heavily fertilized and watered soils is low because of dilution by the abundant plant tissue. Surveys of plants grown in soils throughout the USA and other countries have provided data and have been used to map areas of Se deficiency and excess. Deficient areas include the southeastern, northeastern, midwestern, and far northwestern portions of the US. The prevalence of Se-E deficiency diseases in animals throughout the USA correlates closely with the areas having low (<0.05 ppm dry weight) plant Se concentrations. Animals are able to utilize Se from inorganic salts (selenites and selenates) as well as from the organic forms in plants. Further, the Se of compounds in feedstuffs of plant origin has a much higher biological availability than Se found in compounds of animal products (e.g., fish meals). Other inorganic Se sources, elemental Se, and selenides, have little or no biological value for animals. In most of the studies that compared the efficacy of various chemical forms of Se to prevent deficiency disease in animals, *organic Se was found to have greater protection than inorganic Se (selenite).* However, because selenite is readily available and inexpensive, this form is commonly used as a dietary Se supplement.

Selenium is distributed widely in animal tissues, and the concentration is directly related to dietary intake. Highest concentrations are found in kidney and liver, intermediate amounts are found in heart and skeletal muscle, and low content is found in blood and fat. Animals fed rations in which small amounts (0.1–0.2 ppm) of Se are added to meet their nutritional requirements do not develop large increases in tissue Se content; therefore, human consumption of the tissues of animals so fed offers no risk of causing Se toxicosis.

Selenium deficiency in animals may be induced by the incorporation of high but nontoxic amounts of certain elements that antagonize Se. *Copper, silver, tellurium, and zinc in rations can induce typical lesions of Se-E deficiency in animals fed diets containing amounts of Se ordinarily considered adequate.* Also, a role for high amounts of dietary sulfur as a Se antagonist has been claimed. The importance of Se antagonists in field, rather than laboratory, conditions remains to be established. However, such a mechanism should be considered when animals fed a selenium supplement develop lesions of Se-E deficiency.

Vitamin E content of compounded animal feeds is generally low because many of the feedstuffs used are poor sources of that vitamin. Rich sources of vitamin E include wheat bran, many vegetable oils, and legumes such as alfalfa. The biological activity of vitamin E is concentrated in the α-tocopherol fraction, and thus determinations of total tocopherol content of feeds may be of limited value for determining their vitamin E potency, to prevent deficiency disease. Diets that contain large amounts of polyunsaturated fats (e.g., those in fish oils) will require greater amounts of vitamin E, which limits oxidation and the development of rancidity. Also, if diets with low Se content are fed, vitamin E supplements will need to be increased to prevent deficiency disease.

Of the domestic mammals, *cattle, sheep, and pigs are most susceptible to nutritional myopathy*. Horses and goats are moderately susceptible, and occasional cases have been reported in dogs and cats. Most zoo ungulates should be regarded as susceptible to the disease. Historically, nutritional myopathy has been thought of as a disease of young animals, particularly the very young. *Rapid postnatal growth seems to predispose*, a problem perhaps of outgrowing a scarce resource or of biochemical transition as fiber types develop into the adult patterns. Although nutritional muscle degeneration does occasionally occur in mature animals, it is rare.

In cattle, spontaneous nutritional myopathy may occur in utero in 7-month-old fetuses, and muscle lesions are seen in lambs and calves at birth. However, lesions may not occur in calves or lambs born of cows or ewes, which themselves have extensive lesions of nutritional myopathy prior to, or at the time of, parturition. It is equally true that dams of calves or lambs with extensive lesions seldom show clinical disease or even clinicopathological evidence of muscle fiber breakdown.

Nutritional myopathy occurs in all of the susceptible domestic species on widely variable planes of nutrition. Neonatal disease usually affects the thrifty, well-grown suckling animal and the sporadic disease in yearlings and adults usually occurs in animals in good physical condition. The adult disease affects animals fed marginal-quality rations, such as turnips or poor-quality hay, and can appear as clinical or subclinical disease in animals in very poor condition because of neglect or chronic disease. Animals with nutritional myopathy often lose condition rapidly and appear to be very unthrifty even when the muscle disease is not clinically severe.

One of the most perplexing aspects of these myopathies is the irregularity and unpredictability of their occurrence. Natural disease is seldom a serious problem in consecutive years, yet sometimes it will occur in most years in any given region. A good deal of correlative and circumstantial evidence indicates that climate-related conditions, such as the length and the amount of sunshine of the growing season, and the length of the housing season, may be very important. Since the disease often occurs while animals are consuming stored feeds, the condition and duration of storage of the fodder can be relevant. Detailed investigation sometimes reveals comparable concentrations of vitamin E and selenium in forage from one year to the next, yet the incidence of nutritional myopathy in animals consuming it may be quite different. Grazing of dry pastures may be associated with an increase in the incidence of disease and may also have an influence later through the stored hay or grain harvested from them. On the other hand, ingestion of lush pasture may also cause problems. The leaves of some pasture plants have moderately high levels of polyunsaturated fatty acids and these fatty acids are absorbed largely intact in herbivores. They are almost certainly antagonistic to selenium and vitamin E, thus the requirements for these nutrients may be raised considerably when animals first graze new pasture. *In most parts of the world, nutritional myopathy occurs in late winter or early spring*, but in sheep it may occur more often in the fall, in both pastured and feedlot animals that are immature.

The patterns of nutritional myopathy seem, in a general way, to obey the rules of straightforward deficiency of one or two essential nutrients. The metabolism of vitamin E and selenium is incompletely understood. Understanding of factors involved in membrane integrity and membrane alterations in disease has elucidated the role of the subcellular changes, which seem to be a basic result of deficiency of these substances.

In many cells, *vitamin E- and selenium-containing enzymes are required as physiologic antagonists to a group of chemically varied substances known as free radicals.* **Free radicals** are molecules with an odd number of electrons; they can be either organic or inorganic. Some free radicals are products of normal cell function, and several participate in, or are products of, oxidative metabolism. They may also be produced outside the cell as products of tissue radiation, drug reactions, and inflammation. One of the major sources of free radicals is the cell detoxification process, which renders materials less harmful by converting them to epoxides. Many intracellular and extracellular free radicals contain oxygen, and are involved in electron transfer reactions. They are highly reactive and this is responsible for their rapid alteration (instability), which occurs in oxidation-reduction reactions within a wide range of cellular structures and enzyme systems.

Free radicals may initiate cellular injury by causing peroxidation of membrane lipids and by causing physicochemical damage to protein molecules including those of mitochondria, endoplasmic reticulum, and cytosol. Protection against the effects of free radicals is provided partly by the constant presence of *small scavenger molecules* such as tocopherols, ascorbate, and beta-carotene. These "quench" free radicals, but both free radicals and scavengers are consumed in the process. Protection is also provided in part by selenium-containing enzymes of the *glutathione peroxidase/glutathione reductase system*. This system is capable, under normal circumstances, of more or less constant renewal by a complex sequence that makes use of several enzymes, although some consumption of the selenium-containing component does occur.

From the above, certain conclusions seem to emerge. *Although vitamin E- and the selenium-containing glutathione system perform many similar functions at the cellular level in quenching destructive metabolites and byproducts, they may function independently.* The circumstantial clinical evidence that one can relieve the need for the other in the prevention of muscle disease is also reasonably explained. The need for some of both at all times within the cell, and vitamin E outside the cell, perhaps is explained by the fact that the two mechanisms quench a different array of free radicals and that tocopherol operates both outside and inside the cell while the glutathione system operates only inside the cell. The practical interpretation of deficiency of vitamin E or selenium should relate to the consumption of these elements during a steady intracellular production of free radicals, rather than an interpretation of these nutrients as structural cellular components that may be deficient.

In the absence of sufficient protection by selenium and/or vitamin E, cellular membranes are modified by free radicals, and the ability of those membranes to maintain essential differential gradients for one or more ions is diminished or lost. The normal inward flow of calcium ions from the extracellular compartment to the cytosol, where calcium levels are one quarter to one half as high as extracellularly, causes a greatly increased demand for energy to move calcium away from the calcium-sensitive myofilaments and into mitochondria which act as sumps. As a result, mitochondria may accumulate up to 50 times their normal amount of calcium, and this results in reduction in their ability to produce energy. It also initiates the sequence of events identified earlier (see Degeneration and repair of muscle) as "mitochondrial calcium overload" which proceeds to calcium-induced hypercontraction of myofibrils and degeneration of myofibers.

The explanations of intracellular dynamics and myofiber degeneration outlined above indicate that *segmental muscle fiber degeneration with mineralization is not a specific lesion.* Any event which can trigger the cascade of degenerative events in the muscle fiber could produce a similar lesion. Starvation, or poor-quality rations, or rations in which vitamin E and/or selenium have been destroyed, could produce muscle lesions simply by not providing enough protective scavenger molecules. Metallic toxicants or other chemicals, including therapeutic agents, might produce disease by binding selenium and inducing a higher than normal demand for antioxidant molecules such as tocopherols or glutathione. The relationship between unaccustomed exercise or cold weather and nutritional myopathy, which is frequently observed in all ages and all susceptible species, may be explained by an energy depletion at the intracellular level rather than by membrane alteration, especially in those instances of nutritional myopathy in which levels of tissue vitamin E and selenium are near normal. Excepting those cases exacerbated by driving, transport, or other forced exercise, serum and tissue concentrations of vitamin E and selenium generally correlate well with levels in the feed of the animals concerned. *This by itself seems to indicate that the naturally occurring disease is a true deficiency.*

Once the myofiber cell membrane (plasmalemma) has been altered, the stage has been set for leakage of intracellular enzymes into the extracellular space. *The concentration of activity of muscle enzymes in blood is a rough indication of the extent of muscle fiber destruction.* It is clear that increase in concentrations of enzymes in plasma may accompany extensive athletic use of muscles and indicate physiologic disruption of muscle fibers. When monitored by muscle biopsies in animals and man, however, such increase in enzyme concentrations is associated with degeneration of fibers or at least of some fiber segments (segmental degeneration).

The concentration of creatine kinase in plasma is the enzyme that seems to be most specific in indicating muscle damage although, in animals, plasma concentrations of aspartate aminotransferase is quite specific for muscle as well. Its activity in serum tends to rise later and disappear much later than creatine kinase and therefore, its evaluation is most useful in establishing the time and duration of the muscle injury. Serum glutathione peroxidase activities are reliable indicators of selenium availability. Tissue concentrations of selenium may not reflect metabolic availability if selenium is stored in complex form. Mineral supplements often contain a variety of metals as contaminants. Those that have been shown experimentally to have a myopathy-inducing effect include iron, silver, tellurium, cobalt, copper, zinc, and cadmium. The complicity of such metals in the production of natural disease is undetermined but worthy of examination.

Nutritional myopathy of cattle

Clinicopathologic descriptions of the disease in calves appeared in the 1890s and the disease was well known at that time in Germany, France, Switzerland, and Scandinavia. *Nutritional myopathy occurs, sometimes in endemic proportions, in calves, mostly of beef type and 4–6 weeks old.* It is also common in animals up to 6 months of age and occurs sporadically in older cattle. In calves there is often a typical history indicating that the dam has been housed at least 3–4 months and had been fed poor-quality hay or not enough hay. Similar problems occur in calves in the USA, Australia, and New Zealand when legume hay alone or irrigated legume pasture is fed. Sulfur fertilizers applied to the pasture, and copper deficiency in the dam, may be contributing factors. *The precipitating event in many cases is unaccustomed physical activity that converts subclinical to clinical disease.* The feeding of cod liver oil that has become rancid destroys vitamin E in the ration and has been blamed for producing the disease. The presenting sign in calves is often stiffness or dyspnea, but a shuffling gait, a dropping of the chest between the shoulders, and outward rotation of the forelimbs have also been noted. Some calves become recumbent and die rapidly with signs of respiratory failure. Calves over 3–4 months of age may show myoglobinuria.

Postmortem lesions in calves are usually dominated by marked mineralization of necrotic skeletal and/or cardiac muscle. When the heart is extensively affected, intercostal muscles and the diaphragm are usually also affected, but other skeletal muscle lesions may not be widespread. *Heart lesions in calves usually involve the left ventricle more than the right.* Small lesions just under the epicardium or endocardium may appear as scattered white "brush" strokes. The mineralized lesions are creamy white and opaque. Small streaks of hemorrhage may also be seen. Lungs are often filled with pink frothy fluid and an excess of fluid may be present in the thorax indicating heart failure. Acute pneumonic changes sometimes develop as a complication of pulmonary edema. *In those cases where skeletal muscle lesions predominate, the most extensive lesions can be found in the large weight-bearing muscles of the thigh and shoulder,* but many others are affected and the lesions are bilaterally symmetrical.

Suckling animals often have extensive lesions in the *highly active tongue and neck muscles*, and occasionally in the voluntary muscles of rectum, urethra, and esophagus. Affected muscles are pale, irregularly opaque and yellow to creamy-white. The longitudinal divisions of muscle (primary bundles of 40–200 fibers) often have an indistinct appearance in most severely affected areas and sometimes streaks of hemorrhage and moderate local edema are present. Minimal myocardial lesions are sometimes present, but not accompanied by evidence of cardiac failure.

In older calves and young adult cattle, the patterns of disease vary considerably and are unrelated to age. Dairy and beef calves 6–12 months of age show stiffness and lethargy just after winter housing, or sometimes while they are housed. *The disease is often related to poor-quality feed.* In similar circumstances, extensive myopathy occurs in pregnant heifers and they may suffer a high incidence of abortion, stillbirth, placental retention, and parturient recumbency. Feedlot steers fed high-moisture corn, which has been treated with propionic acid to control fungal growth and stored for 6–8 months, show initial signs of diarrhea and unthriftiness and become recumbent for 2 or 3 weeks. Many have lesions in muscles at slaughter. Most mature animals with extensive skeletal muscle lesions have myoglobinuria.

Young adult cattle with bilateral dorsal scapular displacement ("*flying scapula*") have rupture of the serratus ventralis muscle and this may be of sufficient duration to have extensive fibrosis and multifocal osseous and cartilaginous metaplasia underlying the displaced scapulae. Presumably, nutritional myopathy of the subscapular muscles has preceded rupture and displacement.

Histologic lesions of nutritional myopathy are varied only slightly by age of the lesion. The earliest lesion visible by light microscopy is *hypercontraction*, recognizable in fiber segments with tightly contracted sarcomeres or no striations. In cross-section, hypercontracted fibers are large, round, and stain strongly with eosin. This is the change which is usually described as *hyaline*, implying an amorphous structure, but striations may be visible for 3–4 days when examined by electron microscopy. Eventually these hypercontracted fiber segments will be phagocytosed but not as quickly as other fibers that undergo fragmentation with or without mineralization. In both types of degeneration, the processes of degeneration and repair are essentially those described earlier and allowance needs to be made in histological examination for the incremental changes of chronic deficiency. Thus, the typical histologic findings will be a *polyphasic, polyfocal myopathy*.

By electron microscopy, the earliest detectable change is *degeneration of mitochondria* and this is followed by loss of some parts of the sarcomere and then disintegration of the tubular systems. By histochemical examination it can be determined that *type 1 fibers in the interior of primary bundles degenerate preferentially but not exclusively.* Apart from this preference, the pattern of degeneration appears to be random. This random pattern is helpful in distinguishing this from other muscle diseases such as ischemic degeneration.

Changes in *myocardium* are very similar to those seen in skeletal muscle. Mineralization of hyalinized fibers is often pronounced and the coagulated, mineralized myofibrils appear to be rapidly removed by macrophages. Necrotic myocardial fibers are not regenerated; they are replaced by condensed fibrous stroma.

Nutritional myopathy of sheep and goats

Nutritional myopathy in **sheep** *is probably more prevalent in more areas of the world than the disease in cattle.* The disease was first described in Germany in 1925. The names white muscle disease, rigid lamb disease, and stiff lamb disease were coined to describe the most frequently encountered clinical patterns in 2–4-week-old lambs, which very often are *spring lambs, recently turned out onto the first green pasture.* Congenital nutritional myopathy does occur in lambs, but not often. The typical disease may occur as an outbreak among lambs from 1 day to 2 months of age or beyond. Mortality at this stage may be very low or may reach 50%. The next peak of incidence occurs at 4–8 months of age as weaned lambs are put onto lush pastures following mowing or into feedlots. Mortality is not usually very high, but the incidence of minimal clinical disease may be moderately high and that of subclinical disease may be higher. Beyond these age groups, nutritional myopathy in more mature sheep is clinically apparent sporadically, but subclinical disease may involve from 5–30% of a group. Under special circumstances, the incidence of clinical disease and mortality may rise dramatically. Thus, in various parts of the world, the disease has been precipitated by stress from bad weather, prolonged winter feeding, subsistence on root crops, or forced activity, as well as feeding on stubble, legume pastures, dry pastures, and pastures with too much copper. Outbreaks in lambs and yearlings have also occurred on pastures on which copper had been made unavailable by top-dressing with molybdenum.

Deficiency in sheep is attributed to deficiency of vitamin E or selenium, but seldom of both. In certain regions of the world, one nutrient does not seem to be able to replace the other to any extent, while in others the addition of either vitamin E or selenium is rapidly curative.

Lesions and their corresponding clinical signs are as varied as the circumstances under which myopathy occurs. The lesions may be detectable in lamb fetuses at least 2 weeks prior to parturition. In the congenital disease, *tongue and neck muscles* used in suckling movements often contain the most severe lesions. When the lesions occur in lambs a few days older, they are likely to be much more extensive and involve primarily the *major muscles of the shoulder and thigh but also back, neck, and respiratory (diaphragm and intercostal) muscles.* The gross appearance of affected muscles is similar to that described for calves, although *the likelihood of muscle mineralization is probably greater.* Lambs normally have pale muscles, consequently the recognition of the mineralized flecking in primary bundles is almost essential if diagnosis is to be made grossly with any confidence. It is necessary to confirm the gross diagnosis by histological examination.

In older sheep, lesions are more varied in distribution, location, and extent, but some similarity to the distribution in young animals may exist. For example, bilaterally symmetrical lesions of the thigh muscles may occur but they may predominate in or be confined to the intermediate head of the triceps, or the tensor fascia latae. Lesions in pregnant ewes may be more or less confined to the abdominal muscles subjected to increased work load from supporting the pregnant uterus and may rupture allowing viscera to herniate.

Microscopically, the changes seen in affected muscle over a period of several days follow the expected sequence of *necrosis, mineralization, phagocytosis, and regeneration characterized by satellite cell proliferation and myoblast formation.* Because the basal laminae of myofibers are intact, all of this can be completed within about 16–18 days, leaving little in the way of residual lesions, but often repair lesions of several days evolution can be seen side by side with more recent ones (*polyphasic, polyfocal myopathy*). Repair can take place in the face of continuing deficiency, but even normal activity during the early repair phase is likely to lead to more myofiber necrosis.

Reports of nutritional myopathy in **goats** are relatively few but this may be due to the fact that the goat is less often reared under intensive animal agriculture practices. Caprine nutritional myopathy has been observed in Europe, the Middle East, New Zealand, Australia, and North America. In most instances it appears in goats on pasture, and clinical and pathological changes are similar to those seen in sheep.

Nutritional myopathy of pigs

Nutritional myopathy in swine has been reported as a spontaneous disease wherever intensive pig rearing is practiced, but particularly in northern, central and eastern continental Europe, the UK, the US, and Canada. Classical lesions involving only skeletal muscles are less common than some of the other expressions of porcine vitamin E/selenium deficiency such as hepatosis dietetica and mulberry heart disease, but *systematic microscopic study of muscles has revealed a much higher incidence of muscle lesions than was thought to exist.* Since the pig is an easily managed experimental animal, much more is known about experimental vitamin E/selenium deficiency in this species than in others.

Naturally occurring nutritional myopathy is not known to be congenital in pigs but piglets as young as 1 day may have extensive

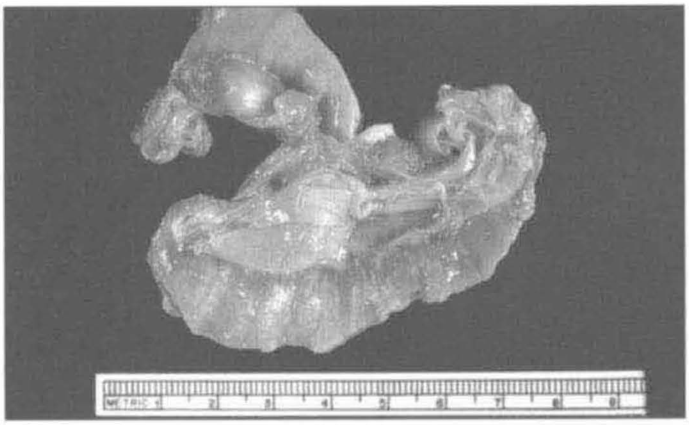

Figure 2.69 White streaks with necrosis radiate in diaphragm from a pig with **nutritional myopathy**.

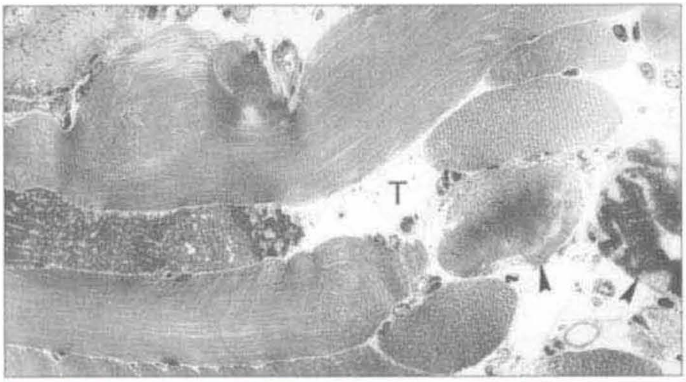

Figure 2.70 Segmental myodegeneration in a pig with nutritional myopathy. T = tube of external lamina; arrowheads = clumps of disrupted contractile material. (Reprinted from Van Vleet JF et al. Am J Vet Res 1976;37:911.)

lesions, which cause paresis or extreme weakness. Growing pigs of all sizes up to 65 kg may be affected in outbreaks, but *most commonly affected are weaned pigs of 6–20 weeks of age.* A special circumstance is that caused in pigs a few days old by the *injection of iron dextran products*, which precipitates acute widespread degenerative and usually fatal myopathy. It is believed that iron acts as a catalyst for lipid peroxidation of cell membranes. Older pigs, and particularly sows that have recently farrowed, are occasionally susceptible to widespread nutritional myopathy of sufficient severity to cause prostration, although more often clinical signs, if present at all, consist of lethargy and slowness of movement.

Apart from deficiencies in diet, the factors that trigger the disease in pigs seem to be relatively few. Unaccustomed exercise does not seem to play a part, but *feeding rancid or oxidized fish liver oils, which destroy vitamin E, may do so.* Newly harvested grain sometimes seems to contain a myopathy-inducing factor, and pigs fed quantities of peas (*Pisum sativum*) may be particularly prone to the disease. Metals occurring as contaminants in ground mineral mixes can induce an increased requirement for vitamin E and/or selenium. Silver, copper, cadmium, cobalt, vanadium, tellurium, and zinc, and possibly other metals, in some way bind selenium or prevent its participation in free radical protective activities. This leads to a two-fold or greater demand for selenium to prevent lesions that can be only partly alleviated by vitamin E supplementation.

Mineralization of degenerate fibers is often not abundant and even when it is present and visible, it is difficult to detect grossly in the naturally pale muscles of the pig. This, and the fact that lesions in heart and liver are much more dramatic, may explain why there are relatively few reports of skeletal muscle lesions in natural outbreaks of vitamin E/selenium deficiency disease. In the experimental disease in pigs, gross lesions may be seen (Fig. 2.69) but many skeletal muscle lesions are microscopic, while the liver and heart lesions are grossly visible.

Microscopic muscle lesions are similar to the polyphasic, polyfocal myopathy seen in lambs and calves (Figs 2.70–2.75). Type 1 fibers are principally affected and in view of the orderly central arrangement of type 1 fibers within muscle fascicles in the pig, this leads to a *distinct central degeneration of primary muscle bundles.* Regeneration is often rapid and complete in surviving piglets, but the mortality rate may be as high as 80% of a litter, and usually 100% when secondary to iron injection. The survival rate for older pigs is higher except in those instances in which the cardiac lesions are prominent. Relatively few pigs survive mulberry heart disease.

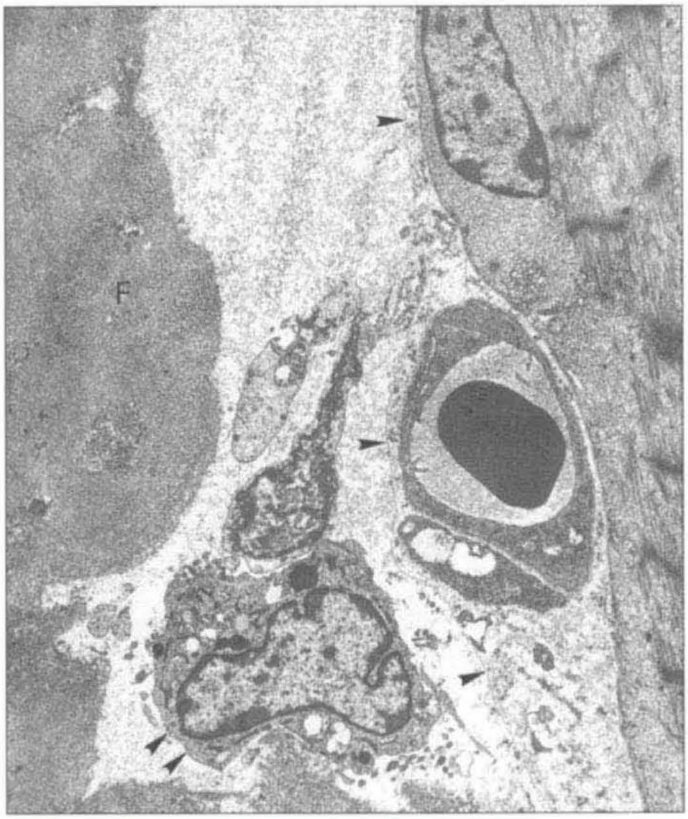

Figure 2.71 Necrotic myofiber is outlined by persistent basal lamina (arrowheads) in a pig with nutritional myopathy. Double arrowhead = invading macrophage. F = disrupted fibrils. (Reprinted from Van Vleet JF et al. Am J Vet Res 1976;37:911.)

Myocardial degeneration (mulberry heart disease) is the most common manifestation of vitamin E/selenium deficiency in growing weaned pigs 6–20 weeks of age. It is described in Vol. 3, Cardiovascular system.

The liver lesions associated with vitamin E/selenium deficiency are referred to as *nutritional hepatic necrosis or hepatosis dietetica* and are described in Vol. 2, Liver and biliary system.

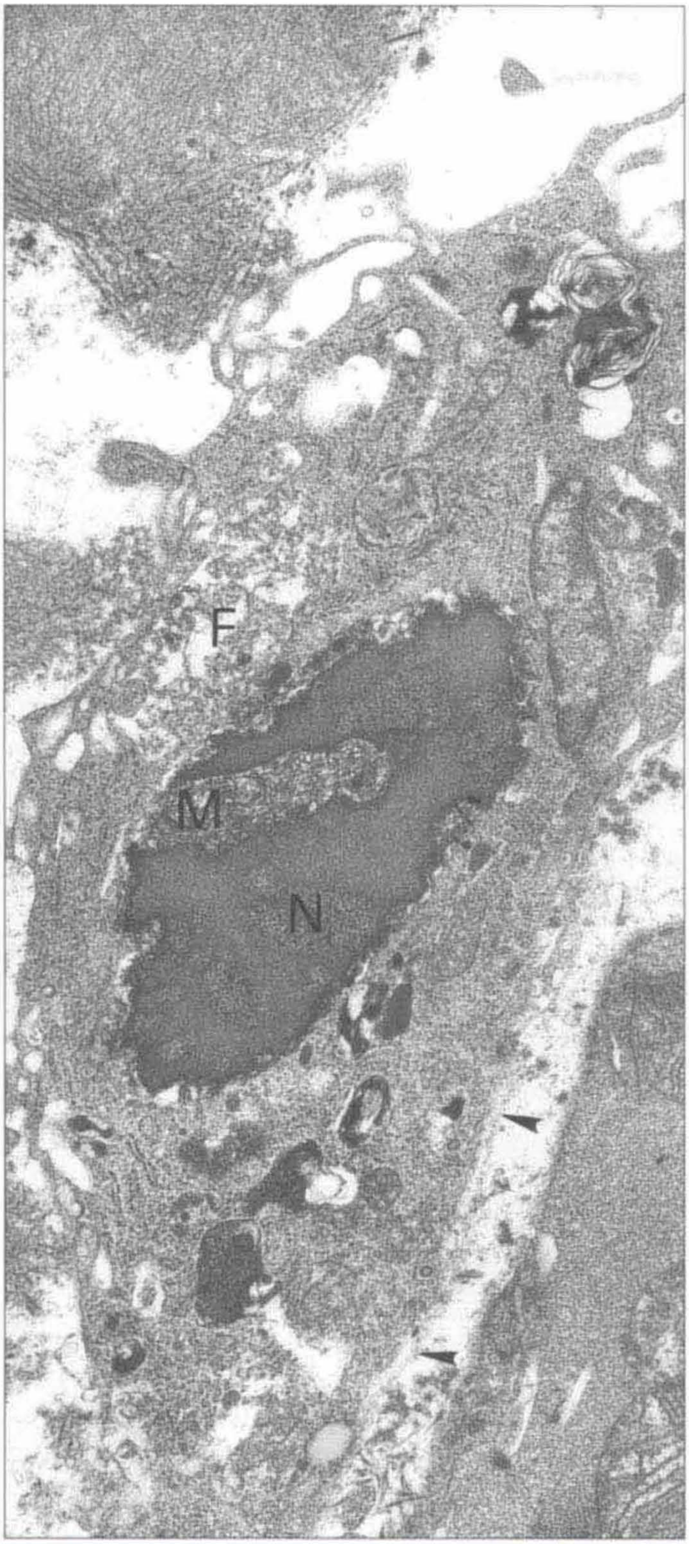

Figure 2.72 Macrophage within necrotic fiber contains engulfed cellular debris, from a pig with nutritional myopathy. Arrowheads = basal lamina, F = disrupted fibrils, M = mitochondria, N = pyknotic myofiber nucleus. (Reprinted from Van Vleet JF et al. Am J Vet Res 1976;37:911.)

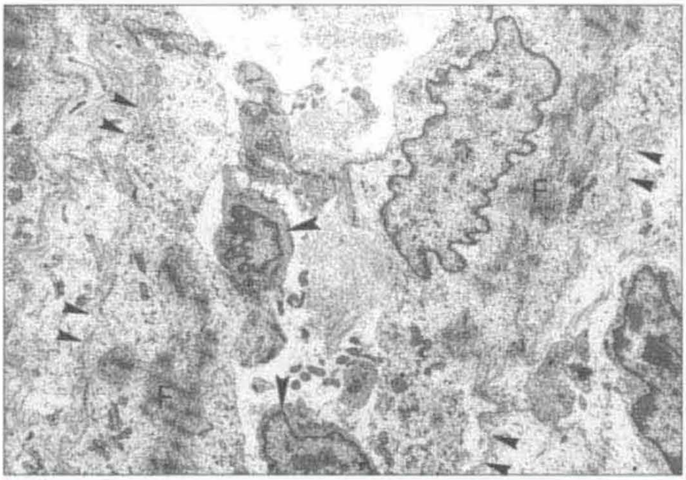

Figure 2.73 Regenerating myofiber, outlined by basal lamina (double arrowheads), has large nucleus and forming myofibrils (F), from a pig with nutritional myopathy. Arrowheads = macrophages. (Reprinted from Van Vleet JF et al. Am J Vet Res 1976;37:911.)

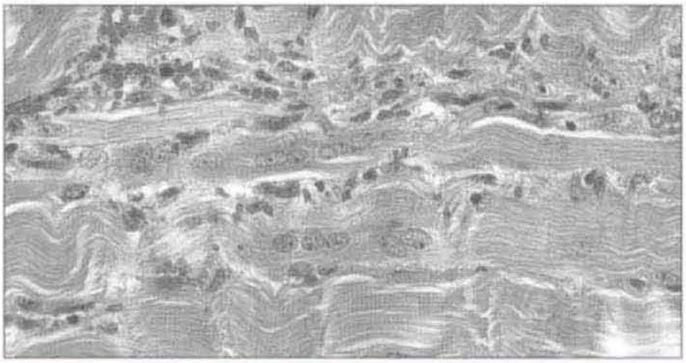

Figure 2.74 Late stage of regeneration has central rows of myonuclei and formed myofibrils in a pig with nutritional myopathy. (Reprinted from Van Vleet JF et al. Am J Vet Res 1976;37:911.)

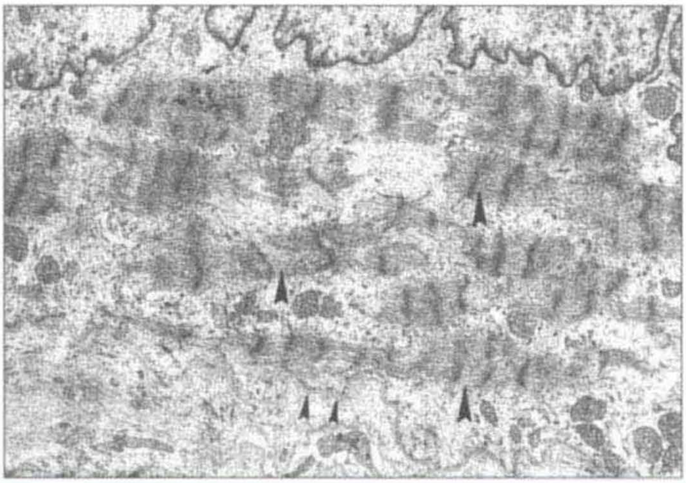

Figure 2.75 Ultrastructural appearance of **late stage of regeneration** in a pig with nutritional myopathy. Forming myofibrils (arrowheads). Basal lamina (double arrowheads). (Reprinted from Van Vleet JF et al. Am J Vet Res 1976;37:911.)

Nutritional myopathy of horses

A disease in *foals* that is, in many respects, comparable to vitamin E/selenium deficiency myopathies in other species, has been recognized for many years. *The usual age range is 1 day to 12 weeks and it may be present at birth.* Cases have been reported from North America, Europe, and Australia in most breeds of light horses and in pony, zebra, and donkey foals. The similarities of lesions and circumstances of the equine syndrome and nutritional myopathy of ruminants has led to the conclusion that the disease is due to a deficiency of vitamin E and/or selenium, but this has not been confirmed. Tissue concentrations of selenium and glutathione peroxidase in affected foals are often no lower than those in healthy foals, however, and selenium and vitamin E appear to have little or no curative value, and in a few instances, little or no preventive value. Serum creatine kinase concentrations may be very high; over 2 million IU/L in nonsurviving cases, and as high as 200 000 IU/L in surviving animals.

Postmortem lesions of nutritional myopathy in foals are similar in many respects to those in calves and lambs – a *polyphasic, polyfocal myopathy*. Foals dying acutely may have myocardial lesions. In the *subacute syndrome, shoulder, neck, and thigh muscles* may be bilaterally and extensively involved and this usually accounts for an inability to rise or to assume certain postures such as the one for suckling. Involvement of the lingual, pharyngeal, and masticatory muscles results in dysphagia. Involvement of digital flexors and extensors may be more frequent than in calves but typical lesions are the *chalky opaque flecking of muscle*. Myoglobinuria may be present and reflects the severity of muscle damage. Sometimes in poorly nourished stabled horses, there is involvement of the masticatory and tongue muscles. This lesion has been called *masticatory myositis* in foals and *polymyositis* in older animals, but based on clinical and pathological grounds *the syndromes seem to be part of nutritional myopathy.*

The distinction between true nutritional myopathy and other diseases is more difficult in older horses than it is for foals, because circumstances and lesions are not as distinctive and because the history is often fragmentary. Nutritional myopathy occurs in animals ranging from less than 1 year to middle age. *The clinical signs may be nonspecific* and may be mistaken for evidence of colic, cardiac failure, wry neck, or general depression, but *the most common features are stiffness and muscular weakness.* Lesions are seldom widespread enough to cause severe crippling although lethargy may be secondary to skeletal muscle degeneration. A willingness to eat is retained in downer animals. *The most convincing indications of nutritional myopathy are the myocardial lesions that are similar to those seen in other species. The presence of myocardial necrosis separates the syndrome from the exertional and ischemic myopathies.*

As is the case with foals, the vitamin E and/or selenium status of older horses with lesions is sometimes difficult to reconcile with information in other species. Low concentrations of blood selenium or glutathione peroxidase are not always present in horses with lesions. Blood tocopherol and glutathione peroxidase concentrations do not seem to be related in individual animals, and selenium and vitamin E may not be curative or, in some cases, not preventive. There is, however, indirect evidence of a need for one or both of the nutrients for optimal muscle function.

Histologic lesions in both foals and older horses are similar to those seen in calves or pigs – a *polyphasic, polyfocal myopathy*. The repair process in foals is rapid and usually complete in 2 weeks if the foal survives, but some lasting retardation of growth may result. Older animals may have the same capability for regeneration especially in subclinical disease, but it is expected that in older animals the repair process may be less effective, and healing will be by scarring in severely affected muscles and this may be responsible for limitations of gait or deviation of the neck.

Steatitis is apparently not seen in older horses with nutritional myopathy but it is common in foals and may, in the healing stage, lead to lumpiness in subcutaneous adipose tissue, especially along the nuchal crest and over the gluteal muscles and the abdominal wall. The affected fat is firm and yellow-brown. Microscopically, neutrophilic infiltration and necrosis and mineralization of fat cells are present.

Nutritional myopathy of other species

Nutritional myopathy is unusual in carnivores and primates. Clinical, morphological, and therapeutic evidence suggests that it does occur. There are several reports in dogs fed prolonged diets unusually low in vitamin E and selenium, and dogs with chronic biliary fistulas developing nutritional myopathy and myocardial damage. *Cats fed vitamin E-deficient diets develop steatitis (yellow fat disease).*

Nutritional myopathy in *ranch mink*, usually accompanied by steatitis, had been a periodic but costly problem. With the introduction of commercial feeds in which vitamin E is added to counteract the effects of oxidized fish oils or other lipid peroxides, it has largely disappeared. It is unlikely that the disease occurs in wild mink.

A number of *zoo animals* appear to be susceptible to nutritional myopathy, but details are scarce and evidence largely circumstantial. A small nocturnal wallaby, the Rottnest quokka (*Setonix brachyurus*), and the nyala (*Tragelaphus angasi*) seem to be exquisitely susceptible. Problems of diagnosis arise in such populations in which capture myopathy is a complication (see Exertional myopathies), but cases that circumstantially appear to be due to nutritional myopathy are reported in several species of gazelle and antelope in Africa, roe deer in Scotland, and white-tailed deer and Rocky Mountain bighorn sheep in North America.

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TOXIC MYOPATHIES

A large group of chemical and biological agents are recognized as producing skeletal muscle degeneration and necrosis, either experimentally in laboratory animals or as sporadic clinical occurrences in human patients treated with various therapeutic agents. However, in veterinary medicine, naturally occurring toxicities are largely limited to disease syndromes associated with ingestion of *ionophores, toxic plants, and plant-origin toxins.*

Skeletal muscle susceptibility to toxicants appears in part related to the specialized metabolism of muscle and the increased demands on the metabolic process from contraction. In addition, sensitivity to toxicants may be enhanced because many of the substances tend to bind to skeletal muscle fibers. Skeletal muscle dysfunction or damage has been induced by altered neurogenic function (increased motor nerve activity, neuromuscular blockade, acetylcholine accumulation, and denervation atrophy); immunologic modulations; alterations in various subcellular sites centered on cell membranes, myofilaments, lysosomes, microtubules, and sarcoplasmic reticulum; altered intracellular calcium concentration; altered protein synthesis; and altered muscle cell differentiation. Some agents, such as oxytetracycline, cause direct local injury at injection sites.

Clinical expression of myotoxicities is highly variable and ranges from lack of overt clinical signs but elevated serum concentrations of skeletal muscle origin enzymes, stiffness and muscle pain with or without myoglobinuria, and severe muscle weakness accompanied by recumbency usually with myoglobinuria. Mortality rates may be high in severely affected animals; death is often caused by concurrent myocardial damage by the same toxin.

Ionophore toxicosis

Ionophores *used in agriculture are monensin, lasalocid, salinomycin, narasin, and maduramicin.* Ionophores are compounds that alter membrane permeability to electrolytes by influencing transmembrane transport. In excess, all of these agents damage skeletal and cardiac muscle but *horses are uniquely susceptible.* Most reports of toxicosis involve monensin, an ionophore used widely for years.

Monensin, an antibiotic produced by the fermentation of *Streptomyces cinnamonensis*, has a growth-promoting effect in ruminants and is an efficient coccidiostat in birds and other animals. Monensin is produced commercially in very large quantities in North America and Europe where it is added, as a concentrated premix, to pelleted or bulk feeds fed to cattle, sheep, and other ruminants. Toxicity develops when monensin is fed to monogastric animals, which have a much reduced tolerance for the drug, or when human or mechanical error leads to concentrations of monensin in the ration that are abnormally high for the species being fed. Toxic effects have been recorded in horses, donkeys, mules, zebras, cattle, sheep, dogs, wallabies, camels, blesbok, Stone sheep, turkeys, and chickens. Many episodes of monensin poisoning have been caused by mixing errors in packaged, pelleted, commercial animal feeds, either concentrates or final mix which has put hundreds or thousands of animals at risk, sometimes over wide geographic areas. In North America alone, such mixing errors have been reported in horse, cattle, dog, and zoo feeds. Some indication of susceptibility of different species is provided by the *estimated LD_{50} for different animals*. Horses and other equids that are sensitive have an LD_{50} of 2–3 mg of monensin/kg body weight. LD_{50} values for other species are: dogs, 5–8 mg/kg; sheep and goats, 12–24 mg/kg; cattle, 50–80 mg/kg; and various types of poultry 90–200 mg/kg. Pigs, which may be given the drug for its coccidiostatic properties or are exposed by mistake, have an LD_{50} of 16–50 mg/kg body weight. The toxic effects of monensin or salinomycin are potentiated by the addition of tiamulin, triacetyloleandomycin, or sulfonamides to the ration, usually for therapeutic purposes.

Ingestion of **maduramicin**, an ionophore antibiotic utilized as a coccidiostat in poultry, has caused *cardiotoxicity in cattle and sheep*. Cases occurred in South Africa and Israel where dried poultry litter was used as a source of protein for ruminants. The clinical and pathological features of this toxicosis are similar to those of monensin cardiotoxicity.

When a single large toxic dose of an ionophore is fed to an animal, clinical signs of *lethargy, stiffness, muscular weakness, and recumbency* occur within 24 hours. Horses and other equids are likely, in the early stages, to show marked signs of colic, apprehension, shifting or fidgeting, sweating, myoglobinuria, and muscle tremors. Dogs show apprehension and progressive weakness. If sublethal doses are fed, the toxic effect will be cumulative, and the clinical onset may be delayed for 2–3 days to weeks depending on the total amount and the period over which it is fed, but the debility is likely to be more pronounced. Animals on low-level toxicity experiments often have delayed progression of the toxic signs because consumption of these feeds is reduced. These animals frequently scour and lose weight. At dose levels capable of inducing clinical signs of toxicity in a few days, many animals show evidence of *progressive cardiac failure due to a high incidence of myocardial lesions*. Animals recovering from the acute disease may subsequently develop within several months signs of progressive cardiac insufficiency from myocardial fibrosis, and sometimes renal failure, in addition to poor growth or poor weight gain, although the signs referable to skeletal muscle injury may disappear.

Postmortem lesions of ionophore toxicity may be difficult to detect in acute cases dying within 24 hours. *In horses, myocardial damage predominates, in sheep and swine the skeletal muscles are the main site of damage and myoglobinuria is generally present* (Figs 2.76, 2.77), *and in cattle skeletal and cardiac muscle are about equally affected*. Skeletal muscle may lack normal rigor, and ill-defined pale streaks may be visible in both myocardium and skeletal muscle. Later, the white streaking and local atrophy of affected skeletal muscles becomes more prominent. Hindlimb muscles may be the sites of major degenerative changes. Cases with terminal cardiac damage will have features of congestive heart failure including fluid accumulations in body cavities, pulmonary congestion and edema, and hepatic congestion.

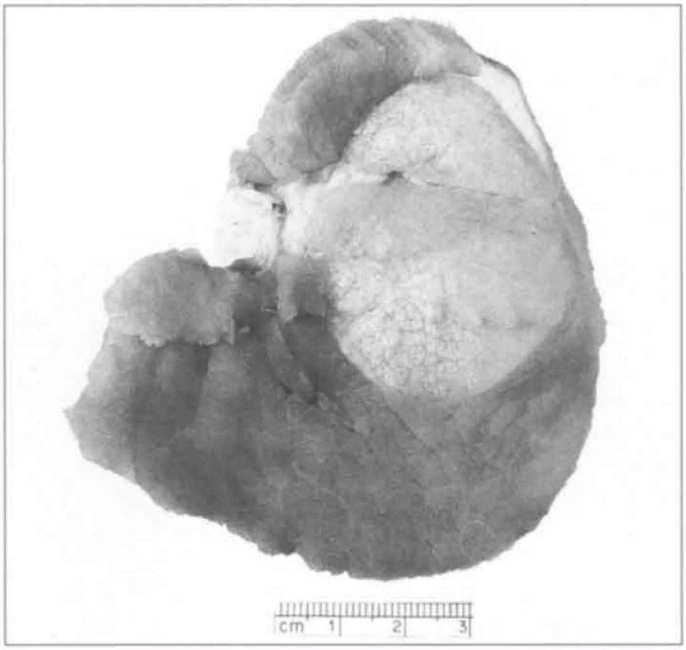

Figure 2.76 Pale area of necrosis in cross-section of caudal thigh muscles from a pig with **monensin toxicosis**. (Reprinted from Van Vleet JF et al. Am J Vet Res 1983;44:1460.)

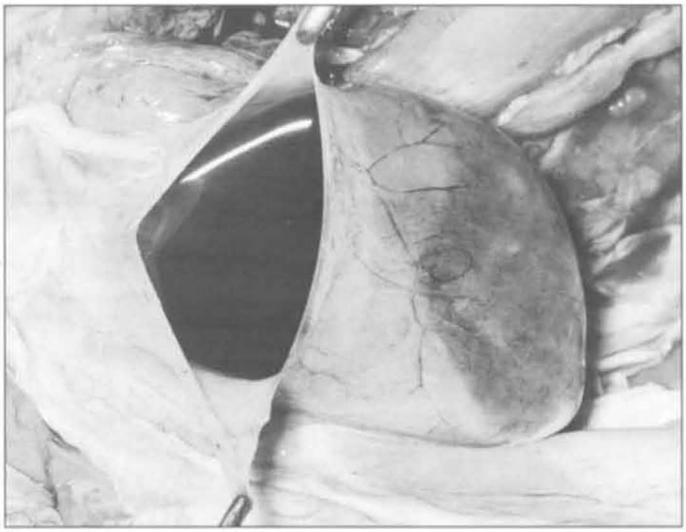

Figure 2.77 Myoglobinuria in a pig with monensin toxicosis. (Reprinted from Van Vleet JF et al. Am J Vet Res 1983;44:1460.)

Microscopic lesions of ionophore toxicity typically are *monophasic, multifocal degeneration* by 48 hours after exposure and thus differ from the polyphasic, multifocal lesions of nutritional myopathy. One of the earliest electron-microscopically visible lesions in muscle fibers is *marked swelling and disintegration of mitochondria*. Monensin is an ionophore that distorts membrane transport of sodium and potassium. This apparently leads to distortions of the electrolyte-modulated calcium gating mechanism and then mitochondrial failure, energy exhaustion, failure of calcium ion removal from the cytosol, and eventually myofibrillar hypercontraction and segmental degeneration. *Both type 1 and type 2 fibers are involved with hyaline necrosis and macrophagic infiltration* (Figs 2.78–2.83).

Myofiber nuclei and satellite cell nuclei as well as endomysial cells apparently survive acute toxicity, and the early stages of regeneration

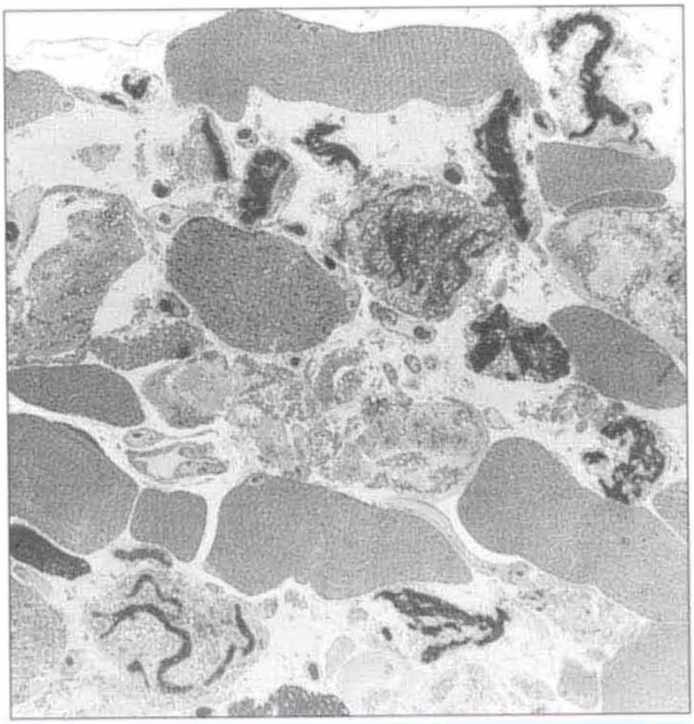

Figure 2.78 Acute myonecrosis of diaphragm of a pig one day after monensin toxicosis. (Reprinted from Van Vleet JF et al. Am J Pathol 1984;114:461.)

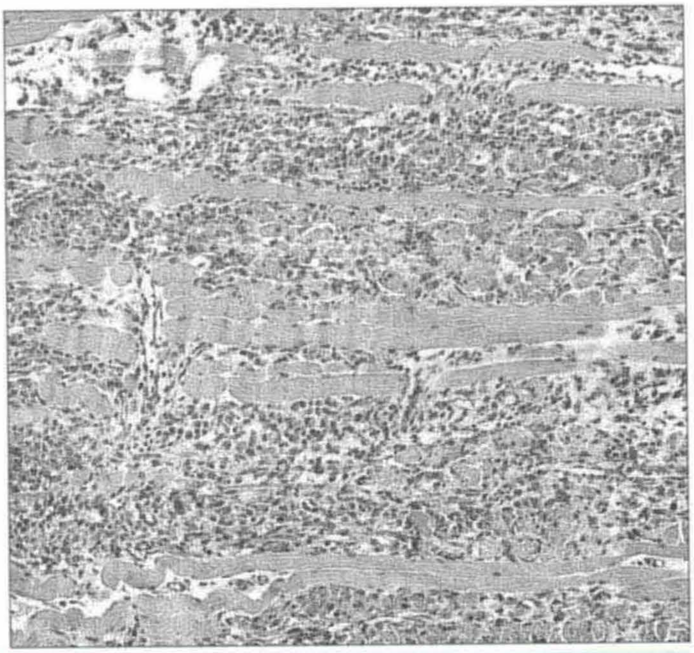

Figure 2.79 Macrophage infiltration into necrotic myofibers of a pig four days after monensin toxicosis. (Reprinted from Van Vleet JF et al. Am J Vet Res 1983;44:1460.)

are initiated during the first few days following exposure (Figs 2.82–2.85).

Myocardial lesions in monensin toxicity are not reparable and, particularly in a growing animal, the probability of lasting cardiac insufficiency is high.

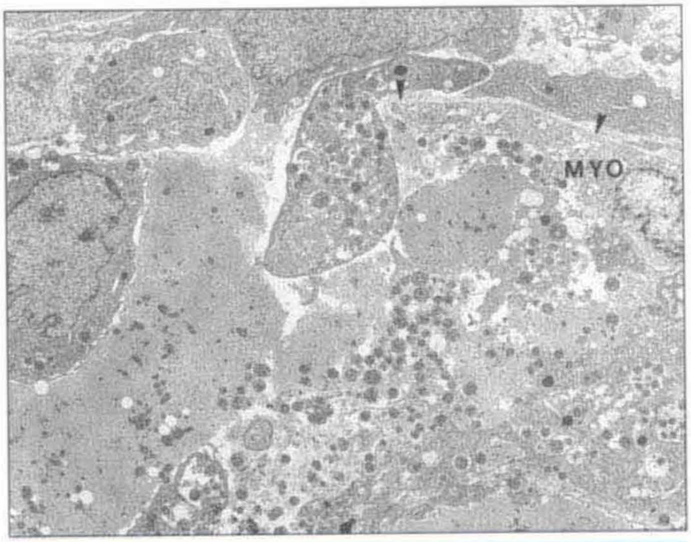

Figure 2.80 Necrotic myofiber with mineralized mitochondria and infiltrated macrophages in a pig with monensin toxicosis. Arrowheads = external lamina, MYO = presumptive myoblast. (Reprinted from Van Vleet JF et al. Am J Pathol 1984;114:461.)

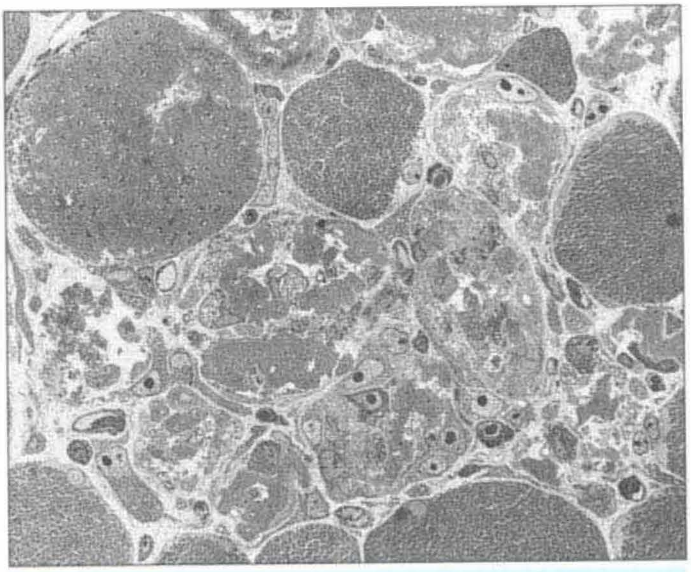

Figure 2.81 Cross-sectioned necrotic myofibers with **macrophage infiltration** in a pig with monensin toxicosis. (Reprinted from Van Vleet JF et al. Am J Vet Res 1983;44:1469.)

Toxic plants and plant-origin toxins

Toxic plants and plant-origin toxins

Cassia spp. toxicity – ruminants

In the southern USA, mature cattle and goats on pasture may ingest the beans of the **senna or coffee senna plant** (*Cassia occidentalis* or *C. obtusifolia*) or **coyotillo** (*Karwinskia humboldtiana*) late in the year, after a killing frost has made the plant more palatable than normal. Horses and pigs may also be affected. After eating the plant for a few days, animals develop diarrhea, show evidence of weakness, and display a swaying, stumbling gait that is related to the developing muscle lesions. The disease progresses rapidly and most of the animals affected become recumbent and develop myoglobinuria and

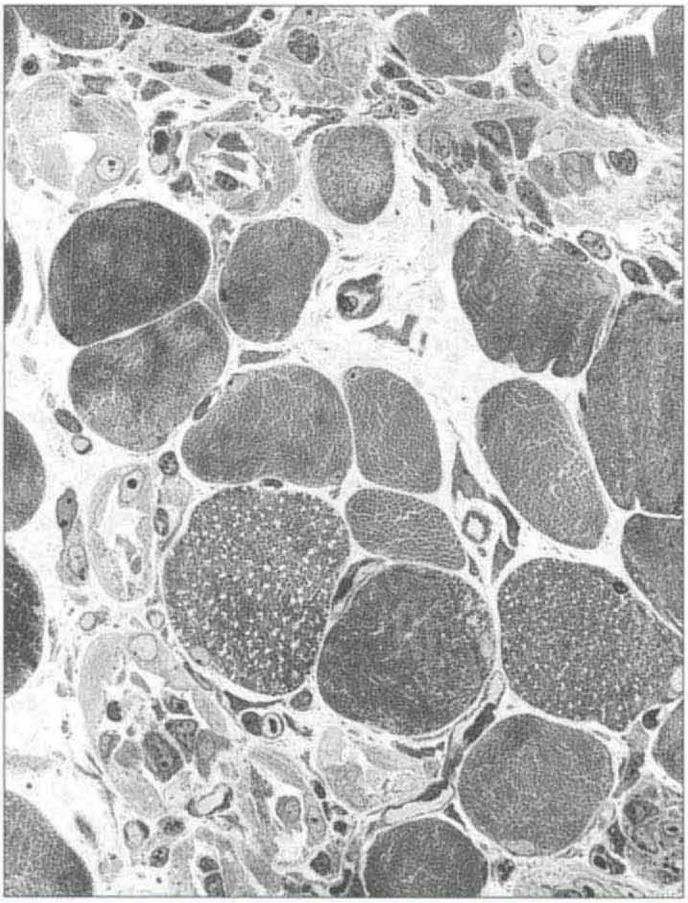

Figure 2.82 Regenerating myofibers have peripheral ring of myoblasts in the diaphragm of a pig with monensin toxicosis. (Reprinted from Van Vleet JF et al. Am J Pathol 1984;114:461.)

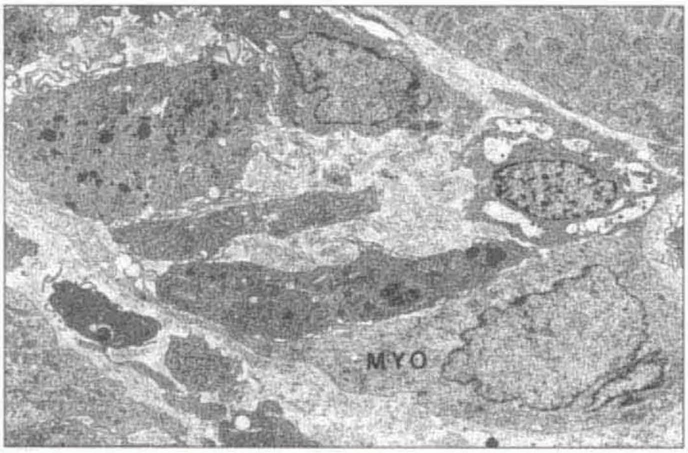

Figure 2.83 Regenerating myofiber has myoblast (MYO) and macrophages in the semitendinosus muscle of a pig with monensin toxicosis. (Reprinted from Van Vleet JF et al. Am J Pathol 1984;114:461.)

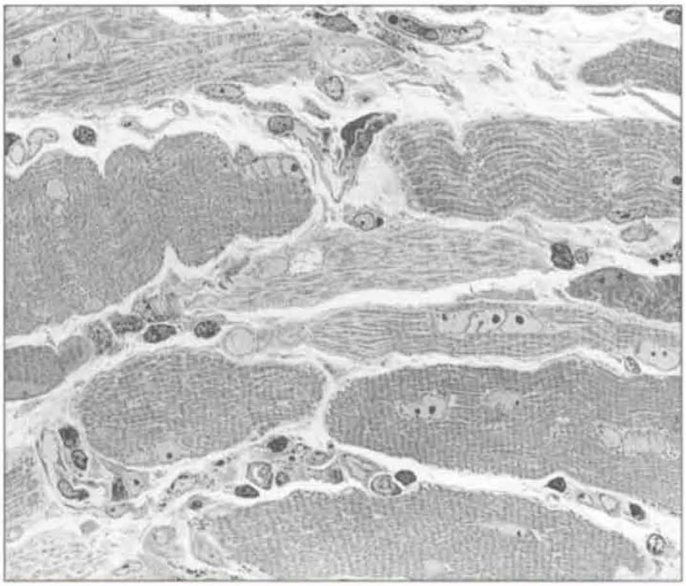

Figure 2.84 Late stage of myofiber regeneration with central rows of nuclei in the diaphragm of a pig with monensin toxicosis. (Reprinted from Van Vleet JF et al. Am J Pathol 1984;114:461.)

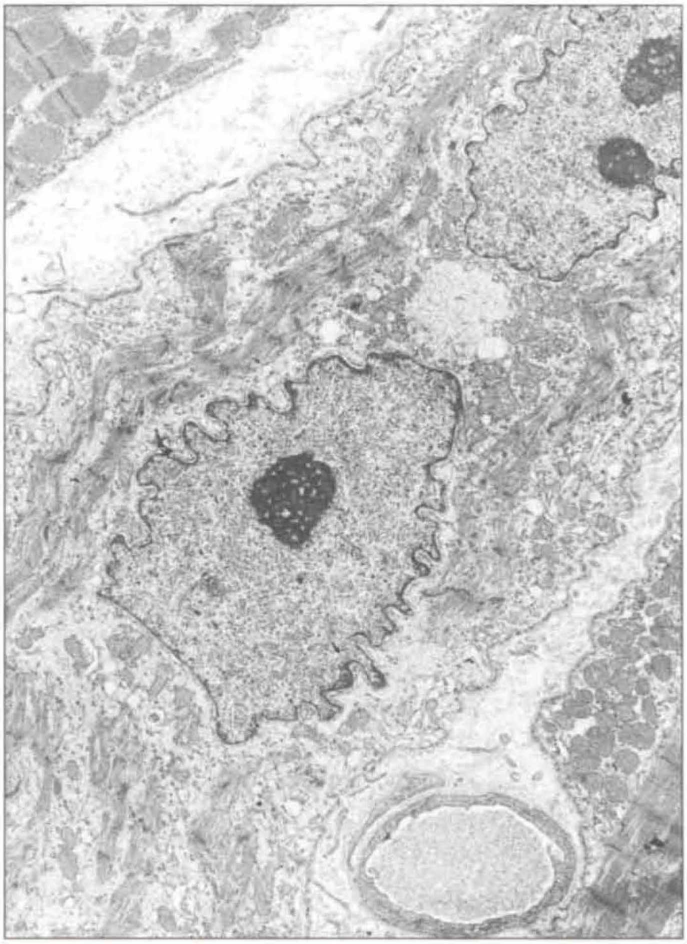

Figure 2.85 Central row of nuclei in **regenerating myofiber** from a pig with monensin toxicosis. (Reprinted from Van Vleet JF et al. Am J Pathol 1984;114:461.)

high concentrations of muscle-origin enzymes in serum. Recumbent animals usually do not recover but may live for several days. The morbidity rate may reach 60%.

Postmortem lesions in recumbent animals consist of *ill-defined pallor of much of the muscle mass*. Histologic changes are typically *monophasic multifocal myopathy*. The destruction of muscle fibers is

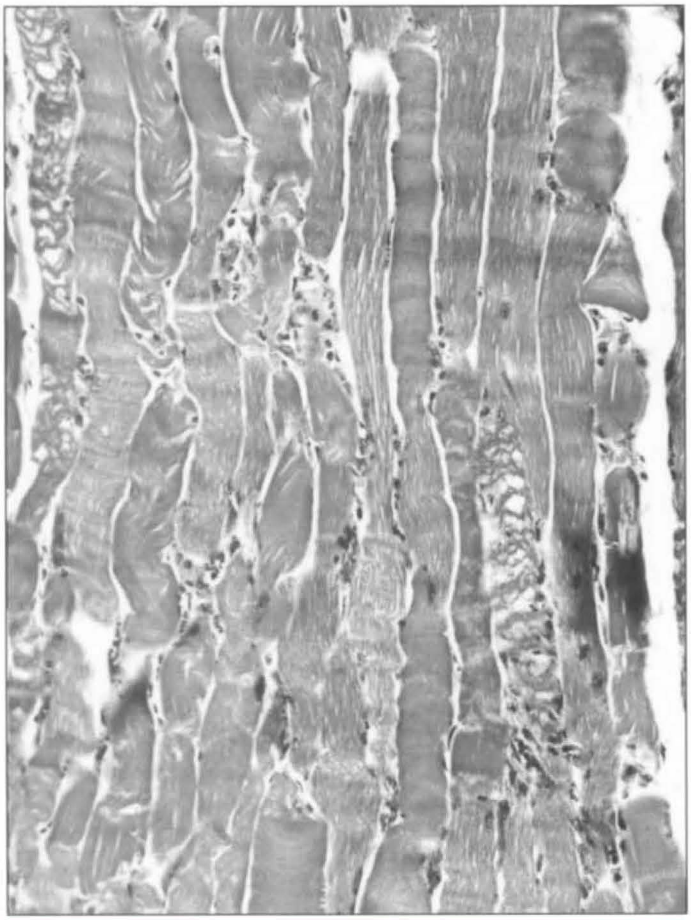

Figure 2.86 Myodegeneration caused by ***Cassia occidentalis*** **poisoning** of a heifer. (Courtesy of KR Pierce.)

segmental, but sarcolemmal sheaths and muscle nuclei remain, enclosing the floccular degenerate contractile elements (Fig. 2.86). Myocardium is not extensively involved but animals dying acutely show some myocardial lesions. Apparently recovered animals have not been examined, and the specific toxin has not been characterized.

Cassia spp. toxicity – swine

Natural outbreaks of toxicity on several pig farms occurred when animals were fed grain contaminated with *C. occidentalis* seeds. Several pigs died after a short period of reduced weight gain and a progressively wobbly, unsteady gait. Experimentally, the clinical disease was reproduced after 40 days with diets with as little as 1% of ground seeds. Postmortem examination revealed no gross lesions, but microscopic degeneration of the myocardium and diaphragm were characterized by *vacuolation and segmental hypercontraction of fibers*. The lesions were unexpectedly limited in the skeletal musculature, considering the marked locomotor clinical signs.

Gossypol toxicity

Gossypol *is a yellow, pigmented, polyphenolic substance present in cottonseeds* (*Gossypium* spp.). It is toxic to swine, and toxicosis occurs when swine are fed cottonseed cake or meal at a concentration of 10% or more of rations to which it is added as a protein supplement.

Lesions occur after feeding such rations for a month or more, which suggests that the toxic effects are cumulative. Gossypol is toxic to experimental lambs and calves at concentrations of less than 450 ppm in the feed (the level of free gossypol permitted in human and some animal foods). Dogs may also be affected. Lesions are present in several organs, including the heart, skeletal muscles, liver, and lungs, and *death is due to cardiac failure*, which causes fluid accumulation in body cavities. Histologically, *segmental necrosis of skeletal muscle and myocardium* is present, the liver has centrilobular necrosis and the lungs are congested and edematous. Affected animals are pot-bellied and poorly grown, and most die acutely. Natural outbreaks of disease in calves and lambs circumstantially linked to cottonseed meal ingestion have similar patterns of poor growth and sudden death. Serum enzyme concentrations are generally not significantly increased, which seems to confirm that the heart is the only striated muscle generally affected. Other circumstantial evidence suggests, however, that in calves, skeletal muscle lesions can be locally extensive but unpredictable in distribution. *Both type 1 and 2 muscle fibers appear to be affected in a selective, segmental myopathy* generally indistinguishable from the lesions of other toxic myopathies except that both myofiber types are equally involved.

Other toxic myopathies

Myopathies are reported in sheep with **lupinosis** (*Diaporthe toxica*), in sheep with **water hemlock** (*Cicuta douglasii*) toxicosis, in calves with **false lupine** (*Thermopsis montana*) toxicosis, in horses with **white snakeroot** (*Eupatorium* spp.) toxicosis, and in pigs, cattle, sheep, and other species with **selenium** toxicosis.

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MYOPATHIES ASSOCIATED WITH ENDOCRINE DISORDERS

Hypothyroidism

Some dogs with hypothyroidism may develop clinical signs of weakness, stiffness, reluctance to move, decreased exercise tolerance, and *muscle wasting* in addition to the traditional signs of alopecia and obesity. The likelihood of development of myopathy is increased if concomitant endocrinopathies are present such as diabetes mellitus and hyperadrenocorticism. Peripheral neuropathy with axonal degeneration may also be produced in hypothyroidism (and hyperadrenocorticism) with subsequent atrophy of type 1 and type 2 fibers.

Muscle biopsies reveal *atrophy and loss of type 2 fibers*. The atrophic fibers appear oval or angular in outline and are present throughout all muscle fascicles. Nemaline rods may be found, especially in type 1 fibers. No evidence of muscle fiber degradation or inflammatory cell infiltration is present. Initiation of thyroid hormone replacement therapy leads to resolution of the lesions. Selective involvement of type 2 fibers has been attributed to alterations in carbohydrate metabolism with subsequent loss of energy from glycolysis and glycogenolysis.

Hyperthyroidism

Affected cats may exhibit muscular tremors, ventroflexion of the neck, disturbances in gait, generalized weakness, and collapse. Microscopic alterations usually are absent, but *nonspecific fiber damage may be present.*

Hyperadrenocorticism

Cushing's disease from pituitary-dependent hyperadrenocorticism, adrenal-dependent hyperadrenocorticism, and iatrogenic hyperadrenocorticism may result in muscular weakness, stiff stilted gait, *muscle atrophy,* and inability to walk in dogs. The pelvic limbs are mainly affected.

In muscle biopsies, there are *selective type 2 fiber atrophy, fiber splitting, and focal necrosis.* Medical or surgical treatment of Cushing's disease may lead to resolution of the muscle lesions unless severe atrophy or contracture was present in the pelvic limb musculature. The muscle fiber alterations are attributed to *increased catabolism and inhibited synthesis of muscle proteins from glucocorticoid excess.*

Hypoadrenocorticism

Addison's disease in dogs and cats often results in muscle weakness presumed to be related to accompanying *hyperkalemia*. Muscle alterations have not been characterized.

Increased growth hormone exposure

Beagle dogs given exogenous porcine growth hormone had hypertrophy of types 1 and 2 fibers. However, skeletal muscle alterations have not been described in dogs or cats with acromegaly.

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MYOPATHIES ASSOCIATED WITH SERUM ELECTROLYTE ABNORMALITIES

Hypokalemia in cats

In 1984, a *polymyopathy* was identified in cats and in over half of the affected animals, there was a coexisting hypokalemia. Subsequently, a distinct syndrome of **hypokalemic myopathy** was characterized and the disorder was also termed *feline kaliopenic polymyopathy-nephropathy syndrome* and *sporadic feline hypokalemic polymyopathy*.

Clinically, the affected cats had generalized weakness, ventroflexion of the neck, a stiff stilted gait, exercise intolerance, reluctance to walk, and muscle pain. Serum activity of skeletal muscle origin enzymes (CK, AST) was elevated and serum potassium levels were low (<3 mEq/L). Hypokalemia was attributed to low dietary intake or excessive renal loss. Low intake occurred in cats fed potassium-depleted regular diets or high-protein vegetarian diets. Excessive renal loss of potassium was present in cats with chronic renal disease and in those fed acidic diets to prevent urolithiasis. Also, the syndrome reported in Burmese kittens 2–6 months of age with intermittent hypokalemia, is presumed to be heritable.

In general, *the skeletal muscle lesions have been either mild or absent*. Lesions have been described as a *polyphasic myopathy with concurrent necrosis and regeneration*. The skeletal muscle damage was attributed to influx of sodium with a decrease in resting membrane potential and hypopolarization, altered glycogen metabolism in skeletal muscle fibers, and ischemic injury from hypokalemia-induced vasoconstriction. Treatment with potassium supplementation is generally successful but the syndrome may recur unless supplementation is maintained.

Hypokalemia in cattle

In 1997, a hypokalemic syndrome with *muscle weakness and myopathy* was reported. Affected cattle were weak, recumbent, and were unable to elevate their heads off the ground and instead held them against their flanks. They were post-parturient and had moderate to severe ketosis with hypokalemia (1.4–2.3 mEq/L). The animals were treated with isoflupredone, a glucocorticoid with high mineralocorticoid activity. The skeletal muscle damage involved the hindlimbs and included vacuolar degeneration, necrosis with macrophagic infiltration, and variably present secondary ischemic injury.

Hypernatremia in cats

Muscle weakness evident as ventroflexion of the neck has been observed with hypernatremia and hypodipsia in the cat.

Hypophosphatemia in dogs

Muscle weakness occurs in dogs with hypophosphatemia. Necrosis of skeletal muscle with myoglobinuria was present in severe acute phosphorus depletion.

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EXERTIONAL MYOPATHIES

The term **exertional myopathy** *is indicative of myofiber damage occurring due to exercise stress.* Acute myofiber injury is precipitated by exercise in a broad group of myopathies, including X-linked muscular dystrophy, nutritional myopathy, metabolic myopathies, malignant hyperthermia, and myopathy due to hypokalemia. In such cases, the initiation of abnormal excitation–contraction coupling, inadequate energy metabolism, ionic imbalance, or simply the mechanical stresses occurring during contraction are thought to lead to myofiber damage of predisposed muscle.

Historically, the syndrome of passage of *myoglobin-pigmented urine (myoglobinuria)* was recognized long before it was determined that massive *skeletal muscle necrosis (rhabdomyolysis)* was the cause. Small wonder that so many names exist for the varying manifestations of exertional myopathy. In a broad sense, *exertional myopathy has included the group of diseases in which acute muscle fiber necrosis is initiated by muscle activity of the major muscle groups but in which the underlying cause is unknown or poorly understood.* Such activity may be intensive or exhaustive, but in susceptible individuals exertional myopathy may occur with only minimal exercise. Continued research into causes of exertional rhabdomyolysis is clearly warranted and, as is true of equine exertional rhabdomyolysis (see below), may lead to better understanding of causes and preventive measures.

Exertional myopathy (rhabdomyolysis) in the horse

Various manifestations of exertional myopathy have been recognized in horses for many, many years. There are numerous names for the disorder, including *azoturia* (presumably named for nitrogen-containing compounds in the urine, and possibly related to the resemblance of myoglobin to the red-purple azo dyes), *black water, paralytic myoglobinuria, Monday-morning disease, set fast, and tying up.* Various classifications have distinguished the disorder in heavy horse breeds that are prone to severe and often life-threatening muscle injury, particularly when worked after a day of rest and full grain ration (hence the term Monday-morning disease), from the often less severe disorder in light horse breeds. Muscle injury severe enough to result in myoglobinuria, profound weakness, and recumbency is common in heavy horse breeds, hence the terms azoturia, black water, and paralytic myoglobinuria. Exertional myopathy in light horse breeds is typically less severe, resulting in episodic muscle pain, sometimes associated with swelling, and reluctance to move, hence the names set fast and tying up. Given the recent recognition of a metabolic myopathy leading to exertional rhabdomyolysis in both heavy and light breeds (see below), it becomes clear that *the same underlying disorder can result in a spectrum of clinical signs.*

It has long been suspected that the clinical disorder represents a syndrome with multiple possible etiologies. It has been proposed that exertional myopathy in the horse be classified as either sporadic exertional rhabdomyolysis or recurrent exertional rhabdomyolysis, with sporadic exertional rhabdomyolysis possible due to muscle exhaustion or electrolyte depletion in any horse, and recurrent exertional rhabdomyolysis occurring in horses somehow predisposed to this disorder. Studies of serum activities of CK and AST following exercise have shown, however, that subclinical exertional myopathy is common in horses. As exertional myopathy can occur without obvious clinical signs other than, perhaps, poor performance, it is possible that so-called sporadic cases really represent recurrent disease, and therefore this classification is difficult to justify based on current knowledge of equine muscle disease.

Previously, *etiologies* proposed for equine exertional rhabdomyolysis have included muscle lactic acidosis, hypothyroidism, electrolyte imbalance, and vitamin E and/or selenium deficiency. Of these, *only electrolyte imbalance, in particular hypokalemia, is still considered possible.* Extensive studies have shown that lactic acid levels in the muscle of exercising horses prone to exertional rhabdomyolysis are no different than those of control horses, removal of thyroid glands results in poor cardiac output and performance without evidence of muscle damage, and vitamin E and selenium status varies widely in affected horses. More recently, a *metabolic myopathy* thought to involve abnormal starch and sugar metabolism (equine polysaccharide storage myopathy, see the Metabolic myopathies section) has been shown to be the most common cause of exertional rhabdomyolysis in many breeds of horses, including Quarter Horse, Warmblood, draft, Arabian, Standardbred, Tennessee Walker, Morgan, and Welsh pony-related breeds. It is possible that a similar metabolic disorder is the cause of recurrent exertional rhabdomyolysis in Thoroughbreds, although this is controversial, with one group reporting evidence for abnormal calcium handling in muscle fibers of affected Thoroughbreds. Defective ryanodine receptor function similar to malignant hyperthermia has not, however, been found in affected Thoroughbreds. Whether the abnormal muscle contracture testing reported in in vitro studies of muscle from Thoroughbreds with recurrent exertional rhabdomyolysis is a primary or secondary abnormality is still unknown. *There is strong evidence that there is an inherited basis for the predisposition to exertional rhabdomyolysis in horses.* Both autosomal recessive and autosomal dominant inheritance have been proposed, depending on the breed studied. Although further studies are needed, *it is now accepted that exertional rhabdomyolysis in horses is most often due to underlying metabolic abnormalities of muscle rather than simply due to poor management of diet and exercise.*

Although diets high in starches and sugars (grains) are associated with increased severity of exertional rhabdomyolysis, clinical signs can still occur in horses fed only forage. Despite differing opinions regarding cause, *almost all horses with recurrent exertional rhabdomyolysis respond positively following a diet change to one that is high in fat, high in fiber, and low in starches and sugars.* Stall rest appears to exacerbate the signs of equine exertional rhabdomyolysis, however the mechanism by which daily exercise benefits such horses is still unknown.

Weakness and/or pain in the hindlimbs occur suddenly, and the animal soon becomes unable or very reluctant to move. This may be accompanied by sweating and generalized tremors. The affected muscles, which are typically those of the *gluteal, femoral, and lumbar groups*, may be swollen and board-like in their rigidity. *Myoglobinuria* can appear early in the disease, causing dark red-brown discoloration of the urine. Severely affected horses become recumbent, a sign that is often a prelude to death from myoglobinuric nephrosis or problems associated with being down and attempting to rise. Considerable variation occurs between cases as to the nature and duration of the initiating exercise and severity of clinical signs. Recovery from mild attacks in quiet animals may occur in a few hours. Recovery from severe episodes may take days. But, if an animal continues to struggle and is unable to rise, death or euthanasia is the most likely outcome. *Atrophy of the gluteal muscles* may be a feature of recovery in moderate to severe cases. Exertional rhabdomyolysis occurs in both males and females, although *females appear to be predisposed.* The activity of the ovarian hormones estrogen and progesterone does not, however, appear to be directly related to onset of exertional rhabdomyolysis in females, and ovariectomy is not an effective therapy.

The apparent pain and muscle swelling associated with many cases of equine exertional rhabdomyolysis is curious, as muscle necrosis per se is neither painful nor does it cause muscle swelling. It is suspected that *increased intramuscular pressure, perhaps exacerbated by oxidative membrane injury, may cause painful muscle injury in this disorder.*

This may explain the often-reported improvement obtained following vitamin E and selenium supplementation in affected horses.

Muscle fiber membrane injury leads to release of sarcoplasmic proteins, most notably myoglobin, creatine kinase (CK), and aspartate aminotransferase (AST). Serum activity of CK peaks at approximately 4–6 hours post-injury and declines rapidly, with a half-life of approximately 6–10 hours. Therefore, if serum CK activity is not reduced by at least 50% every 24 hours this is evidence of continued muscle injury. Serum activity of AST increases more slowly, with a peak at about 48 hours post-injury and a very long half-life that can lead to persistent AST increases for days to weeks. The degree of CK and AST increase does not, however, correlate with severity of clinical signs. It is possible that muscle cramping or stiffness in the absence of overt necrosis may occur in some horses.

Grossly visible changes in muscle may be inapparent. In severe cases they are most obvious in the gluteal, lumbar, and caudal thigh regions but lesions often are widespread. *Muscles may be moist, swollen, and dark, and streaks of pallor may be visible in the more extensively involved muscles.* If ischemic complications occur, the muscles also may show blotchy or linear hemorrhage. In animals that have survived for 2–3 days, muscles may become paler and, although edema may surround larger muscle divisions, the locally damaged areas appear dry compared to normal muscle.

Necrosis affects primarily the strongly glycolytic fibers (type 2B glycolytic and type 2A oxidative-glycolytic fibers; Fig. 2.87), in contrast to the primary involvement of type 1 oxidative fibers in the nutritional myopathies. The timing and sequence of events are not clear, but *damaged fiber segments generally undergo hypercontraction and hyaline degeneration* (Fig. 2.88) *followed by coagulative necrosis.* Mineralization is not typically seen. There is also potential for perpetuation of myofiber injury due to release of free radicals from damaged membranes and subsequent oxidative membrane injury. This sequence of events may reduce the capacity of muscle protein to hold water, consequently water is lost to the interstitial compartment where it raises local pressure and predisposes to ischemia (*compartment syndrome*), particularly if moderate muscle movement is continued (see Circulatory disturbances of muscle). Oxidative injury to muscle capillary endothelium may also contribute to intramuscular edema and increased pressure. Cardiac involvement is rare.

The early irreversible fiber change as monitored by electron microscopy consists of myofibrillar waving and architectural loss, irregular mitochondrial swelling, and interfibrillar edema. After 12 h, more marked sarcomere destruction occurs along with streaming of Z-bands. Histologically, the lesions are characterized by scattered single or small groups of necrotic fiber segments admixed with intact fibers. In the more severe, acute tying-up cases, 1–5% of muscle fibers in a biopsy sample may be affected. In the less severe cases, the amount of fiber damage involves less than 0.2% of fibers in irreversible change but many more fibers can undergo hypercontraction that is potentially reversible. If muscle is examined days after injury, fiber regeneration will be apparent. Lesions are often monophasic and multifocal, but can be polyphasic in horses with repeated bouts of necrosis or on-going injury (Fig. 2.89).

In horses with *polysaccharide storage myopathy*, abnormal inclusions of pale blue-gray complex polysaccharide may be seen within scattered myofibers on H&E stain (see Fig. 2.60). Inclusions may be numerous or very rare, and have been shown to occur only within type 2A and type 2B fibers. *Inclusions stain intensely with periodic acid-Schiff (PAS) for glycogen and resist amylase digestion.* In severe chronic cases, interstitial aggregates of complex polysaccharide-laden macrophages may be seen, indicative of previous necrosis of polysaccharide-laden segments, and marked fatty infiltration is possible (see Fig. 2.60). Subsarcolemmal aggregates of amylase-sensitive glycogen are also a feature of this disorder, and may occur in the absence of complex polysaccharide in some affected horses (see Fig. 2.62). Chronic myopathic changes, including excessive fiber size variation due to fiber hypertrophy and atrophy and increased numbers of internal nuclei are common. Given the high incidence of underlying equine polysaccharide storage myopathy in horses with histories of exertional rhabdomyolysis or postanesthetic myopathy (see below), *PAS staining and careful evaluation of muscle from all such cases for evidence of chronic myopathy and abnormal glycogen and complex polysaccharide storage is warranted.*

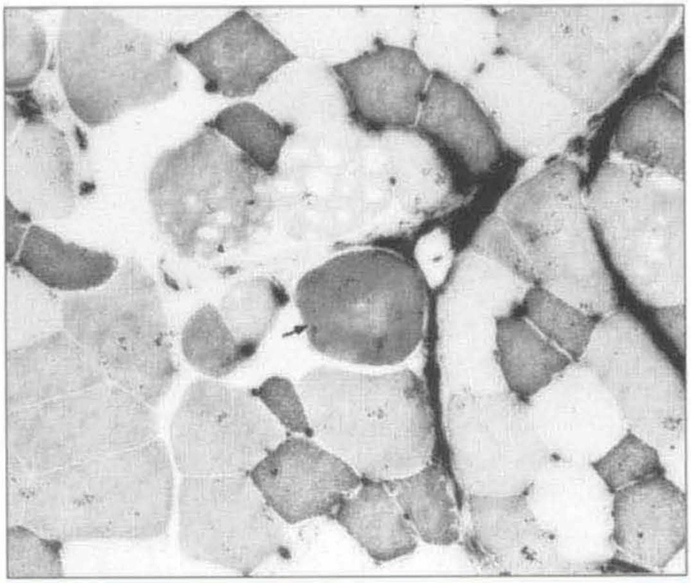

Figure 2.87 Equine exertional rhabdomyolysis with selective necrosis of type 2 (dark staining) fibers (arrow). Frozen section, ATPase, alkaline preincubation. (Courtesy of TJ Hulland.)

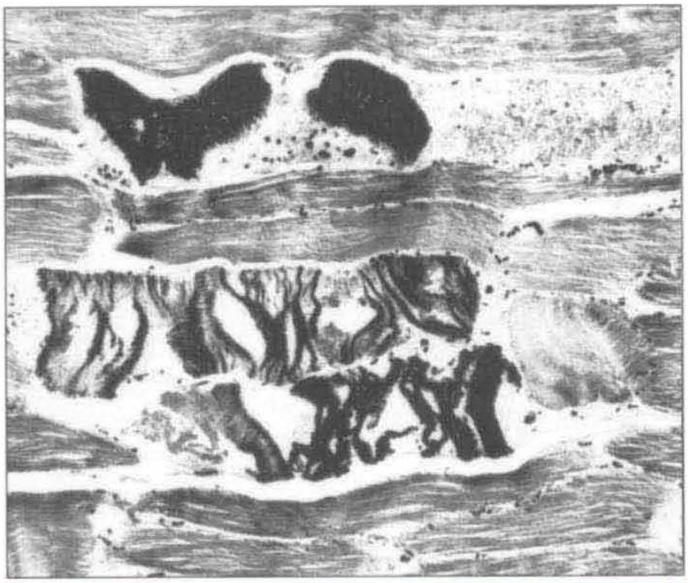

Figure 2.88 Segmental hypercontraction and acute necrosis in myofibers in a horse with **exertional rhabdomyolysis**. (Courtesy of TJ Hulland.)

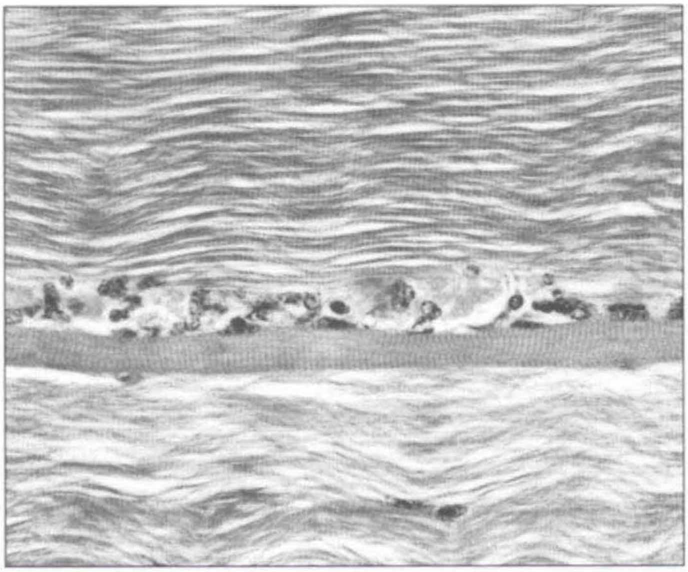

Figure 2.89 Concurrent myofiber necrosis and regeneration in a horse with **recurrent exertional rhabdomyolysis** with episodes 12 days apart. (Courtesy of TJ Hulland.)

Other equine degenerative myopathies

In addition to nutritional myopathy and exertional rhabdomyolysis, *degenerative myopathy can occur in horses recovering from general anesthesia.* This syndrome of **postanesthetic myopathy** has been recognized most often in heavy horse breeds. Although experimental induction of hypotension during general anesthesia will cause postanesthetic myopathy in any horse (see Circulatory disturbances of muscle), recent studies of clinical cases indicate that *many occur due to underlying polysaccharide storage myopathy.* Persistently high CK serum levels can occur in horses with postanesthetic myopathy due to polysaccharide storage myopathy, indicative of on-going muscle injury occurring even after the horse has stood up. A form of malignant hyperthermia has also been proposed as a cause of anesthetic-related myopathy in horses. This may be appropriate for those cases in which hyperthermia occurs during inhalant anesthesia, but most affected horses develop myopathy during recovery rather than during anesthesia. Although such horses may develop dangerously high body temperatures during recovery, this condition is best considered to be a hypermetabolic state rather than malignant hyperthermia. Underlying myopathy of any type may be a predisposing factor in horses that develop life-threatening hyperthermia either during or following general anesthesia.

Nutritional myopathy in horses causes a degenerative myopathy (see Nutritional myopathy), as do various *plant toxins and ionophores* (see Toxic myopathies). A myoglobinuric disease of pastured horses has also been described in Britain, in which extensive myolysis is triggered by an as yet unknown factor.

Exertional myopathy in dogs

Exertional rhabdomyolysis *is recognized as a syndrome affecting racing Greyhounds and racing sled dogs.* Given the vast difference in type of exercise (i.e., sprint vs endurance racing) in these two breeds of dog, there are likely to be differing underlying etiologies.

In *racing Greyhounds*, the disorder primarily affects longissimus, quadriceps, and biceps femoris muscles. Affected dogs may display distress and generalized muscle pain associated with a stiff gait. Myoglobinuria can lead to death due to renal failure. In less severe cases, myoglobinuria is not evident, and muscle pain may only be mild to moderate. Similar to exertional rhabdomyolysis in horses, affected muscles may be swollen as well as painful, indicative of mechanisms besides simple muscle necrosis as a cause of pain and swelling. Predisposing factors proposed include an *excitable nature, lack of physical fitness, hot and humid conditions, and overexertion of physically fit dogs.* Gross lesions are not often seen in affected muscles, although histopathologic evidence of acute degenerative changes is found in susceptible muscles.

Racing sled dogs may undergo massive rhabdomyolysis during racing that can lead to *sudden death.* There is often selective involvement of certain muscle groups; in one case studied there were moderate to severe polyfocal and monophasic necrotizing lesions involving quadriceps, psoas, deep digital flexor, and gastrocnemius muscles, with sparing of triceps brachii, epaxial, and cranial tibial muscles. Gross lesions are not typically seen, and sampling of multiple muscle groups may be necessary to determine the extent of injury. It has been suggested that prolonged endurance-type exercise leads to increased lipid peroxidation and reduced plasma antioxidant concentrations, however vitamin E supplementation does not appear to ameliorate exercise-induced muscle injury in racing sled dogs, and pre-race plasma vitamin E levels do not appear to correlate with risk of exertional rhabdomyolysis. As in horses, dietary management is likely to play a role in canine exertional rhabdomyolysis, as a high-fat and low-carbohydrate diet has been reported to reduce exercise-induced muscle injury in these dogs.

Exertional myopathy in other species ("capture myopathy")

Massive muscle injury associated with exertion reported in other domestic animals such as sheep and cattle can occur due to overexertion. Underlying nutritional myopathy (see the section on Nutritional myopathy) or metabolic myopathy (see the section on Metabolic myopathies) is also possible. *Of special interest is exertional myopathy occurring in wild animals following capture and/or immobilization, an entity known as* "**capture myopathy**." First described in wild ungulates, this disorder has been seen in multiple species, including wild ruminants, captured otters, cetaceans, mustelids, canids, marsupials, and wild birds. Clinically affected animals can exhibit *dyspnea, weakness, muscle tremors or muscle rigidity, hyperthermia, collapse, and, often, death.* Thigh muscle rupture may occur. Those that do not die acutely show myoglobinuria and increased levels of muscle enzymes in the blood and may subsequently die of renal failure. When the animal is down, ischemic complications may be secondary (see Circulatory disturbances of muscle). The muscle lesions are capable of more or less complete repair over a few weeks unless infarction has intervened.

Capture myopathy is associated with stress to the affected animal, and *increased circulating catecholamines may play a role,* particularly in the cardiac damage that can be seen. Hyperthermia and metabolic acidosis have also been associated with capture myopathy. Extreme overexertion likely also contributes. In some cases, marginal selenium status or electrolyte abnormality such as hypokalemia or hyperkalemia may be predisposing factors.

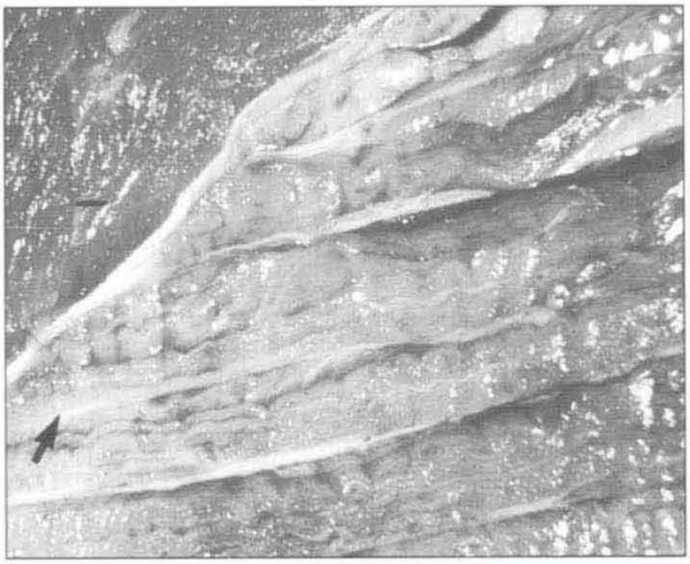

Figure 2.90 Marked pallor of affected muscle (arrow) in **bovine transport myopathy**. (Courtesy of TJ Hulland.)

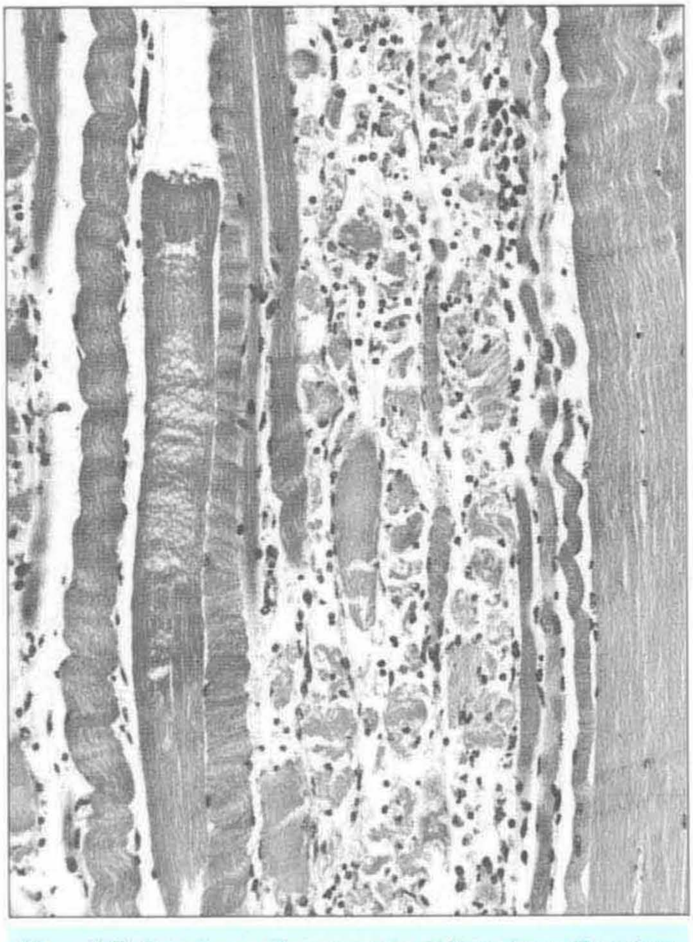

Figure 2.91 Extensive myofiber necrosis in a bighorn sheep with **capture myopathy**. (Courtesy of T Spraker.)

Gross and histologic lesions are often similar to those occurring due to other causes of severe exertional myopathy. They may also resemble those of hyperthermia in pigs, with *prominent muscle edema* in animals dying acutely. Muscles may contain *indistinct pale streaks* (Fig. 2.90), and *hemorrhagic streaking* is not uncommon. Degenerative lesions in skeletal muscle are typically *monophasic and polyfocal*. In most cases, death occurs during the degenerative phase and therefore acute myodegeneration, often with macrophage invasion of affected segments, is seen affecting various muscles (Fig. 2.91). Cardiac lesions, when present, are most often acute, although animals that survive capture myopathy may die acutely at a later date due to myocardial fibrosis. Myoglobinuric nephrosis may be seen.

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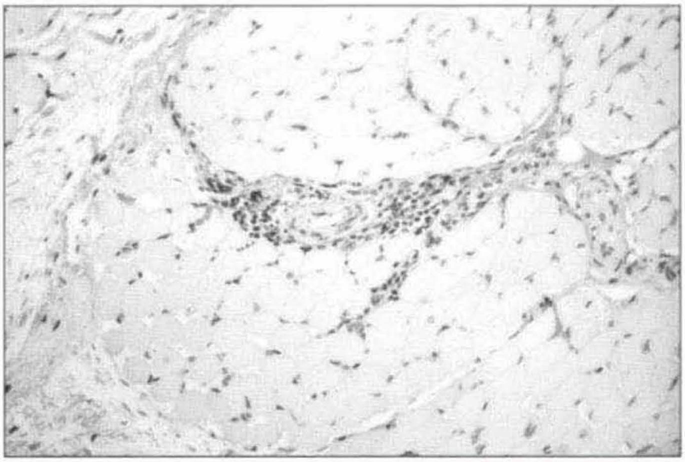

Figure 2.92 Interstitial and perivascular infiltration of mononuclear inflammatory cells characteristic of **immune-mediated myositis** in the temporal muscle of a dog.

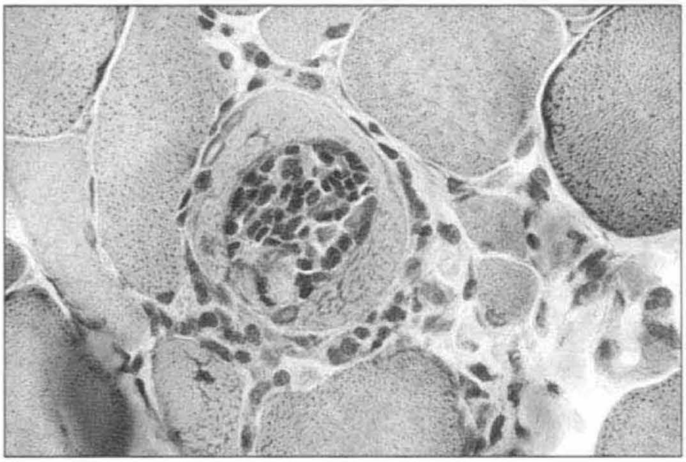

Figure 2.93 Mononuclear inflammatory cell infiltrate at the periphery and "coring out" the interior of an otherwise intact myofiber characteristic of **immune-mediated myositis** in a dog. Frozen section, modified Gomori's trichrome.

IMMUNE-MEDIATED CONDITIONS

Immune-mediated disorders are those in which abnormal activation of the immune system results in tissue damage. *Immune-mediated muscle damage can involve circulating antibodies directed against muscle cell components, cytotoxic T lymphocytes that infiltrate and attack muscle cells, or immune-complex deposition that subsequently exposes muscles to inflammatory mediators and ischemia.*

It is important to distinguish primary immune-mediated myositis from florid degenerative myopathy in which cellular infiltrates, primarily composed of macrophages, can mimic those of true inflammatory disease. It may be necessary to employ special procedures to identify the type of infiltrating cells or to detect specific antibody binding to muscle components. *Immune-mediated disease of muscle is characterized by interstitial and perivascular infiltration of lymphocytes and/or plasma cells* (Fig. 2.92). These cells may be mixed with macrophages and with eosinophils and neutrophils, particularly in cases accompanied by severe myofiber necrosis. *The diagnosis is dependent on determining that cellular infiltrates actually cause the myofiber necrosis and are not secondary to the muscle damage.* The finding of mononuclear leukocytic invasion of otherwise intact myofibers is the hallmark of primary myositis. Infiltrating cells may be seen surrounding intact myofibers, or may be seen centrally, causing a characteristic "coring out" of myofibers with otherwise intact peripheral myofibrils (Fig. 2.93). Lymphocytic myositis must be distinguished from infiltration of skeletal muscle by malignant lymphoma.

Masticatory myositis of dogs

An immune-mediated myositis localized to the masticatory muscles occurs in dogs. The masseter, temporal, and pterygoid muscles are selectively affected. Although previously designated as two separate disorders – *eosinophilic myositis and atrophic myositis – these are now recognized to be two ends of the spectrum of a single disease known as* **masticatory myositis**. Given the thick fascia of the masticatory muscles in dogs, the swelling that can occur in masticatory myositis may be due, at least in part, to ischemic damage due to compartment syndrome initiated by increased pressure within inflamed muscle. *Atrophic myositis is the result of prominent atrophy of temporal and masseter muscles.* The masticatory muscles of dogs have a unique myosin isoform, known as *type 2M myosin*. This unique myosin isoform results in an inability to achieve muscle fiber type reversal of canine masticatory muscle sections with an acid preincubation ATPase reaction. *Antibodies in dogs with masticatory muscle myositis are directed against this unique myosin isoform*, and diagnostic testing for type 2M antibodies in canine serum is available. It has been proposed that various bacterial infections may result in antibodies that cross-react between bacterial antigens and type 2M myosin.

Affected dogs are unable to fully open the mouth (*trismus*). Muscle swelling or atrophy is most obvious in the temporal and masseter muscles and is bilateral. Pain upon opening the jaw is common, and jaw immobility persists during general anesthesia. Swelling of muscle may result in a degree of exophthalmos. Attacks, if untreated, last up to 2–3 weeks and the periods between attacks may be a few weeks, months, or 2–3 years. If left unchecked, *this inflammatory myopathy leads to progressive destruction of the muscles of mastication and permanent inability to fully open the jaw.* Muscle atrophy gradually becomes very obvious and the head appears to have a fine fox-like contour with unusual prominence of the zygomatic arches (Fig. 2.94A, B).

This disorder occurs in many breeds, although the *German Shepherd breed* appears to be predisposed. Serum activities of CK and AST may be normal, or may be mildly to moderately increased. Masticatory myositis with a strong eosinophilic inflammatory component may be accompanied by peripheral blood eosinophilia, but this is not a consistent finding. Clinical response to immunosuppressive doses of corticosteroids may be rapid, but cases may relapse once corticosteroid therapy is discontinued. *Masticatory myositis is progressive*, but now that this disorder is often recognized and treated in early stages, fewer chronic cases are presented for postmortem examination. Affected dogs may, however, develop aspiration pneumonia or complications of corticosteroid therapy leading to death or euthanasia.

Grossly visible changes in the muscle vary with the stage of disease. The acutely affected muscles are swollen, dark red, doughy or hard, and the cut surface reveals hemorrhagic streaks and irregular yellow-white patches. In the late stages, there is advanced atrophy and fibrosis. Some areas consist only of pink-gray, semitranslucent, mature, connective tissue subdivided by the whiter residual tendons (Fig. 2.95).

Histologically, this disorder most often has a *multifocal and polyphasic pattern. Inflammatory infiltrates are interstitial and often perivascular.* Necrotic and regenerating myofibers are common in acute and active stages of the disease. Previous corticosteroid therapy can reduce and alter the inflammatory component present. Eosinophils may or may not be the predominant cell type seen in affected muscle, but *eosinophils will always be admixed with lymphocytes.* Eosinophils can be seen in muscle damaged by a variety of insults. The primary inflammatory cells causing destruction of myofibers in masticatory muscle myositis of dogs are lymphocytes, presumably recruited following binding of antibody. Lymphocytes may be admixed with plasma cells, and plasma cells may predominate in some cases. Macrophages will also be prominent in cases with marked myofiber necrosis. Fiber atrophy is common, and atrophic fibers can be seen associated with inflammation (Fig. 2.96) or with dense fibrosis.

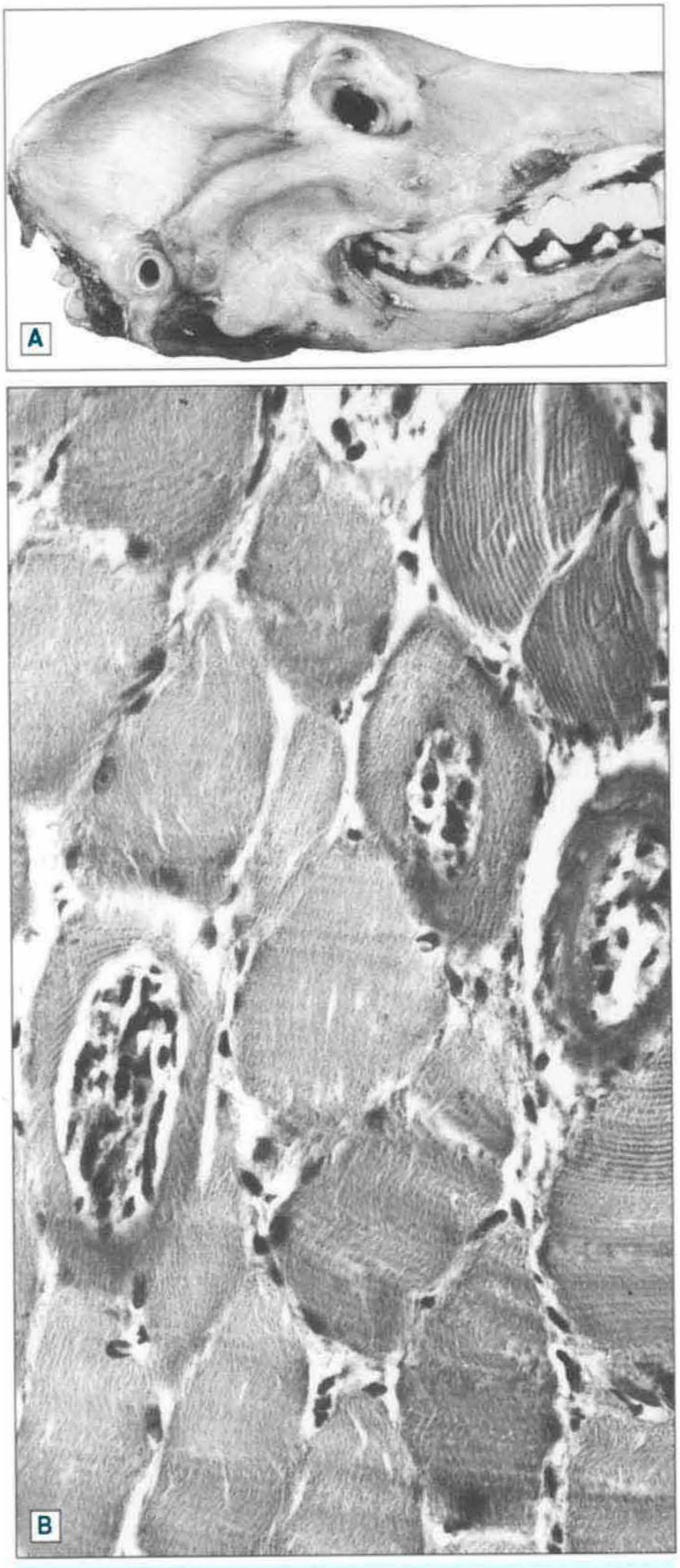

Figure 2.94 Chronic masticatory myositis in a dog. **A.** Severe atrophy of masticatory muscles. **B.** Multiple fibers being "cored out" by mononuclear inflammatory cells. (Courtesy of TJ Hulland.)

Histologic examination of muscle from suspect masticatory myositis dogs can often be frustrating. The inflammatory lesions are patchy in some cases, and biopsy may reveal only generalized fiber atrophy. *Any evidence of fiber degeneration or regeneration, even in the absence of obvious inflammation, should be considered suspicious if the clinical history is consistent with myositis.* Examination for evidence of endomysial and perimysial fibrosis is important, as severe fibrosis will negatively impact on the prognosis for return of full jaw mobility

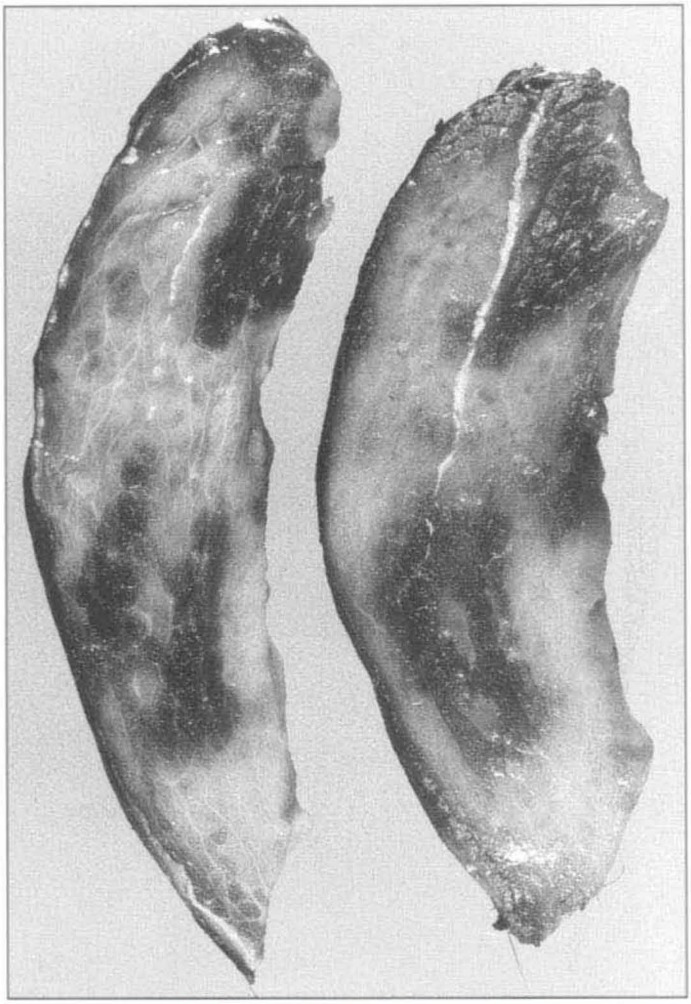

Figure 2.95 Large pale areas of fibrosis in temporal muscle from a dog with **chronic masticatory myositis**. (Courtesy of TJ Hulland.)

and muscle mass. Those cases with numerous inflammatory cells and minimal fibrosis are more likely to recover with corticosteroid therapy than cases in which inflammatory infiltrates are minimal and fibrosis is the predominant finding. An apparent increase in connective tissue may also occur due to loss of damaged myofibers and condensation of existing fibrous stroma.

The masticatory muscles of dogs, especially the temporal muscles, appear to be particularly prone to a variety of generalized myopathic and systemic disorders. Dogs with X-linked muscular dystrophy have prominent atrophy of the temporal muscle and are unable to fully open their mouths, although this condition is not painful. Labrador Retrievers with inherited myopathy also have prominent temporal muscle atrophy. Polymyositis (see below) may appear primarily as a problem with muscles of mastication, although careful evaluation will reveal abnormalities in other muscles. Dogs with any generalized illness often develop rapid atrophy of the temporal muscles that resolves with treatment for the primary problem. Persistent bilaterally symmetric atrophy of masticatory muscles not associated with pain, immobility of the jaw, or generalized disease also occurs in dogs. These latter cases of masticatory muscle atrophy appear to be idiopathic and are not associated with inflammation. If atrophy of

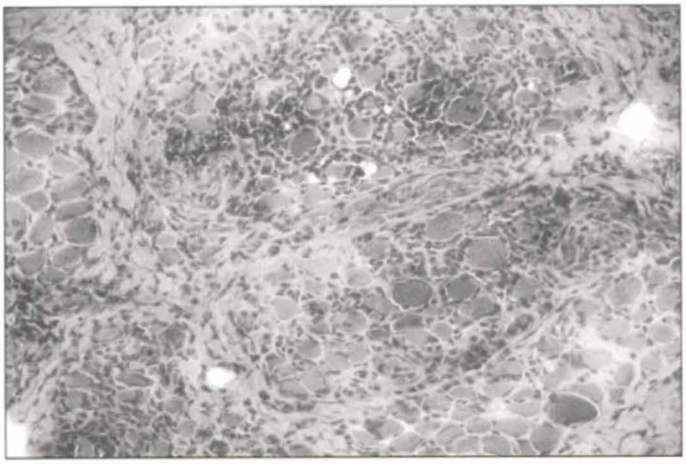

Figure 2.96 Masticatory myositis in the temporal muscle of a dog. Dense infiltrates of mononuclear inflammatory cells are admixed with atrophic and degenerating myofibers. Frozen section, modified Gomori's trichrome.

Polymyositis of dogs

Immune-mediated polymyositis occurs most commonly in dogs. Dogs with polymyositis may be presented with primary signs of masticatory muscle involvement, and both masticatory myositis and polymyositis should be considered in the differential diagnosis of such dogs. Testing for serum antibodies to type 2M myosin may be useful, as *most dogs with polymyositis do not have serum antibodies to 2M myosin.* It is possible, however, for nonspecific myosin antibodies to be found in the serum of dogs with polymyositis or other myopathies associated with myofiber necrosis. *Muscle fiber damage in polymyositis is mediated by T lymphocytes.*

Polymyositis occurs mostly commonly in *adult dogs of various breeds*, although large breeds are over-represented and German Shepherds may be predisposed. *A breed-associated polymyositis has been recognized in Newfoundlands* in which signs of muscle weakness may be apparent in dogs as young as 6 months of age. *Clinical signs of polymyositis are variable*, and include exercise intolerance, overall muscle weakness, stiff gait, and muscle atrophy. Muscle pain, elicited by deep muscle palpation, may be evident. Esophageal muscle involvement leads to regurgitation. Fever may also occur. Increase in serum activities of CK and AST is quite variable, and values may be within normal limits. Polymyositis may occur as part of the spectrum of disease in dogs with *systemic lupus erythematosus*, which is often accompanied by a positive anti-nuclear antibody (ANA) titer. Treatment with immunosuppressive doses of corticosteroids often results in resolution of signs, although if esophageal muscle fibrosis has occurred there will be persistent esophageal dysfunction.

Gross pathologic findings include mild to severe generalized muscle atrophy. In cases with esophageal involvement, *dilation of the esophagus may be evident.* Examination of multiple samples from different sites on the head and limbs may be necessary to detect inflammatory lesions. The disorder is *multifocal and polyphasic. The most common finding is of interstitial and perivascular lymphocytic infiltration in affected muscle. Invasion of otherwise intact skeletal muscle fibers by lymphocytes is*

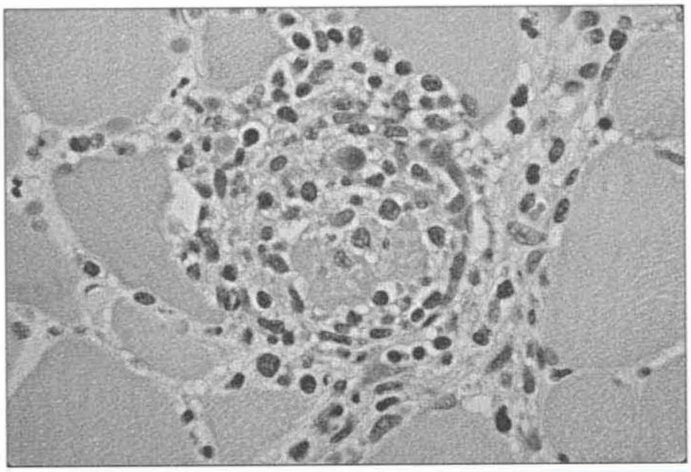

Figure 2.97 Polymyositis in a dog. Mononuclear inflammatory cells surround and invade myofibers causing necrosis. (Courtesy of BJ Cooper)

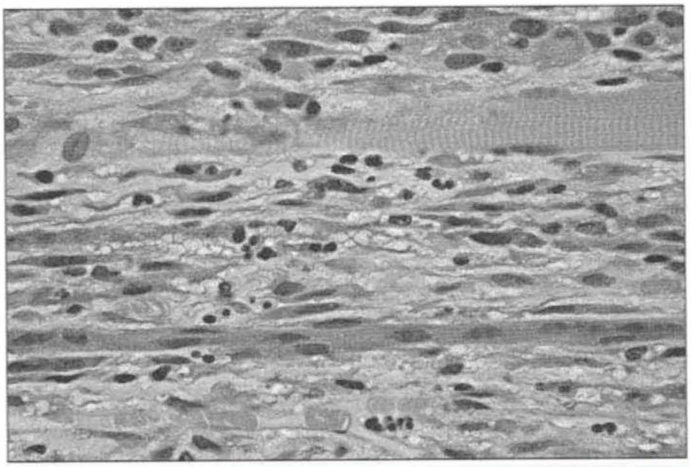

Figure 2.98 Polymyositis in a dog. There is a regenerating myotube as well as active inflammation consisting of eosinophils and mononuclear cells. (Courtesy of BJ Cooper)

characteristic (Fig. 2.97). Regenerating fibers as well as degenerating fibers are common (Fig. 2.98), and severe chronic disease may result in some degree of endomysial/perimysial fibrosis. Fibrosis may be most apparent in masticatory muscles and in the esophageal wall. Immunophenotypic studies have confirmed that, similar to polymyositis in humans, *invading lymphocytes in canine polymyositis are primarily cytotoxic (CD8+) T cells.* An admixture of inflammatory cells, however, including eosinophils and neutrophils, can be seen in particularly florid lesions.

Extraocular muscle myositis of dogs

A bilateral polymyositis involving only the **extraocular rectus and oblique muscles** *has been described in dogs.* The retractor bulbi and all other skeletal muscles are normal. Golden Retriever dogs appear to be predisposed. This disorder affects young to young-adult dogs, with age range of onset from about 6–18 months. *Clinical signs are of sudden onset of bilateral nonpainful exophthalmos.* Gross lesions of swelling and pallor can be seen within extraocular muscles. *Interstitial lymphocytic inflammatory cell infiltrates are associated with myofiber necrosis and regeneration in a multifocal polyphasic pattern.* Given the localization within extraocular muscles, an immune-mediated disorder directed at a unique epitope within these muscles is suspected. Corticosteroid therapy is usually curative, which supports an immune-mediated basis for this disorder.

Polymyositis of cats

Most reported cases of polymyositis in cats have instead proved to be degenerative polymyopathy due to hypokalemia. A lymphocytic polymyositis associated with retroviral disease has been identified in adult cats experimentally infected with *Feline immunodeficiency virus* (FIV). Clinical signs of neuromuscular dysfunction are not apparent, but serum activity of CK is moderately increased. Inflammation has the typical interstitial and perivascular pattern of immune-mediated myositis. Infiltration of muscle and myofiber necrosis of multiple muscles is mediated by cytotoxic CD8+ T lymphocytes. Other lymphocyte subsets are not found. Pelvic limb musculature is more commonly affected than thoracic limb muscles. This disorder in cats is similar to HIV-associated polymyositis in people and SIV-associated polymyositis in monkeys. A severe chronic polymyositis associated with progressive weakness and muscle atrophy has been reported in a captive Bengal tiger. Histopathologic evidence of a multifocal and polyphasic myositis characterized by interstitial infiltrates of lymphocytes and invasion of intact myofibers by mononuclear cells typical of polymyositis were seen. As this case occurred prior to the recognition of FIV infection in cats, it is tempting to speculate that this may also have been viral-associated.

Immune-mediated myositis of horses

Although exertional and postanesthetic myopathies in horses are often called "myositis," this term is not appropriate for these conditions. These disorders are degenerative myopathies and are not primary inflammatory conditions.

Hemorrhagic necrosis of muscle due to vascular injury occurs in horses with immune-complex disease due to *Streptococcus equi*-associated *purpura hemorrhagica.* In some cases, the involvement of skeletal muscle is severe enough for neuromuscular dysfunction to be the primary presenting sign. Affected horses typically have markedly increased serum activities of CK and AST associated with muscle weakness and pain. Dependent edema can also be seen. *Hemorrhagic infarcts can be found within affected muscles, and fibrinoid necrosis of vascular walls and vasculitis are seen in tissue sections* (Fig. 2.99). Inflammatory infiltrates are not seen except at the periphery of necrotic zones. Infarcts in other organs, such as lung and intestine, are common. *Streptococcus equi* can often be isolated from lymph nodes or guttural pouches. Immune complexes in purpura hemorrhagica are composed of IgA and streptococcal M protein.

Another syndrome of degenerative myopathy and rapid muscle atrophy occurs in young to young-adult Quarter Horses following exposure to *Streptococcus equi.* Skeletal muscle appears to be the only tissue affected in these horses. The cause is not yet known, although cross-reacting antibodies to streptococci affecting skeletal muscle myosin have been proposed.

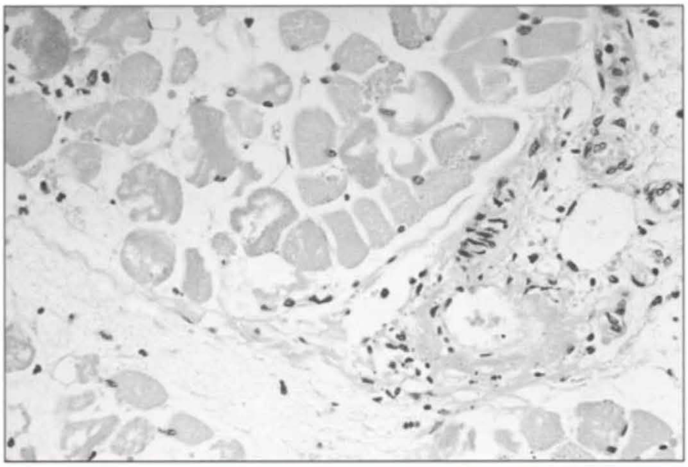

Figure 2.99 *Streptococcus equi*-associated **purpura hemorrhagica** involving skeletal muscle. Acute myofiber necrosis is associated with fibrinoid necrosis of the wall of an interstitial blood vessel. (From McGavin MD, Zachary JF. Pathologic Basis of Veterinary Disease. 2006. with permission of Elsevier Ltd.)

Acquired myasthenia gravis

Two clearly defined types of myasthenia gravis occur in dogs and cats. The *congenital disease* is due to abnormal neuromuscular junction formation and is discussed in the section on Congenital myasthenia gravis. The *acquired disease* occurs when circulating antibodies to motor end plate acetylcholine receptors bind and form immune complexes at the neuromuscular junction. The cross-linking of bound antibodies leads to increased endocytosis of acetylcholine receptors and therefore reduction in receptor density.

As in people, a link between thymic abnormalities and development of acquired myasthenia gravis occurs in dogs and cats. *Thymic follicular hyperplasia or thymoma is associated with development of myasthenia gravis in people, and thymoma is associated with myasthenia gravis in dogs and cats.* The normal thymus contains myoid cells that express muscle proteins and acetylcholine receptors. The presence of these cells is thought to be important for induction of self-recognition of these proteins. Myoid cells are readily seen by light microscopy in the thymus of neonatal or late-term aborted ruminants. Myoid cells occur in the thymic medulla and consist of large round cells with central euchromatic nuclei and abundant eosinophilic cytoplasm. These cells are scattered amongst the epithelial cells of Hassall's corpuscles, and will be found to express muscle actin and myoglobin with immunocytochemical procedures. It is thought that abnormal immune regulation occurs due to thymoma. Removal of the thymic mass often results in resolution of signs of thymoma-induced myasthenia gravis.

Acquired myasthenia gravis in dogs has also been *associated with concurrent hypothyroidism*, and sporadic cases have occurred apparently *associated with malignant neoplasia*. In most cases, myasthenia gravis occurs unassociated with an underlying neoplastic or systemic disease process.

Three clinical forms of acquired myasthenia gravis are recognized in dogs.

- The classic form is the *generalized form*, in which episodic weakness, primarily of appendicular muscles, leads to exercise-induced collapse. Signs of weakness are alleviated by rest. Generalized myasthenia may also appear as selective and persistent weakness primarily of pelvic limbs. Megaesophagus is common in generalized myasthenia gravis in dogs.
- A *localized form* with selective involvement of esophageal, facial, and pharyngeal muscle leads to megaesophagus and regurgitation without generalized weakness.
- The third form is a *fulminating form* in which rapid development of sustained generalized weakness occurs.

Only generalized myasthenia gravis has been described in the *cat*. Breeds at risk for development of acquired myasthenia gravis include German Shepherds, Golden Retrievers, Akitas, terrier breeds, German Shorthaired Pointers and Chihuahuas. An inherited predisposition to myasthenia gravis has been described in Newfoundland dogs. A bimodal age distribution occurs in dogs, with peak incidences of acquired myasthenia gravis occurring at about 3 years of age and at about 10 years of age. There does not appear to be a sex predisposition in dogs. A generalized form of myasthenia gravis occurs in cats, and Abyssinian cats may be predisposed.

Diagnosis of myasthenia gravis can be made in many cases with *electrodiagnostic testing* of repetitive nerve stimulation response, in which a decremental response in the compound motor action potential is demonstrated. A positive response to anticholinesterase therapy (edrophonium) is also considered diagnostic. *Detection of circulating anti-acetylcholine receptor antibodies is considered the most definitive test.*

Abnormalities are not seen in muscle or peripheral nerves by light microscopy, but immune complexes can be detected at neuromuscular junctions using immunocytochemical procedures. Reduction in acetylcholine receptor density due to increased internalization of receptors can be demonstrated with specialized techniques such as bungarotoxin binding assay. Alteration in morphology of synaptic clefts is seen on ultrastructural examination. Dogs with associated megaesophagus often have concurrent aspiration pneumonia.

To date, thymic follicular hyperplasia has not been described as a cause of myasthenia gravis in animals. Thymoma can be associated with either the generalized form or the fulminant form of myasthenia gravis in dogs, and with generalized myasthenia gravis in cats.

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MYOSITIS

Myositis means inflammation of muscle, but it is often difficult to determine whether the process is part of a classical inflammatory response that happens to be active in muscle or whether some components of inflammation are activated by a process that is primarily degenerative. The cause of myositis is often not evident except in the obvious cases in which the inflammatory nature of the reaction is signaled by a suppurative or granulomatous response or where it is a component of a systemic infectious disease, such as *Bluetongue virus* infection in sheep, foot and mouth disease, and toxoplasmosis (see Vol. 2, Alimentary system).

Infectious agents and parasites are frequently the cause of mild myopathy (as distinct from myositis) in animals, in which case, the degenerative muscle changes are an almost incidental and often unnoticed part of systemic infection or toxemia. There are, however, some systemic and local infections that cause myositis rather than myopathy, and they will be considered here. *Living muscle is an inhospitable site for almost all bacteria*, and consequently myositis is rarely a complication of bacterial infections even when they are overwhelmingly septicemic or repeatedly bacteremic. Pyogenic organisms sometimes give rise to solitary muscle abscesses, particularly in pigs and goats, but *bacterial polymyositis is a feature of only a few infections*, such as *Histophilus somni* in lambs and cattle, and *Actinobacillus equuli* in foals. *Clostridium* spp., on the other hand, are very well adapted to growing in muscle once damage to muscle fibers provides them with an opportunity.

Other diseases of muscle with an inflammatory response are presented under immune-mediated conditions and include several canine conditions (masticatory myositis, extraocular muscle myositis, polymyositis) and an equine disease (purpura hemorrhagica).

Suppurative myositis

Abscesses in muscle may sometimes be hematogenous in origin, but more often they result from *inoculation* (penetrating wound, contaminated injection, contamination of surgical site or laceration), or by *extension from a suppurative focus* in adjacent structures, such as joints, tendon sheaths, or lymph nodes. The most common causes of abscesses in muscle are *Arcanobacterium* (*Actinomyces, Corynebacterium*) *pyogenes* in cattle and swine, *Corynebacterium pseudotuberculosis* in sheep, goats, and horses, and *Streptococcus equi* in horses. A variety of streptococci and staphylococci are retrieved from such lesions in many species.

The natural development of suppurative myositis is comparable to abscessation elsewhere. The early stage consists of *local, ill-defined, cellulitis*. Healing may take place after this with a minimum of scarring, or it may proceed to the formation of a *typical abscess with a liquefied center, a pyogenic membrane, and an outer fibrous sheath* (Fig. 2.100). The lesion may slowly organize if it is effectively sterilized, expand if it is not or, alternatively, fistulate to the surface, collapse, and heal. In the healing process, damaged muscle fibers will participate in fiber regeneration to only a very limited extent.

In cats and horses particularly, the early stage of local cellulitis may give rise to a rapidly expanding cellulitis of muscle and adjacent fibrous and fat tissue. This phlegmonous inflammation leads to extensive destruction of muscle fibers that are subsequently mostly replaced by scar tissue. The organisms involved may vary, but *Pasteurella multocida* has been incriminated in cats, while staphylococci appear to be the most common in other domestic species. Acute cellulitis and myositis may occur in *Haemophilus parasuis* infection (Glasser's disease) in swine.

Malignant edema (gas gangrene)

The muscles, especially if devitalized in some manner, are highly susceptible to bacteria of the genus Clostridium, and these organisms, when they proliferate, are highly toxigenic and cause extensive necrosis of muscle, with blood-stained edema and the formation of gas. Once the bacteria become established, the toxins they elaborate provide a suitable and expanding environment for further bacterial growth. Death occurs as a result of *systemic intoxication*.

These bacteria are gram-positive bacilli, to a greater or lesser degree anaerobic, and they exist in the environment as resistant spores. Germination of the spores and vegetative growth requires

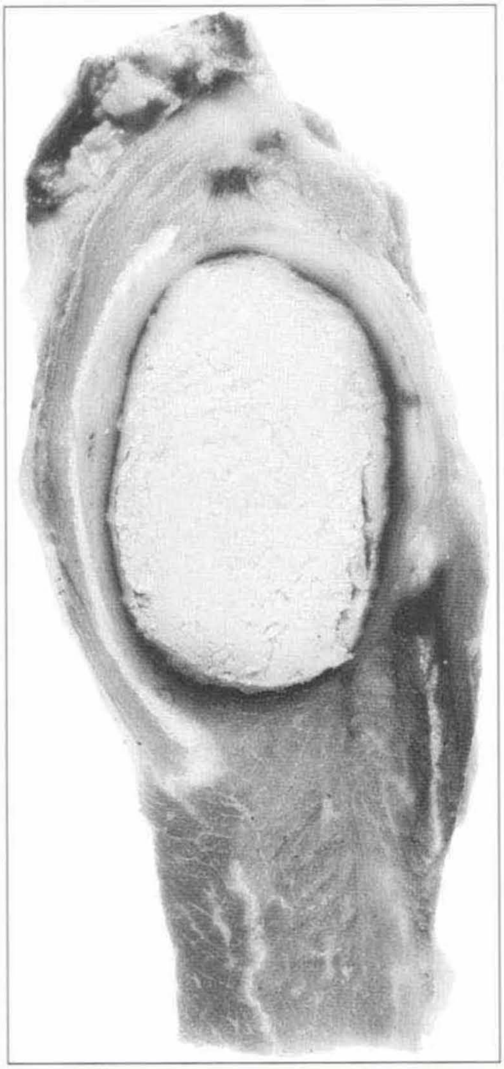

Figure 2.100 Abscess in the gluteal muscles of a goat. (Courtesy of W Hadlow.)

fairly precise local conditions, chiefly a low oxidation-reduction potential and an alkaline pH. These conditions are best produced by deep penetrating wounds and the lesions that result from the activity of the anaerobes are called "*malignant edema*," "*gas gangrene*," and "*anaerobic cellulitis*." Since the pathogenic clostridia are frequently found in soil and feces, *any contamination of an open wound is likely to introduce those potential pathogens*. Although their presence in a wound always carries a threat of gas gangrene, the very great majority of wounds thus infected heal without ill effect; in these, the local conditions in the wound must be regarded as unsuitable for germination, vegetation, or the production of toxins.

The species of the genus *Clostridium* that are of most importance as the agents of gas gangrene are *C. septicum*, *C. perfringens*, *C. novyi*, and *C. chauvoei*. These organisms not only cause gas gangrene, which is usually a mixed infection, but in animals they are, as pure infections, responsible for a number of specific diseases that are not associated with surface wounding and, with one exception, not associated with primary lesions in muscle. The exception is *C. chauvoei, which* causes bacterial myositis – *blackleg* – in ruminants. The other specific diseases include *black disease* caused by *C. novyi* (see Vol. 2, Liver and biliary system), *braxy* caused by *C. septicum*, and the *clostridial enterotoxemias* caused by *C. perfringens* (see Vol. 2, Alimentary system).

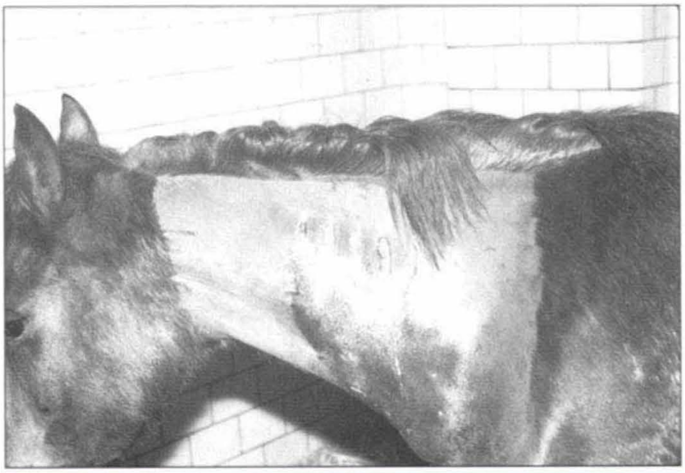

Figure 2.101 Subcutaneous swelling over neck due to **clostridial myositis** in a horse. (Courtesy of W Rebhun.)

Gas gangrene and malignant edema are essentially wound infections in which *C. septicum*, *C. perfringens*, *C. novyi*, *C. sordelli*, and *C. chauvoei* are the principal pathogens acting alone, or in combination with each other or with a variety of other aerobes and anaerobes, the latter being saprophytes with proteolytic and putrefactive properties. *Ruminants, horses, and swine are highly susceptible to these infections*, whereas carnivores are rarely affected with gas gangrene. Since deep wounds are the ones most suitable to the development of gas gangrene, the common causes of such susceptible wounds in animals are castration, shearing, penetrating stake wounds, injuries to the female genitalia during parturition and, especially in swine, inoculation sites. The distinctive characteristics of these local infections are *severe edema* (Fig. 2.101), the formation of *gas bubbles* that give crepitation, *discoloration* of the overlying skin, *coldness* of the affected part, and, in particular, the constitutional signs of *profound toxemia* with prostration, circulatory collapse, and sudden death.

Malignant edema is included in this title to distinguish certain cases of clostridial myositis of which gas gangrene is not a part; indeed, in animals, the non-gangrenous form of clostridial myositis is much more common than is the gangrenous form. *Malignant edema is more typically a cellulitis than a myositis*, and the muscles may escape significant injury even in fulminating, highly toxigenic infections of the sort that are fatal in 48 h. All of the factors that determine whether gangrene develops in these infections are not known. Even when the primary pathogens present are the same, the relative potencies and the amounts of the toxins produced may vary, and it is expected that accompanying nonprimary organisms will, to some extent, influence the local course of the infection. One factor that is probably of much importance in determining whether the inflammation will be confined to the connective tissues (malignant edema) or will directly involve muscle (myositis) is the adequacy of the blood supply to the muscle. If the muscle is devitalized by the initial trauma, or subsequently as a result of toxic injury to the blood vessels, the development of true gangrene is in order; in this manner, *malignant edema or anaerobic cellulitis may develop into gangrene.*

The pathogenesis of clostridial myositis and cellulitis is obviously not simple and is not initiated merely by the presence of spores or vegetative forms in the wound; it may begin only when the organisms have produced enough toxin to immobilize and destroy any adjacent leukocytes and enough toxin to cause death of tissue. Once the bacteria are established and producing toxins, they are capable of creating spreading conditions suitable for their advance. The progression is longitudinal, up and down fascial planes; transverse progression is very limited. The spread is facilitated by increased capillary permeability, the edema fluid separating the muscle fibers and fascia, assisted in this by gas bubbles (Fig. 2.102). This fluid also allows for further diffusion of the toxins and spread of the bacteria. Venous and capillary thrombosis result in local circulatory disturbances and these, in turn, result in further devitalization of tissue. *Once the process is started, it may progress with extraordinary rapidity*. If clostridial myositis is to develop in a wound, there is usually evidence of it within 24 hours.

In gas gangrene, there is extensive disintegration of muscle and saturation of the tissues with exudate that is in part serous and in part profusely sanguineous. When lysis of exuded red cells occurs, the tissues become stained darkly with hemoglobin. The tissues have a rancid odor in the beginning and an exceedingly foul odor in the end.

Histologically, *edema fluid*, poor in protein, separates the muscle fibers from each other and the endomysium. The *degenerating muscle fibers* stain intensely with eosin, the sarcolemma and its nuclei degenerate, but the striations are unduly persistent. Such a histologic picture is always seen at the advancing margin of the lesion. *Neutrophils are never numerous*; a few are loosely scattered at the advancing margin of the lesion and slightly greater numbers in the dermis, but they are rapidly and effectively immobilized and destroyed by the toxins. Deeper within the lesions, muscle fibers are fragmented, but this is probably an indication of physical forces having been applied to necrotic fiber segments at a stage in which the animal is still mobile. Fragmentation is by no means a constant feature of gas gangrene and the absence of it at the periphery of lesions indicates only that, in the later stages of the disease, muscle activity is drastically reduced in a recumbent animal. *Bacteria are seldom numerous in the lesions*, but collections of them may be seen either in muscle fibers or in connective tissue. Involvement of adjacent adipose tissue by the necrotizing process liberates fat droplets that may become embolic.

It is not uncommon for animals to die within 24 h of the onset of local signs of gas gangrene. Invasion, sometimes massive, of the bloodstream occurs shortly before death, or shortly after, and the offending organisms can then be obtained from most tissues. As well as the local lesion, there is at postmortem *severe pulmonary congestion* and evidence of *profound toxic degeneration of the parenchymatous organs*. By very few hours postmortem, there is extensive gas formation in all organs and they crepitate. The liver, especially, may be honey-combed with bubbles, and cut blood vessels continuously release gas bubbles.

There are some variations in the gross appearance of the lesion depending on the species of the principal pathogen. *Putrefaction is the property of contaminating saprophytes* since the primary pathogens are poorly endowed in this regard; the latter are, however, saccharolytic and that is the origin of the *gas bubbles* in the lesions. The exotoxin of *C. novyi* has a rather specific action on vascular endothelium and serous membranes, and relatively pure infections with this organism produce very extensive edematous infiltration of connective tissues. There is no putrefaction, only slight or no discoloration of muscle, and the gelatinous edema fluid is quite clear or at most pink. The toxic potency of this organism is shown by the fact that in fatal infections the organisms are so few as to be difficult to locate even in the primary focus, from which site they show little inclination to move.

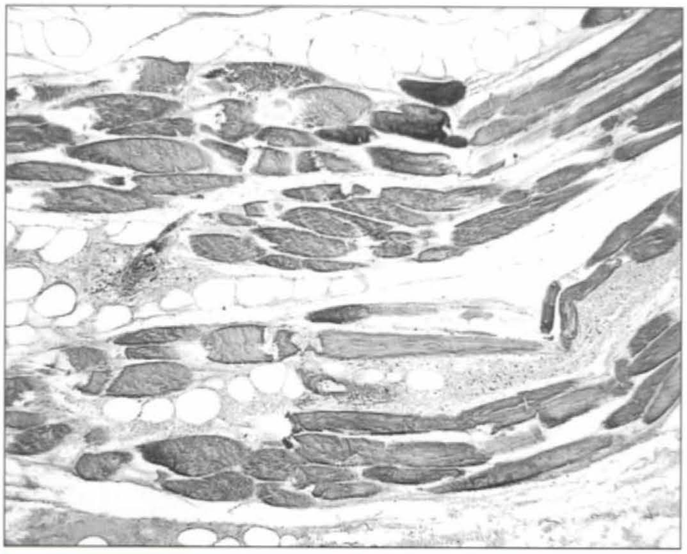

Figure 2.102 Lysed red blood cells and gas bubbles in sublingual muscle in **malignant edema** in a cow. (Courtesy of W Hadlow.)

A typical wound infection by *C. novyi* is that known as "**swelled head**" in rams, the wounds being acquired on the top of the head during fighting. This is a quickly fatal condition, death usually occurring within 48 h. There is extensive infiltration with clear, gelatinous fluid in the tissues of the head, throat, neck, and cranial thorax and sometimes also in the pleural and pericardial sacs. There is no discoloration of muscle and no, or scant, extravasation of erythrocytes. The bacilli are very few in number and cultivable only from the primary focus of infection. *Clostridium novyi* may also predominate in wound infections of horses and, when it does, these lesions too are a nonhemorrhagic variety of malignant edema. *Clostridium chauvoei* as a wound infection always produces heavily sanguineous edema fluid and much gas. *Clostridium septicum* produces large quantities of gelatinous exudate, which is, however, intensely stained with blood. The muscular tissue may be discolored dark red-to-black and, although gas is present, the bubbles are very small. Dark blood exudes freely from veins in the perimysium, and hemoglobin stains narrow collars of surrounding tissue at the margin of the lesions. *Clostridium perfringens* is the usual cause of gas gangrene in man and produces similar lesions in animals. It shows much less tendency than *C. chauvoei* and *C. septicum* to invade the bloodstream terminally or after death. The most frequent cause of malignant edema is *C. septicum*.

Blackleg

Blackleg, also known as black quarter and emphysematous gangrene, is a gangrenous myositis of ruminants caused by C. chauvoei and characterized by the activation of latent spores in muscle. This definition of blackleg separates it from gas gangrene in which, if *C. chauvoei* is involved, it is as a wound contaminant.

Blackleg can sometimes be mimicked closely by the syndrome known as "stable blackleg" or "pseudo-blackleg" caused similarly by germination of latent spores of *C. septicum* in cattle. For diagnostic, prognostic, and epidemiologic reasons, it is important to make a distinction between disease of the blackleg pattern of pathogenesis and disease of the much less complex pathogenesis of gas gangrene and malignant edema. The latter diseases occur sporadically wherever cattle and sheep are raised but appear as a confined outbreak only when groups of animals have been subjected to similar traumatic procedures such as shearing wounds or intramuscular injections. *Blackleg on the other hand is, in spite of a worldwide distribution, peculiarly localized to regions, and within regions to farms.* Within these locales, it is persistently but irregularly enzootic, but rather selective of its hosts, and controllable only by vaccination. *The muscle lesion produced is not distinctive for the blackleg pathogenesis*, nor is the presence of *C. chauvoei* in a typical gangrenous lesion, because *C. chauvoei* can also be a wound contaminant. Identification of the blackleg syndrome should be made on a freshly dead cadaver or preferably on more than one in an outbreak. In the confirmation of blackleg and of pseudo-blackleg particularly, attempts at distinguishing syndromes must be tempered by the knowledge that *C. septicum proliferates rapidly after death while C. chauvoei does not.* The probability of overgrowth is great when a few hours have elapsed between death and bacteriological examination.

Blackleg occurs most often in cattle and sheep and rarely in other domestic animals. Blackleg in **cattle** primarily affects animals 9 months to 2 years of age with a reduced incidence at 6–9 months and 2–3 years, and an even lower incidence in animals over 3 years of age. It affects animals in good condition, and often selectively causes death in the best-grown or best-fattened animals in a group. *Blackleg is chiefly a disease of pastured animals with a tendency to be seasonal in summer.* It is often associated with moist pastures and rapid growth of both forage and cattle, but it is also a problem on some arid ranges. Because the source of *C. chauvoei* organisms appears to be persistent on certain fields, *it has been assumed that the organism is soilborne*, but it is unlikely that it grows in soil. Growth does take place readily in the intestinal tract of cattle, and it is now thought that soil contamination persists by a process of constant replenishment by fecal contamination.

The detailed pathogenesis of blackleg is still somewhat uncertain, but many of the critical points in the following proposed sequence of events have been confirmed in the natural disease and in experimental infections in cattle. *The infection is acquired by the ingestion of spores*, and either these spores, or spores produced following one or more germinative cycles in the gut, are taken across the intestinal mucosa in some way. Macrophages may be responsible for this passage, but it may be possible for the spores to enter natural or transient apertures at tips of villi or in lymphoid crypts or be taken in by lymphoepithelial cells of the ileal domes by endocytosis. *Spores are distributed to tissues where they may be stored for long periods in phagocytic cells.* There may be a certain dynamic turnover, for example in and out of Kupffer cells in the liver, but spores can be found in many tissues of normal animals, including muscle. *The latent spores in muscle are stimulated to germinate when a local event creates muscle damage or low oxygen tension.* This last step is difficult to produce experimentally, but circumstantial evidence seems to confirm its inclusion in the sequence. Parallels exist in other clostridial diseases about which there is much less doubt. It may be that all that is required to establish a medium for the organisms to multiply is a small intramuscular hemorrhage or a degenerative focus initiated by traumatic damage to muscle as part of forced exercise.

The clinical manifestations of blackleg are often not observed and because of the rapid clinical course, *animals are often found dead.* When animals are seen ill, signs consist of lameness, swelling, and crepitation of the skin over a thigh or shoulder if the lesion is superficial, and fever. It is typical for swellings to increase rapidly in size and to be hot initially and cold later. Affected animals subsequently show depression and circulatory collapse. *Death rapidly ensues and seldom does an animal survive more than 24–36 h after the onset of any signs of lameness.* If the muscle lesions are deep within a muscle mass or in the diaphragm, no localizing signs may be evident and no palpable changes detectable. Similarly, lesions in the tongue, heart or sublumbar muscles may escape clinical detection.

An animal that has died of blackleg swells and bloats rapidly, but on incision it is often not as putrid as its external appearance would suggest. Blood-stained froth flows from the nose but not usually from other orifices. Blotchy hemorrhages may be present on the conjunctivae. A poorly circumscribed swelling may be visible on superficial inspection and crepitation detectable on palpation. The skin overlying crepitant swellings is taut and resonant, but normal or dark in color and of normal strength. The subcutaneous tissues and fascia around the lesion are thick with yellow gelatinous fluid that is copiously blood-stained close to the lesion. Gas bubbles may be apparent in the fluid. The affected muscles present slightly different appearances at different distances from the center of the lesion. *Towards the periphery of the lesion, the muscle is dark red, and moist with edema fluid* (Fig. 2.103). *Towards the center, it is red-black, occasionally with putty-colored islands, and the tissue is dry, friable, and porous where gas bubbles separate the primary bundles of fibers* (Fig. 2.104A, B). If this tissue is squeezed, it crepitates and a small amount of thin red fluid oozes out. When the tissue is exposed to air, it takes on a light red

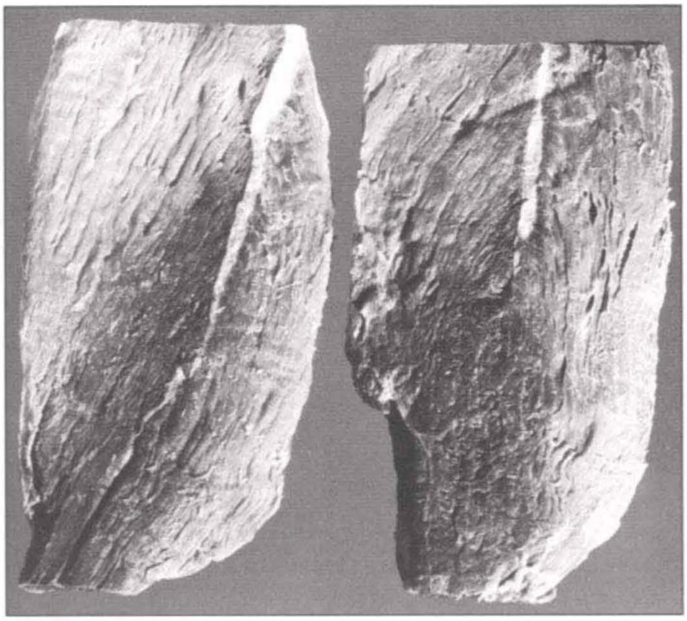

Figure 2.103 Dark areas of hemorrhage on cut surface of muscles in **blackleg** in a calf. (Courtesy of W Hadlow.)

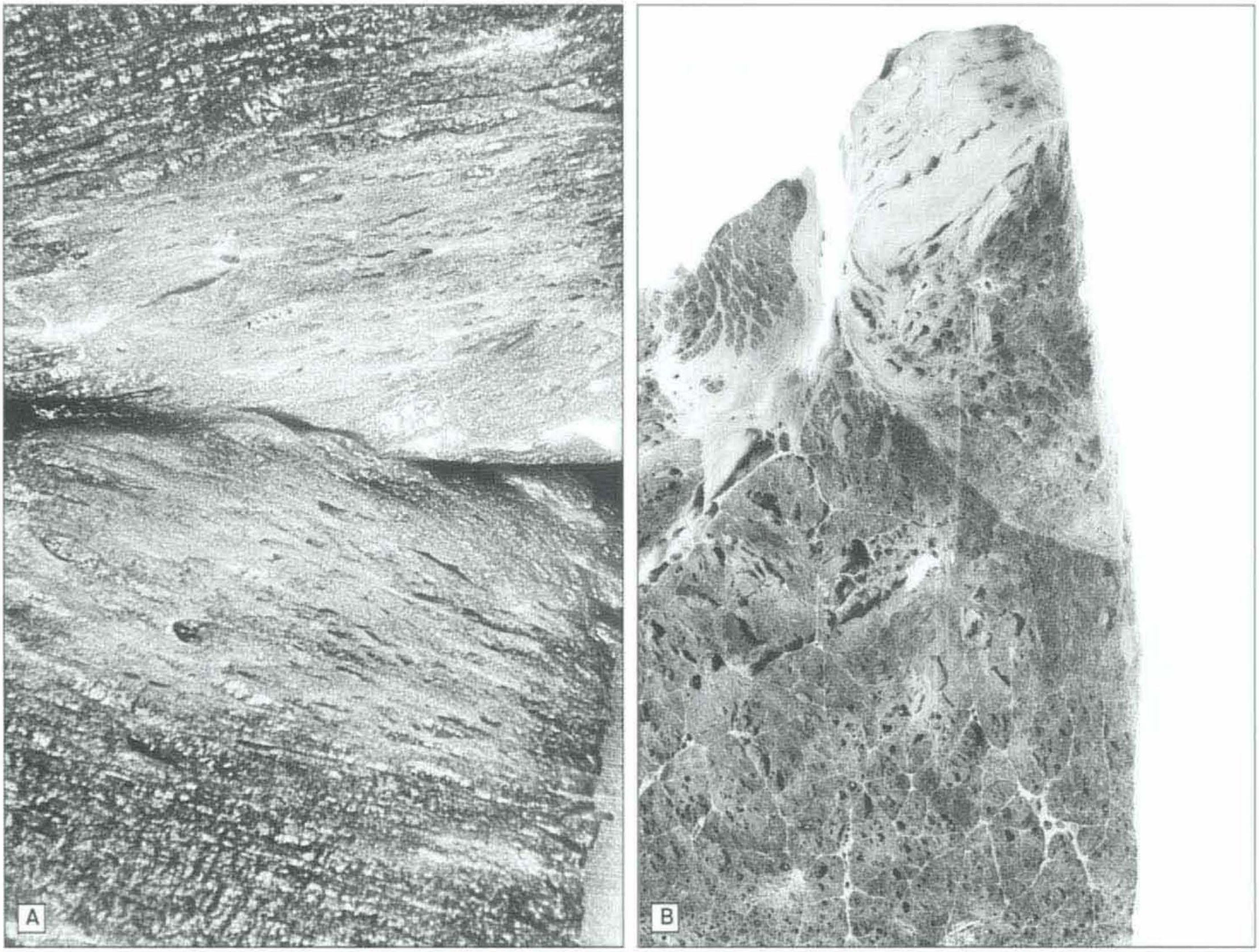

Figure 2.104 Blackleg in a cow. **A.** Dry appearance of affected muscle. **B.** Muscle bundles separated by gas bubbles. (Courtesy of TJ Hulland.)

color and watery exudate drips from cut surfaces. *The odor which emanates from the muscle is sweet and butyric, like rancid butter.*

The initial bacterial lesion in blackleg is cellulitis with copious edema and hemorrhage. Degeneration of the muscle fibers is caused by both diffusing toxin and injury to blood vessels; the extent of ischemic muscle fiber death probably determines how quickly and how extensively tissue gangrene extends through the muscle. Gangrenous lesions expand longitudinally with the long axis of muscles more readily than in a lateral direction, but "skip" areas may create necrotic zones that are highly irregular in contour. The expansion is enhanced by the edema fluid between fibers. At the time of death, animals with a single muscle lesion may have relatively small or very large areas of gangrene. Gas is not produced until muscle fibers die and are penetrated by bacteria and toxins. *The exudate and gas bubbles separate bundles of fibers and individual fibers and these undergo necrosis with preservation of striations in the center of the focus, and fatty and granular degeneration towards the periphery* (Figs 2.105, 2.106). Leukocytes are sparse, being destroyed by diffusing toxins, and only a scattering of debris is found at the periphery of the lesion.

The lesions of blackleg are usually found in the large muscles of the pectoral and pelvic girdles, but they may be found in any striated muscle including

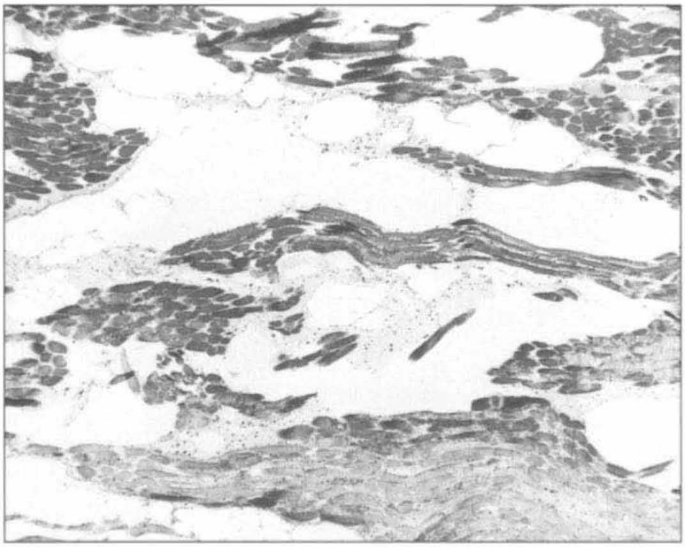

Figure 2.105 Gas bubbles in muscle in **blackleg** in a cow. (Courtesy of W Hadlow.)

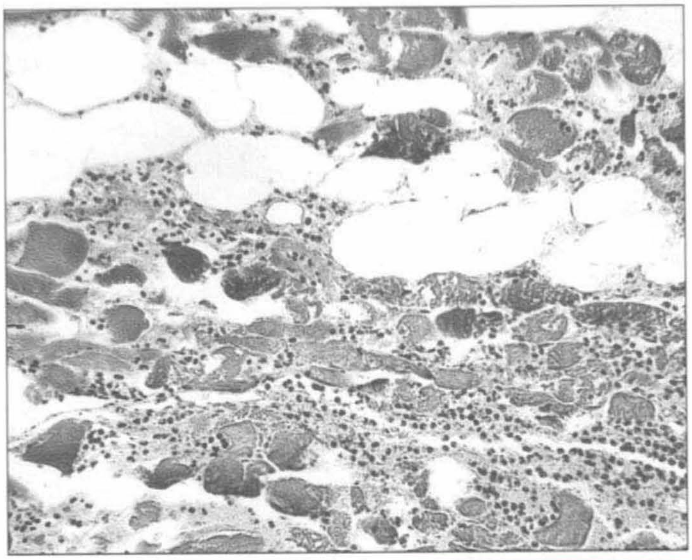

Figure 2.106 Necrosis of myofibers, neutrophilic infiltrate, and gas bubbles in **blackleg** in a cow. (Courtesy of W Hadlow.)

the myocardium. Lesions in the crura of the diaphragm and in the tongue are quite common, and if lesions are present in two or three sites simultaneously, they may be lethal before any of them is very large. This makes their clinical detection more difficult, and their detection at postmortem dependent on detailed examination of many muscles. Even with small widely separated lesions, however, the rancid butter odor may be pervasive.

In addition to the specific muscle lesions of blackleg, changes are present in the rest of the carcass. *There is severe parenchymatous degeneration of liver, kidney, and endocrine glands*, and while this is like conventional postmortem change, the rapidity of its development can suggest blackleg-related toxemia or bacteremia. There is often *fibrinohemorrhagic pleuritis* and, as a generality, when this lesion is present without severe pneumonia, blackleg should be suspected. The parietal pleura is hemorrhagic, and large or delicate blood-stained clots of fibrin overlie the ventral mediastinum and epicardium. Pneumonia is not part of the intrathoracic lesion, but the lungs are congested, and they may be quite edematous. The myocardium may be pale and friable, or dark red; some of the latter areas contain foci of *emphysematous myocarditis and necrosis*. It is these areas that give rise to fibrinohemorrhagic pericarditis; they may be primary or metastatic blackleg lesions. Endocardial lesions sometimes occur, particularly in young animals. The endocardium is hemorrhagic and may be ulcerated or contain built-up fixed, endocardial thrombi in the atrium or on the outer wall of the right ventricle. If there is atrioventricular valve involvement, it is usually on the right side. The peritoneum is intact and normal unless an underlying myositis has extended to it. The spleen may be normal, or enlarged with congested, mushy, pulp. Pale round foci of early putrefactive necrosis may be found in the liver and kidney without reaction; these enlarge with the postmortem interval and become porous. Organisms can often be recovered from many organs and from the blood shortly after death.

Blackleg in **sheep** closely resembles the disease in cattle and the causative organism is the same. *The disease in sheep, however, is much less common than in cattle*, and, although there is some overlap in enzootic distribution, *the disease in sheep usually occurs in locales quite apart from those where it occurs in cattle*. The clinical signs are similar to those in cattle except that crepitation may not be palpable during life, and there is usually dark discoloration of the overlying skin. The lesions resemble those in cattle, but there are usually fewer gas bubbles, and the muscle remains more moist.

Pseudo-blackleg

Pseudo-blackleg mimics blackleg very closely. This disease of cattle produces lesions, which are deep, like those of blackleg. A *diagnosis* of pseudo-blackleg can only be made when there is no detectable wound, the lesion is deeply located, and *C. septicum* is demonstrated in the lesions of a carcass examined immediately after death. This latter precaution is necessary to avoid, as far as possible, misdiagnosis based on the postmortem invasion of *C. septicum* and other organisms from the alimentary tract.

The lesions of pseudo-blackleg differ quantitatively and somewhat qualitatively, from those of blackleg. They tend to be multiple in widely separated muscles. There is very extensive blood-stained gelatinous exudate in the connective tissues, and this exudate contains only occasional small gas bubbles. The lesions may become confluent as very large patches throughout the connective tissues. By contrast, *the muscle lesions, although always present, are less extensive than in blackleg*. The muscles are dark red and moist, and only after examination by multiple incisions is the lack of a distinct primary focus evident. When bubbles of gas are present in necrotic muscle, they are smaller and not as numerous as they are in blackleg.

Pseudo-blackleg is reported in *pigs* in which the muscle lesions are part of a generalized body invasion by *C. septicum* organisms following entry via a primary gastric focus.

Specific infectious diseases with muscle alterations

Muscle lesions are specific findings in *foot-and-mouth disease* of calves (see Vol. 2, Alimentary system), *bluetongue* of sheep (see Vol. 2, Alimentary system), *Teschen disease* of swine, *Coxsackie virus infections* in suckling mice, and leptospirosis (*Leptospira icterohaemorrhagiae*) of dogs. In 5–10% of calves affected with foot-and-mouth disease, pale areas with or without white streaks of mineralization are present (Fig. 2.107) and represent areas of hyaline degeneration histologically. Myocarditis may also be found in calves. Sheep with bluetongue may have scattered small hemorrhagic lesions in skeletal muscle. Microscopic findings are hyaline degeneration, vasculitis, and hemorrhage.

Granulomatous lesions

Various diseases discussed elsewhere may also display granulomatous lesions in skeletal muscle. *Actinobacillosis* ("wooden tongue" in cattle) (see Vol. 2, Alimentary system), *actinomycosis* ("lumpy jaw in cattle") (see Vol. 1, Bones and joints), and *tuberculosis* (see Vol. 3, Hematopoietic system) are examples.

Beef cattle given injections of oil-adjuvant bacterins into paravertebral muscles may develop pyogranulomatous myositis with extension into the vertebral canal. Affected cattle showed lameness and paraparesis.

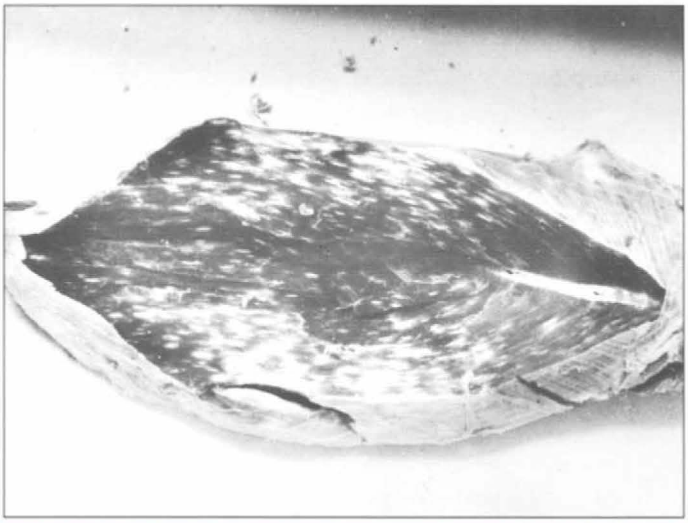

Figure 2.107 Disseminated pale areas of myonecrosis in **foot-and-mouth disease** in a calf. (Courtesy of W Hadlow.)

Other diseases with granulomatous lesions in skeletal muscle are *botryomycosis* and *Roeckl's granuloma* described in detail below.

Staphylococcal granuloma

Chronic granulomas of muscle or connective tissue caused by staphylococci, and referred to as **botryomycosis** in early literature, are much less common than they once were, but they still occur, particularly in the *horse and pig*. They represent a persistent, low-grade infection by *Staphylococcus aureus,* but it is not clear why the same organism at other times produces a conventional abscess or a gangrenous phlegmon. In horses, lesions are most frequent on the neck and pectoral region ("breast boils"), while in the pig, castration wounds and the mammary glands are the most common sites.

The lesion begins as a microabscess around a small colony of organisms and progresses rapidly, sometimes to a very large size. The *fully formed granuloma* is a hard, nodular, gray-white mass of dense, fibrous tissue, irregularly cavitated by small abscesses. The abscesses may be joined by tracts or they may fistulate to the surface. They contain a small quantity of thick, orange-yellow pus, which in turn contains minute granules. The granules consist of a central colony of the organisms while the bulk of the granule mass is made up of "clubs" of reactive protein material; hence the *typical club colony of botryomycosis*. Histologically, the organisms are readily visible in tissues since they stain with hematoxylin and are relatively large. Variable numbers of neutrophils, lymphocytes, and plasma cells are present in the loose fibrous tissue outside of the club colony. Muscle is involved at the periphery of the expanding granulomatous lesion where fibrous septa surround fibers causing atrophy and segmental degeneration.

Roeckl's granuloma of cattle

This nodular lesion of skeletal muscle is apparently specific for cattle, and it may be associated with similar lesions in liver, lungs, lymph nodes, and testes. The lesion is sometimes referred to as "*nodular necrosis*," and it is well known in Europe but rare elsewhere. Included in the list of suggested causes are tuberculosis, pseudotuberculosis, sarcocystosis, blastomycosis, and larvae of *Hypoderma bovis*, but none has been regularly found in typical lesions. Acid-fast organisms are usually not present, and although *Arcanobacterium pyogenes* is sometimes recovered, it is not considered to be the cause of the multiple granulomas.

The lesions occur in cattle of all ages. In any single animal, nodules are all of the same size but the size varies from one animal to the next, from 0.5–5.0 cm in diameter, in or under the skin. *Sites of predilection are the skin around the base of the tail, the limbs, the withers, and the abdominal wall* but lesions are seldom seen deep in muscle masses.

When nodules are cut, surfaces tend to bulge and consist of three zones. The *central zone* is dry, dull, necrotic-looking, and often distinctly yellow. The *reactive zone* is gray-pink, semitranslucent and elastic. The *outer layer* is thin, white fibrous tissue that radiates out along trabecular divisions in adjacent muscle. Larger nodules may be laminated, indicating periodic growth, and small nodules may be reduced to a hard scar with or without mineralization.

Histologically, the early stages of Roeckl's granuloma are small abscesses surrounded by granulation tissue that may contain abundant eosinophils. In other animals, the predominant inflammatory cell may be the lymphocyte. Epithelioid cells and a few giant cells may be present in the reactive zone. The capsule is made up of mature collagen. *These lesions seem to be the modified or exaggerated response of a sensitized animal to a persistent or repetitively introduced antigen.*

Changes in muscle secondary to systemic infections

Lesions in muscle caused by the specific presence of an infectious agent are described elsewhere. More common than these lesions of muscle are the degenerative changes of muscle fibers that occur in acute systemic infections such as pneumonic pasteurellosis of cattle ("shipping fever"); these may be the result of endotoxemia. The lesions, although widespread, cannot be appreciated grossly and consist of *segmental degeneration of a few or many fibers with retraction of fiber segments.* The extent of the degeneration of contractile substance may be very slight, and for this reason floccular or mineralizing changes may be sparse. Some fibers show the distinct hyaline change of Zenker's degeneration with retraction cups visible but not frequent. The fiber breaks lead to *minimal reactive cellularity* since there is little call for extensive clean-up by macrophages or for regenerative proliferation by satellite cells. The changes may be easily overlooked, but a clue to their presence is corrugation of only some fibers or groups of fibers in a longitudinal section of muscle. Although the corrugated fibers may appear otherwise normal, the recoil can be traced to proximal or distal transverse separations. Endomysium and blood vessels are intact, although occasionally there are petechial or larger hemorrhages, and infiltrating lymphocytes or neutrophils are few or absent. *Since the degeneration is almost exclusive to sarcoplasm, survival of the animal should allow complete restoration.*

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PARASITIC DISEASES

Sarcocystosis

***Sarcocystis* spp.** *are protozoal parasites of animal muscle that in many respects resemble coccidia, the main difference being their obligatory development in two hosts.* The sexual stages develop in a predator host, while the asexual phases develop in the prey animal. Some animals, such as the opossum and man, are vehicles for both parts of the *Sarcocystis* spp. cycle but not for the same species, there being considerable specificity on the part of both the intermediate and definitive hosts. There is, however, some latitude. For example, several individual parasites develop in dogs, or coyotes, or foxes, or wolves as a definitive host, and a number of intermediate host species of the same general type may be "accidentally" infected at low level. Currently over 90 *Sarcocystis* species are recognized in mammals, birds, and reptiles, and 14 of these are regularly found in muscles of domestic animals as part of the intermediate host infection. The prevalence of infection in cattle, sheep, and horses approaches 100%. Where it was once thought that the muscle phase of infection was asymptomatic and safe for the intermediate host, it is now known that particularly *the schizogonous phase may cause severe clinical disease and death* under both natural and experimental conditions. The production of enteric disease by the sexual phase in the predator host is considered elsewhere (see Vol. 2, Alimentary system). Fetal infection with abortion and neonatal mortality is described in Vol. 3, Female genital system.

The very substantial differences between *Sarcocystis* species in respect of the intermediate host response appears to be a parasite characteristic, not a difference in host immunologic response, although clinical disease may be modified by immunity.

Consideration here is primarily given to those *Sarcocystis* spp. infections affecting the muscles of domestic animals as intermediate hosts. *Sporocysts* are ingested when herbage contaminated by carnivore or human fecal material is consumed. *Sporozoites* are released and these invade many tissues. One or two generations of *schizogony* take place within endothelial cells, the first in small arterioles, the second (if there is one) in capillaries. The second or third generation of *schizonts* develops within striated muscle fibers as thin-walled cysts initially containing round *metrocytes*, which repeatedly divide to produce numerous banana-shaped *bradyzoites*. The much-enlarged mature cyst persists in muscle for long periods and the cycle is completed when muscle is consumed by the predator host and the *sexual cycle* of the parasite is developed in the intestinal epithelial cells.

Clinical disease in an intermediate host may occur at either of two stages of the developmental cycle. It may take the form of fever, petechiation of mucous membranes, edema, icterus, and macrocytic hypochromic anemia 3–5 weeks after initial infection and lasting for 6–8 weeks during the *schizogonous (parasitemic) stage*. These signs seem to be related to many small episodes of intravascular coagulation, although endothelial schizonts are not the site of thrombus formation. The parasites are also present in perivascular macrophages. The second stage in which clinical signs and death may occur comes as the *schizonts enter muscle at about 40 days*, sometimes with extensive fiber degeneration and marked enzyme release which attracts macrophages and plasma cells. Another wave of muscle disease may be associated with enlargement of the cysts in a massive infestation and this can cause lameness. Maturation of cysts may take 60–100 days, by which time any tissue reaction has subsided; this may account for the earlier impression that the parasite was an innocuous passenger in muscle.

Two species of *Sarcocystis* have been recognized in the muscles of the **horse;** *S. bertrami* (*S. equicanis*) and *S. fayeri*. Both species complete their cycle in the dog. *S. bertrami* seems to cause the greatest tissue response but neither seems to cause clinical disease and both are therefore, judged "non-pathogenic." Cysts in horse muscle are microscopic in size and rarely numerous, but occasionally there is massive involvement of skeletal muscles.

Sarcocystis species in **cattle** are presently considered to be: *S. cruzi* with a cycle completed in domestic and wild Canidae; *S. hirsuta* with a cycle completed in the domestic cat; and *S. hominis* with a cycle completed in man. Two additional species are found in the water buffalo; *S. fusiformis* with a cat–buffalo cycle, and *S. levinei* with a dog–buffalo cycle. Only *S. cruzi* seems to be capable of causing significant clinical disease in cattle. Clinical signs exhibited during the schizogonous stage may include evidence of hemolysis as described above as well as progressive debility, abortion, drooling, lymphadenitis, sloughing of the tip of the tail and, in some outbreaks, death in a high proportion of affected animals. See also Eosinophilic myositis of cattle and sheep.

Four species of *Sarcocystis* regularly affect **sheep,** namely *S. tenella* (*S. ovicanis*) and *S. arieticanis,* which have a sheep–dog cycle, and *S. gigantea* and *S. medusiformis,* which have a sheep–cat cycle. *Sarcocystis tenella* is quite virulent in lambs if infective doses are large, and naturally occurring encephalomyelitis in mature sheep with neurological disease has been blamed on this organism. Chronically debilitated sheep often have an enormous number of cysts in their muscles. This ought to have some effect on muscle performance, but lameness is not apparent. *Sarcocystis gigantea* cysts are very large (1–3 mm) and distend segments of esophageal striated muscle fibers well above the adventitial surface of the esophagus (Fig. 2.108A, B). It is the only *Sarcocystis* of domestic animals that is visible with the naked eye.

Two species (*S. capricanis* and *S. hericanis*) have a goat–dog cycle, and another (*S. moulei*) has a goat–cat cycle. None appears to be pathogenic for **goats** as natural infections but, experimentally, even moderate levels of infectious sporocysts led to death of some animals.

Three species of *Sarcocystis* are found in **swine;** *S. miescheriana* with a pig–dog and pig–wild Canidae cycle, *S. porcifelis* with a pig–cat cycle, and *S. suihominis,* which completes its cycle in humans. Of the three, only *S. miescheriana* is known to produce clinical signs of diarrhea, myositis, and lameness.

Sarcocystis organisms have been observed in the muscles of **dogs** and **cats** from time to time. It was thought that these represented erratically traveling parasites in an abnormal site, but it now appears that such cysts are morphologically unlike the species that originate in the cat or dog and complete their cycle in the sheep, horse, or cow. A new identity has not yet been given to these sarcocysts.

Sarcocystosis occurs in many wild species; for example, deer have at least five distinct species all of which complete their cycle in the dog or in wild Canidae.

Histologically, *Sarcocystis* organisms are rarely accompanied by an acute inflammatory reaction, and *schizonts in endothelial cells cause little or no evidence of endothelial cell destruction.* As the organisms enter muscle, a wide range of change may be encountered. Usually there is no muscle fiber degeneration but there may be thin, linear collections of lymphocytes between fibers in the region. Sometimes the muscle fiber undergoes segmental hyaline change in the region of the invading parasite and rarely, extensive floccular degeneration of muscle fibers occurs. The extent of muscle change bears little relationship to the numbers of developing cysts, but generally, very low numbers of *Sarcocystis* produce no reaction.

As cysts mature, and the contained bradyzoites become more distinct, the cyst capsule within the enlarged muscle fiber becomes

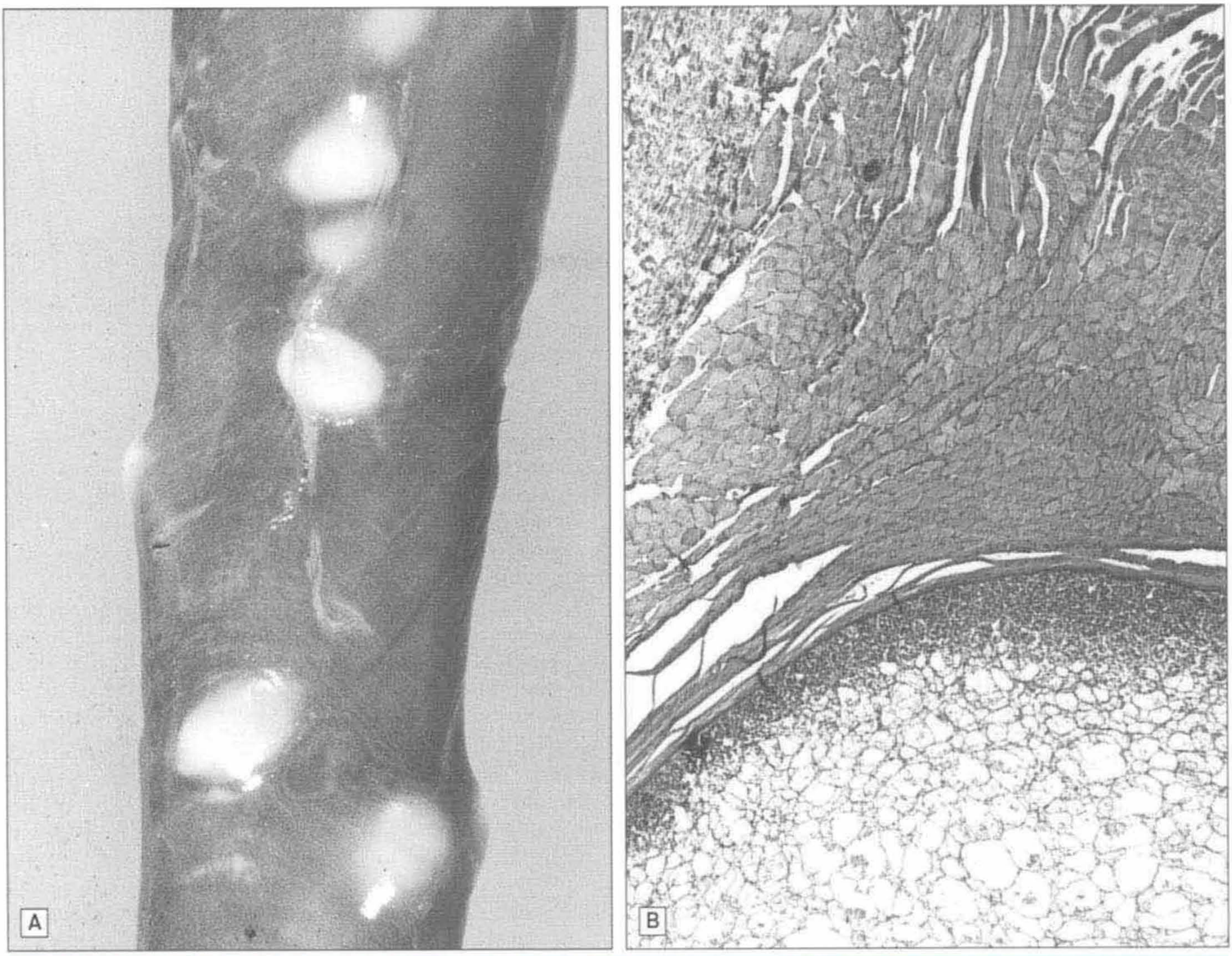

Figure 2.108 **A.** ***Sarcocystis gigantea*** **A.** In the esophagus of a sheep. **B.** Cyst margin in muscle section. (Courtesy of TJ Hulland.)

thicker and more clearly differentiated from the muscle sarcoplasm. Muscle sarcoplasm and muscle nuclei gather around the parasite in the early stages. In some parasitic species, the outer capsular zone develops distinct radial striations, which, on electron microscopy, prove to be complex convolutions of the cyst wall. Small pores allow communication between cyst contents and muscle cell content, but apart from the obvious nutritional dependence of the parasite on muscle fiber, little is known of the biochemical interplay which must take place.

Microscopic inspection of *Sarcocystis*-infected muscle often reveals occasional degenerate parasitic cysts surrounded by variable numbers of inflammatory cells (very few of which are eosinophils), or, at a later state, macrophages and granulation tissue. It is not known whether these represent "over-age" cysts or simply random changes in an easily modified host–parasite relationship. Such reactions in muscle increase with host age.

Eosinophilic myositis of cattle and sheep

Eosinophilic myositis *is a relatively rare condition in cattle and sheep of all ages* that has some significance for meat inspection because the lesions are usually discovered in skeletal muscle and myocardium of animals slaughtered for human consumption. *Sudden deaths in cattle and sheep have been ascribed to the myocarditis.* The disease is included here because *there is good evidence that eosinophilic myositis in sheep and cattle may be caused by degeneration of* Sarcocystis *spp. The converse is not true and, in some cases of eosinophilic myositis, no degenerating parasites can be found. Sarcocystis* spp. remnants are often found in the center of the myositis lesions, and IgE specific for the parasite is associated with degranulated eosinophils. Animals with parasites but not myositis have serum levels of IgE comparable to those in animals with lesions, suggesting that the inflammatory reaction in eosinophilic myositis is stimulated by independent parasite tube rupture or by trauma to muscle fibers. There are reports of traumatic muscle rupture in cattle initiating an eosinophilic response. A heat-stable, eosinophil-chemotactic substance has been isolated from affected bovine muscle in which eosinophilic myositis lesions were present. The substance has a molecular size less than bovine albumin, and its activity seems to correlate with lesion size and severity.

The gross lesions of eosinophilic myositis in cattle are characteristic, being well-demarcated, green, focal stripes or patches that fade to off-white when exposed to air. Some lesions have a brown-green or gray-green color. Single muscles or groups of muscles may be involved or the lesions may be widespread through all muscles, including the heart. Individual lesions may be 2–3 mm to 5–6 cm in diameter in both heart and skeletal muscle, and no part of the muscle mass seems to be exempt.

Histologically, both acute and more chronic fibrous reactions may exist side by side. *The reaction is characterized by large numbers of eosinophils, and it is these that impart the green color to the lesion.* Dense masses of mature eosinophils separate adjacent endomysial sheaths and perimysial trabeculae. Muscle fiber degeneration is not an obvious feature of the disease, but *occasional segments of fibers do undergo hypercontraction and degeneration.* These may be later evident only as endomysial sheaths stuffed with eosinophils rather than muscle fibers because muscle fiber regeneration seems to be impaired or modified.

With time, fibroplasia is evident. In the chronic lesion, dense collagenous tissue is prominent and the inflammatory cell population changes from eosinophils to a smaller population of lymphocytes, plasma cells, and histiocytes.

In some cases, individual lesions may take on some of the characteristics of a *granuloma* in which central muscle fibers along with adjacent eosinophils undergo degeneration. This central necrotic mass acquires a fringe of epithelioid cells and giant cells, and fibrous tissue becomes circumferentially oriented. The central eosinophilic mass may gather calcium salts while the edge of the lesion consists of infiltrating eosinophils and fibroblasts that radiate into adjacent muscle. Granulomatous lesions will leave a small scar, but it is likely that the more diffuse lesions of eosinophilic myositis in cattle can disappear in time with very little residual lesion.

Eosinophilic myositis in *sheep* tends to occur in young animals under 2 years of age. Lesions are comparable in distribution and type to those in cattle although the frequency of the granulomatous type of change may be higher.

Toxoplasma and *Neospora* myositis

In addition to *Sarcocystis,* two apicomplexan parasites, *Toxoplasma* and *Neospora*, produce myopathy in several species. Both infections are dealt with more fully elsewhere (see Vol. 2, Alimentary system; and Vol. 1, Nervous system, respectively).

Toxoplasma gondii infections, particularly in puppies and kittens, or in any species of farm animal naturally immunosuppressed, or in animals on immunosuppressive therapy, may massively involve skeletal muscle fibers. *Myositis with mononuclear leukocytic infiltration, myofiber necrosis, and myofiber atrophy is accompanied by polyradiculoneuritis.* In the majority of toxoplasma infections, however, muscle lesions are rare. In muscle fibers, both tachyzoites and thin-walled cysts (bradyzoites) may be present but the former are generally transient, spherical, and nonreactive.

Neospora caninum infections occur worldwide mainly in dogs, but the organisms are also found in cats, and the disease may have originated in the latter species. *The disease is associated with abortion and perinatal mortality in cattle with nonsuppurative encephalitis, myocarditis, and periportal hepatitis in affected animals.* Neosporosis appears to have been present in the US for over 30 years and many of the organisms originally identified in tissue sections as *Toxoplasma* are now recognized as *Neospora*. The two organisms can be distinguished definitively by immunoperoxidase procedures, and presumptively by microscopic appearance. *Neospora* cysts are thick-walled; *Toxoplasma* cysts are thin-walled and found in several tissues, including muscle. Serologic tests can detect antibodies to *N. caninum* and to *T. gondii.*

Neospora caninum has a much greater tendency to produce *myositis* because the tachyzoites form large fusiform packets in muscle after the second division stage of meronts. When these escape from the muscle fiber in a partially immunized host, an inflammatory reaction, abundant and predominantly histiocytic and plasmacytic, separates individual fibers. There is also segmental muscle fiber degeneration, which may be a response to direct parasite invasion, but is likely the result of a local release of free radicals from dying inflammatory cells. At this stage, clinical signs of muscle weakness may be obvious and at necropsy, muscles over the entire body may be pale, streaky, and atrophic. Inflammatory lesions often also affect nerves and nerve roots and result in denervation atrophy.

The life cycle and source of infection for neosporosis are unknown. The only known mode of natural infection is transplacental in persistently infected bitches, with affected litters having distinctive clinical features of progressive pelvic limb weakness, muscle atrophy, and joint contracture. The disease has been produced experimentally in immunocompetent cats, but the lesions are more severe in cats given corticosteroids.

Trichinellosis

Muscle is the habitat for encysted larvae of the nematode *Trichinella* spp., which may survive there for many years (Fig. 2.109). The muscle belongs to the animal that earlier harbored the adult worm in its duodenum, and *since animal-to-animal transmission of infection is accomplished by the consumption of infected muscle, most of the species regularly involved are carnivores or scavenger species.* Man, dogs, and a variety of wild Canidae, cats and wild Felidae, pigs, rats, mustelids, bears, polar bears, raccoons, and mice become hosts to the adult and their persistent larvae. Other species including horse and birds may become infected when muscle tissue is included in their feed; horsemeat has been a natural source of trichinellosis in man. *Trichinellosis is a zoonotic disease sometimes occurring in spectacular outbreaks in man and animals.* Humans become infected when they consume uncooked or incompletely cooked meat of pigs, bears, or aquatic mammals. In those regions of the world where inspection of meat is routine, the incidence of infection is very low, but even in countries such as Canada and the USA, the incidence in wild carnivores is 1–10%.

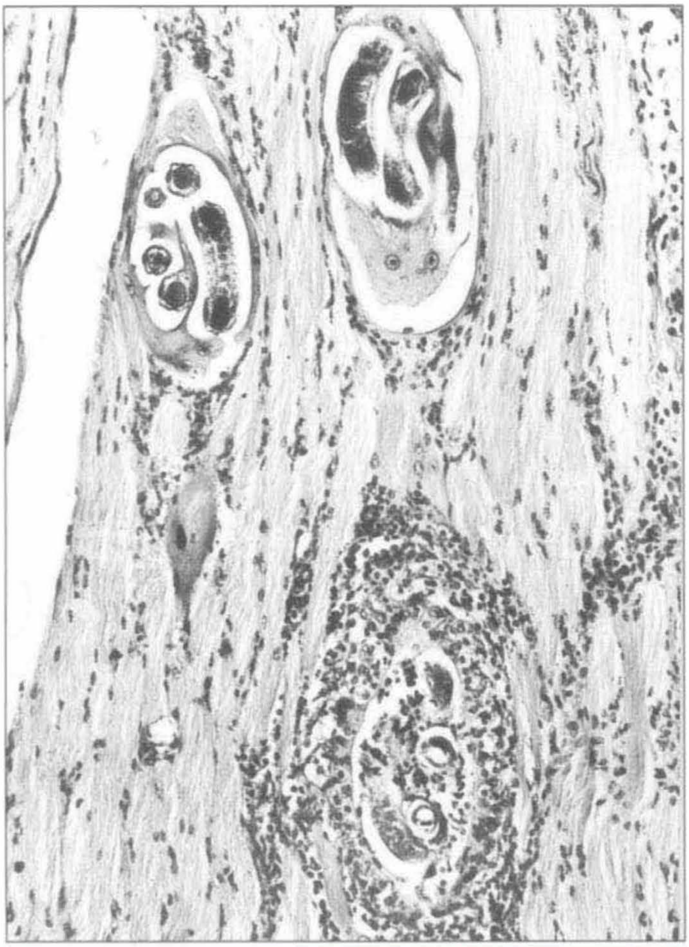

Figure 2.109 ***Trichinella spiralis*** larvae encysted in muscle of a pig.

The parasitic cycle for *T. spiralis* begins with ingestion of infected meat fibers. Gastric juices liberate the encysted larvae, which then molt twice, grow to a length of 1–4 mm as threadlike fourth-stage worms, and molt again. Maturity is reached in about 4 days following ingestion, the adults copulate and the male dies. The ovoviviparous females penetrate via the crypts of Lieberkuhn to the submucosal lymphatics where they deposit 0.1 mm long larvae into lymph vessels. The persistence of females in the duodenum is dependent on the state of surface immunity (probably IgA antibody produced locally in the gut wall) and varies from days to 5–6 weeks. Some larvae may be passed in feces as the female moves out of the duodenal crypts. The remaining larvae migrate with the lymph, then the blood, to reach the pulmonary and systemic circulations. Those that find their way to muscle may achieve a safe haven away from developing immunity by entering a muscle fiber; those that arrive elsewhere may survive for a brief period but are soon destroyed. In a previously sensitized host, few of the approximately 500 larvae produced by each female are able to enter a muscle fiber in time to ensure survival.

Within the muscle fiber, the larva grows, coils and enlarges a segment of the host muscle fiber, which is induced to develop some unusual changes as the "nurse cell." Nuclei enlarge, myofibrils are greatly reduced, the basal lamina is very greatly increased in its thickness and number of folds around the affected segment of muscle fiber, and the sarcoplasmic reticulum, which is in intimate contact with the worm, proliferates. Mitochondria in the immediate vicinity increase in number as they are reduced in size. After a month, the larvae are up to 100 μm long and coiled in a figure of eight. There is usually one per fiber. *Larvae are not normally visible by naked eye inspection of muscles unless they are old and mineralized.* On routine microscopic examination of muscle, larvae lie in bulging glassy segments of muscle fiber that may be loosely encircled by eosinophils, and in due course, by a scattering of lymphocytes, plasma cells, and macrophages. If the parasitized muscle segment degenerates, the larva is exposed and soon dies to become the center of a more acute inflammatory, but still predominantly eosinophilic, reaction. Segments of muscle fiber adjacent to the encysted larva may show evidence of degeneration or subsequent regenerative repair with basophilia and centrally located nuclei. In a heavy infestation, a large proportion of the muscle fibers in predilective muscle sites may be taken up with either the parasite or adjacent reactive zones. Purely physical replacement of functional muscle accounts for most of the clinical signs of infestation when they are present, though usually they are absent.

In 2–3 months, the cellular reaction subsides and the muscle fibers enclosing larvae become further modified to give the impression, on light microscopy, of a fibrous capsule. Since parasite survival can only be assured by intracellular seclusion, the "capsule" is, in reality, modified muscle cell components or, perhaps more correctly, modified satellite cells and basal lamina. Once the larvae are encysted in this way, further change, apart from muscle fiber degeneration, is usually confined to deposition of mineral in the encapsulating muscle structure, but this does not seem to affect parasite viability. *The larvae may survive more than 20 years* although the average life span is probably a good deal less depending on host longevity and the occurrence of fiber degeneration.

Several features of this parasitism are unexplained, including the distribution of larvae within the host. Certain muscles such as the respiratory and masticatory muscles are preferentially and heavily infected while other muscles contain a reduced burden. Activity alone is not responsible because a paralyzed diaphragm is still preferentially susceptible. Concentration of larvae in preferred sites may be influenced by blood distribution, increased larval survival, the presence of some needed nutrient, or preferential shielding from normal body defense mechanisms. Heart muscle is sometimes involved, but not heavily; *the muscles most involved are tongue, masseter and laryngeal muscles, diaphragm, intercostal muscles, and muscles of the eye*, but no striated muscle is exempt. Since some of the selectively involved muscles are small, heavy infestation may have a significant clinical effect in the form of muscle weakness, paralysis, or reduced responsiveness. Usually parasitic infestations of muscle are asymptomatic, and this feature enhances the transfer of infection from animals to man.

Five species of *Trichinella*, with eight genotypes identified by DNA analysis, are parasites of muscle:

- *Trichinella spiralis* is the parasite of pigs, rodents, and man in temperate and tropical climates, and is the most prevalent strain. It is moderately resistant to short-term freezing, and its infectivity is not reduced by freezing and thawing.
- *Trichinella nativa* is found in colder climates and is the species most often encountered in polar bears, bears, aquatic mammals, and the Inuit. Its cycle is similar in most respects to that of *T. spiralis* but the larval form is much more resistant to freezing for long periods.
- *Trichinella nelsoni* is found in carnivores in eastern and southern Africa and also in central and eastern Europe.
- *Trichinella britovi* has been reported in southern Europe.
- *Trichinella pseudospiralis* is found in northeastern Europe and differs from the other species in its failure to encyst in muscle. The species migrates through muscle more or less continuously under experimental conditions, but appears otherwise to have a cycle similar to that of *T. spiralis.*

Cysticercosis

Many of the larval forms of tapeworms of carnivores develop and are temporarily stored in the viscera or other tissues of prey species. A few have a special predilection for skeletal muscles and myocardium, and this much smaller group is dealt with here. The pathological effects of adult tapeworms in the intestinal tract of the carnivorous host are described in Vol. 2, Alimentary system.

Taenia solium is a large tapeworm (up to 8 m long) common in many parts of the world and resident in the intestinal tract of humans and sometimes other primates. The larvae (or metacestode form) usually develop in the pig or wild pig, but for this species of tapeworm humans can sometimes be host to both the tapeworm and the larval cysticercus. Gravid tapeworm segments are passed in feces, and because they are nonmotile, the 40 000 eggs in each segment tend to be concentrated over a small area. Susceptible pigs having access to infected human feces are easily infected. Eggs resist destruction for relatively long periods in soil, moist ground surfaces or sewage sludge, and survive in flowing water for a while. Following ingestion, the outer shell is digested in the stomach releasing and activating the tiny *oncosphere*, which penetrates the intestinal blood vessels and reaches the general circulation. *Most of the larvae in the pig find their way to heart, masseter, tongue, or shoulder muscles.* When they migrate in humans, they are distributed to connective tissues, brain, and viscera. The larvae become *cysticerci* (*Cysticercus cellulosae*), enlarge to a cyst with a single inverted scolex, which, when mature, measure 1–2 cm and are easily visible between muscle fibers. Cysticerci are rapidly ensheathed at first in a loose, and then a more dense, connective tissue capsule derived from endomysium. A few lymphocytes and a few or many eosinophils lie in the outer regions of the capsule. The cysticercus seems to avoid effective immune-mediated destruction for some time by converting elements of the complement system to inactive products, although in a strongly immunized animal the larvae are eventually destroyed, mineralized, and removed (Fig. 2.110A). In order to allow themselves growing room within developing inelastic collagenous capsules, the cysticerci create a crescentic zone of degenerative lysis (presumed to be induced enzymatically); this can often be seen in histologic sections of encysted metacestodes, as can parts of the scolices and hooks in those species which have them. *Cysticercus cellulosae* has rostellar hooks. The survival time in muscle for *C. cellulosae* is not known, but the question is usually not relevant since *pigs are normally slaughtered at a young age when virtually all cysticerci in muscle are viable.* Humans complete the cycle and become infected when they consume raw or incompletely cooked pork.

Taenia saginata is probably the *most common tapeworm in humans; its larvae are in cattle,* and are found in most regions of the world. Transfer of the very resistant eggs from the fecund proglottids to calves or cattle is often enhanced by contamination of open water by sewage, or by use of sewage as fertilizer on fields. It also occurs directly by contamination of animal feeds with human feces or from soiled human hands. The life cycle is similar to that described above for *T. solium* and *the larval form (Cysticercus bovis) similarly preferentially infests heart and masticatory muscles*, although cysts are often widespread throughout the muscles. Histologically the reaction to the parasite involves few eosinophils. *Cysticercus bovis* does not have rostellar hooks. Following a period of growth and development of about 10 weeks the larvae become infective. After about another 30 days the cysticerci begin to die, but some larvae may be viable 9 months after infection. Death of the larvae is probably the result of development of an immune reaction. Cattle that have acquired resistance to the invasion of oncospheres across the gut apparently do not have an enhanced capability to cause degeneration of preexisting muscle cysticerci. Humans acquire infection when they consume inadequately cooked beef or veal.

Taenia ovis is a tapeworm commonly found in dogs and wild carnivores throughout the world that, in its larval form, has a cysticercus (*Cysticercus ovis*) that develops in the heart and skeletal muscles of sheep and goats (Fig. 2.110B). The cycle is similar to that for *T. saginata* but the larvae do have rostellar hooks, which may sometimes be an aid to histologic identification in lesions even after the cysticercus has begun to disintegrate.

Taenia krabbei is a tapeworm in *wild carnivores* in temperate and arctic climates whose larval form (*Cysticercus tarandi*) is found in reindeer, gazelle, moose, and other wild ruminants. Lesions produced in the intermediate host are similar to those seen in cattle with *C. bovis,* and a similar cycle is assumed.

Hepatozoonosis

Myositis caused by *Hepatozoon americanum* (initially classified as *H. canis*) was a consistent feature of 15 natural cases of infection in

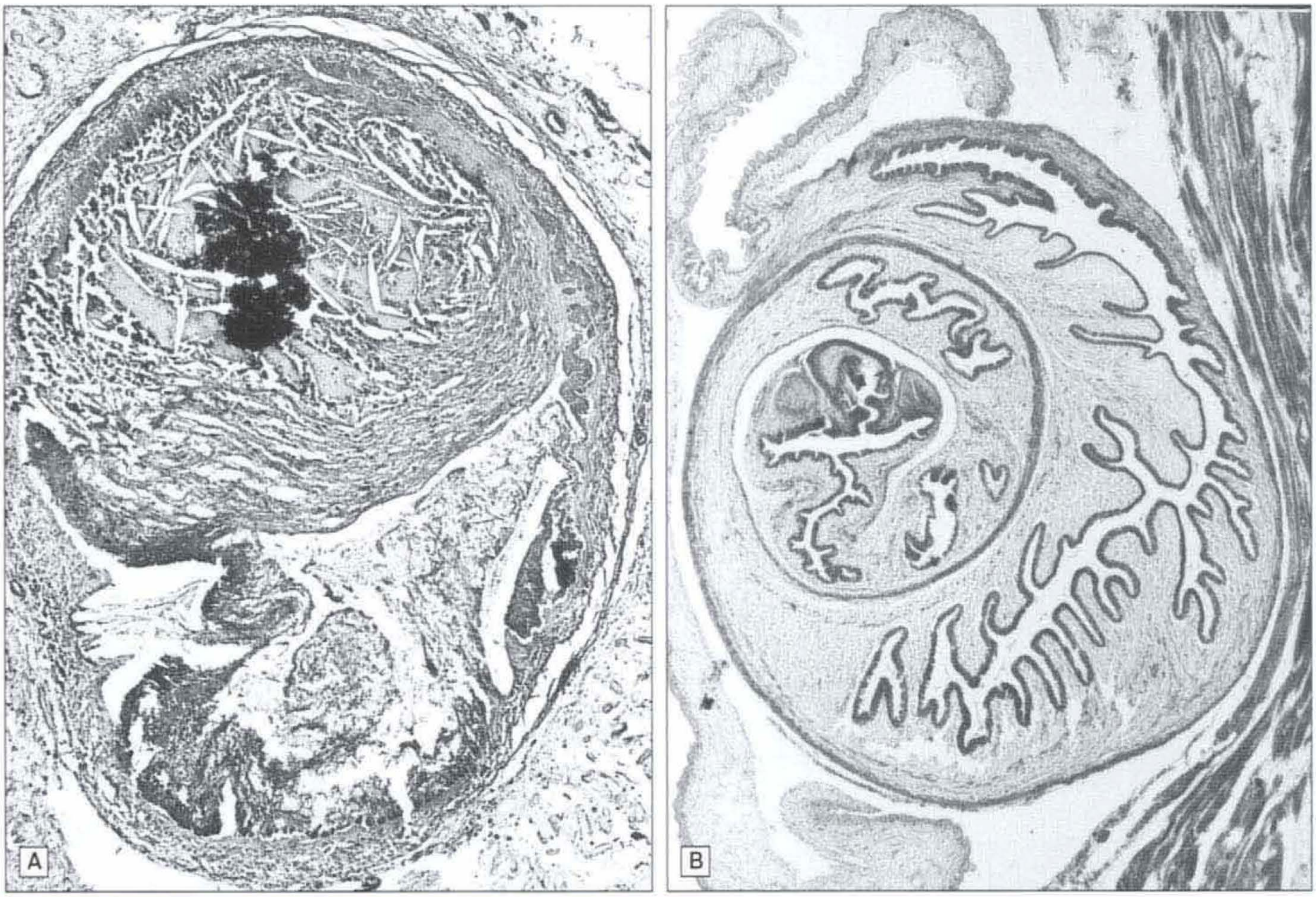

Figure 2.110 A. Degenerating and mineralizing ***Cysticercus cellulosae*** in the brain of a dog. **B.** ***Cysticercus ovis*** in the diaphragm of a sheep.

dogs in the southern USA. Cases have been limited mainly to the Gulf Coast area of Louisiana and Texas and from Oklahoma. The disease occurs in a variety of Canidae and Felidae in South Africa and the Middle East, and has become established in the dog and brown tick (*Rhipicephalus sanguineus*) populations in North America. Animals become infected by ingesting an infected tick or possibly another infected arthropod; sporozoites are released, invade the gut wall, and *undergo schizogony in many tissues of the host, including skeletal muscle*. Rupture of schizonts in time leads to an inflammatory reaction, but the subsequent stage, a large 250 µm, thick-walled, single-celled cyst does not stimulate a body response.

Affected dogs (generally less than 6 months old) show fever, anorexia, weight loss, general body pain, and gait abnormalities, and many show respiratory signs. Concurrent disease may be needed to facilitate infection. The clinical disease is often prolonged by spontaneous remissions. Radiographs may show irregular periosteal proliferations. Most dogs have marked leukocytosis and a slight increase in serum creatine kinase activity. The organism in gametocyte form may be present in neutrophils, and muscle biopsy may demonstrate the cysts, or the typical pyogranulomatous reaction in response to the release of merozoites, or the schizonts themselves.

At postmortem, muscle lesions consist of *multiple acute granulomas* interspersed with rows of neutrophils between muscle fibers. Developing stages of the parasite are usually abundant. Dogs may die with *secondary amyloidosis and glomerulonephritis*.

Chagas' disease (American trypanosomiasis)

The disease in dogs has prominent lesions of myocarditis but may also have skeletal muscle involvement. Affected muscle has *myofiber necrosis with infiltrates of lymphocytes and macrophages*. See Vol. 3, Hematopoietic system for further information.

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NEOPLASTIC DISEASES OF MUSCLE

Primary tumors of striated muscle are uncommon in domestic animals and include tumors of cardiac as well as of skeletal muscle. Myogenic tumors do not arise from fully differentiated muscle fibers and rarely arise from satellite cells. *In most cases, myogenic tumors are thought to originate from uncommitted pluripotential mesenchymal stem cells,* which can also give rise to mixed tumors containing myogenic elements as well as neurogenic, epithelial, or other mesenchymal elements. Such mixed tumors are quite rare in animals.

The cell types encountered in tumors of skeletal muscle reflect the developmental stages of embryonic and regenerating muscle. That is, *tumor cell morphology varies from a round cell resembling a satellite cell or myoblast, to spindle cell, to multinucleate cell.* Historically, the *diagnosis of skeletal muscle tumors has relied heavily on the identification of cross striations within tumor cells.* Cross striations may be identified in elongate multinucleate cells with central chains of nuclei, the so-called "strap cells," or in ovoid cells such as the so-called "racquet cells." Cross striations may be more readily identified following staining with phosphotungstic acid hematoxylin (*PTAH*). Cells with such cross striations are often rare or nonexistent within skeletal muscle tumors, and this approach is time consuming and often frustrating. Identification of primitive sarcomeric structures such as filaments arranged in parallel with associated electron dense Z bands by *electron microscopy* has been somewhat more rewarding. More recently, given the specificity of myogenic markers and relative ease of immunohistochemical identification of muscle tumors, *immunohistochemistry has largely replaced electron microscopy and PTAH staining. Desmin and muscle actin* are proteins expressed early in skeletal muscle differentiation and are expressed by many or most skeletal muscle tumor cells. Desmin will often highlight cross striations in skeletal muscle tumors far better than PTAH stain. These markers will not, however, differentiate tumors of skeletal and cardiac muscle origin from tumors of smooth muscle origin. *Myoglobin and sarcomeric actin* are specific markers of striated muscle differentiation that are expressed later in myocyte differentiation. Typically, fewer tumor cells will express these proteins, and often only the most differentiated skeletal muscle tumor cells will express myoglobin.

When evaluating tumors involving skeletal muscle, *be aware that nonmyogenic tumors infiltrating or metastatic to skeletal muscle can cause extensive damage and bizarre regenerative fibers that often mimic neoplastic cells.* This is especially true of tumors with extensive sclerosis. These bizarre cells should not be mistaken for tumor cells. Entrapped skeletal muscle fibers within a nonmyogenic tumor will also stain with immunohistochemical markers for skeletal muscle and can cause confusion.

Both benign (rhabdomyoma) and malignant (rhabdomyosarcoma) skeletal muscle tumors occur in domestic animals. As in people, rhabdomyoma and rhabdomyosarcoma often occur in young animals. Classification of these tumors, particularly rhabdomyosarcoma, has been difficult and often confusing. The classification presented here is based on the current scheme of classification of myogenic tumors in people.

Rhabdomyoma

Benign tumors of striated muscle origin are most common in the *heart of pigs* and involving the *larynx of dogs*. In pigs, the red wattle pig breed appears to be predisposed. Rare cases of cardiac rhabdomyoma have also occurred in sheep, cattle, and dogs. Females may be predisposed. **Cardiac rhabdomyomas** *are incidental findings* most often involving the left ventricular wall. Tumors can also occur in the septum, and least commonly involve the right ventricular wall. These lesions are present in young animals, and current opinion suggests that they are *most likely hamartomas or dysplastic lesions rather than true neoplasms*. These lesions occur as smooth-surfaced nodular masses up to about 3 cm diameter embedded in the myocardium. These lesions are circumscribed but nonencapsulated, and are formed by *large vacuolated myocytes with pale eosinophilic cytoplasm*. Mitoses are not seen.

Laryngeal rhabdomyomas have been reported in dogs from 2 to 10 years of age. Tumors most often occur as nodular masses protruding into the lumen of the larynx, resulting in clinical signs of dyspnea, stridor, or altered bark. One tumor occurred in the laryngeal pharynx. *Laryngeal rhabdomyoma cells are large, round, and moderately pleomorphic, and contain abundant vacuolated to granular eosinophilic cytoplasm* (Fig. 2.111). Scattered multinucleate and elongate strap cells may be seen. Intracytoplasmic glycogen may be revealed by PAS staining. Ultrastructural features include numerous mitochondria, and these tumors were initially misdiagnosed as laryngeal oncocytomas. Ultrastructural identification of *primitive myofilaments and Z band type material* (Fig. 2.112), and *positive immunostaining for skeletal muscle markers* (Fig. 2.113), has confirmed the striated muscle origin of these tumors. Although there are reports of invasive tumors classified as laryngeal rhabdomyosarcoma, *these tumors typically exhibit minimal invasion and mitotic figures, metastasis has not been seen*, and the vast majority of these tumors are thought to be benign and cured by wide surgical excision.

Rhabdomyoma is rare in other sites and other species. Rhabdomyoma of the *skin* has been reported to occur on the convex surface of the pinna in four white cats aged 6–7 years. Rarely, rhabdomyoma occurs in the skin of the trunk or legs. A cystic rhabdomyoma attached to the *diaphragm* by a stalk has been reported in the mediastinum of a 2-year-old filly.

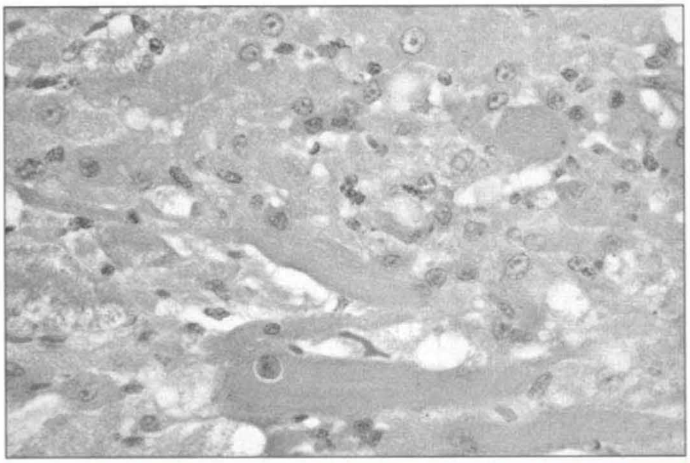

Figure 2.111 Canine **laryngeal rhabdomyoma** with characteristic moderately pleomorphic plump round-to-elongate cells with euchromatic nuclei. (Courtesy of BJ Cooper.)

Rhabdomyosarcoma

Rhabdomyosarcomas are most often fleshy growths that may form within skeletal muscle or grow into the lumen of tubular organs of the urogenital tract. Growth is often rapid, resulting in central areas of hemorrhage and necrosis.

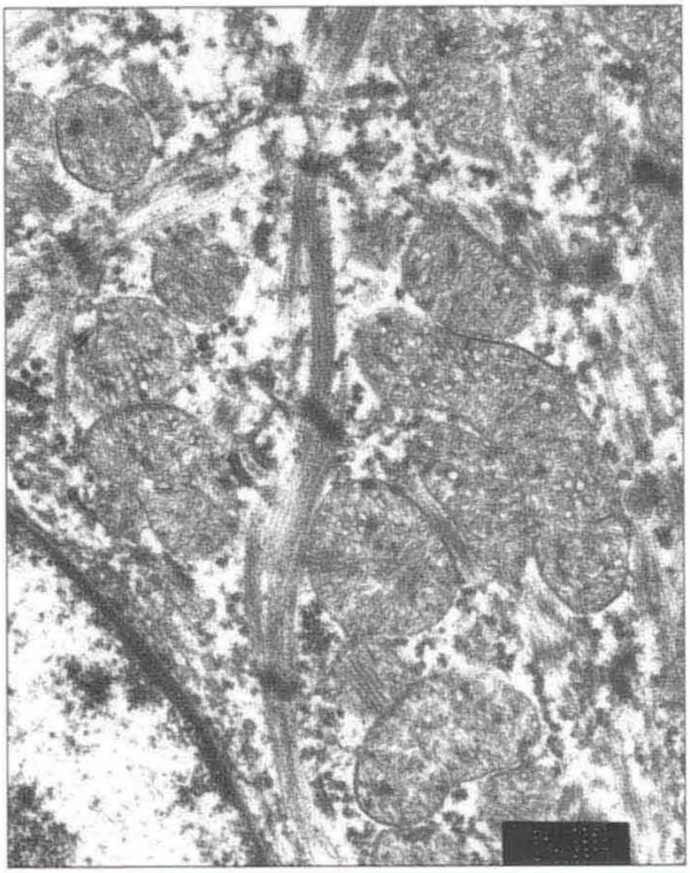

Figure 2.112 Ultrastructural features of canine **laryngeal rhabdomyoma** demonstrating characteristic Z-band type material and associated myofilaments. Mitochondria are also prominent. (Courtesy of DJ Meuten.)

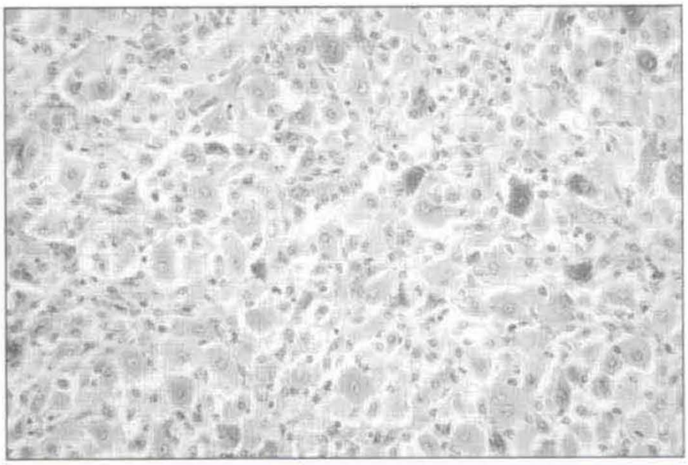

Figure 2.113 Positive myoglobin immunostaining of the cytoplasm of many cells of a canine **laryngeal rhabdomyoma**. (Courtesy of BJ Cooper.)

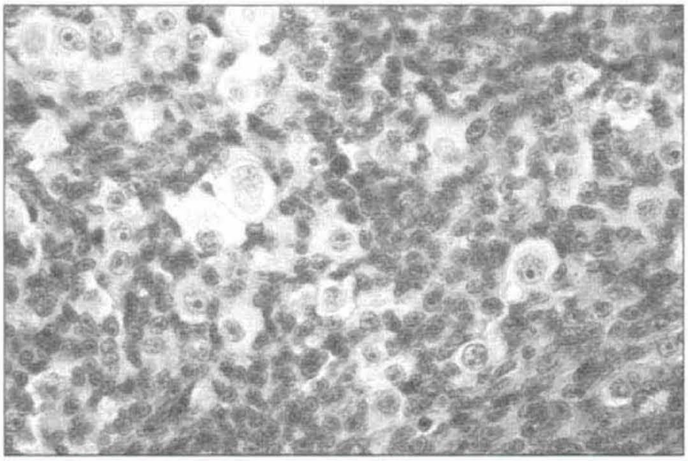

Figure 2.114 Admixed small basophilic cells and larger rhabdomyoblast cells in a feline **embryonal rhabdomyosarcoma**. (From McGavin MD, Zachary JF. Pathologic Basis of Veterinary Disease. 2006, with permission of Elsevier Ltd.)

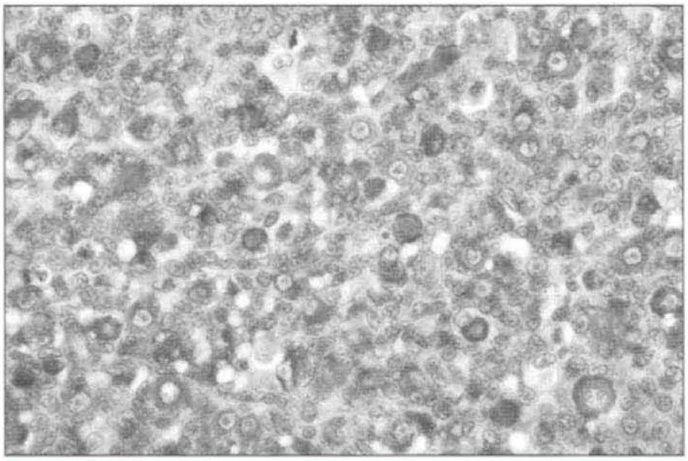

Figure 2.115 Positive immunostaining for desmin in rhabdomyoblast cells of a feline **embryoneal rhabdomyosarcoma**. (From McGavin MD, Zachary JF. Pathologic Basis of Veterinary Disease. 2006, with permission of Elsevier Ltd.)

Rhabdomyosarcoma in people has been classified histologically as *embryonal* rhabdomyosarcoma, *alveolar* rhabdomyosarcoma, and *pleomorphic* rhabdomyosarcoma. *Botryoid* rhabdomyosarcoma, a tumor most common in the urogenital tract, is considered a variant of embryonal rhabdomyosarcoma. *Embryonal rhabdomyosarcoma, and the botryoid variant, are the most common forms in people and, it would appear, in animals.* The alveolar variant is less common in both people and animals, and pleomorphic rhabdomyosarcoma, often thought to be the most classic and most common form, is actually the least common tumor. A review of the veterinary literature revealed that most tumors designated as pleomorphic variants actually had histologic features warranting designation as embryonal rhabdomyosarcoma.

Embryonal rhabdomyosarcoma is composed of primitive cells that may be either round and myoblast-like, or elongate and myotube-like ("strap cells"), and can be binucleate or multinucleate. Tumors may consist entirely of one cell type or may contain a mixture of these cell types. Embryonal rhabdomyosarcoma occurs most commonly in *young animals* and often *involves the head or neck, including the oral cavity*, although occurrence at other sites is possible. The round cell form is the most common, and embryonal rhabdomyosarcoma should be considered in the differential of any nonhistiocytic and nonlymphoid round-cell tumor of the head or neck of a young animal. The *round cell embryonal rhabdomyosarcomas* consist either of small cells with large central euchromatic nuclei, often with a single prominent nucleolus, and indistinct cytoplasm, or of larger round cells with similar nuclei and prominent eosinophilic cytoplasm (*rhabdomyoblast cells*); there is often an admixture of these two cell types (Fig. 2.114). Muscle markers are most often expressed by the larger rhabdomyoblast cells (Fig. 2.115). Scattered cells may have prominent cytoplasmic vacuolation resulting in a "spider-web" cell. The *botryoid variant* – a "grape-like" tumor – occurs most often in the trigone area of the urinary bladder in young large breed dogs. In these tumors the cells consist of elongate multinucleate myotube-like cells, often in a myxoid stroma (Fig. 2.116A, B). A similar embryonal rhabdomyosarcoma with prominent myxoid stroma can occur at subcutaneous sites and can be misdiagnosed as myxosarcoma. Although embryonal rhabdomyosarcoma is most common in dogs, this type of tumor also occurs in cats, sheep, cattle, pigs, and horses.

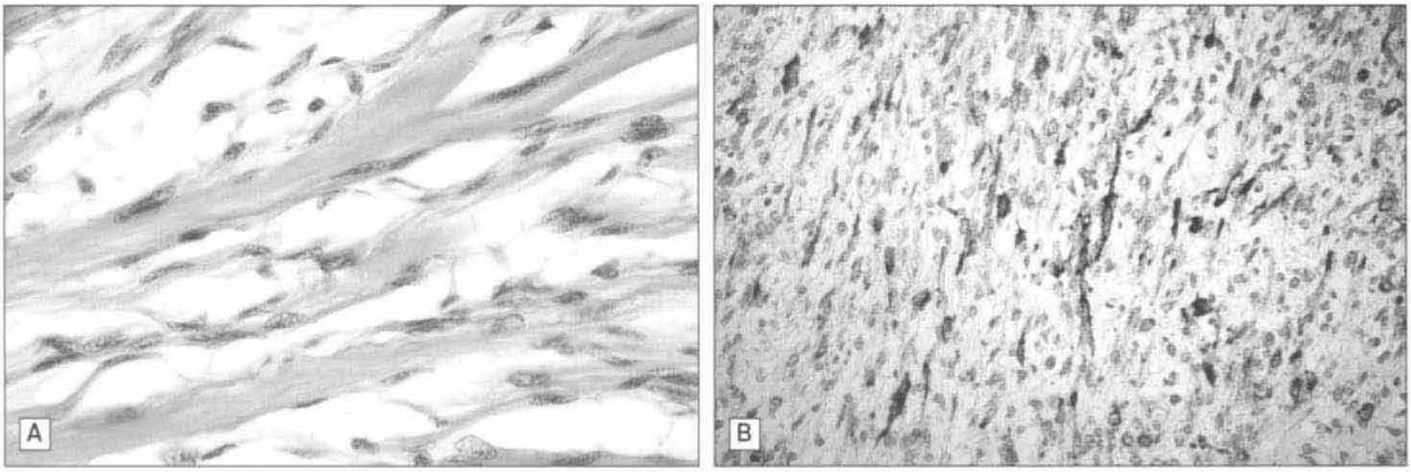

Figure 2.116 Urinary bladder embryonal rhabdomyosarcoma in the trigone of a 1-year-old large breed dog. **A.** Characteristic elongate myotubes. (From McGavin MD, Zachary JF. Pathologic Basis of Veterinary Disease. 2006, with permission of Elsevier Ltd.) **B.** Positive immunostaining for desmin. (Courtesy of BJ Cooper.)

Embryonal rhabdomyosarcoma in all species often occurs in young to young-adult individuals. There appears to be a female predominance, especially for the botryoid variant in the urogenital tract. Cytogenetic abnormalities are commonly associated with childhood rhabdomyosarcomas in people, and a similar situation has been seen in pigs in which a cluster of rhabdomyosarcomas in young pigs was found to have a *genetic basis*.

Alveolar rhabdomyosarcoma is also a tumor of adolescent and young adult people. This tumor consists of sheets of uniformly small, undifferentiated round cells in which tumor cells are supported on a fibrous framework and often *form alveolar-like structures due to loss of cohesiveness in the center of cell nests*. Multinucleate cells can be seen, but cross-striations are rare. A *solid alveolar pattern* is also possible, with sheets of small, undifferentiated round cells that resemble embryonal rhabdomyosarcoma but that lack any differentiation towards the larger eosinophilic rhabdomyoblast cells (Fig. 2.117). This form of rhabdomyosarcoma has been seen in dogs, horses, and a cow.

Virtually all rhabdomyosarcomas exhibit some degree of cellular pleomorphism. Pleomorphic rhabdomyosarcoma is often diagnosed, however *only tumors lacking any areas with features of embryonal or alveolar rhabdomyosarcoma should be designated as pleomorphic variants*. In people, pleomorphic rhabdomyosarcoma is a tumor of adults, most often occurring in major muscle groups such as the muscles of the thigh. Pleomorphic rhabdomyosarcoma has been seen arising in the neck muscle of a 3-year-old dog and in the tongue of a 5-year-old horse. Pleomorphic rhabdomyosarcoma consists of plump spindle cells in a haphazard arrangement that may be admixed with scattered multinucleate cells, strap cells, racket cells, and large, round rhabdomyoblasts (Fig. 2.118A, B).

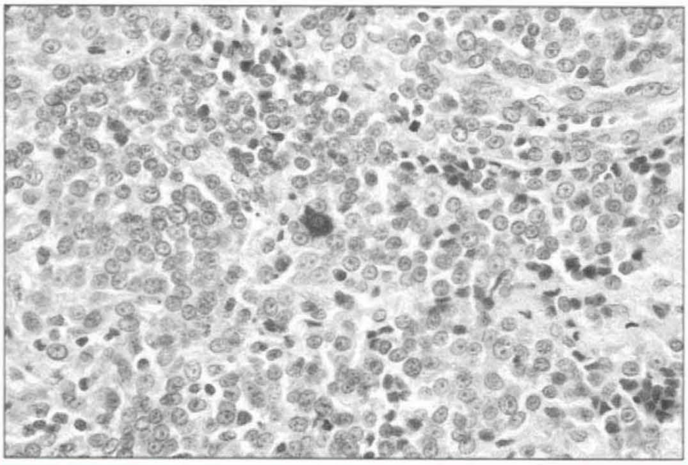

Figure 2.117 Solid sheets of undifferentiated small cells with focal intense cytoplasmic staining for myoglobin in a **solid alveolar rhabdomyosarcoma** in the hip muscle of a 7-year-old dog. (Courtesy of BJ Cooper.)

Cardiac rhabdomyosarcomas occur, but are rare, with all cases to date reported in dogs. The age range of affected dogs was 14 months to 7 years. Clinical signs of cardiac failure can occur, although one tumor was found as an incidental finding. Histologic features of poor differentiation, cellular pleomorphism, mitotic activity, and local invasion warrant the diagnosis of rhabdomyosarcoma rather than rhabdomyoma.

The behavior of rhabdomyosarcoma in animals parallels that of humans, in which aggressive local invasion and frequent metastasis are seen. Although the data regarding pattern of metastasis is scanty in animals, metastasis to atypical sites such as to other skeletal muscle sites, to cardiac muscle, and to spinal cord have been seen.

Nonmuscle primary tumors of muscle

These tumors arise from supporting mesenchymal tissues of muscle. Malignant tumors are far more common than benign tumors.

- *Poorly differentiated sarcomas* and *giant cell sarcomas* occur within muscle, especially at the site of intramuscular vaccinations in predisposed cats. These tumors may be mistaken for rhabdomyosarcoma but rarely express skeletal muscle cell markers, and are most often variants of fibrosarcoma.
- *Hemangiosarcoma* arising within skeletal muscle occurs most frequently in the dog and in the horse (Fig. 2.119). Due to the large amount of hemorrhage and scanty tumor cells, these tumors are often difficult to diagnose from biopsy specimens. Intramuscular hemangiosarcoma is often mistaken clinically for intramuscular hematoma. Intramuscular hemangiosarcoma is an aggressive tumor, exhibiting both local invasion and distant metastasis, such as to lung.

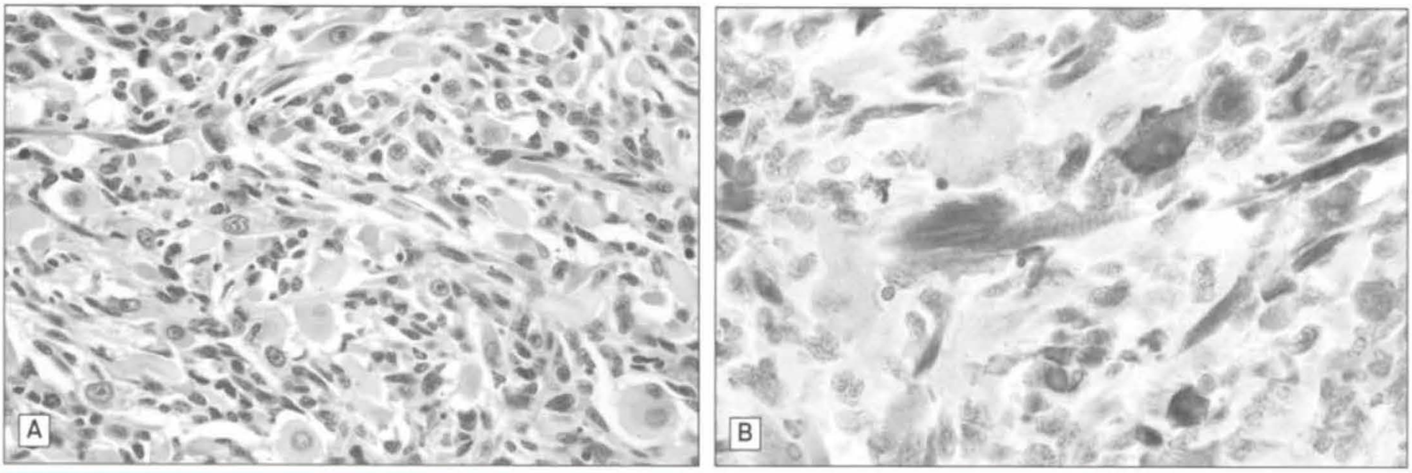

Figure 2.118 Pleomorphic rhabdomyosarcoma in the neck muscle of a 3-year-old dog. **A.** Characteristic mixed plump and pleomorphic spindle cells and round-to-ovoid cells. **B.** Positive immunostaining for desmin that highlights cross striations in spindle cells and in round-to-ovoid cells. (Courtesy of BJ Cooper.)

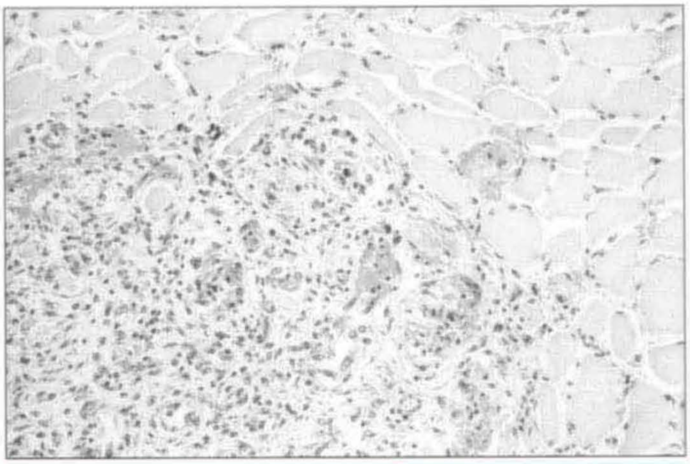

Figure 2.119 Proliferating invasive neoplastic endothelial cells forming irregular vascular channels typical of **hemangiosarcoma** within the skeletal muscle of an adult horse.

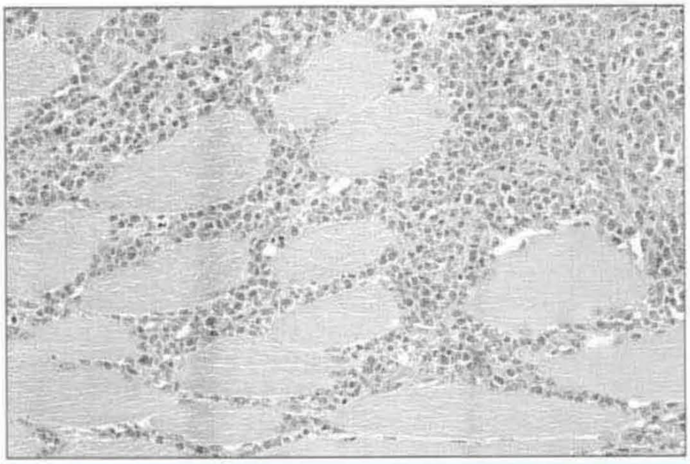

Figure 2.120 Intramuscular invasion of **lymphoma** in an adult cat.

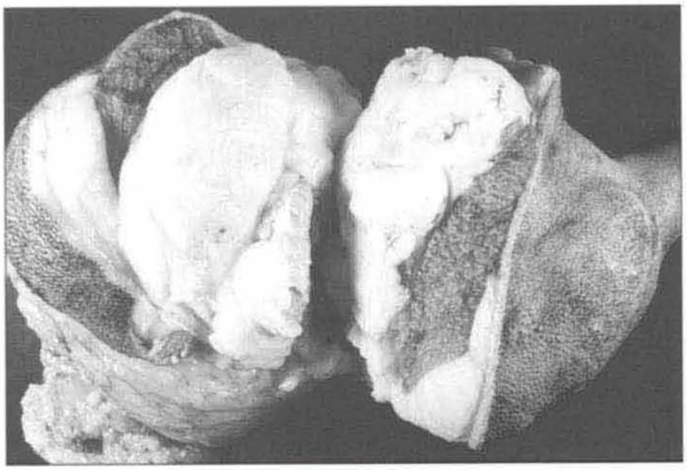

Figure 2.121 **Infiltrative lipoma** invasive into skeletal muscle of an adult dog. (Courtesy of BJ Cooper.)

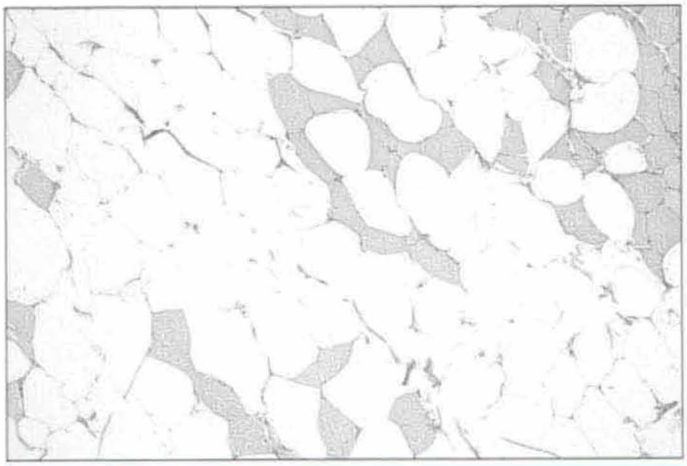

Figure 2.122 Invasion of skeletal muscle by mature adipose tissue typical of **infiltrative lipoma** in an adult dog. (Courtesy of BJ Cooper.)

- *Nerve sheath neoplasms* can arise from major nerve trunks within skeletal muscle but are typically localized to intramuscular nerves without infiltration of adjacent muscle.
- *Granular cell tumor* ("myoblastoma") is a tumor that can occur in the musculature of the tongue of dogs and cats and, rarely, within cardiac muscle. Granular cell tumor consists of closely packed round cells with prominent intracytoplasmic PAS-positive, amylase-resistant granules. Ultrastructurally, these granules consist of secondary lysosomes. Although previously thought to be of skeletal muscle origin, more recent studies have refuted this theory, and granular cell tumor appears to be most often a tumor of Schwann cell origin.
- *Malignant lymphoma* may occur within skeletal muscle, but whether this represents primary intramuscular tumor or local or metastatic spread is not always certain. Infiltration of skeletal muscle by neoplastic lymphocytes must be distinguished from lymphocytic infiltration due to immune-mediated myositis. Lymphoma cells are typically relatively homogeneous and often atypical, and surround myofibers and efface skeletal muscle architecture without obvious myofiber necrosis or "coring out" of myofibers (Fig. 2.120).

Secondary tumors of skeletal muscle

The *infiltrative variant of lipoma* arising within the subcutis is characterized by prominent intramuscular invasion (Figs 2.121, 2.122). This tumor, although locally invasive, does not metastasize, and wide surgical excision is generally curative. Infiltrative lipoma occurs most often in dogs and horses. Although uncommon, *subcutaneous mast cell tumors* can invade underlying skeletal muscle. *Other soft tissue sarcomas* arising in the subcutis, such as fibrosarcoma and hemangiopericytoma, can also extend into adjacent skeletal muscle. *Carcinomas,* such as squamous cell carcinoma, are often seen invading and destroying adjacent skeletal muscle.

In general, tumor metastasis to skeletal muscle is less common than metastasis to other organs. There is evidence to suggest that tumor cells in skeletal muscle microvasculature are destroyed at a higher rate than tumor cells within other organs, such as liver. Dermal melanoma of older gray horses often metastasizes to the muscle fascia, but less commonly involves the muscle itself. Skeletal muscle metastasis of canine prostatic carcinoma can result in foci of intramuscular fibroplasia, sometimes with associated chondroid differentiation, that can mimic focal or multifocal myositis ossificans (see Physical injuries

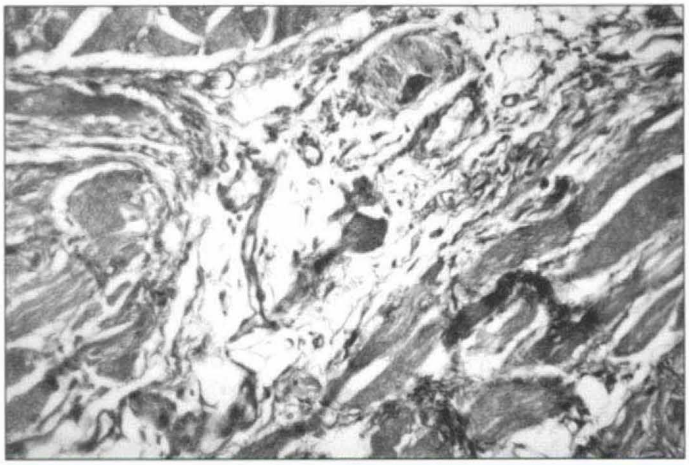

Figure 2.123 Irregular myofiber orientation with associated endomysial fibrosis in a **muscle pseudotumor** from a Great Dane dog. Masson trichrome stain.

of muscle). Diaphragmatic muscle is often a site of intraabdominal or intrathoracic tumor spread by "seeding."

Muscle pseudotumors

Muscle pseudotumors include a group of reactive lesions that can mimic neoplasia. *Localized myositis ossificans* and *musculoaponeurotic fibromatosis* qualify as muscle pseudotumors but are discussed in the sections Physical injuries of muscle and Tendons and aponeuroses, respectively. An *unusual muscle pseudotumor* has been seen in two dogs, both Great Danes, in which a localized swelling in the dorsal scapular area was found to be associated with skeletal muscle. These lesions were composed of irregularly arranged mature skeletal muscle fibers with marked cytoarchitectural alterations and associated endomysial fibrosis (Fig. 2.123). Muscle pseudotumors in man are most often thought to be *associated with muscle trauma*, and a similar etiology seems logical for animals.

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Tendons and aponeuroses

GENERAL CONSIDERATIONS

Tendons are derived from the same pool of embryonic mesenchymal cells as muscle fibers and it is very likely that although commitment occurs early, the differentiation of myoblastic and tenoblastic cells occurs relatively late. In some tendons, notably the suspensory ligaments of the limbs of the foal, the first wave of differentiation is as a muscle and only subsequently does further development produce a tendon. As might be suspected, the two end products have many structural similarities. *The basic structural units of tendons are bundles of collagen that cluster around a central elongated collection of tendon fibroblasts or tenocytes and capillaries.* Multiples of these units combine to form *fascicles* somewhat like primary muscle bundles, and fascicles in turn combine in clusters to form the *complete tendon*, which is ensheathed by a looser fibrous tissue called the *peritenon*.

Tendons are quite completely, though sparsely, supplied with blood vessels but in keeping with low nutritional requirements once the tendon is formed, relatively wide distances may separate adjacent parallel vascular channels in mature animals. Segments of long tendons may appear to be almost avascular at times, if flow volume is used as an indicator of vascularity.

At birth, tendons are cellular and vascular, as tenoblasts or tenocytes elaborate the orderly, synchronously kinked, parallel collagen bundles. The kinking provides a mechanism for absorbing stretch impact and provides an interlocking adhesive strength for adjacent fibers, which is enhanced by the presence of an amorphous, noncollagen ground substance that acts as a "glue." The physical properties of tendons are largely dependent on cross-linking of collagen molecules.

TENDON AGING AND INJURY

As tendons age, they change color from pearly white to yellow-tan and may acquire an even darker brown or red-gray center. Some of these color changes are related to repeated minor episodes of capillary hemorrhage that occur even in normal, unworked horses or cattle. The distinction between normal and abnormal is often a difficult one to make, but *it is normal (in the sense of usual and harmless) for tendons to undergo focal cartilaginous metaplasia.* Osseous metaplasia in tendons, however, is distinctly pathologic.

An important issue in the process of tendon aging and injury relates to predisposing lesions. Cartilaginous metaplasia, ischemia, and local fibroblastic proliferations have been regarded as predisposing changes, but they are frequently found in normal animals. It now seems that preparatory events need not be postulated to explain most of the lesions seen. *The predilection sites for tendon damage are predictably those with anatomic weakness or that receive disproportionate stretch forces.* The subsequent changes relate to stretching of tendon collagen and vessels.

When a tendon is stretched beyond load capacity, fibrils are very likely to be pulled out of kink register and even if no fibrils break, the tendon will be subsequently weaker at that point. Such *stretch lesions* are usually accompanied by ruptured collagen as well, although it may be only microscopically detectable. The sequence of events that follows involves the rupture of capillaries, release of fibrin, stimulation of tenocytes and/or peritenon cells to form myofibroblasts, and the formation of scar collagen that in many ways resembles the original tendon. In the acute stages, this process makes the tendon swollen, warm, and painful; in the chronic stage, the tendon is larger and longer than normal. Original tendon is uniquely constructed entirely of type I collagen. *When tendon repairs, the mature scar will no longer consist only of type I collagen since 20–30% of the replacement tissue will be type III collagen, a form of fiber less able to withstand stretch forces than type I.* It is likely that alterations in the proportions of the component glycosaminoglycans (e.g., hyaluronic acid, dermatan sulfate, chondroitin sulfate) occur following injury and during repair. The differences between a minor sprain and incomplete or even complete tendon rupture are differences in quantity not form. In larger lesions, there is a greater chance for tendon necrosis and sequestration, and a greater chance for the formation of a fibrin clot that is not longitudinally oriented by tension. This may lead to misalignment of the scar fibrils, adhesions to adjacent sheaths, and irregular contours of the tendon. Since myofibroblastic scar tissue is capable of contracting as it matures, the result of tendon repair is not necessarily a longer tendon but, inevitably, a weaker tendon.

PARASITIC DISEASES OF TENDONS AND APONEUROSES

Nematodes of the family Onchocercidae make connective tissue, vessel walls, or tendons of cattle and horses their preferred habitat. Most of these, and especially those that live in tendons, belong to the genus *Onchocerca*, although not all *Onchocerca* parasites have an affinity for tendons. Three different parasites infect domesticated cattle, *Onchocerca gibsoni, O. gutturosa,* and *O. lienalis,* but full investigation may eventually increase the number. Three different parasites, *O. gutturosa, O. reticulata,* and *O. cervicalis* infect horses. *Onchocerca gibsoni* infects cattle in Africa, Asia, and Australia. *Onchocerca gutturosa* occurs in cattle and horses in North America, Africa, Australia, and Europe. *Onchocerca lienalis* is considered to be a separate species by some and synonymous with *O. gutturosa* by others; it is not as widespread as is *O. gutturosa,* but is seen in cattle in Australia and North America. *Onchocerca reticulata* infects horses in Europe and Asia, and *O. cervicalis* infects horses worldwide. The prevalence of onchocerciasis can be very high, ranging from 20–100% of the population in endemic areas, but *infection by these connective tissue parasites has been greatly reduced by widespread use of ivermectin as an anthelmintic.*

The adult worms, less than 1.0 mm thick and 50–80 cm long for the female, much shorter for the male, live in tendons, tendon sheaths, or connective tissues of the brisket or abdominal wall from which site they liberate *microfilariae* over long periods. The larvae make their way to skin where they are picked up by a blood-sucking parasite in which the next phase of development takes place.

Onchocerca gibsoni characteristically provokes and inhabits a "worm nodule" or "worm nest" on the *brisket or external surfaces of the hindlimbs* that may reach 3 cm in diameter. These fibrous lesions are important in the meat industry because the worms must be manually removed and this is time consuming if several dozen are present. The nodules may be palpable or moveable in the skin or they may be fixed to the dermis or ribs. Those in the flank are usually beneath the fascia lata and are not externally palpable. The adult worms inhabit fine tunnels in the discrete nodules of dense fibrous tissue where they form a tangled mass in a milky fluid. Female worms are just grossly visible but the smaller males are not. Sometimes old hemorrhages, or mineralized remnants of worms, are contained in the larger nodules, and microscopically many larvae may also be seen in the capsule as they exit, perhaps via lymphatic vessels. Microfilariae are rarely seen in lymph nodes or blood vessels, but these routes may be used to reach the skin where they collect in large numbers without stimulating a reaction. The intermediate blood-sucking host is probably a simulid or a culicoid insect.

Onchocerca gutturosa is most frequently located on the surface of the *ligamentum nuchae* of cattle adjacent to the thoracic vertebral spines and less frequently on the scapula, humerus, or femur. It is sometimes found in the horse. The adult worms, which are found in pairs, do not stimulate the formation of a nodule as is the case for *O. gibsoni*, but lie in loose connective tissue. They apparently cause no disease or reaction and in spite of their wide distribution around the world they are rarely dissected out or even detected. The intermediate hosts are not all known, but simulids can transfer the infestation.

Onchocerca lienalis lies in delicate tunnels in the gastrosplenic ligament and in the splenic capsule of cattle.

Adult worms of *Onchocerca cervicalis* live between the fibers of the ligamentum nuchae over the shoulder or neck of the horse or in loose connective tissues nearby. A second very similar parasite, *O. reticulata*, resides at a much lower level of frequency in the tissue around tendon sheaths adjacent to the carpus or suspensory ligaments or at the fetlock where a low-grade tissue reaction is initiated by the presence of adult worms. When the worms die, they mineralize and are engulfed by a poorly defined, dense, fibrous reaction. The infestation usually goes undetected and most horses are apparently asymptomatic. The long threadlike female worm is less than 1 mm thick but 50–70 cm long. Larvae are hatched from eggs prior to their release from the uterus, and they make their way through connective tissue to the skin where they presumably travel in the dermal lymph vessels. Larvae (microfilariae), which are about 4 μm wide and 200–250 μm long, tend to aggregate in certain skin regions, particularly the skin of the ventral abdominal midline, the inner thighs, and the eyelids. Living microfilariae seem to stimulate no reaction whatever, but dead larvae are capable of stimulating a response that involves lymphocytes and eosinophils. These local nodular responses may be seen in skin accompanied by pruritus, alopecia, and scaliness, and they may be seen histologically in areas where larvae are particularly numerous. Living larvae can be quite readily detected as loosely curled structures just under the epidermis and adjacent to adnexal skin structures in histological sections. Microfilariae of *O. cervicalis* and *O. reticularis* are transported to another horse by blood-sucking insects in which an obligatory stage of development occurs. Several different insects may be suitable hosts, but only *Culicoides* midges and mosquitoes of the *Anopheles* species have been confirmed in the role.

FIBROMATOUS DISORDERS OF TENDONS AND APONEUROSES

A number of degenerative disorders resulting in proliferation of fibrous, cartilaginous, and osseous tissue within tendons and aponeuroses are described in man and animals. Some such disorders are inherited, whereas others are acquired. For the most part, little is known regarding the pathogenesis of these processes.

Fibromatoses *are defined as progressive, infiltrative, nonmetastasizing fibroblastic lesions.* A variety of such disorders are recognized in humans, and a similar disorder occurs in the horse. These lesions, although considered nonneoplastic by most investigators, can exhibit marked local infiltration and distortion of affected muscle.

Musculoaponeurotic fibromatosis (desmoid tumor) of the horse

A peculiar progressive fibrosing disorder, **musculoaponeurotic fibromatosis (desmoid tumor)**, occurs in the horse. Although most cases involve the *pectoral region*, other sites are possible. Affected horses develop progressive distortion and induration of affected musculature due to proliferation of fibroblasts, myofibroblasts, and subsequent fibrosis dissecting between muscle fibers and fascicles that is often accompanied by foci of lymphocytic infiltration (Fig. 2.124). Although wide surgical excision may be curative, in most cases the extent of the lesion at diagnosis precludes effective surgical intervention. Studies of a small number of horses have revealed fluid-filled pockets of apparently sterile inflammation deep within the lesion, suggesting that this disorder in horses may be a peculiar response to trauma such as from an injection or ruptured bursa.

Fibrodysplasia ossificans progressiva (FOP)

Fibrodysplasia ossificans progressiva *(FOP) is a progressive fibrosing and ossifying lesion of tendons and muscle-associated aponeuroses that occurs in humans and cats.* This disorder was previously referred to as myositis ossificans progressiva, which is incorrect, as the disorder involves muscle-associated connective tissue and is not a primary myopathy (see Physical injuries of muscle). Fibrodysplasia ossificans progressiva in man is a devastating disease of children and young adults. Progressive fibrosis and ossification of muscle-associated connective tissue, including tendons and fascia, result in eventual incapacitation. This disorder is inherited as a dominant trait.

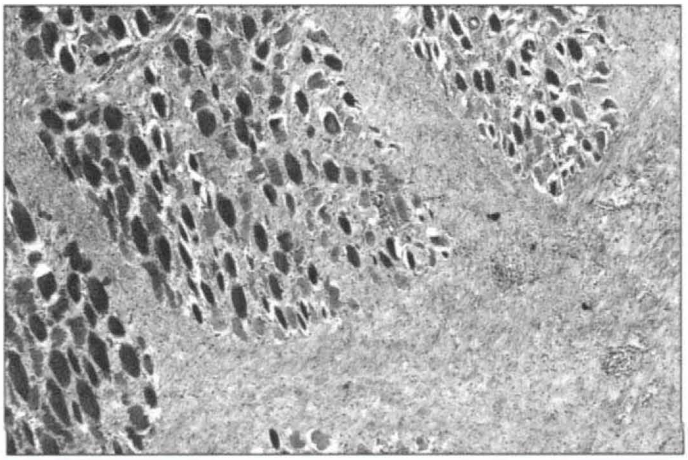

Figure 2.124 Perimysial and endomysial connective tissue dissecting between muscle bundles and myofibers in a horse with **musculoaponeurotic fibromatosis**. Foci of lymphocytes are also visible within the perimysial connective tissue. Masson trichrome stain. (Courtesy of BJ Cooper.)

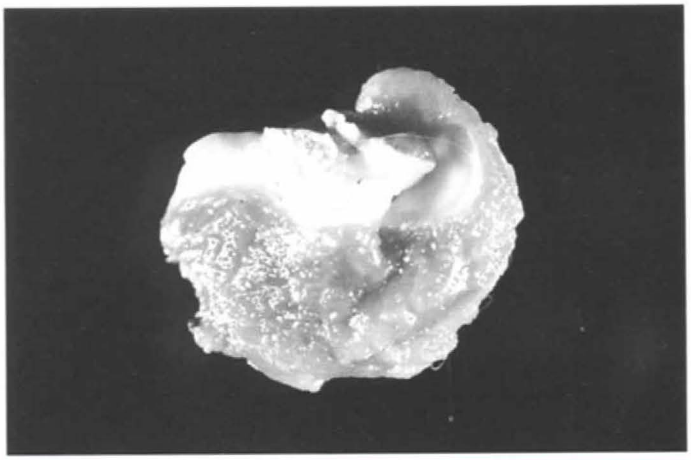

Figure 2.125 Marked thickening of the gastrocnemius muscle fascia of a young adult cat with fibrodysplasia ossificans progressiva. (Courtesy of L. Fuhrer.)

A similar disorder occurs sporadically in young to young-adult *cats*. Age at diagnosis in the cat has ranged from 10 months to 6 years. To date, inheritance of FOP has not been confirmed in the cat. Clinical signs include progressive stiffness of gait, decreased joint mobility, increased bulk and firmness of muscle, and pain upon handling. Radiography and gross pathology reveal *characteristic thickening with mineralization and ossification of muscle fascia* (Fig. 2.125). The histopathologic features are of fibroblastic proliferation within fascia with subsequent cartilage and bone formation. Early lesions may have an associated mild lymphoid infiltrate.

An inherited form of what was described as generalized myositis ossificans has been described in young *pigs*. The lesion in these pigs, however, was a proliferation of periosteal bone involving vertebrae, ribs, and tarsal bones, suggesting that this may be a primary bone disorder.

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3

Nervous system

M. Grant Maxie and Sameh Youssef

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CYTOPATHOLOGY OF NERVOUS TISSUE

Nervous tissue is highly specialized and structurally complex, and neuropathology has always tended to be set apart as an arcane specialist area, to be entered only by a select few. However, the veterinary pathologist cannot escape having to deal with it frequently, and we attempt in this section to provide a contemporary basis for understanding veterinary neuropathology.

The principal cells of the central nervous system (CNS) are *neurons* and *glia*, plus cells of the meninges and blood vessels. *Macroglia* (astrocytes, oligodendrocytes, ependymal cells) are of neuroectodermal origin; *microglia* are derived from bone marrow.

Neuron

The **neuron** *is the fundamental cell of the nervous system, and ultimately all neurologic disease must involve functional disturbances in neurons.* In conventional histopathology, the term "neuron" refers to the cell body of the nerve cell that often is only a small part of the total cell volume. For the purposes of comprehending pathogenetic mechanisms, it is important to remember always that the neuron comprises both the *cell body* ("soma" or "perikaryon") and the cell processes, in particular the *axon*. The whole cell constitutes a structure concerned with the generation, conduction, and transmission of impulses, and in some cases a single cell performs this task over a very long distance. For example, in a lumbar dorsal root ganglion of a horse, the cell body of a sensory neuron may project a process distally to the extremity of the hind foot and centrally to the caudal brain stem, making it without doubt the largest cell in the body. If the soma were enlarged to the size of an orange, the processes would have the dimensions of a garden hose and would be over 20 km in length. Pathologic reactions within one cell may therefore be separated by considerable distance. Also, neurons function in hierarchical chains organized into anatomic systems.

The soma is the metabolic factory for the whole cell and the great bulk of synthetic and degradative operations take place there. The axons are serviced throughout their length by a bidirectional transport system that moves components both away from and towards the soma (anterograde and retrograde transport respectively). The transport mechanisms are fuelled by the consumption of energy along the course of the axon. Many components are moved in the anterograde direction within 25 nm vesicles by a "fast" transport system at a rate of 20–30 mm/h. The motor for this system, intimately associated with the neurotubules, is an ATPase called *kinesin*. Larger vesicles of about 500 nm are transported retrograde by this fast pathway, and the translocator is an isoform of dynein, the ATPase which powers cilia and flagella in other types of cell. Mitochondria are also moved by fast transport, but at a somewhat slower rate. Neurofilaments, neurotubules, and some soluble proteins move by slow transport in the anterograde direction only, at a rate of about 1–3 mm/day, and are degraded when they reach the terminus. Should the operations of the soma or the transport systems be impaired, the health of the axon will suffer and, appropriately, in most instances the largest and longest axons are most vulnerable. Equally, *when a neuron is fatally damaged the whole cell dies*, and the consequent changes will spread over the whole extent of its domain. However, long lengths of the axon may degenerate without compromising the viability of the remainder of the cell and without inducing dramatic morphologic change in the soma, and there is often scope for adequate regeneration of the lost axonal extremity. These issues will be addressed more fully below, under *axonopathy*.

The vitality of individual neurons is sustained by their active relationship with other neurons, or other types of cells with which they interact. If a neuron dies, surviving neurons with which it has synaptic connections may regress due to lack of activation, undergo atrophy, and eventually die. This process is called *transynaptic degeneration* and it will progress along specific anatomic pathways. It is useful also to realize that neurons are extremely diverse pharmacologically and biochemically; this is well illustrated by the highly selective and regionalized effects of different toxins and metabolic disturbances. The selective effects of tetanus and botulinum toxins are good examples.

During organogenesis, vast numbers of neuroblasts proliferate and immature neurons migrate to their final destinations. As large numbers of developing neurons are superfluous, there is a need to select amongst them and eliminate the excess by apoptosis. Discrimination between those that will survive and those that are effete will depend on the transfer of trophic factors between cells including from glia to neurons. Around the time of birth, and for variable periods afterwards, the latter stages of the process may still be extant, particularly in the cerebellar cortex and paravertebral ganglia. It is probable that the signals between cells during growth and physiological activity are the same as those which if overloaded may lead to neuronal injury. The margin of safety between proper levels of transmitter and too little or too much is very narrow.

The mature neuron is a postmitotic cell and there is a net loss throughout adult life, especially during senescence. Thus in a thorough scan of a histologic section from a senile animal, it might be expected that a few degenerate neurons will be found, even when fixation artifacts are minimal. There is however continuous postnatal generation of neurons in some parts of the brain and in at least some species. In species so far examined, neuroblasts generated in the dentate gyrus of the hippocampus and from the progenitor cells of the subependymal zone between the lateral ventricle and the caudate continue to migrate postnatally to the olfactory bulb. These migrations do not depend on guidance from radial glia as in the embryonic brain. Rather the postnatal neuroblasts migrate in a chain designated as the "rostral migratory stream" strictly delineated by rows of astrocytes. There is evidence that new neurons are generated in the olfactory bulb. Axonotomy procedures that sever the pituitary stalk in the rat and some other mammalian species are known to be followed by re-establishment of functional hypothalamic–hypophyseal connections within a few weeks. Cells with neuron-like characters appear to migrate through the neuropil of the median eminence to congregate on its surface. The functional significance of these migrations and the dynamics of these populations are obscure. It is assumed that the olfactory migrants and their proliferation in situ in the bulb may have a bearing on the development of olfactory neuroblastomas.

Degenerative changes of the nerve cell body

Nuclear margination – the neuronal nucleus is usually single and centrally located (Fig. 3.1), and its *margination can be taken to indicate*

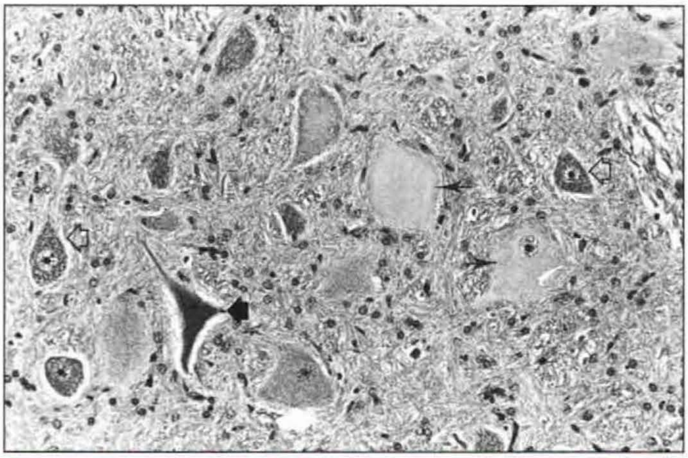

Figure 3.1 Red nucleus in swayback in a goat. Normal neurons (open arrows); **central chromatolysis** (arrows); shrunken, necrotic neuron (closed arrow).

nonspecific degeneration, especially when combined with loss of staining affinity. However, an eccentric nucleus can be normal in some groups of smaller neurons, and is often present in the large cells of the periventricular gray matter such as the mesencephalic nucleus of the trigeminal nerve and the olives. These cells also tend to have a "chromatolytic" appearance.

Chromatolysis is a change in appearance of the soma brought about by the *dispersal of the rough endoplasmic reticulum* (Nissl granules), and is subclassified as *central* or *peripheral* according to its locus within the cell body. Its assessment depends upon an appreciation of the prominence and distribution of Nissl granules seen normally at the particular location. There are no artifactual changes that mimic chromatolysis and, provided it is accurately identified, it can always be regarded as a lesion. A number of special stains, such as cresyl violet, will demonstrate it better than routine hematoxylin and eosin.

Central chromatolysis is best appreciated in large neurons of some of the brain stem nuclei, in the spinal motor neurons, and peripheral ganglia. The chromatolytic cells are swollen and rounded, rather than having the normal angulated appearance, and the nucleus becomes eccentric. Nissl granules clear from the central region of the cell body, leaving this zone with a smooth ground-glass appearance (Fig. 3.1). Central chromatolysis occurs in a number of pathologic situations. It is often seen in bulbo-spinal motor neurons and sensory neurons whose peripherally projecting axons are injured, and especially when the injury occurs close to the cell body. The reaction follows such injury quickly, beginning within 24 hours, and becoming maximal in 1–3 weeks. When the dynamics of axonal flow are remembered, it can be appreciated that such an event will have a major feedback on the soma. In effect, the cell must rearrange its metabolism to adapt to its changed circumstances, and organize for a regenerative effort, involving the reconstruction of an axonal segment greater in volume perhaps than its surviving volume. In this context, *central chromatolysis has been termed the* **axon reaction**, *and represents an anabolic adaptive response*. In this reaction, the nucleus becomes extremely eccentric, and develops a prominent nucleolus and a basophilic cap of RNA on its cytoplasmic aspect. The Nissl substance disperses, and the cytoplasm becomes rich in free ribosomes, lysosomes, and mitochondria. There may also be some increase in the number of neurofilaments. All these changes reflect a shift in metabolic activity, with a switch towards increased synthesis of structural cellular proteins, and a marked decline in synthesis of transmitters. With completion of successful axonal regeneration, the chromatolytic soma returns to normal, sometimes passing through a densely basophilic phase in which it is packed with Nissl granules.

In some circumstances following axonal injury, central chromatolysis may proceed to cell death or permanent atrophy, and this is usually the case in neurons whose axons project entirely within the central nervous system (CNS). *In general, the closer to the cell body the axonal lesion, the more likely is the cell to die.* Those cells destined to die have swollen achromatic cytoplasm depleted of organelles.

Central chromatolysis is also induced in more overtly neuronopathic conditions, most notably the numerous motor neuron degenerations described in various species, and it is a feature of the pathology of perinatal copper deficiency in the sheep and goat (Fig. 3.1). Similarly, it is a striking feature in autonomic ganglia in equine and feline dysautonomias. In all such cases, the affected cells often proceed to necrosis and dissolution (Gudden's atrophy). In many of these degenerative neuronopathies, the cytoplasmic alteration is due to massive accumulation of neurofilaments in the soma (see Progressive motor neuron diseases, p. 372). Nuclear margination is not as marked as in the axon reaction, and the prominent nucleolus and nuclear cap are not evident. These differences distinguish a regressive state from a regenerative one.

Peripheral chromatolysis indicates clearing of the periphery of the soma, with Nissl granules persisting around the nucleus. This change is generally associated with slight cellular shrinkage rather than swelling. It is a nonspecific lesion and can often be regarded as an early stage en route to necrosis.

In both forms of chromatolysis, microglia and astrocytes may proliferate and cover large expanses of the cell surface, thereby separating terminal boutons from the neuronal surface.

Neuronal atrophy – loss of cytoplasmic bulk and reduction in size (Fig. 3.2A) might be expected in situations of permanent loss of synaptic connections (see transynaptic degeneration above), as when central axons undergo Wallerian degeneration but fail to regenerate.

Ischemic necrosis – in this *characteristic acute degenerative change*, the cytoplasm of the neuronal soma becomes shrunken and distinctly acidophilic (Fig. 3.2B), and the nucleus progresses through pyknosis and rhexis to lysis, leaving the coagulated cytoplasmic remnants to undergo liquefaction without undergoing phagocytosis. The remnant "ghosts" may persist for several days. *The reaction is not confined to ischemia*, and may be seen in the cerebral cortex in hypoxia, hypoglycemia, the encephalopathy of thiamine deficiency, and in some chemical intoxications, such as organomercurialism and indirect salt poisoning. Following seizures, it may frequently be found in the dentate gyrus of the hippocampus and the cerebellar Purkinje cells. There are usually obvious proplastic changes in astrocytes and capillary endothelia in the vicinity of the affected neurons.

This raises the concept of **excitotoxicity**, in which neuronal degeneration and death are considered to result from excessive stimulus by an excitatory neurotransmitter. This phenomenon is thought to operate particularly in those neuronal systems utilizing glutamate as a transmitter, such as the cells in the hippocampal areas mentioned above. Paradoxically, glutamate is potentially highly toxic to neurons, and normally is rapidly cleared by the glia following its release. Excessive release or defective clearance of glutamate from the environment of postsynaptic neurons predisposes to excitotoxicity, and

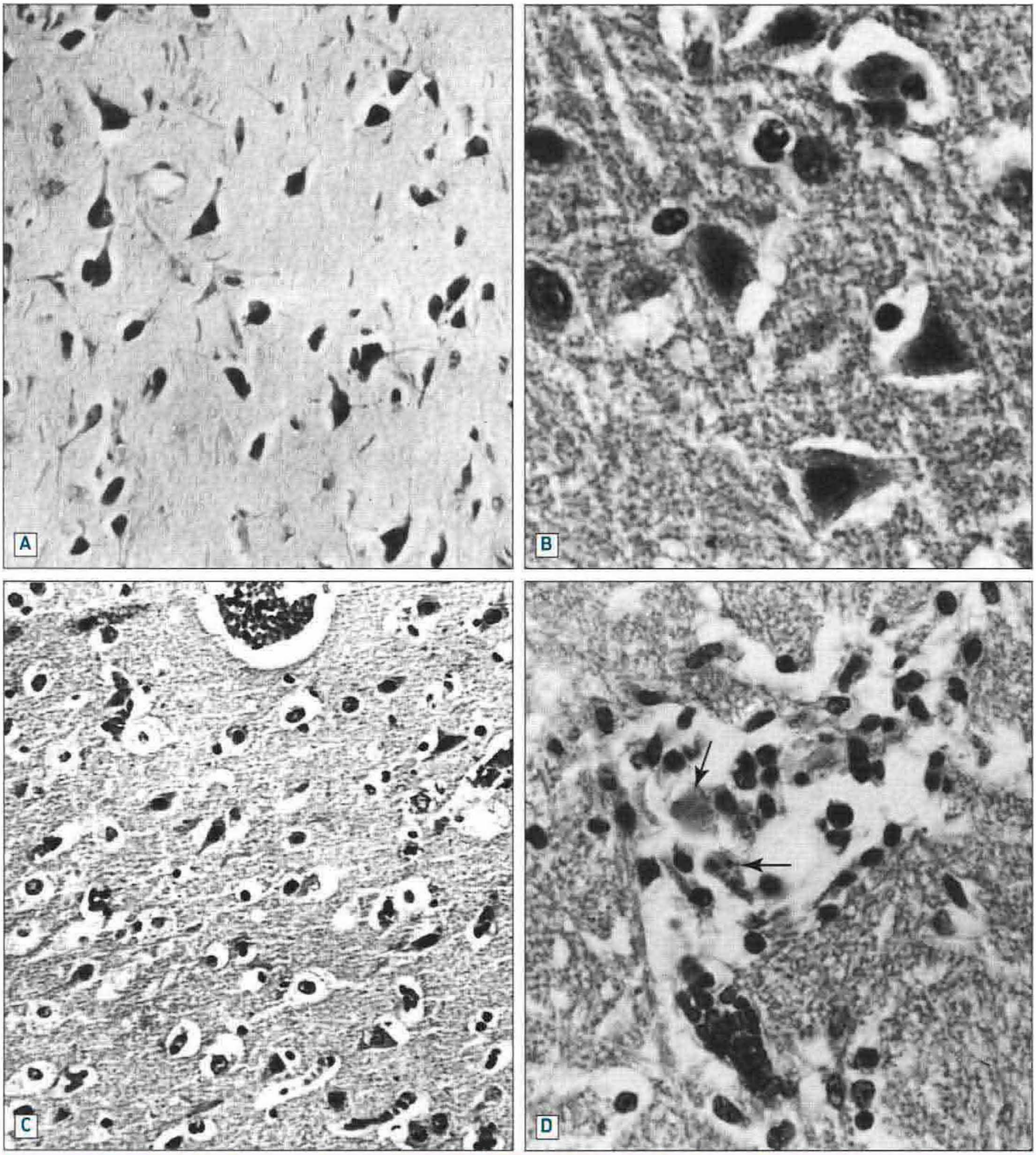

Figure 3.2 A. Chronic nerve cell degeneration. B. Early ischemic nerve cell degeneration. Note cell shrinkage with condensed cytoplasm. **C. Autolyzing cerebral cortex** easily misinterpreted as liquefactive necrosis. **D. Neuronophagic nodule** in equine encephalitis; fragmented neuron (arrows).

such circumstances are provided by hypoxia and hypoglycemia. The pathogenesis is thought to involve ionic overloading of the cell, acute swelling, and then cell death with cytoplasmic coagulation and eosinophilia.

Neuronal necrosis may occasionally be expressed as cytoplasmic shrinkage and basophilia with nuclear dissolution (Fig. 3.1). Shrunken basophilic neurons with normal nuclei ("*dark neurons*") are generally taken to be artifactual, and are often numerous.

Liquefactive necrosis is often at risk of being misinterpreted as autolytic change and fixation artifact, particularly shrinkage artifact (Fig. 3.2C). A lesion can sometimes be distinguished from an artifact by the presence or absence of significant alterations in other tissue cells, for instance the swelling of capillary endothelium or indications of glial proliferation.

Necrosis with neuronophagia – in many viral infections, the death of neurons provokes the gathering of phagocytes around the cell body (**satellitosis**), and removal by them of the debris, forming **neuronophagic nodules** (Fig. 3.2D). This is usually a response of the microglia, although satellite perineuronal oligodendrocytes may also proliferate in response to neuronal injury. Neuronophagia may also be seen in metabolic or toxigenic neuronal degenerations, but is generally not as extensive as in viral infection.

Vacuolar degeneration – neurons in the early stages of acute injury inflicted by viruses, toxins, or metabolic derangements, such as excitotoxicity, may develop numerous small cytoplasmic vacuoles, usually reflecting *mitochondrial swelling*. However, *artifactual peripheral vacuolation is very common*, giving the periphery of the cytoplasm a foamy web-like appearance. This is particularly so in the cerebellar Purkinje cells and large neurons of some of the brain stem nuclei.

Large neuronal vacuoles, few in number per cell, are occasionally observed in otherwise normal brains, and may be seen in the red and oculomotor nuclei of aged cattle and sheep. When they occur at high frequency in the neurons of the medulla and midbrain, they are virtually pathognomonic of *scrapie* in sheep and goats (Fig. 3.3). Widespread neuronal vacuolation with single or multiple large vacuoles, unexplained as to pathogenesis, is occasionally seen in dogs and cattle with progressive neurological dysfunction.

Vacuolar change is strikingly evident in those *lysosomal storage diseases* in which the stored material is extracted during processing, or is unstained by the routine methods. In these situations, the neuron can become dramatically bloated by the accumulation of myriad secondary lysosomes that displace the normal organelles and distend the soma with a foamy mass of apparently empty vacuoles. In most cases, the storage process involves other cell types inside and outside the nervous system, and is accompanied by additional neuropathological manifestations (see Storage diseases).

Storage of pigments and other materials – neurons may accumulate large quantities of **ceroid/lipofuscin** or other pigments, either as a consequence of aging, or in storage disorders involving such substances. The pathogenesis of the *ceroid/lipofuscinoses* remains unclear but, as is discussed elsewhere, there is a genetic basis in some instances, while in others unspecified environmental factors may be involved. The complex storage material accumulates as granules in a manner analogous to the other lysosomal storage diseases, although a clearly defined limiting membrane is not usually apparent ultrastructurally. When the process is intense, rusty-brown discoloration of the gray matter and ganglia may be evident grossly.

Neuromelanins may also accumulate excessively in those midbrain nuclei where they are usually present in modest amounts, and extensive neuromelanosis can occur in sheep chronically poisoned by *Phalaris* sp. In extreme cases, the gray matter and ganglia may have macroscopic green discoloration.

Siderotic pigmentation of neurons, in which the cells become encrusted with basophilic complexes of iron, calcium, and phosphorus, may be found near contusions and hemorrhages. It is not known, however, whether the iron is derived from the hemoglobin or from intracellular iron-containing respiratory enzymes. The neurons may be otherwise normal, or their degeneration may produce small lakes of basophilic deposit. The latter are common in the neonatal cerebellar cortex.

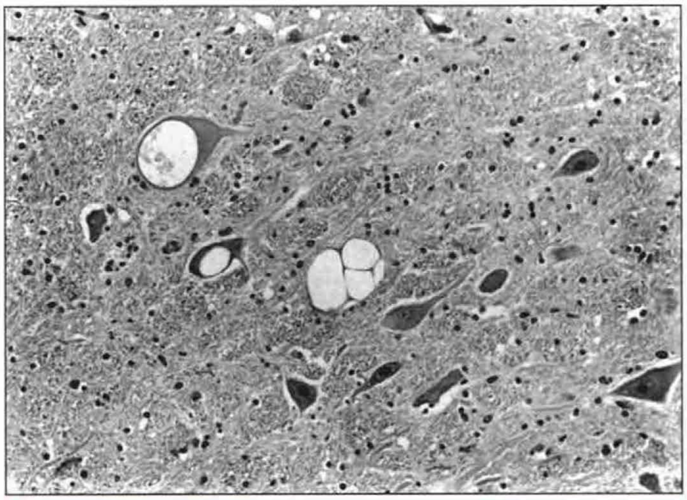

Figure 3.3 Neuronal vacuolation in scrapie in a sheep. (Courtesy of RF Slocombe.)

Viral inclusion bodies – the best known is the *Negri body* of rabies, which is eosinophilic and intracytoplasmic. Herpesvirus inclusions are characteristically intranuclear, while those of the paramyxoviruses such as *Canine distemper virus* may be intracytoplasmic or intranuclear. The use of specific immunostains has greatly facilitated the identification of viral inclusions.

Non-viral eosinophilic cytoplasmic inclusion bodies also occur. Sometimes they are an incidental finding in otherwise normal brains at sporadic locations; in cats, they can sometimes be found in the pyramidal cells of the hippocampus and lateral geniculate nuclei.

More specific inclusions have been described in people and animals in neuronal degenerative diseases. In humans, ultrastructural differences define several types of inclusion: **Hirano**, **Pick**, **Lewy**, **Lafora**, and **Bunina bodies**. *Hirano-like bodies*, appearing histologically as elongated eosinophilic inclusions and, ultrastructurally, as masses of beaded filaments, are reported in horses and dogs, together with other structures having some of the features of Bunina bodies. Further definition of these types of inclusions in animals is required, and their pathogenetic significance is not understood; they are a feature of equine motor neuron disease (Fig. 3.4). *Bunina bodies* are small eosinophilic inclusions of 2–5 μm, sometimes in small clusters or chains that characterize human *amyotrophic lateral sclerosis*; similar inclusions are occasionally observed in degenerative and inflammatory neurological disease in animals.

Lafora bodies are occasionally observed in neurological disease, but most frequently are incidental findings in aged animals. They are basophilic to amphophilic inclusions that are strongly PAS positive and metachromatic, 5–20 μm in size, intracytoplasmic or in processes or free in the neuropil (Fig. 3.5). They represent an abnormality of carbohydrate metabolism producing glucose polymers called *polyglucosans*. In the very rare *Lafora disease* (see Storage diseases), they occur in massive numbers throughout the brain within the neuronal soma, the dendrites, and less commonly the axons; they are associated with severe myoclonus epilepsy.

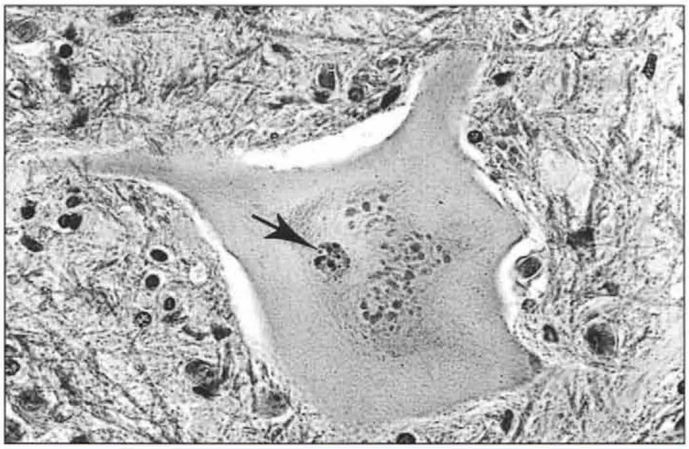

Figure 3.4 Spinal motor neuron in **motor neuron disease** in a horse. Chromatolysis, nuclear dissolution (arrow) and cytoplasmic inclusions.

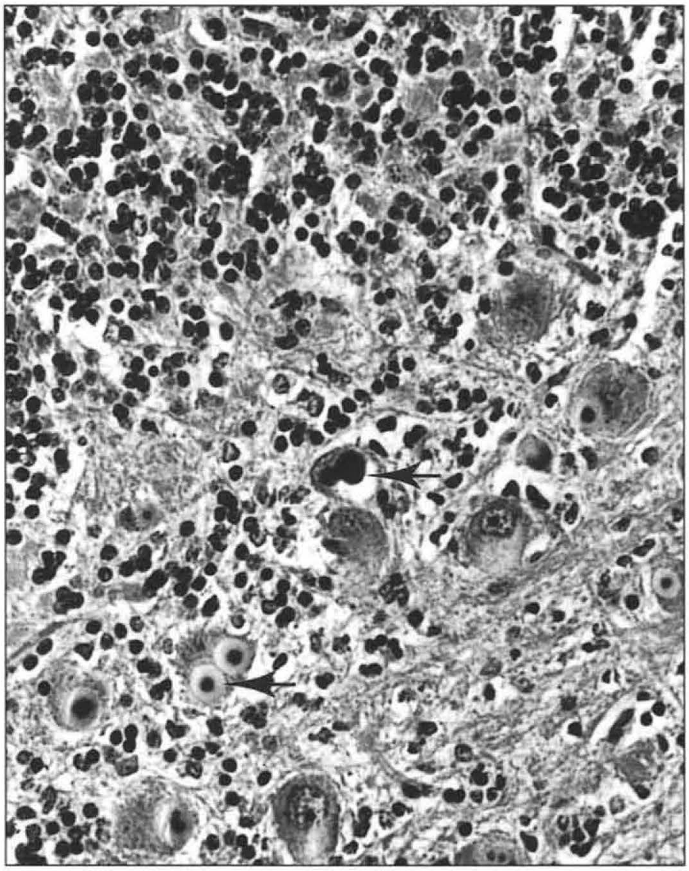

Figure 3.5 **Lafora bodies** in Purkinje cells (arrows) in a dog.

Mucocyte (Buscaino body) is the term applied to glassy, pale gray-blue bodies of variable size and shape, roughly the same size as neurons, seen on routinely processed and stained sections of brains. These are usually dispersed in the neuropil, typically in major white matter tracts and are considered by most to be an *artifact* of fixation and processing. They may be PAS positive, but are not always so, and there are rare reports of the accumulation of mucocytes associated with disease.

Axon

The axon acts as the solitary efferent extension of all neurons except those sensory neurons in the spinal ganglia whose peripherally directed myelinated axons function as dendrites, in that they conduct impulses towards the cell body. Axons may branch extensively towards their terminations.

The first part of the axon is called the *initial segment* and it has distinctive ultrastructure, related to its being the site of membrane ion channels critical for the initiation of a propagated action potential. The axoplasm contains mitochondria, endosomes, intermediate filaments (neurofilaments), microtubules (neurotubules), and secretory vesicles or granules containing neurotransmitters appropriate for the particular cell. The axoplasm will also contain soluble macromolecules such as enzymes.

Axons are sustained by their parent cell bodies and by the cells that invest them along their course. The axoplasm is devoid of ribosomes, and axoplasmic proteins are provided by the soma. Similarly, the lysosomal apparatus is limited in the axon, in terms of digestive capacity, and many obsolete materials and organelles are returned by retrograde transport to the cell body for complete degradation. The role of neurotubules in transport mechanisms has been mentioned above, and is critically important for the maintenance of the axon. Axonal diameter is distinctly reduced at the nodes of Ranvier, and these "strictures" are probably the reason that paranodal swellings filled with transported vesicles and organelles are a feature of many axonopathies in which transport has been disturbed.

Neurofilaments are responsible for the maintenance of axonal size and geometry, and are part of the generic cytoskeletal intermediate filament family. The proteolytic destruction of neurofilaments, triggered by the influx of calcium ions, is a common pathway for the collapse and disintegration of damaged axons. Larger axons are invested in a segmental manner by a *myelin sheath*, interrupted at the *nodes of Ranvier,* and penetrated at intervals by incisures.

All this sophistication is not resolved in routine light microscopic examination of paraffin-embedded tissues. The course of axons can be highlighted by the use of silver staining techniques, which emphasize the shrinkage artifact and distortion produced by routine tissue preparation. Nonetheless, with experience, many lesions in paraffin-embedded tissue can be interpreted but, particularly for peripheral nerves, plastic embedding of specimens is far preferable.

Types of **axonopathy** have been grouped according to whether they begin in the *proximal* or the *distal* portion of the axon, and whether they involve central or peripheral axons or both. Thus, for example, one may distinguish central and peripheral distal axonopathy, or central proximal axonopathy. The principal categories of axonopathy can now be discussed in broad terms.

Wallerian degeneration *denotes the changes that follow acute focal injury to a myelinated axon*, such that distal to the injury it becomes nonviable. When the soma is uninjured, there is potential for regeneration and, in peripheral nerves, this may be complete. Should the acute injury involve death of the cell body, then Wallerian degeneration of the axon will proceed as part of the dissolution of the entire neuron. The classical scenario for Wallerian degeneration is *acute focal mechanical injury in a peripheral nerve*, which effectively transects axoplasmic flow. Within 24 hours, the distal segment begins to degenerate fairly evenly along its length. Focal eosinophilic swellings occur, often containing accumulations of degenerate organelles, and

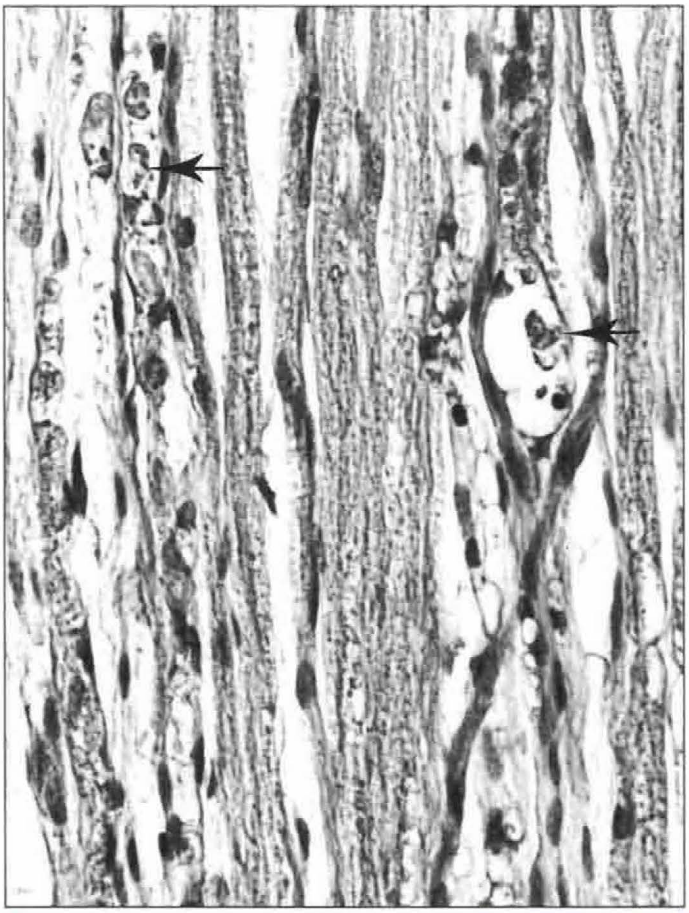

Figure 3.6 Wallerian degeneration in peripheral nerve (arrows) in copper deficiency in a goat.

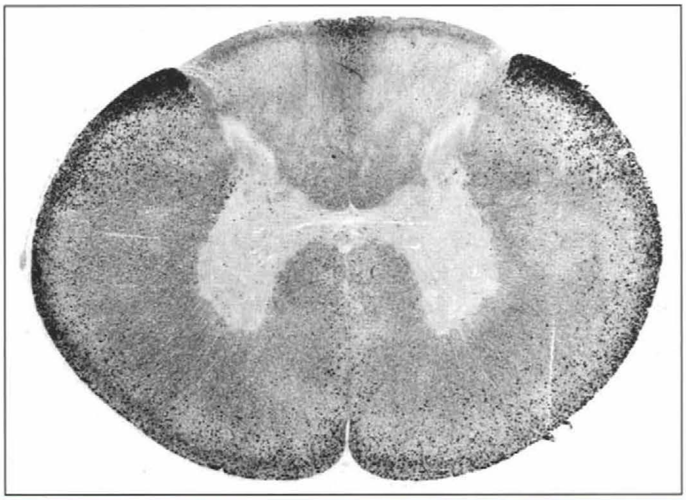

Figure 3.7 Degeneration of myelin in spinal cord in a pig with copper deficiency. Stained with Marchi. Note selective involvement of tracts, namely the fasciculus gracilis and dorsal and ventral spinocerebellar tracts. (Courtesy of MD McGavin.)

then fragmentation becomes evident by 48 hours or so. Schwann cells respond rapidly as myelin sheaths are made redundant by disintegration of the axon. Initially, myelin retracts from the nodes and then forms into *ellipsoids*, regarded originally as "*digestion chambers*" for the enzymic lysis of the axonal fragments (Fig. 3.6). The myelin itself condenses into aggregates and fragments and, together with remaining axonal debris, becomes the target of invading macrophages. Prior to this, the complex myelin lipids are progressively transformed into simpler neutral lipids over a period of 10–20 days, and this is reflected in the reaction to specific lipophilic stains. Macrophages enter the sheath and soon become filled with sudanophilic droplets. These lipid-laden cells may persist in the interstitium for many weeks. Some of the myelin debris is phagocytosed by Schwann cells themselves, and they begin to proliferate. As the debris is cleared away, proliferating Schwann cells form bands along the former course of the myelinated axons (*Bungner's bands*). Similar Wallerian changes occur proximal to the site of injury over several internodes. If conditions are favorable at the site of injury, sprouts from the axonal stump will find their way along the Schwann cell bands and be directed to their correct destinations. In most instances, the *growing axonal sprouts* advance at a rate of 2–4 mm/day, and the new axon will be invested by Schwann cell cytoplasm. Sprouting from individual axons is initially multiple and, by an unknown mechanism, one sprout is selected for the completion of regeneration. Thus a new axon may arise from a different soma than did the original. *The regenerated axon is remyelinated by Schwann cells*, although the new sheath is thinner than the original and nodal length variable and shorter. Should axonal regeneration be prevented, Schwann cell bands persist, and endoneurial fibrosis usually develops. *Abortive regeneration can lead to a tangled clump of neurites, Schwann cells, and fibrocytes at the injury site.* This will happen after transection if too great a distance separates the severed ends of the nerve fibers.

The effectiveness of regeneration in peripheral nerves is related to the comparatively simple axon/Schwann cell relationship, the presence of a basal lamina tube around each myelinated axon, and the replicative ability and metabolic resilience of the Schwann cells.

These conditions do not apply in the **CNS**, where the oligodendrocyte/axon relationship is far more complex – the oligodendrocyte is a relatively poorly regenerative cell type, there is no basal lamina scaffold, and the debris from central myelin is thought to inhibit axonal sprouting. The initial regressive changes of Wallerian degeneration are similar to those described for the peripheral nerves, although they proceed over a longer time-course. This is because the involvement of hematogenous macrophages is slower and less intense in the CNS, and activated microglial cells undertake most of the work. Axonal sprouting and some remyelination can occur. However, the poverty of the regenerative response results mostly in the *permanent disappearance of the axons, myelin, and oligodendrocyte cell bodies*. Some of the myelin debris may be phagocytosed by reactive astrocytes, and their processes extend to fill the vacancy, creating a ramifying network of astroglial scar tissue. Wallerian degeneration in the CNS is most commonly seen in the spinal cord, the optic tract, and the brain stem. Probably the best-known association is with the focal compressive myelopathies in the horse and dog (the wobbler syndromes).

During the active degenerative phase, recently phagocytosed and partially digested myelin debris may be distinguished in paraffin sections by the use of the Luxol fast blue/periodic acid Schiff stain. Degenerate myelin is well visualized by the Marchi technique (Fig. 3.7), or by immunostaining of myelin basic protein (MBP). End-stage plaques of astrogliosis may be demonstrated by traditional gliophilic stains or by the use of immunostaining for glial fibrillary acidic protein (GFAP – intermediate filament protein).

The destruction of myelin in Wallerian degeneration is known as **secondary demyelination** and is to be distinguished from **primary demyelination** in which the axon is initially undamaged (see below).

Distal axonopathy is seen in a number of chronic intoxications and genetically determined entities. It begins with degenerative changes in the distal reaches of the affected fibers and fairly characteristically involves the largest and longest, such as the proprioceptive and motor tracts of the spinal cord, the optic tract, and the recurrent laryngeal and other long peripheral nerves. Implicit in this pattern is a disturbance of anterograde axonal transport, upon which the maintenance of axonal wellbeing depends. In general, the process begins with the formation of focal axonal swellings containing degenerate organelles. These swellings are ovoid or circular eosinophilic structures commonly referred to as **spheroids**. This may progress to axonal fragmentation and attempted regeneration that may be abortive and is succeeded by further degeneration. These changes may develop focally in the distal regions of the axon and may extend more proximally with time. Diagnostically, the key is the recognition of the pattern of degenerative changes towards the terminations of long tracts (see Cycad poisoning, p. 364). The lesion is a feature of intoxication with certain of the organophosphates for example.

Proximal axonopathy is the contrasting situation in which focal swellings and degeneration begin in the proximal axonal regions. It is a pattern less likely to be encountered in natural animal disease than the distal variety. It is perhaps best exemplified by the large fusiform swellings, **torpedoes**, seen on the proximal axonal segments of cerebellar Purkinje cells in the mycotoxicosis of perennial rye grass poisoning and some storage diseases (Fig. 3.8). Such lesions may develop in central or peripheral axons and be associated with genetically determined or acquired disease. Proximal axonal swellings caused by the accumulation of neurofilaments are a feature of several neurodegenerative diseases described elsewhere in this chapter. A defect in the transport of phosphorylated neurofilaments results in the accumulation of masses of them, where they cause large, amphophilic axonal swellings. The fundamental pathogenesis remains undefined.

Axonal dystrophy is a term used to describe an axonopathic process characterized by the occurrence of *large focal swellings, often concentrated in the terminals and preterminals of long axons.* They are therefore frequently seen in and around relay nuclei in the brain, and in peripheral endings. The spheroids in axonal dystrophy can become extremely large, over 100 μm in diameter, and are filled with accumulations of normal organelles, degenerate organelles, and abnormal membranous and tubular structures. In hematoxylin and eosin sections, their appearance can be variable (Fig. 3.9A, B); some are densely eosinophilic, and either smooth or granular or vacuolated; others may be pale with central denser-staining cores; some may have a basophilic hue and evidence of focal mineralization. However, the swellings are not usually associated with any marked reaction on the part of surrounding elements and only rarely are seen to be undergoing fragmentation and dissolution. They are long-lasting, in contrast to the spheroids of acute axonal degeneration. Thinning of the myelin sheath around spheroids will occur as lamellae slip to accommodate the focal axonal enlargement (Fig. 3.10). The pathogenesis is unclear, but evidence points to a *disturbance of retrograde axonal transport.* The lesion is a common finding in the relay nuclei of the caudal brain stem in old age, and is a frequent accompaniment to neuronal storage diseases. It is the principal feature of diseases known as **neuroaxonal dystrophies**, of which several are recorded in the veterinary literature. In several such diseases, the topography of the axonal dystrophy seems to fit the clinical deficits but there is evidence that, in many situations, even intense development of the lesion has no functional significance. This seems generally so in regard to axonal dystrophy in the gracilis and cuneate nuclei.

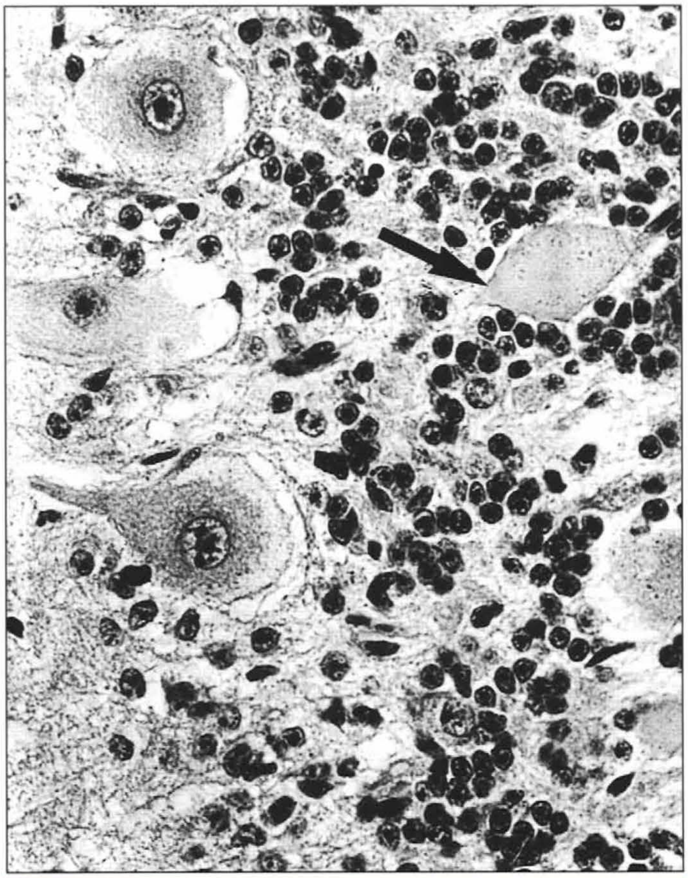

Figure 3.8 Proximal axonal enlargement - "**torpedo**" (arrow) - of a Purkinje cell, in mannosidosis in an Aberdeen Angus calf.

Oligodendrocytes, Schwann cells, and the myelin sheath

The **oligodendrocyte** is one of the close companion cells of the neuron in the CNS. One population of these cells occurs as satellites to nerve cell bodies and may proliferate in the event of injury to the neurons, but the role of the satellites is essentially unknown. *The role of oligodendrocytes is to provide and maintain the myelin sheaths around those axons with a diameter greater than about 1 μm.* They are accordingly located in the myelinated tracts amongst the fascicles of axons and are referred to as *interfascicular oligodendrocytes.* Particularly in neonates and in cases of hypo- or delayed myelinogenesis, distinction histologically between astrocytes and oligodendrocytes can be uncertain. Oligodendrocytes are smaller than astrocytes, nuclear density is greater, and the cells are arranged in rows between fascicles. In immature animals, nuclei of oligodendrocytes are morphologically

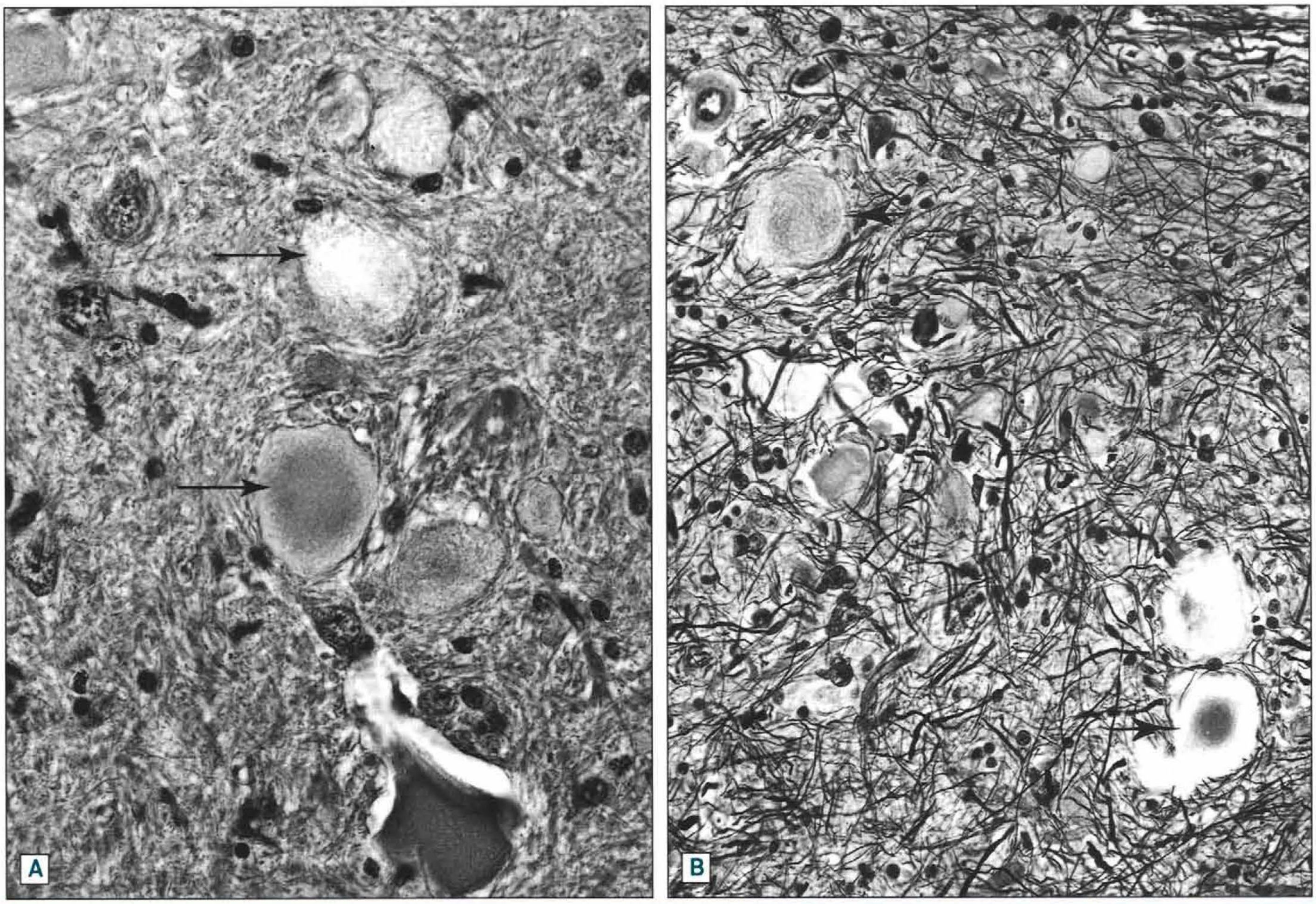

Figure 3.9 Axonal dystrophy in a Rottweiler. **A.** Focal axonal swellings (arrows); H&E stain. **B.** Silver stain reveals varying content of neurofilaments in swellings. (Courtesy of LC Cork.)

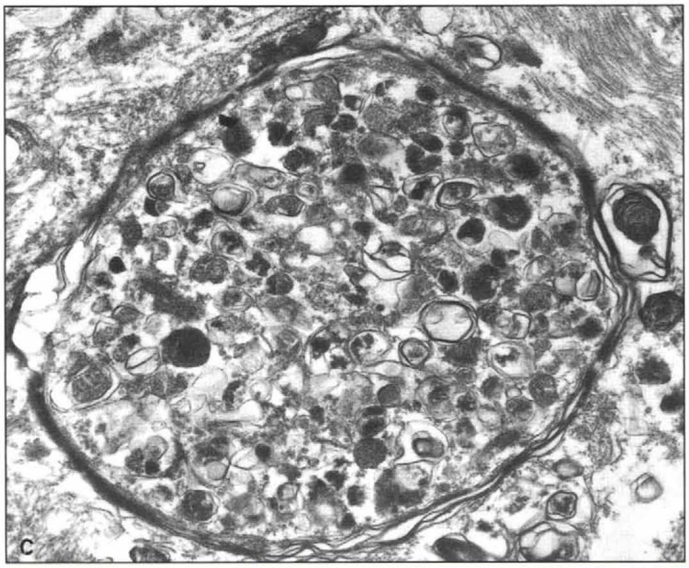

Figure 3.10 Axonal dystrophy in a cat with mannosidosis. Focal axonal swelling containing degenerate organelles; note thinning of myelin sheath. (Courtesy of SU Walkley.)

heterogeneous and those with large nuclei and clear nucleoplasm and not readily distinguished from astrocytes are probably immature and capable of division or synthesis of membrane myelin.

Oligodendrocytes arise from precursor cells in the subventricular zone of the developing forebrain and from the floor plate of the neural tube in brain stem and spinal cord. These progenitors migrate extensively to settle along fiber tracts of the developing white matter where they go through a series of maturation changes to produce myelin.

Each mature oligodendrocyte has a compact cell body of characteristic ultrastructural appearance, and a dozen or so thin processes each of which connects the perikaryon to a segment of myelin some distance away. Each segment of myelin covers one axonal internode, and is an extended and compacted sheet of specialized oligodendroglial plasma membrane bilayers, wound concentrically and spirally around the axon, like a rolled-up newspaper. In the formation of this compacted membrane, both the intracellular and extracellular spaces are obliterated, creating the major and minor dense lines of myelin lamellae as seen with the electron microscope. In some axons, a sheath of 100 or so bilayers may be formed. A portion of intact cytoplasm remains at the innermost and outermost lamellae, known as the "inner" and "outer" tongues respectively. Tracts of uncompacted cytoplasm course through the sheath to form the "incisures," and also occur where the myelin lamellae terminate at the paranodal region, as the "terminal loops." The lamellated myelin sheath is a relatively stable but plastic structure, whose lipid and protein components are supplied and turned over by the oligodendrocyte.

For light microscopy, myelin is well demonstrated by several special stains, with osmium tetroxide being particularly effective. Biochemically, central myelin is largely composed of cholesterol, galactocerebroside, and phospholipids, together with a number of distinctive protein constituents. The most abundant of these is the proteolipid protein (PLP), with lesser amounts of myelin basic protein (MBP), and myelin-associated glycoprotein (MAG). PLP is concentrated at the intraperiod line, MBP on the cytoplasmic face of the major dense line, and MAG at the axoplasmic/myelin interface. They probably play an important role in maintaining the stability of the sheath.

It is thus apparent that one oligodendrocyte myelinates several axonal internodes, that the myelin sheath is part of the oligodendrocyte, and that death of the oligodendrocyte will result in the demise of all the myelin sheath segments supplied by that cell. However, destruction of one or more myelin sheath segments does not necessarily result in death of the parent oligodendrocyte, but may stimulate it to withdraw its remaining myelin. The dynamics of the oligodendrocyte population are still not absolutely clear, but it is becoming accepted that there is a system of undifferentiated reserve cells able to take on to some extent regenerative and reparative tasks. These cells may originate from the perineuronal satellite oligodendroglia.

Myelination occurs relatively late in the development of the CNS, and maturing oligodendrocytes invest axons with myelin by replacing an initial ensheathment of astrocytic processes. Once this is completed, most of the cells assume the characteristics of maturity, while some do appear to remain in a less mature state. The ability of oligodendrocytes to synthesize myelin at specific times and in specific tracts that are specific for the animal species must require signaling mechanisms that wait to be clarified.

Oligodendrocytes do not exhibit a range of reactions for the light microscopist, generally undergoing rapid lysis when injured. Acute injury may be manifested by hydropic swelling of the perikaryon. On occasion, mitotic activity and an increase in numbers may be observed when a primary demyelinating process is operating, but this seems to be very rare in veterinary pathology. Their numbers may be increased by condensation in linear rows in interfascicular gliosis. Inclusion bodies may be present in the nuclei in some viral diseases such as canine distemper. Excepting oligodendroglial tumors, damage to the cells is expressed in disordered myelin.

The process of myelination is dependent on close interaction between the axon and the myelinating cell. The two act as a unit, and signals are exchanged between them for all aspects of the process. While the axon may survive for a long period without its myelin sheath, loss of the axon provokes immediate disintegration and removal of the myelin sheath. *This situation of axonal degeneration with secondary myelin loss is termed Wallerian degeneration*, and has been discussed above under axonopathy.

Myelination in peripheral nerves is the responsibility of **Schwann cells**, and they have a distinctly different relationship with axons to that of oligodendrocytes. Each peripheral internode is myelinated by a single cell, and the myelinated axon is invested by a basal lamina tube of Schwann cell origin, and by endoneurial collagen. Signals from Schwann cells influence the development of all components of the nerve and endo- and epineural connective tissue and the Schwann cells themselves. Schwann cells develop from the neural crest as two different cell types, *myelinating* and *nonmyelinating*. This differentiation is reversible, which is consistent with the absence of precursors in mature nerves regenerating after injury. If mature Schwann cells lose contact with axons, as occurs in nerve injury, the cells undergo regression and the myelin disintegrates. The dedifferentiated cells multiply and provide growth factors that support the regrowth of axons.

The cell body of the Schwann cell directly apposes the axon. Peripheral myelin is also chemically distinct from central myelin and this can be appreciated by the tinctorial difference between the two in appropriately stained sections of the spinal cord/spinal nerve interface. This chemical difference is reflected in antigenic differences; the major protein is termed Po and is distinct from PLP, as is the basic protein P1 from MBP. *Schwann cells are able to replicate prolifically, to phagocytose damaged myelin, and to remyelinate newly regenerated or previously demyelinated axons.* This replicative ability means that the loss of a proportion of the cells may be compensated. The peripheral myelinated axon is therefore a much more resilient structure than its central counterpart. The general principles of the axon/myelin relationship, as outlined for CNS, still apply however. *Destruction of Schwann cells will result in the disintegration of the dependent myelin.* Destruction of axons will cause myelin degradation, Schwann cell proliferation and, in time, endoneurial fibrosis.

In paraffin sections of normal nerve, Schwann cells appear as ovoid nuclei closely apposed to the axon. Proliferating Schwann cells in longitudinal section often appear as bands (*Bungner's bands*) of spindle-shaped cells resembling fibroblasts. In cross-section, they form concentric whorls called "*onion bulbs.*" Nodular proliferations of Schwann cells are referred to as *Reynaud bodies*. Large Reynaud bodies are a common incidental finding in nerves of horses.

A number of diseases primarily involve the myelin sheath, and may frequently leave the myelinating cell body intact. These diseases usually require ultrastructural evaluation for adequate investigation.

In **demyelinating diseases**, the sheath is removed from the axons, leaving them naked over variable lengths and providing potential for serious slowing of impulse conduction. In peripheral nerves, the removal of myelin from randomly scattered internodes gives rise to segmental demyelination, which is best appreciated in teased fiber preparations. Demyelination is frequently carried out by macrophages that insinuate cytoplasmic processes into the intraperiod lines and strip the sheath from the axonal internode, ingesting and digesting myelin debris. In other situations, myelin appears first to be disrupted by humoral factors and undergoes splitting and vesiculation prior to phagocytosis. Myelinophagy can be identified by the use of stains such as the Luxol fast blue/periodic acid Schiff technique. Degenerate myelin can be visualized by the Marchi technique (see Fig. 3.7).

In the CNS, there is some scope for remyelination, but the complex arrangement and limited replicative capacity of the oligodendrocytes limits the reparative potential. Regenerated myelin sheaths are thinner than the originals, appearing to the experienced eye as being too narrow for the diameter of the axon they ensheath. Remyelinated internodes are also shorter. In the peripheral nervous system, the potential for remyelination is much more favorable. Repeated bouts of demyelination may result in "onion bulb" and

Reynaud body formations, and in the production of thin and irregular myelin segments.

In **hypomyelinating diseases**, myelinating cells fail, for various reasons, to provide adequate myelination during the development phase, and the affected individual suffers transient or permanent myelin deficiency, varying in extent and severity according to the particular disease (Fig. 3.11A, B, C, D). The majority of these conditions involve the CNS only (see Myelinopathies). Myelin sheaths are thin or absent, but myelinophagy is generally minimal or nonexistent. Oligodendrocytes may be few, or present in normal numbers, and may exhibit features of immaturity.

In **dysmyelinating diseases**, there is a qualitative defect in the myelin produced, and the quantity may also be reduced. A large variety of these disorders has been produced for research purposes in inbred strains of laboratory mice.

Myelinic edema *is disruption of the lamellar structure by reopening of the extracellular space along the intraperiod line.* It may occur in both central and peripheral myelin. It is caused by a number of chemical agents, e.g., hexachlorophene, and leads to a spectacular state of spongy degeneration of white matter, one form of status spongiosus. With some causal agents, there are associated degenerative and reactive changes, but with other causes there is, remarkably, no apparent response on the part of other tissue elements including the oligodendrocytes themselves. The lesion does not necessarily cause functional disturbances even when well developed; it seems that this may depend on the number of intact lamellae left in place. It may resolve over a period of weeks with no evidence of breakdown of the affected myelin (see Spongiform myelinopathies).

Astrocytes

Astrocytes may be regarded as the interstitial cells of the CNS, as their processes occupy most of the space between and around the neuronal and oligodendroglial elements, and the perivascular and subpial zones. Astrocytes are of two types: the **protoplasmic** (type 1), located mainly within the cerebral gray matter, and the **fibrous** (type 2), located mainly within white matter tracts. Evidence suggests a common progenitor cell, the 011A cell, for the type 2 astrocyte and the oligodendrocyte. Indeed, there is evidence for several different functional types of astrocyte, the functional diversity reflecting diverse locational needs. Radial glial cells, which are the precursors of astrocytes, guide the migration of neurons from the subependymal generative zones to their final positions; astrocytes guide axons into their proper fiber tracts; astrocytes are important in the regulation of ionic exchanges between cells of the nervous system; astrocytes form functional connections by production of molecules that are tropic for other specialized cells of the nervous system.

The intercellular space of CNS tissue is a 20-nm cleft between astrocytes and the other elements, and is interrupted by loose junctions and zonulae adherentia between the former. The astrocytic perikaryon is sparse and barely evident in routine paraffin sections, and the numerous and ramifying processes are invisible. The nuclei appear, therefore, as naked and spherical, and about the size of those of small to medium-sized neurons; they usually lack a nucleolus, but sometimes have a chromatic dot, the centrosome. Immunochemical demonstration of *vimentin* is used to confirm the identity of immature astrocytes, and *glial fibrillary acidic protein* (GFAP) to confirm identity of mature astrocytes including neoplastic astrocytes. However, GFAP is not entirely specific for astrocytes. Ultrastructurally, the astrocytic cytoplasm throughout is relatively devoid of organelles and appears largely empty and watery. The chief features are bundles of intermediate filaments, clusters of glycogen granules, and a few mitochondria and lysosomes.

All capillary blood vessels in the CNS are closely invested by the expanded ends of astrocytic processes, the *end feet*. There is also a dense network of processes at the surface beneath the pia mater, the *glia limitans*. A specialized population of astrocytes occurs in the Purkinje cell layer of the cerebellum and is known as *Bergmann's glia*. These cells have long straight processes that extend out through the molecular layer to the surface.

Astrocytes and their processes are well visualized by the application of special stains, such as the Cajal method (Fig. 3.12A, B), or immunostaining for the intermediate filament glial fibrillary acidic protein (GFAP) (Fig. 3.13A, B). Astrocytes are considered to play an important role in the movement of cations and water, to be much involved in maintenance of conditions favorable for the electrical activity of neurons, and to be a source of cytokines and growth factors that support neurons and neuronal activity. Type 2 cells have a major role in this regard at the nodes of Ranvier where they form a close relationship with both the axon and the terminal myelin loops. They are also involved in the detoxification of ammonia, and perhaps other metabolites.

When lethal astrocytic injury occurs, the cytoplasm swells and becomes visible, albeit faintly, and the nucleus becomes eccentric and pyknotic. Disintegration of cytoplasm and nucleus follows rapidly. Astrocytes, however, are capable of a number of reactive responses when they, or cells around them, are damaged. *A frequent response is swelling and eosinophilia of the cytoplasm, with some cells acquiring two or more nuclei. These plump reactive astrocytes are called* **gemistocytes** (Fig. 3.14). In a mild response, cytoplasmic swelling may be minimal but some proliferation of both cells and processes generally occurs (*astrogliosis*). With cessation of injury, cytoplasmic swelling regresses but there may be a permanent residuum of extra cells and processes. Around the borders of severe lesions, such as malacic foci, the proliferation of processes may become extremely dense (Fig. 3.15). Astrocytic proplasia is limited to surviving tissue however, and post-malacic cavities cannot be filled by astrocytic processes.

Reactive astrogliosis is expected in Wallerian degeneration, following neuronal loss, and in sustained cerebral or spinal edema, and is a feature of many viral encephalitides in which viral infection of astrocytes is probably a prime stimulus. This is certainly the case in canine distemper, in which inclusion bodies are common in reactive astrocytes. Astrocytes are the cells primarily responsible for repair and scar formation in the brain.

In acute cerebral or spinal vasogenic edema, swollen astrocytes and their processes undergo a type of hydropic degeneration. This can only be resolved satisfactorily with the electron microscope, when the clear distension can be seen, particularly in the perivascular end-feet.

An acute astrocytic reaction known as the formation of **Alzheimer type II cells** is best exemplified by the metabolic disturbance in *hepatic encephalopathy* resulting from liver failure (Fig. 3.16). The nucleus becomes distinctly enlarged and vesicular but remains rounded; the cytoplasm swells and may become visible. This change does not involve the generation of large masses of cytoplasmic intermediate filaments, and there is a weak reaction to GFAP

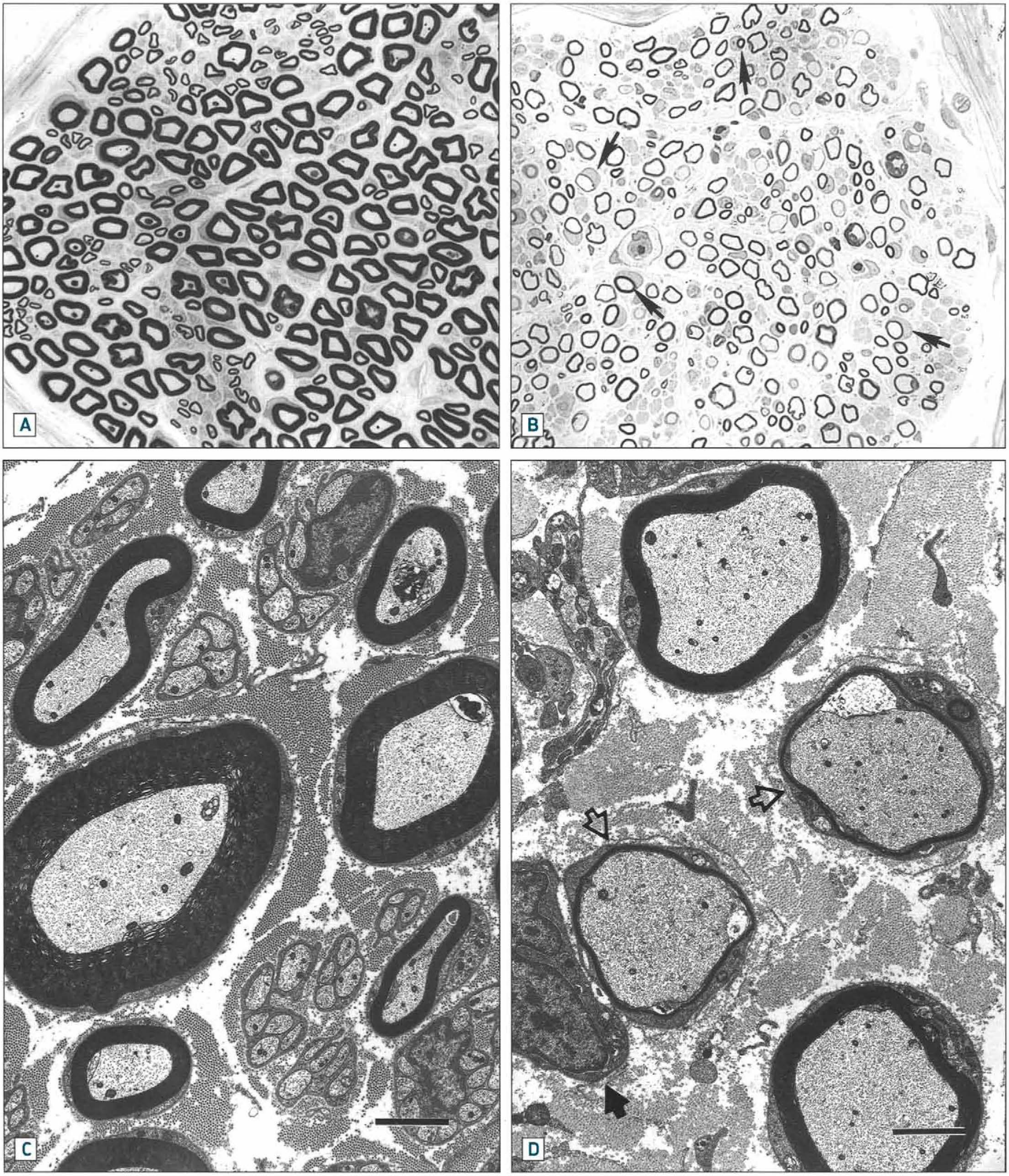

Figure 3.11 Polyneuropathy in a Golden Retriever. Comparison of normal (**A**) and hypomyelinated (**B**) peripheral nerve. Voluminous Schwann cell cytoplasm (arrows). (**C**) and (**D**) corresponding ultrastructure: normal (**C**), hypomyelinated (**D**). Thinly myelinated fibers (open arrow). Schwann cell nucleus (black arrow). Bar = 2 μm. (Reprinted with permission from Braund KG, et al. Vet Pathol 1989:26:202.)

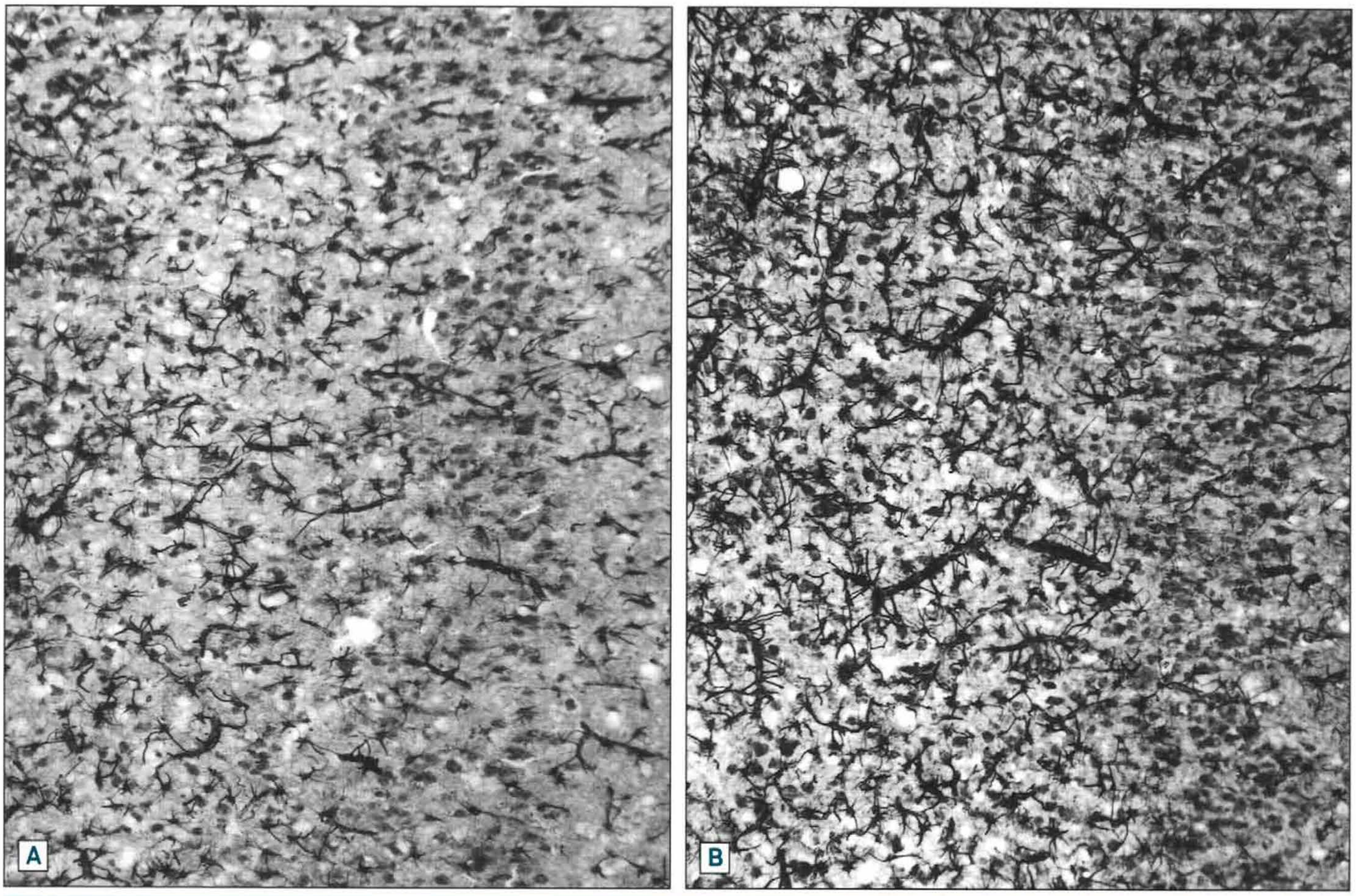

Figure 3.12 Astrocytes in the cerebral cortex of a goat with scrapie, stained by the Cajal method. **A.** Normal. **B.** Fibrous astrogliosis. (Reprinted with permission from Hadlow WJ, Race RE. Vet Pathol 1986;23:593.)

immunostains. It is caused by the accumulation of ammonia and other endogenous toxins, and would therefore also be expected in cases of exogenous ammonia intoxication. In some circumstances, cells suggested to be astrocytes are capable of *phagocytosis of tissue debris*, particularly myelin. This can be seen in Wallerian degeneration in the optic nerves when the bulk of myelinophagy may be performed by astrocytes. It has also been observed in other regions of the brain and cord.

A bizarre and rare astrocytic response is the formation of **Rosenthal fibers**; these are irregularly shaped, hyaline eosinophilic structures of undetermined composition, formed within the cell bodies and processes. The massive production of Rosenthal fibers is a feature of *Alexander disease*, an idiopathic entity described in humans and dogs, now regarded as the only primary genetic disease of astrocytes.

Microglia

Microglia are derived from the mononuclear phagocyte lineage, and function as the *fixed macrophage system* of the CNS. In the normal brain and cord, they appear by routine microscopy as *inconspicuous, small hyperchromatic nuclei, often wedge-shaped, and with no visible cytoplasm*. However, special staining techniques reveal extensive thin cytoplasmic processes and, with the electron microscope, dense perikaryal cytoplasm with elongated strands of rough endoplasmic reticulum and lipofuscin-like granules are characteristic. They are most frequent in the gray matter where they may group in the vicinity of neurons, and in both gray and white matter they are most numerous adjacent to blood vessels. During organogenesis, they are derived from blood monocytes that also give rise to the rich population of leptomeningeal and perivascular histiocytes that can migrate into the neuropil when significant vascular damage has occurred. Microglia are the primary immune effector cells of the central nervous system. Activated microglia at the site of injury express increased levels of major histocompatibility antigens and, like other macrophages, microglia release inflammatory cytokines that amplify the inflammatory response by recruiting other cells to the site of injury. They may also be important in the persistence of some viral infections.

The simplest microglial response to tissue injury is a *hypertrophic reaction* in which the nucleus becomes rounded and the cytoplasm visible as a narrow, often eccentric, eosinophilic rim. In routinely stained preparations, such cells may be difficult to distinguish from astrocytes. They may also proliferate, although their ability to do so seems limited, and many of the cells in proliferative foci are probably derived from immigrant histiocytes. Focal proliferation gives rise to nodules of 30–40 or more cells, while diffuse proliferation creates an overall impression of increased cellularity in the microscopic field (Fig. 3.17). *Microglial nodules are very commonly a feature of viral encephalitides*, occurring in both gray and white matter, but are

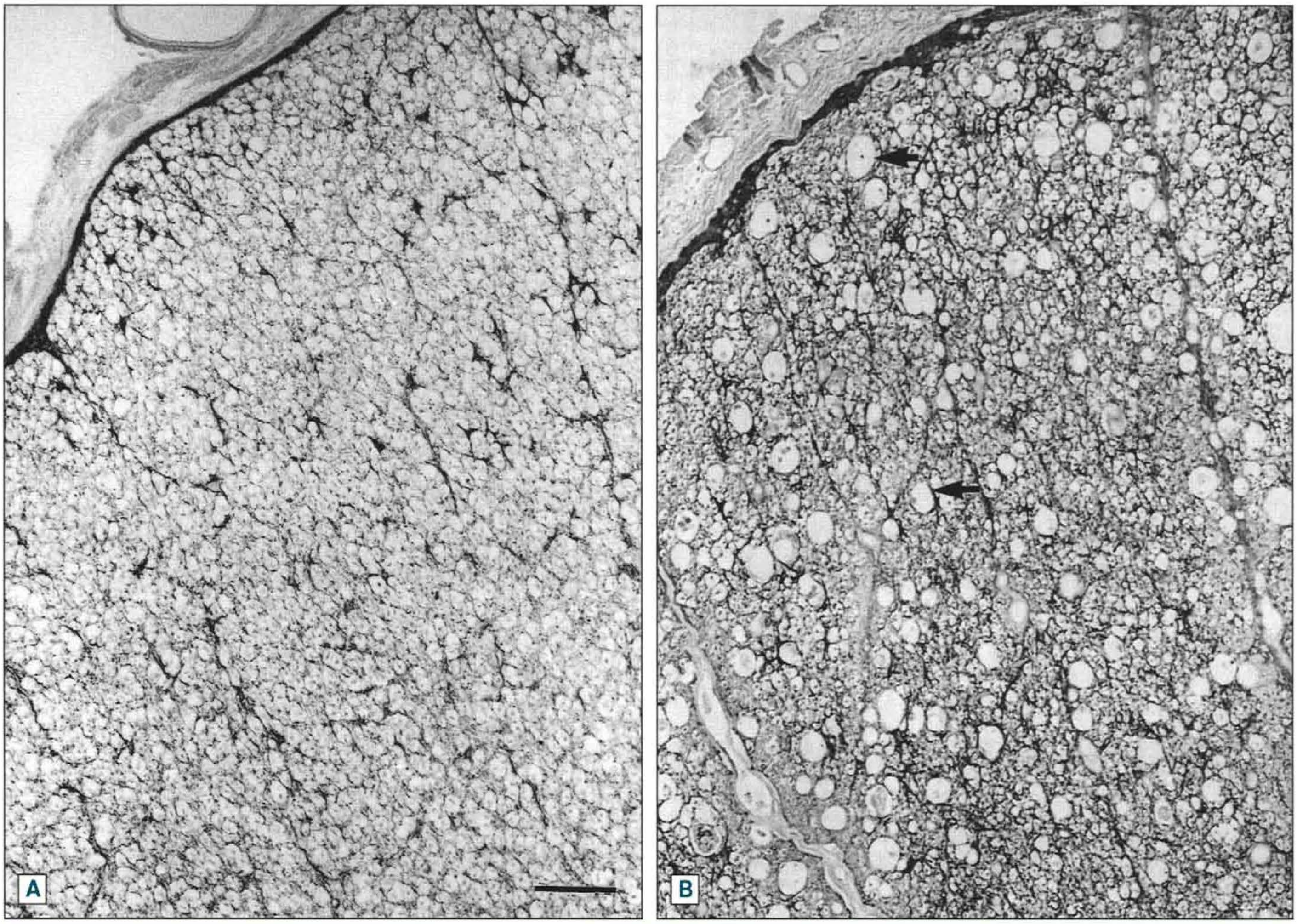

Figure 3.13 Glial fibrillary acidic protein (GFAP) staining of equine spinal cords. **A.** Normal. **B. Wobbler**. Astrogliosis at site of compressive injury; axonal degeneration (arrows). (Courtesy of JV Yovich.)

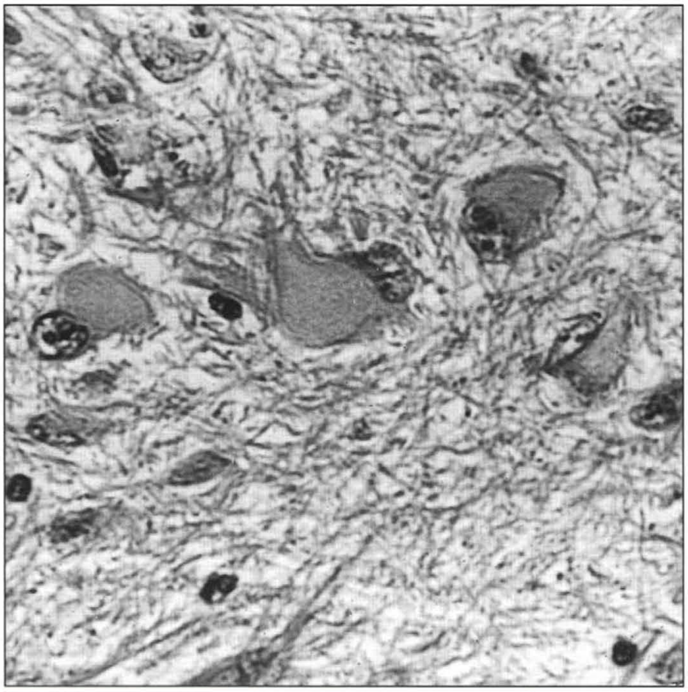

Figure 3.14 **Reactive astrocytes** (gemistocytes) near malacic focus.

not specific for viral infections. Reactive microglia may develop greatly elongated and sometimes tortuous nuclei, in which case they are called *rod cells*. These again are often seen in viral diseases. Activated microglia release cytokines and chemokines that aid in defense against CNS infections, as well as contributing to neurodegenerative conditions.

The most vigorous response of the microglia is their *transformation to macrophages*, when they assume the morphology typical of cells engaged in phagocytosis. When ingesting myelin debris, their cytoplasm becomes foamy as they load themselves with lipid vacuoles. Often the nucleus becomes pyknotic, and they are referred to as **gitter cells, compound granular corpuscles**, or **fat-granule cells** (Fig. 3.18A, B). In severe lesions, many of the gitter cells will have arisen from blood monocytes as well as from microglia. Lipid-laden cells may persist for months around focal lesions, but slow migration to the perivascular spaces and the meninges does take place. Some of the cells may be found at these locations after even longer periods, with the ingested lipid transformed to lipofuscin.

Microglial phagocytes are usually responsible for **neuronophagia**, in which phagocytic cells gather around fragmenting degenerate neuronal cell bodies (see Fig. 3.2D). This response is a feature of many viral infections in which neurons die, but is uncommon in ischemic or other forms of neuronal necrosis.

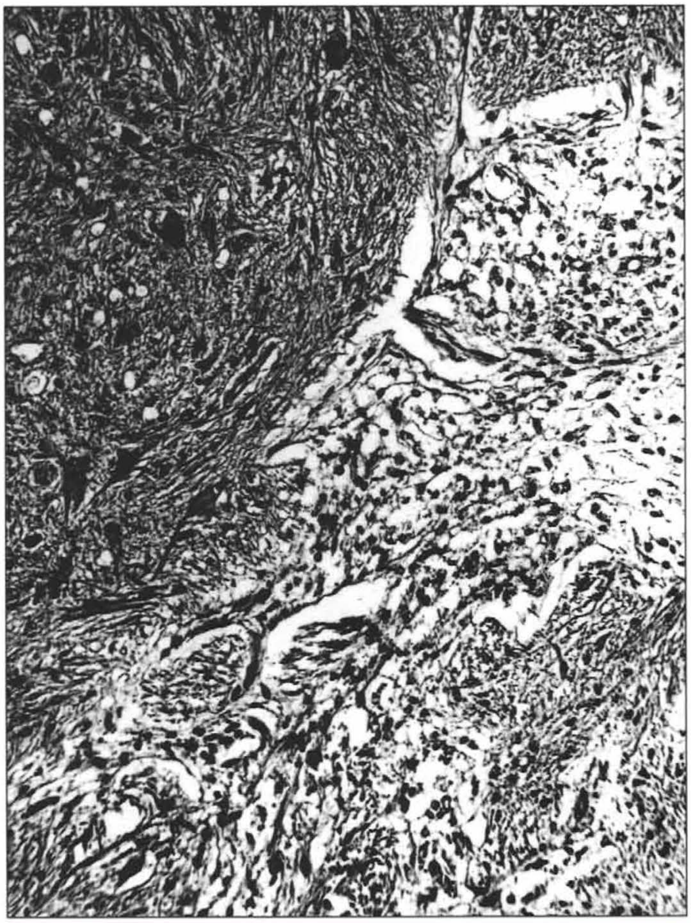

Figure 3.15 **Astrogliosis** forming wall to residual cyst.

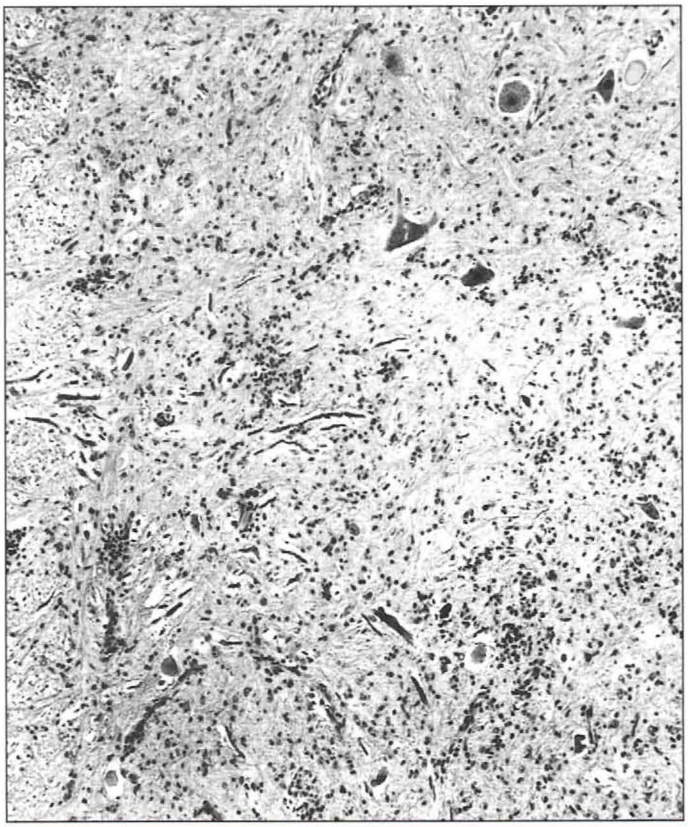

Figure 3.17 Focal and diffuse **gliosis** in the poliomyelitis of louping in a sheep.

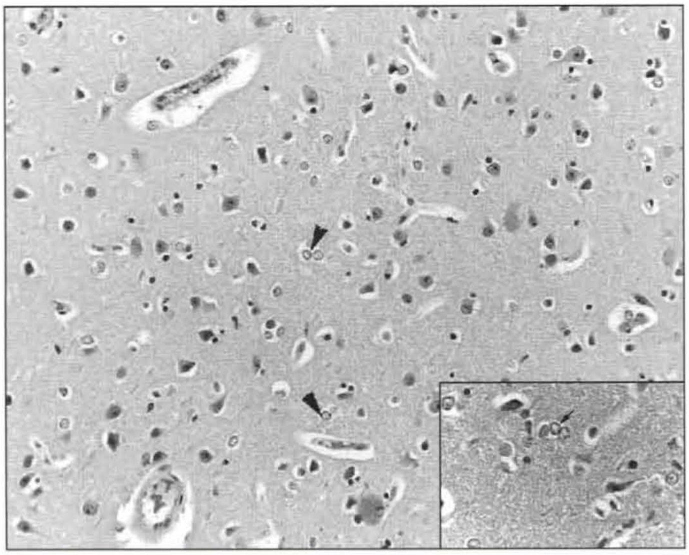

Figure 3.16 Marked **Alzheimer type II astrocytosis** in equine hepatic encephalopathy (arrowheads). Inset: Alzheimer type II cells are often arranged in pairs (cuddling cells) or clusters (arrow): pony.

Microcirculation

There are some structural peculiarities of blood vessels in the CNS that have a bearing on the development of pathologic processes. The capillaries differ from those in other tissues by being surrounded by an investment of astrocytic end-feet. Also, the endothelial cells are sealed together by tight junctions, and the basement membrane divides to incorporate pericytes into the capillary wall. This arrangement in its totality creates the **blood–brain barrier** that selectively limits the entry of many molecules into the neuropil in most of the brain. The barrier is lacking in a few locales, such as the area postrema and certain other periventricular nuclei.

The distribution of brain capillaries varies considerably, but they are more abundant in the gray matter than the white. Their concentration is higher in some parts of the gray matter, such as the supraoptic and paraventricular nuclei and the area postrema, especially where neuroendocrine activity is concentrated. It is in these areas that the blood–brain barrier is lacking.

Regional variations in the concentrations of capillaries do not appear to influence local pathologic processes. The capillary and venular endothelium is highly labile and responds to a variety of injuries by swelling and proliferating (Fig. 3.18B). However, in spite of fairly vigorous proplasia, *cerebral capillaries seem to be almost incapable of budding, so reactive neovascularization is minimal.* The formation of new capillaries is probably limited to situations where granulation tissue is derived from the mesenchyme of the meninges.

Both arterioles and venules of the CNS are thin walled, especially the latter whose walls are composed mainly of a thin layer of fibrous tissue with very little elastica and no muscle. They are thus susceptible to injury and prone to hemorrhage and, in the cerebral white matter, leukocytes tend to sequester in them in bacterial

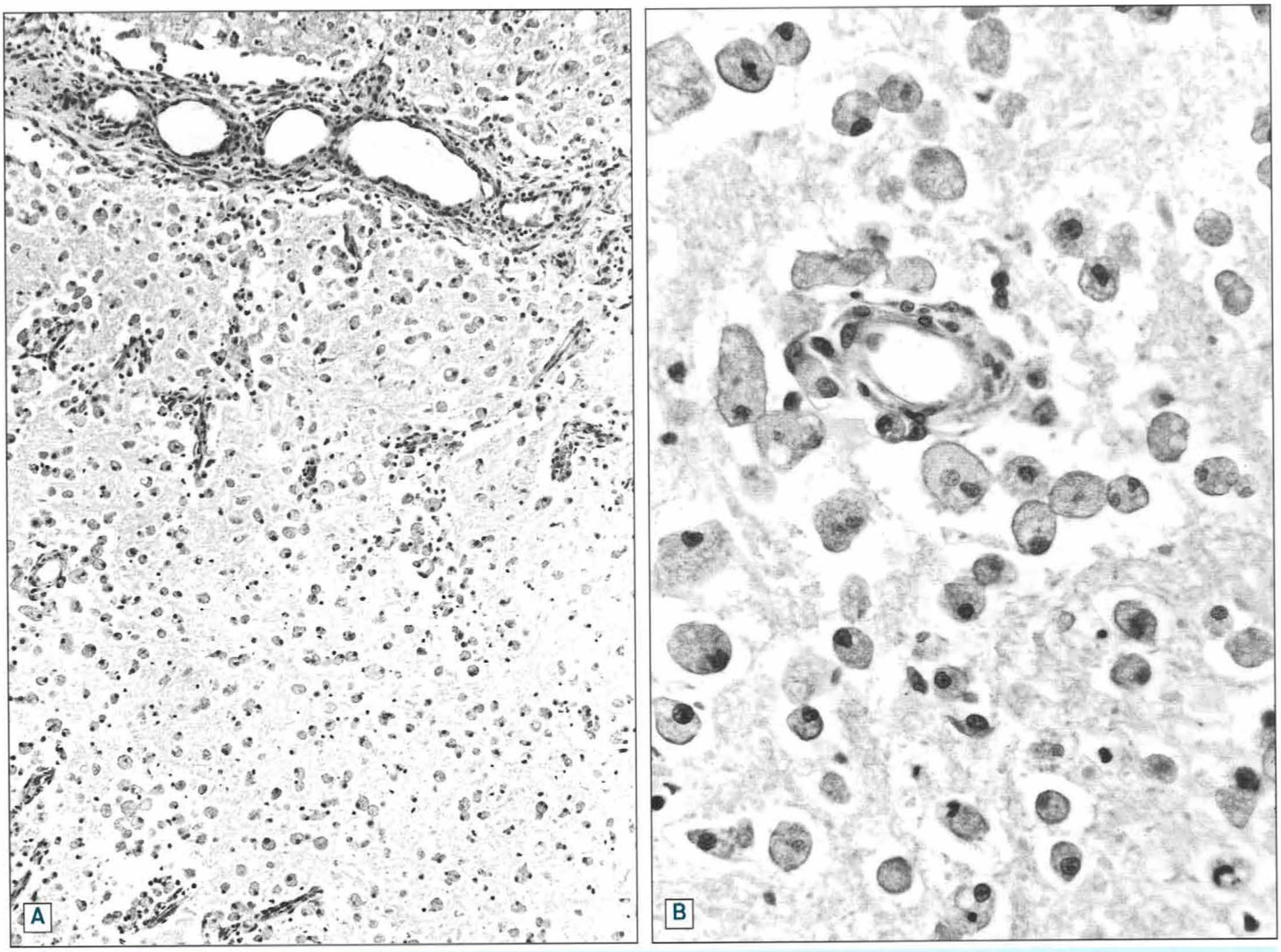

Figure 3.18 A. Gitter cells and reactive capillaries in recent cerebral infarct in a dog. **B.** High-power view of gitter cells.

infections. The veins are valveless and, as backflow of blood after death is usual, cerebral venous congestion can be difficult to assess.

Both arteries and veins have an outer adventitial layer of variable thickness, and a **perivascular Virchow–Robin space**. The space around veins is continuous with the subarachnoid space and is lined by an invagination of the pia-arachnoid. Around cortical arteries however, there is an investment of leptomeninges in a single layer, an arrangement by which the intracortical perivascular space communicates with the perivascular space about arteries in the leptomeninges. Arteries that enter through the rostral perforated substance may differ from those in the dorsal cortex in that the arteries are surrounded by two leptomeningeal layers with a space between them that communicates with the perivascular space around arteries in the subarachnoid space. The varied arrangements may reflect the need to allow passage of interstitial fluid to the local lymphatics.

In diffuse inflammatory or neoplastic diseases, this space, more potential than real under normal conditions, becomes patent and accumulates reactive and invading cells. The tendency for these cells to be confined to the space gives rise to the neuropathologic term **perivascular cuffing** (Fig. 3.19). The size of cuffs is usually related to the size of the space, and they may vary from one cell thick about the smallest venules to 10–12 or more cells thick around the larger vessels. *Perivascular cuffing is classically seen in inflammatory conditions*, and all classes of reactive leukocyte may be seen depending on the cause. While most of the cells are of hematogenous origin, there is no doubt that in some diseases, enteroviral infections for example, they may arise largely from the proliferation of adventitial cells or resident histiocytes. Lymphoid perivascular cuffing is a feature of many viral encephalitides. Some diseases too are characterized by the production of cerebral and/or spinal vasculitis, but these changes are discussed in a following section.

Mention must be made of the *nests of residual glia (islands of Calleja)* that are seen beneath the ependyma of the lateral ventricles and in the dentate fascia of the hippocampus. These cells are relics of the developmental period and possibly act as a supply of replacement cells in the adult. They occur in small aggregations and often as eccentric cuffs around vessels. Generally they are monomorphic and appear as small dark nuclei and may be mistaken for inflammatory cells.

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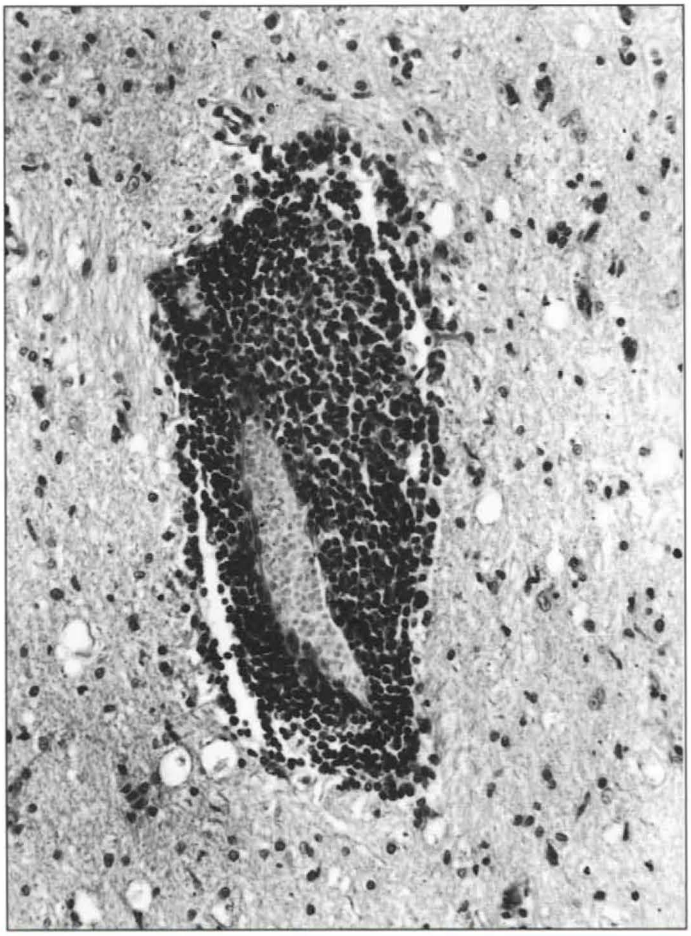

Figure 3.19 Perivascular cuff in a dog with encephalitis. Lymphocytes are confined to the Virchow–Robin space.

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MALFORMATIONS OF THE CENTRAL NERVOUS SYSTEM

Malformations of the central nervous system (CNS) are common in domestic animals, and their variety is perhaps greater than the variety of malformations in other tissues. There is abundant field and experimental evidence that the effects of teratogens are manifested in the nervous system with disproportionately high frequency. One explanation is that the high degree of differentiation and complexity of the CNS give it an increased susceptibility to developmental disturbances. In addition to inherited diseases, a large number of infectious and toxic environmental agents are capable of causing anomalies. The cause of malformation in an individual domestic animal fetus is seldom determined, in part because of the long time lapse between the initiating event and the fetal presentation. The most frequent sporadic anatomic abnormalities are neural tube defects, and these are initiated in a narrow time range: embryonic days 8.5–10.5 in the mouse, days 15–18 in the pig, days 21–28 in humans.

Congenital abnormalities of the CNS consist of deviations in either the nature or velocity of the developmental process. Several main patterns of abnormal development can be recognized.

- The largest category includes those disorders with a morphological basis that are a consequence of *failure or disorder of structural development.* Many such abnormalities are recognizable by distinctive gross changes, but others require microscopic examination.
- Some conditions appear to represent *retardation of normal development* rather than structural aberrations.
- Disturbance of the normal development may also manifest as *premature senescence or degeneration of formed tissues* such as the various forms of neuronal abiotrophy.
- Some congenital abnormalities appear to represent *primary disturbances of function* rather than of tissue structure. The most frequently recognized functional disorders are those that arise as a consequence of inherited biochemical defects and that cause distinctive neurohistologic changes. This category is exemplified by the various lysosomal storage diseases and the leukodystrophies.
- Neither an anatomical nor a biochemical basis has yet been identified for a number of congenital neurological diseases that often *appear to be inherited*, e.g., idiopathic epileptiform conditions in various species.

The initial steps in the formation of the CNS take place early in embryogenesis. As soon as the germ layers are established, the **neural plate**, a thickened band of ectoderm, develops along the mid-dorsal line of the embryo, and is the primordium for the brain and spinal cord. Differential growth at the margins and in the midplane result in folding of the plate to form a *neural groove* bounded on each side by an elevated *neural fold*. The groove continues to deepen, and the neural folds meet dorsally and fuse, likely beginning at multiple sites, to form the **neural tube**, which simultaneously separates from the superficial ectoderm. Physical properties of cells and tissues both inside and outside the neural plate determine the formation of the neural tube (*neurulation*). Cellular proliferative activity within the neural tube is concentrated within an inner subependymal or germinal layer of actively dividing neuroectodermal cells that, with differentiation and outward migration, gives rise first to neurons and later to glia.

The neural tube extends the full length of the embryo and is the progenitor of the entire CNS. **Neural tube defects** (NTD), which result from failure of a portion of the neural tube to close or from reopening of a successfully closed segment, may be cranial or spinal or concurrent in both locations. Shortly after formation of the neural tube, the rudiments of the cranium and vertebrae appear in accord with the fundamental plan of segmental organization. *Segmentation and development of the axial skeletal investment of the nervous system depend on the developmental integrity of the neural tube.* Hence, NTDs also involve malformations of overlying bony or soft tissues. The spectrum of NTDs includes anencephaly, encephalocele, spinal dysraphism, and meningomyelocele. Incomplete closure of a raphe, especially the neural tube, is referred to as *dysraphism.*

The genes controlling neurogenesis are very strongly conserved in evolution and much effort is placed on analysis of responsible genes and gene actions in human embryos and in the many models of NTD in mutant and transgenic mice. Notwithstanding the shared gene arrays of the many mouse models and humans, only one mouse phenotype, the *curly tail* mouse, exhibits the anencephaly/meningomyelocele phenotype that is the most frequent NTD in human fetuses. The phenotype results from failure of closure of cranial and caudal neuropores, the ends of the neural tube. The *Sonic hedgehog gene* (Shh), secreted initially from the notochord and later from the floorplate, is implicated in inducing development of the floor of the neural plate and differentiation of neurons in the ventral sections of the developing cord. Development of the dorsal roof plate of the cord, differentiation of neural crest cells and formation of neurons within the dorsal cord are the responsibility of members of the *bone morphogenetic protein* (BMP) family derived from surface ectoderm.

The normal pattern of development of the CNS is not smoothly progressive but comprises a complicated interdependent series of growth spurts of organs and tissues. Birth does not mark a single stage in the structural development of the brain and there is considerable species variation in the stage of CNS development at birth. *Cattle, horses, sheep, and pigs are born with nervous systems having a remarkable degree of structural and functional maturity; kittens and puppies are born with their nervous systems relatively immature.* After the nervous system is fully formed, the capacity for further production of nerve cells is lost and subsequent development comprises mainly progressive lengthening and myelination of axons and extension of neuronal dendrites and glial processes.

For each species, neural development thus proceeds along a characteristic predetermined pathway. When mature, the nervous system possesses considerable inherent stability, but a time-linked process of decay is also part of the total system of development. Aging neurons tend to accumulate ceroid pigments that almost certainly reduce their functional efficiency. There is also a continual normal loss of neurons, and in normal animals neuraxonal degeneration is an insignificant but not infrequent finding.

Abiotrophy *designates the occurrence of intrinsic premature degeneration of cells and tissues.* In the hereditary abiotrophies affecting the nervous systems of animals, such premature loss of vitality is manifested by accelerated and exacerbated degeneration of neurons and their processes.

Investigation of the etiology of neural effects is complicated by the fact that *the same type of abnormality may be produced by both genetic and exogenous causes,* and in many instances, particularly those of sporadic occurrence, the cause remains unidentified. A variety of transplacental viral infections of the fetal nervous system at critical stages of gestation may result in neurological defects. Some viruses can produce changes such as symmetrical cavitating lesions that lack pathological features that suggest antecedent infection and resemble malformations thought to be of genetic or toxic cause.

The outcome of fetal interactions with agents potentially teratogenic for the nervous system depends on the species and age of the fetus and the nature of the agent. For the various patterns of malformations, there are corresponding critical periods during which developing neural tissue is vulnerable. The teratogenicity of the agent is dependent on its cellular tropism, which is commonly directed towards immature rapidly dividing cells but which often involves specific subpopulations of these cells. Thus, time sets the stage and presents the array of vulnerability, but the agent chooses from among the parts at risk those it has special affinities for damaging.

The most common mode of action for teratogens is selective destruction of cells. Such cytolytic effects have been demonstrated with neural defects induced by *viruses* and *chemicals* as well as by *physical agents* such as hyperthermia. In the case of viruses, cellular destruction can be a direct consequence of infection but may develop as part of the inflammatory reaction. The toxicities of many drugs are mediated not by the compounds themselves but by highly reactive metabolites. If they are not detoxified, these unstable metabolites interact covalently with cell macromolecules and may kill affected cells. Cytolysis seems to be the common operative mechanism in a number of important malformations that often have gross features such as the cavitating cerebral defects and cerebellar hypoplasia. Teratogens may sometimes act by inhibition or distortion of normal cellular development and function to produce more subtle developmental deviations such as hypomyelinogenesis. Infection of calves with *Bovine viral diarrhea virus*, of lambs with *Border disease virus*, and of piglets with *Classical swine fever virus* may provide instances of noncytolytic disturbance of fetal neural development of this type.

Some drug metabolites, rather than killing cells, result in mutations through binding to nucleoprotein. In view of the multiplicity of pathogenetic pathways that have been identified, it is not surprising that a spectrum of pathogenic effects can result from the action of one teratogen. All of the lesions may result from an exposure or any one effect may develop either alone or in combination. Such diversity of teratogenic expression is illustrated by the wide variety of neural and extraneural defects found in kittens born to cats exposed to griseofulvin in pregnancy. *A particular abnormality may arise as an expression of many different causes, sometimes disparate in character.* The cavitating cerebral defects provide examples of this.

Several attributes of fetal neural tissue are pertinent to the interpretation of malformations. Necrosis of groups of cells occurs in normal development and may be seen in association with remodeling processes. The immature nervous system does not react to damage in the same way as adult tissue. Parts may be absorbed without trace of connective tissue or neuroglial repair. Furthermore, severe malformations are unlikely to proceed directly from destructive processes since destruction of parts of the embryonal structure removes the inductor and inhibitor influences on neighboring cell groups. This may, in some instances, lead to arrest of normal processes and, in others, to exuberant reparative proliferation of neuroepithelium that is indicated histologically by the formation of distinctive neuroepithelial rosettes. *Thus, malformations may be compounded of degeneration, necrosis, inhibition, overgrowth, and repair.* There is at best only a general correlation between the nature of an anomaly and the time in development when it was initiated. In retrospective studies of malformations of unknown cause, it is only possible to identify the latest time at which the malformation may have been produced based on the normal development of the nervous system of the species in question.

In the event of infection of the developing nervous system by potentially teratogenic viruses, the outcome is determined primarily by age-related cellular susceptibility. However the immunological status of the fetus, particularly its capacity to produce neutralizing antibody, is likely to be important in determining the character of the neural lesions resulting from viral infection. *Lesions leading to major anomalies tend to occur prior to the fetal age at which the fetus can mount a serum neutralizing antibody response to infectious agents.* The fetal brain, especially in the early stages of development, has limited inflammatory potential, but viral-induced destruction of neural tissue in the early fetus may be accompanied by an intense macrophage reaction despite lack of immunological maturity. Such necrotizing processes typically produce gross anatomical defects with little or no evidence of inflammation because of subsidence of the inflammatory changes. In older fetuses, cytolytic processes are reduced mainly by absence of a susceptible cell population rather than by immune mechanisms, but nonsuppurative encephalitis frequently persists as a residual lesion.

Destruction of developing neural tissue may be effected by mechanisms other than those directly cytopathic for primitive neuroectodermal cells. *Vascular damage* with consequential edema and necrosis is one such mechanism. Although the fetus is resistant to hypoxia, except if prolonged or profound, there is evidence of the potential of circulatory disturbances in the genesis of cavitating CNS malformations such as hydranencephaly.

There are additional poorly understood pathogenetic influences that doubtless contribute to the outcome of fetal interactions with teratogens. Such factors include *maternal and fetal genotype*, and experimental evidence suggests that *pharmacogenetic differences* among fetuses of a particular species are important determinants of the results of in utero drug exposure. With viral infections, teratogenic potential may vary markedly depending on the *strain of the virus* and upon the *immune status* of the maternal host.

The detailed discussions of CNS malformations that follow are organized on an anatomical basis, from cranial to caudal.

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Cerebrum

Cerebral aplasia, anencephaly

True **anencephaly**, which means *absence of brain*, is a rare event, and the term has been misapplied to cases of **cerebral aplasia**, or *prosencephalic hypoplasia*, in which the cerebral hemispheres are absent, but components of the brain stem (midbrain, pons, medulla) have formed (Fig 3.20). Anomalies of the same general or specific pattern vary considerably from case to case in the details of their expression, various

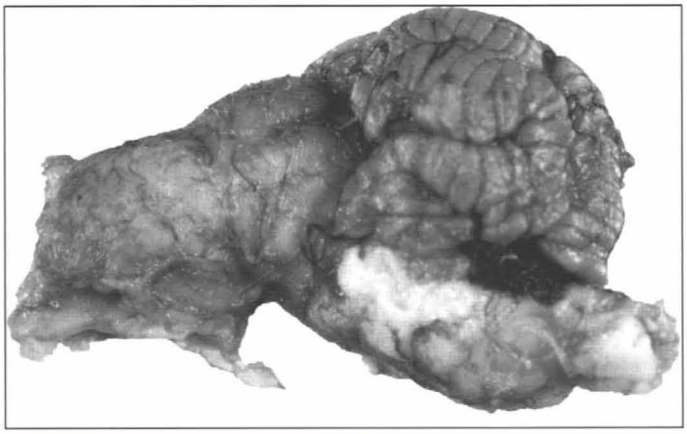

Figure 3.20 Cerebral aplasia ("anencephaly") in a calf. The cerebral hemispheres have failed to form, and only brain stem and cerebellum are present. (Courtesy of RF Slocombe.)

combinations of typical malformations occur, and there is considerable variability in the development of morphological sequelae.

The primary defect in cerebral aplasia is arrest of closure of the rostral portion of the neural tube. There is, in consequence, failure of development of the cranium and, with skin lacking over the lesion, the dysplastic rudiments of neural tissue are exposed to the exterior. Cerebral aplasia is seldom complete from the beginning because the eyes, either well developed or present as rudimentary vesicles, are present. When the eyes are rudimentary, ganglion cells and optic fibers may be missing. The cranial and neural defects are probably more or less proportionate. With severe degrees of cerebral aplasia, or true anencephaly, there may be complete failure of cranial development (**acrania**) or there may be rudimentary development of the occipital and adjacent bones. In those cases of cerebral aplasia in which the rostral portion of the brain stem is present, there may be a greater degree of cranial development but failure of fusion (**cranioschisis**). The base of the cranium is well developed, but there are a variety of anomalies of basal bones and no sella turcica. The neurohypophysis is absent and the adenohypophysis is either absent or unidentifiable. Failure of development of the neurohypophysis can be responsible for the failure of the adenohypophysis to develop normally, and this in turn may be responsible for prolonged gestation of some anencephalics. The caudal extent of the neural tube defect varies greatly. There may be involvement of the cervical vertebrae or of the entire neural tube (**craniorhachischisis totalis**); the defect is always continuous, not segmental. Even when the spinal cord is closed, it may be hypoplastic. When the cord is well developed, it is still small because of the absence of descending tracts.

In cerebral aplasia, the cerebral hemispheres are reduced to a tough formless mass of tissue on the exposed basal bones. The tissue is composed largely of blood vessels with some intermingled neural tissue and is termed the *area cerebrovasculosa*. Choroid plexuses may be recognizable, and the whole may be covered by a thin layer of squamous epithelium.

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Encephalocele, meningocele

Encephalocele *(meningoencephalocele, cephalocele) is protrusion of the brain through a defect in the cranium* (**crania bifida**). The defect is termed **meningocele** if only fluid-filled meninges protrude. In *exencephaly*, the brain may be either exposed or protruding. These anomalies can be inherited in pigs and in Burmese cats, and have been associated with treatment of the pregnant queen with griseofulvin.

The morphogenesis of these defects is not simply a problem of defective ossification of the skull with secondary herniation of preformed intracranial tissue but, instead, *depends on a primary neural tube defect by which there is focal failure of dehiscence of the neural tube from the embryonic ectoderm* and, in consequence, focal failure of development of the axial skeletal encasement. The herniations are related to suture lines and are almost always median. They vary from 2–10 cm in diameter, and the largest diameter is always much larger than the diameter of the cranial opening. The skin forms the hernial sac, and ectopic bits of disorganized neural tissue may be attached to it; the dura mater does not form in the areas of defect. Encephalocele and meningocele occur usually in the frontal regions (Fig. 3.21) but some are occipital (Fig. 3.22), and these latter tend to be located below the

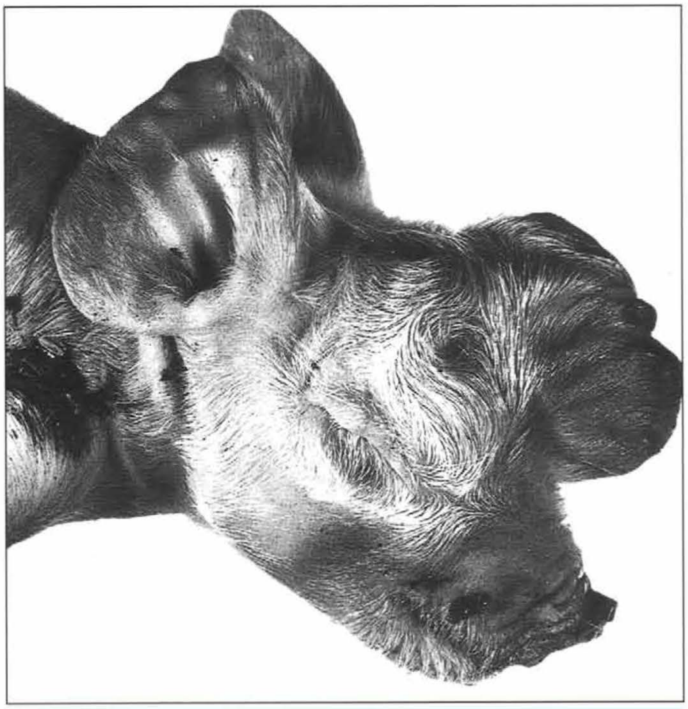

Figure 3.21 Meningocele in a piglet.

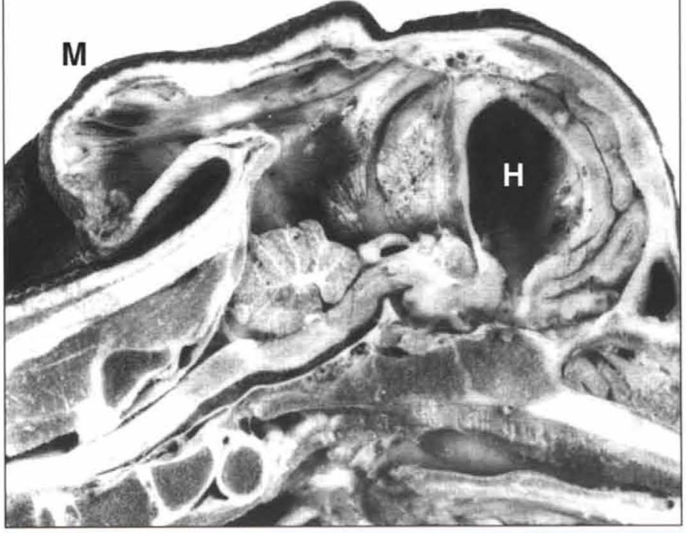

Figure 3.22 Hydrocephalus (H) and occipital **meningocele** (M) in a calf.

occipital crest. Occipital encephaloceles may also be associated with spina bifida of the upper cervical region, sometimes with enlargement of the foramen magnum and absence of the arch of the atlas.

Less well known in animals are *frontonasal or frontoethmoidal encephaloceles* that coexist with facial deformities.

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Defects in cerebral corticogenesis

These malformations have their teratogenetic period early in fetal life following closure of the neural tube, and usually occur in association with other neural anomalies. With the establishment of the telencephalic vesicles, cortical neurogenesis begins from the germinal epithelium lining the neural tube, especially in those parts destined to become the lateral ventricles. Initially, cell division gives rise to similar daughter cells that also act as progenitors so that cell numbers increase geometrically. Later, the cell divisions become increasingly asymmetric as an increasing proportion of progenitors produce postmitotic precursors of neurons. The first postmitotic neurons migrate to the subpial plate along guides produced by radial glia that extend from the periventricular zone to the cortical surface. Successive waves of migratory neurons will leapfrog the preceding waves such that the first waves will reside in the deeper layers of the cortical plate corresponding to layer 6 of the mature cortex and the later waves will migrate to form the more superficial layers. Errors of proliferation of germinal epithelial cells may result in microencephaly or macroencephaly; errors of migration result in various patterns of disorder affecting the cerebral gyri.

Microencephaly *refers to an abnormally small brain.* The diminution affects particularly the cerebrum. The hypoplastic brain is accommodated within the cranial cavity, which is often smaller than normal so that the cerebellum appears relatively large. The gyri are of normal size but simplified pattern, and the brain stem structures are normal. *The deficiency is of cerebral gray and white matter.* It may be an isolated defect or associated with any of a variety of other defects. Microencephaly is manifested externally by an abnormally flattened and narrowed frontal part of the cranium. The cranial bones, particularly the frontal bones, are thicker than normal. Microencephaly occurs in fetal infections by *Akabane virus* in lambs and calves, *Bovine viral diarrhea virus* in calves, *Border disease virus* in lambs, and *Classical swine fever virus* in piglets. The condition has been produced experimentally in lambs exposed to prenatal hyperthermia.

Megalencephaly *refers to an abnormally large brain or excessive volume of the intracranial contents.* Enlargements as a result of excessive germinal cell proliferation are rare and tend to be asymmetrical with exaggerated degrees of heterotopia.

Cortical dysplasia *encompasses defects in the architecture of the cerebral cortex.* In its mildest expression, there is only histological evidence of lack of the normal orderly layered appearance of the cerebral cortex. *Neuronal heterotopia* is the presence of clusters of nerve cells at a site where they are normally absent, such as subcortical white matter. This condition represents incomplete migration of neuroblasts during fetal life and is usually associated with dysplastic development of the cortex. Scattered, rather than clustered, neurons are commonly present in subcortical white matter and are not of clinical significance. *The presence of aggregations of small dark primitive neuroglial cells is a normal finding in periventricular locations and in the rhinencephalic cortex.* Heterotopia may also involve glial elements, here taking the form of aberrant nests of glial cells.

In **microgyria** *(polymicrogyria), convolutions are small and unusually numerous, and the normal gyral pattern is lost in affected areas.* The lesion may be asymmetrical or patchy, abruptly demarcated from normal cortex, but its real extent may only be revealed on cut surfaces. It may be present near the margins of cavitating lesions such as hydranencephaly. **Ulegyria** also imparts a wrinkled appearance to the cortex but arises as a consequence of scarring and atrophy in otherwise topographically normal gyri. It is a *result of laminar necrosis*, particularly affecting sulci, caused by prolonged ischemic/anoxic injury in the perinatal period.

In **lissencephaly** (agyria), the primitive pattern of the telencephalon persists due to arrested migration of neuroblasts from the ventricular zone along radial glial fibers to the appropriate cortical lamina. *Convolutions are almost entirely absent* and the brain surface may be smooth except for slight grooves in which the meningeal vessels are situated. The cerebral cortex is excessively thick. In humans with *LIS1* or *DCX* gene mutations, failure of dynein-mediated nucleus–centrosome coupling leads to abnormal neuronal migration. Lissencephaly occurs in Lhasa Apso dogs.

Pachygyria (macrogyria) is characterized by *excessively broad brain convolutions* resulting from fewer secondary gyri and increased depth of the gray matter underlying the smooth part of the cortex. Pachygyria is a transitional malformation of the cortex, less severe than agyria but akin to it.

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Disorders of axonal growth

As neurons migrate from the periventricular germinal zone along radial glia, they begin to form axonal and dendritic processes. The growth and direction of growth of these processes are guided by complex cues including molecules of the extracellular matrix and there is an extensive literature relating to the molecular genetics that regulate these processes.

Agenesis or hypoplasia of the corpus callosum is an *uncommon anomaly* but is recorded in most domestic species. It is the most studied of the disorders of axonal growth in human and animal models. It may occur alone or in association with other anomalies of the brain (Fig. 3.23). The septum pellucidum is absent as a collateral

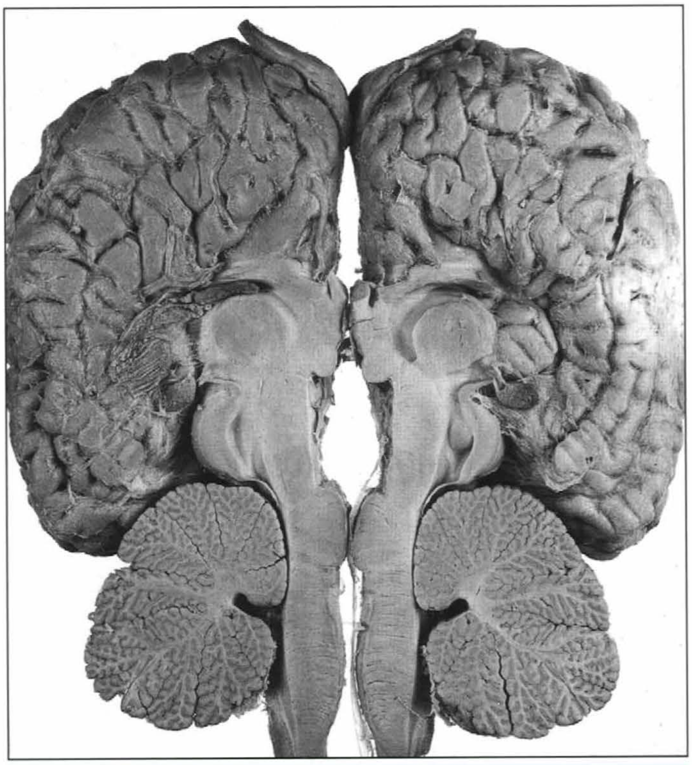

Figure 3.23 Agenesis of corpus callosum in a horse.

defect; the leaves are separated and displaced laterally to the roof of the lateral ventricles. There is no cingulate gyrus, the interhemispheric gyri appearing to radiate from the roof of the third ventricle.

The corpus callosum is formed by the crossing-over of fibers from one hemisphere to the other, a migration that begins at the lamina terminalis, which is the most rostral point of the neural tube. Dysgenesis of the prosencephalic vesicle in its midline may impair the lamina terminalis, or there may be disordered outgrowth of axons from the cerebral cortex. The migration of fibers is guided by a glial scaffold, which may be absent as the primary defect. The corpus may be entirely absent or the commissures may be present and the corpus defective in its central part.

The development of the septum lucidum is linked to that of the corpus callosum and its residue may form a **cystic septum pellucidum**. The cavum septi pellucidi is always present in the fetus as a midline cavity that originates within the commissural plate by tissue resorption. In the normal course, the cavity is obliterated, resulting in a glial midline raphe, but *failure of fusion of the leaflets results in a cystic fluid-filled cavity that varies from a thin slit to a rounder cavity*. The cyst is covered externally by ependyma and lined by a glial mesh. This condition occasionally accompanies defects caused by fetal infections with viruses such as those of Akabane disease, bluetongue, bovine viral diarrhea, and Border disease.

Holoencephaly

Immediately on closure of the rostral neuropore, the rostral end of the tube enlarges to form three vesicles, the rostral of which, or *prosencephalon*, will become the forebrain, the intermediate vesicle will form the *mesencephalon* or midbrain, and the caudal vesicle will form the hindbrain or *rhombencephalon*. The dorsal wall of the rostral vesicle becomes thickened dorsally in the midline to form the commissural plate, eventually the corpus callosum, and the vesicle expands laterally to form the paired cerebral vesicles, which will become the cerebral hemispheres.

Holoencephaly (holoprosencephaly) refers to a spectrum of deformities involving the hemispheres and typically including aplasia of the olfactory bulbs and tracts (arhinencephaly), as a result of failure of cleavage of the prosencephalon. The dysplasia is typically associated with facial deformities, and the extent of the nervous and facial deformities correspond closely. The most severe expression is cyclopia (synophthalmus), but the spectrum ranges through intermediate expressions such as cebocephaly to lesser deformities in which the orbits and nose may be normal or the nares are retruded with variably expressed clefts of lips and palate.

Cyclopia, referring to the presence of a *single large median eye*, emphasizes the most obvious and remarkable abnormality of a very complicated defect. The condition is not uncommon in domestic animals, especially pigs, and it can be reproduced experimentally simply and in many different ways.

The causes of sporadic cases are largely unknown, but many instances in humans are due to chromosomal anomalies. Various degrees of cyclopia are present in Guernsey and Jersey fetuses that are the subjects of *prolonged gestation. Veratrum californicum* is responsible for congenital cyclopian malformations that occur endemically in lambs in the western livestock grazing areas of the USA. The teratogenic agent in the plant is *cyclopamine*, a steroidal alkaloid. Induction of the cyclopian deformity in lamb fetuses is dependent on pregnant ewes ingesting *V. californicum* on day 14 of gestation. Cyclopia has also been produced experimentally in cattle and goats, with a similar narrow interval of susceptibility to maternal ingestion of *Veratrum* on or about day 14 of gestation. Cyclopamine-induced teratogenesis may result from antagonism of Sonic hedgehog gene signal transduction that is necessary for normal dorsoventral patterning of the neural tube and somites. The primary defect occurs at the neural plate stage of development and involves the rostral extremity of the notochord and the mesoderm immediately surrounding it. Failure of proper induction accounts for the changes in the skull, in soft tissues of the face, and in the brain. The optic defect, consisting of greater-or-lesser division of a single anlage, is probably secondary to the defects of the forebrain.

The orbits may be approximated (Fig. 3.24) but, typically, there is one large orbit and a single optic foramen. Several bones, including the ethmoids, nasal septum, lacrimal bones, and premaxillae, are usually absent. The globe may be absent, rudimentary, or may form a single structure of near-normal conformation, or be partially divided or completely duplicated. The nose is a proboscis or tube that does not communicate with the pharynx and that is typically situated above the median eye. The forebrain is always severely malformed; the hemispheres are not fully cleft but are present instead as a single thin-walled vesicle with a smooth surface and common ventricle and lacking olfactory nerves and tracts, corpus callosum, septum pellucidum, and fornix.

The hindbrain is usually normal. The pituitary may be displaced or absent and fetal gigantism associated with prolonged gestation (see Vol. 3, Female genital system) occurs in some deformed lambs and calves. The optic nerve is single, atrophic, or absent. The oculomotor and abducens nerves are hypoplastic or absent. There is internal hydrocephalus.

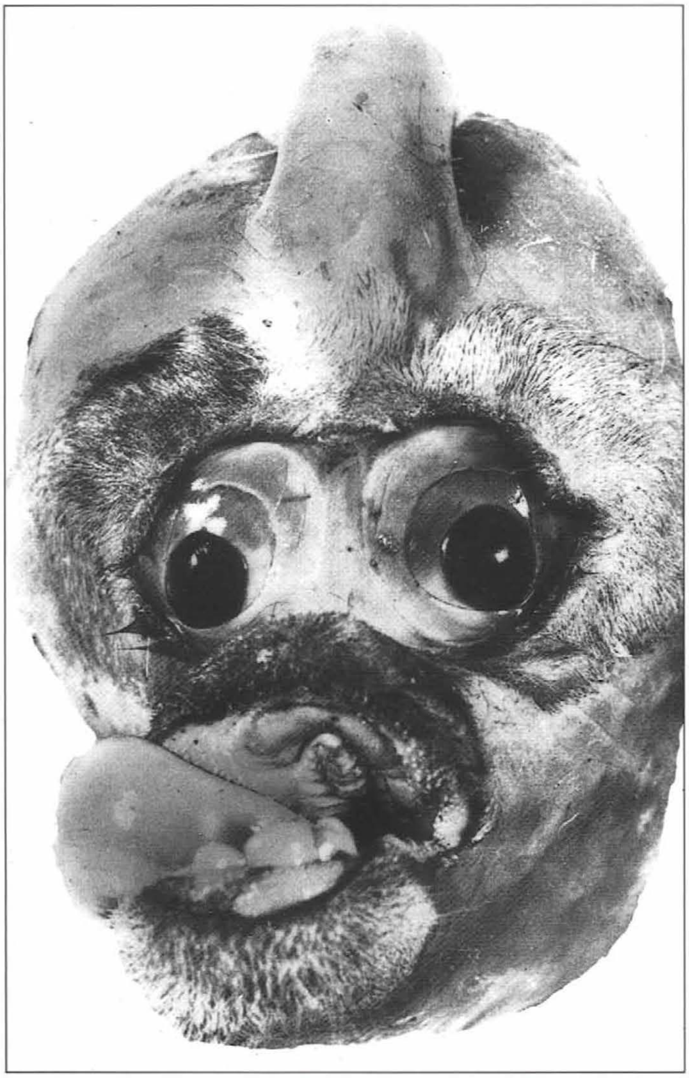

Figure 3.24 Cyclopia (synophthalmus) in a calf with inherited prolonged gestation.

Veratrum californicum also causes embryonic death. A wide range of other defects has been produced experimentally in lambs, with abbreviation of the metacarpal and metatarsal bones being the most distinctive.

Cebocephaly (monkey face) *is anatomically comparable to cyclopia, and probably represents a less severe expression of the same basic defects.* There are two eyes, severely hypoplastic, in separate but approximated orbits. The nose is in the normal position, but deformed and may not protrude. There is a single, small nasal cavity or a proboscis with no communication with the pharynx. The brain appears as in cyclopia but may have a slight median sulcus.

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Hydrocephalus

Hydrocephalus is characterized by abnormal accumulation of fluid in the cranial cavity. In **internal hydrocephalus**, the fluid is within the ventricular system; in **external hydrocephalus**, the fluid is in the arachnoid space; and in **communicating hydrocephalus**, the excess fluid is present in both locations. The communicating and external types of hydrocephalus, the latter to be distinguished from cerebral atrophy, are quite rare in animals. Internal hydrocephalus, which is denoted by variably dilated ventricular cavities lined by ependyma, is quite common, may be congenital or acquired, but both forms may be considered here for convenience.

Cerebrospinal fluid (CSF) is produced by the ventricular choroid plexuses by means of filtration and secretion. There are significant contributions by ependyma and extraventricular structures. Because of the permeable nature of the ependymal lining of the ventricular system, the CSF is in effect an extension of the extracellular fluid of the CNS, and its composition is affected by metabolic and pathological changes within the brain.

The flow of CSF is from the lateral ventricle through the interventricular foramen to the third ventricle and then via the mesencephalic aqueduct to the fourth ventricle. From here, most of the fluid leaves the ventricular system and passes by way of the lateral cerebellomedullary apertures into the subarachnoid space. A small amount of CSF passes into the central canal of the spinal cord from the fourth ventricle. The greater part of the fluid flows forward into the cerebral subarachnoid spaces and basal cisterns; the balance circulates in a restricted fashion in the spinal subarachnoid space. The energy required to circulate CSF is imparted largely by the choroid plexuses through their production of fluid. The arterial pulse also contributes to CSF movement through associated variations in hemispheric volume. Venous resorption of CSF occurs where arachnoid villi form in the walls of the larger meningeal veins. Transfer of fluid is effected by hydrostatic pressure, with reflux being prevented by the valvular nature of the villi. The arachnoid villi are normally highly permeable, being able to permit the passage of red blood cells. Impedance of this resorption appears to contribute to the increased intracranial pressure of hypovitaminosis A in calves. Some outflow in dogs can occur via the cribriform plate and in cats along the subarachnoid space of olfactory, optic, and acoustic nerves, but significant outflow may possibly occur only in association with raised intracranial pressure. The spinal subarachnoid space appears to communicate freely with the lymphatic system opening in the intradural nerve roots.

Familiarity with the normal directional flow of CSF is basic to an understanding of the pathogenesis of hydrocephalus because in most cases it is probably of obstructive origin. Certainly, *an obstruction can be demonstrated in most instances of acquired hydrocephalus in animals.* In congenital hydrocephalus, obstruction is quite often not demonstrable, but stenotic aqueductal malformations may be found (Fig. 3.25). Obstructive hydrocephalus has been induced experimentally in many species of immature laboratory animals with a range of viruses ubiquitous in animals and man. The selective destructive action of these viruses on ependymal cells with subsequent reparative gliovascular

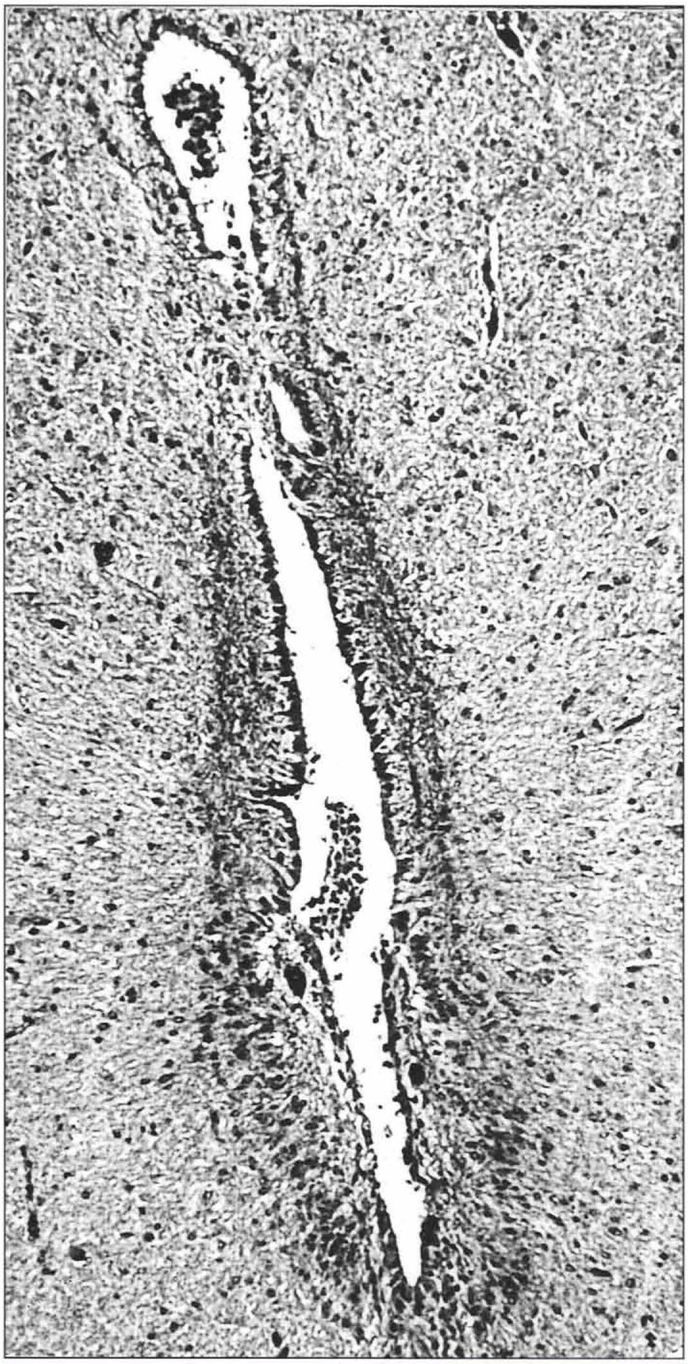

Figure 3.25 Malformation of aqueduct in a calf.

proliferation results in obstruction of CSF pathways, usually in the mesencephalic aqueduct. Thus far in domestic animals, viral-induced hydrocephalus of this genesis has been demonstrated only in experimental canine parainfluenza virus infection in dogs.

Hydrocephalus is "physiological" in the early fetus when the hemispheres are largely thin-walled vesicles. *Congenital hydrocephalus exaggerates this physiological degree of ventricular dilation.* Even in the absence of obstruction, physiological hydrocephalus may persist or be exaggerated in instances of neural dysplasia such as, for example, in cyclopia and cebocephaly. The cavitating cerebral defects, hydranencephaly and porencephaly, are associated with internal hydrocephalus. This is likely to be an *ex vacuo* or compensatory hydrocephalus

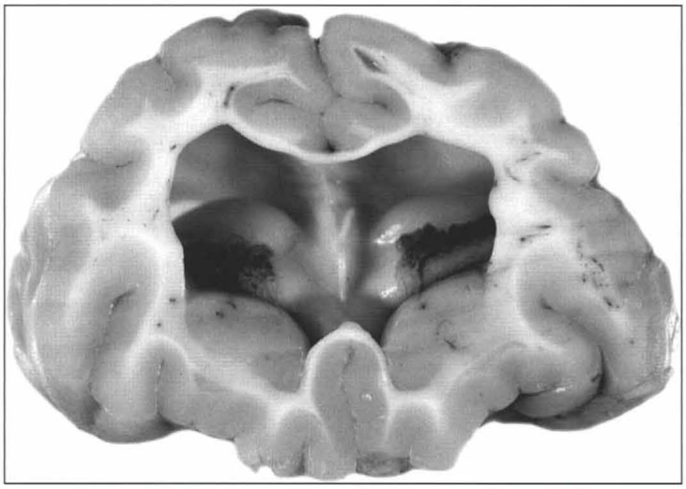

Figure 3.26 Hydrocephalus in a cat, due to vitamin A deficiency. (Courtesy of RF Slocombe.)

occurring secondary to loss of cerebral tissue as in the inherited leukodystrophies and storage diseases, but a hydrostatic component arising from deranged circulation of CSF is also possible. Hydrocephalus may accompany other neural anomalies, but chiefly the rather rare Arnold–Chiari and Dandy–Walker malformations in which hydrocephalus is obstructive in origin and associated with abnormalities of the cerebellum and medulla.

With the exception of the few examples just given, *congenital hydrocephalus remains anatomically obvious but pathogenetically obscure*, and such cases are common. Pups, calves, foals, and piglets are chiefly affected, with a familial incidence, possibly genetic, in pigs. The defect is quite uncommon in cats.

Congenital hydrocephalus is well known in **pups**, especially those of the brachycephalic breeds. This should not imply that the hydrocephalus is correlative to, or a product of, brachycephaly because within breeds there is not much variation in the degree of the skeletal defect, and no obvious relation between it and the presence and severity of hydrocephalus. Malformation of the mesencephalic aqueduct may be a significant pathogenetic factor. The anatomic expression of the hydrocephalus also varies considerably; one lateral ventricle may be involved or both may be dilated symmetrically or asymmetrically, the third ventricle and rostral portion of the aqueduct are usually, but not always, involved, and the fourth ventricle is normal.

Sporadic cases of congenital hydrocephalus occur widely in **cattle**. Many appear to be secondary to aqueductal stenosis. An autosomal recessive gene is considered responsible for many apparently hereditary cases, but the possible roles of fetal infections and nutritional factors must be considered.

Outbreaks of congenital hydrocephalus are recorded in calves in which slit-like deformation of the aqueduct of Sylvius was associated with lateral narrowing of the midbrain, in the peripheral areas of which there was vascular proliferation and perivascular gliosis. Hydrocephalus also occurs in association with chondrodysplasias, especially of the "bulldog" type, but the primary neural defect in these is not known. Several hydrocephalic syndromes have been described in Hereford and Shorthorn cattle; features include cerebellar hypoplasia, microphthalmia, myopathy, and ocular anomalies.

Experimentally, *dietary deficiency of vitamin A* can cause congenital and neonatal hydrocephalus (Fig. 3.26), but spontaneous outbreaks occur only in cattle which have fed for prolonged periods on dry pasture or in feedlots. The hydrocephalus is ascribed to functional impairment of absorption of fluid from arachnoid villi but there is frequently severe compression of the brain and herniations in the caudal fossa, which is expected to provide mechanical obstruction to drainage.

Acquired hydrocephalus is fairly common in animals. It does not approach in severity the congenital defect. The causes are almost always obstructive, but minor degrees of ventricular dilation occur in association with cerebral atrophy in old dogs. Meningeal lesions that destroy the arachnoid villi can lead to external hydrocephalus. This is, however, quite rare, although it has been observed in diffuse meningeal carcinomatosis. Most diffuse meningeal lesions are inflammatory, but these are typically associated with internal rather than external hydrocephalus because meningitis tends to involve the basilar regions and the caudal fossa chiefly and there interferes with the patency of the lateral cerebellomedullary apertures. Most cases of bacterial meningitis are fatal before the changes of hydrocephalus develop unless, as frequently happens, concurrent inflammation of the choroid plexuses extends to the ependyma of the aqueduct and obstructs that channel. Even relatively chronic cases of meningitis, such as may be observed in cryptococcosis, are associated with internal rather than communicating or external hydrocephalus. Additional causes of acquired hydrocephalus are intracranial neoplasms, usually primary but sometimes metastatic, and including papillomas and carcinomas of the choroid plexus, parasitic cysts such as hydatids and coenurids, and the late effects of chronic or healed inflammation that involve the ependyma or cause inflammatory softening or atrophy of paraependymal tissue. The pyogranulomatous ependymitis and meningoencephalitis of feline infectious peritonitis occasionally results in hydrocephalus. In horses, the so-called cholesteatomas or cholesterol granulomas that develop in the choroid plexuses of the lateral ventricles may occlude the interventricular foramen and cause internal hydrocephalus.

Hydrocephalus does not regress. Whether it progresses or not is difficult to determine. Congenital hydrocephalus cannot be easily diagnosed in the newborn in the absence of secondary changes in the cranium, and diagnosable cases seldom live long enough for the course of the defect to be ascertained. Probably, however, congenital hydrocephalus of mild or moderate degree can remain static because, although a severe and fatal defect is common enough in puppies, *hydrocephalus in brachycephalic breeds of dogs is frequently an incidental finding at necropsy*, and the degree of ventricular dilation may be minor or moderate irrespective of the age of the animal. Acquired hydrocephalus in postnatal life tends to be progressive when of obstructive type, the course depending on the site and nature of the obstructing lesion. Compensatory hydrocephalus occurring as a response to cerebral atrophy is static or at the most slowly progressive; it is never severe, except perhaps in familial lipofuscinosis of dogs.

Congenital hydrocephalus is frequently associated with malformation of the cranium. The degree of cranial malformation varies from slight doming, which may be difficult to appreciate, to enormous enlargement, which may cause dystocia. Cranial malformation is not invariable however, and many cases of congenital hydrocephalus of considerable severity may occur with a skull of normal contour. Whether cranial malformation occurs or not probably depends on the time of onset of the hydrocephalus relative to the degree of ossification of the cranial bones and the development and strength of the sutures, and also on the rate at which the fluid is accumulated.

It is often difficult to be certain of the presence of minor degrees of hydrocephalus. The soft brains of the newborn collapse when removed from the skull so that dilation of ventricles may not be apparent. In older, firmer brains, asymmetry of the lateral ventricles and relative dilation of the rostral end of the aqueduct when compared with the caudal end are useful indices. *The septum pellucidum, however, is the structure most sensitive to the effects of fluid accumulation*; it may be fenestrated or may persist as an irregular lacework of connective tissue, but typically, it is absent. Even in mild hydrocephalus, there is usually atrophy in Ammon's horn readily detectable by the ease with which the piriform lobes dimple under slight pressure. With hydrocephalus of greater severity, there is ventricular dilation of corresponding degree. The lateral and third ventricles are most severely affected, and there may be no alteration in the fourth. With ventricular dilation, parenchymal atrophy affects chiefly the white matter and the cerebral cortices; ventrally, the increased pressure is buttressed by the basal ganglia. The corpus callosum is elevated and thinned, and the cerebral cortices over the vertex may be reduced to thin shells of gray matter. The floor of the third ventricle is extremely thinned, the hypophysis is atrophied, and the cerebellum is compressed and displaced caudally.

The extensive cranial malformation of congenital hydrocephalus can occur only if the sutures are ununited. The temporal, frontal, and parietal bones are enlarged and thin and are separated from each other by broad membranes of connective tissue in which accessory bones may form. In these severe cases, the base of the cranium is flattened, the fossae are enlarged and smoothed out, and the orbits are separated but individually reduced in size so that the eyes may protrude.

Gray matter is remarkably resistant to the effects of the pressure exerted by the fluid, but the subcortical white matter degenerates rapidly. It is edematous, the oligodendrocytes and astrocytes are reduced in number, and compound granular corpuscles can be found sometimes in short bands lying deep and parallel to the ependyma. The ependyma, tela choroidea, and meninges are usually not altered significantly.

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Hydranencephaly, porencephaly

In **hydranencephaly**, *there can be complete or almost complete absence of the cerebral hemispheres, leaving only membranous sacs filled with CSF and enclosed by leptomeninges.* The cranial cavity is always complete, in contrast to hydrocephalus, and usually of normal conformation, although occasionally there is mild doming of the skull or thickening of the cranial bones. The dorsal, and often the caudal parts of the hemispheres, are the portions most severely defective (Fig. 3.27A, B, C). The leptomeninges may easily be damaged on removing the calvaria but are in their usual position and form sacs enclosing CSF, the fluid occupying the space normally occupied by parenchyma. Discrete remnants of parenchyma may be present in the meninges. When the leptomeninges are incised, it is apparent that the brain stem is of near-normal conformation with well-developed hippocampus and choroid plexuses. The rostral portion of the corpus callosum and septum pellucidum may be intact although attenuated. Cerebellar hypoplasia may be present as a concurrent defect as may the histologic deficits of hypomyelinogenesis.

Hydranencephaly is the residual lesion of full-thickness necrosis of the cerebral hemisphere. In animals, the lesion develops in early fetal stages and before the mature arrangements of the cortex are present. The marginal tissue is dysplastic, flat, and microgyric or the gyri have a radial arrangement from the defect. Although a diagnosis of hydranencephaly is readily made on macroscopic inspection, histological study

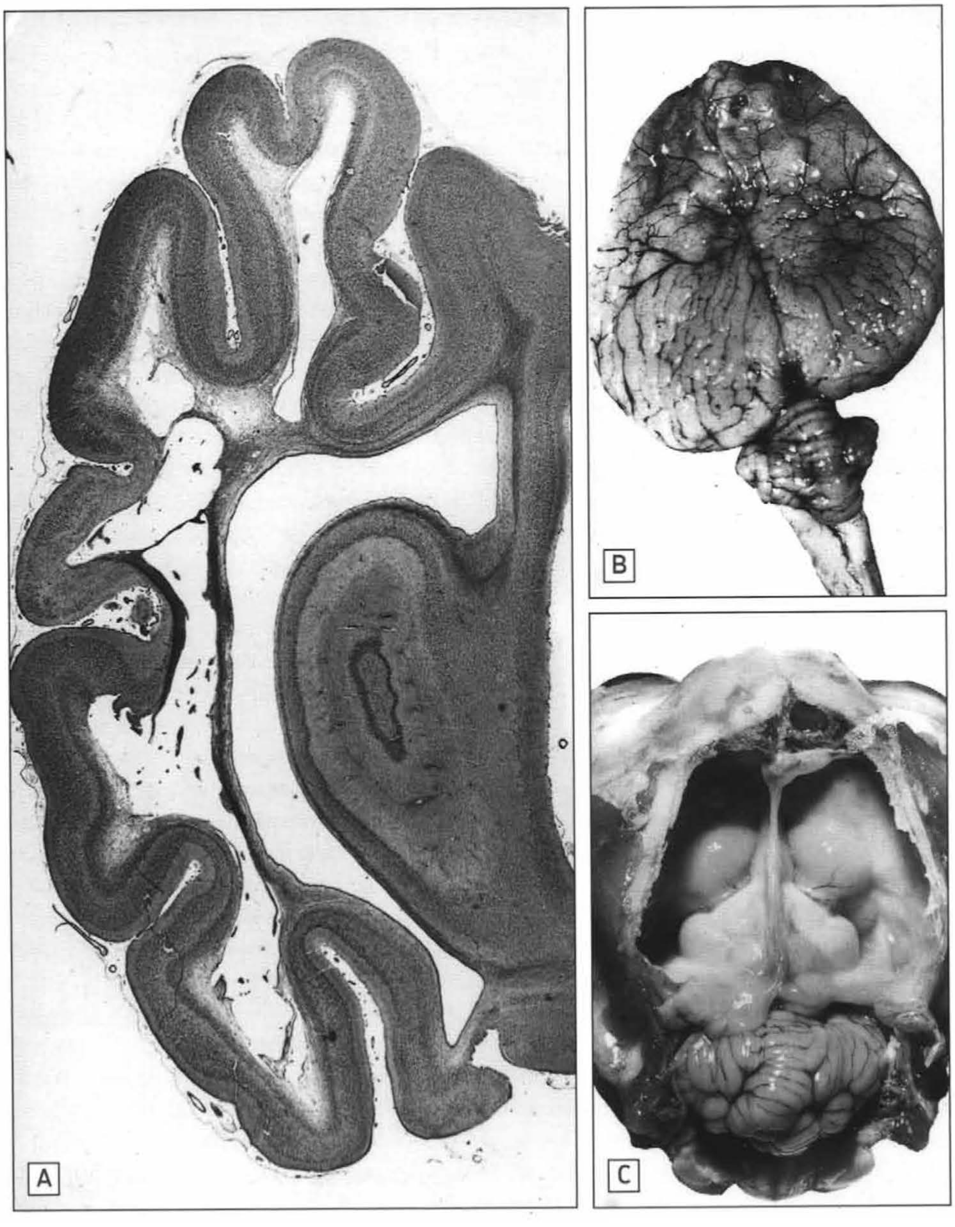

Figure 3.27 A. Hydranencephaly in a lamb with swayback. (Courtesy of J McC Howell.) **B. Hydranencephaly** and cerebellar hypoplasia in a lamb, subsequent to intrauterine *Bluetongue virus* infection. **C. Hydranencephaly** in a cat; meninges removed to expose the brain stem structures.

may provide some insight into the nature of the disease process. The membranous coverings of the remnants of the hemispheres comprise arachnoid, pia, and a thin mantle of residual cortex in normal juxtaposition. The residual cortical tissue may be lined by attenuated ependyma displaced outwards with expansion of the lateral ventricles, but this ependymal lining is frequently incomplete or absent, and the cavity abuts directly on cortical tissue which may be unremarkable except for mild astrocytosis.

Hydranencephaly occurs in all species but is most common in calves, in which it occurs either sporadically or as minor epizootics. The lesion occurs similarly although less frequently in *lambs*. The species occurrence of hydranencephaly reflects its etiological associations with certain viruses infecting the fetus at a critical stage of gestation. In these cases, *hydranencephaly is often part of a spectrum of neural lesions*, the expression of which depends mainly on the age of the fetus at infection. Viruses that are well established as causes of hydranencephaly tend to be either arboviruses, such as *Akabane virus, Bluetongue virus, Rift Valley fever virus* and *Wesselsbron virus*, or pestiviruses, such as *Bovine viral diarrhea virus* and *Border disease virus*.

The *pathogenesis* of hydranencephaly has been clarified through studies of the cavitating encephalopathies caused by fetal viral infections, particularly bluetongue and Border disease in lambs. These infections at critical periods of gestation produce *subventricular zones of necrosis* of the developing cerebral hemispheres. These zones, which may have a vascular basis, involve the neuroblasts in their outward migration and result in cavitation and deprive the cortex of its normal complement of neurons. These cavitations range in size from small cysts with only minor changes in the overlying cortex (porencephaly) to large confluent spaces with the hemispheres reduced to fluid-filled sacs (hydranencephaly). During the necrotizing process, there may be an intense macrophage response, but it is usual for this reaction to have subsided by the time the animal is born and examined.

Mechanical factors also contribute to the development of hydranencephaly. A dissecting effect associated with escape of CSF into the parenchyma may also be important since segmental loss of ventricular ependyma is an early feature of the cavitating process.

Compensatory expansion of the lateral ventricles secondary to loss of brain substance occurs and rapid expansion of the fetal calvaria during the gestation period allows stretching and rupture of residual cortical tissue. In less severely affected areas, the outer rim of cortex overlies a band of subependymal tissue, the intervening cavity being occupied by trabecular parenchymal remnants. Rosette formations of cells that resemble ependymal epithelium may be found in the subependymal area. Accumulations of mineralized debris may be present in the meninges.

Porencephaly *is cystic cavitation of the brain evolving from a destructive process in prenatal life.* The defect typically involves the white matter of the cerebral hemispheres. An affected brain may contain a single cyst or there may be multiple cystic lesions. The temporal portion of the cerebral hemispheres is an area of predilection, but porencephalic change may be found throughout the cerebral hemispheres, although typically sparing the basal nuclei. Occasionally, lesions are found in brain stem and cerebellum. The cysts are usually randomly located, but evidence of bilateral symmetry is sometimes apparent, particularly in well-developed lesions of the cerebral hemisphere. Rarely cysts may communicate with ventricular cavities or with the subarachnoid space. The cysts may be apparent from the meningeal aspect as focal fluctuant areas of attenuated cerebral cortex or as superficial, clear submeningeal cysts. The cysts range in size from microscopic to several centimeters in diameter. The cysts are variable in shape, but roughly spherical or cleft-like outlines are common. On section, they are filled with clear fluid and are smooth walled, but are traversed by variable numbers of trabeculae. Less well-developed lesions may appear as gelatinous softenings.

The porencephalic cavities, particularly the larger defects, are often unremarkable microscopically, being lined by a layer of flattened glia. Apart from some mild marginal astrocytosis, they show surprisingly little evidence of reactive or inflammatory change. In other cases, there is accumulation of hemosiderin-containing gitter cells about the margins of the cyst and within the cavity. The trabeculae comprise residual brain parenchyma, usually oriented about a blood vessel. Some small lesions, evident grossly as focal gelatinous areas, appear as focal leukomalacia comprising white matter in the process of dissolution associated with accumulation of macrophages and gitter cells. Sometimes the inflammatory nature of the lesions is indicated by the presence of mild nonsuppurative meningoencephalitis with gliosis, perivascular cuffing and focal mineralization about the margins of the cysts, and mononuclear infiltration in the meninges overlying the defects.

The etiopathogenesis of porencephaly parallels, but is a less severe expression of, the pathological process seen in hydranencephaly. Both lesions are commonly recorded in the course of outbreaks of cavitating cerebral defects and may occur together in the same brain. In the case of cavitating viral infections of the fetal brain, termination of the disease process in either porencephaly or hydranencephaly is influenced by gestational age at infection, with porencephaly tending to follow infection at a later stage than is the case with hydranencephaly.

Porencephaly is a common manifestation of prenatal infection of *lambs* with some strains of *Border disease virus* and is seen in calves infected in utero by *Bovine viral diarrhea virus*. The cystic or gelatinous transformations of the cerebral white matter that occur in some cases of copper deficiency in lambs (swayback) are porencephalic in nature. The lesion has also been induced experimentally in lambs by exposure of pregnant ewes to hyperthermia during the last two-thirds of pregnancy.

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Periventricular leukomalacia of neonates

Interrelated lesions dominated by intraventricular hemorrhage, ventriculomegaly, and malacia of white matter are quite frequent in neonates. In domestic animals, the lesion has not been distinguished from hydrocephalus and hydranencephaly, which is the reason for considering it here with the malformations from which it needs to be distinguished, rather than with malacic diseases. The application of imaging technology has identified a surprising incidence of the complex in human neonates with later minimal to severe neurological deficits. The lesions in human neonates are most frequently associated with placentitis, prematurity, and growth retardation, suggesting that placental insufficiency may be critically important. In lambs, there is an association with placentitis of toxoplasmosis, tick-borne fever, and chlamydiosis.

Periventricular leukomalacia typically refers to necrosis of white matter adjacent to the lateral ventricles that results in the formation of cysts in the cerebrum or in more extensive lesions simulating hydranencephaly or hydrocephalus *ex vacuo*. In animals, the lesion is not limited to the cerebrum but may extend to affect also periventricular white matter of the cerebellum, cerebellar roof nuclei, and white matter of the cerebellar folia.

Intraventricular hemorrhage may extend caudally as far as the central canal of the spinal cord causing ventricular dilation. The hemorrhage of variable severity may remain intramural in the hemispheral white matter.

Periventricular leukomalacia may allow survival in pups for several weeks. The cavitating cerebral lesion, and the cerebellar lesion if present, is lined internally by ependyma and a thin layer of white matter. There may be local defects allowing communication with the dilated ventricle. Clustered cases are observed in kids and lambs. Affected neonates tend to be small for age, stillborn, or die immediately after delivery, although some with minor neurologic defect may survive and develop normally.

The pathogenesis of these defects is unknown. The mechanisms appear to involve the structural fragility of blood vessels in the periventricular subependymal zone and instability of blood flow in the vulnerable areas. Perivascular hemorrhages may be confined to this zone. The malacic lesion in white matter is coagulative necrosis with vacuolation of the tissue and acidophilic swollen retraction balls on axonal fragments. Microglia provide the only inflammatory cells. Cystic lesions have peripheral sprigs of capillaries and marginal and luminal macrophages expected to contain hemosiderin. In individual cases in lambs and kids, the lesions may be of different ages and, in clustered cases in these animals, some may show hemorrhage only and some may show established cavities.

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Cerebellum/caudal fossa

The cerebellum develops from the lips of the rhombencephalon in the region of incomplete closure of the neural tube. There are *two major germinative zones*. The rostral lips give rise to precursors of the cerebellar granule cells. In their first migration, granule cell precursors cover the whole of the cerebellar pial layer at about the time of closure of the rostral neuropore. This is a germinative layer that produces a second generation of germinal cells, some of which remain as daughter cells and others undergo lateral migration in the developing molecular layer. In a third migratory wave, postmitotic cells send axons vertically down through the molecular and Purkinje-cell layers under guidance of radial glia; the cell body then moves along the axon to the maturing granular layer.

Disorder of the granule cell layer is frequently part of the disruption caused by fetal viral infections. *Focal heterotopias* are frequent in neonates usually without clinical effect. These cells may be the origin of the medulloblastomas of young animals.

The caudal rhombic lips produce a highly proliferative neuroepithelium from which neuronal precursors migrate along several pathways to form the Purkinje cells of the cerebellar cortex, the cerebellar roof nuclei, and to the precerebellar nuclei including the olivary, cuneate, and reticular nuclei of the medulla, and to the pontine and tegmental nuclei of the pons; axons from these nuclei project back into the cerebellum.

The cerebellum has three functionally distinct regions. The *vestibulocerebellum* regulates balance and eye movements. It is the oldest part of the cerebellum (paleocerebellum) and occupies the flocculonodular lobules where it receives afferents from the vestibular nuclei. The *spinocerebellum* regulates limb and body movements, receiving somatosensory information via the spinocerebellar tracts and precerebellar nuclei, and occupies the vermis and medial aspects of the hemispheres. The lateral hemispheres, the *cerebrocerebellum*, receive input from the cerebrum via pontine nuclei and are involved in the composition of complex movements. Within these main cerebellar regions, there is further regional patterning corresponding to body parts.

Disorders of migration and survival of Purkinje cells underlie many of the cerebellar malformations, and premature loss is the most common component of the abiotrophies. Correlative changes may be present in the precerebellar nuclei and in the cerebellar roof nuclei.

Cerebellar defects are among the more important of the developmental anomalies of the CNS because of their frequent occurrence and almost invariable accompaniment by significant and distinctive clinical manifestations. They may be expressions of failure of intercellular communication and support whereby a defect in one cell lineage may adversely affect the organizational pattern and function and viability of other cell types. *Cerebellar defects are quite common in cats and calves, relatively so in pigs, dogs, and lambs, but uncommon in foals.* Most defects of morphogenesis can be divided into two broad categories and the entities are discussed below as being examples of either *cerebellar hypoplasia* or *cerebellar abiotrophy (atrophy)*. This distinction into hypoplastic and atrophic types is somewhat artificial, because it is apparent that many cases are compounded by both processes.

Minor dysplastic lesions of no consequence are quite common in young animals but are fewer or less conspicuous in adults. They are most common in the flocculonodular lobules and where the cortex terminates at the peduncles. The foci are microscopic and consist of tangled islands of germinal, molecular, and granular layers with Purkinje cells distributed haphazardly. The dysplasias of the Arnold–Chiari and Dandy–Walker syndromes, of copper deficiency, and the metabolic storage disorders are discussed separately.

The name of the teratological condition merely emphasizes the major component, in these cases the cerebellar defect. There may, however, be coincident malformations such as agenesis of the corpus callosum or hydranencephaly. *There are always correlative changes*, this term grouping together those structural changes that occur in nuclear masses that send fibers to, or receive fibers from, the cerebellum as well as in the particular tract of fibers themselves. The term implies that there is a causal connection, the cerebellar defects being primary and the subcerebellar defects being secondary, but such an implication is less likely to be valid for the cerebellar hypoplasias than for the cerebellar atrophies. Anatomic classifications of the cerebellar hypoplasias tend to take into account the distribution and severity of correlative changes, especially those in the deep cerebellar nuclei, the pontine nuclei, the inferior olives and in the cerebellar brachia, the restiform bodies, and the spinocerebellar tracts. The pattern of correlative changes varies from case to case, and there is not a quantitative correspondence between the cerebellar defects and the correlative defects. There are also very wide variations in the severity of the cerebellar hypoplasias, which is reasonable if the severity is properly to be related to the time of onset, duration, and severity of the arrest of development. From these viewpoints, *anatomical classification of cerebellar hypoplasias is artificial because the defects are quantitative.*

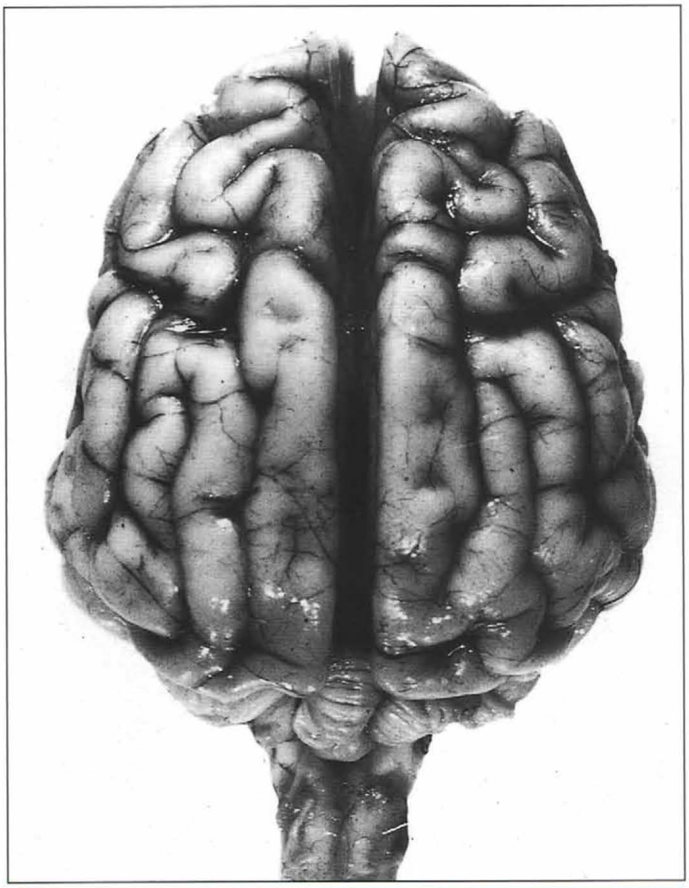

Figure 3.28 Cerebellar hypoplasia in a dog.

Cerebellar hypoplasia, abiotrophy

Cerebellar hypoplasia *is one of the most common congenital nervous system defects of domestic animals and is seen in all domestic species* (Fig. 3.28). There is persuasive evidence for genetically determined occurrence of the disease in calves of various breeds, particularly beef shorthorns, in Arabian and Arabian cross foals, and in Gotland ponies and Chow Chow dogs. A novel form of cerebellar hypoplasia with intraneuronal inclusions is reported from New Zealand in Cocker Spaniel dogs with progressive ataxia and seizures. Cerebellar hypoplasia has been reported in piglets born to sows treated with the organophosphate trichlorfon during pregnancy, and may be detected microscopically in goat kids and lambs affected by hypocuprosis. The most prevalent and best-defined cerebellar hypoplasias are those that follow infection of the developing cerebellum by *Feline panleukopenia virus, Bovine viral diarrhea virus, Border disease virus,* and *Classical swine fever virus.* Canine parvoviral DNA has been detected in cerebellar hypoplasia in dogs. The occurrence of cerebellar hypoplasia as a consequence of viral infections of the developing nervous system is further discussed in the section Viral causes of developmental defects of the central nervous system.

Cerebellar growth patterns determine the gestational or perinatal periods during which cerebellar hypoplasia may follow the action of a teratogen and influence the nature and scope of the structural aberrations marking the hypoplastic process. The cerebellum originates as a dorsal growth of the alar plate of the metencephalon over the fourth ventricle. Germinal cells adjacent to the fourth ventricle differentiate into neurons that migrate into the developing cerebellum to form the deep nuclei and Purkinje neurons. This differentiation occurs prior to mid-gestation. A population of germinal cells migrates to the surface of the developing cerebellum and forms a layer several cells thick. This is the external granular layer that covers the folia as they develop. In this layer, cells proliferate rapidly and differentiate into microneurons such as basket cells, stellate cells and granule cells, which migrate into the folium to their definitive locations. This proliferation, differentiation, and migration begins late in gestation and continues for a few weeks postnatally in most species. Normal cerebellar cytoarchitectural development, including the maturation and localization of Purkinje cells, is dependent upon these microneurons establishing orderly synaptic connections.

It is the actively mitotic germinative cells of the external granular layer that are especially vulnerable to the effects of teratogenic agents. *Feline panleukopenia virus, Bovine viral diarrhea virus, and Border disease virus cause selective necrosis of external granular layer cells.* That the destructive process may be augmented by vascular damage has been demonstrated for *Bovine viral diarrhea virus. Classical swine fever virus* possibly acts through inhibition of cell division and maturation. In puppies, segmental cerebellar dysplasia may result from postnatal infection with canine herpesvirus. Since the growth behavior of the subependymal cell plate has features in common with the external granular layer of the cerebellum, residual lesions attributable to this site of infection, such as cavitating defects, may be associated with cerebellar hypoplasia, particularly in cases of viral origin.

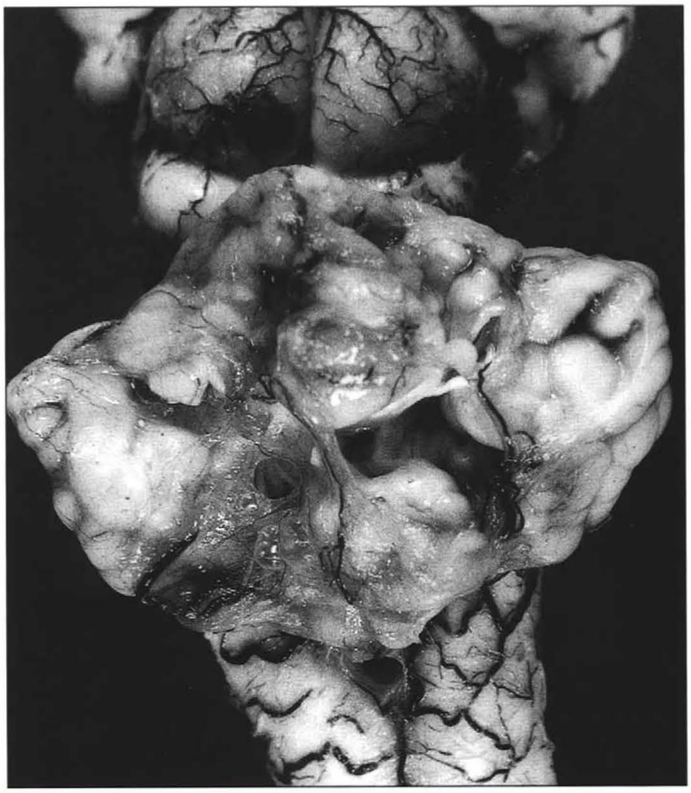

Figure 3.29 Cerebellar hypoplasia (cerebrum removed to expose cerebellar lesion) in a cow; asymptomatic over 5 years.

The anatomic expressions of hypoplasia, both in the cerebellum and subcerebellar structures, are very variable in degree. In some cases, the cerebellum may appear grossly normal, the hypoplastic defects being detectable only on microscopic examination. In such cases, the defects are irregular in distribution although there is a more or less severe loss of Purkinje cells. The granular layer is here and there narrowed and deficient in cells, but the molecular layer is normal. Correlative lesions may be present or absent and when present may be asymmetrical; they occur chiefly in the deep cerebellar nuclei, olives, and cerebellar peduncles. At the other extreme, the cerebellum may be represented only by a small nubbin of tissue or two unconnected nubbins each related to a hypoplastic peduncle (Fig. 3.29). In these severe defects, there is no folial pattern or division into lobes. Intermediate degrees of cerebellar hypoplasia are, however, the most common. It is usually possible to recognize with the naked eye that the cerebellar peduncles are diminished in size, especially the brachium pontis, that the medullary pyramids are flattened and that the small size of the restiform bodies gives the fourth ventricle a flattened appearance. Microscopically, the cerebellar cortex is disorganized. A brief description is not possible because the pattern varies greatly from case to case and place to place in the one animal. The Purkinje cells and the cells of the granular layer are the most obviously deficient and, in those that are present, regressive changes are common.

Cerebellar atrophy (or **abiotrophy**) *refers to premature or accelerated degeneration of formed elements, presumably caused by some intrinsic metabolic defect.* The Purkinje cells appear particularly susceptible to spontaneous degeneration; there may be secondary depletion of granule cells but other cortical layers are normal.

Cerebellar atrophy occurs in lambs, dogs, cats, various breeds of cattle, piglets, and foals. The disease in **lambs**, which are commonly known as "*daft lambs*," is reported from England and Canada. It is not restricted to any breed but is presumed to be inherited. The dams are normal. Affected lambs show signs of cerebellar dysfunction at birth, which include abnormalities of muscle tone, disorders of equilibrium, and tremors. The cerebellum is however normal in size and gross form. The histological changes affect primarily the Purkinje and Golgi cells, and these especially in the median lobe. Degeneration and loss of these cells leave some empty baskets and a replacement astrogliosis. In the early stages of degeneration, the Purkinje and Golgi cells are shrunken and hyperchromatic or swollen and pale with cytoplasmic vacuolation. They ultimately undergo lysis and disappear, leaving a spongy zone between the granular and molecular layers. There is, simultaneously, a diminution in the population of granule cells. Regressive changes with gliosis may be apparent in the deep cerebellar nuclei and olives.

Progressive cerebellar degenerations are common in **dogs,** and abiotrophic defects have been recorded in a variety of breeds including Airedales, Gordon Setters, rough-coated Collies, Border Collies, Finnish Terriers, Beagles, Australian Kelpies, and Bernese Mountain Dogs. Signs in the pups become evident at about 3 months of age. The cerebellum may be normal in size and shape or slightly diminished in size and somewhat flattened. Microscopically, there is degeneration and loss of Purkinje cells and granule cells (Fig. 3.30A, B). The Purkinje cells degenerate, being either shrunken and hyperchromatic or pale, swollen and vacuolated (Fig. 3.31A); most disappear, leaving empty baskets and a fenestrated ground layer (Fig. 3.31B). The atrophic process may show evidence of regional predilection. In Gordon Setters, the dorsal portions of the median lobe and vermis are most severely affected, while in Collie dogs selective involvement of the rostral folia of the vermis is reflected in macroscopic diminution of this area. Collateral changes in the subcerebellar nuclei are minor, but in this regard it may be pertinent that affected dogs are usually euthanized shortly after the defect becomes apparent. Nonprogressive cerebellar ataxia noted at 2 weeks of age is reported in Coton de Tulear dogs.

Hereditary striatonigral and cerebello-olivary degeneration occurs in the *Kerry Blue Terrier.* Pedigrees of affected dogs indicate autosomal recessive inheritance. Clinical signs begin between 9 and 16 weeks of age and are characterized by ataxia and dysmetria. *The inherent defect is neuronal degeneration,* and the brain changes follow a definite anatomical and temporal pattern of development. Degeneration and loss of Purkinje cells in the cerebellar cortex is evident at the onset of clinical signs. Subsequently there is sequential involvement of the olivary nuclei, caudate nuclei and putamen, and finally the substantia nigra. In the cerebellar cortex, chromatolytic degeneration and loss of Purkinje cells is attended by astrocytosis involving Bergmann's glia and depletion of granule cells. In the cerebellar white matter and contained nuclei, there is status spongiosus and axonal swelling. Changes in the olivary nuclei, basal nuclei, and substantia nigra tend to be symmetrical and initially (Fig. 3.32) feature neuronal degeneration, axonal swelling, spongiosus and fibrous astrocytosis, with progression to malacia and cavitation. Olivary neurons undergo chromatolysis, while in the remaining nuclear structures the nerve cells accumulate intracytoplasmic eosinophilic granules and undergo ischemic change.

Macroscopic evidence of the disease process in the cerebellum is limited to a modest degree of folial atrophy but, with progression

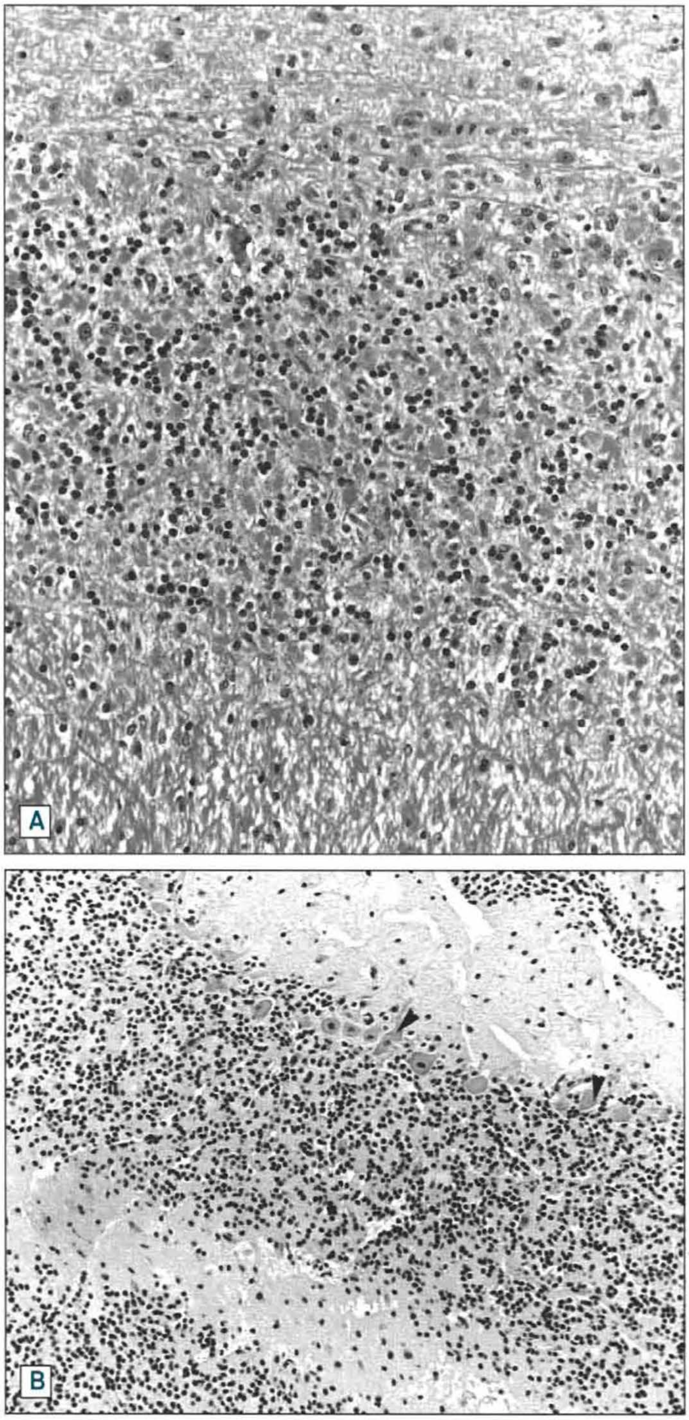

Figure 3.30 A. Cerebellar abiotrophy in a puppy; note severe hypoplasia of the molecular layer with almost complete absence of Purkinje cells. **B.** Cerebellar cortex of an age-matched puppy showing normal molecular layer and abundance of Purkinje cells (arrowheads).

of the condition, involvement of the basal and brain stem nuclei is manifest grossly by bilateral focal malacic lesions. Gross lesions are most severe in the caudate nucleus, which, after 7–8 months of clinical illness, may be reduced to numerous microcystic cavities. *It is proposed that the cerebellum and basal nuclei are the primary areas of involvement*, with the lesions in the olivary nucleus and substantia nigra being attributed to transynaptic neuronal degeneration along glutaminergic neurotransmission pathways.

The Kerry Blue Terrier condition is not the sole cerebellar abiotrophy in which accompanying extracerebellar lesions may be found. Olivary nuclei may be affected in the Bernese Mountain Dog, the cerebral cortex in the miniature Poodle, and spinal Wallerian degeneration may be found in rough-coated and Border Collies and in Merino sheep. In the Swedish Lapland Dog, there is a neuronal abiotrophy involving Purkinje cells; however, the effects of this are overshadowed by motor neuron degeneration and the disease is discussed below with others of that group.

Bovine familial convulsions and ataxia *is a heritable disorder of purebred and crossbred Aberdeen Angus cattle in the United Kingdom.* The clinical syndrome is characterized by intermittent episodic seizures in newborn and young calves and by the gradual development of ataxia with spasticity and hypermetria in calves surviving bouts of seizures extending over 2–3 months. The distinctive microscopic change is Purkinje cell axonal swelling in the cerebellar granular layer. There is also degeneration and loss of Purkinje cells. An apparently similar disorder has been described in Charolais cattle in the United Kingdom.

Sporadic cases of various forms of juvenile and adult-onset cerebellar degeneration, including **olivopontocerebellar atrophy**, have been reported in **cats**.

Cerebellar abiotrophy occurs in **horses**, particularly Arabian and part-Arabian horses, as well as in the Swedish Gotland pony and the American miniature horse, and is an inherited, likely autosomal recessive, condition. Head tremors and ataxia develop in affected Arabian foals between birth and 6 months of age, as a result of loss of Purkinje cells and granule cells from the cerebellar folia. An additional finding in an American miniature horse was degeneration of dorsal accessory olivary and lateral (accessory) cuneate nuclei and focal necrosis in the putamen.

Gomen disease *is a cerebellar degeneration and ataxia of horses in New Caledonia.* Some folial atrophy in the cerebellar vermis may be evident on gross examination. Microscopically, there is thinning of the cerebellar molecular layer and loss of Purkinje and granule cells. There is also considerable deposition of a pigment resembling lipofuscin in many of the surviving Purkinje cells as well as in the neurons of the brain and spinal cord. This material is also present in macrophages seen in areas where Purkinje cells are missing. The cause is unknown, but is thought to be an environmental toxin.

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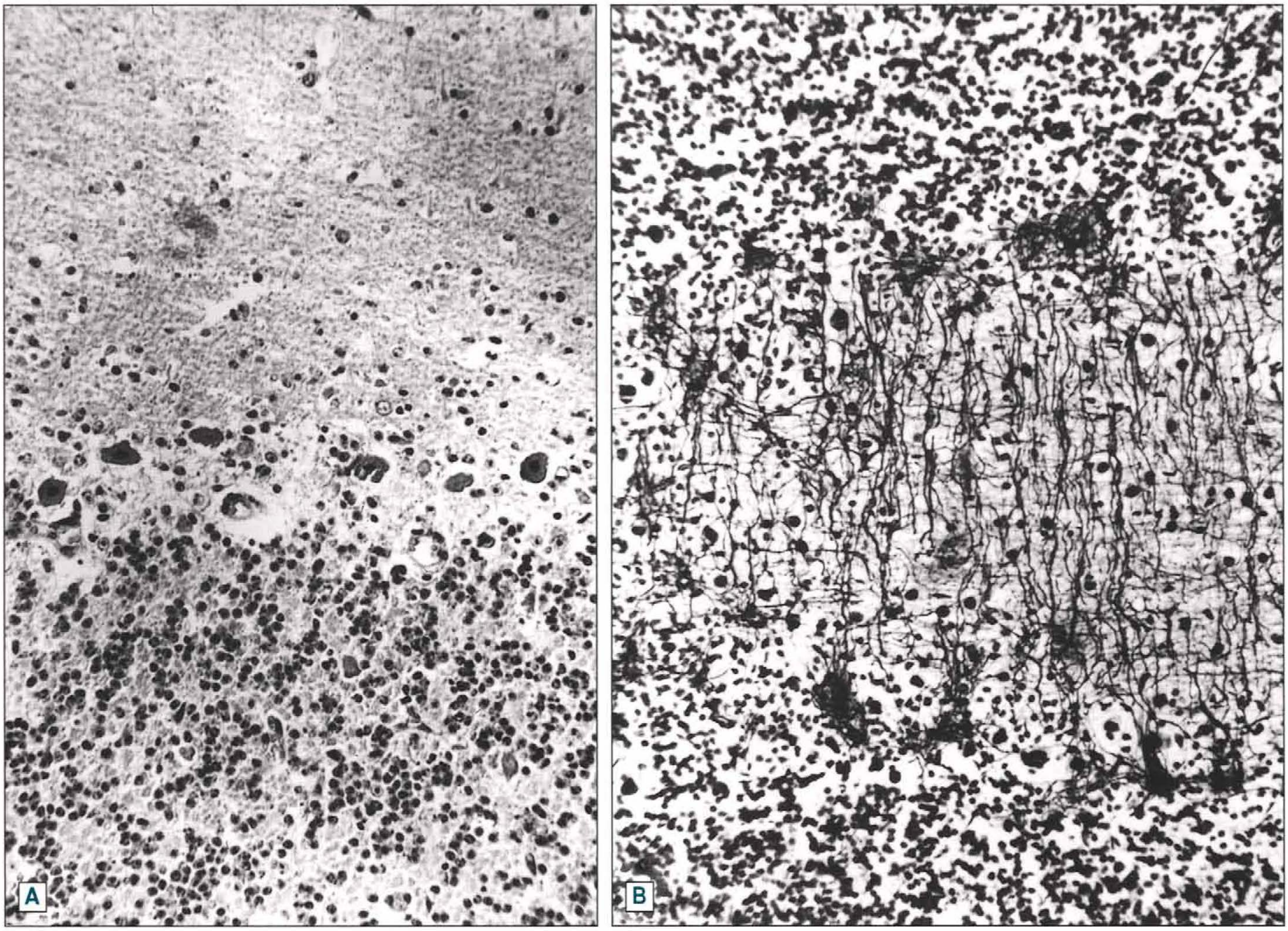

Figure 3.31 A. Degenerating Purkinje cells in **congenital cerebellar atrophy** in a dog. **B. Empty basket cells**; same case as (**A**).

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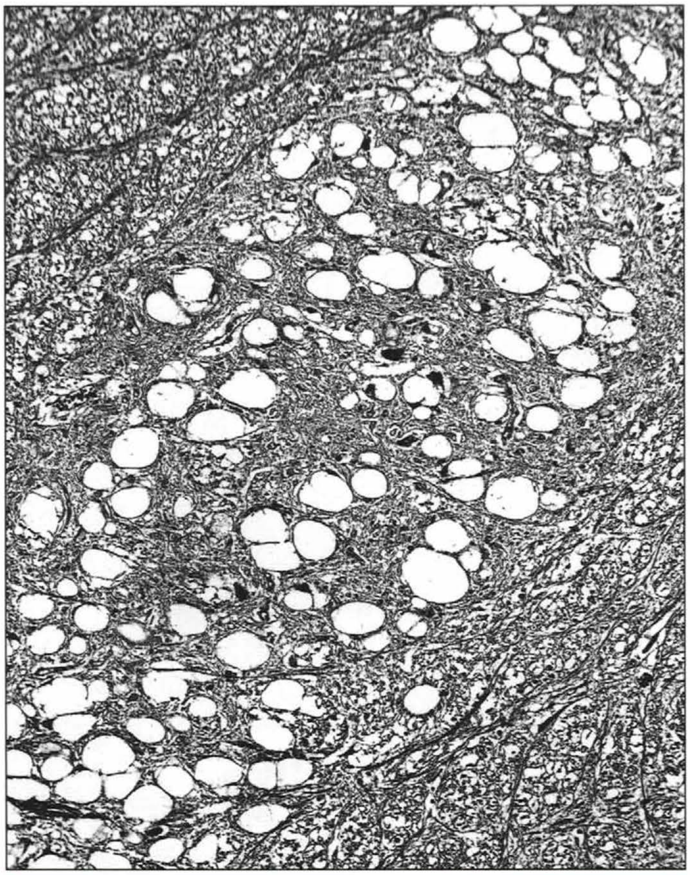

Figure 3.32 Neuronal degeneration and spongiosus, in inferior olive in a Kerry Blue Terrier with cerebellar abiotrophy.

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Arnold–Chiari malformation

In the Arnold–Chiari malformation (Chiari type II malformation), the cerebellar vermis is herniated as a tongue-like process of tissue into the foramen magnum and cranial spinal canal where it overlies an elongated medulla that is also displaced caudally (Fig. 3.33). The cranial nerves from the medulla alter their trajectory. The tentorium is inserted caudally towards the foramen magnum, the tentorial hiatus is shallow and wide, and the pons and occipital poles are displaced through it. The displaced occipital poles show sagittal, parallel gyri. Internal hydrocephalus may be a secondary effect, and spina bifida or meningomyelocele may be concurrent.

The caudal fossa in particular, but perhaps also the rostral fossa, is too small for the normal volume of brain tissue, suggesting that there has been failure of neurogenic induction of osseous growth. The defect is observed occasionally in calves. In the Cavalier King Charles Spaniel dog, occipital bone hypoplasia with obstruction of the foramen magnum and secondary syringomyelia is common, and is similar to human *Chiari type I malformation*.

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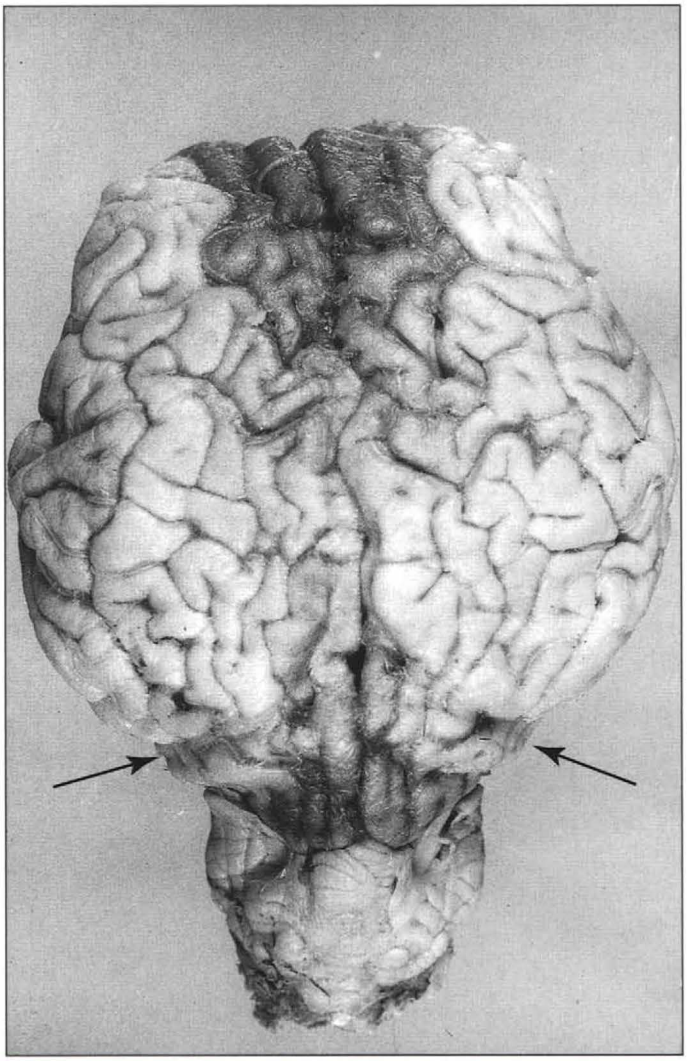

Figure 3.33 Arnold-Chiari malformation in a calf. Cerebellar deformity and coning accompanying spina bifida. Compression and hemorrhage of the occipital lobes (arrows). Pigmentation of rostral brain is normal.

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Dandy–Walker syndrome

In the Dandy–Walker syndrome, seen in various species, *there is a midline defect of the cerebellum with the vermis largely absent and the cerebellar hemispheres widely separated by a large fluid-filled cyst in an enlarged caudal fossa*. The roof of the cyst, which ruptures easily when the brain is removed, consists of ependyma, a disorganized layer of glial tissue, and an outer layer of leptomeningeal tissue. An expanded fourth ventricle forms the floor of the cyst. The vermal malformation is thought to be the result of a primary midline developmental defect.

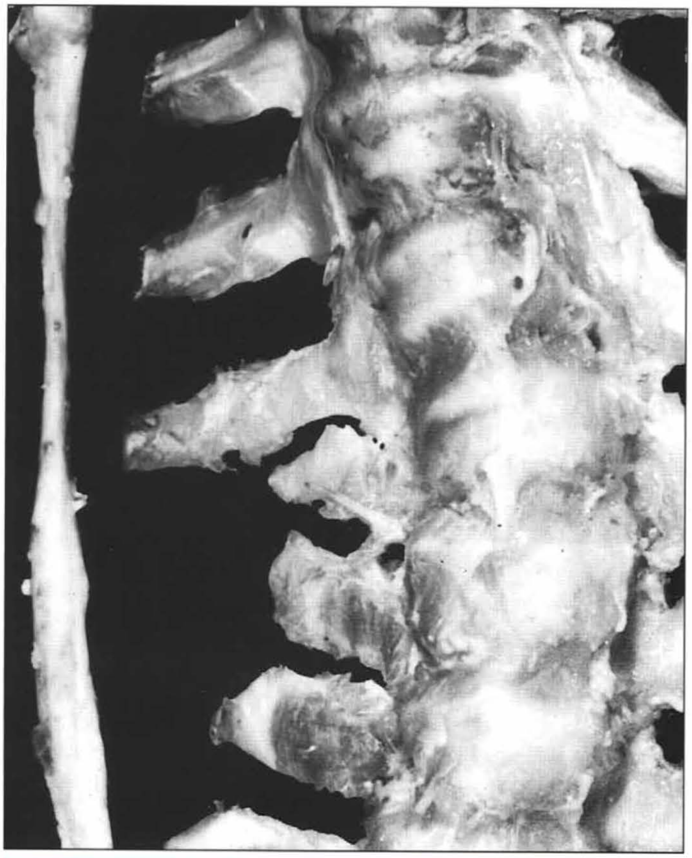

Figure 3.34 Constriction of spinal cord in congenital scoliosis in a calf.

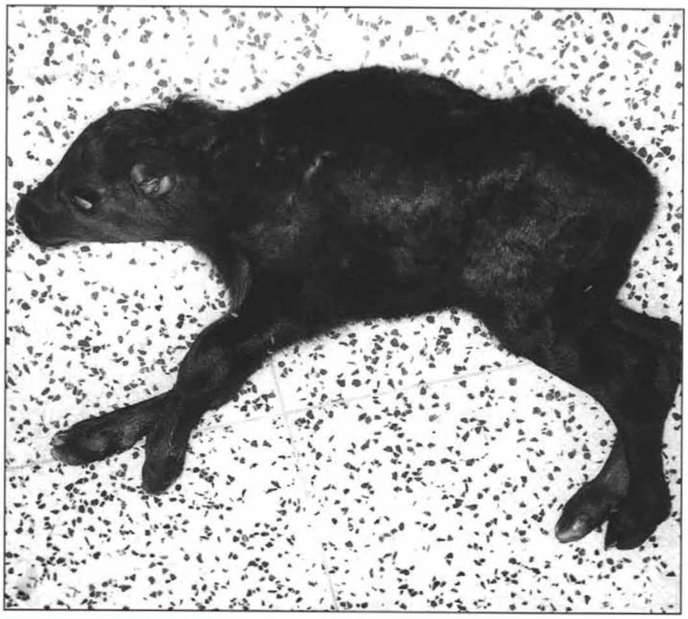

Figure 3.35 Perosomus elumbus in a calf.

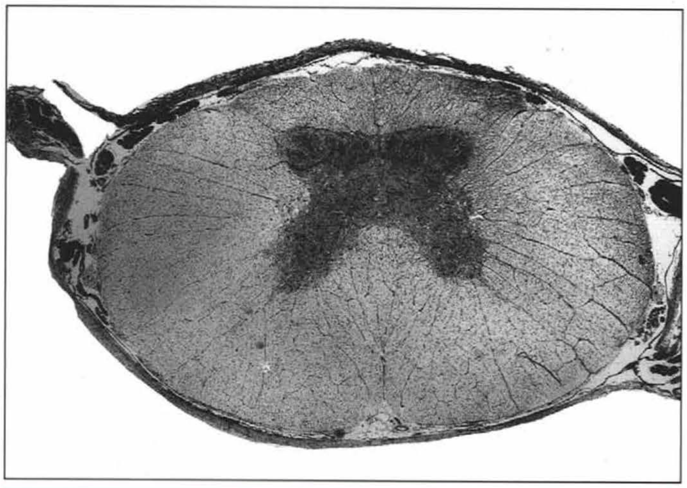

Figure 3.36 Absence of central canal of spinal cord in **dysraphism** in a dog.

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Spinal cord

Myelodysplasia

Myelodysplasia refers to abnormal development of the spinal cord. **Segmental aplasia** and **hypoplasia** may occur at any level of the spinal cord (Fig. 3.34), but *the lumbar region is most frequently affected.* Some cases are associated with fetal *Akabane virus* infection.

Perosomus elumbus, which occurs in calves and lambs, *is characterized by partial agenesis of the spinal cord.* The lumbar segment is involved, and there is failure of induction of the related vertebrae. The cranial part of the body is normal, but *the vertebral axis ends at the caudal thoracic region, and lumbar, sacral, and coccygeal vertebrae are absent* (Fig. 3.35). The spinal cord ends in the thoracic region in a blind vertebral canal. The caudal part of the body remains attached to the cranial part by soft tissue only; the limbs are arthrogrypotic and their muscles atrophic. Atypically, only some lumbar segments are absent, or severely hypoplastic with absence of arches and spinal muscle, and a remnant of cauda equina may be found in the sacral region.

The most severe forms of myelodysplasia, affecting especially the potential dorsal regions of the cord, occur *in association with the forms of spina bifida* described below. Myelodysplasia, not associated with the skeletal manifestations of spina bifida but probably best included in this classification, occurs quite commonly in animals particularly *in association with arthrogryposis in calves and lambs.* The dysplasia, which is readily detectable by the presence of *aberrant central canals* (sometimes as many as six), chiefly involves limited lengths of the lumbar segment of the cord but occasionally is localized to cervical or thoracic regions. *Denervation atrophy of appendicular muscle is constantly present and is the probable basis of the arthrogrypotic changes.* The degree of dysplasia is quite variable, but it is characterized by aberrations of the central canal (Fig. 3.36), by the absence of dorsal or ventral septa, the presence of ectopic septa and clefts, and by distortion, asymmetry, and

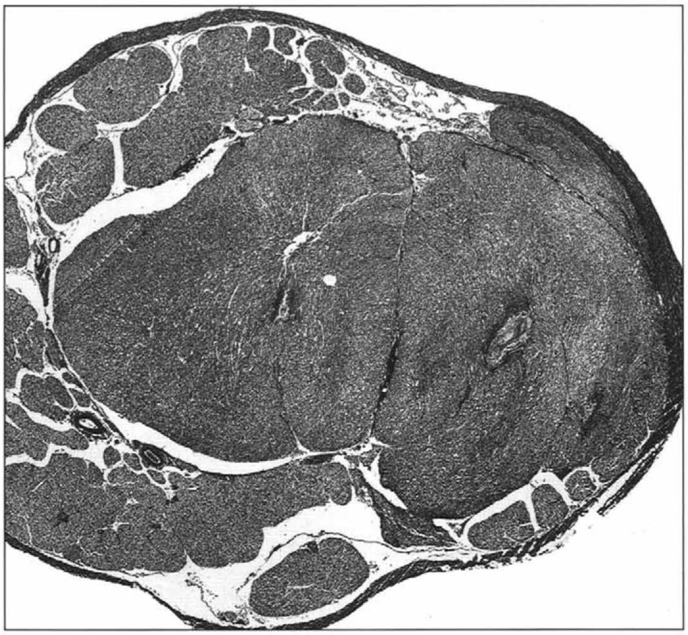

Figure 3.37 Diplomyelia in a dog.

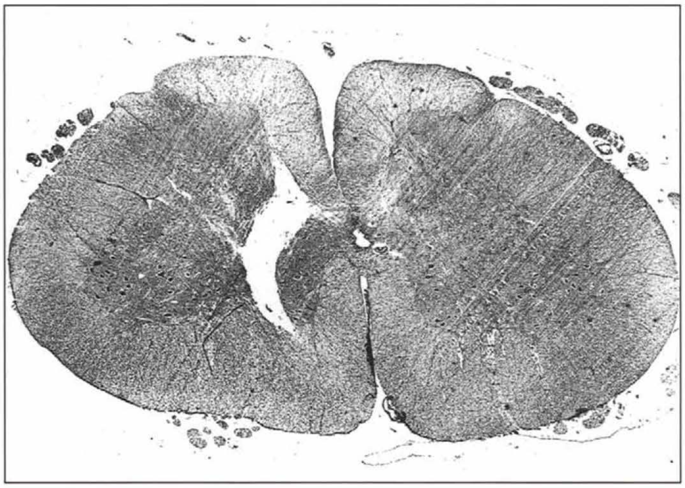

Figure 3.38 Syringomyelia in a dog.

partial duplication of the ventral and dorsal horns of gray matter. In some cases the cord is duplicated completely within common leptomeninges and dura (*diplomyelia*) (Fig. 3.37), or within separate meningeal coverings and vertebral canals separated by a bony partition (*diastematomyelia*).

Syringomyelia *is tubular cavitation of the spinal cord that extends over several segments*; it is an uncommon anomaly in animals. Syringomyelia is rarely acquired and is best known as one of the lesions associated with the familial disorder of young Weimaraner dogs described below. When the cavitation involves the medulla, the defect is termed *syringobulbia*. Syringomyelia and syringobulbia may occur together.

Hydromyelia *is dilation of the central canal of the spinal cord*. It is not commonly detected but occurs in association with spina bifida and may be a precursor lesion for syringomyelia. Both hydromyelia and syringomyelia are found in the dysraphic spinal cord of arthrogrypotic Charolais calves. Hydromyelia may be acquired as a consequence of obstruction of cerebrospinal fluid flow in the central canal.

A syndrome of pelvic limb gait disturbance in young **Weimaraner dogs** is a manifestation of a spectrum of myelodysplastic and dysraphic lesions, and is inherited in a co-dominant mode with variable penetrance. The trait is lethal in the homozygous state. Affected animals exhibit a gait deficit from the time they begin to walk, but clinical signs in some cases may be barely noticeable. Severely affected dogs typically are unable to completely extend the hindlimbs so that their normal attitude is crouched. When walking, the hindlimbs are moved together in a "bunny-hopping" or "kangaroo-gait" fashion. Additionally, affected animals may show thoraco-lumbar scoliosis, depression of the sternum, and abnormal hair streams in the dorsal cervical region. There is no significant progression or regression of clinical signs with age, and there is not a good correlation between clinical signs and the degree of structural malformation.

Pathologically, the exposed spinal cord is grossly normal in size and conformation, but *a range of dysraphic defects may be present* including: anomalies of the dorsal septum, which may be absent; hydromyelia and other anomalies of the central canal, which may be absent; duplication or displacement of the central canal; anomalies of extent and distribution of the central gray matter; anomalies of the ventral horns consisting of deficient delineation and development of medial cell groups, and aberrant collections of neurons and gray neuropil; deficiency of the ventral median fissure, which may be total. Histologic studies on affected embryos suggest that the primary lesion is related to aberrantly positioned mantle cells ventral to the central canal in the floor plate area.

Syringomyelia is not present until about 8 months of age and involves lumbar segments. Cavitation may be barely visible to the naked eye. Microscopically, the cavity is usually found in the central gray matter dorsal and lateral to the central canal (Fig.3.38). There is not much encroachment on the white matter except for that in the white commissures when the cavitation extends from one side to the other. Connection with the central canal is difficult to demonstrate but may be found in serial sections. Ependyma does not line the cavity, the walls of which are formed of frayed nervous tissue with an appearance suggesting that the cord has been squashed or torn at necropsy. The tissue around the cavity is edematous and stains poorly.

Spina bifida and related defects

Spina bifida *refers to absence of the dorsal portions of the vertebrae*. It is, however, a rather imperfect name as the various forms of the defect represent differences in degree of defective closure of the neural tube, its separation from the ectoderm, and its induction of a skeletal investment. It is convenient to divide the defect into several classes on the basis of severity: *myeloschisis, spina bifida occulta, spina bifida cystica with meningocele, and spina bifida with myelomeningocele apply to the vertebral defect; amyelia, diastematomyelia, hydromyelia, and dysraphism apply to the spinal cord.*

In **total myeloschisis**, *neurulation does not occur*, the neural plate remaining open. By total is meant a defect that involves the whole of the vertebral axis with anencephaly an expected accompaniment. There is virtual amyelia (absence of spinal cord), neural tissue being present only as soft red masses in the residual groove. *Local myeloschisis* is a localized defect due to failure of closure of the neural tube. One or more vertebral segments may be defective. The defect may occur in any portion of the vertebral axis but is expected to be lumbosacral.

The defect occurs most frequently in brachycephalic breeds of dogs and is inherited as an autosomal dominant condition in Manx cats. Affected cats are heterozygotes of variable expression, while the homozygous state is lethal. *Sacrococcygeal agenesis* occurs in association with spina bifida in Manx cats, calves, dogs, and sheep.

Spina bifida occulta is perhaps the least rare form of the defect in animals, and it is occult because *it is not apparent except for the presence of dimpling or deeper invagination of the skin.* It may accompany defects in other remote tissues but the defect is otherwise limited to the absence of one or more vertebral arches. The cord may be grossly normal but dysplastic microscopically, usually with diastematomyelia. The spinous processes may be bifid or absent.

In **spina bifida cystica**, a cystic swelling protrudes through the vertebral defect. Because of differential growth of the vertebral and neural axes, the cranial–caudal position of the skin and bone lesions may not correspond, especially in the caudal regions where the defects are expected to occur.

When meninges protrude (**meningocele**), the roof of the cyst is comprised of skin and condensed meninges, including dura mater. The spinal cord may be normal grossly but dysplastic segments are detected microscopically. Macroscopic lesions in the cord include partial duplication and cystic distension of the central canal that communicates with the endodural space via a cleft in the dorsal funiculi. A meningocele may contain a large accumulation of adipose tissue (**lipomeningocele**).

When meninges and spinal cord protrude (**meningomyelocele**), the cyst tends to be broad-based. *Failure of dehiscence of the neural crest from surface ectoderm provides for a central area without epithelial covering.* This *medullovasculosa* corresponds to the cerebrovasculosa of anencephaly and consists of vascularized meninges, heterotopic cord tissue, and connective tissue. The defects in the cord include those mentioned as occurring with meningocele and, in severe cases, duplication or absence of the cord in affected segments. The *tethered cord* syndrome refers to attachment of the meningomyelocele that prevents the normal ascent of the spinal cord as the vertebral column grows.

A **dermoid sinus** is a congenital abnormality, inherited in Rhodesian Ridgeback dogs, in which incomplete separation of the neural tube from the overlying dorsal midline ectoderm allows persistence of a sinus connecting the skin surface to the supraspinous ligament, or it may extend as deep as the dura mater of the spinal cord.

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Viral causes of developmental defects of the central nervous system

Akabane virus

Akabane virus (AKAV), a species of the genus *Orthobunyavirus*, is among the most potent of the viral teratogens of domestic animals, but the infection is otherwise asymptomatic. **Iriki virus**, a strain of AKAV, has similar pathogenic potential. Following maternal infection at critical stages of gestation, AKAV produces a range of predominantly neural abnormalities in calves, lambs, and kids, but *AKAV is best known for producing outbreaks of arthrogryposis and hydranencephaly in calves*. Epizootics of Akabane virus disease in cattle have been reported in areas of Japan, Israel, and Australia. The vector in Australia is the biting midge *Culicoides brevitarsis*, and the virus has also been isolated from mosquitoes in Japan. Buffaloes, horses, and pigs are additional vertebrate hosts. Although arthrogryposis and hydranencephaly may be the most obvious manifestations in field epizootics of bovine AKAV disease, a range of overlapping syndromes is observed in calves in affected herds. The pattern of fetal disease corresponds to the gestational age of the fetus at the time of infection. Infection late in gestation may cause abortion. The initial manifestation of neural abnormality in a field outbreak is the birth of incoordinate calves and, in this group, nonsuppurative encephalomyelitis is evident on histological examination. *Microencephaly* and *cerebellar hypoplasia* occur occasionally as manifestations of late infection. *Arthrogryposis*, sometimes associated with spinal deformities, appears early in the outbreak following fetal infection at 5–6 months of pregnancy. In arthrogrypotic calves, there is loss of spinal ventral horn neurons, loss of myelin in the motor tracts of the spinal cord and in ventral spinal nerves, and denervation atrophy together with fibrous and adipose replacement of skeletal musculature.

At least some strains of AKAV have the capacity to cause *polymyositis*, particularly in the early myotubular phase of skeletal muscle development, suggesting the possible involvement of this process in the arthrogrypotic change. *Severe hydranencephaly*, manifest clinically as blindness and stupidity, is seen towards the end of the epizootic, being the result of fetal infection at 3–4 months of gestation. With increasing age of the fetus at the time of infection, the cavitating cerebral changes are less severe and grade towards *porencephaly*.

The teratogenic potential of AKAV in **sheep and goats** is qualitatively the same as for cattle. Cavitating cerebral defects, arthrogryposis, microencephaly, and agenesis or hypoplasia of the spinal cord have been produced in lambs born of ewes inoculated on days 29–48 of pregnancy. Field observations in Australian flocks suggest that, in sheep, *microencephaly* is relatively more common as a consequence of AKAV infection than it is in cattle.

Aino virus, a serotype of species *Shuni virus*, genus *Orthobunyavirus*, can cause arthrogryposis, hydranencephaly, and cerebellar hypoplasia in bovine fetuses infected experimentally, and is suspected to be the teratogen in field cases of congenital bovine malformations in Japan.

Cache Valley virus (CVV), a serotype of species *Bunyamwera virus*, genus *Orthobunyavirus*, is a mosquito-borne bunyavirus that is endemic in North America. CVV is capable of infecting a variety of mammals, generally as subclinical infections but it is occasionally teratogenic in fetal lambs. The nature and pathogenesis of the abnormalities produced are comparable to those of *Akabane virus* in sheep. Three other members of genus *Orthobunyavirus* – **LaCrosse virus** and **San Angelo virus** (serotypes of *California encephalitis virus*), and **Main Drain virus** – also express bunyaviral tropism for fetal tissue infection and can induce lesions including arthrogryposis, hydrocephalus, fetal death, axial skeletal deviations, anasarca, and oligohydramnios.

Chuzan virus (CHUV), a strain of species *Palyam virus*, genus *Orbivirus*, family Reoviridae, is transmitted by *Culicoides* sp. CHUV is infective for several ruminant species, and in Japan has been incriminated as a teratogen of fetal calves with features similar to those of *Akabane virus* infection.

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Bluetongue virus

Hydranencephaly and porencephaly have been reported in lambs and calves whose dams received a live attenuated bluetongue vaccine or contracted *Bluetongue virus* (BTV, species of the genus *Orbivirus*, family Reoviridae) infection during pregnancy (Fig. 3.27B). The type of congenital anomaly found depends on the fetal age at the time of infection with BTV. Lambs infected with bluetongue vaccine virus at 50–55 days gestation develop severe necrotizing encephalopathy and retinopathy, which at birth is manifest as *hydranencephaly and retinal dysplasia*. Infection of lambs at 75 days gestation causes multifocal encephalitis and selective vacuolation of white matter that is manifest as porencephalic cysts in the newborn; ocular lesions are not observed in these newborn lambs. Lesions in brains of lambs infected after 100 days gestation are confined to mild focal meningoencephalitis. BTV infection of fetal calves can cause hydranencephaly.

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Rift Valley fever virus and Wesselsbron virus

These infections are considered more fully in Vol. 2 Liver and biliary system. In addition to *Wesselsbron virus* (WESSV), there are several other mosquito-borne flaviviruses (genus *Flavivirus*, family Flaviviridae) in South Africa, including *West Nile virus* and *Banzi virus*, to which sheep are experimentally susceptible and which result in abortion, stillbirths, and neonatal deaths; anomalies include *hydranencephaly, porencephaly, and internal hydrocephalus*.

Rift Valley fever virus and WESSV are mosquito-borne and tend to circulate together. They are both primarily hepatotropic, but the wild strain of WESSV and attenuated strains of both are neurotropic, and vaccine strains may be responsible for most outbreaks of congenital neurologic disease. The presenting features are similar to those of Akabane disease, but the high incidence of hydrops amnii and prolonged gestation is especially a feature.

The destructive changes in the CNS produced by one or other of these viruses, or both together, can be more severe than with other teratogenic viruses and result in segmental aplasia of the cord, aplasia of the cerebellum, and anencephaly. It is more usual, however, that the defects include brachygnathia, hydranencephaly or porencephaly, hypoplasia of cerebellum and spinal cord, and varied musculoskeletal stigmata of arthrogryposis.

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Bovine viral diarrhea virus

The diseases caused by *Bovine viral diarrhea virus* (BVDV), genus *Pestivirus*, family Flaviviridae, are considered in detail in Vol. 2, Alimentary system. The ecology of BVDV depends on transplacental transmission and the establishment of immune tolerance and persistent infections. Horizontal transfer of infection occurs readily, but is seldom clinically significant. Persistently infected fetuses that survive to become pregnant transmit BVDV to the conceptus. Although viral strains differ in their pathogenicity, infection of susceptible cows during early and middle stages of gestation is likely to result in either fetal death or a variety of developmental defects, predominantly neural and ocular. Some congenitally affected calves also have erosive lesions in the upper alimentary tract and abomasum resembling those seen in adult cattle; brachygnathism also occurs.

The outcome of infection of the fetal calf is related to gestational age, advancing fetal maturity being associated with increased resistance to the virus. Infections occurring within the first 100 days of fetal life tend to be lethal resulting in *abortion or mummification*. Although the gross pathologic changes seen in these lethal infections lack unique features, the patterns of tissue response are characteristic, but are rarely seen as such fetuses die in utero and undergo autolysis. Necrotizing inflammation can involve a variety of tissues. The reactive changes are dominated by mononuclear, predominantly macrophage, infiltration of hepatic portal areas, myocardium, spleen, and lymph nodes, which is reflected grossly in enlargement, nodularity and mottling of the liver and enlargement of spleen and lymph nodes (Fig. 3.39). The presence of *growth-arrest lines in long bones* suggests that the fetus undergoes one or more intrauterine crises before death. Affected fetuses may have partial alopecia that spares the tail, the lower portion of the limbs, and the head, these being points of initial hair growth during fetal development. The microscopic skin changes that evolve from the initial necrotizing dermatitis and correlate with the alopecia are hypoplasia of hair follicles and cystic distension of adnexal glands.

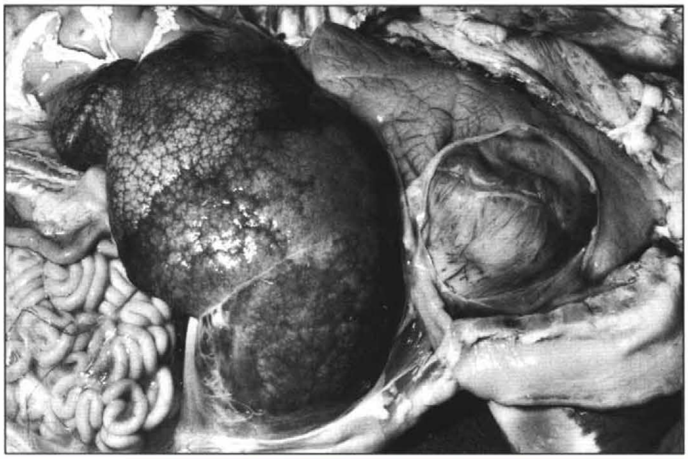

Figure 3.39 Myocardial pallor due to myocarditis and myocardial degeneration, and chronic hepatic congestion with ascites in a bovine fetus due to ***Bovine viral diarrhea virus* infection**, although *Neospora caninum* infection must also be ruled out in such cases.

The teratogenic effects of BVDV are manifest during the 100–170 day period. The period of susceptibility presumably varies with the strain of virus. *Cerebellar hypoplasia is the most characteristic defect* (Fig. 3.40). Gross cerebellar changes range from more or less uniform atrophy to irregular folial atrophy and agenesis accompanied by cavitation. The hypoplastic process is compounded by the effects of necrosis of external granular layer cells and parenchymal destruction as a consequence of folial edema resulting from vasculitis. The relative contribution of these processes is quite variable, but it appears that the vasculopathy may be more prominent in older fetuses. The nature and extent of involvement of individual folia also varies considerably. The evolution of the cerebellar changes has been studied experimentally. Acute lesions are evident 2 weeks after infection. Cellular necrosis in the external granular layer is accompanied by nonsuppurative meningitis. Vasculitis, marked by endothelial proliferation and perivascular leukocytic infiltration, is associated with folial edema, and there may be focal hemorrhages in the cerebellar white matter and cortex.

Folial edema, depending on its severity, may result in total folial destruction, cavitation or focal, often linear, areas of folial white matter deficient in myelin and axons. Where necrosis of the external granular layer predominates, the result is irregular atrophy of affected folia. Features of the atrophic process are marked depletion

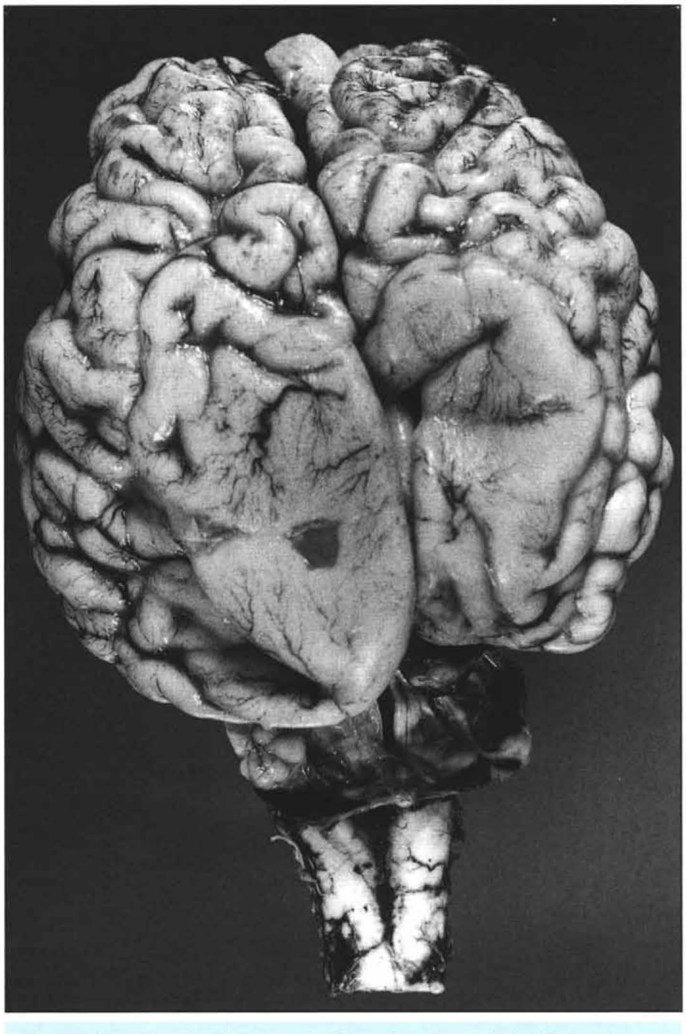

Figure 3.40 **Cerebellar hypoplasia** and **hydrocephalus** in a calf.

of granule cells, ectopia of Purkinje cells, and the presence of swollen Purkinje cell axons in the granular layer. The evolution of the cerebellar lesion in the fetal calf extends over 6 weeks and, by 10 weeks after maternal infection, inflammatory changes are not evident in the brain.

Other CNS defects that may be a consequence of fetal infection are porencephaly, hydranencephaly, microencephaly, hydrocephalus, cystic septum pellucidum, and dysmyelination. Ocular anomalies commonly accompany cerebellar defects, and include retinal dysplasia and atrophy, cataract, optic neuritis and atrophy, microphthalmia, and persistent pupillary membrane.

Infection of the fetus later than 170 days is unlikely to cause either intrauterine death or malformation. This increased resistance coincides with the fetus acquiring the capacity to produce neutralizing antibody to the virus. However, BVDV also has the capacity to induce more subtle developmental aberrations such as *intrauterine growth retardation* and *atrophy of the thymus and lymphoid tissues*. The regressive changes in thymus and lymphoid tissues offer a morphological basis for the immunological suppression and tolerance phenomena associated with congenital infections with BVDV.

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Border disease virus

Border disease (BD) of lambs was first described from the border counties of England and Wales, and is now recognized in many countries. Affected lambs show gross tremors and long hairy birth coats ("hairy-shaker," or "fuzzy" lambs).

BD is caused by *Border disease virus* (BDV), genus *Pestivirus*, family Flaviviridae, which is very closely related to *Bovine viral diarrhea virus* (BVDV) and less closely to *Classical swine fever virus*. These viruses possess a similar host spectrum experimentally, and interspecies transmission, especially between ruminants, does occur naturally but the clinical expression in recipients may be modified or absent. The ecology of BDV and the pathogenesis of BD depend on the ability of the virus to cross the placenta and then either to produce disease in the fetus or a state of immunotolerance and *persistent infection (PI) that allows excretion of the virus continuously in postnatal life*. Strain variations of BDV, differing host responses depending on breed and genotype, and gestational age at which infection occurs contribute to the varied manifestations of the disease. Congenital disease and persistent infection are typically caused by the *noncytopathic biotype* of BDV; mucosal disease-like syndrome is caused by a *cytopathic biotype* of BDV. Sheep can also be infected naturally with BVDV types 1 and 2, and BVDV is capable of producing the BD syndrome in sheep and goats. Pigs can be naturally infected by BDV, e.g., strain Frijters, which can confuse identification of classical swine fever.

Primary infection of postnatal sheep by BDV is usually subclinical, immunity develops and the virus is eliminated. In pregnant ewes, the virus infects the fetus within the first week of exposure and is not then influenced by the immune status of the ewe.

BDV is a potential cause of a variety of developmental disorders, including *hypomyelinogenesis, cavitating cerebral defects such as porencephaly, hydranencephaly, and cystic septum pellucidum; cerebellar dysplasia; arthrogryposis and skeletal defects; mandibular brachygnathism, and thymic hypoplasia*. The pathogenicity of the virus is most freely expressed in experimental infections, the cavitating cerebral lesions and cerebellar dysplasia being common sequelae of experimental infections but only occasionally encountered in the natural disease.

Bovine viral diarrhea virus is capable of producing the Border disease syndrome in sheep and goats, and the syndrome in piglets caused by congenital infection with *Classical swine fever virus*.

The ovine fetus is liable to significant damage if the dam is infected with BDV between days 16–80 of gestation. *The age-related immune capacity of the fetus is the important determinant of the nature of the disease produced*. In the case of infections occurring within the first half of gestation, the result may be *fetal death and abortion*. Alternatively, the fetus may survive, frequently carrying an immunologically tolerated infection. In this event, the virus persists in fetal tissues and postnatally the lamb fails to produce specific antibody. Such PI sheep harbor the virus for prolonged periods, are chronic excretors of the virus, and readily transmit infection. Lateral spread is important in the field and, although the virus can be transmitted experimentally by a number of routes, the mode of lateral transfer of infection under natural conditions has not been identified.

Infections in the second half of gestation elicit both humoral and cell-mediated immune responses and it is this acquisition of immunocompetence that endows the fetus with substantial resistance to infection after day 80 of gestation. In the case of infections initiated between day 90 of gestation and the early days of postnatal life, the cell-mediated immune response is expressed morphologically as *nodular periarteritis*. The periarteritis affects medium to small arterioles, particularly in the meninges and substance of the CNS, but also occurs mildly in a wide range of other tissues.

The developmental anomalies arising from fetal infection are also related to the gestational stage at which the fetus encounters the virus, but lesions produced also vary markedly according to virus strain, dose, route of infection, and host genetic factors. *Hypomyelinogenesis, the characteristic neural lesion*, is diffuse in fetuses infected early in gestation but becomes progressively milder and more restricted to higher, later myelinating regions of the CNS in infections initiated later in gestation. The occurrence of porencephaly and cerebellar dysplasia has been confined to infections initiated at days 45–72 of gestation. The development of the cutaneous lesion requires that infection be initiated before day 80 of gestation.

Hypomyelinogenesis and clinical tremors may substantially resolve during the first few months of life notwithstanding that the animals have persistent replicating infection, suggesting that the normal processes of myelin deposition have been delayed or that cellular injury has been slowly repaired. The virus does infect myelinating oligodendroglia, astroglia, and glial progenitor cells, and it is reasonable to assume a direct effect on the differentiation or maturation of oligodendroglia. However, the virus also infects many non-neural cells, including thyroid epithelium. There are no morphological changes in the thyroid epithelium, but reduction in circulating thyroid hormone levels may contribute to delayed maturation.

The cavitating cerebral defects and cerebellar dysplasia arise from inflammatory destruction of developing neural elements, possibly secondary to vasculitis. These lambs may have severe locomotor and behavioral abnormalities and defects of vision, the latter probably of central origin rather than due to focal retinal dysplasias that may be present. They are serologically positive but not persistently infected.

The *abnormality of birth coats* occurs in fetuses infected before ~90 days gestation. There are no cytological changes in the papillae that can be ascribed to direct viral action. The primary follicles revert to a more primitive type, are enlarged and produce heavily medullated fibers that are most prominent after about 3 weeks. There are fewer secondary follicles and their development is retarded.

BDV infection also interferes with pre- and postnatal development of skeleton, musculature, and viscera. Tissues most affected are those that have their main growth spurt in the fetal period. *Growth-arrest lines in long bones* suggest periods of interrupted intrauterine development.

Lambs that are persistently infected at birth remain so but not all show neurological signs. Growth and viability may be depressed. Some PI sheep develop oculonasal discharges and respiratory distress or severe diarrhea and die in 2–4 weeks with inflammatory lymphoproliferative lesions in many organs. In the brain, these reactions occur particularly in the choroid plexuses and periventricular substance. Proliferative metaplastic changes in the intestinal mucosa affect mainly the cecum and colon, the hyperplastic glands penetrating the muscular mucosa. *The delayed disease has features resembling those of mucosal disease of cattle,* which is considered to be due to superinfection by a different strain of BVDV or by minor mutation of homologous virus in animals that are persistently infected.

Infection by BDV is widespread in **goats** but disease attributable to this virus is not. The characteristics of the natural and experimental disease are similar to those in sheep, but spontaneous neurological disease is not a feature. The fetal goat may be much more susceptible to infection than the fetal lamb; a high incidence of fetal death, mummification, and abortion is reported.

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Classical swine fever virus

The disease is discussed in detail in Vol. 3, Cardiovascular system.

Both vaccine and certain low-virulence field strains of *Classical swine fever virus* (CSFV, hog cholera virus) are teratogenic for the fetal piglet. Fetuses are susceptible to infection regardless of the immune status of the sow. The apparent induction of immune tolerance results in the delivery of chronically infected piglets lacking antibody. The gestational interval during which fetal piglets are susceptible to the teratogenic effects of CSFV extends at least from day 10 to 97 of gestation, but the occurrence of malformation is favored by infection around day 30. The most characteristic anomalies involve the nervous system. The combination of *hypoplasia and dysplasia of the cerebellum and CNS hypomyelinogenesis,* most severe in the spinal cord, comprises one form of the *congenital tremor syndrome* of piglets. *Microencephaly* is also a rather characteristic sequel. The mechanism by which these lesions evolve is compatible with a persistent neural infection resulting in selective inhibition of cell division. Additional effects noted in affected litters include fetal mummification, stillbirth, pulmonary hypoplasia, nodularity of the liver, ascites, anasarca, cutaneous purpura, arthrogryposis, and micrognathia.

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Feline panleukopenia virus

This disease is discussed in detail in Vol. 2, Alimentary system.

Feline panleukopenia virus (FPLV), a species of the genus *Parvovirus,* family Parvoviridae, is pathogenic to the cerebellum of kittens before and shortly after birth, at which time the cerebellum is growing and differentiating rapidly. FPLV has tropism for cells that have a high mitotic rate, and its site of action is the external germinal layer of the cerebellum. The formation of *intranuclear inclusion bodies* is an early feature of the infection; they disappear by day 14 of infection. The infected cells are destroyed and with them the growth potential of the cerebellum; hence *cerebellar hypoplasia* results. The Purkinje cells, which are postmitotic but immature, are also affected although inclusion bodies do not form in them. The nuclei of the Purkinje cells show vesicular ballooning, eosinophilia, and condensation of the membrane. One or more large vacuoles form in the cytoplasm. Some Purkinje cells undergo coagulative necrosis.

In view of the tropism of FPLV for rapidly replicating cells, a wide spectrum of abnormalities might be expected to result from infection of kittens in utero. The virus will cross the placenta and produce generalized infection of the fetus as indicated by the distribution of inclusion bodies. The subependymal cell plate shares growth behavior with the external granular layer of the cerebellum, and, although heavily infected, only one instance of hydranencephaly attributable to damage at this site of infection has been recorded. In visceral organs, only slight degrees of renal hypoplasia have been observed in infected kitten fetuses.

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STORAGE DISEASES

As most storage diseases involve neurons and neurologic impairment, they are discussed here. Within all cells, except mature erythrocytes, normal catabolism directs a steady stream of endogenous macromolecules into vesicular compartments for degradation to simple molecules that may be re-used or excreted. These essentially autophagic pathways, through which each cell recycles its own constituents, may also receive endogenous and exogenous molecules from the extracellular milieu, taken up by endocytosis or phagocytosis.

Storage states, or disorders, are characterized by the accumulation of material(s) resistant to or exceeding the capacity of the machinery of intracellular digestion, disposal, or transport. Hemosiderosis and some types of hepatic lipidosis are common examples of the storage of physiologically normal substances due to the overloading of essentially normal, biochemically competent cells. A storage disease, by contrast, can be regarded as a storage process with a primary pathologic basis within the storing cells, and with potential for the perturbation of their function. The implication is that the catabolic machinery of the cells is fundamentally incompetent. This is generally demonstrable, but it is difficult to provide a neat definition that clearly distinguishes this situation in all circumstances.

Practically all cell types are potentially vulnerable to storage-induction, but those most vulnerable are long-lived postmitotic cells, such as neurons and cardiac myocytes. Cells in dynamic renewal systems, such as enterocytes, scarcely have the chance to become involved before their time is over.

The lysosomal apparatus provides the machinery for a great deal of intracellular degradation; most storage diseases involve intralysosomal accumulation and are hence termed **lysosomal storage diseases**. The 40 or so lysosomal acid hydrolases are capable of digesting completely the complex macromolecules synthesized for cell membranes, organelles, secretory products, etc. This enzymic destruction must be sequestered away from the rest of the cell, and is carried out in vesicles provided with an ion pump in their limiting membranes that maintains an acidic interior, or lysosol, for the optimal activity of the hydrolases.

Newly synthesized lysosomal enzymes are carried from the trans-Golgi region within primary lysosomal vesicles, and are delivered to substrate-containing vesicles (endosomes, heterophagosomes, autophagosomes, secretory vesicles) by fusion of vesicle membranes. The end products of digestion, which are simple lipids, amino acids, and sugars, are transported from the vesicular lysosol back into the cytosol. There may be small quantities of indigestible residues that, in cells such as hepatocytes, may be extruded by a process of exocytosis.

The lysosomal hydrolases tend to be "*exoenzymes*," sequentially breaking linkages at the ends of large molecules, but unable to act on linkages within them. This means that if the sequence is blocked at some point, further digestion cannot proceed. Should the digestive sequence be impaired, the cell will steadily accumulate a mass of vacuoles containing undegraded substrate. One type of molecule will be the major stored substance, but often the stored material will be somewhat heterogeneous, as the enzymes are linkage-specific rather than substrate-specific. Thus, *the morphologic hallmark of lysosomal storage disease is the presence of distended cells, crowded with vacuoles bounded by single membranes containing the stored material*; these vacuoles react enzyme-histochemically for acid phosphatase or other lysosomal hydrolases. They represent the adaptive hypertrophy of the lysosomal apparatus. In some cases, the morphology and histochemical reactivity of the stored substance may fairly clearly indicate its nature, for example glycogen. Lectin histochemistry may also be useful for characterizing stored material, exploiting the avid carbohydrate-binding properties of these agglutinating proteins in combination with a visualizing system.

Lysosomal digestion can be impaired in several ways. The most relevant here is *deficient activity of a specific lysosomal hydrolase because of a* **genetic defect**. This is the basis of the inherited lysosomal storage diseases in humans and animals, most of which are transmitted as autosomal recessive, and some as X-linked, traits. The deficiency in activity may come about via a total absence of enzyme protein, via the production of a defective or unstable enzyme, or via the absence of a specific activator protein required by some enzymes for the initiation of activity. These activators are small-molecular-weight, heat-stable proteins that function as detergents. They may interact with either the enzyme or its substrate. In the category of defective enzyme protein, one could also include the rare mechanism in which the enzyme lacks the specific molecular tag required to direct it, following its synthesis, to the lysosomal compartment; it is therefore immediately excreted from the cell, and the lysosomes remain deficient in that enzyme activity.

In most *autosomally inherited conditions*, the gene dose effect results in heterozygous individuals being generally phenotypically normal, but usually having demonstrably subnormal tissue activity of the particular enzyme in question. Tissues of homozygous affected individuals will contain swollen vacuolated cells, and chemical analysis reveals large amounts of the stored substance, and variable amounts of other metabolically related substances. *Multisystem involvement is likely to occur*, with storage being evident in many cell types in many organs. The cells and tissues most affected will be those most active in turning over the substrate in question, but usually the fixed and mobile macrophages are also prominently involved. This is because they avidly accumulate substrate from tissue fluids and plasma. The process begins in utero and in many cases is well developed at birth, although clinical impairment may be mild at that time. The age of onset and speed of progression of disease can vary, probably on the basis of the amount of residual enzyme activity, and often involving the ratio between various isoenzymes. In several human entities, subtypes are described on this basis.

As pointed out above, the *in vitro* tissue activity of the enzyme involved can usually be demonstrated to be negligible. However, if there was, for example, *deficiency of an activator protein*, the in vitro assay of tissues for the subject enzyme might reveal paradoxically high levels of activity, reflecting the hypertrophy of the lysosomal apparatus. The enzyme, however, would be inactive in vivo in the absence of the activator protein. In addition, and especially if the assay involves synthetic substrates, various isoenzymes may give the impression of adequate activity in vitro, which has no relation to the in vivo situation. In spite of this, assay of enzyme activity in skin fibroblasts or peripheral blood leukocytes has been diagnostically useful in many genetic storage diseases, identifying both homozygous and heterozygous individuals. Molecular genetic methods can be expected to play an increasingly important role in this area.

In an alternative mechanism of storage, *an* **exogenous toxin** *may specifically inhibit a lysosomal enzyme*, and temporarily induce a state analogous to a genetic enzyme deficiency. This is the established basis of at least one plant intoxication, "locoism," and is suspected in others. The morphologic and chemical characteristics are typical, but tissue activity of the subject enzyme may be quite high when it is separated from the inhibitor and assayed.

In a final general mechanism, *substrate that is resistant to a normal and intact enzymic battery may enter degradative pathways*. This may be an exogenous substrate, or a modified endogenous substrate. By this mechanism, several amphophilic drugs, such as chloroquine, have been found to induce storage diseases by complexing with endogenous molecules to produce indigestible products. Theoretically this mechanism could also have a genetic basis, by which an indigestible substrate is produced.

The lysosomal basis of some storage diseases is uncertain, and *non-lysosomal entities*, such as several of the glycogenoses, are clearly defined. Further, although many storage diseases have been defined in molecular terms, some have not and, in most cases, the basis of cellular dysfunction is far from clarified. There is more involved than simply the mechanical crowding out of other organelles. In most instances the storage process seems to have little primary cytotoxic effect. It seems rather that the induction of secondary and tertiary metabolic and structural effects is responsible for functional disturbances.

In many of the **neuronal storage diseases**, the process is multisystemic, and all neurons are involved, including those of the retina and peripheral ganglia, together with cells in most other organs. But neither of the two preceding conditions is invariable. When neurons are involved in storage disease, clinical signs of neurologic impairment eventually become evident, but, in general, this does not correlate with significant neuronal death, and the mechanisms of dysfunction are still largely unresolved. Regional neuronal death begins at an early stage in a few storage diseases and is progressive; it probably contributes significantly to functional disturbance at the end stage of many storage diseases.

In the face of a progressive storage process, neurons have no recourse but to accumulate storage vacuoles until they, or the animal, die. It seems probable that there is some limited capacity to discharge some of the stored load by exocytosis but, *in general, intractable constipation is inevitable as long as enzymic activity is deficient.* In spite of this, the cell limits the sites of storage to the soma and some of the larger dendritic stems. As a result, the soma becomes greatly distended and the cell outline rounded and swollen, rather than angular (Fig. 3.41). However, even within the soma, storage may be somewhat polarized, often adjacent to the axon hillock; differing planes of section may suggest that some neurons are not affected, particularly if examined early in the course of the disease. The multitude of storage vacuoles crowds and displaces other organelles, and the neuron takes on a chromatolytic and foamy appearance. Glial, endothelial, and perithelial cells are generally similarly affected.

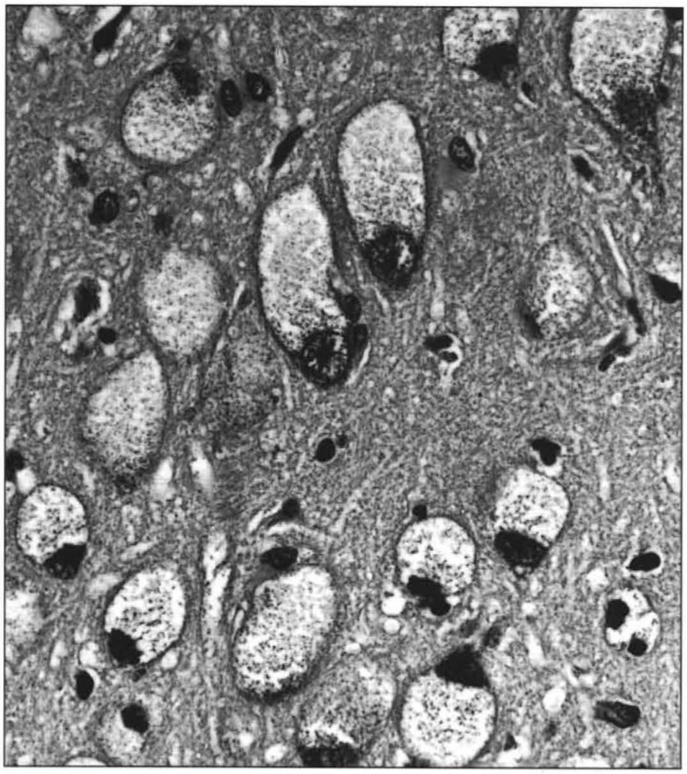

Figure 3.41 Swollen neurons in a **lysosomal storage disease** (GM_1 gangliosidosis) in a sheep. (Reprinted with permission from Murnane RD, et al. Vet Pathol 1991;28:332.)

In several ganglioside storage diseases, certain populations of neurons, particularly in the pyramidal system of the cerebral cortex and thalamic relay nuclei, undergo a form of *focal hypertrophy* in order to generate more "storage space." These cells develop large swollen compartments between the axon hillock and the initial axonal segment, dubbed "*meganeurites*." In addition, cells in these regions may sprout *aberrant dendritic spines*, whether or not they have meganeurites. The spines arise from the axon hillock and from meganeurites and form synaptic contacts. The origin of the presynaptic elements of these contacts has not been determined, but such abnormal neuronal connections could well contribute to malfunction. They may be permanent in the induced storage diseases in which otherwise the stored material may be catabolized and removed if the toxic inhibition of the enzymes is removed. These changes, dramatic as they are, cannot be appreciated without the use of special techniques, most notably the Golgi impregnation method. In addition, focal swellings may develop along the course of axons, appearing as eosinophilic spheroids, similar to those described for axonal dystrophy (see Fig. 3.9). They are often very prominent in some nuclear groups, exhibiting a tendency to form in the terminal presynaptic regions of axons, but they can be seen anywhere in the white matter and also in the peripheral nerves. They do not contain specific storage material, but are crowded with degenerate organelles and/or abnormal tubules and vesicles. They probably reflect a secondary effect of the storage process on retrograde axonal transport. The functional significance of this secondary axonal dystrophy is not resolved, but it is a prominent pathologic feature in many storage diseases.

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Inherited storage diseases

Virtually all the inherited storage diseases of animals are proven or assumed to be lysosomal in nature. This applies to all those to be described below with the exceptions of the canine glycogenoses analogous to types 3 and 7 glycogenoses in humans. They are classified into broad groups, according to the class of macromolecule whose degradation is defective. As many of these diseases were first described in humans, the catalog is replete with eponyms, mostly derived from the names of the eminent people who provided those first descriptions. Within the broad groups, individual entities are defined by the nature of the dominant storage material, which reflects the specific enzymic deficit. Not all the storage diseases documented in humans have been found to have analogs in domestic animals, but the list is growing and the prevalence of inbreeding makes it likely that this will continue. *The general clinical characteristic is the onset of progressive neurologic impairment at a young age.*

In the descriptions of pathology that follow, variations in the patterns of lesions are likely to surface as more cases are described. A useful practical distinction can be made between those diseases in which the vacuoles appear empty in both paraffin and resin sections (Fig. 3.42A), and those in which they contain residual material (Fig. 3.42B). The former reflect water-soluble substrates leached out during processing, and point to certain diseases as described below.

Sphingolipidoses

Sphingolipidoses are lysosomal storage diseases caused by a genetic defect in catabolism of *glycosphingolipids*, which are important normal components of cell membranes.

In general, **gangliosidoses** are characterized clinically by the *onset at an early age of discrete head and limb tremors and dysmetria.* Worsening locomotor deficits and mentation terminate eventually

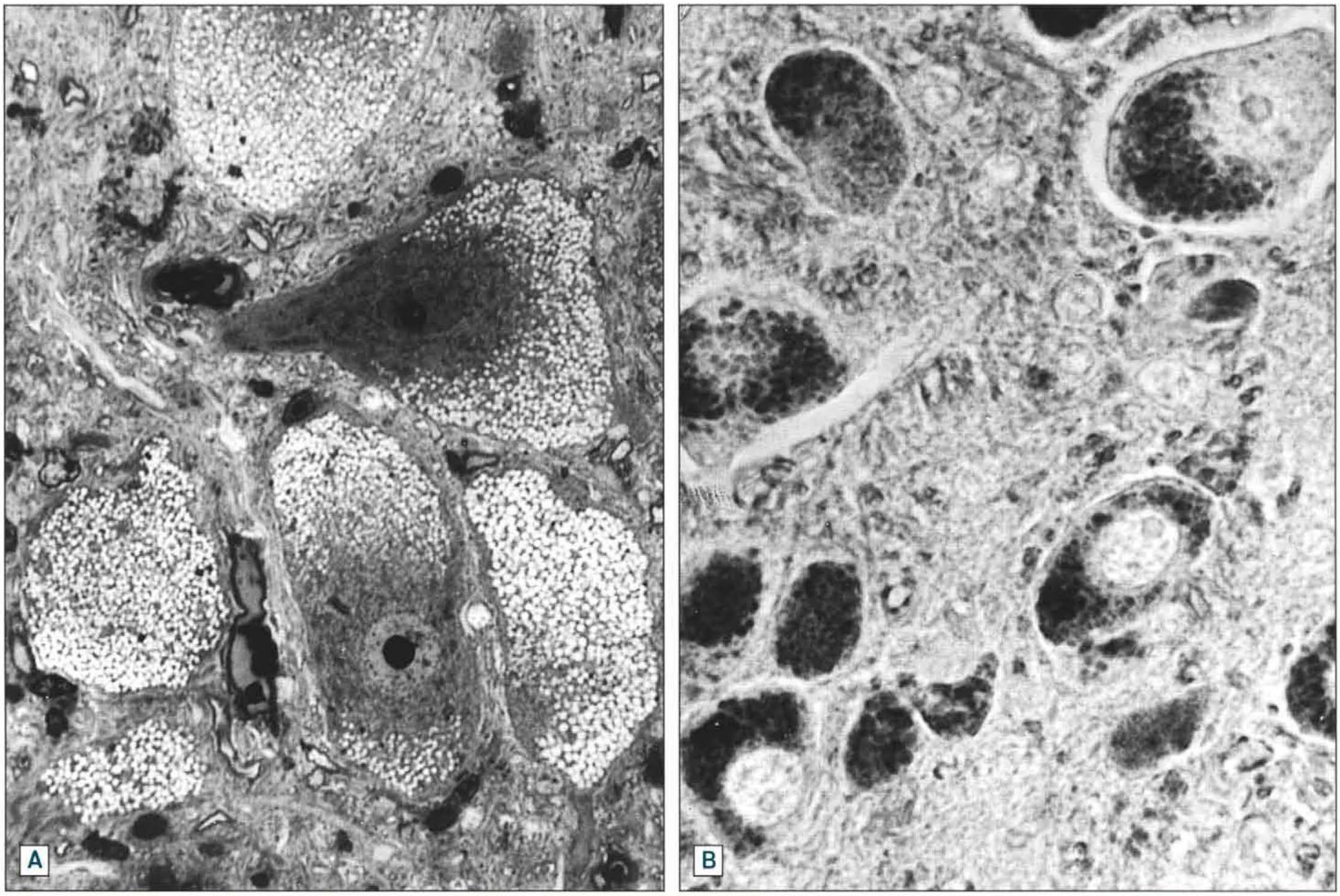

Figure 3.42 Neuronal **lysosomal storage disease**. **A.** Resin section showing "empty" vacuoles in bovine mannosidosis. (Courtesy of RD Jolly.) **B.** Dense granules, Sudan black positive, in gangliosidosis in a German Shorthaired Pointer dog. (Courtesy of E Karbe.)

in blindness, somnolence, seizures, and quadriplegia. Data indicate *autosomal recessive inheritance.*

In **GM_1 gangliosidosis** (generalized gangliosidosis), deficiency of *β-galactosidase* leads to accumulation of GM_1 ganglioside and some oligosaccharides. GM_1 gangliosidosis has been documented in cats (domestic shorthair, Korat, Siamese), dogs (Alaskan Husky, Beagle, English Springer Spaniel, Portuguese Water Dog, Shiba), Friesian cattle, and sheep (Coopworth-Romney, Romney, Suffolk).

In **GM_2 gangliosidosis**, GM_2 ganglioside accumulates due to deficient lysosomal degradative activity of *hexosaminidase*, which exists as a dimer in two forms, αβ (hexosaminidase A) or ββ (hexosaminidase B), or of an *activator protein*. Globoside may also accumulate. GM_2 gangliosidosis has been documented in domestic shorthair and Korat cats (β subunit deficiency), German Shorthaired Pointer dog, Golden Retriever (β subunit deficiency), Japanese Spaniel dog (GM_2 activator protein deficiency), and Yorkshire pigs. Variants of GM_2 gangliosidosis in humans are Tay–Sachs and Sandhoff diseases, and activator deficiency.

Neuronal storage in gangliosidoses is manifested in routine paraffin sections as marked distension of the soma, with *foamy, faintly eosinophilic cytoplasm* (Figs 3.41, 3.43A). The stored material is strongly PAS-positive in frozen sections, and is evident in plastic sections as 1–3 μm osmiophilic granules. Ultrastructurally, these are seen as *characteristic membranous cytoplasmic bodies*, consisting of concentric membranous whorls with a periodicity of about 5 nm (Fig. 3.43B). Vacuolated macrophages may also be found around blood vessels in the CNS, and storage also occurs in glial cells. Axonal spheroids may be reasonably numerous. Gliosis, demyelination and neuronal loss are not apparent until end stage. In those instances where hepatic storage occurs, hepatocytes and Kupffer cells contain large, empty vacuoles, the site of storage of a water-soluble glycopeptide.

GM_1 gangliosidosis in Suffolk sheep is associated with a dual enzyme deficiency, with βl-galactosidase deficiency being profound (5% residual activity), and α-neuraminidase deficiency less so (20% residual activity). Progressive ataxia in affected lambs first becomes evident at 4–6 months of age. Histologically, there is intense storage in most central and peripheral ganglionic neurons (Fig. 3.41), and in the kidney, liver, and lymph nodes, and cardiac Purkinje fibers. Stored material in neurons stains PAS and Luxol fast blue positive, but Sudan black negative. Axonal spheroids are frequent in cerebral and cerebellar white matter.

Glucocerebrosidosis (glucosylceramidosis) has been described in the Sydney Silky Terrier, and is the counterpart to *Gaucher disease* of humans. It results from deficient activity of *glucocerebrosidase*, which

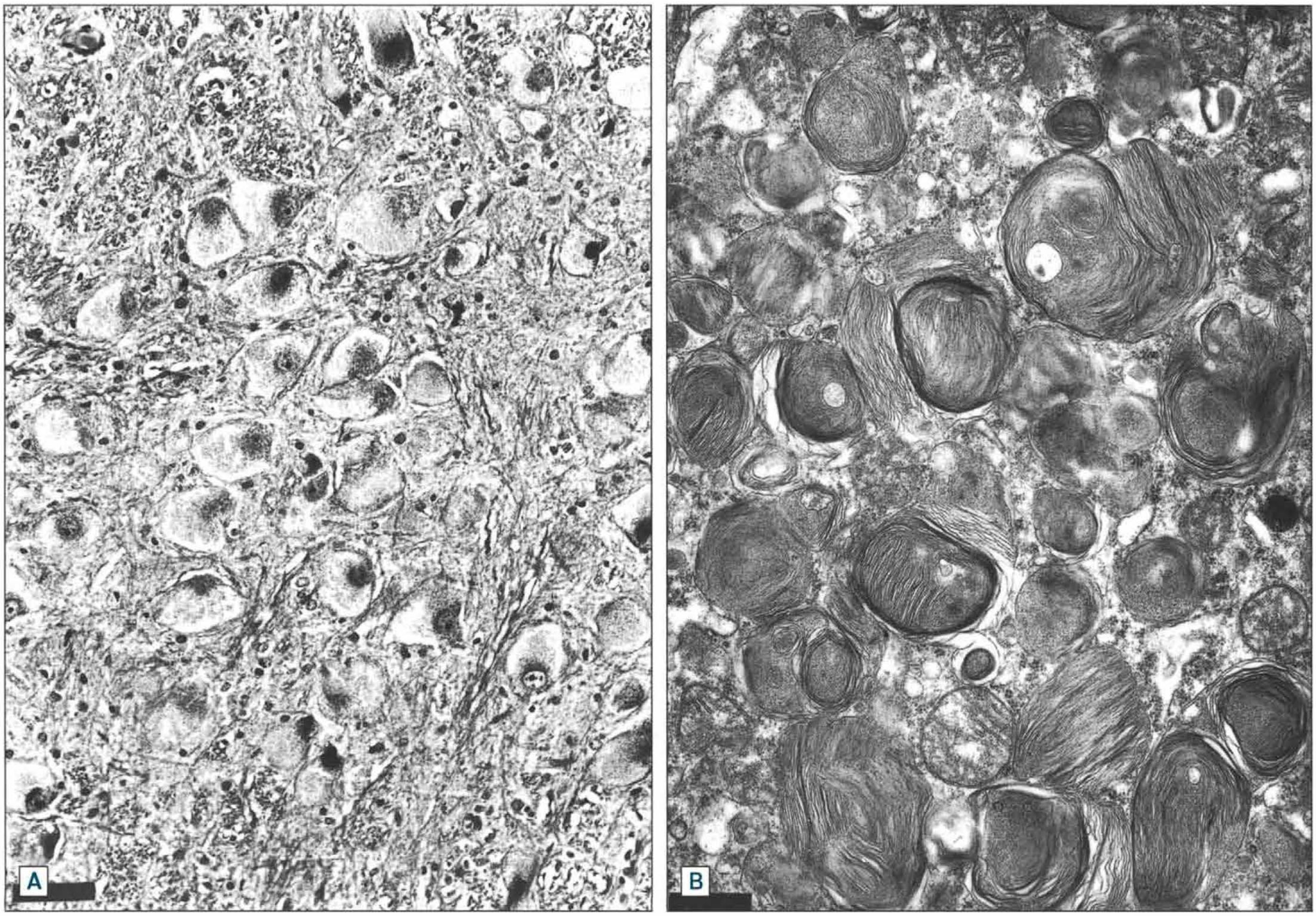

Figure 3.43 GM_1 gangliosidosis in a Portuguese Waterdog. **A.** Pale swollen neurons. Bar = 63 μm. **B.** Storage bodies showing concentric lamellae or parallel stacks of membranes. Bar = 0.6 μm. (Reprinted with permission from Saunders GK, et al. Vet Pathol 1988;25:265.)

catalyzes the conversion of glucocerebroside to ceramide. The former is derived from the catabolism of gangliosides.

Storage in the dog is expressed in macrophages in the hepatic sinusoids and lymph nodes, and in neurons in some parts of the brain but, interestingly, not in Purkinje cells and not in the spinal cord. Swollen cells have weakly eosinophilic cytoplasmic vacuoles, which are PAS-positive in the macrophages but PAS-negative in neurons. Degenerating neurons occur in the cerebral and cerebellar cortices, and Wallerian degeneration in related white matter.

Ultrastructurally, the macrophage storage material has a *characteristic twisted branching tubular structure*, and the cells in the human disease are known as *Gaucher cells*. In neurons, the storage granules are either lamellated membranous cytoplasmic bodies, resembling the Zebra bodies of mucopolysaccharidoses, or bilamellar wisps. In Gaucher variants in humans, storage may not occur in neurons.

Sphingomyelinosis has been recognized in Siamese, domestic shorthair and Balinese cats, and Poodle and Boxer dogs, and is regarded as the counterpart of *Niemann–Pick disease* in people, of which there are several variants. In types A and B, deficient activity of *sphingomyelinase* results in storage of sphingomyelin, cholesterol, and gangliosides in most neurons, and in macrophages in the liver, spleen, lymph nodes, adrenals, bone marrow, and lungs. The type C variant is caused by defective activity of a cholesterol transporter, NPC1 protein, or in the soluble lysosomal cholesterol-binding NPC2 protein. In animals, most cases are analogous to human type A; type C is described in cats and Boxer dogs.

At necropsy there is enlargement and pallor of the liver, splenomegaly, and gray nodules in the lungs. Microscopically, the visceral organs mentioned are packed with foamy macrophages, and the neurons are distended by masses of light gray, 1–2 μm autofluorescent granules. In frozen sections, the reactivity of vacuoles to oil-red-O and PAS stains is variable.

Type C Niemann–Pick disease is also characterized by numerous axonal spheroids in many areas of the neuraxis. Ultrastructurally, neuronal storage granules are concentric multilamellar structures or dense bodies.

Galactosialidosis, which results from combined deficiency of β-galactosidase and α-neuraminidase enzyme activity, is reported in a Schipperke dog. A similar combined enzyme deficiency in inbred Suffolk sheep resembled GM1 gangliosidosis phenotypically.

Galactocerebrosidosis (*galactosylceramide lipidosis, globoid cell leukodystrophy; Krabbe disease*), due to deficiency of *galactocerebrosidase*, is a member of this group, but involves storage in macrophages rather than neurons. It is discussed later under myelinopathies.

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Glycoproteinoses

This group includes diseases in which there is *defective degradation of the carbohydrate component of N-linked glycoproteins*. These carbohydrate moieties are rich in mannose, N-acetyl glucosamine and fucose, and in particular diseases this is reflected in the storage residues that are detectable in the urine.

α-Mannosidosis has been the most economically important of the inherited storage diseases of animals, as it once occurred at high frequency in some populations of Angus cattle and the derivative Murray Grey breed. It is also recorded in the Galloway breed. The characterization and study of the disease in New Zealand led to effective carrier detection and certification schemes for its elimination and the incidence declined significantly. Inheritance is autosomal recessive, and affected calves have retarded growth, increasingly severe ataxia, and behavioral changes. The disease reaches end-stage by about 18 months of age.

Due to the synthesis of a defective enzyme protein, such individuals have *deficient lysosomal α-mannosidase* activity in virtually all cells except hepatocytes. All but the final mannose molecule of the glycans destined for digestion by this enzyme are alpha-linked. As a result, mannose/N-acetylglucosamine oligosaccharides accumulate in storage vacuoles that appear empty by light microscopy (Fig. 3.42A), as the material is extracted during tissue processing. Ultrastructurally, the vacuoles are seen to contain sparse membranous fragments and some floccular material (Fig. 3.44).

Neuronal storage is widespread and severe, but neuronal loss is not conspicuous until the terminal stages. Axonal spheroids are numerous in both gray and white matter, but especially in the cerebellar roof nuclei, the caudal brain stem proprioceptive nuclei, and on the proximal parts of Purkinje cell axons. The striking extent of storage in secretory epithelia, such as in pancreas and kidney, endothelia, fixed macrophages and fibrocytes is best appreciated in plastic-embedded sections.

α-mannosidosis has also been described in Persian and domestic shorthair and longhair cats. In the first two breeds, kittens are clinically affected very early in life, having retarded growth, facial dysmorphism, ataxia, tremor, and hepatomegaly. Intense and universal neuronal storage is accompanied by hypomyelination in the cerebrum, and widespread occurrence of axonal spheroids. Extensive storage in other tissues is as described for the bovine disease.

In the domestic longhair cat, nervous signs are reported to be milder and more slowly progressive, and there are no ocular abnormalities, hepatomegaly, myelin deficiency, or pancreatic acinar cell involvement. However, intense loss of Purkinje cells is evident and there is great diversity in the morphology of the storage cytosomes, with many membranous cytoplasmic bodies in caudal brain stem nuclei.

β-Mannosidosis has been described in the Anglo-Nubian goat, and in Salers cattle. The final mannose residue of the glycoprotein glycans is beta-linked to N-acetylglucosamine; its hydrolysis normally follows that of the alpha linkages described above. Deficiency of *β-mannosidase* leads therefore to the storage of di- or trisaccharides containing one molecule of mannose.

The disease has been most comprehensively documented in Nubian goats; inheritance is autosomal recessive. Clinical signs are severe at birth, affected kids having small palpebral fissures, facial dysmorphisms, domed skulls, joint contractures, intention tremors, and deafness. They are generally unable to stand and, even with intensive care, will survive for only a few months.

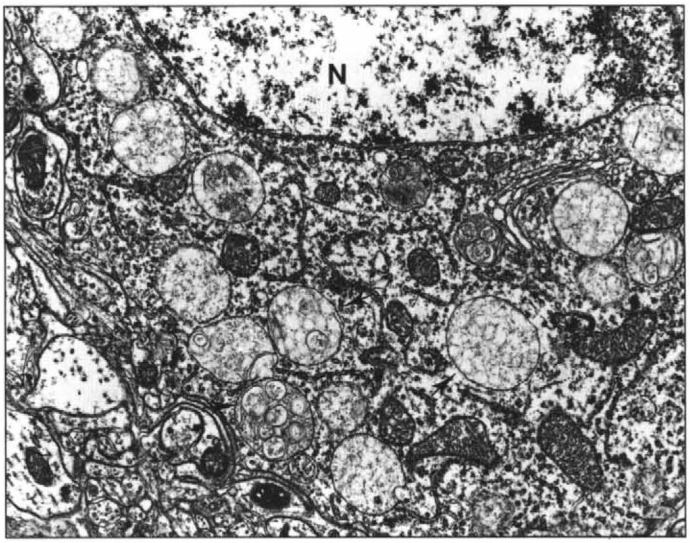

Figure 3.44 Cortical neuron in **feline mannosidosis**; storage vacuoles (arrows) contain either sparse floccular material or membranous arrays. Nucleus (N). (Courtesy of SU Walkley.)

Grossly, there is dilation of the ventricles and hypomyelination of the cerebrum and cerebellum. The latter may be the result of congenital hypothyroidism, due to storage-mediated interference with thyroid function, and it accounts for the congenital syndrome. Microscopically, neuronal vacuolation is ubiquitous and axonal spheroids numerous, especially in the internal capsule, cerebellar white matter, and basal ganglia. Vacuolated macrophages occur around some blood vessels in the brain, and there may be focal mineralization in the cerebellum and cerebrum. Large focal swellings filled with neurofilaments are sometimes present in the proximal axons of spinal motor neurons, and spheroids are numerous in sensory endings in mucous membranes and conjunctivae. Intense storage in most other tissues is similar to that described for α-mannosidosis, as is the ultrastructure of the storage vacuoles.

α-L-fucosidosis occurs in the English Springer Spaniel. As the result of deficient activity of *α-L-fucosidase*, water-soluble, fucose-containing compounds are stored as glycosylasparagines. Autosomal recessive inheritance is established, and the disease has a somewhat delayed clinical onset, with wasting, ataxia and proprioceptive deficits beginning at about 6 months of age, and becoming severe by about 2 years. A striking gross lesion is marked swelling, up to 10 mm diameter, of the cervical portion of the vagus nerves, the cervical nerves, and dorsal root ganglia.

As in mannosidosis, storage is intense in most tissues, cells becoming distended with apparently empty vacuoles. In the CNS, it is present in all neurons and in astrocytes and microglial cells; there are numerous perivascular accumulations of vacuolated macrophages. Axonal spheroids are present in the cerebellar white matter. In the thickened nerves, there is heavy infiltration of macrophages and the accumulation of myxoid perineurial ground substance.

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Mucopolysaccharidoses

This group of diseases is defined by *defective catabolism of glycosaminoglycans (GAG, mucopolysaccharides)* and has major expression in the skeleton and connective tissues. These diseases are therefore also discussed with Bones and joints. Glycosaminoglycans – dermatan sulfate, heparan sulfate, keratan sulfate, chondroitan sulfate – are extremely large and complex molecules, and many enzymes are involved in their degradation. As a result, 11 are entities defined in humans; four have been reported in domestic animals, and in three of these there is major involvement of neurons. General features include facial and skeletal dysmorphisms, degenerative joint disease, corneal clouding, thickening and distortion of heart valves, and thickening of the leptomeninges. Large quantities of heparan and dermatan sulfates are excreted, and are detectable, in the urine.

In animals, deficiency of **α-L-iduronidase** has been observed in the domestic shorthair cat and the Plott Hound, and is regarded as the counterpart of *mucopolysaccharidosis type I* (**MPS I**) *in man*, variants of which include the *Hurler and Scheie syndromes*. The macroscopic features described above are subtended by intense storage in fibroblasts, fixed macrophages, chondrocytes, myocytes, and pericytes in most organ systems. Glandular epithelial cells are consistently less affected than mesoderm-derived cells. The involvement of chondrocytes is associated with dysplasias of endochondral ossification, and with degeneration of articular cartilage.

Neuronal storage is universal, but neuronal loss is not conspicuous. Storage vacuoles are ultrastructurally pleomorphic; they may appear largely empty, or to contain sparse floccular to granular amorphous material, or lamellar membranous structures termed *Zebra bodies*. The latter appear to be lipid in nature, and probably represent induced secondary storage of sphingolipids. There is thus a somewhat variable reaction to tissue staining for GAG and lipid. There is some suggestion that affected cats may have a high incidence of cranial meningiomas.

In the Nubian goat, **N-acetylglucosamine-6-sulfatase deficiency** gives rise to a counterpart of human **MPS III**, *Sanfilippo disease*. Clinical features encompass delayed ability of the neonate to stand and walk, persistently ataxic gait, marked bowing of the forelimbs, and corneal clouding. Gross changes include dwarfism, kyphoscoliosis, scapular hypermobility, and cartilaginous and bony abnormalities. Two main types of lysosomal storage bodies occur microscopically. The primary storage material is *heparan sulfate*, which appears as lucent floccular material in lysosomes in arterial smooth muscle and cardiac myocytes, fibroblasts, macrophages, hepatocytes, Kupffer cells, and chondrocytes. Neurons, in contrast, are packed with PAS-positive multilamellar bodies representing secondary storage of *gangliosides* induced by interference with neuraminidase activity. Mucopolysaccharidoses IIIA and IIIB are reported in several dog breeds.

In Siamese and domestic shorthair cats, **arylsulfatase-B deficiency** produces a counterpart to human **MPS VI** (*Maroteaux–Lamy syndrome*). The disease has the same general features as described above, but neuronal storage does not occur. Storage in peripheral blood neutrophils is detected as metachromatic cytoplasmic granules.

A **β-glucuronidase-deficient MPS** has been described in dogs and cats, and is considered analogous to human **MPS VII** (*Sly disease*). The general features of the clinical disease are as described above, and there is excessive urinary excretion of chondroitin-4-sulfate and chondroitin-6-sulfate. There is *widespread neurovisceral storage*, with cytoplasmic inclusions appearing largely empty or containing sparse granular or lamellar material.

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Glycogenoses

A number of enzymes are involved in the catabolism of glycogen, but only one of these, *α-1,4-glucosidase*, is a lysosomal enzyme. The lysosomal pathway degrades any glycogen that finds its way into autophagic vacuoles, while the other pathways are more related to metabolic mobilization of glycogen. In humans, eight different glycogen storage diseases have been identified, with the lysosomal type being classified as **type II**, *Pompe disease*. As the storage process is very widespread in this disease, it is also referred to as *generalized glycogenosis*. In most other types, storage is concentrated in liver and muscle.

α-1,4-glucosidase deficiency has been well documented in Shorthorn and Brahman beef cattle. The disease has autosomal recessive inheritance, and affected calves have severe clinical signs by about a year of age. The most damaging effects are produced in the skeletal and cardiac muscle; weakness and congestive cardiac failure are clinically dominant, and cardiomegaly and hepatomegaly evident at necropsy. There is *widespread glycogen storage*, much of which occurs in typical lysosomal storage vacuoles, but some of which is intracytoplasmic. Swollen, vacuolated cells contain diastase-sensitive, PAS-positive material, which is ultrastructurally typical of glycogen. Vacuolar myopathy and cardiomyopathy are prominent (Fig. 3.45); *neuronal storage is universal and severe* and is accompanied by glial storage, with numerous axonal spheroids in the vestibular and cuneate nuclei, terminal fasciculus gracilis, and throughout the spinal cord gray matter (see Fig. 3.8). Mild Wallerian degeneration may be present in the lateral and ventral columns of the spinal cord and in some peripheral nerves.

Type II glycogenosis has also been documented in the Lapland dog, and suspected on morphologic grounds in the domestic cat and Corriedale sheep.

Amylo-1,6-glucosidase deficiency is recorded in the German Shepherd Dog, as a counterpart of glycogenosis **type III** (Cori disease). Cytoplasmic glycogen storage occurs in liver, muscle, and myocardium, and in neurons and glia in the brain and spinal cord.

Polyglucosan body disease (glycogen storage disease **type IV**) is reported in a mixed-breed dog as the result of an inherited metabolic defect associated with a deficiency of glycogen-branching enzyme. Periodic acid Schiff (PAS)-positive polyglucosan bodies (abnormal glycogen) were present in neurons, liver, and myocardium.

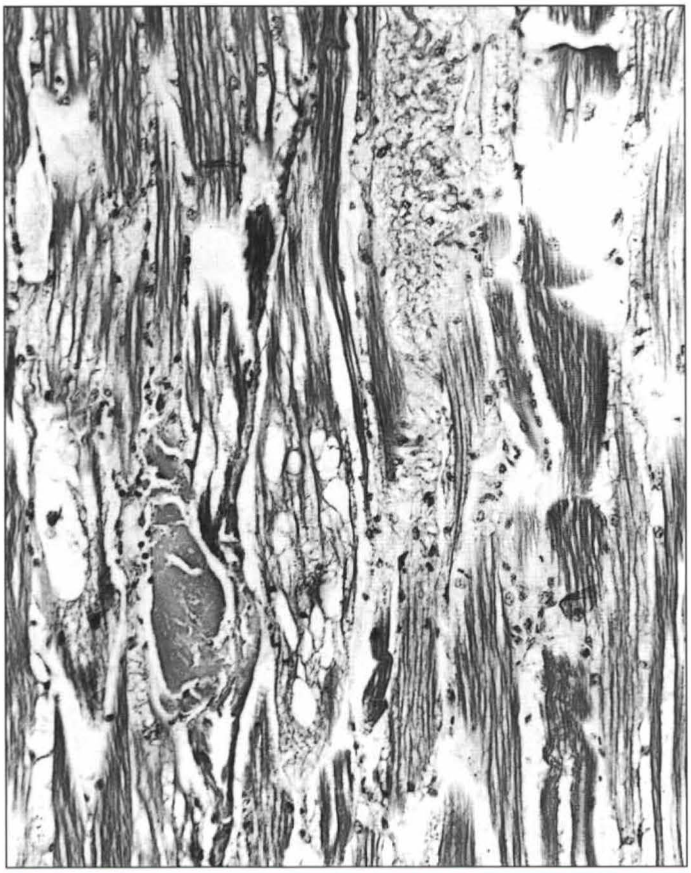

Figure 3.45 Vacuolated and degenerate skeletal muscle fibers in **generalized glycogenosis** in a Shorthorn calf.

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Mucolipidoses

Mucolipidosis describes a disease with the features of both sphingolipidoses and mucopolysaccharidoses. **Mucolipidosis II** (ML II), also called *I-cell (inclusion-cell) disease*, is a lysosomal storage disease caused by deficient activity of *N-acetylglucosamine-1-phosphotransferase*. The condition is inherited as an autosomal recessive in domestic shorthair cats. Affected kittens fail to thrive and have behavioral dullness, facial dysmorphia, and ataxia. Diffuse retinal degeneration leads to blindness. Storage lysosomes containing oligosaccharides, mucopolysaccharides, and lipids are most common in bone, cartilage, skin, and other connective tissues; a few cerebral cortical neurons have lipid inclusions, and some sciatic nerve axons are affected.

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Ceroid-lipofuscinoses

With advancing age and senility, intracytoplasmic granules of the autofluorescent lipopigment, **lipofuscin**, accumulate in neurons, fixed phagocytes, macrophages and muscle cells. This *"wear and tear" pigment* is familiar to all pathologists, and has a characteristic histochemical profile and ultrastructure; the irregularly shaped granules have a high-density component punctuated with vacuoles of low density. For many years, in the group of diseases known as the "ceroid-lipofuscinoses," it has been assumed that a similar pigment is stored in lysosomes. However, other compounds, such as the hydrophobic lipid-binding protein subunit c of mitochondrial ATP synthase are also complexed with lipid and may predominate; hence at least some of these conditions might better be termed **proteinoses**.

In animals, neuronal ceroid-lipofuscinoses (NCL, Batten disease) of proven or presumptive **inherited** nature have been described in Siamese cats, South Hampshire sheep, Devon cattle, Nubian goats, and many breeds of dog including English Setter, Chihuahua, Dachshund, Saluki, Dalmatian, Blue Heeler, Border Collie, Tibetan Terrier, and crossbred. In general, *although storage may be widespread in several organs, it is most damaging in neurons of the cerebral cortex, retina, and cerebellar Purkinje system.* Thus there is frequently extensive cellular loss and atrophy in these regions, and correlating dementia, blindness, and ataxia. Macroscopically, atrophied areas may have a distinctly brown tinge. Microscopically, the storage granules are brightly autofluorescent under ultraviolet light, pale brown-red or colorless with hematoxylin and eosin, weakly acid-fast, magenta with PAS, and intensely positive with Luxol fast blue. Ultrastructurally, they appear as *membrane-bound cytosomes* up to 15 nm in diameter, irregular in outline, and with a variety of forms (Fig. 3.46). Some have membranous material arranged as *"curvilinear bodies" and "fingerprint bodies,"* which are considered characteristic. Others may have *laminated stacks of membranes* akin to zebra bodies, membranous stacks, or dense granular deposits.

The variation in features of NCL is illustrated by comparing the disease in various species and breeds. In Devon cattle, the major clinical deficit is profound blindness at about 14 months of age, with death usually due to misadventure by about 2 years. There is severe retinal atrophy but only mild loss of neurons from the cerebral and cerebellar cortices. In Tibetan Terriers, blindness is again the dominant sign, but the onset is delayed to middle life and is accompanied terminally by stupor. In the Border Collie and some other dog breeds, there are gait and visual deficits by 18–24 months of age, accompanied by increasing aggression and dementia. Retinal lesions are mild and blindness is central in origin. Neuronal loss and gliosis are particularly severe in the cerebellar Purkinje cell layer, and significant in the limbic system.

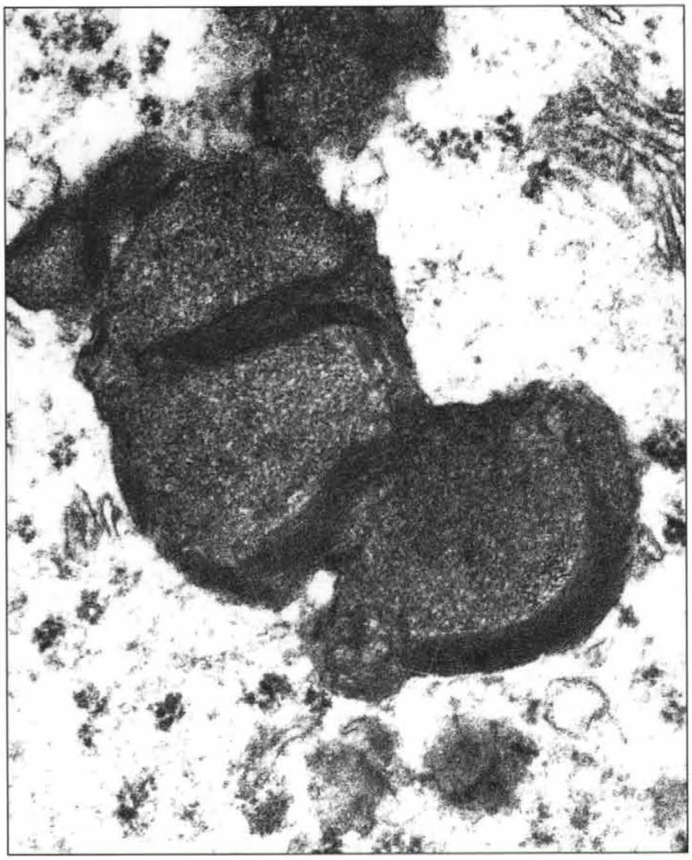

Figure 3.46 Ceroid lipofuscinosis in a Tibetan Terrier. Granular and membranous storage body in a Purkinje cell. (Courtesy of JF Cummings.)

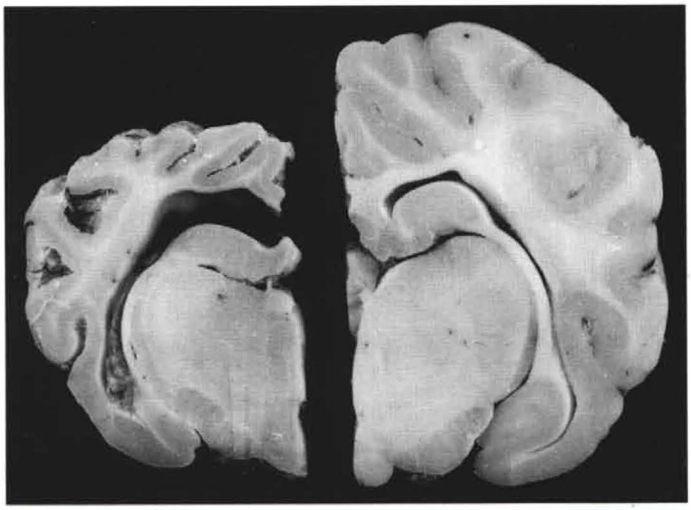

Figure 3.47 Ceroid lipofuscinosis in a South Hampshire lamb. Marked cerebral atrophy at 4 months; control right. (Courtesy of RD Jolly.)

The pathogenesis of at least some forms of NCL may involve a defect in mitochondria rather than a defect in lysosomal catabolism, and may involve accumulation of hydrophobic protein. The NCL in South Hampshire sheep has been thoroughly studied. The brains of affected lambs grow normally until 4 months of age, and then undergo atrophy (Fig. 3.47). Half of the material stored in lysosomes is the lipid-binding protein subunit c of mitochondrial ATP synthase. NCL in Merino sheep is also a subunit c-storing disease, clinically and pathologically similar to NCL in South Hampshire sheep. The NCL in both breeds (OCL6 form) apparently results from a mutation at the same gene locus in chromosomal region OAR7q13-15, and both breeds are potential animal models for the human late infantile variant CLN6. CLN proteins are coded by *NCL* genes.

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Miscellaneous genetic storage diseases

The apparent lysosomal storage of lipid material in spinal motor neurons is described in English Pointer dogs, and is discussed under motor neuron diseases.

A counterpart to the autosomal recessive human **Lafora disease** has been recorded in Basset Hound, Poodle and Beagle dogs. Progressive myoclonus epilepsy is associated with *widespread intraneuronal storage of a complex polyglucosan*, which appears as characteristic *Lafora bodies* (see Fig. 3.5). They are most numerous in Purkinje cells, and neurons of the caudate, thalamic, and periventricular nuclei. They are non-membrane-bound spherical structures with a central basophilic core and a peripheral halo of radiating filaments. They are strongly PAS-positive and are found in both perikaryon and dendrites; they vary greatly in size, ranging up to 15–20 μm. Human LD is caused by mutation in the *EPM2A* gene that encodes for laforin and the *EPM2B* gene that encodes for malin. Lafora bodies are occasionally found incidentally in otherwise normal brains and spinal cords, especially in older dogs.

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Induced storage diseases

Numerous types of storage process have been successfully produced experimentally, and there is a group of naturally occurring disorders related to the ingestion of plants, in which induced storage disease is proven or suspected.

Swainsonine toxicosis

Swainsonine, *an indolizidine alkaloid*, is the active principle of several species of toxic plants that have caused considerable problems for all classes of grazing livestock. These include poison pea (*Swainsona* sp.) of Australia, locoweeds (*Astragalus, Oxytropis* sp.) of North America, broomweed (*Sida carpinifolia*) of Brazil, and shrubby morning glory (*Ipomoea carnea*) of Mozambique. Endophytic fungi, such as *Embellisia,* that grow on locoweed can induce the same toxicity. As a *potent inhibitor of lysosomal* α*-mannosidase*, swainsonine induces a form of **α-mannosidosis** that is a close copy of the genetic disease of cattle and cats. Continued ingestion of toxic material over a period of 4–6 weeks and more results in failure to thrive and, ultimately, ataxia, proprioceptive deficits and behavioral abnormalities ("locoism" in North America, "pea-struck" in Australia). Necropsy examination during, or within a short time after, exposure to the plant reveals microscopic and ultrastructural lesions identical to those of genetic α-mannosidosis. Within 2 weeks of last exposure, much of the storage disease resolves, but axonal spheroids may persist in large numbers in areas such as the cerebellar roof nuclei and caudal brain stem. Swainsonine-induced mannosidosis has been experimentally compared in the cat with genetic mannosidosis in order to determine the reversibility of changes such as meganeurite and aberrant synapse formation in higher neurons (see introductory section). Clinical recovery may or may not occur following cessation of exposure, and the persistence of secondary neuronal changes suggests that they, rather than the storage process, may underlie neuronal malfunction. Exposure of young growing animals is more likely to produce irreversible disease than exposure of adults.

The induced disease is, however, biochemically distinct from the genetic, as *the alkaloid also inhibits Golgi mannosidase II*, an enzyme involved in the post-translational trimming modifications of the glycan moiety of glycoproteins. As a result, abnormal proportions of different types of glycoproteins are produced, and the storage oligosaccharides are larger than those in the genetic disease. No modification of the storage disease appears to result from this difference.

In swainsonine intoxication of pregnant animals, both dam and fetus are affected, and *abortion and terata are well recognized in ovine locoweed toxicosis.* Suppressive effects on fertility are also recognized.

Trachyandra poisoning

Ingestion of *Trachyandra divaricata* or *T. laxa* for several weeks has been associated with severe neurologic disease and **lipofuscinosis** in sheep, horses, goats, and pigs in South Africa and Australia. The clinical syndrome is one of weakness, suggesting a neuromuscular disorder, and is often accompanied by *intense lipofuscin storage in all central and peripheral neurons* and, to a lesser extent, in macrophages of the intestinal lamina propria, hepatocytes, Kupffer cells, and renal tubular epithelium. Pigment storage may be sufficiently intense to cause macroscopic rusty-brown discoloration of central gray nuclei and peripheral ganglia. The pigment granules have all the histochemical and ultrastructural features of lipofuscin. The clinical signs appear to be irreversible and their relationship to the storage process is obscure; the basis of the storage process is unknown.

Phalaris poisoning

Extensive losses in sheep and cattle in Australia, New Zealand, South Africa, California, and Argentina have been due to grazing *Phalaris* sp., principally *P. aquatica* and *P. arundinacea*, but also *P. minor, P. caroliniana*, and *P. angusta*. Several syndromes of *Phalaris* toxicosis affect principally ruminants but occasionally horses – dramatic large-scale sudden mortalities occur in sheep, sudden deaths have been associated in horses, staggers syndromes of acute onset and recoverable or of chronic onset and not recoverable occur in sheep and occasionally in cattle. Onset of staggers syndromes may be rapid on exposure to immature plants or delayed for up to several months. It is probable that resistance to the toxicosis may be produced by catabolism of toxins by adapted ruminal microflora.

There are two acute neurological syndromes. *Sudden death* in sheep and horses without observable tissue changes is probably caused by cardiotoxic compounds, a mix of *methyl tryptamine* and β*-carboline indoleamines* chemically related to 5-hydroxytryptamine (serotonin). The second acute syndrome is in sheep and is referred to as a polioencephalomalacia-like syndrome, the result of edema of deep cortical gray matter laminae. Severe astrocytic edema in the cortex is similar to that seen in citrullinemia, and suggests peracute ammonia toxicity due to toxic impairment of urea-cycle enzymes.

The clinical onset of the staggers syndrome may be delayed for several months after exposure to toxic pasture has ceased. It is characterized by generalized muscle tremors progressing to stiffness, collapse on forced exercise, and tetanic seizures. In most cases, recovery does not occur and apparent recovery is followed by relapses.

In cases examined early in the course of the disease, there may be no gross or microscopic lesions but, in general, pathologic changes are present. Most characteristically, there is *storage of granular pigment within neurons* of the brain stem nuclei, spinal gray matter and dorsal root ganglia, and in macrophages of the cerebrospinal fluid. A similar pigment is also present within renal tubular epithelial cells. When storage is intense, the affected gray matter and kidneys may have distinct green discoloration on gross inspection (Fig. 3.48A). Histologically, the pigment granules are green-brown and present in a perinuclear distribution in neurons (Fig. 3.48B), although some granules may accumulate in dendrites. Ultrastructurally, the storage granules are composed of concentric membranous lamellae, sometimes interspersed with fine granular material. They are membrane bound and are considered to be lysosomal in nature. In most cases, there is also Wallerian degeneration concentrated in ventral, ventromedial, and lateral funiculi throughout the spinal cord, and in the medial longitudinal fasciculus. This distribution suggests selective damage to long descending motor tracts. Severely affected areas may also have intense diffuse astrogliosis. Intense reactive astrogliosis may also be seen in ventral spinal cord gray matter, along with mild neuronal loss.

Solanum poisoning

Ingestion of *Solanum kwebense* or *S. fastigiatum* is associated with neuronal degeneration and loss in the cerebellum, as discussed elsewhere.

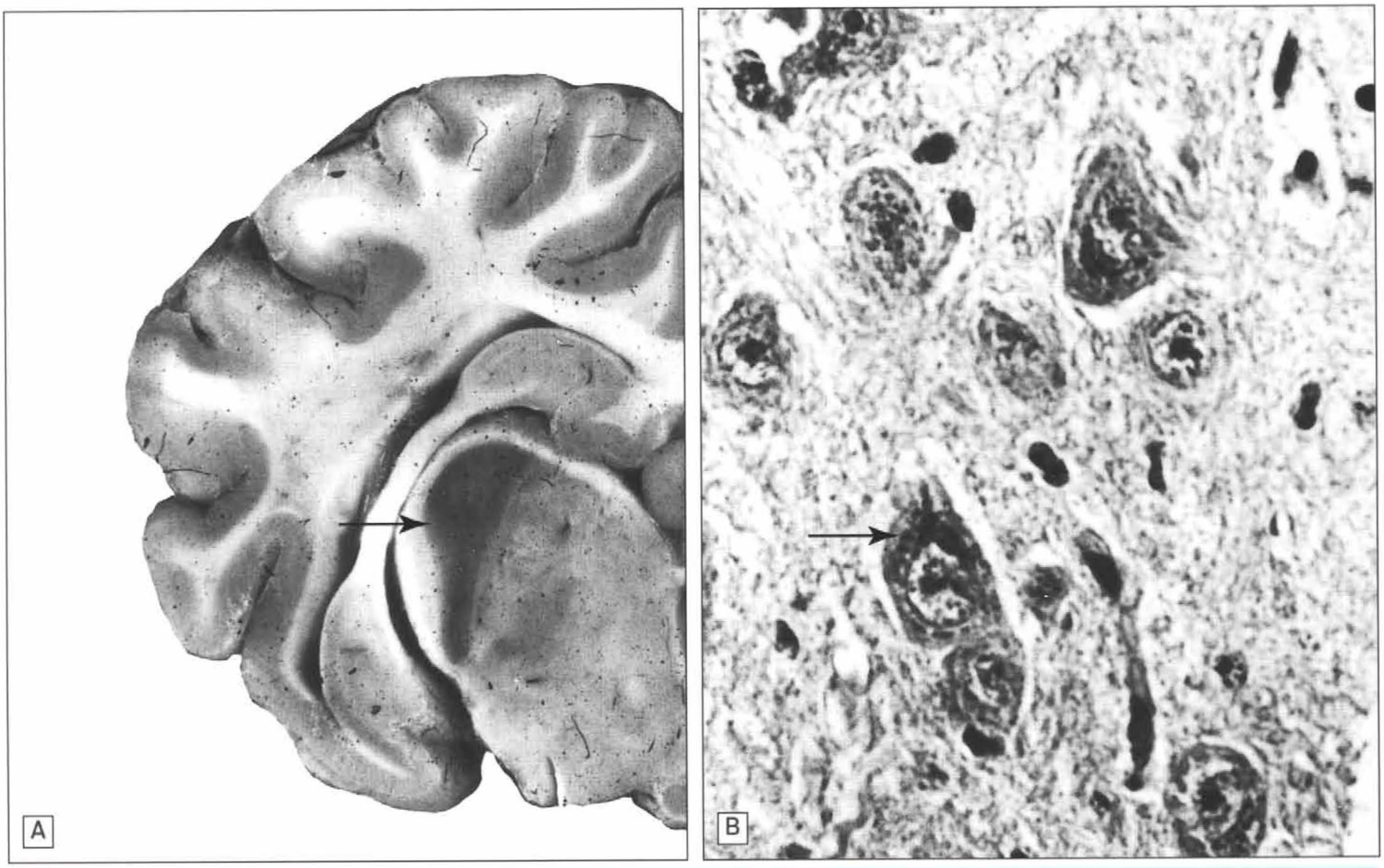

Figure 3.48 ***Phalaris* sp. poisoning** of a cow. **A.** Pigmentation in lateral geniculate body (arrow). **B.** Pigment granules in neuronal cytoplasm (arrows).

Purkinje cells and some other neurons are vacuolated, and lamellated lipid material is stored in endoplasmic reticulum or lysosomes. The lectin-binding pattern is consistent with a glycolipid storage disease.

Gomen disease

As discussed elsewhere, this is a suspected toxicosis of horses in New Caledonia, in which there is cerebellar neuronal degeneration, and associated lipofuscin storage in neurons and phagocytic cells.

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INCREASED INTRACRANIAL PRESSURE, CEREBRAL SWELLING, AND EDEMA

Normally only a narrow space separates the brain from the dura mater. Both the dura and skull are unyielding so that only a relatively small increase in the volume of the intracranial contents is permissible without increasing intracranial pressure. When the pressure is increased, something has to yield. The following refers particularly to the brain, but the principles apply equally to the spinal cord, especially in cases of trauma.

The causes of increased intracranial pressure are many and varied. One component of almost all of them is edema. **Brain edema** is an increase in water content of brain tissue. Brain tissue is about 75% water, a little more for gray matter and a little less for white matter. The edema may be more or less localized and geographically related to local lesions, or it may be diffuse. In addition to the pathogenetic mechanisms to be discussed here, acquired hydrocephalus and vitamin A deficiency in young animals can be responsible for increased intracranial pressure. In vitamin A deficiency, there is increased secretion of cerebrospinal fluid by the choroid plexus and decreased absorption by arachnoid villi, effects that may be quickly repaired by administered vitamin. Also important in domestic animals is impaired growth and remodeling of cranium and vertebrae that leads to disproportion between the volume of the growing nervous system and volume of the cranial cavity and spinal canal.

The local lesions that may result in **local edema** of the brain or spinal cord include: neoplasms, inflammations, parasitic cysts, focal necrosis of various causes, trauma, hemorrhages of parenchyma and meninges, and space-occupying lesions of the meninges that cause pressure on the brain. In each example, the edema may be mild or extensive and may contribute more than the primary or inciting lesion to the clinical signs, and to the swelling of the brain or spinal cord and increase of intracranial pressure.

Generalized cerebral edema and swelling of the brain also occurs in relation to a variety of *systemic conditions*. Some degree of swelling can be anticipated as a *postmortem change*, especially in the brain of young animals, and is probably to be related to the imbibition of fluid in autolysis. Cerebral edema with swelling occurs with diffuse *meningitis*, moderately so in the diffuse *viral encephalitides*, *acute bacterial toxemias* such as clostridial enterotoxemia, and *chemical intoxications* such as lead and organomercurial poisoning, and quite severely in the pathologic syndrome of sheep and cattle known as *polioencephalomalacia*. Generalized edema of moderate degree is expected in the many metabolic and toxic conditions which are characterized by disturbances of cellular osmoregulation, in particular disturbances which interfere with intracellular and extracellular concentrations of sodium and potassium; the acute-onset neurologic disease which occurs in *salt poisoning* of pigs and in *water intoxication* in young ruminants given water *ad libitum* after a period of deprivation would be in this category.

There are differences between gray and white matter in their susceptibility to edema, and different areas of gray substance or of white differ in their vulnerability. The considerations of edema here recognize several types of edema but are limited for descriptive purposes to the cytotoxic and vasogenic varieties. **Congestive brain swelling** is an increase in volume, especially in capillaries and post-capillary venules, as a result of loss of autoregulation of arterial input. In vivo imaging techniques can identify this edematous pattern but it is not easily evaluated at postmortem because of the valveless character of intracerebral veins. **Interstitial edema** that affects the central white matter in hydrocephalus and the spinal cord in hydromyelia and syringomyelia is considered with those diseases, the edema is a result of acutely raised intraventricular pressure. Hypo-osmotic edema follows a reduction of serum osmolality, usually as a result of fluid administration or ingestion.

A distinction is maintained here between **cytotoxic edema** and **vasogenic edema**, notwithstanding that the endothelial barrier between blood and brain may be functionally deranged in each type. *Intracellular* or **cytotoxic edema** depends on direct or indirect noxious injury to cells and interference with the mechanisms that control cell volume. For cells in non-nervous tissues, the swelling would represent hydropic change, the movement of water from interstitial tissues into cell cytoplasm. There is, however, in nervous tissue no significant interstitial tissue or intercellular space. The intercellular space in the brain is not more than 10–20 nm. There is a layer of material that stains as glycoprotein or glycosaminoglycan on cell membranes, but there is doubt as to whether it is the counterpart of other interstitial gels. The net increase in water in the brain in cytotoxic edema must represent a movement from plasma, and the regulatory mechanisms must therefore reside in the capillary endothelium and the brain cells. The basic disturbance is in osmoregulation, which depends mainly on the efficiency of the sodium/potassium pump and adenosine triphosphate as an energy source.

In cytotoxic edema, the swelling is in neurons but mainly in the astrocytes. Simple swelling of astrocytes is the most obvious structural change in gray matter. Swelling of the astrocytes involves the nucleus and processes. Glycogen granules accumulate in the watery protoplasm. If mild edema persists, the astrocytes react as described in an earlier section. If the edema is severe, the astrocyte nucleus is much enlarged, the chromatin is dispersed against the nuclear membrane, and the processes are voluminous. Death of acutely swollen astrocytes may not be accompanied by changes in the adjacent neuropil. Neurons may swell as a brief prelude to lysis. Satellite oligodendroglia are generally spared.

However, in cytotoxic edema, oligodendrocytes in white matter degenerate. The nucleus is swollen and less dense than normal; the nucleolus is hypertrophied, and the cytoplasm is enlarged and often visible in routine material. Changes in oligodendroglial processes that are wrapped as myelin are difficult to evaluate histologically except in those instances of specific cytotoxic edema in which there is splitting of the intraperiod line and the accumulation of water in intermyelinic clefts.

Vasogenic edema *is the term used to distinguish the extracellular accumulation of fluid from cytotoxic edema.* The basis of vasogenic edema is injury to vascular endothelium of sufficient severity to allow leakage or permeation of plasma constituents including, if the injury is sufficient, plasma proteins. The fluid spreads between cells in response to hydrostatic pressures in the cerebral circulation and those in the tissue. Vasogenic edema is a common complication of traumatic, inflammatory, and hemorrhagic lesions of the nervous system. It is not a conspicuous change in gray matter, because the dense tangle of neuropil resists the passage of fluid. There are exceptions such as in the periventricular nuclei where vasogenic edema occurs rather selectively in thiamine deficiency. The structure of white matter offers less resistance to the passage of edema fluid of this origin, and the comparison between the susceptibility of gray and white matter can be seen readily with local lesions near their junction in the cortex. The long fiber tracts such as the spinal cord, internal capsules, and optic tracts tend to be spared. The density and disposition of these tracts probably exert a considerable influence on the spread of edema about local lesions as, for example, the corpus callosum seems effectively to prevent spread from one hemisphere to the other.

The histological appearances are similar to those in cytotoxic edema with the addition of dissecting changes along fiber tracts. The white matter is loosely textured, the myelinated fibers being spread apart. The spaces created may be clear or they may, depending on the degree of permeability, contain homogeneous proteinaceous fluid (Fig. 3.49). Plasma droplets that stain brightly with periodic acid-Schiff are frequently present in perivascular clefts (Fig. 3.50).

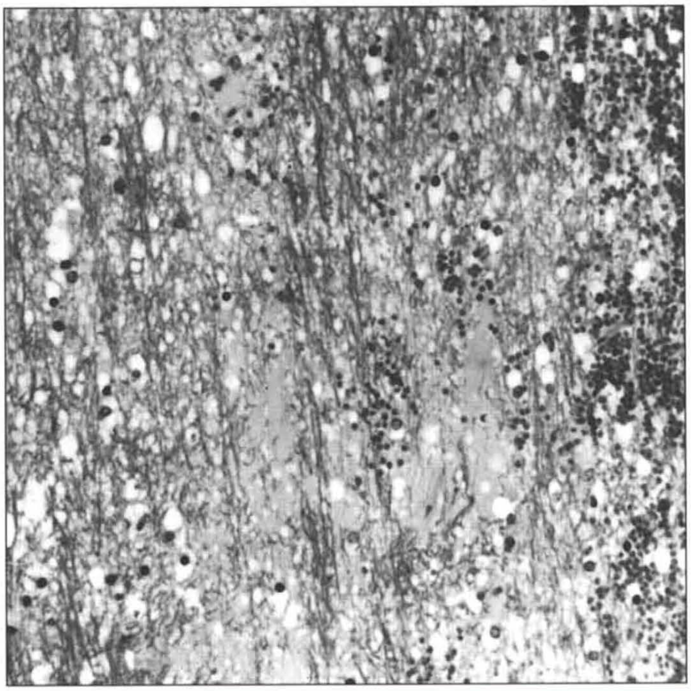

Figure 3.49 Vasogenic edema and hemorrhage with degeneration of white matter.

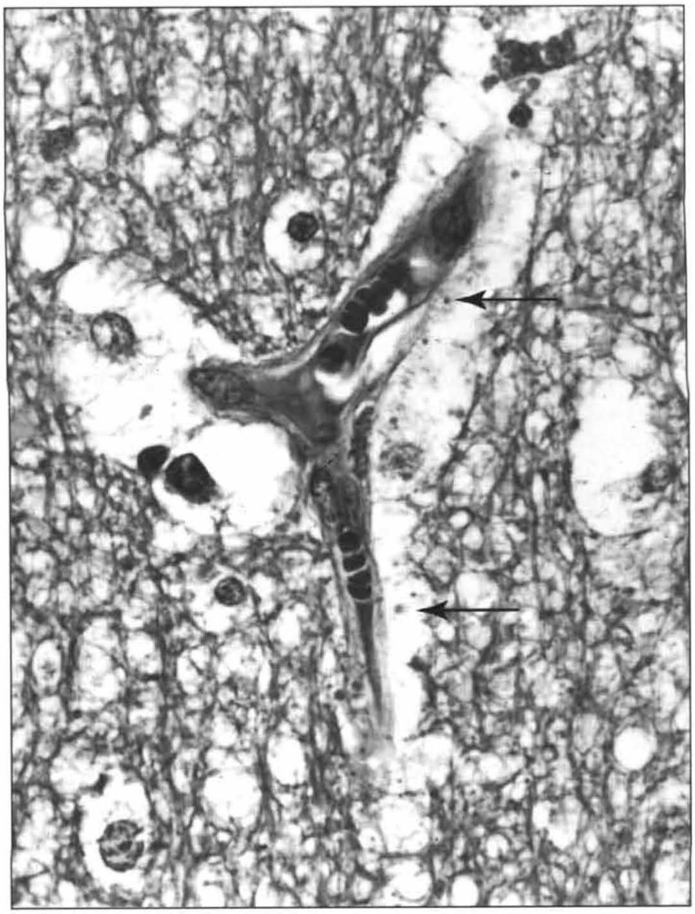

Figure 3.50 Accumulation of clear **edema** with protein droplets (arrows) around a vessel in a pig with mulberry heart disease.

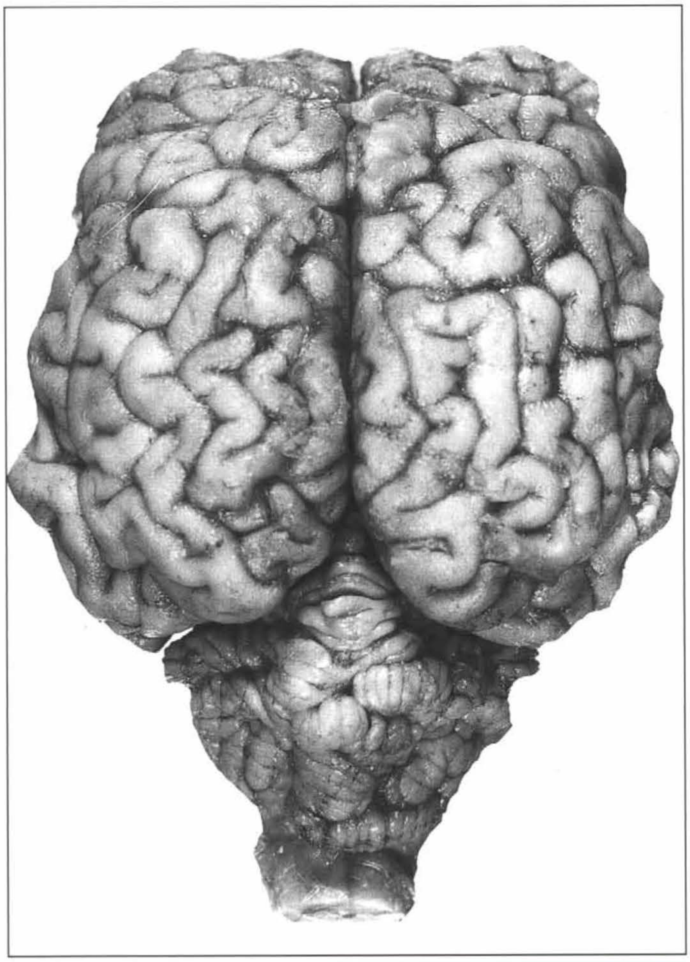

Figure 3.51 Cerebral edema with displacement and coning of cerebellum in a calf.

Diffuse cerebral edema that is mild or moderate may be difficult to recognize grossly. More severe degrees are readily recognized although easily overlooked. *The brain is swollen, pale, soft, and wet* and, because of its softness, the cerebral hemispheres tend to droop over the edges of the parietal bones when the calvaria is removed. With these severe and rapid swellings, the course is short, and there may not be signs of displacement or flattening of the gyri. When the swelling is less severe, and of longer duration, the brain may be relatively firm and dry, the gyri are pale or a faint-yellow color, and the brain is displaced caudally so that it appears unusually elongate. The displacement is most obvious where it involves the cerebellum and medulla (Fig. 3.51). With moderate displacement, the caudal surfaces of the cerebellar hemispheres are depressed by contact with the occipital bones. When the displacement is of greater degree, the *medulla and caudal portion of the vermis are herniated through the foramen magnum.* The displaced vermis is flattened and lies like a tongue over the medulla (so-called lipping of the cerebellum). The rhomboid fossa is flattened. The rostral portion of the vermis is pressed against the rostral medullary velum and may occlude the opening of the cerebral aqueduct to cause internal hydrocephalus. The brain stem is displaced caudally, and this is especially evident in the displacement of the corpora quadrigemina well into the caudal fossa. Perhaps because the tentorium rather closely embraces the brain stem in animals, herniation of the occipital lobes through the tentorial space into the caudal fossa is seldom observed. It may, however, be observed in cerebral edema of long standing in horses and cattle, especially if the brain is fixed in situ so that the pressure grooves produced by the free edge of the tentorium are retained. Impaction of tissue in the tentorial space interferes with the flow of cerebrospinal fluid from the caudal to the rostral fossa and contributes to hydrocephalus.

Displacements in the rostral fossa in diffuse cerebral swelling are of lesser degree and significance. The nerves may be stretched and flattened, but pareses are seldom observed. The vasculature must be compromised in all cases. Occasionally there is thrombosis of the superior sagittal sinus with venous infarction in the dorsal cortex, ischemic necrosis in the caudomedial surface of the occipital lobe referable to compression of the caudal cerebral arteries, and pontine hemorrhage referable probably to occlusion of small veins in this area.

Localized edematous changes reach their most extensive development in the centrum semiovale of the cerebrum and in the deep white matter of the cerebellum surrounding local lesions in these areas. The edematous area may be recognized by the swelling, which may be greater in volume than that produced by the primary lesion and have a soft, depressed and damp or watery appearance on the cut surface. The extent of the edema cannot be appreciated on gross inspection because the margins are indefinite, and the same difficulty in delineating the edematous area is experienced at microscopy. When the lesion is of prolonged duration, the extent of the edematous areas can be appreciated a little better by yellow discoloration that develops.

The combination of edema with a local lesion may displace the brain in one or more directions. Caudal displacement through the caudal fossa may occur as it does in diffuse swelling. The displacements may be more local, involving lateral shifts of the base of the brain or medial displacement of one hemisphere so that the falx cerebri is displaced laterally or the cingulate gyrus is herniated beneath the free margin of the falx and the lateral and third ventricles are depressed.

LESIONS OF BLOOD VESSELS AND CIRCULATORY DISTURBANCES

Diseases of blood vessels are considered in detail with the Cardiovascular system. This section deals with some special features of cerebrospinal circulation and the lesions, usually ischemic or hemorrhagic, which result from vascular injury.

The blood supply to the brain is derived from the *internal carotid and vertebral arteries*, these sources anastomosing under the brain stem and at the *circle of Willis*. The major cerebral vessels, derived from the carotid and vertebral arteries, anastomose quite freely in the pia-arachnoid, but once an artery or arteriole penetrates the substance of the brain, it becomes an *end artery* although there are some anastomoses at the capillary level. Although these anastomoses can be demonstrated readily, even those of arteriolar size in the meninges are probably of little value. Under normal circumstances, the cerebral arteries have rather set fields of supply. If one vessel is occluded, some collateral circulation develops, but it does not take over more than the periphery of the area of supply of the occluded vessel. In the brain, there may be influences governing collateral circulation in addition to those operating in other tissues, but the basic considerations still apply.

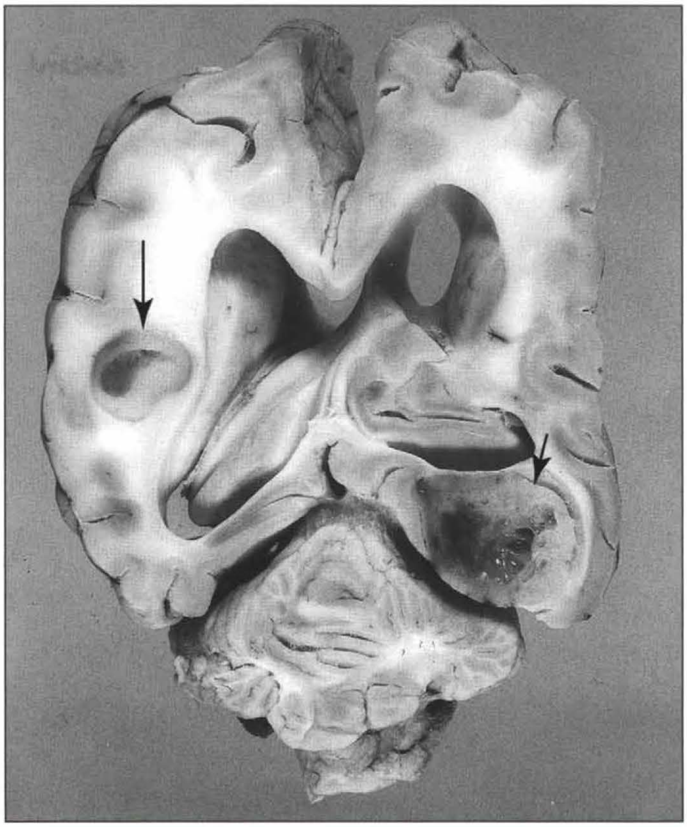

Figure 3.52 Tumor metastases (arrows) at the junction of gray and white matter in a dog.

The development of collateral circulation will be influenced by the anatomic arrangements of vessels, the volume of ischemic tissue, the rate at which the vascular occlusion develops, the size of the occluded vessel, and, importantly, the quantity of blood flow, which is referable to the state of the systemic circulation, and the quality of blood flow, which is referable to such matters as the oxygen tension and viscosity of plasma. For complete cessation of blood flow in an area of brain, it is probably not necessary that there be complete occlusion of the corresponding artery because flow is expected to cease, especially in peripheral twigs, while the intravascular pressure is still above zero. For this reason, ischemic injury in the brain localized to the field of distribution of one or other major cerebral artery may result from occlusion of a carotid vessel. Extracranial anastomoses between the major arterial vessels are, however, effective in the event of vascular occlusion occurring proximal to the circle of Willis.

Arteries entering the substance of the brain are relatively small and arise at right angles from the parent vessels in the pia-arachnoid. There are abrupt changes in caliber when the meningeal vessels divide and this provides an entrapment mechanism for large emboli. The vessels that enter the brain are progressively attenuated to capillaries in both gray and white matter, but many capillaries loop back into the cortex from near the gray–white junction. Many small emboli lodge at the gray–white junction, although expansion of the embolic or metastatic lesions is predominantly in the white matter (Fig. 3.52).

The *arterial supply to the spinal cord* is derived from the vertebral artery in the cervical region and from radicular arteries in the lumbar region anastomosing as the ventral spinal artery. There is some doubt as to the direction of flow in cervical, thoracic, and lumbar portions of the spinal artery and it is possible that there is a border zone in the caudal cervical and cranial thoracic region that is particularly vulnerable to ischemia if flow is impaired in the vertebral artery or the caudal portions of the ventral spinal artery.

In the spinal cord, the central gray matter is supplied by branches of the ventral spinal artery that enter the ventral sulcus, and lesions of these branches affect the gray matter of the cord rather selectively. The white matter is supplied by an anastomotic complex in the meninges that produces many small vessels that penetrate directly as end-vessels and are susceptible to compression or hypotension.

Cerebral veins have abundant and useful anastomoses. Untoward effects are not expected to follow occlusion of single veins because absence of valves permits reflex flow. The venous sinuses of the dura mater empty into the jugular veins, but they also communicate freely through the bones with extracranial veins. Because of these communications, the effects of obstruction of a dural sinus may be relatively slight, unless there is venous stagnation of the head or venous stagnation within the cranium produced by cerebral swelling or occlusion of more than one major sinus. The venous system of the spinal cord is freely anastomotic and drains via the radicular vessels to the paravertebral plexuses. These vessels are also valveless and allow very free communication up and down the spinal canal such that an embolus arising in caudal veins may bypass the caudal vena cava in passing to the lungs. These arrangements of veins may assist in temperature and pressure control in spinal and cerebral vessels. The arrangements have disadvantages. Infiltrating neoplasms may extend from extradural positions to the cord along the venules. In the cranial cavity, infections may extend from the face to the basal meninges. In cattle, sheep, and dogs, pyogranulomatous processes that are occasionally found in the hypothalamic–hypophyseal region are introduced in migrating intravenous foreign bodies that enter the cranial cavity in veins passing through the ophthalmic foramen.

Disturbances in the cerebrospinal vasculature with impaired blood flow may be composed of obstructive and hemorrhagic lesions, or they may be part of global cerebral ischemia with failure of adequate total perfusion. Diseases associated with abnormality of the circulating blood, such as the anoxias, hypoglycemia, and hyperviscosity, are discussed later with neurodegenerative diseases.

Ischemic lesions

The outcome of vascular obstructions depends on the type and size of the vessel obstructed, the degree and duration of ischemia, and the relative vulnerability of the tissues to anoxia. The injury may vary in severity from a temporary functional disturbance to the other extreme of infarction and necrosis. *Neurons and oligodendroglia are the most sensitive of the neural structures to ischemia, astrocytes are moderately resistant, and microglia and the blood vessels are quite resistant and may survive in small areas in which all else dies.* Gray matter, with its high metabolic rate and dependence on oxygen, is more sensitive than is white matter, but there are regional differences in the sensitivity of gray matter.

Obstructive lesions of cerebrospinal vessels are not commonly observed in animals, and ischemic changes may be absent even when the vessels are profoundly altered. On the other hand, lesions which are regarded as being of ischemic type are quite commonly observed in the absence of demonstrable vascular occlusion. These cases tend to be individual in their occurrence and without particular pattern.

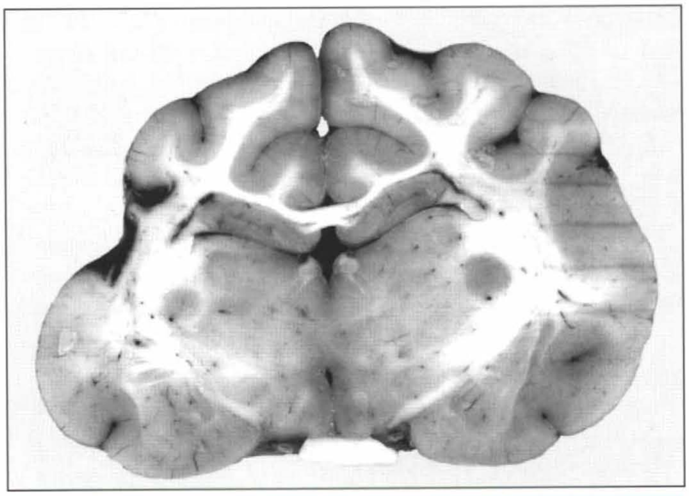

Figure 3.53 Focal loss of gray matter from left parietal cerebral cortex, in **feline ischemic encephalopathy.** (Courtesy of RF Slocombe.)

Global ischemia is occasionally observed in animals suffering cardiac arrest under anesthesia, and in neonatal "barking foals and piglets." Purkinje cells, the hippocampal cortex, and neurons of the cerebral cortex are the most sensitive of all, and, within the cerebral cortex, the deeper laminae are more sensitive than the superficial laminae. Necrosis is accentuated over boundary zones at the margins of distribution of the rostral, middle, and caudal cerebral arteries. Similar regional susceptibility can be demonstrated in the cerebellar cortex. The deeper laminae of the cortex may be selectively destroyed in ischemia, producing a distribution of necrosis that is known as **laminar cortical necrosis**. Lesions are expected to be symmetrical but may not be. In severe and prolonged global ischemia, necrosis may involve all of the cortex and other nuclei such as globus pallidus and lateral thalamus. The sensitivity to necrosis parallels that in severe hypoglycemia except that hypoglycemic injury spares the cerebellar Purkinje cells. The assumption that the injury is a result of anoxia and energy deficit does not recognize the complex biochemical perturbations set in train by cellular anoxia.

Feline ischemic encephalopathy is recognized in mature cats, and results from aberrant migration of *Cuterebra* larvae in the brain. While larval migration tracks may be present, the ischemic lesions may be the result of toxin-induced vasospasm. The extent and distribution of degeneration vary from case to case. Milder lesions occur in superficial cortical laminae as multiple foci. More severe or extensive lesions tend to be in the distribution area of the middle cerebral artery and may be bilateral but asymmetrical (Fig 3.53). In some cases, there may be ischemic lesions in the brain stem.

Ischemic and hemorrhagic cerebral lesions occur in the **neonatal maladjustment syndrome of foals**, also known as the "barker" or "convulsive foal" syndrome, which is discussed with congenital atelectasis in Vol. 2, Respiratory system. The nature of the circulatory derangement is not understood but the lesions are presumed to reflect cerebral ischemia and variably delayed reflow. There is ischemic laminar necrosis of the cerebral cortex (Fig. 3.54), sometimes accompanied by necrosis in the paired gray nuclei of the midbrain and brain stem, and by multiple small hemorrhages. In some foals affected by

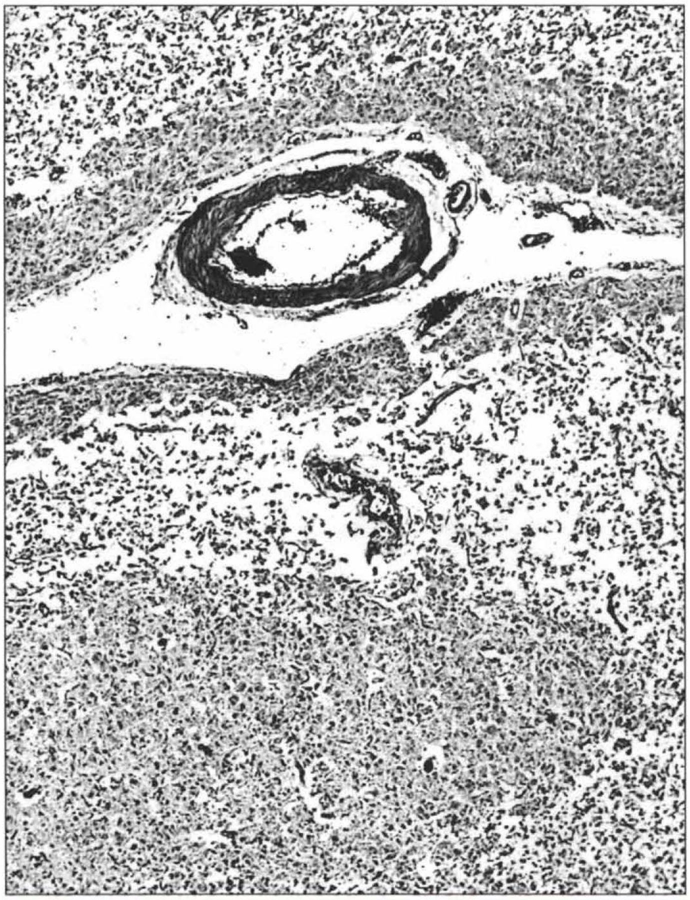

Figure 3.54 Polioencephalomalacia of cerebral cortex in a foal with neonatal maladjustment syndrome.

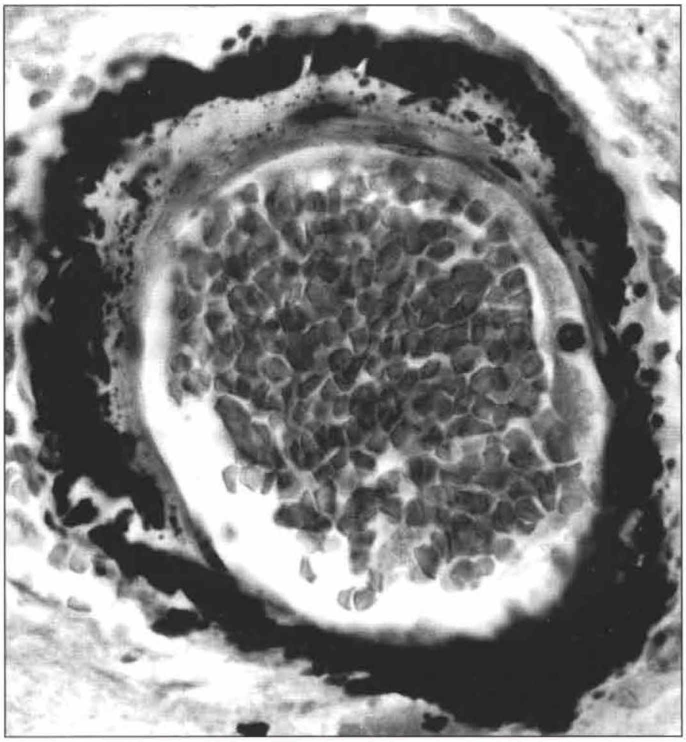

Figure 3.55 Siderosis of cerebral vessel in a horse.

this neonatal syndrome, there is minimal ischemic necrosis but instead a profuse distribution of small perivascular and petechial hemorrhages in the cerebrum, cerebellum, and brain stem.

Degenerative vascular disease of arteriosclerotic type is not important in animal disease. *Atheroma* occurs in some hypothyroid dogs. *Siderosis* of the walls of the arterioles occurs commonly as a change associated with advancing age in horses. The calcium and iron salts may be deposited in amounts capable of converting the vessels to rigid pipes (Fig. 3.55). The patency of the vessels is well maintained. There is no thrombosis, and vascular stenosis probably proceeds slowly enough for adaptive circulatory changes to occur because small areas of softening, presumably ischemic, are unusual.

Hyaline necrosis in meningeal vessels, which is observed in swine with hepatosis dietetica, is not usually associated with secondary lesions. The hyaline necrosis in meningeal vessels in pigs with organomercurial poisoning is associated with severe cerebral injury that may be, in part, due to reduced perfusion (Fig. 3.56). *Amyloid degeneration* of meningeal and intracerebral vessels occurs in aged dogs and the thickenings of the meningeal arteries may be visible grossly. Senile argyrophilic plaques that contain amyloid deposits may be associated with the vascular lesions. The systemic distribution of amyloid is minimal. Petechial hemorrhages occur in relation to the diseased vessels in about 50% of cases and, exceptionally, there is massive hemorrhage.

Cerebrospinal angiopathy is an important cause of neurological disease in pigs. It occurs in the alimentary enterotoxemia known

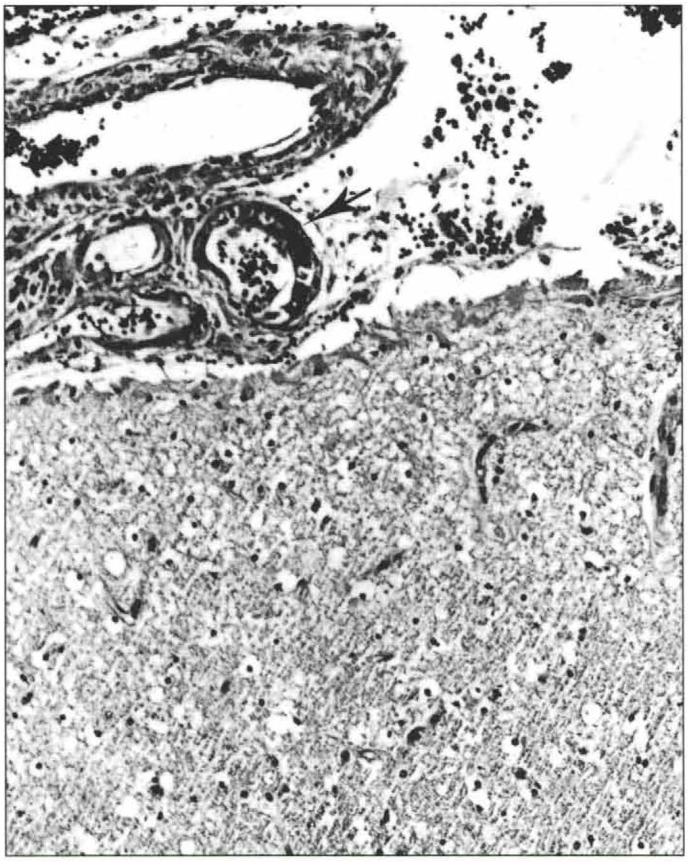

Figure 3.56 Necrosis of meningeal arterioles (arrow) due to organomercurial poisoning in a pig.

as **edema disease** of pigs with similar lesions in vessels of brain and other tissues (Fig. 3.57). The lesions are described in detail in Vol. 2, Alimentary system. In groups of older pigs, arteritis or periarteritis can develop and may represent a chronic or persistent expression of the acute angiopathy.

Vascular permeability changes provide a basis for **annual ryegrass toxicosis** of sheep and cattle in Australia, South Africa, and formerly in the USA. The distribution of the disease is governed in part by the distribution of annual ryegrasses, *Lolium rigidum* and hybrids, in winter rainfall areas, although the disease can occur in other areas by the use of transported fodder. The distribution is also governed by the distribution of toxin-producing *Clavibacter toxicus* (*Corynebacterium rathayi*) and infection by the bacterium of galls produced in the seed head by the nematode *Anguina agrostis*. In addition to *Lolium* spp., known host plants include *Polypogon monspeliensis* (annual beardgrass), *Festuca nigrescens* (chewings fescue), and *Agrostis avenacea* (blowngrass).

The nematode *Anguina agrostis* emerges from fallen galls following first autumn rains, migrates into the growing points of the ryegrass seedlings, and later penetrates the florets to produce galls. The nematodes are harmless to animals but may carry on the cuticle the bacterium that will proliferate in the gall, forming a yellow slime. The active principle, *corynetoxin*, is closely related to the tunicamycin antibiotics produced by some strains of *Streptomyces*. Indeed, tunicamycin-like metabolites have been detected in mycotoxicosis in pigs. Corynetoxins and tunicamycins inhibit lipid-linked N-glycosylation of glycoproteins, which compromises cell membrane integrity.

The toxicosis is characterized clinically by neurologic signs and high mortality. Pregnant ewes may abort. The pathogenesis of abortion has not been examined but the occurrence of hemorrhages in various organs, pulmonary edema and swelling of endoplasmic reticulum of hepatocytes indicates systemic intoxication, notwithstanding the prominence of neurologic signs.

The clinical signs, which are severe, include excitability, aggression in cattle, disturbances of gait and convulsions. The clinical course may be less than 12 hours. Gross changes include pulmonary edema and a pale swollen liver, and occasionally there are hemorrhages in various organs.

Microscopic changes in nervous tissue are subtle, especially following routine fixation by immersion, which may not preserve transudates. Tracer injections show widespread alterations to cerebrovascular permeability in the brain and meninges, indicating endothelial damage and disruption of the blood–brain barrier. The perivascular transudate resembles plasma (Fig. 3.58) in staining properties, and may be present as perivascular lakes or droplets that stain strongly with PAS stain. Only occasionally can fibrin be demonstrated, but extravasation of red cells from capillaries may be present in neuropil. Astrocytes are

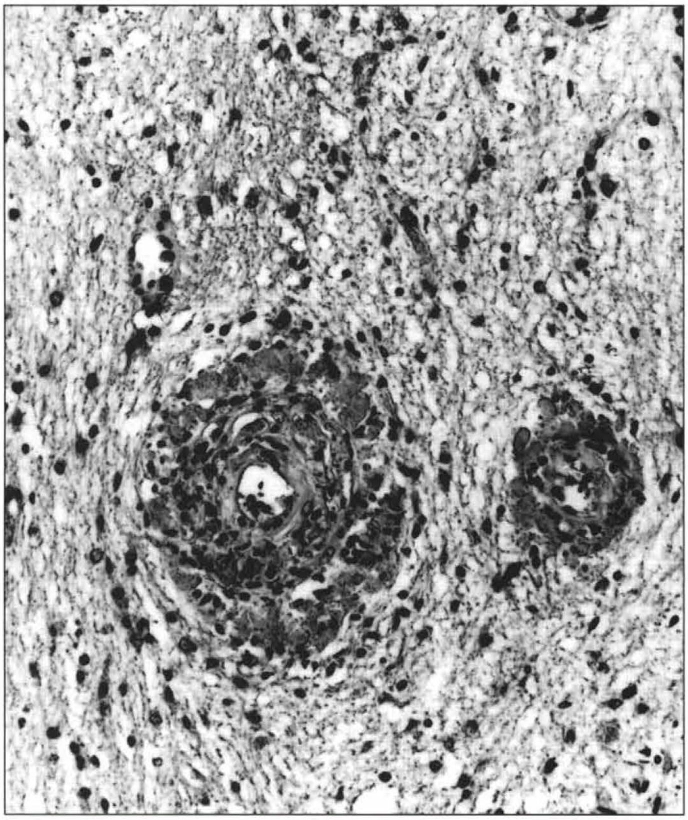

Figure 3.57 Necrosis and inflammation of vessels in brain stem in a pig with **edema disease**.

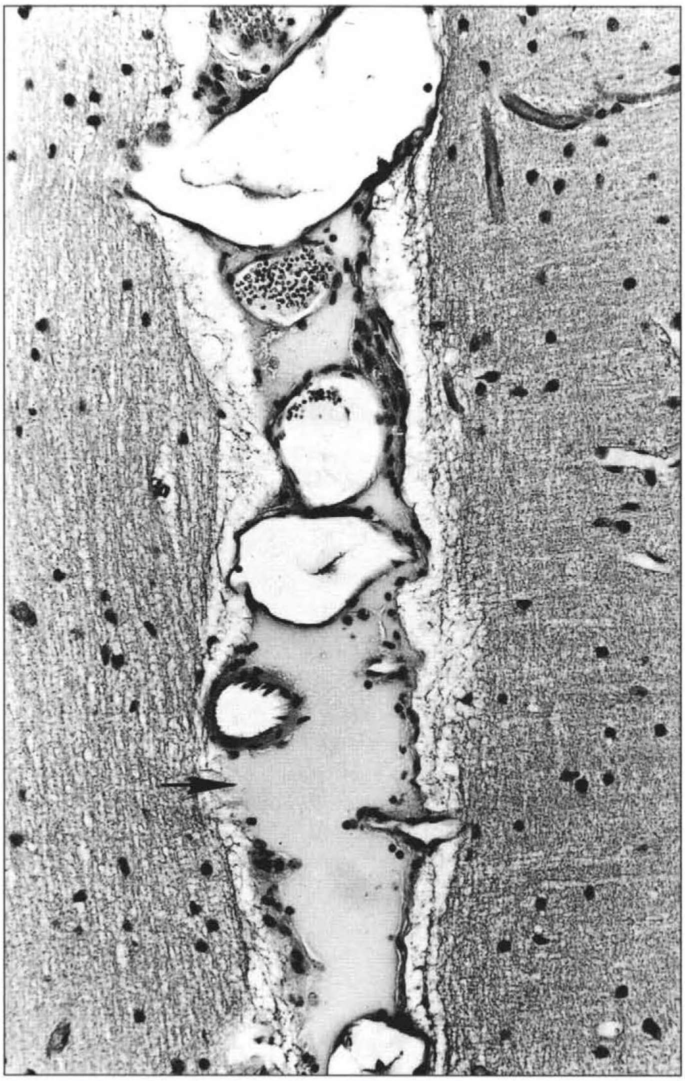

Figure 3.58 **Vascular leakage** (arrow) in the leptomeninges of the cerebellum of a sheep with annual ryegrass toxicity. (Courtesy of J McC Howell.)

swollen with acidophilic cytoplasm, and there may be widespread necrobiosis of oligodendroglia in the cerebral gray matter.

The capillary endothelium shows ultrastructural evidence of injury. Endothelial cells are swollen and electron-lucent, the cisternae of rough endoplasmic reticulum are distended, mitochondria are swollen with disorganization of cristae, and capillary lumina sometimes contain platelet aggregations. The changes are best seen in cerebellar cortex and meninges. Neuronal change is minimal but there may be patchy loss of Purkinje cells and scattered small foci of malacia.

Cerebrospinal vasculitis, affecting both arterioles and veins, occurs in a number of specific diseases. *Polyarteritis* (periarteritis nodosa) appears to have some predilection for the cerebral arteries, especially in the pig, and for the spinal arteries in the dog in which it is cited as **steroid-responsive meningo-arteritis**. Vasculitis is a rather specific feature of classical swine fever (Fig. 3.59), sporadic bovine encephalomyelitis (Fig. 3.60), and malignant catarrhal fever; the agents of these diseases have no predilection for neural tissue, and parenchymal degeneration, which is sometimes observed, is secondary to occlusion of vessels; this occlusion, in turn, is secondary to inflammatory lesions of the vascular adventitia. Adventitial proliferations and perivascular infiltrates are common to many of the encephalitides, both bacterial and viral, and focal softenings of the brain and cord in these inflammatory diseases can frequently be related to occlusive vasculitis. The occlusive lesions are not solely related to the acute phase of inflammation, but adventitial fibrosis and vascular stenosis may be prominent in the healed phase of encephalitis and may lead eventually to ischemic damage and softenings.

Thrombosis and embolism in the cerebrospinal arterioles is very seldom observed in animals. Thrombosis of an internal carotid vessel or ventral spinal artery may accompany atrial and aortic thrombosis, especially in cats (Fig. 3.61). Bone marrow emboli form after trauma and fractures, most commonly in dogs (Fig. 3.62). *Emboli composed of fibrocartilage or nucleus pulposus* occur in spinal arteries and veins in most species, and cause hemorrhagic and ischemic infarcts of sudden onset (Fig. 3.63). The cervical or lumbar segments may be affected. The pathogenesis is discussed with Diseases of bones and joints. *Bacterial thrombi* may develop in a variety of bacteremic diseases such as erysipelas, shigellosis, pasteurellosis, and the septicemias caused by *Histophilus, Streptococcus*, and the coliforms. Abscessation develops rapidly about these bacterial colonies, but in the early stages perivascular zones of softening may be observed.

Perhaps the least morphological expression of arteriolar obstruction is death of neurons in a narrow zone of cortex representing the central portion of a field of distribution of a single arteriole. In the early stage, there is acute ischemic necrosis of neurons and oligodendroglia. There is no softening in these minimal lesions, but they are readily visible at low power as narrow zones of pallor. At higher magnification, the affected zone is seen to be unusually cellular, this being due in the early stages to microgliosis and later to astrogliosis.

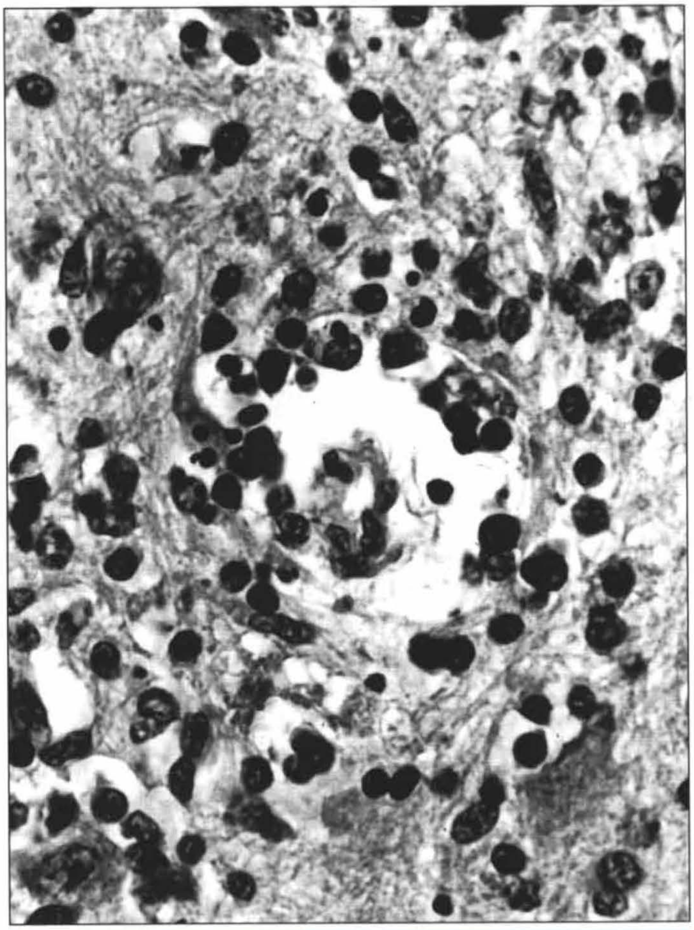

Figure 3.59 Gliosis around a degenerate vessel in classical swine fever.

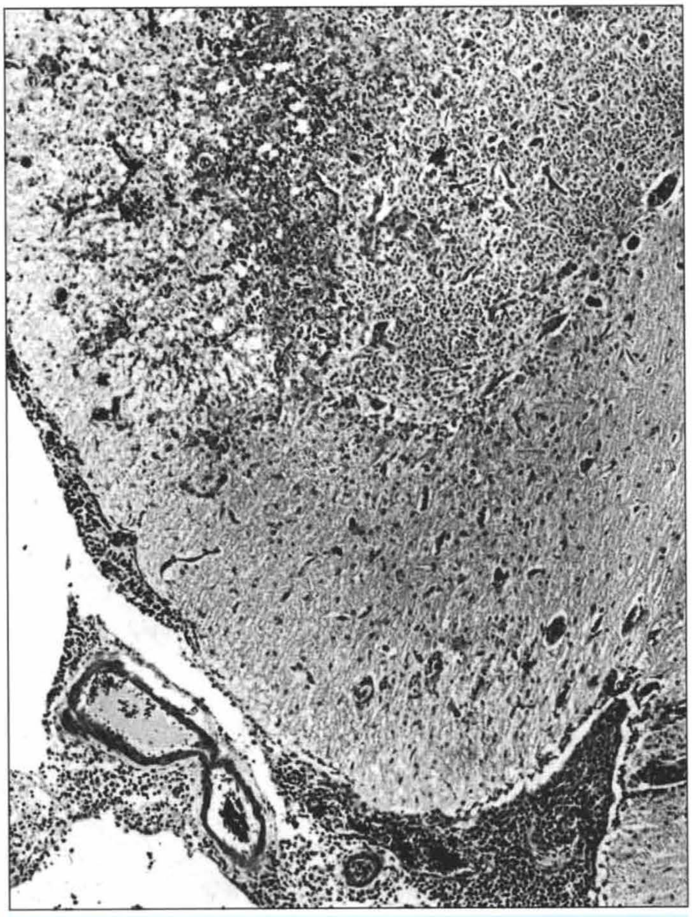

Figure 3.60 Necrosis of cerebellar cortex and meningitis in **sporadic bovine encephalomyelitis**.

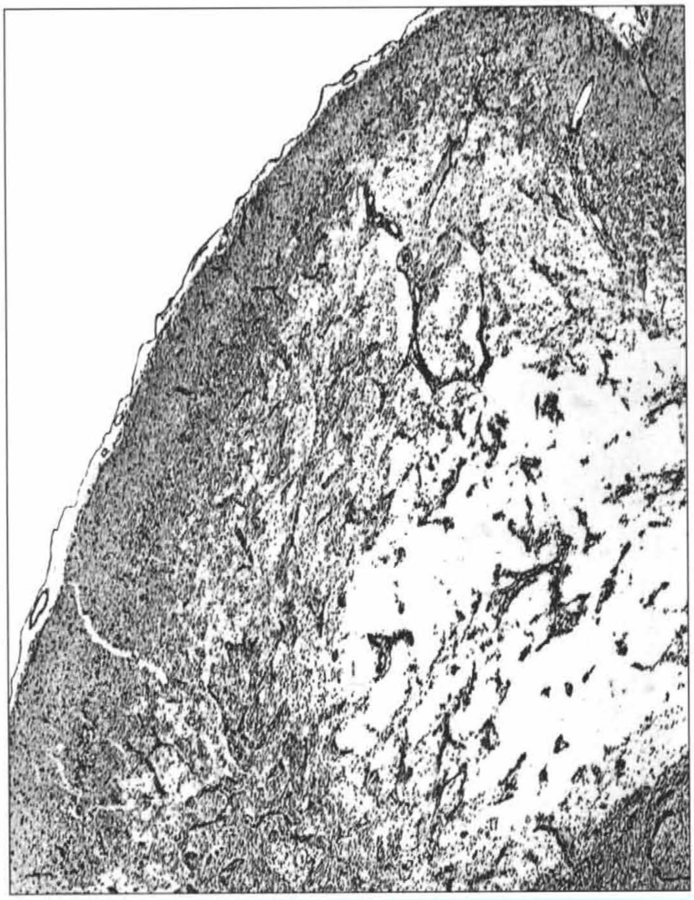

Figure 3.61 Residual cyst in cerebral cortex, the result of carotid thrombosis and ischemic infarction in a cat.

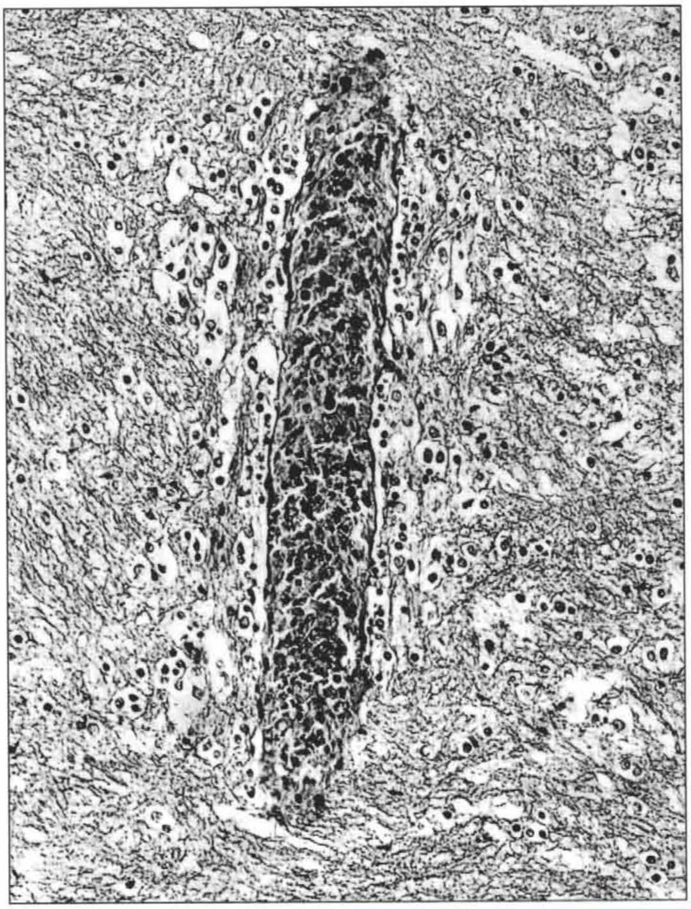

Figure 3.62 Marrow embolus and surrounding intracellular edema following trauma and femoral fracture in a dog.

Obstruction of an artery or arteriole results typically in *infarction*, and infarcts involve both gray and white matter. In the early stages, affected areas swell, and the involved gray matter may be hemorrhagic (Fig. 3.64). At the center of an infarct, there is coagulative and liquefactive necrosis and around the periphery there is a zone of minimal ischemic injury with the characters mentioned above. Many capillary vessels survive, partly because of their relative insensitivity to anoxia and partly because they benefit first from the collateral circulation that develops. Surviving vessels show endothelial and adventitial proliferation, and the astrocytes around the margin of the lesion react. The necrotic tissues are removed from the periphery by liquefaction and microglial phagocytosis, and a cyst remains (Fig. 3.61). The cyst contains fluid, and distended microglia, so-called *compound granular corpuscles*, may be found in it for very long periods. The wall of the cyst is ragged and irregular and is formed by reactive astrocytes. When the defect extends to the meninges, it may be partially filled in by proliferated leptomeningeal tissue.

Obstruction of cerebrospinal veins is the result either of pressure or inflammation with thrombosis, although rarely it may be due to neoplastic invasion and permeation of the dural sinuses. *Thrombophlebitis* is usually bacterial and the associated meningitis is more conspicuous and important than the venous thromboses; isolated venous obstructions are not of much significance.

Intracranial thrombophlebitis can be due chiefly to retrograde spread of inflammation from an extracranial primary focus, but thrombophlebitis of this type is uncommon. Such primary foci may occur in the orbital or nasal cavities, the paranasal sinuses, or the middle ear. Intracranial inflammations have little tendency to cause thrombosis of the dural sinuses. In verminous infestations of the brain, especially those caused by *Strongylus* larvae in horses, there is some tendency for the larvae to migrate to the superior sagittal and transverse sinuses and to cause thrombosis of these. Spinal thrombophlebitis results from ascending infections from docking wounds in lambs and from bite wounds of the tails of pigs.

Noninflammatory thrombosis of the cranial dural sinuses occurs chiefly in the superior sagittal sinus. It occurs in polioencephalomalacia of cattle, in which it is probably secondary to severe and prolonged swelling of the brain. Sinus thrombosis may also be observed following head injuries, even those not accompanied by lacerations (Fig. 3.65).

Venous infarcts differ from arterial infarcts by the more extensive hemorrhage in the affected areas and by the diffusion of blood into the adjacent subarachnoid space. The superficial veins are engorged and the congested area is edematous and swollen. The hemorrhages are perivenular and petechial or larger, and are present in the gray matter and to a lesser extent in the white matter. The subsequent course and reaction are similar to those following arterial obstruction, and the residual lesion is a depressed and shrunken or cystic area that is dark brown-yellow.

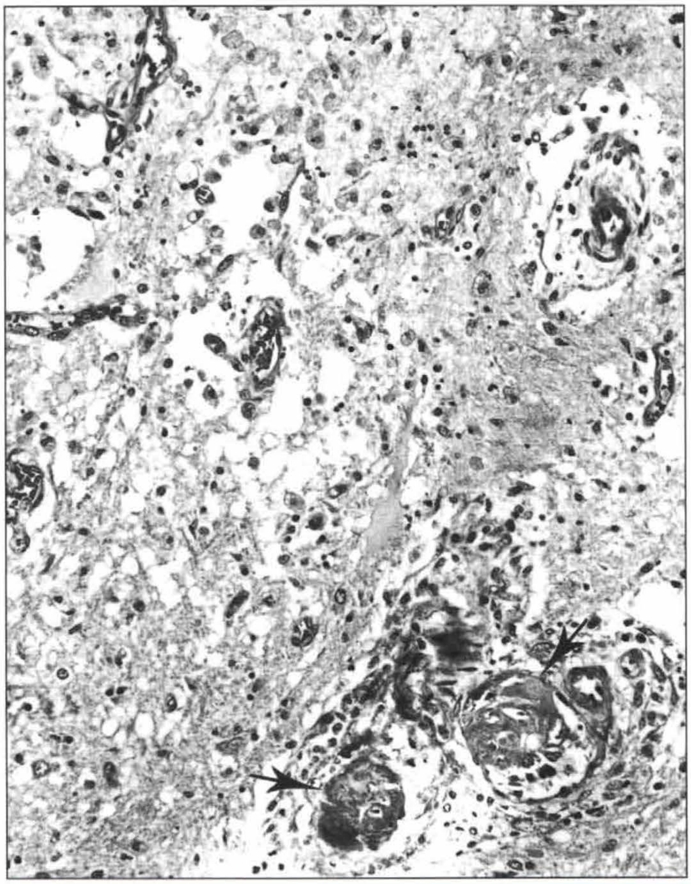

Figure 3.63 Cartilaginous emboli (arrows) causing spinal myelomalacia in a dog.

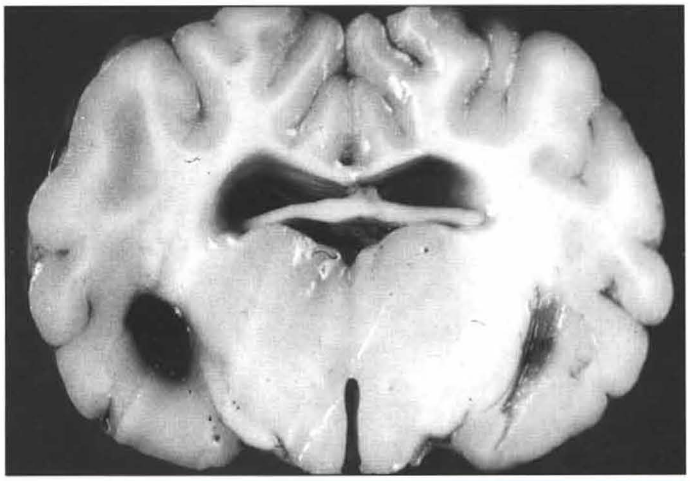

Figure 3.64 Hemorrhage (left) and malacia (right) in cerebrum of a pig with **mulberry heart disease**.

Hemorrhagic lesions

Hemorrhages in the CNS may be *traumatic or spontaneous* and may be restricted to the meninges or the parenchyma or involve both. Hemorrhages accompanying periventricular leukomalacia in neonates are discussed earlier. The causes are, in general, the same and are of much variety. They can be broadly grouped into those affecting the integrity of the vessel wall and those that reduce the coagulability of the blood. Care is necessary in deciding whether observed extravasations are significant because they can be produced readily as *artifacts at postmortem.* Quite extensive spread of blood into the basal meninges commonly occurs from the venous sinuses when the head is removed at the atlanto-occipital junction, and into the spinal meninges when the spinal column is transected. Hemorrhages of petechial size in the parenchyma can be largely avoided as artifacts if the brain or cord is fixed before slicing. The microscopic distinction of antemortem from postmortem hemorrhages can also be difficult because, apart from minor degenerative changes of the parenchyma, terminal hemorrhages provoke very little reaction, even on the part of the microglia.

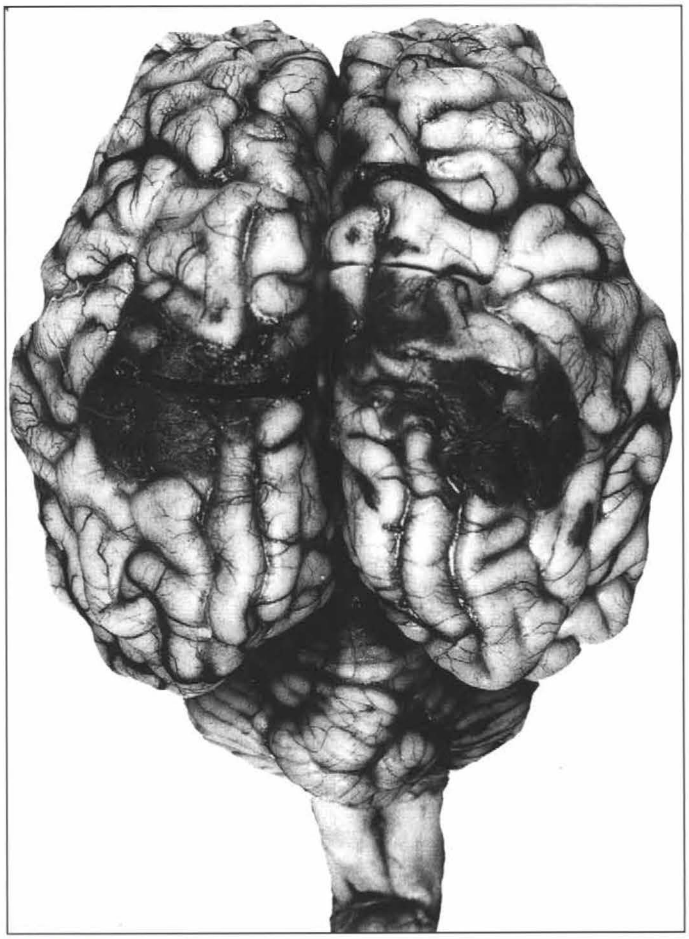

Figure 3.65 Venous infarcts in cortex secondary to thrombosis of sagittal sinus in a calf.

Spontaneous hemorrhages in the brains of animals are almost invariably of petechial or slightly larger size occurring in the hemorrhagic diatheses, especially the symptomatic purpura of septicemic infections (see Vol. 3, Hematopoietic system). Such hemorrhages occur typically from capillaries as well as small venules and are found in meninges and brain. There is no specificity in the geographic distribution of purpuric hemorrhages except in infectious canine hepatitis in which intracranial hemorrhage, although not consistently present, has a predilection for the midbrain and medulla. In "focal symmetrical encephalomalacia" (see Vol. 2, Alimentary system) the lesions are grossly visible only when hemorrhagic; they are regularly bilateral and symmetrical and have a rather specific pattern in which the internal capsules, dorsolateral thalamus, cerebral aqueduct, and pons may all be involved. Hemorrhages also characterize thiamine deficiency in dogs and cats and have a specific distribution and symmetry, being regularly present in the inferior colliculi and less consistently present in the mammillary bodies and other periventricular nuclei.

Isolated hemorrhages or hematomas are quite rare in animals. The hemorrhages may extend to the ventricles and induce hydrocephalus, or involve the central canal of the cord to produce hematomyelia.

Meningeal hemorrhages, both epidural and subdural, are common in lambs and calves that have required obstetric assistance and probably reflect hemodynamic disturbances in dystocia. Hemorrhages of similar distribution occur in calves born unassisted from cows exposed to the coumarins of moldy sweet clover.

Microcirculatory lesions

A range of changes affecting the microcirculation in the nervous system may not produce ischemia or hemorrhage. They are associated with extravasation of formed or fluid elements, are not disease-specific in their character although their distribution may be specific, and result from degenerative changes especially in capillaries and venules. Sludging of leukocytes is frequently observed in venules of cerebral and cerebellar hemispheres in diseases causing leukocytosis, including a range of infectious diseases with neutrophil leukocytosis and the rather rare endotheliotropic lymphoma. Sludging of white cells is frequent in East Coast fever caused by *Theileria parva*, and sludging of red cells infected by *Babesia bovis* and possibly other protozoa; the pathogenesis of sludging may not be the same as for falciform malaria in which adhesion seems to be important in pathogenesis. Viral infections, such as classical swine fever and canine adenovirus, are frequent causes of capillary hemorrhage.

Diapedesis of red cells is a common event in sudden death of many causes and is frequently mimicked in postmortem trauma. The interval between diapedesis and death may be too short for reactive changes, visible by light microscopy, to develop in the vessels. With some delay, swelling of endothelial cells and their nuclei develops. The hemorrhages may be from arterioles, with the red cells remaining in the perivascular space. Diapedesis from capillaries and small venules depends on disruption of endothelium and astrocytic processes and, depending on relative pressures, the red cells may spread in the intercellular spaces.

Diapedesis of red cells occurs in periventricular nuclei in thiamine-deficiency encephalopathy, and in the brain stem in concussion, contrecoup injury, and in hypomagnesemia in sheep and cattle (Fig. 3.66). The white matter of the cerebral and cerebellar hemispheres is susceptible to diapedesis. The hemorrhages are sometimes large enough to be visible as petechiae in diverse conditions including infectious purpuras, anoxia, fat and other microembolism, in diseases associated with erythrocyte sludging, and in disseminated intravascular coagulation.

Leakage of plasma, usually from small venules, may occur in any part of the CNS. It is seen most frequently surrounding areas of traumatic injury or in the conditions noted above that lead to erythrocyte diapedesis. The plasma will contain some fibrinogen, and polymerized fibrin in its usual form may be demonstrated around the vessels. It is more usual, however, for the fibrin to be in its unpolymerized or molecular form, and the plasma both within and outside the vessels to be transformed into a homogeneous gel that stains brightly by the PAS technique. The plasma gel may appear as capillary plugs or as perivascular droplets that appear to incite very little reaction.

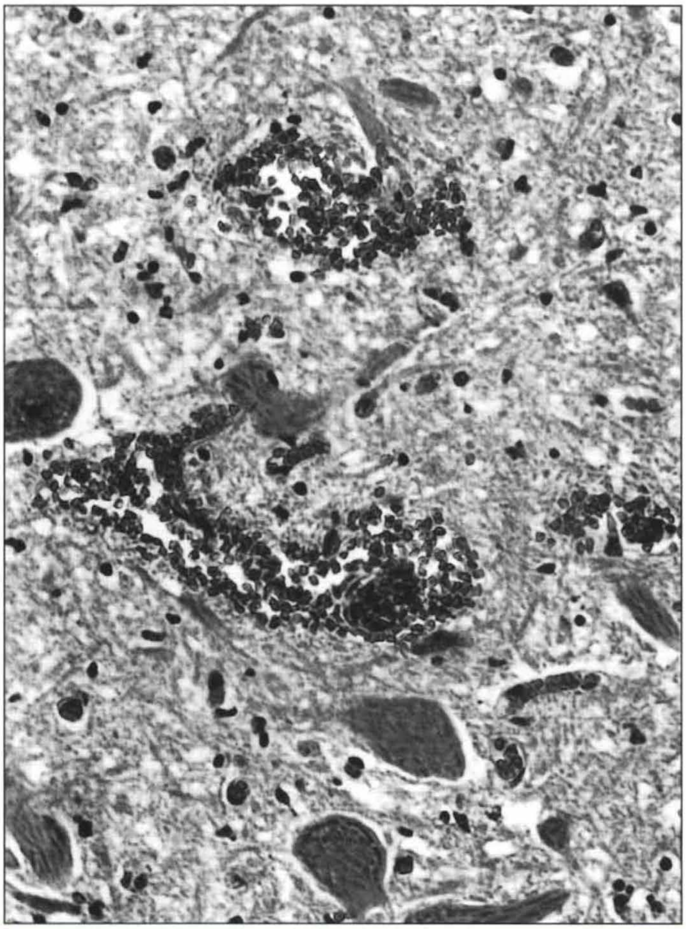

Figure 3.66 Perivascular hemorrhage in a cow with hypomagnesemia.

Vascular injury that allows diapedesis of plasma will also allow passage of serum or protein filtrate (Fig. 3.67). In fields of injury, the differences in levels of permeability change may be evident from the protein content of transudate. While plasma will routinely stain because of its fibrinogen content, the amount of protein in serum may be too low for preservation and staining (see Fig. 3.50). The perivascular changes occur especially about capillaries and venules in the white matter and have the characteristics described earlier for vasogenic edema. Prolonged survival or recovery may leave *focal glial scars* as residue (see Fig. 3.15).

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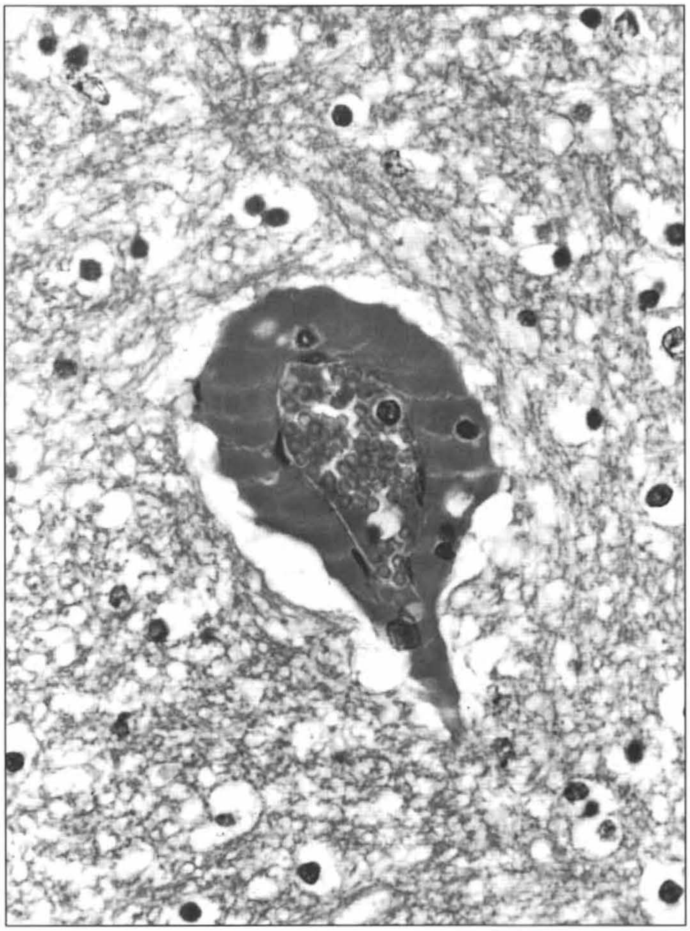

Figure 3.67 Accumulation of proteinaceous fluid around vessel in a sheep with **focal symmetrical encephalomalacia**.

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TRAUMATIC INJURIES

Trauma to the head and vertebral column are of importance because of the effects that such injuries have on the contained brain and spinal cord. Both the brain and spinal cord are well protected from external injurious forces by the bony encasements. The more or less rounded shape of the skull favors glancing blows and the lateral diffusion of lines of force; the diploe and sutures are capable of absorbing considerable amounts of shock; and the internal system of bony ridges directs lines of force to the base of the skull. The spinal column is well protected by the surrounding soft tissues, by its own highly cancellous structure, system of ligaments, and intervertebral disks. The nature of acquired injuries is quite varied and is determined by many factors including: the relative vulnerability of the soft tissues, the direction in which the injurious force is applied, the physical rigidity of the bones, the ability of the part to move in response to the applied force, and the mass and velocity of the force.

Concussion

Concussion is a transient loss of consciousness and reflex activity following a sudden injury to the head. Full recovery is expected, and it is assumed that in mild cases there is no morphological injury. In experimental animals, however, degenerative changes are found in the nuclear masses of the brain stem, and these are probably responsible for the clinical features. Following single injuries, chromatolysis develops in many of the larger neurons of the brain stem, and in a proportion of affected cells the degeneration is progressive. With repeated episodes of concussion, affected neurons have a wider distribution that includes the cerebral cortex, and the proportion of cells irreversibly injured is increased. These changes in the brain stem and reticular formation occur if the impact forces are substantial and the brain is subject to rapid acceleration/deceleration; in these cases direct injury in the hemispheres is to be expected. The mildest degrees of concussion produce no visible structural damage when examined with the light microscope, but there is some experimental evidence that plasma substances including proteins may be transported in vesicles through the endothelial cells, and the brain and cord may lose their capacity for autoregulation of blood flow in local areas of static injury.

While the dynamics of the disturbances in neurons that are responsible for the concussion are unclear, some of the physical qualities of the reaction between the head and the applied force have been elucidated. The vibrations that travel back and forth in the brain from the point of impact appear not to be important. If the head is firmly immobilized, quite a considerable force may be suddenly applied to it with relatively minor effect, the force of the blow (**coup**) being absorbed by the skull. Force of much lesser magnitude will cause concussion if the head is capable of moving in response to the blow. The principle to be obtained from these observations is that *a degree of acceleration or deceleration is necessary to produce concussion,* neural injury being due to displacement of the cranium relative to its contents. The brain of an adult animal is normally a little smaller than the cranial cavity and being suspended in the cavity, is capable of slight independent movement. If the head is freely movable and struck a heavy blow, it moves away from the point of impact and collides at the area of impact (coup) with the brain that is momentarily static. As the brain moves suddenly within the skull toward the point of impact, leptomeningeal blood vessels may be torn as the brain pulls away from the skull (**contrecoup**), and *the contrecoup injury may be greater than the coup injury*. In young animals, the brain closely fits the cranial cavity, so relative displacement of the whole brain is expected to be minimal. However, the brain is plastic, so sudden acceleration or deceleration leads to internal deformation. Displacements and transient deformations of the brain result in neuronal changes and unconsciousness probably through the effect of shearing strains on neurons, axons, dendrites, synapses, and blood vessels, as well as direct pressure effects when the brain is displaced across bony ridges.

Contusion

In contusion, the architecture of the nervous tissue is retained but there is hemorrhage into the meninges and about the blood vessels in the parenchyma. Contusions may be diffuse or focal injuries, although often those coexist.

The pathogenetic factors in diffuse contusions are generally the same as those operating in causing concussion, but the applied force, the displacements, and the induced shearing and direct forces are all of greater magnitude. Typically in diffuse injuries, *some of the most severe hemorrhages occur on the surface of the brain opposite the point of impact*. Hemorrhages of this distribution are known as *contrecoup*, and their development depends on the sudden movement of the brain to the point of impact (*coup*) with tension and tearing of pial and cortical vessels opposite the point of impact, and with direct or rotational displacement of brain over bony prominences, where the vessels become exposed to tensile and shear forces.

Focal contused injuries develop typically at the point of impact, and the mechanism of development is somewhat different from that of the diffuse injuries. In focal injuries, movement of the head is not sufficient to cause significant displacement of the brain. Instead, the applied force, usually with relatively high velocity and relatively low mass, causes fracture or deformation of the skull at the point of impact. The deformation of the skull may only be transient but sufficient to cause bruising of the tissue immediately beneath.

Laceration

Laceration is a traumatic injury in which there is disruption of the architecture of the tissue. The mechanics of lacerations are in general the same as those of contusion. Penetrating injuries are analogous to, but more severe than, those that result in focal contusions. Lacerations may also occur with blunt injuries of the type that cause displacement of the brain. In such cases, contrecoup lacerations occur typically on the surfaces of gyri where these are displaced over bony prominences. Shear forces developed during deformation or molding of the brain may be adequate to cause deep hemorrhages and even the cleavage of the gray from the white matter over small areas of the cortex.

Lacerations caused by penetrating injuries are always liable to *secondary infections*, especially when fragments of skin and soft tissue and spicules of bone from the internal plates are displaced into the brain. In the absence of infection, repair takes place in the manner that is usual for defects of nervous tissue. The detritus of blood and nervous tissue is removed by microglia and meningothelial macrophages. If the defect is small, an astrocytic scar forms, but usually a cyst remains, lined by proliferated astrocytes. Adhesions between the glial tissue and pia mater produce meningocerebral scars that are notably epileptogenic.

Fracture of the skull

Fractures of the skull can be important in terms of the concurrent injuries to the underlying meninges and brain and because they can provide a pathway of infection to the sinuses, meninges, and brain. They are indicative of injuries produced by considerable force and, when the inner plates are fractured, of contusion or laceration of the underlying brain. Fractures of the base of the skull may involve the middle ear and allow the escape of cerebrospinal fluid and the entrance of infection. Frontal fractures involving the cribriform plate may allow cerebrospinal fluid to escape into the nasal cavity.

Fractures of the skull are usually quite easy to detect by virtue of the displacement of bone and meningeal hemorrhage. Some, however, may be difficult to detect. This applies especially to fissures that develop during transient deformation of the skull and to impacted basilar fractures, such as occur in horses that rear backward and strike the nape of the neck and occiput. With these fractures, hemorrhages may be scant and basilar fractures are revealed only when the dura is carefully dissected away.

Injuries to the spinal cord

It is possible for direct injuries to the spinal cord to occur without obvious injury to the vertebrae. Such spinal injuries are necessarily lacerations caused by penetrating foreign bodies. Wandering parasites as causes of traumatic injury are discussed later. Much more common are indirect injuries to the cord acquired in the course of vertebral luxations or fractures with dislocation. *The most common of indirect injuries to the cord are produced by extruded nucleus pulposus in dogs, and by compression of the cervical cord in the syndrome known as "wobbler"* (see Vol. 1, Bones and joints).

Vertebral subluxations are the result of trauma, but *fracture dislocation* may be pathological as well as traumatic, and frequently is in swine and ruminants when the fracture occurs through an area of vertebral osteomyelitis or osteochondrosis. The injuries to the cord occur chiefly at the time of accident and are due mainly to the stresses to which the cord is subjected and partly to impediment to the blood supply. Cumulative injury may occur, especially in dislocations when continued pressure on the cord or stretching of the cord over bony prominences causes intermittent ischemia.

Subluxations are largely restricted to the cervical column, where there is relative mobility of the ligaments. In the thoracic and lumbar spine, comparable forces are more likely to cause fracture because of the brevity of the ligaments. The fractures may involve only a lamina, the odontoid process, or an articular process, and therefore be difficult to detect. *Fracture dislocations of the vertebral column occur chiefly in the caudal cervical region and about the thoracolumbar junction*. Most injurious are those fractures that involve the vertebral body because there is usually displacement of a fragment caudodorsally into the spinal canal. Both in luxation and fracture displacement, pressure may be exerted over several segments of cord by epidural and endodural blood clots (Fig 3.68).

Traumatic injuries to the spinal cord may be slight enough to be satisfactorily recoverable, or they may, at the other extreme, cause transection and an extensive length of necrosis. Early contused

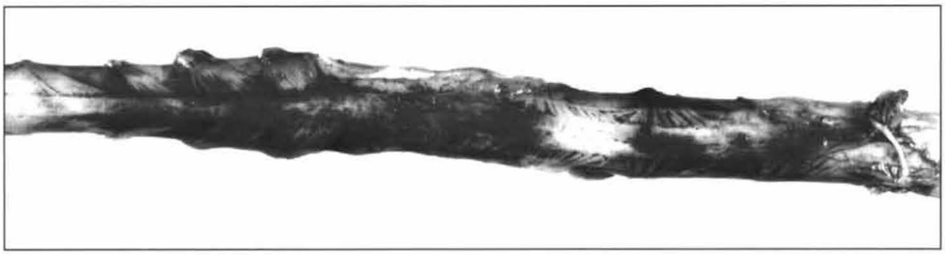

Figure 3.68 Extensive **subdural hemorrhage** of the spinal cord in a dog, caused by physical trauma. (Courtesy of RF Slocombe.)

lesions cause swelling of the cord over one or two vertebral segments. The swelling may be easier to palpate by gently stroking the cord than to appreciate by inspection. Later, the cord is shrunken at the level of injury, and this also may be best detected by palpation. In the swollen zone, dark points of hemorrhage may be detected on section, and these occur usually in the central gray matter and at its junction with the white. If the demarcation of gray from white matter is obscured, it can be expected that softening of some degree is present. Cores of softening may be recognizable grossly, and the fact that they are extending up and down the cord from the point of injury is explained as tracking of exudate, especially serum or edema fluid, along the fiber tracts and the path of least resistance since, because the meninges are usually intact, the amount of local swelling is limited.

With severe compression, the cord may be entirely necrotic at the point of injury and over several segments. *Necrosis is probably due to vascular injury*, and, for the same reason, isolated segments of necrosis may occur apart from the main injury. When the cord is necrotic, it is initially swollen, but after a lapse of some weeks the dura forms a narrowed, collapsible tube containing debris.

Microscopically, in the mildest injuries there is swelling of axons and myelin sheaths. The injured axons become beaded, but they are possibly not irreversibly injured unless fragmentation follows the beading, because functional recovery can follow minor and transient paralytic injuries. Where the axons are severed, there is bulbous swelling of the retracted ends. Degenerative neuronal changes consisting of the clumping of Nissl substance and central chromatolysis are constantly present. Ultimately, there is some loss of neurons. When softening occurs, the sequence of changes is the same as occurs in any neural lesion with loss of substance. The hemorrhage is usually slight and perivascular, but it may enter the central canal and pass along it for a considerable distance.

In long-standing spinal injuries, adhesions may be found between the meninges and between the dura mater and periosteum. In the injured zone of cord, there may be loss of fibers and myelin sheaths with astrogliosis or, in the more severe injuries with loss of substance, there may be cysts, pial-glial or collagenous scars, or the cord may be converted to a thin sclerotic band.

DEGENERATION IN THE NERVOUS SYSTEM

Foregoing sections dealing with cytopathology and the effects of circulatory disturbances and trauma are discussions of changes that are largely degenerative. The same will be true of many sections in later parts of this chapter because neural injury, regardless of cause or severity, is characterized chiefly by degeneration. Discussed in this section are those lesions and diseases of the CNS that are expressed as degenerations of nervous tissue.

Meninges

Age changes in the meninges may advance to such a degree as to be pathological. *Collagenous and osseous metaplasia* occurs commonly in the cranial dura mater of dogs and cats. The process begins in the frontal region and extends to the temporal and occipital areas. The plaque-like thickenings of the surface meninges tend not to extend to the basal or cerebellar meninges. Ossification, which is detectable as small intradural plaques, follows hyalinization of the connective tissues and may or may not be preceded by mineralization. The dura adheres firmly to the periosteum. Perhaps by an independent process, *spherical mineralized nodules* form in the basal dura beneath the medulla in some aging dogs. Histologically, these nodules show a whorled pattern of fibroblastic cells often enclosing small concretions known as **psammoma bodies**.

Hyalinization of dural collagen occurs also in the spinal dura mater with aging and may be preliminary to the osseous metaplasia of the dura observed frequently in large breeds of dogs. The latter condition is referred to as **ossifying pachymeningitis** but, in spite of the frequency of the dural change, little is known of its pathogenesis or effects. *Dural ossification* is found in older age groups of some large breeds and varies in its expression from multiple separate plaques to rigid ossification over several segments of the cord. The changes are best developed in the lumbar region but may involve much of the spinal dura. The basic change appears to be degenerative and metaplastic rather than inflammatory. Locomotor dysfunction in affected animals is more likely to be attributable to concurrent spondylosis and degenerative/reactive changes in joints. The plaques consist of lamellar bone that is mature in appearance and that from the earliest stages contains cancellous spaces and active bone marrow.

Degenerative changes in leptomeninges are less well identified than those in the dura and are limited to collagenization and hyalinization. They are not functionally significant, but the opacities produced, especially the focal thickening in arachnoid granulations in old animals, can be misinterpreted as inflammatory change.

Choroid plexuses

With advancing age, there is *hyaline degeneration of the connective tissues of the choroid plexuses*, especially those of the lateral ventricles (Fig. 3.69). The basement membranes of the capillaries are also involved. The hyaline tissues may become mineralized.

Cholesteatosis of the choroid plexuses in the form of tumor-like nodules, usually termed *cholesteatomas* or *cholesterol granulomas*, occurs in 15–20% of old horses. They are more frequent in the plexuses of the fourth ventricle than in the lateral ventricles, but those in the lateral ventricles are the more important because they may attain large size and, by obstructing the interventricular foramen,

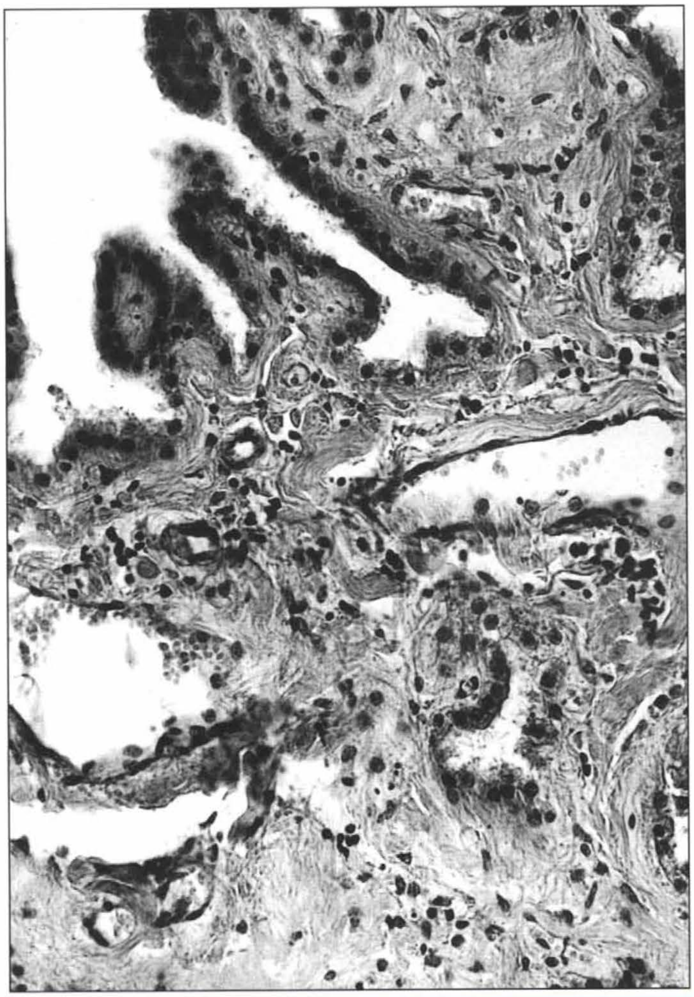

Figure 3.69 **Senile hyalinization** in the choroid plexus of a horse.

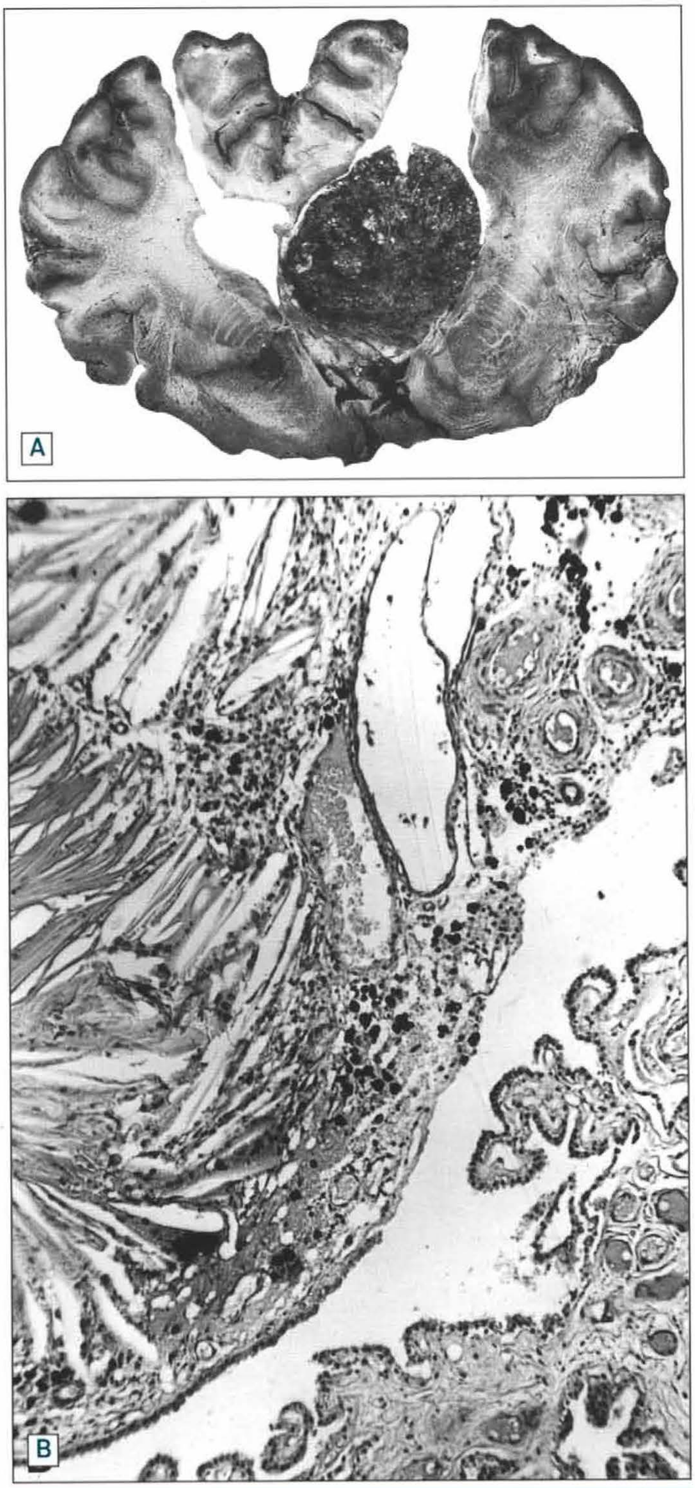

Figure 3.70 **A. Cholesterol granuloma** in the choroid plexus of the right lateral ventricle of a horse. (Courtesy of P Olafson.) **B. Cholesterol granuloma** in the choroid plexus of a horse.

cause hydrocephalus leading to dilation and pressure atrophy of the walls of the ventricles (Fig. 3.70A).

The development of cholesteatosis appears to be related in some manner to chronic or intermittent congestion and edema with congestive hemorrhages in the choroid plexuses. During an edematous episode, the plexuses are slightly swollen, yellow, and soft. The interstitial tissues are edematous and infiltrated lightly by macrophages containing lipid and hemosiderin. The crystals of cholesterol are deposited in the tissue spaces and apparently act as foreign bodies to stimulate low-grade productive inflammation (Fig. 3.70B).

The affected plexus is swollen, and small areas or the whole of it may be occupied by the cholesterol granulomas. These are firm but crumbly, gray nodules with a gleaming, pearly appearance on cut surface. The crystals can be expressed from the cut surface by slight pressure.

Atrophy in the brain and spinal cord

The most spectacular atrophy of the cerebrum occurs in cases of congenital lipofuscinosis and of progressive internal hydrocephalus. **Senile atrophy** of the brain and cord is frequently obvious but seldom severe in animals. The atrophy is most marked when it is associated with the combined influences of senility and marasmus, and it occurs more frequently in sheep than in other species. The pachymeninges are thickened and those of the cranium are partially adherent to the periosteum. The leptomeninges are thickened, tough, and granular. The brain is reduced in volume and weight and may be discolored a darker gray-yellow. The convolutions are narrowed and wrinkled and the sulci are shallow and widened. Both brain and cord are much firmer than is normal. The ventricles are moderately dilated. Histologically, the neurons, especially of the cortex, are shrunken, distorted, and dark staining and their numbers are depleted. The amount of Nissl substance is reduced and the neuronal nuclei stain diffusely. Lipochromes are increased in amount and satellitosis is prominent; macrophages laden with lipochrome are present in the perivascular spaces. There is a diffuse increase in glial fibers, which is responsible for the increased firmness of the tissue. The gliosis is most prominent about Virchow–Robin spaces, and there is a general tendency for protoplasmic astrocytes to transform into fibrous ones. The Virchow–Robin spaces are dilated, and some may be visible to the naked eye. The media of the vessels contain increased amounts of collagen.

Focal atrophy of the brain and cord is the result of slight but long-continued pressure. The loss of function is often disproportionately large as the result of acute pressure lesions compared with the extent of pathologic change. The reverse is often true in chronic pressure, and function may be retained to a surprising degree. Hydrocephalus is common as a cause of chronic pressure, and other sources of pressure are space-occupying lesions of the meninges or bony vault.

The pathogenesis of the changes produced by chronic pressure is unclarified. Because the immediate effects of pressure are more likely to be felt by veins than by arteries or solid tissue, the atrophic changes are usually attributed to venous stasis and edema. More acute pressure effects grade into circumstances and consequences discussed under trauma. Whatever the mechanism, myelin is the most vulnerable component of the tissue and undergoes slow progressive degeneration. There is also slow progressive atrophy and loss of neurons, especially of the Purkinje cells when pressure is exerted on the cerebellum, the neurons of the deeper laminae when pressure is exerted on the cerebrum, and of the ventral horn cells in the spinal cord. The degenerating myelin is replaced by glial fibers.

Segmental cerebellar atrophy is common in pigs following a variety of nervous diseases. The rostral vermis and adjacent portions of the lateral lobes are affected, these being within the distribution of the rostral cerebellar artery. There is no acute change but, instead, a gradual loss of granule and Purkinje cells with secondary changes in the white matter and reactive gliosis. The lesion is asymptomatic.

Anoxia and anoxic poisons

The neuropathological effects of anoxia are seldom observed in animals or perhaps seldom looked for. Since most cases die acutely, there may be very little to see. The pathological effects of anoxia, not only in the CNS, but in general, are expressions of "vulnerability," a property which differs from organ to organ and between parts and cells of one organ. *The components of the nervous system are vulnerable in a rather fixed order,* which is neuron, oligodendroglia, astroglia, microglia, and blood vessels in that diminishing order of sensitivity. The neurons are not a uniform population, and there are regional differences in their susceptibility to anoxia, those of the cerebral cortex and the Purkinje cells being the most sensitive. Within the cerebral cortex, the neurons of the deeper laminae are more sensitive than those in the superficial laminae. Beneath these levels, there is a gradient of susceptibility through the geniculate bodies, hypothalamus, thalamus, paleocortex, and caudate nucleus. The various patterns of vulnerability are relative; they are established largely by experiments in animals and are subject to some variation depending in part on the experimental species and on the type of anoxia induced. They do nevertheless emphasize the problem of selective vulnerability at the cellular level. There are, in addition, broader regional differences in vulnerability to anoxia such as those that allow parts of the cerebral cortex to be necrotic while adjacent parts survive intact and that allow the same agent or anoxic insult to cause, leukomalacia on some occasions, and poliomalacia on other occasions. The basis of these regional differences in vulnerability, and the apparent differences in the patterns of degeneration following different types of anoxia, is still to be established.

The pathological effects of anoxia on nervous tissue are of two types, which are to some extent differences in degree. The least clear morphological expression is *selective neuronal necrosis*. The neuronal necrosis is typically of the ischemic type, and it is followed by glial repair. Greater degrees of anoxia, sufficient to kill astroglia as well as neurons, result in *softening*. These are the expected changes in anoxic anoxias such as might occur with cardiac arrest or periods of apnea in grand mal epilepsy.

As a response in some episodes of anoxia, such as may be induced, for example, in the histotoxic variety of cyanide poisoning, the degeneration and necrosis may affect the white matter with only minimal changes in the gray. The reason for this is not known. The malacic changes in the white matter of the cerebral hemispheres are preceded by edema, which suggests that local circulatory disturbances contribute to its development. Clearly the attribution of selective vulnerability on the basis of local metabolic demand for oxygen is inadequate, and a fuller explanation will probably require, in addition, consideration of vascular architecture, the autoregulation of blood pressure and flow, and local metabolic factors in the maintenance of myelin and axons.

Cyanide poisoning

The anoxia produced by cyanide is classified as *histotoxic* because the action of the poison is to *inhibit intracellular respiratory enzymes*. The cyanides are particularly rapid in their action, and death usually occurs from a few minutes to an hour after the onset of clinical signs. In the most acute cases, the clinical course occupies only a few minutes and is characterized by dyspnea, drooling, trembling, and recumbency; it terminates in convulsions. The *blood is bright red* owing to its high degree of oxygen saturation. In cases that survive for an hour, the clinical signs are the same with, in addition, vomiting, especially in pigs, nystagmus and cyanosis. In these cases, the blood is often dark owing to anoxemia.

The morbid changes are those associated with anoxia. Because most cases survive for half an hour to one hour, the blood is dark and does not clot and the tissues are dark. There is congestion in the lungs and hemorrhages of dyspneic type in the tracheal mucosa. Ecchymoses of the epicardium are usual. In pigs, there is pulmonary edema and hydropericardium, and there may be capillary hemorrhages deep in the myocardium by the coronary grooves. Severe pulmonary edema is present in some dogs.

Descriptions of lesions in the nervous system of animals in cyanide poisoning are largely limited to experimental observations in dogs and cats. In many natural cases of the poisoning, there is no significant alteration in the brain. The degenerative lesions in the brain in experimental poisoning may involve predominantly the gray matter or the white. When in gray matter, there is patchy necrosis and laminar loss of cells in the cerebral cortex, and necrosis in the head of the caudate nucleus, paleocortex, substantia nigra, and thalamus. There is edema in the cerebral white matter, sometimes in the absence of cortical change. On the other hand, single graded exposures of rats may produce the principal lesions in white matter, especially in the corpus callosum. In this species, there are gradients of injury in the callosum reflecting gradients of susceptibility, the most severe degeneration occurring in the caudal core of the callosum. The gradients are attributed to regional differences of blood supply, which are expected to reinforce the histotoxic anoxia of cytochrome oxidase inhibition by cyanide. The earliest change described in the callosal lesion is axonal swelling producing a spongy appearance by light microscopy; alterations of myelin, and oligodendrocytes are second in time.

Plants containing toxic levels of hydrocyanic acid bound as glucosides are common and widespread and are the usual source of cyanide poisoning. The compounds are in general the β-glycosides of α-hydroxynitriles and are activated by endogenous glucosidases in the plant, or in other plants, or by ruminal microorganisms. For these reasons, cyanide poisoning occurs chiefly in ruminants. Toxin generation of this type does not occur at the low pH of the monogastric stomach, but a lethal amount may be absorbed from the large bowel if there has been a large intake of toxigenic material.

Both sheep and cattle are capable of detoxifying cyanide in the liver to form *thiocyanate*. Thiocyanate itself is toxic but in a manner different from that of cyanide; if thiocyanate is present in low concentrations over prolonged periods, it is goitrogenic. The cyanide concentration in a stated species of plant varies considerably with stage and rate of growth and with the fertility of the soil. Plants that are growing rapidly contain the highest concentrations of the glucosides, especially if the growth phase follows one of retardation caused, for example, by wilting, frostbite, or close grazing. The glucosides are regarded as being metabolic by-products.

Plants of many species contain cyanogenetic glucosides but only a few are important in this regard. The majority of cyanogenetic plants are usually harmless either because of low palatability or low concentration of the glucosides. The important cultivated toxic plants are the sorghums (*Sorghum* spp.), and the star grasses (*Cynodon* spp.). Those of the cherry family are also potently cyanogenetic. Linseed concentrates can be dangerous, especially if eaten in large quantities.

Chronic cyanide intoxication is not recorded, but extended periods of grazing the cyanogenetic sorghum and sudan grass may lead to ataxia in cattle and horses, cystitis and urinary incontinence, and abortion in mares. Attempts to link the clinical syndrome to cyanogenetic compounds in the plants are inconclusive, and a causal association with cyanides seems unlikely. There is axonal degeneration and demyelination at all levels of the cord but most prominent caudally in ventral and lateral funiculi. Ataxic syndromes occur in sheep grazing *Sorghum* sp. may involve large numbers of animals in syndromes that differ from those in horses and cattle. Urinary incontinence does not occur. There is limb paresis with knuckling, incoordination and disturbance of equilibrium, and head and body tremors. Pregnant ewes may produce stillborn, weak, or arthrogrypotic lambs. While the lesions in horses and cattle are of Wallerian type in the spinal cord, those in sheep are of an axonopathy with proximal spheroids in the major nuclei of the brain stem and ventral horns of the cord.

Nitrate/nitrite poisoning

Ruminants are also vulnerable to *phytogenous nitrates*, when intraruminal microbial conversion of nitrates to nitrites exceeds the rate of conversion of the latter to ammonia. The nitrate sources again include many crop plants as well as weeds; plant nitrate concentrations are influenced by soil nitrogen level, and physiologic stresses on the plants, such as water stress and herbicide damage. Occasionally, high nitrate concentration in drinking water is the problem. *Absorbed nitrites convert hemoglobin to methemoglobin*, which imparts a *dark "chocolate" color* to the blood, and causes dyspnea, cyanosis, weakness, tremors, collapse, and coma. There are no gross or histological brain lesions. The condition is discussed more fully in Vol. 3, Hematopoietic system.

Fluoroacetate poisoning

Fluoroacetate is toxic by virtue of its ability to form fluoracetyl-CoA, which potently inhibits the Kreb's cycle enzymes *cis*-aconitase and succinate dehydrogenase, thereby paralyzing cellular respiration. Herbivores are usually poisoned by ingesting *fluoroacetate-containing plants*, while other animals may be affected by accident or design by being exposed to sodium fluoroacetate formulated as a *pesticide*. The clinical signs are of the same general character as those described above but, in the dog, frenzied hyperactivity is reported. As with cyanide, the myocardium is highly vulnerable, and multifocal necrosis may be seen. Fluoroacetate poisoning is discussed in Vol. 3, Cardiovascular system.

Carbon monoxide poisoning

This is rarely observed and is discussed in the interests of completeness. Carbon monoxide has an affinity for hemoglobin which is several hundred times the affinity of oxygen for hemoglobin. The carboxyhemoglobin produced prevents oxygen exchange and causes anoxic anoxia. The anoxia is reinforced by a histotoxic component due to the affinity of carbon monoxide for iron-containing respiratory enzymes.

Carboxyhemoglobin and, therefore, the blood and tissues of poisoned animals are *bright pink*. All organs are congested, but especially the brain, in which the veins and capillaries are much dilated. The dilated vessels are observed especially in the white matter and hemorrhages occur from them. Acute fatalities are not expected to be associated with neural lesions. Survival is expected to be followed by complete recovery, but there may be residual lesions of anoxic type, especially neuronal loss.

Carbon monoxide poisoning is reported occasionally in animals in confined quarters that are heated by petroleum fuels. Most of the reported instances are in pigs, and poisoning is associated with a high level of stillbirth and neonatal mortality. Following experimental exposure, patchy or extensive leukomalacia may be present in the hemispheres in the newborn.

Hypoglycemia

It is useful to extend the classification of anoxia to include hypoglycemia, which causes a *disturbance of intracellular respiration*. Oxygen is available to the nervous tissue, but, in the absence or reduction of the amount of substrate, the oxygen is not utilized.

Hypoglycemia may occur as a result of a functioning *tumor of the pancreatic islets* or as a response to insulin overdosage in the treatment of diabetes. It is also part of the metabolic disturbance of *ketosis in cows* and *pregnancy toxemia in ewes*, and arguments have been advanced for regarding the irreversibility of pregnancy toxemia as being due to hypoglycemic encephalopathy. Piglets in the first week of life readily develop hypoglycemia if there is dietary restriction from any cause. Effective gluconeogenesis does not develop in piglets until about the seventh day of life, and during this first week their glycemic levels are rather precise reflections of dietary intake. In spite of the severe convulsions and deep coma that occur in hypoglycemia, conspicuous changes do not occur in the brain. It is probable that biochemical disturbances in the neurons precede histological signs of degeneration by a considerable period. For this reason, acute hypoglycemic death is not expected to produce cerebral lesions. When the period of coma is prolonged, it is probable that all neurons are to some extent altered, but the differentiation of hypoglycemic from autolytic and nonspecific changes can seldom be made with confidence. Although classified with the anoxias, hypoglycemia does not produce the ischemic type of neuronal degeneration characteristic of the usual sorts of anoxia. There is, instead, a tendency for the severe type of neuronal degeneration to occur, characterized by rapid and complete chromatolysis, disappearance of the cytoplasmic margins, and then fading of the cytoplasm, with pyknosis, eccentricity and fading of the nucleus. The regional susceptibility of neurons to hypoglycemia, exclusive of the Purkinje cells, parallels that of susceptibility to anoxia.

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Malacia and malacic diseases

Necrosis of individual elements of nervous tissue is described earlier under Cytopathology. **Encephalomalacia** and **myelomalacia** refer to necrosis in the brain and cord respectively. *Malacia means softening*, and is used interchangeably with that term to signify necrosis of tissue in the CNS. It is sometimes, as imprecise practice, used interchangeably with demyelination. There is no sharp line to be drawn between demyelination and malacia, especially in the early stages of development of the degenerative process, but the term demyelination should probably be restricted to those lesions in which the myelin sheath is primarily or selectively injured to leave the axons naked but intact. When neurons and neuroglia degenerate and die as part of the primary response, the term malacia is applicable.

The *sequence of events* in softening or malacia is specific only for the tissue. The morphological changes in necrosis, in the removal of dead tissue, and in healing are the same regardless of the insult. The insults can be varied, and *malacia, alone or as part of another change, is one of the most common lesions in the brain and cord*. It is discussed in the sections dealing with vascular accidents and trauma, it occurs in many instances of encephalomyelitis, is discussed elsewhere in these volumes as a lesion of mulberry heart disease, of antenatal *bluetongue virus* infections in lambs, and of clostridial enterotoxemia in lambs that escape apoplectic death and live for some days. Malacic lesions are probably the basis of most cases of hydranencephaly.

Although the nature of the malacic process is not specific for cause, there is some specificity in the localization of lesions and in their particular pattern of distribution. These features may allow the recognition of known associations or causes, such as the nigropallidal distribution of lesions in horses poisoned by yellow star thistle, and the focal symmetrical lesions of the internal capsule in lambs with clostridial enterotoxemia. Although these and some other associations have been determined, the pathogenesis of the lesions and the problems of selective injury to certain parts of the brain remain to be resolved.

The sequence of changes in malacic foci is approximately the same in all cases and is outlined below. The rapidity of change is quite variable and depends on the species and age of the animal, the location of the necrosis, the volume of tissue affected, and the inciting cause. The speed of resolution is also affected by the quality of vascular perfusion in adjacent nervous tissue, whether the necrosis is ischemic or hemorrhagic and on the time over which the cause acts.

The malacic process, once initiated, appears to proceed very rapidly in the fetal and immature nervous system, and to leave cystic structures and hydranencephaly without much evidence of continuing reaction at the time these animals are available for necropsy examination (see Fig. 3.27). The reasons for the rapidity of change in immature nervous tissue are not clear, but contributing factors may involve the paucity of myelin that is difficult to remove, the paucity of mature mesenchyme in meninges and about vessels, and the plasticity of vascular arrangements in the developing brain. The rapidity of change in gray matter is generally greater than in the white. Autolytic liquefactive changes depend on continued enzymatic activity that, in turn, depends on availability of oxygen either by diffusion from surrounding tissue or by reflow in the local vessels. Small foci of necrosis are expected to resolve more quickly than large. Malacic lesions in the neocortex where diffusion from collateral vessels is available are expected to resolve more rapidly than those in the paleocortex where the vascular supply is more strictly of the end-artery type. Some causes of malacia, such as vascular occlusion, act promptly and the pathological changes are directly consequential. The cause may act continuously over a period, as in leukoencephalomalacia of horses, and the changes may be incremental. The process, once established, may itself initiate progressive change as in traumatic injury to the cord in which swelling within the confines of the meninges assists the spread of edema and vascular response beyond the site of the original injury.

A malacic lesion that develops acutely may not, in the absence of hemorrhage, be demonstrable before about the twelfth hour of onset. The early change is in texture, with the affected part being soft, and in color, with the affected part being gray. Within 2–3 days, the malacic foci begin to disintegrate, the softness is more evident, and the surrounding tissue is swollen by edema, and pale in gray matter or yellow in white matter. Subject to the above qualifications, *the necrotic area eventually liquefies and a cyst remains.* The cyst may be loculated or traversed by vascular strands that have survived the episode.

Histologically there is, in acute episodes, reduced staining affinity by about 12 hours. The cellular elements show the changes described earlier. The early active response involves circulating neutrophils, which may enter in large numbers, but this response is replaced in 3–4 days by macrophages. The first appearance of macrophages is about blood vessels, their peak activity occurs in about 2 weeks, but a few will survive for a very long time. Astrocytic gliosis replaces or surrounds the resolved necrotic area (see Fig. 3.61).

In the specific syndromes to be discussed below, the degenerative changes tend to be restricted to, or to affect principally, either the gray matter or the white matter. Softening of gray matter is known as **poliomalacia** and softening of white matter is known as **leukomalacia**; each may be qualified as to whether cerebral (encephalo-) or spinal (myelo-). The diseases to be described below are specific, but malacia is not exclusive to them. Necrosis of cerebral cortex, is rare in horses except in the neonatal "barker syndrome" (see Fig. 3.54). In ruminants, poliomalacia is, in the early stages, expected to respond to thiamine, but it also occurs in lead poisoning, in water intoxication, and in other, ill-defined, circumstances. Lead also causes poliomalacia in dogs. Convulsive episodes in dogs, some associated with distemper, may leave malacic changes especially in parietal and temporal cortex. Idiopathic necrosis and malacia of the hippocampus and piriform lobe in cats is reported from Europe as a cause of seizures. Metronidazole intoxication of dogs and cats can result in brain stem leukomalacia. In pigs, polioencephalomalacia is usually due to salt poisoning, but individual cases occur in meningitis or without other association. The isolated case of malacia can be difficult to explain.

Focal symmetrical poliomyelomalacia syndromes

This disease, as described from Kenyan **sheep**, is characterized by focal softening of the gray matter of the spinal cord, most consistent and severe in the cervical enlargement. A similar syndrome occurs in parts of West Africa. There is no information on the cause or pathogenesis, although the distribution of necrosis in relation to the cross-section of the cord and the irregular segmental involvement of the gray matter, especially in the cervical region, are consistent with a *vascular component* in the pathogenesis. Lesions of similar distribution and character are occasionally met with in dogs and cats with inflammatory or thrombotic occlusion of the ventral spinal artery. Affected sheep suddenly develop flaccid or spastic paresis that always involves the forelimbs and sometimes the hindlimbs as well. There are no cerebral signs. Affected animals are lambs or up to 18 months old. There are no gross lesions to be observed except in cases of long standing, in which some brown discoloration may be noted in the malacic areas. Microscopically, there are bilateral lesions of remarkable symmetry in the ventral horns of the spinal cord. The dorsal horns are spared, as is a narrow rim of gray substance around the periphery of the ventral horns, and the commissural gray matter. The affected areas undergo dissolution with the usual reaction on the part of the microglia. At a later stage, proliferating capillaries in small numbers criss-cross the microcavitations, and the astrocytes at the margins proliferate. The malacic foci are found in the cervical and lumbar enlargements as "skip" lesions involving a few segments and, when the necrosis is extensive, similar foci may be found in the medulla.

A similar syndrome has been responsible for heavy losses in native sheep in Ghana and Ivory Coast. All ages are affected but mainly adult ewes. Clinical progression is rapid, from initial stumbling to ataxia and recumbency, opisthotonos and nystagmus and ultimately flaccid paralysis. There are microscopic changes of cytotoxic edema, especially affecting oligodendrocytes widely in the nervous system and also perivascular astrocytes and capsule cells of spinal ganglia. Foci of spongy degeneration and malacia, bilateral but not always symmetrical, are of patchy distribution and most frequent in the spinal intumescence, cerebellar roof nuclei, and large nuclei of the brain stem.

Encephalomyelomalacia of similar character is reported in young individual **goats** in California. The lesions are bilaterally symmetrical and affect particularly the lumbar and cervical enlargements and the inferior colliculi. Other brain stem nuclei are inconsistently involved. The similarity of these malacic lesions to those which can be produced experimentally by nicotinamide antagonists has been noted.

Focal symmetrical poliomyelomalacia of pigs is clinically and pathologically similar to the spontaneous poliomalacias of sheep and goats. The presenting signs are spinal with ataxia progressing to forelimb or hindlimb paresis or quadriplegia in a few days. In field cases, malacic foci are found, symmetrically in the ventral horns of the cervical and lumbar enlargements. The malacic foci are visible grossly as yellow-brown areas of softening or gray depressed areas of liquefaction. The histologic changes are typical of malacia (Fig. 3.71). There is heavy loss of neurons, and endothelial and glial proliferation in older lesions. Similar changes may be present in medullary nuclei.

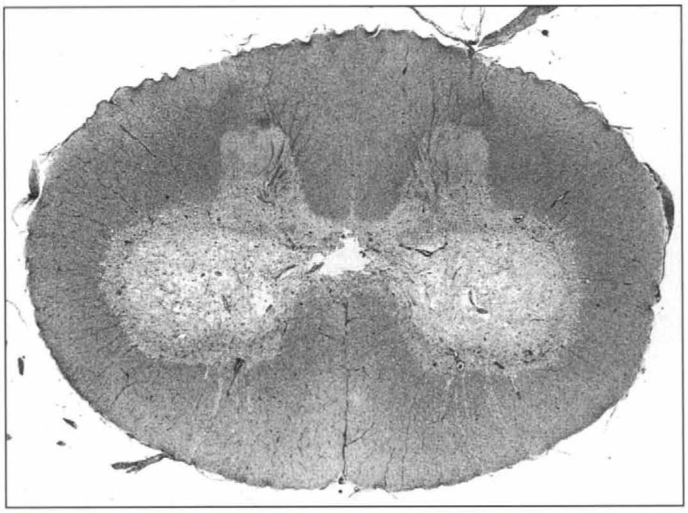

Figure 3.71 Focal symmetrical poliomyelomalacia involving ventral horns of cervical cord in a pig with selenium toxicity. (Courtesy of TM Wilson.)

Affected animals may show changes associated with *selenium toxicoses*, including scurfiness of skin, hair loss and separation of horn at the coronet. Dietary selenium, whether as sodium selenite added to rations or the feeding of selenium-accumulator plants, reproduces the syndrome, although there is some inconsistency in the lesions produced, with degeneration being more frequent in the nuclear masses of the medulla and brain stem. *Astragalus bisulcatus*, a selenium-accumulator plant, will cause concurrent polioencephalomalacia. The mechanism of neurotoxicity of selenium is obscure, but may occur through the generation of reactive oxygen species during reaction of thiols, and is expected to be influenced by other nutritional and metabolic interactions.

Focal symmetrical poliomyelomalacia, the lesion limited to the sacral and lumbar enlargements, is described in **Ayrshire calves**. The malacia affects the ventral horns, sparing the dorsal horns and the central gray matter. The cause is not known.

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Aeschynomene indica is a common weed in irrigated rice fields and may be fed with rice and screenings of broken rice. Signs of neurologic disorder develop in a few days. Lesions are restricted to the brain, are symmetrical, involve especially the vestibular and cerebellar roof nuclei, nuclei of the midbrain, and the paleocortex. The toxic factor is unknown, but pathogenesis may depend on primary damage to microvasculature.

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Polioencephalomalacia of ruminants

Polioencephalomalacia (PEM) as used in practice applies especially to softenings restricted to the cerebrocortical gray matter of laminar distribution. The lesion may alternatively be designated as "cerebrocortical necrosis" or "laminar cortical necrosis." Necrosis of this distribution is the basis of the well-recognized syndrome of cattle, sheep, and goats known as PEM. It is also the lesion of salt poisoning in swine, is occasionally observed in lead poisoning of cattle, and is described among the residual neurologic lesions of cyanide poisoning. Apart from these known associations or causes, laminar cortical necrosis is observed sporadically in swine, dogs, and cats.

The disease, PEM of sheep, goats, and cattle and other managed ruminants, is similar in its clinical and pathologic aspects in the several species, but the course in sheep and goats is, as a rule, shorter, with fewer survivors from the stage of overt brain swelling. Merino sheep appear to be much more resistant than other breeds.

The incidence is highest in sheep in the age group from weaning to 18 months, but sporadic cases occur in older animals. This general statement of incidence applies to pastured animals as well as to those in feedlots or barns. It is a disease of young cattle, the age incidence depending on the population exposure. As originally described in Colorado, affected animals are mainly 1–2 years of age but, elsewhere, the incidence is highest in younger stock of 3–8 months of age. The severity of the disease is rather less in pastured than in feedlot or housed cattle and sheep. Its least clinical expression, occasionally observed in outbreaks in sheep, is blindness and dullness, a tendency to head press against obstacles, and cessation of feeding. There are no ocular abnormalities in this, or in any other, grade of the disease, and the blindness is cortical. Animals showing these mild signs usually recover completely in the course of several days. If they are killed for examination, malacic changes may be found to be minimal or absent but there is widespread neuronal necrosis of ischemic type in the deeper laminae of the cerebral cortex. Animals affected more severely may remain on their feet and show, in addition to the above signs, muscular tremors especially of the head, and intermittent opisthotonos; if they survive, they become partially decorticate and remain blind and stupid.

In severe cases of PEM, the animals are recumbent. There is twitching of the face, ears, and eyelids, intermittent grinding of teeth, drooling, and bulbar paralysis in some. Opisthotonos is present and some animals are convulsive. In the early stages, the neurologic signs are intermittent, but later they become more constant with persistent opisthotonos and nystagmus. Flaccidity is usual, although there may be transient periods of spasticity, and clonic convulsions are intermittent. Death occurs in coma after a course of one to several days. These signs are entirely referable to the nervous system; other systems are undisturbed except for the very occasional case in which massive hemoglobinuria after excess water intake precedes the onset of neurologic signs.

The **lesions** are qualitatively the same in all cases, but they differ in their degree and in the ease with which they may be detected, this depending on severity and duration. Young animals usually die more rapidly than old ones and may show cerebral edema and swelling only. The swelling affects the cerebral hemispheres, which are pale, slightly soft, and droop over the cut edges of the cranium. In these cases of short course, there may be no obvious displacement of the brain. When the fresh cortex is sectioned, it usually shows a laminar paleness about 0.5–1 mm wide following the contour of the gray matter at the junction between gray and white. This change may be most easily visible in the gyri rather than in the depths of the sulci. Its extensiveness varies considerably from case to case but remains within the boundaries of the supply area of the middle cerebral and caudal cerebral arteries. In the rostral distribution of these vessels, the lesions are patchy but are more extensive caudally towards and

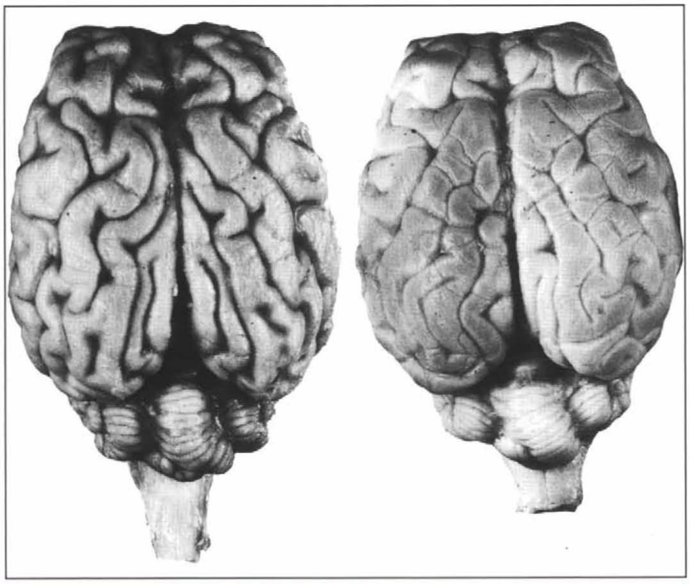

Figure 3.72 Polioencephalomalacia in a sheep. Normal brain (left), and affected brain with flattened gyri (right).

over the caudal poles of the hemispheres. The dorsal surfaces of the hemispheres are involved, and the distribution is approximately symmetrical. In either fresh or formalinized material, the gross lesions of cortical necrosis autofluoresce under ultraviolet light, due to the presence of lipid metabolites in macrophages, or of high-molecular-weight, collagen-like material.

In cases that survive for several days, the necrosis becomes more and more obvious. The brain is grossly swollen (Fig. 3.72), and, almost without exception, it is displaced caudally with herniation of the medulla and cerebellum into the foramen magnum. The dorsum of the hemispheres is palpably quite soft and the normal turgidity of the brain is gone. The surfaces of the gyri are a characteristic yellow-brown and the normal adhesion of leptomeninges to the gyral surfaces may not be present. On the cut surface of the cerebrum, there will be areas of minimal change resembling those described above. The necrotic zones can usually be appreciated to be narrow and pale or yellow instead of gray, and they are friable and shrink away from the cut surface (Fig. 3.73). Lines of cleavage between the gray and the white matter can be discerned. These are present especially over and extending down the sides of the gyri but seldom extend around the depths of the sulci. In some cases, yellow foci of softening can be found in the herniated portion of the cerebellar vermis. Hemorrhage is insignificant and usually not apparent in the cerebrum and cerebellum, but hemorrhagic foci of softening may be found in the collicular region, thalamus, and caudate nuclei. In some cases, there is thrombosis of the dorsal longitudinal sinus.

Animals that survive for 2 weeks or longer are decorticate over more or less extensive areas. The white cores of the gyri are largely intact and project nakedly or with an irregular covering of softened friable gray matter. Over some gyri, the gray matter is intact. The subarachnoid space is widened and the meninges droop over the enlarged sulci. Subpial cysts may be evident where the devastation is not complete and the meninges are adherent. The brain is smaller than normal and not displaced, the lateral and third ventricles are dilated, and there is an excess of cerebrospinal fluid.

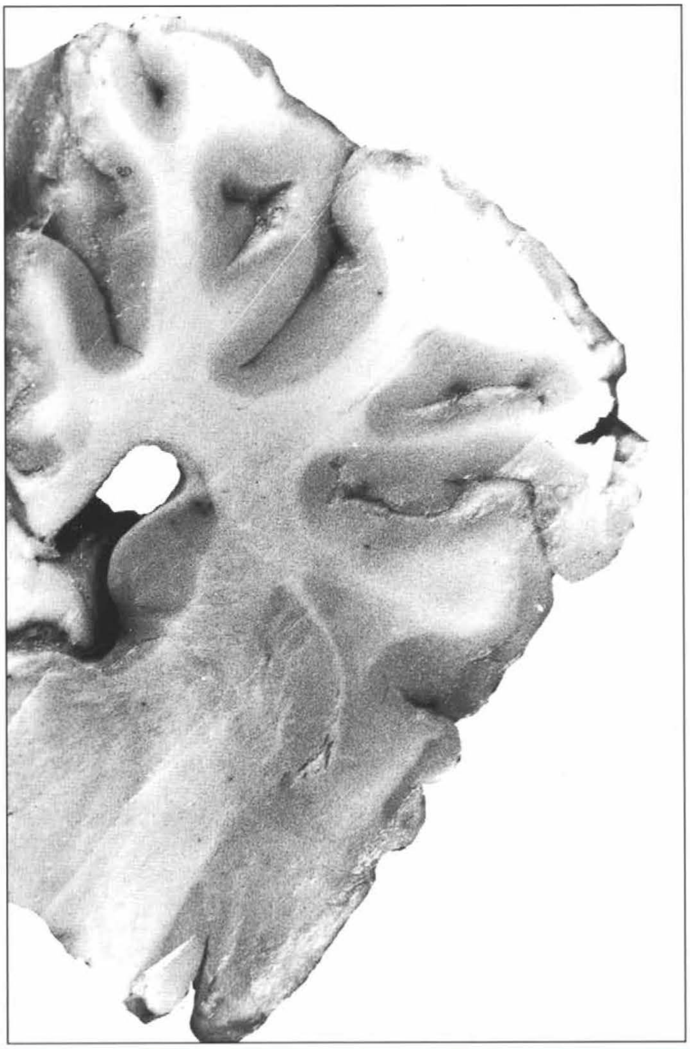

Figure 3.73 Chronic lesion in **polioencephalomalacia** in an ox; note the marked irregular narrowing of cerebral cortex.

The **microscopic changes** are of the same type in all affected parts but vary in distribution and severity. The least change is one of laminar necrosis of neurons affecting especially the deeper laminae. The neurons are shrunken, acidophilic, and surrounded by a clear space; in 2–3 days, many are converted to eosinophilic globules without nuclear remnants. Healing, if it occurs, is with intense astrogliosis.

The malacic areas are laminar (Fig. 3.74). The deepest laminae are consistently involved. Frequently, the superficial lamina is involved. The softened laminae do not necessarily overlie one another although there is usually considerable overlapping. When two distinct laminae of necrosis overlie one another, they frequently merge in the depths of the sulci to produce necrosis of the total width of the gray stratum (Fig. 3.74). Necrosis of the superficial lamina is first recognizable as a rather uniform sponginess. The layer then disintegrates and the middle laminae, if intact, are separated from the pia by a moat of gitter cells in which vessels are very sparse (Fig. 3.75). The leptomeninges are thickened and contain many activated histiocytes.

The middle laminae may remain structurally intact for considerable periods. The neurons therein die acutely, but the glia are rather persistent. The vessels are prominent, with swollen proliferating endothelial and adventitial cells and a perivascular clear space.

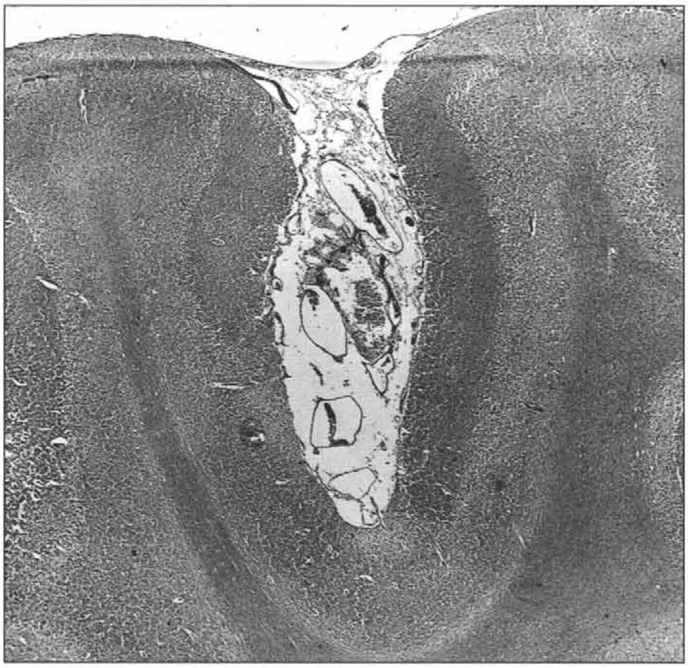

Figure 3.74 **Polioencephalomalacia** in an ox. Pallid areas of degeneration involving full width of cortex in depth of sulcus.

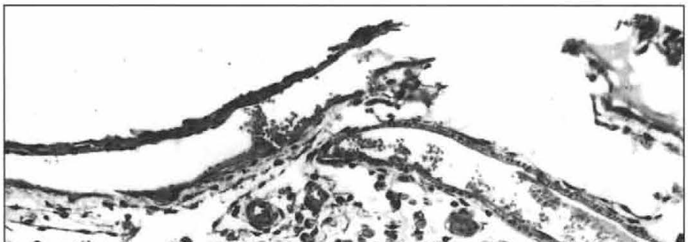

Figure 3.75 Collapse of meninges, with gitter cells replacing necrotic cortex in **polioencephalomalacia** in an ox.

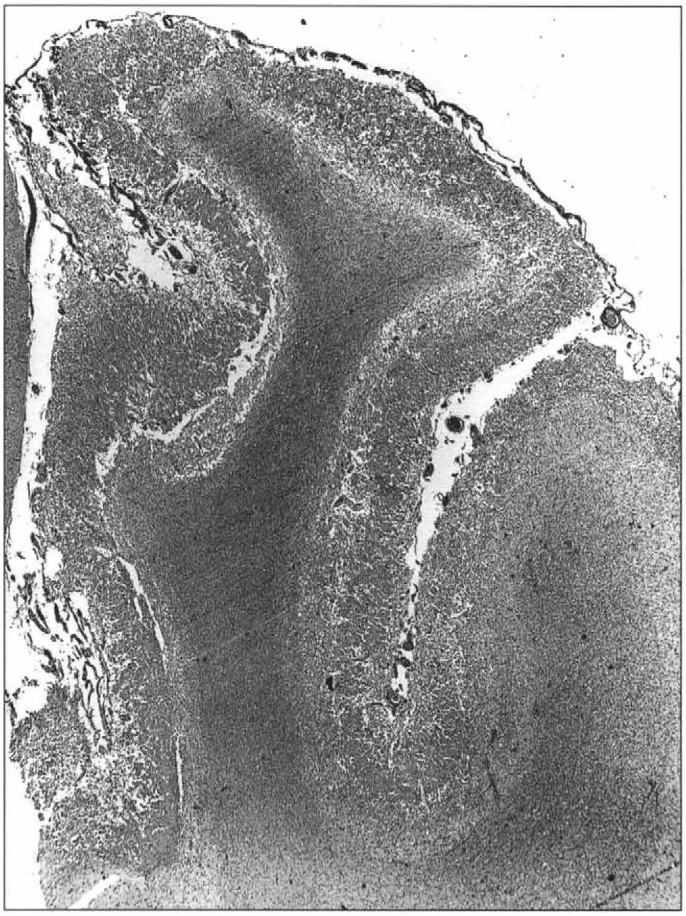

Figure 3.76 Subtotal cortical necrosis with separation in **polioencephalomalacia** in an ox.

There is *early edema and neuronal necrosis in the deepest laminae* and the edema extends to the adjacent white matter, giving the zone a pale washed-out, fibrillary appearance in sections (Fig. 3.74). There is demyelination in the edematous white matter, but the glia survive fairly well, and the astrocytes react and swell. The microglia become active and concentrate in the deep laminae, which disintegrate to cleave overlying cortical remnants from the subjacent white matter (Fig. 3.76).

The macroscopic lesions observed in the cerebellum and subcortical areas are typical foci of softening. Microscopic changes of less severity are more common. They occur in the herniated portion of the vermis and consist of acute degeneration of Purkinje cells with more or less extensive cytolysis in the granular layer.

The chronic lesions of surviving animals resemble those described earlier in ischemic infarcts. The dead tissue is removed by microglia. In zones where the gray matter has been entirely necrotic, the pia may be separated from the white matter by a clear space or be in contact with the white and partially adherent as a glial–pial scar formed by connection with the proliferated astrocytes that line the defect. Where the superficial laminae have remained intact, elongate cystic spaces lined by astrocytes remain where the necrotic parenchyma has been removed.

The areas of softening quite clearly have a distribution related to the field of supply of the middle cerebral artery. When the lesion is of restricted distribution, it is related to the periphery of the field of distribution

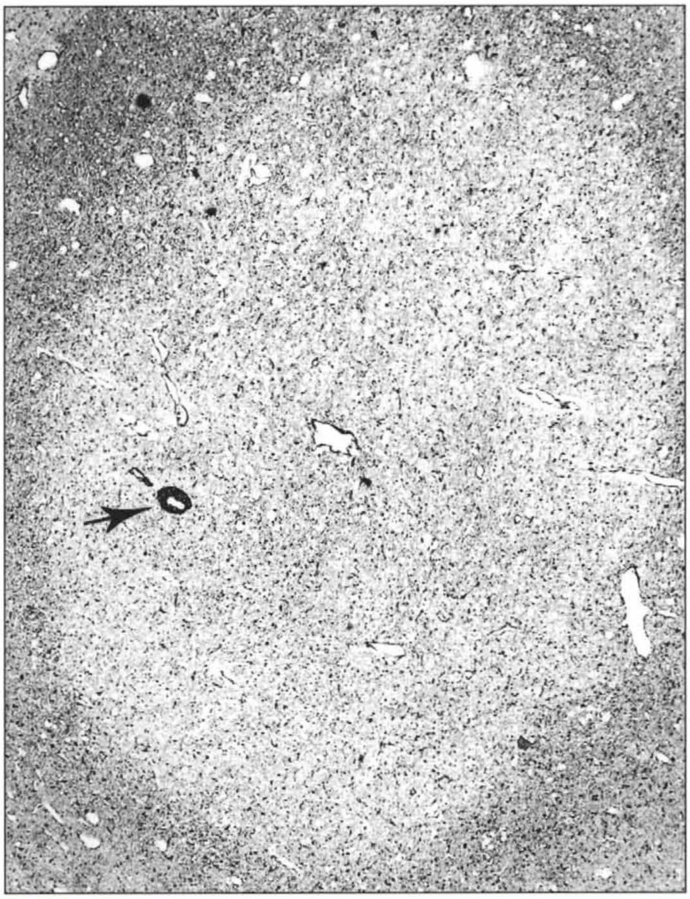

Figure 3.77 Edema in inferior colliculus with ring hemorrhage (arrow) in **polioencephalomalacia** in a sheep.

of this vessel over the dorsal cerebral cortex and, when the lesion is more extensive, it covers more of the field of supply of this vessel, but necrosis is seldom found ventral to the caudal ectosylvian fissure. The cortex rostral to the transverse sulcus and in the field of supply of the rostral cerebral artery escapes significant injury. Necrosis may be observed in the distribution area of the caudal cerebral artery, but only when the swelling and displacement is severe, a fact suggesting that necrosis in this distribution is secondary to tentorial herniation with compression of the caudal vessels against the free edge of the tentorium.

Degenerative changes in periventricular nuclei of type and distribution typical of thiamine deficiency in other species are present in some cases of PEM (Fig. 3.77) and their histologic appearances suggest that their development precedes the cortical necrosis. This observation applies also to the experimental disease produced by amprolium.

The **cause** of PEM in ruminants is often unknown, as the lesion of PEM lacks etiologic specificity. Sporadic cases and outbreaks are observed in animals given access to water after a period of deprivation suggesting that these cases may be expressions of *water deprivation–sodium ion toxicosis* developing in the manner discussed later for salt poisoning in swine. It has been observed in sheep eating the nardoo, *Marsilea drummondi*, and associated with the *thiaminase* present in that fern. The disease has been produced experimentally with analogues of thiamine with impaired activity, and by the feeding of rhizomes of bracken fern, which are known to contain thiaminase.

Deficiency of thiamine or a disturbance in its metabolism is implicated in PEM. Thiamine, if administered early, may lead to prompt clinical recovery. Calves examined early in the course of the disease may have elevated blood pyruvate, reduced blood transketolase, and reduced levels of thiamine in liver and brain. Cerebral edema and laminar necrosis have also developed in lambs and calves given the thiamine antagonist, amprolium. Thiaminolytic enzymes can be found in rumen contents in some cases of PEM, and it is accepted that they are produced as exoenzymes of some rumen microbes. Degradation of thiamine may reduce the amount available and simultaneously produce analogues of the vitamin. The thiamine-responsive disease is sporadic.

Sulfur compounds have emerged as important causes of morbidity. Sulfite, an intermediate in the reduction of sulfate, can cleave thiamine into pyrimidine and thiazole constituents thereby rendering it inactive. Diets and drinking water containing high levels of sulfates can increase the incidence of PEM in cattle, and in sheep fed diets high in sulfur. Sulfur compounds, mainly sulfur dioxide, used to color meat can precipitate a thiamine deficiency syndrome in dogs and cats. The sources of sulfur to ruminants are varied. There is a high incidence of PEM in cattle in feedlots especially when they are fed diets based on molasses, which can be high in sulfur. Inappropriate use of ammonium sulfate for urine acidification in feedlot cattle and sheep is recorded. The high incidence of the disease in animals fed sugarbeet pulp and the occasional outbreak in cattle fed thiocyanate-containing *Brassica* plants may be ascribed to high sulfur content. Groundwater in many areas has a high content of calcium sulfate. The NRC recommended dietary sulfur level for ruminants is less than 0.3%; the maximum tolerated intake is 0.4%; water sulfate concentrations >2000 ppm are associated with the occurrence of PEM. The molecular pathogenesis of sulfur-related PEM is unclear, as is its relationship to thiamine activity and to the malacic lesions in the corpus striatum not seen in thiamine-responsive disease.

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Thiamine deficiency

Thiamine (vitamin B1) is a *dietary requirement of carnivores*. Herbivores are capable of synthesizing their own requirements, the synthesis being microbial and taking place in the rumen, and probably in the large intestine of horses. Experimental deficiency can be produced in calves and lambs, but only when they are very young and before they have established a useful ruminal flora; neurologic signs, including ataxia, cerebellar tremors, and convulsions, occur in these experimental deficiencies (see also Polioencephalomalacia of ruminants). Horses are poisoned by eating bracken fern and horsetail (*Equisetum arvense*), both of which plants contain a *thiaminase*. The plants are unpalatable, but poisoning may occur when they are included in pasture hays. The disease in horses is characterized by incoordination, which may be severe and lead to recumbency and bradycardia. Affected animals respond rapidly to thiamine. The nervous system has never been suitably examined for lesions.

Thiamine deficiency has been produced in most domestic species and in others. It has been an important natural disease in foxes and cats and continues to be of some importance in mink. In these carnivorous species, the deficiency is induced by a *thiamine-splitting enzyme naturally present in many species of fish*. Metabolic analogues of thiamine are known and have been used experimentally to produce the deficiency, but there are no known naturally occurring competitive analogues. Processed foods for dogs and cats can easily be made deficient in thiamine as the vitamin is susceptible to heating at 100°C or above in a medium of neutral or alkaline pH. The feeding to cats and dogs of meat that has been exposed to sulfur dioxide to preserve the fresh appearance can lead to thiamine deficiency; *thiamine is destroyed by sulfates.*

The syndrome, originally described as *Chastek paralysis*, produced by thiamine deficiency in foxes and mink is comparable to that in cats and may develop in 2–4 weeks of being fed the deficient diet. The onset of neurological signs is indicative of severe depletion of thiamine; the clinical and pathological consequences of marginal deficiency are not known. Initially, there is reluctance to eat, and drooling and anorexia may be present for up to one week before distinct clinical signs occur. Neurologic signs first consist of ataxia, incoordination, pupillary dilation, and sluggish pupillary reflexes. Convulsions are easily induced and are characterized by strong ventroflexion of the head. At this stage, animals usually respond rapidly and fully to thiamine, but there may be residual mild ataxia. After 2–3 days of neurological signs, animals pass into an irreversible phase of semicoma, opisthotonos, continual crying, and spasticity.

The *lesions of thiamine deficiency in carnivores* pass through the sequence of vacuolation of neuropil, vascular dilation, hemorrhage, and necrosis. These changes are occasionally observed in the middle laminae of the occipital and temporal cortex as poliomalacia, but *the areas of remarkable vulnerability are in the periventricular gray matter.* The periventricular lesions are always bilaterally symmetrical. The only consistency in the pattern of the lesions in the periventricular system is the involvement of the inferior colliculi (Fig. 3.78A). Next to the inferior colliculi, lesions are most commonly found in the medial vestibular, red and lateral geniculate nuclei, but they may be found in any of the periventricular nuclei. In contrast with Wernicke encephalopathy in man, with which the disease in carnivores can be compared, cats and foxes only irregularly develop hemorrhages in the mammillary bodies.

The initial morphological change is *vacuolation in nuclei of special susceptibility*. This is easiest to detect in the lateral geniculate bodies, inferior colliculi, and red nuclei (Fig. 3.78B, C, D). The vacuolation develops in cats at about the time they become susceptible to the induction of convulsions and at about the time that alterations in the permeability of the blood–brain barrier become demonstrable. This altered permeability, which may result from free-radical injury, is limited to those nuclear masses that are known to be vulnerable to injury in this deficiency. The case may not progress beyond this stage but, instead, pass to recovery. In the event of recovery, *intense astrogliosis* develops and remains to indicate the past presence of thiamine deficiency. With the development of vacuolation it is also possible to recognize vascular dilation especially affecting venules and especially in the susceptible nuclei. *Hemorrhage* occurs from both capillaries and venules, consistently in fatal cases. The hemorrhages may be large enough in the colliculi and vestibular nuclei to be visible to the naked eye (Fig. 3.78A, B).

Thiamine deficiency appears to affect most severely the relay systems from the eye and ear, but the histogenesis of the changes is vague. Phosphorylated thiamine is the coenzyme, *cocarboxylase*, and it participates probably in all oxidative decarboxylations as well as in some other metabolic transformations. It is a cofactor for transketolase, α-ketoglutarate dehydrogenase, pyruvate dehydrogenase, and branched-chain α-keto acid dehydrogenase. It may also have a function in axonal conduction and synaptic transmission. In all animals in which thiamine deficiency has been produced, the deficiency is attended by an *elevation of the pyruvate levels of blood*. This is to be anticipated from what is known of the function of the vitamin, but cannot be connected in any way to the nature of the nervous signs or the nature or distribution of the neural lesions. There is decreased glucose utilization by the brain and shortly before the development of lesions the vulnerable areas suffer a burst of metabolic activity with local production of lactate, and it is possible that the focal lesions, initially, are the result of focal lactic acidosis. The bradycardia is explainable on the basis of impaired carbohydrate metabolism because cardiac muscle depends for its energy supplies on carbohydrate, chiefly pyruvate, and in this differs from skeletal muscle. *Myocardial degeneration* is described in the experimental disease in several species and in the spontaneous disease in cats, dogs, and foxes; focal myocardial necroses are more prominent in the right than in the left ventricle.

Although it is not possible to correlate thiamine deficiency and its lesions with the distribution and activity of pyruvate decarboxylase, there is some correlation between thiamine deficiency and the distribution of transketolase activity in the nervous system. Transketolase is a thiamine pyrophosphate-dependent enzyme active in the hexose monophosphate shunt. The enzyme is active in white matter and apparently important in the metabolism of oligodendrocytes. During progressive thiamine depletion, brain transketolase activity declines before signs of the deficiency develop, and the decline is greater than for pyruvate decarboxylase. Moreover, the decline of brain transketolase is greatest in those areas in which lesions develop in rats.

The relation of transketolase deficiency to the evolution of lesions is unclear, as is the sequence of morphological changes. The spongy change appears to be a primary event, and vascular changes secondary. The hemorrhages are a terminal event. Fine-structural studies suggest that changes in vessels in selective areas of injury are secondary to degenerative changes in regional glia. The glia are edematous – swollen with clear cytoplasm – and their rupture leads to increase of extracellular spaces. Additional studies have shown early damage in the neuropil with degenerative and hypertrophic changes and axonal degeneration with preservation of neurons. Increased release of glutamate in vulnerable brain structures may result in N-methyl-D-aspartate receptor-mediated excitotoxicity; apoptotic cell death may result from increased expression of immediate early genes such as *c-fos* and *c-jun*.

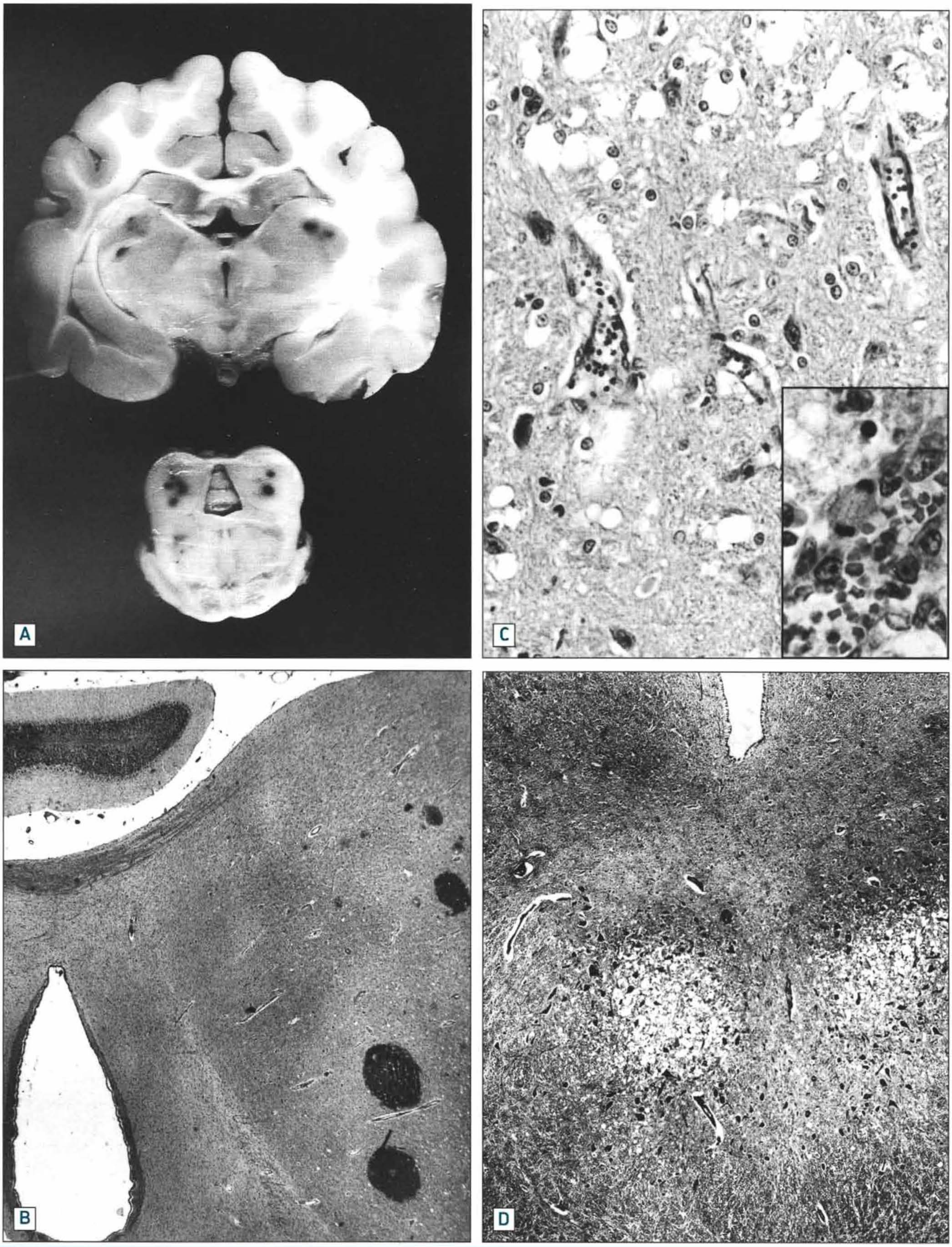

Figure 3.78 Thiamine deficiency in a cat. **A.** Hemorrhage in lateral geniculate bodies and inferior colliculi. **B.** Focal hemorrhages with edema in inferior colliculus. **C.** Edema, gliosis, and vascular reaction in inferior colliculus. Inset: mitosis in endothelial cell. **D.** Edema in oculomotor nuclei.

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Nigropallidal encephalomalacia of horses

Prolonged ingestion of yellow star thistle, *Centaurea solstitialis*, or Russian knapweed, *Centaurea repens*, produces encephalomalacia in horses. It is a disease of dry summer pastures in which the thistle, which remains green, provides most of the forage. The putative neurotoxin is *repin*, a principal sesquiterpene lactone present in aerial parts. Repin appears to cause glutathione depletion, which leads to oxidative damage, mitochondrial dysfunction, and neuronal cell death.

The onset of the disease is sudden and may occur as early as 1 month after initial exposure to star thistle. The syndrome is characterized by idle drowsiness, persistent chewing movements, and difficulty in prehension, the latter apparently related to incoordination of the lips and tongue. Sensation and reflexes are normal. Death is due to starvation, dehydration, or intercurrent disease. *Malacic lesions are consistently present in the brain and affect specifically the pallidus and substantia nigra.* There is some variation of the pattern depending on whether the lesions are symmetrical or not and involve both pallidus and substantia nigra or only one of the structures. Usually both structures are symmetrically involved. The pallidal foci involve the rostral portion of the globus pallidus, are lenticular in form, and 1–1.5 cm in size. The core of necrotic tissue in the substantia nigra is ~0.5 cm in diameter and extends from about the level of the mammillary bodies to the point of emergence of the oculomotor nerve (Fig. 3.79). The affected areas are evident as slightly bulging, yellow, gelatinous foci. The softening progresses rapidly, and in about 3 weeks the lesion is sharply demarcated as a *pseudocystic cavity*. The microscopic changes are those ordinary in malacia and do not give a clue to the immediate pathogenesis.

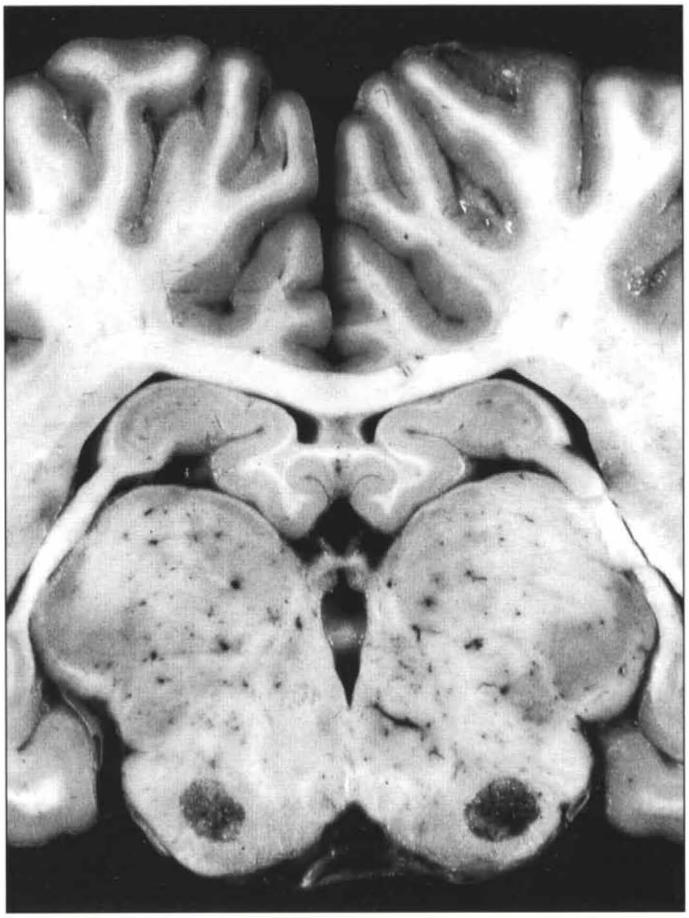

Figure 3.79 Bilaterally symmetric foci of malacia in substantia nigra in **nigropallidal encephalomalacia** in a horse.

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Salt (NaCl) poisoning

Salt poisoning may be a *direct and immediate* result of excessive ingestion of salt, or it may be *indirect and delayed*, developing only after several days of excessive salt intake and restricted water intake. These two types of salt poisoning are pathogenetically dissimilar, and neither will occur if animals are always provided with free access to water of low saline content.

Direct salt poisoning is largely a problem of salinity of drinking water. Excessive saline content of fodder is not a concern, except in terms of palatability, if an abundance of fresh water is available; for example, sheep may take rations containing up to 13% sodium chloride for prolonged periods without ill effects. This high tolerance for salt in fodder is utilized to restrict food intake during periods of scarcity and also to encourage water intake in the control of urolithiasis.

The salinity of drinking water is important, not only in terms of the concentration of sodium chloride or total salts, but also in terms of what salt and what acid radicals are present. It is difficult to provide figures for acceptable salinity of drinking water for livestock because of the variations in the proportions and concentrations of the various salts present, but the usual recommendation is that the concentration of sodium chloride or total salt should be <1.7% for sheep, <1.0% for cattle, and <0.9% for horses, and preferably not more than one half of these concentrations.

Acute, direct salt poisoning occurs chiefly in cattle, especially if they are very thirsty when first given access to saline water. Poisoning may also occur in cattle if they are given free access to salt supplements after a prolonged period of salt restriction such as occurs in cattle grazing on mountain pastures. *Clinical signs in cattle are referable to the alimentary tract and nervous system*, and include vomiting, polyuria, diarrhea, and abdominal pain with paresis, knuckling of the fetlocks, and blindness. Death may occur in 24 hours.

At autopsy, there is severe congestion of the mucosa of the abomasum and excessive, dark, fluid intestinal content. The alimentary changes are probably in large measure due to osmotic disturbances in the gut, but the neurological signs are unaccounted for. Also unexplained is the development of moderate anasarca in animals that survive for some days.

Indirect salt poisoning is a neurologic disease. There are circumstantial reasons for believing that it occurs in cattle and sheep and that it is occasionally responsible for PEM in these species, but *the disease is proven to occur only in swine*. Toxicity is related to the sodium ion; the clinical and pathological features of this disease are not duplicated in any other.

Apparent blindness and deafness initiate the clinical syndrome in indirect salt poisoning in swine. The animal is oblivious to its environment and cannot be provoked to squeal. There is head pressing suggestive of increased intracranial pressure, arching or pivoting, and these signs usually lead to the convulsive syndrome. The convulsions are very characteristic in their pattern and in the regularity of the time intervals in which they recur. They begin as tremors of the snout and rapidly extend as clonic spasms of the neck muscles with jerky opisthotonos that causes the pig to walk backwards and sit down. The animal passes into lateral recumbency and generalized clonic convulsions.

The lesions of this intoxication are restricted to the brain. There is moderate cerebral edema but no displacement. The basic changes in the brain are laminar loss of cortical neurons and laminar (middle laminae) malacia. These are the changes described earlier for PEM of cattle and sheep. The specificity of the lesion is given by the *abundance of eosinophils that are infiltrated into the meninges and Virchow–Robin spaces*. This particular lesion and its frequent relation to excessive intake of salt have been recognized for many decades, but until the cause was established, the syndrome was designated as "eosinophilic meningoencephalitis" on account of the relatively pure infiltrations of eosinophils that were found. Eosinophils tend to infiltrate in the meninges and perivascular spaces in the brains of pigs with other cerebral lesions, such as the leukomalacia of mulberry-heart disease and various encephalitides, but *the combination of laminar change and cerebral eosinophilia is pathognomonic of salt poisoning*.

The amount of salt in the diets of pigs varies considerably, but toxicity does not occur if plenty of water is available. The critical level of salt in the fodder is approximately 2% when water intake is restricted. Signs of poisoning may occur when the water supply is replenished after a period of restriction.

Although excessive intake of salt over a period of several days sets the stage, which is reflected in elevated plasma levels of sodium and chloride, the disease is precipitated by water so that it may equally well be regarded as a *form of water intoxication*. Beyond this, the pathogenesis of the lesions remains obscure. It is noteworthy that they are anoxic in character and distribution.

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Mycotoxic leukoencephalomalacia of horses

A neurological syndrome occurs in horses fed moldy corn for ~1 month or longer. The neurological signs are of fairly sudden onset and consist of drowsiness, impaired vision, partial or complete pharyngeal paralysis, weakness, staggering, and a tendency to circle. The course is from a very few hours to about a month, but death usually occurs on day 2 or 3. Recovery from clinical signs apparently does not occur; chronic cases with static signs are dummies.

The mycotoxin responsible is *fumonisin B1*, a product of *Fusarium verticillioides* (*Fusarium moniliforme*) and *F. proliferatum*, which grow on corn (maize) in warm moist conditions, circumstances in which outbreaks of the disease occur. Sporadic cases occur in circumstances involving moldy fodder. The neurological disease occurs in donkeys and mules as well as in horses.

The neurological signs described in the spontaneous disease are related to the encephalomalacia, which may be due to fumonisin-induced microcirculatory damage as well as impairment of cardiovascular function. In the experimental disease and a small proportion of spontaneous cases, nervous signs may include mania rather than neurological deficit; these dramatic cases may be icteric and the nervous signs are those usual in the horse in acute hepatic failure. In natural and experimental cases, there may be *hepatic lesions* varying greatly in severity. The hepatic lesions when present are similar to those produced in other species by aflatoxin (see Vol. 2, Liver and biliary system).

The lesion usually described is *necrosis of the white matter of the cerebral hemispheres* (Fig. 3.80). The surface of the brain may be unaltered on inspection, but palpable softness may be detected in the cortex overlying large areas of leukomalacia. Grossly, there is no cerebral edema or brain swelling, although the overlying gyri might be slightly flattened and discolored. The foci of softening may be of microscopic dimensions, but usually they are readily visible on the cut surface. The softenings occur at random in the white matter of the cerebral hemispheres. They may be bilateral but are not necessarily symmetrical. The malacic foci are soft, pulpy, gray depressions distinct by virtue of

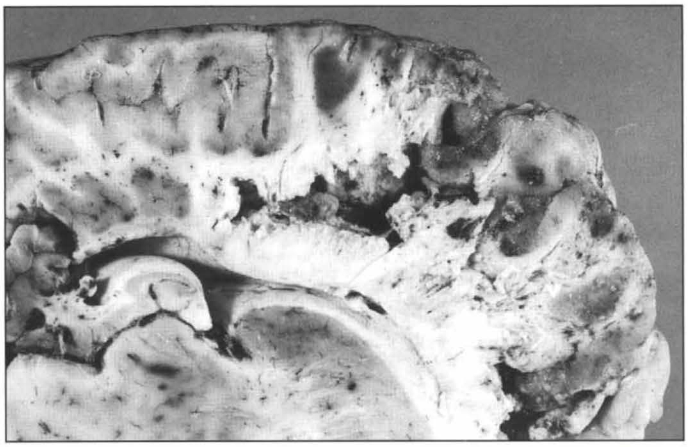

Figure 3.80 Irregular malacia and hemorrhage in cerebral hemisphere in a horse with **mycotoxic leukoencephalomalacia.** (Courtesy of TM Wilson.)

numerous small hemorrhages in a peripheral zone of a few millimeters' breadth. Depending on the duration, there may be diffuse, yellow, edematous swelling of the adjacent white matter.

In small foci, and probably initiating them all, there is severe edema of the white matter. The white matter is spread apart by fluid, and the myelin sheaths, axons, and glia disintegrate to form a structureless, acidophilic, semifluid mass to which the microglia react. The edematous change is not confined to the cerebrum but is reported to occur in all parts of the spinal cord. Foci of softening also occur in the brain stem and spinal cord, but here, in contrast with the brain, the necrosis affects the gray matter chiefly (Fig. 3.81).

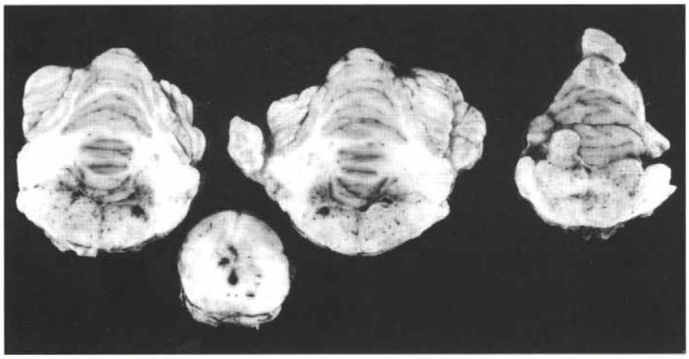

Figure 3.81 Focal hemorrhage and malacia in brain stem of a horse with **mycotoxic leukoencephalomalacia.** (Courtesy of TM Wilson.)

Microscopically, *the areas of malacia are widely distributed and irregular in their form*, mainly in the white matter but a few small foci may be seen in the cortex. The irregular cavitations tend to surround and follow the course of the blood vessels, and there is edematous separation of tissue extending from the periphery of the cavitations (Fig. 3.82A). Cellular infiltrations, mainly of eosinophils but with some plasma cells, are present in the walls of vessels, in the perivascular spaces, and lightly in the edematous parenchyma (Fig. 3.82B). The adventitia of the vessels, especially in the brain stem, may be remarkably thickened. There is abundant lipofuscin pigment in macrophages.

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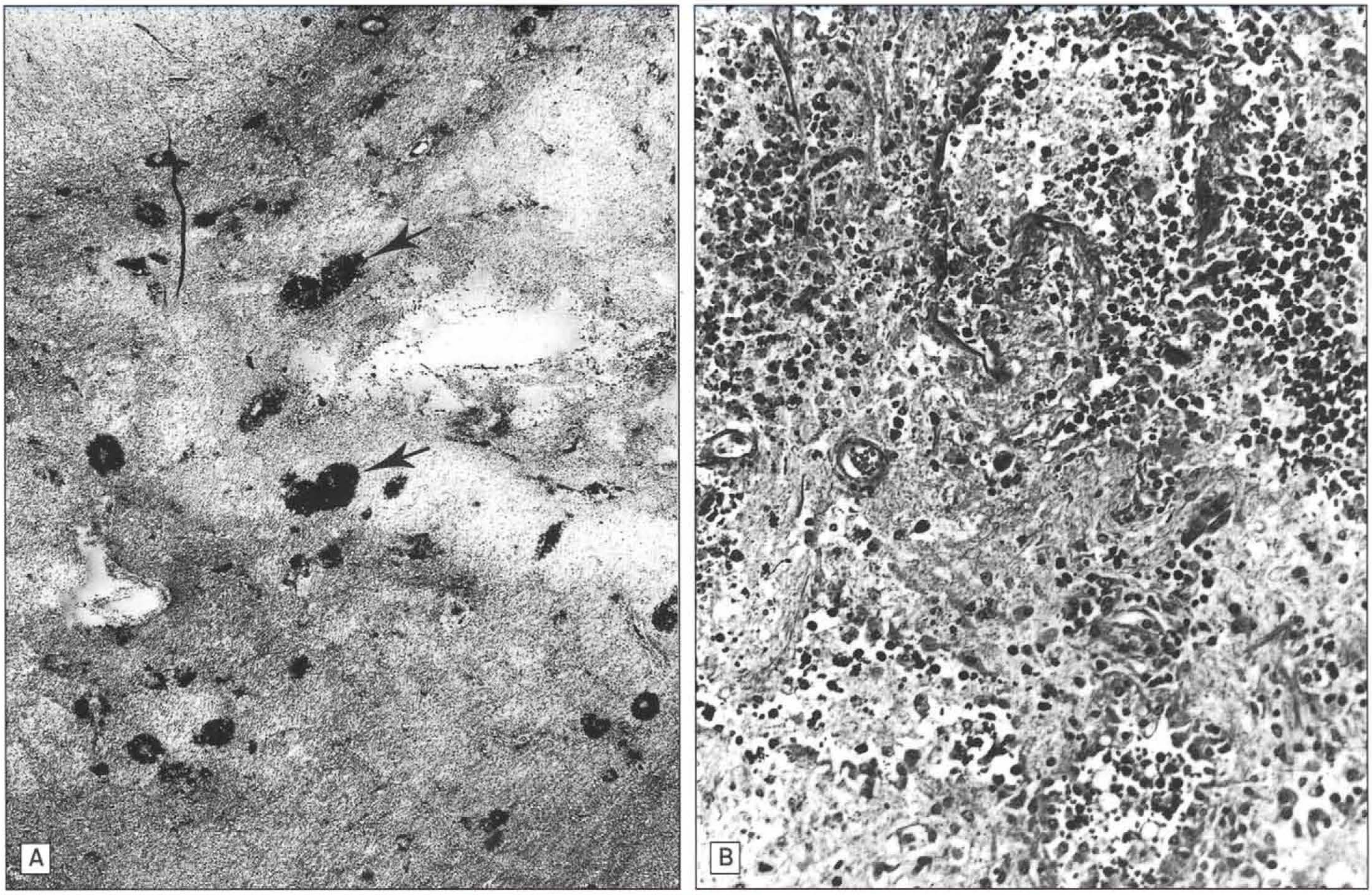

Figure 3.82 A. Malacia and hemorrhage (arrows) in a horse with **mycotoxic leukoencephalomalacia**; myelin sheath stain. (Courtesy of EW Hurst.) **B.** Vascular degeneration in malacic foci in a horse with **mycotoxic leukoencephalomalacia**. Infiltrating cells are eosinophils and hypertrophied microglia (compound granular corpuscles).

Lead poisoning

Lead is perhaps the most consistently important poison in farm animals. Poisoning is common and fatal in cattle, is less common but fatal in sheep, is occasionally observed in horses, dogs, and cats, and is rare in swine. The disease in cattle is probably always acute whereas that in horses is virtually always chronic. The signs are almost exclusively neurologic even though the amount of lead deposited in nervous tissue is relatively small.

The usual sources of lead for cattle are *paint* and *metallic lead in storage batteries*. Adult cattle are most frequently poisoned at pasture by licking paint or putty cans from rubbish dumps. Calves are usually poisoned when from boredom or allotriophagia they lick painted pens, troughs, etc. Metallic lead does not reliably produce poisoning in ruminants under experimental conditions but frequently does so naturally, perhaps because the metal, when well weathered, contains soluble salts on its surface. Lead, chronically ingested, does accumulate in the tissues of ruminants, but it is not a cumulative poison in these species. Under conditions of chronic assimilation in ruminants, large amounts of lead may be stored in tissues, including the brain, without causing lesions or clinical disease. Cows have been shown to tolerate 2 g of lead daily for as long as 2 years without apparent harm. Sheep may accumulate large amounts of lead in the course of grazing over abandoned lead-mining areas that have been converted to pasture; the severe osteoporosis that may develop cannot be attributed to the accumulated lead, but may be related to copper deficiency.

The situation in horses is largely the reverse of that in ruminants because in horses lead poisoning is usually chronic. This probably represents in part species susceptibility, but is in part due to the manner of exposure in that horses are usually poisoned by prolonged inhalation of fumes from lead smelters or by prolonged ingestion of pastures contaminated by such effluent; such pasture exposure of course also occurs in cattle. Contaminated pastures may contain as much as 100–200 mg Pb/kg foliage.

Lead poisoning may occur in circumstances additional to those cited above. Boiled linseed oil contains lead and it is occasionally mistakenly used as a laxative for animals. Dogs are occasionally poisoned by drinking gasoline that contains tetra-ethyl lead. Lead pipes may yield significant amounts of lead to soft water. Lead arsenate, widely used as an orchard spray, is commonly responsible for poisoning of cattle and sheep, but the signs and lesions refer largely to activity of the arsenate.

Lead is usually obtained by ingestion but only a small proportion of the ingested dose is absorbed, something of the order of 1–2% even of a soluble salt such as lead acetate. The limitation of absorption is due largely to formation of insoluble complexes in the alimentary tract. Absorbed lead is slowly excreted in bile, milk, and urine, and is deposited in tissues, especially in liver and kidneys in acute poisoning and in bones in chronic poisoning. The turnover of deposited lead is slow but continuous with gradual elimination in bile and urine; the half-life of lead in blood can range from months to years.

The *relative neurotropism of lead* is not well explained. Most metals exert their most deleterious effects at the sites of absorption and elimination, but lead largely spares these and affects nervous tissue with a high degree of specificity. Lead exerts toxicity by a number of mechanisms, including binding to calcium- and zinc-binding proteins, by random hydrolysis of nucleic acids, and by inducing RNA catalysis through activation of ribosomal 5S RNA, a natural leadzyme.

The various species differ in their sensitivity to lead, and the **clinical syndromes** produced also differ somewhat, although they are always chiefly neurologic. There seems to be remarkably little correlation between the doses of lead that will produce experimental poisoning and the very small amounts that induce toxicity naturally. Young animals are relatively more susceptible than adults.

The acute poisoning in *cattle* usually leads to death in 12–24 hours. Calves stagger, develop muscle tremors, and rapidly become recumbent. Convulsions are intermittent until death, and between convulsions there is opisthotonos, muscular tremors, champing of the jaws, and hyperesthesia to touch and sound. Adults show less tendency to early recumbency. In these there is frenzy, head pressing, and apparent blindness, with death in convulsions. When the poisoning is less acute, cattle may survive for 4–5 days. They are dull and apathetic, apparently blind, and without appetite. There may be drooling, intermittent grinding of teeth, and hyperesthesia, but dullness and immobility predominate. Ruminal agony is fairly constant and dark fetid feces may be passed terminally. Death occurs quietly or in convulsions or from misadventure. The manifestations of lead poisoning in sheep are similar to those of the subacute syndrome in cattle.

The disease in *horses* is paralytic. When horses ingest large amounts of lead, they develop severe depression and general paralysis, sometimes with clonic convulsions and abdominal pain. In chronic poisoning, usually known as *chronic plumbs*, the characteristic signs are those of specific nerve pareses affecting chiefly the cranial nerves and expressed particularly as laryngeal and pharyngeal paralysis. The clinical manifestations in dogs are also neurologic, but their pattern is not characteristic. There is anorexia, emaciation, mental irritability, muscular tremors, ataxia, and intermittent convulsions.

Specific **lesions** are not observed in lead poisoning in cattle. In cases that survive for 4–5 days, the ruminal contents may be foul because of immotility and the lower gut may contain a small volume of dark fetid feces, the color attributed to lead sulfide. There is no gastroenteritis and any clinical signs of colic are probably related to nervous dysfunction. There may be mild degenerative changes in the parenchymatous organs, but these cannot be attributed to lead.

There is moderate brain swelling, but probably never severe enough to cause displacement and not often severe enough to be appreciated grossly. Even microscopically, the cerebral edema is not easy to appreciate or to distinguish from early autolytic change. The capillaries and venules are congested and there may be petechial hemorrhages. The prominence of capillaries in the gray matter is due largely to congestion, but there may be some endothelial swelling and proliferation to make them more conspicuous. Neuronal changes are equivocal. In some subacute cases in which the course is prolonged for several days, there may be *laminar cortical necrosis*. The best explanations of laminar necrosis are still based on ischemia-anoxia, implying that lead is not directly neurotoxic in terms of neuronal injury. Indeed, lead tends not to accumulate in nervous tissue. The capillary alterations may therefore be of considerable functional importance and probably precede the swelling of glia. The capillary changes are best seen in thick sections stained by a Nissl stain and in the cerebellum better than in the cerebrum. In the cerebellum, the vascular lesion in the molecular and Purkinje layer is often accompanied by astro- and microgliosis.

The histological basis of the paralytic changes in lead poisoning in horses probably has the same basis as the peripheral neuropathy of chronic plumbs in man, in which the paralysis affects those muscles that are used most constantly and is due to segmental degeneration of axons and myelin in the distal parts of motor fibers. In humans, the peripheral neuropathy of lead poisoning is purely motor.

In dogs, there is edema of the white matter of the brain and cord. Degenerative changes in myelin sheaths occur almost universally but are somewhat less severe in heavily myelinated tracts such as the optic tract, corpus callosum, and peduncles than in lightly myelinated areas, such as the deep white matter of the cerebellum and cerebrum. There is spongy degeneration in the subthalamus, head of the caudate nucleus, and deep cortical laminae with extensive loss of neurons in such areas. Reaction on the part of astrocytes and microglia is slight.

Mild degenerative changes occur in the liver and kidneys of dogs chronically poisoned with lead, and can be overlooked in routine examinations. Most conspicuous is enlargement and vesiculation of the nuclei in the convoluted tubules. *Irregular, acid-fast, intranuclear inclusion bodies can be found in the renal tubules in some cases.* These inclusions are irregularly present in cases of lead poisoning, but when present, they have diagnostic value. They may develop in any species poisoned, but in dogs, they must be distinguished from the brick-like, acidophilic inclusions that can be present in the nuclei of renal tubules and hepatic cells. These inclusions are also acid-fast, but have no specificity for lead poisoning and are present in the nuclei of apparently healthy dogs. In cattle, the degree of tubular epithelial degeneration is slight in most cases, but there is often a surprising degree of mitotic activity and there may be severe nephrosis with extensive fibrosis in young calves.

The **diagnosis** of lead poisoning is necessarily chemical because lesions are either absent or nonspecific. Because of the variability of pathogenetic factors and species susceptibility discussed above, precise figures cannot be given for the concentration of lead in tissues that can be regarded as indicating lead poisoning. As little as 4–7 μg lead/g liver has been found in horses dying of chronic intoxication. In cattle, levels of lead (on a wet weight basis) of 40 μg/g or more in the kidney and 10 μg/g or more in the liver are accepted as confirmatory of lead poisoning.

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Neurodegenerative diseases

There are some significant neurologic diseases in which dramatic clinical disturbances are not matched by equivalent morphologic alteration in nervous tissue. For instance, *toxins that interfere with synaptic function can have fatal consequences,* yet leave neurons normal in appearance to routine examination. **Botulism, tetanus**, and **strychnine toxicosis** are well-known examples. In the latter two conditions, the release of spinal motor neurons from the inhibitory influence of "Renshaw cells" (inhibitory neurons) leads to the extensor spasms and hyperesthesia that characterize them. The activity of the inhibitory neurotransmitter glycine is blocked – in the case of tetanus by pre-synaptic blockade of its release, in the case of strychnine by post-synaptic blockade of its receptors. An inborn metabolic equivalent of these poisonings is *hereditary myoclonus* of Poll Hereford calves and of Peruvian Paso horses, in which there is an absence of glycine receptors on spinal motor neurons. In the Hereford disease, the calves are born normally, but suffer severe tetanic spasms on stimulation, are unable to stand, and are nonviable.

A distinctive locomotor disturbance in Australian sheep (Coonabarabran staggers, "cathead" staggers) has been associated with grazing of the zygophyllaceous plant *Tribulus terrestris*. The clinical disease is characterized by asymmetric atrophy of pelvic limb extensor muscles, and irreversible asymmetric para- or tetraparesis without ataxia. There are no significant structural lesions in the nervous system. Evidence has been presented to suggest that the syndrome is caused by β-carboline alkaloids acting on dopaminergic upper motor neurons in the nigrostriatum. The long-term nature of the deficits may be the result of the ability of such compounds to form adducts with DNA sequences of genes associated with the synthesis of dopamine. Locomotor disturbances have also been documented in sheep and cattle grazing other zygophyllaceous plants in North America and Africa.

A large number of plant intoxications cause acute neurologic disease without morphologic alterations; **tremorgenic mycotoxicoses** are discussed below.

Inherited **spasticity syndromes** are described in cattle as "spastic paresis" and "spastic syndrome" and in Scottish Terriers as "Scotty cramp." Various inherited **myotonic syndromes** are referred to in Vol. 1, Muscle and tendon. The common **tetanic/paretic syndromes** that accompany hypocalcemia and hypomagnesemia are not associated with significant neural lesions.

This section will deal with noninflammatory diseases in which there is selective neuronal degeneration, involving either neurons in their entirety (**neuronopathies**) or restricted to axons (**axonopathies**). In reality, this division is somewhat arbitrary, as the axon is a wholly dependent part of any neuron, but the concept is useful for the classification of various diseases.

In general, when entire neurons or just their axons are lost in these circumstances, there are reactions by adjacent tissue elements but, as there is often minimal tissue loss overall, the predominant pathologic change is microscopic. At the end-stage of severe chronic diseases, macroscopic atrophy may become evident (see Cytopathology of nervous tissue).

The diseases have been grouped into *three categories of neuronopathies and axonopathies: "central," "peripheral," and "central and peripheral,"* according to the distribution of the lesions in the central and peripheral nervous systems. The choice of category for a particular disease is based on the predominant pattern of morphological change seen in a typical case, and the criteria are not absolute. There will inevitably be some overlap and the naming and lesion topography of each may not necessarily be fully justified or complete. Some of the diseases are rare, and, because they are inherited, are highlighted by research workers aiming to use them as experimental model systems. New entities are progressively being identified. Sampling of the nervous system in diseases such as these should be as comprehensive as possible. The pattern and extent of lesions are important criteria in the diagnosis of known diseases, and in the documentation of new ones.

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Central neuronopathies and axonopathies

Compressive optic neuropathy

Compression of optic nerves in the optic foraminae is a well-known manifestation of vitamin A deficiency in the calf and pig due to stenosis of the foraminae. In poisoning by plants of the genera *Stypandra* and *Helichrysum* and by halogenated salicylanilides, the foraminae are of normal size.

These conditions are discussed fully under myelinopathies, but one usual feature of their pathology is *intense Wallerian degeneration of the optic nerves and tracts*, which may be due to swelling and compression of the nerves within the optic canals. The acute phase of the lesion would be expected to show severe ischemic necro-degenerative changes involving all the nervous tissue elements localized within the optic canals, with acute axonal degeneration extending along the optic nerves, through the chiasm and into the optic tracts of the midbrain. Retrobulbar segments of the optic nerves should be minimally affected. With the passage of time, the site of compression should exhibit malacic alterations, gitter-cell infiltration and astrogliosis, while the progression of Wallerian changes in the optic nerves and tracts should lead to extensive loss of myelinated axons and oligodendrocytes, with reactive astrogliosis. In the long term, retrograde degenerative changes lead to the loss of retinal ganglion cells and their axons, retinal atrophy, and gliosis.

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Organomercurial poisoning

Mercurial compounds are cumulative poisons. The syndromes produced by the organic and inorganic salts are quite different, but the differences are probably not qualitative.

The toxicity of the organic salts depends on their solubility and the syndrome produced depends on the size and chronicity of dosage. Poisoning by inorganic salts is expected to be by ingestion, but percutaneous absorption is possible. *Poisoning by inorganic mercury is quite rare in animals.* Acute toxicity following ingestion is characterized by severe abdominal pain, vomiting, and diarrhea caused by the coagulative effect of mercury in the lining of the gut. Death may occur in a few hours. If the animal survives the initial episode, death may occur several days later from nephrosis with uremia. There may at this stage be ulcerative colitis due to the concentration of mercury in the colonic mucosa by which route it is in part eliminated. Chronic cumulative poisoning probably does not occur in animals.

Mercurialism in animals is usually caused by the organic salts, such as ethyl mercury phosphate and mercury p-toluene sulfonanilide, which are applied to seed grains for the purpose of controlling fungal diseases of the germinating plants. Toxicity may not be manifested if the treated grain is consumed for a short period only. Chronic mercurialism occurs chiefly in swine and is occasionally observed in cattle, but is most unusual in other domestic species. The manifestations are almost solely neurological. Mechanisms of organomercurial toxicity include inhibition of protein synthesis, disruption of microtubules, disturbance of neurotransmitter function, oxidative stress, and triggering of excitotoxicity mechanisms.

"*Minamata disease*" is a mercurialism of cats, birds, and man in the Minamata Bay area of Japan, associated with the eating of fish and shellfish from the bay. The fish contain large amounts of organomercurials, which probably spilled into the bay with industrial effluent. Similar environmental disease occurs in other countries.

The **clinical syndrome** of mercurialism in cattle closely resembles that of lead poisoning, but the signs, which are of sudden onset, may not occur for several weeks after the first of continuous exposures. Because degeneration of the conducting system of the heart is common, cardiac irregularities are to be expected. Signs may occur in swine as early as 15 days after being fed treated grain. There is loss of appetite, wasting, dullness, blindness, severe weakness, and incoordination, progressing rapidly until the animal can no longer stand. The dullness passes to coma, which may last for several days with intermittent episodes of clonic-tonic convulsions. Both swine and cattle may be moderately uremic.

Gross **lesions** are minimal. In both swine and cattle, the kidneys may be moderately swollen, pale, and wet, and in pigs there is often hydropericardium. Poisoned cattle have mild to moderate cerebral swelling and displacement. The cerebral cortices are soft and pale, and occasionally there is juxtasagittal venous infarction. The pallor of the cortex is visible on the cut surface so that there may be no clear distinction between gray and white matter; this change is irregular in distribution. Where the gray matter is distinct from the white, a narrow pallid band separating them may be visible. In swine, the dead white color of the cortex is often striking, especially when it is emphasized by the ischemia that may be present. The appearance of the two hemispheres may be different, that uppermost being pallid and ischemic and that of the side on which the animal is lying having congested meningeal veins. The brain is swollen and the gyri are flattened, although this is difficult to appreciate in pigs in which swelling is never severe.

Microscopically, there is *nephrosis* of mild to moderate degree usually without clear evidence of significant tubular epithelial necrosis although this is dependent on dosage. The tubular epithelium is swollen to such an extent that it may obliterate the tubular lumen. There is microscopic proteinuria. *Degeneration of the Purkinje network in the heart* is observed fairly consistently in cattle, but it may

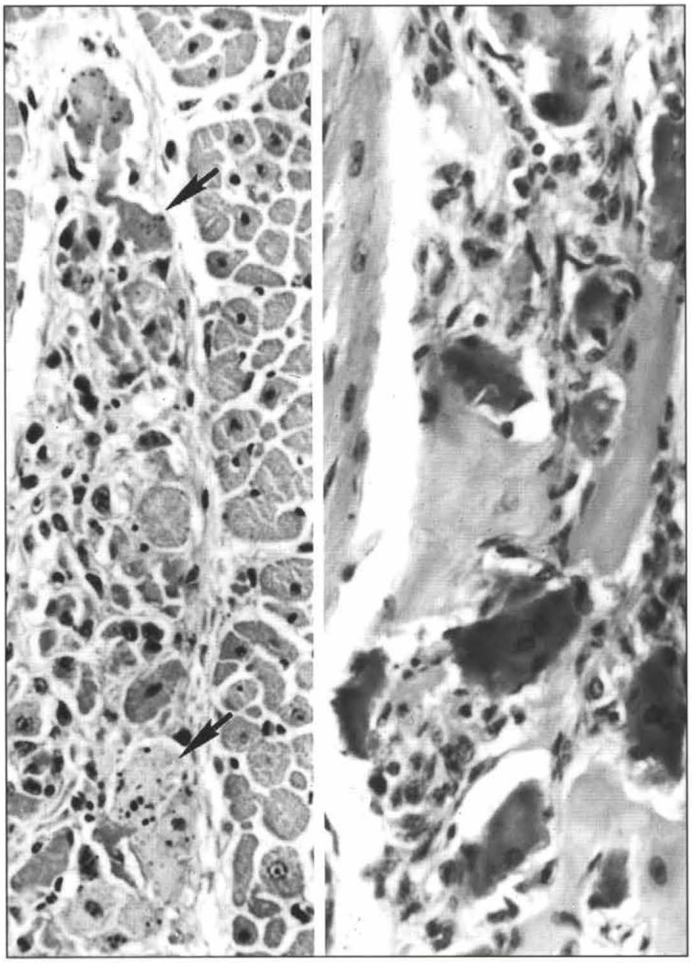

Figure 3.83 Organomercurial poisoning in an ox. Degeneration of Purkinje network in myocardium (left, arrows). Fibrosis and mineralization of Purkinje network (right).

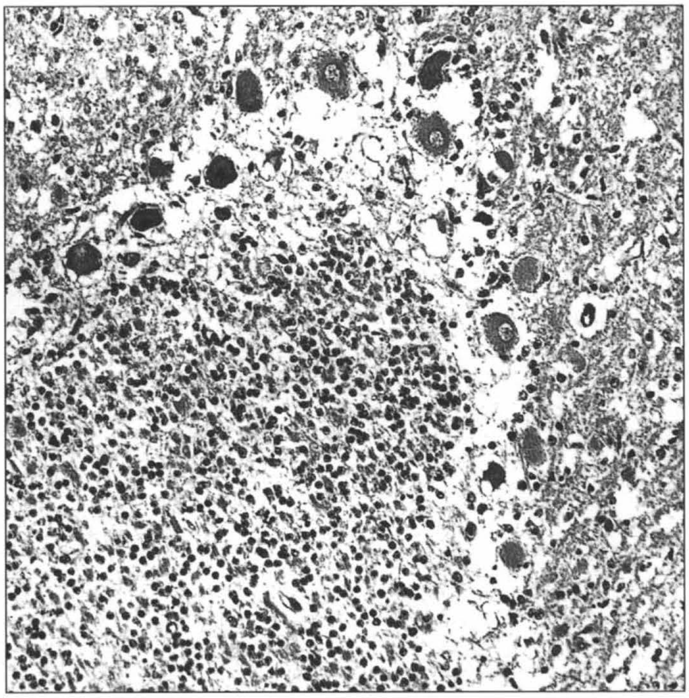

Figure 3.84 Organomercurial poisoning in an ox. Purkinje cell necrosis and degeneration in granular layer of cerebellar cortex.

not be present when the course of the toxicity is short and seems not to occur in swine. There is acidophilic, coagulative necrosis, irregularly but extensively distributed in the Purkinje network (Fig. 3.83A). The degenerate substance fragments and is removed by histiocytes and replaced by connective tissue. Mineralization is common (Fig. 3.83B). There are no clearly demonstrable degenerative changes in the myocardium.

Degenerative changes are consistently present throughout the nervous system in both cattle and swine. They differ quantitatively from case to case but are qualitatively the same. The lesions are rather more rapidly catastrophic in cattle than in swine. There is *acute neuronal degeneration*. This is of ischemic type and largely of ischemic distribution, the neurons of the middle laminae of the cerebral cortex being extensively injured. However, shrunken, acidophilic neurons can be found at all levels of the brain and cord and, with time, there is extensive cell loss from all cortical laminae. Moderate gliosis accompanies the neuronal degeneration and is expected to be most prominent in those areas of the cortical gray matter that, on gross inspection of the cut surface, are pallid. The *gliosis* is in part astrocytic and in part microglial. Compound granular corpuscles are not formed, the reactive microglia being prominently rod-shaped. There is edema in the subjacent white matter. In swine, the edema leads in time to demyelination, but in this species there is no softening. In cattle, the edema of white matter is more severe and, in the multiform layer and middle laminae, frequently produces a spongy degeneration, which may be visible grossly. This occasionally leads to laminar necrosis in cattle.

In both cattle and swine, *fibrinoid necrosis of the media of leptomeningeal arteries* is quite characteristic (see Fig. 3.56). The change probably occurs in all cases, affecting both cerebral and spinal vessels, but it is well marked in a few cases only. Swine are more prone to the vascular lesions than are cattle. Accompanying the vascular lesions, there is an outpouring of much fibrin into the meningeal spaces.

Organomercurials appear rather selectively to damage the *granule cells of the cerebellum* in all species (Fig. 3.84). Cattle are more sensitive to this degeneration than are swine. In swine, the Purkinje cells remain fairly intact, but in cattle they are often lost from about the tips of the folia. The degeneration of Purkinje cells is accompanied by microgliosis in the molecular layer and may be a response to swelling and pressure rather than a direct response to mercury.

Perhaps because the course is usually short in cattle, the spinal neurons are only mildly injured. In swine, there is often extensive ischemic necrosis of individual relay neurons. This, together with peripheral neuropathy, accounts for paralytic phenomena in swine. There is axonal degeneration and demyelination in peripheral nerves, the degenerative changes being more severe when the course is prolonged.

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Solanum poisoning

Several of the many hundreds of species of the plant genus *Solanum* (family Solanaceae; nightshade family) are toxic to livestock by a variety of means. The disease of cattle in southern Africa known colloquially as *maldronksiekte* is associated with grazing *Solanum kwebense*. Affected animals show little abnormality at rest, but when moved exhibit head tilt, muscle tremors, incoordination, and convulsions.

The brain at necropsy may show gross evidence of cerebellar atrophy, uniformly affecting all lobes. Histologically there is diffuse loss of Purkinje cells, gliosis in the molecular layer, and atrophy of both molecular and granular layers. Surviving Purkinje cells are swollen, with strongly acidophilic cytoplasm, which contains numerous small "empty" vacuoles. Neurons elsewhere in the brain may show similar but far less intense vacuolation. Morphologically the vacuolation is suggestive of a lysosomal storage process and, ultrastructurally, there are membranous cytoplasmic inclusions similar to those seen in the sphingolipidoses. The nature of any stored material has not been reported to date.

Similar signs and lesions in cattle have been associated with the grazing of *S. bonariensis* in Uruguay, *S. dimidiatum* in the USA, and *S. fastigiatum* in Brazil, and in goats grazing *Solanum cinereum* in Australia.

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Cycad poisoning

The primitive palm-like plants of this family (genera *Cycas, Zamia, Bowenia, Macrozamia*) occur in tropical and subtropical environments, and have been associated with the intoxication of both people and animals. Cycad toxicity is proposed as a cause of the amyotrophic lateral sclerosis–parkinsonism–dementia complex in humans. The seeds and young fronds of cycads contain the toxic glycosides, *cycasin* and *macrozamin*, and, following ingestion, the hepatic metabolism of the aglycone, *methylazoxymethane*, may cause neuronal apoptosis and acute zonal hepatic necrosis.

In tropical Australasia, chronic exposure of cattle is associated with a neurological syndrome known as "*Zamia staggers*." The syndrome is characterized by pelvic limb ataxia that may become severe. Pathologically there is distal axonopathy in the spinal cord. The ascending fasciculus gracilis and dorsal spinocerebellar tracts have axonal degeneration most intense in the cervical segments, while the same is true of descending ventrolateral tracts (Fig. 3.85A, B, C). Typical Wallerian changes lead eventually to loss of myelinated axons and reactive fibrous astrogliosis.

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Tremorgenic neuromycotoxicoses

Perennial rye-grass staggers is a common mycotoxicosis in parts of Australia, New Zealand, and Europe, and is occasionally reported in the USA and southern Africa. Sheep, cattle, and horses may be affected. It occurs in the summer and autumn on dry, short pastures of *Lolium perenne*, and clinical signs appear 5–10 days following exposure. Animals develop fine head tremors and head nodding and weaving at rest and, if forced to move, have a stiff-legged, incoordinate gait, and are inclined to collapse in tetanic spasms, from which they recover quickly. There is low mortality, and total recovery will take place within 3 weeks of removal from toxic pasture. The disease is a due to indolic *lolitrems* produced by the endophytic fungus *Neotyphodium (Acremonium) lolii*. The toxins are known collectively as *tremorgens*.

There are no gross lesions in lolitrem toxicosis and microscopic change is limited to the occurrence of fusiform enlargement of the proximal axons of some cerebellar Purkinje cells. These axonal swellings are known as *torpedoes* and represent a proximal axonopathy, but their relationship to the clinical signs is unclear, and they have not been reported in any other tremorgenic diseases. The axonal lesions are best demonstrated by the use of silver staining techniques, such as the Holmes method.

Other tremorgenic neuromycotoxicoses are described in which lesions may be absent. *Claviceps paspali*, the ergot of *Paspalum*, produces tremorgenic paspalitrems that cause intoxication in cattle, occasionally in sheep and rarely in horses. Tremorgenic mycotoxin intoxication occurs in dogs from penitrem A or roquefortine produced by *Penicillium* spp. and ingested in moldy food, and in horses consuming mycotoxins in dallis grass.

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Equine degenerative myeloencephalopathy

Equine degenerative myeloencephalopathy (EDM) is a *chronically progressive syndrome of symmetrical ataxia of unknown cause in equids* of several breeds and strains, including zebras. Predisposing factors include *vitamin E deficiency, hereditary predisposition*, inadequate exposure to green pasture, and possible exposure to wood preservatives or insecticides. A role for copper deficiency has not been substantiated. A familial hereditary pattern is recognized for Appaloosa, Standardbred, and Paso Fino horses with EDM.

The average age of horses at initial presentation is about 6 months, but may be as much as 24 months. The clinical presentation is

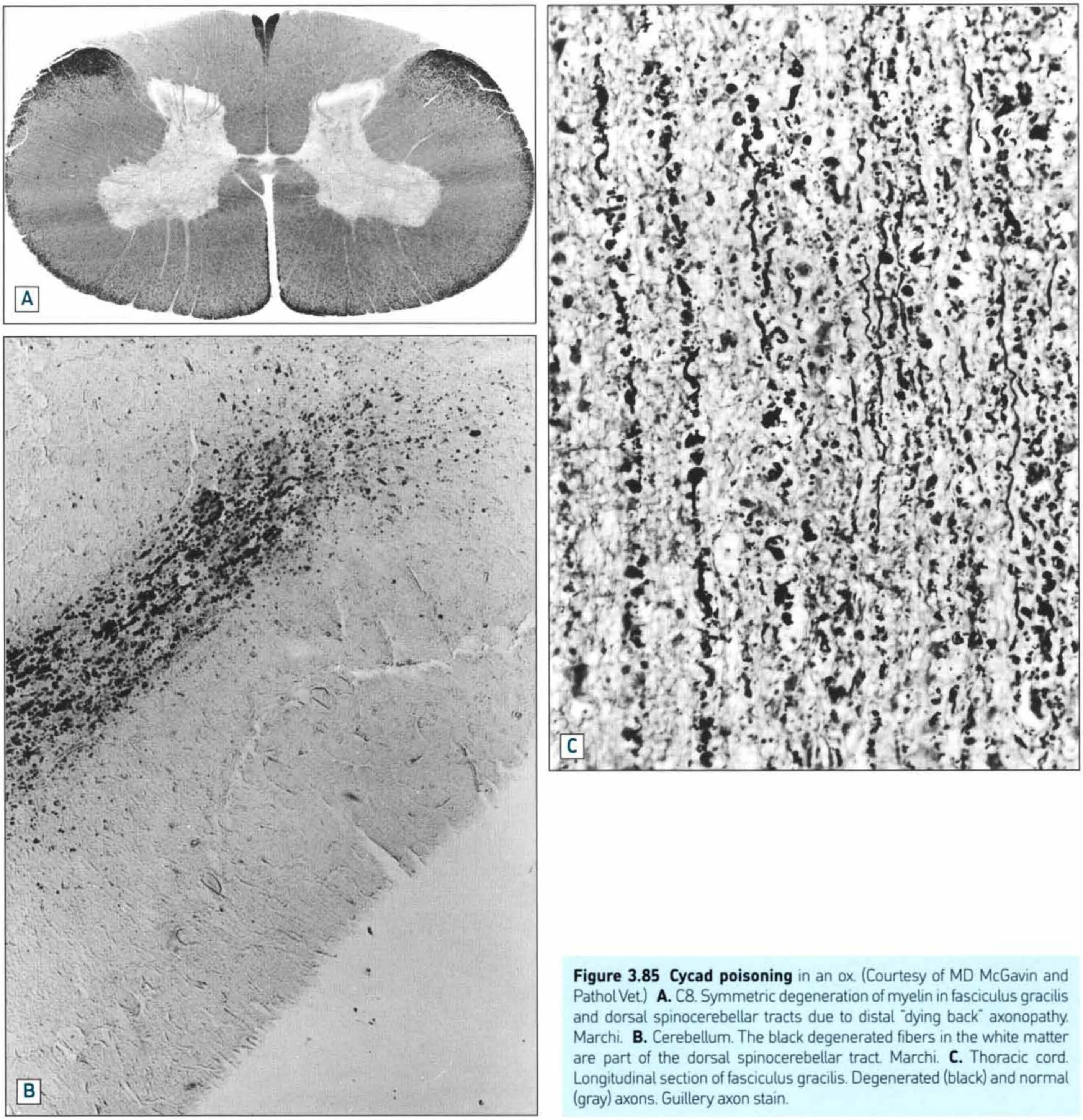

Figure 3.85 Cycad poisoning in an ox. (Courtesy of MD McGavin and Pathol Vet.) **A.** C8. Symmetric degeneration of myelin in fasciculus gracilis and dorsal spinocerebellar tracts due to distal "dying back" axonopathy. Marchi. **B.** Cerebellum. The black degenerated fibers in the white matter are part of the dorsal spinocerebellar tract. Marchi. **C.** Thoracic cord. Longitudinal section of fasciculus gracilis. Degenerated (black) and normal (gray) axons. Guillery axon stain.

dominated by disturbances of general proprioception and upper motor neuron function referable to the spinal cord, and thus closely resembles focal compressive myelopathy of the mid-cervical region. However, no skeletal or other gross lesions can be demonstrated.

Pathologically, there is evidence of *ongoing Wallerian degeneration and post-Wallerian astrogliosis in all funiculi throughout the spinal cord*, but concentrated in the ascending dorsolateral (spinocerebellar) and descending ventromedial (motor) funiculi of the cranial cervical and mid-thoracic segments. The changes are often most severe in the thoracic segments. Similar but mild changes are present in myelinated tracts of the caudal medulla and caudal cerebellar peduncles.

When well established in the thoracic cord segments, the process leads to considerable loss of myelinated axons and dense reactive astrogliosis that also involves the adjacent glia limitans. In some cases, the destructive process seems to reach an endpoint, with dense gliosis but little or no active Wallerian change. Chromatolytic and necrotic neurons, and axonal spheroids, can be found in Clarke's column (the nucleus of the dorsal spinocerebellar tract), which is located just dorsolateral and immediately adjacent to the central canal of the cord between the first thoracic and mid-lumbar segments. The disease involves total destruction of many neurons in this system, and macrophages containing a lipoidal pigment can also be found in this location. Disruption of axonal transport likely plays a crucial role in the pathogenesis of dystrophic axons in EDM.

The origin of the degenerating descending axons in the ventromedial tracts is uncertain, and there have been no reports of neuronal degeneration in the midbrain nuclei likely to be the source of these fibers. It is possible that the descending tract lesion is purely an axonopathy. Axonal spheroids and vacuoles have also been described in the lateral cuneate and gracilis nuclei of the caudal brain stem. These are the relay nuclei receiving the long ascending proprioceptive tracts of the spinal cord. However, even though this disease occurs in young horses, the changes in these nuclei may be nonspecific as they are commonly seen in a variety of situations, including animals with no overt clinical neurologic disease. In contrast to compressive cervical myelopathy (the wobbler syndrome), *there is simultaneous involvement in multiple cord segments of ascending* **and** *descending tracts*. This excludes focal compression of the cord as an etiologic factor. There is no obvious pathogenetic link between the various neuronal systems involved.

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Axonal dystrophies

Diseases in which the pathology is characterized by axonal dystrophy, as described under cytopathology (see Figs 3.9, 3.10), have been described in sheep, horses, dogs, and cats.

One of the earliest axonal dystrophies documented occurred in **Suffolk sheep**. In this disease, described in California, lambs normal at birth develop progressive ataxia at 1–6 months of age. The lambs eventually become recumbent after a course of 10–12 weeks. It is virtually certain that the disease is inherited and appears to be a recessive trait. There are no specific gross changes, and *the diagnostic histologic lesion is the presence of numerous axonal spheroids*, in and adjacent to several gray matter areas. The consistently affected areas are as follows: the entire spinal gray matter, with spheroids in greatest numbers at the base of the dorsal horns, in Clarke's column and in the intermediolateral nuclei; the caudal brain-stem nuclei, gracilis, cuneate, accessory cuneate, inferior olivary, lateral reticular and lateral vestibular; the cerebellar roof nuclei with the exception of the dentate; and the rostral colliculi and lateral geniculate nuclei. The spheroids are the focally swollen terminations of sensory axons, and represent a genuine axonal dystrophy. The pattern of lesions indicates the involvement of two sensory systems, one visual and the other proprioceptive and possibly exteroceptive, and the clinical signs could be accounted for by perturbation of the latter.

An identical condition has been described in **Coopworth, Romney, and Perendale sheep** in New Zealand, while in Australia a disease in **Merino sheep** has been reported as an axonopathy, although the lesions described are consistent with axonal dystrophy. In this disease, as reported from localized areas of eastern Australia, sheep aged 1–4 years develop severe caudal gait abnormalities that are progressive and irreversible. Axonal spheroids are present in large numbers, predominantly in myelinated tracts in the mid- and hindbrain and throughout the spinal cord, being more abundant in the dorsal funiculi than the ventral. Wallerian changes are mild. In the brain, areas of predilection are the cerebellar peduncles, transverse pontocerebellar fibers, dorsolateral thalamic tracts, cuneate fasciculus, median longitudinal fasciculus and corticospinal tracts. Rather than being concentrated at terminal regions, the spheroids appear to be multiple along the course of individual axons. The cause remains undefined but seems likely to be a heritable defect.

In horses, neuroaxonal dystrophy is thought by some investigators to be a localized form of equine degenerative myeloencephalopathy. Affected breeds include Morgans, Haflingers, and Quarter Horses. In the **Morgan horse**, a clinical syndrome of pelvic limb dysmetria and incoordination is accompanied by intense axonal dystrophy in the accessory cuneate nuclei. Breeding experiments suggest a familial component, but no definitive inheritance pattern has been demonstrated.

In **Haflinger horses**, a report describes an ataxia syndrome first evident at about 4 months of age, with ataxia in the pelvic limbs more severe by about 2 years of age. A familial hereditary basis is proposed. Neuroaxonal dystrophy was evident as numerous spheroids in the nuclei gracilis, cuneate, solitary tract and intermediomedialis, and in Clarke's column. These changes were accompanied by astrogliosis and lipofuscin pigmentation of neurons and macrophages, and significantly reduced serum tocopherol values.

Canine diseases classified as axonal dystrophies have been described in a growing list of breeds, including Rottweilers, Collie sheepdogs, Chihuahuas, English Cocker Spaniels, Jack Russell Terriers, and Papillons.

In the **Rottweiler**, neuroaxonal dystrophy is a *familial progressive sensory ataxia*, with a pattern of occurrence suggestive of autosomal recessive inheritance. Abnormal expression of various proteins leads to severe disruption of axonal transport in dystrophic axons. Neurologic signs may be expressed before 12 months of age, often being first noticed as abnormal clumsiness. With time, there is steadily progressing ataxia and distinct hypermetria, particularly in the forelimbs, in which there is also toe-dragging and knuckling. Mild head tremor and incoordination may also become evident. By 4–6 years of age, nystagmus, crossed-extensor reflexes, and a positive Babinski sign may be present, but dogs remain alert and responsive. Strength and conscious proprioception are maintained throughout, and the signs are related mainly to a disturbance of unconscious proprioceptive input to the cerebellum.

At necropsy there may be mild patchy cerebellar atrophy, and the optic nerves appear small. The characteristic histologic feature is the presence of *massive numbers of axonal spheroids* (see Fig. 3.9A, B) in the nerve root entry zone of the dorsal horn throughout the spinal cord, in the vestibular, lateral, and medial geniculate, and sensory trigeminal nuclei, and in Rexed's laminae of the spinal cord. Fewer spheroids are present in the inferior olivary, trochlear, and oculomotor nuclei, and in the spinal cord ventral horn. Occasional spheroids are found in the globus pallidus, hippocampus, thalamus, hypothalamus, caudate nucleus, and reticular substance. In the cerebellum, lesions are concentrated in the vermis. Spheroids are present in the granular layer, white matter, and roof nuclei. There is some loss of Purkinje and granule cells.

Affected **Collie sheepdogs** have progressive ataxia and gait abnormalities first apparent at 2–4 months of age. The disease is strongly suspected to be inherited as an autosomal recessive trait. The lesions are purely microscopic and confined to the deep cerebellar and vestibular areas. Many axonal spheroids are present in the central cerebellar white matter and adjacent roof nuclei, and in the lateral vestibular nuclei. Wallerian degeneration is minimal and the cerebellar cortex unaffected.

In the **Chihuahua**, affected animals are normal until about 7 weeks of age, when there is sudden onset of tremor and gait disturbances. The pathogenesis remains undefined. Large numbers of axonal spheroids are present in the white matter of the internal capsule, cerebellum, lateral geniculate nucleus, rostrodorsal thalamus, acoustic tubercle, olivary nuclei, and the corticospinal and spinothalamic tracts.

In the **cat**, several axonal dystrophies have been reported. A GABAergic neuroaxonal dystrophy occurs in *feline Niemann–Pick disease type C*, an autosomal recessive lysosomal storage disease. An *autosomal recessive hereditary condition* has been as described in domestic shorthaired cats in association with an unusual lilac coat color. Clinical signs first become evident at 5–6 weeks of age as head bobbing. In the ensuing 8–10 weeks, there is progressively worsening ataxia, and possibly visual impairment and vestibular deficits. The process may then stabilize, and animals may survive to adulthood, but are poorly grown. Gross neuropathological change is confined to slight cerebellar atrophy, most obvious in the caudal vermis. Histologically there are numerous spheroids in the inferior olivary and lateral cuneate nuclei. Lesser numbers of spheroids are in the lateral mid-brain tegmentum, lateral and rostral ventral thalamic nuclei, and the cerebellar vermis. An additional feature is diffuse swelling of axons in the above locations and in the medial lemniscus, medial longitudinal fasciculus, central tegmental tract, and spinal nerve dorsal roots. Neuronal loss and gliosis can be found in the inferior olivary and lateral thalamic nuclei, and in the Purkinje and granule cell layers in the cerebellar vermis. Spheroids and neuronal loss are also evident in the spiral ganglion and organ of Corti in the inner ear.

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Congenital axonopathy in Friesian/Holstein calves

A series of cases is described from Australia in which calves were recumbent from birth, and exhibited spastic paresis and a variety of other neurologic signs. The characteristic microscopic lesion is *active Wallerian degeneration in all funiculi throughout the spinal cord*, especially at the periphery. Similar changes are evident in the cerebellar peduncles, the median longitudinal fasciculus, spinocerebellar and rubrospinal tracts, and in the roots of cranial nerves III, V, VII, and VIII. In a few cases, mild degeneration occurs in the midbrain and some peripheral nerves. The Wallerian changes are accompanied by a very mild glial response and it is likely that the disease begins in utero shortly before birth. The pathogenesis is unknown, and an autosomal recessive trait is suspected.

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Myeloencephalopathy in Brown Swiss cattle

The first clinical sign of *bovine progressive degenerative myeloencephalopathy*, known colloquially as *weaver syndrome*, is slight ataxia, appearing at 5–8 months of age and worsening progressively over the ensuing 12–18 months. At the advanced stage, there is severe truncal ataxia and pelvic limb dysmetria, with distinct proprioceptive deficits. This terminates in recumbency with its secondary complications. The condition is inherited as a simple autosomal recessive trait; the weaver gene is closely associated with a microsatellite locus for milk production on bovine synteny group 13.

The major and primary lesion is in the spinal cord white matter. At all stages of the disease, there is *active axonal degeneration, with both axonal lysis and spheroid formation*. Axonolysis imparts an appearance of status spongiosus to affected white matter, and advanced lesions have myelin loss and moderate gliosis. The axonopathy is present in both ascending and descending tracts at all levels of the cord, but is most severe in the thoracic segment. The process may begin in the thoracic segment and extends anterograde and retrograde from this site. Ultrastructural changes indicate disturbed axoplasmic transport and subsequent axonal degeneration. Mild axonal lesions of a similar character occur in the brain stem, but are inconsistent in their location. However, a consistent additional lesion is degeneration and loss of Purkinje cells from the cerebellar cortex.

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Multisystem neuronal degeneration of Cocker Spaniel dogs

In this condition, reported from Switzerland, red-haired Cocker Spaniel dogs of both sexes develop slowly progressive neurologic signs from about 6 months of age. The clinical picture is dominated by behavioral changes, disorders of gait and balance, tremors, and sometimes seizures. Ultimately, the severity of signs necessitates euthanasia. The cause remains undefined, but a genetic basis seems likely.

Neuropathologic changes are bilaterally symmetrical and involve loss of neurons, predominantly in the septal nuclei, globus pallidus, subthalamic nuclei, substantia nigra, tectum, medial geniculate nuclei, and cerebellar and vestibular nuclei. The neuronal loss is accompanied by gliosis and axonal spheroids. In addition, the white matter of the fimbriae of the fornix, central cerebellum, corpus callosum, thalamic striae, and subcallosal gyri have intense gliosis, axonal spheroids, loss of myelin, and perivascular macrophages.

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Neuronal inclusion-body disease of Japanese Brown cattle

An acute neurologic disease of Japanese Brown cattle is described from the island of Kyushu, in which there is hyperexcitability, fever, profuse sweating, and usually sudden death. The cause is unknown, but cases recorded have all been in females. Neuronal cytoplasmic inclusions have been demonstrated in the CNS of humans dying with multiple system atrophy.

There are no gross lesions of significance, but a large percentage of affected cattle have single, or sometimes multiple, *eosinophilic cytoplasmic inclusion bodies in large neurons of the midbrain, pons and medulla*. The inclusions are mostly in the axon hillock region, are oval in shape and about 18 μm in greatest diameter. Ultrastructurally, they appear as sequestrations of degenerate mitochondria, with associated aggregations of rough endoplasmic reticulum and lipofuscin bodies.

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Central and peripheral neuronopathies and axonopathies

Organophosphate poisoning

The toxicity of organophosphates (OPs) is based on their ability to phosphorylate and inactivate a number of esterases for which they can act as substrates. The acute inactivation of acetylcholinesterase is a well-known effect, exploited for pesticidal and anthelmintic purposes. Our concern here is with *delayed neurotoxicity* that follows 2–25 days after exposure and that involves the inactivation of esterases within neurons. Inactivation of esterases tends to be irreversible and recovery depends upon synthesis of new enzyme, and this will vary with the compound and the particular enzyme(s) inactivated. There is also wide individual and species variability in sensitivity to neurointoxication. OPs are derivatives of phosphoric, thiophosphoric, and dithiophosphoric acids, and are used not only as poisons but also in industrial applications in hydraulic systems, as high-temperature lubricants, and as plasticizers. Delayed neurotoxicity, which may be induced by OPs that have no acute effect on acetylcholinesterase, is particularly a property of the *arylphosphates*, the best known being *triorthocresyl phosphate* (TOCP).

The clinical presentation is characterized by the onset of ataxia, weakness and proprioceptive deficits, and ultimately paralysis. Severe dyspnea and loss of vocalization are often present in cattle and pigs, and hypomyelinogenesis is described in piglets and laryngeal nerve degeneration in horses given organophosphate anthelmintics.

The pathology and pathogenesis of delayed OP intoxication have been studied quite thoroughly. OPs are not cumulatively toxic, but a single threshold dose will induce central and peripheral distal axonopathy. *Multifocal degenerative changes develop in distal regions of axons*, with accumulations of organelles in the regions of proximal paranodes producing marked swellings. Axonolysis then follows and there may be cycles of attempted regeneration and further degeneration. In peripheral nerves, these changes are most intense in the intramuscular segments, and involvement of the recurrent laryngeal nerves makes *aphonia* a prominent sign in many cases. In the CNS, axonal degeneration is focused at the distal extremities of the long descending and ascending spinal tracts. The picture is dominated by axonal swellings, microcavitation, and secondary myelin loss.

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Coyotillo poisoning

The coyotillo or buckthorn shrub, *Karwinskia humboldtiana*, is indigenous to the southwest USA, Mexico, and Central America, and its fruits are toxic to many species, including humans. The neurotoxin present in the fruit is *tullidinol*. Goats are most commonly affected, and intoxication is first manifested as hyperesthesia, which is followed by ataxia, gait abnormalities, and weakness, with signs developing over a couple of weeks. If the polyneuropathy is mild, recovery may occur.

Pathologically there is a *severe, predominantly motor, distal peripheral axonopathy* that appears to be the major pathological event, although there is significant segmental demyelination as well. Denervation atrophy becomes apparent in skeletal muscle. In the CNS, numerous swellings may develop in the proximal axons of cerebellar Purkinje cells, appearing in the granule cell layer and the folial white matter. Swollen axons may also be found in the lateral and ventral funiculi of the spinal cord.

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Mesquite toxicosis in small ruminants

Various species of mesquite (*Prosopis*) are widespread on rangeland in the southwestern USA, Mexico, and Central and South America. Goats are more likely to be exposed given their browsing habits. Ingestion of the leaves, pods, and seeds of *Prosopis glandulosa* (honey mesquite) and *P. juliflora* (velvet mesquite) can cause mandibular tremors, tongue protrusion, drooling, dysphagia, and weight loss due to selective toxicity to cranial nerve nuclei. Histologic lesions consist of fine vacuolation of the perikaryon of neurons in the trigeminal nuclei and occasionally the oculomotor nuclei. Wallerian degeneration occurs in the mandibular and trigeminal nerves, with resultant denervation atrophy of muscles that they innervate.

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Aspergillus clavatus toxicosis

An acute clinical syndrome of cattle and sheep has been reported from several countries associated with a mycotoxicosis caused by *Aspergillus clavatus.* Most outbreaks are caused by fungal growth on industrial organic waste or stored products or by plants grown in hydroponics. The fungus is known to produce a variety of toxic metabolites including patulin, kojic acid, cytochalasins, and tremorgenic mycotoxins. The toxic syndromes observed in animals are suggested to result from synergistic action of these toxins. The clinical syndrome is dominated by acute onset of drooling and ataxia that progresses to recumbency and death. The neuropathology is characterized by *acute central chromatolysis of neurons* in the red and vestibular nuclei, and in spinal ventral horn gray matter and ganglia. Wallerian degeneration and myelin edema are evident in all tracts of the spinal white matter.

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Arsenic poisoning

Animals may be poisoned by arsenic by ingestion or percutaneous absorption, the toxic dose by the latter route being considerably less than the toxic oral dose. The commonest sources of arsenic are fluids used as insecticides and herbicides. Most arsenical dips and sprays for animals contain sodium arsenite and, after these operations, a considerable but nontoxic amount of arsenic is absorbed through the skin. Percutaneous absorption is increased if the animals are hot at dipping and kept hot afterwards, and if they are not allowed to dry quickly. Absorption is rapid through shear wounds and through hyperemic skin such as of the thighs and scrotum of rams in the breeding season. Local high concentrations of arsenic on the skin also cause local acute dermatitis.

Herbicides of most importance are those containing *sodium arsenite, lead arsenate, and arsenic pentoxide*, and poisoning occurs usually when animals get access to recently contaminated pasture. A variety of mistakes and accidents commonly expose animals to these compounds. Paris green (cupric acetoarsenite), used as poison for grasshoppers and other parasites of plants, is occasionally responsible for poisoning. Ore deposits frequently contain large amounts of arsenic, and chronic arsenical poisoning can occur when pasture and drinking water are contaminated by the exhaust from smelters.

The toxicity of arsenicals varies considerably depending on the solubility of the salt and, in the case of organic arsenicals, on the rate at which the arsenic is released from organic bondage. The syndromes of arsenic poisoning differ according to acuteness or chronicity and also according to whether the arsenic is organic or inorganic, but these latter differences are probably not qualitative. One, and perhaps the main, *mechanism of action of arsenicals is combination with, and inactivation of, sulfhydryl groups* resulting in general depression of metabolic activity. The organs most susceptible to metabolic decline are the brain, lungs, liver, kidney, and alimentary mucosa. In poisoning by inorganic arsenic, the pattern of signs and lesions is the same regardless of the route of absorption, thus indicating that, although alimentary tract signs may dominate the clinical disturbance, they are part of the systemic intoxications. In poisoning by organic arsenic, which is observed in pigs, the signs are referable entirely to the nervous system.

The ingestion of very large amounts of a soluble **inorganic arsenical** may result in death in less than 24 hours. There is profound depression and peripheral circulatory collapse; at postmortem in such cases, there are usually no lesions, or at most, slight edema of the abomasum.

With poisoning of lesser severity, 1–2 days lapse between the ingestion of arsenic and the onset of clinical signs. The onset is sudden with acute abdominal distress, nervous depression, circulatory weakness, and, after some hours, terminal diarrhea and convulsions. Some cases may survive for several days and show additional neuromuscular signs of tremor and incoordination. In chronic poisoning, the signs are nonspecific, being those of unthriftiness, capricious appetite and loss of vigor. Pregnant cows may abort. Visible mucous membranes and the muzzle may be hyperemic and inflamed.

Lesions produced by *acute poisoning by inorganic arsenicals* can be largely explained on the basis of *vascular injury*. There is splanchnic congestion with petechial hemorrhages of serous membranes. The mucosa or submucosa of the stomach or abomasum is intensely congested, and the abomasal plicae are usually thickened by edema fluid. There are intramucosal and submucosal hemorrhages of patchy distribution, and these lead quickly to more or less extensive ulceration of the stomach and intestine. The intestinal content is very fluid and may contain shreds of mucus and detritus. There is mild, usually fatty, degeneration of the parenchymatous organs, sometimes with hepatocellular necrosis and edema of the kidney. Lesions in the brain develop in the course of 3–4 days and consist of moderate diffuse cerebral edema and petechiation. The hemorrhages, which are of capillary type and apparently due to necrosis of the walls of these vessels, are distributed throughout the white matter.

The anatomical changes in *chronic arsenical poisoning* have the same distribution as in the acute poisoning. The stomach and gut remain mildly congested, edematous, and ulcerated, and there are prominent fatty changes in the heart, liver and kidneys. Neural lesions may not be found in the CNS except for those changes that are secondary to peripheral neuropathy. In both sensory and motor components of peripheral nerves, there is degeneration of myelin and axons.

The application of arsenic to the skin may cause acute and chronic dermatitis if cutaneous circulation is poor, but if the circulation is good the arsenic tends to be absorbed and to cause systemic rather than local toxicity. Dermatitis, when it develops, is characterized by intense erythema, necrosis, and sloughing; the residual ulcerative lesions are indolent.

Organoarsenical phenylarsonic acid derivatives are commonly used as feed additives for swine, for growth promotion and the control of enteric disease. Poisoning is thus largely confined to this species, and is caused by accident or careless management; however, the margin of safety can be quite low when arsanilic acid is used to control swine dysentery. Two syndromes are recognized, caused by p-aminophenylarsonic acid (**arsanilic acid**), and 3-nitro-4-hydroxyphenylarsonic acid (**3-nitro**).

In *arsanilic acid poisoning*, there is usually acute onset of cutaneous erythema, hyperesthesia, ataxia, blindness, vestibular disturbances and, terminally, muscular weakness. There are no gross neural lesions but, microscopically, mild edema of the white matter may be present in the brain and cord, and a few shrunken and degenerate neurons in the medulla. Extensive Wallerian degeneration frequently develops in the optic and peripheral nerves, but may not be present in spite of severe clinical signs.

In *3-nitro poisoning*, there is a syndrome of repeated clonic convulsive seizures following exercise, with progression to paraplegia, but no blindness. Pathologically there is Wallerian degeneration consistent with distal axonopathy in the spinal cord. The lesion is intense in the dorsal proprioceptive and spinocerebellar tracts of the cervical cord, and lateral and ventral funiculi of the caudal cord. Optic and peripheral neuropathy are mild.

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Neonatal copper deficiency (swayback, enzootic ataxia)

It is generally accepted that maternal/fetal copper deficiency is a major factor in a characteristic neurologic disease of lambs, goat kids, and piglets. The syndrome has been most studied in the lamb, and the terms "*swayback*" and "*enzootic ataxia*," used to describe the signs shown by affected animals, are entrenched as names for the disease. The provision of adequate copper supplementation of pregnant animals at risk can effectively eliminate the disease in their offspring, and treatment of affected animals with copper may produce some remission of signs. Supplementation of unaffected lambs at risk has also been claimed to be effective in prevention. Other manifestations of copper deficiency, such as "steely wool," osteoporosis, and hypopigmentation of black wool, could be expected in affected sheep flocks.

The bioavailability of and physiologic requirement for copper are influenced by many factors, and a functional deficiency state is often determined by overall availability rather than actual copper intake. Thus there may be *absolute primary deficiency*, or *conditioned secondary deficiency*, brought about by reduced absorption from the gut, reduced availability in tissues, or enhanced excretion. The interactive roles in copper metabolism of soil and dietary molybdenum, sulfate, iron, and zinc are important. *Molybdenum* is a prime antagonist for copper and, in the presence of adequate sulfate, limits the capacity to absorb copper from the gut and the capacity to store absorbed copper in the liver. This antagonism is unique to ruminants and is provided by the formation in the rumen of *thiomolybdates*, a series of anions in which sulfur progressively substitutes for oxygen in the molybdate ion. Copper complexed to thiomolybdate forms insoluble complexes that are poorly absorbed; this is primarily an effect of maximally substituted tetrathiomolybdate; lesser substituted thiomolybdates may be absorbed and be responsible for reducing copper availability at the local tissue level.

Iron is an antagonist to copper, although the mechanism is not known. Experimental exposure of ruminants to high intakes of iron induces severe hypocupremia, but a role for iron in naturally occurring copper deficiency syndromes is not of known importance.

Copper is required for the catalytic activity of enzymes that are essential for neural function and include tyrosinase for melanin synthesis, cytochrome oxidase for electron transport in mitochondrial respiration, copper/zinc superoxide dismutase for antioxidant activity, dopamine hydroxylase for catecholamine synthesis, and ceruloplasmin for iron homeostasis. Other copper-containing enzymes, such as lysl oxidase, are important in animal disease but anomalous relationships between copper analyses and actions and the occurrence of prenatal and neonatal disease suggest that there are as yet unidentified enzymes or functions required for developing lambs and kids.

Species and breed differences, pregnancy, plant/soil relationships, fiber content of the diet, and seasonal conditions will govern nutritional requirements. Aspects of copper metabolism differ significantly between sheep and goats. It is also possible that undefined factors may bear significantly on metabolic availability. In spite of this, the copper content of CNS tissue in affected neonates tends to be consistently below normal values, and represents the most reliable tissue assay. It is also true that some individuals with similarly low copper values can be clinically normal, although this holds more for goat kids and piglets than lambs. As the ability to metabolize copper is not impaired, tissue concentrations in affected animals may return to normal fairly rapidly after dietary correction, with concentration in the liver recovering more rapidly than in the CNS.

The effects of copper deficiency on the CNS occur in utero and during early neonatal life. Despite intensive investigation, the biological role of copper in the developing nervous system remains unclarified and contentious. Copper is a component of the enzymes cytochrome oxidase and superoxide dismutase, and of the protein ceruloplasmin. Interference with the functions of these has variously been proposed as the molecular basis of the disease via suppression of mitochondrial respiration and phospholipid synthesis or via damage inflicted by superoxide radicals. It should be added that lambs with no clinical signs have been found to have neuronal degenerative lesions, and lambs with clinical signs have minimal lesions.

Clinical swayback in lambs occurs in a congenital form and a delayed form, also called "enzootic ataxia" in which, after being normal at birth, lambs suddenly develop signs at any time between 1 week and several months of age. The clinical signs are dominated by motor

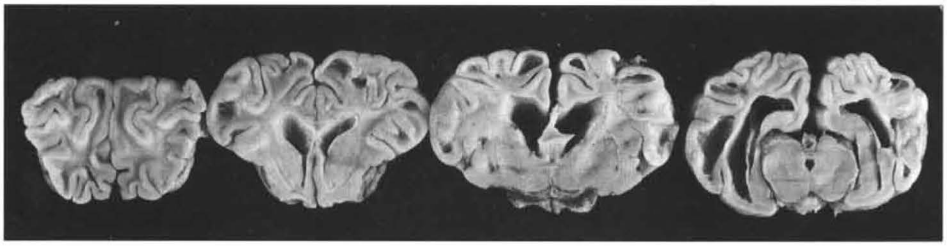

Figure 3.86 Bilaterally symmetrical cavitation of cerebral white matter in **swayback** in a lamb. (Reprinted with permission from Innes JRM, Saunders LZ. *Comparative Neuropathology*. Academic Press, New York, 1962.)

disturbances, with staggering and ataxia. Congenital cases may be blind and unable to stand.

Some lambs with *congenital swayback* have an extensive structural lesion grossly evident in the *cerebral white matter*, but all have some degree of the neuronal degenerative changes described below for the delayed disease. The former lesion is bilateral and symmetrical gelatinous softening or cavitation (Figs 3.27A, 3.86), which may be restricted to the occipital pole or may involve the entire corpus medullare, sparing only a thin rim of white matter adjacent to gray. Histologic and ultrastructural descriptions of the gelatinous lesion are meager, but marked edema with mild fibrillary astrogliosis and a paucity of myelin can be expected. Some myelin degradation products are usually present, but never in great quantity, and gitter cells are sparse. It seems likely that many axons are initially spared, but that rapid dissolution of all elements leads to cavitation. The pathogenesis of this lesion remains obscure; it seems that both hypomyelination and demyelination may be involved but the basis of the tissue lysis is unexplained.

Although lambs with *delayed swayback* do not have lytic lesions in the cerebral white matter, they consistently have changes in both gray and white matter in other parts of the neuraxis. Most investigators consider that these changes largely develop and progress after birth, but this is not absolute, as pointed out above. Extensive Wallerian degeneration is concentrated in dorsolateral and ventromedial tracts throughout the spinal cord. *The pattern of tract degeneration is suggestive of a distal axonopathy*. In addition, conspicuous degenerative changes are present in neurons in the red, lateral vestibular, medullary reticular, and dorsal spinocerebellar nuclei in Clarke's column, and in the spinal motor neurons, particularly in the intumescences (see Fig. 3.1). Many such neurons are undergoing central chromatolysis, and some have nuclear rhexis and lysis. A few may be undergoing neuronophagia. Swollen neurons have been shown by immunocytochemistry to contain masses of phosphorylated neurofilament epitopes. Sites of neuronal loss are marked by fibrous astrogliosis.

Wallerian degeneration may be apparent in ventral spinal nerve exit zones and rootlets, and in peripheral nerves (see Fig. 3.6), although this finding has not been highlighted as much as in goat kids. The pattern of axonal changes is consistent with the degenerating axons being those arising from the nuclei where neurons are also degenerate. Opinion favors the hypothesis that the lesions represent primary neuroaxonal degeneration with secondary myelin loss, although the myelin has been shown to be qualitatively abnormal and therefore theoretically unstable.

A very small number of lambs may have a *cerebellar lesion*, or *cerebrocortical necrosis*, as described below for goat kids. A further variant reported in lambs with delayed swayback is the occurrence of *acute cerebral edema* that is sometimes unilateral and involves both gray and white matter. Small gelatinous or cystic foci may be present at the corticomedullary junction. The pathogenesis is unexplained.

There is no unifying explanation for the spectrum of changes found in swayback, nor for the molecular mechanisms relating to the role of copper. It has been proposed that the spectrum reflects a critical level of copper deficiency, cytotoxic at particular times when different regions of the developing brain/spinal cord are undergoing growth spurts. It has also been suggested that the lesions reflect oxidative damage concentrated in particular vascular fields.

In **goat kids**, the clinicopathologic spectrum is similar, but has different emphasis. The great majority of reports describes delayed swayback, with a high incidence of cerebellar degeneration/dysplasia, and of peripheral motor axon degeneration. The cerebellar changes include necrosis and dystopia of Purkinje cells, depletion of internal granule cells, and Wallerian degeneration in folial white matter. The lesions tend to be multifocal and may involve vermis and hemispheres or be restricted to the vermis. In a very few cases, congenital swayback is reported in kids, and in only two individuals were cerebral gelatinous and cavitating changes found. An additional variant reported in both lambs and kids is the occurrence of diffuse cerebrocortical necrosis.

Lesions in **piglets** have the same general character in regard to Wallerian changes as described above for delayed swayback, but chromatolytic neurons are not evident. A swayback-like disease has also been documented in adult captive *red deer*, but the role of copper is uncertain.

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Progressive axonopathy of Boxer dogs

This progressive disorder is inherited as an autosomal recessive trait, and begins in early life. Defects in slow axonal transport are involved in the pathogenesis of this condition; myelin lesions may then occur in response to primary axonal changes, and represent adaptive remodeling to alterations in axonal caliber and metabolism.

Clinical signs first become apparent at about 2 months of age and progress fairly rapidly until 12 or 18 months, when they may either become static or advance very slowly. The dominant clinical sign is *hindlimb ataxia*, with proprioceptive deficits, hypotonia, and areflexia often being evident.

Degenerative lesions have been described in the spinal cord, caudal brain stem, optic nerves, spinal and cranial nerves, and major autonomic nerve trunks. *Morphologic changes are most obvious in the spinal cord and caudal brain stem*, and their intensity seems to parallel the clinical progression. Axonal spheroids develop in the spinal cord and are concentrated in the ventral and ventrolateral funiculi in the cervical and thoracic segments. A few axons undergoing Wallerian degeneration are also evident, but are always in a minority. Myelin sheaths are thinned around the spheroids, as would be expected, but attenuated sheaths may also occur around axonal segments not obviously swollen. Vacuolation of myelin segments is also a feature. These white matter changes have no obvious tract distribution. In the gray matter, spheroids are found in many nuclei of the caudal brain stem, and to a small extent in the spinal cord. However, from the diencephalon forward, there are virtually no gray matter lesions. Axonal swellings and myelin vacuolation are prominent in the optic nerves in the area of the chiasm.

In spinal nerve roots, there are, early in the disease, focal axonal swellings and a range of myelin abnormalities, including vacuolation, thinning, and segmental loss. With time, the involvement of ventral roots is appreciably more severe than dorsal roots. As the disease advances, such changes progress distally down the nerves, but axonal degeneration is accompanied by regeneration, and denervation of muscle is not significant. Changes of a similar character have been described in cranial nerves and large autonomic trunks.

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Degenerative radiculomyelopathy of German Shepherds and other dogs

This idiopathic condition is a slowly progressive, irreversible, adult-onset disease, characterized by paraparesis and truncal ataxia. The condition is not completely defined, but may be hereditary, and the neurodegeneration appears to have an immune-mediated component. Vitamin E deficiency seems unlikely to be involved.

Some studies describe lesions in spinal nerve roots and cord, whereas others restrict changes to the cord; secondary degenerative changes have been noted in brain nuclei. In about 10–15% of cases, there is loss of patellar reflexes and degeneration in femoral nerves. Arguments have been advanced for and against a distal dying-back type of pathogenesis, and the affliction of dogs of large breeds other than the German Shepherd.

Axonal degeneration and evidence of demyelination are present in the spinal cord and probably, on occasion, in the spinal nerves. While there have been descriptions of symmetrical degenerative changes at the distal extremities of long tracts in the cord, a separate study documented myelin vacuolation, myelin deficiency, a few spheroids and some Wallerian degeneration, distributed randomly and asymmetrically, and most intense in the thoracic segments.

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Giant axonal neuropathy of the German Shepherd Dog

This uncommon disease is a *distal axonopathy* in which disorderly clumps of neurofilaments accumulate towards the extremities of long axons, and their most distal regions eventually degenerate completely. The *neurofilamentous accumulations create the very large argentophilic axonal swellings* that give the disease its name.

The clinical onset is at about 15 months of age and there is progression to paraparesis, ataxia, and megaesophagus within a few months. The disease is apparently inherited in an autosomal recessive manner.

In the CNS, the giant swellings are found in the rostral regions of the ascending fasciculus gracilis and the dorsal spinocerebellar tracts, and in the caudal regions of the descending lateral corticospinal tracts. In the peripheral nervous system, they are found in both myelinated and unmyelinated large fibers in the more distal regions of the major nerves of the limbs and the recurrent laryngeal nerves. Small focal axonal swellings are also scattered through various regions of the brain from the cerebral cortex back to the caudal brain stem, and on to the dorsal and intermediate gray columns of the cord. Some Wallerian changes accompany the small swellings in these areas.

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Progressive motor neuron diseases

There is a group of neurodegenerative diseases, described in several species, which have a common clinical and pathological theme, and which can therefore be dealt with together. They appear to have as a counterpart *amyotrophic lateral sclerosis in humans*. Diseases with this basic theme have been described in Brittany Spaniels, Swedish Lapland Dogs, Rottweiler dogs, domestic shorthaired cats, various breeds of horses, Yorkshire and Hampshire pigs, and in Brown Swiss, Danish Red, Piedmont, and Holstein-Friesian calves.

The clinical presentation in **horses** is dominated by progressive lower motor neuron paralysis which usually ends in tetraplegia and muscle atrophy. There is preferential damage to type 1, high-oxidative fibers, reflected in wastage especially of postural muscles. The pathological findings are dominated by denervation atrophy of pelvic and pectoral muscle groups, and by regressive changes in spinal motor neurons. Other neurons in the motor hierarchy may be afflicted, including the pyramidal cells of the motor cortex. In some instances, neurons in the brain stem and peripheral ganglia are also involved. In the earlier stages of neuronal degeneration, there may be chromatolytic swelling of the soma, with reduced basophilia, and fading of the nucleus, which tends to remain centrally located. In many of the diseases, the swollen cell bodies are often crowded with neurofilaments. Eosinophilic inclusions may be present in the cytoplasm, usually representing clustered remnants of normal organelles trapped amongst arrays of neurofilaments (see Fig. 3.4). In some cases, these inclusions may be found to have the characteristics of Hirano, Lewy, or Bunina bodies described in human neuropathology. The neuronopathy progresses eventually to neuronal death on a cell-by-cell basis, with individual neurons in different stages of the process at any one time. Ultimately there may be considerable neuronal loss, with residual gliosis, and Wallerian change in motor nerves and spinal white matter.

These diseases are considered to be primary metabolic dysfunctions of the nerve cell body; most have an apparently *genetic basis* and an early-age onset. Hereditary spinal muscular atrophy in calves may result from a defect in the survival motor neuron gene. A series of horses is reported from the northeast United States in which the epidemiology strongly suggests an environmental cause. Horses particularly at risk are those that for long periods do not have access to green pasture. Vitamin E status is deficient, which suggests that the lesion is a result of *free radical injury*. The presence of lipofuscin pigments in vascular endothelium and in the retinal pigment epithelium in some affected horses supports the concept of *vitamin E deficiency*. It should be noted also that the neuronal lesions in congenital copper deficiency have many of these features (see Fig. 3.1). Variations on the basic theme are provided in terms of the age of onset and speed of progression of signs, the extent and pattern of frank neuronal degeneration, involvement of nuclei in the mid- and hindbrain, and the presence of Wallerian degeneration in the spinal white matter and peripheral nerves.

Probably the most comprehensively described condition is **hereditary spinal muscular atrophy of Brittany Spaniels**, and it will serve as a generic example. In the Spaniels, there is *autosomal dominant* inheritance and three phenotypic variants: chronic, intermediate, and accelerated. The latter leads to quadriplegia at about 3 months of age, the others over several years, if at all. At the end-stage, there is pronounced denervation atrophy of pectoral and pelvic muscle groups, as well as of the tongue and masseter muscles.

The neuropathology is marked by degenerative change in spinal motor neurons, focused in the intumescences, with similar involvement of the hypoglossal and trigeminal motor nuclei. Particularly in the accelerated, homozygous variant, there are numerous pale, swollen, and chromatolytic motor neurons, with occasional cells undergoing fragmentation and necrosis. Swollen neurons are depleted of ribosomes and a few are packed with neurofilaments (Fig. 3.87). Most characteristically, large argentophilic swellings are present in the proximal segments of many axons close to the affected cell bodies. These have been shown by electron microscopy to be *tangles of disorganized neurofilaments* (Fig. 3.88A). All these changes are much less evident in the more chronic, heterozygous, forms of the disease and clinical weakness is only mild in many cases. Although it is established that there is a defect in the metabolism of neurofilaments as they move from the cell body into the proximal axon of individual neurons, it is not yet clear that this is the primary molecular lesion. Recent studies have shown that there is growth arrest of spinal motor neurons and, initially, affected pups have a greater than normal number of these cells, with a shift to a smaller cell size. Ventral root axons undergo atrophy, which may be followed by loss of entire neurons.

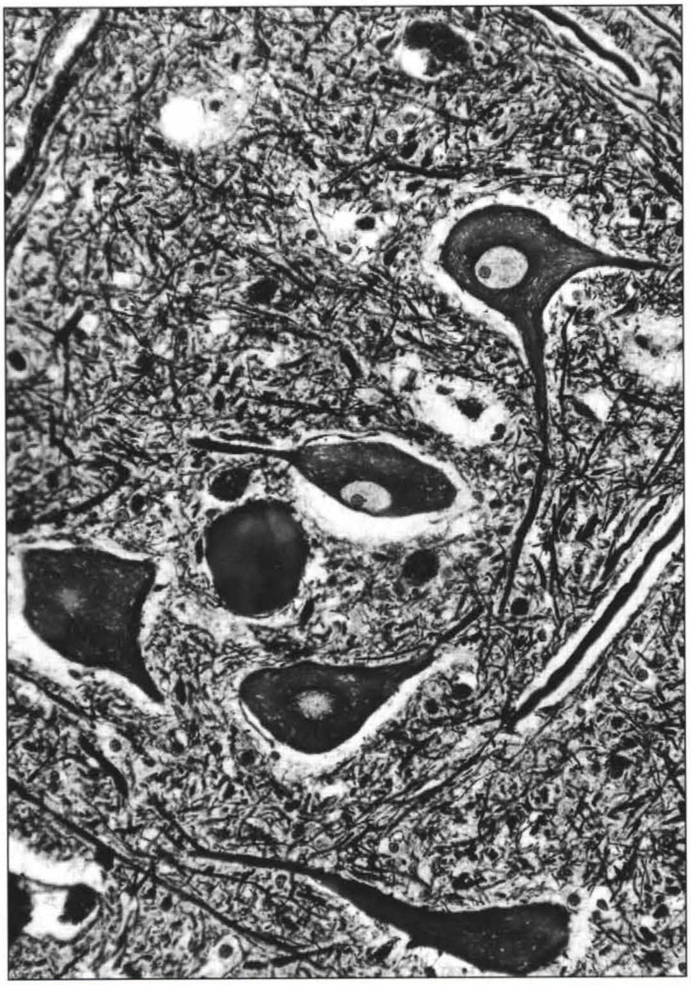

Figure 3.87 Proximal axonal swelling packed with neurofilaments in a spinal motor neuron of a Brittany Spaniel with **motor neuron disease**; silver impregnation. (Courtesy of LC Cork.)

In the **Swedish Lapland Dog**, the pattern of lesions is different, there being degeneration of spinal motor neurons only in the lateral aspects of the intumescences; neuronal degeneration also occurs in spinal ganglia and cerebellar Purkinje cells, but not in brain stem nuclei. The disease has been termed *neuronal abiotrophy*, and is included here because of the dominance of denervation atrophy of muscle in the clinical presentation. The atrophy is concentrated in the more distal muscle groups of the limbs, in keeping with the topography of degenerate spinal motor neurons. Any cerebellar deficits are probably over-ridden by the lower motor neuron impairment.

In the **English Pointer** dog, a strikingly different motor neuron disease has been described in which weakness and muscle atrophy become obvious at about 5 months of age and severe by about 9 months. There is correspondingly severe distal degeneration of peripheral motor nerves and denervation of muscle. However, rather than the changes described above, spinal motor neurons are

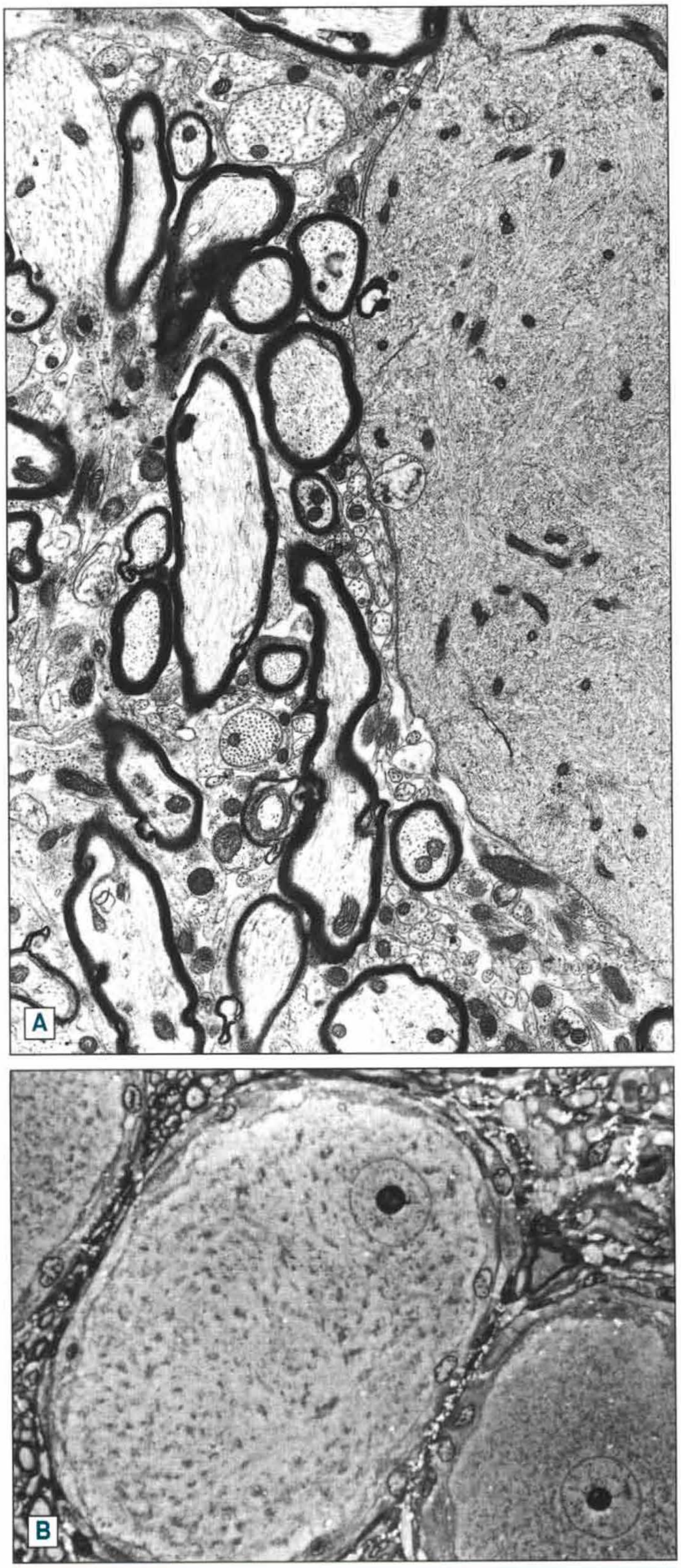

Figure 3.88 A. Proximal axonal swelling packed with neurofilaments in a spinal motor neuron of a Brittany Spaniel with **motor neuron disease**. (Courtesy of LC Cork.) **B.** Swollen neuron in a spinal ganglion of a "**shaker calf**": Nissl substance is dispersed by neurofilamentous masses. (Courtesy of CG Rousseaux.)

filled with *cytoplasmic lipid inclusions* reminiscent of those seen in the gangliosidoses and mucopolysaccharidoses, and in some drug-induced conditions. No significant loss of motor neurons is apparent, and similar inclusions are present in the hypoglossal and spinal accessory nuclei, but not elsewhere.

Focal, asymmetrical spinal motor neuron degeneration with acute onset and a rapid course is described in **German Shepherd** pups. The affected neurons are within the cervical spinal cord intumescence, and exhibit peripheral chromatolysis or vacuolation. There is secondary denervation and wasting of forearm muscles. Progressive lower motor neuron disease has also been reported in **Saluki** and **Griffon Briquet Vendeen** pups.

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Neurodegeneration of horned Hereford calves

The term "**shaker calf**" refers to a disease in which newborn horned Hereford calves are unable to stand without assistance and develop generalized fine tremors and profound muscular weakness. While they often die in the neonatal period because of secondary complications, some, after a short period of apparent remission, show relentlessly progressive spastic paraparesis, but remain alert and may survive for some months. The disease is presumed to be inherited on the basis of pedigree analysis. The fundamental metabolic defect remains to be characterized.

The pathology is characterized by dramatic swelling of the cell bodies and processes of neurons throughout the spinal cord. Swelling is due to the *accumulation of masses of neurofilaments* that impart a faintly fibrillar, amphophilic appearance in routine sections. This lesion involves ventral horn motor neurons, sensory neurons of the substantia gelatinosa, Clarke's column, and the intermediolateral (sympathetic) nuclei. Neuronal necrosis is minimal, although the site of occasional cell loss can be found as nodules of reactive glia. Some

Wallerian degeneration is evident in ventral spinal nerve roots and the ventromedial spinal white matter.

The neuronal lesion is also present in the major motor nuclei of the brain stem, the reticular formation and the cerebellar Purkinje cells, and to a slight degree in the lateral geniculate nucleus and layer 5 of the frontal cortex. Some affected cells are evident in peripheral ganglia (Fig. 3.88B), including the myenteric plexus, and in the retinal ganglion cells.

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Neuropathy in Gelbvieh calves

A familial, and likely hereditary, condition has been reported in Gelbvieh calves that had developed hindlimb ataxia and paresis, and were found histologically to have peripheral neuropathy and proliferative glomerulopathy; skeletal muscle degeneration was prominent in some cases. Degeneration was severe in peripheral nerves, dorsal and ventral spinal nerve roots, and less marked in dorsal fasciculi of the spinal cord.

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Progressive neuronopathy of the Cairn Terrier

Progressive neuronopathy occurs only in Cairn Terriers and is suspected to be inherited. This multisystem chromatolytic degeneration clinically resembles globoid cell leukodystrophy, from which it can be differentiated clinically by the exercise-induced deterioration of neurological signs in progressive neuronopathy. Animals of both sexes develop hindlimb weakness, tetraparesis, ataxia, head tremor, and loss of reflexes between 5 and 7 months of age.

Both central and peripheral chromatolysis of neurons are evident, and occur in Clarke's column and dorsal and ventral horn cells in the spinal cord, in sensory ganglia, and in the cuneate, lateral cuneate, glossopharyngeal, vagus, reticular, lateral vestibular, mesencephalic trigeminal, red and cerebellar roof nuclei. Wallerian degeneration is present in lateral and ventral funiculi of the cord, to various degrees, and also in dorsal and ventral spinal nerve roots.

Ultrastructural studies on one case suggested that the chromatolytic change was associated with depletion of ribosomes and increased numbers of mitochondria in perikarya, and that Wallerian degeneration was probably secondary to the metabolic disturbance in the cell body. This case was also marked by onset of signs at 11 weeks of age, bouts of cataplectic collapse, and thoracolumbar myelomalacia.

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Primary hyperoxaluria in the cat

In this inherited metabolic disorder in cats, profound deficiency in the activity of D-glycerate dehydrogenase is associated with L-glyceric aciduria, hyperoxaluria and the heavy deposition of oxalate crystals in the renal tubules. This leads to *oxalate nephrosis* and renal failure before a year of age.

An accompanying neuronal lesion occurs in the form of large swellings in the proximal axons of spinal motor neurons, ventral roots, intramuscular nerves, and the dorsal root ganglia. These swellings are caused by *neurofilamentous accumulations*, and are accompanied by some Wallerian degeneration in peripheral nerves. There is no obvious metabolic link between the neuronal lesions and the other biochemical disturbances, and the syndrome may represent a dual genetic defect.

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The dysautonomias

The term dysautonomia denotes a profound failure of both sympathetic and parasympathetic functions across several organ systems. Domestic animals affected include horses (**equine grass sickness**), cats (**Key–Gaskell syndrome**), and dogs. *Clostridium botulinum* is thought to play a role in equine dysautonomia and perhaps in the feline syndrome, which may hence be toxico-infectious forms of botulism.

Particularly in the cat, it is considered that there is a single episode of injury to neurons, with acute degeneration and subsequent reparative reactions if the animal survives for a number of weeks. There is a good correlation between the clinical signs and the extensive destruction in autonomic ganglia, and *autonomic denervation would account for the major functional disturbances.* The occasional expression of mild proprioceptive and lower motor neuron deficits may be explained by the lesions in dorsal root ganglia and spinal motor neurons respectively.

In the equine disease, the clinical picture is primarily the result of neurogenic obstruction of the alimentary tract with various parts of the tract involved to differing degrees, with distinct acute, subacute, and chronic forms. Clinical differential diagnosis can be exceedingly difficult. The outcome is usually fatal; some chronic cases may recover with intensive care.

In the cat, there is acute onset of clinical disease that in a few cases may resolve after many months, but many animals die or require euthanasia early in the course. Onset is marked by dilated pupils, prolapsed nictitating membrane, dry mucous membranes, megaesophagus, constipation, vomiting, and dehydration. In the dog, the most severe and consistent loss of autonomic neurons occurs in the pelvic, mesenteric, and ciliary ganglia. Minimal changes are reported in the CNS. Differential diagnoses in dogs include ganglioradiculitis (sensory neuronopathy).

In the acute phase, *extensive chromatolysis and death of ganglion cells* is present throughout the peripheral autonomic ganglia (Fig. 3.89), with axonal degeneration in autonomic nerve fibers. There is also neuronal

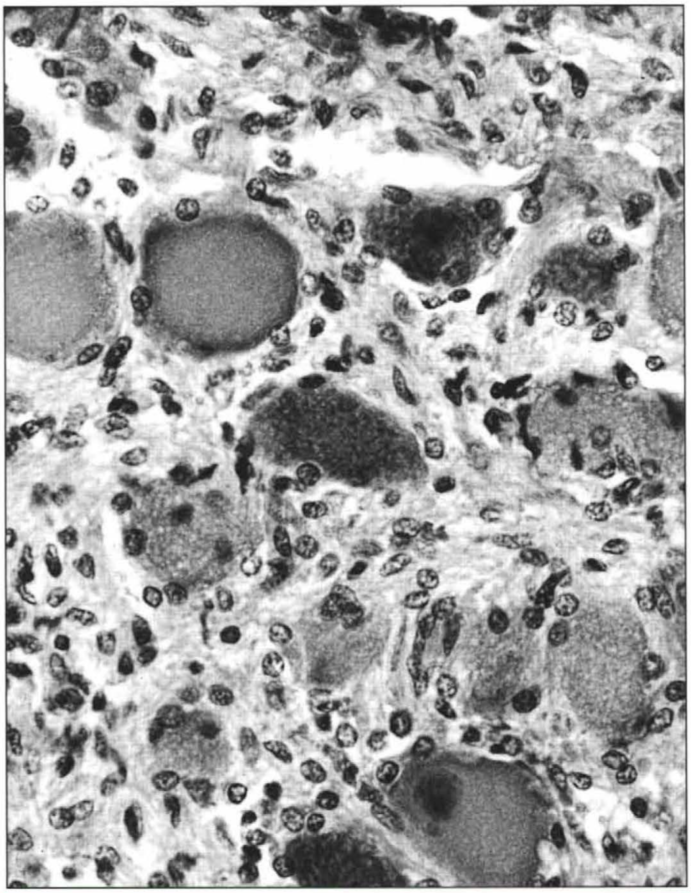

Figure 3.89 Central chromatolysis in ganglionic neurons of a horse with grass sickness.

degeneration in the nuclei of cranial nerves III, V, VII and XII, the dorsal motor nucleus of the vagus, and the nucleus ambiguus. Some neuronal degeneration may be found in dorsal root ganglia, and in the ventral horn and intermediolateral areas of the spinal gray matter. In later phases of the disease, depletion of neurons at the above sites is evident, with reactive and proliferative changes on the part of non-neuronal elements.

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Sensory neuropathies in the dog

Sensory neuropathies are reported in an increasingly long list of various dog breeds. A small number of **English Pointer** pups from a particular mating developed a syndrome of acral mutilation and analgesia. The size of spinal ganglia was reduced, with reduced numbers of neurons, and reduced fiber density in the dorsolateral fasciculus of the spinal cord (Lissauer's tract). These clinical and morphologic findings are consistent with a *specific deficit in nociceptive pathways*. It is suggested that the presumably genetically based disorder involves both hypoplasia of the system and continued degeneration postnatally.

In **longhaired Dachshunds**, proprioceptive, nociceptive, and urinary deficits are associated with a *distal degenerative axonopathy*. Distal cutaneous nerves have marked loss of large myelinated fibers, and degenerative changes in both myelinated and unmyelinated fibers. In the spinal cord there is axonal degeneration in the fasciculus gracilis, of greatest intensity at its distal extremity in the cervical region.

Progressive myelopathy and neuropathy causing progressive ataxia in littermate **New Zealand Huntaway** dogs is described as a *central–peripheral distal axonopathy*, in which there is degeneration of axon and myelin in sensory, proprioceptive, and motor tracts of the spinal cord and to a mild degree in some peripheral nerves.

In dogs of **various breeds**, there are reports of diffuse ganglioneuritis, with destruction of primary sensory neurons and subsequent Wallerian degeneration and axonal loss in peripheral nerves in the spinal dorsal funiculi, spinal tract of the trigeminal nerve, and solitary tract. These cases are mentioned here as the *extensive axonal degeneration* is the more likely lesion to be routinely noted, and its pattern should immediately draw attention to the primary involvement of dorsal root ganglia. The pathogenesis of this ganglioneuritis is not known but may be similar to that of Guillain–Barré syndrome, a post-infectious syndrome in people.

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Peripheral axonopathies

Lesions involving single peripheral nerves are termed **mononeuropathies.** They are usually the result of focal compression or contusion by trauma, tumor masses, or similar lesions, and involve centrifugal Wallerian degeneration about the lesion. If several nerves are randomly involved in such a way, the term **mononeuropathy multiplex** may be applied. **Polyneuropathy** describes bilaterally symmetrical involvement of several nerves, and carries the implication of a systemic disturbance.

From the clinical point of view, most peripheral polyneuropathies occur in association with polyneuritis or demyelination, but in this section our focus is on **noninflammatory axonopathies**, and the other types of disease are discussed elsewhere. Intense Wallerian degeneration of peripheral motor axons will of course accompany degeneration of the ventral spinal motor nerve cell bodies, as occurs in the motor neuron diseases previously discussed. Similarly, in the very rare primary sensory polyneuropathies, peripheral sensory fibers will degenerate, but the degeneration will also extend, with the central projections of these cells, into the dorsal funiculi of the spinal cord. In some intoxications, delayed organophosphate for example, peripheral neuropathy will be accompanied by central axonopathy, as has been illustrated above. All the foregoing emphasizes the unity of the nervous system and the inconsistencies involved in deciding where neurons begin and end, but nonetheless certain diseases have a clear central or peripheral focus.

With the exception of equine recurrent laryngeal neuropathy, *noninflammatory peripheral polyneuropathies are uncommon and are generally to be regarded as distal axonopathies of the spinal neurons*. Motor axon involvement means that *denervation muscle atrophy* is a frequent concurrent lesion. It should be remembered too that with advancing age, subclinical degenerative changes are to be expected in peripheral nerves and spinal roots.

Equine laryngeal hemiplegia

The clinical manifestations of this common and well-recognized disease are the consequence of *denervation atrophy of the intrinsic muscles of the left side of the larynx*. The resultant inability to adduct the arytenoid cartilage and the vocal fold leads to partial obstruction of the airway on inspiration, and inspiratory stridor on exertion, referred to as "*roaring*."

The underlying lesion is *idiopathic degeneration of the left recurrent laryngeal nerve*, suggested to result from progressive degeneration of large myelinated axons, which increases in intensity towards the distal extremities of the nerve. During active degeneration, localized axonal swellings result from paranodal and internodal accumulations of granular dense bodies and degenerate mitochondria, although numerous atrophied axonal segments have also been described. Loss of axons is indicated by the presence of Bungner's bands, which may contain fragments of axonal and myelin debris, and permanent axonal loss is reflected in considerable endoneurial fibrosis. Some axonal regenerative activity may be apparent. There is also evidence of recurrent demyelination and remyelination, considered to be secondary to axonal degeneration.

The cause of this axonopathy remains unknown. Young, tall male horses with long necks seem predisposed and there is a high incidence in Thoroughbred and draft breeds. *Mechanical factors* operating on the left recurrent laryngeal nerve have been proposed, such as stretching of the anchored nerve, or pressure exerted where it reflects around the aorta. However, subclinical involvement of the right recurrent nerve seems to be the rule. Studies of clinically normal horses have revealed bilateral neuropathy with more severe denervation of the laryngeal adductor muscles as compared to the abductors, and this pattern also holds true for horses with laryngeal hemiplegia. The factors determining progression of the subclinical disease, the mechanism for the preferential adductor involvement, and the reason for the greater severity on the left side are unexplained.

One study suggested that similar distal degenerative changes also occur in the long axons of the hindlimbs, but only a few horses were sampled. Such a finding implies a *systemic metabolic or toxic disorder*, producing a polyneuropathy with maximal expression in the left recurrent laryngeal nerve. In New Zealand, laryngeal hemiplegia in association with stringhalt has occurred in seasonal outbreak form in horses grazing the plant *Hypochaeris radicata*. Laryngeal paralysis has occasionally been reported in cases of intoxication with *lead*, or *organophosphates*, as part of widespread axonopathy.

No convincing evidence of lesions in the CNS is available at present, although they have been sought in the nucleus ambiguus from which the recurrent laryngeal axons arise. It has been claimed that axonal spheroids in the lateral cuneate nucleus are more numerous in horses with laryngeal paralysis, but the significance of this is unclear. Such lesions are common and generally nonspecific, and it is difficult to see how they could be related pathogenetically to the rather different lesions in the motor axons supplying the larynx.

Equine stringhalt

Stringhalt is the name given to a clinical condition of horses characterized by *extreme exaggerated flexion of the hindlimbs*. It has been recognized for many years to occur in *sporadic* and *epizootic* forms, the latter associated with the grazing of certain plants, notably *Hypochaeris radicata* (false dandelion, flatweed). Stringhalt may develop subsequent to trauma to the dorsum of the metatarsus. Various degenerative changes have been reported in the spinal cord and peripheral nerves in stringhalt. Selective loss of large-diameter myelinated fibers in peripheral nerves in horses with Australian stringhalt is consistent with distal axonopathy leading to neurogenic muscle atrophy.

Equine suprascapular neuropathy

Mononeuropathy of the suprascapular nerve in the horse has been designated for many years by the old clinical term "*sweeney*." The nerve is prone to injury at its site of reflection around the wing of the scapula, and in many cases *trauma* at this site is probably the initiating cause. Evidence has been advanced to suggest, however, that *entrapment of the nerve* by a tendinous band may lead to degeneration in the absence of additional trauma. In cases of sufficient severity, axonal degeneration, demyelination, and endoneurial fibrosis will be associated with denervation of the spinatus muscle and chronic lameness.

Canine laryngeal neuropathies

Cases are described of neurogenic laryngeal muscle atrophy in old dogs of several breeds but, in the **Bouvier** breed, autosomal dominant inheritance has been proposed for a unilateral or bilateral condition with onset at an early age. Clinical laryngeal paresis/paralysis is evident and is accompanied by denervation atrophy of laryngeal muscles, and Wallerian degeneration of the distal recurrent laryngeal nerves. In this breed, laryngeal paralysis is attributed to an abiotrophic process in nucleus ambiguus in the medulla. Gliosis and neuronal atrophy have been recorded in vagal nuclei in **Siberian Huskies**. In **Dalmatian** dogs, the laryngeal paralysis–polyneuropathy complex is a generalized axonopathy with dying back of laryngeal and peripheral nerves. Neurogenic atrophy of muscles, including those of the larynx, is reported in related **Leonberger** dogs with a spontaneous distal, symmetrical polyneuropathy.

Endocrine neuropathies

Clinical polyneuropathy has been associated with *diabetes mellitus in the dog and cat* and is well recognized in people. In the cat, hindlimb weakness, poor postural reactions, depressed patellar reflexes and plantigrade stance are described. Axonal conduction velocity is reduced in the sciatic and ulnar nerves. Clinical remission often follows therapeutic management of the diabetic state.

The pathogenesis of diabetic neuropathy remains uncertain, but it is generally accepted that there is *distal axonopathy* with cycles of degeneration/regeneration and accompanying demyelination/remyelination. The distal degeneration may be due to impairment of axonal transport mechanisms secondary to reduced availability of glucose for the neuron. Lesions include axonal atrophy of myelinated and unmyelinated fibers, demyelination and, in some animals, intra-axonal accumulation of glycogen.

A peripheral neuropathy has also been associated with *canine hypothyroidism*.

Feline hyperlipoproteinemia

A series of cats has been described with a *genetically based deficiency of lipoprotein lipase activity* and a resulting severe hyperchylomicronemia. Amongst other manifestations, there is a high incidence of *mononeuropathy multiplex*, with clinical palsies related to various peripheral and cranial nerves, including instances of Horner's syndrome. Neurological deficits are related to multiple focal red/brown nodules in the perineurium of nerve trunks, which are organizing hematomas associated with a xanthomatous/granulomatous component. Xanthomatous masses extend between nerve fascicles and appear to compress and distort them, inducing Wallerian degeneration to various degrees of severity. The xanthomas arise from phagocytosis of cholesterol esters by macrophages. These lesions are generally located in loci where trauma is likely and also occur commonly at the emergence of spinal nerve roots.

"Kangaroo gait" of lactating ewes

This condition was first reported from New Zealand, and subsequently from the United Kingdom. There is a low flock incidence and only ewes in lactation or up to 1 month post-weaning are affected. The clinical syndrome is consistent with *bilateral radial nerve palsy*, and there is a characteristic bounding gait during attempted rapid movement. In most cases, there is gradual clinical improvement and eventual recovery. The cause is not known. There have been limited studies of pathology. Extensive Wallerian degeneration with regeneration has been described in the radial nerve trunks of chronically affected ewes, but in some acutely affected animals no radial nerve lesions could be demonstrated. In one study, there were additionally reported spongy change in the neuropil, dorsal root ganglionopathy, and neuronal degeneration in the hippocampus and cervical spinal cord, although these findings were variable.

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Myelinopathies

Myelin sheaths in the central or peripheral nervous system may be the focus of various disease processes, but simultaneous central and peripheral involvement is rare. This is not surprising when one considers the fundamental differences between the two. As previously noted, myelin breakdown and removal in Wallerian degeneration will follow as a **secondary** consequence of axonal degeneration. On the other hand, in some inflammatory diseases, macrophages acting within the orchestration of the immune system will strip myelin from axons, leaving the latter intact for a time at least. In this context, antibodies or inflammatory mediators may bind to and destabilize the structure of the myelin lamellae, causing disruption of the sheath and provoking its phagocytosis. Such **primary demyelination** occurs for example in the CNS in canine distemper, and in the peripheral nerves in "coonhound paralysis." The demyelinating aspects of these entities are discussed elsewhere.

Attention in this section is on *noninflammatory diseases in which some disorder of myelin formation, maintenance, or stability is the primary event*. It should be remembered that the myelin sheath is a specialized extended process of oligodendrocyte or Schwann cell plasma membrane, and myelinopathies are therefore part of the cytopathology of these cells. Within the complex category of myelinopathies, we will discuss *leukodystrophies*, or disorders of myelin synthesis or maintenance; *hypomyelinogenesis,* which may be at one end of the spectrum of leukodystrophy; and *spongy degeneration* or spongiform myelinopathies.

Hypomyelinogenesis

The great majority of the hypomyelinogeneses are restricted to the CNS, and they have been described in most domestic species with the notable exception of the horse. Although in many cases a genetic basis has been demonstrated or strongly implied in hypomyelinogenesis, several viruses have been implicated, and the possibility of toxic or nutritional factors should be kept in mind. A common theme is sex-linked inheritance and affected males.

Myelinogenesis begins sometime after the middle of gestation and continues in the postnatal period for various times depending on the species. It is more advanced at birth in those species in which the young are able to stand and walk soon after, for it correlates with the overall maturity of the nervous system.

The process requires a complex unfolding of events in order to be successful. In the first place, there must be differentiation of competent myelinating cells in sufficient number, and they must migrate to, recognize, and contact the target axons appropriately. Second, the axon itself must send a specific signal to the myelinating cell to initiate its investment. The diameter of the axon dictates whether or not it is myelinated and how thick the sheath will be. The threshold size is about 1 μm in the CNS and 2 μm in the peripheral nerves. Finally, the molecular components of the myelin must be produced and delivered to their correct sites in the membrane. One or several of these processes may be perturbed to give rise to diseases characterized by hypomyelinogenesis.

Myelination does not occur synchronously throughout the nervous system, but in a distinctly regional sequence. Thus lesions of this type may involve some tracts more than others, or completely spare some tracts. There may be complete absence of myelin, or an inadequate amount of myelin that may be either chemically normal or abnormal. For the latter case, the term *dysmyelination* has been coined but, as these conditions are characterized by a reduced quantity of myelin, **hypomyelination** is a suitable generic term. As would be expected, such diseases are manifested early in life, and a very common and dominant clinical feature is the onset of a severe generalized tremor syndrome. This has led to the use of names such as *congenital "tremor," "shaker," or "trembler"* to describe the clinical state. The severity of the clinical signs can vary from life threatening to mild.

The deficiency of myelin may in some instances be permanent, while in others it seems that myelination may be delayed, but eventually proceeds to the extent that clinical deficits resolve. In general, the pathologic picture is dominated by a paucity of myelin with little or no evidence of the breakdown and removal of previously formed sheaths. There may be other accompanying structural defects, notably cerebellar hypoplasia, particularly when a teratogenic virus is the cause.

When routine morphologic evaluations are being made on very young animals, reference should be made to age-matched controls. In the dog for instance, very little myelin is normally present prior to 2 weeks of postnatal age, whereas in the sheep myelination begins at about day 50 of gestation. The investigation of such conditions makes use of traditional stains for myelin and axons, plastic-embedded sections for light and electron microscopy, and immunostaining techniques for marker antigens, such as myelin basic protein. There needs to be some familiarity with the normal morphologic features of early myelination. Some of these are mentioned in the disease descriptions that follow.

Canine hypomyelinogenesis

A number of canine hypomyelinogeneses have been documented and several provisional conclusions can be made. All but one of the diseases involve the central myelin only, and it appears that they are not pathogenetically identical and can be divided into two broad groups according to severity. The most severe are those described in the Samoyed, Springer Spaniel, and Dalmatian breeds.

In the **Samoyed**, severe generalized tremors become apparent at about 3 weeks of age, with a predominance of males being affected. Inability to stand leads to severe incapacitation and a high mortality rate. A genetic basis is suspected. It has been suggested that a central pathogenetic event is *retardation of gliogenesis*, with oligodendrocytes failing to differentiate fully. Profound hypomyelination is suggested on gross inspection of the brain by a lack of contrast between gray and white matter throughout the CNS. The sparing of the peripheral nervous system is well appreciated macroscopically by comparing the myelin-deficient optic nerves to the other cranial nerves, in particular the adjacent oculomotor nerve.

By routine light microscopy, there is normal peripheral myelin but *almost total absence of central myelin, and diffuse microgliosis*. Silver stains suggest axons to be present in normal number. Electron microscopy reveals a few axons to have thin and poorly compacted myelin sheaths, the presence of numerous astroglial processes, and a large number of microglia. Oligodendrocytes are greatly reduced in number and appear immature. Axons, by contrast, are normal in number and morphology but the great majority are devoid of a myelin sheath.

In the **Springer Spaniel**, severe generalized tremor in male pups is evident at 10–12 days of age ("*shaking pups*"). The condition is inherited as an *X-linked recessive*, and severe myelin deficiency is caused by the shaker pup (*shp*) mutation of the myelin proteolipid protein (*Plp*) gene. Central hypomyelination is profound but less severe in the spinal cord than in the brain and optic nerves. In contrast to the Samoyed, microgliosis is not prominent, but in other respects the gross and histologic features are similar. The electron microscope, however, resolves some further differences. For example, some thin myelin sheaths are present that have lamellae of normal configuration. Some axons have a few internodes sheathed, many of which are shorter than would be expected for the axonal caliber, and many of which are single units terminating as "heminodes." Although some immature oligodendrocytes are evident, so too are normally mature ones. In animals 2 months old or more, there are also hallmarks of the early stages of normal myelination that persist well beyond their usual period of the first month of life. These include such features as large amounts of oligodendrocyte cytoplasm in lateral loops, and outwardly terminating lateral loops. In addition, many oligodendrocytes have lysosomal digestive vacuoles thought to relate to the degradation of abnormal myelin components.

The disease in the **Dalmatian** is reported in a male pup that developed severe tremors in the neonatal period and was destroyed at 8 weeks of age. Profound deficiency of central myelin was accompanied by reduced numbers of oligodendroglia, with a few axons having extremely thin and poorly compacted sheaths.

Disorders of lesser severity are described in the Chow Chow, Weimaraner, Bernese Mountain Dog, and in two crossbred ("Lurcher") dogs.

Affected **Chow Chow** dogs have marked impairment from 2 weeks of age, but progressive improvement leads to a virtual absence of clinical deficits by the end of the first year of life. At all stages, the animals remain ambulatory, bright, and responsive, in spite of a pronounced hypermetric gait with a distinctive "rocking horse" motion when trying to initiate movement. Tremors and head bobbing are exacerbated by excitement and disappear at rest. It has not been possible to establish a definite heritable basis for this disease, but it is suggested to be the result of a genetically determined delay in oligodendrocyte maturation.

In pups examined at about 3 months of age, no gross lesions are reported, but profound hypomyelination is evident histologically in the CNS. This is particularly so in the cerebral subcortical white matter, cerebellar folia, ventral half of the cerebral peduncles, optic tracts, and the peripheral zones of the ventral and lateral funiculi of the spinal cord. By contrast, the cerebellar peduncles and fasciculus proprius of the spinal cord are relatively well myelinated. Electron microscopy reveals mostly thin myelin sheaths in the least-affected areas, and mostly naked axons in the worst-affected areas. Oligodendrocytes are present in normal numbers and generally appear morphologically normal, although stellate cells containing intermediate filaments may be found.

In older dogs in clinical remission, the brain is well myelinated apart from some mildly deficient foci in subcortical white matter and corpus callosum. However, myelin deficiency is still marked in the lateral and ventral funiculi of the cord, which also has a few degenerate axons. Ultrastructurally, these areas contain a few well-myelinated or thinly myelinated axons separated by masses of astrocytic processes. Indications of immaturity are provided by poorly compacted sheaths and some massively oversized sheaths that fold away from the axon in redundant loops.

The **Weimaraner** syndrome is closely similar to the above but has only been studied pathologically in the early phase. The tremor syndrome, in evidence at 3 weeks of age, seems to resolve by a year. A heritable basis has been proposed but not confirmed, and delayed oligodendrocyte differentiation suggested as the underlying defect. Pups necropsied at 4–5 weeks of age have no gross abnormalities, but histologic staining reactions suggest myelin deficiency, and this is particularly obvious at the periphery of the lateral and ventral funiculi of the spinal cord. This contrasts with the relatively well-myelinated dorsal columns. Oligodendrocytes are reduced in number. Ultrastructurally, there is considerable evidence of myelin immaturity as described above. Astrocytic fibers are prominent and oligodendrocyte morphology seems normal.

The **Bernese Mountain Dog** "trembler pup" has a fine head and limb tremor that subsides with sleep and improves substantially by 9–12 weeks of age. An autosomal recessive mode of inheritance has been proposed. There are no gross lesions and hypomyelination is concentrated in the spinal cord, where axons are thinly sheathed by morphologically normal myelin. Oligodendrocytes in this case appear to be increased in numbers and morphologically normal. **Lurcher** pups were reported to have unmyelinated and thinly myelinated axons in the peripheral sections of the lateral funiculi of the spinal cord. The changes are subtle and not readily apparent unless plastic-embedded sections are employed. These animals are of interest as they are crossbred, making a genetic cause a more remote possibility.

Hypomyelination has been reported only rarely in **cats**.

The sole reported occurrence of **peripheral hypomyelination** in domestic animals involves the **Golden Retriever** breed. Two littermates, one male and one female, had ataxia, weakness, a crouched stance, and pelvic-limb muscle atrophy at about 2 months of age. Peripheral nerve samples revealed axons of all calibers to be thinly myelinated and Schwann cells to be increased in number and hypertrophied (see Fig. 3.11). There were no indications of demyelination.

Porcine hypomyelinogenesis

Syndromes of **congenital tremor** (CT, myoclonia congenita, dancing pig) are well recognized in pigs. CT has been classified on the basis of the presence (type A) or absence (type B) of myelin deficiency in the central or peripheral nervous system; CT type A has been subdivided into five subtypes, AI–AV. The various forms of CT are similar clinically; affected piglets may bounce on their digits, and have rhythmic whole-body tremors that worsen with excitement and cease with sleep.

CT type AI is caused by transplacental infection in the middle trimester with *Classical swine fever virus*, which causes significant hypomyelinogenesis in addition to other neural lesions in the fetus, most notably cerebellar hypoplasia. The cerebellar changes may be focal and are most likely to be detected in sagittal sections of the vermis and hemispheres. The myelin lesion is generally similar to that seen in lambs with Border disease.

CT type AII may be the result of *Porcine circovirus* type 2 infection, but the role, if any, of this virus remains controversial.

CT type AIII occurs in Landrace or Landrace-crossbreds, and is inherited as an X-*linked recessive* trait, afflicting male neonates, and due to a mutation in the proteolipid protein gene *Plp*. There is myelin deficiency throughout the CNS, but most obvious in the spinal cord, cerebellum, and cerebral gyri. Oligodendrocyte numbers are reduced; many small and medium-sized axons are unmyelinated or only thinly so. Large-diameter axons appear normally myelinated.

CT type AIV occurs in the British Saddleback breed; there is an *autosomal recessive* inheritance mode. Hypomyelination is present throughout the neuraxis with axons of all sizes affected. Many sheaths are poorly compacted and vacuolated. Oligodendrocytes are numerous, and many appear immature ultrastructurally, and sometimes contain cytoplasmic intermediate filaments. Still others contain autophagosomes and dense bodies. The neuropil also contains excessive astrocytic fibers and lipid-laden macrophages. These findings, together with biochemical evidence of myelin degradation, suggest that newly formed myelin is unstable and is rapidly broken down.

CT type AV occurs in piglets of sows exposed to the organophosphate *trichlorfon* between days 45 and 63 of pregnancy.

CT type B is idiopathic, and lacks structural or neurochemical defects.

Ovine and caprine hypomyelinogenesis

Border disease in lambs has become the model for the several pestivirus-induced myelin disorders of animals. *Border disease virus* can cause transplacental infection, which, if on or after day 50, has the potential to produce a range of terata. In the classical syndrome, newborn lambs are often referred to as "*hairy shakers*," due to the combination of generalized tremor and hairy birth coat. There is *diffuse hypomyelination in the CNS,* which is especially prominent in the spinal cord, particularly the more caudal regions. Some myelination is present, but has ultrastructural features of immaturity, in that lamellae remain uncompacted and axons retain the angulated profiles they normally acquire just before myelination and lose soon after. There are also compact sheaths abnormally thin for axonal diameter, and intramyelinic and periaxonal vacuoles.

While glial cell numbers may appear normal by light microscopy, there are in fact reduced numbers of mature oligodendrocytes and increased numbers of microglia. Many microglia are packed with lipid droplets, the origin of which does not appear to be degraded myelin. It is suggested that maturation of oligodendrocytes is delayed, and that although the time of onset of myelination is close to normal, its rate is greatly reduced. The blockade of glial maturation may be the result of virus-induced fetal hypothyroidism; abundant viral antigen can be found in the thyroid, and circulating thyroxin is significantly depressed, but this alternative hypothesis remains unproven.

Severe hypomyelinogenesis is a feature of neonatal goat kids with **β-mannosidosis**. Hypothyroidism has been proposed as the underlying mechanism, as a consequence of the lysosomal storage disease.

Bovine hypomyelinogenesis

Myelination in calves proceeds between gestational week 20 and postnatal week 8. A spectrum of terata and spinal hypomyelinogenesis similar to that in piglets and lambs may develop in calves infected with *Bovine viral diarrhea virus* in utero after day 100 of gestation.

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Leukodystrophic and myelinolytic diseases

This section will address those diseases characterized by degeneration and loss of myelin, not as a result of, or in association with, inflammation but due to some failure of myelinating cells to sustain and maintain their sheaths in an intact and ordered condition. The implication is that sheaths are initially formed, but then deteriorate, leaving their axons intact for a time at least. The use of terminology in this area is of course somewhat arbitrary. For our purposes, **primary demyelination** is taken to imply the removal from around intact axons of structurally and chemically normal myelin, usually by macrophages and often in an inflammatory setting. The term **leukodystrophy** is applied to diseases with a heritable basis, early onset in life, lack of inflammation, symmetry of lesions, and in which some inherent qualitative defect in myelin (*dysmyelinogenesis*) leads to its dissolution and removal. **Myelinolysis** refers to the initial disruption of myelin structure by extensive decompaction of lamellae as a prelude to its breakdown and removal, and as such could be involved in many processes. There is scope for considerable overlap and liberal interpretation of definitions. The diseases discussed in this section will not include any which could easily be classified as demyelinating, in the sense indicated above, or as malacic in the sense indicated elsewhere in this chapter, in spite of the propensity for focal softening and even cavitation in some of the leukodystrophies.

Globoid cell leukodystrophy

This disease, also known as *galactocerebrosidosis*, *galactosylceramide lipidosis*, and *Krabbe disease*, has been documented in the Cairn and West Highland White Terrier, miniature Poodle, Bluetick Hound, Bassett Hound, Beagle, domestic cat, and polled Dorset sheep. It is due to a genetically determined deficiency, usually autosomal recessive, in the activity of lysosomal *galactocerebrosidase*, and as such falls within the *sphingolipidosis group of lysosomal storage diseases*. However, it has special characteristics that make it more appropriate to be discussed as a leukodystrophy, and it was so named before the lysosomal defect was recognized. Neurologic impairment begins at an early age and progresses rapidly to a fatal conclusion. The enzymic deficit blocks the catabolism of the galactocerebrosides (galactosylceramides). These compounds are major components of myelin, and the disorder thus principally affects the metabolic well-being of oligodendrocytes and Schwann cells.

Early in the disease, during active myelination, the myelinating cells store galactocerebroside within lysosomes. However, the enzyme is also involved in the breakdown of other metabolites, most notably galactosylsphingosine (psychosine), which is also normally synthesized by oligodendrocytes. This substance is highly cytotoxic to oligodendrocytes when it accumulates, and causes their extensive degeneration and death. Myelination ceases and formed myelin degenerates. During this process, macrophages accumulate to ingest the degenerate myelin but are also unable to degrade galactocerebroside. They give rise to the distinctive, swollen, PAS-positive "*globoid cells*," which form large cuffs around blood vessels in the central white matter, and are also found in the leptomeninges and in the endoneurium of peripheral nerves (Fig. 3.90). Their presence at the latter site is useful for premortem diagnosis by nerve biopsy. At end-stage, the pathology of the disease is marked by the presence of these cells, together with diffuse demyelination, axonal loss, and dense astrogliosis in the brain and spinal cord.

Dalmatian leukodystrophy

Reported only from Norway, this cavitating leukodystrophy has a probable autosomal recessive inheritance and a clinical onset at around 3–6 months of age. The signs are progressive and variable, and

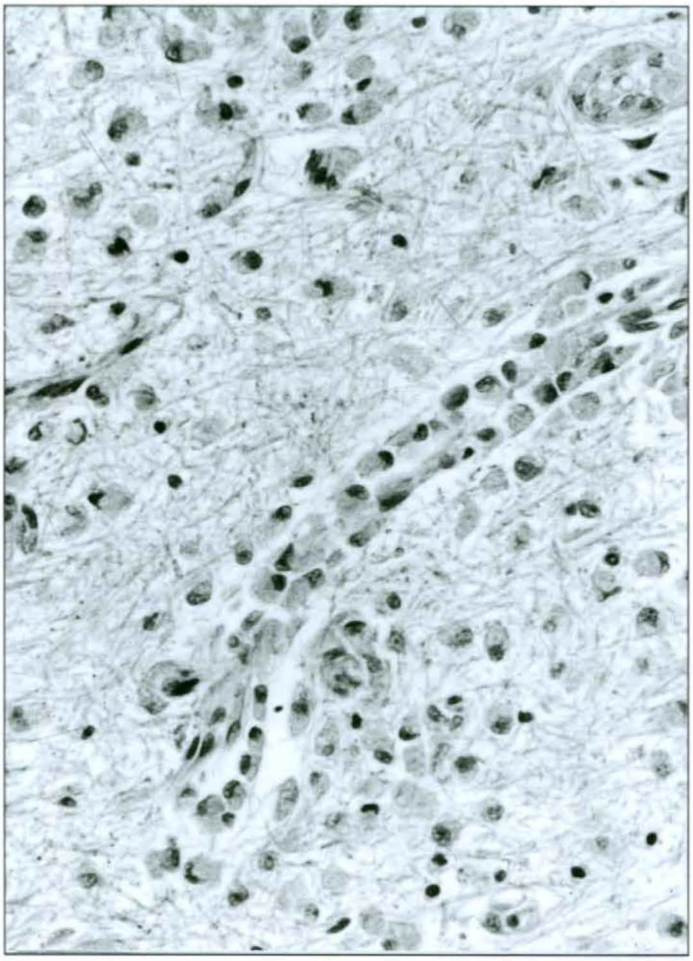

Figure 3.90 Macrophages, filled with degenerate myelin products, aggregating around vessels in **globoid cell leukodystrophy** in a Cairn Terrier.

include either gait deficit or visual impairment or both. The former begins as pelvic and then thoracic limb ataxia and dysmetria, with eventual paraparesis. Mental status remains unimpaired throughout.

The neuropathology is characterized by *bilateral, symmetrical, focal areas of intense loss of myelin* in the centrum semiovale, corpus callosum, internal capsule, caudate nucleus, optic nerves, and thoracic cord white matter adjacent to the ventral horns. Initially axons remain intact. The myelin destruction appears to begin with lamellar vesiculation followed by phagocytosis, and gitter cells are abundant. Fibrous astrogliosis becomes prominent around advanced plaques. The intensity of the process is variable, but lesions are usually visible macroscopically in the central cerebral white matter, appearing as gray depressed areas or, in advanced cases, as cavitations. In some animals, the brain is small and the lateral ventricles enlarged.

Afghan Hound hereditary myelopathy

The initial reports of this disease described acute necrotizing myelomalacia, but subsequent studies indicated a mechanism involving *florid myelinolysis and cavitation*, but essentially sparing axons. It has an apparent recessive inheritance, pre-adult onset, symmetry of lesions, and is noninflammatory, all of which allow for classification as a leukodystrophy. The fundamental pathomechanisms remain undefined. The age of clinical onset is between 3 and 12 months, and caudal ataxia and weakness progress rapidly to paraplegia in a few days. Within 2 weeks there is thoracic limb weakness and phrenic paralysis. A similar syndrome is reported in Kooiker dogs.

The lesions (Fig. 3.91A, B) are focused in the *thoracic spinal cord* where cribriform and spongiotic changes are present in all funiculi (Fig. 3.91B) and are grossly discernible as discoloration and softening or cavitation. Caudally the lesions extend into the lumbar segments, but only in the ventral funiculi; they extend cranially to the midcervical level in the dorsal and/or ventral funiculi (Fig. 3.91A). In some cases there may be lesions in the superior olivary nuclei.

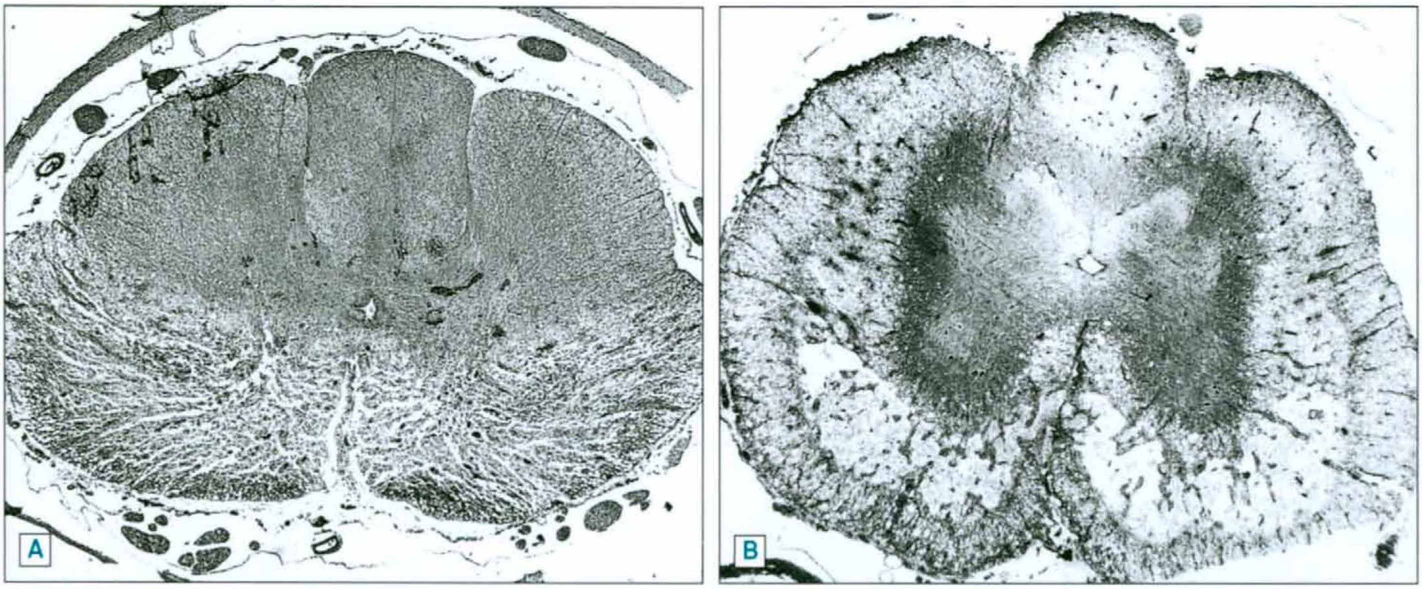

Figure 3.91 Afghan myelopathy. A. Caudal cervical spinal cord. **B.** Thoracic spinal cord.

The sparing of axons is reflected in the paucity of distal Wallerian degeneration in tracts ascending or descending through the lesions.

Light and electron microscopic examination has revealed initial vacuolation of myelin by splitting of lamellae at the intraperiod line and expansion of the extracellular space. Fragmented and degenerate myelin is phagocytosed by gitter cells, leaving surviving axons and reactive astrocytes within microcavities. The cavities are traversed by blood vessels associated with delicate glial strands. Some focal axonal swelling and disintegration are evident, but myelinolysis following vacuolation is the dominant change.

Rottweiler leukoencephalomyelopathy

Some difficulties arise in classifying this disease as a true leukodystrophy, as the onset is delayed until after 12 months of age. However, there is the probability of autosomal recessive inheritance, and the lesions involve progressive noninflammatory symmetrical myelin degeneration and removal. Clinically there are slow, but relentlessly progressive, ataxia, hypermetria, and paresis of all limbs, especially the forelimbs, and ultimately severe proprioceptive loss.

The principal locus of the disorder is the *cervical spinal cord*, which on gross inspection may have extensive dull white discoloration of the dorsal and lateral funiculi. Histologic examination reveals lesions to be present in these funiculi, and also in the deep cerebellar white matter and in other regions of the cord. Lesions are marked by significant loss of myelin (Fig. 3.92), with preservation of most axons, either naked or thinly myelinated, and fibrous astrogliosis. There is some evidence that vesicular degeneration of myelin is an early change. In the cervical cord, there may additionally be focal microcavitation deep within the affected areas, edema, numerous gitter cells, and vascular prominence. A narrow rim of intact white matter usually survives immediately deep to the glia limitans. These severe changes may on occasion extend into the thoracic cord and pyramidal tracts.

Fibrinoid leukodystrophy (Alexander disease)

This rare and unusual *myeloencephalopathy*, analogous to Alexander disease in humans, has been reported in the Scottish Terrier, Labrador Retriever, Miniature Poodle, and sheep. The clinical signs of progressive tetraparesis and ataxia are associated with spongy white matter lesions, astrocytic gliosis, and accumulation of eosinophilic refractile bodies (*Rosenthal fibers*) within the processes of astrocytes disposed around blood vessels in the CNS.

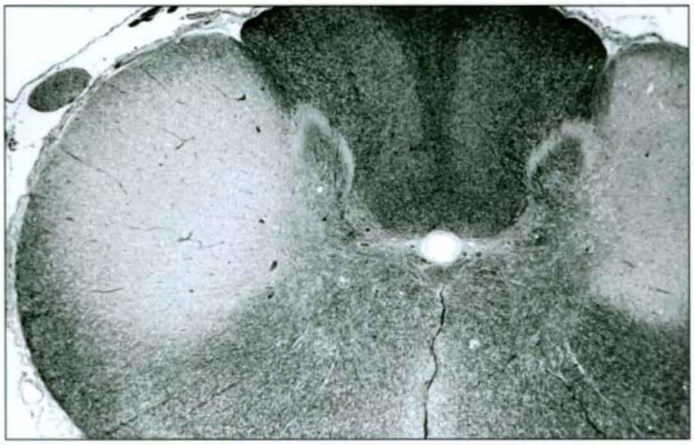

Figure 3.92 Bilateral demyelination of lateral funiculi in the cervical spinal cord in **Rottweiler leukoencephalomyelopathy**. (Courtesy of RF Slocombe.)

Progressive spinal myelinopathy of Murray Grey cattle

Reported in calves of the Murray Grey breed in Australia, this disease may afflict animals at birth, or not become expressed until about 12 months of age. The clinical syndrome is one of spinal ataxia, manifested as incoordination of the pelvic limbs and lateral swaying of the hindquarters at rest. A consistent sign is collapse of one hindlimb, with a tendency to fall to one side. These pelvic limb deficits are progressive and lead to increasing impairment. A similar condition is reported in Simmental calves.

The characteristic lesions are microscopic only, symmetrical, and consistently occur in the *lateral and ventral funiculi throughout the spinal cord*. There is a *distinct deficit of myelin* in the lateral funiculi beneath the dorsal root entry zone, and in ventral funiculi adjacent to the ventral median fissure. In some cases, the fasciculi gracilis and cuneatus are also involved. The process appears to involve ballooning degeneration of myelin sheaths, which may impart a distinctly spongy appearance to the lesion. The progressive loss of myelin is not accompanied by large numbers of myelinophages, and Wallerian degeneration is minimal. There is substantial astrogliosis and thickening of the glia limitans.

In the brain, similar white matter lesions may be found in the spinocerebellar tracts, tectospinal tracts, and medial longitudinal fasciculus. Some neurons undergoing central chromatolysis are present in ventral spinal gray matter, Clarke's column, the red nuclei, lateral vestibular nuclei, reticular formation, and cerebellar roof nuclei.

Progressive ataxia of Charolais cattle

This disease is pathologically unique amongst the domestic species, and appears to represent a progressive inability on the part of some oligodendrocytes to maintain the paranodal extremities of their myelin domains; it may represent oligodendroglial dysplasia. The disease is presumably genetically determined, but has been reported in three-quarter crossbred animals. Both sexes may be affected and clinical signs become evident between 8–24 months of age.

The clinical spectrum involves increasing ataxia and dysmetria, head tremor and some tendency to aggressive behavior. The mental status remains bright and alert. Affected females often urinate in a series of short spurts. The clinical signs progress slowly but steadily, and the animals can be expected to become recumbent by 3 years of age at the latest.

There are no specific gross lesions, but the microscopic findings are distinctive and unprecedented, and are located mainly in the cerebellar white matter and peduncles, the internal capsule, corpus callosum, optic tract, lateral lemniscus, median longitudinal fasciculus, pontine decussation, and ventral and lateral funiculi of the spinal cord. In these white matter tracts, there are *multifocal, granular eosinophilic plaques* about 30 μm in diameter (Fig. 3.93). The plaques are traversed by axons and stain with many, but not all, of the features of normal myelin. There is no sign of myelin degradation or

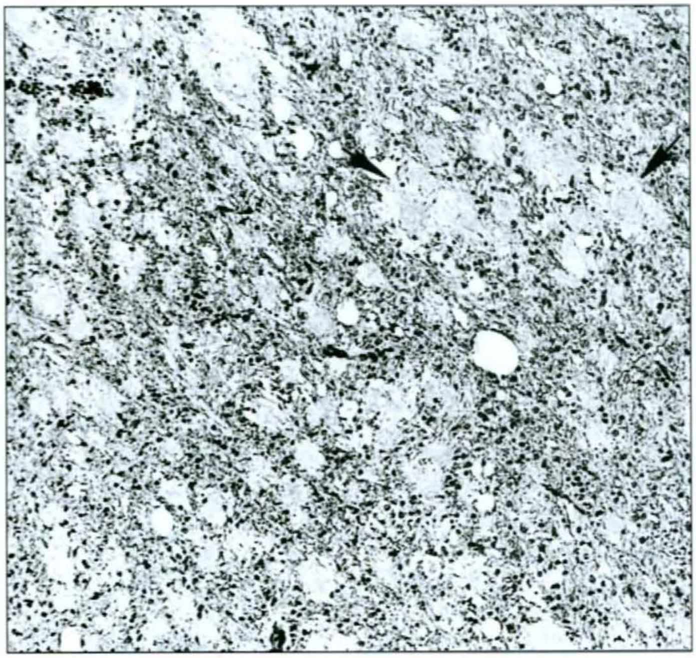

Figure 3.93 Plaques in white matter (arrows) in **progressive ataxia** of Charolais cattle.

phagocytosis. Immediately around the plaques there appears to be an increased number of astrocytes and oligodendrocytes. Some very minor axonal degeneration may be found in some plaques and in normal white matter. It has been concluded that early plaques can be distinguished from old ones. Electron microscopy reveals them to be complex. The ultrastructure suggests great disorder at the myelin paranodes, with relatively normal internodal regions. New plaques reveal axons encompassed, near abnormally long nodes of Ranvier, by hypertrophied oligodendrocyte tongues and processes within a thin myelin sheath. Old plaques contain demyelinated axons (some of which are swollen), surrounded by disorganized myelin lamellae and masses of oligodendrocyte processes. It is suggested that each plaque represents the territory of one oligodendrocyte, and reflects its failure to maintain normal paranodal myelin loops, in the course of which a massive hypertrophy of its processes occurs.

Multifocal symmetrical myelinolytic encephalopathy of Simmental, Limousin, and Aberdeen Angus calves

Reports from Australia, New Zealand, the USA, and Britain describe similar diseases in calves of these breeds, in which *spongy vacuolation of white matter progresses focally to lysis and cavitation*, but with the initial sparing of many axons and nerve cell bodies. In Limousin calves, such foci are present in the cerebellar peduncles and optic chiasm; in Simmental calves, in the internal capsule, caudate nucleus, putamen, periaqueductal white matter, lateral cuneate nucleus, and inferior olive. The distribution extends more widely in Aberdeen Angus and in some cases will include the spinal gray matter. In Limousins and Aberdeen Angus, blindness and dysmetria appear at about 1 month of age, and some animals develop seizures and opisthotonos, while others remain stable. In Simmentals, the clinical onset is between 5 and 8 months of age. There is progressive ataxia, weight loss and eventual dullness and emaciation, with death usual by 12 months of age. The pathogenesis of these diseases is uncertain; similarities exist with Leigh disease of humans, and familial syndromes in Australian Cattle Dogs and Alaskan Huskies.

Feline spinal myelinopathy

An adult-onset condition involving progressive noninflammatory myelin loss in the spinal cord has been reported in cats in California. The pathomechanism is undefined. Myelin deficiency develops in the absence of significant axonal degeneration, and is accompanied by astrogliosis. The lesions occur in all funiculi, and are most intense in the thoracic and lumbar segments.

Tibetan Mastiff hypertrophic neuropathy

This recessively inherited condition affects *peripheral nerves* and is considered to reflect a primary metabolic defect of Schwann cells, with failure to maintain myelin during axonal elongation in postnatal growth. The CNS is not involved. The clinical onset is consistently between 7 and 10 weeks of age. There is rapid progression of generalized weakness with hyporeflexia, slowed nerve conduction velocity, and ultimately tetraplegia in many cases.

In peripheral nerves and their spinal roots, there are changes consistent with *demyelination, remyelination, and endoneurial fibrosis*. Thus many axons are denuded, while some have a thin sheath; proliferating Schwann cells form onion bulbs, and endoneurial collagen is prominent. Axonal degeneration is not a feature. The most characteristic features are revealed ultrastructurally. Schwann cell cytoplasm contains *dense accumulations of 6–7-nm filaments*, occurring at sites where the myelin sheath has separated along the major dense lines, and in the adaxonal cytoplasm. There are also anomalous incisure patterns within the sheaths, with many incisural openings staggered through the entire thickness of the sheath.

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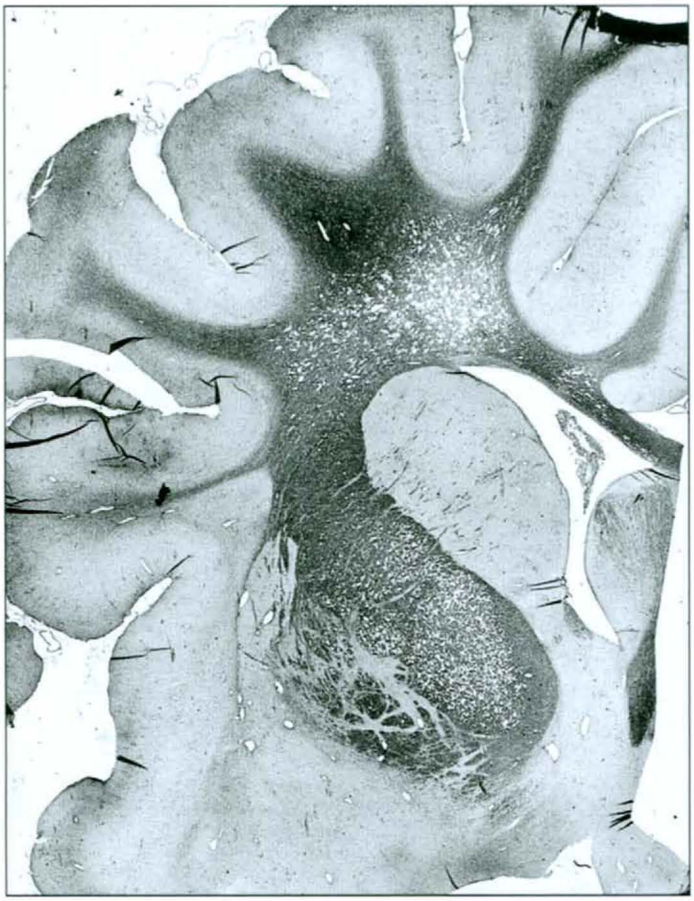

Figure 3.94 Status spongiosus of central cerebral white matter in spongiform myelinopathy in a silver fox. (Reprinted with permission from Hargen G. Bjerkas I. Vet Pathol 1990:27:187.)

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Spongiform myelinopathies

There is a group of diseases in which the dominant pathological feature is *dramatic vacuolation of myelin* that often occurs without any overt indication of large-scale myelin breakdown or phagocytosis. There is still the familiar problem, however, of deciding how to categorize some diseases that have overlapping features of myelin vacuolation and myelin degeneration and it is acknowledged that some of the conditions described below could be placed elsewhere.

Myelin vacuolation is one of several different morphological changes encompassed by the term "**status spongiosus**" of nervous tissue (Fig. 3.94). Although producing a striking light microscopic picture when well developed, electron microscopy is required for its fine definition (Fig. 3.95A, B, C). Histologic changes suggestive of this lesion are a fairly common postmortem artifact.

Vacuolation of myelin may come about in several ways, the most frequent of which involves *separation of lamellae along the intraperiod*

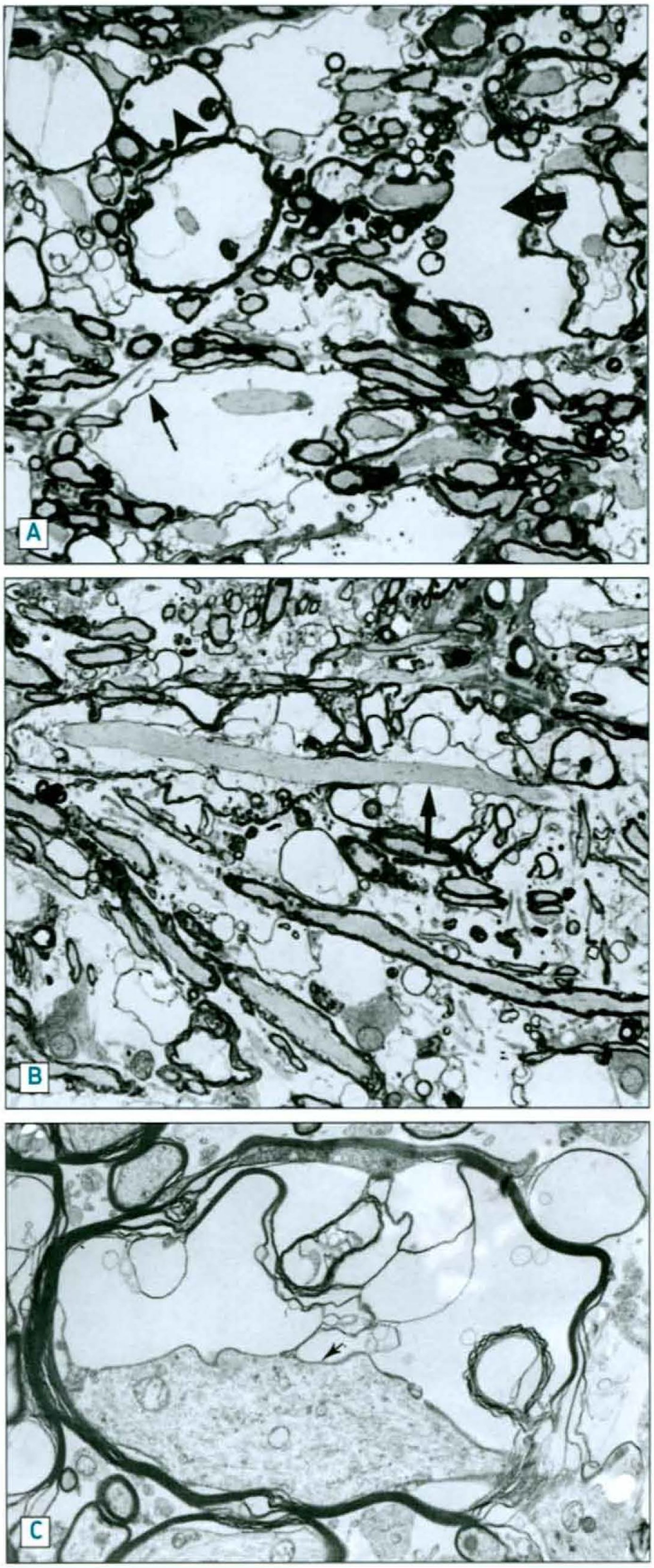

Figure 3.95 Spongiform myelinopathy in a silver fox. **A.** Note thin and dilated myelin sheath around normal axon (small arrow), empty myelin-bound vacuole (arrowhead), and expanded extracellular space (large arrow). **B.** Disrupted myelin around normal axon (arrow). **C.** Electron micrograph demonstrating periaxonal vacuolation and split myelin lamellae around normal axon (arrow). (Reprinted with permission from Hagen G. Bjerkas I. Vet Pathol 1990:27:187.)

line, thereby reopening the extracellular space originally obliterated within the spiraling processes of the myelinating cell. This mechanism can produce vacuoles within the sheath at multiple levels and multiple loci along an internode. In experimental situations, this type of vacuolation has been associated with an increase in tissue water and electrolytes, consistent with simple edema, and accounts for the brain swelling which may occur in severe cases. The vacuoles therefore contain no stainable material. A variety of associated functional disturbances has been described but, remarkably, the lesion may have little functional impact, even when quite intense and prolonged. It seems likely that the myelin lesion is one facet of a complex metabolic disturbance, whose ramifications vary according to basic causes. In humans, the classical disease of this type is *Canavan's disease*, with congenital and infantile forms due to an autosomal recessive trait, and a rare juvenile form with no demonstrable familial association.

Vacuolation may also arise by separation of lamellae at the major dense line, reopening the intracellular compartment of the myelinating cell, or by ballooning of the periaxonal space. Depending on the particular disease, the vacuolation of the myelin may or may not be accompanied by structural changes in other elements of the tissue, including the myelinating cell bodies, and, in general, these are more likely to occur if vacuolation has been prolonged. In some instances too, prolongation of the process is associated with a reduced quantity of myelin. In those cases where there is no indication of myelin degradation, its steady withdrawal by the normal catabolic pathways with concurrent suppression of synthesis is implied.

The spongiform myelinopathies of animals fall into two broad groups: those that are **idiopathic**, and those that have a defined **metabolic** or **toxic** basis.

Idiopathic spongiform myelinopathies

This group is documented in a small number of case reports involving calves, pups, and kittens, and most are suspected to have a heredofamilial basis. Several conditions involving newborn **Hereford** calves have been described over the years, with some conflicting and confusing aspects. Earlier reports proposed a condition, dubbed "hereditary neuraxial edema," to be a single entity with a variable clinical and pathological expression – the pathogenesis of several of these conditions has been unraveled, including *inherited congenital myoclonus* and *branched chain ketoacid dehydrogenase deficiency* (BCKD). The former is recognized in several countries in newborn polled Herefords that have violent myoclonic muscle spasms that prevent them from standing. There are no structural lesions, but biochemical studies have revealed an absence of glycinergic receptors on spinal inhibitory neurons. The functional disturbance is thus analogous to strychnine poisoning and is equally fatal. BCKD is discussed below.

From New Zealand, a disease of **horned Hereford** calves has been named *congenital brain edema*. Newborn calves are unable to stand after birth and have coarse tonic muscle contractions. Vacuolation of myelin is diffuse, extends into the gray matter, and is accompanied by elevated brain water content. Hydropic degeneration of astrocytes and expansion of the extracellular space are detectable ultrastructurally. There is also considered to be a deficiency of myelin.

In Britain, a very similar picture is recorded in **polled Hereford** calves. There may remain an entity in Hereford calves, severely neurologically impaired at birth, in which myelin vacuolation is extensive in the CNS, is confined to the white matter, and is not associated with hypomyelinogenesis. For the time being, such cases could be classified as hereditary neuraxial edema in the absence of any demonstrable metabolic disturbance suggestive of BCKD or other aminoacidopathy.

In **Samoyed** pups, a generalized tremor syndrome occurs in the first few weeks of life, with a severe and ultimately fatal outcome. Myelin vacuolation is diffuse throughout the CNS and there is no change in any other tissue element.

Labrador pups are described to have initial episodes of extensor rigidity, tremor, and dorsal flexion of the neck at 4–6 weeks of age, and then progressive dysmetria. Intense vacuolation of myelin is confined to the white matter in the CNS, but occurs to a mild degree in peripheral nerves as well. Throughout the CNS, the myelin lesion is accompanied by fibrous astrogliosis and prominence of capillary blood vessels. A similar condition with a familial pattern (familial spongiform leukodystrophy) is recognized in **Shetland Sheepdogs**. Clinical signs usually start at 3 weeks of age and include progressive seizures and dysphagia. Spongiform degeneration is most prominent in the cerebellum and corona radiata.

In a **Silky Terrier** pup, a myoclonic syndrome is described, with vacuolation of myelin occurring in the brain but not the spinal cord, together with the presence of Alzheimer type II astrocytes. In **Egyptian Mau** kittens, there is progressive ataxia and hypermetria; vacuolar change extends from white into gray matter throughout the CNS. Spongiform CNS myelinopathy has been reported in a group of related **African dwarf goats**.

Spongiform myelinopathy of silver foxes

A hereditary nervous disease occurs in farmed silver foxes in Norway. The presenting sign is hindlimb ataxia appearing between 2–4 months of age, and progressing over the next 4–8 weeks, after which time clinical improvement seems to occur.

No gross lesions are present, and histologically there is *symmetrical myelin vacuolation* affecting white matter of the cerebrum, cerebellum, brain stem, and spinal cord (Fig. 3.94). The extent and severity of vacuolation vary from case to case. In long-standing cases, vacuolation seems to resolve to a large extent, with residual intense astrogliosis.

Ultrastructurally, early features of the disease include intramyelinic vacuolation due to lamellar separation at the intraperiod line, large cytoplasmic vacuoles in oligodendrocyte cytoplasm, demyelination, expansion of extracellular space, and astrocytic hypertrophy (Fig. 3.95A, B, C). Late in the disease, there is evidence of remyelination in gliotic areas.

Toxic/metabolic spongiform myelinopathies

Hepatic and renal encephalopathy

Endogenous intoxications associated with hepatic and, to a lesser extent, renal failure, are recognized to induce brain lesions of this type, the former quite frequently, the latter less so.

The familiar clinicopathologic term, *hepatic encephalopathy*, designates a complex autointoxication, in which accumulations of ammonia and other metabolites reflect the inability of the liver to carry out its normal detoxifying role. This may in turn reflect reduced liver mass, portosystemic shunting, or both. *The neural lesions of hepatic encephalopathy are several and variable.*

Clinical evidence of nervous dysfunction may be accompanied by extensive and well-developed *spongy vacuolation of myelin* (Fig. 3.96), which tends to be most intense at the junction of the cerebral cortex and adjacent white matter, and often around the deep cerebellar nuclei. The lesion is to be expected in ruminants with subacute or chronic phyto- or mycotoxic liver injury, and in small animals with developmental portosystemic shunts or acquired liver disease. In sheep, copper toxicosis may lead to massive myelin vacuolation. There may also be *Alzheimer type II astroglial cells* (see Fig. 3.16) although, with the notable exception of the horse, this is generally less a feature in animals than in humans. In the horse, hepatic encephalopathy is characterized by the presence of Alzheimer type II cells, with no significant myelin vacuolation.

Although myelin vacuolation may be induced experimentally with ammonia alone, the syndrome is *not simple ammonia intoxication*, but may also involve perturbed monoamine neurotransmission, imbalance between excitatory and inhibitory amino acid neurotransmission, and accumulation of an endogenous benzodiazepine-like substance.

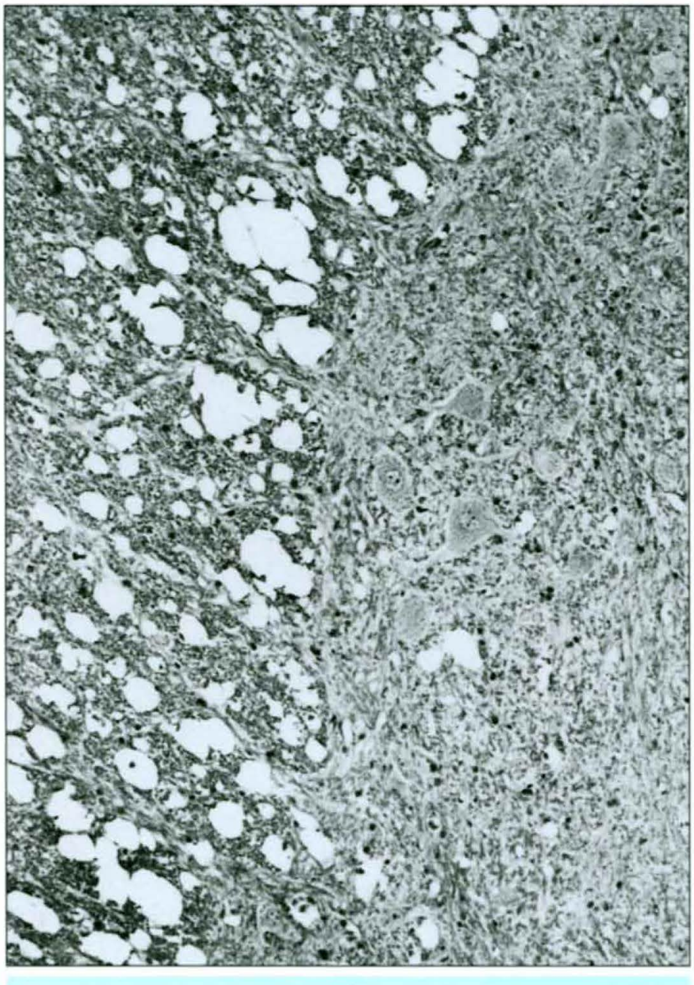

Figure 3.96 Intramyelinic edema and spongiform change surrounding spinal gray matter in **hepatic encephalopathy** in a sheep.

Branched chain α-ketoacid decarboxylase deficiency

This disease, also known as **maple syrup urine disease**, has been described in Hereford, polled Hereford, and polled Shorthorn calves, and is inherited as an *autosomal recessive* character. This mutation causes deficiency of the mitochondrial branched-chain α-ketoacid dehydrogenase (BCKD) complex. Deficiency of BCKD leads to the accumulation of the branched-chain amino acids leucine, isoleucine, and valine and their respective ketoacids, ketoisocaproic, keto-β-methylvaleric, and ketoisovaleric acids. The molecular mechanism of the intoxication remains to be precisely defined, but probably involves toxic metabolites.

The disease may be expressed in utero, and calves become dull and recumbent by 2–4 days of age, and finally develop opisthotonos. The urine in many cases has the smell of maple syrup, the characteristic which gave the disease its common name in affected children. Branched chain amino acids are found to be in abnormally high concentrations in plasma, cerebrospinal fluid, and tissues.

Pathologically, there may be gross evidence of brain swelling expressed as flattened cerebral gyri and, histologically, *severe spongy vacuolation of myelin in the CNS.* The spongiotic change is pronounced in the large myelinated tracts of the cerebral hemispheres and cerebellum (Fig. 3.97), and in myelinated tracts abutting brain-stem nuclei and spinal gray matter. Most of the spinal white matter is not affected. In cases that survive for a week or more, a modest number of axonal spheroids may be found in affected white matter. The

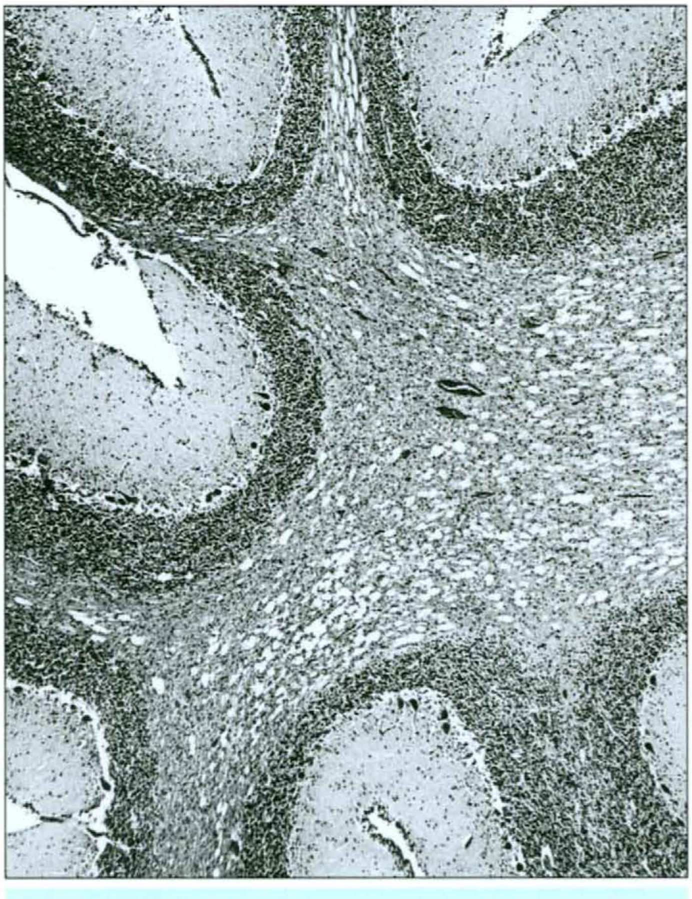

Figure 3.97 Status spongiosus in the central cerebellar white matter of a calf with **maple syrup urine disease**.

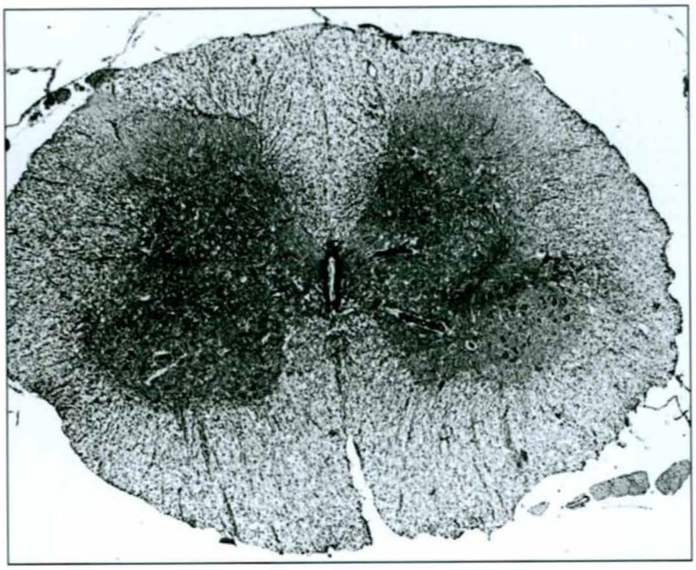

Figure 3.98 Intramyelin edema and diffuse spongiform change in the spinal cord white matter of a dog with **hexachlorophene poisoning**.

brain water content is elevated, and the electron microscope reveals splitting of myelin lamellae at the intraperiod line.

Hexachlorophene toxicosis

Hexachlorophene is a polychlorinated phenolic compound with useful topical antiseptic properties, and is sometimes given orally against *Fasciola hepatica*. Intoxication has usually been associated with repeated application to the skin of very young animals, whence absorption is rapid, and systemic accumulation may cause effects within several days. The clinical features of severe intoxication are shaking, shivering, excitement, tonic-clonic convulsions, and terminal coma.

The major lesion is *spongy vacuolation of central and peripheral myelin* (Fig. 3.98), with lamellar splitting at the intraperiod line, and no associated degenerative or reactive changes. This lesion may persist for many weeks, even after clinical signs have abated. Under experimental conditions, vacuolation of the retinal photoreceptor outer segments is also evident, but this has not been a reported feature of accidental intoxication.

Halogenated salicylanilide toxicosis

The halogenated salicylanilides are long-acting anthelmintics used in sheep and goats, and include closantel and rafoxanide. Accidental overdosing has led to intoxication expressed as ataxia, depression, and blindness, which may be permanent. The response appears to be inconsistent, and unknown predisposing factors are possibly involved.

Microscopically, there is *spongy vacuolation of myelin in both the CNS and peripheral nerves*, without any accompanying reaction or degeneration. The lesion in the brain is most intense in the brain stem. This change may persist for several weeks. Acute degenerative changes may be found in the optic nerves and in the retinal ganglion cell layer, where hemorrhage has also been described. The syndrome has some similarities to stypandrol poisoning, described below.

Stypandra toxicosis

The binaphthalene tetrol, **stypandrol**, is the toxic principle of the Australian liliaceous *Stypandra* sp. ("blindgrass") and also of the Asiatic *Hemerocallis* sp., hence the alternative name **hemerocallin**. Ingestion of toxic strains of such plants by grazing mammals or birds has an acute effect, usually manifested as moderate depression and blindness, although a high dose may produce prostration and death.

In acutely affected animals, *myelin vacuolation* caused by lamellar splitting at the intraperiod line is extensive throughout the CNS, and may persist for many weeks or even months, without apparent functional effect. Blindness, however, tends to be permanent and is associated with Wallerian degeneration of the optic nerves and tracts, and degeneration of retinal photoreceptors. Degeneration of the optic nerves may be the result of compression within the optic canals secondary to swelling caused by the myelin edema, and there may be a separate direct toxic effect on the photoreceptors.

Tylecodon toxicosis

Tylecodon wallichii is the cause of **krimpsiekte, a** neurotoxicosis of small ruminants in South Africa. The toxic principle is **cotyledoside**, a neurotoxic bufadienolide that can produce either acute or chronic poisoning, the latter probably as a cumulative effect of the toxicosis. Affected animals assume a posture with back arched, display torticollis, and then prolonged paralysis. Edema and formation of large vacuoles are particularly prominent in the optic radiations and thalamic white matter. A similar syndrome has been reported in small ruminants poisoned by *Ornithogalum toxicarium* in Namibia.

Helichrysum toxicosis

The many plant species of this genus are widely distributed, and distinct syndromes of toxicity in ruminants occur in Australia and South Africa: *Helichrysum blandoskianum* in Australia produces acute hepatic necrosis, presumptive hepatic encephalopathy, and spongiform change in the white matter of the brain stem, with a high mortality rate; *H. argyrosphaerum* in South Africa is associated with paresis, paralysis, and permanent blindness.

Intense spongy vacuolation of myelin in the brain is sufficient to cause grossly visible swelling of the optic nerves and chiasm, and gelatinous swelling of the periventricular white matter and corpus callosum. Focal hemorrhages may occur in the optic nerves and chiasm. Microscopically, the spongiform myelin lesion in the optic nerves is accompanied by Wallerian degeneration, suggesting compression within the optic canals. Myelin vacuolation is less severe in the spinal cord and peripheral nerves. The syndrome has similarities to stypandrol toxicosis.

Diplodia toxicity (diplodiosis)

Diplodiosis is a mycotoxicosis of cattle and sheep grazing on maize lands towards the end of the growing season in various countries in southern Africa and in Argentina. The causative fungus is *Diplodia maydis*. The fungus is extremely fetotoxic and causes neonatal mortality in lambs of ewes ingesting the toxin during late pregnancy. The spongy change is due to separation of myelin along the intraperiod line and may progress to lysis of myelin.

Bromethalin toxicity

Diffuse white matter spongiosus is described in dogs and cats given a single oral dose of this highly neurotoxic rodenticide.

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Spongiform encephalomyelopathies

The term "**status spongiosus**" applies to a variety of lesions in which the microscopic appearance of the neural tissue is transformed by numerous vacuoles or microcavities. As discussed above, one form of this state results from the vacuolation of myelin, and is the most common. In this section will be discussed diseases in which the change results from either the *swelling of astrocytes* or the *vacuolation of neurons and neurites*. Astrocytic swelling may occur nonspecifically in, or adjacent to, any area of neural tissue acutely injured by trauma, or ischemia, or in which vascular permeability is disturbed. In the absence of such circumstances, its occurrence suggests some direct toxic or metabolic influence upon the astrocytes themselves. It can thus be a feature in hepatic encephalopathy. Spongiform vacuolation of neurons and their processes may occur to a limited degree as an incidental change in the red nucleus and nuclei of the caudal brain stem, and also as an artifact of fixation. It needs to be interpreted against this background, and is usually considered in the context of the diagnosis of scrapie and related conditions. It is distinctly different from the foamy vacuolation seen in the lysosomal storage diseases.

Prion diseases

Prions (the word "prion" is a combined form of "proteinaceous" and "infectious") are believed to be the cause of fatal neurologic diseases in humans and several other mammalian species. Prion diseases (*prionoses*) are characterized by chronic progressive fatal neurologic manifestations, with both sensory and motor deficits and spongiform encephalopathy, and result from accumulation of an abnormal isoform (PrP^{Sc}, Sc refers to scrapie) of the host prion protein (PrP). The gene (*PRNP*) encoding the prion protein is highly conserved in animals and humans. Transmissible prion diseases result from infection with an external PrP^{Sc} and accumulation of host-produced PrP^{Sc}, and are called **transmissible spongiform encephalopathies** (TSEs) to differentiate them from prion diseases originating from genetic mutation, e.g., familial Creutzfeldt–Jacob disease in humans. The prion protein is widely distributed in tissues but concentrated in nervous tissue where normal prion protein (PrP^{c}, c is for cellular) is a glycolipid-anchored glycoprotein located on the surface of neuronal plasma membranes; its exact function in mammals is unknown. PrP^{Sc} is not only membrane anchored, but it can also be demonstrated in lysosomes using immunohistochemistry and electron microscopy. PrP^{Sc} almost never triggers an immune response.

Prion diseases in animals include *scrapie* of sheep and goats, *bovine spongiform encephalopathy* (BSE), *chronic wasting disease* (CWD) of deer and elk, *transmissible mink encephalopathy* (TME), *feline spongiform encephalopathy* (FSE), and *exotic ungulate encephalopathy* of captive wild ruminants. Human prion diseases include *kuru; Creutzfeldt–Jacob disease* (CJD), which has four types, sporadic CJD, familial CJD, iatrogenic CJD, and variant CJD (vCJD); *Gerstmann–Straussler–Scheinker disease* (GSS); and *fatal familial insomnia* (FFI). The inherited prion diseases of humans are all of dominant inheritance and all are associated with coding mutations of the *PRNP* gene. Interest in the TSEs has increased greatly for several reasons. First, the discovery that these diseases may result from an infectious protein agent that is lacking nucleic acids contradicted the central dogma of modern biology that all living organisms use nucleic acids to reproduce. Second, the appearance of BSE in Great Britain and other parts of the world, and its presumed relationship to vCJD, triggered both scientific and public interest in these diseases. Despite extensive work on prions, our understanding of their pathogenesis and etiology is incomplete.

The *protein-only hypothesis* is currently the most accepted hypothesis for the pathogenesis of prion diseases. This hypothesis states that: (1) PrP^{Sc} is the etiologic agent of TSEs and other prion diseases; (2) the transformation of α-helical PrP to its misfolded β-pleated sheet isoform, the PrP^{Sc}, requires either a genetic predisposition or requires the help of unknown protein cofactors (protein X) that act as a molecular chaperone for this conversion; (3) PrP^{Sc} triggers the PrP gene (*PRNP*) to produce more PrP^{Sc} (self-replicating protein); and (4) PrP^{Sc} can be infectious itself and can be transmitted to other hosts and cause disease (transmissible protein). There is a species barrier to transmission of the TSE agent, but it is not complete. Less-accepted theories for the etiology of prion diseases include the virion or virus hypothesis, and the autoimmunity/allergic hypothesis, which states that TSEs could be autoimmune diseases resulting from ingestion of feedstuffs contaminated with certain bacteria, e.g., *Acinetobacter* spp., that show molecular mimicry between bacterial components and CNS tissue. *Spiroplasma mirum*, a cell-wall-deficient bacterium, has also been proposed as a trigger for TSEs.

The mechanism by which PrP^{Sc} causes nerve cell degeneration is complicated and several mechanisms have been proposed including: (1) mechanical destruction of nerve cell membranes from the excessive accumulations; (2) lysosomal accumulation of PrP^{Sc} may trigger apoptosis by release of cathepsin D into cytosol; and (3) PrP^{Sc} may be a direct neurotoxin. There are multiple strains of prions in scrapie of sheep, the strains distinguished by biological properties in defined strains of mice determined especially by incubation periods and lesion profiles, and the nature and patterns of neuropathological changes. In sporadic disease in humans, different strains or types of the agent are beginning to be related to clinical phenotypes, and in some of the diseases in animals, additional to scrapie, there is evidence for strain variation. The agent of BSE is exceptional in being, so far, monotypic. Prions have unusual resistance to physical and chemical agents and this unusual resistance makes inactivation of prion infectivity very difficult.

The pathogenesis of these diseases is further complicated by genetic variation among hosts whereby homozygosity at particular codons is known to confer susceptibility to the natural or experimental disease and heterozygosity confers survival advantage, although this is expressed mainly as prolonged incubation time. In heterozygotes, the outcome of exposure may be further modified by other polymorphisms. For example, sheep carry two alleles of the PrP gene and five different allelic variants. Sheep breeds that are homozygous for the variant VRQ/VRQ (valine (V) at codon 136, arginine (R) at codon 154, glutamine (Q) at codon 171) are the most susceptible to scrapie, whereas sheep with the ARR (alanine (A)) variant are most resistant and do not develop overt disease.

The transmission of prions between individuals or species in spontaneous disease remains a mystery, except for kuru for which there is strong evidence for ritualistic cannibalism, and vCJD for which there is compelling evidence of oral transmission from BSE. It is noteworthy that all cases of vCJD and kuru are homozygous for methionine at *PRNP* codon 129. The oral route is probably important for BSE, vCJD, and TME. The mode of transmission of scrapie and CWD remains unclear.

There are *no gross abnormalities* in the brain other than those expected in older animals with a wasting disease. Some elements of the histological picture are consistently present but variable in their neuroanatomical distribution and degree of expression. The most consistent pattern involves the medulla oblongata. Inflammatory change is absent. *Spongiform change is an important identifying feature.* It consists of small rounded vacuoles in the neuropil sometimes extending into neurites. The vacuoles in the neuropil may become confluent. The extracellular space is normal. The vacuoles are of variable distribution in cerebral gray matter and may be diffuse or clustered. The pathogenesis of the spongiform change is not known.

Neuronal vacuolation has long been the identifier for scrapie. It is a feature of the natural and experimental prion diseases of animals noted above. The vacuolation may occur in the soma, perikaryon, or neurites, but may not be accompanied by the spongy change described above. The empty vacuoles may be single, multiple, or in chains. Loss of neurons is difficult to assess and neuronophagia is absent. Descriptions of abnormal numbers of dark neurons may not be significant.

Astrocytic hypertrophy and hyperplasia varies in severity. Astrocytic nuclei may be paired or densely packed and may be without increase of fibers. Astrocytosis usually involves gray matter affected by spongiform change and may spread to adjacent white matter.

Although frequent and readily detected in kuru and in vCJD of humans, *amyloid plaques are sparse or absent in the animal diseases.* They may be difficult to demonstrate in hematoxylin-eosin-stained sections but are well demonstrated with Congo red and especially the periodic acid-Schiff method. The pathogenic prion isoform is subject to limited proteolysis to produce a smaller molecule, PRP27-30, which is protease resistant and deposited as intraneural amyloid in neurons and neuropil and around neurons and blood vessels.

Electron-microscopic examination of detergent extracts of infected brain reveals characteristic fibrils, termed "*scrapie-associated fibrils*" (SAF), which have PrP^{sc} as their major component. In many cases, plaques of this material may be present in the neuropil, as histochemically detectable amyloid deposits, and it can be detected more sensitively by specific immuno-staining as "scrapie immunoreactive amyloid." Cerebrovascular amyloidosis is also usually demonstrable in ovine scrapie.

Scrapie was the first prion disease to be recognized and described, around 1732 in the UK, and is endemic worldwide, except in Australia and New Zealand where it has been eradicated. The precise route of transmission is unclear but the disease spreads in an infectious-like pattern with rapid lateral communicability in a contaminated field. Infection mostly occurs around the time of birth, and probably via ingestion, but the question of vertical modes of transmission remains unclarified. The likelihood of ewe-to-lamb transmission is highly significant, in that a ewe could give birth to and infect several lambs before showing clinical signs. The scrapie agent can infect goats sharing the pasture with affected sheep; natural spread to species other than goats grazing the same pasture is not reported.

As discussed above, sheep breeds differ in their susceptibility to scrapie. The morbidity of the natural disease is low, except in some closed stud flocks, and clinically affected animals are usually in the 2–5-year age group. There is a long incubation period, and the disease, once clinically manifest, is progressive and ultimately fatal in from 10 days to several months. The agent proliferates initially in the lymphoid tissues and lower intestine, but may take up to 2 years to reach the nervous system. A further 2 years may lapse before clinical signs appear. The methods of spread of the agent between both cells and organ systems are still enigmatic, although there is good evidence that the nervous system is first infected via the splanchnic nerves, which carry the agent to the thoracic spinal cord. Once within the central nervous system, the presence of the agent seems to be of no consequence until it reaches "clinical target areas," where the expression of PrP^{Sc} in select neurons could presumably lead to progressive dysfunction.

Both clinical signs and pathologic changes are somewhat variable but, in general, affected sheep are initially alert but excitable, tremble when excited, and may have seizures. Later, paresthesias may appear, manifested as agitated rubbing against posts and trees, and nibbling at the feet and legs when lying down, behavior that gave rise to the colloquial name "scrapie." Self-trauma can cause extensive loss of wool

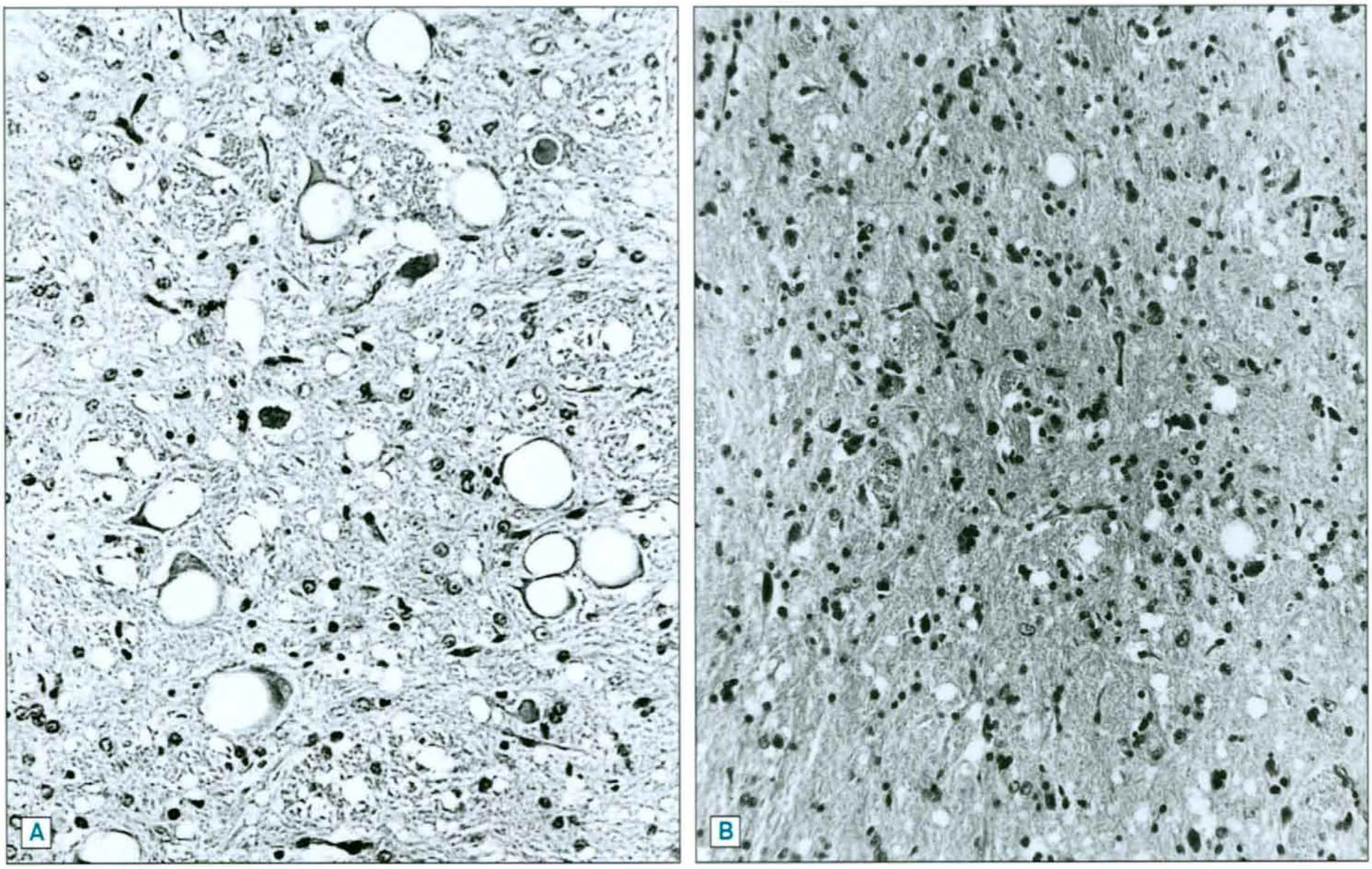

Figure 3.99 Scrapie in a sheep. **A.** Vacuolated neurons in the brain stem. **B.** Vacuolated neurons, loss of neurons, and gliosis in the medulla.

and abrasions of the skin. There is also progressive dysmetria and emaciation, and finally paralysis and death.

Apart from emaciation and self-trauma, there are no significant gross lesions, and no inflammatory changes. The most characteristic finding is the presence of *large intraneuronal vacuoles* in the medullary reticular, medial vestibular, lateral cuneate, and papilliform nuclei. The neuronal vacuolation may however be present throughout the brain stem and spinal cord (Fig. 3.99A). The vacuoles contain no stainable material and may considerably distort and displace the normal organelles. Although the vacuolated neurons do not generally appear degenerate in any other way, shrunken and apparently degenerate neurons may be found in mesencephalic, medullary, and deep cerebellar nuclei, and in the intermediolateral nucleus of the spinal cord. In late stages, neuronal loss may be evident, as well as diffuse fibrillary astrogliosis present mainly in the mesencephalon (Fig. 3.99B) and the molecular layer of the cerebellum. These lesions are bilaterally symmetrical, and the cerebral cortex is rarely involved (see Fig. 3.12A, B). *Spongy vacuolation of the neuropil* in gray matter is due to vacuolation of neuronal processes, but it is seldom a feature of the natural disease in sheep.

Bovine spongiform encephalopathy (BSE) causes cattle 3–6 years of age to become apprehensive, hyperesthetic, and dysmetric. The animals display fear and aggressive behavior, with progressive gait disturbances leading to frequent falling. Eventually, frenzied episodes or recumbency necessitate euthanasia. The origin of the rogue prion is still undetermined; however, ingestion of feed contaminated with infectious prion is the most likely origin for BSE. This contamination may have come from bone and meat meal from scrapie-infected sheep carcasses; however the BSE agent is different biologically than any of the known scrapie strains. Another source suggested is feed contamination with the remains of humans infected with CJD. Sporadic and genetically determined TSEs have not been detected in cattle. BSE is basically a food-borne epidemic; lateral or vertical transmission is highly unlikely. *The pathologic features of BSE resemble those of scrapie*. Scrapie-associated fibrils and immunoreactive amyloid are demonstrable. *Vacuolation of neuronal cell bodies and processes* is particularly prominent in the dorsal vagal, medullary reticular, vestibular, solitary, spinal trigeminal, and red nuclei, and is accompanied by moderate degrees of *spongiform change in neuropil*. The severity of neuropil and neuronal somatic vacuolation varies independently among anatomic locations, and the latter is sometimes accompanied by ceroid-lipofuscin granules. Occasional necrotic neurons and axonal spheroids may be present, as may mild astrogliosis.

Transmissible mink encephalopathy (TME) has caused high levels of morbidity and mortality in ranch mink in North America and northern Europe including Finland and Russia. The source of TME infection was not determined, but contamination of feed materials by the scrapie agent was suggested. The agent was experimentally pathogenic to a variety of wild carnivores, sheep, cattle, and primates. Neuronal vacuolation is sparse in this disease, which exhibits *spongy* *degeneration and astrocytosis* of parallel intensity. Neuronal vacuolation is mainly in brain stem. The lesions are especially well developed in the neocortex and amygdala, corpus striatum, caudodorsal thalamus, and medial geniculate bodies. The pathologic profile may differ between countries, perhaps due to strain differences of the agent or genotype of the captive mink.

Chronic wasting disease (CWD) of cervids affects mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and perhaps other cervids in several states in the USA and in western Canada. Although CWD was first diagnosed in the 1960s in captive cervids, it is now prevalent and possibly increasing among free-ranging and wild susceptible species and is considered to be the only TSE that occurs in free-ranging animals. The source of infection is unknown, but the disease is likely spread horizontally by ingestion of material contaminated with the CWD agent. CWD PrP^{Sc} is widespread not only in the CNS but also in various lymphoid tissues, including gut-associated lymphoid tissue (GALT). The presence of PrP^{SC} in GALT led to the presumption that PrP^{Sc} could be shed in feces causing pasture contamination and a health hazard to other animals or humans. Affected animals show slow progressive weight loss, dullness and depression, polydipsia and polyuria. Deer may show swallowing difficulty and esophageal dilation. Spongiform change, neuronal vacuolation, gliosis, and amyloid deposition are especially well developed in the olfactory tubercle, cortex hypothalamus, and vagal nuclei. Amyloid plaques are more frequently demonstrated in deer than in elk.

Feline spongiform encephalopathy (FSE) has been observed in domestic cats and captive felids in zoos. FSE was likely transmitted to captive felids by ingestion of BSE-infected cattle carcasses, and to domestic cats by ingestion of pet food contaminated with the BSE agent. The lesion pattern emphasizes the spongy change in deep laminae of cerebrum, the corpus striatum, thalamus, medial geniculate body, and cerebellar cortex. Neuronal vacuolation occurs mainly in medullary neurons but is not pronounced.

Transmissible spongiform encephalopathy of captive wild ruminants Several zoo members of the family Bovidae, e.g. nyala, Arabian oryx, and a bison, developed TSE at the time of the BSE epidemic in UK in association with ingestion of BSE-contaminated meat and bone meal.

Spongiform encephalopathy in nonhuman primates Lemurs and a rhesus macaque in France developed TSE naturally. The disease was likely a food-borne infection from ingestion of primate food containing BSE-contaminated beef.

Laboratory **diagnosis** of TSEs can be achieved by immunohistochemistry or by rapid tests such as ELISA or Western blot.

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Bovine citrullinemia

This fulminating neurologic affliction of newborn Holstein-Friesian calves results from a blockade of urea cycle metabolism caused by *deficiency of arginosuccinate synthetase*. The enzyme deficiency leads to hyperammonemia and citrullinemia, and is due to an *autosomal recessive* genetic defect. Increased cross-breeding and screening of sires for the defect have reduced its prevalence to insignificant levels. Citrullinemia has also been reported in dogs.

Calves are affected within the first week of life, the clinical features being depression, head pressing, stupor, convulsions, terminal coma, and death. There are no gross neural lesions, but microscopically there is mild to moderate spongy vacuolation of the deep laminae of the cerebral cortex. *The spongy change is due to astrocytic swelling.* Hydropic astrocytes have enlarged vesicular nuclei, but their cell bodies and processes are vacuolated and give rise to the spongiform appearance of the neuropil, the creation of perineuronal spaces, and the enlargement of pericapillary spaces. The astrocytic reaction is believed to represent a stress on these cells in their attempts to detoxify ammonia. Astrocytes play a major role in ammonia detoxification via utilization of glutamine synthetase and glutamate dehydrogenase activities. In spite of the marked degenerative changes in astrocytes, other elements of the tissue remain morphologically normal, but neuronal function is obviously grossly disturbed.

Affected calves also have pale yellow-ochre discoloration of the liver, which is correlated microscopically and ultrastructurally with hydropic degeneration of hepatocytes.

Neuronal vacuolar degeneration of Angora goats

A scrapie-like neuronopathy is described in young Angora goats in Australia (where scrapie is not present), and is presumed to be genetically determined. Animals become ataxic at about 3 months of age, and progress to severe paresis. No significant gross lesions are present, but there is spectacular vacuolation of large neurons in the red nucleus and other brainstem nuclei, and in the spinal motor neurons. Some Wallerian changes are present in the brain stem, spinal cord and peripheral nerves.

Focal spongiform encephalopathy of dogs

A disease was originally described in **Bull Mastiffs** as familial cerebellar ataxia with hydrocephalus, but the main pathologic feature is spongy vacuolation of several gray matter nuclei. Clinical signs emerge between 6 and 9 weeks of age, and include ataxia and visual impairment, and a number of other variable deficits. An autosomal recessive genetic defect has been proposed.

Macroscopically, there is moderate to severe dilation of the ventricular system. Microscopically, bilaterally symmetrical spongy vacuolation and gliosis are evident in all three deep cerebellar nuclei, the posterior colliculi and, to a lesser extent, the lateral vestibular nuclei. The vacuolation is accompanied by axonal spheroids, but nerve cell bodies appear normal. Some of the vacuoles appear to involve myelin sheaths, but the lesions are essentially confined to the gray matter, and the cytologic basis of the vacuolation is not precisely defined.

Similar vacuolating lesions of these gray matter areas have been described in **Saluki** dogs.

Spongiform encephalopathy of unknown etiology and characterized histologically by neuronal vacuolation is recognized in **Rottweiler** dogs. The condition usually starts at 8 weeks of age and is characterized clinically by progressive laryngeal paralysis, tetraparesis, and cerebellar ataxia. Vacuolated neurons are prevalent in the cerebellar roof nuclei, nuclei of the extrapyramidal system, dorsal nerve root ganglia, myenteric plexus, and other ganglia of the autonomic nervous system.

A hereditary polioencephalomyelopathy of **Australian Cattle Dogs** is characterized histologically by neuronal, neuropilar, and astrocytic vacuolation, particularly in the ventral horn of the spinal cord, cerebellar, and brain stem nuclei. An autosomal recessive trait affecting the astrocytic mitochondria is suspected.

Alaskan Husky dogs can develop a hereditary familial encephalopathy (*Alaskan Husky encephalopathy*) similar to Leigh syndrome in humans and to the multifocal symmetrical myelinolytic encephalopathy of Simmental calves described above. Clinical signs usually start before 1 year of age and include seizures, ataxia, blindness, and behavioral abnormalities. Lesions are more prevalent in the basal nuclei, thalamus, midbrain, pons, and medulla oblongata, and include bilateral symmetrical malacia, status spongiosus, gemistocytic astrocytosis with marked intracytoplasmic vacuolation, gliosis, vascular hyperplasia, and mild infiltration of mixed inflammatory cells, with relative neuronal sparing. Similar but milder lesions occur in the cerebellar ventral vermis and the base of cerebral sulci. Neuronal sparing and marked astrocytic vacuolation suggests a role for astrocytes in the pathogenesis of this condition.

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INFLAMMATION IN THE CENTRAL NERVOUS SYSTEM

Brain and infection

Organs defend against invading infectious organisms either by innate or adaptive immunity. Innate immunity is composed of resident components, e.g., pulmonary alveolar macrophages, or recruited components, e.g., neutrophils. Unlike parenchymatous organs, the brain depends solely on resident innate immunity to recognize and clear pathogens. In other words, under normal nondisease conditions, the brain does not rely either on adaptive immunity or the recruited components of innate immunity for defense. Innate immunity in the brain is diverse and composed of structural units, e.g., blood–brain barrier (BBB), plus cellular and chemical components.

The structural units that separate vulnerable brain cells from the blood stream are the **blood–brain barrier (BBB)** and the **blood–cerebrospinal fluid barrier (BCSFB)**, which is present at the choroid plexuses. The BBB is made up of endothelial cells lining the blood capillaries, pericytes embedded in the capillary basement membranes, and the foot processes of astrocytes. The endothelial cells of the BBB differ from other body endothelial cells, first by having significantly fewer endocytotic vesicles, thereby limiting the amount of transcellular flux, and second, they are connected by both *adherens junction* (e.g., cadherin) and *tight junction proteins*; the latter significantly reduce paracellular flux. Also, BBB endothelial cells are rich in several transport systems that selectively transport essential nutrients to the brain or harmful material back into the blood (efflux transport proteins). One of the most important efflux proteins is *P-glycoprotein*. Inhibition of P-glycoprotein increases the tissue invasiveness of *Listeria monocytogenes*. In addition to these protective properties of the BBB, the CSF side of the BCSFB is also rich in tight junctions, which restricts movement to the CSF. *The ability of organisms to breach these barriers determines their neurotropism*. Neurotropic viruses and bacteria have developed several strategies to breach or to cross these barriers. For example, several neurotropic viruses bypass the BBB and invade the brain via axons (e.g., *Rabies virus*); intracellular bacteria (e.g., *Mycobacterium bovis*) invade the brain as Trojan horses, hidden in infected leukocytes; and finally some bacteria have a direct cytotoxic effect on the endothelial cells of the BBB (e.g., *Histophilus somni*).

The cellular part of the brain's innate immunity is composed of perivascular **dendritic cells, microglia**, and **astrocytes**. These cells are rich in *pattern recognition receptors*, which bind directly and nonspecifically to *pathogen-associated molecular patterns*. For example, all three cell types are rich in *macrophage mannose receptor*, which is a lectin receptor that recognizes "nonself" sugars on the cell wall of gram-negative and gram-positive bacteria, parasites, and yeasts. Microglia cells also express several *tool-like receptors,* e.g., TLR 2 and 6, which have a major role as bridges between innate and adaptive immunity. Activated microglia express several receptors important for phagocytosis, e.g., CR3 and CR4; recognition of pathogens is followed by phagocytosis, largely by microglial cells and to a lesser extent by astrocytes. Finally, glial cells release several *cytokines* and *antimicrobial peptides*, for example, interleukin-1β, tumor necrosis factor-α, and the antimicrobial peptide *cathelicidin*. Inflammation of the brain starts once an infectious agent overcomes the resident innate immunity and recruiting begins of different components of adaptive immunity (leukocytes and humoral immunity), which are more specific and more effective but also more destructive.

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Inflammation in the central nervous system

Inflammation of the brain is **encephalitis**, of the spinal cord **myelitis**, of the ependyma **ependymitis**, of the choroid plexus **choroiditis**, and of the meninges **meningitis**, qualified as **leptomeningitis** when it involves the pia-arachnoid and as **pachymeningitis** when it involves the dura. This is the area of neuropathology that is of most veterinary importance because it embraces many of the transmissible highly fatal infections of animals, because even the sporadic infections are common, and because there is always a pressing need for the pathologist to separate the inflammations into general or specific etiological categories. Most of the specific infectious diseases to be described in this chapter are caused by agents that demonstrate a remarkable or specific neurotropism. These are, however, only a segment of the list of agents that commonly, occasionally, or rarely involve the CNS (Table 3.1); neurologic lesions are also discussed with systemic infections elsewhere in these volumes.

Inflammatory processes in the CNS are basically the same as those in other tissues, but they derive some specific features from the special responsiveness and anatomic arrangements of the CNS. It is important to recognize the criteria of inflammation in the CNS and then to presume from that, as far as is possible, the nature of the infecting organisms because opportunities to intervene clinically are brief and the time frame for clinical intervention is less than required for definitive microbiological diagnosis.

The problem is not only one of why and how the CNS, of known high vulnerability to infection, is so frequently spared in systemic diseases; it is also one of why and how it is infected hematogenously. There is presently no better knowledge of why four out of five cases of Glasser's disease will have meningitis than why one of five will not, even though in the one case the pathologic syndrome is otherwise fully developed.

Infections that, in other organs or tissues, may be inconsequential and even asymptomatic frequently cause death or permanent disability when they involve the nervous system. There are several contributing factors, the most important of which is the *indispensability of most portions of the CNS.* The nervous tissue cannot reconstitute itself, but it may, if the lesion develops slowly, manage a considerable degree of functional compensation. Vascular responses may be more of an impediment than a help in the reaction to inflammation, because they lead consistently to edema and brain swelling that spreads the consequences of the inflammation far from the active focus. Vascular proliferation and fibrous tissue encapsulation may develop only when the inflammatory reaction involves the meninges and the larger blood vessels because these are the only source of reticulin and collagenous tissue. Investigation continues into how viruses spread in nervous tissue; bacteria and fungi can spread rapidly and extensively in the fluid of the ventricular system and meninges. Spread of infection from the ventricular fluid to the periventricular veins occurs readily across the ependyma, and spread from the meninges to the brain, or vice versa, can occur via the Virchow–Robin spaces. The special drainage of CSF is such that exudate rich in fibrin and cells is drained very poorly.

The structure of nervous tissue and meninges limits the anatomical types of inflammation that may occur. **Fibrinous inflammation** is confined to the meninges and larger perivascular spaces. Fibrin is usually indicative of a bacterial infection, but there are exceptions. Fibrinopurulent, largely fibrinous, exudate is caused by the mycoplasmas; fibrinous exudation is typical of the meningeal reaction in malignant catarrhal fever of cattle, and it is occasionally observed in organomercurial poisoning of swine and cattle. **Hemorrhagic inflammation** is not common except as examples of symptomatic purpura in infections such as porcine erysipelas and infectious canine hepatitis and in the infarcts of embolic infections. Hemorrhage is characteristic of helminthic infections, but these lesions are perhaps better regarded as traumatic malacias. **Suppurative or granulomatous inflammation** is the usual response to bacterial or mycotic infections. Viral infections are characterized by **nonsuppurative inflammation**, which is described in more detail later; it is typically composed of neuronal degeneration, perivascular cuffing by mononuclear cells, and focal or diffuse glial proliferations.

Applying these broad criteria, there is seldom much difficulty in deciding on the class of the infecting agent. *There is occasionally some difficulty in distinguishing between the reactions to degeneration and to viral infections.* While suppuration does not occur in either of these processes, early infiltration of neutrophils occurs in acute degenerations, such as the malacias, and a few neutrophils can be found migrating in affected gray matter in the first stages of many cases of viral encephalomyelitis. Acute demyelinating processes are associated with, and probably stimulate, perivascular cuffing. The cuffs tend to be quite distinct from those in viral infections by being relatively very thick (up to ten or more cells thick) and to be composed of pure populations of lymphocytes. *If there are proliferated adventitial cells with plasma cells or other leukocytes mixed in the cuffs of lymphocytes, then the cuffs are likely to be a response to infection.* Only three diseases in animals are caused by viruses and characterized by demyelination and these, namely canine distemper, leukoencephalomyelitis of goats, and visna of sheep, have their own distinguishing characteristics. A possible fourth is so-called "old dog encephalitis." Glial responsiveness occurs in a variety of degenerative and viral lesions, but *glial nodules are characteristic only of an infectious process*, usually viral but occasionally rickettsial or bacterial.

Bacterial and pyogenic infections of the nervous system

Epidural/subdural abscess and empyema

The brain and spinal cord are protected against direct penetration of infection by the skeletal encasement and by the dura mater; the dura is almost impermeable to inflammatory processes. However, infection of the epidural space or the bony encasement with pyogenic bacteria and occasionally fungi can occur and may lead to localized *abscessation* or to the collection of suppurative material in the epidural or subdural space without forming a discrete abscess (*epidural or subdural empyema*). Infection may be hematogenous from *distant sites of infection* (e.g., septic valvular endocarditis, lung abscess), *direct extension* (e.g., osteomyelitis, middle ear and tympanic bullae, eye, paranasal sinuses, ethmoid cells), *trauma* (e.g., bite wounds especially in cats), or by *direct incidental injection* (e.g., contaminated spinal needle). Most cranial epidural abscesses arise by direct extension from one of the paranasal sinuses. Spread from the middle ear and through the cribriform plate is usually directly to the leptomeninges and brain. Occasionally, epidural suppuration is observed to have tracked from a retropharyngeal or nodal abscess through cranial foramina. Epidural suppuration about the base of the brain usually does not become encapsulated.

Tail biting in pigs, docking of lambs, and tail fracture in cats are common etiologies for spinal epidural abscesses that develop either secondary to local venous bacterial invasion or by direct extension from septic osteomyelitis. In cattle, spinal epidural abscesses are usually secondary to osteomyelitis of the vertebral bodies, are more prevalent at the lumbosacral area, and are usually caused by *Arcanobacterium pyogenes* or *Fusobacterium necrophorum*. Migrating grass awns can cause osteomyelitis and epidural abscess in the thoracolumbar regions in dogs. Common bacterial etiologies for epidural abscess or empyema in dogs and cats include *Streptococcus*, *Staphylococcus*, *Brucella*, *Pasteurella*, *Bacteroides*, and *Fusobacterium*, and in horses include *Actinobacillus* and *Streptococcus*.

Clinical signs vary according to the area affected, and can range from mild pain and restlessness to blindness and ataxia. Epidural abscessation at the lumbosacral area in ruminants may lead to circling, which can be confused with encephalitic listeriosis. Spinal epidural abscesses usually cause compression malacia of the cord, if the osteomyelitis from which they originate does not first lead to pathologic fracture with displacement.

Subdural abscessation is seldom observed, but can result from local penetration, perhaps most frequently from the paranasal sinuses. Extension can occur from epidural abscesses or, because subdural suppuration is prone to cause local phlebitis, it may give rise to epidural suppuration. Subdural infection is liable to spread widely via the veins or after penetrating the outer layer of the arachnoid. Spread is also permitted by the slowness with which leptomeningeal fibrous tissue develops to encapsulate the reaction.

Leptomeningitis

Leptomeningitis can be classified according to *etiology* (e.g., bacterial, mycotic), according to *duration* (e.g., acute, chronic), and according to the *type of exudates* (e.g., fibrinous, purulent). Classification by type of exudate is very useful, not only because it indicates the expected histologic lesions, but also clinically because it indicates the possible etiology. *Purulent meningitis* is by far the most common meningitis in domestic animals, especially neonates. *Serocellular meningitis* is described later with viral inflammation. *Hemorrhagic meningitis* occurs in septicemic anthrax and very seldom in other septicemias. Purely *fibrinous meningitis* occurs in malignant catarrhal fever, chlamydiosis, and seldom in anything else. *Mycoplasma bovis* is a rare cause of fibrinous meningitis in calves. *Granulomatous meningitis* occurs in systemic infections of this type, such as tuberculosis and cryptococcosis. The nonsuppurative inflammations of the leptomeninges are described with the diseases of which they may be part. They are mentioned here to draw attention to the wide variety of infectious agents that may localize in and produce inflammation of the leptomeninges. We have also mentioned or discussed purulent leptomeningitis with the many specific diseases of which it can be part, but dwell on the purulent process here because it is common, lacks specificity, and is suitable for a "type" description.

Purulent leptomeningitis may arise by direct extension from an adjacent structure. Extension from an epidural abscess or inflammation may result in diffuse leptomeningitis but, in most of the few cases of this origin, the leptomeningitis is local and overshadowed by the brain abscess that usually forms. Leptomeningitis may arise by local extension from a brain abscess, either by direct permeation or by spread in the Virchow–Robin spaces. Such an origin is observed frequently in listeriosis and in association with very large cerebral abscesses, but most cerebral abscesses, which are usually small, track inwards to the white matter rather than out to the meninges. The reverse sequence in which meningitis spreads to the brain and cord is not unusual in granulomatous infections but is distinctly unusual in suppurative infections, and when it occurs it consists of invasion of the surface of the brain by neutrophils (Fig. 3.100A) rather than of abscessation.

Both purulent meningitis and cerebral abscesses are usually of hematogenous origin, but they are seldom concurrent, and one usually finds meningitis alone or abscessation alone. There are occasional exceptions including thromboembolic lesions and leptomeningitis complicated by choroiditis. Septic emboli are prone to localize in the brain but may localize in meningeal vessels (Fig. 3.100B). Although those in meningeal vessels may lead to diffuse suppurative meningitis, they are usually quickly walled off and prevented from spreading (Fig. 3.100C,D). In the other exception, that of choroiditis concurrent with leptomeningitis, encephalitis or cerebral abscesses may develop because choroiditis leads to exudation into the cerebrospinal fluid and the ependyma is virtually no barrier to infection. Even this combination of pathologic processes is unusual and, when choroiditis is complicated by ependymitis and suppurative encephalitis (Fig. 3.100E), there is usually little or no meningitis. Whether encephalitis develops by spread of meningitis may be largely a question of time, but it is not unusual for animals with purulent meningitis to survive for a week, and sometimes much longer. There is ample time for the inflammation to spread throughout the cranial and spinal meninges – and to the brain if it were going to do so – but it seldom does, and *there is seldom anatomic justification for the common diagnosis of suppurative meningoencephalitis.*

In the section on inflammatory diseases of joints and synovial structures, attention is drawn to the frequent concurrence of polyarthritis with choroiditis and leptomeningitis in fibrinopurulent infections. Erysipelas, in which arthritis is a typical lesion, is an

Table 3.1 Infectious agents/diseases inducing inflammatory changes in the nervous system

Species	Canine	Feline	Porcine	Bovine	Equine	Ovine/Caprine	Camelid	Remarks on brain lesions
a. Viral disease/agent								
Akabane virus	N	N	N	Y	N	S	SC	
Bovine herpesvirus 1	N	N	N	Y	N	N	N	
Bovine herpesvirus 5	N	N	N	Y	N	N	N	
Borna disease virus	Y	Y	N	Y	Y	Y	S	
Bovine paramyxovirus (unclassified)	N	N	N	S	N	N	N	
Canine adenovirus 2	S	N	N	N	N	N	N	Multiple hemorrhages particularly in brain stem/caudate nuclei.
Canine distemper virus	Y	N	N	N	N	N	N	
Canid herpesvirus 1	Y	N	N	N	N	N	N	Necrotizing meningoencephalitis which is most severe in cerebrum and brain stem.
Canine parvovirus 2	S	N	N	N	N	N	N	Vasculitis-induced lesions.
Classical swine fever virus	N	N	Y	N	N	N	N	Vasculitis-induced lesions.
Equine encephalitis viruses (EEEV, WEEV, VEEV)	SC/S	N	S	SC	Y	SC	?	
Equid herpesvirus 1 and EHV-4	N	N	N	N	Y	N	N	EHV 4 rarely causes encephalitis.
Equid herpesvirus 9	?	?	?	?	?	?	?	Natural disease only reported in deer.
Encephalomyocarditis virus	?	?	Y	S	S	?	S	Infection in some species (e.g., bovine) may be limited to myocarditis.
Equine infectious anemia virus	N	N	N	N	S	N	N	Multifocal to diffuse encephalomyelitis is a rare manifestation of EIAV infection; may also cause lymphohistiocytic periventricular leukoencephalitis.
Feline immunodeficiency virus	N	S	N	N	N	N	N	Nonsuppurative encephalomyelitis in some naturally infected cats; secondary *Toxoplasma* or *Feline infectious peritonitis virus* infections.
Feline leukemia virus	N	S	N	N	N	N	N	Non-inflammatory degenerative myelopathy.
Feline infectious peritonitis virus	N	Y	N	N	N	N	N	Fibrinopyogranulomatous periventriculitis, meningitis, and perivasculitis; chronic leukoencephalitis; segmental myelitis; choroiditis.
Hemagglutinating encephalomyelitis virus (HEV)	N	N	Y	N	N	N	N	
Highlands J virus	N	N	N	N	S	N	N	
Japanese encephalitis virus	SC	N	Y	SC	SC	SC	?	
La Crosse virus	S	N	N	N	N	N	?	

Species	**Canine**	**Feline**	**Porcine**	**Bovine**	**Equine**	**Ovine/Caprine**	**Camelid**	**Remarks on brain lesions**
Lentivirus encephalomyelitis	N	N	N	N	N	Y	N	
Louping ill virus	Y	?	Y	Y	Y	Y	Y	
Malignant catarrhal fever	N	N	S	Y	N	SC	?	
Nipah virus	Y	Y	Y	S	Y	Y	?	
Porcine arterivirus	N	N	Y	N	N	N	?	
Porcine circovirus-2	N	N	Y	N	N	N	?	
Porcine paramyxovirus (unclassified)	N	N	S	N	N	N	N	
Porcine rubulavirus	N	N	S	N	N	N	N	
Pseudorabies virus	Y	Y	Y	Y	S	Y	?	
Rabies virus	Y	Y	S	Y	Y	S	Y	
Porcine teschovirus (Teschen disease)	N	N	Y	N	N	N	N	
Tick-borne encephalitis virus	Y	?	?	?	?	?	?	
West Nile virus	Y	Y	SC	SC	Y	S	Y	
b. Protozoal diseases								
Acanthamoeba encephalitis	S	?	?	?	?	S	?	
Babesiosis	S	S	S	Y	S	Y	Y	Sludging of parasitized RBCs in blood capillaries.
Neospora caninum	Y	N	N	Y*	S	S	Y	* in bovine encephalitis, is only observed in aborted fetuses or very young calves infected in utero.
Neospora hughesi	N	N	N	N	Y	N	N	
Sarcocystis canis	S	N	N	N	N	N	N	
Sarcocystis neurona	N	S	N	N	Y	N	N	
Theileriosis	S	N	N	Y	S	Y	Y	
Toxoplasmosis	Y	Y	Y	SC/S	SC	Y	Y	
c. Bacterial diseases								
Listeria monocytogenes	S	S	S	Y	Y	Y	Y	
Histophilus somni	N	N	N	Y	N	Y	?	
d. Chlamydial and rickettsial diseases								
Chlamydophila pecorum	?	?	?	S	?	?	?	

Y	The species is susceptible to natural infection and overt disease is reported regularly.
S	The species is susceptible to natural infection but overt disease is reported only sporadically.
SC	The species is susceptible to natural infection but the disease occurs subclinically without neurologic signs.
N	The species is not susceptible to natural infection.
?	No refereed papers about species susceptibility to natural infection.

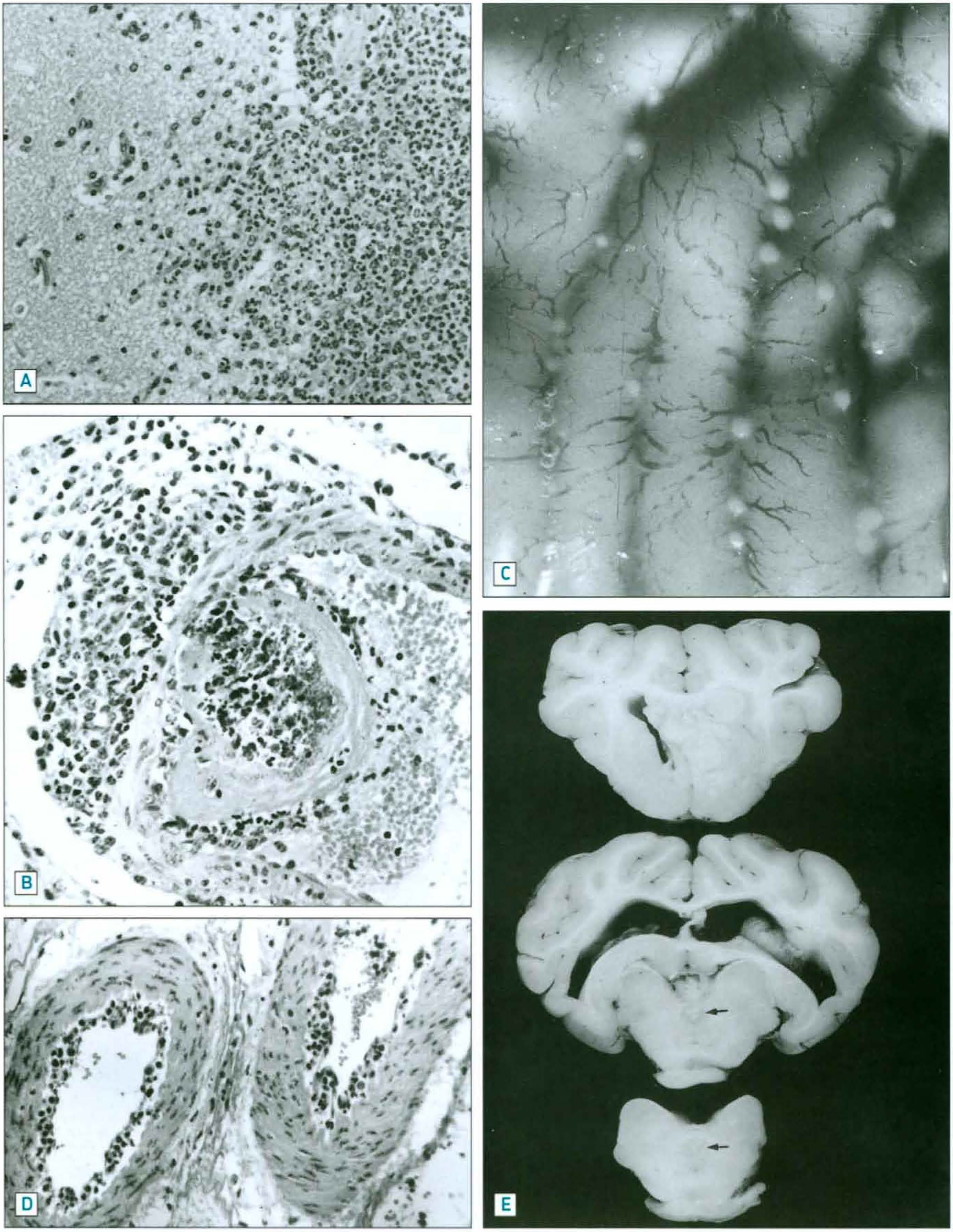

Figure 3.100 A. Suppurative meningitis with extension into cerebellar cortex. **B. Embolic meningeal arteritis** from streptococcal endocarditis in a pig. **C.** Miliary **meningeal abscesses** in a calf due to *Arcanobacterium pyogenes*. **D. Meningeal arteritis** with leukocytes beneath endothelium in coliform meningitis in a calf. **E.** Frontal sections to show periventricular abscess, early hydrocephalus, and occlusive ependymitis of aqueduct (arrows) secondary to **streptococcal choroiditis** in a pig.

exception in that it may produce septicemic and embolic lesions in the brain but it does not cause suppurative meningitis. Several bacteria, such as streptococci and *E. coli*, cause bacteremia and suppurative meningitis in neonates resulting in an important clinical entity, especially in ruminants and pigs, called **neonatal bacterial suppurative meningitis** (NBSM). Streptococcal NBSM in calves, lambs, and piglets (but not in foals), frequently has a combination of polyarthritis, purulent leptomeningitis, choroiditis and, in calves only, endophthalmitis (Fig. 3.101A, B). In pigs, *Streptococcus suis* types 1 and 2 (see Vol. 1, Bones and joints) cause NBSM that is usually accompanied by polyserositis; *S. suis* meningitis is suppurative in the acute stage, and lymphoplasmacytic in pigs that survive. *Streptococcus pneumoniae* usually produces fulminating septicemia in calves characterized only by very acute splenitis but, if the course of this infection is less fulminating than usual, polyarthritis and meningitis can be found. *E. coli*, another cause of NBSM of protracted course, commonly causes well-developed meningitis and polyarthritis in neonatal calves and lambs, but even in fulminating infections the mild changes of early inflammation can be found in these structures. On the other hand, both coliform and streptococcal infections in calves and piglets may avoid the joints and meninges and localize instead in the choroid plexuses and spread from there to the ventricles and brain, or localize in the brain alone. The coliforms and streptococci behave differently in calves with respect to the eyes; a combination of synovial, meningeal, and intraocular localizations is almost invariably of streptococcal origin (and the lesions can be well developed within 12 hours of birth, or even at birth according to farmers); the coliforms can, but only seldom do, cause endophthalmitis.

Mannheimia haemolytica and *P. multocida* are usually regarded only as causes of fibrinous pneumonia and hemorrhagic septicemia, but they are responsible for localized infections in other locations, including the meninges, in ruminants. When polyarthritis is present in septicemic or pulmonary pasteurellosis, *fibrinosuppurative leptomeningitis* can also be anticipated. Isolated cases and limited outbreaks of pasteurellosis that is entirely meningeal are observed in cattle and sheep, usually young ones. The course is asymptomatic until meningitis develops.

Once infectious agents gain access to the leptomeninges there is little resistance to spread in the meningeal spaces, and the inflammatory process becomes more or less diffuse in most cases. When the inflammatory process, excepting some that are granulomatous, remains localized, it is probable that only the inflammatory reaction, and not the infection, reaches the meningeal spaces. Cerebrospinal fluid is an excellent culture medium for many sorts of bacteria and these spread rapidly in the fluid, assisted to some slight extent by normal flow so that, although meningitis may appear grossly to have a limited distribution, its true distribution can be determined only microscopically.

The apparent gross distribution of meningitis varies somewhat with the cause. Thus in listeriosis, the process is confined largely or entirely to the meninges covering the medulla oblongata and upper cord, and in Glasser's disease and in malignant catarrhal fever the exudate is concentrated over the cerebellum and occipital poles of the cerebrum. In pyogenic meningitis, the basal meninges show the most obvious changes.

In the first day or so of suppurative meningitis before exudation is clearly recognizable, the meninges may be faintly opaque and hyperemic (Fig. 3.101C). After a few days, the appearance of the brain and cord is typical. The basal cisterns that accumulate the most exudate are filled with creamy pus or with gray-yellow fibrinopurulent exudate. The extreme exudation in these cisterns is due in part to their large size but in part also to sedimentation of particulate exudate. The exudate is in the arachnoid spaces and there is little if any on the outer surface of this membrane (Fig. 3.101D). The arachnoid appears stretched. *It is easy to overlook even copious exudates because their color is not very different from that of the brain.* A useful clue is that even the largest basilar vessels and the trunk of the oculomotor nerve are partially or completely buried and obscured by exudate and the filling-in of basal sinuses and grooves obliterates the normal topography. Over the hemispheres the exudate is usually confined to the fissures, where the arachnoid space is wide, and spares the surfaces of the gyri, where the arachnoid space is narrow. There is seldom frank pus over the hemispheres except in cases of unusually long survival.

The severe degree of exudation described above is what is usually seen in animals. In very acute or early cases, the exudation may be considerably less, and detectable only as congestion and cloudiness of the basal meninges extending towards the convexity of the hemispheres as fine gray sleeves about the arteries and veins. On careful inspection by naked eye, almost every case of purulent meningitis can be detected but the microscope may be necessary to confirm some cases.

The brain is swollen in every acute case of pyogenic meningitis, and the swelling is frequently severe enough to cause displacement with coning of the cerebellum. The pathogenesis of the edema and swelling is not known. The edema affects the white matter. It is possible that obstruction of the meningeal orifices of Virchow–Robin spaces by exudate and stasis of flow of meningeal fluid may contribute to the edema. The brain itself is normal except for softness and swelling and the rare cortical infarcts in the cerebrum or cerebellum.

Choroiditis commonly complicates leptomeningitis. It is usually quite obvious when it affects the plexuses of the fourth ventricles, but that affecting the plexuses of the lateral ventricle is not apparent until the ventricles are opened, although it may be expected if cloudy fluid escapes from the third ventricle when the infundibular process is opened. The CSF is cloudy, flakes of exudate overlie the plexuses and float in the fluid, and smeary sediments of pus lie on the walls of the ventricles (Fig. 3.101B). The exudate may be impacted in the aqueduct, occluding it and leading to ependymitis. Internal hydrocephalus then develops rapidly.

Microscopically, purulent meningitis does not differ in its character from pyogenic inflammation in other loose tissues, such as the lung. A few mononuclear cells are mixed with a very large number of neutrophils in the arachnoid spaces (Fig. 3.101E). The amount of fibrin in the exudate varies. There may be some infiltration for a short distance along the Virchow–Robin spaces about veins (Fig. 3.102). The pia mater as a rule remains intact, and it is only in exceptional cases that some microbial activity is observed in the adjacent parenchyma, or the pia is eroded to allow neutrophils to invade the surface of the brain. When the choroid plexuses are involved, they are swollen and infiltrated with leukocytes, many of them mononuclear. The plexus epithelium is eroded and covered by fibrin and cells. The ependyma is also eroded, there is edema of subependymal tissues, and the surrounding veins are infiltrated.

Internal hydrocephalus is a sequel to ependymitis and occlusion of the aqueduct, as a result of which the lateral and third ventricles are

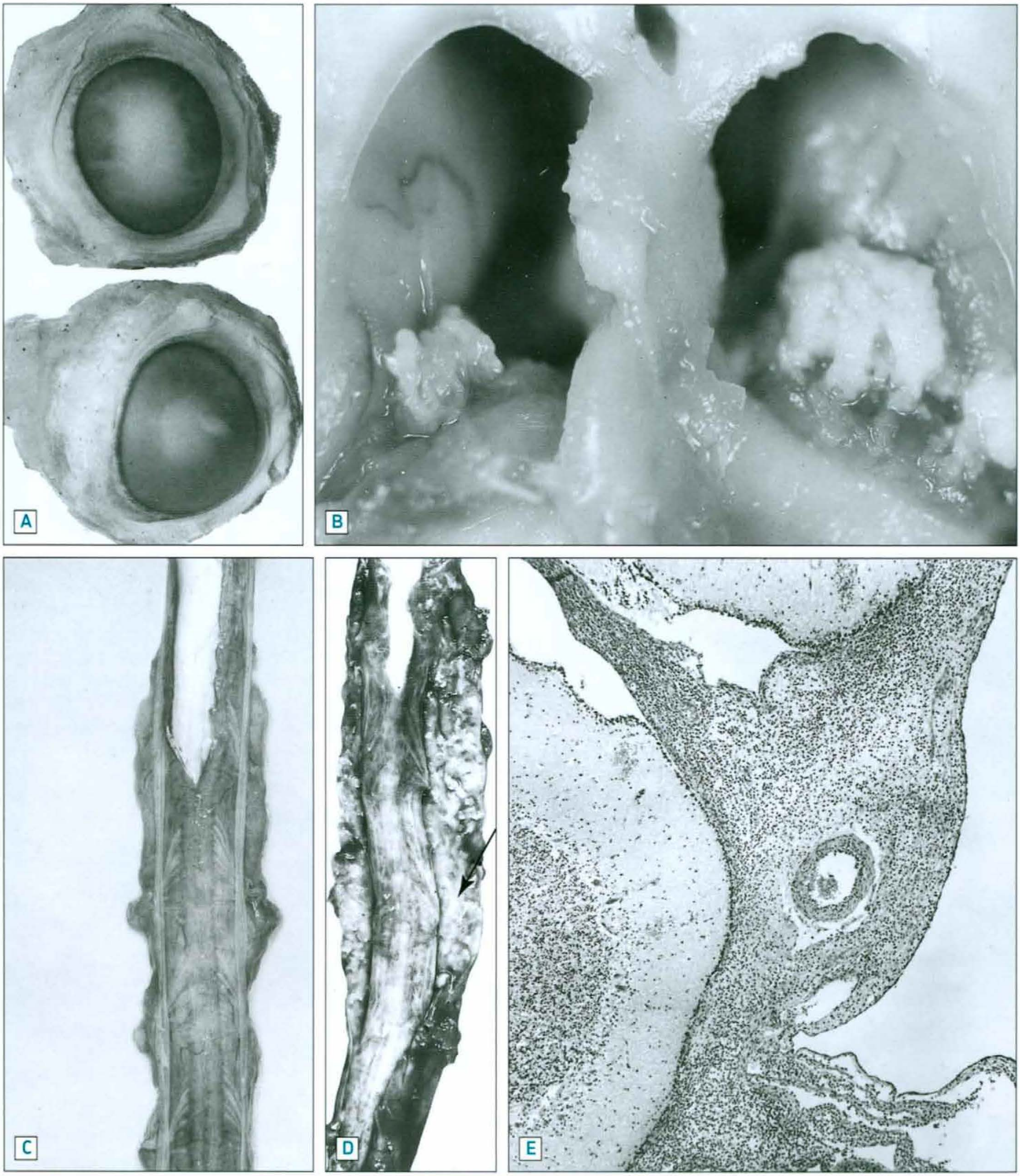

Figure 3.101 A. Hypopyon in streptococcal endophthalmitis in a calf. **B.** Horizontal slice through lateral ventricles to show **streptococcal choroiditis** and acquired hydrocephalus. **C. Spinal leptomeningitis** in a calf, due to *Escherichia coli*. (The dura is incised and reflected full length, the congested leptomeninges are incised at top.) **D. Spinal meningitis** in a dog; dura reflected to expose exudate (arrow). **E. Fibrinopurulent exudate** in leptomeninges in a pig, due to *Haemophilus parasuis*.

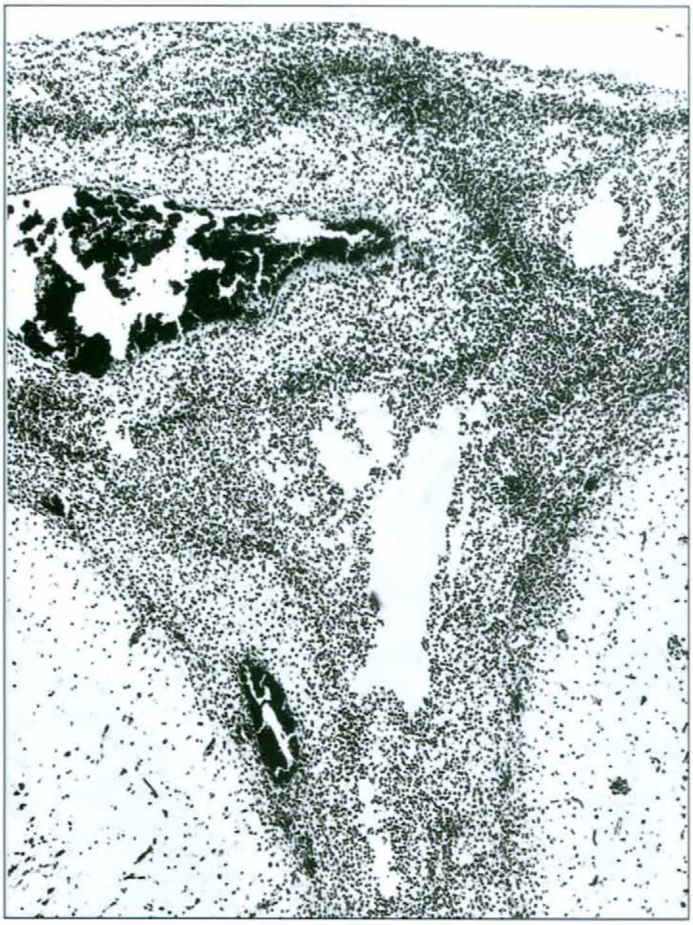

Figure 3.102 **Purulent meningitis** caused by *Haemophilus* spp. in a pig.

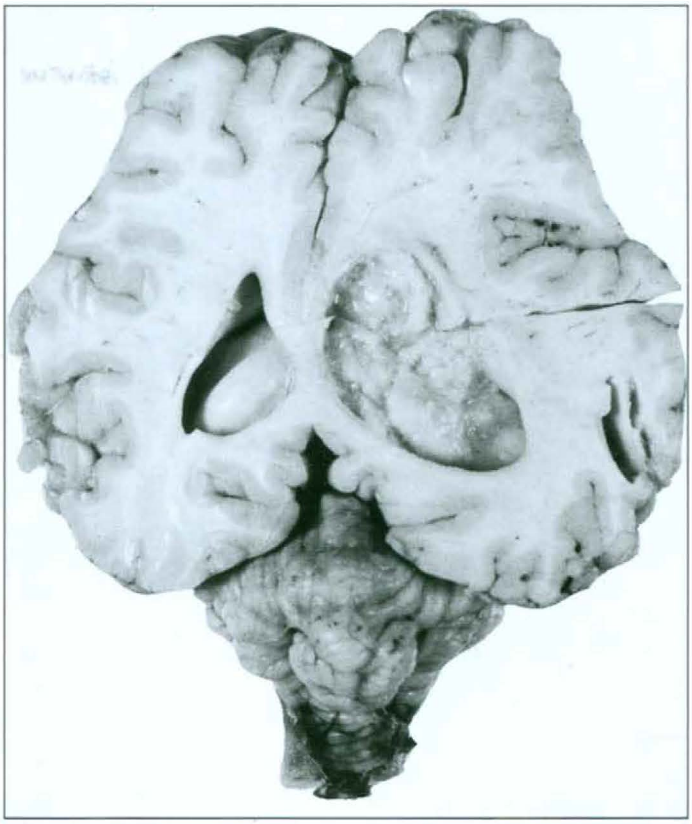

Figure 3.103 Necrobacillary abscess arising in **choroiditis** in an ox: swelling of hemisphere and caudal displacement with coning of cerebellum.

dilated (Fig. 3.103). This condition is a complication of choroiditis, but hydrocephalus may also be a sequel without choroiditis being present. The medullary foramina are frequently occluded as a result of inflammation in the tela choroidea so that CSF cannot escape from the ventricular system to the arachnoid spaces; the hydrocephalus is noncommunicating. Obstruction to the flow of fluid in the arachnoid spaces may occur as a result of brain swelling with impaction in the tentorial incisure, heavy deposits of exudate in the arachnoid space, or as a result of meningeal adhesions and thickenings in chronic inflammations. The hydrocephalus of this pathogenesis is communicating.

Chronic pyogenic leptomeningitis is rarely observed in animals. The process may sterilize itself or be sterilized by antimicrobials, but much of the injury is established in the early stages of the process and, once the diagnosis is evident clinically, *death is the expected outcome*. The early injury is exaggerated by the persistence of exudate even after the infection is controlled because there is no free drainage from the meningeal spaces. Healing occurs only after there has been considerable destruction of the meningeal framework with repair by fibrous tissue. Meningeal adhesions may produce cystic loculations in the arachnoid space, and obliterate the medullary foramen or basal arachnoid space and cause lingering death from hydrocephalus.

In a purulent process, *leptomeningeal vasculitis commonly occurs*. The expected sequelae of venous or ischemic infarcts are seldom observed even in those few cases with vasculitis in which thrombi can be found. This apparent discrepancy is probably due to the rate at which the vascular obstructions develop and on the type and size of vessel. Cases in which suppurative thrombophlebitis of the sagittal or transverse sinuses, or both, can be readily observed may not have venous infarcts in the brain. Thrombosis in the afferent circulation usually involves the smallest of meningeal vessels and usually develops at a rate that allows collateral circulation to develop. Inflammation of larger arteries (Fig. 3.100D) in which the endothelium is dissected from the intima by leukocytes has not been observed to lead to thrombosis.

Septicemic lesions, septic embolism, and cerebral abscess

The brain responds in septic or endotoxemic shock by releasing or controlling the release of several proinflammatory and anti-inflammatory cytokines; this major biochemical role is not always associated with a morphologic change in the CNS. *The CNS is injured to some extent in every episode of septicemia or sustained bacteremia.* The simplest and most common type of injury is inflicted on the venules, especially those of the cerebral white matter and to a lesser extent those of the cerebellar white matter. Injury of this type is not of much significance. There is sludging of leukocytes and probably of erythrocytes in these vessels, associated usually with degenerative and reactive changes in the endothelium. In the symptomatic purpuras, it is frequently possible to find the site of diapedesis in the brain, but not in other organs. Associated with endothelial injury there is often leakage of plasma into the perivascular space and, given time, adventitial proliferation and infiltration of a few lymphocytes or other leukocytes into the Virchow–Robin spaces. Commonly, the injury is more severe, the vessel wall is totally necrotic in its cross-section, and in these lesions a few bacteria may be demonstrable (Fig. 3.104).

Septic embolism in the CNS may be a complication of active endocarditis; the bacteria are usually gram-positive. The bacteria implicated most commonly are *Erysipelothrix rhusiopathiae* in pigs, streptococci in all species, and *Arcanobacterium pyogenes* in cattle.

The toxigenicity and pathogenicity of many infections by gram-negative bacteria in septicemic phase seem to depend largely on the number of organisms present, as has been demonstrated in sheep for septicemic pasteurellosis caused by *Mannheimia haemolytica*. In septicemic pasteurellosis of sheep, in infection of foals by *Actinobacillus equuli*, in infections of sheep and cattle by *Histophilus somni*, and in some coliform infections of calves, the blood literally swarms with bacteria because common to these infections is massive and widespread bacterial embolism. These are the principal infections in which bacterial embolism occurs in the CNS without there being a demonstrable primary focus such as endocarditis, pneumonia, or an abscess. The problem of cerebral embolism in these infections is not solely one of quantitative factors determined by the height of the bacteremia; qualitative factors of unknown nature are also involved.

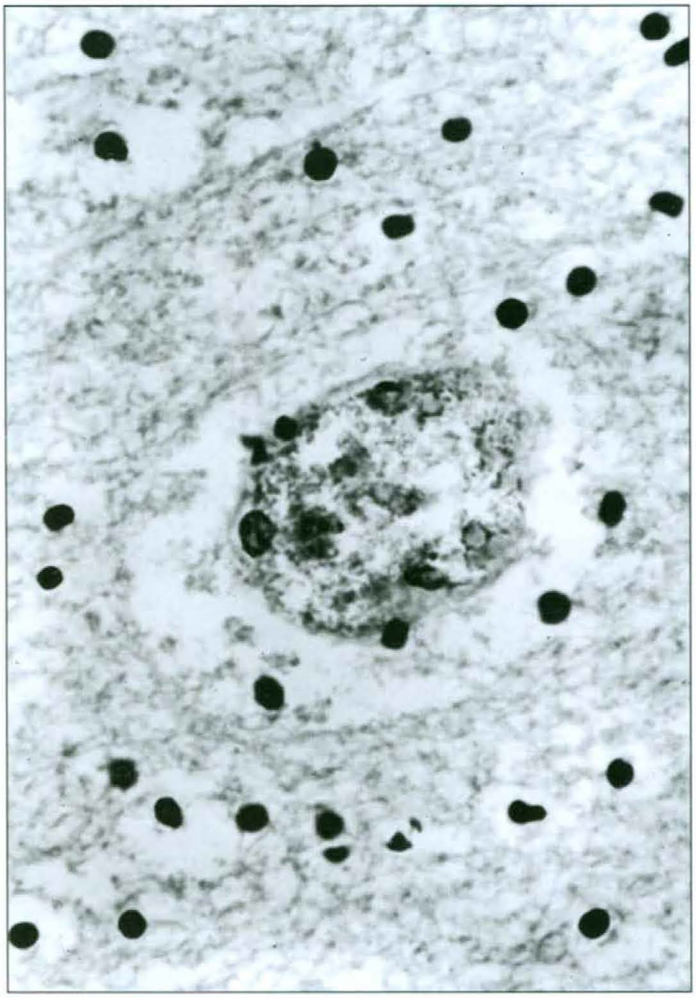

Figure 3.104 Necrosis of cerebral venule in *Escherichia coli* septicemia in a calf.

Septic thromboemboli and **bacterial emboli** lodge in the small cerebral vessels but, whereas thromboemboli tend to lodge particularly in small arterioles, bacterial emboli lodge in capillaries and venules. Consequences depend on the vessels involved, although the consequences are not particularly significant because abscessation occurs in either location. *Arterial emboli frequently cause ischemic infarcts, and venous and capillary emboli cause hemorrhagic infarcts.* The venular lesions also extend rapidly to involve large veins, whereas local spread in arteries does not occur.

Cerebral abscesses may arise in embolism, by direct implantation in wounds, or by direct invasion of the brain from an adjacent structure. Leptomeningitis rarely leads to abscessation, whereas choroiditis commonly leads to periventricular abscess. Abscesses in the spinal cord are seldom sought or observed; they may be hematogenous via arteries or veins (Fig. 3.105), and rarely do they enter through the dura.

Abscesses of hematogenous origin may occur anywhere in the brain, but there are two areas of remarkable predilection – the hypothalamus, and the cerebral cortex at the junction of gray and white matter (Fig. 3.106A, B). *Listeria monocytogenes* is an exception because it always demonstrates an affinity for the reticular formation in the brain stem. There may be only one abscess in the brain, or they may be multiple, especially when due to bacterial embolism. Multiple abscesses of septic thromboembolic origin tend often to be localized in one area of the brain. Some care is necessary in specifying the origin of multiple adjacent foci of suppuration because the *production of satellites*, each of which may be larger than the primary, is a natural attribute of brain abscesses.

Abscesses arising by direct invasion of the brain may develop in any location. There are two common sites of invasion, namely the *cribriform plate and inner ear*, and two somewhat less common, namely the *hypophyseal fossa and the paranasal sinuses*. Hypothalamic abscesses

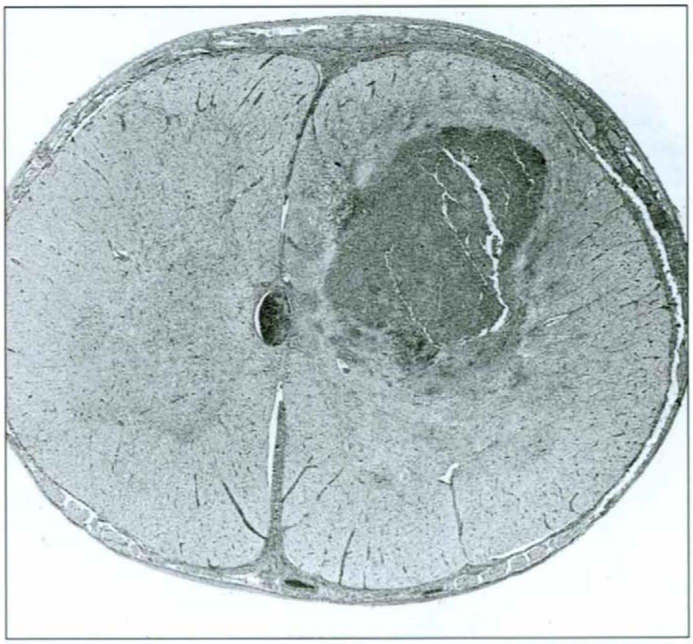

Figure 3.105 Meningitis, tracking abscess in dorsal horn, and ependymitis, complicating docking wound in a lamb.

are observed occasionally in cattle and dogs. At least some of those in dogs are caused by minute foreign bodies migrating with phlebitis through the orbital fissure or foramen rotundum and carrying actinomycetes. The importance of the cribriform plate and internal ear in the development of cerebral abscess is due to the frequency with which infections occur in those locations, that in neither site is there an actual or potential epidural space to protect the brain, and because nerves and vessels enter and leave through both.

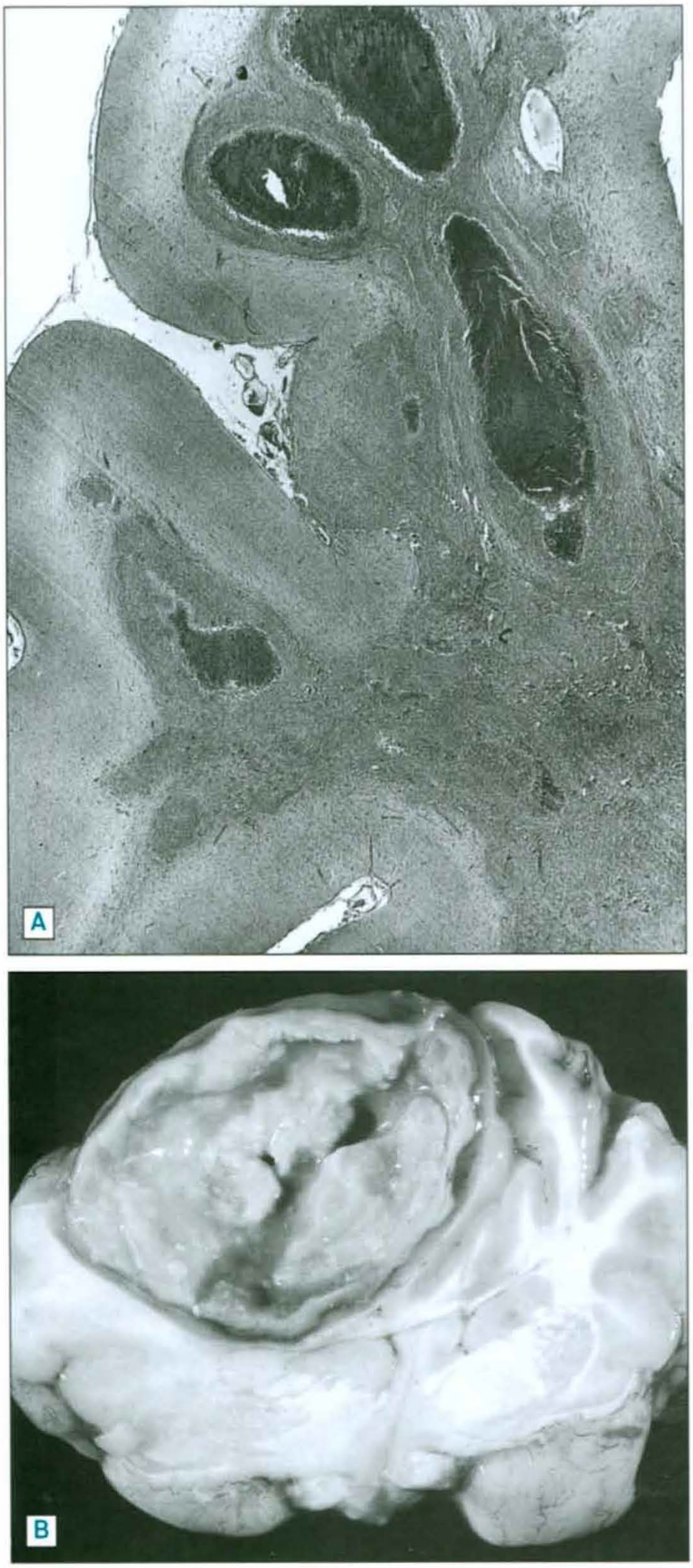

Figure 3.106 A. Hematogenous **abscesses** in cerebrum in a pig. **B. Abscess** distorting left cerebral hemisphere in a goat. *Pasteurella multocida* and *Fusobacterium necrophorum*.

Frontal abscesses of sinus origin occur occasionally in cattle as a complication of dehorning wounds, but abscesses in this site occur most commonly in sheep in which sinusitis, especially in the ethmoid cells, develops as a suppurative complication of myiasis (*Oestrus ovis*). *Arcanobacterium pyogenes* is the organism usually present. The olfactory bulb is destroyed and the first ventricle is opened, infection then spreading to the substance of the hemisphere (Fig. 3.107). There is little tendency to spread into the meninges from the point of entry or to invade the cortical gray matter, although both layers are usually secondarily involved by expansion of the abscess later.

Abscesses commonly develop at about the *cerebellopontine angle* as complications of suppurative otitis media. These are usually problems of pharyngitis, infection spreading via the Eustachian tube to the middle ear and from there to the brain, either by erosion of the bulla or extension along natural foramina.

Cerebellopontine abscesses are rarely, if ever, observed in horses in spite of the frequency of pharyngitis in this species. This exemption of horses may be due to the diversion of exudates from the Eustachian tube to the guttural pouches. Otogenic cerebral infections are very

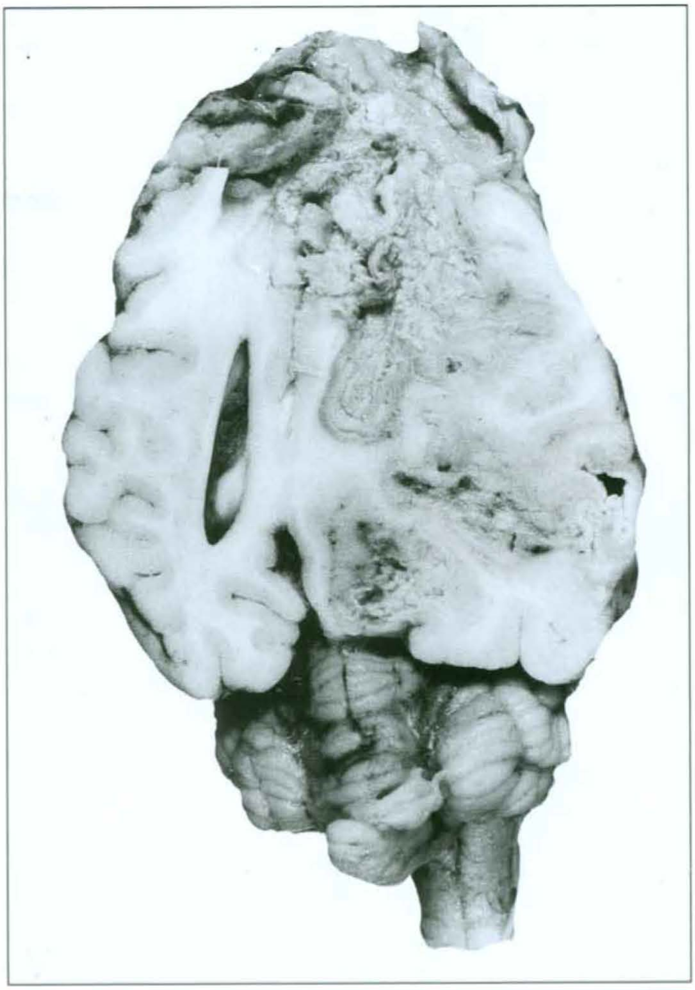

Figure 3.107 Arcanobacterial **abscess** arising from right ethmoid in a sheep.

seldom observed in dogs. Those observed have come via the auditory meatus rather than the Eustachian tube, and the cerebral reaction is nonspecifically granulomatous, usually without pus. Otitis media caused by *Pasteurella multocida* is fairly common as a complication of chronic cases of upper respiratory infection in cats and, when the infection extends to the cranial cavity, it produces diffuse purulent leptomeningitis and not, as expected, a cerebellopontine abscess. Most otogenic infections occur in sheep and swine; a few are observed in cattle, and in each species the outcome is an abscess of the brain.

Otic infections are commonly bilateral so that otogenic abscesses may be also. The usual organism in these abscesses is *Arcanobacterium pyogenes* alone or mixed with *Pseudomonas aeruginosa*, *Pasteurella multocida*, and mixed cocci. Affected pigs usually have several other chronic debilitating infections at the same time so that several animals in the herd may have otogenic abscesses. This pattern of infection in calves is uncommon and sporadic. In sheep on the other hand, the disease may be observed in limited outbreaks and there is an association with grazing on rough, dry, mature summer pastures. The reasons for this association are unknown.

Established abscesses are found much more commonly in the white matter than in the gray, in spite of the fact that they usually begin in the gray. There is no suitable explanation for this tendency to track into white matter, but once within white matter they do permeate along fiber tracts (Fig. 3.108). As a result of this activity, satellite abscesses are to be expected, sometimes chains of them. Connecting purulent tracts may be very thin and difficult to find.

An abscess may begin as an intense accumulation of neutrophils in and around a thrombosed vessel (Fig. 3.109A), or in a focus of septic encephalitis in which neutrophils lightly infiltrate a zone of early softening. The principal differences between a cerebral abscess and an abscess in some other location are the vulnerability of the surrounding nervous tissue to edema, which can destroy it, and the slowness with which encapsulation occurs (Fig. 3.109B). The meninges and larger blood vessels are the only sources of fibroblastic tissue for encapsulation.

In the *early stages*, the abscess cavity contains a liquefying center and the margins are irregular and poorly defined even microscopically. The surrounding brain tissue is edematous and infiltrated by neutrophils. The microglia and vessels are fairly resistant and reactive in a narrow peripheral zone, but the neurons and neuroglia degenerate. Most abscesses develop rather slowly and later become encapsulated. The capsule seems often to be formed more by condensation of vessels around an expanding focus than by proliferating fibroblastic tissue, but both contribute; the result is an irregular capsule thicker on the meningeal than on the ventricular aspect. Most capsules are very distinct and 1–3 mm wide. Their distinctness is due to the paucity of reticulin or collagen spreading out into the surrounding parenchyma or into the abscess cavity. In old abscesses with shrinkage, the core may separate from the capsule, and the capsule may separate extensively from the surrounding cerebral substance. The surrounding tissue is discolored yellow due to edema, the edematous zone often being much larger than the abscess itself. The *microscopic structure of the wall of a chronic abscess* does not differ much from case to case except in the thickness of the capsule. A narrow zone of histiocytes or gitter cells faces the neutrophilic debris. The next zone is a laminated layer of collagenous fibers between which are rows of gitter cells laden with debris. The outer zone is vascular, the vessels being large and usually nonreactive. The surrounding nervous tissue is severely edematous, with degeneration of myelin and fibers. Astrocytes are swollen and reactive about encapsulated abscesses, but it takes a long time for their fibrils to begin to intertwine with capsular reticulin. The veins are heavily cuffed by lymphocytes.

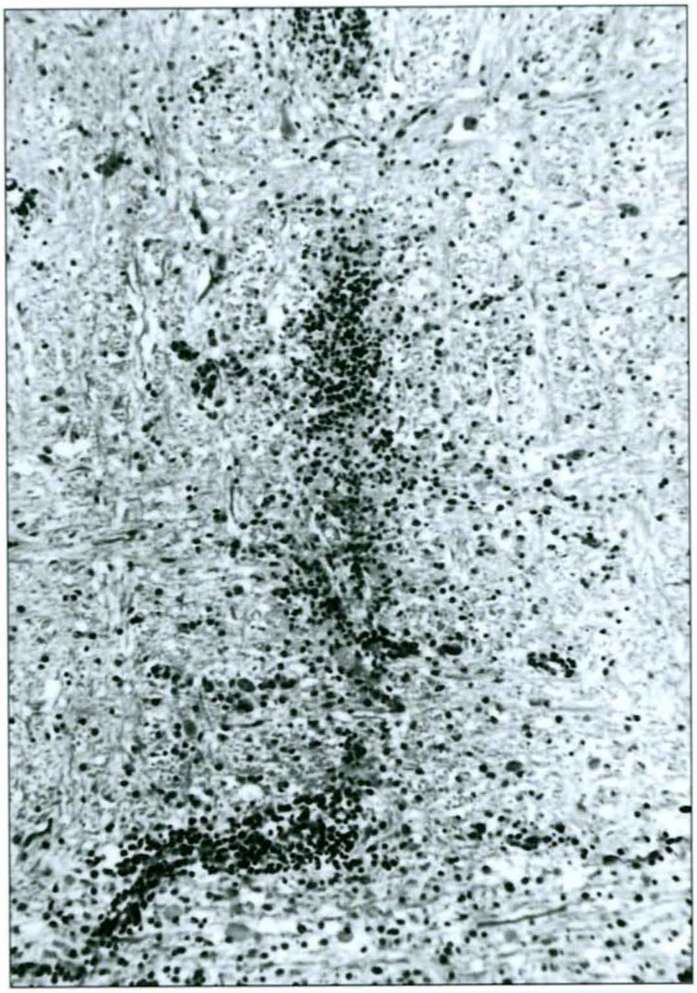

Figure 3.108 Spreading tract in suppurative (streptococcal) encephalitis in a pig.

When abscesses are multiple, death is the outcome after a short course. When they are isolated, they may permit prolonged survival. The course is usually short with medullary abscess, even when small, because it, or more usually the edema it provokes, interferes with vital centers. Abscesses in the hypothalamus or cerebrum may track through the white matter to the ventricles to produce pyencephaly that is quickly fatal (Fig. 3.110A, B, C, D). Large abscesses ultimately expand to the meninges to produce adhesive meningitis. Many abscesses act as space-occupying lesions by virtue of their size or the edema they provoke or both. The consequences of space occupation depend on the site and size of the lesion.

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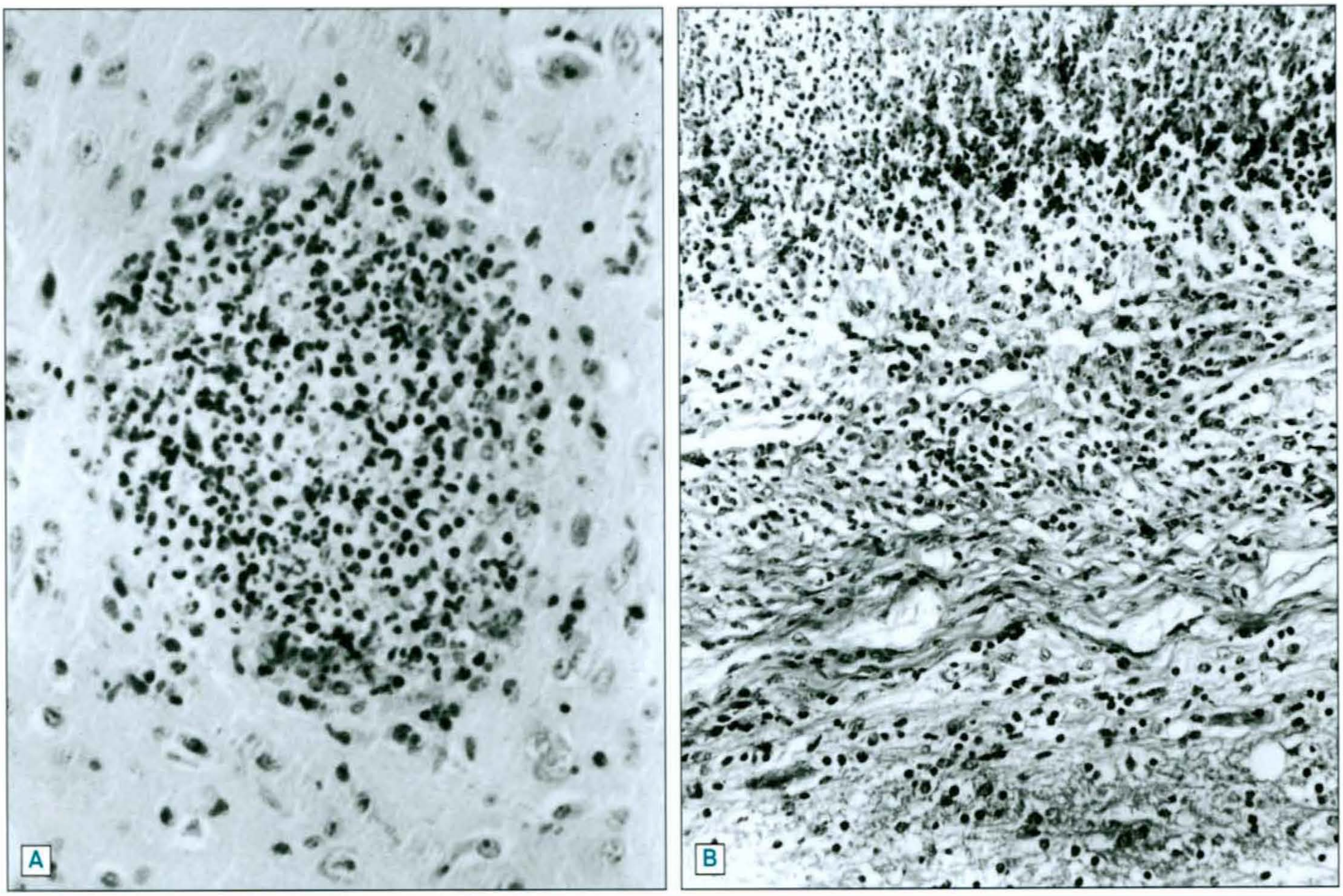

Figure 3.109 A. Early **cerebral abscess** from septic embolus in a pig. **B.** Structure of wall of **cerebral abscess**. Abscess (above) is separated from normal brain by a thin capsule.

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Granulomatous and pyogranulomatous meningoencephalomyelitis

Granulomatous to pyogranulomatous meningoencephalomyelitis is observed in many systemic mycotic (e.g., blastomycosis, cryptococcosis) or algal (e.g., protothecosis) diseases, which are described elsewhere in this book. Granulomatous inflammation can also be caused by bacteria (e.g., *Mycobacterium bovis, Nocardia* sp.) (Fig. 3.111), and migrating helminths or arthropod larvae (see below). The diagnosis and differentiation among these etiologies requires culture or demonstration of the causative pathogen using histochemical or immunohistochemical stains.

Listeriosis

Listeriosis is caused by *Listeria monocytogenes*, a Gram-positive, facultative anaerobic bacillus that is ubiquitous in the environment and can multiply in diverse environmental conditions – it can grow in a temperature range from 4 to 45°C and at a pH range of 5 to 9. It is remarkably viable in the external environment, being able to survive in dried media for several months and in suitably moist soil for ~1 year. The organism is commonly isolated from tissues of normal animals, including tonsils and other gut-associated lymphoid tissue, and in large numbers from the feces of ruminants. *L. monocytogenes* has more than 11 serotypes; almost all animal infections are caused by serotypes 1/2a, 1/2b, and 4b.

Listeriosis is of worldwide distribution, possibly excepting the tropics. The organism has been isolated from diseased mammals and birds of many species and produces septicemia, meningitis, and abortion in humans. In domestic animals, the disease is most important in ruminants. *L. monocytogenes* is an intracellular pathogen of macrophages, neutrophils, and epithelial cells. Important virulence factor include the surface protein *internalin,* which internalizes with *E-cadherin*, an adherans junction protein, to overcome the intestinal, placental, and blood–brain barriers. Also, it relies on another virulence factor, *cholesterol-binding hemolysin*, to lyse phagocytic cell phagosomes and escape to the cytoplasm. The organism proliferates in the host cell cytoplasm and many migrate against the cell membrane to form protrusions that can then be taken up by other cells. It is one of the few organisms known to co-opt the host cell contractile actin to facilitate cell-to-cell transfer.

Listeriosis behaves as three separate diseases or syndromes. They seldom overlap so that each syndrome probably has a separate

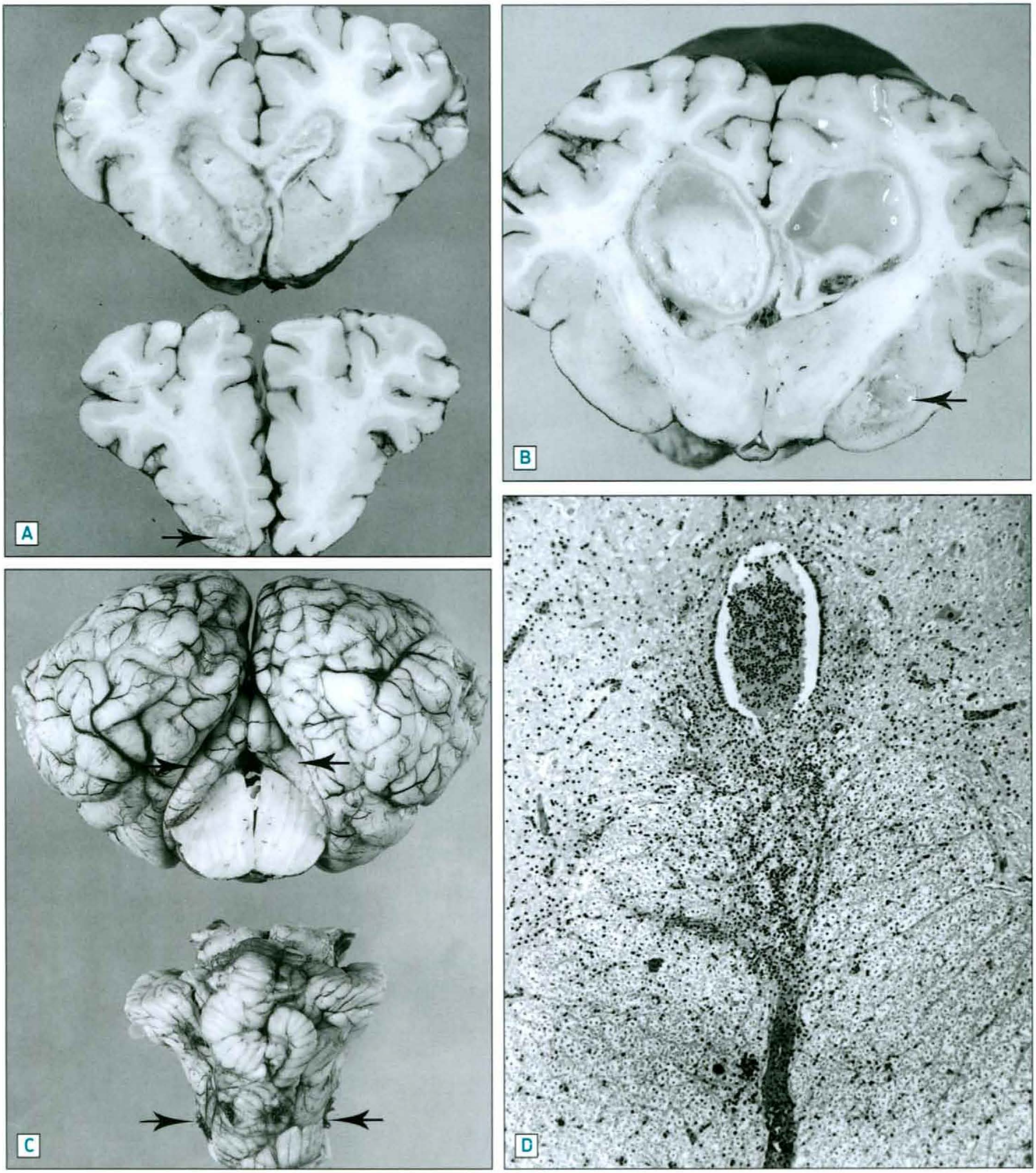

Figure 3.110 Pyencephaly in a calf. **A.** Pus in lateral ventricles (above) and left olfactory ventricle (arrow, below). **B.** Dilation of lateral ventricles by fibrinopurulent exudate (arrow). **C.** Brain swelling with cerebellar coning and hemorrhage (arrows, below) and subtentorial herniation of hemispheres (arrows, above). **D.** Inflammatory exudate in aqueduct.

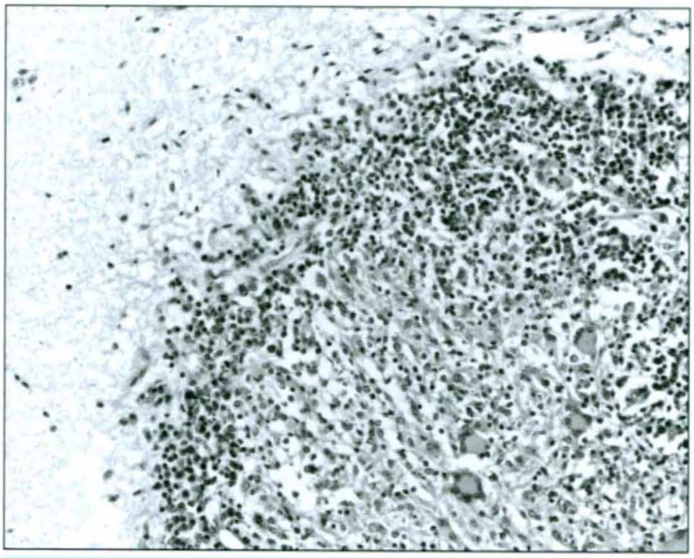

Figure 3.111 Granulomatous meningoencephalitis in bovine tuberculosis. (Courtesy of D Driemeier.)

pathogenesis. *The three recognized syndromes are: infection of the pregnant uterus with abortion, septicemia with miliary visceral abscesses, and encephalitis.* Additional syndromes of clinical significance in ruminants include conjunctivitis, possibly from contaminated silage dust, endocarditis, and mastitis. The uterine infection is discussed with Diseases of the pregnant uterus (see Vol. 3, Female genital system). Aborting ruminants are not usually ill, and abortion is usually late in gestation. The uterine infection is probably hematogenous and the bacteremic phase is asymptomatic, and localization occurs only in the uterus. Infection of the uterine contents can be established quite readily by oral exposure of pregnant animals and by intravenous inoculation.

Septicemic (systemic) listeriosis occurs in aborted fetuses and neonatal lambs, calves, and foals up to 1 week of age and in others that are several months of age, and is characterized by multisystemic bacterial colonization and multifocal multisystemic areas of coagulative necrosis or microabscess formation. The necrotic areas or microabscesses are miliary in distribution, very numerous in the liver, but much less numerous in the heart and other viscera, and characterized by tissue lytic necrosis with infiltration of neutrophils and fewer macrophages. Neonates generally become infected in utero.

Listerial encephalitis occurs almost solely in adult ruminants; its pathogenesis is partially understood. Listerial encephalitis may be sporadic or occur in outbreaks in which the morbidity may be 10% or higher. Outbreaks are usually associated with *heavy feeding of silage*, with disease most likely occurring in winter and early spring when the animals are indoors. This association of outbreaks of cerebral listeriosis with silage feeding is a circumstantial observation, but the association is so common that silage is fed as a calculated risk and removed from the ration when the first case occurs. The association with silage may indicate an acquired susceptibility of the animals or the provision of a growth medium that leads to heavy infection pressure. The organism will multiply in spoiled silage that is incompletely fermented and with a pH of 5.5 or above. Ingested *Listeria* is likely to breach the oral mucosal barrier through pathological or physiological wounds, e.g., erupted teeth wounds. *After invading the oral mucosa, the bacteria invade the trigeminal nerves and travel centripetally via axons to the brain. Listeria* can also breach the blood–brain barrier under experimental conditions; however the specific distribution of listerial encephalitis in the natural disease is inconsistent with a hematogenous infection. In animals, *Listeria has a remarkable affinity for the brain stem*, the lesions being most severe in the medulla and pons and less severe rostrally in the thalamus and caudally in the cervical parts of the spinal cord. Intravenous or oral dosing of pregnant ruminants will regularly produce intrauterine infection but seldom produce intracranial infection and probably never produce encephalitis of the specific distribution. Bacteremias with localization in the CNS regularly cause meningitis, choroiditis, and cerebral abscesses, which is what *L. monocytogenes* does, but only as an experimental hematogenous infection. When a pregnant animal dies of encephalitic listeriosis, the uterine contents are usually sterile.

Conjunctivitis and keratitis follow experimental conjunctival exposure, but this should not imply that the endophthalmitis of the natural disease is produced by local invasion. Rhinitis is clinically apparent in many cases of encephalitic listeriosis, but histological evidence does not support the idea that the olfactory nerves are a route of invasion of the brain. The position with respect to other cranial nerves and the internal ear has not been examined.

The *neurological signs* and lesions of listeriosis in the various ruminants are qualitatively the same, differing only in severity. The signs are combinations of mental confusion and depression, head pressing, and paralysis of one or more medullary centers. Characteristically, there is deviation of the head to one or other side without rotation of the head; when such an animal moves it does so in circles, hence the name "*circling disease*." There is frequently *unilateral paralysis of the seventh nerve* causing drooping of an ear, eyelid, and lips. There may also be paralysis of the masticatory muscles and of the pharynx. *Purulent endophthalmitis*, which is usually unilateral, is often present and has caused listeriosis to be confused with malignant catarrhal fever. The course of the disease in sheep and goats is a few hours to 2 days; survival occurs but usually with neurologic handicaps.

Listeriosis in *swine* is comparable to the disease in ruminants, but is relatively rare. Outbreaks of encephalitis may be observed with lesions of usual distribution in the brainstem. Alternatively, there may be abortion and neonatal death. The usual expression of the disease is visceral with miliary abscesses in the liver and heart.

The patterns of listeriosis in *other domestic species* appear to follow the general scheme but are rarely observed. The encephalitic form in adult horses, the septicemic form in foals, and abortions in mares are reported. Several cases of encephalitis caused by *L. monocytogenes* are reported in dogs.

Gross lesions are usually not observed in the brain in listerial encephalitis. Occasionally, the medullary meninges are thickened by green gelatinous edema, and gray foci of softening may be found in the cross-section of the medulla. The initial lesions are parenchymal; involvement of the meninges, which is almost constant, is secondary to the parenchymal lesions. Mild meningitis commonly affects the cerebellum and cranial cervical cord, and less commonly is found in patches over the cerebrum and down the spinal cord. *The characteristic parenchymal lesion is a microabscess*. It may begin in a tiny collection of neutrophils (Fig. 3.112A), but more usually begins in a minute focus of microglial reaction. The glial nodules may persist as such, the cells taking on the characters of histiocytes, but the tendency is always for the nodules to be infiltrated by neutrophils and for their centers to liquefy (Fig. 3.112B). The focal lesions do not

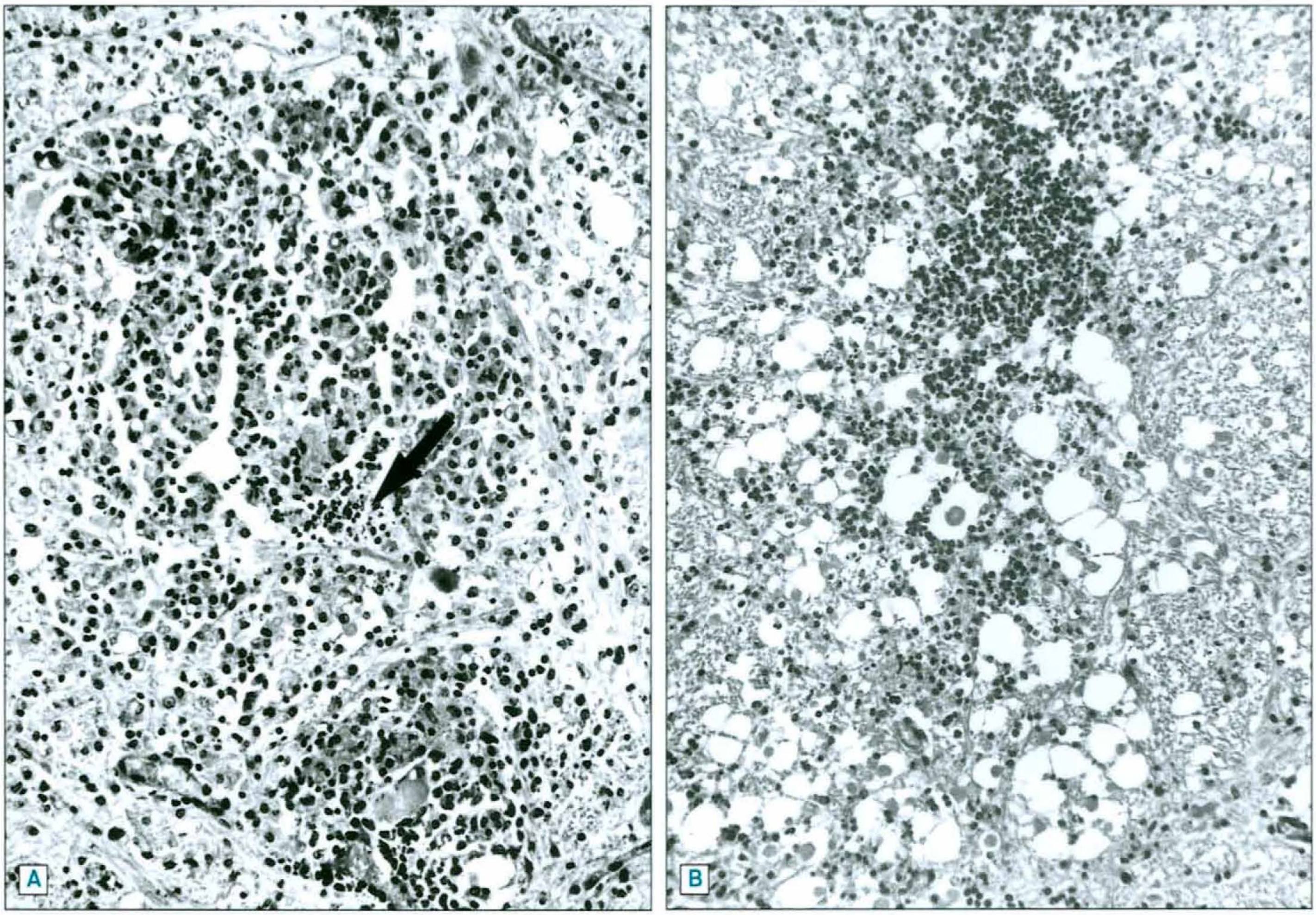

Figure 3.112 Listeriosis. A. Microglial reaction with small focus of **suppuration** (arrow) in a sheep. **B. Suppurative encephalitis** in an ox.

expand much, but suppurative foci may streak through the white matter. Apparently, the organism is not highly toxigenic because the parenchyma surrounding the glial nodules and focal abscesses may be little changed. Commonly however, the white matter is edematous and rarefied. Such areas may be large and lightly, but diffusely, infiltrated by neutrophils and hypertrophied microglia. Focal softening occurs and may coalesce. They are related to vessels that are occluded by inflammatory and thrombotic changes.

Acute vasculitis with exudation of fibrin occurs in the white matter in relation to suppurative foci. The vasculitis is secondary to drainage in the Virchow–Robin spaces from the primary parenchymal foci. It is in this manner that the meningeal infiltrates develop. Perivascular cuffing is heavy. The cuffs are composed mainly of lymphocytes and histiocytes with a few admixed neutrophils and eosinophils; granulocytes predominate in some cases.

Confirmation of the *diagnosis* is by culture, which is usually difficult and needs special procedures. Demonstrating gram-positive intramonocytic or intraneutrophilic bacilli in tissues in association with the aforementioned lesions is pathognomonic for listerial encephalitis.

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Histophilus somni infections (histophilosis, or H. somni disease complex)

Histophilus somni (formerly *Haemophilus somnus*) is the only species of the genus *Histophilus*, family Pasteurellaceae, and encompasses bacteria isolated from cattle and previously described as *Haemophilus somnus*, as well as ovine isolates formerly referred to as *Histophilus ovis* and *Haemophilus agni*. *H. somni* is a fastidious gram-negative coccobacillus, and a facultative anaerobic organism that is considered normal flora of the male and female bovine genital tract and to lesser extent the bovine nasal cavity. Calves are infected by carrier cows in the first months of life, and they in turn disseminate the infection in feedlots. The mechanism, site, and circumstance by which the bacteria invade the bloodstream are not known, but it is possible that organisms in genital discharges or aerosolized urine invade via the respiratory tract. Infection, especially the respiratory form, is usually preceded by stress factors such as transportation.

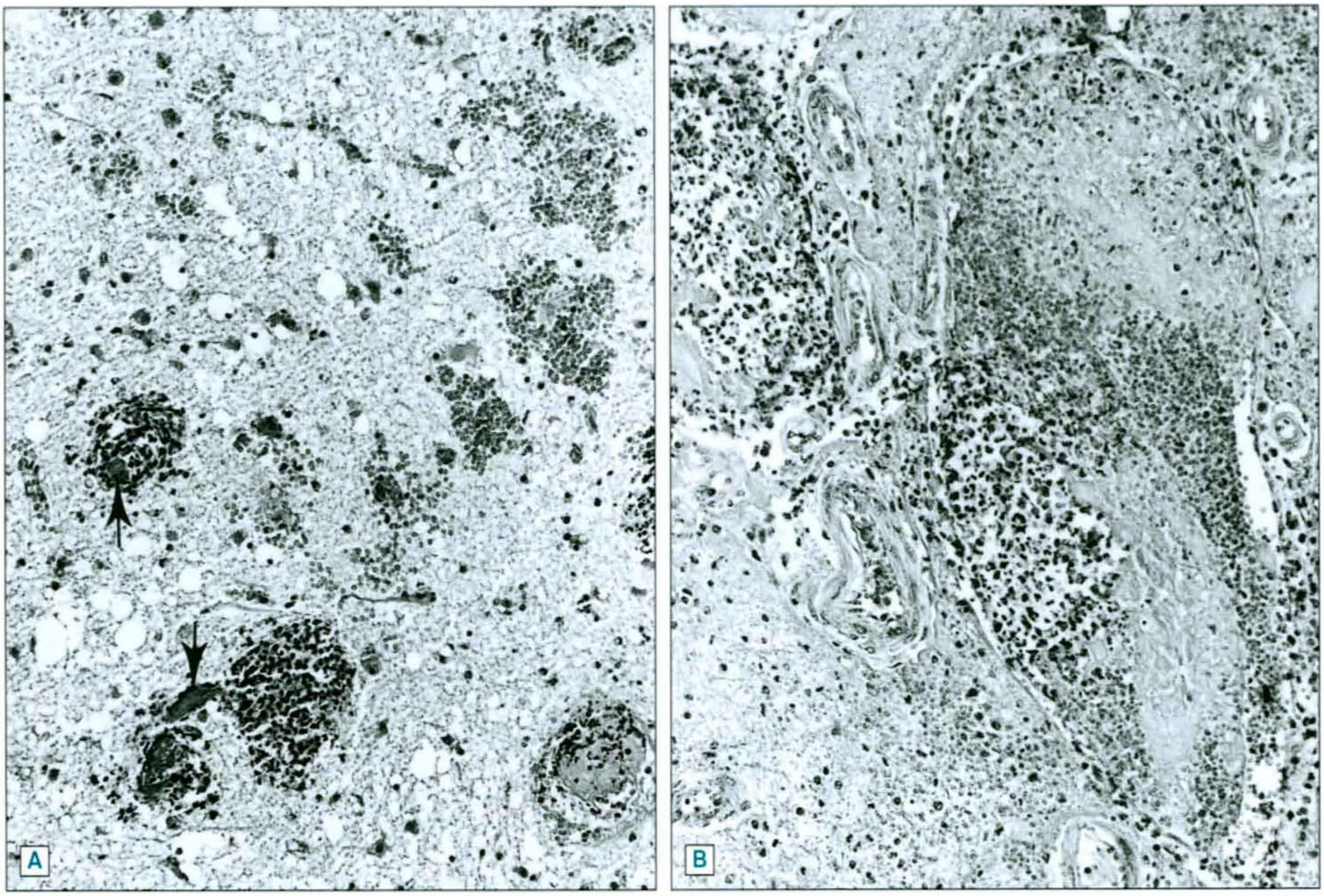

Figure 3.113 ***Histophilus somni*** infection in an ox. **A.** Thrombosis, hemorrhage, and bacterial colonies (arrows) in cerebral white matter. **B.** Thrombosis of meningeal vessels.

These bacteria have virulent and avirulent strains. Important *virulence factors* include lipo-oligosaccharide (LOS), immunoglobulin Fc binding proteins, inhibition of oxygen radicals, and intracellular survival. The mechanism of vasculitis in *H. somni* infection is complex but it can be partially explained by the effect of LSO on endothelial cells. LSO triggers apoptosis of bovine endothelial cells in vitro by caspase-3 activation. This effect is associated with the production of reactive oxygen and nitrogen intermediates. Cerebral blood vessels are particularly vulnerable to damage by the organism, but vasculitis can develop in most organs.

Histophilus somni causes a *septicemia* that may result in acute death or it may localize in one or several organs causing subacute or chronic, fatal or nonfatal disease. The septicemic phase of the disease is brief, and accompanied by fever and stiffness with few other signs. In some cases, the infection is controlled at this stage without localization, but often it leads to cerebral vasculitis with acute thrombosis, hemorrhage, and necrosis (Fig. 3.113A, B).

Histophilus somni can infect several bovine organ systems and cause infectious thrombotic meningoencephalitis (ITME), otitis externa, pneumonia, laryngitis/tracheitis, myocarditis, abortion, metritis/infertility, arthritis, mastitis, orchitis, and conjunctivitis. *Vasculitis with secondary thrombosis is the hallmark of the infection.* Bacterial embolism does not occur but may be simulated microscopically by intravascular proliferation of bacteria at sites of thrombosis. Thus, the former name "thromboembolic meningoencephalitis" is inaccurate. Histophilosis is an important disease of young cattle, but acute and chronic infections involving other organs are also economically significant.

Septicemia caused by *H. somni* develops in cattle of various ages maintained under various management systems, but in North America the disease is more prevalent in feedlots. It is most common in early winter, shortly after susceptible animals are moved there from pastures. Infection occurs in a large proportion of animals, but disease occurs in a minority. *H. somni* induces conditions in sheep identical to the bovine disease, i.e., septicemia, ITME, abortion, etc.

Cerebral localization produces a variety of neurologic signs, and without treatment affected animals become comatose and die within 1–2 days. The cerebral lesions are distinctive, and are visible in a large majority of untreated cases. The cerebrospinal fluid is cloudy and may contain pus and fibrin. *Scattered throughout the brain and spinal cord are multiple foci of hemorrhage and necrosis* (Fig. 3.114A, B). These foci may be 1–30 mm in diameter, and range from the bright red of recent hemorrhage to dark red-brown in older lesions. They have near-random distribution throughout the brain, but there may be some predilection for the thalamus and junction of the gray and white matter of the cerebral cortex. Meningitis is usually visible grossly and is most easily identified over the hemorrhagic foci. In animals that

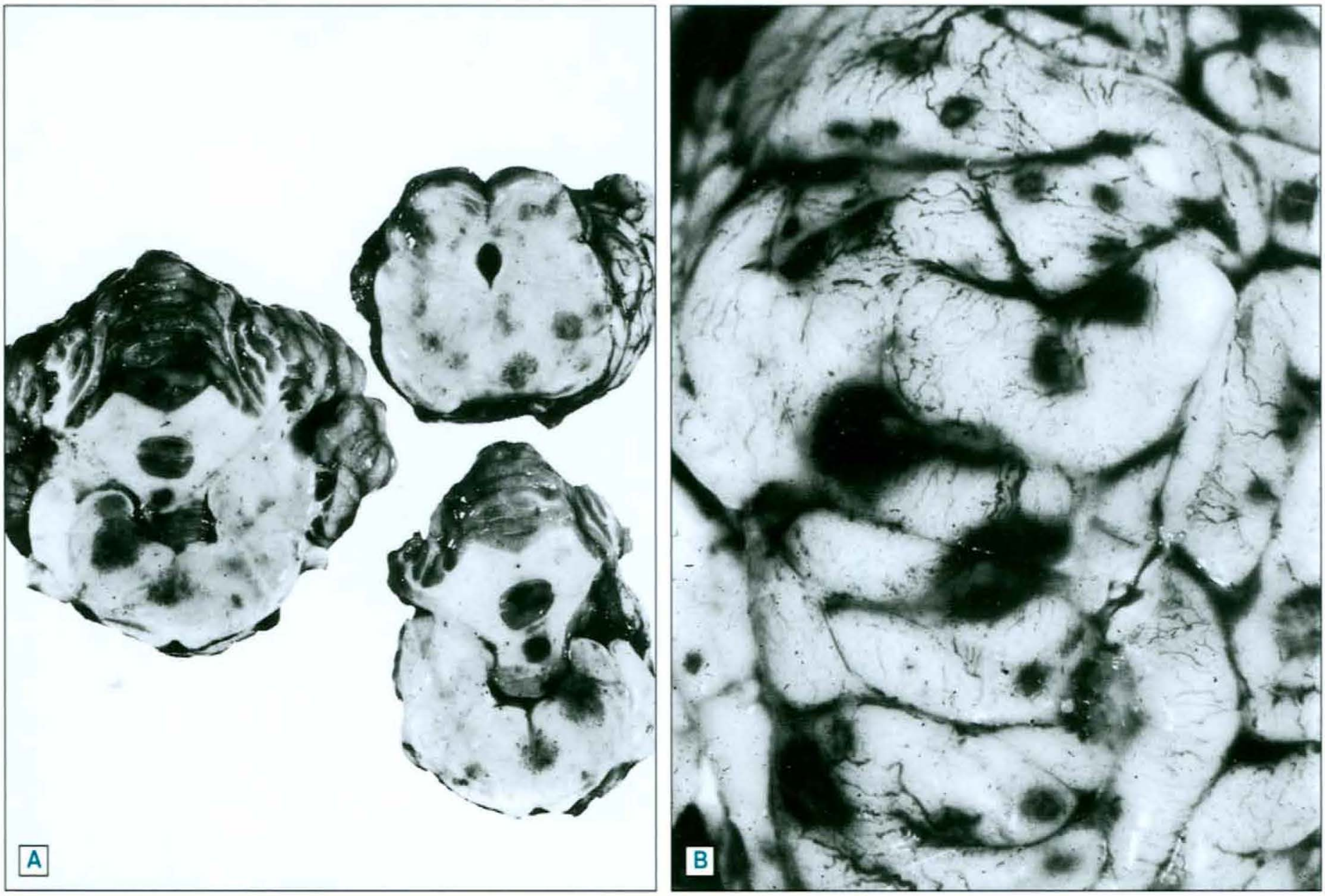

Figure 3.114 ***Histophilus somni*** infection in an ox. Multiple areas of hemorrhage and necrosis in brain stem (**A**) and cerebral cortex (**B**).

survive for a day or more, diffuse purulent leptomeningitis involves the basilar portions of the brain, and in these animals the older parenchymal lesions may have begun to soften.

The *histologic lesions* are similar in all organs but are usually most severe in the brain and consist of *intense vasculitis with thrombosis* and extension of the inflammation into the surrounding parenchyma, with or without infarction (Fig. 3.113A, B). Small venules (thrombophlebitis) are primarily affected, with thrombi often containing colonies of bacteria. The inflammatory response is neutrophilic, and cerebral lesions are quickly converted to abscesses.

Although cerebral vascular localization is a common and most dramatic result of the septicemia, *petechiation and evidence of inflammation are often visible throughout the body*, even in animals that die from fulminating disease. Foci of inflammation are easiest to identify in the renal medulla, skeletal muscle, lung, and laryngeal mucosa, but may also be visible in myocardium, intestine, and urinary bladder. Subacute to chronic disease develops commonly in some organs, especially joints, lungs, and heart, and is an important manifestation of *H. somni* infections.

In the acute disease, there is excess fluid in many *joints*, particularly the atlanto-occipital, where the capsule is distended by fluid that contains fibrin and sometimes blood. The synovial membranes and connective tissues around the affected joints are edematous and petechiated. The organism is sensitive to antimicrobials, and the course of the disease is often modified by therapy. The joints of animals that have survived several days contain thick mats of fibrin and pus, and the periarticular tissues are browned by old hemorrhage. Erosion of articular cartilages is rare.

Histophilus somni has been isolated from cattle with a variety of types of *pneumonia*, and its role in the bovine respiratory complex is discussed in the chapter on respiratory diseases. Pneumonia is an uncommon feature of the acute encephalitic form of the disease, but the lung can be involved as part of widespread vasculitis. *Ulcers of the larynx* occur in other diseases of feedlot animals, but, like atlanto-occipital arthritis, they are a regular enough feature of *H. somni* infection to serve as a valuable clue to the diagnosis and prompt a search for lesions in the brain. Similarly, *retinal hemorrhages* are grossly visible in a percentage of animals, and are of diagnostic assistance clinically and when gross brain lesions are absent.

A major manifestation of *H. somni* infection in some parts of North America, especially in western Canada, is *myocardial localization* following asymptomatic septicemia. Infarction, myocarditis, or abscess formation may result, and can lead to cardiac failure with or without mural and valvular endocarditis. In many animals, myocardial abscesses are found only when the myocardium of animals with chronic pneumonia or pleuritis is incised. Abscesses are most common in the left ventricular free wall, particularly in the papillary muscles.

Histophilus somni causes sporadic disease involving many other organs, including the ear, mammary gland, and those of the male and female genital tracts. It can also colonize the pregnant uterus and produce fetal disease and abortion (see The female genital system). The reported cases have not involved animals with cerebral localization, and may result from asymptomatic septicemia or transcervical invasion.

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Viral infections of the nervous system

Viral infections of the central nervous system are common, usually as part of systemic infections rather than examples of a specialized affinity for nervous tissue. Several viruses are neurotropic, however only a few are neurovirulent. A neurovirulent virus is a virus that multiplies in neural tissue and is able to induce lesions.

The **routes of invasion** of the nervous system available to viruses are *via nerves*, including the olfactory tracts, and *hematogenous*. Centripetal spread in axons does not depend on viral replication in axons, but passage is provided by the mechanisms of fast axoplasmic transport. Invasion by the olfactory route is theoretically simpler since olfactory receptors extend into and beyond the olfactory epithelium, and a cuff of arachnoid extends through the cribriform plate to the olfactory submucosa. This arrangement would allow viruses to spread along nerves without first penetrating to the submucosa or, having penetrated to the submucosa, to reach the CSF directly. This may be the route taken by *Bovine herpesvirus 1, Porcine teschovirus*, and Pseudorabies virus in young pigs. The olfactory route could be used following intranasal exposure or following hematogenous seeding of the olfactory epithelium or mucosa.

For most viruses, spread to the CNS is *hematogenous* after multiplication in some other tissue and the development of viremia of sufficient magnitude and duration. Viruses are susceptible to removal from blood by histiocytes, but viremia can be sustained if the viruses are small, associated with cells of blood, or replicated in endothelium or lymphatic tissues. Some viruses, such as *Canine distemper virus*, infect vascular endothelial cells whereas others infect surrounding glia suggesting transport across endothelium in pinocytotic vesicles. Invasion across the choroid plexus is not impeded by the fenestrated endothelial cells there but would require active infection of the epithelium and ependyma. There are areas of selective permeability to indicator dyes in the brain, such as the tuber cinereum and area postrema, but there is no evidence that these are selectively used by viruses.

The means by which viruses *spread in nervous tissue* are not known. Rapid dissemination can occur in the CSF, but some viruses, such as *Rabies virus*, extend rapidly through the parenchyma. The rate of spread of *Rabies virus*, assuming cell-to-cell growth and transmission, is probably more rapid than its generation time would permit, and permeation of the limited extracellular spaces of the brain by large viruses seems unlikely.

Separation of the specialized cells of the CNS into neurons, oligodendrocytes, astrocytes, and ependymocytes understates the diversity of cell populations in the brain and cord. Different cell populations may show different susceptibilities to infection by different viruses. Thus, Pseudorabies virus is nonselective in the destruction of cells in the rostral cortex of piglets, *Feline panleukopenia virus* selects rapidly proliferating cells of ependyma and cerebellar cortex, and *Porcine teschovirus 1* appears to affect particularly the spinal motor neurons.

Degeneration of neurons, reactivity of the glia, and perivascular reactions are the general hallmarks of viral infection of the CNS, and imply a sequence of viral cytopathogenicity and reaction to cellular degeneration. However, viruses that produce manifestations deviating from this rather simple system are many. *Classical swine fever virus* and *Bluetongue virus* at a suitably early fetal stage of development may produce **malformations** characterized by degeneration of tissue without reaction; later when the animal is immunologically competent, these viruses may produce ordinary encephalomyelitis. *Feline panleukopenia virus* causes cerebellar hypoplasia in the neonate but is without effect at a later stage of development.

Some viruses may produce *persistent tolerated infection* without clinical signs or lesions. At the other end of the spectrum is *Rabies virus*, which can cause death with minimal cytopathic effect and reaction, or no morphological change at all. Some virus infections are characterized by long latency and slow attrition, visna/maedi of sheep being the standard example expressed as either degeneration or chronic inflammation. *Transformation of nervous system cells by viruses* can be demonstrated in vitro, and a variety of tumors of the nervous system can be produced by viruses in experimental animals, but they have, as yet, no natural counterpart. The contribution of an immunological response to both the progress and morphology of encephalomyelitis is very difficult to determine.

General pathology of viral inflammation of the nervous system

Viral infections of the CNS typically induce **nonsuppurative inflammation**, a term that includes quite a variety of quantitative and qualitative changes. The changes are not specific for viral infections, being produced in some bacterial infections such as salmonellosis in swine, in the rickettsial infection, "salmon poisoning," of dogs, and probably in other rickettsioses. Nor are the lesions qualitatively the same in all viral infections, a fact that is of considerable usefulness in differential diagnosis. The differences are due in part to inherent characters of the agents, routes of invasion, patterns of localization, success of viral replication and release, duration and degree of cellular injury, and host defense reactions. As well as the differences, there are also the very considerable similarities that allow them all to be included with the "nonsuppurative encephalomyelitides."

The distribution of lesions in different viral infections is a reflection of the varied affinities of the viruses, and is useful in differential diagnosis but only generally applicable. The distributions of lesions or of inclusion bodies are only very rough guides to the distribution and activity of the virus.

Perivascular cuffing, or accumulation of cells in the adventitia of vessels and in the perivascular Virchow–Robin space, is almost constant in encephalitis, and, when present, is usually the most striking microscopic change. The accumulated cells are usually leukocytes, but in some diseases there may be very few hematogenous elements recognizable, the cuffs being composed instead of histiocytic cells that appear to have proliferated in situ from adventitial elements. These latter frequently fragment to resemble degenerate neutrophils if the postmortem interval is prolonged. Injury to the vessel wall proper is not constant, but when it occurs it may affect the arteries as a hyalinizing or fibrinoid change. This is characteristic of malignant catarrhal fever, for example, and occurs also in equine encephalitis. The endothelium may be selectively injured in classical swine fever and infectious canine hepatitis. The lesions associated with *Equid herpesvirus* infection may be limited to quite subtle endothelial swelling and proliferation in small blood vessels. When, in inflammation, the perivascular cuffs are large enough to disorganize the wall of the vessel and compress it, the endothelium frequently shows signs of swelling and proliferation, and there may also be altered adhesiveness so that leukocytes and red cells tend to stick to it. Thrombosis is incidental and very seldom observed, but compression of the vessels probably accounts for the ischemic parenchymal softenings that occur.

Infiltrating cells are predominantly lymphocytes, and they accumulate in the perivascular spaces (see Fig. 3.19). The earliest cells in the perivascular spaces may be neutrophils; in acute lesions, some of these can be found wandering in parenchyma or grouped in dense clusters. They soon disappear but may be found later for a short time in areas that soften. Lymphocytes remain in the cuffs and are admixed after a week or so with an increasing number of plasma cells and macrophages. A few eosinophils may also be found in pigs. Perivascular cuffing is not specific for viral infections. It is also a reaction to degeneration of neural tissue.

Glial reactions occur if the parenchyma is injured, even though the injury may not be appreciable, but the reaction may be absent in those infections that selectively involve the vessel walls. In inflamed areas, the oligodendroglia degenerate. Astrocytes degenerate or react depending on how strictly and severely they are injured – the stimuli for astrocytic reaction were discussed earlier in this chapter. *The gliosis that is so frequently a feature of nonsuppurative encephalomyelitis is almost solely microglial. The gliosis may be diffuse or focal but is commonly both* (see Fig. 3.17). Diffuse gliosis may be more apparent than real if due more to hypertrophy than to proliferation of these glia. Focal gliosis may occur anywhere in the parenchyma and may be related to small vessels and injury to microvasculature. When there are more than a dozen or so cells in such foci, some of the cells are likely to be lymphocytes, and occasionally there are a few plasma cells. The microglia in the center of a focus are frequently degenerate. There are two specifically named forms of microgliosis: "*neuronophagic nodules*" are foci of microglia about degenerating neurons (see Fig. 3.2D); "*glial shrubbery*" is an accumulation of these cells in the molecular layer of the cerebellum in relation to degenerating Purkinje cells (Fig. 3.115).

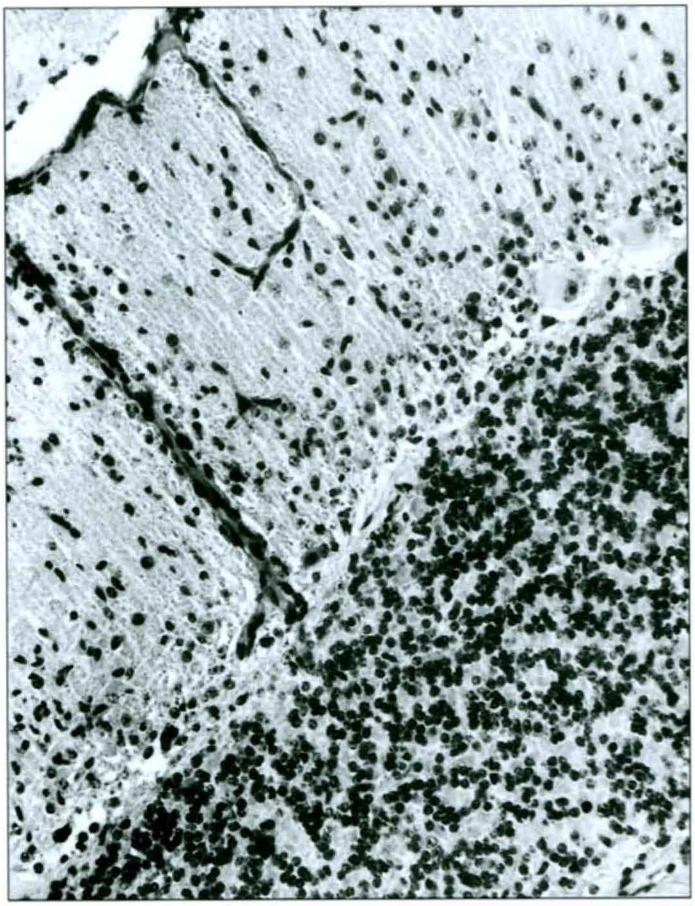

Figure 3.115 Encephalitis in **louping-ill** in a sheep. Destruction of Purkinje cells and gliosis in molecular layer of cerebellum ("glial shrubbery").

Neuronal changes must be the principal determinants of the outcome of the infection. The distribution of neuronal degeneration is to some slight extent specific, but the extensiveness is nonspecific and varies considerably. The morphological features of the degenerate cells are nonspecific. As a rule, the more severe degrees of neuronal degeneration are caused by viruses that are highly neurotropic, such as *Rabies virus*, but the severity of neuronal degeneration is not a dependable "lead" in differential diagnosis. The fact that *Rabies virus* can be lethal without morphological change in the CNS raises the possibility that other viruses may cause severe encephalopathy without inflammatory change. *The usual form of neuronal degeneration is central chromatolysis*, which may extend to completion with the cell then swollen, pale, and devoid of a nucleus. This is typical of the axonal reaction to injury, but the axons are intact. Many neurons appear as if coagulated, being shrunken, rounded, and isolated, and staining darkly with eosin. The nucleus is pyknotic or has disappeared. The coagulated neuron stimulates the formation of the neuronophagic nodule, but not all necrotic neurons elicit the reaction. Usually, intact neurons can be found adjacent to degenerate ones.

Lesions in white matter occur consistently even though many viruses are supposed to be specifically tropic for gray matter. Cuffing reactions are to be expected even if degeneration is limited to adjacent gray matter. *Microgliosis* is focal rather than diffuse and the nodules are small. Some degree of *disintegration of myelin* is inevitable, but conspicuous demyelination is a feature only of canine distemper, lentivirus leukoencephalitis of goats, and visna. Demyelination associated with viral diseases may be due to direct infection of melanin-forming cells, i.e., oligodendroglia, as in infection with *Canine distemper virus*, or may be a bystander lesion due to injury from inflammatory cytokines associated with viral infection.

Meningitis is seldom severe except in local distributions, and these tend to overlie parenchymal lesions. Some agents, of which typical examples are those of sporadic bovine encephalomyelitis (see Fig. 3.60) and malignant catarrhal fever, have a selective affinity for meningeal structures in the CNS. Others, such as *Canine distemper virus*, share the affinities so that leptomeningitis is part of the primary response. In the more purely neurotropic infections, such as rabies, the meningitis, or more precisely the meningeal infiltration, is due probably to drainage of products of reaction via the Virchow–Robin spaces from the brain or cord. The reacting cells in the meninges are of the same type as those in the brain, and they float freely in the arachnoid spaces.

No virus is known to be tropic for peripheral nerves in the sense of selectively producing direct lesions in them. Degenerative changes occur fairly early in the end plates and terminal parts of axons when the central cell body is destroyed. Foci of microglia and lymphocytes occur in the nerve roots quite commonly. Inflammation of the paravertebral ganglia, and especially the trigeminal (Gasserian) ganglia, is characteristic of rabies and of Teschen disease and related infections in pigs, but the frequency and distribution of this lesion in other viral encephalomyelitides and in other ganglia is unknown.

Inclusion bodies may form in neurons, neuroglia, or microglia and other mesenchymal cells. Inclusion bodies in the nervous system are usually acidophilic. They may be found only in neurons as in rabies, in glia, or in both. They may be cytoplasmic, nuclear, or both. Intranuclear inclusions, which must be distinguished from altered nucleoli, have considerable specificity; somewhat less reliance can be placed on intracytoplasmic inclusions. Cytoplasmic viral inclusions must be distinguished from normal inclusions.

Lyssavirus infections

Rabies is caused by *Rabies virus* (RABV), which belongs to the genus *Lyssavirus* of the family Rhabdoviridae. The RABV glycoprotein (RVG), which is a trimeric and surface-exposed viral coat protein, is responsible for RABV neurotropism by binding to several neural tissue receptors including the neuronal cell adhesion molecule (NCAM), and the p75 neurotrophin receptor (p75NTR). Evolutionary studies based on genes encoding the surface glycoprotein *suggest that RABV evolved first in bats*, possibly in vampire bats much more widely distributed than at present, and only later became adapted to terrestrial carnivores. There is a single major antigenic type with minor variations that allow epidemiological surveillance. *Seven genotypes* are defined by phylogenetic analysis. Type 1 is the classical *Rabies virus* of animals and vampire bats, and of all other bat lyssaviruses in North America. Type 2 (*Lagos bat virus*), type 3 (*Mokola virus*), type 4 (*Duvenhage virus*) are African genotypes. Types 5 (EBLV-1) and 6 (EBLV-2) are *European bat lyssavirus* 1 and 2, and type 7 (Ballina virus) is the *Australian bat lyssavirus*. The seven genotypes may further be allocated into *two major phylogroups* based on pathogenicity for mice and cross-neutralization. Phylogroup 1 includes genotypes 1, 4–7, and phylogroup 2 includes genotypes 2 and 3. Excepting *Mokola virus*, bats may be the preferential vector species for genotypes 2–7.

Rabies virus has two biotypes: "fixed" virus and "street" virus. *Fixed RABV*, which is the basis of vaccine strains, is a laboratory biotype stabilized in its properties by serial intracerebral passage. It is highly neurotropic, is not secreted in saliva, and does not produce Negri bodies. *Street RABV* is the feral biotype that circulates in enzootics and epizootics. In addition to neurotropism, it is tropic for salivary glands, in which it reaches high concentration, and possibly in other mucus-secreting epithelia, and produces Negri bodies.

The establishment of infection ordinarily depends on inoculation of the virus into a wound, such usually being inflicted by the bite of a rabid animal. Contamination of a fresh wound by infected saliva or tissues is much less dangerous. The virus replicates in myocytes around a bite wound for a short period of time, and then buds from the plasma membrane. Viral particles invade the local neuromuscular junction through conjugation of the RVG with the nicotinic acetylcholine receptor, and then invade neurotendinous spindles and ascend to the CNS and paravertebral ganglia via axoplasmic flow. Viral replication in the CNS is followed by centrifugal spread to major exit portals, such as the adrenal gland, nasal mucosa, and salivary glands; the virus is secreted with the saliva for a few days prior to the appearance of clinical signs. The incubation period is variable from weeks to months.

Although there are species differences in susceptibility, rabies is one disease to which *all mammals are susceptible*. The disease can be regarded as one of carnivores because it is almost always transmitted naturally only by bites, and man, herbivores, etc. are dead-end hosts. There are exceptions to the rule that RABV is bite-transmitted: *oral infection* can occur in diverse species and the application of modified RABV in "baits" utilizes this potential; *aerosol infection* can occur, as in dense congregations of colonial bats in bat caves, probably as droplet infection from salivary secretions; and a variety of aberrant circumstances may provide transfer opportunities for infectious virus, as has been reported for *corneal transplants*.

Reservoir hosts vary from time to time and from region to region. The principal reservoir vectors are: foxes and skunks in the USA and Canada, with the raccoon of importance on the Atlantic seaboard; foxes and dogs in northern Canada; foxes moving from east to west in Europe; wolves in eastern Europe and Iran; jackals in India and Northern Africa; the mongoose and genet cat in South Africa; and the mongoose in the Caribbean. *Sylvatic vectors* are responsible for most transmissions to man and domestic animals in countries where dog populations are controlled. Oral vaccination of wild carnivores, by using vaccine-laden baits, and routine vaccination of dogs have led to almost complete elimination of canine-transmitted rabies in developed countries. Rarely, vaccination of severely stressed animals with vaccines containing modified-live virus may induce post-vaccinal rabies. In tropical areas where domestic and feral dogs are not controlled, these animals are the principal hazards for man and livestock.

Bats present a special epidemiologic problem. Fructivorous and insectivorous bats as well as vampires are capable of transmitting RABV. Vampire bats inhabit South and Central America extending into northern Mexico and are, historically, responsible for a high incidence of rabies in mammals, especially cattle but including humans. When clinically affected, vampire bats manifest the disease as the furious form and show unnatural daylight activity.

The **clinical course** of rabies is usually acute, from 1–2 days, but can be as long as 10 days. It is seldom that a clinical diagnosis of rabies can be made with confidence. The terms "*furious rabies*" and "*dumb rabies*" place emphasis on particular features within a spectrum of behavioral changes and are inappropriate for noncarnivorous animals. *Aberrant behavioral patterns* can be recognized in affected animals during epizootics. The period of salivary excretion of virus before the onset of neurological signs is expected to be not more than a few days, vampire bats possibly excepted, and the duration of clinical disease to be a few days only. Once expressed clinically as neurologic disease, *rabies is almost invariably fatal*; recovery with or without neurologic deficit is quite rare but has been observed in several species following experimental exposure. Progressive infection and clinical disease do not inevitably follow exposure; up to 25% of feral populations may have specific antibodies as evidence that the infection provoked an immune response without progression to neurologic disease.

Specific gross lesions are not present at necropsy, but self-inflicted wounds and foreign bodies in the stomach of a carnivore should raise suspicion. The **histologic lesions** of rabies, when present, are *typical of nonsuppurative encephalomyelitis, with ganglioneuritis and parotid adenitis*. Inflammatory changes are usually present, but they may be very mild or absent. To some extent at least, the severity of lesions reflects the duration of the clinical disease. In the CNS, inflammatory and degenerative changes are most severe from the pons to the hypothalamus and in the cervical spinal cord, with relative sparing of the medulla. This relative sparing of the medulla appears to apply to all domestic species. The most severe lesions of the disease are generally found in dogs whereas other species, especially ruminants, which are highly susceptible, may show little more than an occasional vessel with a few cuffing lymphocytes and a few very small glial nodules (*Babès' nodules* in this disease), and this in spite of having numerous Negri bodies. These reactive phenomena probably reflect largely the degree of neuronal degeneration, and this may be remarkably slight in herbivores and remarkably severe in dogs.

The reaction is typically one of *perivascular cuffing and focal gliosis*. The cuffs are 1 to several cells thick and composed solely of lymphocytes (Fig. 3.116A); ring hemorrhages confined largely to the perivascular space are common about cuffed vessels. Hemorrhages are occasionally severe enough to be visible grossly in the spinal cord of horses and cattle (Fig. 3.116B). The Babès' nodules are composed of microglia, and they occur in both white and gray matter. The nodules vary greatly in size, some containing only six or seven cells and

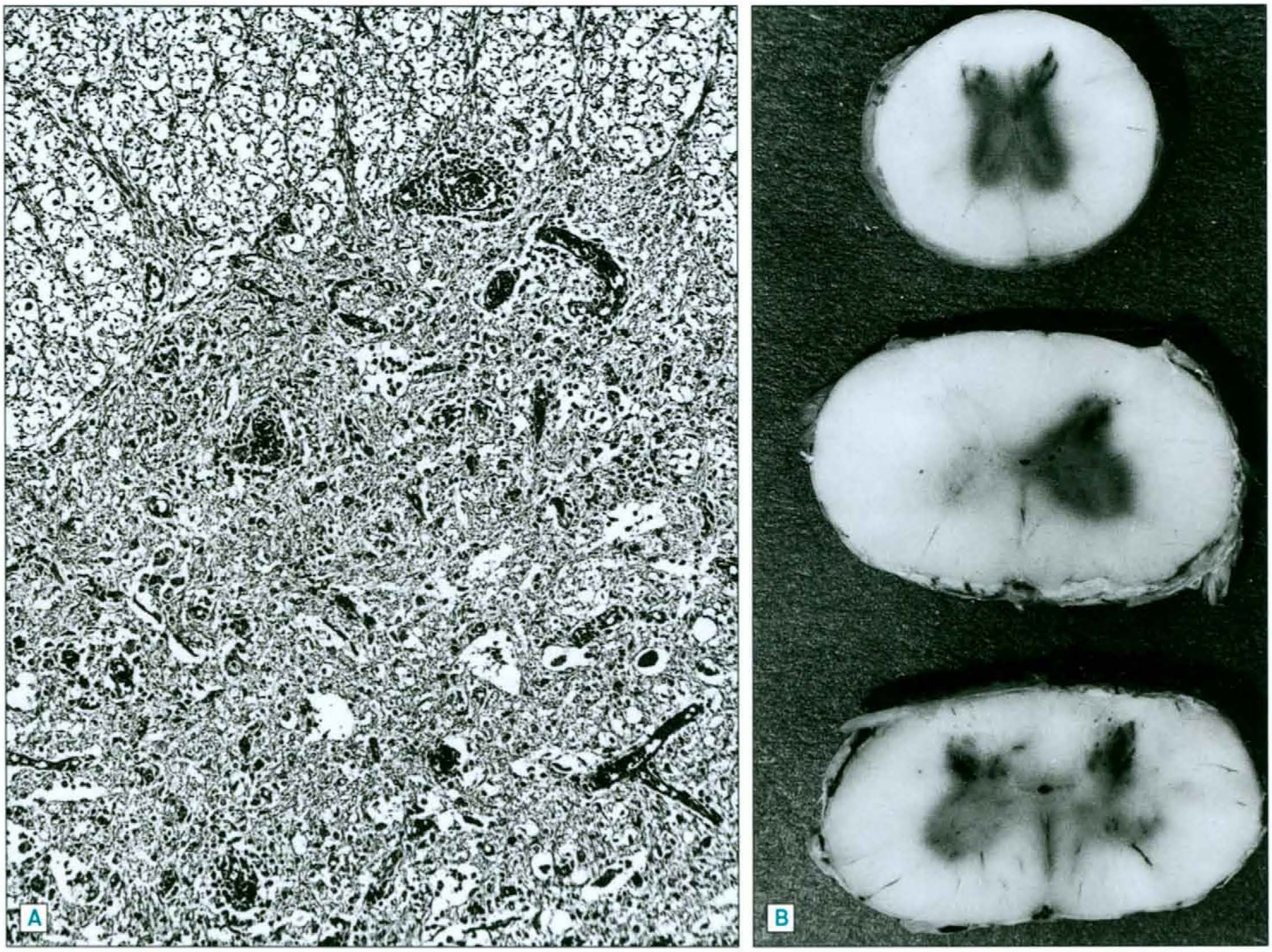

Figure 3.116 Rabies. A. Perivascular cuffs and focal gliosis in a horse. **B.** Hemorrhage in gray matter of spinal cord.

some containing 100 or more. Diffuse as well as focal gliosis occurs in areas of gray matter such as the pons and in the spinal cord, both horns of the latter being involved.

Neuronal degeneration in carnivores may be very extensive and quite out of proportion to the observed reactive changes, but may be very slight in pigs and herbivores. Neuronal and/or gray matter neuropilar vacuolation (*rabies-induced spongiform encephalopathy*) is reported to occur in experimental and natural rabies. The specificity of the neuronal changes and of the whole pathologic picture depends on the *inclusion bodies of Negri*. These are always *intracytoplasmic* and are present most commonly in the hippocampus of carnivores and in the Purkinje cells of herbivores. Fixed RABV does not produce Negri bodies, and street RABV fails to do so in up to 30% of cases. Neurons of any distribution may contain inclusion bodies, but they tend to be scarce where the inflammatory reaction is severe. Indeed Negri bodies may be found only in neurons that are otherwise histologically normal; they are not present in degenerate neurons. They have also been found, but rarely, in ganglion cells of the adrenal medulla, salivary glands, and retina. While the number of Negri bodies has little relation to the length of the incubation period, there is a relation to the duration of the clinical disease. They may not be found if the animal is killed instead of being allowed to die. They are produced consistently in white mice, the usual test animal.

Negri bodies are round or oval structures usually ~2–8 μm in diameter (Fig. 3.117A). They are plastic, their shape being molded to their environment. Those in the dendrites, seldom observed except in Purkinje cells, are oval and those in the cell body are usually rounded. There may be one or more per cell, and affected cells are otherwise only little changed. The inclusions are surrounded by a clear thin halo. Nonspecific homogeneous inclusions may be found in the pyramidal cells of the hippocampus in cats, skunks, and dogs. There may be several such inclusions per cell but each is minute, not measuring more than 1.5 μm. In old sheep and cattle, larger neurons, especially of the medulla and cord, may contain nonspecific inclusions. These have a dust-like distribution, are usually numerous, and are brightly acidophilic, angulated, and ~1.0 μm in size. Nonspecific inclusions that are indistinguishable from Negri bodies by light microscopy occur in the lateral geniculate neurons in cats. *Fluorescent antibody techniques are required for positive identification* and are essential in the rare chronic cases which may not yield virus on mouse inoculation.

If there is no *ganglioneuritis* in the paravertebral ganglia, then the possibility of the animal having rabies is very remote. If there is ganglioneuritis, it may be part of rabies or something else. Pigs, for example, get ganglioneuritis in the Teschen group of infections. Inflammatory changes in the trigeminal (Gasserian) ganglion in rabies may be present without inflammatory or neuronal changes being clearly evident in the brain. The ganglionic changes are of the same character as those in the brain, namely acute degeneration of ganglion cells, proliferation of capsule cells, and microglial nodules (Fig. 3.117B).

The natural transmission of RABV depends on virus being present in the saliva and, therefore, in the salivary glands. Fixed virus

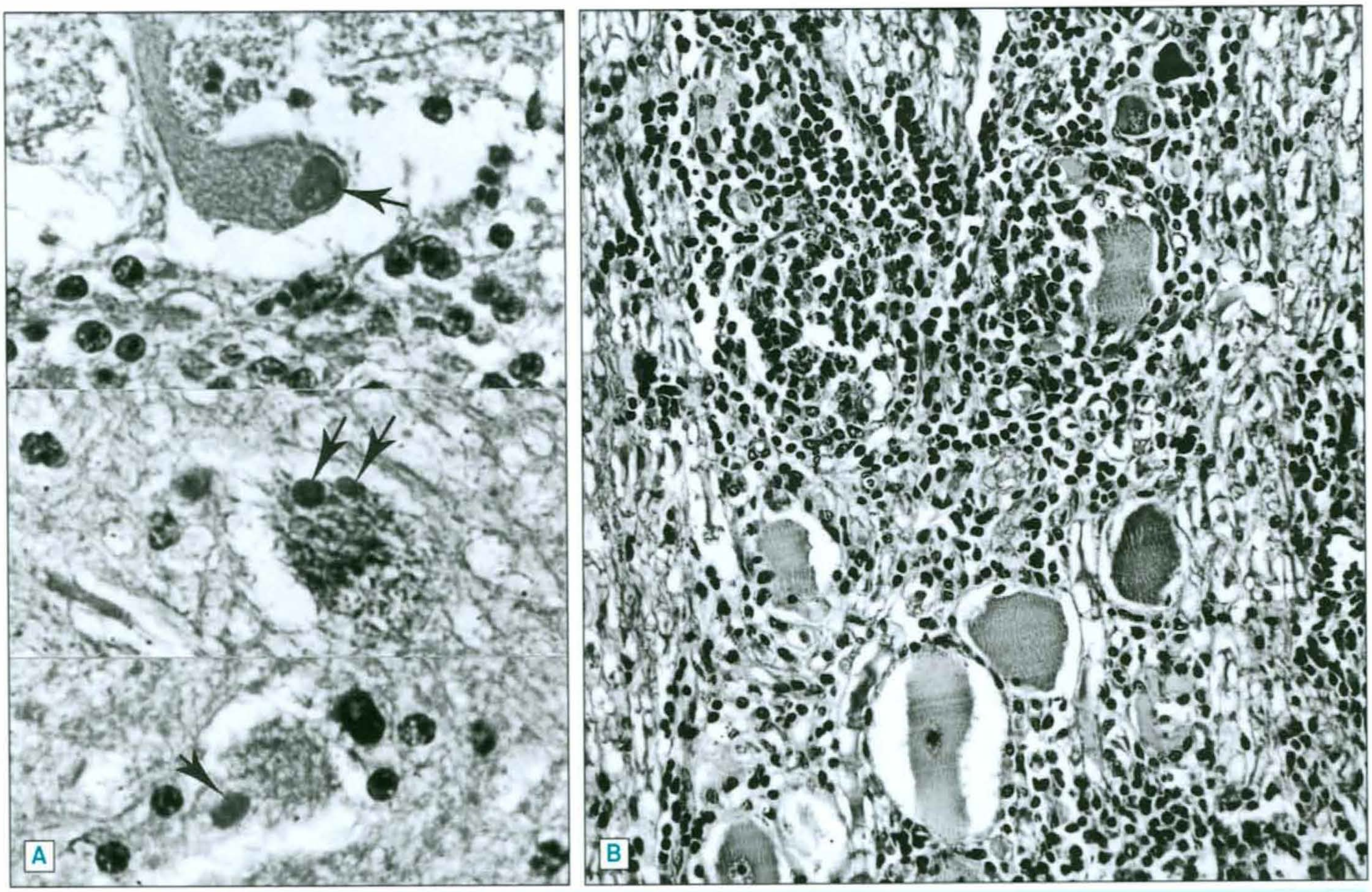

Figure 3.117 Rabies. A. Composite picture showing Negri bodies (arrows) in neuronal cytoplasm. **B.** Severe nonsuppurative inflammation of trigeminal ganglion.

has no affinity for the salivary glands, and none is present in some cases of infection with the street virus. Degenerative changes are reported in the epithelium of the mandibular salivary gland, but not in the parotid, in dogs.

The **diagnosis** of rabies is made by utilizing fluorescent antibody labeling on fresh or fixed tissue, or by virus isolation in cell culture.

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Pseudorabies

Pseudorabies is also known as Aujeszky's disease, mad itch, infectious bulbar paralysis, and porcine herpesvirus infection. The causative agent, ***Suid herpesvirus 1*** (SuHV-1; Pseudorabies virus, PRV), belongs to the genus *Varicellovirus*, subfamily Alphaherpesvirinae, family Herpesviridae, but is unusual for a member of that group in its relative lack of host specificity and by being spread laterally as well as vertically in swine. A number of strains have been identified which exhibit a wide range of virulence. Pseudorabies virus is capable, as are other members of the family Herpesviridae, of establishing latent infection. Trigeminal ganglion, olfactory bulb, and tonsil are the most consistent sites of latency of PRV. In these organs, viral DNA can be detected in the absence of infectious virus. The pig is the only natural host, but the common domestic species are naturally susceptible; there are very few reports in horses and goats. Progressive infections do not occur in humans. The disease is reported worldwide, except for Canada and Australia. Natural infections occur in rats and mice and various species of wildlife and on fur farms. Of the laboratory animals, the rabbit is the most susceptible and is preferred for identification of the virus because of the fairly consistent development of intense local pruritus following subcutaneous inoculation. Guinea pigs are less susceptible and may resist subcutaneous inoculation but succumb to intracerebral and, occasionally, to intraperitoneal inoculation.

The virus is maintained in enzootic areas in wild and domestic swine, for which it is highly contagious but usually asymptomatic, and probably in brown rats. *Transmission* can occur by ingestion, but the usual method of spread between pigs is thought to be by contact of infective secretions with nasal mucosa or abraded skin. Animals are susceptible to intranasal inoculation and, regardless of the route of infection, the *virus can be found in nasal secretions*. The virus may also be present in saliva and urine. It is also present in blood, but this is of no significance for epidemiology or transmission. The infection will occur in pigs by contact very readily and probably by direct nose-to-nose transmission, but it does not appear to be contagious between individuals of other species and they probably acquire their infection by contact with swine or, possibly, rats. Pigs may harbor virus for many months in tonsils and nasopharyngeal secretions after exposure, but in other domestic species the virus is fairly strictly neurotropic, and therefore is not excreted unless given experimentally in large doses. Ingestion of *infected pig meat* is the usual source of infection for dogs and cats. Cattle and sheep may become infected by direct contact with carrier swine or by aerosol exposure, but there is strong circumstantial evidence implicating contaminated feed.

The *pathogenesis* of the infection following local inoculation is well established for the rabbit and is probably comparable in other species. The virus causes a local reaction at the site of inoculation if percutaneous and then spreads centripetally along the related nerve to the spinal cord; it then spreads outwards again along other peripheral nerves as other segments of cord are progressively invaded by spread within the CNS. Because of the progressive advance of infection along the cord, death may occur before demonstrable amounts of virus reach the brain and before lesions have time to develop there. Intracerebral inoculation produces encephalitis, and virus spreads to the cord and centrifugally along peripheral nerves to an extent that depends on survival time. Because the virus also circulates in the blood, there is some possibility but no evidence that it invades the brain directly, the evidence instead suggesting that it localizes in viscera and invades the nervous system along autonomic nerves. Following nasal or intraocular exposure, the virus spreads along the related nerves. The route of invasion following ingestion is by retrograde transneuronal infection. Transplacental infections occur in pigs causing abortion in about 50% of sows pregnant in the first month, and the delivery of macerated, mummified, and normal fetuses when infection occurs at later stages of gestation. The virus is reported to be present in the semen of carrier boars.

The *signs and course* of pseudorabies in pigs are very variable. Most cases are of mild febrile illness without pruritus or nervous signs, and with recovery expected in a week or so. Sows may subsequently produce mummified litters. Age is a very important factor governing the severity of the disease in swine; the mortality rate in nursing pigs and young weaners may be very high. Very young sucklings do not show specific nervous signs but rapidly become prostrate and die in 12–24 hours. In slightly older piglets, incoordination progresses rapidly to paralysis with muscular twitchings, tremors, and convulsions. Some pigs showing severe signs of encephalitis recover. Experimental peripheral inoculation will produce asymptomatic meningitis and encephalitis in swine, the inflammatory lesions being severe. The disease in older pigs is often characterized by fever, rhinitis, and coughing. There may be generalized pruritus in natural cases but it is not severe, being expressed usually by rubbing of the nose or head. Fetal resorption, mummification, stillbirths, and abortions are frequently reported.

The characteristic clinical sign of pseudorabies in animals other than pigs is *intense cutaneous irritation* developing at the point of inoculation or at the terminal distribution of a nerve trunk which passes the point of inoculation. This does not occur until the virus reaches the related segment of cord. Dogs may become frenzied, and besides the intense pruritus (mad itch) there may be jaw paralysis and drooling reminiscent of rabies. The clinical course in these species, which always ends in death, is frequently acute (a few hours) and never longer than 1 week. Pseudorabies may occur in sporadic, although significant, outbreaks in sheep and cattle. The mortality rate is very high. Death may occur without signs of illness or within 1–2 days of the onset of clinical signs. There is fever, and the itching may be on any part of the body but is most frequently about the head or hindlimbs. Other neurological signs are variable but constantly present.

There are *no specific gross lesions* of pseudorabies. At the site of cutaneous infection, there is acute serofibrinous inflammation, ballooning degeneration, and epithelial necrosis with rare intranuclear inclusions. Self-trauma due to intense itching may exacerbate these lesions. The intense pruritus at the site of inoculation is likely due to stimulation of regional sensory nerves by viral spread and multiplication. Gross changes are seen mostly in young pigs. There may be necrosis of tonsils and sometimes of the trachea and esophagus. Rhinitis with patchy epithelial necrosis is common. The lungs may be edematous. Tiny foci (1–2 mm) of hemorrhagic necrosis typical of alpha-herpesviral infection may be seen in liver, spleen, lung, intestines, adrenals, and placenta.

The **histologic lesions** reflect the neurotropic and epitheliotropic nature of the virus. Lesions are similar in all susceptible species, however, epitheliotropic lesions are more commonly seen in young, aborted, or stillbirth piglets and rarely seen in ruminants or carnivores, where the brain lesions are more common. In brain, the gray matter especially is affected, but death may occur before there are clear indications of neuronal degeneration or inflammatory reaction in the brain. With naturally acquired infections, the inflammatory changes are *nonsuppurative*. In addition, focal gliosis and lesions typical of neuronal degeneration (neuronophagia and satellitosis) are usually present (Fig. 3.118A, B). There is severe ganglioneuritis in paravertebral ganglia. The specificity of the reaction in the brain depends on the development of *acidophilic intranuclear inclusion bodies in neurons and astroglia* (Fig. 3.118C). These inclusion bodies occur in all species, including pigs; fixation in a mercurial fixative is helpful for their demonstration. Inclusions in swine are *solid and amphophilic*, but in other species the inclusions are granular and often small and multiple in an affected nucleus. By any route of infection, piglets tend to develop panencephalitis with most severe lesions in the cerebral cortex, brain stem, spinal ganglia, and basal ganglia of the brain (Fig. 3.118B); in other domestic species, the distribution of lesions in the CNS is local to, and determined by, the route of exposure. Lymphoplasmacytic inflammation with neuronal degeneration of the gastric myenteric plexi is also described.

Epitheliotropic lesions include the presence of tiny areas of coagulative or lytic necrosis in the liver, tonsils, lung, spleen, placenta, and adrenals with the presence of the characteristic intranuclear inclusions. Pulmonary lesions may be mild or severe. Edema and mild cellular infiltration may be diffuse and there may be focal or confluent necrotizing, hemorrhagic pneumonia (Fig. 3.118D). Hemorrhage and necrosis is present in lymph nodes, and foci of necrosis may be found in tonsils, liver, spleen, and adrenal. Necrotizing vasculitis is described in natural infections in sheep and experimentally in piglets. In aborted or stillborn piglets, which are suitable for examination, there is usually no evidence of encephalitis, but foci of necrosis may be found in liver and other parenchymatous tissues together with focal bronchiolar necrosis and interstitial pneumonia.

Rapid **diagnosis** can be achieved by fluorescent antibody tests on frozen sections of tissue, e.g., tonsils, liver, or brain. Isolation in eggs or tissue culture is also available.

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Porcine hemagglutinating encephalomyelitis virus

Porcine hemagglutinating encephalomyelitis virus (HEV), a member of genus *Coronavirus*, family Coronaviridae, can be isolated from the respiratory tract of normal pigs, and is present worldwide. Other porcine coronaviruses include *Transmissible gastroenteritis virus*, *Porcine epidemic diarrhea virus*, and *Porcine respiratory coronavirus*. HEV is a group 2 species coronavirus antigenically related to *Bovine coronavirus*, *Human coronavirus OC43*, and *Murine hepatitis virus*. The pig is the only natural host for HEV. Although there is serological evidence of wide distribution in many swine-raising areas, clinical disease is rare because most piglets receive protective levels of colostral antibodies to HEV. Disease occurs in piglets 1–3 weeks of age and follows a clinical course of about 3 days to 3 weeks. The mortality rate is very high and survivors are usually unthrifty.

Following exposure, replication of virus occurs in the epithelium of nose, tonsil, lung, and small intestine, with spread to the CNS along peripheral nerves rather than hematogenously; viremia is not important in the pathogenesis. Viral antigen is detectable in alimentary ganglia during the incubation period of the disease. It is first demonstrable in the brain in trigeminal and vagal sensory nuclei, with later rostral spread in brain stem. Viral replication occurs in the myenteric plexus of the stomach, and involvement of the autonomic system probably can explain the predominant clinical signs of vomition and constipation.

Two *clinical syndromes* are recognized. Neurologic signs occur in 4–7-day-old piglets in some outbreaks and consist of stilted gait, hyperesthesia, progressive paresis, and convulsions in some cases. Clinical signs in 4–14-day-old piglets are dominated by anorexia and vomiting, and this syndrome is called "*vomiting and wasting disease*."

Lesions in the CNS may be found in some affected piglets that do not show clinical signs of nervous disease. The frequency with which inflammatory change is found is quite variable. Lesions when present are those of *nonsuppurative encephalomyelitis* affecting particularly the gray matter of medulla and brain stem. In such cases

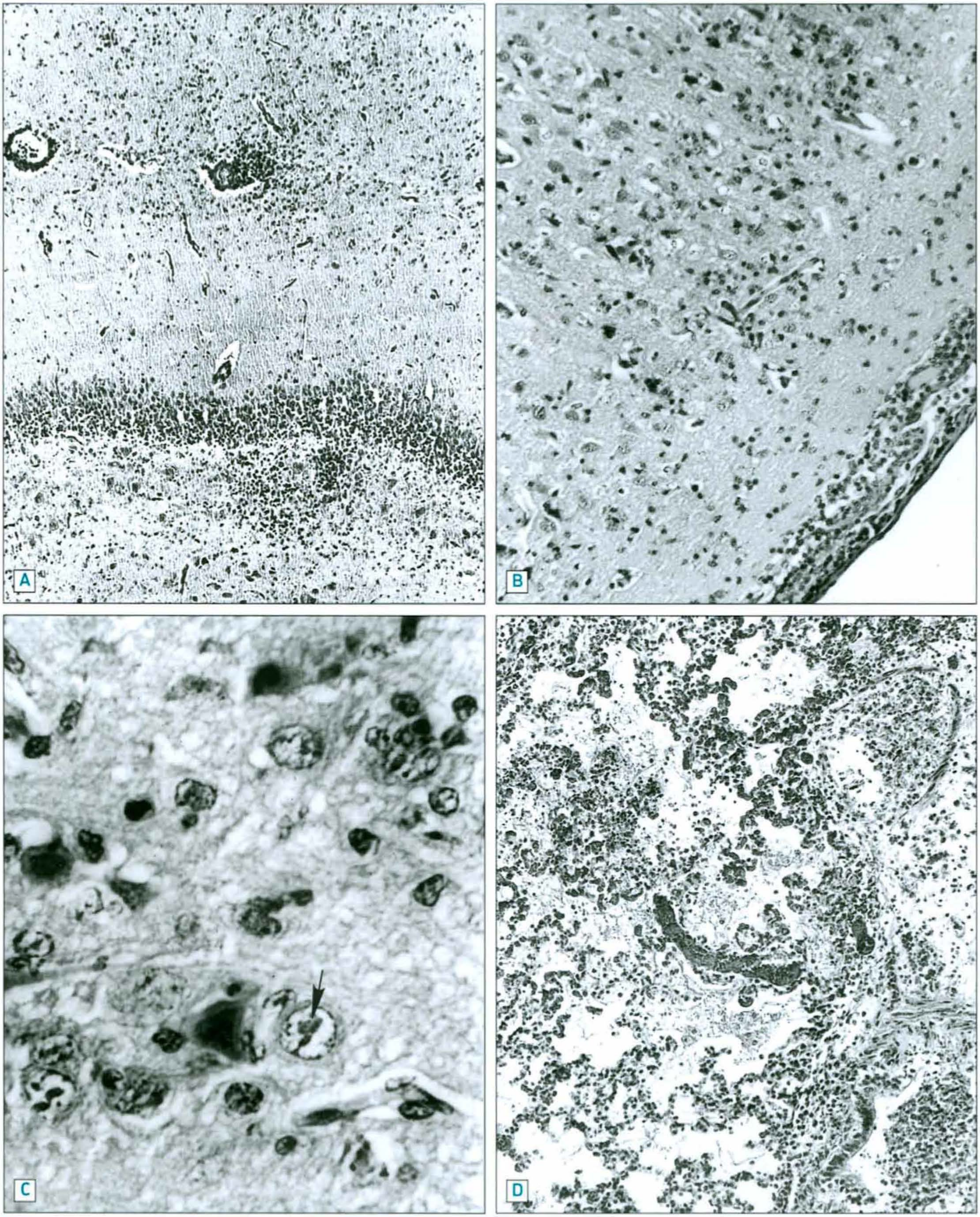

Figure 3.118 Pseudorabies in a pig. **A.** Perivascular cuffing, focal and diffuse gliosis in dentate gyrus. **B.** Meningitis and necrosis of cells in cerebrum. **C.** Neuronal necrosis and irregular inclusion bodies in nuclei (arrow). **D.** Necrotizing bronchopneumonia.

there is inflammation of the trigeminal, paravertebral, and autonomic ganglia. The gastric myenteric plexi are occasionally infiltrated with a few lymphocytes and plasma cells.

Diagnosis of HEV is problematic. Serology on acute and convalescent sera may help in detecting acute infection. Isolation of HEV can be attempted from brain stem of acutely ill piglets. Differential diagnoses of nervous conditions with high mortality in piglets include pseudorabies, classical swine fever, polioencephalomyelitis (Teschen disease), bacterial meningitis, streptococcal septicemia, and hypoglycemia.

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Enterovirus/teschovirus polioencephalomyelitis of pigs

Encephalomyelitis of pigs caused by porcine enteroviruses occurs in many countries and is distinguishable only by a study of the agents. Porcine enteroviruses are ubiquitous but are limited in their pathogenicity to swine. ***Porcine enterovirus*** *A* (PEV-A, formerly PEV serotype 8) and *Porcine enterovirus B* (formerly serotypes 9, 10) are in the genus *Enterovirus*, family Picornaviridae. Porcine enteroviruses serotypes 1–7 and 11–13 have been reclassified as ***Porcine teschovirus*** *1–7, 11–13*, genus *Teschovirus*, family Picornaviridae. Further reclassifications of picornaviruses can be expected. Infection by one enterovirus/teschovirus serotype does not confer protection against infection by another.

Infection is most commonly acquired by pigs after weaning due to waning maternal immunity and mixing of pigs from different sources. Infection follows the fecal–oral route and indirectly through contaminated fomites. Initial replication occurs in the tonsils and the intestinal epithelium, especially of ileum and colon; the enteric phase is not clinically significant or accompanied by tissue change. The enteric phase is followed by viremia and then invasion of the CNS. Viremia by some serotypes may lead to localization in the pregnant uterus and death of fetuses.

Infection with virulent virus may produce nervous signs as soon as 6 days after exposure. The virus is present in large amounts in tonsils and cervical lymph nodes by 24 hours, and in the mesenteric nodes and feces by 48 hours. The disease has two clinical forms: the highly fatal and severe form (**Teschen disease**) and a less virulent milder form (**Talfan disease**, poliomyelitis suum, benign enzootic paresis, Ontario encephalomyelitis, and polioencephalomyelitis.). Teschen disease, first recognized in 1929 in Teschen, Czech Republic, is caused by highly virulent ***Porcine teschovirus 1*** and is limited mostly to Europe, but sporadic epizootics occur in Africa. Talfan disease, caused by infection with less virulent strains of the virus, is more common than Teschen disease and occurs worldwide. Teschen disease is of high morbidity and high mortality affecting all age groups and expressed clinically as convulsions, opisthotonos, nystagmus and coma. Death commonly occurs in 3–4 days. Survivors may have residual paralysis. Talfan disease is characterized by lower morbidity and mortality, and the clinical signs are expressed as paresis and ataxia that seldom progresses to complete paralysis. The infection is asymptomatic in the absence of neurological signs, and up to 95% of exposed pigs develop latent or inapparent infections.

The *pathological changes* in these syndromes are the same although minor differences in severity and distribution of the lesions are reported. There are no gross changes. The histological changes are those of *nonsuppurative polioencephalomyelitis* extending throughout the cerebrospinal axis from the olfactory bulbs to the lumbar cord. Any series of cases of each of the syndromes provides a continuous spectrum of severity and distribution of the lesions. Lymphocytic meningitis is mild in the cerebral meninges and usually overlies areas of parenchymal injury in the cerebrum. In weaners and older animals in which the course is more prolonged than in very young pigs, intense lymphocytic meningitis develops over the cerebellum, usually in conjunction with inflammatory lesions in the underlying molecular layer (Fig. 3.119). Cerebellar meningitis is very slight if the course of the disease is 4–5 days or less, so that, although emphasized in reports of Teschen disease, it is not a feature in very young animals in which the course is short. The most severe lesions occur in the brain stem from the hypothalamus through the medulla and

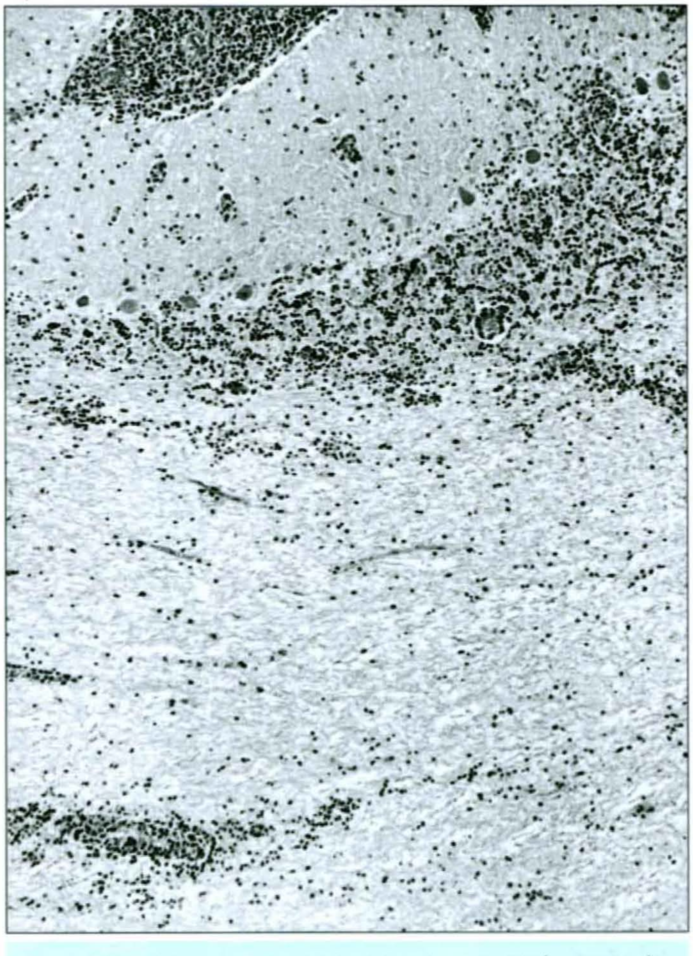

Figure 3.119 Nonsuppurative meningitis and encephalitis (cerebellum) in **enteroviral encephalomyelitis** in a pig.

decrease in intensity and diffuseness down the spinal cord. There is relative sparing of the cerebral and cerebellar cortices, but the deep substance of the cerebellum is consistently and often severely involved. Lesions in the spinal cord in each syndrome are largely confined to the gray matter, particularly the ventral horns, but may selectively involve the dorsal horns in very young pigs. Cord lesions are those of nonsuppurative myelitis and the motor neurons, particularly in gray matter of the ventral horns, experience different stages of neuronal degenerative and necrotic changes (neuronal swelling, chromatolysis, satellitosis, and finally neuronophagia). Lesions are consistently present in the dorsal root ganglia, and especially the trigeminal ganglia (Fig. 3.120).

Diagnosis can be achieved by virus isolation or immunohistochemistry.

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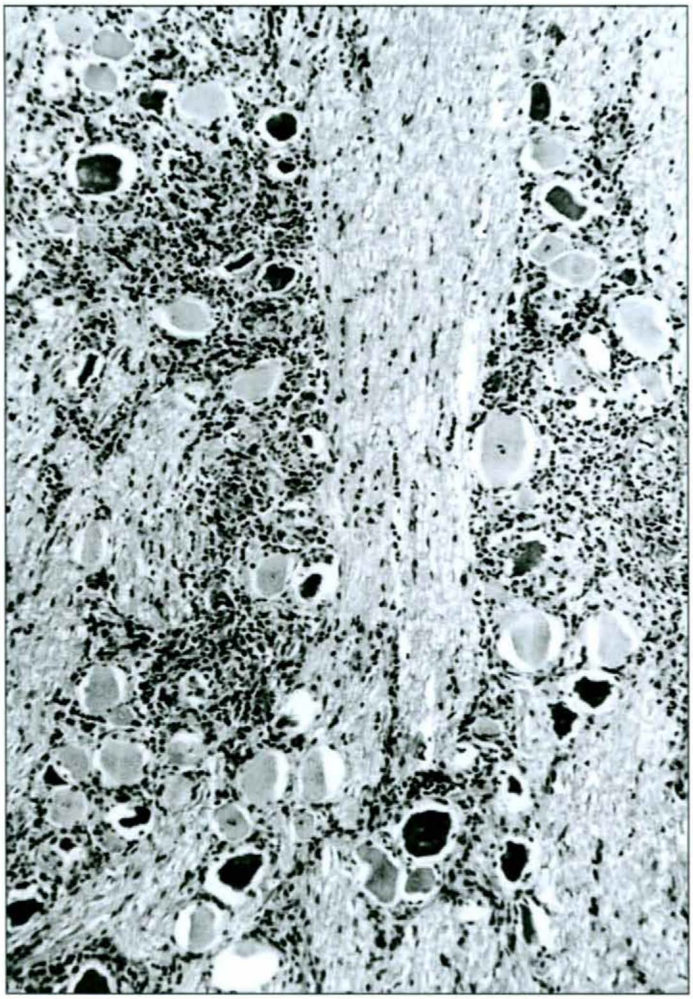

Figure 3.120 Ganglioneuritis in enteroviral encephalomyelitis in a pig.

Flaviviral encephalitides

The genus *Flavivirus* of the family Flaviviridae contains several important viruses that cause encephalitis in domestic and wild animals. Some of these viruses are emerging as very important not only for animal health but also for public health as they are highly zoonotic. The viruses of veterinary importance in this genus belong either to the *tick-borne virus group* or the *mosquito-borne virus group*. Viruses of both groups are maintained in a cycle involving ticks or mosquitoes respectively as invertebrate vectors, and wild vertebrate hosts as reservoirs. In most cases, infections of humans and/or domestic animals are incidental, and are not important for viral transmission or maintenance in the environment.

Diseases caused by tick-borne flaviviruses

Important veterinary viruses in this group include *Louping ill virus* and *Tick-borne encephalitis virus*; these two viruses are very closely related, and may have originated from a common ancestor. The group also includes several other viruses – *Langat virus, Kyasanur Forest disease virus, Omsk hemorrhagic fever virus*, and *Powassan virus* (POWV) – that are primarily of importance in humans. POWV causes severe encephalitis in humans in the USA, Canada, and Siberia, but induces encephalitis in animals only under experimental conditions.

Louping ill (ovine encephalomyelitis)

Louping ill is a tick-borne viral encephalomyelitis of sheep caused by *Louping ill virus* (LIV), which has been enzootic in England, Scotland, and Northern Ireland for more than a century and has been reported in Norway. The disease is named after the leaping (or louping) demonstrated by the diseased sheep. A similar disease has been reported in Spain, Greece, and Turkey affecting sheep or goats but because the etiologic viruses are different by nucleotide sequencing from LIV, each was considered a subtype of LIV but assigned different names: *Spanish sheep encephalomyelitis virus, Greek goat encephalomyelitis virus*, and *Turkish sheep encephalomyelitis virus*. Cattle, horses, goats, and deer pastured with affected sheep sometimes contract the disease, and nonfatal human infections are known. The virus causes fatal encephalomyelitis in red grouse (*Lagopus scoticus*). Outbreaks in piglets have followed the feeding of raw meat from lambs, and a case has been described in a dog.

The tick responsible for transmission in Great Britain is *Ixodes ricinus*, the castor-bean tick. Other species of *Ixodes*, and perhaps other arthropods, are potential vectors. *Ixodes ricinus* is parasitic on a variety of mammals in addition to sheep and on birds, however, the most significant wildlife host is the red grouse. Larval and nymphal ticks acquire the virus when feeding on infected sheep, and transmit the infection to new hosts in the succeeding nymphal and adult phases. Because of its natural mode of transmission, louping ill is most prevalent in early summer and early autumn when the ticks are active. After infection, the virus propagates in regional lymph nodes, proceeds to viremia, and then enters the CNS via the hematogenous route. Alternatively, the virus may localize indirectly from the blood in nasal structures and enter the brain via the olfactory nerves. Under experimental conditions, a number of factors may facilitate entry of virus into the brain, and facilitation is apparently given in natural cases by concurrent tick-borne fever, a rickettsiosis also transmitted by *I. ricinus*, and known to impair humoral and cellular defense mechanisms.

Although tick transmission is the usual mode of infection, the disease can be contracted in humans, monkeys, and mice by inhalation of infective droplets, but this route is not thought to be important naturally. Rabbits and guinea pigs are not susceptible even by intracerebral inoculation.

Louping ill is a systemic infection and, while it remains so, the disease is mildly febrile but otherwise of no consequence. When it invades the CNS and produces signs of encephalitis, the mortality rate is very high. The morbidity in endemic areas is quite low, and the disease is largely confined to either naïve lambs or to older lambs and yearlings whose colostral immunity is not reinforced by natural exposure. The viremic phase of louping ill is clinically silent or a febrile phase with dullness. Recovery may occur in a couple of days and leave solid immunity. If neurologic signs are to develop, they do so at about day 5 and are characterized by incoordination, tremors, cerebellar ataxia, and terminal paralysis.

There are no gross lesions. The disease is an *acute polioencephalomyelitis*. There is mild leptomeningitis corresponding to areas of inflammation of the parenchyma. Inflammation of the cerebellar leptomeninges may be quite severe when the cerebellar cortex is acutely affected (Fig. 3.115). The inflammatory lesions are more obvious than in most viral encephalitides, but are of the usual type, although unusually large numbers of neutrophils may be present in very severe cases, and largely restricted to gray matter, although cuffing and focal gliosis occur in the white matter. Neuronal degeneration may be severe and neuronophagia prominent. Some degree of selective vulnerability of the Purkinje and Golgi cells of the cerebellum is generally accepted and, although it can be demonstrated in many cases, its detection probably depends to a large extent on the duration of active infection. The spinal lesion is poliomyelitis affecting particularly the ventral horns (see Fig. 3.17). Inclusion bodies have not been observed in sheep but acidophilic, intracytoplasmic inclusions in neurons of the brain stem and cord are reported in experimentally affected monkeys and mice. Immunohistochemistry is available, and viral antigen can be identified easily, especially in the Purkinje cells of the cerebellum and their dendritic processes.

Encephalitis caused by *Tick-borne encephalitis virus*

Tick-borne encephalitis virus (TBEV) is a serious human threat that causes thousands of cases of encephalitis every year in endemic areas in Europe and Asia. Several wild and domestic animals are susceptible to infection but the infection is frequently subclinical, however TBEV is pathogenic for dogs and is able to cause fatal meningoencephalitis. The virus is transmitted to humans and animals mainly by *Ixodes ricinus* or *Ixodes persulcatus*. Humans can also become infected through consumption of milk from infected ruminants. Most infected dogs seroconvert without developing TBE, but the peracute disease in dogs has a high fatality rate. Affected dogs are usually euthanized due to associated severe convulsions, tremors, and ataxia. The histologic lesions are those of severe necrotizing lymphoplasmacytic and histiocytic meningoencephalomyelitis with severe glial nodule formation affecting mostly the basal ganglia, thalamus, mesencephalon, neuroparenchyma surrounding the fourth ventricle, and the medulla oblongata.

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Diseases caused by mosquito-borne viruses

The important veterinary viruses in this group include *West Nile virus* and *Japanese encephalitis virus*. Also included in the mosquito-borne, Japanese encephalitis virus group, are *Murray Valley encephalitis virus* and *St. Louis encephalitis virus*.

West Nile virus encephalomyelitis

West Nile virus (WNV) was first discovered in 1937 in the West Nile district of Uganda. In 1999 it was introduced into New York City where it caused a massive outbreak in animals and humans. During the period of 1999 to 2004, the virus spread rapidly to most of the USA and southern parts of Canada. It has been suggested that the virus was imported from the Middle East, due to genetic similarity between strains isolated in the Middle East and New York. The virus is now distributed throughout Africa, central and southern Asia, Australia (where it is called *Kunjin virus*), the USA, Canada, Mexico, and the Caribbean. The virus is divided genetically into two lineages – lineage 1 WNV is present in North America and some other parts of the world, lineage 2 is restricted to enzootic areas in Africa. Lineage 2 WNV strains are either nonpathogenic or occasionally cause mild human and equine disease. Some clades in lineage 1 (e.g., clade 1a) are highly virulent and are believed to be responsible for the recent outbreaks in North America. The virus has a wide host range, but is maintained in the environment mainly by a bird–mosquito–bird cycle. Wild birds, especially corvids, i.e., crows, are the main amplifying hosts. Wild birds usually develop prolonged viremia and the virus is distributed in almost every organ. In contrast, the viral antigen in infected horses, which is by far the most susceptible species of domestic animal, is sparse and limited to the CNS. Infection between birds or between birds and mammals or reptiles is mainly via mosquitoes. Mosquitoes of the *Culex* spp. are the main maintenance vectors. The virus is also found in other vectors such as ticks, but the biological importance of these vectors is yet to be determined. Rare methods of virus transmission include direct contact with infected materials and ingestion. Transplacental WNV transmission is only reported in human.

The *pathogenesis* of the encephalitis induced by WNV is not completely understood, however after the virus is injected by an infected mosquito, it probably propagates in regional endothelial cells and fibroblasts, viremia develops, and the virus reaches the brain hematogenously.

Equids, especially horses, are very susceptible to the infection. In naive areas, the first signs of WNV are the marked increase in cases of equine encephalitis and increased numbers of wild bird, especially corvid, mortalities. WN fever is a seasonal disease, related to the time of the year of mosquito activity, i.e., summer and fall. When naive horses are infected by the virus, mortality can reach up to 50% of affected horses, and clinical signs range from weakness and anorexia to severe acute ataxia or recumbency. However, the mortality rate decreases dramatically in the following seasons. Gross lesions are usually absent, but a few cases may have acute areas of hemorrhage or malacia affecting the thoracic and/or lumbar spinal cord. *Histologic lesions* are present mainly in the brain stem and thoracolumbar spinal cord, and to a lesser extent in cerebral cortex and cervical cord. The cerebellum is usually spared. *Lesions are those of nonsuppurative encephalomyelitis, gliosis, and glial nodule formation with occasional neuronal degeneration and necrosis* (Fig. 3.121A, B). Lesions are more pronounced in the gray matter. The glial nodules usually contain a few neutrophils amid the glial cells. Areas of hemorrhage and malacia are present in severe cases, especially in brain stem and the ventral horn of the thoracic and lumbar spinal cord. Axonal swelling and spheroid formation is frequent. The severity of lesions is greatly variable between outbreaks, and frequently the severity of clinical signs is not correlated with the severity of lesions. In most cases, the lesions are mild and confined to thin cuffs of a few blood vessels in the brain stem. Extraneural lesions, e.g., hepatitis, myocarditis, etc., which occur in avian WNV infection, do not occur in equine WNV infection.

The information about clinical signs and lesions in non-equine domestic species other than birds is very limited. Ruminants, canids, felids, and swine are far less susceptible to the disease than are horses or birds. These species are susceptible to the infection and develop very short viremia with subclinical disease; histologic lesions are very similar to those described in horses.

Diagnosis of WNV infection can be achieved by detection of WNV antigen in the brain using PCR or immunohistochemistry. WNV antigen in many cases can be very sparse, which makes the interpretation of IHC difficult. Positive IHC staining in most cases is limited to sparse axonal immunostaining.

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Japanese encephalitis

Japanese encephalitis virus (JEV) is found through much of eastern, southern, and southeastern Asia, Papua New Guinea, and the Torres Strait of Northern Australia, where the disease is endemic with dramatic annual epidemics. In humans, there is a high ratio of subclinical to overt infections, with a case fatality rate of 10–15% and a high incidence of residual neurologic deficits in survivors. Transplacental infection followed by abortion occurs in humans and this is also the most serious expression of the disease in *pigs, the domestic species most importantly infected.*

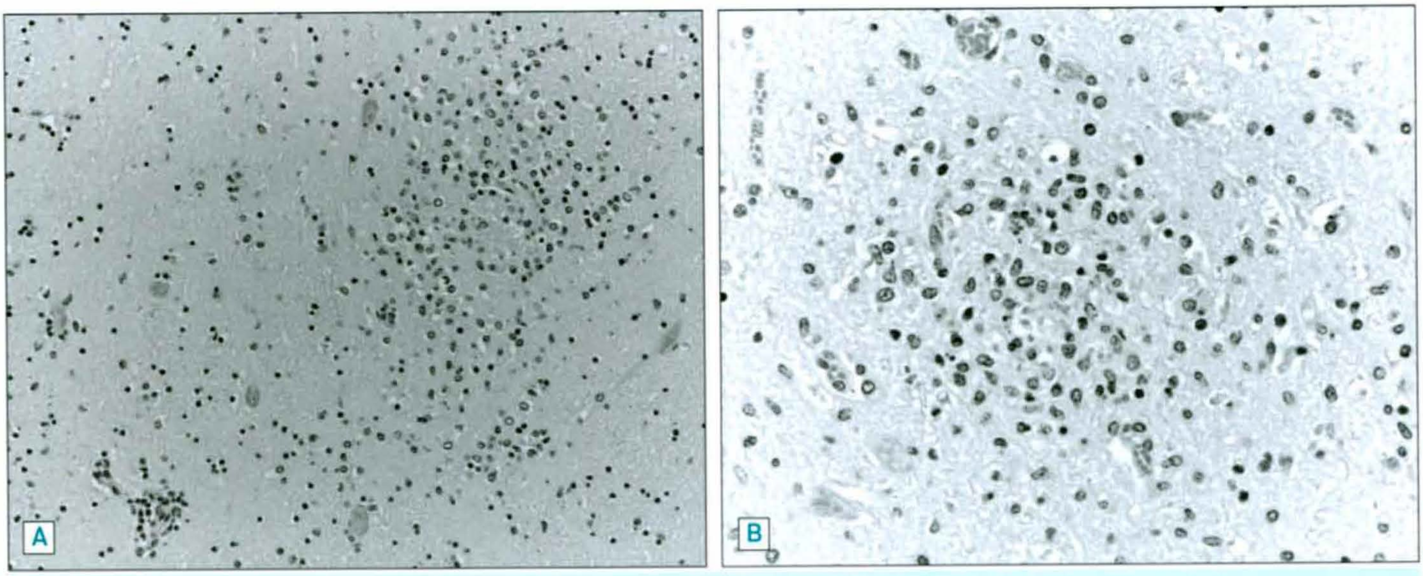

Figure 3.121 Brain stem of a horse with **West Nile viral encephalomyelitis. A.** Perivascular cuffs composed of lymphocytes and plasma cells are usually thin. **B.** Multifocal glial nodules are common and usually contain only a few neutrophils. (Courtesy of MJ Hazlett.)

The virus is maintained through mosquito–bird or mosquito–pig cycles. The virus is transmitted mainly by the mosquito, *Culex tritaeniorhynchus*, but other species of this genus and of the genera *Aedes* and *Armigeres* may be important as the virus is known to be vertically transmitted through some of them. *Ardeid water birds (e.g., herons and egrets) are the main maintenance reservoirs.* Many species of animals and birds are susceptible to mosquito-borne infection and develop antibody responses in timing with suitable climatic and habitat cycles. The pig is a very important domestic animal in many of the endemic areas and it is the most important amplifier host for the virus, developing sustained viremia of sufficient titer to infect feeding mosquitoes and indeed is probably the preferred host for *C. tritaeniohynchus*. Infection of humans and horses is incidental and both species are considered to be dead-end hosts.

Most horses, pigs, and cattle in endemic areas possess neutralizing antibodies against the virus. Intranasal and intracerebral inoculation can produce fatal encephalitis in calves, but natural cases of encephalitis in this species are quite rare. Among animals infected naturally with the virus, *only horses and donkeys develop clinical encephalitis*. There are no clinical signs of encephalitis in pigs, but pregnant susceptible sows may produce *stillborn piglets*. Infected stillborn and neonatal pigs may show hydrocephalus, cerebellar hypoplasia, and hypomyelinogenesis and anasarca; histological changes are restricted to the nervous system and may include *nonsuppurative encephalitis*. The lesions in these neonatal and stillborn piglets probably reflect the timing of infection in relation to the development of immune competence. Diffuse nonsuppurative encephalitis occurs in the brain and cord of piglets up to 6 months of age, but in the cerebellum it affects rather selectively the molecular and Purkinje layers. The histological pattern of Japanese encephalitis in pigs appears similar to that of Teschen and related diseases. In boars, the virus induces orchitis.

Severe epidemics of encephalitis have occurred in horses in Japan. The incubation period in horses ranges from 4–14 days, and case fatality is 5–15%. Lesions are confined to the CNS, are more prevalent in the cerebral hemispheres, and include extensive perivascular lymphoplasmacytic cuffing, gliosis, and areas of malacia with hemorrhage. The lesions in quality and distribution are the same as those produced by the *Eastern and Western equine encephalitis viruses*. Inclusion bodies are not reported in the Japanese disease. *Diagnosis* is best achieved by detection of viral RNA using PCR.

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Alphaviruses – equine encephalitides

Several members of the genus *Alphavirus*, family Togaviridae, cause either overt or subclinical encephalitis in horses or other animals. They all require an arthropod vector (frequently mosquitoes) for transmission.

Eastern, Western, and Venezuelan encephalitis in horses

Western equine encephalitis virus (WEEV), Eastern equine encephalitis virus (EEEV), and Venezuelan equine encephalitis virus (VEEV) are all members of the genus Alphavirus, family Togaviridae. Horses were originally regarded as the primary hosts of the EEVs, but horses are actually accidental and unfortunate hosts; birds for the American viruses (eastern and western type) or rodents for the Venezuelan virus are the most common vertebrate reservoir hosts, and mosquitoes are the principal vectors. Horses and humans, in both of which species the disease is of very considerable importance, are now known to be, in terms of transmission and often literally as well, dead-end hosts in which the titer of virus in blood is ordinarily too low to be a source of infection for mosquitoes.

Not all birds are capable of acting as reservoir hosts. Red-winged blackbirds, cardinals, sparrows, cedar waxwings, and the captive Chinese pheasant are highly susceptible to infection and nearly always die. Many other species, including adult domestic fowl and turkeys, are not sickened by the infection although fatalities can be produced in the young of these species.

EEEV is endemic along the North American Atlantic course, in the Caribbean, Central America, and along the northeastern coast of South America. The virus cycles between water birds and the mosquito, *Culiseta melanura*, which feeds on birds and does not feed on large mammals. Horses are likely to get infected by other mosquitoes, *Aedes sollicitans* and *A. vexans*, which feed on both horses and birds.

WEEV is present mostly in the valleys of western North American states, where the cycle is between wild birds, especially passerines, and the mosquito, *Culex tarsalis*. This mosquito feeds readily on animals, and human and animal cases occur regularly.

VEEV has two major different strain groups, the enzootic strains that are avirulent and cycle between *Culex* spp. mosquito and small rodents in the Caribbean areas, and the epizootic strains that are virulent to human and horse, found mainly in Venezuela, Colombia, and Peru, and circulate between several mosquito species and horses, which produce high titered viremia sufficient to infect the vector mosquitoes. Outbreaks of EEE and WEE occur in seasonal patterns related to the time of the year when mosquitoes are active. VEE outbreaks usually occur in a cyclical pattern approximately every 10 years.

Once infected, mosquitoes are known to remain so for life, and there is evidence that the virus is capable of multiplying in the insects. Arthropods other than mosquitoes may also be of some, but lesser, importance. The virus has been found in chicken mites (*Dermanyssus gallinae*), chicken lice (*Menopon pallidum, Eomenocanthus stramineus*), and assassin bugs (*Triatoma sanguisuga*). The spotted-fever tick, *Dermacentor andersoni*, is capable of transmitting the infection stage to stage and hereditarily. Transmission by aerosolization is reported only in humans; laboratory workers are at high risk of infection by aerosols.

Although humans and horses are the principal mammalian victims, other species are susceptible. Pigs readily develop asymptomatic infections but a few outbreaks have been reported in this species with histology typical of EEE. Pigs are not of significance for natural propagation of the virus because they do not develop significant viremia. Calves are susceptible to intracerebral inoculation but recover in 2 weeks. Guinea pigs and white mice are highly susceptible, rabbits are less so, and sheep, dogs, and cats are refractory.

The three viruses are similar in their pathogenesis. After the mosquito bite, the virus replicates in the regional blood vessels and lymph nodes, viremia develops, followed by secondary replication in lymph nodes and muscles. A second viremia then develops and is followed by brain invasion via the blood. In the CNS, the virus replicates in neurons, glial cells, and blood vessels. The virus causes neuronal necrosis, likely via stimulation of apoptosis.

Young horses are more susceptible than the old. Initially, there is viremia with fever and depression, usually unnoticed. The animal may then recover or the virus may invade the CNS, by which time the fever has subsided. The neurologic signs are characterized by derangements of consciousness and terminal paralysis. There may be early restlessness with compulsive walking, often in circles. There is central blindness. The animal becomes somnolent and assumes unnatural postures. At this stage, the course may remain static and the animal lives as a "dummy," or paralysis may develop, often first affecting cranial nerves but later general and flaccid. *The signs are largely cortical and the cortex is the principal site of the lesions.* The course, if fatal, is usually 2–4 days.

There are no gross changes. The microscopic changes are limited almost exclusively to *gray matter* (see Fig. 3.2D). When the course is short, 1 day or less, the reaction is largely on the part of neutrophils. These infiltrate the gray matter diffusely and may be found in foci suggestive of malacia (Fig. 3.122). There is early microglial reaction to produce rod cells. Endothelial cells, especially of veins are swollen, and hyaline or granular thrombi are common in these vessels. There are narrow cuffs of lymphocytes and neutrophils with perivenous hemorrhage and edema. After a couple of days, the neutrophils disappear, the cuffs are composed of lymphocytes, and there are both focal and diffuse microglial proliferations as in the standard nonsuppurative reactions. Neuronal degeneration and neuronophagia are common findings. Intranuclear inclusions similar to those in Borna disease may be present, but may be very difficult to identify.

The most severe lesions are in the cerebral cortex, especially the frontal, rhinencephalic, and occipital areas, with lesions of lesser intensity in the pyriform lobes. Severe lesions are also present in thalamus and hypothalamus. From the thalamus caudally, the intensity of inflammation diminishes but reveals no selectivity for particular nuclear masses. The cerebellum is less severely injured than other portions, although inflammatory changes may be found in the deep nuclei and spottily in the cortex. Mild changes occur in both dorsal and ventral horns of the cord, but their distribution is irregular. The trigeminal ganglia are not affected. The encephalomyelitis in the Venezuelan type may be purely nonsuppurative. Extraneural lesions are common in humans and birds but are rare in susceptible domestic mammals. Small intestinal lesions in a horse with EEE include multifocal myonecrosis and lymphomonocytic infiltration in the muscular layer and focal mild perivascular lymphocytic infiltration in the submucosa. Myocarditis is not uncommon in pigs suffering from EEE. Horses infected with VEEV can occasionally have some nonspecific extraneural lesions such as myeloid depletion in bone marrow and lympholysis in spleen and lymph nodes.

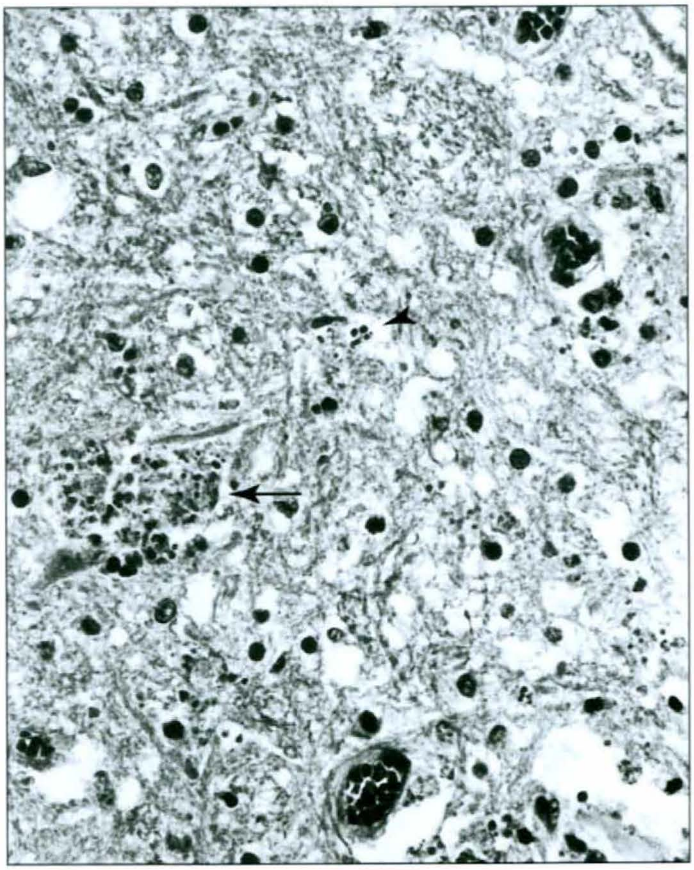

Figure 3.122 Neuronal necrosis (arrow), glial necrosis (arrowhead) in cerebrum in **equine encephalitis** in a horse.

Other *Alphavirus* encephalitides

Highlands J virus of America's east coast, ***Getah virus*** of southeast Asia, and ***Semliki forest virus*** of the Americas are all equine pathogens that are able to induce at least a febrile disease. All of these viruses are maintained in the environment by a mosquito–bird–mosquito cycle. Several mammals and birds seroconvert to these viruses, however overt disease is rare. *Highlands J virus* was reported as the cause of encephalitis in two horses. *Getah virus* does not induce encephalitis but clinical signs and lesions with some similarities to equine viral arteritis.

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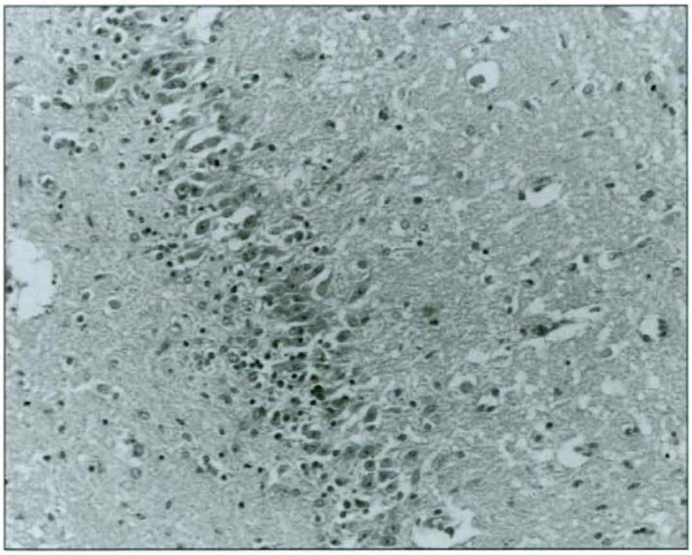

Figure 3.123 Nonsuppurative encephalitis and gliosis in the hippocampus of a horse with **Borna disease.**

Borna disease

Borna disease, named after the village of Borna in Germany, is caused by *Borna disease virus* (BDV), which is the only member of the genus *Bornavirus*, family Bornaviridae. The virus exists worldwide in many vertebrates but most commonly infects horses, sheep, cattle, cats, dogs, and ostriches. The traditional endemic area is central Europe, but antibodies to the virus are found in horses outside Europe, including the USA and Japan. The mortality rate may be high (>80% in horses, 5–40% in sheep); surviving horses may be asymptomatic carriers, or may suffer relapses of disease. The virus has a controversial link to several human neuropsychiatric illnesses. In experimental hosts (tree shrews, rats), infection with BDV not only produces pathoanatomic changes, i.e., nonsuppurative encephalitis, but also behavioral changes and learning deficits.

The virus replicates in the nucleolus of the host cell without cytopathic effect, persistently infects cells, and induces brain lesions by *immune-mediated mechanisms*. In Lewis rats, acute (4–8 weeks post-infection) BDV infection is followed by massive infiltrates in the brain of CD4+ Th1, CD8+ T, and NK cells with a predominance of Th1 cytokines that favors cell-mediated immunity. In the chronic stage (beyond 15 weeks of infection), the aforementioned cellular infiltrates significantly decrease and the predominant cytokines are of Th2 type, favoring the shift to a humoral immune response; the resultant antibodies are not protective and have no significant effect on the disease. Due to this unique feature, infection of neonatal or immunocompromised animals does not lead to disease or to encephalitis.

The epidemiology of Borna disease, including reservoir, methods of transmission, and infection, remains obscure. Inflammation of the olfactory bulbs at the early stages of natural infection in humans suggests an intranasal route of infection followed by transaxonal migration to the olfactory bulb. Vertical transmission in horses and rats with life-long persistent infection is also suggested.

Clinically, the disease occurs sporadically or in clusters, however severe outbreaks are described in different species. *Equids and sheep are the most susceptible animals*, but natural disease is reported in many domestic species including but not limited to cattle, alpacas, cats, dogs, and ostriches. Most infections in horses remain subclinical and BDV-specific antibodies are frequently found in clinically healthy horses. The incubation period is not less than 4 weeks and introduces a clinical syndrome that is purely neurologic but of varied course, death occurring in 1–3 weeks. The mortality rate in diseased horses is 90–100%. Recurrent episodes at time of stress occur in surviving animals. Clinical signs include pharyngeal paralysis, hyperesthesia, standing in awkward positions, circling, muscular tremors, and spasms; blindness is common. Drowsiness and flaccid paresis develop terminally.

There are no gross lesions. The distribution of lesions in Borna disease differs from that in other equine encephalomyelitides and parallels closely the distribution of viral antigen, as displayed by immunohistochemistry, and the distribution of infectivity, as determined by titration in cell cultures. Virus and lesions are present mostly in the gray matter of olfactory bulbs (early stage), hippocampus (Fig 3.123), limbic system, basal ganglia, and brain stem. The dorsal cerebrum and the cerebellum are relatively spared. Lesions may be present in optic nerves and retina. Histologic lesions are those of *nonsuppurative encephalomyelitis* with predilection for the aforementioned areas. Perivascular cuffs can be dramatically thick (>7 cell layer) and usually there are neuropilar clusters of lymphocytes and plasma cells. Other lesions include neuronophagia and focal gliosis. The presence of inclusion bodies (Joest–Degen bodies) is fairly pathognomonic; these are mainly in nuclei, especially in the hippocampus, and are very occasionally cytoplasmic. They stain well and red with Giemsa, and have a clear halo. Commercial PCR and immunohistochemistry kits are available for diagnosis.

Borna disease virus has been proposed as the cause of a chronic, slowly progressive neurologic disease affecting *cats* of all ages, sexes, and breeds. The disease is known clinically as *staggering disease,* and clinical signs include ataxia, paraparesis, and tetraparesis. Behavioral change is not a constant finding. Lesions are more prevalent in the gray matter of brain stem and in dorsal and ventral horns of the thoracic and/or cervical spinal cord segments. Lesions include mild to moderate lymphoplasmacytic cuffing with neuronophagia and neuronal degeneration and astrogliosis. Severe Wallerian degeneration is usually present in ventral and lateral columns of the cervical and thoracic spinal cord.

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Lentiviral encephalomyelitis of sheep and goats

Caprine arthritis encephalitis virus (CAEV) of goats and *Visna/maedi virus* (VISNA) of sheep, in the genus *Lentivirus* of the family Retroviridae, are small-ruminant lentiviruses (SRLV). CAEV is the causative agent of caprine arthritis-encephalitis of goats and VISNA is the causative agent of the visna/maedi disease complex of sheep; at least some strains of the SRLV are transmissible between sheep and goats. In both natural hosts, *four clinical and pathological syndromes are recognized*, namely **mastitis, arthritis, interstitial pneumonia** (maedi and ovine progressive pneumonia) and **encephalomyelitis** (visna of sheep). Within endemic situations, any one or combination of the four syndromes may be present and when in combination one syndrome usually predominates.

Once infected, the virus is never eliminated and, while present, it is active even though there may be no clinical sign of neurologic deficit. Typically for this type of virus infection, the virus is highly cell-associated and replicates only slowly, infection persists for the life of the animal, the incubation period before seroconversion may be several months and before clinical disease may be months or years, the clinical disease is progressive, and the lesions are dominated by active mononuclear inflammatory cells. The pathogenesis and epidemiology of the various conditions are described in detail in Vol. 2, Respiratory system. Described below are the gross and histologic lesions and clinical signs found in the encephalomyelitis form of these diseases.

The encephalomyelitis form in sheep is called **visna** (Icelandic for wasting). As a natural disease, visna occurs in sheep of both sexes but clinical signs are seldom, if ever, observed in animals less than 2 years of age. Disease onset is insidious. The earliest sign may be barely perceptible caudal ataxia and fine trembling of the lips. The first sign to be noticed may be extensor paralysis of the hindlimbs. Once paralytic signs are evident, a fatal outcome appears certain. There is no fever and no sign of cerebral dysfunction, and death is due to starvation or secondary infection. The incidence of visna is relatively low. The course of the infection can be followed fairly well by routine examination of the CSF for lymphocytosis.

Normal sheep are expected to have $\leqslant 0.005 \times 10^9$ cells/L ($\leqslant 5/\mathrm{mm}^3$) of CSF; in visna, cell numbers, chiefly lymphocytes, can be markedly elevated. After intracerebral inoculation, there is a latent period of up to 8 weeks, after which the CSF cell count begins to increase. The cell count may remain high for several months without other signs of disease. Thereafter, the animal may recover, as indicated by a drop in the CSF cell count, or the cell count may remain high, paralysis develops, and death follows.

The disease in the brain is chronic and demyelinating. There are no gross neural changes in this disease, and the histologic change is one of *patchy demyelinating encephalomyelitis*. The distribution of lesions, involving principally the white matter, is unlike the distribution produced by other neurotropic viruses. There is a mild to severe mononuclear type of cerebrospinal meningitis. The parenchymal lesion may be well-established by 1–2 months, and these early lesions are intensely inflammatory with perivascular cuffing and gliosis. They reveal clearly that the process begins in, and immediately beneath, the ependyma diffusely throughout the cerebrospinal axis. In this early stage, the myelinated fibers in the inflammatory foci remain remarkably intact; the gray matter of the cord is irregularly but often intensely affected by a nonsuppurative reaction even 2–3 months after inoculation. In the paralytic and terminal stages of the disease, the periventricular destruction of white matter in the cerebrum and cerebellum is extensive, and in some sections of the brain, especially in the cerebellum, almost every bit of white matter is destroyed leaving the gray matter free.

Destruction of myelinated fibers in the spinal cord is patchy and not due to progressive spread of the pericentral inflammation. The demyelinated plaques are characteristically peripheral and triangular in shape with a base on the pia mater. Although dorsal and lateral tracts are most frequently involved, there is no selectivity for particular fiber tracts and no symmetry. The degenerating foci are almost malacic in their severity, and the plaques contain numerous reactive microglia and astroglia. Spinal nerve roots share in the degenerative process. Germinal centers may form in the choroid plexus. In areas of intense inflammation, liquefactive foci of necrosis occur in the white matter and the loss of myelin is expected to be of Wallerian type. In the spinal cord, evidence of remyelination can be found indicating that oligodendrocytes are not target cells and that demyelination may be primary.

Caprine arthritis-encephalitis (CAE) appears to be widely distributed, but the expression of the infection is highly variable, and many infected goats show little or no clinical disease. Clinical disease of the nervous system affects kids 2–4 months of age and is frequently fatal. Animals that develop the early nervous disease or have early inapparent infections tend to develop synovitis and periarthritis in adulthood (see Bones and joints).

The clinical signs of CAE are referable to motor spinal dysfunction without signs of cerebral disease. Onset is indicated by hindlimb lameness and ataxia with paresis that progresses over several weeks to paralysis. The inflammatory lesions in the CNS may remain active for several years in goats that survive (see Fig. 3.124A, B). In the early clinical phase of the disease, changes are widely distributed in the white matter of the brain and cord, particularly in the subependyma and beneath the pia in the cord. The distribution and character of the lesions in the nervous tissue in the goat are, in general, similar to those in visna of sheep. There is, however, less tendency for the periventricular lesions to progress to gross cavitation of cerebral white matter. Instead, there is a tendency for the inflammatory and myelinoclastic areas to increase in number and severity caudally from the mesencephalon. As in visna, the spinal cord changes are discontinuous and, where present, involve the myelin in subpial plaques or in one or

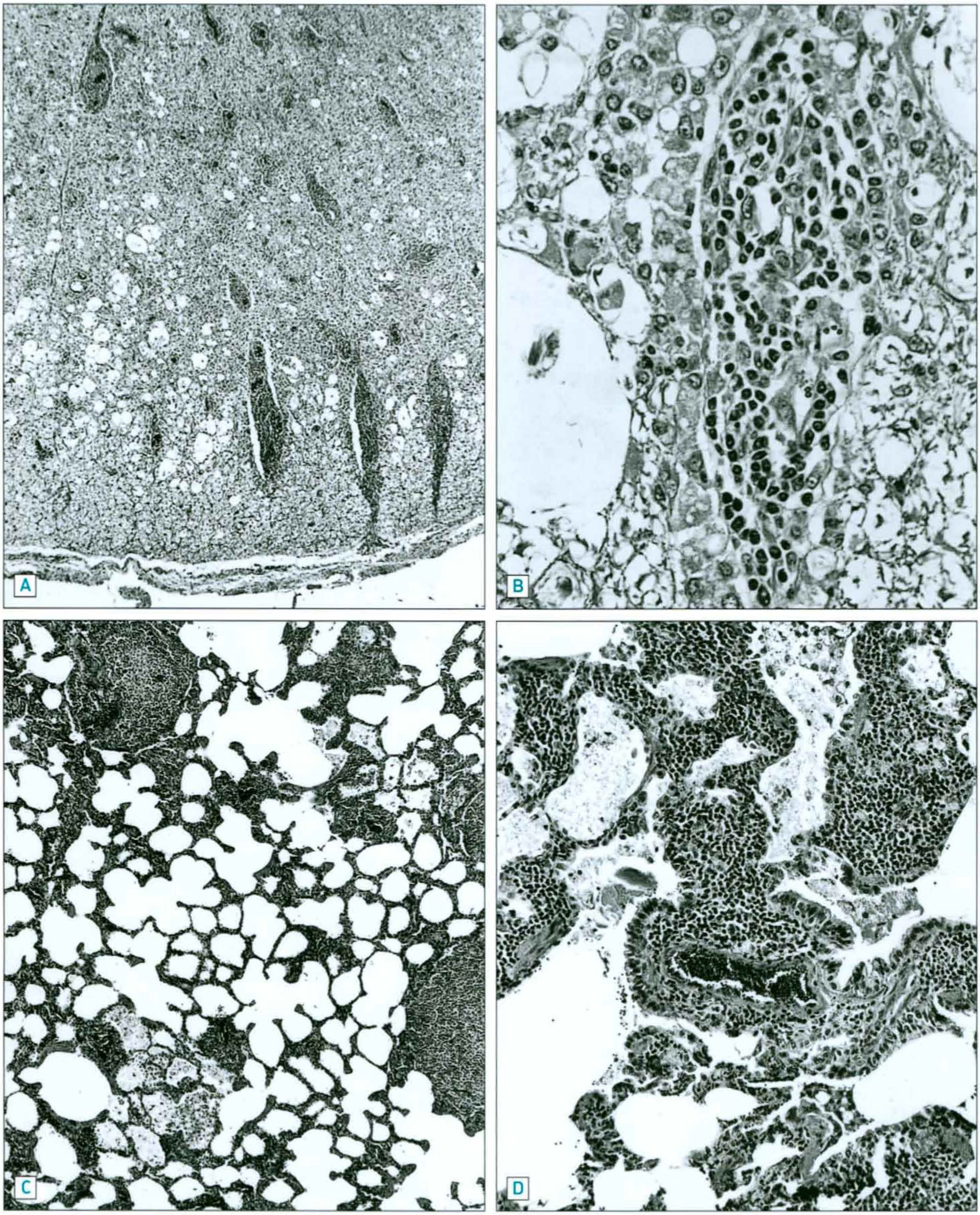

Figure 3.124 Caprine arthritis-encephalitis. A. Leukomyelitis. **B.** Detail of perivascular reaction. Note macrophages bordering vessel. **C.** Prominent perivascular and peribronchiolar lymphoid cuffs and focal interstitial pneumonia. **D.** Detail of (**C**) showing lymphocytes around vessels and bronchioles, and in alveolar walls.

more quadrants of the cord. The extent of perivascular infiltration by mononuclear cells (see Fig. 3.124B) is also greater in kids than in sheep. In addition to the encephalomyelitis, mastitis, and arthritis seen in CAE, interstitial pneumonia occurs in some natural and experimental cases (Fig. 3.124C, D).

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Paramyxoviral encephalomyelitis of pigs

Porcine rubulavirus encephalomyelitis (blue eye disease)

Blue eye disease is caused by *Porcine rubulavirus* (La-Piedad-Michoacan-Mexico virus) of the genus *Rubulavirus*, family Paramyxoviridae. The disease is characterized by encephalomyelitis, reproductive failure, and corneal opacity. Outbreaks have been recorded in Mexico since 1980, but appear to be self-limiting in commercial pigs. In pregnant sows, the infection may be subclinical or responsible for *fetal death, mummification, and stillbirths,* and for the occasional appearance of corneal opacity in the sow. Piglets up to 2 weeks of age are most susceptible with up to 50% morbidity and very high mortality. The clinical signs are of encephalomyelitis leading to death within 2–4 days, although subclinical infections are also frequent and may be manifested only by corneal opacity.

The lesion is a typical *nonsuppurative encephalomyelitis* affecting mainly gray matter of the thalamus, midbrain, and cortex. Inclusion bodies have not been demonstrated. Anterior uveitis is mild, the inflammatory cells congregating in the iridocorneal angle and the corneoscleral junction. The corneal opacity is due to edema, which will resolve if the animal survives.

In mature male pigs, experimental exposure will result in epididymitis in almost all exposed and in a lesser number with orchitis and testicular atrophy. Interstitial pneumonia is part of the description.

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Nipah virus encephalitis

Nipah encephalitis, an emerging disease characterized by severe and rapidly progressive encephalitis, is caused by *Nipah virus* (NiV) of the genus *Henipavirus*, a novel genus in the family Paramyxoviridae. This genus also contains *Hendra virus*. Both are newly emerging viruses that can cause fatal encephalitis and pneumonia in humans and several animal species. The first severe outbreak of NiV encephalitis in humans occurred in 1998 near Ipoh, Malaysia, primarily among pig farmers and their families; the outbreak was preceded by an outbreak of encephalitis and pneumonia affecting pigs on many local farms. *Fruit bats* of the *Pteropid* species were later confirmed to be the natural reservoir host of NiV. Bats shed virus in their urine and saliva, which contaminates fruit that falls into pig pens and is eaten by pigs; bats in these trees also urinate directly on pigs. Initial human infection occurred in pig farmers by direct contact or aerosolization from infected pens. Pig-to-pig or pig-to-domestic-animal infections occur by direct contact with infected pig or mechanically by contact with contaminated utensils or feed. Direct transmission from bats-to-human or from human-to-human is controversial.

Pigs are the animals most susceptible to infection, but natural infection is reported in horses, cats, goats, and dogs; these species were infected by direct contact with infected pigs. Morbidity rates in pigs are ~10%, with case mortality rates of <15%. The incubation period is estimated to be 1–2 weeks. The virus targets two systems, the CNS and the respiratory system. Clinical signs are those of acute dyspnea (labored and harsh respiration, open-mouth breathing, severe cough) and acute nervous signs (trembling, seizures, or tetanus-like spasms). Abortion may occur to pregnant sows.

The lung is diffusely edematous with patchy acute hemorrhage. Meningeal blood vessels are severely congested. The histologic hallmark is *necrotizing vasculitis affecting arterioles, venules, and capillaries* with the presence of binucleated or multinucleated syncytial cells attached to the endothelium of affected blood vessels. Blood vessels undergo fibrinoid degeneration and leukocytoclastic vasculitis. Affected blood vessels are present most commonly in lung, brain, renal glomeruli, and lymphoid organs. Other pulmonary lesions include moderate lymphoplasmacytic bronchointerstitial pneumonia with mild necrotizing bronchiolitis and mild-to-moderate filling of alveoli with neutrophils, even in the absence of significant secondary bacterial infection. Severe lymphocytic and neutrophilic meningitis with mild lymphoplasmacytic encephalitis and occasional gliosis are consistent findings. Due to vasculitis, large areas of hemorrhage and infarction are common in affected organs. Occasionally, *eosinophilic intracytoplasmic and intranuclear inclusions* are present in neurons and syncytial endothelial cells. Syncytial cells can also be found attached within lymphatic vessels and to pulmonary alveolar septa.

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Other porcine paramyxoviral encephalitides

An outbreak of respiratory disease (necrotizing bronchointerstitial pneumonia) and encephalitis was recorded in a large pig farm affecting all ages. The etiologic agent was a previously unknown, as yet unclassified, paramyxovirus different than the other known porcine paramyxoviruses. CNS signs were characterized by recurring episodes of distress, head pressing, tremors, and hindlimb ataxia. No gross lesions were observed in brain. CNS lesions were lymphocytic perivasculitis and diffuse gliosis.

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Akabane viral encephalitis

Akabane disease is caused by *Akabane virus* (AKAV) of the *Simbu virus group,* in the genus *Bunyavirus,* family Bunyaviridae. Iriki virus, a strain of AKAV, causes similar disease. The group also contains *Aino virus,* which causes congenital disease identical to that caused by AKAV. As mentioned earlier in this chapter, AKAV is a common cause of congenital CNS defects in infected bovine fetuses, however the virus has also been associated with nonsuppurative meningoencephalomyelitis in adult cows and young calves. Histologic lesions are more prominent in brain stem, pons, medulla oblongata, and the spinal cord ventral horn. Lesions are those of lymphohistiocytic cuffing with multifocal gliosis, neuronal necrosis, and occasional neuronophagia with microglial cells.

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Bovine herpesviral encephalitis

Bovine necrotizing meningoencephalitis caused by *Bovine herpesvirus 5*

Bovine herpesvirus 5 (BoHV-5), the cause of bovine necrotizing meningoencephalitis, is antigenically related to *Bovine herpesvirus 1* (BoHV-1), the cause of infectious bovine rhinotracheitis. Both viruses are neurotropic, undergo latency in the trigeminal ganglia, and can be reactivated by natural or experimental stress; however, BoHV-1 rarely causes encephalitis. Both of these herpesviruses are in family Herpesviridae, subfamily Alphaherpesvirinae, genus *Varicellovirus.* Outbreaks of severe BoHV-5 necrotizing meningoencephalitis have occurred most frequently in South America, but the disease has been observed in other countries including the USA. Infection is by direct contact or aerosolization. Following intranasal inoculation, BoHV-5 reaches and invades the brain through the olfactory pathway. BoHV-5 envelope glycoproteins E (gE), gI, and Us9 convey viral neurovirulence and neuroinvasiveness by affecting anterograde viral spread via the olfactory pathway.

BoHV-5 encephalitis occurs as a sporadic disease or sometimes as outbreaks in calves and yearlings. The morbidity in herds may be as high as 50%, but is usually much lower; few recognizably sick animals survive. The incubation period is 1–2 weeks, followed by anorexia, apathy, circling, jaw chomping, and finally paddling and recumbency. These clinical signs may be associated with mild to severe rhinitis.

Gross lesions are usually absent, however in the severe form of the disease, bilaterally symmetrical areas of malacia, hemorrhage, and necrosis are described in the gray matter of the rostral cerebrum (Fig. 3.125). The hallmark histologic lesion is *severe cytonecrotizing nonsuppurative meningoencephalitis with marked gliosis* (Fig. 3.126A, B). Lesions are more commonly present in the gray matter of the rostral cerebrum, including olfactory bulb, and to a lesser extent in the cerebellum and diencephalon. Perivascular cuffs can be markedly thick (more than six layers of lymphocytes, plasma cells, and fewer histiocytes). Necrotic neurons are usually swollen, have lost their angularity, are basophilic, and have pyknotic nuclei. Typical *intranuclear alpha-herpesviral inclusions* are occasionally present in degenerate neurons and astrocytes (Fig. 3.126B). Trigeminal ganglioneuritis, neuronophagia, and satellitosis are commonly present. Necrotic or malacic areas can take the laminar cortical necrosis pattern of bovine polioencephalomalacia, however, the latter syndrome is not associated with the severe perivascular cuffing present in BoHV-5 encephalitis. Vasculitis affecting the cerebral microvasculature is only described in rabbits experimentally infected with the virus. The histologic lesions are fairly pathognomonic, however, confirmation of the diagnosis by PCR is recommended.

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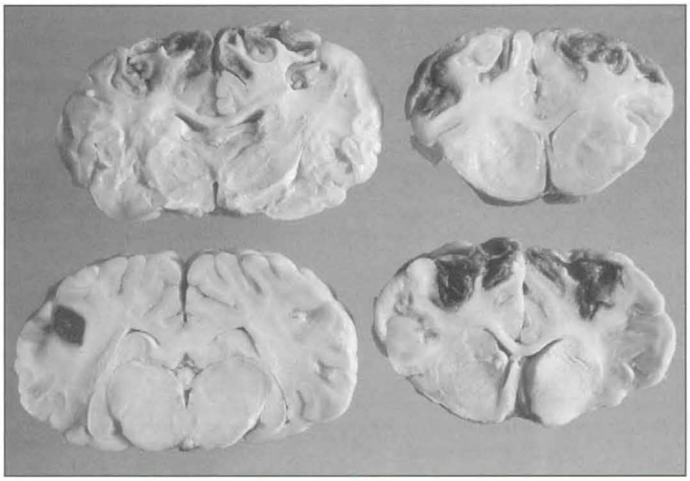

Figure 3.125 Areas of malacia, hemorrhage, and necrosis affecting the gray matter of the rostral cerebrum mostly in a bilaterally symmetrical pattern in **bovine necrotizing meningoencephalitis** caused by BoHV-5. (Courtesy of D. Driemeier.)

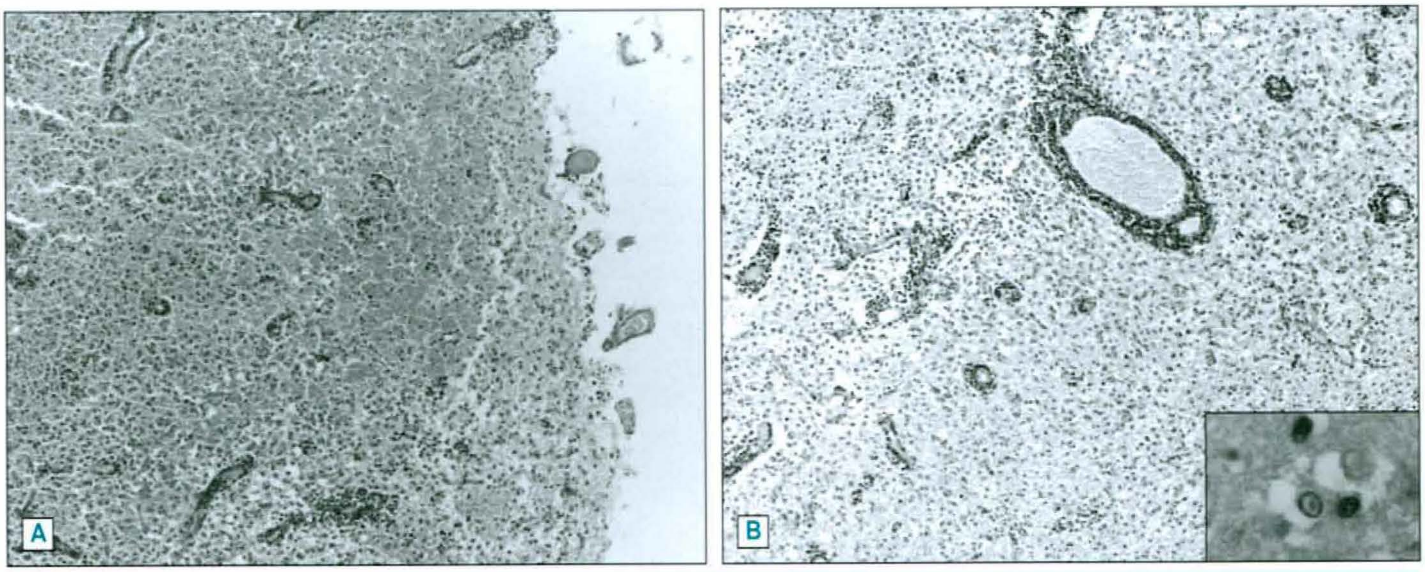

Figure 3.126 Severe hemorrhagic necrosis and malacia (**A**) with perivascular lymphocytic and plasmacytic cuffing (**B**) affecting the external cortical layers of the rostral cerebrum in **bovine necrotizing meningoencephalitis** caused by BoHV-5. Intra-astrocytic intranuclear herpesviral inclusion (inset in **B**). (Courtesy of D Driemeier.)

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Bovine meningoencephalomyelitis caused by *Bovine herpesvirus 1*

Nonsuppurative encephalomyelitis due to *Bovine herpesvirus 1* (BoHV-1) is reported worldwide. BoHV-1 causes multiple and diverse conditions in cattle such as abortion, infectious bovine rhinotracheitis, infectious bovine vulvovaginitis, and balanoposthitis. Some BoHV-1 serotypes are neurovirulent and are able to induce encephalitis. Marked upper respiratory disease typically precedes or occurs concurrently with the encephalitis. The pathogenesis and clinical signs are similar to those described for BoHV-5. Histological lesions are those of *nonsuppurative encephalomyelitis with occasional intranuclear herpetic inclusions.* The massive neuronal necrosis and gliosis described in BoHV-5 are not usually seen in association with BoHV-1. BoHV-1 encephalitis is more prevalent in calves; however sporadic cases can affect adult cattle particularly in the Near and Middle East.

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Malignant catarrhal fever

Malignant catarrhal fever (MCF) is a fatal multisystemic lymphoproliferative and inflammatory disease affecting many ruminant species. The details of MCF, including the lesions associated with its encephalomyelitic form, are described in Vol. 2, Alimentary system.

Nonsuppurative meningoencephalitis is reported in "malignant catarrhal fever" in *swine* in Europe resulting from infection with *Ovine herpesvirus 2*. *Caprine herpesvirus 2*, a gamma-herpesvirus, does not cause overt disease in goats, but causes fatal goat-associated MCF in certain species of deer.

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Bovine paramyxoviral meningoencephalomyelitis

Sporadic cases of nonsuppurative meningoencephalomyelitis due to paramyxovirus are rarely reported. The causative virus is restricted to the European continent and is still not classified within the Paramyxoviridae but is distinct from the other bovine paramyxoviruses such as *Bovine parainfluenza virus 3*. The disease should be differentiated from the sporadic bovine encephalitis caused by *Chlamydophila* spp.

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Porcine circovirus type 2 encephalopathies

Porcine circovirus type 2 (PCV2), the cause of porcine postweaning multisystemic wasting syndrome, produces nonsuppurative or granulomatous encephalitis with gliosis under experimental conditions, either alone or in association with porcine parvovirus. The PCV2 antigen has been identified in brain in these cases demonstrating neurotropism of the virus under experimental conditions. The role of PCV2 in encephalitis associated with natural infection is controversial. PCV2 antigen has been detected in brains of pigs with naturally occurring encephalitis but always in association with other pathogens that can cause encephalitis alone and under natural conditions (e.g., PRRSV or *Streptococcus suis*). Also, the PCV2 antigen was demonstrated in neonatal pigs in association with naturally occurring congenital tremor type A2 (see Porcine hypomyelinogenesis); however, the exact role of PCV2 in this condition is yet to be determined.

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Porcine encephalitis associated with PRRSV infection

Porcine reproductive and respiratory syndrome virus (PRRSV) is neurovirulent especially in young pigs and can cause encephalitis under natural conditions, often in association with other PRRSV syndromes (e.g., interstitial pneumonia) (see Vol. 2, Respiratory system).

California encephalitis virus meningoencephalomyelitis

Several serotypes of species *California encephalitis virus* of genus *Orthobunyavirus*, family Bunyaviridae, usually cause only asymptomatic infections, but are capable of causing encephalitis. These include La Crosse virus (LACV), Snowshoe hare virus, and Jamestown Canyon virus.

La Crosse virus is maintained in the environment and transmitted between susceptible hosts by *Aedes triseriatus* mosquitoes. Chipmunks (*Tamias striatus*) and squirrels (*Sciurus carolinensis*) are the principal amplifying vertebrate hosts. Other wild mammals such as foxes (*Vulpes fulva* and *Urocyon cinereoargenteus*) and woodchucks (*Marmota monax*) may also contribute to virus maintenance. The virus causes encephalitis and secondary neurologic deficits in humans, particularly school-aged children. A few cases have been reported in dogs in Florida and Georgia, USA. The most predominant clinical signs in these dogs were seizures and head tilt. Gross lesions were usually unremarkable, but areas of malacia may be present in cortex. Histologic lesions are predominantly in the cerebral cortex and characterized by *histiocytic and lymphoplasmacytic meningoencephalitis* with fairly thick cuffs and multifocal necrotizing panencephalitis. The necrotic areas in the acute stage are histiocyte and neutrophil rich. The lesions of this disease are similar to those seen in idiopathic granulomatous meningoencephalomyelitis (GME) described below.

Antibodies to **Snowshoe hare virus** have been found in a wide range of wild and domestic mammals, but overt disease is very rare. Encephalitis in association with SSHV was reported in two horses from Canada, but the diagnosis was made based on detection of seroconversion; one horse recovered and the pathologic changes found in the second case were not described.

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Canine herpesviral encephalitis

Canid herpesvirus 1 can cause an acute, highly fatal disease of neonates. Puppies after the age of 3 weeks are resistant to infection. The incidence is low, and the disease is only diagnosed at autopsy. Hypothermia predisposes to disease in neonates, in which the infection would otherwise be asymptomatic. Infection at birth is followed by cell-associated viremia and viral replication in vascular endothelium. This tropism is reflected in large hemorrhages at postmortem most apparent in renal surface, adrenal and serosa of gastrointestinal tract. Focal necroses occur in parenchymatous organs and inclusion bodies may be demonstrated in these foci. *Nonsuppurative meningoencephalitis* is most severe in cerebellum and brain stem. It may be accompanied by necrosis especially in the cerebellar cortex. Vascular endothelial hypertrophy and hyperplasia is accompanied by mononuclear infiltrates. There may be inflammatory changes in the retina, peripheral nerves, and ganglia.

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Equine herpesviral myeloencephalopathy

Equid herpesvirus 1 (EHV-1) and *Equid herpesvirus 4* (EHV-4) are two antigenically related but distinct viruses in the genus *Varicellovirus*, subfamily Alphaherpesvirinae, family Herpesviridae. Both viruses are widespread in horses, have significant economic impact on the equine industry, and are responsible for several clinical conditions including respiratory disease, pulmonary vasculotropic disease, enteric disease, and abortion. *Equine myeloencephalopathy is an important neurological disease* characterized clinically by ataxia, paresis, and paralysis, and caused mainly by EHV-1 and incidentally by EHV-4. Almost all recent outbreaks have been associated with EHV-1 infection. Other members of equine alpha-herpesviruses that are pathogenic but do not cause neurologic disease include *Equid herpesvirus 3* (Equine coital exanthema virus) and *Equid herpesvirus 8*

(Asinine herpesvirus 8), which induces interstitial pneumonia in donkeys. *Equid herpesvirus 9* (Gazelle herpesvirus) causes severe encephalitis experimentally in several species of domestic animals; the natural outbreak of fulminant encephalitis has been reported in a herd of Thomson's gazelles that were in close association with zebra. The natural reservoir for EHV-9 is unknown, but zebras or other equidae have been suspected.

Most horses show serologic evidence of exposure to EHV-1 and EHV-4 but are asymptomatic, and vaccination does not necessarily confer protection from neurologic manifestations. Both viruses contain at least 13 glycoproteins, which are important virulence factors for attachment, entry to the host cell, and cell-to-cell dissemination. The natural spread of EHV-1 is through direct horse-to-horse contact, by inhalation of nasal aerosols from infected horses, or through direct contact with an infected aborted fetus or placenta. EHV-1 replicates first in upper respiratory tract epithelium and local lymph nodes, and then induces T-cell and monocyte-associated viremia that ends with invasion of endothelial cells of the CNS and pregnant uterus. This leukocyte-associated viremia protects the virus from humoral immunity. *The virus is endotheliotropic, epitheliotropic, and neurotropic, but not neurovirulent.* The replication of virus in endothelial cells of the CNS leads to initiation of the inflammatory cascade that ends in *thrombo-occlusive necrotizing vasculitis.* The resultant myeloencephalopathy is due to destruction of CNS tissue secondary to vasculitis. The vasculitis is either due to direct viral cytotoxic effect or due to an immune-mediated (Arthus-type reaction) mechanism. A similar mechanism is responsible for EHV-1-induced abortion and pulmonary vasculotropic disease. There are no genetic or antigenic differences between the EHV strains isolated from neurogenic cases versus abortigenic or respiratory cases.

EHV-1 and EHV-4 have life-long latency in T cells and in neural tissue such as trigeminal ganglia. Latent virus can be reactivated experimentally after very high doses of corticosteroids and naturally after stress (such as castration). In contrast to the extensive studies on EHV-1, the pathogenesis of EHV-4 infection is poorly documented.

The disease occurs sporadically, but in several recent outbreaks, most affected horses either died or were euthanized. The disease is common in late winter and spring, which is also the time of greatest prevalence of EHV-1 abortion outbreaks. The incubation period is 6–10 days and usually occurs in association with abortion and/or respiratory disease but can occur without preceding signs. All ages are susceptible, but *pregnant mares and mares nursing foals are over-represented.*

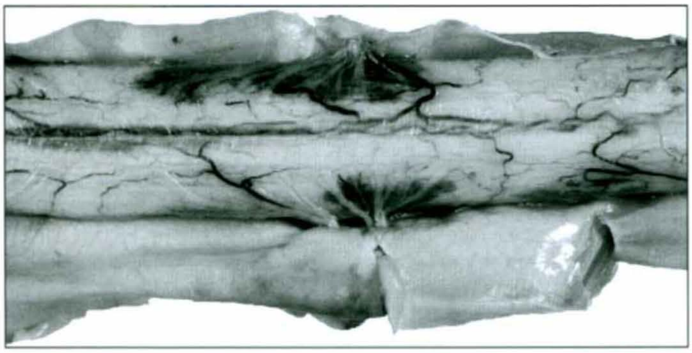

Figure 3.127 Acute spinal cord hemorrhage in a horse with **equine herpesviral myeloencephalopathy**. (Courtesy of RF Slocombe.)

Clinical signs start with fever and mild rhinitis. Neurologic signs are variable and depend on the part of the CNS affected by vasculitis, however common clinical signs include variable degrees of symmetrical ataxia and paresis that are more severe in pelvic limbs. Fecal and urinary incontinence are common, and clinical signs may end in hemi- or paraplegia. Gross and histologic lesions are sequelae to vasculitis. Gross lesions are not always present, but small (0.2–0.5 cm) random multifocal areas of hemorrhage may be present throughout the meninges, brain, and spinal cord (Fig. 3.127). In severe cases, multifocal necrohemorrhagic or malacic areas (up to 1.5 cm in diameter) can be present, especially in the white matter of spinal cord or the white or gray matter of the brain. *The characteristic histologic lesions are nonsuppurative necrotizing vasculitis and thrombosis,* with greater prevalence in the meningeal and parenchymal blood vessels of the brain stem and spinal cord. Perivascular edema, hemorrhage, focal areas of malacia, and infarction are present adjacent to the affected blood vessels. Occasionally, axonal swelling and mild nonsuppurative trigeminal ganglionitis are present. Extraneural lesions include uveal vasculitis and optic neuritis, especially in foals, and testicular and epididymal vasculitis in stallions.

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Canine distemper and related conditions

Canine distemper is discussed in detail in Vol. 2, Respiratory system. Three neurologic conditions are discussed here: multifocal distemper encephalomyelitis in mature dogs, postvaccinal distemper, and old-dog encephalitis.

Multifocal distemper encephalomyelitis in mature dogs

This rare chronic progressive disease occurs when *Canine distemper virus* infects dogs at 4–8 years of age. This disease is not preceded by the classic form of canine distemper, and signs of systemic illness are often absent or transient. Clinical signs have a slow progressive course and include weakness of the pelvic limbs, generalized incoordination, but no seizures or personality changes, and occasionally head tremors. Lesions are restricted to the CNS and are most prevalent in the cerebellum and white matter of the spinal cord. The cerebral cortex is frequently spared. This distribution differentiates this condition from an extremely rare condition, old dog encephalitis, wherein the cerebral cortex is constantly affected. The lesions are those of *multifocal necrotizing nonsuppurative encephalitis* with rare canine distemper intranuclear intra-astrocytic inclusion bodies, and demyelination in the internal capsule and corona radiata.

Post-vaccinal canine distemper encephalitis

This condition occurs in young dogs 1–3 weeks after vaccination with attenuated *Canine distemper virus* vaccines and is characterized by an acute to subacute clinical course (1–5 days). The acute course is characterized by clinical signs reminiscent of the furious form of rabies including aggressive behavior and attempts to attack. It is not completely clear why some dogs develop this condition post-vaccination. Immune stimulation by other canine viruses (e.g., *Canine parvovirus*) at the time of vaccination was suggested. The lesions are not well documented, however, lesions are always restricted to the CNS and are reminiscent of the natural disease, but distinguished by the relative sparing of the white matter.

"Old dog" encephalitis

"Old dog" encephalitis (ODE) is rather rare. Most cases occur in dogs past middle age but it has been observed in dogs as young as 1 year of age. The disease is of insidious onset and is characterized by circling, swaying, and weaving. Compulsive walking with pushing against fixed objects is typical, but there is neither paralysis nor convulsions. The disease progresses over 3–4 months to coma or termination.

ODE is caused by *Canine distemper virus* (CDV), apparently as a consequence of long-term subclinical, persistent infection: CDV appears to persist in a replication-defective state. ODE does not appear to be simply a progression of the encephalomyelitis of canine distemper. Virus can be isolated from affected animals only by explantation of affected brain and then only with difficulty, and the disease is not transmissible by direct inoculation. Inclusion bodies are readily found in some cases, and their structure is identical with paramyxovirus nucleocapsids of CDV in nervous tissue. Antigen that responds to fluorescent antibody prepared against CDV is abundant in cells of the gray matter, and serum antibody titers can be very high.

Lesions are confined to the brain, which appears slightly reduced in size. The ventricles are moderately dilated. Lesions are diffusely distributed throughout the cerebral cortex, thalamus, and midbrain. The reaction is nonsuppurative, qualitatively always the same but varying in degree. The most obvious change is cuffing, and the cuffs are remarkable for their large size and the purity of the lymphocytic populations in them. Plasma cells are present in small numbers. The infiltrating cells are confined to the Virchow–Robin space and seldom spread into the parenchyma. The large cuffs occur in both gray and white matter but are most common at the junction of these two zones. Focal gliosis does not occur in this disease, but there is some proliferation of astrocytes about vessels and neurons. There is uniform and rather diffuse atrophic sclerosis of the cerebral white matter, which gives an impression of gliosis, but astrogliosis is not prominent. There is some demyelination, producing typical punched-out areas in the white matter, and distorted myelin sheaths in the heavily myelinated tracts are quite extensive when specially stained. Lymphocytes may be found in the choroid plexuses where they are inserted into the brain, and about vessels where they enter the parenchyma.

Nerve cells, especially in Ammon's horn and the pons, reveal chromatolysis with only a few remnants of Nissl substance in the periphery of the cytoplasm. The chromatolytic cytoplasm is slightly acidophilic. The neuronal nuclei in the forebrain are remarkably swollen in most of the altered nerve cells. In occasional nuclei, there is pink inclusion-like material. Neuronophagia does not occur. The astrocytic nuclei are remarkably swollen, have an irregular outline, and may contain traces of pink deposit in the nucleoplasm. In a proportion of cases, possibly those of longest duration, prominent *intranuclear and cytoplasmic eosinophilic inclusion bodies* may be found easily.

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Parasitic infections

Protozoal infections

The cerebral complications of infections by protozoa such as *Babesia, Theileria, Trypanosoma,* and *Toxoplasma* are discussed elsewhere in these volumes. *Acanthamoeba*, a free-living ameba, can produce granulomatous meningoencephalitis in dogs as part of an opportunistic generalized infection (Fig. 3.128).

From time to time and in individual cases, pathologists observe *sporozoan parasites* in neural tissues of fetuses, neonates, and adults and lesions presumed to be the consequence of their presence. There are, however, difficulties in specific identification of the parasites and in attribution of pathogenicity. The syndromes considered here are reasonably defined but are subject to revision as the parasites are identified and their lifecycles clarified.

Encephalitozoonosis

Encephalitozoon (Nosema) cuniculi is a microsporidian parasite capable of establishing infection in a wide variety of mammalian species and birds. It is rarely a zoonotic infection. Endemic infection is

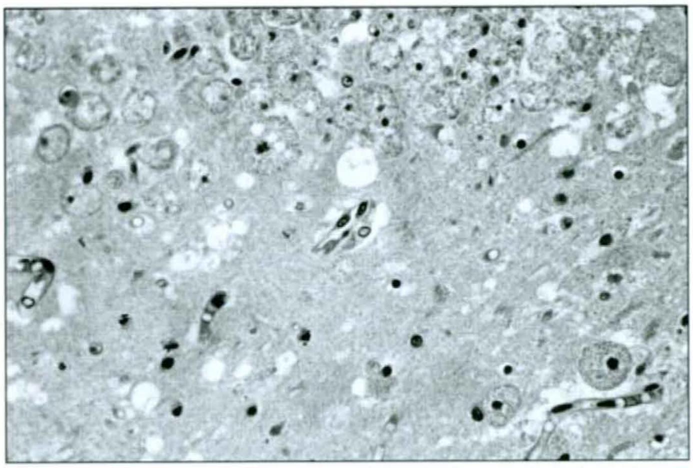

Figure 3.128 Amebic encephalitis in a dog; large numbers of amebae are present in this field, with only minimal reaction. (Courtesy of RF Slocombe.)

common in colonies of laboratory rodents in which the clinical consequences are mild but the pathologic changes may confuse other studies. Amongst domestic species, the disease is of interest in carnivores, especially farmed foxes in which serious mortalities occur, and occasionally in dogs and mink. The incidence of subclinical infection is not known, but a serological survey of an unselected population of asymptomatic stray dogs identified 10–15% to be seropositive.

The organism is an *obligate intracellular parasite* with a direct life cycle. It develops in parasitophorous vacuoles in cells of many tissues, especially endothelial cells, but is most easily found in brain and kidney in acute active infections. In chronic infections, the organisms can be sparse or impossible to find in microscopic sections, although it seems that animals once infected remain permanently so and excrete the organism mainly in urine.

Clinical disease occurs in dog and fox pups; the organism is shed in urine and feces of affected animals. In both hosts, transplacental infections appear to be important, but oral transmission, as by ingestion of infected rabbit carcasses, may occur. Experimental infection of mature dogs does not lead to clinical disease.

Tissue changes in encephalitozoonosis are most prominent in brain and kidney, but the organism selectively parasitizes vascular endothelium, and the *segmental vasculitis* that results is responsible for lesions in many tissues. Gross lesions may be limited to the kidneys as severe, nonsuppurative interstitial nephritis (Fig. 3.129A). Organisms are abundant in sections of kidney early in the disease but they are difficult to find at later stages. They are especially numerous in the epithelium and lumen of tubules, in glomerular capillaries and in the interstitium, and are present in small vessels and in the media and adventitia of intrarenal arteries. Fibrinoid necrosis affects some glomeruli and the arterial lesions resemble those of periarteritis nodosa. Focal hepatic necrosis and nonsuppurative portal infiltrations are associated with organisms in hepatocytes and Kupffer cells and with nodular vasculitis in the triads. Focal myocardial necrosis and inflammation are frequently associated with vasculitis.

The lesions in the nervous system are those of *widespread nonsuppurative meningoencephalomyelitis*. The severity of lesions varies unpredictably in different parts of the nervous system reflecting the random localization of the organism and the irregular distribution of inflammatory vascular change. Focal gliosis and microscopic granulomas surround small vessels (Fig. 3.129B). About larger vessels showing segmental fibrinoid change, mononuclear cells form cuffs involving the adventitia and perivascular space and eventually assume an epithelioid cell appearance. There is astrocytosis in the surrounding parenchyma. The vascular lesions in the meninges in the acute disease resemble those of polyarteritis nodosa and become dominated by sclerotic changes in the chronic disease in which perivascular cuffing and granulomatous reactions persist.

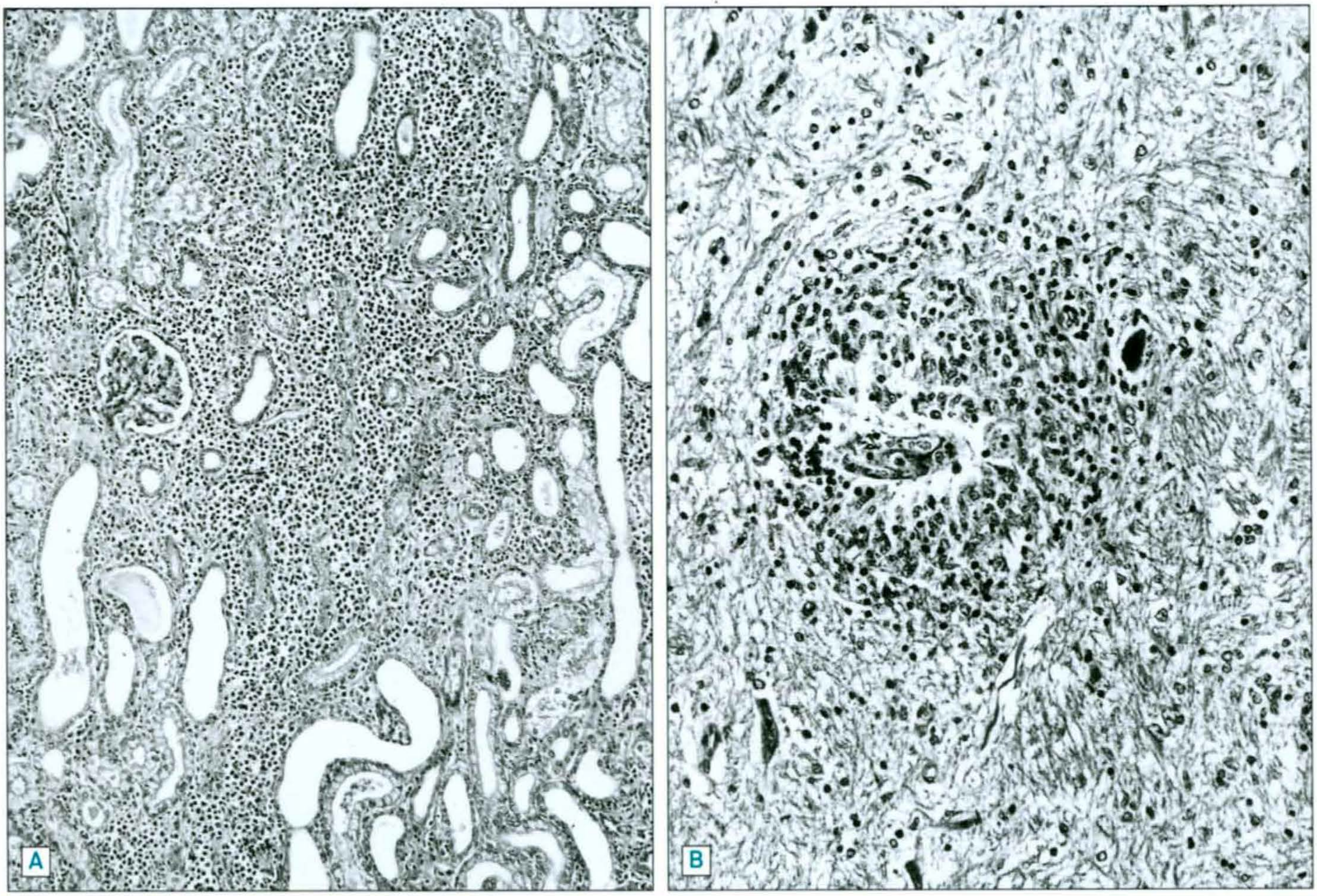

Figure 3.129 Encephalitozoonosis in a dog. **A.** Severe diffuse nonsuppurative interstitial **nephritis.** **B.** Focal granulomatous **encephalitis.**

Puppies which survive the early clinical disease may remain stunted and develop progressive renal disease. It is possible that encephalitozoonosis, as for any sporadic disease, is underdiagnosed especially in chronic infections in which the organism is difficult to demonstrate. Immunohistochemical methods help to identify sparse organisms and to distinguish them from similar parasites, particularly *Toxoplasma* and *Neospora*.

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Equine protozoal myeloencephalitis

Equine protozoal myeloencephalitis (EPM) is caused mainly by ***Sarcocystis neurona***, an apicomplexan protozoan parasite, however identical disease is reported in association with ***N. caninum*** and ***N. hughesi***. Opossums (*Didelphis* spp.) are the definitive host for *S. neurona,* and they are infected by eating intermediate host tissues that contain infective tissue cysts. *S. neurona*-induced EPM is restricted to the Americas in the geographical range of the opossum. Natural intermediate hosts (e.g., armadillos, sea otters, raccoons, skunks, cats) are infected by ingestion of food or water contaminated by sporocysts shed in opossum feces. Horses are assumed to be dead-end hosts, but may also act as intermediate hosts.

Exposure to *S. neurona* is widespread among horses, but the prevalence of the classic progressive disease is much lower. A seropositive horse is positive for exposure but not necessarily for the presence of the disease. However, the presence of seropositivity and the clinical signs of weakness and acute ataxia usually indicate active disease. Infection with *S. neurona* has no age predilection. Most EPM cases due to *S. neurona* infection appear in the summer and fall. Affected horses are presented with ataxia, limb weakness, lameness, and rarely seizures.

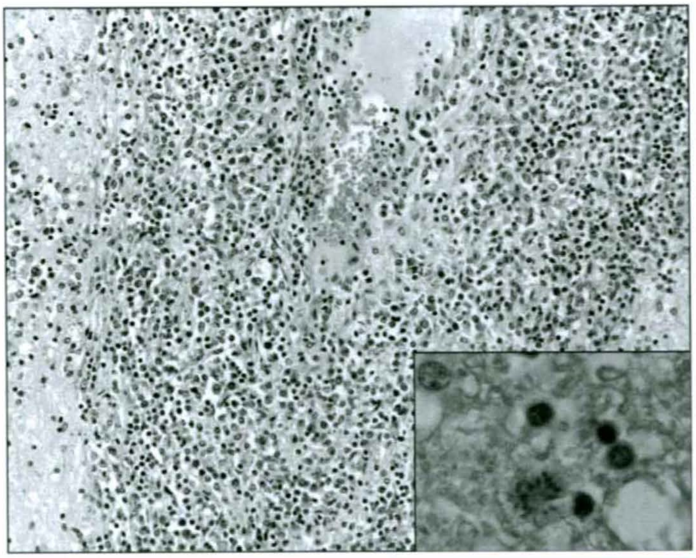

Figure 3.130 Massive mixed inflammatory encephalitis with variable numbers of eosinophils with rare intralesional ***Sarcocystis neurona*** schizonts (inset) in a horse with equine protozoal myeloencephalitis.

Gross lesions are present only in severe cases, and range from multifocal acute hemorrhage to the presence of discrete multifocal gray to dark yellow areas primarily in cross-sections of fixed brain stem, obex, pons, and cervical and thoracic cord. The histologic lesions are usually moderate to severe and characterized by multifocal areas of necrosis, malacia with aggregation of gitter cells, gliosis, and infiltration of large numbers of lymphocytes, histiocytes, plasma cells, and fewer eosinophils and neutrophils with severe involvement of the meninges (Fig. 3.130). The blood vessels in these areas have swollen activated endothelium with thick perivascular cuffs of mononuclear cells and occasional eosinophils. Also and particularly in cord sections, there is axonal swelling or loss besides the appearance of spheroids and some digestion chambers. In chronic cases, the inflammation can be predominantly histiocytic with occasional eosinophils and multinucleated giant cells.

Finding *S. neurona* merozoites or schizonts can be a challenge and serial sections must be examined in most cases. *S. neurona* schizonts are almost always present near areas of inflammation and necrosis, schizonts are oval or irregularly round, have very thin walls ($<0.5\,\mu m$), are up to $20\,\mu m$ in diameter, and contain a few basophilic ovoid merozoites $5\,\mu m \times 1.5\,\mu m$. The stage infective to the definitive host, i.e., sporocysts, can be found in tongue and other skeletal muscles. The *S. neurona* sporocyst is round ($50–100\,\mu m$) or elongate ($500\,\mu m$ long and $40\,\mu m$ wide), and contains a number of bradyzoites.

EPM-like disease can occur in other *S. neurona* intermediate hosts, including the cat, and this disease should be considered in the differential diagnosis of inflammatory encephalomyelitis in this species. Both *N. hughesi* and *N. caninum* can cause identical EPM lesions in horses. The complete life cycle and methods of transmission for *N. hughesi* have not been determined. *Neospora hughesi* tachyzoites are crescent-shaped, approximately $5 \times 2\,\mu m$. Definitive diagnosis of EPM in a live horse is challenging. Detection of *S. neurona* or *N. hughesi* antibodies in serum and cerebrospinal fluid by an immunoblot test is available. Positive results indicate exposure, but do not necessarily indicate that the horse has EPM. The postmortem diagnosis depends on finding characteristic lesions, especially in the presence of the characteristic protozoal stages. Immunohistochemistry kits for *S. neurona* and *N. caninum* are available commercially.

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Neosporosis

Neospora caninum is an apicomplexan coccidian parasite that is a major pathogen of cattle, in which it causes abortion, and for dogs. Other species, such as goats, sheep, deer, and horses, can be infected occasionally. Dogs are the primary definitive host, and they are also considered as an intermediate host. Other canids, e.g., coyotes (*Canis latrans*), may also be important definitive hosts. *N. caninum* has three infectious stages: tachyzoites, tissue cysts, and oocysts. Tachyzoites and tissue cysts are found both in intermediate hosts and the definitive host. However, oocysts are only present in the definitive host. Tachyzoites have been found in neurons, reticuloendothelial cells, hepatocytes, muscle cells including myocardium, and bovine placenta. Tissue cysts have been found in the CNS, muscles, and retina. The exact modes of transmission are not well understood. Dogs become infected by ingesting tissues contaminated with tissue cysts, and then shed oocysts in their feces. Cattle and other intermediate hosts become infected by ingesting sporulated oocyst-contaminated food, water, or soil. However, the principal route for infection in cattle is transplacental (vertical) transmission.

N. caninum does not cause significant clinical disease in adult **cattle**, however it causes abortion in both dairy and beef cows particularly at mid-term, although cows can abort at any time from 3 months to term. Infected fetuses may die in utero, be mummified, stillborn, or born alive with or without clinical signs. Extraneural lesions in bovine fetuses include *lymphocytic, plasmacytic and, to a lesser extent, histiocytic, hepatitis, pancarditis or myocarditis, myositis, and placentitis*. Cotyledonary necrosis may be associated with the placentitis. Intralesional *Neospora caninum* tachyzoites are occasionally present in the aforementioned organs. Tachyzoites appear in groups, either intracellular in neurons, endothelium or epithelial cells, or extracellular. Tachyzoites are spindle-shaped, 4–7 × 2 μm. Tissue cysts are primarily present in the CNS and rarely in skeletal muscles. Cyst diameter is up to 107 μm, with wall thickness of 1–4 μm, and containing numerous bradyzoites 8 × 2 μm.

The most frequent and almost pathognomonic CNS lesion in *bovine fetuses* is the presence of *multifocal discrete foci of necrosis* (~100–300 μm diameter), particularly in the brain and to a lesser extent in the cord (Fig. 3.131A, B). The necrotic areas are fairly well circumscribed, have necrotic centers and are surrounded by a rim of glial cells and macrophages. In advanced lesions, the necrotic area may be completely replaced by macrophages and a few glial cells, which make the lesions appear as discrete granulomas. The recognition of *N. caninum* tachyzoites and tissue cysts in aborted fetal brain or other fetal tissue is usually difficult on H&E stain, and immunohistochemistry must be performed to confirm the diagnosis of neosporosis. Other CNS lesions include mild nonsuppurative meningoencephalomyelitis. Fetal anomalies are not common in association with *Neospora* abortion.

Infection in **dogs** is transmitted either horizontally or vertically (transplacental). Dogs of any age can be affected and the infection can be generalized affecting any organs, including the skin, or can be localized. Infection in adult dogs is usually subclinical. Infection in young congenitally infected dogs is severe and characterized pathologically by encephalomyelitis and myositis/polyradiculoneuritis and clinically by hindlimb paresis that is followed by paralysis. CNS lesions are those of *necrotizing granulomatous, lymphoplasmacytic, and occasionally eosinophilic meningoencephalomyelitis* with diffuse gliosis, occasional axonal swelling, digestion chamber formation, and intralesional *N. caninum* tachyzoites and cysts (Fig. 3.132). These lesions are widely distributed in the brain and cord, however in the cortex the gray matter is affected predominantly. Lesions associated with the neuritis/polyradiculoneuritis is frequently severe, mostly affecting pelvic limbs, and characterized by severe lymphohistiocytic and occasional eosinophilic inflammation with associated secondary degenerative and necrotizing changes either in muscles or nerves with intralesional tachyzoites and rare cysts.

Experimental infection of pregnant **ewes** and **does** produces a disease that is identical pathologically to that observed in cattle, however natural disease is rare.

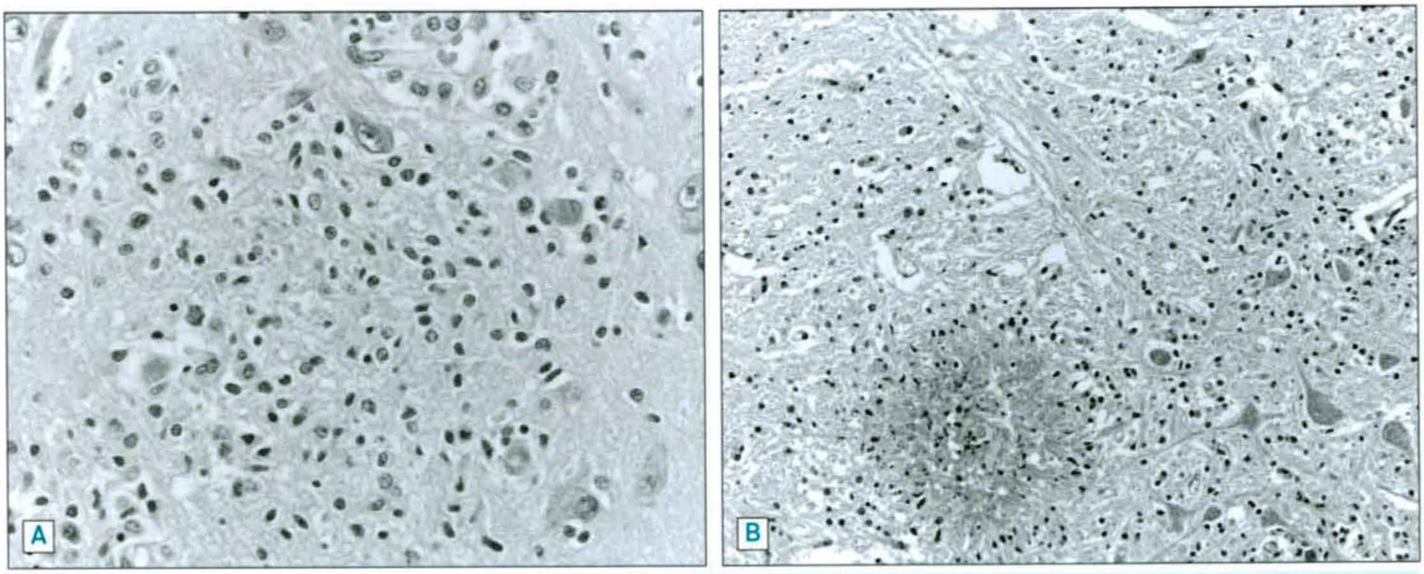

Figure 3.131 Lesions of neosporosis in fetal bovine brains may consist only of focal gliosis (**A**) or may occur as multifocal areas of neuropilar necrosis (**B**) encircled by glial cells and rarely giant cells. *Neospora caninum* cysts are rarely seen.

The epidemiology and methods of transmission of *N. caninum* and other *Neospora* in **horses**, e.g., *N. hughesi*, are not completely understood. Transplacental infection is suggested but not completely confirmed. *N. caninum* has been isolated from a few aborted fetuses. *N. caninum* and *N. hughesi* are rare causes of equine protozoal myeloencephalitis (see above).

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Toxoplasmosis

Toxoplasmosis is one of the most common protozoal diseases affecting humans and animals and is caused by ***Toxoplasma gondii***. Diseases caused by *T. gondii* are very similar in clinical presentation and pathology to those caused by *N. caninum*. Felids are the only definitive host and they also can act as an intermediate host. Other intermediate hosts include humans and other mammals. *T. gondii* has three infectious stages: tachyzoites, tissue cysts, and oocysts. Tachyzoites and tissue cysts are found in both intermediate and definitive hosts, however, oocysts are only present in the definitive host. Tachyzoites and tissue cysts are present more commonly in neural tissue and muscles, but can be present in virtually any tissue. Felids become infected by ingestion of tissues contaminated with tissue cysts, and shed oocysts in their feces. Human and other intermediate hosts including felids can become infected by ingesting sporulated oocyst-contaminated food, water, or soil. Transplacental transmission is important in cats, goats, and sheep. The extraneural pathology of toxoplasmosis is discussed elsewhere in these volumes. The nervous system lesions, including polyradiculoneuritis, are identical to those described above for neosporosis, however *the tissue cyst has a thinner wall ($<0.5\,\mu m$), is 5–70 μm in size, and contains several bradyzoites 0.7–1.5 μm. Tachyzoites are 2–6 μm in size.* The encephalitic form of toxoplasmosis is most likely to occur in immunosuppressed dog and cats or kittens. Toxoplasmosis in pigs is generalized and can cause devastating disease with lesions including nonsuppurative encephalomyelitis with intralesional *T. gondii* stages.

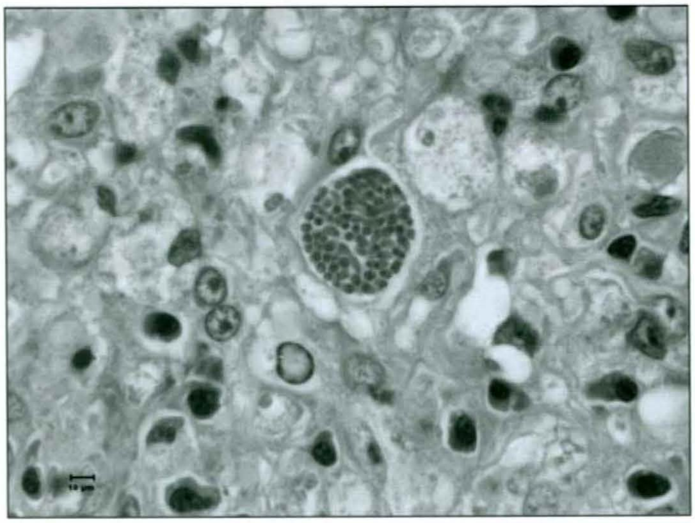

Figure 3.132 ***Neospora caninum* cyst** in the brain of a dog. (Courtesy of MJ Hazlett.)

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Sarcocystis canis encephalitis

A rare and generalized disease affecting dogs mostly in North America is caused by *S. canis* and characterized histologically by multisystemic vasculitis, hepatitis, and necrotizing lymphohistiocytic encephalitis in association with the presence of intralesional *S. canis* schizonts and merozoites (schizonts are 5–25 × 4–20 μm and contain 6–40 merozoites of 5–7 $\mu m \times 1\,\mu m$).

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Helminth and arthropod infestations

Nothing is known of what motivates and directs the migration of larval parasites. Those that migrate somatically are apt to go astray, and this appears especially likely when they wander in an alien host. Aberrant pathways include the nervous system with such frequency as to suggest that nematodes have a special propensity for wandering in the CNS. Whether this is indeed the case remains to be proven, but parasitic migrations in nervous tissue are more likely to be symptomatic than aberrant migrations in other tissues, and there is an impressive list of parasites that have been found in brain or cord. Many of these infestations and the parasites in question are discussed elsewhere in these volumes.

Cestodes

Adult cestodes live almost exclusively in the small intestines of the final host, however certain larval stages can infest the brain of the intermediate host. ***Coenurus cerebralis***, the larval stage of ***Taenia***

multiceps, which infests the small intestines of dogs and wild carnivores, is fairly common in the brains of sheep in Europe, less common in other herbivores, and rather rare in horses and humans. About 40% of pigs harboring ***Cysticercus cellulosae*** (the larval stage of the human tapeworm ***Taenia solium***) have cysts in the meninges and brain as well as in the muscle, and the same species has been identified in the brains of dogs. Possibly ***Cysticercus bovis*** (the larval stage of human tapeworm ***Taenia saginata***) and other cysticerci will invade nervous tissue with comparable frequency. Apparently, hydatids are seldom found in brain.

Nematodes

The term **cerebrospinal nematodiasis** is applied to nervous diseases resulting from aberrant nematode larval migrations. The few nematodes that produce the syndrome with any frequency are discussed below.

Parastrongylus *(Angiostrongylus)* ***cantonensis*** is a metastrongylid lungworm whose only known definitive host is the rat. It is widely distributed in the warm Pacific regions, but its distribution is much more limited than that of the gastropod intermediate hosts and the rat. The parasite resides in the pulmonary arteries of rats, eggs lodge as emboli in alveolar capillaries and the larvae, which hatch in about 6 days, and follow the tracheal–intestinal route to the exterior. First-stage larvae actively penetrate terrestrial and aquatic slugs and snails, which act as intermediate hosts. Transport hosts for third-stage larvae include frogs, crabs, and prawns. In addition to the rat, dogs, humans, and occasionally other species are infected by eating intermediate or transport hosts and, possibly, directly by ingestion of infective larvae that have emerged from intermediate hosts. Ingested larvae enter and are dispersed by the circulation to many tissues, but predominantly to brain, kidney, and muscle. Molting larvae in the brain produce a mild to severe inflammatory reaction before re-entering the venous circulation for return to the pulmonary arteries. Aberrant infections are important in humans and dogs, and are reported in horses and macropods. The human disease, eosinophilic meningoencephalitis, is usually mild and without sequelae, but infection in dogs can be accompanied by ascending paralysis. Larvae that enter the brain in dogs are probably inhibited in their development and destroyed there (Fig. 3.133). The lesions are granulomatous, randomly distributed in the cord and brain and are most frequent and severe in the cord (Fig. 3.134). Rarely, degenerate parasites are present in the granulomas, but apparently viable worms in the tissue are not accompanied by an inflammatory reaction. Eosinophils infiltrate the granulomas but are more numerous in affected meninges.

Parelaphostrongylus *(Pneumostrongylus)* ***tenuis*** is a metastrongylid parasite of white-tailed deer, *Odocoileus virginianus*, in North America. The intermediate hosts are terrestrial slugs and snails. Ingested larvae reach the spinal cord of the deer in ~10 days. They develop for up to 1 month in the dorsal horns of the cord at all levels and then migrate into the meningeal spaces. Some penetrate the dural veins and sinuses and mature. Eggs or larvae are carried in venous blood to the lungs. The larvae do very little to the cord in white-tailed deer, but the reaction is more severe in other species, including red deer, elk, moose, and sheep (Fig. 3.135). ***Elaphostrongylus panticola*** and ***E. rangifera*** of deer in northern Europe and Russia have a life cycle similar to that of *P. tenuis*, but infections are usually subclinical.

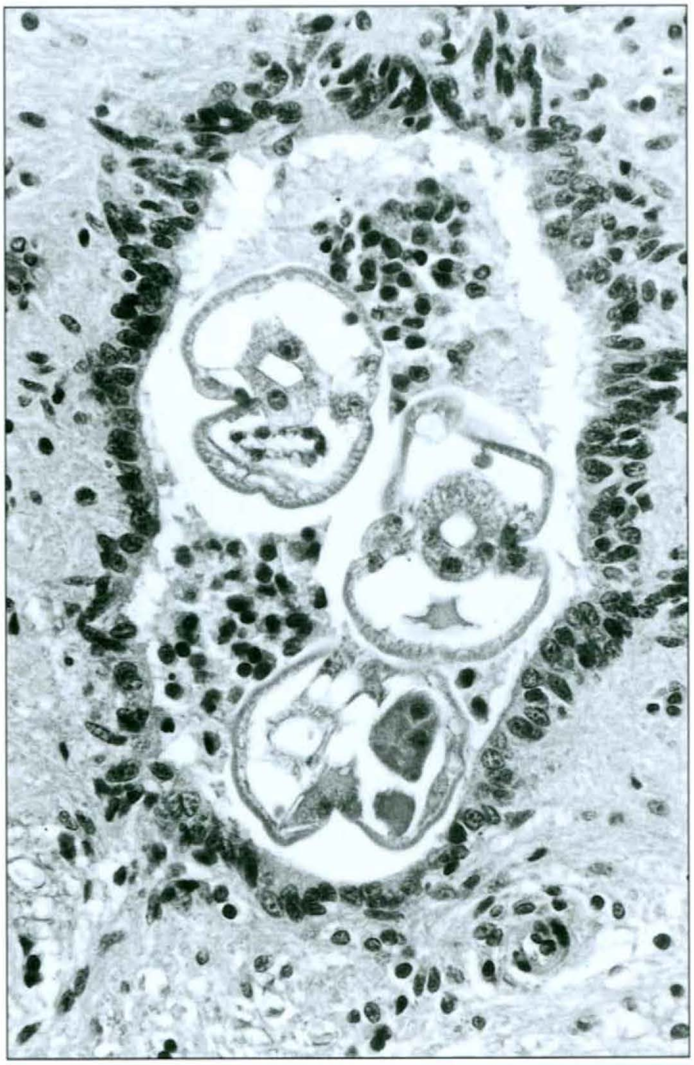

Figure 3.133 Larvae of ***Parastrongylus*** sp. in central canal of spinal cord in a dog.

When larvae of ***Elaeophora schneideri*** – a filarial parasite discussed in the Cardiovascular system – develop in the leptomeningeal arteries of various cervids, sheep and goats, they can cause ischemic necrosis of brain tissue.

Setaria digitata is normally found as an adult in the peritoneal cavity of cattle and buffalo in Asia (see also Peritoneum). Microfilariae can be carried to aberrant hosts such as horses, camels, sheep, and goats by mosquitoes, and larvae wandering in the brain and spinal cord are responsible for the neurological disease known as *kumri* (lumbar paralysis) in Asia. The migrating larvae apparently cause little or no damage in the natural host. The location of the lesions is variable, as are the clinical signs produced. Characteristically, the neurological signs are ataxia, weakness, or paralysis. The severity of the signs varies from slight weakness to quadriplegia, depending on the number and location of the wandering parasites; however, affected animals may remain bright and alert. The CNS lesions produced are fundamentally traumatic, and lead to microcavitation, as described below.

Halicephalobus gingivalis *(Micronema deletrix)* is a free-living nematode that is accidentally, but rarely, a parasite; this nematode is characterized by a rhabditiform esophagus. Massive intracranial invasion is reported in horses. The syndrome is acute and of short

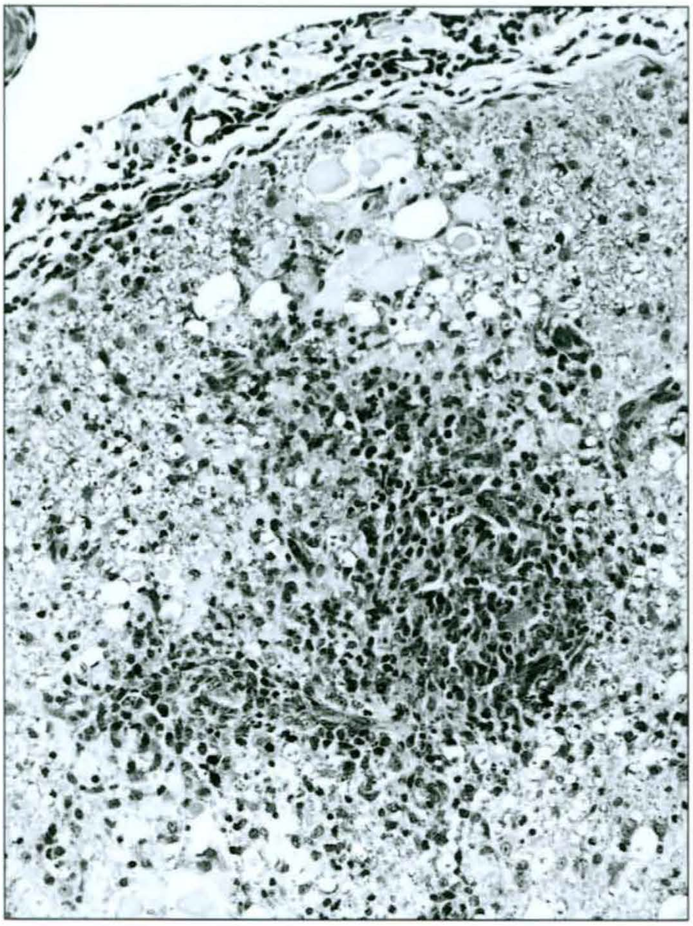

Figure 3.134 Granuloma formation, Wallerian degeneration, and spinal meningitis due to ***Parastrongylus*** infestation in a dog.

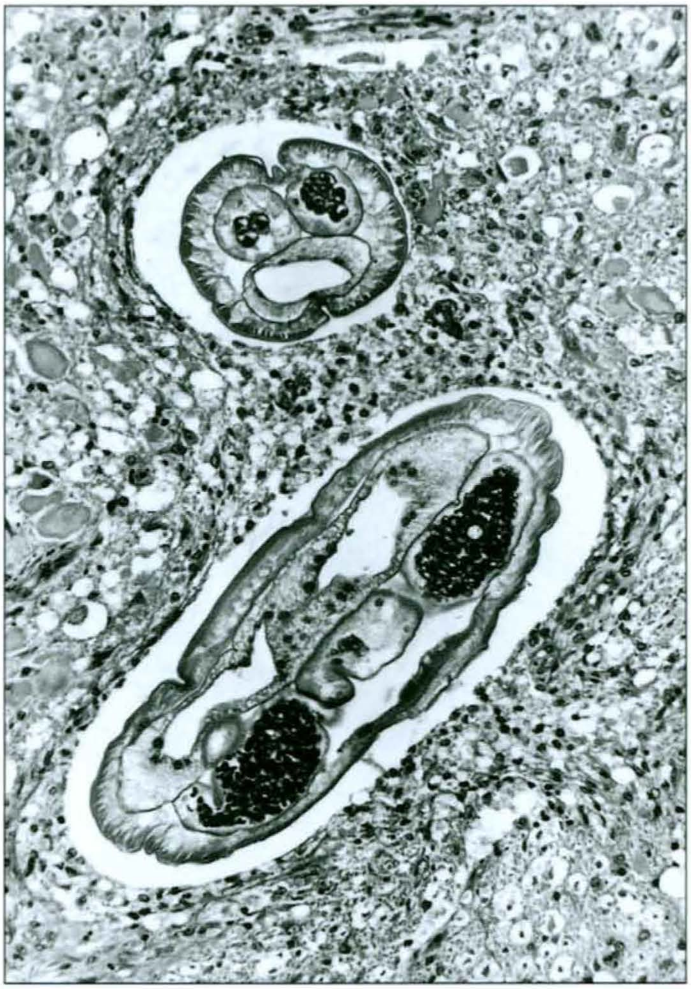

Figure 3.135 ***Parelaphostrongylus*** in spinal cord of a sheep.

duration. There are focal arachnoid hemorrhages and patchy meningeal thickenings. Only parthenogenetic female worms and larvae are found among the specimens in the brain, most easily in perivascular spaces (Fig. 3.136). Depending on the area of the CNS affected, lesions are granulomatous and eosinophilic meningoencephalitis, myelitis, polyradiculitis, or even cauda equine neuritis-like lesions. Parasitic granulomas in the kidney and gingiva may accompany the cerebral invasion. Little is known about the life cycle and method of transmission of this nematode. Oral ingestion or wound contamination then hematogenous distribution is suggested. Also, transmission from infected dam to her foal through milk is described in one case.

Gurltia paralysans, found in the spinal veins of cats, is reported to be responsible for a high incidence of paralysis in this host, and ***Angiostrongylus vasorum*** has caused hemorrhagic malacia in the brains of dogs. Aberrant hosts can develop severe cerebrospinal nematodiasis when they incidentally ingest the eggs of ***Baylisascaris procyonis*** (raccoon ascarid) or *Baylisascaris columnaris* (skunk ascarid).

Larval worms may also migrate aberrantly in the CNS of their natural hosts. ***Stephanurus dentatus*** quite frequently invades the spinal canal and may even encyst in the meninges in pigs. ***Strongylus* spp**. occasionally invade the brains of horses; Figure 3.137 shows the type of lesions that occur, and, although not identified in this case, the larvae were probably of *S. vulgaris* because these were identified in thrombi in the aortic bulb and carotid artery. **Ascarids** have a propensity for wandering in the brain of alien hosts and occasionally do so in their natural hosts.

Trematodes

Trematodes apparently have little tendency to invade nervous tissue. ***Troglotrema acutum*** may invade the brain from its normal habitat in the paranasal sinuses. The eggs of the lung flukes, ***Paragonimus* spp**., have been observed in the brains of dogs, possibly arriving there as emboli.

Arthropods

The only larval arthropods of interest are ***Hypoderma bovis***, which normally migrates through the spinal canal, and ***Oestrus ovis***, which may invade the brain from the nasal sinuses.

Cuterebra spp. (larva of a rodent or rabbit bot) in an abnormal host, i.e., cat and to a lesser extent dog, can undergo aberrant migration and has been reported in many organs including the eyes and CNS. Adult *Cuterebra* are nonparasitic and are seldom observed. Lesions in the brain are characteristic and indicative of vascular compromise and direct toxicity by toxin released from the larvae. These lesions include superficial laminar cerebrocortical necrosis, cerebral

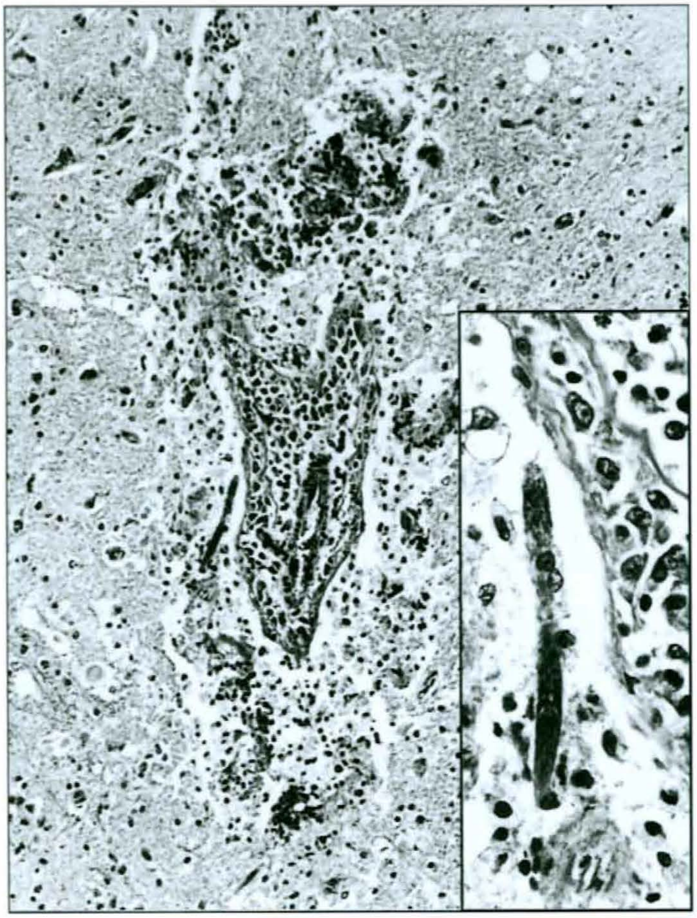

Figure 3.136 Perivascular inflammation in the brain of a horse caused by ***Halicephalobus gingivalis*** (*Micronema deletrix*). Inset: parasite with cellular reaction.

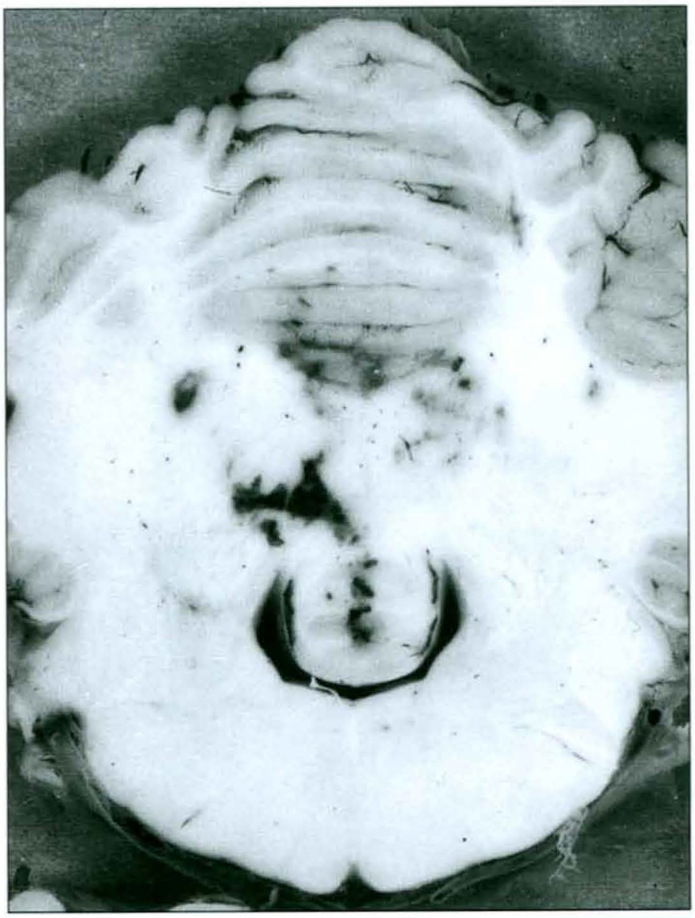

Figure 3.137 Hemorrhagic tracks in cerebellar white matter probably produced by ***Strongylus vulgaris*** in a horse.

(particularly at the olfactory bulbs and peduncles) and subependymal malacia and infarction, and finally larval migratory track lesions that are characterized by focal necrosis, hemorrhage, and infiltration of eosinophils, lymphocytes, plasma cells, and fewer neutrophils. Most of the track lesions are present in caudate nucleus or thalamus. Cuterebral larval migrans in the feline brain is thought to be the cause of *feline ischemic encephalopathy*.

The few parasites specifically mentioned are the most important in terms of neuropathology. Occasionally helminth larvae are discovered accidentally but rarely identified in sections of brain or cord, and it is somewhat more common to find lesions typical of those produced by migratory parasites without being able to locate the parasite. Some parasites, such as *Elaphostrongylus*, usually remain in the CNS whereas others, such as ascarids and strongyles, can be expected to keep moving. Finding the parasite is, therefore, largely a matter of luck, even when it is sought very early after the onset of clinical signs.

The lesions produced in nervous tissue by migratory larvae are mainly *malacic* and, although random, are fairly distinctive in their pattern. *Coenurus cerebralis* produces, in the invasion phase, purulent meningoencephalitis and later acts as a space-occupying lesion, but other invading parasites produce mainly traumatic lesions with very little inflammatory reaction except for a few eosinophils. The lesions produced by nematodes are sometimes grossly visible as *hemorrhagic foci or narrow, slightly tortuous tracks*. Brown, hemorrhagic discoloration depends on the parasite hitting a vein or arteriole, and it appears that some worms have a tendency to migrate along veins. There may be only one or several such tracks in the CNS, and they occur quite at random. Microscopically, the lesion is an irregular focus or pathway of traumatic malacia into which some hemorrhage may have occurred. There may be slight cellular infiltration in the adjacent meninges or nerve roots. The track is liquefied, and its margins not sharp, and apart from lymphocytes, gitter cells, and a few eosinophils, there is no significant reaction in the damaged tissue or in the adjacent vessels. The disruption, which is not selective in the tissues destroyed, leads to *microcavitation*. The disrupted axons, swollen, tortuous and as globose fragments, persist for some time in the microcavitations (Fig. 3.134). Gemistocytic astrocytes may be present in older lesions.

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Chlamydial disease

Sporadic bovine encephalomyelitis (SBE) occurs in calves less than 6 months of age, and is caused by ***Chlamydophila pecorum***. Identical disease is also reported due to infection with *Chlamydophila psittaci*. *Chlamydophila pecorum* also causes a wide range of other conditions in calves, including polyarthritis, metritis, conjunctivitis, and pneumonia. The disease occurs in the USA, Japan, Europe, and Australia; the agent probably has a worldwide distribution and most infections are asymptomatic. Encephalomyelitis is reported to occur naturally only in cattle and buffalo. As a rule, SBE is indeed sporadic, affecting only a few animals in a herd, however outbreaks of the disease with morbidity of 25% are described. Transmission appears to be by direct contact. The clinical syndrome is not particularly characteristic. It is composed of moderate fever and signs of catarrhal inflammation of the respiratory tract. There is some stiffness, weakness of the hindlimbs with staggering and knuckling of the fetlocks, and muscle tremors. There is some dullness; signs of excitement are not present. Death occurs in a few days to a few weeks.

The organism has a tropism for blood vessels, mesenchymal tissue and serous membranes, which make *vasculitis and polyserositis the hallmark of lesions*. Encephalitis is secondary to vascular damage. The gross morbid change that suggests a diagnosis of SBE is serofibrinous inflammation of serous membranes and synoviae. This is most consistently *peritonitis*, and in ~50% of fatal cases there is also *pleuritis and pericarditis*. The meninges appear congested and edematous and occasionally are covered with a few fibrin tags. Microscopically, there is a rather *severe and diffuse meningoencephalomyelitis* (see Fig. 3.60). The leptomeningitis is most severe about the base of the brain. The reactive cells are almost solely histiocytes and plasma cells, with only a few neutrophils. These cells infiltrate the meninges and perivascular spaces and mix with reactive adventitial cells of the vessel walls. The vascular endothelium proliferates secondary to lesions in the vascular walls, and ischemic changes may occur in the parenchyma. Reactive microglial nodules are widespread in the brain.

Cell culture of *Chlamydophila*, the gold standard diagnostic tool, is being displaced by detection by PCR. Elementary bodies produced by this organism occur in the cytoplasm of mononuclear cells in the exudates in the meninges and from serosal membranes and in microglia of nodules, but they are not numerous and their demonstration by special stains or immunohistochemistry is not usually rewarding.

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Idiopathic inflammatory diseases

Necrotizing meningoencephalitis of Pug and other small breed dogs

Canine necrotizing meningoencephalitis (NME), formerly "Pug dog encephalitis," is an idiopathic disease affecting mainly Pug dogs, but also reported rarely in Maltese, Pekingese, Shih Tzu, and Chihuahua. A similar condition, but with lesions more prevalent in the white matter, is reported in Yorkshire Terriers (distinct from *Yorkshire Terrier necrotizing encephalopathy*, which affects brain stem), named necrotizing leukoencephalitis. The cause of NME is unknown; several etiologic agents have been suggested as causes, including alpha-herpesvirus, but none has been confirmed. An autoimmune reaction against canine brain tissue has been suggested as a possible mechanism. A wide age range is affected. Generalized convulsions and their aftermath dominate the clinical picture, which may include lethargy, ataxia, and progression to coma. The clinical signs refer essentially to cortical disease that progresses rapidly over a few weeks, but which may extend to several months.

The lesions are particularly in the cerebral cortex, and are bilateral but asymmetric, often confluent over large areas, and extend to the adjacent white matter with relative sparing of the deeper periventricular tissue. The geography of the lesions therefore helps to distinguish this disease from other encephalitides of the dog. Grossly, localized swellings in the cerebrum contribute to asymmetry, and malacic foci may be seen as typical yellow areas of softening (Fig. 3.138) or, in cases of longer duration, as tiny cystic cavities.

The histological changes are necrotizing and with an affinity for the hemispheres. Numerous foci of meningitis, characterized by infiltrations of lymphocytes, plasma cells, and monocytes (Fig. 3.139), diminish caudally and may be absent in the caudal fossa and spinal cord. These infiltrates breach the pial barrier and destroy the superficial cortex to an extent and severity that is unusual. The evidence of

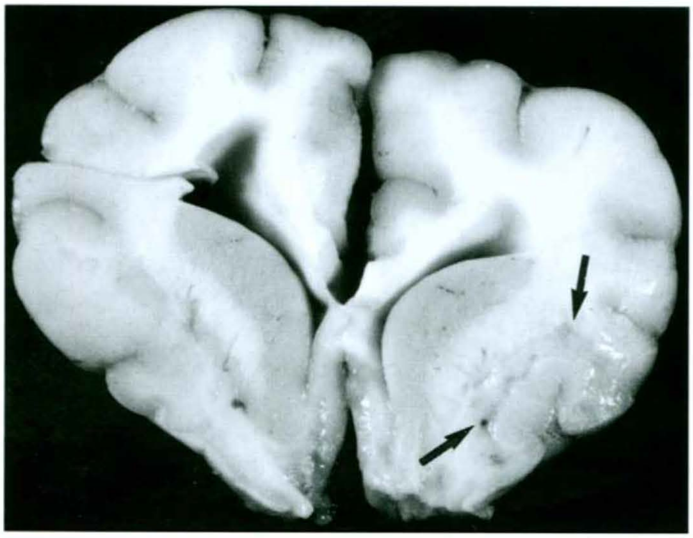

Figure 3.138 Malacic focus in ventrolateral cerebral hemisphere (arrows) in **Canine necrotizing meningoencephalitis**. (Reprinted with permission from Bradley GA. Vet Pathol 1991;28:91.)

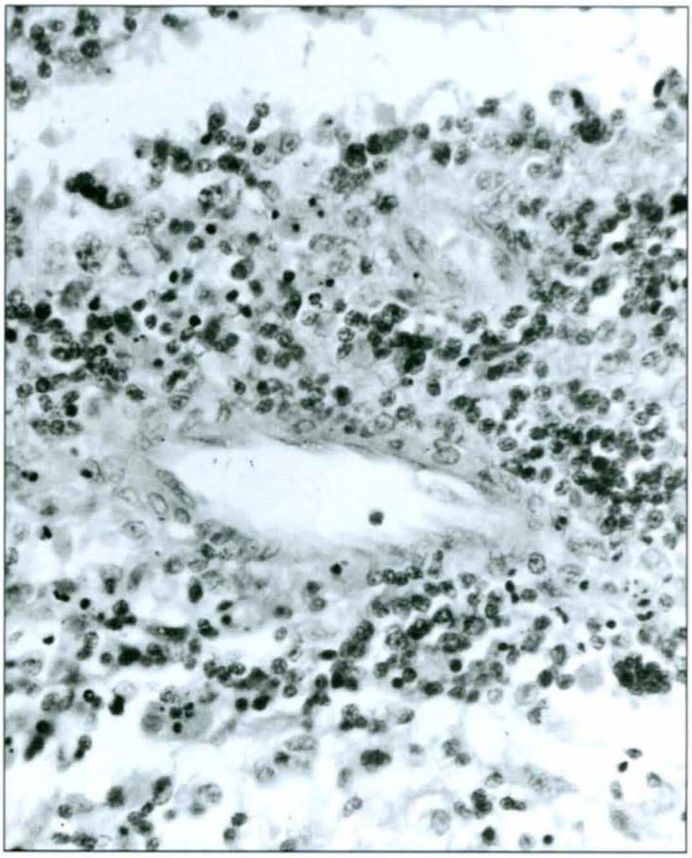

Figure 3.139 Meningeal arteriole surrounded by mixed mononuclear infiltrate in **Canine necrotizing meningoencephalitis**.

cerebral necrosis extends from selective neuronal necrosis to areas of malacia, the latter especially in chronic cases. Vascular endothelium in the cortex is reactive and associated with edema, occasional petechiae, and diffuse accumulation of mononuclear cells in parenchyma and vascular cuffs.

The differential features of this meningoencephalitis, in addition to its nonsuppurative nature, are the malacic degenerations and predilection for the cerebral cortex. This condition must be differentiated also from granulomatous meningoencephalomyelitis (GME). The inflammatory infiltrate in GME contains more histiocytes, which in the chronic stage transform to epithelioid cells that can form discrete cohesive sheets or granuloma-like lesions. Also, in GME, the reaction is predominantly in the white matter and is distributed in almost all parts of the CNS. In contrast, the reaction in NME is mostly in the cortical gray matter.

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Granulomatous meningoencephalomyelitis

Granulomatous meningoencephalomyelitis (GME) is a sporadic disease of the CNS of dogs. GME appears to have a worldwide distribution and to occur mostly in *young to middle-aged dogs of small breeds*, e.g., terriers and toy breeds, however the disease can occur in any breed and in an age range of 6 months to 12 years. The cause of GME is unknown; several infectious causes have been suggested but not confirmed to be the cause of this condition. Based on the predominance of CD3+ T cells and MHC class II antigen-positive macrophages, an immune-mediated mechanism has been proposed.

Variations in the distribution and extent of the lesions result in a variety of clinical signs. Spinal lesions may be associated with ataxia, paresis, or paralysis (Fig. 3.140). Lesions in the brain stem frequently produce signs of vestibular dysfunction. Changes of behavior, forced movement and circling, depression and convulsions occur with supratentorial lesions. Macroscopic lesions, if evident, consist of gray-white discoloration of the white matter of the brain or spinal cord, and in those cases in which the cellular aggregations become confluent, there may be irregular areas of malacia (Fig. 3.141).

The histologic changes are patchy in distribution. There may be very few foci, or they may be disseminated, or they may be localized to one area and confluent, or there may, in the same animal, be both confluent and disseminated distributions. *The essential histologic feature is perivascular aggregation of cells rather selectively in the white matter.* The minimal lesion is cuffing of vessels by lymphocytes and plasma cells with small eccentric clumps of macrophages. The macrophages increase in number and may come to comprise the cuff, appearing as discrete granulomas, which, depending on the plane of section, may appear to be in the parenchyma. Occasional mitoses are present in these cells. Transformation to epithelioid cells occurs later. The perivascular aggregates expand in concentric arrangements and displace surrounding parenchyma (Fig. 3.142). Where the cuffing response is severe, edema and necrosis may occur in the adjacent white matter leading to a spillover of mononuclear cells into the parenchyma and to the usual reactive changes. Large malacic foci are unusual. In cases of prolonged duration, confluence of lesions occurs and reparative responses include the deposition of abundant reticulin and collagen in perivascular arrangements. Involvement of meninges is patchy and often related to lesions of white matter directly underlying.

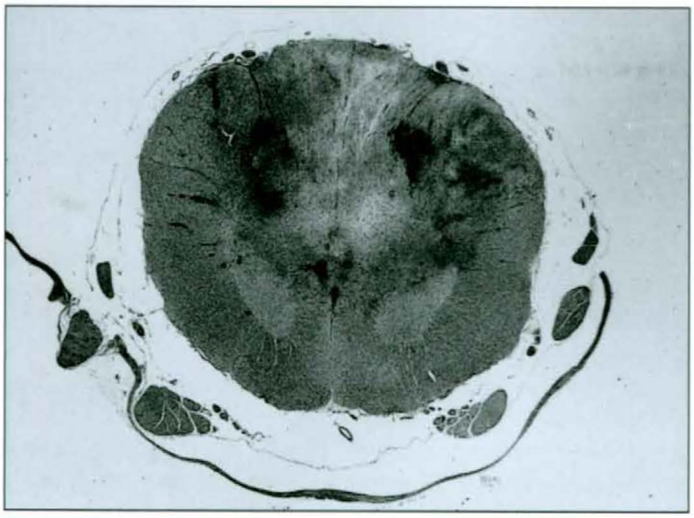

Figure 3.140 Focal lesions in the spinal cord of a dog with **granulomatous encephalitis**. (Courtesy of JB Thomas.)

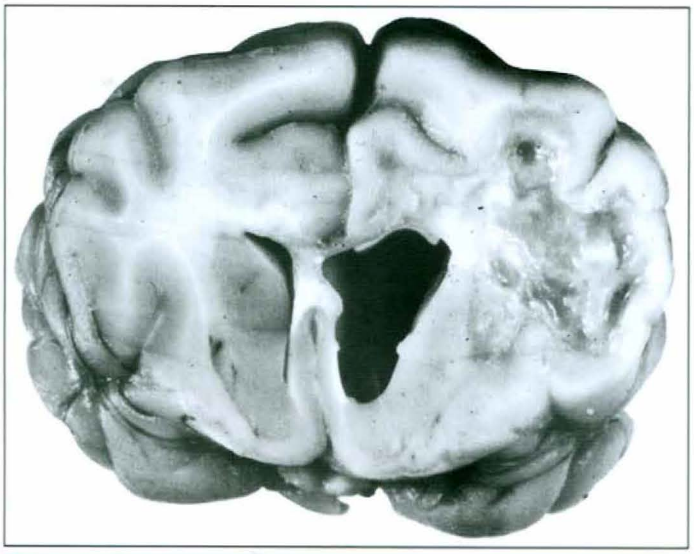

Figure 3.141 Malacia of cerebrum and hydrocephalus secondary to infarction in a dog with **granulomatous encephalitis**.

Cytological characterization of the cell aggregates can be difficult. In many, and perhaps most, cases, the cells are easy to classify, being well differentiated as lymphocytes, monocytes, plasma cells, and histiocytes (Fig. 3.143). Granulocytes may be present but are not numerous, and histiocytes often show epithelioid transformation and may form small syncytial masses. There may also be in some cases large immature cells of reticulohistiocytic type in which mitoses may be few or many. Differentiation between GME and brain malignant histiocytosis (BMH) can be problematic occasionally; BMH is frequently part of a multisystemic tumor and rarely occurs in isolation. Also, the absence of cellular atypia and the predominance of perivascular orientation should favor the diagnosis of GME.

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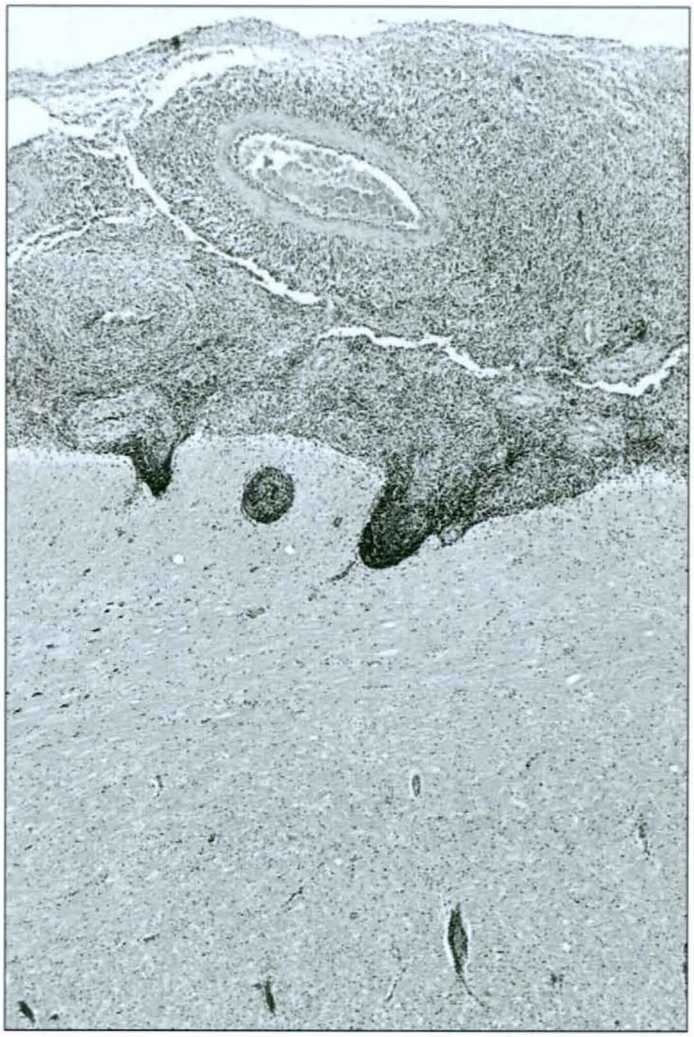

Figure 3.142 Dense perivascular meningeal infiltrate in **granulomatous encephalitis** in a dog.

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Acute polyradiculoneuritis (coonhound paralysis)

Acute polyradiculoneuritis, or polyradiculoneuropathy, affects primarily dogs, occasionally cats, and rarely horses, and has many similarities to Guillain-Barré syndrome in humans. The condition was named coonhound paralysis to reflect the fact that some affected dogs had been bitten or scratched by raccoons, although cases may have no known exposure to raccoons. Within 7–10 days, ascending flaccid paralysis, starting in the hindlimbs and progressing cranially to involve the forelimbs, leads to quadriplegia and rapid atrophy of muscle. There are no cerebral signs. Some dogs die of respiratory paralysis, but most will recover slowly if nursing is adequate. Dogs that have

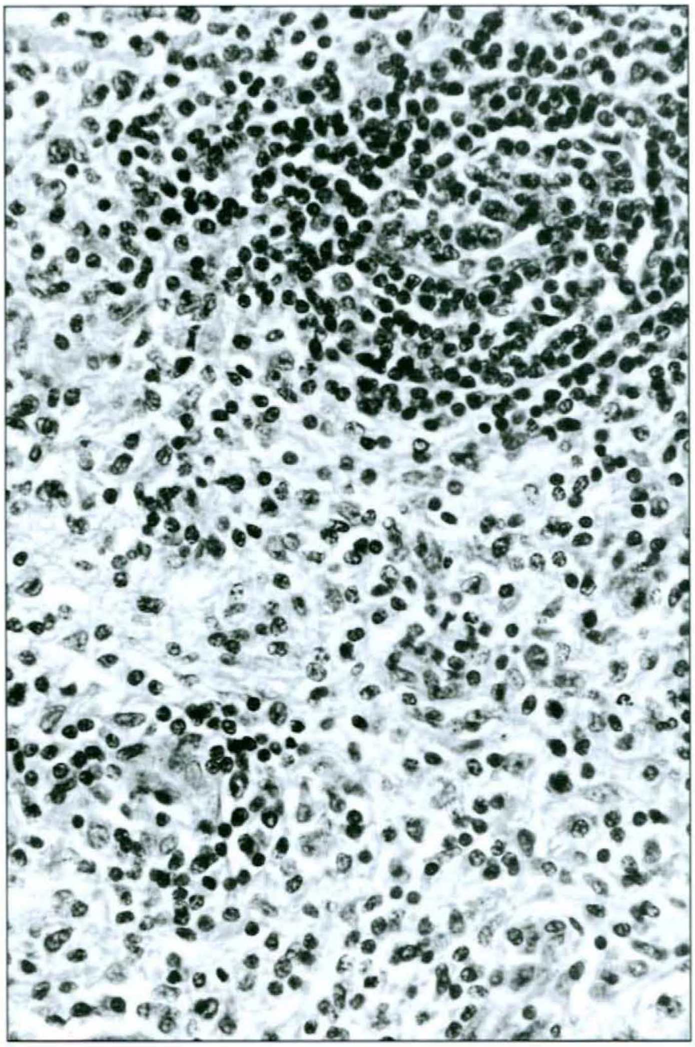

Figure 3.143 Detail of cellular infiltrate in brain in **granulomatous encephalitis** in a dog.

recovered appear to have increased sensitivity to subsequent exposure but may survive several bouts of paralysis. The disease has been transmitted using pooled saliva of raccoons, but the cause has not been identified; an autoimmune mechanism is suspected. Lesions are found in the *ventral roots of spinal nerves and in peripheral nerves*. Mononuclear and plasma cells infiltrate around venules, but the extent of the infiltrate is variable and not correlated with the course of the illness or the severity of nerve degeneration. There is primary and Wallerian degeneration afflicting ventral roots in particular, with axonal reaction in motor neurons and atrophy of denervation in muscle. This idiopathic polyradiculoneuritis is the most common inflammatory condition of peripheral nerves in dogs.

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Neuritis of the cauda equina

Neuritis of the cauda equina of horses is a polyneuritis in which the presenting signs are referable to the sacrococcygeal nerves and include perineal anesthesia, tail paralysis, urinary incontinence, fecal retention, weakness, atrophy of coccygeal muscles and, in longstanding cases, atrophy of the muscles of the hindlimbs. The neuritis is progressive. The cause is unknown, but the nature of the reaction suggests immune mediation, which may follow viral infection. The condition has been compared to experimental allergic neuritis in laboratory animals. *Halicephalobus gingivalis* is reported as a novel cause of cauda equina neuritis in one horse.

Although the pathologic changes emphasize the *sacral and caudal spinal nerve roots*, there may simultaneously be asymmetric pareses of other nerves producing isolated limb pareses, and paresis or paralysis referable to cranial nerves. The lesions in these other nerves are similar in character to, but much milder than, those in caudal nerve roots. Inflammatory changes are present in sensory and some autonomic ganglia, but changes in the spinal cord are limited to those that reflect peripheral nerve injury.

The gross changes affect in particular the extradural parts of the sacral and coccygeal nerves and may extend through the intervertebral foraminae into the adjacent muscle. The roots are thickened and fusiform and usually discolored by recent or old hemorrhage. The intradural segments of affected nerves are discolored but not usually enlarged.

Microscopically there is *granulomatous inflammation with extensive fibrosis*. The thickening and discoloration are attributable to hemorrhage, proliferation of epineural tissue, and inflammatory cell infiltrates. The infiltrating lymphocytes, plasma cells and macrophages are frequently disposed as to form granulomas, often with central epithelioid and giant cells; granulocytes do not feature in the infiltrates. Degenerative and regenerative changes are present in the myelin and axons of affected roots and appear to be more closely associated with endoneurial and perineurial fibroplasia than with leukocytic infiltrates.

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Steroid-responsive meningitis-arteritis (Beagle pain syndrome)

Steroid-responsive meningitis-arteritis (SRMA) is a *polyarteritis of possible immune-mediated origin affecting mainly small to medium-sized leptomeningeal and myocardial arteries*, but arteries in many organs can be affected. Beagles, especially those in laboratory-bred colonies, are at high risk, but the disease is reported in Boxers, German Shorthaired Pointers, Nova Scotia Duck-tolling Retrievers, and rarely in other breeds. Affected dogs have fever, hyperesthesia, severe pain on manipulation, cervical rigidity, and anorexia. The clinical course of SRMA is usually acute, but a chronic form exists. Gross lesions are minimal, but areas of subarachnoid hemorrhage may be present along the brain stem and the spinal cord, especially the cervical part. Histologically, small to medium-sized leptomeningeal arteries, especially of the brain stem and spinal cord, and to a lesser extent heart and cranial mediastinum, have moderate to severe perivascular and transmural infiltrates of lymphocytes, plasma cells, histiocytes, and fewer neutrophils. Occasionally, neutrophils predominate. Also, there is severe fibrinoid necrosis, thrombosis, and occasionally periarterial fibrosis. Mild lymphocytic and histiocytic leptomeningitis is also a constant finding.

Distinct from SRMA, an unusual case of idiopathic vasculitis resembling "*isolated angiitis of the CNS*" in humans has been reported in a mixed-breed dog. The necrotizing vasculitis, with cuffs of mixed cell types including multinucleated giant cells, resulted in localized cerebral necrosis.

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Shaker dog disease

Shaker dog disease is an idiopathic condition characterized clinically by *tremors* that worsen after stress or excitement. The condition was described first affecting solely young adult white-haired small dogs (little white shakers), but now is recognized in dogs of any size or coat color. Histologically, *mild diffuse, nonsuppurative encephalomyelitis* is present. No myelin disease is present.

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Sensory ganglioneuritis (sensory ganglioradiculitis)

Sensory ganglioneuritis (sensory neuronopathy) is a rare idiopathic disease of adult dogs characterized by *nonsuppurative inflammation of dorsal root (sensory) spinal ganglia and cranial sensory ganglia* with degeneration and necrosis of sensory neuronal cell bodies and proliferation of ganglionic satellite cells. The cause may be a cell-mediated immune mechanism. Secondary to sensory ganglion neuronal injury, Wallerian degeneration develops in the dorsal funiculi (fasciculus cuneatus and fasciculus gracilis) and the affected areas appear grossly as white V-shaped or triangular areas throughout the entire length of the spinal cord. Similar but milder lesions can be present in sympathetic ganglia, peripheral nerves, myenteric plexi, motor roots, and the spinal tract of the trigeminal nerve. Breed or sex predilection have not been observed. Clinical signs are variable and include generalized sensory ataxia, depression or absence of spinal reflexes, facial hypalgesia/paresthesia, megaesophagus, and dysphagia. Masticatory muscle atrophy is observed in a few dogs in association with this condition and is attributed to loss of motor fibers as they course through the trigeminal ganglion.

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Post-infectious encephalomyelitis

Neurological disease that follows, after a variable period, common viral infections or vaccination exposure is well known in children as post-infectious or post-vaccinal encephalitis, or acute disseminated encephalomyelitis. The common pathologic basis is a *demyelinating inflammatory process dominated by mononuclear inflammatory cells with a distinctive perivenous distribution*. Examples that meet the criteria have occurred in relation to rabies vaccination in dogs when such vaccines were prepared in neural tissue, and the pathologic process is the same as that in experimental allergic encephalomyelitis. The histologic changes are widely distributed in the brain and cord and affect mainly the white matter. Perivascular infiltrates of lymphocytes, plasma cells, and monocytes/macrophages widely distend the space and spread into the surrounding parenchyma. Proliferation of adventitial cells may be prominent. Although much descriptive emphasis has been given to demyelination, this is restricted to the perivascular areas of infiltration and to surrounding areas showing the usual degenerative and reactive changes.

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Eosinophilic meningoencephalitis

Recognized causes of eosinophilic meningoencephalitis include nematode and protozoan parasites. **Idiopathic eosinophilic meningoencephalitis** has been reported in dogs, with Rottweilers and Golden Retrievers over-represented, and one cat. The condition is characterized clinically by behavioral changes such as inappropriate urination and lack of response to commands. In severe cases, episodes of sternal or lateral recumbency without loss of consciousness are described. Clinical pathology findings are mild to moderate peripheral blood eosinophilia and CSF pleocytosis with predominance of eosinophils. Grossly, there is thickening and green discoloration of the meninges. Histologic changes are those of eosinophilic and granulomatous meningitis of the cortex and cerebellum, and the underlying neural parenchyma appears pallid, occasionally spongiotic, and has mild eosinophilic and histiocytic cuffing with rare areas of axonal swelling and neuronal degeneration. Spinal cord lesions are poorly documented. The condition is usually responsive to steroid treatment, suggesting an immune-mediated mechanism.

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Granulomatous radiculitis of the seventh and eighth cranial nerves of calves

This is a rare condition affecting young calves and characterized clinically by facial paralysis and pathologically by the presence of multifocal granulomas affecting mainly the roots of cranial nerves VII and VIII. The etiology is unknown; however, *Mycoplasma bovis* was suggested as a cause because some affected calves had concurrent mycoplasmal otitis media.

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NEOPLASTIC DISEASES OF THE NERVOUS SYSTEM

Primary neoplastic disease of the CNS of animals is rare in species other than the dog and cat. Neoplasms derived from virtually every cell type in the nervous system are recorded.

The tumors may be *congenital*, and these do not differ in their characteristics from similar tumors in adult animals with the possible exceptions of: medulloblastoma, which is thought to be derived from the residual cells of the external granular layer of the cerebellum; craniopharyngiomas, which are thought to arise from remnants of Rathke's pouch, which forms the adenohypophysis; chordoma, which is thought to arise from remnants of notochord; intracranial teratoma, which is probably derived from germ cells; and cystic epidermoid tumors, which are thought to result from the inclusion of surface ectodermal cells at the time of closure of the neural groove. The relatively high incidence of tumors in Boxers and Boston Terriers may indicate a hereditary predisposition, which these breeds also have for endocrine neoplasia. The introduction of computerized tomography (CT) and magnetic resonance imaging (MRI), in addition to the use of immunohistochemistry, have improved our understanding of CNS tumors in various domestic animals and have helped in reaching more accurate diagnoses. Neoplasms of the CNS rarely metastasize to extraneural tissue, and their clinical importance depends on their destructive effect on host neural tissue and the resulting neurologic deficits.

The conventional tumors of the CNS are discussed here and classified according to the current histological classification published by the Armed Forces Institute of Pathology (AFIP) in cooperation with the World Health Organization (WHO). Tumors of the pituitary and nonchromaffin paraganglia are discussed with the Endocrine system.

Tumors of neuroepithelial tissue

Astrocytoma

Astrocytoma is the most common primary intracranial tumor (Fig. 3.144A–E). Astrocytomas are found most commonly in dogs, but are reported also in cats and cattle, and may occur in the brain or cord but are more prevalent in cerebrum, thalamus, hypothalamus, and midbrain. In dogs, astrocytomas are common in brachycephalic breeds but can occur in any breed. Astrocytomas have no age predilection, but are more prevalent in middle-aged or older dogs. The gross appearance varies, depending largely on the degree of malignancy. These tumors can be very difficult to detect grossly, especially when they involve white matter or grow slowly. They are then white and, because of their firmness, may be more readily palpable than visible. Their presence may be suspected only by deviation of some architectural feature (Fig. 3.144B, D). Larger and more malignant tumors are prone to vascular accidents and necrosis, and they are then easy to see (Fig. 3.144E), but the margins are never discrete, especially not when they are surrounded by edematous tissue. The extent of the tumor is always much greater than can be appreciated grossly.

Histologically, these tumors are very diverse and are classified as *low-grade astrocytoma* (well differentiated), *medium-grade astrocytoma* (anaplastic), and *high-grade astrocytoma* (glioblastoma).

Low-grade astrocytoma appears as an unencapsulated expansile and subtly invasive mass that replaces pre-existing tissues and has low to moderate numbers of bland round to oval cells. In most cases, the neoplasm appears as an increased population of fibrous astrocytes that individually are not clearly malignant (Fig. 3.144A). Variants of this neoplasm include **fibrillary astrocytoma** (neoplastic cells have scant cytoplasm but abundant fibrillary processes and filaments), **protoplasmic astrocytoma** (neoplastic cells have scant cytoplasm and few short processes and filaments), **gemistocytic astrocytoma** (neoplastic cells have abundant acidophilic cytoplasm and eccentric oval to round nuclei; Fig. 3.144C). Another variant, the **pilocytic astrocytoma** (neoplastic cells are bipolar, elongated (piloid or hair-like) astrocytes and have few Rosenthal fibers), has been reported in both dogs and cats.

In **medium-grade astrocytoma**, the population is denser; the nuclei are a little larger and darker and show slight but definite variations in size and shape but no mitoses. The cells are recognizable as astrocytes. The walls of the vessels may be slightly thickened.

In **high-grade astrocytoma**, or *glioblastoma multiforme*, hemorrhage and necrosis are expected and the adventitial and endothelial cells of the vessels proliferate forming glomeruloid blood vessels. Generally, only a few cells are recognizable as astrocytes. Neoplastic cells have a tendency for pseudopalisading around necrotic areas. Pleomorphism, giant nuclei, and multinucleated giant cells are common. Mitotic figures are common and atypical.

Astrocytomas exhibit positive immunostaining for glial fibrillary acidic protein (GFAP), S-100 protein, and vimentin. The staining pattern for GFAP varies from sparse to abundant.

Oligodendroglioma

This is the easiest of the glial tumors to recognize even when growing rapidly. This tumor is reported in dogs especially in brachycephalic breeds and rarely in cats. Grossly, it usually appears well demarcated, being gray, soft, and almost fluctuating (Fig. 3.145A). Hemorrhage and necrosis occur but are unusual. The tumor is densely cellular with almost no stroma. The nuclei are remarkably uniform and like those of normal oligodendroglia in size and shape. The cytoplasm does not stain, but its membrane does, so that the nucleus seems to lie in a clear

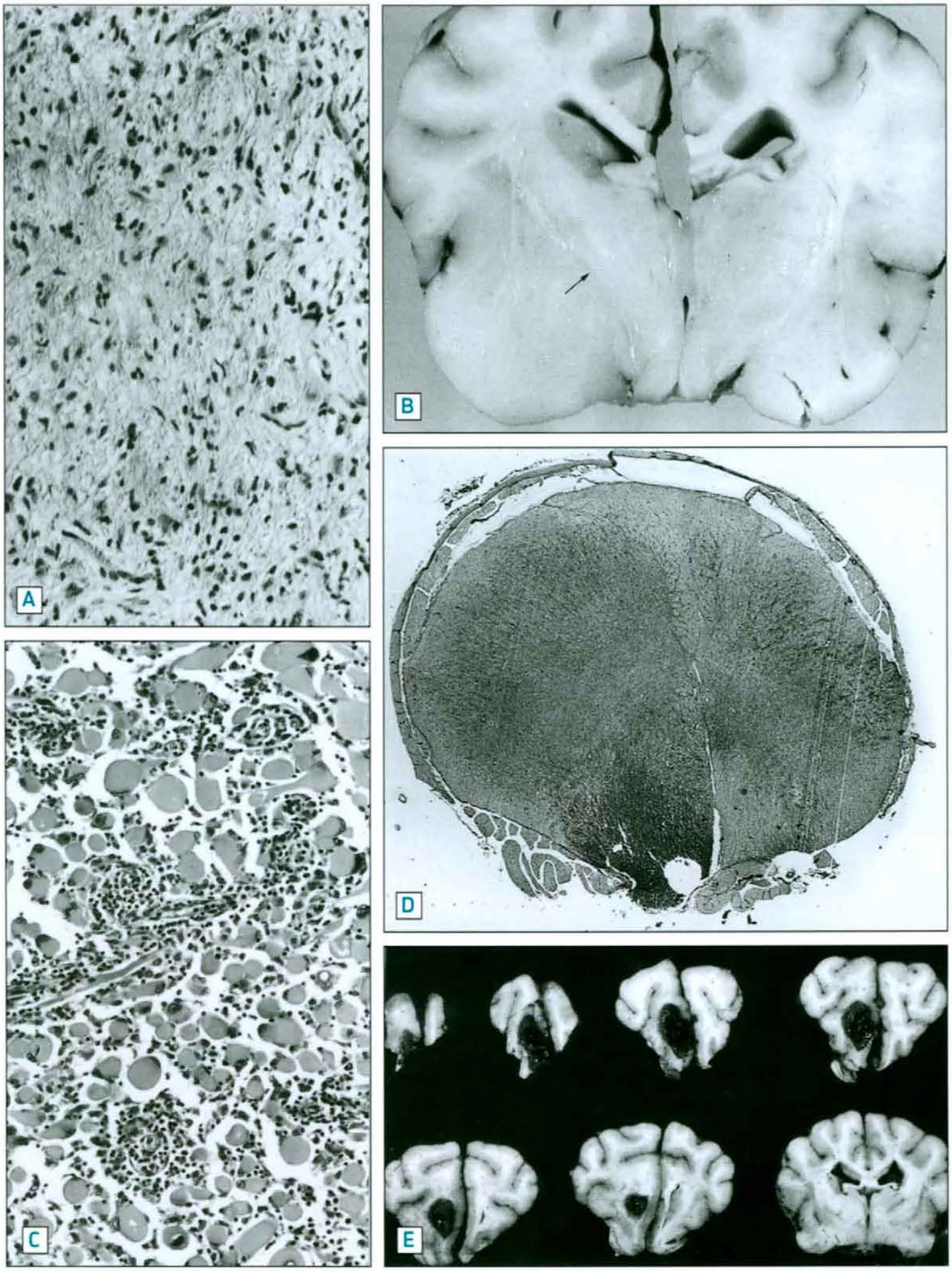

Figure 3.144 **A. Fibrous astrocytoma** in a dog. **B. Astrocytoma** in piriform lobe in a dog. Note lack of definition but displacement of internal capsule (arrow) by the homogeneous tumor. **C. Gemistocytic astrocytoma** in a dog. **D. Astrocytoma** of spinal cord in a dog. **E. Hemorrhagic astrocytoma** of left frontal lobe in a dog.

polyhedral or rounded halo (honeycomb cell pattern) (Fig. 3.145B). These tumors occur in white matter, and those near the third ventricle may contain areas distinguishable as astrocytoma or ependymoma. Blood vessels may proliferate especially at neoplasm margins to form glomeruli-like vessels. Mucinous degeneration and cyst formation may occur in these tumors and mineralization may occur in some of them. There are no clear indices of malignancy, although all must be regarded as malignant. Oligodendrogliomas do not stain with GFAP; however, the neoplastic cells are usually intermingled with some astrocytes that readily stain with GFAP.

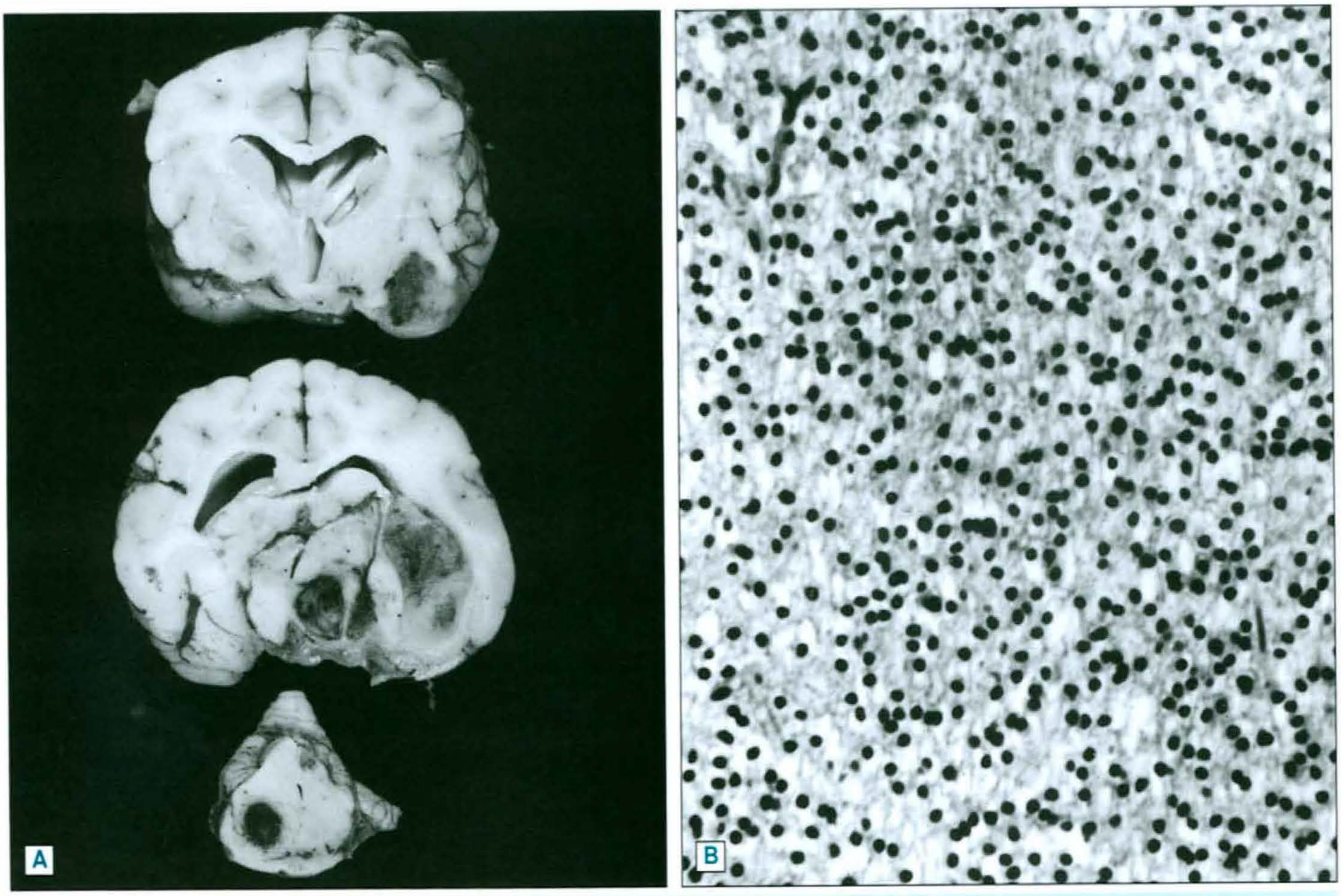

Figure 3.145 A. Oligodendroglioma involving right side of brain stem and piriform lobe in a dog. (Bottom section reversed to show caudal extension in brain stem.). **B.** Histologic pattern of oligodendroglioma.

Oligoastrocytoma (mixed glial tumor)

This glial neoplasm is composed of both neoplastic astrocytes and oligodendroglia.

Gliosarcoma

This rare glial tumor is composed of highly anaplastic glial cells with abundant sarcomatous components. Positive GFAP staining should differentiate this tumor from other spindle cell tumors, e.g., fibrosarcoma.

Gliomatosis cerebri

This is a diffuse infiltrating disease of dogs, predominantly of brachycephalic breeds, and humans characterized by an *infiltrative cell type reminiscent of astrocytes* rather than by the formation of a distinct tumor mass. The infiltrates involve the brain, often bilaterally but asymmetrically, with discontinuous areas also in the spinal cord. There is no tumor "mass," rather diffuse enlargement of affected regions, the cells insinuate among normal structures that remain intact with only slight damage to axons and neurons. Where the infiltrates involve the molecular layer of the cerebrum or the deep white matter, they spread in veils on leptomeninges and ependyma.

Gliomatosis cerebri is composed mainly of cells reminiscent of fibrillary astrocytic cells but there are also oligodendrocytes, cells of transitional character, and small unclassified cells (Fig. 3.146). The origin of the neoplastic cells in the canine cases is still controversial, as they do not stain with glial markers such as GFAP. The pattern of infiltration is unexplained, but involves participation of cell adhesion molecules.

Ependymoma

Ependymomas are neuroglial tumors derived from the lining epithelium of the ventricles and central canal of the spinal cord. Most arise about the third ventricle. They are gray and fleshy but may be dark from hemorrhage if they project into a ventricle. They are more prevalent in dogs and cats, but are reported in horses and cattle.

The tumors are usually densely cellular and those arising about the third ventricle may be difficult to distinguish from undifferentiated pituitary tumors. The nuclei are small, dark, and regular and the cytoplasm has no distinct boundaries. Pseudorosettes form around blood vessels, and are characterized by a perivascular nuclear-free zone. True rosettes are also present and appear as tubular cavities lined by cells of epithelial appearance. The cells forming true rosettes are bound together by desmosomes, have basally located nuclei, and

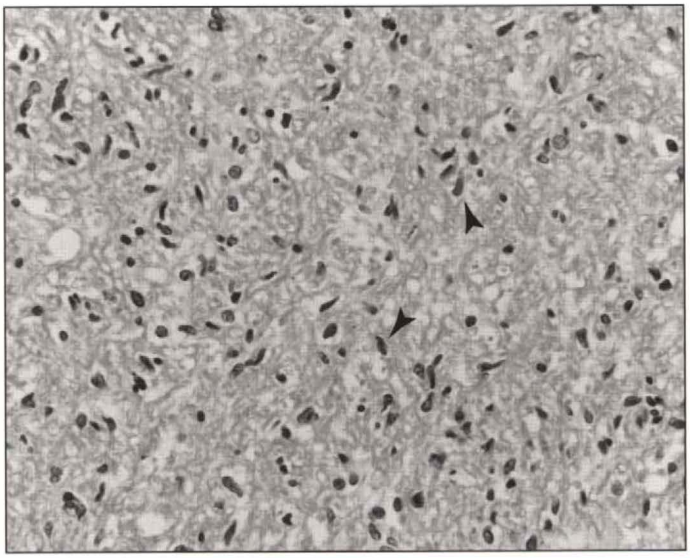

Figure 3.146 Gliomatosis cerebri appears as an increase in the cellularity of cerebrocortical white matter due to diffuse infiltration of neoplastic cells (arrowheads) that do not form a discrete mass or efface the pre-existing tissue (Boxer dog).

have surface cilia anchoring phosphotungstic acid hematoxylin (PTAH)-positive blepharoplasts. A **papillary variant** does exist and is characterized by branching papillary stroma covered by recognizable, usually ciliated, ependymal cells (Fig. 3.147). Ependymomas of the spinal cord may be more papilliferous than those in the brain, with tumor cells attached to fronds that are supported by a delicate stroma and embedded in mucinous intercellular stroma. A **clear cell variant** resembling oligodendroglioma is only reported in humans. **Malignant ependymoma** is characterized by increased cellular atypia and mitoses.

Most canine ependymomas stain negatively for GFAP; however, feline and equine cases are reported to stain positively for this marker. Most ependymomas show slight positive staining for vimentin and cytokeratin. Human ependymomas stain strongly with anti-epithelial membrane antigen antibody (EMA); however, information about immunoreactivity of domestic animal ependymomas to EMA is not available.

Choroid plexus tumors

These are rare, reported in cats, horses, and cattle, and occur with higher frequency in dogs. They may be papillomas or carcinomas. They are vascular papillary growths that implant widely on the meninges. The cells retain recognizable choroidal character (Figs 3.148, 3.149). According to cellular atypia and invasiveness, these tumors can be classified as papilloma or carcinoma. Internal or communicating hydrocephalus is a complication. They express epithelial markers, e.g., pancytokeratin, but not glial markers, e.g., GFAP.

Neuronal and mixed neuronal glial tumors

These tumors of adult animals are composed of neurons and/or neurons admixed with glial cells.

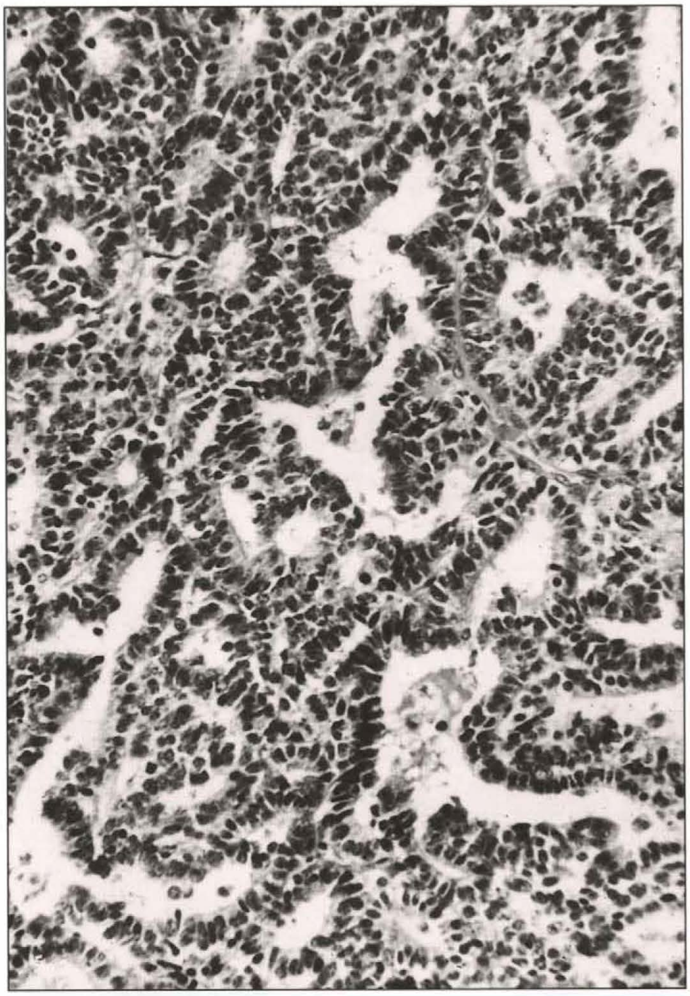

Figure 3.147 Typical branching papillary structure in an **ependymoma** in a dog.

Gangliocytomas

These are extremely rare tumors that are reported in dogs, a cow, and a horse. They appear to have a predilection for the cerebellum. The tumor is composed of cells reminiscent of large ganglion cells or mature pyramidal cells. Neoplastic cells do not stain with glial markers but show different reactivity with neuronal markers such as neuron-specific enolase (NSE) and synaptophysin.

Ganglioglioma

This is another rare bicellular tumor composed of neoplastic neuronal cells and neoplastic astrocytic cells. They have the same immunohistochemical staining pattern as gangliocytomas, but the astrocytic cells stain with GFAP.

Olfactory neuroblastomas (esthesioneuroblastoma)

This rare malignant tumor arises from primitive neurosensory cells present in the olfactory mucosa. They are uncommon but have been identified in various animal species, mainly dogs and cats. The olfactory epithelium is unique amongst neuronal structures in that the basal cells retain the ability to divide and differentiate to become

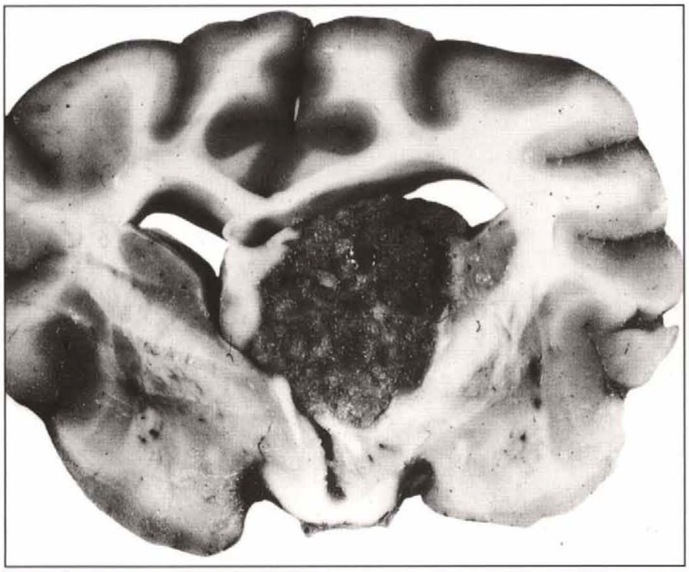

Figure 3.148 Choroid plexus papilloma in a dog.

sustentacular cells or bipolar neurosensory cells. The tumors are locally aggressive and may penetrate the cribriform plate. The dense cellular population is homogeneous, arranged in sheets or clusters but also forming true and pseudorosettes (Flexner-type rosettes and Homer Wright-type pseudorosettes). They may be palisaded on trabeculae, oval in shape with scant cytoplasm. Cytoplasmic processes may form an abundant, delicate fibrillary matrix. The detection of type C retroviral particles identified with feline leukemia in spontaneous olfactory neuroblastomas of cats is of interest. Neoplastic cells stain with different degrees of reactivity for both neuronal and epithelial immunohistochemical markers, e.g., NSE and cytokeratin respectively.

Embryonal tumors

These tumors arise from primitive or progenitor cells present in the nervous system and are capable of differentiating into different lineages including glial or neuronal cell lines.

Medulloblastoma (cerebellar primitive neuroectodermal tumor)

Medulloblastoma, well-known in children, is rare in animals, but occurs in the young of several species (Fig. 3.150), mainly calves and dogs. There is no such cell as a "medulloblast," the name invented for this tumor of unknown parentage. It is currently thought to arise from undifferentiated cells found in neonatal life beneath the cerebellar pia mater and thought to be the precursors of the cerebellar cortex. These tumors grow rapidly. Histologically, they are densely cellular with scant stroma and few vessels (Fig. 3.151A). The cells are small and classically "carrot-shaped" with oval or elongate nuclei and the cytoplasm tapering at one pole. They are also supposed to produce small perivascular palisades and pseudorosettes (Fig. 3.151B). These classical features are not always present, and then there is nothing by which this tumor can be identified except its location in the cerebellum. The term medulloblastoma should be limited to those embryonal tumors originated in the cerebellum. Tumors of this histological appearance in other sites in the brain of calves or other species are called *primitive neuroectodermal tumor* not medulloblastoma. Medulloblastomas stain positively for different neuronal markers including NSE and synaptophysin and some show positive staining for GFAP.

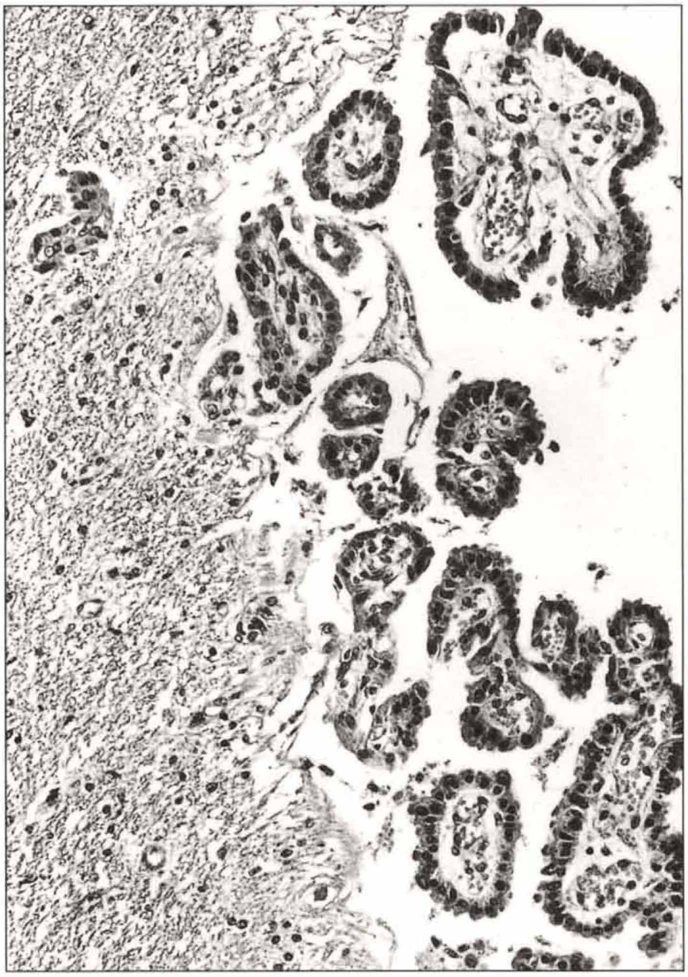

Figure 3.149 Invasion into the periventricular white matter of a dog by a **choroid plexus carcinoma**.

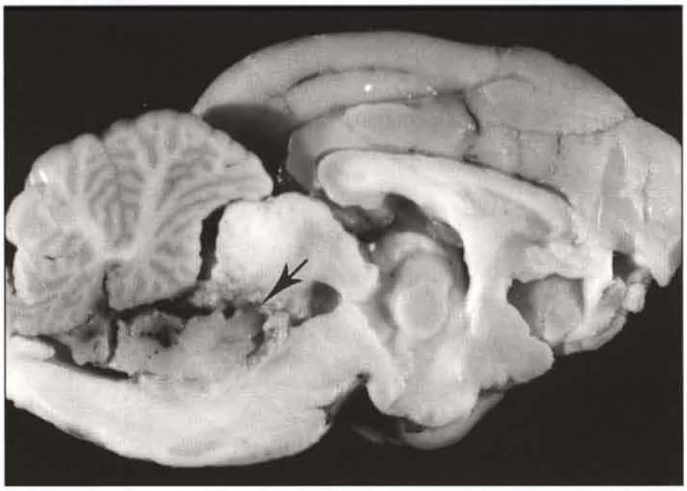

Figure 3.150 Medulloblastoma between cerebellum and fourth ventricle with extension into colliculus (arrow) in a cat.

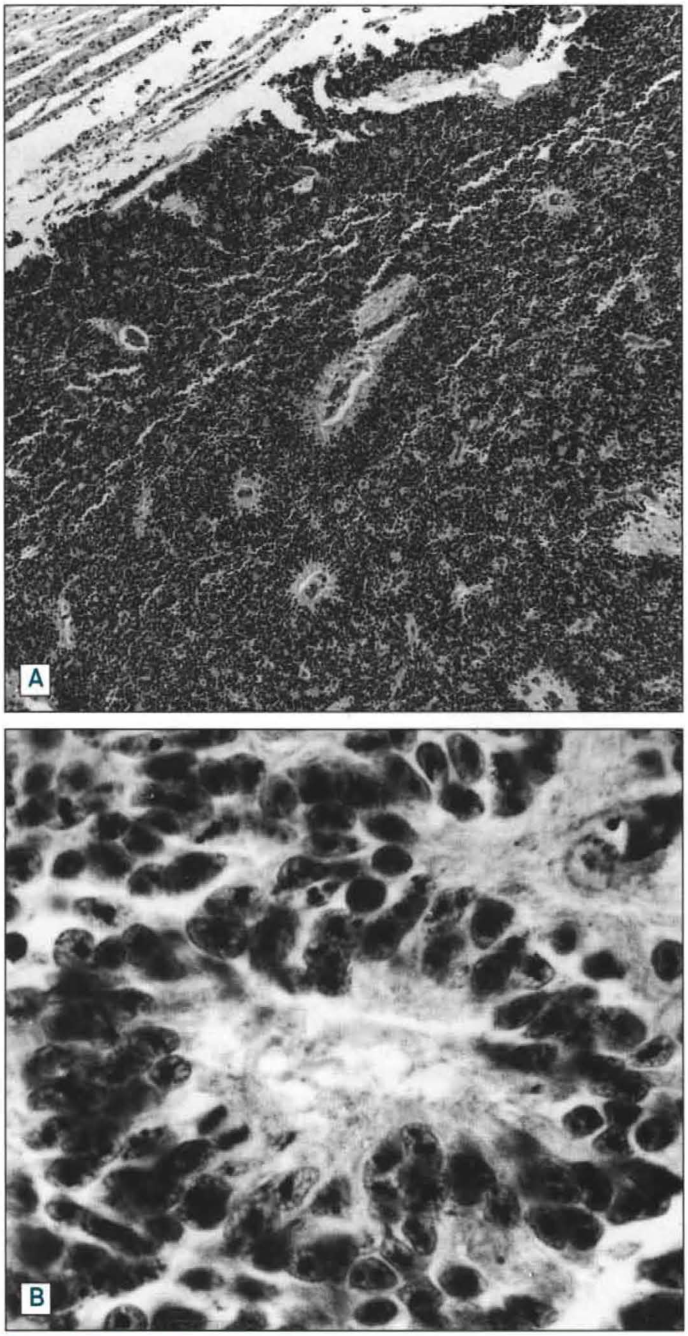

Figure 3.151 Histologic patterns of **medulloblastoma** in a calf. Pseudorosettes are visible in (**A**) and are detailed in (**B**), as circular groupings of dark tumor cells around a central pale area containing neurofibrils. (Courtesy of MD McGavin.)

Neuroblastomas

These tumors are rare and occur in any part of the CNS. Histologic criteria to distinguish them from other cellular neurogenic tumors are not satisfactory and identification must depend on other means. They are thought to arise from primitive neuroepithelial cells with differentiation towards postmitotic neuroblasts. The histologic appearances are similar to those of medulloblastoma, consisting of masses of small rounded cells that resemble lymphocytes, with hyperchromatic nuclei and scant cytoplasm. The presence of rosettes and pseudorosettes is helpful. Neoplastic cells stain positively with neuronal markers and negatively with glial markers such as GFAP.

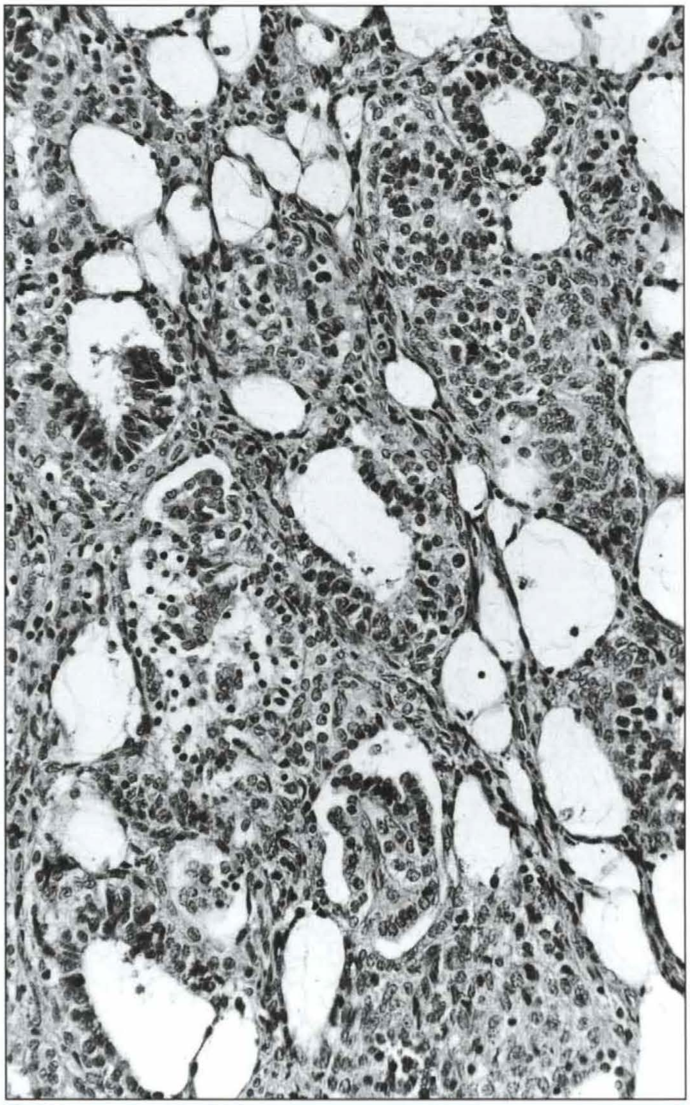

Figure 3.152 Neoplastic cells showing tubular structures with infoldings in **spinal nephroblastoma** in a dog.

Thoracolumbar spinal tumor of young dogs (spinal nephroblastoma)

These are single intradural masses occurring in young, large-breed dogs in the region between the tenth thoracic and second lumbar segments, and affected animals are presented with signs of cord compression. The tumor likely originates from the renal primordium, a renal ectopic embryonic remnant from which diverse nephron cell types are derived, present at this area. The histologic appearance is of glandular areas intermingling with cellular areas. The glandular areas consist of rosettes and tubules, the latter tortuous, branching and sometimes papilliferous, with infoldings reminiscent of embryonic glomerular capsules (Fig. 3.152). The cellular areas contain densely packed cells of blastema appearance with ovoid clear nuclei and indistinct cytoplasm. Some streaming is evident but muscle fibers are not present. In some of these tumors, glomerular structures can be recognized with confidence as can tubular cross-sections strongly

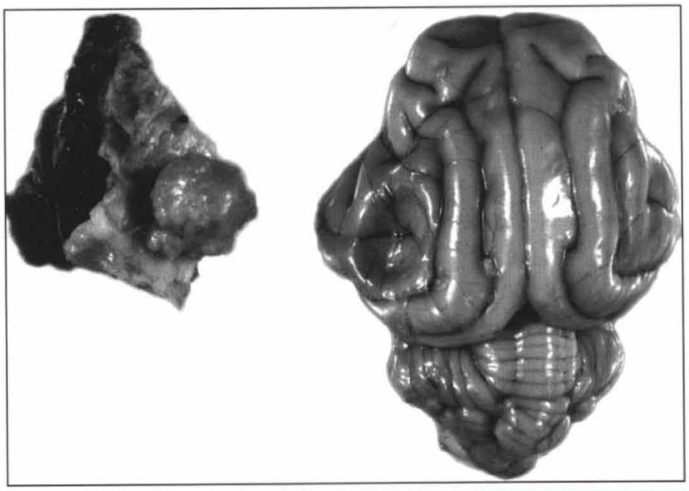

Figure 3.153 Meningioma attached to dura and calvaria (left), and leaving an indentation in the left parietal cerebral cortex of a cat. (Courtesy of RF Slocombe.)

suggestive of distal renal tubules. Presumably these tumors arise from ectopic embryonic remnants but notably the kidneys do not contain neoplasm although nephroblastomas are well recognized in dogs. From time to time, undifferentiated tumors of embryonic type and possibly derived from remnants of pronephros are seen in the cervical region of dogs and sheep. These tumors stain positive for cytokeratin and negative for glial and neuronal markers.

Pineal tumors

These tumors are rare. The benign variant is called *pineocytoma* and the malignant variant is called *pineoblastoma*. They are described in horses, cattle, and dogs. The diagnosis is based on the site of the tumor and its replacement of the pineal body. Other positive identifying characteristics are absent. Microscopically, there is a resemblance to medulloblastoma. The tumor may extend into midbrain and thalamus. Teratomatous tumors with characteristics of the gonadal teratomas are not described in the pineal gland of animals. Specific markers for tumors in animals are not reported.

Tumors of the meninges

These include the only tumor arising from meningothelial cells, **meningioma**, and those arising from nonmeningothelial cells, such as fibrosarcoma and sarcomatosis.

Meningiomas

Meningioma is the most common type of intracranial tumor in the cat and it is one of the commonest of the intracranial and intraspinal tumors in man, and is relatively common in dogs, rare in cattle, and not recorded in horses. It rises within the meninges, usually in close association with the dura, and grows expansively, compressing but seldom invading the brain (Fig. 3.153). Those in humans are presumed, by virtue of their site and structure, to arise mainly from the arachnoid granulations and also from islands of arachnoid cells that lie in the inner surface of the dura mater. A rare variant of meningioma is the **paranasal meningioma**, which arises from meningeal arachnoid cells that are trapped within or outside bone during development of the skull, and is reported in horses and dogs. The meningiomas observed in animals conform in histologic type to those observed in humans.

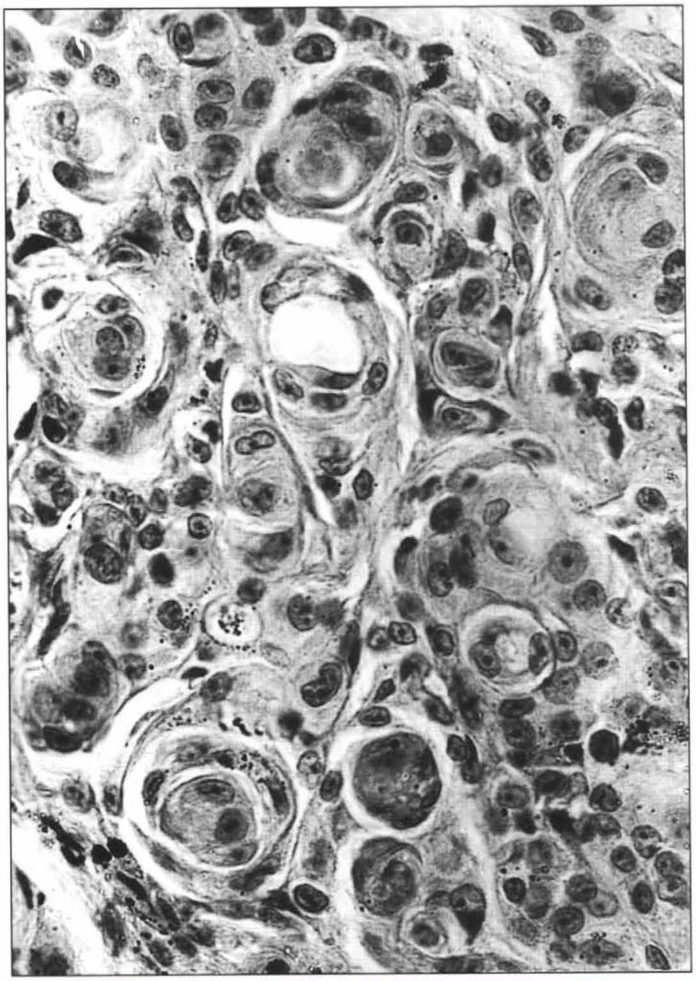

Figure 3.154 Histologic appearance of a **psammomatous meningioma.**

Meningiomas are globular, ovoid or tuberous, sometimes plaque-like, well circumscribed, and have a smooth surface. They are gray, sometimes yellow, on cut surface, firm, and may be gritty. Feline meningiomas are more easily separated from the brain parenchyma than are the canine ones, which are usually more interdigitated into the brain parenchyma. In cats, common locations include the tela choroidea of the third ventricle and the supratentorial meninges. They may be multiple in cats. There are several histologic varieties.

The **meningotheliomatous or epithelioid meningioma** is diffusely cellular with the cells in sheets or pseudo-alveoli. The stroma varies in amount, is richly vascular, and may contain much collagen. The cells are large with abundant finely granular pale cytoplasm without a distinct margin, and the nuclei are spherical or ovoid and rather vesicular. The **psammomatous meningioma** has the same general features as the foregoing, but the cells arrange themselves in whorls (Fig. 3.154). In the center of a whorl, lamellar hyaline tissue forms, derived possibly from cells, stroma, or a blood vessel. As the hyaline focus expands, it tends to be impregnated with salts of calcium and iron to form *psammoma bodies*. The **fibroblastic meningioma** is similar to fibroblastic tumors elsewhere. The **transitional or mixed type** has features of both epithelioid and fibroblastic meningioma.

Angioblastic meningiomas are highly vascular with prominent endothelial cells in formed vessels and lining vascular clefts. The vessels are surrounded by spindle cells giving a distinct resemblance to hemangiopericytoma. **Papillary meningiomas** are extremely rare in animals and composed of meningothelial cells arranged in papillary structures supported by fibrovascular cores. **Myxoid meningioma** is similar to myxoma elsewhere. There may be some difficulty in separating **hemangioma** from highly vascular meningiomas; both contain endothelial cells, pericytes, and stromal cells, the latter of uncertain parentage.

Granular cell meningiomas resemble granular cell tumors that occur elsewhere. They are included here because they do occur in the meninges and spinal nerve roots of dogs and the notion is attractive that some, at least, of the tumors are derived in common with cells of nerve sheaths and other neural crest mesenchyme. The tumors are usually small and well circumscribed but not well encapsulated. The cells are large and rounded, the cytoplasm clear apart from fine granules that may be few or numerous, and the nuclei small and rounded. The cytoplasmic granules are acidophilic and PAS-positive. Delicate stroma, in which amyloid may be deposited, separates the cells individually or in small groups. Stigmata of anaplasia are absent.

Most intracranial meningiomas are benign as indicated by low frequency of metastases and failure to invade the brain. By these criteria, ~2.5% are malignant and may metastasize. In contrast, extracranial meningiomas, which occur mainly in the paranasal region and orbit, are anaplastic and locally aggressive. Meningiomas stain positively for vimentin and negative for synaptophysin and GFAP. Staining for cytokeratin, NSE, and S-100 usually yield positive results but with sparse to moderate expression.

Fibrosarcoma

Fibrosarcomas may provide circumscribed or diffuse involvement of the meninges, or they may form local lesions of the brain or cord. They are not rare. Those of the brain and cord retain a perivascular orientation even though they are infiltrative. Histologically, some are acceptably fibrosarcoma but some are more pleomorphic and anaplastic spindle cell tumors. The histogenesis of these tumors is uncertain. They may arise from fibroblasts, pericytes, or resident reticuloendothelial cells of the monocyte-macrophage series. Some of the more anaplastic astroglial tumors provoke a remarkable degree of capillary endothelial hyperplasia and fibrosis of the vascular adventitia in brain and meninges. The proliferating fibroblastic cells are atypical, their appearance consistent with fibrosarcoma.

Meningeal sarcomatosis

Meningeal sarcomatosis is a rare condition reported only in dogs and characterized by diffuse infiltration of the leptomeninges, especially of the lumbar spinal cord, by pleomorphic neoplastic mesenchymal cells. Tumor may extend along the entire cerebrospinal axis from medulla to sacral cord. Neoplastic cells are present usually circumferentially in the subarachnoid space and may invade the subpial parenchyma of the spinal cord and to lesser extent the brain. Individual cells are pleomorphic, where the main neoplastic cells are large irregularly round cells (35–80 μm) with abundant cytoplasm and large round-to-ovoid hyperchromatic nuclei (20–65 μm). Neoplastic cells usually have a high mitotic rate and up to threefold anisokaryosis. In addition, several lymphoid, plasmacytoid, and histiocytic cells are present amid the neoplastic cells. A few multinucleated giant cells and intact neutrophils may also be present. The neoplastic large cells stain positively for vimentin. They usually stain positive, but with sparse to moderate expression, for CD18 and actin. They stain negatively for lymphocyte markers, S100, cytokeratin, and GFAP.

Hematopoietic tumors

Hematopoietic tumors such as lymphosarcoma, multiple myeloma, and malignant histiocytic tumors that originate in extraneural locations can metastasize to the CNS as part of their multisystemic metastasis, however in the following section we will describe those hematopoietic tumors that originate primarily in the CNS.

Lymphoma/lymphosarcoma

Primary CNS lymphomas are reported mainly in dogs and cats and sporadically in ruminants. They are mostly intraparenchymal and have an angiocentric (perivascular) pattern in contrast to lymphosarcomas metastatic from extraneural areas that are usually arranged diffusely in the meninges. Most primary CNS lymphomas are of T-cell type. Histologically, they follow the same morphology as extraneural lymphomas/lymphosarcomas.

Plasma cell tumor

A primary intracerebral intraparenchymal plasma cell tumor has been reported in a dog. An identical tumor was reported in a cat, but the diagnosis of primary neural plasma cell tumor was not confirmed due to incomplete postmortem examination.

Malignant histiocytosis

Primary neural malignant histiocytosis was reported in the right parieto-occipital lobe of a miniature Schnauzer as a poorly demarcated mass composed microscopically of histiocytic cells with many binucleate and multinucleate giant cells. Neoplastic cells stained positively for lysozyme.

Non-B, non-T leukocytic neoplasm (neoplastic reticulosis)

This controversial malignant neoplasm has a histologic resemblance to necrotizing meningoencephalitis (NME) of Pug dogs and granulomatous encephalitis (GME). The term "reticulosis" came into use because of aggregation of reticular fibers around neoplastic cells. Many cases diagnosed previously as reticulosis have been reclassified as lymphoma and a few as histiocytic tumors. The tumor may be single or multiple and can occur in any part of the CNS but is more prevalent in the cerebral white matter. The exact histogenesis of the neoplastic cells is yet to be determined, however, the neoplasm is composed histologically of angiocentric large (25–50 μm) round cells reminiscent of histiocytes and numerous non-neoplastic lymphocytes and macrophages. Multinucleate giant cells may predominate. Neoplastic cells usually show atypia and a moderate mitotic rate. The neoplastic cells stain positively with CD18 and negatively for lymphocyte markers, proving a leukocytic but non-lymphoid origin. The neoplasm has been reported in dogs and rarely in cats, cattle, and horses. The differentiation between neoplastic reticulosis and GME (formerly called inflammatory reticulosis) is difficult. The predominance of neoplastic histiocytic-like cells, cellular atypia, and high mitotic rate should favor the diagnosis of neoplastic reticulosis.

Microgliomatosis

The histogenic origin of the neoplastic cell in this rare neoplasm is controversial. The neoplasm diffusely infiltrates the cerebral white matter and brain stem with cells reminiscent of microglial cells. The infiltration in some cases can be limited to meninges or can be perivascular. In any case, this is a non-mass-forming neoplasm and the brain is almost unremarkable grossly. The neoplastic cells do not cause significant destruction of the pre-existing tissue. Neoplastic cells stain negatively for GFAP. There is strong overlap between microgliomatosis and gliomatosis cerebri. In human microgliomatosis, most neoplastic cells stain for CD18 and other monocyte/macrophage lineage markers. Similar studies on the immunohistochemical markers expressed by canine microgliomatosis are not available.

Tumors of the sellar region

Suprasellar germ cell tumors

Germ cell tumors are presumed to arise from ectopic embryonic germ cells which, intended for the developing gonad, may become widely distributed. They are responsible for a spectrum of tumors in humans, usually in the midline and according to histological features designated as *seminoma, choriocarcinoma, entodermal sinus tumor, and teratoma.* They are revealed early in life and the preferred locations in the cranial cavity are in the pineal region or in the hypothalamus above the sella. These are rare tumors in dogs. Their location and growth pattern is similar to that of pituitary adenomas and some may be misdiagnosed as craniopharyngioma.

The reported canine cases were suprasellar and classified on the basis of location, admixture of distinct cell types and patterns, and positive immunochemical staining for alpha fetoprotein. Sheets and nests of cells resembling seminoma were admixed with areas of vacuolated hepatoid cells, glandular formations similar to those in gonadal teratomas, and occasional foci of squamous epithelium.

Craniopharyngioma

Craniopharyngiomas are thought to arise from remnants of Rathke's pouch, which forms the adenohypophysis. Aberrant differentiation of Rathke's epithelium is common in dogs, expressed in cystic structures lined by respiratory-type epithelium. The craniopharyngioma, in contrast, consists of clumps of epithelial cells palisaded on collagenous stroma. Keratinization may be present to form pearls, as in well-differentiated squamous carcinoma. Degenerative foci contain cholesterol crystals and blood pigments. Neoplastic cells are positive for cytokeratin.

Other primary tumors and cysts

Epidermoid cysts

Epidermoid cysts in the brain are confined to the fourth ventricle and environs. They are rare, probably represent surface ectoderm misplaced at the period of closure of the neural groove, and are described only in humans, dogs, and a horse. The cystic structure is lined by squamous epithelium, and the cavities contain keratinaceous debris. They occur in young dogs and may develop to several centimeters in diameter. The clinical signs are determined by the location of the tumor.

Hamartoma or meningio-angiomatosis

Hamartoma or meningio-angiomatosis is a rare benign lesion, best regarded as a malformation or hamartoma producing circumscribed plaques on the surface of the brain. Blood vessels are in excess in the lesions and are cuffed by proliferating cells that are considered to be meningothelial. The lesion does extend into the underlying neural substance, which shows mixed degenerative and reactive changes.

Metastatic tumors

Secondary tumors can metastasize from extraneural tissue to the CNS. The most common examples include canine hemangiosarcoma, malignant histiocytosis, lymphosarcoma, and malignant melanoma of dogs and rarely other species.

Tumors affecting the CNS by extension or impingement

There are several tumors that do not arise directly from the CNS, but arise from structures adjacent to the CNS and sometimes impinge on the CNS, for example, vertebral osteosarcomas, nasal carcinomas, and chordomas.

Chordoma

Chordomas are rare tumors in animals with the exception of mink and European ferrets. In ferrets they are usually present at the tip of the tail. In humans, dogs, and cats, they are slow-growing persistent tumors mainly of the sacrococcygeal region with some occurring in paraspinal and cranial regions. On the basis of histological characters, *the tumor is presumed to arise from notochordal remnants*. Grossly, they are gelatinous, gray, friable, and lobulated. Histologically, the lobules are not encapsulated but are well defined by bands of connective tissue. The principal cell (physaliferous cells) types are large, clear, and vacuolated with a botanical appearance and some resemblance to cartilage (Fig. 3.155). The cytoplasm contains large clear vacuoles with distinct boundaries. At the periphery of the lobules there are smaller, stellate cells with eosinophilic cytoplasm; mitotic figures are rare in these but they may be the germinative cells. There may be islands of bone or cartilage. Mucinous substance can be demonstrated in the cytoplasmic vacuoles of the larger cells and between the cells; the smaller peripheral cells may contain PAS-positive granules. Differentiation from mucinous chondrosarcoma can be difficult without assistance of immunochemical methods. Chordomas will stain

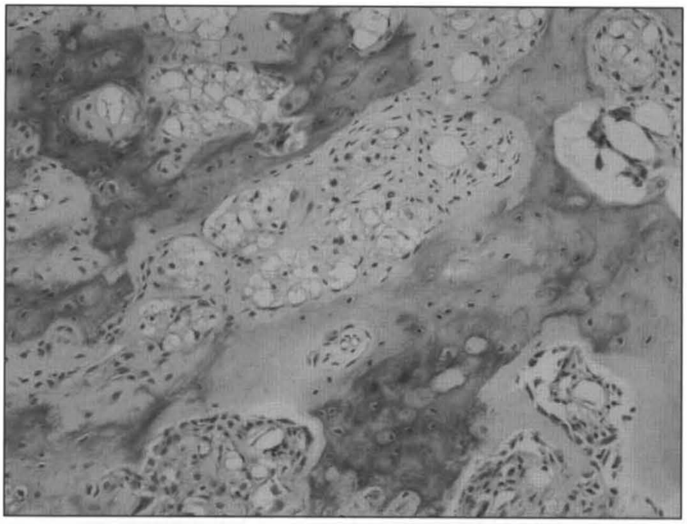

Figure 3.155 A **chordoma** in the tail of a ferret is composed of large, clear, vacuolated principal cells (physaliferous cells) in association with chondro-osteoid metaplasia.

immunohistochemically for cytokeratin, but chondrosarcomas will not. Chordomas also stain positively for vimentin and S-100.

Tumors of the peripheral nervous system

Ganglioneuroma and peripheral ganglioneuroblastoma

These rare tumors originate from the cranial and spinal ganglia and from sympathetic ganglia of the autonomic nervous system. They have been reported in cats, pigs, cattle, dogs, and horses. They can be solitary or multicentric. The few ganglioneuromas observed in cattle have developed in relation to the abdominal sympathetic plexuses. **Ganglioneuroma** is composed of ganglion cells and glial cells.

Ganglioneuroblastoma is composed of poorly to fairly well differentiated ganglion cells with more atypia and high nuclear-to-cytoplasmic ratio. The degree of differentiation of these tumors varies considerably, and they are a mixture of ganglion cells, Schwann cells, and nerve fibers. The ganglion cells show different degrees of differentiation from primitive neuroblasts to some that are remarkably mature. In the more primitive form, **neuroblastoma**, the neoplastic cells may form true or pseudorosettes. The adrenal medullary ganglioneuromas and ganglioblastomas are probably better regarded as hamartomatous malformations rather than as neoplasms.

Paraganglioma

This rare neuroendocrine tumor originates from extra-adrenal paraganglion chief cells associated with autonomic nervous system ganglia. The histologic picture resembles chemodectoma described in Vol. 3, Cardiovascular system. The neoplastic cells stain positive for neuroendocrine markers such as synaptophysin and chromogranin.

Peripheral nerve sheath tumors

These include the benign form (schwannoma, neurofibroma) or the malignant form (malignant schwannoma, neurofibrosarcoma) (Fig. 3.156A–E). The Schwann cell of the peripheral nervous system is accepted as the origin of these tumors. Most of these tumors are seen in the skin. The dermal tumors do pose problems of differentiation from fibrous and perivascular lesions (see the Skin and appendages). In skin, they do show some significant differences from the tumors of nerve trunks and roots.

Schwannomas may be largely solitary infiltrating lesions at any site on a nerve trunk. Those distant from the CNS are not encapsulated or well defined, are difficult to dissect cleanly, and have an ordinary fibrous appearance and texture. Schwannomas of nerve roots tend to be well-defined fusiform tumors. They probably arise from a single nerve and extend proximally and distally in conjunction with the nerve but external to it. In this way they may extend through the intervertebral foraminae and extend also to involve other nerves that have a plexus arrangement such as the brachial plexus. They may arise within and remain within the dura mater, and such tumors may be globose rather than fusiform and soft and discolored from hemorrhage. Expansive intradural growth may be slow and compress the brain or cord, but some of these tumors are malignant and invasive (Fig. 3.156D) and metastasize particularly to the lung.

Any cranial or spinal nerve root may be the site of growth, but in the dog, in which they are not rare, the brachial plexus is most frequently involved. In the thoracic region and on the acoustic nerve (Fig. 3.156A), the tumors tend to originate within the dura. The histologic appearance varies within and between tumors depending on the presence or absence of anaplastic change and the extent of hemorrhage, degeneration, fibrosis, and mineralization. Blood vessels are often prominent but ill formed. The anaplastic tumors consist of closely packed cells with oval or elongate nuclei that give an impression of interlacing streams not supported or guided by reticulin or collagen (Fig. 3.156B). In areas of tumors that are not anaplastic, two patterns are dominant and referred to as Antoni A and Antoni B. *Antoni A arrangements* are repetitive and give the tumor its character. Uniform, fusiform cells are arranged as bands, herring bones, whorls, or palisades (Verocay bodies) (Fig. 3.156C). *Antoni B tissue* is degenerative and may predominate in some sections. It is loose and myxoid, sometimes hyalinized and may be sparsely cellular (Fig. 3.156E). Microcysts may be present in areas of myxoid change. Cartilaginous and osseous metaplasia occur infrequently. A malignant variant is more invasive grossly and occasionally metastasizes to the lung and other organs. The neoplastic cells stain strongly for vimentin and usually stain positively but with varying intensity with S-100, GFAP, nerve growth factor receptor, and myoglobin. They stain negatively with α-smooth muscle actin, which stains hemangiopericytoma and rhabdomyosarcoma positively.

Neurofibromatosis of cattle is a well-recognized Schwannoma. It is common in abattoir material from old animals but has been observed in very young calves. The skin may be affected, but the lesions are usually restricted to deeper nerves of the thoracic wall and viscera. The brachial plexus, intercostal nerves, hepatic autonomic plexus, epicardial plexus, and autonomic nerves of the mediastinum are those most frequently affected in various collective patterns. Sympathetic ganglia, especially the stellate and others of the thorax, are also frequently involved. Affected nerves are thickened, firm, and gray, and may bear yellow-gray nodules. Affected ganglia may be enlarged to several centimeters and appear lobulated on section. The histologic appearances are as described above for Schwannomas, but anaplastic change is rare (Fig. 3.156C).

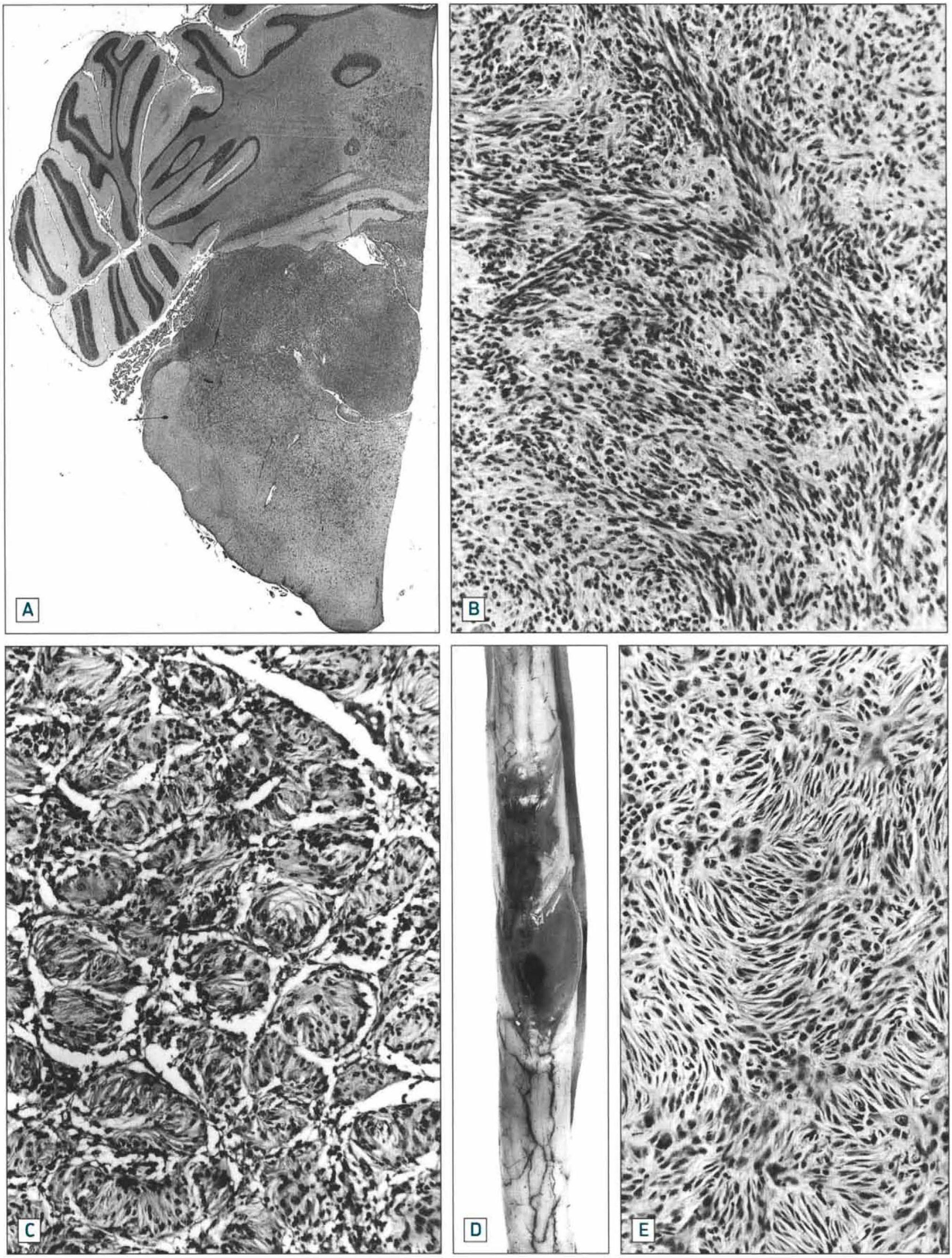

Figure 3.156 A. Acoustic schwannoma at cerebellopontine angle and filling the fourth ventricle of a dog. **B.** Section of (**A**). **C.** Patterns resembling Verocay bodies in neurofibromatosis in an ox. **D. Malignant schwannoma** in the spinal cord of a dog. **E. Schwannoma** in a cat.

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4

Eye and ear

Brian P. Wilcock

Eye

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Eye

GENERAL CONSIDERATIONS

The role of the veterinary pathologist in the diagnosis of ocular disease is usually restricted to histologic examination. Gross pathology of the eye is the realm of the clinical ophthalmologist. The sophistication of ocular clinical examination, including imaging techniques like ultrasound and magnetic image resonance, represents a substantial challenge for the ocular histopathologist. Especially when dealing with globes submitted from ophthalmic specialists, we are being asked to provide very precise histologic counterparts for some very specific macroscopic lesions detected by these increasingly sophisticated clinical examination techniques.

The reluctance of many pathologists to embrace ophthalmic pathology stems from the disappointing quality of sections made from formalin-fixed globes processed by routine methods, from unfamiliarity with ocular anatomy and histology, and from fear of the complex terminology shared by clinical ophthalmologists and ophthalmic pathologists. At least equally daunting is the need to be familiar with the ever-growing list of specific ocular syndromes, the correct identification of which has huge significance in terms of therapy and prognosis. There are many examples in which a perfectly adequate description and general morphologic diagnosis will nonetheless fail to communicate the appropriate prognostic or therapeutic information, simply because the clinician reading the report cannot make the connection between the histologic description and the specific clinical-pathologic syndrome. This is also true when dealing with the histologic manifestations of the innumerable inherited ocular disorders that occur in purebred dogs, the species that indisputably dominates veterinary ophthalmology. In many instances, the correct diagnosis requires the correlation of the lesion with the age, breed, and specific clinical features of the disease.

Ocular fixation

The eye undergoes very rapid postmortem/postenucleation change that not only obscures subtle degenerative lesions but also mimics genuine developmental or degenerative diseases. Even with a globe obtained within minutes of death or surgical removal, improper handling of the specimen frequently results in a section of poor quality. The globe can be speedily and gently removed by grasping the third eyelid with forceps and applying traction to the globe whilst making a circumferential incision at the fornix. Blunt curved scissors inserted through this incision may be used to sever the extraocular muscles and optic nerve, and allow the globe to be removed from the orbit. All orbital fat and extraocular muscles should be gently removed from the sclera to permit rapid penetration of fixative to the retina.

The choice of fixative depends upon the disease suspected and upon the type of examination to which the eye will be subjected. *Formalin* has the advantage of ready availability, ease of shipment, little danger of overfixation, and adequate preservation of color and macroscopic detail for photography. Also, it permits localization in the bisected globe of lesions identified ophthalmoscopically, and the use of electron microscopy should such examination be warranted by the findings of light microscopy. However, formalin penetrates the sclera slowly and there are postmortem changes, including retinal detachment, even in globes fixed immediately after death or surgery. Injection of formalin into the vitreous (0.25 ml for a dog or cat, 2.0 ml for a horse) greatly improves retinal fixation and helps prevent the almost inevitable retinal detachment that follows routine formalin fixation. Rapid-penetrating fixatives such as *Zenker's, Davidson's or Bouin's* are preferred for globes in which preservation of histologic detail (especially retinal detail) is paramount. All render the globe and its refractive media opaque and less suitable for macroscopic assessment and photography than does formalin. All require strict attention to the duration of fixation. *Regardless of the method of fixation, all eyes benefit from hardening in 70% ethanol over about 24 hours to prevent retinal detachment when trimming the globe for embedding.* A mixture of equal parts cold 4% buffered glutaraldehyde and 10% neutral formalin has been recommended as an ocular fixative for both light and electron microscopy.

In all domestic animals, *the preferred section for histology is made from a midsagittal slab which includes optic nerve*, thereby allowing examination of both tapetal and nontapetal fundus in the same section. **Attempting to slice the fixed globe with a scalpel blade is**

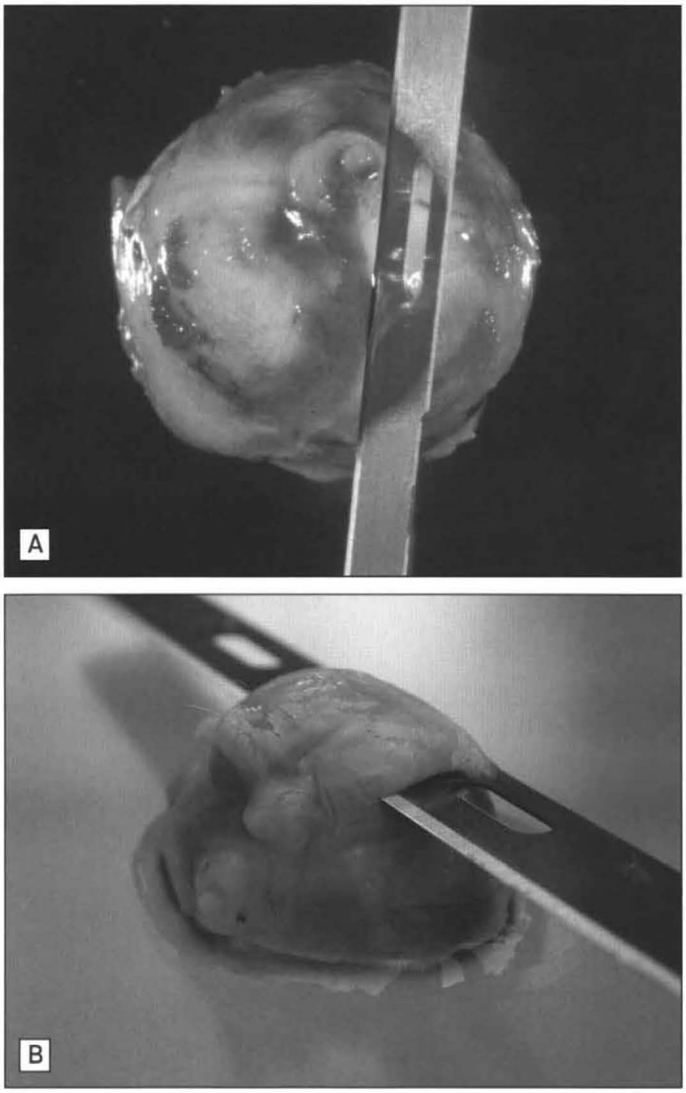

Figure 4.1 A. Trimming a fixed eye. The first incision is made from back to front, perpendicular to the posterior ciliary artery and just adjacent to the optic nerve. **B.** The second cut, made from front to back to avoid detaching the retina, is parallel to the first and far enough to the periphery to miss the lens.

perhaps the single biggest contributor to sections of poor quality. *The optimal cutting instrument is a new disposable microtome blade.* For small globes less than about 1 cm in diameter, an ordinary consumer razor blade is perfectly adequate.

Because there is no easily accessible instruction manual on how to obtain a good histologic section, those details are included here.

- The fixed globe, free of extraocular muscles and eyelids, is opened by a smooth sagittal incision beginning adjacent to the optic nerve and ending with the cornea.
- The correct 6:00–12:00 orientation, needed to capture tapetal and nontapetal fundus, is insured by making the incision at right angles to the orientation of the posterior ciliary artery (Fig. 4.1A).
- The open globe is then inspected for macroscopic lesions.
- A second cut, parallel to the first, is made from the cornea backward through the retina (Fig. 4.1B).
- That second cut should be far enough to the periphery to leave the already-bisected lens undisturbed (for a second cut through the lens will surely dislocate it).
- The resulting slab should be lifted carefully into a thick processing cassette or a tissue bag.
- Even though the piece of tissue is as much as 1 cm thick, it is hollow and thus presents no difficulty in terms of automated tissue embedding procedures.

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DEVELOPMENTAL ANOMALIES

Ocular developmental defects are common in domestic animals, particularly in purebred dog breeds in which extensive linebreeding has been used to increase the predictability of the phenotype. Many of the defects involve the eyelids and result from accentuation of anatomic peculiarities of the breed, such as entropion from deliberate enophthalmos or misdirected hairs from overly prominent facial folds. Such anomalies are clinically obvious and amenable to surgery, and rarely require the attention of a pathologist.

Anomalies of the globe are usually multiple, which reflects the stepwise induction and interdependence of the various parts of the developing eye. Without proper consideration of ocular embryology, any discussion of the lesions found in anomalous eyes threatens to be just a catologue of observations rather than a roadmap to understanding that such lesions are all predictable results of a relatively small number of possible errors in organogenesis. It is also important to recognize the differences in normal ocular structure among the various species, and the different rates at which mature form is attained. For example, the retina of carnivore eyes continues to develop for about 6 weeks postnatally, whereas that of ruminants and horses is mature at birth. Thus, something like retinal dysplasia is necessarily an in utero event in ungulates, but may be in response to early postnatal injury in carnivores.

Review of ocular organogenesis

The primary optic vesicle is an evagination of the forebrain that, with differential growth of brain and surface ectoderm, becomes separated from the presumptive diencephalon by the *optic stalk.* The

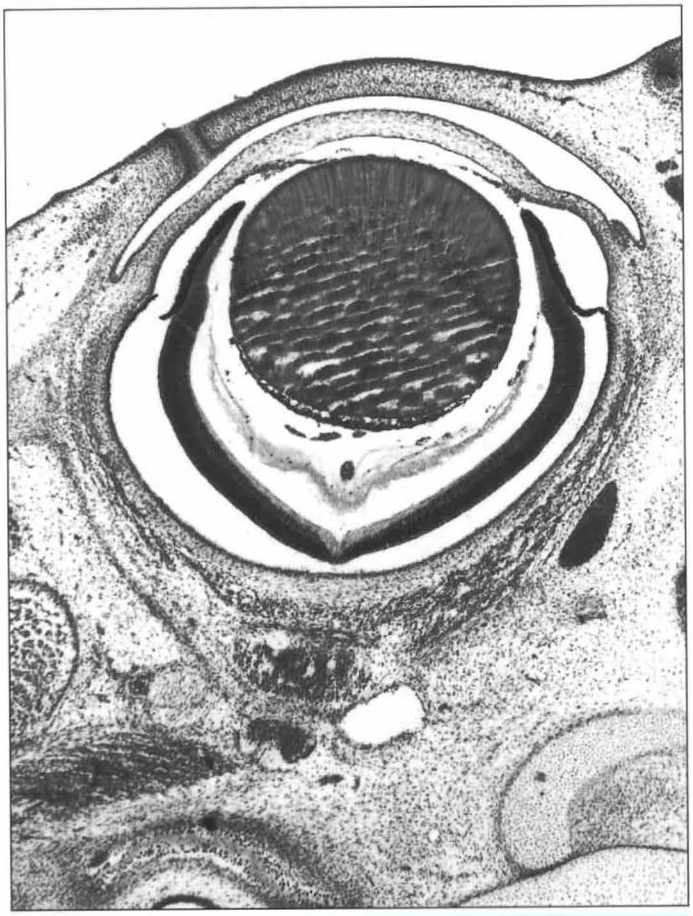

Figure 4.2 Canine embryo at 34 days gestation. Lids fused, cornea fully formed. Large lens surrounded by complete vascular tunic derived posteriorly from hyaloid artery and anteriorly from the future pupillary membrane. Iris not yet formed.

apposition of primary optic vesicle to overlying surface ectoderm induces a focal ectodermal thickening, the *lens placode*. The placode grows to form a primitive *lens vesicle*. It is the developing lens that orchestrates the invagination of the optic vesicle to form the bilayered *optic cup* and bring the lining neuroectoderm into the apposition that provides the future photoreceptor and pigment epithelial layers. Surrounding the optic cup is a mass of *mesenchyme*, derived from neural crest, which will form the vascular and fibrous tunics of the eye (iris and ciliary body stroma, corneal stroma and endothelium, choroid and sclera) under the induction of the differentiating neuroectoderm (Fig. 4.2). *Ocular adnexa and muscles* form independently and seem not to require normal development of the globe, as evidenced by the presence of normal lacrimal gland, lids, and extraocular muscles in most cases of severe microphthalmos.

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Defective organogenesis

Failure of the eye to attain even the stage of optic cup is a rare occurrence and is usually of unknown cause. The defect is usually bilateral but asymmetrical, and the severity of the defect relates to the stage of organogenesis at which the insult occurred. Failure of formation of the primary vesicle, or its early and complete regression, is *true anophthalmos* and is very rare. Failure of optic vesicle invagination gives rise to the very rare *congenital cystic eye*. Incomplete invagination results in *congenital retinal nonattachment*. Failure of division (or subsequent fusion) of the optic primordium as it grows from the telencephalon results in *cyclopia*, or *synophthalmos*, a single dysplastic midline globe.

Anophthalmos and microphthalmos

Anophthalmos, total absence of ocular tissue, is a very rare lesion, and almost all cases described are more correctly termed *severe microphthalmos,* in that some vestige of eye is found in serially sectioned orbital content. The usefulness of distinguishing between the two is questionable, and *many authors have adopted the term "clinical anophthalmos" for all such cases*. Concurrent anomalies of skeletal and central nervous systems are common.

Macroscopic examination of orbital content usually reveals a normal lacrimal gland and vestigial extraocular muscles. The globe is usually recognized as an irregular mass of black pigment, with structures such as cornea or optic nerve variably recognizable. Histologically, there is almost always a mass of pigmented neurectoderm, reminiscent of ciliary processes, and some effort at retinal differentiation (Fig. 4.3). There is frequently some remnant of lens, a finding that suggests regression of an embryonic globe that had reached at least the stage of optic cup. One or more plates of cartilage, presumably derived from third eyelid analog, are common.

Cyclopia and synophthalmos

Damage to the prosencephalon prior to the outgrowth of the optic vesicles may result in improper separation of paired cranial midline structures, including eyes. *Cyclopia is a fetal malformation characterized by a single median orbit containing a single globe.* Most specimens have some duplication of intraocular structures, such as lens, iris, or hyaloid

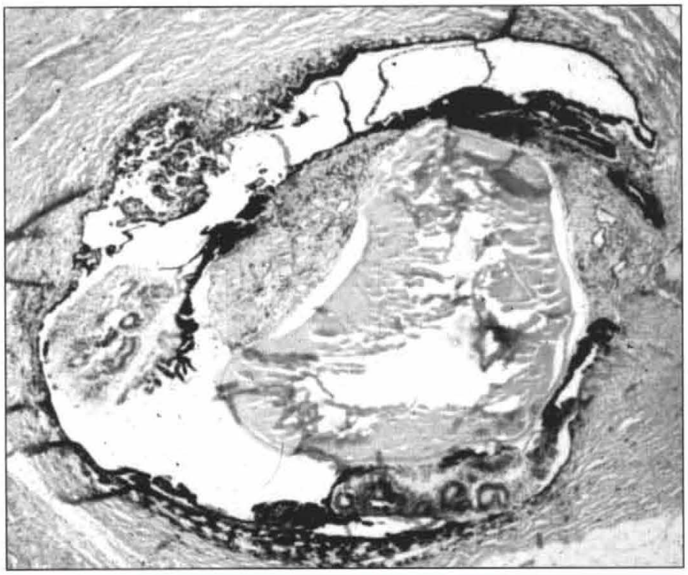

Figure 4.3 Secondary microphthalmia. The ciliary processes tend to be highly conserved even in the most disorganized globes.

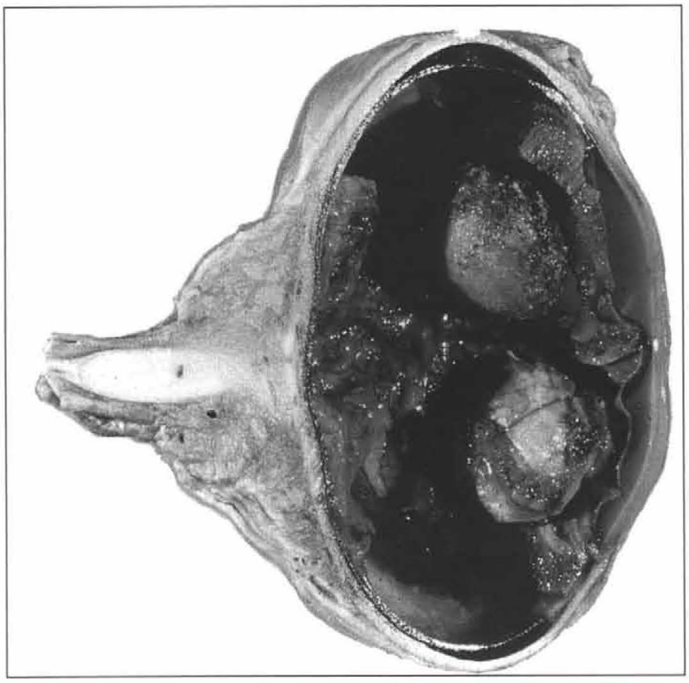

Figure 4.4 Globe from typical "cyclopian" calf. Duplication of lens and pupil indicates **synophthalmos** rather than true cyclopia.

vessels, and are thus more properly considered *incomplete separation or early fusion (synophthalmos)* (Fig. 4.4). Some specimens have two dysplastic globes within a single orbit. Severe cranial anomalies accompany cyclopia and synophthalmos, including absent or deformed ears, a median proboscis, cranioschisis, cleft palate, and brain anomalies ranging from microcephaly to hydranencephaly and hydrocephalus.

Cyclopian-like malformations have been reported in sheep, chickens, and dogs, and as inherited defects in cattle with the most thoroughly documented cases being in sheep grazing alpine pastures rich in the legume *Veratrum californicum*. Fresh and dried plants contain three steroidal alkaloids – jervine, cyclopamine, and cycloposine – capable of damaging the developing neural groove of the fetal lamb. Ewes eating the plant on gestational day 15 have lambs with the cyclopian malformation, for it is at that time that the neural groove has formed and the first cranial somites are forming. A similar syndrome has been produced in kids and calves by maternal feeding of the plant on day 14 of gestation. Ingestion of the alkaloids prior to day 15 in sheep may cause fetal death but no anomalies, and exposure soon after day 15 may cause various skeletal abnormalities but not cyclopia.

In naturally occurring outbreaks, affected lambs have deformities ranging from cyclopia with microcephaly to relatively normal lambs with harelip and cleft palate. Prolonged gestation is common in the case of severely malformed fetuses.

Cystic eye and retinal nonattachment

Failure of apposition of the optic vesicle to the cranial ectoderm results in failure of lens induction, which in turn removes the major stimulus for invagination of the optic vesicle to form the optic cup. *Persistence of the primary optic vesicle is seen as a cystic eye* (Fig. 4.5), consisting of a scleral sheet lined by neurectoderm of variable neurosensory and pigmentary differentiation. The absence of lens and of bilayered iridociliary epithelium distinguishes this rare lesion from the more common dysplastic eye of secondary microphthalmos.

Incomplete invagination of the optic vesicle allows persistence of the cavity of the primary optic vesicle and prevents attachment of the presumptive neurosensory retina to the developing retinal pigment epithelium. *In the postnatal globe, retinal nonattachment cannot easily be distinguished from acquired retinal separation.* In each instance, the retina is extensively folded and may have improper differentiation of neuronal layers. The diagnosis of retinal nonattachment is assisted if there is also lack of apposition between the two layers of neurectoderm covering the anterior uvea (destined to be iridal and ciliary epithelium) and if retinal rosettes are evident. In addition, since nonattachment is an early and fundamental error in organogenesis, such eyes usually lack a lens and probably will be microphthalmic with multiple anomalies.

Coloboma

The mildest and latest defect in organogenesis results from failure of complete fusion of the lips of the optic (embryonic) fissure, a slit-like but normal channel in the floor of the optic cup and stalk through which the vasoformative mesoderm and stromal mesenchyme enter the globe. Failure of closure of the fissure may occur anywhere along its length, but the channel persists most frequently as a notch-like defect of the caudal pole at, or just ventral to, the optic disk. Its exact location can vary substantially. It is lined by an outpouching of dysplastic neurectoderm. If the defect is sufficiently large, the outpouching of neurectoderm induces a similar bulge in the sclera, termed **scleral ectasia** (Fig. 4.6). Occasionally such ectasias are so large as to form a retrobulbar cyst as large as the globe itself (Fig. 4.7). Regardless of size, the lining of the scleral coloboma is formed by neurectoderm that bulged through the defect in the optic cup. Abortive neurosensory differentiation within the cyst wall is common and permits definitive

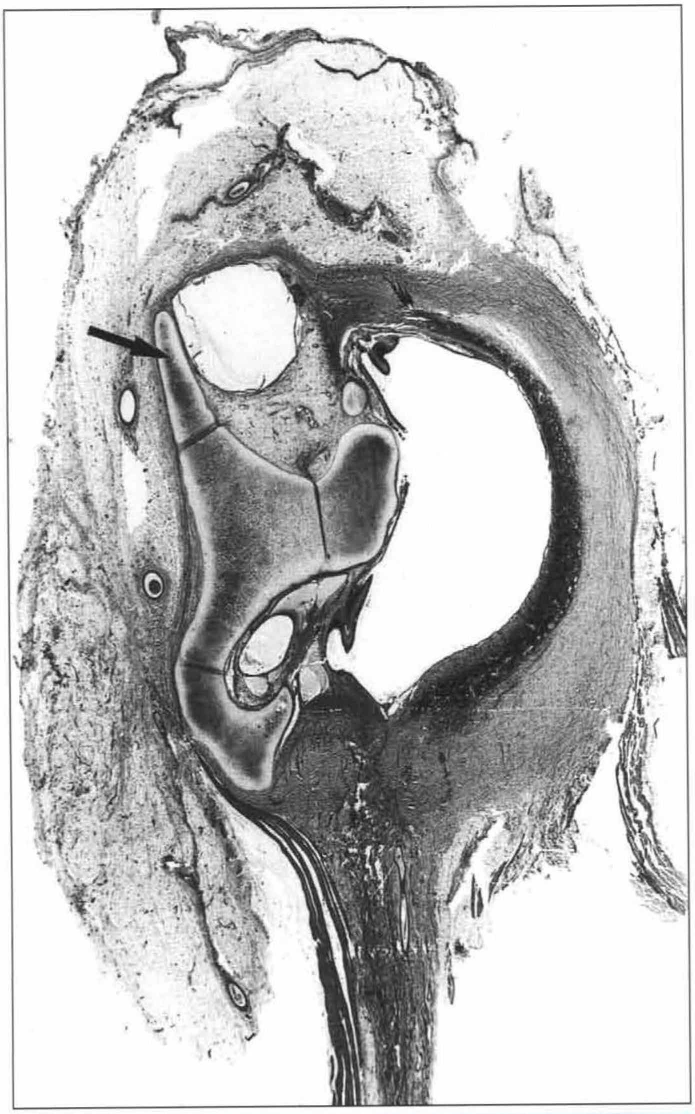

Figure 4.5 Severe **microphthalmos** in a foal. There is no lens, no apparent attempt at invagination, and no neurosensory retinal differentiation. Persistence of the cavity of the optic vesicle qualifies this as a cystic eye. Cartilage plate (arrow) is probably an analog of the third eyelid.

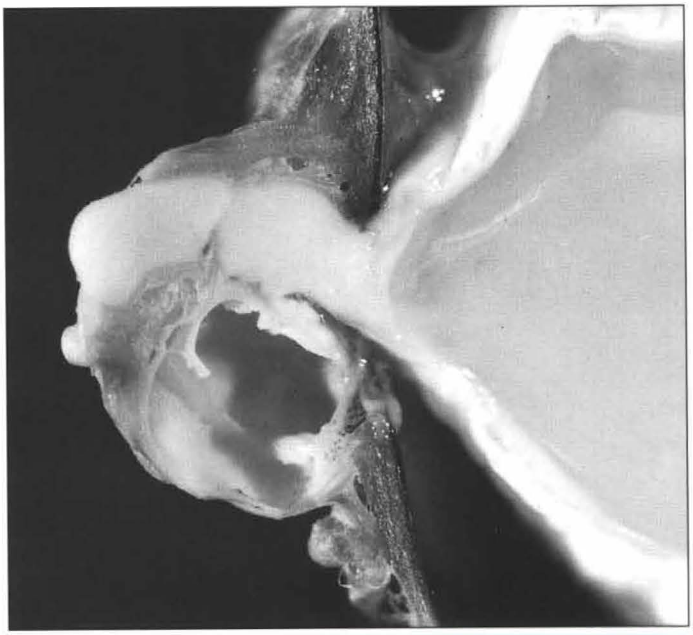

Figure 4.6 Coloboma adjacent to the optic disk, accompanied by retinal separation. Collie eye anomaly.

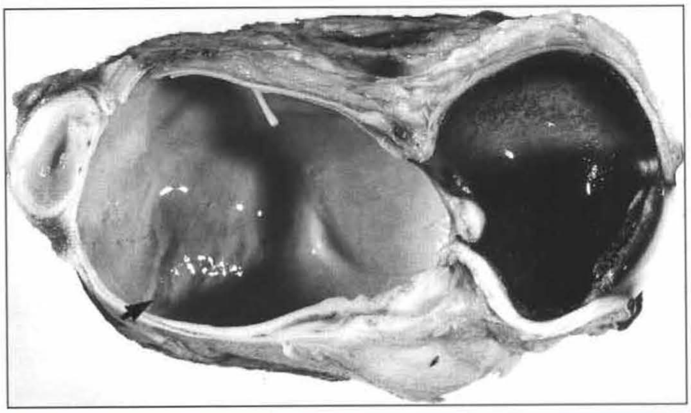

Figure 4.7 Retrobulbar cyst (arrow) formed by coloboma and massive scleral ectasia in a calf. The globe is small and the retina is completely separated.

identification of the retrobulbar cyst as being a **coloboma** (absence or defect of some ocular tissue) in terms of pathogenesis (Fig. 4.8).

Colobomas occur in all domestic species, but are especially frequent in Collie dogs as one manifestation of the Collie eye anomaly. In Collies, they usually arise within or just adjacent to the optic disk. They appear to arise as focal defects in maturation and/or induction of sclera and choroid by the retinal pigment epithelium (RPE) that is forming from the outer layer of the optic cup. Because the exact location of the embryonic fissure is somewhat variable, it is difficult to determine how many examples of coloboma are the result of delayed closure of that normal embryologic structure, and how many represent some more fundamental defect in the proper interaction of RPE and the developing periocular mesenchyme destined to form choroid and sclera. Proper maturation of the RPE, and in particular the normal acquisition of pigmentation, appears to be critical to the induction of normal mesenchymal migration and maturation. Its failure results in such varied anomalies as choroidal hypoplasia, segmental or diffuse iris and ciliary hypoplasia (known clinically as **iris coloboma**), and even microphthalmia. Because of its frequent association with the merle dilution defect in the coat color of dogs, the general syndrome is known as *merle ocular dysgenesis*. It is similar, but not identical, to Collie eye anomaly. The same defect occurs, with much less frequency, in color-dilute (incompletely albinotic) horses, cattle, non-merle dogs, and cats. A similar pathogenesis and spectrum of lesions, not proven to be associated with color dilution, occurs in Collie eye anomaly (see later). In Charolais cattle, colobomas of (or near) the optic disk are inherited as an autosomal dominant trait with incomplete penetrance. The lesion is bilateral but not necessarily equal in severity.

Defective differentiation

Subsequent to formation of the optic cup, ocular differentiation involves continued differentiation of neurectoderm into retinal and

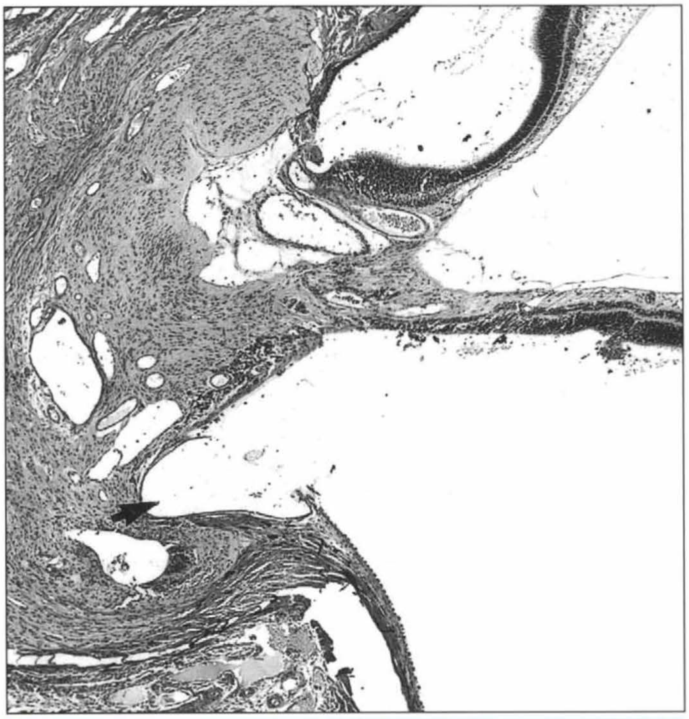

Figure 4.8 **Coloboma** (arrow) at the optic disk in a Collie pup with Collie eye anomaly. Dysplastic neurectoderm lines the defect and attempts to form sensory retina.

uveal neuroepithelium, and induction of primitive periocular neural crest mesenchyme to form the sclera and uvea. The normal development of retinal pigment epithelium from the neurectoderm of the posterior half of the optic vesicle seems prerequisite for these differentiations to occur.

It is traditional to present specific ocular anomalies as they relate to structures of the adult eye, and thus as anomalies of cornea, iris, lens, retina, and so on. This approach correlates well with the clinical examination of the eye but provides no understanding of the fundamental pathogenesis of the anomaly. *Here we will organize these "later" anomalies (occurring after the stage of optic cup formation) on the basis of the presumed pathogenesis: defective migration, proliferation, or remodeling of ocular mesenchyme, defective maturation of neurectoderm, and defective development of ectoderm.*

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Anomalies of mesenchyme

After formation of the optic cup and separation of the lens vesicle, the periocular mesenchyme undergoes a complex series of migrations, differentiations, and atrophies that determines the final structure of the vascular and fibrous tunics of the globe. At the anterior edge of the optic cup, one or more waves of mesenchymal invasion form corneal endothelium, corneal stroma, and the anterior half of the transient perilenticular vascular network, including the pupillary membrane. The posterior half of this perilenticular vascular tunic is formed by invasion of mesodermal endothelial cell precursors and supporting perivascular mesenchyme through the optic fissure to form the extensive but transient *hyaloid artery system* (see Fig. 4.2). Another mesenchymal wave accompanies the ingrowth of the neurectoderm at the anterior lip of the optic cup to form the iris stroma, although it is unclear which layer acts as the primary inducer. Its peripheral portion later atrophies to form the porous *filtration angle* of the anterior chamber, a process that may not be completed in carnivores until 6–8 weeks after birth. The choroid and sclera are induced by the developing retinal pigment epithelium to form from the mesenchyme surrounding the caudal half of the optic cup.

Anomalies of mesenchyme may result from defective ingrowth or differentiation, as with choroidal and iris hypoplasia, incomplete atrophy of the tunica vasculosa lentis or hyaloid artery system, or incomplete remodeling of the filtration angle to cause primary glaucoma.

Choroidal hypoplasia

This is a relatively common lesion in the eye of dogs by virtue of its prevalence in the Collie breed as the hallmark of Collie eye anomaly, and a very similar syndrome occurs in Australian Shepherd dogs and Shetland Sheepdogs. It is also seen in a variety of dog breeds in association with genes for color dilution (merle, dapple, and harlequin). *The hypoplasia is thought to result from induction failure by a defective retinal pigment epithelium.* The basic defect is not clearly established but may be related to defective pigmentation, a suggestion supported by the prevalence of iris and choroidal hypoplasia in white animals of all species, especially those with blue irises. Other anomalies linked to the choroidal hypoplasia/hypopigmentation include optic nerve coloboma, microphthalmia, and (with the merle ocular dysgenesis) cataract and segmental iris hypoplasia. Some degree of retinal dysplasia is also common. At least in theory, all of these defects are predictable outcomes of improper mesenchymal induction by a defective, inadequately pigmented retinal pigment epithelium. Even in otherwise normal (nonwhite) animals with a blue iris, there is usually hypoplasia of the tapetum and choroid.

Collie eye anomaly

This is a common disease of smooth and rough Collies, first reported in 1953 and at one time estimated to have affected 90% of North American Collies. During the period 1975–1979, the defect was still present in over 70% of 20 000 Collies examined in a voluntary screening program. Prevalence in Europe and the United Kingdom is lower (30–60%). *The basic defect, patchy to diffuse choroidal hypoplasia, is inherited as an autosomal recessive trait*, but the numerous associated defects are more unpredictable in their familial pattern. Similar syndromes are reported in Border Collies, Shetland Sheepdogs, and Australian Shepherd dogs, and are probably of similar pathogenesis. The prevalence in these breeds, as in rough Collies, has marked geographic variation.

The *ophthalmoscopic findings* include one or more of retinal vessel tortuosity, focal to diffuse choroidal and tapetal hypoplasia, optic nerve coloboma, and retinal separation with intraocular hemorrhage. Other observations that are occasionally made in eyes of affected dogs are enophthalmos, microphthalmos, and corneal stromal mineralization. The disease is always bilateral but not necessarily equal. Even the mild, visually insignificant lesion of focal choroidal hypoplasia is genetically significant.

Macroscopic examination of the bisected globe reveals abnormal pallor of the posterior segment of the globe. If the globe is transilluminated, the sclera and choroid are focally or diffusely more translucent than normal. The pallor and translucency imply choroidal hypoplasia. Within or adjacent to the optic disk there may be a colobomatous pit of variable size, the lining of which is continuous with the retina. Accompanying the larger type of pit is a bulge in the overlying sclera, called **scleral ectasia** or **posterior staphyloma.** If there is retinal separation, it is usually complete, with the only sites of attachment being at the abnormal optic disk and at the ora ciliaris (Fig. 4.9). In such cases, there may be extensive intravitreal hemorrhage and retinal tears. Almost all Collie eyes with retinal separation have large optic disk colobomas. Detachment from the ora ciliaris (so-called *retinal disinsertion*) may also occur, leaving the folded retina on the floor of the globe (Fig. 4.10).

The fundamental histologic lesion found in all affected eyes is choroidal hypoplasia. It is always diffuse, despite ophthalmoscopic observation of a lesion that may appear only to be focal within the dorsal temporal quadrant of the fundus. The choroid is thin and poorly pigmented, and the tapetum is thinner than normal or even absent (Fig. 4.11A, B). Retinal pigment epithelium is poorly pigmented, even in nontapetal fundus, and may be vacuolated. Because the choroid and tapetum in the normal dog do not reach adult thickness until about 4 months postpartum, age-matched control eyes are essential if overinterpretation of normal choroidal immaturity is to be avoided.

Histologic examination of eyes with optic disk colobomas reveals the bulging of dysplastic neurectoderm, continuous with retina, into the pit in the nerve head. The neurectoderm may show jumbled differentiation into ganglion cells, photoreceptor rosettes, glial cells, or pigment epithelium. Rosettes are common in the neurosensory retina adjacent to affected disks or embedded in the optic disk itself. In some specimens, there are degenerative retinal lesions overlying severely hypoplastic choroid. Edematous clefts are seen in the nerve fiber layer, and ganglion cells may be severely vacuolated.

Other retinal lesions include retinal folds and detachment. The folds are seen on histologic section as tubes of fully differentiated retina cut in cross-section or tangentially, and are thought to represent folds in a neurosensory retina that at least temporarily has grown in excess of the space available for it within the optic cup. These folds correspond to the clinically detectable vermiform streaks in the fundus, and gradually disappear as the dog (and eye) matures, allowing the growth of scleral shell to catch up with that of retina. Presumably it is a similar growth imbalance, but in the opposite direction, that causes retinal separation in about 10% of eyes with this syndrome. In this situation, a retina that is too small attempts to stretch from optic disk to ora ciliaris by the shortest route, rather than following the curvature of the scleral shell.

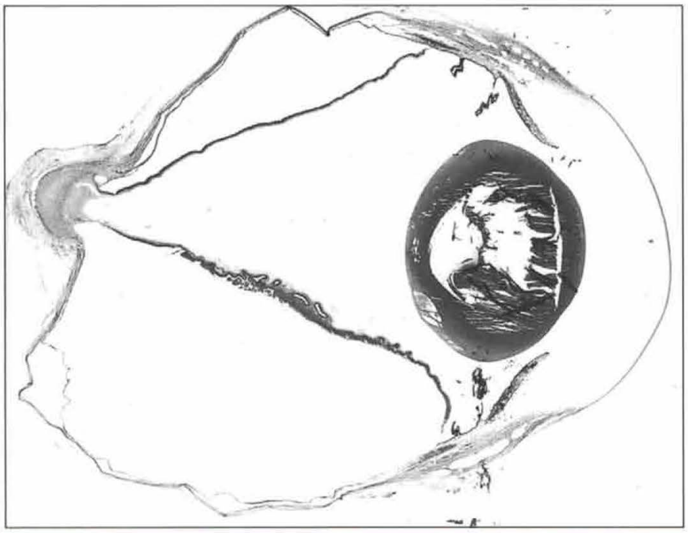

Figure 4.9 Retinal separation in Collie eye anomaly. There is coloboma at the optic disk. Choroid and sclera are thin. Retinal folding does not constitute dysplasia.

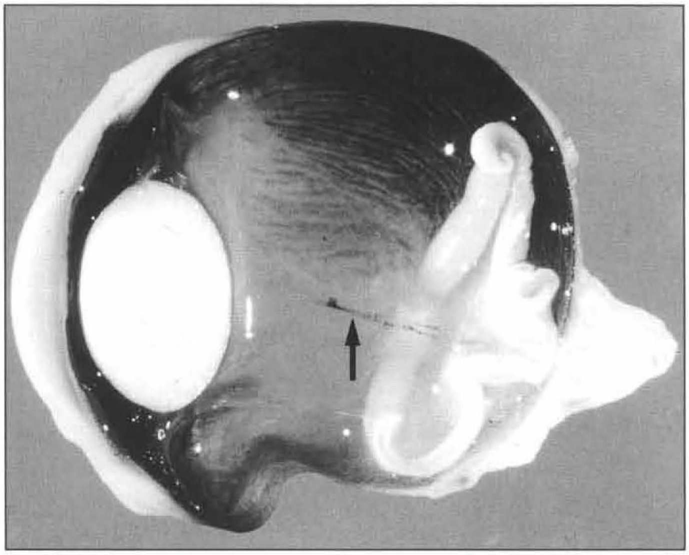

Figure 4.10 Retinal separation from ora ciliaris in Collie eye anomaly. Note hypopigmented choroid and prominent hyaloid artery (arrow).

Focal fibroblastic metaplasia and mineralization are occasionally seen in the subepithelial corneal stroma of dogs with Collie eye anomaly, but a similar defect is seen in anomalous eyes of other breeds; a genetic link to the Collie eye defect is not established. Tortuosity of retinal veins, a controversial clinical lesion sometimes considered part of Collie eye anomaly, has no described histologic counterpart.

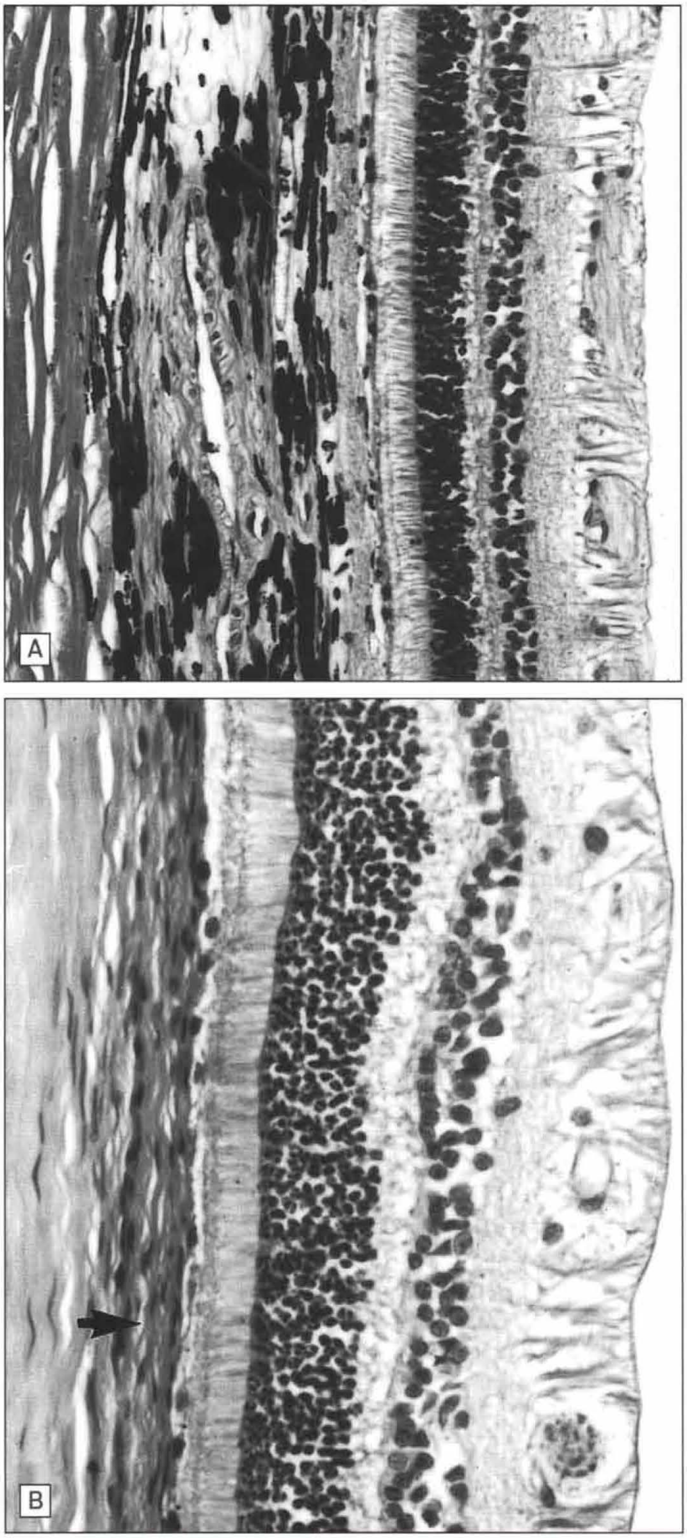

Figure 4.11 A. Normal posterior pole of the globe of a 13-week-old puppy. Tapetum present but thin (normal for puppy). Choroidal thickness approximates that of retina. **B.** Posterior pole just dorsal to the optic disk of a 13-week-old Collie with **Collie eye anomaly**. Tapetum is absent. Choroid (arrow) is severely hypoplastic.

The earliest lesion of this anomaly is defective differentiation of primitive retinal pigment epithelium to form rosette-like structures near the optic disk. Proper differentiation of both pigment epithelium and neurosensory retina requires obliteration of the lumen of the primary optic vesicle, which allows the two neurectodermal layers to come into apposition. Whether the earliest lesion of Collie eye anomaly results from inherently defective differentiation of pigment epithelium or from imperfect apposition of the two neurectodermal layers has not been resolved, but the central role of the pigment epithelium in determining ocular morphology suggests that the primary defect is in maturation of the presumptive retinal pigment epithelium. Anomalous development of choroid and sclera, including coloboma, is not seen in fetuses up to 45 gestational days, but is seen in neonates. This suggests that the defect is in choroidal maturation rather than in initial induction.

Another manifestation of mesenchymal maldevelopment in Collie eye anomaly, rarely noted clinically, is delayed atrophy and remodeling in the anterior chamber. The filtration angle may be closed, iris stroma may be attached to the corneal endothelium by a mesenchymal bridge, and remnants of anterior perilenticular mesenchyme are unusually prominent. Pigmentation of iridal neurectoderm is sparse. As these neonatal anterior segment lesions are not seen later in life (8–20 weeks) when puppies are examined ophthalmoscopically, it is presumed that they reflect only a minor delay in mesenchymal remodelling.

Defects primarily in anterior chamber mesenchyme

Hypoplasia of the iris *is a rare defect that may occur alone or in conjunction with multiple ocular defects.* It is relatively most frequent in horses, where it may be inherited and associated with cataract and conjunctival dermoids. The defect presumably results from incomplete inward migration of the anterior lip of the optic cup, with resultant lack of a neurectodermal scaffold to guide the subsequent migration of mesenchyme destined to form the iris stroma. The hypoplasia is usually severe and most cases are clinically described as *aniridia*. Histologic examination of such eyes usually reveals the vestigial iris as a triangular mesenchymal stump covered posteriorly by normal-appearing pigmented epithelium (Fig. 4.12A). The trabecular meshwork within the filtration angles may be malformed, but the ciliary apparatus is usually normal. The lens often is cataractous (Fig. 4.12B) and sometimes ectopic or hypoplastic. Glaucoma has been described as a sequel in horses (but not in other species), but it should be an expected sequel in severely affected eyes in any species because of the inevitable concurrent trabecular hypoplasia (Fig 4.12A).

Hypopigmentation of the iris may be unilateral or bilateral. When the loss of pigment is patchy in one or both irises, it is known as *heterochromia iridis*. The pigmentation may be diffusely absent in the iris stroma but present in the posterior iris epithelium (*subalbinotic*), or absent in both stroma and epithelium (*true albinism*). The iris is normal except for absence of visible pigment granules in the cytoplasm of otherwise normal stromal melanocytes and epithelial cells. Tapetum and, less reliably, choroid of affected eyes usually are hypoplastic as well as poorly pigmented.

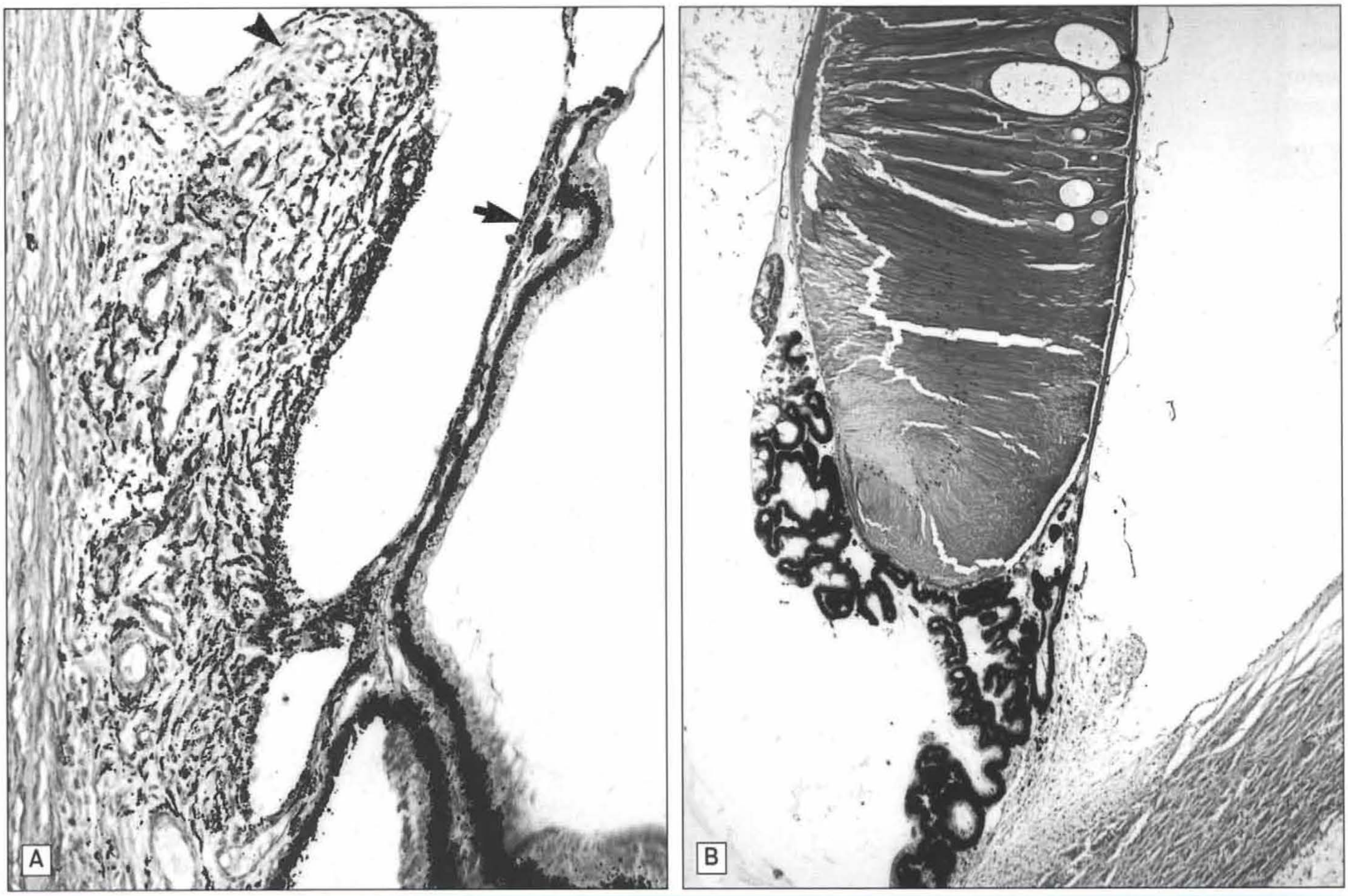

Figure 4.12 A. Iris hypoplasia (arrowhead) with hypoplasia of the trabecular meshwork in a dog. The ciliary processes (arrow) have developed normally. **B. Iris hypoplasia**, congenital cataract and dysplasia of ciliary processes in a piglet; one of three affected. The adherence of ciliary processes to the lens represents an arrest in remodeling rather than improper development.

Incomplete atrophy of the anterior chamber mesenchyme is relatively common in dogs and occurs occasionally in other domestic species. During organogenesis, waves of mesenchyme migrate between the surface ectoderm and the anterior rim of the optic cup. Some of the ingrowing mesenchyme forms corneal endothelium and stroma, while other portions of mesenchyme form the iris stroma and trabecular meshwork. Some of that mesenchyme occupies the anterior chamber and, as it matures, it forms a fibrovascular sheet stretching across the face of the lens and developing iris, known as the *pupillary membrane*. Its vascular component creates the anterior portion of an embryonic perilenticular vascular plexus, known as the *tunica vasculosa lentis*. Both the pupillary membrane and the tunica vasculosa lentis normally disappear late in gestation or in the early postnatal period. Failure of this anterior chamber fibrovascular mesenchyme to atrophy results in the very common anomaly of **persistent pupillary membrane** (persistence of the tunica vasculosa lentis alone is discussed later).

Atrophy of the pupillary membrane is frequently incomplete at birth and, in dogs, persistent remnants are common up to about 6 months of age. These insignificant and usually bloodless strands are seen as short, thread-like protrusions from the area of the minor arterial circle (iris collarette) and they may insert elsewhere on the iris, cross the pupil, or extend blindly into the anterior chamber (Fig. 4.13A). Persistent pupillary membranes achieve clinical significance in two ways. First, the size and number of strands crossing the pupil may be such that vision is obstructed. Second, strands that contact lens or cornea are associated with focal dysplasia of lens or corneal endothelium, clinically seen as opacity (Fig. 4.13B). Because the normal pupillary membrane never contacts the cornea, strands of pupillary membrane that extend from iris to cornea are considered to be minor versions of anterior segment dysgenesis (see below).

Histologic descriptions are mainly from studies in *Basenji dogs*, in which persistent pupillary membrane occurs as an autosomal recessive trait of variable penetrance. In this breed, atrophy of the pupillary membrane is abnormally slow even in dogs free of the defect in adult life, and remnants in puppies up to 8 months old are common. The membranes are seen as thin endothelial tubes, invested with a thin adventitial stroma, extending from vessels in the iris stroma near the collarette. The tubes are usually empty, but in severely affected eyes may contain erythrocytes, and the adventitia may contain melanin. The tubes weave in and out of the plane of section en route to corneal, iridic, or lenticular insertions. At sites of corneal insertion, corneal endothelium is either absent or dysplastic, with the latter manifested as fibrous metaplasia. Descemet's membrane is malformed or absent in the areas of attachment and there is associated deep stromal corneal edema to account for the clinically observed, minute

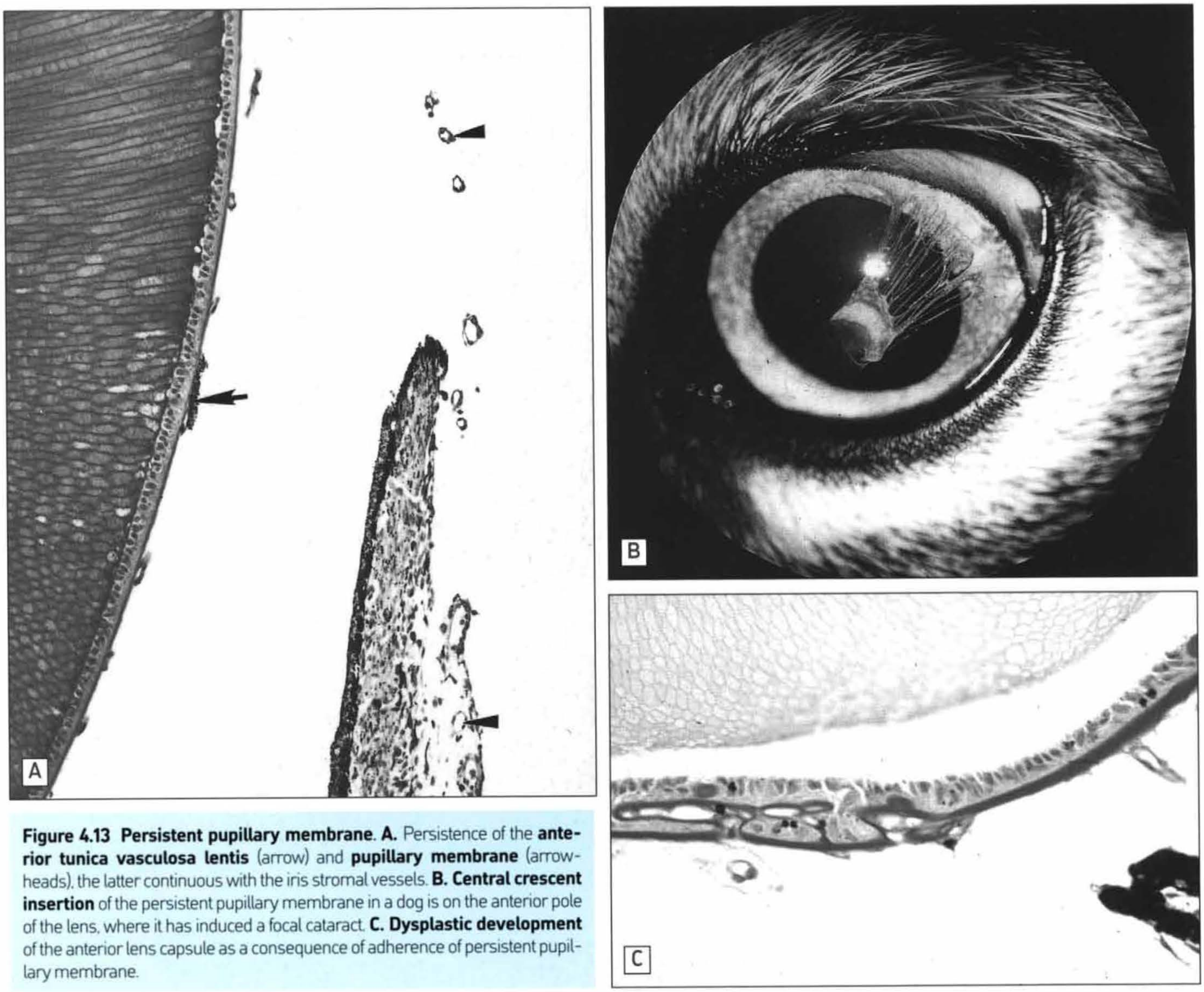

Figure 4.13 Persistent pupillary membrane. A. Persistence of the **anterior tunica vasculosa lentis** (arrow) and **pupillary membrane** (arrowheads), the latter continuous with the iris stromal vessels. **B. Central crescent insertion** of the persistent pupillary membrane in a dog is on the anterior pole of the lens, where it has induced a focal cataract. **C. Dysplastic development** of the anterior lens capsule as a consequence of adherence of persistent pupillary membrane.

gray stromal opacities. *Contact with the lens is accompanied by similar epithelial and basement membrane dysplasia, resulting in one or more epithelial, subcapsular, or polar cortical cataracts* (Fig. 4.13C). Some would classify this latter, more severe form of persistent pupillary membrane as a mild version of anterior segment dysgenesis (see below).

Much less common than persistent pupillary membrane are those defects grouped under the general category of **anterior segment dysgenesis** (or **anterior segment cleavage syndrome**). This group includes multiple anomalies of cornea, lens, and anterior uvea that stem from disordered development of anterior segment mesenchyme and/or improper separation of the developing lens from the overlying corneal mesenchyme. Such eyes are commonly microphthalmic and usually have microphakia, cataract, and congenital corneal opacities at sites of congenital anterior synechiae. The most severe cases have fusion of iris with corneal stroma without observed corneal endothelium or Descemet's membrane (forming a so-called "internal corneal ulcer"), and thus have no detectable anterior chamber (Fig. 4.14). *Most examples in dogs and cats probably result from perinatal corneal perforation from trauma or from progression of suppurative bacterial keratoconjunctivitis (ophthalmia neonatorum).* The

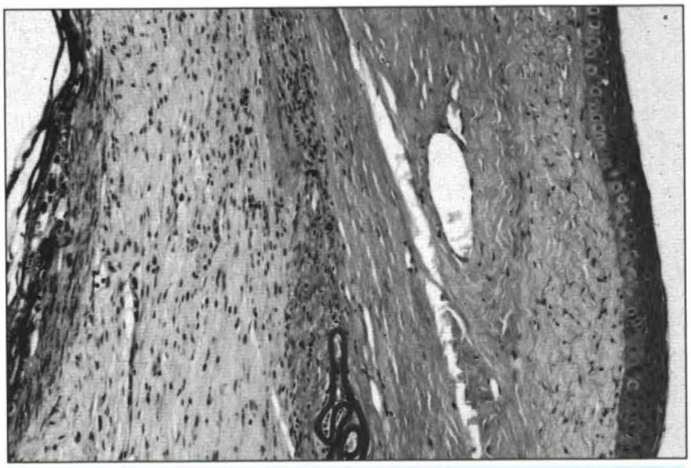

Figure 4.14 Anterior segment dysgenesis. Improper remodeling of anterior segment mesenchyme results in failure of formation of the anterior chamber. The iris is then fused to the cornea.

result is iris prolapse and incorporation of the iris into the developing cornea resulting in obliteration of the anterior chamber. Since the globe of dogs and cats continues to develop for many weeks after birth, this is yet another example of how difficult it can be to precisely distinguish idiopathic developmental disorders from those resulting from postnatal traumatic or inflammatory diseases.

Maldevelopment of the filtration angle (goniodysgenesis) occurs as a prevalent, inherited condition in dogs and in severely anomalous eyes of animals of any species. The defect may result from incomplete atrophy of mesenchyme that normally fills the fetal iridocorneal angle, or from inadequate posterior migration of the iris root. The defect is much more common in dogs than any other species. In the most severe cases, the trabecular meshwork may appear as a solid mesenchymal mass indistinguishable from the adjacent iris stroma (Fig. 4.15A). This rare lesion, known us *trabecular hypoplasia*, is a cause for truly congenital glaucoma. It is often accompanied by iris hypoplasia. Much more commonly, the error in remodeling is less profound and the lesion is seen as an imperforate or inadequately perforated mesenchymal sheet separating anterior chamber from a relatively normal trabecular meshwork (Fig. 4.15B). The only lesion may thus be a pectinate ligament that is thicker, more heavily pigmented and less fenestrated than normal. This has sometimes been referred to as *"pectinate ligament dysplasia."* (For a more complete discussion, see Glaucoma.)

Clinically noted ocular features of **bovine Marfan syndrome**, which are similar to human Marfan syndrome, are ectopia lentis, microspherophakia, and myopia. The human disease is caused by mutations in the fibrillin-1 gene; affected cattle have abnormal fibrillin metabolism. Eyes of affected cattle are characterized by megaloglobus, increased circumlental distance, asymmetrical ciliary processes, intact but fragile zonular fibers, and ectopia lentis. Affected animals have moderately hypoplastic ciliary bodies, compact filtration angles, and long thin irises with decreased fibrous stroma.

Incomplete atrophy of posterior segment mesenchyme

Incomplete atrophy of posterior segment mesenchyme may result in the mild and common lesion of **persistent hyaloid artery,** or in the much rarer but clinically more significant lesions of **persistent posterior perilenticular vascular tunic** with or without concurrent persistence of the primary vitreous. There is a tendency in clinical literature to group all of these defects under the umbrella of **persistent hyperplastic primary vitreous**, but histologically there is quite a wide range in the nature of the defect. Only the most severe qualify as true persistent hyperplastic primary vitreous.

The hyaloid artery and its branches are formed from mesenchyme and pre-endothelial mesoderm that enter the optic cup through the optic fissure prior to its closure. The vessel traverses the optic cup from optic disk to lens, where it ramifies over the posterior lens surface (posterior tunica vasculosa lentis). It joins with the vascular portion of the pupillary membrane (anterior tunica vasculosa lentis) to form a complete perilenticular vascular tunic. *As with its anterior chamber counterpart, the hyaloid system undergoes almost complete atrophy before birth.* Persistence of some vestige into adult life is common and clinically insignificant. In ruminants, the most common remnant is *Bergmeister's papilla*, a cone of glial tissue with a vascular core that extends from optic disk for a few millimeters into the vitreous (Fig.4.16). In calves up to about 2 months of age, the vestigial hyaloid system may still contain blood. In carnivores, it is the pupillary membrane that normally persists for several weeks postnatally. Bloodless remnants of the anterior termination of the hyaloid artery on the posterior lens capsule are known as *Mittendorf's dot*; it is a harmless anomaly, common in dogs and ruminants up to several years of age.

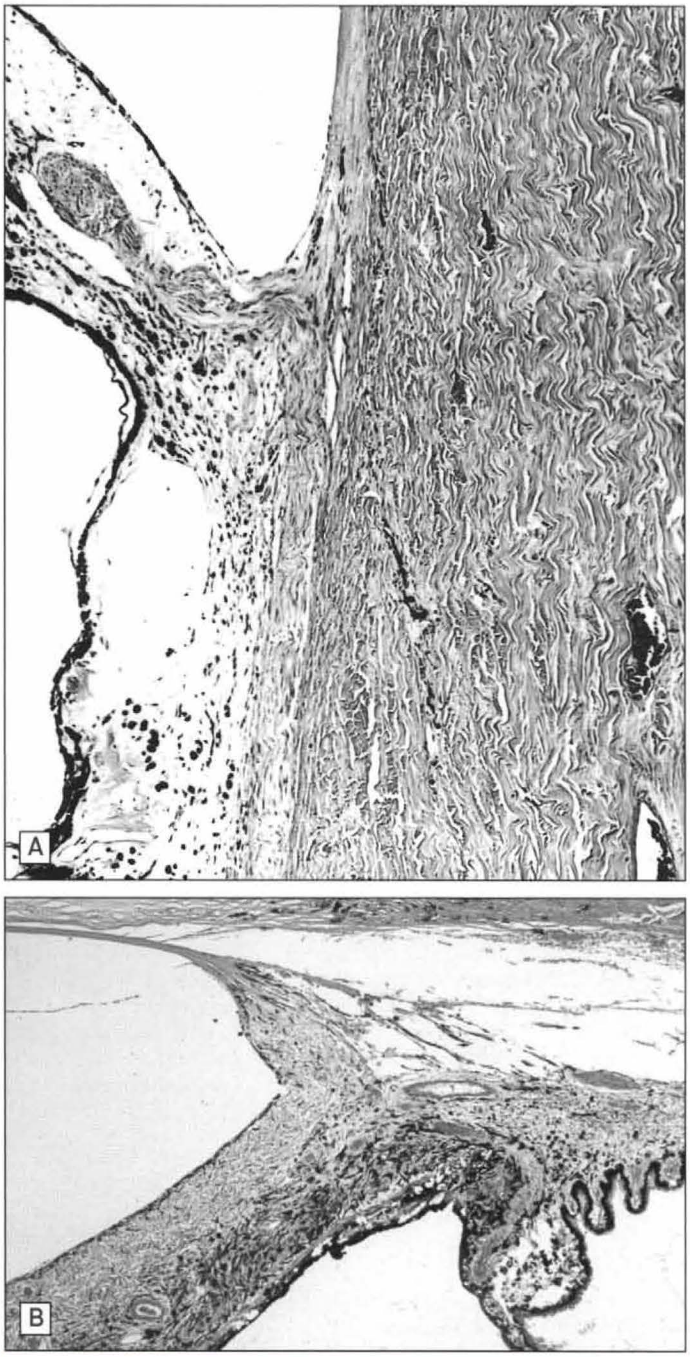

Figure 4.15 A. Goniodysgenesis. A primitive example (**trabecular hypoplasia**) with little maturation of the embryonic mesenchyme destined to form pectinate ligament and trabecular meshwork. **B.** A solid sheet of mesenchyme extends from the termination of Descemet's membrane into the iris stroma, with no obvious pectinate ligament. Other portions of the trabecular meshwork are relatively normal.

Much less common is undue persistence and even hyperplasia of the anterior end of the hyaloid system (**posterior tunica vasculosa lentis**). The normal tissue is a combination of blood vessels and

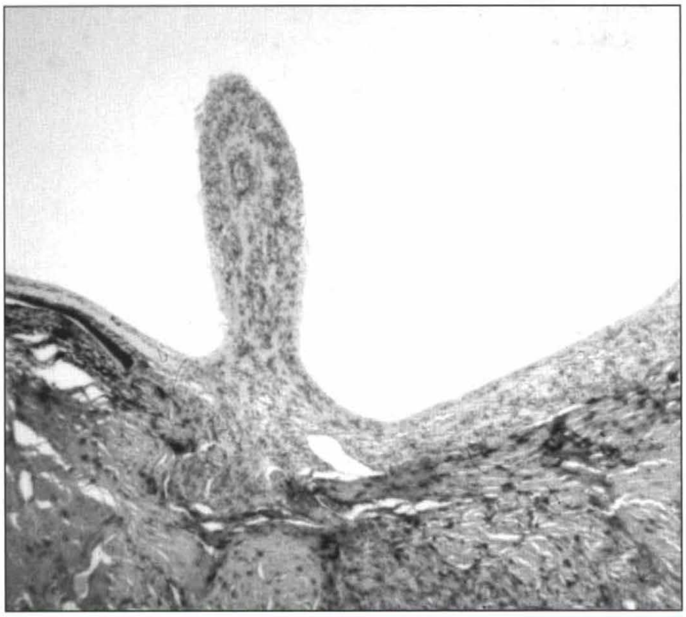

Figure 4.16 **Bergmeister's papilla**, the minimal histologic presentation of persistent hyaloid artery.

perivascular mesenchyme. Surrounding the hyaloid system is some primitive collagen, poorly characterized extracellular matrix, and a few macrophages. The combination of blood vessels and surrounding stroma is known as the *primary vitreous*. At least theoretically, *anomalous retention of the hyaloid artery and/or primary vitreous could therefore be separated into distinct entities of persistent hyaloid, persistent posterior tunica vasculosa lentis, persistent primary vitreous, and persistent hyperplastic primary vitreous*. Of these, the one most frequently reported (perhaps just because it is the most spectacular and significant) is **persistent hyperplastic primary vitreous**. In people, this rare anomaly is typically unilateral and is accompanied by microphthalmos, microphakia, retinal detachment, shallow anterior chamber, and embryonic filtration angles. The many reports of this anomaly in dogs have described a unilateral or bilateral retrolental vascular or fibrovascular network, usually without any other reported anomalies other than the expected posterior polar cataract. Such lesions are better described as *persistent posterior tunica vasculosa lentis*. In Doberman Pinschers, Bouviers and Staffordshire Bull Terriers, the classification as hyperplastic primary vitreous is more credible. In these breeds, the defect is inherited and forms a spectrum that includes persistent pupillary membrane, cataract, lenticonus, and microphthalmia as well as persistence of variable amounts of primary vitreous and posterior tunica vasculosa lentis (Fig. 4.17 A, B). The defects are detected as early as gestational day 30, at which time hyperplasia of posterior tunica vasculosa lentis is already obvious. Posterior polar cataracts and preretinal membranes are observed by day 37. The one report of two cases in cats was not supported by histopathology and its correct classification remains unknown.

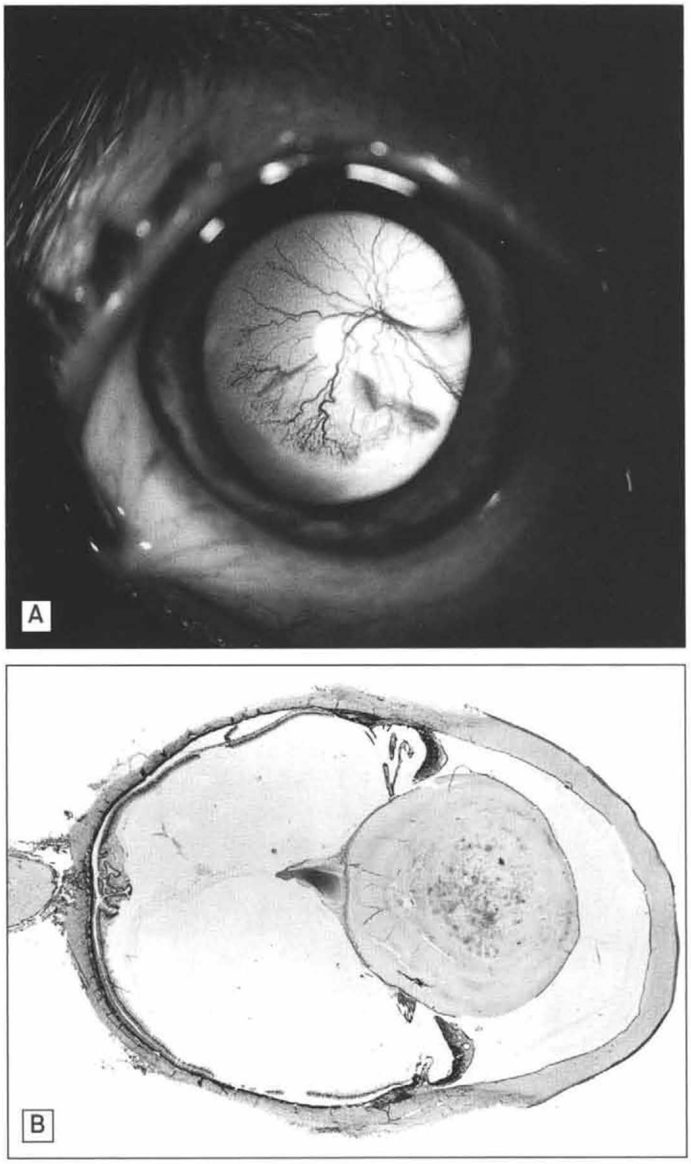

Figure 4.17 **A. Persistence of hyaloid artery and posterior tunica vasculosa lentis** in a dog. Posterior polar cataract is the almost inevitable consequence, as seen here. **B. Persistent hyperplastic primary vitreous.** A fibrocartilaginous plaque adheres to an elongated lens (lenticonus) and extends to the posterior pole. Retina is dysplastic near the optic disk, and the ciliary processes have not retracted from the lens capsule.

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Anomalies of neurectoderm

Included under this heading are anomalies of retina, optic nerve, and of neuroepithelium of iris and ciliary body. Of these, *retinal anomalies are by far the most frequent and most significant.*

Retinal dysplasia

Retinal dysplasia *is a general term denoting abnormal retinal differentiation characterized by jumbling of retinal layers and by glial proliferation.* In clinical practice, the term has been used incautiously to include genuine retinal dysplasia, postnecrotic retinal scarring of the developing retina, and retinal folding. Genuine retinal dysplasia results from failure of proper apposition of the two layers of the optic cup or from failure of proper induction by an inherently defective retinal pigment epithelium. Those examples clinically classified as retinal dysplasia that reflect disordered wound healing of developing retina, or just folding of retina that is otherwise perfectly formed, should not be considered examples of true retinal dysplasia. While there is no formal agreement on the issue, it seems most logical to divide so-called retinal dysplasia into three different categories: *retinal folds* within an otherwise normal retina, *postnecrotic dysplastic retinal wound healing*, and rare *"true" retinal dysplasia* that is a sequel to some fundamental developmental error in retinal induction or maturation.

Those examples of retinal dysplasia in which the only abnormality is **retinal folding** are by far the most common, and are seen primarily in dogs. The anatomic location, ophthalmoscopic appearance, and effect on vision vary from breed to breed but tend to be uniform within each breed, a fact used by clinical ophthalmologists when attempting to distinguish inherited dysplasias from those occurring as isolated anomalies or as sequelae to in utero infections. The most severe examples occur in dogs with retinal nonattachment. These have extensively folded retinas since the distance to be traversed from optic disk to ora ciliaris in a straight line is shorter than the convex route taken by attached retina, and redundant retina is therefore obliged to fold upon itself. Less severe examples, which are seen much more frequently and in the greatest variety of breeds, probably reflect inequity in growth rate between the retina and the outer layer of the optic cup (choroid and sclera). *The most common example is probably seen in Collie eye anomaly and in American Cocker Spaniels*, in which the defect may be transient. Presumably, the folds become flattened and disappear as continued choroidal and scleral growth eventually create a globe that can accommodate the retina. At least in Collies (and other related breeds with syndromes similar to Collie eye anomaly), *the retinal lesion is probably secondary to defective RPE.* Since the other defects of choroidal/scleral formation and maturation are attributed to faulty induction by a defective RPE, it is reasonable to attribute the retinal folding to the same mechanism (Fig 4.18).

A histologically similar retinal folding that may not depend on retinal:scleral growth imbalance is seen in English Springer Spaniels. Changes are seen as early as gestational day 45 and always by day 55. Focal infolding of the neuroblastic layer away from the retinal pigment epithelium and focal loss of the junctions between the neuroblasts (the outer limiting membrane) are the early changes, followed by overt focal retinal separation and extensive retinal folding. In all breeds in which this type of dysplasia has been adequately studied, it is inherited as a simple autosomal trait.

Retinal dysplasia as a sequel to **retinal necrosis** can occur as a sequel to a wide variety of viral and physical-chemical insults to the embryonic eye; naturally occurring examples are almost exclusively viral. Since the carnivore retina continues to develop for about 6 weeks after birth, the opportunity is great for postnatal injury in

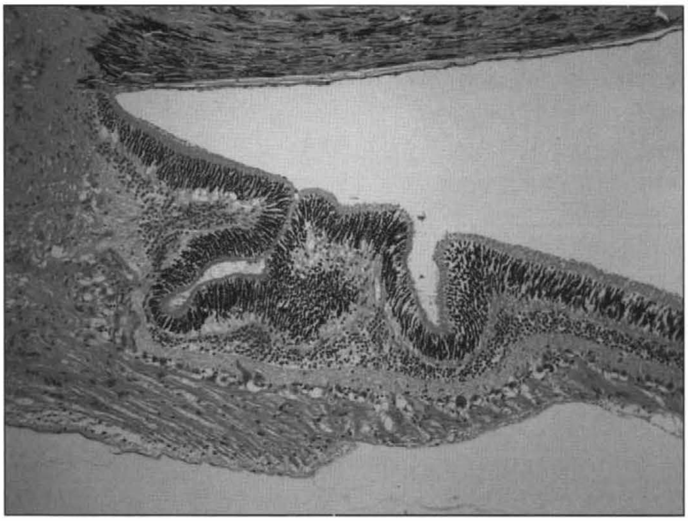

Figure 4.18 Retinal folding, presumably as a result of retinal redundancy that may eventually self-correct as the scleral shell grows to accommodate the retina. Retinal histologic organization is normal, distinguishing this from true retinal dysplasia.

puppies and kittens to produce retinal maldevelopment. Retinal maturation is most rapid in central (peripapillary) retina and progressively less towards the periphery, so that occasionally dysplastic lesions may be encountered only in peripheral retina – suggesting a viral (or other) injury quite close to the 6-week-old limit for dysplasia of this pathogenesis. Mature retina will scar but will not develop lesions of dysplasia.

The specific viruses implicated in domestic animals are *Bovine viral diarrhea virus* in cattle, *Bluetongue virus* in sheep, herpesvirus in dogs, and both parvovirus and leukemia virus in cats. The histologic lesion is similar for all diseases, with variation in lesions caused by the same virus in one species as great as the variation caused by different viruses in different species. *The most significant clue suggesting viral rather than genetic cause is the presence of residual inflammation and postnecrotic scarring in retina, optic nerve and, perhaps subtly, in choroid.* Injured retinal pigment epithelium undergoes one or more of reactive hyperplasia, migration into injured retina as discrete pigmented cells in areas of scarred retina, or metaplastic formation of multilayered fibroglial plaques in place of normal simple cuboidal epithelium (Fig. 4.19A, B). Disorganization of nuclear layers and rosette formation are seen as in other types of dysplasia.

Infection of calves with *Bovine viral diarrhea virus* between 79 and 150 days gestation is the most frequently encountered and thoroughly studied retinal dysplasia of known viral etiology. Work with other viruses has been too limited to allow definition of the susceptible period in fetal development or of the full range of resultant lesions. The limited descriptions of the other viral-induced retinal lesions suggest that the sequence of events is probably quite similar for all such agents.

The initial ocular lesion is nonsuppurative panuveitis and retinitis with multifocal retinal and choroidal necrosis. The acute inflammatory disease gradually subsides over several weeks, and most cases of spontaneous abortion or neonatal death retain scant vestige of previous inflammation. Those ocular structures already well differentiated at the time of the endophthalmitis (cornea, uvea, optic nerve) may undergo atrophy and scarring or be left virtually untouched. Other tissues, such as retina, are actively differentiating and exhibit a combination of the above atrophy and scarring as well as abortive regeneration and arrested differentiation. Retinal pigment epithelium in most examples (*Bluetongue virus* being an apparent exception) is infected and subsequently injured. *The result is a patchy alternation of abortive retinal regeneration, hyperplastic pigment epithelium, and postnecrotic glial scarring* (Fig. 4.19C). The lesions are usually more severe in nontapetal retina and are bilateral but not necessarily symmetrical. It seems reasonable to speculate that those naturally occurring cases in which the dysplasia is confined to peripheral retina represent late viral infection when only peripheral retina is still differentiating.

Because the virus has affinity for other neural tissues, *all calves with retinal dysplasia induced by Bovine viral diarrhea virus also have cerebellar atrophy, and some have hydrocephalus or hydranencephaly.* A similar association with hydrocephalus and other brain anomalies has been described for *Feline panleukopenia virus* infection in cats, *Bluetongue virus* infection in sheep, and in a possibly hereditary syndrome in white Shorthorn and Hereford cattle. In the latter two instances, the involvement of virus could not be excluded based upon published information.

Experimental irradiation of neonatal puppies (and, presumably, kittens) results in retinal necrosis and scarring virtually indistinguishable from postviral retinal dysplasia.

True retinal dysplasia, not associated with exogenous infection or teratogen, is rare. *It is characterized by retinal folds, retinal rosettes, patchy to diffuse blending of nuclear layers, loss of retinal cells and glial scars.* The folds and rosettes are the histologic counterparts of the vermiform streaks seen on the fundus with the ophthalmoscope. *The hallmark of retinal dysplasia is the rosette*, composed of a central lumen surrounded by 1–3 layers of neuroblasts. The three-layered rosette is the most common in naturally occurring cases in animals, and shows more or less complete retinal differentiation. Most such rosettes are probably retinal folds cut transversely (and, as mentioned above, should not be considered true dysplasia if no additional lesions are present). The lumen contains pink fibrils resembling photoreceptors and is bounded by a thin membrane resembling the normal outer limiting membrane. One- and two-layered rosettes are encountered infrequently and consist of a lumen surrounded by undifferentiated neuroblasts.

True retinal dysplasia occurs in combination with chondrodysplasia in several dog breeds, but particularly in Labrador Retrievers and Samoyeds. Cataract and persistent hyaloid remnants may accompany the retinal lesion. In Labradors, all the defects are the result of a single gene, with recessive effects on the skeleton and incompletely dominant effects on the eye.

Optic nerve hypoplasia

Hypoplasia is the most common anomaly of the optic nerve. The defect may be unilateral or bilateral, and usually occurs in eyes with other anomalies and particularly in eyes with retinal dysplasia. *In most instances, the so-called 'hypoplasia' is more likely to be atrophy* as the inevitable result of the destruction of ganglion cells in glaucomatous, viral, toxic, genetic, or idiopathic retinal disease (Fig. 4.20). The only clear example of an alternative pathogenesis is that associated with *maternal deficiency of vitamin A in cattle,* in which atrophy of the developing optic nerve results from failure of remodeling of the optic nerve foramen and subsequent stenosis. A similar lesion occurs in *pigs*, but in that species hypovitaminosis A seems more indiscriminately teratogenic, and optic nerve hypoplasia is accompanied by diffuse ocular dysplasia and multiple systemic anomalies. Hypoplasia is a relatively frequent clinical diagnosis in *toy breeds of dogs*, without apparent visual defects (and thus rarely receives histologic examination). Most examples are probably hypomyelination of the optic disk, which results from premature halt of myelinated nerve fibers at, or posterior to, the lamina cribrosa. The opposite, with myelin extending too far into the nerve fiber layer of the peripapillary retina, is also seen in dogs and is a frequent but insignificant occurrence in horses.

Inherited optic nerve hypoplasia is documented in one strain of laboratory mice, although it may accompany inherited retinal dysplasia or multiple inherited anomalies in any species. Histologic examination of affected eyes reveals few if any ganglion cells and a thin and moth-eaten nerve fiber layer.

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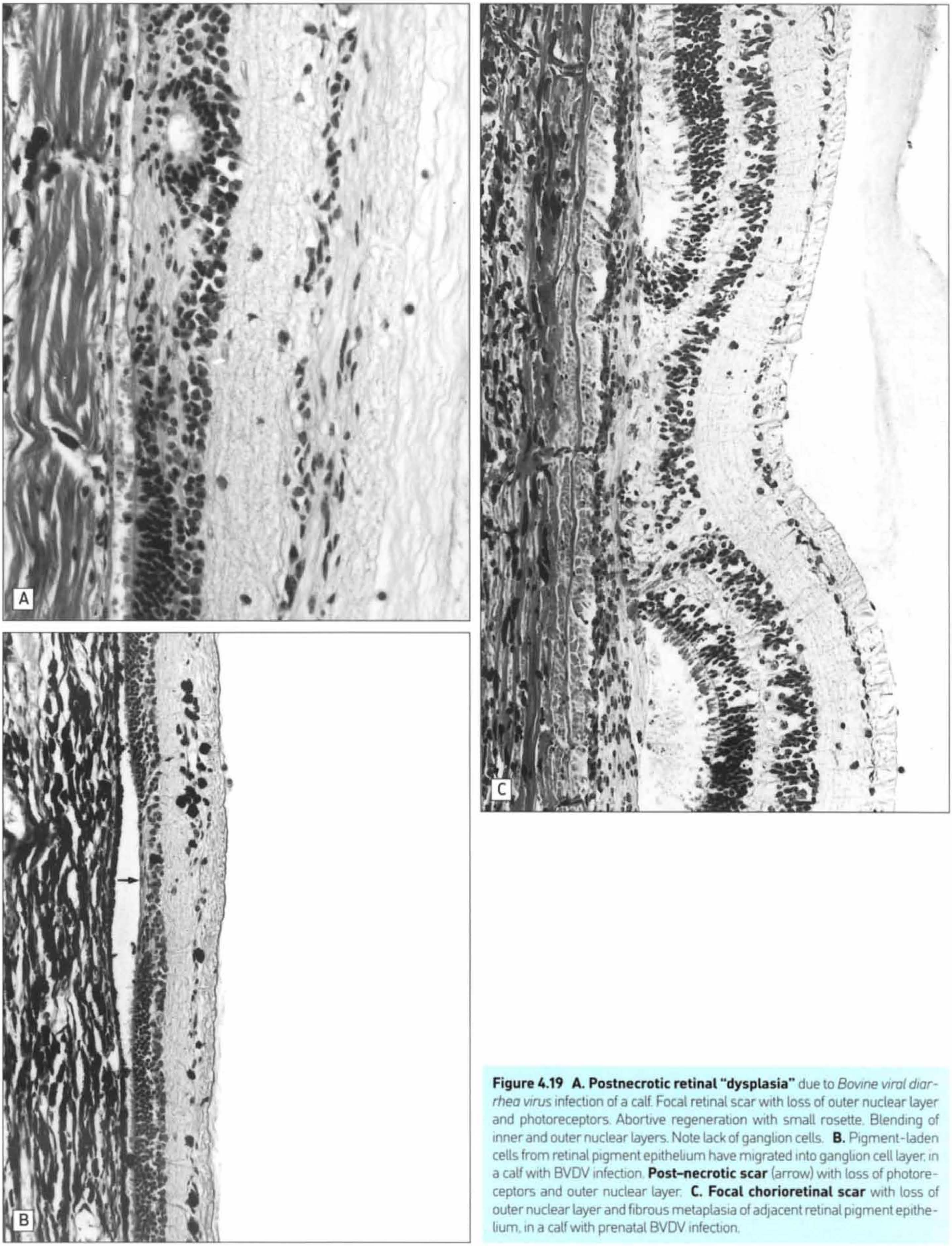

Figure 4.19 A. Postnecrotic retinal "dysplasia" due to *Bovine viral diarrhea virus* infection of a calf. Focal retinal scar with loss of outer nuclear layer and photoreceptors. Abortive regeneration with small rosette. Blending of inner and outer nuclear layers. Note lack of ganglion cells. **B.** Pigment-laden cells from retinal pigment epithelium have migrated into ganglion cell layer, in a calf with BVDV infection. **Post-necrotic scar** (arrow) with loss of photoreceptors and outer nuclear layer. **C. Focal chorioretinal scar** with loss of outer nuclear layer and fibrous metaplasia of adjacent retinal pigment epithelium, in a calf with prenatal BVDV infection.

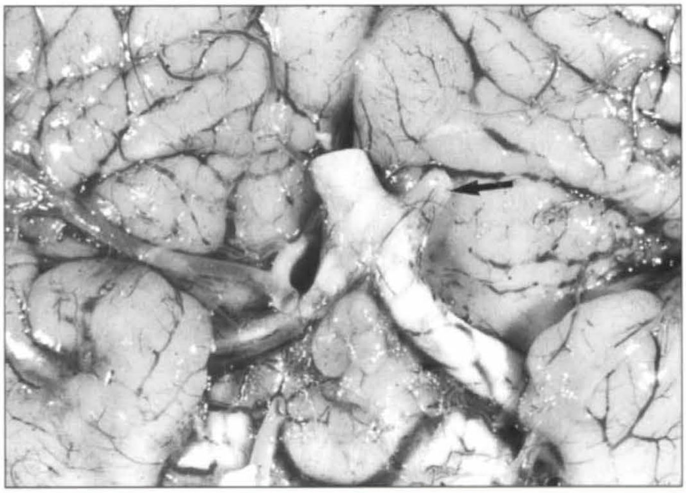

Figure 4.20 Optic chiasm in a foal with unilateral secondary (degenerative) microphthalmos. **Small left optic nerve** (arrow) due to prenatal atrophy following ganglion cell destruction.

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Anomalies of surface ectoderm

From fetal surface ectoderm are derived corneal epithelium, lens, lacrimal apparatus, and the epithelial portions of the eyelids and associated adnexa. Seldom are anomalies of the extraocular structures the subject of histopathologic study inasmuch as they are clinically obvious and of significance only if they result in corneal irritation, impaired vision, or unacceptable appearance.

Excessively large or small palpebral fissures are part of current fashion in some dog breeds. *Micropalpebral fissure frequently leads to entropion* as the lid margin curls inward, and resultant corneal abrasion necessitates surgical correction. Congenital entropion also occurs as sporadic flock epizootics in lambs, but whether this is a structural deformity or the result of eyelid spasm is unclear. Entropion associated with microphthalmos occurs in all species. Other eyelid defects include colobomas, which are focal to diffuse examples of eyelid agenesis, and delayed separation of the eyelid fusion, which is the normal state during organogenesis.

Disorders of cilia are very common in dogs, but uncommon in other species. Congenital defects include one or more of ectopic cilia, misdirected but otherwise normal cilia (**trichiasis**), the occurrence of a second row of cilia from the orifice of normal or atrophic Meibomian glands (**distichiasis**), and excessively large cilia (**trichomegaly**). In each instance, the significance of the anomaly depends on the presence or absence of corneal irritation.

The lacrimal gland and its ducts develop from an isolated bud of surface ectoderm and, while anomalies must surely exist, they have not been investigated. *Failure of patency of the lacrimal puncta* occurs in dogs and horses and manifests as excessive tearing. Ectopic or supernumerary openings have been reported in dogs and in cattle.

Corneal anomalies

Primary corneal maldevelopment is rare in all species. The category may be expanded to include *corneal dystrophies*, defined as bilateral, inherited, and usually central corneal opacities that, despite their typically adult onset, presumably have a congenital basis. These rare lesions will be discussed with degenerative diseases of the adult cornea.

Corneal anomalies may be ectodermal or mesenchymal, and may affect one or more of corneal size, shape, or transparency. **Microcornea** refers to a small but histologically normal cornea in an otherwise normal globe. A small cornea occurring in a microphthalmic globe is expected and does not merit a separate description. Mild microcornea of no clinical significance is reportedly common in certain dog breeds. **Megalocornea** has not been reported in domestic animals, except in predictable association with congenital buphthalmos.

Dermoid is a congenital lesion of cornea or conjunctiva characterized by *focal skin-like differentiation*, and as such is properly termed a **choristoma.** They occur in all species. There is one report of a geographically high prevalence of multiple, and sometimes bilateral, dermoids as an inherited phenomenon in polled Hereford cattle in the American midwest, but ordinarily they seem to occur as single, random anomalies of unknown pathogenesis. Defective induction (skin instead of corneal epithelium) by the invading corneal stromal mesenchyme is the most popular speculation.

The *degree of differentiation varies,* but most consist of stratified squamous keratinized and variably pigmented epithelium overlying an irregular dermis containing hair, sweat glands, and sebaceous glands. Very rarely, cartilage or bone is seen. The degree of adnexal differentiation varies widely but may approach that of normal skin (Fig. 4.21A, B, C). At the edge of the dermoid, the dermal collagen reorients to blend with the regular stroma of cornea, and the epidermis transforms itself to corneal epithelium. Surgical removal may be for cosmetic reasons, or may be required if dermoid hairs irritate cornea, or if the position of the dermoid interferes with vision. In most instances of corneal dermoid, the choristoma is attached to the surface of a corneal stroma of normal thickness, so excision of the dermoid should not risk perforation of the globe.

Congenital corneal opacities are *usually caused by anomalous formation of the anterior chamber, particularly congenital anterior synechiae and persistent pupillary membranes.* Adherence of anterior chamber structures to the corneal endothelium, or perhaps their interposition during ingrowth of the corneal endothelium, results in focal absence of the corneal endothelium and disorganization of adjacent corneal stroma. Grossly, the affected cornea has deep stromal opacity caused by stromal edema or fibrosis in the area of the defective endothelium. Pigment, originating from adherent uveal strands, may be found in the corneal stroma. The opacity may be diffuse or focal, depending on the extent of uveal–corneal adhesion. Many examples are part of a more widespread anterior segment dysgenesis (see under Defects primarily in anterior chamber mesenchyme).

Diffuse, congenital corneal opacity occurs in Holstein-Friesian cattle in England and Germany. The histologic lesion is diffuse corneal edema but its pathogenesis is unknown. The cornea remains permanently opaque.

Corneal opacity caused by noncellular depositions occurs in dogs and is usually of adult onset despite an apparently genetic basis. The exception is multifocal, subepithelial deposition of basophilic, PAS-positive material in the corneas of puppies with Collie eye anomaly or other mesodermal dysgeneses. The material is of unknown origin and may be the histologic counterpart of the transient, multifocal, subepithelial opacities seen quite commonly in 2–3-week-old puppies whose eyes are otherwise normal and thus unavailable for histologic examination.

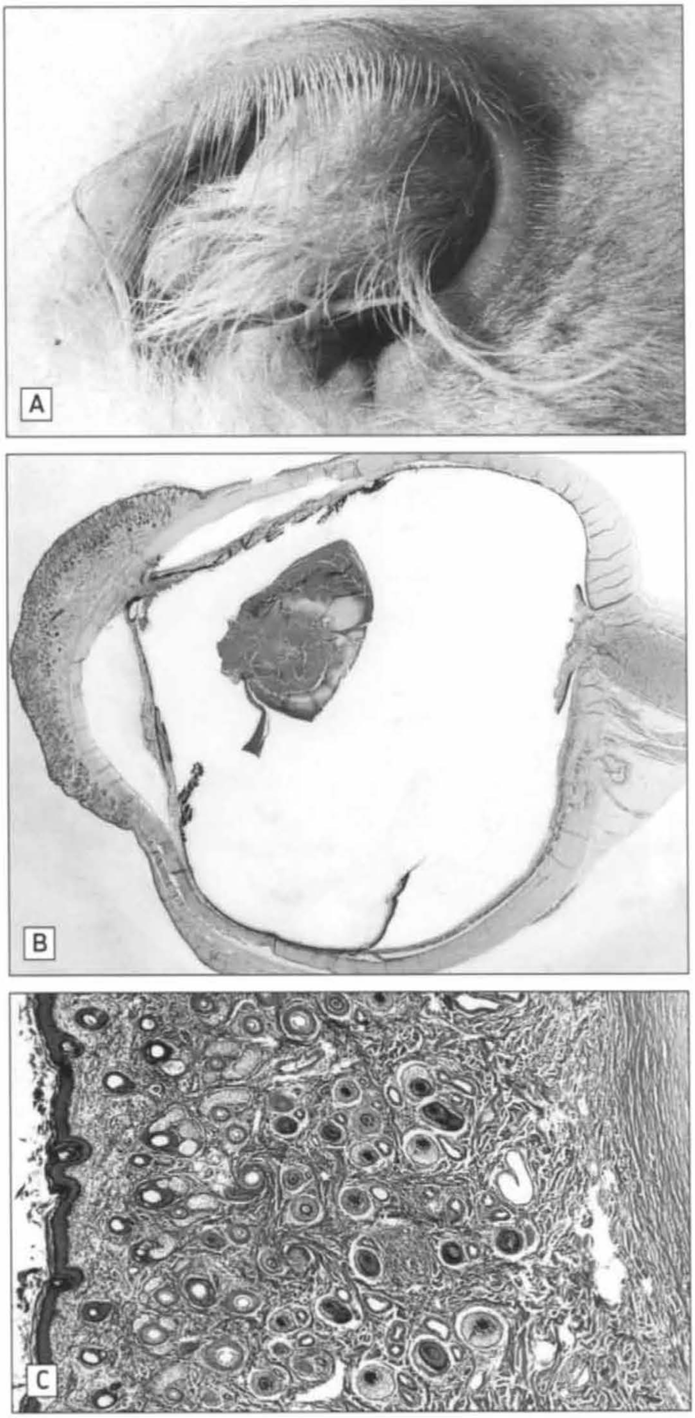

Figure 4.21 Corneal dermoid in a calf. **A.** Notch-like defect in lower lid is a coloboma. **B.** Anterior rupture of lens capsule with well-organized anterior synechia, probably from foreign-body perforation. **C. Corneal dermoid** in a calf; same eye as in **(A)** and **(B)**. Development approaches that of normal skin. Note abrupt transition to dense regular corneal stroma at deep margin of the dermoid.

Anomalies of lens

The lens may be abnormally small, abnormally shaped, ectopic, or cataractous. Of these, *only ectopia and cataract are common.*

Aphakia *is the congenital absence of lens, and it may be primary or secondary.* It is claimed that primary aphakia is possible only in a rudimentary globe because of the central role of lens in the induction of invagination of the primary optic vesicle. Any globe with the structure of optic cup, regardless of how dysplastic, must have had a lens early in organogenesis and its absence later must be the result of degeneration. This assumption is an extrapolation from work done many years ago in chicken embryos; while no work has been published to refute this contention, there is no work in mammals to confirm it. In the one report of aphakia in modern literature that includes histologic examination, several other puppies had small lenses and all had invaginated optic cups with iris and retina. There was no conclusion about the nature of the injury to the developing eyes or the timing of such injury.

Microphakia, *or congenitally small lens*, is reported in dogs, calves, and cats, but is nonetheless rare. Most such reports describe the defect in association with ectopia lentis, microphthalmos, and anterior chamber mesenchymal anomalies. Such lenses are spherical and almost always are cataractous.

Lenticonus and **lentiglobus** *are rare defects of lens shape* characterized by an abrupt change in capsular configuration so that the lens acquires a globular or conical protrusion. The defect is usually polar and, in animals, usually posterior. From scattered and very old descriptions, it is difficult to define the "typical" histology of such lesions or their pathogenesis. The defect usually appears as a focal overgrowth of cortical lens fibers covered by thin posterior lens capsule and retained posterior epithelium. Of four relatively recent descriptions, all of canine eyes, all had congenital cataract but only in one did the cataract involve the protruding lens fibers themselves. Other ocular lesions reported include hyperplasia of tunica vasculosa lentis, rupture of the lens protrusion, and dysplasia of ciliary epithelium. At least in Doberman Pinscher dogs, the posterior lentiglobus or lenticonus accompanying hyperplastic tunica vasculosa lentis appears to be an acquired defect caused by the abnormal fibrovascular elements adherent to lens.

Congenitally ectopic lenses occur in all species, but are relatively common only in dogs and horses. *Much more common than congenital luxations are spontaneous luxations in adult dogs*, which may be associated with acquired lesions of the zonule. The reason for the particular susceptibility of small terriers and Poodles to spontaneous lens luxation is unknown.

Congenital cataract occurs in most severely anomalous eyes, but may occur as an isolated ocular lesion. When cataract is present in eyes with multiple anomalies, it usually results from persistence of some part of perilenticular vasoformative mesoderm, but may also result from intraocular inflammation or toxic degeneration. Persistence of pupillary membrane or hyaloid system frequently results in multiple epithelial defects and subcapsular opacities at the sites of mesodermal contact with the lens.

In dogs, congenital primary cataracts are frequently hereditary but, as with corneal and retinal diseases, most hereditary cataracts are not congenital. Subtle, nonprogressive nuclear or cortical opacities are common but clinically insignificant in dogs and are of unknown pathogenesis. Primary, and usually diffuse, cataract is the most common ocular anomaly of horses. The pathogenesis is unknown, but there is usually no other ocular lesion. Congenital nuclear cataracts have been described as an inherited lesion in Morgan horses in the United States.

Congenital cataract is rare in cattle, swine, sheep, and goats. In cattle, hereditary congenital cataract occurs in Holstein-Friesians and in Jerseys and is thought to be an autosomal recessive trait. It is also seen as an infrequent result of fetal infection with *Bovine viral diarrhea virus*.

There is a single report of bilateral, complete cataracts in a litter of Persian kittens, but there are no examples in swine or small ruminants except in association with multiple ocular defects.

The pathology of congenital cataract is the same as acquired cataract, and is discussed later. It may be nuclear, cortical or capsular, focal or diffuse, stationary or progressive depending upon the timing and pathogenesis of the original injury.

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Ocular adnexa

The adnexa include eyelids, nictitating membrane, lacrimal and accessory lacrimal glands. Developmental, degenerative, inflammatory, and neoplastic diseases of these structures are commonly encountered in clinical practice, but only the neoplasms and proliferative inflammatory lesions are regularly submitted for histologic examination.

Eyelids

Disorders of size and configuration of the palpebral fissure are common in purebred dogs, as are anomalies of number or placement of cilia. None requires histologic evaluation.

Blepharitis is *inflammation of the eyelid. Ordinarily, it refers to inflammation of the haired skin that covers the outer surface of the eyelid.* Diseases primarily affecting the palpebral conjunctiva that lines the bulbar surface of the eyelid would ordinarily be classified as conjunctivitis and are considered later. The diseases of the eyelid skin are, in general, exactly the same as diseases of skin elsewhere, and

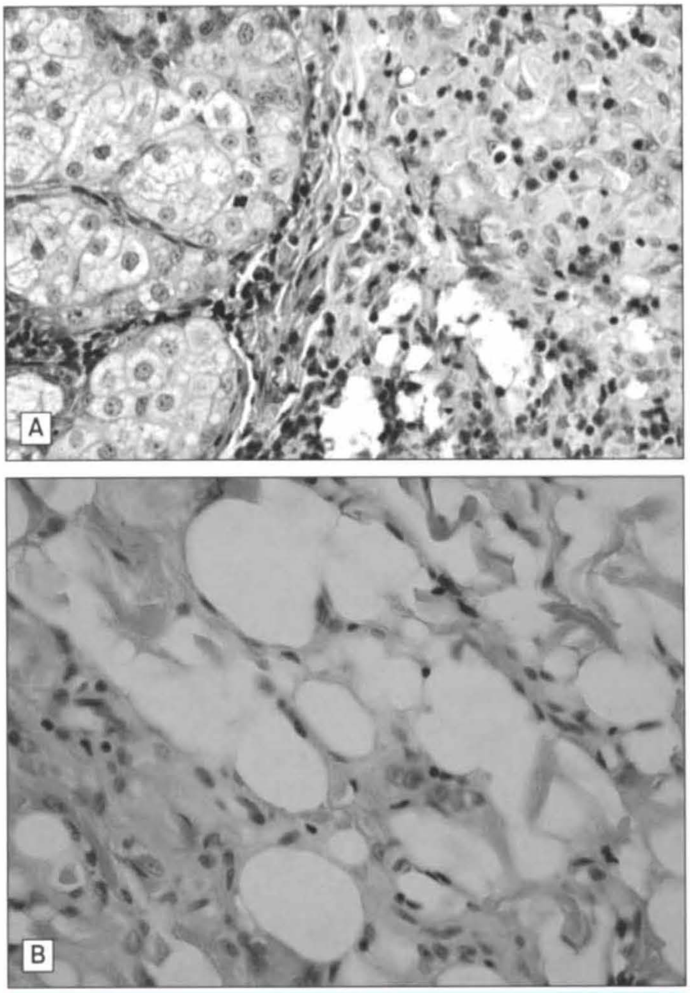

Figure 4.22 Chalazion **A.** Leaking Meibomian gland or, more often, Meibomian adenoma surrounded by foamy macrophages. **B.** In this variant (particularly common in cats), the leaking secretion forms **lipid lakes** with a relatively inconspicuous granulomatous reaction.

are thus not considered here in any detail. Inflammatory lesions more or less unique to eyelid include hordeolum, chalazion, and idiopathic granulomatous marginal blepharitis.

External hordeolum or **stye** is *suppurative adenitis of the adnexal glands of Zeis or Moll.* **Internal hordeolum** is *suppurative inflammation of the Meibomian gland.*

Chalazion is *sterile granulomatous inflammation in response to the leakage of Meibomian secretion into the surrounding dermis.* While it can theoretically occur in response to any type of injury to the Meibomian gland, almost all cases are found adjacent to Meibomian adenomas. The histologic lesion is distinctive and comes in two forms that may occur independently or in conjunction with one another. The more common is an accumulation of large foamy macrophages and multinucleated cells around the abnormal Meibomian gland. The macrophages contain distinctive refractile intracellular slender elongated crystals that have a bright silvery-white appearance when examined with polarized light. Alternatively, the reaction may consist of a mixture of these macrophages and lakes of free lipid. In routine sections, these appear as clear empty spaces that could be confused with dilated lymphatics (Fig. 4.22A, B). This latter variant was originally described in cats as idiopathic lipogranulomatous conjunctivitis (see below), but current opinion is that it is just a feline version of chalazion.

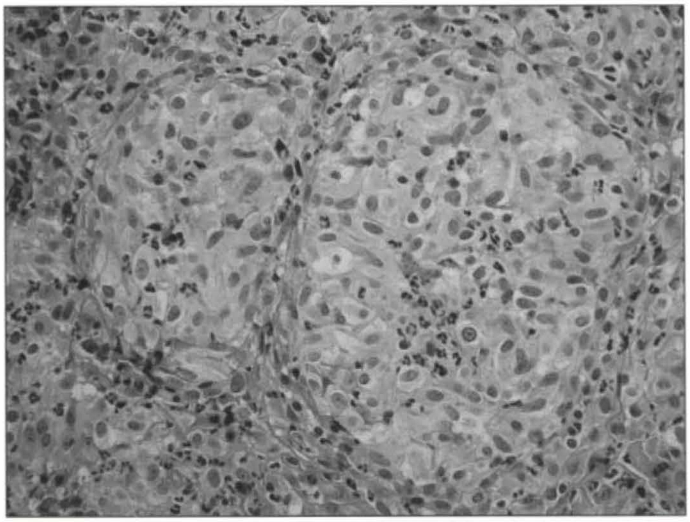

Figure 4.23 Granulomatous marginal blepharitis. Granulomas form around clear lipid vacuoles, sometimes with neutrophils. The location along the palpebral surface of the eyelid margin is critical to the diagnosis.

Idiopathic granulomatous marginal blepharitis is a uniquely canine lesion. The macroscopic lesion varies from a single nodule to a series of coalescing nodules that create virtually diffuse thickening of one or both eyelid margins. *The histologic lesion is a coalescence of suppurating granulomas in the subconjunctival tissue of the eyelid margin, without any proven association with adnexal structures and without any identifiable etiologic agent.* The granulomas often form around a clear central lipid vacuole, with or without neutrophils at the interface between the vacuole and the surrounding macrophages (Fig. 4.23). The lesion bears considerable similarity to cutaneous sterile pyogranuloma syndrome and other idiopathic granulomatous panniculitis syndromes, all of which are equally mysterious in terms of pathogenesis. *The lesions differ from those of chalazion in that the latter does not form discrete granulomas, does not involve neutrophils, and is always found adjacent to Meibomian glands.*

Lacrimal system

Acquired disease of the lacrimal system is probably quite common in dogs if one includes keratoconjunctivitis sicca (see Keratitis) and eversion of the gland of the third eyelid.

Dacryoadenitis *is inflammation of the lacrimal gland,* and may result from involvement in orbital cellulitis or orbital trauma, spread from severe intraocular inflammation, incidental involvement in systemic diseases such as malignant catarrhal fever, feline infectious peritonitis, and canine distemper; or apparently specific immunologic assault. Specific dacryoadenitis caused by a coronavirus is extremely common in laboratory rats in which acute necrotizing inflammation of lacrimal, Harderian and salivary glands results in eventual fibrosis and squamous metaplasia of affected glands. Residual lesions in mildly affected rats are multiple lymphoid aggregates in the glandular interstitium. Similar changes are often seen in dogs with keratoconjunctivitis sicca, and in the absence of demonstrated viral cause, are assumed to represent autoimmune lacrimal adenitis. The analogous lesion in people with Sjögren's syndrome is associated with influx of numerous T-helper cells into the gland, but no studies have yet been published to prove this immune pathogenesis for canine lacrimal adenitis and atrophy. However, the efficacy of cyclosporine, which acts primarily by suppression of T-helper cells, in reversing canine lacrimal adenitis provides evidence for such a pathogenesis.

Protrusion of the nictitans gland is quite common in dogs, and is thought to reflect a congenital laxity in the connective tissue anchoring the gland to the cartilage of the third eyelid. Because the resultant eversion is unsightly and resembles a neoplasm, these lesions frequently are excised even though the membrana nictitans may be normal except for overlying conjunctival inflammation from exposure and abrasion. Since this gland sometimes supplies a significant proportion of total lacrimal secretion, its surgical removal may be followed by keratoconjunctivitis sicca in dogs that have less than optimal function of the primary lacrimal gland. In dogs with keratoconjunctivitis sicca, the gland may suffer the same lymphocytic interstitial adenitis, fibrosis, and atrophy as affects the lacrimal gland itself.

Conjunctiva

At the orifice of the Meibomian glands, the epidermis of the lid undergoes abrupt transition to the pseudostratified columnar mucous membrane typical of the palpebral conjunctiva. Goblet cells increase in number from the lid margin to the fornix, but ordinarily are absent in that portion of conjunctiva that extends from the fornix to the corneoscleral junction (bulbar conjunctiva). Lymphoid aggregates are common in the subepithelial connective tissue, particularly below the bulbar conjunctiva and the inner aspect of the nictitating membrane. These aggregates are more prominent in the conjunctiva of horses than other domestic species. Whether this is normal or a reflection of increased antigenic stimulation of the conjunctiva in the dusty environment of many horse stables is unknown. The transition from conjunctival to corneal epithelium occurs at, or slightly central to, the corneoscleral junction, and is marked by gradual loss of pigment, rete ridges, subepithelial blood vessels, and lymphoid tissue.

The general pathology of the conjunctiva is similar to that of other mucous membranes. Acute conjunctival injury, whether physical, chemical, or microbial, results in hyperemia and severe edema. Evacuation of goblet cells and cellular exudation from the very labile conjunctival vessels add to the excessive lacrimation caused by any ocular irritation. The ocular discharge progresses from serous to mucoid and perhaps purulent with increasing severity of insult. *Chronic irritation* results in epithelial hyperplasia, hyperplasia of goblet cells and lymphoid aggregates, or even squamous metaplasia progressing to keratinization. The goblet cell hyperplasia is a very uncommon lesion when compared to squamous metaplasia and lymphoid hyperplasia. Lymphoid hyperplasia may be so marked as to result in grossly visible white nodules that may require surgical or chemical removal to reduce irritation of the adjacent cornea. Although characteristic of a number of etiologically specific conjunctival diseases, *lymphoid hyperplasia is best considered a nonspecific response to any chronic antigenic stimulation.* Conjunctivitis frequently accompanies other ocular disease, notably keratitis, uveitis, and glaucoma. Conversely, conjunctival inflammation may spread to cornea, uvea, and orbit, although only secondary corneal involvement is common.

The causes of **conjunctivitis** *include every class of noxious stimulus, including allergy and desiccation.* Because conjunctival biopsy is rarely performed until all therapeutic measures have failed, it is rare to identify an etiologic agent associated with conjunctivitis. The etiologic significance of bacterial, fungal, and even viral agents should be considered very carefully in light of the normal bacterial flora, and the high prevalence of conjunctival carriage of several viral agents in clinically normal animals. At least in dogs, the isolation of gram-negative organisms, especially coliforms, *Pseudomonas* and *Proteus*, should be considered significant in light of the almost exclusively gram-positive flora of normal conjuctiva. Conjunctivitis occurs in a wide variety of multisystem diseases, such as canine distemper and ehrlichiosis, equine viral arteritis and babesiosis, bovine viral diarrhea, malignant catarrhal fever, classical swine fever, rinderpest, African swine fever, and others. Conjunctivitis accompanies most viral and allergic diseases of the upper respiratory tract. Only those diseases in which conjunctivitis is particularly prominent are discussed here.

Infectious bovine rhinotracheitis (IBR) is usually accompanied by serous to purulent conjunctivitis, which can be confused clinically with *infectious bovine keratoconjunctivitis* ('pinkeye') caused by *Moraxella bovis* (discussed below). However, corneal involvement with IBR is uncommon and is never the central suppurating ulcer typical of infectious keratoconjunctivitis. In an unpredictable number of animals, multifocal white glistening nodules, 1–2 mm in diameter, may be seen on the palpebral or bulbar conjunctiva. They appear as early as 3 days after instillation of *Bovine herpesvirus 1* into the conjunctival sac, and represent hyperplastic lymphoid aggregates. Overlying conjunctiva may be ulcerated and the defect filled with fibrin. IBR is discussed in Vol. 2, Respiratory system; Vol. 2, Alimentary system; Vol. 3, Female genital system.

In contrast to the situation in dogs, most cases of conjunctivitis in *cats* are probably caused by infectious agents. The agents incriminated include mycoplasma, chlamydia, or herpesvirus. The diagnosis is usually made based upon clinical characteristics, the presence of other clinical signs, and demonstration of the infectious agent via PCR or culture. Histologic lesions are not etiologically specific, and demonstration of the specific infectious agent in histology or cytology samples (even with the aid of immunofluorescence) becomes progressively more difficult as the lesions age.

Felid herpesvirus 1 causes a combination of conjunctivitis, keratitis, and upper respiratory disease when it first infects young cats, but it may cause conjunctivitis alone as a recurring infection in older cats that had recovered from the initial disease.

Mycoplasma felis and *M. gatae* have been reported to cause conjunctivitis unassociated with other signs in immunosuppressed cats, but instillation of organisms into the conjunctival sac of healthy cats without prior corticosteroid administration does not cause disease. In addition, many of the cats with putative mycoplasma conjunctivitis have had concurrent infection with herpesvirus or with chlamydia. It is likely that the mycoplasma, which is a member of the normal feline conjunctival flora, acts as a medically significant opportunist rather than as a primary pathogen. The conjunctivitis is pseudodiphtheritic and initially is unilateral. Histologically there is nonspecific erosive and suppurative conjunctivitis. Diagnosis requires the demonstration of coccoid bodies in the periphery of conjunctival epithelial cells.

Chlamydophila psittaci usually causes unilateral conjunctivitis in cats of any age, without any other associated disease. The conjunctivitis is initially neutrophilic, but rapidly becomes a nonspecific mixed infection with subepithelial neutrophils, macrophages, lymphocytes, and plasma cells. Early in the disease (between days 7 and 14), typical intracytoplasmic inclusion bodies can be seen, and their detection is enhanced by immunofluorescent staining. Because the clinical signs are characteristic and disease is easily treated with tetracycline, histologic assessment is rarely required. In cases that are resistant to therapy, the disease is usually chronic and histologically nonspecific by the time biopsy is eventually done.

Parasitic conjunctivitis

Parasitic conjunctivitis is relatively common worldwide and may be caused by members of the genera *Thelazia, Habronema, Draschia, Onchocerca,* and several members of the family Oestridae. Of these, only *Thelazia* is truly an ocular parasite; the others cause eyelid, conjunctival, or orbital disease incidentally in the course of larval migration.

Members of the genus ***Thelazia*** are thin, rapidly motile nematodes 7–20 mm in length that inhabit the conjunctival sac and lacrimal duct of a variety of wild and domestic mammals worldwide. Their prevalence is much greater than the prevalence of conjunctivitis, suggesting that their number must be greater than usual before signs of conjunctival irritation are observed. The species most commonly associated with conjunctivitis in domestic animals are *T. lacrymalis* in horses; *T. rhodesi, T. gulosa, T. skrjabini* in ruminants; *T. callipaeda* in carnivores and humans; and *T. californiensis* in many species including dog, cat, bear, coyote, deer, and man. Female worms are viviparous, and larvae free in lacrimal secretions are consumed by flies of the genus *Musca* in which they develop for 15–30 days. The third-stage infective larvae migrate to the fly's proboscis and are returned to the conjunctival sac as the fly feeds.

Ocular habronemiasis results from deposition of larvae by the fly intermediate host, usually *Musca domestica* or *Stomoxys calcitrans*, in the moisture of the medial canthus of horses. Larvae of *Habronema muscae, H. microstoma,* or *Draschia (Habronema) megastomum* are the culprits. The burrowing larvae cause an ulcerative, oozing lesion about 0.5–1.0 cm in diameter at the medial canthus, which becomes progressively more nodular as a granulomatous reaction to the larvae mounts. Mineralized granules may be found within the lesion along with caseous debris, liquefaction, and viable larvae. The histologic lesion is similar to that of cutaneous habronemiasis, namely chronic eosinophilic and granulomatous inflammation surrounding live or dead larvae and eosinophils (Fig. 4.24).

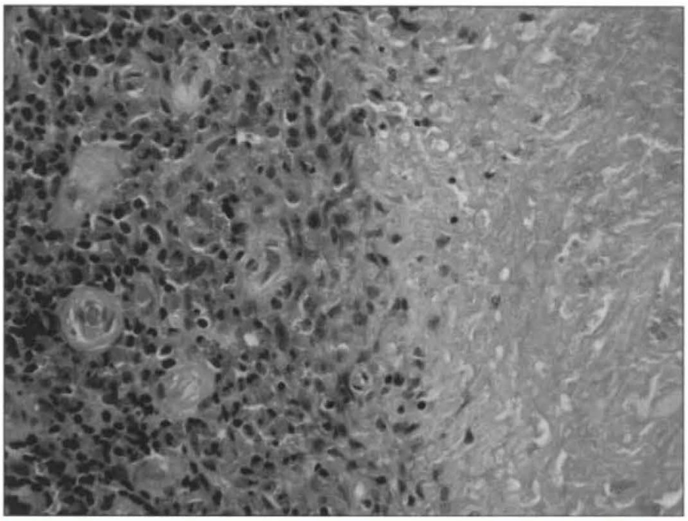

Figure 4.24 Conjunctival habronemiasis. Only rarely would fragments of larvae be detected within the center of these eosinophil-rich granulomas.

Ocular onchocerciasis results in the formation of granulomas and suppurating granulomas around fragmented or viable adult filarids within the sclera and subconjunctival lamina propria of dogs. Dogs are considered abnormal hosts for this parasite, which is much more commonly found in horses, cattle, and other ungulates. In horses, the infection causes a more diffuse eosinophilic and granulomatous conjunctivitis and peripheral stromal keratitis with a character similar to that in skin. Adults and microfilariae can be identified within the reaction.

Ophthalmomyiasis

A syndrome of periocular and even intraocular invasion by *fly larvae* occurs in various species including man. Its various manifestations are known collectively as ophthalmomyiasis. Specific *oculovascular myiasis*, "*uitpeuloog*," or "*gedoelstial myiasis*" is a disease of domestic ruminants and horses caused by invasion and migration of larvae of ***Gedoelstia*** spp. of Oestridae. The *Gedoelstia* are parasites of the blue wildebeest and hartebeest, the larvae being deposited in the eye, rather than in the nares as is the habit of *Oestrus ovis*. The most important member of the genus in terms of frequent aberrant parasitism in domestic species is *G. hassleri*, which in its natural antelope host migrates to the nasal cavity via the vascular system and cerebral meninges and subdural space. The parasitism is not clinically significant in the antelope, but in domestic species that are aberrant hosts, severe ocular and neural disease occurs, sometimes on a large scale. The disease is seasonal and occurs particularly in domestic ruminants in contact with wildebeest.

The ocular lesions vary from *transient mild conjunctivitis to destructive ophthalmitis* with orbital or periorbital edema or abscessation affecting one or both eyes. Neurological signs of varied pattern are common in sheep, partly due to the larvae directly and partly to thrombophlebitis marking their route of invasion. Thrombosis may be very extensive, may involve the jugular vessels and endocardium, and may cause sudden death when coronary vessels are affected.

Larval migration may be into the conjunctival sac, orbital tissues or into the eye itself. In the last instance, *ophthalmomyiasis interna*, the globe is often destroyed by the larval penetration. However, a syndrome of relatively harmless larval migration in the subretinal space or within vitreous has been reported in people. The characteristic subretinal linear tracks may be accompanied by focal retinal separation, preretinal and subretinal hemorrhage, and focal proliferations of retinal pigment epithelium. Two reported cases in cats had similar subretinal tracks, hyperplasia of pigment epithelium, and retinal hemorrhages. In one, the live motile larva was detected either on the face of, or just within, the retina. Subsequent examination failed to detect the larva, and the eye lesions resolved except for the subretinal tracks and pigment clumps.

The penetration is usually by a single larva despite numerous eggs or larvae within the conjunctiva. The larva may die within the globe or continue its migration by uneventful exit from the globe via sclera, optic nerve, or vessel adventitia.

Ophthalmomyiasis interna anterior has also been reported in a cat in association with infection with a first instar larva of ***Cuterebra*** spp. Severe anterior uveitis resolved after prompt surgical removal of the larva, but retinal degeneration and blindness ensued.

Immune-mediated conjunctivitis

Presumed allergic conjunctivitis occurs in all species but is most likely to be investigated in dogs (most examples of conjunctivitis in cats are assumed to have an infectious pathogenesis). Rarely is a specific allergen identified and, like its counterparts in allergic skin diseases, the diagnosis is based upon the failure to demonstrate infectious or mechanical causes, response to corticosteroid therapy, and sometimes a convincing association with environmental changes. Biopsy is rarely warranted, but when taken during the acute disease may show epithelial changes ranging from erosion to hyperplasia to squamous metaplasia, with eosinophils around dilated subepithelial blood vessels and percolating throughout the epithelium. Eosinophils are much more likely to be identified in cats than in dogs, a species difference that is also true of allergic skin disease in general. More chronic lesions, which are the more usual to be biopsied, have squamous metaplasia and lymphocytic-plasmacytic subepithelial infiltrates and the formation of lymphoid nodules.

There are a few histologically distinctive examples of conjunctivitis that are assumed to represent immune-mediated disease, mostly because they respond only to aggressive immunosuppressive therapy. In some dogs with chronic conjunctivitis (perhaps particularly German Shepherd Dogs), the infiltrate sometimes becomes particularly plasmacytic, diffuse, and thick in a fashion resembling an interface dermatitis. The bulbar surface of the third eyelid is the favorite location, and many believe this lesion (sometimes referred to as "*plasmoma*") to be the conjunctival variant of pannus keratitis.

Cats and horses may develop a severe **eosinophilic conjunctivitis** that is thought, by some, to be a conjunctival counterpart of the eosinophilic keratitis syndrome. Lesions may be unilateral or bilateral, and at least in cats there is almost always a concurrent ulcerative marginal blepharitis. Histologic changes include ulceration, epithelial hyperplasia, squamous metaplasia, and a heavy lymphocytic infiltration with a large proportion of eosinophils. Lesions in cats contain no detectable infectious agent, and no herpesviral DNA can be detected (the role of herpesviral infection in the pathogenesis of histologically similar eosinophilic keratitis in cats remains controversial). The clinical syndrome is rapidly responsive to topical corticosteroid administration.

Ligneous conjunctivitis is a distinctive clinical and histologic entity, thus far described only in Doberman Pinscher dogs. The clinical disease is bilateral and characterized by marked thickening and opacity of the palpebral conjunctiva and conjunctiva of the third eyelid. Histologically, the conjunctival lamina propria is thickened by massive deposition of hyaline material and a diffuse scattering of mononuclear leukocytes. Most of the leukocytes are T-lymphocytes, and the hyaline material stains weakly for IgG and IgA.

Feline lipogranulomatous conjunctivitis is probably the feline counterpart of canine chalazion. The lesion occurs almost exclusively in the lamina propria of the palpebral conjunctiva adjacent to the margin of either the upper or lower eyelid. The histology is very repeatable, consisting of a nodular accumulation of clear lipid lakes intermingled with large foamy macrophages and a few small or mononuclear leukocytes. Although the original report contained no mention of adjacent Meibomian lobules, the similarity between this entity and some cases of canine chalazion (or granulomatous dermatitis adjacent to injured cutaneous sebaceous glands) is striking and impossible to ignore (see Fig. 4.22B).

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CORNEA

The cornea of domestic mammals is a horizontal ellipse varying from 0.6–2.0 mm in thickness among the various species. In general, the larger and older the animal, the thicker is the cornea. It appears as a structural and physiologic modification of sclera, and when chronically injured may lose the specialized features of cornea and resemble sclera both ophthalmoscopically and histologically. Embryologically, the epithelium is derived from surface ectoderm; the stroma and corneal endothelium are from neural crest mesenchyme.

The major attribute of cornea is its clarity, and it is the loss of clarity that is the most obvious indicator of corneal disease. The clarity results from several highly specialized anatomic and physiologic features: an unusually regular, nonkeratinized and nonpigmented surface epithelium; an avascular, cell-poor stroma composed of very thin collagen (mostly type I) fibrils arranged in orderly lamellae separated by a critical distance to allow the uninterrupted passage of light (620–640 Angstroms); and a high degree of stromal dehydration maintained by the presence of epithelial tight junctions, endothelial tight junctions, and a Na-K-dependent ATP-ase pump in the cell membrane of the corneal endothelium (Fig. 4.25A).

The cornea exists in a privileged environmental niche, bathed in the nurturing and antimicrobial saline of the tear film and protected from other irritation by the movable eyelids. Within this protected niche, the cornea does therefore not require the protective attributes of skin (keratinization, leukocytes, blood vessels) to protect itself from the harsh external environment. If there is rapid deterioration in any component of this protective environment, the cornea is most likely to respond with ulceration and subsequent wound healing. If, on the other hand, the shift is of gradual onset, then the more likely response is adaptive metaplasia in which the cornea reaches into its embryologic cutaneous heritage and becomes skin-like.

Corneal injury may result from physical or chemical trauma, microbial agents, increased intraocular pressure and, rarely, from inborn errors of metabolism. Specific features of some of these injuries will be discussed later, but the general reactions to most corneal injuries are presented here.

Corneal edema

Corneal edema occurs rapidly following injury and results from imbibition of lacrimal water through damaged corneal epithelium, absorption of anterior chamber water at a site of corneal endothelial damage, or failure of electrolyte (and thus water) extrusion by the corneal endothelium. If the epithelial or endothelial defect is focal, the resultant edema is limited to the stroma adjacent to the defect. The edematous cornea is clinically opaque, and may be up to five times its normal thickness (Fig. 4.25B). At least experimentally, edema subsequent to endothelial damage tends to be more severe than edema secondary to epithelial injury. Edematous stroma stains less intensely than normal, and collagen lamellae are separated into a fine feltwork of pale-staining fibrils by excessive hydration of the glycosaminoglycan ground substance (principally keratan sulfate and chondroitin sulfate). Percolation of stromal fluid into the epithelium results in the intercellular and intracellular edema known as *bullous keratopathy* (Fig. 4.25C).

Edema may also be part of more chronic corneal disease. Corneal vascularization in response to severe injury is accompanied by edema, as the porous new capillaries leak fluid into the interstitial spaces. A small amount of perivascular corneal edema frequently accompanies the deep stromal vascularization seen in chronic anterior uveitis of any cause. Sometimes the edema is unexpectedly diffuse, severe, and may persist even after the uveitis itself has subsided. Such eyes have a neutrophilic or lymphocytic destructive endothelialitis, with leukocytes interspersed among the vacuolated, pyknotic endothelial cells (see later section Uveitis). Other examples of corneal edema are seen in glaucoma and anterior segment anomalies. In the former, it is assumed that the high aqueous pressure drives fluid into the hydrophilic corneal stroma to a degree that overcomes the endothelial ion pump that dehydrates the stroma under normal conditions. In anterior segment anomalies, persistent pupillary membranes or congenital anterior synechiae cause focal defects in endothelial continuity.

Persistent corneal edema seems to predispose to stromal vascularization and fibrosis, but numerous experimental models show that

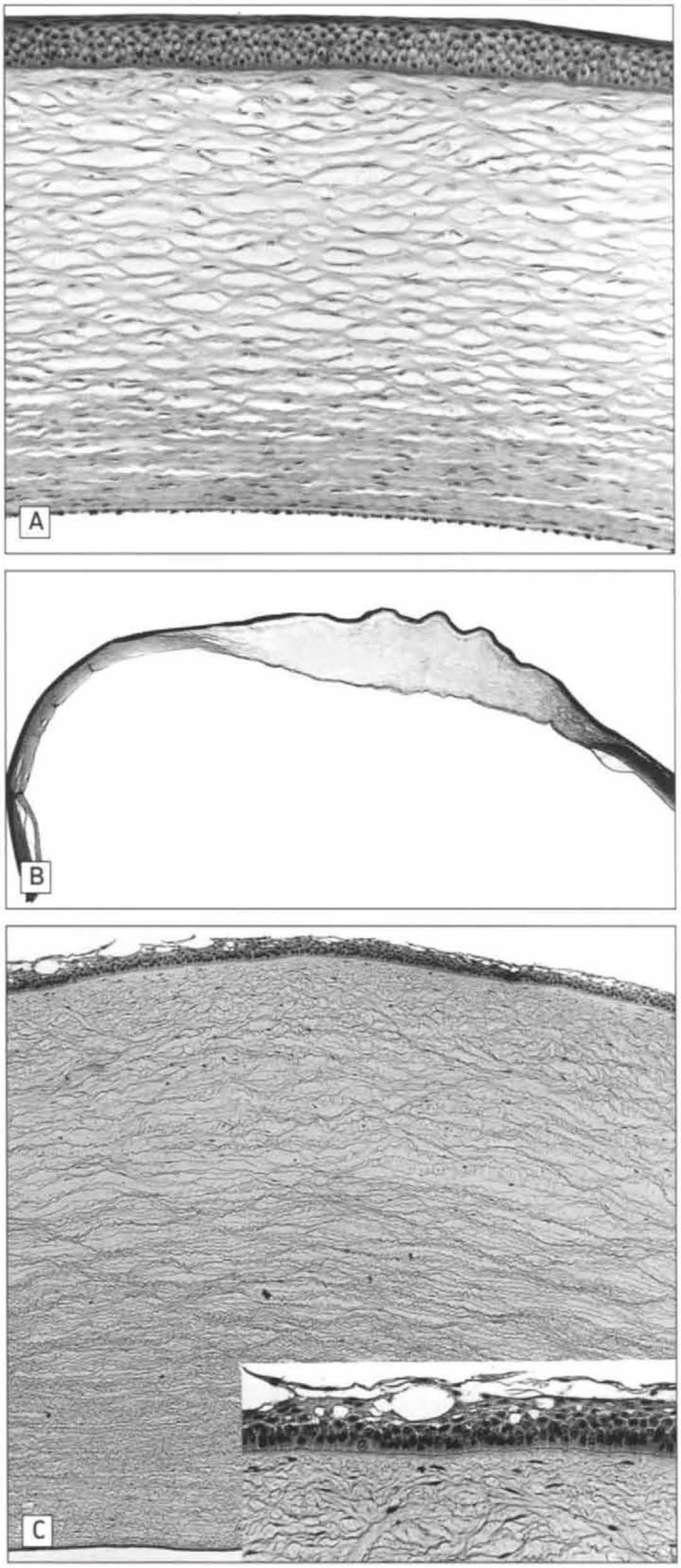

Figure 4.25 A. Normal canine cornea. Uniform, nonkeratinized epithelium without pigmentation or rete ridges. Stroma is poorly cellular. The clefts among the stromal fibers are unavoidable formalin fixation artifacts. **B. Central corneal edema** from abrasion of the corneal endothelium by lens. Foal, with congenital anterior lens luxation. **C. Severe corneal stromal edema**. Percolation of the fluid into the epithelium (insert) creates fluid vesicles known as **bullous keratopathy**.

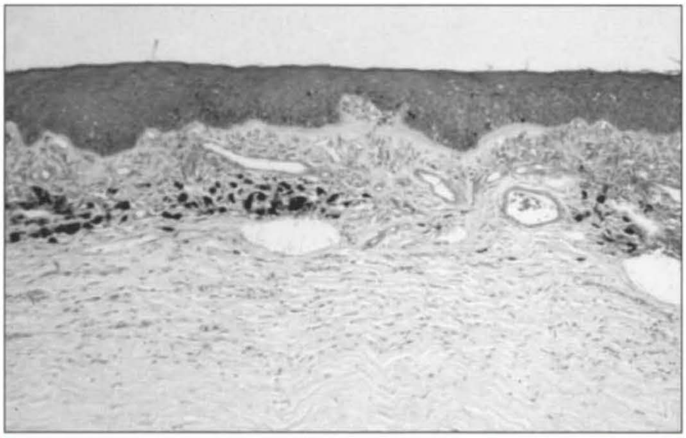

Figure 4.26 Corneal cutaneous metaplasia as a result of chronic keratoconjunctivitis sicca.

edema itself is not the stimulus. Instead, it is probably the infiltrating neutrophils that provide most of the angiogenic and fibroblastic cytokines. Damaged epithelium and stromal fibroblasts are alternative sources. A natural example of virtually permanent corneal edema, without accompanying vascularization, occurs in Boston Terrier and Chihuahua dogs with endothelial dystrophy.

Corneal cutaneous metaplasia

Injury to the corneal surface that exceeds the homeostatic ability of that epithelium resulting in corneal ulceration is described below. Less drastic change to the local environment that results in sublethal injury to the surface epithelium (qualitative or quantitative inadequacy of tears, irritation from misdirected eyelashes or from entropion) will result in adaptive cutaneous metaplasia. *The chronically irritated epithelium undergoes reactive hyperplasia with the appearance of rete ridges, melanin pigmentation, and surface keratinization.* The adjacent stroma undergoes dermis-like irregular fibroplasia and acquires vascularization via capillary migration from the limbus. These changes, while they enable the cornea to survive in a hostile environment and to combat the inflammatory stimulus, also deprive it of its transparency (Fig. 4.26).

Corneal wound healing

Corneal ulceration represents a loss of the surface epithelial barrier. It causes rapid osmotic imbibition of the tear film water, resulting in corneal edema. Neutrophils may be absorbed as well, and if present in large numbers they may initiate stromal lysis as they release their cytoplasmic enzymes. Persistent recruitment of neutrophils is usually a manifestation of sepsis, and is discussed further in the sections dealing with keratitis. In any corneal wound, however, there will be at least a few neutrophils.

The mechanisms of healing of corneal wounds vary with the severity of the injury, and involve an extremely complex interaction of epithelium, stroma, and innumerable cytokine growth factors derived from tear film, infiltrating leukocytes, and resident epithelium and stroma. Only the major elements will be described here, with an emphasis on histologically detectable events rather than on chemical mediators.

It is probably true that virtually any corneal injury results in at least some degree of both epithelial and stromal injury, but *from a purely histologic perspective one can sort corneal injuries into those involving primarily the epithelium and those involving a combination of the epithelium and underlying stroma. Those nonseptic defects involving epithelium alone, or epithelium and superficial stroma, heal by epithelial sliding followed after about 24 hours by mitosis.* One cannot claim that depth alone is the deciding factor, since even very deep wounds will sometimes heal just with epithelial sliding and eventual mitotic rebuilding, as long as the epithelium is satisfied with the quality of the underlying stroma. The sliding begins within a few hours, initially from wing cells from the immediately adjacent, viable cornea. Migration by basal cells rapidly follows. The sliding is preceded by lysis of the hemidesmosomes. Adhesion of the sliding epithelium is initially to adhesion molecules like fibronectin and laminin deposited along the exposed stromal surface. Reformation of the hemidesmosomes and their anchoring filaments may take weeks or even months, during which time epithelial adhesion remains precarious. Healing of shallow, uninfected corneal ulcers is rapid; experimentally induced 7-mm ulcers in horses heal within a mean of 11 days.

Persisting or reoccurring ulcers that cannot be healed just by sliding and replication of adjacent corneal epithelium may require the recruitment of cells from the epithelium at the corneoscleral junction, which is the site of the permanent replicative population. Such cells, when recruited for corneal wound healing, seem prone to retaining a conjunctival phenotype that includes pigmentation and a tendency to form rete ridges. One of the characteristics by which we recognize *chronic, stubborn corneal ulceration* is to observe this *conjunctival "metaplasia" of the surface epithelium.* Another is to recognize a *thickened basement membrane*, as the regenerating epithelium always seems to produce its own new basement membrane even if preexisting basement membrane still seems available. If the injurious stimulus disappears, the conjunctival character of the epithelium slowly fades, being replaced within a few weeks by a normal corneal epithelial configuration. Epithelial adhesion to the underlying stroma remains fragile for 6–8 weeks until the hemidesmosomal attachments of epithelium to basal lamina reform, and until the new epithelium secretes type VII collagen fibrils that anchor the basal lamina to the stroma. In the interim, the cells adhere to a mixture of fibrin and fibronectin derived from the inflamed conjunctival vessels via the tear film or from the injured cornea itself. In many cases, the only evidence of previous shallow ulceration is a thickened basal lamina resulting from secretion by the regenerating epithelium, and gentle undulation of the normally flat epithelial–stromal interface.

Stromal damage may be a direct result of the severity of the initial injury, but more often it is the result of neutrophil-mediated stromal lysis in those corneal injuries that were initially, or later became, septic. Shallow nonprogressive defects in the superficial stroma may be ignored, or may become filled by a thickened plaque of epithelial cells that create an epithelial facet. *Deeper defects that include more than the outer third of stroma will usually require rebuilding of the damaged stroma before epithelial sliding and regeneration can occur.* Within a few hours of the insult, neutrophils enter the wound via the tear film. They migrate into the stroma and control bacterial contamination, degrade damaged collagen, and stimulate both fibroplasia and vascularization via production of various cytokines, especially basic fibroblast growth factor derived from injured epithelium, stromal neutrophils, and injured stromal fibroblasts themselves. Viable stromal cells (keratocytes) adjacent to the wound undergo fibroblastic metaplasia and secrete large amounts of sulfated ground substance, particularly chondroitin sulfate. Most of the new stroma is usually produced by fibroblasts recruited from the limbus. Their ingrowth is always accompanied by a similar ingrowth of new blood vessels. This ingrowth begins about 4 days after substantial corneal injury, and migrates from the limbus centrally at a maximal rate of about 1 mm per day. This 4-day lag time is, presumably, a period of grace in which small or shallow defects can heal with epithelial regeneration alone, without the visual impairment that inevitably follows stromal fibroplasia. Once the epithelium seals the defect, there is immediate cessation of neutrophilic influx and, presumably, a similarly abrupt drop in the production of fibroblastic/angioplastic stimulatory cytokines. *The scarring and vascularization that are the manifestations of stromal rebuilding are permanent*, even though the fibrous tissue gradually becomes less cellular, the collagen fibrils reorient to resemble more closely the parallel arrays of normal stroma, and the ground substance gradually reverts from an embryonic configuration dominated by chondroitin sulfate to the normal predominance of keratan sulfate. Complete restitution of normal stroma, however, never occurs, although the residual scar may be subtle and better detected by clinical examination than by histology. Undesirable though such scarring may be, it is certainly better than the alternative of ineffective corneal healing and inevitable corneal rupture.

Healing of a corneal perforation involves the same events as does healing of a deep corneal ulcer, but there are some added challenges and complications. The cut edges of Descemet's elastic membrane retract from the wound and the transcorneal gap is initially plugged with fibrin and, sometimes, by prolapsed iris. If the gap is not closed by suturing or by a provisional matrix supplied by fibrin and/or iris stroma, there is the risk that the surface epithelium will grow downward along the cut surface of the stroma and into the anterior chamber (Fig. 4.27A). Its migration will be inhibited only by contact with viable corneal endothelium. If it does not encounter that endothelium, there is nothing to stop the epithelium from growing as a layer of stratified squamous epithelium all over the inside of the globe (Fig. 4.27B, C). Obstruction of the filtration angle inevitably causes glaucoma.

As with the surface epithelium, the corneal endothelium at the deep edge of the perforation attempts to bridge the defect by sliding over the fibrin scaffold to restore endothelial continuity. Replacement by mitosis begins within about 24 hours in some experimental models, but *the regenerative capability of the corneal endothelium in adult animals of most domestic species is very limited, and repair occurs by endothelial sliding and hypertrophy.* So potent is this capability that normal stromal dehydration can be maintained even in the face of a 50% reduction in endothelial cell density. The cut ends of Descemet's membrane make no apparent effort at regrowth, but rather the endothelium gradually secretes a new membrane that may eventually fuse with the old or remain separated from it by a layer of fibrous tissue.

The sequence of epithelial sliding and regeneration, remodeling stromal fibrosis and endothelial repair is not uniformly successful. Large gaping wounds fill with proliferating epithelium and stromal fibrous tissue that may protrude into the anterior chamber. The fibroblasts, most of which are probably derived from keratocytes but which may also evolve via endothelial metaplasia, tend to grow along the posterior surface of Descemet's membrane. Regenerating or sliding endothelium is then separated from the coiled remnants of the

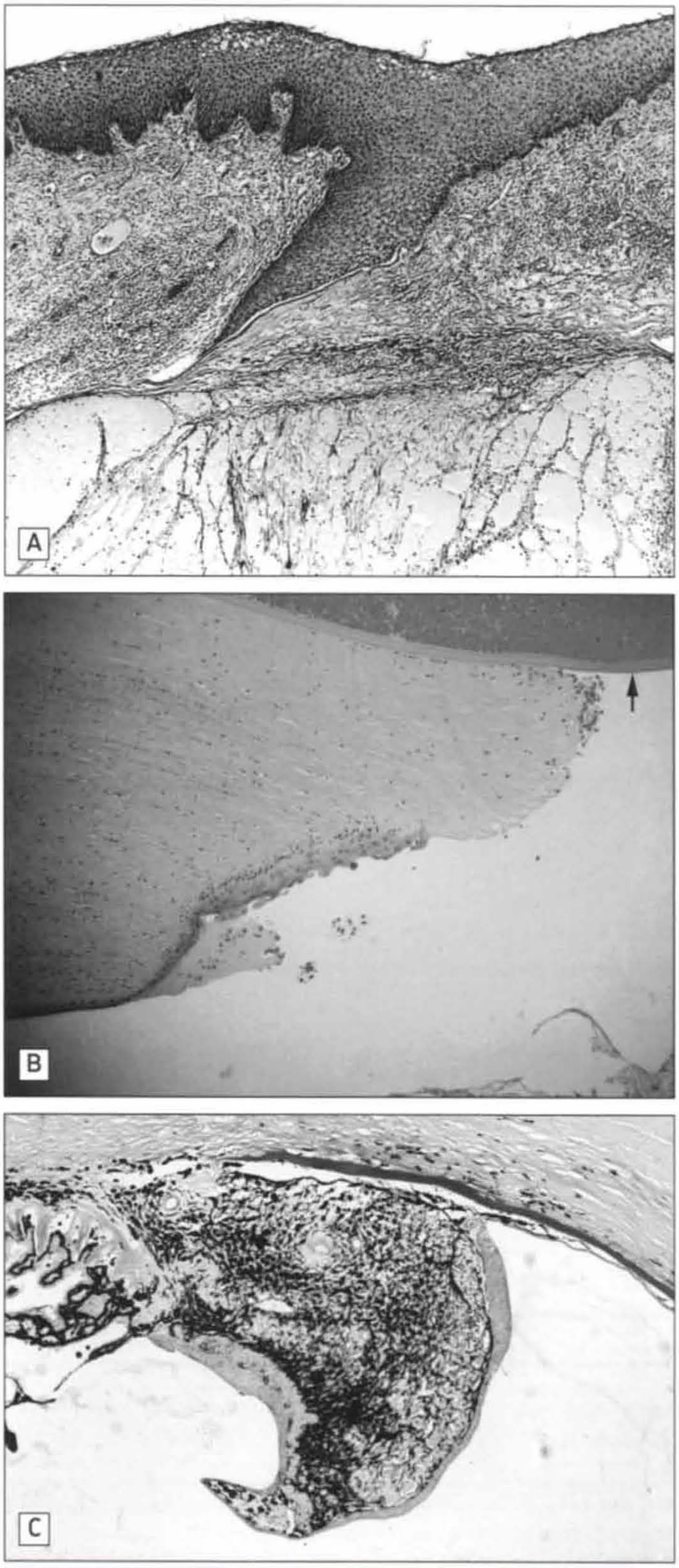

Figure 4.27 A. Corneal perforation in a steer. Defect filled by downgrowth of hyperplastic corneal epithelium. Adjacent stroma is vascularized and chronically inflamed. Edematous iris adheres to the innermost aspect of the lesion, creating focal anterior synechia. **B. Corneal epithelial downgrowth** along the gaping edge of an unsutured corneal laceration. Arrow points to Descemet's membrane that remains intact. **C. Corneal epithelial downgrowth** into the anterior chamber extends over the anterior and posterior surfaces of the iris.

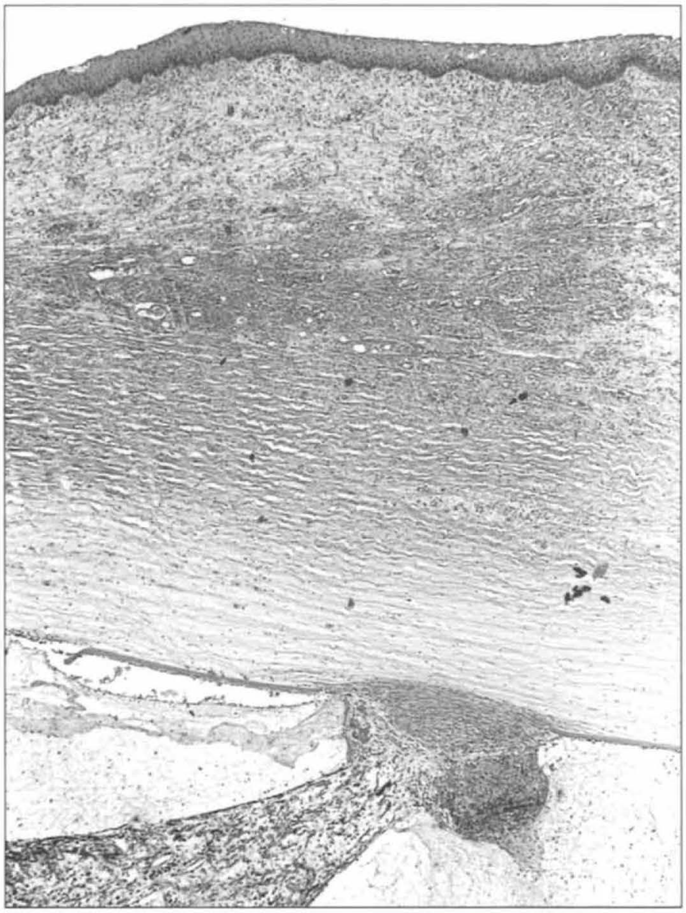

Figure 4.28 Corneal cutaneous metaplasia and chronic superficial stromal inflammation with vascularization. **Anterior synechia** adherent by fibrous plaque that is partly formed by metaplastic corneal endothelium.

original Descemet's membrane by a dense fibrous layer, called a *retrocorneal fibrous membrane*. Eventually, the corneal endothelium may regain continuity on the posterior surface of this membrane, secrete a new Descemet's membrane, and result in a cornea with two separate Descemet's membranes. Those perforations leading to iris prolapse will usually heal with permanent incorporation of the iris into the huge corneal scar, creating a permanent anterior synechia (Fig. 4.28).

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Corneal dystrophy

Corneal dystrophies are bilateral, inherited (but not necessarily congenital) defects in structure or function of one or more corneal components. They are subclassified as epithelial, stromal, or endothelial. They are all uncommon, and almost all examples have been described in dogs. The list grows daily, with more than 30 different breeds affected. The least infrequent are the stromal dystrophies characterized by the deposition of lipids and/or minerals within an otherwise normal-appearing stroma. The deposition of mineral or lipid secondary to inflammatory disease or systemic metabolic abnormality should not be interpreted as corneal dystrophy.

Corneal endothelial dystrophy occurs in Boston Terriers, Chihuahuas, Dachshunds, and several other dog breeds, and causes slowly progressive bilateral corneal edema in mature dogs. The edema usually begins adjacent to the lateral limbus and may initially be unilateral and unaccompanied by other clinical signs. Later, epithelial fluid bullae may rupture to cause painful corneal ulcers and associated inflammation. Despite the persistent stromal edema, fibrosis and vascularization do not occur unless rupture of epithelial bullae initiates keratitis. *The primary lesion is spontaneous necrosis of corneal endothelium followed by hypertrophy, fibroblastic metaplasia, and sliding of viable endothelium.* A marked progressive decrease in overall endothelial cell density results, eventually, in what usually is severe bilateral corneal edema. The reason for the endothelial cell death is unknown. Focal irregularities in Descemet's membrane occur in areas of endothelial loss, presumably a result of new basement membrane production by adjacent reactive endothelium.

Posterior polymorphous dystrophy is characterized by multifocal random degeneration of corneal endothelial cells, accompanied by compensatory endothelial hypertrophy. Cellular loss causes exposure of Descemet's membrane, and adjacent deep stromal patchy edema.

A rare, juvenile-onset, genetically transmitted *endothelial dystrophy in Manx and domestic shorthair cats* is manifest as bilateral, progressive central epithelial and stromal edema. Fluid accumulates within superficial stroma and within the epithelium. Primary morphologic abnormalities are not described in the Manx, but in shorthairs there is irregularity and vacuolation of corneal endothelium.

Corneal stromal dystrophies include a wide range of breed-specific lipid and/or mineral deposits within the corneal stroma, with the exact age of onset, location, and clinical appearance being relatively specific in each breed. Specimens are rarely available for histologic assessment since the disease does not cause blindness and is not associated with any systemic abnormality. In most, the chemical nature of the deposit and character of the metabolic abnormality have not been determined.

Other corneal deposits

Deposition of mineral, lipid, or pigment within the cornea may occur secondary to chronic corneal injury or to systemic metabolic disease in any species.

Corneal hypermelanosis often accompanies *chronic corneal irritation in dogs* and less frequently in other species, particularly horses. The pigment is found in the basal layer of the corneal epithelium and in the superficial stroma. *It is the result of progressive ingrowth of new germinal cells that have retained pigment from the bulbar conjunctiva.* The clinical name, "*pigmentary keratitis*," is purely descriptive. The corneal epithelium is invariably hyperplastic and often has the other features of corneal cutaneous metaplasia, such as rete ridge formation, keratinization, and abnormally thick basement membrane. There is usually evidence of chronic stromal inflammation, including vascularization. Corneal stromal pigmentation without evidence of epithelial cutaneous metaplasia occurs infrequently, associated with previous iris prolapse that has contributed uveal melanin to the corneal stromal scar.

Other types of corneal pigmentation are rare. *Hemosiderin* will be found within corneal endothelial cells subsequent to anterior chamber hemorrhage or within stromal macrophages if there has been hemorrhage into the corneal stroma itself. Similar pigment may occur following implantation of corneal foreign bodies containing iron or other metals.

Diets high in cholesterol produce *diffuse corneal stromal lipidosis*, as well as focal lipid deposits in uveal epithelium and stroma. While hyperlipemia is not a documented prerequisite for most cases of corneal lipidosis in dogs (most of which are spontaneous dystrophies), *circumferential peripheral stromal lipidosis* is reported in dogs with hyperlipoproteinemia resulting from hypothyroidism and other causes.

Regardless of pathogenesis, the histologic lesion is similar. Cholesterol crystals and lipid vacuoles are found principally in anterior stroma, and are sometimes surrounded by lipid-laden macrophages and variable numbers of other leukocytes (Fig. 4.29). Vascularization is often present, but its pathogenesis is unknown. It appears that corneal vascularization can predispose to stromal lipidosis in animals with hyperlipemia, but it is also true that some animals with primary corneal lipidosis will develop secondary inflammation and vascularization.

Mineral deposition occurs primarily in the anterior stroma and the epithelial basement membrane. Predisposing corneal changes include desiccation, anesthesia, edema, or inflammation. *There are many methods for inducing deposition of calcium salts, but stromal edema seems to be the common denominator in almost all cases.* The edema may result from corneal epithelial desiccation (exposure keratitis), uveitis, corneal trauma, or chemical injury. Hypercalcemia from vitamin D toxicity or hyperparathyroidism exacerbates the mineralization and is essential to lesion development in some experimental models.

An unidentified corneal deposition is often seen in canine eyes suffering from multiple anomalies, particularly those involving uvea. Similar deposits are seen, with less regularity, in the horizontal midportion of the cornea of many normal puppies. Fine basophilic periodic acid-Schiff positive linear deposits are associated with the epithelial basement membrane or superficial stroma. There is some disarray of superficial stromal fibers but no inflammation. The nature and pathogenesis of the deposit are unknown, but most disappear after a few months.

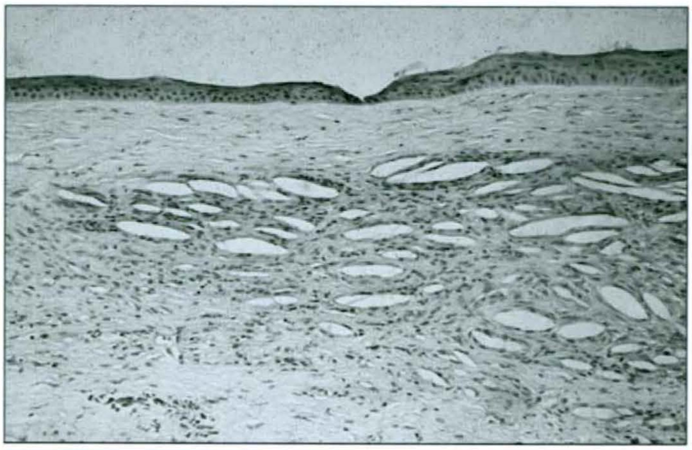
Figure 4.29 Lipid keratopathy. Clefts of cholesterol within the corneal stroma trigger mild nonseptic granulomatous inflammation.

Corneal degeneration

"Corneal degeneration" is a vague term sometimes used to describe those corneal lesions characterized by noninflammatory loss of epithelial or stromal viability. Diseases such as keratoconjunctivitis sicca and pannus keratopathy are sometimes considered primary degenerative lesions but their principal manifestation is inflammatory and they are discussed under keratitis.

The only degenerative, noninflammatory, acquired corneal lesions presented here are corneal sequestrum in cats and horses, and canine persistent erosion syndrome. It is quite possible that all three diseases have a similar pathogenesis, but for the moment they will be listed as three different diseases because of differences in clinical presentation.

Feline corneal sequestrum *is recognized clinically as a discrete orange-brown discoloration of the central cornea, affecting one or both eyes* (Fig. 4.30). Persian and Himalayan cats are more frequently affected than other breeds. Histologically, *the lesion is noninflammatory necrosis of stromal keratocytes, accompanied by pallor, hyalinization, and slight orange discoloration of the affected stroma.* The discoloration may be absent in very early cases. The overlying epithelium may be ulcerated or intact, but in those cases with an intact epithelium there is virtually always histologic evidence of previous ulceration. In older lesions, the periphery of the sequestrum may be marked by a zone of reactive mononuclear leukocytes and, perhaps, a few giant cells. The pigment is derived from porphyrins within the tear film, absorbed into the cornea as part of corneal edema that follows ulceration. *The sequestrum will eventually slough, and the defect heals by granulation* (although most lesions are treated by excision before that stage is reached).

The pathogenesis remains controversial. In flat-faced Persian and Himalayan cats, the pathogenesis probably involves corneal ulceration secondary to desiccation because of abnormal facial configuration. In

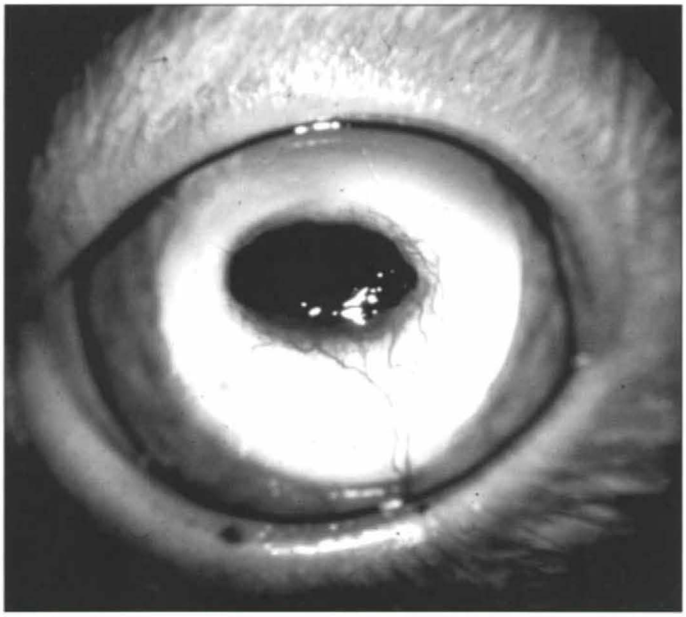

Figure 4.30 Feline corneal sequestrum.

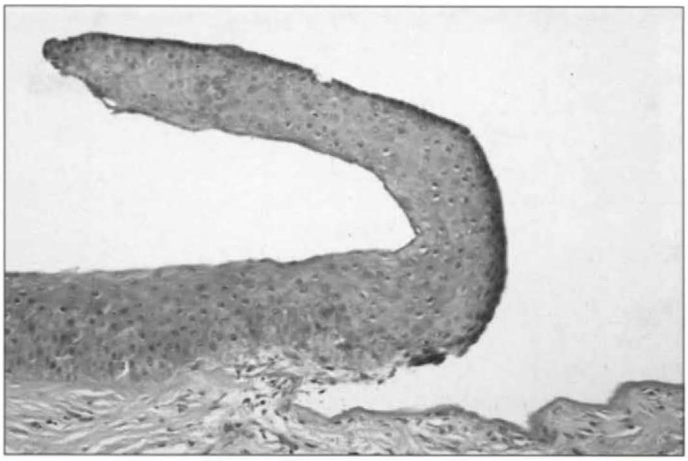

Figure 4.31 Canine persistent ulcer. Dysplastic, strongly regenerative epithelium is unable to adhere to the underlying superficial stroma.

non-Persian cats, there is a loose statistical association with herpesviral infection, and *it is reasonable to propose that corneal sequestrum is an uncommon sequel to any corneal ulceration in cats.* As will become clearer below, the brown discoloration is unique to cats and, for a long time, caused us to overlook the histologic similarity between the feline disease and similar histologic entities in horses and dogs. Not all feline cases acquire the characteristic brown pigmentation, and indeed some examples are virtually indistinguishable from canine and equine persistent ulcers described below.

Canine persistent (recurrent) ulcer syndrome was first described in Boxer dogs (hence the name "*Boxer ulcer*"). Although Boxers and related breeds may be predisposed, similar recurrent erosions are encountered in a wide variety of breeds. *The clinical syndrome is distinctive, characterized by a shallow central corneal erosion with scant edema and (at least initially) no vascularization.* The lesion refuses to heal, or repeatedly re-ulcerates, because of poor adhesion of the epithelium to the underlying stroma. The defect appears not to be in epithelial healing per se, since sliding and mitotic activity are normal in affected dogs. Keratectomy specimens reveal poorly adherent hyperplastic epithelium at the ulcer margins, usually with multiple clefts separating epithelium from stroma even in areas distant from the obvious ulcer (Fig. 4.31). The basal lamina is usually not visible with light microscopy, and the epithelium appears to be attempting to adhere to a thin zone of hypocellular, pale-staining stroma that could correctly be interpreted as a very shallow sequestrum qualitatively similar to what was described above in cats. *The observation of pyknotic and lytic keratocyte nuclei within this superficial zone suggests that the basic defect is degeneration of the superficial stroma,* so that epithelial hemidesmosomes and anchoring collagen fibrils attempting to reform after ulceration have no substrate in which to anchor. Very chronic cases usually acquire superficial stromal granulation tissue appropriate to any chronic ulceration, but its onset is much delayed in comparison to infectious or traumatic ulcers.

The lesion in **horses** is less frequent and less well characterized than in dogs or cats. It is histologically identical to what occurs in dogs, although it seems to be more often complicated and thus disguised by superimposed fungal infection (see Fig. 4.35B).

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Keratitis

Corneal inflammation is called **keratitis** *and has traditionally been divided into epithelial, stromal (interstitial), and ulcerative keratitis.* It is probably time to abandon this arbitrary classification, at least when dealing with ocular histopathology. In realistic terms, almost all corneal inflammatory lesions reaching a pathologist are chronic and severe, and it is difficult to determine how they started. By the time we see them, almost all are ulcerated or show extensive stromal scarring below a healed ulcer. Regardless of cause, corneal inflammation initially follows the stereotyped sequence of edema and leukocyte immigration from tears and distant limbic venules. With severe lesions, corneal stromal vascularization, fibrosis and epithelial metaplasia with pigmentation may occur.

Keratitis usually results from physical, chemical, or microbial injury to the cornea, but the cornea may also be affected by extension of disease from elsewhere in the eye, adnexa, or conjunctiva. The stroma and endothelium may become involved in diseases of the uvea by extension via the aqueous or by direct extension from iris root or ciliary apparatus across the limbus.

Purely **epithelial keratitis** is rarely encountered in histologic preparations, either because the clinical lesion is transient and so mild that eyes are unavailable for histologic examination, or because the lesion progresses to ulceration (as in acute keratoconjunctivitis sicca).

Ulcerative keratitis includes a large group of lesions caused by physical and chemical trauma, desiccation, bacterial, fungal or viral infection, and rarely from primary degeneration of the corneal epithelium itself. *Regardless of cause, the loss of epithelium initiates a predictable series of corneal reactions caused by tear imbibition, local production of cytokines, and opportunistic microbial contamination of the wound.* Imbibition causes superficial stromal edema below the ulcer and is followed by immigration of neutrophils from the tear film and, later, from the limbus. The leukocytes, while somewhat protective against opportunistic pathogens, also add their collagenases, proteases, and stimulatory cytokines to the wound and thereby may contribute to its progression. Epithelial and stromal repair proceeds as already described for corneal wound healing, but the repair fails in those cases in which microbial contamination is well established or in which the cause of the initial ulceration has not been corrected. Common examples of the latter are found in dogs in which corneal trauma by misdirected cilia or facial hair, or desiccation due to lacrimal gland dysfunction, persists.

The usual role of bacteria and fungi in the pathogenesis of corneal ulceration is opportunistic. However, these opportunists contribute significantly to the perpetuation and worsening of the lesion. Proteases and collagenases of microbial, leukocytic, or corneal origin progressively liquefy corneal stroma, a process termed **keratomalacia** (Fig. 4.32). Ulcers contaminated by *Pseudomonas* and *Streptococcus* spp. are particularly prone to rapid liquefaction because of the potent collagenases and proteases produced by these organisms. *Pseudomonas* ulcers have been extensively investigated because of the devastating liquefaction of cornea that commonly accompanies this infection. The bacteria themselves produce numerous proteases and other toxins that may be important in the establishment of the early infection, but most of the characteristic stromal malacia results from the action of proteases originating from leukocytes, reactive corneal epithelium, or injured stroma. The stroma contains a variety of proenzymes (for collagenases, elastases, gelatinases, and other stromal lysins) that are cleaved by the *Pseudomonas* toxins to produce the active enzymes. Which toxins are produced, and in what quantities, is very strain dependent. The stepwise degradation of stroma is seen histologically as a featureless eosinophilic coagulum, which occurs with progressive septic ulcers regardless of the species of bacterium. Neutrophils may encircle the liquefying focus as a thick wall of live and fragmented cells. The resulting localized suppurative keratomalacia is called a ring *abscess* (Fig. 4.33), although that terminology is rarely used today. It is seen more commonly in cattle than any other species, perhaps because of the prevalence of untreated, contaminated corneal ulcers in that species and the prevalence of septic corneal perforation.

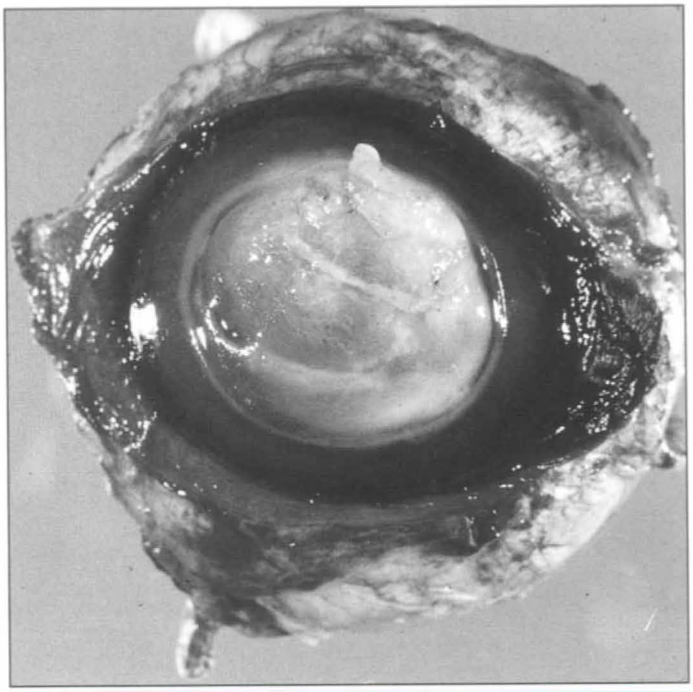
Figure 4.32 Keratomalacia in a horse with *Pseudomonas* keratitis.

The sequelae of ulcerative keratitis involve cornea, conjunctiva, and uvea. *The ulcer itself may heal with vascularization and scarring proportional to the severity of the initial lesion. It may persist as a stubborn but nonprogressive lesion, or it may progress to involve more of the stroma and epithelium.* Stromal liquefaction that reaches Descemet's membrane results in its forward bulging as a descemetocele. This membrane, while resistant to penetration of the microbial agents themselves, is apparently permeable to inflammatory mediators and microbial toxins that diffuse into the anterior chamber. These chemicals, combined with a vasoactive sensory neural reflex from irritated cornea, are responsible for the vasodilation and exudation in anterior uvea that are seen histologically in virtually all globes with deep ulcerative keratitis. Even in nonperforating keratitis, the anterior uveal inflammation may result in sufficient fibrin exudation so as to predispose to focal adhesions of iris to lens or (rarely) to cornea.

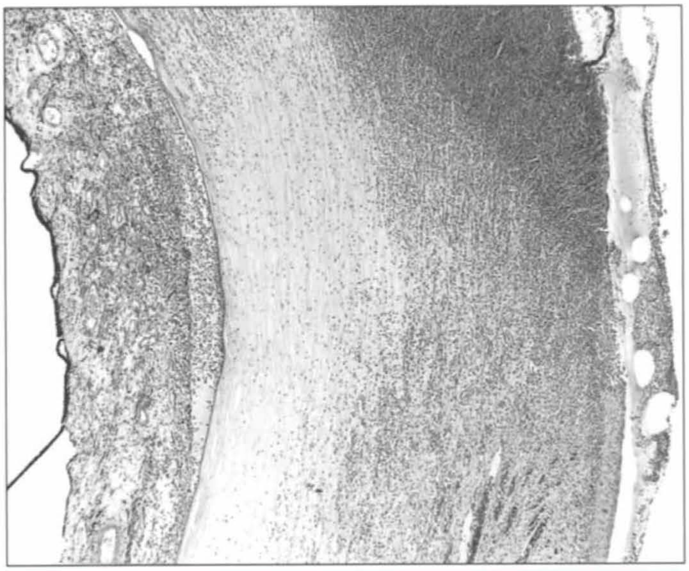

Figure 4.33 Severe suppurative keratomalacia accompanied by massive stromal edema, in a calf. The iris bows forward and almost obliterates the anterior chamber, a manifestation either of iris bombé or of nearby iris prolapse.

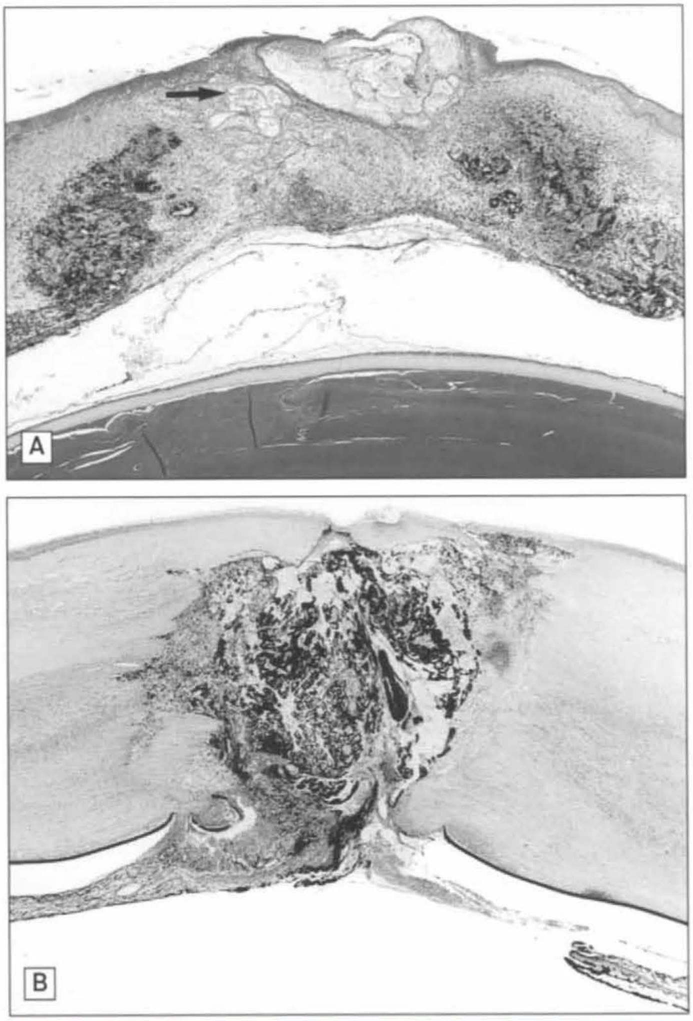

Figure 4.34 A. Healing corneal perforation. Corneal epithelium is attempting to heal across a fibrin mass plugging the defect. Iris is incorporated into the lesion and will form **anterior staphyloma**. Note coiled remnant of Descemet's membrane (arrow). **B.** Iris entrapped within the cornea following perforation of ulcer (**iris prolapse**).

In the case of corneal perforation, the iris flows forward to plug the defect (*iris prolapse*) and may subsequently become incorporated into the corneal scarring. This outcome is usually called **anterior staphyloma**, meaning a focal defect in the ocular fibrous tunic (i.e., cornea) that becomes lined by uvea (Fig. 4.34A, B). The distinction from anterior synechia is of little significance.

The conjunctiva is involved in almost all instances of keratitis, either as a victim of the same injury or as the nearest vascularized tissue to the diseased cornea. Hyperemia, cellular exudation, and lymphofollicular hyperplasia are common as the conjunctiva responds to the diffusion of inflammatory mediators of microbial, leukocytic, and tissue origin from the injured cornea.

There are some instances in which the lesions are found primarily within the stroma. Examples of bacterial or fungal keratitis in which the organisms were implanted into the stroma may cause chronic suppurative keratomalacia with negligible involvement of superficial stroma or epithelium. Alternatively, deep ulcerative septic keratitis may heal superficially, yet persist deep within the stroma. In either of these two situations, the deep lesion is referred to as *stromal abscess*.

Midstromal corneal vascular ingrowth from the limbus is a very common lesion in response to vascular endothelial growth factors elaborated in the course of chronic uveitis of virtually any cause. It appears to be a purely accidental lesion with no obvious purpose, but it does serve as a valuable and permanent histologic marker for previous or ongoing intraocular inflammatory disease. Its liability as a marker for subacute or chronic intraocular inflammation is probably not absolute, because similar midstromal vascularization can probably occur in response to growth factors liberated from detached retina or intraocular neoplasms (see Significance of uveitis).

Pannus keratitis

The only credible candidate for a *genuine stromal keratitis*, in which the primary target for the inflammatory disease is the stroma itself, is **pannus keratitis**. *This is an idiopathic disease seen most frequently in German Shepherds and phenotypically similar breeds.* Its prevalence and severity are directly correlated with altitude, suggesting that *sunlight exposure* is part of the pathogenesis. The clinical disease is distinctive. The early lesion is seen in dogs of either sex, usually in early middle age, as a vascularized opacity growing into the corneal stroma from the limbus. The ingrowth is bilateral although not always of simultaneous onset, and most frequently originates from the ventrolateral limbus. There is no ulceration, but pigmentation is often marked. The untreated lesion eventually infiltrates the entire cornea, converting the superficial stroma to an opaque membrane resembling granulation tissue. At one time, superficial keratectomy was the recommended therapy and so histologic specimens were quite often available. Today, most cases are treated with potent immunosuppressive therapy, and the need to perform a keratectomy to restore vision is rare indeed.

The histologic appearance varies with the duration of the lesion. *The initial lesion is superficial stromal infiltration of mononuclear cells, especially plasma cells.* Subsequently, there is progressive vascularization and fibroplasia in the superficial third of the stroma, accompanied by epithelial hyperplasia and pigmentation that may include the stroma. The deep stroma is never affected.

The pathogenesis of the condition is unknown, but an immune reaction to altered corneal epithelial antigens is hypothesized. Its response to continuous corticosteroid administration supports this hypothesis, as does it striking histologic similarity to discoid lupus and other lupoid dermatoses. Despite the similarity, immunofluorescence tests for intraepithelial or basement membrane immunoglobulin are negative. Infectious agents are not consistently isolated. A histologically similar lesion of the bulbar conjuctiva of third eyelid occurs in the same breed (so-called "*plasmoma*") and may reflect the same mysterious pathogenesis.

Keratoconjunctivitis sicca and desiccation keratitis

The response of the cornea to desiccation depends on the rapidity of onset and the severity of the desiccation. *It is seen as a consequence of inadequacy in the quantity or quality of the tear film* (usually called **keratoconjunctivitis sicca**). It also occurs as a consequence of exophthalmos, improper eyelid closure because of eyelid developmental anomaly, acquired eyelid disease, nerve injury to prevent blinking, profound CNS depression in which the blink reflex is lost, or conditions such as glaucoma or orbital mass that prevent proper eyelid closure because of abnormal ocular size or position. Under such circumstances, the corneal lesion is usually referred to as **desiccation keratitis**, although the effect upon the cornea is exactly the same as in acute keratoconjunctivitis sicca. In those cases in which the desiccation occurs only in a horizontal band not adequately covered by the eyelids for whatever reason, the lesion is sometimes referred to as *band keratopathy*.

If the desiccation is profound and occurs rapidly, the cornea has no time to adapt and the outcome is *acute ulceration*. It can be distinguished from other types of corneal ulceration histologically because *it is the only example of corneal ulceration that occurs in the absence of edema or neutrophilic infiltration* (because there is no tear film to provide either the water or the leukocytes). If the desiccation is only mild, or occurs over a long interval that allows corneal adaptation, the resulting lesion is *corneal cutaneous metaplasia*.

Desiccation keratitis (either acute or chronic) may follow destruction or denervation of lacrimal or accessory lacrimal gland by orbital inflammation, drugs, neoplasia, or trauma. Squamous metaplasia with resultant inadequacy of secretion may be seen with chronic deficiency of vitamin A. Specific lacrimal adenitis with subsequent atrophy is well recognized with coronavirus infection in rats and may be seen in the acute or chronic phases of canine distemper. Similar adenitis probably occurs with other viruses and in other species but such lesions are poorly documented. Transient keratoconjunctivitis sicca may accompany acute herpetic keratoconjunctivitis in cats.

Keratoconjunctivitis sicca, as a specific disease entity, is encountered more commonly in **dogs** than in any other species, with an overall prevalence in North America of about 1%. Most cases are chronic, progressive, and idiopathic. The reason for greater than expected prevalence in certain breeds (English Bulldog, Lhasa Apso, Shih Tzu, West Highland White Terrier, and others) is unknown. Because the disease is amenable to medical or surgical management, few specimens are available for histologic examination until the very chronic stages. At this time, the lacrimal gland is atrophic with interstitial lymphoid infiltration and fibrosis, but provides no clue as to the initial lesion. The ability of certain immune modulators, notably cyclosporine, to reverse the disease, points to some kind of immune-mediated phenomenon, perhaps autoimmunity.

The corneal changes vary with the severity and rapidity of onset of lacrimal deficiency. In acute disease with marked lacrimal deficiency, clinical signs of ulcerative keratitis may occur. The corneal epithelium is thinned, has numerous hydropically degenerate cells, and may suffer full-thickness ulceration. The accompanying stromal changes, including eventual vascularization and fibrosis, are those of ulcerative keratitis. More commonly in dogs, however, the desiccation is not absolute (at least initially) and the epithelial response is protective epidermalization without prior ulceration. Keratinization, marked hyperplasia with rete ridge formation, and pigmentation are commonly seen. Stromal inflammation and vascularization are usually superficial, resulting in a lesion very similar to pannus keratitis. Squamous metaplasia may also occur in the bulbar conjunctiva. The conjunctivitis that clinically is the earliest lesion of keratoconjunctivitis sicca is rarely available for histologic examination.

Herpetic keratitis of cats

Feline herpetic keratitis, caused by *Felid herpesvirus 1,* is seen either as the sole ocular lesion or in concert with conjunctivitis. Clinical signs associated with herpesviral infections in cats include conjunctivitis, keratitis, rhinotracheitis and, in neonates, systemic disease with encephalitis and necrosis in visceral organs. Acquired immunity alters the manifestations of the disease and results in different lesions predominating in different age groups. *Keratitis is commonest in adult cats* and seems to result from activation of latent infection during concurrent immunosuppressive disease or corticosteroid therapy. Concurrent mild respiratory disease may be present, but *in adults the disease is often purely corneal and may even be unilateral.* In contrast, *the infection in adolescent cats causes nonspecific bilateral erosive conjunctivitis without keratitis.* Intranuclear inclusions are numerous within cells prior to sloughing, and leukocytes are sparse until ulceration permits opportunistic contamination. Upper respiratory disease is almost always present.

The corneal lesions fall into two very different categories: *shallow transient erosions and ulcers* that represent the direct cytopathic effect of acute viral infection, and *more severe stromal keratitis* that is probably an immune response to viral antigen in persistent or recurring infections. The typical acute superficial corneal lesions are multifocal minute corneal erosions and ulcers that have a tendency to coalesce into branching dendritic ulcers. Early in the disease, typical herpesviral inclusions may be seen with histology, and herpesviral antigen can be demonstrated with immunofluorescence or other techniques. Severe or recurrent lesions in immunosuppressed cats may result in underlying stromal keratitis with lymphocytic infiltration, persistent edema, and vascularization.

There is much more written about the clinical features and clinical diagnosis of herpesviral keratitis than there it is about its pathology, simply because most cases are never subjected to histologic evaluation. By the time a sample of conjunctiva or cornea is taken for histologic assessment in cases that have been therapeutically resistant, histologic detection of inclusion bodies is futile and immunofluorescence is usually negative. Virus can usually be detected with PCR, but interpretation of that result is almost impossible because of the high prevalence of carriage in asymptomatic, healthy cats. For the same reason, attempts to link persistent herpesviral infection with feline corneal sequestrum or feline eosinophilic keratitis have been less than convincing.

Feline eosinophilic keratitis

Another uniquely feline ocular lesion is seen clinically as unilateral or bilateral proliferative, "fluffy" white stromal keratitis. There is no breed, age, or sex predilection, and no proven association with other ocular or systemic disease. Since the diagnosis is made by cytologic evaluation of superficial scrapings or (occasionally) by histologic examination of surgical keratectomy specimens, this disease is more likely to be seen by pathologists than most other corneal disorders. Scrapings of the surface of the lesion reveal numerous eosinophils and fewer mast cells and other mononuclear leukocytes. Eosinophils may be less conspicuous on histologic examination of keratectomy specimens, perhaps because most seem determined to emigrate through the epithelium and into the tear film rather than remain within the tissue. Instead, the stromal lesion is a *mixture of macrophages, plasma cells, fibroblasts and, unpredictably, mast cells and at least a few eosinophils.* The latter are least frequent in older lesions, either because of time alone or because older lesions are more likely to have received a lot of corticosteroid therapy. A characteristic lesion, not present in every case, is a *dense granular eosinophilic coagulum* along the surface of the keratectomy specimen. It seems to consist of free eosinophil granules. No bacterial or fungal agents have been seen. While there are histologic similarities to cutaneous eosinophilic ulcer and linear granuloma, no statistical association has been proven and the lack of understanding even of the cutaneous eosinophilic lesions makes such attempted comparisons of very limited value. While much speculation exists about the relationship between persistent herpesviral infection and eosinophilic keratitis, there is no proven etiologic link.

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Mycotic keratitis

Mycotic keratitis *is a destructive, suppurative, ulcerative and deep stromal keratitis most commonly seen in horses,* but occasionally encountered in all domestic species. The offending fungus is usually a member of the normal conjunctival flora, and its role in the disease is always that of *opportunistic contaminant. Aspergillus* is the most frequent isolate, but cases caused by other common conjunctival fungi like *Alternaria, Penicillium,* and *Cladosporium* are not rare. *Most cases are probably iatrogenic,* occurring in animals in which a corneal ulcer, laceration, or penetrating wound had been treated with long-term antibiotics and/or *corticosteroids.* The latter is a particularly common villain in this context. Horses seem particularly prone to mycotic keratitis, perhaps related to the mold-laden, dusty environment in which many horses are housed; only rarely does the lesion occur in dogs or cats. Since virtually all stabled horses have fungi as part of their conjunctival flora, *seeing hyphae within the corneal stroma is required for the diagnosis.* Isolation from a corneal swab or shallow scraping is not adequate.

Because the disease is much more prevalent in horses than any other species, most of the description below is derived from equine cases. The histologic changes in other species, however, are very similar to what occurs in horses. There appears to be a difference in the typical lesion seen in temperate climates and what occurs in horses in very warm and humid environments. In the latter, there are cases in which the fungi are found throughout the cornea and are easily identified by even shallow scraping. That is not the case in those examples of the disease diagnosed in cooler climates, which I will consider the "typical" disease.

The typical early lesion is deep ulcerative keratitis with suppurative keratomalacia. Some chronic lesions are exclusively stromal because of successful epithelial and superficial stromal healing of the initial penetration (or perhaps because therapy eliminated the infection in the superficial stroma). For whatever reason, the typical equine eye enucleated for mycotic keratitis has an intense neutrophil-rich deep stromal keratitis with several characteristic features: neutrophils are karyorrhectic, inflammation is most intense immediately adjacent to Descemet's membrane, and frequently there is lysis of the normally resistant Descemet's membrane with spillage of the corneal inflammation into the anterior chamber. *Fungi are numerous within the malacia of the deep stroma and within Descemet's membrane itself,* but rarely if ever are seen within the anterior chamber (Fig. 4.35A). When they occur within the anterior chamber, they are always anchored to the nearby Descemet's membrane. Despite ample opportunity, there has never been a reported case of disseminated intraocular mycosis as a sequel to mycotic keratitis. Fungi are sparse or absent within the superficial half of the stroma, which explains why corneal scrapings or even keratectomy specimens may fail to reveal the agent. The reason for the apparent targeting of Descemet's membrane is not known, but the presence of the tropism even in untreated eyes suggests that it is a

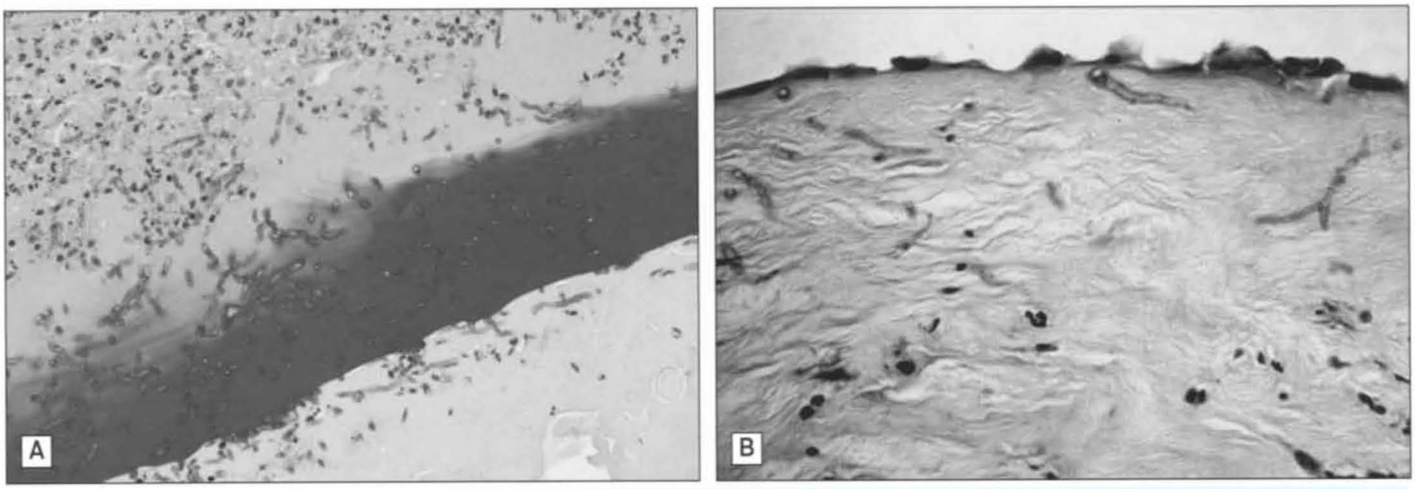

Figure 4.35 A. Equine mycotic keratitis. The fungi typically are found within and adjacent to Descemet's membrane, accompanied by karyorrhectic neutrophils and stromal malacia. **B. Opportunistic fungal contamination** of an equine superficial corneal sequestrum, but distinctly different syndrome from traditional mycotic keratitis. Note the absence of leukocytes.

genuine tropism and not just persistence of a previously generalized stromal infection in the site least likely to be reached by topical fungicides.

In horses in tropical and near-tropical climates (many reports, for example, come from Florida), the fungi are more diffusely distributed within the cornea and are thus more easily captured by routine cytology or culture swabs. While the distribution of the lesion within the cornea is also more diffuse, its fundamental lytic character remains the same.

In horses, cats, and dogs, we will occasionally see *corneal sequestra contaminated with fungal hyphae*; the fungi are easily found on scraping, leading to the mistaken impression that this is true mycotic keratitis (Fig. 4.35B). One could debate the issue, but it seems better to keep this as a separate syndrome quite different from the lesion described above.

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Infectious bovine keratoconjunctivitis

Infectious bovine keratoconjunctivitis ("pinkeye") vies with squamous cell carcinoma as the most important disease of the bovine eye. It occurs worldwide, is most prevalent in summer due to the increase in fly vectors, and has a clinical expression that ranges from initial conjunctivitis and ulcerative keratitis to iris prolapse, glaucoma, and phthisis bulbi. The prevalence of severe sequelae reflects inadequate management of the disease rather than any special virulence of this agent as compared to other infectious causes of keratitis in other species.

The disease behaves as an infectious epizootic within a susceptible population, frequently affecting over 50% of the cattle at risk within 2 weeks of the initial clinical case. Shedding of virulent organisms by a carrier animal is thought to be the usual route of introduction into a previously unexposed group, although a role for various mechanical or biological vectors is also assumed.

Moraxella bovis has been confirmed as the most important causative agent, and the only one for which Koch's postulates have been fulfilled. Concurrent infection with other agents such as *Mycoplasma bovoculi, Mycoplasma conjunctivae, Acholeplasma laidlawii,* and bovine herpesvirus may contribute to lesion severity. Earlier skepticism about the virulence of *M. bovis,* based upon the unreliability of reproduction of the disease, isolation of the organism from apparently healthy cattle, and failure of isolation from some overtly affected cattle, has been overcome by detailed information on the pathogenesis of the disease. *It is now clear that virulence of M. bovis is associated with hemolytic, leukocytolytic, piliated strains that predominate only in the eyes of affected cattle.* Pathogenic isolates of *M. bovis* express a calcium-dependent transmembrane pore-forming cytotoxin. Nonpiliated, nonhemolytic strains predominate in healthy cattle and are probably part of the normal conjunctival flora. The use of immunofluorescence has demonstrated *M. bovis* in many of the naturally occurring cases for which the results of culture were negative. In naturally occurring outbreaks, the number of isolations of hemolytic *M. bovis* falls to almost zero as the outbreak wanes, but a few chronically affected carriers remain as the most important source of virulent bacteria for outbreaks of disease in the next summer.

In addition to variation in the virulence of different strains of *M. bovis*, sunlight, dust and, perhaps, concurrent infection with infectious bovine rhinotracheitis virus (*Bovine herpesvirus 1*) increase the severity of the disease. Calves are usually affected more severely than cattle over 2 years of age, although absolute resistance to infection seems fragile. The protective effect of serum antibody against the disease is controversial. Specific IgA is found in tears of infected calves, and there is substantial evidence that locally produced IgA is strongly protective.

Following experimental inoculation of virulent *M. bovis* onto the cornea, pilus-mediated adhesion and production of bacterial cytotoxin result in microscopic ulceration in as little as 12 hours. Initial adhesion is to older surface epithelium ("dark cells") and results in the development of microscopic pits in the cell surface. *Moraxella* is found within degenerate epithelial cells, but it is not known whether invasion is necessary for subsequent cellular destruction. *In field epizootics, the earliest lesion is bulbar conjunctival edema and hyperemia, followed in 24–48 hours by the appearance of a shallow central corneal ulcer.* The ulcer is a small (<0.5 cm) focus of epithelial necrosis that may appear as erosion, vesicle, or full-thickness epithelial loss. In untreated animals destined to develop the full clinical expression, the ulcer enlarges, deepens, and frequently attracts enough neutrophils to qualify as a *corneal abscess*. Stromal liquefaction ensues, probably as a result of neutrophil lysis, which is itself initiated by *Moraxella*-derived leukotoxins. By the end of the first week, there is extensive stromal edema and vascularization extending from the limbus. As with any severe ulcerative keratitis, the subsequent progression or regression of the lesion varies with each case as modifications by therapy, opportunistic bacterial and fungal contamination, trauma, inflammation, and immunity interact. Keratomalacia frequently leads to forward coning of the weakened cornea (*keratoconus*). In most instances, whether treated or not, the cornea heals by sloughing of necrotic tissue and filling of the defect by granulation tissue. Re-epithelialization may take up to a month, leaving a cornea that is slightly coned and variably scarred. The scarring often is scant and interferes little with vision in spite of the severity of the primary lesion.

Less satisfactory sequelae, while not common in relation to the overall disease prevalence, are still relatively common. Sterile anterior uveal inflammation may result in focal or generalized adherence of iris to cornea (anterior synechia) or lens (posterior synechia). Descemetocele may progress to corneal rupture, which in turn may lead to phthisis bulbi or resolve by sealing with prolapse of the iris. Synechia and staphyloma may lead to impairment of aqueous drainage and thus to glaucoma.

Infectious keratoconjunctivitis (contagious ophthalmia, pinkeye) of sheep and goats

Epizootics of conjunctivitis and keratitis in sheep and goats share many of the features of the bovine disease: summer prevalence, rapid spread, and exacerbation by dust, sunlight, and flies. Feedlot lambs seem particularly susceptible. Unlike bovine keratoconjunctivitis, the range of clinical signs and proposed causes suggests that there may in fact be several different diseases. Many agents including bacteria, mycoplasmas, chlamydiae and rickettsiae have been suggested as causes, but various mycoplasmas and *Chlamydophila psittaci* may be the most important agents. The lesions caused by *Mycoplasma mycoides* var. *capri* in goats and *Mycoplasma conjunctivae* var. *ovis* in sheep are similar but usually milder than those caused by *Moraxella bovis* in cattle. This is particularly true of goats in which deep corneal ulceration is uncommon.

Keratoconjunctivitis associated with *Chlamydophila psittaci* is usually predominantly conjunctivitis. Initial chemosis and reddening are followed by massive lymphofollicular hyperplasia in bulbar conjunctiva and nictitating membrane. Keratitis may occur but ulceration is seldom prominent. Animals with conjunctivitis may have concurrent polyarthritis from which chlamydiae can be isolated.

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LENS

The lens is a flattened sphere of epithelial cells suspended in the pupillary aperture by a large number of transparent elastin-like fibers known as *lens zonules*. These originate from the lens capsule near the equator, and fuse with the nonpigmented ciliary epithelium along the lateral surfaces of the ciliary processes or in the valleys between adjacent ciliary processes. *The range of histologic reaction of lens to injury is very limited due to the simplicity of its structure and physiology, and its lack of vascularity.*

The lens is entirely epithelial. Outermost is a *thick, elastic capsule*, which is the basement membrane produced by the underlying germinal epithelial cells. The capsule is thickest at the anterior pole and becomes progressively thinner over the posterior half of the lens. The capsule in the neonate is thin, but it thickens progressively throughout life.

Below the capsule is a layer of simple cuboidal *lens epithelium* that, in all but fetal globes, is found below the capsule of only the anterior half of the lens. The apex of these cells faces inward toward the lens nucleus. At the equator, these germinal cells extend into the lens cortex as the *nuclear bow*, an arc of cells being progressively transformed from cuboidal germinal epithelium to the elongated spindle shape of the mature *lens fibers* (Fig. 4.36). The bulk of the lens is composed of onion-like layers of elongated epithelial cells anchored to each other by interlocking surface ridges, grooves, and ball-and-socket protuberances. These elongated fibers contain no nucleus and few cytoplasmic organelles, relying almost entirely on anaerobic glycolysis for energy. Since the lens cannot shed aging fibers as does skin or intestine, these cells are compacted into *the*

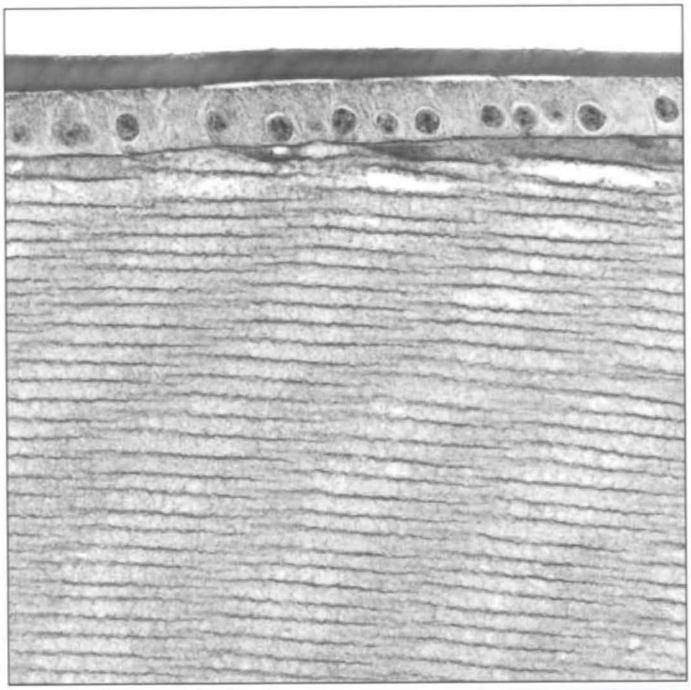

Figure 4.36 Normal canine lens with characteristic regularity of surface epithelium and lens fibers.

oldest central part of the lens, the nucleus. The continuous accumulation of these old desiccated fibers with altered crystalline protein results in the common but visually insignificant *aging change of nuclear sclerosis.*

Although in many ways similar to cornea in structure and function, the optical clarity of lens rests not with the regularity of its fibers but in its high percentage of cytoplasmic soluble crystalline protein and paucity of light-scattering nuclei or mitochondria. The lens is about 35% protein, the highest of any tissue, and over 90% is of the soluble crystalline variety. Insoluble high-molecular-weight protein (albuminoid) is found in the nucleus and cell membranes. Opacity of lens is associated, at least in some cases, with decreasing concentrations of crystalline and increasing albuminoid protein, the latter insoluble in water and optically opaque. *Many of the insults that result in degeneration of lens ultimately interfere with its nutrition.* Since it is avascular in the postnatal animal, *the lens relies entirely upon the aqueous for the delivery of nutrients and removal of metabolic wastes.* Glaucoma, ocular inflammation, metabolic disorders and various toxins share the common feature of altering the amount or quality of lenticular nutrition by altering the flow or composition of the aqueous humor.

Ectopia lentis

The only lenticular defects of importance in domestic animals are those affecting location, configuration, and clarity. Those affecting configuration are usually developmental defects and are discussed earlier. **Dislocations of the lens** may be congenital or acquired, and the latter include spontaneous dislocations and those secondary to trauma and glaucoma. *Apparently spontaneous dislocations* are encountered most frequently in middle-aged (3–8 years) terrier dogs in which an inherited predisposition to bilateral zonular rupture exists. The pathogenesis of the defect has been best studied in the Tibetan Terrier, in which the zonules develop in a dysplastic, reticulate fashion that precedes luxation by several years. *Traumatic dislocation* is usually via blunt trauma, notably automobile accidents. The dislocation may be partial (*subluxation*) or complete (*luxation*), and in the latter instance the free lens may damage corneal endothelium or vitreous causing edema and liquefaction, respectively. Such lenses may be surgically removed but seldom receive histologic examination. Anterior luxation frequently results in glaucoma, perhaps caused by anterior prolapse of the vitreous into the pupil. Lens luxation is inexplicably uncommon in cats. It is seen in middle-aged cats as unilateral and usually anterior luxation. One-third of cases occur in eyes with no other observed lesion, while the remainder occur in eyes with preexistent uveitis or glaucoma, or a history of trauma.

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Cataract

Cataract is the most common and most important disorder of the lens. Cataract means lenticular opacity, and is usually prefixed by adjectives relating to location, maturity, extent, suspected cause, and ophthalmoscopic appearance. Of these adjectives, only those of location and, to some extent, maturity are useful in histologic description. *The simple structure of the lens results in a stereotyped reaction to injury that provides few clues to pathogenesis.* Unless permitted by invasion through a capsular tear, inflammation cannot occur within the avascular and totally epithelial lens.

The main challenge facing the pathologist looking at a histologic section of lens is whether any of the microscopic changes are genuine or not. The lens is difficult to section, and artifacts like fiber disruption and capsular tearing are almost unavoidable. *Histologic changes that distinguish genuine cataract from fixation or sectioning artifact are (in order of overall utility): detection of Morgagnian globules, bladder cells, lens epithelial hyperplasia, posterior migration of lens epithelium, and mineralization* (Fig. 4.37).

Morgagnian globules *are bright pink spherical globules of denatured lens protein.* They are distinguished from artifactual fragmentation of lens fibers because the latter form jagged rectangular fragments, not spheres (Fig. 4.38). With more advanced liquefactive change, fluid lakes appear between fibers, presumably the result of complete liquefaction of fibers. Some of the clefts are probably the result of osmotic fluid imbibition by the cataractous lens. The osmosis results from protein denaturation into more numerous, smaller peptides and from degeneration of the capsular epithelium in which resides the Na–K-dependent ATPase osmotic pump critical to normal lens hydration.

In advanced cataracts, the degenerate fibers may liquefy to the extent that their low-molecular-weight end products diffuse through the semipermeable capsule, resulting in the spontaneous clearing of the opaque lens typical of the *hypermature cataract.* Histologically, such lenses consist of a dense, eccentric residual nucleus in a lake of

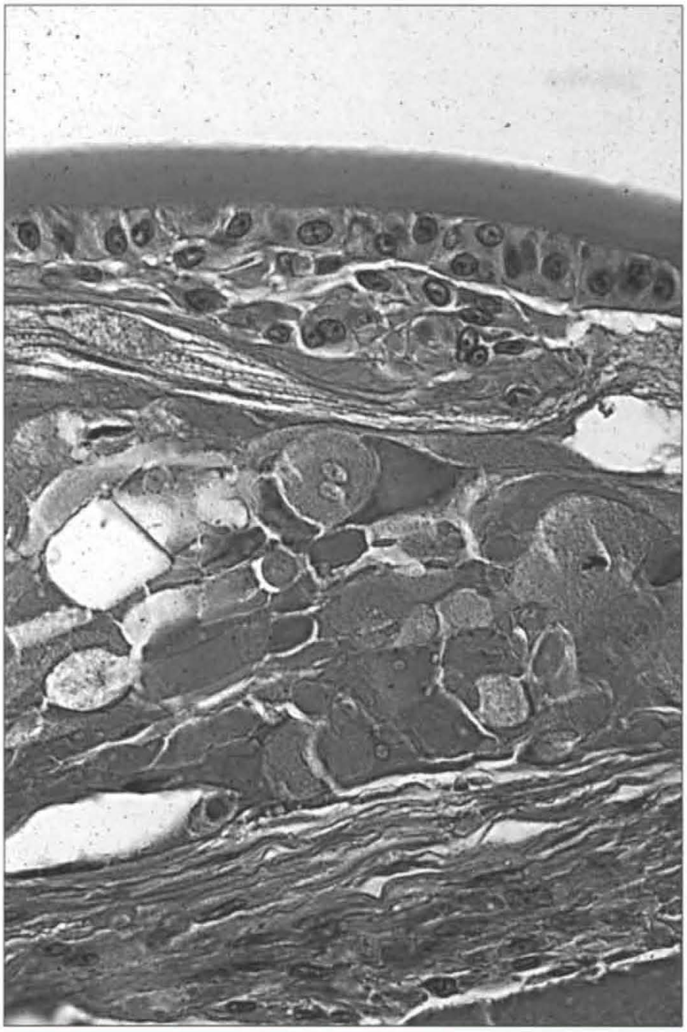

Figure 4.37 The general pathology of **cataract**: fiber liquefaction, reactive hyperplasia of capsular epithelium, and bladder cells.

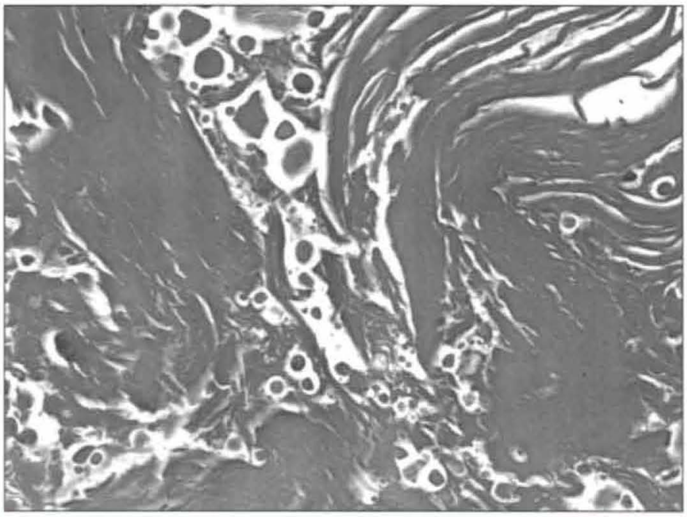

Figure 4.38 Spherical accumulations of denatured lens protein known as **Morgagnian globules**, probably the most reliable histologic criterion for distinguishing cataract from artifactual lens fragmentation.

proteinaceous fluid surrounded by a wrinkled capsule. The adjacent uvea will usually have a moderately severe lymphocytic-plasmacytic infiltration in response to unidentified inflammatory mediators present in that leaking lens fluid. The lesion/process is known as *phacolytic uveitis* (see below).

Bladder cells are claimed to reflect abortive efforts at new fiber formation by lens epithelium. Such efforts are apparently never successful, and the hydropic degeneration of such cells results in the formation of *large foamy nucleated cells called bladder cells* because of their bloated, fusiform shape.

Epithelial hyperplasia or **fibroblastic metaplasia** is the usual histologic counterpart of anterior subcapsular cataract, and is usually seen following focal trauma, or adherence of the iris or of persistent pupillary membranes to the anterior surface of the lens. *Initial epithelial degeneration or necrosis is followed by hyperplasia and sometimes by fibrous metaplasia.* The resultant *epithelial plaque* lies just under the anterior lens capsule. The innermost epithelial layer may remain basilar in type rather than fibroblastic. Each epithelial layer, even if metaplastic, secretes a new basement membrane that separates each layer from the adjacent layers. This characteristic feature is best seen with PAS stains. The end result is a focal plaque formed by multiple, sandwiched layers of flattened epithelium and basement membrane at the anterior pole of the lens. Remnants of adherent iris or pupillary membrane, including pigment, may complicate the histologic appearance.

Posterior migration of lens epithelium is a common change in chronic cataracts. Although it seems to occur more slowly than the changes of Morgagnian globule or bladder cell formation, it is equally reliable (when present) as a histologic indicator of cataract formation. The flattened cuboidal epithelial cells migrate from the equator to line the posterior capsule. Usually, they will also undergo stratification and sometimes fibroblast-like metaplasia. This re-establishment of the fetal morphology is seen most commonly with any chronic cataract in young animals, whose epithelial cells perhaps retain greater migratory ability. Adjacent cortical fibers are usually degenerate (Morgagnian globules, bladder cells, and clefts of granular proteinaceous fluid).

Deposition of calcium salts occurs quite frequently within cataracts of any type, and provides no clue as to pathogenesis.

The sequence of histologic change in cataract is the same regardless of cause, and thus diagnosis of cause can be made only in light of patient data or concurrent disease. In dogs, for example, familial cataracts may be congenital or of later onset. Specific examples may typically occur alone or with other ocular lesions, and occur at an age, in a location and with a progression sufficiently characteristic to allow presumptive diagnosis of a breed-specific syndrome. Cataract also occurs secondary to glaucoma, endophthalmitis, ocular trauma, and anterior segment anomalies, and observation of these latter defects permits presumptive diagnosis of the pathogenesis of the accompanying cataract.

Cataract may result from exposure of the lens to a wide variety of physical and chemical insults such as solar or other irradiation, cold, increased intraocular pressure, toxins, nutritional excesses and deficiencies, nearby inflammation, and direct trauma. The list of potential cataractogenic chemical toxins grows daily and includes food additives, chemotherapeutic agents, and by-products of ocular inflammation. The pathogenesis of the cataract is not determined for more than a few such insults, but a common denominator seems to be the ability to upset the precarious balance between substrate supply and enzymic activity within the almost exclusively anaerobic lens. This imbalance results in degeneration of fibers, accumulation of nonmetabolized substrate or production of abnormal metabolites. The latter two classes of products may be cytotoxic or osmotically active, thus drawing water into the critically dehydrated lens and causing opacity.

Most cataracts in people and animals are not identified as being caused by a single insult, but are assumed to represent the result of years of accumulated and perhaps synergistic cataractogenic activity of environmental, dietary, and inborn insults. *The majority of cataracts seen in veterinary practice fall into one of three categories: inherited, post-inflammatory, and idiopathic.* In reality, the large group of inherited cataracts in dogs is of unknown pathogenesis, although extrapolation from knowledge of similar cataracts in rodents and man suggests that inborn errors of lenticular metabolism are at fault. Postinflammatory cataracts result from injury to lenticular epithelium by adjacent inflammation, interference with aqueous production, composition and flow, and accumulation of toxic bacterial, leukocytic, and plasma by-products in the lenticular environment. Adherence of iris to lens (posterior synechia) inevitably causes a focal subcapsular cataract.

Other than these broad categories, there are a few naturally occurring examples of cataract about which there is some understanding.

Diabetic cataract develops in about 70% of spontaneously diabetic dogs, but is rare in diabetic cats. The opacity is bilateral and begins in the cortex at the equator. Progression to complete cortical opacity usually occurs within a few weeks. The pathogenesis of the cataract has traditionally been ascribed to the excessively high level of glucose within the aqueous. Glucose is normally the major energy source for lens fibers, with most of it used to fuel the Embden–Meyerhof pathway of anaerobic glycolysis. When the rate-limiting enzyme of this pathway, hexokinase, is saturated with glucose, the backup of glucose is shunted to alternative metabolic pathways. Chief among these is the *sorbitol pathway*, activated in the rabbit lens by glucose concentrations of >5 mmol/L (>90 mg/dL). In this pathway, the excess glucose is converted by an aldose reductase to the polyalcohol, sorbitol, which is then slowly reduced to a D-fructose, a ketohexose. Because this second reaction is much slower than the first, *sorbitol may accumulate to very high concentrations within the lens and osmotically attracts water even to the point of hydropic cell rupture.* Under experimental conditions at least, the early cataract may be reversed if aqueous sugar levels are reduced to normal, but the later cataract is irreversible.

However, osmotic events alone are not enough to explain all of the structural and metabolic changes in sugar-induced cataracts. The efficacy of antioxidants in ameliorating such cataracts, the nature of intralenticular biochemical alterations, and detection of increased intralenticular oxidants all point to some kind of oxidative damage as an additional promoter of cataract.

Galactose-induced cataracts probably have the same complex and incompletely understood pathogenesis as the diabetic cataract and are seen in orphaned kangaroos and wallabies raised on cows' milk, as well as in a host of experimental models. Since marsupial milk is much lower in lactose than bovine milk, the enzymically ill-equipped neonate develops osmotic diarrhea from undigested lactose and galactose in the intestine, and some excess galactose enters the aqueous humor. The lens, deficient in the enzymes needed to utilize the galactose by converting it to glucose–6-phosphate for anaerobic glycolysis, shunts the galactose via aldose reductase to its polyalcohol, *dulcitol*, which acts osmotically as does sorbitol to disrupt lens fibers.

Cataract reported in puppies and wolf cubs fed commercial milk replacer, or in kittens on feline milk replacer, has been attributed to *deficiency of arginine*, although in several case reports the specific dietary error was not identified. Cataract due to **dietary deficiency** of any of several sulfur-containing amino acids, zinc, or vitamin C occurs in farmed fish, and many models of nutritional cataract exist in various laboratory animals.

Various forms of **irradiation** cause cataract. The lens absorbs most of the ultraviolet and short-wavelength visible blue light that would otherwise damage the retina. At least in people, the chronic exposure to such irradiation is thought to be important in the pathogenesis of senile cataract. **Sunlight-induced cataract** has been described several times in farmed fish, but not yet for other domestic animals as a naturally occurring phenomenon. Absorption of ultraviolet or near-ultraviolet wavelengths by lens epithelium nucleic acids or lenticular aromatic amino acids results in photochemical generation of free radicals and peroxidative damage to numerous structural components of the lens.

A similar pathogenesis probably explains the development of cataract in animals irradiated as part of cancer therapy. In one study, 28% of dogs receiving **megavoltage X-radiation** for nasal carcinoma developed *diffuse cortical cataract* within 12 months of irradiation. In people, the risk of cataract is dose-related, and reaches virtual certainty with dosages of 800–1500 centigrays or rads, whereas rodents require at least twice that dosage. The dogs in the study cited above received between 3680 and 5000 centagrays. Antioxidants such as vitamin E or C, or hypoxia, are significantly protective against several models of light or other irradiation-induced cataract, providing further support for the common denominator of oxidative stress in the pathogenesis of such cataracts.

The aminoglycoside antibiotic and anthelmintic **hygromycin B** has been shown to induce *posterior cortical and subcapsular cataracts* in sows, but not boars, fed the drug continuously for 10–14 months. The effect is dose-dependent and perhaps even cumulative. Pigs fed the same therapeutic daily dose, but consuming the drug on an 8-week-on/8-week-off basis in accordance with the manufacturer's recommendations, do not develop cataracts. The pathogenesis of the cataract is unknown, but a partial inhibition of hygromycin-induced cataracts in vitro by addition of vitamin E suggests that peroxidative damage to lens fiber membranes may be important. *Deafness* in pigs, and also dogs, caused by hygromycin B is discussed with the Ear.

Traumatic lens rupture results in a spectrum of change much different from the changes of cataract described above. First, massive release of more or less native lens protein at the time of rupture may cause severe perilenticular nonsuppurative endophthalmitis about 10 days after the initial trauma (see **Lens-induced uveitis**). Second, the capsular rent permits leukocytes to enter the lens to speed the dissolution of lens fibers. Fibroblastic metaplasia of lens epithelium may result in cartilage or even bone within the lens. *Even after total destruction of the lens fibers, the durable capsule will be found somewhere in the anterior or posterior chamber as a curled eosinophilic mass*, often encapsulated in fibrous tissue probably derived from surviving lens epithelium or from injured ciliary epithelium. Such remnants distinguish lenticular rupture with subsequent dissolution from true developmental aphakia.

Cataract surgery is nothing more than planned traumatic lens rupture, and it is therefore not surprising that we sometimes encounter the same sequence of events as seen with accidental rupture. The main difference is that, with cataract surgery, the lens is already degenerate and the material that may be released following surgical incision of the capsule is not capable of inducing the same magnitude of inflammatory and proliferative reaction. Nonetheless, *complications of cataract surgery and prosthetic lens implantation are quite common in dogs*, the only species in which the surgery is performed on a regular basis. In most instances, the lens capsule remains within the globe and the degenerate lens material is removed by aspiration. The artificial lens is then implanted within the preexistent capsular "bag." Any lens epithelium left behind after removal of most of the cataractous lens forms the nidus for potential regrowth of lens fibers, usually as a combination of fibroblast-like cells and swollen bladder cells. This postoperative proliferation may use the implanted prosthetic lens (or, in its absence, the lens capsular bag) as the growth substrate, not only causing postoperative opacity but also posing the threat of causing pupillary occlusion. Some degree of postoperative epithelial/fibroblastic proliferation is normal in all such eyes, but the amount can be minimized by religious "vacuuming" of the inner surface of the lens capsule to remove all possible germinal epithelium.

Adhesion of pigmented or nonpigmented cellular material to the anterior lens capsule is a valuable histologic clue. *Pigmented epithelium adherent to the lens capsule is a reliable marker for previous posterior synechia*, even if this synechia has been reversed by surgical or pharmacologic intervention or has been accidentally broken during trimming of the globe. Similarly, fibrous or fibrovascular membranes along the anterior lens capsule most often are derived from previous posterior synechia (but they can represent complications of phacoclastic uveitis, pre-iridal fibrovascular membrane, or persistent pupillary membrane). They are significant causes for pupillary obstruction and secondary glaucoma.

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UVEA

The uvea is the vascular tunic of the eye. It is derived from the primitive neural crest mesenchyme surrounding the primary optic cup (only the vascular endothelium is mesodermal). Its posterior differentiation into *choroid* is guided by the developing retinal pigment epithelium, but its differentiation anteriorly into *iris and ciliary body* is probably orchestrated mostly by lens. The presumptive anterior uveal mesenchyme accompanies the infolding of the neurectoderm at the anterior lip of the optic cup to form the stroma of the iris and ciliary processes. That portion of anterior periorbital mesenchyme not accompanying these neurectodermal ingrowths remains to form the ciliary muscle and trabecular meshwork. Posteriorly it forms the choroid and sclera. In all domestic mammals except the pig, the choroid undergoes further differentiation to produce the *tapetum lucidum* dorsal to the optic disk. Defects in development of the retinal pigment epithelium (including its cranial specialization as iridal and ciliary epithelium) inevitably result in defective induction or differentiation of the adjacent uvea.

The mature uvea includes iris, ciliary body, and choroid, the last divided into vascular portion and tapetum lucidum. The filtration angle is shared by iris, ciliary body, and sclera. Its diseases are discussed in the section Glaucoma.

The **iris** is the most anterior portion of the uveal tract. It is a muscular diaphragm separating anterior from posterior chamber, forming the pupil and resting against the anterior face of the lens. The bulk of the iris is stroma of mesenchymal origin, with melanocytes, fibroblasts, and endothelial cells its major constituents. There is neither epithelium nor basement membrane along its anterior face, but rather a single layer of tightly compacted fibrocytes and melanocytes. *The iris stroma is thus in free communication with the aqueous*, a fact of great importance when we consider the secretion or absorption of inflammatory mediators and growth factors in the anterior half of the globe.

The posterior surface of the iris is formed by the double layer of neurectoderm from the anterior infolding of the optic cup. The two layers are heavily pigmented and are apposed apex to apex, with the basal aspect of the posterior epithelium facing the posterior chamber, and separated from it by a basement membrane. The basilar portion of the anterior epithelium, in contrast, is differentiated to form the smooth muscle fibers of the *dilator muscle* of the iris. These fibers lie along the posterior aspect of the iris stroma immediately adjacent to the epithelium. The *constrictor muscle* is found deeper within iris stroma but only in the pupillary third to quarter of the iris. The iris epithelium is rather loosely adherent between layers and between adjacent cells of the same layer, so that *cystic separation* occurs quite commonly. Numerous spaces reminiscent of bile canaliculi lie between adjacent cells and communicate freely with the aqueous humor of the posterior chamber.

The **ciliary body** extends from the posterior iris root to the origin of the neurosensory retina. Like iris, it consists of an inner double layer of neuroepithelium and an outer mesenchymal stroma. The epithelial cells are oriented apex to apex and are separated from the posterior chamber and vitreous by a basal lamina. Only the outer epithelial layer is pigmented. The ciliary body is divided into an anterior *pars plicata* and a posterior *pars plana*, the latter blending with retina at the *ora ciliaris retinae*. The pars plicata consists of a circumferential ring of villus-like epithelial ingrowths supported by a fibrovascular core, called *ciliary processes*. External to the ciliary processes, the mesenchyme forms a ring of smooth muscle, the *ciliary muscle*, responsible for putting traction on the lens zonules and effecting the changes in lens shape necessary for visual accommodation in mammals (other vertebrates may use different and remarkably effective mechanisms). The muscle in domestic animals, particularly ungulates, is poorly developed and accommodation is thought to be minimal in these species. The lens zonules anchor in the basal lamina of the nonpigmented ciliary epithelium, particularly of the pars plana and within the crypts between ciliary processes. The precise location of this anchoring is highly variable among species.

The **choroid** is the posterior continuation of the stroma of the ciliary body. The posterior continuations of the inner and outer layers of ciliary epithelium are retina and retinal pigment epithelium respectively, with the transition made rather abruptly at the ora ciliaris retinae. *The choroid consists almost entirely of blood vessels and melanocytes*, except for the postnatal metaplasia to tapetum dorsal to the optic disk. The choroid is thinnest peripherally, thickest at the posterior pole, blends indistinctly with sclera externally and is separated from the retinal pigment epithelium internally by a complex basal lamina called *Bruch's membrane*.

Many portions of the uveal tract in carnivores continue to develop for at least several weeks after birth, and many of the uveal anomalies like goniodysgenesis or choroidal hypoplasia probably represent developmental errors in postnatal remodeling and maturation, rather than being truly "congenital."

The **general pathology of uvea** *includes anomalous or incomplete differentiation, degeneration, inflammation, and neoplasia.* Anomalies have been previously discussed, and uveal neoplasms are considered in the section on Ocular neoplasia.

Uveal degenerations, except as a sequel to uveitis, are poorly documented. *Idiopathic atrophy of the iris* is described in Shropshire sheep as a bilateral defect obvious by 1–2 years of age. About 25% of the iris is converted to full- or partial-thickness holes; those of partial thickness are spanned by a posterior bridge of iris epithelium. The eye is otherwise normal except for rudimentary corpora nigra. The pathogenesis of the apparently spontaneous atrophy is unknown. Similar atrophy is extremely common in *old dogs*, particularly those of smaller breeds (Poodles, Chihuahuas, Miniature Schnauzers). It is also seen in *old cats*, although much less frequently than in dogs. In both species, the pathogenesis is unknown and there are no published descriptions of the microscopic lesions.

Uveal cysts probably form as the result of fluid accumulation between the two layers of the posterior iris or ciliary epithelium. Such cyst formation is *common in old dogs* and less common in old cats. These cysts may be seen clinically as one or more translucent black cysts attached to the posterior iris or freely floating in the aqueous. Whether the cysts are truly degenerative, or represent residual lesions of fluid exudation from an undetected anterior uveitis, is unknown. A breed-specific syndrome of *iris cyst formation associated with the development of glaucoma* has been reported in Golden Retrievers and Irish Wolfhounds. In the Retrievers, there is concurrent lymphocytic anterior uveitis, although that uveitis is often barely perceptible when the globe is finally submitted for histologic examination. Whether the subtlety of the inflammation is genuine or just a result of aggressive anti-inflammatory therapy prior to enucleation is unknown. The exact relationship between the uveitis, the cyst formation, and the glaucoma has not been clarified.

Uveitis

Uveal inflammation is common and may result from ocular trauma, noxious chemicals, infectious agents, neoplasia, or immunologic events. In addition, corneal injury may cause hyperemia and increased permeability of anterior uveal vessels either by percolation of bacterial toxins or inflammatory mediators into the aqueous, or by stimulation of a vasoactive sensory reflex via the trigeminal nerve. The uvea may be the initial site of inflammation, as in localization of infectious agents, or may become involved as the nearest vascular tissue capable of responding to injury of the lens, cornea, or ocular chambers. Conversely, the uvea seldom undergoes inflammation without affecting adjacent ocular structures. The pathogenesis of uveitis can be arbitrarily divided into *three broad categories*: those examples associated with the hematogenous localization of infectious agents within the uveal tract, diseases resulting from the uveal response to infectious or noninfectious irritants within the ocular chambers, and "autoimmune" uveitis that results from a failure of immune tolerance or from immune mimicry by some infectious agent.

The vocabulary of uveitis

The vocabulary of uveitis and its sequelae is complex:

Anterior uveitis describes inflammation of iris and ciliary body.

Posterior uveitis involves ciliary body and choroid, with **panuveitis** occasionally used to designate diffuse uveitis.

Chorioretinitis describes inflammation of choroid and, usually less severely, overlying retina.

Endophthalmitis is inflammation of uvea, retina, and ocular cavities, with **panophthalmitis** reserved for inflammation that has spread to involve all ocular structures including sclera.

The usefulness of such terminology is doubtful when one considers the vascular unity of the uvea and its intimate association with other ocular tissues. Disagreement between clinical assessment of the extent of the uveitis and histologic evaluation is common: most cases diagnosed clinically as anterior uveitis would be designated as endophthalmitis by histologic assessment. *By convention, the choice of diagnostic classification is strongly influenced by clinical severity, with anterior uveitis the mildest and panophthalmitis the most severe lesion.*

The significance of uveitis

The major significance of uveitis is its effect on adjacent nonuveal tissues. Some effects result from the accumulation of acute exudates or chemical by-products of inflammation, but most result from the later organization of exudates and proliferative events of wound healing within ocular cavities (Fig. 4.39).

Corneal changes include edema and peripheral stromal hyperemia (*ciliary flush*). The edema results from corneal endothelial damage or from increased permeability of limbic blood vessels responding to inflammatory mediators released from the adjacent uvea. In the former instance, damage may be the direct result of the agent causing uveitis, as occurs in infectious canine hepatitis or feline infectious peritonitis. It may also occur as a result of an immune response to endothelial cells containing antigens of these infectious agents, to cross-reaction between microbial and corneal endothelial antigens (immune mimicry), or as a nonspecific response to the presence of the chemical by-products of inflammation within the anterior chamber. Similar by-products mediate the acute inflammatory response in the nearby limbic and conjunctival vasculature, leading to edema in the peripheral corneal stroma. Hyperemia of this limbic network also results in *the circumferential peripheral corneal stromal hyperemia, resembling*

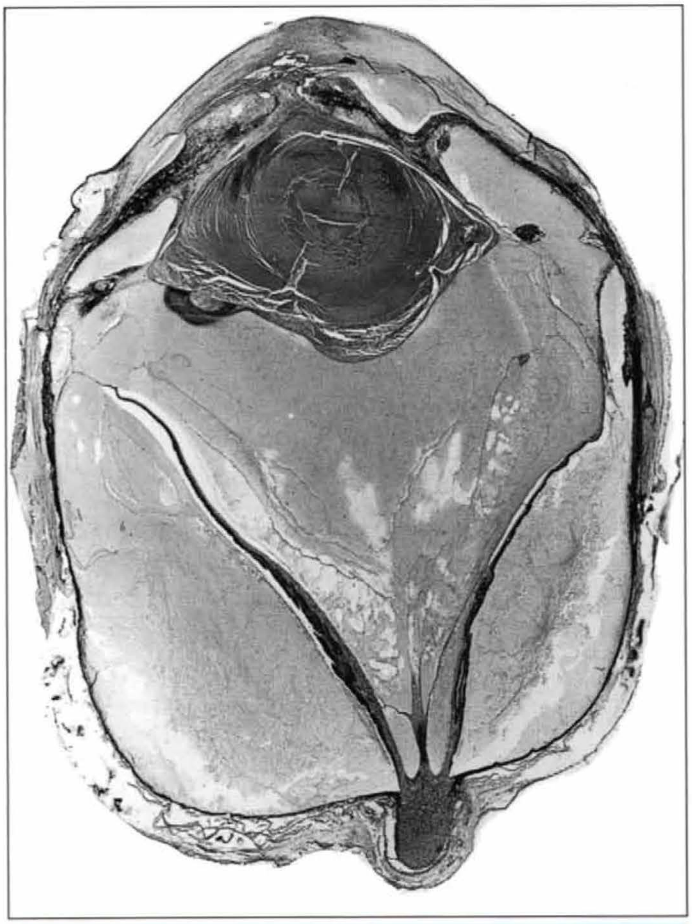

Figure 4.39 Endophthalmitis. Posterior synechia with pupillary block and iris bombé, serous effusion into the aqueous and vitreous humors, and complete serous retinal detachment. Note the healing corneal perforation and the ruptured anterior lens capsule, indicating that this particular example of endophthalmitis is secondary to lens rupture.

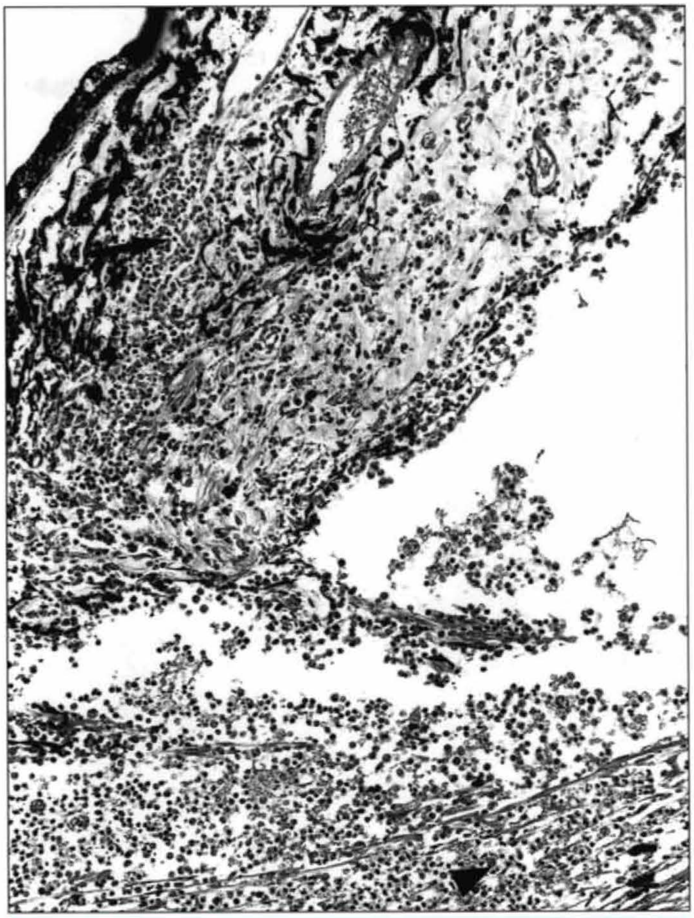

Figure 4.40 Anterior uveitis in a dog. Leukocytes are within the iris stroma and trabecular meshwork. Such exudates rarely if ever obstruct enough of the filtration angle to cause glaucoma. The arrow identifies the pectinate ligament, and the arrowhead is within the corneoscleral portion of the trabecular meshwork.

a brush border, which is a clinical hallmark of anterior uveitis. In eyes with chronic uveitis, corneal edema may also result from glaucoma or from anterior synechia. Persistent edema may lead to stromal fibrosis, vascularization, bullous keratopathy, and the risk of ulceration. *Limbic hyperemia may give way to corneal midstromal vascularization*. This last lesion is extremely common and is a reliable indicator of current or previous uveitis. It is presumed to be an "accidental" and apparently purposeless response to the percolation of angiogenic cytokines from the nearby uveal inflammation.

The accumulation of fibrin, leukocytes, and erythrocytes in the aqueous may result in plugging of the filtration angle and subsequent glaucoma. The infrequent observation of this sequel suggests either unusual potency of the fibrinolytic system within the aqueous, or the inability of exudates to plug more than the most ventral portion of the circumferential angle (Fig. 4.40). Much more common is the organization of inflammatory exudates upon the surface of iris or ciliary body. Adherence of iris to lens (**posterior synechia**) is more common than adherence to cornea (**anterior synechia**) because of the normally intimate association of the lens and iris. If the posterior synechia involves the circumference of the iris, the pupillary flow of aqueous is blocked, posterior chamber pressure rises, and the iris bows forward (**iris bombé**) and may actually adhere anteriorly to the cornea. Glaucoma results from pupillary block, peripheral anterior synechia, or both. In severe and prolonged anterior uveitis, there may be development of a **pre-iridal fibrovascular membrane** on the iris face (Fig. 4.41), which may span the pupil to cause pupillary block. Alternatively, this fibrovascular membrane may extend to cover the face of the pectinate ligament and cause neovascular glaucoma (Fig. 4.42). This membrane may contract on the face of the iris resulting in infolding of the pupillary border to adhere to the anterior (**ectropion uveae**) or posterior (**entropion uveae**) iris surface. Atrophy of iris may follow severe and necrotizing inflammation, and some examples can be distinguished from idiopathic and senile atrophy by the observation of residual lesions of the previous uveitis, such as lymphoid aggregates, focal synechiae, and uveal hyalinization.

The ciliary body suffers the same range of chronic lesions as does the iris. Deposition of PAS-positive hyaline material along the lumenal surface of the ciliary epithelium is particularly frequent in horses. It appears to be deposited in the cytoplasm of the nonpigmented epithelium, and may represent aberrant basement membrane. It is not fibrin; in many instances it has the staining properties of amyloid. Organization of exudate within the posterior chamber or vitreous results in *a retrolental fibrovascular membrane, called a* **cyclitic membrane**, which stretches around the ciliary body and across the back of the lens. Vitreous is almost always liquefied as a result of the severe uveitis, and continued contraction of fibrin in the posterior chamber

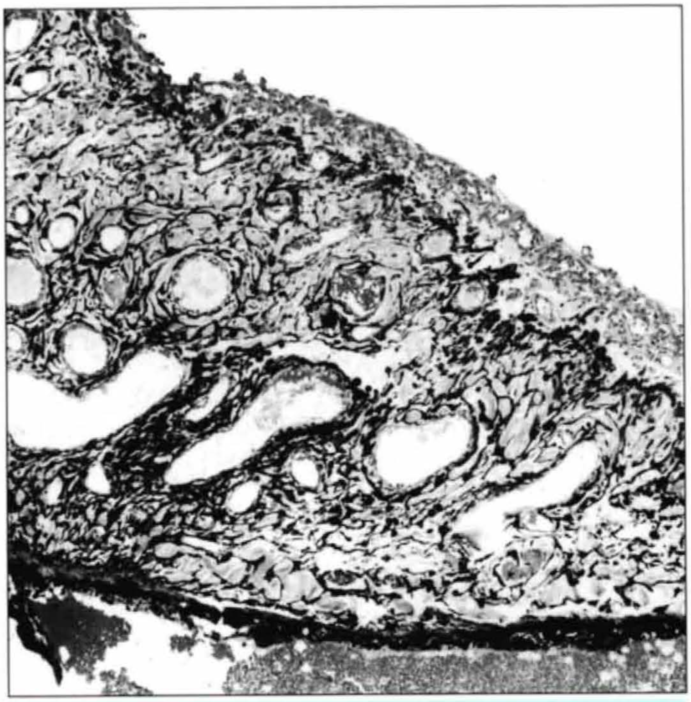

Figure 4.41 **Pre-iridal fibrovascular membrane**.

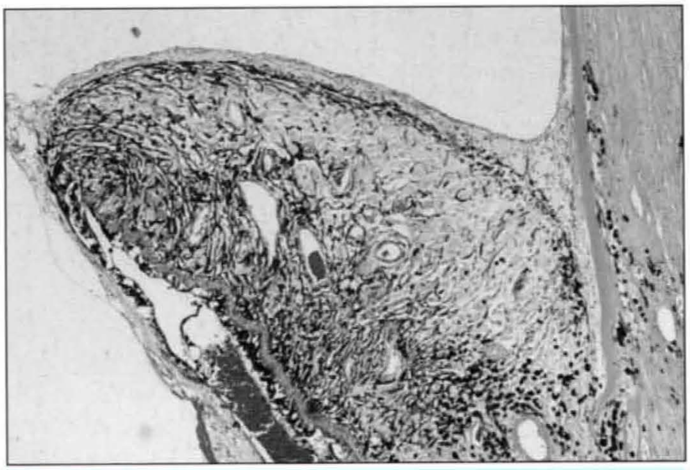

Figure 4.42 **Pre-iridal fibrovascular membrane** crossing the anterior face of the pectinate ligament, causing secondary glaucoma.

and vitreous causes a **tractional retinal separation**. Histologic examination of most cyclitic membranes reveals a fibrovascular retrolental membrane incorporating lens into its anterior face and a folded, degenerate retina in its posterior surface.

The residual lesions *of chronic choroiditis include focal lymphoid aggregates and scarring.* Tapetum usually remains unaffected. As choroiditis severe enough to evoke these lesions will almost invariably have involved retina and retinal pigment epithelium, the residual scar will involve these structures. *Chorioretinal scars are seen as focal fibrous chorioretinal adhesions* in place of normal retinal pigment epithelium. Because these scars prevent the involved retina from separating as part of processing artifact, they frequently appear as "spot welds" along an otherwise artifactually detached retina. Retinal pigment epithelium may be hypertrophic or hyperplastic, particularly if retina has been chronically separated by choroidal effusion. The fibroblast-like cells forming the scar may be derived from retinal Muller cells, choroidal fibroblasts, or metaplasia of retinal pigment epithelium, the last being the major source.

Cataract *is a common sequel to uveitis*, either as a result of uveal adhesions to lens surface, altered aqueous flow with lenticular malnutrition, exposure to injurious inflammatory by-products or increased aqueous pressure in postinflammatory glaucoma.

Phthisis bulbi *describes a hypotonic, shrunken, structurally disorganized eye that is the end stage of severe ophthalmitis*. Phthisis is seen most commonly as a sequel to severe prolonged suppurative septic ophthalmitis from corneal perforation. Cornea and sclera are thickened by fibrosis and leukocytic infiltration and ocular content is barely recognizable. Mineralization and even ossification may occur, but cartilage is absent (unlike congenitally dysplastic globes). *A shrunken, end-stage eye that contains ocular structures with at least recognizable orientation is properly termed* **atrophia bulbi**. The term is seldom used, but atrophia is much more common than true phthisis bulbi.

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The classification of uveitis

There is no truly satisfactory method for classifying uveitis, or for organizing its presentation in a textbook in a manner suitable for all purposes. It does little good to organize classification based upon the innumerable specific diseases capable of causing uveitis, since that approach suffers from the "catch-22" that you need to know what the disease is before you can read about it! Classification based on pathogenesis suffers from the same problem. The clinical classification as anterior vs. posterior uveitis is essentially irrelevant when looking at histologic sections, because virtually all uveitis would be histologically classified as endophthalmitis. In the end, what seems most practical, at least for an audience of pathologists, is to initially classify uveitis on the basis of exudate type, with subdivision based upon histologic evidence of pathogenesis (lens-induced, traumatic, etc.) or etiology as appropriate.

Uveitis is thus classified as serous, suppurative, granulomatous, or lymphocytic-plasmacytic. The usefulness of such classification in predicting causes decreases as the lesion ages and as the events of host immune response blend with the initial inflammation. Furthermore, the reaction may differ among different compartments within the same globe. In general, the highly mobile neutrophils (if present) will be found mostly within the ocular chambers. The less mobile but longer-lived lymphocytes, plasma cells, or macrophages will usually predominate within the uveal tissue itself. As is true in other tissues such as joint and uterus, this may give rise to conflicting terminology depending upon whether the disease is being diagnosed by aspiration cytology of chamber fluid or histologic assessment of the solid tissue.

Acute serous uveitis involves the usual sequence of protein-rich fluid exudation, sometimes with fibrin, followed by emigration of neutrophils. In the iris, the protein-rich fluid readily percolates through the loose stroma to enter the anterior chamber as the clinically observed **aqueous flare**. The ciliary processes are usually distended by inflammatory stromal edema, perhaps a consequence of the initial inability of the serous exudate to pass through the tight intercellular junctions of the ciliary epithelium. Choroid exhibits the most convincing vascular engorgement as well as edema, with the latter frequently seeping through the retinal pigment epithelium to cause **serous retinal separation**. Since such mild uveitis is not likely to result in removal of the globe, observation of serous uveitis is usually restricted to globes enucleated for other reasons, such as severe ocular trauma, neoplasia, or corneal ulceration.

Suppurative uveitis *usually reflects a bacterial pathogenesis*, such as in the neonatal septicemias of calves, foals, and pigs. Neutrophils also predominate in acute mild neurogenic uveitis associated with corneal epithelial injury and in the acute phase of phacoclastic uveitis. In very mild uveitis, they are found marginated along the endothelium of iris and ciliary venules, in perivascular adventitia, adherent to ciliary processes, and in the filtration angle (see Fig. 4.40). Neutrophils rapidly degenerate within the aqueous to assume an unsegmented globular morphology. Clumps may adhere to the corneal endothelium as **keratic precipitates**, or settle ventrally within the anterior chamber as **hypopyon**. The hypopyon may, in histologic section, seem to be plugging the filtration angle, but only rarely would such exudate cause glaucoma, because only rarely would such exudate be able to occlude a significant part of the circumferential filtration angle. Fibrin exudation may accompany the suppurative inflammation, but fibrinolysis is very efficient within the anterior chamber, and glaucoma rarely results.

Granulomatous uveitis *is characterized by the conspicuous presence of epithelioid macrophages and, occasionally, giant cells.* As is true of granulomatous disease elsewhere, only rarely is the exudate purely granulomatous and most involve substantial participation by neutrophils. Ocular localization of some species of dimorphic fungi or of algae, helminths, or mycobacteria may cause granulomatous endophthalmitis, as may lens rupture and the Vogt–Koyanagi–Harada-like syndrome in dogs.

Lymphocytic-plasmacytic uveitis *is by far the most common histologic type of uveitis encountered in enucleated globes.* The leukocytes resident within the uveal tissue are dominated by lymphocytes and plasma cells, with or without the formation of actual lymphoid aggregates and nodules. It may occur simply as a chronic form of what was initially a suppurative uveitis, but is more frequently seen as the typical manifestation of immune-mediated uveitis, viral infection, phacolytic uveitis, or uveitis secondary to intraocular neoplasia. Inasmuch as the inflammation in most of these examples is probably of immunologic pathogenesis, *it is probably more accurate to consider all lymphocytic-plasmacytic uveitis as immune-mediated*, with the prefix "idiopathic" or the name of the inciting antigen. Dogs, cats, and horses are the species most frequently affected, and in these species the inciting agent is usually unknown.

Specific examples of uveitis/endophthalmitis follow.

Bacterial endophthalmitis

Bacteria may enter the eye hematogenously or via penetrating wounds. Those arriving hematogenously cause their initial lesion in ciliary body or, less frequently, in choroid. Those arising via penetration usually incite the initial reaction in anterior chamber, particularly if the penetration is via perforation of an ulcerated cornea. Most are suppurative, and their extent and severity vary with the size of inoculum, virulence of the agent, and host response and its duration.

The list of organisms capable of causing endophthalmitis is long. *It is probably true that any bacterium capable of bacteremia or septicemia can cause endophthalmitis.* Particularly prominent are the streptococci and coliforms in neonatal septicemia. The failure to detect ocular lesions in such animals is more often the result of the brief, fatal course of the disease than of specific ocular resistance. The ocular lesion may be very mild, better detected by opacification of the plasmoid aqueous in Bouin's or Zenker's fixative than by histologic examination. Histology may reveal only edema of ciliary processes with a few neutrophils along the capillary endothelium or enmeshed in filaments among ciliary processes. The well-known bovine *Histophilus somni* bacteremia, infectious thrombotic meningoencephalitis, is seen as focal rather than diffuse chorioretinitis.

Exceptions to the generalization that bacterial endophthalmitis is suppurative occur if infection is caused by bacteria which, in other tissues, incite lymphocytic or even granulomatous inflammation. *Ocular tuberculosis* is largely of historical interest. It occurred as part of generalized systemic disease and the typical tubercles were most numerous in the choroid. *Mycobacterium tuberculosis* var. *bovis* was the usual isolate except in cats where the human strain was common. In cats, ocular tuberculosis may also occur as keratoconjunctivitis without uveal involvement.

Brucella canis may cause chronic lymphocytic endophthalmitis that is probably immunologically mediated. Agglutinating titers for *B. canis* antigen in aqueous exceed those of serum, and the ocular lesions are similar to those of equine recurrent ophthalmitis.

Listeria monocytogenes often causes endophthalmitis in association with meningoencephalitis in ruminants (see Vol. 1, Nervous system). The condition is unilateral and the pathogenesis is obscure.

Uveitis caused by the rickettsias of *Rocky Mountain spotted fever* and *ehrlichiosis* are discussed under Retinitis.

Mycotic endophthalmitis

Fungi may affect the eye as causes of keratitis, orbital cellulitis, or endophthalmitis. Only rarely do the fungi causing keratitis or orbital infection penetrate the fibrous tunic to cause intraocular disease. However, hematogenous uveal localization is rather common in the course of systemic mycoses caused by *Cryptococcus neoformans* and *Blastomyces dermatitidis* and, less regularly, with *Coccidioides immitis* and *Histoplasma capsulatum*. In immunodeficient animals, one might expect occasionally to detect endophthalmitis as part of generalized disease caused by saprophytic fungi such as *Aspergillus* or *Candida*; these same agents will occasionally cause endophthalmitis in conjunction with penetrating plant foreign bodies. The frequency with which endophthalmitis accompanies systemic mycosis is unknown and probably varies with the specific agent, the species affected, and whether the disease is in an endemic area or is a sporadic occurrence. Hematogenous ocular mycosis is found almost exclusively in dogs, except for cryptococcosis, which is more common in cats. *Blastomyces* and *Cryptococcus* are more likely to invade the eye in the course of generalized infection than are *Coccidioides* or *Histoplasma*, and occurrence in nonendemic areas is strongly linked to prolonged systemic corticosteroid therapy. Involvement is bilateral but not necessarily equal. Blastomycosis, cryptococcosis, and coccidioidomycosis are discussed with the Respiratory system, and histoplasmosis with the Hematopoietic system.

Blastomycosis *is the most frequently reported cause of intraocular mycosis in dogs.* In is rare in cats. Between 20–26% of dogs with the systemic disease are blind or have grossly observed ocular lesions, suggesting that intraocular involvement would be recognized more often if histologic examinations were routinely done. The clinical ocular disease is severe diffuse uveitis, frequently with retinal separation.

The histologic appearance is of diffuse pyogranulomatous or granulomatous endophthalmitis with retinitis, exudative retinal separation and, commonly, granulomatous optic neuritis. Choroiditis is often more pronounced than is the anterior uveitis. The greatest accumulation of leukocytes often is in the subretinal space enlarged by exudative retinal detachment. The causative diagnosis depends upon the demonstration of the spherical-to-oval, thick-walled yeasts in vitreous aspirates or in the histologic section. They are usually most numerous in the subretinal exudate, but are rare in anterior chamber or in retina itself (Fig. 4.43). The organisms are free or within macrophages, are 5–20 μm in diameter, and show occasional *broad-based budding*. Extremes in sizes may result in yeasts from 2–30 μm diameter. The eye may, in addition, have the full spectrum of corneal, lenticular, and glaucomatous sequelae as expected of any severe uveitis. Panophthalmitis with orbital cellulitis is seen in about one-third of enucleated globes.

Cryptococcosis is similar to blastomycosis in that *the lesions are predominantly within retina, choroid, and optic nerve.* However, infection of the eye may arise either hematogenously or by extension from the brain via optic nerves, and lesions are often conspicuously lacking in cellular host reaction. *Large collections of poorly stained pleomorphic yeasts, surrounded by wide capsular halos, impart a typical "soap-bubble" appearance to the histologic lesions* (Fig. 4.44). The yeasts vary in size but most are 4–10 μm in diameter. Round, oval, and crescentic forms are seen. In some animals, however, a granulomatous reaction mimicking that of blastomycosis can be found. In such lesions the organisms typically are scarce.

The frequency with which ***Coccidioides immitis*** infects the eye appears to be low (~2%), despite the prevalence of generalized infection in endemic areas. The ocular lesion resembles blastomycosis in that *pyogranulomatous reaction occurs around fungal spherules.* The reaction is predominantly purulent around newly ruptured spherules, gradually becoming granulomatous as the released endospores mature. The lesion tends to be more destructive than other mycoses, usually

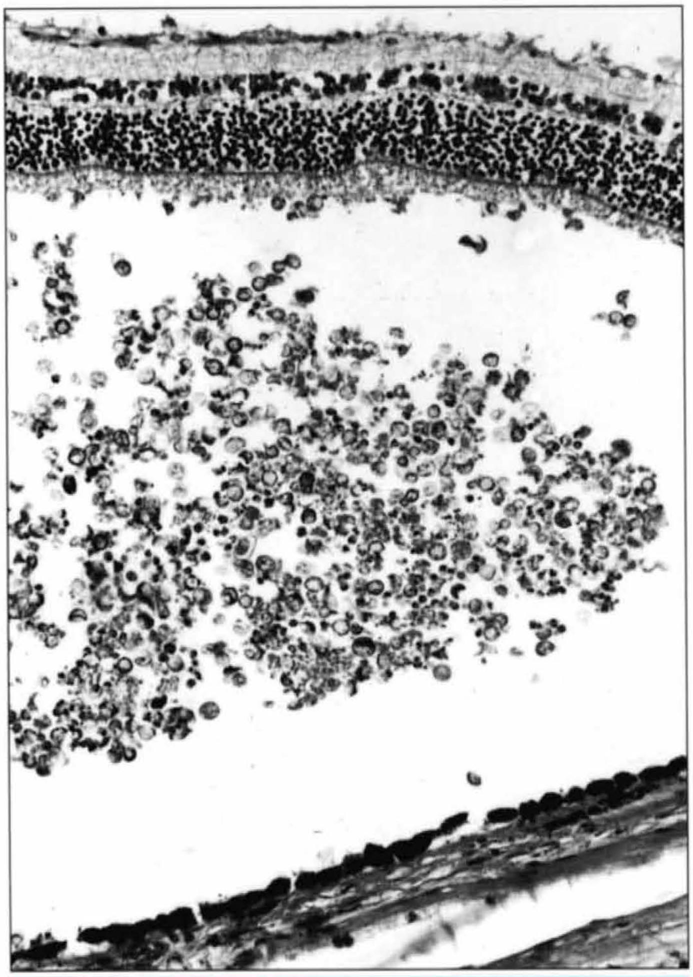

Figure 4.43 Subretinal exudate containing ***Blastomyces dermatitidis***, in a dog receiving long-term corticosteroid therapy.

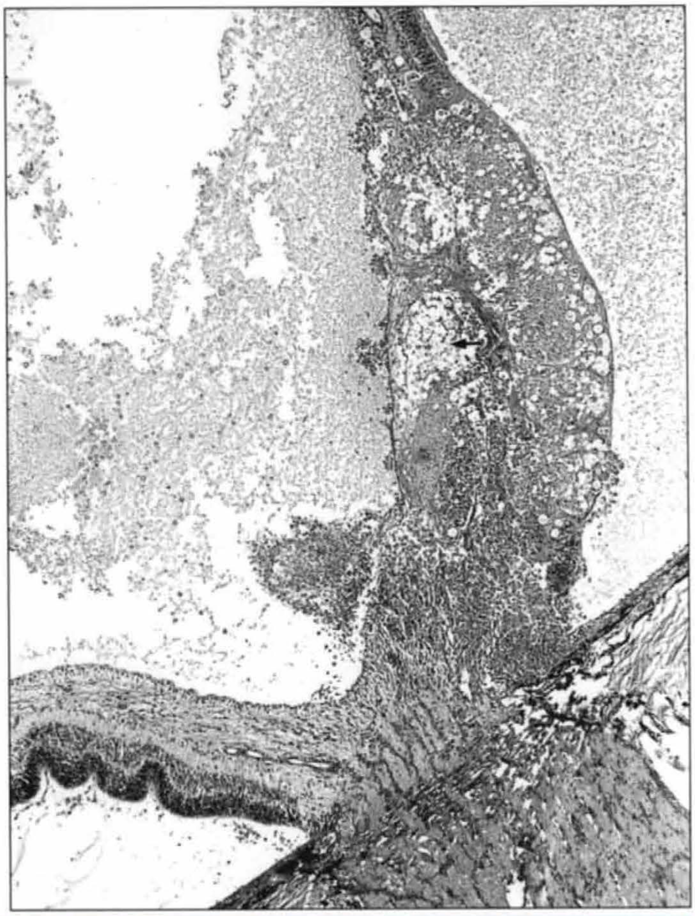

Figure 4.44 Retinal separation and focal necrotic retinitis in a cat with ***Cryptococcus*** infection. Note "soap-bubble" appearance (arrow) and minimal host reaction.

spreading to involve sclera and even episclera in a suppurative panophthalmitis.

Histoplasma capsulatum is a common cause of generalized mycosis in dogs but is rare in other domestic species. It has a predilection for lymphoid tissue and other tissues rich in phagocytes, such as lung and liver, and it is perhaps this preference that accounts for the paucity of ocular involvement in spontaneous disease. In dogs and cats, infiltrative choroiditis or panuveitis occurs and is dominated by plasma cells and by macrophages filled with the organisms. Retinal separation, plasmoid vitreous, and optic neuritis also develop. The reaction tends to target the choroid, and to be less destructive than either blastomycosis or coccidioidomycosis.

Prototheca are not fungi, but they are most easily confused with *Cryptococcus* and *Blastomyces*, and histologic lesions are certainly similar to those occurring in mycotic endophthalmitis. They are *colorless saprophytic algae* capable of causing enteric, cutaneous, mammary, or generalized granulomatous disease in a variety of mammalian species. Ocular lesions have been described only in dogs with the disseminated form of the disease. *The lesions are bilateral and may vary from lymphocytic-plasmacytic to granulomatous panuveitis with optic neuritis and exudative retinal separation.* The host response is usually quite mild. The lesions resemble ocular mycosis, particularly cryptococcosis, and are distinguished only by the observation of the pleomorphic algae. In histologic section, the algae are free or within phagocytes. The organisms are spherical to oval, from 2–20 μm in diameter with a refractile, PAS-positive, and argyrophilic cellulosic cell wall. Each cell consists of granular, weakly basophilic cytoplasm surrounding a central nucleus. *Prototheca* reproduces by asexual multiple fission, so that multiple daughter cells form within a single cell wall. One or two cycles of nuclear division without cytoplasmic cleavage may produce transient multinucleated cells before eventual cytoplasmic division results in up to eight daughter cells. Each daughter cell acquires a capsule, resulting in a parent cell criss-crossed by septations that represent the cell walls of maturing daughter cells. Rupture of the parent cell wall releases the unicellular autospores. Collapsed, crumpled, and seemingly empty cell walls are visible in histologic section. There is no budding as with *Blastomyces* and *Cryptococcus.*

Those canine isolates that were definitively identified were *P. zopfii*. An enteric route of entry is probable inasmuch as necrotic enteritis is a feature of the disease (see Vol. 2, Alimentary system). Immunodeficiency may be a prerequisite for dissemination of the organism. Lesions are found in many visceral organs, skin, and lymph nodes in most cases. The reaction is granulomatous but is usually minimal in comparison to the large number of organisms.

Protozoan endophthalmitis

While ocular lesions have been reported in infections caused by protozoa of the genera *Toxoplasma, Leishmania, Encephalitozoon, Besnoitia,* and *Trypanosoma,* only *Toxoplasma* specifically causes intraocular lesions, although many cases diagnosed as toxoplasmosis were probably caused by *Neospora*. Most of the others cause keratoconjunctivitis that occasionally extends to anterior or generalized uveitis in which the causal agent may be found. The exception is *Encephalitozoon* that may induce periarteritis within the uvea and retina as it does elsewhere.

Toxoplasmosis affecting the eye is, as elsewhere, much more frequently suspected than proven, and clinical diagnoses greatly outnumber those confirmed by histopathology. *The histologic lesion is usually in retina, uvea, or extraocular muscles, and varies from focal, acute coagulative necrosis to granulomatous or lymphocyte-rich inflammation.* The organisms are seen most easily as intracellular pseudocysts during acute disease or as true cysts during remission. The more noxious merozoites are found only with difficulty as 7–9 μm crescentic, basophilic bodies within phagocytes or free amid necrotic debris.

The histologic changes of ocular toxoplasmosis have received various interpretations and valid differences probably exist between species and between individuals of differing immune status. In man, the disseminated disease is usually congenital and the ocular lesion is multifocal necrotic retinitis in which free or encysted *Toxoplasma gondii* are found. There may be lymphocytes and plasma cells in adjacent choroid. In human adults, the ocular lesion is predominantly a lymphocytic-plasmacytic choroiditis, suggesting that the pathogenesis is related more to host immune response to the previously encountered, ubiquitous antigen than it is to local infection.

Lesions analogous to human congenital toxoplasmosis occur in *young cats* as multiple foci of retinal necrosis. Choroiditis may be present and is lymphocytic-plasmacytic, and anterior uveitis is seen in only 20–30% of such cases. *Much more common than this classical retinochoroiditis, however, is lymphocytic-plasmacytic anterior uveitis with serologic evidence of active Toxoplasma infection.* The role of toxoplasmosis in feline anterior uveitis is controversial (see Idiopathic lymphonodular uveitis of cats). Retrospective histologic studies list the majority of such cases as idiopathic and are presumed to be immune-mediated, with antigen or antigens unknown. In the several large published studies, there has not been a single case confirmed by observation of merozoites or cysts in the eye; even serologic evidence in these studies pointed to toxoplasmosis in only 1–2% of cases. In contrast, one study reported evidence of anterior uveal production of toxoplasma-specific antibody in 32 of 69 cats with anterior uveitis, and another report confirmed that anterior uveitis is indeed the most frequent manifestation of toxoplasmosis in cats, seen in 60% of cats with confirmed toxoplasmosis. Nonetheless, evidence remains less than conclusive about the role of *T. gondii* in the prevalent and enigmatic syndrome of anterior uveitis in this species. It may be that the local production of *Toxoplasma* antibody in cats with uveitis is merely the result of nonspecific recruitment of *Toxoplasma*-sensitized lymphocytes into the chronically inflamed uvea, and that lymphoid aggregates in such eyes are producing a whole range of antibody quite irrelevant to the original cause of the uveitis.

The situation in other species is not clear, but the prevalence of ocular lesions seems quite low. Lymphocytic cyclitis and multifocal necrotic retinitis are most frequently described and are usually seen together. *Multifocal choroiditis*, not necessarily adjacent to retinal lesions, is lymphocytic-plasmacytic in most species but granulomatous in sheep. A more common lesion is severe myonecrosis in extraocular muscles associated with free and encysted *Toxoplasma*. Toxoplasmosis is discussed in more detail in Vol. 2, Alimentary system.

Parasitic endophthalmitis

Many parasites are found incidentally in the eye, including: *Echinococcus* in primates and *Cysticercus* in swine, multifocal ischemic chorioretinitis and optic neuritis in elk caused by occlusive vasculitis due to microfilariae of *Elaeophora schneideri*, and uveitis associated with fortuitous localization of larvae of *Toxocara canis* or other ascarids, *Angiostrongylus* *vasorum*, *Dirofilaria immitis*, and *Onchocerca cervicalis*. In addition, adults of *Setaria* spp. are occasionally found within the eye of horses. The long threadlike worms are seen floating within the aqueous, and the uveitis that results seems to be the result of mechanical irritation. The only specific intraocular parasitism is seen with the lens fluke of fish (*Diplostomum spathaceum*), which, after penetrating the skin, seeks the lens with remarkable speed and specificity. The principal lesion is cataract induced by the intralenticular presence of hundreds of larvae awaiting ingestion by fish-eating birds for completion of their life cycle, but infected fish may have larvae arrested in many other ocular or extraocular locations.

Chronic mild anterior uveitis is reported to accompany ectopic localization of immature *Dirofilaria immitis* within aqueous and vitreous cavities of canine eyes. Studies of pathology are sparse, but endophthalmitis is reported in which anterior synechiae, subretinal exudate, and early cyclitic membrane may accompany the numerous vitreal and subretinal larval nematodes.

Ocular onchocerciasis affects people and horses. The *human* disease is endemic in Africa and Central America and is one of the most frequent causes of blindness in the world. The microfilariae of the causal agent, *Onchocerca volvulus*, are transmitted by *Simulium* spp. flies, and affect the skin, eyelids, and corneas of children and young adults. The microfilariae are found throughout the eye, but the lesion of greatest visual significance is diffuse sclerosing superficial stromal keratitis complicated by anterior uveitis with synechiae and eventual glaucoma.

Equine onchocerciasis has some similarities. The parasite, *O. cervicalis*, is of worldwide distribution and surveys from the USA document the prevalence of dermal infection in horses as varying from 48 to 96%. About one-half of the infected horses have microfilariae in conjunctiva or sclera. The microfilariae enter the eye only incidentally in the migration from the ligamentum nuchae to the subcutis. The ocular sites of greatest concentration are the peripheral cornea and the lamina propria of the bulbar conjunctiva near the limbus. The microfilariae in the cornea are associated with superficial stromal keratitis resembling the disease of man, albeit much milder. Some of the horses also have anterior uveitis typical of equine recurrent ophthalmitis, prompting theories that *Onchocerca* is one cause of this disease. The microfilariae can be recovered from the conjunctiva and eyelids of horses with uveitis, keratoconjunctivitis, and eyelid depigmentation, but are recovered with equal frequency from horses with no ocular disease and no microscopic reaction to the worms.

Ocular manifestations of **visceral larva migrans** in people are associated with larvae of *Toxocara canis* or, perhaps more frequently, of the raccoon roundworm *Baylisascaris procyonis*. The unilateral granulomatous fundic lesions are caused by a single wandering larva, and are relatively common in children but have rarely been described in nonhuman subjects despite the rather common occurrence of ascarid-induced granulomas in canine kidneys, lungs, or livers. The paucity of reports may not reflect the actual prevalence of disease in specific canine populations. One large survey of working sheepdogs in New Zealand recorded a 39% prevalence of lesions attributed to visceral larva migrans, contrasted to a 6% prevalence in similar dogs living in urban environments. *The active lesions were lymphocytic and granulomatous uveitis, nonsuppurative retinitis, and peripapillary nontapetal retinal necrosis.* Inactive lesions involved choreoretinal scars and multifocal chronic retinal separations in dogs older than 3 years. Larvae most compatible with *Toxocara canis* were seen in sections of some acutely affected eyes. The high prevalence in these dogs was tentatively ascribed to the feeding of uncooked frozen mutton that may have contained *T. canis* larvae as part of a dog–sheep–dog life cycle. A report of similar lesions in Border Collies in the US was associated with the feeding of raw pork.

Ocular disease may also result from the intraocular migration of *fly larvae*. This syndrome, termed *internal ophthalmomyiasis*, is discussed with diseases of conjunctiva.

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Viral endophthalmitis

The fuzzy distinction between infectious uveitis/endophthalmitis and immune-mediated disease is most obvious when dealing with viral causes of endophthalmitis, even though it is probably also a major factor in mycotic and parasitic diseases. The distinction is therefore arbitrary, and *discussed below are those diseases that are probably best classified as immune-mediated endophthalmitis for which the antigen is identified as viral.*

Canine adenovirus

Canine adenovirus 1 (infectious canine hepatitis virus) is the best-documented cause of virally induced, immune-mediated uveitis in domestic animals. The systemic disease is discussed in Vol. 2, Liver and biliary system. During the acute viral stage of the disease, viral replication within endothelium and stromal phagocytes of the uvea results in a primary mild nonsuppurative uveitis that usually is clinically undetected. Inoculation of field virus into the anterior chamber of dogs and foxes may result in viral inclusion bodies within corneal endothelium and subsequent edema, but edema is not a feature of the active stage of naturally occurring disease. During the convalescent phase of the disease, or 6–7 days after vaccination with a modified live virus, a small percentage of dogs develops anterior uveitis, endothelial damage, and corneal edema ("*blue eye*") that is a manifestation of type III hypersensitivity to persistent viral antigen in which complement fixation attracts neutrophils. The proteases of neutrophils are responsible for the cell injury.

The histologic lesion is bilateral but not usually of equal intensity, thus clinically apparent disease may be unilateral. *Corneal edema results from diffuse hydropic degeneration of corneal endothelium and secondary stromal edema.* In a small percentage of affected dogs, the damage is so persistent as to cause interstitial keratitis and permanent fibrosis. Whether this sequel results from unusually persistent antigen, unusually severe endothelial damage or age-dependent variation in endothelial regenerative ability is unknown. *Intranuclear inclusion bodies* of adenovirus type may be seen in a few degenerate endothelial cells. There is accompanying anterior uveitis, with lymphocytes and plasma cells around vessels in iris and ciliary body, in the filtration angle and adherent to cornea as keratic precipitates. Choroidal involvement is mild or absent. Sequelae such as synechia or angle obstruction with debris are infrequent, occurring in less than 5% of affected eyes. In most dogs, whether recovering from natural or vaccine-induced infection, the ocular reaction subsides within 3–4 weeks.

Feline infectious peritonitis-associated uveitis

The coronavirus of feline infectious peritonitis causes diffuse uveitis that is probably immune-mediated (see Vol. 2, Alimentary system). The frequency of ocular lesions is unknown because the eyes are not regularly examined in cats with the disease. Estimates based upon clinical examination range from about 10% in an outbreak to 50% of unselected clinical cases. *Most cats that die of the disease have ocular involvement as detected by coagulation of aqueous with acidic fixatives (indicating increased aqueous protein).* The histologic evidence for inflammation in some eyes may be subtle indeed, and such eyes usually reach postmortem without clinically detected uveitis. Conversely, some cats develop severe uveitis attributed to this virus by clinical, serologic, or histologic evaluation, without concurrent evidence of the disease elsewhere.

The typical histologic lesion, as is the case elsewhere in the body, varies with time and location. Leukocytic infiltration is most extensive in ciliary body and adjacent limbic sclera, and is usually a rather even mixture of neutrophils, lymphocytes, plasma cells, and macrophages. In some eyes, the infiltrate is purely histiocytic. The inflammatory cell population often becomes more purely lymphoid in the choroid and more neutrophilic in the anterior chamber. Perivascular lymphocytic-plasmacytic aggregates are common in retrobulbar connective tissue and in the optic nerve sheath, and in the retina. In the retina, the accumulations are larger and are more likely to involve a true phlebitis than is the case with the subtle perivascular retinitis that is a frequent and nonspecific accompaniment to most forms of anterior uveitis. Sequelae to the uveitis are rarely seen, either because the cats are in the late stages of the disease when ocular lesions are examined or because euthanasia halts its progression. Retinal separation with serous subretinal exudate is occasionally observed. The presence of large globular accumulations of macrophages and neutrophils adherent to the corneal endothelium (*keratic precipitates*) is an important clinical hallmark of the disease, and is useful histologically as well. Neutrophilic endothelialitis with severe corneal edema may also occur.

Bovine malignant catarrhal fever-associated uveitis

The presence of severe uveitis is an important clue in the clinical differentiation of malignant catarrhal fever from other bovine systemic disorders, particularly from mucosal disease. The histologic lesions within the eye resemble those elsewhere in the body: arterial necrosis and perivascular and intramural lymphocytic accumulations. The presence of mitotic figures among the lymphoid cells is distinctive. The arteritis usually is most obvious in the iris, but may be seen affecting arterioles or venules in the retina, choroid, meninges of optic nerve, or even peripheral cornea. There is *marked corneal edema with a ring of peripheral corneal stromal vascularization*, clinically seen as a dark red circumferential brush border of straight vessels in the perilimbal cornea. Blood vessels in the conjunctiva and even in the newly vascularized cornea may be targets for the disease, so that the edema and hemorrhage of vessel injury are added to the nonspecific lesions of conjunctivitis and peripheral keratitis that accompany uveitis of any cause in all species.

Infiltration of lymphocytes among corneal endothelial cells is associated with patchy necrosis of that layer, which may also contribute to the corneal edema (Fig. 4.45). A layer of mononuclear leukocytes enmeshed in fibrin often is adherent to the aqueous face of the corneal endothelium.

Even the very early lesions are lymphocytic. In vessels, the first changes involve subendothelial and adventitial lymphocytic and lymphoblastic accumulation, with little necrosis. Despite longstanding speculation for an immune-complex pathogenesis for the vasculitis, proof is lacking. Deposition of immunoglobulin or complement is not a significant feature of the vascular lesion within the eye, and a T-cell-dependent, type IV immune pathogenesis has been suggested.

Immune-mediated uveitis

There is no clear distinction between immune-mediated uveitis and uveitis traditionally ascribed to a specific causative agent. Except for rapidly progressing bacterial uveitis following hematogenous localization or penetrating injury, *virtually all uveitis probably has an immune component superimposed on initial nonspecific inflammation.* Even traumatic uveitis probably permits unusually large amounts of endogenous ocular antigens to enter venous drainage and to reach the spleen and other lymphoid tissue. Types III and IV hypersensitivity have been induced in various laboratory animals using tissue-specific antigens of photoreceptor, uveal, lens, and corneal origin. *Lens-induced uveitis* and an *idiopathic uveitis associated with dermal depigmentation in dogs (the so-called Vogt–Koyanagi–Harada syndrome)* are naturally occurring examples of uveitis induced by endogenous ocular antigens. Recent

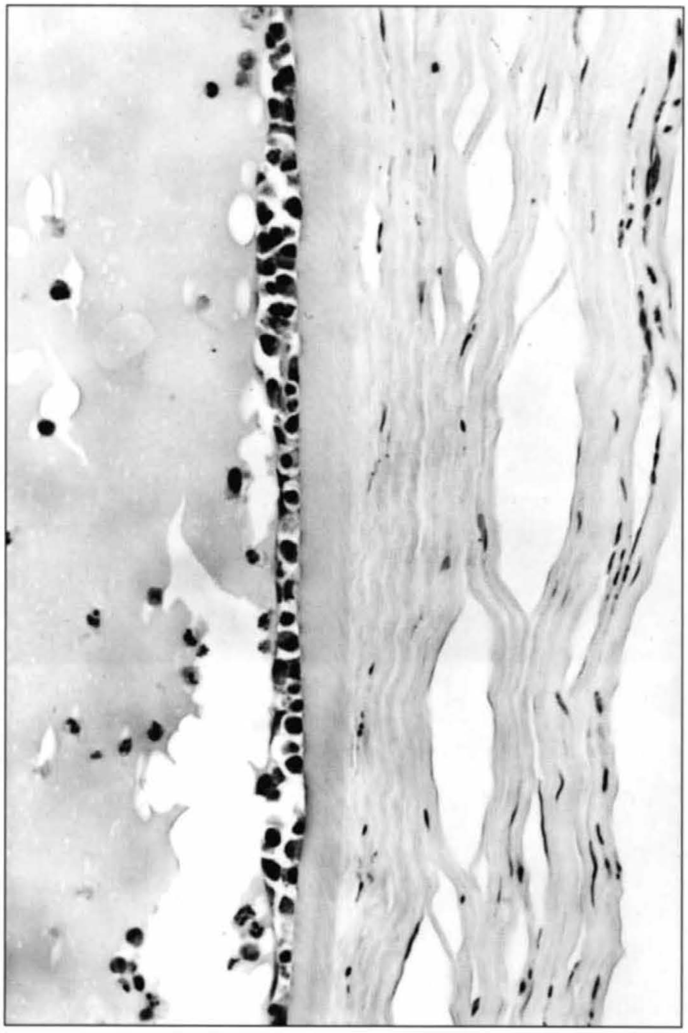

Figure 4.45 Lymphocytes, as part of a presumed **immune-mediated endothelialitis**, obscure the few remaining corneal endothelial cells in bovine malignant catarrhal fever.

demonstration of strong cross-reactivity between *leptospiral antigens* and equine corneal endothelium (immune mimicry) serves to further obscure the distinction between infectious and immune-mediated ocular disease.

Included in this section are those diseases that are exclusively or predominantly immune-mediated, and for which the triggering antigen has not been proven to be an infectious agent. *In general, all are characterized by chronic, lymphocytic-plasmacytic panuveitis, in which the infiltrating leukocytes form lymphoid aggregates and perivascular cuffs.* Clinically, they are either continuously progressive syndromes or subject to periodic irregular clinical exacerbations and remissions.

The normal eye contains almost no lymphoid tissue and, following initial antigenic challenge, must rely on diffusion of antigen to the spleen before effector lymphocytes return to enter the eye. The lymphocytes do so primarily as perivascular aggregates throughout the uvea.

Those examples of uveitis associated with penetrating injury or massive localization of infectious agents cause disruption of the blood–eye barrier. The globe thus responds to the inflammatory stimulus as would any other tissue, and those events will not be further described here.

Hematogenous localization (or experimental inoculation) of less damaging antigen within the ocular chambers that allows the blood–eye barrier to remain intact triggers a remarkable adaptation of the inflammatory and immune response known as anterior segment immune deviation, described below.

The eye was once thought to be an immunologically privileged site, with no resident lymphocytes, no antigen-processing macrophages or dendritic cells, and no lymphatic drainage. These peculiarities, plus the "*blood–eye barrier*" created by the tight intercellular junctions of iris endothelium, ciliary nonpigmented epithelium, and retinal pigment epithelium, fostered the mistaken belief that many intraocular antigens were sufficiently sequestered as to be seen as "non-self" in the event of their release into systemic circulation. The numerous diseases characterized by lymphocytic-plasmacytic uveitis in the absence of an identified infectious agent have thus been incautiously grouped as examples of "autoimmune" reaction to sequestered uveal, lenticular, or retinal antigens.

More recent studies have demonstrated that these supposedly unique and sequestered antigens are neither unique nor completely sequestered, and that antigens of any type introduced into the intraocular environment will trigger a robust immune response. Antigens identical to some of the lenticular or uveal antigens, for example, are found in nonsequestered tissues elsewhere in the body. Antigens inoculated into the anterior chamber induce a systemic humoral and T-cell response, clearly pointing to at least some leakiness in the blood–eye barrier. A variety of experiments have led to the discovery of a carefully regulated system of ocular immunity, termed **anterior chamber-associated immune deviation**. In this system, antigens introduced into the intraocular environment in a relatively controlled fashion are altered by contact with cytokines secreted by trabecular endothelial cells, corneal endothelium, and probably many other resident ocular tissues. The list of specific cytokines and how they alter antigen expression continues to unfold. The first cytokine proven to alter antigen reactivity was TGF-β2, but the list of chemical participants includes TNF-α, vasoactive intestinal peptide, and substance P. These altered antigens are then processed by macrophages and dendritic cells of bone marrow origin that are resident within the uvea. These antigen-presenting cells promote the development, after 5–6 days, of specifically sensitized suppressor T-cells within the spleen. These suppressor cells then return to the uvea where they act to suppress delayed-type hypersensitivity and the production of complement-fixing antibodies. The cells do not blunt the more discriminating noncomplement-fixing antibody production and cytotoxic lymphocytic activation that can destroy unwanted antigen without the messy bystander injury that would be so damaging to the globe.

Whatever their type, the splenic lymphocytes reach the eye about 1 week after experimental introduction of antigen into anterior chamber. Typically the lymphocytes are seen as perivascular aggregates in iris stroma, in the ciliary body and, less obviously, in the choroid and even the retina. In long-standing cases (which are most likely to receive histologic examination) the aggregates may be very large and resemble lymphoid follicles (Fig. 4.46). As in other tissues, amplification of the immune response results in recruitment of lymphocytes that are not necessarily specific for the inciting antigen. The polyclonal nature of these lymphocytes is probably important in the typically recurrent nature of uveitis in all species. Once established in the eye, these cells respond to a diverse range of circulating antigens that enter the eye through a blood–eye barrier disrupted by

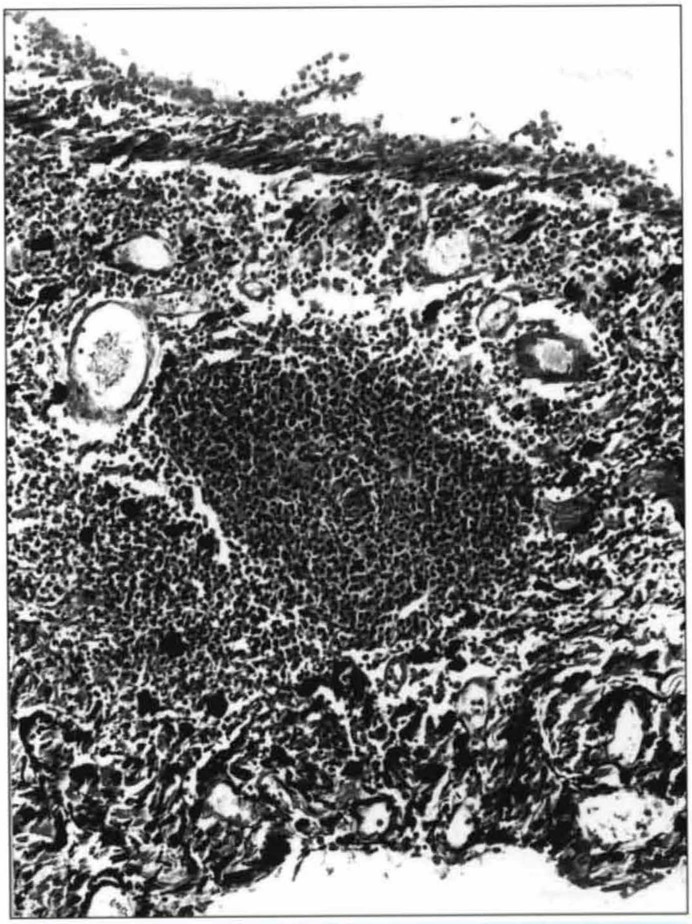

Figure 4.46 Equine recurrent uveitis. Lymphoid nodules in iris stroma.

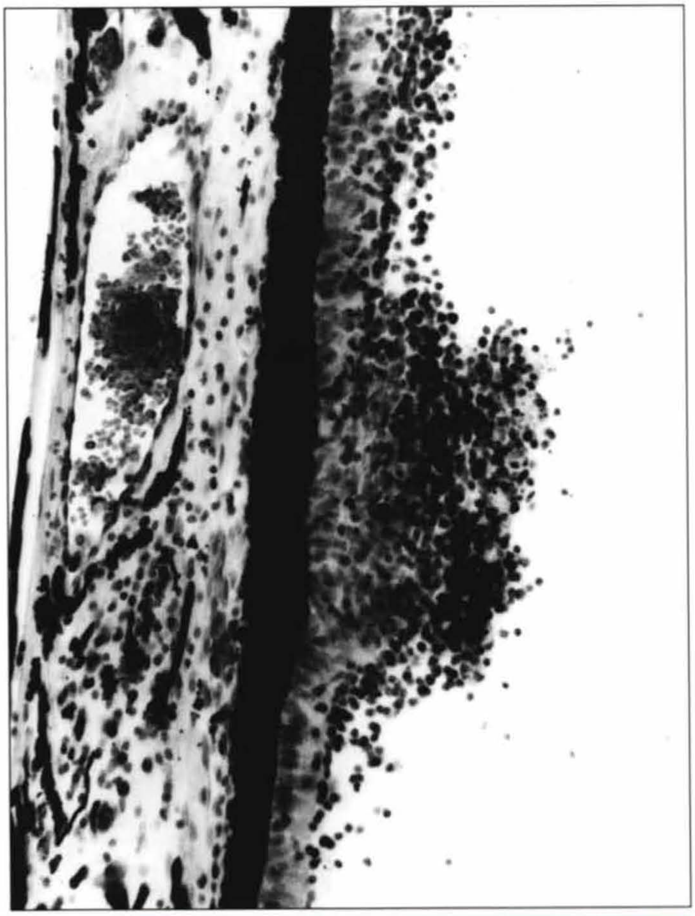

Figure 4.47 Choroiditis and inflammatory infiltrate of pars plana, in **equine recurrent uveitis**.

the previous bout of inflammation. *It is thus possible, or even probable, that chronic, recurrent uveitis results not from persistence of a single antigen, or repeated exposure to the same antigen, but is a stereotyped ocular response to activation of any one of its many acquired lymphoid populations by a variety of circulating antigens or native ocular antigens.*

Specific examples of immune-mediated endophthalmitis are discussed below, and include equine recurrent uveitis, idiopathic lymphonodular uveitis of cats, canine lymphocytic uveitis, canine uveodermatologic syndrome (Vogt–Koyanagi–Harada-like syndrome), and lens-induced uveitis.

Equine recurrent ophthalmitis (periodic ophthalmia)

This is a worldwide and important cause of blindness in horses and mules. The blindness results from repeated attacks of anterior uveitis occurring at unpredictable intervals and with increasing severity. With each attack, there is increasing involvement of the posterior uvea, retina and optic nerve, and increasingly frequent sequelae of cataract, lens luxation, synechiae, retinal separation, and interstitial keratitis. Despite the frequent observation of posterior synechiae, glaucoma is rarely reported. It is speculated that aqueous drainage in horses relies less on the trabecular meshwork and more on uveal resorption than is true of dogs or cats. The disease may initially be unilateral but eventually affects both eyes. Blindness is usually a late sequel, but may occur early in the disease if exudative choroiditis causes retinal separation.

Gross lesions of the acute disease are typical of anterior uveitis in any species: serous conjunctivitis, chemosis, circumcorneal ciliary hyperemia, corneal edema, and plasmoid aqueous and vitreous with fibrin and leukocytes in the aqueous. Clinically, such animals are often systemically ill as detected by fever, decreased appetite, and depression. Lacrimation and photophobia are usually marked. Subsequent attacks tend to become increasingly severe and resolution of the gross lesions between attacks is less complete. Such horses, during the quiescent period, may have one or more of: peripheral corneal vascularization with fibrosis and persistent edema; irregular thickening and pigmentation of iris; multiple posterior synechiae; patchy residual uveal pigment on lens capsule; and peripapillary retinal hyperreflectivity suggesting retinal scarring.

The microscopic lesions depend on the stage of the disease and represent a continuum from anterior uveitis to endophthalmitis with retinal scarring, or even phthisis bulbi. The earliest lesion is anterior uveal inflammation that is transiently neutrophilic but rapidly becomes predominantly lymphocytic. Ciliary processes are most obviously affected. Edema, fibrin, and leukocytes distend the stroma, and leukocytes and fibrin lie in the anterior chamber and in the filtration angle. Neutrophilic-to-lymphocytic pars planitis is fairly common (Fig. 4.47). In the eyes of horses with a history of several attacks of uveitis, the exudate in these acute flare-ups of the disease is almost purely lymphocytic-plasmacytic and is found about vessels of the choroid, retina, and optic nerve as well as the anterior uvea. Peripheral corneal

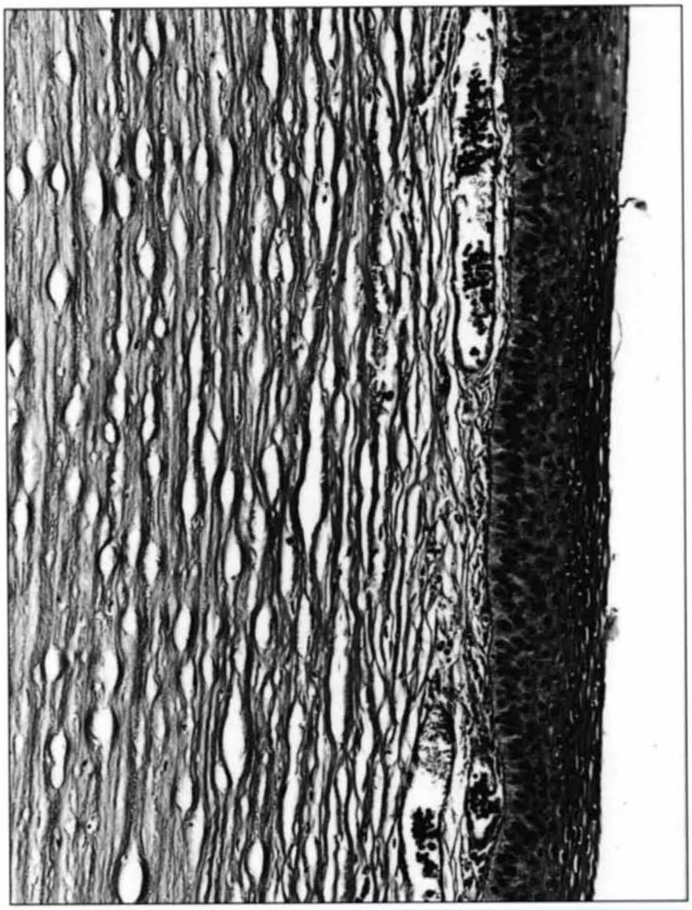

Figure 4.48 Peripheral corneal stromal vascularization and subtle fibrosis in a horse with **equine recurrent uveitis**.

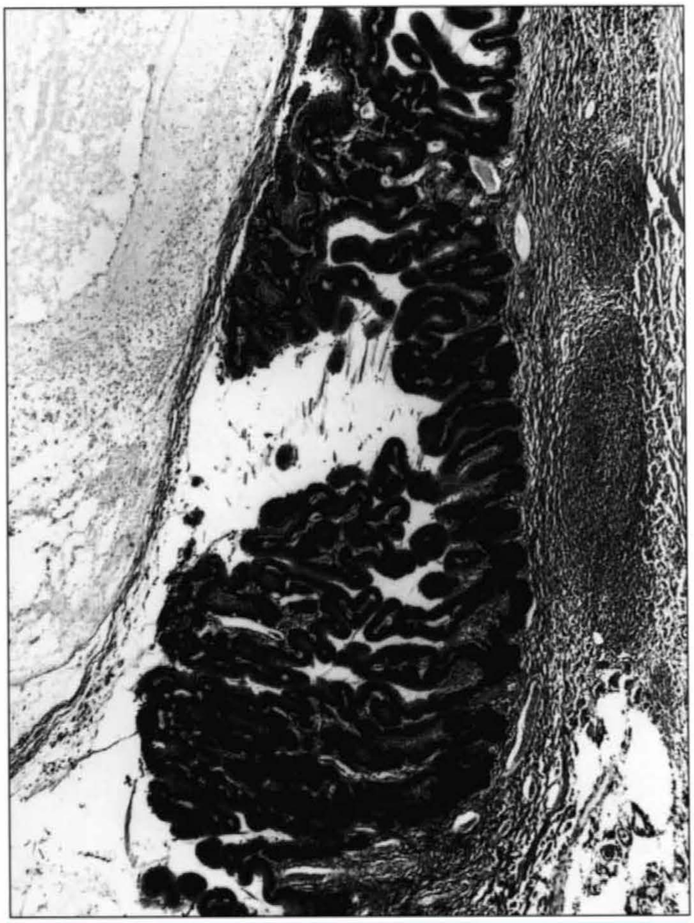

Figure 4.49 Equine recurrent uveitis. Lymphoid nodules may form anywhere in the anterior uvea. Here they coalesce within the stroma just below the ciliary processes.

vascularization, both from conjunctival and limbic ciliary vessels, becomes increasingly prominent and extends further toward the center of the cornea (Fig. 4.48). Edema accompanies the newly formed vessels. The chorioretinitis may be sufficiently exudative to cause multifocal retinal separation. As these severe uveal lesions regress during clinically quiescent periods, they leave behind characteristic residual changes. Relatively early in the disease there is the development of perivascular lymphoid aggregates in the iris and ciliary body that persist and may even form true lymphoid nodules (Fig. 4.49). The ciliary processes may remain thickened by fibrous organization of stromal edema, and a hyaline membrane often seems to cover the ciliary epithelium. This material, in fact, lies within the apical cytoplasm of the nonpigmented ciliary epithelium. In most respects, it resembles amyloid (Fig. 4.50). Small blood vessels persist along corneal stromal lamellae and there is subtle fibrous disorganization of the stroma, the result of previous edema. Peripapillary chorioretinal scarring is seen as focal retinal photoreceptor loss, jumbling of layers and gliosis. Adjacent retinal pigment epithelium may be hypertrophic or hyperplastic, and a focal cluster of lymphocytes in the nearby choroid is common.

Choroidal vessels are unusually thick-walled due to edema or fibrin, the latter probably analogous to the hyalinization described in several reports. Increased vascular permeability persists even in quiescent periods with loss of the blood–aqueous barrier demonstrated by fluorescein angiography. Whether or not this vascular alteration participates in the perpetuation of the uveitis is unknown, but it is known that such alterations predispose to localization of circulating immune complexes and subsequent type III hypersensitivity-induced inflammation.

Focal retinal detachments may reattach by fibrous organization of subretinal exudate or may progress to total separation with a barely recognizable retina adherent to the posterior lens capsule. Gliosis and lymphocytic aggregates may be found within proximal optic nerve. Scarring in optic disk and adjacent retina often is clinically obvious and may occur in horses with no other lesions of uveitis, leading to speculation that it is not really linked to, or at least not specific for, equine recurrent uveitis. Lesions of such sequelae as cataract, chronic conjunctivitis, and glaucoma are described elsewhere.

The *causes and pathogenesis* of recurrent equine ophthalmitis have not been intensively studied, in contrast to the abundance of opinion expressed in reviews or texts. *The almost universal opinion is that the disease is the result of hypersensitivity to exogenous antigen*. The most frequently cited antigens are *Leptospira* and dead microfilariae of *Onchocerca cervicalis*. The demonstration of antigenic cross-reaction between equine corneal endothelium and several common leptospiral serovars suggests that *accidental autoimmunity* may participate in some of the lesions. Cross-reaction with other ocular antigens has not been studied, but the repeated observation that lesion development follows the development of serum or aqueous antibody titers to *Leptospira* makes an immune pathogenesis very likely for this disease syndrome.

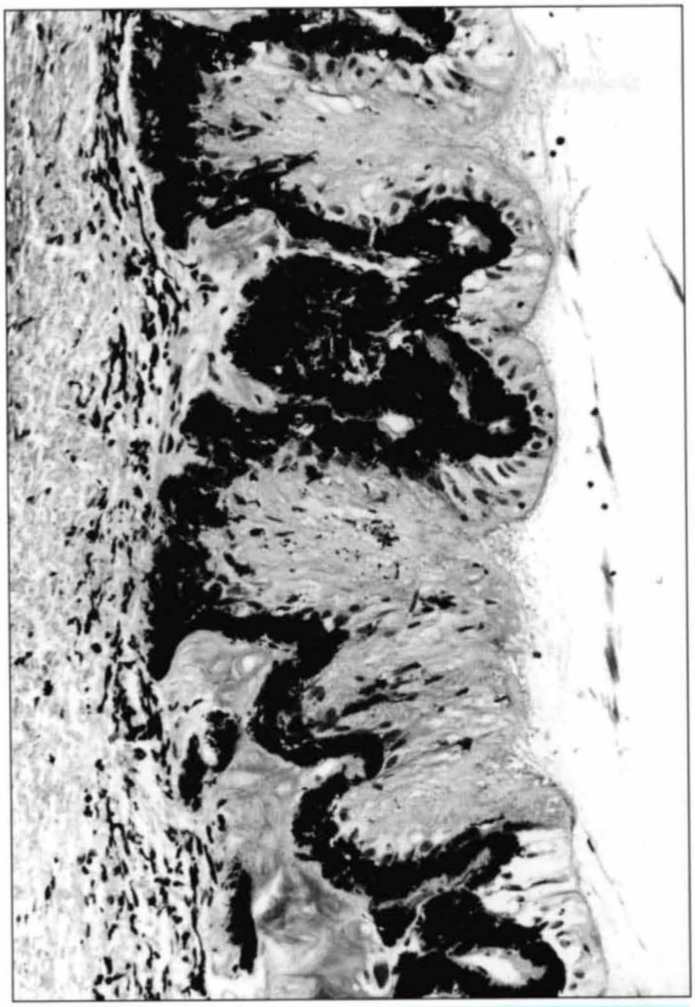

Figure 4.50 Deposition of amyloid-like material within the apical cytoplasm of the inner, nonpigmented ciliary epithelium, unique to **equine recurrent uveitis**.

There seems little doubt that *Leptospira,* particularly *L. pomona,* can initiate uveitis in horses and in man. In both species, the uveitis develops as a sequel to infection, delayed by weeks or years. The initial suspicion of this association was the observation, as early as 1948, that horses with uveitis frequently had very high serum and aqueous humour agglutination titers against *L. pomona.* This observation has since been repeatedly confirmed, and has been supported by the observation of uveitis in 22 of 36 eyes of Shetland ponies inoculated subcutaneously with small numbers of *L. pomona.* All ponies underwent subsequent leptospiremia but none developed ocular lesions until 50 weeks after inoculation. The lesions were typical of anterior uveitis, and six eyes progressed to phthisis bulbi after repeated bouts of uveitis. In 18 eyes, there were central retinal scars typical of the naturally occurring disease. *Although there seems little doubt that hypersensitivity to L. pomona can cause the syndrome of equine recurrent ophthalmitis, it is unlikely to be the only cause.* In a survey in Florida, only one of ten horses with uveitis had a positive microagglutination titer against *L. pomona.* Eight had cutaneous onchocerciasis but that was not significantly different from the overall 60% prevalence of that parasite in the sample population. Typical of the long-standing controversy about this disease is a 1990 survey of horses with uveitis in Virginia and Maryland, in which 52 of 80 affected horses had positive serum titers to one or more leptospiral serovars, and a statistically significant association between uveitis and positive titer was found for the serovars *pomona* and *autumnalis.*

Onchocerca cervicalis infection of the eye is considered briefly with helminthic uveitis. Reaction to dead microfilariae within uveal tissues is considered by some as an important cause of equine recurrent uveitis, although the high prevalence of this parasite in the horse population makes such claims difficult to support statistically. The enthusiasm for this pathogenesis may be generated, in part, by the importance of onchocerciasis as a leading cause of blinding keratitis in people.

Idiopathic lymphonodular uveitis of cats

This is by far the most frequent histologic pattern of the uveitis in cats, and vies with diffuse iris melanoma as the most common cause of glaucoma in this species. It is because of the glaucoma that these eyes are enucleated and thus become available for histologic evaluation. The mechanism by which the uveitis causes the glaucoma is unknown.

Perivascular accumulations of lymphocytes and plasma cells are seen throughout the uvea and, with less regularity, around small vessels in the retina. The iris tends to have the greatest accumulation, and the lymphocytic-plasmacytic perivascular aggregates may become so large as to be clinically visible. Formation of actual lymphoid follicles may occur in chronic and severe cases (Fig. 4.51). The nodules and follicles may also occur within the trabecular meshwork and and within the ciliary body, but choroidal involvement is usually quite subtle.

The syndrome is presumed to be immune-mediated, but the identity of the antigen or antigens is unknown. Its initial clinical presentation is usually unilateral, but the other globe is considered "at risk" for developing subsequent uveitis. There has been no study to document the actual risk. The pattern of serologic reactions in cats with this lymphonodular uveitis is no different than the general population, except perhaps to *Toxoplasma* (see Protozoan endophthalmitis, above). That debate continues, but it seems clear that the syndrome is not associated with the presence of histologically detectable infectious agent within the globe. Studies using immunohistochemistry and PCR to detect infectious agents have been similarly inconclusive.

Idiopathic lymphocytic uveitis in dogs

The histologic lesions are, in general, similar to those described above for cats and horses: *lymphocytic-plasmacytic panuveitis that tends to be more severe in the anterior uvea that in the choroid.* Some cases develop lymphoid nodules and even follicles within the anterior uveal stroma. The disease is bilateral but not necessarily of uniform severity. Unlike the situation in cats, it rarely is associated with glaucoma. The main differential diagnosis is phacolytic uveitis, and indeed in some cases the distinction is impossible.

Uveodermatologic syndrome in dogs (Vogt–Koyanagi–Harada syndrome)

Despite the exotic-sounding name, *this disease is relatively frequent in those areas in which the most susceptible breeds (Akitas, Siberian Huskies, Samoyeds) are popular. The clinical syndrome of facial dermal depigmentation and severe bilateral uveitis is distinctive,* although many dogs examined

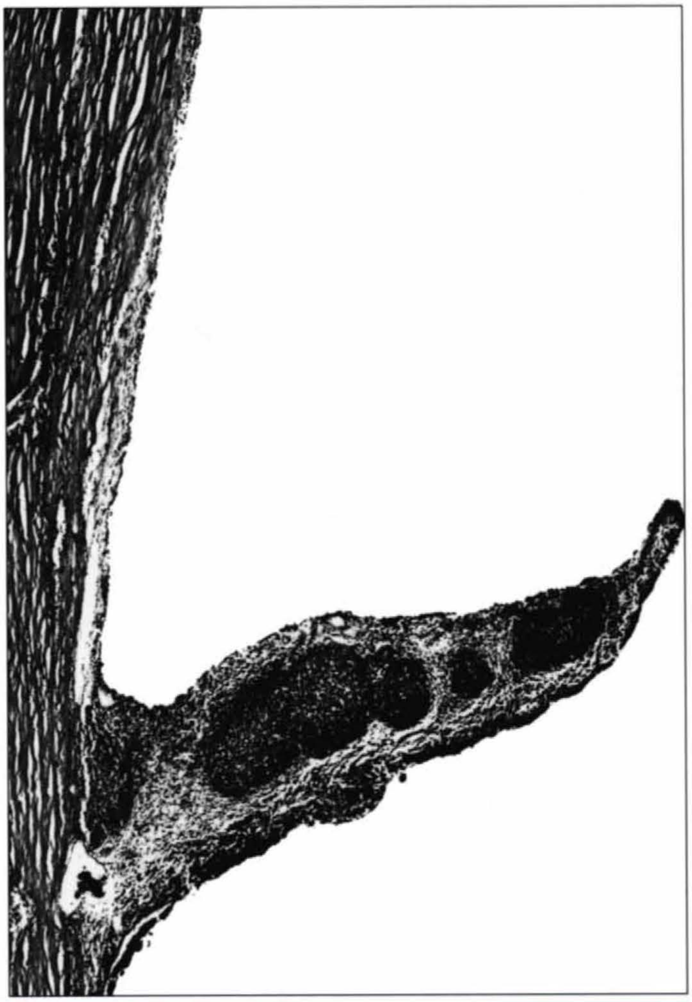

Figure 4.51 Lymphonodular iritis and secondary glaucoma in a cat. The iridocorneal angle has been dislocated far posterior to the termination of Descemet's membrane (angle recession).

for the uveitis are not noted to have skin lesions. The canine syndrome closely parallels the human disease, except for the encephalitis that is the least-frequent part of the human syndrome and has not been confirmed in dogs. The human disease is most prevalent in Orientals; the predilection in dogs for the Japanese Akita is a fascinating but unexplained parallel.

The histologic lesion is a destructive granulomatous endophthalmitis with abundant dispersal of melanin. The melanin-laden retinal pigment epithelium seems to be especially susceptible. Retinal detachment and destructive granulomatous inflammation are seen in advanced cases. The lesion is distinguished from the more prevalent systemic mycotic diseases by the predilection for pigmented tissues within the eye, lack of visceral involvement, and by the distinctive skin lesions if they are present (see Vol. 1, Skin and appendages).

The pathogenesis of the human disease is thought to involve cell-mediated immune reaction to uveal (or epidermal) melanin.

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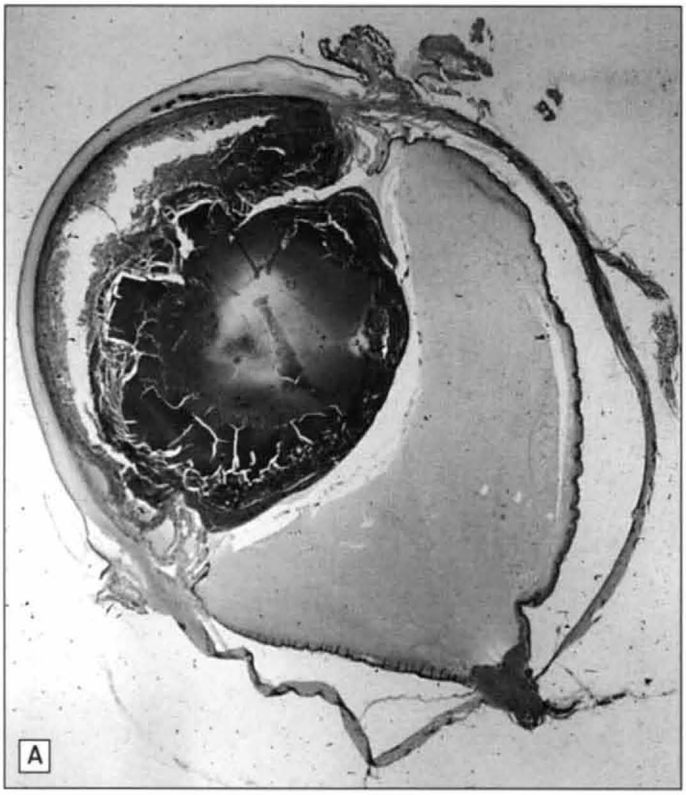
Lens-induced uveitis

Uveitis in response to leakage of lens material is seen in all species, but is most frequent by far in dogs. The term lens-induced uveitis encompasses two very different syndromes – phacolytic and phacoclastic uveitis – that differ markedly in clinical severity, in histopathology, and in pathogenesis.

Phacolytic uveitis is a *mild lymphocytic-plasmacytic anterior uveitis that occurs in response to the leakage of denatured lens protein through an intact lens capsule, which occurs regularly in the course of maturation of cataracts towards total fiber liquefaction.* The inflammation is readily controlled by routine therapy, so the pathologist is likely to encounter this lesion only as an incidental finding in eyes with hypermature cataracts that were enucleated for reasons unrelated to the uveitis. The lesion is identical to that described for idiopathic ("immune-mediated") uveitis, except it is always mild. Its pathogenesis is unknown. The lens leaks small denatured lens proteins that are not immunogenic but are, perhaps, direct inflammatory stimulants with lymphocytic chemotactic properties.

Phacoclastic uveitis *is, at least histologically, a more complicated disease that follows rupture of a normal lens in an unknown percentage of cases.* The rupture is usually from corneal penetration by a thorn, quill, bullet, or cat claw, and thus usually is of the anterior capsule. The clinical syndrome is distinctive: corneal perforation and mild traumatic uveitis that are successfully managed by conventional therapy, followed by the sudden reappearance of a severe, intractable uveitis 10–14 days after the initial injury. Poor response to medical therapy and the eventual development of glaucoma or phthisis bulbi prompt enucleation, such that *phacoclastic uveitis is one of the most prevalent ocular diseases to be submitted for histologic examination.*

The macroscopic changes in the bisected globe are diagnostic: the lens is flattened in its anteroposterior dimension and there frequently is a wedge of opacification extending from the anterior capsule towards the nucleus. Usually there is posterior synechia, iris



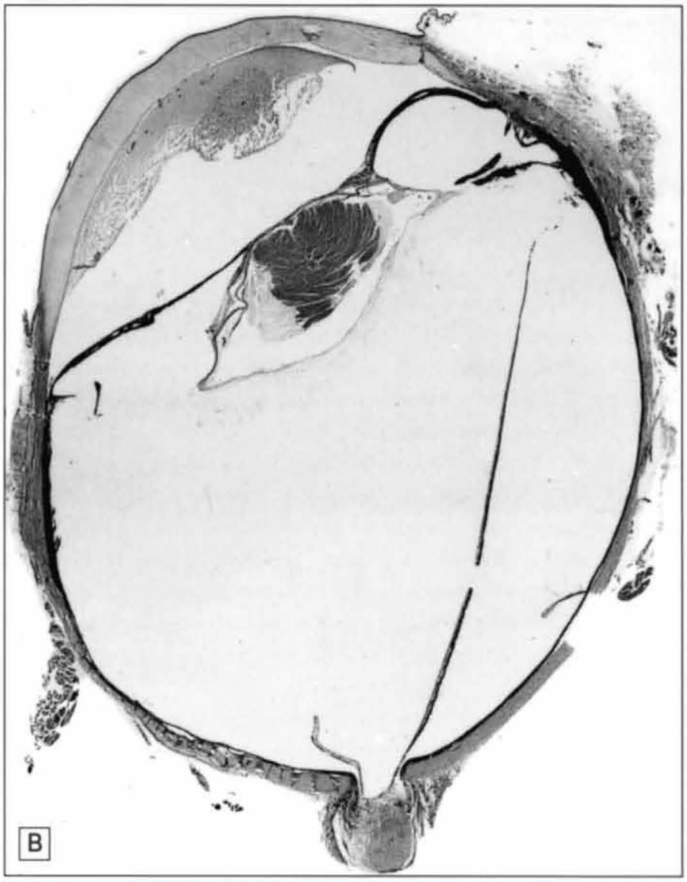


Figure 4.52 A. Acute phacoclastic uveitis following *Encephalitozoon*-induced lens capsule rupture in a rabbit. **B.** Posterior synechia, iris bombé, and glaucomatous cupping of optic disk following **traumatic corneal and lenticular perforation**. The anterior-posterior flattening of the liquified lens is typical.

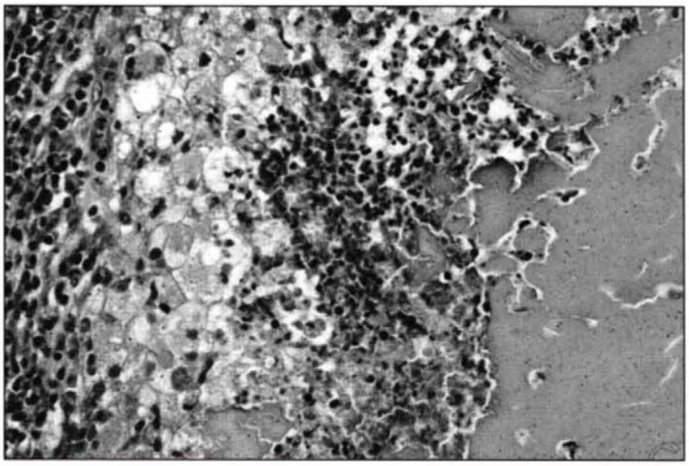


Figure 4.53 Acute phacoclastic uveitis. Laminations of neutrophils, macrophages, and then lymphocytes surround fragments of ruptured lens (arrow).

bombé, and the various other lesions as seen in any severe uveitis (Fig. 4.52A, B).

The histologic lesions vary considerably depending on duration and, probably, on the amount of lens protein that escaped through the rupture site. There are often complex lesions that result from a combination of the direct effects of trauma, immunologic reaction to massive release of lens protein, reparative proliferation of metaplastic lens and/or iridociliary epithelium, and possible contributions by corneal wound healing, sepsis, and glaucoma. It is worth mentioning that some, and perhaps most, of these globes have at least some participation by bacterial infection that occurs concurrently with the original penetrating injury. How much that infection contributes to the histopathology described below remains unknown. Most samples reaching a pathologist are from animals that have been treated extensively with antibiotics, but it is still possible that previous septic inflammation was the significant contributor to the persisting histologic changes.

The simplest and presumably earliest lesion of phacoclastic uveitis occurs at the site of capsular perforation. The edges of the capsule are retracted and coiled outward, and a wedge of neutrophils and liquified lens material extends from the perforation towards the nucleus. The inflammation outside of the lens is usually distinctly perilenticular and involves a mixture of neutrophils and macrophages in the anterior and posterior chambers, and a lymphocyte-dominated reaction within the uveal stroma (Fig. 4.53).

Older lesions (which predominate in most enucleated globes) are dominated by perilenticular proliferative changes of wound repair. There is proliferation and fibroblastic metaplasia of lens epithelium adjacent to the perforation, which escapes from the lens to ramify over the lens surface and frequently incorporates the lens, ciliary processes, and iris leaves into a large fibrous mass that obstructs aqueous outflow. Metaplasia of ciliary epithelium or recruitment of fibroblasts from uveal stroma may contribute to the proliferation (Fig. 4.54). Many such specimens contain little evidence of inflammation other than fibroplasia, probably because of very extensive anti-inflammatory therapy that is, in hindsight, useless against the proliferative events that doom the eye to glaucoma or phthisis.

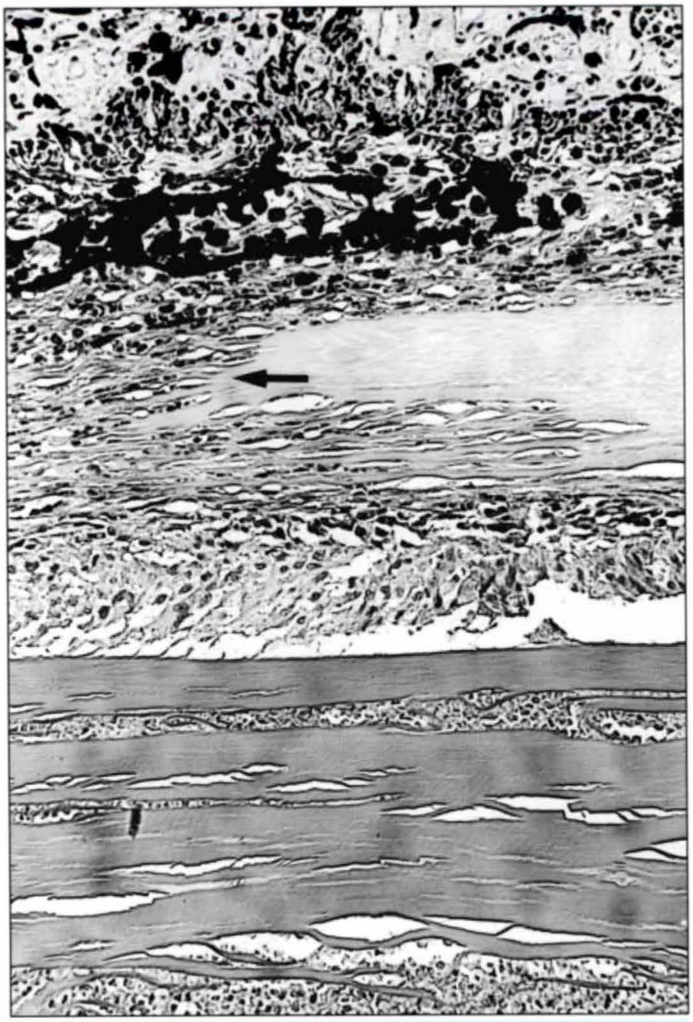


Figure 4.54 Chronic phacoclastic uveitis. Severed ends of anterior lens capsule (arrow) are fimbriated. Typical fibrous proliferation fuses cataractous lens to the (pigmented) iris.

Phacoclastic uveitis is an important complication of cataract surgery in which fragments of lens cortex or epithelium may be left in the eye. These initiate the same inflammatory and proliferative reaction as described above, and the complications are as refractory to conventional anti-inflammatory therapy as is the naturally occurring disease.

The immune pathogenesis of phacoclastic uveitis has been extensively studied, but with no universally accepted conclusion. The current theory is that the release of massive amounts of lens protein antigen overwhelms the splenic T-cell tolerance to small amounts of lens antigen. Recruitment of lens-sensitized lymphocytes into the perilenticular uvea then initiates both the pyogranulomatous perilenticular inflammation and the proliferative events of healing that, unfortunately, doom the eye. This pathogenesis, if true, explains the typical delay between injury and reaction, and why rapid surgical removal of the lens is preventive. It may also explain the unpredictability of phacoclastic uveitis, especially following small perforations in puppies, which seem often to heal uneventfully. Even in adult dogs the disease in unpredictable, so owners' questions about the risk of phacoclastic uveitis as the justification for surgical removal of a perforated lens cannot be answered with certainty. One study found lens removal shortly after perforation prevented serious complications in six of seven dogs thus treated, whereas five of six dogs treated with aggressive medical therapy lost the eye to complications of uveitis.

Two interesting variations on what is basically a canine scheme are seen in rabbits and cats. *Rabbits* suffer what appears to be spontaneous lens capsule rupture of a previously normal lens. The response is well-contained perilenticular granulomatous inflammation very similar to human phacoanaphylactic uveitis. The rupture is associated with the intralenticular presence of *Encephalitiozoon*, and it is assumed that the organisms penetrate and thus weaken the posterior lens capsule. *Cats* occasionally develop lesions similar to those in dogs, but also develop a unique feline primary intraocular pleomorphic sarcoma that may arise from metaplastic lens epithelium or from other transformed epithelial elements in the reparative reaction (see Feline post-traumatic sarcoma).

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Glaucoma

Glaucoma is a pathophysiologic state characterized by a prolonged increase in intraocular pressure. While such increases of pressure may theoretically result from increased production or *decreased removal of aqueous*, only the latter is known to occur. The lesions in glaucomatous eyes include those related to the pathogenesis of the glaucoma and those resulting from the glaucoma itself. *Glaucoma occurs commonly in dogs*, less commonly in cats and horses, and rarely in other species. The medical and surgical control of glaucoma remains problematic despite extensive experimentation with many different therapeutic modalities. Because most affected eyes eventually require enucleation or evisceration, *glaucoma is one of the most frequent ocular conditions submitted for histologic evaluation.*

The lesion predisposing to glaucoma may be the result of *antecedent ocular disease*, particularly intraocular neoplasia and anterior uveal inflammation with posterior or anterior synechiae. Such cases are termed **secondary glaucoma. Primary glaucoma** describes those cases without evidence of prior ocular disease and, in practical terms, is *synonymous with malformation of the filtration angle.* Primary glaucoma is seen almost exclusively in dogs, and vies with neoplasia as the most frequent cause of glaucoma in dogs.

Because the pathogenesis of glaucoma so frequently involves developmental or acquired distortion of the filtration angle, a description of that structure is appropriate here.

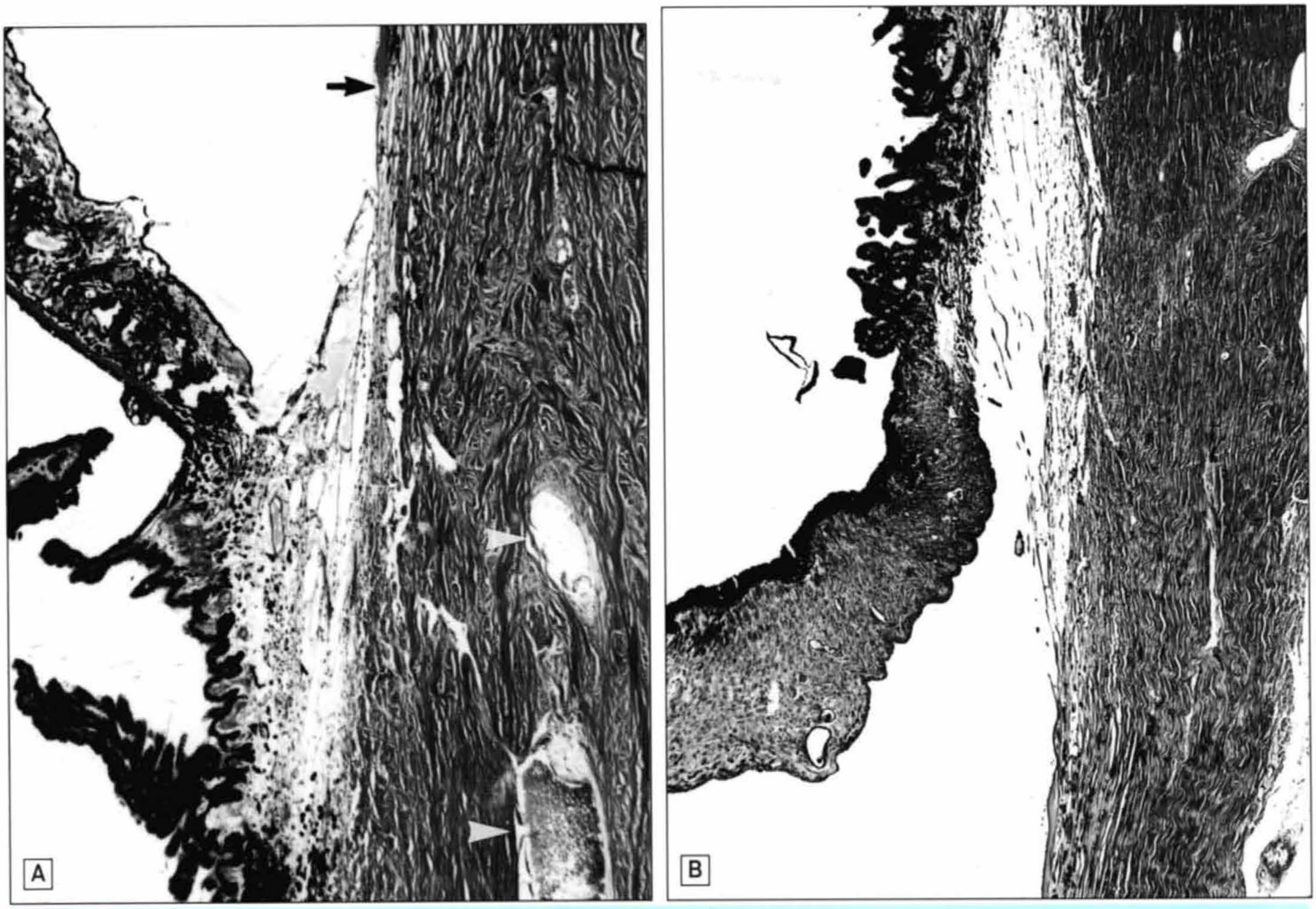

Figure 4.55 A. Normal canine filtration angle showing fenestrated pectinate ligament inserting at the termination of Descemet's membrane (arrow). Large vessels (arrowheads) are part of scleral venous plexus. **B. Normal feline filtration angle**.

The **filtration apparatus** is a series of mesenchymal sieves that occupies the iridocorneal angle, and extends circumferentially around the globe. These sieves appear to form by rarefaction of the same mesenchyme that forms iris stroma, and its rarefaction continues (at least in carnivores) for several weeks after birth. This area of perforated mesenchyme is the **ciliary cleft**, bordered externally by sclera, posteriorly by the muscles of the ciliary body, and internally by the iris stroma. Its anterior border, at least in dogs, extends for about a millimeter into the deep peripheral corneal stroma just external to Descemet's membrane. Its border with the anterior chamber is marked by the **pectinate ligament,** which is visible clinically as a series of cobweb-like branching cords (carnivores) or a fenestrated sheet (ungulates) stretching from the termination of Descemet's membrane to the anterior portion of the iris root. They consist of collagenous cords covered by a very thin endothelium, with a thin intervening layer of basement membrane-like material. The endothelium is continuous with the corneal endothelium, and the collagenous core is continuous with corneal stroma.

Aqueous humor percolating through the pectinate ligament into the ciliary cleft must then pass through mesenchymal sieves consisting of collagenous cords covered by phagocytic and pinocytotic endothelium, called the **trabecular meshwork**. The large, open network of cords occupying most of the ciliary cleft is the uveal trabecular meshwork, while anterior and external to it is a more compressed network called the corneoscleral trabecular meshwork. Ordinarily, aqueous humor produced by the ciliary processes passes through the pupil, through the pectinate ligament, and then through the uveal and corneoscleral trabecular meshworks en route to the *scleral venous plexus* that will return the aqueous to the systemic circulation. Improper development or acquired obstruction of any part of this drainage pathway may result in glaucoma, but one must remember that *the ciliary cleft extends 360° around the iridocorneal angle. Blockage of most of it is required for the development of glaucoma*, and this assessment is virtually impossible with two-dimensional histologic examination. It is quite common to encounter dog eyes with maldeveloped filtration angles in both the dorsal and ventral portions contained in a routine sagittal histologic section, yet with no evidence of glaucoma. Examination of the circumference of the angle with a dissecting microscope or scanning electron microscope in such cases often reveals the maldevelopment to be segmental and thus not a cause for glaucoma.

Differences exist among species in the finer details of angle structure and in the degree to which alternative routes of aqueous outflow are utilized (Fig. 4.55A, B). The horse, for example, has very thick pectinate fibers, and an inconspicuous corneoscleral trabecular meshwork and scleral venous plexus. As implied by these histologic features, *the horse employs alternative routes of aqueous outflow* (into iris stroma and especially posteriorly through ciliary muscle and into choroid) that are much more important than similar routes in dogs or cats. In contrast, the *cat* has extremely delicate pectinate fibers, a very large, open ciliary cleft, a conspicuous scleral venous plexus, and minimal (about 3% of aqueous outflow) reliance on alternative outflow pathways. In dogs, alternative drainage routes account for 15–25% of all outflow. The existence of these alternative routes may explain the absence of glaucoma in some eyes (especially in horses) in which the angle changes would ordinarily have resulted in glaucoma, and may even explain the presence of glaucoma in eyes with apparently normal angles but lesions affecting portions of these other potential drainage routes.

Lesions causing glaucoma

Primary glaucoma is most frequently encountered in dogs. Although theoretically primary glaucoma may have no visible angle lesion (which is frequently the case in people), *in dogs there is almost always a readily detected maldevelopment.* The one exception is primary open-angle glaucoma in Beagle dogs in which there is no visible antecedent lesion. The broad term **goniodysgenesis** encompasses all developmental defects of the filtration angle, of which two types account for most canine cases. *The most prevalent is continuation of mature iris stroma across the trabecular meshwork to insert into the termination of Descemet's membrane.* Some consider this an example of dysplasia of pectinate ligament, and the term **imperforate pectinate ligament** is widely used. The band usually is much thicker than pectinate ligament and lacks the appropriate perforation to allow the drainage of aqueous humor. The trabecular meshwork posterior/exterior to this broad mesenchymal band often seems normal, although that is quite variable (Fig. 4.56A). It is seen as a *breed-related and thus presumably inherited defect* in Bouvier des Flandres, Basset Hounds, American Cocker Spaniels, Dandie Dinmont Terriers, Siberian Huskies, Samoyeds, Chows, and numerous other breeds. The defect, and the resultant glaucoma, is occasionally seen in mixed-breed dogs. The dysplasia is usually bilateral but not necessarily of equal extent, thus the glaucoma is often present initially only in one eye. The prevalence of iridopectinate dysplasia is much higher than the prevalence of glaucoma, and even in dogs with very extensive dysplasia that should seemingly eliminate almost all aqueous drainage, the onset of glaucoma is not until several years of age. It is not unusual to have cases in which clinically detected glaucoma was delayed until 8–10 years of age. Age-related changes in outflow resistance within the alternative routes of aqueous outflow have been postulated as the explanation, as have changes to the angle configuration related to age-related forward movement of the lens. These remain as theories.

Importantly, there appears to be little direct correlation between the severity of the goniodysgenesis and the actual risk of developing glaucoma, its age of onset, or its severity. The extent to which this is true seems to vary by breed, and perhaps the best generalization is that dogs with structurally (i.e., as evaluated by gonioscopy) normal filter angles have no increased risk of glaucoma, while those that have at least some developmental abnormality are at increased risk. *Continuing to breed dogs with abnormal angles increases the prevalence of glaucoma within that line.* It may be that the goniodysgenesis that is visible clinically and histologically is not the direct cause for the glaucoma, but a marker for some other defect in trabecular development that is the real cause of the glaucoma.

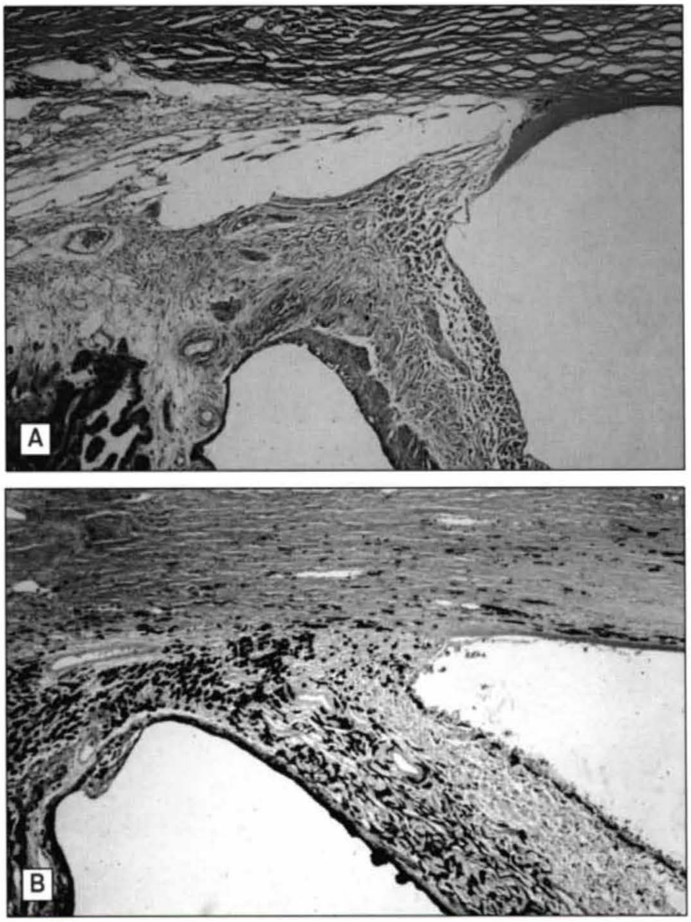

Figure 4.56 A. Goniodysgenesis, with a continuous band of iris-like mesenchyme in place of the normal, perforated pectinate ligament. **B. Trabecular hypoplasia**. This rare, profound arrest in mesenchymal development causes truly congenital glaucoma.

The second major type of goniodysgenesis is seen as a more fundamental arrest in the maturation of the trabecular meshwork such that the ciliary cleft is filled with tissue resembling primitive anterior uveal mesenchyme (Fig. 4.56B). This may occur in conjunction with iris hypoplasia or anterior chamber cleavage syndromes, but it may exist as an isolated defect termed **trabecular hypoplasia**. It has no known familial predilections. Such animals usually have truly congenital glaucoma, in contrast to the adult onset that characterizes the more common breed-associated glaucomas. It is important to note that the remodeling of the trabecular meshwork in dogs and cats continues for several weeks after birth, so that one should avoid overinterpreting apparent angle solidification in very young carnivores.

Secondary glaucoma *occurs most commonly as the result of posterior synechiae as a sequel to anterior uveitis.* The pupillary margin of the iris normally floats along the surface of the lens. Any fibrinous effusion that increases the stickiness of the iris creates a substantial risk of iridocorneal adhesions and resulting pupillary block. Continued production of aqueous humor in the presence of pupillary obstruction results in characteristic forward bowing of the iris known as *iris bombé* (Fig. 4.57). In this condition, the glaucoma is caused not

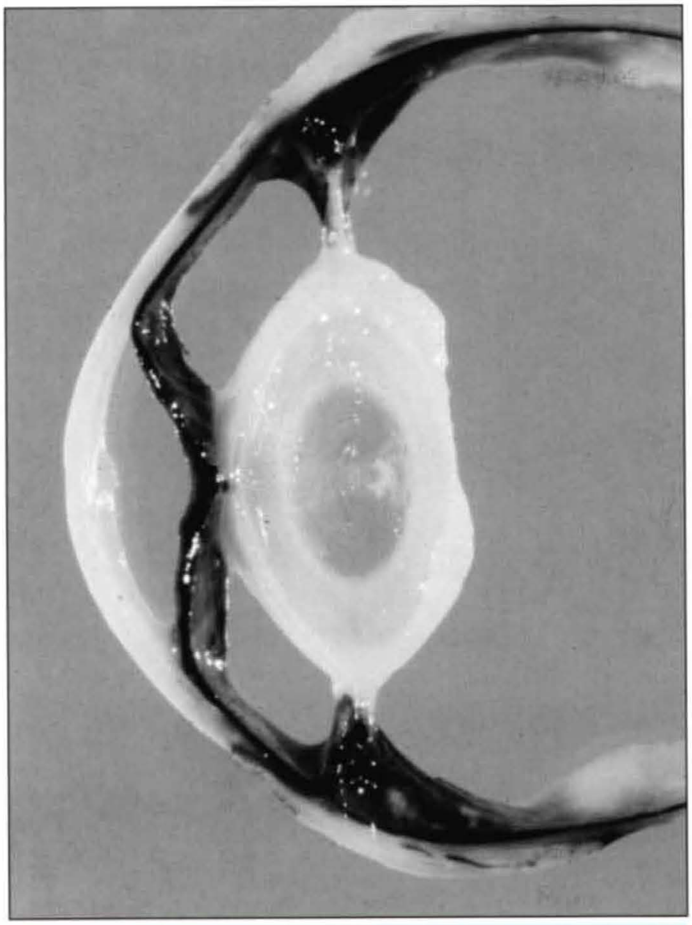

Figure 4.57 Iris bombé, reflecting a combination of posterior synechia, pupillary block, and eventual peripheral anterior synechia.

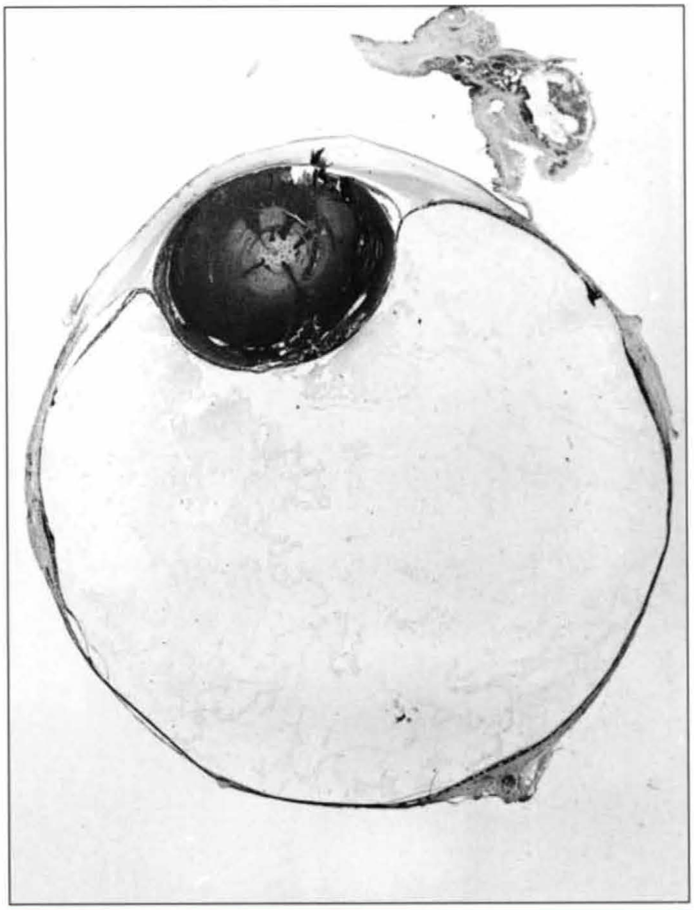

Figure 4.58 Glaucoma caused by swelling of cataractous lens (intumescent cataract) and resultant functional anterior synechia, in a dog. Note thin sclera typical of buphthalmos secondary to glaucoma.

only by pupillary obstruction but by peripheral anterior synechia as the forward bulging of the iris effectively seals the iridocorneal angle. Anterior synechia occurring independently of iris bombé is an occasional cause of secondary glaucoma. Because the iris does not normally contact the cornea, anterior synechia unrelated to iris bombé is frequent only as a consequence of corneal perforation, in which case the iris flows forward as an iris prolapse to seal the defect and may then adhere diffusely to the corneal endothelium (see Fig. 4.34).

Other frequent causes of secondary glaucoma include occlusion of the trabecular meshwork or pupil by pre-iridal fibrovascular membrane or by neoplasia. Posterior migration by corneal endothelium to cover the anterior face of the pectinate ligament and iris is seen quite frequently and may represent an important mechanism for the development of secondary glaucoma, even though the cause for the endothelial migration is usually not obvious. It may be particularly important as a trigger for the still unexplained delayed onset of glaucoma in adult dogs with lifelong goniodysgenesis.

Lens luxation may precipitate glaucoma by allowing vitreous to occlude the pupil, by stimulating anterior uveitis, or by trapping iris against cornea. Rarely, lens swelling with cataract (intumescent cataract) seems to occlude the pupil (Fig. 4.58). In small terrier dogs with an inherited tendency to luxation, there is an unusually high prevalence of glaucoma. Removal of lens prior to the onset of glaucoma prevents the expected glaucoma, seemingly establishing the causal role of the luxation. Posterior synechiae may also occlude the pupil (particularly as part of phacoclastic uveitis), but posterior synechiae usually cause glaucoma via iris bombé and thus a circumferential peripheral anterior synechia.

There are substantial species differences in the prevalence of the mechanisms by which glaucoma may occur. In *dogs*, goniodysgenesis, posterior synechiae with iris bombé, pre-iridal fibrovascular membrane, anterior uveal melanoma, and anterior lens luxation are the leading causes, in approximately that order of prevalence. In *cats*, diffuse iris melanoma and chronic idiopathic lymphonodular uveitis are the only prevalent causes of glaucoma, with posterior synechiae being inexplicably rare. In *horses*, the presence of pre-iridal fibrovascular membranes across the pectinate face is the most frequent cause, although the stimulus for the membrane development is unknown in most reported cases. Many horses with glaucoma have a clinical history of previous uveitis, but histologic assessment of the glaucomatous globe usually reveals very little evidence of that inflammation and no obvious connection between the inflammation and the development of glaucoma. In those horses with glaucoma in which the membrane had not apparently crossed the pectinate ligament, glaucoma may have been caused by obstruction of alternative uveal routes of aqueous outflow, which are more important in horses than in dogs or cats.

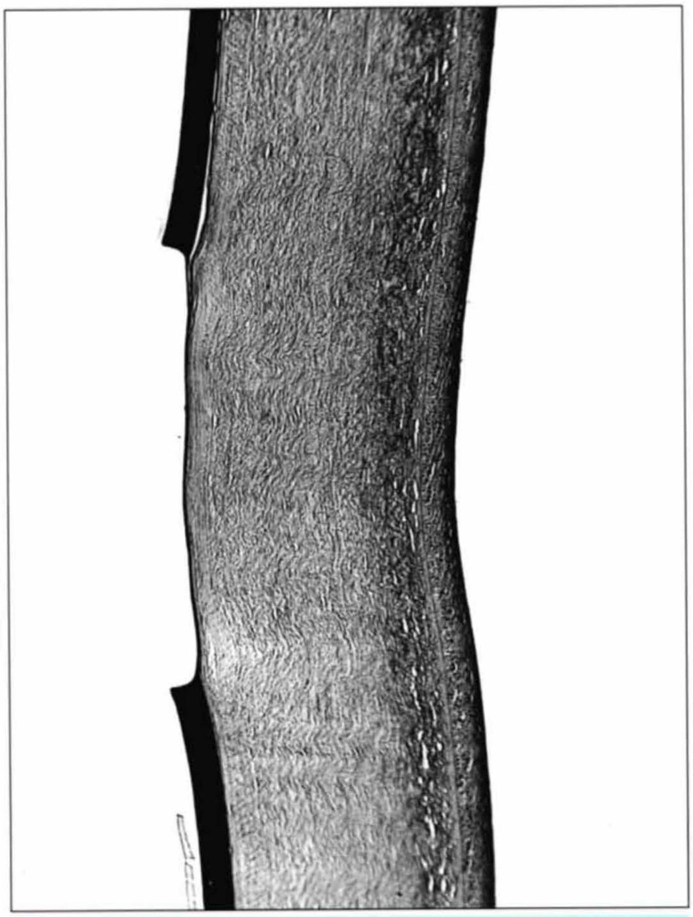

Figure 4.59 Focal break in Descemet's membrane (**corneal stria**) commonly seen in horses and dogs with glaucoma.

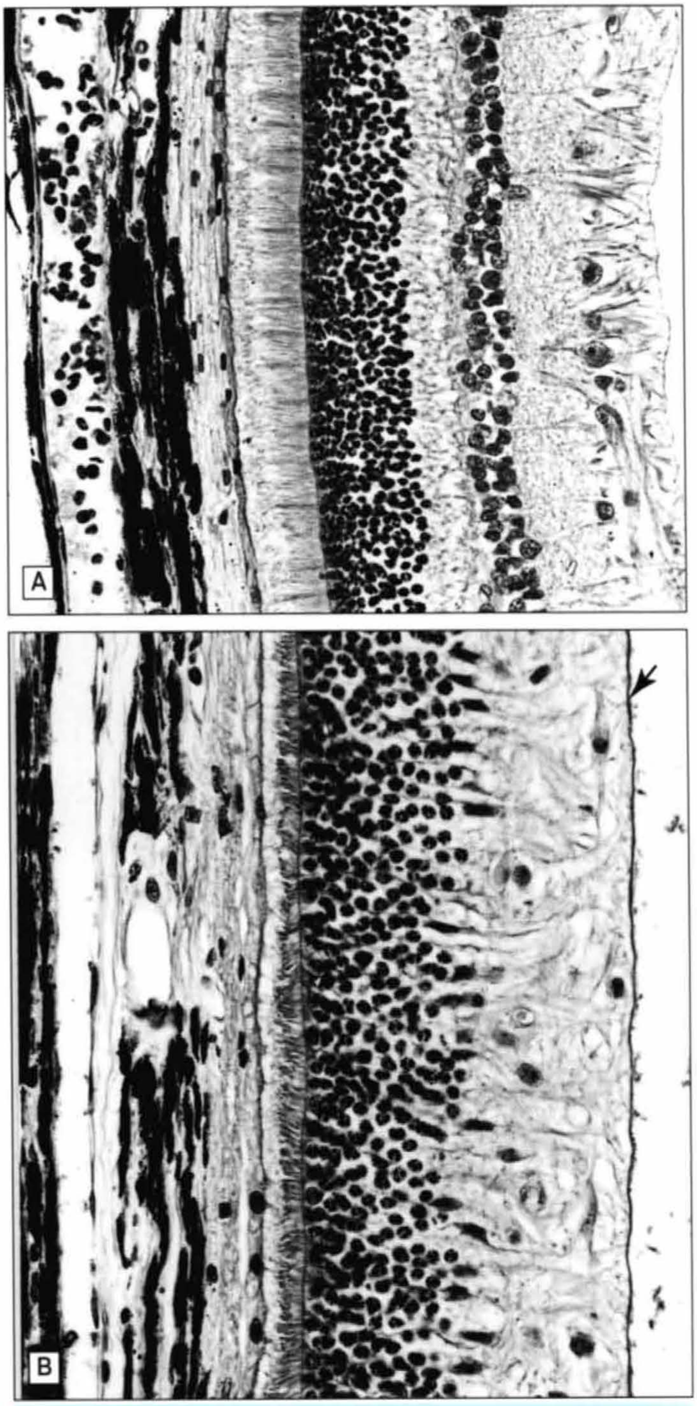

Figure 4.60 A. Normal canine retina overlying tapetum lucidum. **B. Early glaucomatous retinopathy** in a dog. Ganglion cells are absent. Remnant of inner nuclear layer remains. Inner limiting membrane is abnormally prominent (arrow) over atrophic nerve fiber layer.

Lesions resulting from glaucoma

Lesions that develop as a result of glaucoma vary with the duration and severity of the glaucoma and the distensibility of the globe, and affect virtually all parts of the globe. *Enlargement of the globe* (**buphthalmos** or **megaloglobus**) occurs most readily in young animals or in those species with thin scleras such as cats and laboratory animals. In the cornea, increased aqueous pressure injures the corneal endothelium, resulting in *diffuse edema and eventual fibrosis and vascularization.* If buphthalmos occurs, corneal stretching results in rents in Descemet's membrane, visible clinically as **corneal striae** (Fig. 4.59). These are relatively most frequent in horses and least prevalent in cats with glaucoma. Failure of lids to cover the enlarged globe permits *corneal desiccation and eventual ulceration* with all its sequels. If the ocular enlargement has developed only slowly, the cornea may have time to undergo adaptive keratinization and even cutaneous metaplasia. *Cataract is usual*, presumably the result of stagnation of aqueous humor and subsequent lens malnutrition. Iris and ciliary body undergo bland atrophy, most obvious as thinning and flattening of ciliary processes. Collapse of the ciliary cleft and trabecular meshwork itself is frequent and makes evaluation of these structures for possible goniodysgenesis very difficult. *Migration of corneal endothelium across the face of the pectinate ligament and on to the surface of the iris is commonly seen,* but we do not know whether it is part of the pathogenesis of glaucoma, or just a result of that glaucoma.

The retinal lesions are characteristic. Atrophy begins in nerve fiber and ganglion cell layers, making glaucoma the only naturally occurring cause of inner retinal atrophy other than the rare instances of traumatic or neoplastic disruption of optic nerve (Fig. 4.60A, B). *Loss of nerve fibers unmasks the normally inconspicuous Muller fibers*, a lesion that may be more easily seen and more confidently interpreted than the loss of the nerve fibers or ganglion cells themselves (Fig. 4.61). This is

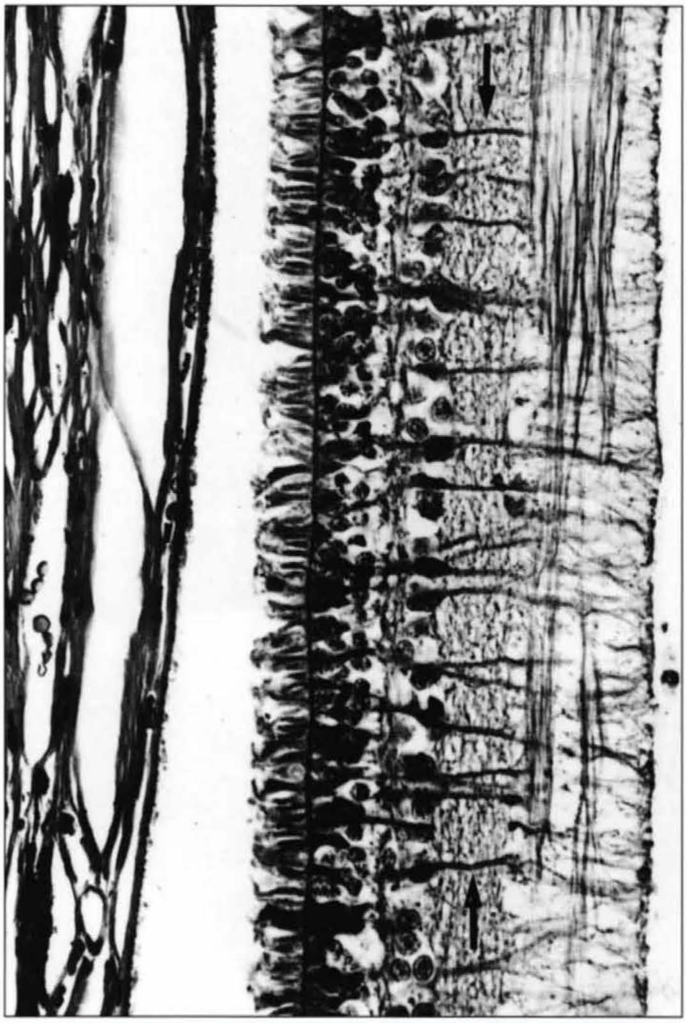

Figure 4.61 Glaucomatous retinal atrophy. Ganglion cells are absent, inner nuclear layer is sparse, and Muller fibers (arrows) unduly obvious due to loss of nerve fiber layer.

particularly true in cats, in which the ganglion cells persist with considerable tenacity under circumstances that would, in dogs, have progressed to a very obvious atrophy. With increasing duration or severity, the inner nuclear layer and its axons and dendrites also atrophy, resulting in thinning of the inner nuclear layer and the blending of this layer with the outer nuclear layer as the plexiform layers (the axons and dendrites of the nuclear layer cells) rarify.

In dogs with high-pressure glaucoma, there is sometimes *full-thickness retinal atrophy* that includes even damage to the RPE. Although the cause is unknown, it may be that the very high pressure causes collapse of choriocapillaris, resulting in ischemic damage to those tissues supplied by this delicate but essential capillary layer. This appears to be a uniquely canine susceptibility, in that the glaucomatous retina of other species rarely if ever shows full-thickness atrophy.

In all species, the dorsal half of the retina is less severely affected than ventral retina. The basis for this sparing remains speculative, but it may be related to the anatomy of the lamina cribrosa and the ease with which increased intraocular pressure compresses axons as they exit through that scleral sieve.

The pathogenesis of the selective ganglion cell loss in glaucoma remains controversial, and there is probably more than one mechanism. The loss may be the result of in situ apoptosis triggered by exposure to elevated retinal and vitreal levels of excitatory amino acids (especially glutamate), a response to pressure-associated ischemic injury, or a result of loss of axoplasmic flow secondary to pressure-induced injury to the optic nerve as it traverses the lamina cribrosa. It is clear that the ganglion cell injury is not directly correlated with the magnitude of the increase in intraocular pressure.

Excavation ("cupping") of the optic disk *is a pathognomonic lesion when present*, but its absence does not rule out the diagnosis. It occurs by at least three mechanisms, any (or all) of which may explain the cupping in an individual eye. Particularly in animals with a thin sclera and lamina cribrosa, the elevation of pressure may cause rapid posterior bowing of the lamina, resulting in visible cupping without apparent nerve atrophy. This is frequently seen in cats and rabbits, but not in ungulates with their thick, rigid lamina. In all species, cupping also occurs by axonal loss from the optic nerve. The pathogenesis for that axonal degeneration is either direct compression of axons or ischemic injury. It has been suggested that the posterior bowing of the lamina cribrosa contributes to the axonal injury by mechanical pinching of axons or blood vessels as they pass through the distorted lamina (Fig. 4.62A, B). This cupping is distinguished from coloboma by the absence of dysplastic neurectoderm lining the defect and by the presence of inner retinal atrophy.

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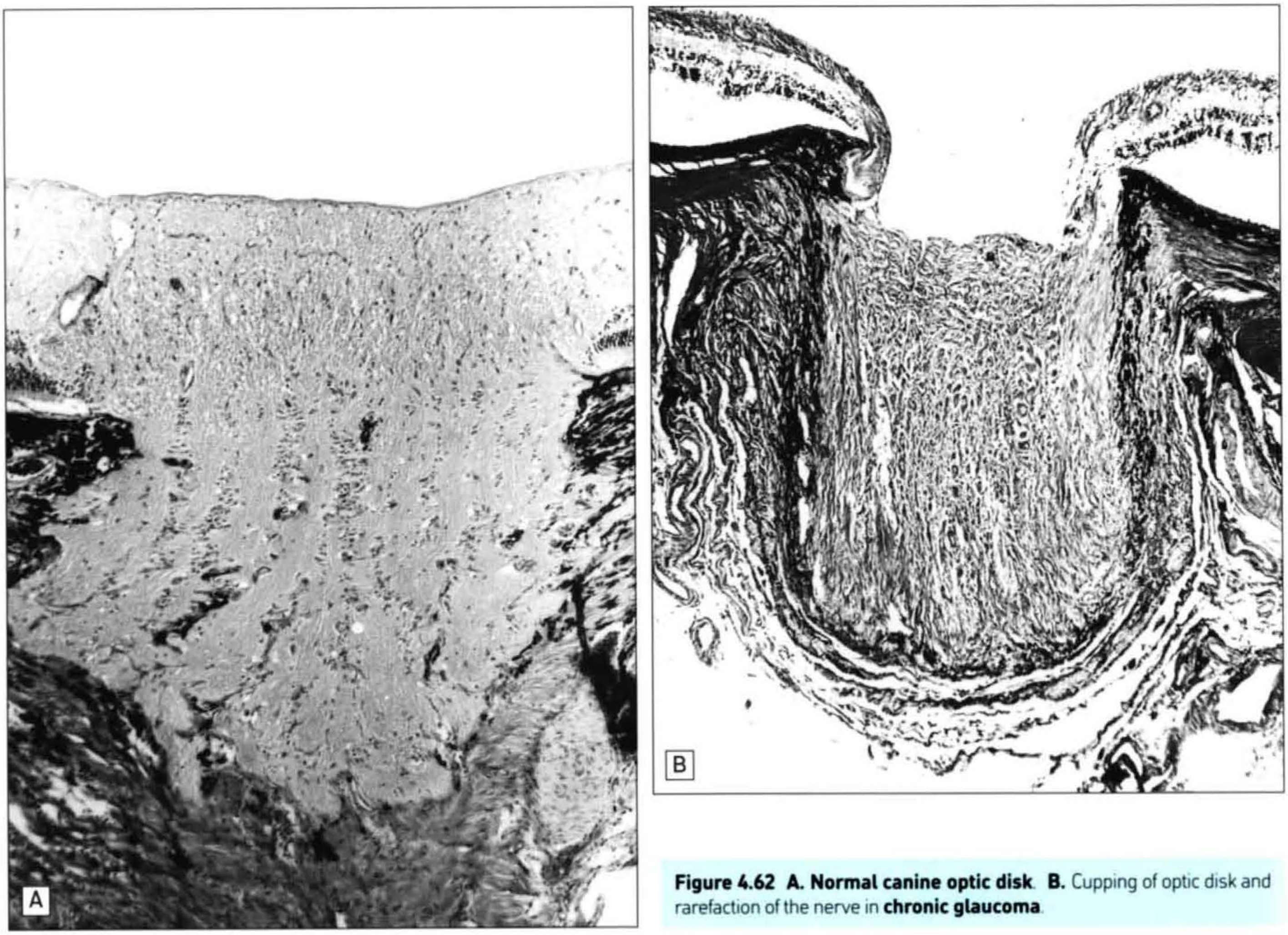

Figure 4.62 A. Normal canine optic disk. B. Cupping of optic disk and rarefaction of the nerve in **chronic glaucoma**.

RETINA

When involution of the primary optic vesicle brings into apposition the anterior and posterior poles, the anterior (innermost) neurectodermal layer undergoes mitotic replication and subsequent specialization to form the *nine layers of the neurosensory retina.* The outermost neurectoderm remains as a relatively unspecialized simple cuboidal layer, the *retinal pigment epithelium.* Although it is traditionally considered the tenth retinal layer, its structure, function, and reaction to injury are unlike those of neurosensory retina and it is best discussed separately. *In this discussion, retina refers only to the neurosensory retina.*

In the fixed, bisected globe, the retina is seen as a thin, opaque membrane lining the posterior half of the globe between vitreous and choroid. It joins the darkly pigmented pars plana of the ciliary body at an abrupt transitional point called the *ora ciliaris retinae.* In all but the best-preserved specimens, the retina is separated artifactually from the retinal pigment epithelium and adjacent choroid, remaining adherent only at the ora ciliaris and at the optic disk.

Histologically, the neurosensory retina begins abruptly at the ora ciliaris as a multilayered continuation of the inner layer of the ciliary epithelium. In dogs and sometimes in horses, the layers here are poorly defined and photoreceptors sparse. The peripheral retina is only about half the thickness (100 μm) and has half the photoreceptor density (250 000/mm^2) of the central retina, with fewer nuclear and plexiform elements and a thin innermost nerve fiber layer.

The retina consists of three structural components: neurons, glia, and vasculature. The neurons are the functional elements and transmit the photoactivated electrical impulse from photoreceptor process to occipital cortex. *The photoreceptor is the raison d'etre of the entire globe.* It is a sensory, apical cytoplasmic process of the neurons forming the outer nuclear layer. These processes, called **rods or cones** based upon their shape and ultrastructural composition, extend from the outer nuclear layer toward the choroid. They are enveloped by a glycosaminoglycan interphotoreceptor matrix, and interdigitate with apical processes of the retinal pigment epithelium, but no actual adhesions exist between the two layers. Within the outer segment of the photoreceptors are stacks of collapsed, disk-like spheres that contain the photoactive chemicals. The disks within rod outer segments are constantly produced basally and shed apically at the rate of 80–100 disks per day, with an outer segment turnover time of 6 days in dogs. Effete disk debris is engulfed and degraded by the retinal pigment epithelium. Such turnover has not been demonstrated in the outer segment of cones which have stacks of lamellae formed by infoldings of the plasma membrane. In addition, cones appear to be of many different types within a single retina. It is probably the ratio of different cones sensitive to different wavelengths of light that permits the visual cortex to discriminate color. *In general, fish,* *amphibia, reptiles, and birds have excellent color discrimination. Ungulates can distinguish yellows, blues, and, variably, green and red. Carnivores have very limited color perception, as far as can be determined.*

Other retinal layers are best described in terms of function. The photoelectric stimulus originating in the photoreceptor outer segment is transmitted through the outer nuclear layer and along the axons of the photoreceptor nuclei to the bipolar and horizontal neurons of the *inner nuclear layer*. The accumulation of outer nuclear layer axons and inner nuclear layer dendrites forms the *outer synaptic or plexiform layer*. The inner nuclear layer contains the nuclei of the bipolar, horizontal, amacrine, and glial (Muller) cells. The bipolar cells receive impulses from the photoreceptors and relay them to ganglion cells. The bipolar cells also stimulate the horizontal cells, which transmit the impulse horizontally to excite adjacent bipolar cells. Amacrine cells counterbalance the bipolar cells in that their stimulation releases an inhibitor of ganglion cell excitation. The glial cells are primarily structural support cells, whose processes traverse the retina to form the retinal scaffold and their anterior and posterior terminations fuse to form the inner and outer limiting membranes. The axons of the bipolar and amacrine cells, dendrites of ganglion and horizontal cells, and glial processes form the thick *inner synaptic or plexiform layer*. The *ganglion cell layer* is the thinnest and innermost of the neuronal layers. Large, granular neurons form a single and often sparse layer, supplemented by a few astrocytes, which become bilayered in the area centralis to accommodate the marked increase in photoreceptor density. *The density of ganglion cells predicts, in a general way, visual acuity.* They are most closely packed in animals requiring fine visual discrimination (most birds, predatory fish, many reptiles). In contrast, they are sparse in ungulates who do not feed by sight but who flee at anything that moves. Their axons form the *nerve fiber layer* that gradually increases in thickness towards the optic disk. In most animals, the fibers are not myelinated until they reach the optic disk. The nerve fiber layer is separated from the vitreous by an *internal limiting membrane* formed by the terminations of the Muller fibers and a true basal lamina.

The organization of the **retinal vasculature** is an important variable in ophthalmoscopic examination, both because vascular abnormalities are frequent signposts of disease and because normal species variation can erroneously be diagnosed as disease. Carnivores, ruminants, and swine have large venules and smaller arterioles radiating from the optic disk to the peripheral retina. The horse has about 60 thin, short vessels extending from the disk for about 5 mm into surrounding retina. In dogs and cats, the major vessels lie within the deep half of the nerve fiber layer and the ganglion cell layer. In ruminants and pigs, the vessels are very superficial and bulge into the vitreous, covered only by a thin layer of nerve fibers and basal lamina. The retinal vessels form an end-artery circulation that supplies the inner layers of the retina. The photoreceptors and outer nuclear layers are avascular and receive nutrients primarily by diffusion from choroid. Such dependence cannot be absolute (except in horses) because degeneration of these outer layers is surprisingly slow (weeks to months) following retinal separation. In contrast, occlusion of a retinal vessel results in focal infarction of the inner retina within less than 1 hour.

The blood vessels also participate in the *blood–eye barrier* similar to that already described for the uvea. The tight endothelial junctions and junctions between adjacent retinal pigment epithelial cells conspire to create a retina that is immunologically isolated from nonocular tissues in a manner similar to that described for the uvea. Like the uvea, such a barrier is likely not absolute and the various retinal antigens are likely to be neither totally sequestered nor absolutely unique to the retina. Saline extracts of retina yield the retinal S antigen, and the interphotoreceptor retinoid-binding protein is another antigen that may be important in the initiation or perpetuation of degenerative (see Retinal degeneration) or inflammatory retinopathies.

The **retinal pigment epithelium** extends from the ora ciliaris to the optic disk as the posterior continuation of the outer layer of ciliary epithelium. It forms a simple cuboidal epithelial layer that is separated from the choroid by a complex basal lamina, *Bruch's membrane*. The apical border interdigitates with the photoreceptors, with an average of about 30 photoreceptors contacting a single pigment epithelial cell, but forms no junctional complexes. The inclusion of the adjective "pigmented" is something of a misnomer in domestic species except the pig, inasmuch as the epithelium overlying the tapetum contains no cytoplasmic pigment granules. This seemingly insignificant layer plays a major role in embryologic induction of the eye as previously described, and also plays a crucial role in the nurturing of the photoreceptors throughout life. The pigment epithelium engulfs and degrades obsolete rod and cone outer segments, absorbs light to protect photoreceptors, synthesizes and degrades part of the glycosaminoglycan matrix enveloping photoreceptor outer segments, and participates in the vitamin A–rhodopsin cycle. Which of these functions are most essential for photoreceptor health is still unclear.

The **ocular fundus** *is a clinical term describing those ophthalmoscopically visible portions of the posterior globe excluding vitreous.* The fundus is commonly divided into dorsal tapetal and ventral nontapetal fundus, with the optic disk usually at the junction of the two. The retina, although almost transparent, does absorb some incident and reflected light to dull somewhat the fundus reflection ophthalmoscopically. Areas of retinal atrophy absorb less light and are seen as areas of increased tapetal reflectivity. Pre- or subretinal exudates, conversely, increase light absorption and are seen as focal fundic opacities. Developmental or acquired absence of tapetum allows black choroidal pigment to be seen. More severe choroidal lesions may be seen as red choroidal vasculature or even pink sclera obscured by variable amounts of residual pigment. Particularly in dogs, cats, and horses, selective breeding has made hypoplastic variations in amount and pigmentation of choroid and tapetum normal for particular breed or color varieties.

The **general pathology of retina** is often said to resemble that of brain. While this is undoubtedly true inasmuch as retina is merely an extension of the brain, the prevalence of such lesions as malacia, nonsuppurative perivascular cuffing, and proliferative microgliosis within the retina is very low compared to the brain. While no actual data are published, *most animals with encephalitis do not, in fact, have concurrent retinitis. Retinal inflammation is most often the result of spread from choroid or across the vitreous from anterior uvea.* Degenerations are much more common than inflammations and are not usually accompanied by inflammatory reaction. The mature mammalian retina has no capacity for regeneration of entire neural cells, although photoreceptor outer segments and glia may be replaced if destroyed in the course of degenerative or inflammatory disease. Even the fetal retina has poor regenerative capacity as evidenced by the prevalence of retinal dysplasia following prenatal or neonatal retinal injury. Retinal repair is by proliferation of inner layer astrocytes that eventually form a dense glial scar. Occasionally the astrocytes proliferate along the vitreal face of the retina, forming a preretinal fibroglial membrane. Similar subretinal membranes are seen with chronic detachments and originate from retinal pigment epithelium or Muller cells. The retinal pigment epithelium retains mitotic ability. When injured, these cells respond with hypertrophy, hyperplasia, and fibrous metaplasia. The presence of pigment in the neuroretina is frequent in instances of retinal atrophy, most probably derived from migration of retinal pigment epithelial cells into the adjacent retina.

Autolytic changes are visible within retina within 30 minutes of death, and within a few hours are of sufficient magnitude to interfere with the diagnosis of retinal degenerations. The earliest histologic change is pyknosis of a few nuclei in outer and inner nuclear layers, and loss of uniform density of the photoreceptor layer. Progressive dissolution of the photoreceptor outer segments results in retinal separation. Nuclear layer pyknosis and ganglion cell chromatolysis are widespread within 4–6 hours. *By 12 hours, retinal separation is complete and the extensively folded retina, with autolytic photoreceptors, may mimic genuine retinal separation.* The extensive pyknosis within both nuclear layers distinguishes the two, being absent in antemortem separation (see below for other criteria). By 18 hours after death, the retina is represented by a barely separable bilayer of pyknotic nuclei suspended in a pale, eosinophilic foamy matrix representing fragmented nerve fiber and plexiform layers.

Overview of retinal histopathology

The large amount of information about the causation and clinical features of the various retinal diseases is intimidating and serves to obscure the *fundamental simplicity of retinal histopathology*. The vast majority of retinal lesions fall into three categories: *inflammatory disease* as part of endophthalmitis, *photoreceptor degeneration* from inherited metabolic disease, detachment, or toxicity, and *inner retinal atrophy* (particularly ganglion cells) as a result of glaucoma. The angst over retinal histopathology can be further reduced by recognizing that many diseases of different pathogenesis share the same histologic appearance. For example, there are dozens of inherited photoreceptor diseases of dogs, yet they all look histologically identical. Most are even ultrastructurally identical. Furthermore, photoreceptor degeneration resulting from inherited retinopathy looks histologically identical to atrophy resulting from excessive exposure to light, from chemical toxicity, and even from retinal separation.

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Retinal separation

The retina is firmly attached in the globe only at the ora ciliaris and at the optic disk. *When the retina separates, it does so by cleaving photoreceptors from their interdigitations with the retinal pigment epithelium.* Separation may occur as the result of accumulation of inflammatory exudates, transudates, tumor cells, or helminths between pigment

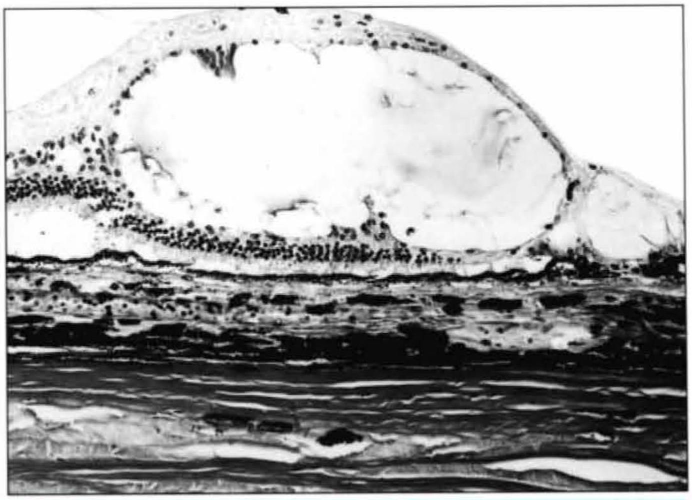

Figure 4.63 Peripheral microcystoid retinal degeneration in a dog. Such change is very common and of no apparent functional significance.

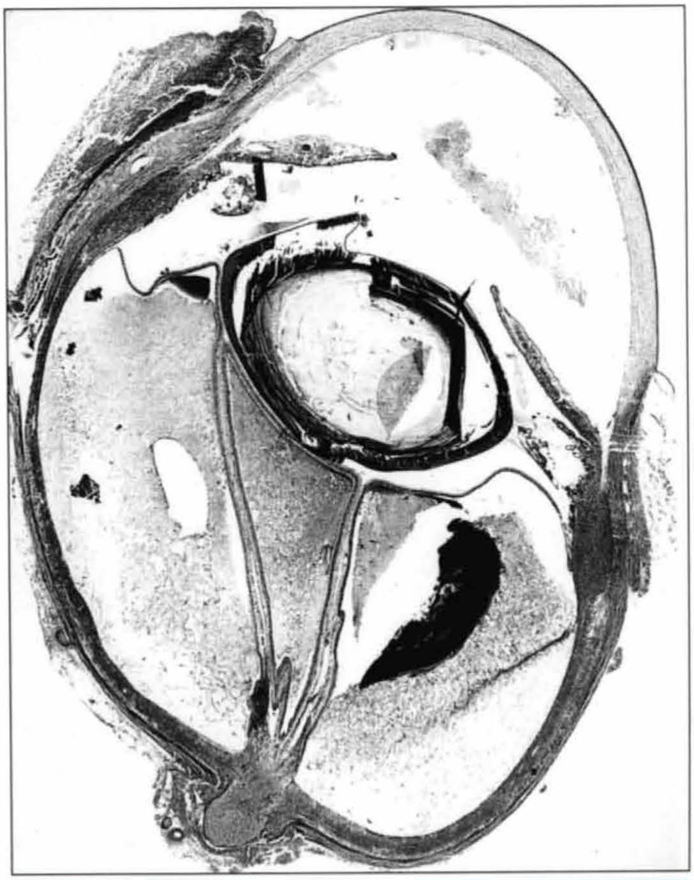

Figure 4.64 Complete retinal exudative separation in a dog with metastatic choroidal melanoma.

epithelium and photoreceptors, by contraction of a cyclitic membrane, or by the subretinal leakage of liquefied vitreous through retinal tears. Such tears may result from orbital trauma or from progression of peripheral cystic retinal degeneration. The latter is relatively common in man but not in domestic animals despite the frequent occurrence of microcystoid retinal degeneration in the peripheral retina of dogs (Fig. 4.63) and, less often, of horses.

The diagnosis of retinal separation in fixed specimens is complicated by the ease with which retinal separation can be induced by delayed fixation or improper handling of globes. *The credibility of the diagnosis is greatly enhanced by the presence of subretinal exudates or cyclitic membranes* (Fig. 4.64), *but in their absence the diagnosis rests upon the observation of photoreceptor outer segment degeneration, hypertrophy and hyperplasia of pigmented epithelium, and the development of marked edema in inner nuclear, ganglion cell and inner plexiform layers* (Fig. 4.65A, B). *Hypertrophy of the retinal pigment epithelium* is the most rapid change, occurring within a few hours of separation. The edematous changes are visible with the light microscope as early as 3 days following experimentally induced separation in owl monkeys. Coalescence of the edema creates a virtual cleavage of inner from outer retina, called **retinoschisis**. The cleavage is spanned by the radial Muller fibers, which seem the only anchor holding retina together. *Photoreceptor degeneration* is slower to appear under the light microscope, with loss of outer segments (probably the most subtle change that can be unequivocally diagnosed with routine light microscopy) visible by about 14 days after experimental saline-induced "exudative" retinal detachment. It is probably much faster than that when the exudate is full of noxious by-products of cell necrosis and inflammation, but no precise figure is available. Inner segments and the cell bodies of the outer nuclear layer are almost unaffected and may remain so for months, suggesting that their maintenance is not so intimately linked to the pigment epithelium as is the case with the outer segments. This temporal hierarchy of change permits reasonably accurate aging of retinal separations, sometimes a necessary or at least interesting assessment in eyes enucleated after numerous clinical examinations or manipulation. The outer retinal lesions are apparently not ischemic inasmuch as there is very little necrosis and no similarity to the lesion induced by retinal artery occlusion. Perhaps the outer layers can survive by diffusion of oxygen and nutrients from the subretinal fluid or from vascularized inner layers, and indeed the speed of photoreceptor atrophy varies with the height of the separation. An exception is seen in horses, inasmuch as the horse retina depends almost entirely on choroidal diffusion for oxygenation. Separation in this species results in rapid, full-thickness retinal infarction. A very

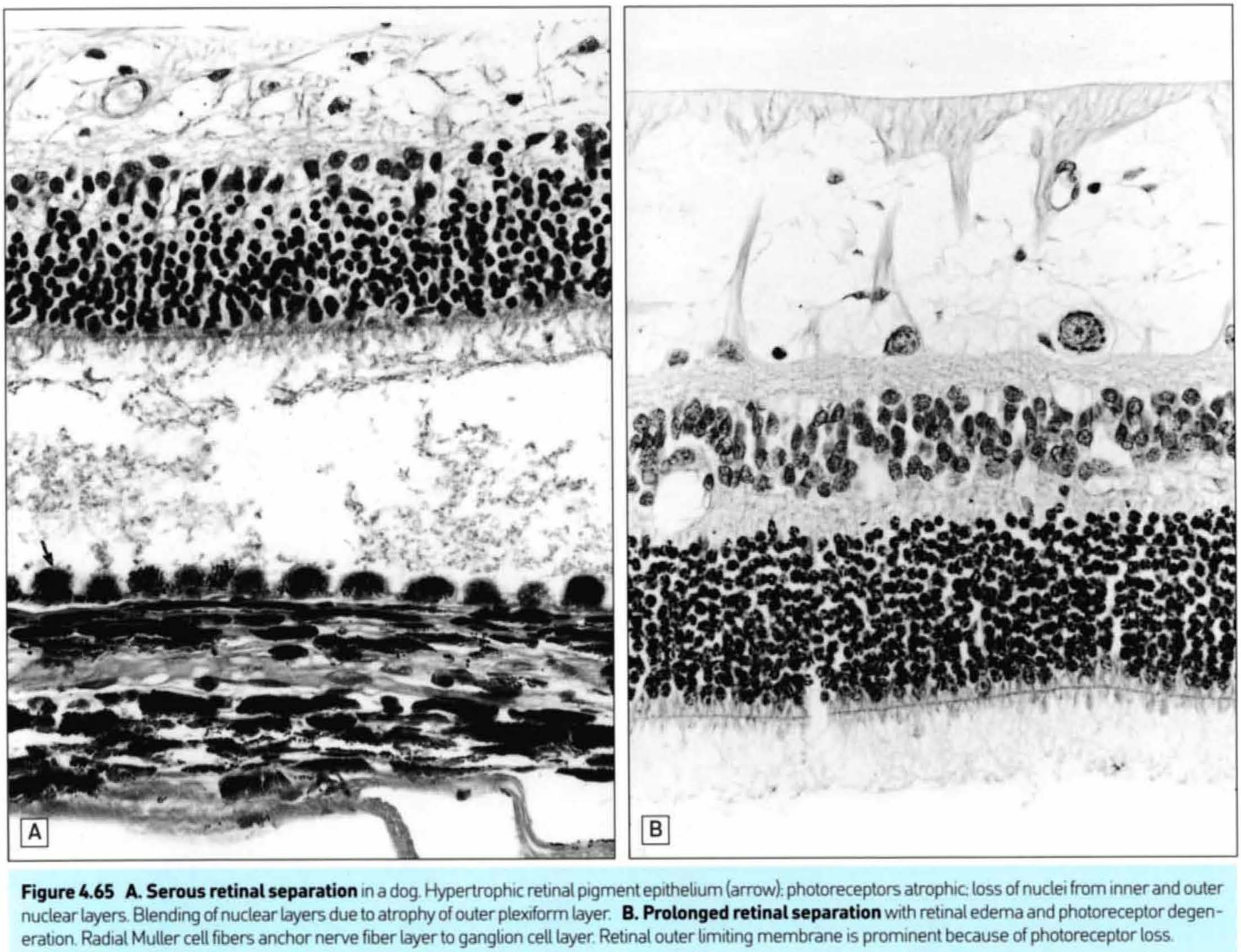

Figure 4.65 A. Serous retinal separation in a dog. Hypertrophic retinal pigment epithelium (arrow): photoreceptors atrophic; loss of nuclei from inner and outer nuclear layers. Blending of nuclear layers due to atrophy of outer plexiform layer. **B. Prolonged retinal separation** with retinal edema and photoreceptor degeneration. Radial Muller cell fibers anchor nerve fiber layer to ganglion cell layer. Retinal outer limiting membrane is prominent because of photoreceptor loss.

frequent lesion in horses is focal, linear, or multifocal chorioretinal glial scarring with pigment migration and fibrous metaplasia of pigment epithelium, a lesion that is probably a healed infarct following traumatic separation or thromboembolic disease.

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Retinal degeneration

Retinal degeneration, more commonly called **retinal atrophy**, *may result from senile change, nutritional deficiency, metabolic disorder, or injury caused by infectious, chemical, or physical agents.* With the exception of the previously described glaucomatous retinal atrophy, virtually all are initially degenerations of photoreceptor outer segments or of retinal pigment epithelium, and many retinal atrophies of different pathogenesis have similar histologic appearance. The similarities become even stronger as the lesions progress to the severity usually encountered in enucleated eyes from clinically affected animals. Nonetheless, it is useful to review the subject and to discuss the differences between some of the best-studied examples of naturally occurring retinal atrophies. Most frequent are the inherited retinopathies of dogs grouped under the name, *progressive retinal atrophy*. Less common are retinal degenerations caused by deficiencies of taurine, vitamin E or vitamin A, by excessive visible light, or by several toxic or metabolic diseases.

Inherited retinal atrophies in dogs

Progressive retinal atrophy describes, admittedly with some inaccuracies, *a large group of bilateral retinal diseases in dogs*. They share the clinical features of being bilateral, progressing to blindness and being unassociated with inflammatory or other ocular disease. More than 100 breeds have been identified as affected, although there is little published information as to the prevalence within various breeds. Almost all of the ones thus far studied are inherited as an *autosomal recessive trait*. Some are juvenile-onset degenerations that result from a congenital biochemical defect and are thus properly termed *photoreceptor dysplasias*. Photoreceptors never reach proper ultrastructural or physiologic maturity, and affected dogs may be blind by a year or two of age. Irish Setters, Collies, Norwegian Elkhounds, and Miniature Schnauzers are the best-studied breeds that are affected, each with slightly different clinical expression and biochemical abnormality. Some initially affect only rods, but most affect both rods and cones. Alaskan Malamute dogs have what seems to be a purely cone dysplasia, leaving dogs visually impaired in daylight but with good night (i.e., rod-dependent) vision.

A quite separate group of diseases are currently considered to be *true degenerations* in that photoreceptor development seems normal. It may be, however, that even these inherited atrophies will be shown to have a developmental biochemical defect. *The trend to date has been to reclassify more and more degenerations as biochemical dysplasias*, a consequence of the use of more sensitive investigative techniques.

All of these different diseases may have significant differences in pathogenesis, but by the time the eyes are removed from impaired or totally blind dogs, the histologic and ultrastructural lesions are similar. *At this stage, the old name of progressive retinal atrophy continues to be used as a catchall.* The light microscopic lesion is degeneration of photoreceptors beginning dorsolateral to the optic disk. Over months or years, the photoreceptor loss extends and there is secondary atrophy of nuclear and plexiform layers of retina (Fig. 4.66). Eventually, in dogs permitted to live long enough, the retina remains as a poorly cellular glial scar. Despite the many similarities in clinical and histologic features, the importance of these retinopathies in the study of retinal disease in general warrants some more detailed explanation of the best-described variants.

Retinal atrophy in Irish Setters is described as *rod–cone dysplasia type 1* (rcd1), inherited as a simple autosomal recessive trait. Dogs homozygous for the defect have arrested differentiation of the rod external segments. Cones are less affected. The defect is detected ultrastructurally as early as 16 days after birth, at which time the

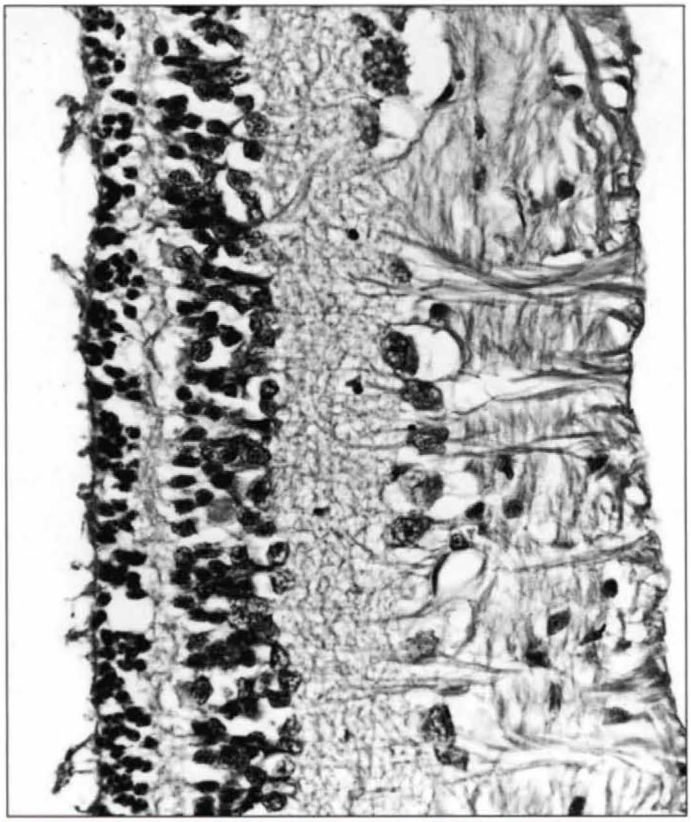

Figure 4.66 Progressive retinal atrophy in an English Cocker Spaniel. Diffuse atrophy of photoreceptors and outer nuclear layer.

outer rod segments should be developing adjacent to the pigment epithelium. Arrested development is followed by degeneration of inner rod segments, so that there is *diffuse loss of all rod photoreceptors by 12 weeks of age.* This is followed by loss of cones and of outer nuclear layer. The central retina is affected earlier and more severely than is peripheral retina. By the time the dog is about 1 year of age, there is diffuse atrophy of the outer nuclear layer, and the inner nuclear layer is in direct contact with the pigment epithelium. The inner retinal layers are unaffected. Dogs show visual deficits in dim light as early as 6 weeks of age and are usually blind by 1 year. *The biochemical defect is a marked deficiency of the phosphodiesterase responsible for the continuous hydrolysis of cyclic guanine monophosphate within outer segments.* While the function of the enzyme in this site is not fully understood, the resultant excess of cGMP is toxic to photoreceptors in vitro, and is known to cause arrested development or degeneration of rod outer segments in vivo. In the Irish Setter, the substrate levels are about ten times higher in affected than in control dogs, and the elevation precedes morphologic change in the photoreceptors. There are other biochemical retinal abnormalities (in rhodopsin and in membrane lipids), but it is not known whether they are primary abnormalities or merely effects of the cGMP phosphodiesterase deficiency. *The basic defect in rcd1 in Irish Setters is a nonsense mutation in the cGMP phosphodiesterase beta-subunit gene (PDE6B).* Also in the Irish Setter breed, there is a late-onset, progressive retinal atrophy that is likely hereditary, and that is distinct from rcd1.

Rough Collies have a *virtually identical photoreceptor dysplasia*, also inherited as an autosomal recessive trait. The progression of the lesion is slightly slower than in Irish Setters, but the biochemical defects are virtually identical. It appears that the defect results from a mutation of different genes than are involved with the Irish Setter dysplasia. In neither instance is it clear how photoreceptor death leads to death of all the outer nuclear layer neurons.

Retinal atrophy in Norwegian Elkhounds *is a historical disease, now eliminated*, that resembled the Collie and Irish Setter diseases in most clinical respects, but the biochemical defect was never identified. It was apparently not related to elevated cGMP. Onset of visual deficits in dim light was early; progression to blindness was only slightly less rapid than that in Setters. Ultrastructurally and biochemically, however, the two diseases were distinct. In Elkhounds, the primary lesion was restricted to rods. The rods were not arrested in their development but developed imperfectly. Light microscopic lesions appeared at about 6 months of age and progressed to complete photoreceptor loss by 1–2 years.

A *second type of rod–cone dysplasia* exists in this breed, with rapid progression to blindness by 12–18 months of age. Photoreceptor growth is erratic and apparently uncoordinated, but there is also dysplasia of rod and cone axonal synaptic junctions in the outer plexiform layer, which results in greatly reduced transmission of impulses from the photoreceptors. Ultrastructural changes are present in both the rod and cone outer segments as early as a few weeks after birth, but progression to complete retinal degeneration takes many years.

Cone degeneration (dysplasia) in Alaskan Malamute dogs *is a progressive and highly selective cone dysplasia*, inherited as a simple recessive trait. Although now rare because of elimination by selective breeding, it remains an interesting example of specific cone dysfunction. Affected dogs are noticed to have poor vision in bright light as early as 8 weeks of age. Night vision is, and remains, clinically normal, as does the ophthalmoscopic appearance of affected eyes. The ultrastructural lesion is disorganization and loss of cones, with rods normal. Adult dogs have no cone outer segments, and atrophic inner segments, but no change in rods or outer nuclear layer.

Rod–cone degeneration in Miniature Poodles *is classified as a true degeneration in that photoreceptor differentiation seems to be normal until 6–9 weeks of age.* After this time, and progressing at an unpredictable rate, there is disk disorganization and plasma membrane fragmentation in rod outer segments, visible as early as 15 weeks of age in the retina adjacent to the optic nerve. Peripheral retina is unaffected at this early stage, but by several years of age, the entire retina is affected. Cones are affected in a similar but milder fashion later in the disease course. The reason for the fragmentation is unknown, but it is known that affected dogs have abnormally slow outer segment turnover (about 40% of normal) prior to any observed structural change. Affected Poodles have evidence for a decreased rate of incorporation of docosahexanoic acid, the major long-chain structural membrane fatty acid, into rod outer segments. It may be that the decreased disk turnover permits older membranes to unduly persist and to be peroxidatively damaged in situ. The histologic lesion is identical to that of Irish Setters or Norwegian Elkhounds. Cataracts are present in many Poodles with retinal atrophy. Because of wide variation in disease progression, dogs may not be noticed to have dim-light deficiencies until middle, or even old, age.

An *almost identical disease* occurs in English and American Cocker Spaniels, Labrador Retrievers and Portuguese Waterdogs, resulting from a *mutation at the same genetic locus.* The diseases differ from breed to breed in the age of clinical onset and geographic distribution within the retina as seen ophthalmoscopically. The list of breeds reported to have some type of progressive retinal dysplasia/degeneration grows daily, and it is hard to determine how many of these represent a purely local phenomenon related to one or two specific lines of dogs, and which ones are truly global problems.

Central progressive retinal atrophy of dogs (pigmented epithelial dystrophy) denotes *a peculiar lesion in retinal pigment epithelium of dogs that apparently results from defective intracellular phagocytosis of shed photoreceptor outer segments.* Normal pigment epithelium engulfs and enzymatically degrades this material, resulting in a gradual buildup of intracellular lipopigments throughout life. In dogs with this pigment epithelial dystrophy, membrane peroxidation is excessive, and lipopigments accumulate to excess. Associated with the pigmentary accumulation, the epithelial cells hypertrophy. Photoreceptor outer segments adjacent to hypertrophic pigment epithelium degenerate. As the lesion progresses, hypertrophy and hyperplasia of epithelium give rise to dysplastic pigmented cell clumps. Within such clusters there may be an eosinophilic, hyaline, periodic acid-Schiff positive concretion resembling drusen. This material, rather frequent in ophthalmic specimens from people with a variety of degenerative retinal or choroidal diseases, is a concretion of excess basal lamina produced by the pigment epithelium. The eventual histologic lesion in affected dogs is a *monolayer of hypertrophic, lipochrome-rich pigment epithelial cells with multifocal hyperplastic clumps.* Retina has atrophy of photoreceptors and outer nuclear layer, and some irregular gliosis. Pigment-laden cells may invade the retina.

The disease is sporadic and of unpredictable clinical progression. The prevalence is, for example, much higher in Great Britain than in

North America, where it is rare. Retrieving and herding dogs are most frequently affected, with particularly high prevalence in Briards. The prevalence seems to vary dramatically from region to region, and over time. The ophthalmoscopic lesion of irregular black mottling begins near the optic disk and may progress to generalized pigment mottling interspersed with the increased reflectivity of atrophic retina. The mode of transmission is autosomal recessive in those breeds for which it is known. An interesting speculation, based upon morphologic similarities, is that the disease represents a defect in vitamin E metabolism within pigment epithelium. In Briards, the disease has been termed congenital stationary (i.e., nonprogressive) night blindness, although in many cases the disease does undergo slow clinical progression so the designation as "stationary" may not be appropriate. The *genetic defect (deletion of RPE65)* allows injurious accumulation of phospholipid esters of photoreceptor origin within the RPE, which eventually can be seen histologically as accumulation of lipid vacuoles.

Sudden acquired retinal degeneration is *an enigmatic, rapidly progressing photoreceptor degeneration that is histologically identical to the inherited progressive retinal atrophies. Blindness occurs very rapidly (over a period of a few days to a few weeks). Affected dogs are adult or even elderly, and the disease can affect any breed or crossbreed.* The fundoscopic lesion is bilaterally symmetrical and diffuse across the retina, but histologic studies of the early lesions are very few. The cause is unknown, but the presence of the retinal disease is linked to systemic signs of polyuria, polydipsia, and elevated serum cholesterol and alkaline phosphatase. Some, but not even the majority, of the affected dogs have adrenal cortical hyperfunction. How this malfunction causes the irreversible retinopathy, if indeed it does, is unknown. One small study demonstrated circulating, complement-fixing antibody to retinal S-antigen and interphotoreceptor retinoid-binding protein, raising the possibility that the disease is a cytotoxic autoimmune phenomenon.

Photoreceptor degenerations and dysplasias in cats

Photoreceptor degeneration is much less commonly observed in cats than in dogs. In the past, the great majority of cases have probably been examples of dietary taurine deficiency causing so-called *feline central retinal degeneration.* This once common disease has now become rare because of changes to virtually all commercial feline diets, but it will still occur in animals fed homemade diets and in those cats habitually eating some types of dog food. It is discussed in greater detail under nutritional retinopathies below.

Inherited retinal dysplasias and degenerations have been reported as sporadic occurrences in a variety of cat breeds, but only in the *Abyssinian breed* has the syndrome been adequately studied. In this breed there are two different diseases: *early-onset rod–cone dysplasia* and *late-onset retinal degeneration* affecting rods much sooner than cones. The early-onset dysplasia is inherited as an autosomal dominant trait. It is histologically and ultrastructurally similar to the disease in Irish Setter dogs, and a similar defect in the activity of cGMP phosphodiesterase has been reported. Affected cats are blind by a few months of age.

The late-onset retinal degeneration is inherited as an autosomal recessive, and affected cats progress slowly to blindness by 5–10 years of age. The earliest structural changes are in rod outer segments in peripheral retina, with jumbling of the rod disks and patchy blunting of the photoreceptors themselves. Only after many years is there histologically detected diffuse photoreceptor loss. A much more prevalent feline retinopathy, caused by deficiency of dietary taurine, is discussed later.

Inherited night blindness in horses

This poorly documented disease affects the *Appaloosa breed* and is probably inherited, and is seen as night blindness with daylight vision that is usually, but not always, normal. No structural lesion is seen in retinas of affected horses, and functional studies point to a defect in intraretinal synaptic transmission in outer plexiform or inner nuclear layers rather than a defect in photoreceptors.

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Light-induced retinal degeneration

Light of various wavelengths has a variety of injurious effects on cornea, lens, or retina that vary with the wavelength, duration, and intensity of the light.

The effects also vary with a large but poorly understood group of animal variables that include ocular pigmentation, habitat, previous experience with photoperiod, nutrition, body temperature, age and, most obviously, species. The wavelength of light has the greatest effect; short wavelengths in the *ultraviolet and blue range* (up to about 475 nm) have the greatest energy per photon and are the most damaging. Fortunately, most of these wavelengths are absorbed by cornea and lens, so that their lethal effects on retina are seldom seen. They may cause corneal epithelial injury or cataract, although these effects are apparently rare in domestic animals (see Cataract).

In people, accidental exposure to light from arc welding, solar eclipses, or ophthalmic examination or operating equipment (including lasers) creates the potential for rapid injury from mechanical disruption or heat. While such damage is certainly possible in other animals, most naturally occurring lesions result from the additive effects of much less intense ultraviolet and short wavelength visible light because of unnatural photoperiods. Animals with poorly pigmented eyes, and those adapted for nocturnal vision, are most susceptible. Susceptibility also increases with age and with temperature.

The initial lesion is disruption of rod outer segment disks, with eventual destruction of all photoreceptors and their nuclei. Because the lesion is identical to most inherited, nutritional, and toxic retinopathies, *the diagnosis is made on the circumstantial evidence of abnormally bright light, abnormally long light photoperiod, or a rapid change in photoperiod.* Most instances occur with rodents or fish kept in continuous fluorescent light. Albino rodents or deepwater fish are, predictably, the most susceptible.

The mechanism by which visible light of moderate intensity damages the retina is still incompletely understood, and different experimental models give rise to different theories. Most studies use blue light in the 400–475 nm range, which, unlike shorter ultraviolet wavelengths, is not filtered out by the cornea or lens. The most popular theory is that of light-induced oxidation of the very abundant polyunsaturated long chain fatty acids of the rod disks, with the generation of free radicals to then cause cell membrane damage. This theory gains support from studies showing a protective effect by vitamin C or E, and enhanced injury under conditions of retinal hyperoxia.

Nutritional retinopathy

Nutritional causes of retinal degeneration include deficiencies of *vitamins C, A, or E*, and the amino acid, *taurine*. Retinal atrophy and cataracts have been seen in fish with a dietary deficiency of vitamin C. The lesions were thought to be light induced, with the fish unusually susceptible because of the deficiency in the antioxidant effects of the vitamin. The ocular lesions of vitamin E deficiency resemble those of retinal pigment epithelial dystrophy and were referred to briefly under that heading. Pups fed severely deficient diets develop night blindness within about 6 weeks, and an extinguished electroretinogram suggestive of diffuse photoreceptor damage. These last two effects are not seen in naturally occurring central retinal atrophy. Retinopathy has been described in primates and dogs fed rations deliberately and severely deficient in vitamin E. *Lipofuscin*, seen as eosinophilic cytoplasmic inclusions, accumulated to excess in the pigment epithelium, and was followed by hypertrophy of the pigment epithelium and degeneration of photoreceptor outer segments. Eventually there was full-thickness central retinal atrophy and some small foci of retinal separation. Retinal degeneration with ceroid-lipofuscin accumulation in the retinal pigment epithelium has also been noted in vitamin E-deficient horses, but did not appear to cause visual impairment.

Hypovitaminosis A

Retinopathy caused by hypovitaminosis A is seldom encountered except in growing cattle or swine kept in confinement and fed a ration deficient in the vitamin over months or years. Grains other than corn (maize) are very poor sources of vitamin A, and the level in corn falls markedly with prolonged storage. Green pasture is very rich in carotene, which is converted to vitamin A by intestinal epithelium. Hay that is excessively dry, leached by rain, cut late in the year, or stored for prolonged periods is a much less adequate source. In most pastured animals, the liver reserves are sufficient to prevent clinical signs of deficiency for at least 6 months and often up to 2 years. Young, rapidly growing animals have greater requirements and smaller stores of the vitamin and are thus more susceptible than adults.

Hypovitaminosis A affects bone remodeling and causes epithelial cell atrophy and defects in synthesis of rhodopsin. Ocular lesions can result from each of these three defects.

As previously discussed, *maternal deficiency of vitamin A causes blindness in offspring due to defective remodeling of optic nerve foraminae and subsequent ischemic or pressure atrophy of the nerve.* In piglets, there may be massive ocular dysplasia and such anomalies as cleft palate, skeletal deformities, hydrocephalus, epidermal cysts, genital hypoplasia, and anomalous hearts. *Optic nerve atrophy is preceded by optic disk swelling (papilledema)*, and followed by atrophy of nerve fiber and ganglion cell layers. This sequence of events may occur if very young animals are on deficient diets, with the optic nerve changes being caused in part by stenosis of optic foramen and in part by increased cerebrospinal fluid pressure that itself results from atrophy and metaplasia of arachnoid villi. The papilledema precedes optic nerve necrosis and is reversible. The corneal lesions of hypovitaminosis A have received scant attention and are seldom seen in natural outbreaks.

The acquired ocular effect of hypovitaminosis A involves photoreceptor outer segments. The ophthalmoscopic lesion is multifocal retinal atrophy and scarring in animals with slow or absent pupillary light reflex and apparent blindness. The histologic lesion is patchy-to-diffuse photoreceptor atrophy which first affects the rod outer segments. Night blindness is thus the initial complaint and is often the chief complaint about a deficient herd. Eventually the atrophy affects all photoreceptors and their nuclei, and may progress to full-thickness atrophy with scarring. The lesions have been produced in all domestic species on specially formulated diets, but naturally occurring retinal lesions are almost restricted to cattle with chronic deficiencies.

The pathogenesis of the photoreceptor atrophy demonstrates the structure–function interdependence of retinal cells. Vitamin A is converted to retinene and then to the glycoprotein rhodopsin. Rhodopsin is stored as a component of the lamellar disks of the outer segment. Light initiates a physicochemical change in rhodopsin, resulting in a cascade of events culminating in the hyperpolarization of the outer segment membrane. The resultant electrical impulse is transmitted to bipolar cells, ganglion cells and then to brain. *Deficiency of vitamin A necessarily results in a deficiency of rhodopsin.* The corresponding ultrastructural lesion is swelling, then fragmentation of lamellar disks that can be reversed by therapy with vitamin A, unless inner segments have also been affected. Regeneration simulates normal development and requires about 2 weeks to rebuild outer segments completely. Vitamin A is discussed further in Vol. 1, Bones and joints.

Taurine-deficiency retinopathy

Retinal degeneration caused by taurine deficiency is seen only in cats, although taurine is the predominant free amino acid in the retina of other species. Among domestic mammals, only the cat seems unable to synthesize taurine from cysteine in amounts adequate for retinal function. Taurine is considered a dietary essential for cats, and its deficiency results in *characteristic central retinal atrophy* and in *myocardial failure* (see Vol. 3, Cardiovascular system). Changes in commercial diets, and better recognition of the risk of diseases related to taurine deficiency, have almost eliminated this disease.

The ocular lesion of taurine deficiency was first detected in cats fed semi-purified diets in which casein was the only protein. After several months, such cats developed focal retinal atrophy adjacent to the optic disk, which progressed to generalized retinal atrophy. Supplementation with taurine halted but did not reverse the lesion, presumably because photoreceptor nuclei or inner segments already had been damaged. The clinical and histologic features of this newly recognized nutritional retinopathy were virtually identical to those that had already been recognized in an idiopathic, naturally occurring disease of cats called "*feline central retinal degeneration.*" It is assumed that the two diseases are actually the same, although that may not be true in every single instance. At least some of the cases of idiopathic central atrophy were associated with the feeding of dry dog food, which (for a cat) is deficient in taurine.

The clinical lesion is a *focal lesion of tapetal hyperreflectivity* that is bilateral, dorsolateral to the optic disk and is usually unassociated with visual impairment. *The histologic lesion is photoreceptor degeneration,* initially targeting cone outer segments but eventually affecting rods as well. The rods of the peripheral retina are the last to degenerate. Taurine also seems essential for membrane integrity of the tapetal reflective rodlets, so that dissolution of the membrane surrounding these crystalline intracytoplasmic inclusions is another characteristic lesion. Less clear is the association of taurine deficiency with diffuse retinal atrophy in cats (Fig. 4.67).

Familial atrophy occurs in Abyssinian and Persian cats, but most cases are of unknown cause. Continued deficiency of taurine leads to diffuse retinal atrophy and thus might be responsible.

Toxic retinopathies

Experimental toxic retinopathies have been caused by many chemicals and toxic plants, but only a few toxic plants cause important diseases of domestic animals.

Bracken fern (*Pteris aquilinum*) causes progressive retinal degeneration in sheep in several areas of Great Britain. The common name "bright blindness" refers to pupillary dilation and tapetal hyperreflectivity of the severely affected sheep. The disease has been seen only in flocks grazing hills rich in bracken fern, and has been reproduced by prolonged feeding of the fern to sheep. A similar syndrome has been noted in cattle during long-term exposure to the fern. The lesion is usually seen in middle-aged or older sheep as bilateral and initially central tapetal hyperreflectivity. Diffuse involvement follows. *The histologic lesion is nonspecific, consisting of photoreceptor outer segment degeneration progressing to depletion of all retinal layers.*

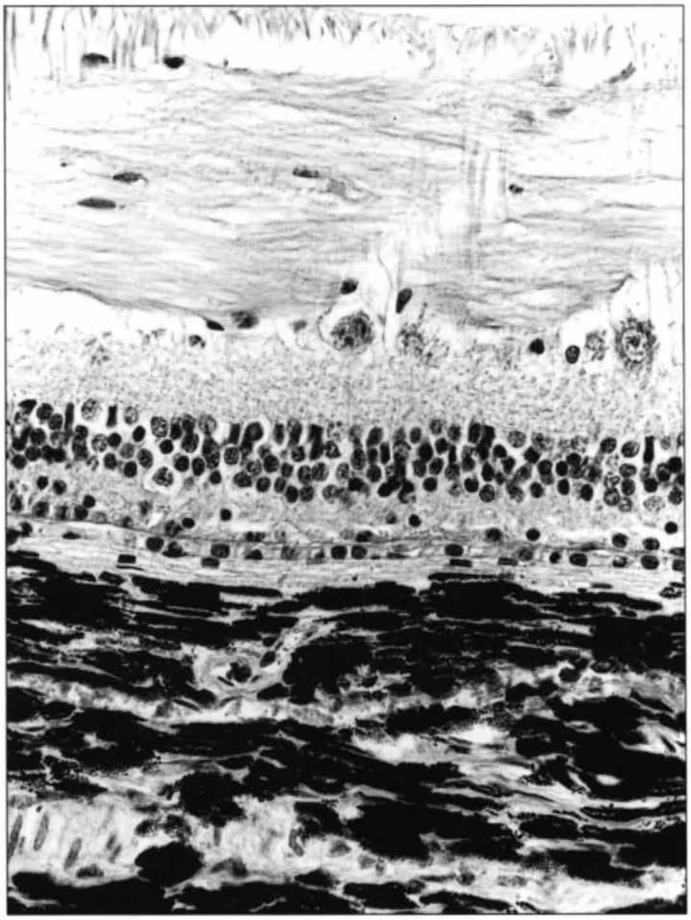

Figure 4.67 Idiopathic diffuse retinal atrophy in a cat. Note complete atrophy of photoreceptors and depletion of outer nuclear layer.

Blindness is one of the features of intoxication with **locoweed,** *Astragalus* and *Oxytropis* spp., in the USA, **darling pea** (*Swainsona* spp.), **blind grass** (*Stypandra* spp.) in Australia, and **selenium indicator plants** worldwide.

Astragalus and *Swainsona* cause a *neurovisceral lysosomal storage disease* analogous to genetically transmitted mannosidosis (see Vol. 1, Nervous system). All members of the genus *Swainsona* contain an indolizidine alkaloid, swainsonine, which is a potent inhibitor of lysosomal mannosidase. At least some *Astragalus* spp. contain a similar alkaloid. Chronic ingestion of the plant occurs in cattle, sheep, and horses forced to eat the plants on dry pastures where nothing more palatable is available. Affected animals develop behavioral abnormalities and defects of gait and vision. The histologic lesion consists of widespread cytoplasmic vacuolation in most organs due to the intralysosomal accumulation of mannose-rich oligosaccharides. Onset of clinical signs may require several months of heavy *Swainsona* ingestion, but ultrastructural vacuolation is seen within a few days. The ocular lesion is, as elsewhere in the central nervous system, vacuolation of neuronal cytoplasm and, later, axonal degeneration. The vacuolation is readily reversible upon cessation of ingestion of the plant and seems not to be the lesion responsible for clinical signs. The axonal degeneration is not reversible and is probably the more important lesion. Whether blindness is retinal or central in origin is unknown.

Poisoning with *Stypandra* spp. occurs in sheep and goats on dry pastures in southwestern Australia. The plant is among the first to reappear after autumn rains end the drought, and is eaten if nothing better is available. Acute intoxication is frequently fatal. Animals surviving the acute stage become blind and ataxic. In retina, there is diffuse photoreceptor atrophy and patchy hyperplasia of retinal pigment epithelium. Axonal degeneration is found within the optic nerve and elsewhere within the central nervous system.

The colloquial term *"blind staggers" refers to chronic intoxication of sheep and cattle with plants known to accumulate organic selenium selectively*. Affected animals wander aimlessly, become weak and ataxic and are finally paralyzed prior to death. There is some question as to whether blindness is genuine or merely the result of stupor. Ocular lesions are not described. The syndrome of blind staggers does not occur in experimental selenium toxicity, and it is possible that the syndrome is of much more complex pathogenesis than simple selenium toxicity. Plants of the genera *Astragalus* and *Oxytropis* are selenium accumulators as well as sources of swainsonine-like alkaloids.

Mycotoxicosis associated with the consumption of *Corallocytostroma* sp. fungus on Mitchell grass is reported to cause "black soil blindness" in cattle in Australia. The disease presents as rapidly progressive blindness and death. The histologic lesion is not described.

Photoreceptor degeneration has been described in cats treated with the antibiotic **enrofloxacin**. Initial reports suggested that this was an idiosyncratic reaction since the toxicity occurred after administration of the drug at recommended dosages. Subsequent analysis suggests that this is a direct toxicity that occurs when the retina is exposed to unusually high levels of this widely used antibiotic, even when administered at the daily recommended dosages (subsequently revised). Retinotoxic levels are likely to be reached when the drug is given by rapid intravenous infusion or when given to cats with impaired renal or liver function that may not properly metabolize and eliminate the drug. *Toxicity is thus most likely to be seen in old cats receiving prolonged therapy*. Similar retinal toxicity is likely to be shared by other fluoroquinolone antibiotics. Most cats had clinically obvious visual impairment within a few weeks of drug administration, and the blindness was permanent in most cases.

Miscellaneous retinopathies

Retinal lesions are found in a number of metabolic disorders and systemic states. Best known among these is diabetes mellitus, but retinal lesions are found also in any of the neuronal storage diseases, coagulation disorders, anemia, disseminated intravascular coagulation, hyperviscosity syndrome, hypertension, and following excessive exposure to oxygen or light.

Diabetes mellitus is *the major cause of blindness in people in North America. The cause of the blindness is chorioretinal vascular disease with subsequent retinal degeneration*. The characteristic lesions are seen only in patients with diabetes of 10–15 years duration. Even though virtually all chronic diabetics develop some retinal lesions, fewer than 10% become blind. Blindness is strongly predictive of the development of fatal diabetic nephropathy. Lesion development is not prevented by insulin replacement. Other ocular lesions include cataract, rubeosis iridis, glycogen-induced vacuolation of iris epithelium, and massive thickening of the ciliary basal lamina. The corneal epithelium may be unduly fragile, and tear production may be reduced.

The retinal lesion in people is mostly the result of microvascular disease. Loss of retinal pericytes, development of microaneurysms, thickening of capillary basal lamina and retinal hemorrhages constitute the early, degenerative phase of the retinopathy. This is followed by a proliferative phase in which more capillary aneurysms, arteriolar–venular shunts and neovascularization occur as the presumed responses to retinal ischemia. The neovascularization is initially bland and confined to the retina, but later there is extension into preretinal vitreous with accompanying fibroplasia (retinitis proliferans). Hemorrhages and hyalinized collections of leaked plasma are common in the retina.

In nonprimates, the naturally occurring retinopathy is seen only in dogs and, even then, infrequently. This low frequency may be due to the fact that affected dogs do not live long enough for the retinal disease to develop. In dogs deliberately made diabetic and kept for up to 6 years, microvascular lesions typical of human diabetes occur. Pericyte loss is accompanied by capillary aneurysms, reactive endothelial proliferation, and perivascular plasmoid exudates or hemorrhages.

Other types of **ischemic retinal injury** are much more frequent in domestic animals than is diabetic retinopathy. *Undoubtedly the most frequent examples are associated with vascular hypertension in cats and, less frequently, in dogs*. Ischemic retinopathy as a consequence of *DIC*, of *tumor metastasis*, or of *bacteremia* is fairly common, with the prevalence varying greatly with species. *Perhaps the most common example of ischemic retinal injury is that resulting from retinal detachment*. The blood vessels of the choriocapillaris supply oxygen and other nutrients to the photoreceptors and outer nuclear layer, hence retinal detachment inevitably results in gradual outer retinal ischemic/malnutritive atrophy.

Hypertensive retinopathy *is in most cases associated with chronic renal failure*. At least 60% of dogs with chronic renal failure are hypertensive. Dogs and cats are most frequently affected. In cats, hypertensive retinopathy is also claimed to be associated with hyperthyroidism, but a study of 100 hyperthyroid cats did not provide any evidence for that anecdotal association.

The macroscopic ocular lesions include retinal or preretinal hemorrhage, retinal edema, and retinal detachment because of serous effusion from injured choroidal blood vessels. *The histologic lesions are primarily in retinal and choroidal vessels*, which have lesions varying from fibrinoid necrosis of tunica media to medial hypertrophy with adventitial fibrosis. Changes that are probably secondary to vessel damage include localized retinal necrosis, exudative retinal separation with resultant atrophy of photoreceptors and hypertrophy of retinal pigment epithelium, and intraretinal hemosiderin deposition (Fig. 4.68). Usually there is ischemic necrosis of RPE, which allows for the leakage of hypertensive edema fluid from the choroid into the subretinal space (which would normally be prevented by the tight junctions between adjacent RPE cells). Vascular lesions and associated necrosis may also occur in anterior uvea. Eyes that are eventually enucleated or obtained at necropsy may have a variety of other lesions that probably occur secondary to chronic retinal detachment and chronic intraocular hemorrhage. Most notable among these is pre-iridal fibrovascular membrane and its resultant hyphema or neovascular glaucoma.

The early lesions, likely to be seen only under experimental conditions, are the result of exaggerated autoregulatory vasoconstriction in response to systemic hypertension. Sustained vasoconstriction leads to ischemic necrosis of the deprived retina, RPE or choroid, as well as necrosis of vascular endothelium distal to the constricted precapillary sphincters. The histologic consequences are focal retinal necrosis, and leakage of

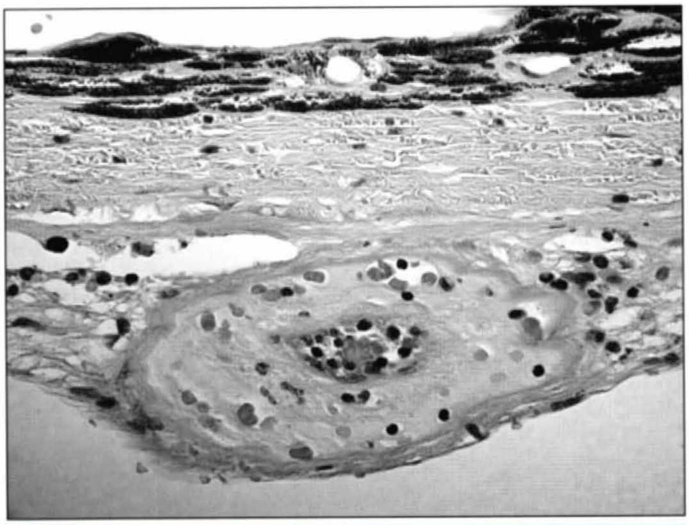

Figure 4.68 Hypertensive retinopathy.

plasma or even erythrocytes through damaged endothelium. This leakage causes intramural fibrinoid change in the vessels and edema or hemorrhage in adjacent retina.

Retinal infarction, usually seen as a combination of hemorrhage and liquefaction, occurs subsequent to retinal vessel thrombosis, occlusion by tumor emboli, or vasculitis associated with immune disease or a few infectious diseases (Fig. 4.69). The most likely to be encountered are thrombotic meningoencephalitis of cattle, and Rocky Mountain spotted fever or ehrlichiosis in dogs. In addition to the vascular lesions themselves, which may have specific characteristics associated with the individual diseases, the retinal lesions vary from focal hemorrhage to areas of hemorrhagic liquefactive necrosis or healed lesions of chorioretinal scarring.

Similar retinal scarring has been seen in horses following massive but sublethal blood loss, as in surgery or from nasal hemorrhage subsequent to severe cranial trauma. Affected eyes have multifocal retinal atrophy and hyperpigmentation. Similar lesions may also result from focal retinal separation. In horses, such separation carries a high risk of causing focal retinal infarction because they have such limited intrinsic retinal vasculature (see Fig. 4.69).

Retinal hemorrhages are seen in a variety of primary clotting disorders, in thrombocytopenia of any cause, and in degenerative or inflammatory vascular disorders. Retinal hemorrhages also recur in cats with profound anemia of any cause; the mechanism is unknown. The lesions heal with scarring if the cat survives the anemia, suggesting that the hemorrhage is only the most visible manifestation of multifocal and probably ischemic retinopathy.

Many of the **neuronal storage diseases** cause retinal lesions identical to those in the brain. The list of those with described ocular lesions probably reflects those in which the eyes have been examined rather than a true reflection of those diseases in which ocular lesions do, or do not, occur. Those interested should consult a useful, referenced table in the text by Slatter.

Senile retinopathy *is characterized by microcystoid degeneration*, which is very common in dogs from middle age onwards (see Fig. 4.63). A similar lesion is found occasionally in horses. The lesion affects peripheral retina adjacent to ora ciliaris and for a variable distance medially. There is formation of small cystic spaces within inner nuclear and plexiform layers, fusion of inner and outer nuclear layers, pigment cell accumulation and haphazard atrophy and mingling of nuclei in a manner simulating peripheral retinal dysplasia. If the cysts rupture to the vitreal face, the retina external to the cyst is seen as an atrophic hole. Such holes are foci of extreme retinal thinning, and only the external limiting membrane separates vitreous from pigment epithelium. The pigment epithelium and choroid are usually normal but they too may show atrophy and fibrosis. Intermingling of cysts and holes in peripheral retina is common. Complete breaks are uncommon and do not usually lead to retinal separation, as occurs in people.

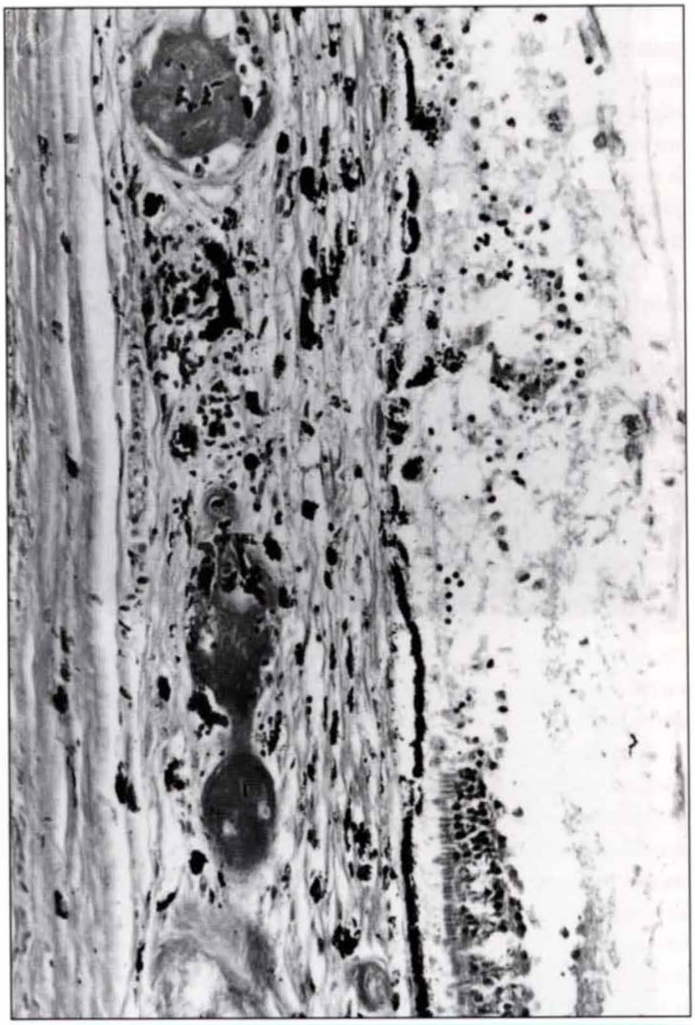

Figure 4.69 Choroidal thrombosis and vasculitis in a horse with idiopathic purpura hemorrhagica. Note **infarction** of adjacent retina.

Multifocal coalescing peripheral retinal atrophy is very frequent in very old dogs and horses, but is of no apparent visual importance.

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Retinitis

Retinitis as the sole ocular lesion is rare, but may occur in animals with neurotropic virus infections, with toxoplasmosis, and with thrombotic meningoencephalitis of cattle (Fig. 4.70). In the latter disease, however, it is more usual to find the typical thrombotic, inflammatory lesions in choroid as well as retina. Their character is identical to the lesions in the brain. The multifocal chorioretinal scars expected as sequelae are seldom seen, perhaps because cattle with neurologic and ocular lesions almost inevitably die. The prevalence of the ocular lesion, useful as an aid in the clinical diagnosis, is estimated at 30–50% in animals with the septicemic form of the disease, and as high as 65% in experimentally infected calves.

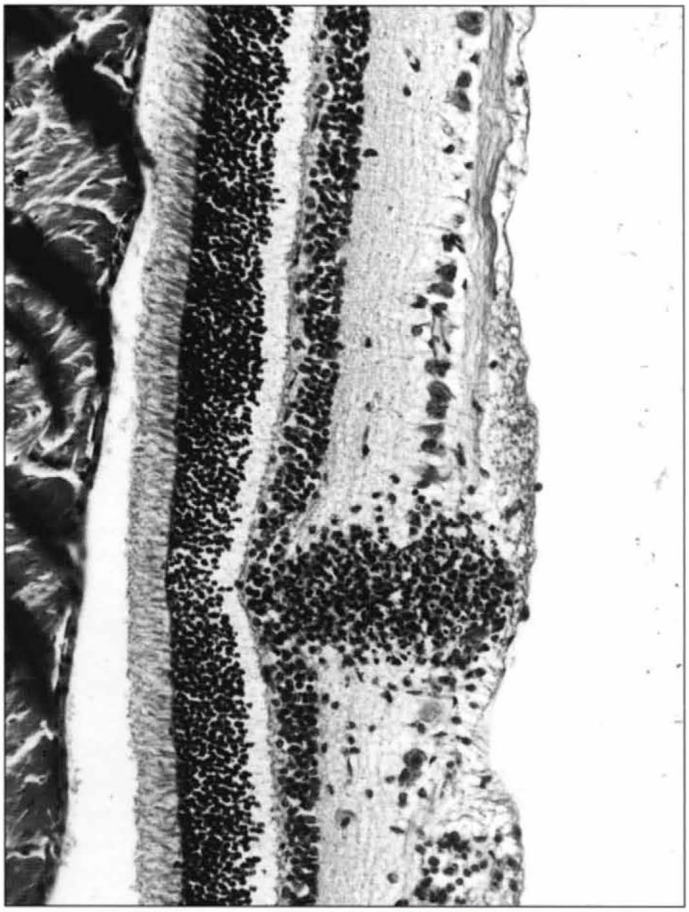

Figure 4.70 Focal destructive retinitis due to *Histophilus somni* infection in a steer.

Multifocal viral retinitis with the same histologic features as the respective brain lesions occurs in animals with classical swine fever, rabies, Teschen disease, Borna disease, pseudorabies in pigs, canine distemper, and scrapie (a prion disease). Undoubtedly the list is incomplete, and is probably limited only by the rarity with which retinas receive histologic evaluation in animals dying with systemic viral, bacterial, and protozoal diseases. *The ocular lesions associated with canine distemper will be described here in some detail as the archetypal example of viral retinopathy* (other aspects of canine distemper are discussed in Vol. 2, Respiratory system).

Retinal and optic nerve lesions occur in most dogs with naturally occurring **canine distemper.** The lesions most often are degenerative rather than inflammatory, although some of the degenerative changes may have been sites of inflammation earlier in the disease course.

Acute lymphocytic-plasmacytic chorioretinitis and optic neuritis are found in about 25% of dogs submitted for laboratory confirmation of the disease. Random perivascular cuffing, edema, focal exudative retinal separation, and hypertrophy of retinal pigment epithelium are present. Eosinophilic intranuclear inclusion bodies occur in ganglion cells or astrocytes in 30–40% of the cases, which is the only etiologically specific change in what is an otherwise nonspecific picture shared by many systemic infections.

The more prevalent lesions are multiple random foci of retinal degeneration and scarring. These usually affect the full thickness of retina, and are most likely sequelae to the previous undetected retinitis. Such foci often contain numerous melanin-laden cells, probably derived from migration of adjacent, injured retinal pigment epithelium. Occasionally only the outer nuclear layer and photoreceptors are missing, probably a sequel to focal exudative retinal detachment.

Optic nerve lesions of one type or another are present in all dogs with ocular lesions. Nonsuppurative neuritis, astrocytic scarring and demyelination similar to that in brain are the three most frequent changes. In those dogs suffering only the demyelinating disease, the ocular lesions may be inapparent, or there may be demyelination of optic nerve and ganglion cell degeneration.

Other infectious examples of retinitis in dogs include **Rocky Mountain spotted fever** (RMSF, *Rickettsia rickettsii*) and **canine ehrlichiosis** (*Ehrlichia canis*). The clinical and histologic ocular lesions are virtually identical and occur in a high percentage (80% for RMSF) of dogs with active infection. Most of the lesions result from injury to vascular endothelium parasitized by the rickettsiae; multifocal hemorrhage, edema, and vascular necrosis occur in all parts of the eye. *Multifocal retinal hemorrhage, perivascular retinal edema, and necrosis of endothelium in retinal venules and arterioles are the characteristic retinal changes.* While often listed along with other agents as a cause of anterior uveitis or endophthalmitis, most naturally occurring infections have clinical signs attributable only to the vascular injury rather than a genuine uveal inflammation. There is one report of unusually severe uveitis occurring 14–28 days after experimental infection with *Rickettsia rickettsii*, following the disappearance of all other signs of the acute systemic disease. Dogs thus affected had neutrophilic and lymphocytic destructive vasculitis, assumed to represent a type III immune reaction to parasitized endothelium.

Viscera larva migrans from *Toxocara canis* has been linked to granulomatous endophthalmitis and chorioretinitis in dogs. It is likely that many of these cases were actually caused by the raccoon roundworm *Baylisascaris procyonis* (see Parasitic endophthalmitis).

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OPTIC NERVE

The optic nerve is a white fiber tract of brain formed by the outgrowth of ganglion cell axons from the eye through sieve-like perforations in posterior polar sclera, called the *lamina cribrosa*. The axons travel within a preformed neurectodermal tube formed by the primary optic stalk to reach the optic chiasm and then the lateral geniculate body. The neurectoderm lining the optic stalk induces the surrounding mesenchyme to form the three meningeal layers, similar to and continuous with those of brain itself. Later differentiation of neurectoderm produces astrocytes and oligodendroglia that, together with the ganglion cell, axons, and fibrovascular septa from pia mater, form the substance of the optic nerve. The *optic disk* is the intraocular portion of the nerve and is the only portion available to ophthalmoscopic examination. It is formed by the convergence of ganglion cell axons prior to their exit via the lamina cribrosa. The axons of the nerve fiber layer are unmyelinated, and at what point (relative to lamina cribrosa) the axons become myelinated determines the ophthalmoscopic appearance of the optic disk. Histologically, the disk is unmyelinated in most domestic species except the dog, contains abundant glia and may have a small paracentral excavation – *the physiologic cup* – from which Bergmeister's papilla originates. A few pigmented cells are commonly seen, as are small neuroblastic clusters, both probably minor anomalies of retinal differentiation but of no significance.

There is considerable variation in the normal histology of the optic nerve among animals of different species and ages. Optic disk myelination has already been mentioned. The lamina cribrosa is formed by heavy fibrous trabeculae in horses, dogs, and cattle and is therefore more obvious than in cats and laboratory animals. Fibrous septa within the nerve are prominent in cattle and horses, and their similarity to the axons in hematoxylin and eosin sections may mask a pathologic paucity of nerve fibers. The fibrous tissue reportedly increases with age.

The general pathology of optic nerve shares features of both retinal and neural disease. Because it is in direct continuity with both structures via its axons, and with brain via the perineural cerebrospinal fluid, it is common for optic nerve to be affected by diseases of either retina or brain. Thus optic neuritis is expected in at least a proportion of animals suffering with inflammation of retina or neural white matter, and optic nerve atrophy inevitably follows loss of ganglion cells. Fortuitous hematogenous localization of infectious agents or tumor cells may occur in optic nerve as anywhere else.

Papilledema *is hydropic swelling of the optic disk.* It may result from extraocular events that cause an increase in cerebrospinal fluid pressure within the optic nerve or from local vascular leakage. The former is usually associated with retrobulbar tissue masses, but is also seen with intracranial neoplasms and with hypovitaminosis A. Ocular hypotony may cause optic disk edema as a result of decreased tissue hydrostatic pressure. Serous inflammation within the nerve also results in papilledema. Papilledema is a common clinical diagnosis that rarely is available for histologic examination.

Optic neuritis *is a term sometimes used rather broadly to describe both inflammatory and degenerative diseases of the nerve.* Optic neuritis is seen clinically as swelling, hyperemia, and focal hemorrhage within the optic disk. Affected animals, usually dogs or horses, are blind when the lesion is bilateral. Although described as a clinical entity not associated with other ocular lesions, histopathologic confirmation is lacking. Optic neuritis may, of course, accompany any case of retinitis or endophthalmitis.

The pattern of inflammation within the nerve may provide clues to the pathogenesis of the neuritis. Perineuritis, or optic nerve leptomeningitis, is typical of meningeal spread of bacterial meningitis from the brain. Toxoplasmosis and cryptococcosis frequently cause multifocal and nonselective lesions within the extraocular nerve, as does canine distemper. Optic neuritis originating as endophthalmitis is usually restricted to the optic disk. Feline infectious peritonitis is frequently associated with perineuritis and optic neuritis in which the mononuclear aggregates are around blood vessels in the meninges and in the extensions of the meninges into the nerve.

Chronic optic neuritis, like its counterpart in the brain, is characterized by focal gliosis, astrocytic scarring, and secondary axonal degeneration. The loss of axons may be partially masked by the increased prominence of glia and pial septa.

Degeneration of the optic nerve *is part of optic neuritis, glaucoma, and chronic, severe retinal atrophy of any cause.* Initiation of gliosis and fibrosis may eventually make the chronic degenerative lesion indistinguishable from that of chronic inflammation. The most frequently diagnosed example is that following *trauma* to one or both nerves in dogs or cats struck by cars (Fig. 4.71). The gross lesion may be avulsion or contusion. Injury to the nerve may be instantaneous as caused by tearing or complete severance, or may result from vascular injury with slightly delayed ischemic necrosis. In severed nerves, there is disintegration of the distal axons back to the lateral geniculate body. The proximal portion of each affected axon dies back to the ganglion cell, which eventually also dies. The inner nuclear layer remains unaffected, a useful criterion to distinguish traumatic, "die back," ganglion cell atrophy from that of glaucoma.

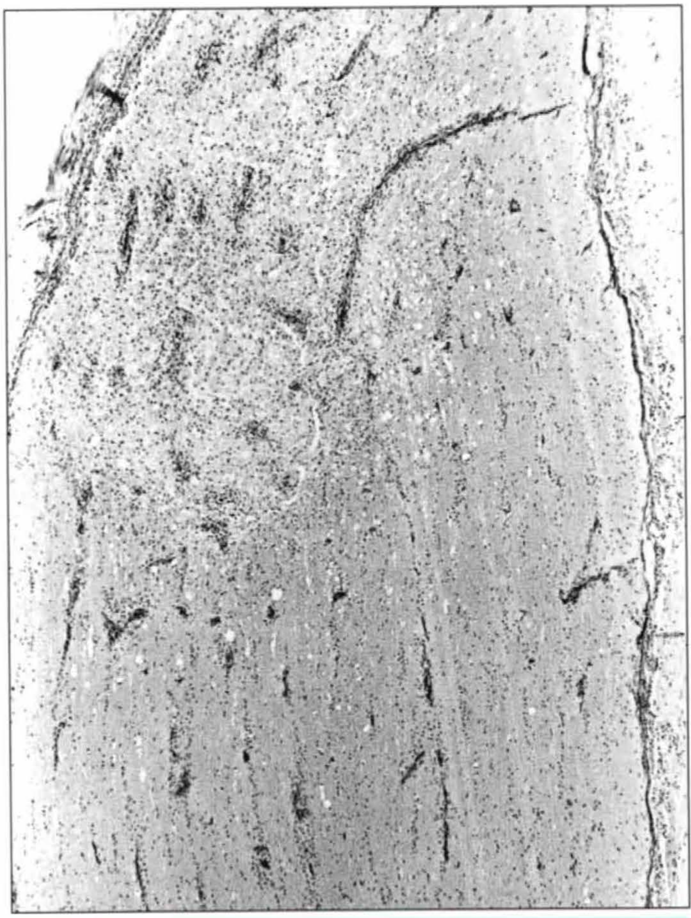

Figure 4.71 Axonal degeneration in the retrobulbar, intraorbital optic nerve in a dog, 11 days after being struck by a car.

Degeneration of optic nerve also occurs in calves *deficient in vitamin A*, and in ruminants ingesting *male fern* or *hexachlorophene*. Ingestion of male fern, *Dryopteris*, on pasture or as a taenicidal extract causes papilledema and subsequent optic nerve demyelination when ingested in large amounts. Retina may be unaffected early, but ganglion cell atrophy occurs eventually. Hexachlorophene administered to calves or sheep as an anthelmintic causes edema and then atrophy and gliosis of optic nerve.

Proliferative optic neuropathy is an unusual lesion of horses. Anecdotal descriptions are numerous, but histologic descriptions are few. The lesion is a raised, gray mass on the surface of the optic disk, unassociated with visual deficit. *The mass is composed of spherical mononuclear cells with hyperchromatic, eccentric nuclei and foamy eosinophilic cytoplasm* (Fig. 4.72). Some of these cells are also found within extraocular optic nerve. The cytoplasmic content may be stored lipid, but its origin is not known. The described lesion bears much resemblance to the proliferation of myelin-laden macrophages that occurs

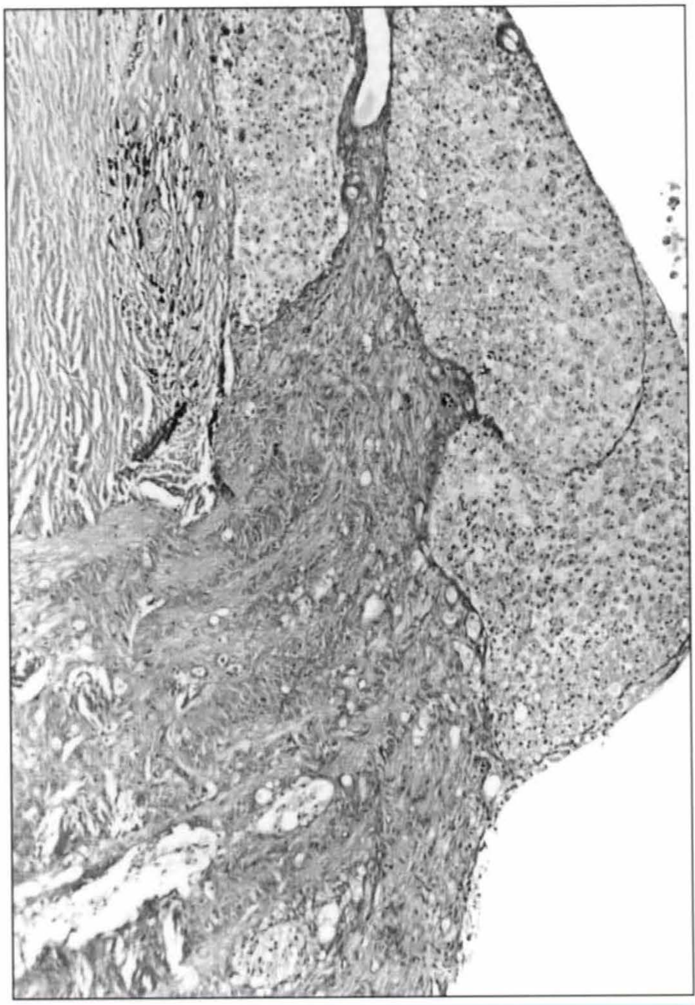

Figure 4.72 Equine proliferative optic neuropathy. The identity of the foamy cells is much debated, but they are probably reactive macrophages full of lipid.

in and on optic nerves injured by trauma or ischemia. Also, the distinction between the proliferative optic neuropathy and gliomas or granular cell tumors described in various reports is unclear.

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SCLERA

The **limbus** *marks the transition from the avascular, nonpigmented and very orderly cornea to the vascularized, pigmented and interwoven fibrous tissue that identifies sclera.* The sclera forms the posterior two-thirds of the fibrous tunic of the eye, blending with choroid on its inner aspect and orbital fascia exteriorly. Its thickness increases with age and varies considerably among domestic species. In cattle and horses, it is thickest at the posterior pole (2.2 mm in cattle and 1.3 mm in horses) and thinnest at the orbital equator (1.0 mm in cattle, about 0.5 mm in horses). In dogs and cats, it is much thinner, about 0.3 mm at the posterior pole and 0.1 mm at the equator, varying somewhat with age and globe size. In carnivores, however, there is a circumferential ring of thickened (1 mm) sclera at the limbus in which is buried the venous plexus receiving aqueous drainage. The sclera is perforated by numerous vessels and nerves, the most notable of which are the optic nerve and limbic scleral venous plexus.

The optic nerve fibers exit the globe through extensive scleral fenestrations called the **lamina cribrosa**. Diseases of the sclera are few in comparison to diseases of other ocular structures. Most are inflammatory and arise by extension from within the globe or from orbital cellulitis. *The efficiency with which the sclera resists inflammatory spread is evidenced by the infrequency of panophthalmitis as opposed to endophthalmitis, and the even greater infrequency of intraocular involvement resulting from orbital inflammation.* When the sclera is involved in inflammatory disease originating within the eye, its initial involvement is seen histologically as leukocytes in perivascular adventitia that is in direct communication with the choroid. A similar phenomenon is seen in scleral extension of choroidal neoplasms, in which collars of tumor cells surround scleral vessels but show little inclination to infiltrate directly into scleral connective tissue.

Nodular granulomatous episcleritis (ocular nodular fasciitis) *is the most prevalent disease of dogs that is primarily scleral.* It occurs rarely in cats. It is the proliferative, nodular lesion of the limbus that has been variously termed nodular fasciitis, nodular scleritis or episcleritis, fibrous histiocytoma, proliferative keratoconjunctivitis, conjunctival granuloma, and Collie granuloma. The various names reflect the spectrum of clinical presentations of this lesion. The variations are treated as a single entity, nodular granulomatous episcleritis (NGE), in this discussion. My decision to do so is purely for the sake of simplicity, since there is no compelling evidence to justify splitting this histologic entity into multiple diseases simply because the lesions may occur in various locations within and below the conjunctiva, and clinical management is similar for all.

The usual macroscopic lesion is a firm, painless, moveable, nodular swelling, 0.5–1.0 cm in diameter, below the bulbar conjunctiva at, or just posterior to, the limbus. Infiltrative extension of the mass into the peripheral corneal stroma is accompanied by edema and vascularization (Fig. 4.73A). Although the temporal limbus unilaterally is the most frequent site for initial occurrence, other common locations include the third eyelid and elsewhere along the limbus. Third eyelid involvement is often bilateral, and occurs almost exclusively in rough Collies. Limbic and nictitans involvement may occur in the same dog and even in the same eye.

Ocular nodular fasciitis behaves as would a locally infiltrative neoplasm. Extension is usually into peripheral corneal stroma and posteriorly into sclera, episclera, and Tenon's capsule. The tissue of origin of this lesion is unresolved. Fibrous tissue of sclera, episclera, and Tenon's capsule have all been suggested. Lesions involving the third eyelid, or the rare case of palpebral subconjunctival origin, probably originate from the fascia native to those structures. Histologic examination distinguishes this lesion from extension of intraocular tumors or the rare scleral sarcomas. Occasionally, infiltration of therapeutically refractory fasciitis is so extensive, both into cornea and sclera, as to require enucleation.

Histologically, the lesion is a proliferative, nonencapsulated mixture of histiocytic cells, spindle cells, and mononuclear leukocytes (Fig. 4.73B). The spindle cells may be fibroblasts, histiocytes, or a mixture of both. The spindle cells are haphazardly arranged and, despite a fibrous appearance to the section, surprisingly little collagen is demonstrated by special stains, except in coarse septa that may dissect the mass into irregular lobules. Reticulin, however, is abundant. The mononuclear leukocytes are found loosely throughout the mass but are usually most numerous near the periphery. *Important histologic features are the absence of collagenolysis and the absence of discrete granulomas.* While the lesion may be quite granulomatous, the macrophages are uniformly intermingled with the other cellular elements. When present in peripheral cornea, the above cell mixture affects stroma but spares the epithelium and an adjacent zone of subepithelial stroma.

Necrotizing scleritis *is a rare lesion seen in dogs as a poorly delineated inflammatory and proliferative lesion of the anterior sclera.* The disease incites much more inflammatory reaction, as measured by clinical criteria, than does nodular fasciitis. *The lesion consists of coalescing scleral granulomas centered on remnants of denatured, refractile collagen.* Eosinophils sometimes are seen in the centers as well. The formation of true granulomas may not be prominent, but at least a few are required to distinguish this lesion from nodular granulomatous episcleritis described above. The lesion tends to slowly spread circumferentially and posteriorly to involve the entire sclera, and involvement of uvea and even retina with granulomas eventually occurs. Bilateral involvement is usual, but not necessarily at the same time. Response to anti-inflammatory therapy is poor unless that therapy is very aggressive, and so an unusually high percentage of eyes with this disease eventually become available for histologic assessment. No etiologic agent has been seen.

ORBIT

Diseases of the orbit are few and relatively uncommon in domestic animals except for those resulting from trauma and, in dogs, from neoplasia. Systemic diseases of bone, muscle, blood vessels, and nerves may incidentally

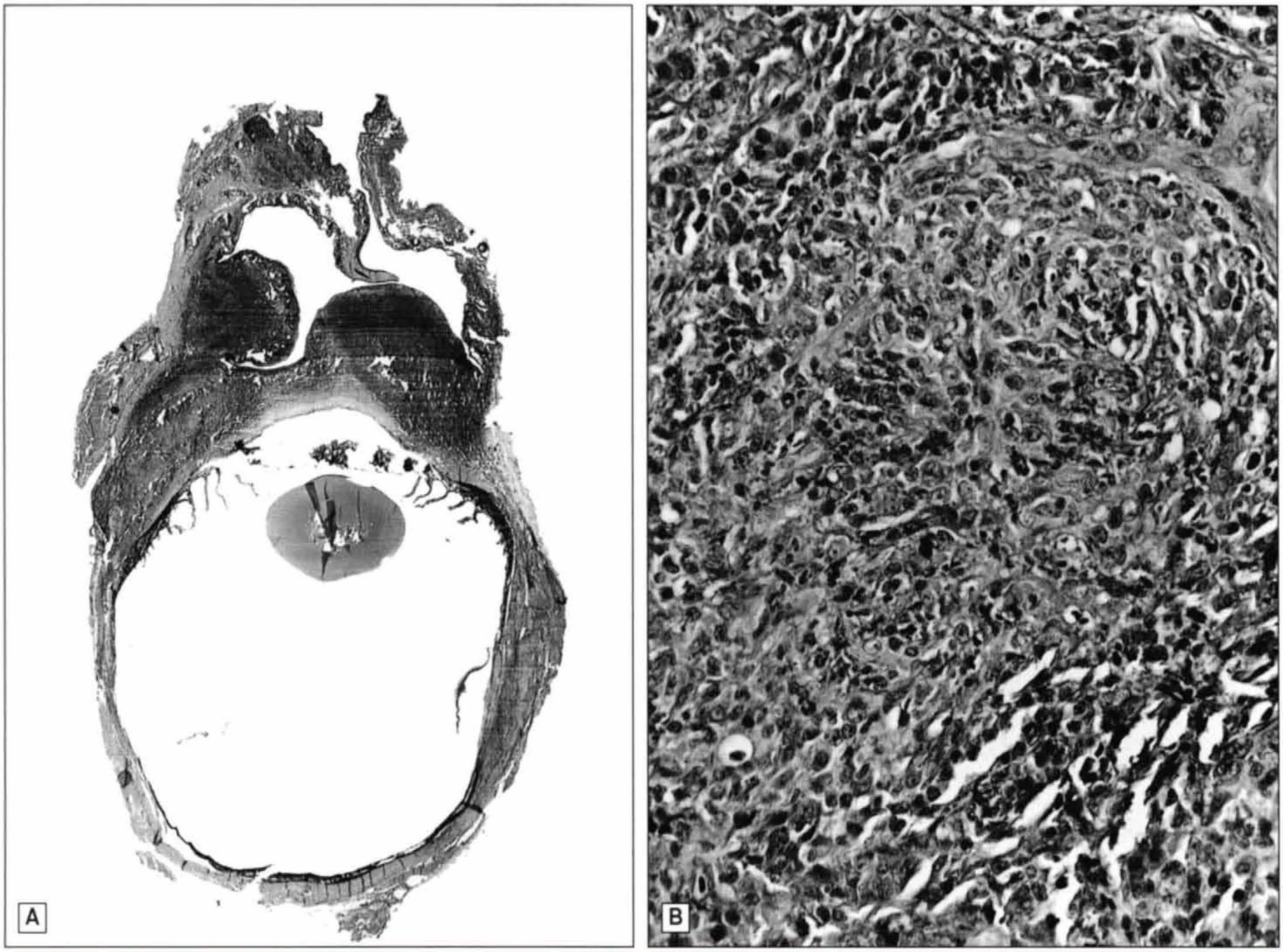

Figure 4.73 Nodular granulomatous episcleritis syndrome. A. Unusually extensive lesion, which progressed for 2 years without adequate therapy. **B.** Higher magnification, demonstrating the characteristic intermingling of lymphocytes, macrophages, and fibroblasts without actual granuloma formation.

affect orbital components. Orbital fat fluctuates with nutritional status contributing to the enophthalmos of malnourished animals. Ordinarily, however, orbital disease arises by extension of inflammatory lesions from the mouth, paranasal sinuses, or from penetrating wounds through periorbital soft tissue. Extension from intraocular inflammation is surprisingly rare, a tribute to the barrier offered by the sclera. Conversely, orbital disease rarely invades the globe. Metastatic orbital neoplasia is rare except for lymphoma of cattle and cats. Direct invasion by malignancies arising in the mouth, facial bone, nasal cavity, or sinus is more common. While theoretically the orbit may suffer from primary neoplasia of any of the bony or soft tissues within it, such occurrences are infrequent. Of these, optic nerve meningiomas, ill-defined spindle-cell sarcomas, and lacrimal gland tumors in dogs are the most common (see Ocular neoplasia).

Orbital cellulitis *is the term commonly used to describe pyogenic orbital inflammation*. The cause is usually bacterial and the pathogenesis involves extension from nearby inflammation of paranasal sinuses, molar tooth socket, or periorbital soft tissue. Only rarely does uncontrolled endophthalmitis spread through the sclera into the orbit. Bacteremic localization within the orbit, while presumably occurring as do such localizations elsewhere, is seldom detected except perhaps for *Streptococcus equi* infection in young horses.

Orbital myositis *occurs as a specific syndrome in dogs, affecting the extraocular muscles.* It affects primarily young dogs of large breeds, and is not associated with masticatory or generalized myositis. The histologic changes are identical to immune-mediated masticatory myositis, with multifocal random interstitial lymphocytic myositis, muscle fiber necrosis, and subsequent fibrosis. Antibodies against type 2M muscle fibers have been demonstrated in affected dogs.

Orbital inflammation most frequently results from *penetrating foreign bodies*, whether by direct penetration or particle migration from conjunctival sac or pharynx. Horses seem particularly prone. Aberrant localization by nematode parasites (*Dirofilaria immitis*, *Ancylostoma caninum*) or *Diptera* larvae is reported.

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OCULAR NEOPLASIA

Although the eye is the site of a wide range of primary and metastatic neoplasms, only a few are of sufficient prevalence or importance to justify discussion here. This is not to say that neoplasia is unimportant, and in fact exactly the opposite is true. *At least in dogs and cats, which are the species from which we are most likely to receive histologic specimens for assessment, neoplasia is second only to glaucoma as a cause for enucleation.*

Primary ocular tumors may arise from the eyelids and adnexa, from optic nerve, or from within the globe. Those arising within the globe may originate from any of the tissues, but only those from uveal melanoblasts and iridociliary neurectoderm are anything other than rare. *Most primary intraocular tumors have negligible potential for metastasis.* Dogs and cats are most frequently affected; primary intraocular neoplasms are inexplicably rare in other domestic species.

Metastatic ocular neoplasia is reported rather infrequently but it is common when sought. Multicentric lymphoma in cats, dogs, and cattle regularly involves the eye, although in cattle the retrobulbar tissue is preferred over the eye itself. *With the exception of malignant lymphoma, carcinomas are reported more frequently than sarcomas.* This probably reflects the greater metastatic potential of carcinomas in general rather than any specific difference in ocular tropism. Uveal vessels are the usual sites of lodgement, and ocular disease may result from vessel occlusion, or from inflammation in response to tumor antigen, or to necrosis of either tumor or damaged host tissue. *Hyphema is more common in eyes with tumor-induced uveitis than with uveitis of other causes and is therefore a diagnostically useful sign.*

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Eyelid and conjunctival neoplasms

Squamous cell carcinoma

Squamous cell carcinoma arises from the conjunctival epithelium of the limbus, third eyelid, or eyelid in cattle, horses, cats, and dogs, in that order of frequency. **Bovine** *ocular squamous cell carcinoma is one of the most common and most economically significant neoplasms of domestic animals.* Its relative rarity in dogs is peculiar and unexplained.

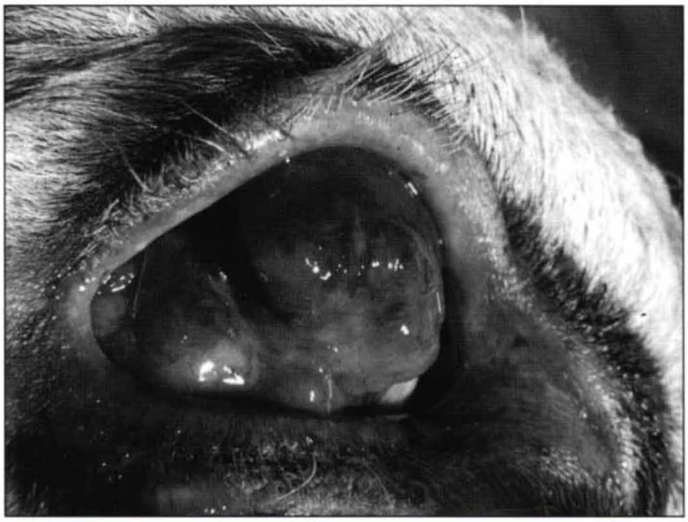
Figure 4.74 Squamous cell carcinoma in an ox.

The prevalence of the disease in all species is related to exposure to ultraviolet radiation and to lack of pigment in lids and conjunctiva. Its geographic prevalence is therefore directly correlated with altitude and inversely correlated with latitude, as well as with the prevalence of animals with poor periocular pigmentation. In cattle, in which this disease is of greatest significance, the prevalence is highest in the "white-faced" Hereford breed and in the high altitude regions of the Canadian and American West. It also occurs in other breeds of cattle, as well as Indian water buffalo, sheep, and cattalo (Fig. 4.74). Variation in prevalence in different lines of Herefords even in the same region has led to speculation that other genetic factors within the breed, other than facial pigmentation, may influence susceptibility. The question of etiology has been further widened by demonstration of papillomaviruses in some of the papillomatous precursor lesions that eventually transform into squamous cell carcinoma. Similar papillomaviruses, as well as being the causative agents of cutaneous warts, have been demonstrated in bovine alimentary papillomas in Scotland, and viral DNA persists in the squamous-cell carcinomas that arise from these papillomas in cattle grazing pastures that contain bracken fern. It remains to be determined whether or not there is any relationship between a viral component of the ocular carcinoma and the fact that in many cases the tumor regresses after immunotherapy. *At least at the moment, no viral particle or viral genome has been consistently demonstrated in ocular squamous cell carcinomas in any species.* Environmental co-carcinogens such as those in bracken fern have not yet been implicated in the induction of ocular tumors.

The tumor in all species develops through a series of premalignant stages, called epidermal plaques and papillomas, before proceeding over months or years to carcinoma in-situ and to invasive carcinoma (Fig. 4.75). Spontaneous regression of the precancerous lesions may occur with an estimated frequency of 25–50%. At least in cattle, plaques are much more common (about 6:1) than papillomas or outright carcinomas. The *epidermal plaque* is characterized by marked acanthosis, with variable presence of keratinization, dyskeratosis, and epidermal downgrowth into the subconjuctival connective tissue. Invasion through basal layer or basement membrane is not seen. *Papilloma* also involves acanthosis but, in addition, there is marked para- and hyperkeratosis with

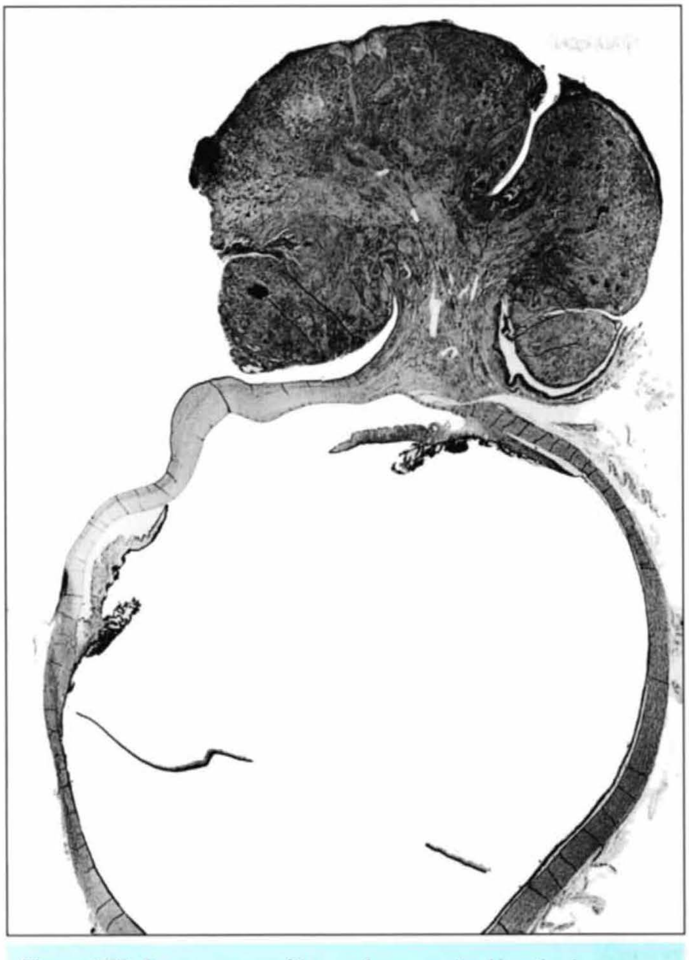

Figure 4.75 Squamous papilloma of corneoscleral junction in an ox.

papillary projections supported by a vascularized connective tissue core. Papillomas may be up to 3.0 cm in diameter, pedunculated or sessile, and are often ulcerated. *Carcinoma in situ* arises by focal or multifocal transformation of increasingly dysplastic cell nests in the deep layers of plaques or papillomas. *Fully developed carcinoma* has squamous cell invasion across the basement membrane. Tumor invasion is almost always accompanied by *intense lymphocytic-plasmacytic infiltration*, presumably the host response to tumor antigen. It is assumed that it is this response that is responsible for regression of some of the precursor lesions, although spontaneous regression of fully developed carcinoma is rare. Stimulation of immune-mediated rejection by intralesional inoculation of antigenic tumor extracts or nonspecific lymphocyte stimulants induces partial or total regression of small tumors.

Histologically, ocular squamous cell carcinoma resembles similar tumors in other sites and ranges from well-differentiated carcinomas with keratin pearl formation to anaplastic carcinomas with marked nuclear size variation and mononuclear tumor giant cells (Fig. 4.76A, B). *Metastatic or invasive potential has not been correlated with histologic criteria, but there is a correlation between site of origin and subsequent behavior.* Most surveys in cattle identify the bulbar conjunctiva of the limbus as the most frequent site of origin, estimated at about 70% of all occurrences. Some surveys consider nictitating membrane as next most frequent, with palpebral conjunctiva of the true eyelid as third. Other reports claim nictitans origin to be uncommon, and eyelid tumors to be as common as those of limbic origin. Tumors arise only very rarely from the cornea because of the limited mitotic capability of that tissue. Apparent corneal tumors are almost always extensions from tumors arising at the limbus. Tumors arising at the limbus are confronted by the dense and poorly vascularized connective tissue of sclera and peripheral cornea, which retards metastasis to extraocular sites. Invasion of corneal stroma and sclera occurs slowly, but intraocular invasion is very uncommon (Fig. 4.77). Tumors arising from the nictitans extend to the root of the membrane and then to the cartilage and bone of the orbit and internal nares. *Metastasis probably will eventually occur in all instances*, with parotid lymph node the initial site. Wide dissemination to thoracic and abdominal organs has been reported and is probably limited only by the limited longevity of the target animals.

Squamous cell carcinoma of the **equine** eye is much less thoroughly documented, but is quite common. In contrast to cattle, *the preferred site is the edge of the third eyelid, followed by limbic bulbar conjunctiva.* This targeting is, again, inversely correlated with the presence of protective melanin pigmentation, and is positively correlated with those factors causing increased exposure to ultraviolet radiation. In some reports, heavy draft horses are predisposed, but all breeds may be affected. The mean age of affected horses is about 9 years. Bilateral involvement is seen in 15–20%. The same range of precancerous lesions occurs in horses as in cattle. Prognosis is strongly influenced by therapy, but even the untreated neoplasm is slow to metastasize and even then it is usually only to local lymph nodes. Retrospective studies document 10–15% of equine ocular squamous cell carcinomas to have regional or distant spread, but the data do not consider duration of the disease prior to therapy.

In **cats,** *ocular squamous cell carcinoma most frequently affects the skin or palpebral conjunctiva of the eyelids.* White cats are particularly susceptible, and squamous cell carcinomas in these animals may occur simultaneously or sequentially on eyelids, ear pinnae, nose, and lips. The early lesion is one of sunlight-induced epithelial necrosis, and even the early neoplasm may be ulcerated and inflamed to a degree that may mask its neoplastic character and delay appropriate therapy. Growth tends to be circumferential around the lid margins, resulting in a palpebral fissure bordered by a thickened, red, and ulcerated tumor. Metastasis to local lymph nodes occurs late in the course of the disease.

In **dogs,** *squamous cell carcinoma infrequently involves the eye.* Proliferative eyelid or conjunctival growths in dogs are much more likely to be Meibomian adenomas, viral and nonviral papillomas, or nodular granulomatous episcleritis. In one study of 202 canine eyelid neoplasms, squamous cell carcinoma accounted for only 2% of lesions. Precancerous changes probably occur but, in contrast to cattle and horses, eyelid papillomas in dogs are usually benign and nonprogressive lesions.

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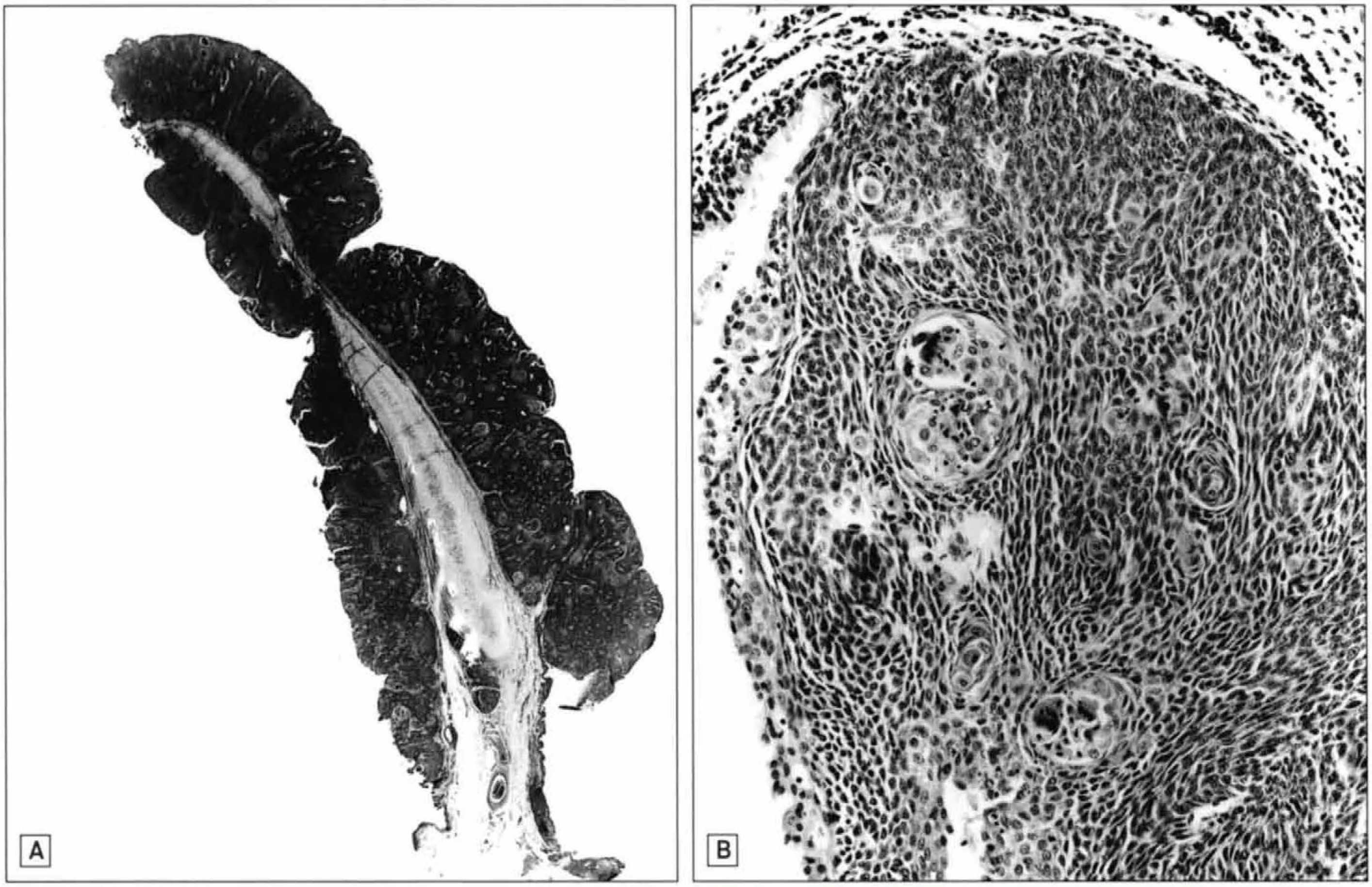

Figure 4.76 A. Early squamous cell carcinoma involving conjunctiva of nictitating membrane in a horse. **B.** Histology of **A**: disorderly and defective maturation, premature keratinization, and invasion across the basement membrane.

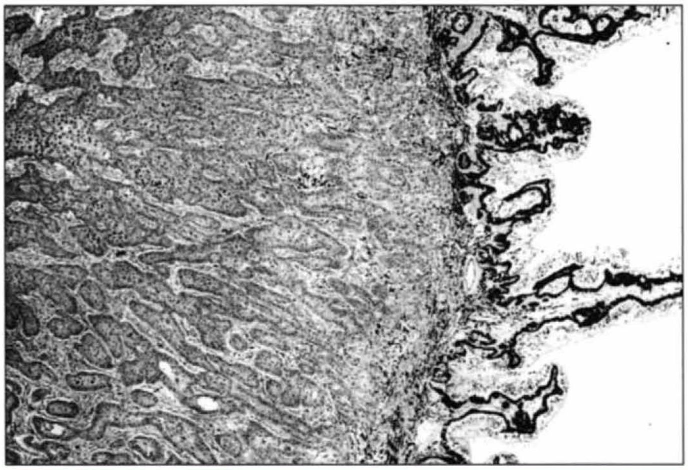

Figure 4.77 Scleral squamous cell carcinoma in a horse has grown inward to approach ciliary processes, an unusual behavior for these normally exophytic tumors.

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Meibomian adenoma

Meibomian adenoma is the most common ocular neoplasm of dogs, accounting for at least 70% of eyelid tumors. It is comparable in many respects to sebaceous adenomas found elsewhere in the skin. However, these tumors originate specifically from Meibomian gland and not from other eyelid sebaceous glands, and they regularly have at least some histologic features that are infrequently seen in their cutaneous sebaceous counterparts. In the eyelid tumors, *the lobules of foamy, eosinophilic sebaceous cells are intermingled with a prominent population of basal (reserve) cells.* The basal cells often account for 50% or more of the tumor cells. In many instances, the basal cell component is so prominent as to cause diagnosis to be made of basal cell tumor with sebaceous differentiation (comparable to cutaneous sebaceous epithelioma) or even Meibomian adenocarcinoma. Many have substantial melanin within the basal cells. None metastasize, and even the so-called carcinomas show little inclination for invasive growth. Other distinctive but not invariable features are papillary hyperplasia of the overlying epithelium of the eyelid margin, a marked lymphocytic-plasmacytic infiltrate among tumor lobules and around the tumor margins, and a localized granulomatous response (chalazion) to leaking secretion (Fig. 4.78A, B).

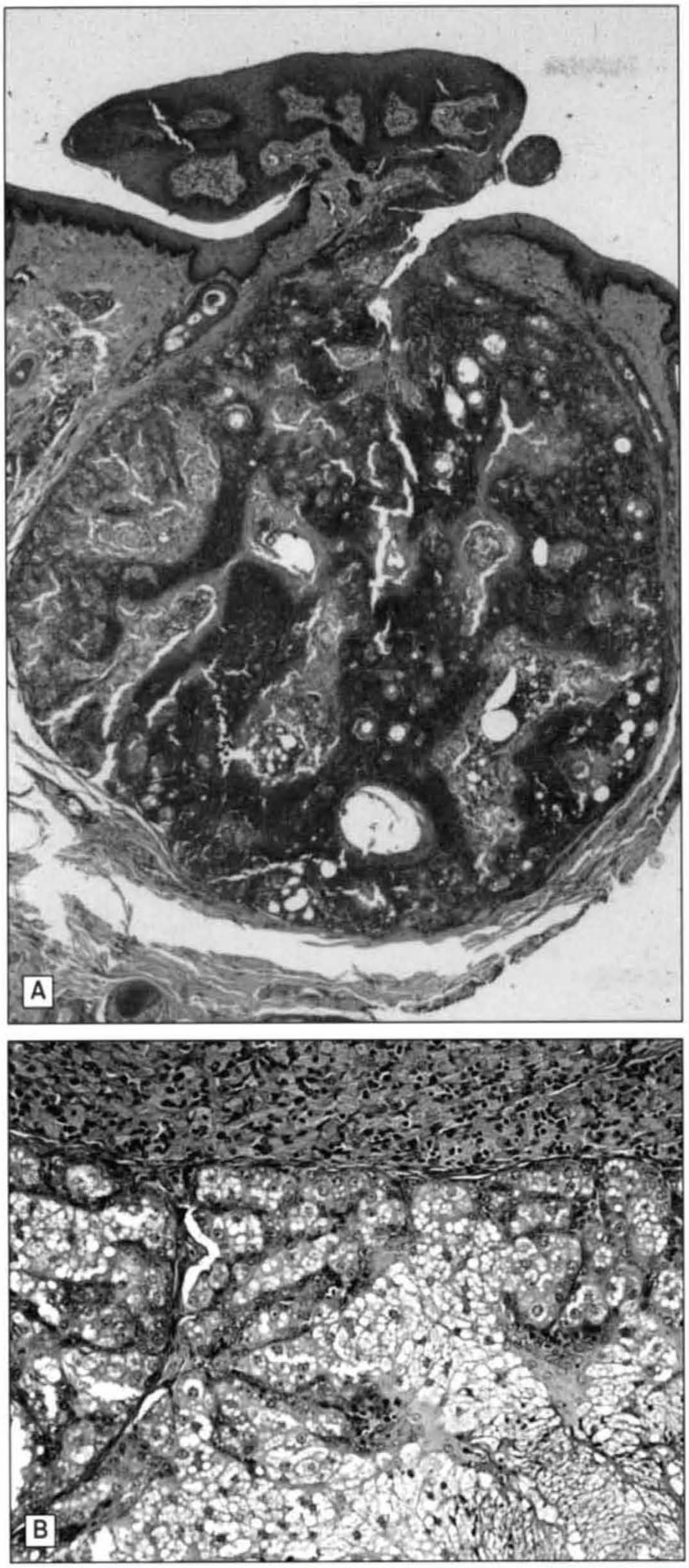

Figure 4.78 Meibomian adenoma. A. The relative proportions of germinal basal cells and mature sebaceous cells vary widely, but without any apparent prognostic significance. Papillary hyperplasia of the overlying eyelid marginal skin is frequently seen. **B.** Meibomian adenoma (below) with a surrounding zone of foamy macrophages (above) typical of chalazion.

Other adnexal and conjunctival tumors

A wide range of neoplasms has been reported to occasionally affect the conjunctiva or adnexa of domestic animals. Most examples are reported primarily in dogs, and they are listed below in order of overall prevalence in that species.

Melanocytoma *is probably the second most common tumor of the eyelid in dogs.* It will occasionally occur in cats and in gray horses. In dogs it is a typical cutaneous benign melanoma, identical to what occur so frequently anywhere else in skin. In cats, they are also similar to those tumors occurring elsewhere in skin, and more than half are both histologically and behaviorally malignant. It is important to note that the behavior of melanocytic tumors associated with the eye is greatly influenced by exact anatomic location, so these comments are applicable only to those melanomas arising in the haired skin of the eyelid.

Other melanomas arising from conjunctiva are described below in the section on Melanotic tumors of the eye. In brief, **limbal (epibulbar) melanocytoma** arises from the melanocytes that inhabit the stroma of the corneoscleral junction. It is benign. In contrast, **primary conjunctival melanoma** arises from the melanocytes of the palpebral or bulbar conjunctiva itself, and most examples are behaviorally aggressive.

Conjunctival papilloma usually arises from bulbar conjunctiva as a series of slender protruding stalks of conjunctival lamina propria covered by bland, often pigmented, stratified squamous epithelium with no cytologic atypia and no ballooning degeneration as is typical of viral papillomas.Viral papillomas associated with *Canine oral papillomavirus*, identical to those affecting the mouth, will occasionally occur in any portion of conjunctiva.

Conjunctival vascular tumors *represent a continuum from telangiectasia to hemangioma and hemangiosarcoma.* They arise with equal frequency in the temporal (lateral) bulbar conjunctiva and along the leading edge of the third eyelid, suggesting that sunlight is important in their pathogenesis.They usually arise in the very superficial subepithelial vascular plexus. *Surgical excision is invariably curative*, with virtually no metastatic risk regardless of cytologic and histologic criteria of malignancy. Some examples are associated with *marked dysplastic pseudocarcinomatous hyperplasia of the overlying conjunctival epithelium that divides the vascular tumor into lobules*, and some use the term **angiokeratoma** to describe this variant. It behaves as do the other conjunctival vascular tumors. Behaviorally malignant examples seem more prevalent in horses, where more than half of the reported cases are solid hemangiosarcomas with very aggressive local infiltration and, usually, distant metastasis. They are easily mistaken for fibrosarcomas because they have such poor channel formation and so little blood. Indeed, some of these might arise from lymphatic endothelium.There is much speculation, but no proof, that sunlight is important in their causation.

Nodular granulomatous episcleritis (also known as ocular nodular fasciitis, ocular fibrous histiocytoma) *is not a neoplasm*, but its clinical appearance is easily confused with neoplasia and samples will be submitted as probable neoplasms. It is more fully discussed under Diseases of the sclera.

Mast cell tumors, with the same histologic range as those rising elsewhere in skin, may occur in the conjunctiva of dogs. Their biological behavior has not been documented. In cats, mast cell tumors more commonly arise within the haired skin of the eyelid.

Adenocarcinoma of the gland of the third eyelid occurs as a nodular swelling in very old dogs (mean age 11.5 years). They occur, albeit very rarely, in cats. *They are locally infiltrative, recur after attempted resection, but are cured by complete removal of the third eyelid.* Only chronically neglected cases metastasize to lung after a very protracted local expansion. Histologically, these are tubular carcinomas with abundant squamous metaplasia. They should not be confused with the prominence of the gland that occurs with prolapse of the gland ("cherry eye") or with lymphocytic interstitial adenitis.

Lymphoma may have several ocular manifestations, the most frequent of which are diffuse uveal metastases as part of generalized lymphoma in dogs or cats, or as retrobulbar tumor in cattle. It may occasionally occur as a conjunctival disease as part of generalized lymphoma or as a mucocutaneous manifestation of epitheliotropic lymphoma. There are several reports of conjunctival or third eyelid lymphoma occurring in horses and in cats as an apparently isolated lesion that can be cured by local excision, suggesting that they have actually arisen in those locations.

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Melanotic tumors of the eye

Our understanding of the biology of primary ocular melanomas in animals has suffered greatly from the premature assumption that they were similar to the much-studied human ocular melanomas. Perhaps because of this error, a great many enucleated globes were available for histologic evaluation, inadvertently allowing the flurry of retrospective studies that eventually established ocular melanomas in dogs and cats as distinct entities quite different in structure and behavior from the human neoplasms.

Primary melanocytic tumors of the eye or adnexa are common in dogs and cats, rare in horses, and almost nonexistent in other domestic species. Even in dogs and cats, the prevalence and behavior of the various types of ocular melanomas and melanocytomas differ markedly, so that generalizations must be studiously avoided.

In **dogs**, this list of distinct clinical/histologic entities includes benign eyelid melanocytoma, malignant conjunctival melanoma, limbal melanocytoma, anterior uveal melanocytoma, and choroidal melanocytoma. In **cats**, the list is the same but it is dominated by diffuse iris melanoma. Malignant cutaneous melanomas occasionally affect the eyelid or arise within the conjunctiva, and there are a few reports of limbal and anterior uveal melanocytomas similar to those occurring in dogs. In **horses**, one encounters cutaneous melanocytomas in the skin of the eyelids, primarily in gray horses. There are a few reports of benign anterior uveal melanocytomas, and one report of a locally invasive, histologically malignant conjunctival melanoma.

Melanocytomas and malignant melanomas arising within the haired skin of canine and feline eyelid, respectively, are identical to those arising elsewhere in the skin and they will not be further discussed here.

Melanomas arising in the conjunctiva are very infrequent compared to most other ocular melanocytic tumors. The few case reports do not allow for generalizations about behavior, but *they often are histologically and behaviorally malignant.* Their relationship to eyelid melanoma seems analogous to those of the lip, where melanomas of the haired exterior lip are benign like most skin melanomas, while those of the mucous membrane of the inside of the lip are malignant like those elsewhere in the mouth. Conjunctival melanomas may appear as well-pigmented tumors of bland, plump melanocytes with little anisokaryosis or mitotic activity, or as cytologically malignant tumors with marked anisocytosis, anisokaryosis, hyperchromasia, and even multinucleation. Primary conjunctival melanomas often are poorly melanotic. The most reliable histologic criterion in such cases is the observation of intraepithelial nests of tumor cells. Local recurrence and spread after excision is frequent, and metastasis to lung has been reported.

Limbal (epibulbar) melanocytoma *is a histologically and behaviorally benign tumor* of the melanocytes normally found in an oblique line that demarcates the junction of corneal stroma with sclera at the limbus. The tumor is composed of large plump melanocytes with a central nucleus and abundant cytoplasmic pigment. Mitotic figures are absent and nuclear variation is minimal. The tumor grows outwardly as a protruding spherical nodule, hence the alternative name of *epibulbar melanoma.* There may be nodular expansion into peripheral cornea, but virtually never into the uvea or anterior chamber. *Except for location, these tumors are identical to anterior uveal melanocytomas.* This creates a problem when one encounters the tumor that occupies both the anterior uvea and the limbus. These should be assumed to be anterior uveal melanocytomas extending outwardly, since it appears that primary limbal melanocytomas have essentially no ability to invade the globe.

Anterior uveal melanocytoma *is the most frequent intraocular tumor in dogs.* It is topographically, histologically, and behaviorally unrelated to human epithelioid ocular melanomas to which it was long compared. The typical tumor arises from melanocytes of the iris root or adjacent ciliary body, and is composed of variable proportions of lightly pigmented spindle cells and heavily pigmented plump melanocytes identical to those of limbal melanomas. The spindle cells are assumed to be the proliferative population, and the plump cells probably represent the mature, end-stage melanocytes with a storehouse of cytoplasmic pigment (Fig. 4.79A, B, C).

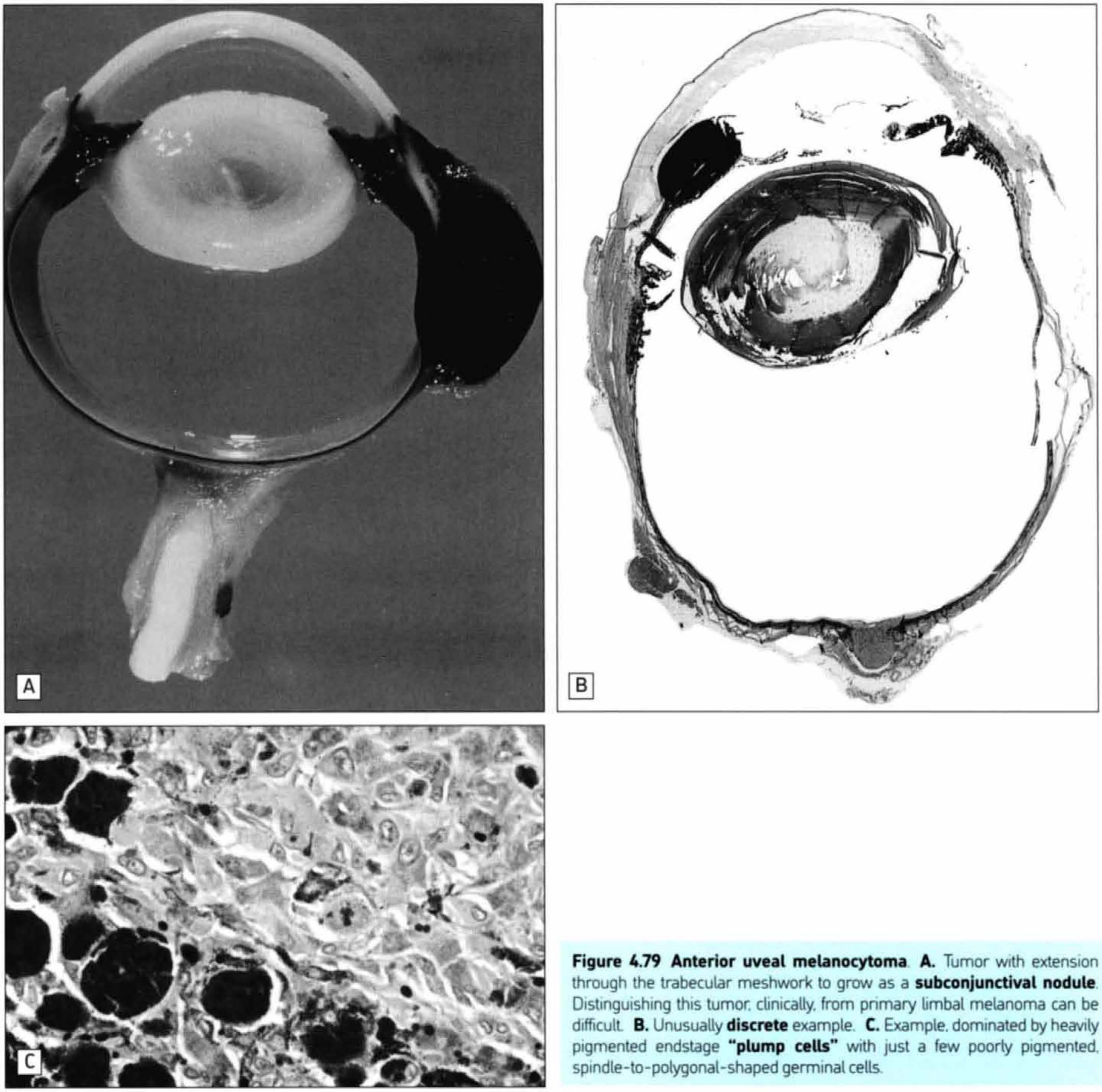

Figure 4.79 Anterior uveal melanocytoma. A. Tumor with extension through the trabecular meshwork to grow as a **subconjunctival nodule**. Distinguishing this tumor, clinically, from primary limbal melanoma can be difficult. **B.** Unusually **discrete** example. **C.** Example, dominated by heavily pigmented endstage **"plump cells"** with just a few poorly pigmented, spindle-to-polygonal-shaped germinal cells.

The diagnosis itself presents no problem, but offering an accurate prognosis is more complex. About 15% of all canine anterior uveal melanocytomas are histologically malignant, and one-third of these (or about 5% of all uveal melanocytomas) have been confirmed to be behaviorally malignant by virtue of extraocular metastases. *This small group of genuine malignancies can be predicted by mitotic index.* Histologically malignant tumors are dominated by the spindle cells rather than the plump cells, are more lightly pigmented, have much more anisokaryosis and more mitotic figures than the benign tumors. Of these, mitotic index is the most reliable predictor of behavior. Benign tumors have virtually no mitotic figures. Those confirmed as behaviorally malignant have three or more (usually many more!) mitoses in ten high-power (40×) microscopic fields; conversely, not all tumors with high mitotic index are destined for metastasis.

Even these benign melanocytomas are eventually significant to the eye, spreading transsclerally and circumferentially within the globe. *Glaucoma* from occlusion of ciliary cleft is probably the eventual fate of all eyes with this neoplasm. *Uveitis* from tumor necrosis or *hyphema* from tumor-induced uveal neovascularization are other frequent accompaniments.

Feline multifocal uveal melanocytoma *is a rare tumor of cats* that looks histologically identical to the canine uveal melanocytomas. They are sufficiently different from the much more common diffuse iris melanoma of cats to justify a separate classification. There is no published information about metastatic risk. They seem to arise as multiple foci randomly throughout the uveal tract, expanding inwardly to create multiple nodules within the ocular cavities, and sometimes outwardly as space-occupying scleral nodules.

Equine anterior uveal melancytomas occur most often in gray horses, many quite young (less than 8 years). Most involve anterior uvea and are histologically similar to the benign uveal melanocytomas of dogs. None has metastasized. Although almost all intraocular melanocytic tumors of cats are diffuse iris melanomas, one will occasionally encounter focal or **multifocal melanocytomas** similar to those of dogs. Other than having a more unpredictable site of origin within the uveal tract, they seem histologically and behaviorally identical to their more common canine counterparts.

Choroidal melanocytomas account for about 80% of all human ocular melanomas, but are rare in other species. The few cases described in dogs were discovered as incidental findings on fundoscopic examination, and grew very slowly. They seem very similar to the benign melanocytomas of limbus or anterior uvea: well pigmented, cytologically bland, and cause clinical signs only by their slow expansion to cause retinal detachment or compression of optic nerve. There is a single report of systemic metastasis from a tumor that looked histologically benign.

Clinically insignificant **iris nevi** or **freckles** occur in dogs as nonprogressive pigmented spots. Their only significance is to cause unnecessary enucleation. Histologically, the lesions are well-circumscribed clusters of bland melanocytes adjacent to the anterior border layer of the iris.

Feline diffuse iris melanomas are unique. The usual clinical presentation is of patchy iris hyperpigmentation that very slowly progresses to diffuse iris hyperpigmentation and thickening over several years. *The eventual outcome is virtually always glaucoma* (Fig. 4.80A).

It was at first considered an interesting but benign lesion. Subsequent retrospective studies have been unanimous in documenting a *moderate metastatic risk*. Because cats seem even more elusive than dogs in terms of follow-up studies, the actual number of cases with good postoperative data is small, and considerable debate persists among ophthalmologists about the real risk of metastasis by these tumors. It seems clear that the probability that a cat will die from metastatic disease is low, but the prevalence of clinically silent visceral metastasis is quite high. This contrast is probably related to the very slow growth rate of the metastatic tumor foci, which is exactly what happens within the globe itself.

Histologically, these tumors diffusely infiltrate the stroma of the iris and the ciliary cleft, and then the overlying sclera, peripheral cornea, and ciliary body. They are notoriously pleomorphic, and are apt to be misdiagnosed by those pathologists not aware of this disease as malignant lymphoma or anaplastic metastatic malignancy. Tumor cells vary from spindle-shaped cells to multinucleated epithelioid cells (Fig. 4.80B, C). Pigmentation often is light and the cytoplasm may be foamy and eosinophilic. Balloon cells with foamy cytoplasm and very distinct cell boundaries are frequent in some tumors. The accurate prediction of tumor behavior is compromised, in all published studies, by the low percentage of affected cats available for follow-up. Metastasis has been correlated with large tumor size, intrascleral spread, and mitotic index.

It remains unclear whether cats also can have focal-to-coalescing hyperpigmentation of the anterior iris stroma that is not precancerous. Some cases, as in dogs, seem nonprogressive and harmless (although follow-up information is anecdotal). Others slowly coalesce and thicken, at which time they are indistinguishable from diffuse iris melanoma. When sampled for histology, virtually all such lesions look like early melanomas, but of course, most of these apparently harmless, nonprogressive lesions do not receive histologic evaluation!

Pigmentary (melanocytic) glaucoma *is included here because it is histologically indistinguishable from a diffuse uveal melanocytoma.* The syndrome was originally described as a bilateral but asymmetrical massive pigmentation of the uveal tract of Cairn Terrier dogs that seemed to be causing glaucoma. Histologically, the anterior uvea and even the choroid are thickened by a heavy, diffuse accumulation of large plump cells histologically indistinguishable from those of ordinary canine anterior uveal melanocytoma. The syndrome has been described in a few other breeds in association with glaucoma, but the syndrome remains controversial because one can encounter equally heavy pigmentation in clinically normal globes from breeds that typically have very heavy pigmentation, such as Kerry Blue Terriers, Scottish Terriers, black Labradors, and others. *The causal relationship between the pigmentation and glaucoma therefore remains somewhat controversial.* Some would prefer to refer to the disease as "canine diffuse uveal melanosis," or even as diffuse uveal melanocytoma. Original claims that these accumulating cells are abnormal melanocytes have been challenged by recent electron microscopy indicating that the majority of the accumulating cells are melanin-laden macrophages. That is perhaps not surprising and it does not rule out the possibility that this is indeed a melanocytic proliferative disorder that, over time, has had a lot of leakage of melanin and thus a lot of macrophage recruitment.

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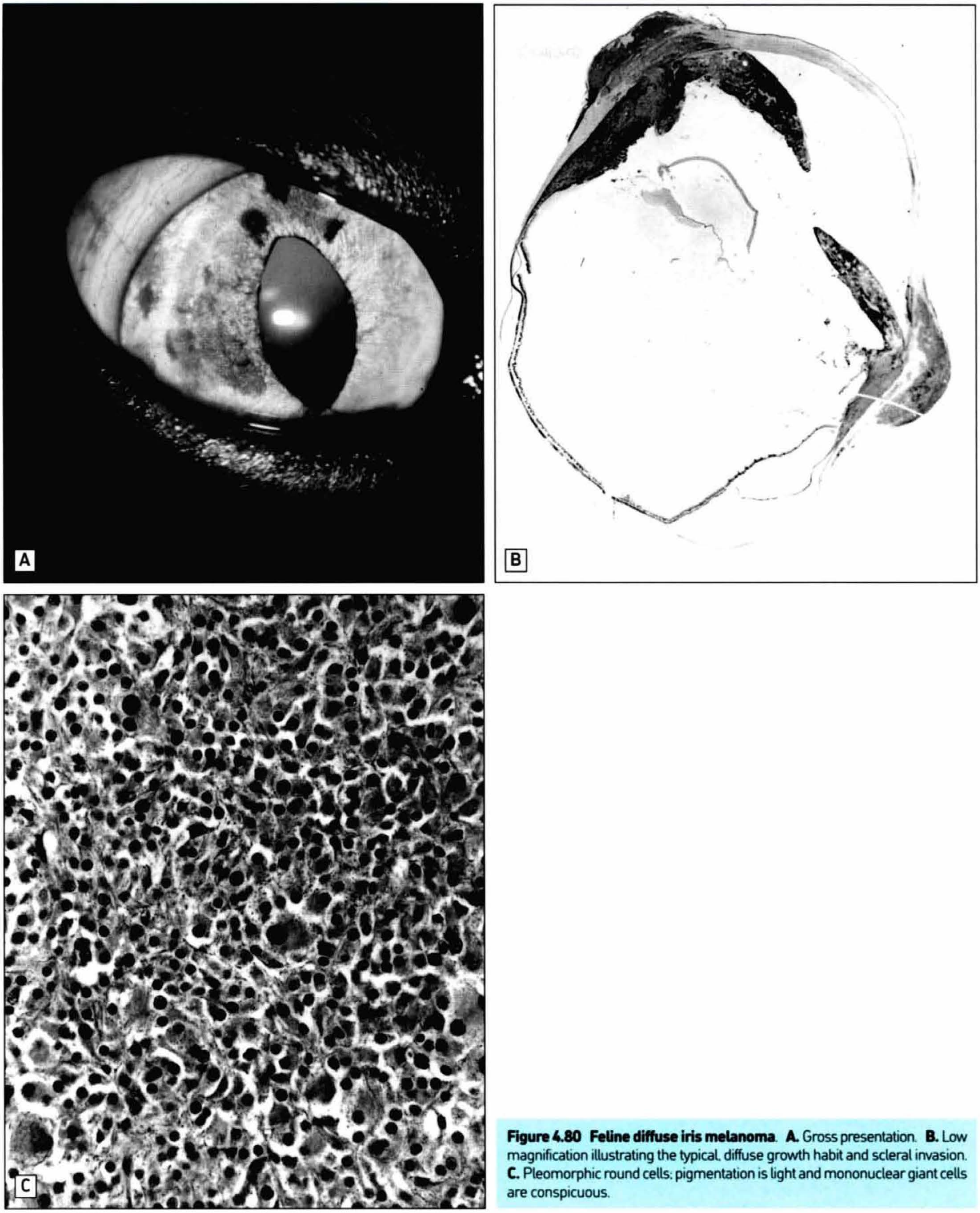

Figure 4.80 Feline diffuse iris melanoma. **A.** Gross presentation. **B.** Low magnification illustrating the typical, diffuse growth habit and scleral invasion. **C.** Pleomorphic round cells; pigmentation is light and mononuclear giant cells are conspicuous.

Roswitha ROM, et al. Abnormal ocular pigmented deposition and glaucoma in the dog. Vet Ophthalmol 2003;6:273–278.
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Tumors of ocular neurectoderm

These tumors include iridociliary epithelial tumors from mature anterior uveal neurectoderm, and medulloepithelioma and retinoblastoma from embryonic neurectoderm. The prevalence of these neoplasms is second to anterior uveal melanomas and melanocytomas, although it is perhaps underestimated because the most common examples are small, slowly expansive tumors within the posterior chamber that are unlikely to cause clinical signs. They are relatively common in dogs, uncommon in cats, and virtually unknown in other species except for medulloepitheliomas in horses.

Iridociliary epithelial tumor (iridociliary adenoma, iridociliary carcinoma) is the most common of this group. Most examples are well-differentiated papillary or tubular adenomas arising from the nonpigmented inner layer of ciliary or iris epithelium (Fig. 4.81). There are certainly examples in which the histologic and cytologic character is more primitive, but metastasis is so rare (if indeed it exists at all) that there is *no justification for diagnosing any of these tumors as iridociliary carcinomas.* Most originate from the pars plicata, but occasionally the histologic evidence points to origin from posterior iris epithelium. The tumor cells usually resemble mature ciliary epithelium and usually have very little associated stroma. Nuclei are basilar, regular, and are surrounded by eosinophilic cytoplasm (Fig. 4.82A). The tumor cells are not pigmented, although melanophages are occasionally seen within tumor stroma. *They make an abundance of basal lamina* oriented, as in normal ciliary epithelium, toward the inside of the eye. Its abundance, easily seen with periodic acid-Schiff reagent, is useful in distinguishing ciliary tumors from carcinomas metastatic to the eye. Examples that have little tubular or papillary organization, or a more primitive cytologic character, may be difficult to recognize as being of iridociliary epithelial origin. They almost always retain abundant basement membrane production that can be accentuated with PAS staining. *The cells also stain for vimentin, S-100, and neuron-specific enolase, which makes them unique among tumors that otherwise look epithelial.* Such additional staining may occasionally be necessary to distinguish primitive iridociliary epithelial tumors from metastatic carcinomas.

Even small iridociliary epithelial tumors may cause hyphema or glaucoma, attributed to this tumor's strong propensity to induce pre-iridal fibrovascular membranes (Fig. 4.82B). This is, presumably, the result of absorption through the porous anterior border layer of the iris of fibrovascular growth factors produced by the tumor cells in an effort to ensure their own survival. Iridociliary tumors are more likely to induce such neovascularization than is any other ocular disease.

Medulloepitheliomas and retinoblastomas *arise from the primitive neurectoderm of the optic cup.* Retinoblastoma is the second most frequent neoplasm of children, yet a critical review of the veterinary literature reveals only a single acceptable diagnosis of this tumor. Conversely, *medulloepitheliomas* are rare in children but many examples have been observed in animals, mainly in the *horse* in which these are probably the *most common primary intraocular tumor.* The neoplasm may originate from any portion of embryonic neurectoderm and may show differentiation into any neurectodermal derivative, i.e., retina, ciliary epithelium, vitreous, or neuroglia (Fig. 4.83A, B). The typical neoplasm is a loose network of branching cords of small basophilic neuroblasts resembling those of embryonic retina. Mitotic figures are numerous. The cords have definite polarity; they rest upon a basement membrane analogous to the inner limiting membrane of retina and some have adjoining apical terminal bars analogous to outer limiting membrane. A typical feature is the clustering of neuroblasts around an empty central lumen defined by the terminal bars, creating a true rosette (Fig. 4.84). The basilar portion of the rosette or cord faces a hyaluronic acid-rich myxoid matrix analogous to the vitreous. This histologic feature may be found in only a few foci within a huge mass that is otherwise composed of poorly differentiated, neuroblast-like cells. Many tumors also contain foci of cartilage, skeletal muscle, or brain tissue and are classified as *teratoid medulloepitheliomas.* Metastases are not recorded, despite many of these being classified histologically as "malignant."

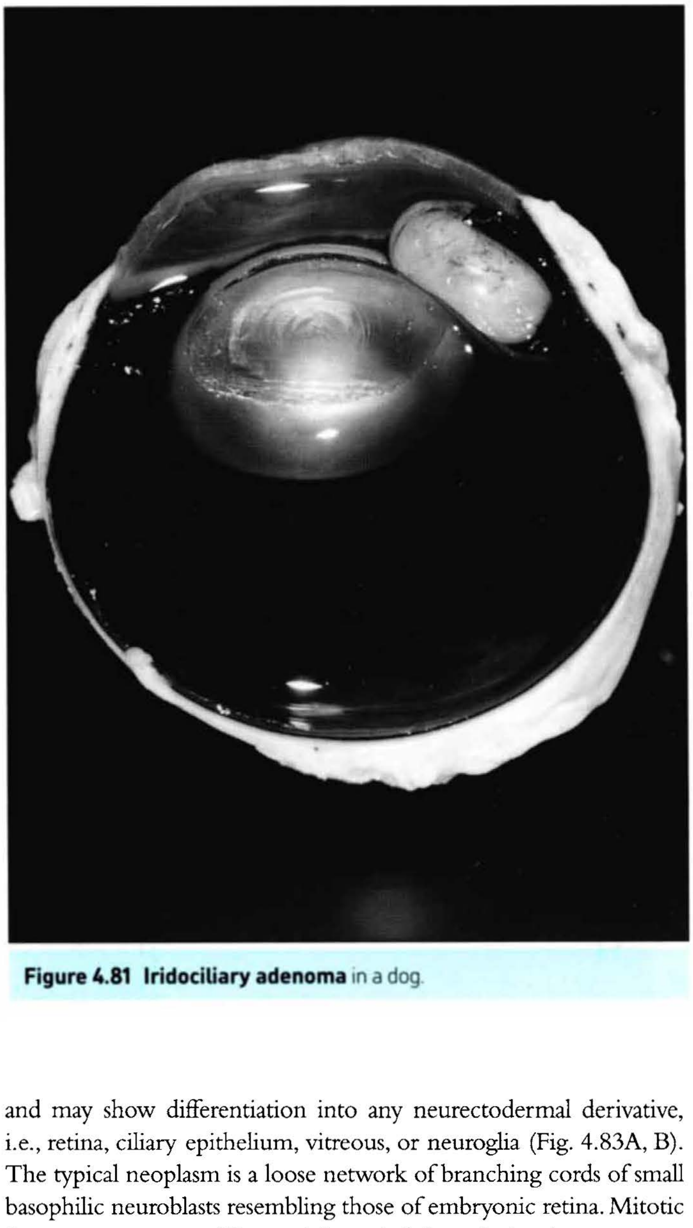

Figure 4.81 Iridociliary adenoma in a dog.

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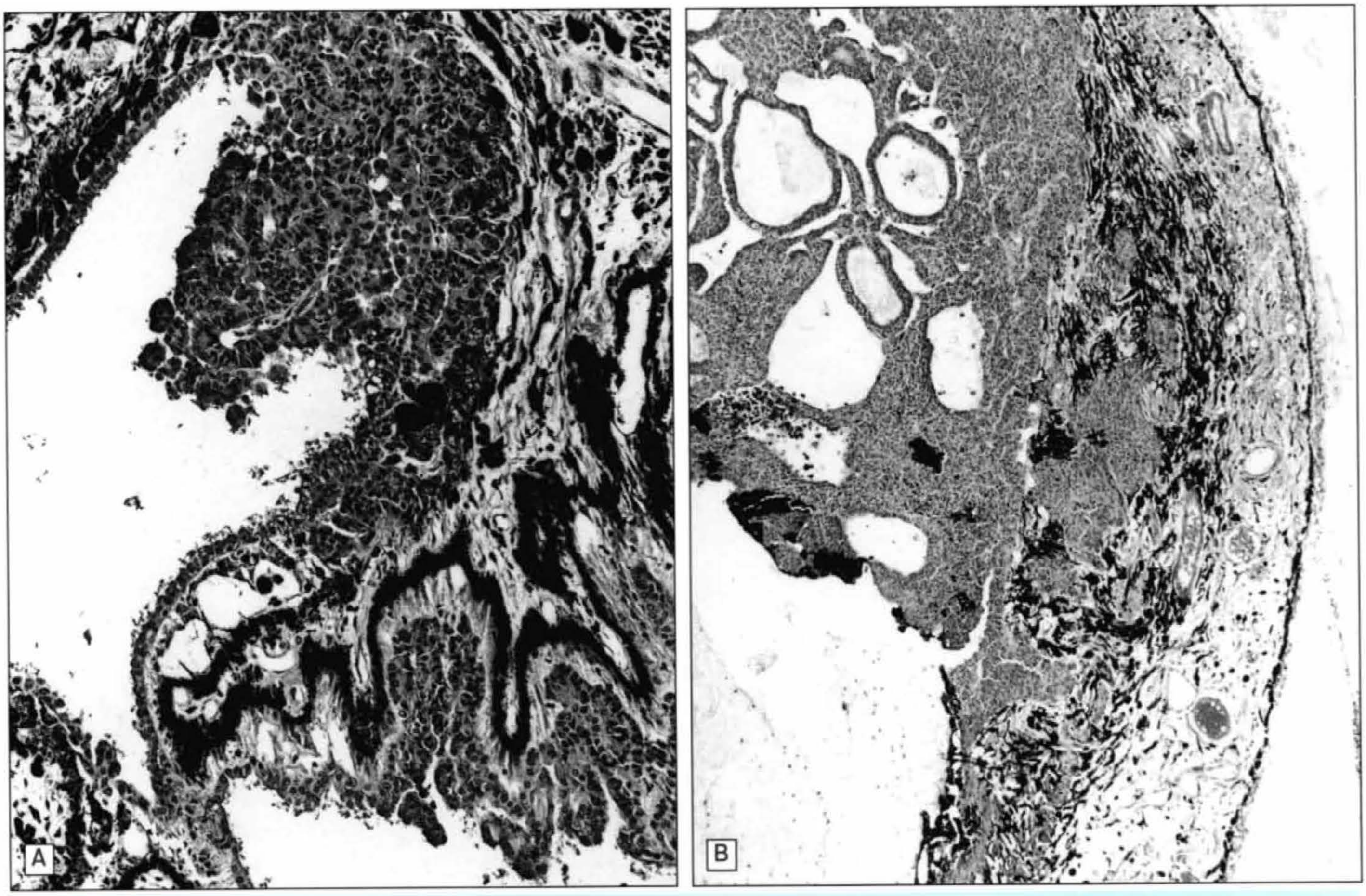

Figure 4.82 Iridociliary adenoma in a dog. **A.** Note well-differentiated tubular proliferation. **B.** Note the presence of tumor within the posterior iris stroma as well as in the posterior chamber, and the preiridal fibrovascular membrane.

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Feline post-traumatic sarcoma

This syndrome seems unique to cats. As the name implies, eyes subjected to *trauma, especially penetrating injury*, are prone to develop pleomorphic spindle-cell sarcomas that destroy the globe and have substantial risk of metastasis. The interval between injury and observed tumor varies from 5 months to 11 years. Those skeptical about claiming such neoplasia to be the result of an injury 10 years previously prefer to call these tumors "primary ocular sarcomas," although such lag times are common in experimental models of carcinogenesis. The risk for injured eyes to develop sarcoma is unknown. Almost all recorded cases have perforated lenses. Most of these tumors appear to be fibrosarcomas, but some have a mixed epithelial–mesenchymal phenotype, and elaborate basement membrane-type matrix as well as express vimentin strongly. Based on immunopositivity for collagen type IV and crystallin alpha A, at least some of these tumors are of lens epithelial origin. Of relevance to ocular surgeons is the development of sarcomas in cat eyes receiving prosthetic lens implants, presumably viewed by the eye as just another form of unwanted lenticular trauma.

The tumor itself varies from fibrosarcoma to osteosarcoma to giant cell tumor, varying even within the same eye. The tumor tends to first surround the lens, then to line the inside of the eye, and finally to extend via scleral venous plexus or optic nerve to involve the orbit (Fig. 4.85). *The inclination to "line the globe" is a repeatable feature that is useful in distinguishing primary ocular sarcoma from rare metastatic sarcomas.* Most cases are presented with advanced disease, and follow-up data to document the prevalence of metastasis are scant. Available evidence documents a metastatic risk of at least 60%, and in many cases there is remarkably rapid development of post-enucleation neurologic signs attributable to invasion of brain via the optic foramen.

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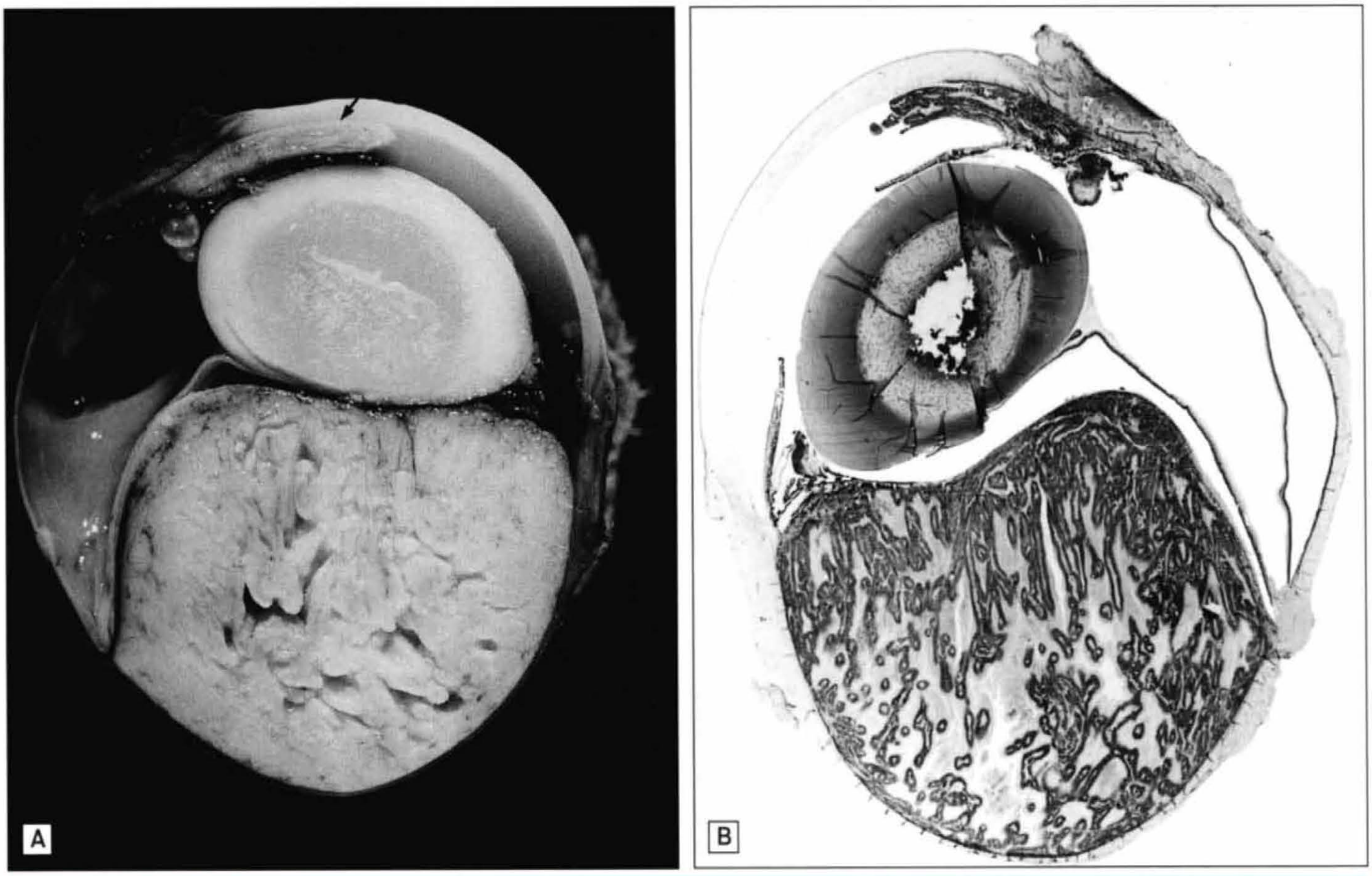

Figure 4.83 Medulloepithelioma in a dog. **A.** The main tumor lies between choroid and retinal pigment epithelium. There is also tumor in anterior chamber (arrow). **B.** Histologic section of (**A**). Note tubular latticework of the tumor.

Optic nerve tumors

While the optic nerve and adjacent retina can presumably develop all of the neoplasms of the central nervous system (excepting those from tissues such as ependyma that are not present in the eye), documented examples are few indeed. Most are reported as individual case reports prior to the era of immunohistochemical markers that would have permitted more precise classification.

Optic nerve meningioma *has a distinctive macroscopic and histologic appearance.* It has been described only in dogs, although it will probably be seen in other species as we begin to look. The tumor probably arises from meningial rests of arachnoid cells that project through the dura mater of the optic nerve into the orbital connective tissue. Tumors arising from these cells create a conical soft tissue mass that surrounds the optic nerve, but usually remains within the cone created by the extraocular muscles. Limited extension into the choroid or posterior vitreous through the optic nerve occurs only occasionally; more often, there is infiltration into the muscle and fat of the orbit. Most often, *the histologic appearance is of large stellate mesenchymal cells that can be confused with epithelial cells.* They have abundant glassy eosinophilic cytoplasm, and may or may not form the *characteristic swirling pattern* typical of central nervous system meningiomas in general (Fig. 4.86). Myxoid, chondroid, or even osseous metaplasia is quite common. Metastasis appears to be extremely infrequent.

Tumors published as **optic nerve astrocytomas** or as less specific "gliomas" were, in retrospect, *more likely to be examples of proliferative optic neuropathy* with the accumulation of reactive astrocytes and macrophages.

Primary orbital neoplasms

Tumors may be primary within the orbit, or arise by extension from adjacent structures or by hematogenous localization. They usually produce deviation or protrusion of the globe with secondary desiccation keratitis. Only those arising as primary tumors within the orbit are considered here. After saying that, *it is not always easy to decide whether the tumor is indeed primary or not,* and the decision is often made only after failure to find any credible primary tumor elsewhere. Of the primary orbital tumors reported in dogs and in horses, *sarcomas are much more prevalent than epithelial tumors.* The sarcomas are a bewildering array of locally infiltrative spindle cell tumors of unknown origin, with the abundance of diagnoses probably reflecting the diversity of pathologists' opinions rather than actual proof of histologic identity. Metastasis is rare but their infiltrative growth habit in this difficult site makes eventual elective euthanasia a frequent outcome.

Among the primary sarcomas, the only ones deserving specific consideration because of a distinctive histologic appearance are

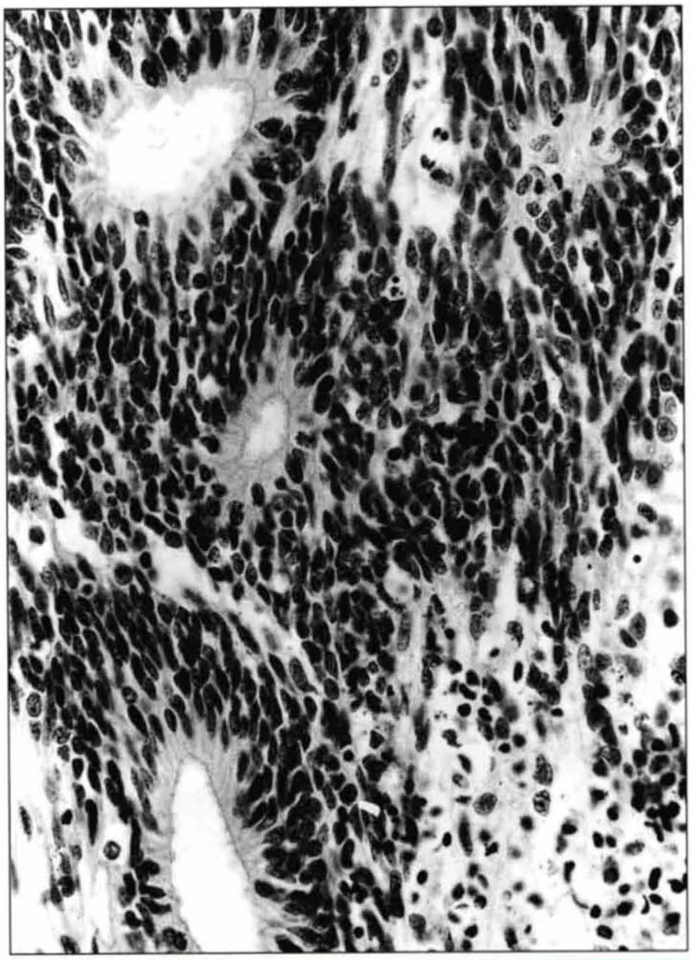

Figure 4.84 Medulloepithelioma in a dog. Note rosette with rudimentary formation of retinal outer limiting membrane and photoreceptors.

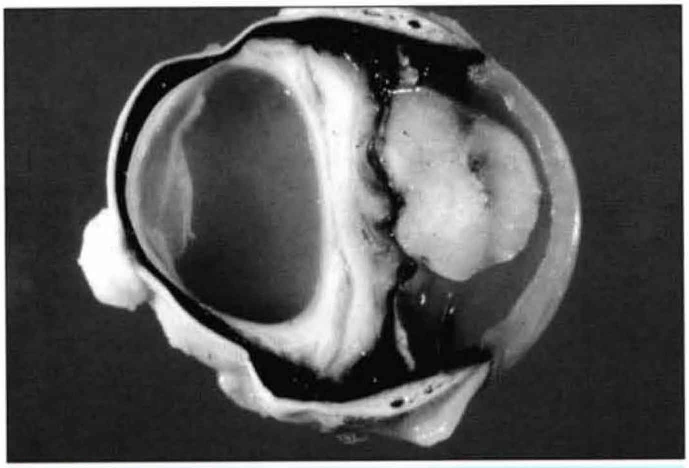

Figure 4.85 Primary feline ocular sarcoma arising from the lens and growing to "line" the posterior half of the globe.

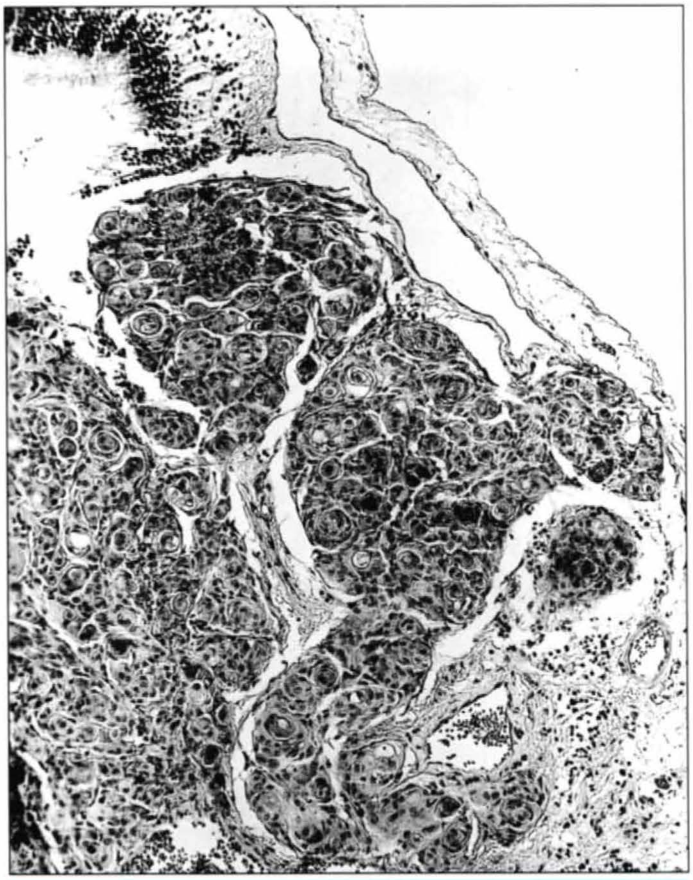

Figure 4.86 Meningioma of optic nerve invading eye in a dog.

multilobular osteochondrosarcomas and *optic nerve meningiomas*. The latter were described in the preceding section. The **multilobular osteochondrosarcomas** arise from the aponeuroses between adjacent bones of the orbit, and are identical in appearance and behavior to this tumor arising elsewhere in the canine skull.

Most primary epithelial tumors of the canine orbit are **lacrimal adenocarcinomas**, *which are locally invasive, recur after attempted resection, but which apparently have little metastatic potential.* In truth, there is so little follow-up information about these uncommon tumors that any statement about metastatic risk is premature. Both adenoma and nodular hyperplasia occur, but seem much less frequent than the malignant tumors. Tumors infiltrating the orbit from the nearby *zygomatic salivary gland* are similar histologically and behaviorally to lacrimal adenocarcinomas. They can sometimes be distinguished on the basis of location alone, but it is also helpful that the zygomatic gland is a mixed salivary gland with a prominent mucinous component that may be retained in well-differentiated tumors. The lacrimal gland is purely serous.

Primitive neuroepithelial tumors of unknown origin are occasionally seen in young dogs and in horses. They are composed of nests, cords, and rosette-like structures formed by small hyperchromatic neuroblastic cells with a very high mitotic index. Very rapid spread throughout the orbit and into brain occurs in affected dogs, but the extremely sparse information on these tumors in horses (four cases in two reports) suggest that they may be much less aggressive in horses than in dogs.

Tumors metastatic within the globe and orbit

The list of tumors that may be found as metastatic localizations within the globe or orbit is the same as any list of metastatic neoplasms in general. Any estimates of prevalence are unreliable because the globe is not routinely investigated in animals dying from disseminated malignancy. *Most of the reported cases are carcinomas*, simply because carcinomas are more likely to undergo hematogenous dissemination than are sarcomas (with the round cell "lymphoreticular" malignancies being a notable exception). In dogs, the most common are probably transitional cell carcinomas and mammary carcinomas, whereas in cats mammary adenocarcinoma and bronchial adenocarcinomas are probably the most prevalent. Across all species, however, *malignant lymphoma* is undoubtedly at the top of the list both as an intraocular and as an orbital metastatic tumor.

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Ear

GENERAL CONSIDERATIONS

Diseases of the ear of domestic animals are of limited interest to most veterinary pathologists. Of the three broad categories – *defects of hearing, otitis media, and otitis externa* – only otitis media in farm animals regularly receives some attention, but even that is usually restricted to macroscopic examination at necropsy. Otitis externa is almost exclusively the realm of the clinical practitioner and microbiologist, although hyperplasia of chronically inflamed epithelium within the external ear canal may simulate neoplasia and be submitted for histologic interpretation. Disorders of hearing certainly occur in domestic animals, but their prominence in the medical literature is the result more of their usefulness as models for human deafness than of their intrinsic importance in animals. The growing availability of equipment for evaluation of hearing may, however, see a resurgence of interest in deafness in specific breeds or colors of dogs and cats. The tedious task of preparing adequate sections of the cochlea and labyrinth when related to the relative importance or prevalence of the diseases makes examination of the inner ear a rare event in most veterinary institutions. The gross and microscopic anatomy of the ear in the various species is reviewed in specialized texts and the appropriate references are included in the bibliography.

EXTERNAL EAR

Diseases of the external ear may be a local manifestation of generalized skin disease or may specifically affect the ear because of some anatomic or physiologic peculiarity. Disorders of skin that only incidentally affect the ear are discussed in Vol. 1, Skin and appendages.

Ear tip necrosis *may result from frostbite, cold-agglutinin disease, ergot poisoning, thrombosis during the course of septicemia, or from trauma inflicted by the animal itself or by cannibalistic herdmates.* Particularly in pigs, the prevalence of atrophic and misshapen ears may be quite high in individual herds. This usually results from septicemia, sarcoptic mange, or cannibalism. In some instances, however, no such history can be elicited, and a syndrome of infectious ear necrosis has been proposed. Culture of ears showing marginal lesions results in isolation of a variety of bacterial agents, and claims for a primary bacterial etiology are not convincing. The one that comes closest is the spirochete *Borrelia suilla*. It usually acts as an opportunistic contaminant of abraded skin in pigs, giving rise to localized (usually ear margins) or generalized ulcerative dermatitis.

Auricular hematoma *occurs as a consequence of trauma, usually from excessive head shaking by dogs with otitis externa.* Dogs and pigs with *pendulous ears* are particularly prone to hematoma formation, but cats are occasionally affected as well. Hematomas usually develop on the concave side of the pinna and are initially fluctuant but become firm as the hematoma (or blood-contaminated seroma) organizes. As it is converted to granulation tissue by fibroblastic and capillary ingrowth, the lesions become hard. Subsequent fibroblastic contraction may result in disfigurement of the pinna.

The location of the initial damage is unclear. Subcutaneous and subperichondrial sites are suggested, but a predominantly intrachondral location seems most likely. The cartilage plate of the pinna is cleaved by a longitudinal fracture and then by the hemorrhage. Granulation tissue forms at the interface of blood and cartilage, and the lesion is eventually converted to a fibrous scar. Late in the reparative process, regeneration of cartilage occurs adjacent to perichondrium or the ruptured edge of the cartilage. The intrachondral location of the hematomas is thought to be the result of rupture of vessels as they pass through minute foraminae in the cartilage plate. The conventional view that the initial lesion results from shear forces or outright trauma caused by head shaking has recently been challenged by one study of 40 dogs and 20 cats with auricular hematoma. No association with ear configuration or otitis externa was found, but most affected animals had serum and local tissue changes interpreted as *evidence for a lupus-like autoimmune disease*. The link between this proposed pathogenesis and the observation of the early cartilaginous fractures and hemorrhages was not made.

Dermatologic disease of the ear pinna may be part of a generalized skin disorder, but a few diseases seem unique to the ear. *Pigs with chronic sarcoptic mange* may have gross lesions only on the ears, consisting of reddening, crusting, and thickening of the pinna. *Short-haired* *dogs with pendulous ears* (Dachshunds, Pointers, Bloodhounds) are predisposed to chronic dermatitis of the ear margin, variously termed *marginal auricular dermatosis* or *ear margin seborrhea*. The gross lesion is a multifocal, greasy, gray, nodular encrustation that may coalesce to cause thickening of the entire ear margin. The histologic lesion resembles that of seborrhea elsewhere, with hyperkeratosis, acanthosis, and mild superficial perivascular dermatitis in which mononuclear leukocytes predominate.

Chronic ulcerative dermatitis of the ear margin of white cats (*feline solar dermatitis*) is discussed below as a premalignant lesion under squamous cell carcinoma, as is a similar disease of sheep.

Alopecia of the pinna occurs in dogs and in Siamese cats. In dogs, the alopecia appears gradually and progresses slowly. There is a strong *breed predilection* for Dachshunds, but it can be seen in other breeds including Italian Greyhounds and Whippets. The cause is unknown. The microscopic lesion is miniaturization of anagen follicles, without follicular destruction and without inflammation. In cats the lesion may wax and wane at irregular intervals throughout life. The histologic changes, in cats, have not been described.

Pinnal vasculitis is surprisingly frequent and resembles the lesion of cutaneous vasculitis elsewhere. In its mildest form, it may cause only pilosebaceous atrophy and thus may mimic the lesions of idiopathic pinnal alopecia when examined clinically.

Otitis externa

Otitis externa is a very common disorder in dogs and cats, and is probably equally common in many other species if they were to be examined. It may involve predisposing, primary, and perpetuating factors. In dogs, the pathogenesis is complex, while in cats, cattle, and goats, the *ear mite* is by far the major initiating factor. In tropical climates, infestation with rhabditiform nematodes (*Rhabditis* spp.) can be an important cause of otitis externa in cattle. *Otitis externa in dogs is most prevalent in breeds with pendulous ears or with abundant hair within the ear canal*, which implies that inadequate circulation of air and entrapment of moisture are important predispositions to the disease. *Foreign bodies*, usually foxtail or grass awns, may be important mechanical irritants, creating a breach for microbial infection, but since otitis is common in city dogs, other factors apparently are involved. The role of microorganisms in the pathogenesis of otitis externa is, as elsewhere, intimately linked to environmental circumstances that permit their uncontrolled proliferation. The bacteria and fungi cultured from diseased ears almost invariably are members of the normal aural flora. *Staphylococcus* spp., *Pseudomonas* spp., *Proteus* spp., and the yeast *Malassezia pachydermatis* are cultured much more frequently or in greater numbers from ears with otitis than from normal ears, but this may be related as much to their resistance to antimicrobial treatment as to their causative role. The lipid-rich environment of the ear canal favors the lipophilic *Malassezia*, which is the best candidate for a primary pathogen in the causation of canine otitis externa inasmuch as it frequently is the only pathogen isolated, and affected ears improve dramatically with antifungal therapy.

Otitis externa in species other than dogs is closely correlated to infestation with ear mites, and even in dogs acariasis can be an important contributor.

Otodectes cynotis *is the ear mite of carnivores*. The importance of the mite in initiating otitis externa is not entirely clear because the ear canal, once inflamed, becomes an environment unsuitable for the mites and thus gives rise to misleadingly low estimates of the prevalence of ear mites in dogs with otitis. As few as five mites can initiate otitis externa in dogs, with bacterial and mycotic opportunists rapidly masking the role of mites in the development of the lesion. Estimates vary from 2–50% prevalence of mites in dogs with otitis externa.

In cats, the role of mites is firmly established. In contrast to dogs, all cats have erect and sparsely haired pinnae and the role of ear carriage and hair is negligible. There is a poor correlation between the number of mites, the severity of inflammation in the external meatus, and clinical signs.

The mites are obligate parasites, spending their entire 3–4-week life cycle on the host. Transmission between animals of the same or different species readily occurs, and spread of the mites from their preferred aural niche to paws as the animal scratches, or tail as it sleeps with tail curled to touch the ear, is occasionally seen.

The mechanism by which the mites initiate otitis is controversial. Some maintain that the mites feed only on epithelial debris, while others claim that the mites pierce the epithelium and ingest blood and lymph. The latter claim is supported by the demonstration of host-specific serum components within the mites, but such components could exist in exudates, and should not be considered proof of penetrating feeding behavior.

The variation in the number of mites necessary to cause clinically obvious otitis, the species variation and some tenuous age resistance to disease (if not infection) suggest an allergic basis for the otitis. Most cats, and perhaps dogs, probably are infected with mites at some time. They may react to subsequent minor infection with immune-mediated inflammation of a severity not predicted by the small number of mites within the ear canal.

Psoroptes cuniculi, *the ear mite of rabbits*, also affects goats, horses, and deer. Its prevalence among domestic animals seems to be greatest in *goats*, ranging from about 20–80%. Careful examination may be required to find the mites. Clinical signs in goats, if present at all, are usually mild, consisting of ear twitching and head shaking. The ear canal of goats is the best site for isolation of several pathogenic *Mycoplasma* species in clinically normal animals. The same mycoplasmas were isolated from ear mites (*P. cuniculi* or *Raillietia caprae*) of infected goats, fuelling speculation that the mites are important vectors of the mycoplasmas in endemic herds. Similar coexistence of ear mites and mycoplasmas has been observed in cattle, but identification of the bacteria within the mites was not reported.

Psoroptes cuniculi may be quite common in horses (20% of horses in one Australian report) but it is unclear how frequently it causes clinical signs. Head shaking and resentment of handling of the ears are the usual signs.

Raillietia auris, *the ear mite of cattle*, occurs in most areas of the world. Infection seems to be common but is rarely of clinical significance. Manifestations of disease may follow extension of the otitis externa through the tympanum, and affected animals show head tilt and circling due to otitis interna. As with so many other agents observed or isolated from the external ear canal, it is difficult to prove a direct causal association between the presence of the mites and lesions or clinical signs of otitis.

Otobius megnini, *the spinose ear tick*, is parasitic on domestic ruminants, pigs, horses, and dogs and cats in restricted geographic areas. Infestation may be so heavy that the ear canals are full of ticks, making diagnosis easy. In light infestations, they occur deep in the folds of the ear, at the bottom of the external meatus. Only the larvae and nymphs are parasitic. The larvae attach themselves to the skin below the hairline and, biting through the skin, suck lymph until they are engorged. The parasites irritate the external auditory meatus and the ensuing exudate may completely fill it. In addition, secondary bacterial infection may occur in the inflamed areas around the bite wounds, and this infection may extend downward and cause otitis media. The microscopic appearance of the ear in this infestation has not been described.

The lesions of **otitis externa** *are nonspecific.* The relationship between type of exudate and causative agent has not been critically evaluated. The initial macroscopic lesion is hyperemia of the external auditory meatus followed by accumulation of serum, cerumen, leukocytes, and epithelial debris. A predominance of leukocytes tends to produce a suppurative exudate whereas cerumen yields a dry, dark-brown crumbly accumulation.

The histopathology of otitis externa varies with the duration of the inflammation and is rather typical inflammation of any epithelial surface. Only chronic lesions are likely to be seen by the pathologist. Such specimens show epidermal hyperplasia with acanthosis, atrophy of hair follicles, hyperkeratosis, and parakeratosis. Crusts of inflammatory debris adhere to the epithelial surface. Ulceration may be present, particularly if *Pseudomonas* is predominant, and *Malassezia* yeasts may be seen in the surface keratin or debris. The dermis contains numerous lymphocytes, plasma cells, mast cells, and neutrophils, the latter usually within dilated venules or adjacent to ulcers. Neutrophils are very sparse in mite-induced otitis. In very chronic and severe examples, dermal fibrosis is marked and ossification may occur. A feature of chronic otitis externa is *increased production of cerumen.* Histologically, there is hyperplasia of the normally very large sebaceous glands and cystic hyperplasia of the coiled tubular apocrine ceruminous glands, which are distended by eosinophilic cerumen. The combination of epithelial hyperplasia, glandular hyperplasia, and stromal fibroplasia may create proliferative lesions that occlude the ear canal and simulate neoplasia.

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MIDDLE EAR

Otitis media

Otitis media *is inflammation of the tympanic cavity within the temporal bone. Its cause is almost always bacterial, the organisms reaching the poorly drained cavity via the Eustachian tube or following perforation of the tympanum.* In swine and lambs, the infection usually ascends from the pharynx via the Eustachian tube. In calves, the pathogenesis may depend on the age of the calves and a variety of management factors. In young dairy calves, *Mycoplasma bovis* causes unilateral or bilateral fibrinopurulent otitis media, sometimes even with bone lysis and remodeling. Affected animals may have no other lesions of *M. bovis* infection, or may have concurrent polyarthritis and/or necrotizing bronchopneumonia. In feedlot cattle, outbreaks of otitis media have been associated with isolation of *Pasteurella multocida.* Those organisms are normal inhabitants of the bovine ear canal, so the significance of these isolates is hard to evaluate: mycoplasma was not sought.

In dogs and cats, chronic otitis externa is the major predisposition, although the tympanum is quite resistant to inflammatory lysis. There are insufficient cases recorded in other species to permit generalization. *In all species, there is circumstantial evidence for hematogenous localization of infection in the middle and inner ear,* perhaps occurring most often in pigs. Otitis media with subsequent vestibular disease can be the presenting clinical complaint in cats with cryptococcosis.

Otitis media as a clinically obvious entity is most frequent in *feeder pigs.* The infection is usually unilateral and associated with hemolytic streptococci, but may involve *Actinobacillus pleuropneumoniae* or *Mycoplasma hyorhinis.* The clinical signs of head tilt, circling, and ataxia suggest involvement of the inner ear in most instances. Otitis media in swine may occur as small epizootics involving a dozen or more pigs. The basis for the clustering of the disease is unproven, but its sporadic association with atrophic rhinitis suggests spread from the upper respiratory tract. In *lambs,* the lesion is usually clinically undetected and unilateral, and often occurs in association with pneumonia. *Mannheimia haemolytica* is the usual isolate from both ear and lung. Bottle-feeding also increases the prevalence of otitis media in lambs. Otitis media/interna is uncommon in *horses,* but can occur as a chronic, insidious infection with the unique sequel of temporohyoid osteoarthropathy in some horses. Head shaking may be the only presenting clinical sign.

Regardless of the route of entry, the lesion and its progression are similar. The epithelium lining the tympanic cavity is hyperemic, edematous, and may be ulcerated. Neutrophils exuding from the reactive vessels under the epithelium enter the tympanic cavity, joining the initially serous or serofibrinous exudate to make it progressively more purulent. Exudate may temporarily drain into the pharynx via the Eustachian tube, which is soon sealed by inflammatory swelling of its epithelium. In severe infections, the exudate escapes via inflammatory lysis of the tympanum or, rarely, the bone on the ventral floor of the tympanic bulla. Chronic inflammation is characterized by inspissation of exudate, lysis of the ossicles and, occasionally, the tympanum, and spread to inner ear and brainstem. Another sequel noted in dogs with otitis media is mineralization of necrotic material within the tympanic bullae to form **otoliths**. Otolithiasis may cause signs of vestibular disease.

The presence of **inflammatory polyps**, either unilateral or bilateral, of the middle ear in dogs is an unusual association with, and possible cause of, otitis externa/media. Surgical removal of aural polyps has resulted in a good prognosis. Nasopharyngeal polyps have also been noted in this location in cats (see Neoplasms and like lesions below).

Primary secretory otitis media (PSOM) has been diagnosed in Cavalier King Charles Spaniels with signs of moderate-to-severe pain in the head or neck, and/or neurological signs. A highly viscous mucus plug filled the middle ear and caused the tympanic membrane to bulge outwards. The prognosis was good following removal of the mucus plug, flushing of the middle ear, and local and systemic medical therapy.

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INNER EAR

Otitis interna

Otitis interna *is almost always the result of infection spreading from the middle ear.* The inflammation is usually of bacterial origin and thus suppurative. Ascension via the eighth cranial nerve is relatively frequent, and results in focal suppurative meningitis or encephalitis in the region of the pons. *The usual clinical syndrome is vestibular dysfunction*, described in a subsequent section.

Deafness

Deafness is difficult to assess in animals, and its diagnosis by simply observing behavioral abnormalities is almost impossible unless the animal is totally and bilaterally deaf. The recent introduction of electrodiagnostic tests has identified a higher prevalence of deafness, especially in dogs, than was suspected. **Conductive deafness** results from interference with the conduction of sound to the sensory end organ (of Corti) by diseases of external or middle ear. Alternatively, **sensorineural deafness** results from maldevelopment or degeneration of the sensory organ, eighth nerve, or auditory pathways within the brain. The last is very unusual and, because of the multitude of possible pathways, is seen only in massive destructive lesions with their neurologic signs overshadowing the hearing loss. *Nerve deafness usually involves the organ of Corti, and is by far the most prevalent type of deafness encountered in animals by virtue of hereditary deafness in several breeds of dogs, and in dogs or cats with color dilution anomalies.* Conductive deafness may result from obliteration of the external auditory meatus by chronic proliferative inflammation or tumor, from inflammatory or traumatic rupture of the tympanum, or from entrapment of the ossicles in exudates or granulation tissue. Rarely there is destruction of the middle or inner ears by osteomyelitis or neoplasia.

The anatomy of the auditory apparatus is complex. The sensory fibers of the eighth cranial nerve terminate at the base of sensory hair cells within the organ of Corti, the latter a sensory specialization of the epithelium lining the cochlear duct. Excitation of the hair cells results from pulsations within the endolymph fluid that fills the entire membranous labyrinth including the cochlear duct. The precise mechanism of such excitation remains unknown. Sound waves in the environment reach the endolymph via the tympanum and ossicles. Vibration of the tympanum is transmitted to the ossicles, and vibration of the ossicles causes vibration of the oval window that separates the footplate of the stapes from the endolymph. Any lesion that interferes with vibration of the tympanum or ossicle interferes with the establishment of fluid waves within the endolymph. Traumatic fracture of ossicles, rupture of tympanum, or dampening of vibration by exudates within the middle ear can do this.

Hereditary cochleosaccular degeneration

Hereditary deafness *in association with incomplete pigmentation of the hair coat and uvea is seen in cats, dogs, mink, and mice. Incompletely documented examples exist in other domestic species.* The pigmentary defect is not true albinism but is white spotting or merling, the distinction being the absence of melanocytes in white areas in the latter instance and functionally defective melanocytes in the former. In some instances, the hearing defect is associated with inheritance of the merling gene. All homozygotes and many of the heterozygotes are deaf and have some degree of iris heterochromia. In other instances there is heterochromia iridis but no apparent coat color dilution (Dalmatian dogs), while in others both eyes and coat are phenotypically normal. Even when the coat color is white, as in deaf Bull Terriers, the genetic basis for the white coat and the associated deafness need not be the merling gene. Out of this confusing picture one should rescue the concept that *color-dilute animals have ocular heterochromia and deafness much more frequently than normal animals, but that other genetic bases for deafness also exist.* Ocular anomalies and deafness commonly are encountered in the same animal, probably the result of the inductive influence of pigmentation on both organs. For example, all white cats with blue irises are deaf as are many Dalmatian dogs with iris heterochromia. Several dog breeds, particularly Dalmatians and English Setters, have a prevalence of early-onset deafness in excess of that predictable from their prevalence in the overall canine population. Prominent also are merle dogs such as Australian Shepherds, Australian Blue Heelers, Old English Sheepdogs and Great Danes.

The fully developed lesion of **cochleosaccular degeneration** *is atrophy of the sensory and supporting cells of the organ of Corti and the saccular macula, collapse of the dorsal or lateral walls of the cochlear and saccular membranous labyrinth, and secondary degeneration of the neurons within the spiral ganglion.* The osseous portion of the labyrinth is unaffected as is the vestibular portion of the inner ear. With minor variations, all cases in dogs and cats are similar. Initial structure and function are normal, but there is failure to achieve normal maturation and, subsequently, degeneration occurs.

The carnivore ear is completely developed at birth and continues to mature for 2 (organ of Corti) to 4 (stria vascularis) weeks. Thereafter, the epithelial lining of the cochlear duct is incapable of mitotic regeneration. In white kittens, the ear is morphologically and physiologically normal at birth but shows arrested development as early as 1 week of age. At this time there is inward sagging of the free dorsolateral wall of the cochlear duct (Reissner's membrane) and saccule, and hydropic change in the stria vascularis. The degeneration within the organ of Corti and adjacent nutritive stria vascularis is so rapid that there is no consensus as to the initial or causal lesion. It may be that degeneration of the stria vascularis results in ischemic degeneration of the avascular organ of Corti, or there may be a primary defect within the sensory cells themselves resulting from an inborn metabolic error.

Senile deafness

Many animals become hard of hearing as they reach old age. This phenomenon, called **presbycusis**, is more frequently observed in old dogs because dogs tend to be kept well into their advanced years and because hearing loss is more readily noticed in them than in other species. In humans, the loss of hearing is progressive from about the fortieth year of life and particularly affects hearing of high tones. The cause seems to be inherent age-related degeneration of the epithelial tissues within the cochlea and of the spiral ganglion, a process that may be accelerated by excessive noise, arteriosclerosis, and nutritional factors. The essential lesions are atrophy of all epithelial structures within the cochlear duct and the associated auditory nerves, as well as neuronal atrophy within the spiral ganglion. Occasionally, the major lesion is atrophy within the stria vascularis or the basal membrane supporting the organ of Corti.

Presbycusis in animals has been studied in guinea pigs, rats, mice, dogs, and cats. *Degeneration of the hair cells in the organ of Corti and loss of neurons from the spiral ganglion are the common denominators in all such instances, and presumably is the aging change common to all mammalian species.* Stria vascularis atrophy has not been seen in these animals.

Acoustic and chemical ototoxicity

Noise, *either as a sudden loud noise or as moderate but prolonged environmental background, causes degeneration of the sensory hair cells within the organ of Corti.* Loud noise causes hair cell necrosis and even outright disruption of the organ of Corti or Reissner's membrane by mechanical trauma mediated via fluid waves within the endolymph, which must be the otic equivalent of tidal waves. Environmental noise is frequently an occupational hazard. The eventual lesion is hair cell necrosis, but whether this results from repeated sublethal microtrauma by noise peaks or by interference with hair cell metabolism is not known. That animals are susceptible to such trauma is well established in experimental models, but investigation of naturally occurring examples is not reported.

The list of chemicals that are ototoxic is very long, and is even longer if idiosyncratic drug injury is included. Only a few major examples likely to be encountered in veterinary practice are included here.

Aminoglycoside antibiotics *(gentamicin, streptomycin, kanamycin, neomycin, and others) are all nephrotoxic and ototoxic in proportion to their blood levels and duration of administration.* Overdosing, or administration to animals with decreased renal function, markedly increases the risk of toxic injury to the inner ear. Clinical signs of vestibular dysfunction precede evidence of hearing impairment. The initial lesion affecting hearing is degeneration of the apical portion of cochlear hair cells. The earliest visible lesion is mitochondrial swelling and increased number of myelin figures. The early lesion is reversible but later becomes permanent, presumably as mitochondrial swelling leads to structural disintegration of cellular respiratory enzymes and cell death. Cats are particularly susceptible and vestibular toxicity (defects of posture, balance, and gait) precedes clinical evidence of hearing loss. Ordinarily, these signs appear only after several weeks of aminoglycoside therapy.

The **diuretics** *furosemide, bumetanide, and ethacrynic acid are chemically related and all are ototoxic to dogs and cats.* Other domestic species have not been tested. Electrophysiologic evidence of hearing impairment occurs within a few hours of even a single high dose but is unaccompanied by structural changes within the cochlea. The exception is ethacrynic acid intoxication in which edema of the stria vascularis is seen ultrastructurally. There is a corresponding alteration in the composition of the endolymph produced by the stria. The hearing impairment, which occurs frequently in people receiving therapeutic doses of the drugs, is assumed to result from structural or physiologic lesions in the hair cells nourished by this abnormal endolymph.

Acetylsalicylic acid (aspirin) and its derivatives are ototoxic for man and several laboratory animal models, but no structural lesion has yet been detected.

The antibacterial-anthelmintic agent, **hygromycin B**, as well as causing cataracts in swine (see Eye) also causes permanent deafness in dogs if therapeutic dosages are given. The drug is not approved for use in dogs. Deafness is reported occasionally in swine receiving the medication in excess of recommended levels. Histologic descriptions of cochlear lesions are not available.

The antiseptic combination of **chlorhexidine and cetrimide** (Savlon7) is a widely used antiseptic that occasionally is used to cleanse the external ear canal. If used in dogs or cats with a ruptured typanic membrane, this solution is *toxic to both vestibular and cochlear cells*, although the clinical signs in affected animals are primarily vestibular.

Other causes of deafness

Deafness is reported in people with various *storage diseases*, but few animal counterparts have been studied for this specific defect.

Goats with β-mannosidosis have deformed ear pinnae, bony exostoses within the tympanic cavity, and deforming accumulation of oligosaccharides within lysosomes of many tissues of middle and inner ear. Epithelial and mesothelial cells of Reissner's membrane, most structural cells of the organ of Corti, and cochlear neurons of the spiral ganglion are all affected, even at a few days of age.

Vestibular dysfunction

Vestibular dysfunction is characterized by head tilt and falling towards the affected side, ataxia without weakness, and nystagmus. Clinical signs are most obvious with unilateral disease. The lesion may be in brain or in the vestibular apparatus, or both. Animals with vestibular dysfunction caused by brain lesions, as in listeriosis or canine distemper, usually show other signs of neurologic dysfunction. Since vestibular signs are more readily detected than is partial hearing loss, *mild lesions of the inner ear are more frequently associated with vestibular abnormalities than with hearing deficits.*

The causes of peripheral vestibular disease are the same as those causing deafness: uncontrolled otitis media, trauma, invasive neoplasia, and a number of drugs. Congenital vestibular disease has been reported in several dog breeds, but no morphologic observations were given. *Nonspecific destruction of part or all of the vestibular apparatus as a sequel to otitis media is by far the most common cause of labyrinthitis in all species.*

Idiopathic vestibular disease occurs in old dogs and in cats of any age. In old dogs, it is often mistakenly diagnosed as an acute cerebrovascular accident (stroke), but there is no demonstrable brain lesion in such dogs, and most have rapid, spontaneous remission of clinical signs unless prevented by premature euthanasia. No histologic examination of the labyrinth has been reported. An equally mysterious acute vestibular dysfunction affects cats of any age. Recovery usually occurs over a few days to weeks. No lesions are reported.

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Neoplasms and like lesions

Neoplasms of the ear include those capable of affecting skin elsewhere, as well as primary tumors of ceruminous glands and, very rarely, tumors of Eustachian or auditory epithelium and of cranial nerves. Inflammatory polyps are included in this section because of their gross resemblance to neoplasia.

Squamous cell carcinoma *is by far the most important skin tumor affecting the ear,* although the predilection of *canine histiocytoma* for the ear pinna probably makes it the most frequent tumor affecting the pinna. Squamous cell carcinoma of the ear occurs commonly in *white cats*, with a frequency more than ten times that of nonwhite cats, and in *sheep* in sunny climates. In cats, the tumor has a long precancerous phase consisting of erythematous, ulcerative dermatitis of the ear margin known as *feline solar, or actinic, dermatitis* because of its association with exposure to sunlight. Other sparsely haired areas, such as the nose, lips, and eyelids, may be affected. Histologically, the lesion consists of multifocal coalescing epidermal necrosis overlying a diffuse superficial dermal infiltrate of lymphocytes and plasma cells. The lesion waxes and wanes with the intensity of sunlight. *Epidermal hyperplasia proceeds to dysplasia, carcinoma in situ and invasive squamous cell carcinoma in a manner analogous to bovine ocular squamous cell carcimona.* The lesion is bilateral but not necessarily of synchronous progression. The progression to invasive neoplasia usually occurs over 3 or 4 years. Metastasis is a late occurrence and prevented (at least in the case of ears) by amputation. Rarely, squamous cell carcinoma occurs within the external ear canal or even within the tympanic cavity of the middle ear. Local infiltration results in damage to cranial nerves VII and VIII with resultant signs of vestibular dysfunction and facial paralysis.

Ceruminous adenomas and adenocarcinomas *occur in dogs and cats.* The tumors represent a continuum from histologically benign to histologically malignant, and are thus very similar to tumors of apocrine sweat glands elsewhere in skin. The ceruminous glands are modified sweat glands within the deep portion of the external auditory meatus. Tumors are relatively more prevalent in cats than in dogs, and in both species they tend to occur in very old animals. In dogs most are benign, but in cats about half are histologically malignant. The adenomas are smooth nodular or pedunculated masses seldom exceeding 1 cm in diameter. The epithelium overlying the tumor is intact unless there is concurrent otitis externa. Ceruminous gland tumors cannot be distinguished in the live animal from cystic dilation with epithelial hyperplasia typical of chronic otitis externa. Carcinomas may occasionally invade from the auditory meatus into the region of the parotid salivary gland or into bone, and when very anaplastic may be confused with salivary carcinoma. The diagnosis of carcinoma usually is based on histologic criteria of anaplasia and local invasiveness rather than on behavioral evidence of metastasis.

Histologically, adenomas are well-differentiated tubular and cystic growths. The epithelial cells are cuboidal and eosinophilic. They may be flattened when the tubular or acinar lumen is dilated. *The most typical feature is the presence within lumina of deeply eosinophilic or orange, colloid-like secretion typical of cerumen.* Mixed ceruminous tumors analogous to mixed apocrine sweat glands or mammary tumors occur infrequently. Carcinomas do not differ markedly from adenomas but have less secretion and more cellular anaplasia, and show invasion by tumor cells into an abundant fibrous stroma rich in mononuclear leukocytes. Mixed tumors with cartilage and bone are described.

Benign cutaneous plasmacytoma occurs with unexplained frequency in the external ear canal of dogs. It has the same histologic appearance here as it does in other locations such as the larynx and skin.

As expected, *various other neoplasms* have been described in the external ear canal or tympanic cavity of dogs and cats. All would seem to be rare, and no prognostic statements are justified by the small numbers. In one small series in dogs, eight of 11 middle ear neoplasms originated in the external ear and perforated the tympanic membrane. Two were papillary adenomas formed by ciliated columnar epithelium and goblet cells, thought to have arisen from the epithelium of the dorsal portion of the tympanic cavity. The final case was an anaplastic carcinoma of unknown origin involving oropharynx and ear.

Nasopharyngeal polyps *occur quite frequently in cats*. The distinctive histologic lesion is a loose mass of connective tissue containing numerous small blood vessels and mononuclear leukocytes, covered by epithelium that may be either stratified, nonkeratinized squamous, or simple-to-bilayered ciliated columnar. *The detection of ciliated epithelium is a prerequisite for the histologic confirmation of nasopharyngeal polyp*. Often the ciliated epithelium is found only focally in protected subepithelial glands, but nonetheless it is the characteristic feature distinguishing this lesion from nonspecific proliferations of glands and connective tissue seen in many cases of chronic otitis externa. The presence of this ciliated epithelium is used to support theories that such polyps originate from the Eustachian tube, but in fact the only proven origin is from ciliated epithelium of the tympanic cavity itself. Some of these polyps are grotesque, protruding from outer ear, hanging into the oropharynx, or even protruding through the nose. A familial occurrence has been seen in Abyssinian and Himalayan kittens, further confusing the debate about the cause of these distinctive lesions.

Under the umbrella of **inflammatory polyp** fall many of the tumor-like proliferations excised from the external ear canal of dogs (rarely cats) with chronic otitis externa. The lesion consists of variable proportions of hyperplastic surface epithelium, hyperplastic or dysplastic sebaceous and ceruminous glands, fibroplasia, and leukocytes. The rupture of the glands often adds the lesions of sterile foreign body periadenitis to the chronic inflammation associated with the bacteria, yeast, or foreign bodies causing the initial otitis itself. Such masses are macroscopically indistinguishable from genuine ceruminous neoplasms; *the histologic distinction is made by observing a mixture of inflammation, fibrosis, and hyperplasia of both sebaceous and apocrine glands within the hyperplastic polyps, whereas the tumor is represented by the proliferation of a single cell type.*

Another nodular lesion, clinically resembling neoplasia, is the **dentigerous cyst** of heterotopic polydontia in foals, seen as a draining nodule on the rostral aspect of the base of the pinna.

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5

Skin and appendages

Pamela E. Ginn, Joanne E. K. L. Mansell, and Pauline M. Rakich

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GENERAL CONSIDERATIONS

The skin is the largest organ of the body and, because of its visibility, it is familiar to all, veterinarians as well as laymen. Consequently, any change in the skin or hair is noticeable and may be a cause for concern. While serious internal organic conditions may be ignored or overlooked because of their covert nature, even mild dermatologic diseases are likely to be brought to veterinary attention. In many veterinary practices, dermatologic cases constitute a major component of the caseload. The diseases range from minor, esthetic problems to life-threatening conditions. Many diseases produce severe economic losses because of damage to wool, hides, and meat or from decreased milk production or growth rates. Even apparently mild hair and skin changes may affect the health of the animal and have serious consequences.

The skin serves numerous functions but one of the most important is protection of the individual from the external environment. The hair and stratum corneum, composed of tightly packed keratinized cells permeated by an emulsion of sebum and sweat, form a physical barrier against potential physical, chemical, or microbial damage. The skin also has a protective chemical barrier composed of inorganic salts, proteins, and fatty acids. A number of proteins have been identified in skin washings from various species and some of these include immunoglobulins of various classes, interferon, albumin, transferrin, and complement. Fatty acids, such as linoleic acid, have antibacterial and antifungal properties. The skin also has *normal microflora* that contribute to skin defense. A mixed population of symbiotic bacteria lives and multiplies permanently on the skin surface and thereby inhibits invading microorganisms. Occasional yeasts and fungal hyphae may also comprise the resident microflora. The skin is also a major sense organ and it protects the organism from desiccation. It is involved in photoprotection, immunosurveillance, nutrient and fat metabolism, and water balance. The skin is involved in temperature regulation by virtue of the hair coat, blood flow, and, in some species, sweating. Lastly, the skin can serve as a window to internal diseases.

As dermatologic cases are more commonly presented to veterinary clinicians for examination, skin biopsies have become a routine diagnostic procedure. An understanding of the pathologic processes in skin, as in any tissue, requires a thorough understanding of the normal structure and function of skin. *As a general rule, the clinician is more familiar with the gross pathology than is the pathologist because the majority of the cases are examined by the pathologist only in the form of a limited number of very small skin biopsies.* This fact underscores the importance of the inclusion of the gross findings in the surgical pathology request. The pathologist must be aware of normal variations in skin components from different body regions and from different species to correctly interpret deviations from normal. The basic architecture is similar in all mammals but skin thickness varies between species and region of the body within the same species. The dermis contributes most to the difference in skin thickness. The epidermis varies less markedly, but tends to be thinner in areas with thick hair and thicker in those areas without hair, such as mucocutaneous junctions. In general, skin thickness decreases dorsally to ventrally on the trunk and proximally to distally on the limbs. The skin is thickest over the dorsal surface of the body and lateral aspect of the limbs and thinnest on the ventral aspect of the trunk and medial limbs.

The integument is composed of epidermis, dermis, hair follicles, adnexal glands, and the subcutis. The appendages include nails, hooves, and claws.

Epidermis

The epidermis is a continually renewing tissue in which keratinocytes move from the basal layer outward to the skin surface where they are ultimately shed. During their migration through the epidermis, cells undergo changes in shape, structure, and composition until they are shed as dead, cornified husks of cells. Protection of an individual from the environment is largely dependent on the structural integrity of the epidermis. *To maintain uniform epidermal thickness and normal barrier function, basal cell proliferation, orderly cell differentiation through the various layers, and cell death must be balanced.* Knowledge of the regulation of this complex, dynamic process is incomplete and primarily based on cultured keratinocytes. Epidermal growth factor, insulin-like growth factor-I, keratinocyte growth factor, basic fibroblast growth factor, interleukin-1, interleukin-6, transforming growth factor-α, vitamin D_3, and retinoids are some of the factors thought to be involved in the growth and differentiation of keratinocytes.

One of the most important and abundant products of keratinocytes is *keratin*. It forms the cytoskeleton of keratinocytes and is thus responsible for the shape of the cells. It is also responsible for structural stability and it contributes to the impervious nature of the stratum corneum. Keratin is composed of acidic and basic pairs of subunits that are differentially expressed by keratinocytes of various layers of the epidermis. Basal cells express keratins K5 and K14, while commitment to differentiation in suprabasal keratinocytes is associated with induction of differentiation-specific keratins K1 and K10.

The epidermis is divided into four layers:

- The **stratum basale** (*basal layer*) is the deep germinative layer of the epidermis and is composed of a single layer of cuboidal to low columnar cells resting on the basement membrane. These cells are mitotically active and their daughter cells continually migrate upward to replenish cells in overlying layers. The basal layer is thought to be *composed of two populations of cells*: (1) *stem cells*, which have a long lifespan, long cycling phase, and short S period; and (2) *transit amplifying cells*, which undergo only a limited number of divisions before terminally differentiating. Mitotic figures and apoptotic cells are occasionally evident. The basal cells are attached to the underlying basement membrane by *hemidesmosomes* and to adjacent and overlying keratinocytes by *desmosomes*. Desmosomes are anchoring structures that mediate adhesion between cells. They have a complex structure that includes *cadherin proteins* of two types, desmocollins and desmogleins. These proteins have different isoforms and they are differentially expressed in different layers of the epidermis. While basal cells express a pair of desmogleins and desmocollins of simple epithelia, desmosomes of the spinous layer express a more varied array of isoforms. These adhesion molecules have been identified as the target in several blistering autoimmune diseases.
- The **stratum spinosum** (*prickle cell layer*) is characterized by *prominent intercellular bridges that are the desmosomal attachments between cells.* The spinous appearance is due to shrinkage artifact that occurs during tissue processing. The cells are polyhedral to

slightly flattened and are arranged in one or two layers in haired skin of dogs and cats and up to four layers in large animals. This layer is much thicker in lightly haired or nonhaired skin and may be up to 20 cells thick in the footpads and nasal planum. Cells of the upper spinous layer synthesize the protein *involucrin*, which is a constituent of the cornified envelope of the stratum corneum.

- The **stratum granulosum** is variably present in haired skin and is one or two cells thick when present. It is usually four to eight cell layers thick in nonhaired skin or at the infundibulum of hair follicles. The stratum granulosum is composed of flattened cells with shrunken nuclei and deeply basophilic keratohyaline granules of variable size and shape. The granules are composed of the precursor of *filaggrin*, a histidine-rich interfibrillary matrix protein that *functions as a biologic glue* that aggregates and aligns keratin filaments together. Stratum granulosum cells also contain *laminar granules* (membrane-coating granules, Odland bodies, keratinosomes), small organelles containing a mixture of lipids that are released to the intercellular space where they coat the cells and contribute to the hydrophobic barrier of the stratum corneum. The cells also synthesize the cysteine-rich protein *loricrin*, a major precursor of the cornified envelope. Dissolution of the nucleus and other organelles begins in upper layers of the stratum granulosum, probably through the action of lysosomal enzymes.
- The **stratum corneum** is the *impermeable, insoluble, highly protective layer* that keeps microorganisms out and essential body fluids in. It is composed of anucleate, dead, flattened eosinophilic cells (*squames*) that are continually shed. Thickness varies greatly according to body region and species. It is thickest in nonhaired areas such as footpads and nasal planum. Routine fixation and processing usually result in loss of approximately 50% of this layer. The cells are arranged in vertical interlocking columns in which intercellular spaces are filled with lipids derived from the laminar granules. The *basket-weave pattern* commonly visible in formalin-fixed sections is an artifact caused by loss of soluble intracellular constituents during routine processing. Structural support for these cells is provided by the cornified cell envelope. The cornified envelope is a complex structure composed of a number of proteins, including involucrin, loricrin, and keratolinin. The proteins are deposited on the inner membrane of the keratinocytes and are cross-linked by transglutaminases to form a highly insoluble, electron-dense band. The keratin in these cells is organized into large macrofilaments that confer mechanical resistance to the cells. The structural stability of the horny layer is derived from the association of the keratinized cells and insoluble extracellular lipids, a structure likened to a wall in which the cells represent the bricks and the lipids the mortar.

Epidermal non-keratinocytes

In addition to keratinocytes, which comprise approximately 85–90% of the cells of the epidermis, *small numbers of dendritic cells also reside in the epidermis*. These include *melanocytes, Langerhans cells, and Merkel cells*. **Melanocytes** are located in the basal layer of the epidermis and outer root sheath of hair follicles; and in HE sections they appear as clear cells with a small dark-staining nucleus due to shrinkage artifact. There is ∼1 melanocyte per 10–20 keratinocytes. They can be visualized more specifically with silver stains, which demonstrate their dendritic processes. Derived from the neural crest, melanocytes migrate into the epidermis during early fetal life. Their processes are intertwined between the surrounding keratinocytes to which they transfer melanin pigment. A melanocyte and its surrounding constellation of keratinocytes is termed the "*epidermal melanin unit*." Melanogenesis occurs in membrane-bound organelles called *melanosomes*, which originate from the Golgi. The melanosomes migrate to the tips of the dendrites and are phagocytosed by adjacent keratinocytes. Most melanin pigment in skin is in the basal layer; but in dark-skinned animals, melanin may be present throughout the epidermis. Melanin is photoprotective and exposure to ultraviolet light increases melanin production, often resulting in a cap of pigment granules over the nucleus. Skin pigmentation is affected by local inflammation because melanocytes respond to inflammatory mediators by increasing or decreasing melanogenesis and by altering melanin transfer to keratinocytes.

Langerhans cells *are bone marrow-derived dendritic cells that are functionally and immunologically related to monocyte-macrophage cells*. They appear as clear cells on routine HE sections and may be distributed from the stratum basale to the stratum spinosum, depending on species and region of the skin. They are usually less numerous than melanocytes, however. Langerhans cells are characterized ultrastructurally by rod- or racket-shaped cytoplasmic granules called *Birbeck granules*. Birbeck granules have been identified in Langerhans cells of the pig, cat, cattle, sheep, goat, horse, and human, but not in the epidermal dendritic cells of the dog. Histochemical markers expressed by Langerhans cells include CD1, class II MHC (major histocompatibility) antigens, CD45, vimentin, and S-100. The long dendritic processes of Langerhans cells traverse the intercellular space to the granular cell layer where *they function in immunosurveillance as antigen-presenting cells*. Langerhans cells trap antigens in the epidermis and migrate via afferent lymphatics to draining lymph nodes where they present antigen to T cells in paracortical areas, resulting in proliferation of a population of sensitized T cells. Exposure to UVB radiation decreases Langerhans cell numbers in the epidermis and interferes with their antigen-presenting capacity. Langerhans cells are involved in development of contact hypersensitivity, and increased numbers of epidermal Langerhans cells have been found in horses with insect hypersensitivity and dogs with discoid lupus erythematosus, hypersensitivities, and deep pyodermas.

A second type of clear cell in the basal layer is the **Merkel cell** whose *origin is unknown*. Unlike melanocytes, Merkel cells are connected to adjacent keratinocytes by desmosomes. Merkel cells are also located in the external root sheath of hair follicles. They are identified ultrastructurally by characteristic *dense-core cytoplasmic granules*. Immunohistochemical markers include cytokeratin, neurofilaments, neuron-specific enolase, and desmosomal proteins. Merkel cells have been identified in the dog, cat, sheep, pig, monkey, various laboratory animals, birds, reptiles, and amphibians. Their density in the epidermis is variable and they are in highest numbers in areas involved with sensory perception. Merkel cells form *Merkel cell–neurite complexes* with axons in tylotrich pads that are thought to function as slow-adapting mechanoreceptors and in sinus hairs. Their exact function in these structures is uncertain but they are thought not to act as sensory cells but rather to function as abutments for deformation of the mechanosensitive nerve endings. Merkel cells are also thought to have various neuroendocrine effects and to be involved in control of the hair cycle.

Basement membrane zone

The basement membrane zone (BMZ) is the structurally and biochemically complex junction between the epidermis and dermis. Both the epidermis and dermis contribute to production of the various components of the BMZ. The area is indistinct in HE sections but visible as a thin, homogeneous band with PAS stain. It varies in thickness in different sites and is most prominent in nonhaired areas of skin and mucocutaneous junctions. In all animals but the pig, the dermal–epidermal junction is straight and the BMZ parallels the skin surface; while in pigs and humans, the dermal–epidermal junction is thrown into undulating folds called rete ridges. *The BMZ has a crucial role in anchoring the epidermis to the dermis; and abnormalities of the BMZ result in serious and potentially fatal bullous diseases.* The BMZ also influences growth and differentiation of keratinocytes and acts as a selective barrier for passage of molecules between the epidermis and dermis.

Ultrastructurally, the BMZ is composed of: (1) the **plasma membrane** of basal keratinocytes with their specialized attachment structures, *hemidesmosomes*; (2) the electron-lucent **lamina lucida**; (3) the electron-dense **lamina densa**; and (4) the subbasal **lamina fibrous zone**. Hemidesmosomes are located on the basal aspect of basal keratinocytes and they consist of a cytoplasmic plaque that connects to the cytoskeleton and a transmembrane portion that binds to the underlying basement membrane. The *cytoplasmic plaque* is composed of a number of proteins, including bullous pemphigoid antigen 230 (bullous pemphigoid antigen 1) and plectin, which connect keratin 5 and 14 intermediate filaments to the plasma membrane. Hemidesmosomes also have a transmembrane portion that includes $\alpha6\beta4$ integrin and bullous pemphigoid antigen 2 (type XVII collagen). The proposed ligand for $\alpha6\beta4$ integrin is laminin 5, which is a component of the anchoring filaments of the lamina lucida, and this binding mediates the stable adhesion of keratinocytes to components of the BMZ. *Anchoring filaments* are 2–4 nm diameter filaments composed of laminin 5 (also called epiligrin, kalinin, and BM600). They pass from the plasma membrane through the lamina lucida to attach to the lamina densa. The lamina densa is composed of multiple molecules, including collagen type IV, laminin, nidogen, and several glycoproteins. The lamina densa is connected to anchoring plaques of the underlying dermis by anchoring fibrils. The subbasal lamina fibrous zone of the superficial dermis is composed of anchoring fibrils, anchoring plaques, and microfibrils. Anchoring fibrils are composed of collagen type VII and form looping arrays with one or both ends of the fibrils attached to the lamina densa, thereby anchoring the BMZ to the dermis.

Dermis

The dermis is involved in maintenance and repair of the skin and is the major component responsible for the tensile strength and elasticity of the skin. In addition, the thickness of the dermis largely determines the thickness of the skin. *The dermis is composed of collagen and elastic fibers embedded in a ground substance, blood and lymphatic vessels, nerves, and low numbers of mixed cells.* Except for pigs, domestic animals have no dermal papillae as occur in human skin. Thus, instead of papillary and reticular dermis, the dermis is divided somewhat arbitrarily into *superficial and deep dermis* in domestic animals.

The **dermal fibers** include collagen, reticulum fibers, and elastic fibers, all of which are synthesized by dermal fibroblasts. Collagen fibers are the most abundant constituent of the dermis and they confer tensile strength to skin. The majority of *dermal collagen is types I and III.* The superficial dermis is composed of fine, loosely arranged collagen fibers. The deep dermis consists of thick, densely arranged collagen fibers that roughly parallel the skin surface. Reticulum fibers represent a special thin type of collagen. *Elastic fibers* are inconspicuous in routine HE sections. They can be visualized with special stains such as orcein stain or Verhoeff–van Gieson elastin stain. They are thicker and less numerous in the deep dermis and arranged parallel to the skin surface. The elastic fibers become progressively thinner near the epidermis.

The **ground or interstitial substance** is an amorphous gel-sol that fills the space between dermal structures but allows electrolytes, nutrients, growth factors, and cells to pass through. It consists of *proteoglycans and glycoproteins.* Proteoglycans are high-molecular-weight complexes composed of glycosaminoglycans linked to proteins and those most abundant in the dermis include hyaluronic acid and various chondroitin sulfates. Proteoglycans bind various chemical mediators and thereby function as storage matrix as well as provide lubrication and structural support. *Fibronectins* are a family of glycoproteins involved in mediating cell–cell and cell–matrix interactions that are required for various cell functions including cell adhesion, phagocytosis, and cell migration. The ground substance is usually not visible in normal HE sections; however, a fine granular to fibrillar basophilic material is occasionally evident between collagen fibers. This material is especially abundant in the dermis of Chinese Shar-Peis as a normal variant for this breed.

The **dermal vasculature** is arranged in three intercommunicating plexuses. The *deep plexus* is located at the junction of the dermis and subcutis and it supplies branches to the *middle plexus*, which is located at the level of the sebaceous glands. It, in turn, supplies branches to the *superficial plexus.* The capillary loops parallel to the skin surface immediately beneath the epidermis arise from the superficial plexus. An unusual vascular arrangement is present in the dermis of the llama. It consists of clusters of capillary-size, thick-walled vessels lined by plump endothelial cells distributed throughout the superficial and middle dermis. Dermal lymphatic vessels are inconspicuous in normal skin and only become visible when they become dilated because of increased lymphatic drainage. The skin is supplied with sensory and autonomic nerves that are usually associated with blood vessels.

The dermis is normally **sparsely cellular**. *Fibroblasts* are distributed in low numbers throughout the dermis. They synthesize most of the fibrillar and ground substance proteins of the dermis as well as various growth factors and cytokines. *Melanocytes* in the dermis are usually located near superficial dermal vessels. In contrast to melanocytes of the epidermis and hair follicles ("secretory melanocytes"), dermal melanocytes do not transfer their melanin to surrounding cells ("continent melanocytes"). Normal dermis also contains small numbers of perivascular *monocytes and lymphocytes,* which are indistinguishable from each other. Dermal lymphocytes are T cells of the helper subtype primarily.

Mast cells are tissue-dwelling cells that are most numerous in sites, such as the skin, that interface with the environment. Mast cell numbers in skin vary greatly depending on body location, with numbers ranging from 0–60 per high-power field in cats, and 0–40 per high-power field in dogs. They are in highest numbers in the skin of the caudal pinna in both dogs and cats. They are concentrated around blood vessels, especially postcapillary venules. Mast cells are not present in normal epidermis. Although mast cells are evident in routine HE sections, they are better visualized with Giemsa and toluidine blue, which stain mast cell granules metachromatically.

Mast cells originate in the bone marrow and are released as unidentifiable precursors into the blood. These *immature mast cell precursors are thought to home to the skin* because of mast cell growth factor expression by dermal endothelial cells. Their final differentiation and maturation is controlled by the local microenvironment, including stem cell factor, which is a fibroblast-derived growth factor, and various T-cell-derived cytokines (IL-3, 4, 9, 10). Mast cells have laminin receptors that mediate their adhesion to the extracellular matrix. The perivascular space is rich in laminin, which contributes to the localization of mast cells to this site. Mast cells are a heterogeneous population based on differences in histochemical, biochemical, and functional characteristics that vary between species and different tissues within a single species. Mast cell heterogeneity has been demonstrated in the skin of cattle, dogs, and sheep. A subpopulation of mast cells that does not exhibit metachromasia following formalin fixation has been demonstrated in the skin of dogs and cattle, and heterogeneity of protein content has been identified in dermal mast cells of sheep.

Mast cell granules contain an array of *preformed mediators*; but they are also capable of *synthesizing mediators* such as leukotrienes (LTC_4) and prostaglandins (PGD_2) following stimulation. Tumor necrosis factor-α, a potent pro-inflammatory cytokine, is both preformed and newly synthesized upon activation of mast cells. Mast cells have long been known to be the *critical effector cell* in initiation of acute type I hypersensitivity reactions and in protection against parasitic infections with helminths and ectoparasitic arthropods. More recently, however, it has become apparent that mast cells also function in persistent chronic inflammatory reactions, tissue repair and remodeling, pathologic fibrosis, angiogenesis, hemostasis, hematopoiesis, antibody production, protection against bacterial infections, response to neoplasms, and possibly in control of the hair cycle.

Dermal muscles

Arrector pili muscles are smooth muscles present in all haired skin. They arise in the connective tissue of the superficial dermis and attach to the connective tissue sheath of the hair follicle below the level of the sebaceous gland duct. They are situated on the obtuse angle of the hair follicle and when the muscles contract, the hair follicles are pulled into a vertical position (*piloerection*). This results in formation of air pockets in the hair coat, which provides insulation. Contraction of arrector pili muscles may also be involved in emptying of sebaceous glands. These muscles are largest in skin of the dorsal midline from the neck to the tail. They may be vacuolated in normal animals, especially aged dogs. In addition to arrector pili muscles, pigs also have **interfollicular smooth muscles**. These muscles span the triad of hair follicles at a level midway between the sebaceous and apocrine glands. Their contraction draws the hair follicles together and rotates the outer follicle of the triad. The functional significance of this muscle is uncertain.

Skeletal muscles may be present in the muzzle, forehead, eyelid, and perianal regions. These muscle fibers originate from cutaneous trunci muscle that penetrates into the dermis to allow voluntary movement of the skin. Skeletal muscle fibers also are associated with the large sinus hairs of the face.

Immunologic function

The skin has been proposed to function as an immunosurveillance organ and the term skin-associated lymphoid tissue (SALT), analogous to gut and bronchial-associated lymphoid tissues (GALT, BALT), has been suggested to describe those cellular elements of the skin that deal with antigenic challenges at the skin surface. However, this concept has been disputed because of presumed differences in immune function between the common mucosal immune system and normal skin. The alternative name, **skin immune system (SIS**), has been suggested as a more appropriate term to encompass the skin-specific immune response-associated cells and humoral factors present in normal skin. Key components of this system *include keratinocytes, Langerhans cells, the dermal perivascular unit, and skin-homing T cells.*

- **Keratinocytes** produce multiple inflammatory cytokines, adhesion molecules, and chemotactic factors following nonspecific stimulation, and thereby have a nonspecific proinflammatory and up-regulating effect.
- **Langerhans cells** are thought to trap antigens in the epidermis, migrate out to regional lymph nodes via lymphatics, and present the antigen to T cells. Thus, induction of the immune response does not normally occur within the skin itself but rather in the skin-draining lymph nodes.
- The **dermal perivascular unit** consists of the mast cells, monocytes and macrophages, tissue dendritic cells, and T cells situated around postcapillary venules. As a result of cytokines such as IL-1 and TNF-α released by injured keratinocytes, endothelial cells increase their expression of addressins ICAM-1 and E-selectin and vascular cell adhesion molecule (VCAM-1). These vascular endothelial molecules promote adhesion of circulating leukocytes, especially granulocytes and memory T cells.
- E-selectin is thought to act as an adhesion molecule or vascular addressin for a specific subset of **skin-homing memory T cells**, which have the ligand cutaneous lymphocyte antigen (CLA) on their surface. A circulating pool of such skin-homing T lymphocytes, identified by CLA antigen, represents the cellular basis of immunologic memory of skin.

Thus, it appears that the proinflammatory, up-regulating effects of keratinocytes prepare the dermis for specific immunologic activity, while migrating antigen-presenting Langerhans cells induce expansion of specific lymphocytes in skin-draining lymph nodes. T cells are then recruited to the skin because of binding of skin-specific adhesion molecules to the vascular addressins of dermal endothelial cells.

Hair follicles

Hair serves a number of functions including protection, thermal insulation, social communication, and sensory perception. Arrangement and type of hair follicles vary with species, breed, individual, and body region. In general, however, hair follicle density is greatest over the dorsolateral aspect of the body and least on the ventral aspect. Hair follicles are classified as *primary or secondary*, and *simple or compound*. **Primary hairs** have a large diameter, are rooted more deeply in the dermis, and are associated with sebaceous and sweat glands and an arrector pili muscle. **Secondary hair follicles** are smaller in diameter, are more superficially rooted, and may be accompanied by a sebaceous gland but lack a sweat gland and arrector pili muscle. Follicles in which a single hair emerges from the follicular orifice are termed **simple follicles**; while those in which multiple hairs emerge from a single opening are called **compound follicles**. Each hair of the compound follicle has its own *papilla*; but at the level of the sebaceous gland opening, the follicles unite to exit from a single external follicular orifice. Horses and cattle have simple hair follicles that are evenly distributed. Pigs have simple follicles that are grouped in clusters of two to four surrounded by dense connective tissue. In sheep, the hair-growing areas consist predominantly of simple follicles whereas the wool-growing areas have many compound follicles and consist of clusters of three primary follicles and a number of secondary follicles. Goats have primary follicles in groups of three with three to six secondary follicles associated with each group. Follicular arrangement in dogs and cats consists of two to five large primary hairs surrounded by groups of smaller secondary hairs. The primary hairs tend to be simple while the secondary hairs are compound. As many as 15 hairs may emerge from a single follicular orifice. In cats, secondary hairs far outnumber primary hairs (10–24 secondary hairs : one primary hair).

The hair follicle is formed by a downward invasion of the surface ectoderm (*primary hair germ*) into the underlying mesoderm of the embryo. As they grow down, the epithelial cells envelop a small group of mesenchymal cells in the underlying dermis. These mesodermal cells eventually become the **follicular papilla** that repeatedly induces and maintains growth of the hair follicle throughout the life of the individual. If the papilla is somehow damaged or destroyed, the hair follicle fails to regrow. The epithelial downgrowth eventually becomes canalized to form the hair follicle. In longitudinal section, the fully developed hair follicle consists of three segments:

- the **lower or inferior portion**, from the base of the follicle to the point of insertion of the arrector pili muscle;
- the **isthmus**, the short section from the attachment of the arrector pili muscle to the entrance of the sebaceous duct; and
- the **infundibulum**, extending from the entrance of the sebaceous duct to the follicular orifice.

The entire hair follicle beneath the isthmus, i.e., the inferior segment, can be considered temporary because it disappears during the involution stage of the hair cycle and reforms again during the active phase. In contrast, *the isthmus and infundibular portions of the hair follicle are permanent.*

The **base of the hair follicle** consists of a terminal bulbous expansion of epithelial cells, the hair bulb, with a concavity at its bottom that is occupied by the connective tissue papilla. The *bulb* is composed of the highly proliferative matrix cells and melanocytes and they are separated from the papilla by a thin extension of the basement membrane. The *matrix cells* give rise to six different cell types arranged in concentric layers. *The three innermost layers form the medulla, cortex, and cuticle of the emerging hair.* The next three layers form the *cuticle, Huxley layer, and Henle layer of the inner root sheath.* These layers are further surrounded by the outer root sheath, which is an extension of the epidermis and becomes continuous with the epidermis in the upper portion of the follicle. *External to the outer root sheath* are the *glassy membrane*, corresponding to the basement membrane of the epidermis, and finally the *connective tissue sheath*. All three layers of the inner root sheath keratinize by means of eosinophilic trichohyaline granules and become fully keratinized and disintegrate at the level of the isthmus. The inner root sheath is responsible for providing the rigid support for the developing hair and its final shape, e.g., twisted hair follicles produce curly hairs. From the base of the hair follicle to the isthmus, the outer root sheath is covered by the inner root sheath and it does not keratinize. In the isthmus, where the inner root sheath is no longer present, the outer root sheath undergoes trichilemmal keratinization, i.e., without keratohyaline granules. In the infundibulum, the outer root sheath is identical to surface epidermis and undergoes keratinization with formation of keratohyaline granules.

Hair does not grow continuously but rather in *cycles* consisting of a growth phase, **anagen**; a transitional or involuting phase, **catagen**; and a resting phase, **telogen**. This cyclic activity is thought to be an adaptive response to seasonal variation in ambient temperature. The hair growth cycle varies between different species, breeds, body sites, and hair follicle type. In domestic animals, *neighboring hairs cycle independently of each other and are in different stages of the hair cycle at any one time.* Hair shaft length is directly related to the duration of anagen phase, which is preordained according to body region and genetics. At the onset of catagen, mitotic activity of matrix cells and melanin production by melanocytes of the hair bulb cease. The keratinocytes of the inferior segment of the follicle undergo a controlled process of involution via a burst of apoptosis. This results in the upward migration of the hair follicle and the lower follicle becoming a thin cord of epithelial cells surrounded by a fibrous root sheath. Growth of the inner root sheath stops so that the lower end of the hair shaft is surrounded by thick trichilemmal keratin. The thin cord of epithelial cells is surrounded by a thickened, corrugated glassy membrane; and as it retracts upward, it is followed by the shrunken, contracted papilla. In telogen phase, the base of the bulb is located at the level of attachment of the arrector pili muscle and is approximately one-third of its former length. The base of the hair is encased in trichilemmal keratin and surrounded completely by outer root sheath (*club hair*). A population of stem cells (secondary hair germ) remains somewhere in the permanent portion of the hair follicle. These cells eventually reform the hair follicle during the next growth cycle. In rodents, the *bulge region of the follicle* appears to be the site that contains slow-cycling relatively undifferentiated cells from which arise a population of transient amplifying cells that become the matrix keratinocytes of the new hair bulb. *The bulge region is an area on the outer root sheath at the base of the permanent portion of the hair follicle to which the arrector pili muscle attaches.* It is not yet clear whether or not hair follicles of other domestic species have a similar bulge region of the hair follicle.

Hair growth in many animals has been shown to be regulated by photoperiod, ambient temperature, various hormones, nutritional status, and general health. However, the exact mechanisms that control the cycle are incompletely understood. Growth and development of hair are influenced by many growth factors; these include fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-β (TGF-β), and keratinocyte growth factor (KGF, same as FGF-7). Both IGF-1 and KGF are produced by the dermal papilla and their receptors are found in the overlying anagen hair follicle matrix cells. Factors from the papilla mesenchymal cells are thought to act on a stem cell population in the permanent upper portion of the hair follicle. These competent cells respond to the signals from the papilla by growing deep into the dermis to form the full-length anagen hair follicle. Transition between anagen and catagen appears to be regulated by FGF-5.

The *histologic appearance* of the hair follicle changes considerably during the hair cycle. The **anagen** stage is characterized by a well-developed flame-shaped plump dermal papilla that is capped completely by the hair bulb. The bulb is located in the deep dermis or subcutis and even bulbs of secondary hairs are surrounded by fat because tracts of fatty tissue extend upward from the subcutaneous fat. A layer of columnar matrix cells lines the papilla and melanocytes are dispersed among the matrix cells in pigmented hair follicles. **Catagen** stage is characterized by a thick glassy membrane that is irregular and undulating above the bulb of the hair follicle. The basement membrane between the dermal papilla and matrix is also thickened. The papilla is smaller, more compact because of decreased extracellular matrix and loss of capillary loops, and it is ovoid or round rather than spindle-shaped. The overlying matrix cells are no longer columnar and they appear disorganized. **Telogen** phase is distinguished by the club hair that has a brush-like appearance produced by keratinized fibers extending between the base of the club hair and the cells of the outer root sheath. The dermal papilla is a condensed ball of cells separated from the matrix cells and surrounded by its basement membrane.

Sinus hairs *(tactile hairs, vibrissae) are highly specialized mechanoreceptors that respond to vibratory stimuli as well as static hair displacements.* They are located on the muzzle (whiskers, vibrissae), face, throat, and palmar aspect of the carpus. They are thick, stiff hairs which are tapered distally. Histologically, they are composed of large single follicles surrounded by an endothelial cell-lined blood-filled sinus situated within the dermal connective tissue sheath. The sinus is supplied with numerous nerves; and skeletal muscles attach to the outer sheath of the follicle to confer some voluntary control of the hairs.

Sebaceous glands

Sebaceous glands are distributed throughout the haired skin and are essential for maintaining normal skin and hair. *Sebaceous glands produce sebum, an oily secretion composed of triglycerides, phospholipids, and cholesterol.* This material combines with sweat to form an emulsion that coats the skin to act as a physical barrier to retain moisture and maintain normal hydration, and as a chemical barrier against microbial pathogens. In addition, the oily film coats the hair shafts to give them a glossy sheen; it also acts as a *pheromone*. Lastly, sebaceous glands also appear to be involved with normal hair development, because, in the absence of sebaceous glands, the hair shaft fails to separate normally from the sheath.

Sebaceous glands consist of a solid mass of epithelial cells surrounded by a connective tissue sheath. The periphery of the gland consists of a single layer of cuboidal mitotically active cells resting on a basal lamina, analogous to basal cells of the epidermis. As the cells move inward toward the duct, they enlarge and accumulate lipid that is lost during routine processing. Sebaceous glands are *holocrine glands* and their secretion is formed by decomposition of cells. This is brought about by release of lysosomal enzymes in cells nearest the duct causing them to disintegrate and form sebum, which empties via a squamous epithelium-lined duct into the upper portion of the hair canal. All primary hairs and some secondary hairs have sebaceous glands. They are usually largest in areas with lowest hair follicle density such as mucocutaneous junctions, interdigital spaces, coronet, and dorsal neck and rump. Sebaceous glands are especially numerous and well developed in the chin of cats (*submental organ*), dorsal surface of the tail in dogs and cats (*tail gland*), base of the horn in goats, and infraorbital, inguinal, and interdigital regions of sheep. The footpads and nasal planum are devoid of sebaceous glands, and they are rare in glabrous skin where they empty directly to the skin surface.

Perianal glands

These are specialized secretory glands in the perianal regions, and they are commonly sites for the development of lesions.

- *Anal glands.* These are *specialized apocrine glands* that open directly onto anal skin and into specialized cystic structures on the lateral aspects of the anal canal, the anal sacs. The anal sacs are usually flaccid cystic cavities containing odoriferous secretions, presumably with some territorial marking function. They are present in many species, including domestic and wild felids, ferrets, raccoons, mink, rodents, pigs, and canids.
- *Perianal glands.* These glands are presumed to be *modified sebaceous glands* based on their typical histology, and are comprised of small glands and nests of cells without a prominent ductular network. Islands of these glands are concentrated in the subcutis around the perimeter of the anus, but foci are commonly present over lateral and ventral aspects of the base of the tail, less commonly on the dorsum of the tail, and in the skin of the prepuce and rump. The glands are distinctive, comprised of lobules of large eosinophilic epithelial cells (*hepatoid cells*) surrounded by low numbers of small basal reserve cells. Larger ducts are often lined by stratified squamous epithelium. *Perianal (circumanal) glands are best described in the dog,* and are most developed in entire males. Their function is uncertain, but they may have a role in steroid metabolism, in production of pheromones, and in territorial marking. Similar glands are also present in cats and in pericloacal glands of reptiles, where they probably have similar roles in territorial marking.

Sweat glands

Two types of sweat glands are present in the skin of mammals; they differ in origin, distribution, and possibly in the mode of secretion. These glands have been called apocrine and eccrine glands; but because of questions concerning the mechanism of the secretory process of these glands, the names *epitrichial and atrichial glands have been proposed for apocrine and eccrine glands, respectively.* **Apocrine (epitrichial) sweat glands** develop embryologically from primary hair germ and they are distributed throughout all haired skin, usually deep to the sebaceous glands. *Apocrine glands are associated with primary hair follicles only* and they tend to be largest in areas with lower hair follicle density, such as mucocutaneous junctions, interdigital spaces, coronet, and dorsal midline. Sweat mixes with sebum to form the protective skin surface film. Apocrine sweat glands function in thermoregulation only in horses and cattle. In other species, the secretion may contribute to scent that is involved in social communication. Apocrine secretion may also provide a means of excreting waste products and secreting immunoglobulins that are present on the skin surface. They are *simple saccular or tubular glands* with a coiled secretory portion and straight duct. The secretory portion is composed of a single row of flat cuboidal to columnar epithelial cells surrounded by a single layer of myoepithelial cells situated between the secretory cells and basal lamina. *The duct empties into the pilary canal usually above the entrance of the sebaceous duct or, rarely, directly to the skin surface.* The name apocrine refers to the mode of secretion, which was originally thought to involve pinching off (apo = off) of a portion of the cell. The existence of an apocrine secretory process has been questioned; however, ultrastructural examination of these glands in humans, pigs, horses, and dogs indicates that *several modes of secretion are involved*, including the apocrine type.

In contrast to apocrine glands, **eccrine (atrichial) sweat glands** are derived from the embryonal epidermis rather then from the primary hair germ and they are located only in specialized areas. They occur in the *footpad of dogs and cats, frog of ungulates, snout of pigs, planum nasolabiale of cattle, and medial surface of the carpus of pigs (carpal glands).* The function of eccrine glands is uncertain. The secretion may be involved with scent signaling and in the footpad of cats it may improve frictional capacity of the paw. Eccrine glands are histologically similar to apocrine glands but their ducts open directly to the skin surface. There is no recent detailed examination of eccrine gland secretion to identify the mode of secretion conclusively.

Subcutis

The deepest layer of the skin is the subcutis. It is composed of lipocytes subdivided into lobules by thin bands of collagen and small vessels. The collagenous septa provide structural support by compartmentalizing the subcutis and anchoring the dermis to the fascial planes deep to the subcutis.

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DERMATOHISTOPATHOLOGY

The pathology of the skin, more than that of any other organ, has a specialized vocabulary because many histopathologic changes are unique to the skin. The following is a glossary of terms used in dermatopathology.

Glossary

Acantholysis is a *loss of cohesion between epidermal cells* resulting in intraepidermal clefts, vesicles and bullae (Fig. 5.1). This process may also involve the outer root sheath of hair follicles. Acantholysis is caused by the breakdown of desmosomes between keratinocytes and is characteristic of the *pemphigus complex*. It can also be the result of the action of proteolytic enzymes released by neutrophils or eosinophils in an inflammatory process.

Acanthosis specifically indicates an *increased thickness of the stratum spinosum*, and is due to hyperplasia and occasionally to hypertrophy of cells of the stratum spinosum. Acanthosis, however, is *often used synonymously with hyperplasia* when referring to the epidermis.

Apoptosis refers to *individual programmed cell death*. It is usually seen in the basal layer but can be seen in any layer of the epidermis. Apoptotic keratinocytes histologically resemble dyskeratotic keratinocytes and are eosinophilic and shrunken. They are sometimes referred to as *apoptotic bodies* (colloid bodies, hyaline bodies, Civatte bodies).

Atrophy, in regard to the epidermis, is decreased thickness of the epidermis due to *decreased thickness of the stratum spinosum*. An early sign of epidermal atrophy is the loss of the rete ridges in areas of skin where they are normally present. *The extremely thin normal epidermis of domestic animals makes atrophy very difficult to detect.* It is

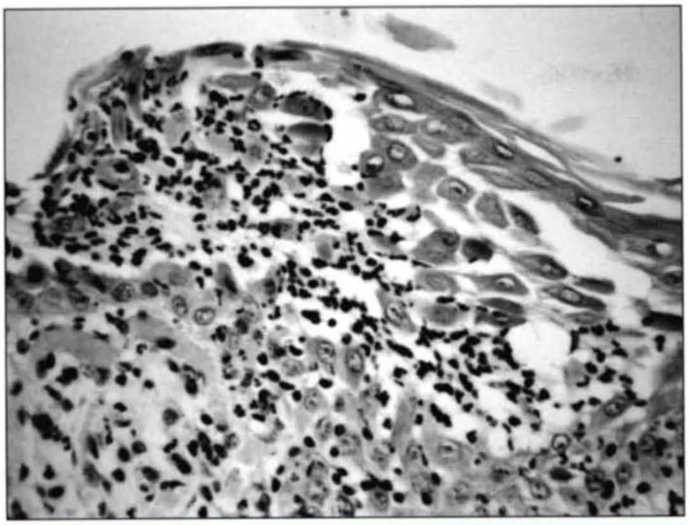

Figure 5.1 Acantholysis in pemphigus foliaceus. Loss of cohesion between keratinocytes leads to individualization of cells. Note cells have normal nuclear morphology.

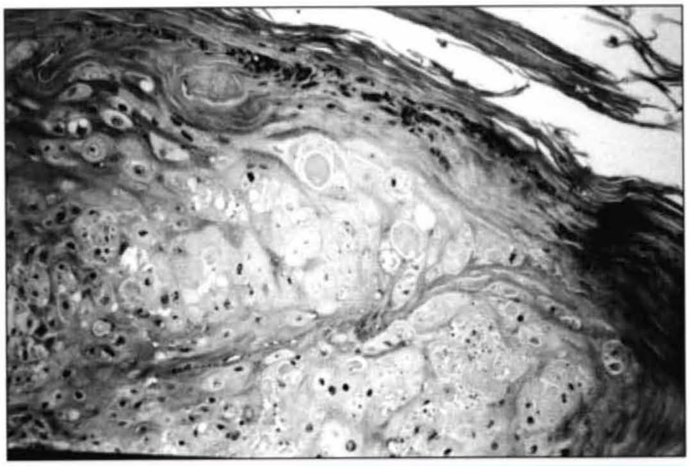

Figure 5.2 Ballooning degeneration in viral dermatitis. Keratinocytes are enlarged and pale due to marked intracellular edema.

seen with endocrine, some immune-mediated dermatoses, and as a result of ischemia. *Dermal atrophy* is thinning of dermal collagen fibrils resulting in decreased dermal thickness, as is seen in some endocrine dermatoses.

Ballooning degeneration of the epidermis is due to *intracellular edema* (Fig. 5.2). It is characterized by swollen eosinophilic cytoplasm, enlarged or condensed nuclei, and a loss of cohesion resulting in acantholysis and sometimes vesicle formation. Ballooning degeneration is a *characteristic feature of viral infections*, particularly of herpesviruses and poxviruses.

Bullae are *collections of fluid* within or below the epidermis greater than 1.0 cm in diameter. They may be caused by severe intercellular or intracellular edema, ballooning degeneration, acantholysis, hydropic degeneration of basal cells, subepidermal edema or other factors resulting in dermoepidermal separation such as the autoantibodies in bullous pemphigoid. Bullae may be *subcorneal, intragranular, suprabasilar or intrabasal, or subepidermal in location.*

Clefts are *slit-like spaces*, which do not contain fluid, within the epidermis or at the dermoepidermal junction. Clefts may be caused by acantholysis or hydropic degeneration of basal cells. However, clefts may also result from handling artifacts.

Collagen degeneration is a *structural and tinctorial change* characterized by slight basophilia, a granular appearance and frayed edges of collagen fibrils, seen with feline, canine and equine eosinophilic granuloma, or other eosinophil-rich conditions, such as mast cell tumors.

Comedo *is a cystically dilated, keratin-filled hair follicle.* Comedones are characteristically seen in Schnauzer comedo syndrome, some endocrine dermatopathies, and actinic dermatitis.

Crusts are consolidated, desiccated surface masses composed of various combinations of keratin, serum, cellular debris and often microorganisms. Crusts are further described on the basis of their composition: *serous* (mostly serum), *hemorrhagic* (mostly blood), *cellular* (mostly inflammatory cells), and *serocellular* (a mixture of serum and inflammatory cells). Crusts should be examined closely for dermatophyte spores and hyphae, *Dermatophilus congolensis*, or large numbers of acantholytic keratinocytes which can be indicators of pemphigus complex. Bacteria are commonly seen in crusts and are not necessarily of diagnostic importance.

Dells are *small depressions or hollows* in the surface of the epidermis independent of adnexal structures. They are usually associated with focal epidermal atrophy and orthokeratotic hyperkeratosis.

Dermal edema is recognized by *dilated lymphatics* (not visible in normal skin), widened spaces between blood vessels and perivascular collagen (*perivascular edema*) or widened spaces between dermal collagen fibers (*interstitial edema*). The dilated lymphatics and widened perivascular and interstitial spaces may or may not contain lightly eosinophilic, homogeneous, proteinaceous fluid. Dermal edema is a common feature of any *inflammatory dermatosis*. Severe edema of the superficial dermis may result in subepidermal vesicles and bullae, necrosis of the overlying epidermis, and predisposition to artifactual dermoepidermal separation during handling and processing of biopsy specimens. Severe edema of the superficial dermis may result in vertical orientation and stretching of collagen fibers, producing the "gossamer" (web-like) collagen effect seen in severe urticaria.

Desmoplasia usually refers to *fibroplasia and collagenous stroma induced by neoplastic processes.*

Dyskeratosis is *premature or abnormal keratinization of individual keratinocytes* in the epidermis or follicular epithelium. Histologically, dyskeratotic cells are eosinophilic and shrunken with condensed, dark-staining nuclei (Fig. 5.3). Dyskeratosis may be seen in a number of dermatoses including lupus erythematosus, erythema multiforme, and graft versus host disease. It can also occur in neoplastic dermatoses, especially papillomas. Dyskeratosis is a feature of the severe epidermal dysplasia that precedes the development of some squamous cell carcinomas.

Dysplasia refers to *faulty or abnormal development* of the epidermis, hair follicles, or any component of the skin. It is an abnormal but nonneoplastic change, however it can accompany neoplastic changes.

Dystrophic mineralization is the deposition of *calcium salts as basophilic, amorphous, granular material along collagen fibrils* as in hyperglucocorticism. Dystrophic mineralization of the hair follicle basement membrane can be seen in hyperglucocorticism, and as a senile change in dogs, especially poodles.

Epidermolytic hyperkeratosis (*granular cell degeneration*) is a change in the stratum granulosum characterized by perinuclear

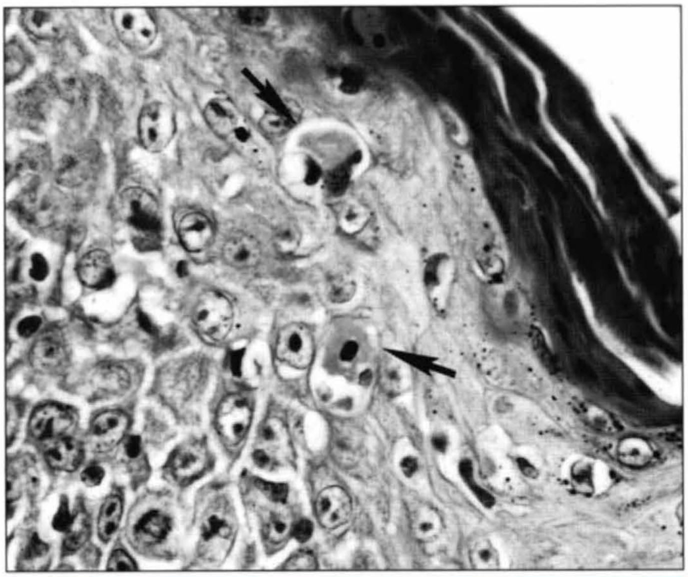

Figure 5.3 Dyskeratosis. Individual keratinocytes have premature or abnormal keratinization. They are shrunken, dark-staining, and have pyknotic nuclei (arrows).

clear halos, large irregular keratohyaline granules, a markedly thickened stratum granulosum, and orthokeratotic hyperkeratosis. It may be seen in certain types of ichthyosis, linear epidermal nevi, or can be an incidental finding in a variety of conditions.

Epidermal mast cells are frequently seen in biopsies from cats with inflammatory dermatoses. They are found within the epidermis as well as the hair follicle outer root sheath, and are most commonly found in diseases associated with tissue eosinophilia, such as feline eosinophilic plaque and feline eosinophilic granuloma.

Exocytosis *is the migration of inflammatory cells and/or erythrocytes through the intercellular spaces of the epidermis.* Exocytosis of inflammatory cells is a common feature of any inflammatory dermatosis. Exocytosis of neutrophils implies an infectious process, whereas exocytosis of eosinophils suggests a hypersensitivity, such as ectoparasitism and feline eosinophilic plaque. Exocytosis of erythrocytes implies purpura, severe vasodilation, or trauma.

Festoons are *dermal papillae, devoid of attached epidermal cells, which project into a vesicle or bulla.* Festoons can be seen in porphyria, bullous pemphigoid and epidermolysis bullosa.

Fibrinoid degeneration is the deposition of fibrin in vessel walls, or in dermal collagen.

Fibroplasia is a reactive process and is the formation and development of fibrous tissue due to increased number of fibroblasts.

Flame figures *are areas of refractile collagen surrounded by eosinophils and eosinophil degranulation products.* In chronic lesions, the eosinophil content decreases, histiocytes increase in number and *palisading granulomas* may be formed. Flame figures may be seen in eosinophilic granuloma, sterile eosinophilic pustulosis, insect/arthropod bite reactions, and other eosinophil-rich conditions.

Flame follicles *are catagen and telogen follicles with pronounced eosinophilic trichilemmal keratin* (Fig. 5.4). These can be seen in endocrinopathies, and are also prominent in normal haired skin of plush-coated breeds of dog, such as the Nordic breeds and Pomeranians.

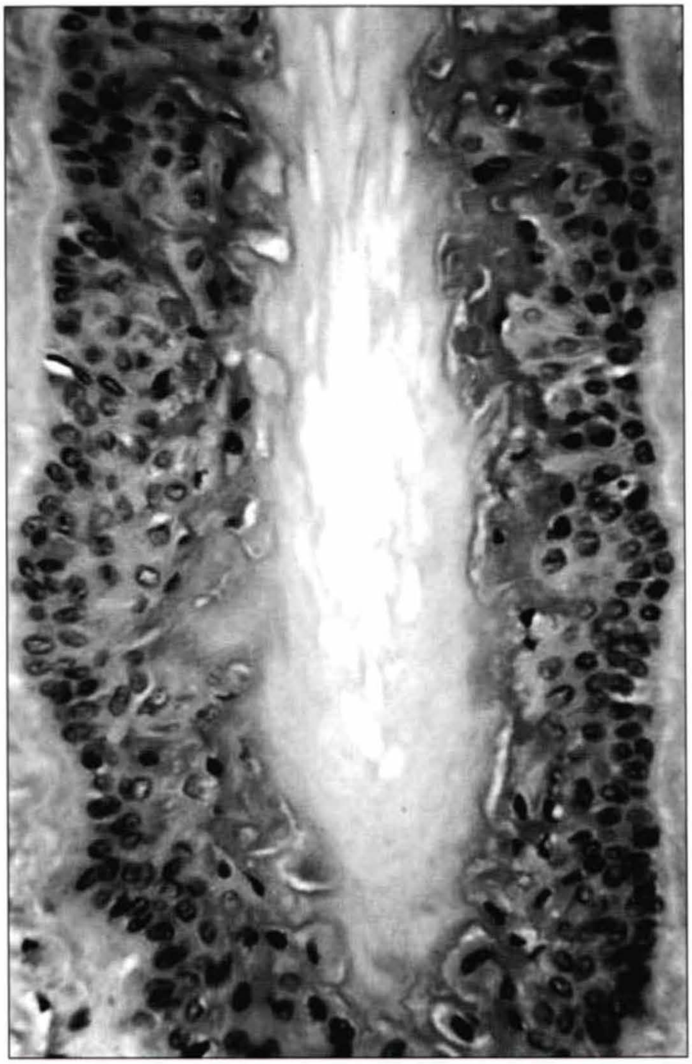

Figure 5.4 Flame follicle. Follicle with pronounced eosinophilic trichilemmal keratinization. The keratin has a serrated border resembling a flickering flame. (Courtesy of B Dunstan.)

Follicular atrophy refers to the *gradual involution and disappearance of hair follicles* characteristic of hormonal dermatoses, follicular dystrophies and ischemia.

Follicular dystrophy indicates *abnormally formed hair follicles.*

Follicular keratosis refers to *keratin-filled hair follicles.* This is a common feature of diverse conditions such as endocrine and many inflammatory dermatoses.

Folliculitis is inflammation of the hair follicle. It can further be divided into *mural folliculitis* (inflammation of the follicular epithelium) (Fig. 5.5A), *luminal folliculitis* (inflammation in the follicular lumen) (Fig. 5.5B), and *perifolliculitis* (inflammation around but not significantly impinging on the follicle) (Fig. 5.5C).

Furunculosis is *inflammation of the hair follicle that has resulted in destruction of the follicular epithelium and release of the luminal contents into the dermis, causing dermal inflammation* (Fig. 5.6). This can be seen in any process that is destructive to hair follicles, such as bacterial infection, dermatophytosis, demodicosis, and trauma.

Granulation tissue formation is a reparative process that is characterized by the presence of fibroblasts, edema, inflammatory cells, and neocapillaries. The blood vessels have plump endothelial cells and

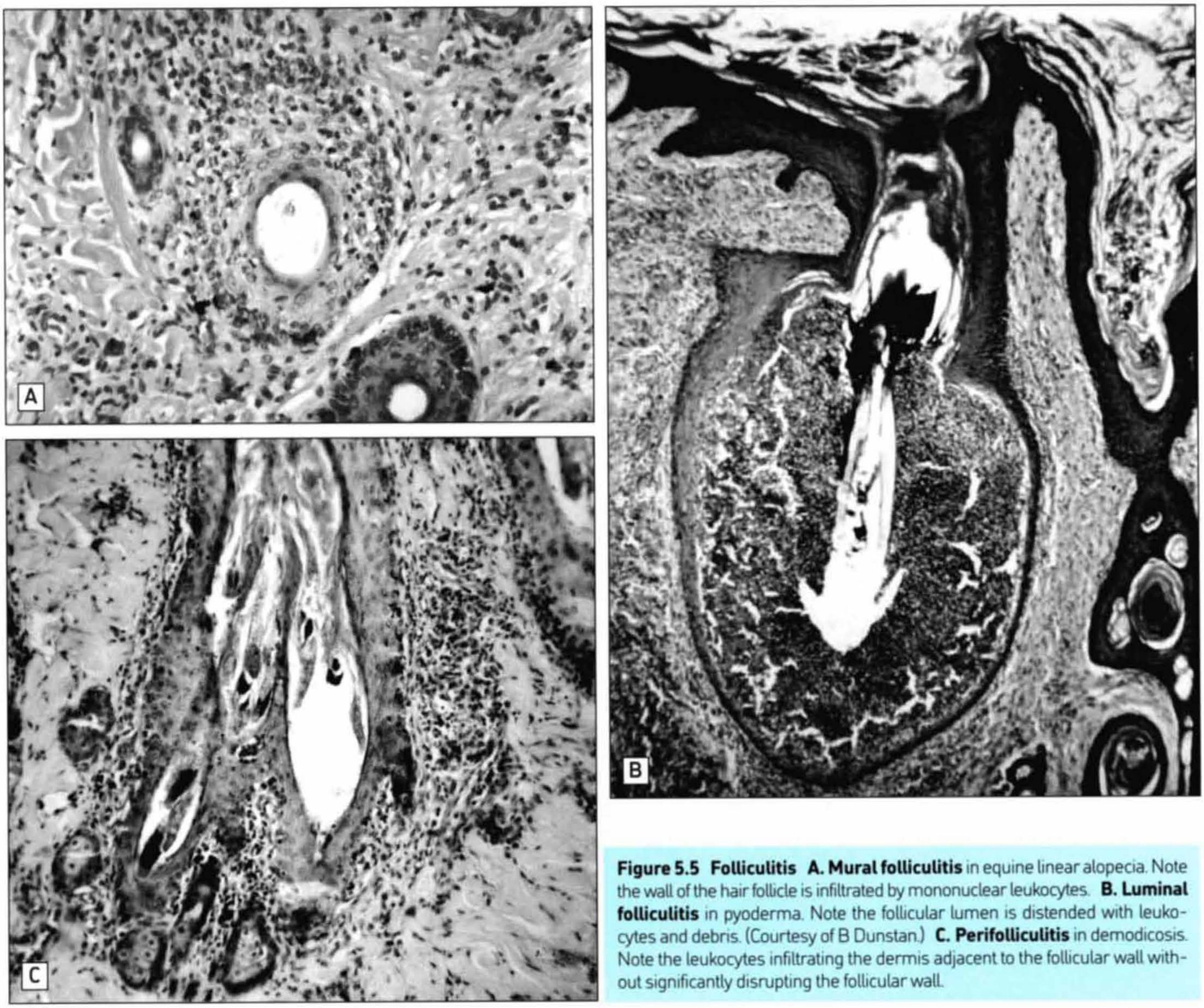

Figure 5.5 Folliculitis A. Mural folliculitis in equine linear alopecia. Note the wall of the hair follicle is infiltrated by mononuclear leukocytes. **B. Luminal folliculitis** in pyoderma. Note the follicular lumen is distended with leukocytes and debris. (Courtesy of B Dunstan.) **C. Perifolliculitis** in demodicosis. Note the leukocytes infiltrating the dermis adjacent to the follicular wall without significantly disrupting the follicular wall.

are oriented perpendicular to the surface of the skin. Fibroblasts and collagen fibrils are oriented parallel to the surface of the skin.

Grenz zone is a *zone of relatively normal collagen that separates the epidermis from an underlying dermal alteration.* A Grenz zone may be seen in neoplastic conditions, such as plasma cell tumors, and granulomatous disorders.

Hamartoma is *a tumor-like malformation composed of an abnormal mixture of normal tissue elements or an abnormal proportion of a single element.* Unlike a **choristoma**, the components of a hamartoma are normal to the location. By definition, hamartomas are congenital lesions, however they may not be detected until later in life and the term is often used interchangeably with nevus, which can be congenital or tardive in onset.

Hidradenitis *is inflammation of apocrine (peritrichial) sweat glands.* These glands commonly become involved secondarily in suppurative and granulomatous dermatoses. Periglandular accumulation of plasma cells is commonly seen in chronic infections, lichenoid dermatoses, and acral lick dermatitis.

Horn cysts (keratin cysts) are circular cystic structures surrounded by flattened epidermal cells. *They contain concentrically arranged lamellar keratin.* Horn cysts are features of a number of follicular and epithelial tumors. **Pseudo-horn cysts** are keratin-filled, cyst-like structures formed by the *irregular invagination of a hyperplastic, hyperkeratotic epidermis.* They are seen in numerous hyperplastic or neoplastic epidermal dermatoses.

Horn pearls (squamous pearls, keratin pearls) are focal, circular, *concentric layers of squamous cells showing gradual keratinization toward the center*, often accompanied by cellular atypia and dyskeratosis. Horn pearls are features of squamous cell carcinoma, keratoacanthoma and pseudocarcinomatous hyperplasia.

Hyalin is an eosinophilic, glassy, homogeneous, refractile material composed of *type IV collagen.* The term is often used as an adjective in descriptions (**hyaline**).

Hypergranulosis indicates *increased thickness of the stratum granulosum*, often accompanied by larger, more intensely stained granules. Hypergranulosis may be seen in any dermatosis in which there is epidermal hyperplasia.

Hyperkeratosis, *increased thickness of the stratum corneum*, is a common finding in many chronic dermatoses. It can be either **orthokeratotic** (without nuclei) (Fig. 5.7A), or **parakeratotic** (nuclei

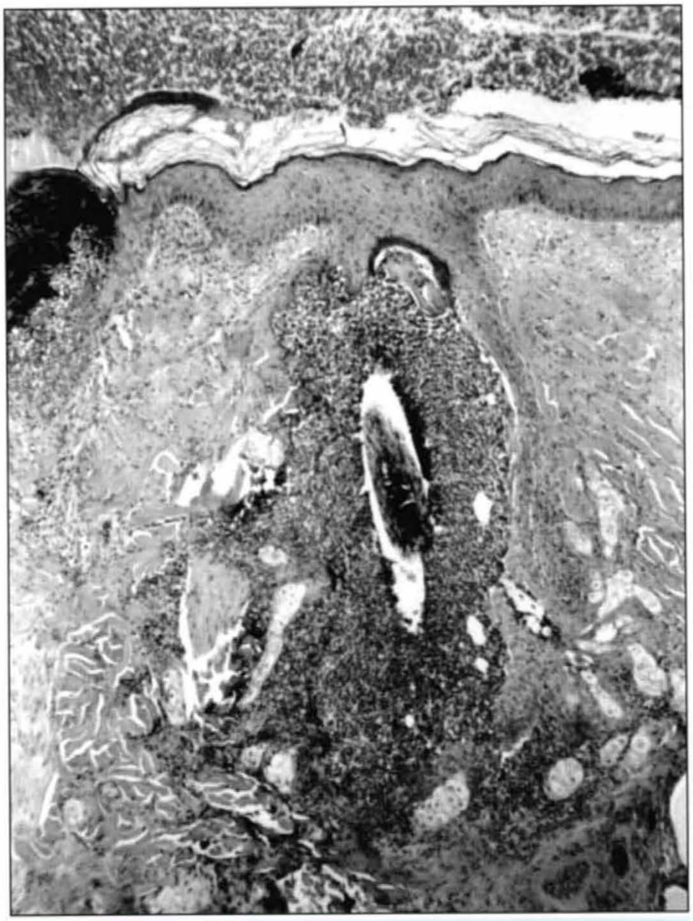

Figure 5.6 Furunculosis in bacterial folliculitis. The wall of the follicle has been perforated and follicular contents are in the dermis.

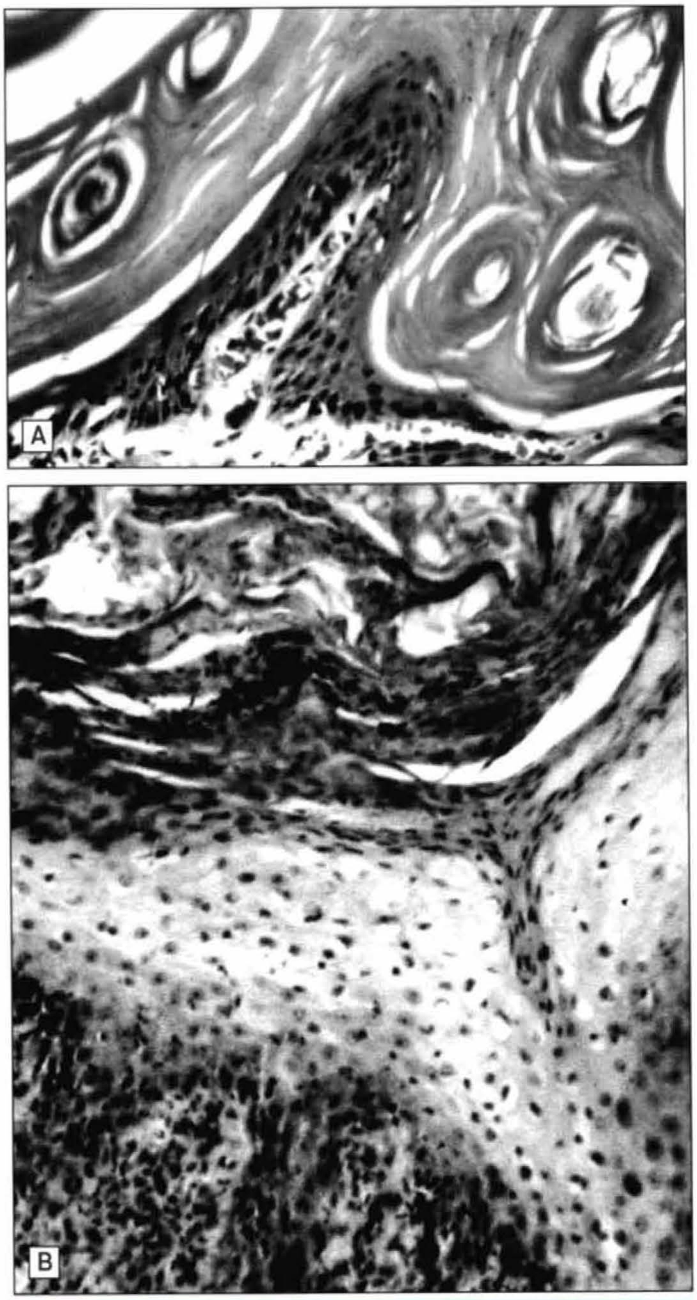

Figure 5.7 A. Orthokeratosis in ichthyosis. Laminated layers of compact anuclear keratin cover the epidermal surface. (Courtesy of B Dunstan.) **B. Parakeratosis** in zinc-responsive dermatosis. A thick layer of keratin with nucleated keratinocytes covers the epidermal surface.

retained) (Fig. 5.7B). *Hyperkeratosis and orthokeratosis are sometimes used synonymously.* Orthokeratosis can be divided into basket weave (the normal pattern in the stratum corneum), compact, and laminated. *Basket weave orthokeratosis* is the most common form, present not only in normal stratum corneum, but also in orthokeratotic disorders such as disorders of keratinization, endocrinopathies and hypersensitivities. *Compact orthokeratosis* can be seen in lichenoid dermatoses, cutaneous horns, and chronic trauma. *Laminated orthokeratosis* characterizes ichthyosis. Orthokeratosis and parakeratosis are not mutually exclusive and are often seen in the same section of hyperkeratotic skin. *Diffuse parakeratosis* can be seen in many chronic dermatoses, especially chronic ectoparasitism, zinc-responsive dermatosis, dermatophilosis, dermatophytosis, or thallotoxicosis. Multifocal parakeratotic caps over the shoulders of follicular ostia is a feature of idiopathic seborrheic dermatitis. Diffuse orthokeratotic hyperkeratosis suggests endocrinopathies, nutritional deficiencies, seborrhea, and ichthyosis. Marked orthokeratotic hyperkeratosis involving the hair follicles suggests vitamin A-responsive dermatosis, acne, Schnauzer comedo syndrome, or endocrine dermatopathies such as hyperadrenocorticism.

Hyperpigmentation refers to *increased melanin within the epidermis and, often, concurrently in dermal melanophages.* Hyperpigmentation may be focal or diffuse, and confined to the stratum basale or present throughout all epidermal layers. It is a common nondiagnostic finding in chronic inflammatory and hormonal dermatoses, as well as in some developmental and neoplastic disorders. Hyperpigmentation must always be cautiously assessed with regard to the animal's normal pigmentation.

Hyperplasia is an increase in the number of cells. In reference to the epidermis, it means an increased thickness due to an increased number of keratinocytes. *Epidermal hyperplasia is a common feature of almost all chronic inflammatory conditions. Acanthosis* is a term often used synonymously with epidermal hyperplasia. Epidermal hyperplasia may be further specified as *irregular* (in which the hyperplastic rete ridges are uneven in shape and height); *regular or psoriasiform* (in which the hyperplastic rete ridges are of even thickness and length); *papillated* (digitate projections of the epidermis above the skin surface); and *pseudocarcinomatous* (extreme, irregular hyperplasia that may

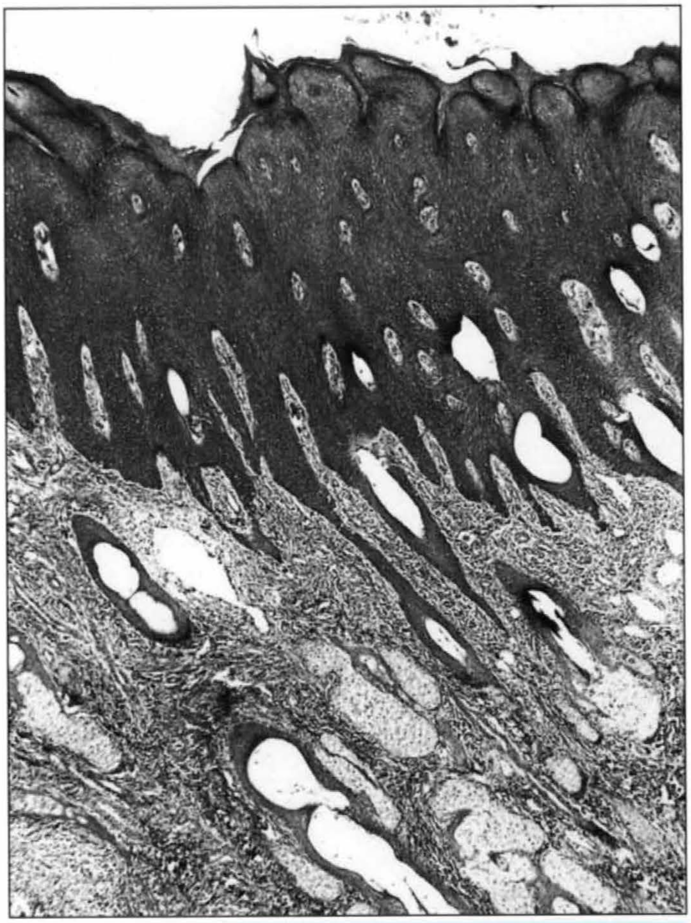

Figure 5.8 Pseudocarcinomatous epidermal hyperplasia. The epidermis has marked, irregular hyperplasia with branched or fused rete pegs. The basement membrane is intact and there is no cellular atypia.

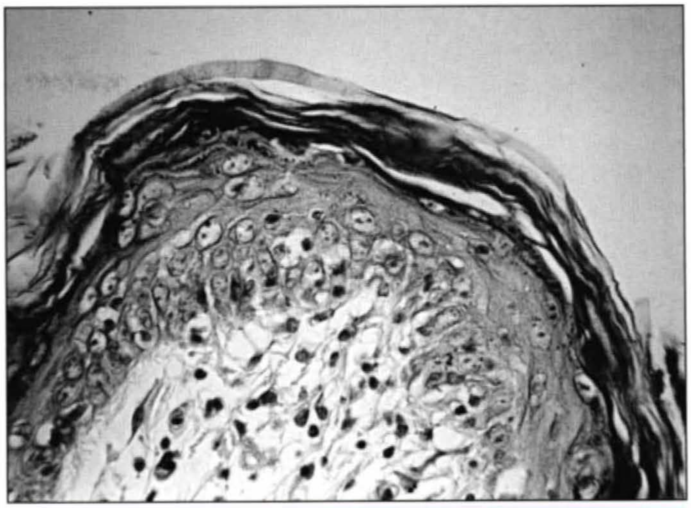

Figure 5.9 Hydropic degeneration. Clear vacuoles are present within and below basal keratinocytes.

demonstrate increased mitotic activity and branched or fused rete pegs) (Fig. 5.8). The process may resemble squamous cell carcinoma; however, there is no cellular atypia, and the basement membrane remains intact. *Irregular epidermal hyperplasia is nonspecific and is the most common type.* Regular epidermal hyperplasia is fairly uncommon and may indicate specific dermatoses, such as lichenoid psoriasiform dermatosis of the Springer Spaniel. Papillated hyperplasia is most commonly seen in seborrheic dermatitis, and in papillomas. Pseudocarcinomatous hyperplasia is most often seen at the margin of a chronic ulcer, or may be associated with underlying chronic dermal suppurative, granulomatous or neoplastic processes.

Hypopigmentation refers to *decreased melanin in the epidermis.* It may be associated with congenital or acquired idiopathic defects in melanization (leukoderma, vitiligo), toxic effects of certain chemicals on melanocytes (e.g., monobenzyl ether of dihydroquinone in rubbers and plastics), inflammatory disorders that affect melanization or destroy melanocytes, hormonal disorders, and dermatoses featuring hydropic degeneration of basal cells (e.g., lupus erythematosus). In those hypopigmented dermatoses associated with hydropic degeneration of basal cells, the underlying superficial dermis usually reveals pigmentary incontinence.

Intracellular edema of the epidermis is characterized by increased size, cytoplasmic pallor, and, sometimes, displacement of the nucleus to the periphery of the affected cell. Intracellular edema of the epidermis may affect cells in a laminar fashion, leading to horizontal layers of edematous keratinocytes. Severe intracellular edema may result in reticular degeneration and intraepidermal vesicles. *Intracellular edema is a common feature of any acute or subacute inflammatory dermatosis.* **Hydropic degeneration** is a specific type of intracellular edema restricted to the basal layer and sometimes the basal cells of the outer root sheath of hair follicles. Hydropic degeneration may result in intrabasal clefts or vesicles, or subepidermal clefts or vesicles due to dermoepidermal separation. It is characterized by clear vacuoles within basal keratinocytes, sometimes accompanied by individual keratinocyte necrosis (Fig. 5.9). **Vacuolar degeneration** is sometimes seen in conjunction with hydropic degeneration and refers to vacuoles above and below the basement membrane zone. Hydropic degeneration of basal cells can be seen in lichenoid dermatitides, drug eruptions, lupoid dermatoses, and dermatomyositis. Caution must be exercised not to confuse freezing artifact or delayed fixation artifact with intracellular edema.

Lymphoid nodules are well-circumscribed, rounded, dense, sometimes perivascular accumulations of predominantly mature lymphocytes in the deep dermis and/or subcutis. They are uncommon and *seen primarily in the cat.* They are seen most frequently in conjunction with immune-mediated dermatoses, dermatoses associated with tissue eosinophilia, and in panniculitis such as injection site panniculitis. They can also be seen in insect-bite granuloma (pseudolymphoma).

Mucinosis (myxedema, myxoid degeneration, mucoid degeneration) is characterized by increased amounts of an amorphous, granular, basophilic material that separates, thins or replaces dermal collagen fibers and surrounds blood vessels and appendages in hematoxylin and eosin-stained sections. Only small amounts of mucin are visible in normal skin, mostly around appendages and blood vessels. Mucin is more easily demonstrated with stains for acid mucopolysaccharides such as Alcian blue and colloidal iron. Mucinous degeneration may be seen as a focal process in numerous inflammatory, neoplastic and developmental dermatoses. Diffuse mucinosis is a feature of normal skin of the Chinese Shar-Pei dog, is occasionally seen in idiopathic mucinosis, and has rarely been reported in hypothyroidism and acromegaly.

Multinucleated keratinocyte. Keratinocytes with multiple nuclei can occasionally be seen in viral infections, such as herpesvirus and FeLV infections.

Munro's microabscess is a small, desiccated accumulation of *neutrophils* within the stratum corneum.

Necrosis is the death of cells or tissues. *Necrotic keratinocytes* are identified by loss of intercellular bridges with resultant rounding-up of the cell, and a normal-sized or swollen eosinophilic cytoplasm. The nucleus becomes pyknotic and cytoplasm becomes eosinophilic and homogeneous, and the cell loses its normal shape. Individual keratinocyte necrosis can occur in drug eruptions, graft-versus-host disease, and interface dermatoses. Necrosis of the epidermis or dermis may be more extensive due to physical and chemical injury, or to interference with vascular supply.

Nests (theques) are well-circumscribed clusters or groups of cells within the epidermis and/or the dermis. Nests are seen in some neoplastic and hamartomatous dermatoses such as melanocytic nevi and melanomas.

Nevus is a focal malformation of the skin, congenital or tardive in onset, consisting of *local excess of one or several of the normal mature constituents of the skin.* The term nevus should always be used with a modifier such as *melanocytic, epidermal, vascular, collagenous, organoid,* etc. The term nevus and hamartoma are often used interchangeably, as, although hamartomas are by definition congenital lesions, they are often not noticed until later in life.

Panniculitis (steatitis) refers to *inflammation of subcutaneous fat.* It can occur without significant involvement of the overlying dermis and epidermis (sterile nodular panniculitis, feline nutritional steatitis, bacterial or fungal panniculitis, lupus erythematosus panniculitis, subcutaneous fat sclerosis), or can be involved by extension of inflammation of the dermis. Fat micropseudocyst formation and lipocytes containing radially arranged needle-shaped clefts can be seen with subcutaneous fat sclerosis and idiopathic sterile panniculitis.

Papillomatosis refers to the projection of dermal papillae and epidermis above the surface of the skin resulting in an irregular undulating configuration of the epidermis. Papillomatosis is often associated with epidermal hyperplasia and is seen with chronic inflammatory and neoplastic dermatoses.

Pautrier's microabscess is a small, focal accumulation of *abnormal lymphoid cells* in the epidermis or follicular epithelium, typical of epitheliotropic lymphoma (Fig. 5.10).

Pigmentary incontinence *refers to the presence of melanin granules free within the subepidermal dermis and within dermal macrophages (melanophages).* It can result from any process that damages the stratum basale, especially hydropic degeneration of basal cells (lichenoid dermatoses, lupus erythematosus, dermatomyositis and erythema multiforme).

Pustules are cavities in the epidermis or follicular epithelium filled with inflammatory cells, usually neutrophils or eosinophils.

Reticular degeneration is caused by severe intracellular edema of epidermal cells. *These cells burst, resulting in multilocular intraepidermal vesicles whose septa are formed by resistant cell walls.* It may be seen with any acute or subacute inflammatory dermatosis, such as acute contact dermatitis.

Satellitosis *refers to individual necrotic keratinocytes in the epidermis surrounded by lymphoid cells (satellite cells)* (Fig. 5.11). It is a characteristic finding in erythema multiforme and occasionally is seen in other interface dermatoses.

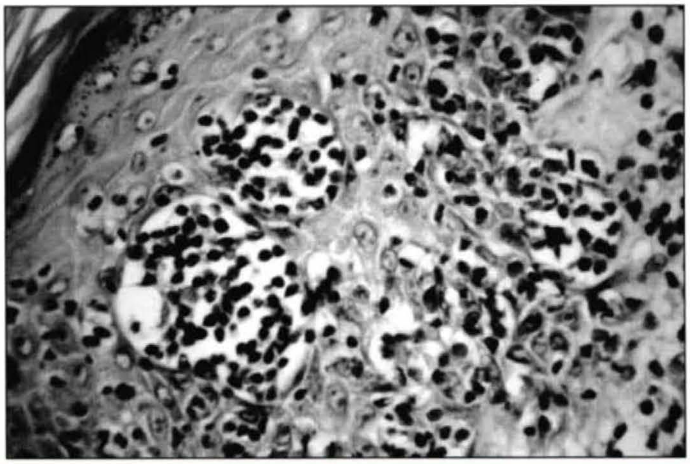

Figure 5.10 Pautrier's microabscess in epidermotropic lymphoma. Nests of neoplastic lymphocytes reside in clear spaces within the epidermis.

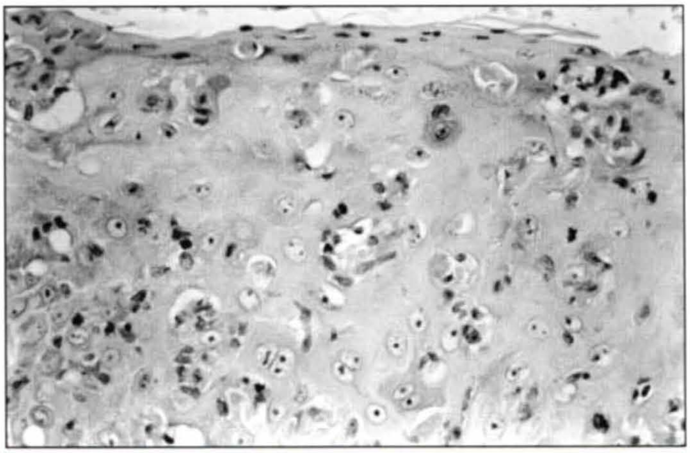

Figure 5.11 Satellitosis in erythema multiforme. Lymphocytes cluster around dyskeratotic, necrotic or apoptotic keratinocytes.

Sclerosis (scar) is the end point of fibrosis. *Increased numbers of collagen fibers* have a thick, eosinophilic, hyalinized appearance and the number of fibroblasts is greatly reduced.

Sebaceous gland hyperplasia refers to increased numbers of sebaceous glands and is common in chronic inflammatory conditions. Nodular aggregates of hyperplastic sebaceous glands are a common senile finding and are frequently multifocal.

Spongiform pustule of Kogoj is *multilocular accumulation of neutrophils within a sponge-like area of the stratum granulosum and stratum spinosum.*

Spongiosis (intercellular edema) of the epidermis is characterized by widening of the intercellular spaces with accentuation of the intercellular bridges, giving the involved epidermis a "spongy" appearance (Fig. 5.12A). Severe intercellular edema may lead to rupture of the intercellular bridges and the formation of *intraepidermal vesicles* (Fig. 5.12B). Severe spongiotic vesicle formation may disrupt the basement membrane zone in some areas, giving the appearance of subepidermal vesicles. Intercellular edema is a common feature of acute or subacute inflammatory dermatoses. Diffuse spongiosis, which also involves the hair follicle outer root sheath, may be seen in

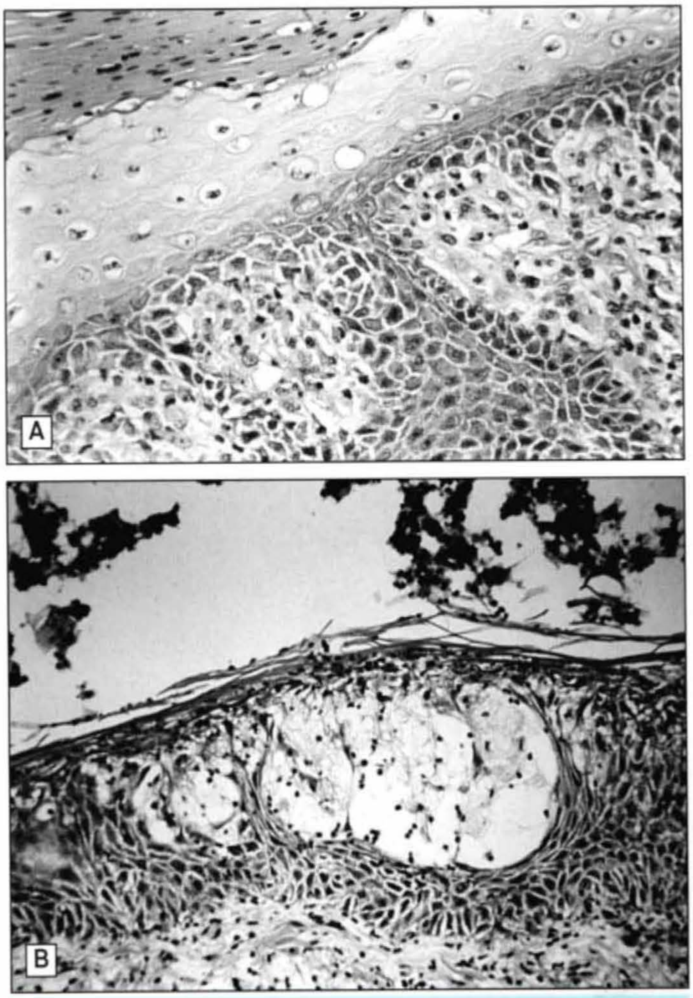

Figure 5.12 A. Spongiosis and laminar intracellular edema in superficial necrolytic dermatitis. Extracellular edema resulting in widening of intercellular spaces between keratinocytes of the deeper epidermis and a linear distribution of pale-staining keratinocytes affected by intracellular edema in the mid-epidermis. **B. Spongiosis with vesicle formation.** Marked intercellular edema has led to breakdown of intercellular bridges and vesicle formation. (Courtesy of B Dunstan.)

other inflammatory disorders including feline eosinophilic plaque or granuloma. Spongiosis of the upper one-half of the epidermis, which is overlaid by marked diffuse parakeratotic hyperkeratosis, is seen in superficial necrolytic dermatitis (hepatocutaneous syndrome).

Squamous eddies *are whorl-like patterns of squamous cells with no atypia, dyskeratosis or central keratinization.* Squamous eddies are features of numerous neoplastic and hyperplastic epidermal disorders.

Transepidermal elimination *is a mechanism by which foreign or altered constituents can be removed from the dermis.* This can be illustrated by the elimination of mineralized collagen across the epidermis and follicular epithelium in calcinosis cutis.

Vesicle is a *fluid filled blister less than 1.0 cm in diameter* in, or immediately below, the epidermis. They may be *subcorneal, suprabasilar, or subepidermal.* When these lesions contain large numbers of inflammatory cells, they may be referred to as **vesicopustules**.

Villus is a dermal papilla covered by one or two layers of epidermal cells that projects into the base of a vesicle or bulla. Villi are seen in pemphigus vulgaris and warty dyskeratoma.

Pattern analysis

The pattern of cell distribution in the skin and the cell types present are extremely important diagnostic clues to many dermatoses. *Pattern analysis is based on recognition of ten different non-neoplastic reaction patterns in the skin at scanning magnification.* At higher magnification, the predominant cell type can be identified and described as *lymphocytic, histiocytic, neutrophilic, eosinophilic, or plasmacytic.* Pattern analysis frequently allows a pathologist to generate a list of differential diagnoses. It is far more efficient to use pattern recognition, and then differentiate between the diseases that demonstrate that pattern, than to learn the histologic features of each disease. It is not unusual to see more than one pattern of inflammation in a biopsy. This can be due to the presence of more than one dermatosis, or due to the presence of both a primary and secondary condition, such as perivascular dermatitis due to a hypersensitivity with a secondary pustular pattern due to pyoderma. Another common combination of patterns would be an overall pattern of perivascular dermatitis (due to hypersensitivity reactions or ectoparasitism) or atrophic dermatosis (due to endocrinopathy) with a subordinate, focal pattern of folliculitis, furunculosis, or intraepidermal pustular dermatitis (due to secondary bacterial infection). It can be difficult to assess the importance of each pattern within the biopsy, and *it becomes very important to recognize which patterns are more specific than others in order to identify the primary condition.* An example would be an interface dermatitis, which is more specific for certain dermatoses than is a perivascular pattern (the least specific).

Perivascular dermatitis

Perivascular dermatitis is the *least specific of the patterns of inflammation,* and it is necessary to observe the type of leukocyte involved and the epidermal changes, in order to create a differential list. In perivascular dermatitis, *the predominant inflammatory reaction is centered around the superficial or deep dermal blood vessels, or both.* Most perivascular dermatitides involve predominantly the superficial dermal blood vessels. Concurrent involvement of the deep dermal blood vessels suggests a systemic disease (infection, immune-mediated). In the horse, most perivascular dermatitides are both superficial and deep. *The primary cause of superficial perivascular dermatitis is a hypersensitivity reaction,* although chronic bacterial infections, viral infections, diseases of altered keratinization, and nutritional deficiencies can all demonstrate this pattern at some point during evolution of the condition. Any perivascular dermatitis containing significant numbers of *eosinophils* should first be suspected of representing an allergic dermatitis such as ectoparasitism, food allergic dermatitis, or atopy. Focal areas of eosinophilic exocytosis and necrosis ("*epidermal nibbles*") are suggestive of ectoparasitism. Other perivascular dermatitides that may contain eosinophils include zinc-responsive dermatosis, equine multisystemic eosinophilic epitheliotropic disease, and chronic pyoderma. Perivascular dermatitis is subdivided on the basis of accompanying epidermal changes into four types:

- In **pure perivascular dermatitis**, there are few or no epidermal changes. The most common dermatoses in this category include acute hypersensitivity reactions and urticaria (Fig. 5.13).
- **Perivascular dermatitis with spongiosis** is characterized by various degrees of spongiosis and spongiotic vesicle formation. Severe spongiotic vesiculation may disrupt the basement

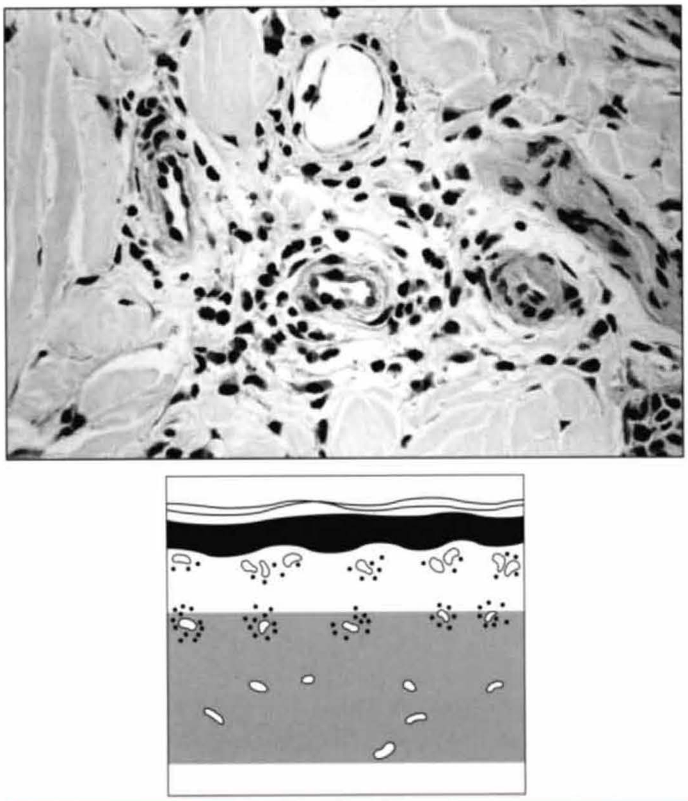

Figure 5.13 Histologic and schematic appearance of **superficial perivascular dermatitis**. *Culicoides* insect bite hypersensitivity. Note leukocytes, in this case eosinophils, surrounding dermal vessels.

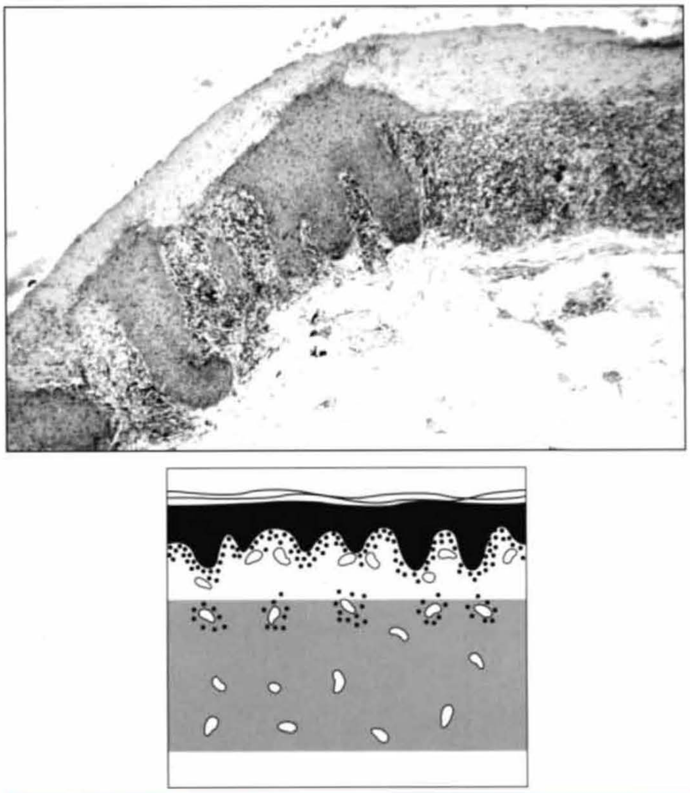

Figure 5.14 Histologic and schematic appearance of **lichenoid interface dermatitis** in discoid lupus erythematosus. Large numbers of lymphocytes and plasma cells abut the epidermal-dermal junction. The basal layer of the epidermis demonstrates apoptosis and vacuolar degeneration of keratinocytes.

membrane zone resulting in subepidermal vesicles. The epidermis usually shows various degrees of hyperkeratosis and hyperplasia. The most common dermatoses in this category include hypersensitivity reactions, acute contact or irritant dermatitis, ectoparasitism, feline eosinophilic plaque, feline miliary dermatitis, and viral infections. When the hair follicle outer root sheath is also involved, feline allergic dermatitides, such as feline eosinophilic plaque and feline eosinophilic granuloma, are suggested.

- **Perivascular dermatitis with epidermal hyperplasia** is characterized by various degrees of epidermal hyperplasia and hyperkeratosis with little or no spongiosis. This is a common, nondiagnostic, chronic reaction pattern. The most common dermatoses in this category are chronic hypersensitivity reactions, diseases of altered keratinization, acral lick dermatitis, and any dermatitis that has undergone chronic irritation and trauma.
- **Perivascular dermatitis with hyperkeratosis** is characterized by various degrees of either orthokeratosis or parakeratosis. The presence of orthokeratosis suggests diseases of altered keratinization (seborrhea) or ichthyosis, and the presence of parakeratosis suggests zinc-responsive dermatosis, chronic ectoparasite hypersensitivity, or *Malassezia* dermatitis.

Interface dermatitis

Interface dermatitis is characterized by damage to the basal layer of keratinocytes, such as basal cell degeneration or necrosis of keratinocytes, that obscures the dermoepidermal junction. This pattern can be divided into interface cell-poor (interface changes with minimal superficial dermal inflammation) or interface lichenoid (interface changes with a lichenoid band of mononuclear inflammation). Pigmentary incontinence and apoptotic bodies are commonly seen in both types.

- The **cell-poor interface pattern** can be seen in diseases such as dermatomyositis, systemic lupus erythematosus, erythema multiforme, drug eruptions, graft-versus-host reactions, bovine viral diarrhea, rinderpest, and bovine pseudo-lumpy skin disease.
- The **lichenoid interface pattern** can be seen with discoid lupus erythematosus, idiopathic lichenoid dermatoses, lichenoid keratoses, Vogt–Koyanagi–Harada-like syndrome (uveodermatologic syndrome), panepidermal pustular pemphigus, lichenoid psoriasiform dermatosis of Springer Spaniels, malignant catarrhal fever, and drug eruptions (Fig. 5.14). The lichenoid band of inflammation is usually lymphocytic and plasmacytic, except in VKH-like syndrome where it is primarily composed of lymphocytes and histiocytes that contain fine melanin granules.

Caution should be exercised in differentiating interface lichenoid inflammation from lichenoid inflammation. The latter refers to a lichenoid band of inflammation, commonly heavily plasmacytic, closely apposed to the dermoepidermal junction, that does not necessarily involve damage to the basal cell layer, such as chronic pyoderma, mucocutaneous pyoderma, and ectoparasitism (scabies or *Cheyletiella*) in dogs. In these cases, the lichenoid band of inflammation can also include neutrophils or eosinophils.

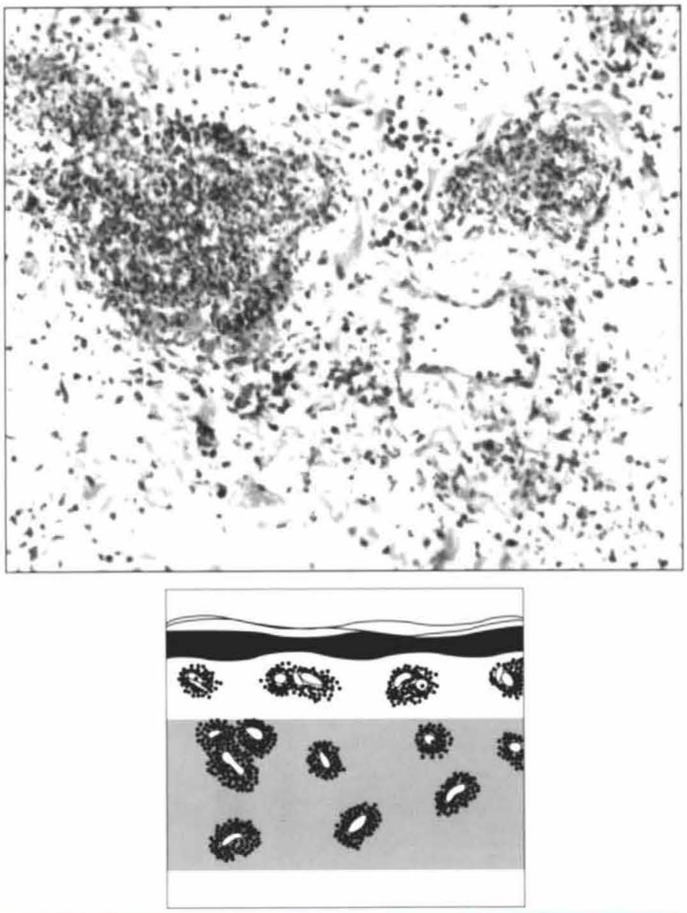

Figure 5.15 Histologic and schematic appearance of **vasculitis**. Leukocytes target vessel walls. Fibrin, erythrocytes and leukocytic debris are in the dermis and vessel walls.

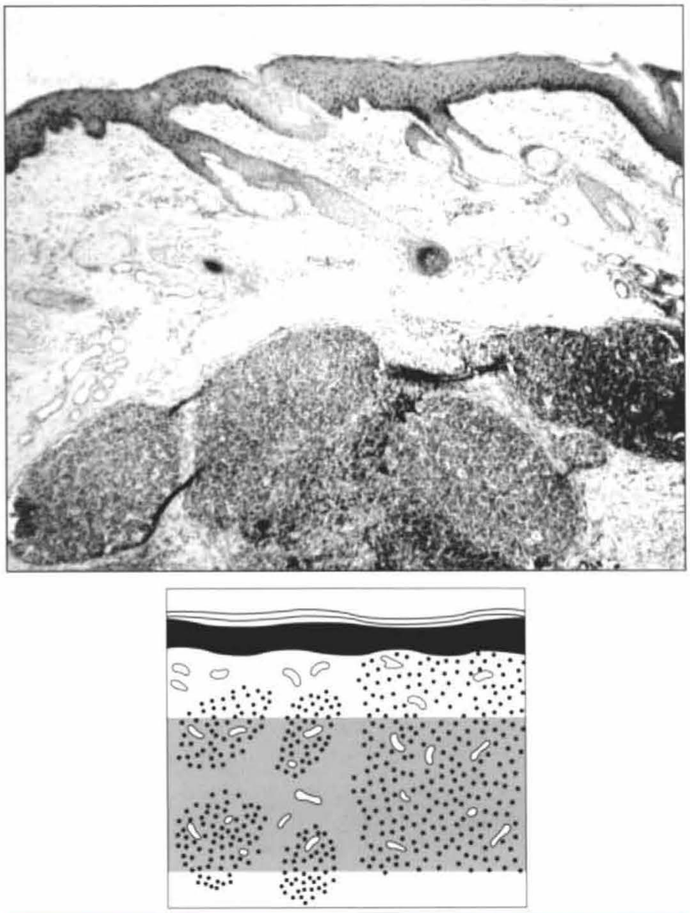

Figure 5.16 Histologic and schematic appearance of **nodular-to-diffuse dermatitis** in fungal dermatitis. Coalescing nodules of lymphocytes, plasma cells, and macrophages obscure the deep dermis.

Vasculitis

Vasculitis is characterized by *inflammation targeting the walls of venules or arterioles*, resulting in at least partial destruction of the vessel wall, sometimes with fibrin deposition (Fig. 5.15). It can be accompanied by fibrinoid necrosis, thrombosis, hemorrhage, and evidence of ischemia. Vasculitis can be immune mediated or septic. *Immune-mediated vasculitis* is due to type III hypersensitivity. The deposition of antigen–antibody complexes in vessel walls activates complement, which results in generation of factors chemotactic for neutrophils. Activation of neutrophils with release of reactive oxygen species and lysosomal enzymes then directly damages vessel walls. *Septic vasculitis* is caused by systemic infection with agents that have a predilection for endothelial cells, resulting in endothelial damage. Vasculitis can also be locally induced by bacterial antigens deposited in vessel walls.

Vasculitis can further be classified on the basis of the *dominant inflammatory cell* within vessel walls. There are neutrophilic, eosinophilic, lymphocytic and mixed types. It should be noted that the inflammatory cell involved often reflects the stage of the disease rather than characterizing a specific disease.

- **Neutrophilic vasculitis** is by far the most common type and may be *leukocytoclastic* (associated with karyorrhexis of neutrophils resulting in "nuclear dust") or *nonleukocytoclastic*. It is seen with connective tissue disorders, hypersensitivity reactions, septicemia, equine purpura hemorrhagica, Rocky Mountain spotted fever, classical swine fever, thrombophlebitis, and as an idiopathic disorder.
- **Lymphocytic vasculitis** may be seen with dermatomyositis, malignant catarrhal fever, vaccine-induced panniculitis, lymphomatoid granulomatosis, and rarely in cutaneous lymphoma. It can also reflect a chronic stage of a vasculitis that was originally neutrophilic.
- **Eosinophilic vasculitis** is rare. It is seen most commonly in lesions induced by arthropod insult, feline eosinophilic granulomas, and rarely in mast cell tumors.

Nodular and diffuse dermatitis

Nodular and diffuse dermatitis is characterized by nodules, or diffuse sheet-like infiltrates of inflammatory cells, in the dermis or subcutis (Fig. 5.16). Nodular and diffuse dermatitis may be characterized by the predominant cell type present (neutrophils, macrophages, lymphocytes, eosinophils, or mixed). The inciting antigen may be an infectious agent, noninfectious material, or the inflammation may be idiopathic. **Neutrophils** predominate in dermal abscesses associated with infectious agents such as bacteria, fungi, algae, and protozoa. They can also be present in sterile lesions, as in foreign body reactions and the sterile pyogranuloma syndrome. **Histiocytes** predominate in *granulomatous inflammation*, which is typically chronic. Granulomatous infiltrates containing large numbers of neutrophils are frequently called *pyogranulomas*. While all granulomatous dermatitis is nodular or diffuse in pattern, not all nodular and diffuse dermatitides are granulomatous.

Granulomas are nodular masses of granulomatous inflammation. They may be subclassified as "tuberculoid" (a central zone of neutrophils and necrosis surrounded by histiocytes, epithelioid macrophages, and giant cells, in turn surrounded by lymphocytes and an outer layer of fibroblasts) or "sarcoidal" (consisting of epithelioid macrophages). *Tuberculoid granulomas* may be seen in tuberculosis, feline leprosy, atypical mycobacterial infection, and *Corynebacterium pseudotuberculosis* infections. *Sarcoidal granulomas* may be seen in sterile sarcoidal granulomas and foreign body reactions. *"Palisading" granulomas* are characterized by the alignment of histiocytes like staves around a central focus of collagen degeneration (feline, canine, and equine eosinophilic granuloma, equine mastocytoma), parasite or fungus (habronemiasis, pythiosis, conidiobolomycosis, basidiobolomycosis, demodicosis), lipids (xanthoma), or other foreign material (e.g., calcium as in dystrophic calcinosis cutis and calcinosis circumscripta). Granulomas and pyogranulomas that track hair follicles resulting in large, vertically oriented ("sausage-shaped") lesions are seen in sterile granuloma/pyogranuloma syndrome of dogs and cats, or can be seen together with folliculitis due to the presence of intrafollicular antigens such as dermatophytes.

Nodular and diffuse dermatitis is often associated with certain unusual inflammatory cell types. **Foam cells** are histiocytes with vacuolated cytoplasm due to their contents (lipid, debris, microorganisms). **Epithelioid macrophages** are histiocytes with elongated or oval vesicular nuclei and abundant finely granular, eosinophilic cytoplasm with ill-defined cell borders. **Multinucleated giant cells** are histiocytic variants that assume three morphologic forms: *Langhans* (nuclei form a circle or semicircle at the periphery of the cell), *foreign-body* (nuclei are scattered throughout the cytoplasm), and *Touton* (nuclei form a wreath which surrounds a central, homogeneous, amphophilic core of cytoplasm that is, in turn, surrounded by abundant foamy cytoplasm). In general, these three forms of giant cells have little diagnostic specificity, although the Touton variety is strongly indicative of xanthomas, and the Langhans type suggests the need for an acid-fast stain. **Eosinophils** may predominate in feline, canine, and equine eosinophilic granuloma, in certain parasitic dermatoses (habronemiasis, elaeophoriasis, parafilariasis, dirofilariasis, dracunculiasis), furunculosis, and in hairy vetch toxicosis. Mixed cellular infiltrates are most commonly neutrophils and macrophages (pyogranuloma), or eosinophils and macrophages (eosinophilic granuloma), or a combination of the three cell types. **Plasma cells** are common components of nodular and diffuse dermatitis in domestic animals and are of no particular diagnostic significance. They may contain eosinophilic, intracytoplasmic inclusions, which are called *Russell bodies*. These accumulations of glycoprotein are largely globulin and may be large enough to displace the cell nucleus. Reactions to ruptured hair follicles are a common cause of nodular and diffuse pyogranulomatous dermatitis in domestic animals, and any such lesion should be examined for keratinous and epithelial debris. All other nodular and diffuse dermatitides should be cultured, examined in polarized light for foreign material and stained for bacteria and fungi. In general, microorganisms are most likely to be found near areas of suppuration and necrosis.

Intraepidermal vesicular and pustular dermatitis

Intraepidermal vesicles and pustules can be caused by intercellular edema which can be seen in any acute or subacute dermatosis, viral infections, hydropic degeneration of keratinocytes, and by acantholysis (Fig. 5.17). It can be useful to subdivide this category on the basis of the site of the vesicle or pustule within the epidermis.

- *Subcorneal pustules and vesicles* are most commonly seen in pemphigus foliaceus, pustules associated with superficial pyoderma, and eosinophilic pustules due to hypersensitivities.
- *Pustules and vesicles in the stratum spinosum* are most commonly seen in the pemphigus complex, viral diseases, and occasionally in hepatocutaneous syndrome.
- *Suprabasilar pustules and vesicles* are a feature of pemphigus vulgaris.

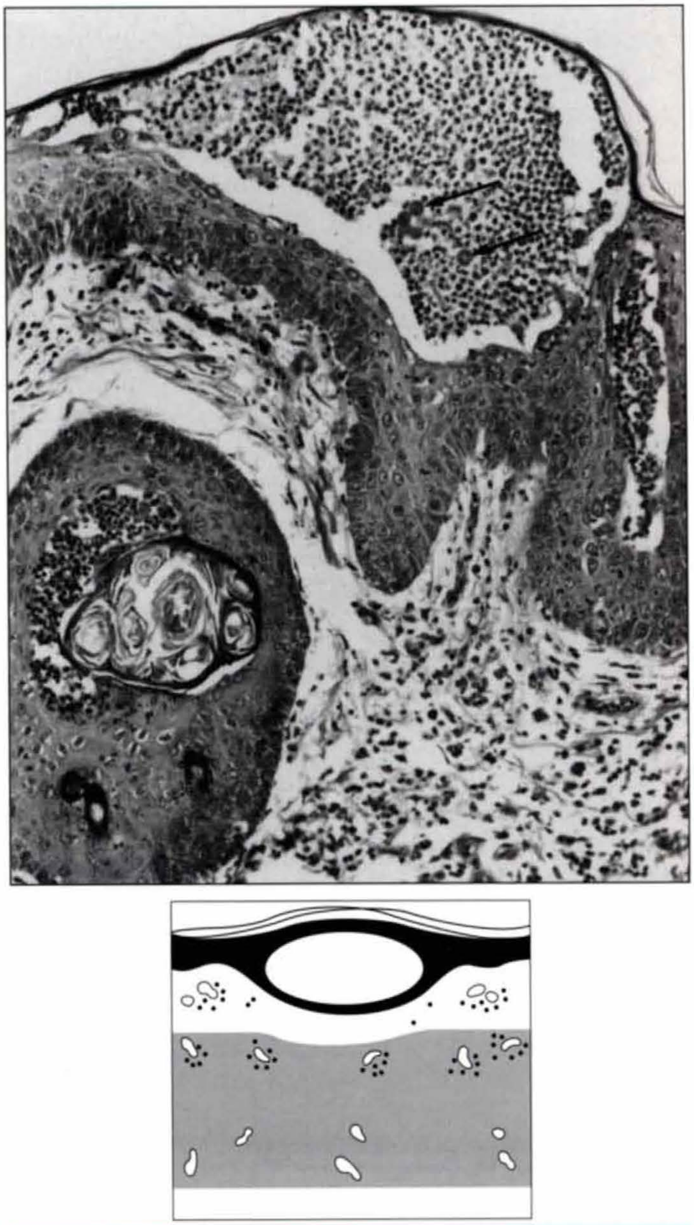

Figure 5.17 Histologic and schematic appearance of **intra-epidermal vesicular/pustular dermatitis** in pemphigus foliaceus. A collection of leukocytes and acantholytic keratinocytes (arrows) are within a subcorneal pustule. The follicular epithelium is also involved.

- *Intrabasilar vesicles* can be seen in lupus erythematosus, dermatomyositis, erythema multiforme, graft-versus-host disease, and toxic epidermal necrolysis.

Subepidermal vesicular and pustular dermatitis

This pattern is characterized by separation of the epidermis from the dermis (Fig. 5.18). Subepidermal vesicles and pustules may be formed through hydropic degeneration of basal cells, dermoepidermal separation, severe subepidermal edema and/or cellular infiltration, and severe intercellular edema with disruption of the basement membrane zone. Caution is warranted when examining older lesions, as re-epithelialization may result in subepidermal vesicles and pustules assuming an intraepidermal location as re-epithelialization forms a pseudo-base to a vesicle. Such re-epithelialization is usually recognized as a single layer of flattened, elongated basal epidermal cells at the base of the vesicle or pustule.

Perifolliculitis, folliculitis, and furunculosis

Perifolliculitis (see Fig. 5.5C) means accumulation of inflammatory cells around a hair follicle in which the inflammation does not significantly impinge on the follicular epithelium. It can be accompanied by mild follicular spongiosis. **Mural folliculitis** (see Fig. 5.5A) is characterized by inflammation which targets the wall of the follicle. **Luminal folliculitis** (see Fig. 5.5B) implies the accumulation of inflammatory cells within follicular lumina. **Furunculosis** (see Fig. 5.6) occurs when the hair follicle ruptures releasing the contents into the dermis. *Perifolliculitis, mural and luminal folliculitis, and furunculosis usually represent a pathologic continuum and all may be present in the same specimen.* Follicular inflammation is a common gross and microscopic finding in dogs, and is less common in other species. It can be caused by intrafollicular bacteria, dermatophytes, and parasites such as *Demodex* spp., *Pelodera strongyloides, and Stephanofilaria* spp. It can also be secondary to pruritus caused by atopy, food allergic dermatitis, ectoparasites, and keratinization disorders such as seborrhea. The folliculitides associated with bacteria, fungi and parasites are usually suppurative initially, whereas those associated with atopy, food allergy, and seborrheic dermatitis are usually predominantly initially spongiotic (small numbers of exocytosing mononuclear cells and/or neutrophils). Any chronic folliculitis, particularly where there is furunculosis, can become pyogranulomatous or granulomatous.

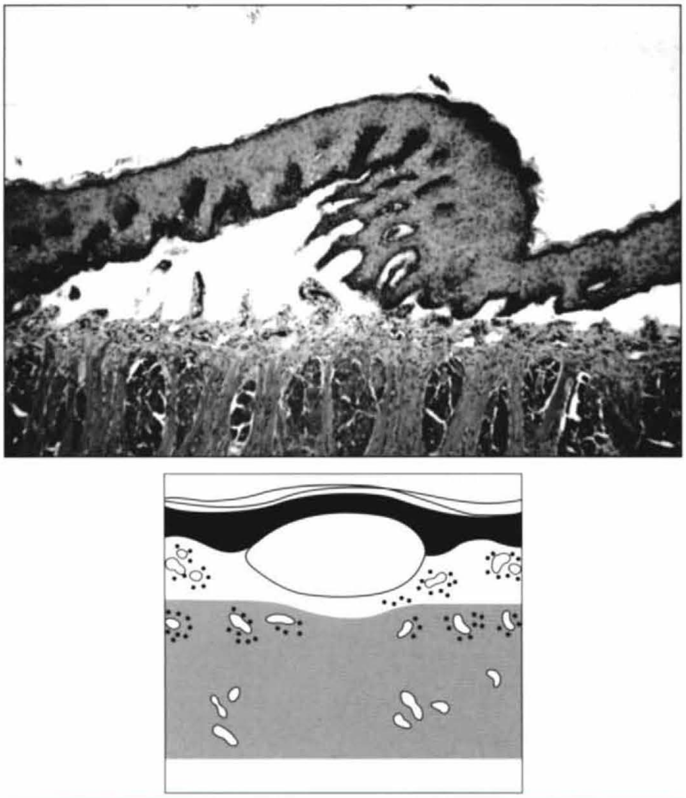

Figure 5.18 Histologic and schematic appearance of **sub-epidermal vesicular dermatitis** in epidermolysis bullosa. The epidermis is separated at the dermal–epidermal junction. (Courtesy of B Dunstan.)

Furunculosis, regardless of the initiating cause, is frequently associated with locally large numbers of eosinophils presumably due to a reaction to released keratin. Idiopathic sterile eosinophilic folliculitides may be seen in cattle and dogs (sterile eosinophilic pustulosis). Insect stings are postulated as the cause of eosinophilic folliculitides affecting the nose of dogs. In cats and horses, sterile eosinophilic folliculitis may be seen in conjunction with hypersensitivity reactions (mosquito bite hypersensitivity, atopy, food allergy, onchocerciasis, equine eosinophilic granuloma, *Culicoides* hypersensitivity, flea bite hypersensitivity). Feline herpesviral dermatitis can also result in eosinophilic folliculitis and furunculosis.

Mural folliculitis can be seen in the pemphigus complex, and these disorders should be considered if there is marked acantholysis. The hair follicle outer root sheath may be involved in hydropic degeneration and lichenoid cellular infiltrates of lupus erythematosus, drug eruptions, erythema multiforme and idiopathic lichenoid dermatoses.

Lymphocytic peribulbitis directed at the bulb of anagen hair follicles is characteristic of alopecia areata. Perifollicular fibrosis is commonly seen in chronic folliculitides, dermatomyositis and chronic granulomatous sebaceous adenitis.

Fibrosing dermatitis

Fibrosis marks the resolving stage of an intense, destructive inflammatory reaction or signifies an ongoing, more insidious, inflammatory process. Fibrosis which is recognizable histologically does not necessarily produce a visible clinical scar. Ulcers limited to the upper portion of the superficial dermis do not usually result in scarring, whereas virtually all ulcers that extend into the deep dermis result in fibrosis and clinical signs of scarring. *Fibrosing dermatitis follows many severe insults to the dermis and is often of minimal diagnostic value.* Common causes of fibrosing dermatitis include furunculosis, equine exuberant granulation tissue, actinic dermatitis, acral lick dermatitis, scleroderma, and morphea (localized scleroderma).

Panniculitis

Panniculitis is inflammation of subcutaneous fat (Fig. 5.19). This inflammation often also secondarily involves the deep dermis. Likewise, the panniculus can be secondarily involved in deep dermal inflammation. Panniculitis may be caused by infectious agents, foreign bodies, vitamin E deficiency, trauma, pancreatic disease, vasculitis, drug eruption, and lupus erythematosus. However, it is often sterile and idiopathic.

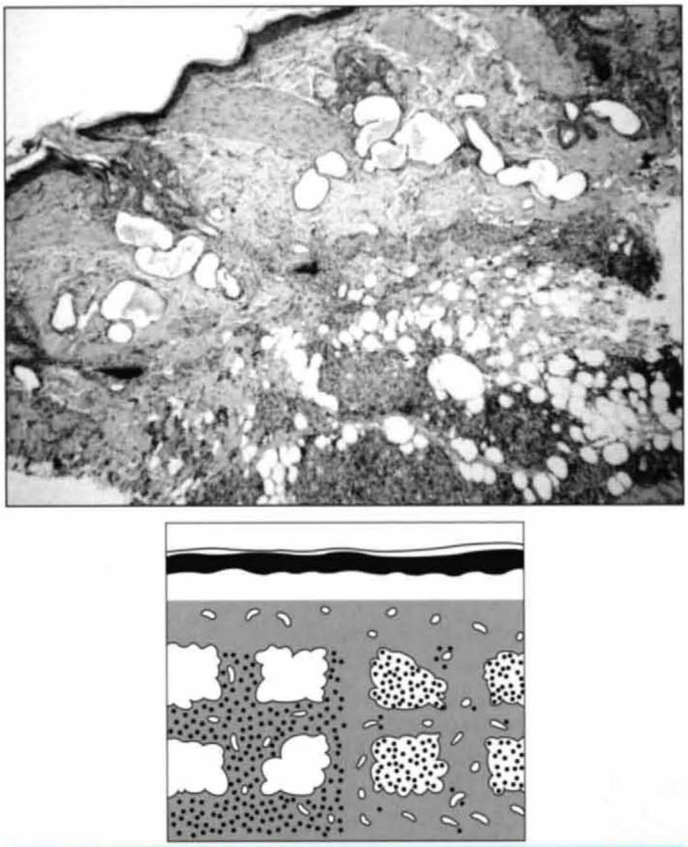

Figure 5.19 Histologic and schematic appearance of **panniculitis**. Inflammation targets the subcutaneous tissues. Note replacement of the adipose tissue with leukocytic aggregates.

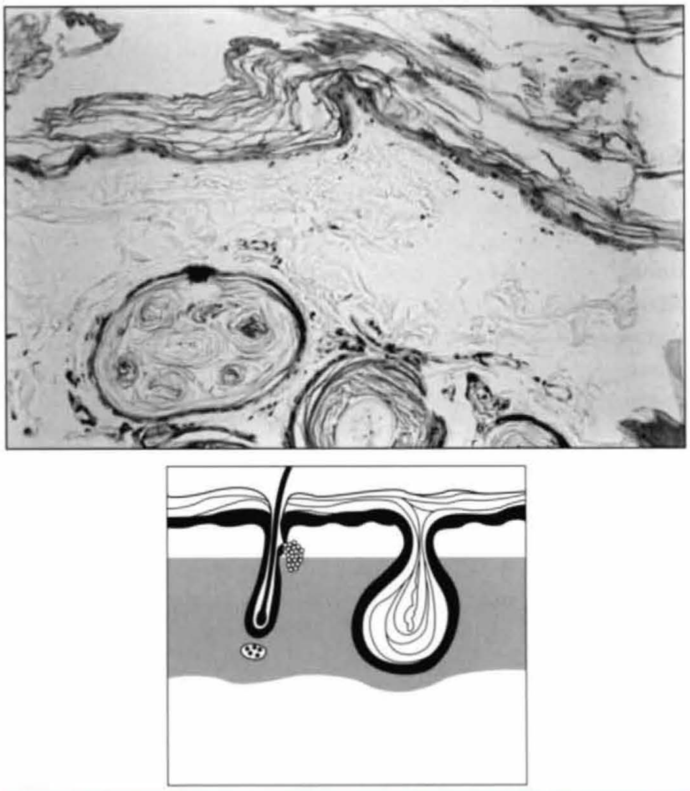

Figure 5.20 Histologic and schematic appearance of **atrophic dermatosis**. The epidermis is 1–2 cells thick, follicles are reduced to small epithelial cell clusters, the dermis has sparse collagen fibers, and sebaceous glands are inconspicuous. In the schematic, note the thin dermis, hyperkeratosis, pilosebaceous atrophy, follicular keratosis and plugging.

Panniculitis can be divided into **lobular** (fat lobules are primarily involved), **septal** (interlobular connective tissue septa are primarily involved), and **diffuse** (both anatomic areas involved) types. However, these anatomical divisions are rarely of diagnostic significance and, in fact, all three patterns may be seen in a single lesion from the same patient. *The majority of inflammatory conditions of the panniculus are granulomatous or pyogranulomatous*, and the histologic appearance of panniculitis due to an infectious agent can be very similar to sterile panniculitis.

In dogs, one form of panniculitis mimics a rare form of lupus erythematosus (lupus erythematosus panniculitis or lupus profundus). It is characterized by lobular hyaline degeneration of fat, lymphocytic vasculitis, lymphoid nodules, and sometimes mucinous degeneration. A similar microscopic lesion occurs in dogs at sites of previous rabies vaccination.

Atrophic dermatosis

Atrophic dermatosis is usually characterized by atrophic changes in the hair follicles and adnexal structures (Fig. 5.20). *It can also refer to atrophy of the epidermis or the dermis. Evaluation of atrophy of the epidermis and dermis should always take into account the site from which the biopsy was taken.* Epidermis is normally thicker on the dorsum than the ventrum, and hair follicles and shafts are larger and closer together on the dorsum than on the ventral or glabrous skin. In addition, familiarity with the various stages of the hair follicle cycle is essential if atrophy of the follicle is to be evaluated. Atrophic dermatosis is often accompanied by some, or all, of the following features: atrophic hair follicles (telogen and catagen follicles are usually predominant), orthokeratotic hyperkeratosis, follicular keratosis, decreased numbers of hair shafts in follicular infundibula, epidermal atrophy, sebaceous gland atrophy, and dermal atrophy (this can be difficult to assess without site-matched controls). Atrophic dermatosis is rarely diagnostic for a specific condition but can suggest a group of diseases such as the *endocrine dermatoses, the most common cause of atrophic dermatosis*. It will almost always be necessary to confirm the identity of the endocrinopathy with endocrinological function tests. Other less common causes of atrophic dermatoses include ischemia, heritable dysplasias such as dermal atrophy in cutaneous asthenia (dermatosparaxis), and paraneoplastic alopecia. Atrophic dermatosis is not an inflammatory pattern, but can be secondarily affected with inflammation, for example, pyoderma superimposed on an endocrine dermatopathy.

Follicular and adnexal atrophy can also be secondary, rather than the primary disease process. An example would be follicular atrophy as a result of a chronic inflammatory process, such as chronic allergic dermatitis, or sebaceous adenitis. In this case there would be histologic evidence of the chronic inflammation previously present, such as scarring.

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CONGENITAL AND HEREDITARY DISEASES OF SKIN

Congenital diseases are those that are present at birth. They may be hereditary or result from other factors that were present during development in utero. Various environmental influences, such as infectious agents, nutrient imbalances, toxic chemicals and plants, and ambient temperature, present during gestation can bring about abnormalities in the skin and hair that are present at birth but are not hereditary. Some congenital diseases are incompatible with life and result in death at birth or shortly thereafter. Congenital abnormalities of the skin may be associated with abnormalities of other tissues or organs. In contrast, although they are genetically determined, some hereditary abnormalities of skin (*genodermatoses*) are not apparent at birth and may instead be manifested later in life, i.e., tardive onset. For example, color dilution alopecia may not be evident until early adulthood. These conditions will be covered elsewhere in the chapter under the appropriate section.

As developments in medical science have reduced the incidence of preventable diseases, an increasing awareness of genetic diseases has developed. New genetic disorders are being recognized at an increasing rate as the degree of diagnostic sophistication of veterinary medicine has grown. This is happening, at least in part, because knowledge from genetic disorders in humans often leads to recognition of similar diseases in animals. Once an analogous genetic disease is identified in animals, affected animals may be used to gain new knowledge regarding the genetics, pathogenesis, or treatment of the condition, thereby producing reciprocal benefits for both human and animal health.

Epitheliogenesis imperfecta

Epitheliogenesis imperfecta (EI) is a congenital condition in which localized or widespread areas of the squamous epithelium of the skin and mucous membranes are absent. It is an uncommon to rare anomaly of calves, piglets, foals, lambs, puppies, and kittens. Lesions consist of sharply demarcated, variably sized defects in the epidermis or mucosa, resulting in exposure of a glistening, red, moist, hairless dermis, or oral or esophageal submucosa (Fig. 5.21). Hooves and nails may be absent or poorly developed; teeth may be malformed; and pinnae may be absent. Congenital defects involving other systems are also present in some affected animals. Fetuses with extensive lesions are generally born dead. Affected animals born live may die in early postnatal life because of infection or septicemia, or secondary to dehydration and electrolyte abnormalities resulting from loss of large amounts of water from the nonepithelialized surfaces. Small lesions heal with cicatrization and may be compatible with life. In all species, *histologic lesions are an abrupt absence of epithelium and a lack of adnexal structures in the dermis or rare rudimentary hair follicles devoid of apocrine and sebaceous glands.*

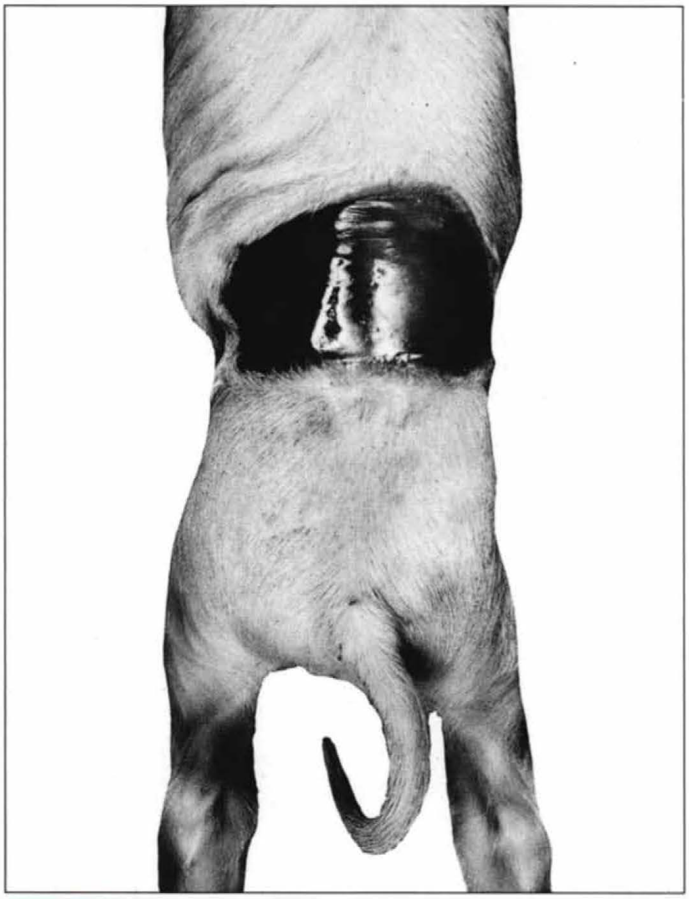

Figure 5.21 Epitheliogenesis imperfecta in a pig.

Epitheliogenesis imperfecta has been reported most often in **calves** and it occurs in many breeds. The condition appears to be an *autosomal recessive trait* and it is seen most often in herds in which there is extensive inbreeding. Both male and female calves are affected. Lesions are usually extensive and involve the extremities most commonly; however, any portion of the body may be affected as well as the squamous epithelium of the muzzle, lips, and oral cavity. Hooves and dewclaws may be missing or incompletely formed. Some affected calves also have deformed teeth and lack pinnae. Brachygnathia and atresia ani have accompanied the cutaneous abnormalities in some calves. Defects in collagen and lipid biosynthesis by dermal fibroblasts have been reported in a calf with epitheliogenesis imperfecta. However, the histologic abnormality seen in this calf was dermal–epidermal clefting; and ultrastructural examination indicated ballooning degeneration and confluent vacuole formation of the basal cells, changes suggestive of epidermolytic epidermolysis bullosa rather than of epitheliogenesis imperfecta.

Epitheliogenesis imperfecta appears to be a recessively inherited trait in **piglets.** Only male piglets were affected in one report and it was thus speculated that the condition is sex-linked. Most piglets have extensive lesions and die shortly after birth. Piglets with small focal lesions may survive but are smaller than normal littermates. At least some affected piglets also have hydroureter and hydronephrosis. Lesions in **foals** usually involve the legs and oral cavity. The proximal esophagus may also be affected and hooves may be lacking. In some instances, teeth are malformed. EI is hereditary in newborn American Saddlebred foals, and is linked to mutation in a gene coding for a laminin subunit protein on equine chromosome 8q. It is unknown, but likely, that EI is genetic in lambs, puppies, and kittens. In **lambs**, epitheliogenesis imperfecta is similar grossly to "red foot," a colloquial term for epidermolysis bullosa, with sloughing of hooves and ulceration of the tongue and hard palate. EI is very rare in **dogs.** In a closed colony of Beagle dogs in which 1500 puppies were born during a 4-year period, only one case occurred, suggesting a spontaneous mutation rather than an inherited anomaly. EI was reported in a litter of Siamese **kittens** with linear ulcers on the tongue.

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Wattles

Wattles (tassels), similar to those seen in goats, occur occasionally in pigs and rarely in sheep. They occur in many breeds of **pigs** with no apparent sex predilection and are inherited as an autosomal dominant trait. Wattles are asymptomatic, cylindrical, teat-like structures that hang from the ventral mandibular region. They are 5–7 cm long and may be unilateral or bilateral. Histologically, they are composed of a core of fibrocartilage, surrounded by fibrous and adipose connective tissue, and covered by haired skin. Wattles have also been reported in Dorset Down, Merino, and Karakul **sheep**. The structures in sheep are smaller than those of pigs and they lack the fibrocartilaginous core seen in wattles of goats and pigs.

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Ichthyosis (congenital disorders of cornification)

Ichthyosis is a heterogeneous group of disorders of cornification that are all characterized by hyperkeratosis and accumulations of scales (Fig. 5.22). The name is derived from the Greek word for fish because of the fish scale-like appearance of the hyperkeratotic skin. Ichthyosis in animals is a rare congenital condition that has been reported in cattle, dogs, pigs, chickens, laboratory mice, and a llama. The disease in humans is classified into four major and several minor forms based on clinical, histologic, cell kinetic, and genetic characteristics. Kinetic studies have shown that two of the major forms, lamellar ichthyosis and epidermolytic hyperkeratosis, are proliferation keratoses resulting from accelerated cell turnover causing abnormal cell maturation and excessive keratin. In contrast, icthyosis vulgaris and X-linked ichthyosis, the other two major forms, are retention hyperkeratoses related to increased cohesiveness between stratum corneum cells resulting in decreased loss of keratin. The biochemical defects in the various types of ichthyosis are still being investigated.

Ichthyosis vulgaris, the most common form in humans, is an autosomal dominant disease characterized histologically by orthokeratotic hyperkeratosis and a decreased or absent granular layer, and ultrastructurally by a delay in dissolution of desmosomes in the stratum corneum and small, abnormal keratohyaline granules. It is produced by a defect in synthesis of filaggrin. **X-linked ichthyosis**, a recessive form in which males have more severe disease than female heterozygotes, is caused by an absence of steroid sulfatase activity. Steroid sulfatase acts on cholesteryl sulfate, a product of lamellar granules or Odland bodies, which is discharged into the intercellular space and is involved in cell cohesion in the lower stratum

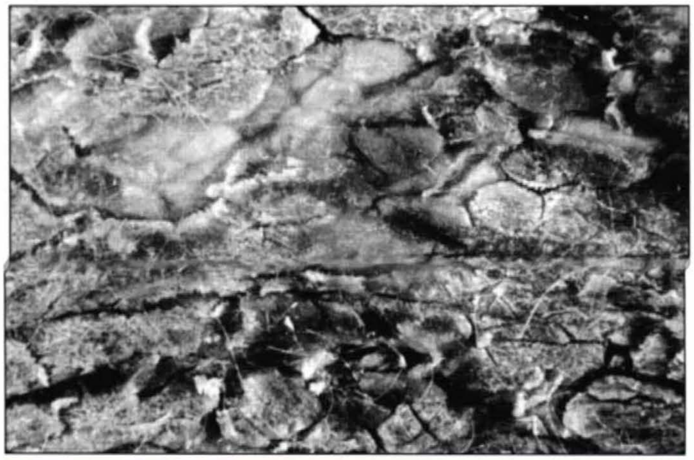

Figure 5.22 Ichthyosis. Note adherent plaques of keratin. (Courtesy of B Dunstan.)

corneum. Failure of the enzyme to inactivate cholesteryl sulfate results in persistent cell cohesion and interferes with the normal process of desquamation. The histologic features of X-linked recessive ichthyosis are orthokeratotic hyperkeratosis with a normal or hyperplastic granular layer. Keratohyaline granules are ultrastructurally normal. **Epidermolytic hyperkeratosis**, also called bullous congenital ichthyosiform erythroderma or bullous ichthyosis, is an autosomal dominant form characterized histologically by orthokeratotic and parakeratotic hyperkeratosis, vacuolation of keratinocytes in the upper stratum spinosum and stratum granulosum, and a markedly thickened granular layer with increased numbers of irregularly shaped keratohyaline granules. Ultrastructural changes consist of increased numbers and clumping of tonofilaments, premature formation of keratohyaline granules, and abnormal association of desmosomes and tonofilaments resulting in acantholysis. The biochemical basis is a defect in the K1 and K10 genes. **Lamellar ichthyosis** is an autosomal recessive ichthyosis characterized histologically by compact orthokeratotic hyperkeratosis and mild acanthosis. It is thought to be caused by a defect of keratinocyte transglutaminase which results in decreased cross-linking of loricrin and involucrin and thus interferes with formation of the cornified cell envelope. **Harlequin ichthyosis** is a rare autosomal recessive, usually fatal form to which some cases of ichthyosis in calves have been compared. In this form of ichthyosis, there is a thick compact stratum corneum with follicular hyperkeratosis and variable appearance of the stratum granulosum. The exact biochemical defect is uncertain but abnormal lamellar granule formation and secretion and a defect in protein phosphatase, which may be involved in processing of filaggrin, have been demonstrated. The majority of cases of ichthyosis in animals have been compared to lamellar ichthyosis of humans based on the clinical and light microscopic features (Fig. 5.23). However, *cases that have been characterized more completely do not correlate well with the human classification system*. The biochemical defect has not been determined in any of the cases of ichthyosis reported in animals thus far.

Ichthyosis has been reported in many breeds of **cattle** and the mode of inheritance appears to be *autosomal recessive*. Both males and females are affected. Two forms of ichthyosis have been described in cattle, *ichthyosis fetalis* and *ichthyosis congenita*. However, the underlying molecular defect(s) is unknown, and it is uncertain whether the forms are distinct diseases or merely represent variations in expression of a single abnormality. *Ichthyosis fetalis* is the more severe form and it has been compared to the harlequin fetus form of ichthyosis in humans. Affected calves are dead at birth or die shortly after birth. The skin is hairless and covered by thick scales divided into plates by deep fissures which represent normal cleavage planes of the skin. The tight, inelastic skin is everted at the mucocutaneous junctions and the ears are usually smaller than normal. *Ichthyosis congenita* is a less severe form compatible with life in which the limbs, abdomen, and muzzle are primarily involved. The skin is dry, hard, and inflexible like old leather and may be prominently folded. There are flat plates of hyperkeratosis in which dense mats of hairs are entrapped. The keratin plaques are separated by shallow hyperemic fissures. The condition is characterized histologically by *prominent laminated orthokeratotic hyperkeratosis of the epidermis and superficial portion of hair follicles*. The epidermal surface is wrinkled or folded and acanthosis is variable.

Ichthyosis has been reported rarely in various purebred and mixed-breed **dogs**. In all cases, parents of affected animals have been clinically normal and thus a *recessive mode of inheritance was suspected*; however, the pattern of inheritance has not been documented by breeding studies. The biochemical abnormality has not been identified in any of the cases, but ultrastructural findings and cell kinetic studies performed in a few dogs with ichthyosis suggested a *proliferative mechanism* for the condition. Lesions are evident at birth; and both male and female pups are affected. The entire body is usually involved but in some cases lesions are limited to the sparsely haired portions of the body. Tightly adherent light tan-gray or parchment-like scales or thick plaques are frequently widely distributed. The skin is variably thickened and may be greasy; and the hair is thin and dull or absent in patches. Some affected dogs are smaller than their normal littermates. Footpads are frequently also hyperkeratotic and appear to be painful. Histologically, there is *marked orthokeratotic hyperkeratosis that includes the infundibular portion of hair follicles*. The epidermal surface is frequently irregular and the epidermis is mildly to moderately hyperplastic. In many of the cases, the granular layer contains an increased number of enlarged and irregular keratohyaline granules and many cells are vacuolated and degenerated leading to microvesicle formation. The dermis is usually normal or has nonspecific inflammation.

Several familial disorders of cornification have been described in dogs. An unusual hereditary condition has been reported in five related *female*

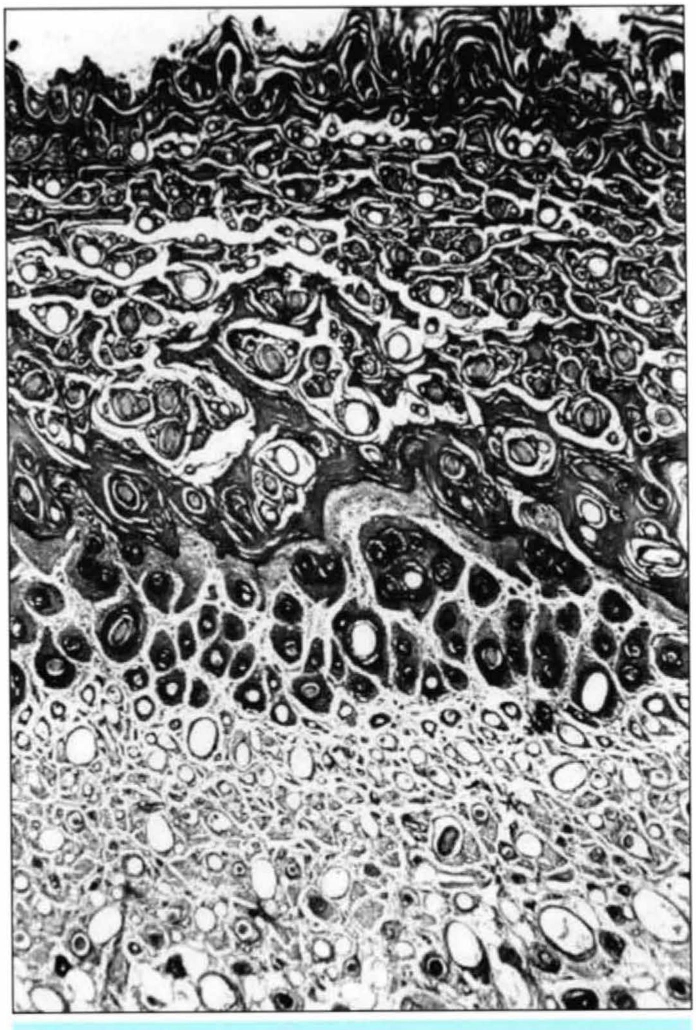

Figure 5.23 Ichthyosis. Note the thick layer of excess keratin adherent to the epidermal surface. (Courtesy of B Dunstan.)

Rottweiler dogs. Since no related males were affected, an X-linked mode of inheritance was suspected. Cutaneous lesions consisted of generalized scaling and hyperkeratotic pigmented plaques. Three dogs also had multiple noncutaneous congenital anomalies. Histologic findings in these dogs included intracellular keratinocyte edema, epidermal vacuoles, and primarily parakeratotic hyperkeratosis of the surface and follicular epidermis. Ultrastructural changes in two of the dogs included large keratohyaline granules, tonofilament clumps, and numerous lipid vacuoles in the stratum corneum and granulosum. The clinical, histologic, and ultrastructural features of this disorder cannot be classified into any single disorder of cornification in humans. *Hereditary footpad hyperkeratosis* has been reported in Irish Terriers and in a family of Dogues de Bordeaux. All footpads become progressively hyperkeratotic, fissured, and painful. Both males and females are affected and lesions are evident by six months of age. The mode of inheritance has not been determined. The histologic features are mild to moderate papillated epidermal hyperplasia and diffuse orthokeratotic hyperkeratosis.

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Hereditary zinc deficiency

Hereditary zinc deficiency occurs in cattle and Bull Terrier dogs and results in multisystemic disease that includes skin lesions. Animals are normal at birth and usually begin developing skin lesions at approximately one to two months of age at which time growth retardation also becomes evident. Skin lesions consist of crusting which is most prominent on the face and distal extremities, dry flaky skin, and hair color fading. A decrease in serum zinc concentration precedes clinical signs; and alkaline phosphatase, a zinc-dependent enzyme, frequently decreases in parallel with the zinc concentration. Affected animals also commonly have diarrhea. Animals with hereditary zinc deficiency have a hypoplastic thymus; and, consequently, secondary infections are common because of associated immune system dysfunction involving both humoral and cell-mediated immunity. The condition is an *autosomal recessive trait* in both cattle and dogs; and it is considered analogous to acrodermatitis enteropathica of humans. *The characteristic histologic lesion is marked diffuse parakeratotic hyperkeratosis*.

Hereditary zinc deficiency in cattle is also called *lethal trait A 46, hereditary parakeratosis, hereditary thymic aplasia*, and *Adema disease*. It affects Friesian cattle and Black Pied Danish cattle of Friesian descent in Europe, and has been reported in Shorthorn cattle in the United States. The condition normally begins with depression, diarrhea, and skin lesions when calves are 4–8 weeks of age. The skin becomes dry and flaky and the hair coat is rough. Patches of erythema, scaling, oozing, crusting, and alopecia begin on the muzzle and then appear around the eyes, ears, and intermandibular space. Similar lesions develop later on the legs; and the skin around the stifles, fetlocks, and coronary bands becomes particularly crusty, fissured, and painful. The flanks, perianal area, and ventral abdomen may also be affected. The hair may lighten in color, a change that may be especially prominent around the eyes and resemble the spectacle lesion of copper deficiency. Affected calves are lethargic, drool, and may have difficulty suckling. They are smaller than unaffected calves of the same age. Conjunctivitis, rhinitis, bronchopneumonia, and other infections are common because of *immune dysfunction*. Untreated calves usually die 4–8 weeks after the onset of clinical disease.

Oral zinc supplementation effects a complete reversal of clinical signs and may restore thymic morphology if instituted early enough. *Intestinal malabsorption of zinc is the cause of the disorder in cattle*. Zinc is absorbed from the intestine by two separate pathways, i.e., a transporter-dependent active system and passive diffusion, and it is the zinc-binding ligand system which is suspected to be defective. A cysteine-rich intestinal protein (CRIP) has been identified in the ligand-dependent pathway and has been suggested as being defective in this condition.

The most striking and consistent gross abnormality is *marked thymic hypoplasia*. Histologic abnormalities include *depletion of small lymphocytes* of the thymus, especially in the cortical region, and hypoplasia of the spleen, lymph nodes, and Peyer's patches. The skin lesions are characterized histologically by *perivascular dermatitis, acanthosis, pallor and vacuolation of the upper spinous and granular layers, and marked diffuse parakeratosis*. Neutrophilic exocytosis and superficial bacterial cocci may be prominent.

Lethal acrodermatitis of Bull Terrier dogs is also thought to be caused by an abnormality in zinc absorption or metabolism; however, zinc supplementation fails to produce clinical improvement. The concentrations of both serum zinc and copper have been found to be lower in affected bull terriers as compared to control dogs, raising the question of the role of copper deficiency in the pathogenesis of the canine disease. The condition is characterized by growth retardation, progressive skin lesions, paronychia, diarrhea, abnormal behavior, bronchopneumonia, and death usually by 18 months. Some affected puppies have lighter pigmentation than their normal littermates and this difference becomes more pronounced with age. By 2 months of age, they are obviously smaller than their normal littermates. Skin lesions usually begin by 6–8 weeks of age and consist of crusty exfoliative lesions involving the distal extremities, footpads, and mucocutaneous junctions, particularly around the eyes and mouth. Digits are prominently splayed and footpads develop cracks and frond-like masses of keratin. The skin is erythematous and moist under the crusts. Interdigital pyoderma and paronychia are common. Many affected dogs also have diarrhea and exhibit abnormal behavior consisting of increased aggressiveness initially, and lethargy and decreased responsiveness later in the course of disease. Respiratory tract infections are common and bronchopneumonia is a common cause of death.

Extracutaneous necropsy lesions consist of a *small or absent thymus* and may also include a high, arched palate and brachygnathia inferior. Histologic changes in the skin are *mild to moderate perivascular dermatitis, moderate to marked acanthosis which may be accompanied by pallor of the superficial epidermis, and marked diffuse parakeratotic hyperkeratosis.* There may also be neutrophilic exocytosis, intraepidermal neutrophilic pustules, and serocellular crusts containing bacterial cocci and/or yeasts. Diagnosis is straightforward if signalment and clinical history are known. If this information is not available, differential diagnoses include superficial necrolytic dermatitis, zinc-responsive dermatosis, and generic dog food dermatosis. However, parakeratosis is less severe in these diseases and superficial epidermal necrolysis is not a feature of lethal acrodermatitis.

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Epidermolysis bullosa

Epidermolysis bullosa is a heterogeneous group of mechanobullous genodermatoses which are all characterized by skin and mucous membrane blistering and ulceration in response to minor mechanical trauma (Fig. 5.24). Lesions are a result of insufficient coherence of the dermal–epidermal junction because of structural defects in the basement membrane zone. The molecular bases for the various forms of epidermolysis bullosa have been discovered only in recent years. The condition is divided into three broad groups based on the ultrastructural level of the skin cleavage, i.e., epidermolysis bullosa simplex (epidermolytic epidermolysis bullosa), junctional epidermolysis bullosa, and dystrophic epidermolysis bullosa (dermolytic epidermolysis bullosa). Epidermolysis bullosa is further classified into more than 20 subtypes in humans based on clinical manifestations of skin lesions, mode of inheritance, and presence or absence of extracutaneous abnormalities, as well as ultrastructural features. The clinical presentation may range from minimal localized involvement of hands and feet to severe, life-threatening generalized blistering with extracutaneous involvement. Corneal erosions, tooth, nail, and hair abnormalities, and tracheal, gastrointestinal, genitourinary, and musculoskeletal involvement occur in various subtypes of epidermolysis bullosa in humans. In animals, epidermolysis bullosa has been reported rarely and, in most instances, has led to the death of affected individuals.

Epidermolysis bullosa simplex is characterized by *cytolysis of the basal keratinocytes,* which produces intraepidermal clefting. This form of the disease is caused by fragility of the epidermal basal cells because of mutations in basal cell-specific keratins 5 and 14. These mutations result in disruption of the assembly, structure, and/or function of the keratin intermediate filaments that act as the skeleton of basal keratinocytes. Ultrastructurally, cytolysis of the basal cells is seen as intra-epidermal clefting. In some forms, cytolysis is preceded by aggregation and clumping of the keratin tonofilaments which are attached to hemidesmosomes. In **junctional epidermolysis bullosa**, clefting occurs within the lamina lucida because of abnormalities of the anchoring filament-hemidesmosome complexes, which may be reduced in number and poorly formed or may be completely absent. Most cases of junctional epidermolysis bullosa are due to a deficiency or abnormality in one of the hemidesmosome-associated proteins laminin-5, collagen XVII (also called BPAG 2 and BP180), or integrin $\alpha6\beta4$, or the extracellular protein LAD-1, which is secreted by epidermal cells and localizes to the upper aspect of the anchoring filaments. **Dystrophic epidermolysis bullosa** is characterized by a split in the superficial dermis below the lamina densa in the region of the anchoring fibrils, which are fewer in number and distorted or completely absent. The molecular cause of dystrophic epidermolysis bullosa is a mutation in the anchoring fibril protein, type VII collagen.

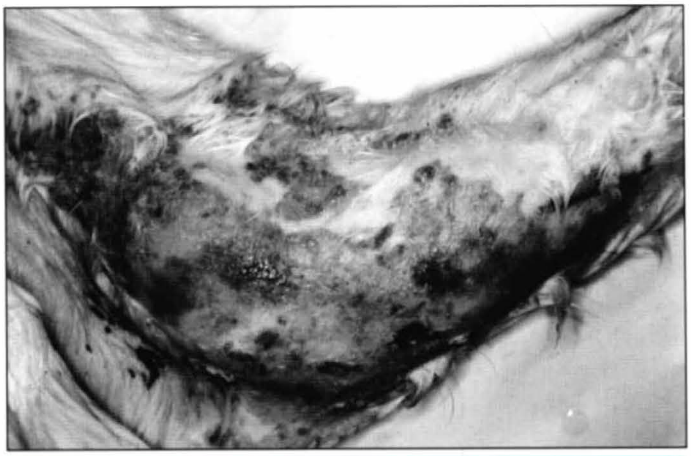

Figure 5.24 Epidermolysis bullosa. An easily detached epidermis led to extensive ulceration in areas exposed to minor mechanical trauma. (Courtesy of B Dunstan.)

Affected animals usually develop lesions shortly after birth; however, in some cases, the disease is not evident until the animal is several months old. *Initial lesions consist of vesicles and bullae, but they quickly rupture and only ulcers may be evident clinically.* Lesions are located in areas of the skin and mucous membranes which are most prone to frictional trauma such as *over bony prominences of the distal extremities, foot pads, lips, tongue, palate, and gingiva.* Hoof sloughing and nail dystrophy and shedding accompany skin lesions in some forms of the disease. Lesions may be induced accidentally by rough handling of an affected animal or intentionally for diagnostic purposes by gentle frictional trauma.

Histologically, most forms of epidermolysis bullosa are characterized by subepidermal clefts and vesicles with minimal inflammation (see Fig. 5.18), changes which are indistinguishable from bullous pemphigoid. Even in the epidermolytic form of epidermolysis bullosa, the cleavage is usually so low in the epidermis that the vesicle appears subepidermal in routine sections. PAS staining to visualize the basement membrane zone may be helpful in determining the level of cleavage, but definitive diagnosis requires ultrastructural examination. Basement membrane antigen mapping by immunofluorescence or immunohistochemistry may be a diagnostic adjunct or alternative to electron microscopy to establish the diagnosis. In epidermolysis bullosa simplex, the PAS-positive basement membrane is at the base of the blister as are type IV collagen, laminin, and BP (bullous pemphigoid) antigen. In junctional epidermolysis bullosa, the PAS-positive basement membrane, type IV collagen, and laminin are at the base of the blister, whereas the BP antigen is primarily on the blister roof. The PAS-positive basement membrane as well as all three basement membrane proteins, i.e., type IV collagen, laminin, and BP antigen, are at the roof of the blister in dystrophic epidermolysis bullosa.

Cattle

A congenital mechanobullous disease, identified as *epidermolysis bullosa simplex*, was described in 25 of 72 calves sired by a single Simmental bull. The condition appeared to be inherited as an *autosomal dominant trait* with high mortality. Lesions were evident in newborn calves and consisted of ulcers of the muzzle, lips, gingiva, dorsum of the tongue, and around joints of distal limbs. Calves were unthrifty, became emaciated after weaning, and developed areas of alopecia, hyperkeratosis, ulcers, and exfoliative dermatitis. Three animals kept under laboratory conditions showed a gradual improvement in severity of lesions but rough handling could still elicit lesions. Histologic changes were typical of epidermolysis bullosa and consisted of *dermal-epidermal separation unassociated with any significant inflammation*. PAS-positive basement membrane was evident on the dermal side of the cleft. In thin sections of skin, cytolysis of the basal keratinocytes was seen but no ultrastructural examination was done to confirm the diagnosis.

A mechanobullous disease suspected to be *dystrophic epidermolysis bullosa* has been reported in Texas Brangus calves with a common bull in their pedigree. The calves developed ulceration of distal limbs and oral mucosa, nasolabial mucosal sloughing, and sloughing of hooves within the first few days of life. The mode of inheritance was suspected to be recessive because of extensive inbreeding. Histologically, the lesions were dermal-epidermal clefts with the PAS-positive basement membrane remaining attached to the basal cell layer, i.e., forming the roof of the bullae. Ultrastructural examination revealed that the lamina densa was attached to the basal layer of the epidermis; inadequate fixation prevented accurate evaluation of the anchoring fibrils.

A *congenital mechanobullous dermatosis of uncertain type* has been described in Angus calves in New Zealand and Murrah buffalo (*Bubalus bubalis*) calves in Brazil. In both reports, skin lesions developed in newborn calves in sites prone to trauma or were induced by trauma. Hoof separation and sloughing were also observed; and in buffalo calves, horns were frequently deformed and partially or completely separated from the underlying corium. Mucous membrane and mucocutaneous involvement was seen in the Angus calves but not in the buffalo calves. The histologic changes seen in the Angus calves were separation of prickle cells and basal cells from each other and sometimes basal cells from the underlying dermis, resulting in *suprabasilar to sub-basilar vesicles* and subsequent shedding of the epithelium. Many basal and prickle cells contained large eosinophilic cytoplasmic bodies. In the Murrah buffalo calves, the characteristic histologic alteration was *suprabasilar clefting* with detachment of the stratum spinosum from the underlying basal layer. Ultrastructural changes in affected areas of skin in the buffalo calves consisted of a loss of desmosomal adhesion between the basal and prickle cell layers. The basal lamina, hemidesmosomes, anchoring fibrils, and anchoring filaments appeared normal. In the Angus calves, desmosomes were lacking or fewer than normal, and many keratinocytes contained a mass of tonofilaments arranged in whorls. Hemidesmosomes were normal. Although the clinical presentation of these cases was typical of epidermolysis bullosa, the histologic and ultrastructural features were unlike those of the common forms of epidermolysis bullosa. However, some variants of epidermolysis bullosa simplex in humans are characterized by cleft formation with acantholysis in the middle or upper epidermis, and in some instances cells contain round clumps within their cytoplasm produced by aggregation of tonofilaments. In both reports, the condition in the calves was suspected to be inherited as an *autosomal recessive* trait.

Sheep

A congenital bullous disease suggestive of *dystrophic epidermolysis bullosa* has been described in Suffolk and South Dorset Down lambs in New Zealand, Scottish Blackface lambs, and Weisses Alpenschaf lambs in Switzerland. Blisters which evolved into ulcers were seen at birth or within the first few weeks of life. Lesions occurred in areas with sparse hair and those prone to frictional trauma such as the muzzle, ears, groin, coronary band, lips, tongue, gums, dental pad, and palate. Lameness and hoof separation and sloughing were common and gave rise to the colloquial name "red foot disease" in Scotland. The lambs grew poorly and were underdeveloped, changes attributed to oral ulceration and reluctance to nurse. Histologic changes were typical of epidermolysis bullosa and consisted of *dermal–epidermal separation with minimal inflammation*. The PAS-positive basement membrane zone remained attached to the epithelium and formed the roof of the vesicle, suggestive of dystrophic epidermolysis bullosa. Only in the Swiss Weisses Alpenschaf has the condition been characterized. Ultrastructural examination of skin from the Swiss lambs indicated that the splitting was below the lamina densa and that anchoring fibrils were absent or rare and rudimentary. Antigen mapping of the lesion identified laminin and type IV collagen at the roof of the cleft, which *confirms sub-lamina densa blistering*; and no type VII collagen, the major structural component of anchoring fibrils, could be identified. These findings are consistent with the dystrophic form of epidermolysis bullosa. The disease was found to have a *recessive mode of inheritance*.

Horses

Junctional epidermolysis bullosa (JEB) has been described in both male and female Belgian foals within the first week or two of life, corresponding to the severe Herlitz form of JEB in humans. Lesions consist of skin and oral mucosal ulceration, most often of the carpi, stifles, hocks, fetlocks, tongue, gingiva, and hard palate. Hoof separation and sloughing (exungulation) are common features. Extracutaneous lesions are rare, and included ocular lesions in one foal and dental dysplasia in another. The histologic lesions consist of *subepidermal clefting with minimal inflammation and PAS-positive basement membrane material evident at the base of the cleft*. Ultrastructurally, the separation is located within the lamina lucida, indicating that hemidesmosomes are underdeveloped. The trait is inherited as an autosomal recessive. The mutation responsible – a cytosine insertion in exon 10 of the LAMC2 gene – has also been identified in JEB phenotype horses in two French draft breeds, the Breton and Comtois.

Dogs

All three forms of epidermolysis bullosa have been reported in dogs. However, the initial cases reported as epidermolysis bullosa simplex in young collies are now thought to represent a mild form of dermatomyositis with unrecognized myositis. *Junctional epidermolysis bullosa* has been described in a newborn Toy Poodle and in three puppies less than 1 month of age from a litter of seven German Shorthaired Pointers. Vesicles, bullae, and ulcers were present on multiple foot pads, in haired skin of frictional sites, and the oral cavity. The lateral hocks, elbows, stifles, and carpi were most commonly involved. No onychodystrophy or dental enamel dysplasia were evident. All of the puppies were euthanized because of the severity of the lesions. A case of *nonlethal junctional epidermolysis bullosa* has been reported in a 4-year-old female mixed-breed dog with a history of vesicles, erosions, crusts, and alopecia since birth. Lesions initially occurred on the lips and ventral abdomen and eventually progressed to involve the face, trunk, tail, and acral regions. Cutaneous atrophy and hyperpigmentation, alopecia, and nail dystrophy developed later. Histologic changes in all affected dogs consisted of subepidermal clefts and vesicles with minimal inflammation and no basal keratinocyte degeneration. The earliest changes seen in sections from the newborn poodle puppy were individual vacuoles in the basement membrane zone. Coalescence of vacuoles led to formation of subepidermal clefts. PAS staining demonstrated the basement membrane at the base of the cleft. Ultrastructural examination in all dogs revealed the separation to be within the lamina lucida. However, changes in hemidesmosomes were variable in the three reports. In the poodle, hemidesmosomes appeared rudimentary in comparison to a normal dog; whereas in the adult mixed-breed dog, hemidesmosomal attachment plaques were hypoplastic and no sub-basal dense plate was apparent. No hemidesmosomal abnormalities were evident in skin sections from the German Shorthaired Pointers. Immunostaining for hemidesmosomal-anchoring filament proteins in the German Shorthaired Pointer puppies indicated that expression of laminin-5 and integrin $\alpha 6\beta 4$ was normal but collagen XVII (BPAG2, BP180) was undetectable. In skin sections from the adult mixed-breed dog, expression of laminin-5, BPAG2, the $\alpha 6$ subunit of integrin $\alpha 6\beta 4$, and type VII collagen was similar to that of normal canine skin.

Dystrophic epidermolysis bullosa has been reported in a 4-year-old female Akita with a life-long history of trauma-induced ulcers and scars over pressure points of limbs and on foot pads. Nail dystrophy was apparent from 1 year of age. The lesions had periodic exacerbations and remissions. The histologic changes were *dermal–epidermal clefting* with minimal inflammation and PAS-positive basement membrane visible at the roof of the cleft. Ultrastructural examination indicated that the separation was beneath the lamina densa and anchoring fibrils were in reduced numbers. Expression of type VII collagen, the major structural protein of anchoring fibrils, was normal. A *dominant mode of inheritance* was speculated in this nonlethal case of epidermolysis bullosa because the dominant form of dystrophic epidermolysis bullosa in humans is typically relatively mild.

Cats

Epidermolysis bullosa appears to be *very rare in cats*. Two cases of dystrophic epidermolysis bullosa have been reported in unrelated cats. The cats were a male domestic shorthair and a Persian female cat. Skin lesions were evident in both cats by the age of 3 months. Lesions consisted of ulceration of multiple foot pads, the palate, oropharynx, gums, tongue, and dorsal spinous skin. Most claws had sloughed and suppurative, ulcerative paronychia affected all four feet. Histologic examination of skin biopsies showed dermal–epidermal separation progressing to ulceration. Type IV collagen was shown by immunohistochemistry to be present at the roof of the blisters. *Sub-lamina densa clefting* was confirmed by electron microscopic examination; and anchoring fibrils were markedly reduced in number and appeared rudimentary and filamentous in skin sections from the Persian cat. Immunofluorescent staining for collagen VII, the primary component of anchoring fibrils, was markedly reduced. *A mutation in the collagen VII encoding gene, COL7A1, was suggested as the cause,* since this gene is mutated in all subsets of dystrophic epidermolysis bullosa in affected human patients.

An undetermined type of epidermolysis bullosa was reported in a 3-year-old male domestic longhaired cat in the United Kingdom. The skin condition had been evident since the cat was 3 months old; a female littermate obtained at the same time showed no skin abnormalities. The affected cat had widespread skin lesions that consisted of scarring, alopecia, crusting, seborrhea, blistering, and ulceration. Several nails had been shed and very few whiskers were present. No lesions were evident in the oral cavity. Histologically, lesions were characterized by dermal–epidermal separation. Ultrastructural examination indicated the cleavage to be above the lamina densa and that hemidesmosomes and anchoring fibrils were fewer and less distinct when compared to skin from a normal cat.

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Canine inherited epidermal acantholysis

A dominantly inherited epidermal acantholytic disease resembling human Hailey–Hailey disease/benign familial chronic pemphigus has been described in English Setters. Hailey–Hailey disease is an autosomal dominant hereditary skin disorder of cellular cohesion characterized by dissociation of epidermal keratinocytes that are subjected to frictional trauma. Lesions in people consist of vesicles and bullae usually located in large skin folds and regions exposed to chronic frictional trauma; mucosal involvement is rare. The histologic characteristics of the condition include *suprabasal clefting and extensive separation of keratinocytes which remain loosely in place*, giving the appearance of a "dilapidated brick wall." Because of the resemblance to autoimmune pemphigus, Hailey–Hailey disease is also called benign familial chronic pemphigus. A widespread intrinsic weakness in keratinocyte adhesion appears to be present but the molecular defect responsible for the disorder is unknown. Desmosomes appear normal in intact skin; structural proteins of desmosomes, adherens junctions, and gap junctions studied so far appear to be expressed normally.

The condition was seen in a 7-month-old male dog and in two of his female offspring, at the ages of 1 and 2 months. Lesions consisted of well-demarcated, alopecic, erythematous, markedly hyperplastic plaques with a rough surface, occasional serous crusting, and peripheral scaling. They occurred on the ventral thorax, head, and stifle. The clinical appearance of the lesions and their location were not typical of the human disease, but the *histologic and ultrastructural features were similar to benign familial chronic pemphigus*. Microscopically, the epidermis was markedly hyperplastic and suprabasal keratinocytes were much larger than those in the perilesional epidermis. Extensive acantholysis resulted in lacuna formation in the suprabasal and upper epidermis and the follicular epithelium. Ultrastructural changes included increased intercellular spaces, perinuclear tonofilament aggregation, and intact desmosome-tonofilament complexes and actin filament in early lesions. Immunohistochemical examination of various desmosomal proteins failed to demonstrate any abnormalities and the molecular defect in these dogs was not identified.

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Congenital hypotrichosis

Congenital hypotrichosis has been described in all domestic species but it occurs most frequently in calves. The hairlessness may be associated with congenital anomalies of other systems such as brachygnathism, dental defects, and thymic or genital abnormalities. Many affected animals are otherwise completely healthy, but some forms of congenital hypotrichosis are associated with ill-thrift and early death. Deliberate propagation of spontaneous mutations producing hairlessness has resulted in development of *specific hairless breeds* such as the Chinese Crested dog, Mexican Hairless dog, American Hairless Terrier, Sphinx cat, and Mexican Hairless pig, among others. The hair coat is an important protective barrier for animals and when it is compromised, as in congenital hypotrichosis, *affected animals are predisposed to sunburn, less tolerant to temperature extremes, and more susceptible to bacterial and fungal infection.*

Hairlessness varies from partial to complete. Partial hypotrichosis is frequently bilaterally symmetrical and the hair that is present is frequently abnormal. It is usually sparse, short and fine, or coarse and wiry, brittle, and easily broken or epilated. Histologic changes are variable, likely a reflection of the different mutations responsible for the hypotrichosis. Some affected animals have only follicular disease; while others have involvement of other skin appendages, in which case the condition is called ectodermal dysplasia.

Genetic hypotrichosis must be differentiated from various causes of non-genetic hypotrichosis. Iodine deficiency can cause goiter and hypotrichosis in piglets, lambs, and calves. Adenohypophyseal hypoplasia in Guernseys and Jerseys, and maternal ingestion of *Veratrum album* by Japanese cattle, have been associated with hairlessness in calves. In addition, alopecia of various degrees has been associated with intrauterine infection with *Bovine viral diarrhea virus* in calves and *Classical swine fever virus* in piglets.

Cattle

Various types of inherited hypotrichosis occur in cattle. Many breeds of cattle are affected and the mode of inheritance varies with the particular form of hypotrichosis. Histologic features are not well characterized for all forms of hypotrichosis in cattle.

A form of **lethal hypotrichosis** occurs in Holstein-Friesian and Japanese native cattle. Calves are born almost hairless and have only small amounts of hair on the muzzle, eyelids, ears, tail, and pasterns. The condition is inherited as a simple autosomal recessive trait, and homozygous calves die within hours after birth. Histologically the skin contains normal numbers of follicles but they are shallow, rudimentary in appearance and do not form hairs. Sebaceous glands and arrector pili muscles appear normal while sweat glands undergo cystic degeneration.

A condition called **semihairlessness** has been reported in polled and horned Hereford calves. Calves have a thin coat of short fine, curly hair at birth and progressively develop a patchy sparse coat of coarse wiry hair which is thicker and longer on legs than elsewhere. The skin is wrinkled and scaly. Animals may not grow well and may have a wild temperament. The condition is a simple autosomal recessive trait. The histologic changes described are dysplastic hair follicles that do not produce hairs. Similar histologic features have been described in **viable hypotrichosis** reported in various breeds of cattle, including Guernseys, Jerseys, Holsteins, Ayrshires, and Herefords. Calves are born with variable degrees of hairlessness. The condition appears to be inherited as a simple autosomal recessive trait in all breeds affected.

Hypotrichosis and anodontia (hypotrichosis anodontia defect, HAD) has been described in male mixed Maine-Anjou-Normandy cross calves and suspected to be a sex-linked recessive trait. Calves are born hairless and toothless and develop a fine, downy hair coat and partial dentition after several months. Affected calves also have a thick protruding tongue, defective horns, and hypoplastic testicles, and they usually do not survive beyond 6 months of age. Histologic changes include small, inactive hair follicles, deformed dermal papillae lacking a vascular network, and degenerative sweat glands. An HAD syndrome, with hypotrichosis, almost complete lack of teeth, and complete absence of eccrine nasolabial glands, has been observed in a family of German Holsteins; similar anomalies in humans are known as X-linked anhidrotic ectodermal dysplasia (ED1). This Holstein phenotype was inherited as a monogenic X-linked recessive trait, and was caused by a deletion in the bovine *ED1* gene.

Hypotrichosis and incisor anodontia (hypotrichosis incisor defect, HID) has been described in Holstein-Friesian cross calves, and inheritance is suspected to be an X-linked incompletely dominant gene. Affected calves have variable areas of thin coat of fine short silky hairs usually involving the face, neck, ears, back, and inner thighs. Eyelashes, vibrissae, and tail brush are usually normal. Calves may become normal with age. Histologically, there are numerous small hairs but large medullated hairs are absent and only telogen follicles are evident in severely affected HID calves.

Inherited epidermal dysplasia, also called **baldy calf syndrome**, is a lethal disease of Holstein- Friesian calves that is likely inherited as a single autosomal recessive trait. The disease causes loss of condition and skin, horn, and hoof lesions that can be confused with inherited zinc deficiency. Calves appear normal at birth; but at one to two months they begin to lose condition despite normal appetite, and develop generalized hair loss and patchy areas of scaly, wrinkled, thickened skin over the neck, shoulders, flanks, and on pressure points. Hooves are elongated, narrow, and pointed and frequently have horizontal rippling. Horns fail to develop and ear tips are curled backwards. Calves have fine slender limbs and drool. They become emaciated and usually die at 6–8 months of age. Histologic examination indicates variable atrophy of adnexa, remnants of hair follicles and sebaceous glands incorporated into the basal layer, and scattered atrophic remnants of apocrine sweat glands.

Congenital hypotrichosis of Hereford cattle is thought to be due to a simple autosomal dominant gene. Alopecia is variable and nonprogressive. Calves have thin, pliable skin, extremely curly facial hair, and may have sparse pellage of thin soft curly, easily broken and epilated hairs, or they may be completely hairless. Some calves also have impaired hoof development. The condition is characterized histologically by hypoplastic or degenerate hair follicles with vacuolation and necrosis of Huxley's and Henle's layers and abnormally large trichohyaline granules in Huxley's layer. Most follicles contain fragmented hair shafts. Arrector pili muscles are reduced in number and frequently not associated with hair shafts. Ultrastructural examination indicates that the giant trichohyaline granules lack normal micro- and macrofilament structures.

A condition consisting of **congenital anemia, dyskeratosis, and progressive alopecia** has also been described in polled Hereford calves in Canada and the USA. Affected calves are often small at birth and have a prominent forehead. They have a hyperkeratotic muzzle with a dirty-faced appearance, and their hair is wiry and kinked or tightly curled and easily epilated. Alopecia is evident initially on the bridge of the nose and ears and it becomes generalized but is most severe on the head, lateral neck, shoulders, and back. The skin of the face and neck is wrinkled and hairless skin is hyperkeratotic. Affected calves also have nonregenerative anemia and fail to grow despite a normal appetite. Histologic abnormalities in the skin consist of orthokeratotic hyperkeratosis and hypergranulosis extending into the infundibular portion of hair follicles and prominent dyskeratosis (apoptosis) of individual epidermal and follicular keratinocytes. Hair follicles are in normal number but many follicles are in telogen phase. There is degeneration of the internal root sheath and atrophy of sebaceous glands. The bone marrow is hyperplastic and characterized by ineffective erythropoiesis with maturation arrest in the late rubricyte stage.

The "**rat-tail syndrome**" is a form of hereditary congenital hypotrichosis that occurs in a small percentage of calves produced by crossing some Continental cattle breeds, e.g., Simmental, with black Angus or Holsteins. The calves have short, curly, malformed, sometimes sparse hair and a lack of normal tail switch development.

Dogs

Congenital hypotrichosis has been reported in *many breeds of dogs*. In most cases, a single puppy of a litter is affected but in some reports several puppies in a litter have been affected. The majority of cases of hypotrichosis in dogs have been in males, and may be referred to as *X-linked ectodermal dysplasia*. Hairlessness may be virtually complete or involve discrete areas that are typically distributed symmetrically. In such cases, areas most commonly affected include the top of the head, ears, ventrum, medial aspect of the legs, and the lumbosacral area. The skin is initially normal but frequently becomes hyperpigmented, hyperkeratotic, and greasy as affected dogs age. Some affected dogs also have sparse, short, wiry whiskers or they may be altogether absent. Eyelashes may also be absent. Some dogs also have missing or misshapen teeth or conjunctivitis. Most cases of hypotrichosis in a single puppy are assumed to be a spontaneous mutation; but X-linked recessive mode of inheritance has been confirmed in a family of Miniature Poodles and German Shepherd Dogs with hypotrichosis. *Histologic changes are variable*, no doubt reflecting differences in underlying abnormalities. In some cases, there are decreased numbers of hair follicles that contain poorly formed or no hair shafts but with normal sebaceous and sweat glands. In other cases, all follicles and associated appendages are absent. These dogs usually also have dental abnormalities and are considered to have a more general abnormality termed a *congenital ectodermal defect*. Footpad biopsies of such dogs also demonstrate a lack of eccrine sweat glands. The underlying molecular defect is unknown in all cases but an abnormality in the epidermal growth factor (EGF) signaling pathway was speculated in the German Shepherd Dogs with hypotrichosis since fibroblasts from these dogs had decreased expression of EGF receptor on their plasma membranes.

Cats

An autosomal recessive form of congenital hypotrichosis has been described in *Siamese and Birman kittens*. The Birman kittens were born virtually hairless and had short fragile wrinkled whiskers or lacked whiskers altogether. Both males and females were affected. Although initially healthy, all affected kittens in one report died by 13 weeks of age from various infections. Necropsy examination of some of the affected Birman kittens revealed *thymic aplasia and lymphoid depletion* of paracortical regions of lymph nodes, spleen, and Peyer's patches, suggesting an immunologic deficiency. Histologic examination of skin indicated reduced numbers of primary hair follicles, which were hypoplastic and devoid of hairs. Sebaceous glands were normal but sweat glands were hypoplastic and in decreased number, and arrector pili muscles were rare.

Hereditary hypotrichosis is recognized in **piglets** and there may be both dominant and recessive forms. The dominant form is thought to be lethal in homozygotes; it is characterized histologically by a decreased number of hair follicles and most appear atrophic. Congenital hypotrichosis is thought to be a simple autosomal recessive trait in polled Dorset **sheep**. Alopecia is most pronounced on the face and legs. Histologic abnormalities consist of hypoplastic hair follicles containing keratosebaceous material but no hairs. Congenital hypotrichosis has been described in a Percheron, but is rare in **horses**. Congenital hypotrichosis is rare in **goats**.

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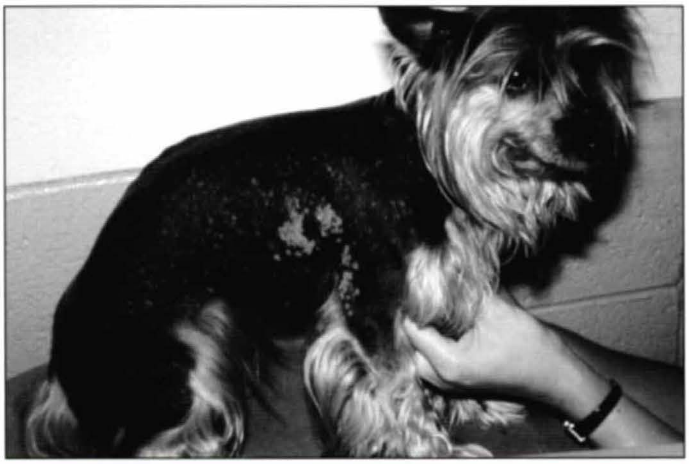

Figure 5.25 Color dilution alopecia in a Yorkshire Terrier. Note thin hair coat and patchy alopecia. (Courtesy of B Dunstan.)

Hypotrichosis associated with pigmentary alterations

Generalized or regional alopecia attributed to follicular dysplasias that include histologic evidence of pigment abnormalities have been frequently described in dogs and less frequently in other species. They have arbitrarily been divided into two categories.

The first of these two categories is **color-dilution alopecia** described in color-dilute animals of breeds such as the Doberman Pinscher, Irish Setter, Dachshund, Chow Chow, Poodle, Whippet, Italian Greyhound, Boston Terrier, Chihuahua, Saluki, Yorkshire Terriers, and mongrels in which the onset of alopecia can be tardive, generally ranging from four months to three years. Puppies are born with normal hair, but develop slowly progressive alopecia (Fig. 5.25). Pedigree analysis in color-dilute Dachshunds suggests this disorder may be inherited as an autosomal recessive trait. The histologic lesions include *misshapen, fragmented anagen hair follicles with pigment clumping in follicular epithelium, hair bulb matrix cells, hair shafts, infundibular keratin, and epidermis* (Fig. 5.26). *Melanin-containing macrophages are frequently present in the dermis around hair bulbs.* There can be some hair follicle atrophy in chronic cases, however this is a secondary change. A condition similar to color dilution-alopecia in dogs has been described in cattle as **cross-related congenital hypotrichosis.** This has been reported in crosses involving Simmental, Gelbvieh and Charolais cattle, most common in the Simmental-Angus and Simmental-Holstein crosses. The condition appears in calves that have color-dilute (gray or chocolate) coats. The affected hair is short, curly and sparsely haired leaving the white haired areas of the coat unaffected. The histologic lesions are virtually identical to canine color-dilution alopecia. **Coat-color-linked hair follicle dysplasia** has also been described in buckskin Holstein cows (color-dilute tan-and-white Holsteins). These animals have short and clinically abnormal hair in the tan areas of the coat.

The second traditional category is **black hair follicular dysplasia**, seen in bi- or tri-color black and white dogs, such as Bassett Hounds, Beagles, Bearded Collies, and mongrels, and in Holstein cattle, in which the alopecia affects only the black-haired areas of the coat, and in which onset is generally in the first few weeks or

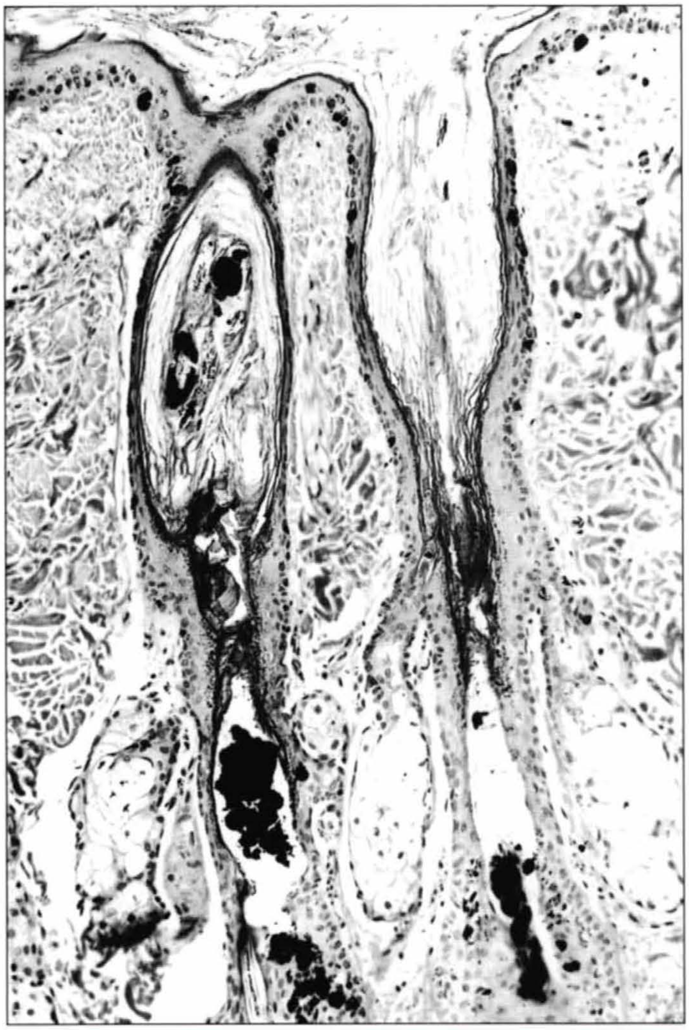

Figure 5.26 Color dilution alopecia in a dog. Note the clumped melanin in the fractured, misshapen hair shafts, follicular hyperkeratosis, and prominent melanocytes in the overlying epidermis.

months of life. Black hair follicular dysplasia is thought to be an autosomal inherited disorder in mongrel puppies, and is thought to have a genetic component in some purebred animals such as Bearded Collies. *The histologic lesions in black hair follicular dysplasias are virtually identical to those of color-dilution alopecia.* A difference in the appearance of the melanin clumps in whole mounts of hair shafts has been suggested.

There are *other follicular dysplasias associated with pigment abnormalities* that do not fall easily into these two categories, but have similar histologic changes. These include follicular dysplasia in the **Portuguese Water Dog** which occurs in the black or red color phase of this breed. These are not color-dilute dogs and the onset can be tardive, generally between 3 months and 5 years. **Black-and-red Doberman Pinschers** that are not phenotypically color dilute, are also reported to have a follicular dysplasia that is generally confined to the caudal dorsum and that has an adult onset. Follicular dysplasia has been described in **Weimaraners**, in which affected young adults had progressive alopecia of the trunk with recurrent folliculitis/furunculosis. In **cattle**, follicular dysplasia has been reported in black Angus and black Angus-Brahman cross; the histologic changes are similar to those in dogs. These cattle are all black animals that are not phenotypically color dilute, however there is adult onset of alopecia that is more commonly associated with color-dilution alopecia. In view of the dysplasias in Portuguese Water Dogs, black-and-red Doberman Pinschers, and black Angus and their crosses that occur in animals not phenotypically color dilute but that can have adult onset, *it is quite possible that separation of follicular dysplasias with pigmentary alteration into color-dilution alopecia and black-hair follicular dysplasia is artificial.* It is likely that these histologically similar follicular dysplasias are subtypes of a single process, however until the mechanism underlying follicular dysplasia is characterized the classification is somewhat arbitrary.

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Hypertrichosis

Congenital hypertrichosis, a condition characterized by an *excessive amount of hair*, is rare. In some instances, the abnormality involves a change in the character of the hair rather than an absolute increase in amount. Excessively long hair is inherited as an autosomal dominant trait in **Friesian cattle** in Europe. The condition results in discomfort during hot weather and decreased productivity. High environmental temperature during gestation has been associated with an unusual hairy appearance of newborn **lambs**. The lambs are small and most do not survive to weaning; the histologic appearance of this abnormality has not been described. *Border disease* is a congenital pestiviral infection of sheep in which lambs are born weak and small, have an abnormal haircoat, and exhibit tonic-clonic spasms (*hairy shaker disease*). Instead of the normal short, fine, closely crimped birthcoat, affected lambs have a long straight coarse coat. The coat abnormality is due to aberrant differentiation of hair follicles that develops only when infection occurs prior to 80 days of gestation. The histologic changes include enlargement of primary hair follicles with an increased degree of medullation and a decreased number and retarded development of secondary follicles.

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Canine dermatomyositis

Dermatomyositis is an idiopathic inflammatory condition of skin, muscle, and occasionally blood vessels of humans and dogs. A familial pattern of occurrence has been found in Collies, Shetland Sheepdogs (Shelties), and their crosses. Dermatomyositis in collies is an autosomal dominant trait with variable expressivity; and it appears to be widespread in Collies in the USA. The disease in Collies has been proposed as a model for a nonfatal form of childhood dermatomyositis, although familial cases are uncommon in humans. The condition in Shelties is assumed to be inherited in a similar manner but the mode of inheritance has not been confirmed by breeding studies. Dermatomyositis has been identified in Shelties in the United Kingdom. The disease has not been characterized as well in this breed; but it appears that myositis is a less prominent feature of the disease in Shelties. Occasional cases of dermatomyositis have been reported in other breeds of dogs; nothing is known regarding the nature of inheritance of the disease in these dogs.

The cause and pathogenesis of dermatomyositis are unknown. Variation in expression of dermatomyositis in dogs suggests that factors other than simple autosomal dominant inheritance are involved in the etiopathogenesis of the disease. Immunologic mechanisms are thought to be involved in this disease in humans and both cell-mediated and humoral immunity have been implicated in the pathogenesis. In Collies with dermatomyositis, serum levels of circulating immune complexes were found to be increased above normal before clinical disease was evident, the onset and severity of dermatitis and myositis correlated with the serum levels of circulating immune complexes and IgG, and circulating immune complex levels decreased to normal as disease resolved. These findings suggest that the immune complexes initiated inflammation rather than resulted from it. IgG was identified as the immunoglobulin component of the immune complexes but the identity of the antigen component was not determined. Dermatomyositis has been associated with viral, bacterial, and *Toxoplasma* infections but infectious agents are generally not isolated from tissues of affected people. Crystalline structures, suggestive of picornaviruses, have been seen in endothelial cells of muscle from severely affected collies. Cases in humans have also occurred following immunization, therapy with various drugs, during pregnancy, and in association with neoplasia.

Skin lesions usually develop in juvenile dogs 7 weeks to 6 months of age. *Earliest lesions consist of small pustules, vesicles, papules, and nodules which evolve into erythematous, crusty, ulcerated, alopecic areas with hypo- or hyperpigmentation* (Fig. 5.27). Lesions are most common on the pinnae, bridge of the nose, lips, periocular skin, over bony prominences of the distal extremities, sternum, and tip of the tail. Mucous membranes and mucocutaneous junctions may be transiently involved early in the course of disease. Footpad ulceration is rare. The disease exhibits a *waxing and waning course* over weeks to months with a variable outcome. In mildly to moderately affected dogs, lesions may resolve spontaneously in 6–12 months; while in severely affected dogs, lesions may regress but do not usually resolve completely and disease may be lifelong and extensive. Lesions heal with no residual scarring in mildly affected dogs; permanent alopecic, hypo- or hyperpigmented disfiguring scars develop, especially on the face, in severely affected dogs. Lesions may be exacerbated by estrus, parturition, or exposure to sunlight. Although cases of adult-onset dermatomyositis have been reported, it is likely that at least some of these dogs had mild transient lesions that were overlooked when they were pups and subsequently developed more obvious disease as adults.

Myositis usually develops several weeks after dermatitis and is proportional in severity to the dermatitis. It is usually first recognized as a bilaterally symmetrical decrease in temporal muscle mass. However, because of the dolichocephalic shape of the collie head, mild temporal or masseter muscle atrophy may be missed. *Myositis principally involves muscles of mastication and extremities below the elbow and stifle*, but it is more generalized in more severely affected dogs. Over time, active myositis is succeeded by muscle atrophy and fibrosis. Generalized symmetrical muscle atrophy, weakness, and exercise intolerance may develop in moderately and severely affected dogs.

Various additional abnormalities may accompany skin and muscle lesions in more severely affected dogs. Peripheral lymph nodes are

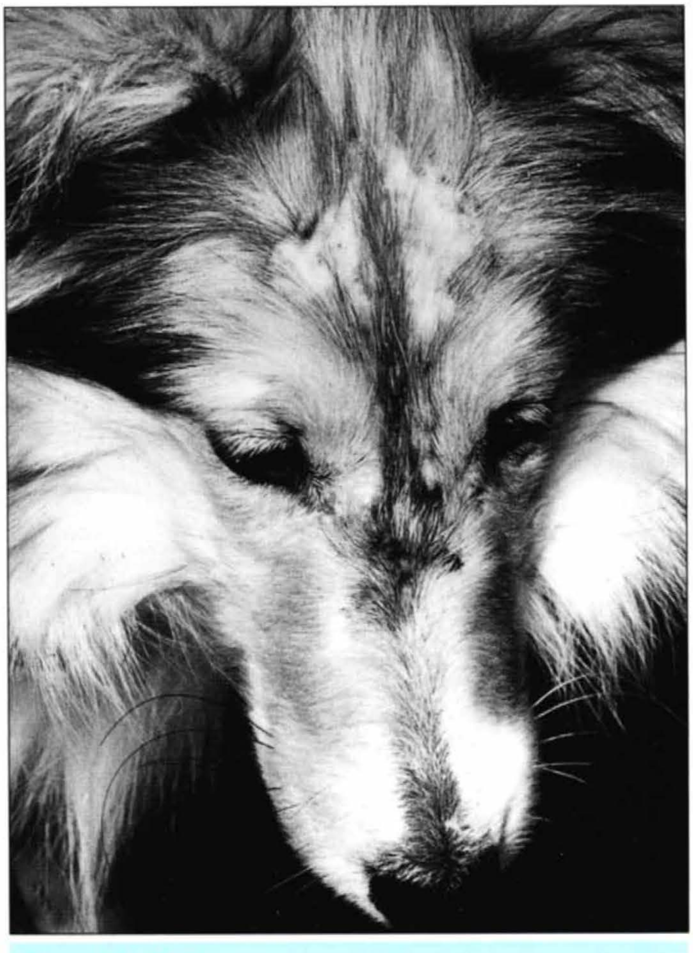

Figure 5.27 Familial canine dermatomyositis in a puppy. Alopecia and crusting of the face.

enlarged because of reactive hyperplasia. Conjunctivitis may develop in dogs with severe periocular skin lesions or because of facial palsy and inability to blink. More severely affected dogs are small and unthrifty in comparison to normal or mildly affected dogs. Fever and joint swelling are noted in some dogs. Secondary bacterial pyoderma, septicemia, or megaesophagus with secondary aspiration pneumonia may develop in more severely affected dogs. Demodicosis may also be present and complicate diagnosis. Severe secondary amyloidosis with resultant renal failure has been described in one affected Collie.

Affected dogs have variable and usually nonspecific *clinicopathologic abnormalities*. Moderately and severely affected dogs commonly have inflammatory leukogram changes which include neutrophilia, with or without a left shift, and monocytosis. Nonregenerative anemia typical of chronic inflammation may develop in severely affected dogs. Serum creatine kinase levels in collies with dermatomyositis were normal or only slightly increased; however, most serum muscle enzyme determinations were done in later stages of disease when active myositis may have been waning. Mild to moderate elevations in serum creatine kinase concentrations were present in several young Shelties with the disease. Occasionally, dogs have positive Coombs' tests, and rarely rheumatoid factor (RF) tests are positive.

The histologic changes in the skin are variable and may be quite subtle or nonspecific. Early lesions consist of scattered individual vacuolated or shrunken, brightly eosinophilic necrotic keratinocytes in the epidermis and infundibular portion of hair follicles. Hydropic degeneration of basal keratinocytes is often present and leads to *dermal-epidermal clefts* that develop into vesicles that contain proteinaceous fluid, erythrocytes, and inflammatory cells. Diagnostically useful artifactual dermal-epidermal separation may be induced at the margins of the section by the shearing action of the punch biopsy. Ulceration and crusting result from lesions with extensive dermal-epidermal separation. Intra-epidermal pustules are uncommon. Hyperkeratosis and acanthosis are variable. In the absence of ulceration, dermal inflammation tends to be mild and consists of mixed cells either surrounding superficial dermal vessels, hair follicles, and glands or distributed in an interface pattern or in some cases diffusely distributed. The infiltrate includes mononuclear cells primarily, fewer neutrophils, and occasional eosinophils and mast cells. *The most consistently present histologic abnormalities are follicular atrophy and perifollicular inflammation that may be accompanied by perifollicular fibrosis* (Fig. 5.28A, B). Variable

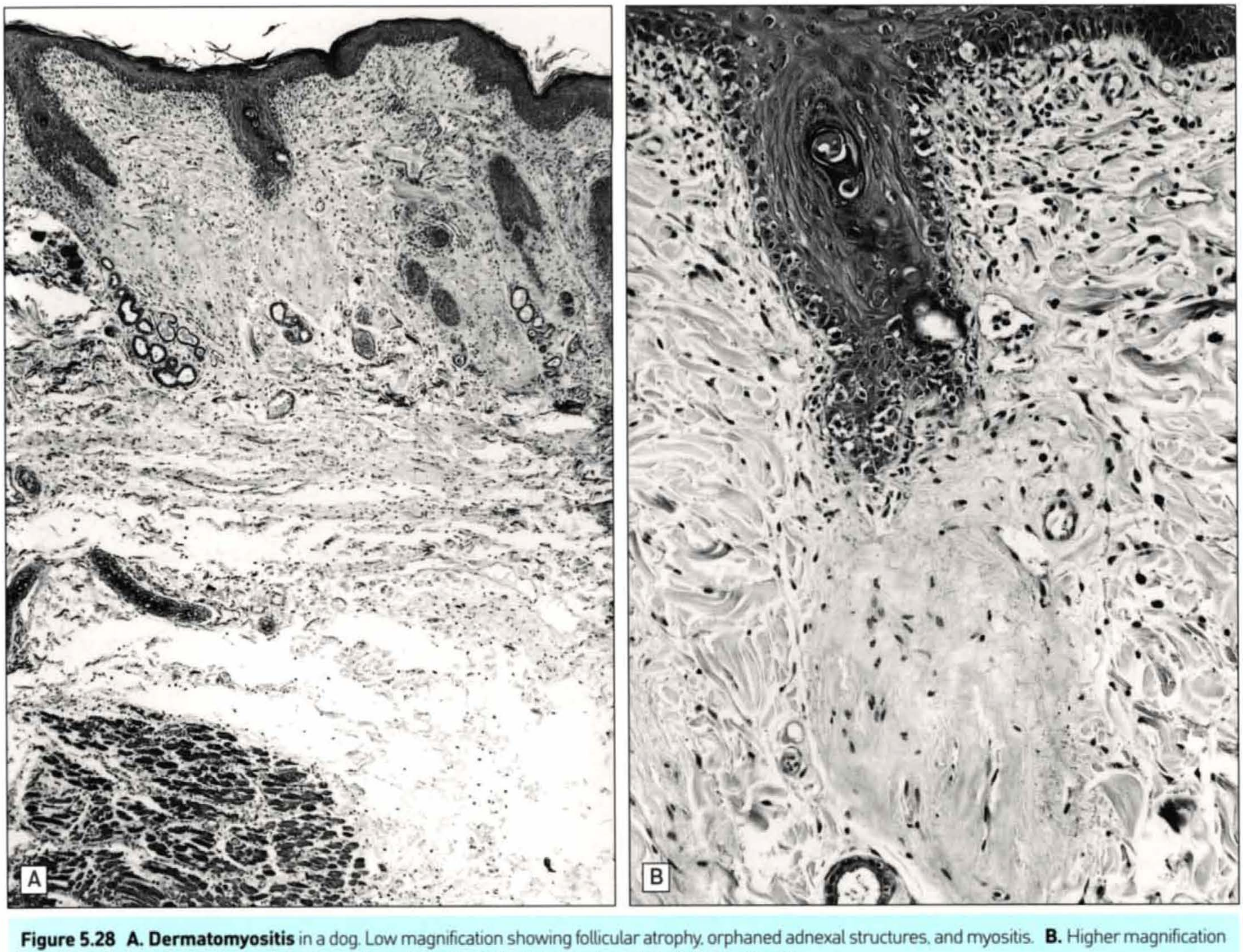

Figure 5.28 A. Dermatomyositis in a dog. Low magnification showing follicular atrophy, orphaned adnexal structures, and myositis. **B.** Higher magnification showing cell-poor hydropic, interface dermatitis and marked follicular atrophy and perifollicular fibrosis.

dermal fibrosis is usually evident in biopsies from dogs older than 6 months. *Muscle lesions* include multifocal interstitial and perivascular infiltrations with mixed cells; myofiber degeneration, regeneration, and atrophy; and fibrosis. *Vasculitis* is an infrequent and subtle finding in the skin, muscle, and occasionally in other tissues.

The principal differential diagnosis is lupus erythematosus and the two diseases may be indistinguishable on individual biopsies. However, lichenoid dermal inflammation is usually prominent in discoid and some cases of systemic lupus erythematosus but is not characteristic of dermatomyositis. Follicular atrophy, a common feature of dermatomyositis, is not usually present in lupus erythematosus. Lupus erythematosus is not typically a disease of young dogs. In some cases, immunologic tests may be required to differentiate the two diseases. Direct immunofluorescent antibody examination, antinuclear antibody test (ANA), and lupus erythematosus test (LE) are frequently positive in lupus erythematosus but negative in dermatomyositis.

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Hereditary connective tissue disorders

The connective tissue components of the skin include collagen (primarily types I and III), elastic fibers, and ground substance composed of glycoproteins and proteoglycans. A defect in any one of these skin molecules can result in structural and functional abnormalities of the entire tissue. Inherited connective tissue disorders of skin may consist of abnormalities involving only one of these components or there may be concurrent alterations in several components. Hereditary collagen dysplasia, the most commonly recognized connective tissue disease, is a complex group of disorders of collagen that results in decreased tensile strength of the skin and may also affect other connective tissues. Alterations in elastic fibers and ground substance may accompany some forms of collagen dysplasia but there are also diseases in which an abnormality of the elastic fibers or ground substance appears to be primary. *Diagnosis of many of these conditions requires ultrastructural examination and biochemical analysis to confirm the presence of structural abnormalities and to identify the molecular defect.*

Hereditary collagen dysplasia

Collagen dysplasia (dermatosparaxis, Ehlers–Danlos syndrome, cutaneous asthenia, cutis hyperelastica) has been reported in humans, cattle, sheep, horses, dogs, cats, mink, and rabbits. Collagen is the major structural protein in skin and other connective tissues and abnormalities in its structure result in skin that is fragile, easily torn, and frequently hyperextensible and loose. In humans, Ehlers–Danlos syndrome (EDS) is divided into at least ten types based on clinical, biochemical, and molecular genetic studies. The natural history of clinical disease and mode of inheritance vary among the different types of EDS. Joint laxity, vascular abnormalities, bowel or uterine rupture, bone abnormalities, ocular abnormalities, and periodontal disease occur in addition to the fragile skin in the various forms of human EDS. Inheritance may be autosomal dominant, autosomal recessive, or X-linked recessive. In animals, clinical disease has been restricted almost exclusively to skin abnormalities and has been characterized in only a few breeding colonies or herd outbreaks. Most instances of collagen dysplasia occur in single animals and the molecular defect and mode of inheritance are usually not determined.

Individuals with inherited collagen dysplasia typically have a *history of frequent skin lacerations following routine handling*, such as shearing or manual restraint, normal activities such as scratching or playing with littermates, or minor trauma. The skin wounds commonly *develop into wide gaping wounds with minimal hemorrhage.* Healing usually proceeds normally but results in *characteristic thin, pale wrinkled scars* resembling cigarette or tissue paper. Extracutaneous signs, such as joint laxity and ocular abnormalities, have been reported in animals only rarely. The skin is usually soft and velvety, hyperextensible, and may hang in loose folds. In some affected animals, the skin laxity becomes progressively more pronounced with age. Severity of clinical signs is variable, even among animals with the same biochemical abnormality. This variability in clinical severity is most pronounced in sheep. One form of the disease described in sheep exhibits such severe manifestations that all lambs die or require euthanasia within the first day or two of life. A second milder form of collagen dysplasia is usually not recognized until later in life when sheep are handled for shearing. *In general, the disease tends to be most severe in sheep, less severe in cattle, followed by dogs and cats, and least severe in horses.*

Diagnosis of collagen dysplasia is based on typical clinical signs and demonstration of morphologic or biochemical abnormalities of the dermal collagen. In some cases, abnormalities are evident at the light microscopic level but frequently none are found or the differences are subtle and difficult to determine except by comparison to a breed- and age-matched control. The dermis may be normal, thinner than normal, or thicker than normal because of an increased amount of ground substance. Collagen fibers may be widely separated, finer and paler than normal, and haphazardly arranged. Rarely, increased numbers of elastic fibers are seen with elastic stains. Fibroblasts are in increased numbers in the dermis of some affected animals. Abnormal collagen fibers may stain unevenly with collagen stains such as Masson's trichrome. Instead of the uniform blue staining of normal collagen, the abnormal collagen may have a red core. This staining feature is not unique to collagen dysplasia, however, as uneven collagen staining also occurs with degenerative disorders of collagen.

In most cases, ultrastructural examination is required to confirm the collagen abnormality. A variety of alterations of collagen fibrils have been found. In longitudinal sections, collagen fibrils may be loosely wound and flat or helical. In cross-section, they may have several irregular thin projecting arms that give them a "hieroglyphic" appearance. This appearance is typical of dermatosparaxis of sheep and cattle and has also been described in a Himalayan cat and a dog. In other forms of the disease, the collagen fibrils are shaped normally but vary markedly in diameter from the normal range. They may all be uniformly larger or smaller or there is a mixed population of fibrils that extend beyond the range of normal minimum and maximum diameter sizes. The fibrils frequently are loose and disorganized rather than being arranged in uniform, compact bundles.

The ultrastructural abnormalities are not specific and biochemical analysis is necessary to determine the particular molecular defect. Collagen synthesis is a multi-step process that includes extensive post-translational processing involving multiple intracellular and extracellular enzymes. Abnormalities in several of these enzymes as well as structural mutations involving the collagen chains have been identified in the various forms of collagen dysplasia in humans and animals. For unknown reasons, the same biochemical defect may produce different clinical abnormalities in different individuals and in different species.

Cattle

Collagen dysplasia in cattle is usually referred to as *dermatosparaxis,* which means "torn skin." The condition is *caused by a mutation in the gene for procollagen I N-proteinase* (also called procollagen aminopeptidase), the enzyme that excises the amino-propeptide of type I and type II procollagens. Each of the three polypeptide chains making up a collagen molecule have short extensions at the amino and carboxy termini. These additional propeptides make the molecules soluble to aid in their transport out of the cell. Subsequent extracellular conversion of the procollagen to collagen requires two enzymes to cleave the amino and carboxy terminal extensions. Following cleavage of the procollagen peptides, the collagen molecules spontaneously assemble into collagen fibrils. *The defect in processing of type I procollagen to collagen results in abnormal precursor molecules with peptide extensions which inhibit formation of uniform fibers and fibers capable of producing normal cross-links.* They assemble instead into abnormal ribbon-shaped collagen fibrils lacking normal tensile strength. Dermatosparaxis in cattle is recessively inherited as is the biochemically analogous condition in humans, EDS type VII C, although clinical signs are not identical.

Most affected calves have thick, wet skin that tears easily and sometimes hangs in loose folds. Associated joint laxity and soft bones have been reported rarely. Light microscopic changes consist of a thicker than normal dermis composed of sparse bundles of fine pale collagen distributed in abundant Alcian-blue-positive ground substance (proteoglycans). Individual collagen fibers are smaller in diameter than in normal skin and their arrangement appears disorganized. There may be an increase in dermal elastin and number of fibroblasts. Ultrastructural examination reveals the collagen to be arranged in loose, twisted flat or helical ribbons rather than being organized in compact parallel arrays. In cross-section, these fibrils have irregular projecting arms which confer a "hieroglyphic" appearance.

Sheep

At least two forms of collagen dysplasia, called *dermatosparaxis,* have been described in sheep, i.e., a *severe form in lambs* noted shortly after birth, and a *milder form* not apparent until sheep are handled as adults. The severe form has been described in Norwegian Dala sheep, Border-Leicester-Southdown crossbred sheep in Australia, and white Dorper sheep in South Africa. The condition is inherited as a simple autosomal recessive trait. Only in the Dala breed has the biochemical defect been identified and it consists of a deficiency in procollagen aminopeptidase activity, as in bovine dermatosparaxis. In Border-Leicester-Southdown crossbred lambs the biochemical abnormality was not determined but it did not appear to be a procollagen peptidase deficiency since there was no increase in dermal procollagen detected. Affected lambs develop skin lacerations during birth or shortly thereafter. The skin is soft and edematous. Lambs frequently die within a few days as a consequence of wound infection and septicemia. Gross examination of the skin indicates that the dermis is moist and thicker than normal with a jelly-like consistency. In some instances, increased friability of internal organs and joint capsules was observed. The histologic and ultrastructural abnormalities are similar to those in cattle with dermatosparaxis.

The *less severe form of dermatosparaxis* has been found in Merino sheep in Australia when adult sheep were handled for shearing. No skin hyperelasticity or joint hypermobility are associated with the skin fragility. The skin lacerations predispose affected individuals to infections and fly-strike. This form is also caused by a deficiency of procollagen aminopeptidase activity. Light microscopic examination reveals a loose, more open appearance to the dermis and a significant increase in the number of dermal fibroblasts in comparison to normal skin. Most collagen fiber bundles are smaller and more lightly stained than those in normal skin. The collagen in some areas is arranged in prominent layers. Transmission electron microscopic examination shows a combination of distorted, hieroglyphic-type fibrils mixed with normal collagen fibrils.

Horses

Collagen dysplasia has been reported in Quarter Horses, a Thoroughbred gelding, and an Arabian-cross filly. An *autosomal recessive* mode of inheritance has been suggested but not proven in the Quarter Horses; *the biochemical defect has not been identified in any of the equine cases.* The condition is not usually recognized until the animal is 6–12 months old and develops frequent skin wounds and scarring on the legs, shoulders, and saddle area. The skin is hyperextensible; but in some cases, the abnormal skin involves sharply delineated areas interspersed among large areas of normal skin. This unusual feature has not been described in other species.

Histologic examination of affected skin may not indicate any dermal abnormalities or the dermis may be thinner than normal and collagen fibers may appear smaller than normal when compared to a control. In some cases, the collagen fibers have abnormal red cores when stained with Masson's trichrome. Ultrastructural changes in two Quarter Horses included disorganized and nonparallel collagen fibril arrangement, increased space between collagen fibrils, and irregular outlines and increased variation in the diameter of collagen fibrils. In contrast, ultrastructural changes in an Arabian-cross filly were suggestive of collagen degeneration. Collagen fibrils were uniform and densely packed in parallel arrays in unaffected areas of skin from this horse; but in sections from abnormal, fragile skin, collagen fibrils were fragmented, widely separated by granular material, disorganized, and associated with phagocytic fibroblasts. These ultrastructural abnormalities are unlike those described in other horses or species.

Dogs

Collagen dysplasia has been described in many purebred and mixed-breed dogs. Affected dogs typically have *soft, easily torn, hyperextensible skin* (Fig. 5.29). In some cases, the skin hangs in loose pendulous folds, a feature that frequently becomes more prominent with age. Thin, white scars are typically the sequelae to skin wounds. Ocular

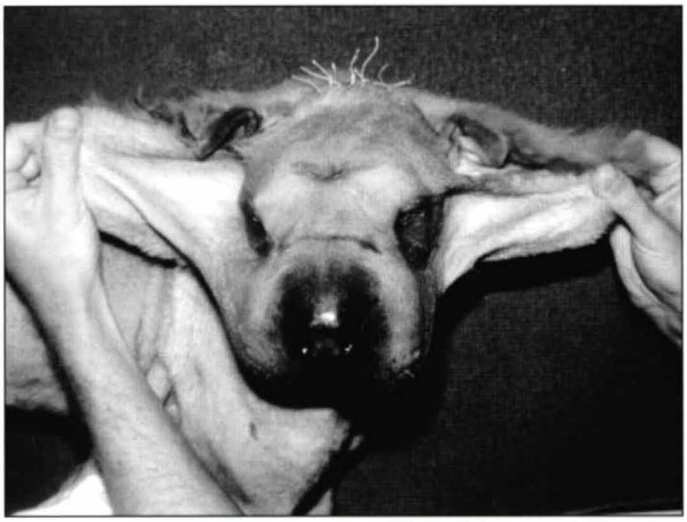

Figure 5.29 Collagen dysplasia in a dog. Note hyperextensible facial skin. (Courtesy of B Dunstan.)

abnormalities and joint laxity, associated problems commonly seen in humans with EDS, have been reported infrequently in dogs, and bone abnormalities are rare. Several breeding studies have shown collagen dysplasia to be inherited in a simple autosomal dominant manner with complete penetrance. *The biochemical abnormality has not been identified in any cases of collagen dysplasia in dogs*; but the skin of Springer Spaniels with collagen dysplasia has been found to have more uncross-linked α-chains than collagen from normal dogs.

Histologic changes may be subtle and consist of dermal thinning evident only in comparison to a section from a normal control. In other cases, a decreased amount of dermal collagen, collagen disorganization, variation in collagen staining, increased number of elastic fibers, or increased amount of extracellular matrix may be seen. The abnormal collagen fibers may stain red with trichrome stain. Ultrastructural abnormalities of the collagen consist of variation in the fibril diameter, shape abnormalities, fibril disorganization, and loose fibril packing. A mixture of normal and abnormal collagen fibers may be present.

Cats

Collagen dysplasia in cats is usually referred to as *cutaneous asthenia*, although in early cases reported it was called dermatosparaxis. The condition has been reported in a number of breeds; *in the majority of the cases, neither the biochemical defect nor the mode of inheritance has been identified*. In a single Himalayan cat with collagen dysplasia, the abnormality was determined to be a defect of the amino-terminal procollagen peptidase, as in dermatosparaxis of cattle and sheep. This cat could not be bred, however, and the mode of inheritance was not determined. A breeding study based on an affected male domestic shorthair cat indicated that the condition is inherited as an autosomal dominant trait and heterozygous individuals synthesize both normal and abnormal collagen molecules.

Cats with cutaneous asthenia have thin, soft, velvety skin that tears easily but with minimal hemorrhage (Fig. 5.30). Lacerations heal to form typical white, tissue paper-like scars. In some cats, loose folds of skin develop as the cats age. No joint laxity has been described in cats with cutaneous asthenia. Histologic examination of the skin yields variable results. No dermal changes are evident in some cases; while in others, the dermis is thinner and collagen fibers are finer and separated by an increased amount of ground substance when compared to skin from an unaffected cat. Normal collagen fibers stain uniformly blue with Masson's trichrome stain while abnormal fibers exhibit segmental red staining areas that are birefringent under polarized light. Ultrastructural examination indicates that normal and abnormal collagen fibers may be present in varying proportions. Abnormal fibers are characterized by disorganized, tangled, nonparallel packing of fibrils. Abnormal "hieroglyphic" fibrils were a feature of the affected Himalayan cat.

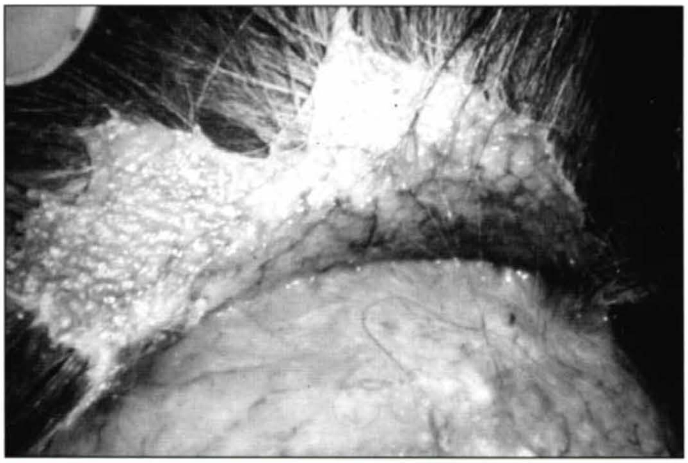

Figure 5.30 Feline cutaneous asthenia. Note tearing of fragile skin with minimal hemorrhage. (Courtesy of R Rosychuk.)

Skin fragility in cats has also been reported as an acquired condition associated with a number of conditions including spontaneous and iatrogenic hyperglucocorticism, diabetes mellitus, hepatic lipidosis, cholangiocarcinoma, and administration of various drugs. The histologic and ultrastructural changes of collagen in cats with acquired skin fragility may be similar or indistinguishable from those seen in cats with cutaneous asthenia.

Abnormalities of elastic fibers

An *excess of elastic fibers* was reported in several piglets of a litter of Large White X Essex pigs with multiple circular to oval shallow depressed skin lesions. The skin was abnormally elastic in these areas and seemed to be bound less tightly to the underlying subcutis. The increase in thick elastic fibers was present only in those areas in which the skin was hyperextensible and the condition was termed **cutis hyperelastica**.

Congenital abnormalities of ground substance

Proteoglycan deficiency

An abnormality of dermal proteoglycan is a rarely documented cause of fragile skin in humans and animals. Proteoglycan is composed of a core protein and glycosaminoglycan (mucopolysaccharide) side chains, and it is the major component of the extracellular ground substance of the dermis. A 4-month-old female Holstein calf with skin fragility, soft and hyperextensible skin, and poor wound healing typical of dermatosparaxis was found to have normal collagen fibers. However, levels of dermatan sulfate proteoglycan in the dermal connective tissue were undetectable. The defect was identified as a mutation involving the gene that codes for the proteoglycan core protein. The mode of inheritance was not determined.

Cutaneous mucinosis of Chinese Shar-Pei dogs

Cutaneous mucinosis is a dermal connective tissue disorder in which *excessive mucin accumulates in the skin.* Mucin is a gel-like substance comprised primarily of acid glycosaminoglycan, hyaluronic acid, and water. *The inherited form of cutaneous mucinosis is considered normal in the Chinese Shar-Pei dog* and is responsible for the thick, wrinkled skin characteristic of the breed. The degree of mucin accumulation is variable. In some dogs, large lakes of mucin form nodules or cysts that may rupture and drip clear, stringy fluid.

Histologically, cutaneous mucinosis is characterized by a variable increase in dermal thickness because of excessive mucin separating collagen fibers. Mucin has great water-binding capacity and thus contains a substantial amount of water, the majority of which is removed during processing of the tissue. What remains in HE-stained tissue sections is fine basophilic granular to fibrillar material separating dermal collagen fibers. Special stains can be used to better visualize the mucin; these include Alcian blue at pH 2.5, which stains mucin blue-green, and mucicarmine, which stains it red. Mucin stains metachromatically with toluidine blue and methylene blue. PAS stain, which stains neutral mucopolysaccharides, does not stain dermal mucin.

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Dermatosis vegetans

Dermatosis vegetans is an inherited disorder of young pigs characterized by vegetating skin lesions, hoof malformation, and giant cell pneumonia. The condition is a simple autosomal recessive trait of Landrace swine in Europe, Canada, and Australia. Clinically affected pigs grow slowly, become emaciated and unkempt, and usually die by 2 months of life. The economic impact of the disease may be considerable since virtually all homozygotes die before reaching slaughter age.

Skin lesions may be present at birth, but more commonly they develop during the first 3 weeks of life, and in rare cases may not arise until 2–3 months of age. *Lesions begin as erythematous papules*, 0.5–2.0 cm in diameter, usually on the ventral abdomen and medial aspect of the thighs. They may extend up the sides and back but do not affect the head. The papules enlarge peripherally over the course of 2 or 3 days and the center becomes depressed. At this stage, the lesions are clinically similar to pityriasis rosea. The papules enlarge to form *plaques* with a depressed center filled with characteristic gray to brown-black granular brittle material. Over a period of weeks, the lesions continue to expand and develop a dry, horny, papilloma-like appearance. They become dark brown to black and *each crusty plaque is surrounded by a hyperemic raised border that sharply demarcates the lesions from the surrounding normal skin* (Fig. 5.31A). As lesions spread peripherally, they coalesce to form extensive areas covered by black crusts. Affected piglets frequently die when lesions reach the typical papilloma-like appearance at 5–8 weeks of age. Skin lesions then begin to resolve if the pig survives.

When they occur, *foot and hoof lesions* are always present at birth (Fig. 5.31B). Usually more than 1 limb is affected and typically all digits, including accessory digits, of an affected foot are involved. The coronary region is markedly swollen and erythematous; and the skin is covered by yellow-brown greasy material. The wall of the hoof is thickened by ridges and furrows parallel to the coronary band. Affected hooves become progressively enlarged, wider, and flatter than normal if pigs survive to 5 or 6 months. Coronary band changes, however, diminish as the pig ages.

At birth, affected piglets seem otherwise normal but over a period of weeks they gradually decline in growth and vitality. Except for animals that die perinatally, virtually all affected pigs show signs of *respiratory dysfunction*, typically increased respiratory rate and labored respiration, several days prior to death. Affected pigs commonly develop anemia and secondary infections, especially bacterial pneumonia.

The *histologic lesions* in the skin vary according to the stage of the condition. Initially, there is superficial dermal edema, vascular congestion, and dermal infiltration with numerous granulocytes, many

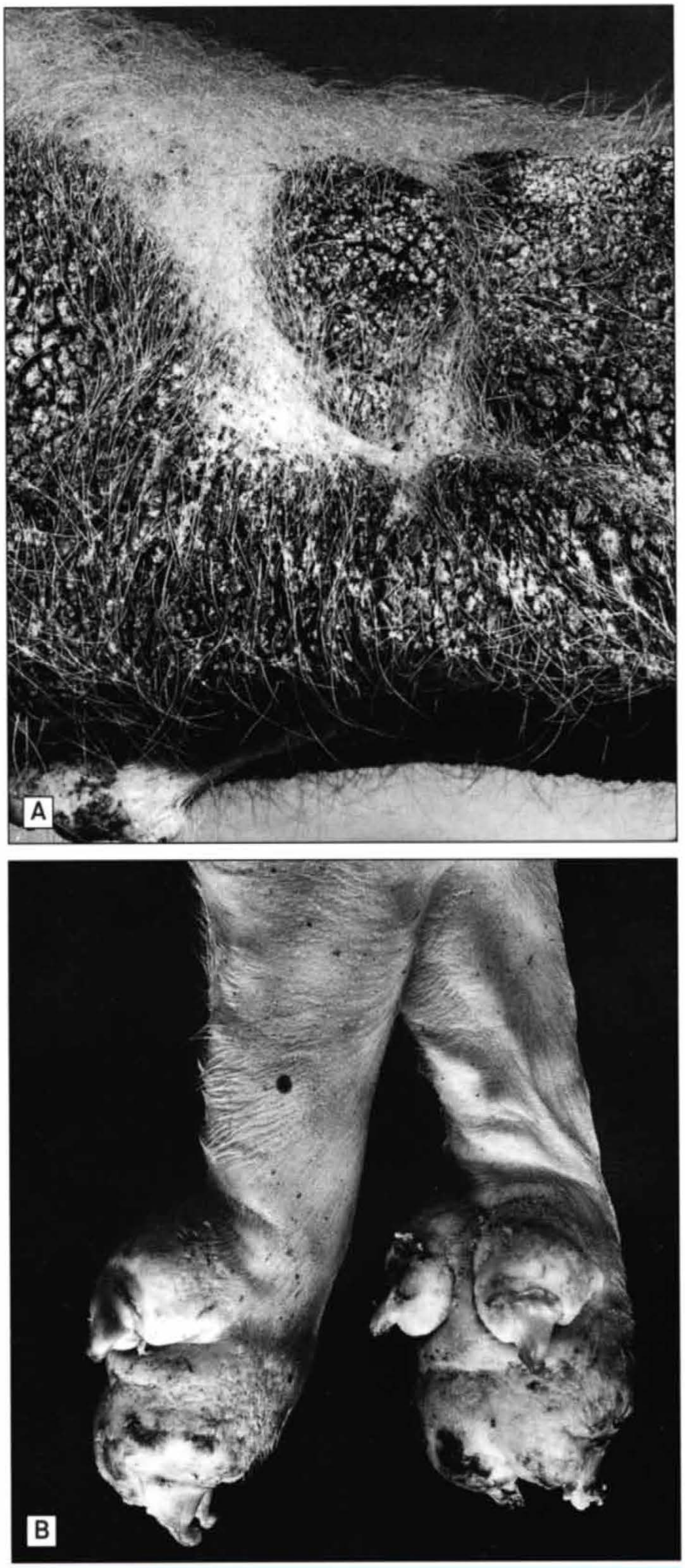

Figure 5.31. Dermatosis vegetans in a pig. **A.** Coalesced, crusted lesions on the abdominal wall. (Courtesy of DH Percy.) **B.** Congenital lesions of the feet. (Courtesy of JA Flatla.)

of which are eosinophils. Fully developed lesions are characterized by *marked orthokeratotic and parakeratotic hyperkeratosis, prominent irregular epidermal hyperplasia, intercellular edema, and intraepidermal pustules and microabscesses containing eosinophils and neutrophils.* The characteristic histologic lesion in the lung is *giant cells in alveoli.* The giant cells have been identified immunohistochemically as originating from monocytes/macrophages. In chronic cases, epithelialization and interstitial fibrosis are evident and multinucleated giant cells may be infrequent when the condition has lasted several months. Typical pulmonary changes may be obscured by secondary infections.

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Dermoid cyst

The dermoid cyst (dermoid sinus) is an uncommon developmental anomaly that has been reported in dogs, cats, horses, and cattle. It is caused by defective epidermal closure along embryonic fissures, which isolates an island of ectoderm in the dermis or subcutis. The majority of dermoid cysts occur on the dorsal midline because of incomplete separation of skin and neural tube during embryonic development; but they also occur in other locations. Although present at birth, dermoid cysts are usually asymptomatic and may not be noticed until they become distended or infected in an older animal. The cyst usually *contains hair, keratin, and sebum* and this material may produce progressive enlargement of the structure so that it becomes clinically apparent. Cysts frequently become infected, producing clinical signs such as purulent discharge, local swelling from cellulitis, or neurologic signs secondary to meningomyelitis.

In **dogs**, dermoid cysts have been reported most commonly in the *Rhodesian Ridgeback*, a breed in which the lesion appears to be inherited as a simple recessive trait. Dermoid cysts have also been reported in a Siberian Husky, Yorkshire Terrier, Shih Tzu, and Boxer. It is unknown whether the lesion is an inherited condition in other breeds of dogs. Dermoid cysts have been associated with multiple vertebral and spinal malformations and hindlimb neurologic deficits in several dogs. The rare condition of *nasal dermoid sinus cyst* results in a discharging sinus over the external nares in dogs; the cyst may extend into the cranial vault and cause cerebral abscessation or recurrent meningitis. In one survey, all cases in *horses* were in Thoroughbreds. Several cases reported in *cattle* all involved Angus. Dermoid cysts are rare in *cats*, and both cases reported were in domestic shorthairs.

Dermoid cysts may be single or multiple. They consist of a well-circumscribed circular or tubular structure in the skin or subcutis and frequently connect to the skin surface by a small pore. A tuft of hair may protrude through this pore and it may be surrounded by a whorl of hair. The cyst may end blindly in the subcutis, it may connect to the dorsal spinous process of vertebrae directly or by a fibrous cord, or rarely it extends down to be continuous with the dura mater of the spinal cord. *Microscopically*, the dermoid cyst is a circular or tubular structure lined by a wall of well differentiated, keratinizing squamous epithelium with associated small but well developed hair follicles, sebaceous glands, and occasional apocrine sweat glands. The hair shafts project into the cyst cavity which also contains keratin and variable amounts of sebum. Bacterial infection results in neutrophilic infiltration into the cyst. Pyogranulomatous dermatitis or cellulitis ensues when the cyst ruptures because of infection, trauma, or obstruction of the pore.

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DISORDERS OF EPIDERMAL DIFFERENTIATION

The epidermis is stratified squamous epithelium that forms a continuously regenerating protective sheet around the body. Basal keratinocytes proliferate, then differentiate, become keratinized and are then sloughed. *In normal canine skin, the migration from the basal to the cornified layer requires 22 days*, as measured by tritiated thymidine incorporation studies. This turnover rate is shortened in some skin diseases; for example, in seborrheic skin disease it is 7–8 days. The basement membrane separates the epidermis from the dermis and basal keratinocytes rest on this membrane anchored by hemidesmosomes and focal adhesions. Basal keratinocytes are the only cells in the epidermis that can undergo mitosis, and once mitosis occurs, the basal cell proceeds to undergo terminal differentiation. These postmitotic cells enter the stratum spinosum, develop intercellular attachments, desmosomes and adherens junctions, and change the keratin composition of the cytoplasmic keratin filaments. In the basal layer, keratins K5 and K14 are expressed, whereas in the suprabasal cells, K1 and K10 are expressed. The intercellular and cell substrate adhesions are complex. *Hemidesmosomes* and *desmosomes* are stable junctions that associate with cytoplasmic keratin filaments, whereas *focal adhesions* and *adherens junctions* connect to actin filaments and are transitory adhesions. *Integrins* are receptors that mediate cell–substrate adhesion, whereas *cadherins* mediate cell–cell adhesion. As the cells are pushed outwards they move into the stratum granulosum. Here they start to make proteins that make up *keratohyaline granules*. As the cells move into the stratum corneum, the cytoplasmic organelles are lost and they become metabolically inactive. These flattened inactive keratinocytes are compacted into a keratin layer that eventually exfoliates. In this way, *the epidermis is continuously regenerating and degenerating by proliferation, differentiation, and keratinization.*

While the cells are moving outward, keratin polypeptides form and polymerize into *keratin intermediate filaments* that are epithelial specific and the major component of the cytoskeleton of epithelial cells. The keratin intermediate filaments aggregate into *tonofilaments* which connect with desmosomes, and therefore indirectly with adjacent cells. The molecular structure of keratin is very important, and genetic mutation can affect keratin filament formation. There are at least 30 keratins in epithelium; K9–20 are acidic (type I), and K1–8 are basic (type II). Two different keratins (one acidic and one basic) pair to form *heterodimers*, for example K1 and K10 in suprabasal cells, and K5 and K14 in basal cells. Hyperproliferative epidermis in skin diseases expresses keratins K6 and K16, not seen in normal skin.

When the cells reach the stratum granulosum they start to synthesize proteins, stored in keratohyaline granules, necessary to form the mechanically strong *macrokeratins* in the stratum corneum. One of these, profilaggrin, dephosphorylates to form *filaggrin*, the major molecule responsible for the glue-like aggregation of intermediate filaments. *Loricrin* is also stored in keratohyaline granules, and this polypeptide contributes to the cell envelope; an insoluble intracytoplasmic barrier. *Other proteins* involved in cell envelope formation include involucrin, cystatin A, CREP (cystine-rich envelope protein), trichohyaline, SPRR (small proline-rich proteins), sciellin, and filaggrin. All of these serve as substrates for *transglutaminases* that polymerize and crosslink these proteins in the formation of the cell envelope. Three different transglutaminases have been identified in the skin. The granular layer also contains small lipid-rich granules – *submembranous lamellar bodies* (Odland bodies, membrane-coating granules) – that contain lipids necessary to form a permeability barrier between cells when they are secreted into the intercellular space. Keratinocytes forming the stratum corneum are dead and can be sloughed when desmosomes are broken down. Hydrolytic enzymes such as cathepsin B-like, carboxypeptidase, and acid phosphatase are thought to be responsible for this desmosomal degradation and subsequent keratinocyte desquamation.

In various epidermal diseases, this orderly epidermal turnover is altered. For instance, increased proliferation and/or decreased dyshesion of epithelial cells will lead to thickening of the epidermis. Nutritional factors such as amino acids, vitamins A or B, zinc, fatty acids, and copper influence proper differentiation and maintenance of the epidermis. Altered expression of different molecules and keratin mutations are the focus of much research.

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Seborrhea

Seborrhea is a term used to describe a broad range of conditions ranging from dry flaky skin to severe oily or scaling, crusty lesions with alopecia. Seborrhea literally means "abnormal flow of sebum," however, the major clinical abnormality in seborrheic skin diseases is *altered keratinization.* Current terminology favors the use of the term *cornification defect* to cover all hyperkeratotic conditions from ichthyosis to flaky skin. The seborrheas can be divided into *primary idiopathic seborrhea* or *secondary seborrhea* where there is an underlying primary dermatosis.

The majority of seborrheic skin diseases are secondary. They can be secondary to disorders such as hormonal imbalances (especially hypothyroidism, hyperadrenocorticism and sex hormone imbalances), ectoparasitism (especially cheyletiellosis, pediculosis and demodicosis), endoparasitism, dermatophytosis, hypersensitivities (inhalant, dietary, drug), abnormal lipid metabolism (malabsorption, liver or pancreatic disease, diabetes mellitus), dietary deficiencies (fatty acids, protein, vitamin A, zinc), chronic catabolic states, environmental factors (especially hot, dry conditions), autoimmune disease (systemic lupus erythematosus, pemphigus foliaceus), and neoplasia (epitheliotropic lymphoma and internal malignancy).

Whatever the underlying cause, seborrheic skin is characterized by certain abnormalities. The first is *altered keratinization with or without altered glandular function.* This is reflected grossly by various degrees of scaling and crusting, with or without greasiness. Second, the surface lipids, whether the seborrheic skin is dry or greasy, have an *increased percentage of free fatty acids and cholesterol, and a decreased percentage of diester waxes.* Third, the altered keratinization and lipid film are accompanied by *a marked increase in the number of surface bacteria per unit of skin.* In addition, the bacterial flora usually switches from the normally nonpathogenic resident micrococci, corynebacteria and coagulase-negative and coagulase-positive staphylococci to a pure, heavy potentially pathogenic population of *coagulase-positive staphylococci.* Thus seborrheic skin, regardless of the underlying cause, is *often complicated by secondary bacterial disease.*

Clinically, seborrheic skin disease is often separated into three morphologic types:

- **Seborrhea sicca** is characterized by dry skin with focal or diffuse flaking and accumulations of white-to-gray nonadherent scales.
- **Seborrhea oleosa** is characterized by focal or diffuse scaling associated with excessive lipid production that produces yellowish to brownish material that adheres to the skin and hair.
- **Seborrheic dermatitis** is characterized by scaling and greasiness with gross evidence of local or diffuse inflammation. Pruritus and/or secondary bacterial infection is often present with all three forms. This clinical categorization has little significance in terms of differential diagnosis, and animals may be dry in one area of the body and greasy in another.

Seborrheic skin disease is reported most commonly in the dog, but also occurs in horses, cats, goats, sheep, cattle, rodents, and primates.

Primary idiopathic seborrhea

Seborrheic skin disease is common in **dogs**. Primary idiopathic seborrheic skin disease occurs in many breeds. The association with particular breeds and also an early age of onset suggest that primary canine seborrhea *may have an inherited basis.* Cocker Spaniels and Springer Spaniels tend to have a greasy, inflammatory form of seborrhea with numerous hyperkeratotic plaques, comedones and follicular casts. *Inflammatory ceruminous otitis externa* is also a constant finding. In Cocker Spaniels, cell proliferation kinetics indicate that seborrheic individuals have increased epithelial cell proliferation of the epidermis, hair follicle infundibulum and sebaceous gland. In addition, recombinant grafting studies have shown that the hyperproliferative epidermis from seborrheic Cocker Spaniels remains hyperproliferative, suggesting that the abnormality may be in the keratinocyte itself. *Other breeds with primary, greasy seborrhea* include the Basset Hound, West Highland White Terrier, German Shepherd, Dachshund, and Chinese Shar-Pei. Breeds having a *dry form of primary seborrhea* include the Irish Setter, Doberman Pinscher, Dachshund, and West Highland White Terrier. Primary seborrhea in German Shepherds, West Highland White Terriers, and Labrador Retrievers is often very inflammatory, lichenified, and pruritic. Seborrheic skin disease is typically more pronounced on the face, pinnae, trunk, pressure points, intertriginous areas, mucocutaneous areas, and paws. In Labrador Retrievers, the distribution is often strikingly ventral ("water-line disease").

Primary seborrhea is uncommon in cats and horses. Primary seborrhea oleosa, presumably of autosomal recessive inheritance, has been described in Persian **cats**. Severely affected kittens show lesions at 3–4 days of age, and develop progressively severe, generalized greasiness, matting of the haircoat, rancid odor, comedones, alopecia, and ceruminous otitis externa. Pruritus is absent. A milder form of the disease is recognized in 6–8-week-old kittens, with mild to moderate greasiness of the skin and haircoat. Primary seborrhea in the **horse** occurs in both dry and greasy forms, and tends to be restricted to the mane and tail. Pruritus is absent. Generalized primary seborrhea is rare in the horse.

Primary idiopathic seborrhea is usually characterized histologically by *superficial perivascular dermatitis. Epidermal hyperplasia* is usually mild to moderate and papillated in configuration. A pronounced keratinization defect is present, typified by *alternating vertical tiers of orthokeratotic and parakeratotic hyperkeratosis.* The parakeratosis is typically found overlying the shoulders of follicular ostia (*parakeratotic "caps"*). The underlying dermal papillae are often edematous, leading to spongiosis and *leukocytic exocytosis of the overlying epidermis ("papillary squirting")* (Fig. 5.32). Spongiform or Munro's microabscesses may be seen in conjunction with the parakeratosis. The perivascular inflammatory cells include variable combinations of lymphocytes, neutrophils, plasma cells, macrophages, and mast cells. Since *secondary bacterial infection is common*, subordinate patterns of suppurative folliculitis, furunculosis, perifolliculitis and intraepidermal pustular dermatitis are frequently seen.

Secondary seborrhea

Secondary seborrheic skin disease is also characterized by a *pronounced keratinization defect, orthokeratotic and/or parakeratotic in nature.* The overlying histologic reaction pattern, however, reflects the underlying disease process (hypersensitivity, ectoparasitism, endocrinopathy, etc.). Occasionally, in predisposed breeds, it is necessary to attempt to eliminate coexisting dermatoses to determine if a primary seborrhea is also present.

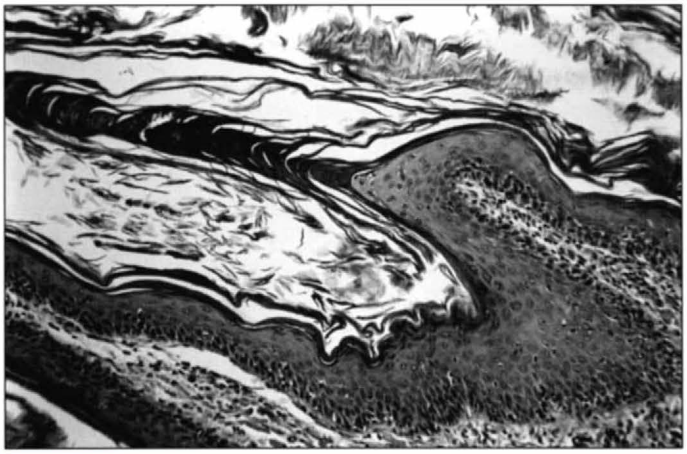

Figure 5.32 Primary idiopathic seborrhea in a dog. Papillated epidermal hyperplasia with vertical tiers of orthokeratotic and parakeratotic hyperkeratosis. (Courtesy of B Dunstan.)

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Acne

Acne is seen in *short-coated breeds of* **dogs**, especially English Bulldogs, Boxers, German Shorthaired Pointers, Great Danes, and Doberman Pinschers. It usually occurs between 3–12 months of age with no sex predilection and occasionally persists into adult life. The *etiology and pathogenesis are unknown*, but, since this is a disease seen in association with puberty in dogs, may be similar to that in humans, involving increased circulating levels of androgens and "acne-prone" skin. The lesions of canine acne are *asymptomatic papules and pustules, which arise from comedones*. Typically, the *chin and lips* are involved. Secondary bacterial folliculitis and furunculosis may occur. Histologically, there is marked orthokeratotic hyperkeratosis, dilation and plugging of hair follicles, and variable inflammation.

Acne is common in **cats** and has no sex or breed predilections. It usually occurs in mature cats, is asymptomatic, and persists for life. It is therefore unlike acne in the dog or humans as it is not associated with puberty. The cause is unknown. The lesions of feline acne are *asymptomatic comedones on the chin and lips*. Occasionally, secondary bacterial folliculitis and furunculosis develop. Histologically, marked orthokeratotic hyperkeratosis, dilation and plugging of hair follicles with variable inflammation are seen.

Schnauzer comedo syndrome

This condition occurs only in the Miniature Schnauzer breed. Either sex may be affected, and the condition usually develops early in life. The exclusive occurrence in Schnauzers and the early onset suggest that this syndrome *may be a developmental dysplasia of hair follicles with an inherited basis*. Clinically, the condition is characterized by multiple asymptomatic comedones over the *dorsal midline*. Occasionally, secondary bacterial folliculitis and furunculosis may develop. Histologically, there is marked orthokeratotic hyperkeratosis, dilation and plugging of hair follicles (comedones), and variable inflammation.

Tail gland hyperplasia

Many **dogs** have an oval area of skin on the dorsal surface of the tail above the fifth to seventh coccygeal vertebrae referred to as the *tail (supracaudal, preen) gland*. Microscopically, large, densely packed *perianal ("hepatoid") and sebaceous glands* characterize this area. In some dogs, especially adult to aged males, this area enlarges. The enlargement is usually firm to slightly spongy, and associated with partial alopecia, scaling and greasiness. At this stage, the lesion is asymptomatic. Occasionally, the lesions become cystic and/or secondarily infected, or neoplastic.

In most instances, canine tail gland hyperplasia is associated with *hormonal imbalances*, especially elevated levels of blood testosterone. Histologically, the lesions are characterized by *marked hyperplasia of the perianal gland component, with a variable inflammatory response*.

The entire dorsal surface of the tail in **cats** is replete with large, densely packed sebaceous glands, recently proposed to be embryonal hepatoid glands. In some cats, especially sexually active males of the Persian, Siamese and Rex breeds, this area becomes clinically seborrheic, whereupon a brown to black, greasy keratosebaceous material accumulates on the hairs of the skin surface. Unless secondarily infected, the condition is asymptomatic. The cause of feline tail gland hyperplasia is unknown, and the colloquialism "stud tail" is misleading, as the condition is also seen in intact females and neutered males and females. Histologically, the condition is characterized by *marked hyperplasia of sebaceous glands, with variable orthokeratotic hyperkeratosis and inflammation*.

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Canine nasodigital hyperkeratosis

Canine nasodigital hyperkeratosis is characterized by *increased amounts of horny keratin on the nasal planum and/or footpads*. This disorder may be seen in association with canine distemper (hard pad disease), pemphigus foliaceus, pemphigus erythematosus, systemic or discoid lupus erythematosus, zinc-responsive dermatoses, superficial necrolytic dermatitis, and as an idiopathic senile change. Digital hyperkeratosis occurs as an hereditary disorder in Irish Terriers and dogues de Bordeaux; nasal hyperkeratosis may occur as a hereditary disorder in young Labrador Retrievers. Histologically, *marked orthokeratotic and/or parakeratotic hyperkeratosis and irregular to papillated epidermal hyperplasia* characterize nasodigital hyperkeratosis. Age, breed, and other histopathologic findings reflect those of the underlying diseases.

Keratoses

Keratoses are firm, elevated, circumscribed areas of excessive keratin production. In humans, keratoses are common and of numerous types. *Keratoses are uncommonly reported in domestic animals.* Actinic keratoses are discussed elsewhere.

Equine linear alopecia (linear keratosis) is a characteristic clinical entity. It occurs in many breeds, however Quarter Horses seem to be predisposed. The age of onset is usually at 1–5 years. The clinical lesions are characterized by one or more *vertically oriented linear areas of alopecia, with variable crusting and scaling.* They are usually unilateral and occur most commonly on the *neck, shoulder and lateral thorax.* The lesions are usually asymptomatic, and may be persistent or permanent. The *etiology of the condition is unknown* but may involve an immune-mediated attack on the wall of the hair follicle. The reason for the linearity is unknown. Histologically the lesion is characterized by *lymphocytic or lymphohistiocytic mural folliculitis*, sometimes with follicular destruction (Fig. 5.33A, B). Multinucleated giant cells are variably present. Sebaceous glands can be secondarily effaced, and there is a variable amount of orthokeratotic or parakeratotic hyperkeratosis, with or without superficial perivascular nonsuppurative inflammation. Linear keratoses with a similar gross and histologic appearance have also been described in *cattle.*

Equine cannon keratosis is a clinically recognizable disease of the horse. It represents a localized form of seborrheic dermatosis, can occur at any age, and has no breed predilection. The colloquial term, "stud crud," is inappropriate as it also occurs in mares. The lesions consist of *vertically oriented, moderately well demarcated areas of alopecia, scaling and crusting on the cranial surface of the rear cannon bones.* The lesions are usually bilateral and persist for life. Pruritus and pain are absent. Histopathologic findings include orthokeratotic and/or parakeratotic hyperkeratosis, irregular to papillated epidermal hyperplasia, and mild superficial perivascular dermatitis featuring lymphocytes and macrophages.

Seborrheic keratoses have been recognized in dogs. They are of unknown cause, and have nothing to do with seborrhea. They may be single or multiple and have no apparent age, breed, sex or site predilections. The lesions are elevated plaques or nodules with a hyperkeratotic, often greasy surface. They are frequently hyperpigmented. Pruritus and pain are usually absent. Histologically, seborrheic keratoses are characterized by orthokeratotic hyperkeratosis, epidermal hyperplasia (basaloid and squamoid), and papillomatosis. In humans, the sudden appearance or enlargement of multiple lesions can be associated with an internal malignancy. This association has not been made in domestic animals.

Lichenoid keratoses have been reported as single or occasionally multiple wart-like papules or hyperkeratotic plaques that may be hyperpigmented on the *inner surface of the pinna in dogs.* Age, breed and sex predilections have not been noted. Histologically, irregular to papillated epidermal hyperplasia, moderate to marked orthokeratotic and/or parakeratotic hyperkeratosis, and a lichenoid inflammatory infiltrate predominantly consisting of lymphocytes and plasma cells characterizes lichenoid keratosis.

Lichenoid dermatoses are rare skin disorders of dogs and cats. There is no apparent age, breed, or sex predilection. The dermatoses are characterized by the usually asymptomatic, *symmetric, grouped, flat-topped papules and plaques that are variably distributed, and that develop a scaly to markedly hyperkeratotic surface.* The lesions are *self-limiting* although resolution may take several years. Histologically these dermatoses are characterized by lichenoid interface dermatitis composed of plasma cells and lymphocytes, marked orthokeratotic hyperkeratosis and follicular keratosis, and moderate epidermal hyperplasia. Apoptotic keratinocytes can be seen primarily, although not exclusively, in the basal layer and there is often hydropic degeneration of basal keratinocytes. If focal areas of suppurative epidermitis and/or suppurative folliculitis are present, a lichenoid tissue reaction in response to staphylococcal infection should be suspected.

Cutaneous horns are recognized occasionally in all domestic species. Some are of unknown cause; *others originate from papillomas, basal cell tumors, squamous cell carcinomas or other keratoses.* In cattle, sheep, and goats, cutaneous horns may arise in lesions of dermatophilosis. In the cat, multiple cutaneous horns on the footpads have been reported in association with *Feline leukemia virus* (FeLV) infection; FeLV was

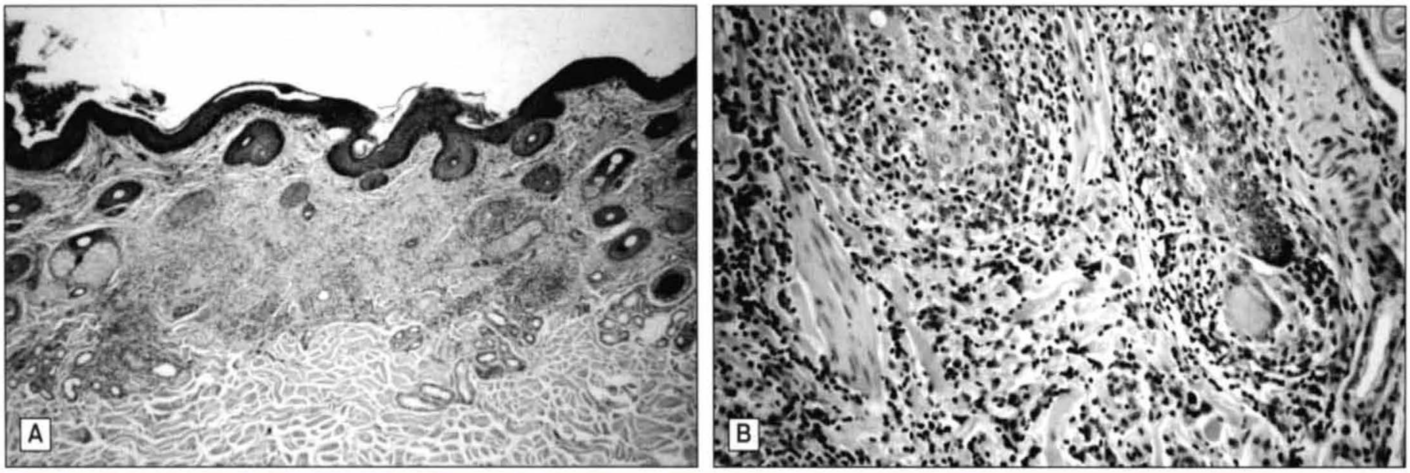

Figure 5.33 Equine linear alopecia A. Low magnification showing a follicular orientation of the inflammatory infiltrate. **B.** Lymphohistiocytic to granulomatous mural folliculitis disrupts hair follicles.

cultured from the horns, and type C viral particles were seen in the lesions with the electron microscope.

Cutaneous horns may be single or multiple, and have no apparent age, breed, sex or site predilections. The lesions are *firm, well-circumscribed horn-like projections from the skin.* They may be small (1 mm diameter × 5 mm length) or quite large (3 cm diameter × 12 cm length). Histologically, cutaneous horns are characterized by *extensive, compact, laminated, orthokeratotic, and/or parakeratotic hyperkeratosis.* The base of the horn must be inspected for the possible underlying cause.

Linear epidermal nevi are characterized histologically by *linear hyperkeratosis with epidermal hyperplasia.* They have been reported in several species. These include linear epidermal nevi in Belgian horses, inflammatory linear verrucous epidermal nevus in dogs, and a hereditary disorder of cornification in Rottweiler dogs (see Congenital and hereditary diseases of skin). The term nevus implies a lesion present at birth and composed of mature elements, and therefore since these linear hyperkeratotic lesions have in common an early age of onset, *they may represent linear epidermal nevi that have tardive onset.* Equine cannon keratosis is grossly and histologically similar to linear epidermal nevi but differs from the linear epidermal nevi of Belgian horses as it can occur at any age and is restricted to the skin of the cannon area.

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Sebaceous adenitis

Sebaceous adenitis is an *uncommon skin disease of dogs* that has also been reported in the cat and rabbit. The condition has been reported in numerous breeds of dog and in mongrels, however there *is breed predilection* for Standard Poodles, Akitas, Samoyeds, and Vizslas, suggesting a genetic basis. In Standard Poodles and Akitas, an autosomal recessive trait is proposed. Onset of the disorder is usually in young adults to middle-aged animals and there is no sex predilection.

The early lesions consist of patches of scaling and alopecia that tend to appear on the *ears and dorsum.* In short-coated dogs, these progress to annular areas of alopecia and scaling on the trunk and head. Longer-coated animals initially develop a thin coat due to loss of the undercoat, and then develop symmetrical multifocal to generalized areas of patchy alopecia and brittle to broken hairs encircled by yellow to brown follicular casts. Secondary bacterial infection is common. Early histopathologic changes are characterized by *granulomatous or pyogranulomatous inflammation targeted on the sebaceous glands and eventually destroying the gland* (Fig. 5.34A). Inflammation can occasionally impinge secondarily on the follicular epithelium causing folliculitis. Orthokeratotic and/or parakeratotic hyperkeratosis together with follicular keratosis can be marked. In the chronic stages, both active inflammation and sebaceous glands may be absent, and there may be perifollicular fibrosis (Fig. 5.34B). After the disappearance of the glands, the hair follicles frequently assume a "stretched out" configuration and are keratin filled. Regeneration of sebaceous glands after variable amounts of time has been reported in occasional cases.

The pathogenesis of this disease has not been fully characterized. Several possibilities include: (1) destruction of the gland due to immune-mediated mechanisms leading to secondary hyperkeratosis; (2) a

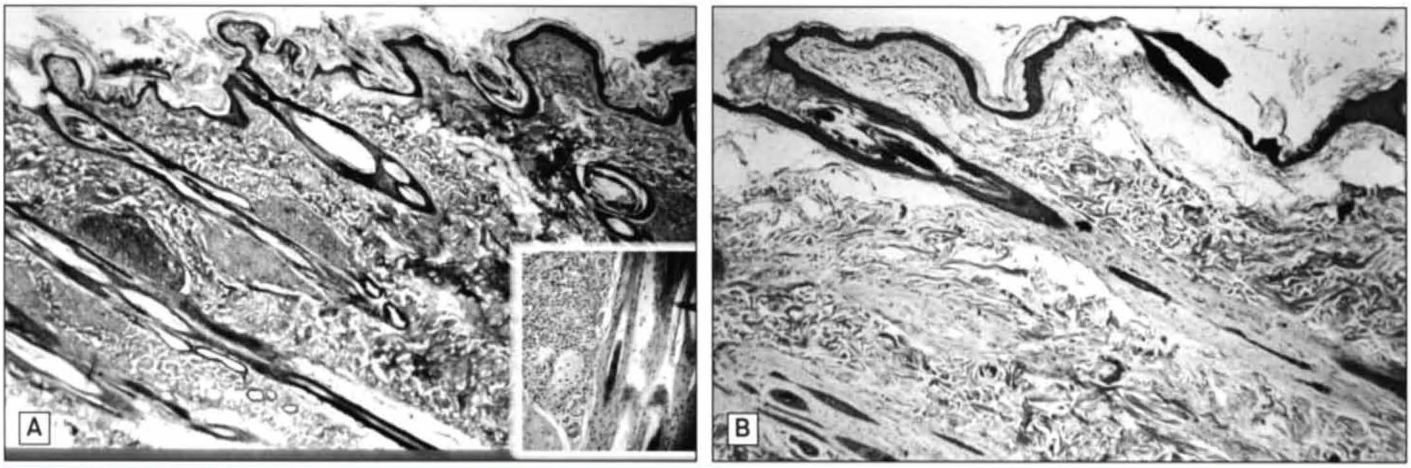

Figure 5.34 Sebaceous adenitis in a dog. **A.** Early lesions. Mononuclear leukocytes target sebaceous glands. Sebocytes are destroyed by the inflammation (see inset). **B.** Late lesions. Inflammation is minimal and areas of fibrosis have replaced sebaceous glands. There is epidermal and follicular hyperkeratosis. (Courtesy of B Dunstan.)

primary keratinization defect resulting in increased amounts of follicular keratin blocking the sebaceous duct and causing inflammation of the gland; or (3) a defect in the structure of the sebaceous duct or gland resulting in inflammation directed at free sebum. In the predisposed breeds such as Standard Poodles and Akita, a genetic basis seems probable together with other factors that would explain the variation in onset and progression of the disease.

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Vitamin A-responsive dermatosis

Vitamin A-responsive seborrheic dermatoses occur most commonly in Cocker Spaniels, but can occur in other breeds (see Nutritional diseases of skin). There is, however, no clinicopathologic evidence to suggest that these animals have vitamin A deficiency.

Lichenoid-psoriasiform dermatosis of Springer Spaniels

This is an uncommon dermatosis that affects young English Springer Spaniels of either sex. Asymptomatic, generally symmetric, hyperkeratotic, erythematous papules usually begin on the pinnae and groin, coalesce to plaques, and progressively involve large areas of the body, especially the ventral abdomen and prepuce. Secondary bacterial infection is common.

Histopathologic findings include *lichenoid dermatitis* composed predominantly of plasma cells, with areas of *psoriasiform epidermal hyperplasia*, occasional apoptotic basal keratinocytes, intraepidermal microabscesses (containing eosinophils and neutrophils), and Munro's microabscesses. Chronic lesions frequently show papillated epidermal hyperplasia, papillomatosis, and moderate to marked orthokeratotic and/or parakeratotic hyperkeratosis. *The histologic findings are very similar to lichenoid keratosis and must be differentiated clinically by knowledge of distribution of lesions and knowledge of the breed.*

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Ear margin dermatosis

This is an idiopathic seborrheic disorder that is localized to the margins of the pinnae. It occurs primarily in the Dachshund, although it can be seen in other breeds with pendulous ears. It may represent a localized primary seborrhea. There is no sex predilection, and the disorder usually begins in young adults. Waxy keratosebaceous accumulations and alopecia follow initial scaling of the ear margins. The disease is symmetrical and asymptomatic. The dermatosis may be complicated by secondary bacterial infection and fissures, at which point ulceration, oozing, crusting, pain and pruritus may be seen. Histopathologic findings are characterized by *marked orthokeratotic and/or parakeratotic hyperkeratosis with follicular keratosis and variable mild superficial perivascular dermatitis.*

Exfoliative dermatoses (exfoliative erythroderma)

This refers to a cutaneous reaction pattern that can be associated with many diseases. Clinically it is characterized by scaling and erythema that can be localized or generalized. Most cases of exfoliative dermatosis have been reported *in association with thymoma, cutaneous lymphoma, visceral malignant neoplasms, drug reactions, or are idiopathic.* Histopathologic findings can reflect the underlying condition and can include parakeratosis, epidermal acanthosis, variable psoriasiform epidermal hyperplasia, variable lymphocytic exocytosis, and perivascular to lichenoid dermal inflammation.

Exfoliative dermatosis resembling human large plaque parapsoriasis has been reported in a dog and a cat. The human disease, to which these cases were compared, is frequently a precursor to mycosis fungoides.

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Hyperplastic dermatosis of West Highland White Terriers

This is a *severe chronic seborrheic disorder* in which there is a marked breed association with West Highland White Terriers. It was previously referred to as "epidermal dysplasia of West Highland White Terriers;" however epidermal hyperplasia is a more common finding. Epidermal disorganization or keratinocyte abnormalities characteristic of dysplasia are not present. Clinical signs begin at <1 year to middle age, with severely affected dogs tending to have an earlier age of onset.

Erythema and scaling with pruritus develop on the trunk, especially axillary and inguinal regions, and progress to involve the whole body. In chronic cases, the skin becomes lichenified, alopecic, hyperpigmented, and greasy. This combination of gross lesions has led to this condition being referred to as the "armadillo Westie syndrome." Histologic epidermal lesions are characterized by *acanthosis with mild to marked orthokeratosis or parakeratosis*. The base of the hyperplastic epithelium can have a scalloped appearance, and there is multifocal spongiosis with lymphocytic exocytosis. *There is commonly secondary* *pyoderma and/or infection with Malassezia.* The absence of *Malassezia* organisms in histologic sections does not necessarily exclude their presence as the organisms may be lost in tissue processing. Cytological evaluation for yeasts is a more reliable indicator of the presence and numbers of yeasts. *The lesions of hyperplastic dermatosis of West Highland White Terriers can be very similar histologically to lesions of chronic allergic dermatitis,* and the two conditions can frequently coexist in this breed. The condition may actually be a tissue reaction secondary to an allergic dermatitis or infectious condition and not a primary disease entity.

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Equine coronary band dystrophy

Equine coronary band dystrophy is a condition of *unknown etiology and pathogenesis.* The condition affects adult horses of any breed, but draft breeds are considered predisposed. Equine coronary band dystrophy is characterized by *marked proliferation and hyperkeratosis of the epidermis of the coronary band and in some cases the chestnuts and ergots.* Usually all four limbs are affected; however the lesion may not encompass the entire coronary band. Clinically, the coronary band is thickened, crusty and scaly. Cracks and fissures may develop and lead to lameness. The chestnuts and ergots are similarly affected and may be ulcerated. Histologically, the epidermis of affected areas is characterized by marked papillary hyperplasia and marked ortho- to parakeratotic hyperkeratosis. In some areas, there is ballooning degeneration of keratinocytes. Dermal inflammation is minimal unless secondary infection is present. *The diagnosis is made by ruling out other differential diagnoses that include pemphigus foliaceus, the hepatocutaneous syndrome, bacterial or fungal infection, selenium toxicosis, and eosinophilic exfoliative dermatitis.* The condition is chronic and treatment palliative.

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Ichthyosis

The ichthyoses are rare disorders of cornification that result in severe generalized scaling. These disorders are congenital and heritable (see Congenital and hereditary diseases of skin).

DISORDERS OF PIGMENTATION

Melanin pigments are responsible for the coloration of the hair, skin, and eyes, and also play an important role in photoprotection. Melanin is synthesized by melanocytes, which are dendritic cells originating as melanoblasts in the neural crest. Melanoblasts develop in the neural crest, migrate to peripheral sites such as skin, hair follicles, and dermis, differentiate into melanocytes, and synthesize melanosomes and melanin. Genetic mutations affecting any of these steps can lead to hereditary hypopigmentation. Many such mutations have been characterized in the murine model but this area has been little studied in domestic animals. Many types of exogenous influences, such as inflammation, UV radiation, endocrinopathies, autoimmune diseases, and nutritional status can affect melanocytes in the skin resulting in acquired hypopigmentation or hyperpigmentation.

Melanin synthesis in melanocytes takes place in *melanosomes,* which are round or elliptic membrane-bound organelles thought to be derived from endoplasmic reticulum and containing enzymes from the Golgi and lysosomal system. Melanosomes are designated type I through type IV, according to their stage of maturation. Type I melanosomes contain no melanin and are electron-lucent, whereas type IV are mature melanosomes that are electron-dense and migrate to the tips of the dendritic processes to be transferred to adjacent epithelial cells. Melanogenesis in round melanosomes produces *eumelanins, the black pigments,* and in elliptic melanosomes produces *pheomelanins, red and yellow pigments.* Pigment types in horses, sheep, goats, and llamas have been analyzed. These melanin pigments arise from the common metabolic pathway of conversion of tyrosine to DOPA and then oxidation to DOPAquinone. *Tyrosinase,* a copper-containing enzyme, is the critical and rate-limiting enzyme in this pathway, catalyzing tyrosine to DOPA. Many gene products are sequentially important to the melanoblast and melanocyte during their development and maturation. Apart from tyrosinase, the molecular role of these gene products has not been completely characterized, but it appears that platelet-derived growth factor (PDGF), and receptors for fibroblast growth factor (FGF-2), endothelin-B, and the Steel factor (cKIT) are crucial.

Cutaneous pigmentary disorders can be divided into disorders of *hyperpigmentation* and *hypopigmentation.*

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Disorders of hyperpigmentation

Acquired hyperpigmentation

Acquired hyperpigmentation of the skin (**melanoderma**) is encountered frequently. It is usually post inflammatory, a result of minor or chronic irritation, and may be accompanied by mild hyperkeratosis. *Both melanosis and hyperkeratosis are common responses to mild injuries by agents as diverse as mites and irradiation.* Hypermelanosis results from an increased rate of melanosome production, an increase in melanosome size, or an increase in the degree of melanization of the melanosome. It is usually associated with an accelerated melanocyte turnover with an increased number of melanosomes, as occurs following trauma and ultraviolet light exposure. Inflammatory mediators likely play a role in stimulating melanocyte production. Activation of pre-existing immature melanocytes by sunlight, estrogen, and progesterone is thought to occur. Endothelin-1, which is produced and secreted by keratinocytes after UV-irradiation, has been shown to accelerate melanogenesis. Basic fibroblast growth factor (bFGF) has been shown to be a mitogen for human melanocytes. Proliferating human epidermal cells in culture produce bFGF, perhaps illustrating the mechanism behind the hyperplastic and hyperpigmented lesions that typify many chronic dermatoses.

Acquired hyperpigmentation may also involve hair (**melanotrichia**). This is usually seen as a result of inflammatory skin disorders, especially those caused by biting insects in the horse and also has been described in white Merino sheep exposed to ultraviolet light.

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Focal macular melanosis

Lentigo simplex has been reported in *cats and dogs*, and is most common in cats with orange, cream, or tricolored coats. The lesions are flat, or minimally raised, pigmented macules and usually occur on the mucocutaneous junctions of the mouth, eye, and nose, and the footpads. Lesions tend to start at less than one year of age and may increase in size and number with age. *The lesions are of no significance except that they can be confused clinically with melanoma or pigmented nevus.* Histologically, lentigines are characterized by minimal to mild epidermal hyperplasia with formation of elongated rete ridges. There are increased numbers of melanocytes, particularly in the stratum basale, and usually increased melanin in basal keratinocytes. Low numbers of melanophages may be present in the underlying dermis. Papillated epidermal hyperplasia and hyperkeratosis are not present. Generalized lesions have been reported in a silver cat.

Merino **sheep** may acquire pigmented macules, particularly after shearing. *Lesions are concentrated on the back suggesting a role for sunlight exposure.* Experimental exposure to ultraviolet light induced lesions as early as 10 days post-irradiation. Histologically, these lesions are characterized by increased numbers of epidermal melanocytes at the dermoepidermal junction and in the normally non-pigmented outer root sheath epithelium.

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Canine acanthosis nigricans

Canine acanthosis nigricans *is an idiopathic dermatitis, characterized by progressive hyperpigmentation, alopecia, and lichenification.* The lesions are roughly bilaterally symmetrical and typically start in the axillae, spreading to involve proximal limbs, ventral abdomen, neck, and inguinal area. Seborrhea, *Malassezia* infection, and bacterial pyoderma are frequent complications. Histologic examination reveals a hyperplastic dermatitis with orthokeratotic and parakeratotic hyperkeratosis, acanthosis and rete ridge formation. All layers of the epidermis are heavily melanized. Spongiosis, neutrophilic exocytosis, and serous crusts may also be present. The dermal inflammatory reaction is mild, pleomorphic in cell type, and superficial perivascular in location.

The primary or idiopathic form of acanthosis nigricans occurs predominantly in Dachshunds. In view of the early age of onset (usually <1 year old) and the strong predilection for dachshunds, it is probable that canine idiopathic acanthosis nigricans is a *heritable disorder*. In man, some forms of acanthosis nigricans are associated with internal malignancies, hyperinsulinemia, insulin resistance, drug administration, endocrine dysfunction, and concurrent autoimmune disease; a similar correlation has not been demonstrated in dogs. The histologic lesions of primary acanthosis nigricans are virtually identical to the common histologic changes associated with chronic pruritic dermatitides (sometimes referred to as pseudo-acanthosis nigricans or secondary acanthosis nigricans) due to several causes, including chronic pyoderma, atopy and seborrheic dermatitis, and some endocrine disorders. *The diagnosis of primary acanthosis nigricans requires clinical correlation together with the histologic findings to support the diagnosis in a young Dachshund with compatible distribution of lesions.*

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Acromelanism

Acromelanism is seen in Siamese and Himalayan cats, rabbits, and mice. It is a condition in which coat color can be influenced by external temperature (high temperatures producing light hairs, low temperatures producing dark hairs) and factors affecting heat production and loss (alopecia, inflammation). *The coat color changes are usually temporary*, and the hair returns to the normal color with the next hair cycle. *These phenomena are due to a missense nucleotide substitution in tyrosinase making the enzyme thermally unstable.*

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Disorders of hypopigmentation

Leukoderma and leukotrichia

Reduction in pigmentation of the skin is **leukoderma**, and of the hair is **leukotrichia**. Leukoderma and leukotrichia may occur independently. They can result from a decrease in melanin (**hypomelanosis**), a complete absence of melanin (**amelanosis**), or from a loss of existing melanin (**depigmentation**). These events result from either an absence of the pigment-synthesizing melanocytes or from a failure of melanocytes to produce normal amounts of melanin or to transfer it to adjacent keratinocytes.

Hereditary hypopigmentation

Hereditary hypopigmentation can be divided into *melanocytopenic hypomelanosis* characterized by the absence of melanocytes in affected areas, and *melanopenic hypomelanosis* in which melanocytes are present but defective. The condition can be localized, focally extensive, or generalized. Melanocytopenic hypomelanosis can be extensive, as is seen in animals with Waardenburg syndromes and in piebaldism. In these cases, there is failure of melanoblasts to migrate from the neural crest into the skin, or failure to survive in the skin. Melanocytopenic hypomelanosis can also be localized, as in vitiligo, in which there is genetically programmed destruction of melanocytes. Melanopenic hypomelanosis is seen in the various forms of albinism.

Melanocytopenic hypomelanosis

Syndromes analogous to the human **Waardenburg syndrome** have been reported in cats, dogs, horses, and rabbits. *Affected animals typically have white coats and blue or heterochromatic irides, and are deaf.* In *cats*, this has been shown to be due to an autosomal dominant mutation with complete penetrance for loss of pigmentation and incomplete penetrance for deafness. In *dogs*, this syndrome has been described in breeds such as the Dalmatian, Bull Terrier, Sealyham Terrier, Collie, and Great Dane. A syndrome analogous to human Waardenburg type-4 (Hirschsprung disease) has been reported in mice with lethal spotting mutation, and in American Paint horses in which white foals from overo mares are born with aganglionic colons. These foals develop colic and die shortly after birth.

Piebaldism is also a form of genetic melanocytopenic hypomelanosis resulting in *multifocal white patches* in which there is absence of melanocytes due to a congenital failure of melanoblasts to migrate from the neural crest to the skin, or by their inability to survive and proliferate in the skin. Piebaldism has been *seen in many species* including horses, dogs such as the Dalmatian, cats, cattle, and rodents. The defect has been shown to be a mutation in the gene encoding c-kit tyrosine kinase receptor, or a mutation in the gene for stem cell factor, which is the receptor ligand. The c-kit tyrosine kinase receptor is associated with proliferation and survival of melanoblasts.

Literally meaning, "blemish," **vitiligo** is a melanocytopenic hypomelanosis of humans and animals, which is characterized by *gradually expanding pale macules that are often symmetrical or segmental in distribution* (Fig. 5.35). Vitiligo has been described in the dog, cat, horse, cattle, and the Smyth chicken (DAM chicken) which has been used as an animal model of the human disease. The immediate cause of vitiligo is the destruction of melanocytes. It is considered to be a *genetic amelanosis inherited as an autosomal recessive trait in animals*. It is thought to be a polygenic disease necessitating simultaneous mutations in several genes resulting in melanocyte destruction or increased risk of immune-mediated destruction of melanocytes. Theories regarding the pathogenesis of this disease include autoimmune destruction of melanocytes, a neurogenic theory involving release of a neurochemical from peripheral nerves that inhibits melanogenesis, a self-destruction theory that involves failure of protection of melanocytes against the toxic effects of melanin precursors, or a combination of factors. Circulating antimelanocytic antibodies have been detected in some studies, lending support to an immune-mediated pathogenesis.

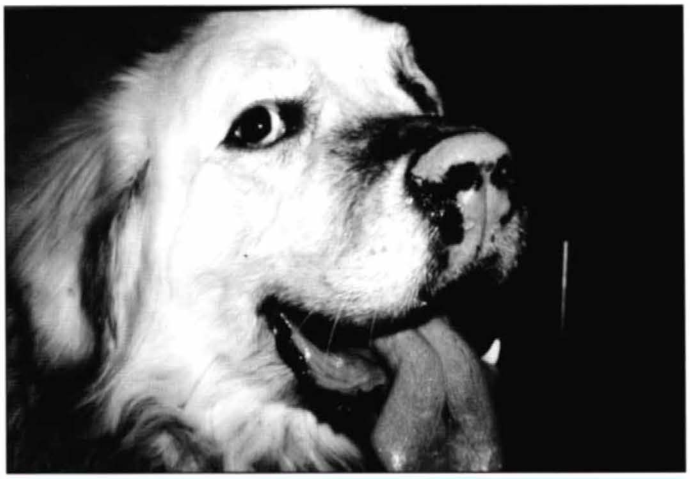

Figure 5.35 Vitiligo in a dog. Coalescing pale macules on the skin of the nasal planum and lips. (Courtesy of B Dunstan.)

Vitiligo in the **dog** has been described in Belgian Tervuren, Doberman Pinscher, Newfoundland, Rottweiler, German Shepherd, Dachshund, German Shorthaired Pointer, and Old English Sheepdogs. Vitiligo in a Dachshund developed concurrently with juvenile-onset diabetes mellitus. The condition best characterized is seen in Belgian Tervurens. The depigmentation in this breed occurs chiefly on the pigmented skin and mucous membranes of the face and mouth in young adult dogs. Histologic examination of affected skin shows an epithelium devoid of both pigment granules and DOPA-positive cells. Electron microscopy confirms the lack of melanocytes in the lesions; their place is taken by Langerhans or indeterminate dendritic cells. Antimelanocytic antibodies have been demonstrated in affected dogs but not in normal animals.

Vitiligo has also been described in **horses**. In one form, the "**Arabian fading syndrome**," affected animals develop round, depigmented macules on the lips, muzzle, around the eyes, and occasionally the anus, vulva, prepuce, and hooves. The disease can start at any age, but is more common in horses under 2 years of age. Circulating antimelanocytic antibodies have been detected in some cases.

In **cattle**, vitiligo-like lesions have been described in Holstein-Friesians, in black Japanese cattle, and in water buffalo.

Siamese cats may develop vitiligo. Antibodies to an 85-kDa surface antigen of melanocytes were demonstrated in four cats with vitiligo. No antibodies were detected in three normal Siamese cats tested.

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Melanopenic hypomelanosis

The various forms of **albinism** are examples of melanopenic hypomelanosis. In albino animals and people, melanocytes are present and normally distributed but are defective in function and fail to synthesize melanin. The extent of the biochemical defect varies, so that *albinism covers a spectrum from amelanosis, oculocutaneous albinism (OCA), through graded pigmentary dilution.* Oculocutaneous albinisms and pigment dilutions are inherited as autosomal recessive traits. In albino animals with white hair and skin, and translucent irides, there is a mutation in the tyrosinase gene resulting in no residual enzyme activity. A mutation resulting in residual enzyme activity produces animals born with white hair but producing blond or pigmented hair as juveniles. The skin remains white but can develop pigmented nevi. This form has been reported in a gorilla.

Chediak-Higashi syndrome in humans, Hereford, Brangus and Japanese Black cattle, Persian cats, mink, blue and silver fox, and various other animal species is an example of *partial albinism and is inherited as an autosomal recessive trait.* While melanin is produced, there is a mutation of the *beige* gene, which plays a major role in generating cellular organelles. This results in a membrane defect leading to the formation of giant melanosomes that are passed with difficulty to the keratinocytes. The clumping of these giant melanosomes produces the color dilution effect. Chediak–Higashi syndrome is discussed with the Hematopoietic system.

Cyclic hematopoiesis (cyclic neutropenia), a lethal hereditary disease of Collie dogs, is caused by an autosomal recessive gene with a pleiotropic effect on coat color dilution. Affected dogs are silver-gray. The abnormal hair pigmentation results from the diminished formation of melanin from its precursor tyrosine rather than from pigment clumping. The normal collie coat color is restored in animals receiving bone marrow transplants to correct cyclic hematopoiesis. The hematological aspects of this disease are considered with the Hematopoietic system.

Coat color dilution has been reported in many species. It occurs in many breeds of dog, in cats, particularly Siamese cats, horses, and cattle. The pale coat coloration is due to clumping of large melanin granules in hair follicles and sometimes in the epidermis. In cats, dilute coat color is thought to be due to an autosomal recessive trait (*Maltese dilution*). **Color-dilution alopecia**, a tardive-onset hypotrichosis associated with color dilution traits in the dog, is discussed under Congenital and hereditary diseases of the skin.

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Acquired hypopigmentation

This follows damage to the epidermal melanin unit by various insults, including trauma, inflammation, radiation, contactants, endocrinopathies, infections and nutritional deficiencies. In general, the severity of the injury determines whether an insult will result in hypo- or hyperpigmentation. *Mild injury results in pigmentary incontinence and epidermal hypopigmentation*; however a mild injury allows accelerated keratinocyte turnover and a subsequent increase in production of melanosomes. *Severe injury results in the death of melanocytes and no subsequent repigmentation.*

Examples of depigmenting diseases in **horses** include onchocerciasis, *Culicoides* hypersensitivity, ventral midline dermatitis, and coital vesicular exanthema. Depigmenting lesions in horses may result from contact with equipment such as rubber bit guards or crupper straps or with feed buckets. Monobenzene ether of hydroquinone, a common ingredient in rubber, inhibits melanogenesis.

In **dogs**, hypopigmentation can occur in immune-mediated diseases such as lupus erythematosus, drug eruptions, bullous pemphigoid, and the various forms of pemphigus. Acquired depigmentation of the lips and/or nose also occurs in dogs as a result of contact with rubber dishes or toys containing dihydroquinone monobenzene ethers. Microbial lesions, such as deep pyoderma, may heal with depigmentation. Depigmenting lesions have been noted also in canine leishmaniasis and in dermatophytosis caused by *Microsporum persicolor*. Epitheliotropic lymphoma often presents with depigmenting, ulcerative lesions of skin and mucocutaneous junctions. Transient depigmentation has been reported in drug eruptions. Subcutaneous injection of corticosteroid or progesterone hormones may lead to focal hypopigmentation in the dog.

Uveodermatologic syndrome (Vogt–Koyanagi–Harada-like syndrome)

A depigmenting condition in dogs, partially resembles an extremely rare condition in humans, the Vogt–Koyanagi–Harada syndrome. *The arctic breeds such as the Akita, Siberian Husky, Samoyed, and Malamute are predisposed to this condition*, however it has been reported in many breeds. The cause is unknown, although an immune-mediated attack on melanocytes, as in the human disease, is presumed. The canine

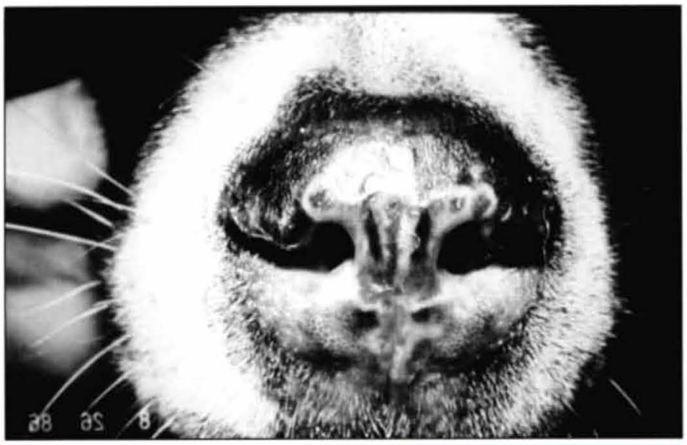

Figure 5.36 Uveodermatologic syndrome in a dog. Note depigmentation of the nose. (Courtesy of K Campbell.)

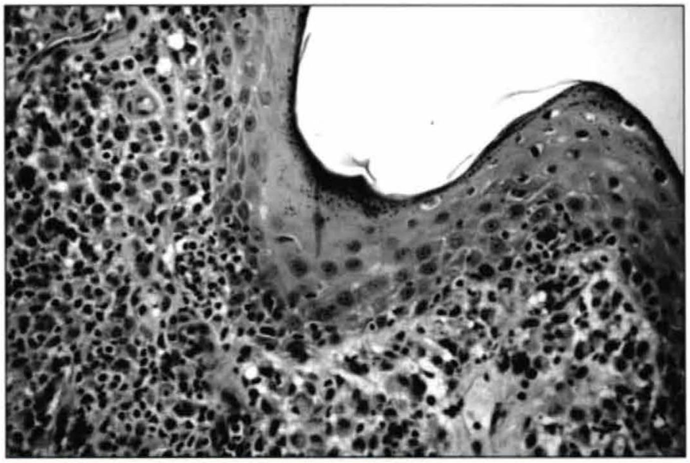

Figure 5.37 Uveodermatologic syndrome in a dog. Lichenoid interface, predominantly histiocytic, dermatitis with rare degeneration of basal keratinocytes and pigmentary incontinence.

lesions comprise *bilateral panuveitis* (see Vol. 1, Eye and ear) and *bilateral cutaneous depigmentation*, chiefly of the lips, nose, and periorbital skin (Fig. 5.36). Ocular lesions most often precede onset of cutaneous lesions. The scrotum, vulva, perianal skin, and footpads are less often affected. Leukotrichia is a common finding around the areas of leukoderma. Occasionally, depigmented lesions become ulcerated, erythematous or crusted. The histologic pattern of the cutaneous lesion is an *interface dermatitis with uncommon degeneration of basal keratinocytes, and a lichenoid inflammatory cell infiltrate in which large histiocytic cells usually predominate* (Fig. 5.37). Pigmentary incontinence is a common finding in areas of depigmentation but vacuolar change of the basal keratinocytes is not. This, and the histiocytic nature of the inflammatory infiltrate, are *major features of differentiation from discoid and systemic lupus.*

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Leukotrichia

Reticulated leukotrichia, colloquially known as "tiger stripe," is recognized in the Standardbred, Thoroughbred, and Quarter Horse breeds. The lesions occur predominantly in yearlings and comprise linear crusts arranged in a cross-hatch pattern on the dorsal midline from the withers to the tail. Transient alopecia and a regrowth of permanently white hair follow crusting. The underlying skin has normal pigmentation. Well-documented precise descriptions of the expected histologic lesions are lacking. Some reports indicate an interface lichenoid dermatitis may be present, while others suggest that a mild superficial dermal mononuclear cell infiltrate and pigmentary incontinence are to be expected. The etiology and pathogenesis are unknown. **Spotted leukotrichia** occurs in the horse as multiple, often somewhat symmetrical, small circular areas of white hair. The spots occur most commonly on the rump and thorax, and Arabians have a predilection. The etiology and pathogenesis are unknown. **Hyperesthetic leukotrichia**, so-called because the lesions are extremely painful, has been reported only in Californian horses. Single or multiple crusted lesions occur on the dorsal midline and heal leaving permanently white hairs. Leukotrichia, also termed *poliosis*, has been reported in **dogs** in association with Vogt–Koyanagi–Harada syndrome, tyrosinase deficiency in Chow Chows, and as an idiopathic, possibly heritable condition in a litter of Labrador Retrievers. In the last example, the condition resolved.

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Alopecia areata and universalis

Leukotrichia can be associated with alopecia areata. These disorders are discussed in more detail under Other immune-mediated dermatoses.

Copper deficiency

This pigmentary disorder is seen primarily in *cattle and sheep*. It has also been reported in *moose*, and experimentally in *dogs*. Copper deficiency may be simple or conditioned by other dietary substances, particularly sulfate and molybdenum. Since copper is an essential constituent of tyrosinase, there is *depressed tyrosinase activity*, and deficient animals show depigmentation of hair or wool. Affected cattle with normally black coats become rusty-brown and develop "spectacle" lesions round the eyes. Black sheep develop intermittent bands of light-colored wool corresponding to periods of restricted availability of copper (Fig. 5.38). The deficiency of copper also affects the physical nature of the wool or hair. In sheep, *the wool has less crimp*, prompting the colloquial name of "string" or "steely" wool. The straightness of the wool is due to inadequate keratinization, probably caused by imperfect oxidation of sulfhydryl groups in prekeratin, a process that involves copper (Fig. 5.39).

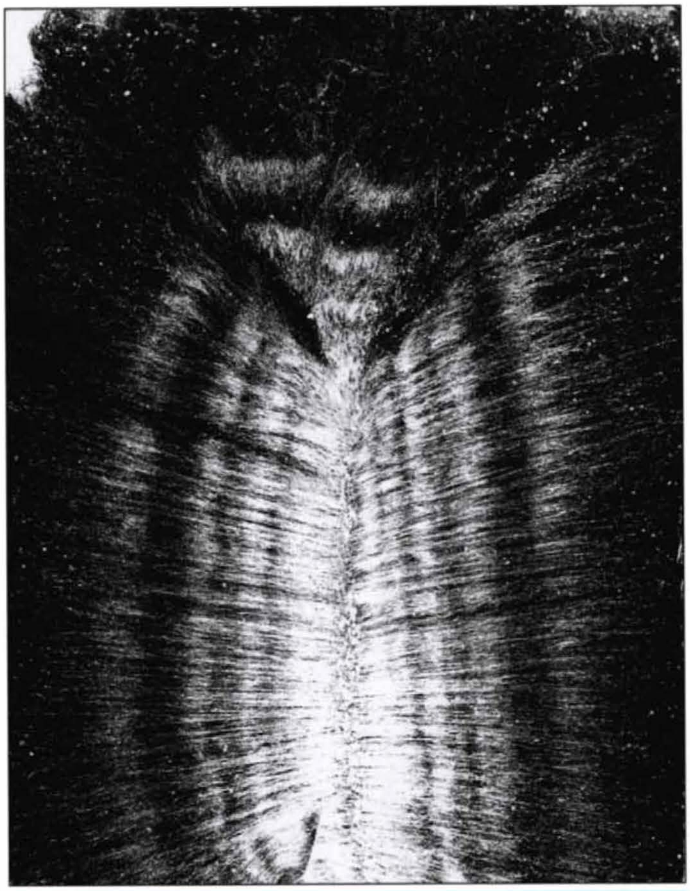

Figure 5.38 Copper deficiency. Black-wooled sheep with bands of achromotrichia corresponding to periods of molybdenum administration. (Courtesy of WJ Hartley.)

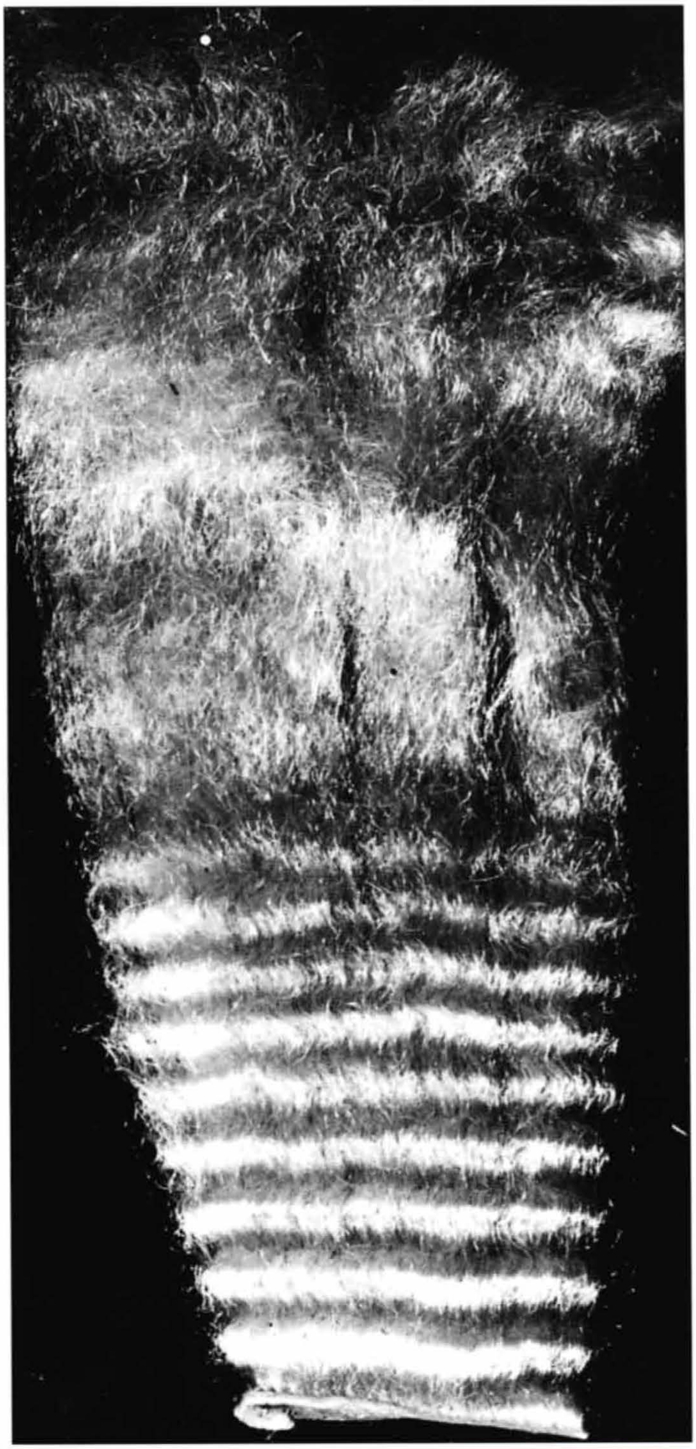

Figure 5.39 Staple from sheep that received 1.0 mg of copper per day, then 10 mg per day. Note stringiness and lack of crimp on deficient regime. (Courtesy of WJ Hartley.)

PHYSICOCHEMICAL DISEASES OF SKIN

The integument has a large surface area in direct contact with the environment and is hence extremely vulnerable to chemical and physical injuries. *Physical stresses* include friction, pressure, vibration, electricity, high and low ambient temperatures, humidity, visible light, and ultraviolet, infrared and ionizing radiation. Cutaneous reactions to visible light are discussed separately. *Chemical toxins* may exert their effect directly as in irritant contact dermatitis or envenomation or indirectly, as in thallium poisoning.

Physical injury to skin

Mechanical, frictional, and traumatic injury

The hair coat of most domestic animals protects from "blisters" that so commonly develop in human skin subjected to prolonged pressure or frictional contact with a hard surface. The following examples tend to occur in heavy animals with skin exposed to repeated or constant pressure such as in animals immobilized by paralysis, or in those exposed to a physically harsh environment.

Dogs

Calluses occur when continual or repetitive pressure or friction is applied to a localized area of skin and represent a protective response of the integument to the physical injury. They tend to occur over bony prominences, particularly the hocks, elbows, lateral surfaces of the digits and on the sternum. Callosities can develop in all domestic animal species, but are most common in dogs, particularly the giant breeds, and in pigs housed on concrete floors with inadequate bedding. They are characterized by epidermal proliferation with prominent epidermal and follicular hyperkeratosis. Dilated hair follicles may lead to furunculosis with secondary pyoderma, severe suppurative to pyogranulomatous dermatitis and eventual fibrosis. Grossly, callosities are *well-circumscribed, lichenified, raised, alopecic, gray, keratinous plaques*. Ulceration may occur. The pig may also develop bursitis.

A **hygroma** *is a false or acquired bursa, which develops subcutaneously over bony prominences.* Hygromas are most common in the giant breeds of dogs at pressure points such as the lateral aspect of the elbow, the greater trochanter of the femur and the tuber coxae. Hip dysplasia in dogs can lead to elbow hygromas as dogs develop an abnormal method of lying down that relies upon dropping to the olecranons to spare the hips. Usually pressure induces a protective callus, but in some animals persistent decubitus ulcers or recurrent hematoma formation eventually lead to the induction of a hygroma. The gross lesion is a variably sized cystic cavity separated from the skin by loose connective tissue. The wall of the hygroma is dense connective tissue that may have a smooth or a villus inner lining. The contents are mucinous and yellow to red, depending on the degree of hemorrhage. *Histologically, the wall is composed of granulation tissue of variable maturity.* A flattened layer of fibroblasts may give the appearance of an epithelial lining. The cavity may contain clumps of fibrin. Organization of fibrin deposits at the margin of the cavity gives rise to the grossly apparent villus projections that occasionally undergo cartilaginous metaplasia.

Decubitus ulcers *are the result of ischemic necrosis that follows application of constant pressure to a localized area of skin.* Studies in Greyhounds suggest that intermittent repeated focal vascular occlusion leads to increased tissue damage from *reperfusion injury*. Thromboxane A_2 and its metabolite, thromboxane B_2, are thought to contribute to vasoconstriction and platelet aggregation. Predisposing conditions include prolonged recumbency, lack of proper bedding, improperly applied bandages or casts, poorly fitting tack, atrophy of muscle, loss of fat, malnutrition associated with systemic disease, contusions, irritation from feces or urine, and body types with large bones, thin skin and low body fat. In large animals, post-anesthetic myopathies, laminitis, and neurologic diseases are predisposing conditions. The "downer" cow is particularly prone to decubitus ulcers. *Decubitus ulcers are graded from I to IV.* In grade I, the lesion consists of focal erythema. In grade II, an ulcer extends into the subcutis. In grade III, the ulcer extends into the deep fascia and the wound edges may be undermined. Grade IV ulcers extend to bone, have undermined edges, and possibly underlying osteomyelitis and septic arthritis.

Intertrigo *refers to localized dermatitis affecting folded areas of skin.* The combined effect of friction, heat, maceration, bacterial or yeast proliferation and irritation by retained secretions leads to superficial inflammation. Examples in dogs include *facial, lip, vulvar and tail fold dermatitis*. Obesity is a predisposing factor. Body fold dermatitis is particularly common in Chinese Shar-Pei puppies. Udder-thigh dermatitis occurs predominantly in first-calf *dairy heifers*. Udder edema is a predisposing factor. Lesions are erythematous and swollen, sometimes ulcerated and often have an unpleasant odor. Histologically, the epidermis is hyperplastic, spongiotic and possibly eroded or ulcerated. Surface pustules, neutrophilic exocytosis, and pigmentary incontinence may be present. The dermis has a dense band of lymphocytes and plasma cells at the dermoepidermal junction. Mucocutaneous pyoderma is the primary differential.

Traumatic injury to the skin is quite common in dogs and is often associated with *compound fractures* sustained in motor vehicle accidents. *Dogfight wounds* tend to be tears rather than punctures, as occur in cats; consequently abscesses are a less common sequel. A plethora of **foreign bodies** may penetrate the canine integument, two of the more dramatic examples being *foxtails* and *porcupine quills*. The external ear canal and interdigital webs are favored sites for grass awn entry and subsequent migration. Retrievers or other animals wounded with *steel shot* may develop fistulous tracts or abscesses as steel shot corrodes when embedded in tissues.

Myospherulosis is a rare form of foreign body reaction in which endogenous erythrocytes interact with an exogenous substance, such as antibiotics or ointments, or with endogenous fat. *The lesions are subcutaneous nodules composed of sheets of large macrophages in which the cytoplasm is filled with homogeneous eosinophilic spherules.* These structures may resemble fungal organisms but are negative with fungal special stains such as periodic acid-Schiff. The spherules stain for endogenous peroxidase, thus establishing their identity as erythrocytes.

Traction alopecia has been reported in dogs. It results from low-grade local ischemia induced by traction on hairs. The traction force is applied by elastic ties or hair barrettes applied to pull the forelock hair into a topknot. The lesions are focal patches of cutaneous atrophy and alopecia that may become eroded and crusted. Histologically, the lesion is an *atrophic dermatosis*. The epidermis is thin and may occasionally show single cell necrosis, erosion or ulceration. The hair follicles are inactive and atrophic, pale staining and appear "faded." Inflammation is minimal and is restricted to areas of surface ulceration.

Pyotraumatic dermatitis or "hot spot" is a common complication of allergic dermatitis or any pruritic dermatosis that leads to an *itch–scratch cycle*. It occurs most often in times of warm, humid weather. Breeds with a thick undercoat, such as German Shepherds, Golden Retrievers, Labradors, and Saint Bernards, are particularly susceptible. Lesions are extremely painful and occur at sites of *self-trauma*, such as the dorsal rump in a flea-infested animal. Grossly, the initial lesions are erythematous, exudative, sharply demarcated patches, spreading extensively if not treated. The surface may be colonized by gram-positive cocci. Alopecia and hyperpigmentation are the typical sequelae. Pyotraumatic dermatitis is characterized histologically by *epidermal ulceration*. A thick serocellular inflammatory crust covers the denuded dermis. The predominantly neutrophilic reaction tends to be restricted to the area beneath the ulcer. Folliculitis and furunculosis are not features of classical pyotraumatic dermatitis but occasionally are concurrent conditions as indicated clinically by scattered papules at the periphery of the lesion. Eosinophils and/or folliculitis were common in one series of cases.

Cats

This species is particularly prone to develop subcutaneous abscesses or cellulitis as a result of fight wounds. The cat bite produces a puncture-type wound that seals over and enables the introduced bacteria (chiefly oral flora) to multiply in the damaged tissue.

Cattle

Tail tip necrosis of feedlot beef cattle is a disease in which slatted-floor housing has been shown to be an important causal factor. The pathogenesis is presumed to be *ischemia*, secondary to compression of, or blunt trauma to, the more proximal parts of the tail. Clinically, there is alopecia, scaling and crusting. Ulceration and suppuration are frequent sequelae. In early lesions, histologic examination reveals only perivascular edema and hemorrhage. Fully developed lesions are characterized by dermal scarring, follicular atrophy, vascular wall hypertrophy, and fragmentation of extravasated erythrocytes.

Pigs

Intensive rearing systems for swine production have increased the occurrence of a variety of traumatic lesions. Those in piglets probably result from contact with *concrete floors* in farrowing crates. The knees are the most common site affected, followed by the fetlocks and hocks. The nipples, particularly the cranial pair, are often involved, as is the tail. The lesions occur within a few hours of birth as circumscribed red macules followed by necrosis, ulceration and crusting. The ulcers heal over 3–4 weeks leaving no permanent defect. The supernumerary digits of the hind legs are subject to trauma in sows housed on concrete slats. Trauma resulting from *vices* such as tail biting, ear biting and flank biting are increasingly common in growing pigs under intensive rearing systems of management. Wounds from a variety of causes, including fight wounds, often develop into subcutaneous abscesses in pigs.

Horses

Cutaneous wounds are common in horses and can be largely attributed to the flighty temperament of the species. *Exuberant granulation tissue, "proud flesh,"* is a relatively frequent and serious sequel to wounds of the distal limbs. Poor circulation, minimal soft tissue, lack of adequate drainage and a tendency for excessive movement predisposes the distal limbs to the development of excess granulation tissue. The gross lesion, *a tumor-like mass of red-brown tissue, must be distinguished from equine sarcoid, cutaneous habronemiasis, mycoses, pythiosis, and squamous cell carcinoma*. Histologically, immature capillaries and capillary loops arranged perpendicularly to the elongated fibroblasts and newly synthesized collagen are distinctive features. A superficial layer of granulation tissue may form in association with any of the above-mentioned conditions, and the entire lesion should be examined before a diagnosis is made.

Injection site reactions are not uncommon in domestic animals. Subcutaneously administered vaccines and therapeutic drugs may be responsible. The nodules may ulcerate or fistulate and are often suspected to be neoplasms. *Histologically, the classic injection site reaction is composed predominantly of nodular aggregates of lymphocytes arranged around a central core of caseous necrosis.* Lymphocytes may form follicles. Irregular refractile or faintly basophilic granular material is often embedded in the eosinophilic debris or found within phagocytic cells. Plasma cells, macrophages and multinucleated giant cells are also present, but in lesser numbers than the lymphocytes. The strong antigenic stimulus provided by the exogenous antigen sometimes results in the formation of germinal centers. The heterogeneity of the cell population and the lack of anaplastic characteristics in the lymphoid cells may differentiate these lesions, sometimes termed *pseudolymphoma*, from genuine lymphoma. Vaccine administration in **cats** has been linked to the development of a variety of sarcomas, including fibrosarcomas, osteosarcomas, malignant fibrous histiocytomas, chondrosarcomas, and rhabdomyosarcomas. **Vaccine-associated sarcomas** have the unique features of a *subcutaneous location, concurrent lymphocytic infiltrates, and possibly macrophages containing granular foreign material.* The proposed mechanism involves an over-zealous reparative response followed by malignant transformation of mesenchymal cells. Antigen load and degree of inflammation present at the vaccination site are possible influencing factors. Aluminum adjuvant particles have been identified in tumor associated macrophages, however, vaccine-associated sarcomas have also been documented to arise from the use of non-adjuvanted vaccines. Injection of killed **rabies vaccine** in **dogs** can lead to focal mononuclear vasculitis and ischemic atrophy of surrounding follicles resulting in a focal area of alopecia and is further discussed under Immune-mediated dermatoses. **Injection site eosinophilic granulomas** with necrotic centers developed in some **horses** within 1–3 days as a response to the use of silicone coated hypodermic needles. The lesion is suspected to be a form of delayed hypersensitivity.

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Psychogenic injury

Several conditions in animals are considered to be similar to **obsessive-compulsive disorders** in humans. In theory, stress causes an increase in the production of endorphins creating reinforcement of the stereotypic behavior characterizing each syndrome.

Psychogenic alopecia in **cats**, a self-induced form of alopecia precipitated or exacerbated by environmental stress, is similar to the obsessive-compulsive disorder of humans, trichotillomania. The condition can occur in any cat but is most common in oriental breeds. Clinically, cats groom excessively, licking and pulling at the haircoat. Psychogenic alopecia/dermatitis has *two clinical forms*. In one, *psychogenic dermatitis*, affected cats lick and chew at a single site creating a well-demarcated erythematous, ulcerated lesion of variable size, which usually is located on an extremity, the abdomen or flank. The lesion grossly resembles those of eosinophilic plaque. The second, or *alopecic form*, is characterized by partial alopecia, broken hairs and normal skin. The lesions often occur as a "stripe" along the dorsal mid-line but may involve perineal, genital, caudomedial thigh, medial elbow, or abdominal areas. This distribution pattern is usually symmetrical resembling feline endocrine alopecia but *the hairs in psychogenic alopecia are broken off rather than easily epilated as occurs in endocrine alopecia*. Diagnosis is largely dependent on history and clinical signs. A trichogram examination of epilated hairs reveals a normal anagen to telogen ratio and fractured tips of hairs rather than tapered ends. *Microscopic examination of skin in the alopecic form is usually normal*, although wrinkling of the outer root sheath and intra- and perifollicular hemorrhage may reflect the trauma applied to the hairs. The inflammatory form has no distinctive histologic features, as it is a nonspecific, ulcerative, hyperplastic, superficial perivascular dermatitis. *Differentials for the alopecic form include endocrine dermatopathy and pruritic skin conditions such as atopy, flea allergic dermatitis, and cheyletiellosis.* As pruritus in the cat is manifest in part by excessive grooming, any degree of perivascular eosinophilic dermatitis should be considered indicative of an underlying hypersensitivity disorder. The condition must also be differentiated from **feline acquired hair shaft abnormality**, a condition resembling *trichorrhexis nodosa* in humans. In this condition, cats with an underlying pruritic skin disease such as flea allergic dermatitis groom excessively. Weakened hair shafts break easily and lead to alopecia. A trichogram reveals white nodes on the hair shafts corresponding to foci of frayed cortical fibers. A biopsy should show evidence of the underlying hypersensitivity condition.

Dogs also develop *psychogenic dermatitis including foot chewing and licking, tail-biting, and flank-sucking*. The gross lesions may be slight but the superficial excoriations often develop into pyoderma. **Acral lick dermatitis**, otherwise known as "lick granuloma" or neurodermatitis, is a relatively common psychogenic dermatitis of dogs, particularly in large active breeds younger than 5 years. Males are affected twice as frequently as females. The areas traumatized by persistent licking and chewing are most commonly the cranial carpus and metacarpus, followed by the radius, tibia and metatarsus. Erythema and epidermal excoriations give rise to a single well-circumscribed, eroded or ulcerated, oval plaque. Occasionally, lesions are multiple. Secondary bacterial infection may result. Re-epithelialization of the lesion leaves a well-circumscribed alopecic plaque often with peripheral hyperpigmentation. Histologically, acral lick dermatitis is a *hyperplastic superficial perivascular dermatitis with marked orthokeratotic and parakeratotic hyperkeratosis, acanthosis and rete ridge formation* (Fig. 5.40). There is a sharp transition between the hyperplastic and ulcerated epidermis, which is covered by a thick scale-crust. Superficial dermal fibrosis is usually marked and collagen fibers in dermal papillae are often arranged perpendicular to the surface epithelium (Fig. 5.41). *This "vertical streaking" of collagen is thought to result from chronic irritation.* Perifolliculitis, folliculitis and sometimes furunculosis are often present. Plasmacytic infiltrates often surround the sweat glands. Sebaceous glands and hair follicles appear hyperplastic. *The primary differential is self trauma due to a pre-existing localized bacterial infection.*

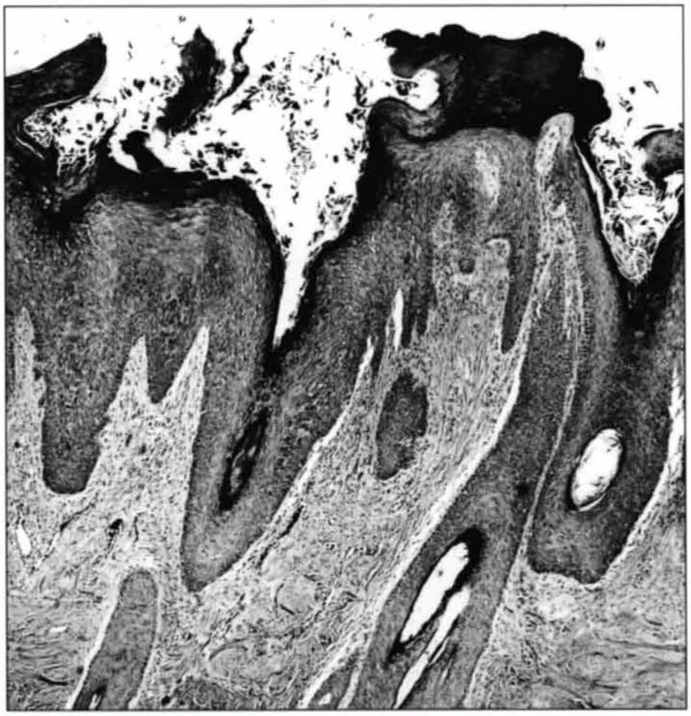

Figure 5.40 Acral lick dermatitis in a dog. Hyperplastic superficial perivascular dermatitis with marked orthokeratotic and parakeratotic hyperkeratosis, acanthosis and rete ridge formation.

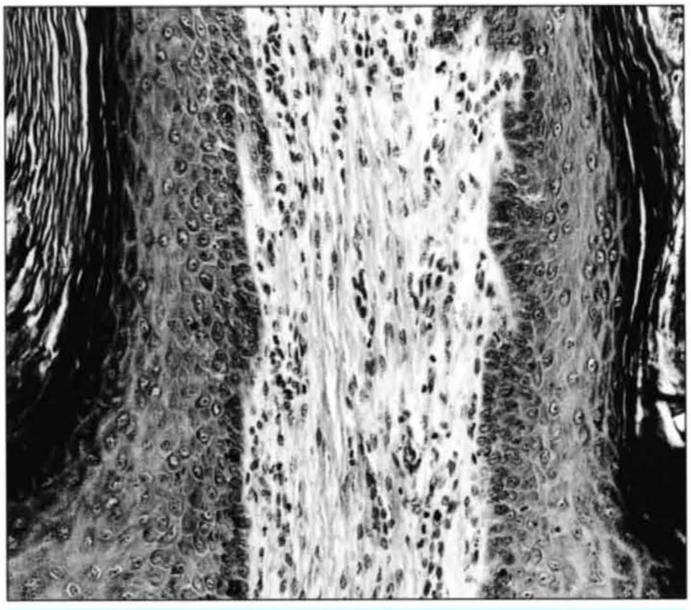

Figure 5.41 Acral lick dermatitis in a dog. Vertical alignment of collagen.

In **horses**, the **equine self-mutilation syndrome** can be misinterpreted as a possible cutaneous disorder as horses bite, kick, or rub the flank or pectoral areas. The behavior is usually accompanied by vocalization, spinning or rolling and is most common in *stallions*.

The condition is thought to be *stress related*. There are no primary cutaneous lesions, however secondary excoriations may be present.

A **self-destructive behavioral condition** primarily affecting **first-calf heifers** is characterized by *excessive licking of the udder and teats* sometimes leading to teat necrosis and culling. The condition is associated with udder edema and increased levels of histamine in the udder tissue leading to pruritus.

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Mineral deposition in cutaneous tissues

Deposition of insoluble calcium salts within cutaneous tissues can occur as a result of injury or degeneration of skin components (**dystrophic mineralization** or calcification), secondary to calcium/phosphorus metabolic alterations (**metastatic mineralization** or calcification), as an idiopathic condition, or may occur iatrogenically. Mechanisms leading to calcium salt deposition are complex and involve such factors as the lower pH of injured tissue, mitochondrial concentration of calcium and phosphorus, and the influx of calcium into injured cells in dystrophic forms. In metastatic forms, loss of mineralization inhibitors, changing of ions into a solid phase, and phosphate ion initiation of crystal formation are implicated in salt formation. Mineralization can be *localized*, as in inflammatory foci (granulomas), in degenerative lesions (follicular cysts), or in neoplasms (pilomatricomas), or may be *generalized* as in the tissue mineralization associated with chronic renal failure.

The most well-known form of cutaneous mineralization is the classic form of **calcinosis cutis** associated with hyperadrenocorticism, which is discussed in Endocrine diseases of skin. For ease of comparison, other known types of cutaneous mineralization are discussed here. **Calcinosis universalis** *refers to widespread areas of calcinosis cutis* and can be seen with hypercortisolism, percutaneous absorption of calcium-containing products, or from the iatrogenic administration of calcium-containing solutions. Some forms of calcinosis cutis can be indistinguishable histologically from calcinosis cutis associated with hyperadrenocorticism. The clinical presentation, concurrent abnormalities, signalment and history should allow distinction of the various forms of calcinosis cutis.

Percutaneous absorption of products containing **calcium chloride or calcium carbonate** has occurred naturally in *dogs*. Multifocal, flat-topped and centrally ulcerated papules and small nodules affected glabrous skin, such as the lips, axilla, inguinal and interdigital skin. Histologically, the lesions comprised granulomas centered on degenerate, mineralized collagen fibers. Ultrastructural examination of experimental lesions indicated that mineral was deposited within the collagen bundles within 24 hours of initial skin contact. The main differential diagnosis was *calcinosis cutis of hyperadrenocorticism*, to which the lesions are histologically identical.

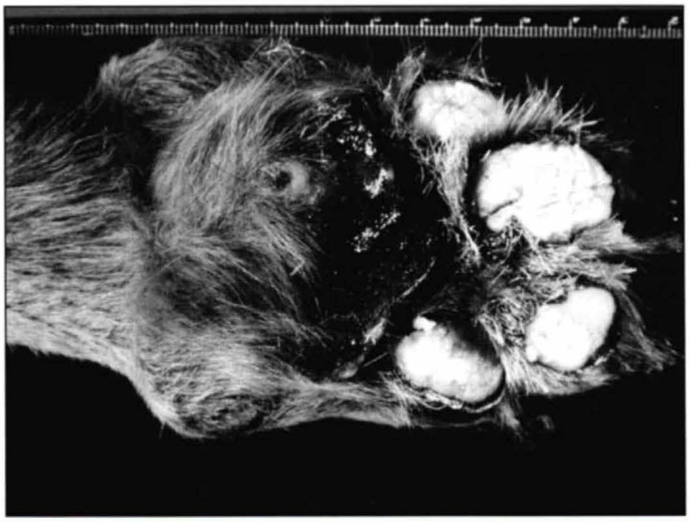

Figure 5.42 Calcinosis circumscripta in a dog. Note chalky material exuding from footpads. (Courtesy of B Dunstan.)

A chemically induced, iatrogenic form of calcinosis cutis has been reported in humans and a dog as a consequence of the subcutaneous administration of a **10% calcium gluconate** solution for treatment of hypoparathyroidism. In the dog, calcium salts formed on basement membranes, dermal collagen, vessel walls and adipocyte membranes. Dermoepidermal separation, pyogranulomatous dermatitis, panniculitis and vasculopathy ensued, leading to marked necrosis and sloughing of the skin. Concurrent hyperphosphatemia was thought to predispose to precipitation of calcium salts.

Dogs with systemic blastomycosis treated with **amphotericin B** developed lesions that resolved over time and were *indistinguishable from calcinosis cutis of hypercortisolism*. Dogs had severe granulomatous cutaneous lesions that may have been predisposed to dystrophic mineralization, but mineralizing lesions were more extensive or separate from primary inflammatory lesions. The dogs had no clinically significant serum calcium/phosphorus abnormalities. Additional factors suspected to contribute to mineralization included mild intermittent serum calcium fluctuations, mononuclear cell production of factors leading to increased osteoclast-mediated bone resorption, and alteration of vitamin D metabolism.

Calcinosis circumscripta (tumoral calcinosis) occurs most often in *dogs, horses* and occasionally *cats* (see also Ectopic mineralization and ossification, in Vol. 1, Bones and joints). Lesions in dogs are most often solitary but can be multiple and occur most often in large breeds <2 years of age. German Shepherd Dogs are predisposed. The *skin over bony prominences of the limbs* is most often affected. The tongue and paravertebral soft tissues, footpads, edges of the pinna in dogs with cropped ears, and cheeks of Boston Terriers are other reported sites. Calcinosis circumscripta of the dorsal thoracolumbar region at a site of previous progestogen injection has been reported in a cat.

The gross and histologic features of the lesion evolve over time. Initially, the lesion may be bulging, fluctuant or cystic, variably ulcerated and contains chalky white material (Fig. 5.42). Histologically,

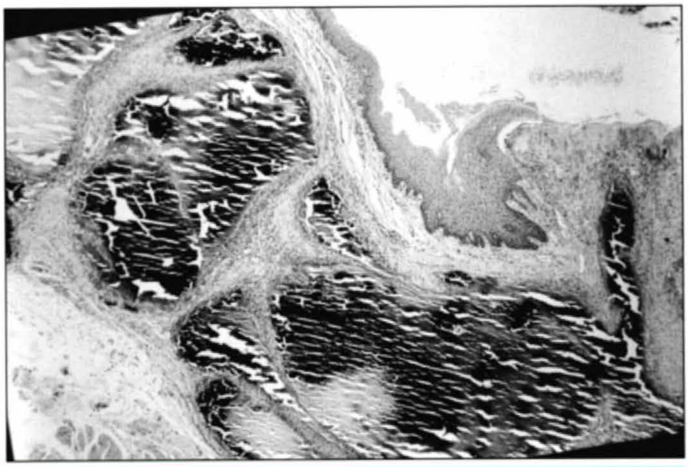

Figure 5.43 Calcinosis circumscripta in a dog. Dermal lakes of basophilic granular material are surrounded by mild fibrosis and giant cells, macrophages and fewer lymphocytes and plasma cells.

subcutaneous to deep dermal lakes of basophilic granular material that stain with von Kossa are surrounded by mild fibrosis and a cellular zone of variable width with giant cells, large macrophages and fewer lymphocytes and plasma cells (Fig. 5.43). Over time, the lesions become firm, progressively more mineralized, and associated with dense fibrous connective tissue bands. Inflammation may subside to some degree over time and osseous or cartilaginous metaplasia may take place. Epidermal sequestration or transepidermal elimination of mineralized material may lead to ulceration.

The pathogenesis of calcinosis circumscripta is not known. Dystrophic mineralization secondary to previous tissue trauma has been proposed based on the predilection for skin covering bony prominences. This explanation is not entirely satisfactory as the lesions do not recur after surgical excision, and trauma to these sites in large dogs would be expected to be repetitive. The name "apocrine cystic calcinosis" was previously applied to calcinosis circumscripta since it has been documented to arise from degenerating, cystic apocrine glands. A relationship to apocrine glands is not evident in most cases. Lesions have also developed at sites of previous injections, or in association with surgical sites sutured with polydioxanone sutures. Cases of calcinosis circumscripta have also been reported to occur in the footpads of dogs and cats with chronic renal failure, and in the footpads of an otherwise healthy German Shepherd Dog and in another German Shepherd Dog with pododermatitis. Symmetrical cases that resolved spontaneously have been reported in young dogs with underlying skeletal disease such as hypertrophic osteodystrophy. It is likely that multiple factors are involved in the pathogenesis of calcinosis circumscripta.

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Cold injury

Most cold-induced cutaneous lesions (frostbite) result not only from direct freezing and disruption of the cells, but more importantly from vascular injury and resultant tissue anoxia. In experimental frostbite lesions in Hanford miniature swine, vacuolation of keratinocytes was the earliest change in the epidermis followed by spongiosis, epidermal necrosis, and separation of the necrotic epithelium from the dermis. Hyperemia and hemorrhage were also early lesions. Inflammatory changes, comprising neutrophilic infiltration and necrotizing vasculitis, occurred 6–46 h postinjury. Thrombosis of small arterioles increased in severity up to 1 week postinjury. By 2 weeks, considerable epithelial regeneration had taken place, either as a complete replacement or as crescents beneath the necrotic epidermis.

Cutaneous injury resulting from cold is uncommon in well-nourished, healthy domestic animals. Well-acclimatized long-haired animals can tolerate temperatures of –50°C for indefinite periods. Cold injury occurs most commonly on the tips of the ears and tail of cats, the scrotum of male dogs and bulls, and the tips of the ears, tail and teats in cattle. The teats are particularly vulnerable if cows are turned out into the cold with wet udders. Affected skin is cool, pale and hypoesthetic. The gross lesions include alopecia, scaling and pigmentary alterations of the skin, hair or both. In severe cases, the ischemic necrosis results in dry gangrene and sloughing of the affected part.

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Thermal injury

Heat may be applied to the skin in a variety of forms and, depending on duration and intensity, will produce *mild to severe necrotizing lesions*. Longer exposure to lower temperatures is more damaging than short exposure to higher temperatures. The lowest temperature at which skin can burn is 44°C (111°F). *Dry heat causes desiccation and carbonization, whereas moist heat causes "boiling" or coagulation.* Thermal injury in domestic animals may be caused by hot liquids, steam, heating pads, hair dryers, drying cages, hot metals such as wood stoves or car engines, fires, friction from rope "scalds," electrical burns from chewing electrical wires, improperly grounded electrocautery units, or lightning strikes. Animals struck by lightning may show a jagged line of singed hair running down a shoulder or flank (Fig. 5.44). This finding is valuable in establishing an otherwise difficult diagnosis. Rarely, small animals incur microwave burns.

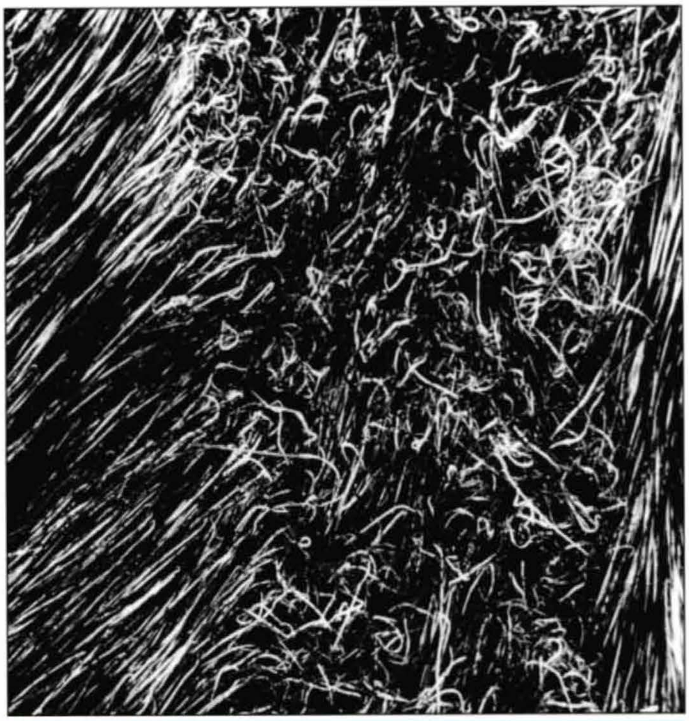

Figure 5.44 Lightning strike. A linear arrangement of singed hairs. (Courtesy of MA Hayes.)

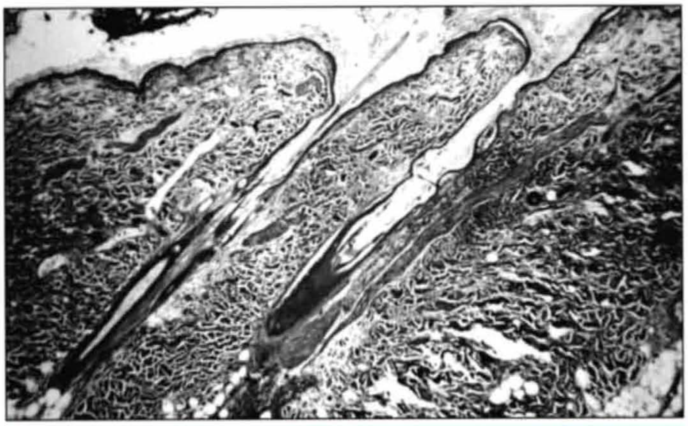

Figure 5.45 Burn. Note diffuse coagulative necrosis of the epidermis. (Courtesy of B Dunstan.)

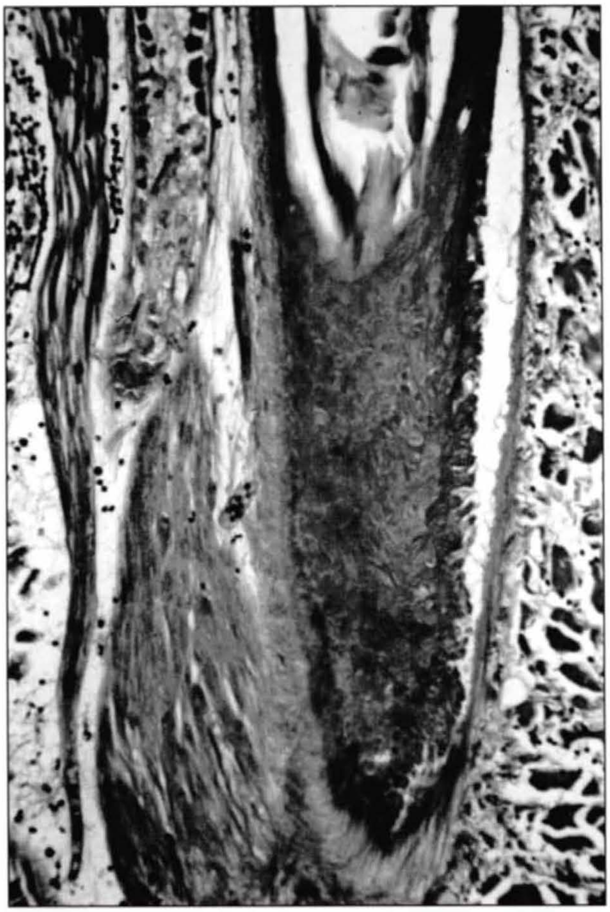

Figure 5.46 Burn. Coagulative necrosis of the epidermis can often "wick" down to involve the follicular epithelium. (Courtesy of B Dunstan.)

Burns are classified into four degrees according to depth of injury.

- **First-degree burns** *involve only the epidermis.* The heated areas are erythematous and edematous as a result of vascular reaction in the dermis, but vesicles do not form. The epithelial cells show no morphological sign of injury although there may be surface desquamation after a few days.
- *In* **second-degree burns**, *the epidermis and part of the dermis are damaged.* The cytoplasm of the epithelial cells is hypereosinophilic, and the nuclei are shrunken or karyorrhexic. Coagulative necrosis of the epidermis (Fig. 5.45) can occur in the absence of substantial dermal injury and often "wicks" down to involve the follicular epithelium (Fig. 5.46). The vascular changes are more prominent than in lesser burns, with marked dermal edema and spongiosis. Vesicles and bullae form in the epidermis, often at the dermoepidermal junction. The bullae contain serum, granular debris, and leukocytes. Healing can be complete if secondary infection does not lead to deeper injury.
- *In* **third-degree burns**, *the destructive effect of the heat extends full thickness through the epidermis and dermis*, causing coagulation necrosis of connective tissues, blood vessels and adnexa. Thermal injury causes thrombosis of blood vessels and vascular leakage leading to the coagulative necrosis of more superficial tissues. Heat of sufficient intensity or duration to penetrate this deeply usually desiccates and chars the outer epidermis. Coagulation necrosis of the dermis produces a swollen amorphous accretion of the connective tissues accompanied by an acute inflammatory reaction. Over time, histiocytes infiltrate the subcutis and fibrosis ensues. Subcutaneous vasculitis may be present. The necrotic tissue sloughs, and the defect is filled in by granulation tissue. Permanent scarring with loss of adnexa results.
- **Fourth-degree burns** *are similar in character to those of third degree but penetrate below the dermis to and beyond the subcutaneous fascia*; their local consequences depend on what lies underneath. Heat in surface tissue is conducted to deeper tissues via the blood and lymph. The degree of injury may not be evident for several days after the insult occurred. Follicular and sweat gland damage continues for 24–48 hours. Once lesions fully develop, the progress of injury should cease, which is helpful in making the diagnosis. Histologically, thermally induced tissue damage is not sharply demarcated and should dissipate gradually with increasing depth of the biopsy. *Clinical differentials* include toxic epidermal necrolysis, erythema

multiforme major, bullous pemphigoid, pemphigus vulgaris, vasculitis, trauma, and other causes of ischemia. Lesions from thermal injury sometimes have an abnormal anatomical distribution or pattern such as drips, angles, lines, or areas of contact that may be helpful in the differential diagnosis.

Microwave burns are unique in that the lesions are sharply delineated histologically without a tapering of the degree of coagulative necrosis in the deeper tissues. The degree of injury is uniform throughout the tissue and inflammation is minimal. The depth of damage depends on the frequency of the microwaves with some frequencies sparing the superficial tissues and coagulating the deeper tissues. Pain perception may not occur until the damage is done and lesions may not be evident for up to 6 days. There is no surface charring, blister formation, or edema. There is thrombosis of vessels and the tissue is mummified. Injury is due to ionizing radiation leading to heat generated from the vibration of molecules in the tissue.

Full-thickness cutaneous burns have been reported to occur in black-haired spots of Dalmatians as a result of **solar radiation**. Normally, cutaneous injury from solar radiation affects lightly pigmented sparsely haired skin rather than areas protected by pigment. *Black skin absorbs approximately 45% more solar radiation than white skin.* The absorption of visible light (400–700 nm) can result in the production of thermal energy resulting in a burn. UV light (100–400 nm) does not penetrate into the dermis and does not produce substantial thermal energy, but has other deleterious effects (see Actinic diseases of skin). Pain, prompting moving to the shade, was likely not perceived by the dogs as the burns were multifocal involving only black-haired areas and Dalmatians are primarily white.

Another unique type of thermal injury, **radiant heat dermatitis**, is similar to *erythema ab igne* in humans. Tissue damage occurs from repeated exposure to moderate heat. Radiant heat dermatitis has been reported as an asymptomatic condition in dogs sleeping next to wood burning stoves or fires, or chronically exposed to heat lamps. The lesions were on the dorsolateral trunk and had a drip-like configuration. Grossly, irregular areas of alopecia were erythematous and hyperpigmented peripherally. Centrally, the lesions are scaly and depigmented. In the acute form, lesions resemble actinic dermatosis with epidermal thinning, basal cell vacuolation, and possible epidermal dysplasia. The dermis has thin fragmented collagen, increased elastin fibers, and melanin and hemosiderin deposits. Over time, epidermal and infundibular hyperplasia, hyperkeratosis and dyskeratosis develop with focal spongiosis and cell necrosis. Karyomegaly of basal cells, dermal edema and mucinosis, hyperplastic sebaceous glands, dilated apocrine glands, and mixed perivascular dermatitis may be present.

Radiation injury

Advances in the treatment of cancer in companion animals have made the possibility of **radiation-induced skin injury** more likely. Clinicians and pathologists need to be able to recognize these lesions in order to provide the best management options and accurate prognosis for resolution of the lesions. *The varieties of radiation modalities available have variable degrees of tissue penetration and potential for tissue injury.* Some forms of radiotherapy penetrate deeper tissues while sparing the skin and others are more concentrated in the superficial tissues or are preferentially absorbed by specific tissues. The type of radiation therapy, source, dose, intensity, and duration of exposure dictate the range of possible side effects. Ionizing photons disrupt chemical bonds in cells leading to injury or cell death. Some cells are not lethally damaged but sustain DNA damage to the extent that replication/replacement are not possible. The effects of radiation damage can be divided into acute and chronic forms.

Acute radiation injury to the skin is a result of damage to rapidly dividing cells. Damage is self-limiting and recovery is associated with rapid cell turnover. Clinical lesions of radiation dermatitis appear 2–4 weeks after exposure. Initially, there is erythema, pain, edema, and heat, followed several weeks later by dry or moist desquamation depending on the degree of injury. Histologically, the lesions resemble a second-degree burn, with suprabasilar or subepidermal bullae formation, dermal edema with fibrin exudation, and a marked leukocytic infiltrate. Re-epithelialization occurs over a period of 10–60 days. The damage sustained to germinal epithelium of hair follicles and sebaceous glands leads to *alopecia* within 2–4 weeks after exposure. Hair re-growth will follow over the next several months but damage to sebaceous glands is not reversible and leads to *permanent scaling*.

The *chronic lesions of radiation injury* are evident months to years after treatment and are primarily *due to damage to the microvasculature*. The epidermis is thin, friable, and in some areas hyperplastic, and may become neoplastic. There is hyperpigmentation and hyperkeratosis. Chronic exudative ulcers may develop but granulation tissue does not form. The dermis is fibrotic with atypical fibroblasts, telangiectasia, and possibly deep arteriolar changes. Endothelial swelling, necrosis and thrombosis lead to occlusion and excessive endothelial proliferation, that when combined with the effects of vascular leakage, leads to vascular collapse. This condition of progressive vessel abnormalities is referred to as *obliterative endoarteritis* and is known to form a "histohematic" barrier to surrounding tissue, leading to continued anoxia and nutrient shortage.

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Chemical injury to skin

Primary irritant contact dermatitis

One of the important functions of the skin is to provide protection against external noxious agents. *The stratum corneum is the major* *protective barrier.* Its integrity is highly dependent on its water content, which in turn is protected by the lipids of the stratum corneum.

Penetration of the skin is enhanced by physical damage to the stratum corneum, chemical alteration of the barrier components, overhydration of the stratum corneum by excess moisture, increased temperature, or alterations of the skin pH. Irritant substances may induce damage by altering the water-holding capacity of the keratin layer or by penetrating the epidermis and directly damaging the cells. Irritant substances vary markedly in their potency. Some, such as strong acids or alkalis, induce immediate and severe tissue damage; others, such as mild detergents or soaps, may require repetitive applications and covering of the area to assist penetration before lesions develop.

Primary irritant contact dermatitis must be distinguished from allergic contact dermatitis (discussed under Hypersensitivity dermatoses). *Irritant contact dermatitis is the more common condition in animals and does not involve prior sensitization.* It may occur in any species, but is most frequent in the horse, cow and dog. The types of agents capable of causing direct cutaneous damage include acids, such as carbolic or sulfuric, alkalis, cresol tars, paints, kerosene, turpentine, antiseptics, and insecticides. An example of the last-mentioned is "flea collar dermatitis" of dogs and cats. Feces and urine are also potential irritants.

The distribution of gross lesions of contact irritant dermatitis in the dog and cat typically involves the glabrous skin of the ventral abdomen, axilla, medial thigh, perianal and perineal areas, footpads (Fig. 5.47A), ventral tail, chin, and inner aspect of the pinnae. The hairy skin is only involved if the irritant substance is in an aerosol or liquid form. Flea collar dermatitis lesions occur as a band around the neck corresponding to the position of the offending collar. In horses, lesions occur most commonly on the muzzle, lower limbs and in areas of contact with the riding tack. Horses with diarrhea may develop severe irritant dermatitis on the soiled perineum.

The *gross lesions*, which typically have a sudden onset, are marked erythema, swelling, and a transient papular-vesicular stage that leads to ulceration and, in severe cases, sloughing of the affected skin. The sequelae include alopecia, scarring, and alteration in skin and hair pigmentation. Hyperpigmentation occurs in most species of domestic animals, but in horses, leukoderma or leukotrichia may be a permanent result of irritant contact dermatitis. Severe lesions can be considered a chemical burn. Differentials include thermal injury and allergic contact dermatitis.

The histologic lesions of irritant contact dermatitis are not pathognomonic, nor can they be distinguished from those of allergic contact dermatitis (Fig. 5.47B). Superficial spongiotic or hyperplastic perivascular dermatitis characterizes both, and vesicles may form. The inflammatory infiltrate is variable in nature, probably reflecting such factors as chronicity, self-trauma and secondary infection. *The diagnosis of irritant contact dermatitis depends largely on the history and the clinical signs, particularly the distribution of the lesions.*

In **swine**, cutaneous erythema and pruritus have been observed within 48 hours of *tiamulin* administration. The most severely affected pigs were recumbent and developed *fatal necrolytic dermatitis.* The areas most severely affected were those in contact with feces and urine. Histologic lesions included full-thickness epidermal necrosis, intraepidermal pustules, and serocellular crusting. It was hypothesized that the lesions represent a severe form of contact

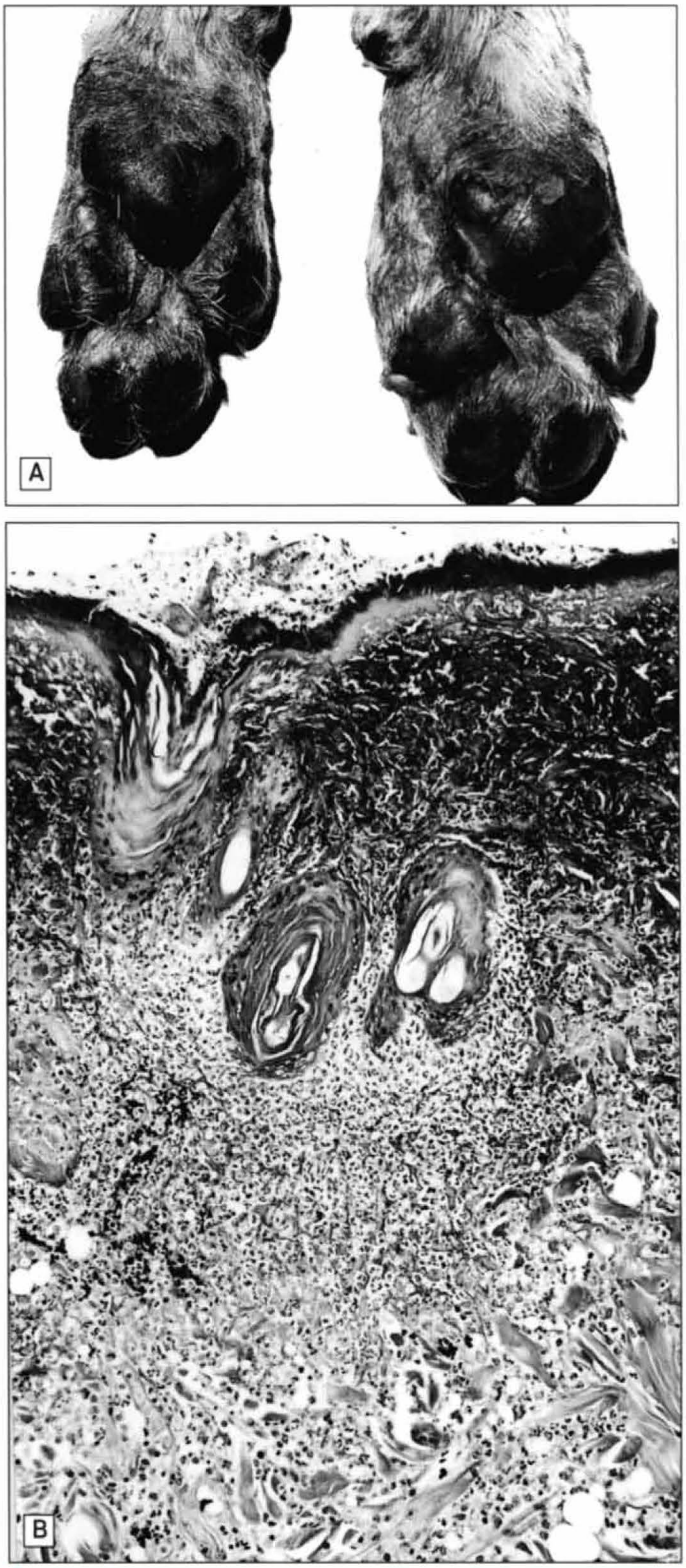

Figure 5.47 Irritant contact dermatitis in a dog. **A.** Footpad swelling and erythema, due to alkali exposure. (Courtesy of RP Johnson.) **B.** Coagulative necrosis of the epidermis and superficial to mid dermis, due to Varsol burn.

irritant dermatitis to tiamulin or one of its metabolites in the excreta. Skin lesions regressed when the drug was withdrawn.

An eosinophilic dermatitis was observed in pigs following heavy salting of pen floors. Lesions were present on the feet.

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Envenomation

The venom of insects, spiders, other arthropods and snakes can cause mild or severe skin lesions with or without systemic signs. Effects are dependent upon composition of the venom, individual victim response, anatomic location of the envenomation, and specific characteristics of the offending organism which may be influenced by season of the year, geographic location, time since last inflicted bite or sting, depth of injury, etc. Different species of animals respond differently to the same venom.

Stings from *Solenopsis invicta*, the **imported fire ant** are common in humans in South America and in most of the southern United States. The ants swarm by the hundreds, covering objects or parts of victims and simultaneously inflict numerous painful stings. The fire ant venom is primarily comprised of an insoluble alkaloid (solenopsin A), shown to be cytotoxic, bactericidal, fungicidal, insecticidal, and hemolytic. Lesions in dogs have been documented and consist of initial swelling progressing to erythematous nodules within 10–20 minutes. Histologically, the sting in dogs produces a vertically oriented zone of coagulative dermal necrosis with variable epidermal necrosis. Necrosis includes the adnexa and may extend into the subcutis. Lesions resolve quickly. Severe type I hypersensitivity (anaphylaxis) reactions are possible.

Stings from **hymenopteran insects** such as *bees, wasps, and hornets* produce effects of a local (angioedema) or possibly systemic type I hypersensitivity reaction due to the histamine, serotonin, and kinins in the venom (see Hypersensitivity dermatoses). Multiple stings dermatoses lead to toxic reactions that can be fatal.

Spider bites are rarely documented definitively in animal or human patients as the initial bite goes unnoticed and the spider is no longer recoverable by the time lesions develop. Bites occur most often on the face and extremities. Most spider bites produce localized pain, erythema and swelling and are not of further consequence. Spiders of importance to the study of the integumentary system are those with venoms leading to *dermonecrosis and eschar formation, a condition referred to as "necrotic arachnidism."* The **brown recluse spider** (*Loxosceles reclusus*) is the spider most well-known to induce dermal necrosis, although there are a number of others. Brown recluse venom contains hyaluronidase and sphingomyelinase-D, which degrade tissues. A blister with a surrounding pale halo and more peripheral erythema characterizes initial reactions documented in humans and some experimental animals. Necrosis and eschar formation occur within 5–7 days. Ulceration may be extensive. Histologically, there is hemorrhage and edema, neutrophilic vasculitis and arterial wall necrosis. The epidermis and dermis undergo necrosis that may extend into the subcutis and underlying muscle. Panniculitis may be present. Eventually, there is dermal scarring and replacement of the subcutis and muscle by hypocellular connective tissue. Brown recluse spider bites in humans can also lead to massive hemolysis. *Differentials include other venomous bites, vasculitis, slough due to iatrogenic injection of irritating substances, thermal burns, necrotizing fasciitis or other cutaneous infection, septic embolization, or trauma.* Compatible lesions, environmental history, and ruling out other conditions producing similar lesions can lead to the presumptive diagnosis of a spider bite.

Snakebite envenomation produces local tissue necrosis and variable systemic effects. Snake venom contains various enzymes, proteins, peptides, and kinins. Systemic effects of snake venom include paralysis, coagulation disturbances, shock, increased capillary permeability, myocardial damage, rhabdomyolysis, and renal failure. Of the five genera of venomous snakes, *crotaline* (rattlesnake, copperhead, cottonmouth and others) *venom* contains the highest concentration of proteolytic enzymes. Local effects include pain, edema, hemorrhage, bullae formation, necrosis, and sloughing of tissue. Bites inflicted in the head or neck region may lead to swelling that interferes with respiration. Bites of pit vipers also introduce potentially dangerous bacteria such as *Clostridial* sp. into the puncture wound. Bites are common in the dog, horse, and, to a lesser degree, cats, and most often are inflicted on the head or legs. The differential diagnoses are similar to those for necrotic arachnidism.

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Thallotoxicosis

The heavy metal thallium is a potent toxin with pharmacological actions similar to lead and mercury. Thallium salts are odorless, tasteless, colorless, and water soluble. Thallium was used extensively as a rodenticide and insecticide prior to 1963 when its sale to the general public in the United States and some other countries was banned. It continued to be used by government agencies as a pesticide and in various industries, such as the manufacture of optical lenses, jewelry, and scintillation counters, until the US Environmental Protection Agency banned thallium manufacture in 1972. Thallium is still used in some parts of the world, and its use may increase as pests develop resistance to more selective poisons.

Accidental or malicious thallium poisoning is rare and is due to the use of undestroyed supplies of old, but newly exposed baits. *It occurs chiefly in dogs*, less often in cats and is reported in sheep, cattle and pigs. The LD_{50} for the dog is 10–15 mg/kg, and the toxin is cumulative. Absorption occurs rapidly from the gastrointestinal and respiratory tracts and skin. The toxin is disseminated widely in the body and is persistent, being very slowly excreted in bile and urine.

The mechanism of toxicity is not fully understood. There are two main hypotheses. The first holds that thallium exerts its toxic effect by combining with sulfhydryl groups, a mechanism common to many heavy metals and leading to disruption of mitochondrial respiratory chain enzymes. The second, which is based on the similarity of ionic radii between thallium and potassium, suggests that thallium may replace potassium in many critical biochemical functions, thus acting as a general cellular poison. The toxic effect may be, in part, due to thallium interacting adversely with derivatives of riboflavin. Thallium can depolarize nerve cell membranes and antagonize effects of calcium on the heart.

The clinical effects depend on the dose and rapidity of administration. Signs of *acute thallotoxicosis* are evident within 12 hours of exposure and are characterized by severe gastrointestinal irritation and neurological signs, including motor paralysis. Glossitis, stomatitis, rhinitis, and bronchitis develop. Death by respiratory failure may occur. Animals may survive the acute episode to develop the chronic syndrome or may bypass the acute disease altogether. Cutaneous, renal and neurological abnormalities, progressive debilitation, and death characterize the *chronic syndrome*.

The *cutaneous lesions* develop 7–10 days after ingestion of thallium and principally affect frictional areas. The pattern of skin involvement in cats and dogs is characteristic, beginning at the commissures of the lips or nasal cleft, occasionally on the ear margins and expanding over the face and head. The mucous membranes are characteristically "brick-red" and may be ulcerated. Lesions also develop on the interdigital skin, footpads, axillae, inguinal areas, perineum and lateral extensor surfaces. The lesions are *marked erythema, scaling, alopecia, exudation, and crusting*. The layers of scale-crust exfoliate with attached hairs to leave a raw, oozing surface. The paws often become very swollen. In more chronic cases, thick scales on the footpads resemble "hard pad" disease conventionally associated with canine distemper. In less severely affected animals, *ease of depilation may be the only clinical indication of thallium poisoning*. The pathogenesis of the alopecia is not fully understood. While thallium enters the hair, as do other heavy metals, by binding to sulfhydryl groups in the keratin, this is unlikely to be destructive to the hair follicle. Thallium may interfere with the energy metabolism of the rapidly dividing matrix cells of anagen follicles. In experimental intoxication of rats, a rapid decline in the mitotic rate is followed by necrosis of the matrix cells within 48 hours. The follicle passes into an abnormal catagen phase followed by complete involution (telogen). However, no club attachment is formed and the hairs are rapidly shed. If the animal survives, hair growth is resumed. Thallium also severely alters the keratinization process and both the surface and external root sheath epithelium demonstrate marked parakeratotic hyperkeratosis.

The *microscopic lesions* in the skin are dominated by *massive, diffuse parakeratotic hyperkeratosis*, which affects both surface and external root sheath epithelia. There is accompanying follicular plugging, hypogranulosis and epidermal hyperplasia (Fig. 5.48). Neutrophil exocytosis and spongiform pustules develop in both surface and follicular epithelia. Partial or full thickness necrosis of the surface epithelium may also occur. The dermal lesions include marked hyperemia, edema, erythrocytic exocytosis and infiltration of neutrophils and mononuclear cells. There may be focal necrosis of sweat and sebaceous glands. Hair follicles are mostly in catagen or telogen. Degenerative changes are noted in anagen follicles.

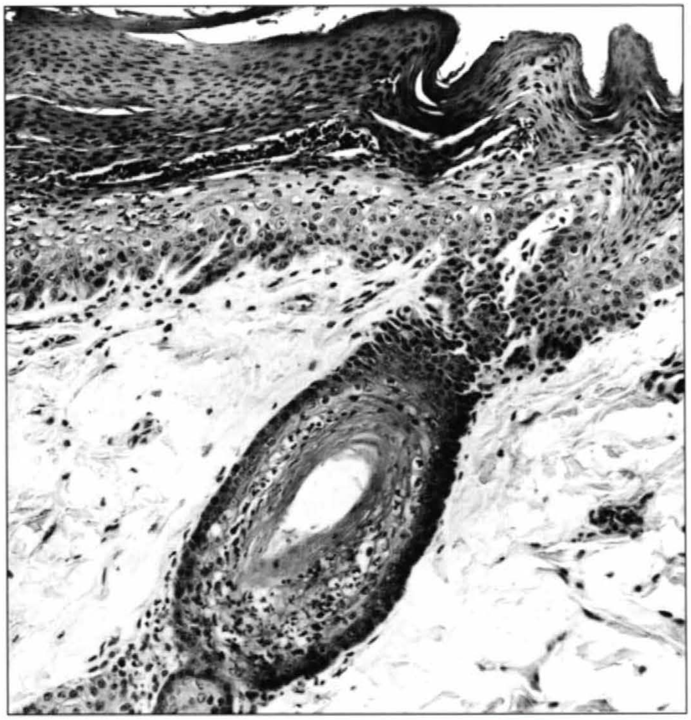

Figure 5.48 Thallium toxicity in a dog. Marked parakeratosis.

Histologic lesions in *other tissues* include multifocal necrosis of myocardial and skeletal muscle fibers, nephrosis, pulmonary edema, reticuloendothelial hyperplasia and lymphoid depletion of spleen and lymph nodes. Secondary bacterial bronchopneumonia may occur as a result of the damage to ciliated epithelia and resultant disturbance of the mucociliary apparatus. Hemorrhagic gastroenteritis occurs in acute thallotoxicosis. Focal suppurative pancreatitis has been described in several animals, but its causal relationship to thallium is not established. Ulcerative esophagitis follows dilation secondary to neuronal damage. Lesions in the central nervous system include neuronal chromatolysis, neuronophagia, and severe edema with little glial reaction. Myelinated peripheral nerves have degenerative lesions including focal distension of myelin sheaths and swelling and occasional fragmentation of axons.

The *differential diagnoses* of acute thallium toxicity include other heavy metal toxicoses, infectious and noninfectious causes of hemorrhagic gastroenteritis, and pancreatitis. Differentials for the clinical lesions include superficial necrolytic dermatitis, zinc-responsive dermatosis, generic dog food dermatosis, toxic epidermal necrolysis, mucocutaneous candidiasis, epitheliotropic lymphoma, and autoimmune diseases such as pemphigus vulgaris and bullous pemphigoid. The *microscopic lesions of thallium toxicosis, zinc-responsive dermatosis, and superficial necrolytic dermatitis are similar* and confirmation requires a compatible history and demonstration of thallium in the urine by the Gabriel–Dubin colorimetric assay or in the stomach contents, suspected bait, urine, liver or kidneys by atomic absorption spectroscopy.

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Arsenic toxicosis

Arsenic is the metal most toxic to the skin and is found as a component of wood preservatives, herbicides, insecticides, insulation materials, paint pigments, feed additives, and some medications, and is a by-product of some mining activities. It is a water supply contaminant in some parts of Mexico, Argentina, Chile, Taiwan, India, the UK, and the USA. Safe standards for levels of arsenic in drinking water have not been established. Arsenic is absorbed by the gastrointestinal tract and skin and is excreted in urine, bile, milk, hair, nails, and exfoliated epidermal cells. Its presence in the skin increases the skin's susceptibility to damage by UV light. Chronic arsenic exposure in humans occurs in environmental (water contamination) and industrial settings and is associated with increased incidence of visceral and cutaneous malignancies, Bowen's disease, palmar and solar hyperkeratoses, and cutaneous pigmentary disturbances. The generation of free radicals with resultant nucleic acid damage is suspected to be involved in arsenic-related carcinogenesis. Arsenic may lead to cellular proliferation through increased production of keratinocyte-derived growth factors. *Urine, hair, and liver are the tissues of choice for establishing arsenic exposure.*

Acute arsenic poisoning is an important toxicosis in domestic animals, particularly cattle and dogs, and is due to sulfhydryl group binding and inhibition of cellular metabolism. Signs and lesions are referable to the gastrointestinal tract, liver, and kidneys. Skin lesions are the result of chronic systemic low-level arsenic exposure or to direct contact. *Arsenic is an established low-grade corrosive that produces irritant contact dermatitis.* Contact lesions occur in animals sprayed or dipped in a concentrated arsenic solution or in dogs lying on heavily contaminated ground. Lesions include erythema and epidermal necrosis leading to the formation of non-healing ulcers. Lesions may affect oral cavity, lips, other mucocutaneous junctions and the feet. Less is known about the effects of chronic systemic exposure of arsenic on the skin in animals, however, chronic arsenic poisoning in farm animals is associated with ill thrift and a dry, seborrheic, alopecic coat.

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Mercury toxicosis

Organomercurial toxicosis in domestic animals is associated chiefly with neurologic and renal disorders and is discussed in Vol. 1, Nervous system and Vol. 2, Urinary system, respectively. In chronic poisoning in **cattle**, skin manifestations including *pustules, ulcers, hyperkeratosis and alopecia* at the tail head are described, but their pathogenesis is poorly understood. **Horses** ingesting mercury-treated seed grain develop *total body alopecia*, followed by loss of the long hairs of mane, tail and forelock. The hooves are not affected and the cutaneous lesion is mild scaling. Experimental chronic methylmercury intoxication in horses produces *exudative dermatitis*, but histologic lesions are not described. Local toxic contact dermatitis follows application of mercurial-containing counterirritants to the legs in horses.

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Cutaneous iodism

Iodides have widespread use as antiseptics, expectorants, intravenous contrast agents, bronchodilators, antithyrotoxicants, and as salt or feed additives. Iodine is readily absorbed from the skin. The majority of reports of adverse effects of iodine occur as hypersensitivity reactions and not toxicoses. *Generalized seborrhea sicca* is reported in horses and cattle accidentally overdosed with iodine-containing drugs or medicated feed. In experimental toxicosis of calves, the cutaneous lesions were limited to scaly patches without alopecia. Conversely, suspected iodism in a horse produced generalized alopecia, sparing only the face, mane and tail.

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Selenium toxicosis

Selenium is a metalloid that acts as an antioxidant with toxic potential. It has chemical properties similar to sulfur. It is excreted from the body in urine, feces, and sweat and integumentary structures. Experimental studies in rodents suggest that selenium may diminish UV radiation-induced skin damage when applied topically. However, some forms are strong contact irritants and vesiculants. The toxic potential of selenium in the diet varies by the chemical form present, nature of the diet, rate of consumption, and by the species and individual animal. The mechanism by which selenium might exert its effects on the integument and appendages is not known, but conceivably, being competitive with sulfur, it modifies the structure of keratin.

Selenium is widely distributed in soils at concentrations ranging from 0.01 parts per million (ppm) or less to 500 ppm or more. Areas of high soil concentration are particularly extensive in parts of the USA (Wyoming, Montana, Utah, Colorado, North and South Dakota, Arizona, Kansas, and Nebraska) and in western Canada, but also occur in parts of Australia, New Zealand, China, Ireland, Mexico, and Israel, among other countries.

Selenium toxicosis occurs in *horses, cattle, sheep, and pigs*, chiefly as a result of the ingestion of plants that have accumulated toxic levels of selenium, but occasionally as a result of accidental overdose of selenium supplements. Plants are divisible into seleniferous and nonseleniferous species. **Seleniferous plants** can selectively concentrate selenium in their foliage and seeds as compared with nonseleniferous species grown under the same conditions. The seleniferous species are subdivided into obligate accumulators, which require high levels of selenium for survival and facultative accumulators. The former, which include members of the genus *Astragalus*, *Machaeranthera* and *Stanleya,* may accumulate over 1000 ppm selenium. Because of their high requirement for selenium, they are known as "indicator" species. Facultative or secondary selenium accumulators such as *Aster, Atriplex, Castilleja,* and *Gutierrezia* take up lesser amounts of selenium. Many nonseleniferous weeds, crop plants, and grasses are capable of passively accumulating selenium if growing on soils with high selenium content. Also, indicator plants increase the availability of selenium to nonseleniferous plants by converting insoluble selenites to soluble selenates and returning these to the soil. Selenium poisoning can occur whenever seleniferous plants are eaten irrespective of levels of selenium in the soil, and it can occur whenever the levels of water-soluble selenium in the soil are high, irrespective of the botanical composition.

Seleniferous plants are not palatable and indigenous stock learns to avoid them. Selenium poisoning occurs chiefly in newly introduced or traveling animals and in indigenous animals forced to eat the seleniferous plants in times of scarcity. Clinically, there are acute and chronic syndromes associated with the ingestion of seleniferous plants such as *Astragalus* and *Oxytropis*. *Acute toxicity* causes severe gastrointestinal and cardiovascular signs with mortality in some instances approaching 100%. Two different syndromes have been described as *chronic selenium poisoning*. "**Blind staggers**," characterized by neurological signs, is probably not due to selenium alone but to other toxic principles in the seleniferous plants. The second syndrome is named "**alkali disease**" because it was originally believed that the pH of the selenium-rich soils was a factor in its pathogenesis. Unlike blind staggers, alkali disease is reproducible in ungulates fed sublethal concentrations of selenium. The presence of internal lesions such as nephrosis, myocardial degeneration, and hepatic fibrosis in chronically poisoned livestock is not found in experimentally reproduced disease, suggesting other factors are involved.

Horses and cattle chronically intoxicated with selenium become emaciated and develop partial alopecia and a general roughness of coat. Foals delivered from affected mares may have lesions. Initially, there is loss of the long hairs in the mane, forelock, and tail of horses (leading to the name "bobtail" disease), and loss of the long tail hairs in cattle. Sheep do not show cutaneous lesions, although in Australia fleece shedding has been attributed to selenium toxicity on some properties. Selenium toxicity is also suspected in alopecias of the beard and flanks of goats in the western United States. In all species, lesions commencing at the coronary band may lead to separation and shedding of the hoof or to the formation of dystrophic grooves, cracks, or corrugations that parallel the coronary band resulting in lameness (Fig. 5.49). Lesions take months to develop. *Histologic lesions* of experimental chronic selenium toxicosis in cattle showed extensive separation of the stratum medium of the hoof with replacement by parakeratotic cellular debris. The germinal epithelium of the hoof wall was disorganized, parakeratotic and hyperplastic. Hair follicles from the tail were atrophic with dyskeratosis and mild hyperkeratosis.

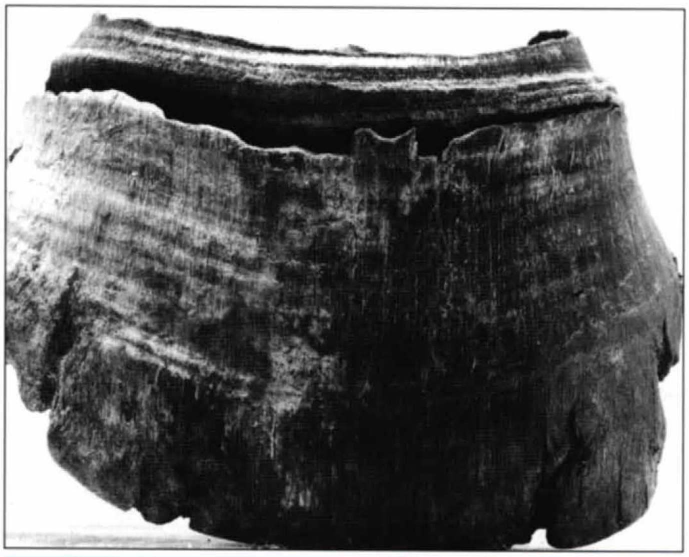

Figure 5.49 Selenium toxicosis in a horse. Hoof wall deformed by rings and grooves. (Courtesy of Queensland Department of Agriculture.)

Diagnosis requires the demonstration of compatible clinical signs, progression of lesions, and identification of a dietary source of selenium with a concentration in the air-dried feed sample of >5 *ppm selenium*. High levels of selenium in the blood (>2 ppm) or integumentary tissues such as hair, hoofwall or sole (>10 ppm) should also be present. Individual animals have a variable response to selenium exposure and some animals with high levels of selenium may not show signs of toxicosis.

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Organochlorine and organobromine toxicoses

Organochlorine and organobromine compounds implicated in toxicities causing, among others, cutaneous lesions, include the *highly chlorinated naphthalenes (HCN), polybrominated biphenyls (PBBs),* and *dibenzofurans. Polychlorinated biphenyls (PCBs)* are the cause of an important industrial dermatitis of humans known as *chloracne.*

Highly chlorinated naphthalene (HCN) toxicosis (X-disease, bovine hyperkeratosis), the result of exposure to tetra-, penta-, hexa-, hepta-, or octachloronaphthalenes, is largely of *historical interest.* HCNs were found to be responsible for high mortality and large economic losses in the cattle industry within the United States, Australia, New Zealand, and Germany during the 1940s and early 1950s. HCN was a common additive in many petroleum products including those used as lubricants for farm machinery such as feed pelleting equipment, wood preservatives, roofing paper and building board. HCNs were a frequent feed contaminant. *These chemicals have not been used in lubricants since 1953.* Recent reports are the result of animals exposed to dumps with stores of old lubricant or abandoned machinery lubricated years ago with HCN containing products. Percutaneous exposure produces cutaneous lesions, whereas parenteral exposure results in both cutaneous and visceral lesions. The poison is cumulative and the disease is chronic. Lesions of HCN toxicity result from interference with the conversion of carotene to vitamin A and resemble the lesions of vitamin A deficiency. The first sign of poisoning is a fall in vitamin A levels in the plasma.

Cattle are the most susceptible species. Initial signs are increased lacrimation and drooling, depression, decreased appetite, and weight loss. Within the first few months, *hallmark cutaneous lesions of marked alopecia with nonpruritic, lichenified, deeply fissured plaques of hyperkeratotic scale are evident on the skin of the neck, shoulders and perineum.* Lesions gradually generalize, sparing only the legs. Marked involvement of the skin of the medial thighs is characteristic. Horn growth may be delayed. The animal may die before the skin lesions are severe, if exposure is high. Concurrent severe secondary infections with *Bovine papular stomatitis virus,* papilloma virus or dermatophytes may be present. The histologic lesions are marked hyperkeratosis of surface and follicular epithelia. Internal lesions are the result of hyperplasia and squamous metaplasia of the epithelial lining of ducts and glands of the body including liver, pancreas, kidneys, and reproductive tract. Bulls may present with epididymal enlargement early in the disease process from hyperplasia and squamous metaplasia of ducts.

Differential diagnoses include other markedly hyperkeratotic dermatopathies such as zinc or vitamin A deficiency, dermatophilosis, dermatophytosis, or toxicosis due to other polyhalogenated aromatic compounds. Definitive diagnosis is dependent upon identification of a source of HCN and extraction of the toxin by capillary gas chromatography and mass spectrometry. Vitamin A levels in the plasma and liver are low, but toxicosis cannot be reversed by vitamin A therapy and the prognosis is poor.

Cutaneous lesions in **cats** exposed to wood preservatives have been ascribed to chlorinated naphthalene toxicity. The lesions include bilateral alopecia and encrustations on the eyelid and around the nostrils.

Pentachlorophenol (PCP)-contaminated wood shavings used as bedding led to chronic toxicity characterized by a *proliferative dermatitis* with crusting, scaling and alopecia accompanied by a *multitude of systemic signs* including peripheral edema, bone marrow hypoplasia, liver disease and wasting in a group of horses. Gross and histologic cutaneous lesions resembled those of HCN toxicity. Wood shavings containing four times the maximal allowable levels of PCP/kg were traced to a lumber company using improper processing techniques. Toxicity was attributed to dibenzofuran and chlorinated dibenzo-p-dioxin isomers found as contaminants of the PCP. *Contaminants of PCP products include a large group of dioxin isomers with a wide range of toxicity that can vary by species exposed and among animals within the species.* PCP compounds are used as antiseptics, disinfectants, herbicides, fungicides, and wood and hide preservatives. The toxicity of the herbicide, agent Orange, widely sprayed in Vietnam, is attributed to *dioxin contaminants.* The compounds enter the body via oral, dermal, or respiratory routes. High exposure leads to rapid death due to uncoupling of mitochondrial oxidative phosphorylation. Chronic toxicity is more common. The contaminants in the PCP compounds are thought to bind to aromatic hydrocarbon receptors in the cell nucleus and influence gene expression. The response may be proliferative or suppressive. Elevated levels of the compounds can be demonstrated in the liver and adipose tissue years after exposure, whereas serum levels are cleared quickly. Similar cases of dioxin isomer toxicity have been reported in *horses* exposed to riding arenas sprayed with contaminated waste oil used for dust control.

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Mimosine toxicosis

Mimosine is a *toxic amino acid* found as a main constituent in the tropical to subtropical, cultivated *leguminous shrubs, Mimosa pudica* and *Leucaena leucocephala* (formerly *L. glauca*). Mimosine and its metabolite, 3-hydroxy-4-(1H)-pyridone (DHP), are toxic. In ruminants, mimosine is a depilatory, whereas DHP is a goitrogen. Poisoning can be acute or chronic and is characterized by *alopecia, poor growth, oral ulcerations,* and *goiter* not prevented by iodine supplementation. Toxicity occurs in a number of countries and goes by a variety of local names: *jumbey* in the West Indies, *lamtoro* in Indonesia, and *koa haole* in Hawaii. A variation of animal susceptibility to mimosine toxicity in different parts of the world is due to the geographic distribution of ruminal bacteria capable of degrading DHP. In vitro antemortem assays for detection of DHP-degrading bacteria can be performed on feces or ruminal contents. A group of bacteria, *Synergistes jonesii,* can be inoculated into the rumen of livestock to prevent toxicity. Mimosine has been shown to reduce DNA synthesis and to block the progression of the cell cycle by chelating iron. DHP prevents iodine binding in the thyroid gland. *Mimosine toxicity occurs in horses, cattle, pigs, and sheep* and has been experimentally reproduced in cattle and laboratory animals.

Horses appear to be most susceptible and lose their hair, especially the long hair of the mane and tail. In severe cases, there is patchy loss of hair above and below the hocks and knees and on the flanks and neck. Disturbed growth at the coronet and periople may produce dystrophic rings on the hooves. There is loss of condition and weakness that perhaps is attributable to malnutrition rather than to mimosine.

Mimosine has a marked depilatory action on the fleece of **sheep**. The fleece becomes easily epilated 14 days after a single oral dose of 400–650 mg/kg body weight. DNA synthesis in the wool follicles is reduced. Mimosine toxicity causing depilation in **pigs** is reported from Indonesia and the Bahamas.

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Gangrenous ergotism and fescue toxicosis

These two conditions can be considered together because the cutaneous lesions of chronic ergotism caused by *Claviceps purpurea* and those of poisoning by tall fescue, *Festuca arundinacea* or *F. eliator*, are identical.

Ergotism is the oldest known mycotoxicosis. The ergot of *Claviceps* spp. fungi is a *compacted mass of hyphae, the sclerotium*, which develops in the seed heads of many species of grasses and cereal grains and completely replaces the ovary. *Ergotism is the disease that results from ingestion of toxic alkaloids produced by the fungi.* The alkaloids are derivatives of lysergic acid and include *ergotamine, ergometrine and ergotoxine*, which is a composite of three alkaloids. The quantity and spectrum of alkaloids in the ergots vary considerably with the strain of fungus, type of plant, season of the year, climatic conditions and other regional factors. The ergots also produce a *variety of amines*, such as histamine, acetylcholine and other nitrogenous compounds with physiological activity.

Of the various pharmacological effects exerted by the ergot alkaloids, the most important in the pathogenesis of gangrenous ergotism is direct stimulation of adrenergic nerves supplying arteriolar smooth muscle. This produces *marked peripheral vasoconstriction.* Arteriolar spasm and damage to capillary endothelium leads to thrombosis and ischemic necrosis of tissues.

Gangrenous ergotism caused by *C. purpurea* is a disease mainly of cattle. It may occur in animals at pasture but is more common in housed animals fed infected grain. Gangrenous ergotism represents the chronic form of intoxication by ergot-producing fungi. Chronic ergotism develops after a week of feeding contaminated grain and begins with *acute lameness with redness and swelling of the extremities*. The hindlegs are more frequently affected than the forelegs. Lesions seldom extend above the fetlock (Fig. 5.50), but ischemic necrosis may extend to the mid-metatarsus. The feet become cold and insensitive with dry necrosis and a prominent line of separation between viable and dead tissue. The necrotic tissue may slough. Ergotism also causes *dry gangrene of the tips of the ears and tail.* Gangrenous ergotism has been described in goats feeding on ergot-infected pasture. The toxicosis can be produced experimentally in sheep, but the syndrome is quite different from that in cattle, being characterized by ulceration of the tongue and of the alimentary mucosae. Sows are relatively resistant but may develop agalactia as a result of central inhibition of prolactin secretion.

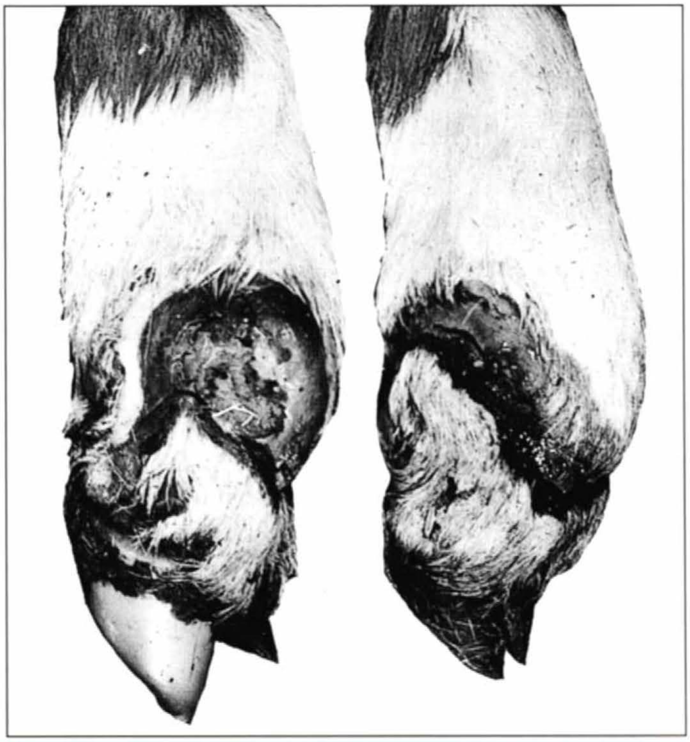

Figure 5.50 Ergotism in a calf. Sharply demarcated ischemic necrosis of distal extremities.

Fescue toxicosis has a variable presentation depending upon the animal species exposed. The most common manifestation, "*fescue foot*," is a disease of *cattle* characterized in the acute form by *dry gangrene of the extremities* commencing 2 weeks after ingestion of the tall fescue grass, *Festuca arundinacea*. This perennial grass is the most common pasture plant in the United States and is usually harmless. The endophytic fungus *Neotyphodium (Acremonium) coenophialum* infects approximately 75% of fescue pastures and imparts increased resistance of the plant to insects and extreme environmental temperatures. Under certain poorly understood conditions, the endophyte-infected plant is toxic. Fescue foot tends to occur with the onset of colder weather indicating that low ambient temperatures may contribute to its development. The alkaloids, *ergonovine, ergotamine, and N-acetyl loline* are responsible for toxicity and act as *peripheral vasoconstrictors*. The acute syndrome in cattle is virtually identical to ergotism.

A chronic disease in **cattle**, known as "summer slump," refers to an increased susceptibility to heat stress seen in certain breeds of cattle in conditions of high environmental temperature combined with intake of endophyte-infected fescue. Cattle have decreased skin temperature and open-mouth breathing suggesting defective thermoregulation. Experimental intravenous injection of alkaloids found in fescue produced lowered skin temperature, heart rate, and prolactin levels, and elevated blood pressure and respiratory rates in heifers. Ingestion of endophyte-infected fescue in **horses** does not lead to the visibly evident effects of peripheral vasoconstriction as in cattle, but experimental studies have demonstrated peripheral vasoconstriction does occur suggesting exposure could lead to foot or leg problems. The more commonly recognized manifestations of fescue toxicosis in the horse are *prolonged gestation, agalactia, thickened placentas, and possible abortion.* Abdominal lipomatosis associated with marked necrosis of abdominal fat and severe weight loss occurs in *domestic ruminants* and some species of wildlife consuming endophyte-infected fescue. A heritable predisposition exists in cattle.

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Tricothecene toxicoses

Macrocyclic trichothecene toxins produced by the fungus *Stachybotrys* spp. cause **stachybotryotoxicosis**. *Ulcerative and necrotizing lesions of the skin and mucous membranes* have been reported in horses, cattle, sheep, and pigs, chiefly from Russia and Eastern Europe. Initial lesions affect the lips, buccal commissures and nostrils. Marked edema of the face may follow. Death follows development of *hemorrhagic diathesis, enteritis, and septicemia*. At necropsy, lesions in addition to the hemorrhagic diathesis include alimentary ulceration, pneumonia, renal infarcts, multifocal hepatic necrosis, and lymphadenitis. In many instances, these lesions may represent secondary mycotic or bacterial involvement.

T-2 toxin is a highly irritant trichothecene mycotoxin from *Fusarium* molds on grain, and causes cutaneous ulceration when applied locally to the skin of pigs. Experimental feeding of T-2 toxin contaminated feed, in combination with aflatoxin, induces crusting and ulceration of the lips, snout, buccal commissures and prepuce. The pathogenesis of the lesion is thought to be *contact irritant dermatitis* due directly to the T-2 toxin or to a urinary metabolite, HT-2 toxin.

Various other trichothecene mycotoxins are also cutaneous irritants and may cause vomition or feed refusal.

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Vetch toxicosis and vetch-like diseases

Hairy vetch *(Vicia villosa* Roth*)* is a cultivated legume used as pasture, hay and silage in most of the United States, and in other countries such as Argentina, Australia, and South Africa. Hairy vetch toxicosis in **cattle** is seen as three unique syndromes: (1) acute neurological disease and hemolysis, followed by death after consumption of seeds; (2) swelling of the upper body accompanied by herpetiform eruptions of the oral mucous membranes, respiratory distress and death after consuming vetch pasture; and, (3) a syndrome characterized by *dermatitis, conjunctivitis, diarrhea, and granulomatous inflammation of many organs*. This third syndrome is the most common form of hairy vetch toxicosis and occurs in cattle and, to a lesser extent, *horses* after consumption of vetch-containing pastures. The clinical syndrome begins two or more weeks after consumption and consists of pruritic dermatitis, diarrhea (possibly bloody), and wasting. Morbidity is low and mortality is high. Holsteins, Angus and cattle 3 years or older are more often affected. Death in cattle occurs approximately 10–20 days after illness begins.

Initial lesions consist of a rough coat with papules and crusts affecting the skin of the udder, teats, escutcheon and neck, followed by involvement of the trunk, face and limbs. The skin becomes less pliable, alopecic, and lichenified. Marked pruritus leads to excoriations. At necropsy, *yellow nodular infiltrates disrupt the architecture of a wide range of organs*, but are most severe in myocardium, kidney, lymph nodes, thyroid and adrenal glands. The kidney may have radially oriented cortical infiltrates that follow the vasculature. Other affected organs may include the mammary and salivary glands, liver, urinary bladder, meninges, and spleen. Histologically, *the infiltrates consist of monocytes, lymphocytes, plasma cells, multinucleated giant cells, and in the cow, eosinophils.* The skin has similar perivascular to diffuse infiltrates, marked hyperkeratosis, and dermal and epidermal edema. This form of the disease has been induced experimentally in an Angus cow that had recovered from vetch toxicosis the previous year. Lesions were evident 11 days after feeding vetch. Death occurred even though vetch was removed from the diet at 12 days. Experimental lesions mirrored those of naturally occurring disease with the additional finding of necrosis of cutaneous apocrine glands.

Other species of *Vicia* and additional compounds are capable of inducing disease indistinguishable from vetch toxicity.

Pyrexia with dermatitis in dairy cows is a syndrome with similarities to hairy vetch toxicity. It has been reported in the United States, England, Wales, France, and the Netherlands. Friesian dairy cows developed pruritic papular eruptions affecting the head and neck, tail head and udder. Secondary lesions were due to self-trauma. In another outbreak, hemorrhages were a prominent clinical sign. The episode in Wales was associated with the introduction of a new silage additive on several farms. The outbreak in the Netherlands was associated with the feeding of *di-ureido-isobutane*

(DUIB) in the seed cake. This condition was reproduced in two cows by feeding a DUIB-containing diet for one month. Histologically, *the lesions of the Dutch outbreak also resembled those of the putative hairy vetch toxicity*. Visceral lesions resembling hairy vetch toxicity occur in dairy cows fed a diet containing *citrus pulp;* the syndrome resolves after removal of citrus pulp from the diet.

Hairy vetch toxicosis in **horses** resembles that in cattle except for the infrequent finding of eosinophils in the infiltrate and lack of heart involvement. Conditions very similar to vetch toxicosis have been reported in horses with no vetch exposure. These cases have been variably referred to as "idiopathic granulomatous disease involving the skin," "systemic granulomatous disease," "generalized granulomatous disease" or "equine sarcoidosis." Organ involvement is variable. Skin lesions include scaling, crusting and alopecia on the face or limbs that progress to a generalized exfoliative dermatitis. Histologically, *the skin has multifocal, sometimes perifollicular to deep dermal nodules of granulomatous inflammation*. Sarcoidosis in man has a genetic basis and is thought to represent a hypersensitivity response to a persistent antigen. These idiopathic conditions in the horse are fairly indistinguishable and should be considered "*vetch- like disease*" until more information is available.

Toxicity from vetch seeds is known to be due to the presence of *prussic acid*. The cause of the granulomatous diseases listed above remains unclear. Nor is it certain whether they represent one entity or a common tissue reaction to a variety of insults. One proposed pathogenesis is that ingestion of vetch or another substance leads to antigen formation in the form of a hapten or a complete antigen that sensitizes lymphocytes and evokes the cell-mediated response upon repeat exposure. Factors that support this hypothesis are the resemblance of the histologic lesions to a type IV hypersensitivity response, age incidence, low morbidity, genetic influence and possible need for repeat exposure. Lymphocyte blastogenesis and cutaneous hypersensitivity studies have not substantiated this hypothesis, however, only a few vetch antigens have been studied.

The diagnosis of vetch toxicity or vetch-like disease is a *diagnosis by exclusion*. It is made after review of the herd history, and character and distribution of the lesions. The combination of lesions is fairly distinctive.

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Quassinoid toxicosis

Quassinoid compounds, such as neoquassin and quassin found in hardwood trees of the genus *Quassia (Simarouba amara)* in the family Simaroubaceae, have been reported to be associated with a *vesiculobullous dermatitis of the skin* around the eyes, nose, ears, anus and lips of horses. *Wood shavings* from these plants have been incorporated in bedding and the outbreaks have occurred in large numbers of exposed horses. Gross lesions develop within the first few days of exposure and most often resolve within a week. Systemic signs such as anorexia and icterus accompany cutaneous lesions in some cases. Hepatopathy and nephrosis have been reported. Similar symmetrical lesions of the oral mucosa, mucocutaneous junctions, and pressure points have been reported in dogs exposed to *Simarouba* shavings.

Quassinoids have been shown experimentally to have insecticidal and anthelmintic properties while their derivatives have antitumor, antiulcer, and cytotoxic activity in vitro. The mechanisms leading to toxicity are not known. Definitive diagnosis is dependent upon a compatible history, exclusion of viral diseases such as vesicular stomatitis and exposure to other toxins, and the positive identification of plants of the *Quassia* genus in the bedding material.

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ACTINIC DISEASES OF SKIN

The radiant energy of the sun includes components that are potentially harmful to mammalian skin. This radiation is known as **actinic radiation** and its acute effect is the well-known *sunburn* reaction. **Photosensitization** is essentially an *exacerbated form of sunburn*, caused by the activation of photodynamic chemicals in the skin by radiation of an appropriate wavelength and is discussed under the heading Photosensitization dermatitis. **Photoallergy** is distinct from phototoxicity; *it occurs when the photoproduct of an exogenous chemical acts as an antigen*. Photoallergic reactions require prior sensitization to the drug or chemical and are more clinically diverse. Photoallergies have not been documented conclusively in animals. **Skin cancers** induced or exacerbated by actinic radiation are considered with Tumors of the epidermis.

Direct effect of solar radiation

Solar energy is a form of non-ionizing radiation comprised of ultraviolet light (100–400 nm), visible light (400–700 nm), and infrared light (700–20 000 nm) rays. *Most of the direct photobiologic reactions in the skin are induced by high energy light in the ultraviolet radiation UVB range (290–320 nm)*. Longer wavelengths of 320–400 nm constitute UVA and may augment UVB-mediated damage. The integument is normally protected against the deleterious effects of ultraviolet radiation by the hair coat, the stratum corneum and melanin pigmentation. The quantity of ozone, smog, altitude, latitude, season of the year and time of day also strongly influence the amount of UV rays reaching the skin. The greatest potential for solar-induced skin damage occurs at high altitudes and temperate latitudes during mid-summer days, and in thin, lightly pigmented, sparsely haired, sun-exposed skin. An increasing prevalence of sun-induced dermatoses and tumors has been noted in humans, coincident with *the depletion of the ozone layer and a consequent increase in the intensity of ultraviolet radiation reaching the earth's surface*. This trend may also become evident in animals. Potentially, all animals are susceptible to the acute and chronic effects of actinic radiation, but the protection afforded by the hair coat, and to a lesser extent stratum corneum and skin pigmentation, is normally sufficient to prevent solar-induced damage. The conditions described below typically affect animals whose anatomical defenses are poor, either by lacking skin pigmentation or hair cover.

UVB radiation stimulates light-absorbing molecules in the skin referred to as chromophores. *Chromophores include keratin proteins, melanin, carotene, nucleic acids, peptide bonds and some amino acids*, to name a few. Light absorbed by chromophores results in electron transfers and free radical production. *Free radical mediated cellular damage* is discussed elsewhere. Energy dissipated from electron transfers induces chemical reactions to form altered cell components referred to as *photoproducts* that include altered DNA molecules, enzymes, and hydrogen and disulfide bonds within proteins. Nucleoprotein is susceptible to ultraviolet radiation damage resulting in mitotic inhibition and, if extensive enough, cell death. Sublethal damage may promote mutagenesis or carcinogenesis by the formation of thymidine dimers between pyrimidine base pairs of DNA. Pyrimidine dimer repair mechanisms normally correct DNA damage prior to cell replication, however repeated or extensive damage may lead to failure of repair mechanisms and cell transformation. The "*sunburn cell*" associated with UV damage is a keratinocyte that has undergone apoptosis. UVB-induced keratinocyte apoptosis is a complex event that involves cytokines such as TNFα and probable p53-mediated induction of apoptosis in cells sustaining substantial DNA damage. Studies in mice have shown that UVB radiation-induced apoptotic keratinocytes are replaced by hyperproliferative keratinocytes leading to epidermal hyperplasia, suggesting apoptosis and hyperplasia are related events. Ultraviolet light also induces mutations of the p53 tumor suppressor gene in keratinocytes contributing to a proliferative advantage to mutated cells that is found in solar-induced actinic keratoses and squamous cell carcinomas in humans. These findings indicate ultraviolet radiation can serve as both a tumor initiator and promoter. UV radiation may also alter immunologic reactivity in favor of the growth of the tumor, through the induction of suppressor T cells and possible impairment of natural killer cell function. UVB radiation exposure reduces the number of Langerhans cells and impairs their antigen-presenting functions. Contact hypersensitivity responses in experimental animals are reduced following UVB irradiation.

Apoptotic keratinocytes, "sunburn cells," arranged singly or in clusters or bands in the outer stratum spinosum are a characteristic microscopic feature of acute sun-induced epithelial damage. These may be induced within 30 minutes of sun exposure. Other lesions include spongiosis, vacuolation of keratinocytes, loss of the granular layer, and, in severe burns, vesiculation. Dermal hyperemia and edema are prominent features. In mild lesions, there is a slight increase in mononuclear cells; in severe burns, there is marked vascular damage, erythrocyte extravasation and neutrophilic exocytosis. The initial lesion of UV-irradiation induced injury consists of transient erythema, probably due to a direct heating effect and the photobiologic effects of UVB acting directly on dermal capillaries. The delayed erythema reaction may be due to direct damage to endothelial cells or the release of cytokines from the radiation-damaged keratinocytes. Ultraviolet radiation has been shown to increase the production of keratinocyte-derived cytokines. Ultraviolet light also induces adaptive responses in the epidermis, in particular epidermal hyperplasia and alterations in melanin pigmentation. An immediate pigment darkening is due to changes in existing melanin, and the delayed or "tanning" reaction to stimulation of melanogenesis and proliferation of melanocytes. *Melanin both absorbs and scatters UV radiation and, being able to trap free radicals, is important in minimizing the deleterious effects of incident photons.* Basal keratinocytes with melanin granules forming protective caps over the nucleus have an increased distribution in sun-exposed skin. In chronically sun-damaged skin, pigment distribution can become irregular due to impaired transfer of melanin from melanocytes to keratinocytes, thereby weakening host defenses. *Long-term effects* of ultraviolet irradiation include variable degenerative changes in the dermis (solar elastosis and fibrosis), adnexa (comedones, cysts), and epidermis (solar keratosis). Lesions vary by species and among individuals within the species.

Solar dermatitis, or *sunburn*, occurs most frequently in *cats, dogs, pigs, cows, and goats*. The lesions in *cats* typically affect the tips of the ears, nose, eyelids, and lips of white, blue-eyed animals. The initial lesion is erythema followed by alopecia, scaling and crusting (Fig. 5.51). The ear tip may curl over. Lesions are exacerbated each summer, often eventuating in malignant transformation into squamous cell carcinoma. Primary phototoxicity in swine occurs in white or light-colored *pigs*. While any age group may be affected, the condition is most severe in suckling and weaner pigs. Occasionally, severely affected ears may slough. Light-colored *goats* and *cows* are also prone to solar dermatitis. The udders are particularly susceptible when does are turned out into strong sunlight after a winter indoors.

Solar dermatitis occurs most often in *short-haired dogs with light pigmentation*. Breeds most often affected include Bull Terriers, Whippets, Beagles, and Dalmatians. Lesions are most often present on the ventrolateral abdomen and thorax, lateral flank, hocks, and bridge of nose in nonpigmented skin. Such lesions are probably related to the basking behavior exhibited by the animals. *Early lesions of erythema and scaling evolve into thick, lichenified, erythematous, crusted patches and plaques.* Hemorrhagic bullae may develop. The most consistent histologic finding in dogs with chronic solar dermatitis is a *narrow, hypocellular, pale-staining band of collagen along the dermoepidermal junction*. This change may be present prior to clinical signs of actinic dermatitis and may be used as an indicator of solar damage if the history, anatomical site, and breed are supportive. Other changes noted in canine sun-damaged skin include epidermal acanthosis, apocrine gland ectasia, and follicular keratosis resulting in follicular cyst formation and possible furunculosis, particularly over pressure points (Fig. 5.52A). A layer of fibrosis often surrounds cystic follicles. A superficial perivascular mixed infiltrate of lymphocytes, plasma cells, monocytes, neutrophils, and rare eosinophils is usually present. Furunculosis leads to a marked foreign-body response. Follicular changes are thought to be due to loss of support of follicles, however elastin studies in dogs have not demonstrated degeneration of elastin fibers supporting the follicular wall. Advanced lesions may have epidermal dysplasia (Fig. 5.52B) or concurrent UV-light induced neoplasms such as a squamous cell carcinoma, hemangioma, or hemangiosarcoma. It is important to note that UV light-induced neoplasms may arise in skin devoid of other changes suggestive of actinic damage. *Actinic comedones* may also be present without lesions suggestive of actinic epidermal damage. *Differentials* are many and include other conditions resulting in comedone formation, acne, various allergies, bacterial or fungal infections, neoplasia or a primary keratinization disorder. Restriction of lesions to nonpigmented, sparsely haired skin and a history of sun exposure should be helpful in differentiation.

Solar elastosis, a hallmark of chronic sun exposure in humans, has been described only rarely in *dogs, cats, sheep and horses* and essentially represents *disorganization of dermal components due to*

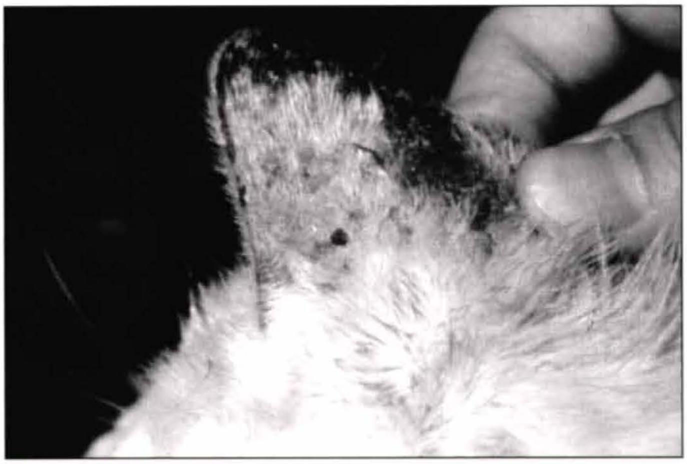

Figure 5.51 Solar dermatitis in a white cat, with erythematous, crusted patches and plaques on the pinnae.

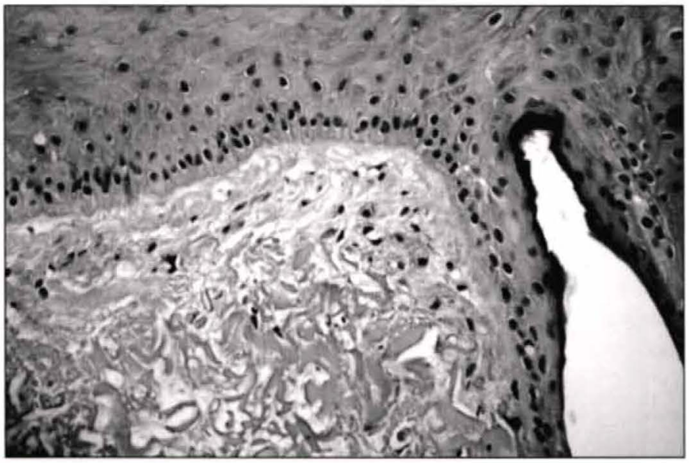

Figure 5.53 Solar elastosis. The dermis is filled with agglomerated, thick, irregular, basophilic degenerate elastic fibers.

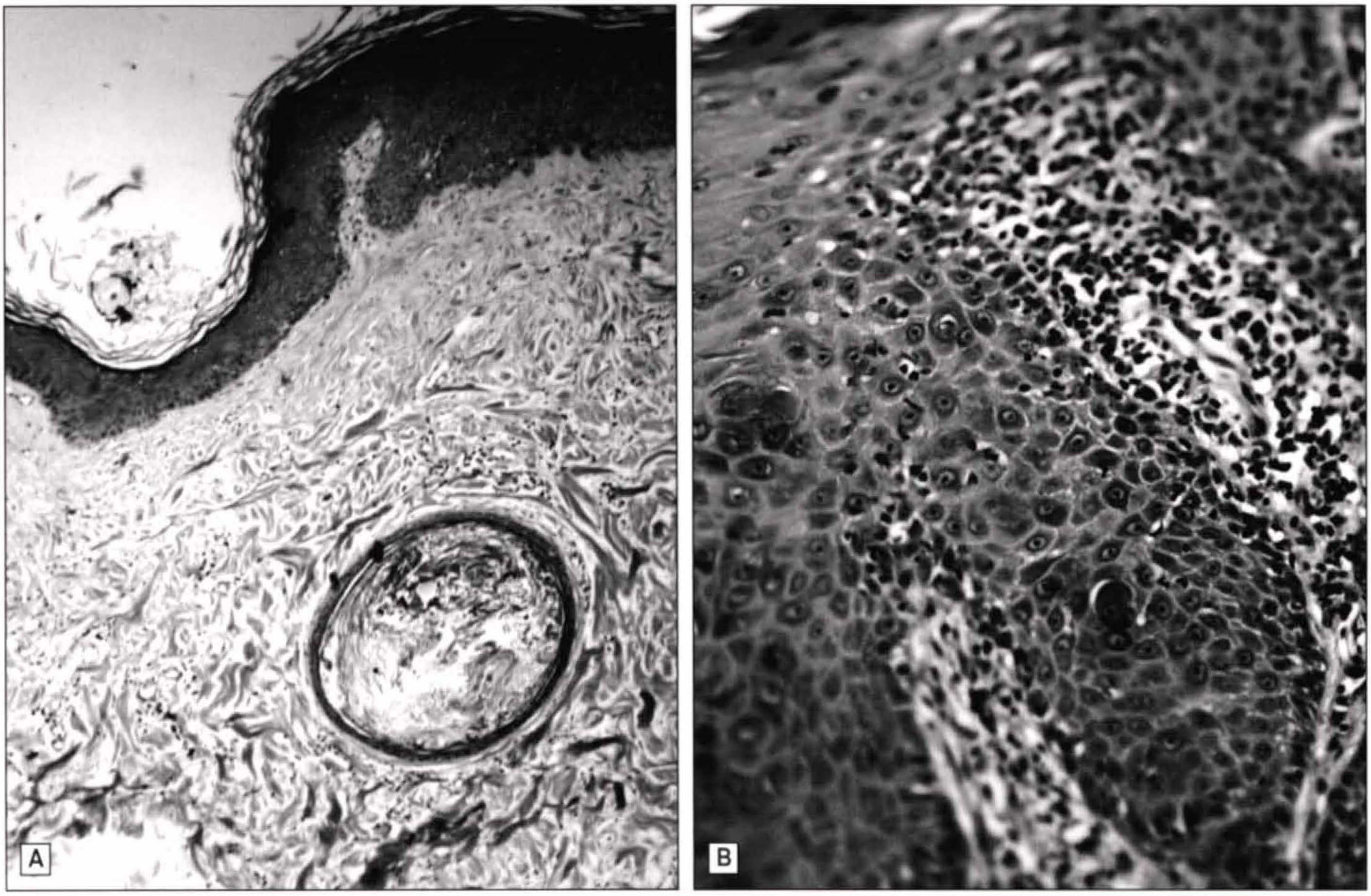

Figure 5.52 Solar dermatitis. A. Note hyperplastic epidermis, superficial layer of pale dermal collagen, and comedone formation. **B.** Hyperplastic epidermis with "**sunburn cells**" and other evidence of dysplasia.

altered fibroblast function. The lesions usually occur in conjunction with solar radiation-associated neoplasms, particularly squamous cell carcinomas. Solar elastosis appears in hematoxylin and eosin-stained sections as *scattered or agglomerated, thick, irregular, basophilic degenerate elastic fibers* (Fig. 5.53). Silver impregnation staining techniques may be needed to demonstrate elastin changes in animals. Solar dermatitis is often present without evidence of elastosis.

Solar keratoses, common *precancerous skin lesions* in humans, occur in cats, dogs and horses. The conjunctiva of horses with white eyelids is a common site. Histologically, the early lesions have many of the features of sunburn, including epidermal hyperplasia, spongiosis, acute dermal inflammation and focal necrotic keratinocytes. More chronic lesions show pronounced epidermal hyperplasia with *dysplasia*, ortho- and parakeratotic hyperkeratosis, perivascular mononuclear cell infiltrates and dermal scarring, but seldom develop significant solar elastosis, as typifies human solar keratoses. *Lesions frequently progress to invasive squamous cell carcinoma.* Solar keratoses may also develop cutaneous horns.

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Photosensitization dermatitis

Photosensitization dermatitis occurs in animals when photodynamic or fluorescent pigments are deposited in sunlight exposed skin. Photodynamic pigments absorb ultraviolet light or visible light in the action spectrum and convert it to light of a longer wavelength, usually beyond the UVB range. The energy from the absorbed light leads to tissue injury by reacting directly with molecular oxygen, producing reactive oxygen intermediates such as superoxide anion, singlet oxygen and hydroxyl radical. Oxygen free radicals may also be formed indirectly, as the result of a calcium-dependent, protease-mediated activation of xanthine-oxidase in the skin. Release of reactive oxygen species initiates chain reactions which lead to mast cell degranulation and damage to cell membranes, nucleic acids, proteins and subcellular organelles, particularly lysosomes and mitochondria.

The photodynamic agent usually reaches the skin via the *systemic circulation*, although *percutaneous absorption* of some photodynamic agents can cause local contact photosensitization. The agent may originate externally or it may be an endogenous substance that has accumulated to an abnormal degree as a result of metabolic dysfunction. Sources include *plant pigments and drugs* or, in the case of metabolic dysfunction, the *by-products of hemoglobin metabolism or chlorophyll degradation products*. The three categories of photosensitization are classified according to the source of the agents.

- In *type I, or primary photosensitization*, the animal *ingests plants or drugs* containing photoreactive substances that are deposited in the skin. Most exogenous sources of photoreactive pigments are found in plants, and therefore foraging animals such as horses, sheep, cattle and goats are most frequently affected.
- In *type II photosensitization*, an inherent inability to properly metabolize heme pigments necessary for erythrocyte production leads to the build up of the photoreactive pigments, *hematoporphyrins*.
- An abnormal build up of *phylloerythrin*, a degradation product of chlorophyll, induces *type III photosensitization*. This is known also as *hepatogenous photosensitization* because it depends upon the failure of a damaged or immature liver to eliminate phylloerythrin. Type III photosensitization occurs most often in animals ingesting large amounts of green forage.
- A fourth group contains those examples of photosensitization for which the pathogenesis is presently undetermined.

The **gross lesions** are similar for all forms of photosensitization. They occur on those areas of the body most exposed to sunlight and which lack protective fleece, hair coat or skin pigmentation. In *cattle*, any area of light-colored skin is susceptible. This is best demonstrated in broken-colored animals such as Holsteins, in which the white skin is affected but the black is spared. The relatively hairless skin of the teats, udder, perineum and muzzle is also affected. The ventral surface of the tongue is frequently affected in cattle if constantly exposed during licking. In *sheep*, the susceptible sites are the ears, eyelids, face, muzzle and coronets, although the back may be affected in animals with an open fleece or which have been shorn closely. The udders and teats of dairy *goats* are predisposed. In *horses*, lesions are most common on the face, perineum, and distal extremities but may affect any white skin. Lesions in *pigs* are uncommon, and have a predilection for the ears, eyelids, udder, and back. Photosensitization is rare in *dogs* and *cats* and causative agents remain obscure.

The initial reaction in photosensitization is erythema, followed by edema, which is more prominent in sheep than in cattle. The very marked edema of the ears in sheep causes them to droop and swelling of the muzzle may cause dyspnea. The disease in sheep is appropriately known as "bighead" or geeldikkop, a South African term meaning "thick, yellow head;" the equivalent term in New Zealand is "facial eczema." The lesions are *intensely pruritic*, causing rubbing, scratching and kicking at affected parts. Vesicles or bullae may develop. There is marked exudation and extensive necrosis. Affected skin becomes dry and sloughs in desiccated sheets (Fig. 5.54). Necrosis is frequently seen on the upper surfaces of the ears

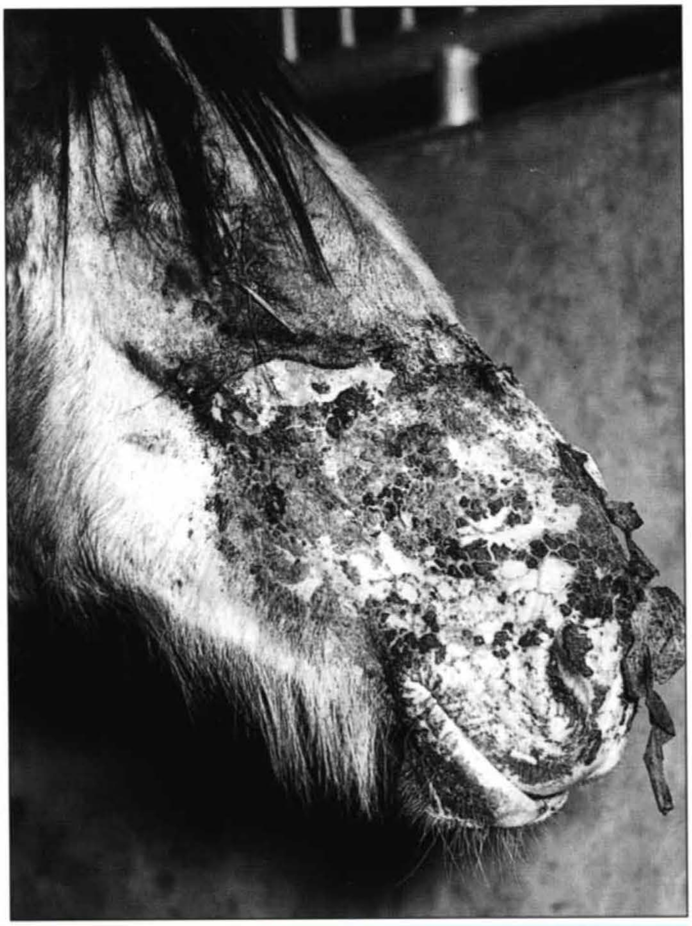

Figure 5.54 Photosensitization in a horse. Note necrosis and sloughing of skin from white areas of the face.

of sheep; the tips typically curl upwards as a result of mummification or may slough entirely. There is swelling of the eyelids and excessive lacrimation. Among the more obscure manifestations of photosensitization is the convulsive reaction of some sheep and cattle, photosensitized by ingestion of St. John's wort, on contact with cold water. *Icterus* typically is associated with hepatogenous photosensitization, but hepatogenous photosensitization may occur in its absence. Economic losses in livestock can be severe due to damaged hides, weight loss, fly-strikes, secondary infections, and reluctance of animals to let the young nurse damaged udders. In severe episodes of photosensitization, animals may die. This is more often the result of concomitant damage to other organs, particularly the liver, than to cutaneous damage alone. Injury to erythrocytes in cutaneous circulation may produce severe hemolysis.

Histologic lesions mirror the gross lesions with *coagulative necrosis of the epidermis* and possibly the follicular epithelium, adnexal glands, and superficial dermis. Subepidermal clefts or vesicles form and the *dermis is edematous*. Endothelial cells of the superficial, mid, and occasionally deep dermal vessels are often swollen or necrotic. Fibrinoid degeneration of vessel walls and thrombosis may be present. Initially, inflammation is sparse but soon the lesions are infiltrated by neutrophils. Secondary bacterial colonization is common.

Differential diagnosis should include other vesicular or necrotizing dermatopathies, including chemical or thermal burns. *Establishing the diagnosis is dependent upon anatomic distribution of lesions in nonpigmented, poorly haired, sun-exposed regions*. Lesions limited to areas of contact such as the extremities, ventrum or muzzle suggests the presence of a contact photosensitizing agent. Multiple affected animals in a herd suggest exposure to a photosensitizing agent rather than a photoallergic reaction. Types I, II, and III photosensitivities can be differentiated by signalment and concurrent clinical signs, such as evidence of liver disease combined with examination of pastures and feedstuffs, and investigating photodynamic drug or chemical exposure. The *Candida albicans* inhibition assay is a simple, inexpensive, quantitative, and relatively rapid assay for screening plants and feedstuffs for potential primary contact or systemic photosensitizers. The test does not detect all phototoxins. Thin-layer chromatography techniques may be useful in identifying phototoxic compounds. Analysis of suspect vegetation for the identification and quantification of fungal spores may be needed to establish fungal organisms and associated mycotoxins as contributing factors.

Primary photosensitization (type I photosensitization)

Plants are the most common cause of primary photosensitization; hence herbivores are most commonly affected. Additional sources include *mycotoxins, molds, chemicals and drugs. The majority of photosensitizing plants contain pigments belonging to either the helianthrone or furocoumarin family of pigments.*

The **helianthrones** include the red fluorescent pigments, *hypericin and fagopyrin*. Some of the most commonly implicated plants include St. John's wort (*Hypericum perforatum*) and buckwheat (*Fagopyrum* spp.), and the resulting diseases are referred to as *hypericism* and *fagopyrism*, respectively. Photosensitization induced by St. John's wort affects horses, cattle, sheep and goats. Hypericin is present at all stages of plant growth but significant amounts are consumed by livestock only when the plant is prolific or other feed is scarce. Other related plants that can lead to hypericism include goatweed, Tipton weed, amber, cammock, and Klamath weed. Photosensitization induced by buckwheat affects sheep, pigs, cattle, goats, and horses.

The **furocoumarin** family of pigments contains the photodynamic agents, *psoralens*. Photosensitization occurs in cattle, sheep, white chickens, and ducks as a result of ingestion of plants such as spring parsley (*Cymopterus watsonii*), bishop's weed *(Ammi majus)* and Dutchman's breeches *(Thamnosma texana)*. The furocoumarins differ from the helianthrone photosensitizing pigments by inducing, additionally, *corneal edema and keratoconjunctivitis*. Primary photodermatitis of pigs in Argentina occurred after consumption of feed contaminated with *Ammi majus* seeds containing the furocoumarin, xanthotoxin. Furocoumarins have also been documented to form *phytoallexins* in fungus-infected parsnips (*Pastinaca sativa*) and celery (*Apium graveolens*), leading to **phytophotocontact dermatitis**. *Psoralens adsorbed onto the skin react with UV light*. These have been associated with phytophotocontact dermatitis in pigs in New Zealand. Lesions were vesicular, affecting only the dorsal aspect of the snout. Rubbing the snouts and feet of white pigs with the leaves of the fungus-infected celery and parsnips before exposing the areas to UV light reproduced the lesions. *Cymopterus watsoni* causes phytophotodermatitis in sheep in Utah and Nevada. Lesions principally affected the non-wooled areas, such as the muzzle, lips and udder. High lamb mortality may be incurred from mismothering. Contact photodermatitis suspected to be caused by giant hogweed (*Heracleum* *mantegazzianum*) occurred in dogs. Giant hogweed contains psoralens and has been documented to cause photodermatitis in man, ducks, and goats.

Texas cattle and deer develop primary photosensitization after consuming moldy leaves of *Cooperia pedunculata*. In addition to skin lesions, keratitis occurs frequently and may lead to blindness.

Ingestion of alsike clover (*Trifolium hybridum*), also known as red clover hay, results in a primary photodermatitis referred to as *trifoliosis* in cattle, sheep, hogs, and some horses. Trifoliosis has been reported in the USA, Canada, Australia, and England. A second syndrome, referred to as *alsike clover poisoning*, is characterized by hepatic dysfunction and photodermatitis and has only been reported in the horse. The toxic mechanism is not known but is thought to be due to a toxin within the plant itself or to the presence of a mycotoxin. The variable presentations are speculated to be related to seasonal changes, stage of plant growth, soil and environmental conditions.

A condition clinically and histologically compatible with a primary photodermatitis occurred in 12 of 30 Harrier Hounds in a kennel in New Zealand. Lesions were limited to the nonpigmented, sun-exposed skin of the tri-colored hounds and resolved within a short period of time with supportive care. The hounds were fed a diet of horse and cattle meat. Although an ingested compound was suspected as the cause, no phototoxin could be identified. Similar cases have been reported in Foxhounds in England and Border Collie dogs in New Zealand.

Phenothiazine photosensitization is characterized by typical cutaneous lesions and in ruminants by the additional lesions of corneal edema and keratoconjunctivitis. The secretion of the ruminal metabolite, phenothiazine sulfoxide, in tears and aqueous humor, has explained the unusual location of the lesions. Phenothiazine photosensitization occurs most commonly in *calves* but also in *sheep, swine, and birds*. Pigs develop cutaneous lesions more frequently than sheep or cattle, probably because the activating radiation is more able to penetrate the integument. The greater susceptibility of calves has been ascribed to a relatively inefficient conversion of the photodynamic sulfoxide metabolite back to phenothiazine in the liver. This conversion depends on effective mixed-function oxidase enzyme activity in the liver.

Photosensitization due to defective pigment synthesis (type II photosensitization)

Photosensitization due to *endogenous pigment accumulation* is the result of a congenital enzyme deficiency causing abnormal heme synthesis with the resultant blood and tissue accumulation of photodynamic agents such as *uroporphyrin I, coproporphyrin I and protoporphyrin III*.

Bovine congenital hematopoietic porphyria *is the result of a deficiency in uroporphyrinogen III cosynthetase*, a key enzyme in heme biosynthesis. The condition is inherited as a simple recessive trait affecting many breeds including Shorthorn, Ayrshire, Holstein and Jamaican cattle. It has also been reported in crossbred cattle. The disease is known as "osteohemochromatosis" and "pink tooth," both suggested by the *red-brown coloration of porphyrin pigments in dentin and bone*. The pigment is also deposited in other tissues but the discoloration may be obvious only in lungs, spleen, and kidney in which it is deposited in the interstitial tissue and tubular epithelium. The pigments are *excreted in the urine*; hence the alternative names "porphyrinuria" and "hematoporphyrinuria." Affected urine is amber to brown, darkens on exposure to light, and fluoresces bright red on exposure to ultraviolet radiation. *Affected teeth and bones also fluoresce.* The anemia of bovine congenital erythropoietic porphyria is discussed in Vol. 3, Hematopoietic system.

The cutaneous lesions result from the photodynamic properties of the accumulated porphyrins, in particular the *uroporphyrins* that absorb UVA radiation. Reactive oxygen species directly induced by the porphyrins or, possibly, via activation of xanthine oxidase in the skin are responsible for the cell membrane damage. The gross cutaneous lesions are typical of photosensitization. The microscopic lesions closely resemble those of erythropoietic porphyrias in man. The chief lesions are subepidermal clefts, hyalinization of dermal capillary walls, and a minimal infiltrate of inflammatory cells. The basement membrane zone lines the base of the subepidermal cleft, in some instances covering small projections of dermal papillae (festoons). Festoons are a more prominent feature of the human lesion since dermal papillae are better developed in human skin.

Bovine erythropoietic protoporphyria is inherited as a *recessive trait in Limousin cattle* in the United States. It differs from bovine congenital porphyria in that photodermatitis is the sole clinical manifestation of the disease. Animals do not have discolored teeth, anemia or urine porphyrin excretion. The enzyme defect is a *deficiency of ferrochelatase*, which allows protoporphyrin IX to accumulate in blood and tissues. Heterozygotes have reduced (50%) ferrochelatase activity and are clinically normal.

Porphyria of **swine** is inherited as a *dominant characteristic.* Although it mimics certain aspects of bovine erythropoietic porphyria, *photosensitization does not occur*, even in white-skinned animals. The defect in porcine porphyria is not known.

Photosensitization occurs in Siamese **cats** with excessive accumulation of uroporphyrinogen I, coproporphyrinogen I and protoporphyrins in blood, urine, feces and tissues. The defect is presumably a *deficiency of uroporphyrinogen cosynthetase III*, as has been established in humans and cattle.

Hepatogenous photosensitization (type III photosensitization)

The most common form of photosensitization in domestic animals occurs in conjunction with primary hepatocellular damage or, less commonly, bile duct obstruction and is due to impaired capacity of the liver to excrete the potent photodynamic agent, **phylloerythrin**. Phylloerythrin is a chlorophyll catabolite formed by microbial action in the intestinal tract and transported to the liver via the portal circulation. Hepatocytes assimilate the phylloerythrin and excrete it into the bile. One of the earliest signs of liver cell damage is a reduced ability to transport and excrete phylloerythrin. Mild renal tubular damage caused by some toxins may further inhibit the excretion of phylloerythrin. The circulating phylloerythrin accumulates in tissues including the skin. *Photodermatitis occurs provided the animal is on a chlorophyll-rich diet and is exposed to sufficient solar radiation of the appropriate wavelength.* High ambient solar radiation and lack of shade are contributing factors. Photosensitization tends to occur most often when the hepatic damage is generalized, even if mild. Severe focal necrotizing lesions of the liver generally do not cause photosensitization because there is enough hepatic reserve to remove the phylloerythrin from the circulation. The cause of hepatic damage may be a plant toxin, mycotoxin, infectious agent, or chemical.

Toxic plants and mycotoxins account for most cases of hepatogenous photosensitization. A few of the many plants implicated in hepatotoxic photosensitization include lantana (*Lantana camara*), bog asphodel (*Narthecium ossifragum*), *Tribulus terrestris*, *Agave lecheguilla*, *Nolina texana*, *Cymadothea trifolii*-infested clover, *Trifolium hybridum* ("alsike clover poisoning"), and *Panicum* spp. grasses such as kleingrass (*Panicum coloratum*). Kleingrass is a perennial grass forage crop with a toxic principle suspected to be a saponin. Cases of kleingrass induced-photosensitization are sporadic, potentially related to environmental conditions and have been reported in Australia, Africa, and Texas. Some plants work in combination; black sagebrush appears to precondition sheep to photosensitization caused ultimately by *Tetradymia* spp.

A number of reports of hepatogenous photosensitization in livestock cite a variety of common forage crops such as alfalfa hay or silage, winter wheat, Bermuda grass pasture, crab grass, oat stubble, or various clover pastures. In the majority of cases, the toxicity was preceded by unusual climatic conditions of drought, increased rainfall, or temperature variations. In many cases, a specific toxic compound cannot be identified. A plausible explanation is the establishment of optimum conditions for the production of mycotoxins, hepatotoxins or photodynamic agents in the damaged plant material.

Forages containing the mycotoxin, *sporidesmin*, from spores of *Pithomyces chartarum* cause **facial eczema**, an economically important hepatogenous photosensitization of sheep and cattle in Australia, New Zealand, South Africa, and the northwestern United States. *Geeldikkop*, a disease characterized by hepatogenous photosensitization, is associated with extensive losses among sheep and goats in South Africa. A hepatogenous photosensitization, secondary to a presumed genetic defect in phylloerythrin transport, has been reported in Corriedale lambs. Hepatogenous photosensitization is discussed in more detail in Vol. 2, Liver and biliary system.

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Photoaggravated dermatoses

In humans, several autoimmune dermatoses are exacerbated by exposure to UV light. These include pemphigus, lupus erythematosus, and bullous pemphigoid. A similar relationship has been proposed in the analogous canine diseases.

A poorly understood disease in the *horse*, **photoactivated vasculitis** affects only the white-haired extremities. The pathogenesis is not currently known. An immune-mediated vasculitis, in which immune complexes may be acting as photodynamic agents has been proposed. Percutaneous absorption of initiating agents has not been ruled out. Affected horses have normal liver function and no known exposure to photosensitizing compounds. In addition, lesions may be restricted to one white extremity, other white areas on the horse are unaffected and the lesions do not always regress with cessation of exposure to sunlight, all indicating the lesions are *not a form of photosensitization*. The lesions often affect the heels and must be differentiated from the other manifestations of the "greasy heel" complex. The acute lesions are well demarcated, erythematous, oozing and crusted; erosion and ulceration may occur and the affected limb may be edematous and painful. The chronic lesions are hyperkeratotic plaques. Histologically, there is intense dermal edema, vascular dilation and subtle small vessel leukocytoclastic vasculitis, affecting only the superficial plexus. Thrombi may be seen occasionally. The epidermis may be eroded or ulcerated but undergoes papillary hyperplasia over time.

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NUTRITIONAL DISEASES OF SKIN

The elasticity of the skin, the orderly maturation of the epidermis and the quality and luster of the horny appendages are an indication of the state of the health of the animal as a whole. This applies equally to nutritional diseases and to diseases of other causes. Many systemic diseases result in cutaneous changes. The general metabolic transformations that take place in the skin are not qualitatively different from those in other tissues but there are some quantitative differences, such as the high requirements and turnover of sulfur-containing amino acids in the elaboration of keratin. *In most metabolic disturbances and deficiencies of essential nutrients, whether from dietary lack, malabsorption, the action of antimetabolites, or the body's inability to properly absorb or utilize nutrients, changes will be reflected in the skin*. The molecular basis for these skin lesions is, however, poorly understood. There are only a few syndromes that occur naturally and are sufficiently clearly defined to warrant discussion here. A larger number can be produced experimentally. Cutaneous manifestations of systemic disease not known to be a result of a nutritional derangement are discussed in other, more appropriate sections of this chapter such as Endocrine diseases of skin and Cutaneous paraneoplastic syndromes.

Protein–calorie deficiency

Starvation or protein–calorie malnutrition results in changes in the skin, the first being the *disappearance of subcutaneous fat*. Even though water intake may not be restricted, there is reduced hydration of the connective tissues of the subcutis and dermis, and the skin wrinkles and loses its elasticity. As hair is 95% protein, *hair growth and keratinization can require up to 25–30% of an animal's daily protein requirement*. Protein deficiencies are rare but can occur in cats fed primarily food formulated for dogs, in young dogs fed a low-protein diet, or in animals with increased nutrient requirements.

An early sign of starvation is the development of a *dull, dry and often brittle hair coat*. Hypotrichosis develops as a thinning of the hair rather than baldness, and seasonal shedding may cease or be prolonged. Lesions may be symmetrical on the head and trunk and spread to the limbs. The skin may atrophy, or hyperkeratosis, hyperpigmentation, and possibly loss of hair pigmentation develop. In pigs, the skin often becomes hyperkeratotic and assumes a dirty, dry appearance and the hair becomes long and shaggy. The skin of a malnourished animal has an increased susceptibility to bacterial infection and parasitic infestations and their effects. Under-nutrition of the pregnant ewe, between 115–135 days of gestation, decreases the number of secondary wool follicles in the developing fetus. Most experimental work on the effects of nutrition on hair growth have been performed in sheep, the purpose being either to improve wool production or to investigate chemical methods of shearing. Changing sheep from a low-protein to a high-protein diet led to a 33% increase in fleece production, due mostly to an increase in the rate of mitosis in the hair bulb. Specific amino acid deficiencies have been investigated as potential replacements for mechanical shearing as wool production is highly dependant upon levels of certain amino acids, specifically cysteine.

The effects of starvation on other organs such as liver, pancreas, bone, and bone marrow are discussed elsewhere.

Fatty acid deficiency

Fatty acid deficiency may occur in all domestic species in association with general dietary deficiency, malabsorption or liver disease. Deficiencies may be evident in animals when the fat has either leached from a diet, has become rancid from improper or prolonged storage, or when the diet was formulated with low fat content to save cost. Animals on specially formulated low-fat diets for therapeutic reasons may develop signs of fatty acid deficiency and require specific types of supplementation. Animals on antioxidant-deficient diets may also develop signs of fatty acid deficiency. Cutaneous lesions take months to develop and begin with *diffuse scaling, loss of haircoat sheen, and alopecia*. The scaliness is initially dry but over months progresses to an oily, often pruritic stage. Otitis externa may be an accompanying lesion and the skin is susceptible to secondary bacterial and yeast infection. Histologic lesions include *epidermal hyperplasia, orthokeratotic or parakeratotic hyperkeratosis and hypergranulosis*. The pathologic mechanisms underlying the epidermal hyperproliferation are not well understood. Experimental studies demonstrated an increase in epidermal DNA synthesis and a decrease in prostaglandin E and F levels in the skin of essential fatty acid-deficient mice. The lower prostaglandin levels likely reflect a lack of precursor arachidonic acid. Deficiency of prostaglandin E_2 could influence epidermal cell kinetics through its effect on ratios of cyclic AMP to GMP. Supplementation of animal diets with balanced omega-6 and omega-3 fatty acids is thought to modulate arachidonic acid metabolism and subsequent production of leukotrienes and prostaglandins that in turn may influence epidermal turnover kinetics and the inflammatory response.

A seborrheic dermatosis in **cats** characterized by dry scaly skin and alopecia is responsive to fatty acid supplementation but is not likely to be the result of a true deficiency. Experimental essential fatty acid deprivation in cats produces dry, scaly coats. Diets containing linoleic acid and linolenic acids as the sole source of essential fatty acids also induce a seborrheic dermatosis. Cats are obligate carnivores, as they lack delta-6-desaturase, the enzyme responsible for converting these 18-carbon fatty acids to longer chain fatty acids. Arachidonic acid is an essential fatty acid for the cat.

Hypovitaminoses and vitamin-responsive dermatoses

Cutaneous lesions may occur as manifestations of deficiencies of *vitamins A, C, and E, riboflavin, pantothenic acid, biotin, and niacin.* Most of these are described in experimentally induced deficiencies. Many of the naturally occurring hypovitaminoses are probably not the result of a single vitamin deficiency but represent the cumulative effect of several inadequacies of the diet.

Vitamin A deficiency

Vitamin A is a fat-soluble vitamin belonging to a group of compounds referred to as *retinoids.*Vitamin A is involved in cellular growth and differentiation, as well as in visual processes and reproduction. Vitamin A has a controlling effect on epithelial differentiation. *Cutaneous lesions of vitamin A deficiency are squamous epithelial hyperkeratosis and squamous metaplasia of secretory epithelia.*Vitamin A oversupplementation is teratogenic and can lead to toxicity if liver storage capacity is exceeded. Signs are manifest primarily in the skeletal system and liver, or as malformations of the fetus in pregnant animals.

Hypovitaminosis A has been reported in all species of domestic animals, although many accounts are anecdotal. It may be secondary to dietary deficiency, decreased intestinal absorption, liver disease or toxicities such as chlorinated naphthalene toxicity of cattle. Bile and pancreatic enzymes are needed for dietary absorption of vitamin A. The cutaneous lesions of hypovitaminosis A in *cattle* are a marked scaling and crusting dermatitis; in the *pig*, follicular hyperkeratosis; and, in *cats*, scaling, follicular plugging and alopecia. A report in the *dog* indicated thickened, hyperpigmented skin with alopecia and follicular hyperkeratosis.

Vitamin A-responsive dermatoses occur in **dogs** and almost exclusively in the Cocker Spaniel. In one syndrome, Cocker Spaniels are predisposed, probably because of a congenital abnormality of epidermopoiesis and keratinization (see Seborrhea). The lesions include hyperkeratotic plaques, follicular plugging and the formation of keratin fronds. Ventral and lateral chest and abdomen are sites of predilection. There may be accompanying otitis externa and pyoderma. Histologically, *the predominant lesion is marked follicular keratosis* (Fig. 5.55). Vertically oriented keratin casts protrude from the follicular ostia. There is mild to moderate epidermal hyperplasia and surface hyperkeratosis of the basket weave type. Dermal inflammation is mild, mononuclear and perivascular, unless secondary bacterial infection intervenes to produce neutrophilic folliculitis and/or furunculosis. There are rare reports of vitamin A-responsive dermatoses in other breeds of dogs. These diseases are not vitamin A deficiencies per se, in that plasma levels of vitamin A are within the normal range. The fact that therapy is effective can be attributed more to the "normalizing" effect of vitamin A (and retinoids) on cellular differentiation in the epidermis. Differentials include primary or secondary seborrhea and late-stage sebaceous adenitis. Oral supplementation with vitamin A may be needed to confirm the diagnosis.

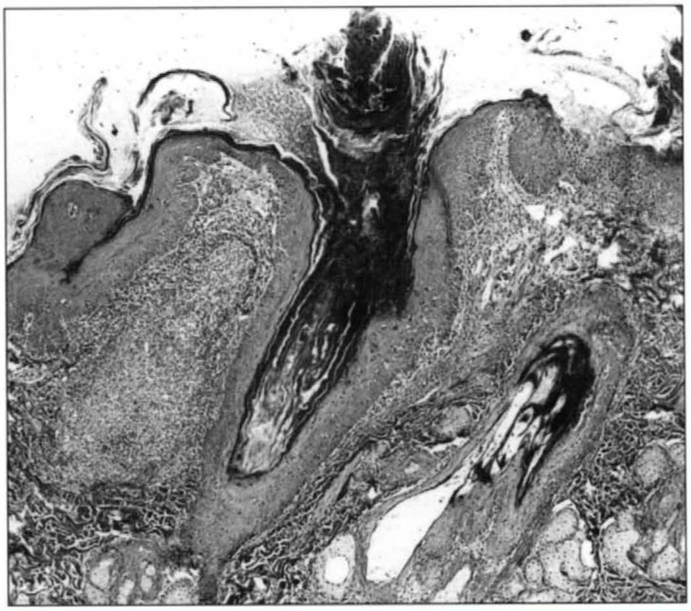

Figure 5.55 Vitamin A-responsive dermatosis. Note the marked follicular hyperkeratosis. (Courtesy of Parker WM, Hardy MH, and J Am Anim Hosp Assoc.)

Vitamin B deficiencies

The **B-vitamin complex** is essential to the maintenance and proper functioning of many important metabolic pathways. B vitamins are water soluble and not stored within the body, necessitating a constant dietary supply. These vitamins interact with each other, with vitamin C, and with fat-soluble vitamins. Single deficiencies of these vitamins are rare. *Deficiencies result in dry, flaky seborrhea with alopecia, anorexia, and weight loss.*

Riboflavin (vitamin B2) deficiency is mostly a problem in *swine and chickens* fed grain rations with borderline concentrations of the vitamin. Ruminants do not become deficient because of rumen synthesis of the B-complex vitamins. Young calves, however, may develop deficiency if deprived of milk or an appropriate replacer. Animals develop hyperemia around the lips, nose and buccal mucosa, diarrhea, weight loss and generalized alopecia. Cutaneous lesions described in hyporiboflavinosis include scaling and ulcerative dermatitis in the pig, erythema, scaling and dry hair coat on the ventral abdomen and hindlegs in the dog, and alopecia in the cat.

Pantothenic acid is a component of coenzyme A, an essential factor for entrance of acetic acid into the Krebs' cycle. Deficiency is documented in *pigs*, but may occur in dogs and calves. Pantothenic acid deficiency in pigs produces progressive alopecia with dermatitis and ulceration, in addition to the general effects of weight loss, diarrhea and neurologic signs. Young pre-ruminant calves may show dermatologic signs that include alopecia, a roughened coat and dermatitis. Leukotrichia has been described in dogs.

Biotin deficiency rarely occurs spontaneously, except in intensively reared *swine*, as the vitamin is widely distributed in feeds. Biotin is essential for proper utilization of fats, glucose, and amino acids. Feeding raw egg whites that contain avidin, a substance that renders biotin unavailable, may induce deficiencies. Cutaneous lesions reported in biotin-deficient pigs include *alopecia, pustular dermatitis and cracked hooves*, causing lameness and significant economic loss. Microscopic lesions include epidermal hyperplasia, orthokeratotic and parakeratotic hyperkeratosis, epidermal necrosis and pustules, and folliculitis. A deficiency attributed, in part, to a lack of vitamin B, probably biotin, was reported in *lambs* reared artificially on reconstituted cow's milk. Alopecia was largely due to a fleece in which individual fibers were thin, weak and straight. Histologically and ultrastructurally, the wool fibers had reduced numbers of cortical cells. Histochemical stains showed a delay in the incorporation and oxidation of sulfhydryl groups. Supplementation with B-group vitamins partially restored the fleece. Biotin deficiency reportedly causes a dry, brittle, hair coat, scaling and leukotrichia in *dogs*. Dogs deficient in biotin may develop periocular and facial alopecia resembling the clinical lesions of systemic lupus erythematosus, discoid lupus, or other dermatoses affecting the face.

Niacin is a component of the pyridine nucleotides NAD+ and NADP+ needed for the function of a number of enzymes involved in nutrient metabolism. Deficiency occurs spontaneously in animals fed diets low in pyridoxine (vitamin B6) and tryptophan as all of these components are needed for pyridine nucleotide synthesis. *Pigs* fed a low animal protein diet that is also high in corn renders niacin unavailable to the animal due to low tryptophan levels. Cutaneous lesions include alopecia and a crusting dermatitis. Niacin deficiency in *dogs* induces reddening and ulceration of the oral mucous membranes, resembling human hyponiacinosis, known as "*pellagra*." Reports of canine cases from the first quarter of the 20th century indicate severe necrotizing glossitis and stomatitis leading to the term "*black tongue*."

Pyridoxine (vitamin B_6) serves as coenzyme in amino acid and protein metabolism. Deficiency produced experimentally in *cats* resulted in a dull, unkempt coat with scaliness and alopecia of the head and extremities. Histologically, hair follicles were in telogen and there was epidermal and follicular hyperkeratosis. Since pyridoxine may be indirectly involved in zinc transport, through its effect on tryptophan metabolism, these effects might be due to alteration in the levels of zinc. Isoniazid has been reported to inactivate pyridoxine.

Vitamin C deficiency

Vitamin C (ascorbic acid) is required for the proper synthesis and structural maintenance of collagen and as a component of a number of essential enzymes. Almost all mammals synthesize vitamin C with the exception of humans, nonhuman primates, guinea pigs, Indian fruit bats, and Indian pipistrels. Insects, invertebrates, and fish also cannot synthesize vitamin C. *Deficiencies (scurvy or scorbutus) are limited to these species of animals*, and signs are related to the inability of fibroblasts to form collagen or osteoid and are discussed in Vol. 1, Bones and joints.

Vitamin E deficiency

Vitamin E deficiency is discussed primarily in Vol. 1, Bones and joints and Vol. 2, Liver and biliary system. Steatitis or "*yellow-fat disease*" involves subcutaneous fat and may be seen clinically as a skin disease. The yellowness of the fat is due to the *deposition of ceroid*. Vitamin E acts as an antioxidant to prevent lipid peroxidation. Dietary requirements vary by species and individual and with the other components of the diet. Naturally occurring vitamin E deficiency has not been reported in the dog.

Nutritional panniculitis occurs in *cats, mink, foals and swine*. The disease is associated with the feeding of *fishmeal, fish offal or other products with a high concentration of unsaturated fatty acids*. Diets with a high content of oily fish such as tuna, white fish and sardines are most often implicated, however, the condition in cats also has been associated with diets containing primarily liver, diets with only a small fish content, or improperly stored or outdated commercial cat food. The practice of feeding high-fish diets to cats is reported to be common in Greece. Destruction of vitamin E also occurs in food that, through improper processing or storage, becomes rancid. The disease is not, however, the result of a simple deficiency, since cats fed diets deficient in vitamin E but also lacking in unsaturated fatty acids, do not develop panniculitis. In *mink and foals*, it may be associated with degeneration of muscles, and in *swine* it may occur alone or be associated with any one or combination of ulceration of the squamous mucosa of the stomach, muscle degeneration, and hepatosis dietetica. The disease has variable mortality in *cats* after a short clinical course in which there is progressive depression, possible fever, hyperesthesia, reluctance to move, palpable thickening and increased firmness of the subcutaneous fat, easiest to detect in the inguinal region. The changes are not confined to the subcutaneous tissues but affect all fat depots. Biopsy reveals fat that varies from gray to lemon yellow to orange and is indurated and sometimes edematous. Hematological changes may include neutrophilic leukocytosis and anemia.

The initial histologic change is deposition of globules of ceroid in the interstitial tissue. This, together with a fishy odor, may be all that is observed in swine in which the fat is soft and gray rather than yellow. In most affected cats, *fat necrosis stimulates inflammation, which is initially neutrophilic but becomes granulomatous*. Numerous macrophages, and occasionally giant cells, ingest the ceroid pigment. Mineralization may be present. Ceroid may be found also in macrophages of the liver, spleen and lymph nodes. In cats, the initial differential should include infectious steatitis, sterile nodular panniculitis, lupus panniculitis, or other noncutaneous diseases such as feline infectious peritonitis, ascites of various causes, and other causes of hyperesthesia. *The clinical signs and the demonstration of ceroid, a variant of lipofuscin, which is acid fast and autofluorescent, should establish the diagnosis*.

Vitamin E-responsive dermatosis has been described in **goats**. Kids and adults, on a selenium-deficient diet, developed periorbital alopecia and generalized seborrhea. The haircoat was dull and brittle. Histologically, the lesions were orthokeratotic hyperkeratosis and mild superficial perivascular dermatitis, in which mononuclear cells predominated.

In **dogs**, a variety of conditions including dermatomyositis, acanthosis nigricans, demodicosis, and discoid lupus erythematosus are variably responsive to topical or systemic vitamin E therapy. Improvement in condition is thought to be due to the antioxidant properties of vitamin E leading to the stabilization of cell membranes and modulation of the inflammatory response through arachidonic acid and prostaglandin metabolism and actions on leukocytes.

Alopecia in **calves** associated with the feeding of milk substitute was attributed, in part, to vitamin E deficiency. The calves had low levels of serum vitamin E and the hair regrew after vitamin E therapy was initiated for concurrent nutritional myopathy.

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Mineral deficiency and mineral-responsive dermatoses

Iodine, cobalt, copper, and zinc deficiencies may lead to integumentary lesions. Iodine deficiency is discussed in Vol. 3, Endocrine glands. The effects of copper deficiency on wool and hair are referred to under Disorders of pigmentation. Cobalt deficiency causes a progressive, wasting disease in ruminants. There are nonspecific changes in the wool or hair coat including lack of growth and increased fragility.

Zinc deficiency

Zinc is an essential trace element. Zinc is a component of many important metalloenzymes and is a cofactor for many others. It exerts its primary effect through zinc-dependent enzymes that regulate RNA and DNA metabolism. Zinc thus plays a role in all metabolic processes involved with tissue growth, maturation and repair, and is involved in vitamin A metabolism and in enzymes needed for free radical scavenging. Zinc also modulates many aspects of the immune and inflammatory responses. The relationship between the changes in particular tissue enzyme activities brought about by zinc deficiency and clinical manifestations of the deficiency syndrome are not, however, well understood. In the integument, a substantial proportion of zinc is in the wool or the hair coat. Naturally occurring true zinc deficiency is extremely rare in cats, and not reported in horses, or dogs. On the other hand, zinc-responsive dermatoses are described in the dog in the following sections.

Zinc deficiency causes anorexia, alterations in food utilization, growth retardation, reproductive disorders, depression of the immune response, hematologic abnormalities, depression of central nervous system development, decreased wound healing, and keratinization defects in epidermis, hair, wool, and horny appendages. True zinc deficiency is of most significance in the pig.

Parakeratosis in swine

Zinc-responsive dermatosis in swine (parakeratosis) became an important clinical entity in the 1950s, coincident with and related to the widespread introduction of dry meal feeding. *The cause is not a simple deficiency.* The availability of dietary zinc is adversely affected by the presence of phytic acid in plant protein rations, a high concentration of calcium, a low concentration of free fatty acids, alterations in intestinal flora, and the presence of bacterial and viral enteric pathogens such as transmissible gastroenteritis virus. Zinc deficiency may induce secondary vitamin A deficiency as a result of its effect on appetite and food utilization. Economic losses are due to depression of growth rate, but, with improved management techniques, *parakeratosis is no longer a major problem.* Parakeratosis occurs in young, growing pigs, 2–4 months of age. The initial gross lesions are erythematous macules on the ventral abdomen and medial surface of the thigh. The lesions develop into papules, which become covered with a gray-brown, dry, roughened scale-crust that may reach 5–7 mm in thickness. Deep fissures penetrate the crust and are filled with brown-black detritus, which is composed of sebum, sweat, soil and other debris. These areas are susceptible to secondary bacterial infection, often leading to pyoderma or subcutaneous abscessation. *Lesions are roughly symmetrical and have a predilection for the lower limbs, particularly over the joints, around the eyes, ears, snout, scrotum and tail.* In severely affected animals, lesions may become generalized. Pruritus is not a feature of parakeratosis. The dorsal surface of the tongue is "furred" and the esophageal mucosa loses its normal smooth sheen and becomes dull and white.

The microscopic lesion in the skin is marked hyperplastic dermatitis with diffuse parakeratotic hyperkeratosis (Fig. 5.56A, B). The oral mucous membranes also demonstrate hyperplastic epithelium. Acanthosis, elongation of rete ridges, and mitotic figures in the basal keratinocytes are regular features of the hyperplastic response of the epidermis. The dermal lesions in uncomplicated parakeratosis include variable vasodilation and a mild to moderate, predominantly mononuclear cell, perivascular infiltrate. With bacterial infection, there may be nodular or diffuse neutrophilic dermatitis, folliculitis, perifolliculitis or furunculosis.

Parakeratosis in swine must be differentiated grossly from sarcoptic mange and exudative epidermitis. The former is usually intensely pruritic, and the latter usually occurs in a younger age group and the scale-crust is greasy rather than dry. Parakeratosis is rarely fatal unless toxemia or septicemia secondary to cutaneous bacterial infection develops or because of exacerbation of intercurrent infections such as pneumonia. Affected pigs recover rapidly upon zinc supplementation.

Zinc deficiency in ruminants

The clinical signs and gross lesions of zinc deficiency in ruminants are similar to those described in pigs. Hyperkeratosis also affects the forestomachs. Naturally occurring zinc-deficiency dermatoses are uncommon in **cattle**, but have been reported, for example, in grazing cattle from Guyana and housed dairy cows in Finland. The

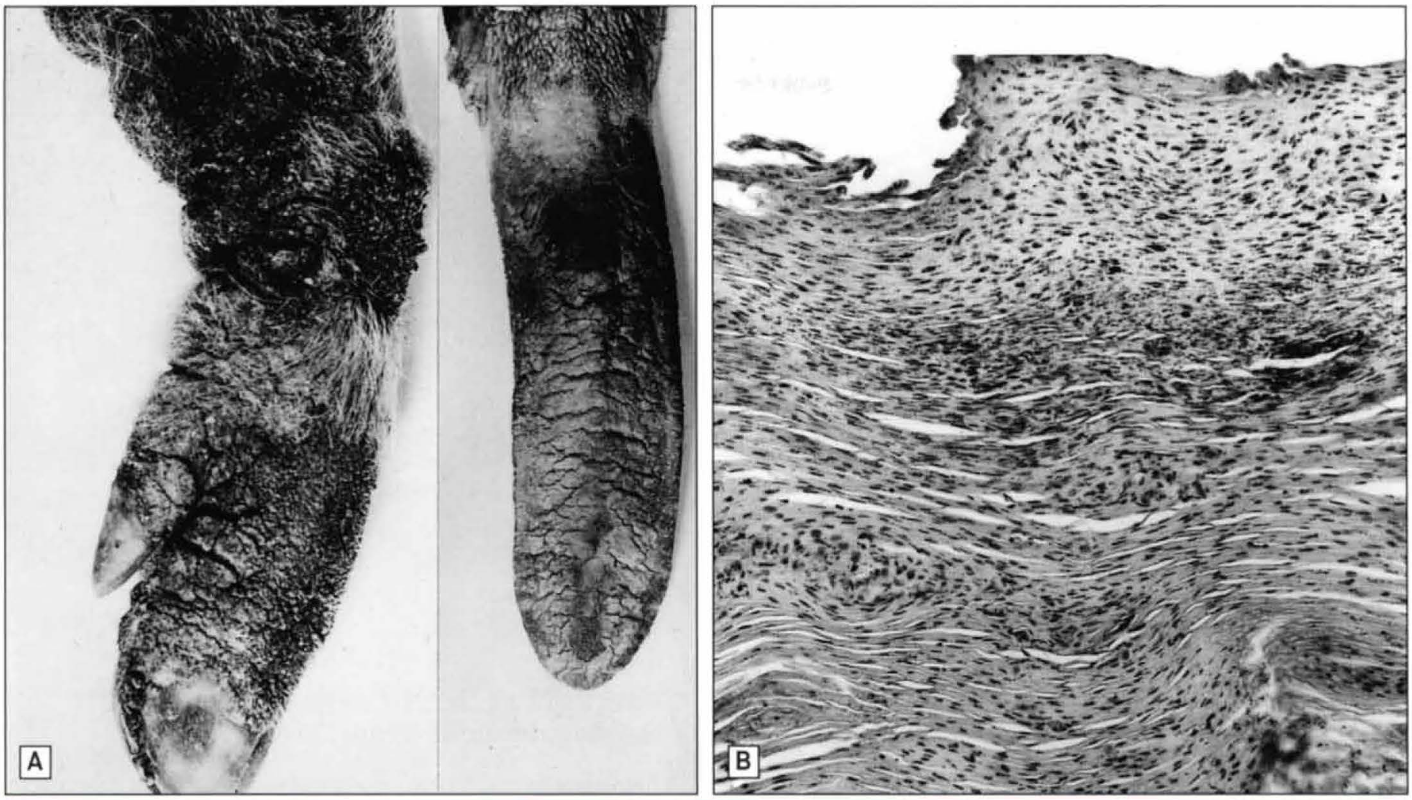

Figure 5.56 Zinc-responsive dermatosis in a pig. **A.** Marked hyperkeratosis of the skin of the foreleg and tongue epithelium. **B.** Marked parakeratotic hyperkeratosis.

hereditary zinc deficiencies of cattle are discussed under Congenital and hereditary diseases of skin.

In **sheep** with naturally occurring deficiency, histologically the hyperkeratosis is predominantly of the orthokeratotic rather than the parakeratotic type. Sheep also have thin, easily epilated, red-brown stained wool and loss of fleece. Wool eating and drooling are prominent clinical signs. Crusting and scaling lesions develop around the eyes, nose, hooves, and scrotum and on pressure points. The normal ringed structure of the horns is lost and the horns are shed. Abnormal growth of the hooves may lead to soreness and the adoption of a kyphotic stance.

Zinc-responsive dermatoses in **goats** are reported from the USA, Europe and Australia, sometimes associated with depressed reproductive efficiency. Experimental zinc deficiency of goats produces the typical weight loss, alopecia and parakeratotic scaling dermatosis described in other ruminant species.

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Canine zinc-responsive dermatoses

Naturally occurring zinc-responsive dermatoses in the dog fall under two syndromes:

- **Syndrome 1** *affects primarily Siberian Huskies and Alaskan Malamutes*, and rarely other breeds not of Arctic origin, such as Great Danes. The cutaneous lesions usually become manifest before the dogs are 1 yr of age. Older dogs may develop lesions during times of stress such as pregnancy, lactation or intercurrent disease. Lesions comprise *scaling and crusting dermatitis* with a predilection for the face, particularly around the eyes, lips and nose, pressure points and footpads (Fig. 5.57A). Lesions may be unilateral initially and progress to a bilateral distribution. Pruritus may be present. Secondary pyoderma is not uncommon. *The pathogenesis of the syndrome is not well established.* Alaskan Malamutes with chondrodysplasia have zinc-responsive spermatozoal defects and reduced zinc absorption from the intestinal tract; possibly malabsorption is responsible for the dermatological disease. Lifetime supplementation of zinc is necessary to alleviate signs.
- **Syndrome 2** *occurs in puppies of any breed and is associated with a relative deficiency of zinc, probably secondary to excessively high levels of calcium and/or phytates in the diet in rapidly growing animals.* In the USA, this disease has been associated with the feeding of *generic dog food*, in Britain and Sweden with the feeding of soy- and/or cereal-based diets. Recovery quickly follows restoration of a diet meeting the National Research Council standards for canine nutrition or zinc supplementation of cereal diets. Now that dog food manufacturers are aware of the problem, *the disease is less common.* The gross

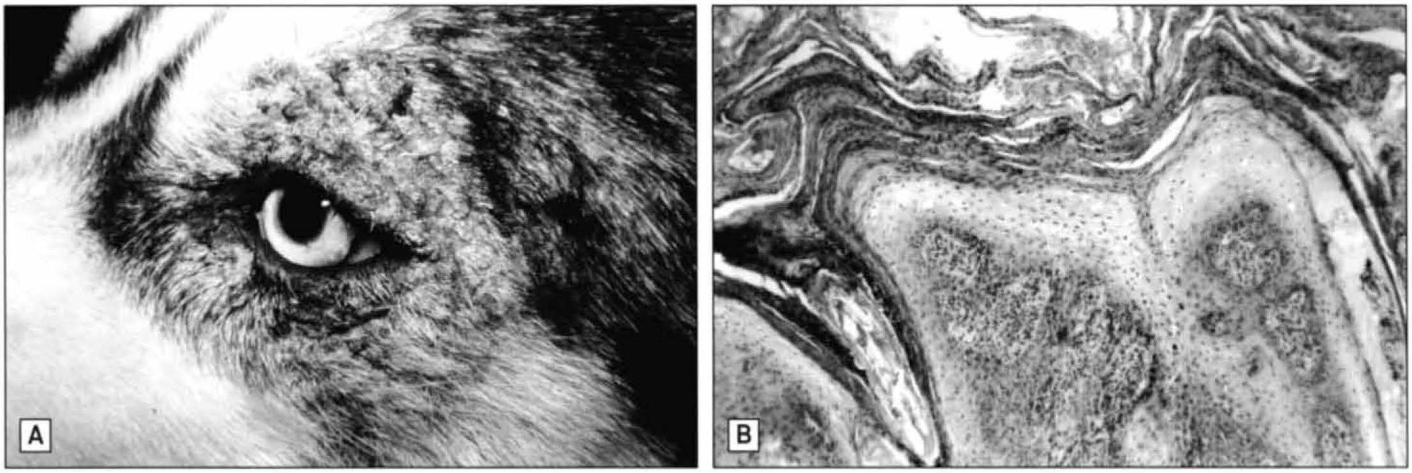

Figure 5.57 Zinc-responsive dermatitis in a dog. **A.** Scaling and crusting dermatitis of the face. (Courtesy of B Dunstan.) **B.** Papillary epidermal hyperplasia, marked epidermal edema and parakeratotic hyperkeratosis affecting the epithelium of the skin surface and follicular ostia.

lesions are multiple, scaling and crusted plaques, particularly affecting the muzzle, pressure points, distal extremities and trunk. There is extreme thickening and fissuring of the footpads and, sometimes, the planum nasale. Secondary pyoderma may develop and puppies often show a local lymphadenopathy.

The histologic lesions of both syndromes are usually typical of zinc deficiency, with papillary epidermal hyperplasia, marked spongiotic parakeratotic hyperkeratosis affecting the epithelium of the skin surface and follicular ostia, and multifocal neutrophilic crusts (Fig. 5.57B). Mild to moderate superficial perivascular mononuclear or eosinophil-rich dermatitis is present. Some reports indicate that the keratinization defect was sufficiently severe to induce dyskeratotic changes at all levels of the epidermis, while in others orthokeratotic hyperkeratosis alone was found.

Differential diagnoses include superficial necrolytic dermatitis, thallium toxicity and chronic hypersensitivity dermatitis that may look very similar histologically. Dermatophytosis, demodicosis, and pyoderma should be considered based upon gross lesions. *The signalment, history, lesion distribution, and pathologic changes should help to establish the diagnosis.* In some cases, response to dietary changes and/or zinc supplementation may be needed for confirmation of the suspected diagnosis. Serum and plasma zinc levels are not an accurate method of assessing zinc status. Values, even in a normal animal, are subject to marked variation according to sex, age, stress, concurrent disease, and collection methods. It is possible that dietary factors other than zinc are involved in some of these diseases, particularly those responding to dietary changes and not just to zinc supplementation.

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Superficial necrolytic dermatitis (hepatocutaneous syndrome)

A histologically distinct cutaneous reaction pattern characterized by the so called "red, white, and blue" *epidermal changes of parakeratosis, epidermal necrolysis or laminar intra-epidermal edema, and basilar hyperplasia* has been recognized most often in the *dog*, and occasionally in the *cat*. The condition resembles necrolytic migratory erythema, a human paraneoplastic syndrome most often associated with alpha cell neoplasms of the pancreas. *The pathogenesis of superficial necrolytic dermatitis (SND) is unknown, but hepatic dysfunction and derangement of glucose and amino acid metabolism are clearly involved.* Elevated glucagon levels alone are unlikely to be directly responsible for the skin lesions, as both dogs and humans may develop the disease in their absence and dermatitis is not an inevitable result of the hyperglucagonemic state. Hypoaminoacidemia, due to sustained gluconeogenesis and increased hepatic catabolism, is documented in both canine and human patients and it has been postulated that it may deplete epidermal proteins and induce epidermal necrosis. Zinc and fatty acid metabolism may also be deranged. *The most likely pathogenesis of abnormal or impaired ability to properly utilize nutrients and the clinical and histologic similarities of this entity to zinc-responsive dermatoses* warrant discussion of SND in this section.

In dogs, SND has been reported to occur in association with a variety of systemic diseases including *glucagon-secreting tumors of the pancreas, hyperglucagonemia, diabetes mellitus, and liver disease.* Over 90% of the reported canine cases are associated with *severe hepatopathy*. The variety of concurrent diseases and the histologic appearance have led to terms for the characteristic cutaneous reaction pattern in dogs. These include superficial necrolytic dermatitis, hepatocutaneous syndrome, diabetic dermatopathy, metabolic epidermal necrosis, and necrolytic migratory erythema. Diabetes and hyperglucagonemia in some cases could be secondary to the hepatic degeneration. A feline case was associated with a pancreatic carcinoma presumably of endocrine origin.

Lesions have a roughly bilaterally symmetrical distribution. The muzzle, lips, periocular skin, edges of the pinnae, distal extremities,

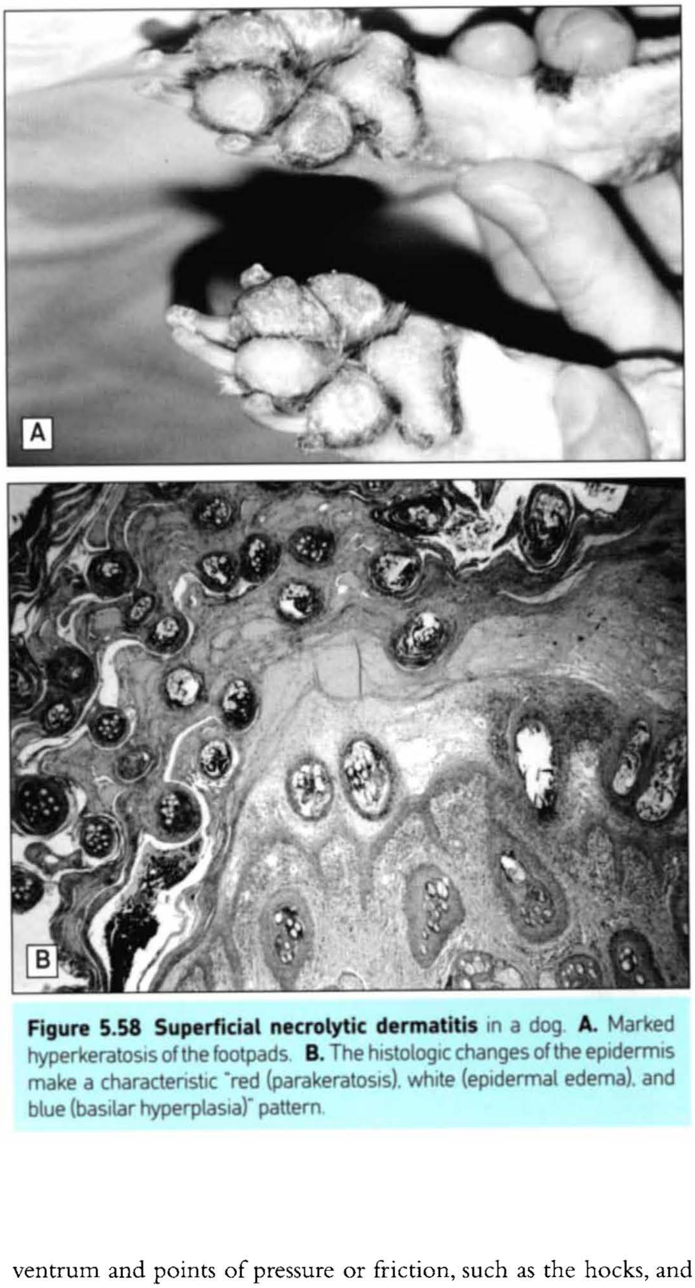

Figure 5.58 Superficial necrolytic dermatitis in a dog. **A.** Marked hyperkeratosis of the footpads. **B.** The histologic changes of the epidermis make a characteristic "red (parakeratosis), white (epidermal edema), and blue (basilar hyperplasia)" pattern.

ventrum and points of pressure or friction, such as the hocks, and the external genitalia are typically affected. Oral and mucocutaneous lesions are occasionally reported. Lesions are erythematous, erosive, ulcerative and crusted. The footpads are markedly hyperkeratotic (Fig. 5.58A). Footpad lesions were not a feature of SND in the cat. *The histologic lesions of the "red, white and blue" epidermis are virtually pathognomonic - the distinctive feature is a band of hydropic, pale-staining keratinocytes in the upper half of a usually acanthotic stratum spinosum* (Fig. 5.58B). Both intra- and intercellular edema contribute to the epidermal pallor. As these cells degenerate, clefts and vesicles may form in the outer stratum spinosum. Neutrophils may accumulate to form subcorneal pustules. The stratum corneum is diffusely and markedly parakeratotic and appears hypereosinophilic in comparison to the subjacent pale-staining stratum spinosum of the epidermis. The epithelium of the follicular infundibulum can also be affected. The basal cell layer is basophilic and hyperplastic, forming small rete ridges. Dermal inflammation is minimal, and predominantly mononuclear and perivascular. In eroded lesions, neutrophilic exocytosis is prominent and inflammatory crust covers the surface.

In cases associated with liver disease, the most commonly associated gross lesion is a *nodular liver with intervening firm, collapsed parenchyma*. Histologically, there is loss of parenchyma and minimal inflammation. Hepatocytes are markedly vacuolated due partially to fatty degeneration. The nodules have been interpreted in some cases to represent regeneration and in others to be comprised of nodular remnants of atrophic hepatic parenchyma.

The diagnosis is based on typical histologic findings. However, the pathognomonic epidermal edema may not be present in every biopsy. *Differential diagnoses* include other parakeratotic, hyperplastic dermatitides, such as the zinc-responsive dermatoses, lethal acrodermatitis of bull terriers, and thallium toxicity. The clinical differential is the same as listed for zinc-responsive dermatoses. The prognosis is generally considered poor since most cases have advanced liver disease. Cases associated with a pancreatic neoplasm may have resolution of skin lesions following tumor excision.

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ENDOCRINE DISEASES OF SKIN

Endocrine disorders frequently manifest clinically as skin disease, but these dermatoses are rarely specific for any one endocrinopathy. While these dermatopathies are more common in dogs, they can occur in any species. **Clinical features** of many endocrine dermatoses include *a dry, coarse, brittle, dull, easily epilated haircoat that fails to regrow after clipping, hypotrichosis and hyperpigmentation, and alopecia that is frequently bilaterally symmetrical.* Secondary pyoderma and seborrhea are common. In addition, endocrine dermatoses share many **histologic features**, *including orthokeratotic hyperkeratosis, follicular keratosis, dilation of follicular ostia and infundibula, hair follicle atrophy, absence of hair shafts in follicles, increased numbers of telogen follicles, variably increased trichilemmal keratinization (flame follicles) of follicles, and epidermal hyperpigmentation.* These histologic changes suggest an endocrine dermatosis but frequently are not pathognomonic for a specific endocrinopathy. *A combination of clinical and histologic features together with clinical testing to demonstrate hormonal deficiency or excess is required for confirmation.*

Hypothyroidism

Hypothyroidism is the most common endocrine dermatopathy in **dogs**, and almost all cases are due to primary hypothyroidism. The most common cause is thought to be *lymphocytic thyroiditis*, an immune-mediated condition similar to Hashimoto's thyroiditis in humans. *Idiopathic thyroid atrophy* is also a common cause and may represent the end stage of lymphocytic thyroiditis. *Other causes* include developmental defects of the thyroid gland and rarely iodine deficiency. Pituitary neoplasia, or hypopituitarism, resulting in decreased secretion of TSH can cause secondary hypothyroidism. It is a disease of middle-aged dogs and there is no sex predilection, although the incidence is increased in neutered males and ovariohysterectomized females. Breeds that are predisposed include the Doberman Pinscher, Golden Retriever, Chow Chow, Great Dane, Irish Wolfhound, Boxer, English Bulldog, Dachshund, Afghan Hound, Newfoundland, Alaskan Malamute, Brittany Spaniel, Poodle, Irish Setter, and Miniature Schnauzer. *Clinical cutaneous changes* include secondary seborrhea, dry, coarse, brittle hair, hair that does not regrow after clipping, hypotrichosis with fine retained hairs, hyperpigmentation and alopecia that tends to develop first in areas of friction and can progress to become bilaterally symmetrical. Pruritus is not a feature unless there is secondary pyoderma or seborrhea. Histologic changes can be nonspecific and simply suggest an endocrinopathy. The most common changes are orthokeratotic hyperkeratosis with follicular keratosis, epidermal hyperplasia, and hair follicles in telogen or atrophied. Changes that are more specific but less common are myxedema, and dermal thickening.

Hypothyroidism occurs less frequently in other domestic animals, and usually in association with iodine deficiency and goiter. In **Merino sheep** and **Afrikander cattle**, a hereditary defect in the biosynthesis of thyroid hormone produces symmetric hypotrichosis and thick, myxedematous, wrinkled skin. In **goats**, hypothyroidism occurs in a mixed strain of Saanen and dwarf goats in association with a hereditary congenital thyroglobulin deficiency. Gross cutaneous changes include bilaterally symmetrical hypotrichosis and thick, myxedematous, scaly skin. Histologic findings include orthokeratotic hyperkeratosis, follicular keratosis, diffuse dermal mucinous degeneration (myxedema) and dermal thickening.

Hyperadrenocorticism

Hyperadrenocorticism is common in **dogs** and can be caused by bilateral adrenocortical hyperplasia resulting from a *pituitary tumor* (pituitary-dependent hyperadrenocorticism), a *neoplasm of the adrenal cortex* (usually unilateral), or exogenously administered glucocorticoids resulting in *iatrogenic hyperglucocorticism*. Of the naturally occurring forms, the pituitary-dependent form is considerably more common. The disease is seen most commonly in middle-aged or older dogs, and the Boxer, Boston Terrier, Dachshund, and Poodle are predisposed. Clinical cutaneous changes can include *bilaterally symmetric hypotrichosis or alopecia affecting primarily skin of the trunk, pendulous abdomen, thin skin that has decreased elasticity, slow wound healing, hyperpigmentation, telangiectasia, scaling, comedones, and calcinosis cutis* (Figs 5.59, 5.60A). Lesions of calcinosis cutis occur most commonly in the axilla, groin, or dorsal neck region and appear as erythematous papules that frequently ulcerate and exude a white, chalky material. Calcinosis cutis is almost always the result of iatrogenic hyperadrenocorticism. Other causes of mineral deposits in the skin are discussed under Physical injury to skin.

Histologically, canine hyperadrenocorticism is characterized by thin epidermal and follicular epithelium, which can be between 1–3 cell layers (Fig. 5.60B). If calcinosis cutis is present, the epidermis is frequently hyperplastic and can be ulcerated. Other changes can include orthokeratotic hyperkeratosis, marked follicular keratosis sometimes with comedone formation, thin dermis, hair follicles

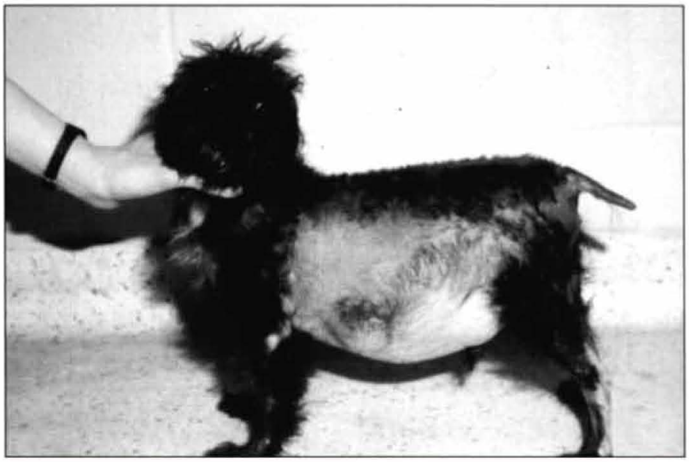

Figure 5.59 Hyperadrenocorticism in a dog. Note pendulous abdomen, truncal alopecia, and redistribution of adipose tissue. (Courtesy of B Dunstan.)

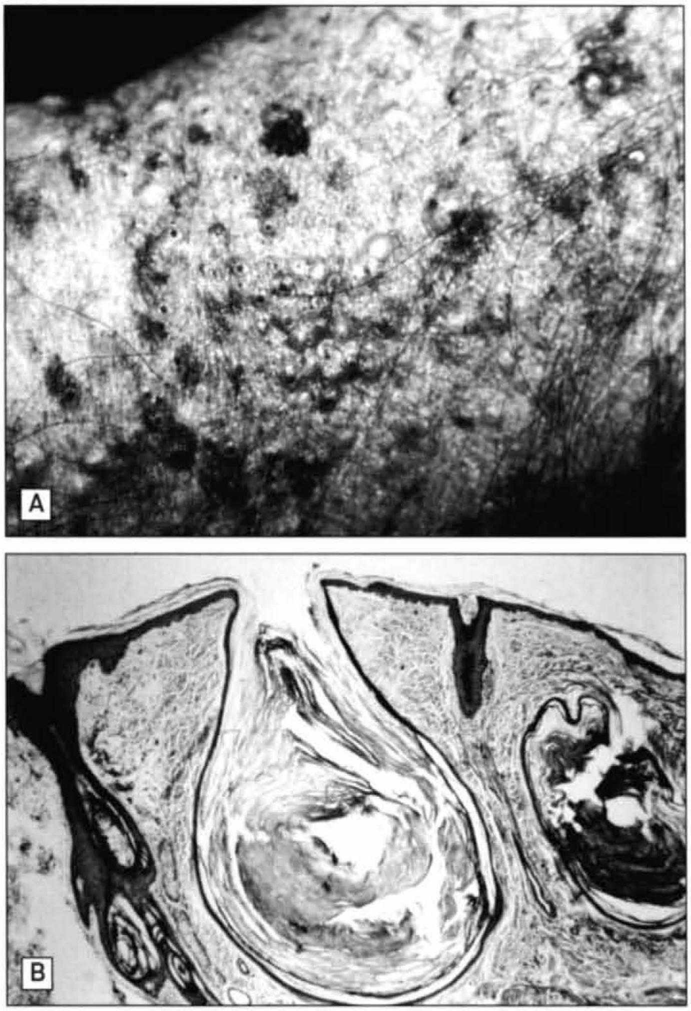

Figure 5.60 A. Comedones in a dog. Note gross appearance of distended follicles. (Courtesy of B Dunstan.) **B. Hyperadrenocorticism** in a dog. Note thin epidermis and hair follicles distended with keratin. (Courtesy of B Dunstan.)

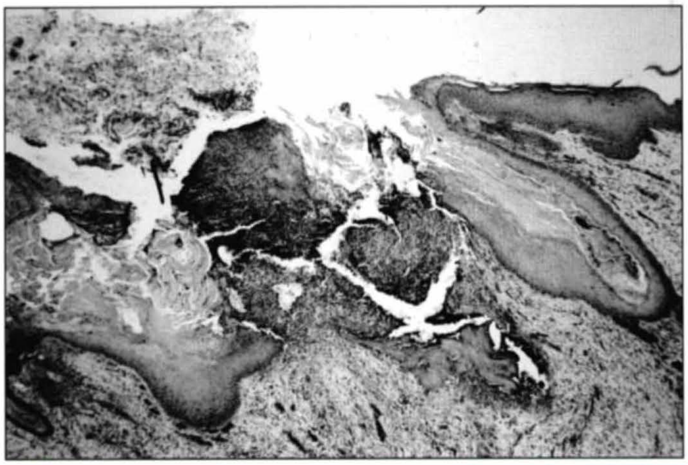

Figure 5.61 Calcinosis cutis in hyperadrenocorticism in a dog. The epidermis is hyperplastic. A large area of mineralized dermal collagen accompanied by granulomatous inflammation is undergoing transepidermal elimination.

that are frequently in telogen or catagen, sebaceous gland atrophy, and variable hyperpigmentation. Phlebectasia has been reported in dogs. *The presence of calcinosis cutis* (Fig. 5.61), *which can occasionally result in osseous metaplasia, is virtually pathognomonic for hyperadrenocorticism*, bearing in mind that mineralization of the external root sheath can be seen in normal old dogs, and in Poodles. Cutaneous histologic lesions of hyperadrenocorticism can occur focally at the site of topical glucocorticoid application.

Hyperadrenocorticism is considerably less common in **cats**. As in dogs, the condition is more commonly pituitary dependent than adrenal dependent or iatrogenic. The clinical presentation in cats is similar to that in the dog, however in addition there is often concurrent diabetes mellitus, and marked skin fragility and friability in which dermal collagen is reduced. This can result in tearing and lacerations of the skin (*feline skin fragility syndrome*). Histologic lesions in the cat are similar to those in the dog, however calcinosis cutis and telangiectasia have not been reported.

Hyperadrenocorticism occurs in **horses** and is almost always seen in association with *hypertrophy, adenomatous hyperplasia, or functional neoplasms of the pars intermedia of the pituitary*. The disease affects primarily aged horses and has no breed or sex predilection. Gross cutaneous changes may include a coarse, brittle, long, shaggy hair coat (hirsutism), an abnormal shedding pattern, episodic hyperhidrosis, poor wound healing, decreased muscle tone, weight loss, and susceptibility to secondary skin infections.

Hyposomatotropism and hypersomatotropism

Hyposomatotropism occurs in young **dogs** with *congenital pituitary dwarfism usually due to pituitary cysts*. In the German Shepherd Dog and Carnelian Bear Dog, pituitary dwarfism is thought to be inherited as a simple autosomal recessive condition and is usually associated with a cystic Rathke's cleft. The pituitary deficiency is often accompanied by deficiencies in thyroid, adrenal and sex hormones. The growth retardation due to the pituitary defect can be accompanied by a puppy coat that fails to shed and the development of endocrine alopecia with typical histologic changes.

Hypersomatotropism (*acromegaly*) is very rare in **dogs**. Excessive somatotropin (growth hormone) production is associated with administration of progestins or with the metestrus (luteal) phase of the estrous cycle in intact female dogs. Cutaneous changes include thick, folded, myxedematous skin, especially on the head, neck and distal extremities. The haircoat may be long and thick and the nails may exhibit rapid overgrowth. Histologic findings in canine hypersomatotropism include thickened dermis due to increased production of glycosaminoglycans and collagen by dermal fibroblasts. Myxedema is present in about a third of cases.

Hyperestrogenism

Hyperestrogenism can occur in male or female **dogs**. In middle-aged to older intact male dogs it is associated with *functional testicular neoplasms*, primarily Sertoli cell neoplasms, and occasionally functional interstitial cell tumors and seminomas. The Boxer, Shetland Sheepdog, Cairn Terrier, Pekingese, Collie and Weimaraners are predisposed. Hyperestrogenism is also seen in middle-aged to older intact female dogs with *polycystic ovaries or functional ovarian neoplasms*. It can also occur in male or female dogs following estrogen administration.

Gross cutaneous changes include *symmetrical hypotrichosis or alopecia*, which typically originates at the perineum and genital region and progresses cranially on the trunk. The hair is dry and dull, is easily epilated, can fail to regrow after clipping, and can be accompanied by hyperpigmentation, especially macular pigmentation. Male dogs may develop a pendulous prepuce, gynecomastia, or prostatomegaly with squamous metaplasia of the prostatic ducts. Females may develop an enlarged vulva. Histologic changes include hair follicles that are primarily in telogen, orthokeratotic hyperkeratosis, and follicular keratosis.

Alopecia X

Growth hormone/castration-responsive dermatosis has many synonyms, including hyposomatotropism of the adult dog, sex-hormone alopecia, pseudo-Cushing's syndrome, testosterone-responsive dermatosis, estrogen-responsive dermatosis, congenital adrenal hyperplasia like-syndrome. This diversity in nomenclature reflects the differences in endocrine values and responses to various treatments and the fact that the pathogenesis of this condition has not been fully characterized. *To simplify the nomenclature, the condition is now being referred to as* **alopecia X**. These dogs have normal thyroid function and adrenal function, and most theories involve a deficiency or imbalance in sex hormones and/or growth hormone deficiency. There may be partial deficiency of 21-hydroxylase or other adrenocortical enzymes necessary for adrenal steroidogenesis.

Alopecia X occurs most often in *plush-coated Nordic breeds*, such as the Pomeranian, Keeshond, Chow Chow, Samoyed, Siberian Husky, Alaskan Malamute, Norwegian Elkhound, American Eskimo Dog, and occasionally in other breeds. Affected dogs have *symmetrical alopecia* of the trunk, perineum, caudal thighs, and neck, sparing the head and distal extremities (Fig. 5.62). Hyperpigmentation is common. Alopecia X usually develops between one and two years of age and either sex can be affected. Initial histologic changes are characterized by *follicular atrophy*; trichilemmal keratinization in primary hairs can be quite prominent. Caution is warranted in making the diagnosis of

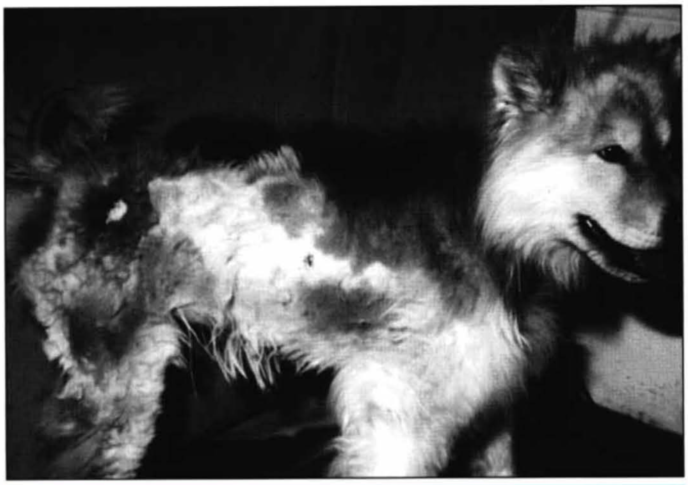

Figure 5.62 Alopecia X in a dog. (Courtesy of B Dunstan.)

Alopecia X based exclusively on this feature as normal primary hair follicles of Nordic breeds can have increased trichilemmal keratinization.

Canine recurrent flank alopecia

Canine recurrent flank alopecia (seasonal flank alopecia, cyclical flank alopecia) is a condition seen most commonly in the Boxer, English Bulldog, Airedale Terrier, Schnauzer, and Griffon Korthal, but can occur in many breeds. It is characterized by alopecia of the skin of the flank that is usually bilaterally symmetrical, rarely unilateral, and occurs recurrently or seasonally. The first episode occurs at approximately 4 years of age but can be variable. In the northern hemisphere, the onset of alopecia is usually between November and March. There is spontaneous hair regrowth after 3–8 months, however occasional dogs have progressively less hair regrowth after each episode. Rare dogs fail to regrow hair after the first episode; and some dogs have one episode of alopecia that never recurs.

Clinically, the bilaterally symmetrical lesions are usually confined to the thoracolumbar regions, have well demarcated margins with abrupt transition from affected to unaffected skin, and are usually hyperpigmented. Histologic changes are those of *non-inflammatory, non-scarring follicular atrophy*. Follicular infundibula are dilated and filled with keratin that can extend into the openings of the primary and secondary atrophic follicles, giving the appearance of an inverted footprint over the remnants of the follicular epithelium (Fig. 5.63).

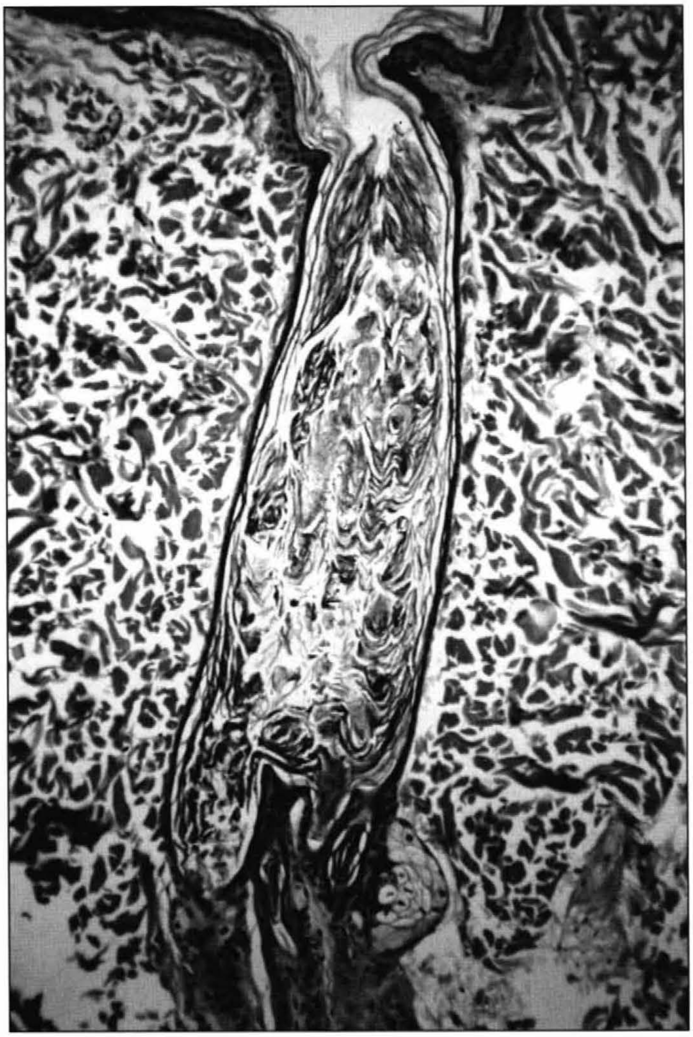

Figure 5.63 Recurrent flank alopecia in a dog. Follicular infundibula are dilated and filled with keratin that extends into the openings of the primary and secondary atrophic follicles, giving the appearance of an inverted footprint. (Courtesy of B Dunstan.)

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IMMUNE-MEDIATED DERMATOSES

Hypersensitivity dermatoses

Contact with exogenous antigens usually leads to induction of a protective response, but when the immune response causes damage to tissues, it is called *hypersensitivity or allergy*. Compounds such as dust, pollens, food, drugs, microbiologic agents, insect components, and various chemicals contain antigens that are normally innocuous but may induce allergic reactions in predisposed individuals. Most cutaneous hypersensitivity reactions are mediated by type I (immediate) hypersensitivity, type IV (cell-mediated or delayed) hypersensitivity, or by a combination of the two types. Hypersensitivity reactions cause a variety of dermatoses that range from annoying and uncomfortable to severely debilitating or life-threatening. Allergic dermatoses are common and important in dogs, cats, and horses and are rarely reported in food animals.

Urticaria and angioedema

Urticaria (hives, heat bumps) and **angioedema** (angioneurotic edema) *are variably pruritic, edematous skin lesions produced by mediators released by basophils and dermal mast cells.* Urticaria and angioedema are most common in horses, uncommon in dogs, and rare in ruminants, pigs, and cats. A wide variety of immunologic and nonimmunologic causes have been implicated, but frequently the specific causative agent cannot be determined for a particular individual. Immunologic causes of urticaria/angioedema are thought to involve type I hypersensitivity reactions primarily; type III hypersensitivity is involved occasionally. *Recognized initiators* in all species include foods and food additives, drugs, biologic agents, stinging and biting arthropods, intestinal parasites, inhalant and contact allergens, and bacterial, fungal, and viral infections. *Nonimmunologic factors* associated with urticaria/angioedema include physical factors such as heat, cold, or pressure; mast cell degranulating agents such as radiocontrast media; and agents that result in perturbation of arachidonic acid metabolism. Deficiency of C1 esterase inhibitor is a genetic cause of chronic urticaria in humans. Aspirin and other nonsteroidal anti-inflammatory drugs, psychological stress, and concurrent febrile illness may be *exacerbating factors*, if not causative ones, in humans with chronic urticaria. Another cause of chronic urticaria identified in humans is IgG autoantibody directed against the IgE receptor of cutaneous mast cells and circulating basophils. Binding of the autoantibody to this receptor induces release of histamine. Irrespective of cause, the *final common pathway is increased vascular permeability and resultant edema produced by histamine, the major mediator, and possibly also by kinins, eicosanoids, and neuropeptides.*

Drugs are probably the most frequent cause of urticaria in **horses**. A wide variety of systemic drugs and biological products have been implicated in initiating urticaria. *Wheals* frequently develop in minutes to hours after exposure to the offending drug and usually subside within several hours. *Cholinergic or heat-reflex urticaria*, induced by exercise or a hot bath, has been reported in a horse. Pruritus in this horse appeared to be exacerbated by pelleted feed. An unusual form of urticaria called *dermatographism*, which is induced by blunt pressure to the skin, has also been described in the horse. In some instances, urticaria is thought to be caused by *overfeeding of grains*, especially those high in protein content ("protein bumps," "feed bumps"). Lesions occur anywhere on the body but are most common on the face, neck, and thorax. In **cattle**, a unique form of urticaria has been described in high-producing dairy cows, especially Jerseys and Guernseys, that become sensitized to casein in their own milk (*milk allergy*). Foods, drugs, biologic agents, and venomous stings are reported most frequently as causes of urticaria and angioedema in **dogs** and **cats**.

Urticaria is characterized by *wheals (hives), which are discrete, well-circumscribed, erythematous, edematous plaques with a flat-top and steep sides* (Fig. 5.64). They are cool and pit upon digital pressure. They vary in size and may coalesce to measure many centimeters in diameter. Wheals are usually round; but in some instances they assume bizarre and irregular serpentine shapes. The overlying hair may stand erect, giving the impression of a follicular disease. The lesions may be localized to a single body region, e.g., lateral neck, head, or thorax, or they may involve the entire body. The individual lesions usually last less than 12–24 hours and disappear leaving no residual skin changes unless pruritus results in self-mutilation. Although individual lesions are transient, new ones may erupt over a period of days or weeks. *Angioedema consists of larger, less well demarcated swellings* that originate subcutaneously. With time, the swellings may gravitate ventrally. Angioedema is a potentially serious condition since involvement of perilaryngeal tissues may cause asphyxiation. Approximately 50% of affected humans develop both lesions concurrently but development of both wheals and angioedema does not seem to be as common in animals.

Microscopic lesions are variable and nonspecific. The epidermis is usually normal in nontraumatized lesions. Urticaria is characterized by *dermal edema,* which is visualized as widening of spaces between collagen fibers in the superficial and middle dermis. The change may be very subtle and can be missed. Edema is typically more severe and involves the deep dermis and subcutaneous tissue in angioedema. Small vessels are congested and lymphatics are dilated. Inflammation is inconsistent. When present, it usually consists of perivascular granulocytes, mast cells, and fewer lymphocytes and macrophages. The reaction involves the upper and middle dermis in urticaria and the deep dermis and subcutis in angioedema. Vasculitis is not a typical feature of urticaria and angioedema.

Diagnosis is usually based on the history and appearance and transitory nature of the clinical lesions. Biopsy is performed to rule out other conditions when the lesions are recurrent or chronic and the diagnosis is uncertain. Identification of a particular inciting

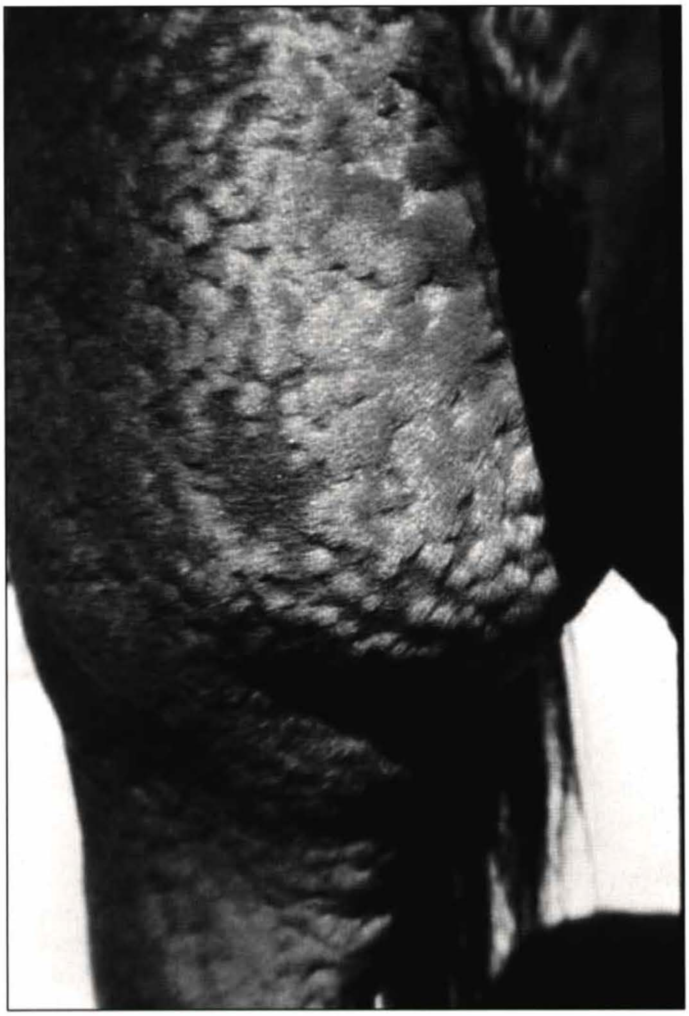

Figure 5.64 Urticaria in a horse. Discrete, well-circumscribed, erythematous, edematous plaques with a flat-top and steep sides. (Courtesy of B Dunstan.)

cause relies on history and a combination of elimination trials, environmental alterations, intradermal skin testing, and insect control measures.

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Atopic dermatitis

Atopy is an inherited tendency to produce IgE antibodies and develop clinical allergy to pollens and other innocuous environmental antigens. Clinical disease can involve the respiratory tract, and consist of allergic rhinitis and asthma, or the skin, and manifest as atopic dermatitis. In animals, atopic dermatitis is much more common than the respiratory manifestations of atopy. Allergic rhinitis occurs in cattle, however.

Atopic dermatitis is a multifactorial skin disease in which there is a complex interrelationship between genetic, environmental, skin barrier, pharmacologic, psychologic, and immunologic factors that contribute to the development and severity of the disease. Although clearly a *genetic disorder*, the genetic mapping of atopic dermatitis is not simple and more than one gene appears to be involved. The *pathogenesis* of atopic dermatitis is incompletely understood but it is thought to be caused by an *underlying immunoregulatory imbalance* producing activation of a subpopulation of T helper type 2 lymphocytes (Th2 cells) that release cytokines important to the pathogenesis of atopic dermatitis. Th2 cells produce IL-4, IL-5, and IL-13 and are associated with IgE-mediated reactions. IL-4 is a critical cytokine in the development of allergic diseases. It signals T cells to become Th2 cells, which are the major source of IL-4 production, thereby initiating a self-amplifying process. IL-4 induces expression of IgE receptors on Langerhans cells, resulting in enhanced antigen presentation capability. IL-4 and IL-13 induce the antibody isotype switch from IgM to IgE. They also up-regulate expression of vascular cell adhesion molecule–1 (VCAM-1), an adhesion molecule expressed by cutaneous endothelial cells and involved in migration of eosinophils and mononuclear cells into sites of allergic inflammation. IL-5 promotes differentiation, vascular endothelial adhesion, and survival of eosinophils. In contrast, Th1 cells produce IFN-γ, which inhibits IgE synthesis and differentiation of Th2 cells. The balance between Th1 and Th2 cells may be modulated by biochemical defects in monocytes. Peripheral blood mononuclear cells of individuals with atopic dermatitis have been shown to have increased activity of phosphodiesterase, which results in increased monocyte prostaglandin E_2 (PGE_2) production. PGE_2 tends to inhibit Th1 production of IFN-γ and accentuate IL-4 secretion by Th2 cells.

IgE appears to have a multifunctional role in the pathogenesis of atopic dermatitis. An immediate type hypersensitivity reaction develops within minutes of allergen exposure when mast cells bearing allergen-specific IgE bind allergen and release a variety of preformed mediators, most notably histamine. The skin histamine content has been found to be increased in humans and dogs with atopic dermatitis. *Activated mast cells* also release cytokines such as IL-4, IL-5, IL-6, TNF-α, and platelet activating factor (PAF) that further promote inflammation. An IgE-dependent late-phase reaction develops subsequently when expression of leukocyte adhesion molecules on postcapillary venule endothelium promotes influx of eosinophils, neutrophils, and mononuclear cells into the skin. Eosinophils release various mediators including PAF, eosinophil major basic protein (MBP), eosinophil cationic protein (ECP), and cytokines that promote inflammation and cause tissue damage. Antigen-specific IgE antibodies also appear to be involved in a non-classical role in antigen uptake and processing. IgE-bearing antigen-presenting cells (APCs), particularly Langerhans cells and dermal dendritic cells, bind, process, and present specific antigen locally to allergen-specific indeterminate T cells (Th0) thereby directing them to the Th2 cell phenotype. Moreover, IgE-mediated antigen presentation increases the presenting capacity of these APCs up to 100-fold, a mechanism known as "*facilitated antigen presentation*." Ultimately, B cells are stimulated to produce more allergen-specific IgE, which is bound to antigen-presenting cells, and a vicious cycle of facilitated antigen presentation is perpetuated.

Atopic dermatitis traditionally has been thought to be triggered by inhaled aeroallergens, hence the synonym **allergic inhalant dermatitis**. However, there is evidence both in humans and animals suggesting that *a transepidermal route of exposure may be involved instead or as well*. In the dog, such a route of antigen presentation is suggested by the typical distribution of skin lesions primarily to ventral, sparsely haired areas. Immunohistochemical studies of lesional skin from humans, dogs, and cats with atopic dermatitis have found increased numbers and focal aggregation of activated Langerhans cells within the epidermis. *These findings are interpreted to represent antigen capture within the epidermis*. Increased numbers of T cells are also seen in the epidermis. Clustering of dendritic cells is also evident in the superficial dermis, suggesting increased local antigen capture and/or antigen presentation to T cells. Furthermore, both the aggregated epidermal Langerhans cells and dermal dendritic cells are positive for surface IgE, findings consistent with the theory that cutaneous allergen capture or presentation is mediated by IgE. In lesional skin of atopic dogs, epidermal eosinophil exocytosis, subcorneal eosinophilic micropustule formation, and eosinophil degranulation have been observed and interpreted to indicate that the interaction between allergen and immunocompetent cells occurs in the epidermis and results in the release of eosinophil chemotactic factors by keratinocytes or epidermal Langerhans cells. In addition, increased expression of adhesion molecules by dermal endothelial cells has been found. Increased numbers of CD4+ and CD8+ T cells are seen in lesional atopic skin of humans, cats, and dogs. Tissue-selective homing of T cells is thought to be regulated at the level of T cell recognition of vascular endothelial cells via interaction of differentially expressed T lymphocyte homing receptors, such as cutaneous lymphocyte antigen (CLA), and their endothelial cell ligands. Therefore, *increased expression of endothelial adhesion molecules promotes migration of increased numbers of CLA+ T cells into the skin*. The tendency to develop atopic dermatitis as opposed to asthma or allergic rhinitis may depend on differences in skin-versus lung-seeking propensity of the memory/effector T lymphocytes.

The characteristic feature of atopic dermatitis is intense pruritus but the pathogenesis is not well understood. It is thought to be driven by local release of pharmacologic mediators, including histamine, neuropeptides, and eicosanoids; however, it does not appear that histamine is a major pruritogen in atopic dermatitis. Of the various chemical mediators, *neuropeptides* may be particularly important in the pathophysiology of itching in the atopic individual. In addition, atopic dermatitis appears to be associated with a decreased threshold for pruritus. The development of clinical lesions is also partially dependent on skin trauma caused by scratching. Once the itch-scratch cycle is initiated, mechanical trauma to the keratinocytes results in release of various proinflammatory cytokines that can maintain and promote inflammation. These include IL-1, TNF-α, and IL-4. It is unclear whether there is also an intrinsic keratinocyte defect. Keratinocytes from humans with atopic dermatitis exhibit enhanced production of GM-CSF in response to IL-1, which can contribute to increased numbers and enhanced antigen-presenting function of dendritic cells.

Atopic dermatitis is associated with a cell-mediated immunodeficiency that appears to be confined to the skin. Consequently, patients with atopic dermatitis have an increased tendency to develop skin infections, which are generally superficial. Superficial infection with *Malassezia* is common in humans and dogs with atopic dermatitis. Lesional skin of humans and dogs with atopic dermatitis has a high incidence of colonization with pathogenic strains of staphylococci. Besides causing superficial pyoderma, *S. aureus* in humans is believed to augment allergen-induced skin inflammation in atopic dermatitis by secreting exotoxins that act as *superantigens*. Superantigens have the capacity to activate large classes of T cells nonspecifically by virtue of their ability to bridge the linkage between the class II major histocompatibility complex molecule on the APC and the T cell receptor without having to be processed and presented by the APC. IgE antibodies directed against these exotoxins have been identified in individuals with atopic dermatitis and the severity of disease correlates with these IgE antibodies, presumably because the locally produced exotoxins are absorbed through the skin surface and cause IgE-dependent mast cell degranulation within the dermis. Other toxins secreted by *S. aureus* organisms have been found to induce release of TNF-α by keratinocytes and cause inflammation by nonspecific mechanisms.

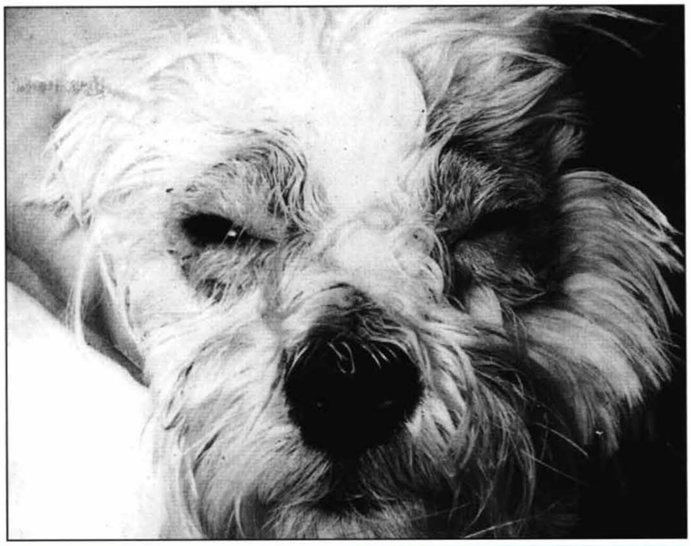

Figure 5.65 Atopy in a dog.

Atopic dermatitis is a common condition in **dogs** and is estimated to affect 10–15% of the population. It is second only to flea-bite hypersensitivity in those geographical areas with fleas. It has a *familial pattern* but the mode of inheritance is unknown. No relationship between dog leukocyte-antigen (DL-A) and canine atopy could be found. Breeds thought to be predisposed include the Chinese Shar-Pei, Cairn Terrier, West Highland White Terrier, Scottish Terrier, Lhasa Apso, Shih Tzu, Wire-haired Fox Terrier, Dalmatian, Pug, Irish Setter, Boston Terrier, Golden Retriever, Boxer, English Setter, Labrador Retriever, Miniature Schnauzer, Belgian Tervuren, and Beauceron. Females are affected more commonly in some studies, while in others no sex predilection has been found. The age of onset is variable but disease usually is apparent between 1 and 3 years of age. It is rare in dogs less than 6 months, except in Chinese Shar-Peis which may develop disease as early as 3 months of age; and, unless there is a change in the environment, most dogs develop clinical signs by 6 years.

The *primary clinical sign is pruritus*, which frequently begins seasonally but eventually becomes perennial. Pruritus most commonly affects the face (Fig. 5.65), paws, distal extremities, and ventrum and is manifested typically as face-rubbing and foot-chewing and licking. Skin lesions are usually due to self-trauma, secondary bacterial pyoderma, *Malassezia*-dermatitis, and secondary seborrhea. Secondary skin changes include variable alopecia, salivary staining of the hair, excoriations, papules, pustules, crusts, hyperpigmentation, lichenification, and pyotraumatic dermatitis. Eventually cutaneous involvement may become generalized. Atopic dogs characteristically have an unpleasant odor that results from a combination of seborrhea, secondary bacterial or yeast infection, and hyperhidrosis. Atopic otitis externa is common. Noncutaneous clinical signs are rare and may include conjunctivitis, rhinitis, asthma, and gastrointestinal disorders.

No breed or sex predilection is apparent in **cats** with atopic dermatitis; and it is uncertain whether there is an inherited predisposition. Clinical signs usually develop at 6 months to 2 years of age. *Pruritus* is the primary clinical sign but it may not be obvious because of the secretive nature of cats. Instead, hair loss, secondary to covert licking and chewing, may be the only abnormality and the skin may appear normal. *Clinical lesions of atopic dermatitis in cats are extremely variable.* They may include, in addition to self-induced alopecia, eosinophilic granuloma complex lesions, miliary dermatitis, recurrent swelling of the lower lip, otitis externa, and pruritus of the face, pinnae, and neck. *Miliary dermatitis is a clinical reaction pattern of cats* that consists of numerous small erythematous crust-covered papules. It is not specific for atopic dermatitis and may also occur in ectoparasite, food, and drug hypersensitivities. Noncutaneous signs associated with atopy in cats include sneezing, conjunctivitis, coughing, and asthma. Lymphadenopathy may be present in cats with miliary dermatitis or eosinophilic granulomas.

Pruritus is the primary clinical sign in **horses** with atopy. Some cases are manifested as recurrent pruritic urticaria. Onset is usually between 1.5 and 4 years of age. Skin lesions usually develop secondarily in response to self-trauma and include alopecia, excoriations, lichenification, and hyperpigmentation. The face, ears, ventrum, and legs are most commonly affected. Arabians and Thoroughbreds may be predisposed. A seasonal pruritic dermatosis typical of atopic dermatitis has been described in two **Suffolk ewes**.

Microscopic changes associated with atopic dermatitis have been considered nonspecific and usually consist of perivascular to interstitial dermatitis. In *dogs*, the epidermis is variably hyperplastic and may have mild, patchy intercellular edema, focal parakeratosis, and crusting. Erosion and ulceration may be present from self-trauma. Exocytosis of lymphocytes or eosinophils and small subcorneal accumulations of eosinophils may be seen. Dermal inflammation is superficial perivascular to interstitial, and consists of lymphocytes and macrophages primarily. Mast cells are in increased numbers and may be numerous. Eosinophils and neutrophils are usually in low numbers, but eosinophils may be missed because of degranulation. Sebaceous glands are hyperplastic and apocrine sweat glands may be dilated in chronic lesions. Superficial dermal blood vessels may be congested and the superficial dermis may be mildly edematous.

Histologic changes in the skin of *cats* with atopic dermatitis vary according to the lesions biopsied. The epidermis varies from normal to variably hyperplastic. Serocellular crusts, foci of intercellular edema and epidermal necrosis, and exocytosis of small numbers of eosinophils are usually present in lesions of miliary dermatitis. The dermis contains a superficial perivascular to interstitial infiltrate of mixed cells. Mast cells, eosinophils, lymphocytes, and neutrophils are typically present but vary in their proportions. Neutrophils are most numerous in areas of erosion or ulceration. In some cases, the lesions are those of an eosinophilic plaque. The microscopic lesion in *atopic horses and sheep* is hyperplastic perivascular dermatitis with a predominance of eosinophils.

Diagnosis of atopic dermatitis is based on compatible history and physical examination findings coupled with demonstration of allergen-specific IgE antibodies. In dogs, measurement of total serum IgE has not been found to be useful because there is no significant difference in IgE concentrations between atopic and clinically normal dogs. *Intradermal skin testing* (IDST) has been considered to be the most reliable method of identifying clinically relevant allergens. In vitro tests that measure concentrations of allergen-specific IgE are commercially available but generally have been found to correlate poorly with IDST results. However, newer procedures for measurement of allergen-specific IgE may be more accurate in identifying clinically relevant allergens. Diagnosis may be complicated by the presence of concurrent hypersensitivity conditions such as flea-bite allergy or food allergy.

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Food hypersensitivity (allergy)

Food hypersensitivity refers to an exaggerated, immunologically mediated reaction to specific food allergens resulting in disease. This hypersensitivity reaction may be mediated by IgE (type I hypersensitivity) and by non-IgE-mediated mechanisms. Food-induced allergic reactions are responsible for a variety of signs involving the skin, gastrointestinal tract, and respiratory tract. The skin may represent the second most frequent target organ, after the gastrointestinal tract, in food hypersensitivity reactions.

Food allergy in humans is thought to develop as a consequence of physiologic and immunologic immaturity resulting in increased absorption of food antigens during the first few months to years of life in association with the inherited tendency for increased production of IgE antibody. Absorption of intact food proteins appears to be limited normally by the intestinal mucosal barrier and by combination of the proteins with food allergen-specific IgA secreted into the gut. However, adult levels of IgA are not generally produced until puberty and this relative IgA deficiency may contribute to increased permeability of the gastrointestinal barrier during childhood. Gastrointestinal infections and parasitism may also contribute to disruption of the mucosal barrier to increase absorption of food antigens. The majority of food allergens are *glycoproteins* with molecular weight between 10 000 and 80 000 daltons, which tend to be resistant to proteolysis and are heat-stable and water-soluble. Even in the mature intestine, approximately 2% of ingested food antigens are absorbed into the circulation in an immunologic form. However, these immunologically recognizable proteins do not normally cause adverse reactions despite being transported throughout the body because tolerance develops in most individuals. Tolerance is thought to involve activation of CD8+ T suppressor cells in GALT to suppress an immune response. The development of tolerance to food appears to have little effect on B cells since antibody production to food proteins is a universal phenomenon; although low concentrations of serum IgG, IgM, and IgA food-specific antibodies are found in normal individuals, they are of no clinical consequence. In genetically predisposed infants, however, ingested antigens result in excessive production of IgE antibodies. These food-specific antibodies bind high affinity receptors on mast cells, dendritic cells, and macrophages in tissues and basophils in circulation. When food allergens penetrate mucosal barriers and reach the IgE bound to mast cells and basophils, mediators are released that induce the signs of immediate hypersensitivity. Activated mast cells also generate various mediators (such as IL-4, tumor necrosis factor α, and platelet activating factor) that may induce an IgE-mediated late-phase response in which eosinophils, lymphocytes, and monocytes are attracted to the site of reaction and release additional inflammatory mediators and cytokines. Mononuclear cells can be induced to secrete "histamine-releasing factors" that interact with IgE bound to basophils and possibly other cells to increase releasibility of these cells. In addition, IgE antibodies bound to Langerhans cells lead to more efficient capture of antigens and preferential activation of Th2 lymphocytes that promote allergic inflammatory reactions. The development of dermatitis rather than respiratory signs in individuals with food allergy may relate to homing of allergen-specific T cells to the skin by way of cutaneous lymphocyte-associated antigen (CLA), a homing molecule that directs these cells to the skin.

The pathomechanism of food hypersensitivity in animals is unknown, but it is assumed to be an IgE-mediated hypersensitivity reaction as in humans. It is not known when the gastrointestinal tract of companion animals becomes mature. Since gastrointestinal parasitism and viral enteritides are relatively common in animals, disruption of the intestinal mucosal barrier may be an important factor in the development of food hypersensitivity. Food allergy, as recognized in animals, is clinically different from the condition in humans, however. In humans, food hypersensitivity is most commonly seen in infants and young children; and in many cases, a loss of clinical sensitivity develops after 1–3 years of an appropriate food elimination diet. Although signs may initially develop in young animals, food hypersensitivity is typically a disease of adult animals and no loss of clinical sensitivity has been observed in affected animals. Furthermore, laboratory and clinical evidence suggests that food-specific IgE antibody directly contributes to the pathogenesis of atopic dermatitis in humans. In animals, food allergy is currently considered a separate entity from atopic dermatitis, although the two conditions may coexist.

Food allergy is thought to be the third most common hypersensitivity in **dogs** after flea-bite allergy and atopic dermatitis. The prevalence is unknown but food hypersensitivity has been suggested to account for 10–15% of canine allergic dermatoses. A significant number of dogs with food allergy, ranging from 23% to 45% in various studies, have concurrent hypersensitivity conditions such as atopic dermatitis, flea-bite allergy, or both. The most common allergens identified are *beef, wheat, dairy products, chicken, eggs, corn, and soy*. Some dogs, up to 64% in one study of 25 dogs with food allergy, react to two or more dog food ingredients, suggesting that multiple food hypersensitivities are not uncommon. Signs of food allergy may develop at any age; but in approximately 50% of dogs, signs are seen before 1 year of age and may occur as early as 4 months. No sex predisposition has been found. Breed predilection is uncertain. None has been found in some studies, while different breeds have been found to be at increased risk in various studies, no doubt reflecting regional differences in genetic pools.

Clinical signs in dogs are extremely variable. *The most consistent sign is pruritus* that is usually nonseasonal and may be generalized or localized to the feet, face and ears; inguinal and axillary regions; or lumbosacral and perineal area. Depending on the location of the pruritus, food allergy may mimic sarcoptic mange, flea allergy dermatitis, or atopic dermatitis. The pruritus may be unresponsive or poorly responsive to glucocorticoid therapy. Primary skin lesions may include erythema, papules, or pruritic urticaria-angioedema but they are frequently obscured by self-trauma as a consequence of chronic pruritus. More commonly, only secondary lesions are seen and these typically include excoriation, alopecia, hyperpigmentation, lichenification, scales, crusts, ulceration, and seborrhea. In a small number of cases, pruritic otitis externa or recurrent superficial pyoderma may occur in the absence of any other clinical signs of food allergy. Bacterial pyoderma and *Malassezia* infection are common secondary complications. Some dogs also exhibit *concurrent gastrointestinal signs*, which include increased frequency of defecation most commonly, and mucus and/or blood in feces, tenesmus, vomiting, or diarrhea less frequently. Neurologic signs, such as malaise and seizures, and respiratory signs have been reported rarely in conjunction with skin lesions in dogs with food allergy.

The prevalence of food allergy in **cats** is unknown. No breed or sex predilection is apparent. Most cases are recognized in young adult cats. The major complaint is *severe pruritus* that is usually nonseasonal and frequently poorly responsive to glucocorticoid therapy. The face, ears, and neck are most commonly involved but pruritus may be generalized (Fig. 5.66). Allergens identified include

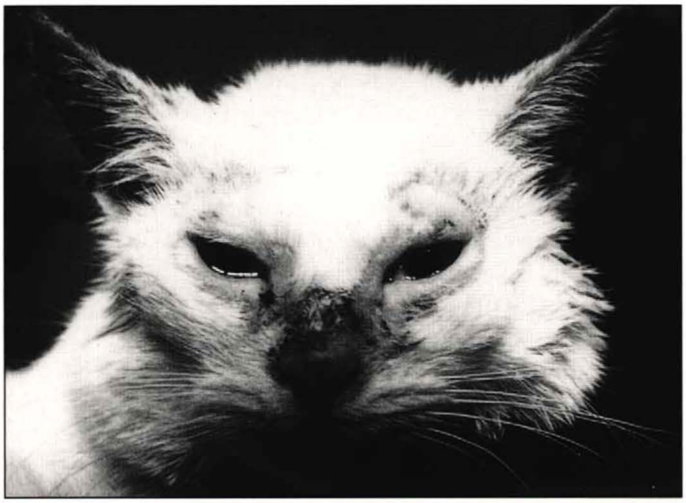

Figure 5.66 Food allergy in a kitten. Periocular and nasal alopecia, crusting, and erythema.

fish, lamb, milk, whale meat, beef, chicken, rabbit, eggs, and pork. Food allergic cats may also have other hypersensitivity conditions such as atopic dermatitis or flea-bite allergy. Skin lesions are extremely variable and include erythema, angioedema-urticaria, self-induced alopecia and excoriations, crusting, seborrhea, or miliary dermatitis. They may be localized to the face and head or generalized in distribution. Eosinophilic granuloma complex lesions may be a manifestation of food allergy. Concurrent gastrointestinal or respiratory signs are uncommon.

Food hypersensitivity has been reported rarely in **horses, cattle**, and **pigs**. Substances that have been incriminated include wheat, barley, bran, oats, concentrates, and tonics in horses; wheat, bran, corn, clover hay, rice bran, and soybeans in cattle; and clover pasture in pigs. Clinical signs in horses and cattle are pruritic papular eruptions, pruritic urticaria, and tail rubbing. A condition described as food allergy in white hogs on new clover pasture consisted of generalized erythema and skin pain, depression, and reluctance to move. The description of this condition is more suggestive of an adverse food reaction, such as photosensitization, than of food hypersensitivity, however.

The microscopic lesions associated with food allergy are variable and are not diagnostic. The epidermis is variably acanthotic and may be multifocally spongiotic. Crusting, erosion, and ulceration may be present. The superficial dermis is mildly to moderately edematous and inflammation is variable. In dogs, inflammation may be perivascular, interstitial, or diffuse. The cells are mixed and include lymphocytes, macrophages, eosinophils, and mast cells. Neutrophils and plasma cells are present in numbers proportional to the degree of self-trauma. Sebaceous and apocrine glands may be hyperplastic in chronic lesions. In cats, dermal inflammation may be a superficial perivascular mononuclear dermatitis but more commonly is characterized by eosinophilic inflammation that is perivascular to diffuse and extends into the subcutis. Mast cell numbers are commonly moderately to markedly increased throughout the dermis. Collagen degeneration may occur in areas of intense eosinophilic inflammation. Eosinophilic folliculitis and furunculosis and lesions of eosinophilic plaque are present in some cases of food allergy in cats. The microscopic lesion ascribed to food allergy in large animals is superficial perivascular dermatitis, with eosinophils comprising a significant proportion of the inflammatory cells.

The *diagnosis* of food allergy is made by a combination of appropriate clinical history, exclusion of other pruritic conditions, and resolution of pruritus with feeding of a hypoallergenic diet. Intradermal skin testing and measurement of serum allergen-specific IgE levels have been found to be unreliable for diagnosis of food allergy in animals.

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Allergic contact dermatitis

Allergic contact dermatitis is an uncommon hypersensitivity condition in domestic animals. Although considered a typical delayed (type IV) hypersensitivity reaction, *there is considerable overlap in pathogenesis between allergic contact dermatitis and primary irritant dermatitis and the rigid distinction between the two conditions is becoming blurred.* It has been estimated to account for 1–10% of dermatoses in dogs. The low incidence, as compared to that in humans, is thought to be due to the natural protection afforded by the hair coat of most animals and by the decreased exposure to potential allergens in cosmetics and industrial chemicals.

Allergic contact dermatitis is caused by contact with a nonirritating concentration of a substance to which an individual has previously become sensitized. The compounds involved are usually lipid-soluble haptens (low-molecular-weight substances) that become immunogenic only after penetrating the epidermis and binding covalently to an autologous structural or cell surface protein to form a complete antigen. This antigen is subsequently internalized and processed by Langerhans cells and presented to CD4+ T cells. Langerhans cells are crucial to development of allergic contact dermatitis as depletion of these cells results in a decreased ability to induce contact sensitization. During the induction phase of the reaction, Langerhans cells present antigen to CD4+ cells in the paracortical region of regional lymph nodes where the specifically sensitized T cells undergo clonal expansion and then circulate as memory cells in blood and home to skin by virtue of adhesion molecules that bind to addressins on endothelial cells. Development of allergic contact dermatitis depends on the nature of the allergen, frequency of contact, and state of the skin. *Factors that damage the integrity of the protective barrier of the skin predispose to development of allergic contact dermatitis.* The induction phase is typically a prolonged process, estimated to require 6 months to 2 years to develop. While strong immunogens can elicit sensitivity after as short a period of contact as 7 to 21 days, most contact allergens are weaker immunogens and require chronic repeated exposure for sensitization to develop. Upon subsequent exposure to the contact allergen, specifically sensitized T cells in skin and circulation are presented the antigen by Langerhans cells (*elicitation phase*), become activated, and elaborate various cytokines that attract and activate other inflammatory cells and stimulate proliferation of the epidermis. Only a very small fraction (<1%) of the infiltrating T cells are specific for a relevant antigen. Thus, various amplification and recruitment mechanisms are involved in induction of the reactions. The activated T cells secrete a Th1 profile of cytokines, including IFNγ and TNFβ, which recruit and activate a wide variety of inflammatory cells, affect keratinocyte function and differentiation, and induce expression of adhesion molecules on endothelial cells and keratinocytes. TNFβ and IFNγ induce expression of ICAM-1 on keratinocytes and endothelium, which promotes homing of T cells to the dermis and epidermis. Unlike the delayed-type hypersensitivity reaction that develops in response to antigen injected into the dermis which primarily involves CD4+ cells, the response to hapten painted on the epidermis involves both CD4+ and CD8+ T cells. Local production of cytokines induces arrival of more T cells and further amplification of proinflammatory mechanisms. Irritated keratinocytes also release a variety of cytokines that induce or augment the inflammatory response.

Allergic contact dermatitis has been reported most frequently in *dogs and horses*, although most cases have not been confirmed by patch testing. Numerous *plants and chemicals* have been suspected or shown to cause allergic contact dermatitis in animals. In dogs, wandering Jew (*Tradescantia fluminensis*), spreading dayflower (*Cornelina diffusa*), doveweed (*Murdannia nudiflora*), leaves and bulbs of plants in the family Amaryllidaceae, dandelion leaves, cedar wood, and Asian jasmine have been implicated in cases of allergic contact dermatitis. Cobalt and nickel ions, pine oil resin found in cleaning products, topical medications such as neomycin, rubber, cement, and various fragrances such as those in shampoos and carpet deodorizers are a few of the chemical causes of allergic contact dermatitis in dogs and cats. In horses, pasture plants, insecticides, various dyes and preservatives of tack items, soaps, and bedding materials have been suggested as causes of allergic contact dermatitis.

The *clinical course* is typically prolonged and lesions in dogs are usually confined to sparsely haired areas of the body such as the lips, chin, ventral cervical and thoracic areas, abdomen, scrotum, perineum, and ventral interdigital skin. Foot pads are usually protected by the thick stratum corneum; however, cracking of foot pads of all four feet was a feature described in a cat with suspected allergic contact dermatitis to carpet deodorizer. Lesion distribution is also dependent on the contactant involved. *Primary lesions include erythema, papules, plaques, and vesicles*, but these are very transient and thus rarely seen. *Pruritus is variable*, ranging from mild to severe. *Chronic secondary lesions are more commonly seen and include alopecia, lichenification, scaling, crusting, excoriations, and pigmentary changes.* Secondary bacterial or fungal infection may complicate lesions.

Microscopic changes described in various reports of allergic contact dermatitis in animals have been conflicting. *Differences in histologic lesions no doubt reflect, at least in part, differences in stage of the reaction.* While neutrophils have been the predominant cell type seen in some cases, lymphocytes or eosinophils predominated in others. The epidermis may be variably spongiotic and may develop vesicles in some areas. In allergic contact dermatitis produced experimentally in dogs by topical application of 1-chloro-2,4-dinitrochlorobenzene (DNCB), histologic changes at 48 and 72 hours post-challenge included mild acanthosis, mild dermal edema, and perivascular and perifollicular infiltrations of lymphocytes and macrophages. Mild and focal to widespread and marked epidermal necrosis may occur, both in patch test reaction sites as well as in spontaneous lesions. Exocytosis of inflammatory cells is a common feature but the cells vary and may be neutrophils, lymphocytes, eosinophils or a combination thereof. The superficial dermis may be edematous and the predominant inflammatory cells may be lymphocytes, neutrophils, or eosinophils. Inflammation may be perivascular, diffuse interstitial, or lichenoid and frequently perifollicular. *The histologic changes of allergic contact dermatitis are frequently indistinguishable from those caused by irritant contact dermatitis.*

The list of *differential diagnoses* is extensive and includes a wide variety of allergic and parasitic conditions, bacterial and fungal infections, and irritant contact dermatitis. When a single animal of a group is affected, allergic contact dermatitis is considered more likely than irritant dermatitis. *Diagnosis is based on history, physical examination findings, exclusion of other dermatoses, and is confirmed by restriction followed by provocative exposure to the suspected allergen or patch testing.*

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Insect hypersensitivity

Insects are cosmopolitan in their distribution and virtually all animals are exposed to them. Many insects are capable of inducing allergic reactions, including various dermatoses. *Antigens of insect* *origin that produce hypersensitivity are usually proteins and sources include venom, saliva, whole bodies, shed skins, egg capsules, feces, and insect hemoglobin.* These antigens can be introduced via the bite or sting of insects or by inhalation, ingestion, or percutaneous absorption. In general, lesions associated with insect hypersensitivity are *seasonal* or seasonally more severe and *involve short- or sparsely haired regions,* such as the nose, muzzle, pinnae, inguinal area, and distal extremities. *Lesions typically consist of pruritic crusted papules and are characterized histologically by eosinophilic inflammation.*

The most well known insect hypersensitivities of veterinary significance are those caused by an allergic reaction to salivary antigens and these are *flea-bite hypersensitivity of dogs and cats* and *Culicoides hypersensitivity of horses.* More recently, a *hypersensitivity to mosquito bites has been recognized in cats* and an *eosinophilic furunculosis of the face of dogs* is suspected to be a hypersensitivity response to the sting or bite of various insects. The first three of these conditions will be discussed in this section. Canine eosinophilic furunculosis of the face is discussed under Eosinophilic and collagenolytic dermatitides.

Flea-bite hypersensitivity is a pruritic dermatitis caused by hypersensitivity to allergens in the saliva of fleas. *It is the most common allergic dermatosis of dogs and cats in flea-endemic regions.* The cat flea, *Ctenocephalides felis felis*, is the major initiator of the condition. Since many animals harbor large numbers of fleas without any apparent skin abnormalities, it is likely that animals develop skin disease as a result of flea infestation only if they are allergic to fleas.

Both type I and type IV hypersensitivity reactions are believed to be involved in the pathogenesis of flea-bite hypersensitivity. Participation of immediate hypersensitivity is supported by the fact that clinically allergic animals develop immediate skin reactions in response to intradermal injection of flea antigen and that IgE antibodies to flea antigen are demonstrable in sera from allergic animals. Histologic evidence also supports a role for immediate hypersensitivity and suggests that cell mediated/delayed hypersensitivity is involved in the pathogenesis of flea allergy as well. Many flea allergic animals also have delayed skin test reactions, further support for a type IV hypersensitivity component. Upregulation of mast cell proteases has been demonstrated during sensitization, with selective release of mast cell tryptase after exposure to flea antigen.

No breed or sex predilections have been reported in flea-bite hypersensitivity; and in cats, no age predilection has been recognized. In dogs, disease occurs most commonly between 3 and 5 years, unless naive dogs are moved to a flea-endemic area at a later age. Disease is rare prior to 6 months. Signs tend to be more severe in summer and fall in animals living in areas with cold winters but are year-round in those living in warm regions or where indoor infestation persists. Affected individuals may have other hypersensitivity conditions, such as atopic dermatitis or food allergy.

Flea-bite hypersensitivity in **dogs** is characterized by pruritus, erythema, wheals, and papules that may become crusted. *Primary lesions are usually obscured by secondary lesions that develop in response to chronic pruritus.* These may include hyperkeratosis, lichenification, hyperpigmentation, alopecia, excoriations, redundant skin folds on the rump and caudal thighs, and seborrhea. Lesions typically involve the dorsal lumbosacral area, flanks, caudal and medial aspect of the thighs, and ventral abdomen. In severely hypersensitive dogs, lesions may become generalized. Pyotraumatic dermatitis ("hot spots") and bacterial pyoderma are common secondary complications. Firm alopecic nodules ("fibropruritic nodules") may develop on the dorsal lumbosacral region secondary to self-trauma in chronically affected dogs. Differential diagnoses include other hypersensitivity conditions (atopic dermatitis, food allergy), sarcoptic mange, cheyletiellosis, and bacterial or yeast infection.

The lesions of flea-bite hypersensitivity in **cats** are extremely variable. The most common manifestation is multiple erythematous pruritic papules covered by brown crust ("*miliary dermatitis*") on the dorsal lumbosacral area, flanks, caudal and medial aspect of the thighs, ventral abdomen, and neck. In some cats, the condition may be manifested as over-zealous grooming rather than scratching, producing alopecia that may be ventral abdominal, bilaterally symmetrical along the lateral aspect of the trunk, or dorsal lumbosacral. The skin may appear completely normal grossly or excoriations, crusts, scales, and hyperpigmented macules may be seen. Fleas or flea dirt may not be evident because of the fastidious grooming behavior typical of most cats. Eosinophilic granuloma complex lesions have also been associated with flea-bite hypersensitivity in cats. Secondary bacterial pyoderma is uncommon. Differential diagnosis is extensive because the manifestations of flea allergy are so varied in cats. *The most common differentials for miliary dermatitis include other hypersensitivity conditions (atopic dermatitis, food hypersensitivity), dermatophytosis, and cheyletiellosis.* Cases characterized by self-induced alopecia with normal appearing skin may resemble an endocrinopathy.

Histopathology is useful in confirming the suspected diagnosis of a hypersensitivity condition, but *the microscopic changes associated with flea-bite hypersensitivity are similar to those seen in other hypersensitivities and the specific diagnosis cannot be made histologically.* The epidermis is variably acanthotic and foci of spongiosis and serocellular crusting are commonly seen. Mixed orthokeratotic and parakeratotic hyperkeratosis and self-induced erosion or ulceration may be present. Foci of epidermal necrosis and intraepidermal eosinophilic pustules may be evident. The superficial dermis is mildly to moderately edematous and a perivascular to interstitial infiltration of eosinophils, lymphocytes, fewer macrophages, and mast cells is present in the superficial to mid-dermis in dogs and may extend into the subcutis in cats. The proportion of eosinophils in relation to mononuclear cells varies, with eosinophils being most numerous in early lesions and mononuclear cells predominating in more chronic reactions. Melanophages are in variable numbers and may be numerous in chronic cases with prominent lichenification. Sebaceous and apocrine glands may be hyperplastic in chronic lesions. Neutrophils are numerous in association with ulceration or bacterial infection. In cats, eosinophilic mural folliculitis and furunculosis have been seen in flea bites as have histologic lesions of eosinophilic granuloma complex.

***Culicoides* (insect) hypersensitivity** *is the most common allergic dermatosis of horses.* It occurs worldwide and *Culicoides* spp. gnats are the most common cause. The condition is known by a variety of colloquial names worldwide, including Queensland itch, kasen, dhobie itch, sweet itch, muck itch, summer itch, and summer eczema. It is a major annoyance to horse and owner and substantial economic losses can be incurred from treatment, prevention, and damage caused by scratching horses. *Culicoides* hypersensitivity is an *intensely pruritic dermatosis* that can render affected animals too restless and anxious to perform.

The insects are also called biting midges, punkies, and "no-see-ums." Hundreds of different species exist throughout the world and they vary in their favored feeding sites and time of activity. They are most active when the ambient temperature is greater than

10°C (50°F), when humidity is high, and when there is no breeze since they are weak fliers. The insects are in highest numbers in wetlands and swampy areas.

The hypersensitivity to salivary antigens of *Culicoides* gnats is thought to be mediated by *both type I and type IV hypersensitivity reactions*. Support for immediate hypersensitivity is provided by immediate skin test reactivity to *Culicoides* antigens, presence of specific IgE in affected horses, and peripheral eosinophilia and increased blood histamine concentrations during periods of insect activity. The presence of increased numbers of primarily CD4+ T lymphocytes and eosinophils in skin test reactions is also consistent with immediate hypersensitivity. Also, sulfidoleukotriene generation from peripheral blood leukocytes in response to *Culicoides* extract was increased in horses with insect hypersensitivity, indicating involvement of IgE-mediated hypersensitivity in the pathogenesis of *Culicoides* hypersensitivity. Delayed reactions (up to 48 hr) to intradermal skin tests suggest that type IV hypersensitivity may also be involved in the pathogenesis of the condition.

Culicoides hypersensitivity is typically a seasonal disease but in warm climates it may be a problem virtually year-round. Horses of any breed and either sex are affected; pedigree studies suggest there may be a genetic basis. Lesions are uncommon in horses less than 2 years old; however, in tropical and subtropical climates with a long insect season, sensitization and mild clinical disease may develop within the first year of exposure. The favored feeding sites vary with the species of *Culicoides* endemic to a particular environment, accounting for the varied distribution of skin lesions. Distribution may be dorsal and involve the head, ears, neck, withers, back, and tailhead; or it may be primarily ventral and involve the intermandibular space, legs, and ventral midline. In some areas, such as Florida in the USA, multiple species of *Culicoides* are active at different times and have different favored feeding sites such that disease may be generalized. The *primary lesions are pruritic papules* that may be recognized initially by clusters of erect hairs and commonly become encrusted. Because of severe pruritus, however, self-mutilation obscures the primary lesions and results in *more commonly observed secondary lesions such as excoriations, crusts, lichenification, pigmentary changes, broken hairs, alopecia, and a short stubbled tail ("rat tail")* (Fig. 5.67). The mane may be rubbed off and the skin over the neck and withers may become thickened and rugose. Lesions typically heal during winter and recur in spring or summer and commonly worsen with age. Affected horses may scratch and bite themselves and rub objects in their environment, thereby causing damage to themselves, their riders, and environmental objects. Constant anxiety and restlessness may prevent severely affected animals from performing as riding or show animals. Also, since sweating exacerbates pruritus, affected horses cannot be worked vigorously. The differential diagnoses include ectoparasitism and other hypersensitivity dermatoses. The *diagnosis* is based on seasonality of the condition, location and appearance of lesions, sporadic occurrence of the condition in a group, eliminating other diseases, and response to therapy.

The *histologic lesions* of *Culicoides* hypersensitivity are typically *superficial or superficial and deep perivascular dermatitis consisting of eosinophils and lymphocytes primarily*. The epidermis is variably acanthotic, spongiotic, and hyperkeratotic with foci of parakeratosis, and may be focally necrotic. Increased numbers of T lymphocytes and Langerhans cells are present in the basal portion of the epidermis. Erosions and ulceration may be present as a result of self-trauma and are associated with neutrophilic inflammation. The dermis is variably edematous and increased numbers of mast cells, which may be degranulated, are present. In some cases, eosinophilic inflammation is diffuse and collagen degeneration or eosinophilic folliculitis may also be seen.

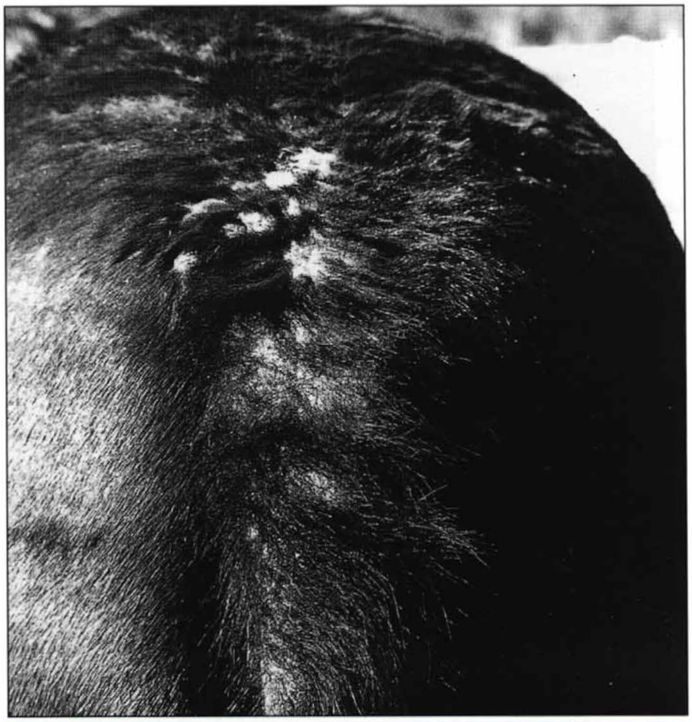

Figure 5.67 ***Culicoides* hypersensitivity** in a horse. Alopecia, crusting and scaling at the tail head ("rat tail").

A seasonal pruritic dermatosis attributed to *Culicoides* hypersensitivity has also been described in *cattle, sheep, and donkeys*. Affected sheep were 2–5 years of age and had an intensely pruritic, exudative dermatitis with loss of wool and marked skin thickening of the abdomen, udder, teats, and legs. Lesions in adult dairy cattle consisted of marked thickening and folding of the skin of the head, ears, and neck. Donkeys developed exudative dermatitis, alopecia, crusting, and skin thickening of the legs and head. Affected animals were restless and often bit at themselves, suggesting that they were intensely pruritic. Histologic examination of biopsies from affected sheep and cows showed primarily an eosinophilic dermal infiltrate.

Mosquito-bite hypersensitivity dermatitis has been documented in the Mosquito-bite dermatitis section.

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Hormonal hypersensitivity

Pruritic dermatitis resulting from hypersensitivity to endogenous sex hormones is recognized in women ("autoimmune progesterone dermatitis") and is *very rare in dogs*. Results of intradermal skin tests with aqueous progesterone in women indicate that type I (immediate) and/or type IV hypersensitivity are involved. *Most canine cases are in intact females, frequently with a history of irregular estrus or recurrent pseudopregnancy*. The condition is characterized by *intense pruritus* that develops or is exacerbated near the time of estrus or pseudopregnancy but tends to become more severe and protracted with each episode; pruritus is generally perennial in male dogs. Bilaterally symmetric erythema and crusted papules typically begin in the dorsal lumbosacral, perineal, genital, and caudomedial thigh areas and progress cranially. In chronic cases the condition is generalized and the skin becomes alopecic and lichenified. Pruritus is usually unresponsive to glucocorticoid treatment. Differential diagnoses include other allergic conditions and sarcoptic mange. The microscopic lesion is a hyperplastic superficial perivascular dermatitis in which neutrophils, mononuclear cells, or eosinophils may predominate. *Diagnostic clues include poor response to glucocorticoid therapy and development or exacerbation of cutaneous signs coincident with estrus or pseudopregnancy*. Gonadectomy is curative.

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Intestinal parasite hypersensitivity

Hypersensitivity to intestinal parasites has been suspected of causing pruritic dermatoses in *dogs, cats, horses, and humans*. The pathomechanism is unknown but a type I hypersensitivity reaction has been proposed. *Ascarids, coccidia, hookworms, tapeworms, and whipworms have rarely been associated with pruritic dermatoses that resolve with elimination of the intestinal parasites*. Lesions that have been attributed to intestinal parasite hypersensitivity include multifocal or generalized papulocrustous eruptions, pruritic seborrheic disease, pruritic urticaria, and pruritus without skin lesions. The histologic changes described are superficial perivascular dermatitis with variable numbers of eosinophils, ranging from few to many. Diagnosis is based on fecal examination and resolution of the skin lesions following appropriate parasiticidal therapy.

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Autoimmune dermatoses

The autoimmune skin diseases are *uncommon to rare* but merit detailed consideration as many are debilitating to life-threatening and require specific therapy. *Their characteristic microscopic lesions combined with clinical information and careful interpretation of ancillary studies often enables a specific diagnosis to be made*. In the vast majority of cases, the stimulus triggering the aberrant T or B cell responses against self-antigens is unknown. Drug therapy, underlying neoplasia (see Paraneoplastic pemphigus, p. 652), tissue injury, infectious diseases, other autoimmune diseases, and genetic makeup are all factors known to be associated with the occurrence of autoimmune diseases. Penicillinamine, for example, may precipitate clinically, histologically and immunologically classical cases of pemphigus in humans, which regress when the drug is withdrawn. Drug-induced forms of pemphigus are thought to be the result of haptenization of keratinocyte antigens rendering them immunogenic. One theory suggests environmental agents, such as drugs, influence T cells by causing DNA methylation abnormalities that, in turn, alter gene expression. Exposure to ultraviolet light is known to exacerbate cutaneous autoimmune disease perhaps by inducing keratinocyte ICAM-1 expression and keratinocyte production of pro-inflammatory cytokines. Still another theory suggests that the structural similarities of peptide fragments of some infectious agents to host proteins may trigger post-infectious autosensitization.

The recognition that one autoimmune disease, tissue injury, inflammatory or neoplastic process in an individual may precede the onset of cutaneous autoimmune disease led to investigation of the phenomenon termed "*epitope spreading*." Epitope spreading refers to the process by which the targets of the autoimmune response do not remain fixed but drift to include other epitopes on the same protein or nearby proteins of the same tissue. This may account for regional variations in pemphigus antigen expression and the clinical variation of cutaneous autoimmune diseases. Epitope spreading may also account for aberrant immune responses developing to tissue antigens after the tissue has been injured, possibly leading to the release or exposure of a previously sequestered antigen.

Selection of fresh lesions is crucial to diagnosis. Demonstration of tissue-bound or circulating autoantibody using appropriate immunological tests may be helpful in confirming the diagnosis of an autoimmune skin disease. However, such tests (e.g., direct and indirect immunofluorescence testing, immunohistochemistry) are fraught with interpretation pitfalls (false-positive or false-negative test results) and should

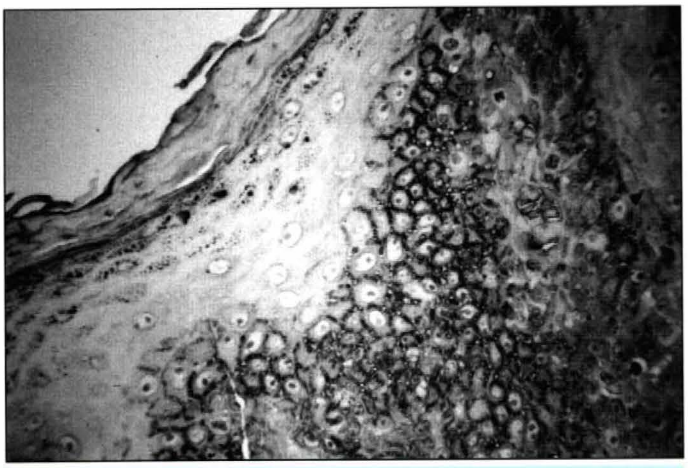

Figure 5.68 Immunoperoxidase stain of **pemphigus complex**. Note diffuse intense labeling of intercellular bridges.

never be interpreted in the absence of histologic examination. Demonstration of autoantibodies does not necessarily confirm causative roles for these antibodies, just as negative results do not necessarily exclude the diagnosis of a cutaneous autoimmune disease. For example, the various entities of the pemphigus complex are characterized by the deposition of immunoglobulin, with or without complement, on the surface of keratinocytes or at the basement membrane zone using the techniques of direct immunofluorescence (IF) testing or immunohistochemistry (IHC) (Fig. 5.68). Unfortunately, false-negative (poor lesion selection, prior glucocorticoid therapy) and false positive (any dermatosis in which spongiosis or numerous lymphocytes and plasma cells are present) reactions are frequent. In addition, normal epithelium of the canine nasal planum and footpad often label nonspecifically. Hence, *these immunopathological tests can only be interpreted in the light of clinical and histopathological findings*. Indirect immunofluorescence testing has variable usefulness in domestic animals with pemphigus as results may be falsely negative or "pemphigus-like" antibodies can be occasionally found in non-pemphigus diseases. Recent studies indicate indirect IF testing for canine PF is useful provided the appropriate substrate (bovine esophagus) is used. In many of the putative cases of autoimmune skin disease in animals, the criteria for autoimmunity have not been met fully.

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Autoimmune diseases characterized by vesicles, pustules, or bullae as the primary lesion

The pemphigus complex

Pemphigus refers to *a group of autoimmune skin diseases characterized clinically by pustules, vesicles, bullae, erosions and ulcers and histologically by loss of adhesion between cells (acantholysis)*. Autoantibodies directed against antigens within various stratified squamous epithelia, including skin, mucocutaneous junctions, oral mucosa, esophagus, and vagina, develop and can be detected via immunologic assays. *Different types of pemphigus are recognized based on the level at which acantholysis occurs within the epidermis, and the clinical and immunological findings*. Autoantibodies are directed against components of keratinocyte transmembrane glycoproteins responsible for keratinocyte cell-to-cell adhesion (desmosomes) or cell-to-substrate adhesion (hemidesmosomes). Desmosomes are more numerous in the more superficial layers of the epidermis and vary in protein subunit composition. Hemidesmosomes are present on the basal surface of the basal layer of the epidermis. Vesicles, pustules, or bullae form within the various layers of the epidermis or at the basement membrane zone according to the location of the target antigen. Severity of clinical disease is related to the depth of vesicle or bullae formation, with the more severe lesions associated with separations deep within or below the epidermis. The mechanisms leading to acantholysis are not completely understood. Antigen-antibody binding may result in a type II immune reaction or promote proinflammatory cytokine release from keratinocytes. The binding of pemphigus autoantibodies to keratinocyte antigens is associated with the synthesis and secretion of urokinase-type plasminogen activator (uPA), a serine protease that activates plasminogen. Activation of plasminogen may indirectly induce the cleaving of intercellular contacts. Anti-uPA antibodies or the specific inhibitor of uPA, plasminogen activator inhibitor type-2, inhibit lesion formation in vitro. Another hypothesis proposes that the binding of autoantibodies to keratinocyte antigens may disrupt the structural integrity of the adhesion molecule. Experimental studies in mice have demonstrated that acantholysis in pemphigus foliaceus (PF) can occur with passive transfer of PF IgG autoantibody as well as with transfer of Fab fragments and $F(ab')_2$ fragments in both complement-sufficient and complement-deficient mice.

Pemphigus foliaceus (PF) is the *most common form of pemphigus in domestic animals* and has been reported in the *dog, cat, horse, and goat*. In humans, PF autoantibodies recognize the desmosomal protein, *desmoglein 1*. Desmoglein 1 autoantibodies have also been identified in dogs, but autoantibodies to other keratinocyte antigens and to the basement membrane have also been identified, indicating that PF, at least in the dog, may be an immunologically heterogeneous disease. Desmoglein 1 belongs to the cadherin family of cell-adhesion molecules, and is found primarily in the upper layers of the epidermis and in only small amounts in the oral mucosa. In PF, the acantholytic process occurs at a superficial level in the epidermis producing clinical lesions that are *typically exfoliative rather than obviously erosive or ulcerative.* There is no sex predilection in **dogs**, but Akitas, Chow Chows, Bearded Collies, Collies, Chinese Shar-Peis, Dachshunds, Newfoundlands, Doberman Pinschers, Schipperkes, English Springer Spaniels, and Appaloosa horses appear predisposed. In dogs, most cases occur prior to 5 years of age. The gross lesions are similar in all species and are usually cutaneous and involve mucosa only rarely, as mucosal epithelium has desmoglein 3 as a prominent superficial adhesion molecule rather than desmoglein 1. Lesions may wax and wane. Erythematous macules give rise to transient pustules that rupture to form very shallow erosions that become covered by thick crust and scale. Alopecia is often marked. *Lesions in the dog and cat tend to appear first on the nose and spread to periorbital areas, the ears, neck and ventral abdomen.* Involvement of the feet may produce marked papillary hyperkeratosis and crusting of the footpads, and there is a *predilection for the nail beds*, occasionally leading to sloughing of nails. In **cats**, lesions are often seen around the nipples in addition to lesions on the face and ears (Fig. 5.69A). PF is the most common autoimmune skin disease in **horses;** lesions often begin on the face or distal extremities, or may be localized to coronets. In any species, the lesions may become generalized (Fig. 5.69B, C). Foals may also be affected with a benign form of PF that responds rapidly to treatment or may resolve spontaneously. Pain and pruritus may be present.

The histologic pattern of pemphigus foliaceus is an acantholytic subcorneal (see Figs. 5.1 and 5.17) *or intragranular pustular dermatitis.* Either neutrophils or eosinophils may predominate. At the base of the pustule, acantholytic keratinocytes continue to detach and enter the pustule. Ruptured pustules form a thick inflammatory crust in which acantholytic cells are prominent. The external root sheath of the hair follicle undergoes a similar acantholytic process. Dermal infiltrates consist of mild perivascular to interstitial neutrophils or eosinophils. There is dermal edema, vascular congestion and occasionally, hemorrhage. Rarely, a lichenoid inflammatory infiltrate is seen. *Deposition of IgG at intercellular bridges in all layers of the suprabasilar epidermis or in the superficial epidermis demonstrated by IF or IHC is characteristic, but not specific, for PF.* Secondary bacterial infection may complicate lesions. *The primary differential for PF is superficial bacterial folliculitis.* Acantholytic cells are more numerous and can more often be found in rafts in PF than in superficial folliculitis. *The "cling-on" stratum granulosum cells are only present in PF.* In PF, pustules also span the interfollicular epidermis encompassing multiple follicles whereas in superficial folliculitis pustules are more likely to be centered on single follicles. Evidence of recornification and reformation of pustules is often prominent in PF. Dermatophytosis and superficial pyoderma (impetigo) can also have similar gross and histologic lesions. Careful examination of skin sections with fungal and bacterial stains and possibly cultures may be needed for differentiation. Impetigo does not involve hair follicles whereas PF may.

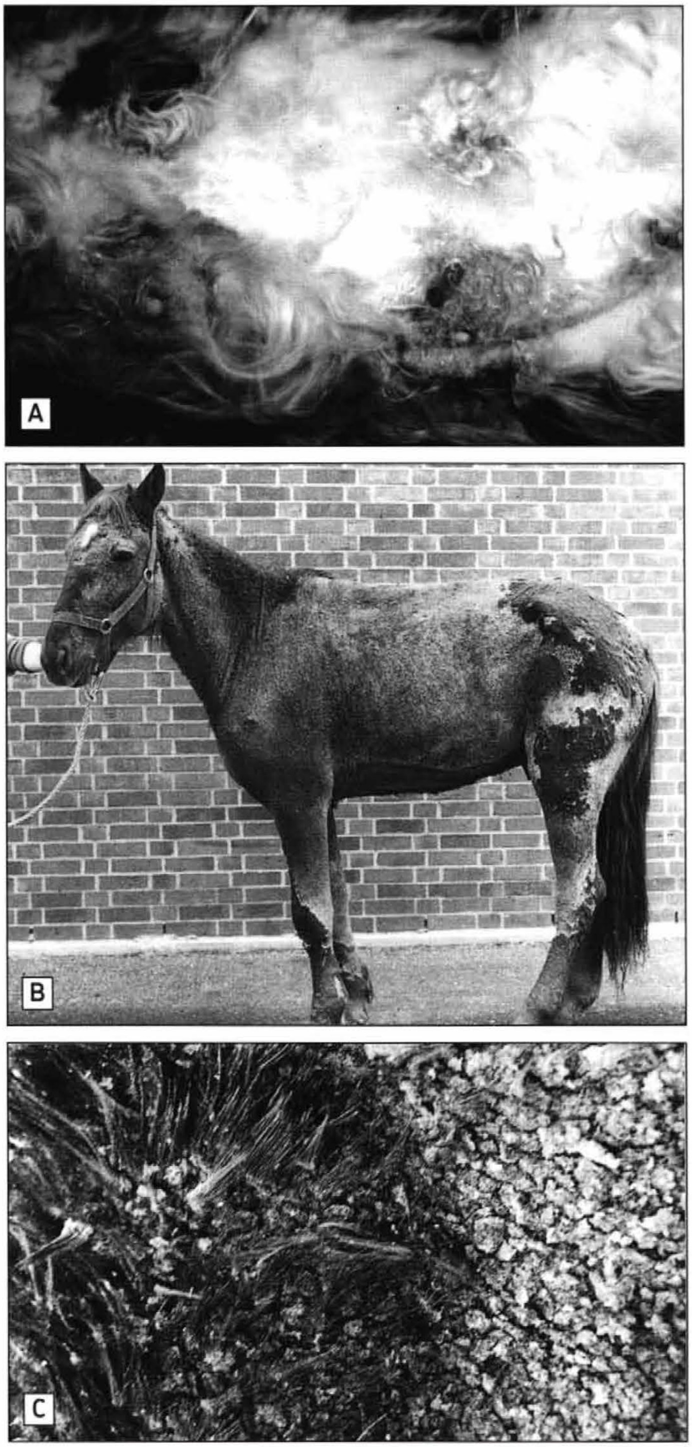

Figure 5.69 Pemphigus foliaceus. A. Note alopecia and crusts of the skin around the nipples in a cat. (Courtesy of K Beale.) **B.** Generalized exfoliative dermatitis and alopecia in a horse. **C.** Detail of skin from (**B**) showing massive build up of scales and crusts.

Panepidermal pustular pemphigus (PPP) refers to a form of pemphigus in the dog that encompasses some of the features of **pemphigus foliaceus, pemphigus vegetans,** and **pemphigus erythematosus**, previously thought to be distinct entities. It is similar to pemphigus foliaceus in that lesions develop in the superficial epidermis, however, lesions are not restricted to the superficial layers of the epidermis so it does not neatly fit the same criteria as PF. Immunological studies have not definitively documented the presence of autoantibodies to desmosomal or hemidesmosomal proteins in PPP, but studies evaluating autoantibody epidermal binding patterns in dogs with PF have identified four main IgG intercellular epidermal patterns. One pattern indicates that antibodies to desmoglein 1 are present as they are in PF. Other IgG autoantibodies have been detected against intracellular keratinocyte antigens in

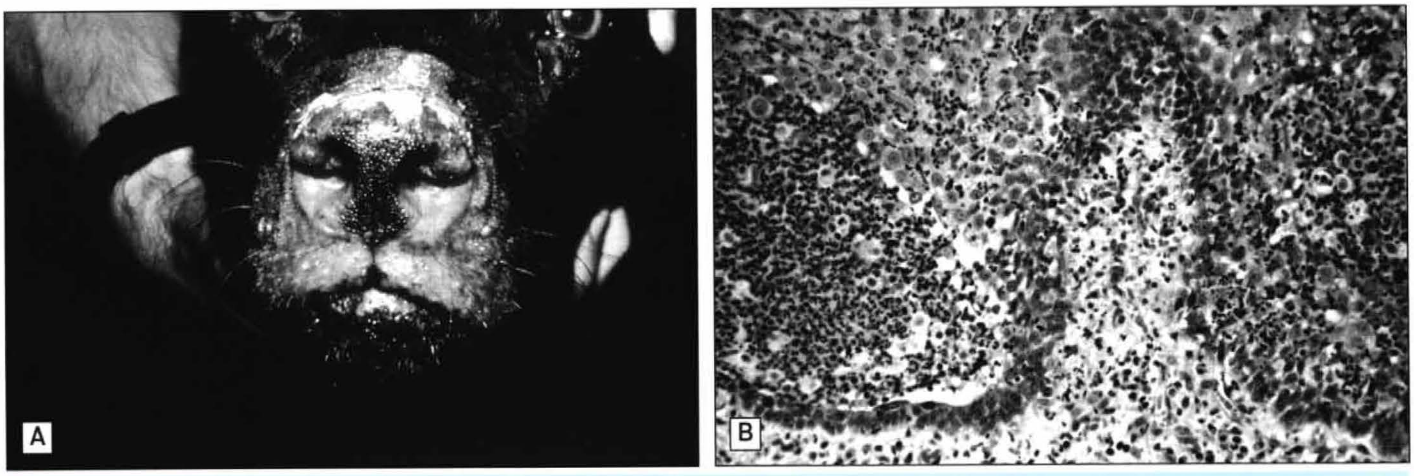

Figure 5.70 Panepidermal pustular pemphigus. (Courtesy of B Dunstan.) **A.** Scaly and crusted plaques and papules of the skin of the face and nasal planum. **B.** Pustules are present at several levels of the surface epithelium, including the suprabasal layers.

the stratum basale, spinosum, granulosum, and corneum, and basement membrane, substantiating the immunologically heterogeneous characteristics of pemphigus subtypes in the dog. These autoantibody binding patterns help to explain the variable location of epidermal pustules in subtypes of canine PF. *Original designations of pemphigus vegetans and pemphigus erythematosus in animals were based on similarities to the human conditions. Further studies suggest the conditions are not directly comparable.* Pemphigus erythematosus was considered to be a variant of PF with a facial or "lupus" lesion distribution occurring in dogs and cats. Criteria for diagnosis and differentiation from PF were dependant upon the immunological demonstration of both a diffuse cell surface IF or IHC pattern typical of the pemphigus group and a linear band of immunoglobulin, with or without complement, deposited at the basement membrane zone. For reasons previously stated, IF and IHC are not dependable. Reported cases of pemphigus vegetans in veterinary patients are very limited and originally thought to be benign variants of pemphigus vulgaris. The lack of clear clinical or histologic distinction between pemphigus vegetans and pemphigus erythematosus in the dog has led to the new inclusive entity of PPP by some veterinary dermatopathologists; however, *classification is controversial.* Some veterinary dermatopathologists also consider PPP to be a subtype of PF. Differentiation of PPP from typical cases of PF is discussed below.

PPP is less common than PF, but more common than PV. Akitas and Chow Chows are predisposed to develop both PPP and PF. In most cases, lesions first appear on the head in a symmetrical distribution involving the medial canthi of the eyes ("lupus distribution") and inner surfaces of the pinnae and nasal planum (Fig. 5.70A). The condition may remain localized to the head or proceed to the prepuce, abdomen, nail beds, and footpads. Some cases become generalized. *Gross lesions* are characterized initially by *erythematous macules* leading to *vesiculopustular lesions* resembling those of PF. Over time however, *the lesions progress to proliferative, scaly and crusted plaques or papules. Verrucous vegetations studded with small pustules are characteristic.* Depigmentation of the nasal planum may develop. The predominant *histologic lesions* are *numerous large, intraepithelial pustules composed of eosinophils, neutrophils and a few to large numbers of acantholytic epithelial cells.* These pustules occur at several levels through the surface epithelium, down to the suprabasal layers and also involve the infundibular outer root sheath (Fig. 5.70B). An eosinophilic to neutrophilic intra-infundibular mural folliculitis may be present. In mature lesions, there is papillated epidermal hyperplasia. The dermis has a perivascular to interstitial mixed infiltrate with eosinophils. Biopsies from the mucocutaneous junction or the nasal planum may be intensely plasmacytic and lichenoid. IFA or IHC may demonstrate immunoglobulin at intercellular bridges and/or the basement membrane zone, however results can be variable. *Histologic differentials* include *predominantly facial PF, discoid or systemic lupus, mycosis fungoides, pyoderma or bacterial folliculitis, dermatophytosis, and the uveodermatologic syndrome.* In PF, pustules are restricted to the stratum granulosum and stratum spinosum; in PPP, they occur in the deeper layers of the epidermis. PPP is also very proliferative compared to PF.

Pemphigus vulgaris *is the most severe and the more rare form of pemphigus in animals.* This life-threatening disease has been reported in the *dog, cat, horse, goat, monkey, and llama.* There are no apparent breed or sex predilections. Middle-aged **dogs** are most commonly affected. In humans and dogs, pemphigus vulgaris autoantibodies recognize the desmosomal protein, desmoglein 3. Desmoglein 3 also belongs to the cadherin family of cell-adhesion molecules and is most prominent in the area of the basal layer of keratinocytes of the epidermis and mucosal epithelium, hence lesions occur deeper in the epidermis and in the oral mucosa.. Desmoglein 3 is not a component of hemidesmosomes. The *fragile vesicles or bullae in the epidermis are extremely transient and readily rupture* to leave the more common presenting lesion of a roughly circular, shallow, flat-based erosion or ulcer. The margins often develop epidermal collarettes. Firm sliding pressure to adjacent unaffected skin may induce fresh vesicle formation or dislodge the skin (*the Nikolsky sign*). Lesions involve mucous membranes (Fig. 5.71A), mucocutaneous junctions and skin in the mechanically stressed areas such as the inguinal and axillary regions. Oral involvement is present in 90% of cases, and in 50% of cases the lesions commence in the mouth. Involvement of the nailbeds occurs also and corneal ulceration may be present. Animals may be febrile, depressed, and anorectic and have leukocytosis. Drooling is often a presenting complaint.

a variant of PV with IgG autoantibodies directed against plakin proteins and desmogleins 1 and 3 has been documented in the dog. Previously, autoantibodies to plakin proteins were thought to be unique to pemphigus associated with occult neoplasia (see Paraneoplastic pemphigus, p. 652).

Clinical differentials include bullous pemphigoid, erythema multiforme, toxic epidermal necrolysis, systemic lupus erythematosus, idiopathic ulcerative dermatosis of Collies and Shetland Sheepdogs, mycosis fungoides, and other diseases resulting in oral ulcers. Histopathologic findings of primary lesions in PV are diagnostic.

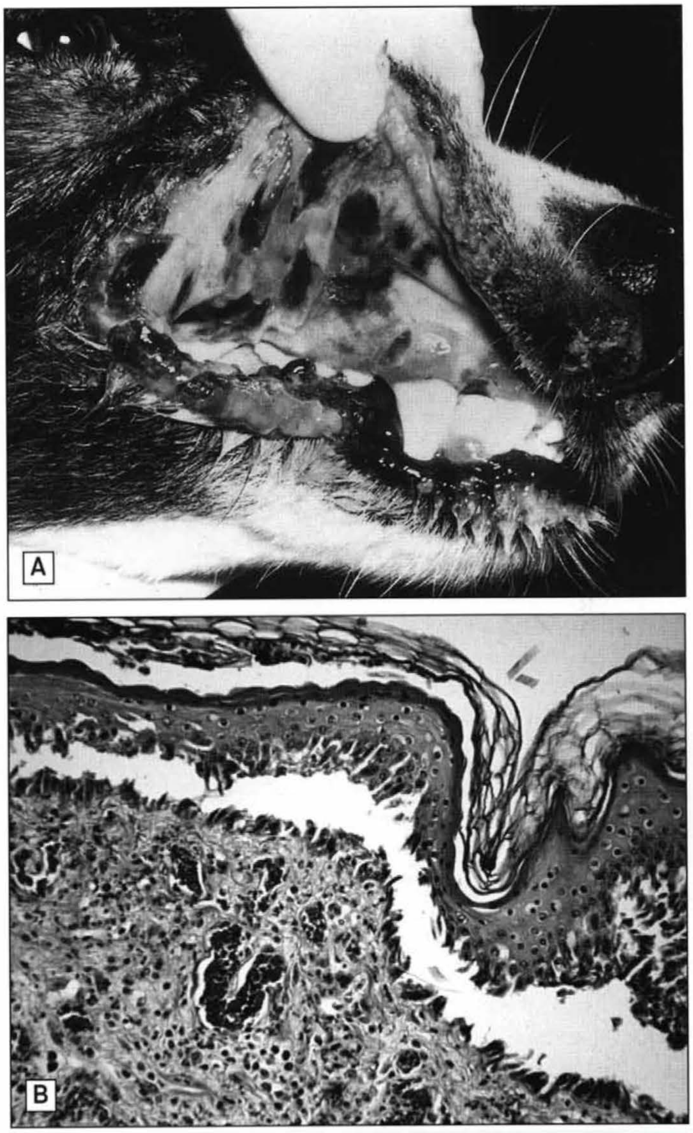

Figure 5.71 Pemphigus vulgaris. A. Flaccid bullae in oral mucosa. (Courtesy of Parker WM. and Can Vet J.) **B.** Separation of the epidermis occurs between the stratum spinosum and stratum basale. Basal keratinocytes also lose intercellular contact leaving the basal layer arranged to resemble a "row of tombstones." (Courtesy of B Dunstan.)

Microscopically, *the earliest lesions consist of spongiosis and vacuolation of the suprabasilar epidermis progressing to suprabasilar acantholysis with intraepidermal clefts, vesicles or bullae between the stratum basale and the stratum spinosum* (Fig. 5.71B). The basal keratinocytes, although separated from each other following disruption of intercellular contacts, are anchored to the basal lamina resembling a "row of tombstones." The outer layers of the epidermis form the roof of the vesicle that may contain a few acantholytic keratinocytes, singly or in clumps, but few or no inflammatory cells. The process may extend into the hair follicle epithelium. The dermal reaction in haired skin varies from a mild superficial perivascular to interstitial accumulation of mononuclear cells and eosinophils. A moderately intense lymphocytic-plasmacytic interface infiltrate may be present in mucosal lesions. The immunological pattern of labeling for autoantibodies with IFA or IHC is similar as that for PF. Recently,

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Bullous pemphigoid

Bullous pemphigoid is *a chronic, autoimmune skin disease characterized clinically by vesicles, bullae and ulcers, and histologically by subepidermal vesicles/bullae containing eosinophils or other leukocytes*. In humans, the autoantibodies are directed against bullous pemphigoid antigen 1 (BPAG1, a 230-kDa intercellular antigen) and bullous pemphigoid antigen 2 (BPAG2, also called type XVII collagen, a 180-kDa hemidesmosomal transmembrane molecule). In animals, only BPAG2 has been identified. The mechanism of dermoepidermal separation is thought to be the result of the release of proinflammatory cytokines IL-1, IL-5, IL-6, IL-8 and others by keratinocytes altered by antigen-antibody interactions. Cytokines recruit neutrophils and eosinophils leading to the release of damaging proteases. Separation of basal cells from the underlying dermis may also be the direct result of

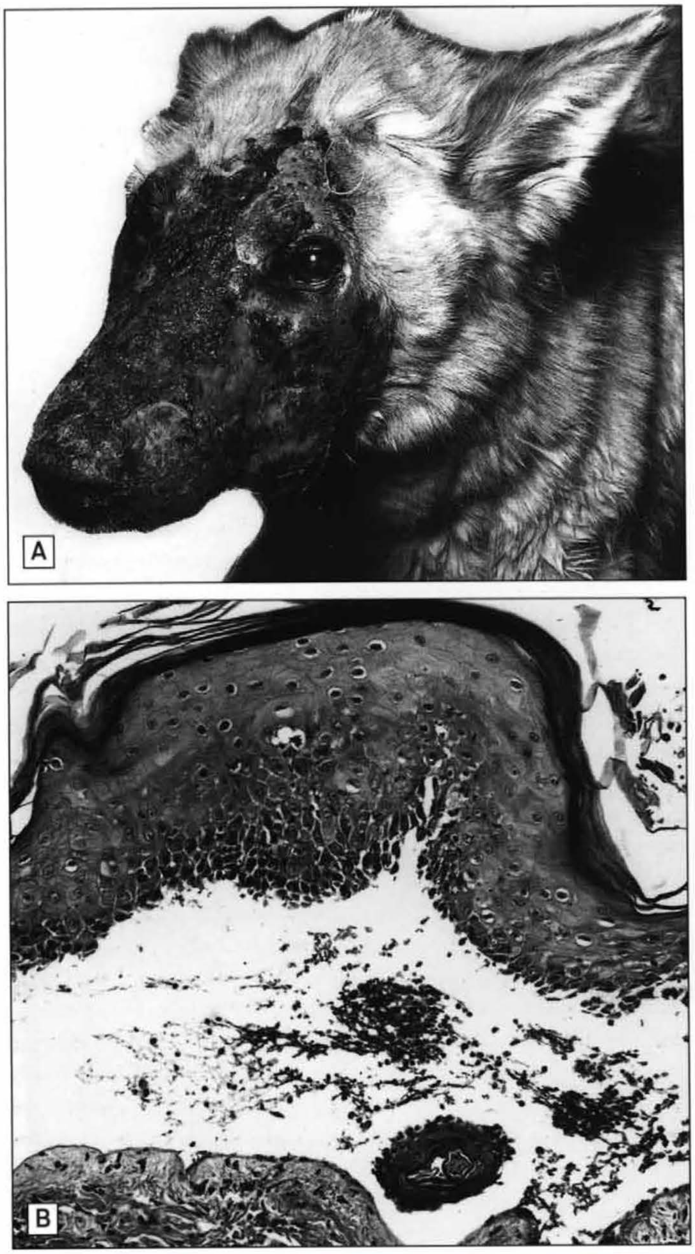

Figure 5.72 Bullous pemphigoid in a dog. **A.** Severe ulcerative facial lesion. **B.** Subepidermal vesiculation. The basement membrane zone forms the floor of the vesicle. Note the lack of acantholysis.

disorganization or internalization of components of the hemidesmosomes. In almost all cases, the stimulus triggering the immune response is unknown, although drugs may precipitate clinically, histologically and immunopathologically classical cases of pemphigoid that regress when the drug is withdrawn. Exposure to ultraviolet light is known to exacerbate bullous pemphigoid.

Bullous pemphigoid occurs in *dogs, cats, pigs, and horses*, with no apparent age or sex predilection. *Collies* appear predisposed. The gross lesions are vesiculobullous, ulcerative and crusted in nature. Grossly, bullous pemphigoid is usually indistinguishable from pemphigus vulgaris. In **dogs**, the lesions affect mucous membranes, particularly of the oral cavity, mucocutaneous junctions, and skin of the face, ears, axilla, groin, and paws (Fig. 5.72A). In **cats**, lesions may be more commonly present on the commissures of the lips, oral mucosa, and hairless regions of the concave aspect of the pinnae. In **horses**, the lesions are often generalized with marked sloughing of the oral mucosa and signs of systemic illness. Lesions on the rump and back characterize the disease in **Yucatan mini-pigs**. Mucosal lesions in pigs have not been described.

Histologically, there is *a subepidermal bulla filled with fibrin and variable numbers of neutrophils, eosinophils and mononuclear cells* (Fig. 5.72B). Eosinophils are not always present as they are in lesions of humans. *Acantholytic keratinocytes are not present.* The basal cells that line the roof of the bulla are not initially degenerate. The basement membrane zone lines the floor of the bulla as separation occurs within the lamina lucida. There is often marked subepidermal vacuolar alteration. The dermis is usually markedly edematous, capillaries are dilated and lined with swollen endothelial cells, and there is perivascular accumulation of neutrophils, eosinophils and mononuclear cells. Mild to moderate lichenoid interface dermatitis may be present, particularly in mucocutaneous regions. *Histologic differential diagnoses* include pemphigus vulgaris, systemic lupus erythematosus, epidermolysis bullosa, toxic epidermal necrolysis, and thermal burns.

Classically, bullous pemphigoid is characterized by the presence of *immunoglobulin (IgG, M, or A) and/or complement deposited at the basement membrane zone* within skin lesions. The BP180 antigen extends to the lateral and apical aspects of basal keratinocytes and labeling may extend to these areas. Serum autoantibodies may be detected in some cases.

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Linear IgA disease in dogs

Linear IgA disease (LAD) *is an acquired autoimmune subepidermal blistering disease occurring in humans and recently characterized in the dog.* IgG and/or IgA autoantibodies against LAD-1, defined as the processed extracellular domain of type XVII collagen, are demonstrable at the basement membrane zone of lesional skin and in the serum of affected dogs and humans. It is interesting to note the bullous pemphigoid (BP) antigen is also type XVII collagen, however in BP, antibodies recognize the transmembrane form of type XVII collagen. Clinically, LAD is characterized by erosions, ulcers, and crusts on the face, extremities, and in the oral cavity. Histologically, dermoepidermal clefting with little or no neutrophilic infiltration is present. *Differential diagnoses are similar to those listed for BP and TEN.* Differentiation of LAD from other subepidermal blistering diseases requires demonstration of linear IgA deposits at the basement membrane zone of skin lesions as well as identification of circulating autoantibodies against the target antigen, LAD-1. Too few cases have been documented in the dog to define the prognosis.

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Epidermolysis bullosa acquisita in the dog

Epidermolysis bullosa acquisita (EBA) is a subepidermal blistering disease characterized by vesicle formation in areas of concurrent neutrophilic superficial dermatitis. The condition has been reported in dogs and humans. Circulating and tissue-bound autoantibodies targeting the distal end of anchoring fibrils in the sub-lamina densa of the lower basement membrane zone have been demonstrated using immunofluorescence and immunoelectron microscopy. The actual target antigen has been shown to be the amino-terminal globular noncollagenous domain of type VII collagen. Clinical lesions in the dog are characterized by erythematous and urticarial eruption with vesicles, bullae, or ulcerations arising in areas subject to friction, including oral ulcerations. Histologically, dermoepidermal separation is present in association with marked neutrophilic inflammation in the superficial dermis and within the vesicles and bullae. Too few cases have been documented to provide information regarding triggering events or clinical outcome. Differentials include those listed for BP.

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Paraneoplastic pemphigus

Paraneoplastic pemphigus (PNP) is an aggressive form of pemphigus most often associated with solid or hematopoietic neoplasia. One case in a dog could not be associated with an underlying neoplasm, so it is possible for the condition to occur in the absence of underlying neoplasia. PNP has been documented in *humans and dogs*. Cutaneous lesions may precede detection of the neoplastic process. The condition is resistant to treatment.

Lesions consist of *severe mucosal and mucocutaneous blistering and erosions*. Histologically, lesions have a combined pattern of erythema multiforme and suprabasilar acantholysis characteristic of pemphigus vulgaris. Lymphohistiocytic, lichenoid interface dermatitis and apoptosis of keratinocytes with lymphocytic satellitosis throughout the epidermis are characteristic. Immunologically, labeling of intercellular bridges and/or basement membrane is detected by IHC or IFA staining. Direct immunofluorescence studies implicate autoantibodies to a 190 kDa protein, the 210 and 250 kDa desmoplakin proteins, and to the 230 kDa BPAGI, one of the bullous pemphigoid antigens. Not all cases with the above described lesions and autoantibodies have underlying neoplasia. Histologic differentials include PPP and those listed for PV and BP.

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Lupus erythematosus

Lupus erythematosus occurs in two forms: *systemic lupus erythematosus*, affecting multiple tissues including the skin, and *discoid lupus erythematosus* that is localized to the skin. While the two conditions share cutaneous lesions and certain immunological abnormalities, it is not certain that the one is a localized form of the other. Progression of discoid lupus to systemic lupus rarely, if ever, occurs.

Systemic lupus erythematosus (SLE) is a multisystem disease characterized by abnormalities in both the humoral and cellular immune systems. In the active state in the dog, lymphopenia with a marked decrease in percentage and absolute numbers of CD8+ cells and a decrease in absolute numbers of CD4+ cells occurs. The resulting CD4+:CD8+ ratio is increased from approximately 2.3 in the normal dog to almost 6 in dogs with active SLE. This ratio corrects with response to appropriate therapy. T-cell subset imbalances result in altered immune regulation and B cell hyperactivity. General failure of immune autoregulation, including defects of suppressor T-cell function and dysregulation of endogenous cytokines such as IL-6, results in the *production of antibodies against a variety of membrane and soluble antigens*. The most characteristic of these are the antinuclear antibodies directed against double-stranded DNA, RNA, nucleoprotein and histone-related antigens. Antibodies are also detected against erythrocytes, leukocytes, platelets, organ-specific antigens, clotting factors and IgG (rheumatoid factor). While some of these antibodies may be responsible for tissue injury, *the main tissue damage is effected by the antigen-antibody complexes that deposit at various sites throughout the body and incite a type III hypersensitivity reaction*. In the skin, the antigen-antibody complexes are located under the basement membrane zone of the epidermis and to a lesser extent in the walls of the dermal blood vessels. *Ultraviolet light exacerbates lesions* presumably by inducing the translocation of antigens normally expressed only intracellularly, such as extractable nuclear antigens, to the keratinocyte cell membrane. Specific autoantibodies to these antigens attach to keratinocytes and induce keratinocyte death by antibody-dependent cellular cytotoxicity mediated by lymphocytes or monocytes. Injured keratinocytes then release a plethora of cytokines including IL-1, IL-2, IL-6, and TNFβ with the resultant localization of the lymphohistiocytic infiltrate and increased B-cell activity. Other studies suggest ultraviolet radiation induces ICAM-1 expression, which in turn promotes release of TNFα. ICAM-1 is the major ligand for LFA-1, an adhesion molecule found on all leukocytes.

Canine and **feline** SLE occurs without clear age or sex predilections. Most dogs are middle-aged at the time of diagnosis. In humans, there is a clear preponderance of SLE in females, whereas one canine study found male dogs were more commonly affected. Collies, Shetland Sheepdogs, German Shepherd Dogs, and Siamese, Persian, and Himalayan cats appear predisposed. SLE is a chronic condition that *may wax and wane*, and clinical signs may appear sequentially rather than simultaneously, confounding the diagnosis. Sex hormones and nutrition are known to modulate disease activity. Drug administration, pregnancy, prolonged exposure to extreme heat or cold, and excessive exposures to UV radiation have been known to trigger onset of SLE. *Polyarthritis, fever of unknown origin, anemia, thrombocytopenia, stomatitis, glomerulonephritis, and dermatitis are the most common manifestations.* Polymyositis, lymphedema, and neurological signs may also be present. Dermatological signs occur in $\sim 1/3$ of affected dogs and cats. Skin lesions tend to occur in areas exposed to sunlight such as the face, ears, nose, lips, and sparsely haired, lightly pigmented, thin skin of other body regions (Fig. 5.73A). Footpads may be hyperkeratotic or ulcerated. Nail beds may be involved. *Gross skin lesions are extremely variable*, from a mucocutaneous, ulcerative dermatitis resembling pemphigus vulgaris and bullous pemphigoid to erythema, scaling and alopecia of little specificity. A bullous form of SLE with IgG autoantibody against collagen VII has been reported in the dog and found to be similar to the condition in humans. Secondary bacterial pyodermas are common. Occasionally, dogs develop a symmetrical scaling, erythematous and alopecic maculopapular lesion extending over the bridge of the nose onto the cheeks, resembling in distribution the classical malar rash of human systemic lupus.

The most common presenting sign in **horses** with SLE is *a sharply demarcated zone of depigmentation of the skin around the eyes, lips, nostrils, genitalia and skin of the perianal and perineal regions.* Depigmentation may be gradual or rapid and may be accompanied by erythema and scaling. Additional lesions reported have included lymphedema of the extremities, panniculitis, alopecia, and scaling of the face, neck, and trunk, and generalized exfoliative dermatitis. Systemic signs such as polyarthritis, anemia, thrombocytopenia, proteinuria, fever and weight loss may also occur in the horse, but are not common.

The microscopic lesions of SLE are variable. The critical diagnostic pattern is lichenoid interface dermatitis with hydropic degeneration of basal cells and a lymphohistiocytic to plasmacytic infiltrate at the dermoepidermal junction (Fig. 5.73B). Lymphocytes predominate. *Apoptosis of basal keratinocytes and pigmentary incontinence, a consequence of the basal cell degeneration, are diagnostically helpful histologic lesions, as is marked subepidermal vacuolar change* (Fig. 5.73C). Occasionally, this change or a leukocytoclastic vasculitis may lead to cleft or bulla formation between the dermis and epidermis. The same changes often affect follicular epithelia. A *subepidermal bullous form of SLE* with documented circulating IgG autoantibodies to type VII collagen as well as IgG deposits at the basement membrane zone has also been reported in a dog. In the acute cutaneous lesions of SLE, there is moderate to marked dermal edema, capillary dilation, extravasation of leukocytes and erythrocytes and variable lymphohistiocytic infiltrates around vessels of superficial and deep capillary plexuses. Mucinous degeneration may be prominent. As the lesion becomes chronic, there is focal thickening of the basement membrane zone reflecting accumulation of antigen-antibody complexes. Fibrinoid deposits may also occur around superficial blood vessels. Occasionally leukocytoclastic vasculitis develops. Other lesions include ortho- or parakeratotic hyperkeratosis, epidermal hyperplasia, dyskeratosis, adnexal atrophy and dermal fibrosis. Although some follicles may atrophy over time, other follicles in anagen are typically present. Immunoglobulin and complement deposition at the basement membrane zone may be detectable by IF or IHC staining. An ANA titer of >256 is present in 97–100% of dogs with SLE but is not

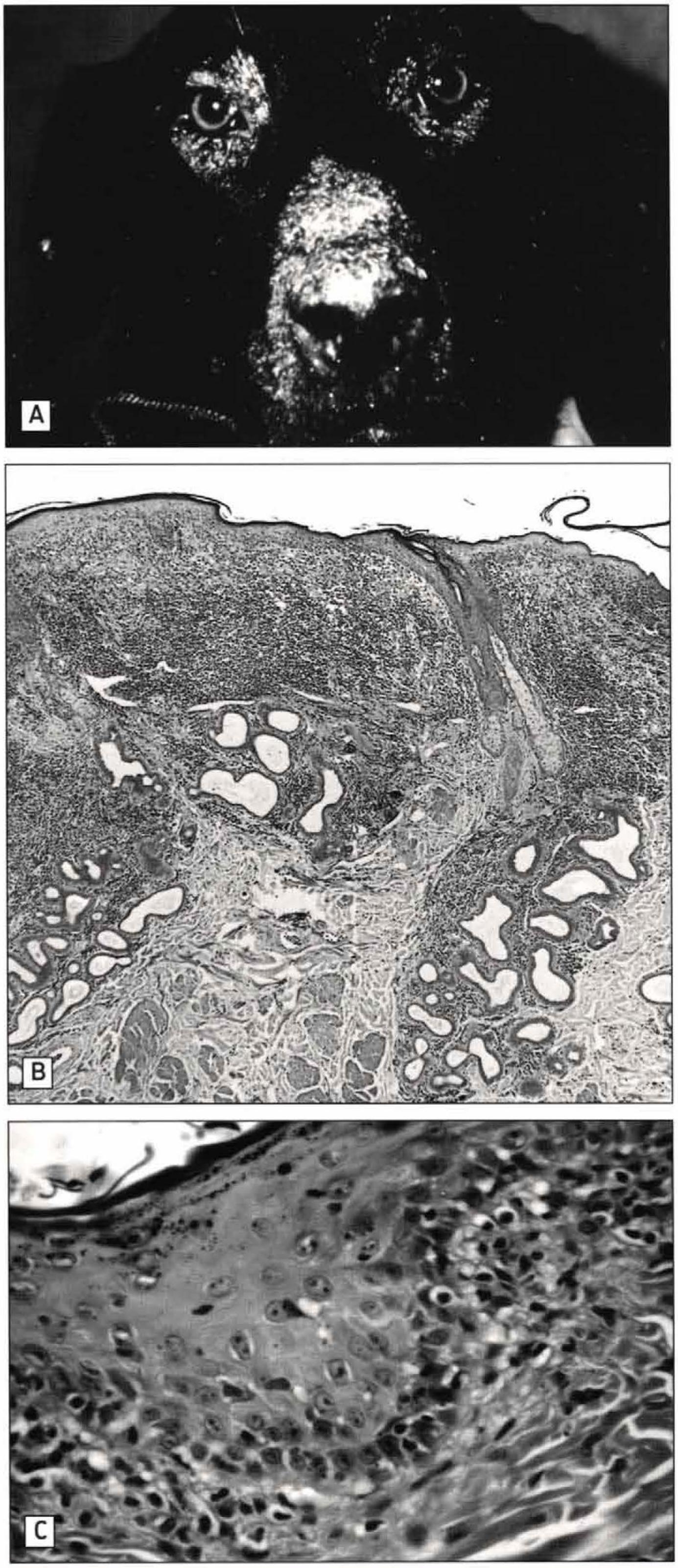

Figure 5.73 Systemic lupus erythematosus (SLE). **A. Symmetrical facial lesions** in SLE. (Courtesy of K Beale.) **B. Lichenoid interface dermatitis** in SLE. **C.** Hydropic degeneration of basal cells, apoptosis, and a lymphohistiocytic-to-plasmacytic infiltrate at the dermoepidermal junction in SLE.

entirely specific for the disease. *Differential diagnoses* vary with the type of lesions present but may include other autoimmune skin diseases, drug eruption, vasculitis, mycosis fungoides, dermatomyositis, demodicosis, dermatophytosis, and seborrhea. In the horse, other depigmenting conditions such as idiopathic leukoderma and the breed-specific, juvenile Arabian leukoderma should be considered.

The localized cutaneous form or **discoid** form of **lupus erythematosus** (DLE) is described most commonly in the *dog*, and rarely in *horses and cats*. DLE as a distinct entity in animals is currently under close scrutiny, and terminology for this clinicopathologic condition is likely to change as the pathogenesis becomes understood and may be found to be different from SLE. The relationship of DLE to SLE is obscure, as DLE does not progress to SLE. The human form of DLE is not comparable to the current condition referred to as DLE in animals. **Photosensitive nasal dermatitis** *is a more general term proposed for use for these conditions in animals until the pathogenesis can be determined.* In **dogs**, the disease is more prevalent in bitches and there is a marked breed predilection in Collies, Shetland Sheepdogs, Siberian Huskies, and German Shepherds. *Sunlight exacerbates the lesions*, which typically affect the nasal planum in dogs and cats, and the face and neck in horses. In the dog, perioral, periocular, and pinnal skin may also be affected. The lesions include erythema, depigmentation, scaling, crusting, alopecia and occasionally ulceration. Rarely, dogs are presented with only nasodigital hyperkeratosis. *Histologic findings are very similar as previously described for systemic lupus.* The epidermis may be more hyperplastic and the infiltrate is predominantly comprised of lymphocytes and macrophages with fewer plasma cells, and is often denser than in SLE. If a secondary mucocutaneous pyoderma is present, plasma cells may be more numerous. IF or IHC staining may reveal IgM, IgG, IgA, and/or complement at the basement membrane zone, however false-positives of the nasal planum are common. *Clinical differential diagnoses* include nasal solar dermatitis, PF, PPP, uveodermatologic syndrome, contact dermatitis, SLE, trauma, mycosis fungoides, drug eruption, dermatomyositis, and vitiligo. *A major histologic differential is a mucocutaneous pyoderma.* Mucocutaneous pyodermas lack evidence of basal cell damage and are predominantly plasmacytic. Both conditions may be present simultaneously.

Vesicular cutaneous lupus erythematosus is a disorder formerly known as *ulcerative dermatosis of the Collie and Shetland Sheepdog*. This condition was originally thought to be a variant of dermatomyositis, but recent immunological studies indicate the presence of autoantibodies that target nuclear antigens similar to that seen in cutaneous lupus erythematosus. Antinuclear antibody testing is negative. Lesions develop in middle-aged to older dogs. The Shetland Sheepdog and Collie appear predisposed to lesion development. Clinical lesions consist of vesicles and bullae that evolve into erosions and ulcers. The inguinal and axillary regions are most commonly involved, but lesions may also occur on mucocutaneous junctions. Histologic changes consist of interface lymphocytic dermatitis with hydropic degeneration of basal cells, keratinocyte apoptosis, and extensive vesicles and bullae at the epidermal–dermal junction.

Exfoliative cutaneous lupus erythematosus, formerly known as *lupoid dermatosis of the German shorthair pointer*, occurs in young (3 months to 3 years) German Shorthair Pointers. Gross lesions consist of scaling and crusting commencing on the head and back but becoming generalized. The most severe lesions are often present at pressure points and the scrotum. Pyrexia, peripheral lymphadenopathy and lethargy may be present. Histologic changes mimic cutaneous lesions of systemic lupus erythematosus. Some affected dogs may have a positive antinuclear antibody test.

Lupus panniculitis (lupus profundus) is *characterized by well-circumscribed subcutaneous nodules over the trunk and proximal extremities.* It is reported rarely in dogs. Histologically, it is initially a septal panniculitis with dense nodular infiltrates of lymphocytes and plasma cells and fewer macrophages. The fat lobules may undergo necrosis, often represented by hyalinization without mineralization. Usually *the dermis and epidermis show the typical microscopic features of lupus*, including hydropic degeneration of basal cells, pigmentary incontinence, thickened basement membrane zone and sclerosis of the dermis. Abundant mucinous degeneration is usually prominent. Leukocytoclastic vasculitis may be present in the interlobular septa. Histologically, lupus panniculitis is distinctive, but a rabies vaccine-induced lymphocytic panniculitis, that is virtually indistinguishable histologically, occurs in dogs.

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Other immune-mediated dermatoses

Drug eruptions

Drug eruptions are occasionally reported in *dogs, cats, and horses* and rarely in other domestic animals. Drugs responsible for skin eruptions may be administered *orally, topically, or by injection or inhalation.* Any drug may cause an eruption, and any one drug consistently produces no specific type of reaction. Thus, *drug eruption can mimic virtually any dermatosis. Erythema multiforme, toxic epidermal necrolysis, and vasculitis are well-recognized dermatoses that can be manifestations of cutaneous drug eruptions* and are described individually later in this section. *Criteria* for establishing a cutaneous reaction as a confirmed drug eruption include: (1) elimination of other causes of the skin lesions, (2) timing of onset of reaction with administration of a suspect drug, (3) improvement upon drug withdrawal, (4) recognition that the suspect drug has been associated with similar reactions in other animals or species in the past, and (5) recurrence of lesions upon rechallenge of the patient with the drug. Understandably, all five of these criteria are not often met, particularly rechallenge as this can be associated with high morbidity or mortality. Time period between drug administration and onset of an adverse reaction varies widely from hours to months and lesions can result from a single or repeated administration. *Drug-induced gross and histologic lesions are not pathognomonic* for an adverse cutaneous drug eruption although histopathological changes may often point to a limited list of differentials. *Histologic patterns that have been recognized as forms of adverse drug reactions include: urticaria; perivascular dermatitis (allergy-like); hydropic and/or lichenoid interface dermatitis (erythema multiforme, toxic epidermal necrolysis, lupus erythematosus-like); perforating folliculitis; vasculitis; intraepidermal vesiculopustular dermatitis (pemphigus-like); and subepidermal bullous reactions (pemphigoid-like).* The pathogenesis of lesion formation in many types of drug eruptions is not known with certainty. Drug hypersensitivities are believed to involve all four types of hypersensitivity reactions, and in some cases are not thought to be immunologically mediated.

The most common drugs recognized to produce hypersensitivity reactions in domestic animals are the *sulfonamides (especially trimethoprim-potentiated) and penicillins.* Erythema multiforme and toxic epidermal necrolysis (discussed later in this section) have been seen most commonly with trimethoprim-potentiated *sulfonamides, cephalosporins and levamisole. Diethylcarbamazine* and *5-fluorocytosine* have been associated with fixed drug eruption on the scrotum of dogs. The ulcerative lesions healed with hyperpigmentation when the drug was withdrawn but recrudescence at the same site, with vesiculation, occurred when the dog was rechallenged. *The mechanism underlying fixed drug eruption is unknown.* In people, the epidermal invasion of T cells in fixed drug eruptions is associated with the expression of the intracellular adhesion molecule ICAM-1 on the surface of lesional keratinocytes. *Cyclosporin* has been reported to cause a lymphoplasmacytoid dermatitis with malignant features, usually manifest as a solitary plaque or nodule. *Urticaria and angioedema* have been associated with levamisole, barbiturates and some antibiotics. Drug eruptions manifesting as *exfoliative erythroderma* have been seen in dogs treated with levamisole and lincomycin. Drug eruptions resembling bullous pemphigoid clinically, histologically and immunohistochemically have been associated with *triamcinolone.* It is quite possible that some cases described as bullous pemphigoid actually represent drug eruptions. Pemphigus foliaceus-like drug eruptions have been described in cats treated with ampicillin or cimetidine and dogs receiving trimethoprim-sulfonamide. Lesions consisted of classical subcorneal pustules with acantholytic cells, with an additional feature of vasculitis in some cases. Systemic lupus-like drug reactions have been reported in dogs and in cats. Cutaneous vasculitis caused by immune complex deposition may be initiated by drug administration. Sulfadiazine administration in Doberman Pinschers causes a poorly defined skin rash as well as ocular, joint, kidney, and hematological abnormalities suggestive of systemic vasculitis.

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Cryopathies

Cryopathies are cold-related hypersensitivity syndromes that include **cold agglutinin disease**, *a condition in which erythrocyte autoantibodies react at lower temperatures to produce microvascular thrombosis in superficial dermal vessels.* Other cryopathies are small vessel vasculopathies associated with abnormal serum proteins (paraproteins) that precipitate out of the serum at cooler temperatures and redissolve upon warming. *Paraproteins include cryofibrinogens, cryoglobulins, macroglobulins, and gamma heavy chains.* Cryoglobulins may be monoclonal IgG or IgM (type I cryoglobulinemia), or mixed mono- and polyclonal with one antibody directed against the other, in which case, immune complexes are in the circulation (type II cryoglobulinemia). In type III cryoglobulinemia, the immunoglobulins are polyclonal. Type I cryoglobulinemia is often associated with underlying disease such as multiple myeloma, leukemia, or lymphoma where as in type II or III, connective tissue disease such as SLE, or a systemic infection is present. Type II and III may also be idiopathic. *Most cases reported in* *dogs and cats are the result of cold-reacting anti-erythrocyte antibodies.* Cold agglutinin disease has been reported rarely to cause skin disease in dogs and cats. Cold agglutinin disease as a post-infectious event has been reported in sheep, horses, and pigs. One report in Birman cats occurred in association with neonatal isoerythrolysis and suggested that group B blood group cats may be predisposed. Lead exposure and hemobartonellosis have also been implicated as predisposing factors in dogs and cats. Cryopathy due to cryofibrinogens and cryoglobulins has also been reported in the dog. A monoclonal cryoglobulinemia associated with multiple myeloma has been reported in a cat.

Cutaneous signs associated with cryopathies result from vascular insufficiency (obstruction, stasis, spasm, and thrombosis). Lesions include erythema, purpura, cyanosis, necrosis, ulceration, and occasionally sloughing of extremities, and are precipitated or exacerbated by exposure to cold. The paws, pinnae, nose and tip of the tail are typically involved. Skin biopsy usually reveals necrosis, ulceration and often secondary suppurative changes. *Microvascular thrombosis* may be evident in cold agglutinin disease. In other types of cryopathies, paraproteins are precipitated out and deposited throughout vessel walls and within vascular lumens. The proteins stain pink with HE and bright red with PAS. *Hemorrhage* may be present. In type I cryopathies, inflammation is usually absent, whereas a leukocytoclastic vasculitis is often present in types II and III. *Diagnosis requires a test for cryoprecipitates and analysis of serum proteins.* Erythrocyte agglutination on a cooled slide that reverses upon warming and a positive Coombs' test at 4°C should be present in cases of cold agglutinating anti-erythrocyte antibodies. *Differential diagnoses include frostbites, disseminated intravascular coagulation, SLE, dermatomyositis, and other causes of vasculitis.*

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Graft-versus-host disease

Graft-versus-host disease (GVH) occurs as a complication of bone marrow transplantation. It has been recorded in *humans, dogs, cats, and horses.* The disease results from donor T-lymphocyte responses to recipient histocompatibility antigens in the *acute phase*, and from immunocompetent recipient lymphocyte responses to transplantation antigens in the *chronic phase*. Principal target organs are the *skin, liver and intestinal tract.* GVH is considered the *classic example of cell-mediated attack upon the epidermis.* Proposed target cells in the skin include basal keratinocytes, follicular stem cells, and/or Langerhans cells. Studies indicate both CD8+ and CD4+ lymphocytes are active in the disease process, with CD8+ T cells found more often in the epidermis and CD4+ T cells in the dermal infiltrate. NK cells may also play a role. T cell production of IFNγ results in keratinocyte expression of ICAM-1, the binding molecule for LFA-1 found on infiltrating T cells. TNFα production by activated keratinocytes and Langerhans cells and IFNγ lead to keratinocyte production of IL-8 attracting more lymphocytes.

Skin lesions in the acute phase include generalized erythematous macules, multifocal alopecia, and ulcerative dermatitis. Oral lesions, epidermal detachment and follicular papules may be present. In the chronic phase, erythema, irregular hyperpigmentation, dermal fibrosis, cutaneous atrophy, and cicatricial alopecia from chronic ulceration may occur. *Histologic findings* include various degrees of hydropic and/or lichenoid interface dermatitis with marked lymphocytic satellitosis of necrotic keratinocytes in all layers of the epidermis and the follicular epithelium. There are lymphocytic exocytosis and spongiosis. Dermal lymphocytic infiltrate is variable. Dermal–epidermal cleft or ulceration may also be present. Over time, epidermal atrophy and dermal sclerosis with loss of adnexa may occur. Basal layer vacuolation and inflammation persist. *Differential diagnoses* include erythema multiforme, toxic epidermal necrolysis or other drug eruption, alopecia areata, and radiation dermatitis. *The history and the triad of cutaneous, hepatic and intestinal signs should lead to the proper diagnosis of GVH.*

Erythema multiforme

Erythema multiforme (EM) is an uncommon cutaneous reaction reported in the *dog, cat, horse, and cow.* EM is *usually triggered by drug therapy or infection* (viral, fungal, or bacterial), but may also occur in conjunction with underlying *neoplasia*, or may be *idiopathic*, particularly in older dogs. A few of the drugs reported to trigger EM in animals include *d-limonene-based dips, levamisole, cephalexin, trimethoprim-sulfa, gentamicin, and penicillin.* The pathogenesis is not completely understood, however studies support a *combined type III and IV immune reaction* as the mechanism of lesion formation. The possibility also exists that a drug or infectious agent may induce keratinocytes to undergo apoptosis without involvement of the immune system. The *histologic lesions*, cellular infiltrates and types of lymphocytes present mirror that of GVH disease, lending further support for a cell-mediated immune response. IF or IHC results are variable but may show deposits of immunoglobulin and/or complement in the dermal microvasculature or at the dermoepidermal junction. Circulating immune-complexes may also be present. It is unclear whether these findings indicate a role for humoral immunity in skin lesions or are epiphenomena. Antibodies to the desmosomal proteins, desmoplakin I and II have been documented in cases of EM major (see below) in humans but it is not yet determined whether antibodies formed secondary to keratinocyte damage and antigen exposure or as a primary event.

EM occurs in *two forms* that may overlap one another and with toxic epidermal necrolysis. **EM minor** *is characterized chiefly by an acute onset of symmetric erythematous macules and papules* (Fig. 5.74A). These spread peripherally and clear centrally to produce annular, serpiginous to arciform and polycyclic patterns (Fig. 5.74B). *Classic "target" lesions may be present* (5.74C).

Urticarial plaques with similar configurations and vesiculobullous lesions also occur. Lesions tend to involve mucocutaneous junctions,

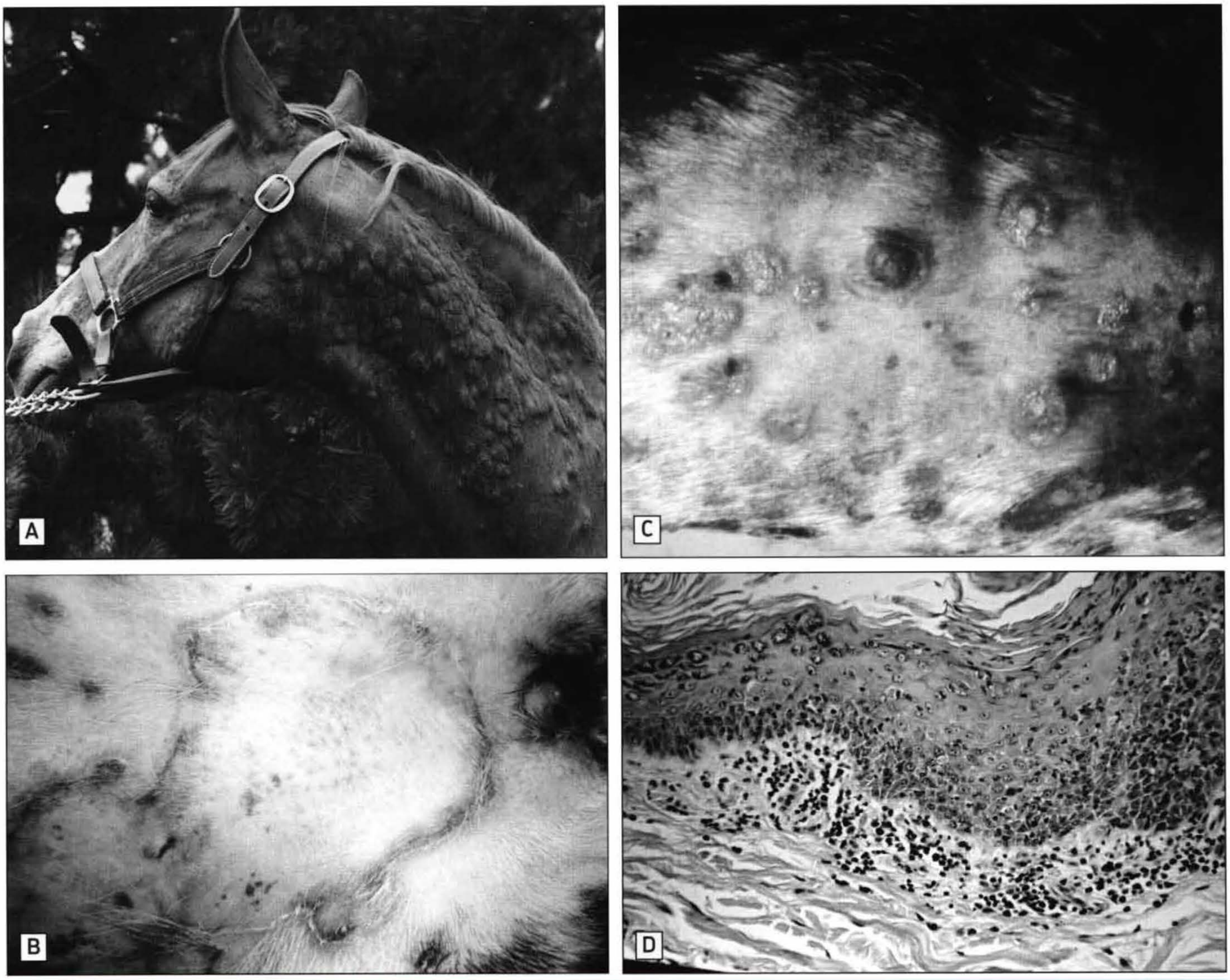

Figure 5.74 Erythema multiforme (EM). **A.** In a horse, **multiple irregular plaques** that are not transient as they are in urticaria (see Fig. 5.64). **B.** Erythematous and erosive **serpiginous to arciform lesions** in EM in a dog. (Courtesy of University of Florida Clinical Dermatology Service.) **C. Classical target lesions** with central clearing in EM in a dog. (Courtesy of University of Florida Clinical Dermatology Service.) **D.** Characteristic **interface dermatitis** with necrotic keratinocytes, often with lymphocytic satellitosis, scattered throughout the epidermis in EM in a dog.

the ventral trunk, proximal limbs and occasionally the nose and footpads. Pruritus is usually not a feature and there are no systemic signs, other than those attributable to the underlying cause. In **EM major** (Stevens–Johnson syndrome), *widespread mucosal lesions, extensive necrotizing and vesiculobullous skin lesions and signs of systemic illness such as pain, lethargy, pyrexia, and anorexia are present.* Histologically, the lesions of EM show the *characteristic interface dermatitis* (Fig. 5.74D). Necrotic keratinocytes, often with lymphocytic satellitosis, are scattered throughout the epidermis and the adnexal epithelia (see Fig. 5.11). A sparse lymphohistiocytic infiltrate occurs at the dermoepidermal junction and around superficial blood vessels. Vasculitis is not a feature. As the lesion ages, the infiltrate may become more dense, subepidermal bullae may develop and the epidermal necrosis may become confluent. Secondary exudation follows necrosis. *Clinical differentials* include TEN, burns, SLE, urticaria, vasculitis, other immune-mediated dermatoses, mycosis fungoides, superficial necrolytic dermatitis, and necrotic arachnidism. The *histologic pattern* is shared by GVH disease, acute lupus erythematosus, and less severe forms of toxic epidermal necrolysis. Occasionally, lesions may histologically resemble mycosis fungoides (MF). Important distinguishing features are atypical lymphocytes (MF) and apoptosis accompanied by lymphocytic satellitosis in all levels of the epidermis (EM). A less common finding in erythema multiforme is severe dermal edema with vertical orientation of dermal collagen and subepidermal vesiculation. *EM may be self-limiting, resolve with elimination of underlying triggering event, or as in some idiopathic cases, become chronic or recurrent.* Chronic EM is more commonly seen in older dogs and is characterized by the additional changes of a proliferative epidermis with marked parakeratosis and extensive lymphocytic exocytosis.

Toxic epidermal necrolysis

Toxic epidermal necrolysis (TEN) is characterized by disseminated erythema, widespread bullous necrosis of epidermis and mucous membranes,

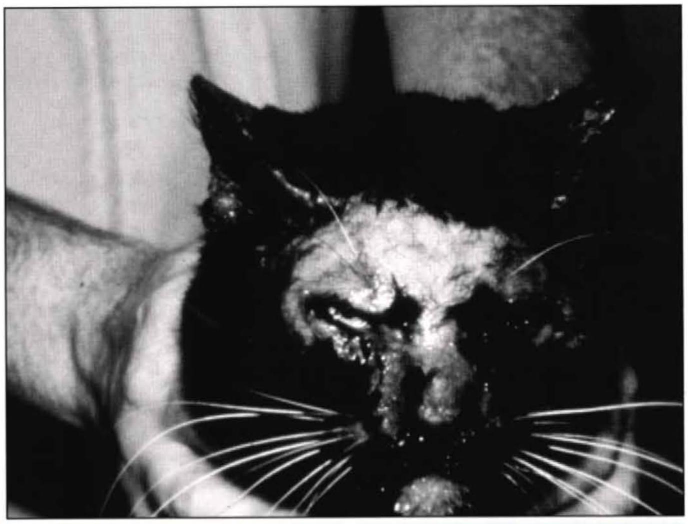

Figure 5.75 Complete loss of the epidermis of the skin of the face in **toxic epidermal necrolysis.** (Courtesy of University of Florida Clinical Dermatology Service.)

and severe toxicity. It is most often associated with drug intake or concurrent infections, but many cases are idiopathic. TEN and EM major (Stevens–Johnson syndrome) are very similar in clinical presentation, and one may be the extension of the other. The lesions of erythema multiforme major show areas of full-thickness epidermal necrosis as in TEN, but differences in the clinical course, response to therapy, and fatality rate suggest that they are separate entities.

The pathogenesis of TEN is not known. Cell-mediated immune mechanisms have been postulated as the basis for necrosis. Lymphopenia and alterations in the T-helper and T-suppressor lymphocyte ratios occur in human patients. Drugs associated with TEN, however, do not induce lymphocyte transformation in vitro. Furthermore, the widespread confluent epidermal necrosis, in the virtual absence of infiltrating inflammatory cells, argues against an effector role for cytotoxic lymphocytes. As in EM, lesional deposition of antibody and complement may represent epiphenomena.

Toxic epidermal necrolysis is a serious, acute-onset and life-threatening disease in dogs and cats. It has been associated with concurrent infectious disease or neoplasia, and *is a form of drug eruption*. Drugs implicated include penicillins, cephalosporins, levamisole, and 5-fluorocytosine. Affected animals are painful, anorectic, febrile, depressed and lethargic. Mortality is high. Lesions are usually generalized and involve mucous membranes, mucocutaneous junctions, and haired skin (Fig. 5.75). There is ulceration, often with an epidermal collarette, crusting, scaling and secondary pyoderma. The skin is extremely painful and fragile and may slough with routine handling. A *positive Nikolsky sign* is often present and large sheets of epidermis may detach. Vesiculation may occur but is often transient. Lesions may also involve the respiratory and digestive tracts. *Grossly*, toxic epidermal necrolysis may be difficult to distinguish from EM major, superficial burns, pemphigus vulgaris, bullous pemphigoid, systemic lupus erythematosus, thallium poisoning, mycosis fungoides, necrotic arachnidism and candidiasis.

Histologically, *TEN is characterized by full thickness coagulative necrosis of the epidermis, which may extend into the external root sheath of the hair follicle*. Separation of the necrotic epidermis occurs at the dermoepidermal junction and leads to subepidermal vesiculation. Dermal inflammation is minimal until the epidermis becomes detached. The reaction, when present, is perivascular or rarely lichenoid and chiefly lymphohistiocytic. Lesions with less than full-thickness epidermal necrosis and more intense dermal inflammation may be indistinguishable from cases of erythema multiforme with confluent zones of keratinocyte necrosis.

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Vasculitis

Cutaneous vasculitis, a condition in which dermal vessels are the principal target of injury, can be associated with a primary immunopathogenic event, or may be secondary to drug therapy, infectious agents, systemic connective tissue disease, underlying malignancy, toxins, or, often may be idiopathic. Classic examples include infection with an endotheliotropic organism (*Rickettsia rickettsii*), immune-complex vasculitis (systemic lupus erythematosus) and septicemia (*Erysipelothrix rhusiopathiae*). Although cutaneous vasculitis has many causes, many cases fall into the category of type III hypersensitivity reactions. Cutaneous vasculitis may be a secondary event associated with cutaneous diseases such as staphylococcal infections. Drug-induced vasculitis is thought to be the result of haptenization of host proteins, direct drug toxicity against vessel walls, autoantibodies against endothelial cells, or possibly cell-mediated cytotoxic reactions against vessels.

Cutaneous vasculitis is seen most often in the *dog and horse* and is considered to be rare in cats, pigs and cattle. Approximately 50% of cases of vasculitis in dogs and horses are idiopathic. *Gross lesions suggestive of vasculitis include erythematous plaques or macules, palpable*

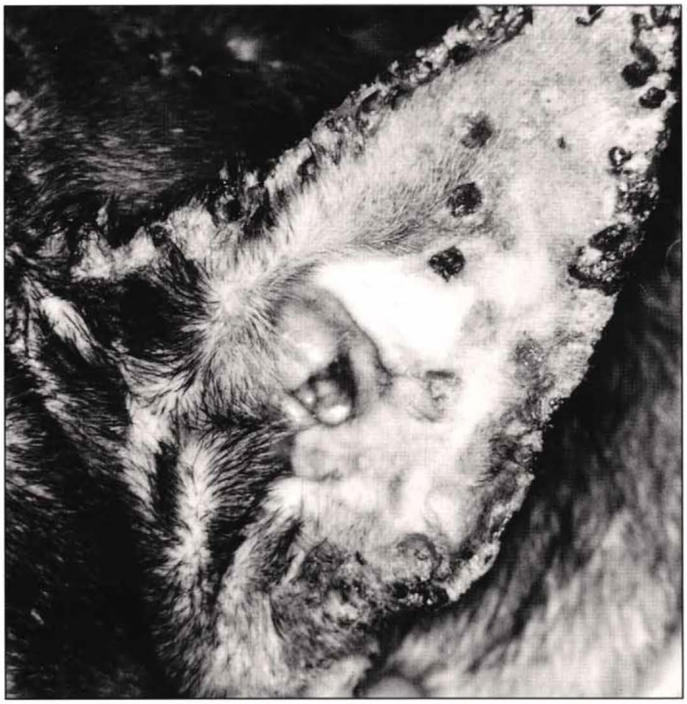

Figure 5.76 Typical punched-out ulcers on the margins of the ears, due to **vasculitis** in a dog.

purpura, hemorrhagic bullae, edema, necrosis, and well-demarcated ulcers (Fig. 5.76). Ischemic necrosis may occur leading to eschar formation and sloughing of the skin or distal extremities. Paws, pinnae, lips, tail, and oral mucosa are most commonly affected. Ischemic atrophy of folliculosebaceous units may lead to areas of alopecia and scaling in long standing lesions of more subtle vasculitis.

Small arterioles, capillaries and post-capillary venules are most often affected in cutaneous vasculitis. Histologic lesions in the classic case feature evidence of damage to the vessel wall such as *necrotic cell debris and fibrinoid necrosis within the vessel wall, mural infiltrates of either neutrophils or lymphocytes, and intramural or perivascular edema, hemorrhage or fibrin exudation* (see Fig. 5.15). Subtle cases of vasculitis may be characterized by edema, rare necrotic cells in the vicinity of vessels and a mild interstitial mononuclear cell infiltrate. Microscopic evidence of vasculitis in the dog is often subtle and may lead to underdiagnosis of the condition. Some pathologists prefer to refer to very cell-poor cases of vasculitis as a *vasculopathy* rather than true vasculitis when degenerative lesions of vessel wall and evidence of ischemia are detected in the absence of inflammation of vessel walls. Edema, hemorrhage, and evidence of ischemia and homogenization of dermal collagen are helpful changes. *A preponderance of leukocytes in vessels walls rather than in the dermis suggests that vessels are the primary target of inflammation and not just serving to deliver leukocytes.* Vessels will stand out in the section due to the attraction of leukocytes to vessel walls.

Some generalizations regarding the pathogenesis of the vasculitis can be made from types of leukocytes present within vessel walls; however, the types of leukocytes present does not necessarily point to a particular etiology and may simply reflect the stage of the disease process.

- *Neutrophilic vasculitis* is most suggestive of a type III immune-complex hypersensitivity reaction and is often referred to as *leukocytoclastic vasculitis* if neutrophil degeneration and nuclear karyorrhexis are evident. Examples include the vasculitis associated with staphylococcal dermatitis in dogs, immune-mediated vasculitis as in SLE, some drug eruptions, and septicemia.
- *Lymphocytic vasculitis* may suggest a cell-mediated immune basis, such as rabies vaccine-induced cutaneous vasculitis and some drug eruptions.
- *Eosinophilic vascular infiltrates* are most suggestive of a type I hypersensitivity reaction. Eosinophils may be the predominant cells in some cases of equine vasculitis and in association with the markedly eosinophilic dermatitis seen in some arthropod bite lesions, mast cell tumors, or in lesions of the eosinophilic granuloma complex in the cat.

Involvement of deep dermal blood vessels in all cases may suggest systemic disease.

In **dogs**, cutaneous vasculitis has been associated with drugs, systemic or localized infections (Rocky Mountain spotted fever, staphylococcal pyoderma), malignancies and connective tissue diseases. Familial or inherited forms of vasculitis have also been recognized.

- A syndrome of cutaneous ulceration in conjunction with limb edema and/or acute renal failure, referred to as *cutaneous and renal glomerular vasculopathy (CRGV) of Greyhounds* or in layman's terms "Alabama rot" has been reported sporadically in young greyhounds throughout the United States. Multifocal cutaneous ulcers, renal afferent arteriolar and dermal arterial thrombosis, azotemia, microangiopathic hemolytic anemia and thrombocytopenia with normal coagulation times characterize CRGV. Cutaneous lesions may occur in the absence of renal or other systemic disease. The syndrome has many features in common with the hemolytic-uremic syndrome in people caused by a Shiga-like toxin induced endothelial cell necrosis. *Studies have failed to identify infectious agents, toxins, or evidence of an immune-mediated reaction in CRGV.* Exposure to Shiga-like toxin-producing *E. coli* has not been completely ruled out. Affected dogs may be genetically predisposed to develop CRGV as outbreaks have occurred in related groups of dogs. Hemorrhage, fibrinoid arteritis, thrombosis with infarction and ulceration characterize the skin lesions which are most often found on the limbs. Renal glomerular necrosis and tubular necrosis are present in the kidneys.
- A genodermatosis affecting *German Shepherd Dog puppies* characterized by swelling, depigmentation and frequent ulceration of footpads, ear and tail tips and nasal planum depigmentation has been reported in Canada. A neutrophilic to mononuclear nodular dermatitis, dermal collagenolysis, and subtle vasculitis are present histologically. Nasal lesions correspond to an hydropic interface dermatitis. The cause is unknown but presumed to be immunologically mediated as lesions are temporally related to vaccination dates. Dogs recover by 5–6 months of age. The condition may also be related to an underlying collagen disorder, as musculoskeletal abnormalities are also present.
- Familial vasculitis has been reported in a litter of *Scottish Terriers*. Lesions were limited to the nasal epithelium and mucosa and were characterized by leukocytoclastic vasculitis and pyogranulomatous inflammation leading to ulceration and destruction of the nasal planum.
- Cutaneous vasculitis leading to alopecia and ulcerative lesions of the extremities and bony prominences with histologic lesions

virtually indistinguishable from dermatomyositis of Collies and Shetland Sheepdogs (see Congenital and hereditary diseases of skin) has been reported in *Jack Russell Terriers*.

- Lymphocytic to mixed mononuclear vasculitis, often associated with cutaneous infarction, has been noted in dogs with the systemic lymphoproliferative disorder, *lymphomatoid granulomatosis*.

In **horses**, cutaneous vasculitis has been seen with or following numerous infections, such as *Streptococcus, Influenza A virus, Equine arteritis virus, Equine infectious anemia virus, Ehrlichia, Rhodococcus equi,* and *Corynebacterium pseudotuberculosis*.

- *Equine purpura hemorrhagica* is an acute, usually streptococcal infection (strangles)-associated, leukocytoclastic vasculitis characterized clinically by urticaria and extensive edema of the distal limbs, ventrum and head. These swellings may progress to exudation and sloughing.
- *Pastern leukocytoclastic vasculitis* is a syndrome unique to the horse that affects the unpigmented, sun-exposed skin of distal extremities, hence ultraviolet radiation is thought to play a role in the pathogenesis. Adult horses are most often affected. There is no evidence of liver disease or exposure to photosensitizing compounds. Interestingly, a single limb may be affected although other limbs lack pigment as well. Lesions are crusty, eroded or ulcerated, sharply demarcated and may be associated with extensive edema and pain. Histologically, a leukocytoclastic vasculitis of the superficial dermal vessels is present. Chronic cases develop papillary hyperplasia of the epidermis giving the lesion a verrucous appearance.

Cutaneous vasculitis in **pigs** is most commonly associated with *Erysipelothrix rhusiopathiae* infection. Systemic vasculitis affecting primarily the skin and kidneys has been reported recently in pigs and has been called the *dermatitis/nephropathy syndrome*. Skin lesions consisted of irregularly enlarging hemorrhagic macules and papules on the ears, limbs, abdomen, thorax and perineum. *Leukocytoclastic vasculitis* was present in the dermis, panniculus, synovium, and renal pelvis. Pigs had concurrent pneumonia and immunological studies suggested *Porcine reproductive and respiratory syndrome virus* might be associated with the condition. Direct immunofluorescence testing or immunohistochemistry may demonstrate immunoglobulin and/or complement in vessel walls and occasionally at the basement membrane zone in suspected cases of vasculitis. Positive tests are most likely in lesions less than 24 hours old. False positives occur also. Adverse drug reactions, infection, familial vasculopathies, cryopathies, and systemic autoimmune diseases such as SLE should be ruled as possible causes. In many cases, a specific cause cannot be determined.

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Rabies vaccine-induced vasculitis and alopecia in dogs

An ischemic dermatopathy leading to alopecic, hyperpigmented patches of atrophic skin (Fig. 5.77A) is a recognized form of injection-site reaction associated with the subcutaneous administration of rabies vaccines in dogs. All breeds are affected, but Poodles, Yorkshire and Silky Terriers are predisposed. Lesions most often develop at sites of previous vaccination, but occasionally multifocal lesions may occur. Lesions may appear months after the vaccine was administered. The *histologic appearance is highly characteristic*, although the vascular lesions are subtle. Venules, arterioles and small arteries develop a *very mild chronic lymphocytic vasculitis*, characterized by thickening of the vessel wall, a few intramural mononuclear inflammatory cells, scattered nuclear debris and variable perivascular mononuclear infiltrates (Fig. 5.77B). Occasionally a more florid leukocytoclastic vasculitis is seen. A cell-poor interface dermatitis with vacuolar change in the basal epithelial layer and pigmentary incontinence and mural folliculitis may be present in some cases. The dermis is atrophic and hyalinized, sometimes mucinous and hair follicles are in telogen and pale staining ("faded") (Fig. 5.77C). A diffuse increase in mononuclear cells throughout the dermis is accompanied by lymphocytic panniculitis. The additional lesions of erosions and ulcers of the oral mucosa and skin of the extremities and bony prominences and ischemic myopathy in subjacent musculature have been reported in cases with multicentric involvement. Immunofluorescence staining has identified rabies antigen in the vessels and epithelium of the hair follicles. A low-grade immune-mediated vasculitis with resultant tissue anoxia leading to the atrophic changes in the overlying skin has been suggested as the pathogenesis. Lesions may remain for months to years. Differential diagnoses include dermatomyositis, traction alopecia, idiopathic vasculopathy, and lupus profundus.

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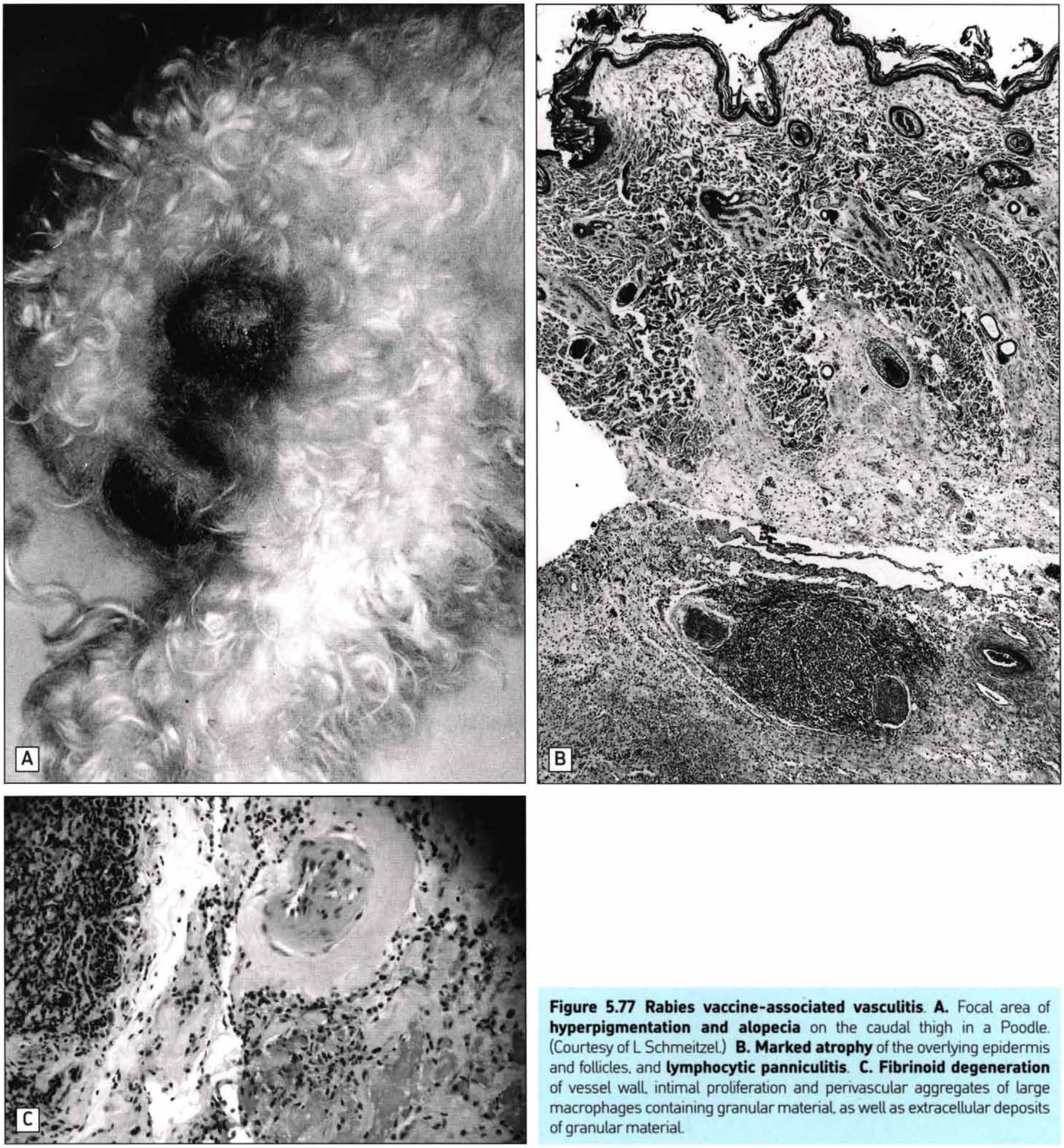

Figure 5.77 Rabies vaccine-associated vasculitis. A. Focal area of **hyperpigmentation and alopecia** on the caudal thigh in a Poodle. (Courtesy of L Schmeitzel.) **B. Marked atrophy** of the overlying epidermis and follicles, and **lymphocytic panniculitis. C. Fibrinoid degeneration** of vessel wall, intimal proliferation and perivascular aggregates of large macrophages containing granular material, as well as extracellular deposits of granular material.

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Canine uveodermatologic syndrome (Vogt–Koyanagi–Harada (VKH) syndrome)

This syndrome, seen only in dogs and humans, is characterized by the concurrent *acute onset of bilateral uveitis and depigmentation of the nose, lips, eyelids and occasionally the footpads and anus.* Although the cause of the disorder is unknown, a cell-mediated hypersensitivity to melanin has been hypothesized. It is discussed further under Disorders of pigmentation.

Plasma cell pododermatitis

This is a *rare disorder of cats* of all breeds, ages and sexes. A recent study found 50% of cases were positive for feline immunodeficiency virus. Although the pathogenesis is unknown, the tissue plasmacytosis, hypergammaglobulinemia, and beneficial response to immunomodulating drugs *suggest an immune-mediated basis*. In addition, occasional cats have other abnormalities, such as renal amyloidosis, plasmacytic stomatitis, positive anti-nuclear antibody tests, Coombs-positive anemia, polyclonal gammopathy, or glomerulonephritis with positive direct immunofluorescence testing (immunoglobulin deposited at the basement membrane zone). Clinically, plasma cell pododermatitis begins as soft, nonpainful swelling of multiple footpads on multiple paws. The central metacarpal or metatarsal pads are usually most severely affected. Affected pads feel soft due to collapse of the underlying fatpad. Footpad surfaces are crosshatched with white scaly striae but may become ulcerated or develop fleshy granulomatous proliferations which may hemorrhage. Lameness develops with progression of the lesions. Some cases spontaneously resolve or may recur seasonally.

Histologically, early lesions are characterized by *superficial and deep perivascular dermatitis with plasma cells predominating*. Later, there is a diffuse plasmacytic dermatitis. Russell bodies can be numerous. Leukocytoclastic vasculitis is rarely seen. Ulcerated or proliferative lesions show various degrees of superimposed suppurative-to-pyogranulomatous inflammation. The gross and histologic features are diagnostic.

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Cutaneous amyloidosis

The physical structure of amyloid gives it special properties, such as apple green birefringence when Congo red-stained sections are polarized. The amyloid fibrils (AL amyloid) are derived from monoclonal immunoglobulin light chains. Cutaneous amyloidosis occurs rarely in *horses and dogs*. The cutaneous lesions are not usually associated with known triggering factors in horses; but in dogs, cutaneous amyloidosis has been associated with monoclonal gammopathy, dermatomyositis, and is seen occasionally in the stroma of plasmacytomas of skin and oral cavity (see Cutaneous plasmacytoma). In **dogs** with monoclonal gammopathy, purpuric lesions are seen, and cutaneous hemorrhage is easily induced by minor external trauma. The superficial dermis contains an amorphous, homogeneous, eosinophilic substance, and the walls of blood vessels in the involved area are thickened by deposition of the same substance.

In **horses**, the lesions are multiple, asymptomatic papules, nodules, and plaques that are most commonly seen on the head, neck, shoulders and pectoral region. There is no established breed, age, or sex predisposition. The lesions are firm, well-circumscribed and 0.5–10 cm in diameter. The overlying skin and haircoat are normal. The initial lesions may be urticarial in type. The cutaneous lesions may be accompanied by similar nodules in the respiratory mucosa and regional lymph nodes but are seldom associated with systemic amyloidosis. Lesions may regress, become recurrent or progressively enlarge. Histologic findings include *nodular-to-diffuse granulomatous dermatitis and panniculitis. Large extracellular deposits of amyloid appear as variably sized areas of homogeneous, amorphous, hyaline, eosinophilic material, which may contain clefts or fractures*. Multinucleated histiocytic giant cells are usually numerous. *Clinical differentials* include infectious and non-infectious granulomas, cutaneous neoplasms, and eosinophilic granulomas.

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Alopecia areata

Alopecia areata (AA) is a non-scarring, asymptomatic, inflammatory alopecia occurring in humans, nonhuman primates, dogs, cats, horses and cattle. Alopecia areata may be focal, multifocal or generalized (alopecia universalis). Lesions occur most commonly on the face, neck and trunk. Leukotrichia may be seen in some animals initially. Areas of alopecia are usually hyperpigmented and areas of alopecia may exhibit sparse numbers of short, dystrophic hairs (Fig. 5.78A). Lesions may be bilaterally symmetrical. There is no apparent age, breed, or sex predilection. In horses, the mane and tail are often affected. Currently, there are two rodent models available to study the condition, the C3H/HeJ mouse and the Dundee experimental bald rat.

Early, clinically active lesions are characterized histologically by an accumulation of lymphocytes ("swarm of bees") in and around the inferior segment of anagen hair follicles – the classic example of *peribulbar lymphocytic folliculitis* (Fig. 5.78B). Unfortunately, the classic lesion is not always evident and more subtle bulbar inflammation is the rule. Bulbar keratinocytes are frequently vacuolated, apoptotic or karyorrhectic. Pigmentary incontinence and melanophagia in the peribulbar region are also typical findings. Telogen follicles are unaffected and dystrophic hair follicles may be present. Demonstration of the lymphocytic peribulbitis may be difficult and require examination of multiple sections from different levels of the paraffin-embedded biopsy specimen. Biopsies from the periphery of early, expanding lesions are most rewarding. *Histologic findings in chronic, clinically static lesions are nondiagnostic*, revealing a predominance of telogen hair follicles and follicular atrophy, that may be misdiagnosed as an endocrine skin disorder. Immunologic studies in dogs and horses have indicated that the intrabulbar lymphocytes are primarily cytotoxic CD8+ lymphocytes. CD1+ dendritic antigen-presenting cells and both CD8+ and CD4+ lymphocytes are found in the peribulbar infiltrate. In addition, various autoantibodies targeting trichohyaline, hair keratins, and other components of the hair follicle have been demonstrated in various species, indicating a role for humoral immunity. In dogs and horses, *AA frequently spontaneously reverses itself*, however sometimes the hair regrowth is white. *Clinical differentials* include telogen effluvium and many other causes of lymphocytic mural folliculitis such as EM, SLE, GVH, ischemia, and demodicosis, to name a few. *The distinguishing feature of AA is the fact that the hair bulb and inferior segment of anagen hairs are the primary*

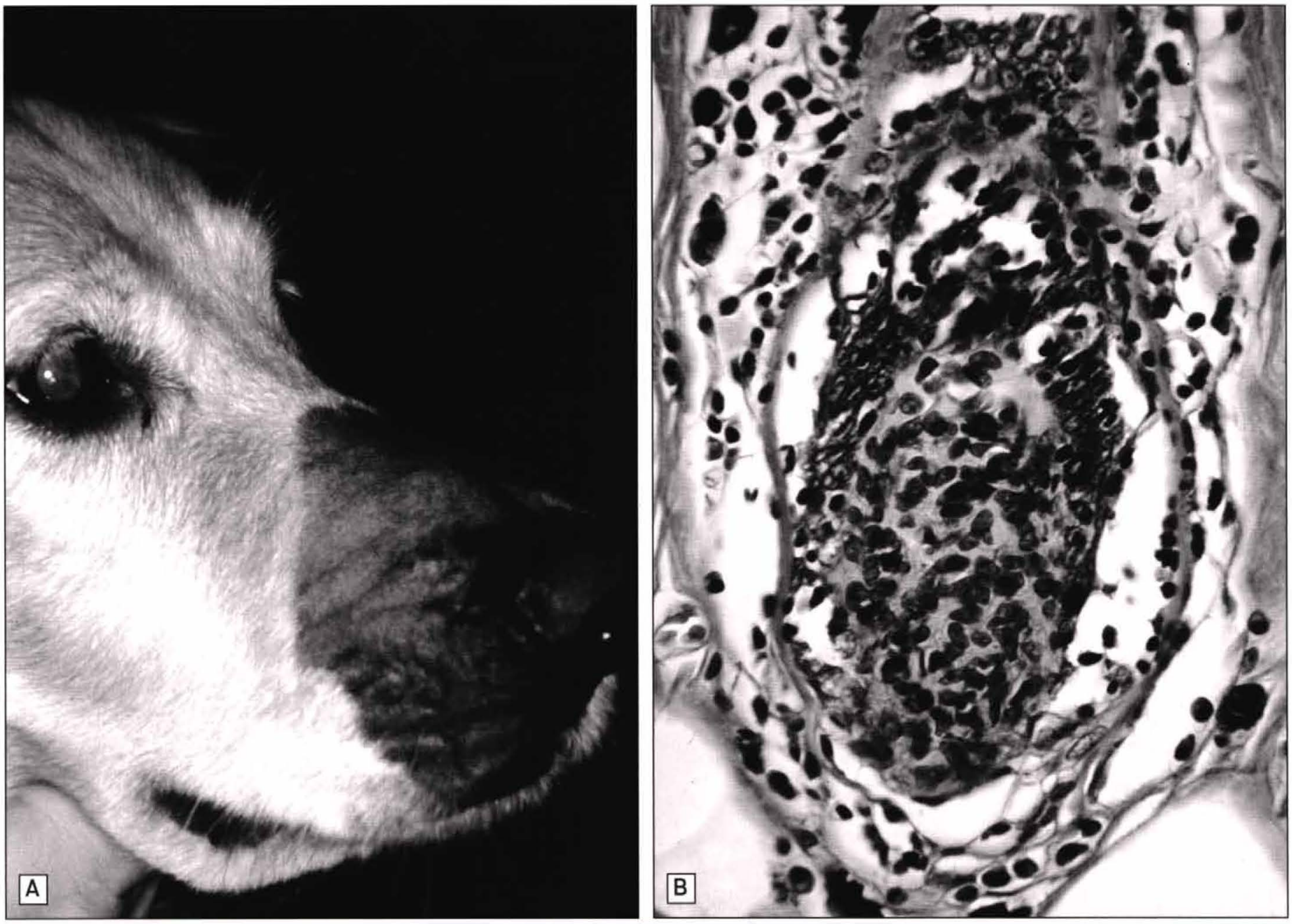

Figure 5.78 Alopecia areata in a dog. **A.** Note discrete area of non-inflamed, **hyperpigmented alopecic skin.** (Courtesy of K Beale.) **B. Peribulbar lymphocytic folliculitis.** Lymphocytes are in and around the hair bulb. (Courtesy of B Dunstan.)

targets of inflammation rather than the infundibulum or the isthmus, although the isthmus has been reported to be affected in a horse.

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VIRAL DISEASES OF SKIN

Cutaneous lesions occur in the course of a number of viral diseases in domestic animals. Viruses may induce skin lesions upon *local infection*, but the intact integument is resistant to viral penetration; injection via an arthropod bite or introduction through a cutaneous wound is a prerequisite for infection. Examples of local viral infection include papillomas induced by the Papovaviridae, bovine mammillitis induced by a herpesvirus, and the so-called milker's nodule in humans caused by a parapoxvirus. More often, viruses localize in the skin during the viremic phase of a *systemic infection.* Examples include some poxvirus infections, malignant catarrhal fever and the vesicular diseases, such as vesicular stomatitis, and foot and mouth disease. Pantropic viruses, such as *Canine distemper virus* and *Classical swine fever virus*, may cause cutaneous lesions; but *most viruses causing cutaneous lesions are epitheliotropic.* Some epitheliotropic viruses, in particular the Poxviridae, have a predilection for the epithelium of the skin. Others, including the viruses associated with the mucosal diseases, cause primary lesions in the alimentary tract with lesser involvement of the skin.

A "rash," comprising erythematous macules due to long-lasting dilation of dermal blood vessels, is often associated with systemic viral disease in humans. Such lesions are uncommon in animals, but may be hidden by the haircoat. Exceptions are the *cutaneous erythema* occurring in classical swine fever and African swine fever, and hemorrhagic diathesis of skin occurring with disseminated *Porcine adenovirus* infection. In addition, a condition known as *dermatitis/nephropathy syndrome of pigs*, seen clinically as erythematous macules, papules, and plaques, due to cutaneous and systemic necrotizing vasculitis, may be associated with *Porcine circovirus 2* (PCV2) infection, with possible roles for *Porcine reproductive and respiratory syndrome virus* (PRRSV) and *Pasteurella multocida*.

Cutaneous viral diseases are *more common in food-producing animals than in pets*. Some of these diseases, notably sheeppox, cause significant mortality. Others have an economic impact because of their deleterious effect on production. Herpes mammillitis and pseudocowpox, for example, reduce milk yield in dairy cattle; contagious pustular dermatitis affects the growth rate of lambs by interfering with their ability to suckle. A few of the large animal viral dermatoses are extremely mild, for example molluscum contagiosum in the horse. Systemic viral diseases with cutaneous manifestations are rare in dogs and cats. *Canine distemper virus* is associated with nasodigital hyperkeratosis, so-called "hard pad" disease, and pustular dermatitis. In cats, rare occurrences of cutaneous disease occur with *Feline calicivirus* infection. Cutaneous lesions caused by *Felid herpesvirus 1* can occur in the absence of respiratory disease, a presumed recrudescence of a latent herpesvirus infection.

Traditionally, viral diseases have been *diagnosed* by light and electron microscopy, serology, and viral culture. However, the development of monoclonal antibodies to specific viruses for use with immunofluorescence and immunoperoxidase techniques is increasingly being used for specific and rapid diagnosis.

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Poxviral infections

The Poxviridae is a large family of complex DNA viruses. *Highly epitheliotropic, they cause cutaneous and systemic disease in birds, wild and domestic mammals and humans. Some members of the Poxviridae, including Sheeppox virus, Ectromelia virus, Monkeypox virus, and the now eradicated Variola virus (human smallpox), cause severe systemic disease.* Others cause mild, localized disease, for example pseudocowpox that chiefly affects the teats of milking cows. A few poxviruses are associated with hyperplastic or neoplastic conditions, such as molluscum contagiosum in horses and Shope fibroma of rabbits.

The Poxviridae share group-specific nucleoprotein antigens. Animal poxviruses are in the subfamily Chordopoxvirinae. The genera (*species names italicized*) include:

- *Orthopoxvirus – Camelpox virus, Cowpox virus, Ectromelia virus* (mousepox virus), *Monkeypox virus, Vaccinia virus* (buffalopox virus, rabbitpox virus). Unassigned member of the genus: Uasin Gishu disease virus.
- *Parapoxvirus – Bovine papular stomatitis virus, Orf virus* (contagious pustular dermatitis virus, contagious ecthyma virus), *Parapox virus of red deer, Pseudocowpox virus* (milker's nodule virus). Unassigned members in the genus: Auzduk disease virus (Camel contagious ecthyma virus), Chamois contagious ecthyma virus, Sealpox virus, and a virus that causes papillomatous dermatitis and pododermatitis in cattle.
- *Avipoxvirus – Fowlpox virus, Pigeonpox virus*, and many other avian poxviruses.
- *Capripoxvirus – Goatpox virus, Lumpy skin disease virus, Sheeppox virus.*
- *Leporipoxvirus – Myxoma virus; Rabbit fibroma virus* (Shope fibroma virus).
- *Suipoxvirus – Swinepox virus.*
- *Molluscipoxvirus – Molluscum contagiosum virus.*
- *Yatapoxvirus – Tanapox virus,* Yaba monkey tumor virus.

Many of the poxviruses are host-specific; but the orthopoxviruses, such as *Cowpox virus* and *Vaccinia virus*, affect a wide range of species. Some poxviruses, for example *Pseudocowpox virus*, are zoonotic. *Infection is usually achieved by cutaneous or respiratory routes.* Poxviruses, whether acquired by the subcutaneous or respiratory routes, usually gain access to the systemic circulation via the lymphatic system, although multiplication at the site of injection in the skin may lead to direct entry into the blood and primary viremia. Secondary viremia disseminates the virus back to the skin and to other target organs.

Poxviruses induce lesions by a variety of mechanisms. Degenerative changes in the epithelium are caused by virus replication and induce *vesicular lesions* typical of many poxvirus infections. Degenerative lesions in the dermal or submucosal tissues sometimes result from ischemia secondary to vascular damage caused by viral multiplication in endothelial cells. Poxvirus infections also *induce proliferative lesions*. Poxviruses, such as *Orf virus*, replicating in the epidermis typically induce hyperplasia along with degenerative changes. Host cell DNA synthesis is stimulated before the onset of cytoplasmic virus-related DNA replication. Proliferative changes may be explained by a gene, present in several poxviruses including *Molluscum contagiosum virus*, whose product has significant homology with epidermal growth factor. Poxviruses also encode for functions that may counteract host defenses. These include genes related to those encoding the SERPINs (a superfamily of related proteins important in regulating serine protease enzymes that mediate kinin, complement, fibrinolytic and coagulation pathways) and genes encoding anti-interferon activities.

Pox lesions have a typical developmental sequence. They commence as erythematous macules, become papular, and then vesicular. The vesicular stage is well developed in some pox infections, such as sheeppox, and is transient or non-existent in others, such as contagious pustular dermatitis. Vesicles develop into *umbilicated pustules* *with a depressed center and a raised, often erythematous border.* This lesion is the so-called *"pock."* The pustules rupture and a crust forms on the surface. This crust may become very thick, as in lesions of contagious pustular dermatitis. Lesions heal and often leave a residual scar. The mucosal lesions are briefly vesicular and develop into ulcers rather than pustules.

Histologically, pox lesions begin as epidermal cytoplasmic swelling and vacuolation, usually first affecting the cells of the outer stratum spinosum. There is evidence, from experimental studies with the virus of contagious pustular dermatitis, that post-injury proliferating keratinocytes are the target for viral replication. Rupture of the damaged keratinocytes produces multiloculated vesicles, so-called *reticular degeneration*. The early dermal lesions include edema, vascular dilation, a perivascular mononuclear cell infiltrate and a variable neutrophilic infiltrate. Neutrophils migrate into the epidermis and aggregate in vesicles to form microabscesses. Large intraepidermal pustules may form and sometimes extend into the superficial dermis. There is usually marked epithelial hyperplasia and sometimes pseudocarcinomatous hyperplasia of the adjacent epithelium. This contributes to the raised border of the umbilicated pustule. Rupture of the pustule produces an inflammatory crust, often colonized on its surface by bacteria.

Poxvirus lesions often contain *characteristic intracytoplasmic inclusion bodies*. These are single or multiple and of varying size and duration. The more prominent inclusions are designated *type A*. They are eosinophilic, reflecting their high protein content, and weakly Feulgen-positive. *Smaller, basophilic Feulgen-positive type B* bodies also occur and represent the site of virus replication.

Diagnosis of poxvirus infections is usually based on *typical clinical appearance* and may be supported by *characteristic histologic lesions*. Parapoxviruses are ultrastructurally distinct from the other poxviruses that are morphologically similar to each other when viewed by electron microscopy.

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Parapoxviral diseases

Contagious pustular dermatitis

Contagious pustular dermatitis is a poxviral disease of *sheep and goats*, with incidental infections occurring in humans, camels, cows, and many wild ruminants, and very rarely dogs. The disease is caused by *Orf virus*, a *Parapoxvirus* closely related to *Pseudocowpox virus* and *Bovine papular stomatitis virus*. Synonyms for contagious pustular dermatitis include contagious ecthyma, orf, infectious labial dermatitis, soremouth, and scabby mouth.

The disease is geographically widespread and occurs wherever sheep or goats are raised. The virus can repeatedly infect sheep and goats; and while live-virus vaccines control the disease and decrease the severity of the disease, they also ensure its continuance by perpetuating infection in the environment. The economic significance of contagious pustular dermatitis results chiefly from loss of condition, since *affected animals neither suckle nor graze*. Morbidity in a susceptible population may reach 90%, but mortality rarely exceeds 1% unless secondary infection intervenes, or unless the animals are immunosuppressed or stressed in which case mortality can be high. Mortality often results from the invasion of primary lesions by the larvae of the screwworm fly (*Cochliomyia hominivorax*) or by bacteria such as *Fusobacterium necrophorum* and occasionally *Dermatophilus congolensis*. Cellulitis may complicate pedal lesions, mastitis may complicate mammary lesions, and necrotizing stomatitis and aspiration pneumonia may complicate oral lesions.

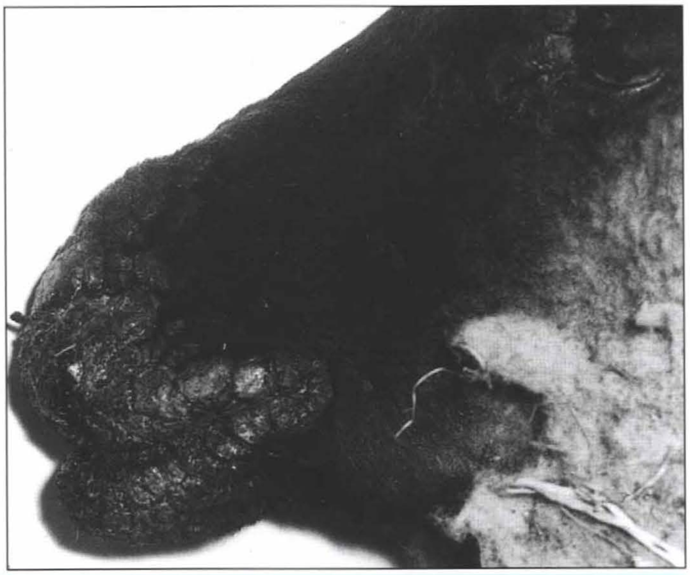

Figure 5.79 Contagious pustular dermatitis (orf) in a sheep. Scabby lesions at the margins of the lips.

Contagious pustular dermatitis affects sheep and goats of all breeds. *It is predominantly a disease of lambs and kids*. Infection is established through cutaneous abrasions, particularly those associated with dry and prickly pasture or forage. Clinically affected lambs may transmit the virus to the udder of the ewe. The virus is hardy and probably persists in a dry environment indefinitely in crust material shed from affected animals. Chronically infected, reinfected or, possibly, latently infected carrier animals may allow the virus to persist in a flock for several years.

Gross lesions usually commence at the commissures of the lips and spread around the lip margins to the muzzle (Fig. 5.79). Primary lesions sometimes occur on the face about the eyes. In severe cases, lesions may develop on the gingiva, dental pad, palate and tongue (Fig. 5.80A). Lesions mainly confined to the tongue must be differentiated from those of foot and mouth disease. The buccal lesions are raised, red or gray foci with a surrounding zone of hyperemia. Very rarely, lesions extend to the esophagus, rumen and omasum in the lower alimentary canal, causing ulcerative gastroenteritis, and in lungs and heart. Lesions on the limbs are less common than on the lips and tend to involve the coronet, interdigital cleft, and bulb of the heels. They may extend, in severe cases, to the knee or hock on the caudal aspect of the leg. Lesions of the mammary gland affect the teats and adjacent skin of the udder. Lesions may develop in other areas of sparsely wooled skin such as the inner thigh, axilla, and the edge of wounds in recently earmarked lambs, or tail-dock sites. Proliferative lesions affecting predominantly the head, neck and body have been described in Nubian goats.

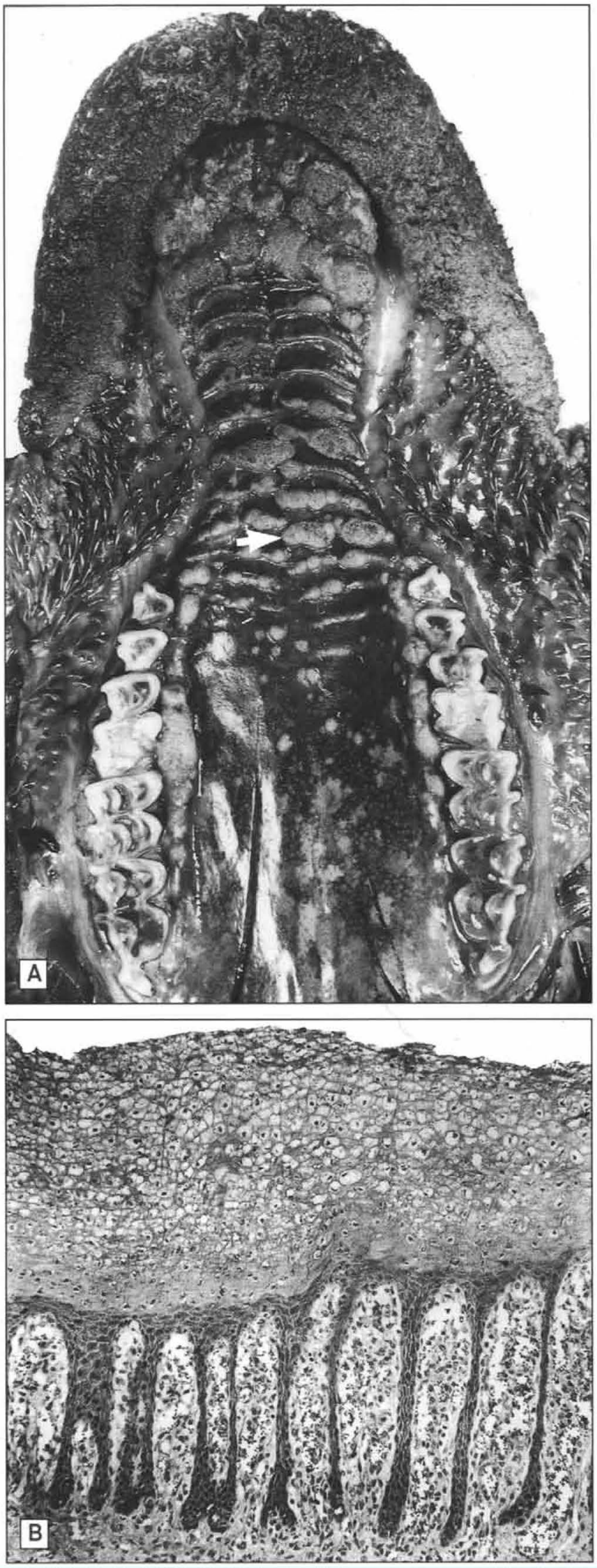

Figure 5.80 Contagious pustular dermatitis in a sheep. **A.** Crusty, proliferative lesions on the lips, dental pad, and palate (arrow). **B.** Note marked epithelial proliferation and increased vascularity and intracellular edema of keratinocytes.

The lesions develop through the typical pox phases but are much more proliferative. The vesicular stage is transient and pustules are flat rather than umbilicated. The most significant feature of the gross lesion is the layer of thick brown-gray crust that may be elevated 2–4 mm above the skin surface. Depending on the degree of secondary infection, regression is usually complete by 4 weeks. Papillomatous growths, resulting from continued epidermal proliferation, sometimes occur.

The microscopic lesions of contagious pustular dermatitis are characterized by vacuolation and swelling of keratinocytes in the stratum spinosum, reticular degeneration, marked epidermal proliferation, intraepidermal microabscesses, and accumulation of scale-crust. In experimentally abraded sheep skin, the active site of viral replication was found to be the newly proliferative keratinocyte population, growing up under the superficial necrotic layer. By ~30 hours postinfection, keratinocyte swelling and vacuolation commences in the outer stratum spinosum. It is accompanied by cytoplasmic basophilia, which corresponds ultrastructurally to an increased number of polyribosomes presumably active in viral protein synthesis. Basophilic intracytoplasmic inclusion bodies are reported as early as 31 hr post-infection. By 72 hours postinfection, the keratinocytes show nuclear pyknosis and marked hydropic change, leading to reticular degeneration. The term ballooning degeneration is often used, but the keratinocytes do not have the homogeneous eosinophilic cytoplasm typical of this condition. At this time intracytoplasmic eosinophilic inclusion bodies appear. The inclusion bodies persist for 3–4 days, as long as the hydropic cells are found. The proliferative reaction in the epidermis is underway by 55 hours postinfection with mitotic figures numerous in the stratum basale. By 3 days postinfection, the epithelium is 3–4 times normal thickness and rete ridges are markedly elongated (Fig. 5.80B). Pseudocarcinomatous hyperplasia is common.

Dermal lesions include superficial edema, marked capillary dilation, and an early influx of neutrophils, followed by a marked accumulation of MHC class II dendritic cells, with CD4+ T cells, CD8+ T cells, and B cells. A thick layer of scale-crust is built up, composed of ortho- and parakeratotic hyperkeratosis, proteinaceous fluid, degenerating neutrophils, cellular debris and bacterial colonies. The subsequent microscopic appearance of the lesions depends on the degree of secondary bacterial infection.

Contagious pustular dermatitis occurs in camels and a variety of wild and captive ungulates. Dogs may acquire infection by eating infected lamb carcasses. Lesions resemble those in sheep. It is a *zoonotic disease* and is a recognized occupational hazard amongst goat and sheep handlers.

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Ulcerative dermatosis of sheep

Ulcerative dermatosis is a disease of the epidermis of sheep that has been reported in the literature as being caused by an *unclassified poxvirus*, which is similar to *Orf virus*, but the viruses do not cross-protect. The disease has been reported in South Africa where it is known as "pisgoed" or "pisgras," in the United Kingdom as a contagious venereal infection, and in the USA where it is known as "lip and leg ulceration," "anovulvitis," "infectious balanoposthitis" and "ulcerative vulvitis." The various names indicate the *essential features of the disease, which are ulcerative papules on the lips, face, legs, feet, vulva, prepuce and, occasionally, the glans penis.* The genital lesions are transmissible by coitus.

Presumably, infection results from viral contact with damaged skin. *The pathologic process is primarily ulcerative*, with ulcers of up to 4–5 cm in diameter and 3–5 mm deep. Pus covers the granulation tissue at the base of the ulcer and underlies a scab that is thin, brown and bloody, and unlike the thick parakeratotic crusts that develop in contagious pustular dermatitis. The underlying dermis is diffusely swollen, especially in distensible parts such as the vulva and prepuce. The lesions on the hairy parts of the face tend to be fairly well circumscribed, but those of the feet tend to spread, especially on the interdigital skin. The vulval lesions usually begin on the tip and spread around the margins of the lips. An ulcerative ring tends to form around the preputial orifice, but the preputial mucosa is spared. Lesions on the glans penis remain moist. The urethral process may become necrotic. The labial and pedal lesions must be distinguished from those of contagious pustular dermatitis and foot-and-mouth disease, and the preputial lesions must be distinguished from noncontagious forms of balanoposthitis. *Detailed descriptions of the histopathology of ulcerative dermatitis are lacking*; the lesions are supposedly distinguishable from those of contagious pustular dermatitis by the *lack of epithelial hyperplasia.*

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Pseudocowpox

Pseudocowpox is caused by *Pseudocowpox virus*, a *Parapoxvirus*. The virus is closely related to *Bovine papular stomatitis virus* and *Orf virus. It is a common endemic infection in cattle throughout most of the world.* It affects chiefly milking herds and occasionally beef herds. Morbidity in a herd approaches 100% but only 10–15% of cows are affected at any one time. The economic significance lies in the effect on milk production, either as a result of sore teats or because of secondary bacterial mastitis. Lesions, which affect the teats (Fig. 5.81), udder, and perineum, commence as erythematous macules and papules, but do not form the umbilicated pustules seen in cowpox and vaccinia infections. Instead, a characteristic ring or horseshoe-shaped crust forms that may become umbilicated as it expands, but infrequently ulcerates. *The histologic appearance of lesions is typical of other poxviral infections*. The lesions usually heal within 6 weeks. Occasionally, lesions develop in the mouth and on the muzzle of suckling calves, and the infection can be spread by cross-suckling as well as poor hygiene in milking sheds. Transmission to people induces "milker's nodule."

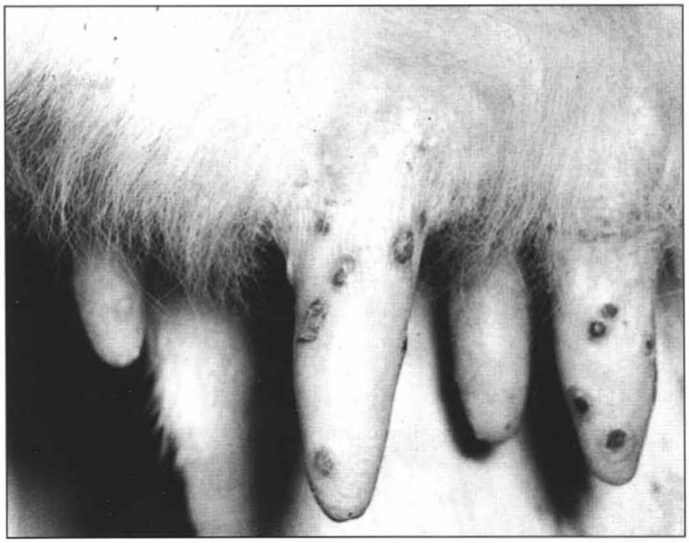

Figure 5.81 Pseudocowpox on the teats of a cow.

Bovine papular stomatitis

Bovine papular stomatitis virus is distributed worldwide, and although it causes disease more commonly in *cattle <2 years old* it can occur at any age and in any breed. Lesions occur on the *muzzle, nostrils, lips, and mouth* (see Vol. 2, Alimentary system), and cows with suckling calves can develop *teat and udder lesions*. The development and appearance of the lesions are similar to pseudocowpox, with resolution of lesions in days to weeks. A chronic form has been described in which exudative necrotic dermatitis involved the trunk as well as the mouth, and in which the animals died in 4–6 weeks. *Transmission to humans induces lesions identical to "milker's nodule"* caused by *Pseudocowpox virus* infection. The histologic appearance of lesions is typical of other poxviral infections.

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Parapox of red deer

A new addition to the genus *Parapox* is the *Parapox virus of red deer*, first noted in 1986 in farmed red deer in New Zealand. Morbidity is high, but mortality is low unless secondary infections are present. The virus causes crusty lesions on the lips, muzzle, face, ears, and neck, and on the antler velvet in stags. Removal of the crusts leaves a red raw ulcer. Histologic lesions are characterized by *epithelial hyperplasia with ballooning degeneration, dyskeratosis, and eosinophilic cytoplasmic viral inclusions.*

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Orthopoxviral diseases

Cowpox

Cowpox virus and cowpox-like viruses belong to the genus *Orthopoxvirus*. They are closely related to, but antigenically different from, *Vaccinia virus*. Cowpox affects a range of species including *wild and domestic Felidae, cattle, dogs*, rodents, humans, and several zoo and circus animals including elephants and rhinoceros. Cowpox occurs in the *UK and Europe*. Outbreaks reported from other geographical locations can be ascribed to *Vaccinia virus* infection; the lesions induced by the two orthopoxviruses are clinically indistinguishable. In cattle, *Cowpox virus* infection is manifested by poxvirus lesions that develop on the *teats and udder*, and occasionally at other sites such as the muzzle of suckling calves. *Cowpox virus* is not endemic in cattle and *infections in cattle are uncommon*. Re-evaluation of the epidemiology of *Cowpox virus* relegates cattle to a minor role, and it has become increasingly clear from serologic surveys that *small wild rodents, such as voles and mice, are the virus reservoir*, and it is from this reservoir that other mammals can become infected accidentally. Domestic cats, and rarely dogs, in UK and Europe, can be infected by hunting wild rodents, and cats occasionally have been shown to be able to transmit the infection to humans. The evidence suggests, however, that the virus is of low infectivity for humans. Since the first report in 1978, *Cowpox virus* infection has been documented with increasing frequency within its geographical range of the UK and Europe. Epidemiological studies show a higher prevalence in free-ranging country-dwelling cats and a seasonal incidence in the autumn, a time when the small mammal population is at its highest. These features further support the serologic evidence of a wild rodent reservoir of virus.

Domestic cats develop *cutaneous* and, occasionally, *respiratory lesions*. The primary lesions in cats have a predilection for the *face and forepaws*. Approximately 50% of cats show a single primary cutaneous lesion, usually on the head, neck, forelimbs, or paws. The initial lesion is ulcerative and has been described as "bite-like." Lesions heal by granulation, but may occasionally develop into abscesses or cellulitis. The secondary cutaneous lesions develop 4–16 days after the onset of the primary lesion. Generalization from primary inoculation sites in the skin has also been demonstrated in experimentally infected cats. Mucocutaneous junctions, oral mucosa and the tongue may also be involved. *Secondary lesions* are multiple (usually more than ten lesions). They commence as firm 2–3 mm nodules, enlarging over 2–3 days to form 0.5–2 cm, circular, ulcerated papules or plaques. Lesions are rarely vesicular except on the oral mucosa and inner aspect of the pinna. The ulcers heal with thick gray crusts over 2–3 weeks. Cellulitis may develop but generally secondary bacterial infection is not a significant complication. Corticosteroid or progesterone therapy may be responsible for converting a local infection into a generalized one or exacerbating the secondary lesions in some animals. Although fatal *Cowpox virus* infection has been seen in cats with concurrent *Feline immunodeficiency virus* (FIV) infection, there is no convincing evidence to show a direct relationship. Approximately 30% of *Cowpox virus*-infected cats concurrently infected with FIV have uneventful recoveries.

The disease is rarely fatal. Approximately 20% of cats show some signs of malaise in the acute stage of secondary lesion development, presumably associated with a viremic phase, and a few exhibit clinical signs of upper respiratory tract disease. Lower respiratory tract lesions, which are rare in domestic cats, include pleural effusion and localized areas of cream-colored consolidation in the ventral lung lobes. They are thought to develop from systemic spread rather than from primary respiratory infection.

The *microscopic lesions* in naturally infected cats are *focal, sharply demarcated ulcers covered by fibrinonecrotic exudate*. The ulcers may extend to the deep dermis, subcutis or even muscle. An intense dermal inflammatory cell infiltrate of neutrophils and mononuclear cells may be associated with the base of the ulcers. *Eosinophilic, homogeneous, intracytoplasmic inclusion bodies*, 3–7 μm in diameter, occur in keratinocytes in the hyperplastic epithelium at the margin of the ulcers and in the epithelium of the external root sheath and sebaceous gland. Epidermal lesions typical of poxvirus infections develop in cats experimentally infected intravenously or by skin scarification. These lesions include focal hyperplasia, with reticular degeneration and multilocular vesiculation. Epidermal cells bordering the vesicles contain eosinophilic intracytoplasmic inclusion bodies. Viral inclusions also occur in macrophages, fibroblasts and endothelial cells. The extensive necrosis is probably ischemic in origin following viral damage to endothelial cells. The pulmonary lesion is a necrotizing alveolitis in which eosinophilic inclusion bodies are present in the degenerating cells.

Electron microscopic examination of scabs reveals typical orthopox virions. *Cowpox virus* infection may be confirmed by immunohistochemical staining of lesions, PCR, or virus isolation. Serological tests may be helpful in establishing retrospective diagnoses, as virus neutralizing antibodies are persistent for several years.

There have been outbreaks of severe orthopox infections in several species of **nondomestic Felidae** in English and Russian zoos. Lions, cheetahs, pumas, jaguars, and ocelots have been affected. Rats were implicated as the source of infection in the Russian outbreaks. Two forms of the disease are recognized. The cutaneous form is rarely fatal. The lesions are ulcerative and crusted as described for domestic cats. The respiratory form is uniformly fatal and consist of severe fibrinous and necrotizing bronchopneumonia and pleuritis. A virus closely resembling *Cowpox virus* is responsible.

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Vaccinia

Vaccinia virus, the type species for the *Orthopoxvirus* genus, *does not cause natural infection in domestic animals. The origin of the virus is controversial.* One theory is that it represents the laboratory survival of horsepoxvirus, which is now extinct in nature, and that horse-derived material was the source of vaccine material used by Edward Jenner in 1817 to protect against variola or smallpox. Another theory is that *Vaccinia virus* was derived from *Cowpox virus* by repeated passage on the skin of cows, sheep, and other animals, and that "horsepox" was due to the infection of horses with *Cowpox virus*. Incidental infections in cattle, horses and pigs were transferred from vaccinated people. The lesions in cattle are indistinguishable from those of cowpox and in swine are indistinguishable from those of swinepox. When vaccinia is inoculated onto scarified skin of horses, papular lesions resembling a naturally occurring poxvirus infection described in horses in the USA result. Furthermore, inoculation of the skin of the flexor surface of the pastern produces lesions resembling the classical "grease heel" form of horsepox. *Vaccinia virus infections of animals are rare* now that smallpox has been eradicated and routine vaccination has been discontinued.

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Buffalopox

Buffalopox virus, an *Orthopoxvirus* closely related to *Vaccinia virus,* is the cause of buffalopox, an economically important disease of domestic buffaloes. It is thought that Buffalopox virus is a subspecies of *Vaccinia virus* resident in the water buffalo population. In India it is considered to be an emerging enzootic virus and can occur in epidemic form, with significant economic impact. It has been reported in Pakistan, Indonesia, Egypt, Italy, and Russia. Zebu cattle are apparently refractory to infection. The lesions predominantly affect the teats, udder, medial aspects of the thighs, lips and muzzle but may be generalized, especially in calves. *Buffalopox virus can be zoonotic*, causing lesions primarily on the hands. Human-to-human transmission has been postulated following the occurrence of disease in children who had had no contact with infected animals. Experimentally, the virus can be transmitted to cattle, rabbits, guinea pigs, and mice.

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Camelpox

Camelpox virus, a distinct species of *Orthopoxvirus*, causes severe disease in *dromedary camels*. It is characterized by *high morbidity and a relatively high mortality rate* in young animals. A major effect is a fall in milk production and loss of condition. The disease is widespread in northern and eastern Africa and mid-eastern Asia but has not been reported in Australia. Lesions affect both *skin and mucous membranes* and follow the usual pattern of pox lesions. Lesions tend to be most concentrated around the mucocutaneous junctions of the face but may be found on the neck and forelegs. Rarely, the infection can involve the respiratory system or become systemic. Generalized skin lesions occur more typically in calves. Fatalities are usually associated with *secondary bacterial infection leading to septicemia*, a phenomenon more prevalent in the rainy season. Clinically, *the lesions can be identical to parapox* (camel contagious ecthyma) and laboratory tests such as ELISA, PCR, and immunohistochemistry, as well as electron microscopy, can be used to differentiate the infections.

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"Horsepox" and Uasin Gishu disease

Horsepox was a common disease of horses in the 18th and 19th centuries; however, *it became naturally extinct at the end of the 19th century*. It is thought that Jenner, in his "cowpox" vaccination experiments to protect humans from smallpox, was actually using horse-derived material as a source of the vaccine, and that *Vaccinia virus* may be the long-lost agent of horsepox. Experimental infection of horses with *Vaccinia virus* reproduces the "grease heel" lesions of Jenner's horsepox and the more generalized form known as *equine papular dermatitis*. Orthopoxviruses have been isolated from equine papular dermatitis; however, the virus has not been fully characterized. *Orthopoxvirus* has also been isolated from Uasin Gishu disease in Kenya, and Uasin Gishu disease virus is closely related to *Vaccinia virus* and *Cowpox virus*.

Poxviral lesions in the horse take *several clinical forms*. Jenner originally described an exudative dermatitis of the flexor aspects of the hind pasterns. The condition is colloquially named "*grease heel*" because thick, yellow grease-like exudate mats the hair. Unfortunately, poxvirus infection is only one manifestation of this clinical entity, sparking considerable controversy as to the true nature of equine pox.

A second form has a predilection for the *muzzle and buccal cavity*. The lesions, which develop in the typical sequence of a pock, affect the inner surface of the lips and cheeks, the gums, and the ventral surface of the tongue. Following the development of the buccal eruptions, crops of pocks may appear in the rostral nares, on the face and on other parts of the body. It is a benign infection, sometimes seriously complicated by bacterial contamination.

Another manifestation takes the form of generalized papular eruptions. In the USA and Australia, the disease is known as

equine papular dermatitis. This is a highly contagious disorder, spreading by direct contact and through infected harness, bedding and grooming tools. The lesions are firm papules, up to 0.5 cm diameter, which tend to develop on the lateral neck and shoulder and thorax but become generalized. The papules become crusted and the crusts slough to leave circular alopecic patches. Resolution may take 6 weeks.

Uasin Gishu disease from Kenya is also characterized by generalized skin lesions but the disease differs clinically from equine papular dermatitis. Lesions begin similarly as small papules but develop into crusted papillomatous proliferations, up to 2 cm diameter. Lesions eventually resolve but the disease may continue for 2 years. The histologic lesions of Uasin Gishu are identical to molluscum contagiosum.

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Equine molluscum contagiosum

Equine molluscum contagiosum is another proliferative poxvirus infection in the horse, and *the causal virus is now thought to be identical to, or closely related to, human Molluscum contagiosum virus.* In horses this is a mildly contagious, self-limiting cutaneous infection. In situ hybridization experiments indicate there is very close homology between the equine and human *Molluscum contagiosum virus*. Attempts to grow both the human and equine *Molluscum contagiosum virus* in culture have failed, and this can help differentiate the virus from the orthopoxvirus of Uasin Gishu, which produces histologically and clinically similar lesions, but which can be grown in culture. Cutaneous lesions resembling the human and equine condition clinically, microscopically and ultrastructurally occur in macropods (kangaroos and quokkas) and chimpanzees. The small, self-limiting lesions are easily overlooked and usually are found incidentally at autopsy or surgery. The equine lesions may be localized to the *penis, prepuce, axillary and inguinal areas, and muzzle.* Concurrent systemic disease, such as granulomatous enteritis, may predispose to more widespread distribution. Commencing as multiple, circular, smooth-surfaced, gray-white 1–2 mm papules, the lesions become umbilicated and develop a central pore from which a tiny caseous plug is extruded.

The microscopic lesions of molluscum contagiosum are highly characteristic. *Well-demarcated foci of epidermal hyperplasia and hypertrophy form pear-shaped lobules in the superficial dermis.* The individual keratinocytes are markedly swollen and contain *large intracytoplasmic inclusions known as "molluscum bodies"* (Fig. 5.82). These occur initially as eosinophilic, floccular aggregates in the cells of the inner stratum spinosum. As the keratinocytes move towards the surface, the inclusions grow in size and density, compressing the nucleus against the cytoplasmic membrane until it is a thin crescent. The inclusion becomes increasingly basophilic so that cells of the stratum corneum contain deep-purple molluscum bodies. These exfoliate through a pore that forms in the stratum corneum and enlarges into a central crater. There is usually no dermal reaction. Molluscum bodies are easily identified in cytological preparations. The inclusions contain myriads of poxvirus particles at various developmental stages, but to date no attempts have been made to culture these viruses, presumably because the diagnosis has been post-mortem.

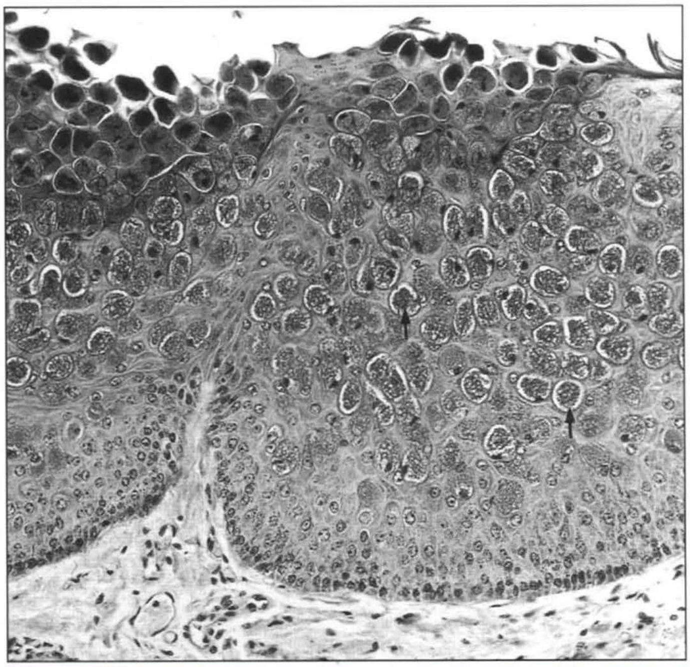

Figure 5.82 Molluscum contagiosum in a horse. Focal epidermal hyperplasia. Note large intracytoplasmic inclusion bodies referred to as "molluscum bodies" (arrows).

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Capripoxviral diseases

Sheeppox, goatpox and lumpy skin disease of cattle are caused by viruses of the genus *Capripoxvirus* and cause significant economic losses in countries where they are endemic. The exact relationship between these viruses has been controversial. *It is believed that they represent strains of a single virus.* The evidence includes antigenic and biochemical similarity, a high degree of nucleotide sequence homology, lack of absolute host specificity in most strains, evidence of recombination in the field, and demonstration of cross-infection and cross-protection. The viruses are indistinguishable by conventional serology; nevertheless the geographic distributions of the

3 are different indicating the viruses are distinct, and most strains of *Capripoxvirus* show definite host preferences. PCR methods of diagnosis of capripoxvirus have been developed so that classical virology methods based on live virus need not be used in areas of the world where the virus is exotic.

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Sheeppox

Sheeppox is the most serious of the pox diseases of domestic animals. It exists in Africa, Asia, and the Middle East where, despite attempts at vaccination, it is responsible for cycles of epidemic disease followed by periods of endemic maintenance with low morbidity. The disease is exotic to the Americas, Australia, and New Zealand. Eradication measures eliminated the disease from Britain in the mid-19th century but have only recently been successful in Eastern European countries. *Sheeppox causes extensive economic loss* through high mortality, reduced meat, milk or wool yields, commercial inhibitions from quarantine requirements, and the cost of disease prevention programs.

Transmission of infection is by direct contact with diseased sheep or indirect contact via contaminated environment. Insect transmission has been demonstrated experimentally. *Sheeppox virus* is resistant to desiccation and remains viable for up to 2 months on wool or 6 months in dried crust. There are breed differences in disease susceptibility. Fine-wooled Merino sheep are particularly sensitive whereas breeds native to endemic areas, such as Algerian sheep, are comparatively resistant. Sheeppox occurs in all ages of sheep with high morbidity, and mortality as high as 50%; but the disease is most severe in lambs, with mortality reaching 80–100%. A high level of background immunity, such as occurs in endemic areas of Kenya, is associated with low mortality, even in the young.

Sheeppox is a *systemic disease*. Infection is usually by the respiratory route but may occur through skin abrasions. The incubation period is 4–7 days and is followed by a leukocyte-associated viremia. The virus localizes in many organs including the skin where the virus concentration is highest 10–14 days postinfection. The initial clinical signs are fever, lacrimation, drooling, serous nasal discharge, and hyperesthesia. Skin lesions, which develop 1–2 days later, have a predilection for the sparsely wooled areas and typically involve eyelids, cheeks, nostrils, vulva, udder, scrotum, prepuce, ventral surface of the tail, and medial thigh.

The macroscopic lesions follow the typical pattern for pox infections. *Sheeppox lesions have a prominent vesicular stage* (Fig. 5.83A, B). The vesicles are umbilicated and, being multilocular, yield only a small amount of fluid if punctured. Occasionally a large vesicle forms as a result of cleavage of necrotic epidermis from underlying dermis. The *pustule stage* is characterized by the formation of a thin crust. In severely affected animals, the lesions coalesce. There may be marked, gelatinous dermal edema. Highly susceptible animals often develop hemorrhagic papules early in the course of the disease and, later, ulcerative lesions in the gastrointestinal and respiratory tracts. Approximately one-third of animals develop multiple pulmonary lesions that comprise *foci of pulmonary consolidation*.

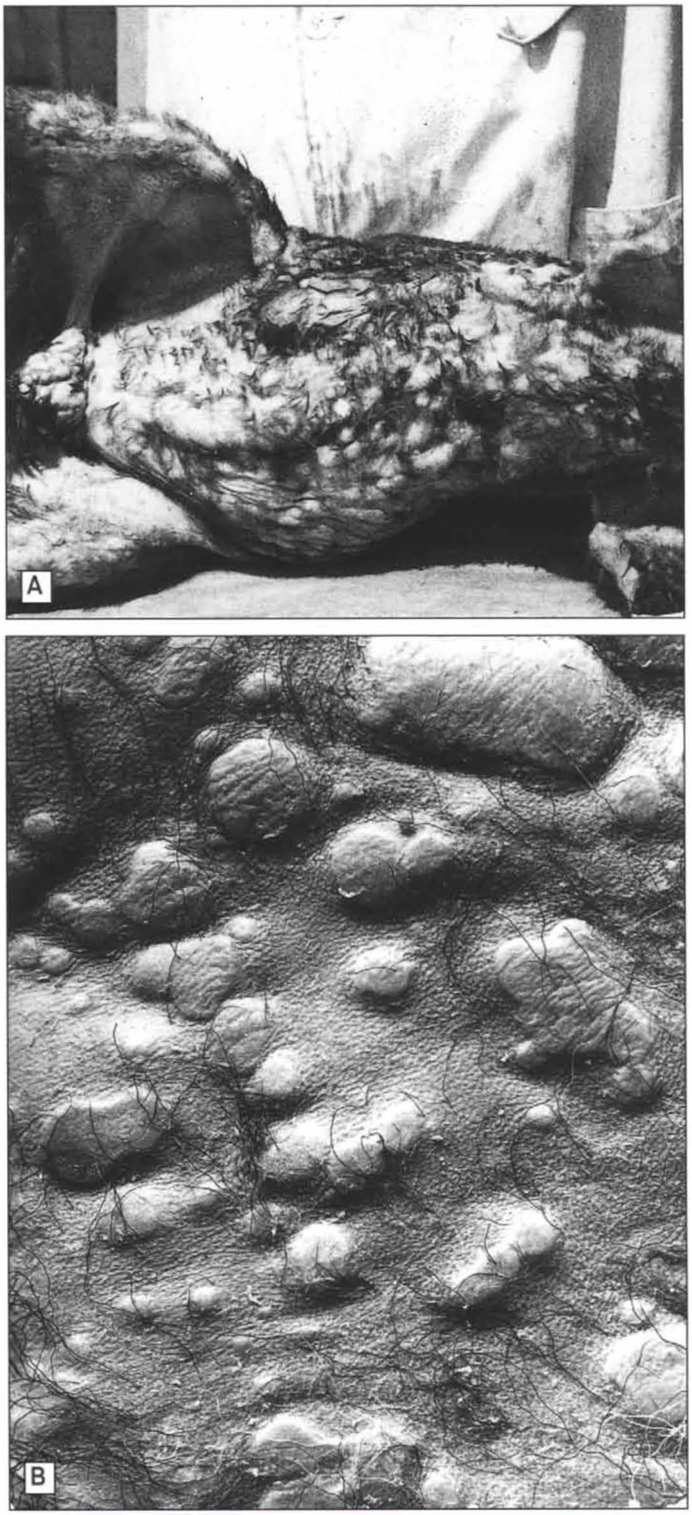

Figure 5.83 Sheeppox. (Courtesy of X Ivanov). **A.** Disseminated irregular swellings involving ventral abdomen and scrotum **B.** Close-up of papules.

The kidneys have multifocal, circular, fleshy nodules throughout the renal cortices.

Healing of the skin lesions is slow, taking up to 6 weeks and a scar may remain. In the milder form of the disease, seen in endemic areas, the full range of pox lesions does not develop. Instead, epidermal proliferation produces papules covered by scale-crust, which heal with desquamation in a few days. Such lesions often occur on the ventral surface of the tail.

Sheeppox lesions have the typical epithelial changes for the group, including marked vacuolar degeneration of stratum spinosum keratinocytes, microvesiculation, eosinophilic intracytoplasmic inclusion bodies, and epidermal hyperplasia. The lesions affect both surface epithelium and that of the hair follicles. There are, in addition, *marked dermal lesions* reflecting the systemic route of cutaneous involvement and possibly implicating immune-mediated lesions in addition to those caused by direct viral damage. The initial dermal lesions, corresponding to the macroscopic erythematous macule, are marked edema, hyperemia and neutrophilic exocytosis. During the papular stage, large numbers of mononuclear cells accumulate in the increasingly edematous dermis. These cells, first described by Borrel, are called "cellules claveleuses" or "*sheeppox cells*" and are characteristic of the disease. The nuclei of sheeppox cells are vacuolated and have marginated chromatin. The vacuolated cytoplasm contains single, occasionally multiple, *eosinophilic intracytoplasmic inclusion bodies.* Sheeppox cells are virus-infected monocytes, macrophages and fibroblasts, but not endothelial cells. Approximately 10 days post-infection and corresponding with the most prominent epithelial lesions and peak of skin infectivity, severe necrotizing vasculitis develops in arterioles and post-capillary venules. Virus particles have not been identified in endothelial cells, and the vasculitis may be due to immune-complex deposition. Ischemic necrosis of the dermis and overlying epidermis follows.

The pulmonary lesions are proliferative alveolitis and bronchiolitis with focal areas of caseous necrosis. Alveolar septal cells contain intracytoplasmic inclusion bodies. Additional histologic lesions, characterized by the accumulation of sheeppox cells, may involve heart, kidney, liver, adrenals, thyroid and pancreas.

The course and outcome of sheeppox depend not only on the usual host-virus relationship but also on the nature and location of secondary infections. The virus itself may cause death during the febrile, eruptive phase of the disease. Of great importance, however, are the secondary bacterial infections that rapidly develop in the necrotic tissue of the pocks. Death is often due to bacterial septicemia or pneumonia.

Goatpox

Goatpox, caused by *Goatpox virus*, occurs in North Africa and the Middle East. A benign form of goatpox occurs in California and Sweden. The clinical signs of goatpox vary in different geographic areas. The disease has *many parallels with sheeppox, but is generally milder* with a low mortality rate (5%), although generalized eruption with mortality rates approaching 100% may occur. The cutaneous lesions have a predilection for the same areas as for sheeppox. In nursing kids, lesions may appear on the buccal mucosa or rostral nares. In animals with higher levels of resistance, the lesions may be confined to the udder, teats, inner aspects of thighs or ventral surface of the tail.

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Lumpy skin disease

Lumpy skin disease, caused by *Lumpy skin disease virus* of the *Capripoxvirus* genus, is a disease of cattle, buffalo, and occasionally other wild species of hoofstock, characterized by the *eruption of multiple, well-circumscribed skin nodules, accompanied by fever, ventral edema, and generalized lymphadenopathy.* Lumpy-skin disease is found throughout the African continent and Madagascar, with sporadic reports from the Middle East and Israel.

Cattle of all ages, sex and breeds are affected, although the disease is more severe in Channel Island breeds. Both *Bos indicus* and *Bos taurus* cattle are susceptible, however the disease can be less clinically severe in Zebu breeds. The disease occurs in epidemics – a notable one in 1944 affected 8 million cattle. Infection is transmitted mechanically by a variety of biting insects. Epidemics tend to follow periods of prolonged rainfall, which favor population increases in vector species. A forest maintenance cycle, probably involving Cape buffalo, is thought to be the reservoir of infection in the inter-epidemic periods. No reservoir host apart from cattle has been identified.

The morbidity is extremely variable and inapparent infection is not uncommon. *Mortality is usually low*; around 1% but may be >50%. Economic losses are due to debilitation, loss of milk and meat production, damage to hides, and reproductive wastage due to fever-associated abortions and temporary sterility in bulls.

The natural incubation period of lumpy-skin disease is 2–4 weeks, but this may be halved in experimental infection. In severely affected animals, the development of large numbers of cutaneous lesions over most of the body is preceded by fever, marked weight loss, profuse drooling, oculonasal discharge, ventral edema and generalized lymphadenopathy. In the mild disease, there may be few isolated nodules and no prodromal fever. *The cutaneous lesions are firm, circumscribed, flat-topped nodules 0.5–5.0 cm in diameter* (Fig. 5.84). They may coalesce. The nodules have a creamy-gray color on cut section and involve the full width of the cutis, extending into the subcutis and occasionally adjacent muscles. Nodules affecting the scrotum, perineum, udder, vulva, glans penis, eyelids and conjunctiva are usually flatter, and in non-pigmented tissue are surrounded by a zone of intense hyperemia. *The fate of the nodules varies. Typically, they undergo central necrosis and sequestration, but some may resolve rapidly and completely, and others may fail to separate but, instead, become indurated and persist as hard intradermal lumps for many months.* Sequestration is preceded by central necrosis in the nodule and occurs rapidly. Separation of the epidermis around the margin of the nodule exposes a rim of dermal granulation tissue. As the process of separation extends into the dermis, the nodule comes to contain a *core or sequestrum of*

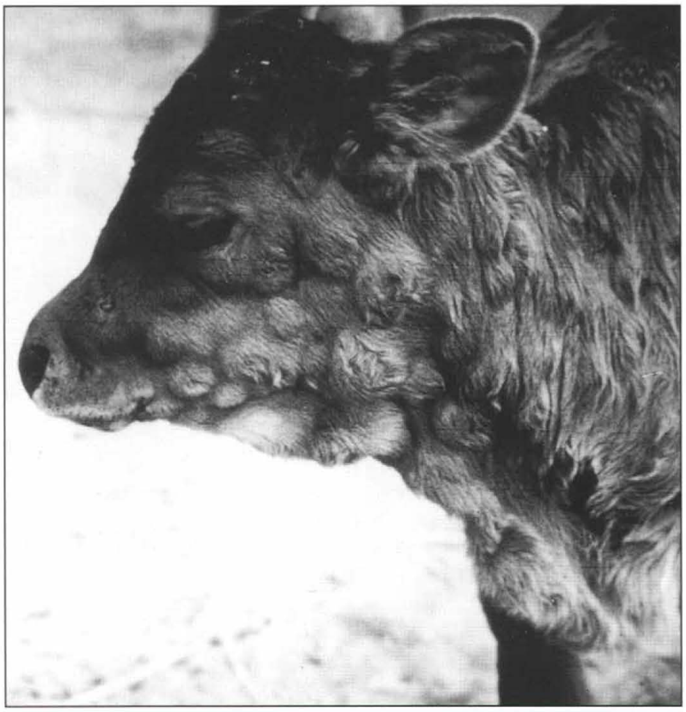

Figure 5.84 Circumscribed, nodular lesions of the skin in **lumpy skin disease** in a calf. (Courtesy of CC Brown.)

necrotic material ("sit-fast"), which is cone-shaped and flat-topped. When the sequestrum is removed, a deep ulcer remains which is slowly filled with granulation tissue. *Secondary bacterial infections* develop in the necrotic cores of the nodules and contribute very significantly to the seriousness of the disease. Large craterous ulcers develop which lead to lymphangitis and lymphadenitis. Local extension of lesions causes blindness, tenosynovitis, arthritis or mastitis.

The mucous membranes of the upper respiratory and upper alimentary tracts often develop multiple, discrete ulcerative lesions, irrespective of the number of cutaneous nodules. Those in the respiratory tract may cause swelling sufficient to result in severe dyspnea and asphyxia. Aspiration may lead to pneumonia or, if the animal recovers, scarring may cause stenosis of the cranial portion of the trachea. Nodules occasionally occur in parenchymal organs including kidneys, lungs and testes.

Although the virus is introduced percutaneously, *the infection is systemic*. A leukocyte-associated viremia disseminates the virus to various tissues, including the skin where greatest virus concentration occurs 9–12 days postinfection. The virus infects a wide range of cells, including keratinocytes, mucous and serous glandular epithelium, fibrocytes, skeletal muscle, macrophages, pericytes, and endothelial cells. *Damage to endothelial cells causes vasculitis that is central to the pathogenesis of the lumpy-skin disease lesions.*

Acute lesions consist of vasculitis, lymphangitis, thrombosis, marked dermal edema that sometimes induces dermoepidermal separation, and infarction. The epidermis shows the typical vacuolar changes associated with poxvirus infection. *Intracytoplasmic, eosinophilic, homogeneous, and occasionally granular, inclusion bodies* occur in endothelial cells, pericytes, keratinocytes, macrophages and fibroblasts. Virions in various stages of development are present in these inclusion-containing cells and in peripheral nerves. Neutrophils, macrophages and occasionally eosinophils migrate into the dermis in the acute lesions to be replaced as the lesion ages by a predominantly mononuclear cell population. The infarcted tissue is sequestered and surrounded by granulation tissue. Inclusion bodies are absent from the resolving lesions but may be present in adjacent skin or sebaceous glands. Lymph nodes are edematous and hyperplastic.

The chief differential diagnosis is **pseudo-lumpy skin disease** *caused by a herpesvirus identical to the bovine herpes mammillitis virus but originally known as the Allerton virus.* Pseudo-lumpy skin disease is a milder condition clinically and the nodules are superficial, resembling only the early stage of lumpy-skin disease. Confirmation of the latter is best achieved by demonstration of poxvirus particles in fresh or formalin fixed tissue.

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Suipoxviral disease

Swinepox

The host-specific *Swinepox virus*, the sole member of genus *Suipoxvirus*, is the chief cause of pox lesions in swine. In the past, *Vaccinia virus* was also responsible. The disease occurs worldwide and is endemic in areas of intensive swine production. The disease has received relatively little attention as it is *usually mild and mortality is negligible. It chiefly affects young, growing piglets* but occurs in neonates promoting speculation that transplacental infection may be possible. Normally, *swinepox virus* is transmitted by contact. The virus is resistant and persists in dried crust from infected animals. The sucking louse *Haematopinus suis* often acts as a mechanical vector and also assists infection by causing skin trauma. *The gross lesions typically affect the ventral and lateral abdomen, lateral thorax, and medial foreleg and thigh* (Fig. 5.85A). Occasionally, lesions on the dorsum predominate. In severe infection, lesions may be generalized and rarely involve the oral cavity, pharynx, esophagus, stomach, trachea and bronchi. The morphology of the gross lesions follows the typical pattern of pox infection. The erythematous papules usually transform into umbilicated pustules without a significant vesicular stage. The inflammatory crust eventually sheds to leave a white macule. *Grossly, swinepox must be differentiated from the vesicular diseases, classical swine fever, pityriasis rosea, dermatosis vegetans, and sunburn.*

The histologic lesions also follow the pattern for pox infections (Fig. 5.85B). The eosinophilic, intracytoplasmic inclusion bodies are quite transient and are not found in older lesions. Vacuoles develop in the nuclei of infected stratum spinosum keratinocytes early in the course of swinepox infection.

Vaccinia virus causes lesions in swine that are clinically difficult to differentiate from *Swinepox virus* infection. However with the eradication of smallpox and the cessation of smallpox vaccination, swinepox is now the primary poxviral disease of swine. Vaccinia viral infections have a shorter incubation period and smaller, more

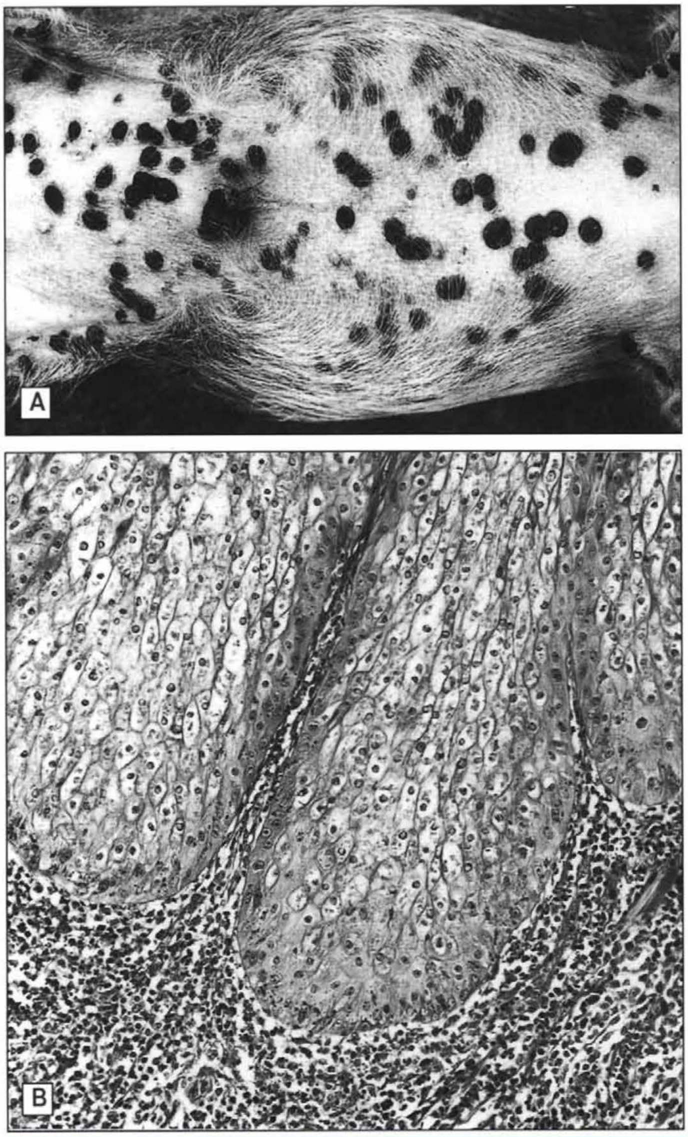

Figure 5.85 Swinepox. A. Gross lesions on ventral body. **B.** Marked acanthosis and intracellular edema and diffuse dermatitis.

transient lesions. Histologically the lesions are very similar except that nuclear vacuoles are not observed. The diseases are definitively differentiated only by virological examination.

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Herpesviral infections

The Herpesviridae is a large family of *enveloped DNA viruses* responsible for important animal diseases, of which relatively few are primary skin diseases. *Bovine herpesvirus 2* causes primary cutaneous infections and is widespread in many parts of the world. It is responsible for 2 distinct clinical forms of skin disease; *bovine mammillitis of cattle*, which is the major cutaneous infection, and *pseudo-lumpy skin disease*. Skin lesions occur in systemic herpesvirus infections such as *malignant catarrhal fever*, and *infectious bovine rhinotracheitis* in cattle, and *pseudorabies* in pigs. Skin lesions associated with *Felid herpesvirus 1* infection are also recognized, usually in the absence of respiratory lesions. Vesicles, pustules, ulcers and depigmentation are seen on the genital organs and occasionally the lips and nostrils of horses with *Equid herpesvirus 3* infection (equine coital exanthema), and in *Bovine herpesvirus 1* infectious vulvovaginitis and balanoposthitis.

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Bovine herpesvirus 2 diseases

Pseudo-lumpy skin disease

Bovine herpesvirus 2 (BoHV2) is a member of the Alphaherpesvirinae subfamily and is antigenically related to human Herpes simplex virus 1. First isolated in Africa in 1957 from lesions resembling lumpy-skin disease, it was named the *Allerton virus*. The associated disease was subsequently named "pseudo-lumpy skin disease" to differentiate it from the more serious disease caused by *Lumpy skin disease virus*. Pseudo-lumpy skin disease is widespread in southern Africa with sporadic reports from Australia, the UK, and the USA. It is characterized by a generalized eruption of superficial cutaneous nodules that develop a central depression but that heal without scar formation and do not produce the deep necrotic sequestra of true lumpy skin disease.

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Bovine herpes mammillitis

Bovine herpes mammillitis, or ulcerative mammillitis, is a localized form of BoHV2 infection seen sporadically in the USA, Canada, Great Britain, Europe, Africa, and Australia. Serological surveys indicate that *infection is much more common than disease*. In Africa and Asia, several species of wild animals have antibody titers to the virus, although clinical disease is not seen.

Herpes mammillitis is *chiefly a disease of lactating dairy cows* but occurs in heifers about to calve and in beef animals. The incidence is usually sporadic with occasional local epidemics in fully susceptible herds. In previously exposed herds, the disease affects only the recently introduced nonimmune, first-calf heifers. There is no mortality. The economic significance of teat lesions lies in the effect on milk production or secondary bacterial mastitis, which complicate ~20% of cases.

Intact teat skin is refractory to virus penetration, indicating that *some form of teat trauma precedes infection*. Transmission of the virus is presumed to involve mechanical vectors, particularly the milking machine, but biting flies, such as *Stomoxys calcitrans*, have also been implicated. In the latter instance, it is difficult to explain the localization of lesions to the teat and mammary gland. Local tissue temperature has, however, been shown to be critical in the pathogenesis, and environmental conditions have been associated with the incidence of outbreaks. Experimental cutaneous inoculation of BoHV2 results in higher virus titers and larger and more persistent lesions when the site is kept cold. The increased prevalence of disease in the autumn months may also relate to the temperature sensitivity of the virus. The source of infection within a herd is not known, but latency, a characteristic of the Herpesviridae, is likely important. Latency has been demonstrated in experimental BoHV2 infections.

The macroscopic lesions affect the teats, less frequently the udder and occasionally the perineum of lactating cows. Transmission of infection to nursing calves may result in ulcerative lesions of the muzzle, chin, lips, and occasionally the oral cavity. Teat lesions develop after a 3–7-day incubation period. *The teat becomes very swollen and painful and develops 1–2 cm diameter plaques*. Vesicles are rare. The epidermis in the center of the plaque becomes necrotic and sloughs to expose an erythematous, irregularly shaped ulcer. Exuded serum mixed with blood forms a thin brown crust, which is easily displaced by the teat cups. The lesions heal beneath the crust and there is no residual scar. Lesions on the udder are often diffuse, giving rise to the term "gangrene of the udder." Regional lymph nodes are swollen in the early stages.

The microscopic lesions are characterized by the formation of epithelial syncytia containing prominent intranuclear eosinophilic inclusion bodies. The inclusion bodies are typical Cowdry type A. The nuclear chromatin is marginated and the eosinophilic inclusions are surrounded by a clear halo. The inclusions are numerous from the time of the first macroscopic lesion until the fifth day. Thereafter, they are very difficult to find. Syncytial cell formation commences early in groups of cells in the stratum basale and inner stratum spinosum. The process extends to involve the full thickness of the epidermis, the outer root sheath of the hair follicle infundibulum, and the sebaceous gland. By day 5 after macroscopic lesions develop, the epidermis is necrotic, although the outlines of syncytial cells are apparent. The inclusion bodies lose their sharp outline and fill the entire nucleus. Some are free in the necrotic epidermis, released from fragmenting nuclei. The necrotic epidermis and adnexa are infiltrated with large numbers of neutrophils. Loss of the necrotic epithelium leaves an ulcer, which is covered by hemorrhagic and fibrinous exudate and a band of degenerating neutrophils. Deeper, there is dermal edema and a predominantly mononuclear cell infiltrate. In the healed lesion, the epithelium is re-established; there is superficial dermal fibrosis, and a perivascular infiltrate of predominantly mononuclear cells.

The diagnosis of herpes mammillitis is confirmed by isolation of the virus. A rapid provisional diagnosis may be made by examining clinical material by electron microscopy. Biopsy is diagnostic if lesions are collected before the fifth day. Cytology smears prepared from an early lesion are diagnostic if syncytial cells containing eosinophilic intranuclear inclusion bodies are found. Serological tests allow a retrospective diagnosis provided paired samples are collected and a rising titer identified.

Experimental intravenous inoculation of **sheep** with BoHV2 results in lesions resembling those of similarly inoculated cattle. Naturally occurring outbreaks of dermatitis of the pasterns caused by BoHV2 have been described in captive Dahl sheep. Goats will only develop skin lesions following intravenous inoculation.

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Bovine herpesvirus 4 diseases

A strain of *Bovine herpesvirus 4* (BoHV-4) can cause udder lesions on dairy cows (**mammary pustular dermatitis**); the teats are not involved. The macroscopic lesions are *vesicles, pustules, ulcers and crusts*, originally 2–4 mm in diameter, becoming larger with coalescence. The microscopic lesion is *intraepidermal pustular dermatitis*. The etiologic association between BoHV-4 and the cutaneous disease is controversial however, as the virus is an ubiquitous herpesvirus of cattle that can produce no disease when experimentally inoculated into susceptible cattle and can be isolated from cell cultures prepared from clinically normal cattle. While not a primary mastitis pathogen, BoHV-4 may prolong cases of bacterial mastitis. BoHV-4 infection is considered a risk factor for abortion in cows, and is reported as a cause of endometritis in postparturient dairy cows.

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Felid herpesvirus 1

Felid herpesvirus 1 (FeHV-1, Feline viral rhinotracheitis virus), a well recognized pathogen of the upper respiratory tract, is commonly associated with oral ulceration. It can also be associated with *focal ulcerative lesions primarily on the haired skin of the face or on the nasal planum*, with rare reports of lesions on the feet and trunk. The lesions generally occur in the absence of clinical respiratory signs. In common with other herpesviruses, FeHV-1 can establish latency in the trigeminal ganglion. As affected cats often have a history of previous respiratory disease or recent stress, recrudescence of a latent herpesvirus infection is likely. *The macroscopic lesions consist of crusts, ulcers, and vesicles, frequently on the face or nasal planum, which can be persistent or recurrent* (Fig. 5.86A). Microscopically, the lesions are

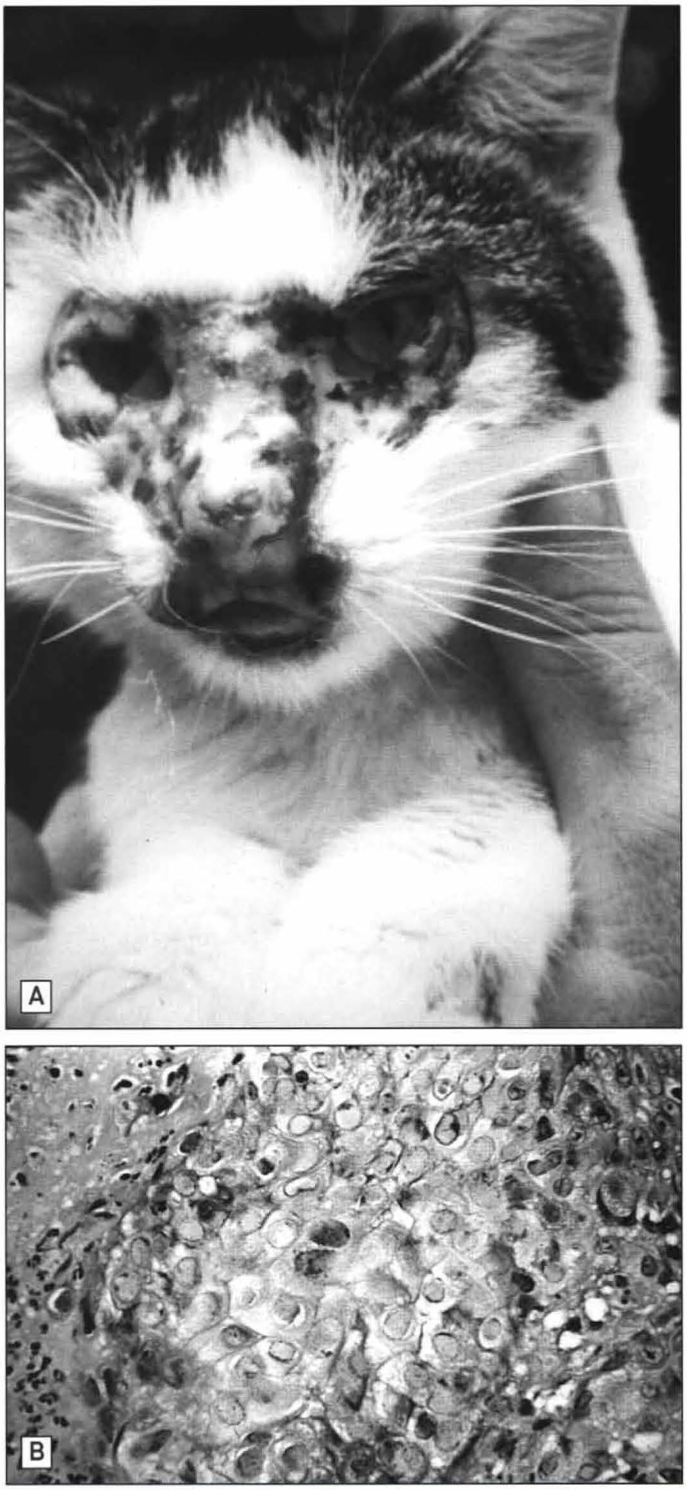

Figure 5.86 Feline herpesvirus dermatitis. A. Marked ulceration and crusting lesions of the face. (Courtesy of University of Florida Clinical Dermatology Service.) **B.** Note marked intracellular edema and **intranuclear inclusions** within keratinocytes.

ulcerative and necrotizing, and the mixed dermal inflammation frequently includes numerous eosinophils. Adnexae can be destroyed and free keratin in the dermis is associated with eosinophils and foci of collagen degeneration. *Large amphophilic or glassy intranuclear inclusions* are present in the surface and adnexal epithelium (Fig. 5.86B). They are variable in number and sometimes hard to find in small rafts of epithelial cells surrounded by necrotic debris. The similarity of the inflammatory component in this condition with that of the feline hypersensitivity conditions, such as mosquito bite hypersensitivity or feline eosinophilic ulcer, warrants close scrutiny of eosinophilic necrotizing cutaneous lesions for intranuclear inclusions or examination by molecular techniques.

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Retroviral infections

Several skin disorders have been associated with retroviral infections caused by ***Feline leukemia virus*** (FeLV) and ***Feline immunodeficiency virus*** (FIV) in **cats**. Since both viruses are immunosuppressive, *most dermatological conditions are considered secondary*. However, primary viral infection of keratinocytes has been reported. Scaling, crusting and alopecic lesions affecting primarily the head and face with occasional involvement of trunk and extremities is seen uncommonly in FeLV-infected cats. Histologically, syncytial keratinocytes are seen in the epidermis and superficial follicular epithelium, accompanied by dyskeratosis, pustules, and ulcers. Immunohistochemical staining demonstrates FeLV antigen in epithelial cells and giant cells. In addition, cutaneous keratin horns seen in FeLV-infected cats have been represented as a primary effect of viral infection of keratinocytes. Recurrent pyoderma and paronychia have been attributed to the immunosuppressive effects of FeLV. Bacterial skin disease affecting the face and cellulitis associated with ear tags are described in the acute stage of experimental FIV infection in specific-pathogen-free kittens. Dermatological diseases associated with naturally occurring chronic FIV infection include generalized demodectic mange, notoedric mange, cowpoxvirus infections, pustular pyoderma, atypical mycobacteriosis, miliary dermatitis and abscesses. Cats that are FIV-positive are more frequently diagnosed as having subcutaneous abscesses and cellulitis than are noninfected cats. Since the virus is shed in high titer in the saliva, and bite wounds are thought to be an important mode of transmission, this finding may simply reflect a greater tendency for cats that fight to become FIV-positive. Many of the associations made between retroviral infection and specific diseases are anecdotal.

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Parvoviral infections

More typically associated with reproductive problems, ***Porcine parvovirus*** (PPV) has been implicated in outbreaks of vesicular and ulcerative dermatitis and glossitis in 1–4-week-old piglets in the midwestern USA. Various clinical signs, including diarrhea and sneezing, accompanied the skin lesions. *Small slit-like erosions, ruptured vesicles, and extensive ulceration were seen on the tongue, lips, snout, coronary band and interdigital spaces.* Severe lesions sometimes led to separation and sloughing of the hoof wall. Intact vesicles were seen rarely. Sporadic cases have been reported in which the lesions were exudative rather than ulcerative. They were grossly indistinguishable from those of exudative epidermitis. PPV has been cultured from skin and internal organs of affected piglets and PPV antigen has been detected in hair follicles of lesional skin. Skin lesions were reproduced with tissue culture-origin PPV, but were not as severe as those induced with crude suspensions prepared from the skin lesions. In the sporadic cases, *Staphylococcus* spp. and *Swinepox virus* were also recovered. It is likely that the severe clinical disease results from dual bacterial and viral infection.

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Papillomaviral infections

Papillomaviruses are associated with a variety of proliferative skin lesions, typically benign epithelial neoplasms, although they have also been shown to be associated with proliferative cutaneous plaques and papules. Although these lesions are almost always benign and often self-limiting, there is evidence that in some cases, both in humans and animals, the virus can be a factor in the development of malignant tumors. Papillomaviruses tend to be species-specific viruses that affect stratified squamous epithelium in all domestic, and numerous wildlife, species. Papillomavirus-associated lesions are discussed under Tumors of the epidermis.

BACTERIAL DISEASES OF SKIN

Normal skin of healthy individuals is highly resistant to invasion by bacteria due to natural defense mechanisms, consisting of physical, chemical, and microbial components. The stratum corneum is composed of tightly packed keratinized cells and intercellular substance derived from lamellar granules that form an impermeable physical barrier. Furthermore, this layer continually desquamates and any adherent bacteria are lost as the outer cells are shed. The sebum-sweat emulsion, which spreads along the skin surface, contains a variety of antibacterial substances including fatty acids, inorganic salts, and proteins such as complement components, transferrin, and immunoglobulins. Finally, the normal skin microflora prevents pathogenic bacteria from multiplying and becoming established on the skin. These normal resident bacteria are a mixture of organisms that live in symbiosis and maintain static, consistent populations restricted to the superficial layers of the stratum corneum and hair follicle infundibula. They inhibit colonization of invading organisms by competition for limited nutrients and production of antibacterial substances. The protective effect of bacterial interference is well documented. For example, the development of exudative epidermitis has been prevented by exposing piglets to avirulent strains of *Staphylococcus hyicus*.

The normal skin flora is established shortly after birth and it is difficult subsequently to introduce other bacteria. The normal resident flora of skin has been investigated most extensively in dogs. Coagulase-negative staphylococci, *Micrococcus* spp, some aerobic gram-negative species, and *Clostridium* spp. are most numerous and are probably residents of canine skin. The presence of coagulase-positive staphylococci in the normal canine microflora is controversial. *Staphylococcus intermedius*, the most common canine skin pathogen, is commonly isolated from the anus and nares and less frequently from the skin and hair of dogs. The mucous membrane sites may act as carrier sites for seeding of the skin, where *S. intermedius* may be a transient organism taking advantage of temporary changes in local microenvironment that permit its short-term proliferation. In all species, skin surface humidity and temperature are important factors in determining the composition and density of skin microflora, with hot humid conditions being associated with increased numbers of skin bacteria. Regional variation in numbers and types of bacteria occurs, and moist intertriginous areas and oily skin have the highest numbers of bacteria.

Cutaneous bacterial infections are typically pyogenic and are thus commonly called **pyodermas**. They can be categorized as *primary and secondary* or *superficial and deep*. Primary pyodermas are those in which no underlying cause can be found. However, *it is now thought that the vast majority of pyodermas are secondary to underlying cutaneous, endocrine, or immunologic abnormalities*. Localized disruption of normal host defenses may be produced by maceration, biting ectoparasites, scratching, abrasions and other skin wounds, or introduction of foreign bodies such as plant thorns or awns; such disruption promotes development of clinical infection. Allergic, seborrheic, and follicular disorders are the most common predisposing causes of bacterial skin infection in dogs. Hypothyroidism and spontaneous and iatrogenic hyperadrenocorticism are common metabolic conditions associated with pyoderma. Primary immunologic abnormalities are uncommon predisposing factors.

Bacterial skin disease is seen much more frequently in dogs than in any other mammalian species, and pyoderma is one of the most common skin diseases in dogs. This apparent increased susceptibility of dogs to pyoderma has been attributed to the relatively thin, compact stratum corneum, the small amount of intercellular lipids in the stratum corneum, lack of a protective lipid seal at the entrance of canine hair follicles, and the relatively high pH of canine skin. *S. intermedius* is the predominant bacterial isolate from canine pyodermas. The factors that promote proliferation of *S. intermedius* on the skin and development of pyoderma are poorly understood. Pathogenicity of staphylococci in humans correlates with various proteins and toxins thought to act as virulence factors. Similarly, a role has been suggested for *S. intermedius* exotoxins (superantigens) in the pathogenesis of canine pyoderma.

Coagulase-positive staphylococci are also the most common bacteria isolated from pyoderma in horses (*S. aureus*, *S. intermedius*), in goats (*S. intermedius*, *S. aureus*), and in cattle and sheep (*S. aureus*). *Staphylococcus hyicus* causes exudative epidermitis in piglets and has been associated with superficial pyoderma in several other species.

Many other bacteria cause skin infections. *Dermatophilus congolensis* is responsible for superficial pyoderma in many species. Many gram-negative bacteria are opportunistic pathogens that can invade already diseased or compromised skin. Organisms that are typically associated with infections of other organ systems occasionally cause skin disease. *Listeria monocytogenes* has been found to be the cause of pyoderma in a small number of humans and a dog. Although bacterial infections are typically associated with neutrophilic inflammation, certain bacteria such as mycobacteria, *Actinomyces,* and *Nocardia* typically produce *granulomatous dermatitis or panniculitis.*

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Superficial bacterial pyoderma

Superficial pyodermas involve the epidermis and/or superficial portion of hair follicles. They occur more commonly than deep pyodermas. Superficial pyodermas usually are of short duration, heal without scarring, and are not usually associated with systemic illness. *Gross lesions are extremely variable* and include papules, pustules, crusts, circular scaling areas of alopecia (**epidermal collarettes**) (Fig. 5.87), hyperpigmented or erythematous macules, a moth-eaten appearance to the hair coat, diffuse erythroderma, and hyperpigmented lichenified plaques. *Microscopic lesions* consist of subcorneal or loosely organized, spongiotic superficial epidermal pustules, superficial folliculitis, and crusts. Neutrophils are the predominant inflammatory cell. Bacteria are not always visible histologically and culture may be necessary to confirm the etiology.

Impetigo

Impetigo is a superficial pustular dermatitis that does not involve hair follicles. It is most common in dogs but also occurs in kittens, piglets, cows, sheep, and goats. Impetigo is usually caused by *coagulase-positive staphylococci in association with predisposing causes.* Moist and dirty environments, cutaneous abrasions, parasitism, stress, and poor nutrition are common predisposing factors in most species. Impetigo in adult dogs is frequently seen in conjunction with diabetes mellitus, hypothyroidism, and natural or iatrogenic hyperglucocorticism. Lesions develop in young kittens as a result of excessive wetting of the skin by the queen as she transports the kittens.

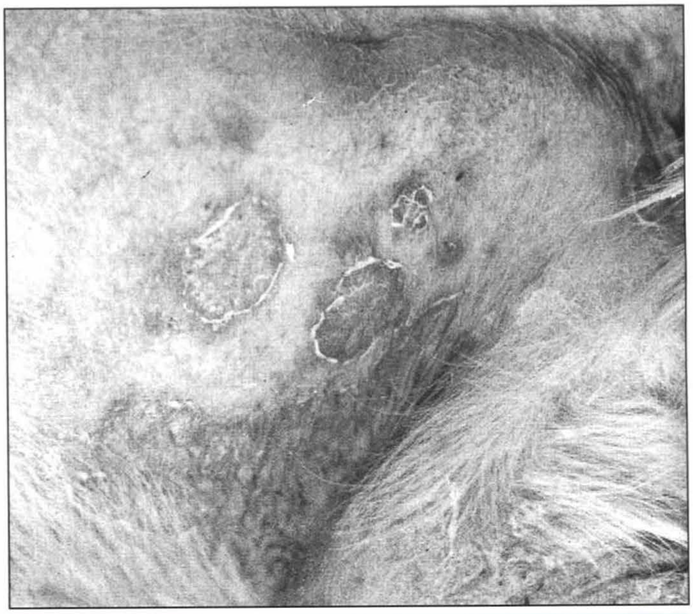

Figure 5.87 Superficial pyoderma. Annular erythematous lesions with epidermal collarette.

The lesions begin as small erythematous papules that develop into superficial pustules (Fig. 5.88A). They are fragile and rupture easily, leaving a honey-colored crust adherent to a shallow erosion. A *bullous form of impetigo*, consisting of large flaccid pustules, is more common in adult dogs. In *puppies*, the lesions are most common on the glabrous skin of the inguinal and axillary areas. In *kittens*, the lesions are usually confined to sites which are most commonly in contact with the queen's mouth; these include the back of the neck, head, and shoulder areas. Lesions in *cows and does* are usually on the udder, especially at the base of the teats and the intermammary sulcus. Occasionally, the infection extends to involve the teats, ventral abdomen, medial thighs, perineum, and ventral surface of the tail. The lesions are infectious and may be spread by the milker to other cows or does and to the hands of milkers. One outbreak of contagious impetigo in a herd of dairy cattle was associated with crowding and intensive showering of the cows to decrease heat stress during the heat of summer.

The microscopic lesion of impetigo is a subcorneal pustule composed of neutrophils primarily (Fig. 5.88B). The pustules usually extend above the skin surface and are located between hair follicles. Bullous impetigo consists of larger pustules that span several hair follicles. Acantholysis may be mild. Gram-positive cocci are present within intact pustules. The epidermis is mildly to moderately acanthotic and variable intercellular edema is common beneath pustules. The superficial dermis is edematous and superficial perivascular to interstitial inflammation is composed of neutrophils primarily. *The principal differential diagnosis is pemphigus foliaceus.* However, in contrast to pemphigus, acantholysis is absent or minimal in impetigo and bacteria are present in intact pustules of impetigo.

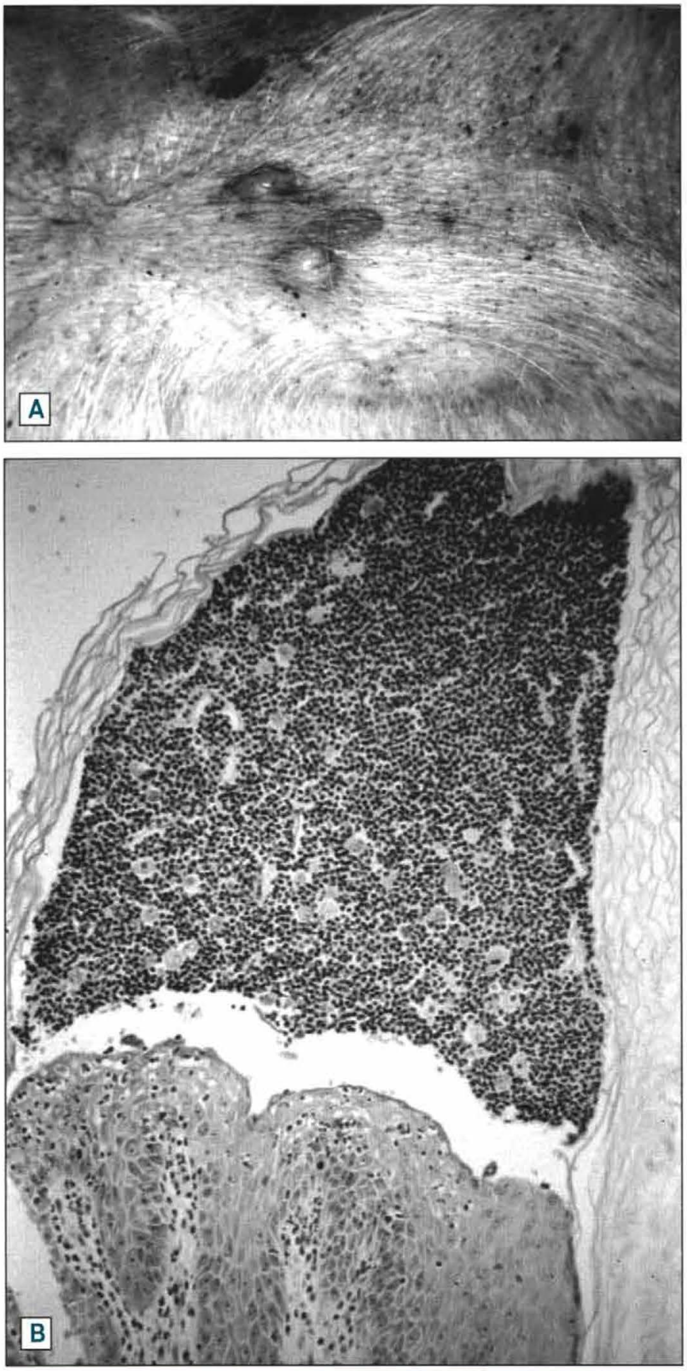

Figure 5.88 Superficial pyoderma (impetigo) in a puppy. **A.** Note pustules. (Courtesy of University of Florida Clinical Dermatology Service.) **B. Subcorneal pustule** in superficial pyoderma (impetigo).

Exudative epidermitis of pigs

Exudative epidermitis is an acute, exudative, superficial pyoderma of young pigs caused by Staphylococcus hyicus. The disease has also been called **greasy-pig disease**, impetigo contagiosa suis, and seborrhea oleosa. The infection occurs worldwide wherever intensive pig production is carried out. It is most common in *piglets 5–35 days of age*, but mild cases occur in older pigs also. The morbidity ranges from 10–90% and mortality from 5–90%. Mortality is higher in young pigs with lower resistance. Usually, when a litter is affected, all piglets develop the disease. *The infection may cause significant economic loss*; and in some countries, such as Denmark, the incidence appears to be increasing. Autogenous vaccines prepared from toxigenic strains of bacteria are used to prevent infection.

The *pathogenesis* of exudative epidermitis is incompletely understood. Both virulent and nonvirulent strains of *S. hyicus* are part of the normal skin flora of healthy pigs. The highest rates of carriage of the organism are found in the youngest piglets, suggesting that the organisms are acquired at birth. However, the presence of virulent organisms is not sufficient to produce disease. It is thought that infection develops as a result of *trauma* that breaches the skin barrier. Other factors that may predispose piglets to developing clinical disease include *agalactia of the sow, concurrent infections, and nutritional deficiencies. S. hyicus* strains are virulent by virtue of their ability to produce an *exotoxin* which, when injected in purified form or in cell-free extracts, is capable of producing exfoliative skin lesions in pigs which are typical of exudative epidermitis. Initial characterization of the exotoxin indicated that it has a molecular weight of approximately 27 kDa, is heat labile, appears to target epidermal cells of the stratum granulosum, and thereby produces intra-epidermal cleavage between the stratum corneum and stratum granulosum. More recent studies have identified at least three, and possibly four, antigenically distinct exfoliative toxins and shown them to be *metalloproteases*.

Exudative epidermitis has been compared to staphylococcal scalded skin syndrome of humans, which is also caused by several serologically distinct types of exfoliative toxins (epidermolysins). However, the presence of cocci in the lesions of piglets makes exudative epidermitis *more comparable to human bullous impetigo*, a superficial vesiculopustular infection that is also caused by staphylococcal exfoliative toxins and which contains gram-positive cocci within intact pustules. In contrast, staphylococci are present at a distant, usually extra-cutaneous, site in staphylococcal scalded skin syndrome rather than in the skin lesions. The toxins in both of these human conditions cause separation at the interface between the stratum spinosum and stratum granulosum by an unknown mechanism. However, it appears that the toxin acts primarily on intercellular substance, and binding to filaggrin may be involved in the pathogenesis. The disease affects children primarily, because the exfoliative toxin is excreted more slowly by the immature kidneys of young children. In addition, most adults are protected by anti-toxin antibody which young children do not have. Less rapid toxin excretion and lack of protective antibodies may also explain the greater incidence and severity of exudative epidermitis in the youngest piglets.

The disease can be divided into peracute, acute, and subacute forms.

- In the *peracute form*, most common in piglets only a few days old, there is abrupt onset of lesions around the eyes, snout, chin, and on the ears with extension to the medial aspect of the legs. Lesions then rapidly spread to the thorax, abdomen, entire legs, and hooves. Lesions begin as peeling of small areas of the stratum corneum leaving red, glistening, moist areas. These areas are quickly covered by *greasy, dark brown exudate*. Lesions become generalized in 24 to 48 hours and the entire body is erythematous and covered with brown, greasy, malodorous exudate (Fig. 5.89). Erosions of the coronary bands and heels commonly develop. Conjunctivitis also occurs frequently and typically causes matting together of the eyelids and results in an inability to see.

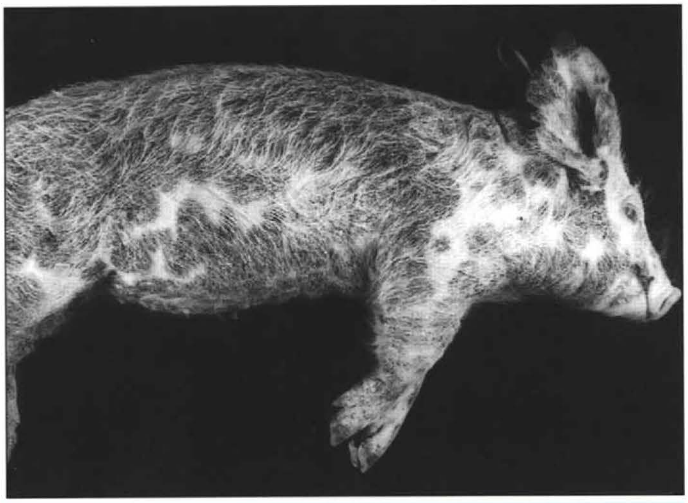

Figure 5.89 Exudative epidermitis ("greasy-pig disease").

Death occurs within 3–5 days as a result of dehydration, electrolyte imbalance, negative energy balance, and septicemia.

- In the *acute form*, the course is more protracted. The skin becomes thick and wrinkled and the exudate covering the entire body becomes dry, hard, and cracked, producing a generalized furrowed appearance. The underlying skin visible in the furrows is red.
- The *subacute form* occurs in older piglets and skin lesions are milder and usually confined to the head and ears. The subacute disease may appear as a dandruff-like scaling or as red-brown macules. Older piglets with less severe forms of disease frequently survive, however recovery is slow and the piglets are severely stunted. Additional lesions that may also occur in affected piglets are subcutaneous abscesses, necrosis of the ears and tail, and polyarthritis.

The earliest *microscopic lesion* is a subcorneal vesicular to pustular dermatitis. Extension of infection to hair follicles results in a superficial purulent folliculitis. In fully developed lesions, the skin is covered with a thick crust composed of ortho- and parakeratotic keratin, lakes of serum, accumulations of neutrophils, necrotic debris, and microcolonies of gram-positive cocci. The epidermis is variably acanthotic and rete ridges are elongated. Cells in the outer stratum spinosum exhibit variable intracellular edema. Neutrophilic exocytosis, intercellular edema, and spongiotic pustules may be seen in the epidermis and infundibular portion of hair follicles. The dermis is edematous, dermal vessels are congested, and there is a perivascular to interstitial neutrophilic infiltrate. Dermal inflammation is more intense and diffuse in areas of ulceration. In subacute cases, exudation is less severe and there is more marked epidermal hyperplasia, hyperkeratosis, and parakeratosis. Inflammation in the dermis becomes primarily mononuclear.

Microscopic lesions may also be seen in *other tissues*. Lymph nodes draining severely affected areas of skin contain foci of hemorrhage, purulent inflammation, and occasional microcolonies of bacterial cocci. Renal lesions are common and bacteremia is not required for them to be present. The lesions are distinctive, with early vacuolation of the epithelium of collecting ducts and renal pelvis progressing to epithelial degeneration and exfoliation. Intratubular casts of desquamated epithelium may be sufficiently severe to be evident macroscopically, leading to linear striations of the renal pelvis, and accumulation of cellular sediment in the pelvis and ureters. The process may be sufficiently severe to occlude the ureters. In cases in which bacteremia is present, purulent pyelonephritis is common. Animals with greasy pig disease may also have lesions in the oral cavity and conjunctiva, and the causative organism has been associated with abortion in sows.

The *differential diagnoses* for exudative epidermitis include zinc deficiency/ parakeratosis, sarcoptic mange, streptococcal pyoderma, and viral infections. In young pigs, the lesions are sufficiently distinctive that the disease is not confused with other conditions.

Staphylococcus hyicus has also been proposed as the cause of the so-called *flank-biting and necrotic ear syndromes of pigs*. Both conditions are characterized by large erosive to ulcerative crusty skin lesions in early weaned pigs. *S. hyicus* has been associated with skin lesions in *other species*. The organism is not uncommon in cattle and is frequently secondary to parasitic skin disease. The organism was isolated from skin lesions of a young pygmy goat with chronic generalized seborrheic dermatitis and alopecia. The microscopic lesion was a purulent exudative epidermitis similar to that in pigs. *S. hyicus* has been cultured from a number of horses with exudative crusty alopecic skin lesions on the distal limbs that resembled "grease heel" clinically.

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Dermatophilosis

Dermatophilosis (cutaneous streptothricosis, mycotic dermatitis, cutaneous actinomycosis, lumpy wool, strawberry foot rot, rain scald, rain rot, Kirchi, Gasin-Gishu, Senkobo disease, Drodo-Boka, Savi, Ambarr-Madow) is an *acute, subacute, or chronic superficial exudative dermatitis caused by the actinomycete Dermatophilus congolensis*. The disease occurs worldwide and has a wide host range but it is *most* *common in the hot humid tropics and subtropics and in areas with heavy prolonged rains.* In hot monsoon climates, cattle are the primary animals affected and the disease is endemic in some portions of Africa. In more temperate climates, sheep and goats are involved primarily. The disease is occasional in horses and it is rare in dogs, cats, pigs, and humans. Cases of dermatophilosis have also been reported in camels, mules, donkeys, zebras, giraffes, Thompson's gazelles, woodchucks, striped skunks, raccoons, hedgehogs, gerbils, foxes, ground squirrels, seals, owl monkeys, captive polar bears, chamois, tortoises, and Australian bearded lizards. There is no apparent sex or age predilection; and congenital infections have been reported in calves and lambs.

In cattle and sheep, dermatophilosis causes important economic losses by virtue of decreased meat and milk production, damaged hides, wool loss, and devaluation, infertility, and early culling. Severe udder and teat lesions may interfere with suckling by calves and result in decreased growth rate. Affected animals are predisposed to secondary infections and cutaneous myiasis. Severely affected animals of any species may become emaciated and die.

Dermatophilus congolensis is a gram-positive pleomorphic bacterium whose natural habitat is unknown. The organism is probably not free-living since attempts to culture it from soil have been unsuccessful. Clinically normal carrier animals and crusts from infected animals probably serve as sources of infection. *D. congolensis* has a distinctive life cycle in which *coccoid bodies germinate to produce branching filaments* (Fig. 5.90A). These filaments undergo transverse and longitudinal septation to *form parallel rows of coccoid bodies.* The cocci are resistant to unfavorable conditions and are reproductively dormant until the appropriate wet conditions occur and they are activated to become motile zoospores.

Dermatophilosis cannot be reproduced experimentally to resemble natural disease, even with large doses of the organism. *Multiple factors* appear to be involved in pathogenesis of the natural disease. Two factors that appear to be most important are trauma to the skin and prolonged wetting. Zoospores are unable to overcome the protective barriers of the hair, surface lipid film, and stratum corneum, and their entry is facilitated by breaks in the skin surface. Trauma from ectoparasites, shearing, dipping, barb wire injuries, sharp stones, and scratches from sharp vegetation can act as portals of entry for the organisms. Besides producing skin trauma, external parasites such as flies, mites, lice, ticks, and mosquitoes also act as mechanical vectors. *In addition to skin trauma, prolonged moisture is needed for the activation, proliferation, and spread of the zoospores.* Wetting may also act to breach skin barriers by dissolving the surface lipid film and softening the stratum corneum. Outbreaks of dermatophilosis are frequently associated with periods of unusually heavy rainfall, housing in pastures or paddocks with standing water or mud, or after intensive high-pressure washing of animals.

Other factors involved in development of disease are less well understood. Genetic factors may be involved, since some breeds of cattle appear to be more resistant to disease than are others. Skin color appears to have an effect because some light-skinned breeds or light-skinned areas may be more susceptible to infection. A definite association between infestation with the tick *Amblyomma variegatum* and occurrence of dermatophilosis in cattle has been found. In herds with effective tick prevention, there is a much lower incidence or diminished severity of the disease. Since the lesions of dermatophilosis do not correspond to sites of tick attachment, a systemic effect on the animal's immune system has been suspected. Experimental support for tick-induced immunosuppression has been found in studies comparing in vitro lymphocyte stimulation responses wherein tick-infested cattle had lower mitogen-induced lymphoblast transformation responses than tick-free cattle, and addition of their serum to lymphocytes from tick-free cattle had an inhibitory effect on the response. In addition, concurrent diseases or stresses may contribute to the development of dermatophilosis by compromising the host's immune system. Intestinal parasitism, nutritional deficiencies, stress of pregnancy or migration, viral infections, and other infectious diseases may increase susceptibility to the condition.

Once the normal skin surface has been disrupted and activated zoospores gain access to the epidermis, infection can develop. The zoospores are apparently attracted to the low carbon dioxide concentration of the normal epidermis and there they germinate to *form mycelia that invade the viable epidermis and outer root sheaths of hair follicles.* Only rarely do the bacteria proliferate in the dermis or deeper tissues. The means by which *D. congolensis* invades the epidermis is unknown but recent investigations have found a variety of enzymes released into the culture fluid of the bacteria suggesting that *the ability of the organism to produce exoenzymes may be the pathogenetic mechanism.* As the filaments invade the epidermis, keratinocytes at sites of penetration begin to cornify and numerous neutrophils accumulate beneath and migrate into the epidermis, which subsequently separates from the underlying dermis. The neutrophils inhibit further invasion by the organism, and the epidermis reforms from cells in adjacent external hair follicle sheaths. This new epidermis is again invaded by organisms arising from the hair follicles to initiate another cycle of epidermal penetration, neutrophilic exocytosis, and epidermal detachment. *These repeated sequences of bacterial invasion, inflammation, and epidermal regeneration produce the thick laminar and parakeratotic crusts characteristic of dermatophilosis.*

Why some animals develop mild localized disease that resolves rapidly and spontaneously while others develop chronic widespread and debilitating disease is unknown. Skin commensal bacteria, primarily *Bacillus spp.*, have been shown to produce substances inhibitory to growth of *D. congolensis* in culture. It has been suggested that if a similar effect is present in vitro, bacteria normally present on skin produce substances that are bacteriostatic or bactericidal and thus prevent *D. congolensis* from proliferating. Differences in microflora of different individuals or breeds of animals could explain differences in response to infection. Furthermore, during the rainy season these bacterial inhibitors could be leached out or diluted to ineffective concentrations, permitting proliferation of *D. congolensis*. Differences in individual immune function or responses have been sought to explain why some animals recover quickly while others develop chronic infections. Chronically infected sheep have been found to have decreased numbers of dendritic cells in the dermis, to produce lower antibody titers in response to a novel antigen injected subcutaneously, and to have differences in an allele of a molecule which forms a portion of the T-cell receptor complex when compared to sheep which recovered spontaneously. The conclusions drawn from these studies were that poorer antigen presentation, differences in T-cell function, and a less vigorous antibody response to bacterial cell surface antigens may have allowed more widespread establishment of lesions and decreased ability to resolve lesions by chronically infected sheep. However, in other studies no correlation between serum antibody level and resistance

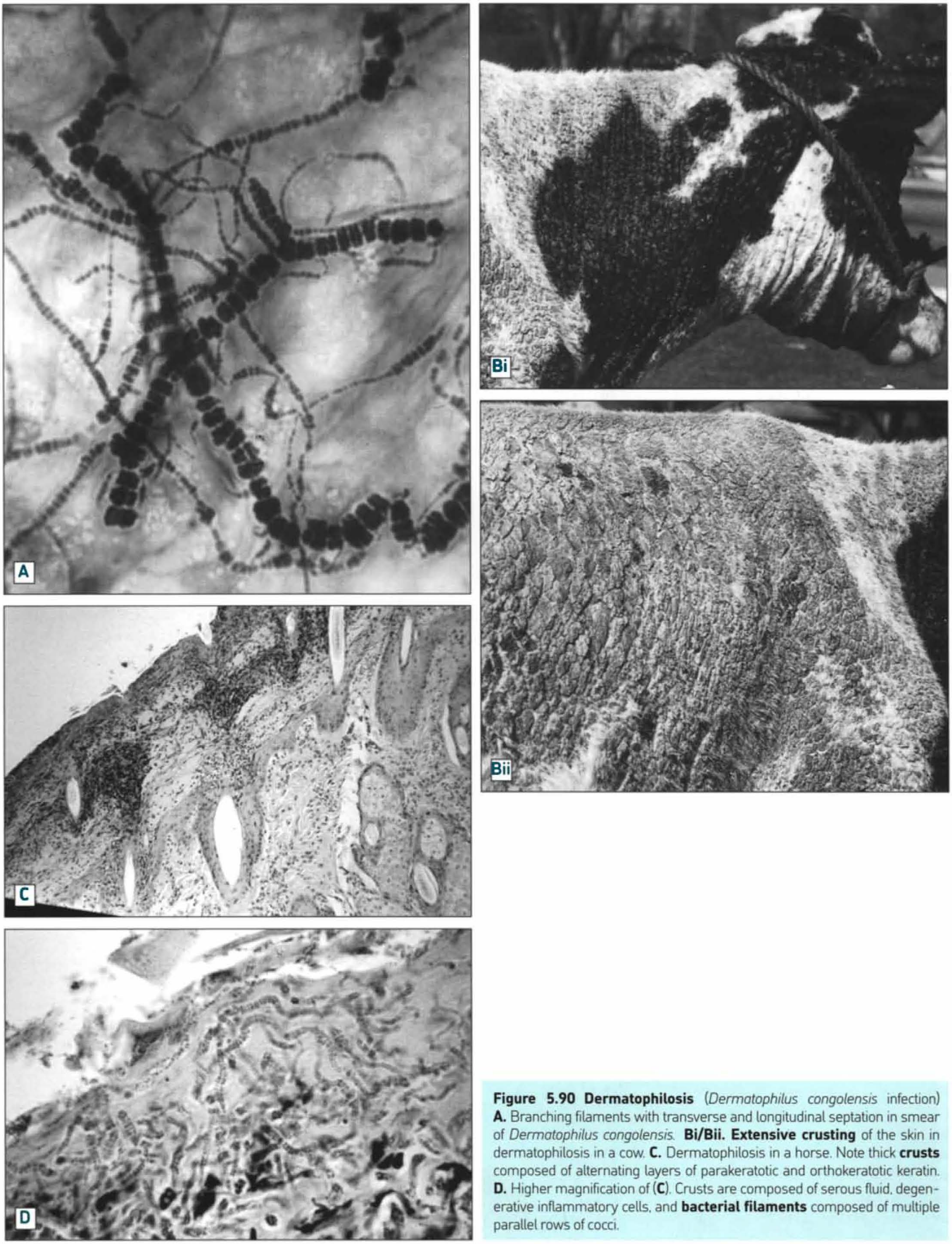

Figure 5.90 Dermatophilosis (*Dermatophilus congolensis* infection) **A.** Branching filaments with transverse and longitudinal septation in smear of *Dermatophilus congolensis*. **Bi/Bii. Extensive crusting** of the skin in dermatophilosis in a cow. **C.** Dermatophilosis in a horse. Note thick **crusts** composed of alternating layers of parakeratotic and orthokeratotic keratin. **D.** Higher magnification of (**C**). Crusts are composed of serous fluid, degenerative inflammatory cells, and **bacterial filaments** composed of multiple parallel rows of cocci.

to infection was found. Specific antibodies of various classes have also been detected on the skin surface but their role is unknown. Studies of vaccines against *D. congolensis* in sheep have shown that vaccines based on one strain of *D. congolensis* gave some protection against homologous challenge but were ineffective against challenge with a different strain of the bacterium. The involvement of antibody in resolution of disease is supported by histologic observations in which a chronological correlation was shown between lesion resolution and the presence of increased numbers of dermal plasma cells, many of which contained IgA, suggesting a role for this immunoglobulin in humoral immunity at the skin surface.

The *earliest lesions* of dermatophilosis consist of patches of slight erythema that are visible only in unpigmented areas. Very small papules and pustules develop next and are more evident by palpation than by visual inspection. As the lesions become covered by exudate and hairs become entrapped within the developing crust, they form small tufts that resemble paintbrushes. *As these small lesions coalesce, they form the typical large oval to circular domed yellow-brown adherent scabs which when removed leave a moist hyperemic base that may bleed.* The lesions are painful and nonpruritic. In chronic cases, lesions progress to form thick layers of dry spongy material involving extensive areas of long hair or hard wart-like crusts that protrude above the hair surface, usually in areas with a short haircoat (Fig. 5.90B). *Differential diagnoses include dermatophytosis, staphylococcal dermatitis, mite infestation, various viral infections, zinc-responsive dermatosis, and pemphigus foliaceus.*

The distribution of lesions in **cattle** is variable. Lesions confined to the face and ears are most often seen in young suckling calves. In other forms of the disease, lesions occur on the brisket, axillae, and inguinal areas or on the udder and teats of cows and scrotum and prepuce of bulls. Cattle standing in deep water or mud develop lesions on the legs. Lesions located in the perineum and tail are assumed to result from trauma caused by mounting by other animals. In many cases, lesions are diffusely distributed over the head, topline of the neck and body to the tail, and dorsal sides. *Animals with greater than 50% of body involvement often show weight loss, dehydration, and death.* Rare instances of subcutaneous abscesses, lymphadenitis, and oral lesions have been reported.

In **sheep**, infection of the wooled areas is frequently missed in early stages and some animals recover spontaneously during the acute stage of infection. If the disease continues to progress, the wool of the neck, back, and flank becomes matted with exudate to form dense pyramidal masses (lumpy wool, mycotic dermatitis) which may last for months to years in some individuals. *Strawberry foot rot* is an infection that begins at the coronet regions and may progress to involve the skin to the carpi and/or tarsi. In some cases, crusts may be confined to the ears, nose, and face, a clinical form common in lambs. Concurrent infection with contagious ecthyma has been reported.

Dermatophilosis in **goats** frequently consists of 2–3-mm crusty lesions on the pinnae and tail of kids and large pyramidal crusts on the dorsal midline, sides, caudal thighs, and scrotum of adults. Lesions involving the distal limbs resemble strawberry foot rot of sheep. Concurrent infection with contagious ecthyma may occur and secondary infections with staphylococci, streptococci, or corynebacteria may develop.

In **horses**, lesions of dermatophilosis are frequently located on the dorsal aspect of the body and look as if large drops of liquid have scalded the skin (*rain scald*). Horses kept in wet, marshy, or muddy enclosures, and undergoing trauma to the legs develop lesions of the distal extremities primarily (grease heel, scratches, mud fever). Lesions on the legs may be associated with swelling, pain, and lameness. In some instances, only the head is affected. Unpigmented skin may be more susceptible to infection and lesions on these areas are typically very erythematous. Outbreaks in show horses have been associated with frequent high-pressure washing.

Dermatophilosis cases reported in **cats** are notable in that they have all been subcutaneous or extracutaneous infections. The lesions consisted of draining nodules involving or in the area of the popliteal lymph nodes or the subcutaneous tissue of a paw and masses on the tongue and serosal surface of the urinary bladder. In at least one of the cats with subcutaneous infection, superficial wounds suggestive of a recent cat fight were seen, prompting speculation that the infection was traumatically introduced.

The earliest *histologic lesions* of dermatophilosis are superficial dermal congestion, edema, and neutrophil infiltration of the superficial dermis. Exocytosis of neutrophils becomes more pronounced as lesions progress and intraepidermal or subcorneal pustules may develop. Eventually, repeated cycles of bacterial invasion and inflammation result in *thick crusts composed of alternating layers of parakeratotic and orthokeratotic keratin, serous fluid, degenerate inflammatory cells, and bacterial filaments composed of multiple parallel rows of cocci* (Fig. 5.90C, D). The epidermis is acanthotic with orthokeratotic and parakeratotic hyperkeratosis. Purulent folliculitis with intralesional bacterial filaments is also usually present. Dermal inflammation is usually mild and superficial perivascular. In cases of dermatophilosis in cats and rare instances of subcutaneous and lymph node infection in cattle, the lesions consist of granulomas or pyogranulomas with scattered necrotic foci which contain typical *D. congolensis* filaments.

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Deep bacterial pyoderma

Deep pyodermas are serious bacterial infections that involve the hair follicle, dermis, and/or subcutis. They are usually chronic or recurrent, heal with scarring, and are commonly associated with regional or generalized lymphadenopathy and systemic signs. The clinical appearance of deep pyoderma is tremendously diverse. Lesions commonly seen in deep pyodermas include dark red or violaceous raised nodules, poorly demarcated areas of tissue swelling, hemorrhagic bullae, fistulous tracts, abscesses, purulent or serosanguineous exudate that dries to form crusts, and necrotic or ulcerated skin covered by crusts. Pain may be severe. Microscopic changes associated with deep pyodermas include folliculitis, furunculosis, nodular to diffuse dermatitis or panniculitis, and variable fibrosis. Bacteria may not be seen microscopically even with special stains.

Staphylococcal folliculitis and furunculosis

Staphylococcus spp. are the most common cause of folliculitis in domestic animals. Other organisms less frequently or rarely associated with folliculitis include *Streptococcus* spp., *Corynebacterium* spp., *Pseudomonas* spp., *Bacillus* spp., and *Pasteurella multocida*. Inflammation of the deep portion of hair follicles frequently results in rupture of the follicular wall (furunculosis) and extension of infection to the surrounding dermis and panniculus. *Staphylococcal folliculitis and furunculosis are very common in the dog, common in horses, goats, and sheep, and uncommon in cats, cattle, and pigs.* Skin lesions associated with folliculitis and furunculosis are extremely variable. The earliest skin lesion is a follicular papule with one or several hairs protruding from the center. The papule develops into a pustule but these are very fragile, break easily, and are thus very transient. Consequently, crusted papules are seen more commonly than are pustules. In short-haired dogs, horses, and cattle, the earliest clinical sign is a dishevelment of the haircoat produced by small groups of hairs tufting together above the skin surface, an appearance that can be confused with urticaria. As the hairs fall out of infected follicles, multiple small foci of alopecia and scaling develop. The hair coat develops a moth-eaten appearance as the areas of alopecia become progressively larger. Lesions often enlarge and develop a central ulcer that discharges purulent or serosanguineous exudate that dries to form a crust. When furunculosis occurs, dark red to violaceous nodules, draining fistulae, ulcers, and extensive tissue swelling develop (Fig. 5.91). Hemorrhagic bullae may be prominent in some cases. Scarring, alterations in pigmentation of the hair and skin, and lichenification may result. Regional and generalized lymphadenopathy are common. Constitutional signs, such as fever and anorexia, may occur when infection is severe or extensive.

The *microscopic changes* of staphylococcal folliculitis and furunculosis are as varied as the gross lesions. The epidermis is variably acanthotic and may be ulcerated. Serocellular crusting is common. Staphylococcal folliculitis is characterized by *neutrophilic inflammation* but the relative number of neutrophils is extremely variable, depending on the duration of the infection and presence of furunculosis. Pustules composed of neutrophils and variable numbers of eosinophils may be seen within the infundibulum or ostium of hair follicles. Neutrophils accumulate within the lumen of hair follicles, distending the lumen and frequently causing rupture of the hair follicle wall (see Fig. 5.5B). Neutrophils, eosinophils, macrophages, and plasma cells form dense sheets in the dermis and may extend to the panniculus. Hemorrhagic bullae consist of large pustules with hemorrhage in the interfollicular dermis. Large dermal pustules may form cavitary lesions. Release of hair and keratin into the dermis or panniculus induces an *intense foreign body reaction* that may completely efface the follicle (see Fig. 5.6). Fragments of hair or keratin may be surrounded by discrete granulomas composed of multinucleated giant cells, epithelioid macrophages, and neutrophils

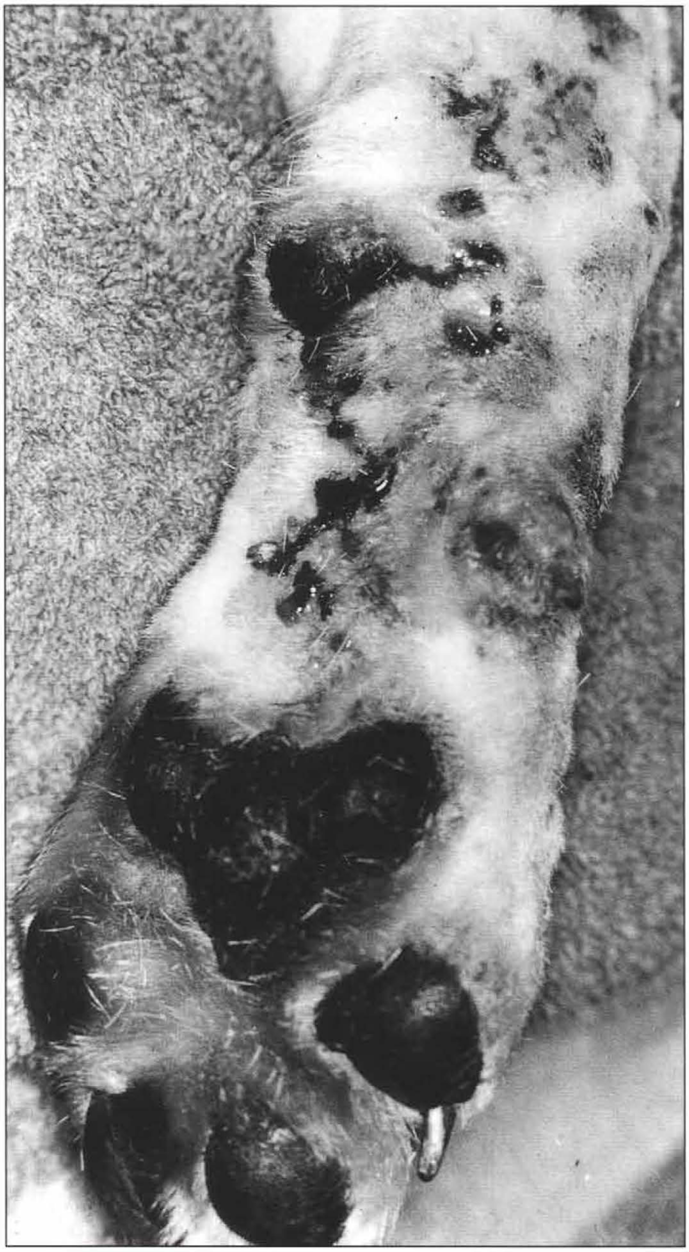

Figure 5.91 Deep pyoderma in a dog. Swollen paw with multiple draining tracts.

(*trichogranuloma*). Eosinophils may be numerous around the fragments of hair and keratin. Inflammation may become diffuse as adjacent furuncles coalesce and replace normal dermal structures completely. Hemorrhage and fibroplasia are variable. Bacteria may not be evident even with special stains such as tissue Gram stain or Giemsa stain.

Deep pyoderma is not as common as superficial pyoderma but is still a relatively common disease in **dogs**. *Staphylococcus intermedius* is usually the primary pathogen in bacterial folliculitis and furunculosis in dogs. However, gram-negative bacteria such as *Proteus*, *Pseudomonas*, and *E. coli* are not uncommon secondary invaders. Infection may be localized or widespread. *Localized forms* of staphylococcal deep pyoderma commonly involve the bridge of the nose, the chin ("canine acne"), feet (pedal folliculitis-furunculosis), and pressure points. A particularly refractory form of staphylococcal pyoderma has been recognized in German Shepherd Dogs. *German Shepherd pyoderma* is poorly understood; but because of the breed predilection, an inherited component has been suggested. The condition typically begins as pruritus involving the lumbosacral, caudal abdominal, and medial thigh regions. Lesions initially resemble pyotraumatic dermatitis and the lesion distribution is suggestive of flea allergy dermatitis. As the condition progresses, lesions assume an appearance typical of chronic pyoderma and frequently become more widespread. An underlying predisposing cause is frequently not apparent, and an immunologic abnormality is suspected. Neutrophil chemotaxis and killing capacity appear to be normal. Limited studies in a small number of affected German Shepherd Dogs have found a cell-mediated immunodeficiency by the lymphocyte transformation test and a marked increase in the CD8+ T lymphocytes in peripheral blood. Whether these changes are a cause or effect of the disease is unknown. Examination of T and B lymphocytes in histologic sections found a marked paucity of T lymphocytes in lesions of deep pyoderma of German Shepherd Dogs as compared to similar lesions from dogs of other breeds.

Staphylococcal pyoderma in **horses** most often involves the harness, saddle (saddle scab, saddle boils), neck, and dorsal lumbosacral regions. *Friction from tack* is considered an important initiating factor. It is most common in the summer, coincident with excessive sweating, higher environmental temperature and humidity, and increased numbers of biting insects. Lesions are usually painful rather than pruritic. *Staphylococcal pyoderma may be one of many causes of "grease heel,"* in which lesions involve the caudal aspect of the pastern and fetlock areas of one or more legs. In tail pyoderma, the dorsal surface of the tail is particularly affected and infection is usually secondary to skin abrasions caused by tail rubbing associated with insect bites, mange, biting lice, pinworms, or vice. Although much less common, *Corynebacterium pseudotuberculosis* can cause folliculitis and clinical lesions ("contagious acne") similar to those with staphylococcal folliculitis.

Staphylococcal pyoderma is common in **goats** and frequently begins on the udder and spreads to the ventral abdomen, medial thighs, perineal area, face, pinnae, and distal limbs. Severe infections, particularly those with secondary mastitis, may produce pyrexia, anorexia, depression, and septicemia. In **sheep**, staphylococcal pyoderma may occur as a benign pustular dermatitis on the lips and perineum in otherwise healthy 3- to 4-week-old lambs. Lesions usually regress spontaneously within 3 weeks. A more severe form of infection involves the face (facial eczema, eye scab), particularly of adult ewes just prior to lambing. Facial dermatitis appears to be contagious, and spread is thought to result from head abrasions sustained while feeding at troughs or by fighting.

Staphylococcal pyoderma occurs occasionally in cattle, pigs, and cats. Infection in **cattle** is seen most commonly in young bulls on the tail, perineum, scrotum, and face. Trauma and poor hygiene may be initiating factors. Staphylococcal pyoderma in **pigs** usually occurs on the hindquarters, abdomen, and chest of piglets less than 8 weeks of age and spontaneously regresses. Pyoderma is uncommon in **cats** and may be mistaken for other diseases. Lesions may consist of a crusted papular eruption on the face, head, or over the dorsum that clinically resembles miliary dermatitis associated with various hypersensitivity conditions. Scaling, crusting, or ulceration of footpads or nailbeds may be confused with pemphigus foliaceus or dermatophytosis.

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Abscesses and cellulitis

Abscesses *are well-circumscribed accumulations of pus.* **Cellulitis** *is a severe, deep, suppurative infection that is poorly defined and tends to dissect through tissue planes. The lesions are usually painful and overlying skin is often friable, dark, devitalized, and may be sloughed.* The wounds frequently have a putrid smell and may be emphysematous if the organism is a gas-producer (*Clostridium* spp., *Bacteroides* spp.). Pyrexia and regional lymphadenopathy may be present. Abscesses and cellulitis are fairly common in large animals and are *one of the most common disorders in cats*, where they are a frequent sequel to fight wounds. Other predisposing causes include traumatic puncture wounds, foreign bodies, injections, and shearing and clipping wounds. Microscopically, abscesses consist of central accumulations of neutrophils and/or necrotic debris, frequently surrounded by a wall of granulation tissue or dense collagenous connective tissue, depending on duration. In contrast, cellulitis is poorly circumscribed and consists of extensive purulent to pyogranulomatous dermal and subcutaneous inflammation that may be accompanied by hemorrhage, necrosis, and thrombosis. Bacteria may or may not be visible histologically.

A wide variety of aerobic and anaerobic bacteria have been associated with abscesses and cellulitis. The most common organisms include staphylococci (dogs, Thoroughbred horses, cattle), *Clostridium* spp. (malignant edema, gas gangrene, and big head in horses, cattle, sheep, goats, pigs, and dogs), *Pasteurella multocida* (cats), and *Corynebacterium pseudotuberculosis* (abscesses in horses, goats, cattle, and sheep; ulcerative lymphangitis in horses, cattle, sheep, and goats; caseous lymphadenitis in sheep and goats). *Rhodococcus equi,* most commonly associated with pneumonia in young horses, has been isolated from cutaneous abscesses and cellulitis in young horses and rarely in cats. *Pasteurella granulomatis* has been associated with a disease in southern Brazil called *lechiguana* which is characterized by large subcutaneous fibrosing eosinophilic abscesses. Lesions are most commonly located in the scapular region and the condition is frequently fatal if untreated. Various *mycoplasmas* and mycoplasma-like organisms have been recovered from abscesses in cats and from decubital abscesses in calves.

Streptococcus canis has been isolated from dogs with an intensely painful **necrotizing fasciitis.** The infection begins as cellulitis, frequently 12–48 hours after minor trauma, that rapidly progresses to shock and necrotizing fasciitis. The condition is similar to streptococcal toxic shock syndrome of humans caused by *S. pyogenes.* The condition is characterized grossly by *extensive exudation along fascial planes and necrosis of subcutaneous fat and fascia,* resulting in extensive sloughing of necrotic skin. The microscopic lesions of necrotizing fasciitis include severe necrosis, suppuration, fibrinous exudation, and hemorrhage of the dermis and subcutaneous fat, fascia, and muscle. In some instances, the epidermis and superficial dermis are infarcted as a result of thrombosis of dermal and subcutaneous blood vessels. Colonies of bacterial cocci may be evident in the inflamed subcutaneous tissue.

Conditions that resemble **toxic shock syndrome** in humans have been described in dogs. *Streptococcus canis* has been documented as the cause in most dogs; however, other bacteria, especially *Staphylococcus intermedius,* that produce exotoxins – including toxic shock syndrome toxin-1 – could potentially play a role. The site of infection can be the skin, as seen with necrotizing fasciitis; however, the primary site of infection in some dogs has been the lung or urinary tract. In dogs with toxic shock-like syndrome, but without necrotizing fasciitis, clinical skin lesions include multicentric to generalized cutaneous erythema. Some dogs also have edema and vesicles or pustules that progress to ulcers. The ears, extremities, and ventrum are frequently affected, and dogs can be depressed and have fever, anemia, thrombocytopenia, and neutrophilia. Histologically, these dogs have superficial dermatitis with apoptotic keratinocytes bordered by neutrophils. The lesions can progress to full-thickness necrosis and ulceration of the epidermis. As in necrotizing fasciitis, lesions can be fatal without early therapy with appropriate antibiotics. The cause of the skin lesions in the dogs with toxic shock-like syndrome, but without necrotizing fasciitis, has not been determined. In humans, the skin lesion is thought to result from nonspecific stimulation of lymphocytes by exotoxins with superantigen activity and the release of cytokines, including TNF-α.

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Cutaneous bacterial granulomas

A wide variety of bacteria are capable of producing granulomatous inflammation of the skin. The organisms are frequently of *low virulence* and are introduced by *traumatic implantation.* These infections are typically *slowly progressive* and produce *cutaneous or subcutaneous nodules.* Inflammation is nodular or diffuse, granulomatous or pyogranulomatous, and involves the dermis, panniculus, or both. *Diagnosis commonly requires special stains such as tissue Gram stain and routine or modified acid-fast stains.* Staining frozen sections of formalin-fixed tissue may be necessary to demonstrate the bacteria in some atypical mycobacterial infections. Organisms are so infrequent in some cases that confirmation of the bacterial etiology cannot be made histologically and depends instead on cultural isolation of the agent.

Actinomycosis and nocardiosis

Actinomycosis and nocardiosis are uncommon subacute-to-chronic opportunistic cutaneous, pulmonary, and disseminated infections that develop secondary to wound contamination, inhalation, or ingestion. The diseases are discussed together because of their clinical and histologic similarities. *Actinomyces* and *Nocardia* are the most common actinomycetes, so-called higher bacteria, which cause disease, but occasional reports of infections in animals by other actinomycetes include *Streptomyces* and *Actinomadura.* The infections occur sporadically and are worldwide in distribution. They are not considered of public health significance.

Actinomyces species are gram-positive, non-acid fast, filamentous anaerobic or microaerophilic rods that are *commensal inhabitants of the oral cavity, intestine, and upper respiratory tract.* Cutaneous infection is usually secondary to bites, penetrating wounds caused by foreign bodies such as quills or grass awns, and wounds contaminated by licking. Cutaneous actinomycosis occurs in dogs, cats, horses, and cattle. Infection in pigs usually involves the mammary gland. Several retrospective studies in dogs indicate that young, large breed, male dogs that are used or housed outdoors are most commonly affected. *Actinomyces viscosus* is cultured most commonly from animal infections; but the organism is fastidious and difficult to culture and frequently it is not speciated even when cultured.

Nocardia species are common *soil saprophytes* that are ubiquitous in the soil and water and on plants. They cause infection by wound contamination, inhalation, or ingestion. They are aerobic, gram-positive, filamentous organisms that may be acid fast. There appears to be considerable variation in the degree of pathogenicity among various strains of the organism. Experimental infections suggest that defense against nocardial infections involves both neutrophils, which may have an immediate role in inhibiting the growth of the organisms, and cell-mediated immunity, which is responsible for the ultimate clearance of infection. Cutaneous nocardiosis is reported most frequently in dogs, cats, horses, and cattle. The most common isolate is *Nocardia asteroides.* Cutaneous nocardiosis in cattle, called *bovine farcy*, is caused by an organism originally called *N. farcinica* but is now thought to be *N. asteroides*. Farcy occurs in Africa, Asia, and South America.

The *gross lesions* of actinomycosis and nocardiosis are usually indistinguishable. They consist of abscesses, cellulitis, ulcerated nodules, draining fistulous tracts, and dense fibrous masses. *When the triad of clinical signs consisting of tumefaction, draining sinuses, and tissue grains is present, the lesion can be termed an* **actinomycotic mycetoma**. Lesions progress slowly by local extension. They occur most commonly on the head, neck, and extremities. Pleural and retroperitoneal infections may extend to involve the subcutaneous tissues of the lateral thoracic wall and flank area, respectively. The exudate is variable and ranges from thin serosanguineous to thick purulohemorrhagic. It may be odorless or foul-smelling and contain white, yellow, tan, or gray "**sulfur granules**." Sulfur granules are more common in actinomycosis than in nocardiosis. Regional lymphadenopathy frequently accompanies skin lesions. In cats, lesions on the ventral abdomen resemble mycobacterial infections. Actinomycosis in cattle usually involves the mandible or maxilla causing proliferative osteitis ("*lumpy jaw*"); the infection may extend from the bone to the overlying skin to form firm nodules, abscesses, and draining sinus tracts with extensive fibrosis. The cutaneous lesions of bovine farcy are typically associated with lymphangitis and lymphadenitis and are clinically similar to those caused by tuberculosis.

Microscopically, actinomycosis and nocardiosis are characterized by *pyogranulomatous dermatitis and panniculitis*. The epidermis is variably acanthotic and may be ulcerated. The dermis and subcutis contain central accumulations of neutrophils surrounded by a wall of epithelioid macrophages and variable numbers of multinucleated giant cells. Necrosis may be prominent within the central abscess. The pyogranulomas are separated by granulation tissue or dense fibrous connective tissue containing lymphocytes and plasma cells. Fibrosis tends to be more common and severe in actinomycosis than in nocardiosis. Organized masses, measuring 30–3000 μm or more in diameter, of basophilic or amphophilic-staining organisms, may be seen in the centers of the abscesses (Fig. 5.92). They are commonly bordered by a clubbed corona of brightly eosinophilic *Splendore-Hoeppli material. These structures correspond to the sulfur granules seen grossly in the exudate.* Granule formation is a more common feature of actinomycosis; in nocardiosis, the bacteria tend to be distributed singly among the neutrophils or within the macrophages and are difficult or impossible to see in HE-stained sections. However, *Nocardia* species may form granules in cutaneous lesions that are morphologically indistinguishable from those of *Actinomyces*. In Gram-stained sections, the bacteria are evident as delicate, branched, and beaded filaments, from 10–30 μm or more long and 0.5–1.0 μm wide. Fragmentation of filaments produces coccobacillary forms. The filamentous nature of the bacteria is most apparent at the periphery of granules; while coccobacillary forms constitute the centers of granules. The beaded appearance is due to alternating gram-positive and gram-negative regions within the filament and is less prominent with *Actinomyces* than with *Nocardia* species. *Actinomyces* are acid-fast negative with most acid-fast stains. Some, but not all, filaments of *Nocardia* may stain strongly acid-fast with modified acid-fast stain. However, *Nocardia* species are not invariably acid-fast and, if negative, cannot be differentiated from *Actinomyces*. Thus culture must be done to identify the organism. *Nocardia* species may resemble opportunistic mycobacteria but they do not stain with routine acid-fast stain as do mycobacteria. A single random section may not contain granules and serial sections may be necessary to find organisms. However, small biopsies may not contain organisms at all. Since granules or individual organisms may be rare, a wide *wedge biopsy* including deep subcutaneous tissue is more likely to contain organisms than are punch biopsies.

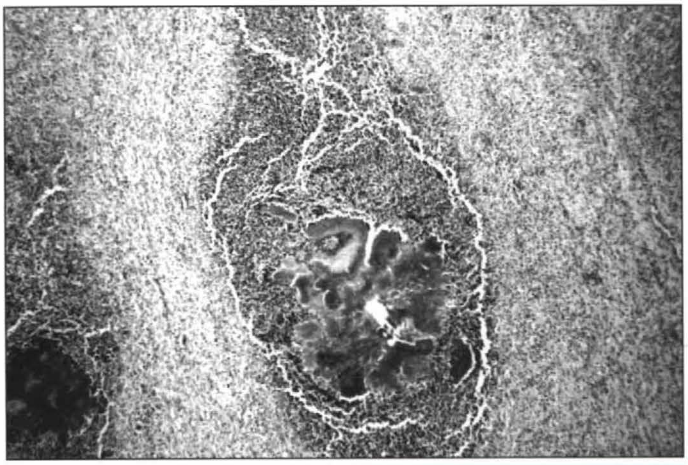

Figure 5.92 Abscess with clusters of organisms in the center in **actinomycosis** in a cow.

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Mycobacterial infections

The genus *Mycobacterium* is a large group of acid-fast organisms that includes both obligate pathogens and many saprophytic organisms that are occasionally opportunistic pathogens. They cause chronic infections of various organ systems, including the skin. The members of the *tuberculosis complex*, *M. tuberculosis*, and *M. bovis*, are obligate pathogens that cause tuberculosis in humans and a wide range of domestic animals worldwide. Pulmonary and gastrointestinal lesions are most common in tuberculosis, *but the skin can become involved in disseminated infections* and, rarely, infection may be confined to the skin. In contrast, *infections with saprophytic mycobacteria frequently involve the skin and they are usually acquired by wound contamination or traumatic implantation.* Cutaneous mycobacterial infections are reported most frequently in cats and less often in dogs. *Mycobacterial lesions are all characterized by granulomatous or pyogranulomatous dermatitis and/or panniculitis with variable necrosis.* These infections can be diagnosed by cytologic or histologic examination and organisms are best visualized with acid-fast or modified acid-fast stain. The organisms may be rare to numerous and the number of acid-fast bacilli seen within the lesion cannot be used to identify the specific organism involved.

Culture is necessary for definitive identification of the organism so that appropriate therapy and the zoonotic potential of the infection may be determined. *Many of the organisms are extremely fastidious and difficult to culture and isolation may require many weeks to months.* The need for culture is becoming increasingly obviated by molecular-based techniques which can identify the organisms in tissue section and can be completed within a few days.

Feline leprosy

Feline leprosy is a rare localized cutaneous infection caused by acid-fast bacilli that are not culturable by standard mycobacteriological methods. The infection has a widespread distribution, with cases reported in New Zealand, Australia, North America, and Europe. Cases are frequently associated with temperate maritime regions. Feline leprosy has no zoonotic potential.

Based on successful transmission to rats and mice and comparison of delayed type hypersensitivity skin reactions in cats, the causative agent of feline leprosy has been speculated to be *Mycobacterium lepraemurium.* This is the organism that causes rodent leprosy; it is a slow-growing organism that is isolated only with difficulty using enrichment techniques. The means of transmission is unknown, but *bites of cats or rodents, contact with rodents, and soil contamination of cutaneous wounds are thought to be involved.* Since the agent of human leprosy, or Hansen's disease, has been found in viable form in mosquitoes, bed bugs, ticks, and fleas, it has been suggested that transmission of feline leprosy may involve similar vectors. *Immunosuppression* has been suggested as contributing to development of infection, but such factors have not been identified in the vast majority of reported cases. Recently, the organism was identified by molecular analysis as *M. lepraemurium* in tissue specimens from four of eight cases of feline leprosy examined. However, two cases were found to be infected with a mycobacterium that had 16S ribosomal RNA gene sequence sharing closest nucleotide sequence identity with that of *M. malmoense.* These findings suggest that *feline leprosy may not be caused by a single agent.*

Feline leprosy occurs most commonly in young adult cats 1–3 years old; no breed or sex predilection is evident. Several studies have found an increased incidence of disease in the winter. Lesions may be located anywhere but are most frequent on the head and limbs. They consist of single or multiple, nonpainful cutaneous or subcutaneous nodules which are haired, alopecic, or ulcerated. They may be soft and fleshy or firm and measure up to 3–4 cm diameter. *The nodules are frequently mistaken for neoplasms.* Affected cats may also have regional lymphadenopathy but they are usually otherwise healthy. A second syndrome has been identified in cats >9 years old, with generalized skin involvement and a slowly progressive clinical course, caused by a novel mycobacterial species that is present in large to enormous numbers.

The *histologic changes* consist of granulomatous inflammation involving the dermis and frequently the subcutis. The epidermis is variably acanthotic or ulcerated. The predominant cells are *large, pale, foamy epithelioid macrophages* arranged in focal aggregates or diffuse sheets that replace normal architecture (Fig. 5.93). Lymphocytes, plasma cells, and neutrophils are in variable numbers. Multinucleated giant cells and small foci of caseous necrosis are present in some lesions. *Acid-fast organisms are demonstrable by routine acid-fast stains, such as Ziehl–Neelsen and Kinyoun's,* and are usually in moderate numbers within macrophages or in necrotic foci. However, acid-fast bacilli may be in massive numbers in some cases or only rare in others. Mineralization and encapsulation are not typical of feline leprosy. The *histologic features* of feline leprosy have been compared to human leprosy, in which the particular histologic reaction is associated with the host's resistance. Whether this is also true for feline leprosy has not been determined. Reactions consisting of epithelioid granulomas with central caseous necrosis surrounded by a zone of lymphocytes usually contain numerous organisms within the necrotic foci. These lesions are compared to the *tuberculoid response* of human Hansen's disease, which is considered a *high-resistance form.* A second type of reaction in cats consists of solid sheets of large foamy macrophages and many multinucleated giant cells, with acid-fast bacilli being numerous in both cell types. Perivascular lymphocytes and plasma cells vary from few to many. This latter type of reaction has been compared to the *lepromatous form* of human leprosy which is considered to be the *low-resistance form* associated with a deficit in cell-mediated immunity. Neutrophils are present in both types of lesions but are most numerous in the lepromatous form, often resulting in a pyogranulomatous reaction. Nerve involvement, the most important histologic feature of Hansen's disease, is only sporadically observed in feline leprosy. Regional lymph nodes are characterized by variable architectural disruption by infiltrates of macrophages. Acid-fast bacteria are rare within lymph nodes.

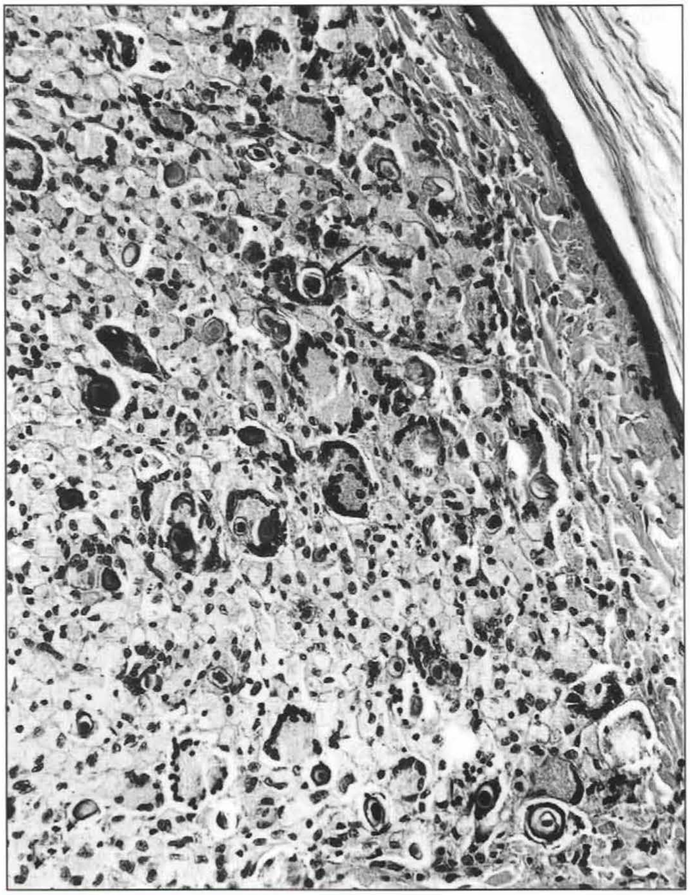

Figure 5.93 Macrophages, concretions (arrow), and multinucleate giant cells in **feline leprosy**.

Differential diagnoses include other mycobacterial infections, mycotic infections, and cutaneous xanthoma/xanthogranuloma. Xanthomas are sterile granulomatous lesions composed of foamy macrophages, which resemble the lepromatous form of feline leprosy. However, this form of feline leprosy is usually associated with numerous acid-fast bacteria and acid-fast staining will allow differentiation from xanthomas. Mycotic infections can usually be differentiated by demonstration of fungal elements with special stains and culture of a compatible fungal agent. Diagnosis of feline leprosy is complicated by the fact that it cannot be reliably differentiated from other mycobacterial infections histologically. Traditionally, feline leprosy has been diagnosed when acid-fast bacteria were identified in a granulomatous skin lesion from which mycobacteria could not be cultured. However, failure to culture mycobacteria does not confirm a diagnosis of feline leprosy since there are other mycobacterial species that are difficult to culture, organisms may not be cultured because of technical difficulties, or because organisms may be rare or unevenly distributed within the tissue and the sample may not be representative. As molecular-based diagnostic tests become more common, the diagnosis of feline leprosy as well as other mycobacterial infections will become more rapid and accurate than when based on histologic examination and culture.

Opportunistic, or atypical, mycobacteria

Opportunistic, or atypical, mycobacteria are widespread saprophytes commonly found in *water, soil, and decaying vegetation.* They include rapidly growing and slow-growing organisms. Human and animal infections caused by opportunistic mycobacteria are being reported with increasing frequency. Cutaneous lesions with rapidly growing opportunistic mycobacteria have been reported primarily in cats and less frequently in dogs. Isolates associated with skin infections include *Mycobacterium chelonae, M. fortuitum, M. phlei, M. smegmatis, M. thermoresistible,* and *M. xenopi.* Although cutaneous disease has also been caused by slow-growing atypical mycobacteria, these organisms are more commonly associated with disseminated disease that is indistinguishable from tuberculosis. *M. avium-intracellulare* complex (MAC) is the most common slow-growing opportunistic mycobacterium isolated from cutaneous disease in cats and dogs. *M. kansasii* has been cultured from skin lesions in cattle. Cutaneous infections are thought to be acquired from the environment following trauma such as bite or fight wounds, abrasions, and puncture wounds. Cutaneous disease occurs most commonly in adult cats that hunt or fight frequently and may be more common in obese individuals. After inoculation of organisms into a wound, the lesion develops slowly over a period of weeks to months. Experimental evidence indicates that introduction of acid-fast bacilli into oil or adipose tissue enhances their pathogenicity, possibly by providing nutrients for their growth or by protecting them from the host immune response. Animal-to-animal transmission is not thought to occur. Underlying predisposing diseases or immunologic compromise are often present in humans with these infections but are usually not identified in animals. Affected cats tested for feline leukemia virus and feline immunodeficiency virus infections have been negative.

The clinical course of opportunistic mycobacterial infection is typically prolonged. *Lesions include single or multiple cutaneous and subcutaneous nodules, plaques, purpuric macules, or diffuse swellings.* Multiple punctate ulcers or large fistulous tracts frequently develop and discharge serous, serosanguineous, or purulent exudate that is not usually malodorous. Lesions in cats caused by rapidly growing opportunistic organisms are characteristically located in the subcutaneous fat of the inguinal area. The tissue becomes progressively thickened, board-like, and firmly attached to the body wall. Lesions slowly enlarge and may involve the entire ventrum or extend up the lateral aspect of the trunk. Regional lymph nodes may become enlarged. Despite extensive cutaneous involvement, affected animals usually remain active and normal and exhibit no systemic signs. Skin lesions in cattle are frequently located on the distal limbs.

The *microscopic lesions* of atypical cutaneous mycobacteriosis are usually *multinodular-to-diffuse pyogranulomatous dermatitis and panniculitis.* Since inflammation is generally most intense in the subcutaneous fat, a *deep wedge biopsy* is preferable to a punch biopsy for diagnosis. Infections caused by rapidly growing mycobacteria typically contain circular clear vacuoles surrounded by a rim of neutrophils and a wider outer zone of epithelioid macrophages and variable numbers of neutrophils among more diffuse inflammation composed of macrophages, neutrophils, and lymphoid cells (Fig. 5.94A). Multinucleated giant cells are infrequent. *Organisms are characteristically rare and difficult to find but are usually located in small clumps within the clear vacuoles* (Fig. 5.94B). Small numbers of organisms may also be found within macrophages. The bacilli may stain more intensely with modified acid-fast stain than with routine acid-fast stain. In some instances, staining frozen sections of formalin-fixed tissue may result in enhanced acid-fast staining. The organisms are long rods to short filamentous bacilli. Intracellular bacteria tend to be shorter rods. The organisms stain unevenly positive with tissue Gram stains, resulting in a beaded appearance to the bacilli. *Because of the scarcity of organisms, any single section may lack organisms and the diagnosis will be missed.* The clear vacuoles characteristically seen among inflammatory cells in lesions with rapidly growing opportunistic mycobacteria are not typical of lesions caused by MAC organisms. Moreover, acid-fast bacilli are frequently numerous and are demonstrable by routine acid-fast stains in MAC infections. Lesions in cats caused by MAC organisms may be indistinguishable from feline leprosy. In rare instances, fibroplasia may be marked and the lesion may be confused for a spindle cell tumor. *The specific diagnosis of opportunistic mycobacterial infection requires identification by culture or molecular-based techniques.* Because of the scarcity of organisms in some lesions, diagnosis may require multiple attempts at biopsy and culture.

Miscellaneous mycobacterial infections

Although tuberculosis caused by *M. bovis* typically produces pulmonary or alimentary lesions, in some cases the skin may be the only apparent site of infection. In a survey of 57 cats with *M. bovis* infection in New Zealand, *nonhealing skin lesions* were the most common clinical sign and no evidence of internal disease was evident in the cats with skin disease. Lesions occurred in various body locations, including the legs, shoulder, flank, and inguinal area. The majority of cases occurred in regions in which *M. bovis* is endemic in the Australian brush-tailed possum and it was speculated that skin infections were more common than the classical forms of tuberculosis because they were acquired from *bite or scratch wounds from infected possums.* The histologic features consisted of poorly circumscribed pyogranulomatous inflammation extending along fascial

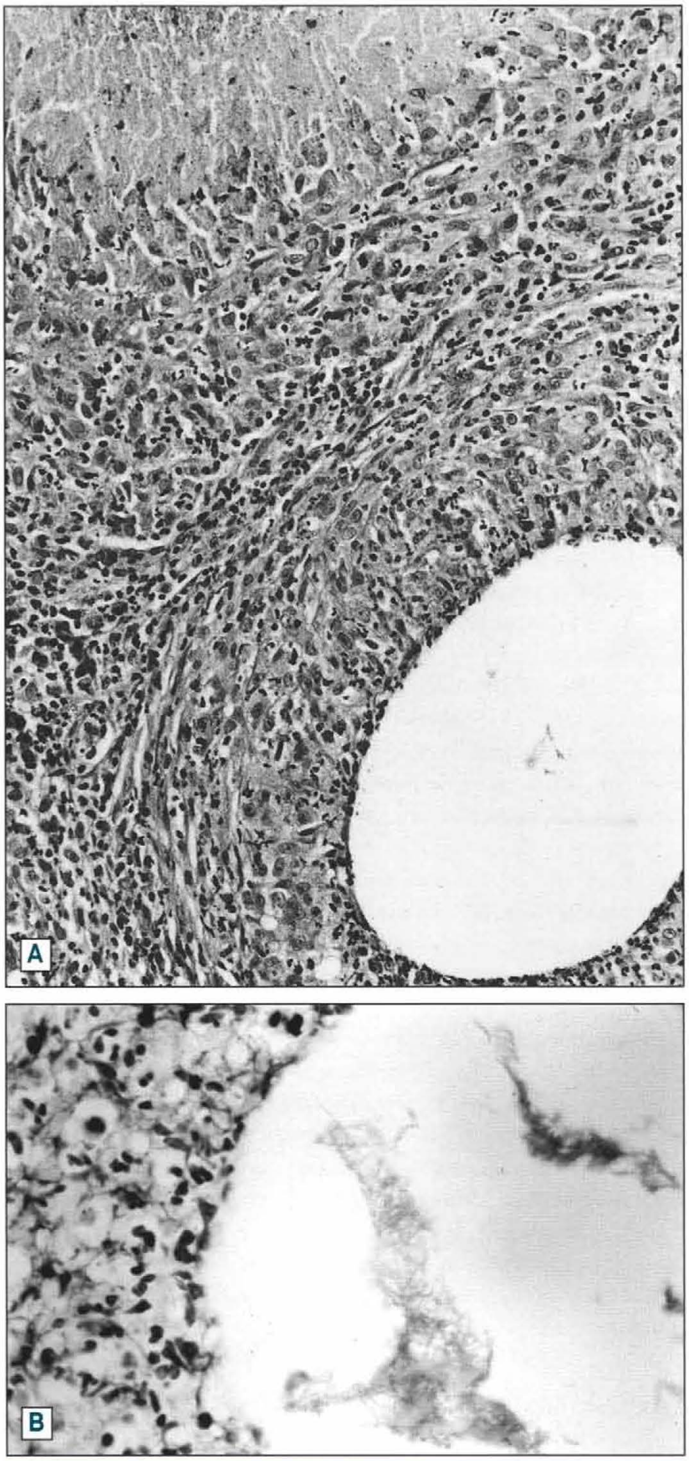

Figure 5.94 Atypical mycobacteriosis in a cat. **A.** Accumulations of mixed leukocytes with neutrophils, bordering a fat globule. **B.** High magnification of fat globule containing **filamentous organisms**.

planes. Coagulative necrosis was moderate to extensive. Acid-fast bacilli varied from rare to innumerable. Without cultural identification of the organisms, the histologic lesions could be confused for feline leprosy and other mycobacterial infections. No evidence of a zoonotic risk was found, since no pet owners became infected despite the presence of large numbers of organisms in some of the skin lesions.

Infection with an as yet unidentified mycobacterium with characteristics intermediate between *M. tuberculosis* and *M. bovis* has been described in cats in the United Kingdom and Channel Islands and termed **feline tuberculosis syndrome.** Adult cats with a history of hunting may be predisposed to the infection. All cats tested for feline leukemia virus and feline immunodeficiency virus were negative and no evidence of immunosuppression was found. *The primary clinical signs were cutaneous lesions*; while lesions more typical of classical tuberculosis, such as generalized lymphadenopathy and respiratory signs, were evident in only a few of the cats. *Skin lesions included swellings, firm raised nodules, and draining sinuses.* Some of the lesions arose in sites of previous bite wounds. Regional lymphadenopathy was frequently present. Microscopic lesions consisted of *discrete and coalescing pyogranulomatous dermatitis and panniculitis.* Granulomas composed of epithelioid macrophages and fewer neutrophils were surrounded by more diffuse infiltrates of macrophages, neutrophils, plasma cells, and lymphocytes in a proliferating granulation tissue stroma. Necrosis was variable and ranged from a few scattered foci located within some of the granulomas to extensive coalescing foci. Organisms were visible with routine acid-fast stain and were usually in low numbers but were numerous in a few cases. The zoonotic potential of this mycobacterial infection is unknown.

A condition referred to as **canine leproid granuloma syndrome** has been described in dogs in Australia, Africa, and North America. The disease is characterized by *cutaneous lesions in which acid-fast bacteria are visible histologically but cannot be cultured.* Short-coated breeds, particularly Boxers and their crosses as well as Doberman Pinschers and Bullmastiffs, appear to have a predilection for the infection. Lesions consist of *single or multiple, firm, painless nodules primarily on the head and especially on the dorsal surface of the pinnae.* The frequent location on the head led to speculation that transmission involves biting flies or insects. In some cases, lesions spontaneously resolved after weeks to months. In other cases, lesions persisted indefinitely and progressively enlarged or additional lesions developed and became ulcerated. Lymph node and internal organ involvement was not seen. The microscopic features of the condition were not described but acid-fast bacteria were reported to vary from numerous to infrequent, widely scattered, and very difficult to find in tissue sections. Preliminary molecular-based identification of the organisms suggests that the etiological agent(s) is *a new mycobacterial species grouped within the saprophytic mycobacteria.*

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Botryomycosis

Botryomycosis (bacterial pseudomycosis, bacterial granuloma) is *a chronic infection caused by nonfilamentous bacteria that form colonies visible as tissue grains or granules within lesions*. Clinically and histologically, the condition resembles actinomycotic and eumycotic mycetomas. The disease occurs in humans and all domestic animals. Although reported rarely, the condition is probably not uncommon. Lesions are usually localized in the skin and subcutis but may extend deep to involve underlying bone and muscle. Disseminated infection with visceral involvement is rare. Coagulase-positive staphylococci are involved most commonly; but streptococci, *Pseudomonas, Actinobacillus, Pasteurella, Proteus, Escherichia*, and other organisms have also been isolated from lesions. Infection is thought to develop as a result of wound contamination or trauma such as bites, lacerations, or puncture wounds with foreign bodies.

Lesions typically consist of *firm, nonpruritic single or multiple nodules that ulcerate and develop draining fistulae*. They discharge purulent material which frequently contains *white to yellow sand-like grains*. The microscopic lesion is characterized by the presence of *basophilic granules within the center of neutrophilic abscesses or pyogranulomas in the dermis or subcutis.* These granules correspond to the grains seen grossly in the discharge. They are surrounded and separated by fibrous tissue which may be quite extensive. Plant debris or other foreign material may also be seen in the lesions and indicate the means of infection. The granules consist of compact bacterial colonies which are usually surrounded by amorphous, deeply eosinophilic, radially arranged Splendore-Hoeppli material. The name of the condition is, in part, derived from the appearance of the granules which was thought to be suggestive of a *bunch of grapes*. The bacteria are not well delineated in H&E-stained sections, thus *special stains are usually necessary to distinguish the organisms from actinomycotic bacteria and fungi*. Individual cocci or bacilli within the granules are seen most clearly in sections stained with tissue Gram or Giemsa stains. The GMS procedure also stains bacteria and allows differentiation from fungi.

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Bacterial pododermatitis of ruminants

Footrot

Footrot is a highly contagious bacterial infection of the digits of *sheep, cattle, and goats*. It has also been reported in *pigs*. The multistrain, gram-negative, obligate anaerobe *Dichelobacter* (formerly *Bacteroides*) *nodosus* is the essential causal pathogen. *Footrot should not be confused with necrobacillosis of the ruminant foot*, which is a noncontagious, mixed bacterial infection in which *Fusobacterium necrophorum* is the principal pathogen. Footrot occurs worldwide in areas with temperate climate and moderate to high rainfall. The condition is particularly important in sheep because of the economic impact it has on both the wool and meat industries. *Footrot is a major disease in high rainfall areas*, such as Australia and New Zealand, and *can produce severe lameness* because of necrosis of the sensitive laminae and separation of the hoof. The reduced mobility and reluctance of affected animals to eat results in weight loss and decreased wool quality and growth rate, which correlate in a linear manner with the number of feet infected. In addition to the production losses, the cost of treatment and control contribute to significant economic losses.

The pathogenesis of footrot is complex and has been studied most extensively in sheep. The disease is initiated by *maceration of the interdigital skin* brought about commonly by extended exposure to lush, continually wet pasture. Disease is thus most common in rainy seasons. Minor wounds, abrasions from stones, sharp plants, or long coarse grasses, and damage caused by migrating *Strongyloides* larvae are additional factors that can predispose to development of clinical footrot. The damaged epidermis then becomes colonized by *Fusobacterium necrophorum*, aerobic diphtheroids, coliforms, and other common bacteria originating from the soil, skin, and feces. *Footrot develops if the dermatitis is subsequently complicated by infection with D. nodosus.* The bacterium is an obligate parasite of the ruminant hoof and carrier animals serve as the source for uninfected stock. Transmission of infection requires adequate moisture and a mean ambient temperature greater than 10°C. Direct contact between susceptible and infected sheep is not required for transmission of infection. Contact with contaminated substrate for less than 1 hour has been shown to be adequate for transmission; and outbreaks have been associated with contact with environment contaminated by affected animals as long as 4 days previously. *F. necrophorum* and *D. nodosus* act synergistically to produce clinical disease. *D. nodosus* produces a variety of *extracellular proteases* that are thought to digest the horn and allow bacterial invasion of the epidermal matrix of the horn. It also produces a *heat-stable soluble factor* that enhances the growth and invasiveness of *F. necrophorum*. *F. necrophorum* produces a *leukocidal exotoxin* that reduces phagocytosis.

The separation of horn is caused by lysis of the epidermal matrix as a result of the local inflammatory response rather than from direct bacterial attack. *Arcanobacterium pyogenes* and other aerobic bacteria are usually located superficially in the lesion where they remove oxygen, destroy hydrogen peroxide, and create the anaerobic environment necessary for the growth of the two anaerobic pathogens.

Footrot in sheep is a complex clinical disease syndrome. The spectrum of clinical disease is divided into three major forms: *virulent, intermediate, and benign*. Factors associated with severity and persistence of infection include virulence of the particular strain of *D. nodosus*, the environment, and host resistance. Considerable variation exists in the natural resistance to the disease and there is evidence to suggest a genetic basis for the variation in resistance. A possible association between alleles of the major histocompatibility complex (MHC) class II region and resistance to footrot has been found. Most Merino flocks are considered to have a high proportion of susceptible animals. Virulence of *D. nodosus* varies widely from strain to strain. Virulent strains of the bacterium produce more extracellular proteases, including elastase; and proteases from virulent strains tend to be more thermostable than those from benign strains. Two genomic regions of *D. nodosus* are preferentially associated with more virulent isolates of the bacterium. These regions have been named *vap*, for virulence-associated protein, and *vrl*, for virulence-related locus. Although useful as indicators of virulence, the function of these two regions has not been determined. The *virulent form of footrot in sheep* is the serious, persistent form of the disease characterized by severe necrotic damage to the hoof and extensive separation of hoof horn in more than one foot in a high percentage of the sheep. The infection may persist for more than 1 year if not treated, and chronically infected sheep may die of emaciation as a result of severe pain and lameness. Affected animals are also predisposed to flystrike. *Benign footrot*, also called "*foot-scald*," is a milder, less persistent form of the condition. It is typically characterized by moderate interdigital dermatitis which causes mild lameness and minimal production loss. The benign form has a greater propensity for self-cure.

Footrot in sheep develops approximately 10–20 days after exposure and *begins as interdigital dermatitis in the axial bulbar notch*. At this early stage, the interdigital skin is pale, swollen, and moist. Inflammation progressively extends forward and caudad around the bulb of the heel. Separation of the horn usually occurs 7 days later, beginning at the skin-horn junction and spreading to the bulb and sole. The animal is severely lame at this stage. From the sole, the process spreads to the hoof wall to involve the axial and abaxial surfaces of the digits. Exudation is minimal and consists of a small amount of gray, greasy malodorous material in the cleft beneath the underrun horn. Since the infection does not destroy the germinative layers of the horn, new horn is continually being regenerated but is rapidly destroyed as long as the infection continues. Chronically infected feet become long and misshapen.

Benign footrot is indistinguishable from the early stages of virulent footrot. However, *in benign footrot the erosions usually remain confined to the caudal aspect of the interdigital cleft*. Occasionally there is separation of the soft horn of the heel and caudal portion of the sole but there is no separation of the hard horn. Affected sheep may appear normal or exhibit mild, temporary lameness. The effect of benign footrot on production is minimal.

Diagnosis of footrot in sheep is based on clinical signs, demonstration of D. nodosus in smears from lesional material, and culture and biochemical characterization of isolates to identify antigenic and virulence variants. Because the organism is slow to grow, culture and biochemical testing is time-consuming and may require up to 10–14 days. Gene probes have been developed to identify the virulence-associated loci vap and vrl in lesional material. This testing can be completed within 48–72 hours.

Footrot of **cattle and goats** is typically a *less severe disease* and resembles the benign form of footrot of sheep. Infection with *D. nodosus* is more common than is clinical disease. Direct transmission can occur between sheep and goats in the same environment but the innate resistance of goats appears to be greater than that of sheep. The lesions in goats tend to stay confined to the interdigital skin. Underrunning lesions are not common in goats and are usually restricted to a portion of the soft horn. *D. nodosus* isolates from *cattle* with footrot tend to be relatively benign strains. Footrot in cattle usually begins at the caudal aspect of the interdigital space, spreads laterally on the bulbs of the heels, and eventually involves the entire interdigital space. *Lesions usually remain confined to the interdigital skin* which may be eroded, ulcerated, or become deeply fissured. The fissures are covered with necrotic material with a characteristic fetid odor. Lesions may extend to the heels and mild separation of the soft horn may develop. Extensive undermining of the horn, as occurs in sheep, does not typically occur in cattle. Affected animals stand on their toes. In rare severe cases, fever, anorexia, recumbency, and decreased milk production may develop.

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Papillomatous digital dermatitis

Papillomatous digital dermatitis is a painful, contagious dermatitis of the feet of *cattle* that occurs worldwide. The condition is also called *footwarts* and *hairy heelwarts*; and clinically and histologically, it appears to be the same disease as *digital dermatitis, interdigital papillomatosis, verrucose dermatitis, and digital papillomatosis*. Papillomatous digital dermatitis is economically important because it frequently *causes moderate-to-severe lameness* that results in weight loss, decreased milk production, and poor reproductive performance. A similar condition has been reported in *sheep and a horse*. The condition in horses is not as rare as might be supposed from the literature, and is recognized in diagnostic laboratories where horses are commonly submitted for disease investigations.

The *vast majority of cases are in dairy cows* but it has also been reported in beef cattle. Although the disease occurs in all ages, the highest incidence appears to be in *replacement heifers*. The etiology of papillomatous digital dermatitis is unknown but it is *suspected to be bacterial* based on the favorable response to antibacterial therapy, presence of numerous *intralesional spirochetes*, lack of demonstrable viruses and other known pathogens, and evidence of a specific humoral response in affected animals to spirochetes isolated from lesions of papillomatous digital dermatitis. The spirochetes have been identified as *Treponema phagedenis*-like. Genetic analysis indicates that the organisms are related to pathogenic human treponemes associated with periodontitis. The condition is also usually associated with management conditions in which the feet of cattle remain wet for prolonged periods, such as following a rainy season, during indoor confined housing in the winter, or in muddy corrals. The condition tends to disappear when cattle are on pasture, but some outbreaks are unassociated with any apparent predisposing factors.

Papillomatous digital dermatitis most commonly affects the skin proximal and adjacent to the interdigital space at the back of the hindfeet. The front feet are less frequently involved and usually only one foot is affected. The cranial aspect of the foot and the interdigital skin are rarely involved. Lesions are painful, forcing the animal to shift its weight to the toe of the foot and resulting in clubbing of the affected foot and atrophy of the bulbs of the heels. Early lesions are well-circumscribed, round-to-oval, red plaques up to 6 cm in diameter with a moist granular surface prone to bleeding and with a very strong, pungent odor. They are partially to completely alopecic and may be bordered by hypertrophied hairs two to three times longer than normal. The lesions become progressively more proliferative and less painful with time. Mature lesions are irregular wart-like growths or filamentous papillae which measure 0.5–1.0 mm in diameter and 1 mm to 3 cm in length and are pale yellow, gray, or brown.

The early *microscopic changes* of papillomatous digital dermatitis consist of mild epidermal hyperplasia with foci of erosion, necrosis, ballooning degeneration, and microabscesses. The basal layer exhibits an increased mitotic index. The dermis contains minimal perivascular inflammation. Mixed bacteria may be present in the outer necrotic debris but only spirochetes are present in the deeper viable epidermis. Two morphologically distinct spirochetes have been demonstrated to be in large numbers in early lesions. One is a long thin type with few twists and the other is a short, thick spirochete with many twists. The spirochetes are oriented perpendicular to the epidermis and appear to invade the keratinocytes. The older lesions are composed of frond-like projections or plaques of markedly hyperplastic epidermis with parakeratosis and hyperkeratosis. Foci of necrosis and hemorrhage, ballooning degeneration, and aggregates of neutrophils are scattered throughout the hyperplastic epidermis. At this later stage, inflammation is more intense in the dermis and plasma cells may be numerous.

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Skin lesions in systemic bacterial disease

Skin lesions may develop during the course of systemic bacterial infection. They frequently arise *as a result of vasculitis and/or thrombosis*. This occurs most commonly in **pigs**. Swine erysipelas, caused by *Erysipelothrix rhusiopathiae*, is a generalized septicemia that is commonly associated with *blue-purple rhomboidal plaques (diamond skin lesions)* (Fig. 5.95). These lesions are obvious on light-skinned pigs but may require palpation for detection on pigmented animals. The lesions either regress spontaneously or undergo necrosis,

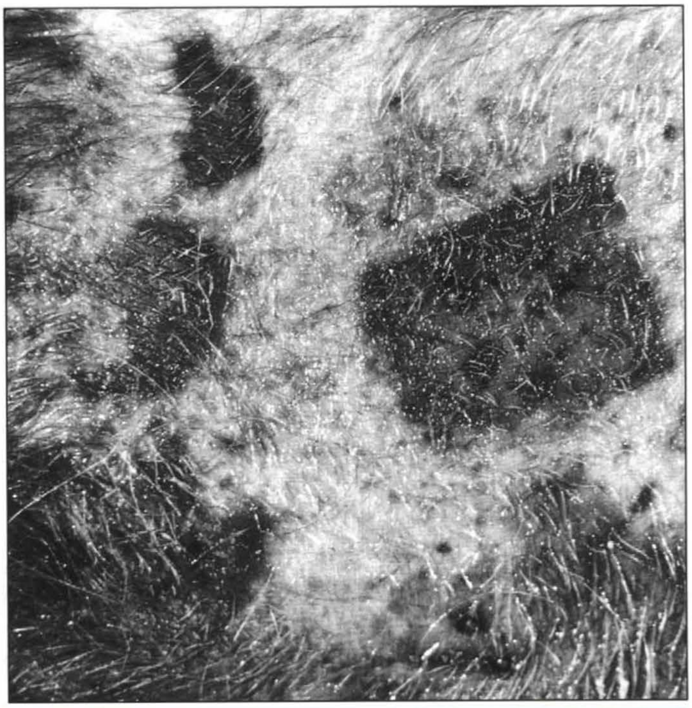

Figure 5.95 Classical "diamond skin" lesions in ***Erysipelothrix rhusiopathiae*** infection in a pig.

become hard and dry, and eventually slough. Severely affected ears and tail may also be sloughed. Microscopic findings include marked dermal congestion, neutrophilic vasculitis, cutaneous necrosis, and suppurative hidradenitis. Various gram-negative septicemias, in particular septicemic salmonellosis caused by *S. choleraesuis*, may produce blue-purple discoloration of the skin of the ears, ventral abdomen, snout, and tail primarily as a result of *endotoxin-induced venous thrombosis*. *Pasteurella multocida* may cause similar skin lesions in pigs. *Edema disease*, caused by a hemolytic *E. coli* may be associated with subcutaneous edema secondary to vascular hyaline degeneration, fibrinoid necrosis, and mural edema.

Skin lesions occur less commonly in other domestic species with systemic bacterial infection. *Salmonella dublin* infections in **cattle** may produce gangrene of distal extremities, tail, and pinnae as a consequence of vascular thrombosis. Cutaneous lesions are uncommon in **dogs** with systemic infections. Rocky Mountain spotted fever, caused by *Rickettsia rickettsii*, is most often associated with skin lesions. These consist primarily of edema and hyperemia; petechial and ecchymotic hemorrhages may also occur but typically involve mucous membranes rather than the skin. Acral dermal necrosis occurs rarely. Microscopic findings consist of necrotizing vasculitis, which may result in cutaneous necrosis. Dogs with Lyme borreliosis, caused by *Borrelia burgdorferi*, may develop a small, reddened skin lesion at the site of tick attachment. This lesion usually disappears within a week and is very inconspicuous as compared to the striking expanding, annular erythematous rash (erythema chronicum migrans) typical of the infection in humans.

FUNGAL DISEASES OF SKIN

Fungi are ubiquitous in the environment but, of the thousands present, only a few are capable of causing infection in animals. *The vast majority of fungi in nature are incapable of causing infection because they are unable to breach two major physiologic barriers to fungal growth in tissue: temperature and oxidation-reduction potential.* Many fungi have an optimal growth range considerably below the temperature of the body and cannot survive the relatively high temperature of the body. Other fungi, even if thermotolerant, cannot survive in the reduced state of living tissue. The propensity of fungi to cause infection is dependent not only on their ability to adapt to the tissue environment and temperature, but also to withstand the lytic activity of the host's cellular defenses. Some fungi are *true pathogens* with the ability to cause disease in normal individuals while many more organisms are *opportunistic pathogens* that infect individuals who have become immunologically or otherwise compromised and are unable to resist and suppress the fungal invasion. Immunocompromise by various infectious agents, such as *Human immunodeficiency virus, Feline leukemia virus, Feline immunodeficiency virus,* and *Canine distemper virus,* as well as pharmacologic immunosuppression, are important predisposing factors in development of some fungal infections. However, many mycotic infections occur in individuals without any clinically overt immune impairment. These infections are likely a consequence of an overwhelming exposure to the infectious propagules or mild immunologic defects that are not readily apparent. Mycotic infections are commonly divided into three categories: cutaneous, subcutaneous, and systemic.

- **Cutaneous mycoses** are the most common fungal diseases in veterinary medicine. The infections are generally confined to the nonliving cornified layers of the skin, hair, and claws.
- The **subcutaneous mycoses** are fungal infections that involve the skin and subcutaneous tissues. They are caused by a wide variety of saprophytic fungi that gain entry by traumatic implantation. Infection is a very indolent process and usually remains localized to the site of entry with slow spread to surrounding tissue.
- The **systemic mycoses** are infections of the internal organs. Cutaneous involvement usually occurs as a result of hematogenous dissemination. Skin lesions from direct cutaneous inoculation in systemic mycoses are rare. These infections include blastomycosis, cryptococcosis, coccidioidomycosis, and histoplasmosis. Occasionally the diagnosis of a systemic fungal infection/mycosis, particularly blastomycosis in dogs and cryptococcosis in cats, is made by biopsy of skin lesions.

Histopathology is an important diagnostic tool for fungal infections. It is a relatively rapid and inexpensive means to make a definitive or presumptive diagnosis of mycotic infection. In many cases, fungal infections are mistaken for neoplasms and the entire lesion is fixed and submitted for histologic examination. Consequently, only fixed tissue is available and the pathologist has the responsibility for the final diagnosis because the organism cannot be cultured. Some fungal organisms have distinctive enough morphologic features that, if present in sufficient numbers, they can be identified with a high degree of certainty, e.g., *Blastomyces, Cryptococcus,* and *Coccidioides*. Other mycotic infections are caused by a variety of fungi that are similar in appearance in tissues and cannot be specifically identified, although the disease can be named, e.g., dermatophytosis, phaeohyphomycosis, and eumycotic mycetoma. In some cases, fungi may be detectable in tissues but may be impossible to identify and only the conclusion that mycotic infection exists can be made. Although immunofluorescence and immunohistochemical methods for identification of fungi in formalin-fixed, paraffin-embedded tissue sections have been developed, the specific antisera are not readily available, and *culture remains the mainstay for identification of fungal pathogens.* Some fungi stain with H&E but many fungi do not stain or stain very poorly with H&E. However, even in these cases, fungal infection can be suspected because of the presence of clear circular or linear structures, representing unstained fungal hyphae, within the lesion. *Special fungal stains,* such as Gomori methenamine silver (GMS), which stains fungi black, and periodic acid-Schiff (PAS), which stains fungi pink-red, delineate the fungi so their morphology can be examined. In general, GMS is better for screening tissue because some fungal-like organisms (e.g., *Pythium*) stain poorly with PAS. Fungal stains may mask the natural color of fungi, however. To determine whether a fungus is pigmented, it can be examined in unstained cleared and mounted sections or a melanin stain, such as Fontana-Masson, can be applied to the tissue.

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Cutaneous fungal infections

Cutaneous mycoses are infections in which the fungal organisms are generally confined to the nonliving keratinized tissues, i.e., *stratum corneum, hair, claw, and horn.* Pathologic changes, which occur in response to the infectious agent and its metabolic products, involve the epidermis and dermis and may vary from minimal to severe and extensive. These infections include candidiasis, *Malassezia* dermatitis, and dermatophytosis.

Candidiasis

Candidiasis (candidosis, moniliasis, thrush) is a rare opportunistic infection of skin, mucocutaneous junctions, external ear canal, and the claw bed. *Candida* spp. yeasts are normal inhabitants of the upper respiratory, alimentary, and genital tracts but are not normally present on the skin except at mucocutaneous junctions of body orifices. Factors which alter the superficial keratin barrier (such as maceration, chronic trauma, or burns), upset normal flora (such as prolonged broad spectrum antibiotic therapy), or produce immunosuppression (such as diabetes mellitus, hyperadrenocorticism, neoplasia, viral infections, cytotoxic chemotherapy, or prolonged glucocorticoid treatment) predispose to infection with the organism. Since neutrophils are a major defense mechanism against *Candida*, prolonged neutropenia renders an individual more susceptible to candidiasis. The other major mechanism for eliminating *Candida* from the skin surface is T-cell-mediated immunity.

Infections have been described in *dogs, cats, pigs, horses, and goats.* Lesions are variable and may begin as papules, pustules, and vesicles which evolve into characteristic *sharply delineated ulcers with erythematous borders and a malodorous surface with moist gray-white exudate.* Chronic lesions consist of thickened alopecic hyperkeratotic skin with prominent folds. Histologic changes include hyperkeratosis, parakeratosis, serocellular crusts, subcorneal or superficial epidermal neutrophilic pustules, and spongiosis. The dermis is edematous and contains a superficial perivascular to interstitial mixed infiltrate. *Yeasts, pseudohyphae, and hyphae may be numerous but are best visualized with PAS or GMS stains.* Generally, yeasts are most numerous on the surface of the lesions, while hyphae and pseudohyphae extend into the epidermis.

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Malassezia dermatitis

Malassezia pachydermatis (formerly Pityrosporum canis) is a constituent of the normal resident flora of the skin and mucous membranes of dogs and cats and a common cause of ceruminous otitis. Its role as a cause of dermatitis has been controversial. Evidence that the organism is capable of acting as an opportunistic pathogen is provided by cases of chronic dermatitis in which large numbers of yeasts are evident on the skin surface and lesions resolve following antifungal therapy. Predisposing factors are thought to be required for the yeast to cause clinical disease. Excessive surface lipid or cerumen, high humidity, or failure of host defense mechanisms to control overgrowth of the yeast may predispose an animal to develop clinical disease. Many dogs with *Malassezia* dermatitis also have allergic skin disease, seborrhea, or bacterial pyoderma as well as a history of long-term corticosteroid or antibiotic treatment; it is unclear whether these concurrent disorders are involved in development of yeast infection or are merely coincidental. Humans with atopic dermatitis have an increased incidence of *Malassezia* skin infections. Dogs with *Malassezia* dermatitis develop high serum titers of specific IgG antibody and exhibit significantly greater skin test reactions in response to intradermal injection of *M. pachydermatis* extracts than dogs without evidence of *Malassezia* dermatitis, suggesting that *hypersensitivity may be involved in the pathogenesis of clinical disease.* Various breeds of dogs have been found to be at increased risk for *Malassezia* dermatitis in different reports. These differences in affected breeds may reflect regional variation in breed popularity or differences in regional gene pool. In several reports, however, the Basset Hound has been found to have a predilection for this disorder. When numbers of *M. pachydermatis* yeasts on the skin were examined, normal Basset Hounds were found to have higher carriage levels of the yeast as compared to normal mixed-breed dogs, a fact that may contribute to the increased incidence of the disorder in this breed. No sex predilection is evident and most cases are in adult dogs. *Malassezia* dermatitis is reported rarely in cats and has been associated with various neoplasms and other conditions that could alter the host immune response.

Lesions associated with Malassezia dermatitis include erythema, alopecia, greasiness, yellow-gray scaly plaques, lichenification, and hyperpigmentation (Fig. 5.96A). A rancid offensive odor is typical; and moderate to severe pruritus is a common feature. The face, ears, ventral neck, axilla, inguinal area, cranial aspect of the legs, caudal thighs, and feet are most commonly involved. Involvement of one or several of these areas is more common than generalized disease. *Malassezia* dermatitis in cats is seen as black ceruminous otitis externa, chronic chin acne, or generalized erythematous scaly dermatitis.

Histologic features of *Malassezia* dermatitis consist of variable hyperkeratosis, parakeratosis, scale-crusts, and irregular epidermal hyperplasia with intercellular edema (Fig. 5.96B). Exocytosis of lymphocytes, neutrophils, or eosinophils has been found in various reports. Rarely, intra-epidermal eosinophilic pustules were found in dogs without any apparent concurrent dermatosis. There is variable edema and perivascular to interstitial infiltration of mixed cells in the superficial dermis. *Malassezia* organisms are *oval to footprint- or peanut-shaped yeasts located in the stratum corneum or in crusts.* They are usually seen in focal aggregates rather than diffusely distributed and they can best be visualized with PAS or GMS stains. The organism will be missed if the skin surface was scrubbed prior to obtaining the biopsy or if the surface debris is lost during processing.

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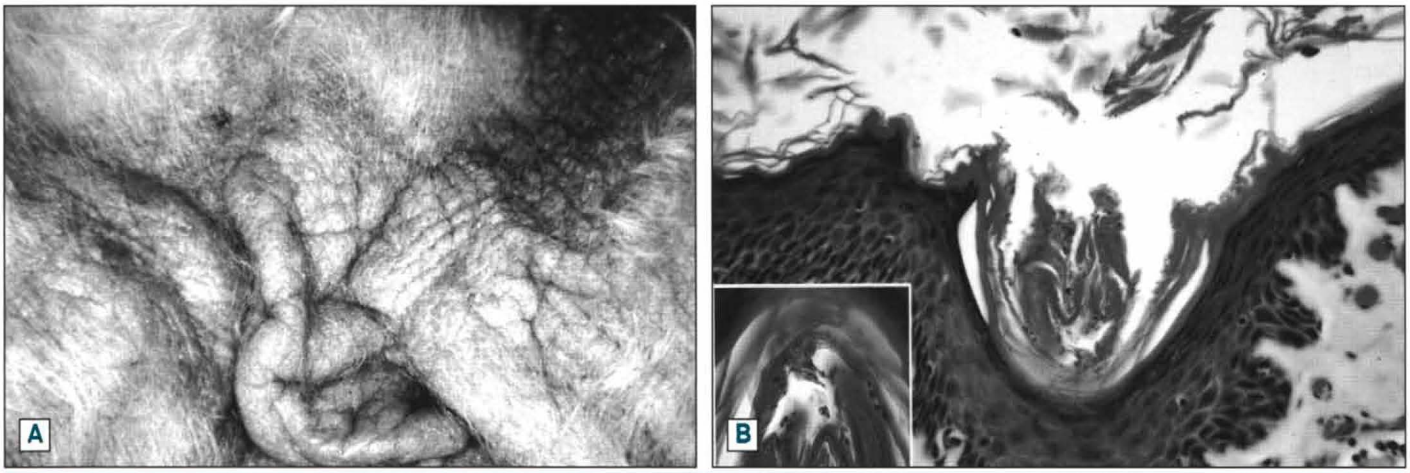

Figure 5.96 ***Malassezia* dermatitis** in a dog. **A.** The skin is erythematous, alopecic, greasy, lichenified, and hyperpigmented. (Courtesy of K Beale.) **B.** The epidermis is irregularly hyperplastic with moderate parakeratosis. Yeasts can be seen within the keratin (see inset). (Courtesy of B Dunstan.)

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Dermatophytosis

Dermatophytosis ("**ringworm**") *is a superficial fungal infection generally confined to the keratin layers of the skin, hair, and nails.* In rare instances, deeper tissues are involved. The infection is caused by a group of fungi that are capable of using keratin as a source of nutrients and are among the few fungi that cause communicable disease. Infection can vary from mild to severe as a consequence of the host's reactions to metabolic products of the fungus, virulence of the particular species or strain, location of the infection, and local environmental factors. In general, the infection has no effect on growth rate or other measures of productivity, but economic losses may result from hide damage or inability to show infected animals.

The infection is caused by fungi of the genera *Microsporum*, *Trichophyton*, and *Epidermophyton*, which are cosmopolitan in distribution. They are commonly divided according to the host preference and natural habitat of the fungus. The **anthropophilic** dermatophytes are primarily adapted to humans and rarely infect other animals (e.g., *T. rubrum*, *T. mentagrophytes* var. *interdigitale*). Animals occasionally develop infections with these organisms as a reverse zoonosis from infected humans. The **zoophilic** species are those dermatophytes that have become adapted to animals and typically cause less inflammation in their adapted hosts than do geophilic or anthropophilic dermatophytes (e.g., *M. canis* in cats, *T. equinum* in horses). They occasionally infect humans. **Geophilic** species (e.g., *M. gypseum*) normally inhabit the soil in association with keratinous debris in the process of decomposition but they may cause human and animal infection. *M. canis*, *M. gypseum*, and *T. mentagrophytes cause the majority of infections in animals.*

Incidence and prevalence of dermatophytosis vary with individual host factors, health status, climate, season, natural reservoirs, and local environment. A variety of local and systemic factors may predispose an individual to infection. Areas of chronically warm, moist skin are more likely to become clinically infected. Long-term corticosteroid administration, cytotoxic drugs, diabetes mellitus, hematologic malignancies, and other causes of natural or iatrogenic immunosuppression are associated with susceptibility to dermatophytosis. Incidence of infection is increased with hot, humid weather and when populations of stable flies and mosquitoes are large. In confined animals, infection is common in fall and winter when animals are crowded together in wet, poorly ventilated, unsanitary conditions with decreased exposure to sunlight. Young animals appear to be more susceptible to infection than adults. Reasons for this apparent predisposition include immaturity of host immunity, lack of previous exposure, and age-related differences in biochemical properties of the skin, such as qualitative or quantitative differences in antifungal fatty acids in sebum secretions. Transmission of dermatophytosis occurs by direct contact with infected animals or indirectly by exposure to infective hair and scales in the environment (contaminated grooming equipment, bedding, saddles, cages, etc.). *Hair fragments containing infectious arthrospores are the most effective means of transmission.* They can remain infectious for more than 18 months if protected from the deleterious effects of ultraviolet light. This material is the major source of persistent environmental contamination. Certain dermatophytes are associated with specific sources. *M. canis*, despite its name, is most commonly associated with cats, and the cat is considered the reservoir for this dermatophyte. *T. mentagrophytes* dermatophytosis is typically acquired from small rodents; infection with *M. gypseum*, a geophilic dermatophyte, is assumed to be acquired from digging or rooting in contaminated soil. An animal's habitat is an important factor in the incidence and type of infection contracted. In a survey of dermatophytosis in Madras, India, a predominance of *M. gypseum* was cultured from stray dogs, which would be expected to have frequent contact with soil. In contrast, pet dogs were infected primarily with zoophilic (*T. mentagrophytes*) and anthropophilic (*T. rubrum*) dermatophytes, the latter infections suspected to be reverse zoonoses since there was a history of exposure to people known to be infected with *T. rubrum* in most of the cases.

Normal skin is relatively inhospitable to fungal growth because of low moisture conditions, antifungal substances in the surface film, and normal resident flora. Sebum contains fatty acids which are fungistatic and play an important role in resistance to infection. The process by which the stratum corneum is continually renewed may also present a form of defense against organisms because the process results in continuous shedding of the stratum corneum and thus removes infecting organisms with the sloughed keratin. Disruption of the stratum corneum, either by microabrasions or maceration, appears to be important in facilitating invasion by the fungus. Fungal cells adhere to keratinocytes and migrate to the follicular orifice. Dermatophytes produce keratinolytic enzymes, keratinases, which hydrolyze keratin and enable them to penetrate and invade the hair shaft. They grow downward within the hair shaft toward the hair bulb until they reach the keratogenous zone where they stop since they cannot grow in viable tissue. Infection continues as long as the downward growth of the fungus is in equilibrium with keratin production; if not, the fungus is sloughed and the hair is cleared of infection. When the hair enters telogen phase, keratin production stops and fungal growth ceases. Hair shafts are weakened as a result of penetration by the fungi and they become brittle and easily broken.

Dermatophytosis in healthy individuals is *usually self-limiting*, with lesions resolving in several weeks to two or three months. An inverse relationship appears to exist between the degree of inflammation produced by the fungal pathogen and chronicity of infection. Defense against infection involves both immune and non-immune mechanisms. The epidermis represents more than just a passive barrier against entry of invading microorganisms, because keratinocytes produce a variety of eicosanoids, growth factors, chemotactic cytokines, interleukins, and colony stimulating factors that can mediate inflammation. In addition, increased epidermal proliferation occurs early in infection, even before an immunologic response develops, and helps expel the organisms by desquamation. Cell wall components of the organisms activate complement via the alternative pathway. Chemotactic factors generated in the complement cascade as well as those produced by keratinocytes attract neutrophils, which are the chief effector cells. They can kill fungi with products generated in the oxidative burst and also by nonoxidative microbicidal substances within their granules. Neutrophils also have fungistatic activity via *calprotectin*, a calcium- and zinc-binding protein. Macrophages are involved to a lesser extent and produce nitric oxide, which inhibits fungal growth. Nonspecific mechanisms prevent invasion into deeper tissues even in the absence of immunity. Substances in the dermis may prevent growth of organisms. Some of these include unsaturated transferrin, which may interfere with fungal growth in the dermis by competition for iron, and α_2-macroglobulin which inhibits keratinases. Both humoral and cell-mediated immunity develop in response to infection with dermatophytes. Antibodies develop against keratinase as well as fungal glycoproteins but they apparently do not help eliminate infection since the highest titers are found in patients with chronic infections. In contrast, development of cell-mediated immunity is associated with clinical cure and elimination of the organism. In experimental *M. canis* infections in cats, there was no relationship between antibody titers and recovery; but a close temporal relationship was found between the development of increased lymphocyte proliferative responses and regression of lesions. Cell-mediated immunity is the major immunologic defense in clearing dermatophyte infections. Immunity post-infection is relative, so re-infection is possible; however, lesions are smaller and resolve more quickly when an animal is re-infected with the same organism.

Impaired T-cell function has been associated with chronic or recurrent dermatophyte infections. An increased incidence of dermatophyte carriage has been found in cats infected with feline immunodeficiency virus (FIV), and severe dermatophyte infections are common in people with AIDS. However, even apparently healthy individuals can have persistent infections. *Inhibitory factors*, which can interfere with lymphocyte blastogenesis in vitro, have been found in serum of people with chronic dermatophyte infection. In addition, *T. rubrum* cell wall mannan has been shown to inhibit lymphoproliferation in vitro and is thought to be involved in the pathogenesis of chronic infections. Chronic dermatophyte infections have also been reported in dogs. *Trichophyton* spp. and *M. persicolor* dermatophyte infections, lasting up to 5 years in some cases, have been described in dogs without any other evidence of an immunodeficiency disorder, suggesting that these organisms may also produce inhibitory substances which prevent development of an effective immune response and elimination of the organism.

The *clinical signs* of dermatophytosis are highly variable and depend on the host-fungus interaction. Well-adapted species, such as *M. canis* infection in cats, produce minimal inflammation while less-adapted species, such as the zoophilic dermatophyte *M. gypseum*, produce more significant inflammation and more prominent lesions. *Expanding circular patches of scaling and alopecia or stubbled hairs are considered the classical lesion of dermatophytosis but these are frequently the exception rather than the rule* (Fig. 5.97A, B). Follicular papules and pustules, more extensive inflammation caused by furunculosis, and crusting are prominent in many cases. Lesions are typically non-pruritic, but occasionally pruritus is intense. *Infection of nails is called* **onychomycosis** and is characterized by misshapen, crumbly or easily broken, and split nails that may be sloughed. **Kerions** are rapidly developing tender erythematous alopecic nodules that may ulcerate. They are usually solitary and most common on the face and forelimbs of dogs that dig in the dirt. These lesions result from *severe furunculosis* producing locally extensive inflammation that may be confused for a tumor. A rare form, the **dermatophyte pseudomycetoma** seen almost exclusively in Persian cats, occurs as *subcutaneous nodules* (Fig. 5.98A, B).

The microscopic lesions of dermatophytosis are as variable as the clinical lesions. *Histopathology is not considered as sensitive as culture for diagnosis*; but it can be used to confirm infection when the significance of a cultured organism is in question. It is most useful in the nodular forms, such as kerion and pseudomycetoma, which are frequently negative when superficial material is cultured. Biopsies should be taken within the outer border of expanding alopecia as this is the most active site of infection and organisms are most likely to be present. In some cases, fungal organisms are evident in HE-stained sections, but *in many instances, fungal stains (PAS, GMS) are necessary to demonstrate infection*. Dermatophytes occur as septate hyphae that

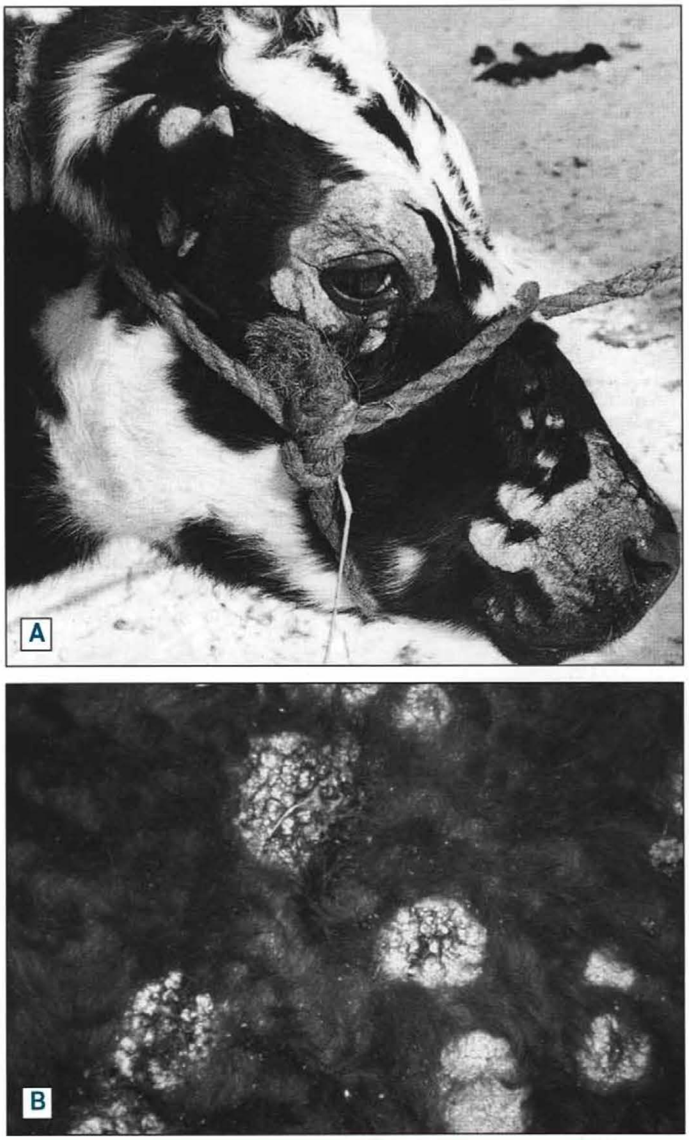

Figure 5.97 Dermatophytosis in a calf. **A.** Gray-white, rough, hairless lesions on the ear, muzzle, and periorbital skin. **B.** Classical "ringworm" lesions of expanding circular patches of scaling and alopecia.

break up into chains of round-to-oval arthrospores in the surface and follicular keratin. *Hyphae are also usually present in the hair shafts and arthrospores are formed on the outside of the hairs (ectothrix) or within the hairs (endothrix).* In rare cases, fungal organisms are present only in the surface keratin; if this material was removed in preparing the skin for obtaining the biopsy or is lost during tissue processing, the diagnosis will be missed. *Ortho- and parakeratotic hyperkeratosis* is a typical feature, and acanthosis is variable, ranging from mild to marked (Fig. 5.99A). Inflammation may be very mild and consist of low numbers of perivascular and perifollicular lymphocytes and macrophages. This is the case when infection is caused by a species of dermatophyte that is well adapted to its host (Fig. 5.99B). *Neutrophilic luminal folliculitis* is a common lesion in dermatophytosis and may result in *follicular rupture and development of discrete granulomas* surrounding fragments of hair at the base of the follicles (Fig. 5.99C). *Eosinophils* may be numerous in these trichogranulomas.

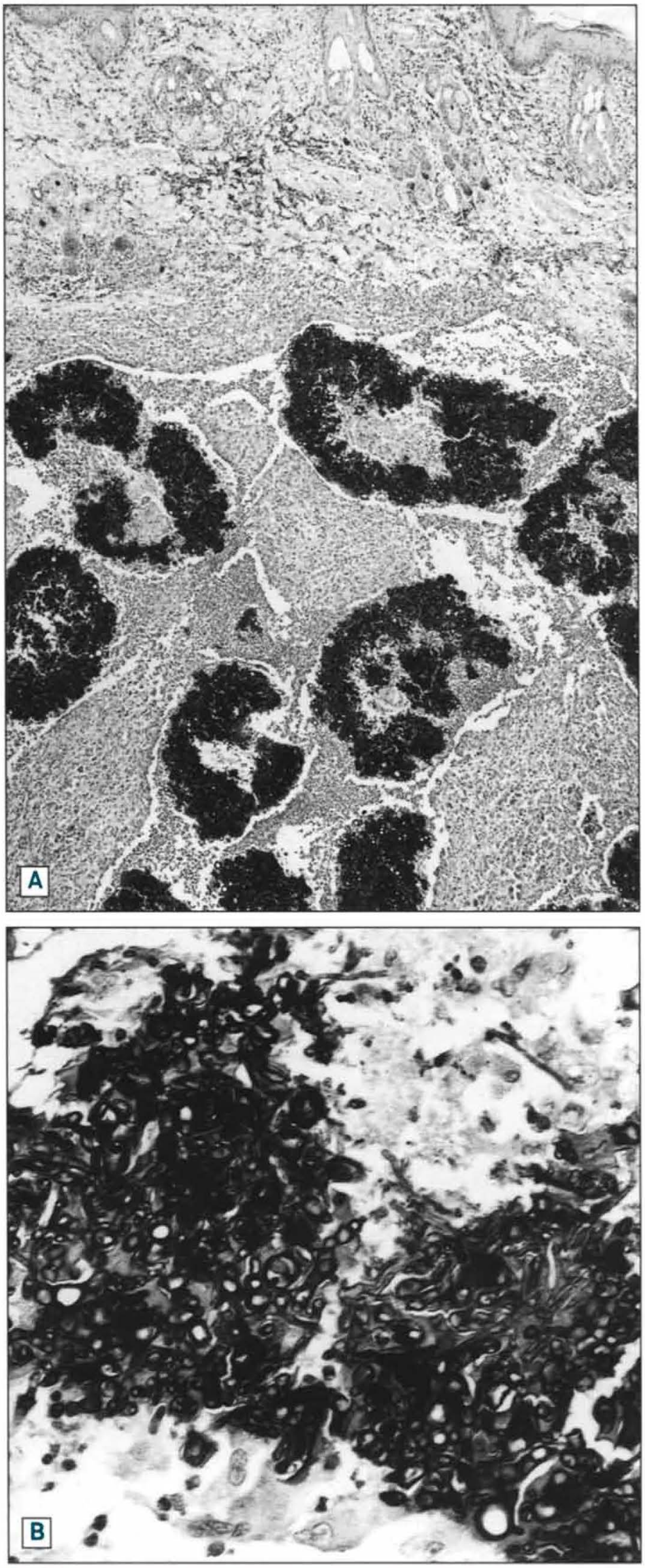

Figure 5.98 ***Microsporum canis*** **pseudomycetoma** in a Persian cat. **A.** Myriads of fungal hyphae (PAS-positive) in the panniculus surrounded by granulomatous inflammation. **B.** Higher magnification of **(A)** showing the appearance of the **hyphae**.

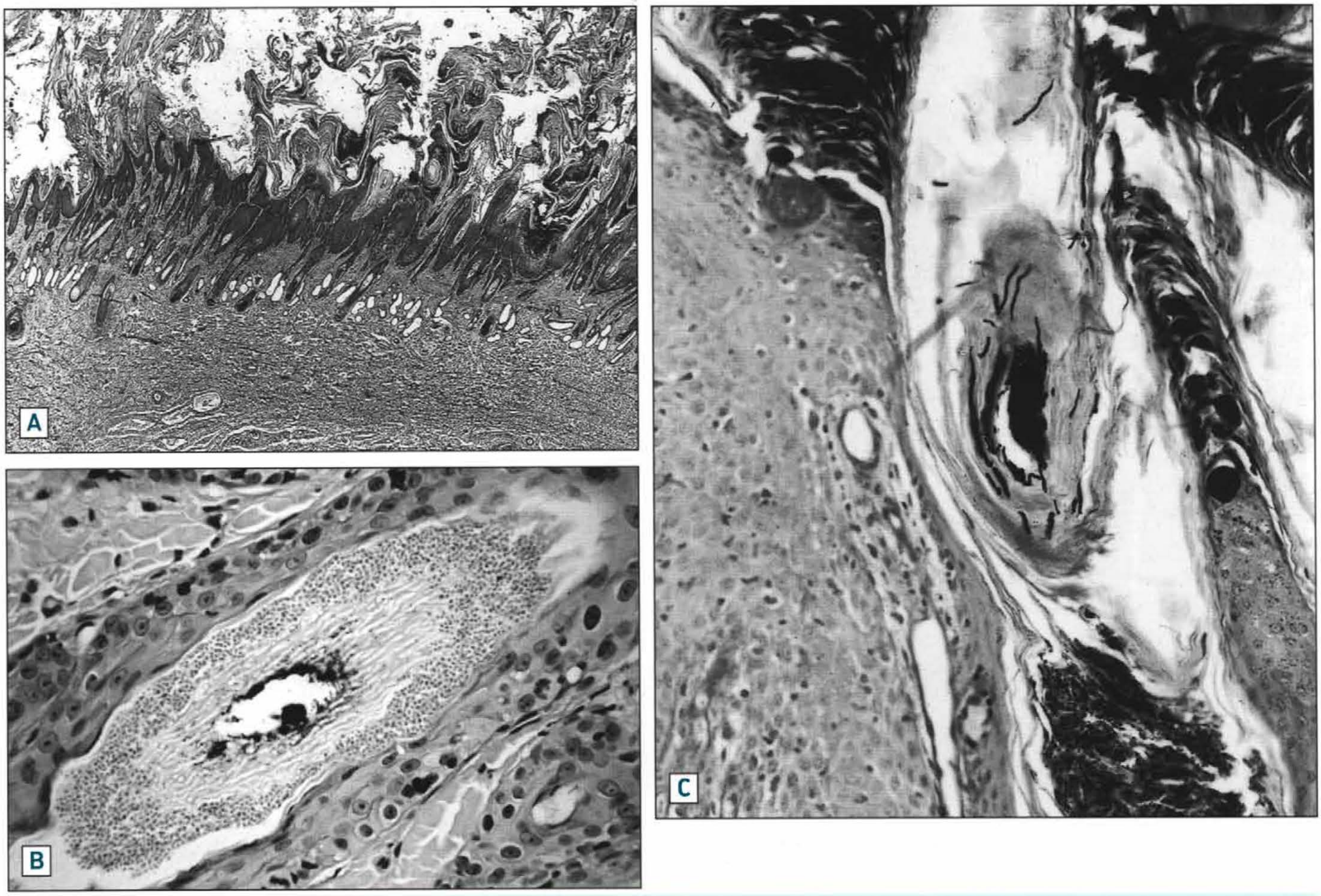

Figure 5.99 Dermatophytosis. A. Acanthosis and hyperkeratosis in a calf. **B.** Hair follicle heavily colonized by **fungal spores**, with minimal inflammation. **C. Filamentous organisms** in a follicle accompanied by leukocytes, in dermatophytosis in a calf; silver impregnation.

Kerions consist of diffuse pyogranulomatous inflammation in the deep dermis produced by extensive furunculosis. This form of inflammation is most commonly caused by poorly adapted organisms, such as the geophilic dermatophyte *M. gypseum*. Hair fragments containing hyphae and arthrospores are frequently present among the inflammatory cells but may be destroyed by the intense inflammatory reaction. *In some cases, the pattern of inflammation may be confused with autoimmune diseases.* Lymphocytic lichenoid interface dermatitis and subcorneal pustules containing neutrophils and acantholytic cells have been reported in several cases of dermatophytosis in dogs and horses; however, organisms were evident with fungal stains. The *dermatophyte pseudomycetoma* is characterized by masses of fungal elements surrounded by granulomatous or pyogranulomatous inflammation in the deep dermis and subcutis.

Cattle

Dermatophytosis is *common* in cattle and is most prevalent in calves. *T. verrucosum* is the most frequent isolate; others include *T. mentagrophytes, T. equinum, M. gypseum, M. nanum*, and *M. canis*. Outbreaks are associated with *crowding and confinement indoors during fall and winter.* Lesions occur on the *neck, head and ears, and pelvic area* most commonly. The intermaxillary space and dewlap are common sites for lesions in bulls. Lesions consist of *circular areas of alopecia with variable crusting or scaling*. In calves, the entire neck may become alopecic, crusty, thickened, and corrugated and lesions may be intensely pruritic. The disease may be of some economic significance because of damage to hides or restrictions on showing or marketing of infected animals. In very severe cases, loss of condition and decreased milk production or weight gain may result. A live attenuated vaccine for *T. verrucosum* has been effective in control of bovine dermatophytosis in Europe.

Sheep and goats

Dermatophytosis is *rare* in goats and sheep. *T. verrucosum* is the most common isolate. Lesions are usually on the *face, ears, neck, and limbs* in goats. In sheep, lesions are usually on the haired areas of the face and ears and may be intensely pruritic. *M. gypseum* infection in show lambs in the United States, called "*club lamb fungus*" by laypersons, involves wooled skin anywhere on the body but lesions are especially common on the lateral thorax. This condition is of zoonotic significance since, in approximately 50% of families with affected lambs, at least one family member had skin lesions consistent with ringworm. Outbreaks of *M. canis* dermatophytosis in sheep also involve the wooled parts of the body. In infections involving the fleece, lesions consist of circular areas of matted, discolored wool, several millimeters to up to 6 cm in diameter. Pruritus is variable.

Pigs

Dermatophytosis is *rare* in pigs. *M. nanum* is the most common isolate and infection produces little effect on the general health of the animal. *T. verrucosum* occurs in pigs housed in premises previously occupied by cattle. Infections also are also caused by *T. mentagrophytes, M. canis,* and *M. gypseum*. The lesions of *M. nanum* are especially common behind the ears and on the trunk, and consist of irregular dark patches with fine brown scales or crusts. No alopecia or pruritus occurs. Lesions may resolve spontaneously or spread slowly to involve extensive areas. An outbreak of dermatophytosis caused by *M. canis* in pigs was characterized by red alopecic patches of variable size on the backs and flanks. Pruritus was mild. The source of the infection was suspected to be rabbits, which had skin lesions clinically consistent with ringworm, or possibly from cats on the farm.

Horses

Dermatophytosis in the horse (*girth itch, tinea*) is caused by a variety of organisms but most commonly by *T. equinum*. Infections with *T. mentagrophytes* and *M. gypseum* are also common; *T. verrucosum* and *M. canis* infections are less frequent. Lesions occur most often in *areas in contact with the saddle and tack* when infection is spread by contaminated equipment or riders. In some cases, the caudal aspect of the pastern may be the only site affected and resemble "grease heel" or "scratches." Outbreaks of dermatophytosis caused by *M. gypseum* have been associated with periods of humid weather and a high incidence of mosquitoes and stable flies. In some training establishments, over 30% of the horses have ringworm and the causative agent is *M. gypseum*. The mane and tail areas are not usually affected. Initial lesions may be papular and resemble fly bites or urticaria but within several days they become the more typical scaly or crusty circular alopecic foci. The lesions of *M. gypseum* tend to be smaller than those of *T. equinum*. Pruritus varies from severe to absent. Lesions may continue to expand for one or two months and then regress as an immune response develops. Several horses with *T. equinum* dermatophytosis that resembled pemphigus foliaceus have been described. Two adult horses developed rapidly progressive painful widespread dermatitis consisting of papules, pustules, crusting, scaling, and erosions. Microscopically, lesions were characterized by prominent acantholysis. However, fungal arthrospores and hyphae were also evident and lesions resolved with antifungal therapy.

Dogs

M. canis is considered the most common cause of dermatophytosis in dogs; but in some areas, infections caused by *M. gypseum* predominate. *T. mentagrophytes* infection is less frequent. Infections with anthropophilic species *Epidermophyton floccosum* and *T. rubrum* have been reported and suspected to have been acquired from infected humans. Initial lesions are frequently on the face and forelegs. The lesions of dermatophytosis are grossly indistinguishable from demodicosis or pyoderma. Dogs with dermatophytosis frequently exhibit classic expanding annular patches of alopecia, scaling or crusting with an erythematous border. Scaling and crusting vary from mild to severe. Follicular papules and pustules are also common. Severe folliculitis and furunculosis may result in permanent alopecia and scarring. In some cases the lesions consist of generalized greasy scaling resembling seborrhea. Pruritus is usually absent but may be severe in some cases. Cases of dermatophytosis caused by *T. mentagrophytes, T. terrestre,* and *M. persicolor* with a very long duration (1–5 years) have been reported. These infections suggest that there is *little tendency for spontaneous resolution in some infections*, a situation analogous to chronic dermatophytosis in humans. Kerions are often associated with *M. gypseum* or *T. mentagrophytes* infections. Infection by *M. persicolor*, a rare zoophilic dermatophyte associated with voles, may produce lesions characterized by prominent scaling but minimal alopecia. This dermatophyte does not invade hair and thus fungal organisms are seen only in the surface keratin. *Onychomycosis is rare* and may consist of chronic inflammation of the ungual fold or infection of the claw alone, producing deformity or fragility of the claw. *Several cases of Trichophyton dermatophytosis with clinical and histologic features resembling pemphigus erythematosus have been reported.* These dogs had scaling, crusting facial dermatitis and alopecia. Microscopically, lesions included interface dermatitis accompanied by intraepidermal pustules with acantholytic cells, suggestive of an immune-mediated disease. However, fungal elements were demonstrable with fungal stains.

Cats

Dermatophytosis is a *common* disease in cats and infection may be endemic in large catteries, with up to 35% of cats being culture positive. The vast majority of cases (greater than 90%) are caused by *M. canis*, which is of considerable public health significance because of its zoonotic potential. Despite the name, this dermatophyte is well adapted to the cat and induces minimal host response. In some surveys, long-haired cats have a higher incidence of positive cultures than do short-haired cats. Geographic differences may influence the prevalence of various dermatophytes. A survey of dermatophytes isolated from cats in shelters in the United States found *M. canis* isolates to predominate in the southeastern United States; while in cats from the northern United States, anthropophilic organisms were cultured and *M. canis* was not isolated from any cat. An *M. canis* fungal cell wall vaccine developed for use in cats has been shown to induce both humoral and cell-mediated immune responses, but it does not appear to be protective against challenge exposure with a large number of fungal spores.

Lesions are extremely variable. They frequently begin on the face, ears, and forelegs and may become generalized. Pruritus is uncommon but may be moderate. Classical circular foci of alopecia and crusting or scaling are more common in kittens. In adult cats, lesions may be extremely subtle and consist of patchy mild alopecia or broken hairs with little skin change. Scaling and crusting may be absent or severe. Some cases appear as papulocrustous miliary dermatitis or as feline acne. Folliculitis with alopecia is uncommon. Microscopic findings are variable. In some cases, organisms are infrequent and only a single infected hair may be found in a biopsy, while in other cases organisms may be numerous. Pseudomycetomas are subcutaneous nodules that occur in Persian cats. They are an atypical form of dermatophytosis caused by *M. canis* in which the deep dermis and subcutis are involved. There is frequently also a history of previous or concurrent superficial dermatophyte infection. Lesions are usually focal and measure 2–3 cm in diameter; but in some instances, they are multiple and involve extensive areas. They consist of discrete masses of bizarre distorted septate fungal hyphae, larger thick-walled fungal cells resembling *chlamydospores*, and chains of round structures surrounded by a hyaline eosinophilic Splendore-Hoeppli reaction. These granules are surrounded by granulomatous or pyogranulomatous inflammation. Fungal organisms are not necessarily present in the hair and keratin of the overlying skin.

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Subcutaneous fungal infections

The subcutaneous mycoses are a heterogeneous group of infections caused by a wide variety of fungi of low virulence that require mechanical introduction into tissue before they can cause disease. The organisms are common saprophytes that normally exist in soil, vegetation, or on normal skin and hair. Their ability to adapt to the tissue environment and elicit disease is variable. The clinical course is usually insidiously progressive over a period of months to years. Infection usually remains localized to the site of entry and surrounding tissues. Slow extension by way of lymphatics occurs in some diseases; but widespread dissemination is rare. Granulocytes are involved in control of many subcutaneous fungi, and granulocytopenia may render an individual more susceptible to subcutaneous mycoses.

Eumycotic mycetoma

Mycetomas are tumor-like infections of skin and subcutaneous tissues caused by two different types of organisms: actinomycetes (**actinomycotic mycetoma**) and fungi (**eumycotic mycetoma**). They are caused by a diverse group of organisms, many totally unrelated to each other, all of which elicit similar clinical disease. *Mycetomas are characterized clinically by the triad of tumefaction (swelling), draining tracts, and grains in the discharge.* Eumycotic mycetomas are those caused by fungi and they have been reported worldwide in humans, occasionally in dogs and horses, and rarely in cattle and cats. A wide variety of fungi that exist as saprophytes in the soil or on plants have been associated with mycetomas. The organisms involved may also cause other clinical diseases, e.g., phaeohyphomycosis and mycotic granulomas, when the three criteria are not present for a diagnosis of a mycetoma. Some cases reported in the veterinary literature as mycetomas are actually phaeohyphomycosis. Mycetomas usually involve skin and subcutaneous tissues and sometimes extend to involve underlying bone. *Curvularia* spp. and *Scedosporium apiospermum* (*Pseudallescheria boydii*) are most commonly involved in mycetomas in animals; *Cladophialophora bantiana* was identified as the cause of eumycetoma in one dog. These organisms are introduced by *wound contamination or implantation* by thorns and other plant material. Lesions often do not develop for many months after inoculation of the fungus. This long incubation period is thought to represent the time needed by the organism to adapt to the environment of the host tissues. All cases have become infected from sources in nature and thus *the condition is not contagious.*

Lesions in animals are widely distributed on the body but they frequently occur on the extremities and the face. In rare cases, subcutaneous lesions develop in dogs as extensions of abdominal infection. Disseminated infections have been reported rarely. Lesions are usually solitary and begin as small dermal or subcutaneous papules or nodules that enlarge gradually over a period of months to years. As the lesion slowly enlarges, deeper tissues such as muscle and bone may become involved. The reaction to the fungus is suppurative and inflammation is accompanied by prominent fibrosis that produces progressive swelling. Abscesses evolve into fistulas that emerge to the surface and give rise to the characteristic appearance of a mycetoma. The fistulas discharge serous, purulent, or hemorrhagic exudate containing grains. *The grains or granules are composed of aggregates of fungal organisms* and vary in size from 0.1 mm to several millimeters in size and may be white, yellow, pink-red, brown, or black. The color, size, shape, and texture of the grains may be sufficiently characteristic to suggest the etiologic agent, but *definitive identification of the fungus requires culture. Curvularia* spp. and *Madurella* spp. have been associated with black-grained mycetomas, while *P. boydii* is usually associated with white grains.

Microscopically, the lesions are similar regardless of the etiologic agent. *The salient histologic feature is the presence of granules in a tumor-like mass of chronically inflamed tissue.* Granules are spherical, lobular, or scroll-shaped masses of fungal hyphae that are 2–6 μm in diameter, septate, and branching. They are pigmented or nonpigmented. Chlamydospores are also frequently present, especially at the periphery of the mass. The fungal hyphae may be embedded in an amorphous eosinophilic "cement-like" substance. The granules of some mycetomas are surrounded by an amorphous eosinophilic radially arranged or smoothly contoured Splendore-Hoeppli reaction. The granules are located within abscesses composed of neutrophils and necrotic debris. *Fragments of plant material* may be seen within the lesion adjacent to the granules and are suspected to be the vehicle of infection. The abscesses vary in size and are present in the dermis and subcutis. Abscesses are surrounded by a chronic inflammatory reaction consisting of epithelioid macrophages, multinucleated giant cells, and fewer plasma cells and lymphocytes. Fibrous connective tissue of variable maturity separates the inflammatory foci. In some cases, *special stains may be needed to determine if the grains are composed of bacteria or fungi.* If the grains are hard, they may be displaced or removed altogether from the tissue because of drag on the microtome blade during sectioning. Multiple sections may be needed to find granules. The internal configuration of a granule may be suggestive of a specific fungus or group of fungi. Definitive identification of the organism can be made on tissue sections by immunofluorescence techniques but the specific antisera are not readily available and *culture is the usual means of identification.*

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Phaeohyphomycosis

Phaeohyphomycosis is an uncommon opportunistic subcutaneous, cerebral, or systemic infection caused by a wide variety of fungi that are all characterized by formation of dematiaceous, or pigmented, hyphae in tissue. The pigmentation is due to the presence of melanin and results of experimental studies suggest that the pigment may act as a virulence factor in development of infection. The organisms responsible for these infections are worldwide in distribution and are widespread in the soil, on wood, and in vegetation. Some of the organisms can also be cultured from the skin of healthy people and animals. Subcutaneous infection is thought to result from wound contamination or traumatic implantation of wood slivers, thorns, sticks, and the like. The taxonomy of the organisms as well as their names and the diseases they cause have been changed frequently, causing confusion in the literature. The list of organisms identified as causative agents of phaeohyphomycosis is continually increasing. Some of the fungi that have been associated with phaeohyphomycosis in animals include *Alternaria alternata, Bipolaris* (Drechslera) *spicifera, Xylohypha bantiana (Torula bantiana, Cladosporium bantianum, Cladosporium trichoides), Curvularia geniculata, C. lunata, Exophiala jeanselmei, Ochroconis* (Dactylaria) spp., *Phialophora verrucosa*, and *Wangiella* spp.

Phaeohyphomycosis has been reported most frequently in *cats* and occasionally in *horses, dogs, cattle, and goats.* The disease also occurs in humans. Some of the cases reported in the veterinary literature as mycetomas, in which small groups of dematiaceous fungi are present within tissues but which do not otherwise fulfill the clinical criteria of mycetomas, are actually cases of phaeohyphomycosis. No underlying immune deficiency is apparent in most cases of subcutaneous phaeohyphomycosis; whereas disseminated infections have been associated with immunologic compromise or debilitating disease. Subcutaneous infection with *Staphylotrichum coccosporum*, a fungus previously thought to be nonpathogenic was described in a cat positive for *Feline leukemia virus*. Subcutaneous infection is insidiously progressive and evolves over months to years.

Lesions of pheohyphomycosis consist of *single or multiple subcutaneous nodules.* The *fungal pigmentation may be grossly visible in the tissue* and, in some cases, the nodule is so dark it may be mistaken for a melanoma. In *cats*, lesions are usually single and occur most commonly on the face and feet. Firm to fluctuant subcutaneous nodules grow slowly and may be ulcerated or have draining tracts. Multiple recurrences following surgical excision are common. The clinical course in some reported cases has been several years. Lesions in *horses* are frequently multiple and located on several different parts of the body. Occasionally nodules may be generalized. Multiple ulcerated cutaneous nodules are the usual form of the infection described in *cattle*, and lesions may also be present in the nasal mucosa. Lesions in *dogs* are also frequently multiple and may be extensive. They consist of poorly circumscribed ulcerated or fistulating nodules or plaques on the feet and legs. In one affected dog, lesions consisted of multiple nodules in the area of ascending lymphatics of a leg and an enlarged regional lymph node, a clinical appearance similar to the cutaneous-lymphatic form of sporotrichosis.

The histologic diagnosis of phaeohyphomycosis is made by demonstrating pigmented hyphae within the tissue. The lesions consist of multifocal-to-diffuse pyogranulomatous dermatitis and panniculitis. The overlying epidermis is acanthotic or multifocally to diffusely ulcerated. Microabscesses with foci of necrosis are frequently prominent. Necrosis may be extensive. Fungi are distributed throughout the lesion as scattered small aggregates and individual hyphae. *The hyphae are septate, 2–6 μm wide, and branched or unbranched.* They are often constricted at their prominent thick septations and may contain single or chains of thick-walled vesicular swellings, 25 μm or more in diameter, that resemble chlamydospores. *The innate brown pigment may not be readily apparent*, but at least some pale yellow-brown to black hyphae will be found in unstained (cleared and mounted) or HE-stained tissue sections.

If pigmentation is not readily apparent, a melanin stain , such as Fontana-Masson, has been suggested to confirm the presence of melanin in the hyphae. However, some nondematiaceous fungi have been found to be Fontana-Masson positive, calling into question the usefulness of the procedure. Fungal stains demonstrate the organisms well but can mask the natural color. The etiologic agents are so similar in appearance within tissues that they cannot be identified on the basis of their morphology. Culture is always needed for specific identification of the fungi. Phaeohyphomycosis has been confused with eumycotic mycetomas caused by dematiaceous fungi. *The fungi in mycetomas form discrete organized granules, whereas those of phaeohyphomycosis appear as individual hyphae and small aggregates scattered throughout the lesion.* The aggregates of hyphae in phaeohyphomycosis may be surrounded by Splendore-Hoeppli material but this does not constitute a granule. The fungal elements of pheohyphomycosis are frequently intracellular within epithelioid macrophages and multinucleated giant cells; granules of mycetomas are nearly always extracellular.

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Sporotrichosis

Sporotrichosis is an uncommon chronic infection usually limited to skin and subcutaneous tissue caused by the opportunist fungal pathogen Sporothrix schenckii. The organism is a dimorphic fungus, growing as hyphae at environmental temperatures and in yeast form in tissue. *S. schenckii* exists as a saprophyte distributed worldwide in the soil, on plants, and on various plant materials. The organism is considered only weakly pathogenic and a great deal of variability occurs in virulence of strains isolated from soil. Disease is most common in temperate and tropical zones. Sporotrichosis has been reported in horses, mules, donkeys, cattle, goats, swine, dogs, cats, rats, mice, hamsters, domestic fowl, camels, dolphins, armadillos, chimpanzees, and humans. The infection has been *reported most often in horses, cats, and dogs*. Infection is usually acquired by wound contamination or inoculation of the organism into tissue by puncture wounds caused by thorns, wood splinters, or contaminated claws. Pulmonary infection is acquired by inhalation of spores. In humans, the disease is considered an occupational hazard for those who work with the soil, plants, or plant materials.

Sporotrichosis is subdivided into *three clinical forms*.

- The **primary cutaneous form** consists of multiple scattered raised alopecic, ulcerated, crusted nodules or plaques that remain confined to the point(s) of entry of the organism. It is thought that this form results from a *high degree of host immunity*, preventing spread of infection. Nodules may become ulcerated and associated with seropurulent exudate and crust formation. The normal grooming behavior of cats may result in autoinoculation and spread of lesions to distant sites. The cutaneous form may have a very chronic course. An unusual case of sporotrichosis in a dog consisted of otitis externa characterized by multiple cutaneous nodules which persisted for more than 5 years. A donkey with sporotrichosis had multiple slowly progressive facial lesions for 2 years before the disease was diagnosed.
- The **cutaneous-lymphatic form** involves the skin, subcutaneous tissue, and associated lymphatics. Lesions begin as firm round nodules at the site of entry, usually on an extremity, and spread proximally along lymphatics. Lymphatic vessels become thick and corded and a series of secondary nodules forms as the infection progresses. The nodules may break open and discharge seropurulent material. Lesions may cavitate and expose extensive areas of underlying muscle and bone. Regional lymphadenopathy is common. This is the most common form in *horses*. Lesions generally involve the proximal forelimbs, chest, and thigh but usually no regional lymph node involvement is evident. *Dogs* usually have the cutaneous or cutaneous-lymphatic form. The head, pinnae, and trunk are involved most frequently. In *cats*, lesions are usually located on the head, distal limbs, and base of the tail. The initial draining puncture wounds may be indistinguishable from cat-inflicted fight wound infections.
- The **extracutaneous/disseminated form** may involve a single extracutaneous tissue, such as osteoarticular sporotrichosis, or multiple internal organs. It develops as a sequela to cutaneous-lymphatic infection or following inhalation of the fungus. The disseminated form of sporotrichosis *occurs most frequently in cats*, and no immunosuppressive factors are usually identified. In experimentally induced sporotrichosis in cats, organisms were shown by culture to have disseminated to viscera in 50% of the cases. Cats with disseminated sporotrichosis are often febrile, depressed, and anorexic.

Microscopically, sporotrichosis is usually a *nodular to diffuse pyogranulomatous or granulomatous inflammatory reaction involving the dermis and subcutaneous fat*. The epidermis is acanthotic or ulcerated. Neutrophils, epithelioid macrophages, multinucleated giant cells, and fewer lymphocytes and plasma cells form discrete granulomas or extensive sheets of inflammation replacing dermal and subcutaneous tissues. Fibrosis is variable; and necrosis may be extensive. *Yeast(s) surrounded by a stellate radial corona of brightly eosinophilic material (asteroid body/Splendore-Hoeppli reaction) are seen in some cases.* The yeasts appear as round, oval, or elongated ("cigar"-shaped) single or budding cells which measure 2–6 μm or more in diameter for the round and oval forms and 2 × 3 to 3 × 10 μm for the cigar form

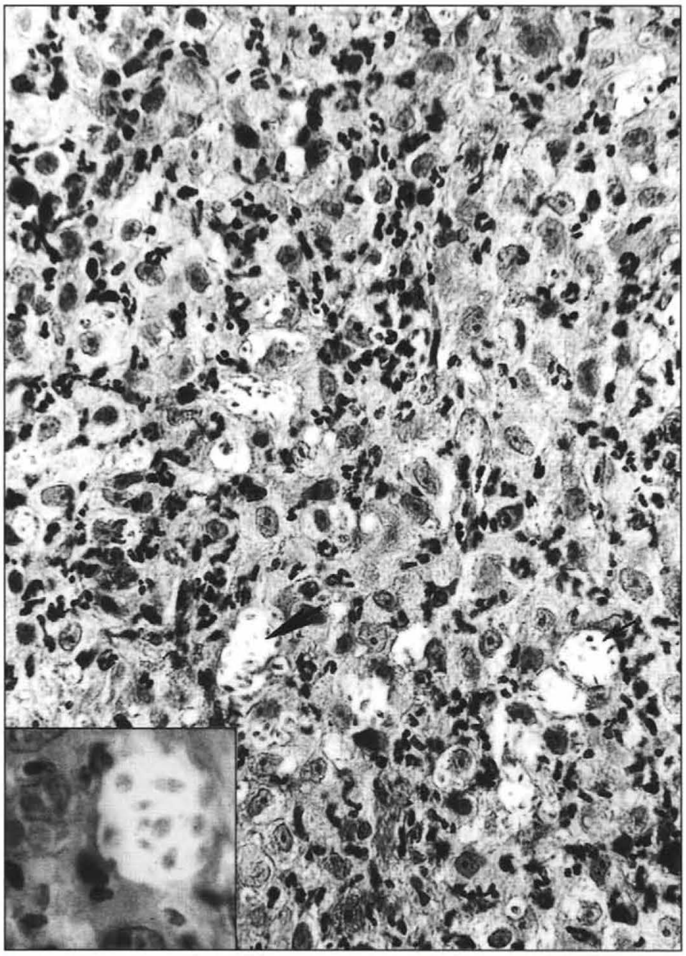

Figure 5.100 Pyogranulomatous inflammation with organisms in vacuoles (see inset) in **sporotrichosis**.

(Fig. 5.100). The cigar forms are considered characteristic of *Sporothrix* but they may not be found regularly. *In general, organisms are numerous in lesions from cats and rare in tissues from dogs and horses.* However, in immunosuppressed dogs, yeasts may be numerous. The yeasts may be extracellular or within neutrophils and macrophages. The yeasts have a refractile cell wall from which the cytoplasm may shrink during processing and give the appearance of a capsule. In such instances and when no cigar forms are evident, the organism may be mistaken for *Cryptococcus neoformans* or *Histoplasma capsulatum*. Yeasts stand out clearly with fungal stains, but serial sections may be needed to demonstrate even a single organism. Without a significant number of cigar-shaped forms, identification may not be possible. In these cases, *immunoperoxidase or fluorescent antibody staining* using specific *S. schenckii* antiserum is necessary for definitive identification of the organism. Organisms may not be found despite the use of serial sections and special stains, and diagnosis in such cases requires culture. The yeast can usually be cultured from tissue obtained from areas of active inflammation even in dogs and horses, which typically have very few organisms in tissue. In cats, cytologic examination of exudates and material obtained by fine needle aspiration of nodules is frequently diagnostic because sufficient numbers of yeasts are usually present to identify the organism.

Sporotrichosis is considered a zoonotic disease. Transmission of the infection from cats to humans has been reported many times and *infected cats pose a significant public health danger.* Veterinarians, other veterinary professionals and students, and owners exposed to ulcerated wounds or exudates from infected cats have developed infections. The organism may be able to penetrate intact skin since not all patients who develop lesions can recall having wounds or being bitten or scratched by infected cats. The large number of organisms typically present in lesions from cats is thought to be the reason for the transmission of disease from cats to humans. However, even when the lesions in cats had few organisms, transmission to humans has occurred. In contrast, infection was not transmitted from a dog with multiple cutaneous lesions containing relatively numerous organisms to several adults and children with whom the dog had had frequent contact for the 2 year duration of infection. These cases suggest that factors in addition to absolute numbers of yeasts are involved in the apparent ease of cat-to-human transmission.

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Cutaneous oomycosis (pythiosis and lagenidiosis)

Oomycosis refers to infection by *Pythium insidiosum* (formerly *Hyphomyces destruens*) or *Lagenidium* sp. organisms, both of which are aquatic dimorphic water molds and members of the Oomycetes in the kingdom Protista. These opportunistic pathogens live in warm stagnant water and are most often reported in regions with tropical to subtropical environments. The organisms are thought to enter the skin through cutaneous wounds, and infection may involve the dermis, subcutis or distant tissue as described below.

Pythiosis (leeches, swamp cancer, bursattee, phycomycosis) is *a chronic cutaneous-subcutaneous, gastrointestinal, or multisystemic granulomatous infection of horses, dogs, cattle, cats, and humans living in tropical, subtropical, and temperate regions* throughout the world. *Pythium* spp. occur in the soil and aquatic environments worldwide, and some are important plant pathogens. *P. insidiosum* is unique in that it has adapted to colonize mammalian tissues. The organisms require warm weather and moisture for reproduction and thus the majority of cases occur in late summer and fall in tropical and subtropical areas. Water lilies, grasses, and debris of plants found in aquatic or humid habitats appear to support the propagation of *Pythium* spp. The infective stage of the organism is a biflagellate aquatic zoospore that is released seasonally in association with warm weather and moisture. Infection is thought to be acquired from prolonged contact with stagnant fresh water containing the newly emerged zoospores that are motile and are attracted chemotactically to injured tissue. The zoospores produce a substance that enables them to attach to host tissues during initial stages of infection and they appear to play an important role in producing infection. However, the zoospore stage is not known to form in tissue and the infection is thus not considered to have zoonotic potential.

Not all animals with pythiosis have a history of contact with permanent bodies of water, suggesting that the organism may proliferate in temporary stands of water or even on wet grasses.

Cutaneous pythiosis has been reported most frequently in **horses**. No age, breed, or sex predilection is recognized. Lesions occur most commonly on the *limbs, distal to the carpus and hock*, and on the ventral aspect of the thorax and abdomen, sites that are most likely to be in contact with stagnant water and would be traumatized by aquatic plants or vegetation (Fig. 5.101A). Affected horses frequently have a history of prolonged contact with water in lakes, ponds, swamps, or flooded areas. Lesions are usually single but occasional horses develop lesions in several separate sites. Lesions begin as nodules that enlarge very rapidly to become circular masses of granulation tissue. The masses ulcerate or develop multiple fistulous tracts that drain thick purulohemorrhagic material. The sinuses also contain characteristic gray-white to pale yellow coral-like concretions (called *leeches* or *kunkers*) that may be extruded at the skin surface (Fig. 5.101B). The colloquial name leeches is based on the initial misidentification of the masses. *These structures are unique to the horse* and are not seen in other animals with pythiosis. Lesions are frequently extremely pruritic, and biting or rubbing the lesion contributes to tissue damage. The largest lesions usually develop on the thorax and abdomen and may attain a size of 45 cm or more in diameter. In chronic cases, underlying bone may be invaded. Regional lymph nodes may be involved but visceral spread is rare. *The clinical appearance of the lesions may resemble basidiobolomycosis, cutaneous habronemiasis, excessive granulation tissue, and neoplasia (particularly sarcoid and squamous cell carcinoma).*

Cutaneous pythiosis has been reported less frequently in **dogs** than has the gastrointestinal form. The disease occurs most often in young adult, large breed dogs, and German Shepherd Dogs may be predisposed. The disease in dogs is relentlessly progressive with no proven medical treatment. Most lesions are on the extremities but they may also occur on the face, ventral abdomen and chest, and in the perineal area. In most cases, a single body region is involved. *The initial lesion is a poorly circumscribed dermal nodule that rapidly expands peripherally and extends into the subcutis to form multiple secondary nodules* (Fig. 5.101C). The nodules develop into spongy masses that ulcerate, become necrotic, and develop multiple fistulous tracts that drain purulohemorrhagic exudate. Early lesions on the legs may resemble acral lick granulomas. Pruritus is not as constant a feature in dogs as it is in horses with pythiosis. A common clinicopathologic abnormality is absolute eosinophilia.

Pythiosis has been reported infrequently in **cattle**. Most cases have been in beef calves <12 months of age. Calves of both sexes have been affected. The majority of the lesions are located on the distal extremities. They consist of irregular swellings with ulceration and multiple draining tracts. Although no concretions have been present, the tissue contains numerous yellow punctate foci. Reports of pythiosis in **cats** are rare. As in other species, lesions usually are located on the extremities. In at least two cats, the lesions progressed very slowly and the clinical course was much more protracted than is typical of the disease in dogs and horses. Moreover, nodules remained confined to the subcutaneous tissues and there was no involvement of the overlying skin.

Microscopically, the lesions consist of *granulomatous inflammation with foci of liquefactive necrosis and extensive fibrosis* involving the dermis and subcutis. The epidermis is acanthotic or focally to diffusely ulcerated. The necrotic foci consist of eosinophilic coagula containing necrotic eosinophils and sometimes degenerate collagen and small necrotic blood vessels. Necrotic foci are most expansive in lesions from horses and they correspond to the concretions seen grossly (Fig. 5.101D). The necrotic material is surrounded by viable eosinophils, epithelioid macrophages, and occasional multinucleated giant cells. The organism is located in the necrotic foci but it does not stain with H&E. However, the presence of hyphae may be detected by occasional clear, unstained circular or linear spaces within the necrotic debris. Pythium stains poorly or not at all with PAS, but it is readily stained with GMS and appears as *thick-walled, sparsely septate hyphae, 4–10 μm in diameter, with occasional branching* (Fig. 5.101E). Organisms are usually most numerous in necrotic foci and may also be seen in walls of small arterioles. They are rare in areas of granulomatous inflammation and they are not usually present in intervening connective tissue. The hyphae are similar to those of the zygomycetes *Basidiobolus* and *Conidiobolus*. The hyphae of these organisms tend to be broader, ranging from 5–15 μm in diameter, however the differentiation of *Pythium* sp. from the zygomycetes in tissue is frequently impossible.

Diagnosis of pythiosis is based on the appearance of the gross lesions, microscopic lesions, and culture of the organism. Pythium is readily cultured in most instances; but in some cases, it cannot be grown. Failure to culture the organism may be related to sample handling because refrigeration decreases its survival and freezing kills the organism. Definitive identification of the oomycete, however, requires that the cultured organism be induced to produce the diagnostic zoospores, a process that is complicated, time-consuming, and not always successful. Because of the rapidly progressive course of the disease in many animals, a more rapid means of diagnosis is preferable so that an accurate prognosis may be made and specific therapy instituted. To this end, PCR assays, immunohistochemistry, and immunoblot analyses have been developed for identification and differentiation of *Pythium insidiosum, Lagenidium* and the zygomycetes. Not all assays are available for each organism, but a combination of assays and evaluation of the clinical information and laboratory results can lead to a definitive diagnosis.

Cutaneous lagenidiosis is remarkably similar to cutaneous pythiosis in geographic occurrence and clinical and histologic lesions. Lagenidiosis has been reported only in dogs. Gross lesions consist of nodular and necrotizing lesions of the limbs and trunk that drain and may be accompanied by lymphadenitis. The disease is also very aggressive, and affected dogs frequently develop lesions of the great vessels, mediastinum, lungs, and esophagus. *Histologic lesions are indistinguishable from those of pythiosis and zygomycosis.* Again, clinical presentation, PCR assays, immunohistochemistry and immunoblot analyses are needed to differentiate these infections.

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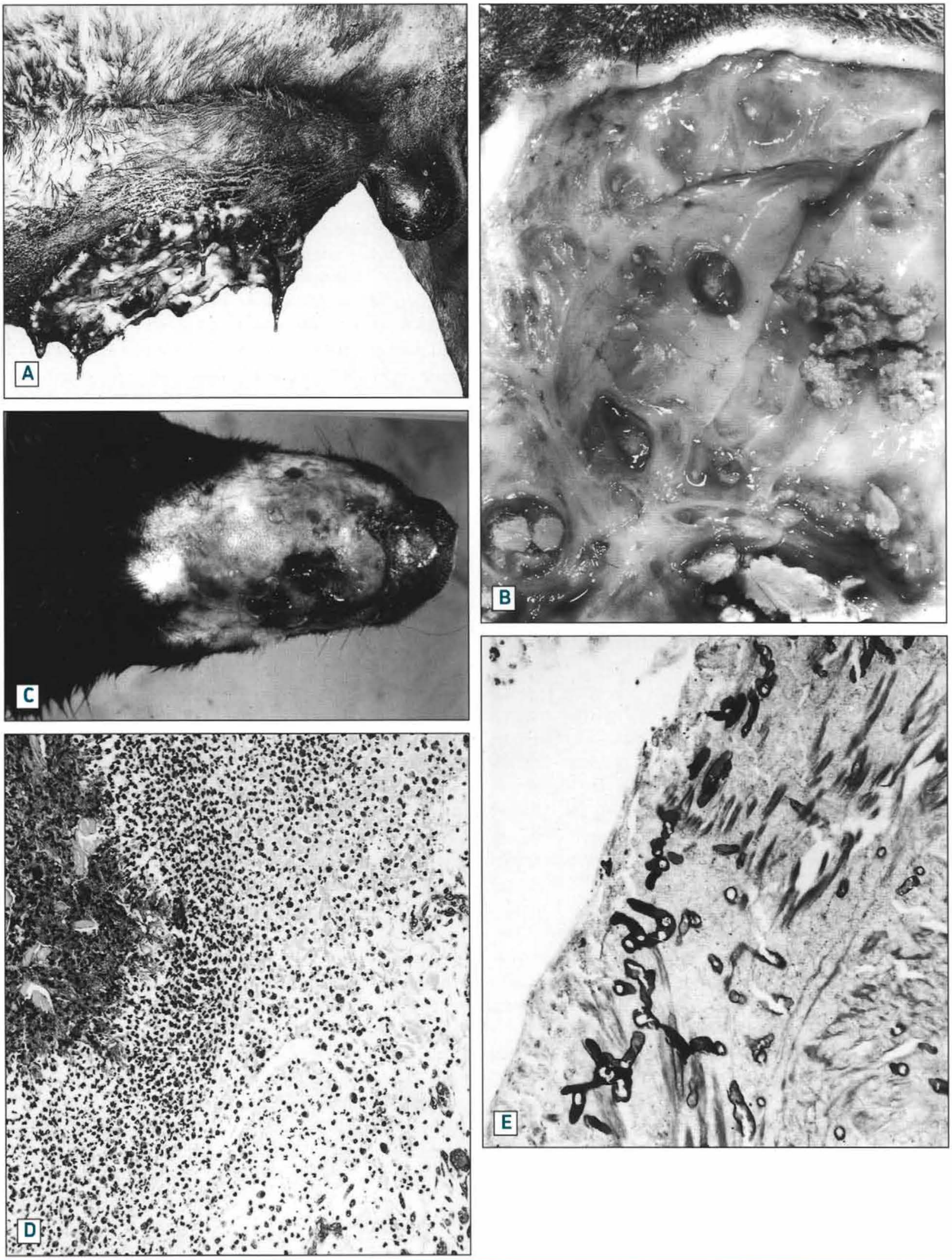

Figure 5.101 Pythiosis. **A.** Ulcerated, draining lesion on the ventral abdominal skin in a horse. **B.** Cut surface reveals granulation tissue and multiple, light-colored cores of necrotic debris ("**kunkers**") in equine pythiosis. **C.** Multiple, ulcerated and draining tracts on the skin of the face in pythiosis in a dog. **D.** Section through edge of necrotic core (above, left) in equine pythiosis. **E.** **Hyphae-like structures** of organism at the edge of the lesion in equine pythiosis.

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Zygomycosis

Zygomycosis refers to subcutaneous, systemic, or rhinocerebral infections caused by a wide variety of zygomycete fungi. The unifying diagnostic feature of zygomycosis is that the organisms all form infrequently septate hyphae that are typically significantly broader than other fungi with filamentous tissue forms, such as *Aspergillus* or the agents of phaeohyphomycosis. The hyphae are unpigmented and commonly range from 6–25 μm in diameter. The class Zygomycetes is composed of two orders. The order Mucorales includes *Rhizopus, Mucor, Absidia, Mortierella, Rhizomucor*, among other genera; and diseases caused by these organisms have been called **mucormycosis**. The order Entomophthorales contains the genera *Basidiobolus* and *Conidiobolus* and disease caused by these fungi is called **entomophthoromycosis**. The term phycomycosis was used when zygomycetes and oomycetes, such as *Pythium* spp., were grouped together in a single division. However, as the oomycetes have been determined not to be true fungi and their taxonomy has been reclassified, the term has become obsolete because the taxonomic equivalent no longer exists. The diseases should be referred to as zygomycosis or pythiosis. Zygomycetes are cosmopolitan in distribution and they are widespread in nature. They all occur as soil saprophytes, agents of decay, insect pathogens, or as components of normal skin and hair flora. They are common laboratory contaminants and are thus sometimes ignored when cultured from clinical specimens. The portal of entry may be cutaneous, gastrointestinal, or respiratory. Zygomycosis is not a contagious disease, the environment being the source of all infections.

Zygomycosis is a *rare disease of humans and animals*, including dogs, cats, horses, llamas, sheep, and pigs. The disease in humans usually occurs in debilitated or immunocompromised individuals. In animals, however, immune compromise is usually not evident and disease may be the result of exposure to a large number of organisms. *Traumatic implantation* is thought to be the portal of entry for the subcutaneous form. Inoculation by biting insects has also been suspected to be a means of entry for the organisms. Members of the order Mucorales usually cause disseminated disease, and *angioinvasion by hyphae is a characteristic feature*. Zygomycosis in dogs and cats is usually in the form of *fatal gastrointestinal mucormycosis*. Zygomycosis caused by *Rhizopus oryzae* has been reported in pigs in which the disease consisted of subcutaneous granulomas with draining tracts as well as lesions in the stomach, liver, and lymph nodes. *In general, fungi of the order Entomophthorales cause localized subcutaneous granulomas.* These infections occur most often in tropical and subtropical regions. The fungus *Conidiobolus* is distinguished by its predilection for the nasal mucosa, nares, and surrounding skin. Infections by various species of this organism have been reported in dogs, horses, llamas, and sheep.

Zygomycosis occurs most frequently in **horses** and infections are usually caused by *Basidiobolus haptosporus* (basidiobolomycosis) and *Conidiobolus coronatus* (conidiobolomycosis). The lesions consist of circular ulcerated nodules or swellings with serosanguineous exudate. Small irregular gritty masses of yellow-white material (kunkers, leeches) may be discharged to the surface along with the exudate. Basidiobolomycosis usually involves the chest, trunk, head, or neck. Solitary lesions are the rule and they may grow rapidly and become very large. Pruritus is a common feature. *A discontinuous undulating band of yellow-white material that sharply demarcates the superficial hemorrhagic, edematous tissue from the underlying fibrogranulation tissue characterizes the cut surface of granulomas.* The clinical lesions are similar to those of pythiosis but may sometimes be differentiated in the horse by differences in anatomic location, number and appearance of kunkers, and epidemiology. The granulomas of zygomycosis usually affect the lateral aspects of the trunk, neck, and head, and the kunkers are less numerous, smaller, and of no particular shape. In contrast, lesions of pythiosis are commonly located on the lower limbs and contain numerous, large, coral-shaped kunkers. Furthermore, pythiosis is typically associated with access to standing water. *No such association with water exists for basidiobolomycosis or conidiobolomycosis*, which are caused by agents found in soil and decaying vegetation.

Unlike the protracted clinical course typical of most cases of zygomycosis, nasal zygomycosis in **sheep** caused by *C. incongruus* results in loss of condition and death within a period of 7–10 days after initial clinical signs. This infection produces prominent asymmetrical swelling of the face, extending from the nostrils to the eyes, and marked thickening of the skin and subcutaneous tissue. In advanced cases, the nasal skin is alopecic and necrotic.

Subcutaneous zygomycosis caused by *Conidiobolus* of unknown species has been reported in a young adult **dog**. Lesions involved the skin of a hind leg and the thoracic wall but not the nasal mucosa or skin surrounding the nose. The lesions on the leg began as an ulcerated mass that progressed to circumferential swelling and induration with numerous coalescing ulcers draining serosanguineous fluid. The condition exhibited some waxing and waning, but new lesions continued to develop. No immunologic compromise or predisposing factors were apparent.

Microscopically, zygomycosis is characterized by *multifocal-to-diffuse eosinophilic and granulomatous dermatitis and panniculitis with multifocal necrosis*. Necrotic foci consist of eosinophilic coagulated material corresponding to the kunkers seen grossly. Eosinophils, neutrophils, epithelioid macrophages, and multinucleated giant cells surround the eosinophilic coagula and are separated by fibrovascular connective tissue. Fungi are usually located in necrotic foci and may be seen as clear linear or circular hyphal "ghosts" often surrounded by Splendore-Hoeppli material (Fig. 5.102). Basophilic granular protoplasm may be visible with H&E stain, but the organisms are usually better visualized with GMS stain. They frequently stain poorly with PAS. The hyphae range from 5–20.5 μm diameter for *B. haptosporus* and 5–12.8 μm for *C. coronatus*. Hyphae are thin-walled, vary moderately in diameter, and have occasional septations and uncommon branching. Folded, twisted, or compressed hyphae may be seen. The lesions of nasal zygomycosis caused by *C. incongruus* in sheep are characterized by segmental necrosis and thrombosis of subcutaneous blood vessels. Many fungal hyphae are visible in necrotic foci, in thrombi, and within vessel walls. This propensity to invade blood vessels is common with Mucorales but is an unusual feature for the Entomophthorales.

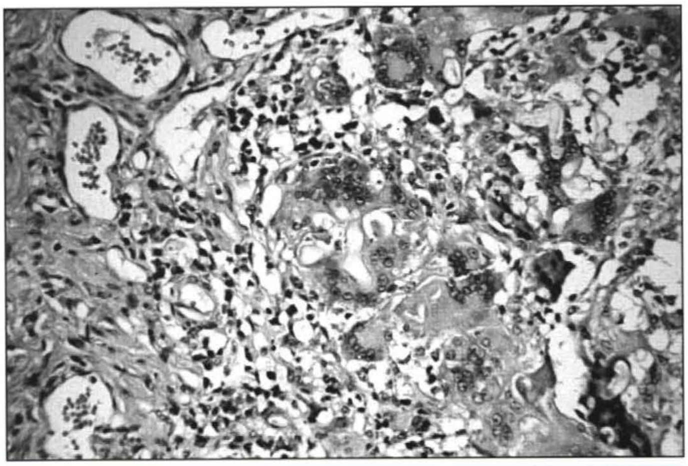

Figure 5.102 Zygomycosis. *Conidiobolus* sp. dermatitis in a dog. Note clear linear and circular hyphal "ghosts" amid granulomatous inflammation.

The microscopic lesions of basidiobolomycosis and conidiobolomycosis are similar to those of pythiosis and the diseases may be histologically indistinguishable, especially when the width of hyphae is at the narrow end of the range. All members of the zygomycetes are morphologically similar in tissue, and consequently culture is necessary for specific identification. Although immunohistochemical identification of fungi in tissue sections has been reported, the specific antisera are not readily available and culture remains the usual means of identification.

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Miscellaneous fungal infections of skin

Infections with various opportunist fungi are reported sporadically in animals. These organisms are ubiquitous in the environment, common contaminants of laboratory cultures, and frequently also a component of skin and hair flora of normal animals. Thus, *diagnosis of infection is difficult to make by culture alone and requires histologic demonstration of tissue invasion by morphologically compatible organisms.* In humans, these infections are usually associated with immunosuppression and neutropenia, but predisposing factors are not usually identified in animals. A convenient means of classifying opportunistic subcutaneous mycotic infections is based on the presence or absence of pigment in mycelia. Those caused by *pigmented ("phaeoid") fungi are called phaeohyphomycosis* and have already been discussed. *Infections caused by colorless or hyaline fungi are called* hyalohyphomycosis. Organisms that cause infection with some regularity or have some particularly distinctive feature are assigned to a separate category, e.g., aspergillosis, pythiosis, etc. Reports of infection with dematiaceous fungi and the number of fungi causing phaeohyphomycosis are more numerous than those caused by unpigmented fungi. The system is artificial in that completely unrelated organisms fall into the same category. However, most of these organisms have similar tissue morphology and elicit the same pathologic response, i.e., (pyo)granulomatous dermatitis and panniculitis. Culture or immunofluorescence or immunohistochemical staining of tissue sections is necessary for specific identification of these organisms.

Cutaneous **hyalohyphomycosis** caused by *Acremonium* and *Fusarium* has been described in dogs and cats. *Aspergillus* species are not commonly associated with skin infections. Subcutaneous infection with *Aspergillus versicolor* producing a nodular mass on the upper lip was reported in an adult Saddlebred mare. The lesion recurred once following surgical excision but had not recurred one year after the second surgical excision. *A. terreus* has been associated with subcutaneous granulomas in an adult Holstein cow without any lesions elsewhere. Dogs with disseminated aspergillosis may rarely develop cutaneous lesions secondarily.

Geotrichosis is a *rare* disseminated, bronchopulmonary, oral, vaginal, cutaneous, or alimentary infection caused by *Geotrichum candidum*. Cutaneous infections have been reported in dogs, snakes, humans, flamingos, horses, and a giant tortoise. *G. candidum* is a ubiquitous soil saprophyte and is also part of normal flora of the oral cavity, intestine, skin and hair. Lesions were on the head and back in dogs and on the head and neck in horses. Gross lesions varied from well-circumscribed ulcers covered by exudate to nonulcerated nodular masses with variable alopecia and desquamation. Infection appeared to be pruritic in several affected horses. Geotrichosis has also been associated with paronychia and onychodystrophy in several dogs. Microscopically, branching septate hyphae were present in the surface keratin and exudate, necrotic epidermis, and extended into the dermis. The organism may be confused histologically with *Candida, Aspergillus*, and *Trichosporon*.

Paecilomycosis is a *rare* subcutaneous or disseminated infection of cats and dogs caused by *Paecilomyces* fungi. The organism is a common airborne contaminant that may be resistant to many sterilizing techniques. Skin lesions typically consist of focal masses or edematous swellings, frequently on the feet and legs. Lesions have also been described on the face and nasal cavity. One disseminated infection in a young adult German Shepherd was thought to originate from an external ear infection.

Trichosporon yeasts cause white piedra, cutaneous infections, systemic infections, pneumonitis, valvular endocarditis, and have been associated with abortions. In humans, disseminated systemic infections usually occur in immunocompromised patients with granulocytopenia. White piedra is a mycotic infection of hair shafts caused by *T. beigellii* (*T. cutaneum*) in humans, monkeys, and horses in temperate to tropical climates. The infection is characterized by *firm, irregular white or pale brown nodules on hair shafts*. In horses, the long hairs of the mane, tail, and forelock are affected. *The nodules consist of tightly packed septate hyphae that are held together by a cement-like substance.* Cutaneous trichosporonosis is *rare* and has been reported only in cats. One cat also had multicentric lymphosarcoma that may have caused immunosuppression. Lesions reported in cats consisted of a nodule in a nostril and an ulcerated mass on the distal leg at the site of a previous cat bite wound. Microscopically, the organisms consist of budding yeast cells and hyaline, branched septate hyphae.

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PROTOZOAL DISEASES OF SKIN

Cutaneous lesions occur in several **systemic or localized protozoal** infections. Differentials for protozoal dermatitis should include *Besnoitia* sp., *Leishmania* sp., *Caryospora* sp., *Neospora* sp., *Toxoplasma* sp., and *Sarcocystis* sp. The majority of these cutaneous protozoal infections have been reported in **dogs**, however some cause serious disease in other domestic species as described below. *Toxoplasma* sp. is considered an opportunistic pathogen most often seen in dogs concurrently infected with canine distemper virus. Cutaneous infection with toxoplasma organisms has not been reported, however the morphological similarities to other protozoal organisms reported to cause dermatitis in animals warrant considering it in the differential of protozoal dermatitis. Cytologic, histologic, immunohistologic and ultrastructural studies can be used to differentiate organisms that are histologically similar. Cutaneous lesions have also been associated with *Theileria* and *Babesia* infections (see Vol. 3, Hematopoietic system). *Trypanosoma equiperdum* and dourine are discussed in Vol. 3, Female genital system.

Besnoitiosis

Members of the genus *Besnoitia* are responsible for a serious disease of *cattle and horses* and, more rarely, donkeys, goats, and sheep. In North America, opossums, caribou, reindeer, and mule deer may serve as intermediate hosts. The genus is currently classified under the family Sarcocystidae, in the subfamily Toxoplasmatinae.

Besnoitia have a *two-host life cycle*. The *definitive hosts are felids* and the intermediate hosts vary with the parasitic species. For *B. besnoiti*, the intermediate host is the ox and for *B. bennetti* it is the horse. These two organisms are morphologically identical by light microscopy and are distinguished only by their host range. Sexual reproduction occurs in the intestinal tract of the definitive host and the sporulated oocysts are passed in the feces. *The exact mode of infection of the intermediate hosts is not known*, but contamination of watering places and mechanical transmission by blood sucking insects may be involved. The life cycle in the intermediate host is characterized by release of sporozoites from sporulated oocysts and the subsequent dissemination of tachyzoites that lead to bradyzoite cyst formation. Cysts occur mostly in the dermis, subcutis, fascia, muscle, mucosa of the upper respiratory tract, pharynx, and conjunctiva. Infection, however, may be generalized and is typically so in some infections in rodents and wild animals. *The tissue cysts represent parasitized host cells* – the fibroblast in *B. wallacei* infection of rodents and the histiocyte in *B. besnoiti* experimental infection of rabbits. The bradyzoites multiply in cellular vacuoles and induce hyperplastic and hypertrophic changes in the host cells. These often divide to form multinucleated cells. The enlarging mass of crescent-shaped bradyzoites compresses the cell cytoplasm and nuclei into a thin rim forming an inner coat to the cyst. A hyalinized collagenous cyst wall is laid down around the parasitized cell. The cysts, which measure up to 500 μm with a 10–30 μm thick wall, are visible to the naked eye. Ingesting parasitized tissue from the intermediate host infects the definitive host.

Bovine besnoitiosis is a serious disease in South Africa and is associated with severe loss of condition in affected cattle, mortality up to 10% and marked damage to the hide. The disease also occurs in central and northern Africa, southern Europe, Asia, South America, and Russia. After an incubation period of ~1 week, a *pyrexic phase* develops in which animals become anorexic, depressed and reluctant to move. Approximately 1–4 weeks after the onset of the pyrexic phase and corresponding with cutaneous cyst formation, generalized lymphadenopathy, edematous swellings of the extremities and severe systemic signs develop. Pregnant cows may abort at this time. The second stage is known as the *depilatory stage* since it is characterized by marked alopecia, thickening of the skin, exudation and fissuring. *The cysts may be visible macroscopically in the scleral conjunctiva or nasal mucosa as small, round, white foci*. Animals lose condition and up to 10% mortality may occur at this point. The third phase is characterized by *dry seborrhea*. Animals remain unthrifty for an extended period and rarely regrow a normal hair coat. The skin remains alopecic, lichenified and scaly.

Histologically, epidermal hyperplasia, marked hyperemia, dermal edema and perivascular accumulations of lymphocytes, plasma cells and large histiocytes that will become hosts to the parasites accompany the acute febrile stage. *Crescent-shaped trophozoites occur in arterioles and lymphatics and free in tissue spaces*. Occasionally they may be detected in macrophages. As the parasites become encysted, inflammation and edema diminish. *The mature cysts incite little or no cellular reaction unless they rupture*; a necrotizing or granulomatous response ensues around the collapsed hyaline capsule. Numerous eosinophils are present in these reactions. The mature cyst wall has *four distinct layers*. Outermost is a condensed, hyalinized, laminated, birefringent layer of collagen fibers. Next is a very thin homogeneous intermediate zone. The third layer is the cytoplasm of the host cell and in this layer lie the several giant, vesicular but compressed host cell nuclei. A thin inner membrane, probably condensed cell cytoplasm, encloses the dense mass of 5–7 μm crescentic bradyzoites. These may be separated from the wall by an artifactual shrinkage space. *Lesions in other tissues* include focal disseminated myositis, keratitis, periostitis, endosteitis, lymphadenitis, pneumonia, periorchitis, orchitis, epididymitis, arteritis, and perineuritis.

Besnoitiosis in **horses, donkeys**, and **burros** has cutaneous lesions similar to those described in cattle. **Caprine** besnoitiosis occurs in Iran in both wild and domestic species. The *Besnoitia* cysts are observed in the skin, blood vessels, epididymis and testes. The pathological changes are comparable to those of *B. besnoiti* infection in cattle. In an outbreak affecting more than 500 domestic goats in Kenya, ocular cysts were the most common finding, but cysts were found in many body systems. Dorper **sheep** were also affected in that outbreak. A disease resembling besnoitiosis was reported in New Zealand lambs.

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Leishmaniasis

Members of the genus *Leishmania* are responsible for systemic disease in man, dogs, cats, horses, and other mammals. Members of the *L. donovani* complex cause most infections. This intracellular parasite of the mononuclear-phagocytic system is *transmitted to animals and man by blood sucking sandflies*. The parasite's life cycle and manifestations of visceral disease are discussed in Vol. 3, Hematopoietic system. This zoonotic disease is endemic in Mediterranean countries and in parts of Africa, India, and Central and South America. Past reports of the disease in North America primarily involved animals with a history of foreign travel, however endemic foci of leishmaniasis now exist in Texas, Oklahoma, and Ohio. Recent reports indicate cases of canine visceral leishmaniasis have been confirmed in 21 of the United States and in southern Canada. The geographic distribution of the disease can be expected to continue to rise with the advent of increased international travel and the existence of immunosuppressive viral infections and therapeutics.

Studies in mice and dogs indicate that resistance to infection is dependent upon a Th1-type of immune response, while susceptibility is associated with a Th2-type of immune response. The alopecic form of the cutaneous disease as described below in the dog has been shown to be associated with fewer organisms and a more appropriate cellular immune response in terms of number of antigen-presenting Langerhans cells, MHCII-positive keratinocytes and infiltrating T cells. In contrast, dogs with the nodular form lacked antigen-presenting cells and had more numerous macrophages containing large numbers of organisms. It has been suggested that the clinical and histologic lesions may be useful in establishing a prognosis for remission in that the character of the lesions that develops reflects epidermal immunocompetence. Leishmaniasis is often associated with concurrent dermatoses such as opportunistic infections, parasitism, autoimmune disease, or neoplasia. This association and documented peripheral blood lymphocyte subset abnormalities *suggest that leishmaniasis may induce immune dysfunction. Cytology, immunohistochemistry, PCR, and serologic testing can help confirm diagnosis of the disease.* Currently, there is no cure for canine leishmaniasis, however, remission is possible.

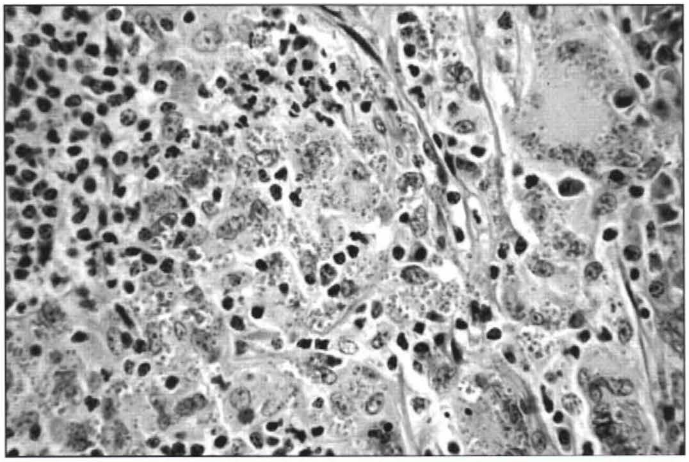

Figure 5.103 Leishmaniasis in a horse. Granulomatous inflammation. Macrophages and many multinucleate giant cells contain multiple *Leishmania* amastigotes.

The *skin* is one of the main organs affected in systemic leishmaniasis in **dogs**. Gross and histologic lesions are highly variable depending on host response and concurrent disease. Gross lesions may be alopecic, ulcerative, nodular or pustular. Onychogryposis and paronychia have also been reported. Alopecia with dry scaling on the head or entire body and multiple areas of ulceration of the skin of the head and limbs are seen most frequently. Initial lesions of alopecia are often periocular and referred to as *"lunettes."* Nonpainful, nonpruritic, variably ulcerated nodules varying from <1.0 cm to several cm in size may also be present but are less common. Nodules are most common on the ears, eyelids, and face but can be anywhere on the body. *Nodular mucosal leishmaniasis* affecting the oral cavity, tongue, nose, and penis has been reported in the dog. Unusual cases of leishmaniasis may present as a *generalized subcorneal pustular dermatitis*. Histologically, *the more common lesions consist of hyperkeratotic, nodular to diffuse superficial and deep, granulomatous dermatitis* (Fig. 5.103). Foci of inflammation may be perivascular, perifollicular, or interstitial in orientation. Perifollicular lesions frequently replace sebaceous glands. Some animals have infiltrates consisting primarily of large, foamy macrophages with numerous organisms and fewer plasma cells and lymphocytes while other animals have larger numbers of lymphocytes and plasma cells, indicating a more effective cellular immune response. Multinucleated giant cells may be present. The infiltrate in areas of ulceration includes neutrophils. *Leishmania* amastigotes are most commonly identified within macrophages, however they can occasionally be found within other leukocytes, endothelial cells or fibroblasts. In areas of necrosis, amastigotes may be free within the interstitium.

Clinical differentials for the skin lesions are numerous and vary with the types of lesions and include sarcoptic and demodectic mange, seborrhea, pemphigus foliaceus, systemic lupus erythematosus, bacterial infections, and superficial necrolytic dermatitis and zinc-responsive dermatitis, to name a few. The nodular form must be differentiated grossly from various cutaneous neoplasms, and grossly and histologically from infectious or sterile granulomas. *Leishmania* sp. *can be distinguished from other protozoa via light microscopy by recognition of the kinetoplast oriented perpendicular to the nucleus.*

Cutaneous leishmaniasis has been reported in **cats** in Europe and South America, and in a cat living in Texas; the cat was presented repeatedly with progressive, multiple, non-ulcerated cutaneous nodules on the ears, face, and nasal mucosa. Histologic lesions were described as dermal infiltrates of macrophages containing *Leishmania* amastigotes. No evidence of systemic infection was found at necropsy 7 years after the initial cutaneous lesions containing amastigotes were identified.

Cutaneous leishmaniasis has been reported in **horses, mules, and donkeys** in Europe, South America, and in a horse in North America and another in Puerto Rico. Lesions consist of crusted or ulcerated nodules on the pinna, head, and neck or less commonly the legs, scrotum and penis. Histologically, the dermis contains nodular to diffuse infiltrates of macrophages and lymphocytes or distinct granulomas with organisms identified in macrophages or free within the interstitium. Cutaneous lesions may not be associated with systemic infection.

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Miscellaneous coccidian parasites

Caryospora spp., apicomplexan parasites whose primary hosts are reptiles and raptors, rarely cause *pyogranulomatous dermatitis in puppies*. Immunosuppression and concurrent disease, such as canine distemper, likely play a facilitatory role. Lesions involve skin and draining lymph nodes and comprise diffuse pyogranulomatous dermatitis. Macrophages contain large numbers of intracellular organisms, including schizonts, gamonts, oocysts, and caryocysts. Caryocysts have a thin cyst wall enclosing the host cell nucleus and contain up to three sporozoites. Not all stages of the life cycle may be present in the tissue sections, precluding a microscopic diagnosis in some cases. Immunohistochemical studies identified the agent in one case as *C. bigenetica*. Experimental oral infection of immunosuppressed puppies with *C. bigenetica* induced typical skin lesions affecting muzzle, periocular skin, footpads, ears and abdomen within 10 days of inoculation.

Neospora caninum is a cyst-forming protozoal parasite of *dogs, cattle, sheep, goats, horses, and deer*. It is best known as a cause of bovine abortion capable of transplacental transmission. It typically causes systemic and neurologic disease. *N. caninum* is considered a primary pathogen in dogs with the majority of cases occurring as neurological disease in puppies. Cutaneous lesions occur in mature dogs often with underlying immunosuppression from drug therapy or concurrent disease. It is not known whether the disease in adult dogs results from a reactivated congenital infection or from a recently acquired infection. Lesions have been described as *multifocal-to-generalized, ulcerative and nodular dermatitis*. Histologically, there is pyogranulomatous and eosinophilic, to necrotizing and hemorrhagic, dermatitis. Numerous tachyzoites, 4–7 μm × 1.5–5 μm, may be seen in macrophages, keratinocytes, and neutrophils, and rarely in endothelial cells and fibroblasts. Tissue cysts are not present in the cutaneous lesions. Identification of *N. caninum* can be confirmed by immunohistochemistry.

An unidentified ***Sarcocystis*-like protozoan** was associated with *multiple cutaneous abscesses and disseminated visceral lesions in a dog*. The skin lesions were diffuse, necrotizing, hemorrhagic and suppurative. Large numbers of protozoa were present, primarily in macrophages and neutrophils, and occasionally in fibroblasts and endothelial cells. Some vessels contained thrombi and there was associated dermal and epidermal infarction. The organism did not stain with antisera to the other apicomplexan parasites so far identified as causing dermatitis in dogs, namely *Neospora caninum* and *Caryospora* sp.

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ALGAL DISEASES OF SKIN: PROTOTHECOSIS

Protothecosis is an uncommon disease caused by *Prototheca* spp. organisms, which are thought to be *colorless, nonphotosynthetic variants of the green alga Chlorella*. The organism is ubiquitous in organic matter and in fresh and marine waters. Reports of infection in humans and domestic and wild animals are worldwide, but the majority of cases are from the southeastern United States. The two most common species associated with infection are *Prototheca wickerhamii* and *P. zopfii*. *Mastitis in cows and disseminated disease in dogs are more common than cutaneous-subcutaneous infection*. In humans, the cutaneous form is the most common and is thought to be initiated by traumatic implantation, resulting in a protracted and indolent clinical course. *Cutaneous protothecosis has been reported in dogs and cats* from the USA, Australia, England, and Spain. In those cases in which the organism was speciated, *P. zopfii* is most often associated with disseminated disease, whereas the cutaneous form has been caused by *P. wickerhamii.*

Despite widespread distribution of *Prototheca* spp., *the prevalence of infection is very low* and attempts to reproduce infection experimentally have met with mixed success. This has led to speculation that the organism is an *opportunist with low pathogenicity* that requires an underlying immunologic dysfunction for the development of disease. Most humans with cutaneous protothecosis have a concurrent disease condition that may alter the immune response to the organism. Except in one dog with disseminated protothecosis, no immunologic deficit has been identified in animals with cutaneous or disseminated disease. However, there is evidence to suggest that infection may become established in an individual with minor immunosuppression and that the algae may further compromise the host immune response.

The cutaneous lesions are variable and consist of small crusty ulcerative lesions, multiple miliary nodules, firm raised nodules, or poorly demarcated subcutaneous swellings anywhere on the body. The tissue is soft and uniformly pale tan or white. Histologically, the masses consist of *diffuse infiltrates of primarily epithelioid macrophages* in the dermis and subcutis. Multinucleated giant cells, neutrophils, and lymphoid cells are in variable numbers, and foci of necrosis may be present. *Organisms are typically numerous but they are only lightly stained with H&E and therefore are poorly visualized.* The cell wall and internal contents are readily stained with fungal stains, e.g., Gridley (GF), Gomori methenamine silver (GMS), and periodic acid-Schiff (PAS). The organisms vary from 1.3–25 μm in diameter and consist of cells called *sporangia* that divide by internal cleavage to form multiple *endospores*. Identification of *Prototheca* can be made reliably by examination of tissue sections, but species identification usually requires culture or immunofluorescence methods because differences in the two species are subtle. *P. wickerhamii* sporangia are smaller and round in comparison to the larger and oval or cylindrical shape of most *P. zopfii. Protothecae must be differentiated from Chlorella algae*, whose natural green pigmentation is removed in fixation and routine tissue processing. In contrast to *Prototheca* cells, *Chlorella* sporangia contain many large starch granules that are stained by GF, GMS, and PAS.

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ARTHROPOD ECTOPARASITES

Of the parasitic arthropods, only small a fraction are parasites of domestic animals, but the harmfulness of these is quite out of proportion to their number. Some, such as the mites, are pathogens in their own right, but *the majority of them owe their immense importance to their ability to act as mechanical or biological transmitters for many pathogenic viruses, bacteria, protozoa and helminths.* Their role as vectors is discussed in relation to the specific diseases throughout these volumes.

The parasites of concern to us here belong to the two large classes, Insecta and Arachnida. The class Insecta contains four important orders, Diptera (*flies*), Siphonaptera (*fleas*), Mallophaga (*biting lice*) and Siphunculata (*sucking lice*). The class Arachnida contains the order, Acarina, in which *ticks and mites* are classified. For information on biological characters and classification, reference should be made to texts on entomology.

Many **arthropod bites or stings** go unreported and are of minimal consequence. The type and number of arthropods inflicting the bite or sting and the individual host response determine the severity of the injury. In general, most arthropod bites initially appear as circular, erythematous lesions, 0.5–2.0 cm in diameter. Lesions may progress to focal areas of necrosis with ulceration, alopecia and crust formation. Histologically, the area of necrosis and inflammation may have a triangular outline with one point of the triangle in the deep dermis or panniculus. In early lesions, the inflammation is perivascular to diffuse and includes variable numbers of eosinophils, neutrophils, lymphocytes, and macrophages. Edema and hemorrhage may be present. As the lesion ages, the area may become nodular to form "*arthropod-bite granuloma*" comprised of macrophages, lymphocytes, mast cells, eosinophils and plasma cells. Lymphoid follicles may form. More specific details concerning injury inflicted by various arthropods, if known, are discussed in the following sections.

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Flies

Flies belong to the insect order, Diptera. Different species have various degrees of adaptation to a parasitic existence. Adult flies feed on blood, sweat, tears, saliva, feces, urine, and other body secretions. Non-biting, nuisance flies accomplish this by feeding only at the body surface on wounds or natural body orifices, whereas biting flies puncture the skin to feed. *Musca* are facultative feeders. Some, such as the Simuliidae and parasitic species of Culicidae and Ceratopogonidae, are obligate bloodsuckers, although usually only the females draw blood. At the other end of the spectrum are the Oestridae, whose larvae are obligate parasites, and some members of the Hippoboscidae that are obligate parasites in the adult stage.

Because of the variety of parasitic modes, it is not possible to generalize on the effects of flies on domestic animals nor, with the exception of a few obligate parasites, is it possible to be specific because there is little information available on primary pathogenicity. Flies adversely affect domestic animals by *causing annoyance*, by *direct toxicity* that may be fatal following massive insect attack, by *indirect toxicity* due to the deposition of larva into damaged skin (myiasis), by *local irritant effects* causing dermatitis that may predispose to secondary bacterial infection or to myiasis, by *injection of antigens* that induce hypersensitivity reactions, by *blood-feeding activities* that cause anemia, and by the biological or mechanical *transmission of other pathogens*. It has been estimated that tabanid flies could transmit 35 pathogens including *Equine infectious anemia virus* and trypanosomes. *Musca* spp. have been implicated in the mechanical transmission of anthrax, mastitis, and conjunctivitis in animals. *Stomoxys calcitrans*, the stable fly, is thought to be the primary transmitter of habronemiasis in horses.

Animal annoyance, so-called "*fly-worry*," is an important source of economic loss to the cattle, sheep and, to a lesser extent, swine industries. Fly worry refers to the behavioral disturbances in animals brought about by the attempted feeding of flies. Biting flies inflict pain whereas non-biting flies cause annoyance by clustering around the eyes and nostrils where they feed on lacrimal or nasal secretions (*Musca autumnalis* and *Hydrotaea irritans*) or by other means such as simulating the sound of a bumble bee (*Hypoderma* spp.). Fly-worry occasionally induces such apprehension that the animals run aimlessly ("*gadding*"), and severe injury or death may result from misadventure. Deaths are usually sporadic but high levels of mortality have been reported. Of much greater economic importance is the *loss of production* associated with fly worry. When the insects are numerous, they cause very considerable annoyance to livestock, interfere with feeding and resting and cause reduced milk production and reduced weight gain. Fly worry has been attributed to biting flies such as the horn fly (*Haematobia (Lyperosia) irritans*), the stable fly (*Stomoxys calcitrans*), and horse flies (several genera in the family Tabanidae), and to non-biting species such as house flies (*Musca* spp.), *Hypoderma* spp. and the sheep-head fly (*Hydrotaea irritans*). *Culicoides* spp. biting midges can cause pruritus and restlessness in horses. Horses that develop a hypersensitivity response to *Culicoides* spp. insect bites may suffer weight loss as a result of severe pruritus and irritation (see Immune-mediated dermatoses).

Mortality may arise from direct toxic effect as well as from misadventure. Death may be the result of urticarial swelling of the head and neck or of shock. Many flies are attracted to exhaled carbon dioxide and can occasionally cause *death by suffocation* of cattle, horses, or other animals when large numbers of flies are inhaled. Mosquitoes, especially aggressive species such as *Aedes vigilex*, may cause significant mortality amongst piglets and puppies. The Simuliidae (black flies or buffalo gnats) are responsible for massive animal mortalities, particularly in temperate latitudes and river valleys following extensive flooding when the insect population expands. They feed on cattle, horses, sheep, goats, poultry, wild mammals and birds. *Simuliid flies exert systemic effects through inoculation of a heat-stable toxin*, which causes increased vascular permeability and abnormalities in cardiorespiratory function, which may cause death.

The hematophagous flies seldom cause serious loss of blood, however anemia may result from heavy infestations by *Haematobia irritans*, mosquitoes, the sheep ked *Melophagus ovinus* and *Stomoxys calcitrans*, which may ingest as much as 16 mg blood per feeding.

In dogs, stable flies (*Stomoxys calcitrans*) typically attack the face and tips or folded edges of the pinnae producing erythematous to hemorrhagic dermatitis with variable pruritus. *Simuliidae* spp. can also feed on small animals. Lesions are most often found on the head, ears, legs, or hairless areas of the abdomen and appear as papules, ulcers, or circumscribed areas of necrosis. Occasionally annular macular lesions with a central puncture surrounded by an edematous zone with a peripheral erythematous rim may be evident.

Local irritant effect results from *injection of salivary fluids into the host*. Very little is known of the nature of the cutaneous lesions produced in animals by these insects. The character and severity of the local lesions vary. Pruritus is often intense, resulting in secondary traumatic lesions. The primary lesions are usually erythematous papules or wheals often surrounding a central bleeding point (mosquitoes) or small puncture wounds (biting flies). The wheals are usually transient but may persist for several weeks. The puncture wounds often develop an exudative crust. Some flies (Simuliidae) feed by lacerating the skin until a pool of blood forms on the surface from which they feed. Histologically, there may be intraepithelial eosinophilic spongiform pustules or focal areas of epidermal necrosis indicating the penetration point. The dermal reaction is superficial perivascular in pattern and contains predominantly eosinophils, lymphocytes and plasma cells. Occasionally there is acute necrosis of the surface of the papules, including both dermis and epidermis.

The injected salivary substances are irritant and many are allergenic, and hypersensitivity reactions probably contribute to the severity of the local lesions caused by a variety of biting flies. Hypersensitivity reactions to *S. calcitrans* are recognized in cattle; affected animals develop coalescing blisters on the forelimbs. An important allergic dermatitis of horses is caused by hypersensitivity to *Culicoides* spp.

Rather more important than the bloodsucking or biting flies are those species whose larvae are highly destructive facultative or obligate parasites. Infestations with such larvae cause *myiasis*, which is discussed below.

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Myiasis

Myiasis is the infestation of the tissue of living animals with the larvae of dipterous flies. The larvae are referred to as *maggots* or *grubs* and may be facultative or obligate parasites. The important families are Cuterebridae, Sarcophagidae (*Wohlfahrtia* spp.), Gasterophilidae (stomach bots of horses), Oestridae (nasal bots, warbles), and Calliphoridae (blowflies). Only those flies whose larvae cause cutaneous or subcutaneous lesions are discussed here. Nasal and stomach bots are described elsewhere. Agents of facultative myiasis affecting the skin live in decaying organic matter, and the females oviposit in wounded, infected, or heavily soiled skin of warm-blooded vertebrate hosts. The larvae feed on host tissues and eventually drop to the

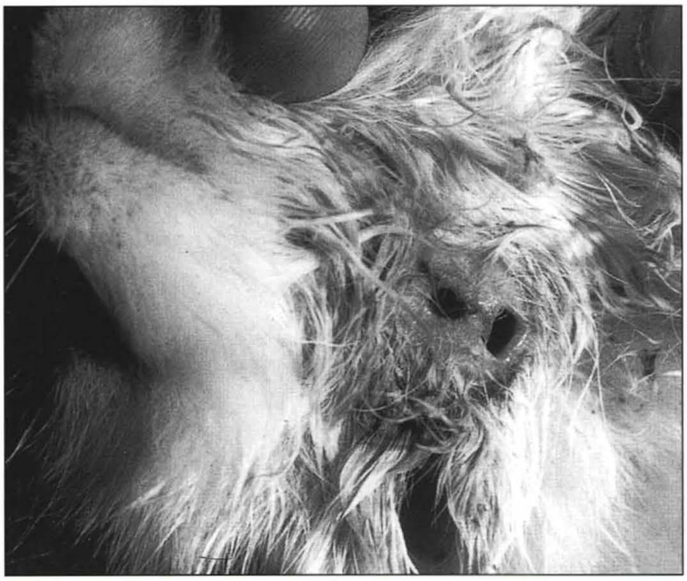

Figure 5.104 ***Cuterebra* infestation** with draining lesions in a cat.

ground to pupate. *Cochliomyia hominovorax,* the screwworm fly, produces larvae that are obligatory parasites and are discussed separately.

Cuterebra

The larvae of ***Cuterebra*** spp. (Diptera; Family: Oestridae; sub-family: Cuterebrinae) are obligate parasites of rodents and rabbits but occasionally aberrant infestations occur in cats and rarely in dogs, pigs, and humans. Larvae attach to the host's fur and either enter via ingestion through grooming, direct skin penetration or through natural orifices to migrate to the subcutaneous tissues to produce an initially firm, then fluctuant, cyst-like subcutaneous abscess in which the larvae mature. The larvae breathe through a fistulous pore in the skin through which they are visible, and feed off tissue debris. Wounds heal slowly after larvae are removed or released and secondary bacterial infection may occur. The majority of infections occur in late summer or fall. *In cats, the larvae have a predilection for the neck area* (Fig. 5.104), often over the submandibular salivary gland, but swellings also occur in the scrotal region. Larvae may locate in aberrant locations such as the pharynx, nasal cavity, eye, and brain.

In South and Central America, ***Dermatobia hominis*** (Diptera; Oestridae; sub-family: Cuterebrinae) affects cattle, sheep, goats, pigs and people and, rarely dogs and cats. It is also known as the *human bot fly*. The adult fly captures another carrier insect to which it attaches its eggs. The eggs hatch when the carrier insect visits a host. The larvae then attach to the host and penetrate the skin to form local cutaneous nodules that can be pruritic. The mature larvae exit the nodule through holes that leave the host susceptible to fly strike. A case report of an infected dog from the Netherlands described lesions as painful, erythematous, exudative nodules with a central pore.

Wohlfahrtia

Wohlfahrtia magnifica (Diptera; Superfamily: Oestoidea; Family: Sarcophagidae) is an *obligate larval parasite of warm-blooded vertebrates* in the Mediterranean basin, eastern and central Europe and Asia minor. The female fly deposits larvae on the host near body orifices or in wounds. Larvae mature and drop to the ground to pupate in 5–7 days. *W. magnifica* causes myiasis in sheep, camels, poultry and to a lesser extent in cattle, horses, pigs, and dogs. Fecal soiling in sheep is a predisposing condition. *W. vigil* is a parasite of mink, foxes, rabbits, and occasionally dogs and cats in North America. The larvae can penetrate the tender skin of young animals, hence the young are most often affected. *W. nubia* is a secondary facultative invader of wounds in camels in North Africa and the Middle East.

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Warbles

Warbles caused by ***Hypoderma bovis*** and ***Hypoderma lineatum*** (Diptera; Family: Oestridae; sub-family: Hypodermatinae) *occur chiefly in cattle*, although the parasite is not host specific; horses, sheep and humans are affected occasionally. *H. bovis, H. lineatum*, and *H. sinense* are present in Europe and Asia. Intensive eradication programs have eliminated *Hypoderma* spp. from the UK and Ireland and have greatly reduced their populations in much of the USA and Canada. *Przhevalskiana silensus* affects sheep and goats in Asia and Eastern Europe. *H. diana* is an important parasite of deer and possibly sheep in the Palaearctic. *H. tarandi* parasitizes reindeer in northern Eurasia and North America. There are no warble flies in Australia.

Warble flies are also known as heel flies because the eggs are deposited predominantly on the hair of the legs. Larvae emerge 4–6 days later and burrow directly into the skin or into hair follicles causing minimal irritation. Larvae migrate along fascial planes leaving tracks of green gelatinous material known as "butcher's jelly." The first instar larvae of *H. bovis* overwinter in the epidural fat, whereas those of *H. lineatum* develop in the esophageal submucosa. In the esophageal lesions, collagen bundles around the first instar larvae of *H. lineatum* appear fragmented, as if undergoing enzymatic digestion. A collagenase has been isolated from *H. lineatum*. In the epidural lesions of *H. bovis*, it is the fat tissue that appears necrotic. In the spring, the larvae migrate dorsally to the subcutaneous tissue of the back to form subcutaneous nodules ~3 cm diameter with a central pore for respiration. The lesions, which are known as "warbles," last for 4–6 weeks, during which the larvae undergo 2 molts. The mature third-instar larvae emerge from the breathing hole and pupate in the soil. In horses, the lesions occur in the saddle region and are often "blind" in that the larvae do not complete their development. Fatalities resulting from aberrant migration into the central nervous system are reported in horses.

Histologically, the cellular reaction is predominantly eosinophilic and lymphocytic. It is the eosinophilic infiltrate that gives "butcher's jelly" its green coloration. However, the most intense inflammatory reactions occur at sites of previous migration rather than around the viable larvae, suggesting that in naive hosts the parasites depress any effective host responses. Proteinases with the capacity to cleave the third component of bovine complement have been isolated from the first instar larvae of *H. lineatum*. Such enzymes could well ablate the host's inflammatory responses. The actual "warble" is lined by a wall of granulation tissue that matures to form a connective tissue capsule in which lie islands of eosinophils. The cystic cavity between the cuticle of the parasite and the granulation tissue fills with fibrin and a few inflammatory cells, chiefly eosinophils. Cuticle sloughed during ecdysis, or remnants of dead larva, incite a marked foreign body giant cell reaction. Once the larvae emerge, the cavity is repaired by fibrosis, but small foreign body granulomas may persist for months.

Warbles are economically important. The buzzing of the adult *H. bovis* (*H. lineatum* is silent) disturbs cattle causing considerable loss in milk and meat production. Larval tracks in the tissues decrease carcass value and the larval-induced holes markedly depreciate the value of the hide. Larval rupture, either accidental or deliberate, may induce a fatal anaphylactic reaction. This may result from systemic effects of the warble toxin, from type I hypersensitivity reactions, or a combination of both.

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Calliphorine myiasis

This occurs in all animal species but is *most common in sheep*, particularly in Australia where it is of major economic importance. The flies involved are members of the subfamily Calliphoridae (**blowflies**). Important blowfly genera are *Lucilia, Calliphora, Protophormia, Phormia* and *Chrysomia*. The larvae (*maggots*) adopt a *facultative parasitic mode*, which is an adaptation of their beneficial and important role in the breakdown of carrion. Adult flies lay eggs on moist, warm skin of weakened or debilitated animals, in wounds or areas of heavy soiling with feces, urine or other body fluids. Hence, any species of animal can be susceptible.

Moisture, whether provided by rain, dew, urine, sweat or inflammatory exudate, predisposes to bacterial proliferation. These bacteria are often of fecal or urinary origin. The odor induced by the bacterial proliferation and resultant inflammatory exudate attracts the primary flies, which deposit batches of 50–200 ova. The larvae of primary flies emerge within 12–24 hours and grow rapidly, feeding on inflammatory exudates. *The primary larvae secrete proteolytic enzymes*, including collagenases, which liquefy the host tissues and provide predigested nutrients. The *cutaneous necrosis* that results attracts the secondary flies to oviposit. The resulting larvae *tunnel into the adjacent viable tissue* and markedly expand the lesion. The putrefactive odor attracts more flies, and the process is further exacerbated. The lesions of fly strike are often extensive and *leave large areas of undermined skin with punched-out holes*. The subcutaneous tissues may become cavitated. Muscle may be destroyed and body cavities invaded. Lesions may result in death from *shock, debilitation, toxemia or bacterial septicemia*.

The species of fly involved in fly strike differs with geographic location: *Lucilia cuprina* is the most important primary fly in Australia, *Phormia regina* in USA and Canada, and *Lucilia sericata* in Great Britain. Several different species of flies are involved in the development of the lesion of cutaneous myiasis. Primary flies, such as *L. cuprina*, are capable of initiating a strike on living sheep. Secondary flies, such as *Chrysomia rufifaces*, are not able to initiate a strike, but greatly exacerbate the lesions initiated by the primary fly. They may also displace the maggots developing from the eggs laid by the primary fly. Tertiary flies, such as the housefly *Musca domestica*, attack at a later stage and do not contribute significantly to the skin damage.

The development of fly strike in sheep depends upon abundance of primary flies, susceptible sheep, and moisture. The prevalence of the disease tends to follow the rise and fall in the population of primary flies, which in turn depends upon the climatic zone. In general, the flies require warm and moist but not hot conditions. Thus there is usually a double wave of primary flies, peaking in the spring and autumn. Certain breeds of sheep, in particular the fine-wooled Merino, have an inherent predisposition to attract fly strike. The character of the fleece and conformational features, such as skin wrinkling, allow retention of moisture or predispose to fecal or urinary soiling which are all initiating factors in fly strike. *Pseudomonas aeruginosa* (of "fleece rot") proliferation in soiled wool of sheep is of major importance. *Dermatophilus congolensis* may be involved occasionally.

In sheep, the lesions are most common in the perineum ("*breech strike*"), particularly in sheep with a narrow conformation and/or marked skin wrinkling which favor urine or fecal soiling. Lesions may affect the preputial orifice ("*pizzle strike*") particularly in animals with narrow urethral orifices, which predispose to urine soiling. Rams with deep head folds may develop "*poll strike*," possibly predisposed to by fight wounds. "*Wound strike*" occasionally follows castration or tail docking and "*body strike*" follows prolonged wetting, which in turn predisposes to "fleece rot" or dermatophilosis.

The initial *gross lesion* is a patch of dark-brown, moist wool, which has a foul smell. The wool is often very hot as a result of putrefaction and inflammation caused by the larvae. As the process advances, the maggots burrow under the skin causing irregular ulcers with scalloped edges. The lesions are irritating and pruritic. Occasionally the maggots migrate deeper into the muscle.

The economic losses of fly strike in sheep result from death, disfigurement, and depreciation of the fleece and costs associated with prevention and treatment.

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Screwworm myiasis

Members of the Calliphoridae also cause myiasis; however, they differ from the blowflies in that *screwworm fly larvae are obligate parasites, invading edges of fresh, uncontaminated wounds on live animals*. The American species of screwworm flies are ***Cochliomyia hominivorax*** and ***Cochliomyia macellaria*** (the secondary screwworm fly), which is usually a carrion breeder but can act as a secondary invader of fly strikes. The African and Asian screwworm fly is *Chrysomyia bezziana*. The disease occurs in Africa, Asia, Central and South America and Mexico but has been virtually eradicated from the USA and Mexico, following a program in which release of massive numbers of irradiated male flies rendered the annual breeding a sterile one. An outbreak of screwworm in Libya in 1988, seen as a major threat to the livestock of southern Europe and Africa, was dealt with in a similar fashion. International air travel proffers a source of spread to susceptible countries.

Screwworm myiasis affects all domestic animals and humans and is an important cause of mortality in wildlife. The flies oviposit in cutaneous wounds, such as those caused by castration, dehorning, branding or accidental injuries. The navel of neonatal calves, the perineum of recently calved cows and tick bites are also favorable sites for oviposition. The larvae feed in groups, to penetrate and liquefy fresh, live host tissue with the aid of proteolytic enzymes. Blood-stained fluid, often containing incompletely digested shreds of tissue, oozes from the wound, which contains clusters of voraciously feeding larvae. A distinctive and particularly evil odor emanates from the lesion. The lesions are extremely painful and may expand rapidly, leading to death in untreated animals. Screwworm infestation is a reportable condition in many countries.

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Sheep ked infestation

Melophagus ovinus (Diptera: Hippoboscidae) *is a wingless fly that causes a chronic, pruritic dermatitis of sheep*. Goats are also affected. Of worldwide distribution, the disease's chief economic importance is the associated *loss of wool production*.

Melophagus ovinus is an obligate ectoparasite. The eggs develop into larvae within the female until they are ready to pupate. After parturition, the female attaches its larva to wool fibers with the aid of a sticky substance. The immotile larva transforms into a chestnut-brown pupa ~3–4 mm long. The pupal stage lasts 3–5 weeks and the adult keds live 4–5 months. They prefer the sides of the neck and body and are difficult to detect in fully-fleeced animals. The adults feed actively on blood. While anemia may develop in severe ked infestations, the more significant lesions are the result of the *severe pruritus*, which causes the sheep to rub and bite, and thus damage the fleece. The adult fly's excreta stains the wool, further reducing its value. Wool loss and vertical ridging of the skin leads to a condition referred to as "cockle." The irritation induced by the bites also affects weight gain. The US sheep industry has estimated losses attributed to sheep keds to be about $40 million per year.

Histologic lesions reported are superficial and deep perivascular dermatitis with eosinophils and lymphocytes predominating. Fibrinoid necrosis of small arterioles is also described.

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Hornfly dermatitis

While mainly an obligate parasite of cattle, the **horn fly (*Haematobia irritans*)** is one cause of *seasonal ventral midline dermatitis in the horse*. In **cattle**, the flies feed in groups primarily on the back, withers, and head. They leave the animal only briefly to mate and lay eggs. Horn flies require fresh bovine feces to lay eggs. Large numbers of horn flies in cattle can result in significant loss of blood, wounds that attract other flies, and in loss of production. *H. irritans* is also thought to transmit the skin parasitic nematode of cattle, *Stephanofilaria stilesi*.

In **horses**, horn flies, cluster on the ventral abdomen (and occasionally on the neck or periocular region), producing bites marked by tiny drops of dried blood. A few days later pruritic, scaling, alopecic patches develop. These become lichenified and heal with either leukoderma or melanosis. The lesions are often single, usually well-circumscribed and occur near the umbilicus. Histologically, lesions are perivascular and eosinophilic, typical of many insect bite-induced dermatopathies. If ulcerated, lesions predispose to infection by *Habronema* spp. nematodes. Lesions of *Culicoides* hypersensitivity (see Immune-mediated dermatoses) and onchocerciasis may also occur on the ventrum, but these are diffuse, often extending from the axillae to the groin. Both *Culicoides* hypersensitivity and onchocerciasis are sporadic diseases whereas up to 80% of horses in a group may be affected with horn-fly bite dermatitis.

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Mosquito-bite dermatitis

Mosquito bites in animals are common and the bite itself is of little consequence most of the time. Mosquitoes serve as *vectors* for a number of important diseases, including malaria in humans, canine and feline heartworm disease, equine viral encephalitis and equine infectious anemia, and rabbit myxomatosis. In **cats**, mosquito bites can induce a severe papular, crusting dermatitis characterized by dense eosinophilic infiltrates. Experimental studies in cats utilizing intradermal skin tests and Prausnitz–Kustner tests indicate that these lesions develop only in cats hypersensitive to mosquito bite antigens and are initiated by a type I hypersensitivity reaction. The disease is seasonal and often pruritic. Clinically, cats initially develop wheals progressing to erythematous papules and plaques that eventuate into crusted, ulcerated and sometimes hypopigmented lesions (Fig. 5.105). Sparsely haired regions of the body are most often affected such as the bridge of the nose, the pinnae, and footpad margins. The pinnae may develop symmetrical lesions of miliary dermatitis. The severity of lesions varies by individual, leading to scar formation in the more severe cases.

Histologic lesions include intraepidermal eosinophilic microabscessation and perivascular to diffuse interstitial infiltrates of eosinophils, mast cells, macrophages and lymphocytes. The epidermis is often spongiotic and acanthotic. Eosinophilic granulomas around focal areas of collagen degeneration, and perifollicular to intrafollicular eosinophilic infiltrates with furunculosis, may be present. Cats may have regional lymphadenopathy and peripheral eosinophilia. *Clinical differential diagnoses* include lesions of the eosinophilic granuloma complex, other cutaneous hypersensitivities such as atopy or food allergy, actinic dermatitis or squamous cell carcinoma, and pemphigus foliaceus. *Histologic differentials* include *Felid herpesvirus-1*-associated dermatitis, other cutaneous hypersensitivities mentioned above, and feline eosinophilic granulomas. Eosinophilic folliculitis and furunculosis are not usually seen in feline eosinophilic granulomas and are less prominent in food allergy than in mosquito bite hypersensitivity. Ballooning degeneration, viral inclusions, and marked necrosis are usually present in herpesvirus-induced lesions. Careful evaluation of histologic lesions and circumstantial evidence are essential keys to the diagnosis.

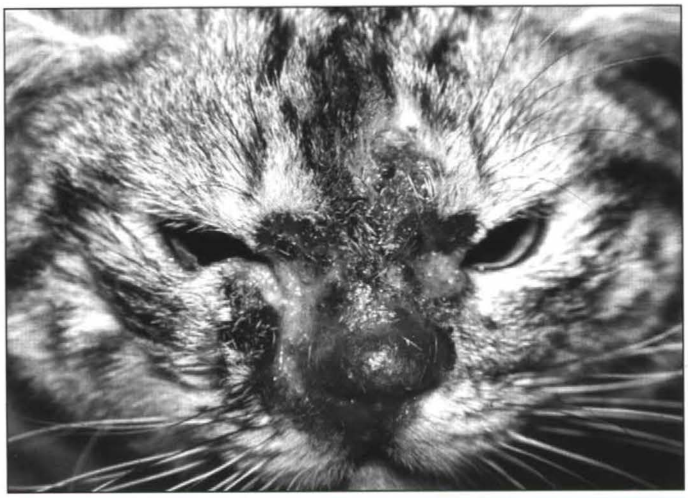

Figure 5.105 Mosquito-bite dermatitis in a cat. (Courtesy of B Dunstan.)

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Lice

Lice are host specific, obligate parasites of the class Insecta. Two orders of lice are recognized. The Mallophaga are *biting lice,* which have mouth parts specially adapted for chewing the epithelial scales, feathers, and sebaceous secretions of birds and mammals. The Anoplura have piercing mouth parts and are the *blood-sucking lice* of mammals. Lice cannot live away from their hosts for more than a few days. Consequently, spread of infestation occurs mainly by direct contact among hosts. Pigs and humans are parasitized only by sucking lice, birds and cats are parasitized only by biting lice, and both types parasitize other domestic animals. Because various species of lice have adapted to different microenvironments within the host pelage, it is possible for an animal to carry several species at once.

Infestation with lice is called **pediculosis**. It tends to be a seasonal problem, being worse in winter. The signs associated with pediculosis are extremely variable. In most instances, lice do not pose a significant threat to the host. *Heavy infestations signal an underlying contributing condition such as poor sanitation, overcrowding, ill thrift, or poor nutrition.* Low infestations may be unaccompanied by clinical signs in carrier animals. *Most lesions result from skin irritation and resultant pruritus.* They include alopecia, papules, crusts, and damage to wool or hide caused by rubbing or biting. *Sucking lice may induce anemia,* which is occasionally fatal in heavily infested animals. Weight loss and decreased milk production are associated with the constant irritation seen in some lice infestations.

Lice are almost always host specific, but *Heterodoxus longitarsus*, normally parasitic on kangaroos, has become an important ectoparasite of Australian dogs. *Phthirus pubis*, the human crab louse, has been reported to infest dogs living with infested humans. Because of host specificity, it is advantageous to consider pediculosis of the different hosts in turn.

Louse infestation occurs in **cattle** more often than in other domestic species. *Haematopinus eurysternus*, the short-nosed louse; *H. quadripertusus*, the tail-switch louse; *H. tuberculatis*, the buffalo louse; *Linognathus vituli*, the long-nosed louse; and *Solenoptes capillatus* are sucking lice of cattle. *Damalinia bovis* is the one biting species. The various species have preferred habitats. *Damalinia bovis* tend to cluster about the poll, forehead, neck, back, and rump; *Linognathus* and *Solenoptes* prefer the head, neck, and dewlap. There may be antagonism between the species; in dual infestation with *D. bovis* and *L. vituli*, the former tends to occupy the dorsal half and the latter the ventral areas. Some lice are widely distributed, others tend to cluster in groups. Infestations are quite common, particularly in colder weather or seasons, but, unless heavy, are not particularly deleterious. Poorly fed, overcrowded, and unthrifty animals are more susceptible to heavy infestations. Conversely, heavy infestations may indicate underlying disease as debilitated or ill animals cease normal grooming activities. Lesions reflect pruritus. Heavy periorbital infestation of heifers by *Haematopinus quadripertusus* has caused keratoconjunctivitis and periorbital papillomatosis. Bovine pediculosis usually has little deleterious effect on weight gain and other production parameters. Economic consequences are due to deterioration of hide quality, damage to fences, and costs of treatment. The exception is *Hematopinus eurysternus* infestation, which may cause anemia and death in some uniquely susceptible cattle. *H. eurysternus* feed most often around the poll, neck, brisket, and tail but may become generalized.

The species of sucking lice affecting **sheep** include *Linognathus ovillus* (the face or blue louse), *L. africanus* (also called the blue louse) and *L. pedalis* (the foot louse). *L. ovillus* is not very pathogenic. *L. pedalis* characteristically infests the hairy skin on the legs. Infestations are frequently light and may be confined to one limb, as the lice form localized clusters. Rarely, heavy infestations spread to adjacent scrotal or abdominal skin, causing irritation and dermatitis secondary to self-trauma. While goat and sheep lice are considered host specific, there are reports of naturally occurring transmission of the goat louse *Damalinia caprae* to sheep and experimental transmission of *D. ovis* to goats.

Damalinia ovis, a biting louse, is a common and serious ectoparasite in sheep. Populations of *D. ovis*, on an individual or in a flock, can build up very quickly. The numbers of lice fluctuate with the season, increasing usually in late winter and early summer. The population declines in the summer probably because the lice, which are sensitive to heat and low humidity, cannot survive in the body fleece where temperatures at the tip of the staple may reach 48°C. There are also unexplained individual differences amongst sheep in their susceptibility but, in general, unthrifty sheep carry the heaviest infestation. The size of louse populations may be in part regulated by the sheep's immune response. The lice feed on loose skin scales and sebaceous secretion and there is a correlation between the degree of scurf and the size of the louse population. The highest concentration of lice occurs along the dorsal midline, chiefly over the withers, but the parasites range over the entire body. Eggs are attached to the wool fibers close to the body surface. The infestation has serious consequences because of the *marked pruritus* it induces. The cause of the pruritus is unknown but is thought to be more than simple mechanical irritation. Affected sheep rub, scratch and bite at the skin resulting in severe damage to the fleece. Focal crusting may be associated with some louse infestations. Economic loss is due to reduced fleece quality, cost of prevention, and mortality from secondary myiasis. There is, however, no evidence to support the claim that sheep louse infestation leads to unthriftiness.

In **goats**, the sucking louse, *Linognathus stenopsis*, is more pathogenic than the biting lice, *Damalinia caprae*, *D. limbata* and *Holokartikos crassipes*. The haircoat of Angora goats may be seriously damaged by the irritation induced by pediculosis.

Two species of lice occur on **horses**, namely *Haematopinus asini*, a sucking louse, and *Damalinia equi*, a biting louse. The populations of lice fluctuate considerably, being highest when the hair is long as in winter or in debilitated animals which have not shed their hair. In warm weather, the populations decline but some lice persist in the long hair of the mane and tail. Both species of louse induce skin irritation. The lesions of pediculosis, rough coat, variable alopecia and self-excoriation, result from the animal rubbing or biting at the irritated areas.

One species, *Haematopinus suis*, a sucking louse is parasitic on **pigs**. Preferential sites include the ears and skin folds of the neck, axillae and inguinal areas. Infestations are often severe but, with the exception of nursing piglets, anemia is not severe. In white-skinned animals, numerous small puncta may be seen, especially in scalded carcasses. Constant irritation from lice also interferes with growth rate and efficiency of food conversion. The lice are vectors for *Swinepox virus*, *African swine fever virus*, and *Eperythrozoon suis*.

Linognathus setosus is a sucking louse and *Trichodectes canis* and *Heterodoxus spiniger* are biting lice of **dogs**. *Trichodectes canis* may serve as an intermediate host for the tapeworm, *Dipylidium caninum*. Pediculosis is a rare disease in pet dogs. Breeds with moderately long, fine hair may provide a more favorable environment for lice, and the disease is more prevalent in the cooler winter months. The biting lice cause pruritus, which may be associated with mild to moderately severe dermatitis with papules and crusts or with patchy alopecia. Infestation in the absence of pruritus may be an incidental finding.

Only one species, the biting louse, *Felicola subrostratus*, occurs on **cats**. Infestation may be an incidental finding or it may be associated with mild pruritus in the absence of lesions. Occasionally there is generalized scaling (seborrhea sicca) or multifocal or generalized papular, crusting dermatitis.

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Fleas

Fleas are ubiquitous and obligate parasites. They are intermittent parasites and their survival depends upon temporary episodes of feeding and a habitat where the host is periodically available. Fleas primarily parasitize hosts that return on a regular basis to a nest, burrow, bedding, or lair. Hence, animals such as ungulates rarely have fleas and carnivores, rabbits, rodents, and bats often do. Most fleas can parasitize a range of hosts. An exception to this general rule, is the more recent finding that cat fleas spend more of their lifetime on the cat than fleas infesting other species. Fleas are chiefly a problem in cats, dogs, pigs, and humans. Flea bites damage the host from the irritation, pruritus, blood loss, and possible transmission of infectious agents. *Ctenocephalides felis*, the cat flea, *Ctenocephalides canis*, the dog flea, and *Pulex irritans*, the human flea, are vectors for the dog tapeworm, *Dipylidium caninum*. Fleas are also vectors of tularemia, bubonic plague, and rabbit myxomatosis. Flea saliva is injected into the host as the flea feeds leading to hypersensitivity reactions in some animals.

Fleas are the most common ectoparasites of **cats** and **dogs**. The most important species are *Ctenocephalides felis*, the cat flea, and *Ctenocephalides canis*, the dog flea. However, infestations also occur with *Pulex irritans* (human flea), *Leptopsylla segnis* (rat flea), *Echidnophaga gallinacea* (chicken stick-tight flea), *Spilopsyllus cuniculi* (European rabbit flea), and *Ceratophyllus* spp. (bird and hedgehog fleas). *C. felis* is the most common flea found on both dogs and cats in North America and northern Europe.

The clinical manifestations of flea infestation are highly variable. Some animals, despite heavy infestations, remain asymptomatic carriers. Some animals may develop **flea bite dermatitis**, which is a reaction to the many irritant substances in the flea's saliva, but the vast majority of animals that develop lesions do so because of hypersensitivity reactions to allergenic components of the flea saliva. **Flea allergy dermatitis** is an extremely common and very important disease of the dog and cat; it is discussed in detail under Immune-mediated dermatoses. Finally the blood-sucking activities of fleas may induce blood-loss, iron deficiency anemia in heavily infested animals, particularly in kittens, puppies or debilitated adults.

Lesions affecting the pinna of cats may be caused by *Spilopsyllus cuniculi*, the flea of rabbits and hares. Typically, hunting cats acquire the infestation from their prey. Macroscopic lesions are crusted, alopecic patches on both aspects of the pinna. Histologically, eosinophils are prominent in the dermal inflammatory cell infiltrate.

The two fleas most commonly associated with **swine** are the human flea (*P. irritans*) and the chicken stick-tight flea (*E. gallinacea*). Infestation with *C. felis* and *C. canis* have also been reported. In Africa, *Tunga penetrans*, the chigoe flea, has been associated with swine infestations although it is chiefly a human parasite. The female flea burrows into the skin causing ulcerative lesions. The skin around the coronary band, on the scrotum and snout are favored sites. Infestations of the teat canal have been associated with agalactia in sows. Fleas may act as vectors for *Swinepox virus*. Heavy infestations with *Ctenocephalides* spp. in Africa lead to anemia, reduced weight gain and even death in **sheep** and **goats**, particularly in the young. Fleas may also trigger allergic dermatitis in sheep. *C. felis* has been rarely reported in **cattle**. Heavy infestation with *C. felis* was reported to cause mortality in calves, lambs and kids in Israel. Rarely, **horses** may become infested with *Echidnophaga gallinacea* or *Tunga penetrans*. Papules, crusts, pruritus, and alopecia may develop.

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Mites

Sarcoptic mange

Sarcoptes scabiei (Acarina: Sarcoptidae) is responsible for scabies in man and sarcoptic mange in domestic animals worldwide. Scabies has also been reported to cause severe disease in a number of wildlife species. *It is a common ectoparasite in swine.* The disease occurs in *cattle* and goats but is not as important as psoroptic mange. The disease is rare in horses and sheep. Ovine sarcoptic mange has not been reported in North America. Sarcoptic mange is *common in the dog* and may be underdiagnosed. It occurs rarely in the cat. So-called feline scabies is caused by *Notoedres*. The economic importance of sarcoptic mange in food-producing animals is due to depressed growth rate and decreased rates of food conversion. Production studies in experimentally infected pigs and sheep demonstrating the effect of therapy, support the contention that *the disease is of economic significance*. Sarcoptic mange is a notifiable disease in many countries.

Through host-adaptation, *S. scabiei* has become divided into morphologically indistinguishable varieties, which rarely cross infect. Each variety is named for its host; thus *S. scabiei* var *equi* is usually confined to the horse but may live temporarily on cattle or humans. People are quite readily parasitized by most of the animal-adapted varieties. However, in most cross-infestations, the parasites remain on the skin surface and do not complete their life cycle.

In the normal host, the parasite completes it life cycle in tunnels burrowed into and under the stratum corneum. After mating in a "molting pocket" close to the surface, the female burrows through the stratum corneum to feed on cells of the stratum granulosum and stratum spinosum. Cutting mouthparts and tarsal claws on the legs of the mite achieve the excavation. In swine, this phase of infestation takes ~3 weeks. Epidermal cell damage induces proliferative changes in the surrounding keratinocytes so that the surface openings of the tunnels become sealed with thick parakeratotic scale-crust. In swine this process takes a further 3–4 weeks. After 7 weeks of infestation, the crust falls off and the mites vacate the tunnels. Approximately 40–50 ova are laid in the burrows at a rate of 1–3 per day. They develop through the larval and nymphal stages in the same tunnel or in new ones, to reach maturity in 10–15 days depending on the host species. In general, both parasites and ova have poor viability in the external environment, however, low temperature and high humidity may allow some mites and nymphs to persist in the environment up to 21 days. The disease, which is *highly contagious*, is transmitted largely by direct contact, but may occur following indirect contact with contaminated objects such as bedding.

The *pathogenesis* of lesions in *S. scabiei* infestation is due to *direct damage* inflicted by the parasite mechanically, by the *irritant effects* of its secretions and excreta, and by an *allergic reaction* developed against components of the mite or one or more of the extracellular products of the parasite. Evidence for an allergic pathogenesis in animals comes chiefly from experimental infestations of swine. Initial lesions in pigs are due to parasite invasion and are localized and non-pruritic. After 7–11 weeks, there is generalized urticarial eruption associated with extreme pruritus. This eruption coincides with the development of immediate and delayed hypersensitivity reactions and peripheral eosinophilia. The delayed hypersensitivity response is dependent on antigen dose, whereas the immediate hypersensitivity response is independent of the degree of antigen exposure. Lesions regress between 12–18 weeks after the initial infestation.

The variability of the clinical manifestations of sarcoptic mange probably reflects individual variations in the duration and intensity of the hypersensitivity reaction and in the related capacity of the host to limit parasitic multiplication. Asymptomatic carriers exist. Most *S. scabiei*-related diseases are caused chiefly by the allergic reaction, and the lesions are the result of self-trauma induced by severe pruritus. However, animals with a weak hypersensitivity reaction may exhibit severe crusting dermatitis characterized by the presence of large numbers of mites. This type of disease is typically seen in poorly nourished animals or animals debilitated by coexisting disease. An

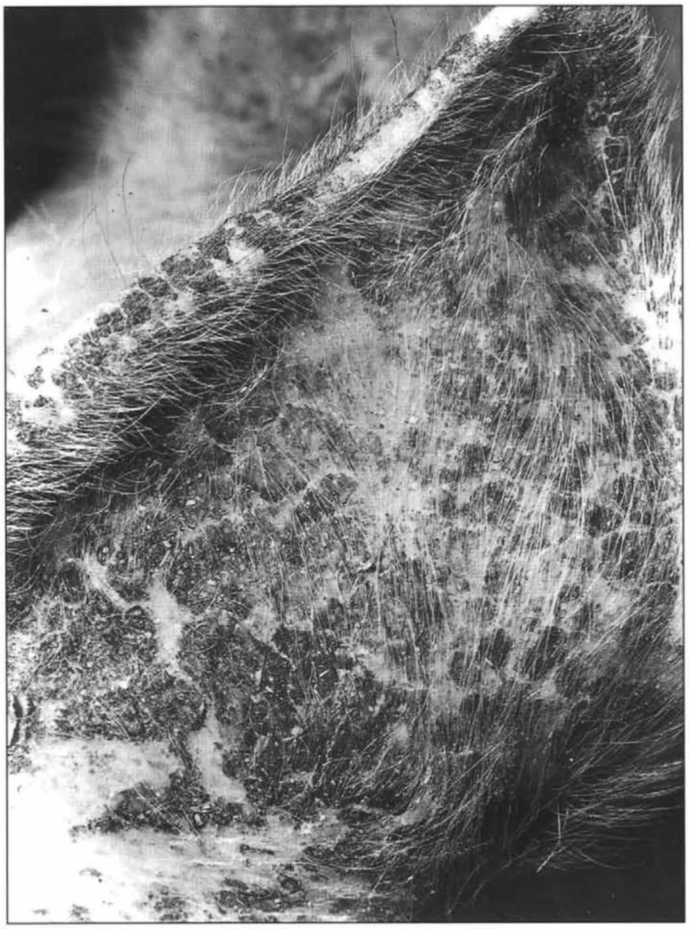

Figure 5.106 Sarcoptic mange in a pig.

example is the chronic form of scabies in dogs associated with long-term corticosteroid therapy. This has been referred to as *Norwegian-type scabies*, following the human nomenclature for severe scabies in immunosuppressed people. Diminished levels of hypersensitivity are correlated also with the development of chronic infestations in swine, known as *hyperkeratotic mange*. This manifestation of mange is sporadic and chiefly affects breeding adults.

The primary parasite-related lesions of sarcoptic mange are erythematous macules or papules, which develop a local scale-crust in reaction to the burrowing mites. The thickness of the overlying scale-crust is proportional to the number of tunnels beneath. In poorly nourished or immunosuppressed animals, which develop massive mite infestations, the lesions are characterized by alopecia, marked lichenification, accumulation of thick gray scale-crust and fissuring. Excoriations, hemorrhagic crusts and patchy alopecia follow the early lesions of erythematous papules. Chronic hypersensitivity lesions include marked alopecia, scaling and lichenification.

The distribution of the lesions is characteristic in the various species. In **pigs**, the mites have a predilection for the *inner surface of the pinna* where they cause primary lesions (Fig. 5.106). The papular lesions associated with the allergic reactions are located chiefly on the rump, flank and abdomen, and the secondary changes due to chronic self trauma follow a similar pattern. In the chronic hyperkeratotic form of the disease, heavily crusted lesions develop over the whole body but are most severe on the head, neck, and legs. In **dogs**, the preferred sites are the lateral elbows, hocks, ventral thorax, and lateral margin of the pinna. There may be an associated peripheral lymphadenopathy. The lesions may become generalized in untreated dogs or dogs inappropriately treated with corticosteroids. In **cattle**, the lesions chiefly affect the neck, head and sacral areas but may become generalized. The disease in **goats** also has a predilection for the head but may involve the whole body. In **sheep**, only the haired areas develop lesions, particularly the lips, nostrils, external surface of the pinna and occasionally the legs. Generalized lesions occur in the more hairy desert sheep of the Sudan. Lesions in the **horse** begin on the head and neck and may extend to involve most of the body, but seldom the legs or mane.

Histologically the lesions vary with the balance between allergic reaction and parasitic infestation and chronicity. *Definitive diagnosis requires demonstration of the parasite.* Rarely, sections of mites or ova may be present within the epidermis or surface crusts. Lesions consist of a mild to severely acanthotic epidermis with variable orthokeratosis to patchy parakeratosis. In fully developed lesions, there is marked spongiosis, mixed leukocytic exocytosis, serocellular crusts, and possibly intraepidermal eosinophilic pustules. Vasodilation, endothelial swelling, and edema may also be present. Immunosuppressed animals with large numbers of adult mites in epidermal burrows often have a markedly parakeratotic stratum corneum. Dermal lesions consist of a mild-to-moderate superficial to mid-level perivascular infiltrate with a variable ratio of lymphohistiocytic cells and eosinophils. The chronic allergic lesions reflect continued trauma with dermal fibrosis, epidermal hyperplasia with prominent rete ridge formation, hyperpigmentation and a predominantly mononuclear cell perivascular infiltrate.

Diagnosis of the typical allergic form of the disease depends chiefly on the clinical signs of extreme pruritus and the nature and distribution of the cutaneous lesions. Mites are characteristically difficult to demonstrate, either in skin scrapings or in microscopic section. Approximately two-thirds of affected dogs fail to yield parasites even when multiple scrapings are performed. Mites are more commonly recovered from puppies than adult dogs. *The microscopic lesions are not diagnostic, being indistinguishable from other allergic dermatoses.* The most useful diagnostic procedure is response to appropriate therapy. In the chronic form of sarcoptic mange associated with poorly developed hypersensitivity reactions, mites are plentiful in scrapings and in tissue section. *Clinical differential diagnoses* include other causes of pruritic dermatitis such as atopy, contact, food, or flea-bite hypersensitivity, infestation by other parasites, *Malassezia* dermatitis, or superficial pyoderma.

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Notoedric mange

Notoedres cati (Acarina: Sarcoptidae) is the cause of *feline scabies* and is also a parasite of the rabbit, and occasionally foxes, dogs, and humans. *The disease in cats is uncommon to rare*, although there are some endemic areas of higher prevalence. The mite has a life cycle similar to that of *S. scabiei*. The infestation is *highly contagious*, with transmission chiefly by direct contact. The major clinical sign is *pruritus*. The lesions in cats commence on the head and ears, particularly on the margin of the pinna but may extend to the neck, paw or become generalized. Lesions include partial alopecia, thickening and wrinkling of the skin and, in chronic cases, the formation of tightly adherent yellow-gray crusts (Fig. 5.107A). There may be accompanying regional lymphadenopathy. Lesions in dogs are indistinguishable from sarcoptic mange. Histologically, the lesion is a hyperplastic, eosinophil-rich, superficial perivascular dermatitis with focal parakeratosis. *The diagnosis is based on history, clinical signs and demonstration of typical mites in section* (Fig. 5.107B) *or skin scrapings*. Mites are readily found in skin scrapings and histologic sections from cats.

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Psoroptic mange

The psoroptic mites (Acarina: Psoroptidae) infest *sheep, cattle, horses, rabbits, and goats* as well as other nondomestic species. Human beings are not susceptible. There are actually fewer species of ***Psoroptes* mites** than originally thought, as mites from one host can often infect another host, and morphologic distinctions overlap. Based on critical literature review and molecular genetic analyses, the *Psoroptes* mites are conspecific, and, by rule of priority, belong to the one genus *P. equi* (Hering, 1838). Traditionally, *Psoroptes ovis* infests sheep, cattle and horses; *P. natalensis* infects cattle in South Africa, South America, and Europe; and *P. cuniculi* affects the ears of several species including rabbit, horse, donkey, goat and sheep.

Psoroptic mange is a serious disease in cattle and sheep and is a reportable disease in several countries. Bovine psoroptic mange showed a recrudescence in North America during the 1970s and early 1980s but has been brought under control by the effective use of ivermectin. Ovine psoroptic mange has been eradicated from many countries including Australia and New Zealand. The *economic importance* in sheep and cattle results from a marked decrease in weight gain, reduced milk production, reduced fleece weight and quality, occasional mortality and costs related to prevention and eradication campaigns.

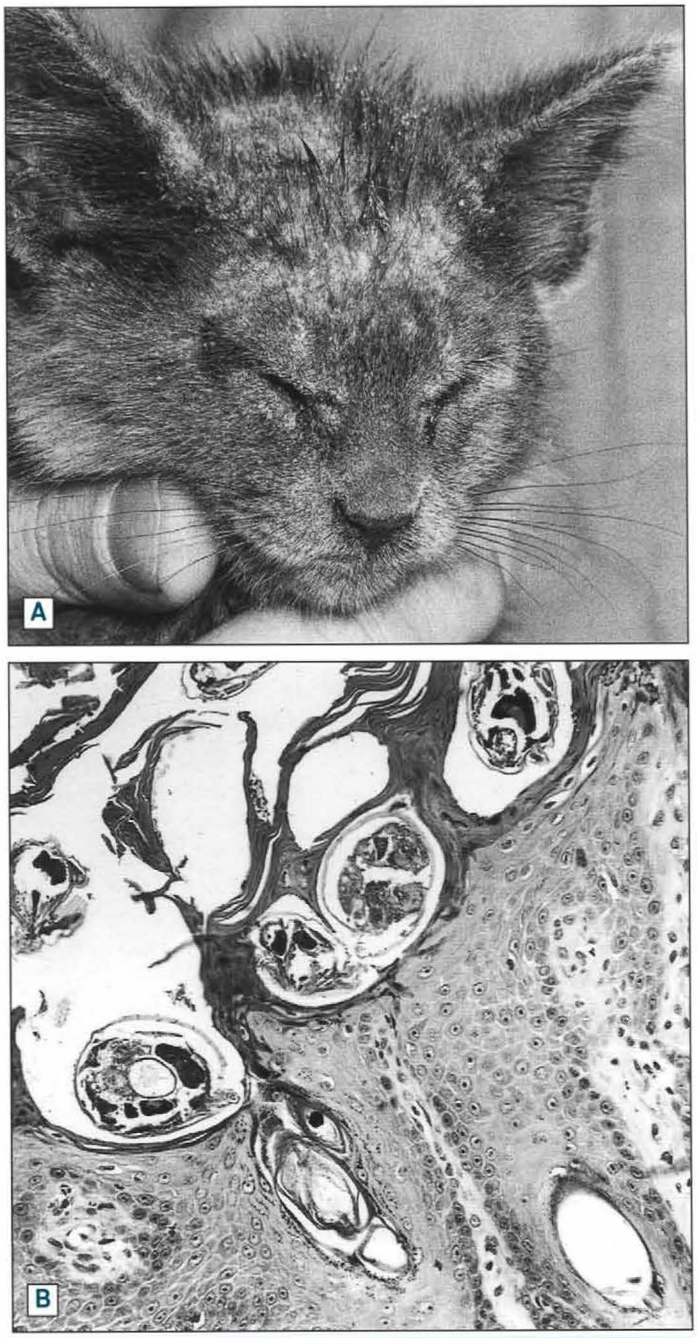

Figure 5.107 Notoedric mange in a cat. **A.** Gross photo **B.** Mites embedded in the surface keratin of the hyperplastic epidermis.

Psoroptic mites do not burrow into the outer epidermis, as do the sarcoptic mites, but instead complete their life cycle on the skin surface. Lipids from the stratum corneum provide a major source of nutrients in the early stages of infestation, probably supplemented by serous and hemorrhagic inflammatory exudates in the later stages.

Psoroptic mange is characterized by *intensely pruritic dermatitis*. The pathogenicity of the mite has been attributed to its local irritant effect on the epidermis, but this does not readily explain the marked loss of condition induced by *Psoroptes* infestation in some species, particularly cattle. The detrimental systemic effects may derive directly or indirectly from a *chronic hypersensitivity reaction* rather than from local dermatitis. The histologic lesions, in which the predominant inflammatory cells in the superficial dermis are eosinophils, mast cells, and lymphocytes, are consistent with an *allergic pathogenesis*. Constant pruritus resulting from allergy markedly reduces feed intake, and secondary bacterial infection or myiasis may further contribute to loss of condition. There is little evidence to support the hypothesis that the mites inoculate a toxic compound along with their saliva.

Histologically, the lesions are similar in all species. The pattern of inflammation is superficial perivascular dermatitis with predominantly spongiotic, exudative or hyperplastic reactions depending on chronicity. The eosinophil is the most numerous of the infiltrating leukocytes, followed by lymphocytes, other mononuclear cells and mast cells. Dermal edema is usually marked. Mites are present both on top of and under the surface scale-crust. Sebaceous gland hyperplasia has been described in lesions in sheep and cattle.

Psoroptic mange in **sheep**, also known as "*sheep scab*," may occur as a latent infection in which mites persist in the ears, infraorbital fossae, inguinal and perineal folds and at the base of the horns. In rams, mites may be found on the scrotum or prepuce in small, dry lesions. Latency occurs in the summer months when the fleece microclimate is less favorable to parasite proliferation. In autumn and winter or with debilitation of the host, the parasitic population explodes and lesions are induced. The withers and sides are particularly affected. The initial lesions are papules ~0.5 cm in diameter covered with a yellow serous crust, which may mat the fleece. The individual lesions expand at the periphery and may coalesce to become diffuse over most of the body surface. The main damage to the fleece is caused by self-trauma induced by the severe pruritus. Affected sheep scratch, kick, rub and tear out the fleece with their teeth.

In **cattle**, psoroptic mange is sometimes referred to as "*cattle scabies*." Lesions also diminish in the summer months as a result of decreasing mite populations, attributable to increased self-grooming. Previous exposure to mites, while not conferring solid resistance to reinfection, also limits the mite population, chiefly by reducing the rate of oviposition in the females. Lesions in reinfested cattle, while occurring earlier, progress more slowly, reflecting the decreased mite population. Lesions in naturally affected cattle usually commence about the poll, withers or at the base of the tail (Fig. 5.108) and chiefly result from persistent licking, rubbing and scratching induced by pruritus. Infested areas are fairly well defined as areas of alopecia. Alopecic areas become lichenified and covered by dry gray crusts and scales. Severely affected calves may develop mild anemia, lymphopenia and marked neutropenia.

In **goats**, *P. cuniculi* is known as the *"ear canker" mite* because of its predilection for the external auditory meatus. Mite infestation induces head shaking and occasionally crusted lesions on the inner surface, and alopecia on the outer surface of the pinna. In debilitated or stressed animals, thick brown-yellow, dry scale-crust accumulates on the inner aspect of the pinna and, rarely, spreads to involve the poll, body and the legs. Concurrent *Mycoplasma* and *P. cuniculi* infections have been described in the ears of goats; however, *Mycoplasma* may be cultured from the ears of clinically normal animals, placing some doubt on the significance of the finding.

In **horses**, *P. cuniculi* is found quite frequently in the ear. Infestations have been associated with "head shakers" in the United Kingdom and Australia, but are often subclinical. *Psoroptes ovis* infestations are rare; lesions are crusted papules with alopecia and the preferred sites are at the base of the mane, forelock and tail.

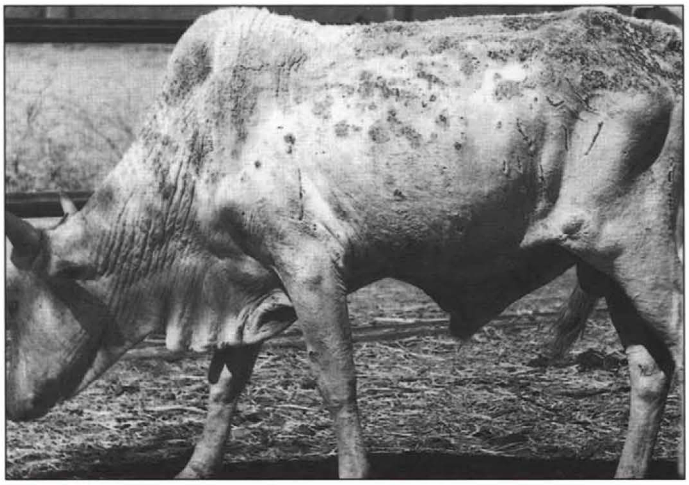

Figure 5.108 Psoroptic mange in a cow. (Courtesy of FI Awad.)

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Chorioptic mange

Chorioptic mange, caused by ***Chorioptes bovis*** *(Acarina: Psoroptidae), affects horses, cattle, sheep, and goats. Not host specific, C. bovis is an obligate parasite that lives on the surface of the skin.* The mite populations tend to fluctuate considerably as a result of host and environmental factors. Inapparent infections allow persistence in the population. Clinically affected animals are pruritic and have papular, crusted, scaly, alopecic and/or lichenified lesions depending upon the duration of the disease and the degree of self-trauma inflicted.

The disease in **cattle** predominantly affects *housed dairy cows* and is most prevalent in winter. Subclinical infections are probably quite common. The major clinical sign is *pruritus*, but this is not as severe as in sarcoptic or psoroptic mange. Chorioptic mange is, in general, a less serious condition than psoroptic mange in cattle, although a syndrome of highly irritant coronitis was associated with falling milk production. The typical distribution of lesions is perineum, tail, scrotum, udder, and caudal areas of thigh, hindlimbs and rump. Lesions are predominantly alopecia, erythema, lichenification, and wrinkling of the skin.

The disease in **horses** is uncommon. As indicated by the colloquial name "leg mange," lesions occur preferentially on the lower limb around the fetlock but may extend proximally to the thigh and ventral abdomen. Draft horses, with thick-feathered fetlocks are affected more often. The lesions are most severe in winter, as in cattle.

In **goats**, lesions originally were described as commencing on the neck and spreading to the back, base of tail, and lateral body. Another pattern, in which lesions affect the coronet, pasterns, and lower limbs, appears to be more typical. The face, udder, and scrotum also may be affected.

In **sheep**, *C. bovis* mites prefer the distal extremities, particularly the pastern and interdigital skin of the hind limbs. The scrotum may be affected and the resultant scrotal dermatitis may lead to temporary infertility. Chorioptic mange has been eradicated from the sheep population in the United States. It is a reportable disease in some countries.

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Otodectic mange

Otodectes cynotis (Acarina: Psoroptidae) is an obligate parasite of the external skin surface of *dogs and cats*. While the mite may be found at several body sites, its preferred habitat is the *external ear canal*. The major lesion is thus *otitis externa* (see Vol. 1, Eye and ear). Focal, erythematous, alopecic, or excoriated lesions occur occasionally on the face, feet, neck, or tailhead. Self-trauma and head shaking can lead to aural hematomas. The mites are contagious, particularly in young animals and can live off the host for extended periods of time. *Diagnosis is by direct visualization*; however, mites may be difficult to demonstrate in some cases.

Cheyletiellosis

Members of the genus ***Cheyletiella*** (Acarina: Cheyletidae) affect *dogs, cats, rabbits, wild animal species, and, incidentally, humans*. There are three species involved: *C. parasitivorax* is chiefly a parasite of rabbits although formerly considered as a canine pathogen; C. *yasguri* is the major canine cheyletiellid; and, the species most commonly associated with feline infestation is *C. blakei*. Host specificity is weak and cross infestations are not uncommon. The mites are obligate parasites, completing their life cycle on the skin surface in ~35 days. The infestation is transmitted by direct contact, frequently from a carrier female to her litter. The mites originally were thought to survive for only 1–2 days away from the host, but more recent observations suggest longer survival may permit indirect transmission.

The pathogenicity of cheyletiellid infestation is controversial. The presence of the mite on naive hosts usually, but not always, induces hyperkeratosis, which may or may not be associated with pruritus. Adults, possibly because of acquired immunity, usually have asymptomatic infestations.

The gross lesions in both the dog and cat reflect the mite's *predilection for the dorsal midline*. Lesions often commence over the caudal back and progress cranially but may become generalized. The typical lesion is a moderate to marked exfoliation of small, dry, white scales (seborrhea sicca). The mites crawl in "pseudotunnels" in the loose keratin debris and their movement has produced the colloquial name "*walking dandruff*" for cheyletiellosis. Cats may develop, in addition, focal, multifocal or generalized erythematous papules or crusted lesions. Occasionally animals show pruritus in the absence of scaling. *Diagnosis depends upon demonstration of the mites* via skin scraping, acetate tape, vacuum cleaning, or brush techniques. Biopsy, which shows a spongiotic hyperplastic superficial perivascular dermatitis with variable numbers of eosinophils, is nondiagnostic since mites are usually not demonstrable. *Clinical differentials* should include other pruritic dermatoses and idiopathic or primary seborrhea. Humans in contact with affected animals often develop a pruritic maculopapular rash on the arms and trunk. As the mites do not complete their life cycle on human skin, these lesions regress once the animal is treated.

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Psorergatic mange

Psorergates ovis (Acarina: Cheyletidae) is a parasite of the integument of *sheep*. The disease occurs in Australia, New Zealand, South Africa, and South America. It is thought to have been eradicated from the USA and has not been reported in Europe. The mite, which is much smaller than sarcoptid mites, is an *obligate parasite and goes through its life cycle on the skin surface in the loose keratin debris*. The mite does not penetrate deeper than the stratum corneum. Infestations occur predominantly *on the dorsum*. Seasonal influences greatly affect mite populations, with lowest numbers occurring in the summer and highest in the spring.

Mite infestations induce *pruritus*; the pathogenesis of which is not known, although hypersensitivity reactions have been postulated. Lesions result from the sheep biting and pulling at the fleece. Fleece damage is more severe in the fine-wooled Merino than in the coarse-wooled breeds. The lesions occur on the flanks, thighs and lateral body wall, and comprise bleached, twisted tufts which give the fleece a ragged, tasselled appearance.

A mite named *P. bos* has been isolated from nonpruritic, scaling and alopecic lesions in cattle in the USA, Canada, Australia, New Zealand, and South America.

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Demodectic mange

Demodex mites *(Acarina: Demodicidae) are normal inhabitants of the hair follicles and sebaceous glands in most species of domestic animals and in humans.* The cat also hosts a species of *Demodex*, *Demodex felis*, that lives in the superficial stratum corneum rather than in the follicles. The mites found in the different hosts are regarded as separate species, although they are similar morphologically. The mites are generally named for the host species, as in *D. canis* of dogs and *D. bovis* of cattle. *D. phylloides* of swine, *D. ghanensis* of cattle, *D. caballi* of horses and *D. aries* of sheep are exceptions in the nomenclature. A short form of Demodex species mite is also reported in dogs. It has been considered unusual for an animal to host more than one species of *Demodex*, but synhospitality may in fact be the rule rather than the exception.

Demodex spp. are *obligate parasites*, completing their life cycle in the hair follicle or its adnexae. They are rapidly killed by desiccation on the surface of the skin, but mites move from follicle to follicle and it is probably at this time that transmission to another host takes place. Transmission usually occurs by direct contact from the dam to her offspring during nursing in the neonatal period.

Demodex mites are part of the normal fauna of the skin in most, if not all, mammalian species. This implies that small numbers of mites exist in harmony with the host, and it is only when the equilibrium between the host and parasite is altered in favor of the mite that excessive proliferation occurs and lesions of demodectic mange are produced. The most severe expression of demodectic mange is seen in the dog as *generalized dermatitis* (Fig. 5.109A), which is, on occasion, fatal. Multiple lesions occur commonly in cattle, less commonly in pigs and goats, but with no systemic consequences. The demodectic mites of sheep rarely assume pathogenicity.

Demodicosis is one of the most common skin disorders of **dogs** *in North America.* In active infections, mites are first observed on skin scraping from the muzzles or feet of newly infected animals. Lesions may remain localized and self-limiting or become generalized. Generalized demodicosis is more prevalent in particular breeds of purebred dogs and often affects successive litters of certain clinically normal bitches or sires. *It has been postulated that puppies genetically predisposed to develop demodectic mange have a form of cell-mediated immune dysfunction that alters the body's recognition and reaction to the mites allowing massive proliferation of the mite population.* Immunophenotypic and histopathologic studies in the dog indicate active lesions of demodicosis produce *mural lymphocytic folliculitis with infiltration of the follicular wall by primarily CD3+ and CD8+ T lymphocytes.* The mural folliculitis is often associated with apoptosis or degeneration of keratinocytes and perifollicular pigmentary incontinence consistent with a lichenoid tissue reaction similar to epidermal lesions of lupus erythematosus or graft vs. host disease. Current research suggests lymphocyte-mediated follicular wall injury may be directed against keratinocytes expressing either altered self-antigens or *Demodex* antigens. It is not known whether or not the presence of cytotoxic T lymphocytes indicates an appropriate host reaction to eliminate the parasite or if it represents an inappropriate and self-damaging host response. *The disease itself, in its complicated form, appears to induce a secondary immunodeficiency state*, especially in the presence of *S. intermedius*. Dogs with chronic generalized pustular demodicosis show reduced cell-mediated immune responsiveness as measured by depressed lymphocyte blastogenesis reactivity. Evidence of humoral immunosuppressive factors also exists in dogs with generalized demodicosis. It is not yet clear whether this change is attributable to the secondary bacterial infection or to the mite infestation itself. A dog exhibiting generalized demodicosis at the time of rabies vaccination later developed rabies after exposure to a rabid skunk. This dog also failed to receive the recommended rabies vaccine booster but nevertheless suggests the dog may not have been able to mount an appropriate immune response to the vaccine.

Demodicosis in the dog has two forms, *squamous or pustular* and may be *localized or generalized*. Erythema, alopecia, scaling, and comedones characterize the squamous form. *Pustular demodicosis* occurs when secondary bacterial infection ensues and furunculosis and cellulitis develop. Papules, pustules, draining tracts, and edema may be evident. Perifollicular hypermelanosis manifest as pinpoint hyperpigmentation is suggestive of demodicosis.

Localized demodicosis (limited to one body region) occurs in young dogs 3–10 months of age and is usually self-limiting. These dogs do not have depressed cell-mediated immune responsiveness and can respond to intradermal challenge with a crude *Demodex* antigen. The lesions are single or multiple, well-circumscribed, erythematous, scaly, and alopecic patches usually affecting the head around the lips, eyes, ear canals, or on the extremities. Approximately 10% of cases of localized juvenile demodicosis progress to the generalized form of the disease.

The *generalized form* may be of juvenile onset (dogs 3–18 months of age) or adult onset (dogs >12–18 months of age). *Juvenile-onset generalized demodicosis* is familial in many breeds. *Adult-onset generalized demodicosis* has no breed predispositions. Adult-onset, severe demodicosis has been reported to occur concurrently with hyperadrenocorticism, corticosteroid administration, hypothyroidism, and chemotherapy and other serious internal or infectious diseases. It is not known if these conditions predispose the dog to generalized demodicosis or if the affected dogs were initially already immunologically predisposed to develop demodicosis. The lesions of generalized demodicosis may be particularly severe on the face, forelimbs and feet. In some dogs, lesions may be confined to the feet (Fig. 5.109B). Lesions include patchy to diffuse alopecia, erythema, scaling and crusting, with or without lesions of secondary bacterial pyoderma. Peripheral lymphadenopathy occurs in ~50% of affected dogs. Often the dogs with generalized pustular demodicosis are depressed, febrile and debilitated, and may die.

Histologically, demodicosis has a variable appearance depending on the stage of the disease and presence of secondary bacterial infection. Early, uncomplicated lesions are characterized by a predominantly *lymphocytic mural interface folliculitis*. Lymphocytic infiltration of the isthmus and infundibulum accompanied by various degrees of vacuolar degeneration and apoptosis of keratinocytes of the outer root sheath, follicular melanosis or pigment clumping in the outer root sheath, and perifollicular pigmentary incontinence are present.

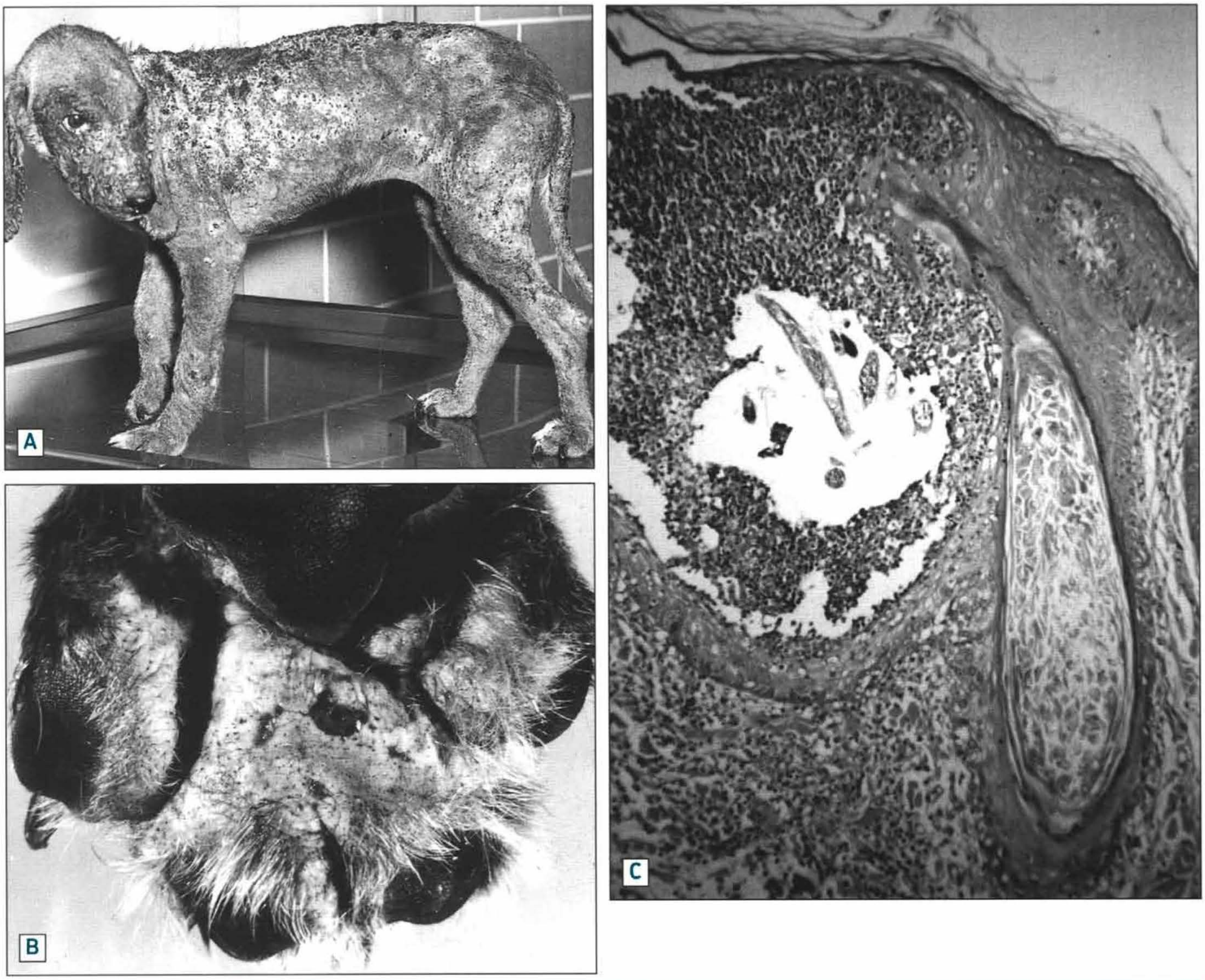

Figure 5.109 Demodicosis in a dog. **A.** Generalized demodectic mange. **B. Pododermatitis** in demodicosis. **C.** Folliculitis and **furunculosis** releasing *Demodex* mites into the dermis.

Immunophenotyping has identified infiltrating lymphocytes as CD8+ cytotoxic T cells, while the perifollicular infiltrates was found to be comprised of approximately equal numbers of CD4+ and CD8+ lymphocytes. The external root sheath may be hyperplastic. *Perifolliculitis* is also a consistent feature with infiltrates of plasma cells, macrophages, and lymphocytes in periadnexal regions. Mast cells and eosinophils may be present in smaller numbers. Marked follicular hyperkeratosis is associated with variable numbers of mites in the upper third of the follicle. Mural folliculitis is also consistently present at later stages and in cases with secondary bacterial infection but may not be the predominant pattern. In some cases, follicles may contain numerous mites but have no evidence of folliculitis of any type. This is especially true in cats. *Suppurative folliculitis and furunculosis* may be present depending on the presence and extent of secondary bacterial infection and the generation of deep pyoderma. Typically, large numbers of mites occupy the hair follicles at all levels and also occlude the opening of the sebaceous gland into the pilar canal. Marked follicular hyperkeratosis and build up of mite products causes follicular plugging. Bacterial proliferation within the plugged follicle often induces neutrophilic folliculitis. The combined effects of *follicular keratosis*, mite proliferation and folliculitis lead to *follicular rupture* and release of mites, bacteria, keratin, sebum, and other irritant products into the dermis (Fig. 5.109C). The bacteria, chiefly *Staphylococcus* spp., induce neutrophilic dermatitis often with abscessation. The keratin and other irritant substances stimulate a granulomatous reaction, chiefly of epithelioid macrophages, but a few multinucleated giant cells may be present. This *pyogranulomatous furunculosis* of demodectic mange differs from most other types of furunculosis in that eosinophils are rare or absent in the reaction. Epidermal lesions include hyperplasia, orthokeratotic and parakeratotic hyperkeratosis and variable spongiosis, neutrophilic exocytosis, ulceration and inflammatory crusting. Longer-standing lesions consist of perifollicular mid-to-deep dermal or occasionally subcuticular granulomas sometimes containing remnants of mites. Chronic lesions also have marked dermal fibrosis, often with obliteration of adnexa. Mites or fragments of mites are found in the subcapsular zone of regional lymph nodes associated with a local granulomatous inflammatory response. These do not indicate active invasion but rather passive transport to the node, via lymphatic channels.

Demodectic mange in **cattle** occurs worldwide. Three species are responsible: *D. bovis, D. ghanensis,* and *D. tauri.* Economic significance lies largely in the damage that mite infestation produces in hides. In some parts of Africa and Madagascar, demodectic mange in cattle may become generalized and fatal, this outcome contributed to by other debilitating conditions such as malnutrition, tick-worry and tropical heat.

Typical gross lesions are *multiple cutaneous papules or nodules*, usually between 2–4 mm diameter and occasionally reaching 1 cm or more. Nodules vary in number from a few to several hundred (Fig. 5.110A, B). The nodules are visible in smooth-coated cattle, often indicated by overlying tufts of erect hairs. In rough-coated cattle such as Herefords, detection usually requires palpation. The preferred sites are the shoulders, neck, dewlap, and muzzle but, in heavy infestations, nodules may be present over most of the body. The content of the nodules is thick, waxy or caseous material, sometimes stained with blood. The contents may liquefy and discharge to the surface forming a thick crust, or rupture of the nodule into the dermis may generate an abscess or a granulomatous reaction.

Histologically, the nodules are follicular cysts lined by a flattened squamous epithelium and filled with keratin squames and large numbers of demodicid mites. Adult parasites occur occasionally in sebaceous glands and rarely in apocrine sweat glands. A mild mononuclear cell infiltrate may occur around the epithelial lining. Rupture of a follicular cyst induces a marked nodular granulomatous reaction in which degenerating and occasionally mineralized segments of parasites and keratin debris are surrounded by epithelioid macrophages, multinucleated giant cells, lymphocytes, plasma cells and eosinophils.

The lesions in **goats**, caused by *D. caprae,* are similar to those described for cattle in both distribution and morphology. Some affected goats are mildly depressed, inappetent and have decreased milk production. Generally, the chief economic significance of caprine demodicosis lies in damage to the hides.

Demodectic mange is rare in **sheep**. *D. ovis* infestation is probably not uncommon in the medium to coarse-wooled sheep, affecting the Meibomian glands of the eyelid and the sebaceous glands of the primary follicles on the body, particularly the neck, flank and shoulders. *D. aries* infests the large sebaceous glands of the vulva, prepuce and nostrils. Grossly, lesions may be papular, nodular and rarely pustular. *D. ovis* infestation has been associated with matted fleece ("stringy wool"). Histologically, the mites are present in sebaceous glands or the pilar canal, occasionally inciting folliculitis or furunculosis.

While *Demodex* spp. are found commonly in the eyelid glands of the **horse**, demodectic mange is a very rare disease. *D. caballi* parasitizes the pilosebaceous units of the eyelids and muzzle, while *D. equi* infestation occurs over the body. Lesions associated with the latter occur on the head, neck and shoulder but may become generalized. They include papules, nodules and occasionally pustules. A patchy to diffuse alopecia with marked scaling occurs in the "squamous" form of the disease.

Demodex phylloides of **pigs** is not uncommon but is relatively unimportant in comparison to sarcoptic mange. The mites reside in the pilosebaceous units on the eyelids and snout with no clinical effect. The principal economic loss results from extensive trimming of affected carcasses. The lesions typically involve the ventral abdomen, ventral neck, eyelids, and snout. They commence as small red macules, developing into cutaneous nodules covered by surface scale. Incision of the nodules releases thick white caseous debris that is full of mites. The histologic lesions are as described in cattle.

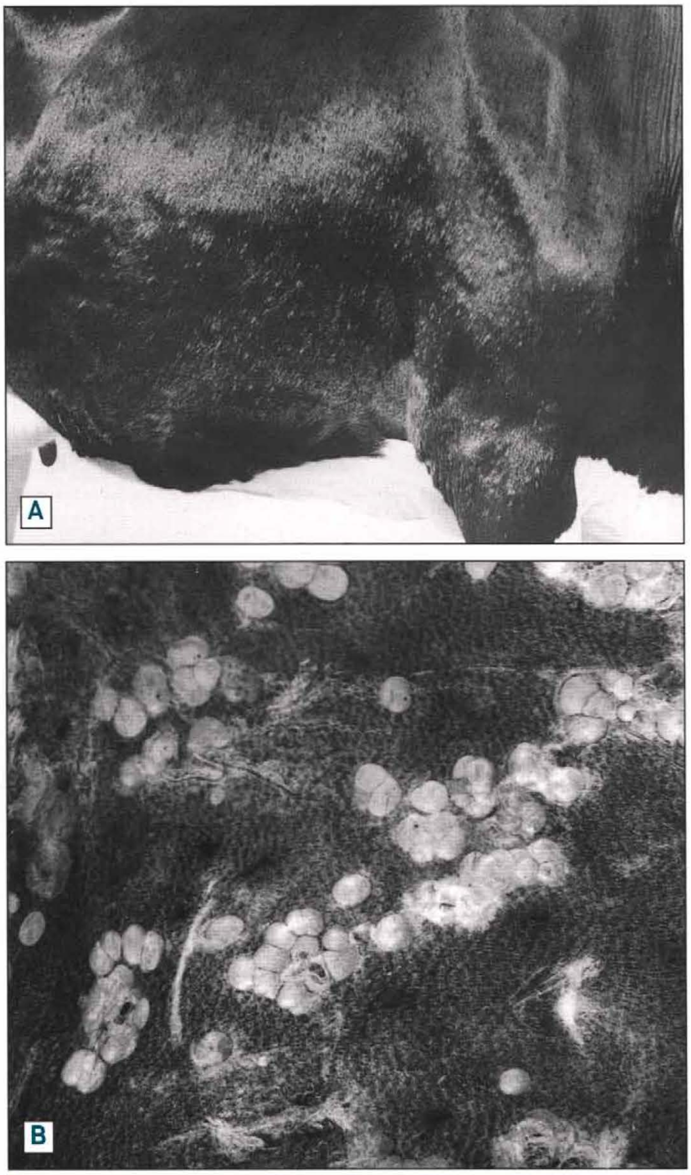

Figure 5.110 Demodectic mange in a cow. **A.** Multiple cutaneous nodules. **B.** *Demodex bovis* nodules beneath the hide.

Cats may develop demodectic mange. Two species of *Demodex* mites have been associated with feline demodicosis. One is *D. cati* that resides in the hair follicles and sebaceous glands; the other, *D. gatoi*, is found on the skin surface in pits in the stratum corneum. *D. gatoi* is shorter and broader than *D. cati*. Unlike dogs, there is no age or clear breed predisposition.

Demodex gatoi is associated with alopecia, scaling, hyperpigmentation, erythema, and excoriations due to pruritus and excessive grooming. Lesions are most often on the head, neck, groin, and extremities. Ceruminous otitis may be present. Clinical reports suggest this species of mite may be transmitted horizontally between cats. *Differentials* include atopy, food allergy, flea-bite hypersensitivity, dermatophytosis, feline psychogenic alopecia, or other ectoparasitism.

Demodex cati infestation results in single or multiple areas of alopecia, erythema and scaling that resemble the localized form of canine demodicosis. Lesions have a predilection for the chin, eyelid, head, and neck. Generalized lesions may occur in association with systemic disease, such as feline leukemia virus or feline immunodeficiency virus infection, hyperadrenocorticism, or diabetes mellitus, which presumably suppress normal cell-mediated immune responses. *D. cati* infestation of lesions of multicentric squamous cell carcinoma in situ (Bowen's disease) suggest that a localized alteration in the cutaneous immune response may allow mite proliferation. The lesions of *D. cati* are multifocal to generalized areas of alopecia and erythema, with variable degrees of scaling, and papules and crusts. Secondary pyoderma is not a common accompaniment. A third syndrome, otitis externa, has also been associated with *D. cati* infestation.

Histologic lesions tend to be limited to follicular and surface hyperkeratosis, sometimes with follicular atrophy. Inflammatory cell infiltration is minimal in *D. cati* lesions. Some eosinophil, mast cell and neutrophil infiltrates have been described in infestations with *D. gatoi,* but the reaction was never severe and concurrent allergic disease could not be ruled out.

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Trombiculiasis

The nymphs and adults of the trombiculid mites are free-living or parasitize plants or other arthropods; the larvae are parasitic and are known as "harvest mites," "chiggers," or "red bugs." The parasitic larvae are six-legged and resemble minute red or yellow spiders just visible to the naked eye. The mites attach themselves to the skin and make a channel into the epidermis, called a *stylostome*, through which salivary enzymes are injected and digested tissue fluids are withdrawn. The mites engorge to twice their original size over a period of 3–5 days, after which they drop off and complete their life cycle in the soil. *Intensely pruritic dermatitis* develops at the sites of attachment, probably as a result of an allergic reaction to the salivary secretions delivered through the stylostome; the allergic reaction caused by larval trombiculid mites is known as *trombidiosis*.

Wild vertebrates are the usual hosts for the trombiculid mite larvae, but food-producing domestic animals, pets and people may be accidentally infested. The disease tends to have a seasonal incidence, occurring in the *late summer and autumn* when climatic conditions favor an expansion of the mite population. Factors such as soil-type also influence the prevalence of trombiculiasis in different geographical regions. Animals exposed to fields and woodlands are more often infested.

Trombicula autumnalis, the European harvest mite, attacks most domestic species. *T. sarcina*, an Australian species known as the leg-itch mite, is an important parasite of sheep although its principal host is the gray kangaroo. *T. alfreddugesi* (North American chigger), and *T. splendens* are some of the species implicated in trombiculiasis in cats, dogs and horses. *Straelensia cynotis* normally reside on foxes, but can infest dogs.

Lesions tend to occur in areas close to ground contact in animals exposed to wild or semi-wild areas. Lesions are extremely pruritic. In **sheep**, the larvae attach preferentially on the skin of the caudal pastern. The interdigital web is the predilection site in the **dog**. In a massive infestation reported in two dogs, temporary hindlimb paresis developed. The mite in **horses** is known as the "heel-bug" because of its tendency to parasitize the feathered area of the pastern. Lesions may also occur along the mane and at the tail-head. Face, particularly lip, involvement is not infrequent. The lesions are intensely pruritic. In **cats**, lesions affect the paws, head and ears, but an atypical generalized form of the disease may occur in association with *W. americana* infestation.

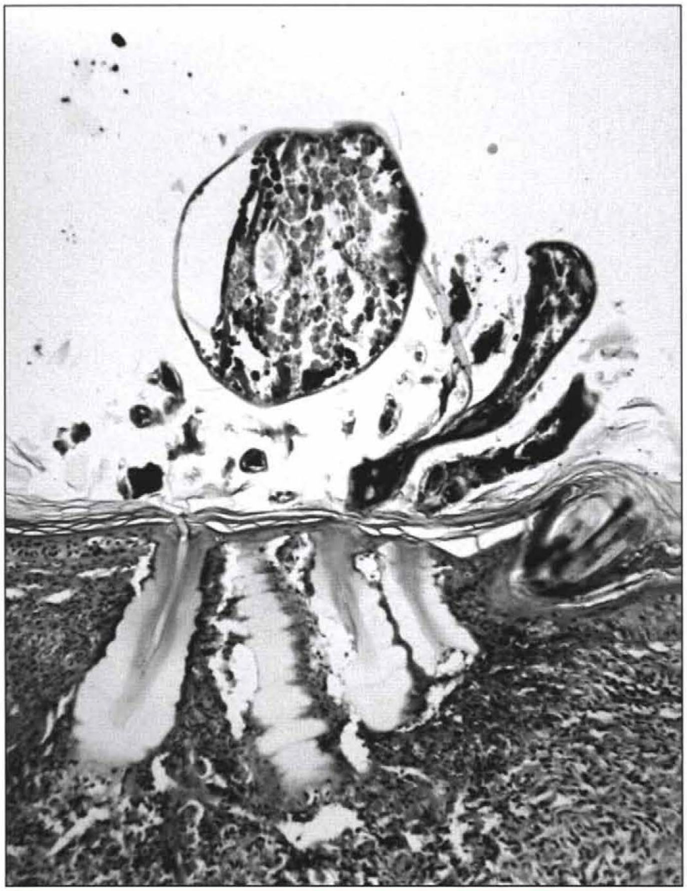

Figure 5.111 Trombiculiasis in a black bear. Section of skin showing mites within the surface keratin and multiple stylostomes oriented vertical to the skin's surface and extending into the dermis.

The gross lesions in all species are small, erythematous papules on which are clustered tiny (0.2–0.4 mm) bright red, orange or yellow mites. The mites leave a small, shallow ulceration that oozes a serous discharge and becomes crusted. Pruritus induces marked self-trauma, which may incite secondary bacterial infection. Histologically, *the mite is present in tunnels within the stratum spinosum or in the stratum corneum*. The presence of the mites induces both hyperplastic and degenerative changes in the epidermis. The stylostome appears as a pale staining, hyalinized tube with undulating margins oriented vertical to the skin's surface and extending into the dermis (Fig. 5.111). A large zone of necrotic cellular debris usually surrounds the stylostome. The surrounding dermis has a superficial perivascular infiltrate, with eosinophils and mast cells predominating.

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Other mite-induced dermatoses

The **cat fur mite**, *Lynxacarus radovsky*, infests cats in the USA, Australia, Fiji, South America, and the Caribbean. The mite attaches to the hair shafts rather than to the skin surface. Predilection sites include the tail tip, tail head, and perineum. The mite infestation mimics seborrhea sicca, giving the coat a characteristic "salt and pepper" appearance. The infestation is not usually associated with lesions, although crusted, exudative and pruritic lesions and alopecia have been described.

The **straw-itch mite** (*Pyemotes tritici*) is normally found in straw or grain where it parasitizes the larvae of soft-bodied grain insects. The parasite may occasionally infest humans and other mammals causing pruritic dermatitis. Mildly pruritic lesions may develop in horses fed infested hay. Multiple papules and wheals may occur on the neck, withers and thorax. *Acarus (Tyroglyphus) farinae* and *A. (Tyroglyphus) longior* may cause a pruritic, exudative, crusting and alopecic dermatosis in horses exposed to contaminated grain or hay.

Cats and dogs may be infested on rare occasions with the **poultry mite**, *Dermanyssus gallinae*. The infestation resembles that of cheyletiellosis but pruritic eruptions have been described.

Sheep may, on rare occasions, become infested with the **stored product mite**, *Sancassania berlesei*. These mites cannot infest dry skin; infestations are secondary to other conditions such as myiasis.

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Ticks

Ticks belong to the class Arachnida, sub-class Acari. They are divided into two families, the Argasidae and the Ixodidae. *The*

Argasidae are the so-called soft ticks, lacking the scutum that characterizes the Ixodidae. Included in this group is *Argas persicus*, a complex of tick species that are important parasites of birds. *Otobius megnini*, known as the "spinose ear-tick," is parasitic to all domestic animals causing severe parasitic otitis externa and predisposing to bacterial infection or myiasis. Most of the pathogenic species of tick are found in the Ixodidae.

Ticks are most important as vectors for a large number of serious viral, bacterial and protozoal diseases of domestic animals. Babesiosis, Rocky Mountain spotted fever, Lyme borreliosis, heartwater disease, Q fever, louping ill, and anaplasmosis are a few examples of tick-transmitted diseases. Ticks also are small, attach firmly to their hosts, utilize multiple hosts, live for prolonged periods of time, and can often survive without feeding for long periods of time. These factors make ticks important in the possible transport and transmission of diseases from one host to another and between countries or continents. Importation of animals harboring ticks can pose a threat to animals in areas free of certain diseases. Ticks also harm their hosts more directly by causing *local injury* at the site of attachment. If infestation is heavy, fatalities may result. The local injury may predispose to myiasis and secondary bacterial infection, particularly to staphylococcal cutaneous abscesses or septicemia. Heavy infestations are also capable of causing *anemia* as a result of the bloodsucking activities of the ticks, but the Ixodidae, which engorge only once at each instar, are much less important in this respect than are the argasid ticks, which as adults engorge repeatedly. Tick bites may also induce hypersensitivity reactions. Several species of ixodid ticks have neurotoxins in salivary secretions that can cause *paralysis* of the host. These species include *Ixodes rubicundus* and *Rhipicephalus evertsi* of South Africa, *I. holocyclus* of Australia, *Dermacentor andersoni* in western North America, and *D. variabilis* in eastern North America.

The *local reaction to ticks is variable*, depending on properties of the tick, for example its ability to secrete prostaglandins, and on host factors such as the level of tick resistance. Primary tick bite lesions are papules and wheals, which proceed to focal areas of necrosis with crusts, erosions and ulcers and lead to focal alopecia. In contrast, tick-sensitized hosts produce extremely erythematous reactions. Histologically, the primary lesions are less severe and develop more slowly than those of sensitized animals. For example, in experimental *Ixodes holocyclus* infestations of cattle, the inflammatory cell reaction to the tick mouthparts embedded deeply into the dermis is not marked, even at 40 hr post-attachment. The lesions are restricted to the immediate feeding site and are predominantly neutrophilic. By contrast, previously sensitized cattle show epidermal spongiosis and vesiculation at some distance from the actual point of penetration as early as 1 hour post-attachment. By 12 hours, intraepidermal vesicles, bullae and microabscesses containing basophils with eosinophils and neutrophils are prominent. Basophils, many degranulated, are numerous in the edematous dermis from 1 hour post-attachment, reflecting the important role cutaneous basophil hypersensitivity plays in tick immunity. Severe hypersensitivity reactions to *Boophilus microplus* have been described in horses in Australia. Within 30 min of reinfestation, sensitized horses develop intensely pruritic papules and wheals, chiefly on the lower legs and muzzle. As tick bite lesions progress, they are often typified by the "arthropod-bite granuloma" described earlier in this section.

Tick infestation in lambs leading to secondary staphylococcal septicemia can result in high mortality.

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HELMINTH DISEASES OF SKIN

The skin is the natural portal of entry of a number of metazoan parasites that have their final habitat in the gastrointestinal tract or elsewhere. As a rule, those infective larvae that can invade percutaneously are not host specific, thus infection of aberrant hosts occurs. Such parasites are quite varied in their nature and include infective larvae of trematodes such as *Schistosoma*, and of the nematodes of various genera including *Strongyloides, Gnathostoma, Ancylostoma, Bunostomum, Uncinaria*, and others. Infective larvae of the filariids such as *Dirofilaria* and *Setaria* are deposited in the skin by the biting insects that are their vectors. The first percutaneous invasion of one of these parasites in its natural hosts takes place very quickly (for example, the larvae of *Strongyloides* and *Bunostomum* reach the dermis in 15 min) and provokes little reaction. Repeated invasions in a natural host or single invasion in an unnatural host are met with some resistance that is manifested as acute dermatitis limited to the invaded area. The cutaneous lesions are, except under experimental circumstances, seldom observed in animals. It is on the glabrous nonpigmented skin of people that they are easily observed and well recognized as the so-called "creeping eruption." There the larvae produce acutely inflamed, serpiginous, vesicular to papular tracts that may advance several centimeters a day. Usually, aberrant larvae die in the skin, but some enter the vessels and become lodged in the lungs or other tissues. Nematode larvae most often incriminated are *Ancylostoma braziliense* and *A. caninum*, the canine hookworm larvae. *Uncinaria stenocephala, Bunostomum phlebotomum, Gnathostoma spinigerum, Dirofilaria* sp., *Strongyloides procyonis*, and *Strongyloides westeri* are nematode parasites of other domestic and wild animals that have also been reported to cause cutaneous larval migration in humans.

Hookworm dermatitis occurs in dogs kept on grass or dirt and subjected to poor sanitation. Lesions consist of pruritic red papules on parts of the body exposed to the ground. The footpads and interdigital regions of the feet may be erythematous, edematous and painful. Claw deformities may occur. Histologically, hyperplastic spongiotic perivascular dermatitis with eosinophils and neutrophils is present. Larval tracts may be present in the epidermis or dermis lined by degenerating leukocytes. Inflammation is thought to be due to hypersensitivity reaction to migrating larvae.

The cutaneous lesions produced by the blood flukes and those nematodes that pass through the skin on the way to the gut are discussed elsewhere with the mature parasites (Vol. 2, Alimentary system). To be discussed in more detail here are those helminthic infestations that remain more or less localized to the dermis. The dermatitis produced by the larvae of *Elaeophora* is discussed in Vol. 3, Cardiovascular system.

Cutaneous habronemiasis

The aberrant deposition of the larvae of the spirurid nematodes *Habronema majus* (*microstoma*), *H. muscae* and *H. megastoma* by transmitting flies at cutaneous or mucocutaneous sites causes this *common disease of* **horses**. The adult worms normally develop in the stomach of horses following the ingestion of infective larva (see Vol. 2, Alimentary system). The cutaneous disease occurs sporadically in horse populations in temperate or tropical climates during the summer when the transmitting flies, *Musca* spp. and *Stomoxys calcitrans*, are active. Horses with a hypersensitivity response to the larvae are affected and the condition is recurrent each summer, hence the term, "*summer sores*." Lesions are consistently pruritic.

The location of the lesions is in the moist exposed areas of the body that attract flies. The most common sites are the *medial canthus of the eye* (Fig. 5.112A), *the glans penis and prepuce, and any cutaneous wound*. Since lacerations are more common on the distal extremities, these too are predilection sites for habronemiasis. Fly bites alone are sufficient to initiate an infestation. Ulcerative diseases initiated by other causes can be complicated by habronemiasis.

The gross lesions are rapidly progressive and proliferative in nature, comprising *ulcerated, tumorous masses of red-brown granulation tissue*. The surface is friable and hemorrhages readily. Lesions may be single or multiple and range in size from 5–15 cm in diameter and from 0.5–1.5 cm in depth. They are often irregular in shape in the early stages but become circular as they enlarge. On cut section, multiple small (1–5 mm) yellow-white, caseous, and occasionally gritty foci are scattered through the granulation tissue. These are often confused with the "kunkers" of pythiosis, but lack the characteristic branching pattern of the true kunker. In the deeper parts of the lesion, the more mature connective tissue has a dense, white appearance. The lesions on the conjunctiva and eyelids do not usually exceed 2 cm. Commencing with a serous conjunctivitis, small ulcerated, proliferative nodular lesions develop on the mucous membrane of the third eyelid and at the medial canthus. Lacrimal duct involvement characteristically produces a lesion 2–3 cm below the medial canthus. The entire conjunctiva may be affected resulting in profuse lacrimation, photophobia, chemosis, and inflammation of the eyelid. On cut section, *the nodular lesions of brown-red granulation tissue contain the typical caseous or mineralized foci*. Involvement of the penis and prepuce may cause prolapse of the urethral process and dysuria. On rare occasions, *Habronema* granulomas are found in the lung.

Histologically, multiple aggregates of degenerate eosinophils are scattered randomly throughout a collagenous connective tissue stroma of variable maturity. Epithelioid macrophages, multinucleated giant cells and degenerating eosinophils often surround longitudinal or cross-sections of degenerating larvae, which may be mineralized (Fig. 5.112B). However, *larvae may be rare or absent in many caseous foci*. The fibrous connective tissue is heavily and diffusely infiltrated with eosinophils and with fewer numbers of mast cells, lymphocytes and plasma cells. The surface of the lesion is usually covered with fibrinonecrotic exudate overlying highly vascular granulation tissue infiltrated with neutrophils.

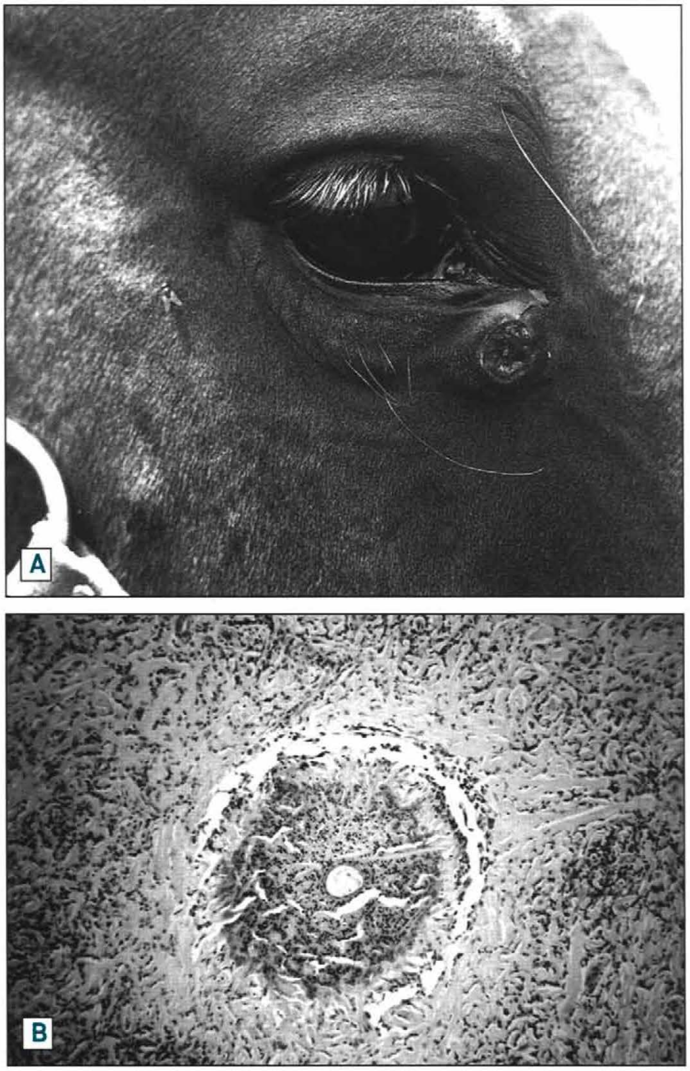

Figure 5.112 Cutaneous habronemiasis in a horse. **A.** Ulcerated nodule below medial canthus. **B.** Clusters of degranulating eosinophils surround a cross section of a *Habronema* sp. larva.

Since the gross lesions of cutaneous habronemiasis may resemble those of exuberant granulation tissue, botryomycosis, pythiosis, equine sarcoid and squamous cell carcinoma, the diagnosis requires biopsy and histologic evaluation. Squamous cell carcinoma and equine sarcoid are readily distinguished histologically. Exuberant granulation tissue lacks the caseous foci of degenerating eosinophils and larvae. Special stains will reveal the causative agents in botryomycosis (see Bacterial diseases of skin) and pythiosis (see Fungal diseases of skin). Nodular collagenolytic granuloma (nodular necrobiosis) presents a different clinical appearance but may be confused histologically with habronemiasis (see Miscellaneous skin conditions). Habronemiasis may complicate a pre-existing lesion; secondary *Habronema* infestations occur with pythiosis, *Corynebacterium pseudotuberculosis* infection and in skin tumors, particularly squamous cell carcinoma of the penis.

Cutaneous habronemiasis has been reported in a **dog**. Lesions developed on the face but, unlike the equine disease, were not characterized by rapid proliferation of granulation tissue. The dog was housed under unsanitary conditions in the company of several heavily parasitized ponies.

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Stephanofilariasis

Members of the genus ***Stephanofilaria*** (Spirurida: Setariidae) are parasites of **cattle**. All stephanofilarid parasites cause similar cutaneous lesions, but the species are geographically separated and the lesions occur on different parts of the host's body. *Stephanofilaria stilesi* occurs in North America, affecting the abdominal skin near the midline; in Australian cattle, initial lesions develop at the medial canthus of the eye; *S. dedoesi* in Indonesia causes dermatitis, known locally as "cascado," on the sides of the neck, dewlap, withers and around the eyes; *S. assamensis* causes "hump-sore" of Zebu cattle in India and the Soviet Union; S. *kaeli* causes dermatitis of the legs of cattle from the Malay Peninsula; *S. dinniki* affects the shoulder of black rhinoceros; *S. boomkeri* causes severe dermatitis in pigs in Zaire; *S. zaheeri* causes dermatitis of the ears of buffalo, and *S. okinawaensis* causes lesions on the teats and muzzle of cattle in Japan. *Stephanofilaria kaeli, S. assamensis,* and *S. dedoesi* cause crusting dermatitis in **goats.**

Flies transmit stephanofilariasis. The hornfly, *Haematobia irritans*, is the vector of *S. stilesi* and the buffalo fly, *Haematobia irritans exigua*, is the major vector for stephanofilariasis in Australia. *Musca conducens* transmits *S. assamensis* and *S. kaeli*. The flies ingest microfilariae when feeding on cutaneous lesions. After a period of development in the fly, the infective larva is deposited on the skin by the biting species of fly or onto cutaneous wounds in the case of the nonbiting vectors such as *M. conducens*. The adults of *S. stilesi* live in cystic diverticula off the base of the hair follicles. The parasites are very small, with males reaching 3 mm and females 8 mm in length.

The *initial macroscopic lesions* in *S. stilesi* infections are circular patches 1 cm or less in diameter in the skin of the ventral midline, in which the hairs are moist and erect and the underlying epidermis is spotted with small hemorrhages and droplets of serum. These initial foci enlarge and coalesce, sometimes to produce a lesion 25 cm or more in diameter. As the foci enlarge, new spots of hemorrhage and exudation develop at the periphery while, in the central areas, the hair is shed and the exudate builds up into scabs or rough dry crusts through which the few remaining hairs penetrate (Fig. 5.113). The lesions are mildly pruritic and rubbing may aggravate them. In the healing stage, the affected areas remain as alopecic, lichenified plaques (Fig. 5.114).

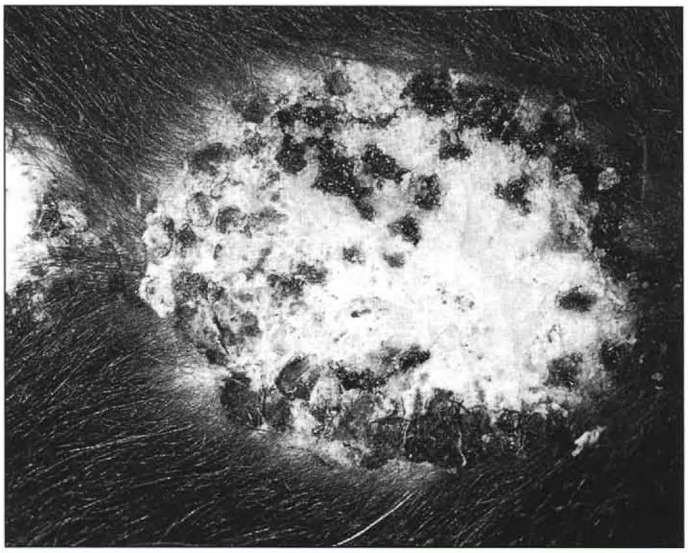

Figure 5.113 Early lesion on the ventral midline in **stephanofilariasis** in a cow.

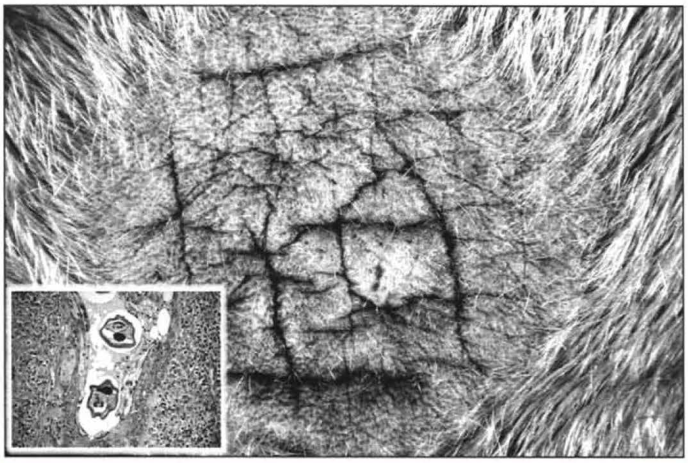

Figure 5.114 Chronic lesion of lichenified, alopecic plaque in **stephanofilariasis**. Nematode within a hair follicle (inset).

Histologically, sections of adult parasites may be seen in the cystic diverticula from hair follicles or lying free in the adjacent dermis. They may be identified as *Stephanofilaria* spp. with some confidence if microfilariae are seen in the uteri of females since this parasite is viviparous; if ova rather than larvae are found, it is more likely for the parasite to be of the genus Rhabditis. Microfilariae occur free in the dermis or in dermal lymphatics, enclosed within their own vitelline membranes (*S. stilesi*), or may be found free or unhatched in surface exudate (*S. kaeli*). There is little dermal reaction to the microfilariae or to the adults enclosed in cystic hair follicles, but the presence of adults in the dermis stimulates a mononuclear inflammatory reaction. There is accompanying superficial and deep perivascular dermatitis characterized by accumulations of eosinophils and mononuclear cells, chiefly lymphocytes. The epidermis is hyperplastic, spongiotic and often covered by orthokeratotic and parakeratotic hyperkeratosis and inflammatory crust. Spongiform microabscesses containing eosinophils and mononuclear cells are also described; such lesions are more typically associated with the bites of arthropod ectoparasites. It is difficult to assess the relative contributions made to the lesion by the stephanofilarial parasite and by the bites of the fly that acts as the vector.

Diagnosis is usually made on the basis of the typical gross lesions, by histologic examination of biopsy specimens or by demonstration of microfilariae by deep scrapings.

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Onchocerciasis

Onchocerca spp. are filarial nematodes (Spirurida: Onchocercidae) which affect cattle, horses, sheep, goats and man. *The adult worms reside in various connective tissue locations*, chiefly ligaments, tendons and subcutaneous nodules, but also perimysial sheaths, cartilage and the aorta. The microfilariae migrate to the dermis where they lie free in tissue spaces or lymphatics. In man, blindness caused by migration of microfilariae into the chambers of the eye has been a serious health concern in Africa and Central America. Microfilariae in the dermis of any host are aspirated by the intermediate hosts, which are insects of the families Simuliidae (black flies, buffalo gnats) and Ceratopogonidae (biting midges). The prevalence of infection is very high in cattle and horses, ranging up to 100% in some studies.

Onchocerca cervicalis occurs worldwide and is the major species affecting the horse in North America; adults reside in the ligamentum nuchae and range from 27–75 mm in length. *Onchocerca reticulata* occurs in horses in Europe and Asia; adults live in the flexor tendons and suspensory ligaments, particularly of the forelimbs. *Onchocerca cervicalis* and *O. reticulata* are primarily transmitted by *Culicoides* sp.

Onchocerca gutturosa more commonly affects cattle but also is recovered from horses. *Onchocerca gibsoni* infects cattle in Australia, Asia, and Africa; *O. ochengi* infests cattle in Africa. *Onchocerca armillata* occurs in cattle, sheep, and goats in Africa and Asia; the adults live in the aorta.

Most animals show no reaction to the presence of living microfilariae in the dermis. The reason for the lack of host reaction to the living microfilariae is unknown. Dead microfilariae are, however, highly phlogistic and an intense inflammatory reaction may occur after treatment with microfilaricides in some animals. *Onchocerca* microfilariae cause cutaneous lesions in some horses; in cattle, the adults cause the cutaneous lesions.

In dogs, onchocerciasis has been reported involving the palpebral conjunctiva, third eyelid, sclera, cornea, and retrobulbar space. Dogs were presented for evaluation of periocular or ocular masses. Degenerating or mineralized adult *Onchocerca* spp. parasites were found in the center of pyogranulomas comprising the masses.

Equine cutaneous onchocerciasis

The pathogenicity of *Onchocerca cervicalis* and *O. reticulata* microfilariae, originally questioned because the parasites could be readily demonstrated in the skin of clinically normal horses, became accepted when it was shown that ivermectin treatment induces rapid and concurrent disappearance of cutaneous lesions and microfilariae. The prevalence of adult parasites in horses has not diminished despite the widespread use of avermectins. It has been postulated that individual hypersensitivity to microfilarial antigens is the reason for the sporadic occurrence of equine skin lesions.

Areas affected are the face, neck, medial aspects of the forelimbs, ventral thorax, and abdomen. Lesions on the face may be diffuse, periorbital or central. *The central lesions, which often involve the facial markings, are very characteristic of onchocerciasis.* The lesions on the neck are focal and tend to occur at the base of the mane. Lesions on the ventral thorax and abdomen are often diffuse, in comparison with the localized lesions of horn fly-induced ventral midline dermatitis. The ventral lesions are indistinguishable grossly from those of *Culicoides* hypersensitivity, but onchocerciasis does not cause lesions at the base of the tail. It is likely that onchocerciasis and *Culicoides* hypersensitivity can exist simultaneously.

Cutaneous lesions include partial or complete alopecia, scaling, crusting, and leukoderma. Secondary excoriations or ulcerative dermatitis are induced by self-trauma in pruritic animals. Ocular lesions are discussed in Vol. 1, Eye and ear. The microscopic lesions associated with microfilarial infection vary from none to superficial and deep perivascular lymphocytic and eosinophilic dermatitis. Microfilariae may be present in large numbers or may be very sparse. They are best recovered from unfixed biopsies that are minced and incubated in saline at 37°C. *Differential diagnoses* include other fly-bite-associated dermatitides, staphylococcal folliculitis, occult sarcoid, and infestation with lice, mites, or ticks. Lesion distribution should be helpful.

Viable adult parasites in the nuchal ligament are not associated with significant lesions; in older horses, there is an increased frequency of caseated, mineralized and granulomatous lesions, associated with death of the parasite. *Onchocerca gutturosa* is also found in the nuchal ligament of horses in Australia. Histologic examination revealed that this parasite does not penetrate the elastic tissue of the ligament and the inflammatory reaction is localized around the worm. Mineralization is the fate of degenerate *Onchocerca gutturosa* worms, in contrast to caseation with *O. cervicalis.*

Bovine cutaneous onchocerciasis

Cattle can be infected by three species of *Onchocerca*. Cattle infested with *O. gibsoni* develop multiple subcutaneous papules and nodules of ~2 cm diameter, although lesions up to 9 cm are recorded. Lesions may be hard or soft depending on the degree of mineralization and fibrosis or the degree of caseation and suppuration. Lesions predominantly affect the brisket but also the stifle and hip. Histologic assessment of *O. gibsoni* nodules revealed dead worms and associated degenerative changes such as mineralization in ~30% of nodules. Eosinophilic infiltration was more marked around viable worms, with eosinophils apparently adherent to the cuticle. In *O. gutturosa* infections, the worm is found in the nuchal ligament and connective tissue around tendons and ligaments of the shoulder, hip, and stifle. It does not form nodules and is thus easily overlooked. In *O. ochengi* infections, scrotum, udder, flanks, lateral thorax, and head are affected.

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Pinworms

Oxyuris equi infection of the equine large intestine can be associated with *pruritic dermatitis of the perineal region* leading to self-induced excoriations and alopecia of the tail known as "*rat tail*." The adult parasite crawls out of the anus to deposit eggs on the hair and skin using a gelatinous material that can induce pruritus. *Diagnosis* is made by using the tape method in the perianal region for identification of characteristic operculated eggs.

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Parafilariasis

Parafilaria multipapillosa occurs in horses in Eastern Europe. *Parafilaria bovicola* is endemic in cattle in Africa, India, and parts of Europe. *Parafilaria bassoni* has been reported in African buffalo and springbok in Namibia. The parasites are thin, thread-like worms, 2–7 cm long. The adults inhabit subcutaneous and intermuscular connective tissues producing nodules 1–2 cm in diameter. In the spring and summer, the nodules rapidly enlarge, burst open, hemorrhage, and heal. This coincides with the migration of the fully gravid female into the more superficial dermis to oviposit and to release the infective microfilariae. The vectors, flies such as *Haematobia atripalpis* in Russia and *Musca* spp., are infected when they feed from these bleeding points, known as "blood nodules." Secondary subcutaneous abscesses may occur.

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Pelodera dermatitis

Some of the biological characteristics of the Rhabditidae have been discussed with the principal parasitic genus, *Strongyloides*, in Vol. 2, Alimentary system. Here it is necessary only to describe the cutaneous lesions produced occasionally by the small free-living worms of this family. Those worms that are found in the lesions are usually classified as ***Pelodera (Rhabditis) strongyloides***.

Pelodera dermatitis occurs most commonly in dogs, occasionally in cattle and rarely in horses and sheep. The condition has been reported in humans. The development of dermatitis to a clinical degree probably requires exposure to many larvae. These worms live as saprophytes in warm moist soil that is rich in organic matter, and significant infections probably require that the host's skin should be continually moist and filthy. The lesions develop on contact areas, particularly at the margins of areas caked with dirt. Affected **dogs** often have a history of being bedded on straw. Acute dermatitis develops, commonly with neutrophilic folliculitis and is due to the combined stimuli of the parasites, bacterial folliculitis, and of the scratching, rubbing, and licking prompted by severe pruritus, probably induced by an allergic reaction to the parasite. The gross lesions include erythema, papules, excoriations, scaling, exudation and crusting with partial to complete alopecia. Pustules may occur, particularly in dogs. Histologically, the worms are in the lumina of the hair follicles or in the dermis, surrounded by an intense eosinophilic inflammatory reaction (Fig. 5.115). *Differential diagnoses* based on gross lesions in dogs include hookworm dermatitis, dirofilarial dermatitis, dermatophytosis, demodicosis, bacterial folliculitis, scabies, strongyloidiasis and contact dermatitis. Definitive diagnosis is based upon environmental history and deep skin scrapings or biopsy. *Pelodera* dermatitis in **sheep** may result in complete loss of wool, hyperkeratosis and lichenification in affected areas.

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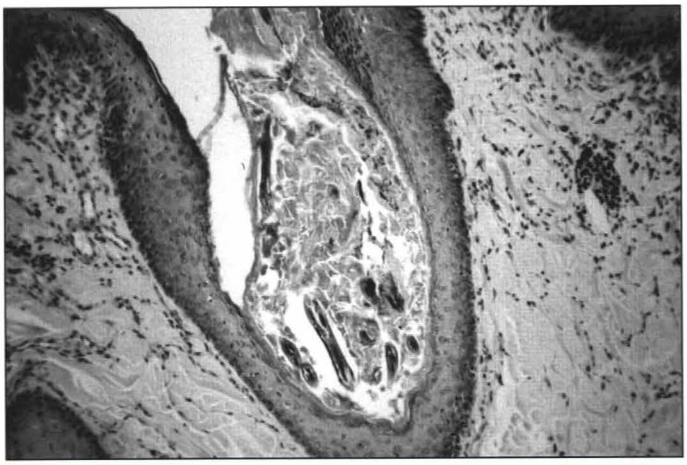

Figure 5.115 ***Pelodera (Rhabditis) strongyloides* folliculitis** in a dog. Adult rhabditiform parasites within distended hair follicle.

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Miscellaneous helminths

Dracunculus medinensis (Spirurida: Dracunculidae) is the "guinea worm" of humans in Asia and Africa. It has been introduced into America, the West Indies, and Fiji. The parasite has been reported in *dogs, cats, horses, and cattle* as well as other species in endemic areas. *Dracunculus insignis* is the species of the nematode that occurs in dogs and wild carnivores in North America. Infection is particularly prevalent in raccoons and mink that appear to be the natural definitive species. The intermediate host is a crustacean copepod but frogs may act as paratenic hosts. Ingesting the intermediate host, infected frogs, or water containing infected copepods infects the final host. The larvae are released during digestion and proceed to migrate through the body. The adult worms mature in the connective tissue of the host in approximately 1 year. The parasite occurs typically in the subcutaneous tissues of the limbs. The mature female may measure up to 70 cm in length, resulting in the formation of a 2–4 cm nodule. The gravid female produces an intraepidermal bulla with her anterior end by means of toxin secretion. Rupture of the bulla forms a shallow ulcer from which a milky exudate drains. When these lesions contact water, the worm is stimulated to release very large numbers of larvae that are ingested by copepods in the water. Fine needle aspiration of the cutaneous nodules reveals rhabditiform *Dracunculus* larvae, approximately 500 μm long and covered by a striated cuticle. The adults lie in a pseudocyst lined by fibrous connective tissue and infiltrated with eosinophils, lymphocytes and multinucleated giant cells. Ulcerated lesions are seen as draining fistulae that may be painful or pruritic. *Differentials* include foreign body, neoplasia, infected wound, or cutaneous dirofilariasis. The parasite can be identified by morphologic features.

Dirofilaria immitis microfilariae can occasionally lead to cutaneous lesions in dogs harboring the adult parasite. A series of five cases document a scabies-like, papular to nodular, variably ulcerated, pruritic dermatitis of the skin of the head, trunk, and extremities in dogs infected with *D. immitis*. Histologic examination of affected skin revealed angiocentric and pyogranulomatous dermatitis with intralesional microfilariae. A type II hypersensitivity response was proposed as a pathogenic mechanism based on positive immunoreaction of microfilariae with anti-IgG serum. Lesions resolved with antiparasitic treatment. Ectopic adult *D. immitis* have been recovered from cutaneous abscesses and interdigital cysts in parasitized dogs. In addition, a generalized cutaneous syndrome characterized by a pruritic papular and crusting dermatitis without intralesional microfilariae has been apparently associated with infection with *D. immitis* and was thought to be a manifestation of an unusual hypersensitivity reaction. Parasites are not present in the cutaneous tissues. Lesions also resolved with antiparasitic treatment. *Dirofilaria repens* occurs in the subcutaneous connective tissues of dogs in Mediterranean countries and tropical parts of the Orient. The intermediate hosts are probably mosquitoes. The microfilariae occur in dermal lymphatics. This parasite is not known to be pathogenic. *Dipetalonema reconditum* occurs in the subcutis of dogs and coyotes. Its only significance is in the differential diagnosis of *D. immitis* infections by identification of microfilariae in peripheral blood.

Dermatitis associated with nematode microfilariae of **undetermined species** has been associated with a pruritic, papular, or plaque-like dermatitis of the head and limbs in dogs determined by multiple assays to be free of adult *Dirofilaria immitis* and *Dipetalonema reconditum*. Lesions were single to multiple, erythematous, alopecic, ulcerated, and crusted. Histologic changes included ulceration and perivascular, periglandular, or interstitial mixed inflammatory cell infiltrates with variable eosinophils and plasma cells. Microfilariae were free within the dermis or subcutis and within microgranulomas. No microfilariae were present within vessels. One adult nematode was also present in the cutaneous tissues and was determined to be *Acanthocheilonema* sp.

Incidental subcutaneous parasitism by the free-living form of the helminth, ***Gordius robustus*** has been reported in the cat. The cat developed a 1–2 cm firm subcutaneous nodule on the back. Histologically, the nodule consisted of an adult nematomorph surrounded by granulation tissue and pyogranulomatous to eosinophilic to lymphoplasmacytic infiltrates. *G. robustus* has a free-living adult form as well as a parasitic larval form that resides in the body cavity of freshwater or terrestrial insects. *Differentials* include many causes of solitary cutaneous nodules. Definitive diagnosis is dependent upon recognizing morphologic features of the parasite.

Suifilaria suis occurs in pigs in South Africa. The worms are 2–4 cm long and live in the subcutaneous and intermuscular connective tissues, sometimes producing small white nodules. The female is oviparous and the eggs are released to the surface via small vesicular eruptions in the epidermis. The remainder of the life cycle is unknown.

Parelaphostrongylus tenuis, a common parasite of white-tailed deer, is an occasional cause of neurological disease in goats. Some affected goats also develop an unusual dermatitis, often restricted to one side of the body. Lesions are vertically oriented, alopecic, ulcerated, crusted or scarred, linear tracks on the shoulder, thorax or flanks. The lesions have been explained tentatively on the basis of ganglioneuritis leading to irritation of dermatomes. Histologically, the lesion is a fibrosing dermatitis with focal areas of basal keratinocyte hydropic degeneration.

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MISCELLANEOUS SKIN CONDITIONS

Canine juvenile cellulitis

Canine juvenile cellulitis (juvenile sterile granulomatous dermatitis, puppy strangles, juvenile pyoderma) is an idiopathic disease typically affecting puppies less than 4 months of age. The condition can affect one or more puppies from a litter of any breed but golden and yellow Labrador Retrievers, Dachshunds, Lhasa Apsos, and Gordon Setters are most often reported. A condition clinically and histopathologically identical to juvenile cellulitis has also been reported in adult dogs. Immunologic, infectious, traumatic, parasitic and environmental conditions have all been proposed as possible etiologies. The fact that the lesions are sterile when cultured, nontransmissible, and respond to corticosteroids better than antibiotics alone eliminates infectious agents as probable causes. Special stains and electron microscopy have also failed to identify intralesional infectious agents. Immune system tests have not revealed abnormalities in affected animals. Depressed in vitro lymphocyte blastogenesis responses have been reported but likely represent the result not the cause of the disease.

Cutaneous lesions comprise papules, pustules, crusts, alopecia, and very marked edema (Fig. 5.116). Skin of the muzzle, face, ears, and occasionally the feet, abdomen, vulva, prepuce, and anus are affected. Otitis externa is common. Lymphadenopathy of the mandibular nodes is common and may precede the onset of the skin lesions. Lymphadenitis may also occur in nodes distant to the skin lesions and may occur in the absence of skin lesions. The cutaneous lesions are bilaterally symmetric, painful but not pruritic. Anorexia, fever, malaise and arthritis affecting multiple joints are not uncommon. *Histologically, the lesions are pyogranulomatous nodular to diffuse dermatitis with furunculosis and pyogranulomatous lymphadenitis.* Inflammation often extends into the panniculus. The differential diagnoses include severe bacterial dermatitis, demodicosis, and adverse drug reactions or other cause of angioedema. The condition resolves with proper treatment in 1.5–6 weeks and rarely recurs.

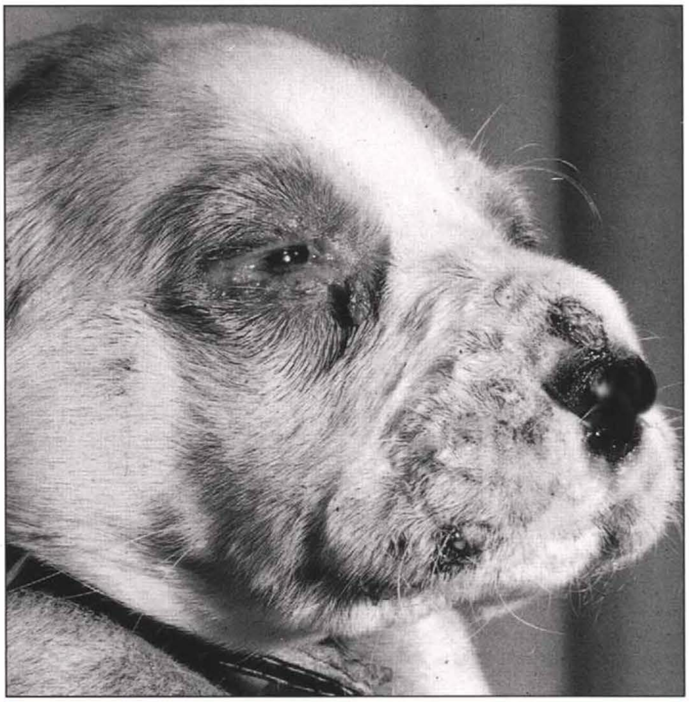

Figure 5.116 Marked soft tissue swelling in **juvenile cellulitis** in a puppy.

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Cutaneous paraneoplastic syndromes

Cutaneous paraneoplastic syndromes are dermatoses associated with internal neoplasms. The neoplasms may be malignant or benign but are most often malignant. In keeping with the definition of a paraneoplastic syndrome, *these conditions cannot be directly attributed to the anatomic location of the neoplasm.* The skin or mucosal lesions may precede, concur with, or follow the diagnosis of the underlying neoplasm. Removal or elimination of the tumor should alleviate the cutaneous lesions, while relapse of the dermatosis signals recurrence of the underlying neoplasm. The majority of cutaneous paraneoplastic syndromes in animals do not have a defined pathogenesis and often have only been reported a limited number of times. These syndromes are better documented in humans, but the increased recognition and clinical significance in domestic animals warrants mention in this chapter. The well-recognized cutaneous lesions seen with functional pituitary, adrenal or testicular tumors are discussed elsewhere (see Endocrine diseases of skin). Cutaneous lesions associated with the cryoglobulinemia associated with multiple myelomas have been discussed elsewhere (see Other immune-mediated dermatoses).

A unique dermatosis reported in aged cats is characterized by symmetrical ventrally distributed alopecia affecting the trunk and limbs (Fig. 5.117A) has been reported to be associated with a concurrent **pancreatic carcinoma** or **bile duct carcinoma**. The alopecia is due to *marked follicular atrophy* (Fig. 5.117B). Adnexal structures are also markedly atrophic. The epidermis lacks a stratum

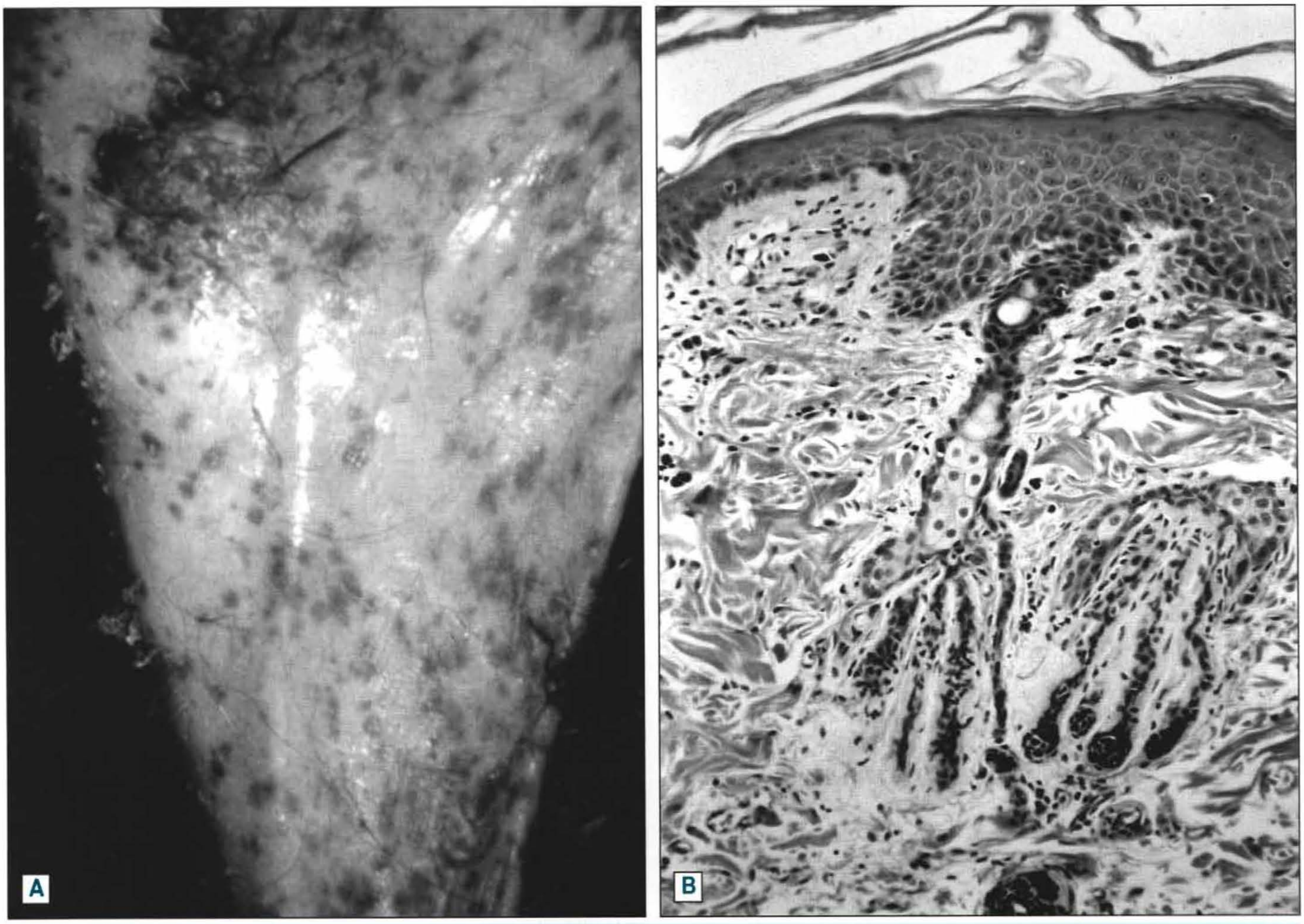

Figure 5.117 Feline paraneoplastic alopecia. (Courtesy of B Dunstan.) **A.** Glistening appearance of the skin on the medial aspect of the limb. **B.** Marked follicular atrophy.

corneum, giving the regions of affected skin the characteristic "glistening" or moist appearance. The lack of the stratum corneum may be due to licking by the cat. Inflammation is not a feature unless complicated by secondary infection. Footpads may be softened and hyperkeratotic. The pancreatic carcinoma is usually in an advanced stage, often with hepatic metastasis at the time of diagnosis and the prognosis is grave. The condition must be differentiated from hyperadrenocorticism and feline psychogenic alopecia.

Thymoma and concurrent **exfoliative dermatitis** has been reported in cats. Cats are usually aged and have signs such as dyspnea and coughing referable to an intrathoracic mass. Cutaneous lesions may be well developed prior to onset of respiratory signs. The cutaneous lesions consist of generalized, dry exfoliative dermatitis with multifocal ulceration, easily epilated hairs and variable pruritus. Histologically, marked orthokeratotic hyperkeratosis with patchy parakeratosis is usually present. The epidermis is hyperplastic with multifocal spongiosis and exocytosis of lymphocytes and variable neutrophils into the surface and follicular epithelium. The dermal component has been reported as either a perivascular mixed infiltrate or a lichenoid, lymphocytic dermatitis with hydropic changes in the basal epidermis and outer follicular root sheath. Epidermal and follicular keratinocyte apoptosis with lymphocytic satellitosis may be present. Some cases have been reported to also have a overgrowth of *Malassezia pachydermatis* and bacteria. It has been speculated that the presence of a thymoma initiates abnormal immunological responses responsible for the dermatitis, which at times can resemble erythema multiforme or graft vs. host dermatitis histologically.

Superficial necrolytic dermatitis (hepatocutaneous syndrome) has been reported in dogs and occasionally the cat. The condition is sometimes associated with **endocrine tumors of the pancreas** (see Nutritional diseases of skin).

Multiple **nodular dermatofibromas** of the distal limbs have been reported in middle-aged German Shepherd Dogs and occasionally other breeds (Golden Retriever, Boxer, Belgian Shepherd, mixed breed) in association with **renal cysts**, **renal cystadenocarcinomas** and/or **uterine leiomyomas** in the bitch. Both kidneys are often affected. The cutaneous lesions range from 2 mm to several centimeters and are comprised of mature, sparsely cellular bundles of collagen within the subcutis or occasionally the dermis (Fig. 5.118A, B). Animals may be presented for examination of the cutaneous lesions prior to signs referable to the internal neoplasms. Death occurs most often due to complications of the renal lesions. Nodular dermatofibrosis is suspected to be inherited in an autosomal dominant manner in German Shepherd Dogs.

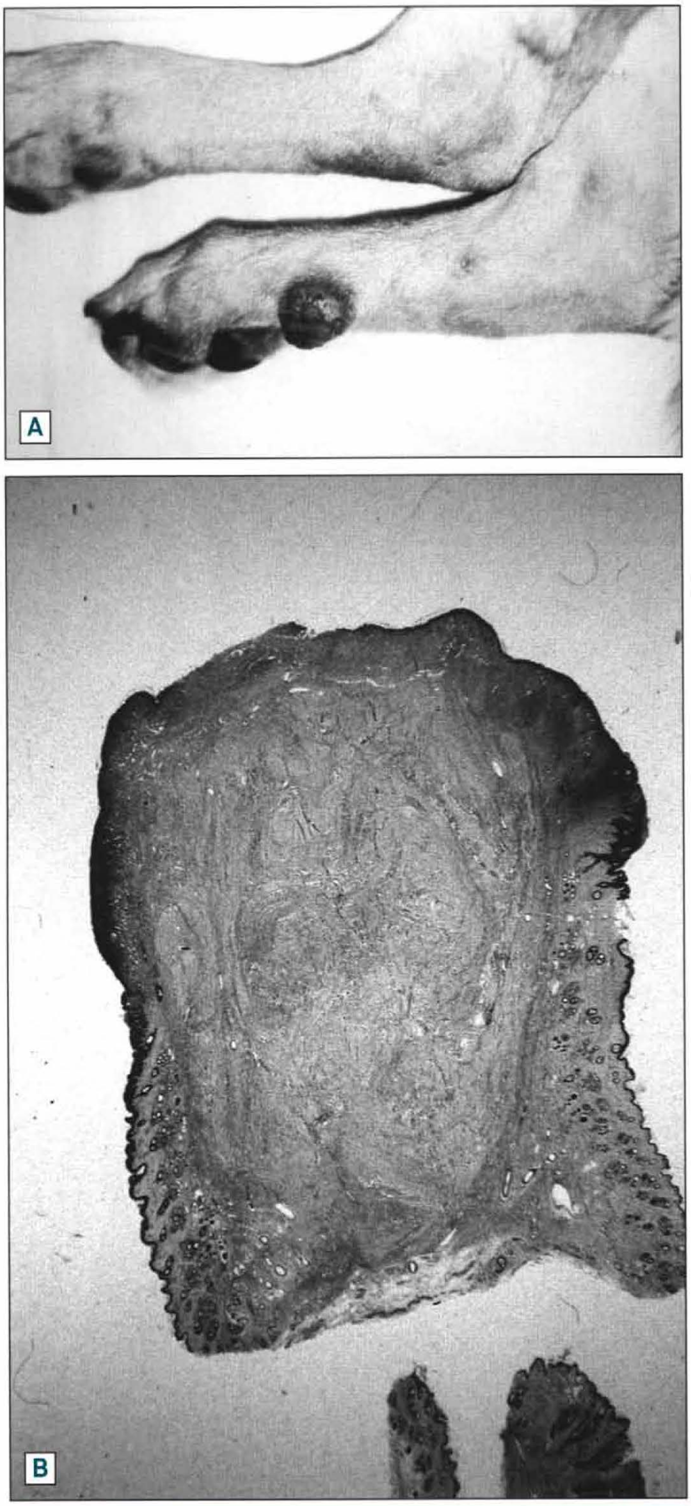

Figure 5.118 Dermatofibrosis in a German Shepherd Dog. (Courtesy of University of Florida Clinical Dermatology Service.) **A.** Protruding fibrous nodule on the distal limb. **B.** Nodule from **(A)** comprises sparsely cellular bundles of mature collagen.

Severe **generalized pruritus** without primary gross or histologic cutaneous lesions or other identifiable cause for pruritus has been reported in the dog and horse in association with underlying **malignant lymphoma**. Self-trauma can lead to extensive excoriations and secondary infection. This condition is also reported in humans with lymphoma and a variety of other internal malignancies. The mechanism of the development of pruritus is not understood but is speculated to be related to tumor-induced release of chemical mediators. Pruritus can only be relieved by successful treatment of the malignancy.

Paroxysmal **cutaneous flushing** of extensive areas of the skin without pruritus has been reported in a German Shepherd Dog with an intrathoracic mast cell tumor and pulmonary adenocarcinoma. Functional pheochromocytomas upon rare occasion can lead to cutaneous flushing in the dog. Flushing is due to vasodilation and is usually caused by vasoactive mediators.

Occasionally, vesicular, pustular or bullous dermatoses virtually identical to idiopathic autoimmune skin diseases may be associated with an underlying malignancy. The tumor is suspected to trigger the production of autoantibodies. These types of paraneoplastic dermatoses must be differentiated from true autoimmune skin diseases as the prognosis may vary. Differentiation is based on ruling out the presence of an underlying neoplasm and resolution of the skin lesions with tumor removal. A **subepidermal bullous stomatitis** microscopically identical to bullous pemphigoid has been reported in a horse in association with a hemangiosarcoma. Immunoprecipitation studies indicated the horse had antibodies to desmoplakin I and II, the bullous pemphigoid 230 antigen, and a 190 kDa antigen. This immunoprecipitation profile was consistent with that reported for **paraneoplastic pemphigus** as reported in humans and the dog. Paraneoplastic pemphigus is a form of pemphigus that is sometimes associated with an underlying malignancy and has been previously discussed (see Immune-mediated dermatoses).

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Eosinophilic and collagenolytic dermatitides

The presence of eosinophils in inflammation is usually considered to be an indication of a hypersensitivity reaction or parasitic infestation, however eosinophils can be seen in many diseases and complete understanding of the role of this leukocyte remains to be elucidated. Eosinophils produce lipid mediators that contribute to an acute allergic response, and also TGF-β that is associated with chronic inflammation and fibrosis. Eosinophil cytoplasmic granules contain four cationic proteins that are essential in host defense against parasites, however in the process of this defense the contents of the granules also cause severe tissue damage and contribute to the collagen degeneration and collagenolysis which are so common in many eosinophilic dermatitides. Eosinophilic inflammation in the horse and cat is a common tissue reaction and it seems that the chemotactic stimuli that attract neutrophils in most species attract both eosinophils and neutrophils in these two species.

Feline eosinophilic dermatoses

Cats have several distinct eosinophilic dermatitides, some of which have been previously collected under the term **eosinophilic granuloma complex**. These conditions include *feline eosinophilic granuloma, eosinophilic plaque, and indolent ulcer.* The collective term has been useful as a grouping since these conditions respond to similar treatments. Histologically these three conditions are distinct, although occasionally the features overlap. In addition, allergic dermatitis in cats, such as hypersensitivity to ectoparasites, atopy, and food allergic dermatitis, can demonstrate overlapping features, especially with eosinophilic plaque and occasionally with feline eosinophilic granuloma. Indeed eosinophilic plaque has been established as a hypersensitivity, and although not as yet fully characterized, eosinophilic granuloma and indolent ulcer may well eventually be shown to have an allergic basis. Cats with eosinophilic granuloma can also have eosinophilic plaque or indolent ulcer.

Feline eosinophilic plaque *is a common condition in cats and is a hypersensitivity reaction to food, fleas, or may be associated with atopy.* The condition can occur alone or together with miliary dermatitis, and in some cases the two conditions can form a continuum. It occurs most commonly in the inguinal, axillary, perineal or lateral thigh areas, although it can occur anywhere. *Lesions are pruritic, and affected cats lick them constantly*, resulting in raised, red, ulcerated plaques and patches that can develop peracutely. *Histologically the lesions are characterized by epidermal acanthosis and spongiosis with exocytosis of eosinophils* (Fig. 5.119A, B). In some cases there is epidermal and follicular epithelial mucinosis characterized by pale basophilic or gray mucin between keratinocytes. Inflammation in the dermis is diffuse or perivascular, markedly eosinophilic, and may extend into the subcutis. Mast cells, lymphocytes, and macrophages are present in smaller numbers.

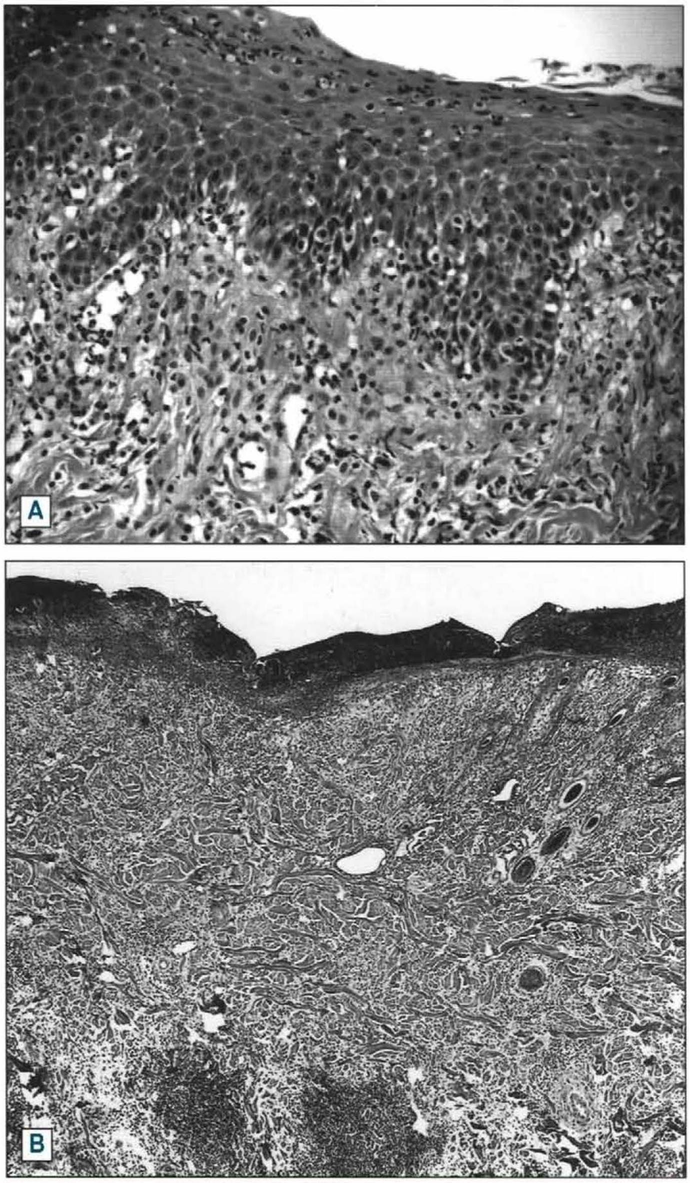

Figure 5.119 Feline eosinophilic plaque. A. Hyperplastic, spongiotic epidermis. **B.** Dermal infiltrate of eosinophils and mast cells.

Feline eosinophilic granuloma (feline collagenolytic granuloma, linear granuloma) is a common condition in cats. It can occur at any age, however is *more common in young cats.* Spontaneous regression can occur. An underlying hypersensitivity has been suggested as the cause however the condition may also occur in cats with no other evidence of allergic disease. A genetic predisposition has been documented in some cases. The lesions can be nodular or linear, and occur on the skin, footpads, mucocutaneous junctions, and in the oral cavity. Linear lesions are more common on the caudal or medial thigh and nodular lesions are more common on the lips, chin, oral cavity and face. The lesions are raised, pink and frequently alopecic. Histologically the lesions are characterized by *diffuse dermal inflammation composed primarily of eosinophils*, with fewer mast cells, macrophages, and occasional lymphocytes. *Within the inflammation are large irregular foci of collagen fibers and degranulated an degenerating eosinophils (flame figures)* (Fig. 5.120). These foci may be surrounded by macrophages and multinucleated giant cells. The epidermis may be acanthotic or ulcerated.

Indolent ulcer (rodent ulcer) is a common and clinically distinct condition in cats. The cause of these lesions is unknown. In

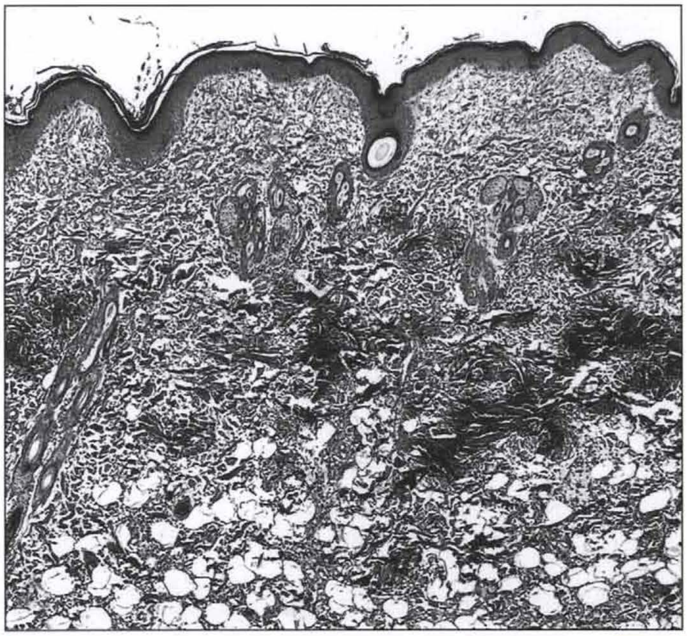

Figure 5.120 Eosinophilic granuloma in a cat. The dermis contains multiple foci of collagen fibers (arrow) surrounded by degranulated and degenerating eosinophils and mononuclear leukocytes (flame figures).

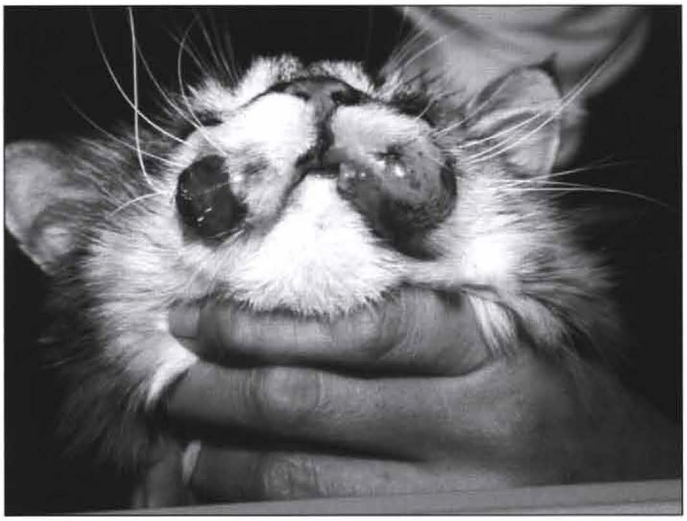

Figure 5.121 Feline indolent ulcer. Ulcerated lesions on the upper lips. (Courtesy of B Dunstan.)

some cats they are reported to be a manifestation of allergic disease, although in other cases an allergic etiology cannot be found. Affected cats can have concurrent eosinophilic granuloma or eosinophilic plaque, increasing the index of suspicion of an allergic etiology. *Clinically the condition is characterized by an ulcerated lesion on the upper lip adjacent to the philtrum that can be unilateral or bilateral* (Fig. 5.121). The ulcers are not pruritic or painful. Histologically the acute lesions are characterized by diffuse infiltrates of eosinophils with neutrophils, mast cells, and macrophages, however the lesions are more often biopsied in the chronic phase when the inflammation is composed almost entirely of lymphocytes, plasma cells, macrophages and neutrophils, together with fibrosis. The numbers of neutrophils vary according to the degree of ulceration.

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Canine eosinophilic granuloma

This is a *rare condition in dogs* that histologically has many similarities to feline eosinophilic granuloma. Any breed and age can be affected, however the condition appears to be more common in Siberian Huskies, in dogs under 3 years of age, and males. There may be a genetic basis for the disease in Siberian Huskies and Cavalier King Charles Spaniels. The etiology is unknown although a hypersensitivity has been proposed, due to the fact that the lesions are corticosteroid-responsive, the eosinophilic nature of the lesions, and that there is occasionally circulating eosinophilia. *The lesions can be nodules or plaques, and occur most commonly in the mouth and on the tongue*. Cutaneous lesions can be multiple and there is one report of a single lesion occurring in the external ear canal. *Histologically the lesions are composed of diffuse dermal eosinophilic inflammation within which are foci of degranulating eosinophils and degenerating collagen sometimes surrounded by epithelioid macrophages*. The overlying epithelium or epidermis may be acanthotic or ulcerated.

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Equine eosinophilic nodular diseases

Eosinophilic inflammation is a common reaction pattern in the horse, and cutaneous nodules, either single or multiple, are common skin lesions in the horse. Nodular conditions of unknown etiology and pathogenesis include collagenolytic granuloma (nodular necrobiosis), axillary nodular necrosis, and unilateral papular dermatosis. Other eosinophilic nodular conditions with known etiologies, such as cutaneous habronemiasis, can look histologically similar (see Helminth diseases of skin). Cutaneous mast cell tumors in the horse can also be seen clinically as cutaneous nodular lesions, and, as histologically they may contain significant numbers of eosinophils and areas of necrosis, care should be taken to differentiate this tumor from an inflammatory eosinophilic lesion.

Collagenolytic granuloma (nodular necrobiosis, equine eosinophilic granuloma) is the *most common* of the equine cutaneous eosinophilic nodular diseases. The etiology is unknown. Hypersensitivity, especially to insect bites, has been suggested,

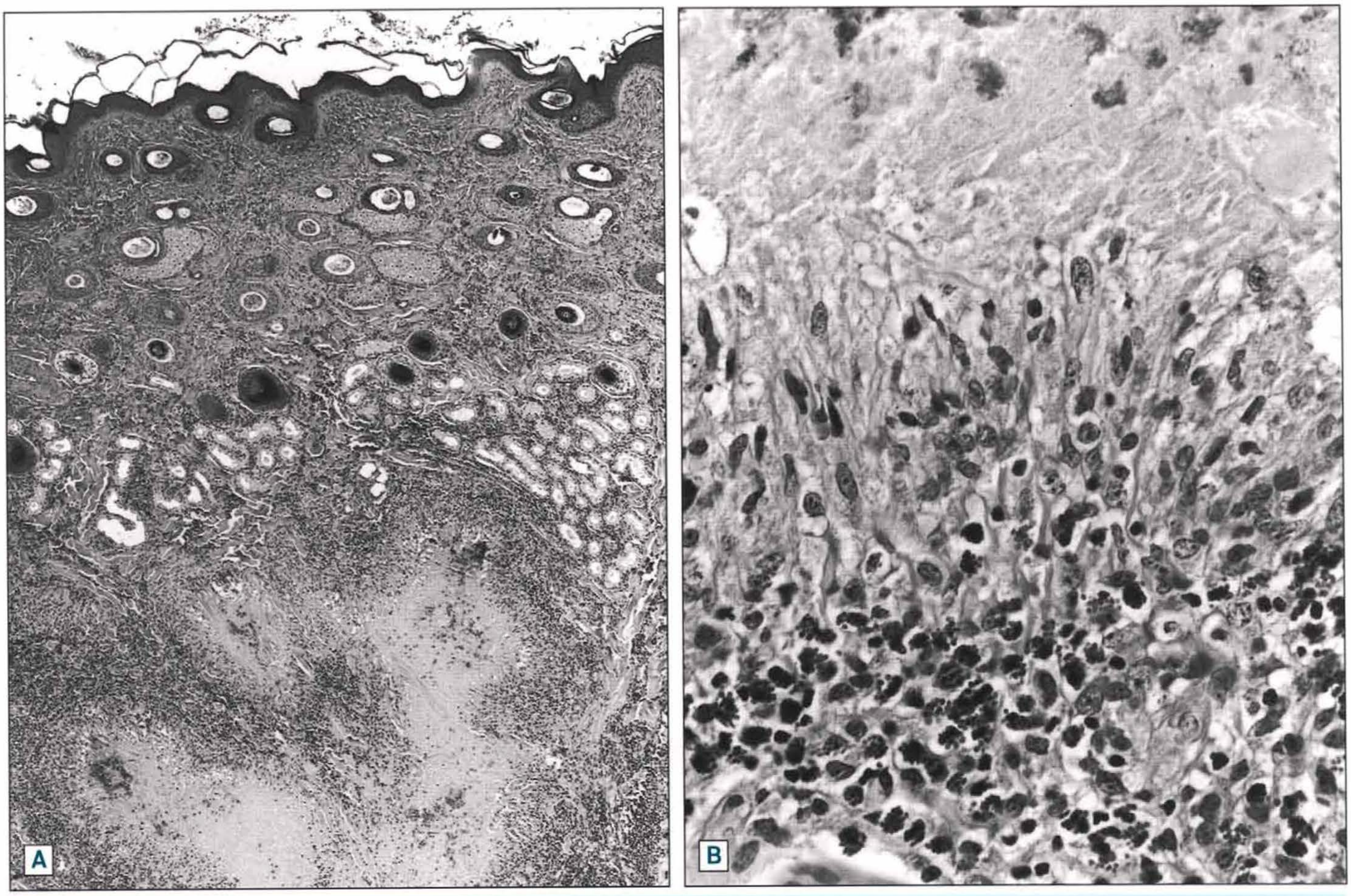

Figure 5.122 Equine nodular collagenolytic granuloma. A. Foci of collagen degeneration deep in the dermis. **B.** Degenerating collagen and degranulating eosinophils are surrounded by epithelioid macrophages and additional eosinophils.

however this has not been fully established. The lesions occur as single or multiple firm nodules measuring 0.5–5 cm in diameter, most commonly on the withers and back but can occur anywhere on the body, and can be generalized. *Histologically the dermal lesions are characterized by foci of dermal collagen degeneration that are surrounded by granulomatous inflammation, sometimes forming a palisading granuloma, and inflammation that is markedly eosinophilic* (Fig. 5.122A, B). Eosinophilic granulomas have also been reported associated with the silicone coating on needles at injection sites, however these lesions are clinically distinct as they tend to be linear, and can usually be documented to be at an injection site.

Axillary nodular necrosis is considerably less common than collagenolytic granuloma. The cutaneous and subcutaneous nodules are 1–5 cm in diameter and can be single or multiple. They are usually unilateral and occur on the trunk behind the axilla, which explains the colloquial term "girth galls." Trauma from tack can explain the ulceration of nodules but is not considered to the cause, which is currently unknown. *Histologically the lesions are characterized by foci of coagulative necrosis that may contain eosinophils, surrounded by eosinophilic and granulomatous inflammation.* Eosinophilic vasculitis can sometimes be found. The collagen degeneration that is a feature of collagenolytic granuloma is not usually a feature of axillary nodular necrosis.

Unilateral papular dermatosis is an uncommon condition in horses seen primarily in the United States, and is more common in spring and summer. It has been seen in many breeds although Quarter Horses appear to be over-represented. *Clinically the lesions are multiple cutaneous nodules and papules, measuring 2–10 mm, which occur in a unilateral distribution, usually on the trunk.* The etiology is unknown, however the seasonality of the lesions together with the eosinophilic nature of the histologic findings *suggests ectoparasite hypersensitivity*. It has further been suggested that the unilateral distribution may indicate direct contact hypersensitivity to an ectoparasite. *The histologic lesions are characterized by small foci of eosinophilic coagulative necrosis that are often folliculocentric, surrounded by eosinophilic dermal inflammation.* Histologic differentiation of this condition from the two preceding nodular diseases in horses, especially in the absence of folliculocentricity, can be problematic without a clinical description of the lesions.

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Multisystemic, eosinophilic, epitheliotropic disease in the horse

This rare disease typically occurs in young horses and is characterized by eosinophilic infiltration of many organs, with intestine and skin being most commonly affected. The cause of multisystemic, eosinophilic, epitheliotropic disease is unknown. Proposed etiologies have included viral, bacterial, and toxic mechanisms, however none of these has been confirmed. Recurrent episodes of type I hypersensitivity due to dietary, inhaled or parasitic antigens has been suggested in several reports. There is one report of this condition occurring concurrently with intestinal lymphosarcoma, and several other unpublished cases that appear identical to the case described; the authors suggest that the clonal proliferation of T lymphocytes triggers proliferation of eosinophils by secretion of cytokines such as IL-5. This mechanism has also been proposed in humans who have concurrent abnormal T-lymphocyte proliferations and hypereosinophilic syndrome. Affected horses are presented clinically with weight loss, pitting edema, and often with exudative, exfoliative dermatitis that usually originates at the coronary bands or head and becomes generalized. Eosinophilia is generally absent. Histologically the condition is characterized by *eosinophilic and lymphoplasmacytic infiltration of multiple organs, sometimes with eosinophilic granuloma formation.* Organs most commonly affected include pancreas, gastrointestinal tract, salivary glands, and skin. In the skin the inflammation may be perivascular and interstitial, with epidermal acanthosis and hyperkeratosis, and sometimes with spongiosis and eosinophilic exocytosis into eosinophilic subcorneal pustules.

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Sterile eosinophilic folliculitis and furunculosis (eosinophilic furunculosis of the face)

This is an uncommon condition in **dogs** characterized by the sudden onset of painful folliculocentric nodules and papules with erosion or ulceration. The etiology of this condition has not been fully characterized, but *a hypersensitivity reaction to arthropod bites is strongly suspected.* The condition is most common in young, large breed dogs, and lesions are primarily seen on the dorsal muzzle, around the eyes, ears, and less commonly in the axillae, inguinal areas, and ventrum. Histologic lesions are characterized by predominantly folliculocentric eosinophilic inflammation. Eosinophilic luminal inflammation can lead to furunculosis, with free hair shafts surrounded by eosinophils and degranulating eosinophils. The dermal inflammation may be accompanied by edema and mucin deposition, and occasionally dermal collagen necrosis is present.

In **cats**, sterile eosinophilic folliculitis and furunculosis may be seen as a bystander lesion in any allergic dermatitis, however is more commonly seen in mosquito-bite hypersensitivity and feline herpesvirus dermatitis, both of which are most commonly located on the face and head. In **horses**, sterile eosinophilic folliculitis and furunculosis can be seen in any of the eosinophilic nodular conditions or hypersensitivities. However, other than unilateral papular dermatosis, the follicles are affected secondarily and the lesions are not folliculocentric. Sterile eosinophilic folliculitis has been reported in **cattle** and is characterized by multiple crusting or alopecic lesions primarily on the head, neck, and trunk.

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Eosinophilic dermatitis with edema

This is a rare condition in dogs. The cause is unknown, although hypersensitivity to drugs, arthropods, or diet is suspected. *It is characterized by erythematous macules and plaques primarily involving the ventral abdomen and ears,* and has been compared to Wells' syndrome (eosinophilic cellulitis) in humans. The histologic lesions are marked perivascular to diffuse eosinophilic dermatitis with edema, and occasional foci of collagen degeneration.

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Feline relapsing polychondritis

Feline relapsing polychondritis is a rare condition in the cat characterized by bilateral dorsal or medial curling of the pinnae. The pinnae are discolored purple and markedly edematous. Histologically, lymphoplasmacytic infiltrates surround areas of necrosis of the auricular cartilage. The condition was named after a similar human condition thought to be an immune-mediated attack directed against type II collagen and known to affect other cartilaginous sites such as the nose, trachea, and cardiac valves. The pathogenesis in cats is not defined and despite the name, the condition in cats is not known to be recurrent. Too few cases have been studied to accurately document the clinical course or define association with other diseases.

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Follicular lipidosis

Regional alopecia of areas of mahogany-colored points of the hair of the feet and face of young Rottweilers has been reported. Histologically, the lesions are characterized by vacuolation and nuclear pyknosis of the hair matrix cells of primary anagen hair follicles. Matrix cells of the bulb are most severely affected while cells of the internal and external root sheath are affected to a lesser degree. The vacuoles are clear or contain fine granular or clumped melanin. Hair shafts also contain occasional vacuoles and have irregular, thickened, or frayed cuticles. Ultrastructural examination and oil-red-O staining of additional sections confirm the vacuoles to be lipid. *This condition may represent yet another type of color-associated follicular dysplasia.* Too few cases have been recognized to clearly predict the clinical course of the alopecia, or to identify associated abnormalities or the mode of inheritance, if any. The cause has not been determined.

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Follicular mucinosis (alopecia mucinosa)

Follicular mucinosis is characterized by collections of mucin in the outer root sheath of the follicular epithelium and of sebaceous glands. Intraepithelial cysts filled with mucin may form. Grossly, the lesions consist of patchy alopecia and scaling. A disease resembling follicular mucinosis in humans has been described in the **cat** and **dog**. The condition described in 2 cats chiefly affected the head, neck, and shoulders. Histologically, there was mucinous degeneration of the outer root sheath of the follicular infundibulum. Both cats developed epitheliotropic lymphoma within several months of the initial biopsy. Follicular mucinosis has been reported in a 10-year-old male Labrador Retriever. The skin of the head, limbs, and some areas of the trunk were affected. In addition to the histologic lesions described above, there was a perivascular and perifollicular lymphocytic to plasmacytic infiltrate. The epidermis was mildly acanthotic with compact orthokeratosis and multifocal spongiosis with lymphocytic exocytosis. The basal cell layer demonstrated hydropic degeneration, mild apoptosis and prominent pigmentary incontinence. No follow-up information was available. In humans, follicular mucinosis can resolve spontaneously or become a chronic relapsing condition. Some cases are thought to progress to epitheliotropic lymphoma, but this is controversial. It should be noted that epidermal and epithelial mucinosis can be seen in various allergic dermatitides of the cat.

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Laminitis

The hoof wall is a complex structure composed of an epidermis and dermis that attaches to the underlying distal phalanx. The distal phalanx is held in place by interdigitation of the epidermal lamina of the inner hoof wall and the dermal lamina of the corium that is attached to the third phalanx. When these laminae fail, the forces of body weight, motion, and tendons lead to sinking and rotation of P3, shearing of vessels that supply these tissues, and damage to the corium of the sole and coronet. *The separation of the distal phalanx (coffin bone, P3) from the inner hoof wall is responsible for the severe clinical signs in laminitis*, rather than a primary inflammatory process. Laminitis occurs in all hoofed species, but particularly affects horses and cattle. *Laminitis is one of the most devastating of equine diseases, often leading to chronic debilitation or euthanasia.*

Precipitating events include trauma and generalized metabolic disturbances. In the horse, laminitis is sporadic, although fat, underexercised ponies are especially predisposed. Colic, sepsis, toxemia, episodes of diarrhea or shock, excessive water (after exercise), or lush pasture intake, carbohydrate overload, drug therapy, and intense training with repeated foot trauma ("road founder") have all been implicated as precipitating events in the horse. Black walnut (*Juglans nigra*) heartwood shavings toxicity causes natural disease in the horse.

Laminitis occurs sporadically in *dairy cows, heifers, fattening cattle, and young bulls*. In cattle, carbohydrate overload is also an important predisposing cause of laminitis. Others include metritis, mastitis, and ketosis. A heritable form has been reported in Jersey cattle in South Africa, the USA, and the UK. *Laminitis, not related to traumatic and metabolic episodes, occurs in all species but is important only in ungulates.* Erysipelas in lambs, the various causative types of footrot in pigs, sheep, and cattle, and bluetongue in sheep are examples of diseases in which degenerative and inflammatory changes occur in the laminae of the hoof.

The *pathogenesis* of metabolic laminitis remains incompletely understood although progress has been made. Past studies suggested that a *primary ischemic event* precipitated laminitis. Recent studies using an equine model of carbohydrate overload-induced laminitis indicate that degeneration of primary epidermal laminae and the basement membrane are responsible for the architectural changes in the hoof wall and the onset of clinical signs. Vascular events leading to ischemia also play an important role in laminitis but are now thought to occur as a consequence of the initial laminar degenerative changes. Events triggering the degeneration are still not completely defined, however bacterial-derived soluble factors generated in the equine large intestine have been shown in vitro to activate laminar enzymes capable of lysing components of the epidermal laminae. Two of these enzymes, metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) are present in normal laminar tissues and levels are increased in laminar tissues affected by laminitis. MMP-2, MMP-9, and inhibitors of MMP enzymes are thought to work in concert to regulate epidermal cell-to-cell detachment/attachment and cell-to-basement-membrane attachment during regular growth and maintenance of the laminar tissues. Cultured human keratinocytes increase production of MMP enzymes under the influence of TNF, IL-1 and TFG-1. Factors leading to increased production of MMP-2 and MMP-9 in the epidermal laminae during laminitis are not yet known.

The earliest changes evident in the laminar tissue anatomy during acute laminitis are elongation and disorganization of basal and parabasal keratinocytes and attenuation of the tips of the secondary epidermal laminae. The basement membrane of the secondary laminae detaches first in the region of parabasal cell attachment and subsequently in the region of the basal cells leaving aggregates of degenerating basement membrane components within the connective tissue space between the laminae. *Loss of the basement membrane leads to separation of the dermal and epidermal laminae of the hoof wall and* *is key in loss of structural integrity of the P3/hoof wall attachment.* Collapse of secondary laminae leads to loss of capillaries normally present within the connective tissue between the epidermal laminae. The loss of capillaries leads to increased resistance to blood flow, arteriovenous shunting, and eventual ischemic damage. This early stage is characterized clinically by a bounding digital pulse. Current research suggests that the progression of laminitis in the very early stages prior to degeneration of the epidermal laminae and basement membrane may possibly be slowed or halted by attempts to produce vasoconstriction in the foot (ice-packing). This slowing of circulation would theoretically decrease the delivery of cytokines or other factors responsible for induction of MMP activity.

Acute laminitis is seen as sudden lameness and severe pain affecting most commonly the forefeet but may affect all feet, or just the hind feet. An increase in the hoof wall temperature and a bounding digital pulse indicate marked vascular engorgement in the hoof tissues. In horses, pain is often severe enough to provoke systemic disturbances. A section through an acutely affected hoof reveals little gross alteration beyond congestion of the laminar dermis and occasionally hemorrhage. There is no hoof deformity, although the skin above the coronary band may be swollen. Horses with diffuse swelling and depression along the coronary band are often found to have acute separation and displacement of P3 and are referred to as "sinkers," a manifestation of acute laminitis. Microscopic changes are as described above. In addition, the dermis may be congested, edematous, and have mild hemorrhage and mild infiltrates of mononuclear cells. In time, coagulative necrosis of the secondary laminae may be evident.

Chronic laminitis is defined clinically as the phase commencing after several days of lameness or when *rotation of the third phalanx is first radiographically evident.* The rotation has been attributed to loss of the interlocking force normally supplied by the epidermal laminae. In chronic cases, the ventral deviation is caused also by irregular hyperplasia of epidermal laminae placing a wedge of epidermis between the phalanx and the immovable hoof wall. The weight of the animal, the leverage forces placed on the toe and the pulling forces of the deep digital flexor tendon contribute mechanically to the rotation. *In severely affected animals, the third phalanx may penetrate the sole*, which becomes convex. A mid-sagittal section through the hoof wall at this point will show obvious separation of P3 from the dorsal hoof wall, sinking and various degrees of rotation of P3. The corium at the coronary band and sole may be edematous or hemorrhagic. In long-standing cases, the space between the dorsal hoof wall and P3 is filled with firm white tissue (proliferative epidermis). The toe usually turns up and the cranial aspect of the hoof becomes concave and wrinkled by encircling horizontal ridges.

The chief microscopic lesion in chronic equine laminitis is marked irregular hyperplasia of the epidermal laminae. The regenerating secondary laminae may not regain their orderly arrangement and instead form irregular and anastomosing epidermal cords. The epidermal laminae, both primary and secondary, become markedly hyperkeratotic. The reason for the hyperkeratosis is not known. Both physical and physiologic influences on keratogenesis are likely altered in chronic laminitis. Similar epidermal lesions occur in chronic bovine laminitis, although parakeratotic hyperkeratosis develops in addition to the orthokeratotic hyperkeratosis. Alterations in the dermal vasculature are most prominent in cattle. Moderate-to-marked arteriolosclerosis and arteriosclerosis occur in chronic bovine laminitis, especially in the solar dermis. Other changes in laminitis in cattle include chronic dermal granulation tissue, organized and recanalized vascular thrombi, perineural fibrosis and perivascular accumulations of macrophages, often containing hemosiderin.

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Localized scleroderma

Localized scleroderma (morphea) is a rare disease of dogs and cats. *Asymptomatic, well-demarcated, sclerotic plaques that are alopecic, smooth and shiny characterize the condition.* Lesions tend to be linear and occur on the trunk, limbs, and head. Diffuse fibrosing dermatitis is seen histologically. Dense collagen bundles replace the entire dermis and subcutis. Pilosebaceous units are essentially absent. A mild superficial and deep perivascular accumulation of lymphohistiocytic cells is present. The pathogenesis is not defined. Affected animals are otherwise healthy and lesions have been reported to regress spontaneously in some cases.

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Porcine juvenile pustular psoriasiform dermatitis

This disease of weanling pigs was originally named **pityriasis rosea**. Because the clinical signs and gross lesions bear little relationship to those of the human disease for which it was originally named, the new designation, porcine juvenile pustular psoriasiform dermatitis, has been suggested. *The disease is of no significance, except esthetic. The cause is not known.* A hereditary predisposition has been suggested but not proven, particularly in Landrace pigs. Lesions develop most often in weaned pigs 3–14 weeks of age. Entire litters or just a few piglets may be affected.

The disease begins with nonpruritic, small scaly, erythematous papules on the skin of the abdomen and inner thighs. The papules expand centrifugally to produce at first scaly plaques and later, when the central areas return to normal, ring-shaped, erythematous lesions (Fig. 5.123). As the rings expand, they coalesce to produce mosaic patterns and may extend to the sides and perineum. *The clinical differential diagnoses include swinepox, dermatosis vegetans, and ringworm.* The acute histologic lesion is superficial and deep perivascular dermatitis with eosinophils, neutrophils and mononuclear cells. Spongiosis and leukocytic exocytosis lead to the formation of spongiform pustules, and there is superficial dermal mucinous degeneration. Superficial epidermal necrosis may extend into the ostia of the hair follicles. As the lesions heal, marked psoriasiform hyperplasia and parakeratotic scale-crusts are predominant. The condition spontaneously resolves within about 4 wk.

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Spiculosis

Spiculosis is a dysplastic and dyskeratotic condition of the hair follicle that results in 1–2 mm diameter and 0.5–2.0 cm long brittle spicules (hair shafts) protruding from hair follicles. The condition has been reported in humans and Kerry Blue Terriers. Clinically, the condition is characterized by coarse, brittle, and long hair shafts and pruritus, scaling, and alopecia of the skin of various areas of the body. Histologically, hair follicles have enlarged hair bulbs that may have two dermal papilla. Enlargement of the hair bulb is due to collections of heavily pigmented matrix cells that keratinize prematurely to form hyperpigmented keratinized amorphous masses or columns of unpigmented keratin in place of normal hairs.

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Sterile granulomas and pyogranulomas

Sterile granulomatous or pyogranulomatous dermatoses have been reported in dogs, cats, and less commonly in horses. Absence of microbial agents and foreign material and good response to glucocorticoid therapy has suggested an *immune-mediated pathogenesis.*

Sterile pyogranuloma syndrome

The sterile granuloma/pyogranuloma syndrome (idiopathic periadnexal multinodular granulomatous dermatitis) occurs in **dogs** of all ages and sexes but Collies, Boxers, Great Danes, Weimaraners, English Bulldogs, Doberman Pinschers, Dachshunds, and Golden Retrievers appear to be predisposed. The condition is clinically and, in many respects, histologically very similar to cutaneous reactive histiocytosis as described in dogs (see Histiocytic proliferative disorders). Future studies may indicate that these two entities are a single disorder. Most

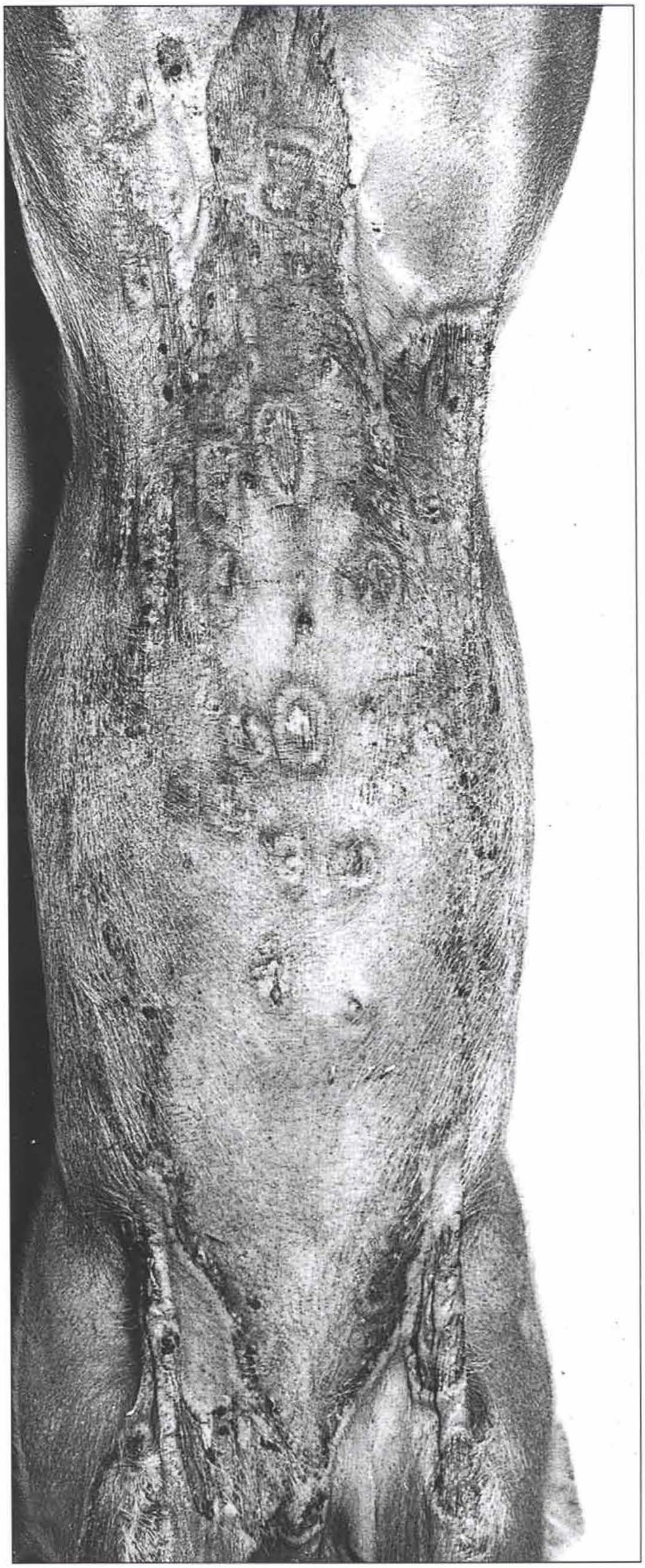

Figure 5.123 Porcine juvenile pustular dermatitis. Circular, ring, and coalescing lesions of the ventral abdomen and proximal limbs. (Courtesy of JA Flatla.)

dogs have multiple asymptomatic haired to partially alopecic papules, nodules or plaques on the face and/or feet. Pedal lesions are frequently secondarily infected, ulcerative and fistulous. The pinnae, periocular region, trunk and abdomen may also be affected and lymphadenopathy may be present. Dogs are not systemically ill and the condition may spontaneously resolve or wax and wane. Histologic findings include *large perifollicular granulomas or pyogranulomas* that are elongated and vertically oriented that track hair follicles but do not invade them. Older lesions may become diffuse; obliterating adnexal structures and extending extend into the subcutis. Histiocytes, lymphocytes and neutrophils predominate, with occasional plasma cells or multinucleated giant cells.

Sterile granulomatous or pyogranulomatous dermatitis has also been reported in **cats**. Older male cats had multiple, pruritic, erythematous-to-violaceous papules, nodules and plaques, especially on the head and pinnae. Histologically these lesions were characterized by perifollicular pyogranulomatous dermatitis. Middle-aged female cats had pruritic, bilaterally symmetric, erythematous-to-purpuric plaques in the temporal regions. Histologically these lesions were characterized by nodular-to-diffuse granulomatous inflammation wherein multinucleated histiocytic giant cells, unexplained purpura and erythrophagocytosis, and a superficial dermal Grenz zone were prominent features.

The histologic differential diagnoses includes inflammation due to the presence of infectious agents or foreign material, sarcoidosis, sebaceous adenitis, and cutaneous reactive histiocytosis. Negative special stains for bacterial and fungal organisms, negative cultures, lack of polarization of foreign material on microscopic examination and failure to respond to antibiotics are supportive. Sebaceous glands are clearly the target of inflammation in sebaceous adenitis leading to somewhat regular and mid-dermal perifollicular location of inflammatory nodules. Lesions of cutaneous histiocytosis may be difficult to distinguish and both conditions may have a waxing and waning course. Distinct, organized granulomas are typically not present in cutaneous reactive histiocytosis whereas they may be present in sterile nodular pyogranuloma syndrome. Histiocytes are often larger, possibly atypical and may have numerous mitotic figures in cutaneous reactive histiocytosis.

"Sarcoidal" granulomatous disease

Sarcoidosis in humans is a systemic granulomatous disease of undetermined etiology. The granulomas are characteristic, being composed predominantly of epithelioid macrophages with few lymphocytes ("naked" granulomas).

A sterile "sarcoidal" granulomatous dermatitis has been described in **dogs** and **horses**. Dogs develop multiple erythematous papules, nodules and plaques that were neither pruritic nor painful. The lesions most commonly affected the neck and trunk. Nodular-to-diffuse sarcoidal granulomatous inflammation was present histologically.

Equine generalized granulomatous disease is characterized by exfoliative dermatitis, wasting, and sarcoidal granulomatous inflammation in multiple organ systems and is discussed in more detail in Vetch toxicosis and vetch-like diseases.

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Sterile nodular panniculitis

Panniculitis refers to inflammation of the subcutaneous adipose tissue. Panniculitis has many causes, most of which have been discussed in previous sections (bacteria, fungi, immune-mediated disease, trauma, injection of irritant substances, foreign bodies, nutritional disorders, adverse reactions to vaccines or other injections, pancreatic disease). *The syndrome of sterile nodular panniculitis currently is considered to be idiopathic and the diagnosis is largely based on elimination of known causes of panniculitis, particularly panniculitis due to infectious agents.* It is likely that future studies will provide insight into the pathogenesis of lesion formation and define specific diseases now grouped within this syndrome.

In sterile nodular panniculitis, adipose tissue is the primary target of inflammation. Excisional biopsy is necessary to establish the diagnosis as the lesions are often confined to the subcutis and deep dermis. It is a rare condition affecting **dogs, cats**, and **horses**. There is no age or breed predisposition in cats or horses but, in dogs, affected animals are usually young (<1 yr) and Dachshunds and Poodles are over-represented.

The gross lesions are subcutaneous nodules that may become cystic, ulcerate or develop fistulous tracts. The exudate may be oily, serosanguineous, or hemorrhagic. Lesions are often multiple, 0.5 to several centimeters in diameter and may be grouped or distributed widely (Fig. 5.124). There is a tendency for the canine lesions to affect the trunk. Lesions in the horse are most often found on the neck, thorax, abdomen, and proximal limbs and may elicit pain upon palpation. *The microscopic lesion is lobular panniculitis that varies from necrotizing, to pyogranulomatous or granulomatous, to fibrotic, depending on the stage of the lesion.* Lipid released from damaged

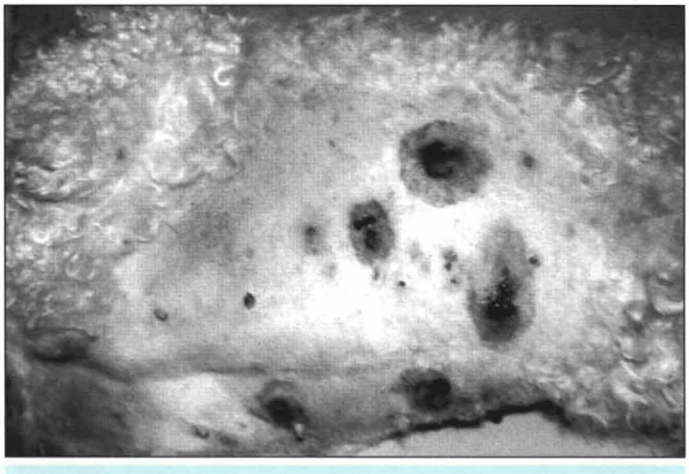

Figure 5.124 Sterile nodular panniculitis. Note multiple draining lesions on the trunk.

lipocytes hydrolyzes to glycerol and fatty acids. Fatty acids incite further inflammation that perpetuates the lesions. Affected animals with single lesions may be asymptomatic while animals with multiple lesions often have pyrexia, lethargy, and anorexia. A normochromic, normocytic nonregenerative anemia is seen in chronically affected animals with multiple extensive lesions. *Sterile panniculitis is indistinguishable histologically from the panniculitides of infectious cause.* The lesions fail to respond to antibiotics but resolve with corticosteroid therapy, sometime leaving residual scars. Some cases spontaneously regress. The condition can be recurrent.

Necrotizing panniculitis

Panniculitis secondary to pancreatitis and pancreatic neoplasia is well-recognized in humans. *Necrotizing cutaneous lesions are rare complications of pancreatic disease in the dog.* Cases have been reported in a dog with pancreatic carcinoma and one with pancreatic nodular hyperplasia. The pathogenesis likely pertains to the release of pancreatic lipases.

The lesions are multifocal, erythematous, nonpruritic cutaneous nodules, which may ulcerate centrally and discharge seropurulent exudate. Histologically, the lesion is necrotizing panniculitis in which mineralization of the lipocytes is a predominant feature. The initial inflammatory reaction is neutrophilic but may become granulomatous with time.

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Symmetrical lupoid onychodystrophy

Symmetrical lupoid onychodystrophy (SLO) is a degenerative disease of the nailbed in **dogs**. A wide variety of breeds and both male and females are affected. Dogs between 1–8 years are more commonly affected. German Shepherd Dogs, Rottweilers, Miniature Schnauzers, Golden and Labrador Retrievers, Boxers, and Greyhounds may be predisposed. The condition is characterized initially by brown discoloration of the proximal nailbed followed by separation and sloughing of one or more of the claws of multiple feet in otherwise healthy dogs. The condition is painful and often leads to lameness. Amputation of the third phalanx of affected digits to include nailbed epithelium is often needed to establish the diagnosis. Histologic lesions are most pronounced on the dorsal aspect of the claw. *The most common changes are a lichenoid interface dermatitis with lymphocytic exocytosis and spongiosis and multifocal hydropic degeneration of the basal epidermis.* Pigmentary incontinence may be severe and there may be dermal hemorrhage or fibrosis and mucinosis of the deep dermis. Direct immunofluorescence for deposits of immunoglobulin have not revealed specific labeling. Nails regrow but are misshapen, dry, soft or brittle and discolored. Secondary bacterial infection may occur. *The pathogenesis of SLO is not known* and, in fact, the possibility that SLO is not a specific disease entity but merely an inflammatory reaction typical for the nailbed has not been ruled out. Amputation of the third phalanx and histopathological evaluation of this tissue is a relatively new procedure. *The clinical differential diagnoses includes pemphigus, lupus erythematosus, vasculitis, keratinization disorders, and bacterial or fungal infection.*

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NEOPLASTIC AND REACTIVE DISEASES OF THE SKIN AND MAMMARY GLANDS

Epithelial tumors of the skin

Epithelial tumors of the skin are classified according to the predominant pattern of differentiation and the biological behavior. Recent refinements of the classification of a number of these tumors according to the World Health Organization International Histological Classification of Tumors of Domestic Animals have led to changes in nomenclature. These changes may not be widely accepted, as justification for reclassification based on prognosis or response to therapy has not been forthcoming. The decision to classify a given tumor as one or another of the epidermal or adnexal tumors is often quite arbitrary. A neoplasm of the multipotential germinal cells of the epidermis may differentiate into a number of types of epithelial cells characteristic of mature cells of the various components of the epidermis or adnexa. Sometimes the differentiation results in a distinctive group of tumor cells, making precise identification unequivocal. At other times, the tumor cells may differentiate toward several skin structures forming squamous cells, sebaceous cells or components of the hair follicle. The tumor can then be named according to the most aggressive or dominant cell type within the tumor. Other than squamous cell carcinomas, the majority of tumors derived from the epidermis or adnexa exhibit benign behavior.

Tumors of the epidermis include squamous papilloma, squamous cell carcinoma, and basal cell tumors. *Adnexal tumors of follicular origin* include infundibular keratinizing acanthoma (intracutaneous cornifying epithelioma, keratoacanthoma), tricholemmoma, trichoblastoma, trichoepithelioma, and pilomatricoma. *Adnexal tumors arising from glandular structures* include sebaceous gland tumors, apocrine gland tumors, and eccrine gland tumors. Also included in this section are varieties of tumor-like or keratin-filled cystic lesions that can be confused with true neoplasms. Mention will be made also of tumors that metastasize to the skin.

Cysts, hamartomas, and tumor-like lesions

Cysts

Infundibular cysts (epidermoid cyst, epidermal cyst, epidermal inclusion cyst) are seen as single or multiple, smooth, most often unilocular, spherical dermal nodules seldom larger than 1 cm in diameter. *Each nodule consists of an orderly wall of stratified squamous epithelium identical to epidermis, with gradual keratinization at its luminal surface* (Fig. 5.125A, B). *The cyst lumen is filled with concentric laminations of keratin.* There is usually neither mineralization nor inflammation within the cyst. The basal layer of the cyst wall abuts dermal collagen in a smooth line analogous to the normal dermal–epidermal junction. In longstanding lesions, the epithelial wall may become very thin, and *rupture may release entrapped keratin to stimulate pyogranulomatous dermal inflammation.* Infundibular cysts may arise from dilatation of the infundibulum of occluded hair follicles, and indeed occasionally one can detect superficial dermal scarring that may support this hypothesis. As well, such cysts may contain fragments of mature hair shafts. Other cysts arise from traumatic, developmental or surgical implantation of epidermal fragments into dermis or subcutis. Penetrating grass seeds cause implantation of epidermal fragments in **sheep**. Occasionally, an epidermal inclusion cyst (*subungual epidermal inclusion cyst*) can be found within the bone of the third phalanx of the **dog**. One report in the dog documents multiple squamous cell carcinomas arising from multiple infundibular cysts in a dog. In the **horse**, epidermal inclusion cysts are most commonly reported to occur in the nasal diverticulum (*atheromas*) or at the base of the ear (*dentigerous cysts*).

Isthmus cyst is a cyst lined by a keratinizing epithelium that lacks a granular layer. The inner layers of the epidermis lining the cyst have large amounts of pale eosinophilic cytoplasm and inconspicuous intercellular bridges.

Panfollicular cyst (trichoepitheliomatous cyst, hybrid cyst) is lined by stratified squamous epithelium that includes areas resembling both the infundibular cyst and isthmus cyst. In addition, areas of squamous epithelium comprised of primitive basal cells with abrupt keratinization to shadow cells may be present.

Dilated pore of Winer is a flask-shaped epidermal cyst on the head or neck of middle-aged or old cats and rarely, dogs. It is connected to the skin surface by a pore. It differs from the infundibular cyst in that the stratified keratinizing epithelium of the cyst wall near its base is hyperplastic with very regular rete ridges in parallel columns. For practical purposes, *it is yet another variant of epidermal/follicular cyst.*

Keratoma (horn cyst, horn tumor, keratin cyst) is a keratin-filled cyst arising from the coronary band. They are seen in cloven-hoofed animals and have been most often reported in the **horse**. The cysts are 1–5 cm in diameter and grow distally, between the third phalanx and the hoof wall. Keratomas are painful, causing lameness and bulging of the affected area of the hoof wall. *Histologically, the cyst is lined by squamous epithelium of the primary epidermal lamellae and filled with laminated keratin that in some cases may mineralize.* Trauma to the hoof wall and infection have been suggested, though not proven, causes of keratomas.

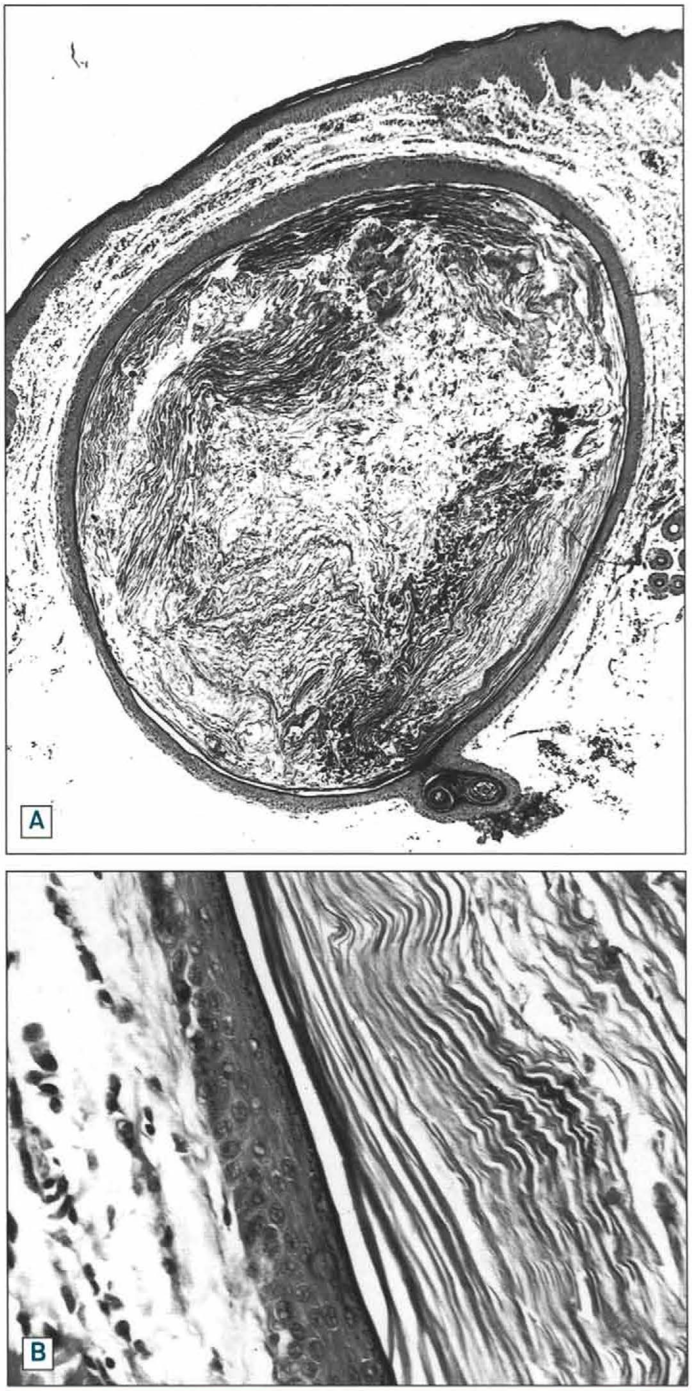

Figure 5.125 Infundibular cyst. A. Cyst filled with laminated keratin. **B.** Simple wall formed by an epidermis with gradual keratinization.

Dermoid cysts (dermoid sinus) are congenital lesions found in young animals along the dorsal midline. They arise by developmental failure of epidermal closure along embryonic fissures that maroons an island of multipotential ectoderm within the subcutis. Some dermoid cysts may extend deep into the tissue and be connected to the dura mater of the spinal cord. Some, such as those occurring in Rhodesian Ridgeback **dogs**, actually retain a sinus pore to the skin surface (see Congenital and hereditary diseases of skin). Dermoid cysts are common in some families of Boxer dogs and affected animals have multiple cysts along the midline of the skin over the forehead. In the **horse**, dermoid cysts occur most often on the midline between the withers and the rump. Dermoid cysts contain keratin and hair fragments and sometimes sebaceous secretions. The dermis abutting the cyst wall has numerous hair follicles, sebaceous glands and occasionally, apocrine glands. Specimens with only hair follicle differentiation may be confused with unilocular trichoepitheliomas, but the dermoid cyst has gradual keratinization rather than abrupt trichilemmal keratinization.

Apocrine cysts may be single or multiple and are filled with clear secretions and lined by a single layer of apocrine cells. The lining may form intraluminal papillary projections. In some animals, particularly dogs, apocrine cysts can be multiple and numerous. The observation of very orderly proliferation, especially if in multiple sites, should lead to a diagnosis of hyperplasia. This condition is referred to as *apocrine gland cystomatosis* (cystic hyperplasia of apocrine glands) and currently is of undetermined etiology. It is most common in middle aged to older male dogs. Expression of epidermal growth factor was detected in the epithelial cyst lining in one case of apocrine gland cystomatosis in an Old English Sheepdog.

Hamartomas

Hamartomas are *disorganized and excessive amounts, or enlarged components, of mature tissue elements indigenous to the site in which they arise.* Most hamartomas are congenital. Hamartomas grow independent of the growth of the animal and hence may enlarge later in life and become a problem. **Epidermal hamartomas** (pigmented epidermal nevus) grossly appear as focal hyperkeratotic and hyperpigmented plaques. Histologically, there is epidermal papillary hyperplasia and hyperpigmentation of the lower epidermis. The stratum granulosa has enlarged keratohyaline granules. In the dog, an association with papilloma virus infection has been documented in some cases. **Follicular hamartomas** have been reported in the dog and consist of collections of very large primary hair follicles and associated glands surrounded by variable amounts of collagen. The hair follicles extend into the subcutis. Clinically, the lesions may be focal or form rugose, disfiguring nodular to plaque-like lesions with large protruding hairs. **Fibroadnexal hamartomas** (adnexal nevus, focal adnexal dysplasia, folliculosebaceous hamartoma) consist of aggregates of markedly distorted and variably inflamed folliculosebaceous units surrounded by dense collagen and lacking a connection to the skin surface. Some pathologists believe this type of lesion is a result of trauma and is not a true hamartoma.

Tumor-like lesions

Idiopathic squamous papillomas *(warts) are 1–5 mm papillary masses comprised of hyperplastic stratified squamous epithelium supported by dermal projections.* The maturation of the epidermis is orderly and does not show viral-induced cytopathic effects as seen in viral papillomas. These lesions are most often seen on the eyelids, face, conjunctiva, or footpads of older dogs and occasionally cats.

Cutaneous horns *are exophytic cylindrical formations of compact keratin* a few millimeters in diameter and 1–2 cm in length formed by an underlying markedly hyperplastic epithelium. Orthokeratotic hyperkeratosis predominates. Cutaneous horns may arise from squamous papillomas, infundibular keratinizing acanthomas, or dilated pores of Winer. Cutaneous horns of the footpad of cats have been associated with feline leukemia virus infection. One report in a cat cites a concurrent cutaneous horn and multiple squamous cell carcinoma in situ.

Warty dyskeratoma is an endophytic growth comprised of a markedly hyperplastic epidermis demonstrating extensive dyskeratosis and suprabasilar acantholysis.

Xanthomas (*xanthogranulomas*) are single to multiple nodular lesions occurring most often in the cat and occasionally in the dog. Clinically, the lesions are *asymptomatic yellow to white nodules, plaques or dermal papules.* In cats, xanthogranulomas are most often found on the face, legs, trunk, or footpads while in the dog the lesions appear on the face, ears, and ventrum. Histologically, the xanthogranulomas are dermal to subcutaneous collections of large foamy macrophages and multinucleated giant cells interspersed among deposits of cholesterol and triglycerides that appear as arrays of clear lenticular clefts and lakes of extracellular pale staining material. Disorders of metabolism such as hyperlipidemia, diabetes mellitus, or high cholesterol diets lead to the formation of xanthogranulomas in most cases, while other cases, particularly solitary xanthomas, are idiopathic. *The histologic differential diagnosis includes granulomatous inflammation associated with infectious agents, such as feline leprosy.* The presence of cholesterol clefts and free lipid and lack of detection of infectious agents should lead to the diagnosis of xanthogranuloma.

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Tumors of the epidermis

Papilloma and fibropapilloma

Cutaneous papillomas are benign proliferative epithelial neoplasms that have a complex etiology and pathogenesis. The differences in site preference, clinical course and histology of such lesions have been made more understandable by the discovery that *most papillomas are caused by infection with a host- and often site-specific papillomavirus* of the family Papovaviridae.

Not all papillomas are caused by or contain viruses. The nonviral lesion is considered to be an idiopathic squamous papilloma and is described in tumor-like lesions. Papillomaviruses are associated with skin papillomas in all domestic and many nondomestic species. Immunocompromised animals have increased susceptibility to papillomavirus infection. Cattle, horses and dogs are most frequently affected. In each species, there are several viruses distinguishable by gene sequencing, and in most instances each identified virus type has a preferred site for replication and, except for bovine papillomaviruses, seems highly species-specific. *In general, papillomaviruses induce two types of cutaneous lesions – squamous papilloma and fibropapilloma* – but lesions of intermediate type are common. While some of the variation in anatomic localization or histologic pattern appears to result from management practices and routes of inoculation, the different viral strains themselves seem to have site preferences and typically induce one or the other histologic reaction (papilloma or fibropapilloma).

Advances in immunohistochemical localization of the viral antigens or detection via gene probes has greatly increased the sensitivity of viral detection, permitting retrospective studies on fixed tissues for the presence of the viral genome. Viral papillomas may regress due to cell-mediated immune attack, may persist, or may progress to squamous cell carcinomas. While some viral and host factors are known to influence the behavior of viral-induced papillomas and fibropapillomas, many other factors are not yet defined.

The typical papilloma is a 1–2 cm wart-like, filiform, exophytic, and hyperkeratotic mass composed of hyperplastic epidermis supported by thin, inconspicuous dermal stalks with dilated capillaries. Lesions can be anatomically extensive and multiple (Fig. 5.126A). The stratum corneum exhibits variable degrees of ortho- to parakeratotic hyperkeratosis. Most of the hyperplasia is due to marked expansion of stratum spinosum cells, which have pale basophilic cytoplasm (Fig. 5.126B). Cells of the spinous and/or granular layer have ballooning degeneration of their cytoplasm and eccentric pyknotic nuclei (*koilocytes*). Degenerating keratinocytes may have condensed *eosinophilic cytoplasmic inclusions that represent aggregates of keratin*, a result of the viral cytopathic effect. These inclusions should not be confused with the cytoplasmic inclusions associated with poxvirus infections. The stratum granulosum has large, irregularly shaped eosinophilic keratohyalin granules. Cells of the stratum spinosum and granulosum may have vesicular nuclei with intranuclear pale basophilic viral inclusions that contain virus particles visible with electron microscopy and viral antigen detectable by immunohistochemistry, but these may not be numerous. A variant of the typical squamous papilloma is the **inverted papilloma** that shares the same morphological features, as the typical squamous papilloma except the lesion is endophytic, residing in an invagination of the epidermis. Inverted papillomas have been described as arising in the nailbed of dogs as well as on the skin.

Fibropapillomas *appear as nodules or plaques covered by a variably hyperplastic and hyperkeratotic epidermis*. Classic examples of fibropapillomas are the *equine sarcoid* and *feline fibropapilloma* (see Spindle cell tumors). Microscopic lesions typical of fibropapillomas include the features of acanthosis, hyperkeratosis and downgrowth of rete ridges, but the dermal proliferation predominates. The proliferating cell is a large, plump fibroblast. The cells are arranged in haphazard whorls and fascicles rather than in perpendicular sheets as in granulation tissue. In some the epidermal proliferation is minimal and is seen only as slight acanthosis and accentuation of rete pegs, while in others the hyperplasia resembles full-fledged papillomas.

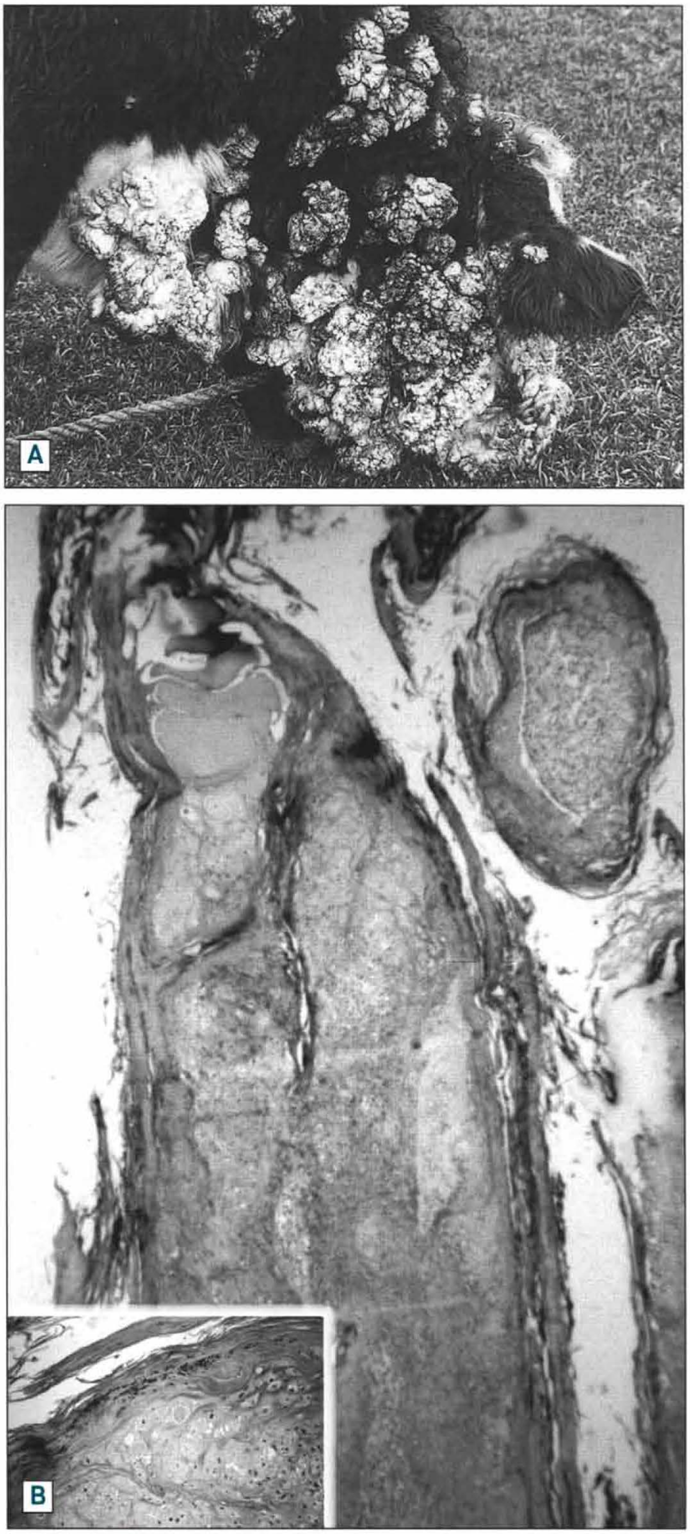

Figure 5.126 A. Multiple **papillomas (warts)** in a steer. **B. Papilloma** in a puppy. Marked hyperplasia is due to marked expansion of stratum spinosum cells. Cells of the spinous and/or granular layer have ballooning degeneration of their cytoplasm (see inset).

Cattle

Six different papillomaviruses occur in cattle. Three (*Bovine papillomavirus* (BPV) types 1, 2, and 5) cause cutaneous fibropapillomas, whereas the other three (BPV-3, -4, -6) cause papillomas of skin (BPV-3, -6) or alimentary tract (BPV-4). For both lesion types, affected cattle are less than 2 years of age and have single or multiple lesions that usually spontaneously regress within a year of appearance. Bovine papillomatosis may be a herd problem in that the virus is easily transmitted by animal-to-animal contact and by fomites. *BPV-1, -2, and -5 cause "teat frond" warts, common cutaneous warts, and "rice grain" fibropapillomas.* These three conditions are characterized by a fibrous core with an overlying variably hyperplastic stratified squamous epithelial lining and occur most often on the udder, teats, head, neck, shoulders and to a lesser extent on the omasum, vagina, vulva, penis, and anus. Lesions may be small nodules or large cauliflower-like lesions subject to trauma and possibly fly strike. Teat papillomas that appear during lactation and regress during the nonlactating period are not uncommon. Interdigital papillomas can lead to lameness. *BPV-3, -4, and -6 cause lesions that are grossly flattened and sessile.* Histologically there is epithelial hyperplasia but not fibroblast proliferation. These lesions tend to be more persistent. In some parts of Scotland and England, BPV-4 causes lesions in the alimentary tract and urinary bladder that may progress to squamous papillomas. Ingested bracken fern (*Pteridium aquilinum*) acts as a co-carcinogen in the progression from benign papilloma of the bladder mucosa to an invasive squamous cell carcinoma. Infection with papillomavirus and repeated exposure to strong sunlight appears to accelerate the development of squamous carcinomas arising in the sclera and lid margins of cattle, particularly those with poorly pigmented periocular skin such as Simmental and Hereford cattle. Papilloma viral infection is therefore one of the contributing factors in the development of the condition known as *cancer-eye*. BPV-1 and BPV-2 have a broader host range and tissue tropism than other papillomaviruses in that they are responsible for both bovine cutaneous fibropapillomas and equine sarcoids.

Horses

Horses develop *typical viral squamous papillomas*. They occur as single or multiple masses in young horses and usually regress within a year (Fig. 5.127). *Muzzle* is the most prevalent site, but tumors may occur anywhere including genital mucous membranes. They are histologically identical to squamous papillomas in calves. Two different viral genome sequences have been described in horses. Unlike the bovine viral strains, however, *the equine virus appears to be species specific. Congenital papillomas* have been reported in foals, and may be hamartomatous rather than viral induced.

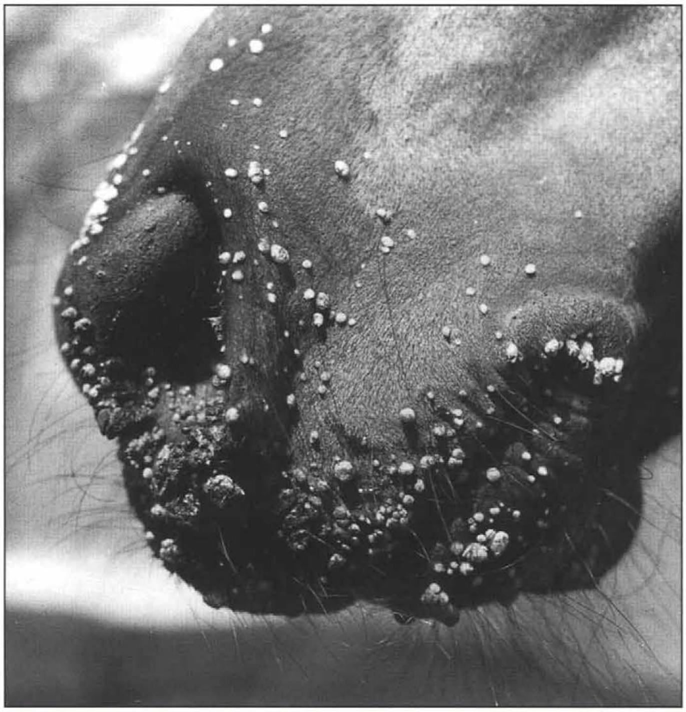

Figure 5.127 Multiple viral papillomas on an equine muzzle.

The most important papillomavirus-induced lesion in the horse is the *sarcoid*, known to be caused by BPV-1 and BPV-2. The lesions are primarily fibroblastic (see Spindle cell tumors) and have not been shown to be transmissible by direct contact.

Sheep and goats

Ovine papillomavirus 1 causes *fibropapillomas* in sheep. There is one report of multiple cutaneous papillomas in a sheep in England in which papilloma-like virus was observed and subsequently transmitted to other sheep. Multiple cutaneous papillomas have been described in goats but circumstantial evidence points towards an actinic rather than viral cause. No virus has been demonstrated by immunofluorescence, electron microscopy or in situ hybridization.

Dogs

Papillomaviruses in dogs cause papillomas of the oral, ocular and genital mucous membranes and of haired skin. Studies indicate that more than one papillomavirus infects the dog and that each virus is responsible for papillomas at a specific body site. *Canine oral papillomavirus* (COPV) has been cloned and characterized and is known to be responsible for canine oral papillomatosis, a contagious and self-limiting condition of young dogs. Occasionally, young dogs with this condition will also develop papillomas on the esophagus, conjunctiva or cornea. Idiopathic sporadic and solitary papillomas can occur on the haired skin of dogs of any age. Eyelid papillomas may appear as part of juvenile papillomatosis or as solitary lesions in older dogs. A report documenting COPV as the cause of multiple papillomas of the haired skin and oral cavity of a dog with corticosteroid-induced immunosuppression suggests that the tissue tropism of the various papillomaviruses is influenced by the immune system. Generalized cutaneous papillomatosis has also been reported in a dog with chemotherapy-induced immunosuppression. A novel papillomavirus was also documented as the cause of multiple foci of endophytic epidermal hyperplasia in a dog on long-term corticosteroid therapy. The lesions resolved with cessation of corticosteroid therapy. Only the oral papillomatosis has been well studied. *The typical transient squamous papillomas contain virus-like basophilic intranuclear inclusions* that are typical of papillomavirus with electron microscopy and immunohistochemistry. The virus is similar to *Bovine papillomavirus 1*. Evidence of viral pathogenesis for the other papilloma-like lesions is not as strong. Some solitary cutaneous papillomas and venereal papillomas contain papillomaviral genome, but the relationship of the viral genetic material to lesion development or to the virus of oral papillomatosis are unknown. Oral or ocular papillomas in young dogs have been reported to progress to squamous cell carcinoma in situ or to invasive squamous cell carcinomas upon rare occasion. A variant of the typical papilloma, the **inverted papilloma** has been reported in the dog. The tumors were single-to-multiple and grossly resembled intracutaneous cornifying epitheliomas (keratoacanthomas). They were keratin-filled cyst-like structures lined by stratified squamous epithelium. The epithelium, however, had intraluminal papillary projections and typical keratinocyte viral cytopathic effects, intranuclear inclusions, and papillomavirus virions present ultrastructurally. Molecular studies suggested the virus was different from COPV.

Cats

Feline fibropapillomas with features virtually identical to the equine sarcoid have been described and found to be associated with papillomavirus DNA via PCR. The implicated virus is most similar to *Bovine papillomavirus* (see Spindle cell tumors). Papillomavirus antigens have also been detected in a large number of cases of multicentric squamous cell carcinoma in situ in cats and in non-neoplastic, proliferative lesions of the epidermis and outer root sheath of hair follicles in immunosuppressed cats. One cat with the non-neoplastic proliferative lesions was infected with FIV and the others were Persians with coat color characteristics suggestive of Chediak–Higashi syndrome. Progression to overt neoplasia and malignancy of these lesions has not been established. At least eight feline papillomaviruses infect the oral cavity and skin of domestic and wild felids.

Rabbits

Domestic **rabbits** develop oral papillomatosis due to infection with *Rabbit oral papillomavirus*. Papillomas, which are multiple and found most often under the tongue, are grossly and histologically typical of viral-induced squamous papillomas.

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Squamous cell carcinoma

Squamous cell carcinoma *is a relatively common, locally invasive and occasionally metastatic neoplasm of most domestic species.* The behavior of squamous cell carcinoma of the skin is usually that of locally destructive spread. Its metastatic potential is low, with certain qualifications depending on location. Those initiated by sunlight are slow to metastasize, then usually only to local lymph nodes. In contrast, those originating on the canine digit may be more prone to metastasize, but even these are cured by amputation in virtually all but the most neglected cases.

Sunlight is probably the most important carcinogenic stimulus for these tumors and accounts for the preference of squamous cell carcinoma for the eyelid and conjunctiva of cattle and horses, the ear pinna of cats and sheep, and the vulva of cattle, goats, and recently sheared sheep (Fig. 5.128A, B). Chronic exposure to sunlight has also been proven to cause squamous cell carcinoma of the relatively hairless, poorly pigmented abdominal and juxtanasal skin of dogs (see Actinic diseases of skin). The progression from solar keratosis to carcinoma occurs over several years and in many instances never attains the full status of carcinoma. The action of sunlight may be related to overexpression of p53 protein as a result of UV-induced mutations of the *p53* tumor suppressor gene.

In addition to sunlight, *carcinogens* contained in tobacco, coal tar and soot, arsenic, and smegma have been shown experimentally or by epidemiologic inference to cause squamous cell carcinoma of skin and other tissues. *Genetic factors and papillomaviruses* also influence the occurrence of squamous cell carcinomas. The influence of *epidermal injury* per se in the initiation of cancer is still unsettled, but there is support for this theory in the greater risk of squamous carcinoma at sites of ear notching, branding, burns, and chronic inflammation. Squamous cell carcinomas of the nasal planum arising in chronic cutaneous lesions of discoid lupus erythematosus have been reported in dogs.

Squamous cell carcinomas are usually firm, white, poorly demarcated dermal masses that are ulcerated and streaked with red. In some locations (eye, penis) they are raised and papillary even though the surface is ulcerated. Chronic inflammation accompanies many of these tumors, particularly those of the ear pinna and nail bed. In these instances, the reddening, crusting and ulceration of the inflammatory lesion may hide the tumor. Microscopic examination of such lesions is often dominated by hyperkeratosis, parakeratosis, ulceration, acanthosis and superficial dermal scarring, with only a few foci of unequivocal neoplasia in which atypical squamous cells have invaded across the basal lamina of the hyperplastic rete pegs. The precancerous plaque and papilloma lesions of ocular squamous cell carcinoma are described under Ocular neoplasia (Vol. 1, Eye and ear).

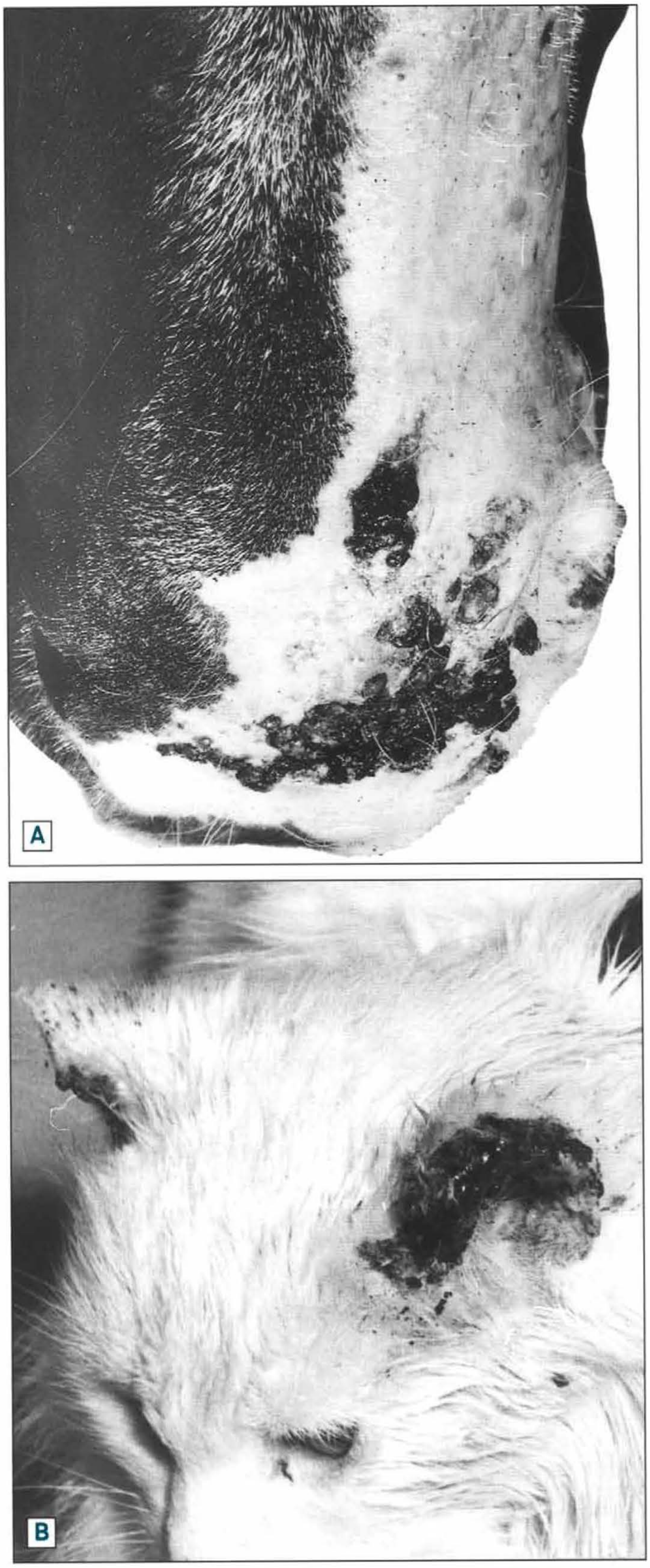

Figure 5.128 Squamous cell carcinoma. A. Involving the unpigmented skin of a horse's nose. **B.** Involving the unpigmented skin of a cat's ears.

Squamous cell carcinomas are recognized microscopically by identifying malignant epithelial cells demonstrating various degrees of differentiation towards keratinocytes. **Squamous cell carcinoma in situ** refers to a malignant tumor of cells with squamous differentiation that is *confined by the basement membrane.* These tumors are often seen in association with ultraviolet light exposure and are interpreted by some pathologists as marked dysplasia (solar keratosis, actinic keratosis), a precancerous stage in the process of malignant transformation of cells. In **invasive squamous cell carcinomas**, *tumor cells breach the basement membrane* to form trabeculae, nests and cords within the dermis that are usually contiguous with the epidermis in at least one area. The tumor cells most resemble those of normal stratum spinosum but have vesicular nuclei with one or multiple very prominent nucleoli. Cytoplasm is usually abundant and eosinophilic. Keratinization within such cords or islands results in laminated keratin "pearls" surrounded by tumor cells. With increasing anaplasia, tumors exhibit wide variation in nuclear size, decreasing cytoplasmic mass, increased basophilia, and disappearance of intercellular bridges. Keratinization may be limited to only individual tumor cells and mononuclear or even multinucleated giant tumor cells may be present. In the typical squamous cell carcinoma, the tumor cells spread through the dermis as slender anastomosing cords, with some cells falling off the cords to remain as apparently isolated islands in the dermal stroma. Tumor cell invasion is usually associated with marked desmoplasia. Mitotic figures are usually numerous and are in proportion to the degree of anaplasia. Some tumors, especially those of actinic origin with a long precancerous phase, are accompanied by abundant mononuclear leukocytic inflammation. Others, such as those of the digit, are both inflamed and very scirrhous. In such tumors, the neoplastic cells may be obscured by inflammatory cell influx, difficult to distinguish from the reactive fibroblasts, or largely destroyed by the ulceration of the inflamed mass.

Acantholytic squamous cell carcinomas are histologic variants demonstrating marked dyscohesion and degeneration of neoplastic keratinocytes resulting in pseudocyst formation within the tumor. This type of tumor must be carefully distinguished from an adenocarcinoma by identifying tumor cell keratinization. **Spindle cell squamous cell carcinomas**, in which the neoplastic epithelium is distinguishable from the desmoplastic stroma only by immunohistochemistry, have been reported. **Basosquamous carcinomas** are low-grade malignant tumors comprised primarily of dermal lobules of basaloid cells with centralized foci of atypical abruptly keratinized cells. The basaloid population may have foci of dyskeratosis or melanization. Tumor lobules may or may not be connected with the epidermis.

Subungual squamous cell carcinomas in **dogs** may be multiple and are seen most often in black dogs, particularly, Labrador Retrievers and Standard Poodles. **Carcinoma of the horn of cattle** is almost exclusive to castrated male adult cattle in India and neighboring countries. The tumor gradually infiltrates and destroys the horn core and may invade adjacent sinuses and cranial bone. The histology is of well-differentiated squamous cell carcinoma but neither the site of origin nor the reason for its peculiar site and sex preference is understood. Theories about chronic trauma to the huge horns are not now in vogue.

Multicentric squamous cell carcinoma in situ (Bowen-like disease) is a type of squamous cell carcinoma confined by the basement membrane. These lesions may progress to invasive squamous cell carcinomas. This tumor is not associated with exposure to ultraviolet light but instead has been associated with papillomavirus infection. The condition has been described in middle-aged to older mixed-breed **cats**. One case reports an association with a cutaneous horn in a cat with no evidence of a viral infection. Tumors appear as 0.5–5 cm in diameter, single to multiple irregular, slightly elevated plaque or papillary lesions of haired pigmented skin. Microscopically, sharply demarcated areas of the epidermis and follicular outer root sheath are irregularly thickened and disorganized by neoplastic keratinocytes. The basal and spinous layers are most affected. Mitotic activity is present in all layers of the epidermis and keratin pearl formation may be present. Tumor cells often have large hyperchromatic nuclei and may have vacuolated cytoplasm, cytoplasmic pallor, or occasionally are multinucleated. The tumors are often pigmented and may have papillomatous epidermal projections with marked hyperkeratosis.

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Basal cell tumor

Basal cell tumors are *common in dogs and cats but are rare in other domestic species. They are the most common skin tumor of cats.* Current classification schemes limit the category of basal cell tumors to tumors *comprised only of proliferations of undifferentiated basaloid cells.* Those that exhibit differentiation towards basal cell-derived structures such as sebaceous glands, hair follicles or even keratinized epidermis are given a wide variety of names depending on the predominant direction of such differentiation. This new reclassification of cutaneous tumors has now placed some of the tumors that used to be considered basal cell tumors in the category of trichoblastomas (see Tumors with adnexal differentiation). Human basal cell tumors most frequently occur in lightly pigmented skin exposed to sunlight, the latter known to favor the development of the tumor. No such predilection is proven for dogs and cats.

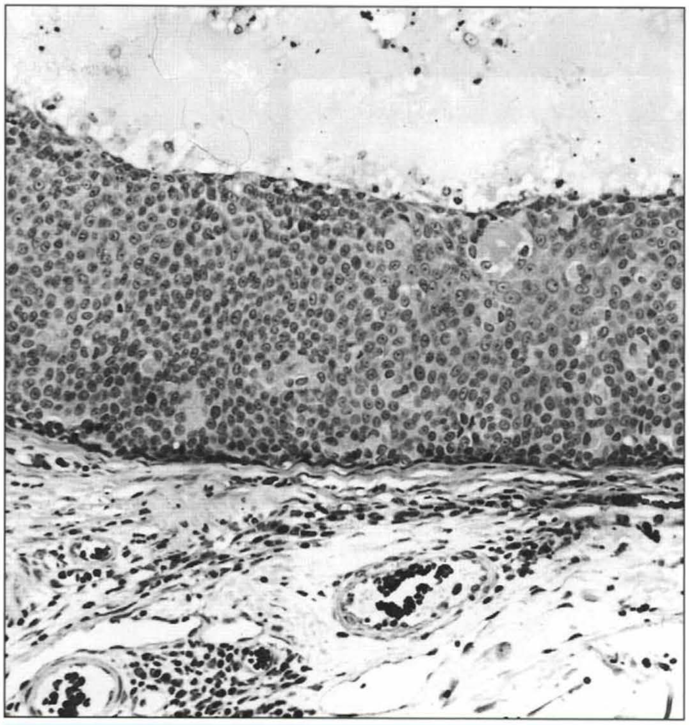

Figure 5.129 Cystic basal cell tumor.

Histologically, basal cell tumors are composed of cords or nests of cells resembling those of normal stratum germinativum. Each has an oval, deeply basophilic nucleus, a single nucleolus, and scant, eosinophilic cytoplasm with indistinct cell boundaries. In contrast to normal stratum germinativum, there are no intercellular bridges. The cells extend into the dermis as islands, or grow expansively as solid sheets, multiple small cysts lined by basal cells, or less frequently as a single large cyst (Fig. 5.129). The latter form is most common in the cat. Several layers of undifferentiated basal cells with no inner limiting membrane and no adnexal or squamous differentiation form each cyst. The cysts are usually filled with eosinophilic debris, and most probably form by central necrosis of an initially solid mass of basal cells. The islands of tumor cells may or may not connect with the overlying epidermis. Mitotic figures and nuclear pleomorphism are variable. Benign tumors have no stromal invasion or associated desmoplasia. Tumor cells may contain melanin, and melanophages may be present in the intervening stroma.

Basal cell carcinomas have two subtypes. **Infiltrative basal cell carcinomas** are comprised of cords of primitive basaloid cells that penetrate the surrounding dermis and possibly subcutis. Mitotic figures may be numerous. The tumor is usually associated with desmoplasia. **Clear cell basal carcinomas** are more rare and consist of large, polygonal cells with clear or finely granular cytoplasm. Nuclei lack pleomorphism and mitoses may be variable.

Tumors with adnexal differentiation

Tumors arising from hair follicles

Hair follicle (pilar) neoplasms are common in dogs but apparently are very rare in other domestic species. Their classification is rather complex; however, the majority of tumors arising in animals share a benign biological behavior. Exceptions exist as described below.

Intracutaneous cornifying epithelioma (*infundibular keratinizing acanthoma*) is a benign cystic tumor of the skin of dogs. Common sites are the dorsum of the neck and back. Usually there is only a single tumor, but in Norwegian Elkhounds, a breed predisposed to these tumors, they may be multiple or occur in succession. Tumors of this description arising in the subungual region are called **keratoacanthomas**.

The gross lesion is a slightly raised firm 1–3 cm nodule that may have a central craterous pore representing the follicular isthmus or infundibulum from which keratin can be expressed. *The histologic lesion is a simple or multiloculated cavity filled with keratin and lined by squamous epithelium with large, pale eosinophilic squamous cells multifocally containing keratohyaline granules.* The periphery of the cyst wall consists of basal cells (Fig. 5.130A, B). The basal cells have gradual and complete maturation to stratum corneum, but may become somewhat disoriented and form keratin pearls within the cyst wall as well as shedding keratin into the lumen. The basilar layer is orderly, and while it may show blunt cord-like expansion into surrounding compressed dermis and anastomosing cords of epithelial cells forming small horn cysts in the wall of the central cyst, there is no breach of the basement membrane by tumor cells. Focal rupture is common, inciting a granulomatous or pyogranulomatous reaction in surrounding dermis.

Trichoepithelioma is *a tumor of primitive hair germ that exhibits rudimentary differentiation towards all three segments of the follicle: the infundibulum, isthmus, and the inferior segment.* Trichoepitheliomas are single or multiple skin tumors occurring anywhere on the body with a slight preference for the back. The typical macroscopic appearance is of a very firm, white, multilobulated and encapsulated tumor that may infrequently be mineralized. Histologically, the usual pattern consists of multiple islands of basal cells with differentiation into the external or internal sheath surrounded by a thickened basement membrane; the center of each island undergoes abrupt keratinization without interposition of a granular layer (Fig. 5.131A, B). Occasional tumor cells containing trichohyaline granules and foci of matrical keratinization are present.

The outer layer of basal cells may abut the fibrous stroma or send out basal cell ribbons into a mucinous stroma. Some trichoepitheliomas have several large primary cystic areas surrounded by many smaller cysts. Rupture of some of the keratinaceous cysts stimulates pyogranulomatous inflammation within the stroma and even within the cyst, sometimes accompanied by mineralization and foreign-body giant cells. Mineralization and inflammation are more typical of the closely related pilomatricoma. **Malignant trichoepitheliomas** have the additional features of mitotic activity, cystic degeneration and necrosis, and invasion of subcutis sometimes accompanied by desmoplasia. Lymphatic invasion is considered an essential finding to confirm malignancy by some pathologists. Regional and pulmonary metastasis is possible. *Trichoepitheliomas are distinguished from all other epidermal or appendage tumors by the abrupt (matrical) keratinization and the follicle-like basal cell nests that surround it.* Other tumors or cysts simulating trichoepithelioma lack one or both of these features.

Tricholemmoma (also trichilemmoma) is a rare, *benign and nonrecurring,* pilar tumor of dogs in which tumor cells differentiate into cells characteristic of either the inferior segment of the hair follicle (**inferior tricholemmoma**), external root sheath, or the isthmic (**isthmic tricholemmoma**) section of the external root sheath. In *inferior tricholemmomas,* tumor cells form nests separated by a fine collagenous stroma. The tumor cells have abundant eosinophilic cytoplasm that is pale (clear cells) peripherally and more intensely eosinophilic toward the center of the nests (Fig. 5.132). Pallor of the outermost layer of cells is due to marked cytoplasmic glycogen storage, seen in histologic section as cytoplasmic clearing. These are thought to represent differentiation toward

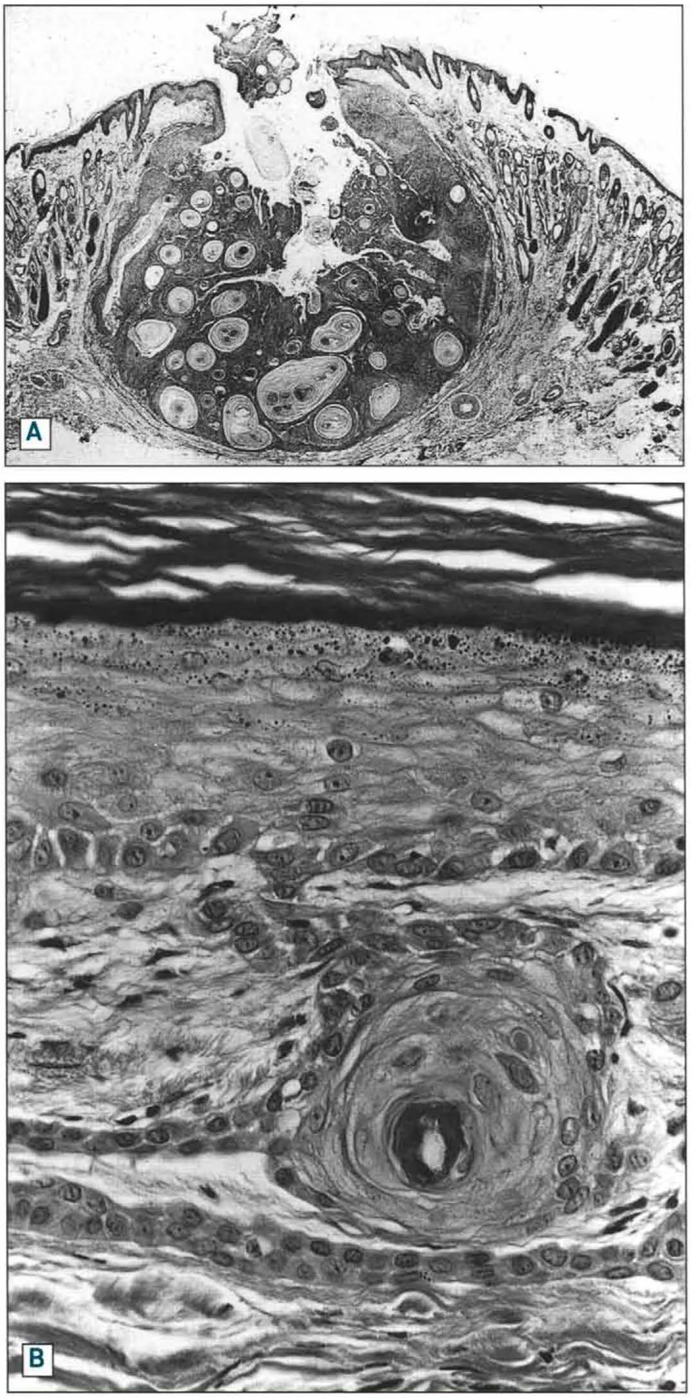

Figure 5.130 Intracutaneous cornifying epithelioma. A. With pore opening to the surface of the skin. **B.** Detail of wall showing gradual keratinization and keratin pearl formation.

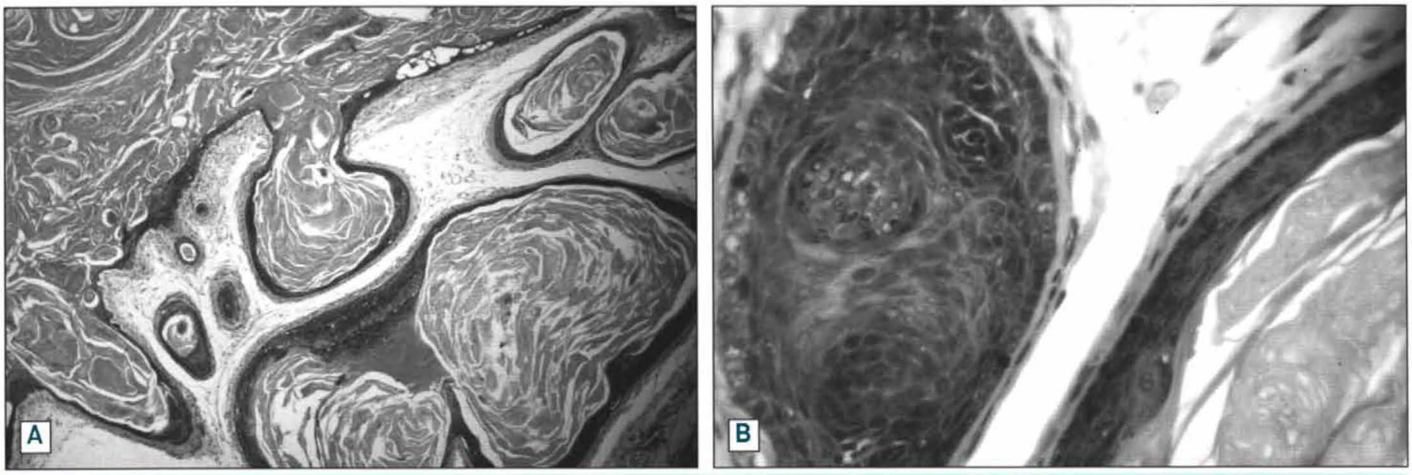

Figure 5.131 Trichoepithelioma. A. Note multiple keratin-filled cysts. **B.** Note rudimentary hair follicle formation.

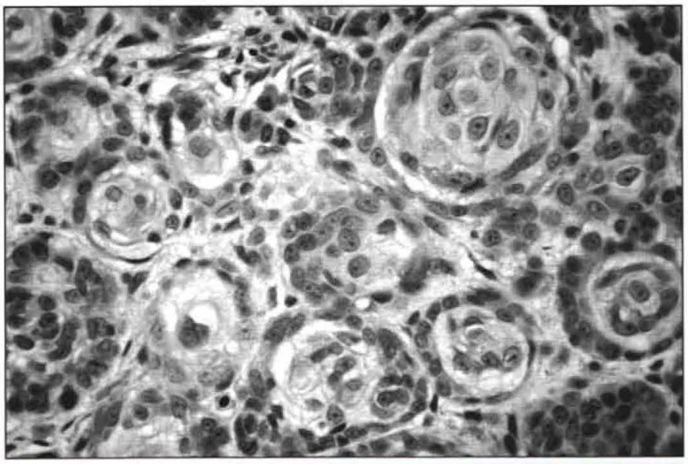

Figure 5.132 Tricholemmoma. Tumor cells have abundant eosinophilic cytoplasm that is pale (clear cells) peripherally and more intensely eosinophilic toward the center of the nests.

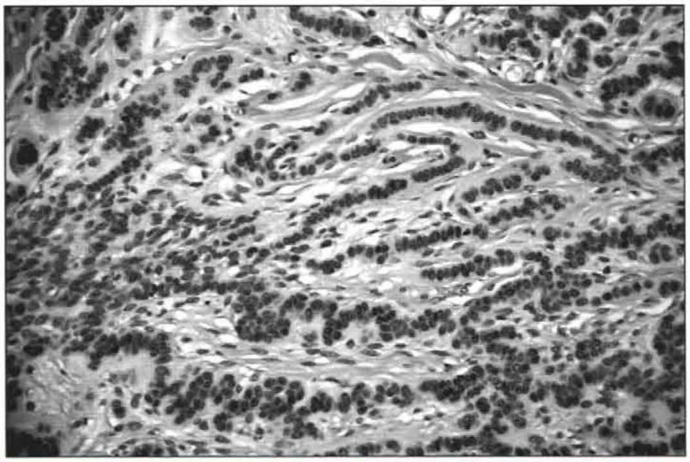

Figure 5.133 Trichoblastoma. Long ribbons of pale epithelial cells extend into the dermis.

outer root-sheath cells. Each tumor follicle is surrounded by a thick, homogeneous basal lamina resembling the vitreous sheath of the normal follicle. The epithelial cells in the *isthmic tricholemmoma* are arranged as islands and radiating cords that may intersect or be associated with the epidermis. The tumor cells show central trichilemmal keratinization.

Trichoblastoma is *a benign tumor derived from the primitive hair germ of embryonic follicular development.* There are ribbon, trabecular, granular, and spindle cell trichoblastomas. In dogs, these tumors were formerly classified as basal cell tumors.

- The **ribbon trichoblastoma** is a dermal to subcutaneous tumor formed by long, narrow, branching cords of pale eosinophilic epithelial cells (Fig. 5.133) that may join or radiate from a large central aggregate (medusoid pattern). A mucinous stroma is frequently associated with the medusoid pattern. Cell borders are indistinct. Nuclei of tumors arranged in cords often palisade perpendicular to the long axis of the cord. Mitotic figures may be numerous. Abundant collagen that may appear hyalinized accompanies the tumor cells.
- **Trabecular trichoblastomas** occur in the dermis and consist of multiple lobules of pale eosinophilic epithelial cells with scant poorly demarcated cytoplasm and euchromatic nuclei. The cells at the periphery of the lobules palisade while centrally located cells have more abundant cytoplasm.
- **Granular trichoblastoma** tumor cells have large numbers of intracytoplasmic eosinophilic granules and eccentrically placed nuclei.
- **Spindle cell trichoblastomas** are most often seen in cats and consist of trabeculae and lobules of elongated, streaming, basaloid epithelial cells within a fibrous stroma. Tumor cells have scant cytoplasm, oval nuclei with inconspicuous nucleoli, and rare mitoses. Melanin may be present in the tumor cells and accompanying melanophages.

Pilomatricoma (*pilomatrixoma, epithelioma of Malherbe, necrotizing and calcifying epithelioma*), macroscopically resembles trichoepithelioma (Fig. 5.134A) but is often more heavily mineralized and consists of fewer, larger cysts than does trichoepithelioma. Pilomatricomas are believed to be derived from primitive hair

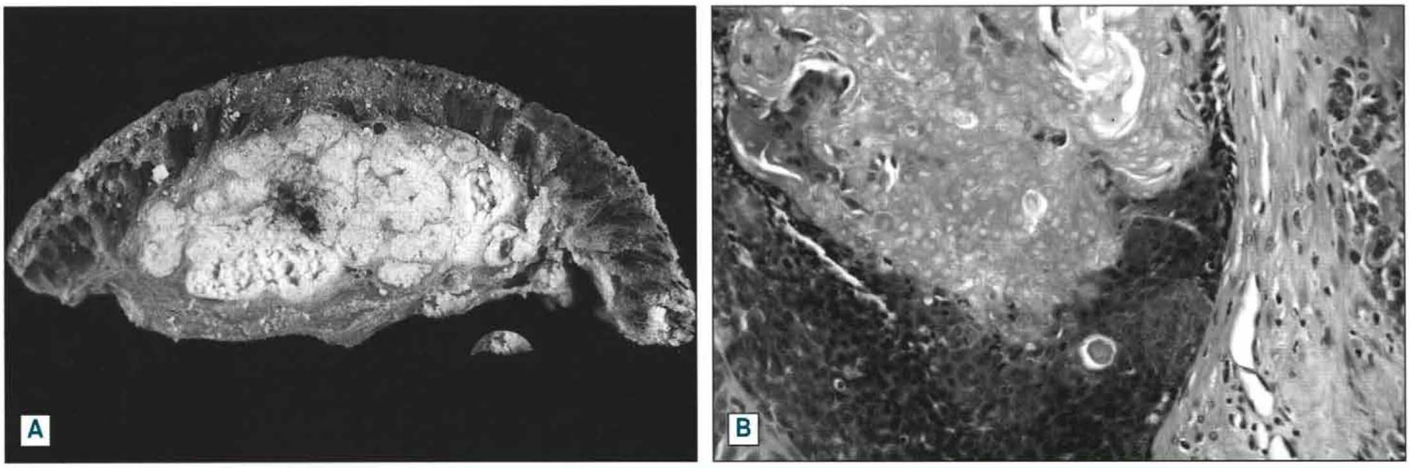

Figure 5.134 Pilomatricoma in a dog. **A.** Multilobulated, mineralized, and well-demarcated keratinizing dermal tumor. **B.** Multiple lobules of basal cells with abrupt keratinization. Note shadow cells in the center of the lobules.

matrix and thus show incomplete differentiation towards hair cortex. *The typical microscopic morphology is of one or more large, thick-walled cysts partially filled with so-called shadow or ghost cells* (Fig. 5.134B) *typical of matrical differentiation.* These cells are flattened eosinophilic epithelial cells with a central empty halo in place of the lysed nucleus. The cyst wall is composed of multiple layers of basal cells, showing a sudden zonal degeneration to form the central laminations of shadow cells. Mitoses may be present. Mineralization of shadow cells is very common, and cyst rupture results in the pyogranulomatous inflammation that is very typical of these tumors. Foci of necrosis within the tumor are frequently seen, and mineralization or even ossification within the stroma occurs occasionally.

Pilomatricomas are almost always single and benign. They occur most often on the back, neck and tail. One report indicates that Miniature Poodles and Kerry Blue Terriers develop these tumors more frequently than other breeds. The very few reports of behaviorally malignant tumors describe tumors with greater mitotic activity and nuclear pleomorphism; however, some of these tumors were not readily distinguished at initial presentation from benign tumors and the diagnosis of malignancy was made in retrospect after documented metastasis. Metastases to the regional lymph nodes, lung and bone have been documented.

Tumors arising from sebaceous or modified sebaceous glands

Sebaceous glands give rise to *sebaceous gland hyperplasia, sebaceous adenoma, sebaceous epithelioma, and sebaceous carcinoma.* **Modified sebaceous glands** include the Meibomian glands of the eyelid, which give rise to *Meibomian gland adenomas, epitheliomas, and carcinomas* that are histologically identical to the corresponding sebaceous gland lesion and will not be described separately. The **hepatoid (perianal) glands** are also modified sebaceous glands that give rise to hepatoid gland adenomas, epitheliomas, and carcinomas. *Epitheliomas are considered to be lesions intermediate between an adenoma and carcinoma.*

Sebaceous gland hyperplasia is seen as single or multiple, raised, multilobulated masses within the superficial dermis below focally hyperplastic, hyperkeratotic epidermis. Many are ulcerated by continued tumor growth or by trauma. Most are less than 1 cm in diameter and are most frequently submitted with clinical diagnoses of "multiple papillomas." Histologically, each tumor consists of rather symmetrical hyperplasia of a single sebaceous gland clustered about a keratinized sebaceous duct. In some specimens, more than one gland is involved. The sebaceous cells (sebocytes) are fully mature and the peripheral rim of basal (reserve) cell population is inconspicuous. Surrounding dermis and adnexa are compressed. The hyperplasia is multiple in about 40% of affected dogs.

Sebaceous adenoma is *comprised primarily of mature sebaceous cells, but differs from hyperplasia in that there are more basal (reserve) cells than in hyperplasia, and the lobular proliferation is greater, less symmetrical, and not necessarily related to a single sebaceous duct.* The lesion compresses adjacent structures. Melanin within tumor cells, and stromal melanocytes and melanophages may be present and cause the tumor to be grossly pigmented. **Sebaceous ductal adenomas** are similar tumors but are comprised primarily of large numbers of haphazardly arranged ducts with intervening clusters of sebocytes and reserve cells.

Sebaceous epithelioma *is a term used by some to refer to sebaceous gland tumors of low-grade malignancy.* These tumors have mostly basaloid reserve cells with numerous mitoses and only scattered single or small clusters of well-differentiated sebocytes. Horn cysts suggesting ductal differentiation may be present (Fig. 5.135). Melanocytes and melanophages may be present within the intervening stroma and the basaloid cells may contain melanin also.

Sebaceous carcinomas are occasionally encountered in dogs and rarely in cats. *Sebaceous carcinomas are locally infiltrative, solitary, poorly circumscribed tumors made up of pleomorphic cells that show some evidence of sebaceous differentiation.* The sebaceous carcinoma lacks complete or orderly sebaceous cell maturation, and distinct reserve cell populations are not present. Lobule formation is often present but is not prominent. Mitotic figures, anisocytosis, anisokaryosis and hyperchromasia are present as the cytologic counterparts of malignancy. The prognosis following wide surgical excision seems to be good, although no large series has been published. Differentiation from liposarcomas and balloon cell melanomas may be difficult.

Perianal gland adenomas (*hepatoid gland tumor, circumanal gland tumor*) are common in intact, aged male dogs. They occur occasionally in females and rarely in castrated males. They have not

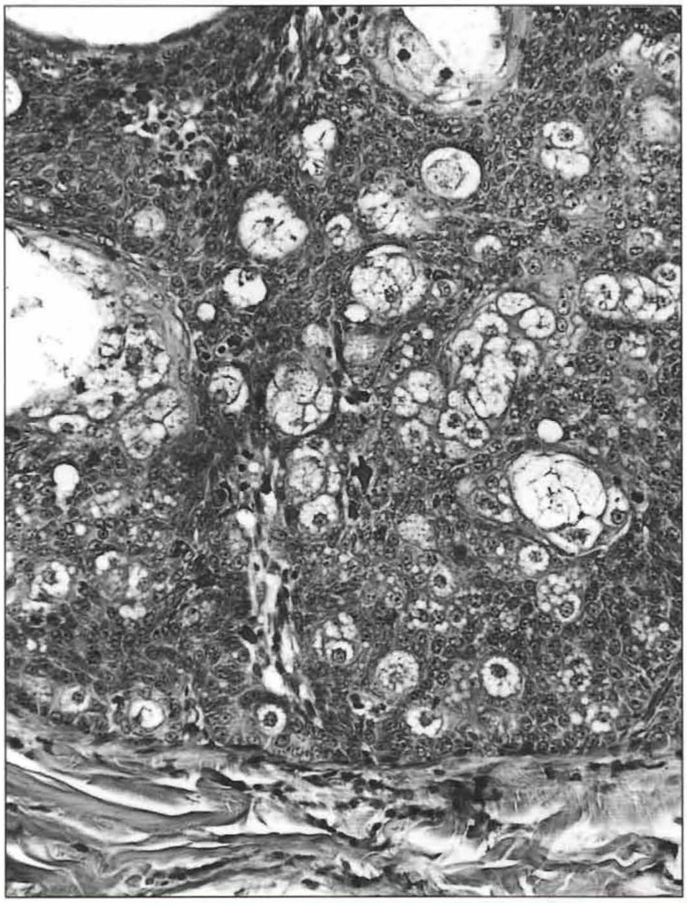

Figure 5.135 Sebaceous epithelioma in a dog.

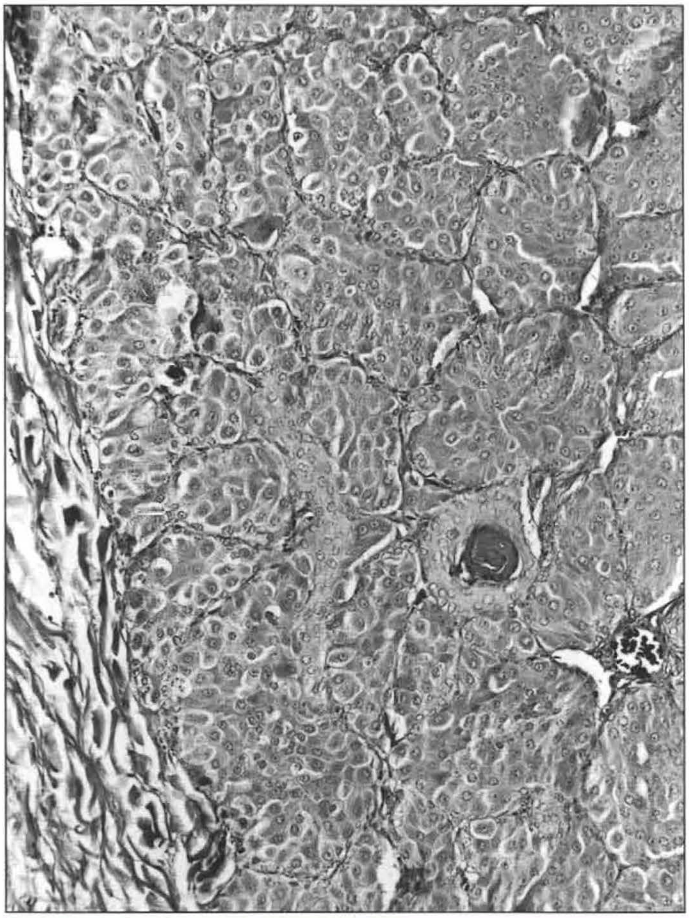

Figure 5.136 Perianal gland adenoma. Lobular arrangement of hepatoid cells and foci of squamous metaplasia.

been reported in other domestic species. *Most perianal gland tumors are called adenomas, even though the lobular development is so uniform that the tumor is more likely to be hormone-dependent hyperplasia.* The strong predilection for intact male dogs and the regression of about 95% of these tumors following castration also points to hyperplasia rather than true neoplasia. Nonetheless, "perianal adenoma" seems firmly entrenched and the difference in terminology is of no practical importance. The tumor growths most commonly occur in the perianal area, but about 10% occur in sites of ectopic perianal gland tissue such as tail, flank, back, prepuce and even chin. Of these, the ventral skin of the tail is the most frequent location.

The *gross appearance* of perianal gland adenomas is of one or more raised rubbery masses that may grow to 10 cm or more in diameter. Ulceration and secondary infection are common, and can serve to confuse the diagnosis. The typical tumor consists of multiple tan lobules separated by a delicate but definite collagenous stroma. *The histologic lesion is similar to normal perianal gland.* Well-differentiated tumors consist of islands, cords, and lobules of polygonal cells with abundant, finely vacuolated eosinophilic cytoplasm and a central, small, round, vesicular nucleus. The cells resemble hepatocytes and these tumors are sometimes called "hepatoid gland" tumors (Fig. 5.136). At the periphery of each lobule is a rim of basal reserve cells, and in some tumors these cells may predominate over the fully differentiated hepatoid cells. Mitotic activity of reserve cell, necrosis, hemorrhage, secondary inflammation, squamous metaplasia and squamous pearl formation (ductal differentiation) are common and are no cause for concern. Some cells may resemble sebocytes. Aggregates of tumor cells are separated by a fine fibrovascular stroma and tumors are often encapsulated. *Local excision and castration is curative and prevents the development of new perianal gland tumors.*

Perianal gland epitheliomas are tumors of low-grade malignancy comprised of mostly reserve cells. Mitotic activity is increased in comparison to the adenomas but nuclear atypia is not present.

Perianal gland carcinomas account for no more than 5% of all perianal tumors if rigorous diagnostic criteria are used. They are relatively more common in castrated males and in females. They exhibit the usual features of malignancy: variation in nuclear and cytoplasmic mass, poor cytoplasmic differentiation, frequent mitotic figures, and lack of organized lobule formation. Local tissue invasion should be evident. Lymphatic invasion must be diagnosed with caution since compression of reserve cells by lobular expansion may simulate a tumor embolus surrounded by lymphatic endothelium. Direct extension into the pelvic canal or metastasis to regional lymph nodes occurs as a late event in chronically neglected tumors. Castration has no demonstrated effect on tumor behavior.

Tumors arising from apocrine and modified apocrine glands

Tumors of the *apocrine (paratrichial) sweat glands* are the least frequent of the adnexal skin tumors in dogs. They are relatively more common

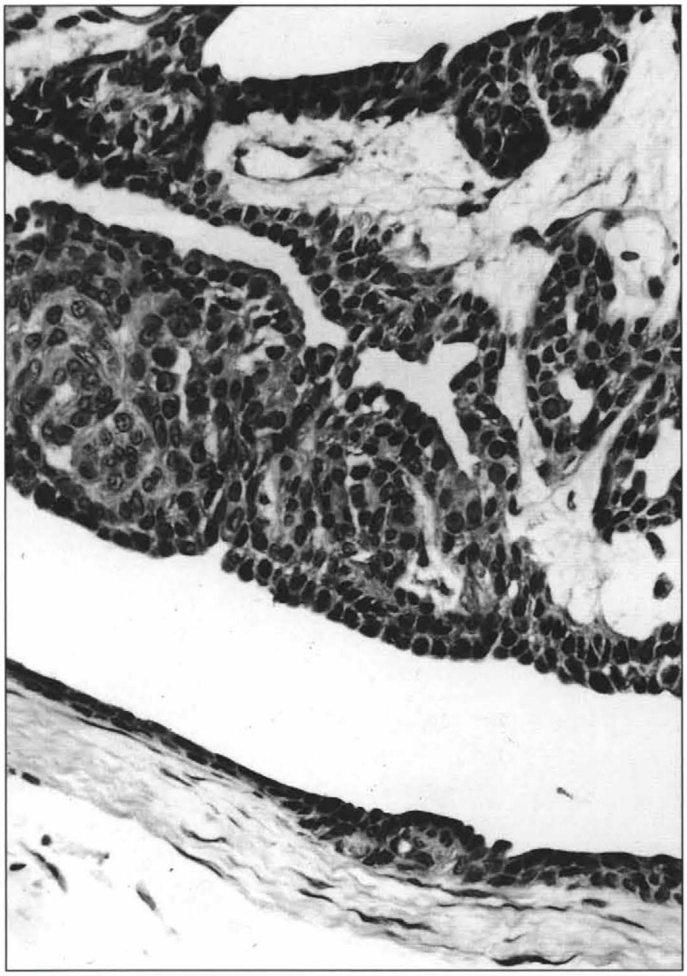

Figure 5.137 Papillary apocrine adenoma.

in cats. There is a single report of a mixed sweat gland tumor in a bull. Tumors of the eccrine sweat glands are rare but histologically distinctive tumors occurring in the footpads.

Apocrine gland adenomas include *cystic and papillary types*, the latter merely a variant of the former. The cystic lesion consists of one or more cavities lined by well-differentiated cuboidal-to-columnar epithelium with eosinophilic cytoplasm and basally located nuclei, often with the apical secretory blebs typical of normal apocrine glands. Many tumors have at least some tubules lined by an orderly bi-layer of cuboidal epithelium, perhaps an effort by the tumor cells to recreate the structure of the duct of the normal gland (Fig. 5.137). Proliferation of epithelium may result in intraluminal papillary growths supported by a delicate fibrous stroma. The proliferation may fill the cyst lumen, or lumens may contain eosinophilic secretions.

Apocrine gland carcinomas include *solid, cystic or tubular types*, the latter clearly predominating. The cystic tumors have intraluminal papillary growths. Apocrine carcinomas are locally aggressive growths, spreading through the dermis, subcutis and muscle from the primary focus. Invasive tumor cells are often accompanied by desmoplasia. Lymphatic invasion and regional lymph node and pulmonary metastasis are common. The histology of carcinomas differs from their benign counterparts primarily in the criteria of loss of polarity and local stromal invasion, as mitotic figures and nuclear and cellular pleomorphism may be variable. The tubular carcinomas have focal cystic or solid areas and are typically very scirrhous. Identification is based upon tubules retaining differentiation to resemble sweat glands, particularly the presence of secretory blebs at the luminal surface. The sweat gland carcinoma is the only primary tubular adenocarcinoma of skin.

Complex and mixed apocrine gland tumors are rare. The proliferation involves both epithelial cells and the periglandular myoepithelial sheath, and may be analogous to the mixed mammary tumor of dogs. Mucinous, chondroid and osseous metaplasia within the tumor stroma is allegedly of myoepithelial origin. The malignant version of this tumor varies from the benign tumor in that the glandular epithelial component demonstrates features of anaplasia and stromal invasion. On rare occasions, both glandular and myoepithelial components demonstrate features of malignancy and the tumor is referred to as an **apocrine gland carcinosarcoma** or **mixed malignant apocrine gland tumor**.

Benign and malignant tumors with differentiation towards apocrine ducts may occur. **Apocrine ductal adenomas** and **ductal carcinomas** are characterized by multilobular and sometimes cystic dermal-to-subcutaneous masses formed by a double layer of basophilic epithelial cells with scant, clear cytoplasm. Cells adjacent to the basement membrane zone may be fusiform. Squamous differentiation may be present. Malignant tumors have nuclear and cellular pleomorphism and stromal invasion. Lymphatic invasion and metastasis are not common.

Ceruminous glands *can give rise to adenomas, complex and mixed adenomas and the malignant versions of these tumors*. Morphologic features are very similar to those described for the apocrine gland counterpart with the additional distinguishing feature of *brown luminal secretions* on H&E-stained sections. In addition, foci of tumor cells may be found within the overlying epithelium and secondary inflammation is more common.

Carcinoma of the apocrine glands of the anal sac is an uncommon but very malignant tumor of *old female dogs*. Males are rarely affected. The tumor histologically varies from solid, rosette, to tubular types. The solid form consists of sheets of relatively monomorphic polygonal cells with scant eosinophilic cytoplasm and round-to-oval hyperchromatic nuclei. In the rosette form, tumor cells with peripheral nuclei are arranged radially, sometimes surrounding small amounts of eosinophilic secretory material. The tubular arrangement has larger cells with abundant eosinophilic cytoplasm and hyperchromatic nuclei. Tubules are filled with eosinophilic secretions. These tumors are invasive, induce a desmoplastic response, and almost invariably spread to regional lymph nodes and then various viscera. Over half have already metastasized prior to diagnosis. Tumor growth is often directed inward through the pelvic canal so that a grossly visible perianal mass is seen in only about 50% of cases. The unusual feature of the tumor is its ability regularly to *induce hypercalcemia in affected dogs* (see Vol. 3, Endocrine glands).

Tumors of **atrichial (eccrine) glands** are rare but do occur as *adenomas or scirrhous tubular carcinomas* within the loose connective tissue of the pad of dogs and cats. Tumor cells have very pale eosinophilic cytoplasm and basally located nuclei. **Eccrine gland carcinomas** are morphologically similar to apocrine gland carcinomas. Differentiation is dependent upon knowing the site of origin.

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Melanocytic tumors

Tumors derived from melanocytes or melanoblasts are of *neuroectodermal origin*. They have been reported in most species of domestic animals and many wildlife species, although they are most common in dogs, horses, and some breeds of swine. There are some species differences in melanocytic tumors. In **dogs**, *melanomas in the oral cavity and non-haired skin of the lip are almost invariably malignant.* Those of the nailbeds also are frequently malignant, whereas cutaneous tumors are more commonly benign. In **horses**, melanomas occasionally occur as congenital tumors, however they are most common in *older gray horses with a site predilection for the perineum, genital area, and distal limbs* (Fig. 5.138). The behavior of melanomas in horses is difficult to predict based on histologic features. They can be clinically malignant and aggressive from the outset, or they may demonstrate slow growth for years with a sudden onset of malignant behavior, or growth can be slow for many years without evidence of metastasis. In some breeds of **swine**, such as Sinclair miniature swine and Hormel crosses (MeLiM), melanomas can occur as *congenital tumors*, and these breeds have been extensively used in biomedical research. Melanomas are uncommon in **cats** and are often *amelanotic*. Melanomas in **cattle** may occur as a congenital lesion, or they may occur at any age. Most are benign in cattle, although occasional tumors are malignant. Melanocytic tumors are uncommon in **goats** and **sheep** and are generally pigmented.

The *histologic diagnosis* of melanocytic tumors is complicated by the fact that they can display various degrees of pigmentation, from heavily pigmented to amelanotic. In addition, neoplastic melanocytes can be pleomorphic, and melanocytic tumors can display a variety of cell shapes, including *spindle cell, balloon cell (clear cell), epithelioid cell, and signet-ring cell*, thus making them difficult to distinguish from poorly differentiated sarcomas and carcinomas. *Junctional activity can be helpful in identification* (Fig. 5.139), as the only other tumors to display this activity are epitheliotropic lymphoma, and rarely, cutaneous histiocytomas. The advances in development of immunohistochemical detection of melanocytic markers has aided greatly in diagnosis of these tumors, particularly in the diagnosis of amelanotic melanomas. While antibodies against vimentin, S-100, and neuron-specific enolase are sensitive for tumors of melanocytic origin, they are not specific for these tumors and react with many other tumors. Currently *Melan A is the most specific immunohistochemical marker for melanomas*, albeit not 100% sensitive, and has been shown to be useful in many species, including the dog, cat, and horse.

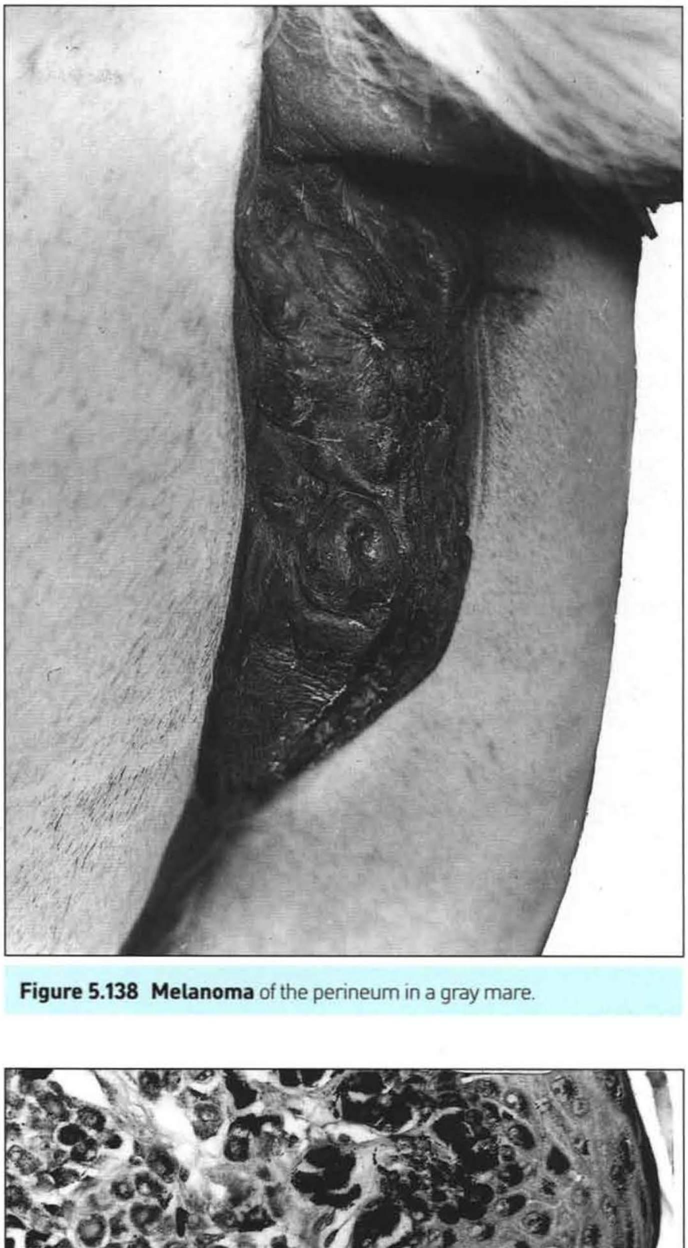

Figure 5.138 Melanoma of the perineum in a gray mare.

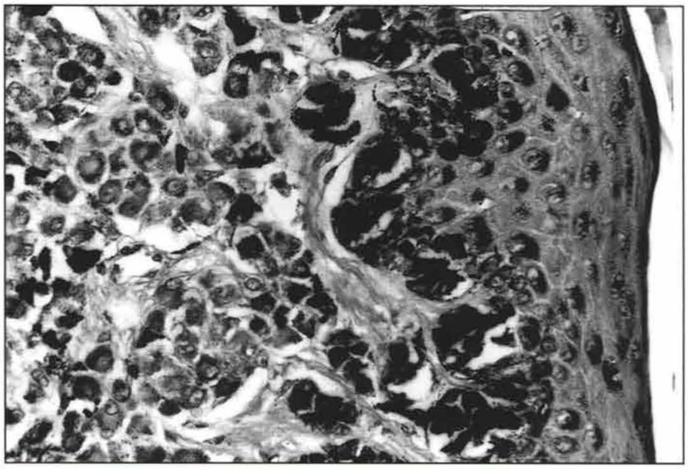

Figure 5.139 Benign melanoma demonstrating junctional activity.

Various studies on canine melanomas have attempted to establish criteria on which to base the prediction of biologic behavior of melanomas. These studies suggest *that the most significant factor predicting the clinical behavior of a melanocytic tumor in the dog is mitotic index* (total number of mitotic figures in ten randomly selected high-power (×40) fields). In one study, a mitotic index of three or greater, notwithstanding the cytologic features, was regarded as indication of malignancy, whereas in another study mitotic activity together with atypical cytologic features was regarded as predictive of clinical behavior. Several proliferation markers have been examined as an aid in predicting survival. It is reported that Ki67, but not PCNA, is a useful prognostic factor in melanomas of dogs and cats, however Ki67 is not useful in predicting clinical behavior in equine melanomas. The degree of pigmentation and cell type are reported not to be associated with prognosis.

Benign melanocytic tumors

Lentigo (lentigo simplex)

A lentigo is a proliferation of melanocytes that is usually confined to the epidermis, resulting clinically in a pigmented circumscribed macule. This lesion is considered to be a hyperplastic melanocytic lesion rather than neoplastic (see Disorders of pigmentation).

Melanocytoma (benign melanoma, melanocytic nevus)

Cutaneous melanocytomas are usually solitary, black, brown, or gray cutaneous nodules. Histologically, melanocytomas can be *junctional* (confined to the epidermis and dermoepidermal junction), *compound* (involving both epidermis and dermis), or *dermal*. Of these, the most common in domestic animals are compound and dermal melanocytomas. They are moderately circumscribed but not encapsulated and may be composed of any melanocytic cell type, i.e., spindle, epithelioid, balloon cell (clear cell), or signet ring. Most commonly they are composed of spindle cells, epithelioid cells, or a mixture of these cell types. The epithelioid cells often occur in nests in the dermis or in the epidermis and along the dermoepidermal junction, or in follicular epithelium. The spindle cell component may form whorls and fingerprint patterns in the dermis. The degree of pigmentation can vary, with the epithelioid cells commonly being darkly pigmented, and the spindle cells lightly pigmented or amelanotic. Mitotic figures are rare, and in the dog a mitotic index of less than three is reported to predict benign behavior.

Balloon-cell melanocytoma is a well-recognized variant in which the circumscribed dermal mass is composed of large epithelioid to polygonal cells with plentiful pale amphophilic to eosinophilic cytoplasm that has a faintly granular appearance. Fine melanin granules can sometimes be detected in low numbers of tumor cells. The nuclei are small, hyperchromatic, and uniform.

Melanocytoma-acanthoma

This tumor is composed of both melanocytic and epithelial cells. It is well described in humans but is rare in domestic animals and has been reported only in dogs. The tumors are seen as solitary pigmented nodules, generally 1 cm or less in diameter. Histologically, the tumor is a *combination of a junctional melanocytoma together with a benign epithelial tumor*. The epithelial population forms a mass in the dermis composed of cords and nests with occasional small cystic structures containing keratin. Melanocytic cells form nests in the epidermis and sometimes in the cords of epithelial cells within the dermal mass; melanocytic spindle cells can form whorls and bundles between the epithelial cords and nests.

Malignant melanoma (melanosarcoma)

Malignant melanomas are generally tumors of older animals, however they have been reported in juvenile animals of many species. Criteria for malignancy and prognosis are described in the introduction to this section; *in the dog, a mitotic index of 3 or greater appears to be the most accurate predictor of a poor prognosis*. In addition, several studies have indicated that *the degree of pigmentation and the histologic pattern are not correlated with prognosis*. Malignant melanomas can be composed of a variety of cell morphologies including spindle cells, epithelioid cells, a mixture of spindle cells and epithelioid cells, signet-ring cells, or balloon cells (clear cells). In addition, the cells can be heavily pigmented or amelanotic and form bundles, sheets, nests, and whorled patterns. Focal areas of chondroid or osseous metaplasia within the tumor may be seen on rare occasion. The most common types of malignant melanoma are composed of epithelioid cells, a mixture of epithelioid and spindle cells, or spindle cells alone, forming an unencapsulated mass in the dermis or subcutis. Various degrees of junctional activity may be present in the epithelioid cell form and in the mixed epithelioid-spindle cell form. The epithelioid cells tend to form clusters and nests, and the spindle cells tend to form sheets, bundles, or whorls. The neoplastic cells have variable nuclear pleomorphism, usually single prominent basophilic nucleoli, three or more mitotic figures per ten high-power (×400) fields, and mitotic atypia (Fig. 5.140A). Cytoplasm is generally moderate to abundant, and the degree of pigmentation is highly variable, from darkly pigmented to amelanotic. An infrequent form of spindle cell melanoma, composed entirely of amelanotic spindle cells, can be impossible to distinguish from fibrosarcoma or neurofibrosarcoma without the aid of immunohistochemistry. While the epithelioid and spindle cell forms of melanoma are usually pigmented to some degree, the uncommon balloon cell form and signet-ring cell form are usually unpigmented or poorly pigmented.

Balloon cell melanosarcomas (Fig. 5.140B) are dermal masses that are sometimes multilobulated, and exhibit no junctional activity. The cells are large, with large vesicular nuclei, prominent nucleoli, a relatively low mitotic index, and plentiful clear cytoplasm without visible melanin. Rare cells may have fine pale cytoplasmic dust-like granules. Occasional multinucleated cells can be present.

Signet-ring melanosarcomas (Fig. 5.140C) are composed of round to polygonal cells, that have eccentric nuclei compressed by abundant faintly eosinophilic or amphophilic cytoplasm that is not visibly pigmented. Occasional cells have fine pale brown granules. Nucleoli are prominent. Occasional multinucleated cells can be present.

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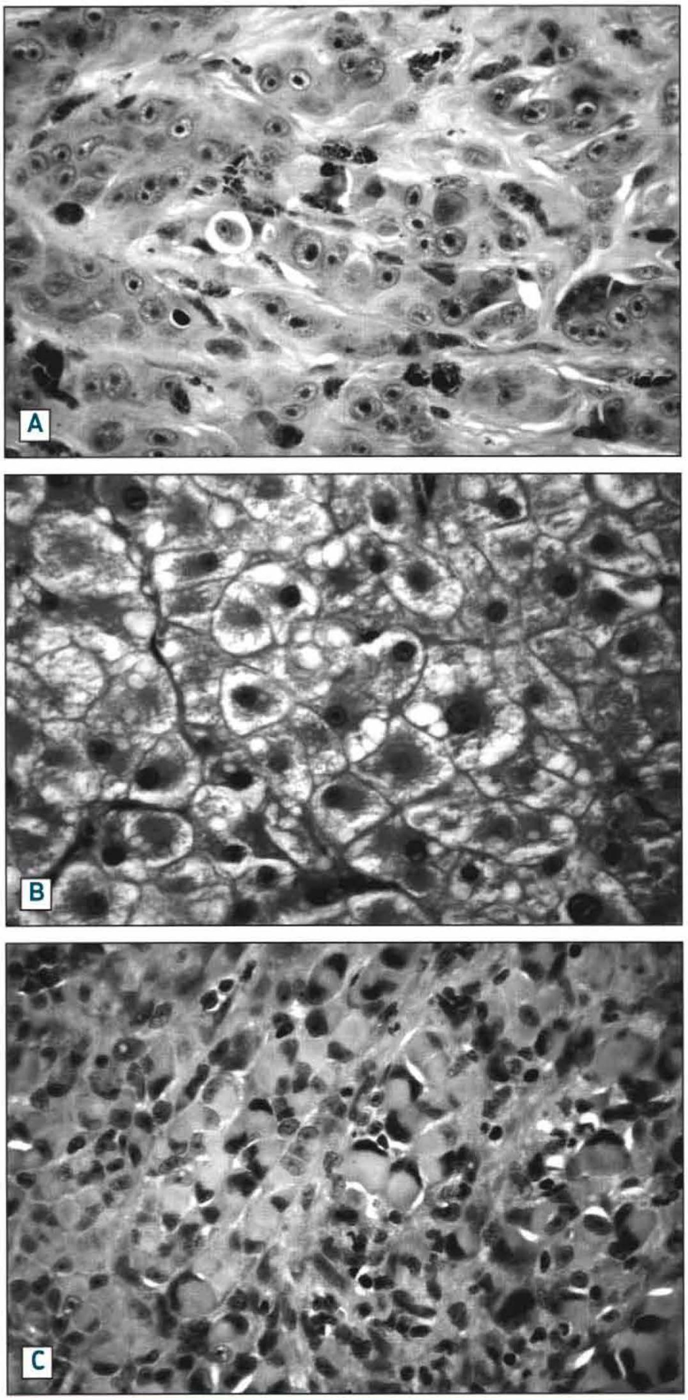

Figure 5.140 Malignant melanoma (melanosarcoma). **A.** Note large prominent nuclei and cellular atypia. **B.** Balloon cell type. **C.** Signet-ring cell type.

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Spindle cell tumors

Tumors arising from spindle-shaped cells of the skin are common in dogs and cats, sporadic in horses, and uncommon to rare in other domestic species. *Classification of these tumors may be difficult and the nomenclature is inconsistent and controversial.* The tumors are classified according to the mature tissue they resemble but histologic differences are frequently subtle and morphologic appearance may not be specific enough to reflect histogenesis, particularly for malignant spindle cell tumors. Consequently, *accurate diagnosis by morphologic features alone may not be possible for many tumors.* An immunohistochemical study of canine cutaneous fibrosarcoma, hemangiopericytoma, and schwannoma found poor correlation between morphologic diagnosis and tumor cell differentiation. Although electron microscopy and immunohistochemistry may be helpful in determining the line of differentiation exhibited by tumor cells, some tumors defy identification because of conflicting results owing to loss or alteration in antigens normally present or acquisition of novel antigens. Classification of neoplasms is considered important because of the expectation that it will provide a prediction of the biologic behavior of the tumor. Exact identification of spindle cell sarcomas of the skin may not be essential, however, because *most soft tissue spindle cell tumors exhibit similar behavior and prognosis. They are typically locally invasive, recur frequently after surgical excision, but metastasize infrequently.* The *mitotic index* is more important than the tumor type in predicting the biologic behavior of most soft tissue sarcomas in dogs. Although soft tissue sarcomas may appear well-circumscribed or even encapsulated, *finger-like microextensions of tumor commonly infiltrate into the surrounding tissue to give rise to satellite lesions not visible grossly.* The apparent tumor circumscription commonly results in incomplete excision, leaving microscopic foci of tumor tissue that result in recurrence. Wide surgical excision may be curative, however. Because determination of adequacy of excision is prognostic, *surgical margins should be marked.*

Benign spindle cell tumors

Skin tags (*fibrovascular papilloma, acrochordon, skin polyp*) are benign, fibrovascular lesions of middle-aged and older dogs that may be a *proliferative response to trauma or inflammation rather than actual neoplasms.* They are solitary or multiple soft, polypoid or filiform hairless masses up to 1 cm in diameter and 2–3 cm long that occur most commonly on the *trunk, sternum, and bony prominences* of the limbs. Microscopically, skin tags are composed of mature collagenous tissue that is more highly vascular than normal dermis and covered by an irregularly hyperplastic, hyperkeratotic and hyperpigmented epidermis. Adnexal structures are absent and mononuclear inflammatory cells may be present in low numbers. Ulceration and neutrophilic inflammation are common sequelae to trauma.

Collagen nevi *are focal nodular accumulations of excessive dermal collagen that are relatively common in middle-aged and older dogs.* They are typically solitary, alopecic, firm, dome-shaped nodules up to 1 cm in diameter. Microscopically, they are composed of haphazardly arranged bundles of collagen and low numbers of mature fibroblasts that entrap adnexal structures and result in their distortion or atrophy. Collagen nevi are usually located in the dermis but large masses may extend into the subcutis. The primary differential is fibroma, which tends to be larger and located deeper in the dermis, displaces and compresses adjacent structures, and is more cellular than a collagen nevus. Some veterinary pathologists believe that collagen nevi are actually fibromas of low cellularity. A syndrome called **nodular dermatofibrosis**, characterized by *multiple cutaneous collagenous nodules*, has been reported as *a marker of renal epithelial neoplasia in German Shepherd Dogs.* The condition is thought to be inherited in an autosomal dominant manner. Affected dogs are adults and most have bilateral renal cystadenocarcinomas. Nodules may number in the hundreds and are located anywhere on the body. Individual cases in several other breeds have also been reported. Histologically the lesions are similar to collagen nevi and are differentiated primarily on the basis of the large number of lesions present and the breed affected.

Fibromas *are uncommon benign tumors of fibroblasts and collagen that occur in adult and aged animals of all species.* Fibromas are usually solitary, soft-to-firm, well-circumscribed, round, dome-shaped or pedunculated masses that vary from 1 to 50 cm in diameter. They are usually alopecic and may be hyperpigmented. Large tumors may be ulcerated secondary to trauma. Microscopically, fibromas are well-circumscribed, nonencapsulated dermal or subcutaneous nodules composed of fibroblasts and abundant collagen. The fibroblasts have uniform, oval-to-elongate bland nuclei that may be slightly larger than fibroblasts in the normal dermis and have fine chromatin, inconspicuous nucleoli, and rare mitotic figures. The cytoplasm merges imperceptibly with collagen that is arranged in whorls and interwoven bundles that are thicker and more dense than those in the normal dermis. Adnexal structures are usually displaced and compressed peripherally. Some tumors contain substantial amounts of *mucinous or myxomatous matrix material in addition to the collagen*, in which case the term **fibromyxoma** may be used. In contrast to collagen nevi, fibromas are typically larger, more highly cellular, and displace rather than incorporate adnexal structures.

Myxomas (myxofibromas) are *rare cutaneous neoplasms arising from fibroblasts or multipotential mesenchymal cells and containing abundant glycosaminoglycan stroma.* They usually occur in adult or aged animals as solitary, infiltrative soft masses that are poorly circumscribed and may extend along fascial planes. The tissue is pale and exudes a clear viscous fluid on cut surface. A report of a myxoma developing at the site of a subcutaneously implanted pacemaker in a dog raised the question of whether the implant may have induced the tumor. Microscopically, myxomas are nonencapsulated dermal or subcutaneous masses composed of small stellate to spindle cells randomly distributed within abundant basophilic mucinous stroma with scant, fine collagen fibers. Cellularity is typically low and the cells have small hyperchromatic nuclei and rare mitotic figures. *Recurrence is common because of the infiltrative growth pattern.* Myxomas are difficult to differentiate from myxosarcomas because both are poorly circumscribed, locally infiltrative, and have low mitotic activity. However, nuclear and cellular pleomorphism is more apparent and atypical mitotic figures may be seen in myxosarcomas.

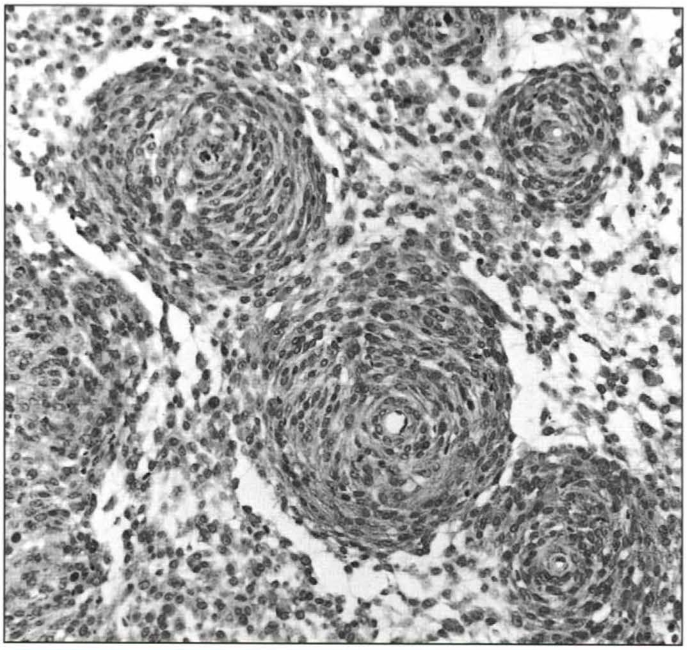

Figure 5.141 Hemangiopericytoma from a dog. Concentric whorls of plump spindle cells around capillary lumina.

Locally infiltrative or malignant spindle cell tumors

Hemangiopericytomas *are relatively common, histologically distinctive, spindle cell tumors of dogs.* Occasional tumors with similar microscopic features are seen in horses and cats. Some ultrastructural and immunohistochemical findings have supported a pericyte origin for this tumor in dogs but *histogenesis is still uncertain.* They usually occur on the *limbs of older dogs* of either sex and any breed but are most common in large breed (>30 kg) dogs. They are usually solitary, slow growing multinodular masses that appear grossly well circumscribed and measure up to 25 cm in diameter. The tissue is usually pale and the consistency varies from soft to very firm. The tumors *frequently recur following excision* owing to the difficulty in identifying tumor margins and inability to perform wide surgical excision because of anatomic constraints. *Metastasis is uncommon*, however.

Hemangiopericytomas are usually located in the dermis and subcutis and are primarily composed of uniform plump spindle cells with oval nuclei, fine chromatin, and small central nucleoli. The characteristic microscopic feature of hemangiopericytoma is *plump spindle cells arranged in concentric layers to form whorls, sometimes with small vessels within the centers* (Fig. 5.141). Sheets and interlacing bundles of similar spindle cells, occasional polygonal cells and multinucleated cells, and variable amounts of collagen also comprise most tumors. Densely cellular areas alternate with loose, myxomatous-appearing areas. Aggregates of lymphocytes may be scattered within some tumors and are usually most prominent along the periphery. At the margins of the tumor, *finger-like microextensions* of tumor cells commonly infiltrate along fascial planes and are the reason many tumors are incompletely excised despite the clinical impression that they are well circumscribed. The mitotic index is usually low but it may not be a reliable prognostic indicator of metastasis. Recurrent tumors may exhibit more prominent pleomorphism and a higher mitotic index. *Tumor cells typically express vimentin intermediate filament and are also frequently positive for actin but are negative for desmin.* A small number of tumors has been positive for S-100 protein, suggesting that some tumors diagnosed as hemangiopericytoma by morphologic features may actually be of neural origin.

Cutaneous tumors of neural origin are uncommon in domestic animals but are likely under-diagnosed because of their histologic similarity to other more common tumors of the skin. They can be composed of one or more elements of a nerve, i.e., axon, Schwann cell, and perineurial fibroblast. Consequently, these tumors are histologically heterogeneous and the histogenesis is frequently uncertain, resulting in varied and confusing classification and terminology in the literature. They have been called neurofibromas/neurofibrosarcomas, neurilemmomas, neurinomas, and schwannomas/malignant schwannomas. The name **peripheral nerve sheath tumor** is a broad term proposed to include all tumors arising from peripheral nerves; however, since most tumors are composed of Schwann cells, the term **schwannoma** is appropriate for the majority of the tumors. Both benign and malignant forms occur; however, tumors that appear histologically benign commonly recur. Schwannomas are common in **cattle** but occur primarily in the heart and rarely involve the skin. A condition termed **neurofibromatosis** has been observed in cattle of all ages and may occur congenitally. It is characterized by multiple neural tumors that usually involve deep nerves and viscera but sometimes also the skin.

Cutaneous peripheral nerve sheath tumors are usually solitary, well-circumscribed, slow growing, soft to firm nodules in middle-aged to aged animals. In cats, the head and distal limbs may be involved most frequently. Schwannomas are usually subcutaneous in dogs; in cats they may be confined to the dermis. Microscopically, *schwannomas are most commonly composed of small spindle cells characterized by oval, spindle-shaped, or wavy nuclei, fine chromatin, small inconspicuous nucleoli, and pale indistinct cytoplasm. The spindle cells form whorls, interlacing fascicles, and palisades reminiscent of nerve* (Fig. 5.142). A delicate collagenous stroma is moderately abundant and a mucinous matrix may be prominent in some tumors. Delicate finger-like projections of tumor cells commonly extend into adjacent tissues and between fascial planes, accounting for frequent recurrences. Cellularity is increased, cellular pleomorphism is more prominent, and there is a decreased tendency to form whorls and palisades in malignant tumors. Mitotic figures are uncommon in benign tumors but may be moderately numerous in malignant peripheral nerve sheath tumors. Histologically and behaviorally, peripheral nerve sheath tumors may be difficult to differentiate from fibromas, well differentiated fibrosarcomas, and hemangiopericytomas. However, *neural tumors express S-100 protein, myelin basic protein, neuron-specific enolase, and glial fibrillary acidic protein, whereas the other more common cutaneous tumors do not.* Immunohistochemical staining of **granular cell tumors** (granular cell myoblastomas) has demonstrated S-100 protein, myelin basic protein, and neuron-specific enolase within tumor cells, suggesting that they also represent a form of peripheral nerve sheath tumor.

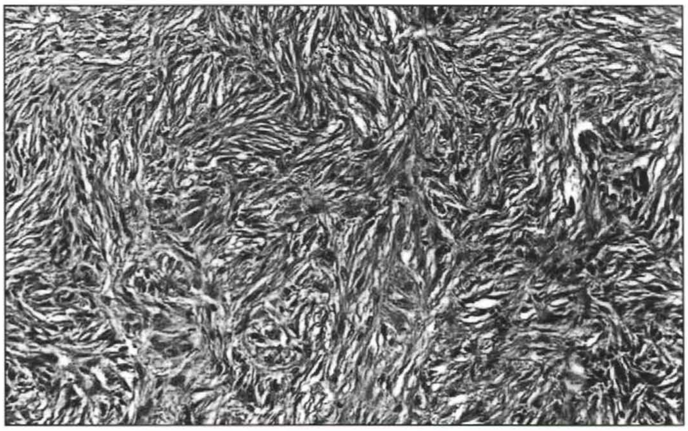

Figure 5.142 Peripheral nerve sheath tumor.

Traumatic neuroma (*tail dock neuroma, amputation neuroma*) is considered an exuberant but non-neoplastic proliferation of the proximal nerve stump occurring in response to injury or surgery. In veterinary medicine, this lesion is rare and occurs most frequently as a result of tail docking in **dogs**. The tumors develop in young dogs, usually within 1 year after caudectomy. They are typically painful, self-traumatized, alopecic, hyperpigmented, lichenified lesions adherent to the underlying deep tissues at the tip of the tail. Microscopically, traumatic neuromas are well-circumscribed nodules composed of *haphazardly arranged myelinated nerve bundles of varying size randomly distributed within a relatively abundant connective tissue stroma.*

Sarcoids *are locally aggressive, non-metastatic fibroblastic skin tumors of horses, mules, and donkeys.* They are the *most common skin tumor of horses*, accounting for up to 30% of tumors, and occur in any breed, sex, or age. However, there appears to be a higher incidence in Quarter Horses, Arabians, and Appaloosas and a lower incidence in Standardbreds. Young adult horses 3–6 yr of age are most commonly affected. A combination of factors appears to be involved in development of the tumors, including *exposure to a viral agent, cutaneous trauma, and a genetic predilection.* Viral etiology has been deduced based on reports of epizootics of cases, transmission studies, detection of virus particles in cultured tumor cells, and demonstration of DNA sequences very similar or identical to that of *Bovine papillomavirus-1* or *-2* genome in tumor cells but not in normal tissues, granulation tissue, or other tumors of horses and donkeys. Sarcoids frequently develop in areas subjected to trauma or at sites of wounds 6 to 8 months after wound healing. A genetically determined predisposition is suggested since an association between susceptibility to sarcoid and certain major histocompatibility complex (MHC) alleles has been found.

Sarcoids develop anywhere but are most common on the *head, legs, and ventral trunk.* They may be single or multiple. The tumors are classified according to their gross appearance as verrucous, fibroblastic, mixed, or occult. The *verrucous type* is a small wart-like growth, usually measuring less than 6 cm in diameter, with a dry, rough surface and variable alopecia. The *fibroblastic type* of sarcoid is more

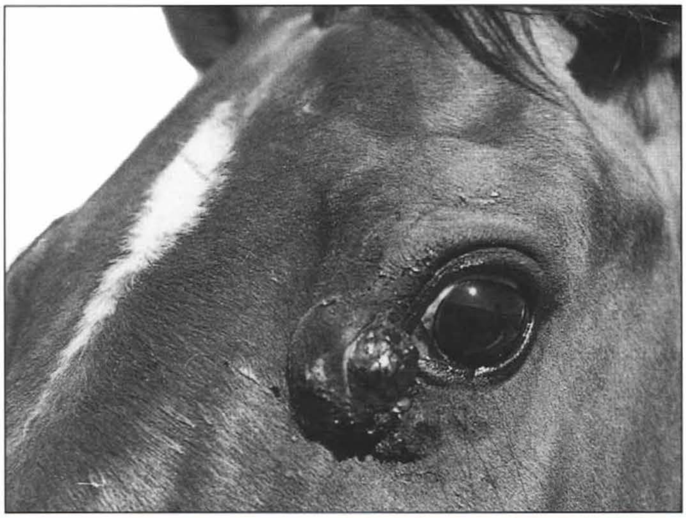

Figure 5.143 Sarcoid from a horse.

variable in appearance and may range from a well-circumscribed firm nodule with intact surface to large masses, greater than 25 cm in diameter, with an ulcerated surface prone to hemorrhage and resembling exuberant granulation tissue. The *mixed type* is a transitional form in which a verrucous sarcoid becomes a fibroblastic type as a result of trauma or biopsy. The *occult form* consists of a slow-growing, slightly thickened area of skin with slight surface roughening and alopecia that remains static for long periods. *Spontaneous remission* may occur after several years in up to 30% of cases. The tumors are characterized by a high rate of recurrence, up to 50%, following surgical excision.

Grossly, sarcoids may resemble many inflammatory and neoplastic diseases including bacterial and fungal granulomas, exuberant granulation tissue, habronemiasis, papilloma, squamous cell carcinoma, fibroma, and other less frequent mesenchymal tumors (Fig. 5.143). Consequently, *histopathology is necessary for definitive diagnosis of a sarcoid.* Wide surgical excision is preferable to a biopsy because the lesions are frequently associated with ulceration, inflammation, and granulation tissue proliferation and the diagnosis may be missed in a small biopsy. Additionally, the trauma associated with obtaining a biopsy of a sarcoid may dramatically stimulate the growth rate and aggressiveness of the tumor. Sarcoids are typically *biphasic tumors composed of both epidermal and dermal components.* The epidermal component may be minimal or absent in some tumors, however. When the epidermis is intact, hyperkeratosis, parakeratosis, and acanthosis with thin rete pegs extending deep into the dermis are common features (Fig. 5.144). The dermal component consists of fibroblasts and collagen in various proportions. The fibroblasts have plump nuclei and nucleoli may be prominent. The mitotic index is usually low. *Fibroblasts at the dermal-epidermal junction are frequently oriented perpendicular to the basement membrane in a "picket fence" pattern, which is a distinctive histologic feature seen in most sarcoids.* The cells are arranged in whorls, interlacing bundles, or haphazard arrays of variable density. Tumor margins are typically indistinct and adequacy of excision is frequently difficult to determine. When the tumor is ulcerated, it may not be possible to differentiate a sarcoid from granulation tissue, fibroma, well-differentiated fibrosarcoma, and peripheral nerve sheath tumor. Immunohistochemical staining for S-100 protein may be useful in confirming a peripheral nerve sheath tumor. Additional biopsies to include intact epidermis may be required to make a diagnosis.

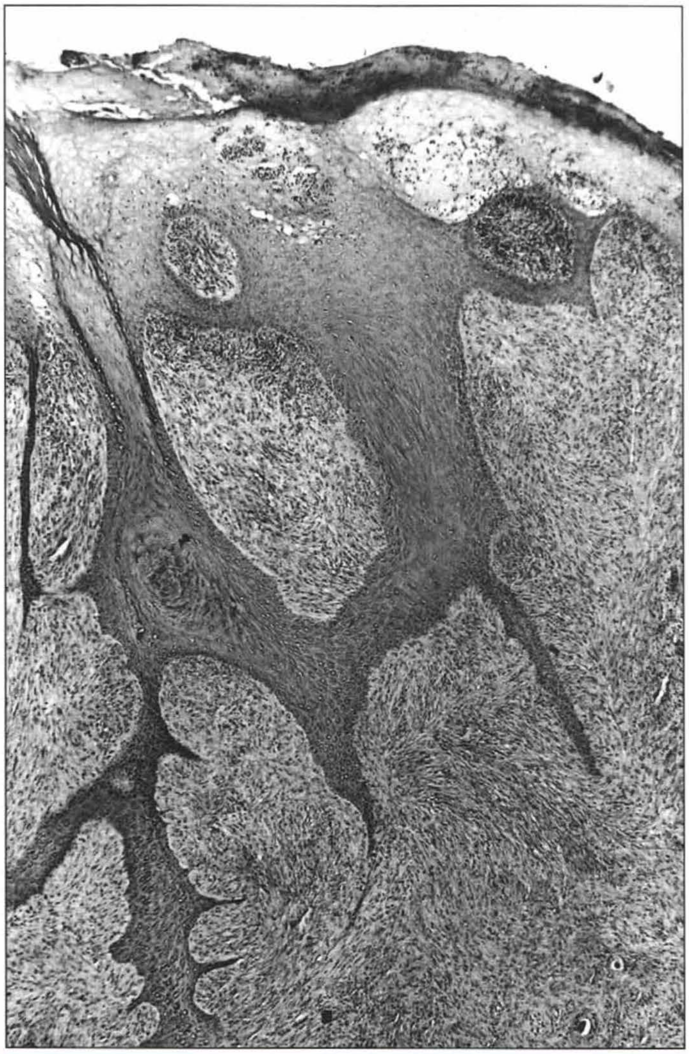

Figure 5.144 Sarcoid from a horse. Note epithelial proliferation and associated dermal fibroblastic proliferation.

A tumor with histologic features similar to equine sarcoid has been described in cats and termed **feline fibropapilloma.** The cats are frequently young, 5 years or less, and have one or several tumors that may be ulcerated. The tumors have been reported in various sites, including the lip, nose, digits, pinna, tail, and gingiva. Recurrence was common but metastasis has not been documented. As in equine sarcoids, papillomavirus DNA has been identified in some of the feline tumors by polymerase chain reaction testing. The nucleotide sequence was similar to a bovine papillomavirus and many of the affected cats had known exposure to cattle.

Fibrosarcomas are malignant tumors of fibroblasts that show no other evidence of cell differentiation. They commonly recur and may metastasize. *Fibrosarcomas are undoubtedly over-diagnosed*, as virtually any anaplastic highly cellular spindle cell sarcoma containing collagen is diagnosed as a fibrosarcoma when more specific histogenesis is not apparent. However, as *immunohistochemistry* has become a routine technique in most diagnostic laboratories, fibrosarcoma can be separated from other spindle cell tumors such as peripheral nerve sheath tumor, leiomyosarcoma, rhabdomyosarcoma, amelanotic malignant melanoma, spindle cell carcinoma, and others.

Fibrosarcomas are common in dogs and cats and uncommon in other domestic species. In **dogs**, they usually occur in older animals and are most common on the trunk and limbs. They usually arise in the subcutis and are poorly circumscribed masses of variable size that may be soft to firm in consistency. Large tumors are often ulcerated and alopecic. Most canine fibrosarcomas are low-grade malignancies that commonly recur locally and metastasize infrequently. Similarities have been noted between canine fibrosarcomas from presumed injection sites and feline post-vaccinal fibrosarcomas, suggesting that post-injection sarcomas may also occur in dogs.

Fibrosarcoma is the most common malignant mesenchymal tumor of **cats**, and three forms of fibrosarcoma have been recognized – virus-induced, solitary in older cats, and post-vaccinal.

Virus-induced fibrosarcoma is rare. *Feline sarcoma virus* (FeSV) is the cause of multicentric fibrosarcoma in cats usually less than 5 years of age. FeSV is a replication-defective retrovirus that requires *Feline leukemia virus* (FeLV) as a helper virus. The genetic recombination of the two viruses produces an acutely transforming virus that induces multiple simultaneous rapidly growing fibrosarcomas after a short incubation period. FeSV-induced fibrosarcomas are typically locally invasive and metastasize to lung and other sites.

Solitary fibrosarcomas in older cats are much more common that virus-induced multicentric tumors. These may arise in the dermis or subcutis. Subcutaneous tumors are usually on the trunk and limbs; dermal tumors primarily involve the digits and pinnae. The clinical appearance and behavior of this type of fibrosarcoma are similar to those in dogs.

Epidemiologic evidence supports a relationship between vaccine administration in cats and the development of *post-vaccinal fibrosarcomas* and, to a lesser extent, other sarcomas in the injection sites. The interval between vaccination and tumor development is 3 months to 3.5 years. The mechanism of tumor development is unknown but persistent injection-site induced inflammation leading to deranged fibrous connective tissue repair response and eventual neoplastic transformation in genetically predisposed cats has been postulated. Alterations in oncogene and growth factor expression may be involved in the pathogenesis. Post-vaccinal fibrosarcomas typically develop in the subcutis of the dorsal cervical and interscapular area, dorsal and lateral thorax, flank, and musculature of the thigh, locations that are all *common vaccination sites*. These tumors arise in cats that are younger (median age, 8 years) than those with fibrosarcomas at nonvaccination sites (median age, 10.5 years) and are larger than nonvaccination site fibrosarcomas. The masses frequently have cystic centers, firm attachments to dorsal spinous processes or other deep structures, and ill-defined margins. *They are biologically aggressive and commonly recur multiple times within a period of weeks to months when removed.* The metastatic rate is not well characterized, but was found to be as high as 24% in one study. The lungs and regional lymph nodes are the most common sites of metastasis. Wide surgical excision prolongs the interval to recurrence but often fails to remove the tumor completely. Radiation therapy and chemotherapy are under evaluation as adjunctive treatment to prevent local recurrence and systemic spread, respectively.

Microscopically, *fibrosarcomas consist of interlacing and intersecting bundles of immature fibroblastic cells.* The tumors show a great deal of variation with respect to cellular pleomorphism and density, mitotic activity, and amount and maturity of collagen. Multinucleated giant cells are seen in many fibrosarcomas but are not numerous. The tumors are dermal, subcutaneous, or both. They are usually nonencapsulated, and local invasion is evident as finger-like projections of tumor cells extending along fascial planes and into surrounding tissues. These microscopic extensions of the tumor account for the difficulty in excising the tumor completely and resultant recurrences. Although collagen is the primary stromal element, mucin may also be produced in small amounts. Immunohistochemical staining is *positive for vimentin* only. *Retrospective studies have found only the mitotic index* (total number of mitotic figures in ten high-power (×400) fields) *to be significant in predicting tumor behavior.* In dogs, a mitotic index less than 9 was associated with greater survival than a mitotic index of 9 or greater. Likewise, cats with solitary fibrosarcomas with a mitotic index of 5 or less had significantly greater survival time than cats with tumors with mitotic index of 6 or greater. Poorly differentiated fibrosarcomas may be difficult to differentiate from a number of other mesenchymal and nonmesenchymal tumors including malignant schwannoma, malignant fibrous histiocytoma, leiomyosarcoma, and spindle cell forms of amelanotic melanoma and squamous cell carcinoma. In such cases, immunohistochemical staining is necessary to exclude other tumors by showing lack of immunoreactivity to antigens typical of other cell types. In small biopsies with a limited amount of tissue to evaluate, it may be difficult to differentiate fibrosarcoma from reactive granulation tissue.

Post-vaccinal fibrosarcomas are typically subcutaneous and have large cavitated centers as a result of extensive necrosis. An inflammatory reaction, consisting of lymphocytes and macrophages that sometimes contain gray-brown or blue globular material typical of a vaccine reaction, is commonly present at the periphery of the tumor. Mitotic activity and cellular pleomorphism tend to be high; however, histologic grade does not appear to be associated with outcome. Tumor cells of vaccination-site fibrosarcomas have been reported to be consistently strongly immunoreactive for platelet-derived growth factor and its receptor, epidermal growth factor and its receptor, and transforming growth factor-β, while nonvaccine-associated fibrosarcomas are negative or only slightly positive.

Malignant fibrous histiocytoma *(giant cell tumor of soft parts, extraskeletal giant cell tumor) is an uncommon tumor of controversial histogenesis.* It was originally believed to be derived from malignant cells of monocyte-macrophage origin capable of acting as "facultative" fibroblasts. Ultrastructural and immunohistochemical findings, however, indicate that malignant fibrous histiocytoma may not be a distinct entity but rather a *collection of anaplastic mesenchymal and nonmesenchymal tumors.* Nevertheless, the diagnosis of malignant fibrous histiocytoma is made for those neoplasms characterized histologically by the *combination of fibroblast-like spindle cells, vacuolated histiocyte-like cells, and variable numbers of pleomorphic multinucleated giant cells along with a collagenous stroma.* The tumor is uncommon to rare in dogs and cats and has been reported in horses and an adult cow. Most cases occur in older animals. They occur on the *legs and shoulders* most commonly. The tumor is usually solitary, firm, and poorly circumscribed and the skin surface may be ulcerated. *The tumors typically have a moderate rate of growth, exhibit locally invasive growth, and recur commonly after surgical excision.* The potential for metastasis is disputed but has been found to be low in most reports. Microscopically, the giant cell type of malignant fibrous histiocytoma is most common and is composed of fibroblastic-like spindle cells arranged in a storiform (or cartwheel) pattern or in interwoven bundles with variable amounts of collagen resembling a fibrosarcoma. Polygonal cells that resemble histiocytes are distributed throughout the tumor or form focal aggregates. Multinucleated giant cells with 30 or more nuclei and abundant eosinophilic cytoplasm usually form aggregates rather than being diffusely distributed throughout the tumor. The giant cells resemble osteoclasts but are not associated with any osteoid production. Mitotic activity is variable but usually moderate to high and atypical mitotic figures are common. There is no immunohistochemical profile unique to malignant fibrous histiocytoma and, in fact, immunohistochemical staining may result in classification of tumors diagnosed as malignant fibrous histiocytoma by light microscopy as various other malignant tumors.

Other mesenchymal tumors

Tumors arising from subcutaneous lipocytes occur in all species. **Lipomas** are most common in dogs and may be multiple. In all species, they usually occur in adult and aged animals and are most commonly located on the trunk and proximal limbs. *Lipomas are masses of well-differentiated lipocytes indistinguishable from normal fat* except for a compressed boundary of delicate stroma that normally serves to delineate the margin between tumor and adjacent normal adipose tissue. Some lipomas contain cartilage, bone, collagen, or blood vessels. In addition, some mesenchymal tumours contain roughly equal proportions of adipose tissue mixed with other mesenchymal components. These form distinctive variants such as *angiolipomas, fibrolipomas* and *leiomyolipomas*. Occasionally hemangiosarcomas and mast cell tumours may be found arising within lipomatous masses, suggesting that lipomas may sometimes provide a local environment suitable for the emergence of these more sinister neoplasms. Necrosis, hemorrhage, fibrosis, and mild macrophage inflammation may occur as a result of trauma to lipomas.

Infiltrative lipomas are uncommon tumors of dogs. *They are usually large, poorly delineated, deep subcutaneous masses composed of well differentiated lipocytes that infiltrate subcutaneous muscle and fascia.* They may cause pain or interfere with limb function and may invade through the body wall.

Liposarcomas are malignant tumors of lipocytes and are uncommon in all species. Liposarcomas in cats have been associated with retrovirus infection and with vaccination sites; a liposarcoma in a dog was associated with a glass foreign body. *Liposarcomas may recur but rarely metastasize. They are usually well-circumscribed, nonencapsulated, highly cellular masses of round to polyhedral cells primarily and fewer stellate, spindle, and multinucleated cells.* Lipocyte origin is recognized by the presence of *cytoplasmic vacuolation* that may be numerous fine vacuoles or a single large clear vacuole that displaces the nucleus peripherally. Mitotic figures are generally infrequent. *Myxoid liposarcomas* have prominent mucinous ground substance. Liposarcomas may be difficult to differentiate from anaplastic sebaceous carcinomas and balloon cell or clear cell melanomas. The tumor cells of liposarcoma are usually arranged in solid sheets of vacuolated cells while in sebaceous carcinoma the cells tend to be subdivided into nests and lobules. The presence of rare cells with cytoplasmic dusting of very fine brown granules can differentiate melanoma from the other two tumors; however, immunohistochemical staining for cytokeratin, vimentin, and a melanocytic marker (such as Melan A) may be needed to differentiate the tumors.

Tumors originating from muscle are rare in the skin of animals. Smooth muscle tumors, **leiomyomas** and **leiomyosarcomas**, can arise from arrector pili muscles (*piloleiomyomas*), cutaneous blood vessels (*angioleiomyomas*), and specialized muscles of genital skin. These tumors are usually solitary, firm, and well-circumscribed in animals. In humans, piloleiomyomas are commonly multiple; and multiple piloleiomyomas have been reported in an old cat. Microscopically, leiomyomas are small, well-circumscribed dermal masses adjacent to and surrounding hair follicles. They are composed of uniform long spindle cells in whorling and interlacing bundles. The cells have bland elongate nuclei with blunt ends ("cigar-shaped") and moderately abundant pale eosinophilic cytoplasm. Mitotic figures are rare. Leiomyosarcomas exhibit nuclear pleomorphism and low to moderate mitotic activity. Angioleiomyomas consist of interlacing bundles of smooth muscle cells between numerous vascular channels. Smooth muscle tumors may be confused with fibromas, fibrosarcomas, or malignant schwannomas. In such cases, *immunohistochemical staining for actin and desmin can confirm muscle origin.*

Cutaneous tumors of skeletal muscle, **rhabdomyomas** and **rhabdomyosarcomas**, are extremely rare. Rhabdomyomas of the ear pinna have been reported in four white-eared cats. The tumors were thinly haired, red-purple, nonulcerated discoid nodules 1–2 cm in diameter on the convex surface of the pinna. Histologically, they were well-circumscribed masses composed of whorls and bundles of long spindle cells with cross-striations evident in a few tumor cells. Mitotic figures were rare. Surgical excision was curative.

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Vascular tumors

Cutaneous vasoformative tumors are common in dogs, occasional in cats and horses, and uncommon in other species. The majority are benign in dogs and horses whereas malignant tumors are more common in cats. Vascular tumors may be associated with thrombocytopenia, disseminated intravascular coagulation, and other hemostatic abnormalities.

Hemangioma *is a benign neoplasm of blood vessel endothelium that can originate in the dermis or subcutis.* The tumor is usually a solitary, well-circumscribed, fluctuant to firm, blue to red-black, slow-growing mass. Cutaneous hemangioma in **dogs** usually occurs in older animals (mean, 9–10 years) without apparent sex predilection. The tumor can occur anywhere but lightly pigmented, sparsely haired ventral abdominal and inguinal skin may be predisposed. Chronic solar damage has been suggested as a cause of dermal hemangiomas in this location and they may be multiple. Surgical excision is curative.

Benign cutaneous vascular lesions in **horses** frequently occur in animals less than 1 year of age and some are congenital, raising the question of whether the lesions are true neoplasms or vascular malformations (hamartoma or nevus). The confusion in categorizing these vascular lesions in young horses has resulted in *inconsistent nomenclature* and they have been referred to as **lobular capillary hemangioma** and **vascular nevus** in the literature. The lesions are most commonly located on the limbs and present as a cauliflower or nodular mass or diffuse skin thickening which may become alopecic and ulcerated. The lesions may recur following excision. In some instances they are too extensive to be excised, necessitating euthanasia of the affected animal. Cutaneous hemangiomas usually occur in adult and aged **cattle**; congenital hemangiomas have been reported also and these are frequently multiple.

Microscopically, hemangiomas consist of nonencapsulated dermal or subcutaneous masses composed of blood-filled channels lined by a single layer of flattened, mature endothelium (Fig. 5.145). Mitotic figures are rare. Subcutaneous tumors are usually well-circumscribed, while those in the dermis may not be as well defined and may incorporate adnexal structures within the mass. The tumors are classified as *capillary* or *cavernous* depending on the size of the vascular spaces. The cavernous type is more common in dogs. The vascular channels are usually separated by collagenous septa that contain variable numbers of mast cells, lymphoid cells, and hemosiderin-laden macrophages. Occasional vascular channels contain fibrin thrombi. *Solar-induced dermal hemangiomas* of glabrous skin are located in the superficial dermis and may be associated with solar elastosis. A variant of hemangioma, called **angiokeratoma**, is a raised superficial dermal hemangioma with prominent, irregular hyperplasia of the epidermis which extends down to separate and partially surround the vascular channels. The *lobular capillary hemangioma/vascular nevus of young horses* consists of multiple discrete dermal lobules of closely packed, haphazardly arranged vascular structures of small caliber. The vessels are lined by endothelial cells with plump oval nuclei and low mitotic activity. **Cutaneous angiomatosis** is a multinodular hemangioma with intervening inflamed connective tissue stroma that extends above the skin surface and bleeds spontaneously. It occurs in cattle and horses most commonly and also in cats and goats. This lesion has been compared to the human "pyogenic granuloma."

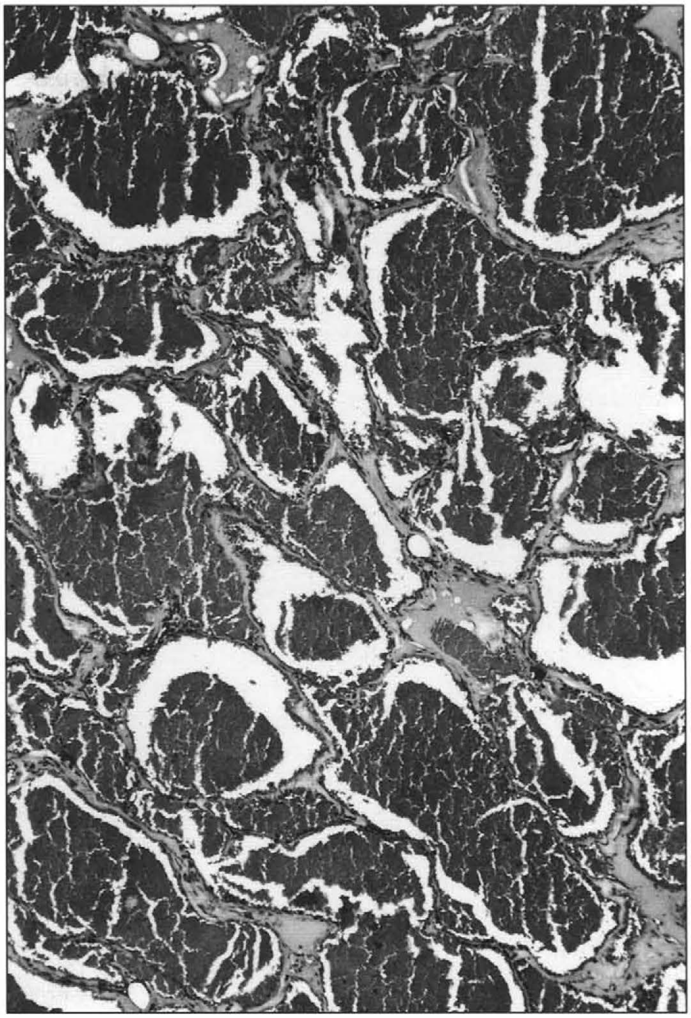

Figure 5.145 Cutaneous hemangioma from a dog.

Hemangiosarcoma, the malignant form of a tumor of blood vessel endothelium, can occur in the skin as a primary site or as a result of metastatic disease from a primary visceral tumor. These tumors usually occur in adult and aged animals. In one study of cutaneous vascular tumors of cats, hemangiosarcomas occurred most frequently in white-haired sites and most commonly involved the pinnae and head. Cutaneous hemangiosarcoma commonly recurs following excision and has the potential for widespread local invasion and metastasis; but in general it appears to be less aggressive, has a longer clinical course, and prolonged survival when compared to visceral hemangiosarcomas. In contrast to the subcutaneous form, dermal hemangiosarcoma may be cured by wide surgical excision alone.

The degree of differentiation is extremely variable, ranging from well-differentiated tumors with well-defined vascular channels to poorly differentiated tumors with minimal lumen formation. *Usually hemangiosarcomas consist of plump pleomorphic endothelial cells arranged in single or multiple layers along trabeculae of collagen or preexisting dermal collagen fibers to form a poorly circumscribed meshwork of blood-filled spaces.* Collagenous trabeculae lined by endothelial cells commonly protrude blindly into the vascular spaces. Mitotic activity may be high. Frequently, portions or entire tumors are composed entirely of sheets or intersecting and anastomosing bundles of pleomorphic spindle cells and they may be indistinguishable from fibrosarcoma or other poorly differentiated spindle cell sarcomas. The presence of slit-like spaces containing erythrocytes between tumor cells may be the only clue to the diagnosis. Immunohistochemical staining may be required to identify the cells in anaplastic tumors. Endothelial cells express vimentin, factor VIII-related antigen (von Willebrand factor), and CD31 (platelet-endothelial cell adhesion molecule/PECAM). However, *immunostaining must be interpreted with knowledge of limitations of the procedure*; in poorly differentiated tumors, very anaplastic cells may not express typical antigens and immature reactive stromal endothelial cells may be mistaken for tumor cells.

Tumors arising from endothelium of lymphatic vessels are rare in domestic animals. Both the benign, **lymphangioma**, and malignant, **lymphangiosarcoma**, forms occur in young animals and it has been suggested that they represent lymphatic malformations resulting from a failure of connection between lymph vessels and the venous system rather than being true neoplasms. *The skin and subcutis appear to be the most common site of tumors of lymphatic endothelium and the caudal ventral abdomen and inguinal areas appear to be predisposed.* They typically present as poorly defined fluctuant or edematous masses that are present for many months and drain clear serous to milky fluid. Rarely they occur as multiple clear turgid vesicles or bullae. It may be difficult to distinguish between lymphangioma and lymphangiosarcoma histologically as the histologic appearance may not correlate well with the biologic behavior of the tumor. Tumors that appear histologically benign commonly recur and may be locally invasive. Lymphangiosarcomas commonly invade local tissues extensively and metastasize to distant sites. They are poorly defined and difficult to remove completely, resulting in poor wound healing and recurrence. In contrast to tumors of vascular endothelium, *lymphangiomas/lymphangiosarcomas are composed of spaces largely devoid of material.* They may contain only small amounts of proteinaceous fluid and low numbers of erythrocytes and/or lymphoid cells. The connective tissue stroma separating the vascular spaces is loose or edematous and may contain aggregates of lymphoid cells. These tumors have been reported to be immunoreactive for factor VIII-related antigen (von Willebrand factor); CD31 reactivity is variable. Ulceration, hemorrhage, granulation tissue proliferation, and inflammation are frequently present because of the chronic clinical course and may result in misdiagnosis when a small biopsy is taken. It may not be possible to differentiate a poorly differentiated lymphangiosarcoma from hemangiosarcoma. Further complicating diagnosis is a condition called **lymphangiomatosis**, which is considered a developmental disorder composed of bland dilated lymph channels involving skin, soft tissue, bone, and parenchymal organs.

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Histiocytic proliferative disorders

Proliferations of histiocytic cells in the dog comprise a complex and incompletely understood category of disease. Fortunately, advancement in the characterization of the canine histiocytic proliferative disorders in terms of morphologic characteristics, clinical behavior, and phenotype has led to enhanced diagnostic and prognostic capabilities. The cause and pathogenesis of these conditions remain largely undetermined. Currently, histiocytic proliferative disorders in the dog comprise the entities of **cutaneous histiocytoma, cutaneous histiocytosis, localized histiocytic sarcoma**, and **disseminated histiocytic sarcoma** (formerly known as malignant histiocytosis). These conditions have only been well characterized in the dog.

Histiocytes arise from a CD34+ precursor cell found in the bone marrow and differentiate into cells of the mononuclear-phagocyte system or the dendritic cell system. Dendritic cells and macrophages have overlapping functions in that both can function as antigen-presenting cells and can act as effector cells. *Macrophages* are primarily effector cells and act as elements of the innate immune system, whereas *dendritic cells* function primarily in antigen presentation and hence provide a strong influence on the adaptive immune system. The morphological features of neoplastic or abnormally reactive histiocytes are similar and can mimic tumor cells of different histogenic origin or be confused with granulomatous inflammation. Differentiation often requires immunophenotyping and sometimes ultrastructural characterization.

Canine cutaneous histiocytoma

Canine cutaneous histiocytomas (CCH) (Langerhans cell histiocytosis) are tumors arising from proliferations of the intra-epidermal dendritic cell, the Langerhans cell. Langerhans cells acquire and process foreign cutaneous antigens, migrate to regional lymph nodes and present the antigen to T cells. Immunophenotypic markers of Langerhans cells and therefore cells comprising CCH include MHC I and II, CD1a, CD1c, CD11a, CD11b, CD11c, CD18, CD44, CD45, CD45R, CD49d, ICAM-1, and E-cadherin. Expression of the above markers may vary with the state of activation of the tumor cells and clinical stage of the tumor. CCH should also express vimentin. Lysozyme is a useful but not a reliable marker for these tumors. Histiocytoma cells are negative for Thy-1 and CD4. Unfortunately, at the time of writing, the majority of the above markers are not commercially available and/or require the use of cryopreserved tissues. Establishing the diagnosis may rely on attempting to rule out other round cell neoplasms for which commercially available antibodies for formalin-fixed specimens are available.

Canine cutaneous histiocytomas are most often solitary, benign tumors but can be multiple and on very rare occasion have been noted to lead to regional and distant metastasis. Greater than 70% of CCH occur in *dogs <4 years of age* and are most often found on the *head (pinna), or limbs* but can occur anywhere on the body. There is no sex predilection. Grossly, the tumors are raised, red, frequently ulcerated, 0.5–4 cm nodules that grow rapidly. The tumors are well demarcated but unencapsulated and located primarily in the dermis with the base of the tumor smaller in configuration than the more superficial aspect ("top heavy"). Microscopically, *tumor cells form diffuse sheets that displace adnexal structures and dermal collagen, and extend from the dermoepidermal junction into the subcutis.* Epidermotropism may be evident in some CCH. A clear zone usually surrounds intra-epidermal tumor cells. Care must be taken to differentiate the lesion from an epidermotropic lymphoma. The epidermis is often attenuated or ulcerated and associated edema may lead to *vertical rowing of tumor cells* near the surface. Ulceration is associated with infiltrates of neutrophils. The intact epidermis may be hyperplastic. Individual tumor cells are round to oval with moderate to abundant eosinophilic, slightly foamy cytoplasm. Cell borders are fairly distinct in areas of low density but obscure when cells are arranged in diffuse sheets. Nuclei are centrally located, oval, to indented and vesicular with most often a single nucleolus that may be inconspicuous. *Mitotic figures are variable but most often numerous* with up to 10–15/hpf. The base of the tumor may have infiltrates of CD8+ cytotoxic T lymphocytes, a sign of *host-mediated spontaneous tumor regression.* Infiltrates of lymphocytes may also be found in perivascular or periadnexal regions. In older lesions, the lymphocytic infiltrates may be more extensive than the remaining tumor cell infiltrates, leading to a misinterpretation of primary inflammatory process. *Tumor cell apoptosis and zones of necrosis are common findings,* particularly in regressing lesions but are not present in all CCH. As the function of Langerhans cells is to capture antigen and migrate to regional lymph nodes, regional lymph nodes may be enlarged but are not painful and should regress to normal size after tumor removal. Histologic changes in regional lymph nodes have not been well documented but in rare cases have been shown to be due to infiltrates of Langerhans cells.

The histologic appearance of tumors in dogs with *multiple cutaneous histiocytomas* is the same as described above, however spontaneous regression may be delayed and secondary bacterial infection may be a complication. Shar-Peis, Shar-Pei crosses and the English Cocker Spaniel are reported to have a higher incidence of multiple cutaneous histiocytomas. At least one author has proposed the term; "*progressive Langerhans cell histiocytosis*" for cases characterized by multiple, treatment-refractory canine cutaneous histiocytomas shown to be of Langerhans cell origin.

The overwhelming majority of CCH are benign and cured by surgical excision or by the spontaneous regression that usually occurs within a few weeks. Immunosuppressive drugs are contraindicated as this may delay natural regression of the lesions. Morphological features that suggest possible malignant behavior of a CCH include aggregates of tumor cells that are primarily in the deep dermis and subcutis, lack of lymphocytic infiltration, presence of plasma cells, and plugging of lymphatic vessels by tumor cells. The histologic differential diagnosis includes inflammatory lesions and other round cell neoplasms such as lymphomas, plasma cell tumors, and mast cell tumors. The deep dermal lymphocytic infiltrates, presence of typical histiocytes in the superficial regions, high mitotic index, infrequent presence of eosinophils, signalment and anatomical location are useful features in confirming the diagnosis of a histiocytoma. Immunophenotyping may be needed in some cases to distinguish CCH from other round cell tumors such as cutaneous lymphoma, particularly if epidermotropism is present.

Cutaneous reactive histiocytosis and systemic reactive histiocytosis

Cutaneous reactive histiocytosis (CRH) is *a reactive disorder targeting the skin that is characterized by single to multiple, non-painful plaques or nodules comprised of proliferations of histiocytes accompanied by lymphocytes and neutrophils.* **Systemic reactive histiocytosis** (SRH) is *the term used to designate a similar condition that affects not only the skin, but also lymph nodes and other body organs.* Cutaneous reactive histiocytosis does not necessarily lead to systemic reactive histiocytosis and not all cases of systemic histiocytosis have cutaneous lesions. The histiocytic cells in CRH and SRH are immunophenotypically CD1+, CD11b+, CD11c+, MHCII+, CD4+, and CD90+ (Thy-1+), ICAM-1+ activated dendritic cells. The lymphocytes are CD3+, CD8+, TCRαβ+ T lymphocytes, and the neutrophils label for CD11b+. Cultures and special stains fail to reveal causative agents and the condition responds favorably, at least for a time, with immunomodulatory therapy, hence it is thought to be the result of immune dysregulation.

Gross lesions of CRH are restricted to the skin and subcutis, may be alopecic or haired, and are most often on the *head, neck, perineum, scrotum, and extremities* (Fig. 5.146). The condition occurs in dogs 2–11 years of age with no sex or breed predilection. Systemic histiocytosis can have similar cutaneous lesions in addition to infiltrates of other tissues and organs such as the nasal cavity, sclera, retrobulbar tissues, lung, spleen, liver, lymph nodes, and bone marrow. Systemic reactive histiocytosis can occur in any breed, but Bernese Mountain Dogs, Rottweilers, Golden and Labrador Retrievers are predisposed. The age range for SRH is 1–9 years.

Histologically, cutaneous lesions are *"bottom heavy" multinodular infiltrates of large round-to-oval, rather bland histiocytes* mixed with

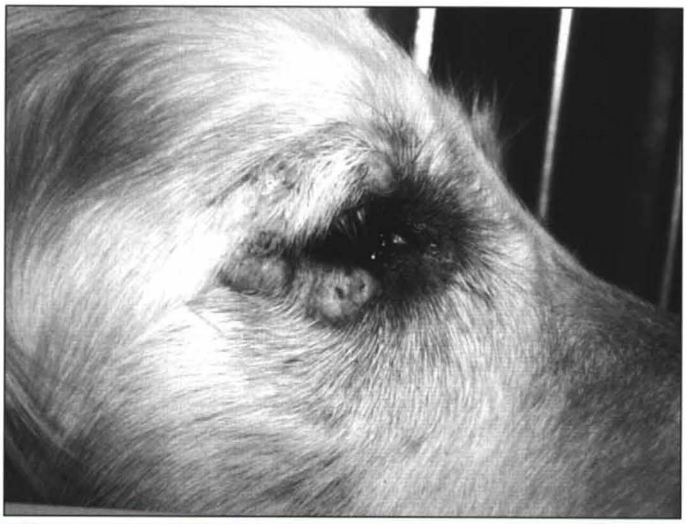

Figure 5.146 Cutaneous histiocytosis from a dog.

lymphocytes and neutrophils in the deep dermis and subcutis. In some cases, the histiocytes may be vacuolated, giving the infiltrate a lipomatous appearance. Lymphocytes may comprise 50% of the infiltrate. Eosinophils and rare plasma cells may occasionally be present. Vessels are often surrounded and invaded by the infiltrate leading to thrombosis and tissue necrosis. Older lesions extend into the more superficial dermis and may have a periadnexal distribution. Epitheliotropism is not a feature. Infiltrates of other tissues and organs in SRH are morphologically similar to the cutaneous infiltrates.

The *histologic differential diagnoses* include a response to infectious agents, other causes of periadnexal granulomas such as idiopathic sterile pyogranulomas, drug reactions, cutaneous histiocytoma, and lymphomatoid granulomatosis.

The prognosis for CRH and SRH is guarded. Lesions may wax and wane and the minority of cases of CRH may spontaneously regress. Many times, the lesions are slowly progressive and require long term management with immunosuppressive therapy and often lead to death, particularly if there is systemic involvement.

Localized histiocytic sarcoma

Localized histiocytic sarcomas are rapidly growing malignant neoplasms occurring most often in the skin, subcutis, and associated soft tissues of the extremities. The tumors are often found in periarticular regions and invade the joint capsule, tendons and muscles of the region. Other reported sites for LHS include the spleen, brain, liver, gastric wall, and tongue. LHS are most often reported in Bernese Mountain Dogs, Golden, Labrador, and Flat-coated Retrievers, and Rottweilers, but can occur in any breed. There is no sex predilection and reported age range is 2–11 years.

Immunophenotypically, the histiocytic cells comprising these lesions are variable but most consistent with a *dendritic cell origin or occasionally a macrophage origin (hemophagocytic histiocytic splenic sarcoma).* Splenic histiocytic sarcomas are comprised of cells phenotypically characteristic of interdigitating dendritic cells of the white pulp (CD1+, CD11c+, MHCII+) while histiocytic sarcomas arising in periarticular regions have phenotypic evidence of interstitial dendritic cell origin.

Grossly, the tumors are comprised of white, multinodular tissue that invades and destroys surrounding tissues. Metastasis to regional lymph nodes has been reported. Splenic histiocytic sarcomas metastasize to the liver. Histologically, histiocytic sarcomas consist of a mixture of pleomorphic, anaplastic, plump, round histiocytic cells and pleomorphic spindle-shaped cells. Tumor cells have abundant eosinophilic cytoplasm and large oval-to-indented or twisted vesicular nuclei. Multinucleated giant cells are common. Neutrophils and CD8+ lymphocytes may be present as well. Mitotic activity is high and phagocytosis may be evident. Immunophenotyping reveals positive labeling for CD1, CD11c, MHCII, ICAM-1, variable reactivity to CD90 (Thy-1) and negative reactivity for CD4. Histologic differential diagnoses include a wide variety of other sarcomas. Definitive diagnosis often requires immunohistochemistry. The prognosis is guarded but best when early, wide resection is performed (amputation) and the regional lymph nodes are determined to be uninvolved.

Disseminated histiocytic sarcoma

Disseminated histiocytic sarcoma is the term now used to refer to the previously described condition of malignant histiocytosis. Disseminated histiocytic sarcoma is a term used to reflect the multicentric form of histiocytic sarcoma described above. Histologically and immunophenotypically, the lesions are identical. It is unclear whether the disseminated histiocytic sarcoma represents metastasis of a primary lesion or multicentric malignant transformation of histiocytes. The condition was originally described in Bernese Mountain Dogs but has since been documented in Golden and Labrador Retrievers and Rottweilers. It is likely that cases in other breeds will be reported as well. The condition has been reported in the spleen, lung, liver, lymph nodes, bone marrow, central nervous system, kidneys, skeletal muscle, stomach, vertebral bodies, and adrenal glands. Cutaneous involvement is rare. The age at presentation ranges from 3–11 years and there is no sex predilection. The prognosis is extremely poor and the condition is rapidly progressive and there no known successful therapy.

Histiocytic diseases in cats

Histiocytic diseases in cats are inadequately characterized at this time to provide details regarding morphologic features, immunophenotyping, and clinical behavior. To date, a small number of cases of epitheliotropic cutaneous histiocytic proliferative conditions identified as Langerhans cell histiocytosis, histiocytic sarcomas, and an angiocentric pleocellular dendritic antigen presenting cell proliferative disorder have been documented. Further studies are needed before conclusions regarding histiocytic proliferative disorders in cats can be drawn.

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Mast cell tumors

Single or multiple nodular dermal proliferations of mast cells occur in all domestic species but are *most common in dogs* (Fig. 5.147A). When the mast cells are present in large numbers and as an essentially pure population, a diagnosis of mast cell tumor (*mastocytoma*) is usually made. Some, more cautiously, consider such growths as *cutaneous mastocytosis* until invasive growth or morphologic evidence of anaplasia confirms their neoplastic nature. As heavy accumulations of mast cells may occur in a variety of parasitic, mycotic, allergic and idiopathic inflammatory syndromes, such caution is warranted; however, sheets of mast cells are not present in these other conditions. Multiple spontaneously regressing mast cell tumors have been reported in young dogs, cats, pigs, calves, foals, and humans suggesting the underlying process may have been mast cell hyperplasia rather than a true neoplasm. Diffuse dermal and subcutaneous infiltrates of well-differentiated mast cells occurring over large areas of the body have been reported in cats, dogs and a foal. The condition has been compared to *urticaria pigmentosa* described in humans.

The histologic lesion is seldom a diagnostic challenge except in the very poorly differentiated tumors. *The mast cells form diffuse loose sheets or densely packed cords of round cells with a central round nucleus, abundant granular basophilic cytoplasm, and distinct cell membrane* (Fig. 5.147B). Scattered diffusely among the tumor cells are mature eosinophils. Even solid tumors tend to have some areas composed of cords of tumor cells alternating with collagen bundles, particularly at the infiltrative border of the tumor. They are never encapsulated or even well demarcated except at their superficial edge. A Grenz zone typically separates them from an intact epidermis. Ulceration in large or traumatized tumors may bring the skin surface in contact with the tumor. Eosinophils are few to numerous. Edema may be very severe, giving the tumor an appearance that macroscopically and microscopically resembles acute inflammation. Foci of tumor necrosis, eosinophilic or mononuclear leukocytic vasculitis, vascular necrosis, mineralization, mucinous extracellular matrix, collagen degeneration or tumor cells with giant or multiple nuclei are all occasionally encountered. Degenerating collagen may incite granulomatous inflammation.

In **dogs**, *mast cell tumors account for 15–20% of skin tumors and are the most frequent malignant or potentially malignant tumor of the skin.* The mean age of affected dogs is ~9 years with a range of 3 weeks

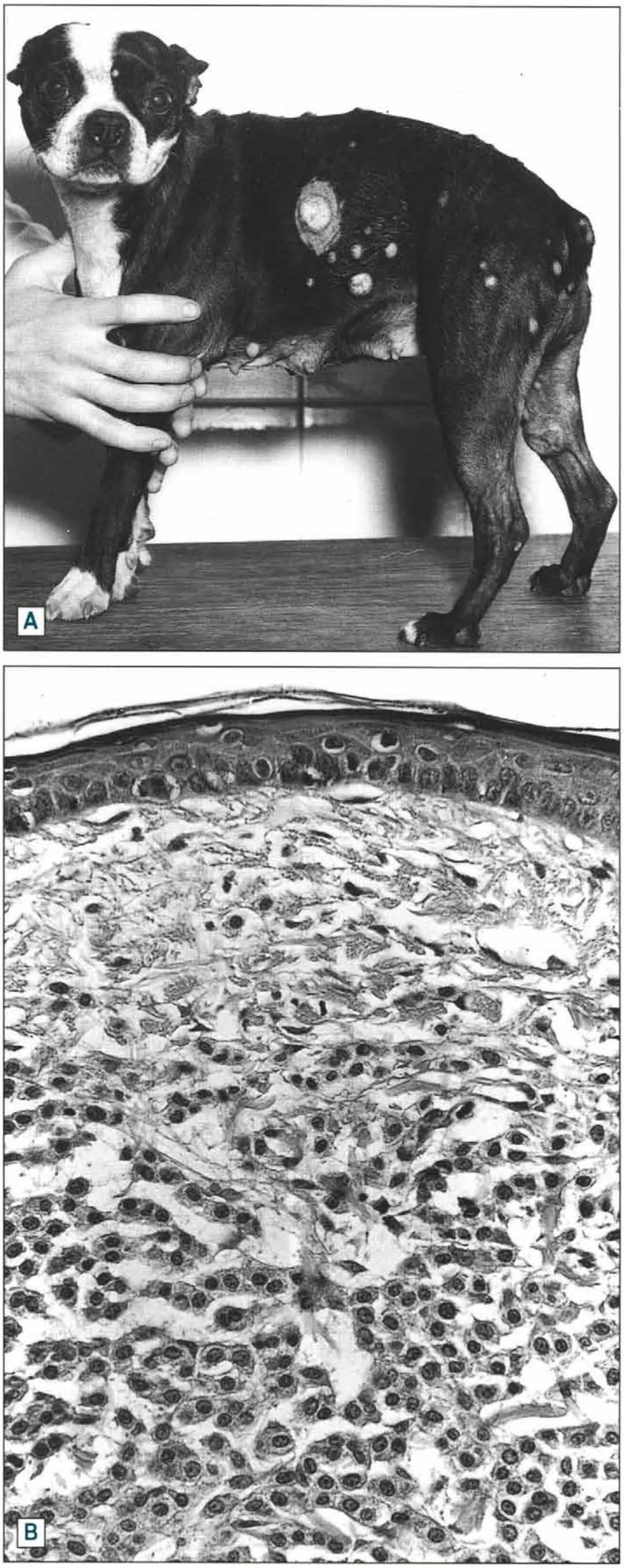

Figure 5.147 Cutaneous mast cell tumors. A. Multiple tumors in a Boston Terrier. **B.** Well-differentiated cutaneous mast cell tumor from a dog. Short cords of uniform round cells with sparing of the superficial dermis.

to 19 years. Boxers, Terriers, Boston Terriers, Labrador Retrievers, Beagles, and Schnauzers are reportedly predisposed. Shar-Peis are also predisposed and in this breed, mast cell tumors often occur at an earlier age than in other breeds. Site predilections vary with the breed. Studies investigating mast cell tumors in the dog have attempted to correlate clinical outcome with histologic grade, breed, tumor location, growth rate, clinical stage, argyrophilic nucleolar organizer region (AgNOR) counts, DNA ploidy, *p53* tumor suppressor gene product alterations, and proliferating cell nuclear antigen reactivity. Analysis of matrix metalloproteinases and expression of stem cell factor receptor and their correlation with histologic grade has also been investigated. Alterations in stem cell factor receptor expression are postulated to contribute to the proliferation of mast cells. To date, the most reliable criterion upon which to base a prognosis remains the histologic grade, however, establishing the histologic grade is subjective and less useful in tumors determined to be of intermediate differentiation. All mast cell tumors in the dog maintain a degree of unpredictability and should be regarded as potentially malignant.

There are currently two accepted grading systems for canine mast cell tumors, *the Patnaik system* (most commonly used) and *the Bostock system*. Well-differentiated tumors are considered Patnaik grade 1, while well-differentiated tumors in the Bostock system are considered grade 3. To avoid confusion, it is best to designate mast cell tumors as *well-differentiated, intermediate, or poorly differentiated*. Well-differentiated tumors carry an approximate metastatic potential of 10% while poorly differentiated mast cell tumors have a 55–96% rate of possible metastasis. Mast cell tumors metastasize first to regional lymph nodes and then most often to the liver and spleen. Mast cells may be detected in the peripheral blood and bone marrow in cases of widespread dissemination. Some mast cells may be present in the blood, bone marrow, and peripheral lymph nodes in the absence of disseminated disease, in normal animals and in animals with other diseases and these findings must be interpreted with caution.

- The **well-differentiated mast cell tumor** consists of round cells with a central small nucleus with a single inconspicuous nucleolus, and abundant, well-granulated cytoplasm. Cytoplasmic borders are distinct. There is very little cellular variation and mitotic figures are almost never seen. Tumor cells are arranged in rows or nests separated by collagen bundles. The tumor is confined to the dermis. Tumors of this type account for ~50% of the mast cell tumors of dogs and their behavior is usually benign. Excision is usually curative, with over 90% of affected dogs surviving >4 years.
- **Mast cell tumors of intermediate grade** are comprised of closely packed cells with distinct cytoplasmic boundaries, an increased nuclear-to-cytoplasmic ratio and fewer granules than a well-differentiated tumor. Giant, binucleate or occasional spindle-shaped cells may be present. Mitotic figures may be present in small numbers. The tumor cells are arranged in rows or nests separated by a collagenous stroma that may be thick or hyalinized with areas of edema and necrosis. The tumor may infiltrate the lower dermis and subcutis. The 3.5 year survival rate is ~55%.
- The cells of **undifferentiated (anaplastic) mast cell tumors** exhibit poor cytoplasmic granulation, large nucleus in relationship to cytoplasmic volume, irregular nuclear shape, moderate variation in nuclear size, several nucleoli and occasional binucleate, multinucleated or giant cell. Mitotic figures may be numerous. The cytoplasmic granulation may be inapparent or visible only as fine cytoplasmic dusting, and may require staining with Giemsa or toluidine blue to demonstrate the metachromatic granules. Tumor cells infiltrate the subcutis and deeper tissues. About 20% of the canine tumors fall into this prognostic category. Dogs with poorly differentiated tumors have a 3.5 year survival rate of approximately 15%.

The *macroscopic appearance* of canine mast cell tumors varies widely with their stage of progression and degree of histologic differentiation. Well-differentiated tumors most often appear as a rubbery, nodular nonencapsulated variably alopecic dermal mass 1–4 cm in diameter that clinically resembles a lipoma. Poorly differentiated tumors tend to achieve a large size more quickly, are less circumscribed and often associated with inflammation and edema of the surrounding dermis and possible satellite lesions. Tumors of intermediate grade have a gross appearance that varies between those described above.

Other clinical signs may occasionally result from the release of histamine or other vasoactive products from the mast cells. *Gastroduodenal ulceration* is relatively frequent in dogs with disseminated disease, occurring in 35–83% of cases. Histamine release stimulates the specific H2 gastric parietal cell receptors, resulting in increased acid secretion and perhaps local mucosal ischemia. Ulceration follows and may lead to fatal exsanguination. *Hypotensive shock* from massive synchronous degranulation, as may occur with cryosurgery, is a rarely reported complication.

Mast cell tumors in **cats** exist as *primary cutaneous mast cell tumors* and as *visceral mastocytosis*. They are separate diseases. Mast cell tumors are the second most common cutaneous neoplasm in the cat and account for 20% of skin tumors of cats in the USA. The metastatic potential of feline cutaneous mast cell tumors is very low (~5%) and those destined for behavioral malignancy are easily detected by anisocytosis, hyperchromasia and mitotic activity. Apparent recurrence at the surgical site or elsewhere in the skin is seen in 25–50% of the cases, but most of these probably represent multicentric origin. Cats with multiple mast cells tumors are often FIV positive. There is *no widely accepted grading system* for feline mast cell tumors.

Mast cell tumors in cats appear as one or several firm, raised pink, alopecic papules varying in size from millimeters to several centimeters. Less commonly, the tumor appears as a poorly defined area of swelling due to an infiltrative rather than nodular lesion. The head and neck are preferred sites. Histologically the cells are usually extraordinarily uniform, polygonal to round, and grow in a diffuse sheet interrupted only by small clusters of lymphocytes and rare eosinophils. The cytoplasm is clear, eosinophilic or only faintly basophilic. Obvious granularity is infrequent. The nucleus is round, central and relatively hyperchromatic in comparison to that of canine mast cells. Even with metachromatic stains such as Giemsa or toluidine blue, cytoplasmic granules may stain poorly, yet ultrastructurally they are abundant. Occasionally, binucleate, multinucleated or cells with giant nuclei measuring up to 25 μm may be seen. Eosinophils are seen in only a small number of feline mast cell tumors. The tumor is not encapsulated and is most often confined to the dermis. Collagen degeneration and stromal proliferation are rare. Much less frequent is a *second histologic type* seen most often in young cats as multiple, simultaneous or sequential, tumors located at the junction of the dermis and subcutis. Siamese cats are predisposed. The tumor mast cells resemble histiocytes and the lesion may be mistaken for granulomatous inflammation. Toluidine blue or Giemsa staining may be equivocal. The cells are confirmed as mast cells on electron microscopic examination. Eosinophils, lymphoid aggregates, well-circumscribed growth habit and benign behavior are similar to the more usual mast cell tumor of cats described above.

Mast cell tumors in **horses** have been cautiously termed *cutaneous mastocytosis*, but the growths are comparable to the cutaneous tumors of other species. They most commonly present as solitary nodules on the head, trunk, neck, and limbs. The tumors may be hyperpigmented, alopecic or ulcerated. Collagen degeneration, large aggregates of eosinophils, necrosis, and focal mineralization are more prominent. While in some respects resembling such lesions as cutaneous onchocerciasis, habronemiasis or collagenolytic granulomas, none of these three is characterized by sheets of mast cells. Multiple congenital mast cell tumors that spontaneously regress have been reported. There are no reports of metastasis of mast cell tumors in the horse.

Mast cell tumors in **ferrets** resemble the well-differentiated feline mast cell tumor grossly, histologically and in terms of biological behavior.

In **pigs**, mast cell nodules have been described as tumors and as multifocal inflammatory aggregates, perhaps in response to *Eperythrozoon*. Their morphology resembles that of well-differentiated feline tumors. In the pigs with multiple skin lesions, visceral aggregates were also found.

In **cattle**, scant data suggest the cutaneous tumors are usually multiple and are associated with visceral mast cell aggregates, although purely cutaneous tumors have been reported. Congenital cutaneous mast cell tumors consisting of well-circumscribed 1–7 cm nodules randomly distributed all over the body have also been reported. Histologically, the tumors had features of well-differentiated mast cell tumors. There are more reports of cutaneous metastases of multicentric visceral tumors than there are of primary skin disease. Cattle of any age, including calves, may be affected.

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Cutaneous lymphoma

Lymphomas in the skin can be a primary cutaneous neoplasm or can be part of a multicentric lymphocytic neoplasm. Cutaneous lymphomas have been reported in most domestic animals and in many wildlife species, however the tumor has been most closely studied in the dog, cat, horse, and in cattle. The body of information about cutaneous lymphomas in veterinary dermatopathology has expanded rapidly in recent years, and is still a subject under close study. The development of *monoclonal antibodies directed against lymphocyte antigens* has greatly enhanced immunophenotypic analysis of tissue sections and needle aspirate preparations of these tumors. There are currently several antibodies that are routinely used in formalin-fixed tissue to characterize cutaneous lymphomas. As in humans, *T lymphocytes* in many domestic species consistently express the cell surface antigen CD3, and *B lymphocytes* can be identified in fixed tissues in many species by expression of CD79a. Many other monoclonal antibodies have been developed to identify lymphocyte subsets and others to identify lymphocyte antigens in fresh-frozen tissue. A description of these is beyond the scope of this chapter and the reader is referred to more detailed information on this topic in the bibliography. Classification schemes to predict behaviour of cutaneous lymphomas have been controversial. Several classification schemes devised for human lymphomas have been used, however they were based on lymphomas in lymph nodes. These schemes have included Rappaport, Lukes-Collins, Kiel, National Cancer Institute working formulation, and more recently, the Revised European-American Classification of Lymphoid Neoplasms (REAL). Since primary cutaneous lymphomas differ histologically and immunophenotypically from primary nodal lymphomas and often carry a better prognosis, *classification of primary cutaneous lymphomas using lymph node schemes has been difficult and unsatisfactory.* A new classification scheme devised for primary cutaneous human lymphomas has been

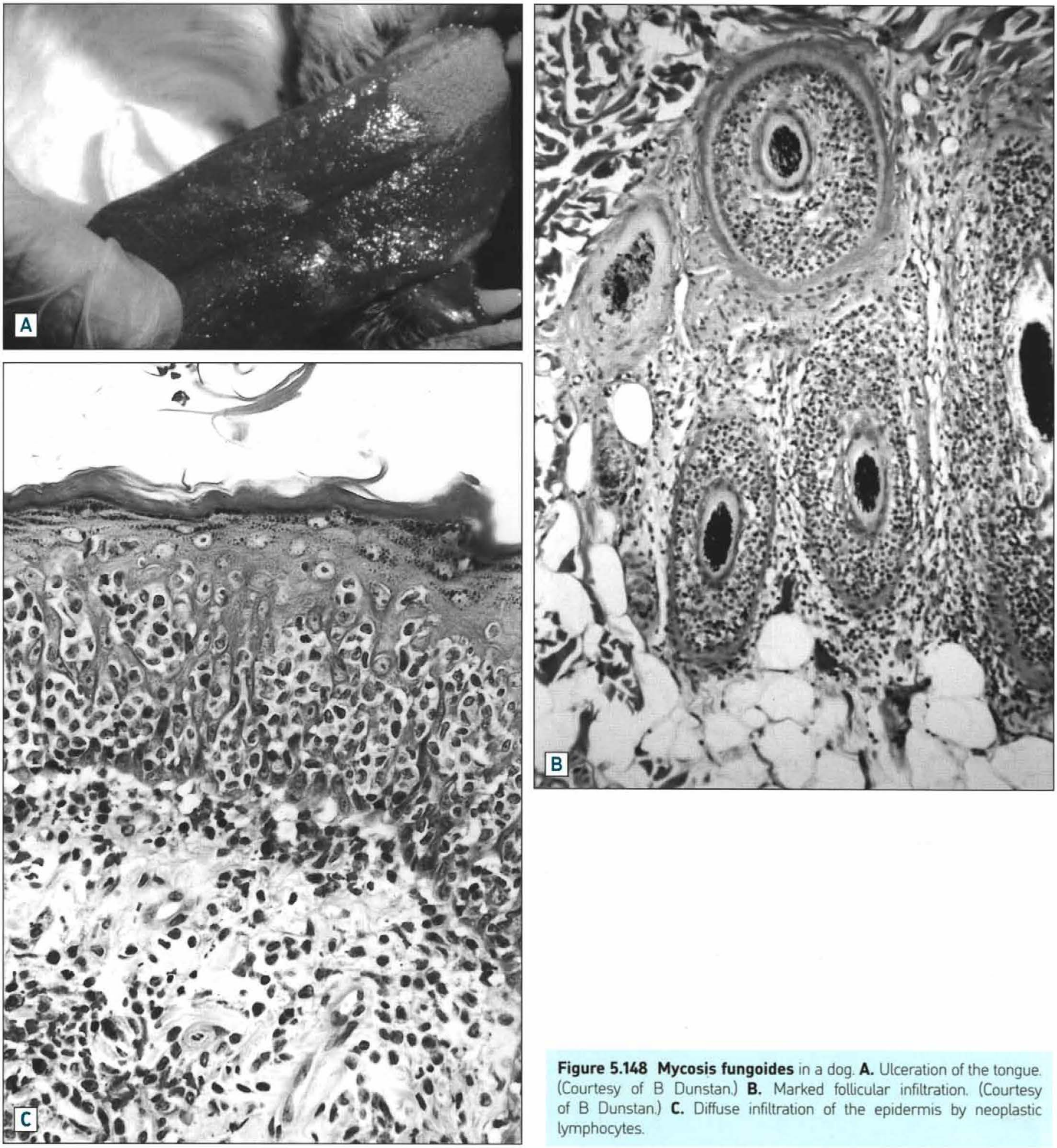

Figure 5.148 Mycosis fungoides in a dog. **A.** Ulceration of the tongue. (Courtesy of B Dunstan.) **B.** Marked follicular infiltration. (Courtesy of B Dunstan.) **C.** Diffuse infiltration of the epidermis by neoplastic lymphocytes.

proposed by the European Organization for Research and Treatment of Cancer (EORTC). It is based on a combination of clinical, histologic, immunophenotypic, and genetic criteria. The EORTC and REAL schemes use similar terminology, and these two systems may allow for recognition and comparison of similar tumors in cutaneous and non-cutaneous sites. Currently there have been no studies using the EORTC system on primary cutaneous lymphomas in domestic animals.

Cutaneous lymphomas in domestic animals are usually divided into two groups; *epitheliotropic cutaneous lymphomas*, in which the neoplastic lymphocytes invade the epidermis and/or adnexal epithelium, and *nonepitheliotropic lymphomas* that involve the dermis and subcutis. With the increasing use of immunohistochemical characterization of cutaneous lymphomas in domestic animals, division into *T-cell cutaneous lymphomas* (which would include mycosis fungoides, Pagetoid reticulosis, and angiocentric lymphomas/lymphomatoid granulomatosis), and *B-cell lymphomas*, will become more useful.

Epitheliotropic cutaneous lymphoma in domestic animals is represented primarily by **mycosis fungoides**. Mycosis fungoides has been reported in the *dog, cat, horse, rabbit, and cattle*. Mycosis fungoides is a disease of *older animals* and frequently there is a history of chronic erythematous, exfoliative dermatitis or multiple plaques and nodules. Lesions can be anywhere, however mucocutaneous junctions of the mouth, nose, eyes, anus, vulva, or the gingiva and palate are often involved (Fig. 5.148A). Histologically the neoplastic lymphoid infiltrate involves epidermis but can also involve the epithelium of adnexal structures (Fig. 5.148B). The lymphoid infiltrate in the epithelium can be diffuse (Fig. 5.148C), scattered, or form aggregates ("*Pautrier's microabscesses*") (see Fig. 5.10). Dermal involvement varies from a mixed perivascular cellular component that contains low numbers of neoplastic lymphocytes to a more fulminating diffuse monomorphic neoplastic infiltrate. Epidermotropic cutaneous lymphomas frequently ulcerate and verification of the epitheliotropic nature of the neoplasm can be difficult, however, a neoplastic lymphocytic component that abuts the ulcerated epidermis suggests epidermotropism. The neoplastic lymphocytes can vary from a small cell type, with a low mitotic index, hyperchromatic nucleus and scant cytoplasm, to a large cell type with moderate mitotic index together with moderate amounts of pale basophilic to clear cytoplasm. Although less well characterized than the human form of mycosis fungoides, epitheliotropic lymphomas in domestic animals also express T-cell surface antigens such as CD3. However, in contrast to human epitheliotropic lymphomas, which are reported to express CD4 in approximately 90% of cases, 80% of epitheliotropic lymphomas in domestic animals express CD8. Mycosis fungoides with a leukemic blood component (**Sezary syndrome**) has been reported in the dog, cat, horse, and cow. **Pagetoid reticulosis (Woringer–Kolopp disease)** is a localized solitary plaque form of epidermotropic lymphoma that is well recognized in humans and has been reported rarely in the dog. In this form, the neoplastic lymphocytes are confined primarily to the epidermis and the dermal component is a mixture of mature lymphocytes and macrophages.

Angiocentric lymphoma (lymphomatoid granulomatosis) in the skin is usually a cutaneous manifestation of pulmonary lymphoma, however, it is thought to occur rarely as a primary cutaneous disease. Although well described in humans, this form of lymphoma has been reported infrequently in domestic animals. It is characterized by *angiocentric, angioinvasive, and angiodestructive neoplastic lymphocytes in the dermis and subcutis, resulting in intramural and extravascular neoplastic infiltrates*, in contrast to the luminal accumulations of lymphocytes in malignant angioendotheliomatosis. The neoplastic cells are medium- to large-sized lymphoid cells that can have a histiocytic appearance, with a cleaved or reniform nucleus. Inflammatory cells such as eosinophils, plasma cells, and small lymphocytes may accompany the neoplastic cells. In the small number of cases reported, the neoplastic lymphocytes have been identified as T lymphocytes by immunophenotyping. Clinically the presentation is one of multiple alopecic and frequently ulcerated dermal or subcutaneous nodules. The angiodestructive nature of the neoplasm can result in multifocal necrotic or infarctive lesions.

Nonepitheliotropic cutaneous lymphomas appear to be less common than epitheliotropic lymphomas in most domestic animal species. Clinically the animals have multiple dermal and subcutaneous nodules that infrequently ulcerate. Histologically the neoplastic cells form *"bottom-heavy" nodules in the dermis and subcutis*, often with a Grenz zone. The neoplastic cells may be large cells with a histiocytic appearance, and immunophenotyping is often necessary to distinguish them from the histiocytic proliferative diseases. The neoplastic cells can efface adnexal structures, but invasion into the epidermis or adnexal epithelium is very unusual.

Nonepitheliotropic lymphomas that have been studied are usually of B-lymphocyte origin, with the exception of the angiocentric lymphomas discussed previously. An interesting form of nonepitheliotropic lymphoma is the **T-cell-rich B-cell lymphoma,** which is well recognized in humans and has now been reported in the *cat, pig, dog, and horse*. In the horse, it appears to be the most common form of cutaneous lymphoma. In this form, the nodular masses are composed of neoplastic large B lymphocytes accompanied by a background population of smaller reactive T lymphocytes that usually comprise the majority of the cell population. The large neoplastic B cells exhibit cellular atypia, with large vesicular nuclei and numerous mitotic figures that are often atypical. Since the neoplastic population is often the minority population, mitotic index should be evaluated carefully taking into account the fact that the majority population is non neoplastic small T lymphocytes with normal morphology and low mitotic index. These tumors also usually contain variable numbers of macrophages, epithelioid macrophages, and occasionally multinucleated giant cells. The mixed nature of the population can lead to this tumor being mistaken for inflammation, or a neoplasm of T lymphocytes. T-cell-rich B-cell lymphomas in the horse appear to be histologically the same as the neoplasm referred to in the older literature as *equine cutaneous histiolymphocytic lymphosarcoma*.

Intravascular lymphoma (malignant angioendotheliomatosis, intravascular lymphomatosis, angiotropic large-cell lymphoma) is *characterized by intravascular neoplastic lymphocytes in the vessels of the skin and other organs in the absence of a primary mass or circulating neoplastic cells*. This condition is rare in humans and was originally thought to be a proliferation of endothelial cells, which lead to the original name of malignant angioendotheliomatosis. At present it has been reported rarely in the dog and cat. Clinically the skin lesions appear as plaques and nodules, and histologically vessels in the dermis and subcutis are partially or completely filled with *large atypical lymphoid cells*. In humans, the intravascular neoplastic cells are of B-lymphocyte origin, however in dogs the majority of cases so far have been of T-lymphocyte origin.

Cutaneous pseudolymphomas *are benign reactive proliferations of lymphocytes that mimic cutaneous lymphomas histologically and sometimes clinically*. These proliferations are poorly characterized in the veterinary literature, but are well recognized in humans as forming both a band-like infiltrate in the superficial dermis (*T-cell pattern*) and nodular to diffuse infiltrates in the dermis and subcutis (*B-cell pattern*). Pseudolymphomas in humans can be due to many antigenic stimuli, such as drug eruptions, arthropod or tick-bite reactions, and contact dermatitis. *Differentiation between benign inflammatory lesions and early cutaneous lymphoma can be extremely difficult*. Although immunophenotyping can distinguish between T-cell and B-cell lymphomas, methods to distinguish between inflammatory and malignant lymphocyte proliferations are unreliable at present.

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Cutaneous plasmacytoma

Cutaneous plasmacytomas are common in dogs and rare in cats. Most cases are benign however malignant cutaneous plasmacytomas demonstrating local invasion and tissue destruction do occur. It has not been described in other species. It occurs most often in *middle-aged or old dogs* with a marked predilection for *feet, ear canal, and mouth.* The typical tumor is a small spherical mass grossly similar to benign cutaneous histiocytoma, but its histology is distinctive. *Sheets of pleomorphic round cells are divided into solid lobules by a fine fibrous stroma.* The cells often have marked variation in nuclear size and degree of basophilia, with binucleation or multinucleation and numerous mitotic figures. *At least some of the cells retain a perinuclear halo (suggesting a Golgi zone), "clockface" nucleus, and basophilic cytoplasm considered typical of plasma cells.* Russell bodies are sometimes seen even in very atypical cells, and the cytoplasm of most cells is pyroninophilic. Cells recognizable as plasma cells are most easily found near the periphery (Fig. 5.149). A Grenz zone is usually present and there is no epithelial invasion. Electron microscopy or immunohistochemistry for canine immunoglobulin confirms the diagnosis but seldom are these tests necessary. Occasionally a particularly anaplastic example must be distinguished from amelanotic epithelioid melanoma or lymphoma. The distinction is critical, since even bizarre plasmacytomas are cured by excision. Extracellular amyloid (AL) is found in a small percentage (perhaps up to 10% of plasmacytomas). They bear no apparent relationship to multiple myeloma. Multiple tumors, rarely > 2–3, have been reported, as has a very low prevalence (~5%) of local recurrence.

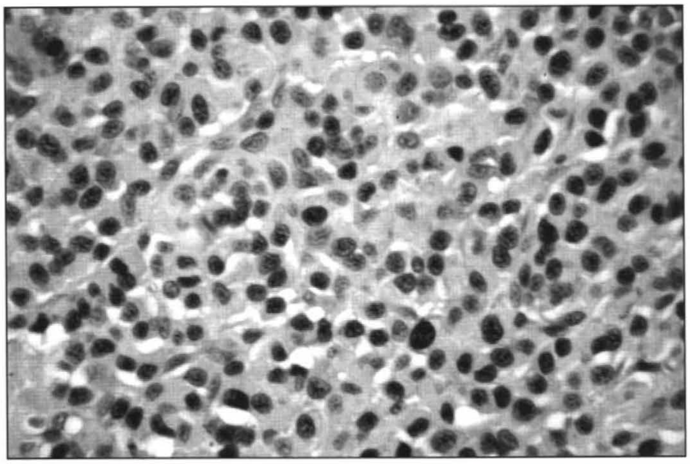

Figure 5.149 Cutaneous plasmacytoma from a dog.

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Merkel cell tumor

Merkel cell tumors have rarely been reported in the **dog**. Clinically, the tumors appear as nodular lesions that may resemble a histiocytoma. Too few cases have been reported to establish age, site or sex predilections. Microscopically, the tumors are comprised of *dermal infiltrates of round cells arranged as solid nests or clusters separated by collagenous stroma.* Individual tumor cells are reported to have indistinct margins and moderate amounts of pale cytoplasm. The nuclei are oval or spherical with dispersed chromatin. The nucleolus is not conspicuous. A small number of mitotic figures may be present. *Differential diagnoses* include cutaneous plasmacytoma and basal cell epithelioma. Definitive diagnosis requires ultrastructural examination or immunohistochemistry. Merkel cell tumors label for cytokeratin and chromogranin A and do not label for immunoglobulins.

Electron microscopic evaluation demonstrates *electron dense-core granules typical of neuroendocrine cells.* Merkel cell tumors in the dog are most often benign with only one report documenting metastasis. A malignant neuroendocrine carcinoma of the skin (Merkel cell tumor) has been reported in a Maine coon **cat**.

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Tumors of the mammary gland

Mammary masses are *very common in dogs and to a lesser extent cats,* but are rare in other domestic species. In the dog, mammary tumors vary greatly in their morphologic appearance and biological behaviour. The high prevalence of canine mammary tumors, the need to identify dogs that would benefit from aggressive therapy, and the potential usefulness as an animal model has led to many investigations into the factors governing mammary tumor development and behavior in the dog. These studies have attempted to correlate histologic features and clinical outcome with characteristics such as hormone receptor expression, oncogene and tumor suppressor gene alterations, intratumoral vessel density, argyrophilic nucleolar organizer counts, tumor cell kinetics and intermediate filament expression. Results of these studies have added to the current state of knowledge regarding mammary gland tumors in the dog but have not completely defined parameters upon which to base a prognosis. **Invasion** *by the tumor into surrounding tissue is considered one of the most important predictors of behavioral malignancy.* In dogs, 80% of animals with invasive tumors will have a survival time of <2 years. Metastases to lung and lymph nodes are frequently found at necropsy. Conversely, 80% of dogs with non-invasive tumors – regardless of any other histologic features – will be alive at 2 years post-diagnosis. *The presence of tumor emboli in lymphatics or veins is absolute evidence for local invasion and metastatic potential.* In its absence, care must be taken to ensure that the invasion is really into surrounding normal tissue and not just into the abundant stroma accompanying the tumor itself.

Classification schemes for mammary tumors may be based on histogenesis of the neoplastic cells, morphologic appearance, or prognostic factors. *No scheme is completely satisfactory, and correlation of histopathological diagnosis and clinical outcome vary.* The scheme utilized in this text follows that outlined by the current World Health Organization International Histological Classification of Tumors of Domestic Animals, and considers both morphologic features and known indicators of prognosis for the dog. One exception to the WHO classification scheme that is included here is the findings of a recent study documenting the morphologic features and biological behavior of all mammary neoplasms occurring during the lifespans of 1343 Beagles. This study found a specific tumor type, the **ductular carcinoma** (arising from interlobular or intralobular ductules) had a higher fatality rate than other types of adenocarcinomas arising in the mammary gland. The ductular carcinoma is not a specific morphological subtype currently recognized by the WHO classification scheme but it will be included in this discussion as *it carries a poorer prognosis that other types of mammary gland adenocarcinomas.* The Beagle study also found that there was no difference in the frequency of metastasis between simple and complex carcinomas or between these tumors and adenocarcinomas in mixed tumors. They also reported that carcinosarcomas metastasized more frequently than adenocarcinomas in mixed tumors.

In **cats**, *mammary tumors are 85–90% behaviorally malignant* and are classified as subtypes of carcinomas based on morphologic features alone. Hyperplastic and dysplastic lesions of the mammary gland will also be considered here.

Mammary hyperplasia and dysplasia

Mammary ductal hyperplasia occurs frequently in bitches and occasionally in queens. Single or multiple extralobular ducts are distended with proteinaceous fluid and some cellular debris. Their leakage may induce sterile granulomatous inflammation rich in ceroid-laden macrophages. Histologically, the lesion consists of *patchy intraductal hyperplasia of epithelial cells.* Papillary proliferations of epithelium may fill the duct lumens. Proliferative cells are small, uniform and associated with an orderly myoepithelial layer. They may spontaneously regress, and ovariectomy reliably causes their regression, whereas progestogen administration induces their development. It is not known whether they predispose to, or even progress to neoplasia although neoplastic transformation would seem likely.

Mammary lobular hyperplasia *consists of patchy hyperplasia of secretory acini indistinguishable from the normal lactating gland.* Proliferation involves intralobular ductular epithelium, myoepithelium, and fibrous tissue in varying proportions. The lobular architecture of the gland is maintained. It occurs in intact, nonpregnant and non-lactating bitches, and occasionally in queens. Serial sectioning of the mammary glands reveals numerous small (1–4 mm diameter) hyperplastic foci in 50% of clinically normal bitches even at 3 years of age. They are most numerous in the caudal two glands, paralleling the greater prevalence of neoplasia in these glands. Circumstantial evidence supports the view that such lesions should be considered *preneoplastic changes.* Indeed, many hyperplastic lobules contain dysplastic foci with some degree of epithelial disorganization and hyperchromasia, blurring the distinction between hyperplasia, dysplasia and in situ neoplasia.

Mammary fibroepithelial hyperplasia *(fibroadenomatous change) occurs in young cycling, or pregnant cats.* It also can be induced in old unspayed females and males by treatment with *megestrol acetate.* Progesterone receptors are present in high concentrations. The condition appears most often 1–2 weeks post-estrus, usually affects one or more glands, and may regress spontaneously or require ovariectomy. Grossly, the mammary tissue is markedly swollen and erythematous. Necrosis and ulceration may be present. Edema may involve the rear limbs. Histologically there is *branching and proliferation of mammary ducts lined by simple columnar epithelium, surrounded by massive and concentric proliferation of edematous stroma compatible with myoepithelium* (Fig. 5.150). The prominence of the stroma is in sharp contrast to mammary neoplasia in cats, which almost always is solid or alveolar carcinoma with little stroma.

Mammary neoplasia in dogs and cats

Mammary tumors have been reported to comprise 50% of all neoplasms in the **bitch**. They occur most often in bitches 10–11 years old and are

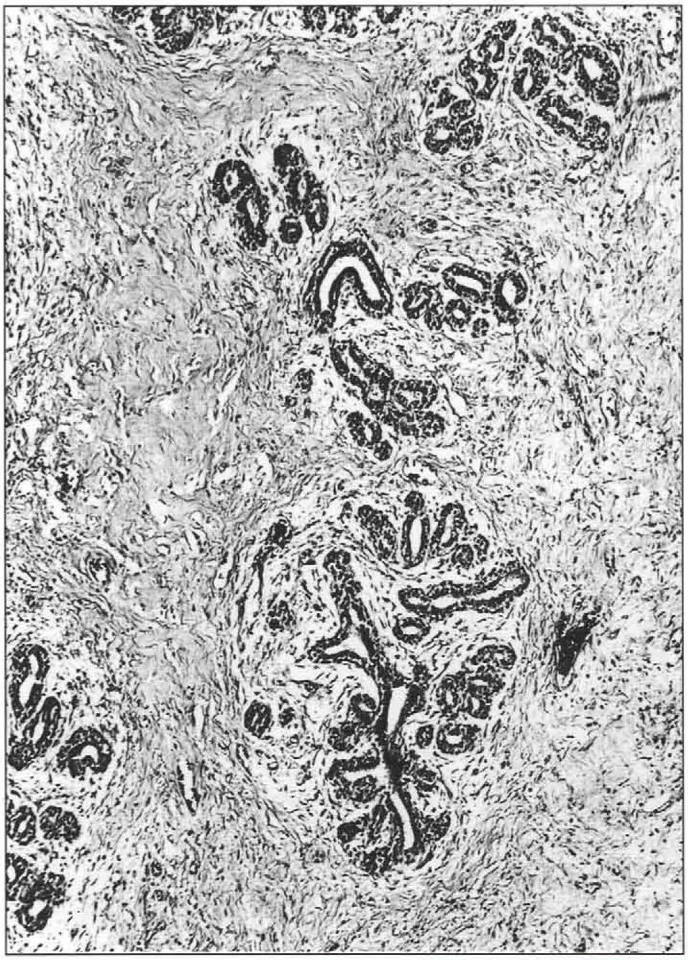

Figure 5.150 Fibroepithelial hyperplasia in the mammary gland of a cat.

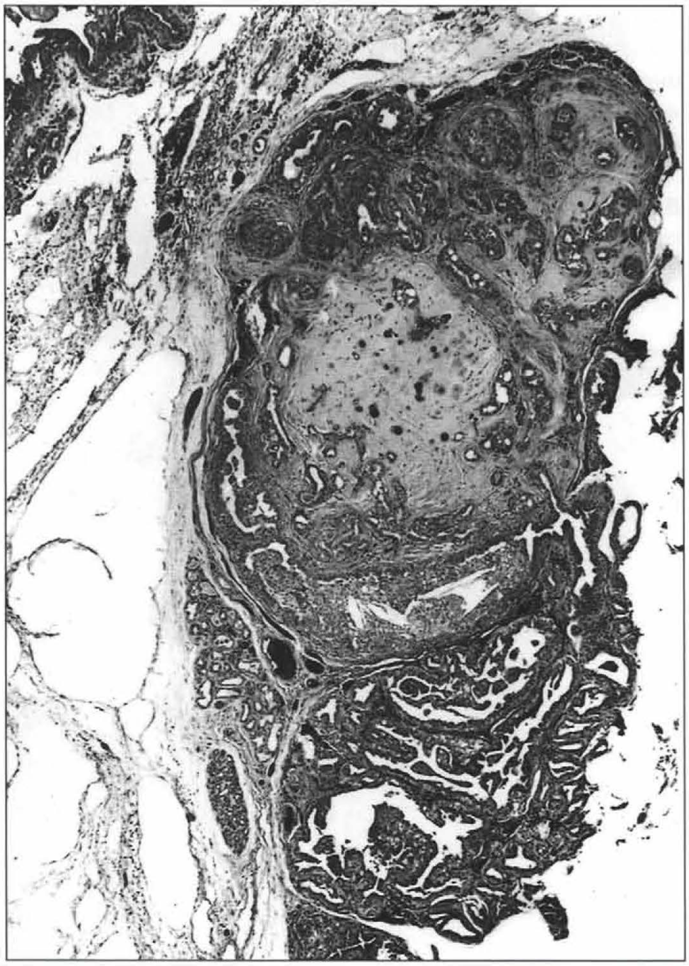

Figure 5.151 Benign mixed mammary tumor with typical mixture of tubular, papillary, and cartilaginous elements.

very rare in dogs less than 5 years of age. Dogs have five pairs of glands and 70% of all tumors develop in glands 4 and 5. Tumors can involve the lobular tissue or the nipple. Mammary tumors in the dog are hormonally dependent. Ovariohysterectomy at or prior to first estrus dramatically decreases the risk of development of mammary tumors. Between 41–53% of mammary tumors in the dog are considered malignant. *Metastasis occurs via hematogenous or lymphatic routes to seed the lung and regional nodes, respectively*. Carcinomas that metastasize to the inguinal lymph nodes may spread to the internal iliac nodes and lead to colonic compression. Many tumors with histologic criteria of malignancy such as cellular atypia or invasion, do not exhibit malignant biological behavior. In addition, the morphologic appearance of the tumor can vary significantly within the section and the lack of consensus regarding criteria for the diagnosis of malignancy leads to a variation in interpretation of lesions.

Benign tumors of the canine mammary gland include simple adenomas, complex adenomas, basaloid adenomas, fibroadenomas, and benign mixed tumors. The *malignant tumors* include ductular carcinoma, carcinoma in situ, complex carcinoma, simple carcinoma, spindle cell carcinoma, squamous cell carcinoma, mucinous carcinoma, lipid-rich carcinoma, and a variety of sarcomas.

- **Simple adenomas** are comprised of either well-differentiated luminal epithelium arranged as simple acini with or without secretions, or as solid nodules of spindle cells (**myoepithelioma**).
- Tumor comprised of both myoepithelium and secretory epithelium that are encapsulated and demonstrate no cellular atypia are classified as **complex adenomas**.
- **Basaloid adenomas** are comprised of solid lobules of monomorphic basaloid epithelium that palisade against a basement membrane. Central cells may show squamous or secretory epithelial differentiation.
- **Fibroadenomas** are tumors comprised of luminal epithelium and possible myoepithelium mixed with stromal elements. The stroma may be sparsely cellular or highly cellular.
- **Benign mixed tumors** are comprised of well-differentiated luminal and/or myoepithelial cells accompanied by a mesenchymal population that has produced cartilage, bone, or fat in combination with a fibrous component (Fig. 5.151).

Carcinomas occurring in the mammary glands of dogs will be discussed in order of increasing malignancy.

- **Carcinomas in situ** (noninfiltrating carcinoma) consist of cerebriform or solid clusters of neoplastic epithelium confined by the basement membrane of a duct, lobule, or cyst. The tumor may be necrotic in the center. These lesions are often multicentric.
- **Complex carcinomas** are comprised of both luminal epithelium and myoepithelium. Complex carcinomas demonstrate

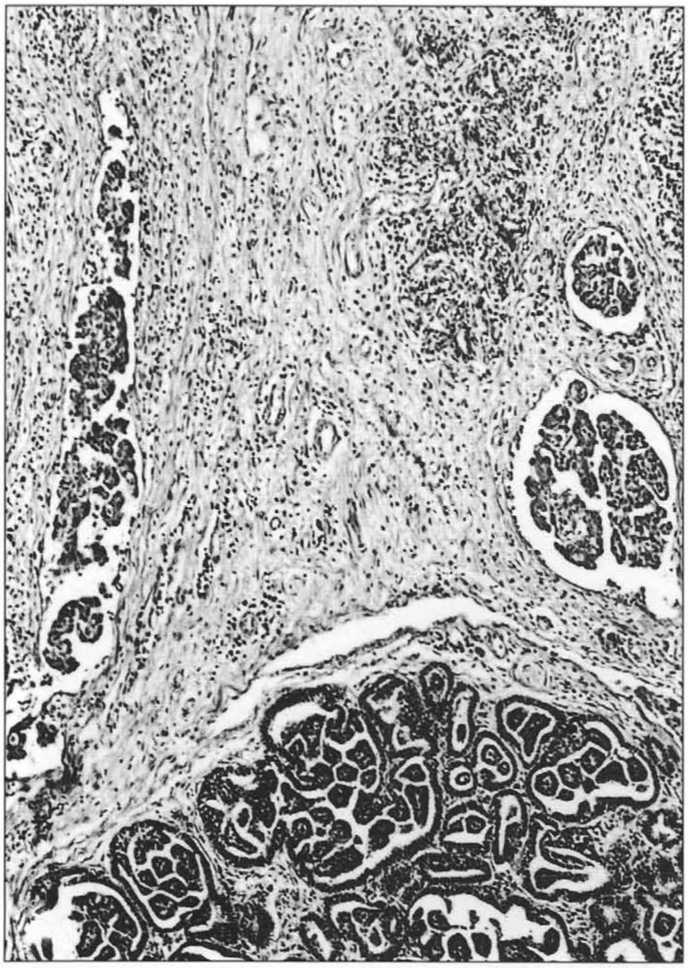

Figure 5.152 Mammary gland papillary adenocarcinoma with lymphatic invasion from a bitch.

infiltrative growth, lack of encapsulation, high cellularity, necrosis and numerous mitotic figures. Squamous metaplasia is frequently present. Lymphatic invasion is present in about 10% of the tumors.

- **Simple carcinomas** are comprised of luminal epithelium **or** myoepithelium. They may have a tubulopapillary or solid arrangement (Fig. 5.152). If the tumor cells are highly pleomorphic and defy histologic recognition as luminal or myoepithelium, the tumors are classified as **anaplastic carcinomas.** Anaplastic carcinomas may be positive for vimentin and cytokeratin with immunohistochemistry. Simple carcinomas are highly invasive and frequently metastasize.
- **Spindle cell carcinomas** are rare malignant tumors comprised of spindle cells arranged as solid or tubular patterns, and are thought to arise from myoepithelium as the cells label for vimentin and cytokeratin.
- **Ductular carcinomas** are comprised of branching duct-like structures filled with solid sheets, papillary projections or cystic aggregates of neoplastic epithelium. They may be simple or complex.
- **Squamous cell carcinomas** arising in the mammary gland are usually highly invasive and frequently invade lymphatics. The periphery of the tumor has predominantly basal cells with cellular atypia. Care must be taken to distinguish these tumors from cutaneous squamous cell carcinomas as the former are more aggressive.
- **Mucinous carcinomas** are of the simple or complex type and produce large amounts of mucin.
- **Lipid-rich carcinomas** are comprised of highly vacuolated epithelial cells that are positive for neutral lipids.

Primary mammary sarcomas *are infrequent in dogs and very rare in cats.* They presumably arise from the fibrous stroma of the gland or of the adjacent subcutaneous tissue. Histologic changes that suggest malignancy are occasionally seen in the myoepithelial stroma, or in the metaplastic bone or cartilage of mixed mammary tumors (so-called malignant mixed tumors). If such tumors metastasize, it is usually the epithelial component that spreads. **Osteosarcomas, chondrosarcomas, liposarcomas, fibrosarcomas,** and **carcinosarcomas** (malignant luminal or myoepithelial cells admixed with malignant stromal cells) have all been reported to arise in the mammary gland.

Mammary tumors in the **cat** represent the third most commonly reported tumor in the cat. Tumors develop most often in intact female cats with a mean age of 10–12 years. Tumors occasionally develop in spayed females or male cats. Spaying decreases the incidence of tumors but does not have the dramatic protective effect as seen in dogs. *At least 85–90% of the tumors are malignant* (Fig. 5.153A, B) *and metastasis to regional lymph nodes, lungs, pleura, liver, adrenal glands, and kidneys is common.* Tumors are firm, nodular and may be ulcerated. Any gland may be affected and tumors are usually multiple. Differences in biological behavior based on histologic subtype of mammary gland carcinomas in the cat have not been shown. **Carcinoma in situ, tubulopapillary carcinoma, solid carcinoma, squamous cell carcinoma, mucinous carcinoma,** and **carcinosarcomas** have all been reported in the cat. These tumors are morphologically similar to the same tumor described in the dog. In addition, a subtype referred to as a **cribriform carcinoma** comprised of malignant epithelial cells arranged in a solid pattern with many small apertures is common in the cat.

Mammary tumors in other species

In **mares**, the few reported tumors have been *scirrhous solid carcinomas* that have been extremely invasive locally, and all have had widespread metastasis.

In **cows**, the literature is old and of variable quality in that many diagnoses had no histologic confirmation. Most descriptions are of fibropapilloma-like growths in the teat canal or large ducts, but everything from fibroma, squamous cell carcinoma, osteoma to fibrosarcoma has been diagnosed. Many of the polypoid teat or cisternal growths were probably hyperplastic inflammatory polyps. **Goats** and water buffalo are cited in review articles as having a very low prevalence of mammary neoplasia.

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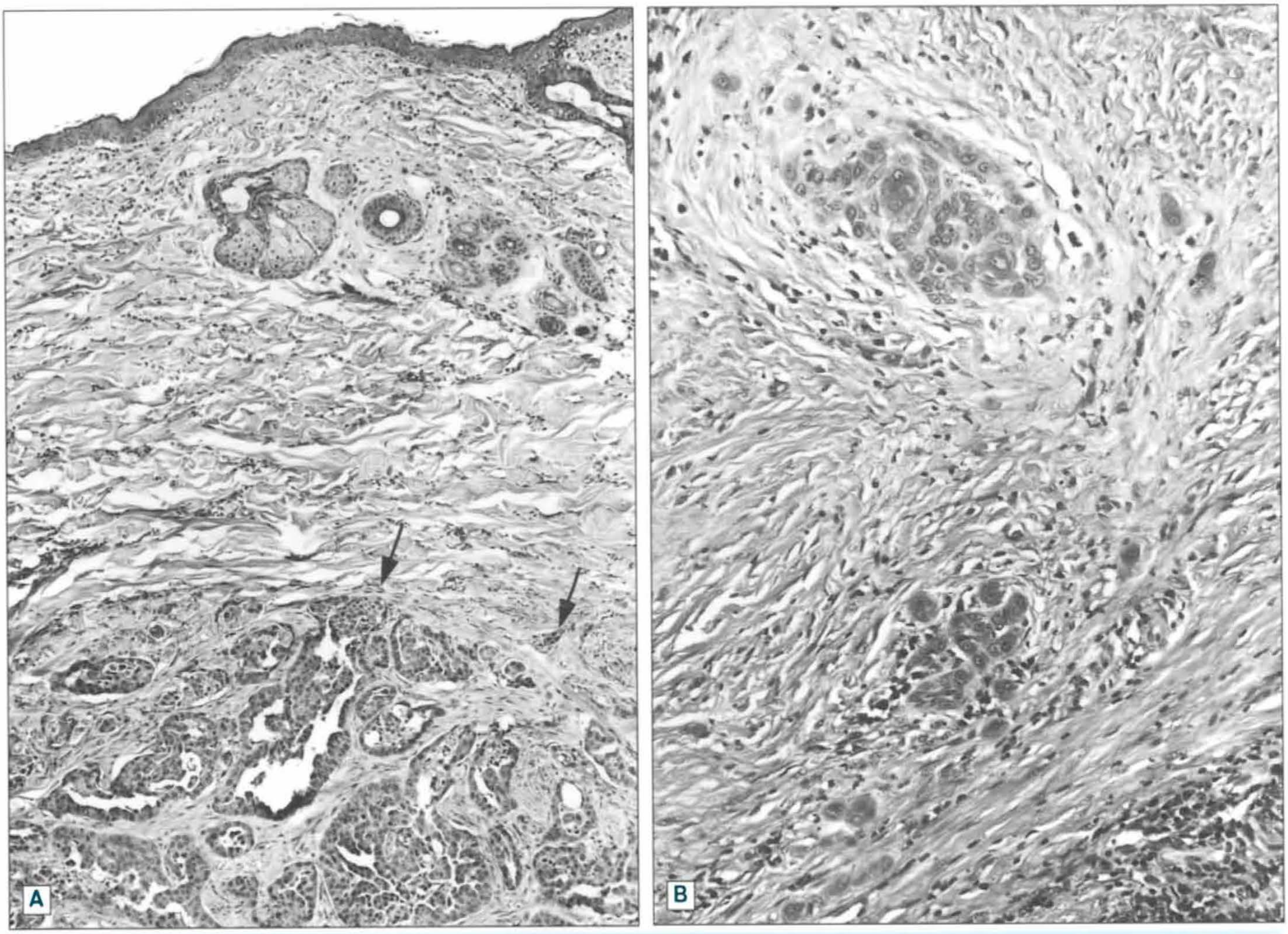

Figure 5.153 Mammary gland carcinoma from a cat. **A.** Note stromal invasion (arrows). **B.** Higher magnification of **(A)** showing poorly cohesive islands of malignant epithelial cells invading the dermis.

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Tumors metastatic to the skin

A variety of tumors can metastasize to the skin, but *the process is uncommon*. The usual routes of lymphatic, hematogenous, or implantation from a surgical procedure apply. A thorough search for a primary tumor, knowledge of prior history of a tumor, and an awareness of a few unique tumor patterns of metastasis are necessary to help establish the condition as a metastatic process.

Pulmonary carcinomas in the **cat** have a propensity to metastasize to the digits, often prior to onset of clinical signs referable to the primary tumor. Clinically, the lesions are suggestive of paronychia. Histologically, nests, solid sheets and glandular structures formed by malignant epithelial cells are found in the dermis and subcutis. Bony lysis of the third phalanx may be evident radiographically and histologically. Tumor cells have abundant eosinophilic cytoplasm, basally oriented nuclei and, frequently, apical cilia. Squamous differentiation is also common. Desmoplasia is usually present.

Visceral **hemangiosarcomas** may metastasize to the subcutis in the dog. Subcutaneous hemangiosarcomas, particularly if multiple, should prompt the clinician to search for a primary visceral tumor. Metastatic tumors may be fairly well-differentiated or anaplastic.

Mammary gland carcinomas in the dog may metastasize to the dermis of the inner thigh. Spread of tumor cells occurs via direct invasion of dermal lymphatics or from retrograde metastasis of tumor cells from the external inguinal lymph node. Mammary gland carcinomas in the cat may also metastasize to the ventral abdominal tissues using similar pathways. Primary apocrine gland carcinoma of the skin may be difficult to distinguish.

Abdominal surgeries to remove **transitional cell, colonic** or **prostatic carcinomas** have the potential for implantation of tumor cells in the skin at the surgical site. Tumor cells may also reach the skin via retrograde lymphatic metastasis.

1

Alimentary system

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ORAL CAVITY

Examination of the oral cavity should be standard procedure during any postmortem examination. To obtain a clear view of the mucous membranes of the buccal and oral cavities, teeth, tongue, gums, and tonsils, it is essential to split the mandibular symphysis and separate the mandibles as far as possible. A thorough examination of all structures will reveal not only local lesions, but often those that may be due to systemic disease. Lesions may be associated with congenital anomalies; trauma (physical and chemical); bacterial, mycotic, viral, and parasitic infections; metabolic and toxic diseases; and immune-mediated, dysplastic, or neoplastic disease. The poor physical condition of an animal may be directly related to oral lesions that result in difficulties of prehension, mastication, or swallowing of food.

Congenital anomalies

The development of normal facies and the oral cavity requires the integration of many embryonic processes. The complexity and duration of this development may lead to a great variety of aberrations. These are usually expressed in the newborn in the form of *clefts resulting from failures of integrated growth and fusion.* A common failure of fusion is that of the maxillary processes to the frontonasal process. This may leave facial fissures, cleft lip (harelip, cheiloschisis) and uni- or bilateral primary cleft palate involving the area rostral to the incisive papilla.

Facial clefts may involve the skin only, or the deeper tissues as well. They are variously located, and not all are obviously related to normal lines of fusion. All are rare. The least uncommon is a complete cleft from one angle of the mouth to the ear of that side. This results from failure of fusion of the lateral portions of the maxillary and mandibular processes. A defect extending from a cleft lip to the eye results from failure of fusion of the maxillary and frontonasal processes; its least expression is superficial and a failure of closure of the nasolacrimal duct.

Primary cleft palate (harelip, cheiloschisis) includes developmental anomalies of the lips rostral to the nasal septum, columella, and premaxilla. They may be uni- or bilateral and superficial or extend into the nostril. The defect arises from incomplete fusion of the frontonasal process with the maxillary processes.

Secondary cleft palate (cleft palate, palatoschisis) (Fig. 1.1) is often associated with primary cleft palate. The normal hard palate is formed, except for a small rostral contribution from the frontonasal process, by the bilateral ingrowth of the lateral palatine shelves from the maxillary processes. At the midline, they fuse with each other and with the nasal septum, and undergo intramembranous ossification, except in their caudal part, which becomes the soft palate. Inadequate growth of the palatine shelves leaves a central defect, in either or both of the hard and soft palates, which communicates between the oral and nasal cavities. Other manifestations of disordered palatogenesis include unilateral defects in the soft palate; bilateral hypoplasia of the soft palate; or dorsal displacement of the soft palate, with excess soft tissue on the caudal portion. Affected animals have difficulty sucking, may have nasal regurgitation, and usually die within the first few days of life from aspiration pneumonia.

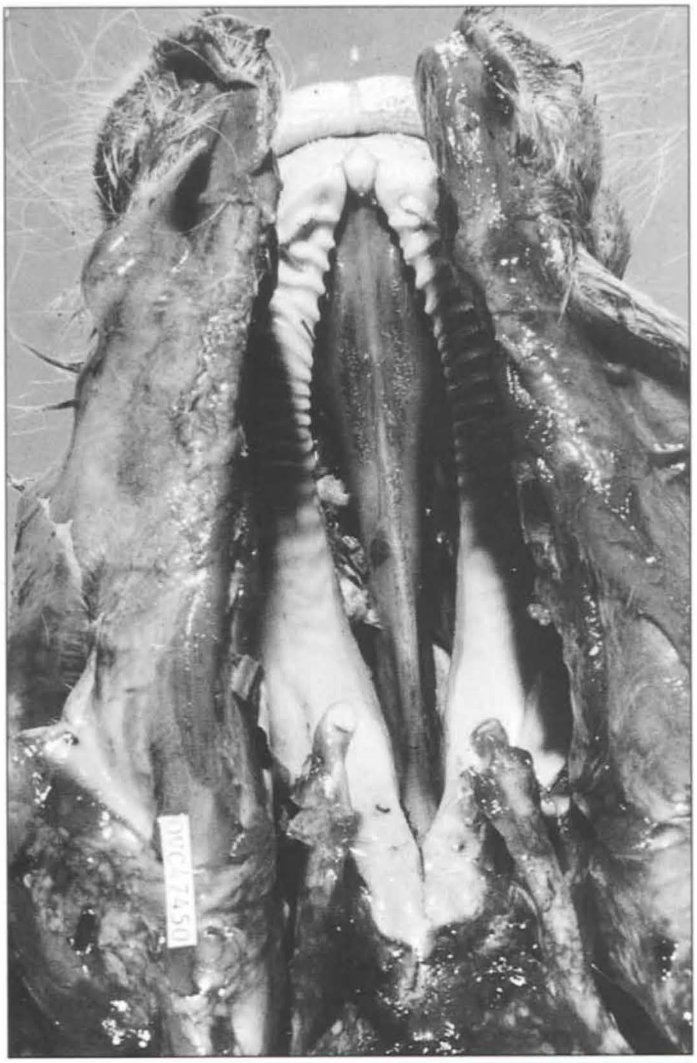

Figure 1.1 Secondary cleft palate exposing the nasal cavity in a calf.

Cleft palates have been reported in most species of domestic animals. In one extensive survey of Thoroughbred foals, 4% of congenital defects were secondary cleft palates. Most of these foals had a complete cleft of the hard palate; a few had clefts or hypoplasia of the soft palate only. In calves, cleft palate is one of the most common anomalies, but is very uncommon in sheep. Primary cleft palate is less common than secondary cleft palate in swine, although the two anomalies often occur together.

The etiology of cleft palate is usually unknown, but examples of *hereditary causes*, maternal ingestion of certain *drugs*, or maternal consumption of *teratogenic plants* during pregnancy have been demonstrated. Secondary cleft palate and arthrogryposis frequently occur together in Charolais calves, and appear to be hereditary (probably simple autosomal recessive), as in Hereford cattle. These two anomalies have also been associated with the ingestion of certain lupines by cows during gestational days 40–70. Cleft palate in lambs may be genetic in origin, but also is associated with the ingestion of *Veratrum californicum*. Secondary cleft palates have been induced experimentally in newborn pigs by feeding gilts seeds or plants of poison hemlock (*Conium maculatum*) during gestational days 30–45. Both tree tobacco (*Nicotiana glauca*) in western USA and tobacco stalks (*N. tabacum*) when fed to gilts early in pregnancy can induce a high incidence of cleft palate and arthrogryposis in newborn pigs. Piperidine alkaloids (coniine, coniceine, and anabasine) in hemlock and tobacco plants are responsible for the teratogenic effects of these plants. Lupines (*Lupinus formosus, L. arbustus*) produce piperidine alkaloids, including the teratogen ammodendrine, which can cause cleft palate and arthrogryposis (crooked calf disease) in calves born of dams fed the lupine at days 40–50 of gestation. Palatoschisis in piglets has also been associated with consumption of feed contaminated with *Crotalaria retusa* seed by sows during gestation. Primary and secondary cleft palate of German Boxer dogs appear to be hereditary, probably due to a single autosomal recessive gene. Secondary cleft palate occurs in Siamese and Abyssinian cats and is thought to be hereditary, although the mode of inheritance has not been determined. Griseofulvin treatment of the pregnant queen and mare will result in palatoschisis in the offspring. The defect has also been reported in both parts of the doubled face in diprosopus cats.

Anomalies in the growth of jaws are quite common. **Brachygnathia superior**, *shortness of the maxillae*, is an inherited breed characteristic among dogs and swine. It has been reported in the Large White or Yorkshire breed. The condition is progressive with age, resulting in malapposition of the incisor and cheek teeth, which interferes with prehension and mastication. In swine, brachygnathia superior may be confused with atrophic rhinitis. In Angus and Jersey cattle, brachygnathia superior occurs as a hereditary trait. It may, in any species, be associated with chondrodysplasia and is also present with other facial defects.

Brachygnathia inferior or micrognathia, *shortness of the mandibles*, may be a mild to lethal defect in cattle and sheep and is a breed characteristic of long-nosed dogs. Micrognathia is a common defect in calves. It is inherited, probably as a simple autosomal recessive trait. There is a higher incidence in males. In Aberdeen Angus cattle, the defect may occur concurrently with cerebellar hypoplasia, and with osteopetrosis in this and other breeds (see Vol. 1, Bones and joints). Mild brachygnathia inferior, termed *parrot mouth*, is a common conformational defect in horses.

Prognathism refers to *abnormal prolongation of the mandibles*. It is rather common, especially in sheep. It may develop with recovery from calcium deficiency in this species (see Vol. 1, Bones and joints). The malformation is relative, and it is not always easy to determine whether the jaw is absolutely long or merely apparently so, relative to a mild brachygnathia superior.

Agnathia is a mandibulofacial malformation characterized by *absence of the lower jaw*, due to failure of development of the first branchial arch and associated structures. The defect is one of the most common anomalies in lambs but is rare in cattle. Associated malformations in lambs may include ateloprosopia (incomplete development of the face), microglossia or aglossia, and atresia of the oropharynx. Concurrent anomalies affecting other body systems may also be evident.

A lethal glossopharyngeal hereditary defect, termed **bird tongue** and caused by a simple autosomal recessive gene, has been reported in dogs. Affected pups have a narrow tongue, especially the rostral half, where the margins are folded medially on to the dorsal surface. The pups are unable to swallow. The muscle fibers of the affected tongues are normal histologically. **Hypertrophy of the tongue** occurs as a congenital anomaly in pigs.

Persistent oropharyngeal membrane in a Hereford calf resulted in separation of the oral cavity from the pharynx, and inability to swallow milk. The cause was unknown, and the lesion is very rare.

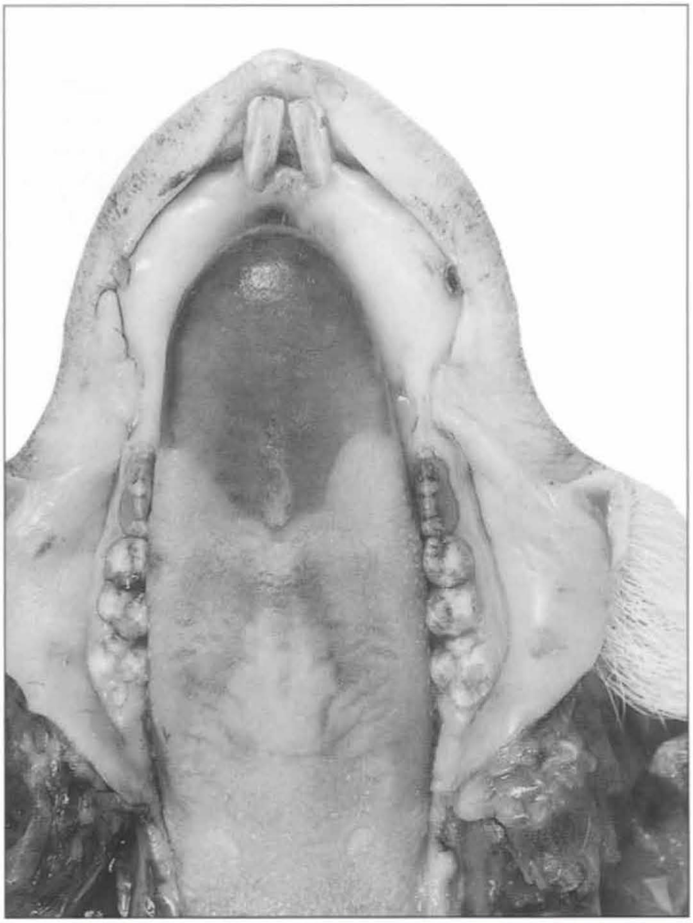

Figure 1.2 Epitheliogenesis imperfecta of the tongue of a pig.

Epitheliogenesis imperfecta is an anomaly causing *widespread defects in cutaneous epithelium, and also affects the epithelial lining of the oral cavity, especially the tongue* (Fig. 1.2) (see Vol. 1, Skin and appendages). The condition is characterized by irregular, well-demarcated, red areas from which the epithelium of the oral mucosa is absent. Histologically, these consist of abruptly defective areas in the squamous mucosa with inflammation of the submucosal connective tissues. The anomaly occurs in most species and is inherited as a simple autosomal recessive character in cattle, horses, and pigs; the mode of inheritance is unknown in the other species. There are several hereditary skin conditions in animals, such as epidermolysis bullosa simplex in collie dogs, ovine epidermolysis bullosa in Suffolk and South Dorset Down sheep, and familial acantholysis of Aberdeen Angus calves, which have minor involvement of the lips and oral mucosa (see Vol. 1, Skin and appendages).

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Diseases of teeth and dental tissues

Dental examinations in animals are usually cursory, except to assess age, but dental disease is common and often is the factor that limits useful lifespan, especially of sheep. The comments on dental development and anatomy are intended to assist the understanding of dental disease.

Teeth develop from horseshoe-shaped thickenings in the oral ectoderm called *dental laminae*. Neural crest cells beneath the laminae induce formation of *tooth buds*, which generate the *enamel organs*. These epithelial structures grow into the underlying ectomesenchyme and organize it to form *dental papillae*, which they enclose like a cap. Surrounding both is another mesenchymal condensation, the *dental sac*, which has a collagenous inner layer, the dental follicle, and an outer fibrovascular layer. Developing teeth erupt through a combination of root elongation, alveolar bone remodeling, and periodontal ligament formation. *Periodontal ligament formation* may be more important for species in which the teeth continuously erupt during life. Tooth eruption is induced by the enamel organ that secretes mediators transforming growth factor-α and interleukin-1β to recruit monocytes in the dental follicle for osteoclast resorption and formation of an eruption pathway.

The *inner enamel epithelium* of the enamel organ induces differentiation of odontoblasts from the mesenchyme of the papilla. Odontoblasts produce *dentin*, which in turn induces enamel formation by the inner enamel epithelium. Formation of dentin is essential for the formation of enamel. These inductive interactions of epithelium and mesenchyme are considered important in the histodifferentiation of some tumors of dental tissues.

The free edge of the enamel organ extends beyond the enamel–dentin junction, and this extension is called *Hertwig's epithelial root sheath*. It molds the dental papilla to form the root or apex of the tooth. Subsequently it fragments, allowing mesenchymal cells from the dental sac to contact the root dentin, differentiate into cementoblasts, and deposit cementum on the dentin. Remnants of the root sheath are called *epithelial rests of Malassez*. They persist in the periodontal ligament, and may give rise to tumors or cysts. They may be important in the induction or repair of cementum, and in periodontal reattachment following injury. In pigs and sheep, the rests may be incorporated into the junctional epithelium as it migrates apically in chronic periodontal disease. Cells of the root sheath that adhere to the dentin can produce *enamel pearls*.

Once the dental lamina has produced the buds of the permanent teeth, it degenerates. Epithelial remnants persist as *epithelial pearls or islands* in the gingiva and jaws. These remnants also may give rise to tumors and cysts.

There are important differences between the **brachydont teeth** of humans, carnivores, and swine, in which the enamel is restricted to the tooth crown, and the **hypsodont teeth** of herbivores. In hypsodont teeth, enamel extends far down on the roots, and is invaginated into the dentin to form *infundibula*. Also, the hypsodont teeth of herbivores, except the mandibular premolars of ruminants, are covered by cementum, which more or less fills the infundibula. Exceptions to these rules are provided by the tusks of boars, which are hypsodont, but not covered by cementum, and by ruminant incisors, which are brachydont but do have enamel covering part of the root dentin and cementum covering the root enamel.

The three hard tissues of teeth are dentin, enamel, and cementum. **Dentin** is light yellow and constitutes most of the tooth. It consists of ~35% organic matter and ~65% mineral. Thus its composition is similar to that of bone, and like bone, it contains type I collagen. Dentin is produced by columnar cells with basal nuclei called *odontoblasts*, which differentiate from mesenchyme of the dental papilla. It is formed as unmineralized predentin. The odontoblasts move away from the dentin–enamel junction, gradually encroaching on the pulp cavity as they produce dentin. Each odontoblast has a process extending into the dentin, encased in a dentinal tubule, which arborizes at the dentin–enamel junction. The process also anastomoses with the processes of other odontoblasts. Dentinal tubules are visible in histologic sections, but the anastomoses are not. Except for the processes, and nerve endings in the dentinal tubules near the pulp, dentin is acellular.

Normal dentin contains incremental or *imbrication lines of von Ebner*, which are fine basophilic lines running at right angles to the dentinal tubules. They represent normal variations in the structure and mineralization of dentin. Sublethal injury caused by certain infections, metabolic stresses, or toxic states may injure the odontoblasts, which then produce accentuated incremental lines known as the *contour lines of Owen*. Sometimes irregular zones of unmineralized or poorly mineralized dentin form between foci of normal mineralization. These are zones of *interglobular dentin*, which may be caused by hypophosphatemia.

Odontoblasts normally are active throughout life, producing layers of secondary dentin, which often contain fewer dentinal tubules than primary dentin. *Reparative dentin* is produced locally in response to injury to dentinal tubules, and contains a limited number of twisted tubules and sometimes a few odontoblasts, which soon die. Reparative dentin may resemble bone and is sometimes called *osteodentin*. *Sclerotic* (*transparent*) *dentin* is formed when dentinal tubules are occluded by calcium salts. The junctions between primary, secondary, and reparative dentin are usually demarcated by basophilic lines.

Enamel has ~5% organic matter and ~95% mineral. It is produced by the tall columnar *ameloblasts* of the inner enamel epithelium. Enamel is produced in the form of prisms or rods, cemented together by a matrix. Mineralization begins as soon as it is formed and is a two-stage process, somewhat similar to that in bone, but much more rapid. The cells of the inner enamel epithelium also move away from the dentin–enamel junction as the tooth is formed, but, unlike odontoblasts, they do not have processes. Enamel is hard, dense, brittle, and permeable, and is translucent and white. Mature enamel is not present in demineralized sections, but some of the matrix of immature enamel may be visible near ameloblasts of developing teeth.

Ameloblasts are very sensitive to environmental changes. Normal enamel contains *incremental lines of Retzius*, which are analogous to the incremental lines of von Ebner in dentin, and also reflect variations in structure and mineralization. The incremental lines are accentuated during periods of metabolic stress. More severe injury, as in fluorosis, or infections by some viruses can produce focal **hypoplasia or aplasia of enamel**.

Formation of enamel ends before tooth eruption. The inner enamel epithelium then merges with the cells of the underlying stratum intermedium and the outer enamel epithelium to form the *reduced enamel epithelium*. It protects the enamel of the formed tooth prior to eruption. Degeneration of this protective layer permits connective tissue to contact the enamel, and there may be resorption of enamel or deposition of a layer of cementum on it. This normally occurs during odontogenesis in horses.

Cementum is an avascular, bonelike substance, produced by *cementoblasts*; it contains ~55% organic and ~45% inorganic matter. In general, the dentin of brachydont teeth is covered by cementum wherever it is not covered by enamel. When dentin formation has begun in the root, degeneration of Hertwig's epithelial root sheath begins and permits mesenchymal cells from the dental sac to contact dentin. They differentiate into cementoblasts, which produce *cementoid*, and later mineralize it. Some layers of cementum do not contain cells (acellular cementum), but in other layers, *cementocytes* are enclosed in lacunae. Sharpey's fibers from alveolar bone are embedded in the cementum. Cementum is more resistant to resorption than is bone, and unlike bone, normally is not resorbed and replaced as it ages; instead a new layer of cementum is deposited on top of the old layer. In some pathologic conditions cementum is resorbed; subsequently, cellular or acellular cementum is deposited, and more or less repairs the defect.

Hypercementosis is abnormal thickening of cementum and may involve part or all of one or many teeth. When extra cementum improves the functional properties of teeth, it is called *cementum hypertrophy*; if not, it is called *cementum hyperplasia*. Extensive hyperplasia is often associated with chronic inflammation of the dental root.

The **periodontal ligament** is very cellular, well-vascularized connective tissue that develops from the dental sac. The **periodontium** comprises the periodontal ligament, gingival lamina propria, cementum, and alveolar bone. The ligament supports the tooth and adjusts to its movement during growth. It is well supplied with nerves and lymphatics, which drain into alveolar bone. The periodontal ligament is also a source of the cells that remodel alveolar bone and, in disease, cementum.

Epithelial rests of Malassez are present in the periodontal ligament and are particularly numerous in the incisor region of sheep. In all species, they may proliferate and become cystic when there is inflammation of the periodontium. The periodontium is also a site of origin of tumors. The periodontal ligament is normally visible in radiographs as a radiolucent line between tooth and alveolar bone. In prolonged hyperparathyroidism, alveolar bone is resorbed, and the ligament is no longer outlined radiographically, a change referred to as *loss of the lamina dura*.

Developmental anomalies of teeth

Anodontia, *absence of teeth*, is inherited in calves, probably as a sex-linked recessive trait in males, and is associated with skin defects. **Oligodontia**, *fewer teeth than normal*, occurs sporadically in horses, cats, and dogs, and also as an inherited trait in dogs. In brachycephalic breeds, the cheek teeth are deficient; in toy breeds, the incisors are deficient. *Pseudo-oligodontia* and *pseudoanodontia* result from failed eruption. These defects may be associated with bone-modeling defects in gray lethal mice with osteopetrosis. Delayed eruption of permanent teeth occurs in Lhasa Apso and Shih Tzu dogs. **Polyodontia**, *excessive teeth*, occurs in brachycephalic dogs; the incisors are involved, and the defect is probably related to breeding for broad muzzles. A high incidence of canine polyodontia, involving particularly an extra maxillary premolar, is reported from the Netherlands. Polyodontia also occurs in horses and cats, involving either incisors or cheek teeth. **Pseudopolyodontia** *is retention of deciduous teeth after eruption of the permanent dentition*. It occurs in horses, cats, and dogs, especially in the miniature breeds.

Heterotopic polyodontia *is an extra tooth, or teeth, outside the dental arcades*. The best-known example is the *ear tooth of horses*, which develops in a branchiogenic cyst. The cysts originate from failure of closure of the first branchial cleft, or from the inclusion of cellular rests in this area. They are lined by a stratified mucous or cutaneous-type epithelium, and may contain one or more teeth, either loosely attached in the cyst wall or deeply embedded in the petrous temporal bone. The tooth is derived from misplaced tooth germ of the first branchial arch, which is displaced toward the ear with the first branchial cleft. The cysts form in the parotid region and may fistulate to the exterior. They are occasionally bilateral.

Rarely the tooth may form a pedunculated mass enclosed by skin, and attached by a pedicle to the skin of the head. Heterotopic polyodontia also occurs in cattle, dogs, pigs, and sheep.

Developmentally **misshapen teeth** are classified as **geminous** (dichotomous) when there is a single root and partially or completely separate crowns; **fused**, when the dentin of two teeth is confluent; and **concrescent** when the dentin is separate but the roots are joined by cementum. Gemination represents the embryologic partial division of a tooth primordium. It occurs in dogs, usually involving the incisors, and the affected tooth usually has a groove dividing the crowns, whose pulp chambers can be seen radiographically to merge in a common root. Misshapen teeth and missing teeth have also been reported in dogs as an X-linked recessive trait of ectodermal dysplasia. Fusion and concrescence represent the joining of two adjacent tooth primordia, one of which may be supernumerary. *Malformation and malpositioning of teeth* accompany abnormalities of the jaw bones. Aberdeen Angus and Hereford calves with congenital osteopetrosis have brachygnathia inferior, malformed mandibles, and impacted cheek teeth. Impacted molars occur as an inherited lethal in Shorthorns; an association with osteopetrosis apparently has not been investigated in this breed.

Odontogenic cysts are epithelium-lined cysts derived from cell rests of Malassez, cell rests of dental laminae, reduced enamel epithelium, or malformed enamel organs. **Dentigerous cysts** are, by definition, *cysts that contain part or all of a tooth*, which is often malformed. Of the odontogenic cysts listed, all except those derived from cell rests of Malassez are potentially dentigerous. (The rests of Malassez are the probable source of periodontal cysts.) Dentigerous cysts originating in malformed enamel organs should include malformed teeth, since development of enamel is incomplete until the organ degenerates. Those teeth in cysts of reduced enamel epithelium or rests of dental laminae are not necessarily abnormal. The affected teeth probably erupt into the preformed cysts. Dentigerous cysts enclose at least the crown of the tooth, but may include it all. The most common forms of odontogenic dentigerous cysts in animals are those involving the *vestigial wolf teeth* of horses and the *vestigial canines*, especially of mares. The smaller cysts appear as tumors of the gums, whereas some of the larger ones may cause swelling of the jaw or adjacent maxillary sinus. Dentigerous cysts of animals are not as destructive as those in humans, in which species they are regarded as the most common benign destructive lesion of the skeleton.

The ear tooth of horses is probably the most common non-odontogenic dentigerous cyst. Occasionally true dentigerous cysts form when a tumor prevents normal eruption or when there is maleruption due to odontodystrophy.

Cystic dental inclusions about vestigial supernumerary teeth also occur in juxtamolar positions in cattle, but are insignificant. These too may be dentigerous, or they may be primordial cysts developed before the stage of enamel formation, and hence contain no mineralized tooth structures. Either type of cyst may give rise to ameloblastomas.

A high incidence of dentigerous cysts involving incisors occurs in some sheep flocks in Scotland, Australia, and New Zealand. A congenital disease involving the jaws and teeth of calves in Germany (*odontodysplasia cystica congenita*) is characterized by massive fibro-osseous enlargement of the maxillae and horizontal rami of the mandibles. Some teeth are malformed, misshapen, or absent. Cystic spaces in the jaws are lined by fibrous tissue or epithelium, the latter probably derived from enamel organs. The dental changes are thought to be secondary to those in bone. Most affected calves are aborted or stillborn, and many have ascites and hydrocephalus. The disease may be caused by environmental influences.

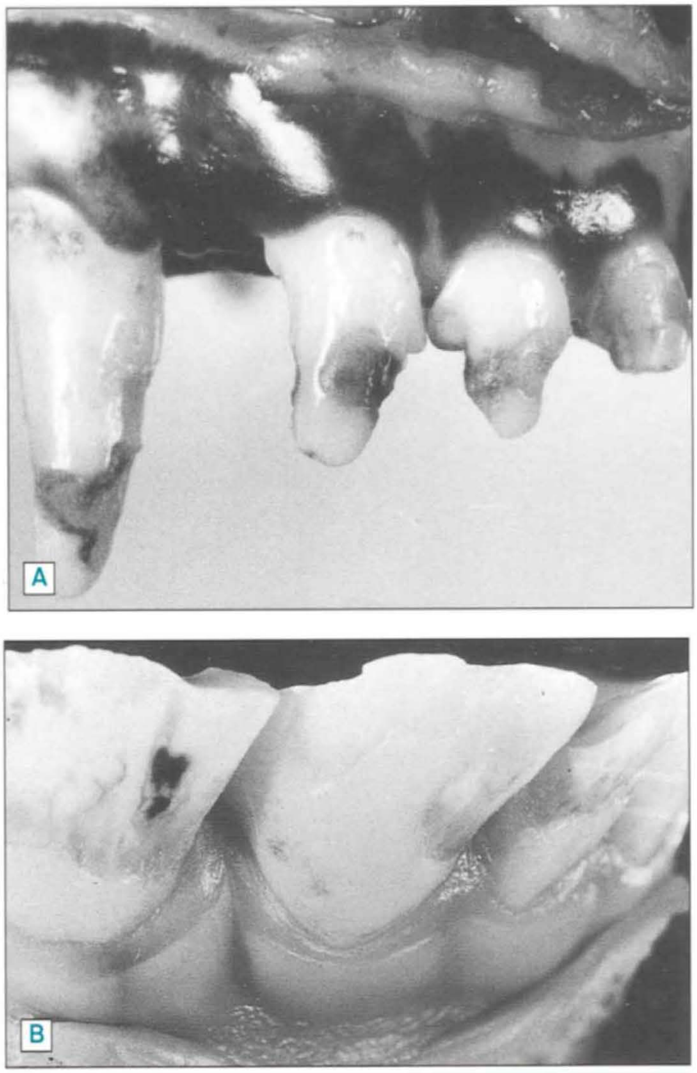

Figure 1.3 Enamel hypoplasia. A. A sequel to *Canine distemper virus* infection in a dog. **B.** Subsequent to intrauterine infection by *Bovine viral diarrhea virus* in a calf. (Courtesy of RB Miller.)

The permanent teeth are unique in that they continue to develop for a long time after birth. Thus, inflammatory and metabolic disease of postnatal life, for instance *Canine distemper virus* infection (Fig. 1.3A), can produce hypoplasia of dentin and enamel. Hypoplasia of the enamel of deciduous teeth occurs in some calves with intrauterine *Bovine viral diarrhea virus* infection (Fig. 1.3B). It has also been described in calves and pigs following irradiation of the dam during gestation. Dysplastic proliferation of dentin and enamel involving mandibular premolars and molars has been seen in young uremic dogs. Extreme fragility of deciduous teeth is a feature of bovine osteogenesis imperfecta (see Vol. 1, Bones and joints). Dental dysplasia, characterized by normal dentin, absence of enamel matrix, and excess irregular cementum, is described in a foal with epitheliogenesis imperfecta involving the oral mucosa.

Degenerative conditions of teeth and dental tissue

Pigmentation of the teeth

Normal enamel is white and shiny, but normal cementum is off-white to light yellow, and normal dentin is slightly darker yellow. Depending on the tooth, or the part of the tooth being examined, the normal color may be any one of these. Normal enamel is never discolored. Hypoplastic enamel of chronic fluorosis is discolored yellow through brown to almost black. Discoloration of brachydont teeth results from pigmentation of dentin, which is then visible through the semitransparent enamel, or pigmentation of the cementum of the root. Dentin may be colored red-brown by pulpal hemorrhages or inflammation, gray-green in putrid pulpitis, and yellow in icterus. Congenital erythropoietic porphyria of calves, cats, and swine discolors the dentin red in young animals (pink tooth) and darker brown in adults, although in swine, the discoloration may disappear with aging. Transient porphyria with pink discoloration of teeth is reported in a dog.

Yellow to brown discoloration of teeth, and bright yellow fluorescence in ultraviolet light, due to deposition of *tetracycline* antibiotics in mineralizing dentin, enamel, and probably cementum, occurs in all species. Treatment of the pregnant dam may cause staining of deciduous teeth in the offspring. Tetracyclines are toxic to ameloblasts in late differentiation and early secretory stages and, at high dose rates, may produce enamel hypoplasia. Black discoloration of ruminant cheek teeth is extremely common, and is caused by impregnation of mineral salts with chlorophyll and porphyrin pigments from herbage.

Dental attrition

Dental attrition is loss of tooth structure caused by mastication. The mature conformation of teeth is largely the outcome of opposed growth and wear, and the degree of wear depends on the type of tooth, the species of animal, and the material chewed. Wear is most evident in herbivores, and irregularities of wear are perhaps the most common dental abnormalities. In general, with normal occlusion and use, the extra-alveolar portion of the tooth does not shorten. Its length is maintained initially by growth, the period of growth depending on the species, then by hypertrophy of the root cementum and/or dentin and by proliferation of alveolar bone, which serves to push the tooth out. Finally, senile atrophy of the alveolar processes and gingival recession may maintain or increase the length of the clinical crown. Cementum hypertrophy and alveolar atrophy may also result in loss of teeth in senility, or, if combined with subnormal wear, produce teeth that in old age are excessively long. Normal wear of the complicated cheek teeth of horses and cattle causes smoothing of the occlusal surfaces. As soon as wear of enamel exposes the dentin, which, being softer, wears more rapidly, secondary or irregular dentin is deposited to protect the pulp. In time, this may fill the pulp cavity and cause death of the tooth. Abnormalities of wear are most common in herbivores (Fig. 1.4). Excessive wear of the deciduous and permanent central incisors occurs in certain sheep flocks in New Zealand. The wear is intermittent and may be severe enough to expose the pulp cavity. The cause is unknown but may be related to delayed eruption of adjacent teeth, leading to increased use of the affected pairs.

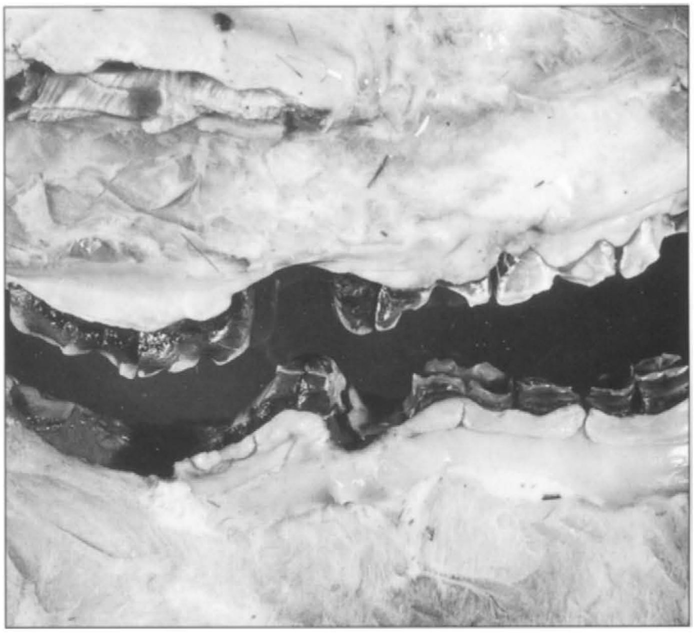

Figure 1.4 Irregular wear of the teeth of a horse.

Subnormal wear, due to loss of the opposing tooth, occurs in oligodontia, abnormal spacing of adjacent teeth, and acquired loss of teeth; it results in *abnormal lengthening*. Such elongated teeth may grow against the opposing gum or, if deviated, into an adjacent soft structure such as cheek or lip. These teeth usually wear in abnormal places because complete loss of antagonism is unusual, since the upper and lower arcades do not coincide exactly, and the coincidence is further reduced by the displacement of chewing. Incomplete longitudinal coincidence of the molar arcades allows irregular wear and hook formation on the first and last cheek teeth.

Abnormal wear due to abnormal chewing is caused by voluntary (as in painful conditions) or mechanical impairment of jaw movement. Lateral movements of the jaws without the normal rotary grinding movements allow the ridges of the teeth of herbivores to become accentuated. Steep angulation of the occlusal surfaces results from inadequate lateral movement of the jaws, and sharp edges form on the buccal aspect of the maxillary teeth and the lingual aspect of the mandibular teeth. This may be unilateral when the animal chews with only one side of its mouth, the other side then being affected. The teeth wear progressively sharper, and pass each other like shear blades; hence the term **shear mouth.** Subnormal resistance to wear on the part of the molar teeth is common, and results in **wave mouth** ("weave mouth") or **step mouth**, in which successive teeth in an arcade wear at different rates. The wave or step form of the antagonistic arcade is reversed, so that the teeth of the two arcades interdigitate. This pattern of attrition is caused by variation in the hardness of opposing teeth, and is usually caused by intermittent odontodystrophy. Opposing teeth of the upper and lower jaws do not develop at the same time; thus, discontinuous nutritional deficiencies often result in unequal wear. Certain vices, such as crib biting, also produce abnormal wear. In severely worn ruminant incisors, a central black core may be visible, which is secondary dentin deposited in the pulp cavity. It is not carious, but stains darker than the surrounding primary dentin.

Odontodystrophies

Odontodystrophies are diseases of teeth caused by nutritional, metabolic, and toxic insults. They are manifest by changes in the hard tissues of the teeth and their supporting structures. Lesions of enamel and dentin are emphasized here. The most prominent effects of odontodystrophies appear in enamel, and lesions of enamel are most significant because they are irreparable.

Formation of enamel occurs in a set pattern. It begins at the occlusal surface and progresses toward the root. Mineral maturation occurs in the same sequence, but for each level, it begins at the dentin–enamel junction and moves toward the ameloblast. Deleterious influences have their most severe effects on those ameloblasts that are forming and mineralizing enamel. Depending on the severity on the insult, ameloblasts may produce no enamel, a little enamel, or poorly mineralized enamel. Removal of the insult permits those ameloblasts that were not yet active to begin making normal enamel. Thus, enamel defects vary in severity from isolated opaque spots or pits on the surface to deep and irregular horizontal indentations. These defects are most clearly seen on the incisor teeth and canine teeth and are usually bilaterally symmetrical. Similar lesions are also produced by infectious agents that injure ameloblasts, such as *Canine distemper virus* and *Bovine viral diarrhea virus* (Fig. 1.3).

Odontoblasts are susceptible to many of the same influences as ameloblasts, but they can be replenished from the undifferentiated cells of the dental pulp. Thus, lesions in actively forming dentin may be repaired, whereas those in enamel are permanent.

Because of their close anatomical association with the jaws, teeth are very susceptible to disruption in the harmony of growth. This harmonious arrangement is often upset in the odontodystrophies and osteodystrophies, and leads to malocclusion and anomalous development of teeth.

Several nutritional and toxic conditions produce odontodystrophy. **Fluorine poisoning** is exemplary (see Vol. 1, Bones and joints). In **vitamin A deficiency**, ameloblasts do not differentiate normally, and their organizing ability is disturbed. As a result, odontoblastic differentiation is abnormal. Several lesions develop, including enamel hypoplasia and hypomineralization, cellular, vascularized dentin (osteodentin), and retarded or obviated eruption.

Calcium deficiency retards eruption, and causes enamel hypoplasia and mild dentin hypoplasia. Teeth formed during the period of deficiency are very susceptible to wear. In sheep, recovery from prolonged calcium deficiency results in malocclusion due to inferior prognathia. This reflects inadequate maxillary but normal mandibular repair during the recovery phase.

Phosphorus deficiency, combined with vitamin D deficiency, depresses dentin formation slightly, but has virtually no effect on enamel, at least not in sheep. Hypophosphatemia is associated with formation of interglobular dentin in humans. Malocclusion and abnormalities of bite in rachitic sheep are secondary to mandibular deformity.

Severe, experimental **malnutrition** also produces malocclusion. Recovery from malnutrition does not correct the lesion, and in addition, is associated with misshapen, malformed teeth, oligodontia, and polyodontia.

The major **effects of odontodystrophies** in herbivores are *malocclusion*, and/or accelerated *attrition*. Sometimes a high incidence of these abnormalities is attributable to one of the causes previously discussed, but often they are idiopathic. Most of the lesions described in experimental odontodystrophies also occur in natural diseases.

A syndrome of dental abnormalities of sheep in the North Island of New Zealand is characterized by excessive wear of deciduous teeth, maleruption and excessive wear of permanent teeth, periodontal disease involving permanent teeth, and development of dentigerous cysts involving permanent incisors. Mandibular osteopathy is also present. Animals older than 5 years are culled for dental problems. The odontodystrophy (and osteodystrophy) is possibly caused by deficiencies of calcium and copper, and perhaps other nutrients, such as protein, and energy. This syndrome exemplifies the naturally occurring odontodystrophies, in that it probably has a complex pathogenesis, and is associated with an osteodystrophy. The latter association is to be expected, since *bones and teeth are usually susceptible to the same insults.*

While dental lesions are not described, tooth loss due to periosteal dysplasia and osteopenia occurs in Salers cattle afflicted with hereditary hemochromatosis.

Infectious and inflammatory diseases of teeth and periodontium

The role of viruses in enamel hypoplasia is mentioned above. Bacterial plaque, along with other tooth-accumulated materials, is discussed here.

Bacterial diseases involving tooth surfaces are caused by the development of supragingival and subgingival **plaque**. *Supragingival plaque* is located on the exposed crown of the tooth and causes dental caries. *Subgingival plaque* is found in the crevicular groove and causes periodontal disease. Tooth enamel is covered by a translucent pellicle, the *acquired enamel pellicle*, which is formed by selective adsorption of complex salivary proteins, and that is essential to the development of supragingival plaque. This is a dense, nonmineralized, bacterial mass, firmly adherent to tooth surfaces, which resists removal by salivary flow and prevents the buffering capacity of saliva from influencing plaque metabolites. Formation of this plaque involves adhesion of bacteria to the pellicle, and adhesion of bacteria to each other, producing a *biofilm*. Only organisms with the ability to adhere to the pellicle can initiate the formation of supragingival plaque; those that cannot are removed by oral secretions and mechanical action. Pathologic reduction of salivary flow increases the prevalence of caries in some species.

The bacteria in supragingival plaque are members of the indigenous oral flora and are usually gram-positive aerobes. Most are streptococci and *Actinomyces* spp., which form an organized array on the tooth surface. Some plaque-forming bacteria synthesize extracellular polymers, which constitute the matrix of the plaque and permit adhesion between organisms of the same species. Some utilize polymers derived from host secretions to adhere to the pellicle, whereas others attach to bacteria of a different species that are already fixed to the tooth. Plaque increases in mass with time, and its composition becomes more complex as anaerobic gram-negative bacteria join the streptococci and actinomycetes that initiated plaque formation.

Supragingival plaque is metabolically active. It utilizes dietary carbohydrates to produce the adhesive polymers and the acids needed to demineralize enamel, and as energy sources for maintenance and for production of various enzymes and stimuli for inflammation. Extensive deposits of supragingival plaque are virtually invisible unless treated with a disclosing solution.

Subgingival plaque is less organized than supragingival plaque, and many of the organisms involved are gram-negative anaerobes that are asaccharolytic, weakly adherent, and motile. They derive their nutrients from the crevicular fluid. The flora of subgingival plaque is less well characterized than that of supragingival plaque. Culture results vary with sample collection technique, site of collection, and selectivity of media, and appear to under-represent the flora detected by molecular means in humans. At least *Porphyromonas* spp. (formerly *Bacteroides*) and spirochetes are likely periodontal pathogens in animals.

Dental calculus (*tartar*) *is mineralized plaque.* It is formed by the deposition of mineral, mainly from saliva, in dead bacteria. In horses and dogs, calculus is predominantly calcium carbonate. Calculus is often found in old dogs and cats, occasionally in horses and sheep, and rarely in other species. The distribution is often uneven, but it is *usually most abundant next to the orifices of salivary ducts.* Calculus on horses' teeth is chalky and easily removed. In dogs, it is hard, firmly attached, and often discolored. Red-brown to black calculus with a metallic sheen develops in pastured sheep and goats. It usually involves all the incisors, principally on the neck of the buccal surface. Minor amounts are common along the gum–tooth junction of the molar teeth, but occasionally larger (up to 2 cm) hard, black, rounded concretions may protrude from between opposed surfaces of the premolars. A high prevalence of calculus in sheep on the Scottish island of North Ronaldsay was related to their predominantly seaweed diet. Calculus was most severe around the cranial cheek teeth, increased in severity with age, was associated with periodontal disease, and contained large amounts of calcium, magnesium, and phosphorus.

Materia alba, which adheres to teeth, is a mixture of salivary proteins, desquamated epithelial cells, disintegrating leukocytes, and bacteria. The bacteria are not organized, and materia alba is easily removed. It is distinct from dental plaque, and from food debris, which also accumulates between uncleaned teeth.

Dental caries

Dental caries is a disease of the hard tissues of teeth, characterized by *demineralization of the inorganic part and enzymatic degradation of the organic matrix.* **Erosions** of teeth are characterized by removal of hard tissues layer by layer. These definitions permit the inclusion of equine infundibular necrosis as a form of caries (see following sections). Caries is common in horses and sheep but rare in dogs. Cats are commonly subject to caries-like odontoclastic resorptive lesions of uncertain etiopathogenesis.

There are two types of caries:

1. **Pit or fissure caries** develops in irregularities or indentations, which trap food and bacteria, usually on the occlusal surface of the tooth. Plaque is not essential for initiation of this form of caries, of which *equine infundibular necrosis* is an example.
2. **Smooth-surface caries** usually occurs on proximal (adjacent) surfaces of teeth, typically just below contact points, or around the neck, and requires dental plaque for its initiation.

The organic acids, principally lactic, which initiate demineralization, are produced by bacterial fermentation of dietary carbohydrates. In smooth-surface caries, plaque produces the acid and maintains a low pH on the surface of the tooth. Progression of lesions depends on various factors such as salivary pH, hardness and resistance to demineralization of enamel, and frequency of access to carbohydrate. Demineralization of enamel often occurs in the subsurface enamel but progresses to caries only with prolonged exposure to acid. Infrequent exposure allows remineralization of enamel between meals. The enzymes that lyse the organic matrix are probably produced by plaque, but may be derived from leukocytes, for which plaque is chemotactic. Carious enamel loses its sheen and becomes dull, white, and pocked. When dentin is exposed, it becomes brown or black. Dentin is softer and more readily demineralized than enamel, and a pinpoint lesion in enamel may lead to a large defect when the carious process reaches the dentin. Nerve endings have not been identified at the enamel–dentin junction, and the pain of caries is thought to be caused by chemical or pressure changes in the dentinal tubules. Spread of infection along the tubules to the pulp cavity may result in formation of secondary or reparative dentin, pulpitis, or periapical inflammation and tooth loss.

In **horses, infundibular necrosis** develops most often on the occlusal surface of the maxillary first molar. The enamel invaginations (infundibula) in the cheek teeth of horses are normally filled with cementum before the teeth erupt. Filling proceeds from the occlusal surface toward the apex, but often is not completed before eruption. At this time the blood supply is cut off, and ischemic necrosis of any residual cementogenic tissue in the infundibula occurs. The deficiency of cementum is called *hypoplasia.* Rostral infundibula are affected more frequently than are caudal, and the first molar more often than other teeth (Fig. 1.5A).

Teeth with incompletely filled infundibula may accumulate food material and bacteria (Fig. 1.5B), and, in some animals, the cavitated area expands to involve all the cementum and the adjacent enamel and dentin. Decay of the mineralized tissues sometimes progresses to coalescence of the cement lakes, fracture of the tooth, root abscess, and empyema of the paranasal sinuses. The incidence of infundibular necrosis increases with age, and 80–100% of horses older than 12 years may have the lesion. Most are without signs, and in most, the lesion does not progress. Inflammation of the dental pulp, in horses and in other species, may result from direct expansion of caries from penetration of bacteria and bacterial degradation products along the dentinal tubules. Production of reparative dentin in the pulp cavity is expected.

In **sheep**, the proximal surfaces of mandibular teeth are usually affected by caries, which is commonly accompanied by periodontitis. Erosions of the neck region of the deciduous teeth occurred in sheep in New Zealand. The lesions were mainly located apical to the enamel–dentin junction on the labial or lingual surface. They did not seem to be related to the usual causes of localized tooth destruction.

Cattle develop loss of dentin just below the crown of incisor teeth at increasing frequency with age. This usually follows recession of the gingiva, and is not considered to be a form of caries, but proteolytic digestion of dentin by chyme in an alkaline pH.

In **dogs**, caries most commonly involves the fourth premolar and the first and second molars. Although relatively uncommon, when caries occurs, defects are often multiple and advanced, leading to therapeutic extraction.

Cats, whose teeth do not have centers where food can collect, very commonly develop multiple caries-like **odontoclastic resorptive lesions**, initially involving the subgingival neck or upper root,

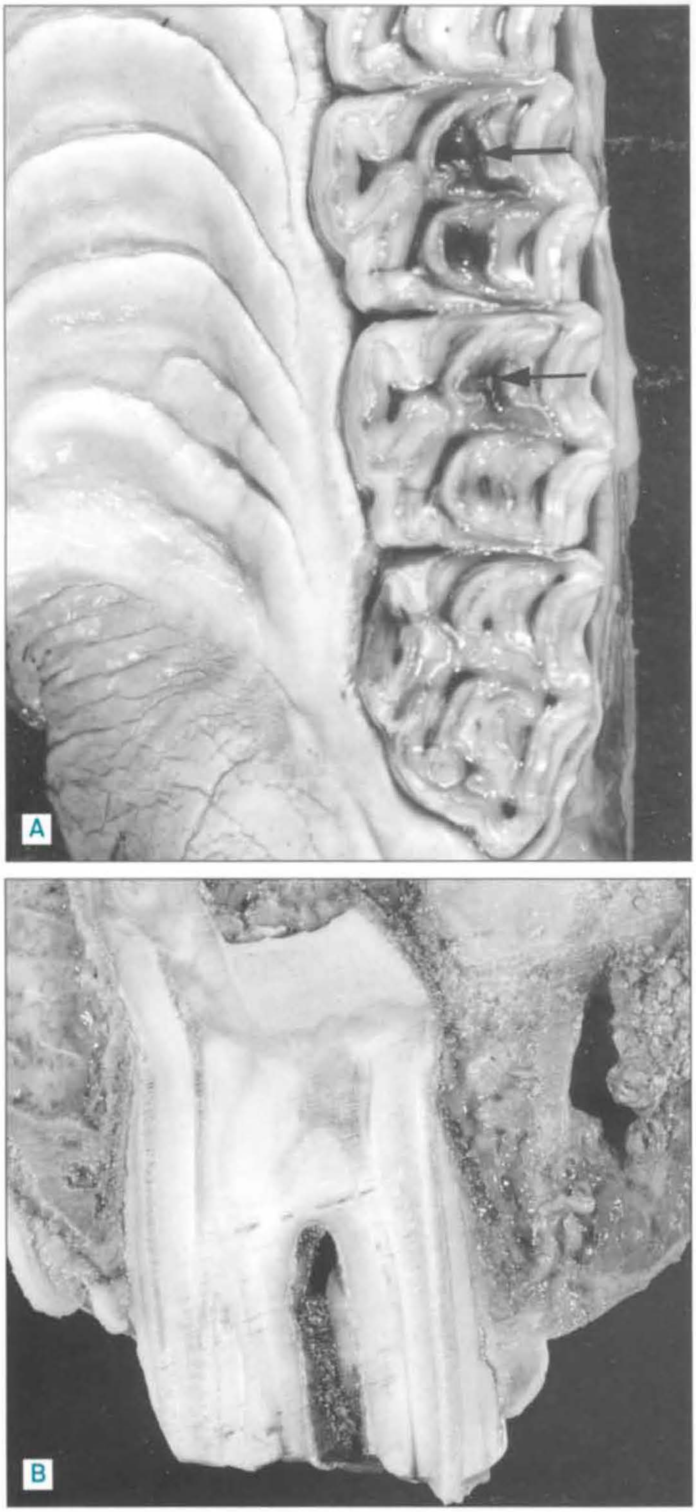

Figure 1.5 A. Infundibular necrosis of first and second maxillary molars (arrows) in a horse. Necrosis is confined to cement lakes. **B.** Section through **(A)** showing black discoloration of infundibulum.

most often of cheek teeth, and increasing in prevalence with age. A reddened swollen area of gingiva or granulation tissue often lies over the lesion, which may be on the labial or buccal aspect, and frequently is painful to touch. The resorptive lesions begin as shallow defects in the cementum, lined by odontoclasts, facing a somewhat disorganized periodontal ligament. They progress into the underlying dentin, and, with time, into the root canal. Either or both coronal and apical extension of lesions may occur. Extension of the process coronally more superficially undermines the enamel, which is resorbed or breaks off, causing destruction or loss of the crown. Extension of the dentinal lesion apically leads to resorption of the root. Remnants of the root may persist, often overgrown by gingiva, following loss of the crown. Conversely, destruction primarily of the root may result in obliteration of the periodontal ligament, resorption of adjacent alveolar bone, but with odontoalveolar ankylosis by reparative hard tissues, retaining the crown in the dental arcade. In addition to the odontoclasts that line the resorbing cementum or dentinal surfaces of the lesion, more advanced defects contain a mixed leukocyte population, macrophages, and disordered granulation tissue. Repair is often superimposed, with cementoblastic or osteoblastic cells producing new mineralized tissue of varied osteoid, bone, cementum, or osteodentin morphology. Pulpitis may occur in the affected root canal, and reparative dentin may be deposited there. The prevalence of such lesions has increased markedly in the past 40 years, suggesting an association with changes in form of diet, but the lesion is idiopathic, with no clear relationship to periodontitis, mechanical trauma, viral infections, nutritional or metabolic disturbances. However, it is suggested that lack of masticatory stimulation may lead to loss of the functional integrity of the periodontal ligament, triggering the lesion.

Pulpitis

The dental pulp is derived from the dental papilla. It is surrounded by odontoblasts and dentin, except at the apical foramen, through which vessels and nerves pass. Pulp is a loose syncytium of stellate fibroblasts, and contains histiocytes and undifferentiated mesenchymal cells. The latter are odontoblastic precursors.

The apical foramen is narrow, and this predisposes to vascular occlusion, ischemic necrosis of the pulp, and death of the tooth. Production of abundant secondary dentin and reparative dentin can cause occlusion, but the usual cause is inflammation. Normally, pulp is the only vascular tissue of the tooth, and, along with the periodontium, the only site of conventional inflammation. *Pulpitis is always related to infection*, the effector bacteria or their products entering through the surface of fractured teeth, carious perforations (especially in teeth with enamel defects), perforations resulting from abnormal wear or trimming, from periodontitis, and possibly hematogenously. In herbivores, in which the pulp is divided by enamel foldings, inflammation is usually limited to one division, and is usually purulent. Very mild pulpitis may heal, but usually it terminates in tooth necrosis, periapical abscessation, perhaps with fistula formation, osteomyelitis, or gangrene as inflammation of the pulp extends to the periodontium and the jaws.

Periapical abscess and osteomyelitis of the jaws are complications of pulpitis that may follow clipping the tusks ("needle teeth") of piglets. Trimming of the incisor teeth of sheep to avoid the effects of broken mouth often exposes the pulp cavity, but the pulpitis that ensues is rarely chronic. The exposed pulp canal is healed in 30–50 days by deposition of reparative dentin and secondary dentin. Similar healing presumably occurs in most piglets. *Maxillary (malar) abscess of dogs* involves the periapical tissues usually of the carnassial tooth, and may cause a discharging sinus beneath the eye. The pathogenesis of the abscess is obscure, but it may be a sequel to crown fractures or to pressure necrosis of periapical tissues. Some chronic inflammations of the pulp are confined to the periodontium and become slowly expansive spherical granulomas about the root apex (*root granulomas*). Occasionally these granulomas are enclosed by an epithelial cyst (*periodontal cyst*) derived from cell rests of Malassez. The epithelium contains plasma cells, and the combination may have a protective role in periapical sepsis.

Periodontal disease

Periodontal disease is the *most common dental disease of dogs and sheep*, and an important problem in other ruminants, horses, and cats. Although there are minor differences among species, in general, periodontal disease *begins as gingivitis associated with subgingival plaque*, and may progress through gingival recession and loss of alveolar bone to chronic periodontitis and exfoliation of teeth.

The gingival sulcus, or crevice, is an invagination formed by the gingiva as it joins with the tooth surface at the time of eruption. Clinically normal animals have a few lymphocytes, plasma cells, and macrophages under the crevicular epithelium of the gingiva, which forms the outer wall of the crevice, and under the junctional epithelium, which is apposed to the enamel of the tooth.

Clinical **gingivitis** is usually initiated by accumulation of plaque in the crevice, but may be associated with impaction of feed, especially seeds, between teeth. Gingivitis is initially characterized by increased numbers of leukocytes and fluid in the gingival crevice, and then by acute exudative inflammation and accumulation of neutrophils, plasma cells, lymphocytes and macrophages in the marginal gingiva. If the disease progresses, marked loss of gingival collagen occurs in a few days, probably related to the activity of prostaglandins and matrix metalloproteinases generated in inflamed tissue, or possibly enzymes from plaque bacteria, such as *Porphyromonas gingivalis*, which also produce enzymes (gingipains) thought to damage junctional epithelium. *Porphyromonas* spp. are implicated as obligate pathogens for canine gingivitis, and as probable participants in feline gingivitis/periodontitis. Grossly the gingiva is red and swollen, due to the hyperemia and edema of inflammation. Acute gingivitis may become quiescent, with lymphocyte aggregations beneath the junctional epithelium. Halitosis is associated with gingivitis in small animals.

Continuation and exacerbations of the inflammation cause apical recession of the tooth–gingiva junction, and resorption of alveolar bone (Fig. 1.6). Alterations in the periodontal flora may be responsible for these exacerbations. A major part of chronic periodontal disease is *resorption of alveolar bone*, which modifies the attachment site of the periodontal ligament. If concomitant bone loss precedes gingival recession, the sulcus is deepened to form a periodontal pocket, which is lined by transformed junctional "pocket" epithelium, and becomes the site of chronic active inflammation. When gingival recession precedes loss of alveolar bone and gingival collagen, pockets do not form, but tooth roots are exposed. In either case, destruction of the periodontium and periodontal ligament, and resorption of alveolar bone, cementum, and root dentin, *lead to exfoliation of teeth*.

Gingivitis is common in **dogs**. Usually it is proliferative, the gingiva being replaced by collagen-poor, highly vascular granulation tissue, which appears as a red, rolled edge next to the tooth. In dogs, gingival pocket formation is quite unpredictable and may be present on one root of a tooth and absent on the other. Bone loss in dogs is often more severe at the bifurcation of two-rooted teeth than in interproximal areas. Resorption of bone is associated with osteitis as the inflammation extends from the periodontium into alveolar bone. In dogs, the premolars and, to a lesser extent, the first molars and central incisors are most severely affected, whereas the second molars and mandibular canines are quite resistant.

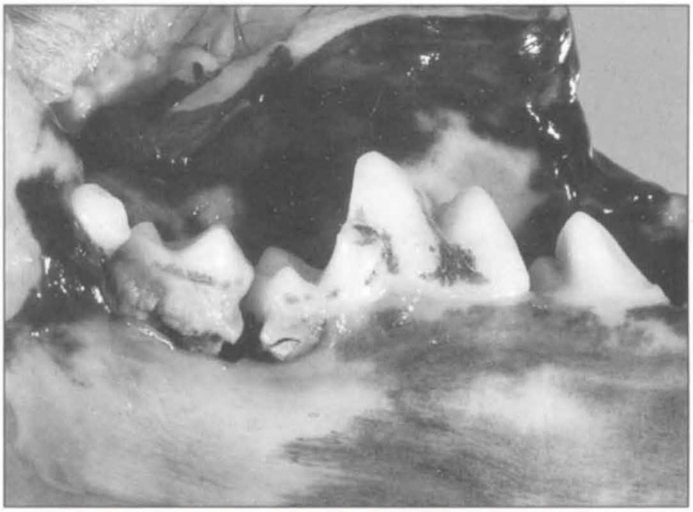

Figure 1.6 Marked gingival recession with exposure of roots of the molar teeth in advanced **periodontal disease** in a dog.

Gingivitis is among the most common veterinary problems in **cats**. In general, it resembles that in dogs.

In **sheep**, periodontal disease may involve all teeth, but the effects are most severe on the incisors, and periodontal disease is a major cause of premature exfoliation. Sheep develop acute gingivitis during tooth eruption, in association with accumulation of subgingival plaque around the tooth. In some sheep, chronic gingivitis involving the lingual aspect of the incisors ensues, and on farms with a high incidence of broken mouth (lengthening of the incisor crown, forward protrusion, and loosening of the teeth), this progresses to chronic active periodontal disease.

Cara inchada (swollen face) is an epidemic periodontitis of **cattle**, formerly common in the west-central part of Brazil. Animals of 2–14 months were mostly affected, and herd prevalences of more than 50% were recorded. When progressive, cara inchada causes loss of teeth, leading to malnutrition. It is associated with dental eruption, and ingestion of forage thought to contain low levels of antibiotics derived from soil actinomycetes that permit colonization of the periodontal space by a variety of gram-negative bacteria, including *Prevotella (Bacteroides) melaninogenica*.

Severe periodontitis and tooth loss are an important part of the syndrome associated with *bovine leukocyte adhesion deficiency*.

The sequelae of suppurative periodontitis are many, being mainly variations on a theme of *osteomyelitis*. The osteomyelitis of actinomycosis is discussed in Vol. 1, Bones and joints. If the mandible is involved, the fistula usually develops on the ventral margin. If the maxillary molars are involved, fistulation may occur into the maxillary sinus. If the premolars are involved, fistulation may develop into the nasal cavity or externally. In dogs, involvement of the canine teeth may produce internal or external fistulae, and involvement of the maxillary carnassials usually produces a fistula beneath the eye, and orbital inflammation. Fistulation may be prevented for some time, or permanently, by ossifying periostitis over the involved bone. Fistulae in the upper jaw tend to be persistent. In the lower jaw, they may heal, usually with extensive deposition of new bone. Occasionally, especially in horses, chronic mild periodontitis may be confined by the periodontium, which is, however, expanded by granulation tissue to form a root granuloma. Under the same circumstances there may be hyperplastic exostosis of the cementum.

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Diseases of the buccal cavity and mucosa

Pigmentation

Melanotic pigmentation is normal and common in most breeds of animals and increases with age. It may be irregular, or the mucosa may be entirely pigmented. Diffuse yellow discoloration may be seen in icterus.

Circulatory disturbances

Examination of the mucous membranes is an essential detail in any clinical or necropsy examination. Pallor may indicate anemia. In cyanosis, the mucosa is dark red-blue. The mucosa are muddy in methemoglobinemia. Acute congestion and cyanosis, associated with ulceration, are common in dogs and sometimes in cats in chronic uremia.

Hemorrhages are indicative of septicemia, and larger ones may accompany local inflammation, trauma, and the hemorrhagic diatheses. Petechiae on the ventral surface of the tongue and frenulum in horses are consistent with equine infectious anemia, or other thrombocytopenic or purpuric conditions. The active hyperemia that gives the diffuse pink coloration to the mucosa in diffuse stomatitis disappears immediately at death, so that at autopsy the inflamed mucosa is disappointingly blanched.

Foreign bodies in the oral cavity

The presence of feed in the mouth of a cadaver is abnormal, except in ruminants, which may eructate and have feed in the caudal pharynx at the time of death. In most cases it is attributable to disease, which results in paralysis of deglutition or semiconsciousness. It is common in horses with encephalitis, leukoencephalomalacia, and hepatic encephalopathy. The food in such cases is usually poorly masticated and readily differentiated from that refluxed postmortem. Bones or other large foreign bodies lodged in the pharynx of cattle suggest pica of phosphorus deficiency. They may cause asphyxiation or pressure necrosis in the wall of the pharynx. Large portions of root crops may also lodge in the pharynx. Dogs often have bones and sticks that tend to be wedged across the palate behind the carnassial teeth.

In dogs, **foreign-body stomatitis** occurs, caused by plant fibers, burrs, or quills (Fig. 1.7). In mild cases, gingivitis surrounds the incisors and canine teeth. Small papules or vesicles and shallow ulcers may be evident on the tongue. Plant fibers may protrude from the lesions. Chronic cases are characterized by *exuberant granulomas* associated with lingual ulceration and gingival hyperplasia with plant fibers deeply embedded in these lesions. Long-haired dogs are especially prone to develop this type of lesion when they attempt to remove plant material that is trapped in their hair coat. The granulomas must be differentiated from neoplasms.

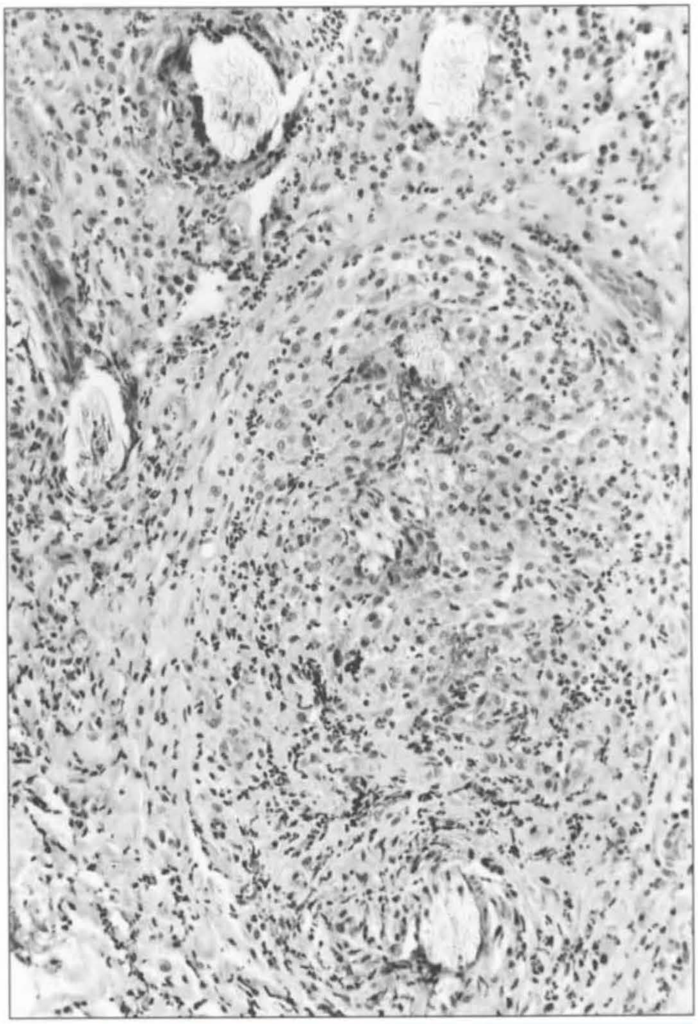

Figure 1.7 Granulomatous reaction to plant material in the tongue of a dog: **foreign-body glossitis** or "burr tongue."

Sharp foreign bodies which cause laceration of the mucosa predispose to necrotic and deep stomatitis. Grass seeds and awns frequently impact between the retracted gingival margin and teeth in periodontitis of ruminants and exacerbate the local initial lesion, perhaps predisposing to the development of osteomyelitis. Horses fed dry triticale hay may develop severe oral ulceration, with masses of awns embedded in the ulcers. The ulcers vary in size from 1 mm to 5 cm in diameter and are mainly located at the junction of the labial and gingival mucosa adjacent to the upper corner incisors, the lingual frenulum, the sublingual folds, the base of the dorsum of the tongue, and the soft palate. Similar lesions in horses have been associated with contamination of hay by foxtail.

Swine have a diverticulum of the pharynx in the caudal wall immediately above the esophagus, and barley awns and other rough plant fibers occasionally lodge here and penetrate the pharynx. This occurs mainly in young pigs, and death follows pharyngeal cellulitis. Similar problems occur in sheep following improper use of drenching guns, and in cattle injured by balling guns.

Inflammation of the oral cavity

Inflammatory processes of the oral cavity may be diffuse (*stomatitis*) or localized predominantly in certain regions to produce, if (1) the pharynx is involved, *pharyngitis*; (2) the tongue, *glossitis*; (3) the gums, *gingivitis*; (4) the tonsils, *tonsillitis* (Fig. 1.8); and (5) the soft palate, *angina*. Lesions limited to the mucosa of the oral cavity are termed *superficial stomatitides*. Processes seated in connective tissues of the mouth, the *deep stomatitides*, are usually sequelae to transient superficial lesions.

Superficial stomatitis

Inflammatory changes may be associated with ingestion of irritating chemicals such as caustic or toxic compounds. An example is paraquat, a herbicide, which may cause severe erosive stomatitis in dogs. Dogs and cats that chew on the plant *Dieffenbachia* may develop oral erosions and ulcers. Electrical burns are occasionally seen in puppies or kittens that chew through electrical wires. It is often not possible to differentiate the cause of diffuse stomatitis, but an attempt to do so is important because it may indicate a systemic disease state. Viral diseases causing stomatitis will be considered in detail in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Inflammatory disease, localized to the buccal cavity, and not part of systemic viral disease, is also common and important. It is generally due to the indigenous bacterial flora. The oral microbiota

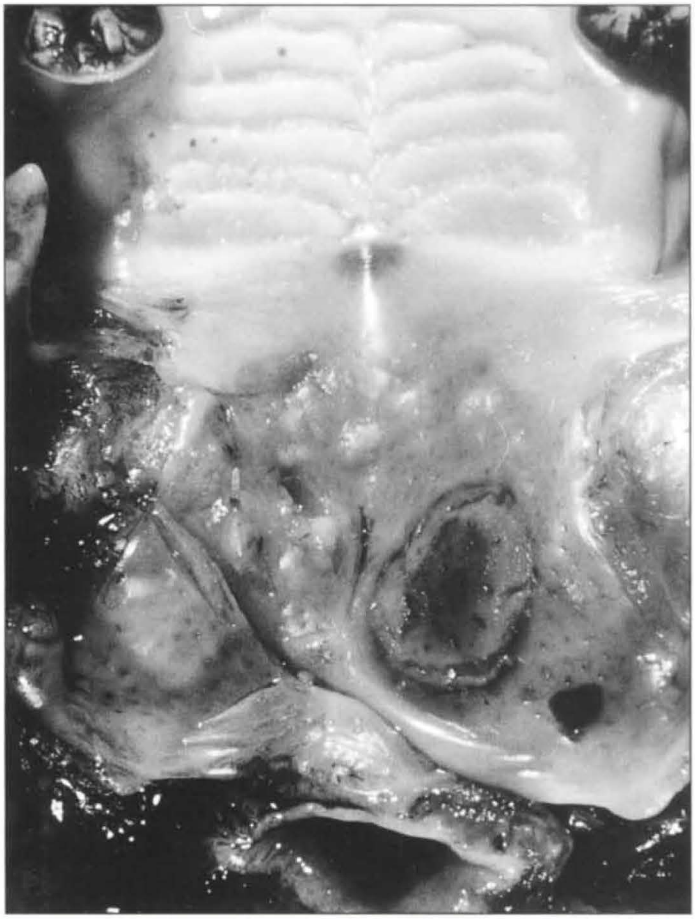

Figure 1.8 Necrotic palatine **tonsillitis** in a pig.

ordinarily contains many microbial species, mainly anaerobes such as *Actinomyces, Fusobacterium*, and spirochetes, which exist in balance with each other and in harmony with the host. Disruption of this microfloral balance may lead to stomatitis. The oral mucosa is quite resistant to microbial invasion for several reasons. These include the squamous mucosal lining; antibacterial constituents of saliva such as lysozyme; immunoglobulins, especially immunoglobulin A (IgA), in oral secretions; and the presence of a rich submucosal vascular network and inflammatory cells. Factors altering the balance of indigenous organisms are not well delineated. Systemic illness, stress, and nutritional and hormonal imbalances may alter the microbial population by altering the amount, composition, and pH of saliva. The integrity of the oral epithelium depends on a high rate of epithelial regeneration to balance loss due to a high rate of abrasion and desquamation. Rapid epithelial replication promotes quick healing of superficial lesions.

The lamina propria of the oral epithelium is well vascularized, but generally dense and relatively inelastic. For this reason, there is little distension of lymphatics and tissue spaces with fluid exudate, and therefore, swelling due to edema is not a significant part of stomatitis involving hard palate and gums, with the exception of the gingival margins.

Catarrhal stomatitis

Catarrhal stomatitis is superficial inflammation of the oral mucosa, which usually involves the caudal fauces and may be associated with mild gingivitis. It is a common nonspecific lesion, which often develops in the course of debilitating diseases. The mucosae are hyperemic, and the loose texture of the submucosa in the fauces permits development of edema. The swelling is aggravated by edema and hyperplasia of the abundant lymphoid tissues of the soft palate, tonsil, and pharyngeal mucosa. The epithelium accumulates, producing a dull gray mucosal surface. Palatine glands produce excessive mucus. Catarrhal stomatitis resolves with the return of normal oral function.

Thrush, or **oral candidiasis**, occurs most commonly in foals, pigs, and dogs. It involves the proliferation of yeasts and hyphae in the parakeratotic superficial layers of the oral epithelium. It appears grossly as patchy pale-gray pseudomembranous material on the oral mucosa and back of the tongue, and probably reflects alterations in epithelial turnover and oral flora (see section on Infectious and parasitic diseases of the alimentary tract, later in this chapter). Mold products of *Stachybotrys alternans* cause catarrhal and necrotizing stomatitis, as well as colitis, if feed is contaminated. Gingivitis and ulceration of the oral mucosa may rarely be associated with infection due to *Nocardia* spp. in dogs.

Vesicular stomatitides

Stomatitis characterized by the formation of *vesicles* occurs in most species of domestic animals. The vesicles develop as accumulations of serous fluid within the epithelium or between the epithelium and the lamina propria. These may coalesce to form *bullae*, and the elevated epithelium is easily rubbed off during chewing to leave raw eroded patches with bits of epithelium adherent. *The transition from vesicle to erosion occurs rapidly*, so that, in individual animals, vesicles may not be seen. This is especially so in dogs and cats because the oral mucosa is very thin. Because the basal epithelium or basement membrane remains intact, regeneration and healing are complete in a few days unless the local lesions are complicated by bacterial or mycotic infections. However, foci of previous erosion may be identifiable for some months by their slight depression and lack of pigmentation.

Traditionally, vesicular stomatitides in animals were associated with *viral infections*, and these are still important causes. Vesicular stomatitis and foot-and-mouth disease are associated initially with vesicle formation; however, rinderpest, bovine viral diarrhea and malignant catarrhal fever produce sharply demarcated erosive/ulcerative lesions without initial vesicle formation. *Oral erosions and ulcers in horses, ruminants, and swine should be regarded as indicating one of the vesicular diseases to which the species is susceptible, until proven otherwise* (see section on Infectious and parasitic diseases of the alimentary tract, later in this chapter). Sunburn, photoirritation associated with grazing on celery and related crops, and lesions associated with parvovirus infection in swine may cause lesions of the snout resembling vesicular diseases. Animals exposed to irritant chemicals in feed or bedding may develop vesicles and erosions of the face and oral cavity, for example, toxicity in horses and dogs associated with irritant quassinoids found in wood shavings derived from Simaroubaceae species.

Bullous immune skin diseases are recognized with increased frequency, especially in dogs, and some of these have severe oral lesions, which will be described here (see also Vol. 1, Skin and appendages).

Pemphigus vulgaris is a severe, acute or chronic, vesiculobullous autoimmune disease mediated by autoantibodies to the desmosome protein desmoglein 3. It is characterized by acantholysis of the epidermis, which results in formation of flaccid bullae and erosions involving mainly mucocutaneous junctions, oral mucosa and, to a lesser extent, skin. Clinically affected dogs and cats have erosions/ulcerations of the oral mucosa and may drool. The oral lesions are generally more prominent than, and precede, the skin lesions. They are most obvious on the dorsal surface of the tongue, which is bright red, with a few scattered pink raised areas representing islands of normal mucosa. The lesions vary greatly in severity and distribution, although the hard palate is often severely ulcerated. Bullae are rarely seen in the oral cavity, because they ulcerate rapidly.

Microscopically, the earliest lesion consists of *suprabasilar acantholysis*, which is followed by the formation of clefts. These lead to ulceration of the mucosa. The basal cells of the epidermis remain attached to the basement membrane and form a so-called "row of tombstones." A few neutrophils and eosinophils may infiltrate the epithelium. There is a variable lymphocytic and plasmacytic lichenoid reaction in the propria. The presence of suprabasilar clefts and bullae due to acantholysis is considered to be diagnostic of pemphigus vulgaris. However, extensive erosion and ulceration of the mucosa and secondary bacterial infections frequently obscure these clefts and bullae. Several biopsies from different areas of the oral mucosa may be required to demonstrate the characteristic lesions. A presumptive histologic diagnosis should be supported by direct immunofluorescence tests that show autoantibodies (usually IgG) and complement in the intercellular spaces of stratified squamous epithelium.

Bullous pemphigoid is a term that has been applied generically to superficial autoimmune vesiculobullous or ulcerative disease of mucous membranes (including the oral mucosa) and skin, characterized by subepithelial clefting; acantholysis is not a feature. It has been reported in humans, horses, dogs, and cats. It is now recognized that there is a *complex of autoimmune subepidermal blistering diseases*, varying in their target antigen, clinical manifestations, and prognosis. Those involving the oral cavity of cats and dogs include bullous pemphigoid, mucous membrane (cicatricial) pemphigoid, and canine epidermolysis bullosa acquisita. All are characterized by circulation of IgG and IgE autoantibodies against specific basement membrane antigens. The characteristic microscopic lesions of all are transient subepidermal blisters, which may contain fibrinocellular exudates with variable numbers of neutrophils and eosinophils. Diagnosis of each is confirmed by the detection of circulating antibody directed at appropriate antigens, using enzyme-linked immunosorbent assay (ELISA) or immunofluorescent tests, or by identification of immunoglobulin fixed to basement membrane. Paraneoplastic bullous stomatitis characterized by subepithelial clefting has been reported in a horse with a hemangiosarcoma.

Bullous pemphigoid is retained as the name for the second most common autoimmune subepidermal blistering disease in dogs and cats. The lesions mainly occur on haired skin, and a minority of cases involve the mucocutaneous junctions or mucosae, including the mouth, which is affected about a third of the time. Microscopically, there is a rich neutrophilic and eosinophilic dermal infiltrate adjacent to and sometimes spilling into the subepidermal bullae. The targets for the autoimmune response are epitopes on canine collagen XVII, a component of the basement membrane.

Mucous membrane pemphigoid is the most common autoimmune subepidermal blistering disease of small animals, causing about half of all cases. Adults are predominantly affected, and among dogs, German Shepherds are overrepresented. Typically, subepidermal vesicles in mucous membrane pemphigoid are associated with a relatively sparse inflammatory infiltrate. The antigen targeted by autoantibodies is collagen XVII or laminin-5, and basement membrane-fixed immunoglobulin is detected by direct immunofluorescent or immunoperoxidase staining of formalin-fixed paraffin-embedded tissue.

Epidermolysis bullosa acquisita is a rare disease of dogs, representing about 25% of cases of autoimmune basement membrane diseases, with a poor prognosis. The associated autoantibodies are directed against collagen VII. The lesions are most common on skin, and advance rapidly to erosions at points of friction, but the oral epithelium often sloughs extensively. Intact subepidermal vesicles may contain no inflammatory cells, or neutrophils may accumulate at the basement membrane, sometimes forming subepidermal microabscesses. The results of serum or cutaneous immunofluorescent tests resemble those in bullous pemphigoid or mucous membrane pemphigoid, but the autoantibodies may be recognized binding to the lower part of the basement membrane.

The oral lesions of pemphigus vulgaris and of the subepidermal blistering diseases must be differentiated from lesions due to trauma, toxic epidermal necrolysis, drug eruptions, chronic uremia, mucocutaneous candidiasis, and lymphoreticular malignancies.

Feline calicivirus causes mainly a respiratory infection in cats. The disease is complicated by *lingual and oropharyngeal ulcers*, which start out as vesicles. They are 5–10 mm in diameter, smooth, and well demarcated from the surrounding normal mucosa. They occur mainly on the rostrodorsal and lateral surfaces of the tongue and each side of the midline of the hard palate. The palatine lesions are apparently more severe in cats fed dry food. Microscopically, the earliest lesions consist of foci of pyknotic cells in the stratum corneum and superficial stratum spinosum. They progress to foci of necrosis with vesicle formation and subsequent erosion and ulceration of the mucosa. Regeneration of the oral mucosa in the ulcerated areas generally occurs within 10–12 days. A single layer of squamous epithelial cells extends from the margins of the ulcer beneath a layer of exudate. Active viral replication also takes place in the tonsillar crypt epithelial cells, and virus may be recovered from these areas for weeks postinfection. Viral inclusions have not been observed in oral epithelial cells. The virus is isolated from a high percentage of cats with chronic stomatitis. Concurrent infection with *Felid herpesvirus 1* may also occur.

Erosive and ulcerative stomatitides

This form of stomatitis is characterized by local epithelial defects of the oral mucosa and nasolabium and is usually associated with acute diffuse stomatitis and pharyngitis. *Erosions* are circumscribed areas of loss of epithelium which leave the stratum germinativum and basement membrane more or less intact. They are usually associated with acute inflammation in the underlying propria. The erosions vary in size and shape. Although they are often a nonspecific development in a wide variety of conditions, they are also an essential part of a number of important diseases. They heal cleanly and quickly, but if secondarily infected or complicated, may develop into ulcers.

Ulcers, in contrast to erosions, are deeper deficiencies that extend into the substantia propria. They too vary greatly in size and shape;

the edges tend to be elevated and ragged, and when they heal, it is with scar formation.

The causes of ulcerative stomatitis are in general those of erosive stomatitis. There are, however, a number of recognized syndromes and specific diseases in which the predominant change is ulceration. *Phenylbutazone* intoxication in horses may cause oral ulcers in concert with ulcers of the stomach, intestine, and colon; the syndrome is discussed with ischemic diseases of the gut (p. 99).

Feline ulcerative stomatitis and glossitis, or **lymphocytic-plasmacytic stomatitis**, is an ulcerative and chronic inflammation of the mucosa of the fauces, the angle of the jaws and, less commonly, the hard palate, gingiva, and tongue. Microscopically, there is chronic active inflammation of the oral mucosa and submucosal connective tissues, dominated by lymphocytes and plasma cells. The syndrome is more common in older cats and may accompany periodontitis. The cause is unknown, but is probably multifactorial, involving imbalance in the oral microbial flora, with predominance of gram-negative anaerobes and spirochetes. Some have reported the isolation of *Feline calicivirus* and *Felid herpesvirus 1* from more cats with lesions of chronic stomatitis compared to those without, but the role of these viruses in the etiology is unresolved. *Feline calicivirus* can persist as a sequel to previous disease episodes and in the face of prior vaccination. *Feline leukemia virus* and *Feline immunodeficiency virus* may predispose some cats to chronic stomatitis because of their immunosuppressive effects, but evidence of infection is not consistently found.

Feline plasma cell gingivitis-pharyngitis or **feline chronic gingivostomatitis** is characterized by raised erythematous, proliferative lesions, mainly in the glossopalatine arches, extending caudally to the palatopharyngeal arch and rostrally to the gingiva. The lesions may involve Eustachian tubes and also can affect the conjunctiva. Histologically, the mucosa is hyperplastic and frequently ulcerated, with a marked submucosal inflammatory cell reaction, mainly plasmacytes, including binucleate cells and cells containing Russell bodies. Neutrophils, lymphocytes, and histiocytes are scattered among the plasma cells. Affected cats have elevated polyclonal serum gamma-globulin levels. The polyclonal gammopathy and the plasmacytic, lymphocytic reaction are suggestive of an immune-mediated lesion, and differentiation from mucosally associated lymphoid neoplasia may be challenging. The etiology and the relationship of this syndrome to feline ulcerative stomatitis and glossitis (see above) are unclear; the two syndromes probably form a continuum, although the plasma cell predominance and the hypergammaglobulinemia attributed to plasma cell gingivitis-stomatitis are distinguishing. Similar stomatitis occurs in dogs.

Eosinophilic ulcer (eosinophilic granuloma, lick granuloma, labial ulcer, rodent ulcer) is a chronic, superficial ulcerative lesion of the mucocutaneous junctions of the lips, and, to a lesser extent, the oral mucosa and skin, in cats of all ages. The cause is unknown but the lesions may respond to corticosteroid, oral progestagens, cryosurgery, or radiation therapy, although recurrences are common. Typically, well-demarcated, red-brown, shallow ulcers, often with elevated margins, occur on the *upper lip on either side of the midline*. They are usually a few millimeters wide and several centimeters long. Occasionally, ulcers are present elsewhere in the mouth, such as on the gums, palate, pharynx, and tongue. Skin lesions are located in those areas that are frequently licked, such as the neck, lumbar area, and abdomen. Microscopically, the squamous mucosa is ulcerated, with large areas of necrosis of the underlying connective tissues and accompanied by a marked inflammatory cell reaction. The cellular reaction consists predominantly of neutrophils at the periphery of the ulcers, with a mainly mononuclear cell reaction (plasma cells and mast cells) in the propria, eosinophils and histiocytes being only occasionally seen. In some cases, the eosinophils and mast cells may predominate, but this difference may be a reflection only of the evolution of the lesion.

Eosinophilic ulcer is one of the three different types of lesions that have been associated with the so-called *eosinophilic granuloma complex*. The other two conditions, eosinophilic plaque and linear granuloma, cause mainly skin lesions, which are different clinically and morphologically from eosinophilic ulcer (see Vol. 1, Skin and appendages).

Oral eosinophilic granuloma (linear granuloma) in dogs occurs as a familial disease in young Siberian Huskies. Sporadic cases have been reported in other breeds, especially Cavalier King Charles Spaniels. Affected dogs have single or multiple firm, often ulcerated, raised plaques, which are covered by yellow-brown exudate, on the *lateral or ventral surfaces of the tongue*. Lesions on the soft palate are less common, and here they tend to be oval to circular ulcers with slightly elevated borders. Cytologic preparations made from scrapings of the oral lesions show many eosinophils, a few neutrophils, occasional macrophages, and epithelial cells.

Microscopically, *foci of collagenolysis* (necrobiosis) in the mid and deep zones of the lingual submucosa are surrounded by a mainly histiocytic granulomatous inflammatory reaction, with giant cells, lymphocytes, plasma cells, and mast cells. *Eosinophils* are a constant feature, but their numbers vary from few to many. The lesions are identical to those seen in linear granuloma of cats.

The cause is unknown, although the morphology of the lesion and the response to corticosteroid therapy suggest hypersensitivity. The familial tendency in Siberian huskies indicates that hereditary factors are involved. *Eosinophilic granuloma must be differentiated from oral mast cell tumors*, which also affect the tongue in dogs. Necrobiosis of collagen fibers is often a feature of mast cell tumors; however, in mastocytoma, the characteristic mixture of mast cells and eosinophils infiltrates the tongue and connective tissues more diffusely. The mast cells may be in various stages of degranulation, and inflammation is minimal or absent in mast cell tumors.

Horses with **eosinophilic epitheliotropic disease** (see Vol. 1, Skin and appendages and section on Eosinophilic enteritis in cats and horses, below) may also have eosinophilic stomatitis and lingual ulceration.

Feline viral rhinotracheitis is a common upper respiratory tract infection of cats caused by *Felid herpesvirus 1* (see Vol. 2, Respiratory system). This virus may cause ulcerative lesions in the mouth, especially on the tongue. Rarely, oral and skin ulcers may occur, without evidence of concurrent respiratory tract infection. Microscopically, foci of cytoplasmic vacuolation in squamous epithelium evolve into areas of necrosis and ulceration. The ulcers are often covered by a layer of fibrinocellular exudate. Herpetic inclusions may be present in epithelial cells at the periphery of the ulcers.

Uremia associated with chronic renal disease often causes *fetid ulcerative stomatitis* in dogs, and less commonly in cats. Dirty gray-brown ulcers occur on the gums, lateral surface, and margin of the tongue, and on the inner surface of the lips and cheeks, often adjacent to the openings of salivary ducts. The margins of the ulcers are swollen and hyperemic.

The pathogenesis of the oral lesions in uremia is still poorly understood. Elevations in blood and salivary urea in combination with urease-producing bacteria, normally present in the oral microflora, may generate ammonia from salivary urea. Ammonia has a caustic effect on the oral mucous membranes. Experimental antibody production against urease renders some intestinal bacteria nonpathogenic and prevents uremic colitis, providing evidence of the importance of urease. However, there is poor correlation between the levels of blood urea and the development of uremic stomatitis, suggesting other important factors. Uremic vasculitis and impaired microvascular perfusion may contribute to the pathogenesis of uremic stomatitis.

Salivary glucose levels may be elevated in dogs and cats with *diabetes mellitus*, resulting in an imbalance of the oral microflora and predisposing to chronic gingivitis in diabetic animals.

Ulcerative glossitis and stomatitis in **swine** is commonly part of **exudative epidermitis** (greasy pig disease) of preweaning pigs (see Vol. 1, Skin and appendages). In addition to the characteristic skin lesions, about a third of the piglets may develop ulcers on the dorsum of the tongue. Erosions and ulcers of the hard palate occur in a small number of piglets. Microscopically, there is ulceration of the squamous mucosa with coagulative necrosis, and vesicle and pustule formation in the superficial epithelium over the rete pegs. A pleocellular inflammatory reaction is evident in the connective tissue below the ulcers.

Oral erosions and ulcers have been reported in pigs with *congenital swinepox*. Microscopically, there is swelling and degeneration of squamous epithelial cells with numerous eosinophilic intracytoplasmic viral inclusions. The central areas of the lesions are necrotic. There is a mixture of lymphocytes, neutrophils, eosinophils, and histiocytes in the submucosa. Poxvirus may be demonstrated by electron microscopic examination of the lesions.

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Deep stomatitides

Lesions of the oral mucosa may permit the entry of pyogenic bacteria, often normal oral flora, into the connective tissues of the submucosa and muscle. Purulent inflammation or cellulitis may develop in the lips, tongue, cheek, soft palate, and pharynx. Abscesses may form and may fistulate through the mucosa or skin. Abscesses in the wall of the pharynx may result from necrosis of retropharyngeal lymph nodes. Necrotic stomatitis with simple necrosis of the epithelium and lamina propria may be produced by thermal or chemical agencies, but in animals, it is usually caused by *Fusobacterium necrophorum* and other anaerobes.

Oral necrobacillosis *Fusobacterium necrophorum* is the principal cause of **oral necrobacillosis** or **necrotic stomatitis** in animals. It is also associated with necrotizing lesions elsewhere in the upper and lower alimentary tract, and liver. Wherever it occurs, it is usually a *secondary invader* following previous mucosal damage. The organism produces a variety of exo- and endotoxins; among the latter are leukocidins, hemolysins, and a cytoplasmic toxin, all of which probably enhance the necrotizing ability of the organism. Once established in a suitable focus, *F. necrophorum* proliferates, causing extensive coagulative necrosis.

The best-known form of necrobacillary stomatitis is **calf diphtheria**, an acute necrotizing ulcerative inflammation of the buccal and pharyngeal mucosa, and also of the laryngeal mucosa (**necrotic laryngitis**). The predisposing lesions may include trauma, infectious bovine rhinotracheitis, and papular stomatitis. Necrosis of palatine and pharyngeal tonsils may be seen. The incidence of diphtheria in slaughtered beef cattle may be as high as 1.4%. The same syndrome is rather common in housed lambs as a complication of contagious ecthyma. The infection may also be initiated in the gums about erupting teeth in any species, and by the trauma produced in baby pigs by removing the needle teeth (Fig. 1.9A). It is frequently fatal in young animals, in which extension often occurs to other organs. In adults, oral necrobacillosis tends to remain localized to the oral cavity, where it may complicate vesicular and ulcerative stomatitides. It is not unusual, however, for the infection to spread down the alimentary tract.

The early lesions are large, well-demarcated, yellow-gray, dry areas of necrosis, surrounded by a zone of hyperemia (Fig. 1.9B). They are found on the sides or dorsal groove of the tongue, on the cheeks, gums, palate, and pharynx, especially the recesses beside the larynx. Primary foci may occur in the laryngeal ventricles. Death may be associated with asphyxia. The necrotic tissue projects slightly above the normal surface and is friable but adherent and is not easily detached. In time it may slough and leave deep ulcers, which may heal by granulation. The necrotic tissues are histologically structureless and are surrounded at first by a zone of vascular reaction, later by a dense but narrow rim of leukocytes, and finally by thick encapsulating granulation tissue. The bacteria are arranged in long filaments, particularly at the advancing edge of the lesions. The submucosal extension of the lesions may take them deeply into the underlying soft tissues and bone.

Spread from the oral foci occurs down the trachea (causing aspiration pneumonia), down the esophagus, and via blood vessels. Death may occur acutely in septicemia with only multiple small serosal hemorrhages as evidence, or metastases may occur in other tissue. Venous drainage from the face to the vascular sinuses of the meninges may lead to pituitary and cerebral abscessation.

Fusobacterium necrophorum has also been associated with a *syndrome of necrotic stomatitis, enteritis, and granulocytopenia in calves.* Affected calves have nonregenerative anemia, leukopenia, neutropenia, hypoproteinemia, and increased fibrinogen levels. In addition to the characteristic oral lesions, there is marked depletion of lymphoid tissues and necrotic enteritis. *Fusobacterium* organisms are present in large numbers in a variety of organs, including the bone marrow. Possibly very virulent strains of *F. necrophorum* produce enough leukotoxins, especially in immunodeficient calves, to suppress bone marrow activity. However, granulocytopenia associated with exposure to bracken or treatment with nitrofurans may also facilitate the development of calf diphtheria.

A gross diagnosis of oral necrobacillosis is ordinarily possible, but may be confirmed by a smear from the margin of the lesion. The organism is difficult to cultivate because it is a *strict anaerobe*.

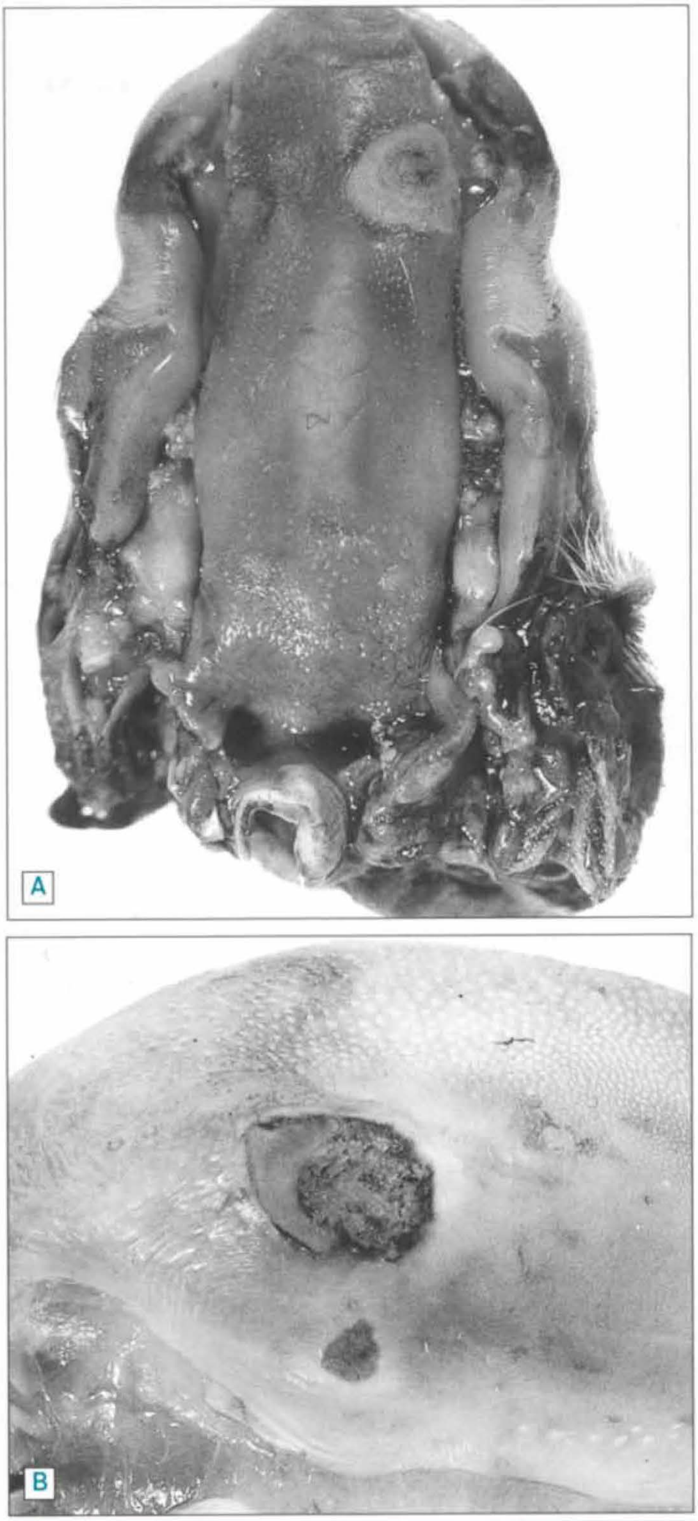

Figure 1.9 A. Necrotic **glossitis and stomatitis** in a pig, subsequent to trauma by needle teeth and *Fusobacterium necrophorum* infection. **B. Oral necrobacillosis** in a calf.

Noma Noma (cancrum oris) is a rapidly spreading *pseudomembranous or gangrenous stomatitis*; it is not caused by a specific pathogen but is associated with tissue invasion by the normal oral flora, particularly fusobacteria and spirochetes. The predisposing factors are unknown, but they are probably nonspecific and associated with mucosal trauma and debility. The disease, which is occasionally observed in horses, dogs, and monkeys, is in many respects similar to oral necrobacillosis. In the lesions, the spirochetes can be found in large numbers at the advancing margins as well as in peripheral viable tissue. In the deep layers of necrosis, fusiforms predominate, and toward the surface, there is a variety of other organisms, chiefly cocci. The initial lesion is a small tattered ulcer of the cheek or gum, which spreads rapidly and may involve much of the buccal surface of the gums and the mucosa of the cheek. It is intensely fetid and consists of a dirty necrotic pseudomembrane surrounded by a zone

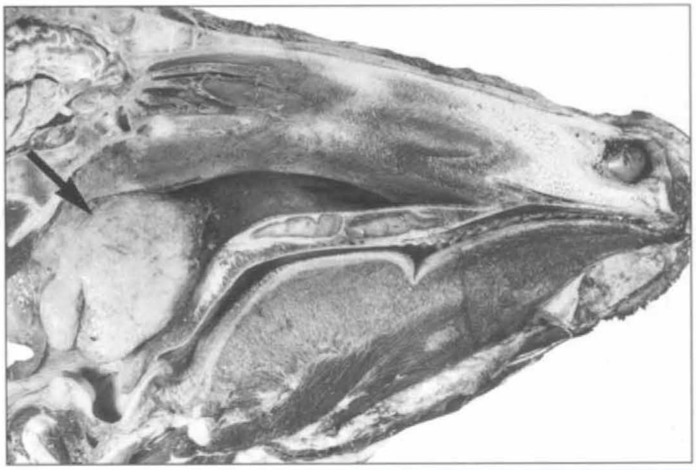

Figure 1.10 Pharyngeal actinomycosis in a cow. Fleshy mass in pharynx (arrow) that resembles actinobacillosis.

of acute inflammation. The necrotic tissue may slough to leave deep ulcers; the cheek may be perforated to leave a gaping defect, or gangrene may supervene.

Actinobacillosis This is a disease mainly of cattle, sheep, and pigs, leading to stomatitis, glossitis, lymphadenitis, and sometimes pyogranulomas in the wall of the forestomachs of ruminants. *Actinobacillus lignieresii* is part of the normal oral flora, and in cattle is associated with deep stomatitis. When introduced into the submucosa, it causes pyogranulomatous inflammatory foci centered on club colonies containing gram-negative coccobacilli. Morphologically similar lesions may be caused by a variety of organisms (Fig. 1.10). *Arcanobacterium pyogenes* may be isolated from lingual ulcers and granulomas in lambs. Microscopic examination of these lesions reveals well-demarcated submucosal granulomas with plant fibers in the center, surrounded by a marked neutrophilic reaction. The organisms most likely gain entry after the mucosa is damaged by hard fibrous plant fibers from the weed lambsleeve sage (*Salvia reflexa*), present in the bedding. *Actinomyces bovis*, a gram-positive filamentous organism, causes pyogranulomatous mandibular and maxillary osteomyelitis in cattle, and mastitis in sows. Staphylococci may cause pyogranulomatous lesions (botryomycosis) in any species, especially mastitis in sows. Less common causes of similar microscopic lesions include *Nocardia* and the various agents associated with mycetomas (see Vol. 1, Skin and appendages).

Actinobacillosis is typically a *disease of soft tissue*, spreading as a *lymphangitis* and usually involving the regional lymph nodes. This distinguishes it from actinomycosis, which causes bone lesions. The tongue is often involved in actinobacillosis, and the chronic condition produces clinical "*wooden tongue.*" Entry of actinobacilli to the tongue may be gained through traumatic erosions along its sides, but often the primary lesion is in the lingual groove. Here, trapped grass seeds and awns may provoke the initial trauma. Lesions elsewhere in the soft tissue of the mouth may be attributed to disruption of the mucosa by similar types of insults, and eruption of, or abrasion by, teeth.

Microscopically, the lesion is a *pyogranuloma*, centered on a mass of coccobacilli, surrounded by radiating eosinophilic clubs made up of immune complexes (Fig. 1.11). The *club colonies*, in turn, are surrounded by variable numbers of neutrophils, and are invested by macrophages or giant cells. Lymphocytic and plasmacytic infiltrates are present in the surrounding reactive fibrous stroma or granulation tissue. An individual inflammatory focus appears grossly as a nodular, firm, pale, fibrous mass a few millimeters to 1 cm in diameter, containing in the center minute yellow "sulfur" granules, which are the club colonies.

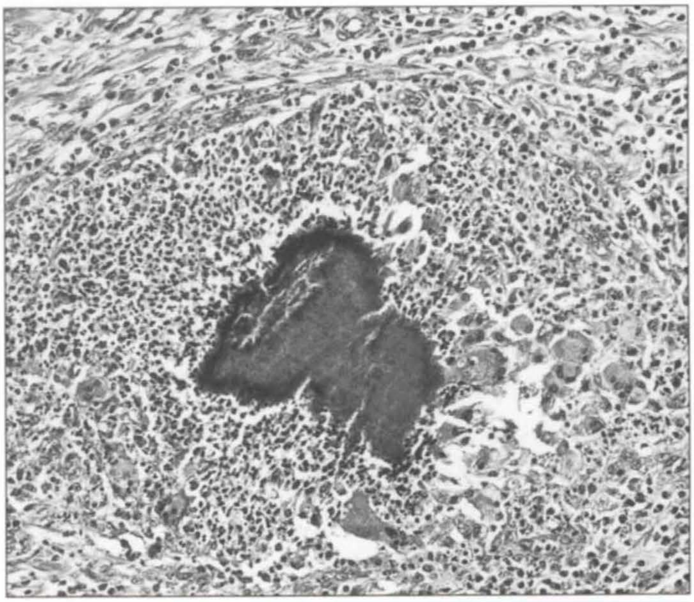

Figure 1.11 Pyogranulomatous focus containing club colony of *Actinobacillus lignieresii* in **actinobacillosis** in a cow.

Lymphogenous spread is common. Affected lymphatics are thickened, and nodules are distributed along their course. This distribution is best seen beneath the mucosa of the dorsum and the lateral surface of the tongue and often can be traced through to the pharyngeal lymphoid tissue (Fig. 1.12). Some of these more superficial nodules erode the overlying epithelium, and coalescence may produce quite large ulcers. The most common form of lingual actinobacillosis consists of granulation tissue in which are embedded many small abscesses surrounded by a dense connective tissue capsule. The epithelium overlying these large granulomas may be intact or ulcerated. Diffuse sclerosing actinobacillosis of the tongue (wooden tongue) is firm, because of extensive proliferation of connective tissue, which replaces the muscle fibers. Granulomatous nodules are sparsely scattered in the fibrous stroma.

Although actinobacillosis in cattle is best known as a disease of the tongue, the infection may occur in any of the exposed soft tissues, especially those of mouth and esophagus; occasionally it involves the wall of the forestomachs, the skin, or the lungs. Lesions in these sites resemble those described in the tongue.

Actinobacillosis causes *regional lymphadenitis*. The cut surface of the node reveals small, soft yellow or orange granulomatous masses, which project somewhat above the capsular contour and which contain "sulfur" granules. There is also sclerosing inflammation of the surrounding tissues, which may cause adhesion to overlying skin or mucous membranes. The retropharyngeal and submaxillary nodes are most often affected, as well as the lymphoid tissues of the submucosa of the soft palate and pharynx. Involvement of the pharynx and the retropharyngeal lymph nodes may cause dyspnea and dysphagia.

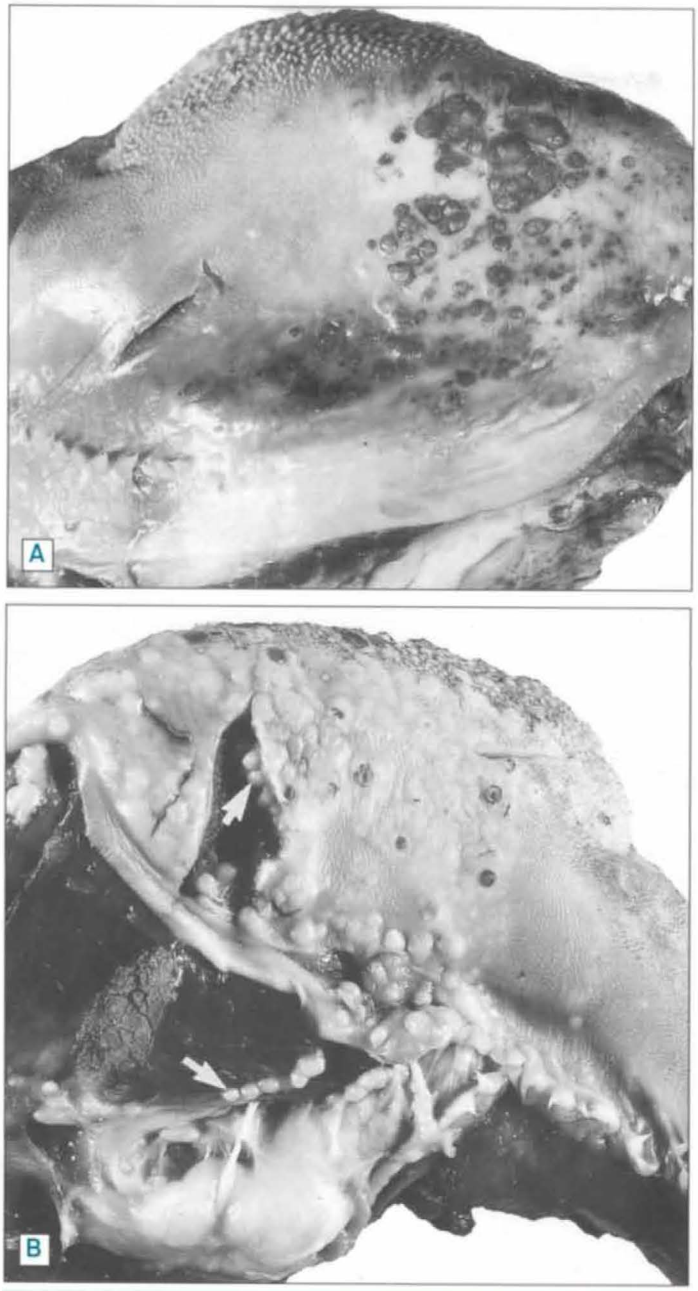

Figure 1.12 Actinobacillosis in a cow. **A.** Granulomas bulging on lateral surface of tongue. **B.** Granulomatous nodules (arrows) along course of lymphatics.

Oral actinobacillosis in *swine* causes lesions similar to those in cattle, including glossitis. Actinobacillosis may also occur sporadically or as outbreaks in *sheep*, but in this species the tongue seems to be exempt. The characteristic lesions in sheep occur in the subcutaneous tissue of the head, especially of the cheeks, nose, lips, and submaxillary and throat regions, and on the nasal turbinates. They may also occur on the soft palate and pharynx as complications of wounds received at drenching. The organism has been isolated from a *horse* with a greatly enlarged tongue.

Oral dermatophilosis of cats This is caused by *Dermatophilus congolensis*, a bacterium that commonly causes exudative dermatitis in a wide variety of species (see Vol. 1, Skin and appendages). In cats, the organism is uncommonly associated with oral granulomas, especially affecting the tongue and tonsillar crypt. Large numbers of gram-positive, filamentous, branching organisms, with longitudinal and transverse divisions, may be demonstrated in the necrotic centers of submucosal granulomas. The organisms probably enter through damaged mucosa. The lesion must be differentiated from the more common squamous cell carcinomas of the tongue. In cattle, *cutaneous streptothricosis* involving the muzzle may extend into the oral cavity.

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Parasitic diseases of the oral cavity

These are of minor significance. Sarcosporidiosis and cysticercosis occur in the striated muscles of the tongue and produce the same lesions as they do elsewhere (see Vol. 1, Muscle and tendon). *Trichinella spiralis* may be found in muscles of the tongue and mastication. *Gongylonema* spp. are found in the mucosal lining of the tongue, especially in swine allowed to graze, and less commonly in cattle and sheep. They evoke little or no inflammation of the mucosa, but a mild-to-moderate lymphocytic and eosinophilic reaction may be evident in the underlying lamina propria.

The larvae of *Gasterophilus* spp. in the horse and of *Oestrus ovis* in sheep are found attached to the pharyngeal mucosa, where they may cause focal ulceration and excite mild inflammation. The larvae of *G. nasalis* migrate from the lips and invade the gums around and between the teeth and behind the alveolar processes to cause small suppurating pockets.

Halicephalobus gingivalis has been observed in proliferative granulomas of the mandibular gingiva as well as bone, skin, kidney, central nervous system, mammary gland, and eye of horses.

Parasitic leeches of the genus *Myxobdella* may infect the oral cavity of cattle and humans, leading to minor dysfunction and hemorrhage. Known colloquially as the "tongue worm," the pentastome *Linguatula serrata* infects the nasal cavity and nasopharynx of dogs and humans.

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Diseases of the tonsils

The tonsils are normally prominent and protrude slightly from the tonsillar fossa in the dog and cat. In these species tonsils are compact, fusiform structures with a finely stippled, pale pink mucosal surface. In swine, tonsillar lymphoid tissue is concentrated in the caudal soft palate, and forms a plaque of raised and pitted thickened mucosa. In horses tonsillar tissues are dispersed over pharyngeal and epiglottic mucosal surfaces and consist of a series of plaques and nodules in the mucosa. In other species, the tonsils are diffuse. They are subject to the usual conditions involving lymphoid tissue, and undergo progressive atrophy with age.

Tonsils are constantly exposed to antigenic stimuli, by virtue of their function in immune surveillance in the oropharynx. As a result, they are a site of functional lymphoid hyperplasia and physiologic inflammation. Many bacteria native to the oropharyngeal mucosa probably inhabit the tonsillar crypts. Consequently they may serve as portal of entry for a variety of bacterial agents and viruses. A significant percentage of swine may carry *Erysipelothrix rhusiopathiae* and *Salmonella* spp. in the tonsils, and *Streptococcus suis* type 2 can be cultured from tonsils.

Desquamated epithelium, bacteria, necrotic debris, and neutrophils may normally be present to moderate degree in *tonsillar crypts*. This reaction is exaggerated, and may be associated with ulceration of the crypt and suppuration of involuted tonsillar lymphoid tissue, in certain bacterial infections, causing the formation of visible yellow nodules. Conditions in which such bacterial tonsillitis may occur include pasteurellosis in sheep and pigs, *Actinomyces* and *Tonsillophilus* in tonsils of swine, and necrobacillosis in all species (see Fig. 1.8). In porcine anthrax, hemorrhagic necrotizing tonsillitis is reported.

Scrapie-associated prion protein is consistently detected by immunohistochemistry in the center of primary and secondary lymphoid follicles of sheep with clinical and histologic lesions of scrapie, and this may be a useful antemortem diagnostic approach in this and other prion-associated diseases of animals.

The tonsil is the site of primary virus multiplication in pseudorabies (Aujeszky's disease) in swine. The virus causes necrotizing tonsillitis, and intranuclear viral inclusions may be seen in cryptal epithelial cells.

Involution of B-dependent tonsillar lymphoid follicles due to viral lymphocytolysis may occur during the early phase of a number of lymphotropic diseases such as feline panleukopenia, canine parvoviral enteritis, canine distemper, bovine viral diarrhea, rinderpest, and swine vesicular disease. Numerous karyorrhectic nuclei, lymphocyte depletion, and prominent histiocytes signal such damage. In distemper, involuted tonsils are susceptible to secondary bacterial invasion and suppuration. Compensatory lymphoid hyperplasia may occur during the postviremic phase of parvoviral infections and distemper. The tonsil often appears to be the preferred organ of viral persistence in a symptomatic carrier infected with *Feline calicivirus*. In *Porcine circovirus 2* infections (postweaning multisystemic wasting syndrome), involution of lymphoid tissue and accumulation of macrophages and giant cells containing basophilic cytoplasmic circoviral inclusions occur, as they do in other lymphoid organs.

Inflammatory polyps of the tonsils occur infrequently in old dogs. They are flat to pedunculated rubbery masses (1–3 cm in length), attached to the tonsillar sinus, with a smooth to verrucous surface. Histologically, the lesions are composed of mature, sometimes edematous, highly vascularized connective tissue that is covered by squamous epithelial cells. Aggregates of lymphocytes and plasma cells are scattered throughout the connective tissue. The lesions are probably the result of chronic recurrent episodes of subclinical tonsillitis. They are usually asymptomatic but may cause gagging and retching.

Tumors, especially lymphosarcomas and squamous cell carcinomas, are common causes of tonsillar enlargement in dogs. Bilaterally enlarged and soft, pale swollen tonsils are usual with lymphosarcoma, and these may become clinically obvious prior to the development of peripheral lymphadenopathy.

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Neoplastic and like lesions of the oral cavity

Some of the lumps, bumps, and cysts that develop in and around the oral cavity are malformations, hyperplasias, and neoplasias originating in tooth germ or teeth. Malformations of dental origin have been considered previously under developmental anomalies of teeth. Gingival masses of all types, many of which are of tooth germ origin, are common in dogs, but occur much less frequently in other species. In this discussion we follow revisions in the nomenclature of lesions that formerly were included with epulis, placing those that clearly are of dental origin with other such tumors, and consigning the remainder to hyperplastic and reactive lesions. Prognostic implications for such masses have not changed, despite some reclassification.

Epulis *is a generic clinical term for tumor-like masses on the gingiva.* Epulis has, in the past, been used to describe developmental, inflammatory, and hyperplastic lesions, as well as several neoplastic lesions of tooth germ origin, which are numerous in dogs, and develop occasionally in cats. It has no specific pathologic connotation, and preferably, *the term should not be used in a morphologic diagnosis, except in the context of fibromatous epulis of periodontal ligament origin*, discussed with Tumors of dental tissues, below.

In evaluating lesions of the gums and jaws, a potentially confusing element is the nature of the epithelium and of the hard tissues that are often found in the stroma. In interpreting these, it is important to recall the inductive pattern of tooth development. Epithelial remnants from tooth organogenesis occur commonly in the gingiva and the periodontium, and nests of dental epithelium can be found incidentally in any proliferative lesion in this region. Characteristically, dental epithelial cells show reverse polarity, their nuclei being located at the apex of the cell, distal to the basement membrane. Often the cell nests are surrounded by a relatively clear halo that contains a few strands of collagenous tissue. Also, gingival epithelium commonly proliferates extravagantly in response to irritation, and may form complex plexiform patterns. Unless there is neoplastic change; the epithelial component is not of primary significance. Similarly, many stromal masses in the regions of the jaws include mineralized tissues or amorphous, apparently mineralizable tissues, which may develop by metaplasia of fibrous tissue or by de novo cellular differentiation. It is often difficult to determine whether this is bone, cementum, dentin, or their precursors, but in any case this is prognostically irrelevant, as is the abundance of such tissues themselves. *In describing lesions of the gums and jaws, the adjective "peripheral" refers to an origin in the gingiva, while "central" implies a deeper origin in the jaw bone.*

The oral and pharyngeal mucosa is a common site of malignant tumors in the dog, as it is in cats. Malignant oral tumors account for ~6% of all canine and ~7% of all feline neoplasms. Large domestic animals have a low prevalence of malignant oral tumors, and when they do occur in ungulates, they are usually relatively nonaggressive.

There are regional geographic differences in the prevalence of certain oral tumors, especially in dogs and cattle. Such differences may be related to the distribution of carcinogens in the environment, and warrant further investigation from the point of view of comparative oncology.

The most common types of malignant oral tumors in dogs and cats are squamous cell carcinomas, fibrosarcomas, and, in dogs only, malignant melanomas. They vary somewhat in their behavior depending on the species in which they occur, and the type and location of the tumor. Dogs and cats with mean ages over about 6 or 7 years of age are mainly affected. The canine population with fibrosarcomas has a mean age of about 8 years. Melanomas in dogs tend to occur in a notably older age class than the other tumors, with the mean of the distribution at about 11–12 years. Typical clinical signs, determined by the location, behavior, and stage of the tumor, are drooling, halitosis, pain, dysphagia, anorexia, weight loss, loose teeth, mandibular fractures, oral bleeding, noisy respiration, coughing, and a change in voice.

Boxers, Cocker Spaniels, German Shorthaired Pointers, Weimaraners, and Golden Retrievers apparently have a higher prevalence of malignant oral tumors than other breeds of dogs, while Dachshunds and Beagles apparently have a very low prevalence. The male-to-female ratio has been reported to be as high as 6:1 for melanomas, 3:1 for tonsillar carcinomas, and 2:1 for fibrosarcomas. The ratios have to be interpreted with some caution since they may be partly related to differences in the ratio of males to females in the general population.

All types of malignant oral tumors in dogs and cats tend to progress rapidly, and regardless of the type of malignancy, the prognosis is generally poor, unless the lesion is completely resected early in its clinical course, prior to metastasis.

Reactive and hyperplastic lesions

Pyogenic granuloma *is a bright red or blue mass on the gums of dogs.* It is composed of extremely vascular granulation tissue covered by gingival epithelium, and is not genuinely granulomatous, despite the name. It ulcerates and bleeds easily. Pyogenic granuloma is probably an exaggerated response to local irritation and infection. In horses, exuberant granulation tissue of periodontal origin sometimes develops at the site of extracted teeth to produce a tumor-like mass in the dental arcade. Epithelial remnants and proliferative bone may be associated with the granulation tissue in such cases.

Peripheral giant cell granuloma (*giant cell epulis*) occurs in dogs and cats as *gingival masses*, often red, that may be smooth and sessile, or pedunculated. Very occasionally, similar lesions, described as *central giant cell granulomas*, occur more deeply in the jaw. The gingival epithelium is hyperplastic or ulcerated and extends deeply into the underlying mass, which is well vascularized and often contains hemosiderin-laden cells. Characteristic of the tumor are numerous giant cells, with multiple central nuclei and abundant eosinophilic cytoplasm, which are located in a densely cellular stroma. Foci of hard tissue may be present. Giant cell granulomas are regarded as hyperplastic or granulomatous lesions, and have occurred at the site of tooth extraction.

Fibrous hyperplasia (formerly part of fibrous or fibromatous epulis, *gingival hypertrophy*) is common in dogs, and may be generalized and diffuse, or focal, localized to one or more teeth. When focal, it is a discrete tumor-like mass and, whether local or general, may cover part of the crown (Fig. 1.13). The stromal component of the mass consists of mature fibrous tissue with low cellular density. Foci of hard tissue and epithelial nests may be present. Local enlargement may be promoted by chronic, probably painless, inflammation. It may be associated with periodontal disease, in which case it is often accompanied by a more-or-less extensive chronic or chronic-active inflammatory

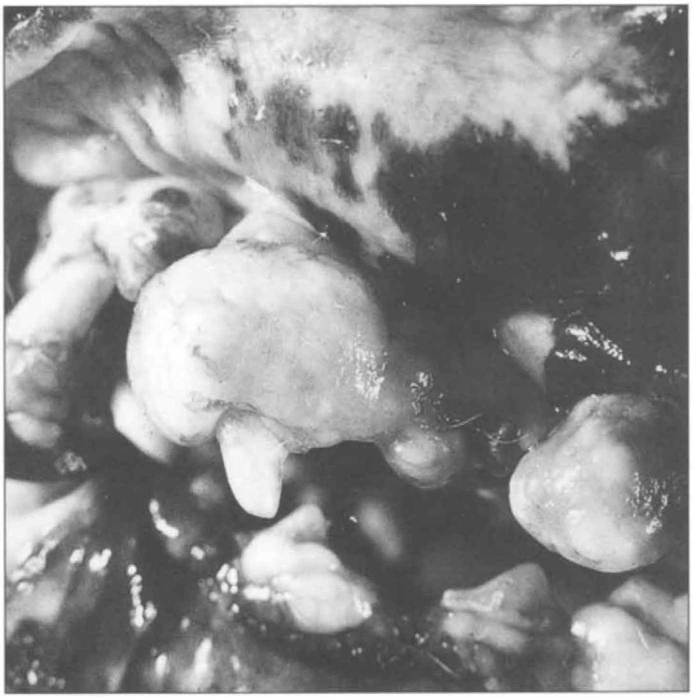

Figure 1.13 Fibrous hyperplasia in a Boxer dog.

reaction. Characteristically there is a band of mononuclear cells, predominantly plasma cells, in the gingival stroma adjacent to the epithelium, which is often hyperplastic, sometimes markedly so. Where the epithelium is ulcerated, neutrophils may be prominent, marginating in vessels, in the stroma, and migrating through the epithelium.

Diffuse fibrous hyperplasia is familial in Boxer dogs, and a more severe overgrowth, termed *hyperplastic gingivitis*, occurs as a recessive inherited disease in Swedish silver foxes. In the foxes, both jaws are affected and the lesion causes displacement and malalignment of teeth, eventually reaching such proportions that the mouth cannot be closed.

Oral papillomatosis

These benign epithelial tumors ("warts") in dogs, cats, and cattle are caused by papillomaviruses.

In **dogs**, they are caused by *Canine oral papillomavirus* (COPV), in genus *Lambdapapillomavirus*, and mainly occur in young animals, although older dogs in close contact may become infected. The lesions are unsightly, and may be traumatized and bleed, but only unusually heavy infections that interfere with swallowing or respiration cause other than cosmetic problems.

The virus is host- and fairly site-specific. Infection of basal epithelium of the squamous mucosa stimulates increased mitosis, while viral genome replicates in the differentiating keratinocytes of the stratum spinosum and granulosum, which degenerate, with viral assembly and expression in superficial squamous layers. The incubation period is generally about 2 months. Spontaneous recovery, mediated by CD4 and CD8 T cells, usually occurs within 1–2 more months, followed by *solid antibody-mediated immunity to reinfection.* The warts first develop as single, smooth papular elevations which are pale or the color of the mucosa. These lesions progress to multiple, proliferative cauliflower-like, firm, white to gray growths (Fig. 1.14). They develop on the lips, gingiva, buccal mucosa, tongue, palate, and walls of the pharynx. The esophagus and the skin of the muzzle also may be involved. Persistent and progressive infections, which may also involve haired skin remote from the mouth, are attributable to immunocompromise of the host, rather than an unusually virulent strain of virus.

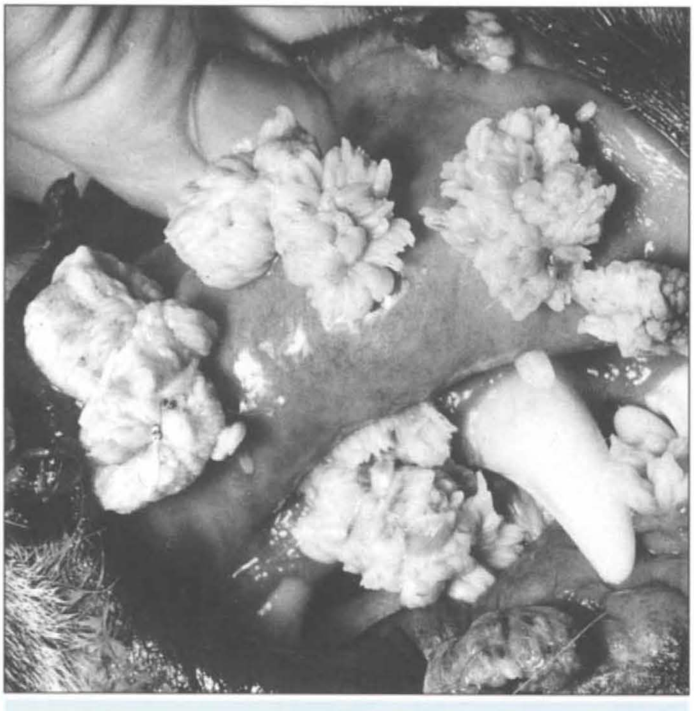

Figure 1.14 Oral papillomatosis in a dog. (Courtesy of WR Kelly.)

The *microscopic structure* of fully developed lesions is typically verrucous, with very thick keratinizing squamous epithelium covering thin, branching, often pedunculated cores of vascularized proprial papillae. There is also marked acanthosis. Individual epithelial cells or small groups in the upper areas of the stratum spinosum and granulosum degenerate, developing koilocytic atypia, with clear cytoplasm around or adjacent to an often somewhat condensed nucleus, and large cytoplasmic granules or inclusions, with loss of intercellular bridges. *Basophilic intranuclear viral inclusions* may be found in cells in the outer spinose layers, but they can be rare. Regressing lesions are infiltrated by moderate numbers of T lymphocytes. Viral antigen can be demonstrated in nuclei of koilocytes and in intranuclear, but not cytoplasmic, inclusions by specific immunohistochemistry. Electron microscopy reveals intranuclear 50–55 nm viral particles, sometimes forming paracrystalline arrays.

Malignant transformation of infected papillomavirus-infected epithelium to produce squamous cell carcinomas can apparently occur, but its importance in the etiology of canine oral squamous cell carcinomas is unclear. Injections of live COPV vaccine into the skin of dogs may result in a spectrum of lesions, including epidermal hyperplasia, papillomas, epidermal cysts, basal cell tumors, and squamous cell carcinomas, and some cutaneous squamous cell carcinomas in dogs contain COPV genetic material.

In domestic and wild species of **cats**, oral and cutaneous papillomas and fibropapillomas are very occasionally identified. In the oral cavity they are most common on the ventral aspect of the tongue, and typically they are multifocal, pink, and more sessile than verrucous. Microscopically, raised areas of thickened mucosa are comprised of hyperplastic keratinocytes with occasional fibrovascular stalks. Typical degenerative changes are seen in infected keratinocytes, and papillomavirus antigen can be detected by immunohistochemistry in a high proportion of such cases.

In **cattle**, oral papillomas, caused by *Bovine papillomavirus type 4* (a strain of *Bovine papillomavirus 3*, genus *Xipapillomavirus*), can occur commonly in endemic areas. Their morphology and distribution in the oral cavity are similar to the papillomas of dogs, but since they infect the esophagus and forestomachs extensively, they are considered more fully in the section on neoplasia of the esophagus and forestomachs, below.

Tumors of dental tissues

Tumors of dental tissues are classified either as epithelial (with or without odontogenic mesenchyme) or mesenchymal neoplasms, and malformations. Tooth development provides the classic example of epithelial–mesenchymal interactions, and inductive influences appear to be active in some tumors. Familiarity with dental embryology assists an understanding of the origin, appearance, and classification of the tumors discussed below.

Tumors of dental tissues, other than fibromatous epulis of periodontal ligament origin, and canine acanthomatous ameloblastomas (acanthomatous epulides), *are rare. All are nonmalignant, and infiltrative or expansive.* However, their location predetermines destruction of bone and displacement of teeth, and they are difficult to remove.

Ameloblastoma is a slowly progressive invasive but nonmetastatic tumor, consisting of proliferating odontogenic epithelium in a fibrous stroma. The proportions of epithelium and stroma vary widely. The terms "adamantinoma" and "enameloblastoma" are obsolete synonyms. These tumors are more common in dogs than in cats and horses, and seem to occur more often in the mandible than the maxilla. Tumors formerly described as ameloblastomas in young cattle are now considered to be ameloblastic fibromas, discussed below.

Ameloblastomas occur at any age. They originate from the dental lamina, the outer enamel epithelium, the dental follicle around retained unerupted teeth, the oral epithelium, or odontogenic epithelium in extraoral locations. They are *predominantly intraosseous* and, because of their location, they may destroy large amounts of bone, and extend into the oral cavity or sinuses. Large tumors may undergo central degeneration and become cystic.

Odontogenic epithelium, which is the criterion for diagnosis of ameloblastoma, may form any one of several patterns (Fig. 1.15). Follicular and plexiform patterns are most common, consisting of discrete islands, or irregular masses and strands of epithelium, respectively. Many tumors contain both patterns. In both, central masses of cells, often resembling the stellate reticulum of the enamel organ, but sometimes with an acanthomatous appearance, are surrounded by a single layer of cuboidal or columnar cells that resemble inner enamel epithelium. Cysts originate from degeneration of the centers of epithelial islands, or from stromal degeneration. Small cysts may coalesce to form grossly evident cavities. Ameloblastomas occasionally undergo keratinization. In some, stromal osteoid and bone develop, which may be an epithelial inductive effect.

Amyloid-producing odontogenic tumors are rare tumors that occur as unencapsulated gingival masses in dogs and cats. They are characterized by dental epithelium, with deposits of amyloid, and sometimes prominent trabeculae of osteoid/dentinoid. The epithelium may be arranged in strands, nests, or masses and may contain areas of stellate reticulum. Occasionally there is mineralization of epithelium or stroma, in the form of small nodules or amorphous masses. The amyloid may also be nodular or amorphous and may be intermingled with the mineral. Distinguished histologically, but not prognostically, from ameloblastomas by the presence of amyloid, these tumors are cured by surgical excision.

Acanthomatous ameloblastoma (acanthomatous epulis, peripheral ameloblastoma, adamantinoma, basal cell carcinoma) *is a common tumor arising from the mucosal epithelium or epithelial rests of the gingiva of dogs*; it does not occur in other species. It is easily confused clinically with the benign stromal masses found in the canine gingiva, but *it often behaves aggressively*, invading local alveolar bone (Fig. 1.16), causing tooth loss, and recurring in many animals following conservative treatment. Grossly they are gray-pink papillary to sessile gingival masses in the vicinity of the alveolus

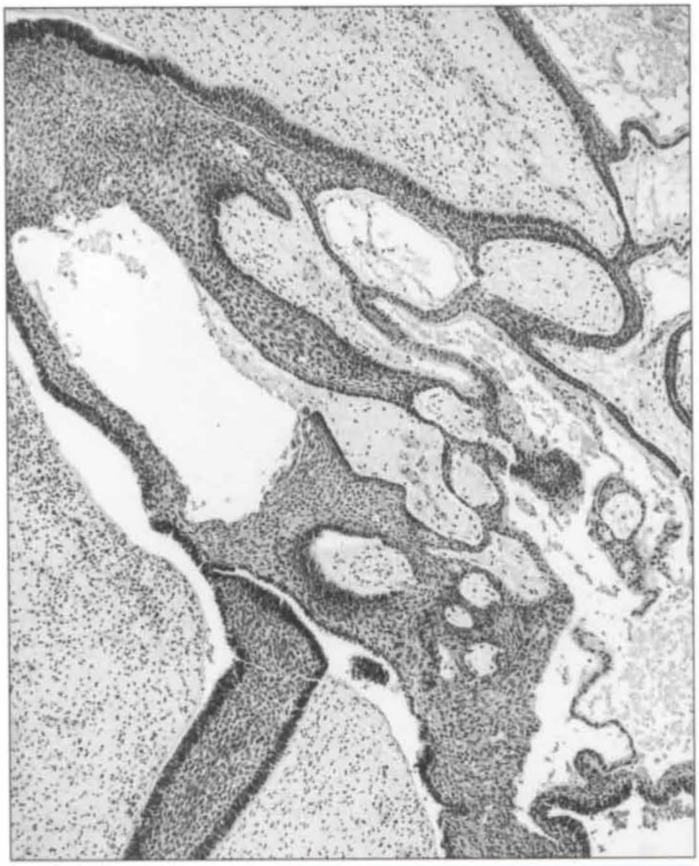

Figure 1.15 Ameloblastoma. Tall enamel-type epithelium and cyst formation.

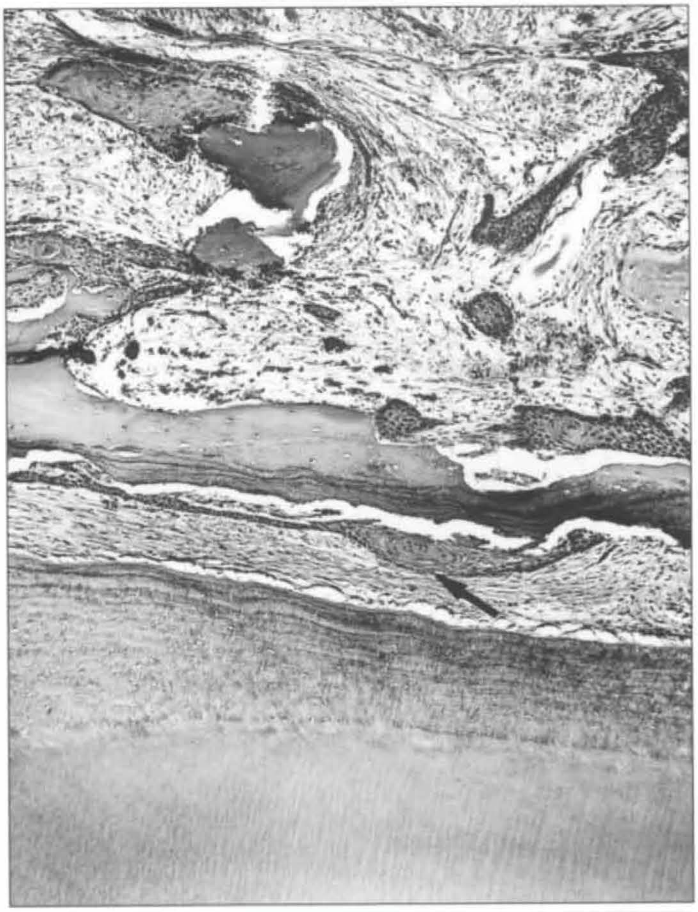

Figure 1.16 Acanthomatous ameloblastoma invading periodontal ligament (arrow) and resorbing alveolar bone (center). Cementum and dentin (below).

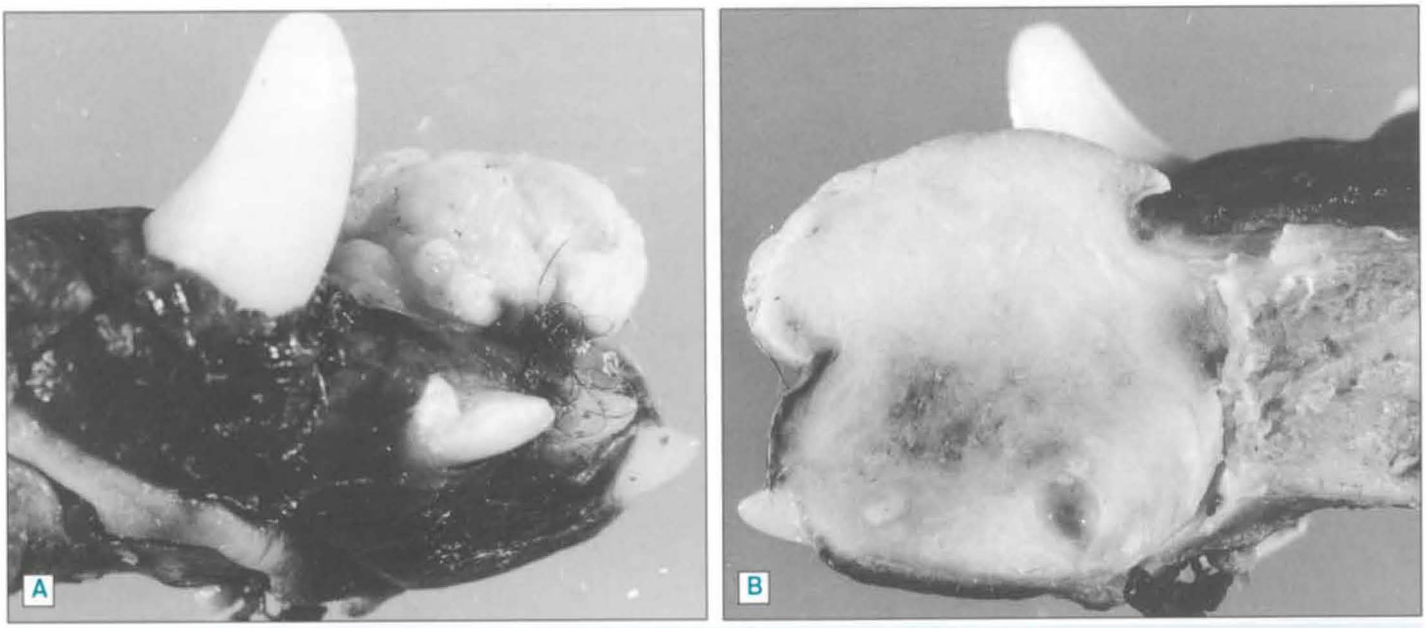

Figure 1.17 Acanthomatous ameloblastoma in a dog. **A.** Lateral view. Exophytic tumor that is also invading and destroying mandibular symphysis and displacing teeth. **B.** Medial view, with the symphysis split.

(Fig. 1.17). Histologically the tumor is composed of sheets, nodules, and anastomosing cords of polyhedral epithelium bordered by a row of cuboidal to columnar cells with round to oval nuclei and moderate amounts of cytoplasm. *Prominent intercellular bridges*, the defining feature of the lesion reflected in the name, are present between many of the central polyhedral cells. In some tumors, intraepithelial cysts contain vacuolated, otherwise structureless, eosinophilic material and cellular debris (Fig. 1.18). These cysts probably form from degenerate epithelium. Small masses of hard tissue may develop in the stroma between the epithelium.

It is sometimes difficult to distinguish an acanthomatous ameloblastoma developing in the gingiva from the epithelial proliferation accompanying gingivitis. Besides the usual prudence required when identifying neoplastic cells in areas of inflammation, other criteria for differentiation include the *predominance of broad sheets of epithelium and the mitotic figures sometimes present in the acanthomatous ameloblastoma*. Evidence of invasion of bone is clearly relevant to the diagnosis, care being taken to differentiate alveolar bone from stromal hard tissues.

Some acanthomatous ameloblastomas show characteristics of squamous cell carcinoma when they escape the influence of the subgingival stroma and invade bone, but metastases are not recorded, and complete surgical resection is curative. Squamous cell carcinoma, and occasionally fibrosarcoma or osteosarcoma, have been reported from the site of irradiated acanthomatous epulides, several months to many years after treatment.

Among the rare tumors containing both dental epithelial and mesenchymal tissue, the **ameloblastic fibroma** (fibroameloblastoma) occurs in horses, and dogs, often in young animals, but is the *commonest odontogenic tumor of cattle*, where it is found in the vicinity of the mandibular incisors of calves. It consists of cords of epithelium resembling dental lamina, intimately associated with spindle cells resembling dental pulp. Where the epithelium is well differentiated, resembling the enamel organ, it may be associated with

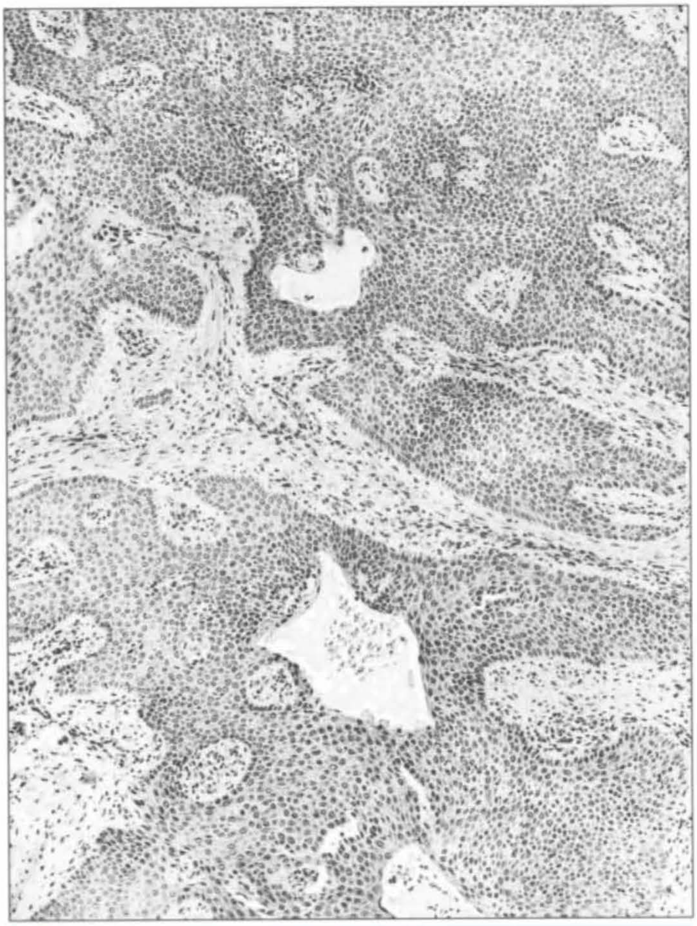

Figure 1.18 Acanthomatous ameloblastoma in a dog, illustrating invasive cords of cells.

enamel and dentin; such variants are termed **ameloblastic fibro-odontomas**, in contrast to ameloblastic fibromas, which contain no mineralized tissue. These tumors generally behave like ameloblastomas, although malignancy has been reported once in a dog.

Feline inductive odontogenic tumor is a rare tumor specific to cats, but the commonest odontogenic tumor in kittens, where they occur as osteolytic masses in the rostral maxilla, causing tooth loss or facial distortion. They are distinguished by the presence of aggregates of neoplastic dental epithelium partially enveloping somewhat spherical masses of cellular mesenchyme, forming structures resembling the cap stage of tooth development, when dental epithelium invests the dental papilla but odontoblasts have not yet differentiated.

Complex and compound odontomas are malformations in which fully differentiated dental tissues are represented. In *complex odontomas*, the tissues are disorganized, and in dogs, cementum is not evident, though it is in horses. In *compound odontomas*, tooth-like structures (denticles) are present, each containing enamel, dentin, cementum, and pulp, arranged as in a normal tooth. Distinction of the two may be arbitrary. Separate areas of ameloblastic epithelium are not present in complex and compound odontomas. A tumor that contains ameloblastic epithelium and separate areas of complex or compound odontoma is an **odontoameloblastoma**.

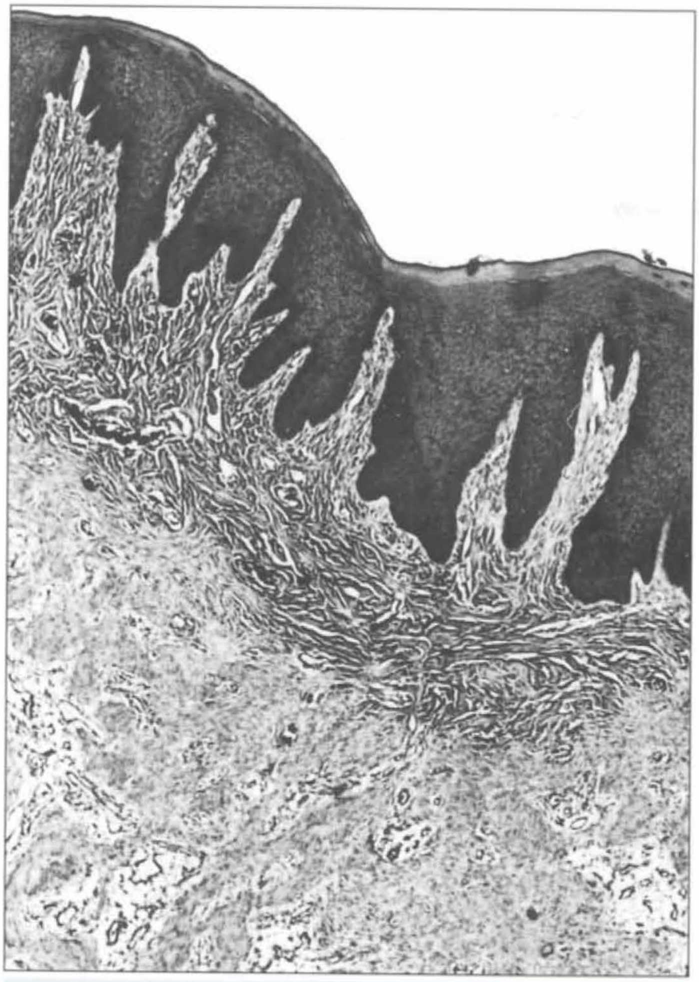

Figure 1.19 Fibromatous epulis of periodontal ligament origin in a dog. Note demarcation between tumor (below) and gingival stroma.

Odontomas are usually located in the mandibular or maxillary arch, and are less rare in cattle and horses than in other species. They are connected with existing dental alveoli and are detected when they bulge the contour of the host bone or interfere with other teeth. They may originate from normally or abnormally placed dental anlagen as well as from a supernumerary dental anlage.

Fibromatous epulis of periodontal ligament origin *is a peripheral odontogenic neoplasm, indistinguishable clinically from fibrous hyperplasia, and most common in dogs.* The distinction is academic since prognosis following surgical removal is good for both lesions. Fibromatous epulis of periodontal ligament origin is a neoplasm, however, comparable to the rare human tumor called peripheral odontogenic fibroma. Similar tumors occur rarely in cats. Fibromatous epulides of periodontal ligament origin are firm to hard, gray-pink neoplasms, often projecting from between the teeth or from the hard palate near the teeth. Often they are mushroom-shaped and have a smooth, lobulated surface. They are attached to the periosteum, and may displace teeth mechanically, but do not invade bone. Fibromatous epulides are most common around the carnassial and canine teeth of brachycephalic breeds, and usually occur in dogs over about 3 years of age.

Fibromatous epulides of periodontal ligament origin are *stromal tumors* consisting of interwoven bundles of cellular fibroblastic tissue that is often well vascularized (Fig. 1.19). They are distinguished from fibrous hyperplasia by the immaturity of this stroma, which is comprised of small stellate to fusiform fibroblasts dispersed in a dense collagen matrix, and by their tendency to contain less inflammatory tissue and more hard tissue, which may resemble bone, cementum or dentin. About 60% contain branching cords or islands of epithelium which may be continuous with the gingiva, or originate in epithelial rests of Malassez. The epithelium is bordered by a row of cuboidal cells somewhat resembling odontogenic epithelium (Fig. 1.20). Though

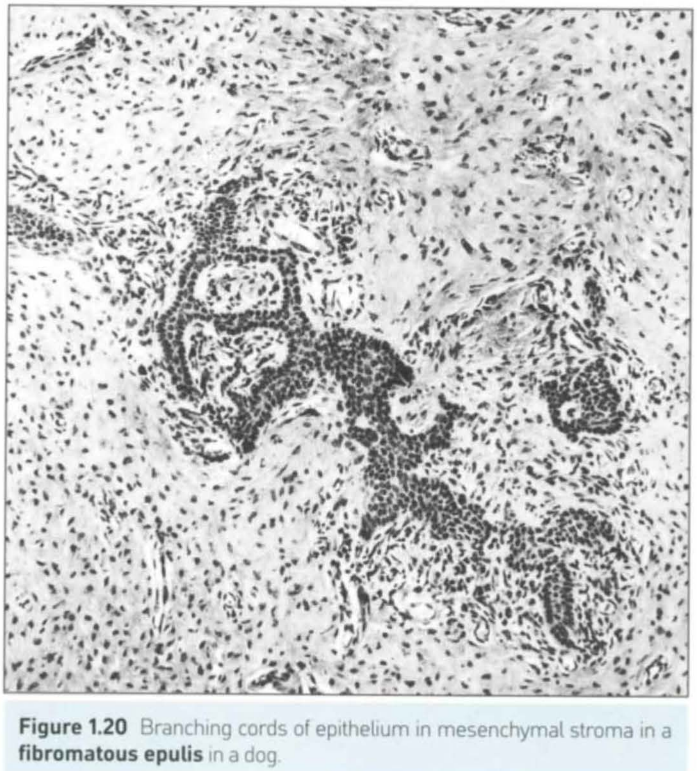

Figure 1.20 Branching cords of epithelium in mesenchymal stroma in a **fibromatous epulis** in a dog.

such masses are sometimes categorized as fibromatous or ossifying, depending on the abundance of the hard tissue, there is no prognostic value in this distinction, since these are all benign tumors that are cured by excision.

Squamous cell carcinomas

Squamous cell carcinoma (SCC) is by far the most common oral malignancy of **cats**, and, although there seems to be some geographic variation, it is generally most frequently located on the ventral surface of the tongue, on the midline near the frenulum. The gingiva is the next most common site, and other locations, including the tonsils, are generally much less frequently involved. In the early stages gingival SCCs are often mistaken for gingivitis. Oral SCCs in cats are generally advanced at presentation. *The tumor is locally invasive*, especially into bone and local soft tissues, but at presentation is less frequently metastatic to regional lymph nodes, and rarely to the lungs, although the survival rate of animals following surgical resection alone is only about 20%. Larger tumors at presentation signal a poorer prognosis. Paraneoplastic hypercalcemia has been reported. Grossly, these tumors are irregular, slightly nodular, red-gray, friable masses, often with an ulcerated surface that bleeds easily. Microscopically the tumor is a conventional SCC in appearance. Infection with *Feline immunodeficiency virus, Feline leukemia virus* or *Feline sarcoma virus* is not clearly a factor in the etiology, which is presumably multifactorial and in some cases may involve exposure to environmental tobacco smoke.

In the **dog**, most studies have found that SCC is second to melanoma in prevalence in the oral cavity. It most frequently involves the tonsils, although the gums are also common sites. Lips, tongue, and other oropharyngeal mucosae are involved much less often. The etiology is likely multifactorial, including epithelial hyperplasia associated with chronic gingivitis, and carcinogens associated with smog and smoke in the case of tonsillar tumors in urban dogs, but, as alluded to above, infection with *Canine oral papillomavirus* may have a part to play in some cases.

Grossly, tonsillar carcinoma usually appears unilateral. The earliest lesion visible is a small, slightly elevated, granular plaque on the mucosal surface, but it is rarely recognized. In the advanced stages, the affected tonsil is replaced by neoplastic tissue, 2–3 times normal size, nodular, firm and white, and the surface often is ulcerated. There is usually extensive infiltration of the surrounding tissues by microscopically typical SCC. Histological examination of the grossly unaffected tonsil may also reveal early carcinoma. SCCs originating in the tonsils of dogs often metastasize early to the regional nodes, initially the retropharyngeal node, and to the thyroid, with distant metastases to many visceral organs, but especially the lungs, and to bone. Tonsillar SCCs must be differentiated from involvement of the tonsil in lymphosarcoma. Those of the tongue may be difficult to resect fully locally, as they infiltrate among muscle bundles, and invade veins and lymphatics. They also tend to metastasize early to regional nodes, though more distant metastases are less common.

Gingival SCCs may be associated with chronic periodontitis in dogs. It is not always clear whether they have predisposed to periodontitis, or resulted from chronic irritation of the gingiva. It is assumed that some originate in the gingiva and others in subgingival or periodontal epithelial rests. They appear as pink or white nodular masses around the mandibular or maxillary dental arcade, often around canine or carnassial teeth, extending to form larger masses or ulcerative plaques; sometimes they involve adjacent hard palate. Their microscopic appearance is conventional, though the degree of keratinization, and formation of keratin pearls, may be lower than in similar cutaneous tumors. They may often be obscured by chronic active inflammation, as well. They are locally invasive, and may invade bone (Fig. 1.21), maxillary tumors sometimes extending into the sinuses and orbit. However, they are much less likely to have metastasized at presentation than SCC of the tonsil.

In **horses**, SCC are found rarely on the gums and hard palate, possibly arising in chronically irritated hyperplastic alveolar epithelium in cases of chronic periodontitis. They are slow-growing, exceedingly destructive, and metastatic mainly to the regional lymph nodes. Such tumors are large when first observed and may project from the palate or gums as gray extensively ulcerated masses, or appear as craterous ulcers. The large ones are extensively necrotic, and the teeth are lost or loosely embedded in the tumor. These tumors of the maxilla rapidly fill the adjacent sinuses and cause bulging of the face and may extend further, into the nasal, orbital, and cranial cavities. The microscopic appearance of the tumors varies considerably, from well differentiated with keratinization of individual epithelial cells and formation of keratin "pearls," to poorly differentiated with little evidence of keratinization.

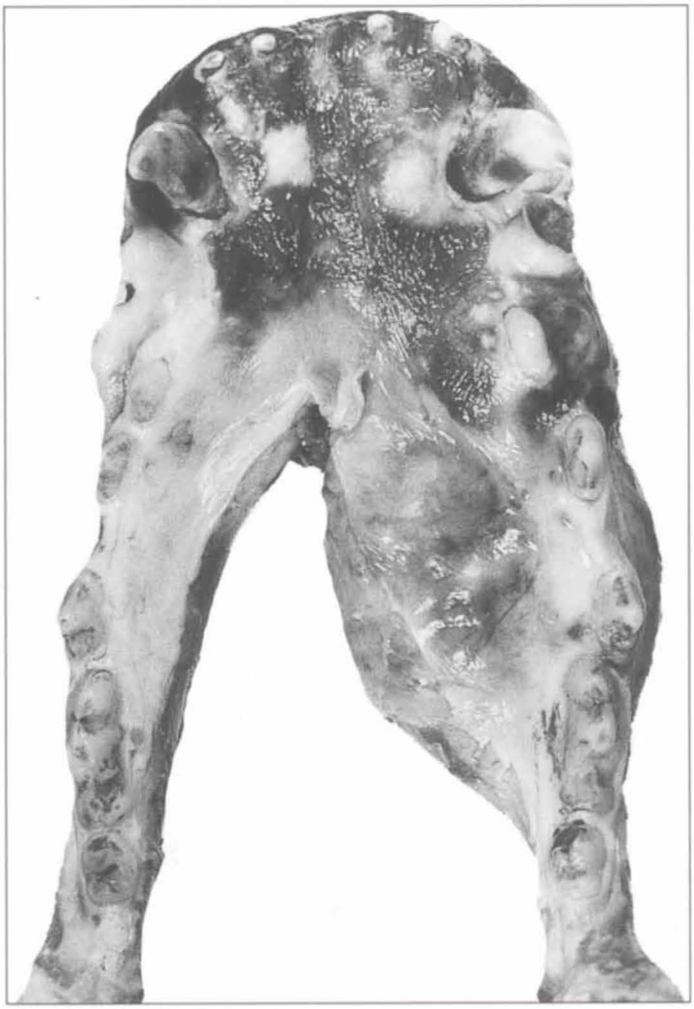

Figure 1.21 Squamous cell carcinoma arising from alveolus of right canine tooth and invading mandible in a dog.

In **cattle**, oral SCCs are very rare, with the exception of a few geographic areas where they are associated with oral papillomatosis and ingestion of bracken fern. A similar association is made in the etiology of SCC of the esophagus and forestomachs in cattle, and is considered more fully in that section. There are sporadic reports of this tumor on the lower lip of **sheep**.

Melanomas

Malignant melanomas *are the most common oral tumors in dogs in many parts of the world.* Although cutaneous melanomas are common in gray horses and certain breeds of swine, these species have no tendency to develop oral melanomas, though rare cases are reported. These tumors are also rare in cattle, sheep, and cats.

Most oral melanomas in **dogs** are malignant, in contrast to cutaneous melanomas, which are usually benign, and the majority have metastasized by the time they are diagnosed. They arise from melanocytes in the mucosa or superficial stroma, mainly on the gingiva and labia, and less often on the buccal mucosa, palate, tongue, and pharynx. There is variation in the outcomes of studies of sex and breed association. Males have been overrepresented in some studies, but not others. Some consider that melanomas are more common in smaller breeds or in those with a dark hair coat and/or pigmented mucous membranes, such as Scottish and Boston Terriers, black Cocker Spaniels, Dachshunds, Miniature Poodles, and Chow Chows, but not all studies have found size or breed associations. *The degree of pigmentation of these tumors varies considerably, and many are at least partially amelanotic, but there appears to be no relationship between the amount of pigment and biological behavior.* Metastases are usually pigmented, but in some cases the primary tumor is pigmented and the metastases are not, and vice versa.

Symptomatic lesions usually are about 3 or 4 cm in diameter when discovered. They grow rapidly; necrosis and ulceration are common (Fig. 1.22A), as is invasion of bone by gingival tumors. Over 70% metastasize to the regional lymph nodes. An approximately equal number spread via hematogenous and lymphatic routes to more distant sites, especially the lungs, where they may be evident at necropsy, but too small to be detected radiographically. The median survival time for untreated dogs is reported as 65 days, and survival following treatment is little better, at about 3 months. The median survival time of dogs with no bone, lymph node or distant involvement detected at diagnosis is about 8 months after surgical resection.

The histological appearance of melanomas varies greatly, from a fairly well-differentiated heavily pigmented type, to a highly anaplastic amelanotic type. The diagnosis of the latter is often difficult. However, certain features are evident in most of these tumors. *Anaplastic melanocytes show*

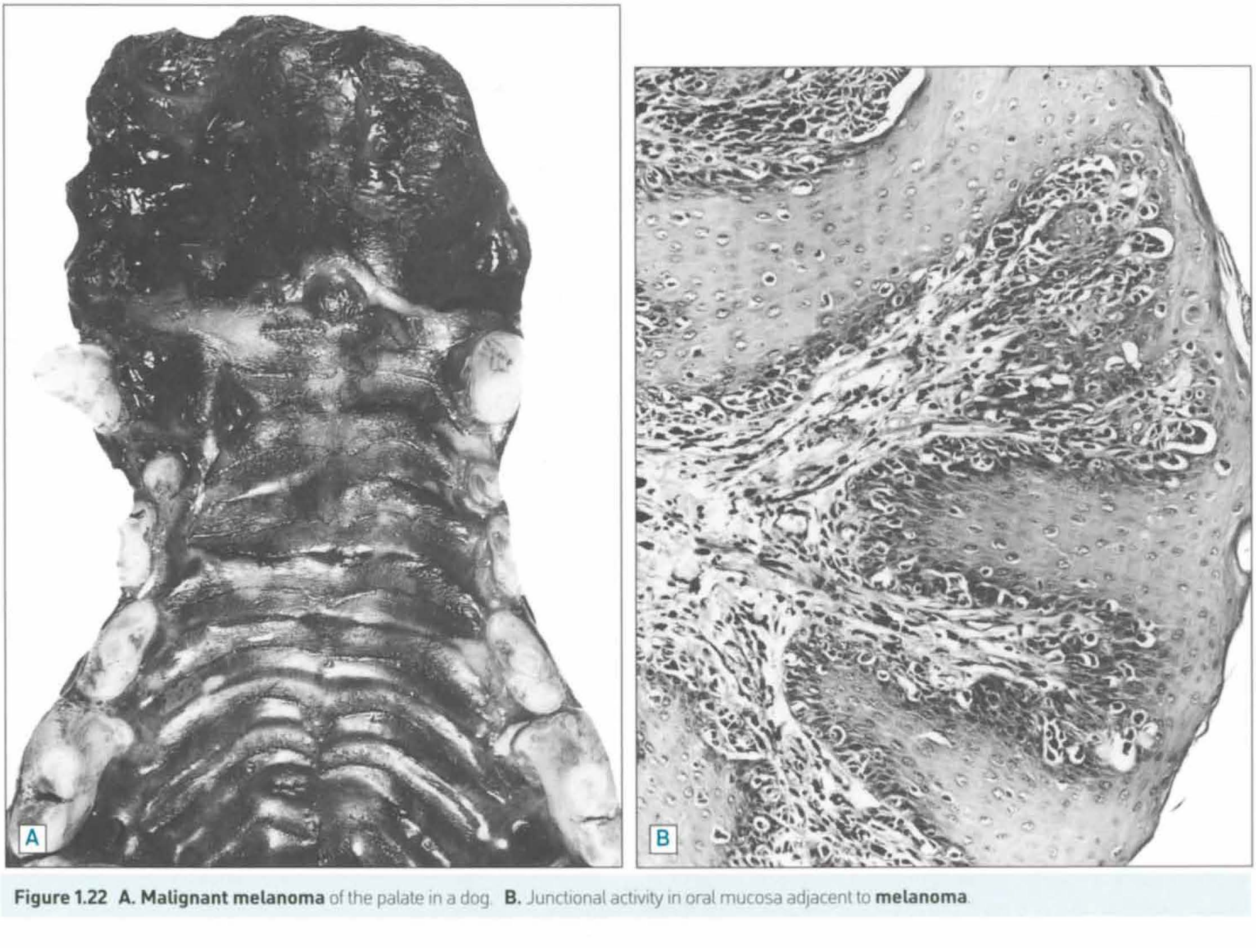

Figure 1.22 A. Malignant melanoma of the palate in a dog. **B.** Junctional activity in oral mucosa adjacent to **melanoma**.

junctional activity, infiltrating the junction between the basilar epithelial cells and the submucosa (Fig. 1.22B). Round or polyhedral cells with a large nucleus and extensive cytoplasm with well-demarcated borders predominate in some tumors. Others are comprised of spindle-shaped cells with oval nuclei containing small nucleoli. Most frequently there is a characteristic mixture of epithelial-like and spindle-shaped cells, which have a marked tendency to form nests supported by a light stroma, extending deep into the submucosa. Multinucleated giant cells may also be present. Osteoid has been reported in the stroma of one canine melanoma. The majority of melanomas have melanin pigment, but detection of this pigment, which may also be concentrated in melanophages, often requires careful examination of individual tumor cells. Cytologic evaluation of melanotic tumors may require bleaching, but any difficulty in interpretation is more likely to involve poorly pigmented variants. They are DOPA-positive (DOPA, 3,4-dihydroxyphenylalanine) in frozen section, and react immunohistochemically (vimentin 100%, melan A >90%). *Melan A is considered sensitive and specific for melanocytes,* differentiating them from melanophages in oral tumors. The mitotic rate and apparent degree of anaplasia may vary throughout the tumor, but since *almost without exception oral melanomas are malignant,* including some that appear benign histologically, all must be considered with suspicion. Prognosis is unrelated to any criterion investigated: demographic; tumor location or mass; pattern or cytologic characteristics.

In **cats**, oropharyngeal malignant melanoma is rare. It occurs in older animals, involves the same regions affected in dogs, resembles the mixed tumor cell phenotype of dogs, and has a short median survival time.

Pigmented basilar epithelial cells are frequently present in superficial areas of the submucosa in a variety of nonneoplastic lesions resulting from irritation to the mucosa. *This so-called "pigmentary incontinence" must be differentiated from malignant melanoma.*

Fibrosarcomas

In **dogs**, fibrosarcoma is the third most common oral malignant tumor, comprising about 15–25% of such lesions, but the most common sarcoma of the oral cavity. It frequently occurs in younger dogs, e.g., one report indicated 25% occurred in dogs less than 5 years of age, and the median age is about 7 years. Larger breeds appear to be predisposed, perhaps especially the Golden Retriever. It occurs mainly on the gums of the maxilla and adjacent palate and the rostral mandible, and less often in the buccal mucosa, lips, and tongue. *Despite a relatively benign histological appearance in large breeds, it grows rapidly, invading maxillary and mandibular bone in the majority of cases, and frequently recurs after surgical removal.* About 20–35% have metastasized to regional nodes, and pulmonary metastases have occurred in about 10–20% at diagnosis.

The tumors are solitary gray to red, firm, irregularly shaped to nodular fleshy masses over about 4 cm in diameter, that may ulcerate and become secondarily infected. They are usually fixed to any underlying bone. Microscopically the submucosa is diffusely infiltrated by densely cellular sheets of pleomorphic fusiform fibroblasts arranged in interwoven bundles with variable, but often relatively small, amounts of collagen. The mitotic index is high in high-grade tumors, and there may be multinucleate giant cells scattered throughout.

In **cats**, this tumor is the second most frequent oropharyngeal malignancy, but it is not common. The gingiva and palate have been reported as sites of predilection by some but others did not observe any specific location. The tumor resembles that in dogs, and invasion of bone is common.

Mast cell tumors

This tumor occurs occasionally in the oral cavity of middle-aged dogs, and, less frequently, in cats. Most commonly they are found on the lip of dogs, but they may arise in the submucosa of the tongue, gum, and hard palate. The tumor is diagnosed using the same criteria as cutaneous mastocytomas, and *all should be considered potentially malignant,* with metastasis to regional lymph nodes a possibility. Mast cell tumor should be considered in the differential diagnosis of oral lesions resembling granulation tissue or eosinophilic granuloma in dogs and cats.

Granular cell tumors

This rare tumor occurs in older (mean age 9 years) **dogs**, mainly in the base of the tongue, but also in the gingiva, lips, and palate. Most granular cell tumors are thought to be derived from neuroectodermal precursor cells. They are elevated, usually less than about 2 cm in size, red, and granular or smooth on the mucosal surface. The cut surface is white and firm. Microscopically, the mass consists of *large, polyhedral to round epithelioid cells that have abundant acidophilic granular cytoplasm.* The cytoplasmic granules are strongly periodic acid–Schiff-positive, and ultrastructurally are *phagolysosomes.* The nuclei are round to oval, centrally or eccentrically located, and have 1–2 nucleoli. Mitotic figures are rare. The tumor cells have a marked tendency to form nests or cords that are separated by a delicate network of reticulin fibers, though some are arranged as sheets of cells. None of these tumors in dogs have recurred after excision, and there is only one record of metastasis, to thoracic organs. There are a few reports of this tumor in the tonsil, tongue, gingival, and palate of **cats**. In **horses**, granular cell tumors are located in the lungs (see Vol. 2, Respiratory system).

Neuroendocrine carcinomas of the oral cavity and pharynx

Neuroendocrine cells of the dispersed neuroendocrine system occur rarely at most levels of the gastrointestinal tract, as well as at other sites, in several mammalian species. In older literature they may be referred to as Merkel cells.

Very rarely, they form tumors of the skin and the oral/nasal pharyngeal mucosa in the dog. In the latter site they tend to be pedunculated, and are located on the gums, lips, and pharynx. *Their behavior is aggressive, with metastasis to local nodes and distant sites,* and local recurrence if margins around the resected tumor are not adequate. Histologically, they consist of well-circumscribed densely packed nests and sheets of polygonal to round cells in the submucosa, resulting in an organoid appearance. The cells have a moderate amount of pale basophilic cytoplasm. The nuclei are pleomorphic, the nuclear membrane is indented, and there are 1–2 centrally located nucleoli. The mitotic index varies from few to 2–3 mitotic figures per high-power field. Multinucleate giant cells are often present. The cells stain negatively with the periodic acid–Schiff stain but have a positive argyrophilic reaction. These are useful features to differentiate this tumor from granular cell tumors. The tumor must also be differentiated from malignant melanoma. Immunohistochemical reactions are usually positive for synaptophysin and neuron-specific enolase, and more variably for chromogranin-A and cytokeratin. Ultrastructurally, the tumor cells contain *cytoplasmic secretory granules characteristic of neuroendocrine cells*.

Plasmacytomas

Extramedullary plasmacytomas are uncommon tumors of the oral mucosa and skin of mainly older dogs (mean age 9–10 years). They arise as primary tumors from plasma cells in the soft tissue, or rarely as metastases from primary osseous myeloma.

This tumor has probably been underdiagnosed in the past, being mistaken for undifferentiated round cell tumor, reticulum cell sarcoma, or a variant of dermal lymphoma. In the oral cavity it has been misdiagnosed as malignant melanoma. Grossly the tumor is a red, lobulated raised mass usually located on the gingiva or lips. It can invade bone, rarely. Plasmacytoma also occurs sporadically in the stomach, colon, and rectum.

Histologically, the tumor is well circumscribed, nonencapsulated, and the overlying mucosa is usually intact, unless it has been traumatized in large tumors. The tumor cells are pleomorphic with variable amounts of amphophilic to basophilic cytoplasm. The nuclei are round to oval and the nuclear membrane is indented. The cells are densely packed into nests and sheets that are divided by scant fibrovascular stroma. Anisokaryosis, bi- and multinucleated cells are frequently present in the center of the tumor, and the most differentiated plasma cells are usually evident at the periphery. The mitotic index varies widely from one tumor to another. Immunoglobulin, especially IgG, can frequently be demonstrated in the neoplastic cells, and AL amyloid is occasionally present among the tumor cells (AL, amyloid immunoglobulin light chain). Electron microscopic examination reveals features typical of plasma cells.

In spite of the anaplastic appearance and the presence of mitotic figures, the biological behavior of these tumors is *benign*, and they are cured by simple resection.

Vascular tumors

Lesions variously considered *vascular hamartomas* or *hemangiomas* have been described in the gums of neonatal **calves**. Hamartomas are focal disorganized overgrowths of mature tissue endogenous to the organ involved. Because of their location, they usually have an inflamed surface, and may resemble, superficially, granulation tissue or pyogenic granuloma. They are pink to red lobulated masses, up to several centimeters in diameter, often pedunculated, on the rostral mandibular gingiva adjacent to the incisors, which may be displaced. They may also be located in the tongue. Microscopically, the tumors consist of irregular thin-walled vascular channels containing erythrocytes or proteinaceous material, and lined by well-differentiated endothelial cells. The vascular spaces are separated by loose fibrous stroma. Vascular hamartomas are benign, but a single hemangiosarcoma in a young calf has been described that involved the palate and gums, as well as distant sites.

Hemangiomas occur rarely in the oral cavity of mature horses, dogs, and cats. Rare hemangiosarcomas have been recorded in the oral cavity of cats and a few dogs, the latter with cutaneous hemangiosarcomas.

Miscellaneous tumors

Various benign and malignant neoplastic and like lesions in the oropharynx have been reported sporadically. In the dog, benign masses include dermoid cysts, histiocytoma, lipoma, lymphangioma, hemangioma, rhabdomyoma, ganglioneuroblastoma, and calcinosis circumscripta (of the tongue). Malignant tumors that have been reported in the oral cavity, mainly in the lip and tongue, are hemangiosarcoma, leiomyosarcoma, histiocytoma, ectopic thyroid carcinoma, hemangiopericytoma, schwannoma, osteosarcoma, rhabdomyosarcoma, and highly malignant undifferentiated tumors.

In cats, benign tumors include hemangioma and fibroxanthoma, and malignant examples are oral and tonsillar lymphosarcoma, schwannoma, and osteosarcoma. Pharyngeal, lingual, and gingival lymphomas have been described in horses.

Salivary tumors are described in the section on salivary glands, below.

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SALIVARY GLANDS

Salivary glands are a complex set of secretory structures, present both as large discrete glands in the head and cranial neck region, and as an extensive series of submucosal minor salivary glands in the oral cavity, including the tongue, and in the oropharynx and larynx. Gland structure is variable, depending on whether the gland produces serous or mucous secretion, or both. Common *acute reactions* to injury include hypersecretion, acute necrosis, edema, and inflammation. Gland atrophy, ductular fibrosis and obstruction, and squamous metaplasia may develop following *chronic injury*. A lesion associated with increasing *age* may be oncocytic metaplasia of salivary duct epithelium.

The most common conditions of the salivary glands are functional, *ptyalism* being an increased secretion of saliva and *aptyalism* being a reduced or ceased secretion. Ptyalism (to be differentiated from failure to swallow) is seen as abnormal accumulation of saliva in the mouth. It occurs in a variety of conditions, including heavy-metal poisoning, poisoning with organophosphates, encephalitis, and, most often, stomatitis. Decreased secretion of saliva is less common, but accompanies fever, dehydration, and salivary gland disease.

Ptyalism in cattle and horses may be an expression of a mycotoxicosis. *Rhizoctonia leguminicola* infestation of legumes is associated with "slobbers syndrome." On well-cured legume hay, the mycelial growth is not visible grossly. The fungus has a wide geographical distribution. *Rhizoctonia leguminicola* produces two biologically active alkaloids, *slaframine* and *swainsonine*. Slaframine, a parasympathomimetic alkaloid, induces excessive salivation, as well as anorexia, excessive lacrimation, diarrhea, frequent urination, and bloat. Milk production is reduced and there is loss of body weight. No specific lesions have been associated with slaframine toxicosis. Guinea pigs are extremely sensitive to the toxin. Presumptive diagnosis may be based on feeding trials in that species, if chromatographic analysis for slaframine is not readily accessible.

Ptyalism may be associated with *neurointoxication*, particularly those agents that affect the trigeminal nuclei. Known causes include ingestion of *Paspalum destichium* and *Prosopis glandulosa* (honey mesquite), poisoning with cholinergic stimulants, neurotropic viruses especially *Rabies virus*, and in dogs exposure to the toad *Bufo marinus* will cause neurologic abnormalities and ptyalism.

Foreign bodies are occasionally present in the ducts, usually the parotid duct, but sometimes the submaxillary. They are usually plant awns or fiber. They invariably cause some degree of inflammation with secondary infection; if the duct epithelium is destroyed, local cellulitis occurs. **Salivary calculi** (*sialoliths*) may also cause obstruction and inflammation. They are more common in horses than other species. Calculi are composed largely of calcium carbonate, and are white, hard, and laminated. They are thought to originate from microliths, which are formed routinely and undergo regular turnover. Secretory inactivity can cause microliths to accumulate more material, leading to calculi that may be obstructive. Calculi are usually single and cylindrical, and they may be quite large. Most of them lodge at the orifice and cause some degree of salivary retention, glandular atrophy, and predisposition to infection and further inflammation.

Dilations of the duct are due to stagnation of flow, and this in turn results from obstruction due to congenital atresia or by foreign bodies, calculi, and inflammatory strictures. The dilated ducts appear as fluctuating cords, sometimes with local diverticula. **Ranula** *is the term* *applied to a cystic distension of the duct in the floor of the mouth.* These present a smooth rounded prominence with a blue tinge and fluctuations, and the lining epithelium is more or less intact. The contents may be serous or thick tenacious mucus. Rupture of a duct or a gland to an epithelial surface results in a permanent fistula as the continued flow of saliva prevents normal restoration, the duct epithelium fusing with that of the surface.

Salivary mucocele or sialocele *is an accumulation of salivary secretions in single or multiloculated cavities in the soft tissues of the mouth or neck.* These cystic formations do not have an epithelial lining. Sialoceles can occur in dogs of any breed, where they may become large enough to require surgery. There may be a history of an antecedent ranula-like swelling in the mouth. Many sialoceles are probably the result of trauma to the duct. They may be located anywhere from the mandibular symphysis to the middle of the neck, the latter due to gravitational displacement. Most are ventrolateral, sometimes bilateral or midline, at the angle of the mandible. They arise most commonly from the sublingual salivary gland, either from individual units of the polystomatic portion or from the duct of the monostomatic portion. Zygomatic salivary mucoceles also occur, associated with local swelling and exophthalmos. Most sialoceles are subcutaneous and may be up to 10 cm in diameter, the larger ones being pendulous. The wall is of soft pliable connective tissue, well vascularized, with a glistening lining. The contents are brown and mucinous, becoming progressively inspissated and tenacious with time.

Small sialoceles, seldom exceeding 0.5 cm in size, are occasionally observed on the side of the bovine tongue. They presumably originate from rupture of the fine tortuous ducts of the dorsal part of the sublingual gland.

The histological appearance of sialoceles varies greatly, apparently depending on the stage of development. Initially, the wall consists of an outer, highly vascularized layer of immature connective tissue and an inner zone of loosely arranged fibroblasts. A pleocellular inflammatory reaction, which may be very mild, is evident in the central area, which also contains much amorphous amphophilic material. More mature fibrous connective tissue forms the wall in later stages when inflammation is mainly mononuclear, and plasma cells often predominate, and the material in the center becomes progressively more basophilic.

Cysts of other origins do occur in this region. Cysts of the **thyroglossal duct** are midline and readily distinguishable when they contain thyroid follicles. Cystic salivary adenomas are rare. **Branchial pouch cysts** may be located ventrolaterally, as are the salivary cysts, or dorsolateral on the neck. Their distinction is probably valid when no demonstrable connection occurs with a salivary duct and a pseudostratified or stratified squamous or columnar lining epithelium is present.

Sialoadenitis, *inflammation of the salivary glands,* is uncommon in animals, but it is, after sialoceles and malignant neoplasms, the most frequently diagnosed lesion involving salivary glands of dogs and cats. The submandibular gland is usually affected. Inflammation of the zygomatic gland in dogs is a cause of retrobulbar abscess. The infection usually gains entrance via the excretory duct, although it may be hematogenous or locally traumatic. Inflammation of the duct results in its obstruction by exudate, desquamated epithelial cells, and mucus. Some of this may be expressed from the ductal orifice as pus; the orifice is usually acutely inflamed. Obstruction of the duct, whether partial or complete, produces secondary atrophic changes in the glands, although there is initial enlargement due to the combined effects of retained secretion and inflammation. The ducts throughout the gland dilate and leukocytes infiltrate the lumen and stroma. The acini undergo compression atrophy or swell and rupture from retained secretion. In acute infections this often leads to suppuration, and in chronic ones only remnants of atrophic epithelium remain in a mass of inflamed scar tissue.

Specific inflammations of the salivary glands in domestic animals are unusual. Examples include rabies, where there is often focal lysis of acinar cells, mononuclear infiltration and, uncommonly, Negri bodies in the ganglionic neurons; strangles in horses; and distemper in dogs. *Eosinophilic sialoadenitis* is found in an eosinophilic epitheliotropic syndrome in horses discussed in the section on Eosinophilic enteritis in cats and horses, below. *Sjögren's-like syndrome* has been diagnosed in cats and dogs, with plasmacytic infiltrates in the salivary glands. Sialoadenitis occurs in **vitamin A deficiency** in calves and pigs and in cattle poisoned with highly chlorinated naphthalenes. In these conditions, inflammation, often purulent, is secondary to squamous metaplasia of the ducts, with stasis of flow and secondary infection; *squamous metaplasia of interlobular ducts is an early and rather specific lesion of vitamin A deficiency.*

Necrotizing sialometaplasia is a distinct, rare disease of dogs and humans, characterized by ischemic necrosis of salivary gland lobules with inflammation and squamous metaplasia of the ducts. The underlying reason is thought to be vascular compromise induced by trauma, although immune-mediated destruction of blood vessels or infection have also been hypothesized. In dogs, the disease is seen in a number of small breeds, primarily terriers. The mandibular gland is affected preferentially. Dogs with this condition are presented in extreme pain, with enlarged hard salivary glands and with recurrent vomition. The reasons for vomition are unclear; however it may be centrally mediated, as administration of anticonvulsants is effective in controlling this clinical sign.

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Neoplasms of salivary glands

Neoplasms of the salivary glands are rare in all species; they have been reported in cattle, sheep, goats, horses, dogs, and cats, but apparently not in swine. Only in dogs and cats do salivary tumors occur often enough to permit generalization. They may arise from either the major or the minor salivary glands, involvement of the major glands being three times as frequent; the parotid and mandibular are the most susceptible. The tumors develop almost exclusively in aged animals; the majority are epithelial, malignant at diagnosis, grow rapidly, and become fixed to the overlying skin. Signs are related to swelling, pain, halitosis, dysphagia, and exophthalmia. Metastasis to regional nodes and distant sites, especially the lungs, is common.

Salivary tumors express the phenotype of ductular or glandular epithelium, either because they arise in these structures, or because they derive from pluripotential stem cells. Duct neoplasms are generally papillomatous if benign, and squamous or mucoepidermoid carcinomas if malignant. Tumors with glandular phenotype are usually adenomatous, their malignant potential sometimes only manifest by carcinomatous areas at the periphery of the neoplasm (Fig. 1.23).

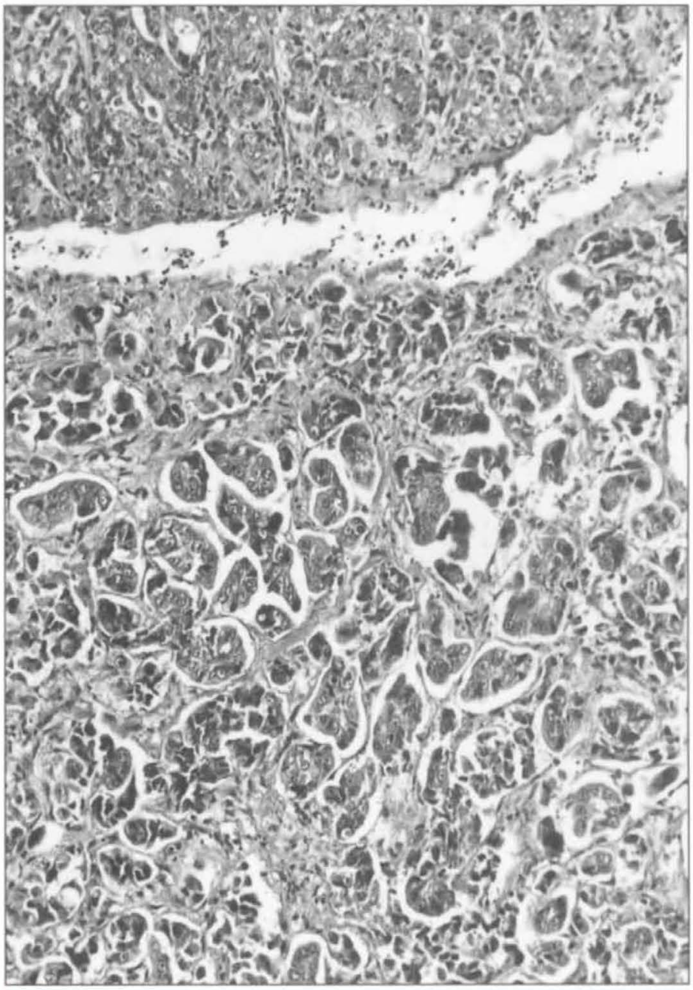
Figure 1.23 Salivary adenocarcinoma of glandular origin in a cat.

The histological structure of salivary tumors in animals is as diverse as in humans and the accepted classifications apply. However, the phenotype of salivary tumors has little bearing on prognosis, with the exception of acinic cell tumors, which are less aggressive, and pleomorphic adenomas, which are benign with potential for malignant transformation. Adenocarcinomas that have metastasized to the nodes or beyond at diagnosis have a worse prognosis than those with tumor localized to the gland. In one study, adenocarcinomas in cats seemed more aggressive than those in dogs.

The most frequent variety in dogs is *adenocarcinoma*, and although there are various structural patterns, sometimes within the same tumor, an acinar or nested arrangement is usually evident somewhere, embedded in an often extensive fibrous stroma. Some adopt a mucoepidermoid pattern, containing squamous epidermoid cells, mucus-producing cells, and intermediate phenotypes, which may line cysts, sometimes in a papilliform pattern. The rupture of such cysts initiates a granulomatous response, including giant cells. Salivary carcinomas infiltrate locally at the periphery, and metastasize via lymphatic and vascular invasion.

Acinic cell carcinomas are less common than adenocarcinomas, occurring mainly in dogs and sporadically in the cat, horse, and sheep. They are at least partially encapsulated masses with potentially infiltrative margins, composed of usually well-differentiated epithelial cells with a low mitotic index, that form a pronounced acinar pattern, or resemble cells of the intercalated duct, including clear cells. The presence of cells of normal acinar salivary epithelial phenotype is an essential criterion for diagnosis of this tumor. They are locally invasive; metastases to the regional nodes are rare.

Pleomorphic adenomas, or mixed tumors, have been described, rarely, in the dog, cat, bovine, and horse. They are similar to the comparable mammary tumors, with areas of myoepithelial cells embedded in a mucinous matrix that also contains neoplastic epithelial cells. Bone and cartilage form in these areas. These tumors grow by expansion, and may develop malignant foci, usually carcinomatous rather than carcinosarcomatous.

Rarely, mesenchymal malignancies, including fibrous histiocytoma, osteosarcoma, and fibrosarcoma, appear to arise in the salivary glands.

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ESOPHAGUS

The esophagus is comprised of an inner circular layer and an outer longitudinal layer of skeletal muscle for much or all of its length. In the pig, there is a short segment near the cardia that is comprised of smooth muscle, and in horses and cats, smooth muscle is found in the distal third of the esophagus. The esophagus is lined by a stratified squamous mucosa, with a variable number of submucosal mucous glands, depending on the species and location along the esophagus. Esophageal protection against aggressive insults resides mainly in mucus and bicarbonate in salivary secretion and is produced by esophageal submucosal glands.

The esophagus merits particular attention during the examination of animals with inadequate growth rate, cachexia, ptyalism, dysphagia, regurgitation, vomition, and aspiration pneumonia. In the ruminant, tympany may be a sequel to esophageal disease. The presence of a "*bloat line*" in the esophagus at the thoracic inlet may indicate a condition causing increased intra-abdominal pressure such as gastric dilation or ruminal tympany. The squamous mucosa is frequently eroded or ulcerated in viral diseases that cause similar lesions elsewhere in the upper alimentary tract. Conditions of striated muscle, such as nutritional myodegeneration and eosinophilic myositis in the ruminant, or polymyositis, systemic lupus erythematosus, and trypanosomiasis in the dog, will involve the esophageal muscle. Envenomation following snake bite may lead to myopathy of the esophageal musculature in dogs. Diseases of the neuromuscular junction, as in myasthenia gravis, and peripheral neuropathies, such as giant axonal neuropathy and polyneuritis, will result in esophageal disease.

Anomalies, epithelial metaplasia, and similar lesions

The mucosa of the distal portion of the feline esophagus normally has a herringbone pattern of superficial folds. **Hypertrophy of the smooth muscle** of the distal esophagus, most commonly the circular layer, occurs in horses. It is usually found incidentally at autopsy, although some cases also have concurrent terminal ileal muscular hypertrophy. Speculatively, defects in the pacemaker cells normally found in smooth muscle at these sites may be the basis for these lesions.

Congenital anomalies of the esophagus are very rarely recorded in domestic animals, and their interpretation as such can be difficult, since similar defects may develop as sequelae of esophageal trauma or inflammation.

Congenital duplication of the esophagus is rare in domestic animals. It may result in a cystic mass, usually in the vicinity of the esophagus, lined by variably columnar to squamous epithelium, and with a double muscle layer in the wall; such cysts may become manifest as space-occupying lesions as they fill with cellular debris and secretion. Tubular duplications of the esophagus are usually clinically silent; they are contiguous with the wall of the true esophagus, with which they communicate.

Rare **segmental aplasia** of the proximal esophagus may be apparent in the neonate. A short blind pouch communicates with the pharynx, and a thin fibrous band connects it to the distal patent esophagus which follows a normal course to the stomach. Esophageal atresia and congenital esophagorespiratory communications result from anomalies occurring when the respiratory primordium buds from the embryonic foregut.

Esophagorespiratory fistulae without esophageal atresia are more commonly recognized in animals; in calves and dogs strong circumstantial evidence suggests that some of these are congenital. Short fibrous bands with a narrow mucosa-lined lumen, connecting an esophagus of normal diameter with the trachea or bronchus, are reported, as are small apertures connecting the lining of esophageal diverticula with the respiratory tree. The lining of such defects changes from stratified squamous to columnar respiratory epithelium in the fistula or wall of the diverticulum. Gastric distension by air in calves, and pneumonia due to aspiration, have been associated with esophagorespiratory fistulae.

Esophageal diverticula are irregular outpouchings or herniations of the esophageal mucosa through the muscularis. They communicate with the esophagus by variously sized, often slit-like, apertures. Most are probably acquired, and they are commonest in the lower cervical esophagus, or in the distal thoracic esophagus, just cranial to the diaphragm. Increased intraluminal pressure, associated with foreign bodies, obstruction or stenosis, is considered the cause of "*pulsion*" *diverticula*, in which the mucosa is forced out through the distended or ruptured muscularis. Such diverticula may be large spherical structures, with a narrow neck. They are most common in the horse and dog. The rare "*traction*" *diverticulum* is the result of contraction of a paraesophageal fibrous adhesion, following perforation and inflammation, drawing with it a pouch of esophageal mucosa that is usually small and inconsequential. In contrast to pulsion diverticula, which have a layer of epithelium lining the inner aspect of a wall of fibrous connective tissue, traction diverticula have a wall comprised of all layers of the esophagus. Ingesta and foreign bodies may accumulate in diverticula, causing gradual enlargement, with the potential for local esophagitis, ulceration, and perforation or formation of a fistula.

Rare *anomalies* of the mucosa include epithelial inclusion cysts, the presence of papillae resembling those of the rumen in the distal esophagus of cattle, and gastric heterotopia.

Hyperkeratosis and thickening of the epithelium may be signs of vitamin A deficiency, common in birds and also seen in herbivores, or with chlorinated naphthalene toxicity. Squamous metaplasia in the ducts of submucosal esophageal mucous glands, and in ducts and glands elsewhere, should be sought. Mild hyperkeratosis may be difficult to assess since in herbivores some degree of keratinization may be normal, and anorexia or failure to swallow results in loss of the abrasive effect of food passage, with accumulation of keratinized squames. Parakeratotic thickening and basal hyperplasia of the epithelium should be considered indicative of response to epithelial injury, and in the distal esophagus of pigs is a concomitant of ulceration of the pars esophagea of the stomach. Hyperkeratosis and thickening of the epithelium leading to raised striations in the distal esophageal mucosa occur following repetitive injury through reflux of gastric acid, usually accompanied by linear erosions. Parakeratosis of the esophagus occurs in pigs with cutaneous parakeratosis caused by zinc deficiency.

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Esophagitis

Erosive and ulcerative esophagitis is a common finding associated with viral diseases causing similar lesions in the oropharynx or reticulorumen. Bovine viral diarrhea, rinderpest, bovine papular stomatitis, infectious bovine rhinotracheitis, and calicivirus in cats may produce necrotizing esophageal lesions. Healing esophageal ulcers repair by granulation. Local epithelial proliferation and thickening produce an opaque pearly appearance of the margin of the lesion or surface of the scar.

Caustic or irritant chemicals, ionizing radiation, electrochemical reactions, and hot ingesta may cause mucosal injury, the severity of which depends on the nature of the insult and duration of exposure. Mild acute insult may result in diffuse or local reddening of the mucosa. Deep sloughing of the mucosa, liquefactive necrosis associated with alkalis, and coagulative necrosis following acid and toxins (paraquat, oak toxicosis) reflect more severe insult and may result in ulceration extending to deeper layers of the esophagus.

Superficial epithelial damage heals uneventfully, though repeated insult may cause thickening of the epithelium with the development of prominent rete pegs. Ulcerated mucosa will granulate, and raised islands of surviving pearly proliferative epithelium may be present over the surface. The inflammatory reaction in ulceration frequently involves muscularis and adventitia. The ultimate development of a contracted fibrous scar causes stricture or stenosis, if the original mucosal defect involved a significant portion of the esophageal circumference.

Reflux esophagitis results from the action on the esophageal mucosa of gastric acid, pepsin, probably regurgitated bile salts, and possibly pancreatic enzymes (Fig. 1.24A). Stratified squamous epithelium appears more susceptible to the corrosive effects of gastric secretion than other types of mucosa in the lower gastrointestinal tract. Relatively short duration of exposure to refluxed gastric content

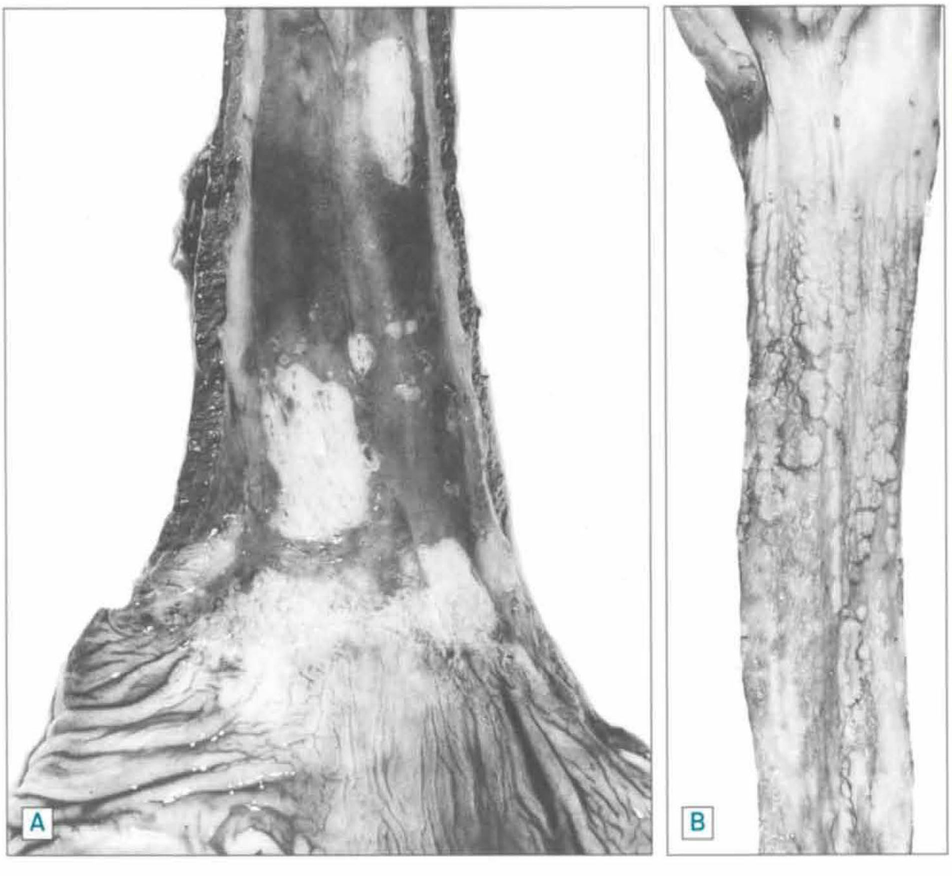

Figure 1.24 A. Reflux esophagitis following chronic vomition in a dog. Islands of squamous epithelium remain surrounded by ulcerated mucosa. **B. Reflux esophagitis** in a pig, secondary to esophagogastric ulceration.

is required to induce epithelial damage, which is signaled by hyperemia or linear erosions and ulcers, perhaps with superficial fibrinonecrotic debris, and erythematous margins. Such damage is most common in the distal esophagus, but may extend well forward, in some instances nearly to the pharynx. The expected microscopic basal epithelial activation, rete peg elongation, and epithelial transmigration by neutrophils occur in response to mild superficial epithelial necrosis. A thinned epithelium following recent moderate insult, or granulation of an ulcerated surface, may be evident. Re-epithelialization with a columnar mucous cell type may occur in distal esophagus adjacent to the cardia. *Papillomatous esophagitis* of unknown etiology has been reported in the distal esophagus of the cat, which normally has a somewhat corrugated or herringbone pattern to the mucosa.

Functional integrity of the lower esophageal sphincter may be compromised or overwhelmed by airway occlusion and increased intra-abdominal pressure, the pharmacologic effects of preanesthetic agents, or abnormality of the hiatus. Reflux esophagitis is thus most common in dogs and cats as a sequel to surgery involving general anesthesia, though it may follow chronic gastric regurgitation or vomition for any cause. In swine and horses, it may be associated with ulceration of the squamous esophageal portion of the stomach (Fig. 1.24B). In dogs, it is associated with rare hiatus herniation.

Hiatus hernia usually involves sliding herniation of all or part of the abdominal esophagus, cardia, and stomach into the thoracic esophagus, rather than periesophageal herniation. It is generally self-reducing, and its effect is usually lower esophageal sphincter failure and reflux, rather than gastric herniation and obstruction. *Gastroesophageal intussusception* is a rare event, most reported in puppies of large breeds of dogs, and is associated with recurrent vomition that may lead to aspiration pneumonia.

Thrush, or mycotic esophagitis caused by *Candida albicans*, is seen in piglets and weaner swine, where the lesions may involve squamous mucosa of the entire upper alimentary canal. The condition is probably secondary to other intercurrent problems, including antibiotic therapy, inanition, and esophageal gastric reflux, and is considered more fully in the section on Mycotic diseases of the gastrointestinal tract, below. Similarly, secondary *phycomycotic* granulomatous involvement of the esophagus is a rarely recorded complication of debilitating systemic disease states and heavy use of glucocorticoids and antibiotics.

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Esophageal obstruction, stenosis, and perforation

"*Choke*," obstruction, or impaction of the esophagus occurs when large or inadequately chewed and lubricated foods, masses of grain or fibrous ingesta, or medically administered boluses, lodge in the lumen of the esophagus (Fig. 1.25). This often occurs where the esophagus deviates or is slightly restricted normally, at the area over the larynx, the thoracic inlet, the base of the heart, and immediately cranial to the diaphragmatic hiatus.

Complications of obstruction include *pressure necrosis and ulceration of the mucosa*, which may progress to perforation. Sharp objects, such as bones, are most liable to cause *perforation*. Usually fatal cellulitis of the periesophageal tissue ensues, which may involve the

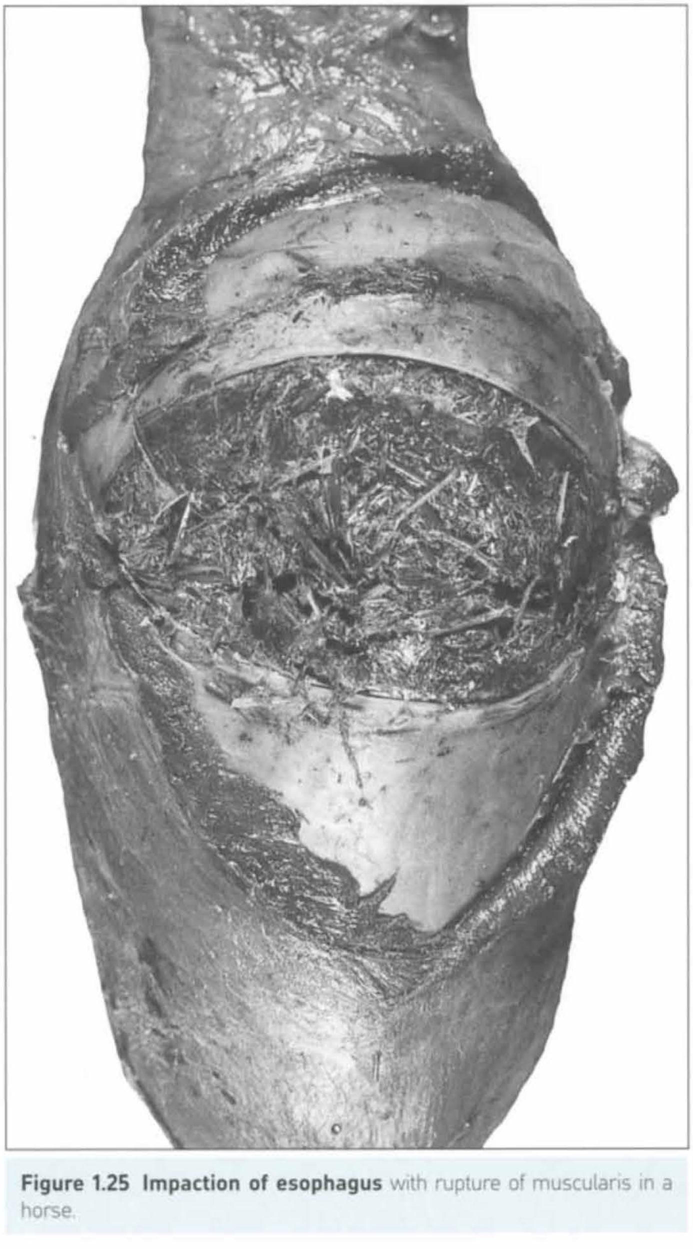

Figure 1.25 Impaction of esophagus with rupture of muscularis in a horse.

mediastinum directly, or by extension along fascial planes from the cervical region, depending on the site of perforation. Alternatively, perforation of the thoracic esophagus may lead to pleuritis.

Sharp objects, such as needles, quills, grass seeds, and awns, may penetrate and track from the esophagus through adjacent tissues. Diverticula, and esophagorespiratory or esophagoaortic fistulae, may also ensue following obstruction by foreign bodies.

Removal or dissolution of an obstructing object may be followed by *scarring* of the segmentally ulcerated esophagus, resulting in narrowing of the lumen, stricture, or stenosis. Esophagitis, especially due to gastric reflux, may have a similar sequel. Although hypertrophy of internal and external muscle layers is seen occasionally in the distal esophagus of cattle and horses (in which species the distal esophageal muscle is normally relatively thick), it is usually not clearly the result of obstruction.

Stenosis may also result from rare intramural or intraluminal neoplasia, or commonly by external compression. Among causes of external compression may be enlarged hyperplastic or neoplastic thyroids, and neoplasia of the thymus and of cervical and mediastinal lymph nodes.

"Vascular ring" anomalies, seen in dogs, occasionally in cats, and rarely in other species, are the most common causes of external constriction of the esophagus. *Dextroposition of the aorta*, the development of the aortic arch from the right, instead of the left, fourth arch, is the most common of these anomalies. This results in entrapment and constriction of the esophagus between the heart and pulmonary artery ventrally, with the anomalous aortic arch on the right, and the ligamentum arteriosum or remnant of the ductus arteriosus dorsally on the left. *Other vascular anomalies* that may constrict the esophagus, and reported only in the dog, are persistence of both right and left aortic arches; persistent right ductus arteriosus; aberrant left subclavian artery, in association with persistent right aortic arch; and aberrant right subclavian artery arising distal to the left subclavian artery, and passing dorsally over the esophagus. The Irish Setter, German Shepherd, and Boston Terrier are the breeds most commonly afflicted with vascular ring anomalies.

Esophageal deviation and stenosis have been associated in English Bulldogs with "thoracic shortening" due to hemivertebra and esophageal compression between the left subclavian artery and the brachiocephalic artery. The site of stricture, with its narrowed esophageal lumen, is readily identified at necropsy.

Constricting mural fibrosis or other causative internal or external obstructive lesions will be obvious. The mucosa at the site of stricture may be ulcerated, as the result of impaction of ingesta, or as a sequel to antecedent esophagitis, pressure necrosis, or neoplasia. The esophagus proximal to the stenotic area is dilated, may contain retained ingesta, and itself may have evidence of esophagitis.

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Dysphagia

Swallowing is a complex and highly coordinated physical act, which may be conveniently divided into three phases – oral, pharyngeal, and esophageal. **Oral-phase dysphagias** are the product of painful physical lesions of the oral cavity and tongue, such as stomatitis, glossitis, gingivitis, or lesions such as hyoid damage that limit movement of the tongue or delivery of the bolus to the pharynx. Loss of hypoglossal nerve function associated with hydrocephalus, trauma, or myasthenia gravis impairs lingual function. Cleft palate results in nasal regurgitation at this phase.

Pharyngeal dysphagia may be associated with painful pharyngitis, tonsillitis, retropharyngeal abscesses, and granulomas. Abscesses, granulomas, and neoplastic processes involving the tonsils and regional lymph nodes may physically intrude on the pharyngeal space. Encephalitis involving the medulla oblongata and the nuclei or tracts of the major cranial nerves involved in pharyngeal contraction and lingual function (V, IX, X, XII) should be sought in pharyngeal dysphagia, unexplained on physical grounds. Rabies and brain abscess in all species, infectious bovine rhinotracheitis and listeriosis in ruminants, are candidate central causes of pharyngeal paralysis. Retropharyngeal abscesses and lesions of the equine guttural pouch may cause peripheral nerve damage and paralysis. Idiopathic myodegeneration and myasthenia gravis have been reported as causes of impaired pharyngeal muscle function. Dysphagia is reported in Bouviers in association with muscular dystrophy.

Cricoesophageal incoordination, or **achalasia** (failure to relax), may impede the first stage of the esophageal phase of swallowing – the opening of the upper esophageal sphincter to accept the approaching bolus. This condition is recognized in dogs. The underlying deficit is unknown but may be muscular as removal of part of the cricopharyngeal muscle is curative.

Megaesophagus, or *esophageal ectasia*, is the result of atony of the esophageal muscle, flaccidity, and luminal dilation (Fig. 1.26). This is the product of segmental or diffuse motor dysfunction of the body of the esophagus. This results in failure of peristaltic propulsion of the food bolus to, and through, the lower esophageal sphincter to the stomach. Ingesta accumulates in the esophageal lumen, with eventual regurgitation of undigested food. Retention of some ingesta in the esophagus may lead to putrefaction and esophagitis in dilated or dependent areas. The volume of the dilated thoracic and cervical esophagus may greatly exceed that of the stomach, and the intrathoracic trachea and heart may be displaced ventrally. Animals with esophageal hypomotility or megaesophagus may have signs of marked malnutrition, including emaciation, dehydration, and osteopenia, often in association with rhinitis and aspiration pneumonia resulting from regurgitation.

Congenital idiopathic megaesophagus (CIM) is relatively common in dogs; it may improve functionally to some extent with time. CIM has its highest prevalence in Great Danes, German Shepherds, and Irish Setters, and appears to be *heritable*, with a pattern in Miniature Schnauzers compatible with a simple autosomal

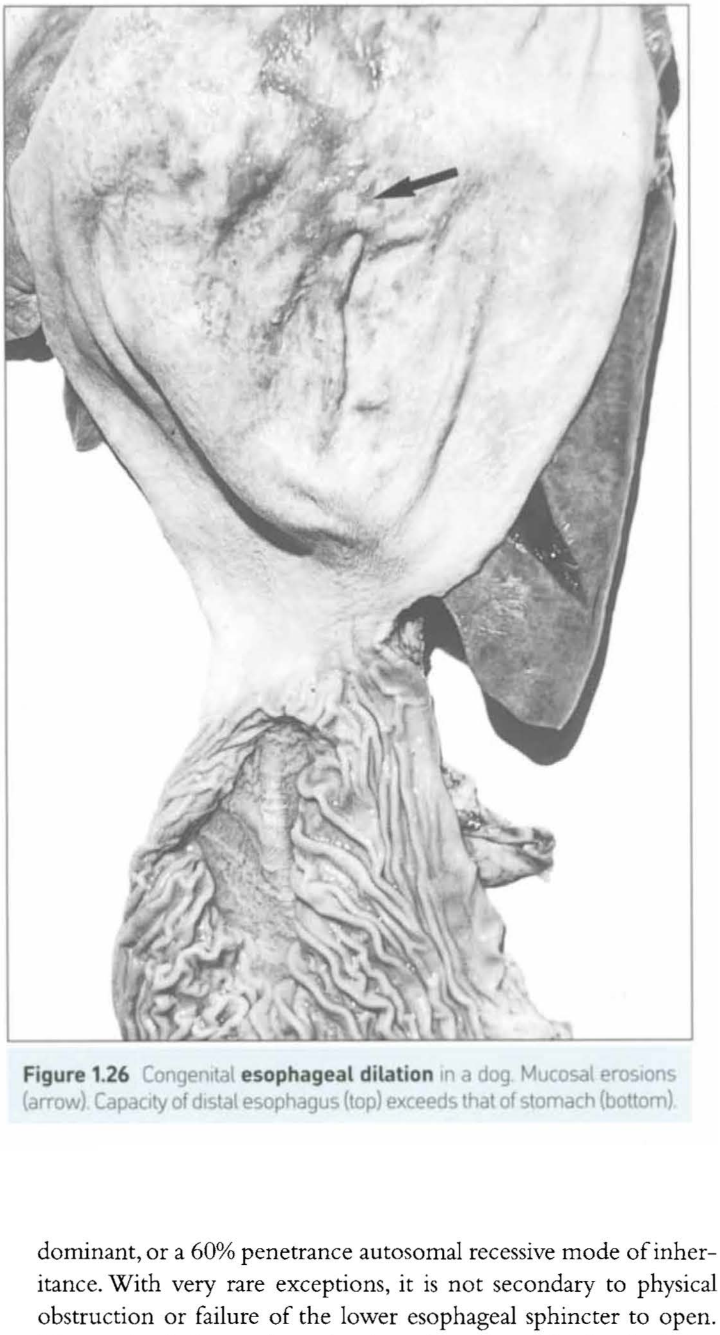

Figure 1.26 Congenital **esophageal dilation** in a dog. Mucosal erosions (arrow). Capacity of distal esophagus (top) exceeds that of stomach (bottom).

dominant, or a 60% penetrance autosomal recessive mode of inheritance. With very rare exceptions, it is not secondary to physical obstruction or failure of the lower esophageal sphincter to open. Motor stimulation to the striated esophageal muscle, despite being carried in the vagus, is not autonomic, and seems intact. There is strong evidence that CIM results from a selective defect in the distension-sensitive afferent autonomic arm of the reflex that coordinates esophageal function.

Most cases of idiopathic megaesophagus in dogs are not comparable to **esophageal achalasia** (failure of the gastroesophageal sphincter to relax) in humans. One case of true esophageal achalasia without esophageal motility disorder has been reported in a dog.

Idiopathic megaesophagus may also develop in *mature dogs*, in which, in some studies, the majority of cases of megaesophagus are reported to occur. In addition, megaesophagus may be acquired secondary to glycogen storage disease in Lapland dogs, myasthenia gravis, administration of cholinesterase inhibitors, hypoadrenocorticism, canine giant axonal neuropathy, immune-mediated polymyositis, polyradiculoneuritis, canine distemper, systemic lupus erythematosus, lead poisoning, Chagas disease, and snake envenomation.

Megaesophagus in the **cat** may be congenital, with signs appearing about weaning, and it seems most common in the Siamese breed. The pathogenesis is unclear. Neuronal degeneration or neurogenic atrophy of muscle is not recognized in the esophageal wall. Megaesophagus in cats has also been associated with functional pyloric stenosis, hiatus hernia, and lead poisoning.

Several **foals** have been reported with esophageal dilation or ectasia, apparently congenital. Dilation of the proximal portion, with a normal or thickened distal thoracic esophagus, was found in two cases. Examination of the wall, which is smooth muscle in the distal half, revealed equivocal muscle abnormalities, and no anomalies of the autonomic ganglia in those animals. However, aganglionosis has been implicated in megaesophagus in a third case. Megaesophagus may also be acquired in foals with ulceration and fibrosis of the cardia and lower esophagus, associated with ulceration of the pars esophagea of the stomach.

Acquired megaesophagus in **cattle** has been associated with hiatus hernia, and with pharyngeal trauma presumably causing vagus nerve damage. Segmental megaesophagus is reported in the cervical region, in one case associated with marked fatty replacement of esophageal myofibers. Megaesophagus is rarely reported in small ruminants.

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Parasitic diseases of the esophagus

Sarcosporidiosis occurs in the striated esophageal muscle of sheep. Esophageal sarcocysts appear as ovoid white thin-walled nodules ~1 cm long projecting from the esophageal muscle. *Sarcocystis gigantea*, the species producing large esophageal cysts and similar large cysts in skeletal muscle, is spread by cats. Microscopic sarcocysts of other species may also be encountered in esophageal striated muscle of a variety of hosts (see section on protistan infections, below, and Vol. 1, Muscle and tendon). Sarcocysts in esophageal muscle normally incite little or no local inflammatory reaction and are only of significance in meat inspection. However, lesions of *eosinophilic myositis*, possibly associated with rupture of cysts, are found in the esophageal muscle, and megaesophagus has been reported in a sheep with esophageal myositis associated with sarcocysts.

Larvae of ***Gasterophilus*** spp. may be temporarily attached to the caudal pharyngeal and cranial esophageal mucosa, and to the mucosa cranial to the cardia, in horses. Insignificant focal ulceration may occur at the sites of attachment.

The larvae of the warble fly ***Hypoderma lineatum*** reside for some time in the submucosa or adventitia of the bovine esophagus before they migrate to the dermis of the back. The easily overlooked translucent larvae may be only 2–4 mm long, but instigate local hemorrhage and neutrophil infiltration. *Hypoderma* assume significance in a small proportion of animals treated with systemic organophosphate insecticides while larvae reside in the esophageal wall. Acute inflammation, probably an allergic response to products of dead larvae, develops in the esophageal submucosa. This leads to swelling, hemorrhage, and necrosis, causing fatal esophageal obstruction, tympany, and esophageal perforation.

Spirurid nematodes of the genus ***Gongylonema*** may be encountered in the stratified squamous mucosa of the upper alimentary tract, including the esophagus, in ruminants and swine. These white threadlike worms up to 10–15 cm long burrow in the epithelium, and occasionally the propria, of the esophageal wall. They usually produce white or red blood-filled zigzag tracks in the mucosa (Fig. 1.27). Their presence is inconsequential to the host.

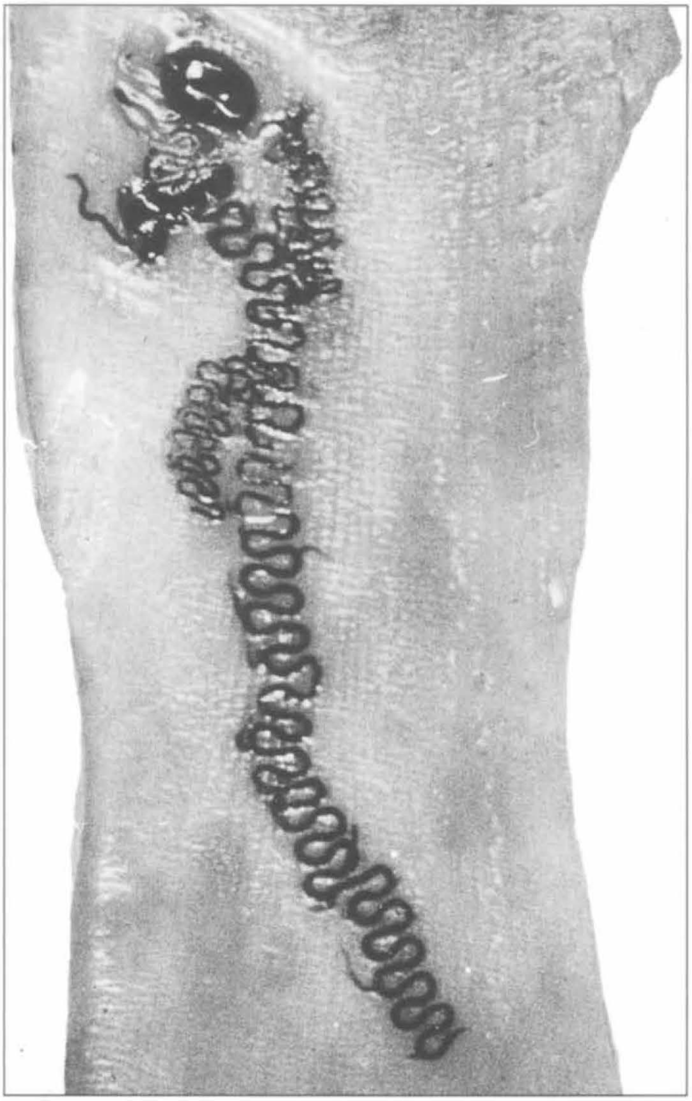

Figure 1.27 Blood-filled tracks and small hematoma in esophageal mucosa caused by ***Gongylonema pulchrum*** migration in a cow.

Spirocerca lupi is a spirurid nematode that parasitizes the esophageal wall of Canidae and some other carnivores. It is most common in warm climates where appropriate species of dung beetles act as intermediate hosts, and where the opportunity for dogs to obtain access to larvae in vertebrate transport hosts is high. The normal site for the adult nematode is in large, thick-walled cystic granulomas in the submucosa of the distal portion of the esophagus or gastric cardia, where one or more pink worms, surrounded by purulent exudate, are found. A fistula leading to the esophageal lumen is usually present, through which the tail of the female worm may protrude, and which provides the outlet for ova to the gastrointestinal tract (Fig. 1.28).

Third-stage larvae of *Spirocerca*, ingested with dung beetles or encysted in the insectivorous transport hosts, penetrate the gastric mucosa and move along arteries to the aorta. Here they migrate, often subintimally, forward to the caudal thoracic area, which they attain within several weeks of infection. Following 2–4 months in an inflammatory granuloma in the aortic adventitia, worms migrate to the subjacent esophagus where they develop to adulthood in the submucosa and perforate the epithelium. Larvae that adopt aberrant migratory pathways may be found in granulomas in sites such as the subcutis, bladder, kidney, spinal cord, as well as stomach and intrathoracic locations.

Aortic lesions associated with *Spirocerca* are described in Vol. 3, Cardiovascular system, but include intimal and medial hemorrhage and necrosis, with eosinophilic inflammation; intimal roughening with thrombosis; aneurysm with rare aortic rupture; intimal and medial mineralization and heterotopic bone deposition. The presence of persistent aortic lesions in the dog, even in the absence of esophageal granuloma, is evidence of prior infection with *S. lupi*. Spondylosis of the ventral aspects of vertebral bodies T5–10 occurs in some cases. Exostoses or bony spurs arise from one or both ends of the vertebral bodies, and are presumably instigated by the local irritant effects of migrating worms.

In some animals with *S. lupi*, *mesenchymal neoplasms* develop in the wall of the esophageal granuloma (Fig. 1.29), and pulmonary fibrosarcoma has been associated with an ectopic worm. The granulomas around *Spirocerca* contain highly reactive pleomorphic fibroblasts with large open nuclei and numerous mitotic figures. Neoplasms arising from such lesions have cytologic characteristics typical of

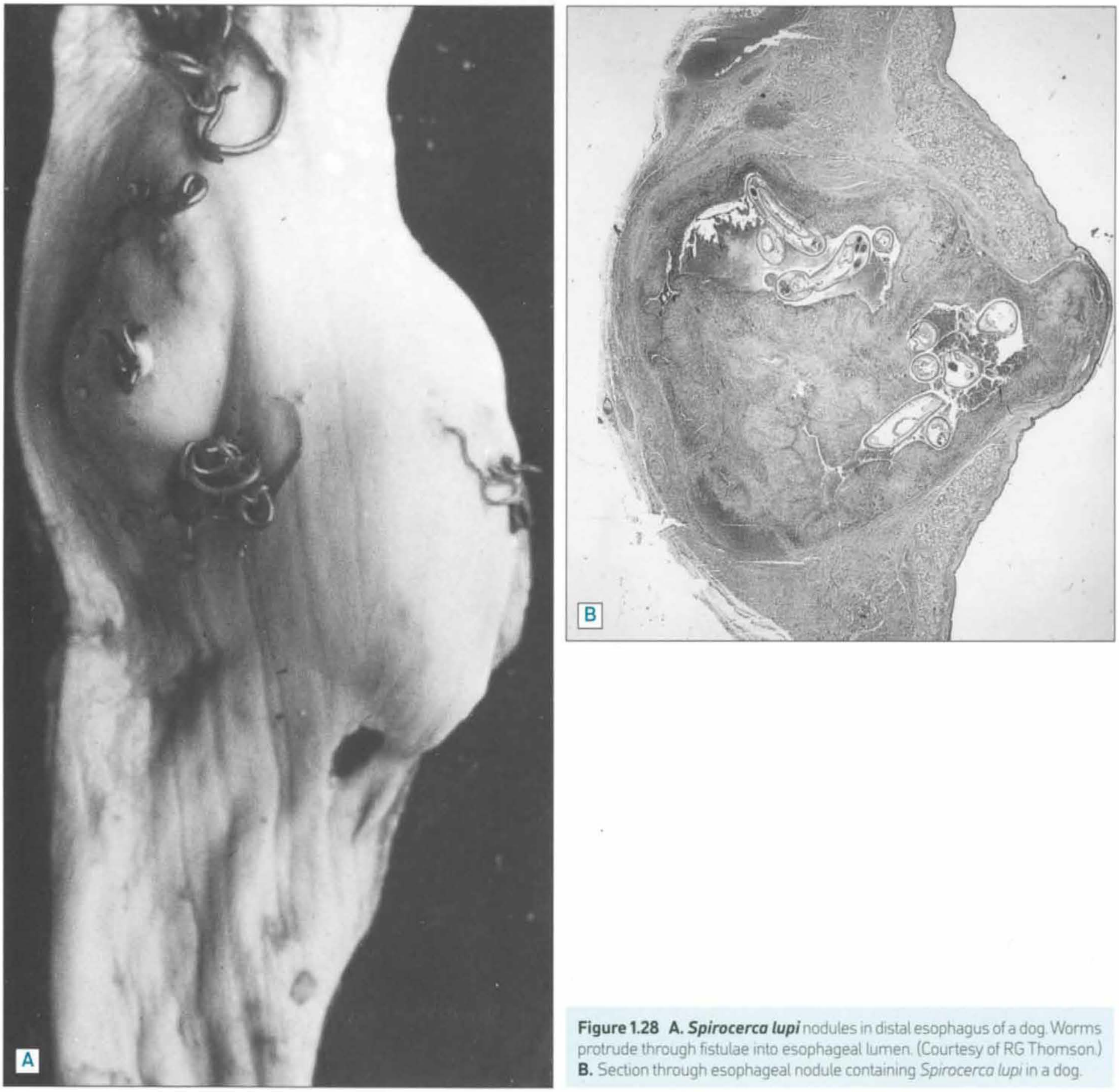

Figure 1.28 A. ***Spirocerca lupi*** nodules in distal esophagus of a dog. Worms protrude through fistulae into esophageal lumen. (Courtesy of RG Thomson.) **B.** Section through esophageal nodule containing *Spirocerca lupi* in a dog.

fibrosarcoma and osteosarcoma, with local tissue invasion, and in many cases, pulmonary metastasis. The carcinogenic stimuli associated with the development of these tumors are unknown. Hypertrophic pulmonary osteopathy is occasionally found in animals with *Spirocerca*-associated sarcoma and, rarely, granuloma.

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FORESTOMACHS

The rumen represents the central processing unit for ingesta and as such is critical to the animal's well-being. However, since most of this occurs as a chemical process with minimal morphologic

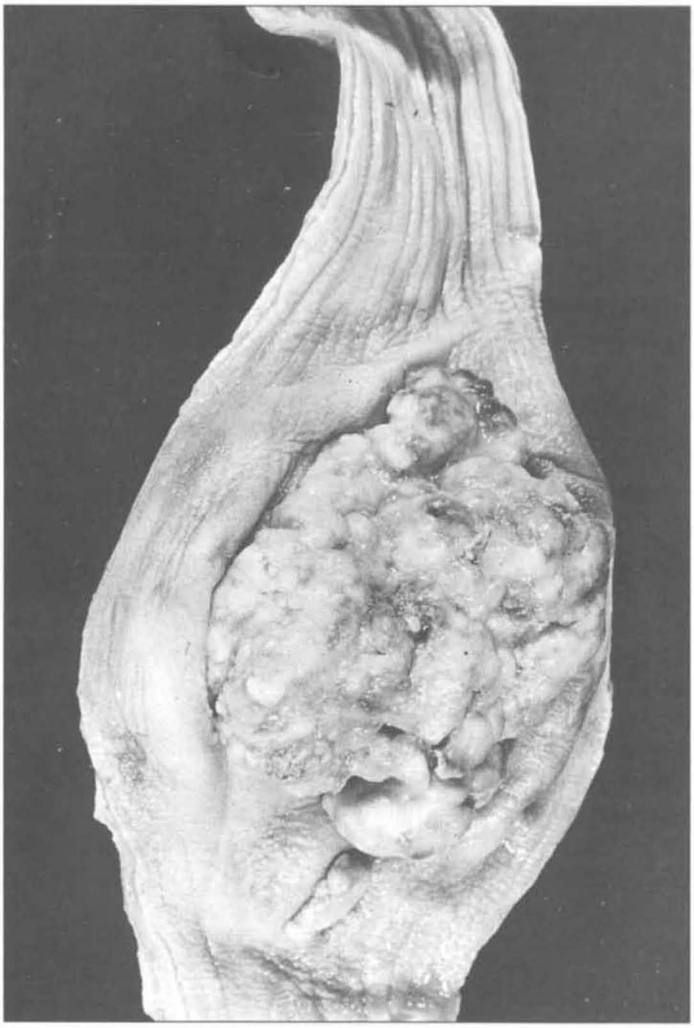

Figure 1.29 Ulcerating **fibrosarcoma** associated with ***Spirocerca*** granuloma in the distal esophagus of a dog. (Courtesy of RG Thomson.)

correlates, it is an organ that often seems to be overlooked by pathologists. Examination of ruminal contents may provide critical cues with respect to general metabolic states. Overly dry contents are an excellent indicator of dehydration. Voluminous frothy contents occur with primary bloat. Urea toxicity can be detected by an ammoniacal odor and alkaline pH. An odor of cooked turnips or a pungent insecticidal smell is suggestive of organophosphates. In grain overload, contents have a fermentative odor and pH may be less than 5.0. With putrefaction and release of toxic amines into the rumen, pH in cases of acidosis may return to near-normal levels. In these animals, the ruminal epithelium remains adherent to the submucosa despite any intervening postmortem changes, and it is an important clue to mucosal disease. Vagus indigestion, often associated with dysfunction of the esophageal groove, leads to accumulation of large volumes of watery fluid within the forestomachs. *Taxus* spp. toxicity will have an aromatic odor like cedar oil and needles will be present in the ingesta. Recognition of characteristic foliage in rumen content may lead to a diagnosis of poisoning by cyanogenic plants of the genus *Prunus*. Motor oil, paint flakes, or metallic lead present in the rumen would help to support a diagnosis of lead poisoning.

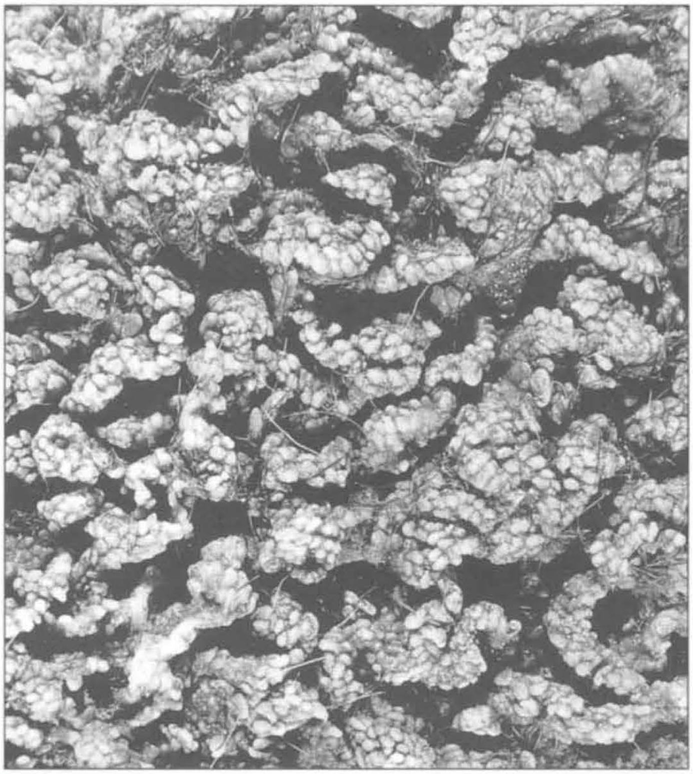

Figure 1.30 Clubbing and adhesion of rumen papillae with parakeratotic epithelium, associated with feeding a ration high in **barley**. Plant fibers and hairs are among the matted papillae.

Dystrophic and hyperplastic changes in the ruminal mucosa

The ruminal mucosa is unique in that it is a stratified squamous epithelium that functions in absorption as well as protection from the vat of fermenting bacteria contained within. Langerhans cells are distributed within the mucosa throughout all compartments of the rumen. T lymphocytes are found individually, and in aggregates, in both intraepithelial and subepithelial locations, with most in the cranial sac and relatively few dorsally.

Ruminal papillae in newborn calves are rudimentary and their subsequent development is dependent on diet, possibly as a consequence of stimulation by insulin-like growth factor-1. The end products of ruminal carbohydrate fermentation, propionate and butyrate, stimulate papillary growth. Papillae can take a variety of shapes, including long and flat, conical, spade-shaped, or hair-like. High-concentrate rations, which provide abundant propionate and butyrate, tend to produce papillae that are club-shaped, clumped, and may be dark. Microscopically, these papillae are covered with epithelium displaying acanthosis, hyper- and parakeratosis, and hyperpigmentation. Secondary papillae may be hyperplastic as well, creating the clumped appearance or rosette-like configurations. Rumens in animals fed barley rations have similar changes. Hairs from the rachilla of the barley adhere to the mucosa, especially in the interpapillary areas, giving it a distinct matted appearance (Fig. 1.30). These penetrate the mucosa and lamina propria, where they evoke a leukocytic reaction, often causing microabscesses. A diffuse pleocellular reaction is evident in the thickened fibrotic wall.

Roughage also plays a role. When animals receive adequate levels (approximately 15%) of coarse roughage, propionic and butyric acids decrease, while acetic acid levels increase, precluding hyper- and parakeratosis.

Hyperkeratosis of the ruminal epithelium also occurs in calves deficient in vitamin A.

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Postmortem change

The ruminal mucosal epithelium usually sloughs within a few hours after death. It separates from the lamina propria in large gray patches, which cover the ingesta when the rumen is opened. *Persistent firm attachment of the ruminal epithelium is abnormal.* This undue adhesion occurs in dystrophic changes, described earlier, in acute rumenitis, especially if caused by fungi, and about healed lesions of necrobacillary rumenitis. Adhesion may not occur in the early stages of ruminal acidosis.

Dilation of the rumen

Tympanitic distension of the forestomachs (tympany, hoven, bloat) may be acute, or chronic and recurrent. The acute or primary tympany of cattle fed legumes or high-concentrate rations is characterized by foaming of the rumen contents, which prevents gas from being eructated, whereas in chronic or recurrent (secondary) tympany, the gas is free but retained because of some physical or functional defect of eructation.

Primary tympany is also called *frothy bloat.* Foam production in ruminal contents occurs normally. However, the amount of foam produced is small and unstable. There is apparently a delicate balance between pro- and antifoaming factors. These factors are multiple, and there is considerable controversy as to the extent to which each one influences the production of the foamy, viscous ruminal content so characteristic of frothy bloat, the most common cause of rumen distension.

The formation of foam is dependent on *soluble proteins*, especially fraction I proteins, which are present in high levels (to 4.5%) in bloat-inducing legumes, such as alfalfa and clover, especially in the pre-bloom stage of growth. Other legumes, notably sainfoin, trefoil, and cicer milk vetch, are not associated with bloat. Soluble proteins, released from chloroplasts, are degraded by the rumen microflora, and they rise to the surface where they are denatured, become insoluble, and stabilize the foam. The optimal pH (isoelectric point) for foam production by soluble proteins ranges from 5.4 to 6.0. *Pectins* are considered to increase viscosity of ruminal fluid and may act as foam-stabilizing agents. Plant lipids may act as antifoaming agents by competing for metal ions with the soluble proteins, thus inhibiting the denaturation of these proteins and resulting in decreased foam production.

Excessive foam production causes distension of the rumen because it prevents formation of a free gas cap, and the clearing of the cardia, which is essential for normal eructation to take place. When foam enters the esophagus, it stimulates the swallowing reflex, which also interferes with normal eructation. Gassy froth accumulates in the rumen as a consequence.

The variation among animals in their susceptibility to bloat may be determined in part by variations in the amount and composition of *saliva*. Saliva apparently has properties which may promote or prevent foaming in the rumen. When secretion of saliva decreases, the viscosity of ruminal contents increases, which in turn promotes foaming. Cows that have high susceptibility to bloating produce less saliva than cows that have low susceptibility. Succulent and high-concentrate feeds reduce salivary secretion, thus increasing viscosity of rumen contents. The composition of saliva affects foam production in several ways. Combination of salivary bicarbonate with organic acids such as citric, malonic, and succinic acids, which are present in high levels in legumes, results in the production of large amounts of *carbon dioxide*, enhancing bubble formation. Carbon dioxide accounts for 40–70% of the total gas produced in the rumen.

High and low susceptibility to bloat can be temporarily transferred between animals by exchange of total reticulorumen contents. The understanding of the full role played by these various factors in bloat is still incomplete, and other factors may be involved.

Rations high in concentrate and low in roughage not only reduce saliva secretion, but also change the ruminal microflora. They promote the growth of large numbers of encapsulated bacteria, which increase the concentration of polysaccharides, and these, in turn, increase the viscosity, promoting foam. These bacteria are also often mucinolytic and may destroy salivary mucins. Perhaps this explains the more gradual onset of **feedlot bloat**, since it takes time for the ruminal flora to change. Particle size of the grains fed may be one factor in feedlot bloat, with smaller particle size predisposing more to bloat.

The *cause of death* in bloat is probably a combination of physical and metabolic effects. Increased intra-abdominal pressure on the diaphragm inhibits respiration, and adversely affects cardiac function. Hypoxia may be caused by respiratory embarrassment. Increased intra-abdominal pressure also has a marked effect on the hemodynamics of the abdominal viscera, which are compressed, driving blood out of them. The caudal vena cava is also compressed, decreasing venous return to the heart. Distension also affects mucosal permeability and alters vagosympathetic reflexes.

The bloated animal is often found dead and distended with gas; blood exudes from the orifices, and because of the gaseous distension, the carcass often rolls on its back and assumes a sawhorse posture, with forelegs extended forward and rear legs pointing backward. The blood is dark and clots poorly; both features are indicative of death due to anoxia. Subcutaneous hemorrhages are prominent in the cranial extremities, which are congested. There is marked edema, congestion, and hemorrhage of the cervical muscles and of the lymph nodes of the head and neck. An inconsistent but significant finding is the so-called *bloat line* in the esophageal mucosa (Fig. 1.31A). This lesion is formed due to congestion with petechial and ecchymotic hemorrhages in the mucosa of the cervical esophagus, which changes

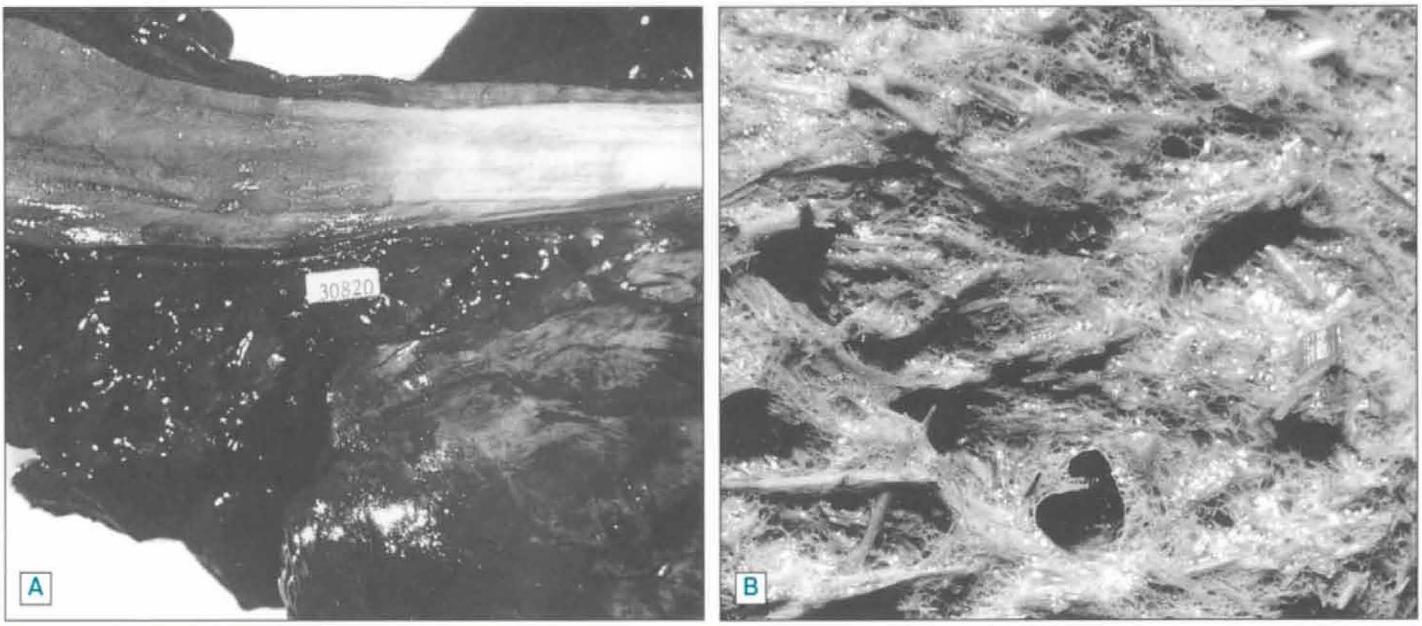

Figure 1.31 **A. "Bloat line"** in the esophagus of a cow with ruminal tympany. Congestion of esophagus and connective tissue cranial to thoracic inlet and blanching of esophagus caudal to thoracic inlet. **B. Frothy bloat** in a cow. Fine bubbles are evident in the rumen content.

abruptly or gradually to a pale mucosa at the level of the thoracic inlet. The tracheal mucosa is hemorrhagic, especially cranial to the thoracic inlet. Blood clots are frequently seen in the bronchi, and paranasal and frontal sinuses. The lungs are pale and compressed into the cranial thorax by the bulging diaphragm. There is pressure ischemia of the abdominal viscera, especially the liver, though the extreme margins of the hepatic lobes may be congested. Lymph nodes and the muscles of the hind legs are pale. There may be marked subcutaneous edema, particularly of the vulva, inguinal region, and perineum, and intestines may herniate through the inguinal canals. If the autopsy is done soon after death, the ruminal contents are bulky and foamy (Fig. 1.31B). The foam gradually disappears after death and is usually absent if the autopsy is delayed for 10–12 hours. Inguinal hernia and diaphragmatic rupture may occur after death.

Secondary tympany (free gas or secondary bloat) may be acute, but is generally chronic, with periods of acute exacerbation. It is usually the result of a physical or functional *defect in eructation of gas* produced by normal rumen fermentation. The more common physical problems include internal or external obstructions of the esophagus or esophageal groove by tumor, papilloma, or foreign body, and esophageal stenosis of any cause. Reticular adhesions, abscesses, peritonitis, or tumor masses that interfere with contractions of the forestomach can result in bloat. Functional causes of secondary tympany include organophosphate intoxication, and vagal damage due to adhesions, lymphosarcomatous infiltrates, or right-sided abomasal displacement and volvulus. It is a component of the syndromes collectively termed *vagus indigestion*. Bloat due to muscular dystrophy of the diaphragmatic muscles has been described in both Meuse–Rhine–Yssel and Holstein–Friesian breeds of cattle.

Secondary tympany, sometimes fatal, occurs in *bucket-fed calves*. They ingest large amounts of milk, which escapes the reticular groove and flows into the rumen, where it putrefies because of digestion by proteolytic bacteria. Failure of the reticular groove to close may be a persistent problem in some veal calves, which are known as *ruminal drinkers*. The clinical signs are characterized by inappetence, unthriftiness, recurrent tympany, abdominal distension, and clay-like feces. The presence of partly digested milk in the rumen results in hyper- and parakeratosis of the mucosa and perhaps mild rumenitis. Casein clot formation in the abomasum is inhibited.

Animals fed rations that have too much undigestible roughage may have recurrent episodes of bloat. Feed must contain adequate portions of protein, starch, and/or sugars, and cellulose to stimulate growth of the cellulytic microflora. Undigestible roughage accumulates in the rumen and reticulum when the intake of digestible nutrients (starches and sugars) is inadequate. As a result, the forestomachs become dilated, which in turn inhibits reticuloruminal contractions that are required for clearance of the cardia and subsequent eructation.

A *diagnosis of secondary bloat* at autopsy is based on physical findings like those described in primary bloat, but without frothy rumen content, and with the addition of any physical causes of impaired eructation. Postmortem distension of the rumen must not be mistaken for antemortem tympany. Extraruminal lesions must be present to establish a diagnosis of bloat.

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Foreign bodies in the forestomachs

Cattle are notoriously lacking in alimentary finesse, a deficiency that allows an amazing variety of foreign bodies, prehended with the food, to be deposited in the forestomachs. Sheep are largely immune because of their more selective eating habits. Foreign bodies are rarely found in the rumen of goats, despite their reputation for indiscriminate feeding habits. In consequence, a large proportion of adult cattle, and very few goats or sheep, have metallic, wood, or plastic foreign bodies in the rumen and reticulum, but rarely in the omasum.

Spherical masses consisting largely of hair or wool (**trichobezoars**) or plant fibers (**phytobezoars**) may also form in these compartments. Hairballs are most common in younger ruminants, the hair being swallowed after licking, particularly by animals deprived of dietary fiber. They may have some other foreign body as a nucleus and contain a proportion of plant fibers, the whole mass concreted by organic substances and inorganic salts. The same general comments apply to phytobezoars. Being smooth, neither are important unless regurgitated to lodge in the esophagus or passed on to obstruct the reticulo-omasal orifice, the pylorus, or the intestine, which is very infrequent. Otherwise, these bezoars are an incidental finding.

The important foreign bodies are those (such as lead) that cause *intoxication* when dissolved, and those that (being abrasive or sharp) *penetrate the mucosa*. In calves on diets low in roughage, ingestion of wood shavings or straw may lead to diffuse cellulitis of the forestomachs and sometimes the abomasum. A mixed bacterial flora containing clostridia is responsible, presumably following mucosal trauma. The sequel to penetration by sharp objects in adult cattle is traumatic reticuloperitonitis.

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Traumatic reticuloperitonitis and its complications

Perforation of the forestomachs by foreign bodies is virtually always caused by a sharp foreign body, usually a *wire or nail* over 4 cm long, penetrating the reticular wall. Incomplete perforation is usually inconsequential, though in some cases focal suppurative or granulomatous inflammation develops in the wall of the reticulum, with minor overlying peritonitis. There are no adequate answers as to why perforation occurs, but it is probably caused by forceful contraction of the reticulum, and many cases seem to be predisposed by the increased intra-abdominal pressure of late pregnancy and parturition.

The prophylactic use of *magnets* has become common in many herds, and this probably contributes to the marked decrease in fatal cases of traumatic reticuloperitonitis. Frequently, these magnets are found incidentally in the reticulum, completely covered by metal foreign bodies, including nails and wires, which might otherwise have penetrated the reticular wall. The replacement of baling wire with twine is another reason for the apparent decline in the prevalence of this disease.

The outcome of complete perforation is consistent, but variations in the pattern are common. The perforation is usually in the cranioventral direction and is followed immediately by *acute local peritonitis*. If the foreign body is short or bent, it may progress no further, and some foreign bodies are apparently withdrawn with the next reticular movement; in such instances, *chronic local peritonitis* with adhesions develops. The foreign body may advance to perforate the diaphragm and pericardium, resulting in *traumatic pericarditis*, but this advancement may be delayed.

A ventral penetration may result in subperitoneal and subcutaneous abscess near the xiphoid. Rare perforation of one of the larger regional arteries may result in sudden death from hemorrhage, and sudden death may also occur if there is penetration of the myocardium or rupture of a coronary artery. Penetration of the thoracic cavity may occur without perforation of the pericardium, causing pneumonia and pleuritis. Right lateral deviation of the penetrating agent involves the wall of the abomasum. It is unusual for the liver or spleen to be penetrated, but metastatic abscesses in the liver are common.

As soon as the foreign body penetrates the serosa, local fibrinous peritonitis develops, which later leads to dense adhesion of variable extent between the reticulum and adjacent structures. Further progression of the foreign body is ordinarily slow and produces a canal surrounded by chronic granulation tissue and containing, besides the foreign body, ingesta, purulent exudate, and detritus. The bacteria commonly active in the tract are *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, and a variety of putrefactive types. In many cases, a foreign body cannot be found, perhaps because it has rusted away or been withdrawn into the reticulum.

Traumatic pericarditis is a less common sequel now, perhaps because many of the initial penetrations are diagnosed and the foreign body removed surgically. The pericardial reaction is copious, fibrinopurulent, and putrid. There are usually additional lesions of traumatic pneumonia and pleurisy.

One of the variants in the usual pattern of migration of the foreign body is penetration of the side of the reticulum, leading to suppurative inflammation in the grooves between the reticulum, omasum, and abomasum. The acute local peritonitis causes immediate cessation of ruminal movements; however, persistent ruminal atony or irregular motility with gradual onset of bilateral abdominal distension, inappetence, and decreased milk production may ensue. Clinically this is referred to as **vagus indigestion**, which may also be a sequel to abomasal displacement. At autopsy, there are very characteristic changes in the stomachs with this syndrome. The rumen is distended with enough fluid to cause sloshing if the carcass is jolted. There is no ruminal fermentation or odor, and bits of unmacerated straw and food particles float on the watery fluid; the more-normal ingesta has sedimented. The omasum can be very large and impacted with dehydrated ingesta. The abomasum may be distended and impacted with dry ingesta, presumably because of functional pyloric stenosis or abomasal stasis.

The question of the importance of vagal nerve damage in the pathogenesis of vagus indigestion remains unresolved. The consensus is that this syndrome is associated with mechanical or functional impairment of outflow of ingesta from the forestomachs or abomasum, but in some cases the primary defect appears to reside in flaccidity of the reticular groove and in degeneration of its muscle and intramuscular nerve plexus. The rumen and reticulum are dependent on intact vagi for normal movement, and a minority of cases of vagus indigestion appear to be associated with damaged nerves.

Vagal lesions may be in the pharyngeal and cervical areas, or intrathoracic, such as lymphosarcomatous infiltration, or abdominal. The latter are usually investment of the nerve in adhesions following reticular perforation, or trauma following abomasal volvulus.

In other cases, degeneration of the vagus is not evident. In these, the dysfunction and lesions are more likely to be due to peritonitis and the subsequent abscessation, or adhesions which disrupt the normal tension–receptor activity, or cause a pain response that interferes with normal motility of the forestomachs and abomasum.

In **failure of omasal transport** (*type II vagus indigestion*), there is impairment of movement of ingesta from the reticulorumen to the omasum, associated with abscesses adjacent to the reticulo-omasal orifice. There, lesions probably result in mechanical or neural interference to emptying of the reticulorumen.

A *diagnosis* of vagal indigestion at autopsy is ordinarily dependent on evidence of abnormal abomasal, omasal, or reticuloruminal motility, in association with morphologic lesions of the vagus nerves, adhesions, or neoplasms involving the forestomachs and abomasum.

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Rumenitis

Inflammatory lesions in the forestomachs occur in a number of *viral diseases* of the alimentary tract in ruminants. In neonatal calves, necrosis of ruminal mucosa is an important sequel to *Bovine herpesvirus 1* infection. Bovine papular stomatitis and contagious ecthyma rarely cause rumen lesions. Ruminal erosions and ulcers are present in some cattle with acute bovine viral diarrhea and mucosal disease; they are reported to be less common in rinderpest. Extensive hemorrhage and ulceration of the reticuloruminal mucosa may be seen in bluetongue in sheep. Adenoviral infection occasionally causes multifocal fibrinohemorrhagic rumenitis. These conditions are described fully in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter. Mycotic rumenitis can occur subsequent to chemical rumenitis, primary viral rumenitis, sepsis, or intensive antibiotic treatment.

Mild inflammation of the forestomachs occurs in some young calves fed *milk* from a pail, when, because of laxity of the reticular groove reflex, the milk spills into the rumen and reticulum in large quantity. A similar problem occurs with feeding by stomach tube. Putrefaction in these compartments leads to mild rumenitis, with edema and mild neutrophil infiltration of the mucosa.

Accidental consumption of excessive quantities of *urea*, in the form of nonprotein nitrogen supplement, or fertilizer, in liquid or powder form, results in the production of ammonia in the rumen. The toxic effect is accelerated by urease in soy-based rations, and is based on the production of high blood levels of ammonia. Rumen contents smell ammoniacal when the organ is opened; the content is alkaline; and there may be congestion or coagulative necrosis of the cranioventral wall of the rumen. Elevated ruminal and abomasal pH values (7.0), without specific lesions, have been associated with ingestion of boron fertilizer by cattle and goats.

Ingestion of toxic levels of *sulfur* results in chemical rumenitis due to the conversion of sulfur to H_2S, and possibly sulfurous acid, in the rumen. Large amounts of yellow sulfur particles are usually found in the rumen and abomasum. Eructation and subsequent inhalation of H_2S result in acute alveolitis. Absorption of sulfides from the lungs leads to marked depression of the respiratory and cardiovascular centers in the central nervous system. Affected animals also develop acidosis, probably due to the absorption of acids, and impaired renal function associated with the direct toxic effects of sulfur metabolites on tubular epithelial cells.

Inflammation of the forestomachs may be associated with certain *plant toxicoses*, mainly in Australia and Africa. Examples are Kikuyu grass (*Pennisetum clandestinum*), prickly paddy melon (*Cucumis myriocarpus*), and several species of *Bryophyllum*, an ornamental garden plant.

Feeding rations deficient in fiber, which is increasingly the case in developed countries, can alter the microflora, predisposing the animals to metabolic disorders or rumenitis. In addition, acute chemical rumenitis develops after overeating on rapidly fermentable carbohydrate, usually grain.

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Rumenitis and acidosis caused by carbohydrate overload

Ruminal acidosis and rumenitis associated with ingestion of excess carbohydrate are problems mainly of *intensive beef and dairy production*. Sheep, and especially goats, are also susceptible to this problem. Its importance lies partly in loss of production and partly in mortality due to the acute disease, in which rumenitis is of minor significance and lactic acidosis is the major cause of morbidity and mortality. Rumenitis assumes significance in subclinical disease or in survivors of acute episodes, by providing a portal for the entry for fungi and *Fusobacterium necrophorum*, which cause secondary infections. These complications are discussed below. Other complications include coexisting primary tympany (frothy bloat), which may be the fatal partner of grain overload in feedlot cattle.

Ruminal acidosis usually follows the *ingestion of excess carbohydrate* in the form of grain, or other fermentable feedstuffs occasionally used, such as root crops, bread, waste baked goods, brewers' waste, and apples. There is wide variation in the amount of carbohydrate necessary to kill an animal, because tolerance to rations high in starch does develop if they are introduced gradually. Sudden increments in the amount of carbohydrate ingested are of more importance than the actual amount. Sudden changes from concentrates with lower energy values to those with higher values may predispose to acidosis. Extreme environmental temperature changes, either hotter or cooler, may result in temporary reductions in feed consumption, and acidosis may develop once such animals return to full feed.

Shortly after the ingestion of a toxic amount of carbohydrate, ruminal pH begins to fall. The decrease in pH during the first few hours is mainly due to an increase in dissociated volatile fatty acids, not lactic acid. The production of the latter increases after there has been a marked change in the ruminal flora, which is very responsive to the substrate available for fermentation. *In cattle and sheep, the normal pH of ruminal fluid varies between 5.5 and 7.5*, depending on the diet fed.

The gram-negative bacteria that predominate in the normal flora, and the protozoa, are very sensitive to changes in the pH; most die at a pH of 5.0 or less. Once the pH of the ruminal contents starts to fall, streptococci, mainly *Streptococcus bovis*, proliferate rapidly, acting as a major source of lactic acid. When the pH reaches 5.0–4.5, the numbers of streptococci decrease, with a concomitant increase in lactobacilli. The pH of rumen content may fall as low as 4.0–4.5 in fatal cases.

As ruminal pH drops, ruminal atony develops, mainly as the result of an increase in the concentration of the nondissociated volatile fatty acids, lactic, propionic, and butyric. They act on receptors that mediate inhibition of reticuloruminal motility via a vagovagal reflex. Loss of forestomach motility in ruminal acidosis is apparently not dependent on the development of systemic acidosis. There is also cessation of salivary secretion, so that the buffering effect of saliva is absent.

The increase in ruminal organic acids, mainly lactate, causes an increase in ruminal osmotic pressure. This results in movement of fluid from the blood into the rumen, producing bulky and liquid ruminal contents and severe dehydration. Plasma volume is reduced; hemoconcentration, anuria, and circulatory collapse follow. Serum protein levels, urea, inorganic phosphorus, lactate, pyruvate, and liver enzymes are all elevated. The osmotic pressure of the intestinal contents also increases when the ingesta with the high lactate concentrations arrives there. Loss of fluid at this level probably contributes further to the dehydration, and it may also play a significant role in the development of the diarrhea that is commonly seen clinically. In addition to the osmotic effects, there is acidosis due to the absorption of lactate from the rumen, and possibly from the intestine.

The low ruminal pH is lethal to much of the normal flora and fauna. The protozoa appear to be particularly sensitive, but many types of bacteria are also lost. In those animals that survive the acute phase of ruminal acidosis, recovery is not complete until a normal ruminal flora is re-established through contact with other animals. Temporary recovery may be followed by what appears clinically to be a relapse in acidosis, but which is a developing mycotic rumenitis. If treatment of the initial fluid imbalance is delayed, death may occur in a week or so from ischemic renal cortical necrosis.

The **gross findings** in this metabolic disease are not specific, and a practical diagnosis requires knowledge of access to fermentable carbohydrate and a clinically observed circulatory failure. At autopsy, the eyes are sunken, the blood may be thick and dark due to dehydration and hypoxia, and there is general venous congestion. The appearance of the ruminal contents varies with the time interval between ingestion of the carbohydrate and the autopsy. In the early stages, there is a copious amount of porridge-like rumen content, which has a distinct fermentative odor. The amount of grain, corn, or other source of starch varies considerably and is an unreliable indication of acidosis, and the presence of finely ground concentrate may be overlooked. Ruminal pH is only helpful when it is low (<5.0), since it may rise in later stages of the disease. Although the ruminal contents may appear relatively normal in more advanced cases of acidosis, intestinal contents tend to remain very watery. Absence of protozoa is consistent with chemical rumenitis, but is also influenced by the interval between death and the postmortem examination.

The **diagnosis** of ruminal acidosis at autopsy can be difficult. The most suggestive abnormality is the *rumenitis*. It is probably chemical and dependent on the low pH, and is not readily discerned grossly. There may be a slight poorly defined blue coloration in the ventral sac of the rumen and reticulum and in the omasum, visible through the serosa. When the epithelium is detached, the lamina propria is seen to be hyperemic in patches. In some cases, the epithelium appears to have undergone fixation due to low pH, and is difficult to peel.

Microscopic examination of the ruminal mucosa is the most reliable way of confirming a diagnosis of chemical rumenitis. The ruminal papillae appear enlarged. There is marked cytoplasmic vacuolation of the epithelial cells, often leading to vesiculation. A mild to marked neutrophilic reaction is evident in the mucosa and submucosa (Fig. 1.32). Focal areas of erosion and ulceration may or may not be present.

Fusobacterium necrophorum is a normal inhabitant of the anaerobic ruminal environment. This bacterium is commonly responsible for complications of ruminal acidosis, producing characteristic lesions in the forestomachs (Fig. 1.33A, B) and in the liver. Invasion of the wall of the rumen takes advantage of the foothold provided by the superficial necrosis and inflammation of acidosis. Inflammatory changes favor the adherence of *F. necrophorum* to ruminal lining cells.

Necrobacillary rumenitis is common in feedlot cattle, probably a product of mild acidosis following a too-rapid introduction to a high-concentrate ration. It affects the papillated areas of the ventral sac and occasionally the pillars. On the mucosal surface, the early lesions are visible as multiple irregular patches 2–15 cm across, in which the papillae are swollen, dark, slightly mushy, and are matted together by fibrinocellular inflammatory exudate. Affected papillae are necrotic, but ulceration may be delayed if there is ruminal atony and stasis. If the animal recovers from the immediate effects of overeating, the necrotic epithelium sloughs, the ulcer contracts, and epithelial regeneration begins from the margins. The regenerated epithelium is flat and white, and the papillae do not return completely. A stellate scar often remains, but many of the smaller lesions

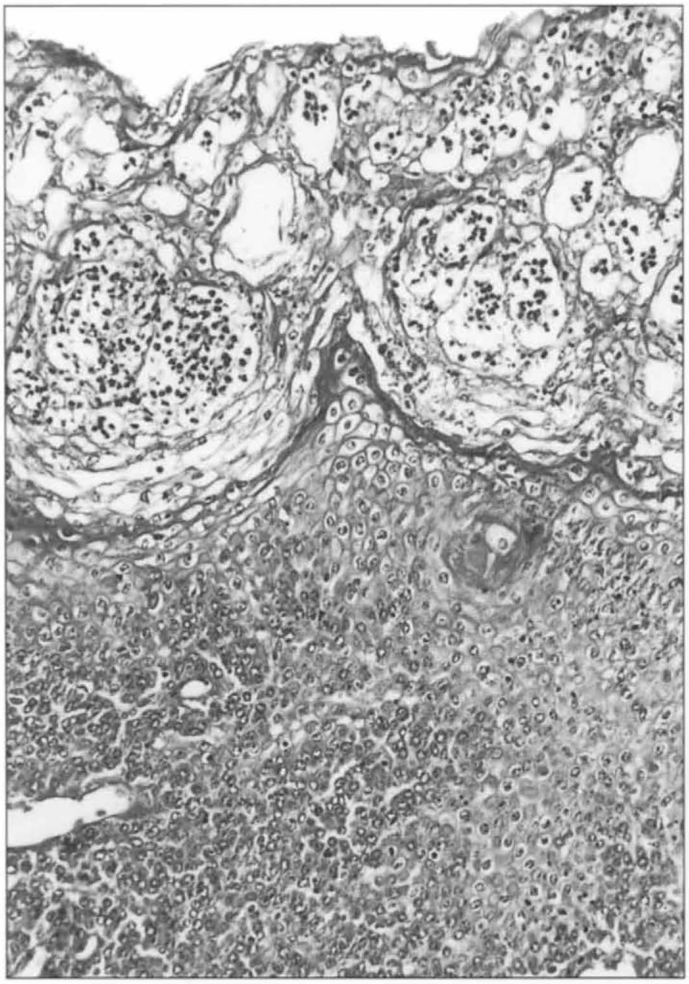

Figure 1.32 Vacuolation and neutrophil infiltration into superficial epithelium of rumen papilla in **chemical rumenitis** (ruminal acidosis) due to excess carbohydrate intake.

may disappear completely (Fig. 1.33C). *Hepatic lesions* are initially the typical coagulative necrosis of necrobacillosis, but in time they liquefy to form abscesses, and these often persist long after the initial ruminal lesions have cicatrized or disappeared.

It is unusual for ruminal necrobacillosis in cattle to be more than a superficial infection, and although the muscle layers are involved in the inflammation, they are not ordinarily invaded by the organism. However, perforation of the omasal leaves is common. In sheep, the infection is more aggressive than it is in cattle.

Mycotic infection should be suspected when inflammation in the wall of the forestomachs extends to the serosa and is *hemorrhagic.* The fungi, which, like *F. necrophorum*, are opportunists, are usually zygomycetes of the genera *Mucor*, *Rhizopus*, and *Absidia*, which cannot be differentiated from each other in histologic sections.

Mycotic rumenitis is much more severe and extensive than necrobacillary rumenitis, and is often fatal. *The basis for the lesion is submucosal venular thrombosis due to fungal invasion*, causing venous infarction of the tissue field involved. The inflammation extends to the peritoneum, causing hemorrhagic and fibrinous peritonitis that mats the omentum to the rumen. In fatal cases, most of the ventral sac and parts of the reticulum and/or omasum are involved. The lesions are very striking and suggest on initial inspection that the walls have been massively infarcted, which in part they have (Fig. 1.34A). The margins are well demarcated, usually by a narrow zone of congestive swelling. The affected areas are red to black, thickened to 1 cm or more, firm and leathery. There is acute fibrinohemorrhagic inflammation of the overlying peritoneum, and beneath it in the grooves there is blood-stained, inflammatory edema.

On the inner surface of the rumen, the lesions are more hemorrhagic than those of necrobacillosis, and more irregular in outline (Fig. 1.34B), and the necrotic epithelium is difficult to detach. Histologically, the rumenitis is characterized by hemorrhagic necrosis of all structures in the wall; by copious fibrinous exudate; and by rather scant leukocytic reaction. *Fungal hyphae* are readily visible in the necrotic tissues and the lumina of the thrombosed blood vessels. More chronic cases are characterized by granulomatous inflammation in the deeper parts of the mucosal lesion.

Mycotic rumenitis and omasitis may occur in cows that do not have a history of acidosis. It has been suggested that these cases may be a sequel of sepsis, with reflux of abomasal fluid into the forestomachs, and therapy with broad-spectrum antimicrobials acting as predisposing factors for mycotic infections. It can also be a sequel to ruminal damage in survivors of *Bovine viral diarrhea virus* infection.

Metastases sometimes occur in the liver and cause necrotizing thrombophlebitis of the portal radicles visible as small irregular tan areas of infarction surrounded by a deep red margin (Fig. 1.35).

Other conditions which have been associated with ruminal acidosis are *laminitis* and an *encephalopathy* which morphologically resembles the lesions of early polioencephalomalacia (see Vol. 1, Skin and appendages; and Vol. 1, Nervous system).

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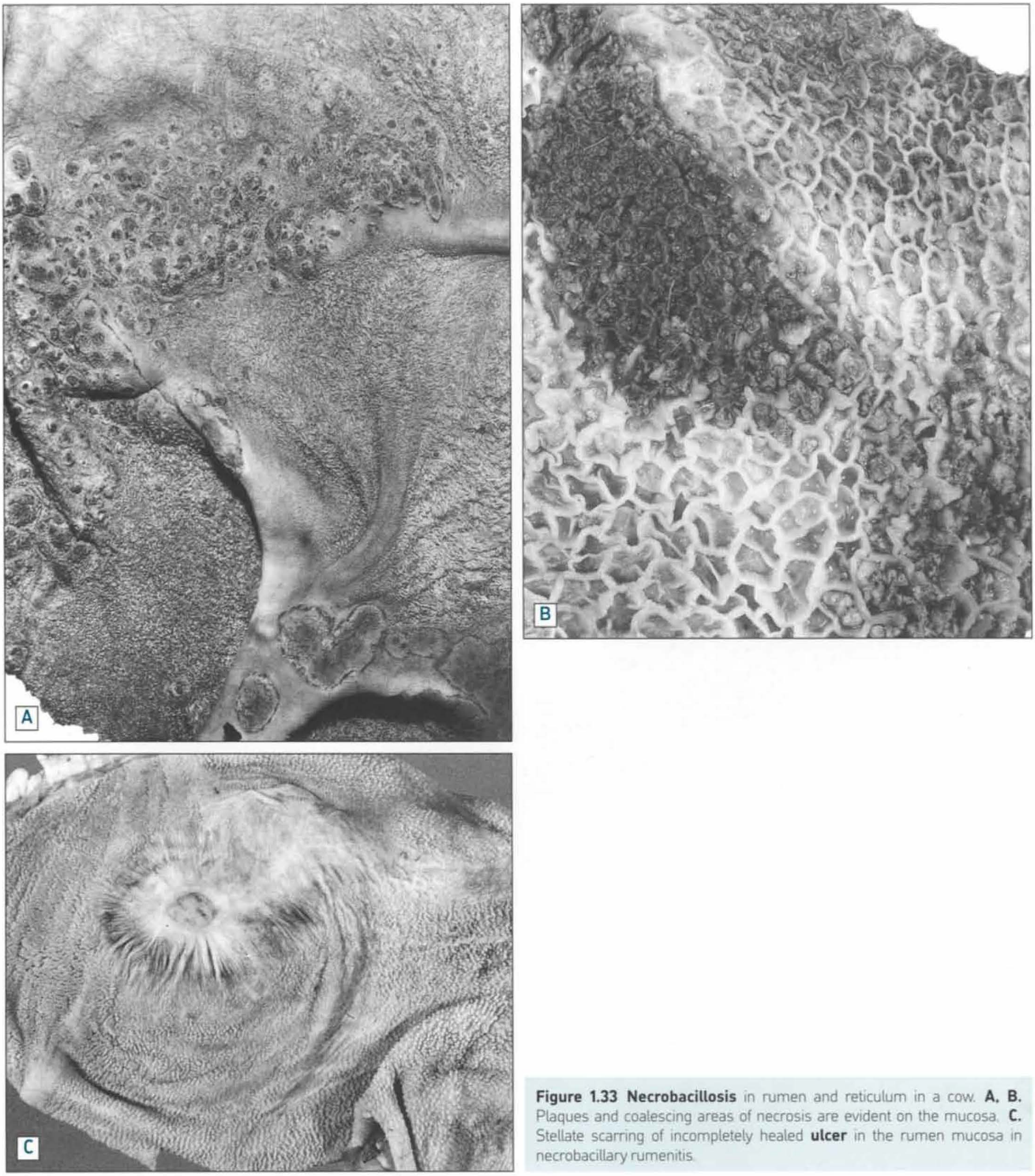

Figure 1.33 Necrobacillosis in rumen and reticulum in a cow. **A, B.** Plaques and coalescing areas of necrosis are evident on the mucosa. **C.** Stellate scarring of incompletely healed **ulcer** in the rumen mucosa in necrobacillary rumenitis.

Parasitic diseases of the forestomachs

Gongylonema spp. occur in the epithelium of the rumen. They appear as described in the esophagus, and are not pathogens.

More important parasites are the conical **flukes** belonging to the family Paramphistomatidae. They are found in cattle and sheep in warm temperate, subtropical, and tropical regions. These red, plump, droplet-shaped flukes are about the size of the papillae between which they reside in the rumen, where they are usually nonpathogenic (Fig. 1.36). If present in very high numbers, they can cause atrophy of papillae and excessive cornification of the stratum corneum and granulosum. Larval paramphistomes in the duodenum

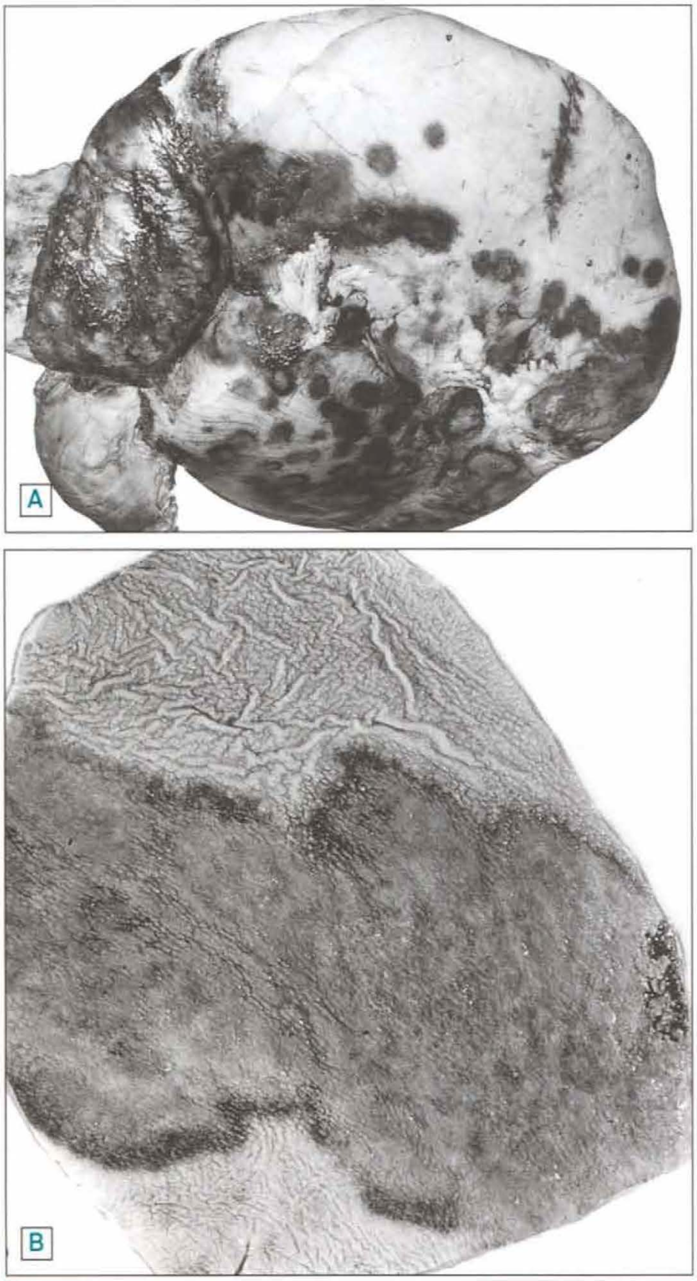

Figure 1.34 Mycotic rumenitis (*Aspergillus* and *Rhizopus*) following acidosis in a cow. **A.** Dark areas of infarction involving rumen and reticulum. **B.** Appearance of mucosal surface of rumen; superficial necrosis overlies congested submucosa.

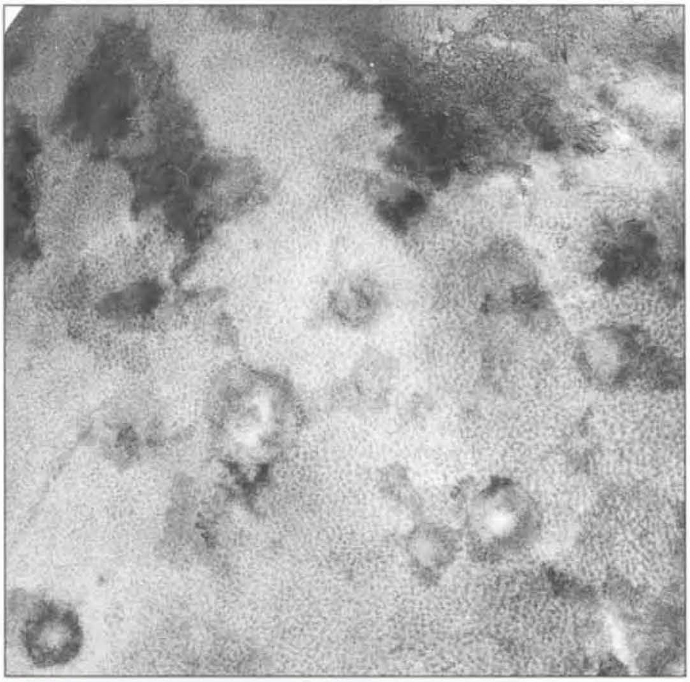

Figure 1.35 Hepatic necrosis in a cow, due to metastasis of fungi via portal circulation from primary foci of zygomycotic rumenitis.

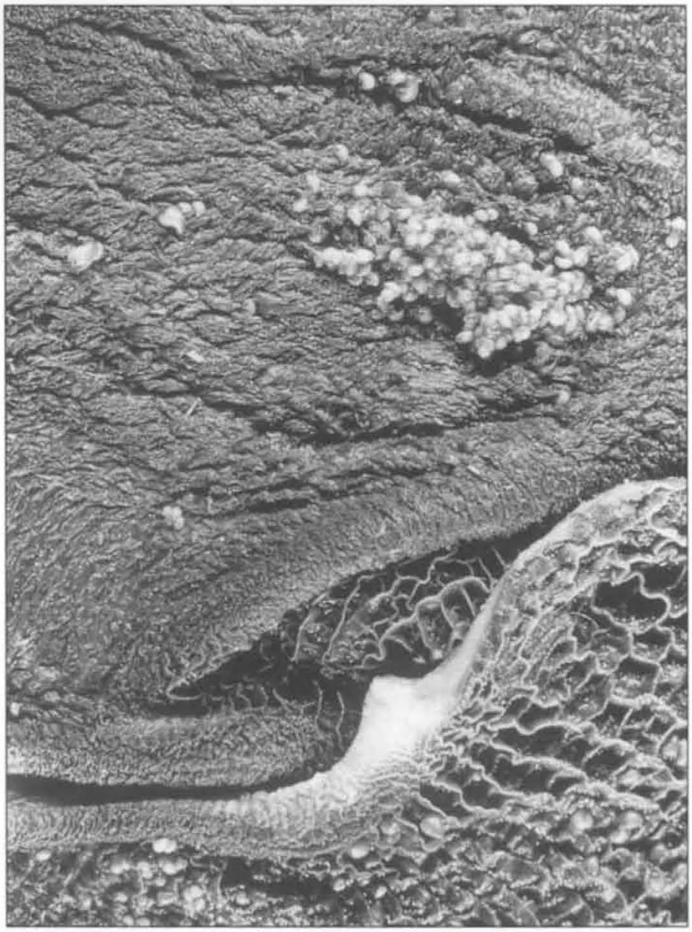

Figure 1.36 *Paramphistomum* spp. **flukes** on the mucosa of the reticulorumen.

can cause disease. The biology and pathogenicity of paramphistomes are discussed in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Myiasis of the rumen caused by larvae of the **"screwworm" fly** *Cochliomyia hominivorax* is occasionally a cause of mortality in young calves in South America. The larvae are presumed to be licked from cutaneous wounds and swallowed. They lodge in the rumen and perforate it.

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Neoplasia of the esophagus and forestomachs

Neoplasia of the esophagus and reticulorumen is, with the exception of papilloma, rare in domestic animals.

Papillomas of the esophagus in **dogs** are uncommon and may be associated with oral papilloma. In **cattle**, papillomata of the esophagus and reticulorumen are common in some areas. They are caused by *Bovine papillomavirus type 4* (BPV-4), which infects only squamous mucosa of the mouth, pharynx, and upper alimentary tract. Bovine alimentary papillomata are usually solitary, though a minority of infected animals may have multiple lesions. Most are small (<1 cm), broadly pedunculate tapering acuminate masses. They are composed of a number of closely packed fronds of squamous epithelium, each supported by a light core of fibrous stroma, and arising from a common fibrous base. Viral replication and the microscopic changes in infected epithelium are typical, as described earlier for canine oral papillomas, although the characteristic koilocytes and intranuclear inclusions can be sparse.

Fibropapillomas, limited to the esophagus, esophageal groove and rumen, are caused by *Bovine papillomavirus type 2* (BPV-2), normally associated with cutaneous papillomas and fibropapillomas. Alimentary fibropapillomas are smooth nodular pearly white masses, usually about 0.5–1.0 cm in diameter, but occasionally up to 3.0 cm and plaque-like. They are comprised of fibromatous stroma covered by acanthotic epithelium, which may occasionally be ulcerated. No evidence of expression of BPV-2 is found in alimentary fibropapillomas, the viral genome being identified by molecular probes.

Papillomas and fibropapillomas are normally asymptomatic, though large lesions of the reticular groove and esophagus may interfere with eructation and deglutition, causing bloating or loss of condition.

Malignant neoplasms of the esophagus and forestomachs in ruminants are ordinarily extremely rare. However, in several localities, **squamous cell carcinoma** is relatively common in **cattle**, associated with BPV-4-induced papilloma (but not with fibropapilloma due to BPV-2). However, BPV-4 viral antigens or genome are not detected in these carcinomas. An interaction between papillomavirus and ingestion of carcinogens in bracken fern predisposes to the development of squamous cell carcinomas of the esophagus and forestomachs in the hill country of Scotland and northern England. Immunocompromise due to bracken fern intoxication is permissive of chronic papillomatosis, and a cofactor in neoplastic transformation to squamous carcinomas. In Brazil and Bolivia, a similar association is made with carcinomas of the oropharynx and esophagus. A high prevalence of carcinoma of the esophagus and forestomachs has also been reported from a single valley in Kenya, in association with papillomata, not confirmed as viral, and with a carcinogen apparently ingested with or derived from native forest plants.

Esophageal and ruminal carcinomas are associated with dysphagia or difficult deglutition, ruminal tympany, and apparent abdominal pain with progressive cachexia. Concurrent papillomas, carcinomas, and hemangiomas of the bladder, like those causing enzootic hematuria, but associated with BPV-2, are often found in cattle with esophageal or ruminal cancer. In Scotland, intestinal adenomas or adenocarcinoma also occur in many cases.

Esophageal and ruminal carcinoma may be seen developing from recognizable papillomas; as brown irregular roughened hyperplastic epithelium; or as ulcerated or irregular proliferative fungating lesions. Distal esophagus, reticular groove, and the adjacent ruminal wall are the sites most commonly affected with carcinoma. Microscopically, they are typical squamous cell carcinomas, and invade locally, their scirrhous nature causing induration of the wall of the organ. They may metastasize to local lymph nodes, and to distant sites such as liver and lung.

In **sheep**, papillomas and squamous cell carcinomas are rare, and little is known of their etiology.

Squamous cell carcinomas may also be encountered rarely in the esophagus of aged **cats**, where they develop in the mid-thoracic portion, forming proliferative plaques of neoplastic cells that eventually ulcerate and invade the wall of the esophagus and adjacent mediastinum. In **horses**, squamous cell carcinoma of the stomach may also involve the adjacent terminal esophagus. Rare squamous cell carcinoma, and adenocarcinomas arising from the esophageal glands, are reported in **dogs**. Invasion of, or metastasis to, the canine esophagus by thyroid, respiratory, and gastric carcinomas is also reported.

Mesenchymal tumors of the esophagus, with the exception of the *Spirocerca*-associated fibrosarcomas and osteosarcomas in **dogs**, referred to previously in the section on parasitic diseases of the esophagus, are very rare. However, leiomyoma, osteosarcoma, and plasmacytoma have been reported in dogs. Connective tissue tumors of the **ruminant** forestomachs are similarly rare, though fibromas of the reticular groove have been reported. Occasional involvement of the rumen, omasum, and reticulum may occur in cattle with lymphosarcoma, usually also involving the abomasum and more distant sites.

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STOMACH AND ABOMASUM

Normal form and function

The stomach should be carefully examined in animals of any species with a history of inappetence or anorexia, cachexia, hypoproteinemia, diarrhea, regurgitation, or vomition. Abdominal distension may be associated with gastric dilation or displacement. Hematemesis, melena, or anemia may signify gastric bleeding. Many infectious diseases, with major systemic or alimentary tract signs elsewhere, produce gastric lesions. Systemic states such as uremia and endotoxemia cause characteristic gastric lesions in some species.

In the horse and pig, an obvious smooth white or yellow **esophageal region** is present. It is covered by stratified squamous epithelium, with susceptibility to insult and reparative capacity similar to that of the esophageal lining. Chronic inflammatory infiltrates and lymphoid follicles are normally present in the lamina propria and submucosa of the **cardiac gland mucosa** abutting the esophageal region, especially in the pig. The cardiac gland zone is gray and is particularly well developed in this species, lining the gastric diverticulum, fundus, and about half the body of the stomach. In the dog, cat, and ruminant, cardiac glands are limited to a narrow zone at the cardia or omasal opening. Cardiac glands are branched tubular structures, lined almost exclusively by columnar mucous cells.

The **fundic, or oxyntic, gland acid-secretory mucosa** in the horse and pig is red-brown and slightly irregular but not highly folded. More prominent longitudinally oriented rugae, or plicae, are present in the dog and cat, and in the abomasum of ruminants. Gastric secretion undiluted by ingesta in the dog or cat normally should be <pH 3.0. Abomasal content should be <pH 3.5–4.0.

Tall columnar mucous cells cover the gastric surface, and line pits or foveolae. The junction of the base of the foveola and the upper portion of the neck of the fundic gland proper is termed the isthmus. Cuboidal or low columnar pluripotential stem cells in a narrow zone in this area undergo mitosis. Three lineages are identified: (1) pit (foveolar) cells; (2) parietal cells; and (3) zymogen (chief) cells. Daughter cells of the pit cell lineage differentiate into **foveolar mucous cells**, migrating up on to the gastric surface, where they are lost, probably in about 4–6 days. The neck of the oxyntic gland below the isthmus is lined by pyramidal, peripherally located acid- and intrinsic factor-producing parietal cells. Interspersed are inconspicuous mucous neck cells, mainly in the upper neck and probably stages in the differentiation of chief, and perhaps, parietal cells, and scattered endocrine cells. In the base of the gland the pepsinogen-producing zymogen, or chief, cells are concentrated.

Mucous neck cells, like foveolar mucous cells, contain periodic acid–Schiff-positive mucus. **Parietal cells** differentiate from cells proliferating at the isthmus, and appear to be relatively long-lived, that is, of the order of weeks to months. A complex tubulovesicular/canalicular structure upon which the hydrogen ion-secretory proton pump is inserted opens at the luminal apex of the cell in the secretory state. A number of long-lived **enteroendocrine cells,** derived from proliferative cells at the isthmus, are recognized in the oxyntic gland, secreting histamine, serotonin, and somatostatin, among other endocrine/paracrine agents. Endocrine cells usually abut the basement membrane of the gland, lack exposure to the gland lumen, and have characteristic basal granules. The **chief cells** are apparently long-lived cells, derived from stem cells at the isthmus. Ultrastructurally they have extensive rough endoplasmic reticulum, a prominent Golgi zone, and numerous zymogen granules.

Normally, mitotic figures are not common in cells at the isthmus of fundic glands, and are virtually never seen at any distance from the isthmus. However, the fundic mucosa of newborn ruminants and especially piglets may be relatively poorly differentiated and proliferative. The proliferative compartment, if active, is sensitive to radiomimetic insults, such as cytotoxic agents and parvoviral infection. This is reflected by narrowing of the isthmus and upper neck and attenuation of the epithelium lining the gland.

The **pyloric mucosa** forms a slightly pitted or irregular surface in the distal portion of the stomach; it extends further cranial along the lesser than the greater curvature. The knob-like torus pyloricus at the pylorus of the pig is a normal structure. The tubular glands of the pyloric mucosa open into deep gastric pits that may extend half the thickness of the mucosa. The glands are lined by pale mucous cells, with interspersed endocrine elements, mainly G (gastrin) and D (somatostatin) cells. Scattered parietal cells may be present, especially in glands in the zone intergrading with fundic mucosa.

The stromal elements of the gastric lamina propria are relatively inconspicuous, in fundic mucosa in particular. Normally, a few lymphocytes and plasma cells, and scattered mast cells are present, mainly deep between glands. Occasional lymphocytic nodules or follicles may be present, usually near the muscularis mucosae. Lymphoid infiltrates are more common in antral mucosa. A thick band of amorphous hyalinized connective tissue, sometimes termed the *lamina densa*, is seen sporadically on the luminal aspect of the muscularis mucosae in the stomach of cats. The cause and significance are unknown.

Rapid fixation of the gastric mucosa is desirable to avoid postmortem artifacts.

Hydrolysis of protein in preparation for subsequent intestinal digestion and absorption is accomplished in the stomach by **acid** and by **pepsin**, activated by autocatalysis from pepsinogen at low pH. *Secretion of acid is the function of the oxyntic, or parietal cells*, about one billion of which are present in the stomach of a 20 kg dog. Regulation of the volume and acidity of gastric secretion is physiologically complex and highly integrated, involving neurocrine, endocrine, and paracrine mechanisms.

The parietal cell secretes hydrochloric acid in response to stimulation by histamine, acetylcholine, and gastrin. All three agonists are probably continuously present and involved in basal acid secretion. However, the effects of acetylcholine and gastrin are largely dependent on concurrent stimulation by the permissive agonist, histamine.

Histamine is a paracrine stimulant, continuously present in the environment of the oxyntic cells, and secreted by mast cells and enterochromaffin-like cells. Occupation of the H_2 histamine receptor on the oxyntic cell causes enhanced generation of cyclic adenosine monophosphate. This in turn stimulates protein kinase cascades culminating in translocation of the proton pump to the apical cell membrane, where acid is secreted.

Acetylcholine, the neurocrine agonist, is released near the oxyntic cell from processes of parasympathetic postganglionic neurons. Its release is enhanced by vagal activity during the central stimulation of the cephalic phase – the pavlovian response. Gastric distension also stimulates the parietal cell via vagovagal and short intramural reflex pathways. Acetylcholine acting on muscarinic M_3 receptors elevates intracellular Ca^{2+}, which stimulates acid secretion.

Gastrin is released into the bloodstream by G cells, many of which are in the pyloric antrum. Calcium, amino acids, and peptides in ingesta, impinging on G cells, stimulate gastrin release. Vagal stimulation during the cephalic phase, and fundic-pyloric vagovagal reflexes, in concert with local pyloric reflexes, initiated by distension, also cause G cells to release gastrin. Gastrin acts mainly by a receptor-mediated process to release histamine from the enterochromaffin-like cells. Gastrin alone is a weak stimulant of acid production, but it synergizes secretion by oxyntic cells exposed to histamine and acetylcholine. In addition, gastrin has an important trophic effect, increasing the number of parietal and endocrine cells in fundic mucosa. In turn, parietal cell mass seems to impact on chief cell differentiation.

Acid production during the gastric phase of secretion is depressed by the negative-feedback effect of acid in the antrum, through the inhibitory effect of somatostatin on the G cell below pH 3.0. Acid, fat, and hyperosmolal solutions in the proximal small intestine also inhibit acid secretion, perhaps by the mediation of neural reflexes, secretin, gastric-inhibitory polypeptide, epidermal growth factor, transforming growth factor-α, or other enterogastrones. Prostaglandin E_2 also inhibits acid production by parietal cells.

The chief cell is probably susceptible to the same general stimuli for secretion as the parietal cell.

Gastric motility is the outcome of an interaction among myogenic, hormonal, and neuronal factors, the latter two impinging directly or indirectly on smooth muscle. Mediating at least part of the neural component of this control system, by as yet uncertain mechanisms, are the interstitial cells of Cajal, pacemakers that are variously found between the layers of gastric smooth muscle, or among smooth-muscle cells.

Gastric mucosal barrier

The gastric mucosal barrier to acid backdiffusion and autodigestion resides largely in the *single layer of foveolar and surface mucous cells and its products*. Integrity of the gastric mucosal barrier implies continuity of the mucosal surface epithelium. The capacity of these cells to maintain tight junctions, to migrate rapidly to fill defects, and to secrete mucus, bicarbonate, and a hydrophobic phospholipid surface layer, is central to protecting the gastric mucosa against progressive injury by insults arising in the lumen.

Gastric mucus is freely permeable to hydrogen ions and has little innate buffering capacity, but it resists hydrolysis by intraluminal pepsin, protecting the integrity of the mucosal surface. Cardiac gland mucosa in the pig, and pyloric mucosa, secrete *bicarbonate* in considerable quantities, and normally resist acid attack. Fundic surface mucous cells also actively secrete bicarbonate into a thin unstirred layer of surface mucus. Bicarbonate and mucus secretion by mucous cells is stimulated by prostaglandin E_2. Acid is buffered by bicarbonate in the thin unstirred layer of mucus, preventing backdiffusion into the mucosa. In addition, the "alkaline tide" of bicarbonate, released into the gastric interstitial space during the course of acid secretion, further buffers the mucosa against acid backdiffusion.

Prostaglandins, particularly of the E series, ubiquitous in gastric mucosal lamina propria, may have protective effects other than by stimulation of bicarbonate and mucus secretion by mucous cells, and by inhibition of histamine-stimulated acid secretion by parietal cells. They cause proliferation, resulting in an increased mass of foveolar mucous epithelium. They may promote incorporation of surfactant molecules into the apical cell membrane of surface mucous cells, increasing its hydrophobicity and imparting greater resistance to water-soluble insults. They may also be involved in gastric mucosal cytoprotection by sulfhydryl compounds, which may neutralize free radicals and other toxic metabolites.

Prostaglandins and basal nitric oxide production cause vasodilation and increased blood flow, in addition to inhibiting acid secretion by parietal cells, and promoting bicarbonate secretion by foveolar mucous cells. The high metabolic rate of the gastric mucosa requires a high blood flow to maintain an intact, functional surface epithelium. Bicarbonate in the local circulation, resulting from the alkaline tide generated by acid secretion in glands deeper in the mucosa, is probably important in buffering the superficial lamina propria against backdiffusion of acid; adequate blood flow flushes injurious free radicals from the vicinity of surface cells. Experimentally, high blood flow is protective against many mucosal insults, whereas ischemia is ulcerogenic.

Epidermal growth factor, originating in salivary glands, and transforming growth factor-α, produced locally in the gastric mucosa, also appear protective, in that they may promote cell proliferation and migration to fill defects, and suppress acid production. Bombesin is probably protective through stimulation of gastrin-mediated nitric oxide release, promoting gastric mucosal blood flow.

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Response of the gastric mucosa to injury

Restitution of acute erosive physical or chemical trauma to the mucosal surface is by rapid (minutes to hours) immigration of surviving attenuated surface and foveolar cells. Under the initial control of transforming growth factor-β and later influenced by epidermal growth factor, repair by proliferation of cells in the isthmus follows, if the erosive lesion is superficial and spares the progenitor cells. A cap of mucus, exfoliated epithelium, and fibrin over a mucosal defect may form a protective barrier conducive to effective restitution of the mucosal epithelium. An acute inflammatory reaction demarcates severely eroded or superficially necrotic mucosa, and hemorrhage may be evident on the surface and in adjacent mucosa. Mitoses become common in the upper gland.

During the early phase of repair, cells lining shallow foveolae and covering the surface are basophilic, poorly differentiated, and flattened, cuboidal, or low columnar. Sites of epithelial exfoliation and neutrophil transmigration or effusion into the lumen may be evident. Congestion, edema, mild neutrophilia, and fibroplasia are seen in the superficial lamina propria. The evolution and repair of gastric ulceration, to which erosion may be antecedent, are discussed later. The progenitor cells of the fundic mucosa have the potential to produce tall columnar mucous cells of the foveolar or surface type, to produce mucous neck cells, and by further differentiation, to evolve into parietal cells.

Atrophy of parietal cell mass without extensive mucous cell hyperplasia occurs in animals, particularly ruminants, which have signs of gastrointestinal disease, including *inappetence*. The change is not evident grossly. Microscopically, fewer parietal cells are seen in the upper neck of fundic glands, and often in the depth of the gland. This is accompanied by epithelial proliferation, indicated by moderate numbers of mitotic figures at the isthmus and in the neck of the gland. Mucous neck cells become the predominant cell in the upper gland. In extreme cases, mucous neck cells are present to the base of glands, and *achlorhydria* occurs. Similar findings occur in animals with a wide variety of syndromes involving loss of appetite. Starvation of moderate duration does not produce comparable lesions. This lesion may be the result of *reduced trophic stimulation of the fundic mucosa*, and is not to be confused with atrophic gastritis, in which loss of parietal cell mass and atrophy of mucosal thickness are accompanied by usually chronic inflammation.

Mucous metaplasia and hyperplasia of glands in the fundic stomach in all species are associated with *chronic inflammation* of the mucosa. As the lesion evolves, parietal cells seem to be lost at an accelerated rate, since they are only present in the basal portion of the glands, and they appear to be progressively displaced from above by hyperplastic mucous cells. Mitotic figures may be numerous throughout the neck of the gland, which elongates. The metaplastic epithelium in early lesions tends to resemble mucous neck cells. In established lesions, columnar mucous cells with regular nuclear polarity, similar to foveolar mucous cells, may be present. When inflammatory infiltrates are local, the mucous change is limited to a few surrounding glands. More diffuse inflammation is associated with the development of widespread epithelial mucous metaplasia. Focal or diffuse, superficial or mucosal, proprial infiltrates of plasma cells and lymphocytes are typical. Often, neutrophils, eosinophils, and Russell-body cells will be present in the lamina propria, and lymphocytes may be between epithelial cells in glands. Globule leukocytes may be present in the epithelium of glands, especially in the parasitized abomasum.

This mucous metaplasia, hyperplasia, and chronic inflammation is associated with a *variety of causes*, including chronic traumatic insults, such as those due to implanted foreign bodies, as well as in the abomasa of ruminants infected with lentiviruses. The specific agency most commonly recognized is *gastric parasitism by nematodes* such as *Ostertagia* spp., *Trichostrongylus axei*, *Hyostrongylus* spp., and *Ollulanus tricuspis*, where the distribution of the lesion is often closely related to the physical presence of nematodes and to the interstitial inflammatory reaction that they incite. Mucous metaplasia and hyperplasia are also typically present around the healing margins of chronic ulcers, perhaps in response to local inflammation.

The mucosa affected in these circumstances is grossly thickened, as on the overhanging margin of an ulcer, or in an *Ostertagia* "nodule," with a pebbled or convoluted surface if the lesion is widespread. Gastric rugae or plicae are thickened, partially as a result of mucosal hypertrophy, perhaps with submucosal edema. The surface of the stomach is usually paler than normal in affected areas; however, local congestion or hyperemia may be evident. Though the surface may be glistening, profuse mucus secretion is not usually obvious. *Achlorhydria* is the consequence of widespread change of this type. Mucous metaplasia and hyperplasia are differentiated from fundic atrophy associated with the loss of appetite on the basis of the degree of mucous cell hyperplasia and differentiation, and the presence of inflammatory cells.

The cause and functional significance of gastric mucous metaplasia are unclear. Presumably loss of parietal cells and hyperplasia of mucous neck cells is partly a response to soluble local immune-mediated stimuli or products of inflammation, since it is localized to the vicinity of glands containing larvae in gastric parasitism, but, as a general phenomenon, seems independent of parasite-specific factors. Tumor necrosis factor-α, interleukin-1, and interferon-γ are candidate cytokines involved in inflammatory mucous metaplasia of the fundic stomach. In vitro, interleukin-1β and tumor necrosis factor-α inhibit acid secretion by isolated parietal cells, and tumor necrosis factor-α induces apoptosis of parietal cells. Replacement of parietal cells by mucous neck cells, or an apparently more fully differentiated mucous cell in chronic gastritis, may be a protective response. It may eliminate the threat of local acid corrosion, and promote the transfer into the lumen of protective soluble factors such as lysozyme and IgA or its analogues.

Achlorhydria ensues in severe chronic gastritis and mucous metaplasia. The pH of gastric secretion approaches or exceeds neutrality under some circumstances, as sodium ion replaces hydrogen ion in gastric content and bicarbonate is secreted. With diminished gastric acid concentration, progressive microbial colonization of the stomach and upper intestine ensues. Parietal cell atrophy and replacement by mucous neck cells in ruminants with anorexia due to enteric disease may predispose to mycotic invasion of the mucosa, if it is physically disrupted. Mucous metaplasia and hyperplasia, as seen in chronic gastritis or conditions like ostertagiosis, do not seem to render the mucosa prone to mycosis. Loss of the hydrolytic effects of acid and pepsin, in achlorhydria, seems to have little effect on digestion of protein and uptake of nitrogen, at least in animals with ostertagiosis, and the effect on protein digestion of atrophic gastritis in humans appears to be minimal. Rather, at least in parasitic gastritis, inappetence diminishes nutrient intake on the one hand, while mucosal permeability permits protein-losing gastropathy; together, these impact on the nitrogen economy of the animal to cause reduced productive efficiency, or loss of body condition, depending on whether the animal enters negative nitrogen balance or not.

Evaluation of *gastric biopsies* is governed by the same caveats discussed below under interpretation of intestinal and colonic biopsies (p. 71). Gastritis, and the pathogenesis of gastrointestinal parasitism, are discussed later in this chapter.

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Pyloric stenosis

Pyloric stenosis is a functional and sometimes anatomic problem, which in part represents probably the only *anomaly* of the stomach recognized in animals. It is relatively common in dogs, and rare in cats and horses.

Recurrent vomition and poor growth in recently weaned animals suggest the clinical diagnosis of a congenital lesion. Contrast radiographic studies will confirm *delayed gastric emptying*. There is limited critical functional information on this problem. An analogous condition in humans is associated with a lack of the interstitial cells of Cajal, but this association has not been clearly demonstrated in domestic animals, nor have morphological or significant immunohistochemical neural abnormalities been identified. In some dogs there may be hypertrophy of pyloric muscle, which appears grossly thickened. Tonic stenosis of the pyloric sphincter may occur in dogs, perhaps due to unconfirmed lesions of the myenteric plexus or due to gastrin excess. In cats, no gross alteration in the diameter of the pylorus or the thickness of its muscle is recognized. An association with esophageal dilation has been made in the cat. Congenital pyloric stenosis in a foal was associated with signs of abdominal pain and reluctance to consume solid feed. In all species, the clinical problem is usually abolished by pyloromyotomy.

Acquired functional pyloric obstruction may be a component of abomasal hypomotility, resulting in abomasal impaction, discussed below.

Acquired pyloric stenosis or obstruction due to physical causes occurs following ulceration, granulation, and stricture of the pyloric canal in any species; due to foreign bodies; as a complication of polyps and tumors in the area; and due to chronic hypertrophic pyloric gastropathy in dogs.

Chronic hypertrophic pyloric gastropathy is the term coined for a syndrome of pyloric obstruction in dogs, associated with *mucosal hypertrophy, hypertrophy of circular smooth muscle, or a combination of the two*. Mucosal hypertrophy alone is the most common lesion; muscular hypertrophy alone is the least common, though some degree of muscular hypertrophy is seen in about half the cases. Affected animals are typically of small breeds, and middle-aged or older. Males outnumber females. The pathogenesis is speculative, and it is not clear whether muscular hypertrophy is primary, as it seems to be in some cases, or whether it is secondary, in response to obstruction related to excess mucosa. Since mucosal and muscular lesions can be present independently, they may have separate causes. The mucosal hypertrophy has features in common with hypertrophic gastritis, described elsewhere. The cardinal presenting sign is *chronic intermittent vomition*, perhaps with weight loss, and with gastric distension in a few cases. Gross examination, by gastroscopy, at gastrotomy, or necropsy, in most cases reveals *enlarged mucosal folds* surrounding and obstructing the pyloric canal. This reflects hypertrophy of glands, which may involve foveolar or deeper glandular elements alone, or in combination, perhaps with cystic dilation of deeper portions of glands. There is usually a concomitant chronic inflammatory infiltrate in the mucosa and occasionally submucosa, and small erosions of the mucosal surface may occur. If there is muscular hypertrophy, this is reflected on the cut surface of the pylorus by irregular firm thickening of the circular muscle. Microscopically, smooth-muscle fibers in affected fascicles are irregularly hypertrophic. Recognition of smooth-muscle hypertrophy requires a full-thickness biopsy, which will not be obtained by endoscopy.

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Gastric dilation and displacement

Gastric dilation in the horse is often *secondary* to obstruction of the small bowel, or of colic with ileus, and is also part of the syndrome "grass sickness," discussed elsewhere in this chapter. Ingestion of *Datura* sp. seeds, which contain a parasympatholytic alkaloid, can also cause ileus, leading to gastric dilation. *Primary gastric dilation* in horses is a sequel to consumption of excess fermentable carbohydrate, sudden access to lush pasture, or excessive intake of water. Dilation associated with intake of fermentable feed is likely analogous to grain overload in cattle. Ingesta may swell through absorption of saliva and gastric secretion. Evolution of gas and organic acids, including lactic acid, by bacterial fermentation of carbohydrate, occurs in the cranial portion of the stomach. An influx of water follows as the result of increased osmotic pressure in the stomach, contributing to increased distension and to systemic dehydration. Animals surviving for any time with acute gastric dilation of this type may develop laminitis.

Gastric rupture may follow primary or secondary dilation of the equine stomach; it may be idiopathic, in that no clear cause is identified. Gastric rupture is diagnosed in about 5% of horses with colic admitted to veterinary hospitals. Rupture usually occurs along the greater curvature, parallel to the omental attachment, releasing gastric content into the omental bursa or the abdominal cavity. Death ensues acutely as the result of shock and peritonitis. The margins of the laceration show evidence of antemortem hemorrhage, which differentiates the lesion from postmortem rupture of a dilated stomach. There may also be congestion of the cervical esophagus and blanching of the thoracic esophagus, producing a prominent "*bloat line*." This, and compression atelectasis of the lungs in some cases, attests to the tremendous increase in intra-abdominal and intrathoracic pressure exerted by the dilated stomach prior to rupture. **Perforation**, as distinct from rupture, of the stomach in the horse is rare, and is associated with parasitism, gastric ulcer, or neoplasia.

Gastric dilation and volvulus (Fig. 1.37) occur relatively commonly in dogs, and uncommonly in cats. In **dogs**, gastric dilation and volvulus are usually problems associated with eating, and probably aerophagia, especially in the *deep-chested breeds*, such as Great Danes, St. Bernards, Irish Setters, Wolfhounds, Borzois, and Bloodhounds. Predisposing factors are controversial but may include: increased laxity of the hepatogastric ligament; a diet of small food particles; recent kenneling; having a raised feed bowl; and infection with the nasal mite *Pneumonyssoides caninum*, which causes "reversed sneezing."

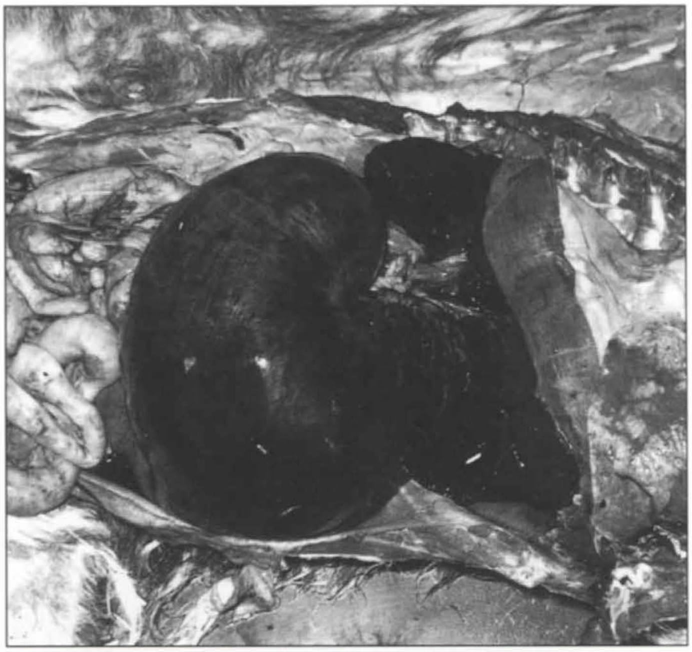

Figure 1.37 Gastric volvulus in a dog. The stomach has undergone venous infarction due to strangulation of its vascular outflow, and is extremely distended and congested.

The gas that contributes to the development of dilation is probably the result of aerophagia, and possibly the evolution of carbon dioxide by physiologic mechanisms. Inability to relieve the accumulation of food, fluid, and gas in the stomach causes the organ to dilate and alter its intra-abdominal position, so that its long axis rotates from a transverse left–right orientation to one paralleling that of the abdomen. In simple dilation, the esophagus is not physically completely occluded, the spleen remains on the left side, and the duodenum is only slightly displaced dorsally and toward the midline. Repeated episodes of gastric dilation probably compromise splenic venous return during periods when the stomach is distended, and eventually lead to episodes of splenic ischemia and segmental infarcts.

For reasons that are unclear, gastric dilation may be converted to gastric volvulus. The stomach rotates about the esophagus in a clockwise direction, as viewed from the caudal aspect. The greater curvature of the distended organ moves ventrally and caudally, and then rotates dorsally and to the right. This forces the pylorus and terminal duodenum cranially to the right and clockwise around the esophagus. Ultimately they lie to the left of midline across and ventral to the esophagus, compressed between the esophagus and the dilated stomach. Depending on the degree of volvulus, the *spleen*, which follows the gastrosplenic ligament, usually ends up lying in a right-ventral position, between the stomach and liver or diaphragm. It is bent into a "V" shape by tension on its ligaments, becomes extremely congested, and may undergo torsion, infarction, and rupture. The esophagus becomes completely occluded in volvulus, which may involve rotation of up to 270–360°. Venous infarction of the gastric mucosa ensues, as volvulus progressively constricts outflow of blood from the stomach. The mucosa, and usually the full thickness of the gastric wall, are edematous and dark red to black, and there is bloody content in the lumen of the stomach. Ischemic mucosa becomes necrotic, and the stomach may rupture. Hemoperitoneum may occur, due to avulsion of gastric blood vessels.

Obstruction of veins by volvulus, and pressure exerted by the distended stomach, result in decreased venous return via the portal vein and caudal vena cava, causing reduced perfusion of intra-abdominal organs, reduced cardiac output, and *circulatory shock*. Increased intra-abdominal pressure impinges on the diaphragm and compromises respiration. A variety of acid–base and electrolyte abnormalities ensue in dogs with gastric dilation and volvulus, contributing to the physiologically precarious state. Cardiac arrhythmias as a sequel to gastric dilation and volvulus have been associated with putative release of *myocardial depressant factor* from an ischemic pancreas, and with myocardial necrosis, resulting from ischemia. Death is inevitable in dogs with acute gastric volvulus that are not treated early. Rare cases of chronic gastric volvulus are reported, with fixation of the spleen in the right side of the abdomen by omental adhesions.

In **swine**, gastric volvulus is a cause of sudden death in *adult sows*, perhaps with a brief premonitory period of anorexia, abdominal distension, dyspnea, and salivation. It is associated with excitement in anticipation of feeding among pigs that are fed at regular, often long, intervals, and may be a sequel to unduly rapid ingestion of feed, water, and air. The twist may occur in either direction about the long axis of the stomach, though clockwise torsion predominates.

Abomasal displacement and volvulus is a common clinical problem in high-producing, intensively managed **dairy cattle**, particularly around the time of parturition, but it also occurs in animals that are predominantly pasture-fed. *The displacement is usually ventrally and to the left of the rumen*. Many affected animals have concurrent problems, including ketosis, hypocalcemia, metritis, and retained placenta. Affected abomasa have decreased sensitivity to acetylcholine, and abomasal atony has been implicated as a prerequisite to displacement. Evolution of gas in the abomasum is directly related to the amount of concentrate in the ration. Left displacement of the gas-filled abomasum is amenable to treatment, and is rarely encountered at autopsy. Handling of an affected animal postmortem may correct displacements in any case. Other than possible scarring of the lesser omentum, the abomasum may be unremarkable. *Abomasal fistulae*, draining in the right paramedian area, may ensue if, during abomasopexy to prevent recurrent displacement, nonabsorbable sutures fixing the abomasum to the abdominal wall penetrate the abomasal mucosa.

Simple right displacement, which accounts for about 15% of abomasal displacements, is probably caused by similar agencies. However, right displacement may be complicated in about 20% of cases by progression to abomasal volvulus, which is clinically serious.

Abomasal volvulus is probably the sequel to rotation of a loop formed by a distended abomasum and attached omasum and duodenum. The volvulus is counterclockwise about a transverse axis through the lesser omentum, when viewed from the right side.

Rotation, buoyed by the gas-filled body of the abomasum, may be in the sagittal plane. With a 360° volvulus, the pylorus ends in the cranial right portion of the abdomen dorsal to the twisted omasum, with the duodenum trapped medial to the omasum and lateral to the partially rotated reticulum (Fig. 1.38). Alternative modes of displacement and rotation are possible, but all may end in this relationship.

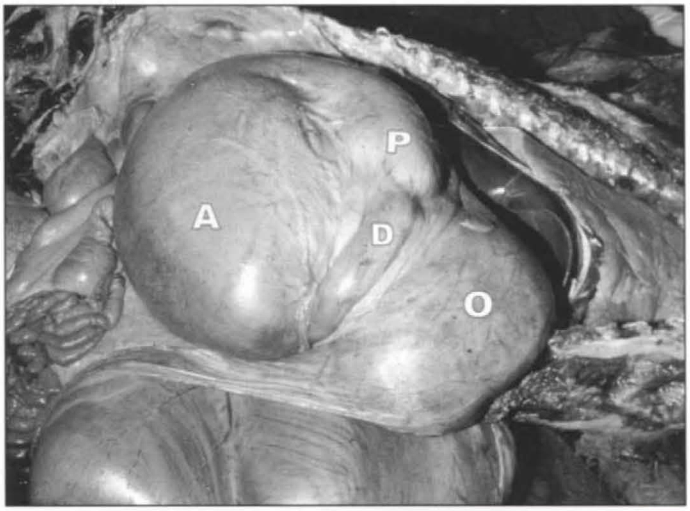

Figure 1.38 Right displacement/volvulus of abomasum. Pylorus in upper right quadrant of abdomen, with duodenum trapped between omasum laterally and reticulum (not visible) dially. A, body of abomasum; P, pylorus; D, duodenum; O, omasum.

Obstruction of duodenal outflow in volvulus results in sequestration of chloride in the abomasal content and the development of *metabolic alkalosis*. Severe volvulus causes obstruction of blood vessels at the neck of the omasum, as well as causing trauma to the vagus nerves in the region. The abomasum becomes distended with blood-stained fluid and gas. Infarction of the deeply congested mucosa may result in ultimate *abomasal rupture*, often near the omasoabomasal orifice, and peritonitis. Damage to the vagal branches may prohibit return of normal abomasal motility in animals successfully withstanding surgery, resulting in vagus indigestion. Cases of abomasal torsion are also occasionally reported in preruminant calves; these are usually fatal.

Sarcina-like bacteria have been associated with abomasal diseases in **lambs**. Pathologic findings include various combinations of abomasal bloat, hemorrhage, and ulcers.

Gastroesophageal intussusception, gastroduodenal intussusception, and **pylorogastric intussusception** are uncommon displacements, involving the stomach of dogs with signs of upper alimentary tract obstruction that require surgical correction.

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Gastric foreign bodies and impaction

A variety of **foreign bodies** may be encountered in the stomach and in the abomasum. Most are incidental findings, or at worst, associated with vomition, mild acute or chronic gastritis, or occasionally with ulceration. *Trichobezoars* (hairballs) are often found in the stomach of long-haired cats, and in calves reared on diets low in roughage, where most are in the rumen, with a few in the abomasum. *Phytobezoars* and trichophytobezoars have been implicated as the cause of pyloric obstruction and death in young lambs on pasture and, in some regions, in cattle grazing fibrous plants. *Fine sand* may accumulate in the abomasum in considerable amounts, usually with no detrimental effect.

Gastric impaction by inspissated content in horses is related to factors such as consumption of fibrous roughage and persimmons, inadequate water intake, and poor mastication. It may cause anorexia, mild colic, and loss of body condition, and is to be differentiated clinically and at autopsy from gastric impaction secondary to pyrrolizidine alkaloid poisoning, from dilation secondary to intestinal obstruction, and from primary gastric dilation.

Primary abomasal impaction in cattle may result from restricted water intake and coarse, high-roughage feed, such as wheat stubble or straw. Secondary abomasal impaction may follow pyloric stenosis, physical or functional, of any cause. It is perhaps most common as a functional abomasal stasis in one of the manifestations of "*vagus indigestion.*" Loss of abomasal motility may be the product of intrathoracic inflammatory or neoplastic vagal lesions; vagal involvement in adhesions following traumatic reticuloperitonitis; vagal trauma in surgically corrected abomasal volvulus; adhesions of the abomasum and omasum that may physically impair motility; or systemic disease that causes abomasal stasis.

The abomasum is impacted with thick porridge-like or inspissated coarse fibrous digesta, despite an apparently patent pylorus. **Abomasal rupture** may ensue, particularly in primary impaction associated with coarse feed, resulting in diffuse peritonitis. Most commonly, the laceration is near the omasal–abomasal orifice, but it may be elsewhere. Omasal dilation and ruminal distension are also found in many of these cases; omasa usually contain inspissated digesta, while rumen content tends to be fluid. Metabolic derangement due to sequestration of chloride in the rumen following regurgitation from the obstructed abomasum, and hypokalemia due to decreased intake in feed in the face of continued normal renal excretion, place these animals in perilous physiologic circumstances, often before inanition becomes a significant factor.

A syndrome known clinically as **abomasal dilation and emptying defect** occurs in Suffolk and Hampshire **sheep**. The animals develop chronic inappetence and weight loss, and at necropsy they have a markedly distended abomasum containing digesta resembling rumen contents. No morphologic gross or microscopic lesions of the stomach, vagus nerve, or other organs have been found, except scattered chromatolytic and necrotic neurons in the celiacomesenteric autonomic ganglion. The cause is unknown, but it is suggested that it may be an acquired dysautonomia, possibly toxic, requiring a genetic predisposition for expression. Though rumen chloride levels are elevated, few animals become hypochloremic and alkalotic, as do cattle with abomasal impaction.

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Circulatory disturbances

Edema of the gastric rugae occurs with hypoproteinemia in any species, in portal hypertension, and is found in the abomasum of cattle poisoned by arsenic, and sheep ingesting tannic acid. Edema fluid collects in the submucosa of the folds, and is particularly obvious in the normally thin abomasal plicae. Edema may contribute to the thickening of rugae seen in gastritis. Edema of the submucosa of the stomach is a common and important lesion in edema disease of swine. It is best appreciated by making several slices through the serosa and external muscle to the submucosa on the greater curvature over the body of the stomach. Edema disease is considered fully in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Hyperemia of the gastric mucosa occurs with ingestion of chemicals, which usually also cause superficial erosion and necrosis, discussed later under chemical gastritis. Focal hyperemia may be related to local irritation of the mucosa by foreign bodies, and with focal acute viral lesions of the abomasum in cattle. **Congestion** of the mucosa can occur in conditions causing portal hypertension, including cirrhosis and shock in the dog.

Uremic "gastritis," seen as severe congestion and hemorrhage of the body of the stomach, associated with signs of hematemesis and melena, is found in some dogs, and occasionally in cats and horses, with renal disease. In such animals, the mucosa is thickened and deep red-black. Lesions vary in severity from case to case, and premonitory changes without severe hemorrhage and necrosis are present in animals euthanized earlier in the course of disease. In such dogs there may be no gross gastric lesion, or variable edema and thickening of rugal mucosa, perhaps with focal ulceration, is evident.

Microscopically, the lamina propria between glands is edematous, and there are increased mast cells. Deposits of basophilic ground substance and mineral are found, especially on the basement membrane of vessels and glands, or on collagen fibrils and in degenerate smooth muscle. These changes occur particularly in the middle and deeper portions of the mucosa. Parietal cells in this area are usually mineralized as well. Such mineral deposits may be seen at autopsy in gross cross-sections of mucosa. More extensive mineral deposition also involves the muscular coats and arterioles of the submucosa and serosa. Such vessels also show evidence of endothelial damage, medial necrosis, and, in some cases, thrombosis. Severe mucosal congestion, edema, and necrosis are possibly related to ischemia secondary to the vascular lesions, though perhaps not directly associated with arterial thrombosis and obstruction, which is often not readily found. Microvascular lesions in the lamina propria, and systemic states in uremia, may be contributory. Impaired renal degradation and excretion of gastrin promote hyperchlorhydria, exacerbating mucosal damage.

The cause of the vascular lesions may be a poorly characterized circulating toxic peptide associated with uremia. Mineral deposition is probably the product of altered systemic metabolism of calcium in renal failure, perhaps coupled with the local microenvironment resulting from bicarbonate moving across the basal border of secreting parietal cells. Membrane lesions in metabolically compromised parietal cells may also act as foci of mineral deposition (see Vol. 2, Urinary system, for a discussion of uremia). Mineralization is also a feature of vitamin D intoxication.

Gastric venous infarction is a common lesion in swine, and is also encountered in ruminants and horses. It is related to endothelial damage and thrombosis in venules, usually associated with *endotoxemia or other bacterial or toxic damage*. Salmonellosis and *Escherichia coli* septicemia in all species, and in addition, in swine, postweaning coliform gastroenteritis, erysipelas, swine dysentery, and Glasser's disease are associated with the lesion. The fundic mucosa is bright red or deep red-black and may have excess mucus or perhaps fibrin on the surface. Occasionally the superficial mucosa is obviously necrotic, adopting a yellow-brown caseous appearance, and may lift off with the ingesta. In section there is thrombosis of venules in the mucosa and often at the mucosal–submucosal junction, usually with prominent fibrin plugs. Thrombosed capillaries and venules may be present at any level of the mucosa, along the base of the ischemic zone of superficial coagulative necrosis, with local hemorrhage and edema. There may be an acute inflammatory reaction delineating the necrotic area in the mucosa. Sometimes the full thickness of the gastric mucosa, focally or diffusely, may be necrotic.

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Gastritis

Gastritis is a term used clinically as a presumptive diagnosis for those cases of vomiting that are thought to arise from gastric irritation rather than disease elsewhere. In pathology, the term is often used with equal imprecision, referring to a wide range of gastric injury in which histologic criteria of inflammation may not be particularly prominent. Under the broad umbrella of "gastritis" fall several different types of lesions: gastric mucosal necrosis, erosion or ulceration caused by mechanical, chemical, or ischemic insults, among which is "uremic gastritis," described earlier; true gastritis, in which the lamina propria contains the leukocytic and vascular changes characteristic of inflammation; and finally, lesions of gastric mucosal atrophy, fibrosis, and lymphofollicular hyperplasia that arguably are the residual lesions of previous active inflammatory disease.

Chemical gastritis or abomasitis, reflected in diffuse gastric congestion, hemorrhage, necrosis, and ulceration, may be induced by chemicals such as arsenic, thallium, formalin, bronopol, steroidal and nonsteroidal anti-inflammatory drugs, phosphatic fertilizers, and by the toxic principal in bitterweed (*Hymenoxon odorata*). **Blister beetle (*Epicauta* spp.) intoxication in horses**, induced by the cantharidin contained in these insects, may cause necrosis and ulceration of the distal esophagus and pars esophagea, and intense hyperemia of the glandular mucosa of the stomach, and in some cases gastric ulcers and rupture. In addition, enterocolitis, nephrosis, hemorrhages of the urinary bladder, and myocardial hemorrhage and necrosis are reported with regularity in blister beetle toxicosis. **Zinc** may cause acute mortality, in which the mucosa of the abomasum and duodenum is a distinctive lime green and necrotic, with an underlying congested, edematous submucosa. Microscopically, radiating crystals are evident in the necrotic tissue. Subacute zinc intoxication may be reflected in abomasal damage characterized by exfoliation of glandular epithelium, ablation of glands in some areas, and reparative proliferation of mucous neck cells, in addition to fibrosing pancreatitis and mild nephrosis. **Mechanical "gastritis"** is most commonly seen in dogs after ingestion of coarse foreign materials and in cats with hairballs. The lesion is discovered as an incidental finding when careful clinicians biopsy the stomach via endoscopy when making the diagnosis of gastric foreign body, or at the time of surgical removal of that foreign material. Hairballs in the abomasum of ruminants are associated with lack of roughage in young animals, but virtually never with disease.

Eosinophilic infiltrates occur in the squamous stomach of horses with an eosinophilic epitheliotropic syndrome, discussed in the section on eosinophilic enteritis in horses, below, and with dermatitis.

The majority of examples of genuine gastritis are seen in dogs and cats with more generalized gastrointestinal inflammatory disease, such as food allergy or idiopathic inflammatory bowel disease, and will be discussed further with those topics (see section on Idiopathic inflammatory bowel disease, below).

Infectious agents in small animals appear to be minor causes of gastritis, when compared with chemical, mechanical, or idiopathic insults. Following the discovery of *Helicobacter pylori* as a major cause of gastric ulceration in humans, there was a flurry of activity to document its significance as a cause of gastritis and/or gastric ulceration in other monogastric species. The picture that has emerged is a confusing one because of substantial flux in the taxonomy of the various gastric spiral organisms, and because of the existence of many different species of *Helicobacter* (only some of which have been successfully cultivated). At least 20 different *Helicobacter* species, distinguished by mRNA sequencing, have been reported from various mammalian species. It is safe to say that probably all mammals have one or more species of *Helicobacter* as part of the normal gastric flora, and thus determining a causal relationship between *Helicobacter* and gastric disease has proven very difficult.

Helicobacter pylori is a common cause of acute and chronic gastritis affecting antrum, corpus, or both, in humans and in other primates. In humans it is the leading cause of chronic peptic ulcers. This may progress in some cases to gastric mucosal atrophy, with loss of antral and/or oxyntic glands. Chronic *Helicobacter* infection in humans also significantly increases the risk for the development of gastric carcinoma and lymphoma. On the other hand, *H. pylori* appears to be a very uncommon infection in other species, and has never been proven to cause disease in nonprimates. Among nonprimates, the most convincing evidence for clinically significant gastric *Helicobacter* infection is in ferrets. Although virtually all adult ferrets have *H. mustelae* as part of the normal gastric flora, heavily colonized ferrets develop diffuse lymphocytic-plasmacytic gastritis with lymphofollicular hyperplasia and sometimes with erosions. Progression to neoplasia has not been documented.

The role of Helicobacter in the development of gastritis or gastric ulceration in other species has not been convincingly documented. Claims for the significance of *Helicobacter acinonychis* as a cause for gastritis in captive cheetahs remain unproven, in that antibiotic treatment improves the clinical disease but does not eradicate the gastric *Helicobacter* infection. Furthermore, wild cheetahs have the same *Helicobacter* colonization, but not the gastritis. In dogs and cats, it is routine to see various spiral organisms colonizing the gastric surface and within canaliculi in parietal cells in normal animals and in those with gastrointestinal disease. Depending on detection methods, the prevalence of colonization is at least 50% and may be nearly universal in adults. The prevalence of such colonization as determined by direct visualization or by culture is as high in clinically healthy animals as in those with clinical signs of gastritis. On the other hand, there are several studies claiming that dogs or cats with natural or experimental *Helicobacter* infection have a higher prevalence of mucosal mononuclear leukocytes and mucosal lymphoid follicles, and faster mucosal turnover, than is seen in animals in which *Helicobacter* infection could not be demonstrated. The problem is that the modest histologic changes have not been correlated with the development of clinical disease. The changes described resemble the kind of changes seen elsewhere in the intestinal tract with, for example, acquisition of the normal flora by germfree animals, or even as part of normal "immunologic" maturation of the gastrointestinal tract. Improvement in clinical signs following antibiotic therapy appropriate for the elimination of *Helicobacter* has not been documented convincingly.

Chlamydophila have been recognized in surface mucous cells of otherwise normal fundic mucosa in cats with no signs of disease. Experimental infection produced conjunctivitis, respiratory disease, but only mild gastritis.

Gastric mucosal hypertrophy is an occasional lesion within the stomach of *dogs*. It may be focal or diffuse. The **focal lesion** is much more prevalent, occurring as an intraluminal papillary proliferation of pyloric antral mucosa causing clinical signs of pyloric obstruction. The proliferation is mostly by mucus-containing cells of the mucosal surface and foveolae, forming papillary projections supported by a lamina propria that is either normal or that contains an increase in plasma cells and eosinophils. The significance of the eosinophilic inflammation in causing the regional mucosal hypertrophy is unknown, but many examples do not have any change in mucosal leukocytes. Surgical excision is curative. The diagnosis is usually obvious on the basis of endoscopic examination. Endoscopic biopsies may or may not permit the diagnosis, depending on the depth of mucosa captured by the biopsy forceps. The key to the diagnosis in an endoscopic biopsy is that *the entire depth of the sample is taken up by surface epithelium and foveolae*, whereas in a normal stomach a biopsy of similar size would capture at least some of the deeper gastric pyloric glands.

Diffuse gastric mucosal hypertrophy (chronic hypertrophic gastritis), similar in many respects to Ménétrier's disease in humans, is rare. Vomition and weight loss, in some cases associated with inappetence or diarrhea, are described in the history. The characteristic lesion is marked gastric rugal hypertrophy involving part or most of the fundic gland mucosa in the body of the stomach. Grossly thickened folds of mucosa over an area 4–10 cm in diameter are thrown up in a convoluted pattern that may resemble cerebral gyri.

Microscopically, these areas are composed of hypertrophic/hyperplastic mucosa that may or may not include secondary folds of muscularis mucosae and submucosa. Findings are variable in the few cases reported. There may be foveolar and glandular epithelial hyperplasia with progressive or total loss of parietal cells, which are replaced by mucous cells of various degrees of differentiation. Marked cystic dilation of mucous glands may occur, which may be evident grossly. Mononuclear cells infiltrate the lamina propria between glands and near the muscularis mucosae, and the propria may be edematous, especially superficially. If the gross appearance of the mucosa in animals with hypertrophic gastritis is not seen by endoscopy or at surgery, biopsies that do not sample the full thickness of the mucosa may be misdiagnosed as chronic superficial or diffuse gastritis.

The cause of chronic hypertrophic gastritis is unknown, but in part it may be mediated by immune events in the mucosa. The condition in humans is associated with protein-losing gastropathy. Significantly, chronic gastritis and chronic hypertrophic gastritis have been reported in the Basenji, a breed of dog in which a syndrome of protein-losing gastroenteritis and diarrhea is well recognized (see Idiopathic inflammatory bowel disease, below).

Hypertrophic antritis, producing a thickened, sometimes convoluted, mucosa in the antrum, is part of the syndrome of chronic hypertrophic pyloric gastropathy, associated with pyloric stenosis in dogs, considered earlier. Etiologic factors are unknown.

Braxy, or bradsot, is an acute abomasitis of sheep and, less commonly, calves, due to infection with *Clostridium septicum* (Fig. 1.39). It is a sporadic disease of young animals, usually occurring in cooler climates. It is reported from many temperate parts of the world. The factors initiating bacterial invasion are unknown, though local tissue damage in the abomasum is implicated. Cold weather is usually associated with the disease, but it is difficult to imagine feed being cold enough, by the time it attains the abomasum, to cause significant mucosal trauma or hypothermia and necrosis. Production of exotoxin by *C. septicum* causes the signs and death, which usually ensues quickly. A similar gastritis has been associated with *C. sordellii* infection.

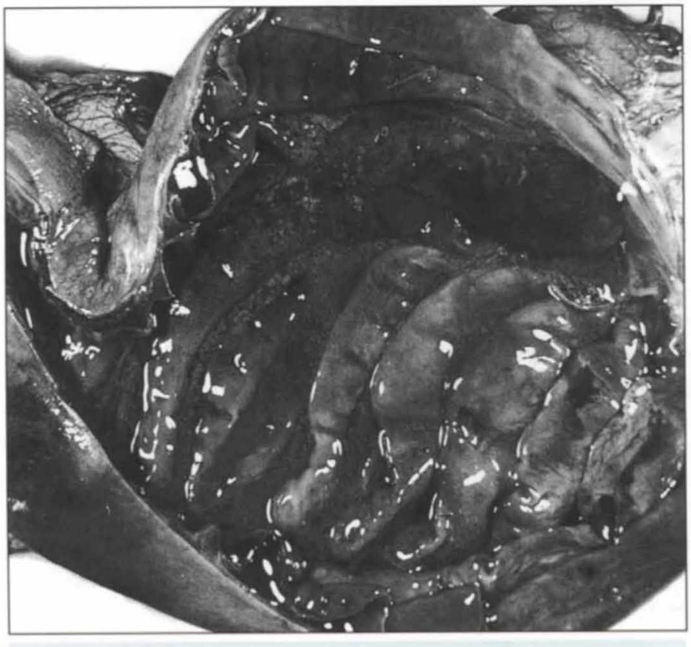

Figure 1.39 Braxy-like **clostridial abomasitis** (*Clostridium septicum*) in a calf. (Courtesy of M Bergeland.)

At autopsy there may be blood-tinged abdominal fluid and the serosa of the abomasum may be congested or fibrin-covered. Mucosal lesions may be diffuse, or involve demarcated foci of variable size and shape. Abomasal folds may be thickened, reddened, and occasionally hemorrhagic or necrotic. Most notable is the presence of extensive gelatinous edema and emphysema in the submucosa. Diffuse edema, and extensive areas of suppurative infiltrate demarcating areas of coagulative necrosis, with prominent pockets of emphysema, are evident in tissue sections. These involve mainly submucosa, and extend into adjacent mucosa and external muscle. There may be venous thrombosis and hemorrhage. Gram-positive bacilli are usually evident as individuals or colonies in affected tissue.

C. perfringens type A has been associated with a syndrome of **tympany, abomasitis, and abomasal ulceration in calves** in the western USA. Animals have abdominal tympany and pain, depression, or may die suddenly. Grossly, there is variable congestion, hemorrhage, erosion, and ulceration of the abomasal mucosa, usually in the fundic area. Circular or linear perforating ulcers may develop. In association with the expected microscopic changes in erosion or ulceration, there is exfoliation and necrosis of mucosal epithelium, edema of the submucosa, dilation and thrombosis of submucosal lymphatics, and mild acute inflammatory infiltrates in the submucosa. Gram-positive bacilli may be on the mucosal surface, or in inflamed submucosa. Proliferation of *C. perfringens* in the rumen of calves with overflow of milk from the reticular groove is believed to promote colonization of the abomasum, and production of necrotizing exotoxin. In both calves and lambs, clostridial gastritis may be preceded by the development of superficial peptic micro-ulcers, which subsequently become colonized and progress to typical clostridial lesions.

Abomasitis associated with viral infection occurs in a number of the systemic viral diseases affecting the gastrointestinal tract, including infectious bovine rhinotracheitis in calves and, rarely, older animals, herpesviral infections of small ruminants, bovine viral diarrhea, rinderpest, malignant catarrhal fever, and bluetongue. Abomasal lesions are rarely the sole manifestation of these diseases, but form part of a picture at autopsy that may suggest an etiologic diagnosis. The appearance and pathogenesis of abomasitis in these diseases vary with the conditions (see Infectious and parasitic diseases of the alimentary tract, later in this chapter).

Mycotic gastritis or abomasitis is a sporadic problem almost invariably secondary to insults that cause achlorhydria, focal atrophy, necrosis, or ulceration under conditions where mycotic colonization can occur. Compromised resistance, perhaps associated with endotoxemia, septicemia, endogenous or exogenous steroids, neoplasia, lympholytic viral disease, and altered gastrointestinal flora due to antibiotic therapy, may further promote mycosis. Fungal hyphae attaining the submucosa typically invade venules and arterioles, causing *thrombosis and hemorrhagic infarction*. The agents involved are usually zygomycetes (phycomycetes) such as *Rhizopus, Absidia*, or *Mucor*; rarely, *Aspergillus* may be implicated. The lesions are areas of necrosis, with an intensely congested or hemorrhagic periphery, ranging in diameter from 1 to 2 cm, to confluence over much of the body of the stomach (Fig. 1.40A). Affected mucosa is thickened, red or pale in the necrotic zone, and may be covered by hemorrhage. Edema and hemorrhage are evident in the submucosa. The lesion may penetrate to the serosa where it is typically seen as a roughly circular area of hemorrhage in the external muscle and subserosa. *Hyphae*, usually broad

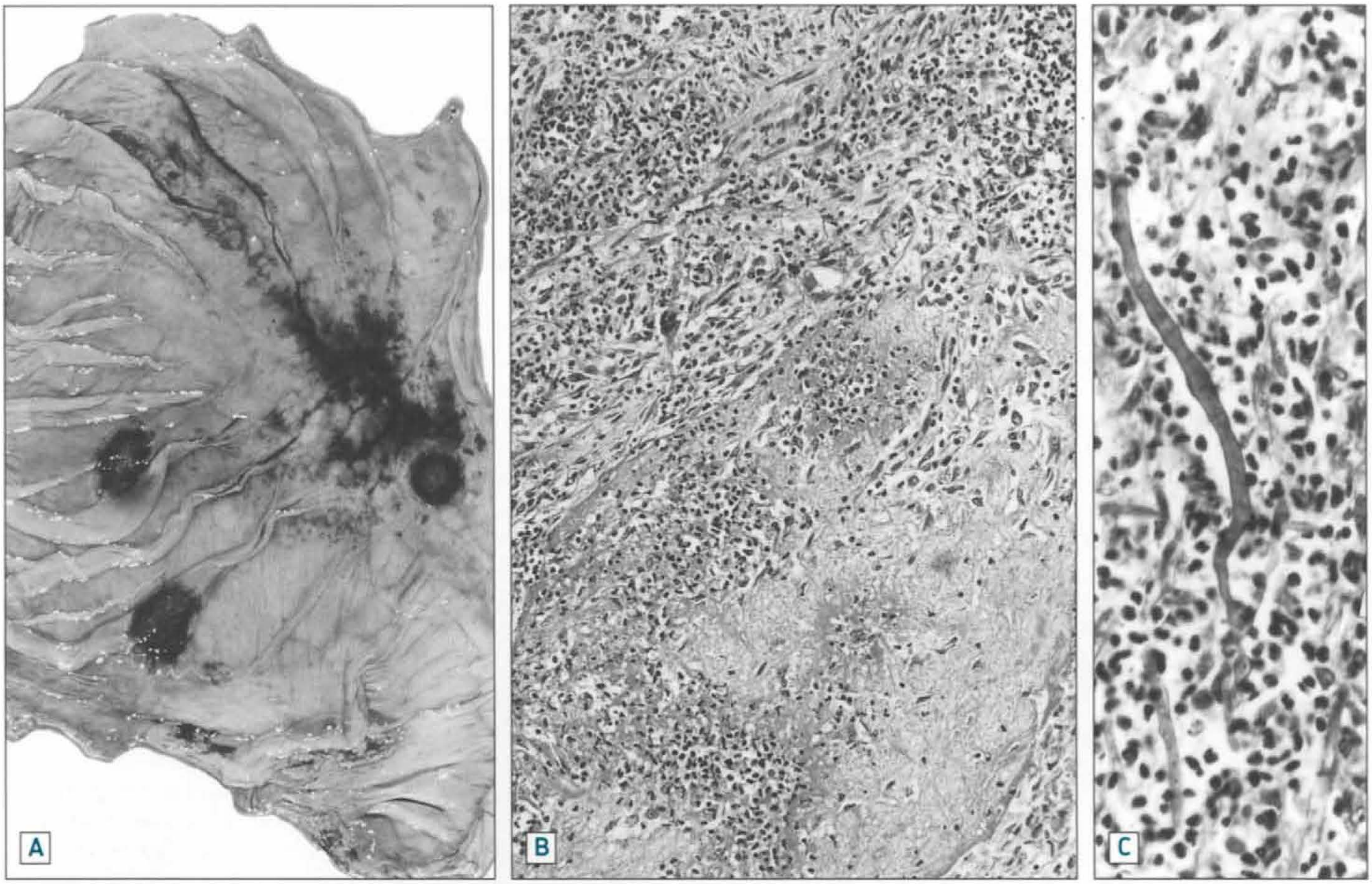

Figure 1.40 Mycotic abomasitis in a calf. **A.** Focal lesions are surrounded by deep red areas of infarction and hemorrhage due to thrombosis of mucosal and submucosal vessels. **B. Thrombosis** of a venule in submucosa of abomasum due to hyphal invasion. **C.** Nonseptate **hyphae** of zygomycete invading the submucosa.

and nonseptate zygomycotic in type, are present in sections of the necrotic mucosa, submucosa, and invading vessels, where they initiate thrombosis (Fig. 1.40B, C).

Candidiasis of the pars esophagea may occur in swine, often in association with preulcerative epithelial hyperplasia and parakeratosis. For an overview of mycosis of the digestive system, and its sequelae, see the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Parasitic gastritis is generally of little significance in small animals. Members of the genera *Physaloptera* and *Gnathostoma* are found in dogs, where the former cause focal ulceration and the latter are the cause of submucosal inflammatory cysts containing suppurative exudate and worms. In cats, *Physaloptera* spp. may attach to mucosal ulcers, whereas *Gnathostoma* spp. and *Cylicospirura felineus* are found in nodules in the gastric wall. *Ollulanus tricuspis* is found on the mucosa of the stomach in cats, where it may cause mild to, rarely, severe chronic gastritis. *Cryptosporidium* infection of the gastric mucosa occurs rarely in cats, with uncertain significance.

In horses, *Draschia megastoma* is found in inflammatory nodules in the submucosa of the cardiac zone, especially along the margo plicatus. *Habronema muscae* and *H. majus* are found on the mucosa and have been associated with mild ulceration. *Trichostrongylus axei* may cause chronic gastritis in the horse. Bots of the genus *Gasterophilus* are found attached to small erosions and ulcers in the esophageal and glandular mucosa, which very rarely become complicated or perforate.

In swine, the spirurids *Ascarops* spp., *Physocephalus* spp., and *Simondsia* spp. are associated with mild gastritis in heavy infections. *Gnathostoma* may be embedded in inflammatory cysts in the submucosa. *Ollulanus tricuspis* may be encountered. *Hyostrongylus rubidus* can cause chronic gastritis and wasting in pigs.

In cattle, sheep, and goats, members of the genera *Haemonchus* and *Mecistocirrus* are large abomasal blood-sucking trichostrongyles, capable of causing severe anemia and hypoproteinemia. *Ostertagia* spp. and related genera, including *Camelostrongylus, Teladorsagia, Marshallagia,* and *Trichostrongylus axei*, in various ruminants, cause chronic abomasitis with mucous metaplasia, achlorhydria, diarrhea, and plasma protein loss. *Cryptosporidium* sp. occasionally causes subclinical abomasitis in cattle, associated with mucosal hypertrophy, attenuation of epithelium lining the neck of fundic glands, and dilation of glands. The small basophilic organisms are present on the surface of epithelium from the base of glands to the mucosal surface. Large schizonts of undetermined coccidia in sheep and goats produce harmless pinpoint pale foci in the abomasal mucosa; the name *Eimeria (Globidium) gilruthi* has been applied. Gastric parasitism is considered more fully in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

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Gastroduodenal ulceration

Gastroduodenal ulcer produces signs much less often in animals than in humans. The pathogenesis of **peptic ulcer** in both humans and animals in general seems to resolve into a relative imbalance between the necrotizing effects of gastric acid and pepsin on one hand, and the ability of the mucosa to maintain its integrity on the other. Impairment of mucosal integrity in the face of normal acid secretion is probably the predominant mechanism, although there are clear instances when hypersecretion of acid is causative.

Factors implicated in hypersecretion of acid include abnormally high basal secretion, possibly associated with an expanded parietal cell mass, perhaps the result of increased trophic stimulation by gastrin. Gastrinomas cause *Zollinger–Ellison syndrome*, characterized by elevated gastric acid secretion and severe gastroduodenal ulceration. Increased histamine levels associated with mastocytosis or mastocytoma also cause acid hypersecretion and ulceration.

Ulceration due to compromise of mucosal protective mechanisms is attributed to *nonsteroidal anti-inflammatory drugs* (NSAIDs), such as aspirin, phenylbutazone, and indomethacin. Orally administered NSAIDs that are weak organic acids, such as aspirin, have a direct deleterious effect on the stomach. Active bicarbonate secretion is responsible for a pH gradient from acid in the lumen to near neutrality at the epithelial cell surface. Stimulation of this bicarbonate secretion is induced by prostaglandin E_2 and nitric oxide. Inhibition of bicarbonate secretion occurs in the presence of atropine and various NSAIDs. Phenylbutazone may also have a direct toxic effect on vascular endothelium in the mucosa, which compromises circulation and predisposes to ulceration.

In humans, gastritis associated with *Helicobacter pylori* infection, and duodenal colonization with this agent, are associated with development of duodenal ulcer. *Helicobacter*-associated gastritis extending cranially in the stomach is associated with gastric ulcer. Similar associations between *Helicobacter* infection, gastritis, and peptic ulcer have not been demonstrated convincingly in domestic animals.

Reflux of duodenal contents containing bile salts has been implicated in the induction of gastritis and gastric ulcer. Under some experimental conditions, acid backdiffusion into the gastric mucosa, and morphologic damage, have been caused by the application of bile salts. The effects are dependent on the pK_a of the bile salt, which must be soluble at acid pH, and on the concentration of hydrogen ion. Lipid solubility of bile salts, and associated damage to surface cell membranes, may mediate these effects. Alcohols, also lipid-soluble compounds, alter permeability of gastric mucosa and permit backdiffusion of acid. Lysolecithin, formed when pancreatic lipase hydrolyzes lecithin in bile, increases gastric mucosal permeability too.

Glucocorticoids and "*stress*" have been implicated in the genesis of ulcer, though the role of steroids is controversial. Experimentally, gastroduodenal hemorrhage and ulceration occur in some species of animals stressed by restraint or social factors, and they are a feature of "trap death syndrome" in small mammals. Severe gastric hemorrhage or ulceration may occur following neurosurgery, trauma to the spinal cord and burns, and it is considered by some to be stress-related. Administration of methylprednisolone sodium succinate to dogs was clearly acutely ulcerogenic. However, some experimental studies have demonstrated that glucocorticoids released during stress are more gastroprotective than ulcerogenic, although the rate of healing in preexisting ulcers was decreased in the presence of steroids.

Reduced mucosal perfusion or ischemia may be a principal factor interacting in stress-associated ulceration, and in that initiated by other modalities, discussed previously. Reduced blood flow to the mucosa in local areas, under a number of circumstances, precedes mucosal hemorrhage or erosion. Ischemia will result in hypoxemic compromise of surface mucous cells. In combination with the effects of other insults, this may initiate mucosal permeability and backdiffusion of acid. Neutralization by blood-borne bicarbonate of acid diffusing into the mucosa also may be reduced in ischemia. Mucosal ischemia may result from reduction in local prostaglandin or nitric oxide concentration, as well as local or systemic hypotension. Ischemia may be more significant in the induction of fundic, rather than antral, ulcers.

Whatever the cause, the results of a breach of the gastric glandular mucosa have the potential to follow a **common pathway to ulceration** in all species. Acute superficial lesions, such as those associated with stress or following administration of aspirin, are often seen as areas of reddening and hemorrhage, especially along the margins of rugae in the fundic mucosa. Acid treatment of hemoglobin gives blood on the surface or in the gastric lumen a red-brown or black color. In some instances, melena, presumably the result of a recent episode of gastric bleeding, may be present in the lower intestine, with minimal gross evidence of hemorrhage or ulceration in the stomach. The microscopic lesion associated with hemorrhage of this type is often subtle; bleeding seemingly results from diapedesis, with minimal mucosal damage. Usually there is superficial erosion of the mucosa, often difficult to differentiate from autolysis, with granules of brown acid hematin in debris on the surface. Inflammation is usually absent. Evidence for healing mild gastric erosion is the presence of basophilic, poorly differentiated, flattened, cuboidal or low columnar cells on the mucosal surface, with mitotic cells in the upper neck of the glands.

Lesions of any genesis proceeding to gastric ulcer do so by progressive, often rapid, *coagulative necrosis of the gastric wall.* Ulcers vary in microscopic appearance depending on their aggression, and the point in their development at which they are intercepted. Acute gastric lesions appear as erosions with superficial eosinophilic necrotic debris and loss of mucosal architecture to the depths of the foveolae, or as a depression in the mucosal surface with necrotic debris at the base. Necrosis usually extends rapidly to the muscularis mucosae, causing ulceration. Once the superficial portion of the mucosa is destroyed, natural local buffering is lost, and the proliferative compartment of the gland, which is near the surface, is obliterated, preventing a local epithelial regenerative response. Ulcers attaining the submucosa impinge on arterioles of increasing diameter, multiplying the risk of significant *gastric hemorrhage.* The ulcer may progress through the muscularis and serosa, culminating in *perforation* of the gastric wall. Severe gastric hemorrhage or perforation are relatively common sequelae of gastroduodenal ulceration in domestic animals.

Ulcers that come into equilibrium with *reparative processes* may do so at any level of the gastric wall below the mucosa, but usually at the submucosa. Subacute to chronic ulcers have a base and sides composed of granulation tissue of variable thickness and maturity, infiltrated by a mixed inflammatory cell population, and overlain by a usually thin layer of necrotic debris. Chronic ulcers wax and wane. Depending on the relative dominance of reparative processes and aggressive ulceration, the layer of granulation tissue may be thick and mature, or thinner, less mature, and with superficial evidence of recent necrosis. There is mucous metaplasia and hyperplasia in glands at the periphery of the ulcer, which, with time, overhang the edge of the lesion, whence epithelial cells may gradually migrate across, closing the defect. Restitution of mucosal integrity is promoted by local activity of cytokines and growth factors such as transforming growth factor-β, basic fibroblast growth factor, epidermal growth factor, interleukins-1β and -2, and interferon-γ, some of which may be secreted by subepithelial myofibroblasts. The mucosa of healed ulcers, even in the fundic zone, is comprised of mucous glands. Excessive scarring of healed ulcers located near the pylorus may lead to pyloric obstruction in any species.

Duodenal ulcers, which usually occur proximal to the opening of the pancreatic and bile ducts, resemble gastric ulcers in their microscopic appearance (allowing for their intestinal location), evolution, and sequelae.

Peptic ulcer in dogs is reported relatively infrequently in the literature. Signs associated with peptic ulcer include variable appetite, abdominal pain, vomition, melena, and anemia. Ulcers, a few millimeters to 3–4 cm in diameter, are found most commonly in the pyloric antrum or proximal duodenum. The gross and microscopic appearance of ulcers varies with their aggressiveness and duration, as previously described. Thrombosed arterioles and venules cut by the ulcerative process are often seen, and should be sought in the bed of gastric and duodenal lesions associated with anemia or obvious hemorrhage.

Perforation of gastric or duodenal ulcers may lead to massive hemorrhage or release of gastric contents into the abdomen. Perforating duodenal ulcer may instigate pancreatitis. Some ulcers perforate silently, the serosal lesion healing by granulation, or adhesion by, and fibroplasia in, the omentum. The irritant nature of gastric contents released in these circumstances may lead to chronic inflammation, granulation, and thickening of the serosa, even when previous perforation cannot be appreciated. A search for microscopic particles of food such as plant material or muscle fibers in the serosal inflammatory response confirms perforation in this circumstance. Chronic peptic ulcers with thickened mucosal margins, scirrhous bases, and perhaps serosal thickening associated with perforation or near perforation, must be differentiated from gastric adenocarcinoma in the dog.

Syndromes resulting from hypersecretion of acid occur in dogs. *Mastocytoma is associated with peptic ulcer,* presumably due to histamine-stimulated acid hypersecretion and microvascular effects. The tumor and mastocytosis do not involve the stomach directly, and ulcers may occur in animals with solitary skin tumors. In one series of 24 dogs with recurrent or metastatic mastocytoma, gastric and duodenal erosions or ulcers, frequently multiple, were present in 20. In many cases such lesions are clinically silent, and they should be looked for at autopsy in animals with mastocytoma. Mast cell tumors have rarely been associated with gastric ulceration in the cow, and in the cat, where gastric ulcer is very uncommon.

Zollinger–Ellison syndrome, *peptic ulcer due to gastrin-secreting pancreatic islet cell tumors or gastrinomas,* has been reported in a few dogs and fewer cats. The history usually includes inappetence, vomition, weight loss, and possibly diarrhea or melena. Reflux esophagitis and gastric or duodenal ulcer are present in most cases. Small nodular masses histologically confirmed as islet cell tumors may be found in the pancreas, and in most animals, metastases to the liver, spleen, or hepatic lymph nodes are present.

Firm diagnosis rests on demonstration of elevated serum gastrin levels by radioimmunoassay; by identification of gastrin-bearing cells in fixed or frozen tumor tissue by immunohistochemistry; or by demonstration of gastrin in extracts of frozen tumor.

The microscopic appearance of these islet cell tumors is not diagnostic for gastrinoma, nor is the ultrastructural appearance of tumor cells necessarily characteristic of the G cell. Pancreatic islet cell neoplasms may be difficult to find, and should be sought assiduously in suspect cases.

The cause in dogs of gastroduodenal ulcer possibly associated with decreased resistance to backdiffusion of acid is less clear. Hepatic disease is often present in dogs with gastric ulcer but the basis for a causal association is obscure. Some ulcers are obviously associated with administration of **glucocorticoids** in high doses as anti-inflammatory, immunosuppressive, or antineoplastic therapy. **Nonsteroidal anti-inflammatory drugs** such as aspirin, naproxen, indomethacin, ibuprofen, flunixin meglumine, and pyroxicam, sometimes given in excessive quantity, are also associated with spontaneous ulcers. Gastric hemorrhage and gastroduodenal ulceration are occasionally seen in dogs following **trauma or major surgery**. A syndrome of gastric hemorrhage, pancreatitis, and colonic ulceration and perforation is recognized in dogs following **spinal trauma**. The pathogenesis of this problem is obscure and undoubtedly complex.

Abomasal ulcers in cattle are common (Fig. 1.41); duodenal ulcer is rarely encountered in this species. Acute ulcers or erosions considered to be the result of stress are frequently seen incidentally in animals, of any age, dying of a variety of causes. They are usually present as linear areas of brown or black hemorrhage or erosion along the margins of abomasal rugae, or as punctate hemorrhages and erosions scattered over the mucosa, especially of the fundus.

Ulcers may be present anywhere in the abomasum. They are common in the pyloric region in cattle, and especially at the torus pyloricus in veal calves, but they may scallop or perforate abomasal rugae and excavate the mucosa in the fundus as well. Often more than one ulcer is present.

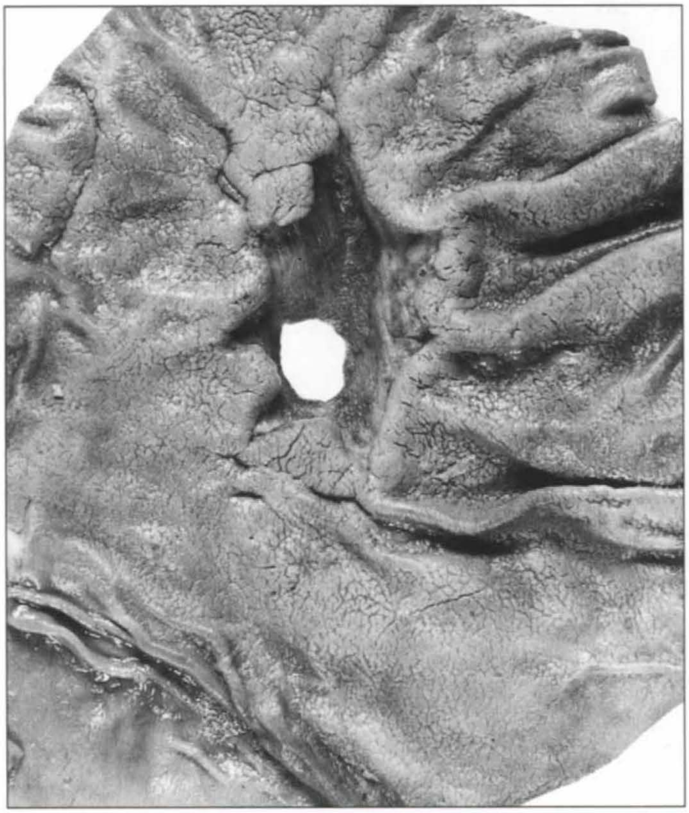

Figure 1.41 Perforated abomasal ulcer in a calf.

The causes of abomasal ulcer are usually unclear, but they are most common in young calves, dairy cows, and feedlot animals. Abomasal ulcer occurred in about 3–4% of feedlot cattle in one study, with about half the cases symptomatic, while there are reports of over 6% of European dairy cows slaughtered with evidence of active or previous abomasal ulcers. A very high proportion, often in excess of 50%, of veal calves may have abomasal ulcers at slaughter. Ulcers often appear to be subclinical, and apparently without effect on growth or performance. However, about one-third of suckling calves dying under 4 months of age in western Canada had perforating abomasal ulcers. They were often associated with hairballs, which, it was concluded, were probably not causal.

Ulcers often seem to occur under *stressful circumstances*, as in recently weaned and veal calves, postparturient cows, animals with concurrent disease such as abomasal displacement or mastitis, or after transportation. Lactic acid and histamine entering the abomasum from the forestomachs in animals poorly adapted to high-concentrate rations may contribute to mucosal damage. In veal calves, consumption of straw, shavings, or other roughage has been associated with an increased prevalence of ulcers, and there appears to be an increase in thickness and altered mucus production in the pyloric mucosa. Abomasal ulcers in range calves in western North America have been associated with *Clostridium perfringens* gastritis, consumption of roughage at pasture, and possibly copper deficiency. Perforating abomasal ulcers may develop in calves secondary to mycotic infection. Abomasal stasis may play a part in animals with physical or physiological abomasal obstruction or displacement. Ulceration of the abomasal mucosa infiltrated by lymphosarcoma will occur, and it may occur as a sequel to ingestion of toxins such as arsenic.

The presenting sign in many cases of abomasal ulceration is *melena*. Hemorrhage causing exsanguination, or perforation and septic peritonitis, is the usual cause of death due to abomasal ulcers. Bleeding abomasal ulcer should be looked for in cattle with melena or anemia, and perforating abomasal ulcer in animals presented with septic peritonitis, especially if digesta is in the abdominal cavity. Perforation may occur into the omental bursa, localizing contamination, and occasionally, points of perforation will be adherent to the abdominal wall, or occluded by superficially adherent omentum.

Gastric ulcer in swine is usually restricted to the *pars esophagea*; in a small proportion of affected pigs lesions extend into the contiguous esophagus. Rarely are significant ulcers of the cardiac, fundic or pyloric mucosa encountered in swine, sometimes in association with ulcer of the pars esophagea, occasionally with gastric parasitism or systemic disease. Venous infarcts in the body of the stomach in swine are not to be confused with gastric ulcer.

Under conditions of modern pig husbandry, the prevalence of ulcer and associated abnormalities of the pars esophagea is high. Weaned growers and feeders are commonly affected. Most lesions are subclinical; however, some prove fatal. Pigs die without premonition, or with a short history that may include anemia, weakness, inappetence, vomition, and melena. Other animals are affected chronically, with signs of anorexia, intermittent melena, and weight loss that may culminate in death or slow recovery with runting.

There is little disagreement over the morphology of ulceration of the pars esophagea, although its etiopathogenesis has been controversial. Many factors have been implicated in the etiology. Stressful husbandry practices have been considered to contribute to development of ulcer, though glucocorticoid administration results in lesions of the fundus, not pars esophagea, in pigs. High dietary copper levels, feeding of whey, starchy diets low in protein, high levels of dietary unsaturated fatty acids, and microbial production of short-chain fatty acids have been associated with the development of ulcers. Experimental infection with *Ascaris suum* has been associated with ulcer, but natural infection is not considered causally associated. While an association between the presence of *Helicobacter heilmannii* and ulceration has been proposed in pigs, this has not been strongly substantiated, and gastric ulcers in pigs involve squamous, rather than glandular, mucosa. Experimentally, factors stimulating acid secretion, especially histamine, consistently cause ulcers of the pars esophagea, suggesting that gastric acidity may play an etiologic role. Repeatedly, *finely ground rations have been found to be ulcerogenic*, and this may be the single most important predisposing factor.

Squamous epithelium has no innate buffering capacity, and it is highly susceptible to attack by gastric acid, pepsin, and refluxed bile, as occurs in reflux esophagitis. Similar events may initiate ulceration of the pars esophagea. Swine with gastric ulcers usually have abnormally fluid stomach contents. Feeding of finely divided rations and prolonged fasting are associated experimentally with increased water in stomach content. Normally there is a declining gradient of pH from esophagus to pylorus in stomach ingesta. Abnormally fluid gastric content fails to partition properly, and the pH gradient from esophagus to pylorus is not established. Relatively low pH occurs at the esophageal end of the stomach, where hydrochloric acid, pepsin, and refluxed bile, along with short-chain fatty acids produced by microbial fermentation of carbohydrate, synergistically attack the squamous mucosa. Meanwhile, the pH at the pylorus is higher than normal. Under conditions of prolonged gastric distension and relatively high antral pH, gastrin-stimulated acid secretion may be excessive, promoting that insult.

Lesions of the pars esophagea may involve only a small part, or virtually all of the gastric squamous mucosa. The lesion evolves through parakeratosis, to fissuring and erosion, with ultimate ulceration in severe cases. All stages in this progression will be encountered at necropsy in pigs. The epithelium of the pars esophagea often appears yellow and is thickened, irregular, roughened, and may flake or peel off readily. *Candida* may be present over the epithelial surface, with hyphae invading the parakeratotic epithelium, perhaps due to favorable cystine or glycogen levels. Rete pegs and proprial papillae are elongate.

Erosion of the epithelium progresses to ulceration and exposure of papillae and deeper propria, which bleed as small vessels are disrupted. Such lesions begin as fissures in the hyperplastic parakeratotic epithelium, but advance to ulcerate the entire pars esophagea. They usually spare only a microscopically visible margin of squamous epithelium adjacent to the cardiac gland mucosa. Ulcers of the pars esophagea, like peptic ulcer, have a floor of necrotic debris overlying exposed connective tissue (Fig. 1.42). Depending on the stage and aggression of the ulcer, there may be a well-developed inflammatory margin to the necrosis and a bed of granulation tissue. *Fatal gastric hemorrhage* often occurs.

Grossly, fully developed ulceration of the pars esophagea is apparent as a punched-out lesion with elevated rolled edges, obliterating the entire pars esophagea and obscuring the esophageal opening (Fig. 1.43). The floor of the ulcer may be so smooth that it is misinterpreted as normal by the inexperienced. Pigs with gastric ulcer at any stage of evolution tend to have fluid content in the stomach. Those with hemorrhagic ulcer may have red-brown gastric content, or massive hemorrhage into the stomach with large blood clots in the lumen, and smaller clots adherent to the base of the ulcer and its exposed bleeding points. Melenic content will often be present in the intestine, and the colon may contain firm black pelleted feces. The

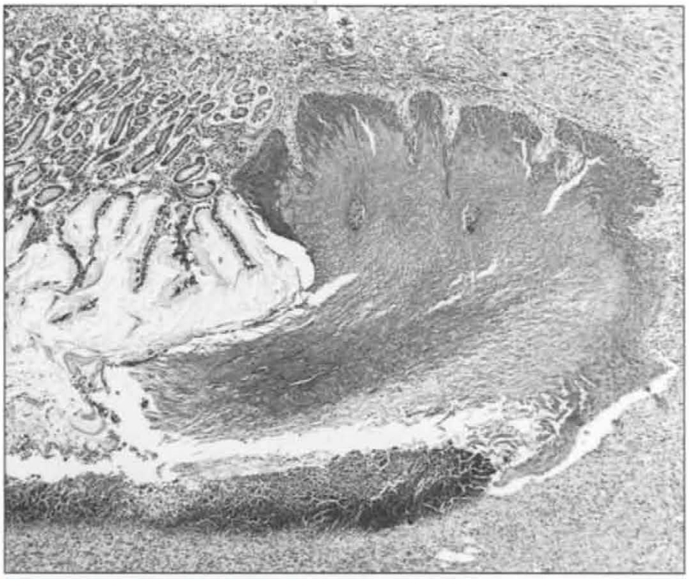

Figure 1.42 Margin of **ulcer of the pars esophagea** in a pig. Cardiac glandular mucosa and a marginal remnant of squamous mucosa overhang the margin of the ulcer, the floor of which is at the bottom.

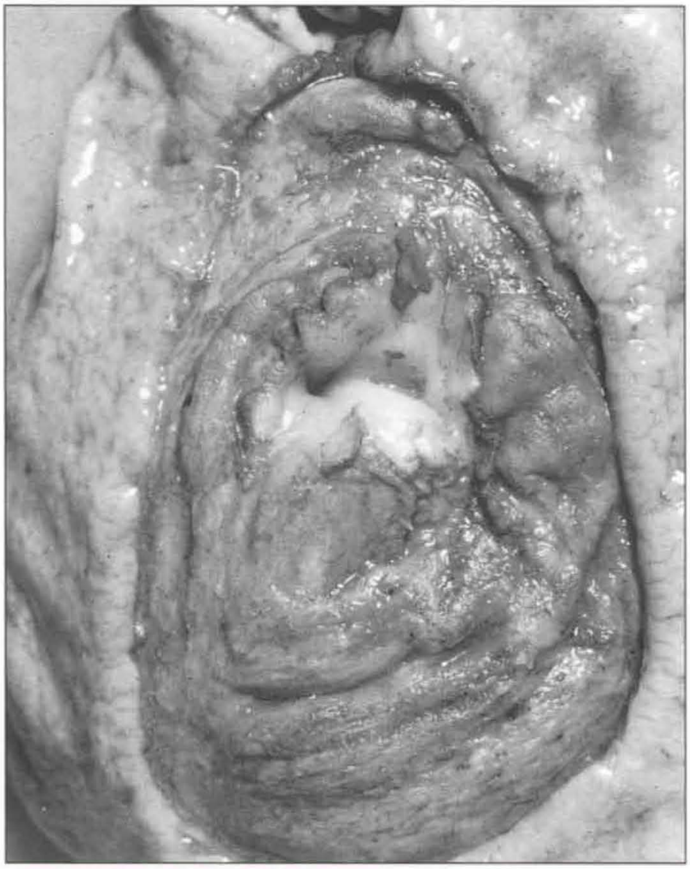

Figure 1.43 Ulceration of the pars esophagea in a pig. Squamous mucosa is ulcerated, but adjacent cardiac glandular mucosa is unscathed. Esophageal mucosa is visible opening into the upper part of the ulcer.

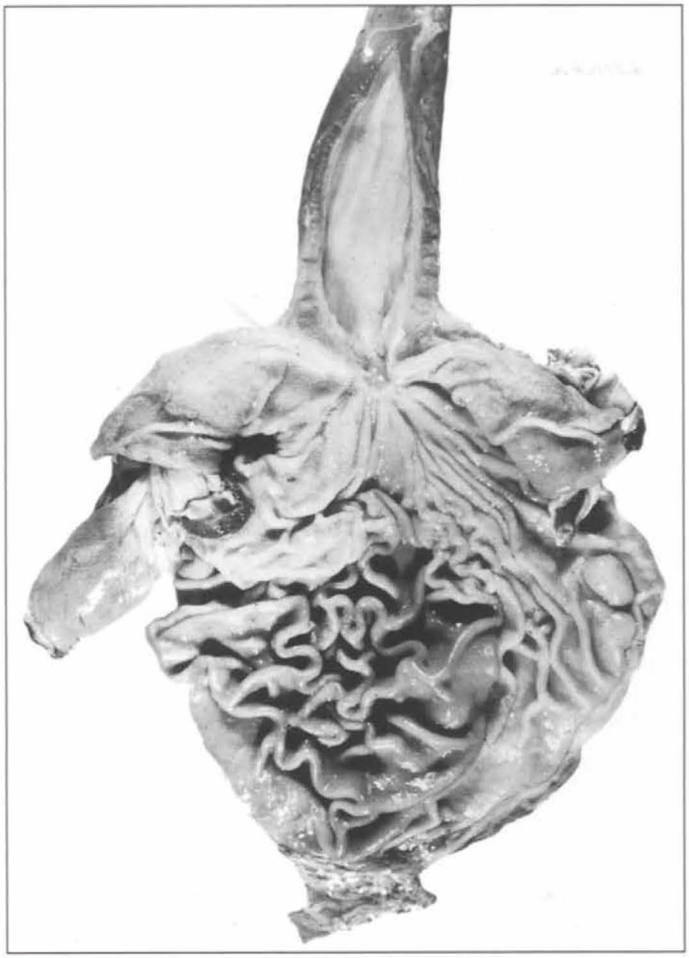

Figure 1.44 **Scarring and stricture** of distal esophagus and pars esophagea following ulceration in a pig.

carcasses of animals that exsanguinate with gastric ulcer are very pale. Blood in the intestine associated with gastric ulcer in pigs must be differentiated from mesenteric torsion and from proliferative hemorrhagic enteropathy associated with *Lawsonia*. A few pigs with parakeratosis, erosion, and ulceration of the pars esophagea have esophageal lesions suggestive of gastric reflux.

Gastric ulcers in some pigs resolve by granulation, and they may become re-epithelialized. Such lesions usually become scirrhous, puckered, and contracted as the ulcer closes from the periphery, and scarring may be visible from the serosa (Fig. 1.44). In these circumstances, stenotic occlusion of the esophageal opening into the stomach may occur, and pigs with this problem can develop muscular hypertrophy of the distal esophagus.

In **horses**, *ulcers in the stomach of foals and adults* are often found at autopsy incidental to some other disease process. Gastric ulcer as a clinical entity is less commonly recognized, though a syndrome of abdominal pain, sometimes associated with gastric reflux, has been described in foals, and gastric ulcers are associated with colic in older horses. Ulcers are common in foals under 4 months of age; about half of a group of foals without signs of gastric disease had ulcers visible by endoscopy. Ulcers have been detected endoscopically in over 65% of performance horses, in training, racing, or on endurance rides. They are often multiple, and while most frequent in the esophageal region, they can simultaneously involve all four mucosal zones of the stomach, and the duodenum, with no relationship apparent in the pattern of distribution.

Ulcers of the *esophageal zone* are common. They are usually most severe at or adjacent to the margo plicatus, involving the edge of the squamous epithelium. They are often large and irregular in shape. There may be extensive fissuring, erosion, and ulceration of the squamous mucosa on the remainder of the pars esophagea, and in the esophagus, sometimes nearly to the pharynx, in foals with reflux. Often islands of thickened white proliferative mucosa are scattered as plaques on a predominantly ulcerated mucosa (Fig. 1.45). Microscopically,

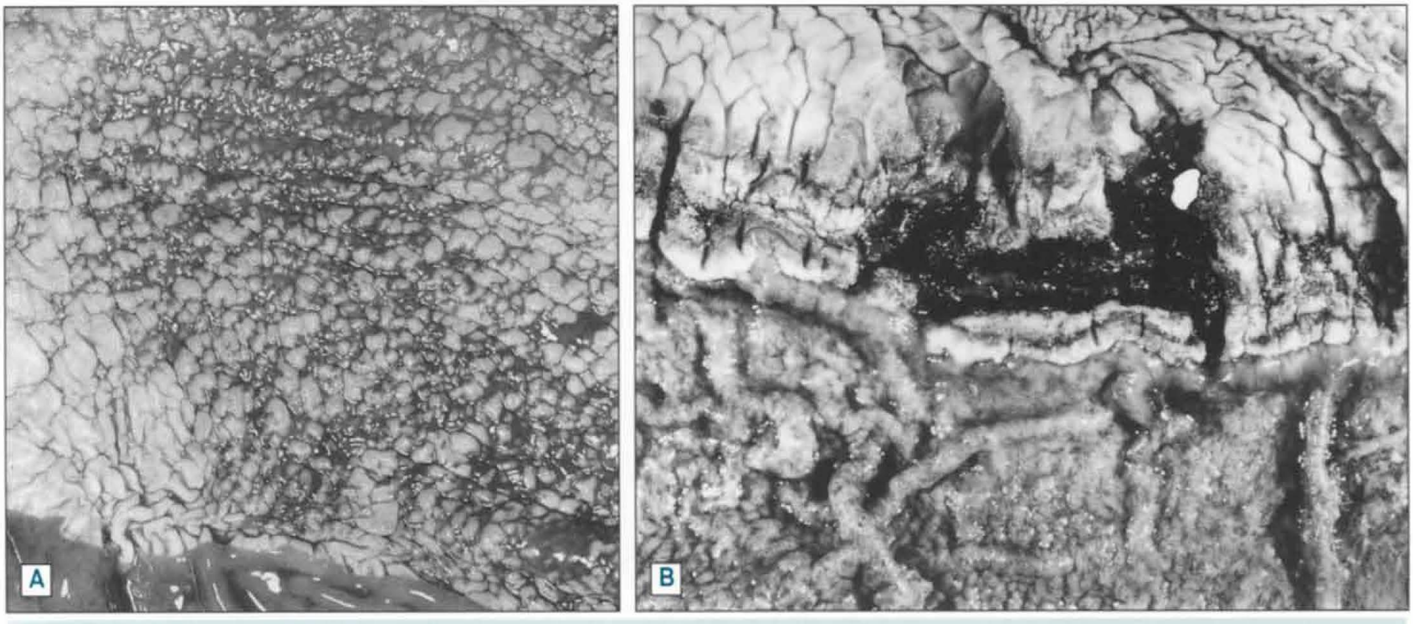

Figure 1.45 **A.** Multiple confluent areas of **ulceration** of the squamous mucosa of the stomach of a foal, with smooth nodular islands of surviving hyperplastic mucosa scattered over the ulcerated area. **B. Ulceration with perforation** of squamous gastric mucosa at the margo plicatus of a foal's stomach.

there is epithelial hypertrophy and hyperplasia in response to insult, marked in some islands of surviving mucosa, with prominent rete pegs and edematous proprial papillae. Lesions then grade through increasing degrees of epithelial erosion to ulceration, with evolution of the ulcer and ulcer bed, as described earlier in swine.

Ulcers in the secretory stomach are less common. These ulcers are also often large and multiple, though a full range from focal punctate to extensive deep lesions may be seen.

Perforation may occur at any site of ulceration, and in one series represented 1% of 600 autopsies on foals. Some foals may exsanguinate due to bleeding ulcers, and occasionally clotted blood will fill the stomach, forming a cast.

Pyloric and duodenal stenosis have been associated with healing ulcers in horses. Ulcerative lesions involving the circumference, or the antimesenteric mucosa, of the proximal duodenum have been associated with gastric ulcers in foals, and duodenal stricture may represent a more chronic phase of this process. Severe esophagitis occurs in foals with ulcer and gastric reflux.

Many cases of gastric ulcers in horses are associated with enteric disease, colonic impaction, ileus, surgery, or other circumstances that can be considered stressful or could cause gastroduodenal reflux. Ulcers are common in horses held in stalls, and alternating periods of feeding and feed deprivation have been shown to induce ulceration of the gastric squamous epithelial mucosa. The pathogenesis of ulceration in the pars esophagea in horses probably resembles that in swine. Abnormally fluid content associated with feeding patterns and feedstuffs is permissive of acid, pepsin, and bile reflux to the cranial part of the stomach, where, at pH levels <4.0, volatile fatty acids generated locally may predispose further to damage to the squamous mucosa. A diet of bromegrass hay has been implicated in causing lower gastric pH, and thus facilitating ulcer formation. Exercise increases intra-abdominal pressure, resulting in gastric compression and reflux of acidic content into the cranial stomach, possibly contributing to ulceration associated with intensive training.

It is unclear whether the *ulcerative duodenitis* seen in some cases is a product of peptic ulceration, or whether it represents a process such as "proximal enteritis," which in turn results in stricture, gastric reflux, and ulceration. *Clostridium perfringens* has been considered as a potential cause of the associated duodenitis. Administration of NSAIDs is commonly associated with gastroduodenal ulcers, and lesions of the glandular stomach, squamous stomach, and pylorus, among other lesions, have been induced in horses intoxicated with phenylbutazone.

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INTESTINE

Normal form and function

The microtopography of the **small bowel** is extensively modified, to increase its surface area, by *spiral mucosal folds* in some species, and by **villi** projecting into the lumen. The villi, projections of lamina propria covered by a layer of epithelium one cell thick, expand the absorptive surface of the small bowel 7–14-fold. In most species, villi are tallest in the duodenum, and decline somewhat in height toward the ileum. The length and shape of villi in "normal" animals vary with the species, age, intestinal microflora, and immune status. In general, villi in dogs, cats, neonatal piglets, and ruminants tend to be tall and cylindrical; those in horses and in young ruminants tend to be moderately tall and cylindrical; villi in weaned ruminants and swine may be cylindrical, leaf or tongue-shaped, or rarely ridge-like, with their broad surface at right angles to the long axis of the gut. Length of villi typically declines somewhat after weaning.

Opening on to the mucosal surface around the base of each villus are several *crypts of Lieberkühn*. These are straight or somewhat coiled (depending on the species and the proliferative status) tubular gland-like structures, lined by a single layer of epithelium. The progenitor compartment of the enteric epithelium resides here, producing cells that differentiate and move up on to the surface of villi, mainly as absorptive enterocytes, ultimately to be extruded as effete cells from the tips of villi. Sloughed cells contribute to the enzyme content and complexity of the intestinal luminal content.

Stem cells are present in the crypts; depending on species and the position in the gastrointestinal tract, they are near or at the base of the crypt. They divide to produce *four main lineages of cells*. **Poorly differentiated enterocytes**, which are cuboidal or low columnar, with relatively few, short microvilli, are the predominant type of cell lining crypts. Especially in the lower half of crypts, these cells form a population that cycles rapidly, undergoing amplification division. One of the ensuing daughter cells usually differentiates and moves into the functional compartment of absorptive enterocytes on the villus. Undifferentiated crypt epithelial cells also secrete electrolytes and water.

Oligomucous cells, derived by mitosis from the basal stem cells, also form a population undergoing amplification division. They contain mucous granules, and are intermediate in structure between undifferentiated crypt epithelium and goblet cells, into which they mature. Well-differentiated **goblet cells** are present in crypts and on the surface of villi, with variable prevalence and distribution at various levels of the intestine, and in the different species. They have basal nuclei and *secrete mucus*, apparently by exocytosis, from the luminal border of the cell. **Intestinal mucus** is a gel that lubricates and probably serves to "insulate" the surface from organisms, which it may entrap or immobilize. It contains *trefoil factor*, also produced by the goblet cell, which promotes epithelial restitution or gliding after erosion; *lysozyme* and *defensins* produced by Paneth cells; and *immunoglobulin A* secreted into it by epithelial cells. Goblet-cell hyperplasia and mucus secretion are promoted by a variety of noxious stimuli, by inflammatory mediators, and by cell-mediated immune events in the gut. Production of trefoil factor may be related to the preponderance of mucous cells often present in healing mucosa.

Paneth cells, a population of enigmatic cells turning over slowly (~20 days) in the base of the crypts in small intestine, are most obvious in horses, among the domestic animals. They are not found in dogs, cats, or swine, and they are not prominent in the intestine of ruminants. Eosinophilic secretory granules are present in the apical cytoplasm of Paneth cells. They contain a number of *antimicrobial proteins and peptides* active in innate host defense, including lysozyme, phospholipase, DNAse, ribonuclease, and alpha-defensins. Adaptive increases in Paneth cell numbers and activity are initiated by T cells and proinflammatory cytokines.

The fourth type of cell found in intestinal crypts is the **enteroendocrine** cell. They too are derived from crypt stem cells, and comprise a heterogeneous population of over a dozen amine- or peptide-secreting endocrine/paracrine cells, representing about 1% of the crypt cell population. These are the cells variously recognized as *enterochromaffin, argentaffin, or argyrophil*; the specific cell type is defined by immunocytochemistry and the ultrastructure of secretory granules. Hormones with relatively clearly understood functions, such as secretin and cholecystokinin, as well as peptides or amines whose endocrine or paracrine implications are still being revealed, are secreted. With the exception of carcinoid tumors of serotonin-secreting cell origin, and rare functional neoplasms of other enteroendocrine cells in humans, the pathologic implications of this class of cells are still very poorly defined.

Enterocytes, which are responsible for the final digestion and absorption of nutrients, electrolytes, and water, are by far the predominant cell type on intestinal villi. They are normally tall columnar cells, polygonal in cross-section, with a regular basal nuclear polarity. A tight junction, which is nevertheless "leaky" to small molecules and water, joins the apical margins of adjacent cells. The barrier to transepithelial macromolecular movement is essentially maintained even at sites of extrusion of effete enterocytes at the tips of villi. Basal to the tight junction, the lateral cell membranes interdigitate loosely, and a long, narrow potential space exists between enterocytes. The basolateral cell membrane is the site of sodium–potassium-dependent adenosine triphosphatase that drives the sodium pump, and of carrier systems exporting monosaccharides from the cell. Absorptive epithelial cells lie on a basal lamina with which they interact via integrins, which may be involved in communications with the underlying mesenchyme. Immediately beneath the basal lamina lies the sheath of syncytial **myofibroblasts** that produce the basal lamina, and with which enterocytes also appear to interact. Myofibroblasts seem to be involved in intestinal organogenesis, and epithelial proliferation and differentiation, as potent sources of growth factors.

The apical surface of normal enterocytes is highly modified into **microvilli**, about 0.5–1.5 μm long and 0.1 μm wide, which are regularly arrayed in close apposition to each other at right angles to the surface of the cell. They are visible as the "brush border" by conventional microscopy. Microvilli increase the surface area of absorptive epithelium by a factor of about 15–40 times. The plasmalemma of microvilli is studded with massive numbers of enzyme molecules, including aminopeptidases and disaccharidases involved in terminal digestion of peptides and carbohydrates. These protrude as minute "knob-like" structures into the glycoprotein "glycocalyx" that coats the surface of microvilli.

In neonatal swine and ruminants, vacuolation of absorptive enterocytes is normal, and the nucleus is often also displaced into the apical cytoplasm. In piglets vacuolation is usual in the ileum, but not in the duodenum, and seems to be a function of cell age. Such

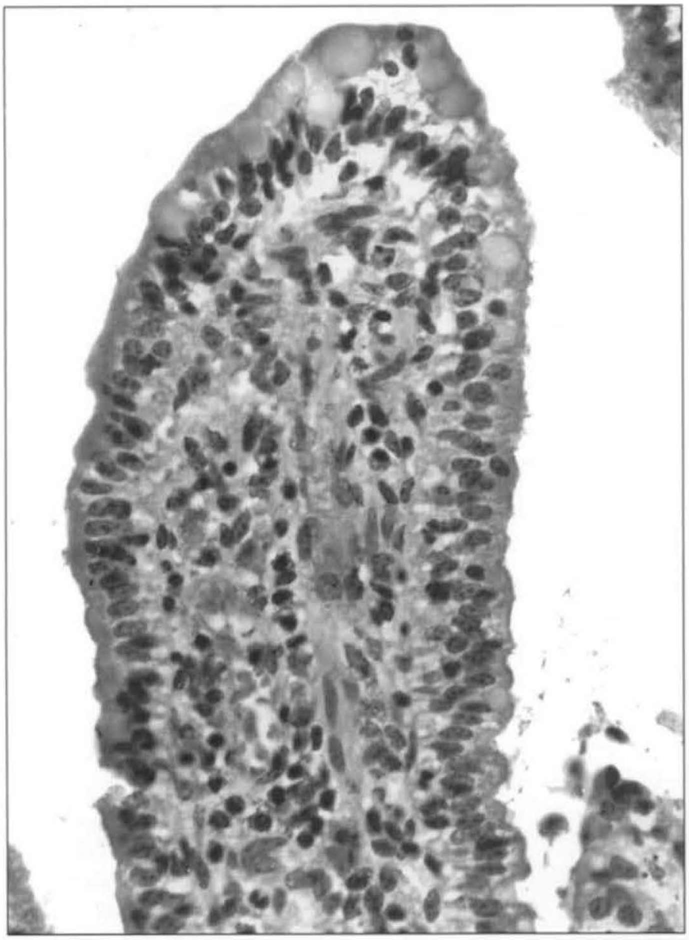

Figure 1.46 Distended vacuoles containing eosinophilic **colostral protein** in apical cytoplasm of enterocytes at the tip of the villus of a 2-day-old calf.

vacuolation should be differentiated from the presence of eosinophilic colostrum present in cytoplasmic vacuoles in the epithelial cells of neonates (Fig. 1.46).

The cytoplasm of absorptive enterocytes is stabilized at the apical border by the filaments of the terminal web. Smooth endoplasmic reticulum is most prominent in the upper half of cells, while cisternal elements of rough endoplasmic reticulum are more uniformly distributed. The Golgi zone lies above the nucleus. Free ribosomes and polyribosomes are numerous in differentiating cells of the upper crypt and lower villus, and are relatively fewer in mature absorptive enterocytes.

The complex of endoplasmic membranes and Golgi apparatus is particularly active in handling *absorbed lipid*, which diffuses from micelles at the cell surface, through the apical membrane, in the form of long-chain fatty acids or monoglyceride. These are re-esterified to triglyceride, appearing in the smooth endoplasmic reticulum, and are complexed with apoproteins produced in the rough endoplasmic reticulum, to be excreted, via the Golgi apparatus, through the basolateral cell membrane as *chylomicrons*. Chylomicrons enter the extracellular space, and leave the villus via the lacteal.

The **lamina propria**, a highly plastic mesenchymal stroma, supports the epithelium of the small intestinal mucosa. It is composed of loose fibrous tissue, through which course blood vessels, and in which smooth-muscle, inflammatory, and immune-active cells are interspersed. In addition to functioning in defense against microorganisms, macrophages phagocytose inert particulate matter reaching the lamina propria from the lumen. Bile pigment, perhaps derived from meconium, is sometimes seen in macrophages in the tips of villi in neonates. Macrophages may become iron-laden and, by loss from the mucosa, may play a role in iron homeostasis. Apoptotic bodies, ceroid, hemosiderin, and bile pigment may also be present in histiocytes in the lamina propria at tips of villi, which are particularly prominent in horses, and should not be mistaken for foci of necrosis. These may represent detritus resulting in part from apoptosis of upwardly migrating subepithelial myofibroblasts at the villus tip. Myofibroblasts are probably largely responsible for the remarkable plasticity of the villus during adaptive changes expressed as villus atrophy and reconstitution of mucosal three-dimensional morphology.

Lymphocytes, neutrophils, and eosinophils are scattered in the lamina propria of villi and between crypts. Eosinophils are especially common in the intestine of ruminants and horses, with no specific pathologic connotation, but they are highly variable in prevalence in the intestine of small animals. Intraepithelial lymphocytes are frequently found between epithelial cells on villi, and, less commonly, in crypt lining. Globule leukocytes may be found in the epithelium of crypts and low on villi, or sometimes in the lamina propria between crypts. Plasma cells are normally not numerous in villi, but are concentrated in the lamina propria between the upper portions of crypts.

The **vascular supply to the small intestinal mucosa** arises in submucosal arteries that give off arterioles at right angles, some of which send branches to a capillary plexus around crypts of Lieberkühn. The majority pass up the centers of villi, arborizing near the villus tip into a dense capillary plexus which lies immediately beneath the basal lamina of the epithelium. Capillaries in villi have fenestrations facing the basal lamina, which may be more permeable than the remainder of the endothelium. One or more venules drain blood from the capillaries in villi and between crypts, and flow into larger veins in the submucosa, which drain into mesenteric veins and the hepatic portal circulation. The *lacteal*, or central lymphatic vessel of the villus, is sufficiently permeable to permit the entry of macromolecules and chylomicrons, and is the main route of lipid transport from the villus.

The cecum and colon vary widely in anatomy and size among domestic animals, depending largely upon the significance of microbial fermentation of carbohydrate in the hindgut. *Production of volatile fatty acids* from carbohydrate by colonic flora occurs in all species. In the horse, this is a primary source of energy, and it is significant in swine and ruminants as well. Extensive movement of electrolytes and water occurs across the colonic wall. In the horse, a volume of fluid approaching that of the extracellular fluid space of the animal may be in the large bowel, which must maintain a fluid medium for microbial fermentation; daily fluid absorption from the hindgut may exceed the extracellular fluid volume. *Absorption of electrolytes and water*, an electrolyte-conserving mechanism, is probably the major function of the colon in dogs and cats, and of the distal colon of herbivores.

The mucosa of the cecum and colon in all domestic species lacks villi, though there are ridges or folds on the mucosal surface. The surface of the hindgut is lined by a single layer of columnar **absorptive epithelial cells** with basal nuclei. These cells generally have more sparse and less regular microvilli in comparison with absorptive cells of the small bowel, and numerous glycoprotein-laden vesicles are in the apical cytoplasm in most species. Typical goblet cells are also interspersed on the colonic surface in variable numbers, depending on the species and a variety of other factors.

Colonic crypts, or glands, are straight tubular structures. The architecture of colonic glands and their cell population resembles somewhat that of small intestinal crypts. *Epithelial cells*, which contain small glycoprotein-laden vesicles, differentiate progressively from stem cells in the crypt to absorptive epithelium as they approach the surface. *Oligomucous cells*, derived from basal stem cells, form a second proliferative population in the lower half of the colonic gland. Well-differentiated *goblet cells* are usually present in the upper half of glands in the large bowel, as well as on the surface. *Enteroendocrine cells* of about a half-dozen types have been recognized, scattered in the cell column lining glands in the large bowel.

The lamina propria of the colon is minimal between closely packed glands. It contains a cell population similar to that in the small bowel. Normally, relatively few inflammatory and immune-active cells are present in the superficial mucosa in small animals and young herbivores; most plasma cells and lymphocytes are between deeper portions of glands. Older herbivores may have somewhat heavier superficial proprial inflammatory infiltrates, and macrophages containing phagocytosed debris may be present below the surface epithelium between the mouths of glands, especially in the horse. In the equine colon, terminal arterioles that enter the mucosa branch at right angles from the submucosal plexus. Capillaries ramify to surround colonic glands, and form an anastomosing network at the luminal surface. Sparsely distributed venules drain the superficial capillary plexus.

The connective tissue of the **submucosa** lies between the mucosa and the **external muscle** of the gut, which is comprised of inner circular and outer longitudinal layers, made up of fascicles of smooth-muscle cells. An extensive **enteric nervous system**, with submucosal (Meissner's), and myenteric (Auerbach's) plexuses marked by ganglia, modulates external autonomic neural regulation and coordinates gastrointestinal motility and function. The neurons of the enteric system equal in number those in the spinal cord, and their ramifications sense and influence epithelial absorption and secretion, local endocrine/paracrine secretion, blood flow, immune events, and motility in the gut. Secretomotor neurons ramify axons that extend to individual crypts of Lieberkühn, where they stimulate secretion of electrolyte, water and mucus. Excitatory and inhibitory effects are mediated by acetylcholine and by amine and peptide neurotransmitters, such as substance P, adenosine triphosphate and vasoactive intestinal peptide, which in some cases are also produced by endocrine cells of the gut and pancreas. In addition, *myofibroblastic pacemaker cells of the intestine*, known as **interstitial cells of Cajal**, are distributed throughout the intestinal musculature, integrated with the extrinsic and enteric nervous systems. Abnormalities of these cells are thought to lead to disorders of motility, and to gastrointestinal stromal tumors, a specific entity described in horses and dogs. Disorders of motility associated with enteric neural lesions, such as the dysautonomias and grass sickness, are discussed in the section on intestinal obstruction, below.

Interpretation of intestinal and colonic biopsies is often subjective, unless clear-cut diagnostic criteria can be met: by recognition of an etiologic agent; a specific cell type; a characteristic pattern of inflammation or anatomic abnormality, such as lymphangiectasia; or cytologic and morphologic abnormalities indicating malignancy. There are significant variations in the microscopic morphology of the gut among individuals and between species, and with age; at different levels of the small and large intestine within species and within the same individual; and as a result of factors influencing "physiologic" inflammation in the mucosa. Limited morphometric data are available, describing in a quantitative manner the "normal" morphology of the intestinal mucosa and its epithelial and inflammatory cell populations in domestic animals. However, until more such information is available, and its application, particularly in the diagnosis of inflammatory bowel disease, is validated, pathologists would do well to expand their experience of "normal" intestinal morphology by close examination of tissues from animals in which gastrointestinal disease is not indicated clinically, and to view critically their reliability in diagnosing such entities. The various grading schemes suggested for interpretation of inflammatory lesions of the stomach and intestine leave much to be desired in terms of objectivity and potential for interobserver variation.

Biopsy interpretation is enhanced by increased size of specimen; increased numbers of specimens; the sampling of all layers of the bowel wall; minimal trauma, rapid fixation, and optimum orientation. Sacrifice of any of these attributes increases the subjectivity with which a biopsy is interpreted. Hence, in dealing with endoscopic and other small biopsies, care must be taken in handling to reduce traumatic artefact and to optimize orientation. Clinicians and pathologists dealing with endoscopic, capsule, and forceps biopsies must remember the limitations in interpretation imposed by small sample size; difficulties in orientation; the frequent failure to sample the deep mucosa; and the usual failure to sample the submucosa and muscularis. These costs are offset to some degree by the opportunity to obtain a greater number of samples than may be possible by other means, at arguably lower risk and cost, and possibly more "focused" on a lesion by direct endoscopic examination.

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Immune elements and the gastrointestinal mucosal barrier

The gastrointestinal tract is presented continually with antigens in food; ingested toxins; viruses; bacteria and their products; and parasites and their excretions and secretions. The epithelial barrier of the gut is but one cell thick and has enormous surface area; the enteric mucosal surface of the average human is about 400 m^2. Therefore, it is not surprising that the epithelium and associated lymphoid and inflammatory cells in the mucosa and submucosa have evolved a complex system for excluding, blocking, sampling, tolerating, or neutralizing and eliminating antigens. At birth, intestinal immune elements are sparse and quiescent; immune activity in the gut is probably stimulated in response to colonization by normal bacterial flora beginning in the early neonatal period. In the mature animal, *lymphoid tissue has been estimated to comprise 25% of the intestinal mucosal mass*, and to exceed that of the spleen in volume.

Innate and adaptive elements of the gastrointestinal mucosal barrier include, physiologically, volume of secretion and peristalsis, which dilute and flush the contents, and gastric and bile acids and pancreatic secretions that break down ingested antigens. The indigenous microflora competitively inhibit, or actively exclude, intruding bacteria. Mucins on the luminal surface form a secretory barrier, and retain trefoil factor, antibody and soluble components of innate resistance, including those produced by Paneth cells. The epithelium provides a physical barrier; participates in innate resistance by production of proinflammatory cytokines; enables passive immunity by antibody uptake in the neonate; and by antigen uptake and presentation may contribute to active immunity. Intraepithelial lymphocytes presumably act as a first line of defense. Soluble antibody in plasma and interstitial fluid neutralizes antigens penetrating the mucosal barrier and contributes to opsonization/phagocytosis, as do endogenous components of the innate immune system, such as complement. The organized elements of the mucosal immune system, Peyer's patches, and other mucosal lymphoid follicles, are sites of induction for intestinal immune responses, generating antigen-activated B and T cells that ultimately home back to the mucosa. Within the lamina propria reside populations of such B and T cells, macrophages, and dendritic cells. Regional lymph nodes receive and trap free and phagocytosed antigens, and antigen-presenting cells activate more B and T cells, promoting mucosal and systemic immunity. Escape of viable infectious agents past the mesenteric lymph nodes permits systemic disease.

The **epithelial cell** of the neonate is capable of uptake and transport of macromolecules from the intestinal lumen to the basolateral cell surface. In all species of domestic animals, **colostral transfer of immunoglobulins** by this route provides the neonate with passive humoral immunity during the early postnatal period. The period of active uptake of macromolecules is short, usually only 24–48 hours in ungulates, and "closure" precludes further bulk transport of macromolecules.

Although bulk transport does not occur, nutritionally inconsequential amounts of macromolecules continue to be transferred by enterocytes in mature animals. For uptake to occur, molecules must escape intraluminal hydrolysis, and pinocytosis must exceed the rate of lysosomal degradation to permit molecules to be exported from the cell. Fully differentiated small intestinal absorptive epithelial cells express on their basolateral membranes major histocompatibility complex class II molecules, as do antigen-presenting macrophages and dendritic cells. Enterocytes are probably capable of directly presenting antigen to T cells. As well, via *pattern recognition molecules* – surface Toll-like receptors and cytosolic nucleotide-binding oligomerization domain (Nod) molecules – they detect highly conserved molecular structures (pathogen-associated molecular patterns) produced by luminal bacteria, and some viral components, activating cellular production of proinflammatory or immunomodulatory cytokines.

Intestinal intraepithelial T lymphocytes form a large population strategically located between intestinal epithelial cells, adjacent to the basement membrane. They may comprise up to 10–20% of the cells within the epithelial layer. In domestic animals they are comprised mainly of cytotoxic T cells, with a small minority of CD4+ and $\gamma\delta$ T cells. They presumably function as a front line of defense, reacting, as part of the innate response, to bacterial and plant products such as amylamines, and to damaged enterocytes. They also presumably participate as components of the adaptive response, responding to antigens entering from the lumen and in tumor surveillance. Some may kill compromised epithelial cells. Most, but not all, intraepithelial lymphocytes appear to originate in Peyer's patches.

Globule leukocytes are visible in hematoxylin and eosin-stained tissue sections as *mononuclear cells with large eosinophilic cytoplasmic granules*, in the epithelium of the crypt and lower villus, and sometimes in the lamina propria. Their origin has been uncertain. Formerly postulated to be derived from mast cells, the weight of evidence suggests that they are *granular lymphocytes*.

Specific epithelial cells called **"M" cells**, associated with Peyer's patches and intestinal lymphoid follicles, actively "sample" particulate matter and macromolecules impinging on the mucosal surface. M cells are interspersed among other cells of the follicle-associated epithelium resembling absorptive enterocytes, on the surface of the "dome" in the mucosa overlying lymphoid aggregates in the submucosa. They often adopt an inverted "cup" shape, with one or more lymphocytes and occasional macrophages, in the basal concavity, in contact with the membrane of the M cell. Material taken up by M cells is transmitted to the associated lymphocytes or macrophages. Despite, or perhaps because of, its role in adaptive immunity, the M cell is exploited as a likely portal of entry to the mucosa by certain pathogenic bacteria, including *Salmonella, Yersinia*, and *Listeria* in some species, and for some viruses. Neutrophils are seen transmigrating the epithelium of the dome, and are found in the lumen over the dome, in enteric bacterial infections of calves in particular.

The **aggregated lymphoid follicles**, or **Peyer's patches**, are scattered in the mucosa of the small intestine, and lymphoglandular complexes or solitary proprial lymphoid nodules may be grossly visible, studding the colonic mucosa. Peyer's patches are present throughout the length of the small intestine in all species, though they tend to be larger distally. They are grossly visible, usually as oval or elongate structures up to several centimeters wide, thickening the antimesenteric wall of the intestine. They may project slightly above the mucosal surface, or appear as cupped depressions, which must not be mistaken for ulcers, especially in dogs. In neonates of some species, including swine, they may be poorly developed and not visible grossly. Elongate **continuous Peyer's patches** are found in the distal ileum, involving the terminal 15–20% of the small intestine, in calves, lambs, and piglets. This structure is different morphologically and functionally from other gut-associated lymphoid tissue, and may be a primary site of B-cell generation. Continuous Peyer's patches involute as the animal matures.

Peyer's patches are comprised of *follicular aggregates of B lymphocytes in the submucosa*, underlying a discontinuous muscularis mucosae. Between the upper borders of adjacent lymphoid follicles are *aggregates of T lymphocytes*. Overlying the lymphoid follicles is a mixed population of T and B lymphocytes extending into the lamina propria in rounded mucosal projections, the *domes*, which lie between villi. Short crypts provide epithelium to domes and adjacent villi. Cell populations of Peyer's patches in newborns and gnotobiotes of most species tend to be sparser than those in older or bacterially colonized animals, though those in neonatal calves appear relatively well developed.

B and T immunoblasts gain access to Peyer's patches via permeable *postcapillary venules*, mediated by specific receptors in the postcapillary venules. The major cell populations in Peyer's patches appear to be B lymphocytes committed mainly to IgA production, while among the T cells is a large proportion of T helper cell precursors. Antigen is processed and presented to lymphocytes in Peyer's patches largely by **dendritic cells**. The cytokine context within which dendritic cells function may determine whether, in response to presentation of a soluble antigen, they induce a persistent clone of activated lymphocytes, or it is deleted, causing tolerance. Alternatively, under some conditions dendritic cells may induce clones of regulatory suppressor T cells, mediating tolerance. Mesenteric lymph nodes play a crucial role in the induction of mucosal immunity and tolerance. Antigen presentation by dendritic cells trafficking to the nodes from the gut, or trapping antigen, perhaps associated with apoptotic enterocytes, draining via lymphatics, is more important than in Peyer's patches. Dendritic cells in the liver may also play a role in inducing tolerance. **Macrophages** are less common in Peyer's patches than in lamina propria, and their role in the interaction among M cells and T and B lymphocytes is unclear. They probably play an effector role in cell-mediated reactions to bacteria entering via Peyer's patches, and certainly in response to agents such as *Mycobacterium avium paratuberculosis* and *Histoplasma capsulatum*, found in the lamina propria.

In the **lamina propria**, **dendritic cells** scattered beneath the epithelium throughout the gut can sample the intestinal luminal content directly by extending dendrites between enterocytes, without compromising the integrity of the mucosal barrier. They may also sample the environment by taking up antigen presented by enterocytes, or by phagocytosing apoptotic enterocytes. **Macrophages** and **dendritic cells** may present antigen to sensitize proprial lymphocytes, and promote cytokine production. Macrophages also sequester iron, inhibiting bacterial metabolism. Macrophages in colonic lamina propria are highly phagocytic, but have a low ability to activate T cells and promote T-cell-mediated immune responses. Proprial T cells are mixed CD4+ and CD8+, most numerous between upper crypts and in the propria of villi, and are likely antigen-activated cells. Cytokine production by these cells is probably determined by the cytokine milieu, but evolution of interferon-γ, tumor necrosis factor-α, and interleukins-1, -4, -5, -8 and -13, activating macrophages and promoting inflammation, are likely.

IgA lymphoblasts leave the Peyer's patch for the mesenteric lymph node and, via the thoracic duct, the general circulation, whence they home on the intestinal mucosa and other mucosal surfaces, including the respiratory tract, mammary gland, and salivary glands. In the lamina propria of the intestine they differentiate into IgA-secretory plasma cells, found mainly in close apposition to columnar epithelium of the upper crypt. Dimeric IgA binds via the J chain to glycoprotein "secretory component" present on the basolateral border of columnar crypt epithelial cells. With secretory component, it is transported in vesicles through the cytoplasm to be released from the apical border of the cell into the lumen of the crypt. It then spreads over the intestinal surface, partly bound to mucus.

IgA-secreting cells are the predominant class of plasma cell in the lamina propria in most species. However, **IgM-secreting plasma cells** are prevalent in young calves, swine, and dogs. IgM is also taken up by secretory component in some species and transported to the intestinal lumen. This may be significant in the young piglet and calf. Although IgA and IgM are secreted, IgG1 is the major antibody class in intestinal secretion in cattle; it appears to be selectively secreted by the gut and in the bile in that species.

The function of IgA in the gut lumen probably lies mainly in blocking attachment by bacteria and viruses to epithelial cells, neutralizing intraluminal toxins, and in limiting absorption of antigens originating in food and produced by microorganisms in the gut. It thereby reduces the likelihood of reaginic and other forms of immune response in the propria. Secretion into the bile, by hepatocytes, of IgA complexed with antigen may, in the species in which it occurs, be a significant means of clearing the circulation of antigen absorbed from the gut. IgA deficiency is reported in Beagle, German Shepherd, and Shar-Pei dogs, where it is associated with increased susceptibility to parvoviral infection, and chronic small intestinal and respiratory disease.

Plasma cells containing IgG are relatively uncommon in the intestinal lamina propria in species other than ruminants. However, locally produced and systemically circulating IgG may assume significance when vascular permeability and inflammation occur, due to its ability to fix complement, facilitate antibody-dependent cell-mediated cytotoxicity, and to opsonize.

Plasmacytes producing IgE are present in the lamina propria, in small numbers in most species. This class of immunoglobulin has been particularly implicated in immune responses to some intestinal parasites. Its significance may be in IgE-dependent cytotoxicity by eosinophils and perhaps by mast cells, as well as in mediating immediate (type I) hypersensitivity reactions in the mucosa.

Intestinal mucosal mast cells differ histochemically and physiologically from mast cells in most other tissues. They are not as demonstrable using standard stains after formalin fixation as are connective tissue mast cells from other tissues; they stain well in tissue fixed in basic lead acetate or Carnoy's fluid. They contain only tryptase, but no chymase, in contrast to connective tissue mast cells, and their granules are few, and variably, rather than uniformly, electron-dense under the electron microscope. Proliferation of intestinal mast cells is T-cell-dependent, and is prominent in some parasitisms. Histamine, serotonin, and other mediators released by mast cells have many and complex effects on vascular tone and permeability; on motility, chemotaxis, and effector function of leukocytes; on immune-active cells; and possibly on mucus release. Mast cells interact with the enteric nervous system, sensing antigen with their IgE immune probe, and communicating with the sensory arm of the enteric nervous system by degranulation, releasing stimulatory soluble mediators. They undoubtedly play a central role in regulation of physiologic, immune, and inflammatory processes in the gut. The enteric nervous system also modulates cytokine release by T cells, through interactions involving a variety of neuropeptides for which T cells have receptors. Peptide release by neuroendocrine cells may also impact on T-cell activity, as well as on intestinal function.

Intestinal **eosinophils** probably do not differ functionally from eosinophils in other sites, being cytotoxic effector cells and modulators of local inflammation.

Immunoinflammatory events in the large bowel are less well understood than those in the small intestine. Presumably, similar principles prevail. **Lymphoglandular complexes**, consisting of submucosal follicular lymphoid aggregates penetrated by glands extending from the mucosa, occur in the cecum and proximal colon of the dog; in the porcine colon; at the cecocolic junction, beginning of the spiral colon, and in the terminal rectum, in ruminants. Epithelium lining the glands is in close contact with lymphocytes. **Solitary mucosal lymphoid nodules**, normally without penetrating glands, and generally restricted to the lamina propria and superficial submucosa, are also scattered throughout the cecum and colon in all species.

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Gastrointestinal microbiota

After birth, no part of the gastrointestinal tract is sterile. Hundreds of species of commensal bacteria, many of them unidentified, inhabit the stomach and intestine, forming an ecosystem of enormous complexity, in which yeasts, fungi, and protists participate in many circumstances. This ecosystem ferments exogenous and endogenous substrates, often those not within the competence of host epithelium to absorb, producing energy in the form of short-chain fatty acids, synthesizing vitamins, and converting amino compounds to ammonia. Four niches are recognized: *(1) the lumen; (2) unstirred surface mucus; (3) mucus deep in crypts; and (4) the surface of enterocytes.*

Generally, bacterial populations are least in the stomach and upper small intestine of ruminants and carnivores, being limited by the acid gastric environment and by peristalsis. The anaerobes and facultative anaerobes increase to about 10^7 per gram of content in the lower small intestine, and total bacterial populations in excess of 10^{10} or 10^{11} per gram of content are present in the cecum and colon. Prominent among colonic bacteria are coliforms, *Lactobacillus,* and strict anaerobes, including *Bacteroides*, *Fusobacterium*, *Clostridium*, *Eubacterium*, *Bifidobacterium*, and *Peptostreptococcus*. Spirochetes are found in swine and dogs. Anaerobic bacteria outnumber facultative anaerobes by a thousand-fold in the large bowel.

The complex ecology of the gut flora imparts upon it considerable stability, and if disturbed it tends to return toward the original state. It is relatively resistant to the intrusion of new inhabitants and this is one of the major factors protecting against the establishment of pathogenic bacteria. It is no coincidence that bacterial diarrhea occurs most commonly in the neonate with a poorly established flora, or after changes in husbandry or antibiotic therapy which may disturb the enteric bacterial population.

The normal flora acts as a barrier to colonization by pathogens through several means. The secretion of proteins such as colicins has little significance in modulating enteric bacterial populations; more important is the production of acetic and butyric acids by the anaerobes. Under the pH and redox conditions in the large bowel, short-chain fatty acids are highly detrimental to members of the Enterobacteriaceae. The high population of lactobacilli in the gut of milk-fed animals probably reduces the establishment of Enterobacteriaceae by this means. Facultative anaerobes are important in maintaining the redox environment for strict anaerobes, by scavenging oxygen. Competition for energy, and the effect of metabolites other than short-chain fatty acids produced by the native flora, militate against establishment by exogenous bacteria. *Host factors* influencing gut flora include composition of the diet; peristalsis, which continually flushes the small intestine of a large proportion of its bacterial population; lysozyme; lactoferrin; gastric acidity if unbuffered or undiluted; and, in the abomasum of suckling calves, perhaps a lactoperoxidase–thiocyanide–hydrogen peroxide system.

The use of probiotics, deliberate oral administration of specific microbial cultures, has been shown to improve body weight gain and decrease diarrhea in newborn calves and pigs, as well as to protect adult animals from colonization with certain pathogens, including *Escherichia coli* O157:H7 and some *Salmonella* spp.

The enteric microbial flora promotes the development of a population of immune and inflammatory cells in the lamina propria, by antigenic stimulation. Mucosal epithelial kinetics are also sped up in conventional animals, in comparison with those which are germfree. IgA secretion into the lumen may influence populations close to the mucosa, and immune activity as a whole must limit establishment on and ingress by microorganisms and their products into the mucosa, as well as establishing tolerance for normal members of the ecosystem. Lactogenic immunity similarly has an inhibitory effect on enteric organisms to which specific antibody is present in ingested milk.

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Electrolyte and water transport in the intestine

Water movement in the bowel is passive, following osmotically the transport of electrolyte and nutrient solutes. The small intestinal mucosa is highly permeable to the passive movement of small ions and water and is therefore considered "leaky," despite the presence of "tight" junctions at the apical margins of absorptive enterocytes. This ensures that the content of the small bowel is approximately isosmolal with the interstitial fluid space. The permeability of junctional complexes appears to be sensitive to Starling forces, influenced by intravascular hydrostatic and oncotic pressure, so that fluid and solute actively absorbed may leak back into the lumen, thus modulating net absorption by the mucosa.

Sodium absorption takes place by a number of active transcellular mechanisms, which vary in importance at different levels of the gut, and with the physiologic circumstance. Fundamentally, Na^+ absorption depends on electrochemical forces established by the adenosine triphosphate-dependent Na^+ pump on the basolateral cell membrane of the absorptive enterocyte. This pump moves Na^+ up a concentration gradient from the cell into the lateral intercellular space.

The concentration of solute, especially Na^+, in the lateral intercellular space, causes water to follow from the intestinal lumen. Since cell membranes and junctional complexes are highly permeable to water, movement is rapid via both transcellular and paracellular routes, and differences in osmotic pressure between lumen and lateral intercellular space are small. Absorbed solute and water in isotonic proportions move into the interstitium of the villus, where, within a few micrometers, they encounter a subepithelial capillary or lacteal. The tight junctions also appear to become permeable during Na^+-nutrient cotransport across the apical cell membrane, resulting in solvent drag of large nutrient molecules into the lateral intercellular space.

The colon of carnivores, the spiral colon of ruminants and swine, and the small colon of the horse are charged with the task of reducing the volume of electrolyte and water lost to the animal in the feces. In contrast to the small intestine, *the colonic epithelium is relatively restrictive to the free movement of sodium and chloride*, though not to potassium. Therefore it is capable of maintaining differences in osmotic pressure, ionic composition, and electrical potential between luminal and proprial surfaces, which make it more efficient than the small bowel in absorbing some electrolytes and water. Ultimately, fecal water may be hypotonic with respect to plasma. Absorption of volatile fatty acids also accounts for considerable water absorption from the colon. Potassium increases in concentration in colonic content as Na^+ concentration declines; this is due to an active secretory process, and a passive response to transepithelial electrochemical gradients.

The mucosa of the small and large intestine, under some conditions, also *secretes chloride, potassium, bicarbonate, and water*. This process, which can be a function of both surface and crypt cells, is important in some pathologic states; but it is also physiologic, and segmental, maintaining the fluidity and buffering capacity of the intestinal content.

Solute movement across the intestinal epithelium is regulated by a number of *hormones and neurotransmitters*, which act through intracellular second messengers. Activation of adenylate cyclase and guanylate cyclase, and increased intracellular Ca^{2+}, all depress nutrient-independent absorption of Na^+ by enterocytes, and open Cl^- channels in crypt cells, promoting secretion. Some products of intrinsic and extrinsic neurons, such as vasoactive intestinal polypeptide and acetylcholine, may be secretory, while others, such as somatostatin and norepinephrine, are absorptive or antisecretory. Local paracrine effects are mediated by the products of enteroendocrine cells, such as somatostatin, and neurotensin (secretory). Serotonin is secretory, possibly directly, and also through stimulation of local prostaglandin production. In addition, guanylin, a peptide secreted from epithelial cells, acts to increase local fluid secretion. Mesenchymal elements in the lamina propria, including myofibroblasts, lymphocytes, mast cells, macrophages, and other inflammatory and connective tissue cells, also produce locally active substances with a direct or indirect effect on epithelial function. Circulating hormones also influence mucosal function. Aldosterone and glucocorticoids enhance Na^+ absorption by the colon, and the latter may inhibit local production of eicosanoids.

Hence, immunoinflammatory events are integrated with the systemic and local neural and hormonal regulation of intestinal absorption and secretion. Dysfunctions of absorption and secretion will be considered in the section on the pathogenesis of diarrhea, below.

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Epithelial renewal in health and disease

Small intestine

The intestinal surface is lined by a population of cells ultimately derived from *stem cells* that are present at or near the base of crypts or glands, but with its proximate source in amplifier populations of undifferentiated columnar or oligomucous cells in the lower half of the crypts. These cells lose their ability to undergo mitosis, and differentiate into *goblet cells* and the functional population of *enterocytes* as they move from the crypt to the villus. In most species, they are *shed from the tips of villi* in about 2–8 days; apoptosis is a minor contributor to physiological enterocyte loss. Generally, cells move off the villus more quickly in the ileum than in the duodenum, perhaps related to the decline in height of villi with distance down the gut in most species.

Normally, the mass and topography of the mucosa are quite stable. This steady state is the product of a dynamic equilibrium between the rate of movement of cells out of crypts and on to villi, and the rate at which they are lost from the tips of villi. Equilibrium is based on interaction of the microflora with the epithelium, and presence of cell cycle regulators, especially transforming growth factor-β and other mediators, including glucagon-like peptide 2, and urokinase, which may facilitate cell shedding.

In the young animal the intestine grows by generation of new crypts, and with them, new villi. As the bowel attains mature size, the number of villi stabilizes, and apparently remains relatively constant. The number of crypts also stabilizes, but some adaptive variation in the ratio of crypts to villi may occur. Adaptive responses to a variety of factors alter the size and rate of turnover of the proliferative and functional epithelial cell populations and with them the microtopography of the gut. The appearance of the small intestinal mucosa is a compromise, achieved by the equilibrium between the rate of cell production and rate of loss. At one extreme lies the intestine of the germfree animal, with a short crypt containing a small proliferative compartment, and tall villi supporting a large functional compartment with a low rate of cell loss. At the other end of the spectrum is the animal suffering from severe intestinal helminthosis, with long crypts reflecting an increased proliferative compartment, yet a flat mucosal surface with relatively few functional enterocytes and an apparently high rate of cell loss.

Although quantitative description of epithelial kinetics is possible experimentally, in the diagnostic situation it is necessary to make a subjective or semiquantitative assessment of the status of the proliferative and functional compartments in tissue sections. The size of the proliferative compartment is reflected in the length and diameter of the crypts, and in the location of the uppermost mitotic cell. The prevalence of mitotic figures can be assessed subjectively, but beyond calculating a mitotic index, no inferences can be drawn about the proportion of the crypt cell population that is replicating, or the duration of the cell cycle.

The *degree of differentiation*, and hence functional status, of enterocytes on villi can be inferred from their appearance. Cytoplasmic basophilia; loss of regular basal nuclear polarity; low columnar, cuboidal, or squamous shape; and an ill-defined brush border all point to a poorly differentiated population of surface enterocytes, which is possibly turning over more rapidly than normal.

Fasting reduces the mucosal epithelial mass; the atrophy is related to prolongation of the postmitotic phase of the cell cycle in the proliferative compartment. Villi do not regress severely, however, since cells on the surface persist for twice as long, moving off the villus more slowly. The effect is immediately reversed by refeeding. Weaning also has similar profound effects on the structure of the intestine, resulting in marked reduction in height of villi in most species.

Restitution of epithelial integrity following minor loss of mucosal surface cells is by *lateral migration of adjacent intact epithelium*, and occurs within minutes. If epithelium on villi is obliterated, for instance by transient ischemia or by viral cytolysis, the villus core contracts, probably due to myofibroblast activity mediated by the enteric nervous system, and stromal elements rapidly undergo apoptosis. Surviving epithelial cells become attenuated, and extend across the denuded intercrypt surface. Restitution is positively influenced by local growth factors and especially by trefoil factors present in mucus.

Regeneration of the mucosa follows, as the three-dimensional architecture is restored within a few days, during which the proliferative compartment undergoes hyperplasia, generating new epithelium, and the stroma is rebuilt. Extensive mucosal ulcers will form a bed of granulation tissue, and with time (weeks to months) may become covered by a neomucosa, with crypts and villi, which evolves following the centripetal immigration of flattened epithelium from the periphery of the lesion. A similar process repairs the mucosal gap in healing intestinal anastomoses.

Villus atrophy

Atrophy of villi is a common pathologic change in the intestine of domestic animals. It results in malabsorption of nutrients, and is sometimes associated with increased plasma protein loss into the gut. Mucosa with short villi can be categorized morphologically into two broad types, recognition of which has implications with respect to pathogenesis and prognosis. The first category includes intestine with apparently normal or hypertrophic crypts, associated with atrophy of villi to various degrees. The second category is comprised of gut with some evidence of damage to the proliferative compartment, and variable villus atrophy. The recognition, evolution, and interpretation of each will be considered in turn.

Villus atrophy with an intact or hypertrophic proliferative compartment takes two forms in domestic animals. A **primary increase in rate of loss of epithelium** from the surface of villi is one mechanism initiating such a lesion. This is the major pathogenetic action of a number of important viral diseases, including coronaviral and rotaviral infection; of coccidial infections which damage surface enterocytes predominantly; of some enteroinvasive bacteria; of transient ischemia, in which the effect is limited to the functional compartment; and, in some circumstances, of necrotizing toxins released by clostridia in the lumen of the bowel. The effect of these agents is to cause significantly increased loss of surface epithelium over a relatively short period of time, while sparing the proliferative compartment in crypts. Villi contract as the size of the functional compartment is diminished, and they may become very stubby. If the animal survives the metabolic sequelae to the malabsorption that results from the damage to surface cells, *compensatory expansion of the proliferative compartment in crypts* permits complete recovery. Epithelium emerging from crypts regenerates villi, resulting in a normal mucosal topography within a few days, and full function returns.

The microscopic appearance of the mucosa depends partly on the number of functional cells lost, which determines the initial degree of villus atrophy, and partly on the amount of regeneration that has occurred by the time the gut is examined. During the early phase of cell loss, damaged epithelium may be seen exfoliating into the lumen of the gut, and villi are shorter or blunter than normal, or sometimes more or less of normal length, but somewhat pointed. Subsequently, the atrophic villi are covered by poorly differentiated, low columnar, cuboidal or squamous cells. There may be fusion of the lateral surfaces or tips of villi in some areas. In severe atrophy there may be mild erosion if inadequate epithelium is available to cover even the much-reduced mucosal surface area. In the acute phase, crypts appear of normal size, but within 12–24 hours, proliferative activity is noticeably increased. Crypts enlarge in diameter and length to accommodate more mitotic cells, which are basophilic, crowded, and obviously dividing, sometimes very close to the surface of the mucosa. The lamina propria appears moderately hypercellular, perhaps due to condensation, possibly due to a mild mononuclear cell infiltrate. As regeneration occurs, progressively longer villi with increasingly well-differentiated epithelium are evident, and hypertrophy of the proliferative compartment gradually subsides.

Atrophy of villi and hypertrophy of crypts is also associated with **chronic or persistent processes** such as nematode parasitism; chronic coccidial infection of surface epithelium; giardiasis in some species; response to dietary components in some species, including soybean protein in calves, kidney bean protein in pigs, and wheat in dogs; idiopathic or specific granulomatous enteritis, such as Johne's disease and histoplasmosis; and chronic inflammatory bowel disease characterized by heavy lymphocytic/plasmacytic infiltrates in the mucosa. Epithelial kinetics have not been investigated in most of these situations in domestic animals. However, they all have in common chronic antigenic exposure, parasitism, or a persistent infectious process in the lumen, epithelium, or lamina propria, usually associated with a significant lymphocytic and plasmacytic infiltrate in the mucosa. Where the etiology can be eliminated, the lesion usually remits, implying that it requires active interaction between host and parasite for its induction and maintenance. The situation is entirely analogous to that of celiac disease in humans, which clearly is an immune-mediated response to specific dietary antigens, through activation of mucosal T cells secreting γ-interferon, among other cytokines. Strong evidence implicates Th2 lymphocyte-driven nitric oxide production in the pathogenesis of villus atrophy in experimental helminth infections in mice. The source and effect of the nitric oxide are uncertain, as is its possible interaction with cytokines such as interleukin-12, interferon-γ, and tumor necrosis factor-β, also implicated in villus atrophy of this type in experimental models. T-cell activity seems to be associated with villus atrophy of this type in parasitisms such as giardiasis as well.

Immune reactions in the gut are associated with increased epithelial cell proliferation. Hypertrophy of the proliferative compartment in these conditions *precedes* the development of villus atrophy, and *is not a response to it.* Evidence points to local stimulation of the proliferative compartment, and perhaps the associated myofibroblast sheath, by cytokines or nitric oxide released by activated T lymphocytes in the mucosa. Epithelial cells leaving the crypts usually do not differentiate fully. They exfoliate prematurely, low on the villus or near the crypt opening, and as the pre-existing enterocytes are shed, villi undergo atrophy over a period of several days. Epithelium that reaches the surface subsequently fails to differentiate fully, though it may become mucous metaplastic, and is rapidly lost into the lumen, matching the rate of cell production.

Hypertrophy of crypts is the early and outstanding change in this lesion, and is consistently present. In its milder forms, the lesion may be better characterized by elongate crypts than by obvious atrophy of villi. The proliferative compartment is expanded and active, and mitotic figures are numerous; goblet-cell hyperplasia may also occur. Elongation of crypts may be so great that even with severe atrophy of villi the total mucosal thickness will not be much reduced from normal. Close observation may reveal poorly differentiated enterocytes exfoliating prematurely from intercrypt ridges on the mucosal surface, or from buttress-like folds around the base of stubby villi. The lamina propria has a prominent population of lymphocytes, plasmacytes, and associated inflammatory cells, and intraepithelial lymphocytes are common. The etiologic agent, in the form of parasites, intracellular bacteria, or yeasts, may be evident. Removal of the causal stimulus, where that is possible, usually results in a return to "normal" within days or weeks.

However it is induced, atrophy of villi with hypertrophy of crypts is associated with local malabsorption of nutrients and water; elongate crypts and perhaps poorly differentiated surface epithelium may secrete electrolytes and water; and if there is proprial inflammation and microerosion of the mucosa, effusion of tissue fluid may ensue. Increased turnover of epithelium may contribute to enteric loss of endogenous protein.

Villus atrophy associated with damage to the proliferative compartment is also commonly seen in domestic animals. It is the sequel to insults that cause necrosis of cells in crypts, or impair their mitotic capacity. The agents that produce these lesions usually have a propensity for damaging dividing cells in any tissue. Since ionizing radiation was recognized early as a cause of such lesions, they are often termed "*radiomimetic.*" Other causative agents include cytotoxic chemicals and mitotic poisons, such as cancer chemotherapeutic agents, T-2 mycotoxin, and pyrrolizidine alkaloids in large doses; viruses that infect proliferating cells, particularly the parvoviruses, and *Bovine viral diarrhea virus* and *Rinderpest virus*. Ischemia of sufficient duration to cause necrosis of some or all cells lining the crypts also causes a lesion of this type.

The microscopic appearance of affected mucosa depends on the severity and extent of the insult, and the interval since it occurred. The primary event is damage to the proliferative compartment, and, except in ischemia, lesions will be evident in crypts well before significant atrophy of villi occurs. Apoptotic epithelial cells and neutrophils may be present in the lumen of damaged crypts, which tend to dilate. If crypt cell necrosis is severe, remaining cells become extremely flattened in the course of attempting to maintain the integrity of the crypt lining. Following radiation, cytotoxic damage, and parvoviral infection, bizarre irregular epithelial cells with large nuclei and nucleoli may be present in crypts and will migrate on to the surface.

Pre-existing surface epithelium continues to move off the tips of villi at an apparently normal rate, even though few or no new cells emerge from crypts. Villi eventually become atrophic or collapse as the surface cell population shrinks. If most proliferative and stem cells have been damaged, crypts stripped of epithelium will also collapse, or "drop out," perhaps leaving a few scattered cystic remnants, lined by attenuated epithelium, in the deeper lamina propria. The overlying surface will be covered by squamous epithelial cells derived from surviving crypts, or will be eroded; the mucosa may eventually ulcerate, to be lined by granulation tissue. Crypts that have not been so severely damaged will undergo hypertrophy within a week or so of the original insult, as compensatory hyperplasia of lining cells occurs.

In viral diseases, the severity and appearance of the lesion often vary considerably at different sites in the gut, and even within an individual tissue section. Lesions due to ischemia tend to be uniform in severity but may be localized; acute or subacute lesions are often hemorrhagic, if there has been reflow.

Extensive lesions due to crypt cell necrosis lead to erosion or ulceration, with severe malabsorption, and effusion of tissue fluid and hemorrhage. The mucosa is often invaded by the enteric flora, sometimes including fungi. Local ulceration may lead to persistent plasma loss, and, if circumferential, to stricture and stenosis. Small ulcers in areas where a few crypts have dropped out will heal as epithelium from adjacent crypts moves over the surface, but crypts may not regenerate for some time, and local villus atrophy will persist.

Large intestine

Epithelial turnover in the cecum and colon is fundamentally similar to that in the small intestine, though villi are not present on the surface. Cells lose the ability to divide after leaving the proliferative compartment in the lower part of the gland. In the upper portion of the gland they differentiate into goblet cells or columnar absorptive cells that emerge and move out over the surface. They are lost into the lumen, probably within about 4–8 days of being produced, though no studies of colonic epithelial turnover have been made in domestic animals. Fasting reduces, and refeeding restores, proliferative activity in colonic glands in experimental animals, and physical distension and certain types of dietary fiber in the colon also appear to be trophic for the mucosa. The colon of gnotobiotic animals has a small number of proliferative cells, limited to the lower portion of the glands. With conventionalization of the gut flora, the proliferative compartment expands.

Restitution of the epithelial integrity of the surface of the large bowel, following insult to, and exfoliation of, surface cells, resembles that in the small bowel. Within a few minutes of injury, surviving surface epithelium, and cells emerging from crypts, become attenuated and migrate at a rate of several micrometers per minute to extend over mucosal defects. Ulcers, surgical incisions, or anastomotic sites in the colon heal by immigration of a single layer of epithelium from the periphery of the defect, with gradual differentiation of crypts, and eventual restitution of normal mucosal architecture. In the horse, depending on the type of anastomosis, epithelium may take over 2 weeks to bridge the granulation tissue in the gap in the mucosa, and restitution of mucosal architecture may require about 2 months.

Lesions in the large bowel presumed to be associated with *increased epithelial turnover* include alterations in both surface and glandular epithelium. The number of goblet cells on the surface and in the upper portion of glands is often diminished, and epithelium in these areas appears poorly differentiated, is more basophilic than normal, and may be low columnar, cuboidal, or squamous. In severe diseases, microerosion of the surface is present. The proliferative compartment in the gland may hypertrophy, causing glands to elongate and dilate. Crowded mitotic cells are present over a greater proportion of the length of the gland, sometimes virtually to the surface of the mucosa, though in some situations goblet-cell hyperplasia may occur. Such changes may be associated with acute, chronic, or chronic-active inflammation of the lamina propria. It is uncertain whether such changes are caused by primary damage to surface epithelium, or by cell-mediated immune effects on the proliferative compartment, analogous to those that occur in the small intestine in celiac disease of humans.

The *proliferative compartment* in the cecal and colonic glands is damaged by the same insults that attack cells in the crypts of the small bowel. However, cytotoxins and parvoviruses tend not to produce severe lesions so commonly in large bowel as they do in small intestine, perhaps because a lower proportion of the proliferative compartment is in mitosis at the time of maximum availability of drug or virus. Additions to the list of agents damaging the proliferative compartment in large intestine include coronavirus in calves, cattle, and dogs, and several species of coccidia in ruminants, which develop in the cells lining glands in the large bowel.

The *evolution and sequelae of lesions* resulting from damage to proliferative epithelium in the large intestine are similar to those in small bowel. Dilation of crypts containing necrotic debris, and lined by attenuated epithelium, indicates such damage. Severe lesions will lead to loss of glands, erosion, and ulceration of the mucosa, perhaps with hemorrhage. Stricture and stenosis may ensue. Following milder damage that spares some stem cells in each gland, the mucosa has the potential to recover fully after a period of reparative hyperplasia.

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Pathophysiology of enteric disease

Protein-energy malnutrition, due to intake of too little feed, or of feed deficient in quantity or quality of nutrients, results in *depletion of fat and muscle mass*, emaciation, and ultimately, death by starvation. In addition, immune function is impaired, predisposing to infection.

Inappetence or, in extreme cases, **anorexia**, are commonly associated with gastrointestinal diseases. Appetite is modulated by a complex and incompletely understood meshwork of neurologic, neuroendocrine, and systemic cytokine inputs impacting centrally on satiety. The potential for physical impairment of gastrointestinal function to affect appetite seems relatively obvious. Less apparent, but highly significant, are the effects on appetite of chronic inflammatory diseases affecting the alimentary system, or distant sites. Possible direct or indirect mediators of this effect are tumor necrosis factor-α and interleukin-1 released from macrophages and other cells. In addition to simply reducing food intake, they may play a role in the complex metabolic puzzle that is the **cachexia**, characterized by active erosion of adipose tissue and muscle mass, associated with chronic inflammatory disease and neoplasia.

Protein-energy malnutrition must be differentiated from the effects of endogenous conditions resulting in malassimilation or protein-losing enteropathy, from cachexia induced by chronic inflammatory and neoplastic disease, and from other problems that may result in recumbency.

In neonates, several factors predispose to death by starvation within the first few weeks of life. These include increased energy demands due to cold and exposure; fetal malnutrition with poor fat depots at birth; and postpartum hyponutrition, which in calves is often associated with poor-quality or insufficient milk replacer. Piglets are born with negligible fat reserves and die quickly of hypoglycemia if not fed adequately. Neonatal ruminants usually have fat depots sufficient to compensate for longer periods of inanition (2–4 days for lambs; 6–10 days for calves), if cold stress is not severe.

In animals that have died of inanition, muscle mass is reduced, due to mobilization of amino acids for gluconeogenesis. Fat in the bone marrow, coronary groove, on the pericardial sac, and around the kidneys is completely depleted, and has the gelatinous clear pink appearance of **serous atrophy**. The liver may appear small, with sharp margins, presumably due to reduced trophic stimuli. A diagnosis of starvation is supported by a history of conditions compatible with reduced quantity or quality of feed, and by steps to rule out problems causing malassimilation and protein-losing gastroenteropathy.

Malassimilation

Digestion and assimilation of nutrients have an *intraluminal phase*, mediated by the biliary and pancreatic secretions, and an *epithelial phase*, carried out by enzyme systems on the surface and in the cytoplasm of absorptive enterocytes. The final step is delivery of the nutrient by the enterocyte to the interstitial fluid, and its uptake into the blood or lymph.

Exocrine pancreatic insufficiency *is the major cause of intraluminal maldigestion*, and it is usually due to juvenile pancreatic atrophy in dogs, or to pancreatic fibrosis and atrophy following repeated episodes of pancreatic necrosis (see Vol. 2, Pancreas). It is occasionally seen in cats. This condition may be complicated by bacterial overgrowth of the small intestine. Bile salt deficiency is rarely seen as a cause of intraluminal maldigestion in domestic animals.

The **epithelial phase of digestion** *is impaired by loss of functional epithelial surface area*. This occurs in short-bowel syndrome following intestinal resection, in which greater than 75–85% of the small bowel has been lost, and, more commonly, in *villus atrophy*. Neonates and ruminants have low levels of maltase; ruminants lack sucrase. In most species, lactase levels decline with age, and malabsorption in dogs fed milk has been attributed to low levels of lactase. The poorly differentiated surface epithelium present on atrophic villi may lack the full complement of enzymes on the brush border and in the cytoplasm necessary for nutrient digestion and assimilation. Lectins present in uncooked beans attach to and damage microvilli on enterocytes, which may explain the malabsorption and diarrhea associated with their use in feeds; lectins also promote bacterial adhesion and overgrowth in the small intestine. A heritable syndrome of *gluten-sensitive enteropathy* has been reported in Irish Setter dogs. This entity is characterized by villus atrophy, increased intraepithelial lymphocytes, and abnormal levels of mucosal microvillar hydrolases. *Cobalamin malabsorption* has been described as a hereditary defect in Beagles, Giant Schnauzers and Border Collies, resulting in anemia and failure to thrive. Delivery of nutrients, especially lipid, to the circulation, may be impaired in lymphangiectasia. The pathogenesis of malabsorption of the major classes of nutrients will be considered briefly.

Assimilation of fat is susceptible to interference at all three phases of digestion and absorption. Lipolysis is impaired if insufficient lipase is available. Most commonly this is a result of pancreatic atrophy or fibrosis. It may also be due to failure by atrophic intestinal mucosa to release the cholecystokinin/pancreozymin necessary for pancreatic secretion. The availability of bile salts for micelle formation is reduced in intrahepatic cholestasis, biliary obstruction, and by depletion of the bile salt pool due to reduced ileal absorption following resection or atrophy. As a result, fatty acid and monoglyceride are not incorporated on to micelles and emulsified; they are therefore not as accessible to absorptive enterocytes. Reduced surface area for lipid uptake will contribute to malabsorption of fat. Poorly differentiated enterocytes on atrophic gut may be less able than normal epithelium to re-esterify long-chain fatty acids to triglyceride and to produce chylomicrons for export from the cell. In lymphangiectasia, granulomatous enteritis, and intestinal lymphosarcoma, lymphatic drainage may be obstructed, and with it the flow of chylomicrons to the systemic circulation.

Malabsorption of lipids may cause *steatorrhea* (excess fat in the feces). It is seen in monogastric animals, especially dogs. Severe fat malabsorption may result in deficiencies of fat-soluble vitamins. Malabsorption of calcium, magnesium, and zinc occurs due to their sequestration in soaps formed by combination with malabsorbed luminal fatty acids. Increased absorption of oxalate, and consequent nephrolithiasis, may be a sequel to reduced concentrations of calcium in the lumen due to soap formation. Malabsorbed lipid may cause *colonic diarrhea*, by mechanisms that will be discussed subsequently.

Maldigestion of polysaccharides occurs if levels of pancreatic amylase are reduced; this is most commonly encountered in dogs with severe loss of functional exocrine pancreatic tissue. Calves and older ruminants normally lack significant amounts of pancreatic amylase and digest starch poorly in the small intestine. Mucosal oligosaccharidase deficiency occurs in villus atrophy, with reduced mucosal surface area. Poor differentiation of enterocytes results in irregular, short, and sparse microvilli, and a reduced complement of oligosaccharidases. The result is impaired membrane digestion of disaccharide and malabsorption of carbohydrate, much of which is subsequently fermented by colonic flora. The osmotic effect of malabsorbed disaccharide augments intraluminal fluid accumulation in the small intestine, and this may be compounded by hydrolysis in the colon. Carbohydrate malabsorption is an important component of disease in neonatal diarrhea due to rotavirus and coronavirus, and in other conditions in which there is extensive villus atrophy in the small intestine.

Protein digestion in the lumen is reduced if pancreatic protease activity is decreased to ~10% of normal, as may occur with exocrine pancreatic insufficiency. Loss of gastric proteolytic activity is of little nutritional significance. In conditions with villus atrophy, reduced mucosal surface area and poor differentiation of enterocytes result in malabsorption of small peptides and particularly of amino acids by mechanisms similar to those involved in carbohydrate malabsorption.

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Diarrhea

Diarrhea is the presence of water in feces in relative excess in proportion to fecal dry matter. Diarrhea usually reflects increased absolute fecal loss of water, but may not, if absolute fecal dry-matter excretion is markedly reduced. Loss of solute and water in diarrhea may lead to *severe electrolyte depletion, acid–base imbalance, and dehydration*, which are life-threatening if not corrected.

Large volumes of fluid derived from ingesta, and from gastric, pancreatic, biliary, and enteric secretion, enter the small bowel; in addition, considerable passive movement of water occurs into the upper small bowel from the circulation in response to osmotic effects. Overall, the bulk of the fluid entering the small intestine is absorbed by the enterocytes, so that the volume leaving the ileum and entering the colon is but a small fraction of the total fluid flux through the small bowel. The large size of this flux implies that relatively small perturbations in unidirectional movement of electrolyte and water may have significant effects on the net movement of fluid.

The colon, in addition to its fermentative function, has the ultimate responsibility for conserving electrolyte and water by absorption from the digesta, thereby minimizing fecal loss. It has a finite capacity for absorption, and if this is exceeded by the rate at which content enters from the small bowel, diarrhea occurs. This is important in "small-bowel" diarrheas, where the lesion is in the small intestine. Since the colon has reserve absorptive capacity, the excess volume entering from the ileum must be considerable for diarrhea to occur. The large capacity and fermentative function of the equine colon may mitigate to some extent the expression of "small-bowel" diarrhea in mature animals of that species.

"Large-bowel" diarrhea, on the other hand, reflects an intrinsically reduced capacity of the colon to handle even normal volumes of fluid and electrolyte presented to it by the small intestine.

Small-bowel diarrhea is classed as "secretory," "malabsorptive," or "effusive," but the mechanisms are not mutually exclusive.

Secretory diarrhea is due to an excess of secretion over absorption of fluid, resulting from derangement of normal secretory and absorptive mechanisms. It is best exemplified by the effects of *diarrheagenic bacterial enterotoxins. Vibrio cholerae* and *Escherichia coli* are the most important sources of such toxins, though only the latter occurs in domestic animals; some *Salmonella* serotypes, *Yersinia enterocolitica*, *Shigella*, and *Campylobacter jejuni* also produce enterotoxin. Cholera, heat-labile *E. coli*, and *C. jejuni* enterotoxin act through the mediation of cyclic adenosine monophosphate (cAMP). Toxin-stimulated cAMP shuts down sodium chloride cotransport at the luminal cell membrane of enterocytes, reducing passive water absorption. Meanwhile, cAMP-stimulated chloride secretion is promoted, and water follows. The resultant increase in secretion and decrease in absorption increase the solute and water load passing from the small bowel to the colon. Heat-stable *E. coli* and *Y. enterocolitica* enterotoxins stimulate cyclic guanosine monophosphate-mediated secretion by the mucosa.

In addition to bacterial enterotoxins, *other factors* may potentially cause secretory diarrhea. Prostaglandins, other eicosanoids, histamine, kinins, and various cytokines directly or indirectly stimulate secretion, often by local stimulation of enteric nervous reflexes, and they may contribute to diarrhea in inflammatory bowel disease. Vasoactive intestinal polypeptide secreted by pancreatic islet cell tumors causes severe diarrhea in humans with such tumors, and is probably the final mediator in secretion stimulated by the enteric nervous system. Thyroid C-cell tumors secrete calcitonin; carcinoids secrete serotonin, bradykinin, and substance P; while mast cell tumors secrete histamine, which is also diarrheagenic. Peptide YY has been identified as a powerful inhibitor of intestinal secretion; its presence is mediated by prostaglandin profiles.

Malabsorptive diarrhea is exemplified by the osmotic retention of water in the gut lumen by poorly absorbed magnesium sulfate, used therapeutically as a laxative. *Malabsorption commonly results from villus atrophy, no matter what the cause.* Electrolyte and nutrient solute, malabsorbed as the result of reduced villus and microvillus surface area, are retained in the lumen of the bowel, along with osmotically associated water. The additional solute and water are passed on to the colon. A secretory component probably contributes to diarrhea due to villus atrophy, at least in transmissible gastroenteritis in pigs. Here, since the villus limb of the postulated crypt–villus fluid circuit is diminished or missing, fluid secreted by the crypts may not be absorbed. Poorly differentiated cells emerging on to the intestinal surface from crypts may also retain some secretory capacity. Malabsorptive diarrhea also occurs in *short-bowel syndrome*, due to reduced absorptive surface.

Increased permeability of the mucosa may contribute to diarrhea by permitting increased retrograde movement of solute and fluid from the *lateral intercellular space* to the lumen, or by facilitating transudation of tissue fluid. **Filtration secretion** is characterized by increased fluid movement through the epithelial membrane via the *paracellular route*; the force for secretion is provided by the transepithelial hydrostatic pressure gradient. Elevated capillary hydrostatic pressure, or decreased plasma oncotic pressure in the villus, alters Starling forces, resulting in edema. This overrides the hydrostatic pressure in the interepithelial space, and permits leakage of interstitial fluid and large protein molecules into the lumen through enlarged pores at the tight junctions. Portal hypertension, right-sided heart failure, hypoalbuminemia, and expansion of plasma volume establish such conditions. Effusion may occur in lymphangiectasia, and in inflamed lamina propria, with increased vascular permeability, proprial edema, and enteric plasma protein loss. Increased exfoliation of epithelium and transient microerosions may provide further potential sites for effusion of interstitial fluid.

Severe necrosis of the epithelium and vascular damage in the mucosa cause obvious malabsorption, and effusion of tissue fluid and blood, evident as fibrin and hemorrhage, in the lumen.

Large-bowel diarrhea *is due to a reduction in the innate capability of the colon to absorb the solute and fluid presented by the more proximal bowel.* A reduction in net absorption by the colon that is relatively small in absolute terms may be sufficient to cause fluid feces. Colonic diarrhea is characterized by *frequent passage of small amounts of fluid feces*, perhaps with mucus and blood.

The colonic mucosa is not as "leaky" as the small intestinal mucosa due to the nature of the tight junctions between epithelial cells. As a result, it is relatively resistant to alterations in permeability due to increased hydrostatic pressure in the propria, in comparison with the small intestine.

Bile acids mediate diarrhea associated with ileal disease. When normal enterohepatic circulation of bile acids is interrupted by ileal damage or resection, *excess bile acids* enter the colon where they stimulate chloride ion secretion through a cAMP-dependent mechanism, resulting in diarrhea. *Fatty acids* enter the colon in increased quantities in steatorrhea resulting from bile salt depletion. Both dihydroxy bile acids and long-chain fatty acids, especially hydroxy fatty acids produced by bacterial action, alter mucosal permeability and cause mild damage to the surface epithelium. The result is net fluid secretion by the colon, and diarrhea. This is also the mode of action of a number of laxatives, including senna and castor oil, which contains the hydroxy fatty acid ricinoleic acid.

Although colonic secretion stimulated by bacterial enterotoxin is not clearly implicated in diarrhea, alterations in the flora in the large bowel may be detrimental to normal function. Absorption of volatile fatty acids produced by bacterial fermentation is responsible for considerable concurrent absorption of water in the large intestine. Reduced production or absorption of volatile fatty acids, secondary to *imbalance of the bacterial flora* in the cecum and colon, may explain some problems of wasting and diarrhea in horses in which no morphologic abnormality of the mucosa can be found.

Osmotic overload of the large bowel results from the delivery by the small bowel of a large volume of fermentable substrate. This may result from excessive intake, but is usually due to malabsorption in the small intestine. Of the malabsorbed nutrients, carbohydrate is the only one of significance in initiating *colonic osmotic overload*. Bacterial fermentation of carbohydrate results in the production of excess volatile fatty acid. This is readily handled by the colon under normal circumstances, by rapid absorption and by bicarbonate buffering. However, a heavy carbohydrate load may overwhelm the colonic buffering capacity, and cause reduced pH. This results in an *altered gut flora* dominated by organisms producing lactic acid, which is absorbed at a slower rate than the volatile fatty acids. Further acidification causes mucosal permeability, permitting an influx of water and solute into the lumen from the tissue, as a result of the increased osmotic pressure generated by lactic acid in the lumen. Diarrhea follows.

Increased intestinal motility probably does not have a primary role in the pathogenesis of diarrhea. Often the small intestine of animals with diarrhea is flaccid and fluid-filled, rather than hypermotile. Increased colonic motor activity is often segmental and antiperistaltic, and probably unrelated to increased transit. Hypermotility, if it does occur, may be in response to, rather than a cause of, increased volumes of fluid in the gut.

Small intestinal bacterial overgrowth (SIBO) may be a consequence of ileus or hypomotility, partial small-bowel obstruction, and radiation injury. Achlorhydria or hypochlorhydria and pancreatic exocrine insufficiency in the dog may also predispose to this problem. Not associated with any histologic changes other then the presence of bacterial colonies in mucus on the mucosal surface in some cases, SIBO is thought to result in increased intestinal permeability that translates clinically to diarrhea.

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Protein metabolism in enteric disease

Disorders of protein metabolism attributable to enteric disease are responsible for significant economic loss in the form of reduced weight gain, wool growth, and milk production. Severe derangement, resulting in negative nitrogen balance in any species, may lead to cachexia, hypoproteinemia, and death.

Decreased protein intake is the most obvious threat to the nitrogen economy, and it is probably the most important in many chronic gastrointestinal diseases. Subclinical inefficiency in production, reduced growth, and emaciation may be the product of various degrees of inappetence, or difficulty in prehension, mastication, and swallowing, or recurrent regurgitation. Low feed quality compounds the effect of reduced feed intake.

A sharp decline in appetite, or *anorexia*, is a common sign of indigestion, obstruction, or systemic disease. In ruminants, loss of appetite to various degrees is an especially important component of the pathogenicity of gastrointestinal parasites, including those infecting the abomasum (*Ostertagia*), small intestine (*Trichostrongylus*), and large bowel (*Oesophagostomum*). Interactions among cholecystokinin, parasympathetic efferent and afferent reflexes, and systemic or gastric leptins, appear to mediate satiety. How they are disrupted in disease states is unclear.

Malabsorption of peptides and amino acids may occur locally in the small intestine as a result of significant *villus atrophy*. However, unless the lesion is widespread, or low in the small bowel, net absorption of nitrogen over the length of the small intestine may not be reduced, due to the compensatory capacity of more distal normal mucosa. Overall, the contribution of malabsorption to disordered nitrogen metabolism appears to be *minor* in most situations.

Protein-losing gastroenteropathy, increased catabolism and loss of endogenous nitrogen via the gastrointestinal tract, is important in many diseases. Excess endogenous protein entering the intestine is derived from two main sources, namely *increased turnover of cells lining the gut*, and *effusion of plasma protein into the lumen of the bowel*; the latter is probably the more important.

Plasma protein loss into the gut presupposes abnormal permeability of the mucosa to large molecules. This may be the product of the blood-sucking activity of nematodes such as *Haemonchus, Ancylostoma,* and *Bunostomum*, or hemorrhage from sites of trauma in the mucosa caused by the feeding activity of worms such as *Oesophagostomum columbianum, Chabertia*, and *Strongylus*. Erosive lesions result in considerable loss of red blood cells and plasma protein. Such lesions may be due to infarction, severe cryptal necrosis, or acute inflammatory damage to the mucosa associated with bacteria, viruses, and coccidia, causing fibrinohemorrhagic enteritis. Microscopic "leaks" in the mucosa also permit plasma loss into the gut. These may result from increased exfoliation of enterocytes into the lumen in villus atrophy, as transient gaps in the mucosa at the site where the cell sloughs. In villus atrophy with a high rate of enterocyte turnover, temporary micro-erosions may develop when flattened cells fail to maintain the integrity of the surface epithelium. The permeability of tight junctions between epithelial cells may be sufficiently altered to permit transit of plasma protein molecules, associated with **filtration secretion** when the hydrostatic pressure in the proprial interstitium is elevated in congestive heart failure and portal hypertension; by decreased plasma oncotic pressure; by increased vascular permeability in acute or chronic inflammation; and in lymphatic obstruction or lymphangiectasia. Tumor necrosis factor-α, prevalent during chronic inflammation, may cause increased paracellular epithelial permeability to large molecules entering the mucosa, and such conditions may permit plasma protein molecules to move in the luminal direction.

Plasma protein loss into the gut is nonselective. Albumin, immunoglobulins, clotting factors, and a variety of transport or carrier proteins, including transferrin, ceruloplasmin, and transcortin, are lost. The physiologic consequences of protein-losing enteropathy may reflect increased catabolism of any of these molecules, but are most obviously related to increased turnover of the albumin pool. Plasma albumin loss into the gut, when expressed as a proportion of the total body pool, will vary in absolute terms, depending on the size of the pool. This concept of **fractional catabolic rate** is essential to understanding the kinetics of plasma protein turnover.

In protein-losing enteropathy, albumin turnover may pass through three phases. During the first phase, the *fractional catabolic rate increases* and, with it, the absolute amount of protein lost into the bowel; as the size of the circulating pool of albumin shrinks, so does the absolute rate of loss of protein, even though the fractional rate remains the same. During the second phase, the size of the circulating pool stabilizes as the rate of albumin synthesis by the liver increases to match in absolute terms the rate of loss. The plasma albumin pool is then in a state of *hyperkinetic equilibrium*; the pool is smaller, with a higher than normal fractional catabolic rate compensated by an increased rate of hepatic synthesis. Provided that the amount of albumin lost does not exceed the synthetic capacity of the liver, this equilibrium may persist for a considerable period. In the third phase, *hypoalbuminemia* *develops as the fractional catabolic rate exceeds in absolute terms the synthetic capacity of the liver.* Alternatively, hypoalbuminemia occurs in the third phase if the rate of synthesis declines, due to deficiency in amino acids derived from the diet and by catabolism of other body protein. The hypoalbuminemia in enteric plasma loss is often associated with *hyperglobulinemia*, since compensatory synthesis of immunoglobulin is remarkable. Several times more immunoglobulin than albumin may be lost into the gut as a result.

The progress and clinical manifestations of protein-losing enteropathy vary with the rate of onset of plasma loss and the fractional catabolic rate. A sudden onset of severe plasma protein loss may cause death during the first phase, before there is time for compensatory synthesis. If the fractional catabolic rate is gradually and only slightly increased, so that compensation occurs with the albumin pool in equilibrium at only a marginally reduced state (perhaps near or within the normal range), subclinical protein loss occurs. Though the fractional catabolic rate is only slightly elevated, the relatively large size of the albumin pool may mean that the absolute loss of protein exceeds that in a hypoalbuminemic animal with a higher fractional catabolic rate, but a smaller albumin pool.

Plasma protein leaking from the stomach or upper small intestine, and protein derived from exfoliated cells in villus atrophy, may be digested and absorbed in the small intestine. This is dependent on luminal proteolysis by pancreatic enzymes, and compensatory membrane digestion and absorption making up for any malabsorption by proximal atrophic mucosa. But the efficiency of protein digestion, even if it is not reduced, is not total. Therefore, a proportion of the increased endogenous protein entering the lumen will be added to the protein escaping digestion in the small bowel, and will enter the large intestine. Here, most of this protein may be converted to ammonia by the colonic flora and absorbed, so that in animals losing protein high in the gut, there may be little or no increase in fecal nitrogen excretion. On the other hand, much of the protein lost into the colon from lesions at that level is passed in the feces, often as protein, and is lost. Ammonia nitrogen absorbed from the colon is converted in the liver mainly to urea. Animals with increased endogenous protein loss into the stomach or small intestine tend to have slightly raised levels of plasma urea, and an elevated rate of urinary urea excretion.

Elevated hepatic synthesis of albumin due to increased turnover of the plasma albumin pool, and increased enteric protein synthesis in support of elevated epithelial turnover in conditions with chronic villus atrophy, is at the expense of anabolic processes elsewhere. Dietary amino acid is diverted to preferential synthesis of plasma and enteric protein. If protein intake is poor due to inappetence or a low-quality ration, or if the rate of protein loss is high, the animal moves into **negative nitrogen balance**. Catabolism of peripheral protein then assumes an increasingly important role in maintaining the pool of amino acids available for plasma and intestinal protein synthesis. This explains in part the reduced growth rate, decreased muscle mass, and reduced bone matrix in sheep with subclinical or mild parasitism, and the cachexia and osteopenia of severe parasitism. These principles probably hold for all syndromes causing enteric loss of endogenous protein in any species.

Loss of enteric protein, and especially plasma, should be suspected in cachectic or hypoproteinemic animals. Diarrhea is often, but not invariably, present. *The two major routes of occult abnormal loss of plasma protein are the kidney in glomerular disease, particularly amyloidosis, and the gastrointestinal tract.* Weeping skin lesions are another source. Anemia and hypoproteinemia may be due to hemorrhage externally or into the gastrointestinal tract. Advanced liver disease may cause hypoalbuminemia, in which case other signs of hepatic failure will likely be concurrent (see Vol. 2, Liver and biliary system). Inability or reluctance to eat, inadequate nutrition or starvation also cause emaciation, usually without profound hypoalbuminemia. The cachexia of malignancy must also be differentiated.

Provided it is adequately hydrated, the hypoalbuminemic animal shows evidence of subcutaneous, mesenteric, or gastric submucosal edema, perhaps with hydrothorax or ascites. Wasting of muscle mass may be marked if the protein loss has been severe and chronic. Unlike starvation, protein-losing gastroenteropathy may be associated with the presence of internal fat depots, since assimilation of energy is not necessarily severely impaired, especially in ruminants.

Anemia

The development of anemia and the kinetics of the erythron following blood loss into the gastrointestinal tract are similar to those of albumin in plasma loss. *Blood loss of any origin, including that due to hematophagous parasites, may cause anemia.* Erythroid hyperplasia in the marrow or in extramedullary sites may not compensate for the continued bleeding. Resolution of the hemorrhage results in eventual restoration of normal red cell numbers and an ultimate decline in erythrocyte production. Chronic blood loss may culminate in depletion of iron stores and development of a nonresponsive hypochromic microcytic anemia.

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Congenital anomalies of the intestine

Segmental anomalies of the intestine are commonly encountered. In early embryonal life the intestine consists of a simple tube, the lumen of which is lined by epithelial cells of endodermal origin. An outer layer of connective tissue from the splanchnic ectoderm surrounds and supports the tube. As the intestines grow with the developing fetus, they form coiled loops that herniate into the umbilicus. In later stages of fetal development, the intestines withdraw, in a cranial to caudal direction, from the umbilicus into the abdomen. The most plausible cause of segmental defects in intestinal continuity is *ischemia of a segment of gut during early fetal life*, resulting in necrosis of the affected area. In addition, an association has been postulated between pressure on the amniotic vesicle during palpation of the embryo for pregnancy diagnosis prior to 42 days of gestation, and the development of atresia coli in calves. The mechanism of this effect is uncertain.

The segmental anomalies of the intestine may vary in degree. **Stenosis** *implies incomplete occlusion of the lumen; complete occlusion is referred to as* **atresia**. Atresia is further subdivided into *membrane atresia*, when the obstruction is formed by a simple membrane or diaphragm; *cord atresia*, in which the blind ends of the gut are joined by a cord of connective tissue; and *blind-end atresia*, in which a segment of gut and possibly the corresponding mesentery are missing, leaving two blind ends. All types of segmental anomaly can be produced experimentally by ischemia to a portion of the fetal intestine.

Atresia coli *is the most common segmental anomaly of the intestine in domestic animals*. It is seen particularly in the spiral colon of Holstein calves, and in the large and small colon of foals; it occurs rarely in cats. Atresia coli may be an autosomal recessive trait in Holsteins. A predisposition for male calves has been described, but this is not consistent among studies. Atresia of the small intestine is less common. **Atresia ilei** is most prevalent in calves, and rare in foals, lambs, piglets, and pups.

These obstructions prevent the normal movement of gut content and meconium in the fetus and neonate. Therefore, they lead to dilation of the proximal segment, with progressive abdominal distension, which may become so extensive prepartum as to cause dystocia. The bowel beyond the discontinuity is small in diameter, and devoid of content other than mucus and exfoliated cells. Animals fail to pass feces after birth.

Atresia ani (*imperforate anus*), *overall, is the most common congenital defect of the lower gastrointestinal tract*. It may affect all species, but is most often encountered in calves, and in pigs, in which it is considered to be hereditary. The defect may consist only of failure of perforation of the membrane separating the endodermal hindgut from the ectodermal anal membrane, or both anus and rectum may be atretic. Atresia ani may be an isolated abnormality, or it may be associated with other malformations, especially of the distal spinal column (spinal dysraphism, sacral or coccygeal vertebral agenesis), of the genitourinary tract (rectovaginal fistula, renal agenesis, horseshoe kidney, polycystic kidneys, cryptorchidism, duplication of scrotum), and occasionally, with intestinal atresia or agenesis of the colon. *Caudal spinal syndromes* occur in humans and animals commonly associated with atresia; this association is presumably because of a reduction in the available pool of neuronal cells in the spinal region during intestinal development. Atresia ani may occur with congenital vitamin A deficiency.

Short colon, probably the result of abnormal rotation of the midgut and failure to lengthen during fetal life, has been reported in several cats and dogs. Signs were mild, and possibly unrelated to the colonic lesion. The cecum was on the left side, and ascending, transverse, and descending colon were not demarcated on contrast radiographs. In several cases, anorectal or urogenital abnormalities were concurrent. **Anomaly of the colonic mesenteric attachments**, presumably congenital, has been associated with the development of colic in a horse, due to distortion and convolution of the large colon.

Hypoplasia of the small intestinal mucosa, with short villi and sparse crypts, has been reported in foals with failure of passive transfer of immunoglobulin. This may represent a defect in fetal organogenesis.

Congenital colonic agangliosis, somewhat analogous to Hirschsprung's disease in humans, occurs in *white foals* that are the offspring of "frame overo" spotted parents. It has not been convincingly demonstrated in other species of domestic animals. Clinically, the foals, which are predominantly white with perhaps a few pigmented dots on the muzzle, abdomen, and hindquarters, develop colic, and die generally within 48 hours of birth. There is stenosis mainly of the small colon, but the entire colon and rectum may be involved. The intestine proximal to the stenotic segment is distended with gas and meconium. The descending colon is contracted but patent. Microscopically, *ganglia of the myenteric plexus are absent in the walls of the terminal ileum, cecum, and colon*, though occasional nerve fibers are evident. Except for the few pigmented spots, melanocytes are absent in the skin. The condition, which appears to be an autosomal recessive trait, is similar to aganglionic colon in Piebald and Spotted Mutant mouse strains. Cutaneous melanoblasts and the myenteric plexus are both derived from the neural crest, which may explain the association between unpigmented skin and lack of the myenteric ganglia. A similar condition of megacolon due to segmental colonic aganglionosis has been reported in pigs. Megacolon associated with few myenteric ganglion cells in Clydesdale foals is not clearly congenital, and is discussed with intestinal obstruction, as is megacolon in other species.

Persistent Meckel's diverticulum is an uncommon anomaly of the lower small bowel, mainly in swine and horses (Fig. 1.47). It is derived from the omphalomesenteric (vitelline) duct, which is the stalk of the yolk sac. Although usually an incidental finding, it may be associated with impaction or intussusception. Also, an increased incidence of tumors has been noted at this site. **Persistent vitelline duct cysts**, distinct from Meckel's diverticulum, have been described on the ileal serosa and in a mesodiverticular band, as an incidental finding in a dog.

Intestinal diverticula, not always clearly remnants of the vitelline duct, occur rarely in dogs, cats, and horses. They may be incidental, or are associated with obstruction, diverticuloumbilical fistula, or with diverticulitis, perforation, and peritonitis. Intestinal adenocarcinoma, smooth-muscle tumors, and heterotopic gastric fundic and pyloric mucosa may occur in the wall.

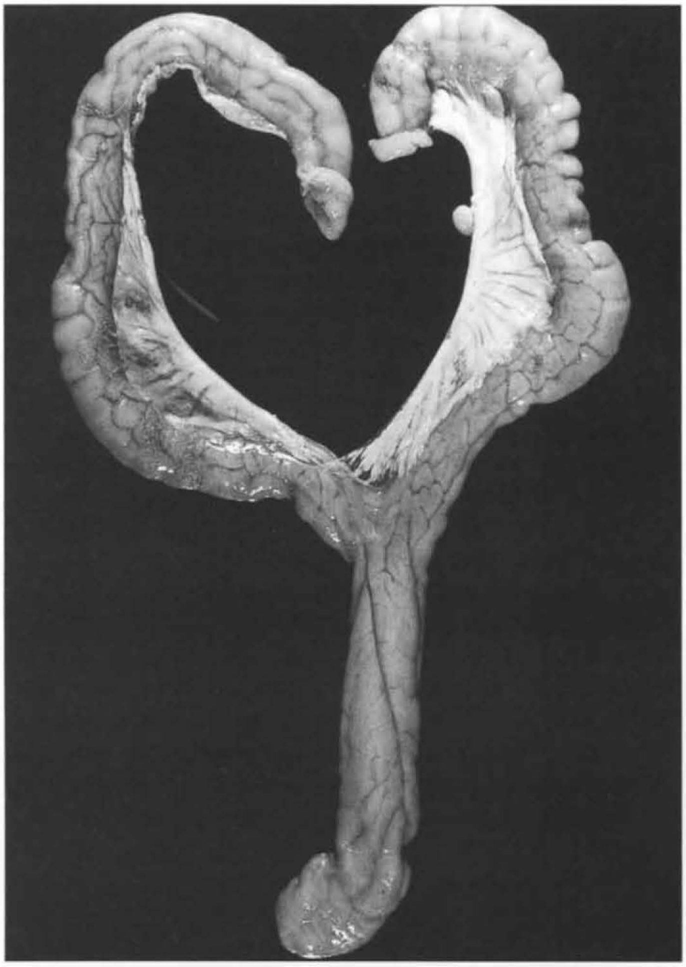

Figure 1.47 Persistent **Meckel's diverticulum** in the mid-jejunum of a pig.

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Intestinal obstruction

Clinically, acute obstruction typically involves the upper or middle small intestine; chronic blockage usually involves the ileum and large bowel. Intestinal obstruction may be the sequel to a physical blockage of the lumen resulting from **stenosis** (narrowing, stricture) due to an intrinsic lesion involving the intestinal wall, **obturation** (occlusion) by an intraluminal mass, or **extrinsic compression**.

Failure of the intestinal circular smooth muscle to contract blocks the peristaltic wave, causing **functional obstruction**, or **pseudo-obstruction**, a clinical syndrome in which there is no physical occlusion of the lumen of an impacted intestine. **Adynamic ileus**, or intestinal obstruction resulting from inhibition of bowel motility, is a common sequel to peritonitis and pain.

Circulatory embarrassment of a segment of bowel, through embolism or venous infarction, will also cause functional obstruction without a physical blockage. Many of the displacements of gut that produce obstruction, such as volvulus, incarceration, strangulation of a hernia, or intussusception, may cause *ischemia*. The term "**strangulation obstruction**" is applied to an event that simultaneously causes ischemia and physically blocks the intestine. Mucosal hypoxia may also be a sequel to venous occlusion resulting from distension of gut proximal to a site of obstruction; it may result from local pressure caused by an adjacent mass; or it may be a sequel to circulatory failure. Ischemia in any circumstance is a serious complication, the pathogenesis of which is dealt with later.

Proximal to the point of obstruction there is *accumulation of fluid*, derived from ingesta, gastric, biliary, pancreatic, and intrinsic intestinal secretion, and *gas*, swallowed or originating with bacterial activity in the gut. Intestinal distension results in sequestration of water and electrolytes in the intestinal lumen, edema of the mucosa, further secretion into the gut, perhaps associated with contractile stimuli, and, in extreme cases, transudation from the peritoneal surface. Upper small-bowel obstruction progresses rapidly to cause *vomiting* in those species that can vomit, with dehydration, hypochloremia, hypokalemia, and metabolic alkalosis due to loss of acid in vomitus, or sequestration of fluid in the forestomachs.

Obstruction of the lower small intestine may result in distension with dehydration. But there is usually less acute electrolyte and acid–base imbalance, since vomition is less severe and absorption of fluid proximal to the obstruction may prevent serious distension and its associated secretion, for some time. Metabolic acidosis eventually ensues following dehydration and catabolism of fat and muscle due to cessation of food consumption and assimilation.

If ischemia and its complications, including rupture, do not ensue, the animal with acute obstruction succumbs to the *systemic effects of hypovolemia, electrolyte, and acid–base disturbance*. Lower intestinal or colonic obstruction may result in eventual metabolic acidosis and loss of body condition following a chronic course. In the horse particularly, obstruction or impaction of the cecum or colon may result in *local ischemia and rupture*.

In obstruction the outstanding gross alteration in the bowel is distension proximal to the point of blockage, due to ileus and the accumulation of fluid contents and gas. The volume of accumulated content may be remarkable in distal intestinal obstruction, such as rectal stricture in the pig, which causes overt abdominal distension. The location, degree, and duration of the obstruction determine the segment and length of bowel involved and the degree of distension. As distension increases, interference with venous return may develop and the mucosa and submucosa become congested. Devitalization of severely dilated gut, or pressure necrosis of the mucosa at the site of lodgment of intraluminal foreign bodies may occur, leading to gangrene or perforation and peritonitis. Distal to the point of obstruction, the bowel is collapsed and empty.

Stenosis and obturation

Intrinsic obstruction due to congenital segmental atresia and imperforations is considered under congenital anomalies of the intestine, above. **Acquired stenosis** due to pathological processes arising within the wall of the intestine may be partial or complete. The primary lesions include intramural abscesses, intramural hematomas, neoplasms (Fig. 1.48), and scarring following ulceration. Many of these develop slowly with a course as described above for simple chronic obstruction.

Foreign bodies of all kinds are commonly found. Small rounded foreign bodies and even some sharp-edged objects may pass through the intestines uneventfully, but for these and many large foreign bodies the course is unpredictable. Some may reside in the intestine for long periods and produce no disturbance until they act as a nucleus for the development of an enterolith. Sharp-pointed foreign bodies may become impacted in the intestine and cause pressure necrosis with ulceration and possibly perforation. Blunt foreign bodies that become impacted cause acute or chronic obstruction depending on their size and location; often they cause local pressure necrosis, and perforation may occur in some cases. **Linear foreign bodies**, such as strips of cloth or string, which are not infrequently ingested by dogs and cats, may pass through the intestine. However, if they become immobilized they produce a typical lesion. One portion of a string becomes fixed, commonly around the base of the tongue, or by impaction at the pylorus. A free end is then stretched taut distally by peristaltic movements, which pleat the gut on the string (Fig. 1.49). It progressively cuts into the lesser curvature of intestinal loops, causing first a puckering of the mesentery and finally perforation and peritonitis.

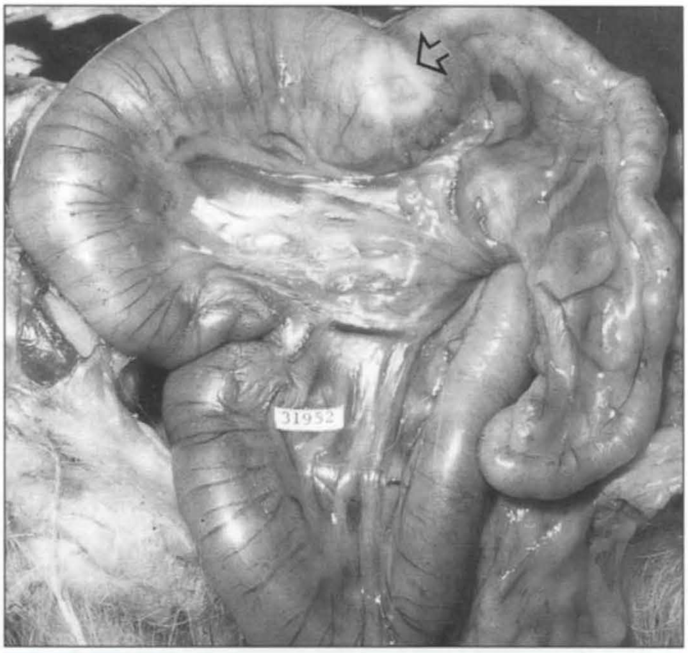

Figure 1.48 Scirrhous adenocarcinoma (arrow) infiltrating wall of small intestine, causing obstruction in a dog. Dilation is present proximal to the obstruction, with contraction of the empty intestine distally.

Enteroliths (mineral concretions) were common in the colon of horses in the past, and they seem to be re-emerging as a problem in some areas, such as California and Florida. Arabians, Morgans, American Saddlebreds, and donkeys are overrepresented with respect to this problem. The mean age for equine enterolithiasis is about 11 years. The stones are comprised of *magnesium ammonium phosphate* deposited in concentric lamellae, at the center of which there is a *nucleus* – a foreign body such as a nail, wire, stone, or particle of feed. They vary greatly in size, some weighing as much as 10 kg, and there may be one or more large ones or many small ones. They are usually smooth and often spherical, but contact with other liths, and abrasion, may smoothly flatten some surfaces. Irregular mineralized masses may also occur, usually based on a fibrous nidus, such as twine, rope, or netting. The source of the magnesium phosphate is probably grain, bran, alfalfa, or alkaline water. The mineral salts deposit around a nidus under poorly understood circumstances, but colonic pH above 6.6 seems to contribute to their formation.

Fiber balls (**phytobezoars** or phytotrichobezoars), which consist largely of plant fibers impregnated with some phosphate salt, may especially be found in the colon of horses. They are not as heavy as enteroliths, are moist, and have a velvety surface. They are usually round and smooth, but some are convoluted like the surface of the cerebrum (Fig. 1.50). Hairballs (**trichobezoars**) sometimes occur in dogs and cats and in ruminants; in ruminants they occur in the forestomachs and abomasum. They may obstruct the pylorus, but rarely cause problems in the intestine.

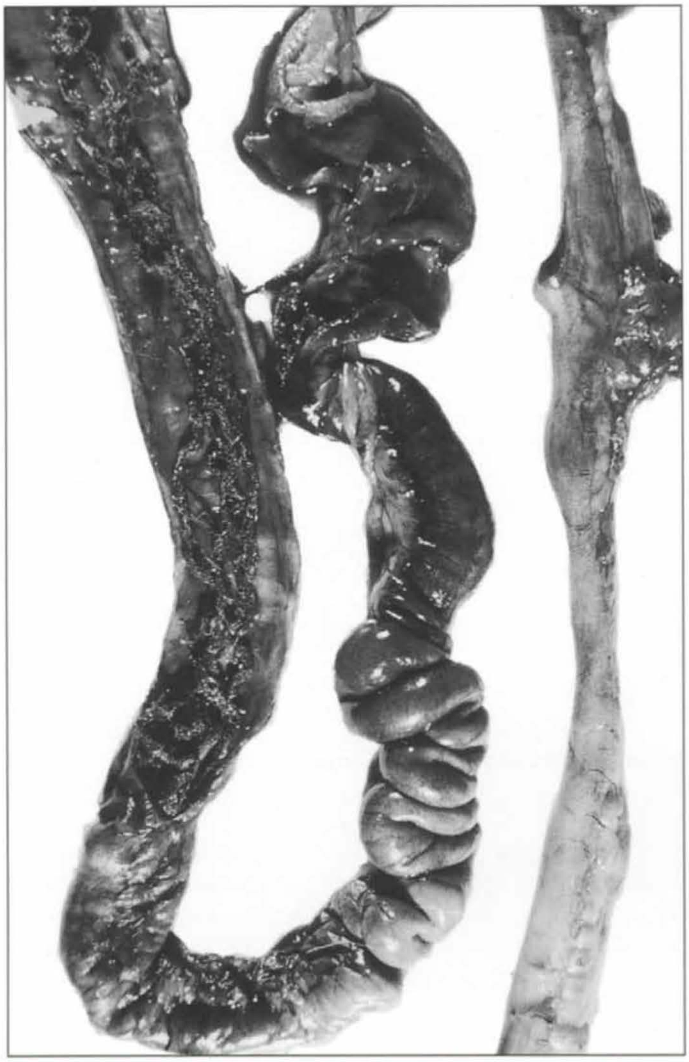

Figure 1.49 Pleating of the small intestine along a **linear foreign body** in a cat. The lower segment of bowel is normal (right).

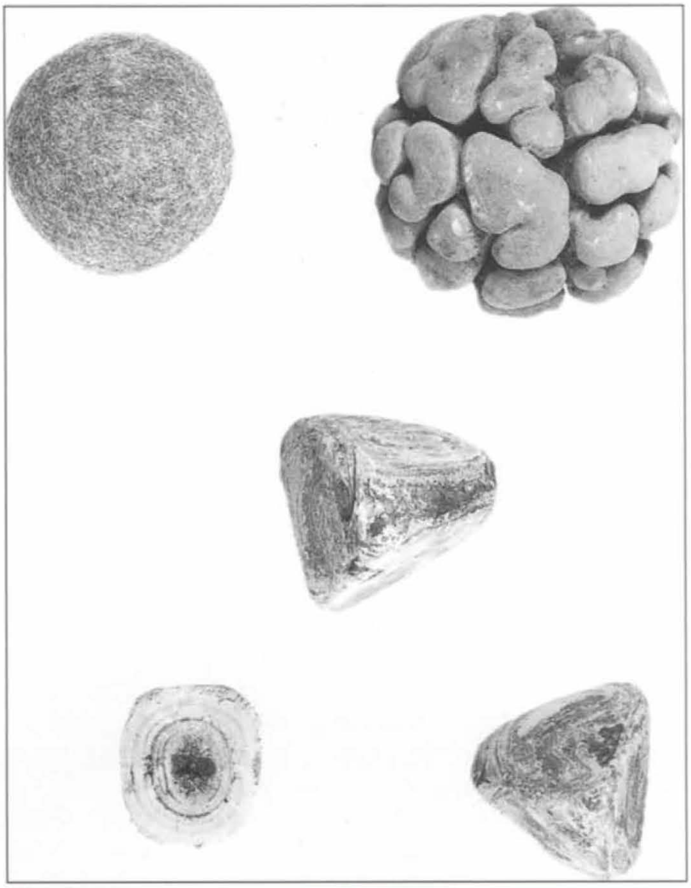

Figure 1.50 **Trichobezoar** (top left). **Phytobezoar** (top right). **Enteroliths** (below).

Enteroliths and bezoars in horses are usually not significant, and small ones may pass in the feces. Apparently they are moved about enough by peristalsis to avoid pressure necrosis. They may obstruct the gut when they are impacted, usually where the colon narrows, in the pelvic flexure and transverse or small colon.

Small intestinal obstruction may be caused by parasites that can form rope-like tangled masses in the lumen. This may occur in pigs and foals infested with large numbers of *ascarids*. It also occurs rarely in sheep heavily infested with tapeworms. Impaction of the ileum is the most common cause of small intestinal, nonstrangulating obstruction in horses. Feeding of a high-fiber diet, such as coastal Bermuda grass hay, predisposes to this condition. Gravel has been reported as a cause of duodenal obstruction in cattle.

Impaction of the colon, by feces in dogs and cats, and by digesta, fibrous foreign material, sand, or feces in horses, is not uncommon and causes simple intestinal obstruction, complicated in the horse by intestinal tympany from fermentative gases if the obstruction is complete. In dogs, obstipation of the colon may be the result of voluntarily suppressed painful defecation, as in prostatic enlargement and inflammation of the anal sacs. Occasionally, it is due to impaction with foreign bodies, especially hair or fine bones in the colon or rectum, or due to stenosis caused by tumors or strictures. It may also complicate trauma to the pelvic area, and paralyzing lesions of the spinal cord; it may occur in Manx cats due to sacral spinal cord anomalies. **Megacolon** may ensue, if the obstruction persists. In small animals, megacolon is frequently idiopathic. Examination of the syndrome in cats reveals no histologic abnormalities and it is suggested that the underlying problem is disturbance in the activation of smooth-muscle myofilaments.

Impaction of the cecum or colon in horses may be precipitated by water deprivation, a change of diet from something soft and lush to hay or chaff, or poor dentition. In animals with dental problems, impaction of the cecum may be recurrent, and cecal smooth muscle may become hypertrophic. Abnormal motility due to altered colonic or cecal pacemaker activity may explain impaction in the absence of other predisposing causes. Deficiency of neurons in the myenteric plexus at the base of the cecum may cause chronic uncoordinated hypercontractility and result in hypertrophy of the circular muscle layer of the cecum. In the colon, fibrous digesta usually impacts at the pelvic flexure, transverse or small colon. Ingestion of indigestible *synthetic fibers* has been associated with colonic impaction. *Sand* may sediment in the colon of horses that have grazed on poorly covered sandy soils. Sand may cause chronic colitis with diarrhea and weight loss, or it may accumulate at any level of the large or small colon, causing one or more points of impaction obstruction, often associated with concurrent displacement or torsion. Ingestion of large numbers of *acorns and leaves* may also cause impaction of the intestinal tract in ruminants. Rupture of the viscus may occur if impactions are not treated, and the bowel wall becomes ischemic and devitalized.

Cecal rupture in horses occurs as a complication of impaction, and of parturition in mares. It has also been reported secondary to ulceration as a result of phenylbutazone therapy. Idiopathic cecal rupture, associated with "overload" of normal digesta, rather than impaction, is also reported, sometimes as a complication of general anesthesia. Also termed cecal "dysfunction," **cecal overload** is differentiated from impaction by the more fluid consistency of the contents. The cecum is distended, and the mucosa may be hyperemic and somewhat thickened at surgery. Disordered motility with increased intracecal pressure has been invoked speculatively to explain subsequent cecal rupture. The lacerations of the cecum in this circumstance are reported on the medial aspect at some distance from the base, but this is not consistent.

Extrinsic obstruction

Compression of intestine causing obstruction is rather common and is caused by tumors, abscesses, peritonitis, and fibrous adhesions. **Neoplasms** involve the intestine by extension from adjacent viscera, particularly pancreas. Therefore, many tumors involve the craniodorsal part of the abdominal cavity and impinge on the duodenum. Inflammatory peritoneal **adhesions** are common, and fibrous bands may stretch from the wall of the bowel to some fixed point, or between two or more points along the bowel or mesentery; the obstruction develops gradually as scarring ties the bowel down or puts kinks in the mesentery. Large firm masses of **abdominal fat necrosis** cause compression stenosis of small intestine, coiled colon, and particularly descending colon and rectum of cattle. **Pedicles of some tumors**, especially *mesenteric lipomas in older horses*, occasionally become wound about loops of intestine and cause obstruction and strangulation. Similarly, the **suspensory ligaments of the ovary** may entrap the equine colon if the ovary is enlarged by neoplasia or other phenomena. Incarceration in hernias, discussed with other displacements of the bowel, is also a common cause of compression obstruction of the gut.

Functional obstruction

Adynamic (paralytic) ileus is in itself not of specific interest to the pathologist but is a rather common condition. It frequently follows abdominal surgery in transient episodes, especially when the intestines are handled roughly, or traumatized. It also occurs in peritoneal irritation of any cause, especially in peritonitis. It is the result of neurogenic reflexes that interfere with control of the inhibitory neurons of the myenteric plexus. Continual tonic discharge by these neurons inhibits contraction of circular smooth muscle and prevents peristalsis.

The intestines are distended with a mixture of gas and fluid, and the wall is flaccid. The defect may be segmental, involving less than a meter of the intestinal length, but there may be many such segments involved, especially in diffuse peritonitis. Gastric rupture may occur in the horse as a complication of obstructive or postoperative ileus of the small intestine.

Pseudo-obstruction, a clinical syndrome in which there is *no physical occlusion of the lumen* of an impacted intestine, may result from segmental or diffuse neuromuscular dysfunction in the gut. It overlaps in part with paralytic ileus, and with elements of impaction and megacolon related to intestinal dysmotility, discussed below. Pseudo-obstruction associated with neuronal hypocellularity or ganglioneuritis involving autonomic ganglia in the gastrointestinal tract, systemic dysautonomia, and intrinsic disease of intestinal smooth muscle are recognized among domestic animals, in addition to congenital aganglionosis of spotted foals, discussed earlier. Occasionally a segment of contracted bowel is noted, causing obstruction; more commonly, in neurogenic disease, affected bowel is dilated, flaccid, and incapable of maintaining tone.

Megacolon in Clydesdale foals, in association with *hypoganglionosis of the myenteric plexus*, has been reported from the USA and Australia in animals 4–9 months of age. The dorsal colons were markedly dilated and the intestinal wall thin. Small colon was patent but devoid of content. Ganglion cells of the myenteric plexus in the transverse and small colons were reduced or absent. There was variation in ganglion cell density in the dorsal colon; in two cases they were present, while in the third, they were markedly reduced in number. Inflammation was not noted. The time of clinical onset of these cases suggests that they may not have been congenital. The common breed suggests a possible genetic basis for the syndrome. If the lesion is acquired, the factors causing myenteric ganglion cell loss are unknown.

Grass sickness in horses, the prototypic **dysautonomia** in domestic animals, occurs chiefly in parts of the UK and western Europe, usually in animals at pasture. Although horses of any age may be afflicted, the disease is more common in those 3–6 years of age. Affected animals are dull but show occasional bouts of colicky restlessness. In the acute disease, there is progressively severe tympany, swallowing is avoided, and saliva drools freely. Any attempt to swallow is apparently painful and stimulates reverse peristalsis in the esophagus. Fine muscular tremors develop over the shoulders and there is sweating. The course in acute cases is from 12 to 72 hours. Some survive the acute phase to live much longer, but may die in due course of the disease. With careful nursing, recovery may be complete but take months to years.

Acute lesions at necropsy are confined to the alimentary tract. The esophageal wall is sometimes edematous, and the mucosa may show longitudinal bands of congestion and ulceration, which may extend into the stomach. The stomach is distended with alkaline mucinous fluid, often of khaki color and pea soup consistency, although sometimes more watery with fibrous material. Sometimes the stomach is ruptured. There may also be excess fluid in the small intestine. The large intestine is impacted with dry contents and the fecal pellets in the small colon are small and dry. These masses may have a surface blackened by a small amount of exuded blood. In chronic cases, the volume of alimentary content is reduced to scant amounts in the stomach. There may be hypertrophy of intestinal musculature, often most prominent in the jejunum.

Degenerative lesions, marked by often subtle chromatolysis, nuclear eccentricity, and neuronal necrosis, and the formation of perineuronal eosinophilic axonal spheroids, have been described in many autonomic ganglia, and a central neuropathy has also been described, as has a reduction in the number of interstitial cells of Cajal. Significant inflammation is not evident. Severity of lesions has been shown to correlate with decreased functional cholinergic responses. *Clostridium botulinum* is thought to play a role in equine dysautonomia, which may hence be a toxicoinfectious form of botulism.

Feline dysautonomia, or Key–Gaskell syndrome, is an autonomic dysfunction of unknown etiology, most common in the UK and continental Europe, with sporadic cases reported elsewhere. A similar syndrome has also been reported in a few dogs. All age groups are affected. Signs include depression, anorexia, reduced lacrimation and salivation, bradycardia, pupillary dilation with delayed pupillary light reflex, megaesophagus, constipation, and, rarely, ileal impaction. Diarrhea is reported in some dogs with dysautonomia. The gastrointestinal signs suggest *disordered motility*, and animals often succumb to the effects of regurgitation, inanition, or aspiration pneumonia, among other problems. Cases tend to occur in clusters, suggesting an environmental toxin or infectious agent, and exposure to botulinum toxin has been suggested, although no conclusive etiologic associations have been made.

Involvement of functions controlled by both sympathetic and parasympathetic divisions of the autonomic nervous system, and of some functions under voluntary control, is reflected in the distribution of neuronal lesions in ganglia of the autonomic system, and in cranial nerve nuclei III, V, VII, and XII, ventral horns of the spinal gray matter, and in dorsal root ganglia. Chromatolysis-like lesions of affected neurons seen by light microscopy have a *distinctive ultrastructural appearance in dysautonomia*. Autophagocytic vacuoles, large cisternae, and complex stacks of smooth endoplasmic membranes are in the cytoplasm of affected cells. Neuronal lesions and subsequent drop-out may be transient, and in the later stages of the disease diagnosis may need to be based on comparative cell counts in ganglia of affected and unaffected cats, unless neuronal loss has been obviously severe.

Intrinsic disease of intestinal smooth muscle may produce a syndrome of **intestinal sclerosis**, somewhat resembling that in progressive systemic sclerosis or *scleroderma* of humans. However, in dogs the lesions are restricted to the intestinal tract, where they usually appear to involve the small and large bowel diffusely. There is typically mild to obvious dilation of the bowel. In section, there is a mononuclear inflammatory infiltrate among smooth-muscle fibers in the circular and longitudinal layers of the bowel wall. Associated with this is interstitial fibrosis, and atrophy of smooth-muscle cells. Fibrosis of the small intestine has also been reported in horses, concentrated in the submucosa and accompanied by arteriolosclerosis. A geographic cluster suggests an environmental basis.

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Displacements of the intestines

Eventration

Eventration is displacement of a portion of the gut, usually the small intestine, outside the abdominal cavity. This is commonly *congenital*, as in schistosomus reflexus, patent umbilicus, and congenital diaphragmatic hernia. Acquired eventrations result from *trauma*, and therefore are varied. The displaced intestine herniates into the abdominal muscle or subcutis, or it may be completely exteriorized. Vaginal eventration may occasionally occur in females following trauma.

Cecal and colonic dilation, tympany and torsion

In ruminants, cecal dilation and torsion is an uncommon condition. It mainly occurs in animals fed *high-concentrate rations*, but it has been associated with late gestation, and ileus from other causes. It usually occurs within 2 months postpartum in cattle. It is rare in other ruminants. About 30% of the carbohydrates in the ration are digested in the cecum of ruminants. Sudden change from a roughage to a grain-based ration results in an increase in the concentration of volatile fatty acids, with only a slight decrease in pH of the cecal contents. An increase in the concentration of dissociated volatile fatty acids, especially butyric acid, causes atony of the cecum, and dilation follows. Once the cecum is dilated and distended with watery digesta, various degrees of clockwise or counterclockwise rotation can occur, which may incorporate adjacent terminal ileum or proximal colon.

In horses, cecal and colonic tympany has a similar pathogenesis. Readily fermentable carbohydrate, following a sudden change in feed, results in an increase in volatile fatty acid production, which exceeds the buffering and absorptive capacity of the organ. As the pH drops, and fermentation shifts to production of the less well-absorbed butyric and lactic acids, water is drawn into the lumen by the osmotic effect. The large bowel dilates with fluid digesta and gas, and motility is reduced by the effects of the volatile fatty acids. Severe abdominal distension, compression of intra-abdominal organs, reduced cardiac return due to postcaval compression, and reduced respiratory capacity due to compression of the diaphragm may follow, with attendant severe pain. Death due to hypovolemia and acidosis may occur before the large bowel ruptures. In recovered horses, laminitis may occur, due to absorption of endotoxin through the cecal mucosa, which becomes eroded and permeable as a result of local acidosis.

Displacements of the equine colon

The large colon of the horse is comprised of a loop of capacious bowel joined along its length by the short mesocolon, and folded upon itself at the sternal, pelvic, and diaphragmatic flexures. The loop is only fixed at its base, by the cecum, the transverse colon, and mesenteric root. Its volume and lack of attachment make the large colon prone to displacement or torsion.

Right dorsal displacement of the colon involves displacement of the left colons to the right of the cecum. Consequently, when the carcass is opened, the left colons are visible first, with the pelvic flexure located near the sternum. It presumably results from displacement and wedging of the large colon due to tympany. The left colon may become displaced clockwise (in the standing animal viewed from above) to the right of the cecum as the pelvic flexure migrates cranial to the cecal root, caudad and to the left across the pelvic inlet, and craniad on the left side, coming to rest at the sternum. This is termed *right dorsal displacement with flexion*. Alternatively, the left colon may move in the opposite direction, caudal and to the right of the base of the cecum, with the pelvic flexure again lying at the sternum. This is termed *right dorsal displacement with medial flexion*. Some degree of torsion may also occur, and obstruction, with mild to severe colic, ensues. Surgery is required to correct the displacement.

Left dorsal displacement of the colon, variously known as entrapment of the colon by the nephrosplenic, renosplenic, or phrenicosplenic ligament, or by the suspensory ligament of the spleen, is also encountered as a cause of obstruction and colic in horses (Fig. 1.51). The left dorsal and ventral colon move laterally and dorsally between the spleen and the left body wall, and become trapped, with the spleen to the left and below, the suspensory ligament of the spleen below, the left kidney on the medial aspect, and the abdominal wall dorsally. The sternal and diaphragmatic flexures may be in their normal position, ventral to the stomach, or they may have moved cranially and become displaced dorsal to the stomach. The colon caudal to the entrapment may become curved cranially, with the pelvic flexure rotated through 180°, due to tension on the taeniae. The stomach and spleen may be displaced caudally and ventromedially, if the flexures have become displaced dorsally into the space cranial to the stomach, in apposition with the left lobe of the liver. If the colon has moved dorsal to it, the stomach often becomes grossly distended, as a result of compression of the esophagus and the duodenal outflow by the incarcerated colon. Compression of the colon at the point of entrapment can cause local bruising or edema and partial ischemia of the displaced organ, and results in obstruction, which may be incomplete. If the gut remains patent, clinical signs are intermittent. The cecum and small intestine may be distended as a result of the colonic obstruction. The cause of left dorsal displacement is unknown, but may be related to an anatomic predisposition resulting from a large cleft between the spleen and the left kidney.

Colonic torsion and volvulus *is one of the most common and grave colic conditions in horses.* The loop of large colon, with or without the cecum, may rotate around itself at some point along its length, or around the root of the mesentery. The condition is life-threatening because of severe vascular embarrassment, and is discussed further under Intestinal ischemia and infarction, below.

Internal hernia

This is a displacement of intestine through normal or pathological foramina within the abdominal cavity without the formation of a hernial sac. It is *uncommon*.

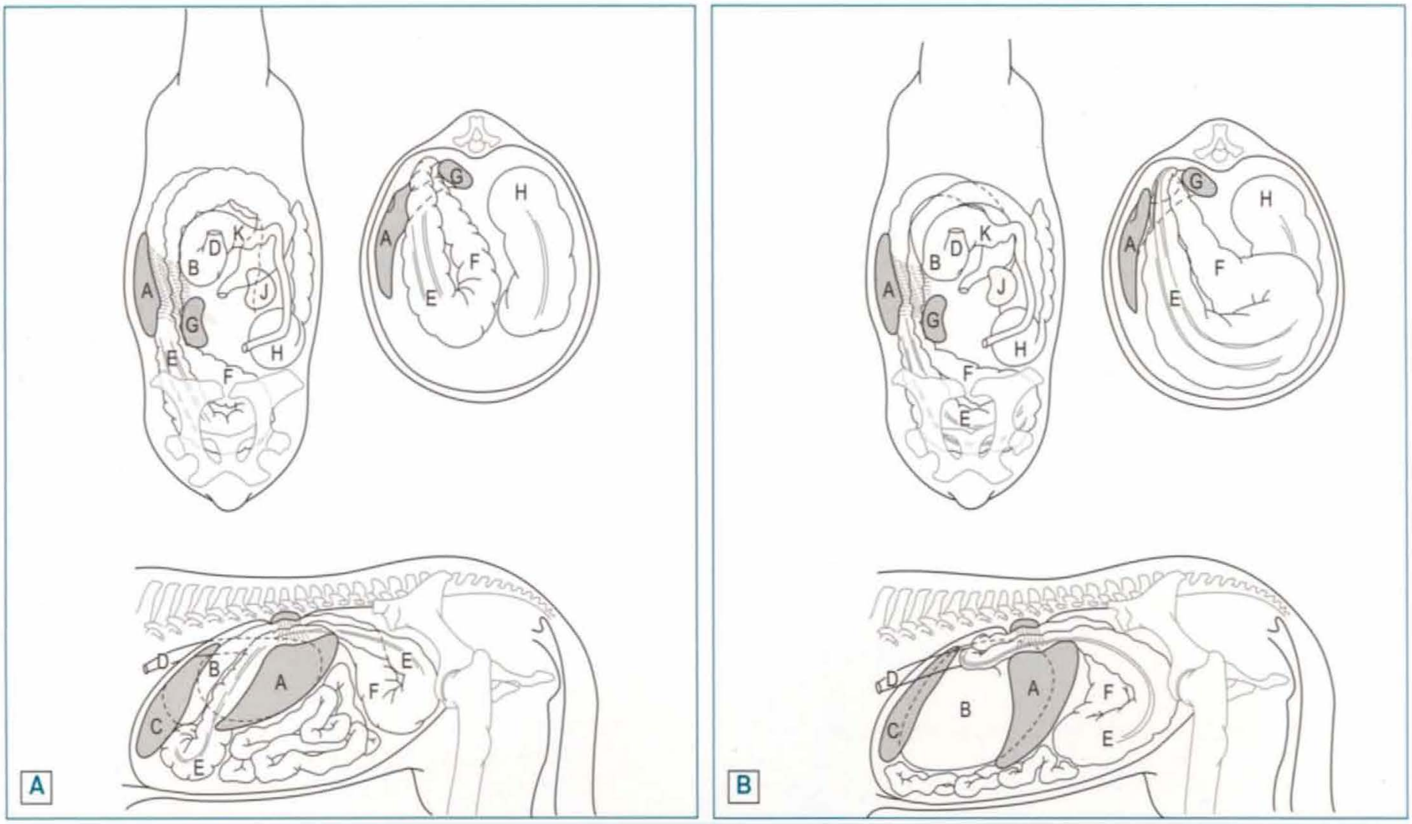

Figure 1.51 A. Left dorsal displacement (nephrosplenic entrapment) of the equine colon. Entrapment with the sternal and diaphragmatic flexures of the colon below the stomach. **B. Entrapment** with the sternal and diaphragmatic flexures displaced dorsal to the stomach. A. spleen; B. stomach; C. liver; D. esophagus; E. left ventral colon; F. left dorsal colon; G. left kidney; H. base of cecum; J. right kidney; K. duodenum. (Reprinted with permission from Livesey MA, et al., Can Vet J 1988;29:135–141.)

Herniation through a natural foramen occurs mainly in horses. In **incarceration in the epiploic foramen**, a portion of small intestine, usually distal jejunum and ileum, may pass down into the omental bursa and become incarcerated, if the normally short and slit-like epiploic foramen of Winslow is dilated for any reason. In these circumstances, the wall of the omental bursa often ruptures.

Omental hernia occurs when a loop of intestine passes through a tear in the greater or lesser omentum. **Mesenteric hernia** is due to passage of intestine through a tear in the mesentery. These are probably traumatic defects and usually involve the mesentery of the small intestine, but occasionally, that of the colon.

Pelvic hernia occurs in young ruminants, and rarely in other species, following castration. During the operation, excessive traction on the spermatic cord may tear the peritoneal fold of the ductus deferens which fixes the duct to the pelvic wall. A hiatus is formed between the ductus deferens and the lateral abdominal or pelvic walls and, through it, loops of intestine may become incarcerated.

Gut may also become incarcerated by passing through lacerations in the lateral ligament of the bladder, or through entrapment by the remnant of a persistent urachus, by the gastrosplenic ligament, and by mesodiverticular or vitelloumbilical bands in the horse.

External hernia

External hernia typically consists of a *hernial sac* formed as a pouch of parietal peritoneum; a *covering* of skin and soft tissues; depending on the location of the hernia, a *hernial ring*; and the *hernial contents*. The hernial ring is an opening in the abdominal wall, and this may be acquired, or it may be natural as, for example, the vaginal ring at the inguinal canal. The hernia usually contains a portion of omentum, a freely mobile portion of the intestine and occasionally, other viscera.

Ventral hernia of the abdominal wall occurs uncommonly in horses, rarely in cattle, and exceptionally in other species, except perhaps following blunt trauma or biting injuries in small animals. These hernias, through the abdominal musculature into the subcutaneous tissue, may be spontaneous in heavily pregnant females, especially older animals, or be a consequence of blunt trauma, bite wounds, horn injuries, surgical scars, or inflammations, which cause weakening or perforation of the muscle. In pregnant mares, they often occur in the lower flank lateral to the mammae, where there is only a single layer of muscle, the transverse abdominal. They may become very large in herbivores because of the weight of the alimentary viscera and pregnant uterus. The lesion must be differentiated from rupture of the prepubic tendon, and from postmortem rupture of abdominal muscle in bloated animals.

Umbilical hernia is *common* and is often present as a congenital and perhaps inherited defect. It is most frequent in pigs, foals, calves, and pups and depends on persistent patency of the umbilical ring. It is the *most common congenital defect in cattle* and has a hereditary component in the Holstein breed. It is also significantly predisposed by umbilical infection. The hernial sac is formed by peritoneum and skin; the contents depend on the size of the ring and of the sac.

Incarceration of enclosed intestine is uncommon. Formation of an *enterocutaneous fistula*, a rare complication of umbilical hernia, is seen most frequently in the horse.

Inguinal hernia may evolve to **scrotal hernia** when the herniated viscera pass down the inguinal canal. The internal, or deep, inguinal ring remains patent in intact male animals, but its diameter and the tendency to herniation in the neonate may be inherited. Inguinal hernias are classified as direct or indirect.

In **direct inguinal hernia**, which is less common, abdominal contents pass through the internal inguinal ring and come to rest in a subcutaneous position, through a tear in either the peritoneum or the fascia around the deep inguinal ring. This is most commonly seen in foals and is thought to result from increased intra-abdominal pressure during passage through the birth canal. These direct inguinal hernias often cause necrosis of overlying skin, can become fixed by adhesions, and strangulate. They are life-threatening.

Indirect inguinal hernias, by contrast, *consist of abdominal contents contained within the tunica vaginalis*. These are by far the *most common form*, occurring as a congenital problem in the young of many species, and as an acquired problem in older animals. While size of the inguinal rings may be a factor in neonates, there is usually no apparent cause in acquired cases. The herniated viscus passes through the inguinal and vaginal rings within the vaginal sheath, coming to lie in the scrotum inside the cavity of the tunica vaginalis. If the hernia is scrotal, there may be degeneration of the testicles. Routine castration of such animals can lead to eventration through the scrotal incision, and closed castration may cause infarction of the herniated loop of gut.

Congenital inguinal hernia is rare in dogs; West Highland Whites, Pekingese, and Basenjis may be predisposed, and it is more common in males than females. However, overall, inguinal hernia is much more common in female dogs. The bitch differs from females of other species in having a patent inguinal ring and canal, through which the omentum and the uterus may pass. The herniated uterus may become incarcerated when pregnant, or if pyometra develops. In horses, indirect inguinal hernias may rupture through the inguinal canal, coming to rest in a subcutaneous location. These ruptured indirect hernias have a similar prognosis as the direct inguinal hernias described above.

Femoral hernias develop as an outpouching of peritoneum through the femoral triangle along the course of the femoral artery. They contain omentum and small intestine.

Perineal hernias *occur principally in old male dogs in association with prostatic enlargement and obstipation*. They are precipitated by abdominal straining and are probably predisposed to by weakening of perineal fascia and muscles from some unknown cause, possibly hormonal. They are very unusual in females. Retroperitoneal pelvic fat bulges through a defect between the coccygeus medialis muscle and the cranial border of the anal sphincter. Usually this is the only tissue to prolapse, and the lesion consists, essentially, of a loss of support on one side of the anal ring. Concomitant with the loss of pelvic support the rectum deviates, and the prostate and the bladder may move into the pelvis. Further displacement may occur occasionally and then the latter organs are forced through the ruptured perineal fascia, causing acute urethral obstruction. Perineal hernias are most commonly unilateral, but bilateral hernias may occur.

Diaphragmatic hernias are *common*. The defect in the diaphragm may be congenital, but most often it is acquired, generally as the result of increased abdominal pressure. Though abdominal viscera pass into the thoracic cavity, strangulation of displaced gut is rare. Acquired diaphragmatic hernias are considered more fully in the section Abnormal contents in the peritoneal cavity.

The sequelae of hernias depend largely on their location and content, but some generalities apply to all. As long as the hernial contents remain freely movable and reducible, there may be no untoward sequelae. Fixation of the hernial contents (*incarceration*) is a serious development. Incarceration may result from stenosis or tension of the hernial ring, adhesion between the contents and the sac, or distension of the herniated viscus. This distension may be due mainly to mural congestion and edema in any incarcerated viscus, accumulated gas or ingesta in the intestine, urine in a herniated bladder, and fetuses or pus in a herniated uterus. Incarcerated intestine may become obstructed, or undergo ischemic necrosis and perforate, causing peritonitis. Small intestine fixed by incarceration may be predisposed to volvulus.

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Intestinal ischemia and infarction

Inadequate or interrupted circulation of blood to the gut is a common problem, particularly in the horse. Obstruction of the efferent veins, blockage of afferent arteries, and reduced flow through an open circulation cause hypoxic damage to the intestine. Whatever the initiating cause, the effect of hypoxia at the level of the mucosa is similar.

In the **small intestine**, within 5–10 minutes of the onset of ischemia, changes are observed at the tips of villi in tissue sections examined under the light microscope, and lesions are well advanced by 30 minutes. Separation of the epithelium from the basement membrane, beginning at the tip of the villus, and progressing with time toward the base, causes the formation of the so-called Gruenhagen's space. Epithelial cells appear relatively normal, but may separate from the villus in sheets. The core of the villus contracts toward the base. Within 1–3 hours, the villus is completely denuded of epithelium, and the mesenchymal core is disintegrating or collapsed and stumpy, with hemorrhage from capillaries.

That this lesion is largely a function of hypoxia is indicated by the mitigating effects of intraluminal oxygen perfusion. The putative countercurrent exchange of oxygen between the afferent arteriole and efferent venules in the villus, and an associated progressive decline in oxygen tension distally in the villus, may render the tip prone to early damage in hypoxia. Villus smooth-muscle contraction, exacerbating the epithelial exfoliation on the distal villus, may be mediated by sympathetic stimulation in ischemia.

Dissociation and necrosis of cells in the crypts of Lieberkühn begin about 2–4 hours after the initiation of ischemia, and within 4–5 hours the epithelium appears completely necrotic or has sloughed, leaving a mesenchymal ghost of the mucosa. The muscularis mucosae may undergo necrosis, but the muscularis externa remains viable for 6–7 hours. During acute ischemia, there is initial hyperexcitability of muscle in affected areas, followed by progressive loss of contractility, which will fail to recover if ischemia persists beyond 4–6 hours. Mesothelial cells on the serosa undergo necrosis within 30–60 minutes of ischemia, and exfoliate; there is a local acute inflammatory response, which may predispose to the development of adhesions. *The speed with which lesions develop in hypoxia emphasizes the significance of rapid fixation of intestinal mucosa if artifact is to be avoided.*

The **colon** of the dog and horse seems less sensitive to short-term ischemia than the small intestine. Mild morphologic damage, characterized by mild edema, and separation and exfoliation of surface epithelium between crypts, is found after 1 hour. However, by 3–4 hours, crypt epithelium is becoming necrotic, dissociating, and exfoliating, and from that point events progress as in the small intestine.

Reperfusion injury, superimposed on the effects of ischemia alone, may enhance the severity of the lesion, if hypoxia is only partial, or ischemia is transient, and reflow of blood raises the tissue oxygen tension. Reperfusion injury implies that the effects of the hypoxic episode have not progressed to complete necrosis of the mucosa, and that further damage is possible. Hence, there is a relatively short duration of hypoxia, and a concomitant mild-to-intermediate degree of mucosal damage, permissive of this phenomenon. In cases of enteric ischemic disease encountered in veterinary medicine, this threshold may frequently have been crossed by the time that the animal is presented for therapy. In addition, there is evidence that in swine, and in the horse, where intestinal ischemic events are most common, reperfusion injury is less likely to occur than in other species.

Reperfusion injury is the result of an interplay among free-radical-mediated damage to the microvasculature and probably stromal elements and epithelium; neutrophil margination and diapedesis into tissue; complement activation; and release of proinflammatory proteins and cytokines, some of which may have systemic effects.

Free radicals in reperfusion injury are largely generated through xanthine oxidase mechanisms in the intestinal mucosa, and through NADPH oxidase mechanisms in leukocytes, mainly neutrophils, already resident in or recruited to the damaged tissue. Hypoxia stimulates the conversion of xanthine dehydrogenase to xanthine oxidase in epithelial cells. In the presence of xanthine oxidase, hypoxanthine accumulating from adenosine triphosphate degradation in hypoxia is converted to xanthine, and molecular oxygen is reduced, generating hydrogen peroxide, superoxide radical and hydroxyl radical intermediates, which inhibit the accumulation of protective nitric oxide. The hydroxyl radical is likely most significant in damaging proteins and initiating a cascade of lipid peroxidation, resulting in metabolic and structural lesions and culminating in cell death.

The acute inflammatory response that accompanies reperfusion results in production of free radicals by the respiratory burst from neutrophils, release of proteolytic enzymes, and physical impairment of the microcirculation. Reduction in tissue nitric oxide permits dismutation of oxygen free radicals to H_2O_2, which promotes activation of phospholipase, leading to the accumulation of proinflammatory mediators, and production of cytokines (tumor necrosis factor-α and -β, interleukins) and adhesion molecules by endothelium. Complement activation is mediated by adhesion molecules, promoting inflammatory events and release of cytokines such as tumor necrosis factor-α and interleukin-1, as well as other cytokines, which increase during reperfusion.

Sequelae of ischemia vary with duration, and hence severity, of insult. Short-term ischemia (maximally, ~3–4 hours), with preservation of at least the base of the crypts of Lieberkühn, will permit repair, as *cells proliferating in the crypts re-epithelialize the mucosal surface within 1–3 days*. Normal architecture is re-established after up to 1–2 weeks, though necrotic muscularis mucosae is not replaced. Effusion of tissue fluid and acute inflammatory cells prevails until epithelium extends to cover the eroded surface fully. Partial damage to the proliferative compartment initially results in dilation of crypts, lined by flattened epithelium resembling that seen after radiation injury (Fig. 1.52). If the amplifier population of progenitor cells can be regenerated, hyperplastic basophilic cells will populate crypts until the mucosal architecture is reconstituted.

Ischemic necrosis of the full thickness of the mucosa will be bounded by an acute inflammatory reaction in the submucosa, which,

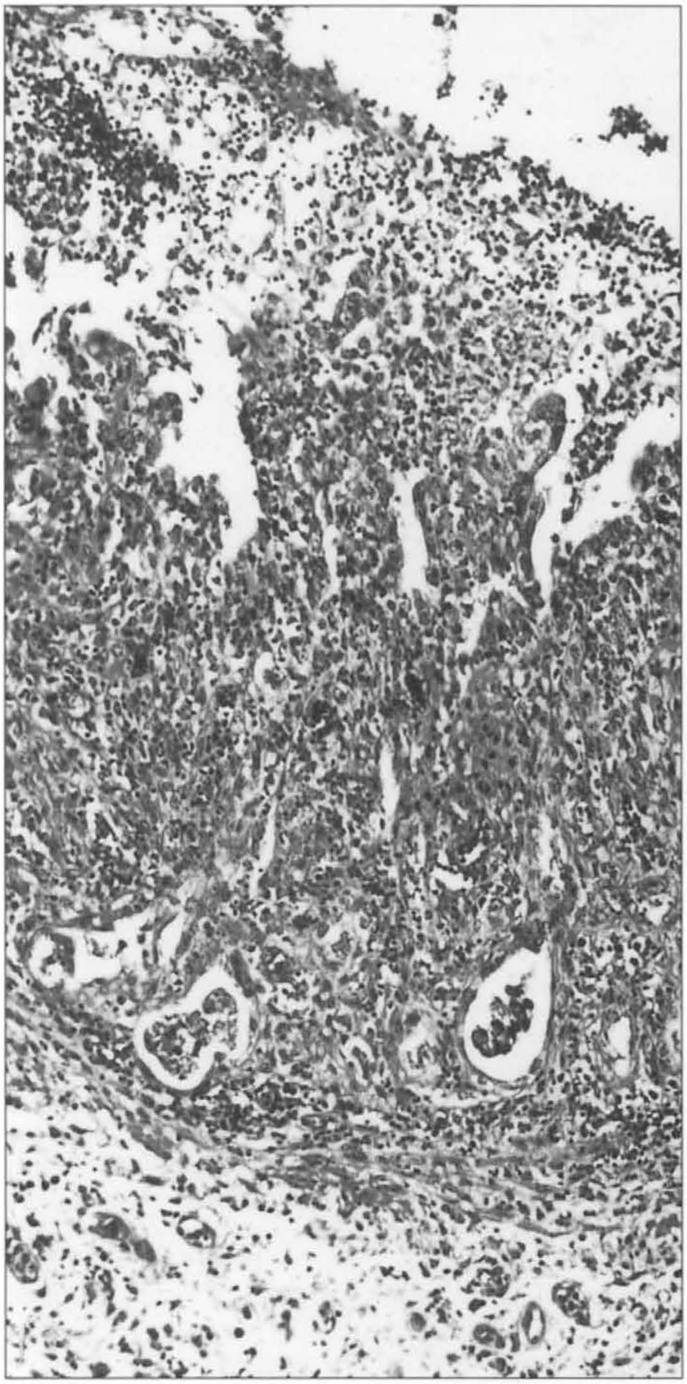

Figure 1.52 Small intestine, **sequel to transient ischemia** 2 days previously. Ulcerated mucosa and dilated crypts are lined by attenuated epithelium and contain necrotic debris. Inflammatory cells and fibrin exude from the mucosa, which is devoid of villi.

under favorable conditions, evolves into a granulating ulcerated surface. Focal ulcerative lesions may ultimately heal by epithelial migration, over the bed of granulation tissue, from surviving crypts within the lesion and around the periphery.

Extensive *mucosal ulcers* that form following severe ischemia have little chance of resolution, due to their large surface area. Chronic ischemic ulcers in the small bowel tend to develop a depressed, fairly clean granulating surface, occasionally with some fibrinous exudate. Ischemic ulcers in the large bowel, especially of horses, develop a dirty yellow-gray fibrinonecrotic surface, perhaps due to the effects of anaerobic bacteria. If the animal does not succumb to the effects of malabsorption and protein loss from the defect, or to transmural bacterial invasion, scarring and stricture may occur. The sequelae of ischemia with reflow are mainly seen in strangulated segments of gut that have been reduced without, or with inadequate, resection, and in some cases of presumed thromboembolic infarction of the equine colon.

Persistent ischemia results in necrosis involving all mural elements. The full thickness of the gut wall ultimately becomes gangrenous, green-brown or black, flaccid and friable.

The *consequences of ischemic lesions* are partly a function of the species, and of the level of bowel affected. Strangulation, volvulus, and similar lesions cause physical obstruction at the site, and ileus proximal to it. Reduced arterial perfusion or thromboembolism causes functional obstruction and ileus. Loss of mucosal integrity results in cessation of electrolyte and water absorption, and ultimately in effusion of tissue fluid and blood into the lumen. Proliferation of anaerobes occurs in the lumen of the stagnant ischemic area, with accumulation of gas, and extreme distension of the closed loop in strangulation obstruction. Toxin production by anaerobes, particularly clostridia, plays a large part in gangrene and ultimate rupture of ischemic gut, as well as having systemic effects. Absorption of endotoxin from the lumen may occur through devitalized mucosa via the portal flow, lymphatic return, or peritoneum. Toxins have a severe detrimental effect on cardiovascular function, contributing to circulatory failure. If death from some other cause does not supervene, transmural invasion by enteric bacteria or perforation of the devitalized wall results in septic peritonitis that is ultimately fatal.

Venous infarction

Obstruction of efferent veins is by far the most common cause of intestinal ischemia. This is a sequel to *incarceration* of herniated loops of bowel; *strangulation* by pedunculated masses, such as lipomas in older horses; *torsion* (twist about the long axis of the viscus); *volvulus* (twist across the long axis of the gut); and *intussusception.* In these circumstances compression of thin-walled veins tends to occur before the influx of arterial blood is obstructed.

In venous infarction, the affected tissue field, often including involved mesentery, becomes intensely edematous, congested, and hemorrhagic, so that the hypoxic bowel wall is thickened and eventually assumes a deep red-black appearance (Fig. 1.53). It has been estimated that 40 liters of fluid may accumulate in the wall of a horse colon that has undergone volvulus. Bloody fluid content and gas distend the lumen of the infarcted segment. As gangrene of the intestinal wall proceeds, the tissue becomes green-black, and septic peritonitis eventually ensues, with or without perforation of the bowel. Advanced venous infarction involves the full thickness of the intestinal wall, and the initiating intestinal accident is commonly evident, except in cases subjected to surgery. Even if a displacement, volvulus, or strangulation has been reduced, the limits of the infarcted segment are usually relatively sharply demarcated, and the affected bowel remains edematous and hemorrhagic. Microscopically, severe transmural edema, congestion, distension of veins, sometimes venous thrombosis, and hemorrhage are present, initially most severe in the mucosa and submucosa. With time, the full thickness

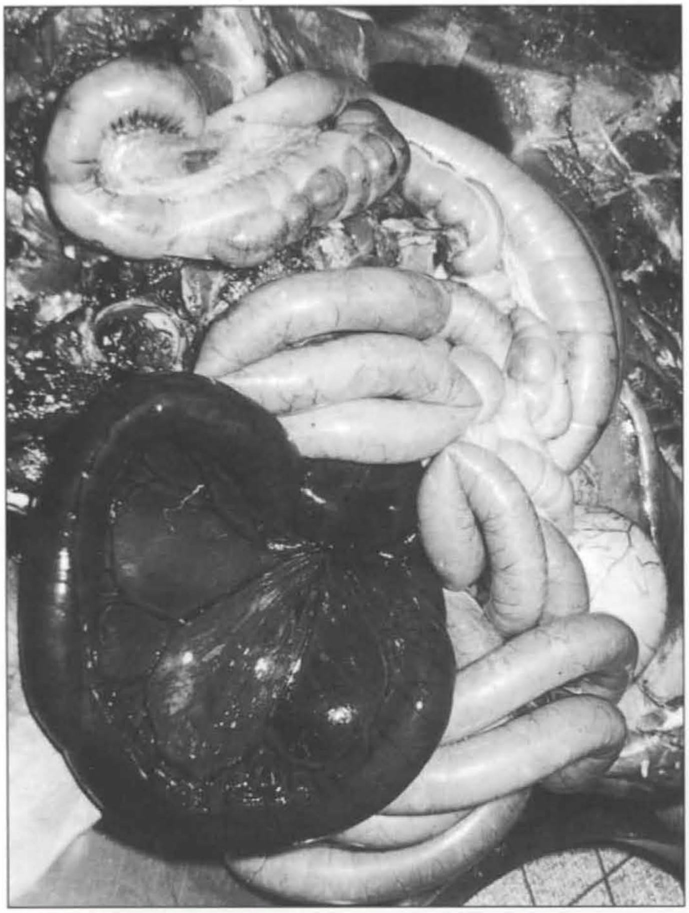

Figure 1.53 Venous infarction of a segment of equine ileum that has undergone volvulus.

of the mucosa becomes necrotic, and the deeper layers of the muscular wall are also devitalized, with invading enteric flora present throughout. Lesions which have advanced to significant necrosis or effacement of the crypt epithelium are associated with failure of the animal to survive, due to either euthanasia on the grounds of the degree of damage, or systemic complications despite correction of the strangulation, or resection.

Displacements of intestine that may progress to incarceration or volvulus with strangulation and infarction have been discussed in the previous section. They are a common cause of colic and mortality in horses.

Torsion of the long axis of the mesentery occurs commonly in suckling ruminants and in swine, uncommonly in horses, and rarely in cats and dogs. The abdomen is distended and, upon opening the cavity, tensely dilated deep red-to-black loops of bowel are usually immediately apparent.

In *swine*, the mesentery of the small intestine and sometimes the large bowel is often involved in a torsion that is usually counterclockwise, when viewed from the ventrocaudal aspect. In torsion involving the small and large intestines, the apex of the cecum may be pointing cranial in the cranial left quadrant of the abdomen, reflecting the rotation of about 180°. In swine, mesenteric torsion may be due to gas production from a highly fermentable substrate in the colon, and its subsequent displacement, with progression to mesenteric torsion. Mesenteric torsion is a common cause of sporadic sudden death in swine but may occur as a recurrent problem in a herd. Many cases of so-called "intestinal hemorrhage syndrome" in that species are probably misdiagnosed mesenteric torsion.

Death due to mesenteric torsion is common in suckling or artificially reared *calves and lambs*. In these species, vigorous ingestion of large amounts of feed over a short period may predispose to gas formation in the gut, or perhaps hypermotility, which induces torsion. Usually only the mucosa of the proximal duodenum and terminal ileum, cecum, and colon is spared from infarction, though occasionally volvulus is restricted to shorter segments of intestine. Similar lesions are occasionally encountered in other species.

In *dogs*, mesenteric torsion has been associated with ingestion of large quantities of food, and with exocrine pancreatic insufficiency in German Shepherds. It is occasionally accompanied by gastric volvulus.

Volvulus of various lengths of the small intestine may occur in any species, but is perhaps most prevalent in the horse, where it is a common cause of strangulation obstruction of the bowel.

Volvulus of the large colon of the horse is predisposed by its lack of mesenteric anchorage, and potential mobility. It may be due to dorsomedial (ventral colon rotating dorsally and medially relative to the dorsal colon) or dorsolateral rotation of the colonic loop along its long axis. Rotation of the left colon commonly progresses to volvulus at the sternal and diaphragmatic flexures. The right colon may rotate in its middle, cranial to the level of the cecocolic fold. Part of the cecum may be incorporated in the volvulus, the right colon and cecum rotating in the region of attachment of the cecocolic fold. Or almost the entire large colon and cecum may be involved in a rotation at the cecal base and transverse colon. The equine cecum alone rarely undergoes torsion, and if so, it may be related to hypoplasia of the cecocolic fold.

The most common direction of rotation of the right colon is *dorsomedial*. The direction and sites of rotation suggest that lesions may begin with tympany and dorsomedial torsion in the left colon, with progression of the twist, from left to right colon. The sternal and diaphragmatic flexures presumably provide some resistance to rotation, causing some twists to be found there. The first site of fixation of the large colon is the cecocolic fold, which may explain a tendency for twists to be localized there; progression beyond this level bases the twist at the mesenteric root of the large bowel. Clinical signs are associated with rotations of over 180°, and torsions involving the cecal base are usually at least 360°.

At surgery or necropsy, the usual signs of strangulation obstruction are evident, including dilation and devitalization of the infarcted segment, and distension of the cecum if it is not twisted. Postmortem rupture of the diaphragm or abdominal wall may occur, due to tympany.

Intussusception involves the telescoping of one segment of bowel into an outer sheath formed by another, usually distal, segment of gut (Fig. 1.54). Any level of the gut with sufficient mesenteric mobility may be involved. The *cause* is usually not apparent, though linear foreign bodies, heavy parasitism, previous intestinal surgery, enteritis, and intramural lesions such as abscesses and tumors may be associated. It may also be a *terminal, agonal, or postmortem event*. The history is that of partial or complete intestinal obstruction, perhaps with bloody feces, and it is most common in young animals.

Intussusception is common in *dogs*, where most frequently it is ileocolic. It is much less common in cats. Intussusception is also

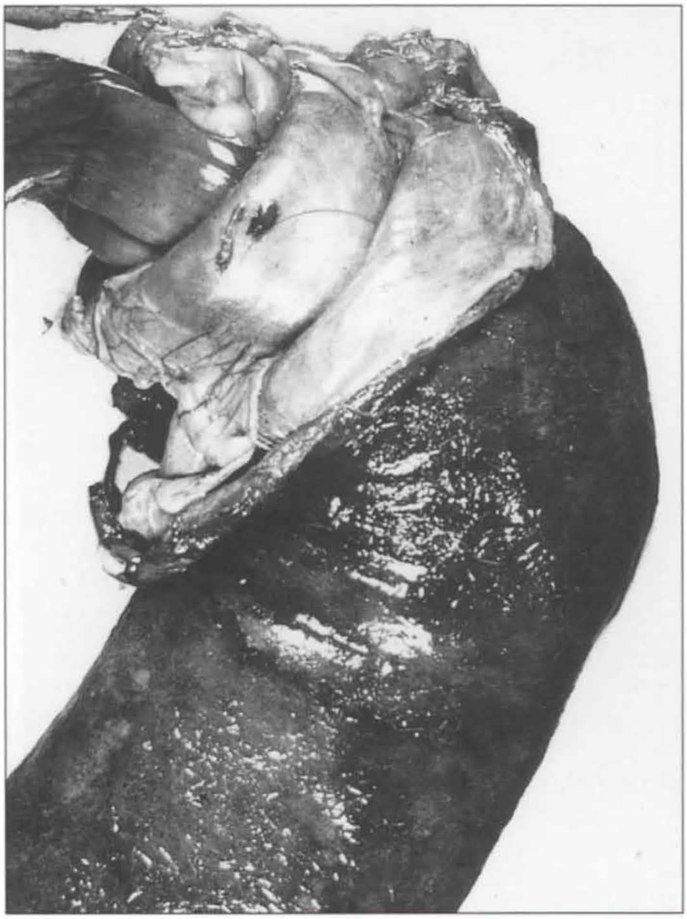

Figure 1.54 Intussusception of the intestine in a dog. Outer intestinal layer has been cut away to expose the edematous and congested infarcted mucosa of the strangulated portion of the intussusception.

moderately common in lambs, calves, and young horses, where it may involve small intestine, cecum, and colon.

The progressive invagination of the leading edge of the intussusceptum into the distal segment results in the *wall of the intussusception being comprised of three layers*: (1) the inner entering, and (2) middle returning, segment of invaginated bowel, and (3) the outer wall of the receiving segment of gut. It is limited in length by the increasing tension on the mesentery drawn into the lesion, to about 10–12 cm in small animals, and about 20–30 cm in large animals. This tension along one edge of the gut may cause the mass to become bowed or spiraled.

Tension and compression of mesenteric veins cause the intussusceptum, or a portion of it, to undergo *venous infarction*. It swells, with edema and congestion, and the adjacent apposed serous surfaces become adherent as fibrin and inflammatory cells effuse from the affected bowel. Adhesion quickly renders the intussusception irreducible. Necrosis and gangrene of the invaginated intestine usually develop, but sometimes the intussusceptum will slough, and the remaining viable segments will maintain continuity of the gut, or rarely, will form two adjacent blind ends. Intestine above obstructing intussusceptions may be dilated, and that below contracted and devoid of content. If obstruction is chronic or partial, there may be hypertrophy of the smooth muscle of proximal bowel. In horses, chronic ileocecal intussusception involves a relatively short (<10 cm) length of bowel. Incidental terminal, agonal, or postmortem intussusception is recognized by the relative absence of congestion, edema, and adhesion of the involuted segment of gut.

Cecal inversion, and cecocolic intussusception in the horse, with inversion of the cecum into itself, or into the right ventral colon, may result in ischemia of the cecum and possibly part of the involved colon; if partial, ischemia usually involves the more distal cecum.

Segmental ischemic necrosis of the small colon may occur in pregnant or postpartum mares, due to mesenteric tension from intussusception and rectal prolapse of the distal large bowel, or perhaps due to laceration of the mesocolon and associated vessels by the feet of the foal during parturition. Colic, and intestinal obstruction, necrosis, rupture, and peritonitis may follow.

Arterial thromboembolism

Ischemia of the gut due to arterial thrombosis and embolism is rare in domestic animals other than the horse. Mucosal and occasionally transmural focal or segmental infarctive lesions are seen in *Pasteurella* septicemia in lambs and in *Histophilus somni* bacteremia in cattle. Most cases of embolic disease are associated with bacterial infections that cause softening and lysis of thrombi and facilitate formation of emboli. This is particularly true for the lesions associated with strongyle migrations in horses, where lesions remain localized unless thrombi induced by the parasite become secondarily infected.

In **horses** it is associated with **endoarteritis**, mainly at the root of the cranial mesenteric circulation, caused by migrating larvae of *Strongylus vulgaris* (see Vol. 3, Cardiovascular system). Effective worm control programs have rendered this problem increasingly rare.

Candidates for a diagnosis of nonstrangulating infarction are animals in which the anatomic distribution of an ischemic lesion is incompatible with volvulus or other strangulation, or where physical evidence for incarceration or strangulation obstruction is not present in the surgical history or at autopsy, and where there is evidence of verminous arteritis. Typically, ischemic lesions of this type are limited to the "watersheds" at the periphery of the colic and cecal arterial circulatory fields – the pelvic flexure and the distal cecum – since collateral circulation within these circulatory fields is extensive.

Lesions limited essentially to the mucosa usually appear to be subacute, the result of ischemia of relatively short duration, and are ulcerative or fibrinonecrotic, usually with a hyperemic margin. They may be tens to many hundreds of square centimeters in area.

Transmural lesions represent ischemia of longer duration. Devitalized gray-brown intestine of normal thickness is interpreted to represent arterial obstruction without significant reflow, except along the boundary with viable tissue. Large edematous, congested, or hemorrhagic, full-thickness lesions, physically or anatomically inconsistent with strangulation, are interpreted as severe arterial obstruction, with subsequent reflow either by relief of the obstruction or by way of collaterals. Ischemic damage to vessels of the mucosa, submucosa, and perhaps deeper structures results in hemorrhage and edema when blood flow returns (Fig. 1.55). Ulcerative or fibrinonecrotic mucosal lesions are probably the result of transient ischemia, and superficial or mucosal damage, with subsequent reflow. Similar lesions may occur following relief of strangulation of short duration, and in nonsteroidal

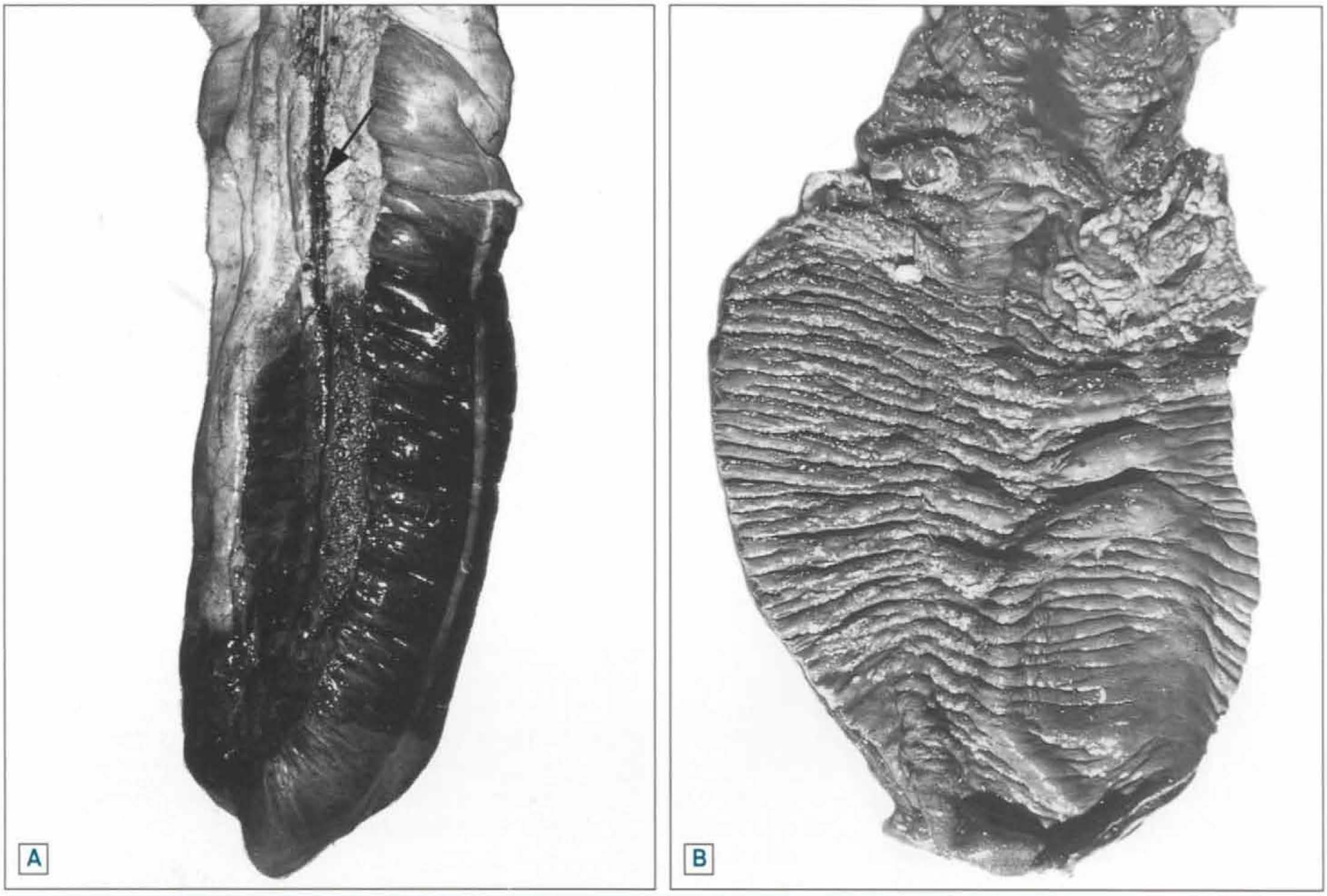

Figure 1.55 **A. Infarction of pelvic flexure** of the large colon of a horse. The colic artery contains a thrombus (arrow). Hemorrhage and edema of the serosa suggest that arterial thrombosis and infarction have been followed by reperfusion, with extravasation of blood from damaged vessels in the affected tissue. **B.** Mucosa of equine **cecum** that has undergone **ischemic necrosis** due to reduced arterial perfusion. Subsequent reflow has occurred and the infarcted mucosa is covered with fibrinonecrotic exudate. Irregular ulcers and foci of exudation are present on the mucosa along the proximal margin of the lesion (toward top).

anti-inflammatory drug toxicosis or salmonellosis, both of which, in part, involve mucosal microthrombosis.

Reduced perfusion

Ischemia due to reduced perfusion of the intestinal vascular bed is a *difficult and uncommon diagnosis*. Circumstances where it may be expected to occur include severe hypovolemic states, such as *hemorrhagic shock* in the dog, cat, and possibly other species; in animals, particularly dogs, with *disseminated intravascular coagulation* (DIC); in dogs with hepatic disease and *portal hypertension*; in *hypotensive shock* due to heart failure; and in animals with reduced mesenteric arterial perfusion, mainly horses with severe *verminous endoarteritis*.

In **"shock gut"** in dogs, and rarely, other species, associated terminally with heart failure, hemorrhage, hypovolemia, and DIC, part or all of the mucosa of the small intestine is deeply congested and the content is hemorrhagic. The pathogenesis of the lesion is related to reflex vasoconstriction in the mucosa and submucosa, shunting of blood away from the mucosa, dilation of mucosal capillaries, and reduction in rate of flow of blood through the villus. Countercurrent transfer of oxygen from the afferent to efferent vessels in the villus aggravates hypoxemia in the villus by increased shunting of oxygen to the efferent venule. Splanchnic pooling of blood, systemic arterial hypotension, and intestinal vasoconstriction occur in endotoxic shock in dogs, causing similar mucosal lesions. Microthrombosis associated with sluggish flow, DIC, and endotoxemia may contribute to mucosal ischemia by obstructing capillaries in the villi, and mucosal and submucosal venules. Microthrombi in these vessels in association with hemorrhagic mucosal necrosis suggest the possibility of ischemia due to "slow flow."

Transient or incomplete reduction in perfusion due to obstruction of the arterial blood supply has a similar effect on the mucosa. The obstruction may be due to *arteriospasm*, perhaps induced by vasoactive mediators such as thromboxane. Mucosa devitalized by hypoxia will become hemorrhagic with continued blood flow. Since the primary problem may not involve a systemic state as complicated as severe shock, the animal may survive long enough to develop an effusive ulcerated or pseudomembranous mucosa, with some prospect of stabilization or repair, if the lesion is not widespread. "Slow flow" due to reduced arterial perfusion with inadequate collateral flow may be expected to affect the "watershed" of a circulatory field preferentially. In the horse this may be the explanation for mucosal lesions at the pelvic flexure and apex of the cecum in which thromboembolism cannot be implicated, but in which mural thrombi in the cranial mesenteric root could have caused significantly reduced perfusion or flushed vasoconstrictive thromboxane into circulation.

Transient or noninfarctive "slow flow" has been proposed as a cause of intermittent colic due to verminous arteritis. It may also play a role in the development of functional obstruction and volvulus in horses with cranial mesenteric arterial lesions.

Ischemia at the periphery of the circulatory field of the caudal mesenteric artery may possibly predispose to rectal perforation in horses. The precarious perfusion of the mucosa at this site may contribute to ischemic ulceration and the development of *rectal stricture in swine*. In many cases this condition appears to be associated with *Salmonella* infection, and it is discussed further with porcine salmonellosis (p. 198).

Acute acorn poisoning in the horse may cause severe gastrointestinal edema and focal hemorrhage, with infarction and ulceration in the cecum and colon. Microscopic lesions in the small and large intestine are consistent with an ischemic pathogenesis, and microthrombi have been associated with mucosal infarcts in the large bowel, as well as in other organs.

Nonsteroidal anti-inflammatory drugs cause ulceration of the upper small intestine and colon, as well as oral and gastric ulceration, which seem to be related to ischemia, in horses and dogs. In horses, phenylbutazone, even at therapeutic dosages, can result in ischemic damage to the intestinal mucosa. It may be that intercurrent stress or dehydration contributes to the pathogenesis. The right dorsal colon is affected preferentially, resulting in the term "*right dorsal colitis.*" Lesions here are characterized by ischemia, often with marked edema and avascular necrosis. Lesions may be focal, linear, or extensive and segmental, involving the entire circumference of the bowel (Fig. 1.56). Depending on the duration and severity of the lesion, the mucosa may be congested and edematous, with superficial necrosis and fibrin exudation, or extensively eroded and ulcerated, with fibrinonecrotic exudate. Early in the process, superficial epithelial necrosis and progressive mucosal necrosis and inflammation are evident. *Microvascular injury*, with subsequent microthrombosis and ischemic ulceration, is considered by some to be the cause of the lesions in the stomach and intestine. This may be the result of direct phenylbutazone toxicity to the microvasculature. Vasoconstriction or depression of other cytoprotective effects, mediated by phenylbutazone inhibition of prostaglandin synthesis, could be the cause of the lesions. Animals may develop diarrhea and hypoproteinemia as a result of the extensive mucosal defects. Minor mucosal lesions may resolve; a sequel to severe colonic damage in horses is colonic stricture.

Lesions in the oral cavity associated with nonsteroidal anti-inflammatory drug administration are deep crateriform ulcers, with a clean granulating base. Concurrent with punched-out ulcers in the glandular mucosa, there may be chronic gastritis and atrophy of the mucosa with loss of differentiation of the cells in the fundus. In the upper small intestine, ulcers may be focal, linear, or segmental and annular. Microscopic lesions that may precede ulceration of the small intestine include mild to severe atrophy of villi, epithelial necrosis, mucosal inflammation, and fibrin exudation. Renal papillary necrosis is often concurrent, if animals are dehydrated.

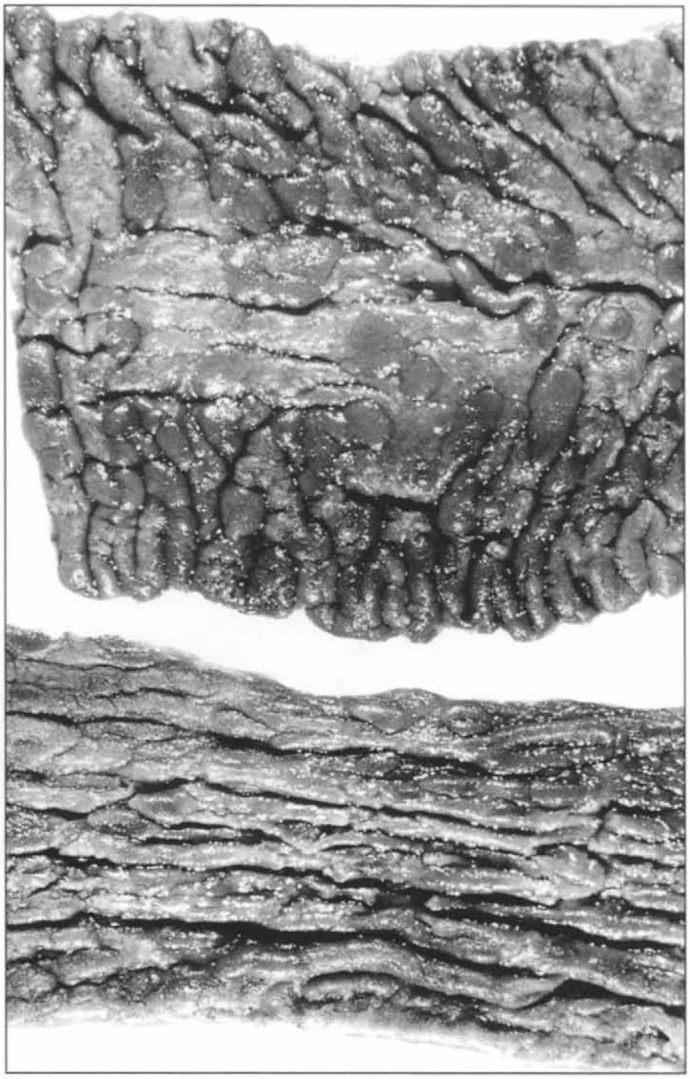

Figure 1.56 Fibrinonecrotic enterocolitis in a foal treated heavily with nonsteroidal anti-inflammatory drugs.

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Miscellaneous conditions of the intestinal tract

Intestinal lipofuscinosis ("brown gut") in dogs is characterized grossly by *tan-brown discoloration of the intestinal muscularis* (Fig. 1.57). It may involve all areas of the gut, but is most common in the lower small intestine. The bladder and mesenteric and peripheral lymph nodes may also be affected grossly. Although the lesion may be incidental, it is usually associated with *chronic enteric and/or pancreatic disease*. Lipofuscinosis has been reported in Boxer dogs with histiocytic ulcerative colitis, but a definite correlation between the two conditions has not been established. A high prevalence of lipofuscinosis has also been reported in dogs that were fed rations high in polyunsaturated fats with a relative *deficiency of vitamin E*, and it is prevented by vitamin E supplementation. Any condition, such as exocrine pancreatic insufficiency, causing a reduction in the absorption of fats, and consequently of the fat-soluble vitamins, especially in the presence of polyunsaturated fatty acids in the diet, may predispose to lipofuscinosis.

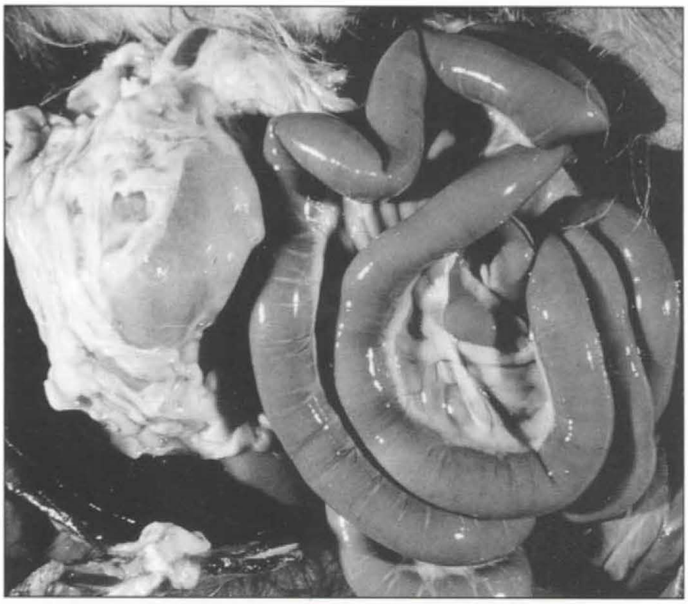

Figure 1.57 Intestinal lipofuscinosis (brown gut), discoloring the small intestine to a tan hue in a dog.

The microscopic lesions of brown gut are gray-to-brown granules in the perinuclear regions of smooth-muscle cells in both inner and outer muscle layers (Fig. 1.58). The granules stain as lipofuscin (periodic acid–Schiff-positive, sudanophilic, weakly acid-fast with Ziehl–Neelsen), and fluoresce dim yellow in paraffin section. The granules, termed *leiomyometaplasts*, are oxidized polymerized phospholipids which are highly resistant to further endophagocytic degradation. They are derived from excess cell membrane lipid peroxidation in vitamin E deficiency. Their propensity to accumulate, especially in gastrointestinal smooth muscle, is unexplained.

A distinct syndrome occurs in *Cocker Spaniel dogs* affected with generalized ceroid-lipofuscinosis, an inherited storage disease. In affected dogs, discoloration of intestinal smooth muscle is evident but, in addition, they have progressive hindlimb paresis and incoordination.

Muscular hypertrophy of the intestine was formerly a common finding in **swine**, but it appears to have diminished in prevalence in most areas. It may be found in apparently healthy animals at slaughter as a uniform thickening of the muscular coats of the *terminal ileum*. The segment involved always includes the most caudal portion, but it may extend a variable distance forward, usually 25–50 cm. The affected area is thickened and has the turgid feel of a rubber hose. The lumen is small and the mucosa is thrown into thick folds, but it is the muscle that thickens the wall. This condition must be differentiated from adenomatosis and necrotic ileitis, manifestations of enteropathy associated with *Lawsonia* in swine.

Rupture may occur, in association with impaction of dehydrated feed in the hypertrophic segment. The actual rupture may be a

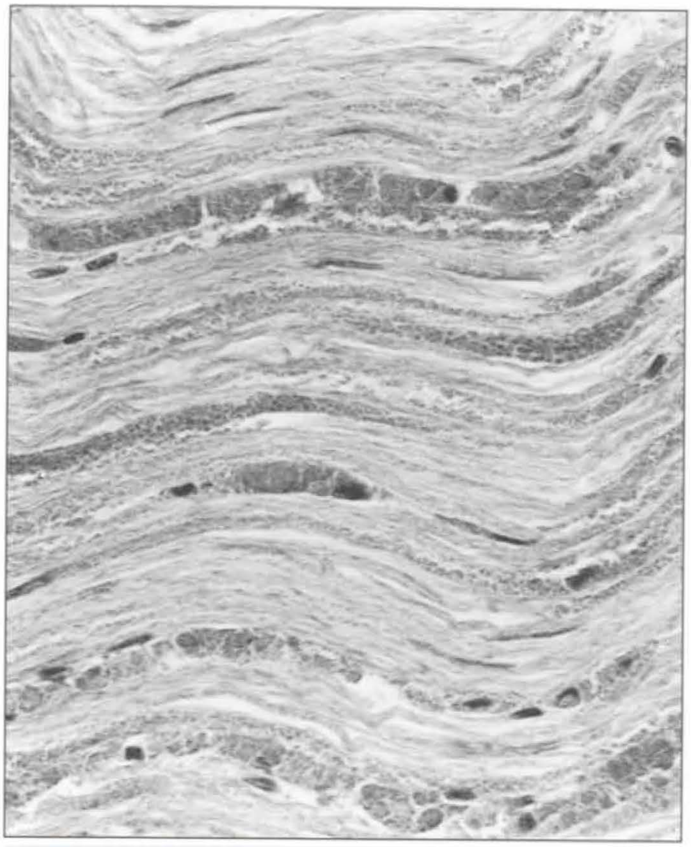

Figure 1.58 Granular accumulations of ceroid-lipofuscin pigment (leiomyometaplasts) in smooth-muscle cells in the small intestine of a dog with **intestinal lipofuscinosis**.

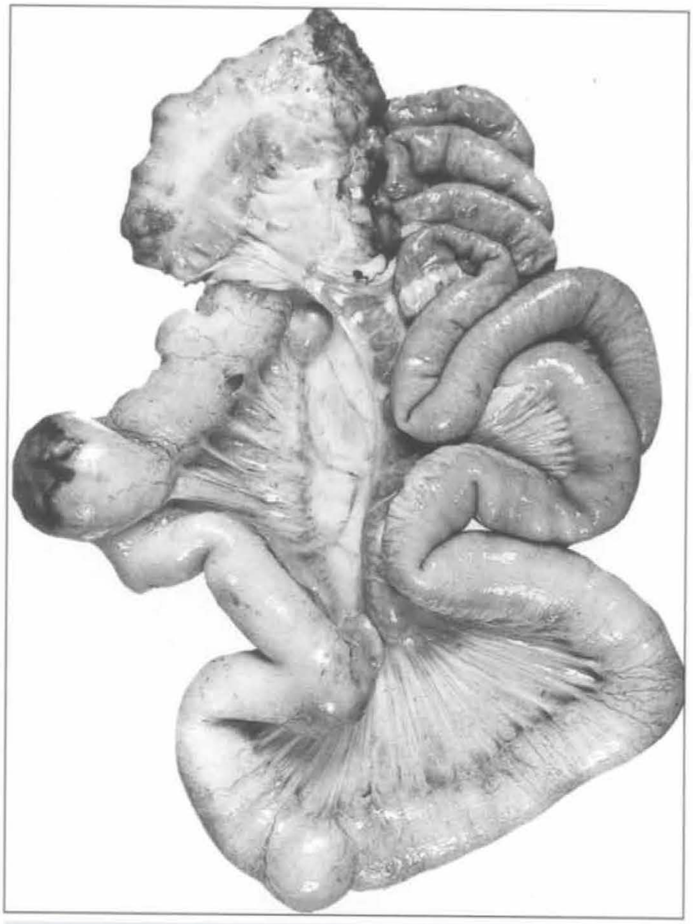

Figure 1.59 Multiple saccular dilations with impending perforation in **muscular hypertrophy of ileum** in a pig. (Courtesy of S Nielsen and J Am Vet Med Assoc.)

result of violent peristalsis, or diverticula may develop (Fig. 1.59), the mucosa undergoing necrosis with secondary bacterial inflammation. Perforation occurs at these weakened areas. While the underlying basis of this condition is obscure, it is likely that the muscular hypertrophy is secondary to a functional obstruction of the ileocecal orifice.

Muscular hypertrophy of the small intestine, of uncertain cause, but sometimes associated with *Anoplocephala* sp. tapeworms at the ileocecal orifice, also occurs in **horses** (Fig. 1.60). The association with tapeworm infection is uncertain because a considerable proportion of cases also show muscular hypertrophy of the *terminal esophagus*. The lesions are similar to those described in swine, except that the affected segment may occur at any point along the small intestine, and occasionally in the large intestine, although the *ileum is the most common site*. Horses with this condition may have chronic mild colic and anorexia or intermittent diarrhea with progressive loss of weight. Diverticula, and perforation or laceration of the thickened intestinal wall, may also occur.

Intestinal smooth-muscle hyperplasia has been described in a goat. Unlike the condition in horses and swine, the increased muscular thickness was jejunal rather than ileal.

Diverticulosis of the small intestine is a rare lesion that is sometimes associated with muscular hypertrophy in pigs and horses. It is characterized by the presence of saccular dilations, which are lined by intestinal mucosa, in the muscularis and subserosa of the small intestine. The diverticula tend to follow the pathway of blood vessels and are mainly located adjacent to the mesenteric attachment. Rupture of the diverticula causes peritonitis.

Intestinal emphysema is a rare condition found mainly in weaned *pigs*, in which it is usually an incidental finding in slaughtered animals, with no economic significance. It is characterized by numerous thin-walled, gas-filled cystic structures, a few millimeters to several centimeters in diameter, in the gut wall and on the serosal surface (Fig. 1.61). These are mainly located in the small intestine, although the large intestine, mesentery, and mesenteric lymph nodes may be involved. Microscopically, the cystic structures appear to be *dilated lymphatics* that are located in the lamina propria, submucosa, muscularis, subserosa, mesentery, and mesenteric lymph nodes. A pleocellular inflammatory reaction may be evident in the walls of the cysts. Although production of gas by bacteria has been implicated, the cause remains obscure.

Rectal prolapse most commonly occurs in *swine, sheep, and cattle*. It may occur in any animal that has *prolonged episodes of tenesmus or straining*, usually associated with colitis or urinary infection or obstruction. In pigs, rectal prolapse occurs as a herd problem when the ration contains *zearalenone*, an estrogenic mycotoxin produced by fungi of the genus *Fusarium*. The toxin causes marked swelling and congestion of the vulva and vaginal mucosa that may be followed by vaginal prolapse. Affected pigs strain continuously and rectal prolapse is a common complication. Rectal prolapse in sheep

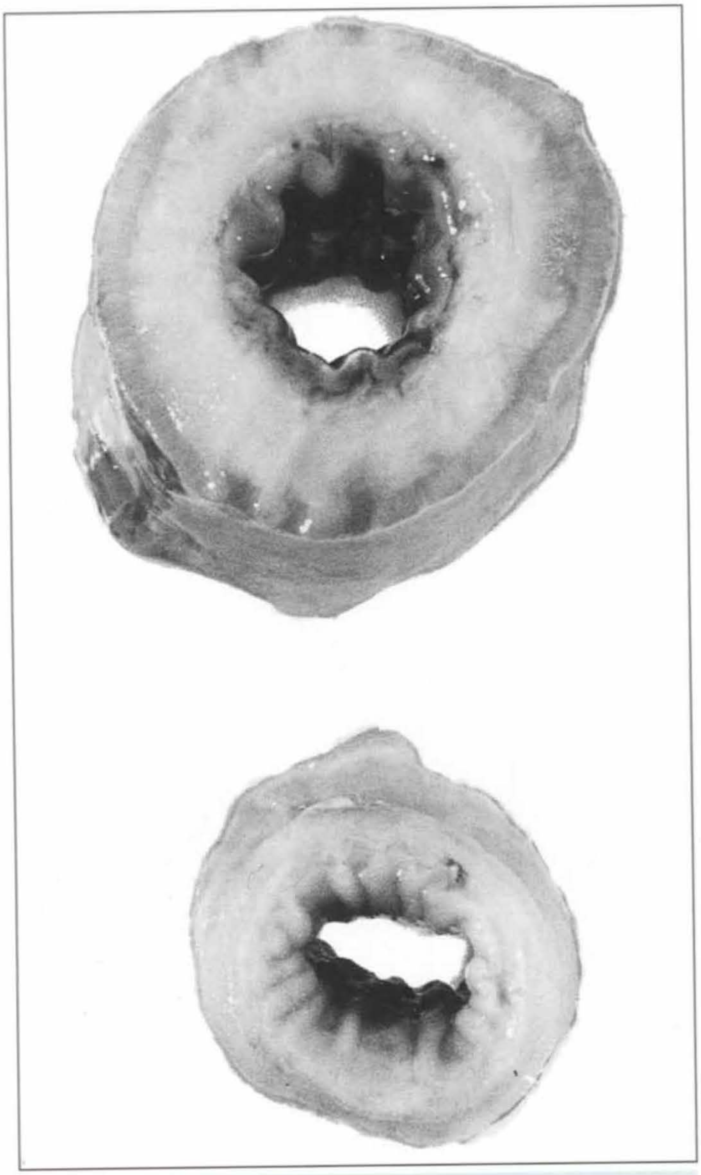

Figure 1.60 Ileal muscular hypertrophy in a horse. Normal equine ileum below.

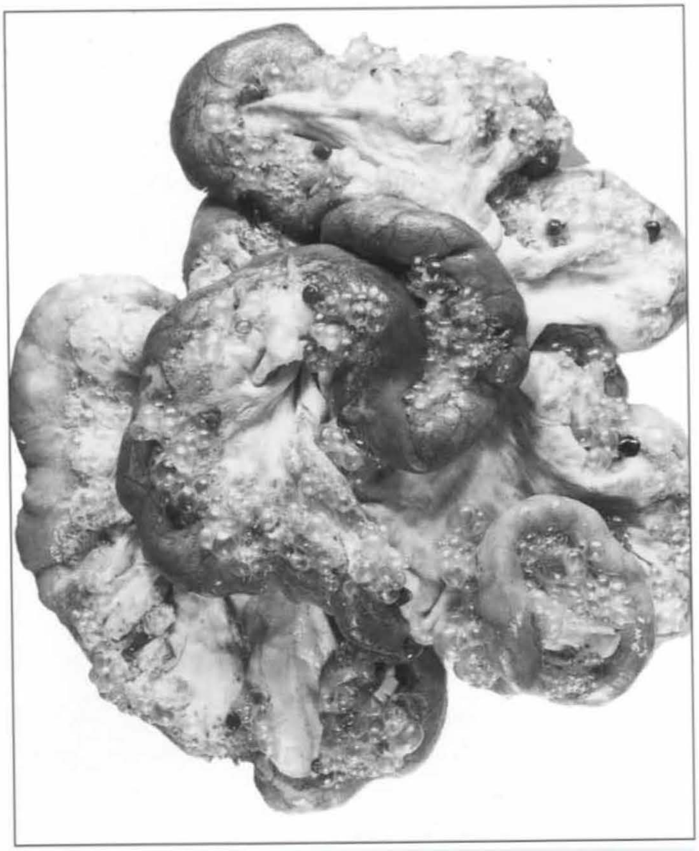

Figure 1.61 Intestinal emphysema in a pig.

may be the consequence of ingestion of estrogenic pastures, and is accompanied by other signs of hyperestrogenism (see Vol. 3, Female genital system).

The prolapsed rectum is edematous, congested, and there may be necrosis and ulceration of the everted mucosa. These lesions are ischemic in origin due to interference with venous blood flow from the prolapsed section. Only the mucosa, or all layers, may be involved in the prolapse. In swine surviving slough or amputation of the prolapsed tissue, *rectal stricture* may ensue. Rectal stricture is discussed further in the section on salmonellosis, below.

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Malassimilation and protein-losing syndromes

In dogs, and to a lesser extent in horses, cats, and other species, usually idiopathic syndromes occur, variably signaled by *chronic diarrhea, weight loss, hypoproteinemia, and malabsorption. Intestinal biopsy, where feasible, is usually necessary to make a diagnosis and establish a prognosis.* These syndromes are typically characterized by abnormal lamina proprial inflammatory infiltrates (eosinophils, lymphocytes, and plasma cells, or granulomatous inflammation), neoplasia, especially lymphosarcoma, or amyloid, perhaps associated with villus atrophy. Lymphangiectasia may also produce a similar syndrome. Infectious causes of erosion or ulceration need to be considered, but are less common. In dogs giardiosis, and sequelae to severe parvoviral infections in animals recovered from the acute phase, may cause such a syndrome. In horses, chronic salmonellosis and *Lawsonia* are potential infectious causes of malassimilation and protein loss.

The limitations on the interpretation of biopsies noted in the section on normal form and function of the intestine must be

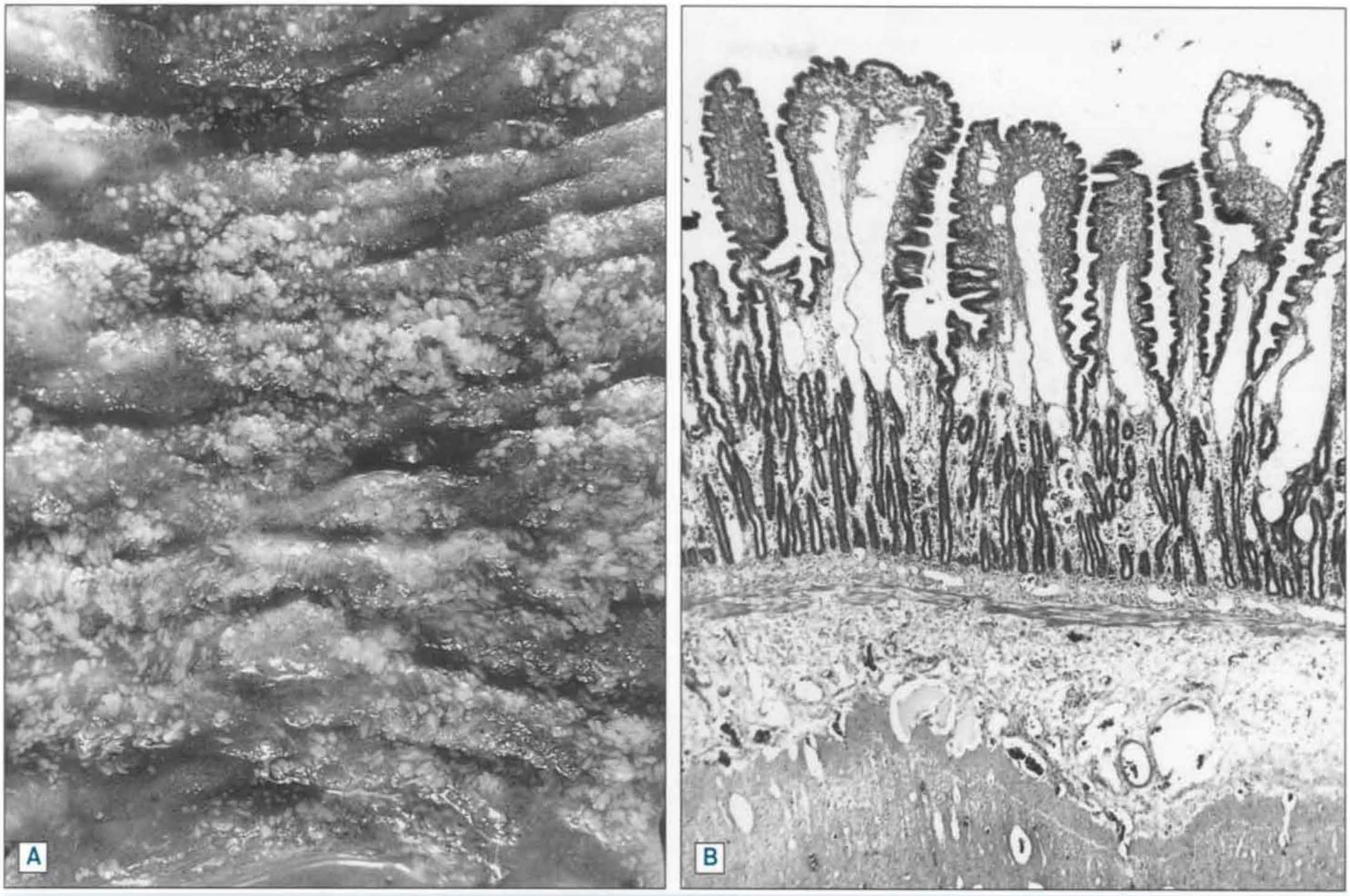

Figure 1.62 Lymphangiectasia in the small intestine of a dog. **A.** Mucosa, thickened by edema, is thrown in folds. Many villi contain white chyle-filled lacteals. **B.** Lacteals are dilated, lamina propria and submucosa are edematous, and lymphatics in submucosa and muscularis are open.

borne in mind. Neoplasia will be differentiated from inflammation, amyloidosis, and lymphangiectasia, and, rarely, characteristic lesions or an agent may be recognized, implicating an infectious process. If inflammatory bowel disease is recognized, associated or complicating problems, such as *Giardia* infection or bacterial overgrowth, should be sought. Associations with the dietary habits and history of the animal should also be investigated, since some cases may be associated with inappropriate responses to dietary antigen.

Lymphangiectasia

Lymphangiectasia has been described most commonly in the dog, where it is among the more common causes of malabsorption/protein-losing enteropathy; it has not been reported in cats. Breed predisposition seems limited to Yorkshire Terriers and the Norwegian Lundehund, in which lymphangiectasia is part of a syndrome of protein-losing enteropathy with inflammatory bowel disease and in which gastritis and gastric neoplasia may coexist. The disorder has also been reported in the horse. Lymphangiectasia is associated with a syndrome variably characterized by *chronic diarrhea, wasting, hypoproteinemia, lymphopenia, hypocalcemia, and hypocholesterolemia.* Peripheral edema, ascites, and hydrothorax result from hypoalbuminemia.

The lesion in the small intestine is *dilation of the lacteals, and often lymphatics* of the submucosa, muscularis, serosa, and mesentery (Fig. 1.62). Villi containing dilated chyle-filled lacteals may stand out grossly as white papillate foci in a thickened, transversely folded edematous mucosa. Serosal and mesenteric lymphatics may be prominent, white, and dilated. *Nodular white masses* up to 5–10 mm in size may be present on the serosa at the mesenteric border and along lymphatics; rarely, they are found on the liver, diaphragm, other abdominal organs, and pleura.

In section, villi may be of normal length or somewhat blunt or stubby, with some hypertrophy of crypts. The surface epithelium may appear normal or perhaps slightly attenuated, and lateral interepithelial spaces are often dilated. The lacteals in many villi are distended, and lymphatics in deeper portions of the mucosa, submucosa, and muscularis usually are. Occasional lipid-laden macrophages are present in and around lacteals and lymphatics; large focal accumulations of lipophages around lymphatics, sometimes with a local granulomatous response to lipid or saponified fat, form the white masses that may be seen grossly. A similar reaction may be present in the draining lymph nodes. The lamina propria is edematous, and the submucosa and deeper portions of the gut wall usually are. The proprial inflammatory cell population may be normal, or the numbers of lymphocytes, plasma cells, and eosinophils may be increased, as in chronic inflammatory bowel disease.

The cause of lymphangiectasia may be lymphatic obstruction. Many cases appear to be acquired, and some may be due to lymphosarcomatous or granulomatous infiltrates obstructing flow in mesenteric lymph nodes. Lipogranulomas along the lymphatic drainage are inconsistent features of lymphangiectasia, and are probably in response to chronic leakage of lipid-laden chyle, rather than a cause of lymphatic obstruction. Usually, no congenital or acquired obstruction of the lymphatic system is obvious, though several dogs with lymphangiectasia have had chylothorax associated with thoracic duct obstruction. Experimental obstruction of mesenteric lymphatics produces hypoproteinemia and lymphangiectasia, but not diarrhea and weight loss, suggesting that *the etiology of the clinical syndrome may be more complex than simple lymphatic obstruction.* Increased vascular permeability associated with chronic inflammatory bowel disease may also contribute to mucosal edema and lymphatic dilation.

Moderate malabsorption of lipid, and plasma protein loss into the gut, causes the signs associated with lymphangiectasia. Malabsorbed lipid may contribute to diarrhea via the effects of fatty acids on colonic secretion. Mucosal permeability associated with increased proprial hydrostatic pressure may cause filtration secretion and contribute to plasma protein loss. It has been proposed that dilated lacteals may rupture, releasing lymph into the lumen of the intestine. Hypocalcemia may be related to loss of the mineral bound to plasma albumin, and perhaps to vitamin D malabsorption, or formation of soaps with malabsorbed lipid in the gut lumen. Hypocholesterolemia is due to lipid malabsorption and effusion of plasma. Lymphopenia is thought to be the result of the loss of lymphocyte-rich lymph into the gut.

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Amyloidosis

Amyloid deposition in the small intestine and stomach may occasionally be encountered in animals with systemic amyloidosis. Sometimes the gastrointestinal lesions predominate and contribute to the clinical syndrome. Significant intestinal amyloidosis leads to signs consistent with malabsorption and enteric protein loss. Usually there is no gross indication of the deposition of amyloid in the intestine. However, occasionally focal ulceration or hemorrhage may be noted. Microscopically, amyloid is seen beneath the epithelium or throughout the propria in villi, and perhaps around or within vessels in the submucosa (Fig. 1.63). It must not be mistaken for collagen deposition, which is most unusual in these locations, though a band of collagenous material is sometimes present at the base of the mucosa in cats. The pathogenic effects of amyloid in the intestine seem to involve either impaired movement of interstitial fluid into lacteals or perhaps increased permeability of capillaries, possibly explaining protein loss into the lumen.

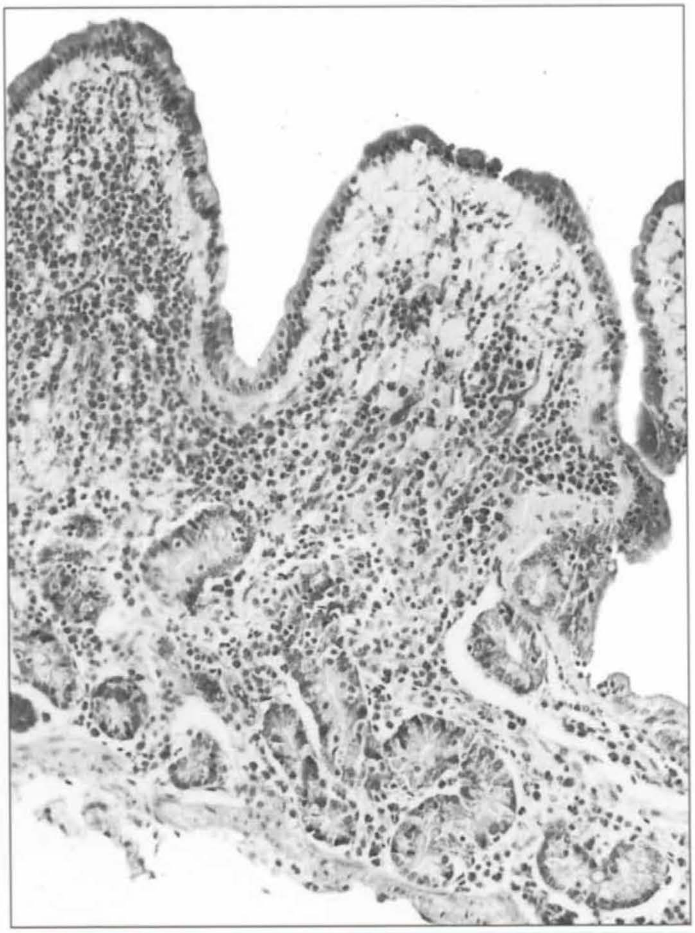

Figure 1.63 Intestine of a goat with deposits of pale amorphous **amyloid** beneath the epithelium on villi, and scattered in the lamina propria. (Courtesy of JR Duncan.)

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Idiopathic inflammatory bowel disease

Some animals, mainly dogs, but less commonly cattle, cats, and horses, showing signs consistent with malabsorption and/or plasma loss into the gut, have microscopic lesions in the mucosa of the small intestine described as *chronic inflammatory bowel disease, lymphocytic-plasmacytic enteritis, filled-villi syndrome,* or *eosinophilic gastroenteritis.*

The latter phenomenon in cats and horses is often part of systemic eosinophilic syndromes affecting those species, considered separately below. In dogs, there is no proof that differentiation of eosinophilic gastroenteritis from chronic inflammatory bowel disease is clinically relevant, nor are there clear criteria for such differentiation, so the two will be considered together. In both dogs and cats, idiopathic mucosal colitis and gastritis may be present as components of chronic inflammatory bowel disease, perhaps with predominant signs reflecting gastric or colonic dysfunction; both are considered here. In contrast to the mucosal pattern of inflammation evident in these entities, inflammatory lesions of the large and small intestine of any species that include a significant population of macrophages usually adopt a transmural pattern, and may be associated with a specific etiology (e.g., *Mycobacterium*, *Histoplasma*) or represent a distinct syndrome (histiocytic ulcerative colitis of Boxers). They are described below with transmural granulomatous enteritis and with typhlocolitis.

Inflammatory bowel disease is a clinical syndrome for which it is difficult to develop a valid, objective histologic counterpart, and it should be a *diagnosis of last resort* made by the clinician after alternatives such as food intolerance, motility disorders, and infectious disease have been ruled out. However, the thoroughness of the clinical and laboratory investigation prior to the use of endoscopic biopsy is influenced by the amount of time and money available to evaluate what are often elusive functional entities. Endoscopic biopsies are often done early, after symptomatic medical therapy has failed to control clinical signs. It is probably not appropriate for a pathologist to issue a diagnosis of "inflammatory bowel disease;" it is more appropriate simply to list the histologic findings and to indicate that *the changes could be "compatible with" a clinical diagnosis of that syndrome.*

Regardless of the portion of the gastrointestinal tract under consideration, the histologic abnormalities are grouped under three broad headings: (1) *changes in mucosal architecture reflecting active or recent epithelial abnormality*; (2) *increased numbers of proprial leukocytes*; and (3) *fibrosis within the lamina propria.* Of these, the epithelial changes are the most reliable, yet the least prevalent. Subjective impressions of increased numbers of leukocytes within the lamina propria are the least reliable, but are the most widely used criterion for a diagnosis, simply because most biopsy samples do not have any other mucosal abnormalities. The pathologist, faced with substantial pressure to make some kind of diagnosis, may reach for the one observation that cannot be disproven (or proven): too many leukocytes.

In the **small intestine**, the cardinal finding is abnormally intense infiltrates of well-differentiated lymphocytes and plasma cells, and sometimes eosinophils, in the lamina propria of villi, between crypts and perhaps in the submucosa. However, normal intestine contains these types of cells and the distinction between normal and abnormal infiltrates is subjective, based on the position and number of cells in the villus. *A layer of lymphocytes, plasma cells, and perhaps neutrophils or eosinophils more than about four cells thick, in the deep mucosa, below the crypts and above the muscularis mucosae, is abnormal* (Fig. 1.64).

Villi may be normal, clubbed, or moderately to severely atrophic, and occasionally fusion of villi may be prevalent (Fig. 1.65). The surface epithelium may appear relatively normal, mucous metaplastic, or low columnar to cuboidal with an indistinct brush border. Intraepithelial lymphocytes may be common. Crypts may be hypertrophic and lined by numerous goblet cells; in other cases goblet cells are less obvious. There may be edema of the lamina propria and dilation of lacteals, suggesting concurrent lymphangiectasia, but usually the edema is not as severe as occurs with that lesion. In this and other conditions with increased inflammatory infiltrates or edema in the lamina propria, including lymphangiectasia, crypts may be obstructed and dilated, and contain mucus and a few exfoliated epithelial cells. This has been termed *cystic mucinous enteropathy*; it is merely a severe variant of lymphocytic-plasmacytic enteritis, and we see no benefit in expanding the nomenclature in an already-confused area. Occasionally, rupture of such crypts will be seen; lakes of mucus, reactive histiocytes, and occasional giant cells are present in the lamina propria (Fig. 1.66). Other distended crypts may contain casts of eosinophilic glycoprotein.

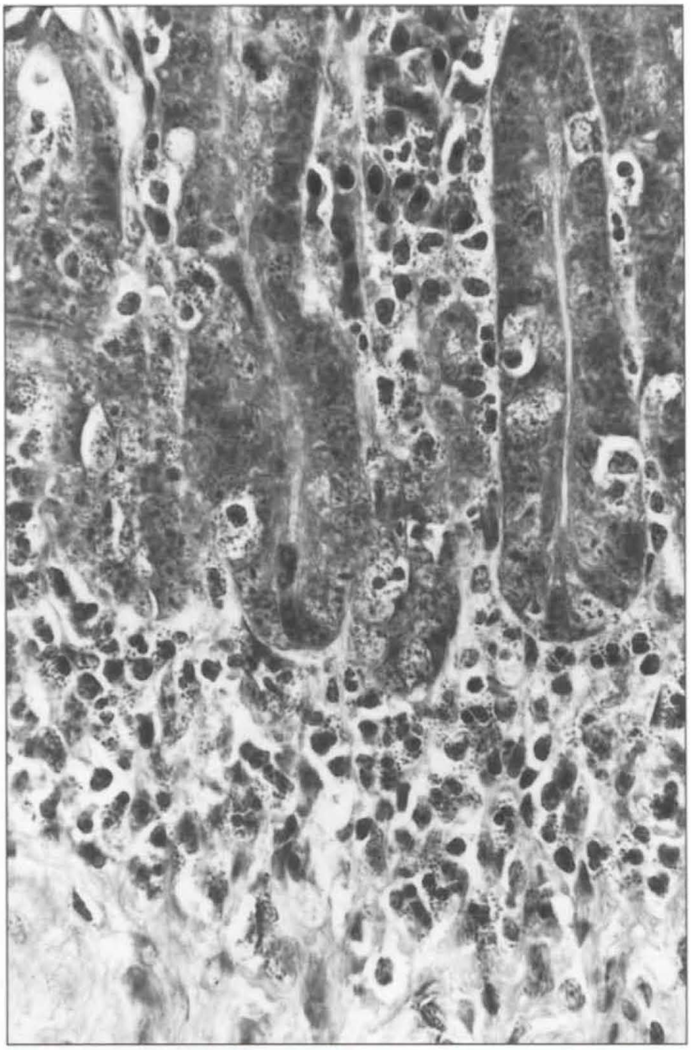

Figure 1.64 Eosinophilic enteritis in a dog. Numerous eosinophils infiltrating the epithelium of small intestinal crypts, adjacent lamina propria, and between the base of crypts and underlying muscularis mucosae.

Care should be taken to differentiate chronic inflammatory bowel disease from early intestinal lymphoma (see discussion of Malignant lymphomas, below). Some suggest that, in the Basenji at least, the former can evolve into the latter.

Idiopathic mucosal colitis is the colonic manifestation of chronic inflammatory bowel disease, and the *commonest form of colitis recognized in dogs.* It is etiologically nonspecific, occurring as chronic or chronic-active lymphocytic-plasmacytic or eosinophilic mucosal inflammation. Histiocytic ulcerative colitis is the distinctive pattern

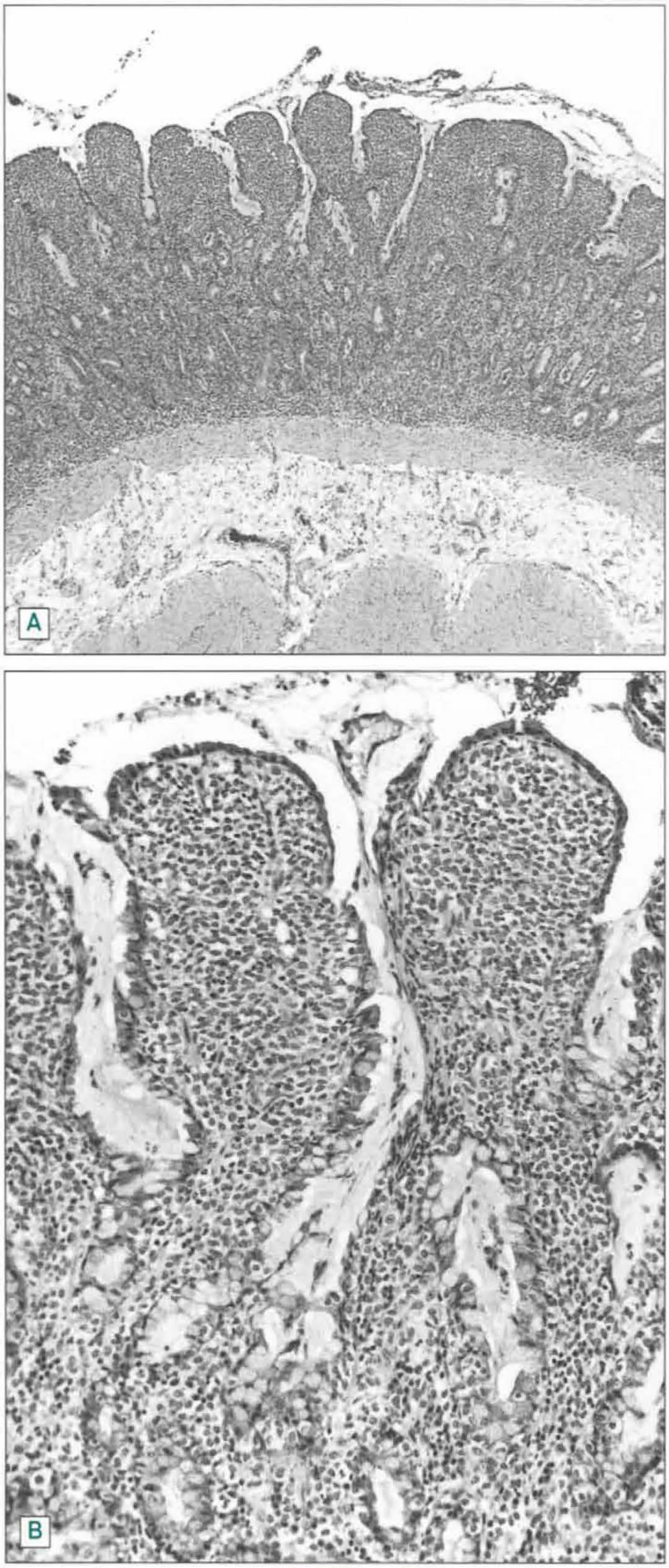

Figure 1.65 Lymphocytic-plasmacytic enteritis in a dog. **A.** Villi are stumpy, club-shaped, or fused. Excessive mononuclear infiltrate at all levels of the mucosa, including between the base of crypts and the muscularis mucosae. **B.** Blunt and club-shaped villi, cuboidal and attenuated surface epithelium, excess mucus secreted from crypts, and abnormal infiltrate of lymphocytes, plasma cells, and histiocytic cells in lamina propria.

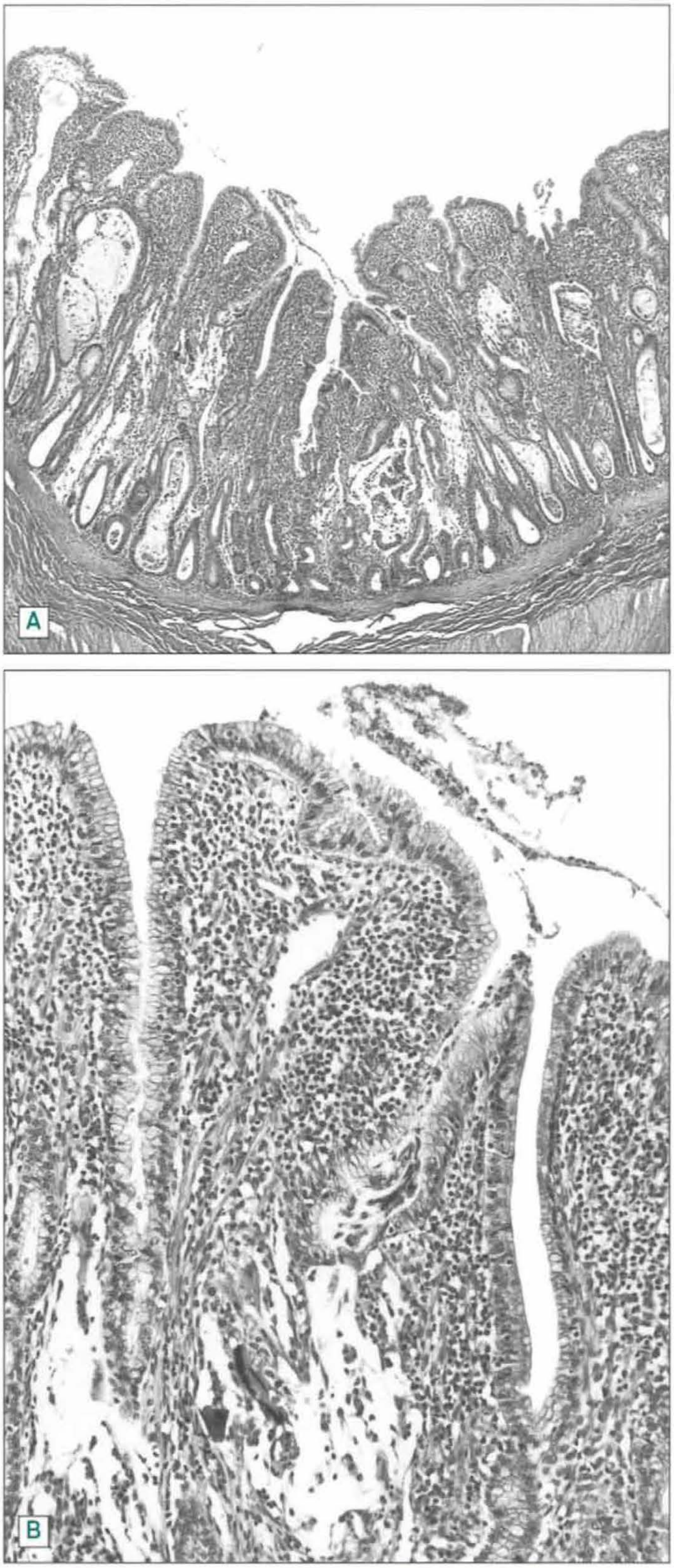

Figure 1.66 Small intestine of a dog with **malabsorption and intestinal protein loss. A.** Blunt and occasionally fused villi, mononuclear cells in lamina propria, and elongate, dilated, and mucus-filled crypts. Some distended crypts have ruptured, releasing mucus into lamina propria. **B.** Detail of (**A**) showing mucus in lamina propria due to rupture of a dilated crypt. Leak of cells and mucus from lamina propria and abnormal numbers of bacteria in lumen.

differentiated from it on microscopic grounds, along with rare cases of *Histoplasma* or protothecal colitis. Colitis is usually diffuse, so that even two or three endoscopic biopsies are probably enough to determine whether or not it is present.

Mild acute mucosal colitis, reflecting a grossly reddened friable surface visible on endoscopy, is characterized by congestion of superficial capillaries and venules, and proprial edema. Neutrophils infiltrate the superficial lamina propria around vessels, and transmigrate or pass between surface epithelial cells into the lumen. The population of lymphocytes and plasma cells in the lamina propria may not differ from normal, but there is generally a moderate increase in mononuclear cells, and perhaps eosinophils, which are usually rare in the superficial colonic mucosa, between glands. There are often few goblets on the surface and in glands, probably due to mucous discharge, rather than cell loss. Surface epithelium may be basophilic, low columnar, or cuboidal (Fig. 1.67A, B). Hyperplasia of epithelium in glands may be evident, and glands dilate. Inflammatory cells, mainly neutrophils, but perhaps lymphocytes, plasma cells, and eosinophils, may accumulate excessively along the mucosal side of the muscularis mucosae, as in inflammatory disease involving the small intestine. The lesions in mild acute colitis often seem mild in proportion to the severity of the clinical syndrome.

The *spectrum of inflammation* in colitis grades from acute towards an increasingly chronic infiltrate, which, along with edema, separates colonic glands and may accumulate deep in the mucosa between glands and muscularis mucosae (Fig. 1.67D). Neutrophils and eosinophils may be scattered among round cells in the propria, and transmigrating surface and glandular epithelium. Globule leukocytes may be prevalent. Accumulation of granulocytes and necrotic debris in the lumen of glands forms so-called *crypt abscesses*.

Greater severity of the lesion is reflected in attenuation and exfoliation of surface epithelium, and the development of *microerosions* on the mucosal surface (Fig. 1.67C). Inflammatory cells, mainly neutrophils, and tissue fluid effuse into the lumen through defects in the epithelium. Persistent erosion, or previous erosion in a healed mucosa, is marked by the development of a thin, horizontally arrayed layer of connective tissue in the superficial lamina propria. With increasing chronicity in colitis of mild or moderate degree, there may be deposition within the lamina propria of a collagenous stroma, throughout which inflammatory cells are interspersed, which separates glands abnormally throughout the mucosa. Downgrowth of glands into often-involuted submucosal lymphoid follicles may occur in chronic colitis.

Severe erosion and ulceration are usually associated with local acute inflammation and with a heavy, mainly mononuclear cell infiltrate in the lamina propria, and often in the submucosa. The ulcerated areas extend usually no further than the muscularis mucosae, and have a base of granulation tissue heavily infiltrated by neutrophils that effuse into the lumen of the bowel. The margin of surviving mucosa may overhang the ulcer. Crypt abscesses may be present in remaining mucosa, and all degrees of erosion and partial ulceration may be present. Idiopathic ulcerative colitis is uncommon, and does not seem as severe as histiocytic ulcerative colitis of Boxers; it rarely comes to autopsy. Severely affected dogs may be cachectic, probably due in part to enteric loss of plasma protein. The mucosa in ulcerative colitis is usually deep red, swollen, folded, and granular due to edema and cellular infiltrates; the depressions may be punctate or up to several centimeters across, roughly round or oval, irregular or elongate. Their margins may be tattered or puckered. Colonic lymph nodes may be enlarged and edematous.

In canine colitis, there is a broad three-dimensional spectrum: (1) in relative chronicity and density of the inflammatory infiltrate; (2) in the distribution of the infiltrate within the wall of the bowel; and (3) in the severity of the epithelial and mucosal change. Generally, milder lesions of superficial epithelium are associated with mild or moderate mucosal inflammation, which may be acute or chronic. In many cases of mild chronic mucosal colitis, the glands do not appear particularly hyperplastic. However, defective repair in the face of severe or ongoing injury may result in crypts that are tortuous or even nearly horizontal, and papillary hyperplasia of the epithelial surface. Severe erosion and ulceration are usually related to a more intense or heavy chronic inflammatory process, which may be limited to the mucosa, but which can extend into the submucosa. Truly granulomatous colitis is rare; when fully developed, perhaps as a component of regional enteritis involving the ileocecocolic area, or in histiocytic colitis of Boxer dogs, considered later, it is ulcerative and transmural. Occasionally, a granulomatous response to barium, or to other foreign material breaching the epithelium, may be observed in the mucosa and submucosa. *Atrophic colitis*, in which the mucosa is markedly thinned, with relatively inactive crypts and modest chronic or chronic-active interstitial inflammation, perhaps with notable interstitial fibrosis, is occasionally encountered.

Gastric changes in dogs or cats with clinically suspected inflammatory bowel disease are challenging to interpret, because of variation in normal microscopic anatomy within the stomach, and because gastric lesions are often patchy. As well, ingestion of chemicals and foreign bodies can create gastric lesions misinterpreted as being those of inflammatory bowel disease.

The stomach has a variety of *normal anatomic features and common background lesions* that have not been proven to have any functional significance. These include a dense band of hyalinized fibrous tissue (*lamina densa*) between the muscularis mucosae and the base of the crypts in cats, lymphoid nodules within the deep lamina propria of both dogs and cats, and a substantial amount of fibrous tissue within the lamina propria of the pyloric antrum. It is not clear whether some of these have any significance as signposts of previous disease. Background lesions that may or may not be correlated with some previous specific stimulus include globule leukocytes in the gastric epithelium of cats, and a combination of gastric proprial fibrosis, glandular atrophy, and glandular nesting within the fundic mucosa. While we assume (without proof) that such fibrosis and glandular atrophy are sequelae to previous inflammation or necrosis, such changes have not been correlated with gastric dysfunction or clinical illness.

Unlike the situation in the intestinal and colonic mucosa, *epithelial injury is not a prominent part of gastritis in dogs or cats.* It is rare to encounter erosion or ulceration as part of gastritis, and acute ulceration associated with chemical or mechanical injury to the stomach has little mucosal cellular infiltrate. It is therefore prudent to distinguish gastric ulceration from gastritis. Lesions compatible with a diagnosis of gastritis include leukocytic infiltrates in the superficial third of the mucosa, mucous metaplasia and hyperplasia in fundic glands, extensive lymphoplasmacytic and/or eosinophilic infiltrates deep in the mucosa, interstitial fibrosis, and associated atrophy of glands. The diagnosis of **gastric mucosal atrophy** must be made with great care because there is substantial difference in mucosal

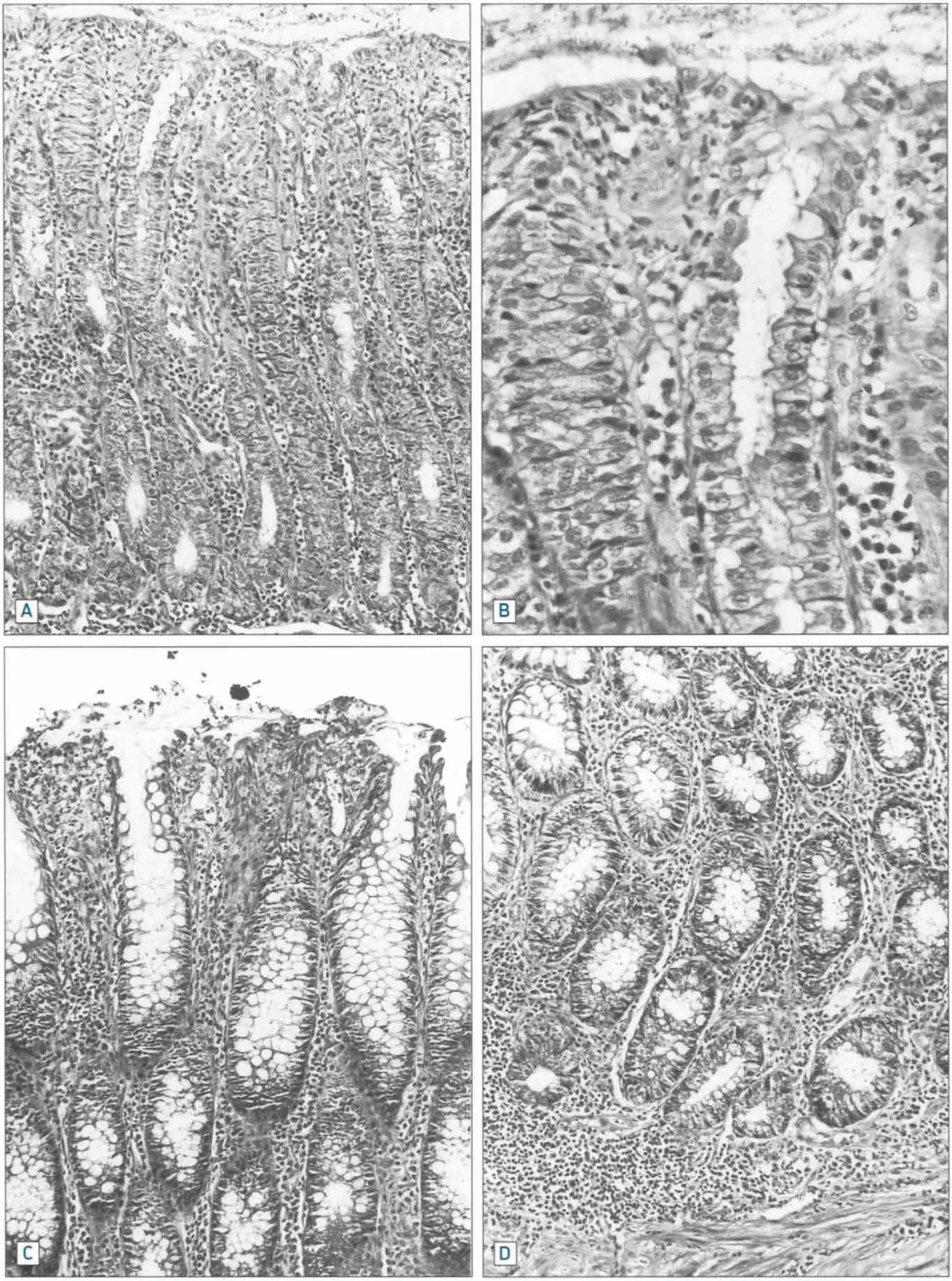

Figure 1.67 Mild acute colitis. **A.** Goblet cells are sparse or absent in glands and on the surface. Superficial epithelium is cuboidal and exfoliating in focal areas. **B.** Epithelium in glands is hyperplastic and crowded. The superficial lamina propria is edematous. **Chronic erosive colitis. C.** Hypertrophic glands are lined by goblet cells. The mucosal surface has widespread erosion and effusion of tissue fluid and neutrophils. **D.** Edema of superficial lamina propria. Deeper in the mucosa there is a moderately increased population of mononuclear cells and increased fibrous stroma. Cells extend between base of glands and muscularis mucosae.

thickness between various portions of the stomach, and even within the same anatomic region.

Most discussions of inflammatory bowel disease assume that the change in mucosal cellularity is primary and that characterization of that cellularity should provide the greatest insight into pathogenesis and therapy. However, assessment of mucosal cellularity is almost entirely subjective and has exceedingly poor interobserver agreement. There are no useful objective reference intervals for small intestinal mucosal cellularity because the range is so broad, and accurate identification of the different cell types is problematic. Characterization of the immunophenotype of lymphocytes in the small intestine has produced variable results that have yet to be usefully associated with the presence or absence of clinical signs.

Attempts to create more *objective grading systems* for the histologic assessment of inflammatory bowel disease have stressed the significance of architectural changes within the surface epithelium and lamina propria rather than relying exclusively on shifts in total or relative leukocyte populations. Such grading criteria include detection of villus or cryptal enterocyte injury (villus fusion, superficial ulceration, enterocyte flattening and basophilia, cryptal hyperplasia), as well as remodeling of mucosal architecture (separation of the crypts from one another or from the muscularis mucosae by edema, fibrosis, and/or leukocytes). However, these schemes still fail to resolve the dilemma of substantial overlap between "normal" and subjectively increased leukocyte numbers in mild inflammatory bowel disease in which there is no epithelial injury or architectural change.

Skepticism about the utility of estimating leukocyte numbers or identifying shifts in phenotype among mucosal leukocytes may not be so appropriate when looking at colonic and gastric inflammatory disease. Perhaps because of its lesser overall proprial cellularity in comparison with small intestine, or perhaps because of its greater accessibility to biopsy, the colonic mucosa was the first part of the canine intestinal tract to be subjected to morphometric grading of architectural changes and objective leukocyte counting, although application of such data is difficult. In comparison with small intestine, the normal range in leukocyte numbers is narrower and the overall population much smaller in both colon and stomach. This is particularly true of the granulocytes. *The presence of neutrophils within the lamina propria of any part of the intestine is probably abnormal*, and is probably a reliable marker for recent epithelial damage or permeability. Eosinophils have more variation, and often are present in very large numbers throughout the small intestinal mucosa. However, they are very sparse within the superficial half of the canine colonic mucosa, and are infrequent within the gastric mucosa.

Since the normal stomach has relatively few mucosal leukocytes, the pursuit of a diagnosis of gastritis based upon objective assessment of leukocyte numbers may be a credible goal. While it has been possible, by counting different types of leukocytes identified by routine histology and immunohistochemistry, to establish reference intervals for eosinophils, plasma cells, B cells, and T cells within various parts of the gastric lamina propria of healthy dogs, more than half of the round cells in the normal canine gastric lamina propria could not be precisely identified. Healthy dogs had an eightfold range in total mucosal cellularity, and the pyloric antrum was three times more cellular than the fundic mucosa. Although dogs with clinical signs of gastritis had an objective increase in eosinophils, plasma cells, and/or intraepithelial lymphocytes in many different combinations, it was difficult to correlate clinical severity or response to therapy with cellular or other parameters of gastric mucosal abnormality. There was no significance to whether the inflammation was limited to the superficial third of the mucosa or was found throughout, although the former was more common than the latter. In a second study of idiopathic gastritis, all cases were classified as lymphocytic, and T cells predominated. Eosinophils were not a significant component, and there was no observed regional variation in severity.

While it is certainly possible (or even probable) that precise identification and quantitation of the infiltrating leukocytes will emerge as significant when we have more precise ways of measuring clinical severity and response to therapy, currently there is no pragmatic clinical significance to distinguishing eosinophilic from lymphocytic gastritis, commenting on whether the lesions are superficial or more diffuse, or making any statement about histologic severity with respect to leukocytes or other aspects of mucosal architecture.

The etiopathogenesis of idiopathic inflammatory bowel disease is not understood in any animal species. The nature of the inflammatory infiltrate suggests that loss of tolerance to dietary antigen or antigens produced by the enteric microflora may be implicated. The common morphologic changes in the mucosa – of cryptal hypertrophy, villus atrophy, and, in severe cases, mucous metaplasia of surface enterocytes, may be side-effects of T-cell-mediated activity in the mucosa. Similar lesions occur in humans with celiac disease (gluten-sensitive enteropathy), which is T-cell mediated. An immunophenotypic investigation of inflammatory bowel disease in cats noted outcomes consistent with dysregulation of immunoinflammatory events. Major histocompatibility complex class II expression by proprial round cells and enterocytes was more pronounced than in the control population.

Lymphocytic-plasmacytic enteritis associated with *familial sensitivity to wheat protein* has been demonstrated in Irish Setters, but not in Basenji dogs, which as a breed seem predisposed to the problem. In Basenjis and in the Lundehund, syndromes of hypoalbuminemia, chronic diarrhea, and wasting occur with high prevalence, primarily attributable to lymphocytic-plasmacytic enteritis, with lymphangiectasia in some dogs. In the Basenji, chronic gastritis or hypertrophic gastritis may be associated, and malassimilation and plasma protein loss into the gut have been documented. Hypergammaglobulinemia commonly occurs in the late stages of the syndrome in Basenjis, and lymphosarcoma develops in some affected animals. In this sense, the syndrome resembles immunoproliferative small intestinal disease (α-heavy chain disease, or Mediterranean lymphoma), which is a disorder of IgA immunoblasts in humans. IgA plasmacytes do not dominate in the Basenji intestinal mucosa; though high circulating levels of IgA are present, it is not known if they are associated with abnormal α-heavy-chain protein. In cats, dogs, and horses, submucosal or transmural lymphoplasmacytic infiltrates may signal a precursor to lymphoma, and the infiltrating cell population must be carefully evaluated; a monomorphic population of lymphocytes, blurring of the proprial–epithelial interface by lymphocytic infiltrates, and mitotic activity suggest lymphosarcoma.

Animals with the gray Collie syndrome (cyclic neutropenia) may also have lymphocytic-plasmacytic enteritis (see Vol. 3, Hematopoietic system).

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Eosinophilic enteritis in cats and horses

Eosinophilic enteritis in cats is rare, and appears to be one manifestation of a hypereosinophilic syndrome that may involve many organs in middle-aged or older cats. It is much more severe than eosinophilic gastroenteritis in dogs. Diarrhea, sometimes bloody, vomition, loss of appetite, and loss of condition may occur. Clinically, *intestinal thickening*, hepato- and splenomegaly, and enlarged mesenteric lymph nodes may be present, in association with circulating eosinophilia and hyperplasia of the eosinophil series in the marrow.

The postmortem picture reflects the clinical findings. Enlargement of the various organs, including liver, spleen, lymph nodes in many locations, and tan nodularities on the kidneys, is associated with *heavy infiltrates of usually well-differentiated eosinophils*. In the small intestine, the eosinophilic infiltrate may be transmural and is accompanied by grossly visible hypertrophy of the muscle layers (Fig. 1.68).

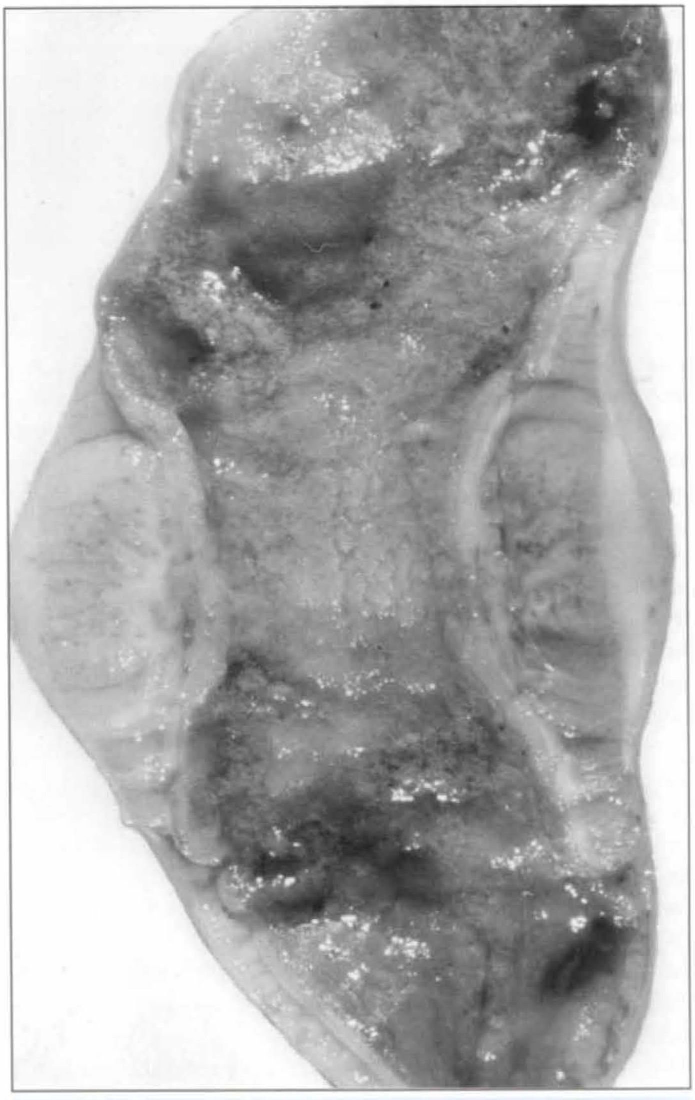

Figure 1.68 Eosinophilic enteritis in a cat. Foci of mucosal congestion, and segmental transmural thickening and hypertrophy of smooth muscle in an area heavily infiltrated by eosinophils.

Eosinophilic colitis may occur in some cases. Lymph nodes may have hyperplastic follicles and many mature eosinophils in sinusoids. Alternatively they may vary through eosinophilic lymphadenitis with fibrosis to complete obliteration of normal architecture and replacement by eosinophils in a fibrillar stroma extending through the capsule into surrounding tissue.

Chronic eosinophilic enteritis in horses has been described as part of a distinct *multisystemic epitheliotropic syndrome*, associated with eosinophilic granulomatous pancreatitis and eosinophilic dermatitis, among other lesions. Affected animals have weight loss, and diarrhea or unformed feces, associated with hypoalbuminemia, suggesting enteric loss of plasma protein. Reduced absorption of glucose occurs, but peripheral eosinophilia is absent. At autopsy, *mucosal and sometimes transmural thickening* may occur at any level of the alimentary tract from esophagus to rectum. Esophageal and gastric squamous mucosa is hyperkeratotic. Thickened mucosa is thrown into turgid transverse folds, or occasionally is fissured and roughened. Focal or diffuse ulcers may be present on the small and large intestine and focal caseous lesions up to 1.5 cm in diameter may be in the submucosa of the gut and common bile duct, as well as in an enlarged fibrotic pancreas.

Microscopically there is diffuse infiltration of the mucosa, submucosa, and often deeper layers of the enteric wall by eosinophils, mast cells, macrophages, lymphocytes and some plasma cells. Moderate to severe villus atrophy, fibroplasia in the lamina propria, and hypertrophy of the muscularis mucosae occur. Caseous foci in the mucosa and submucosa consist of central masses of eosinophils, sometimes surrounded by macrophages, giant cells, and occasionally fibrous tissue. Eosinophilic interstitial infiltrates and granulomas are described in the biliary and pancreatic ducts, pancreas, salivary glands, capsule, and outer cortex of enlarged firm mesenteric lymph nodes, and near portal tracts in the liver. The skin may be thickened and hyperkeratotic and the limbus of the hoof thickened and ulcerated. The eosinophilic dermatitis is described in Vol. 1, Skin and appendages.

Villus atrophy is common, but if large-bowel lesions are absent, there is no diarrhea. Chronic inflammation in the mucosa may explain protein loss and hypoalbuminemia. The cause of this syndrome is unknown. A hypersensitivity response to migrating parasitic larvae has been suggested, as has the possibility that undetected T-cell lymphoma secreting interleukin-5 may be responsible.

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Granulomatous enteritis

The presence of chronic inflammatory infiltrates, including aggregates of histiocytes, and perhaps giant cells, in the lamina propria is the criterion for a diagnosis of **granulomatous enteritis**. With time, the inflammatory reaction typically follows lymphatics transmurally into the submucosa and through the muscularis to the serosa. The submucosa is usually edematous, and lymphatics are prominent. Granulomas may be present in the submucosa or at intervals along lymphatics. The affected lymph nodes are hyperplastic, usually with prominent sinus histiocytosis. Giant cells may be present in sinusoids, or granulomatous foci of various sizes may be evident. Sinusoids contain numerous neutrophils and perhaps eosinophils, and neutrophils may accumulate in the center of granulomas.

Granulomatous enteritis can potentially occur in *all species*. Johne's disease, other intestinal mycobacterioses, and *Histoplasma* enteritis are specific examples (see section on Infectious and parasitic diseases of the alimentary tract, below). Often the cause is not identified.

Transmural granulomatous enteritis is occasionally seen in **dogs** and **cats**. It is generally segmental and perhaps discontinuous in distribution, usually affecting the lower ileum, colon, and draining lymph nodes. Due to the extent of the attendant fibrosis, these lesions may be stenotic, and must be differentiated from invasive carcinoma. In dogs, there may be marked necrosis in the centers of granulomas and considerable fibrosis.

Idiopathic granulomatous enteritis as a cause of wasting and protein-losing enteropathy is most commonly seen as a sporadic problem in **horses**. Depending on the duration of the disease, animals may be markedly cachectic, have subcutaneous edema, especially of dependent areas, and there may be hydrothorax, hydropericardium, and ascites. Lesions in the horse usually affect the small intestine; stomach and large bowel are occasionally also involved. Mesenteric lymph nodes are usually enlarged, edematous, with mottled firm gray areas, fibrotic nodules, or, rarely, caseous or mineralized foci on the cut surface. Granulomatous pale, caseous, or calcified foci may be scattered in the liver.

The *microscopic lesion* may be patchy, regional, or diffuse, and it may be mucosal, or transmural, ultimately gaining the draining lymph nodes. Transmural inflammation is characteristically granulomatous. Villi are mildly to markedly atrophic with hypertrophy of crypts. The epithelium may vary from apparently normal to low columnar or cuboidal with an indistinct brush border. There may be leaks between cells on the surface, or microerosions may be present, through which neutrophils and proteinaceous exudate pass into the lumen. The lamina propria is edematous and contains scattered aggregates of histiocytes and perhaps giant cells, or, less commonly, more organized granulomatous foci. Neutrophils and eosinophils are distributed diffusely throughout the lamina propria, and may be concentrated in or near granulomatous foci. A heavy population of lymphocytes and plasma cells inhabits the lamina propria, and the infiltrate and edema may separate crypts abnormally from each other.

Rarely are agents identified in such lesions. *Mycobacterium avium* or environmental mycobacteria are occasionally incriminated. *Anaerobiospirillum* has been implicated as the cause of ileocolitis in cats.

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Inflammation of the large intestine

The general reaction to injury of the cecal and colonic epithelium was considered earlier in the section on Epithelial renewal in health and disease.

Ischemia, obliteration of the proliferative epithelium by viruses or coccidia, severe inflammation in the mucosa, and necrotizing toxic insults from the lumen are responsible for the development of focal or diffuse ulceration of the large intestine. Inflammatory infiltrates in the lamina propria may be broadly classified as acute, chronic, or chronic-active, and granulomatous. They may be limited in distribution to the mucosa or be transmural, involving submucosa, muscularis, serosa, and frequently the draining lymph nodes. Typhlitis and colitis may be manifestations of a generalized or systemic disease; they may be part of an enterocolitis involving both small and large intestine; or they may be regional and limited to a segment of the intestine, often terminal ileum, cecum, and colon, or some shorter part of the large bowel.

The damaged colonic mucosa may provide the portal of entry for systemic bacterial invasion and for uptake of toxins. Increased mucosal permeability in the colon may permit enteric loss of plasma protein or of blood. Disordered large-bowel flora in hindgut fermenters may compromise uptake of volatile fatty acids and water. In any species, damage to the colonic mucosa may result in malabsorption of electrolytes and water, and perhaps net secretion. Colitis in each of the species will be considered in turn.

Colitis cystica profunda, the presence of dilated colonic glands protruding through the muscularis mucosae into the submucosa, is seen in several species, most commonly in *swine*. The cause is unknown; the lesion may be a sequel to colitis and local damage to the muscularis mucosae, or it may represent herniation into the space left by an involuted submucosal lymphoid follicle. Though the lesion has been seen with colitis in a variety of circumstances, especially in swine dysentery, it may also be found incidentally, with no specific etiologic association.

Typhlocolitis in dogs

Inflammation of the large bowel in dogs is usually associated with *diarrhea*, typically frequent, small in volume, mucoid or bloody, and often accompanied by tenesmus. By far the most common form encountered in dogs is **idiopathic mucosal colitis**, considered above in Idiopathic inflammatory bowel disease.

Severe acute necrotizing colitis and, less commonly, typhlitis, leading to *ulceration and perforation*, with subsequent peritonitis, has been associated with glucocorticoid administration, functional adrenal cortical tumors, and with trauma or surgery involving the spinal cord. Gastric ulceration may occur concurrently. The perforations usually occur in the antimesenteric border of the left colonic flexure or proximal descending colon, though they are reported from the cecum, ascending, transverse, and distal descending colon. The pathogenesis of the colonic lesions developing in these circumstances is unclear, but it does not seem to be associated with the interface between the cranial and sacral fields of autonomic nervous control, as has been proposed.

Necrotizing colitis, ulceration, and perforation may occur rarely in dogs in **uremia**. The mechanism is uncertain, but colonic damage may be the effect of high concentrations of ammonia evolved by urease-producing colonic flora from urea diffusing into the gut from the blood. The lesions are often associated with arteritis, and fibrinoid necrosis, mineralization, and destruction of the media are seen, especially in ruminants.

Trichuris vulpis, the whipworm of dogs, may cause mucosal colitis, which rarely evolves into a granulomatous transmural condition. Clinical trichuriasis is generally associated with a population of worms that extends from the usual site of infection in the cecum and proximal ascending colon into more distal parts of the large intestine. Ulcerative colitis in dogs is also rarely caused by ***Entamoeba histolytica***. Ulcerative, granulomatous transmural colitis is more common as one of the enteric manifestations of **histoplasmosis**. ***Prototheca*** is also a cause of distinctive but rare enterocolitis in dogs, and colitis characterized by a heavy mucosal infiltrate of macrophages, in which the agent may be found, is reported in **leishmaniasis**. ***Canine parvovirus*** causes colonic damage, but in virtually all cases, there are more severe lesions elsewhere, notably in the small intestine. ***Canine coronavirus*** has also been implicated as a cause of colonic, as well as small intestinal, lesions. These conditions are discussed fully in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Spirochetes may be present in the canine colon and their association with diarrheal disease is controversial. There is some indication that predisposing factors, particularly giardiasis, may result in heavy colonization of the mucosal surface, with resulting diarrhea. ***Campylobacter jejuni*** is isolated from dogs both with and without diarrhea, but has been implicated as an etiology of enterocolitis in some situations of outbreaks of diarrhea in dog colonies. In these cases, large aggregates of organisms can be visualized within crypt lumens.

Histiocytic ulcerative colitis is a distinctive histologic syndrome, which has been recognized in Boxers and the related French Bulldog. It is a chronic transmural ulcerative colitis characterized by the presence of large numbers of macrophages containing periodic acid–Schiff-positive granules, in the deep mucosa and submucosa, and in lymph nodes receiving drainage from the colon. Clinically affected animals are usually under 2 years of age. This condition causes typical large-bowel diarrhea, with mucus and blood; weight loss occurs and chronic cases may become cachectic, probably due to protein loss into the gut.

Grossly, the colon of dogs with advanced disease is variably thickened, folded, and perhaps dilated and shortened with some segmental or focal areas of scarring and stricture. Lesions on the mucosa may vary from patchy punctate red ulcers to more extensive irregular circular or linear lesions that may coalesce, leaving only a few islands of persistent mucosa on a granulating colonic surface (Fig. 1.69A).

Early *microscopic lesions* are those of mild nonspecific inflammation. Goblet cells disappear from the surface and glands. Microerosion of epithelium in the upper glands and on the surface is associated with local acute inflammation, migration of neutrophils into the epithelium, and effusion of neutrophils and tissue fluid into the lumen.

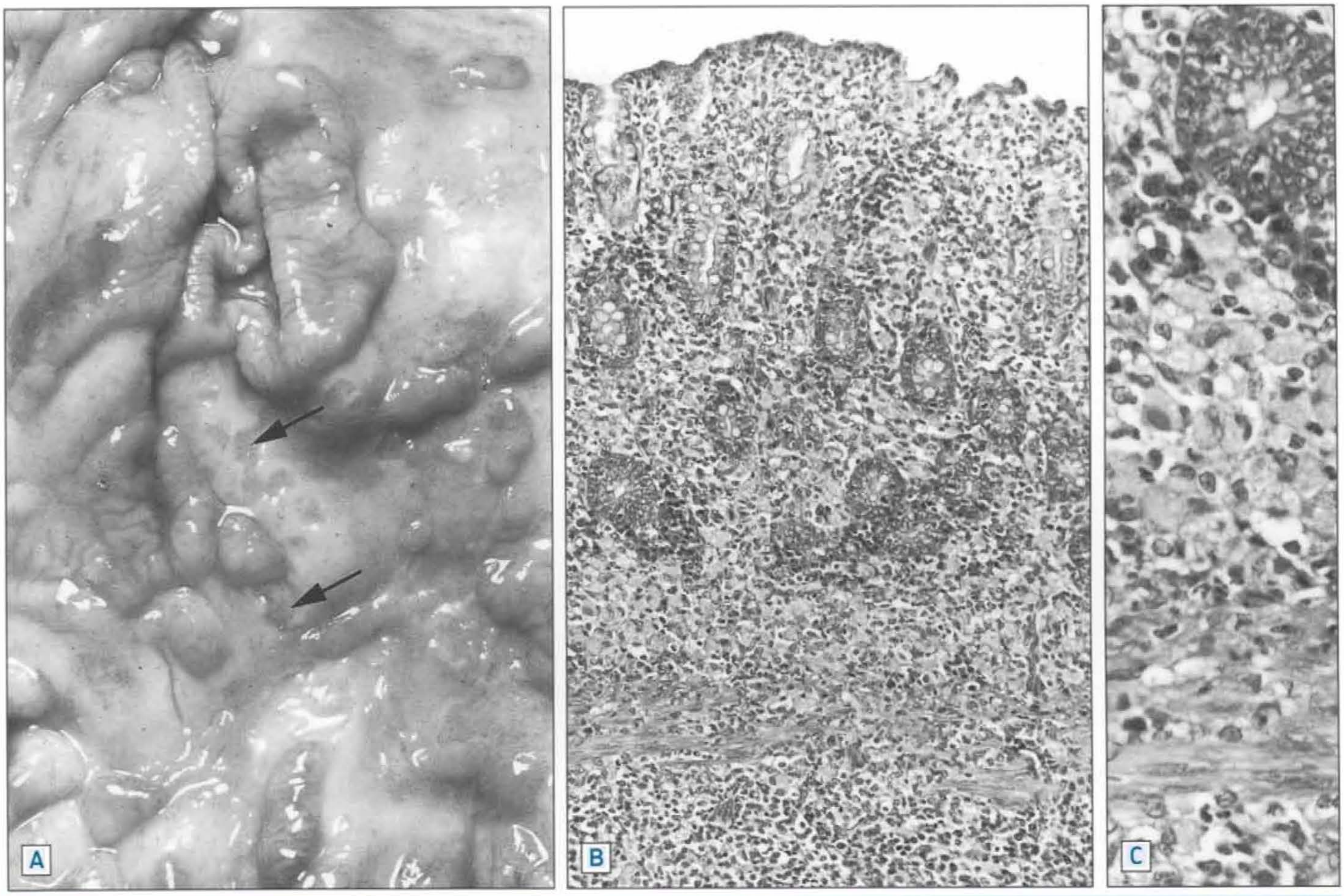

Figure 1.69 Histiocytic ulcerative colitis in a Boxer dog. **A.** Mucosa is thickened and edematous. There are numerous erosions (arrows). **B.** Accumulation of macrophages with abundant cytoplasm throughout mucosa, between base of glands and muscularis mucosae and in submucosa. **C.** Detail of macrophages in mucosa deep to crypts.

Macrophages in these areas may contain phagocytized necrotic debris and bacteria. In some areas the mucosa is thinned, and glands are relatively shortened, though lining epithelium appears hyperplastic. Macrophages with cytoplasmic vacuoles, which contain periodic acid–Schiff-positive material, are mainly deep in the lamina propria, raising the base of crypts from the muscularis mucosae, and in the submucosa (Fig. 1.69B, C). Sometimes they may be relatively sparse in the mucosa, and may be missed in small biopsy specimens if the submucosa is not sampled. These same cells may be found about lymphatics in the muscularis and the serosa, and they may be numerous in subcapsular, cortical, and medullary sinuses in the draining lymph nodes. The cecum is often involved, to a lesser degree, with similar lesions. Ulceration seems to progress from the superficial epithelial erosion and destruction of the basement membrane seen in early lesions. Ulcers usually do not progress beyond the submucosa, and they are lined by granulation tissue. The bed of the ulcer is necrotic, and numerous neutrophils and erythrocytes may be passing into the lumen.

The *cause* of the condition is unknown, as is the origin of the material in the characteristic vacuoles in macrophages. Ultrastructural study suggests that these are digestion vacuoles containing mainly remnants of phospholipid membranes. The material being digested may be phagocytized cell debris and microorganisms picked up in the superficial lamina propria and carried in "constipated" macrophages to deeper structures. The involvement of a variety of infectious agents has been postulated. It seems that a *defect in lysosomal function* may exist in some Boxer dogs that can lead to the accumulation of partially digested phospholipid membrane in macrophages, since similar histiocytes do not accumulate in ulcerative colitis in other breeds of dogs.

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Colitis in cats

Colitis in cats is uncommon. **Idiopathic mucosal colitis**, similar to that described in dogs, occurs occasionally in cats, and has been included in the discussion of Idiopathic inflammatory bowel disease above. ***Tritrichomonas foetus***, associated with persistent large-bowel diarrhea and idiopathic mucosal colitis in cats under about a year of age, is considered in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Feline panleukopenia virus causes colonic lesions in about half the cases. They are similar to, but rarely as severe or widespread as, the lesions found in the small intestine. The relative paucity and mildness of lesions in the colon are related to the lower rate of epithelial proliferation in comparison with the small intestine. Panleukopenia is considered in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Mycotic colitis is occasionally seen in cats as a hemorrhagic ulcerative colitis in which focal or diffuse mucosal invasion by *Candida*, zygomycetes, or *Aspergillus* has occurred, sometimes causing microvascular thrombosis. These invaders occur mainly secondary to colonic damage and leukopenia caused by panleukopenia.

Clostridium piliforme caused mild mucosal colitis in several kittens with soft feces and vague central nervous disorder, but no other obvious intercurrent disease. The lesions were characterized by hypertrophy of crypts; exfoliation of epithelium into the lumen of crypts, which were dilated and lined by flattened epithelium; and a moderate chronic interstitial inflammatory infiltrate. Vegetative forms and spores of *C. piliforme* were recognized ultrastructurally in crypt epithelium. Lesions were not evident elsewhere. It has also occurred in kittens presumably immunocompromised by panleukopenia.

Necrotic colitis, a condition seen predominantly in older animals, is characterized by severe damage to colonic glands (Fig. 1.70). The cause is thought to be ischemia.

Transmural acute ulcerative colitis, with a heavy infiltrate of neutrophils, is the hallmark of ***Salmonella* Typhimurium** infection in cats, considered more fully with salmonellosis in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Anaerobiospirillum, associated with colitis in cats, is discussed in the section on Bacterial diseases of the alimentary tract, below.

Ulcerative colitis, grossly and microscopically similar to idiopathic ulcerative colitis of the dog, occurs very rarely in *cats*. Granulomatous or pyogranulomatous foci in the subserosa or submucosa may cause regional enterocolitis, characterized by fibrosis and serosal nodularity of affected segments, usually without severe mucosal defects. Fibrinoid arteritis causing hemorrhage and edema in the colonic submucosa, and ischemic necrosis of the mucosa, is also reported. The granulomatous syndrome is attributed to *Feline infectious peritonitis virus*, and there may be characteristic lesions in other organs.

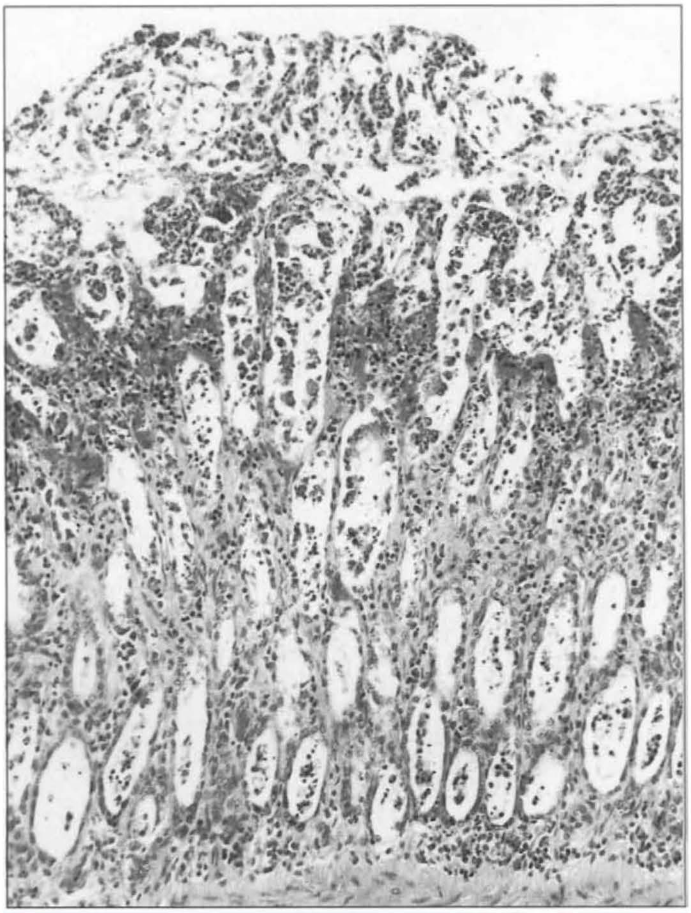

Figure 1.70 Necrotic colitis in a cat. Active necrosis on surface of mucosa. Glands are dilated, contain necrotic cellular debris, or are lined by extremely flattened epithelium. (Courtesy of JS Nimmo-Wilkie.)

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Typhlocolitis in horses

The diagnosis of acute colitis in horses resolves into the differentiation of peracute and acute salmonellosis, Potomac horse fever (equine monocytic ehrlichiosis), and intestinal clostridiosis. Colitis must also be differentiated from the sequelae of intestinal accidents, nonsteroidal anti-inflammatory drug intoxication, and thromboembolism involving the large bowel.

Equine intestinal clostridiosis is increasingly well defined as an entity. Previously, many of these cases were lumped into an umbrella

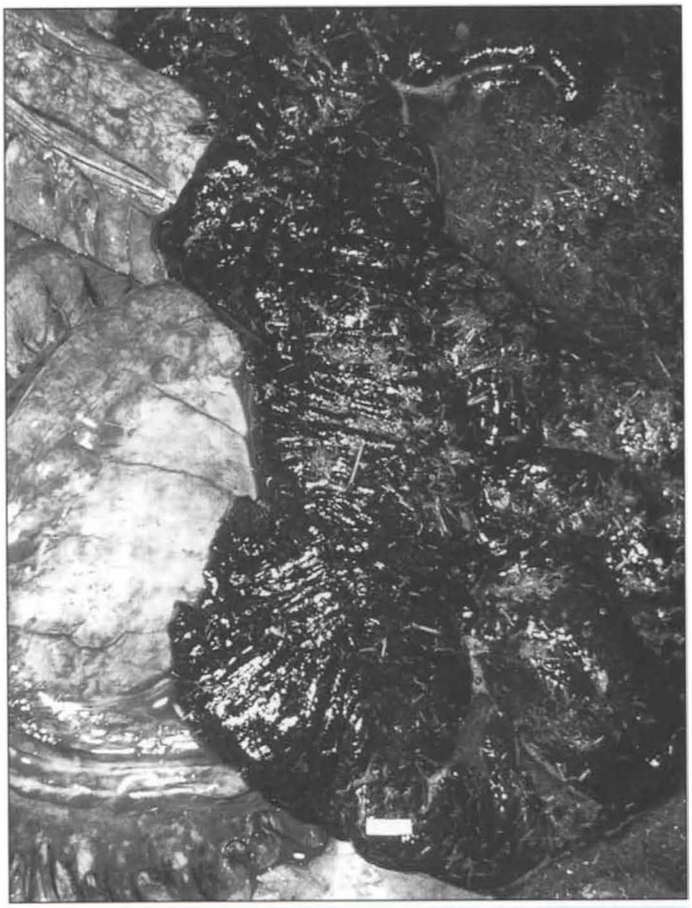

Figure 1.71 Acute colitis, probably clostridial, in a horse. The mucosa is very congested and edematous.

category, "*colitis X*," a severe acute colitis in which all known etiologic agents were ruled out. At necropsy the animal is dehydrated, and there may be subcutaneous and serosal petechial hemorrhage. The blood is dark and clots poorly. Enteric lesions are virtually limited to the large bowel, which is distended, with abnormally fluid content. The serosa of the cecum and large colon may appear cyanotic from congestion and hemorrhage in the mucosa (Fig. 1.71) and perhaps submucosa. The deeper tissues of the intestinal wall are not compromised, as is usually the case in volvulus and often in thromboembolic infarction. The mucosa and submucosa are commonly markedly edematous, and edema is often present at the mesenteric attachment of the gut and in the cecal and colic lymph nodes. The mucosa may appear brown and necrotic with focal fibrinohemorrhagic exudate on the surface. More commonly, it is deeply congested with focal hemorrhage, but blood is rarely present to significant degree in the contents. Gross lesions in other organs are those consistent with circulatory or endotoxic shock.

The microscopic lesions in the large bowel include superficial or full-thickness necrosis of the mucosa, associated with dilation and perhaps thrombosis of small mucosal and submucosal venules. There is hemorrhage and edema in the mucosa, and the submucosa is markedly edematous, with dilated lymphatics. Variable numbers of bacilli are associated with these lesions. Some neutrophils may be evident in the mucosa or submucosa, and fibrin may be effusing from the damaged mucosal surface in less advanced cases. Submucosal lymphoid follicles show evidence of recent severe lymphocytolysis. Congestion, microvascular thrombosis, and hemorrhage may be found in a variety of other organs, especially the adrenal cortex.

Clostridium perfringens type A is the most common clostridial species associated with this syndrome. Most horses carry this organism within their intestines. In addition, *C. difficile* has been associated with equine clostridial colitis. Equine intestinal clostridiosis may occur after *dysbacteriosis*, instigated by antibiotic therapy (especially the tetracyclines), stress, or changes in feeding regimens. With overgrowth of *C. perfringens* type A, β_2 toxin-secreting *C. perfringens* type A or *C. difficile* exotoxins cause severe acute mucosal damage. There is a positive association between the disease and the presence of *C. perfringens* enterotoxin and/or *C. difficile* toxin.

Subacute and chronic diarrhea in horses almost always involves the large intestine, with or without concomitant small-bowel involvement. ***Salmonella*** typhlocolitis must be suspected in such cases. Salmonellosis in horses may have an extremely variable course and pathologic manifestations (see section on Infectious and parasitic diseases of the alimentary tract, later in this chapter). **Potomac horse fever** usually results in diarrhea that does not exceed 10 days in duration; at necropsy there is congestion and ulceration of the mucosa of the large bowel, and enlargement of mesenteric lymph nodes. Suppurative ulcers involving lymphoid tissue in the typhlocolic mucosa, and cecal and colic lymphadenitis, characterize enteric infection with ***Rhodococcus equi*** in foals. **Histoplasmosis** has been reported once in a horse with salmonellosis and ulcerative colitis. Extensive mucosal involvement by **larval cyathostomes and strongyles** and, rarely, ulcerative typhlitis due to **anoplocephalid tapeworms**, may also cause diarrhea and wasting; they are discussed under specific parasitisms.

Ciliate protozoa may be seen in the colonic mucosa of horses dead of a variety of enteric and nonenteric problems; the tissues are frequently autolytic, are rarely inflamed, and the protozoa are terminal or postmortem invaders. Intralesional protozoa have, however, been reported in a horse with diffuse eosinophilic colitis.

Chronic diarrhea and possibly cachexia may also result from persistent ulceration of the cecum or colon due to **ischemic mucosal lesions**. These may be the product of arterial thromboembolism and slow flow, or less likely, corrected strangulation with reflow. Use of *nonsteroidal anti-inflammatory drugs* has also been associated with cecal and colonic ulceration and plasma protein loss. "*Right dorsal colitis*," in which ulcerative lesions are limited to the named part of the large bowel, may be associated with colic, and acute or chronic diarrhea. This syndrome and others associated with the use of nonsteroidal anti-inflammatories are considered with ischemia due to reduced perfusion (see section on Intestinal ischemia and infarction, above).

The specific cause of extensive ulceration may be difficult to determine. Smaller chronic ulcers and widespread subacute erosion and ulceration are most likely the result of salmonellosis, rather than ischemia. A history of administration of nonsteroidal anti-inflammatories, and the presence of lesions in the renal papilla, mouth, and upper alimentary tract, suggests intoxication by those agents.

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Typhlocolitis in swine

The differential diagnosis of typhlocolitis in swine mainly revolves around identifying swine dysentery and other spirochetoses, *Salmonella* enterocolitis, and *Lawsonia intracellularis* infection.

Swine dysentery, caused by *Brachyspira hyodysenteriae*, involves only the cecum and spiral colon. It is a catarrhal to mildly fibrinohemorrhagic erosive mucosal typhlocolitis. The colonic content is fluid and usually blood-tinged. A similar but milder disease, **intestinal spirochetosis**, is associated with *B. pilosicoli*, which causes distinctive microscopic lesions as it colonizes the apex of surface epithelium. ***Salmonella* enterocolitis**, mainly due to *S.* Typhimurium, is a fibrinous, erosive to focally ulcerative condition, mainly of the cecum and colon, but perhaps involving the small intestine, especially terminal ileum. The content is fluid but usually not bloody. Mesenteric lymph nodes are prominent. ***L. intracellularis*** infection is readily recognized by the consistent concurrent involvement of the terminal ileum by adenomatosis, with or without hemorrhage, or by necrotic ileitis.

Postweaning colibacillosis is characterized by catarrhal to mild fibrinohemorrhagic enterocolitis in piglets after weaning.

Fibrinohemorrhagic typhlitis is caused by heavy infestations with ***Trichuris suis***, especially in weaned pigs with access to pastures and yards. Under similar circumstances, ***Eimeria*** infection may rarely cause ileotyphlocolitis.

Rectal stricture appears to be a product of ischemic proctitis, probably related in many cases to infection with *S.* Typhimurium.

Details of these conditions are discussed in the section on Infectious and parasitic diseases of the alimentary tract, later in this chapter.

Typhlocolitis in ruminants

Diagnostic considerations in cattle over 2–3 months of age with acute to subacute fibrinohemorrhagic typhlocolitis include salmonellosis, bovine viral diarrhea, rinderpest (in enzootic areas or populations at risk), coccidiosis, adenoviral infection, and winter dysentery (coronavirus). Lesions of the oral cavity and upper alimentary tract may be expected, but are not necessarily present, in **bovine viral diarrhea** and **rinderpest**. ***Bovine coronavirus*** causes microscopic lesions in colonic crypts in cattle with **winter dysentery**; mild fibrinous typhlocolitis may be seen grossly. **Salmonellosis** affects all age groups from neonate to adult and may frequently involve both small and large intestine in catarrhal to fibrinohemorrhagic enteritis; mesenteric lymph nodes are usually enlarged. **Coccidiosis** may involve ileum and large intestine; it can usually be diagnosed by mucosal scraping at autopsy. ***Bovine adenovirus*** infection may cause severe hemorrhagic colitis, with few lesions elsewhere. **Arsenic**, other **heavy metals**, and **oak or acorn poisoning** may also cause hemorrhagic typhlocolitis and dysentery. Rarely, **trichuriasis** causes hemorrhagic mucosal typhlitis in calves.

Chronic fibrinous or ulcerative typhlocolitis may occur in salmonellosis, bovine viral diarrhea, and coccidiosis.

Granulomatous typhlocolitis associated with chronic diarrhea and wasting may occur in **Johne's disease**, concurrently with granulomatous ileitis and mesenteric lymphadenitis. The mucosa of the large bowel in these cases is thickened and rugose. Impressions of affected mucosa or ileocecal lymph node will contain acid-fast bacilli. Johne's disease in sheep and goats is usually associated with wasting, but often not diarrhea. The large bowel may be involved in a minority of cases; the ileum is consistently affected.

In **sheep**, hemorrhagic typhlocolitis may be present in animals with **bluetongue** and **peste des petits ruminants**; it is rarely the only lesion. **Salmonellosis** may cause fibrinohemorrhagic enteritis in lambs and pregnant ewes, and typhlitis caused by **trichuriasis** will occur rarely, although the presence of parasites in small numbers is common in weaners. **Coccidiosis** may be implicated in hemorrhagic ileotyphlocolitis in lambs and kids, though the small intestine is usually more commonly and severely involved. In goats, enterotoxemia due to *C. perfringens* type D may cause mild to moderately severe fibrinohemorrhagic typhlocolitis. Uremia may cause hemorrhagic lesions associated with vasculitis in the cecum and colon.

Neoplastic and proliferative lesions of the stomach and intestine

Tumors of the lower gastrointestinal tract are *not common* in domestic animals. However, they are relatively prevalent among surgical biopsy submissions from dogs and cats, in sharp contrast to their rarity in horses and food-producing animals. Malignant neoplasms are more common than benign tumors, and, excepting lymphosarcoma, *most are carcinomas*. Polyps are generally hyperplastic rather than neoplastic. The exceptions are rectal polyps in dogs, which are adenomas or carcinomas. Highly malignant scirrhous adenocarcinomas of the stomach and intestine occur in all species. *Lymphosarcoma* is the most common malignant tumor of mesenchymal origin in most species. It may arise in the gut, although involvement of this area is often part of multicentric disease (see Vol. 3, Hematopoietic system). The third major category of gastrointestinal tumors is *stromal*, mainly phenotypically smooth muscle or undifferentiated.

Elaborate classification schemes have been proposed based on histologic or immunophenotypic characterization with, in many cases, little evidence that they are relevant to the biological behavior of the tumor or its response to therapy. Furthermore, there is often so much variation within these lesions that it is difficult to make them conform to arbitrary categories. However, it is important to recognize the range in appearance inherent in the various neoplasms, so that they may be diagnosed correctly, in a clinically meaningful way.

Table 1.1 Major neoplasms and tumor-like proliferative lesions of the lower gastrointestinal tract of domestic animals[a]

Epithelial
- Benign
    1. Papillary adenomatous hyperplasia
    2. Gastric pyloric mucosal hypertrophy
    3. Canine rectal papillary adenoma
- Malignant
    1. Gastrointestinal adenocarcinoma
    2. Neuroendocrine carcinoma (carcinoid)
    3. Gastric squamous cell carcinoma

Stromal
- Benign
    1. Leiomyoma
- Malignant
    1. Leiomyosarcoma
    2. Gastrointestinal stromal tumor

Round cell
- Benign
    1. Plasmacytoma
- Malignant
    1. Malignant lymphoma
    2. Mast cell tumor
    3. Malignant plasmacytoma

Neoplasms metastatic to the gastrointestinal tract

[a] *Since most of these tumors are histologically and behaviorally similar regardless of where they occur in the lower gastrointestinal tract, where appropriate the tumor name can simply be preceded by the adjective* gastric, intestinal, or colonic.

The 2003 World Health Organization *Histological Classification of Tumors of the Alimentary System of Domestic Animals* lists over 50 specific neoplastic entities and over 15 additional tumor-like lesions of the lower gastrointestinal tract. *The pragmatic classification of neoplasms and proliferative lesions outlined in* Table 1.1 *is considerably simpler.* This classification, and the discussion arising from it, is based upon several fundamental principles:

Any classification of neoplasms should be:

- the simplest that is compatible with our current understanding of tumor biology, including therapeutic and prognostic information;
- based upon easily observed and objective features to allow a high degree of interobserver agreement when in use; and
- easily adaptable in response to new information about significant differences in behavior or response to therapy.

There appears to be no significant difference in the histologic appearance or the biological behavior of the various neoplasms based on their site of origin in the lower gastrointestinal tract, i.e., there is no reason to consider colonic adenocarcinomas (or lymphomas) separately from small intestinal or gastric adenocarcinomas. Prefixes such as "gastric" or "colonic" are used as descriptive anatomic terms to aid in communication of results.

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Gastrointestinal adenocarcinomas

The prevalence and distribution of gastrointestinal adenocarcinomas vary among species, although they are common in none. In dogs, gastric carcinomas predominate; small intestinal and colonic carcinomas are relatively uncommon. In contrast, gastric carcinoma is almost unknown in cats, but small intestinal and colonic adenocarcinomas are relatively common. In other species, where they are generally rare, gastrointestinal adenocarcinomas also involve mainly small intestine.

The microscopic appearance is similar, regardless of species or location. A few develop as tubular and papillary proliferations of differentiated columnar epithelial cells projecting into the intestinal lumen. This is perhaps most commonly seen with colonic carcinomas in cats. Such lesions are distinguished from rare papillary adenomatous hyperplasia because *they have at least some invasion into the lamina propria, submucosa, or tunica muscularis.* Such invasion cannot be appreciated with endoscopic biopsies, and these papillary carcinomas sometimes cannot be distinguished from benign adenomatous hyperplasia or papillary adenomas without full-thickness surgical biopsies. This becomes particularly relevant when trying to distinguish the very common rectal papillary adenoma from the occasional, truly malignant, papillary carcinoma arising in the distal colon or rectum of dogs.

The earliest recognizable histologic lesion, regardless of the location, is *local effacement or obliteration of glandular mucosal architecture at the site of origin by proliferating polygonal mucus-producing epithelial cells.* These cells infiltrate the lamina propria, and then invade sequentially through the submucosa and tunica muscularis, infiltrating into lymphatics, and sometimes veins. They penetrate the serosa and exfoliate into the peritoneal cavity to establish neoplastic implants on omentum and mesentery. The tumor also spreads early via lymphatic and venous routes, so that even surgical excision on discovery is rarely curative, because it is too late to prevent metastasis.

The microscopic appearance of the invading tumor can be quite variable, and usually more than one histologic subtype occurs within the same neoplasm. There is no proven prognostic or therapeutic significance to the different histologic patterns. *Most are scirrhous, mucus-producing carcinomas* that can create mucus-filled epithelial lakes throughout the intestinal wall, accompanied by what is often a great deal of fibrous stromal proliferation. The degree of cytologic maturation may be high, yet the diagnosis of carcinoma is obvious, given the transmural invasive behavior. Less commonly, the tumor cells invade in a tubular or acinar pattern, which tends to be less scirrhous, or as scattered individual anaplastic epithelial cells, accompanied by a great deal of desmoplastic fibrous tissue, so that recognizing carcinoma (in contrast to stromal malignancy or postinflammatory reactive fibrosis) may be challenging. In almost all cases, however, the tumor cells produce mucus, which may be revealed by periodic acid–Schiff staining. Formation of *signet ring cells* (an epithelial cell with the nucleus displaced to the periphery by a single large clear cytoplasmic vacuole) is relatively common.

The histological diagnosis is easy with a *full-thickness biopsy.* The submucosal and transmural portions of the tumor are routinely larger and more readily identified as malignancy than the mucosal portion of the tumor, which endoscopic biopsies may not capture.

Alternatively, especially when large deep ulcerative lesions are biopsied endoscopically, only the necrosis, inflammation, and fibrosis that accompany neoplastic cells may be captured. On the other hand, a false-positive diagnosis may be made when attempting to distinguish early carcinoma (especially gastric carcinoma) from dysplastic repair of recent ulceration; in such cases, a full-thickness biopsy to detect invasion is more reliable than the best endoscopic sample.

Adenocarcinoma of the stomach is most frequently reported in **dogs**, usually in animals <10 years of age. It is the most common gastrointestinal adenocarcinoma in dogs, and the most common gastric neoplasm in that species. Males predominate in the population with gastric cancer, and over half of gastric adenocarcinomas in dogs occur in the pyloric region. In the Lundehund, there may be a relationship to the chronic gastritis common in that breed, but otherwise no causal association is recognized in dogs.

Grossly, some gastric neoplasms appear as nonulcerating, firm thickenings involving most of the gastric wall and causing loss of the normal rugal pattern on the mucosal surface. Others are more localized plaque-like thickenings that tend to obliterate rugae and ulcerate centrally. Ulceration occurs in over half of canine gastric adenocarcinomas. Surface proliferation or irregularity other than ulceration is very uncommon in gastric carcinoma in dogs. Cut sections through the stomach wall invaded by carcinoma reveal edema and pale, firm fibrous tissue. Induration or plaque-like pale masses may be evident on the serosa, where the pale outline of infiltrated lymphatics may be prominent. Widespread gastric mural fibrosis and thickening, the result of desmoplasia induced by the malignant epithelium, cause "*linitis plastica*," the so-called "leather bottle" appearance.

Gastric carcinomas in dogs infiltrate the stomach wall aggressively, invading lymphatics, and they have usually metastasized to the local lymph nodes, and often to distant organs, particularly lung, liver, and adrenal, by the time they are diagnosed.

Rare cases of gastric adenocarcinoma are described in **cats**. They adopt tubular and diffuse patterns, and behave typically, but are so rare that a diagnosis of carcinoma should only be made on a full-thickness surgical biopsy or necropsy specimen. Endoscopic samples suggestive of gastric carcinoma in cats are almost always foci of adenomatous hyperplasia upon further investigation.

Gastric adenocarcinoma in **cattle** is exceptionally uncommon, but when it occurs it resembles similar tumors in other species.

Intestinal adenocarcinomas are uncommon in **dogs**; they occur most frequently in the proximal small intestine and large bowel of animals averaging 8–9 years old. Some investigators have reported a slightly higher prevalence of intestinal carcinomas in males, with a breed predisposition in Boxers, Collies, Poodles, and German Shepherds. Weight loss, persistent vomiting, anorexia, emaciation, and abdominal distension are the most common signs when the tumor is located in the small intestine. Dogs with colorectal tumors have large-bowel diarrhea, tenesmus, hematochezia, and dyschezia. Many dogs with intestinal carcinomas are anemic on account of hemorrhage from ulcerating tumors.

Macroscopically, the tumors appear as gray-white, firm, sometimes annular, stenotic areas that commonly affect the entire thickness of the intestinal wall (see Fig. 1.48). These tumors often do not ulcerate, and they usually do not project into the lumen of the gut. Papillary or polypoid intestinal adenocarcinomas do form intraluminal masses, which tend to involve larger segments of the intestine, suggesting horizontal spread. There is dilation of the gut proximal to stenotic and obstructive tumors and there may be hypertrophy of the intestinal muscularis proximal to such neoplasms.

These tumors metastasize widely, mainly via the lymphatics (Fig. 1.72). Involvement of the small intestine leads to metastases mainly in the mesenteric lymph nodes, less commonly to other abdominal nodes, liver, spleen, and lungs. Histological evidence of metastasis at the time of resection of small intestinal tumors predicts a markedly reduced postsurgical survival time, but even dogs without such evidence may succumb to metastatic disease, albeit with longer survival.

Colonic adenocarcinomas metastasize to colonic, iliac, and other pelvic and abdominal nodes. Metastases may also occur in most abdominal organs and the lungs. Implantation on serosal surfaces may result in obstruction of omental and diaphragmatic lymphatics, leading to ascites. In a few cases, malignant cells may spread retrograde in the lymphatics of the abdomen and pelvic limbs, causing edema of the abdominal wall and legs. Dogs with annular colorectal

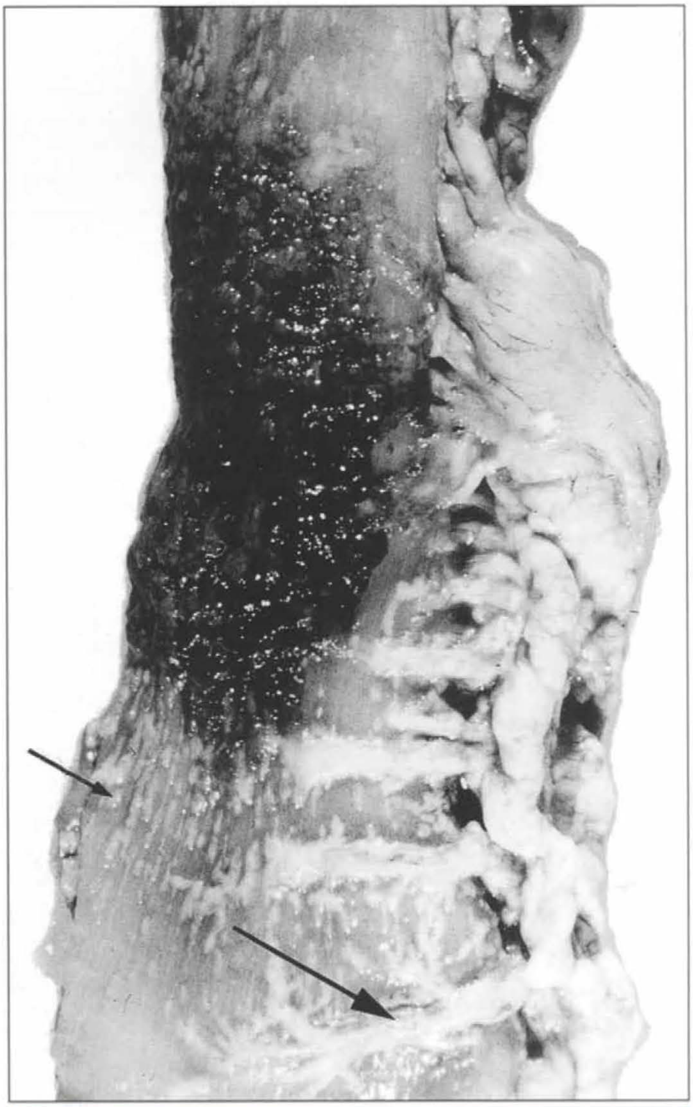

Figure 1.72 Adenocarcinoma of rectum in a dog. Serosal hemorrhage, and plaque-like masses of desmoplastic fibrous tissue and neoplastic cells on the serosa (small arrow) and along serosal lymphatics draining to the mesentery (large arrow).

carcinomas have a much shorter survival period compared to dogs that have a single, pedunculated polypoid tumor in this location. The etiopathogenesis of colonic carcinomas in dogs is uncertain, but there appears to be progression to malignancy from benign adenomatous lesions in at least some instances. β-catenin and cyclooxygenase-2 are overexpressed in malignant colonic epithelial cells in some cases, as they are in humans.

In **cats**, intestinal adenocarcinoma is less prevalent than lymphosarcoma, but it is relatively more prevalent in cats than in dogs. Carcinomas of the intestine are more prevalent in Siamese cats than in other breeds. As in dogs, a higher prevalence of this tumor has been reported in males than females. The mean age of cats with intestinal carcinomas is 10–11 years.

The ileum is the most common site affected, followed by the jejunum. Carcinomas occasionally arise in the large intestine, and when the tumor is located at the ileocecal junction, both the large and small intestine are usually involved. The clinical signs and gross appearance are similar to those in dogs.

The microscopic appearance of intestinal carcinomas in cats is typical (Fig. 1.73), except that osteochondroid metaplasia of the stroma may be a feature. The rare carcinomas involving the large intestine have tended to be papillary, better differentiated, and less scirrhous than carcinomas involving the small intestine.

Intestinal adenocarcinoma is relatively common in **sheep** in New Zealand, the UK, Iceland, Scotland, Norway, and southeastern Australia; in New Zealand and Australia, there is a high prevalence in breeds used for fat lamb production. The cause of the high prevalence is unknown, but may be related to exposure to bracken fern or other unidentified carcinogens. Other associations, such as heavy use of certain fertilizers, and pastures with the weed *Cynosaurus cristatus*, have been reported from New Zealand. Tumors occur mainly in animals 5 years of age or older. Clinically, affected sheep lose weight and have abdominal distension due to ascites, but most cases are incidental findings at slaughter.

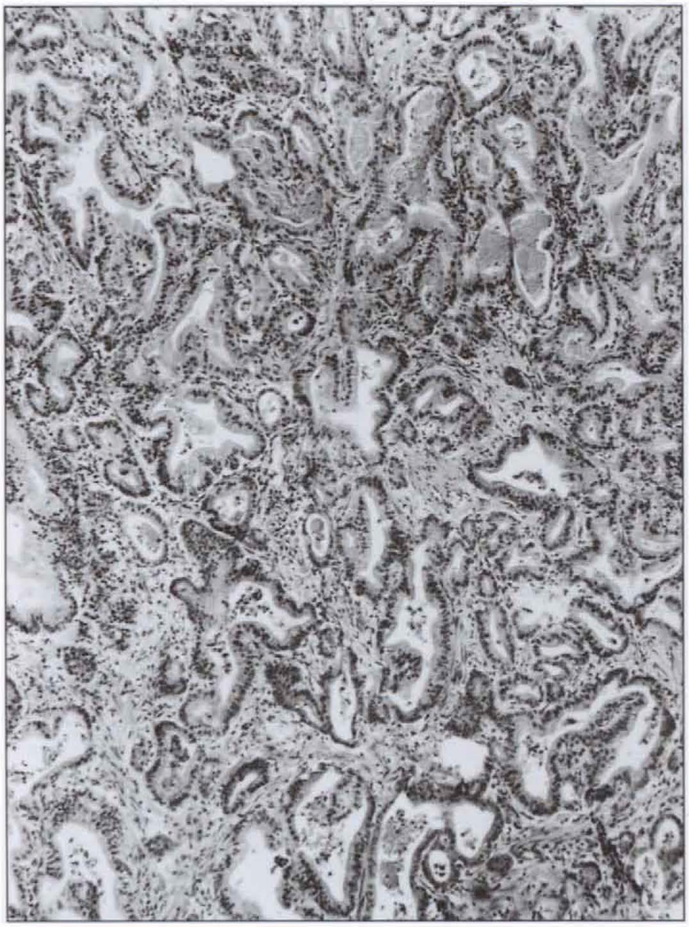

Figure 1.73 Well-differentiated **intestinal adenocarcinoma** in a cat.

The tumors are usually located in the middle or lower small intestine, rarely in the colon (Fig. 1.74). They are dense, firm, white masses, 0.5 to several centimeters long and up to 1 cm thick, which may form annular constrictive bands at the affected site. Cauliflower-like growths may be evident on the serosal surface. Polyps or plaques may protrude into the lumen, but ulceration of the mucosa is uncommon. The distal edge of the tumor is generally well demarcated. There is dilation of the intestine proximal to the lesion. Metastatic implantations on serosal surfaces are common and these appear as opaque to white plaques or diffusely thickened areas, which must be differentiated from mesothelioma. Obstruction of serosal lymphatics by tumor emboli may lead to ascites. Lung and liver metastases are rare.

Microscopically, the tumor is characterized by solid sheets or nests of well-differentiated to highly anaplastic polyhedral, cuboidal, or columnar epithelial cells that may form irregular acinar structures. They may be distributed singly, or in small aggregates, and are often difficult to detect in the heavy fibrous desmoplastic response (Fig. 1.75). The neoplastic cells infiltrate the bowel wall along lymphatics, vessels, and nerve trunks, through to the serosal surface, whence they spread to the mesenteric lymph nodes. This is apparently followed by retrograde lymphogenous metastasis to the gut wall proximal to the primary tumor. These secondary tumors are particularly responsible for constriction of the gut lumen. Sclerotic masses with anaplastic epithelial cells, many of which are periodic acid–Schiff-positive, are located on the serosal surfaces of the abdominal organs but rarely infiltrate the parenchyma. Argentaffin cells may form part of some intestinal carcinomas, especially in lymph node metastases. Mineralization and osseous metaplasia may develop in the stroma.

Intestinal carcinomas are generally rare in **cattle**, **goats**, **horses**, and **swine** (Fig. 1.76), with the exception of those associated with bracken fern, papillomavirus, and upper alimentary cancer in cattle (see section on Neoplasia of the esophagus and forestomachs, above). A high prevalence of intestinal **adenomas** is associated with these tumors in cattle. Three types are recognized: (1) a sessile plaque; (2) an adenomatous polyp; and (3) a more proliferative adenoma of the ampullae where the bile and pancreatic ducts open into the duodenum. Intestinal carcinomas are usually an incidental finding at meat inspection. The location, morphology, and routes of metastasis are similar to those described for sheep, except that serosal lesions are less obvious. Hematogenous spread to the liver, lung, kidney, uterus, and ovaries may occur in cattle.

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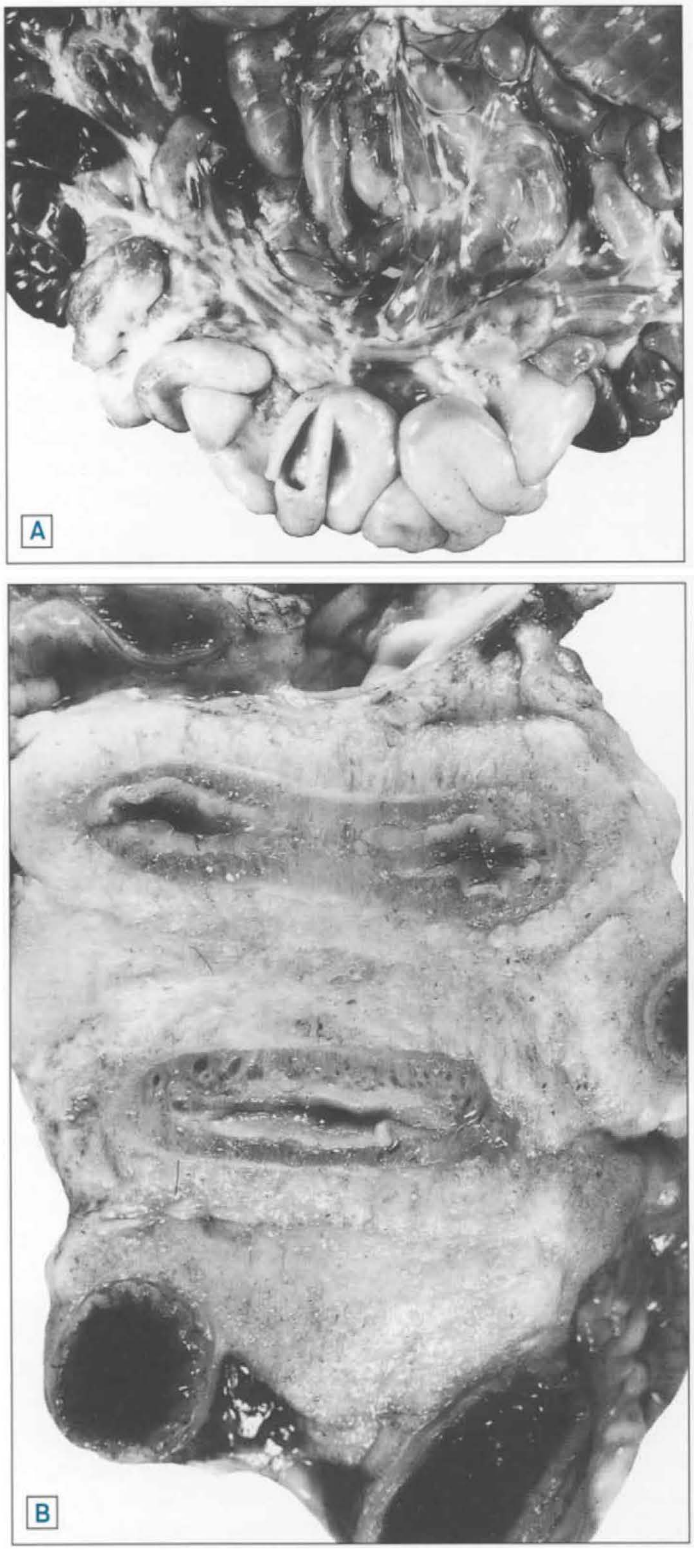

Figure 1.74 Scirrhous **intestinal adenocarcinoma** (white areas) in a sheep **A.** Invading ileum **B.** Invading colon.

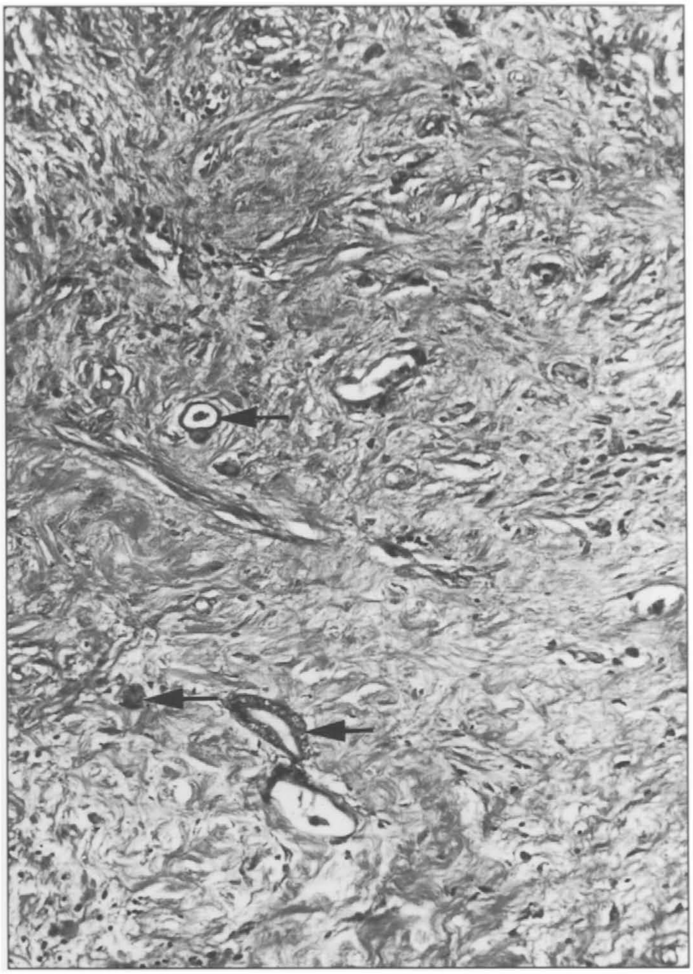

Figure 1.75 Intestinal adenocarcinoma in a sheep. Islands of neoplastic epithelium (arrows) scattered in extensive desmoplastic reaction.

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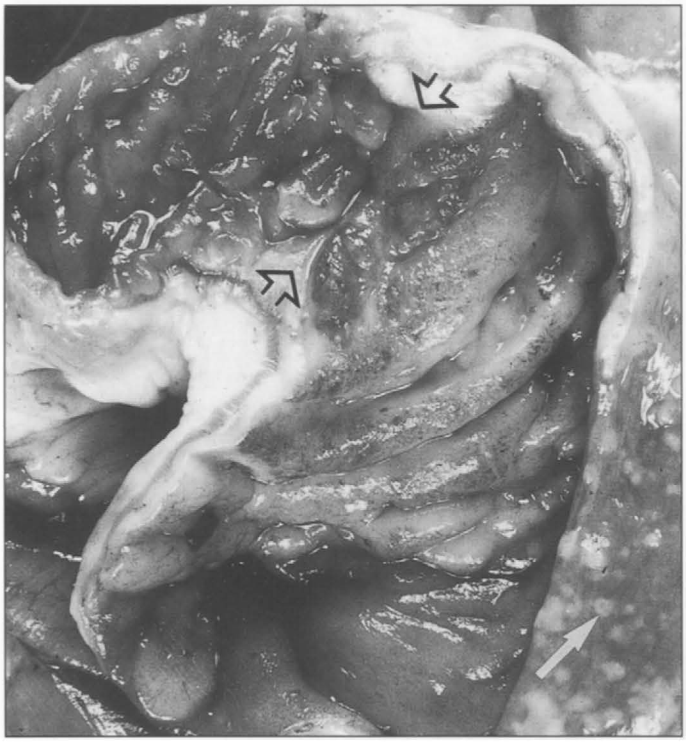

Figure 1.76 Intestinal carcinoma in a cow. There is annular thickening of intestine (open arrows) with carcinomatous serosal plaques (arrow).

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Adenomatous hyperplasia/papillary adenoma/polyps

Adenomatous hyperplasia occurs as focal papillary or papillotubular proliferation of surface epithelium anywhere in the gastrointestinal tract. In the stomach (Fig. 1.77), it is to be distinguished from chronic hypertrophic pyloric gastropathy, discussed with pyloric obstruction.

The most common site is the distal rectum of middle-aged **dogs**, within 10 cm of the anal–rectal margin. Given a variety of names, such as *adenomatous hyperplasia, papillotubular adenoma, colorectal polyp, or adenoma*, and *polypoid carcinoma in situ*, we refer to it as **rectal papillary adenoma**. It occurs in dogs of almost any age, and is usually a single nodule easily managed by adequate surgical excision. Tenesmus, prolapse of the polyp, rectal bleeding following defecation, chronic dyschezia, and diarrhea are the most common signs. There is a single case of paraneoplastic leukocytosis associated with such a tumor. Some, but not all, surveys indicate that this tumor is more common in males.

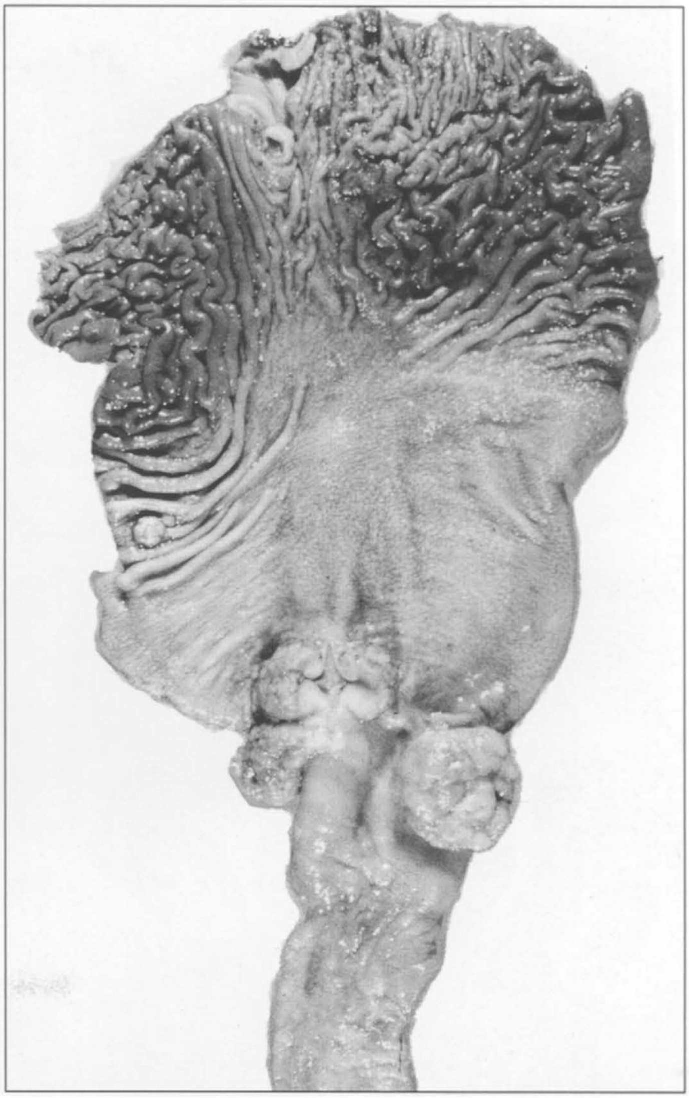

Figure 1.77 Mucosal polyps at the pylorus and along the greater curvature of the stomach of a dog.

Macroscopically, the tumor is usually sessile or slightly pedunculated (Fig. 1.78), varying from 1 cm to several centimeters in diameter. It may be firm or friable and hemorrhagic; the mucosal surface is often ulcerated.

Microscopically, the polyp may have a predominantly tubular or papillary growth pattern (Fig. 1.79). In well-oriented specimens, the tubular pattern is characterized by branching crypts that are lined by generally well-differentiated columnar-to-cuboidal pseudostratified epithelial cells. The papillary type consists of villus-like projections of proprial connective tissue that are covered by a layer of pseudostratified columnar epithelial cells. There may be cytoplasmic basophilia, loss of nuclear polarity, and prominent nucleoli in epithelium in both types of polyps. The number of mitotic figures varies, often within the same polyp. These tumors often appear to originate in the superficial

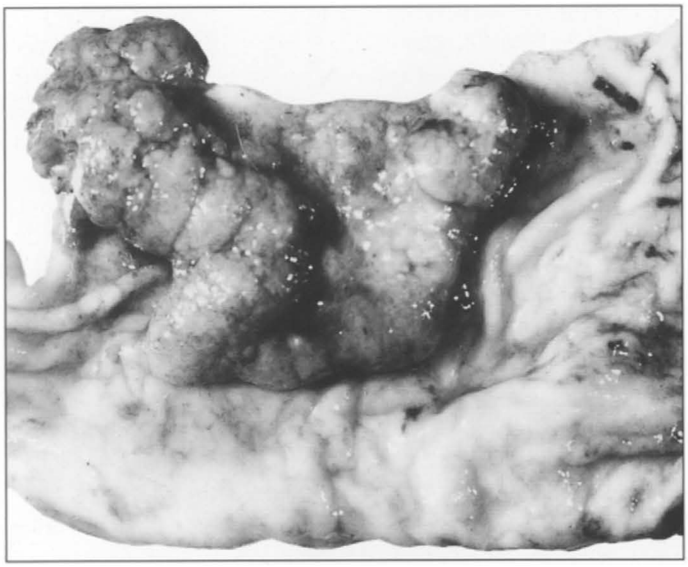

Figure 1.78 Rectal papillary adenoma in a dog.

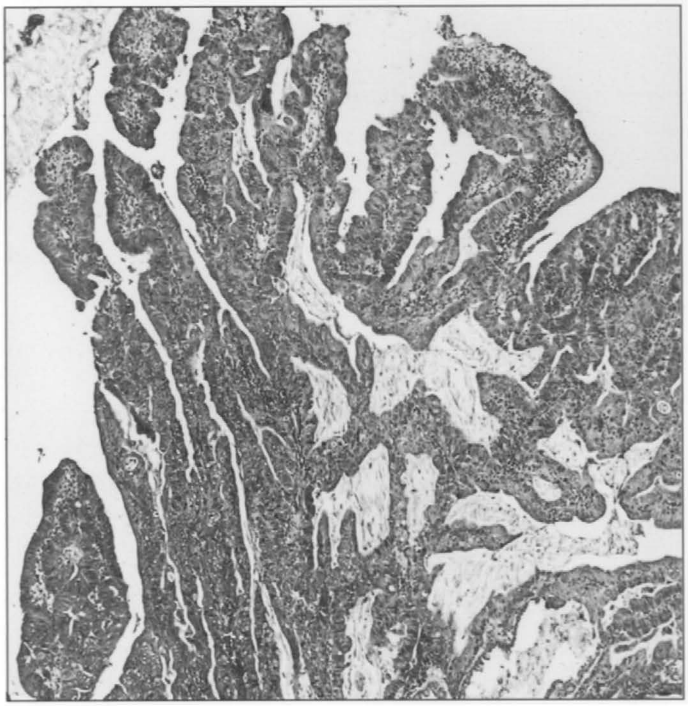

Figure 1.79 Rectal papillary adenoma in a dog, showing tubulopapillary microscopic architecture.

portion of the crypts, deeper portions of which may remain normal, with tubules or slender papillae of hyperchromatic tumor cells above them. The stalk of the tumor is highly vascular and is continuous with the lamina propria or submucosa of the rectum. The tumors are generally well demarcated from the adjacent normal mucosa. The amount of mucin in the epithelium varies considerably, and it is often absent, especially in more dysplastic cells. Some polyps have a malignant appearance histologically. These are characterized by the presence of anaplastic epithelial cells in situ in the mucosa, and, in some cases, invading the propria and adjacent submucosa (Fig. 1.80).

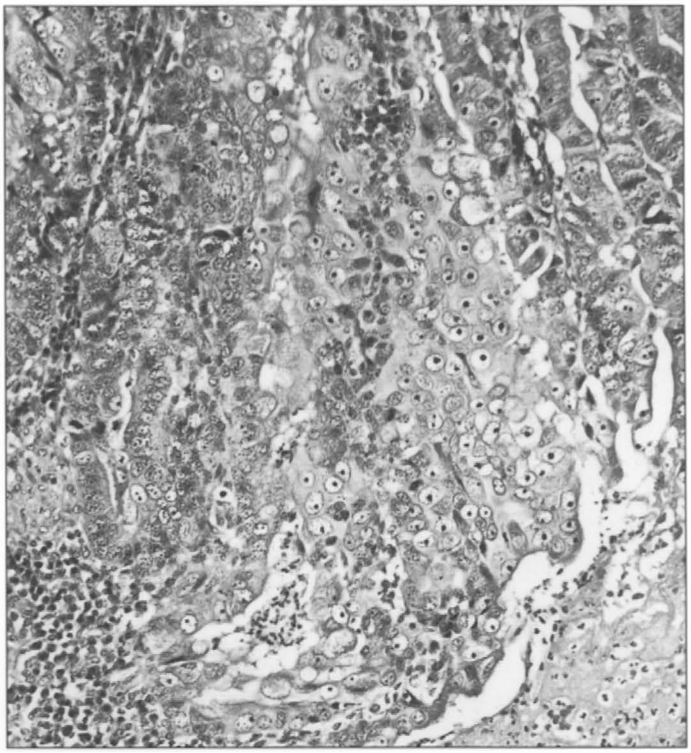

Figure 1.80 Invasive carcinoma arising from the base of a rectal polyp in a dog.

There is limited information on the *biological behavior* of these tumors. In dogs, adequate surgical removal usually results in complete recovery. Some are interpreted at presentation as carcinoma in situ, based on cytologic characteristics, and deep biopsies and/or complete excision of these polyps are essential to rule out local invasion or infiltration of lymphatics, which should be sought in specimens with epithelial morphology suggesting malignancy. Polyps that are more than 1 cm in diameter tend to have cells with a more anaplastic appearance, and recurrence is most probable in cases interpreted as carcinoma in situ, those with multiple masses, or those with more diffuse colonic involvement.

In other species, polypoid and adenomatous tumors are uncommon. Benign adenomatous polyps are reported in the stomach and duodenum of **cats**, predominantly middle-aged males of Asian breeds. They are presented with vomition, hematemesis, and possibly anemia. Excision is curative. These lesions can be confused with papillary adenocarcinomas if sampled only by endoscopic biopsy.

In **cattle**, intestinal polyps are usually an incidental finding, except in those cases where they are large enough to cause partial obstruction. The tumors are raised, often pedunculated, gray-to-brown masses on the mucosal surface. They may occur singly, in grape-like clusters, or they may be scattered. Microscopically they resemble benign rectal polyps in the dog, but they may be capable of malignant transformation (see above).

Adenomatous polyps frequently occur in **sheep** with intestinal carcinoma. Hyperplastic polyps occur in the small intestines of **lambs** and **goats** with chronic coccidiosis (see section on Infectious and parasitic diseases of the alimentary tract, later in this chapter).

Multicentric small intestinal polyposis has been associated with protein-losing enteropathy in a **horse**. The proliferative lesions caused by *Lawsonia intracellularis* in horses and swine may impart a polypoid appearance to the intestinal mucosa grossly, and microscopically should not be mistaken for adenomatous neoplasia.

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Gastrointestinal neuroendocrine carcinomas (carcinoids)

Carcinoids arise from neuroendocrine cells that are scattered in the mucosa of a wide variety of organs, including the stomach and the intestine. These cells secrete low-molecular-weight polypeptide hormones such as secretin, somatostatin and cholecystokinin, or they are part of the amine precursor uptake decarboxylation (APUD) group, producing compounds such as serotonin (5-hydroxytryptamine).

Carcinoid tumors of the gastrointestinal tract are *rare* in domestic animals. They have been reported mainly in aged dogs and very rarely in the cat, cow, and horse. In **dogs**, most carcinoids are located in the duodenum, colon, and rectum, and only rarely in the stomach and lower small intestine. Clinically, they may cause intestinal obstruction, and anemia due to ulceration and hemorrhage. Associated diarrhea in some cases can speculatively be attributed to hypersecretion of functional polypeptide hormones. Rectal carcinoids may protrude from the anus and resemble adenomatous polyps.

Macroscopically, carcinoids are usually lobulated, firm, dark-red to cream-colored masses a few millimeters to perhaps 2 cm in diameter. They seem to arise deep in the mucosa, often forming submucosal or subserosal nodules, perhaps with ulceration of overlying mucosa, and infiltrating transmurally and into the mesentery.

Microscopically, carcinoids have a *distinct endocrine appearance*. Round or oval to polyhedral cells have abundant finely granular eosinophilic or vacuolated cytoplasm and vesiculate nuclei with prominent nucleoli. They form nests, ribbons, rosettes, or diffuse sheets in the mucosa, submucosa, and muscularis. A fine vascularized fibrous stroma often divides the tumor masses into pseudoalveolar arrays. Amyloid may be present in intercellular and perivascular spaces. Uninuclear megalocytes and multinucleate giant cells are present occasionally.

A *diagnosis* of carcinoid is based on the endocrine histologic pattern, cytoplasmic argentaffinic and argyrophilic granularity, immunohistochemical identification of specific secretory products, and the typical ultrastructural appearance. The histochemical reactions may be negative, especially in rectal carcinoids, and they may also be lost during fixation in formalin or by autolysis prior to fixation, as may immunohistochemical reactivity. These cells are routinely positive with immunohistochemical staining for neuron-specific enolase and chromogranin, as are most neuroendocrine tumors, and then with one or more of the specific immune probes related to the peptides being produced. An increased concentration of specific secretory products may be detected in circulation.

Electron microscopic examination helps to differentiate carcinoids from intestinal mast cell tumors. Carcinoid cells have dense, round-to-oval, membrane-bound secretory granules in the cytoplasm, which vary in diameter from 75 to 300 nm. They have abundant rough endoplasmic reticulum and the plasma membrane forms interdigitating processes. Carcinoid cells are Schiff-negative and do not show metachromasia with Giemsa stains.

Data on biological behavior of intestinal carcinoids in dogs are limited. Most cases reported have been malignant. There may be extensive invasion of the gut wall and veins, with metastasis, especially to the liver. The few cases that have been described in other species have features similar to those described in dogs.

Goblet-cell carcinoids (adenocarcinoids, mucinous carcinoids) are tumors that are most commonly found in the appendix of humans, with features of both carcinoids and adenocarcinomas. There is a single report of such a tumor in the rectum of a dog. Microscopically, goblet-cell carcinoids have distinct areas of mucinous adenocarcinoma and carcinoid that may merge. The carcinoid component is most prominent in the primary tumor and the metastases.

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Gastric squamous cell carcinomas

Squamous cell carcinomas derived from the mucosa of the pars esophagea are the most common gastric tumor in **horses**. They occur predominantly in middle-aged or older animals, which are usually presented in an advanced state, with unexplained anorexia, occasionally dysphagia, and weight loss sometimes progressing rapidly to emaciation. At autopsy there may be peritoneal effusion, and there is usually evidence of the neoplasm as plaques of proliferative or scirrhous tissue on the serosa of the stomach. There may also be peritoneal implants, especially on intestine, testes, omentum, parietal abdominal surfaces and diaphragm; direct extension to adjacent organs including liver, spleen, and diaphragm, with progression to the pleural space; and sometimes distant metastases, usually in liver and lung. The appearance of the tumor on serosal surfaces resembles mesothelioma, with smooth creamy plaques or nodules up to 2–4 cm in diameter.

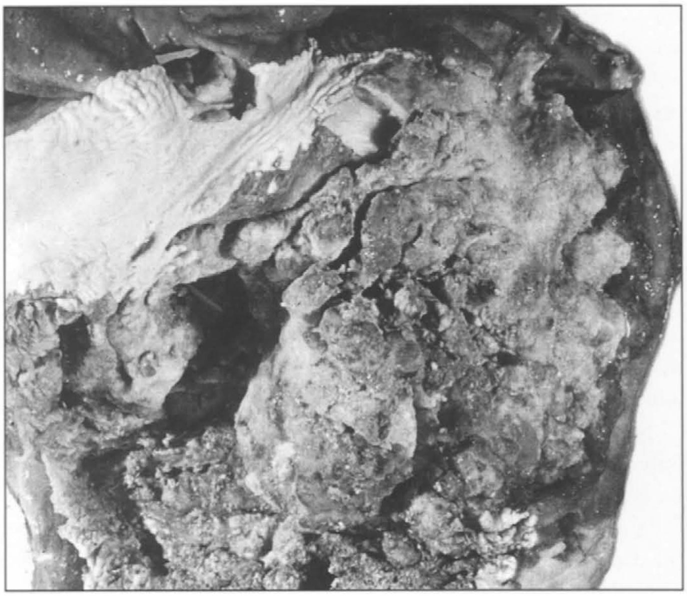

Figure 1.81 Fungating and ulcerative **squamous cell carcinoma** arising from the gastric squamous mucosa of a horse.

The origin of these lesions is usually a fungating cauliflower-like mass 10–40 cm in diameter, with superficial fissures, projecting above the surface of the pars esophagea (Fig. 1.81). Sometimes these lesions are superficially more ulcerative than proliferative. Necrosis and hemorrhage are evident in the tumor mass, which is usually well demarcated from adjacent normal squamous mucosa. Occasionally the tumor extends into the distal esophagus, and may obstruct it. Microscopically, these neoplasms are typical squamous cell carcinomas, invading in cords or nests of cells through the gastric wall. They induce desmoplasia, imparting a scirrhous, firm texture and appearance to the thickened gastric wall and to the peritoneal and pleural implants. One such tumor has been reported as a cause of pseudohyperparathyroidism in a horse.

A squamous cell carcinoma is reported arising from the pyloric gland mucosa in a dog.

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Malignant lymphomas

Gastrointestinal lymphomas occur in most species and are common in **cats**. These neoplasms may be primary, or part of the systemic or multicentric form of the neoplasm (see Vol. 3, Hematopoietic system). Primary lymphoma of the intestine includes cases having malignant lymphocytic infiltrates in the intestine, with or without involvement of the abdominal organs or bone marrow, but with no lesions in the thorax or peripheral sites.

Malignant lymphoma is the most common gastrointestinal neoplasm in companion animals, more prevalent in cats than in dogs. It can be segmental within any portion of the gastrointestinal tract, or diffuse. While the gastrointestinal tract may be involved in advanced multicentric lymphoma in dogs, such cases are rarely submitted for biopsy because the diagnosis has already been made based on lesions elsewhere. Most samples submitted for histologic assessment are from cases suspected of having primary gastrointestinal lymphoma. In cats, almost all cases examined at biopsy are primary within the stomach or intestine.

In both species, spread outside intestine is usually to mesenteric lymph nodes and to liver, and less commonly beyond. In liver, the neoplasm infiltrates in a characteristic portal pattern that should not be mistaken for chronic portal hepatitis or lymphocytic cholangiohepatitis. In lymphoma, the tumor cells often form layers 10–20 deep in the portal tract, and cholangiolar proliferation, fibrosis, and hepatocellular atrophy are absent. In advanced hepatic disease, the cells will also surround central veins. Although scattered tumor cells may occur within the sinusoids, a primarily sinusoidal distribution of lymphocytes signals lymphocytic leukemia rather than intestinal lymphoma.

The classification of lymphomas in animals is complex, integrating distribution, histologic and cytologic appearance, and immunophenotype. Most categories are based on lymphomas discovered in lymph nodes or skin; as a group, intestinal lymphomas have received little detailed investigation in this context. Most of the controversy about intestinal lymphoma is related not to its diagnosis, but rather to the relationship (if any) between its classification and subsequent behavior, including response to therapy; currently no clear association is evident.

In **dogs**, intestinal lymphoma can affect animals from adolescence to old age, with a male sex bias. Nonspecific signs of enteric disease and weight loss may be acute or occur over a period of weeks to months. Hypoproteinemia, probably associated with enteric protein loss, occurs in ~30% of affected dogs.

The tumors are located in the small intestine, stomach, and colon in that order of frequency. They are soft-to-firm, cream-colored masses located in the submucosa that may protrude into the gut lumen. The overlying mucosa may be ulcerated. The masses can be nodular to diffuse and several sections of the gut are usually affected. The mesenteric nodes are often enlarged and the liver is frequently involved at the time of presentation.

The majority of primary gastrointestinal lymphomas in dogs are *epitheliotropic T-cell tumors* based on immunohistochemical reactions, the remainder being mixed, B-cell, or indeterminate. Microscopically, in typical cases of epitheliotropic intestinal lymphoma, the mucosal, submucosal, and muscular architecture is effaced by a population of monotypic large lymphocytes with variable nuclear morphology and obvious mitotic activity. The crypts literally "disappear" as they become infiltrated by neoplastic cells.

The lesions of *very early lymphoma* are more challenging. They are distinguished from chronic inflammatory bowel disease by the *relatively monotypic lymphocyte population* and by the *intraepithelial infiltration of lymphocytes* that obscures the distinction between lamina propria and epithelium. Even with these criteria, differentiation of early neoplastic from inflammatory disease can be problematic, since residual plasma cells, eosinophils, and benign lymphocytes of the normal lamina propria persist among the neoplastic lymphocyte population. Similarly, not all cases of intestinal lymphoma exhibit epitheliotropic growth, and there is overlap between neoplastic epithelial invasion and the increase in intraepithelial lymphocytes that is commonly seen with inflammatory disease. The key is not just the presence of the lymphocytes, but the *obliteration of the proprial:epithelial boundary*. Once the lymphoma effaces crypts or invades submucosa, diagnosis should not be problematic, but full-thickness biopsies from several areas of the intestine may help differentiate chronic inflammatory bowel disease from intestinal lymphoma.

Lymphoplasmacytic enteritis in the dog, particularly the Basenji, may represent a prelymphomatous stage similar to immunoproliferative small intestinal disease in humans. The latter is characterized by diffuse lymphoplasmacytic infiltration of the small intestinal mucosa that results in malabsorption, and predisposes to the development of primary enteromesenteric lymphoma.

In **cats**, the situation is substantially more complicated. There are at least three histologic categories of gastrointestinal lymphoma. However, no correlation is evident between immunophenotype and ultimate biological behavior or response to treatment. It is also fairly common in cats to have sharply segmental disease, and some such cases can be cured by surgical excision.

Small-cell lymphocytic villus lymphoma is an epitheliotropic T-cell lymphoma that typically begins at the base of the villi within the small intestine of old cats. Clustering of suspiciously monotypic small hyperchromatic lymphocytes within the lamina propria just at the base of villi is an exceedingly common observation in cats 15 years of age and older. It is not clear whether this is incipient lymphoma or a normal finding. The number of such cells may vary substantially from villus to villus. As these clusters seem to expand, the lymphocytes infiltrate into the overlying epithelium, obliterating the distinction between lamina propria and epithelium. Unlike inflammatory bowel disease, such lesions may be very patchy, and there is no concurrent increase in plasma cells or eosinophils. With time, the lymphocyte population appears to expand throughout the lamina propria and then transmurally. The syndrome shares substantial clinical overlap with inflammatory bowel disease in that it is often slowly progressive and it is usually not accompanied by clinical signs related to neoplastic infiltration of liver, spleen, or lymph node, even though there is often microscopic tumor within those organs. The extent of microscopic disease within the intestine is usually much greater than the macroscopic impression at the time of surgery.

Large-cell/lymphoblastic lymphoma, in contrast, is characterized by a rapidly progressing transmural lesion that is usually accompanied by clinically palpable intestinal masses and by markedly enlarged mesenteric lymph nodes. Cats of any age may be affected. Most of these are reported as B-cell tumors, although not all investigators concur, and the majority of these high-grade lymphomas have not been subjected to immunophenotyping. The tumor resembles canine lymphomas, in that there is usually transmural effacement of intestinal architecture, and obliteration of the structure of affected mesenteric lymph nodes. Many cases differ from canine lymphomas, however, in having marked cellular pleomorphism. The tumor cells may have fairly abundant cytoplasm, nuclear cleavage, and up to twofold anisokaryosis. Descriptive names like "histiocytic" lymphoma for some of these more anaplastic variants are no longer appropriate, since immunophenotyping is available to identify the cells more precisely. Lesions are often sharply segmental and the primary lesion can be completely excised. However, the high prevalence of metastatic disease makes surgical cures rare.

Large granular lymphoma is usually a rapidly progressive intestinal lymphoma of cats. The tumor cells are either T cells or natural killer cells. These are medium-to-large lymphocytes with pleomorphic nuclei with frequent clefting. The distinctive features are the large red cytoplasmic granules, and perforin immunoreactivity. In the presence of cleaved nuclei, the large granules may be mistaken for those of eosinophils, but they are larger, rounder, and are more intensely eosinophilic. The cytologic characteristics have also caused such tumors to be attributed to globule leukocytes in the past. The granular cells are not present as a pure population. They are often intermingled with other lymphocyte types and with macrophages, so these invasive and destructive transmural lesions are sometimes confused with transmural granulomatous disease or mast cell tumors. These tumors progress rapidly, with widespread dissemination and even leukemia, and they may perforate affected areas of intestine.

In the **horse**, alimentary lymphoma is a relatively common tumor; most are attributed to *B lymphocytes*. It occurs mainly in young adults. Affected horses lose condition, likely due to malabsorption and protein loss; in addition they may be anemic, icteric, and have mild intermittent bouts of colic. Diarrhea is inconsistent. Serum albumin can often be decreased, but they are frequently hypergammaglobulinemic.

Macroscopic lesions in horses are usually located in the small intestine, and these are characterized by local to diffuse thickening of the gut wall with prominent rugae on the mucosa. Nodules or plaques with fibrous adhesions may be evident on the serosa. The mesenteric nodes are markedly enlarged. Other nodes may be enlarged, but to a lesser extent. Microscopically, there is diffuse lymphoid infiltration of the lamina propria and submucosa, usually extending transmurally to the serosa. There is marked villus atrophy to the point of complete loss of villi and crypts. Plasmacytoid cells are regularly present in the lamina propria but less numerous in the submucosa. Similar lymphoid cell infiltration involves the mesenteric nodes and the perinodal connective tissue. Other lymph nodes are involved in about half the cases. A plasmacytoid or plasmacytic reaction and occasional giant cells are also present in the nodes. The neoplastic lymphoid cells are probably of B-cell origin and "home" into the gut-associated lymphoid tissue and the lamina propria of the small intestine.

An immune-mediated type of *dermatitis* has been associated with both chronic inflammatory (granulomatous and eosinophilic enteritides) and lymphomatous lesions in the gut in horses.

Epitheliotropic T-cell lymphomas resembling those in dogs, and ulcerating large granular lymphomas caused by T cells, somewhat resembling those described in cats, also occur infrequently in horses. A T-cell lymphoma occurred concurrently with multisystemic eosinophilic epitheliotropic disease in a horse, prompting the suggestion that undetected T-cell lymphoma secreting interleukin-5 may underlie that syndrome. Paraneoplastic eosinophilia has also been associated with intestinal lymphosarcoma in the horse.

In **cattle** and **sheep**, alimentary lymphomas are generally part of the adult multicentric form of the disease, and the intestinal lesions resemble the effacing tumors described in other species. More important in cattle is lymphosarcoma of the abomasum, which is common in adult cattle. Diffuse mucosal and submucosal lymphocytic infiltrates or nodular proliferations may occur. Strategically placed pyloric tumor may cause obstruction. Diffuse lesions, thickening the gastric wall, frequently ulcerate, and hemorrhage from such ulcers, as melena, is a common sign. The lymphoid infiltrates are recognizable as firm gray-white tissue in the submucosa and mucosa. Involvement of abomasal lymph nodes is disproportionately slight.

In **swine**, diffuse gastric lymphosarcoma occurs. The wall of the stomach is thickened by submucosal lymphocytic infiltrates, which sometimes invade the mucosa locally in many areas, producing nodular elevations that may ulcerate. B-cell lymphomas involving the Peyer's patches have also been detected at meat inspection in swine.

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Gastrointestinal mast cell tumors

These tumors are *uncommon*. They occur in aged dogs, and occasionally in cats, with signs such as vomition, diarrhea, and melena. They are seen in biopsies as invasive round cell tumors that can resemble carcinoids, lymphomas, and gastrointestinal stromal tumors; *definitive diagnosis requires histochemical and possibly immunohistochemical investigations*. Primary mast cell tumors of the gastrointestinal tract are comprised of mucosal mast cells, which differ from connective tissue mast cells, such as those found in skin, in having few granules, which usually do not stain well in formalin-fixed tissue. Abnormal mast cells do not appear in circulation in animals with these tumors.

Mast cell tumors are most commonly seen in stomach, and least common in the colon. Affected areas in the mucosa are tan-colored, firm, thickened, possibly ulcerated, and may be 1 cm to several centimeters in size. The tissue architecture is effaced by a population of granular round cells accompanied by variable numbers of eosinophils. They generally grow in infiltrative cords, but occasionally form endocrine-like packets surrounded by a delicate fibrovascular stroma. There is a wide range in the cytologic appearance. Some tumors are populated by fairly mature mast cells with abundant cytoplasmic granularity easily demonstrated with toluidine blue. Others are populated by anaplastic and pleomorphic round cells or spindle-shaped cells, sometimes with giant nuclei or forming syncytia, in which cytoplasmic granules with metachromatic staining, while present, may be difficult to observe. Those with abundant metachromatic granules must be differentiated from connective tissue mast cell tumors metastatic to the gastrointestinal tract, in which case lesions should be sought in skin and elsewhere. There can be marked variation in the number of eosinophils in the tumor, and they are not definitive, since eosinophils are also common in some intestinal lymphomas, and as part of the background cell population in normal lamina propria.

Gastrointestinal mucosal mast cell tumors are differentiated using a suite of histochemical and immunohistochemical characteristics. They have at least a few *metachromatic granules* when stained with toluidine blue, and many tumors have at least some cells that stain with alcian blue. They stain *immunohistochemically positive* for mast cell tryptase and c-KIT (CD117), but negatively for CD3 (distinguishing them from most T-cell lymphomas), cytokeratin (distinguishing them from carcinomas), and chromogranin and synaptophysin (distinguishing them from carcinoids). Gastrointestinal stromal tumors should be positive for c-KIT, but cytology and lack of metachromatic granules when stained with toluidine blue differentiate them.

Ultrastructurally, the cytoplasm of gastrointestinal mucosal mast cell tumor cells contains many membrane-bound granules that appear as single or fused vesicles. Fine fibrillar material forms a loose network within these vesicles. A few tumor cells contain electron-dense fibrillar granules or intermediate forms. The crystalline electron-dense granules that are present in connective tissue mast cells, such as those from the skin, are rarely evident.

Metastases occur most often in the mesenteric lymph nodes, followed by the liver, spleen, and, rarely, the lungs. Ulceration of the gastrointestinal mucosa occurs commonly with systemic tissue mast cell tumors in cats and with large cutaneous mastocytomas in dogs, due to histamine stimulation of acid production by gastric parietal cells. Gastrointestinal ulceration is not a feature of mucosal mast cell tumors of intestinal origin, except perhaps as a result of mucosal effacement by the tumor.

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Extramedullary plasmacytomas

Plasmacytomas of the lower gastrointestinal tract are *uncommon neoplasms in dogs*, and rare in cats and other species. They are encountered most frequently in the submucosa of the distal colon and rectum of dogs, where they are associated with signs of large-bowel diarrhea and bleeding.

Histologically, they resemble plasmacytomas of the skin, oral cavity, or larynx. The tumor is formed by solid packets of pleomorphic round cells with various degrees of plasmacytoid maturation, especially at the periphery of the tumor. There is frequent nuclear hyperchromasia and convolution. The cells are typically arranged in solid endocrine-like packets of 10–20 cells surrounded by a delicate fibrovascular stroma, and there may be AL amyloid deposition among the tumor cells. A few syncytial plasmacytoid or histiocytic cells may be evident. The majority of the tumor growth is submucosal, with a little bit of overflow into the deep half of the lamina propria. Most tumors have a discrete local growth habit amenable to surgical cure. A small proportion exhibit more aggressive behavior, including invasion of tunica muscularis, and some spread to regional lymph nodes and spleen, perhaps producing a monoclonal gammopathy.

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Intestinal stromal tumors

The intestinal stromal tumors include leiomyoma, leiomyosarcoma, gastrointestinal stromal tumor, and very uncommon examples of almost every other type of stromal neoplasm, including hemangiosarcoma, nerve sheath tumors, fibroma and fibrosarcoma, osteosarcoma, and even lipoma. Ganglioneuromas have been described in dogs, a sow, horse, and a kitten. Other mesenchymal tumors include fibrous histiocytoma in a cow and histiocytic sarcoma in a dog.

Leiomyoma, **leiomyosarcoma**, and **gastrointestinal stromal tumor** are considered together, since recognition of the latter prompted re-evaluation of the criteria for diagnosis of leiomyosarcoma; older literature should be read with this in mind. Together, they are more common in dogs than other types of mesenchymal tumors except lymphosarcoma, and they also occur with some frequency in horses. Smooth-muscle tumors occur uncommonly in cats, equally prevalent in stomach, small intestine, and colon. They occur only rarely, if at all, in other species.

Leiomyoma and leiomyosarcoma represent the two extremes of a morphologic continuum, which included, toward the malignant end of the spectrum, tumors now classified as gastrointestinal stromal tumor. The latter are thought to be derived from interstitial cells of Cajal, or from a stem cell precursor to both those cells and smooth-muscle cells, and they are distinguished by expressing KIT (CD117) antigen. While some may contain smooth-muscle actin, they rarely express desmin. Leiomyomas and leiomyosarcomas, on the other hand, do not express KIT, and should express smooth-muscle actin, and desmin. *Immunohistochemical reactivity is the definitive means of differentiating these tumors*, but may be adversely affected by autolysis and duration of fixation, which must be considered in such studies. However, leiomyomas can be recognized based on their histology and cytology.

In **dogs**, as a group these tumors occur in older animals, in many of which they may be asymptomatic and incidental. Signs can include weight loss, lethargy, anorexia, anemia due to intestinal hemorrhage, abdominal pain, palpable abdominal masses, diarrhea, vomition, and dehydration or other signs of gastrointestinal obstruction. Based on limited studies in which the immunophenotype of stromal tumors of the gut has been determined, leiomyomas are most common and tend to occur in the esophagus and stomach of predominantly male dogs. Gastrointestinal stromal tumors are the next most common, and may occur in the stomach and intestine, with a tendency to predominate in the large bowel; sex distribution is equal. They have a spectrum of behavior from benign to malignant, and include tumors that were diagnosed as leiomyosarcomas prior to revision of terminology. Leiomyosarcomas are seemingly least common, and tend to occur in the intestine, with no apparent sex predisposition.

The majority of symptomatic tumors of this type are expansile nodules arising in the muscularis externa and can be cured by surgical excision, as is certainly the case with leiomyoma. However, a minority, perhaps up to 30%, of the other types have metastasized by the time that they are discovered.

Leiomyomas are sharply circumscribed masses of well-differentiated smooth-muscle cells, and generally lie beneath an intact mucosa, although they may deform the serosal or mucosal profile, and ulceration, perhaps related to pressure necrosis, can occur. They are comprised of swirling bundles of uniform fusiform cells, with abundant eosinophilic cytoplasm, and a central nucleus with blunt ends; usually no mitotic figures are evident. Areas of coagulative necrosis may occur in the tumor mass.

Leiomyosarcomas often resemble leiomyomas grossly, but they are invasive rather than circumscribed, and may ulcerate mucosa. The cells resemble those in leiomyomas in general cytology, but there is usually some degree of anisokaryosis, multiple nucleoli, scattered bizarre nuclei, and obvious mitotic activity. There may be necrosis in the mass and a mild lymphocytic or eosinophilic inflammatory infiltrate.

Gastrointestinal stromal tumors may be demarcated and grow by expansion, sometimes forming exophytic nodules on the serosal surface, or they may be transmural and invasive. Overlying mucosa, while usually intact, may ulcerate. They are usually comprised of spindle cells arranged as interlacing fascicles, or in a storiform (whorling or matted) pattern, with oval nuclei, and a somewhat basophilic cytoplasm with indistinct boundaries. A minority of cases may have somewhat epithelioid tumor cells arranged in trabeculae or solid sheets, perhaps with a somewhat myxoid intercellular matrix, often infiltrating between normal smooth-muscle fascicles. Epithelioid tumors are thought to reflect some degree of neural differentiation, while a storiform pattern is considered to reflect a poor degree of differentiation. There may be nuclear pleomorphism in all patterns of tumor, and mitotic figures are usually evident, and may be common. Hemorrhage or necrosis may be present within the tumor, and lymphocytic and/or eosinophilic infiltrates can occur.

The difficulty in determining the *prognosis* of these tumors in dogs has not changed with the recognition that the great majority are gastrointestinal stromal tumors, rather than leiomyosarcomas. No objective criteria or nomenclature differentiate benign from malignant gastrointestinal stromal tumors. In humans, larger tumor size and high mitotic activity are considered to be poor prognostic indicators, but there are no data substantiating these tendencies in animals.

Invasion into the mucosa causes ulceration and cavitation on the luminal surface (Fig. 1.82), which may lead to perforation of the

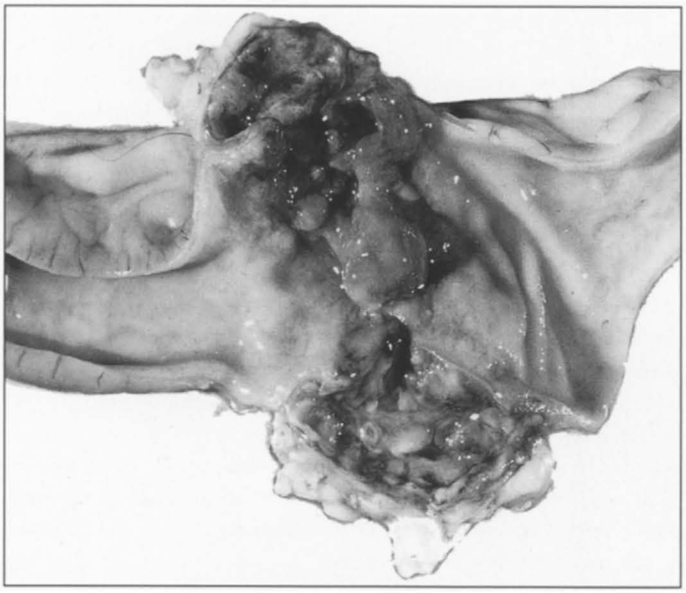

Figure 1.82 Annular ulcerating **leiomyosarcoma** in a cat.

bowel. Even in the absence of perforation, microorganisms from the gut lumen may cause local abscessation and secondary septic peritonitis in which there is intermingling of suppuration, granulation tissue, and tumor cells. Based on studies carried out prior to revision of the nomenclature, tumors in cecum and colon, where gastrointestinal stromal tumors predominate, were determined to have a relatively high metastatic risk. They may metastasize to the mesenteric nodes, mesentery, and liver, but in general, these tumors have a more favorable prognosis after complete resection than other malignant neoplasms of the lower gastrointestinal tract.

The large intestinal tumors may also cause *paraneoplastic hypoglycemia*, due to the production of insulin-like growth factors, that may precipitate the search for a neoplasm. Production of erythropoietin, resulting in erythrocytosis, has also been documented.

In old **horses**, tumors of the intestine have been described that in many cases meet morphologic or immunohistochemical criteria for gastrointestinal stromal tumor, as it has been described above. Most are encountered incidentally, at surgery, meat inspection, or necropsy, but tumors of this type, or previously diagnosed as leiomyomas, may cause intermittent bouts of colic that become more frequent with time. They occur in the stomach, small intestine, and most frequently in the cecum and colon, as encapsulated, moderately firm, multinodular, pale tan, or hemorrhagic masses in the muscularis or subserosa, protruding on the serosal surface, or rarely, as somewhat pedunculated tumors projecting from the serosa or into the lumen. They may vary from 1 to >10 cm in diameter, and are usually solitary, but occasionally may be multiple. Malignancy, if it occurs, must be rare; it is not described.

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APPROACH TO THE DIAGNOSIS OF GASTROINTESTINAL DISEASE

The diagnosis of gastrointestinal disease is facilitated by knowledge of the entities that may be expected in a particular species and age group, and the clinicopathologic syndromes with which they are associated. *In the next section, etiologic entities associated with various syndromes in the domestic species are briefly summarized.* The salient diagnostic features of some conditions, or the steps required at necropsy to confirm a diagnosis, are mentioned. The diseases causing gastritis, and those causing typhlocolitis in all species, have been discussed in previous sections. They are only briefly reiterated here in a general context. Further information on the pathogenesis and diagnosis of most infectious and parasitic conditions is found in the next section.

Diarrhea in neonatal ruminants, swine, and horses

Diarrhea causing dehydration, metabolic acidosis, and electrolyte depletion is an important cause of morbidity and mortality in neonatal piglets, calves, lambs, and kids, and to a lesser extent in foals. Some classes of agents occur in most species of large animals, and mixed infections may occur; these include *Escherichia coli*, coronavirus, rotavirus, and *Cryptosporidium*. These, and some other less common agents, produce diarrhea in neonatal animals, the etiology of which cannot be readily differentiated on clinical grounds or on the basis of gross examination at necropsy.

Undifferentiated diarrhea of neonatal animals requires etiologic diagnosis if appropriate advice is to be rendered on prevention and management of the problem. Many of the agents involved are transiently present, or produce villus atrophy that is obscured by autolysis, hindering diagnosis. To overcome these obstacles, *one or more live untreated animals in the early phase of clinical disease, and representative of the herd problem, must be examined.* They should be killed and examined immediately using a necropsy procedure modified so that specimens of small intestine are fixed within a few minutes of death, and so that appropriate samples of tissue and content are quickly collected for etiologic investigation.

The presence of specific microscopic lesions, and their distribution in the intestine, may suggest an etiology. Culture or application of rapid immunologic methods to gut content and fixed or frozen tissues may identify a bacterial or viral agent. *Cryptosporidium* and coccidial agents may be identified in Giemsa-stained mucosal scrapings as well as in tissue sections. The prevalence of diarrhea in neonates, and the main agents associated, can be expected to vary considerably on the basis of locality, climate, and season, and the management system, as well as by species.

In all species, neonatal animals succumbing to the effects of undifferentiated diarrhea are overtly *dehydrated.* The eyes are sunken in the orbits, the skin lacks elasticity, and the subcutis is tacky. There is usually fecal staining on the perineum, but a rare acute case may dehydrate without significant diarrhea. Scouring animals may continue to suck during the early phase of their illness, a milk clot or curd may be present in the stomach, and internal fat depots may be adequate. Animals that lose interest in feed due to more chronic disease, and those taken off nutrients, may have serous atrophy of fat and appear cachectic. The small intestine is flaccid, dilated, and often thin-walled, and fluid content is present throughout the full length (Fig. 1.83). The content is usually watery and clear green-yellow. It often separates into two phases, the more solid of which appears to consist of small masses of clotted milk or mucus. Sometimes the content is more homogeneous and creamy in texture. In animals examined some time after spontaneous death, this appearance is due to postmortem slough of epithelium. The large intestine similarly contains fluid, creamy or pasty content, usually white or yellow. The mucosa of the small intestine may appear glistening and mildly congested; that in the large intestine is usually unremarkable. The forestomachs in ruminants that have been tube-fed, or in which the reticular groove has apparently failed to close, may contain sour fermented milk or milk replacer and the mucosa may be mildly reddened. The abomasal mucosa of some calves may contain scattered focal stress-associated hemorrhages. In piglets, urates, precipitated as the result of dehydration, may be present in the renal medulla and pelvis.

In each species, undifferentiated diarrhea of neonates may be caused by other agents, in addition to the common bacterial, viral, and protozoal causes cited above. Conditions distinguishable at necropsy from undifferentiated diarrhea, and caused by a further array of pathogens, are also found in most species. The spectrum of agents associated with diarrhea in neonates of each species will be considered below.

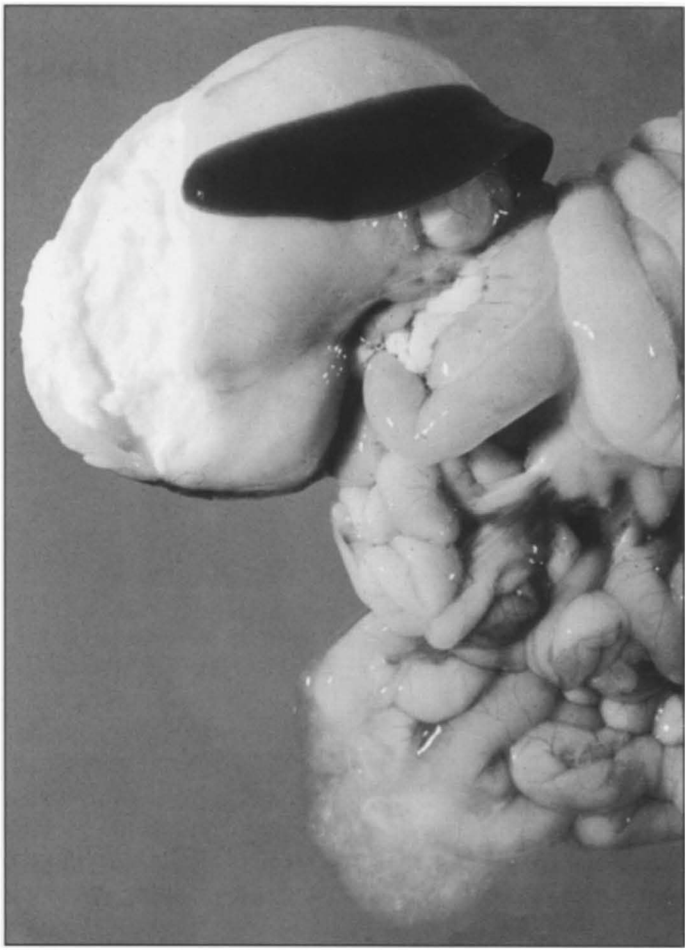

Figure 1.83 The stomach and intestines of a piglet with **undifferentiated neonatal diarrhea**; clotted milk fills the stomach, the small intestine is flaccid, and filled by floccular fluid content, with no gross evidence of inflammation.

Diarrhea in calves

About 10 infectious agents have been associated with undifferentiated diarrhea in calves under about 3 weeks of age, and a further four or five syndromes associated with diarrhea in neonates can often be recognized at necropsy. Frequently, combinations of several of these agents occur together, or in sequence. The consequences of combined infections experimentally often appear to be more severe than infections with individual agents. The diagnosis of these infectious problems in a herd can be complex, and examination of more than one animal early in the course of disease is desirable. Noninfectious diarrhea and emaciation, resulting from the use of nutritionally inadequate milk replacers containing heat-denatured protein, must also be considered and eliminated when examining artificially reared animals.

Enterotoxigenic *E. coli* *is probably the most common single cause of undifferentiated neonatal diarrhea in calves.* Little or no overt morphologic change may be evident in tissue section, though bacteria are seen attached to the surface of enterocytes on villi. The disease usually occurs within the first week of life, and is confirmed by specific immunofluorescent techniques in smears and frozen sections, or by culture and characterization of the organism as enterotoxin-producing. Enterotoxigenic colibacillosis is distinct from the less-common **enteropathogenic colibacillosis**, caused by attaching-effacing organisms, and from **septicemic colibacillosis** and other gram-negative infections occurring in hypogammaglobulinemic neonates. Occasional cases of **enterohemorrhagic colibacillosis**, caused by **Shiga toxin-producing *E. coli*** and producing fibrinohemorrhagic enterocolitis, occur in calves, usually in the second week of life.

Bovine coronavirus and ***Rotavirus*** are responsible for a significant proportion of neonatal calf diarrhea, alone, together, or in combination with other agents. Disease due to these viruses may begin at any time in the first 1 or 2 weeks of life. Both produce villus atrophy by causing lysis or exfoliation of surface enterocytes. *Bovine coronavirus* also commonly causes microscopic, and occasionally gross, lesions in the colon. Both agents may be confirmed by demonstration of virus in feces, or viral antigen in infected epithelium.

Cryptosporidium, a minute apicomplexan protist, is commonly associated with undifferentiated diarrhea and villus atrophy in calves. It is most common in animals about 4–20 days of age. Cryptosporidia are recognized in the brush border of epithelium on villi, and in mucosal smears and fecal smears or flotations.

Giardia, a flagellate protist, has been implicated as an etiologic agent in diarrhea of calves and other young ruminants, but giardiosis is infrequently diagnosed in this context.

Bovine torovirus (Breda virus 2), in genus *Torovirus*, family Coronaviridae, has been recovered from diarrheic calves; though antibody is common in some surveys, disease seems rare. The pathogenesis and lesions caused by the Breda agent are similar to other coronavirus infections, though some crypt cell necrosis is reported in the small intestine.

Astrovirus infections usually appear to be subclinical, and their implication as a cause of diarrhea in calves is rare. Astrovirus infects enterocytes on the villus surface, causing lysis or exfoliation of cells, and transient villus atrophy. Several **noroviruses** (family Caliciviridae, genus *Norovirus*; formerly "Norwalk-like viruses"), or bovine enteric caliciviruses (BEC), have been identified from the feces of diarrheic calves, including norovirus Jena, Newbury agents 1 and 2, and the Nebraska BEC.

Enteroviruses are commonly isolated from the feces of clinically normal animals, and they are likely not associated with significant disease, though diarrhea has occurred in some experimental studies. **Birnavirus** has also been isolated from the feces of diarrheic calves, but with no proof of pathogenicity.

A **bovine enteric syncytial virus**, a group B rotavirus, was identified as a cause of diarrhea in calves in a beef herd. Experimentally, it causes diarrhea, and distinctive syncytia formed by enterocytes on the sides of villi are present in tissue sections of the small intestine.

Bovine parvovirus infects cells in crypts of Lieberkühn in the small and large bowel, and may cause mild to moderate diarrhea, particularly in the postweaning period. Mild necrosis and exfoliation of cells in crypts may occur in the small intestine, with some villus atrophy and fusion.

Bovine viral diarrhea virus can be implicated in enteritis of calves as young as a week or so of age, on the basis of microscopic lesions in the small intestine and colon similar to those found in older animals, and by virus isolation, or antigen or genome identification.

Chlamydophila* infection** is very rarely recognized in association with enteritis. It may be acutely necrotizing and exudative microscopically. ***Strongyloides infection may also cause villus atrophy and diarrhea in calves as young as 2–3 weeks of age. Enterotoxigenic ***Bacillus fragilis*** has been suggested as a cause of diarrhea in neonatal calves, but its role is unproven.

A number of other syndromes are usually distinguishable on the basis of gross lesions at necropsy in diarrheic calves. ***Clostridium perfringens* types B and C** are usually associated with acute hemorrhagic enteritis and dysentery; infection with these organisms is rare.

Salmonellosis, usually due to *Salmonella* Typhimurium, or perhaps *S.* Dublin or *S.* Muenster in enzootic areas, occurs in calves as young as 4 or 5 days of age. It is especially common in stressed calves weaned to milk replacer and moving through sales barns as vealers. Salmonellosis may mimic undifferentiated neonatal diarrhea, perhaps in association with rotavirus, coronavirus, or *Cryptosporidium*. Or it may cause acute fibrinous enterocolitis, with septicemia in a proportion of cases.

Bovine herpesvirus 1 (infectious bovine rhinotracheitis virus) in calves under 2 weeks of age may produce gastrointestinal lesions associated with generalized infection; more commonly the enteric lesions occur alone or dominate the picture. Characteristic foci of necrosis in the mucosa of the forestomachs, perhaps associated with focal ulceration of the abomasum and crypts of Lieberkühn, are the typical findings.

Mycotic rumenitis and abomasitis are complications secondary to infectious bovine rhinotracheitis in calves, or possibly following abomasal venous infarction in salmonellosis.

Diarrhea in other neonatal ruminants

Undifferentiated diarrhea in neonatal lambs is mainly associated with enterotoxigenic ***E. coli***, ***Rotavirus*** and ***Cryptosporidium***; ***Giardia*** may contribute, but its significance is poorly defined. **Astrovirus** has been found in lambs and experimentally produces diarrhea. An **adenovirus** antigenically related to *Ovine adenovirus-2* is reported as a cause of enteritis in goat kids. **Salmonellosis** may occur rarely in young lambs. **Septicemia** is more commonly associated with certain serotypes of ***E. coli***, which may be the cause of "watery mouth" in lambs. ***Bacteroides fragilis***, producing enterotoxin, has been implicated as a cause of diarrhea in neonatal lambs. Causes of undifferentiated diarrhea in young goats are poorly defined, but the problem appears to be minor under usual conditions of husbandry. A similar spectrum of agents may be expected and sought.

Lamb dysentery, due to *Clostridium perfringens* type B in lambs and kids under 8–10 days of age is usually recognized at necropsy as severe hemorrhagic enteritis, occasionally with necrotic ulcers.

Coccidiosis due to *Eimeria* spp. in lambs and in kids may occur in animals as young as 3 weeks of age. Raised white plaques or polypoid masses of infected epithelium are found in the terminal ileum, perhaps associated with hemorrhage.

Strongyloides may also be associated with diarrhea in ruminants only a few weeks old.

Diarrhea in neonatal swine

The agents commonly implicated in undifferentiated diarrhea in piglets under 3 weeks of age are enterotoxigenic ***E. coli***, coronavirus (transmissible gastroenteritis), *Rotavirus*, and *Isospora suis*.

Enterotoxigenic colibacillosis typically occurs in piglets under a week of age, and causes no specific gross or microscopic lesions, other than organisms adherent to the brush border of enterocytes. **Rotavirus** and ***Transmissible gastroenteritis virus*** (TGEV, coronavirus) infections cause villus atrophy, in contrast to most types of *E. coli*. ***Isospora suis*** tends to occur in piglets over 5–6 days of age. It is associated with villus atrophy, and in a minority of cases, necrotic enteritis in the distal small intestine. Meronts or gamonts may be found in mucosal smears, and in epithelium on atrophic villi in section.

A number of other agents are less commonly associated with undifferentiated neonatal diarrhea in pigs. ***Porcine epidemic diarrhea virus*** (coronavirus 777) infection is similar to TGEV infection. **Nongroup A rotaviruses**, antigenically distinct from group A *Rotavirus*, produce similar lesions experimentally; group B cause epithelial syncytia, in addition. **Adenovirus** infection, most commonly evident as intranuclear inclusion bodies in epithelium on the dome over Peyer's patch in tissue sections, may be implicated as a cause of villus atrophy in occasional cases. **Enteroviruses**, **astroviruses,** and **calicivirus** have poorly defined significance as pathogens in piglets, though a calici-like virus causes villus atrophy in gnotobiotic pigs. ***Cryptosporidium*** infects the large and small intestine in swine, where it seems to play a minor role as an enteric pathogen. ***Salmonella*** and ***Klebsiella***, though rarely implicated as pathogens, can cause villus atrophy and diarrhea in neonatal pigs, and some strains of ***E. coli*** with the same capacity are rarely encountered. ***Bacteroides fragilis*** is also rarely associated with diarrhea. None of these bacterial infections are common in piglets. ***Strongyloides ransomi***, transmitted in the milk, may infect young piglets, causing villus atrophy, malabsorption, protein loss, and diarrhea.

Hemorrhagic and necrotizing enteritis due to ***Clostridium perfringens*** type C in piglets in the first week of life is readily recognized at necropsy. ***C. difficile*** also causes diarrheal disease in piglets in this age group, with a distinct suite of gross and microscopic lesions.

Diarrhea in foals

In the young foal, undifferentiated diarrhea may be associated with ***Rotavirus***, ***Cryptosporidium***, and ***Strongyloides***. Enterotoxigenic *E. coli* and coronaviruses are not proven pathogens in foals. ***Actinobacillus equuli*** may cause severe diarrhea and hemorrhagic enteritis, with lesions of bacteremia evident in other organs at necropsy. **Salmonellosis** in foals may be seen as fatal diarrhea with few gross lesions, or as fibrinous enterocolitis and septicemia, similar to that seen in older horses. ***Escherichia coli*** and ***Klebsiella pneumoniae***, with occasional gram-positive infections, are common causes of neonatal septicemia, predisposed by failure of passive transfer of immunoglobulin. Fibrinonecrotic enterocolitis in foals under a week of age may be due to ***Clostridium perfringens* type B or C**, or ***C. difficile***. ***Rhodococcus equi*** may cause chronic diarrhea and wasting in foals. Pyogranulomatous ulcerative lesions of the large bowel, and purulent mesenteric lymphadenitis, often associated with chronic purulent bronchopneumonia, are characteristically found in *R. equi* infection. ***Enterococcus* (*Streptococcus*) *durans***, an enteroadherent coccus, has been associated with diarrhea in foals. Moderate atrophy of villi covered with adherent gram-positive cocci is characteristic; the large bowel is not colonized. ***Strongyloides westeri*** is capable of causing diarrhea in young foals occasionally. **Tyzzer's disease**, which is restricted to foals under about 6 weeks of age, may be associated with diarrhea. However, the liver lesion dominates the pathologic picture.

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Gastroenteritis in cattle over 3 weeks of age

Acute or subacute gastroenteritis associated with systemic disease and, usually, upper alimentary lesions, may be due to **bovine viral diarrhea/mucosal disease**, **rinderpest**, and **malignant catarrhal fever**. In the latter disease, lymphadenopathy occurs, and gross lesions may be expected in other organ systems. **Acute salmonellosis** occurs as enterocolitis with enlarged mesenteric lymph nodes, but without upper alimentary lesions. **Arsenic poisoning**, **oak poisoning**, and consumption of **mustard seed** or **superphosphate fertilizer** may cause acute hemorrhagic gastroenteritis. Acute diarrhea and enterocolitis, sometimes hemorrhagic, may be caused by **adenovirus** infection in calves and feeder animals. **Coccidiosis** in calves over 3 weeks of age and older cattle usually causes dysentery and fibrinohemorrhagic typhlocolitis. **Winter dysentery**, an erosive colitis caused by *Bovine coronavirus* infection, is only occasionally fatal. Diarrhea due to **grain overload** is associated with lesions of chemical rumenitis and usually with a history or evidence of excess carbohydrate intake. **Hemorrhagic bowel syndrome** (jejunal hemorrhage syndrome) with segmental jejunal mucosal necrosis and hemorrhage, and functional or mechanical obstruction of the gut causing severe abdominal discomfort of sudden death, is discussed with *Clostridium perfringens* type A, with which it has been circumstantially associated.

Gastrointestinal parasitism may be associated with acute or chronic diarrhea and wasting in calves and young cattle at pasture. The nematode fauna is usually mixed, but certain genera of helminths may dominate in particular epizootiologic circumstances. Most important is ***Ostertagia***, which causes abomasitis with mucous metaplasia and hyperplasia. Intestinal nematodes such as ***Strongyloides*** and ***Nematodirus*** may cause diarrhea in calves at pasture or in semiconfinement. ***Trichostrongylus*** and ***Cooperia*** in adequate numbers are commonly pathogenic for pastured animals. ***Toxocara vitulorum*** may have some significance in warm climates.

Enteritis is often not grossly recognized in the small bowel at necropsy of animals with intestinal helminthosis. The pathologist needs, therefore, to have a high index of suspicion to come to a diagnosis in some cases. To make a firm diagnosis of intestinal helminthosis it is necessary to demonstrate substantial populations of nematodes by worm count in intestinal content or digests, and ideally to observe villus atrophy in tissue sections of infected intestine. These findings should be associated with a compatible syndrome – in the case of *Ostertagia*, *Strongyloides*, and the intestinal trichostrongyles, inefficient weight gain or wasting, and usually diarrhea. The hematophagous genera ***Haemonchus*** and ***Bunostomum*** cause anemia and hypoproteinemia, and associated poor growth but without diarrhea. ***Oesophagostomum radiatum***, in the colon, may result in anemia, hypoproteinemia, and diarrhea as well as causing nodule formation in the gut wall. ***Trichuris*** may also cause occasional significant hemorrhagic typhlitis, wasting, and diarrhea.

Subacute-to-chronic diarrhea and poor growth or emaciation in cattle may also be due to **copper deficiency**, **chronic bovine viral diarrhea**, **salmonellosis**, **yersiniosis**, **chronic coccidiosis**, and, in cattle over about 2 years of age, **Johne's disease** (paratuberculosis). **Congestive heart failure** and other causes of portal hypertension may also cause chronic diarrhea in individual animals.

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Gastroenteritis in sheep and goats over 3 weeks of age

The major causes of diarrhea and ill-thrift in sheep and goats at pasture are parasitic. The main helminth species causing this syndrome are ***Ostertagia***, ***Nematodirus***, and ***Trichostrongylus***. The genera and species involved vary with the geographic area and climate, and perhaps seasonally, as well as with the age group and other local epizootiologic factors. Larval **paramphistomes** will also cause enteritis in lambs in enzootic areas. ***Strongyloides*** may affect lambs up to several months of age, even those held in confinement. Where it occurs, ***Nematodirus*** is usually a disease of lambs 1–3 months of age. **Trichostrongylosis** usually affects lambs 3 months to yearling age. ***Oesophagostomum columbianum*** may cause significant disease in sheep, as well as causing nodule formation in the intestinal submucosa. ***Trichuris*** typhlitis is an uncommon problem in sheep, as is ***Chabertia*** infection of the colon. ***Bunostomum*** and especially ***Haemonchus*** cause marked anemia and hypoproteinemia without diarrhea. Hemonchosis is a particular problem in lambs and kids at pasture or held in semiconfinement in warm humid climates. It is also important in ewes and does during the postparturient period, or spring, when retarded larvae emerge synchronously from the abomasal mucosa, and mature.

Coccidiosis is a problem mainly in lambs and kids reared in confinement, on feedlot or heavily stocked on pasture. Villus atrophy due to mixed-species infections may be responsible for ill-thrift and diarrhea in older animals, while *Eimeria arloingi* and *E. christenseni* or *E. ovina* and *E. ahsata* may be responsible for mortality in very young kids and lambs respectively.

Acute enterocolitis and septicemia due to ***Salmonella* Arizonae** infection may occur in lambs and sheep. **Yersiniosis** causes fibrinous enterocolitis and caseous mesenteric lymphadenitis. **Grain overload** may cause acute diarrhea in feedlot lambs and in sheep offered supplemental feed. Diarrhea may also be associated with ***C. perfringens* type D enterotoxemia** in sheep and goats, though animals may die very acutely. Where it occurs, ***Peste-des-petits-ruminants virus*** causes acute severe enteritis with upper alimentary lesions in sheep and goats.

Johne's disease causes a syndrome of wasting, usually without diarrhea, in sheep and goats; in goats, caseous mesenteric lymphadenitis may occur. **Schistosomiasis** may cause ill-thrift in sheep in enzootic areas.

Terminal (regional) ileitis of lambs is a syndrome characterized by reduced weight gain, diarrhea, and apparent abdominal pain, usually in 1–4 month-old animals. It is reported from the UK, continental Europe, and North America. At necropsy, animals are typically in poor body condition, with evidence of diarrhea. There is marked enlargement of the caudal small intestinal mesenteric lymph node. The terminal small intestine is thickened, with transmural edema, and a corrugated appearance on the serosal aspect. The mucosa is reddened, and thrown into thick transverse nodular folds. There may be superficial erosion and fibrin exudation. The mucosa of the cecum and spiral colon may also be grossly thickened.

Microscopic lesions are always evident in the ileum, decreasing in frequency and severity with distance cranial and caudal to the ileocecal orifice. In the small intestine, there is moderate to subtotal villus atrophy, associated with hypertrophy of crypts, hyperplasia of crypt epithelium, and a heavy mixed inflammatory cell infiltrate in the lamina propria. Microerosions or ulcers may be present, through which neutrophils exude. In the large intestine, the crypt epithelium is hyperplastic, causing thickening of the mucosa, which is infiltrated by a mixed, mainly mononuclear, inflammatory cell population, and is thrown into folds superficially. Peyer's patches and submucosal lymphoid aggregates are hypertrophic, with lymphoid hyperplasia.

The lesion is compatible with a chronic immunoinflammatory response in the bowel, and it may be due to *Border disease virus*.

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Gastroenteritis in swine over 3 weeks of age

Rotavirus and ***Transmissible gastroenteritis virus*** may be responsible for villus atrophy and diarrhea in susceptible pigs of any age, though significant mortality is generally restricted to neonates and nursing pigs. Considerable morbidity but low mortality occur in the later suckling, weanling, and feeder age groups. **Postweaning diarrhea** is associated with villus atrophy of uncertain etiology; it is discussed with villus atrophy in the section on epithelial renewal in health and disease, above. ***Escherichia coli*** causes postweaning enterocolitis, and Shiga toxin-producing strains of *E. coli* produce "**gut edema**," causing sudden death or nervous signs in weaner and feeder pigs. No mucosal lesions are evident, but edema of the gastric submucosa and mesocolon may be found, among other lesions.

Enterocolitis due to ***Salmonella***, ***Lawsonia intracellularis*** infection, **swine dysentery**, and **intestinal spirochetosis** generally occur in weaner and feeder swine, though proliferative hemorrhagic enteropathy, swine dysentery, and intestinal spirochetosis occasionally involve suckling animals older than about 3 weeks of age. The differential diagnosis of these conditions and **trichuriasis** has been discussed previously in the section on typhlocolitis in swine. **Coccidiosis**, other than that in neonates due to *Isospora suis*, is rare in swine. Occasional ileitis or, less commonly, typhlitis is caused by infection with several species of *Eimeria*.

Porcine circovirus type 2 (PCV2) has been associated with watery diarrhea and weight loss, often accompanied by pneumonia, in weaned pigs. Grossly, the mucosa of the distal small intestine and colon is thickened. Microscopically, a prominent lymphoplasmacytic/histiocytic infiltrate is evident in the lamina propria and submucosa. There is lymphocytolysis and atrophy of follicles in gut-associated lymphoid tissue, and irregular basophilic intracytoplasmic inclusions characteristic of PCV2 infection are evident in macrophages in lamina propria and lymphoid tissue. Immunohistochemical staining for circoviral antigen stains these inclusions, which are presumably phagocytosed viral inclusions from lytic-infected lymphocytes. Similar inclusions are evident in bronchiolar epithelium and alveolar macrophages in lungs affected with bronchointerstitial pneumonia. Other lesions characteristic of PCV2 infection, including lymphadenopathy, hepatitis, and interstitial nephritis, are also present.

Hemorrhage into the intestine may occur in **proliferative hemorrhagic enteropathy** and **mesenteric torsion**, and should be differentiated from the melenic content found in animals with **esophagogastric ulcer**.

Ascaris suum may contribute to diarrhea and ill-thrift in weaner and feeder pigs. The thorny-headed worm ***Macracanthorhynchus hirudinaceus*** may, under rare circumstances, cause ill-thrift or intestinal perforation. Submucosal nodules in the intestinal wall may be caused by ***Oesophagostomum*** infections, which are otherwise usually relatively inconsequential in swine. Thin-sow syndrome has been attributed to **gastric hyostrongylosis**.

Enteritis in horses

Most of the significant gastrointestinal diseases of horses are discussed elsewhere, including salmonellosis, *Lawsonia*-associated proliferative enteropathy, clostridial colitis, antibiotic-associated diarrhea, Potomac horse fever, granulomatous enteritis, and parasitism. The reader should consult portions of the chapter dealing with displacements, obstruction, ischemic disease of the bowel, syndromes associated with malassimilation and protein-losing enteropathy, and typhlocolitis, for discussions putting these conditions into a diagnostic context. The capacity of the equine large intestine probably compensates for the additional fluid load posed by diseases of the small intestine that in other species might be expected to produce diarrhea. Horses presented with a syndrome reflecting hypoproteinemia, usually without diarrhea, may have *Lawsonia* infection, chronic salmonellosis, chronic inflammatory bowel disease or granulomatous enteritis, or enteric lymphosarcoma.

"**Proximal enteritis**," also known as *ulcerative duodenitis*, is characterized by signs of upper small intestinal ileus, including depression and nasogastric reflux. There is debate about including ulcerative duodenitis of foals, associated with gastric ulcers, in the syndrome. We do so here, on the grounds of similar lesions in advanced cases.

At surgery or necropsy, there is fibrinous enteritis or ulceration involving the duodenum, and a variable amount of more distal small bowel. The lesions may be segmental and circumferential, only a few centimeters long, or more diffuse, involving much of the small intestine, but sparing the ileum. The affected segment of bowel may be thickened, congested, or hemorrhagic on the serosal aspect, and there may be fibrinous peritonitis with adhesions, and pancreatitis. Perforation may occur. If the lesion is of some standing, there may be significant stricture at the site. Affected animals often have a distended stomach, with abnormally fluid content, and gastric ulcers, particularly of the pars esophagea. Perforation or rupture of the stomach may occur. Duodenal lesions should always be sought in foals with gastroesophageal ulceration.

Microscopically, there is edema and congestion, with progressive necrosis from the tips of villi in early lesions, advancing to mucosal or transmural acute fibrinonecrotic enteritis, grading to the development of granulation tissue and fibrosis in the bed of more chronic ulcerative lesions.

The etiology is undetermined. Clostridia, *Salmonella*, mycotoxicosis, and ischemia associated with the use of nonsteroidal anti-inflammatory agents have been proposed, but not firmly implicated. The segmental, often circumferential, nature of the lesions is compatible with ischemia, perhaps complicated by secondary bacteria.

Fibrinous enteritis in the small bowel may also occur in blister beetle intoxication. Enterocolitis associated with use of nonsteroidal anti-inflammatory drugs is discussed in the section Intestinal ischemia and infarction.

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Enteritis in dogs

Rotavirus and ***Canine coronavirus*** infection appear to be causes of uncommon, generally nonfatal, diarrhea in young dogs. A variety of other viral agents have been associated with diarrhea in dogs, usually on the basis of electron microscopic observations or virus isolation from feces. These include **astroviruses**, **adenovirus**, and **paramyxovirus**, as well as **calicivirus** and **herpesvirus** antigenically related to *Feline calicivirus* and *Feline herpesvirus*. The pathogenic significance of these agents is unclear.

Canine parvovirus 2 is by far the most significant cause of gastroenteritis in the dog. It is distinguished by the severe fibrinohemorrhagic enteritis usually evident at necropsy, and the radiomimetic microscopic lesions in the gastrointestinal mucosa, lymphoid, and hemopoietic tissues. Dogs with **canine distemper** often develop diarrhea; *Canine distemper virus* apparently does infect the intestinal cryptal epithelium. However, the enteric lesions of canine distemper have not been well described.

Salmonella infection is common, but salmonellosis and enteritis due to other gram-negative bacteria such as ***Yersinia*** is rare in dogs. **Proliferative enteritis** associated with intracellular bacteria is discussed under *Lawsonia*. ***Enterococcus durans*** has been associated with diarrhea in dogs. "**Salmon poisoning**" in the Pacific Northwest of the USA is due to rickettsial infection transmitted by the fluke *Nanophyetus*.

Hemorrhagic enteritis is associated with **clostridial overgrowth**, and may also be due to **reduced perfusion**, and **coagulation defects**, which may be congenital, part of disseminated intravascular coagulation, or due to anticoagulant rodenticide intoxication. **Castor beans** (*Ricinus communis*) contain ricin, a glycoprotein known to cause gastroenteritis, vomiting, diarrhea, and occasionally death.

Ascarid infection in puppies may be associated with ill-thrift, diarrhea, and, in heavy infestations, occasionally with death. **Hookworms** may cause anemia, hypoproteinemia, and ill-thrift, or, in neonatal puppies, occasional acutely fatal infection. ***Isospora*** infection may cause diarrhea in puppies, and occasional mortality. **Giardiasis** is associated with chronic diarrhea in some dogs.

Syndromes of malabsorption and protein loss associated with granulomatous or eosinophilic enteritis, "filled villi," and lymphangiectasia have been discussed elsewhere. The differential diagnosis of colitis, including idiopathic colitis, parasitic colitis, protothecosis and histoplasmosis, *Campylobacter*, and spirochetal colitis, has been discussed previously in the section on typhlocolitis in dogs. Ulcerating or widely infiltrating **colonic neoplasms** may be associated with diarrhea.

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Enteritis in cats

With the exception of **feline panleukopenia**, enteritis in cats is relatively rare. ***Feline leukemia virus*** has been associated with a syndrome of cryptal necrosis in the intestine, resembling panleukopenia, but without lymphoid or bone marrow depletion. **Astrovirus**, enteric **coronavirus**, **calicivirus**, **rotavirus**, and other viruses are reported from the feces of cats, but are only rarely associated with diarrhea, and even less frequently associated with mortality. A **torovirus** has a questionable association with a syndrome of diarrhea and bilateral protrusion of the nictitating membrane. Diarrhea is a common sign in cats with ***Feline immunodeficiency virus*** infection, and may occur in cats with *Feline leukemia virus* infection. Bacterial infections such as **salmonellosis**, **shigellosis**, **yersiniosis**, **helicobacteriosis**, and **Tyzzer's disease** are unusual, as is significant parasitic enteritis due to **helminths**, ***Cystoisospora***, ***Toxoplasma***, ***Giardia***, or ***Cryptosporidium***. Chronic inflammatory small-bowel disease and the forms of colitis occasionally encountered in cats have been considered in previous sections.

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INFECTIOUS AND PARASITIC DISEASES OF THE ALIMENTARY TRACT

Viral diseases of the alimentary tract

Foot-and-mouth disease

Foot-and-mouth disease (FMD) is a highly contagious viral infection of all cloven-hoofed animals. It is a problem of worldwide concern, being enzootic in large areas of Africa, Asia, and parts of Europe and South America. Presence of the virus imposes serious trade restrictions that effectively thwart the development of a healthy agricultural economy.

FMD is an acute febrile condition characterized by the formation of vesicles in and around the mouth, on the feet, teats, and mammary glands. The disease is not notable for high mortality, except in sucklings, but morbidity is very high, with a concomitant loss of productive efficiency.

FMD virus (FMDV) belongs to the genus *Aphthovirus* (aphtha = ulcer) in the Picornaviridae family. The virus is highly resistant under many circumstances, but is inactivated by direct sunlight, due to drying and increase in temperature, and by moderate acidity (pH < 5.0). The acid production that accompanies rigor mortis in carcasses and meat inactivates the virus. However, the alteration in pH is not dependable and the virus survives in offal, viscera, lymph nodes, and bone marrow for an indefinite period under refrigeration. Next to the movement of infected animals, contaminated animal products are likely the most common mechanism of spread. It may survive on hay and other fomites for several weeks.

The resistance of FMDV is of epidemiological significance, especially where control policies involve slaughter rather than vaccination. The importance of a carrier state in the epidemiology of FMD is uncertain. The carrier state has been observed in cattle, sheep, goats, and African buffalo (*Syncerus caffer*), but not in pigs. The carrier state may persist for up to 2 years postinfection in cattle, even in animals with a significant level of serum-neutralizing antibody. Sheep and goats are considered to be a frequent inapparent source of dissemination of the virus. African buffalo can carry FMDV for at least 5 years. Field outbreaks have been associated with buffalo–cattle contact in Africa, but these appear to be rare. Although Asian water buffaloes (*Bubalus arnee*) may be affected in FMD outbreaks, it is not known whether they remain carriers.

Of equal importance to the persistence of the virus is its *antigenic heterogeneity and instability*. There are seven principal antigenic serotypes, namely, the classical A, O, and C types, and SAT-1, SAT-2, SAT-3, and Asia-1. These can be distinguished by serologic tests. Six of the seven serotypes (O, A, C, SAT-1, SAT-2, SAT-3) are known to occur in Africa, four (O, A, C, Asia 1) in Asia, and three (O, A, C) in Europe and South America, although recent pandemics are blurring these geographic distinctions. These serotypes are sufficiently different immunologically that *infection with one type does not confer resistance to the other six*. Within these seven major types there are antigenic subtypes, each different, to variable degrees, from the parent type. Generally, the subtypes cross-immunize to a useful degree, but exceptions do arise and become recognizable, especially when vaccination fails. *Antigenic drift* can also be demonstrated experimentally; new subtypes can be produced by passing the virus in immune or partially immune animals, or by growing the virus in vitro in the presence of immune serum. There are presently over 70 distinct antigenic strains of the virus of natural origin.

As well as differences and variability in antigenic characters, strains of the virus differ in virulence, and a given strain is probably able to vary in virulence. Certainly, comparing different outbreaks, there is considerable variation in the severity of the disease produced in a given host species. Virulence also varies among species. Although the vast majority of strains will affect a wide range of species, there have been occasional viruses that show a distinct predilection for one species. An example is the porcinophilic strain that originated in China in the late 1990s and spread to Taiwan, destroying the swine industry there.

The main portal of entry and primary site of viral multiplication are the pharynx and lung. Subsequent to the first round of replication, there is widespread viremic dissemination to surface epithelium, with subsequent development of lesions in sites of mechanical or physiologic stress, such as oral and pedal epithelium, or teats in lactating animals. FMDV probably gains entry to these areas via Langerhans cells, with replication in a contiguous group of cells in the stratum spinosum. The resulting cellular degeneration and lysis result in an **epidermal vesicle**, *which is the hallmark of the disease.*

Virus is present at high titer in the vesicular fluid, and is present in large amounts in expired air from acutely infected animals, which is the main source of spread – pigs in particular liberate large quantities of airborne virus. Virus persists in lesions for 3–8 days after the appearance of significant neutralizing titers in serum, but seldom beyond day 11 of clinical illness. It is believed that FMDV is localized in epithelial cells of the oropharynx during persistent infection, so that virus may be found in esophagopharyngeal fluid for a considerable period of time.

Within a week of development of neutralizing antibody, the titer of virus in circulation declines. Ordinarily, serum antibody titers decline progressively and fairly rapidly. The duration of persistence of antibody is correlated with the initial titer. In general, animals are resistant to reinfection with homologous strains by natural exposure for about 2–4 years; susceptibility increases as the antibody titer declines.

The characteristic **lesions** of FMD are only seen in those animals which are examined at the height of disease. Lesions heal or are obscured by secondary bacterial infection. Lesions develop mainly in areas subject to trauma: the *oral mucosa*, especially the *tongue*; the *interdigital cleft*; and the *teats* in lactating animals. In cattle, there is appreciable loss of weight and the buccal cavity may contain much saliva. In the living animal, there is diffuse buccal hyperemia and mild catarrhal stomatitis, but the hyperemia disappears at death. Vesicles form on the inner aspects of the lips and cheeks, the gums, hard palate, dental pad, and especially on the sides and rostral portion of the dorsum of the tongue. Sometimes they form on the muzzle and exterior nares. The primary vesicles are small, but coalesce to produce *bullae* which may be 5–6 cm across; these bullae rupture in 12–14 hours, leaving an intensely red, raw, and moist base to which shreds of epithelium may still adhere (Fig. 1.84A). The eroded to ulcerated area may be replaced by regenerated epithelium in less than 2 weeks. Secondary infection may complicate this course.

Foot lesions occur in the majority of cases. There is inflammatory swelling with blanching of skin of the interdigital space in ruminants, coronet in swine, and heels in all species a day or so before vesicles form. The swellings persist until the vesicles rupture and the resultant erosions heal; healing may be considerably delayed on the feet. Vesicles may also occur in the other sites, but much less frequently.

A *malignant form of the disease*, without vesiculation, does occur in young animals and occasionally in adults. In these, death is common, due to *myocarditis*. Poorly defined pale foci of variable size are seen anywhere within the ventricular muscle. Although historically referred to as "tiger-heart," these gross lesions are no different from those generated in any other syndrome of severe, acute myocardial damage, but necrosis of fibers may be striking. Chronic lesions include myocardial necrosis and scarring, pancreatitis with acinar necrosis and regeneration. Diabetes mellitus occurs in experimental cases, as does hypophysitis, leading to a constellation of endocrinopathies because of a range of expressions of pituitary dysfunction.

Sheep are, in general, less susceptible than cattle, and the infection runs a milder course, though there may be exceptions. Lesions may not develop. When they do, the *dental pad* is the preferred site in the oral cavity. Lingual lesions tend to occur on the caudal dorsal portion as underrunning necrotic erosions rather than vesicles. These are small and easily missed, and they heal within a few days. *Lameness* may be prominent in acute outbreaks. Typical vesicles develop in the interdigital cleft, on the coronet and bulb of the heel. They may occasionally involve the entire coronet and lead to eventual shedding of the hoof. Vesicles also occasionally occur on the teats, vulva, prepuce, and on the pillars of the rumen. The peracute form with myocardial necrosis may occur in lambs.

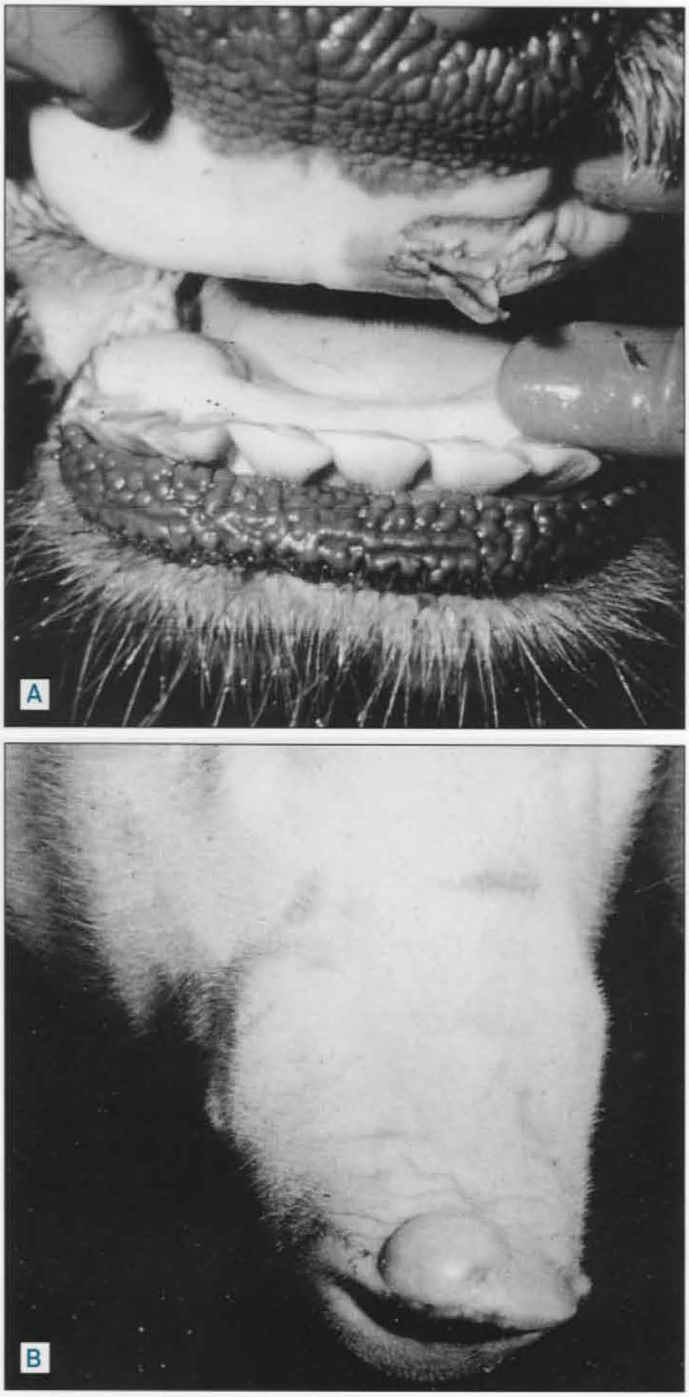

Figure 1.84 Foot-and-mouth disease. A. Ruptured vesicle on the dental pad of a cow. **B.** Vesicle on the snout of a pig.

The disease in **goats** is, in general, similar to that described for sheep. Both species may be inapparent carriers, and many outbreaks worldwide have been due to transport of inapparently infected small ruminants.

In **pigs**, lesions occur in the usual sites, although more commonly on the feet than in the mouth. They may be present on the snout and behind its rim (Fig. 1.84B), and on the teats of lactating sows. Abortion and stillbirth of infected piglets are recorded. The peracute form, with high mortality due to myocarditis, occurs in sucklings, often before vesicle formation is noticed in sows.

FMD must be differentiated from other viral vesicular diseases such as vesicular stomatitis, vesicular exanthema, and swine vesicular disease in susceptible species, and in the latter stages, from diseases producing erosive/ulcerative lesions of the oral cavity. The disease must be considered in the differential diagnosis in cases of sudden death among cloven-footed animals, especially the young. Definitive **diagnosis** requires virus isolation and characterization, demonstration of viral antigen by enzyme-linked immunosortbent assay, or detection of viral genome by polymerase chain reaction or reverse transcriptase-polymerase chain reaction in lesional material. The regulatory status of FMD dictates that this be carried out in accredited laboratories.

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Vesicular stomatitis

Vesicular stomatitis (VS) affects horses, cattle, and pigs, and may also affect wildlife species such as white-tailed deer, raccoons, feral swine, and some rodents. In humans, the virus may cause an inapparent infection or a mild influenza-like condition. Experimentally, various rodent species are susceptible and persistence has been demonstrated in hamsters. Also, persistence of viral RNA has been shown in both experimentally and naturally infected cattle, but the reservoir of the virus is unknown. *The disease is important because it causes a loss in production, especially in dairy herds, and it must be differentiated from foot-and-mouth disease in cattle and pigs.* VS is the only vesicular disease naturally occurring in horses. Sheep and goats do not appear to be susceptible to the disease, but a severe, though nonfatal, influenza-like syndrome may occur in infected humans.

VS virus (VSV) belongs to the Rhabdoviridae family, genus *Vesiculovirus* (type species: *VS Indiana virus* (VSIV)). It is an enveloped single-stranded RNA virus, bullet-shaped and about 80 × 120 nm. Apart from being inactivated by pasteurization temperatures, it shares qualities of resistance with the aphthoviruses. There are several serologically and immunologically distinct types of virus based on epitopes of the surface glycoproteins, including New Jersey, Indiana, Piry, Isfahan, and Chandipura. The two most common serotypes of VSV infecting domestic animals in the Americas are New Jersey and Indiana.

VS is enzootic in Central and South America and occurs sporadically elsewhere in the Americas. It has a seasonal occurrence; outbreaks occur in the warmer seasons and usually cease with the onset of cold weather. The seasonal nature of the disease suggests that it is transmitted by *insects*; however, insect transmission is not essential and *contact transmission* has been proven experimentally. VSV has been isolated from both biting and nonbiting insects and black flies have transmitted the virus to pigs. Biting insects most likely become infected from feeding on lesions rather than blood, since viremia is transient, if present. Nonbiting insects act as mechanical carriers of the virus. It is not known how the virus spreads from one geographic area to another. There is some indication that VSVs adapt to specific regions, developing distinct genotypes based on specific vectors or reservoirs in an ecological area. The intact mucosa is resistant to infection, but abrasions in a susceptible site readily result in infection when contaminated with saliva or exudate from a lesion. Environmental factors that increase the chance of causing abrasions to the skin, teats, or oral mucosa may predispose to infection.

Morbidity in lactating dairy cows may be as high as 100%, although only about 60% of the affected animals drool or froth around the mouth. The lesions of VS occur mainly on the *oral mucosa*; occasionally they do occur elsewhere, including on the *feet*, and in swine, foot lesions are common. This is by no means a dependable feature, and outbreaks of the disease in cattle have been described in which the lesions were predominantly on the *teats*.

The incubation period following exposure by abrasion is 24–72 hours and the viremic phase seems to be short-lived, because the virus cannot be cultured from blood. Secondary lesions are rare. In cattle, intramuscular injections will not initiate the disease, a distinguishing feature from foot-and-mouth disease. After experimental infection of swine, infectious viral particles can be recovered from a wide variety of tissues within 6 hours postinfection, including salivary gland, tonsils, snout, skin, and lymph nodes. However, infective virus, viral antigens, and nucleic acids cannot be demonstrated 6 days postinfection.

Specific serum neutralizing antibodies persist for months in swine and years in cattle. There is no evidence that animals with persistent antibodies may act as a source of infection to herd mates. Animals are immune against the homologous but not the heterologous strains of the virus.

The lesions of VS are indistinguishable from those of foot-and-mouth disease (Fig. 1.85A). Initially, in cattle, there is a raised flattened pale-pink to blanched papule a few millimeters in diameter in or near the mouth. These papules rapidly become inflamed and hyperemic. In the course of a day or so, they develop into vesicles 2–3 cm in diameter and by coalescence may involve large areas. The shallow erosions which follow rupture of vesicles heal within 1–2 weeks unless secondary infections occur; in the mouth, the latter are common. Oral and mouth lesions heal rapidly in swine, but coronary band lesions often become secondarily infected to the point where the claw may separate and slough. Serous rhinitis, with the development

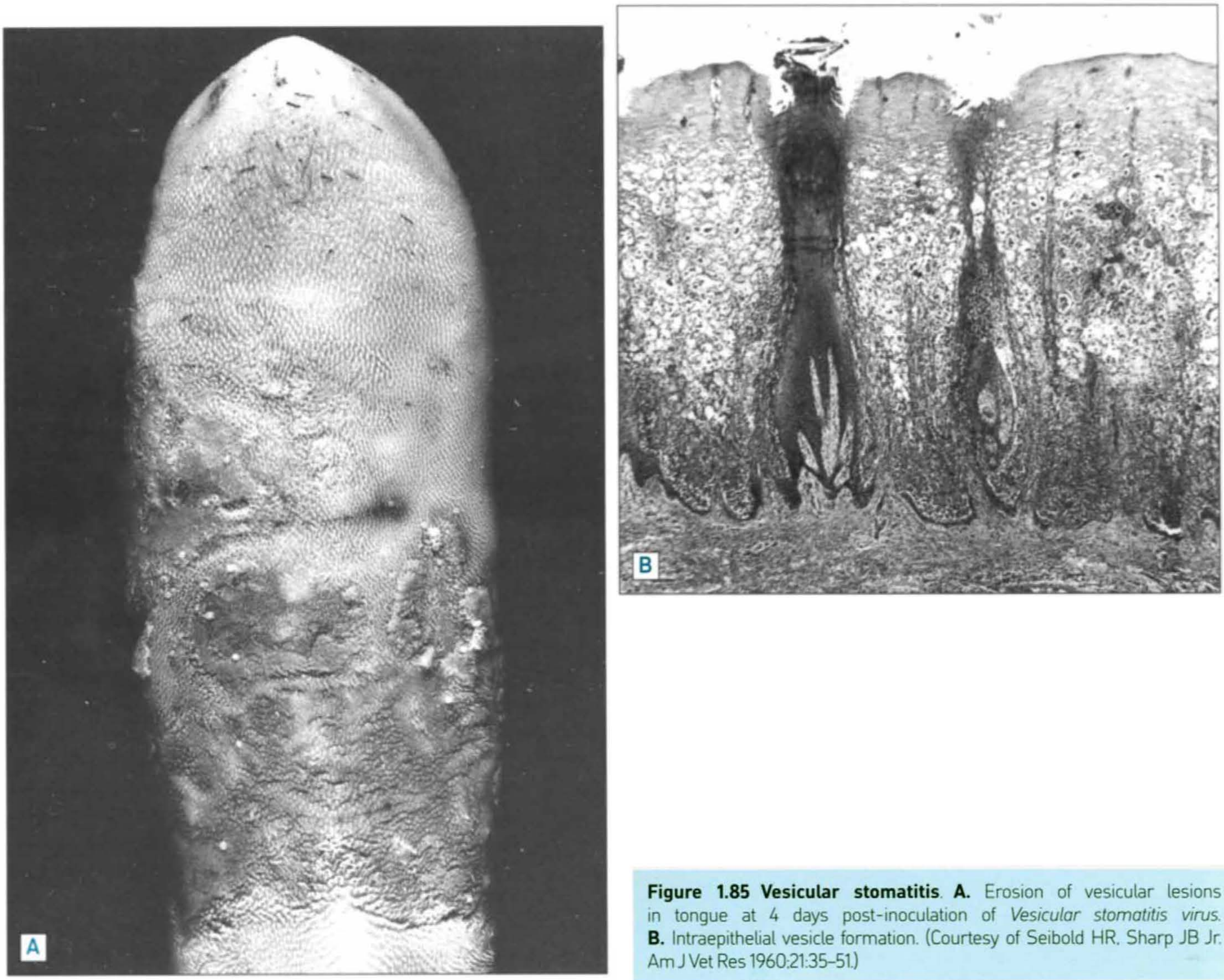

Figure 1.85 Vesicular stomatitis. A. Erosion of vesicular lesions in tongue at 4 days post-inoculation of *Vesicular stomatitis virus*. **B.** Intraepithelial vesicle formation. (Courtesy of Seibold HR, Sharp JB Jr. Am J Vet Res 1960;21:35–51.)

of tags of necrotic mucosa, has been described in experimentally infected swine.

The first microscopic changes are seen in the deeper layers of the stratum spinosum, where the virus replicates. Increasing prominence of the intercellular spaces and stretching of the desmosomes are accompanied by a reduction in volume of the cell cytoplasm (Fig. 1.85B). This dissociation of cells proceeds to distinct intercellular edema (*spongiosis*) followed by further cytoplasmic retraction until the affected epithelial cells float freely in enlarging vacuoles which in turn are loculated by strands of cytoplasmic debris (Fig. 1.86). There is no hydropic degeneration of the epithelial cells and the nuclei until now remain normal. *There are no inclusion bodies.* With the onset of epithelial cell necrosis there is a pleocellular inflammatory reaction in the mucosa and underlying lamina propria. Electron microscopic examination of epithelial cells adjacent to the vesicles confirms the intercellular edema and keratinocyte necrosis seen under the light microscope. The microscopic appearance of the lesions is not diagnostic.

In light of the similarity of VS to foot-and-mouth disease, *laboratory confirmation of VS is essential.* Vesicular fluid and mucosa from the tongue are good sources of the virus. *Diagnosis* is accomplished through virus isolation in tissue culture or embryonated eggs, fluorescent antibody techniques, complement fixation to identify viral antigen, polymerase chain reaction, and inoculation of suckling mice.

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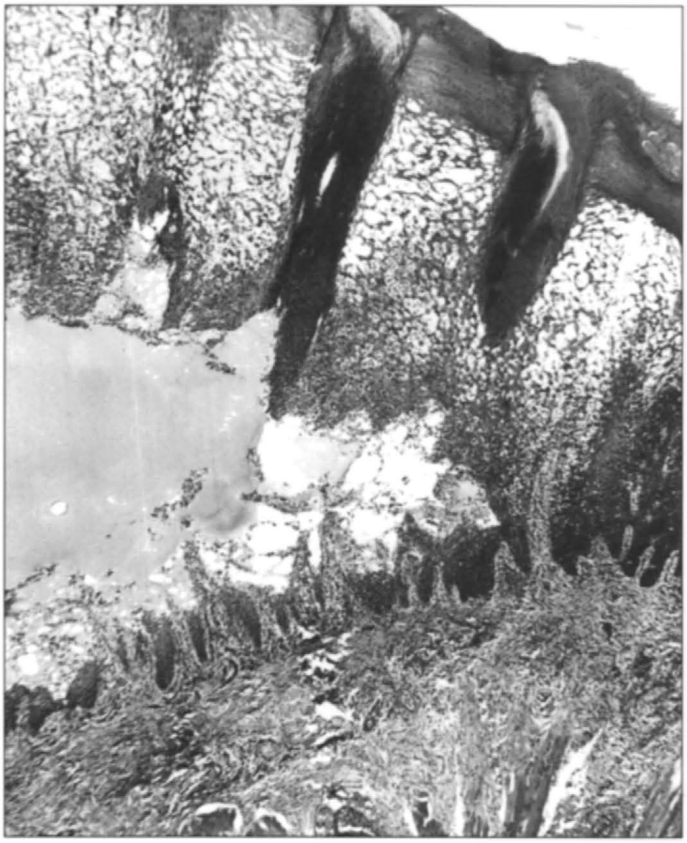

Figure 1.86 Edge of gross vesicle in **vesicular stomatitis**. (Courtesy of Seibold HR, Sharp JB Jr. Am J Vet Res 1960;21:35–51.)

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Vesicular exanthema of swine

Vesicular exanthema (VE) of swine is an acute, febrile disease of swine that is characterized by formation of vesicles on the snout, mouth, nonhaired skin, and feet. *The lesions are indistinguishable from those of foot-and-mouth disease, vesicular stomatitis, and swine vesicular disease.* VE of swine was first diagnosed in California in the 1930s and eventually spread to most swine-producing states in the USA. An eradication campaign was undertaken and the last reported outbreak of VE was in New Jersey in 1956.

VE of swine virus (VESV) is the type species of the genus *Vesivirus*, family Caliciviridae. It has a single-stranded RNA genome and has only one major polypeptide. It is about 35–40 nm in diameter and characteristic cup-shaped structures (calyces) are evident in electron microscopic preparations. There are 13 immunologically distinct serotypes, which vary in virulence.

In 1973, a virus that is biophysically and morphologically similar to VESV was recovered from sea lions (*Zalophus californianus*) with vesicles on their flippers, off the coast of California near San Miguel island. Several strains of this virus, called *San Miguel sea lion virus* (SMSV), produce milder but otherwise identical lesions to those of vesicular exanthema when inoculated into swine, and SMSV is classified as a serotype of VESV. The host range of SMSV is very broad. One serotype, SMSV-7, has been isolated from opaleye fish (*Girella nigricans*) and it produces lesions identical to VE when inoculated into swine, with horizontal transmission to contact swine. It is now thought that VE of swine arose through the feeding of ocean fish to swine, with some adaptation of the virus allowing for very efficient spread through swine.

Most outbreaks of VE were associated with feeding of raw garbage containing pork waste, indicating that the disease was transmitted by direct contact and fomites. VESV now exists only as viral stocks archived in freezers and should be considered a *disease of historical significance only*. The possibility of a recurrence remains, however, if swine are fed uncooked tissues from ocean-origin fish or marine mammals.

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Swine vesicular disease

Swine vesicular disease (SVD) is a highly contagious viral disease of pigs that is characterized by formation of vesicles around the coronary bands and heels of the feet, and, to a lesser extent, on the mouth, lips, tongue, and teats. *Clinically, the disease is indistinguishable from the other vesicular diseases of swine*, including foot-and-mouth disease, vesicular stomatitis, and vesicular exanthema of swine.

The disease was first recognized in Italy in 1966 and it has since been reported from Hong Kong, the UK, continental Europe, and Asia. The economic importance of SVD is related less to the rather limited losses in production and more to the fact that it is difficult to differentiate from other vesicular diseases and hence restricts trade.

Swine vesicular disease virus (SVDV) is a small RNA virus that belongs to the family Picornaviridae, genus *Enterovirus*. It is a porcine variant of Human coxsackievirus B5, which is a serotype of *Human enterovirus B*. SVDV is highly resistant to environmental factors. Unlike foot-and-mouth disease virus, it is not inactivated at the low pH in muscle commonly associated with rigor mortis.

Many outbreaks of SVD appear to originate by feeding raw garbage containing pork products. Transmission within and among affected herds is by direct contact, especially during the early stages of the disease, or by exposure to the virus in the environment, where it is very persistent. The portal of entry is most likely oral or by exposure of excoriated skin. Following contact with infected pigs, vesicles develop within 2 days and consistent virus isolation from tonsil is possible for 1–7 days. Viremia lasts for 2–3 days. SVDV has a strong affinity for the epithelial cells of the coronary band, tongue, snout, lips, lymphoid follicles of the tonsils, myocardial cells, and brain. Virus titers in tissue decrease with the appearance of circulating antibodies, which peak after 2–3 weeks and apparently persist for years. Secretions and excretions have high viral titers for a period of 12–14 days. Feces may contain virus for up to 3 months.

There is some evidence that pigs may become carriers, with stress reactivating viral shedding several months postinfection.

Clinically, *vesicles are most common on the feet*. Oral lesions occur in only about 10% of affected pigs. The foot lesions appear first at the junction between the heel and the coronary band. Initially, there is a 5-mm wide, pale, swollen area that encircles the digit. In later stages a 1-cm wide band of necrotic skin is located along the coronet. Vesicles on the mouth, lips, and tongue occur in clusters and they are small, ~2.0 mm in diameter, white, and opaque. They coalesce and rupture within 36 hours and may be covered by a pseudodiphtheritic membrane due to secondary bacterial infections. Affected pigs usually recover in 2–3 weeks.

The development of vesicles tends to follow a similar course as that reported for foot-and-mouth disease. The virus infects individual epithelial cells in the stratum spinosum, which leads to focal areas of keratinocyte degeneration and vesicle formation. There is an intense leukocytic reaction in the necrotic areas, which is mainly neutrophilic. As with the other vesicular diseases, after 1 week there are indications of epithelial regeneration.

Nervous signs and lesions of *nonsuppurative meningoencephalomyelitis* have been reported in field outbreaks and reproduced experimentally in SVD. Lesions involve most areas in the brain and sometimes spinal cord, and are centered on ganglia and spinal nerve roots. Clinically, the severe lameness tends to overshadow any nervous signs that might be present.

Laboratory diagnosis depends on demonstration of the agent by virus isolation, antigen capture enzyme-linked immunosorbent assay or polymerase chain reaction, in accredited laboratories, on account of its regulatory status.

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Bovine viral diarrhea

Bovine viral diarrhea virus (BVDV) is an RNA virus of the *Pestivirus* genus in the family Flaviviridae. It is widespread in cattle populations and it or closely related viruses can infect most even-toed ungulates, including swine. As an RNA virus, BVDV is highly mutable, due to the error-prone nature of the RNA polymerases responsible for replication of viral RNA. As a result, "swarms" of viral mutants form "quasispecies" that circulate within an infected individual and among individuals in a population. While most quasispecies lack a selective advantage, or suffer deleterious point mutations, preventing them from becoming dominant, the ability to generate mutants enables BVDV to adapt to host responses, and to establish chronic or persistent infections in some circumstances. While low virulence would seem to promote prolonged viral shedding, there may be advantages in high virulence that favor the emergence of quasispecies capable of causing severe disease and high virus shedding.

Point mutation and recombination have produced a number of genotypes of *Pestivirus*, including *Classical swine fever virus* and *Border disease virus*. Two genotypes of BVDV occur, **type 1** (BVDV-1) and **type 2** (BVDV-2), each of which has a number of closely related subgenotypes, many of which circulate widely. Viral genotype may be linked to specific manifestations of BVDV infection; noncytopathic (NCP) BVDV-2 has been associated with thrombocytopenia, for instance. However, there is a range of virulence among both type BVDV-1 and BVDV-2 isolates, varying from subclinical infections or mild clinical disease to severe fatal syndromes.

Recombination of RNA from homologous (BVDV) or heterologous (other viral or host) sources, usually involving the region encoding the nonstructural protein NS2–3 of noncytopathic virus, results in a shift in biotype of either genotype of virus, from the more common **NCP biotype**, in which inapparent persistent infection is produced in cultured cells, to a **cytopathic (CP) biotype**, capable of inducing cytoplasmic vacuolation and apoptotic death of cells in tissue culture. Recombination splits NS2–3, resulting in a small NS3 protein, which induces apoptosis, and is a marker for CP BVD viruses. Reversion of CP viruses to an NCP biotype also occurs, less commonly. *Cytopathogenicity in vitro is not directly related to virulence in vivo.*

BVDV gains access to the oropharyngeal mucosa by ingestion or inhalation, and primary replication is in oropharyngeal lymphoid tissues, including tonsils. The outcome of the ensuing viremia is a product of the genotype and virulence of the virus, the immune status of the host, whether or not the animal is pregnant, and, if so, the stage of pregnancy.

Infection of immunocompetent, seronegative, nonpregnant animals usually results in subclinical infection or mild clinical disease. Affected animals develop a slight fever, leukopenia, and specific neutralizing antibodies, the outcome in 70–90% of BVDV infections. In a few situations, animals, mainly over 6 months of age, develop a more obvious clinical syndrome, with a high morbidity and low mortality – classical **bovine viral diarrhea (BVD)**. The infecting agent is usually an NCP BVDV. After an incubation period of 5–7 days, the affected animals develop a fever, leukopenia, and viremia that may persist up to 15 days. The virus is present in leukocytes (buffy coat), especially lymphocytes and monocytes, and in plasma. There is a transient decrease in the number of B and T lymphocytes and a decline in responsiveness to mitogen stimulation. Clinically, the disease is characterized by lethargy, anorexia, mild oculonasal discharge, and occasional mild oral erosions and shallow ulcers. Diarrhea may occur. In dairy herds there is a transient drop in milk production. Affected animals develop neutralizing antibodies that peak in 10–12 weeks, and probably are immune for life.

A syndrome of **severe acute BVD**, characterized by high morbidity and mortality in all age groups of susceptible animals, has been recognized since the early 1990s. Sometimes termed "BVD type 2," since it is caused mainly, though not exclusively, by primary infections with type 2 virus, this syndrome usually has a peracute to acute course, with fever, sudden death, diarrhea, or pneumonia. It should be noted that not all BVDV-2 isolates are highly virulent. In some cases a **thrombocytopenic syndrome**, characterized clinically by epistaxis, hyphema, mucosal hemorrhages, bleeding at injection sites, and bloody diarrhea, is superimposed on the alimentary syndrome caused by type 2 BVDV, or occurs independently. The pathogenesis of BVD type 2 is most frequently linked to increased strain virulence. However, production of inflammatory cytokines, in response to widespread infection of mononuclear phagocytes, has also been postulated as a cause for the severe disease seen clinically. The mechanism of thrombocytopenia is not completely defined, although infected megakaryocytes in the bone marrow undergo necrosis.

Fetal infections may occur in pregnant immunocompetent seronegative acutely infected females, and in persistently infected counterparts. The outcome of fetal infection is primarily dependent on the stage of gestation. The most serious consequences occur if an NCP BVDV crosses the placental barrier during the first 4 months of gestation. It may result in fetal resorption, mummification, abortion, congenital anomalies, or a persistently infected calf. If the calf survives, it remains viremic for life, and it is also immunotolerant to homologous NCP BVD viruses, due to failure of the immature fetal immune system to recognize the infecting viral antigens as "not-self" or foreign.

Persistently infected (PI) calves may be clinically normal, weak, or undersized at birth. They may appear normal, but are often unthrifty, and may have a rough or curly hair coat. The prevalence of these calves in a herd is usually less than 2%, but may be as high as 25–30% in herds where a large number of naive cows, early in pregnancy, have been exposed to NCP BVDV. *Most PI calves succumb to mucosal disease* (see below), usually between the ages of 6 months and 2 years. The offspring of the few animals that reach sexual maturity and become pregnant are also persistently infected, which can result in families of animals persistently infected with BVDV. PI animals are viremic, and lack antibody to the infecting virus, which they shed constantly, acting as the *most important source of infection in the population*.

In PI animals, virus is present in a wide variety of tissues, and antigen can be demonstrated by immunohistochemistry in skin biopsies – in keratinocytes, hair follicle epithelium, hair matrix cells of the hair bulb, and dermal papillae. Use of skin biopsies for diagnosis of persistent infections by immunohistochemistry or enzyme-linked immunosorbent assay has been exploited diagnostically, but it should be recognized that acutely infected animals may have virus in skin biopsies as well. Lesions in PI animals are minimal and subclinical, in spite of the widespread infection of virtually all tissues.

Mucosal disease is a clinicopathologic syndrome occurring in PI animals that subsequently become infected with a closely related CP strain, or probably more commonly, when the virus causing persistent congenital infection spontaneously develops a recombination encoding NS3. The result is an overwhelming infection that destroys cells, and to which the animal is incapable of responding. Characterized by *low morbidity but very high mortality*, mucosal disease most commonly occurs in cattle that are 6 months to 2 years of age. While deaths may occur within a few days of illness, and almost always within 2 weeks, some cases may survive for months. The incubation period after experimental infection with a CP strain in an animal persistently infected with an NCP BVDV is usually 7–14 days, but may be considerably longer.

Basically, there are *two forms of clinically severe BVDV infection*: mucosal disease in persistently infected animals, and the more recently recognized severe acute form of BVD caused by primary infections with very virulent strains of virus. At necropsy, one cannot confidently differentiate spontaneous cases of severe acute BVD due to BVDV-1 or BVDV-2 from each other, or from cases of mucosal disease, other then by the more hemorrhagic character of some cases of severe acute BVD due to type 2 isolates. Tentative differentiation of mucosal disease from severe acute BVD rests on the epidemiologic picture, and antigenic or molecular characterization of the involved viruses is required for definitive diagnosis of the various syndromes.

Fulminant severe acute BVD or mucosal disease closely resembles rinderpest. At the onset the animal is febrile, with serous to mucoid nasal discharge. Discrete oral lesions are preceded by acute stomatitis and pharyngitis, the mucosae being hyperemic and pink and covered by a thin gray film of catarrhal exudate. There is severe diarrhea and tenesmus with feces containing little or no blood or mucus. Affected animals become lethargic, anorexic, and dehydrated; they have ptyalism, polypnea, and tachycardia, and may die quickly.

In more chronic cases, the development of the oral lesions is like that found in acute cases; however, by the time they die there is usually some evidence of healing. The watery diarrhea of the early phase gradually gives way to feces that are passed frequently, are scant in volume, and contain a large proportion of mucus flecked with blood. Late in the clinical course, there is lethargy, emaciation, ruminal stasis, and frequent attempts at defecation accompanied by severe tenesmus. Interdigital dermatitis, coronitis, and laminitis affecting all four feet may be present in chronically affected animals (Fig. 1.87A), resulting in lameness. In these too, the skin is dry and scurfy, especially over the neck, withers, back, perineal and preputial areas, and vulva, while that on the medial aspect of the thighs and forelegs becomes moist and discolored a dirty yellow.

At necropsy, the **gross lesions** vary considerably, especially in acute cases, in which either upper alimentary or intestinal lesions rarely may be absent, and less so in the chronic disease, in which a broader pathologic picture is often present, perhaps partially obscured by healing or evolution of lesions.

Crusts, erosions, and shallow ulcers are present on the muzzle and nares of many affected cattle. There is loss of epithelium from much of the oral cavity. The most conspicuous oral erosions are on the palate, on the tips of the buccal papillae (Fig. 1.87B), and on the gingiva. Many, especially on the papillae, the hard palate, and in the pharynx, are sharp punched-out ulcers, and expose a denuded, intensely hyperemic lamina propria. In more chronic cases, ulcers may have a margin of thickened proliferative epithelium. The tongue is not always affected; when present, lesions may be evident on all surfaces (Fig. 1.88A, B).

Esophageal lesions are usually present, most commonly in the upper third. In some acute cases, the lesions are shallow erosions, rather than ulcers. The erosions are more or less linear but otherwise irregular, have a dirty brown base, and little or no reactive hyperemia, and may be covered by shreds of necrotic epithelium in animals that have not been swallowing (Fig. 1.88C). In more advanced cases, discrete ulcerations occur. In many chronically affected animals, the ulcers are beginning to heal and have yellow-white slightly elevated plaques of proliferative epithelium at the periphery of the mucosal defect.

Lesions are found in the *reticulorumen and omasum*, but usually not in the esophageal groove. The ruminal content in chronically affected animals with prolonged anorexia is usually scant and dry. In most acute cases, the ruminal content is unusually liquid and putrid. The lesions on the wall of the rumen resemble those present elsewhere in the upper alimentary tract and, although they occur anywhere, they

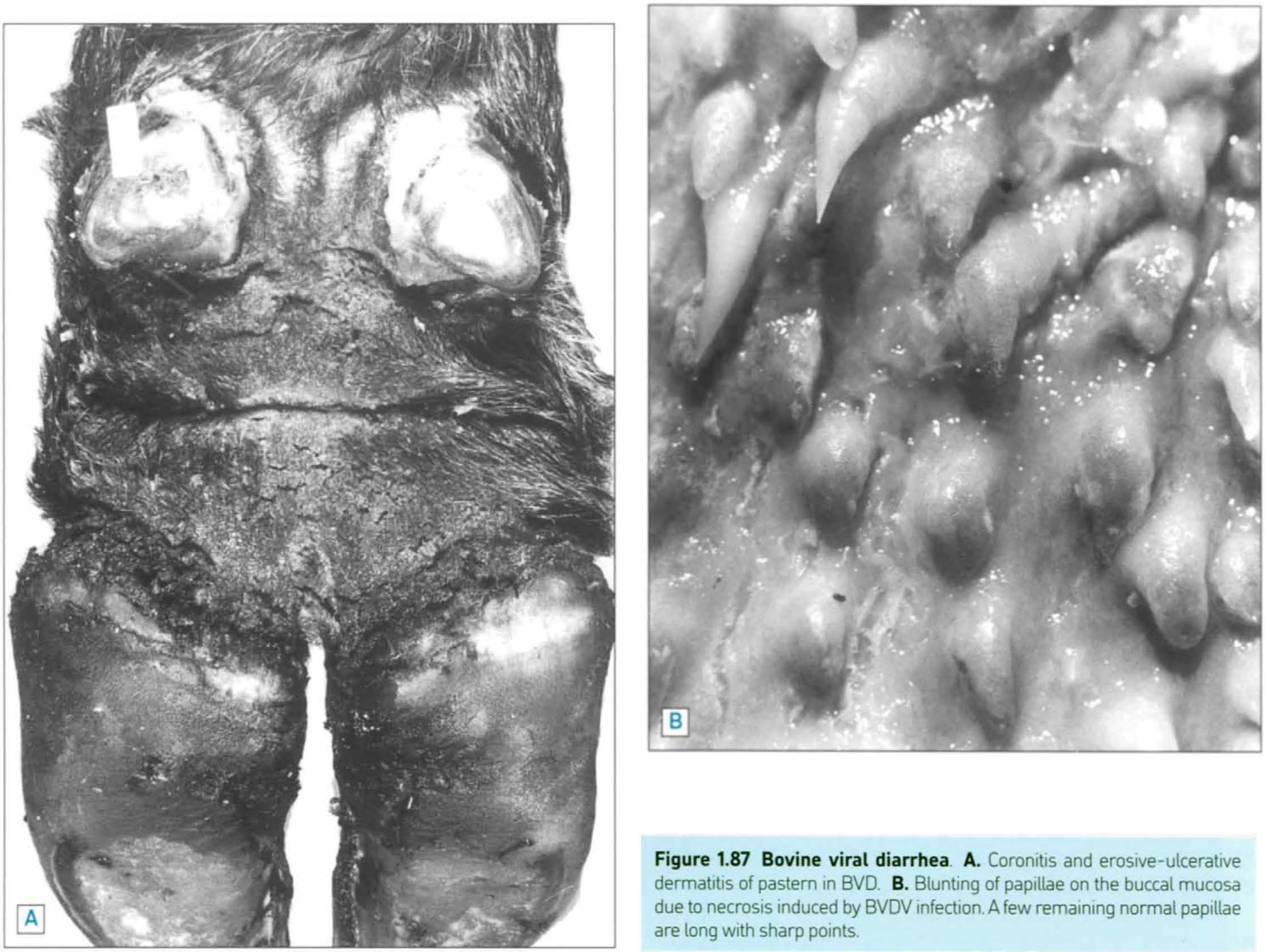

Figure 1.87 Bovine viral diarrhea. A. Coronitis and erosive-ulcerative dermatitis of pastern in BVD. **B.** Blunting of papillae on the buccal mucosa due to necrosis induced by BVDV infection. A few remaining normal papillae are long with sharp points.

are best seen on the pillars and other smooth or nonvillus portions of the mucosa (Fig. 1.89). The omasal lesions are most numerous along the edges of the leaves, sometimes causing a scalloped margin or perforation.

The morphogenesis of the lesions in the squamous mucosa of the upper alimentary tract begins with necrosis of the epithelium (Fig. 1.90). Individual cells and groups of cells deep in the epithelium are eosinophilic and swollen, with pyknotic nuclei. These foci enlarge progressively and form areas of necrosis that extend to, and may involve, the basal layer. In the early stages there is little or no inflammation of the lamina propria, but leukocytes infiltrate the necrotic epithelium. These necrotic foci enlarge progressively and by coalescence, and may form small cleavage vesicles along the proprial–epithelial junction (Fig. 1.91A), leading to erosions or ulcers as necrotic epithelium is abraded away. The ulcerations of the squamous epithelium of the upper alimentary tract are accompanied by inflammation in the lamina propria, especially where this forms papillae (Fig. 1.91B).

Changes are regularly present in the *abomasum*. The sides of the rugae bear ulcers that may be punctate to 1 cm or more in diameter (Fig. 1.92). The histological changes in the glandular epithelium of the abomasum are characterized by epithelial necrosis, mainly in the depths of the glands, and accompanying interstitial inflammation.

The mucosa of the *small intestine* often appears normal over much of its length. However, in some cases the mucosa of the small intestine may have patchy or diffuse congestion. In rare cases, fibrin casts may be in the lumen of the small bowel.

In acute cases, it is usual to find *coagulated blood and fibrin overlying and outlining Peyer's patches*, the covering of which is eroded. This, when present, is a very distinctive lesion that is only paralleled in rinderpest. Severely affected Peyer's patches are often obvious through the serosa as red-black oval areas up to 10–12 cm long on the antimesenteric border of the gut (Fig. 1.93A). Less acutely affected Peyer's patches may be overlain by a diphtheritic membrane, while in milder or more chronic cases the patches may be depressed and covered by tenacious mucus. Mesenteric lymph nodes may or may not be enlarged.

Lesions in the *large bowel* are highly variable. The mucosa may be congested, often in a "tiger-stripe" pattern following the colonic folds, a reflection of tenesmus. In acute cases there may be fibrinohemorrhagic typhlocolitis (Fig. 1.93B). In more chronic cases, fibrinous or fibrinonecrotic lesions and focal or extensive ulceration may be present at any level of the large bowel, but particularly in the cecum and rectum.

The characteristic **microscopic lesion** in the intestinal mucosa is *destruction of the epithelial lining of the crypts of Lieberkühn*. In the

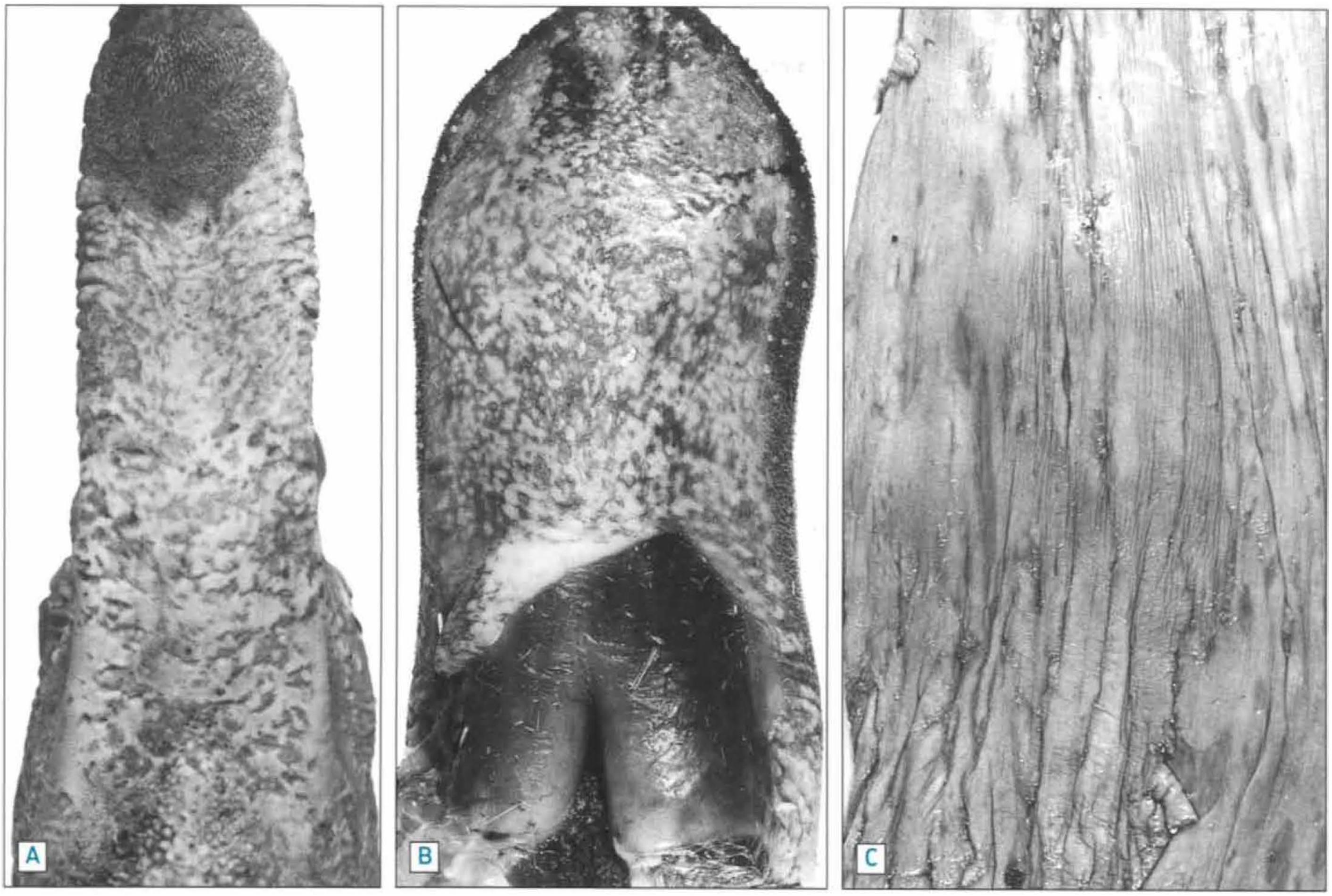

Figure 1.88 Bovine viral diarrhea. A. Dorsal surface of tongue showing multiple confluent ulcers. **B.** Ventral surface of tongue showing multiple confluent ulcers. **C.** Longitudinal erosions and ulcers on the esophagus.

duodenum, only a few crypts are affected, but more crypts are affected more severely in the lower reaches of the small intestine and in the cecum and colon. Affected crypts are dilated and filled with mucus, epithelial debris, and leukocytes. Remaining crypt-lining cells are attenuated in an attempt to cover the basement membrane. Reparative hyperplasia of crypt lining is rarely encountered. Crypt drop-out may be evident microscopically. In the cecum and colon, extensive damage to crypts and attendant collapse of the lamina propria are the probable cause of ulceration seen grossly (Fig. 1.94A). Congestion of mucosal capillaries, and in acute or ulcerated cases, effusion of fibrin and neutrophils from the mucosal surface may be evident.

The microscopic lesions of Peyer's patches are distinctive in BVD, comparable lesions being caused only by rinderpest. In the acute phase of the disease, severe acute inflammation in the mucosa over Peyer's patches accompanies almost complete destruction of the underlying glands, collapse of the lamina propria, and lysis of the follicular lymphoid tissues (Fig. 1.94B). Later in the course of the disease, dilated crypts, lined, at least in part, by cuboidal epithelium and filled with necrotic epithelial cells, mucus, and inflammatory cells appear to *herniate* into the submucosal space previously occupied by involuted lymphoid follicles. Peyer's patches should be sought assiduously at autopsy since their gross and microscopic appearance may provide useful evidence for diagnosis.

Microscopically, the mesenteric and sometimes other lymph nodes show a diminished population of lymphocytes and necrosis of germinal centers. By immunohistochemistry, there is a marked decrease in most lymphocyte subpopulations.

An important microscopic lesion is *hyaline degeneration and fibrinoid necrosis of submucosal and mesenteric arterioles* (Fig. 1.95A). A mild-to-moderate mononuclear inflammatory cell reaction is frequently present in the walls of the vessels and in perivascular areas. The vascular lesions may also be present in a variety of other organs, such as the heart, brain, and adrenal cortices, which may make it difficult to differentiate the disease from malignant catarrhal fever. The vascular lesions in acute mucosal disease are less consistently present and are usually milder, and there is *involution of lymphoid tissue in BVD*, in contrast to the lymphoproliferation characteristic of malignant catarrhal fever.

Coronitis may extend completely around the coronary band, with some separation of the skin–horn junction causing disturbance and overgrowth of the horn (see Fig. 1.87A). Dermatitis may extend from the coronet up the back of the pastern. Milder dermatitis is generalized, with scurfiness, especially from the ears to the withers. In sections of the skin of animals with chronic mucosal disease, there is hyper- and parakeratosis with focal accumulations of necrotic epithelium with intense hyperemia of the adjacent superficial dermis. The epithelial lesions are basically similar to those in the squamous mucosa of the

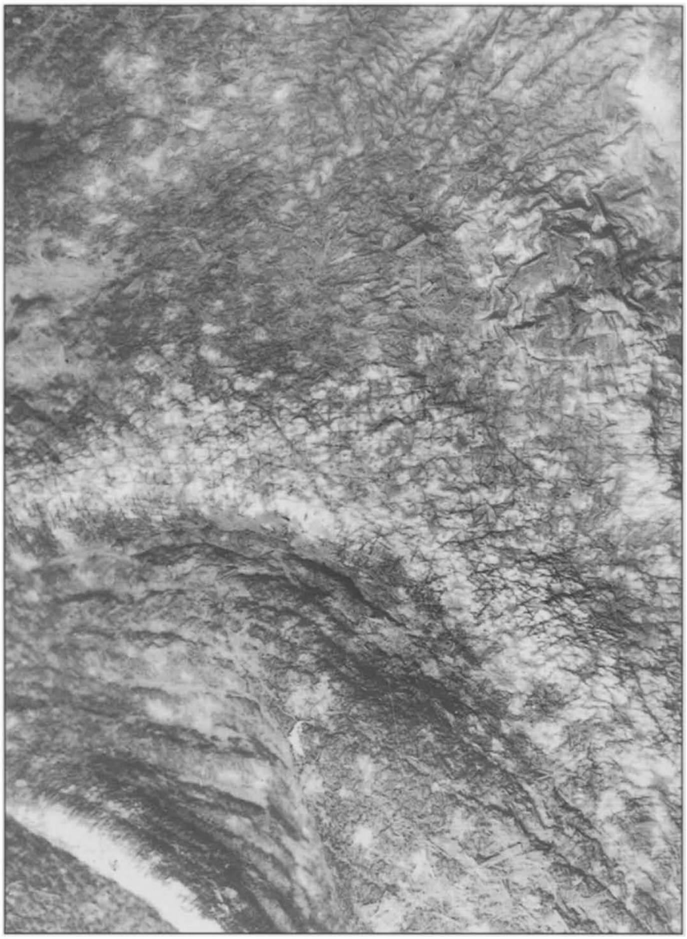

Figure 1.89 Focal and confluent often preulcerative, plaque-like lesions on mucosa of dorsal sac of rumen in **bovine viral diarrhea**.

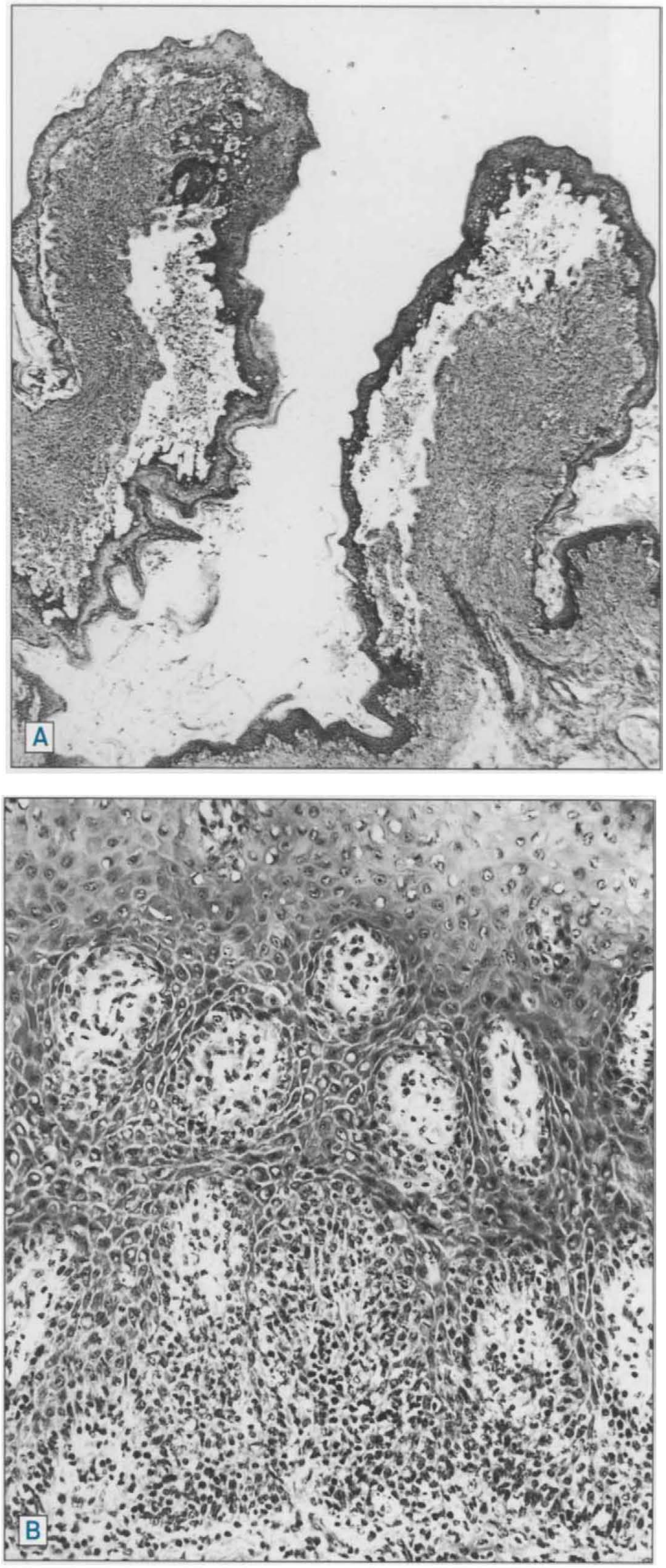

Figure 1.91 Bovine viral diarrhea. **A.** Cleavage vesicles in rumen papillae. **B.** Edema of proprial papillae, acute focal inflammation of papilla and propria. There is necrosis of scattered cells deep in the epithelium.

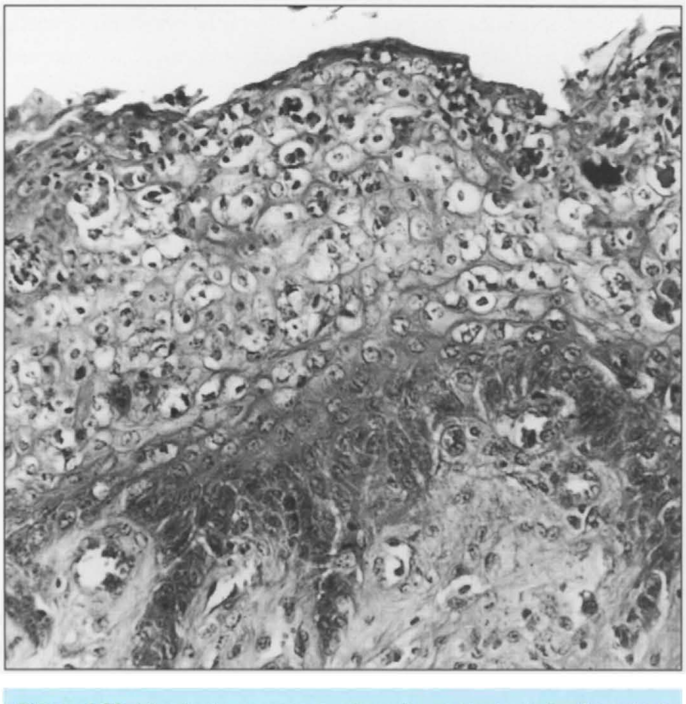

Figure 1.90 Histologic appearance of esophageal lesion in **bovine viral diarrhea**.

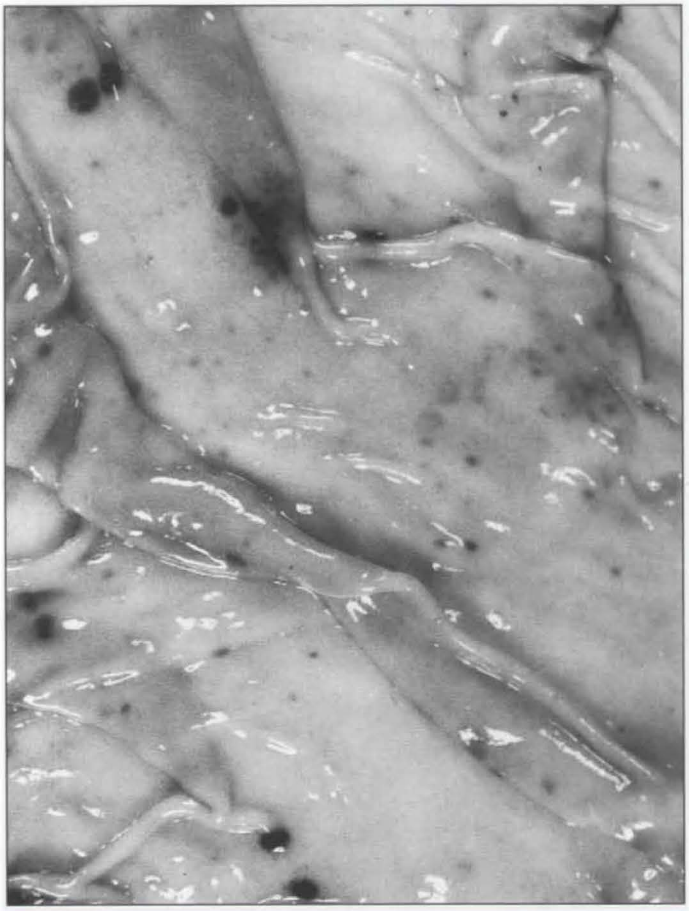

Figure 1.92 Hemorrhage and ulceration in the abomasum in **bovine viral diarrhea**.

upper alimentary tract (Fig. 1.95B). Necrosis often extends deeply to or through the basal layers; it results in minute erosions or ulcerations. There is massive infiltration of macrophages and some lymphocytes in the underlying dermis. These deeper lesions occur in the inner aspects of the legs and the perineum, and there is exudation of serum in these areas. The overlying degenerate epithelium becomes disorderly and eventually is lifted off.

Some animals with chronic disease develop *mycotic infections* secondary to lesions in the forestomachs, abomasum, and Peyer's patches. The lesions are areas of hemorrhagic necrosis involving the mucosa, submucosa, and sometimes deeper layers of the wall. Fungal hyphae are found invading the stroma and causing thrombosis in venules.

Infection of oocytes and cumulus cells in the ovaries has been well-documented, causing speculation concerning ovarian dysfunction and reduced fertility in animals surviving BVDV infection.

After *experimental inoculation with virulent BVDV-2*, animals are febrile by 7 days postinfection. Prominent clinical signs are anorexia, depression, and episodes of profuse watery and blood diarrhea that persist until the animal is moribund at 13–14 days postinfection. Pregnant animals may abort. Leukopenia and thrombocytopenia are often marked. In cases with severe thrombocytopenia, hemorrhage may be evident clinically.

Lesions are found in the digestive and respiratory systems. There is mild tracheitis, bronchitis, and bronchiolitis, which can progress to secondary bacterial pneumonia. Strains may vary in their ability to infect the pulmonary tree and result in disease. Intestinal lesions strongly resemble those seen in mucosal disease, with severe lymphoid depletion and necrosis of epithelial cells. However, with these BVDV-2 infections, there is often also a significant amount of hemorrhage evident externally, as described above, and there may

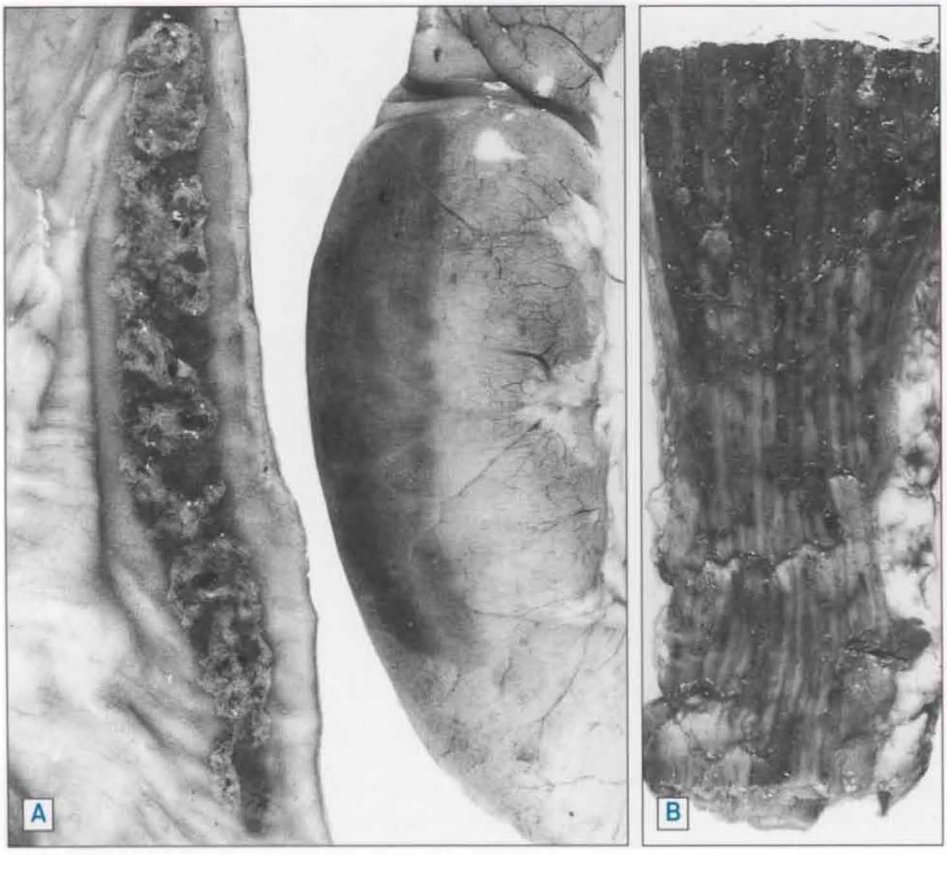

Figure 1.93 Bovine viral diarrhea. A. Fibrinohemorrhagic exudate over Peyer's patch in the ileum (left). Deep red Peyer's patch visible through serosa of small intestine (right). **B.** Fibrinohemorrhagic colitis.

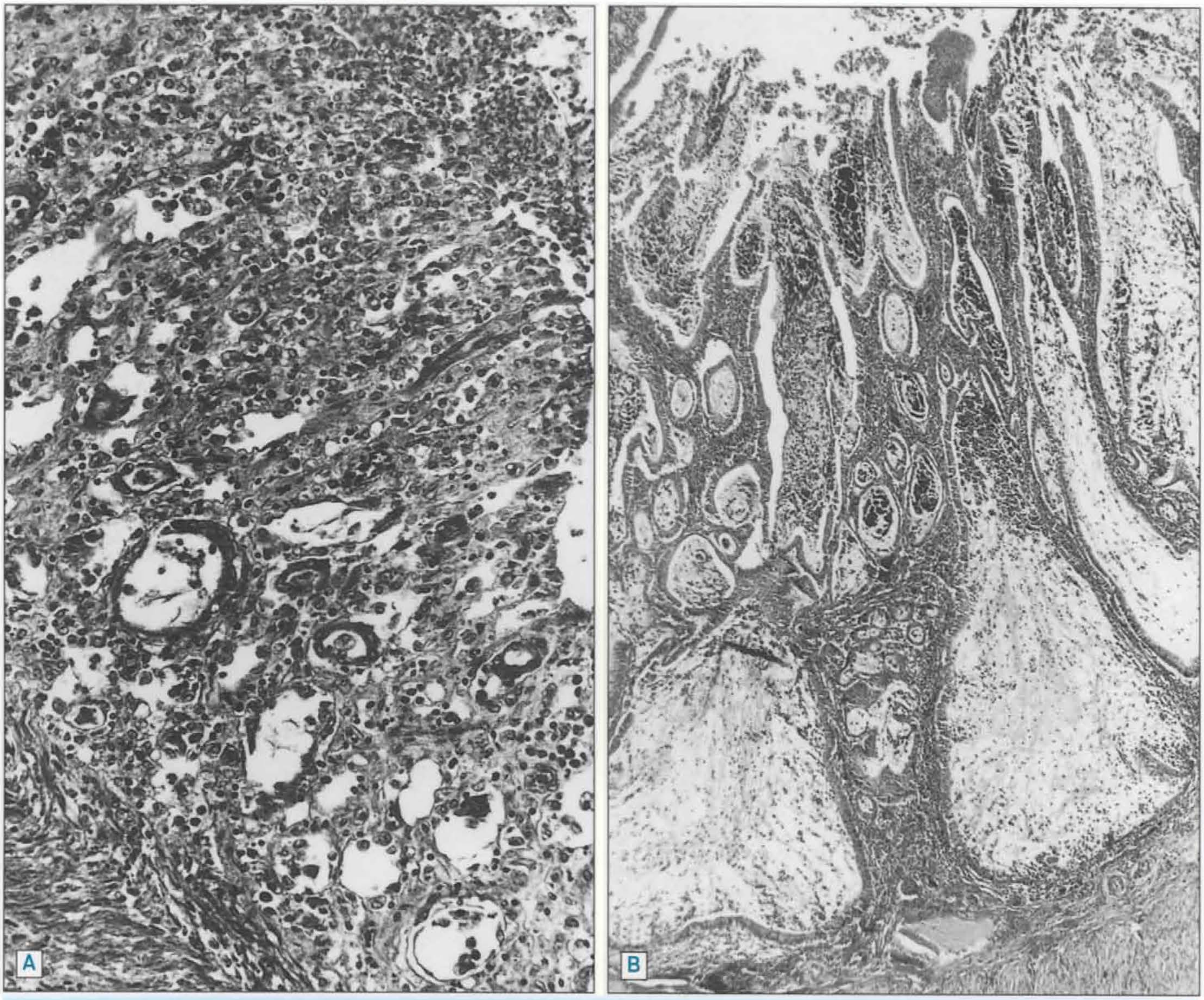

Figure 1.94 Bovine viral diarrhea. A. Colon, with dilated and denuded glands, collapse of lamina propria, and pseudomembrane formation. **B.** Herniation of crypts of Lieberkühn into the submucosa replacing necrotic lymphoid follicles in Peyer's patch. Mucus and inflammatory exudate are in the cystic glands and on the surface of the mucosa.

be extensive subserosal hemorrhages in the thoracic and abdominal cavities (Fig. 1.96). Similarly, edema may be more noteworthy in this form than in mucosal disease. Severe necrotizing vasculitis, especially arteritis, is noted in multiple organs but is most readily identifiable in lymphoid tissue. Meningoencephalitis associated with neuronal infection by BVDV-2 has been reported.

By immunohistochemistry, there is widespread viral antigen within epithelial cells (including oral and esophageal epithelium), smooth-muscle cells, and mononuclear phagocytes in multiple organs, although lesions often do not correspond to sites of antigen staining.

Bovine viral diarrhea virus and secondary infections

BVDV infection suppresses interferon production and impairs lymphocyte function, monocyte proliferation and chemotaxis, humoral antibody production, neutrophil function, and bacterial clearance. These changes are fairly persistent in chronically infected animals and in animals with mucosal disease. The failure of immunogenic response may be associated with immunotolerance, or destruction of immunocompetent cells, which is reflected in lymphopenia. In addition to a lack of humoral antibody response, there is also depression of cell-mediated immunity, as indicated by a poor response of cultured peripheral lymphocytes to various mitogens. The impairment of neutrophil function in cattle infected with BVDV may explain in part the apparent susceptibility of such cattle to *secondary bacterial infections.*

Fetal infection with *Bovine viral diarrhea virus*

In addition to the early embryonic death and abortions that can be ascribed to BVDV, infections of seronegative immunocompetent

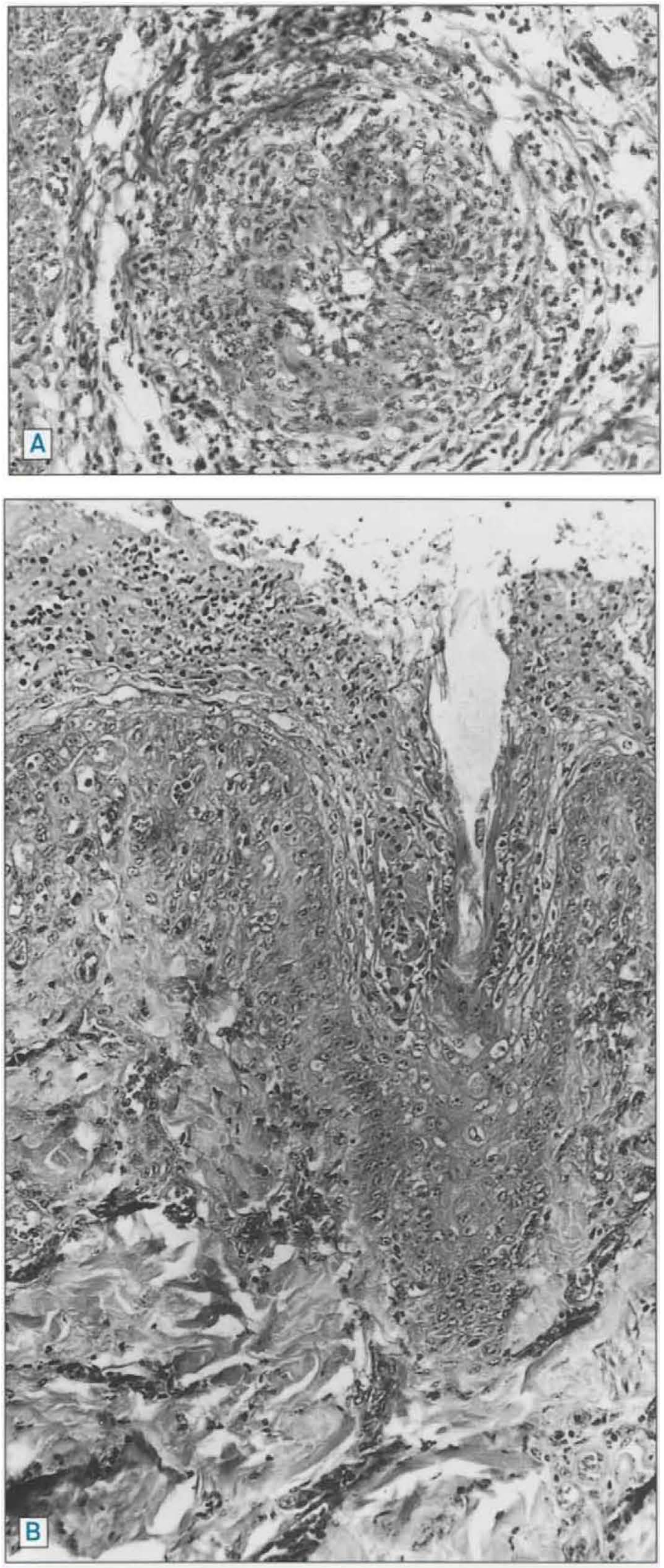

Figure 1.95 Bovine viral diarrhea. A. Fibrinoid necrosis and mild periarteritis of a mesenteric arteriole in the colon. **B.** Skin, with superficial epidermal necrosis extending into hair follicle and hyperplasia of the stratum germinativum.

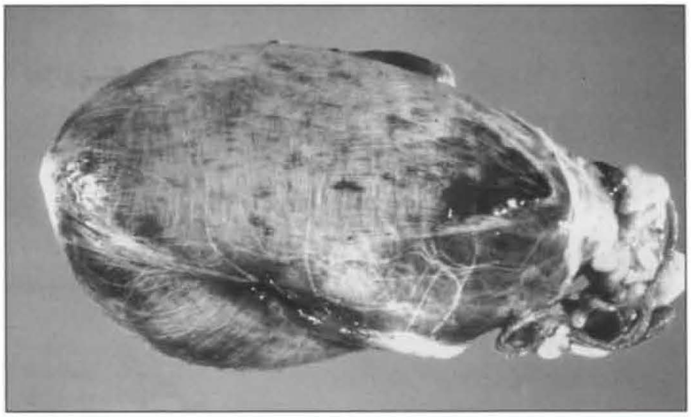

Figure 1.96 Hemorrhages on the serosal aspect of the bladder in the **thrombocytopenic form of bovine viral diarrhea.**

dams, usually between 90 and 120 days of gestation, may result in a wide spectrum of *teratogenic lesions*, including microencephaly, hypomyelinogenesis, cerebellar hypoplasia and dysgenesis, hydranencephaly, hydrocephalus, and defective myelination of the spinal cord. Ocular lesions, such as microphthalmia, cataracts, retinal degeneration, atrophy and dysplasia, and optic neuritis, have all been associated with fetal infections by BVDV (see Vol. 1, Nervous system; Vol. 1, Eye and ear; Vol. 3, Female genital system). Infections of the immunocompetent fetus, usually after 135 days of gestation, result in antibody production that is detectable in precolostral serum samples of the newborn calf.

Fetal infection later in gestation may produce lesions unrelated to teratogenesis. Alimentary tract lesions of the disease may be observed in the fetus. Punctate hemorrhages with ulcers 1–3 mm in diameter may be profuse in the oral cavity, excepting the dorsum of the tongue, and in the esophagus, larynx, trachea, conjunctiva, and abomasum. The fetal lesions of squamous epithelium evolve in somewhat the same manner as those described earlier, with focal hemorrhages in the lamina propria and epithelial necrosis beginning in the basal layer.

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Bovine viral diarrhea virus infection in pigs

The prevalence of naturally occurring antibodies to BVDV in swine in different countries varies between 2 and 40%. The antibodies may complicate the diagnosis of classical swine fever, especially in those countries considered to be free of this disease. Cattle, and modified live virus vaccines containing contaminated fetal bovine serum, are considered to be common sources of infection for swine. There are sporadic reports of disease associated with BVDV infection, including stillbirth, and poorly viable piglets, some showing tremors. Some 2–4-week-old pigs in infected herds are anemic, have a rough hair coat, growth retardation, wasting, and diarrhea. Affected pigs fail to develop neutralizing antibodies to the infecting homologous BVDV. Littermates that remain normal develop neutralizing antibodies. The suggestion is that the infections are congenital. Experimental in utero BVDV infection of sows may result in prenatal and perinatal deaths, persistently infected immunotolerant or normal pigs. Many of these conditions resemble the effects of in utero infection with NCP BVDV in cattle.

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Border disease

Border disease is a congenital infection of sheep and goats, usually with one of several NCP genotypes of *Border disease virus* (BDV), a pestivirus antigenically related to BVDV and *Classical swine fever virus*, but apparently also with some BVDV-2 strains. The disease was first reported in lambs from border areas between England and Wales. It is characterized by embryonic and fetal death, abortion, mummification, and birth of weak lambs or kids. The affected animals have an abnormal body conformation, long hairy fleece, clonic rhythmic tremors ("*hairy shakers*"), unthriftiness, and poor viability (see Vol. 1, Nervous system; Vol. 1, Skin and appendages; Vol. 3, Female genital system).

A syndrome resembling mucosal disease has been reported in lambs that survived the initial border disease; they were persistently infected with an NCP BVDV. Immunohistochemical examination of tissues from persistently infected sheep reveals viral antigen in smooth-muscle cells of hollow organs and blood vessels, epithelial cells in the gastrointestinal tract, lymphocytes, neurons, and glial cells. When cytopathic BDV is superimposed on persistent infection, affected sheep develop chronic diarrhea, wasting, nasal discharge, and polypnea. Macroscopic lesions are particularly present in the cecum and colon and in a few sheep also the terminal ileum. There is marked thickening of the gut wall due to subserosal and mucosal edema and diffuse polypoid hyperplasia of the mucosa, which is hemorrhagic and focally ulcerated.

The *microscopic lesions* in the gut are similar to those described for mucosal disease in cattle. Lymphoid cell reactions are evident in the choroid plexus, portal triads of the liver, kidney, myocardium, thyroids, lungs, spleen, and lymph nodes. In addition, some lambs have marked hypertrophy and edema of the muscularis of the terminal ileum. The lesions in the terminal ileum resemble "terminal ileitis," and BVDV should be considered as a possible cause of that syndrome.

The pathogenesis of fetal infections, resultant border disease, and related enteric lesions in sheep appear to be similar to the multitude of conditions associated with NCP BVDV prenatal infections in cattle. Seronegative ewes infected before 80 days of gestation may produce persistently infected, immunotolerant, chronically viremic lambs.

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Rinderpest

Otherwise known as "cattle plague," rinderpest is an *acute or subacute highly contagious disease of cattle, characterized by erosive or hemorrhagic lesions of all mucous membranes*. The distribution of the disease is progressively shrinking, but it is still enzootic in parts of Africa, the Middle East, and the Orient, to which places it is restricted by limitations on animal movement and the use of highly efficacious vaccine. Pandemics have occurred in the Middle East, sub-Saharan and equatorial Africa, and some of these were apparently related to a relaxation in vaccination programs.

Rinderpest virus (RPV) belongs to the family Paramyxoviridae, genus *Morbillivirus*. It is a highly pleomorphic single-stranded RNA virus with a core diameter of 120–300 nm and a spiked envelope. The virus is highly fragile under ordinary environmental conditions; it is incapable of surviving more than a few hours outside the animal body under normal circumstances.

Probably all cloven-hoofed animals are naturally susceptible to infection, but the expression of infection varies considerably. Goats and sheep do respond, but inconsistently, to experimental inoculations of RPV. Infection in Asiatic pigs may be severe but it tends to be mild in European breeds, which are considered "dead-end" hosts for rinderpest.

Although different strains of RPV vary considerably in their pathogenicity, they are grouped in a single serotype and, when suitably modified, make effective vaccines.

Control of the disease in endemic areas is impeded by difficulties inherent in systems of husbandry and by the coexistence of cattle with large populations of susceptible ungulate wildlife. The infection impacts heavily on wildlife populations in close contact with cattle, and they are important in virus spread. However, wildlife in east Africa are not clearly implicated as reservoirs of the infection. The lability of the virus is such that the *spread of infection* from endemic areas is most likely to be by live animals with mild or subclinical disease.

The disease in cattle may be mild, especially in endemic areas, but will probably be acute or peracute and severe in new foci. The different degrees of severity are in part due to real differences in virulence of strains, and largely due to differences in susceptibility of breeds or races of cattle. Rinderpest may also persist for prolonged periods as a very mild disease in endemically infected cattle and wild ungulate herds and may remain stable for years before the emergence of more pathogenic strains that induce the characteristic disease.

The *nasopharyngeal mucosa* appears to be the main portal of entry in rinderpest. The virus uses glycoproteins expressed on activate lymphocytes and monocytes and on dendritic cells as receptors, and destruction of such cells may be a means by which it causes immunocompromise. It localizes and replicates initially in the palatine tonsils and regional lymph nodes. This is followed after an 8–11-day incubation by a 2–3-day period of viremia that coincides with the fever seen clinically. In circulation, the virus is associated with mononuclear cells. After the viremic stage, the virus replicates in all lymphoid tissues, the bone marrow, and the mucosa of the upper respiratory tract and the gastrointestinal tract. Nasal, oral, and ocular secretions, as well as feces, contain high titers of the virus. In general, excretion of virus ceases by about day 9 of the clinical disease, with the onset of neutralizing antibodies. Recovered animals do not appear to be carriers, although there are reports to the contrary.

Fever and its attendant signs usher in the **clinical syndrome**, with early leukopenia. Fever reaches its peak in about 3 days and falls with the onset of diarrhea, which may be bloody. There is severe abdominal pain, anorexia, ocular and nasal discharge, tachypnea, fetid breath, occasional cough, lethargy, severe dehydration and emaciation, and prostration. Death occurs in 5–8 days. Explosive outbreaks with high morbidity and mortality are more likely to occur in naive populations. Vaccinated or recovered animals usually have lifelong immunity. Secondary bacterial, viral, protozoal, and rickettsial infections are common.

The **gross morbid anatomical changes** in rinderpest are characteristic but not pathognomonic, and are similar to mucosal disease (Fig. 1.97). The lesions in the upper alimentary tract are *necrotizing and erosive-ulcerative*.

RPV has an affinity for the alimentary epithelium. Most severely affected areas in the oral cavity are those contiguous with lymphoid aggregates. Consequently, the caudal part of the oral cavity is affected preferentially. There is some strain variation with respect to presence of oral lesions. In nonfatal cases, there is rapid regeneration of the oral mucosal lesions. Esophageal erosions are usually mild and affect the proximal portion. The forestomachs rarely exhibit any lesions.

The **histologic lesions** of stratified squamous epithelium originate in the stratum spinosum. Entrance into the epithelium may be via infected Langerhans cells that then pass virus along to adjacent cells. Immunohistochemically, irregularly shaped rafts of acanthocytes are infected with virus. These same cells then undergo degeneration and necrosis. Multinucleate syncytia form in the epithelium (Fig. 1.98) and these may have *cytoplasmic and nuclear inclusions*. Abrasion causes the necrotic tissue to lift off and produce shallow erosions or ulcers. This occurs so readily that they are usually the first lesions observed. Their margins are sharp, and the bases are reddened by the underlying congested capillaries. The initial minute erosions enlarge and coalesce to form extensive defects.

The abomasum is often severely reddened, which may just be a reflection of generalized stress for, although immunohistochemically abomasal epithelium is infected with virus, the extent of infection and resulting necrosis is far less than that seen in intestinal mucosa.

Lesions in the intestine are severe and severity correlates with amount of lymphoid tissue in subjacent areas. Consequently, greatest mucosal damage is seen in ileum and the proximal colonic patch. Peyer's patches are almost universally involved. These areas become hemorrhagic and necrotic, and are associated with necrosis of the overlying mucosa, leaving deep ulcers (Fig. 1.99A).

Microscopically and immunohistochemically, there is replication of virus at all levels of intestine, with both crypt and villus epithelium involved. Replication is associated with formation of inclusion bodies, both nuclear and cytoplasmic, degeneration, necrosis, denuding of epithelium, formation of crypt abscesses and, if prolonged enough, villus atrophy. The formation of syncytia within gut epithelium is a rare event, in contrast to the oral cavity lesions, where it is seen with some regularity.

Receptor affinity dictates that *RPV is tropic for lymphoid tissues*. Infection and replication have been documented in both lymphocytes and macrophages. Necrosis of follicular lymphocytes is extreme, and gross inspection, which reveals little abnormality of

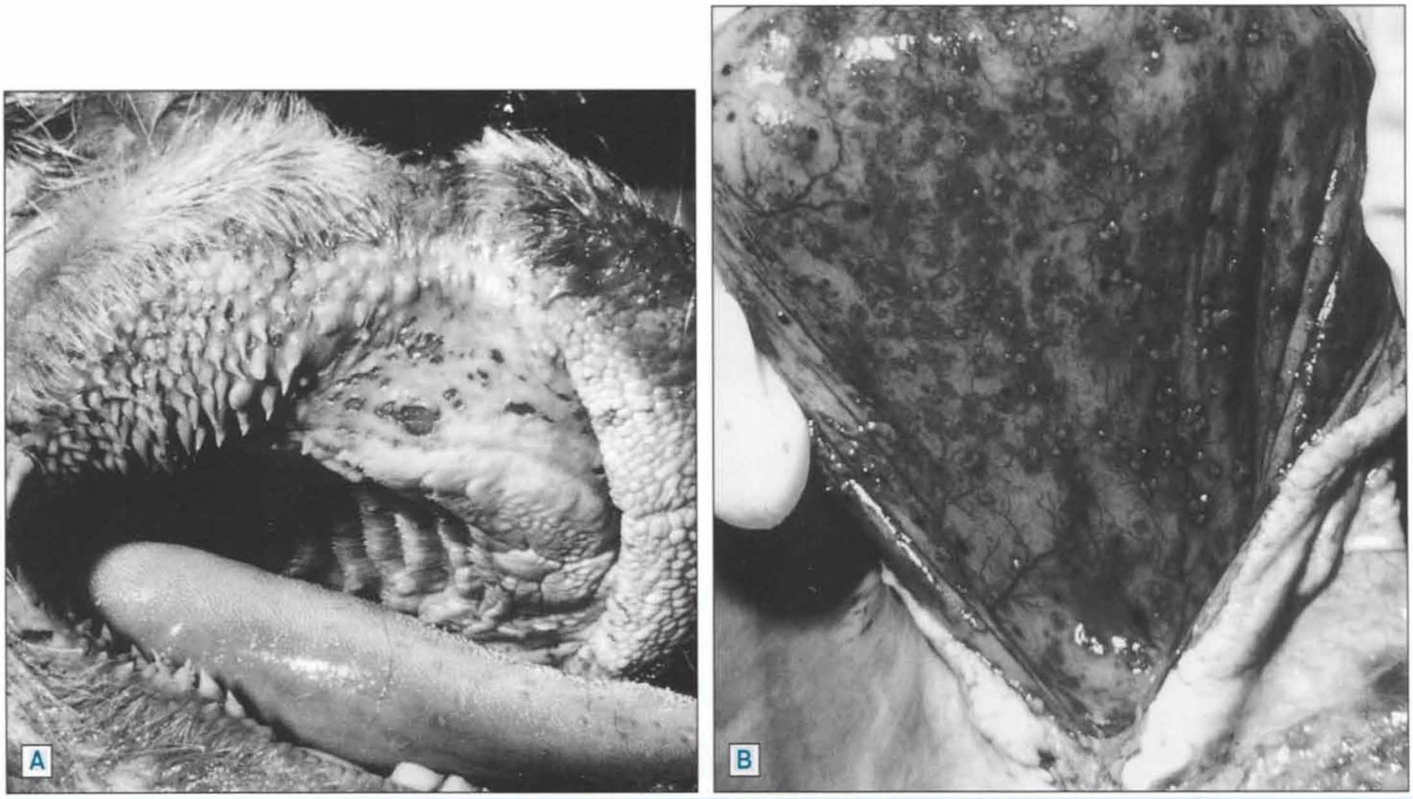

Figure 1.97 Rinderpest in a cow. **A.** Ulcers on the buccal mucosa, and small incipient ulcers on the underside of the tongue. **B.** Multifocal to coalescing fibrinohemorrhagic colitis.

nodes, is misleading (Fig. 1.99B). Multinucleate cells, similar to those in the oral mucosa, occasionally form in the lymph and hemolymph nodes. All or only some follicles may be involved and there is often an increase of other leukocytes in the sinuses. Similar lesions occur in the spleen, tonsils, and, as already noted, in the Peyer's patches.

Acute congestion and edema of the conjunctiva may be followed by purulent conjunctivitis and corneal ulceration. Petechiae are common in the mucosa of the upper respiratory tract, which is usually covered with mucopurulent exudate.

The gross lesions resemble those of severe acute bovine viral diarrhea and mucosal disease, but *rinderpest is distinguished microscopically most readily by the presence of syncytia and inclusion bodies.* Its regulatory status demands laboratory diagnosis in accredited laboratories, by virus isolation, antigen identification in fresh or fixed specimens, or polymerase chain reaction.

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Peste des petits ruminants

Peste des petits ruminants is an acute viral disease of sheep and goats that closely resembles rinderpest and is also known as kata, *stomatitis–pneumoenteritis complex*, goat plague, and pseudorinderpest. The disease was first recognized in West Africa, and is now distributed in sub-Saharan Africa, the Arabian Peninsula, Anatolia, and the Indian subcontinent, including Nepal and Bangladesh. *Peste-des-petits-ruminants virus* (PPRV) is in genus *Morbillivirus*, family Paramyxoviridae, closely related to *Rinderpest virus*, with which it shares common antigenic determinants. The virus cross-reacts with RPV in the immunodiffusion and complement fixation tests. It may be differentiated from RPV using monoclonal antibody techniques and cDNA probes.

The clinical signs, pathogenesis, and lesions of the disease in sheep and goats in general are similar to those of rinderpest (Fig. 1.100), except that the disease is more acute in onset, especially in goats, and follows a more rapid course.

Another difference is the marked involvement of the respiratory tract; affected animals have dyspnea, hyperpnea, and cough. There is

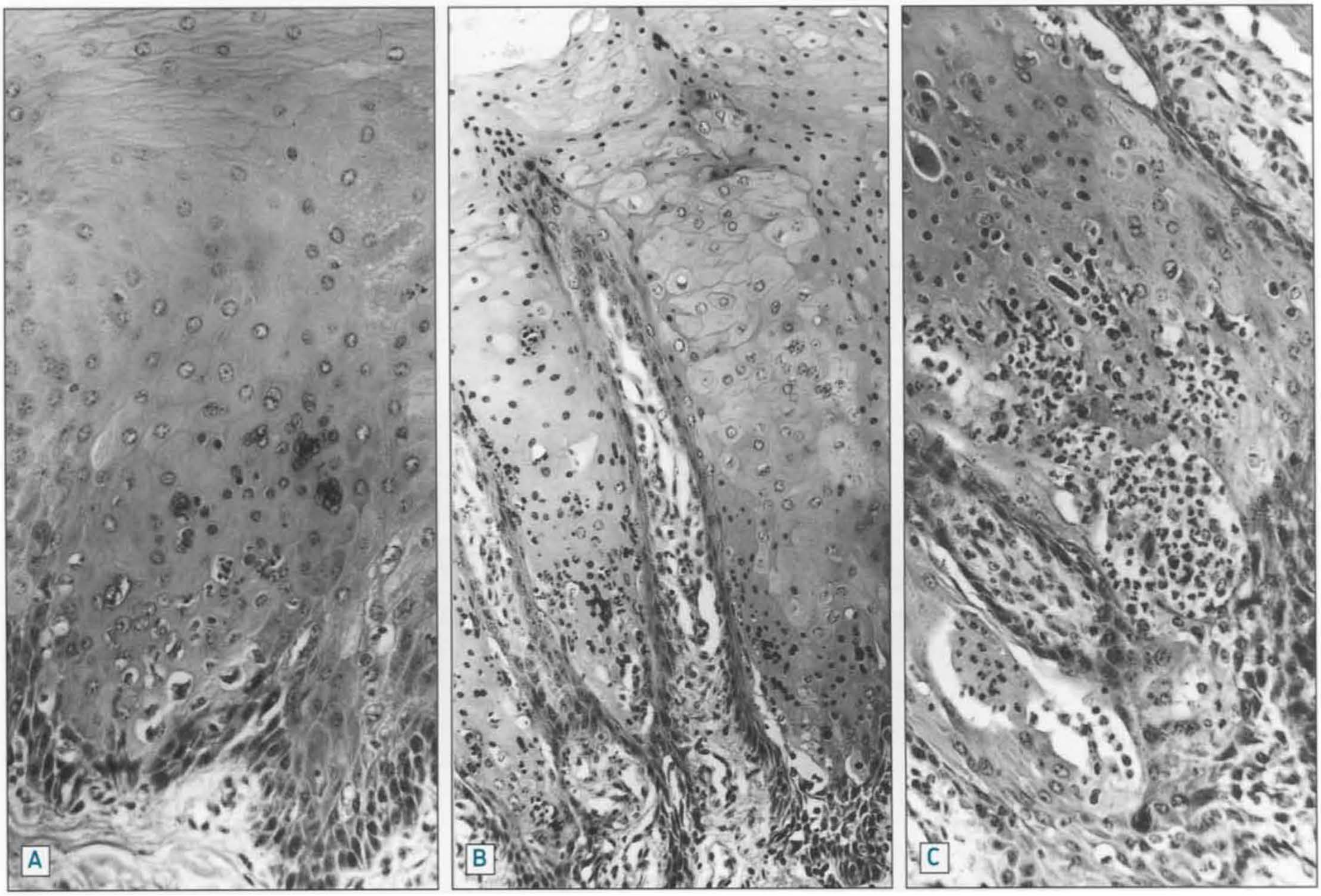

Figure 1.98 Rinderpest lesions in the tongue of an ox. **A.** Early stage of oral lesion showing disorganization of epithelium above the basal layer and formation of syncytial cells. **B, C.** Slightly later stage of (**A**) with beginning separation sparing basal cells.

also a marked serous to mucopurulent nasal and ocular discharge, and erosion/ulceration of the pharyngeal epithelium may be diffuse. The pulmonary lesions of peste des petits ruminants are similar to pneumonia due to *Canine distemper virus* in dogs and *Measles virus* infections in humans. The macroscopic pulmonary lesions include consolidation, atelectasis, and dark-red discoloration of the cranioventral lobes. Some animals have fibrinous pleuritis (see Vol. 2, Respiratory system). Microscopically, there is mild multifocal tracheitis, bronchitis and necrotizing bronchiolitis, and diffuse proliferative *interstitial pneumonia*, with formation of alveolar syncytial cells. Eosinophilic cytoplasmic and nuclear inclusions are present in the epithelial cells of the air passages, type II pneumocytes, and syncytial cells. Viral antigen may be demonstrated in the same cells with appropriate immunohistochemical techniques. The primary viral lesions are often complicated by secondary bacterial infections.

Experimental inoculation of PPRV into cattle and pigs does not produce clinical disease, but these animals will resist subsequent challenge with *Rinderpest virus*. These species are considered to be "dead-end" hosts, since they do not seem to spread the infection to other species. Natural infection or vaccination of sheep and goats with *Rinderpest virus* will protect them against PPRV.

A zoo outbreak of peste des petits ruminants that involved several species of wild ungulates has been reported, and Indian buffalo are also susceptible. The distribution of the virus in free-ranging wild ungulates has not been investigated.

Peste des petits ruminants must be distinguished from rinderpest in the laboratory based on use of monoclonal antibodies to distinguish the agents immunologically in wet or fixed tissue, or by molecular techniques.

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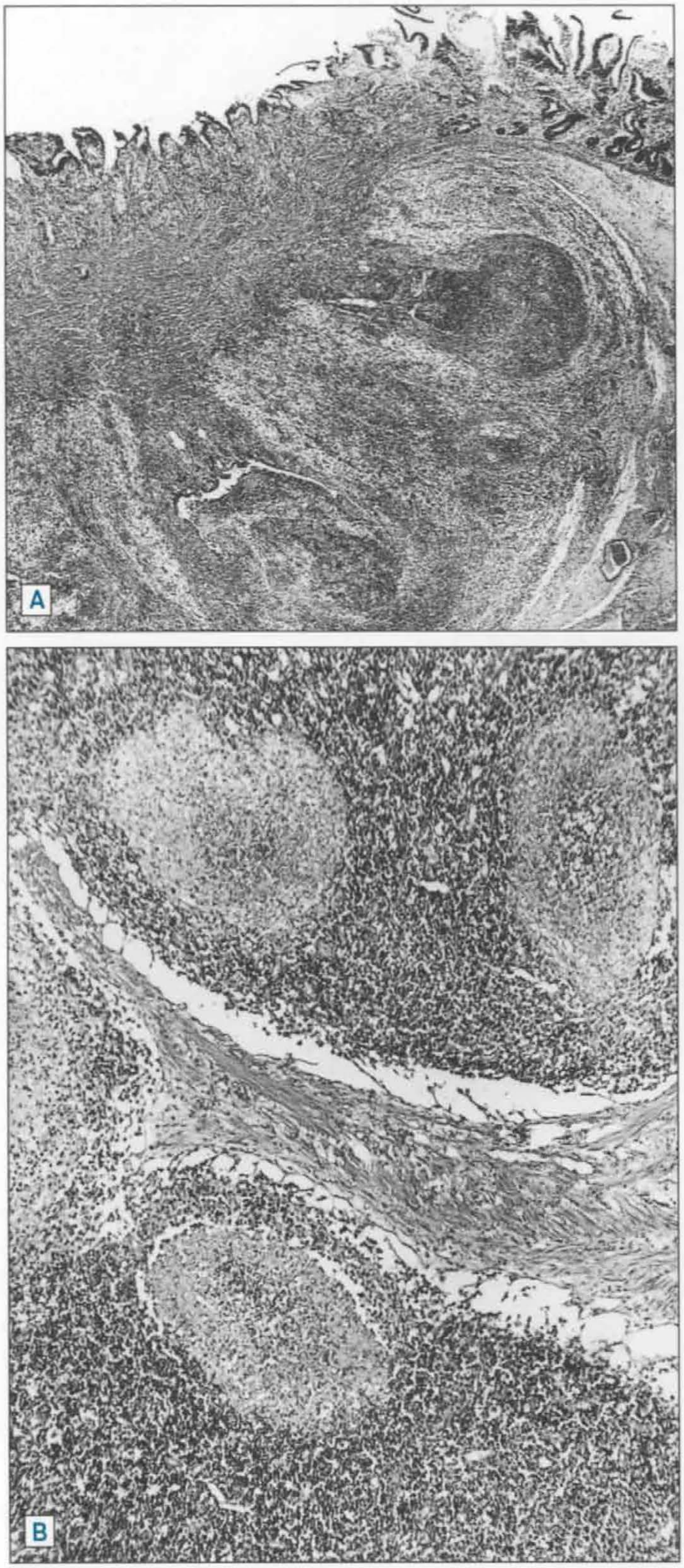

Figure 1.99 Rinderpest in an ox. **A.** Necrosis of Peyer's patch in ileum. **B.** Necrosis of germinal centers in a lymph node.

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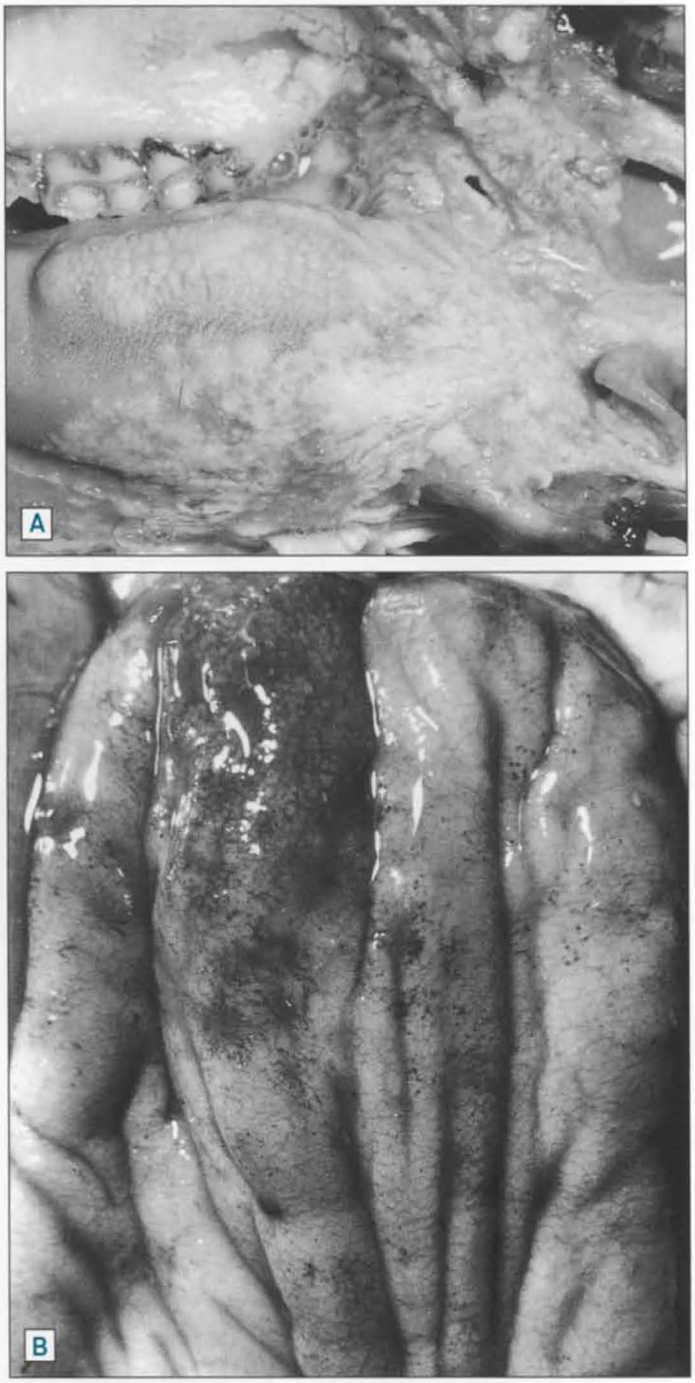

Figure 1.100 Peste-des-petits-ruminants in a goat. **A.** Palatine ulcers, and raised plaques on the mucosa of the side of the tongue and oropharynx. **B.** Diffuse edema, and focal congestion and ulceration of the cecal mucosa.

Malignant catarrhal fever

Malignant catarrhal fever (MCF) is an infectious disease of domestic cattle, some wild ruminants, and occasionally pigs, also known as malignant head catarrh, and snotsiekte. The disease is characterized by *lymphoproliferation, vasculitis, and erosive-ulcerative mucosal and cutaneous lesions.*

MCF is distributed worldwide. It is generally sporadic, although severe herd outbreaks have been reported in feedlot, dairy and range cattle, in farmed bison and deer, and in zoos. Among the Cervidae, all species except fallow deer are probably susceptible. Other susceptible species of ruminants include banteng, Cape buffalo, and greater kudu. *Mortality in susceptible species approaches 100%,* although there are rare recorded cases of chronic infection and also of recovery from the disease, especially in infected goats, bison, cattle, and pigs. Although the agent is transmissible, the disease is apparently not contagious among cattle or bison by direct contact.

MCF is caused by cross-species infections with members of the MCF virus group of ruminant rhadinoviruses (genus *Rhadinovirus*, subfamily Gammaherpesvirinae), four of which are associated with clinical MCF: (1) ***Alcelaphine herpesvirus 1*** (AlHV-1), carried by wildebeest (*Connochaetes* sp.); (2) ***Ovine herpesvirus 2*** (OHV-2), endemic in domestic sheep; (3) ***Caprine herpesvirus 2*** (CpHV-2), endemic in domestic goats; and (4) a virus of undetermined origin causing *MCF in white-tailed deer* (MCFV-WTD). Related rhadinoviruses, as yet unassociated with MCF, have been detected in a variety of members of the Bovidae. Rhadinoviruses of ruminants are highly cell-associated lymphotropic herpesviruses, difficult or impossible to isolate, which are typically transmitted from adults to offspring within the first 2–3 months of life, probably via free virus shed in nasal secretions. In the natural host, infection is latent or inapparent, with intermittent virus shedding, although disease has been incited in sheep by experimental aerosol inoculation of a large dose of OHV-2.

Formerly, MCF was described as the "African" or *wildebeest-associated* (**WA-MCF**) form if it was presumed to be due to AlHV-1, and "North American" or *sheep-associated* (**SA-MCF**) if it was presumed to be due to exposure to sheep. There is no clinicopathologic difference in the disease induced by the various rhadinoviruses, and these terms have only broad epidemiologic connotations. The blue, brindled, or white-bearded wildebeest (*Connochaetes taurinus*) carries AlHV-1. Wildebeest calves become infected during the first 2–3 months of life, when they are also viremic and shed cell-free AlHV-1 in nasal and ocular secretions. Most wildebeest older than 7 months are serologically positive for AlHV-1. In utero infections have also been reported. Wildebeest are infected for life and transmit AlHV-1 to their calves without showing clinical signs. *Wildebeest calves are considered to be the main source of infection for cattle in East Africa.* They may shed infective cell-free virus, in nasal and ocular secretions, for several days. Transmission to cattle may occur even without intimate contact, suggesting aerosol spread. Viremia apparently ceases with the development of active neutralizing antibodies in animals >6 months old. It may be reactivated during late pregnancy or periods of stress, e.g., transportation. Although AlHV-1 produces MCF in many captive exotic species of ruminants, apparently most species that are exposed in their native habitat do not develop disease. AlHV-1 has been transmitted and adapted to domestic rabbits, hamsters, rats, and guinea pigs, in which it produces MCF-like lesions.

The etiologic agent of SA-MCF has never been isolated from sheep; however, polymerase chain reaction probes have permitted its differentiation. *Most sheep have polymerase chain reaction-detectable specific OHV-2 sequences in cells,* and identical sequences are detectable in spontaneous cases of SA-MCF. Experimental transmission of OHV-2 between sheep has been accomplished using an aerosol of virus-infected nasal secretions, and natural transmission from adults to offspring probably takes that route, producing very high rates of infection in the sheep population, where it can be considered ubiquitous. The other two rhadinoviruses associated with MCF have also been identified and implicated by molecular diagnostic techniques.

Attempts to reproduce MCF in cattle by inoculating sheep tissues and secretions have been unsuccessful, but MCF-like disease can be induced in rabbits, hamsters, and guinea pigs by transfer of lymphocytes or T lymphoblast cell lines derived from MCF-affected cattle and deer. The domestic rabbit is the most commonly used model to study the pathogenesis of both forms of MCF. MCF due to OHV-2 occurs spontaneously and experimentally in pigs, and the relative rarity of the disease in swine may be due to lack of exposure to ruminant rhadinoviruses under most conditions of husbandry.

SA-MCF occurs where bovids and deer come in contact with sheep. There is considerable variation in the susceptibility of various ruminant species to SA-MCF. Domestic cattle (*Bos taurus* and *B. indicus*) appear to require high levels of exposure to induce disease. Bali cattle or banteng (*B. javanicus*), the domestic water buffalo (*Bubalus bubalis*), American bison (*Bison bison*) and most species of deer, with the exception of fallow deer (*Dama dama*), seem to be highly susceptible. MCF is one of the most serious diseases of farmed deer in New Zealand, Australia, and the UK. Multiple case outbreaks have also been reported in captive North American cervids.

The *mucosa of the upper respiratory tract and/or the tonsil* is the most likely natural route of entry for the agents of MCF. Both WA-MCF and SA-MCF can be transmitted to susceptible hosts with large volumes of whole blood or lymphoid tissues administered intravenously, but not by cell-free filtrates, indicating that the agents are cell-associated, probably with lymphocytes. The incubation period of MCF is usually 2–10 weeks, but may, on occasion, be very much longer than this.

Antibodies against the rhadinovirus involved can be detected in animals with MCF, and often in herdmates, implying subclinical infection. Development of antibodies does not prevent a fatal outcome.

The **pathogenesis**, clinical signs, and lesions are similar, whatever the agent inducing MCF. Viremia in WA-MCF usually starts about 7 days before the onset of fever, and persists throughout the course of the disease. MCF is characterized by *marked T-lymphocyte hyperplasia.* A population of large granular lymphocytes appears to be infected and transformed by rhadinoviral infection, and OHV-2 genome has been detected in CD8+ T cells, the predominant cell infiltrating around vessels in the brains studied. These cells are probably cytotoxic T lymphocytes or T-suppressor cells, but the mechanism by which they mediate the lesions of MCF is unclear. Dysfunction of this cell population may result in de-repression of T-lymphocyte replication, permitting lymphoproliferation. Deranged cytotoxic T-cell activity may then create the epithelial and vascular lesions, through a type of graft-versus-host response, attacking epithelium of the respiratory and gastrointestinal systems, as well as medium-sized arteries throughout the body. This is a unifying, but unproven, hypothesis explaining the lymphadenopathy, mucosal epithelial lesions, and vasculitis characteristic of MCF. Vascular lesions may mediate infarction of some affected tissue fields, as well.

There is wide variation in the presenting **clinical syndromes**, which are potentially pansystemic. Quite consistently, affected animals have enlarged lymph nodes, although this may be less the case in bison, and there is usually some degree of ocular and oral disease, and exudative dermatitis. There is edema of the eyelids and palpebral conjunctivae and congestion of the nasal and buccal mucosae.

Photophobia is accompanied by copious lacrimation. There is conjunctivitis and an increasing rim of corneal opacity, starting at the limbus and progressing centripetally. Corneal ulceration occurs in some cases, but in those that die quickly, the infiltration of the filtration angle may be all that is seen, and this is easily overlooked. Hypopyon may be seen. In some cases there are nervous signs, such as hyperesthesia, head pressing, trembling, nystagmus, incoordination, and behavioral changes. Other animals may have gastroenteritis with diarrhea, which may in acute cases be bloody. This is most commonly seen in deer. The disease may take an acute course of about 1–3 days, particularly in animals with hemorrhagic enteritis. Those with less severe gastroenteritis, central nervous signs, or generalized disease may linger for as long as 9–10 days. Mortality in MCF has been considered to approach 100% of clinical cases, but recovery may occur, although chronic ocular lesions and vasculitis persist.

Gross morbid changes may not be present in occasional animals that die of peracute MCF, and in these the diagnosis must rest on the detection of the characteristic histologic changes, and demonstration of the genome of an implicated rhadinovirus in tissue.

The carcass is dehydrated, and may be emaciated if the course has been prolonged. Conjunctivitis may be evident. The muzzle and nares are heavily encrusted and, if wiped, often reveal irregular raw surfaces, although in some cases there may be only a slight serous discharge. Cutaneous lesions, especially in SA-MCF, are common, but often overlooked. Affected areas include the thorax, abdomen, inguinal regions, perineum, udder, and occasionally the head. There may be, acutely, more or less generalized exanthema with sufficient exudation to wet and mat the hair, and to form detachable crusts; in unpigmented skin there is obvious hyperemia. The crusts may become several millimeters thick, and there is patchy loss of hair. Sometimes these cutaneous changes begin locally about the base of the hooves and horns, the loin, and perineum; they may remain localized or become generalized. In severe cases, the horns and hooves may slough. *Caprine herpesvirus-2* has been associated with syndromes in deer that include dermatitis and alopecia, alone, or in combination with gastrointestinal or neurological disease.

The respiratory system may have minor or severe lesions (Fig. 1.101A). When the course is short, the nasal mucosa may only have congestion and slight serous exudation. Later, there is a copious discharge. Lesions are most severe in the rostral third of the nasal cavity, corresponding to the zone of stratified squamous epithelium. In some cases, fibrinous tracheobronchitis may occur (Fig. 1.101B).

The lower alimentary mucosae may have no significant lesions in the peracute disease, although *oral lesions are present in most cases of MCF*. Minor erosions are first observed on the lips adjacent to the mucocutaneous junction. Sometimes apparently normal epithelium on the surface of the tongue peels off in sheets (Fig. 1.102). Later, erosive and ulcerative lesions may involve a large area of oral mucosa, frequently occurring on all surfaces of the tongue, the dental pad, the tips of the buccal papillae, gingivae, both areas of the palate, and the cheeks. In some areas, the cheesy or tattered necrotic epithelium may not be sloughed at the time of inspection. Esophageal erosions or

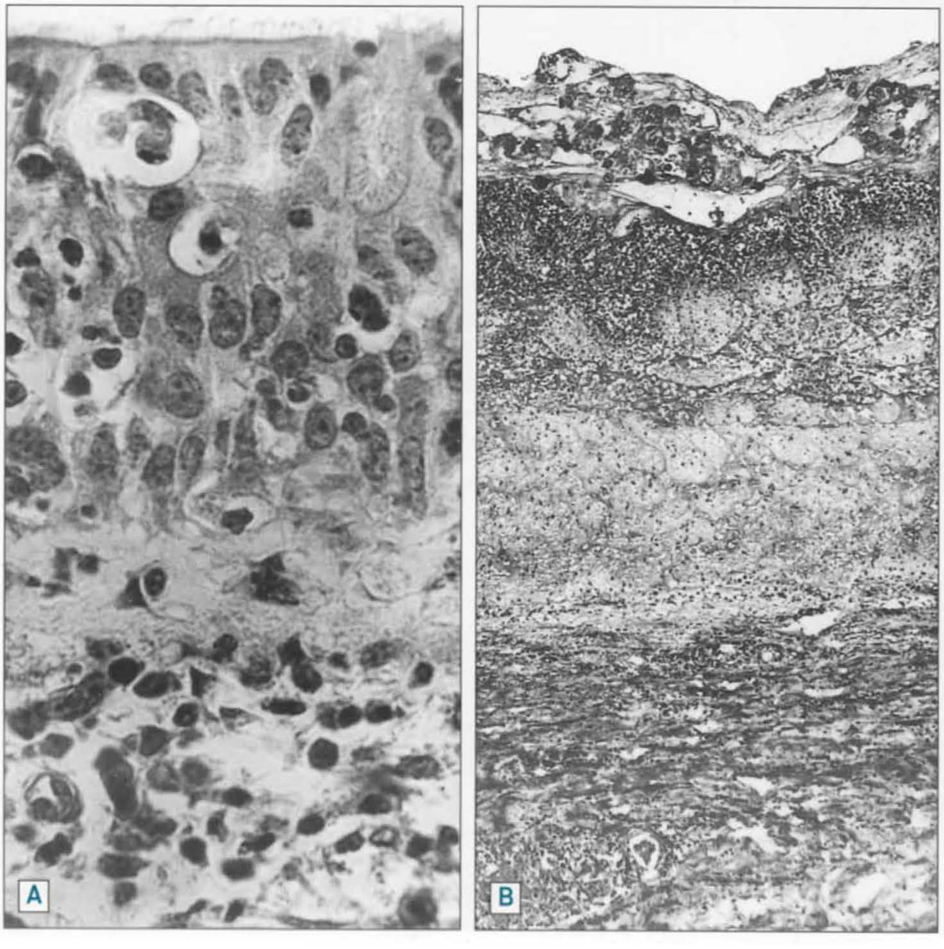

Figure 1.101 Malignant catarrhal fever. **A.** Nasal mucosa, with degeneration of epithelium and infiltration of lymphocytic cells in uncomplicated rhinitis. **B.** Pseudomembranous tracheitis.

ulcers, similar to those that occur in the other diseases causing ulcerative stomatitis, occur in MCF, and, as in rinderpest, are most consistent in the cranial portion. Lesions of the same sort may be present in the forestomachs. Focal ulceration or generalized hyperemia may be evident in the abomasal mucosa. In deer, especially, hemorrhagic or fibrinohemorrhagic typhlocolitis may be a prominent finding.

The *liver* may be slightly enlarged. Close inspection will reveal, in some cases, diffuse mottling with white foci, which are periportal accumulations of mononuclear cells (Fig. 1.103). There may be numerous small hemorrhages and a few erosions of the mucous membrane of the gallbladder.

Characteristic lesions may occur in the *urinary system.* Renal changes are not always present. They are infarcts or 2–4 mm foci of nonsuppurative interstitial nephritis (Fig. 1.104). They may be numerous enough to produce a mottled appearance, and may form slight rounded projections from the capsular surfaces. The pelvic and ureteral mucosa frequently has petechial and ecchymotic hemorrhages. Similar lesions are present on the mucosa of the urinary bladder, or there may be more severe hemorrhage erosion and ulceration of the epithelium, and hemat Superficial lesions may occur in the vagina and vulva of the oral cavity and skin.

Enlargement of lymph nodes is a characteristic le most species, perhaps except bison. All nodes may some may appear grossly normal. Affected nodes ma the normal size, and some, including hemolymph usually too small to recognize, may become qui 1.106). There is edema of the affected nodes and connective tissue. On cross-section it is apparent th size is due to *lymphocytic hyperplasia.* Some of the no or hemorrhagic. The spleen is slightly enlarged, a follicles are prominent.

Most animals may have *meningoencephalitis,* as a tis, perhaps reflected grossly by meningeal edema, the most consistent histologic lesions of the diseas

The **histologic changes** must usually be relied nosis of MCF, and its differentiation from similar access to molecular diagnosis of specific rhadinov increasing. The characteristic histologic changes a phoid tissues and in the adventitia and walls of medi especially arteries in any organ, and these will be

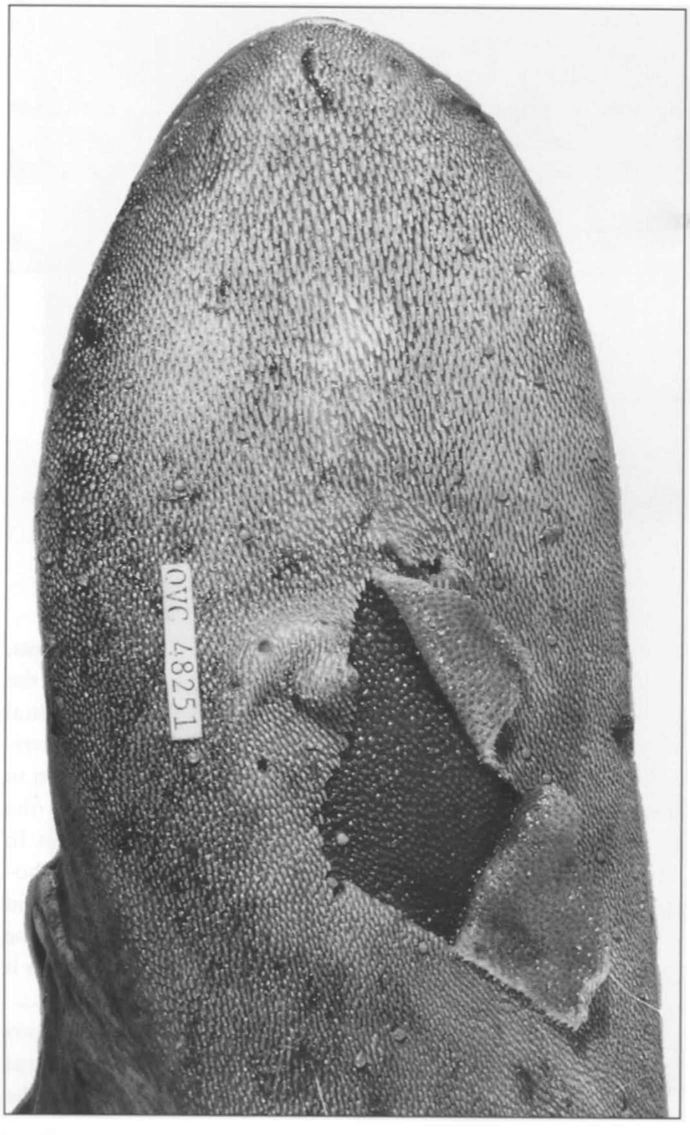

Figure 1.102 Separation of necrotic lingual epithelium from underlying propria in an ox with **malignant catarrhal fever**.

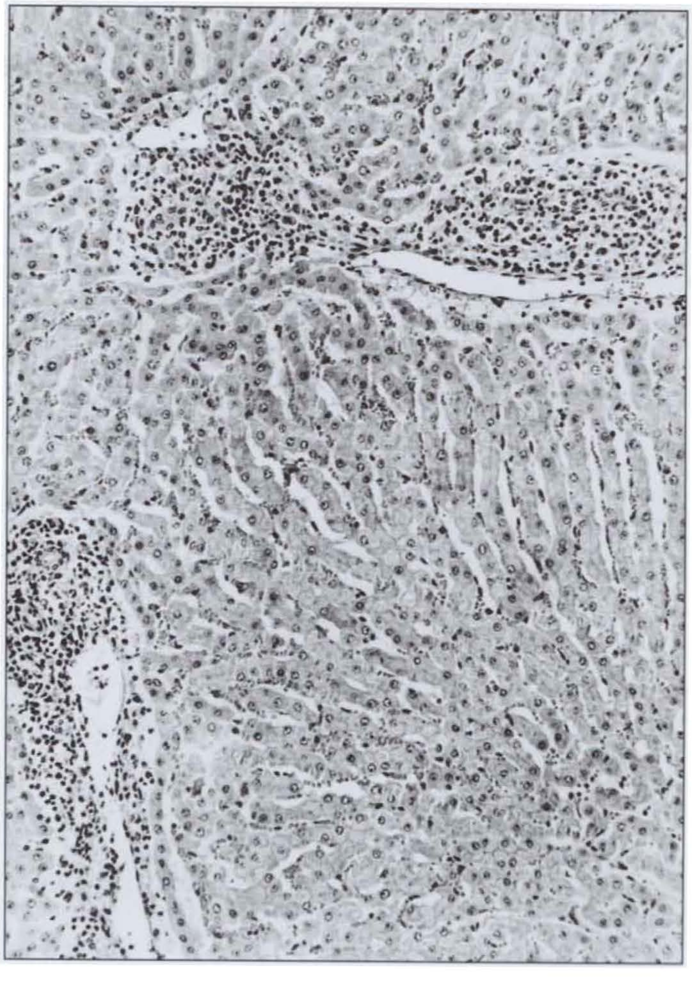

Figure 1.103 Accumulations of lymphocytes in portal triads in **malignant catarrhal fever**.

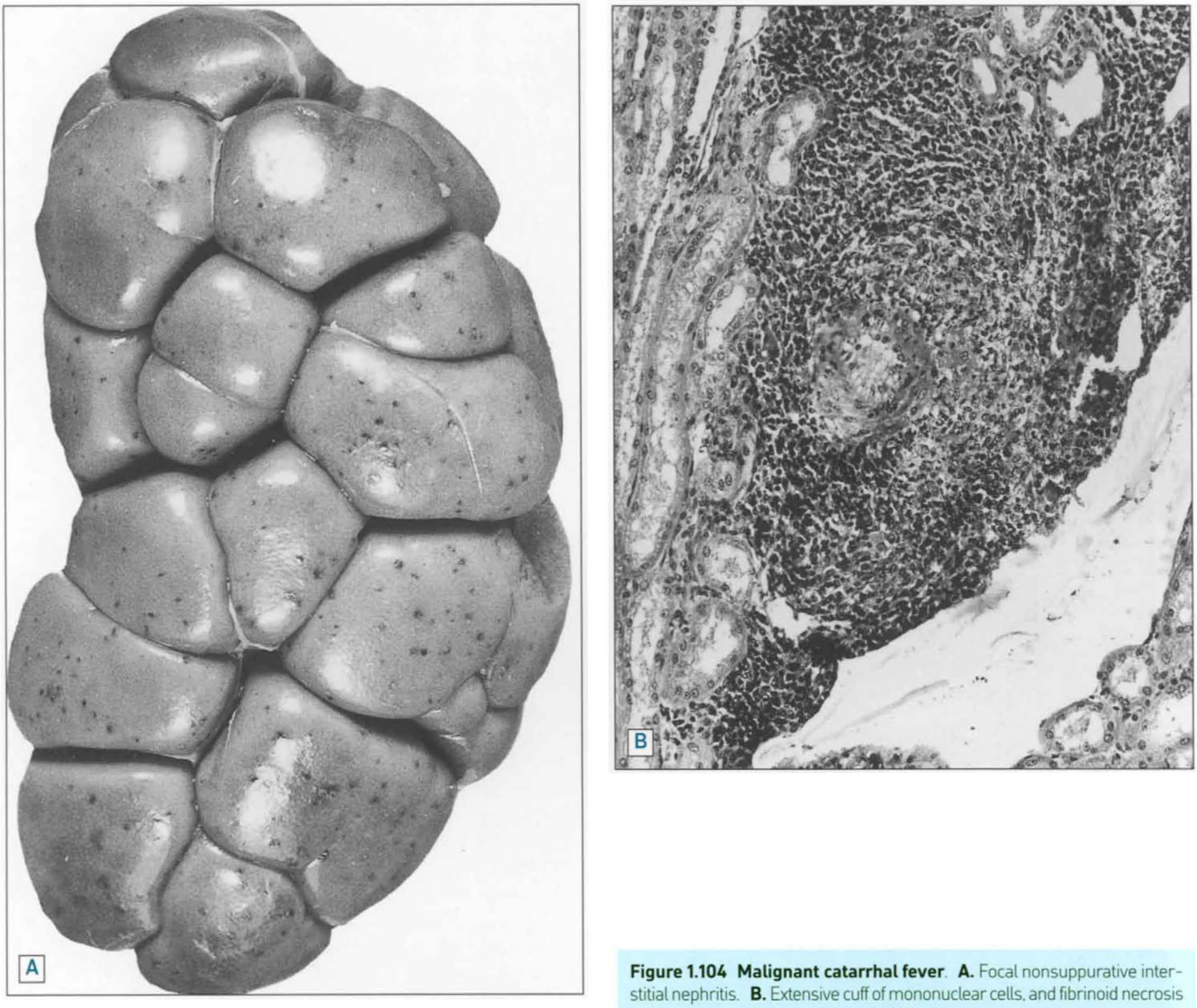

Figure 1.104 Malignant catarrhal fever. **A.** Focal nonsuppurative interstitial nephritis. **B.** Extensive cuff of mononuclear cells, and fibrinoid necrosis in the wall of a small arteriole in the kidney.

other lesions. They are characterized by perivascular accumulation of mainly mononuclear cells, and fibrinoid necrotizing vasculitis (Figs 1.107 and 1.108). These changes may be focal or segmental, and may involve the full thickness of the wall, or be confined more or less to one of the layers. When the intima is involved, there is often endothelial swelling. Thrombi are difficult to demonstrate in damaged vessels. The media may be selectively affected, or perhaps the adventitia alone. Severely affected segments of vessel are replaced by a coagulum of homogeneous, eosinophilic material, in which fragmented nuclear remnants are seen. The perivascular accumulation of cells is particularly characteristic. They are mainly lymphoid cells with large open nuclei and prominent nucleoli; small lymphocytes and plasma cells may be present occasionally. The vascular lesions may be more subtle in other species (especially deer and elk) than in cattle. Cattle that recover from SA-MCF also have distinctive vascular lesions 90 days after clinical onset. Concentric fibrointimal plaques, disrupted inner elastic lamina, focally atrophic tunica media, and vasculitis of variable severity are evident in many organ systems.

In *lymph nodes* there is active proliferation of lymphoblasts, which form extensive homogeneous populations of cells in the T-cell-dependent areas of the interfollicular cortical and paracortical zones. Focal areas of hemorrhage and necrosis associated with arteritis may be seen in all areas of the nodes. The lymphoid reaction in the spleen varies from marked lymphoid cell hyperplasia, in the periarteriolar sheaths, to atrophy and depletion of lymphocytes. In addition, there is marked proliferation and infiltration of lymphocytic and lymphoblastic cells, mainly perivascular in distribution, in a variety of organs. The lymphoreticular proliferation may become so severe in some organs that it is difficult to determine whether it is hyperplastic or neoplastic.

Microscopic arteritis similar to that present in other organs occurs in the nervous system of many cases. Necrotizing arteritis, plasma exudation into the meninges or Virchow–Robin space, and the predominantly adventitial lymphocytic response are, in the brain of cattle, *unique to MCF*, and allow it to be differentiated from other nonsuppurative encephalitides. Degenerative changes in nervous

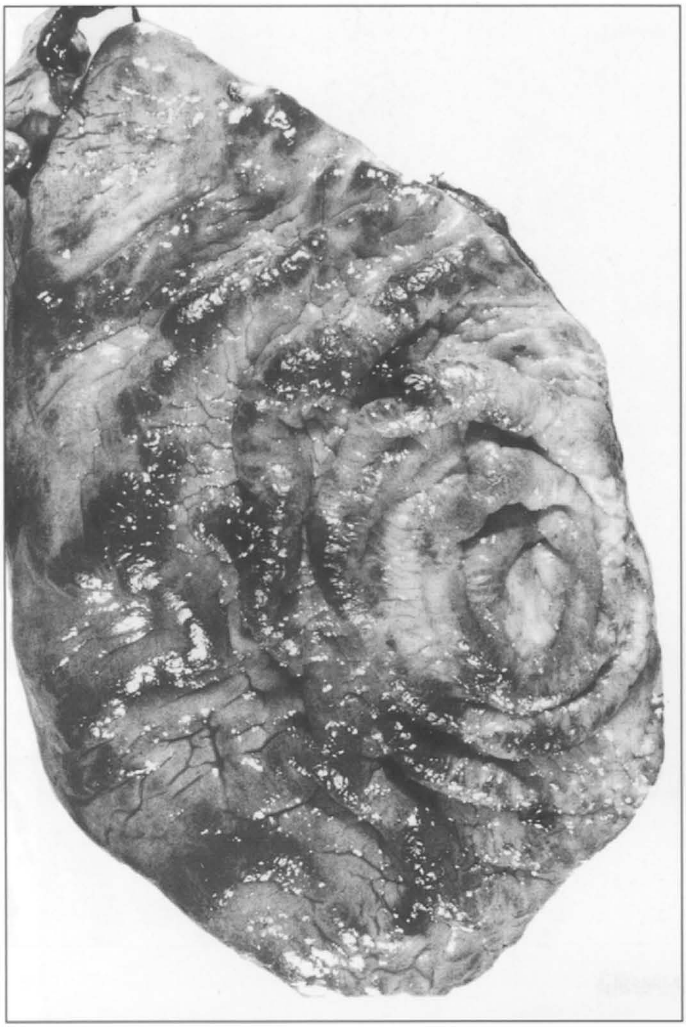

Figure 1.105 Hemorrhages in mucosa of urinary bladder in **malignant catarrhal fever**.

parenchyma can be explained on the basis of the vascular changes.

The lesions in *skin and squamous mucosae* of the alimentary tract consist of a lichenoid infiltrate, as the altered and proliferating lymphoid population moves into the upper dermis and then the epidermis (Fig. 1.109). Often, typical arteritis, involving small and medium-sized vessels, is present in underlying tissue. Groups of epithelial cells become necrotic, with swollen, strongly acidophilic cytoplasm; ultimately the full thickness of epithelium in affected areas undergoes necrosis and ulcerates (Fig. 1.109). Granulomatous mural folliculitis was the prominent microscopic finding in alopecic sika deer infected with CpHV-2.

The mucosa of the abomasum may be infiltrated by lymphocytes, and undergo mucous metaplasia or focal ulceration. The mucosa of the lower alimentary tract, especially the cecum and colon in deer, may similarly be heavily infiltrated by lymphocytes, often with fibrin and blood exuding into the lumen where surface and glandular epithelium has undergone necrosis and collapsed, sometimes over wide areas (Fig. 1.110). Submucosal arterioles in affected areas of abomasum and intestine are affected by the characteristic arteritis.

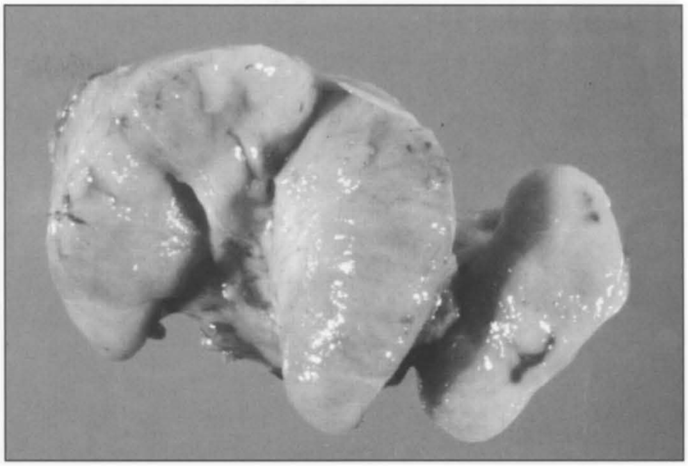

Figure 1.106 Hyperplastic enlarged lymph nodes bulging on cut surface, in cow with **malignant catarrhal fever**.

The *mottling of liver and the focal nephritis* seen grossly are due to the perivascular accumulation of mononuclear cells in the portal triads of the liver (Fig. 1.103) and in the cortices of the kidney. In the liver, these cuffs may be very large and invest the branches of the hepatic artery, which may undergo fibrinoid necrosis. Microscopic lesions are frequently present in the kidneys, even though gross lesions are not; they consist of vasculitis involving the smaller arteries and afferent arterioles. Extensive diffuse lymphocytic infiltrates disrupt the normal renal cortical architecture, and in some cases, infarcts appear to be associated with vasculitis involving arcuate arteries.

Ophthalmitis often occurs, and its presence is a useful differential criterion from other ulcerative diseases of the alimentary tract. Corneal edema, secondary to vasculitis, is responsible initially for the opacity (Fig. 1.111). Later there may be lymphocytic infiltration of various structures within the globe. There is retinal vasculitis and, in some cases, hemorrhagic or inflammatory detachment of the retina in focal areas. Lymphocytic optic neuritis and meningitis may be seen (see Vol. 1, Eye and ear).

Differentiation of acute severe BVD and mucosal disease from MCF is sometimes difficult, but *MCF usually affects one or more organ systems or tissues (liver, kidney, bladder, eye, brain, tracheobronchial tree) not involved in mucosal disease, and typically produces lymphoid hyperplasia in cattle, whereas lymphoid tissue in BVDV infections is expected to be atrophic.* Arteritis may be seen in some cases of BVDV infection, mainly in the submucosa in the lower alimentary tract. Fortunately, arteritis is present in one tissue or another in all cases of MCF, whether peracute, acute, or mild with recovery, though it may be necessary to examine many sections to find it. The best organs to examine for vascular lesions are the brain and leptomeninges, carotid rete, kidney, liver, adrenal capsule and medulla, salivary gland, and any area of skin or alimentary tract showing gross lesions. *A combination of arteritis, lymphoid hyperplasia, and lymphocytic infiltrates into affected epithelia is very characteristic of MCF.*

Bali cattle as well as other species of cattle and buffalo are affected with a disease that closely resembles MCF, and both diseases occur geographically together in Indonesia. **Jembrana disease** is caused by a *lentivirus* distinct from *Bovine immunodeficiency virus*, and has associated mononuclear proliferation and thrombosis in vessels of multiple organs, seemingly similar to MCF. Proliferation of T lymphocytes

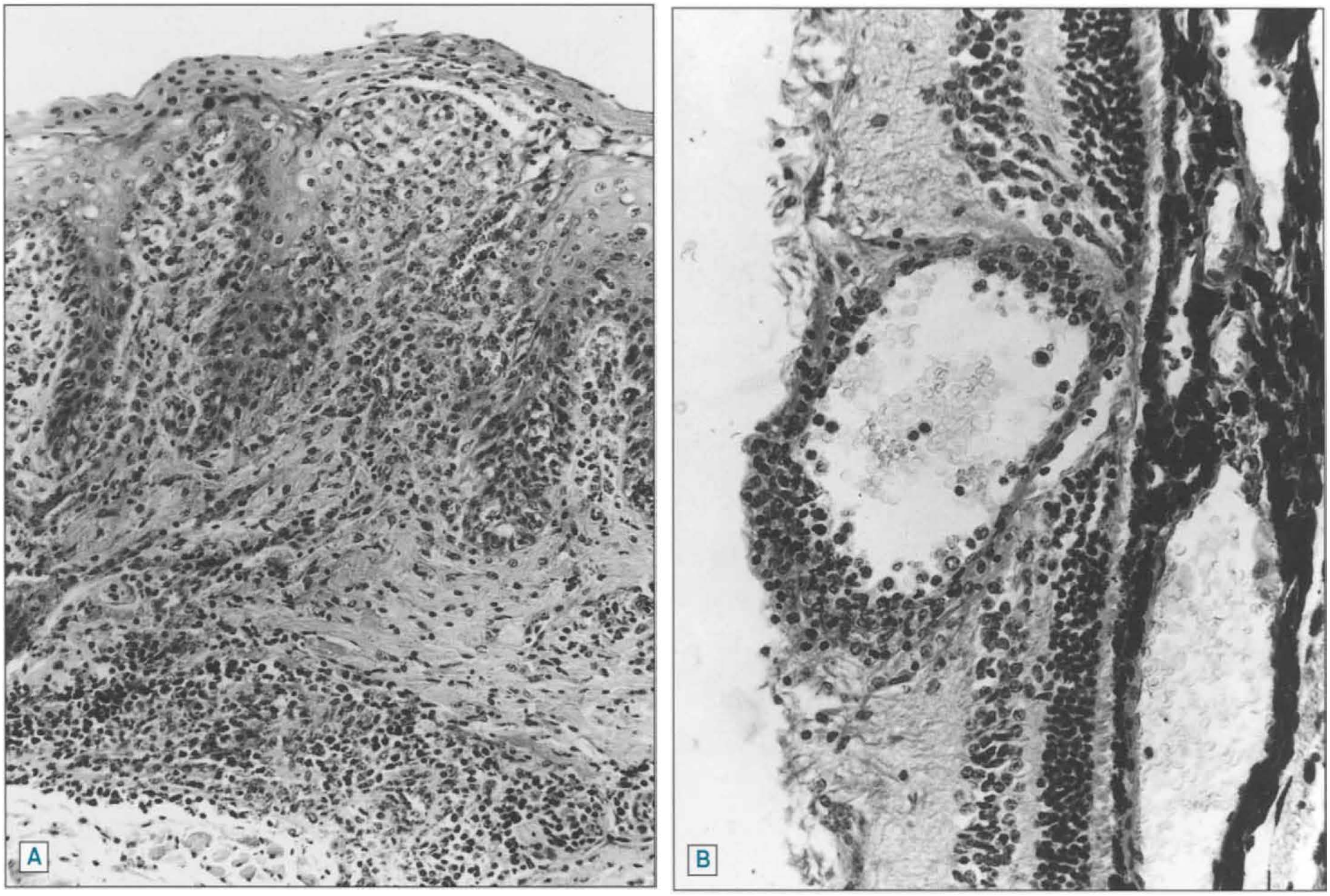

Figure 1.107 Malignant catarrhal fever. **A.** Vasculitis, with infiltration of lamina propria by lymphocytic cells with developing ulcer over papilla in the tongue. **B.** Vasculitis in retina.

and atrophy of follicles in lymph nodes and spleen, with lymphoid infiltrates in multiple organs in Jembrana disease, appear similar to lesions of MCF.

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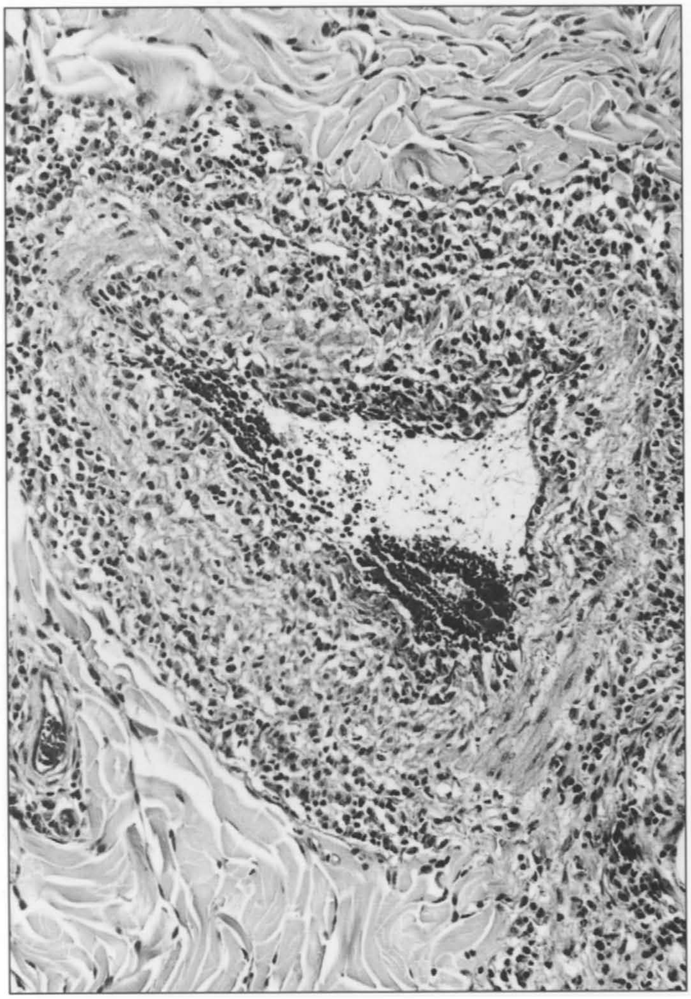

Figure 1.108 Periarteritis in the deep dermis of a cow with **malignant catarrhal fever**. (Courtesy of JA Yager.)

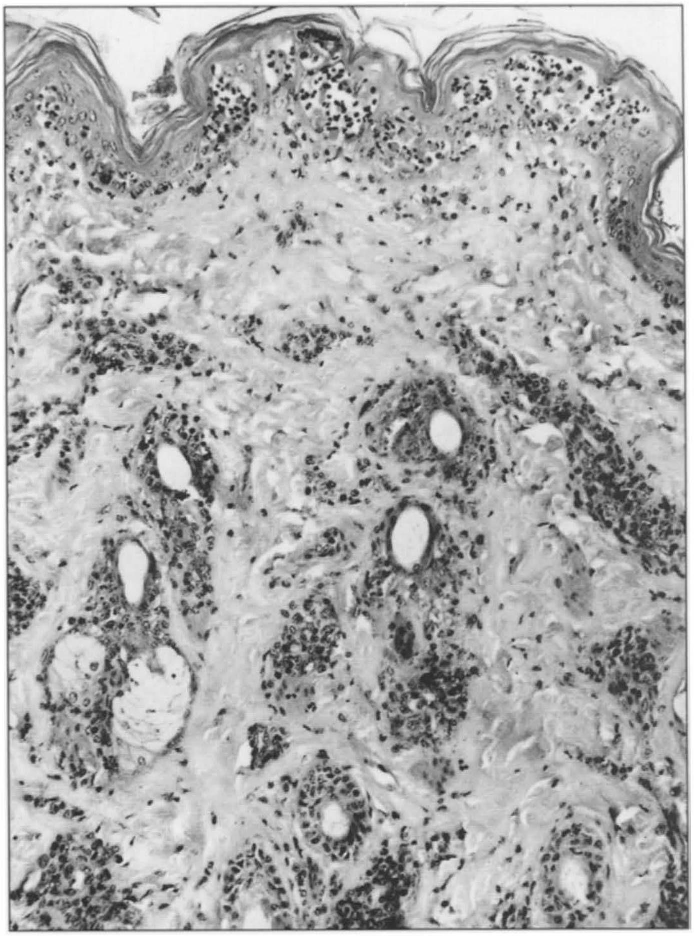

Figure 1.109 Necrosis of epithelium of skin in **malignant catarrhal fever**.

Bluetongue and related diseases

Bluetongue is caused by a reovirus of the genus *Orbivirus,* family Reoviridae. There are at least 24 recognized serotypes of *Bluetongue virus* (BTV), distinguished by serum neutralization tests. Immunity to one serotype does not confer resistance against another, and may cause sensitization, with a more severe syndrome following infection by a second type. Apparently, not all serotypes are pathogenic.

Epizootic hemorrhagic disease of deer is caused by a virus which represents another serogroup of *Orbivirus.* The virus causing *Ibaraki disease,* recognized in cattle in Japan, is a variant of *Epizootic hemorrhage disease virus* (EHDV); seropositive animals have also been found in Taiwan and Indonesia, and an identical virus has been isolated in Australia.

BTV, EHDV, and related viruses are spread by vector-competent *Culicoides* spp., also known as midges or gnats. The virus multiplies by a factor of 10^3–10^4 in the *Culicoides* within a week of the infected blood meal being ingested, and transmission can occur, following infection of the salivary glands, 10–15 days after the initial blood meal. Transovarial transmission of virus in *Culicoides* does not occur.

BTV circulates in a broad belt across the tropics and warm temperate areas, from about latitude 40°N to 35°S, with incursions or recrudescence during the *Culicoides* season, annually, or at irregular longer intervals in cooler temperate areas. The condition is enzootic or seasonally epizootic in most of Africa, the Middle East, the eastern Mediterranean basin, the Indian subcontinent, the Caribbean, and the USA. It appears sporadically in the Okanogan valley of western Canada. Recently it has made persistent incursions into the Iberian peninsula, Corsica and Sardinia, Italy, and the Balkans, perhaps associated with climate change. Seasonality of infection toward the periphery of its distribution probably reflects the unavailability of vectors, since virus may be able to overwinter in latently infected $\gamma\delta$T cells in the skin of sheep, which express virus once vector feeding occurs again.

Sheep, goats, and cattle are the susceptible domestic species, wherever bluetongue occurs. Sheep are the domestic species most highly susceptible to bluetongue, but there is considerable variation in expression of the disease, depending on the breed, age, and immune status of the sheep, the environmental circumstances under which they are held, and the strain of virus. Typically, indigenous breeds seem more resistant to clinical disease than do exotics. Goats, though susceptible to infection, rarely show signs; however, disease has occurred in goats in the Middle East and India. Infection in cattle usually produces only inapparent infection or mild clinical disease. In Africa, a wide variety of nondomestic ungulates and some small mammals may be inapparently infected; mortality has occurred in naturally or experimentally infected topi, Cape buffalo, and kudu. In North America, wildlife species, particularly white-tailed deer, black-tailed or mule

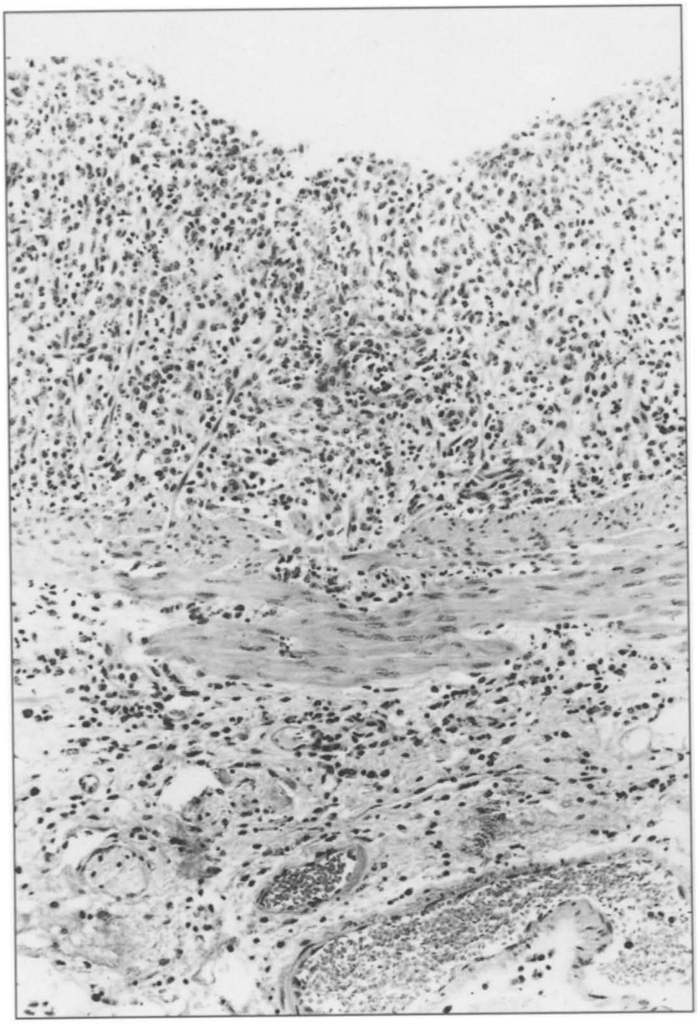

Figure 1.110 Colitis with collapse of glands and edema of submucosa in **malignant catarrhal fever**.

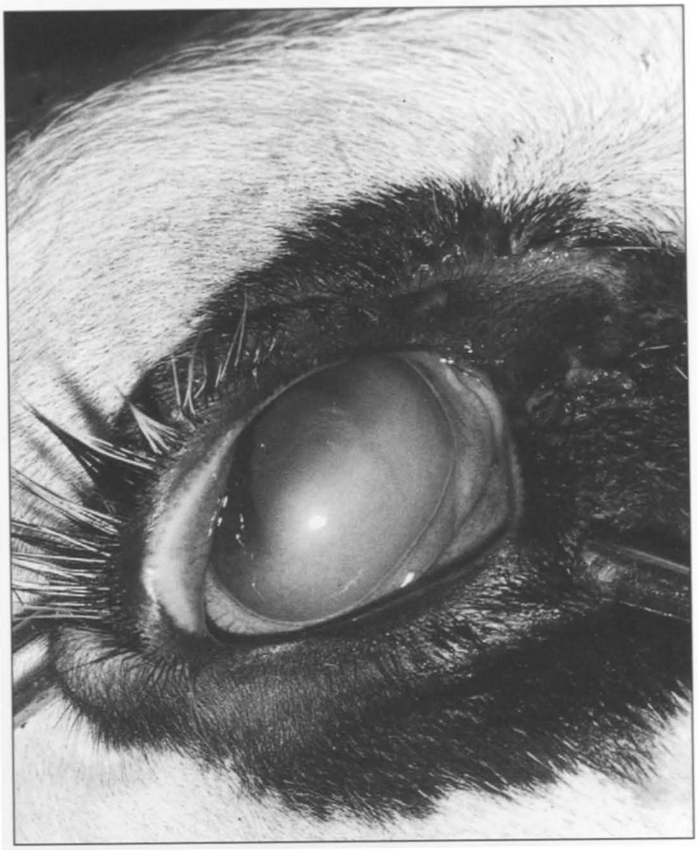

Figure 1.111 Corneal edema in **malignant catarrhal fever**.

deer, elk, bighorn sheep, bison, and pronghorn antelope are also infected. Bluetongue is responsible for significant mortality in all these species except elk, which usually develop mild or inapparent infection, and bison, which are infrequently demonstrated to be serologically positive. Vaccine inadvertently contaminated with BTV and administered to dogs caused significant mortality, but BTV is not normally considered a pathogen in dogs.

Epizootic hemorrhagic disease serogroup virus has been isolated in Nigeria, but the natural vertebrate hosts there are not known. In North America, the white-tailed deer is extremely susceptible, and widespread epizootics have occurred among this species in the USA. A single outbreak has been recognized in Alberta, Canada. The rate of survival is much higher among black-tailed deer and pronghorn antelope, and elk are only very mildly affected. Clinical disease similar to that produced by bluetongue may occur rarely in cattle. Though sheep are not considered to develop disease when infected with BTV, occasional mild clinical signs and lesions resembling bluetongue have been reported in sheep inoculated with some Australian isolates. In Japan, the *Ibaraki virus* strain of EHDV produces a clinical syndrome resembling bluetongue in cattle, but not in sheep.

BTV and EHDV circulate together in North America. Both viruses may be involved simultaneously in outbreaks of hemorrhagic disease in wild ruminants, and both have been isolated from *Culicoides* in a single locality at the same time. The role of cattle as reservoirs of BTV is uncertain. Cattle may act as reservoirs in that the virus will be associated with the erythrocytes for the lifespan of that cell. Detectable viremia in cattle is thought to be less than 9 weeks.

The **pathogenesis** of bluetongue, epizootic hemorrhagic disease, and Ibaraki disease is fundamentally similar in all species in which disease is seen. Primary viral replication following insect bite occurs in regional lymph nodes and spleen. Viremia about 4–6 days after inoculation results in secondary infection of endothelium in arterioles, capillaries, and venules throughout the body, but especially in lung microvascular endothelium. Microscopic lesions, fever, and lymphopenia begin a day or so later, about a week after inoculation. BTV in the blood appears to be closely associated with, or in, both leukocytes and erythrocytes, and it may co-circulate with antibody.

Endothelial damage caused by viral infection initiates local *microvascular thrombosis and permeability*. This is reflected microscopically by the presence of swollen endothelium, and fibrin and platelet thrombi in small vessels, with edema and hemorrhage in surrounding tissue. These lesions in turn mediate the full spectrum of gross findings. These are fundamentally *ischemic necrosis* of many tissues; *edema* due to vascular permeability; and *hemorrhage* resulting from vascular damage compounded, in severe cases, by consumption coagulopathy due to thrombocytopenia and depletion of soluble clotting factors. Differences in the expression and activity of vasoactive and pro- and anticoagulant mediators by infected pulmonary endothelium may explain the greater propensity of sheep to show signs, in comparison with cattle.

Bluetongue in sheep is highly variable; it may cause inapparent infection or an acute fulminant disease. Typically, leukopenia and pyrexia occur, even in mild infections, coincident with viremia. The degree and duration of fever do not correlate with the severity of the syndrome otherwise.

In the early phase there is hyperemia of the oral and nasal mucosa, drooling, and nasal discharge within a day or two of the onset of fever. Hyperemia and edema of the eyelids and conjunctiva may occur, and edema of lips, ears, and the intermandibular area becomes apparent. Hyperemia may extend over the muzzle and the skin of much of the body, including the axillary and inguinal areas. Focal hemorrhage may be present on the lips and gums, and *the tongue may become edematous and congested or cyanotic*, giving the disease its name. Infarcted epithelium thickens and becomes excoriated; erosions and ulcerations develop along the margins of the tongue opposite the molars, and the mucosa of much of the tongue may slough.

Excoriation and ulceration also occur on the buccal mucosa, the hard palate, and dental pad. Affected areas of skin may also become encrusted and excoriated with time, and a break in the wool can result in parts or much of the fleece being tender or cast. The coronet, bulbs, and interdigital areas of the foot may become hyperemic. Coronary swelling and streaky hemorrhages in the periople may be evident as a result of lesions in the underlying sensitive laminae. These hemorrhages may persist in the hoof as brown lines that move down the hoof as it grows. A defect parallel to the coronet may also be evident in the growing hoof in recovered cases.

Internally, in acute cases, there is *subcutaneous and intermuscular edema*, which may be serous or suffused with blood. Superficial lymph nodes are enlarged and juicy. Bruise-like gelatinous hemorrhages and contusions, which may be small and easily overlooked if not numerous, are often present in the subcutis and intermuscular fascial planes. Focal or multifocal pallid areas of streaky myodegeneration may be present throughout the carcass, perhaps partly obscured by petechial or ecchymotic hemorrhage. Resolving muscle lesions may be mineralized or fibrous. Stiffness, reluctance to move, and recumbency seen clinically are due to these muscle lesions.

Necrosis may be present deep in the papillary muscle of the left ventricle, and elsewhere in the myocardium. The lesion which is perhaps most consistent and closest to pathognomonic for bluetongue is *focal hemorrhage*, petechial or up to 1 cm wide × 2–3 cm long, in the tunica media at the *base of the pulmonary artery*. These hemorrhages are visible from both the internal and adventitial surfaces, and may be present in clinically mild cases with few other lesions. Petechial hemorrhage may also be present at the base of the aorta and in subendocardial and subepicardial locations over the heart.

There may also be edema and petechial or ecchymotic hemorrhage in the pharyngeal and laryngeal area. In severe cases the lungs may assume a purple hue, with marked edematous separation of lobules, and froth in the tracheobronchial tree, probably due to pulmonary microvascular damage and heart failure. Animals with pharyngeal or esophageal myodegeneration suffer from dysphagia, or regurgitate, and may succumb to aspiration pneumonia.

Hyperemia, occasionally marked hemorrhage, or in advanced cases, ulceration of the mucosa may occur on rumen papillae, the pillars of the rumen, and the reticular plicae. In convalescent animals, stellate healing ulcers or scars on the wall of the forestomachs may be apparent.

Microscopically, acute lesions are characterized by microvascular thrombosis, and edema and hemorrhage in affected sites recognized at autopsy. In squamous mucosa and skin, capillaries of the proprial and dermal papillae are involved, resulting in vacuolation and necrosis of overlying epithelium. There is a mild, local neutrophilic infiltrate acutely, and a similarly mild mononuclear reaction in the dermis or propria in uncomplicated chronic lesions, which may granulate if widely or deeply ulcerated. Similar microvascular lesions are associated with necrosis and fragmentation of infarcted muscle. Muscle during the reparative phase follows the usual course of regeneration of fibers or fibrous replacement, depending on whether or not the sarcolemma retains its integrity.

In **cattle**, *clinical bluetongue is rarely apparent*; in endemic areas it may never be evident. Mortality is low, and it is often attributed to secondary infection. Clinical disease may be a function of hypersensitivity in previously exposed animals, and disease in experimentally infected animals is poorly defined. Fever, loss of appetite, and leukopenia are usually seen after an incubation period of 6–8 days, and there may be a drop in milk production in dairy cattle. There is reddening of the epithelium of the mucous membranes, and of thin exposed skin, especially notable on the udder and teats. Edema of the lips and conjunctiva may be present. Drooling may become profuse, and as the disease progresses over the next several days, hyperemia and congestion of the mucosae become more intense. Ulcerations of the gingival, lingual, or buccal mucosa occur, most consistently on the dental pad. There may be necrosis of epithelium on the muzzle. Muscle stiffness is a feature of the disease in some animals. Laminitis, characterized by hyperemia and edema of the sensitive laminae at the coronet, may be apparent, and in some cases, hooves on affected feet may eventually slough. Sloughing or cracking of crusts of necrotic epithelium may also occur on affected parts of the skin, but the ulcerative or erosive defects heal readily. Viral antigen and thrombosis are present in small vessels in affected tissues during the acute phase.

The signs and lesions of **Ibaraki disease** are similar to those of bluetongue in cattle, though more severe in some cases. As well as the signs and lesions described in cattle with bluetongue, there may be difficulty in swallowing in 20–30% of clinically affected animals, and the swollen tongue may protrude from the mouth. At necropsy, in addition to the lesions observable externally, there may be congestion, erosion, or ulceration of the mucosa of the abomasum, and less commonly, the esophagus and forestomachs. Ischemic necrosis and hemorrhage of the striated muscle in the tongue, pharynx, larynx, and esophagus cause the difficulty in swallowing seen clinically, and similar changes are seen in other skeletal muscles. Necrotizing aspiration pneumonia is a sequel to dysphagia in some animals.

The *hemorrhagic diseases* in bighorn sheep, pronghorn antelope, and white-tailed and black-tailed or mule deer in North America resemble bluetongue in sheep. White-tailed deer may develop a particularly severe and fulminant hemorrhagic disease, with high mortality. There may be necrosis of velvet antler, and hooves may slough in survivors. Bluetongue in goats, though usually inapparent, can resemble bluetongue in sheep.

Bluetongue in sheep must be differentiated from foot-and-mouth disease, peste des petits ruminants, contagious ecthyma, and photosensitization in particular. In cattle, the condition must be differentiated from foot-and-mouth disease, vesicular stomatitis, bovine viral diarrhea, rinderpest, malignant catarrhal fever, and photosensitivity. In Japan, Ibaraki disease of cattle must in addition be differentiated clinically from ephemeral fever virus.

In addition to the systemic disease described, *abortion*, perhaps unobserved, and birth of progeny with various *congenital defects* may follow BTV infection of pregnant sheep and cattle. In sheep, BTV infection of ewes early in gestation may result in hydranencephaly. Anomalous calves produced by BTV-infected cattle have excessive gingiva, an enlarged tongue, anomalous maxillae, dwarf-like build, and rotations and contractures of the distal extremities. Porencephaly, hydranencephaly, and arthrogryposis are also reported in calves infected in utero with BTV. Antibody may be sought in neonates that have not sucked, and attempts should be made to isolate virus, since some prenatally infected animals may have immune tolerance, and persistent infection. Anomalies of the brain are considered further in Vol. 1, Nervous system.

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Parapoxviral infections

Bovine papular stomatitis

Papular stomatitis of cattle occurs worldwide. *It is generally an insignificant infection*, but needs to be differentiated from other more serious diseases affecting the oral cavity and skin. It is caused by *Bovine papular stomatitis virus* (BPSV), a member of the family Poxviridae, genus *Parapoxvirus*, that is closely related to *Pseudocowpox virus* that causes pseudocowpox in cattle and milker's nodules in humans. It is morphologically similar to, and shares antigens with, *Orf virus* of sheep and goats (see Vol. 1, Skin and appendages). However, analysis of the genome indicates that these viruses are distinct.

BPSV is relatively host-specific. As with many of the poxviruses, neutralizing antibody is not readily demonstrated. Infection does not confer significant immunity, and successive crops of lesions and relapses can occur. The disease is more common in calves than in older animals, although the susceptibility of, or recrudescence in, the latter may be increased by intercurrent debility, disease such as bovine viral diarrhea, infectious bovine rhinotracheitis, or other stressors.

The *papular lesions* of this disease occur on the muzzle and in the rostral nares, on the gums, the buccal papillae, the dental pad, the inner aspect of the lips, the hard palate (Fig. 1.112A), the floor of the oral cavity behind the incisors, the ventral and lateral (not dorsal) surfaces of the tongue, and, occasionally, in the esophagus (Fig. 1.112B) and forestomach.

The initial lesions, which are likely to be detected on the muzzle or lips, are erythematous macules, ~2 mm–2 cm in diameter. Shortly, the central portion becomes elevated as a low papule, although the elevation is not easy to see, and by the second day a gray central zone of epithelial hyperplasia has developed on which there is superficial scaliness and necrosis. A central necrotic area may slough to form a shallow craterous defect surrounded by a slightly raised red margin. Lesions may coalesce. The course of individual lesions is about a week.

Histologically, there is focal but intense hyperemia and edema in the papillae of the lamina propria, with accumulation of a few mononuclear leukocytes. The epithelium is thickened, sometimes to twice its normal depth, by hyperplasia and ballooning degeneration in the deeper layers (Fig. 1.112C). The cytoplasm of affected cells is clear, and the nucleus may be shrunken. *Dense eosinophilic inclusion bodies lie in the vacuolated cytoplasm*, especially in cells at the active margin of the lesion. In the central, more advanced part of the lesion, a mainly neutrophilic infiltrate into the superficial propria and epithelium is associated with erosion of the upper layers of necrotic cells. The basal layer survives and may be very flattened in eroded areas. Vesicles do not form.

A *chronic form* has been reported, with necrotic and proliferative stomatitis, represented histologically by extensive parakeratotic hyperkeratosis, pseudoepitheliomatous hyperplasia, and occasional intracytoplasmic inclusion bodies.

Papular stomatitis is probably more common and widespread than reports indicate. Variation in the extent and gross appearance of the lesions is to be expected, depending on the usual host–parasite factors and the nature of superimposed infections. They may predispose to the development of necrotic stomatitis, and must be differentiated from the lesions of bovine viral diarrhea, alimentary infectious bovine rhinotracheitis, and other causes of ulcers and erosions in the upper alimentary tract. The infection can be transmitted to humans to produce small papules that may persist for several weeks on the skin, usually of the fingers or forearms.

Rapid diagnosis is readily accomplished by demonstration of characteristic parapoxvirus particles in negatively stained material from lesions examined under the electron microscope.

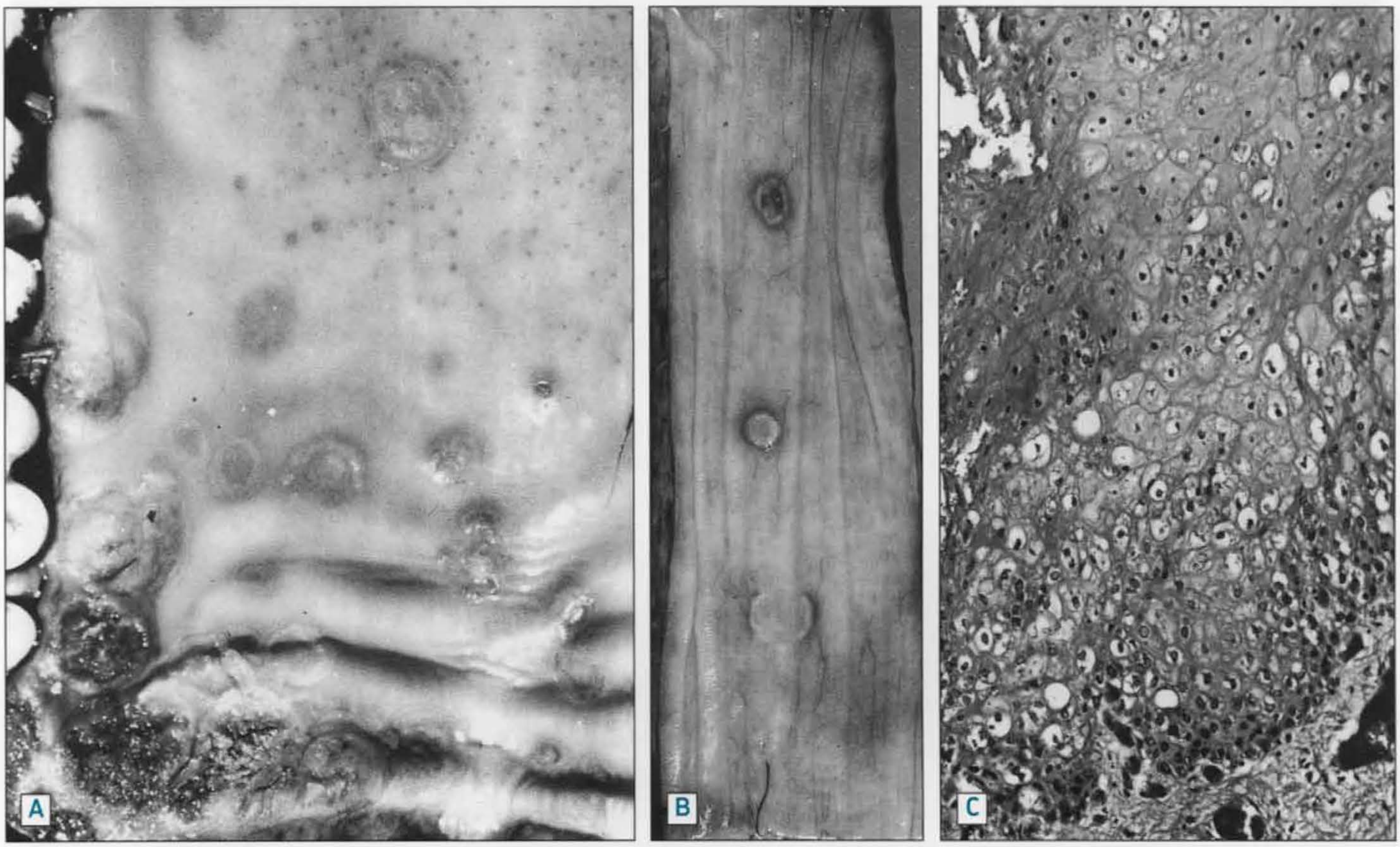

Figure 1.112 Bovine papular stomatitis. A. Lesions at various stages of evolution in palate. **B.** Lesions in esophagus. **C.** Thickened epithelium at the margin of a lesion, with ballooning degeneration of cells in the deeper layers.

Contagious pustular dermatitis

Contagious pustular dermatitis, also called **orf** or **contagious ecthyma**, is a parapoxviral disease of sheep and goats that is characterized mainly by *proliferative scabby lesions on the lips, face, and feet* (see Vol. 1, Skin and appendages). The disease has also been reported in camels and a gazelle. Lesions may extend into the oral cavity, involving the tongue, gingiva, dental pad, and palate. Involvement of the esophagus and forestomachs occurs, but is very unusual. In general the evolution of the lesions is similar to papular stomatitis of cattle, though they are more exudative and usually much more proliferative. Morbidity may be high, and death can occur in suckling animals. In the upper alimentary tract, lesions may consist of focal red, raised areas, which coalesce to form papules followed by pustules. The latter rupture, and on the muzzle and in the mouth they may become covered by a gray to brown scab, although scab formation may not occur in the mucosa of the upper alimentary tract. Diagnosis is by demonstration or isolation of *Orf virus*.

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Infectious bovine rhinotracheitis

Bovine herpesvirus 1 (Infectious bovine rhinotracheitis virus, BoHV-1), which is in genus *Varicellovirus*, subfamily Alphaherpesvirinae, has been associated with a wide range of clinicopathologic syndromes in cattle. These include necrotizing rhinotracheitis, conjunctivitis, infectious pustular vulvovaginitis and balanoposthitis, vesicular lesions of the udder, abortions, and latent infection (see appropriate chapters). Clinical significance of BoHV-1 in other species such as bison that have serological evidence of exposure is not known.

A *systemic form* of the disease, which usually involves the alimentary tract, may occur spontaneously in neonatal calves (in which it may be congenital, or acquired shortly after birth) and in feedlot cattle. It has been reproduced experimentally in young calves.

The pathogenesis of systemic infection with BoHV-1 is poorly understood. Colostrum-deprived calves are especially susceptible,

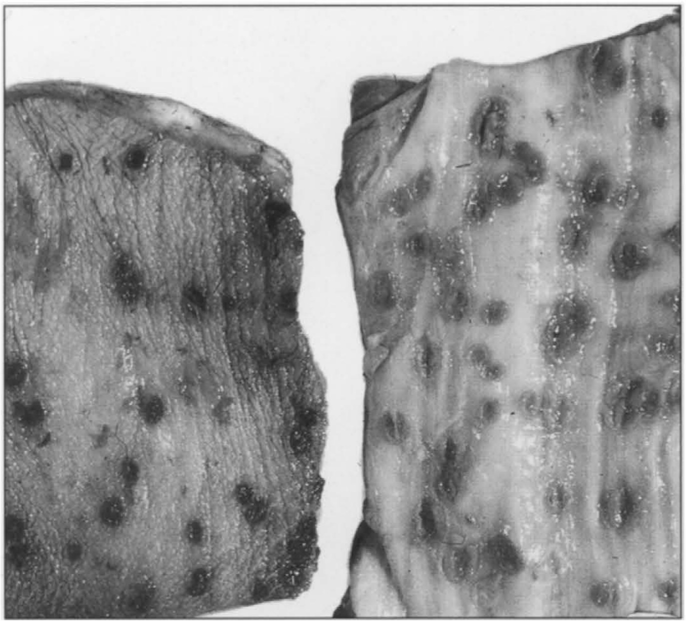

Figure 1.113 Foci of necrosis on mucosa of esophagus and rumen in a neonatal calf in **infectious bovine rhinotracheitis**.

and the disease can be prevented by feeding colostrum from actively immunized dams. The virus probably spreads from the mucosa of the upper respiratory tract to other tissues by circulating leukocytes. Peripheral blood mononuclear leukocytes may exhibit apoptosis in response to BoHV-1, but the significance of this is unknown. Experimental infection of calves with noncytopathic *Bovine viral diarrhea virus* (NCP-BVDV) followed by BoHV-1 inoculation results in dissemination of the latter to a variety of tissues. BVDV impairs cell-mediated immunity, and this may allow BoHV-1 to escape from the respiratory tract and lead to a systemic infection. Dual infections of BVDV and BoHV-1 occur under field conditions, but coinfection of these two viruses is not a prerequisite for the disease to develop.

Clinically affected animals have hyperemic oral and nasal mucosae, and focal areas of necrosis, erosion, and ulceration on the nares, dental pad, gums, buccal mucosa, palate, and the caudal, ventral, and dorsal surfaces of the tongue. Characteristically, the lesions tend to be punctate with a slightly raised margin; the necrotic areas are covered by a gray-white layer of fibrinonecrotic exudate, which leaves a raw red base when removed.

The *lesions* may extend into the esophagus, usually only the upper third, and the forestomachs. In the esophagus, the erosions and ulcers may be irregular, circular, or linear, and often they have a punched-out appearance and a hyperemic border (Fig. 1.113). The ruminal lesions, which are most commonly located in the dorsal and cranioventral sacs, vary considerably. The earliest lesions consist of foci of necrosis and hemorrhage, a few millimeters in diameter. In some cases, the necrosis may involve almost the entire surface of the ruminal mucosa, which becomes covered by a thick, dirty gray layer of exudate, resembling curdled milk, which adheres tightly to the wall (Fig. 1.114A). Similar lesions may be evident in the reticulum. Focal areas of necrosis result in the formation of holes, as large as 1.5 cm in diameter, in the leaves of the omasum. In addition, these calves may have focal areas of necrosis in the abomasal mucosal folds, which may coalesce to form areas of necrosis 2–3 cm in diameter. The intestines are red and dilated, and the serosal surface may be covered by a thin layer of fibrinous exudate.

The enteric lesions may be accompanied by changes in the upper respiratory tract. When present, the respiratory lesions are similar to those described for older cattle, although they are milder and generally limited to the nasal mucosa, larynx, and upper third of the trachea (see The Respiratory System, Volume 2, Chapter 5).

Gray to yellow necrotic foci 2–5 mm diameter may be evident macroscopically on the capsular and cut surfaces of the liver, the adrenal cortices, the spleen, and in Peyer's patches.

Microscopically, the lesions in the squamous mucosa are characterized by *focal areas of necrosis* (Fig. 1.114B), *erosion, and ulceration*. Severe necrosis may involve the entire papilla or mucosa more diffusely. *Nuclear inclusions* may be present in epithelial cells in the periphery of the lesion, although these are an inconsistent finding. They are more likely to be found if tissues are collected in the early stages of the disease and fixed in Bouin's fluid. The abomasal lesions consist of necrosis of glandular epithelial cells. Affected glands are dilated, and filled with necrotic debris. Focal necrotic lesions involving crypts and lamina propria may be present in both the small intestine and large bowel (Fig. 1.115). Abomasal and intestinal lesions may predispose to the development of *secondary mycosis*, which is a common complication.

Foci of coagulative necrosis may occur in the liver, lymph nodes, thymus, Peyer's patches, spleen, and adrenal cortices. Typically, there is little inflammation associated with the necrosis. Herpesviral inclusions are inconsistently seen in cells at the periphery of the necrotic foci.

The lesions in the upper alimentary tract of cattle associated with BoHV-1 infection must be differentiated from those of calf diphtheria, bovine papular stomatitis, and bovine viral diarrhea. The ruminal lesions must be differentiated from those of *Bovine adenovirus* infection and nonspecific rumenitis, described elsewhere in this chapter. The liver lesions may be confused with focal necrosis associated with septicemias, for example, listeriosis or salmonellosis (see Vol. 2, Liver and biliary system).

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Caprine herpesvirus

A herpesvirus that shares some antigens with, but on the basis of restriction endonuclease analysis is distinct from, *Bovine herpesvirus 1*, has been isolated from neonatal goat kids in various parts of the world. This virus has been designated *Caprine herpesvirus 1* (CpHV-1),

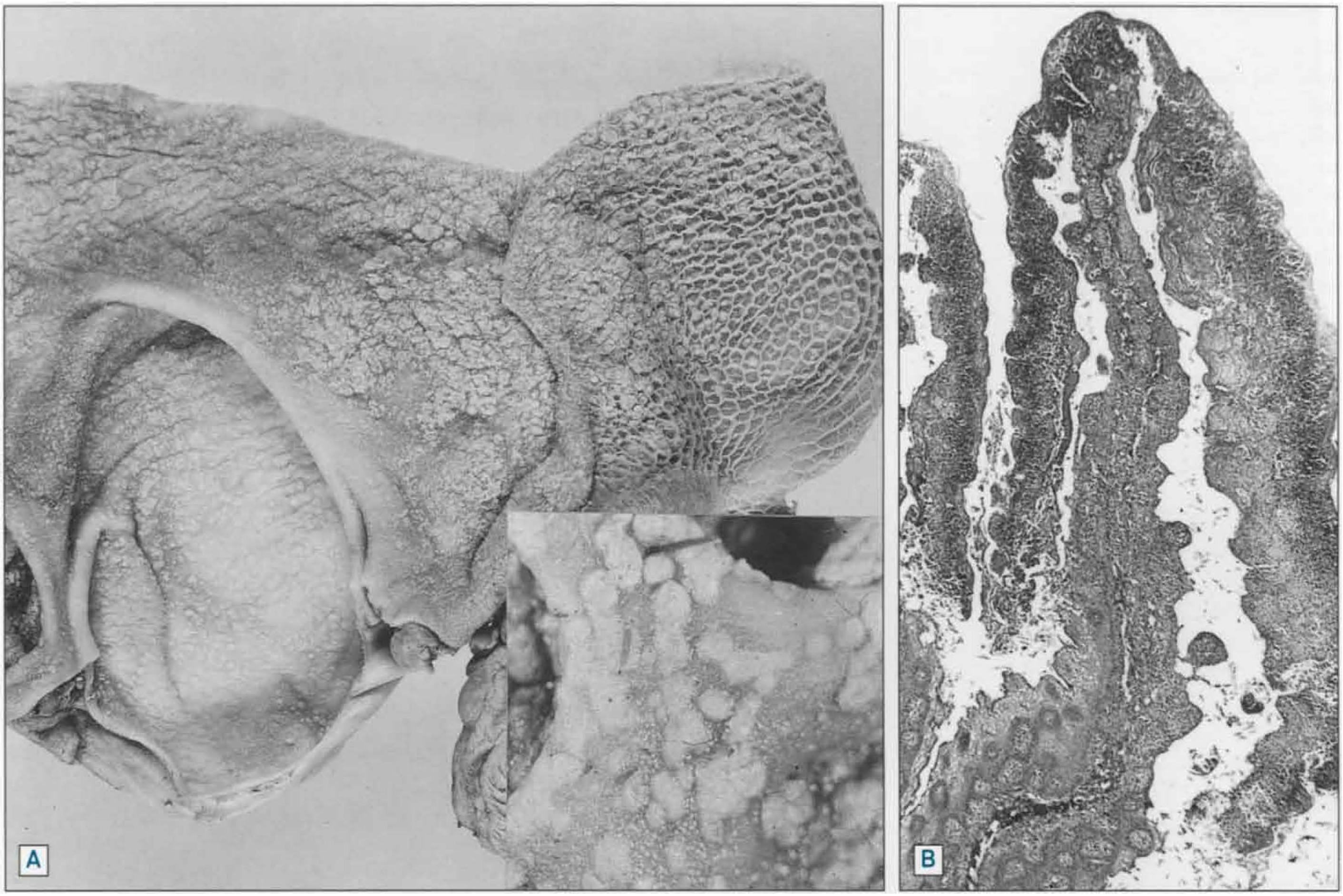

Figure 1.114 Infectious bovine rhinotracheitis in a neonatal calf. **A.** Cheesy necrotic debris in rumen and reticulum. Inset: Detail of rumen lesion. **B.** Necrosis of omasal fold.

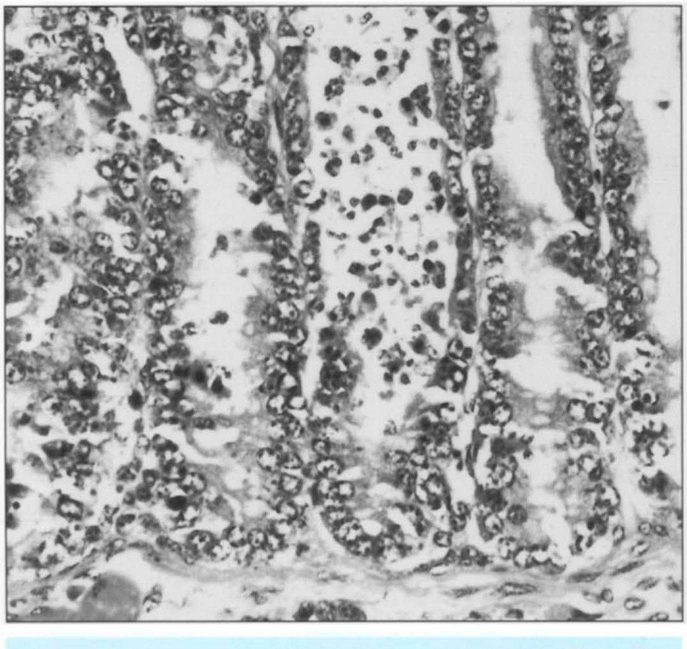

Figure 1.115 Necrosis of epithelium in the crypts of Lieberkühn in the small intestine of a neonatal calf with **infectious bovine rhinotracheitis**.

genus *Varicellovirus*, subfamily Alphaherpesvirinae. Although capable of infecting sheep, cattle, and goats, severe infection is restricted to goats. *CpHV-1 causes severe systemic disease with erosions and ulcerations of the alimentary tract in neonatal goats.* Adult goats may be latent carriers, developing vulvovaginitis or balanoposthitis (see Vol. 3, Female genital system). Infection of pregnant does causes abortion.

The disease in neonatal kids is characterized clinically by fever, conjunctivitis, ocular and nasal discharges, dyspnea, anorexia, abdominal pain, weakness, and death, usually within 1–4 days after onset of clinical signs. Affected kids have leukopenia and hypoproteinemia.

Macroscopic lesions are most obvious throughout the entire alimentary tract. Round or longitudinal erosions, which have a hyperemic border, are evident in the oral mucosa. These are particularly prominent on the gums around the incisor teeth and to a lesser extent in the pharynx and esophagus. Focal red areas of necrosis, which may be slightly elevated above the surrounding mucosa, occur in the rumen. In the abomasum, numerous longitudinal, red erosions are located in the mucosa. The most severe lesions occur in the *cecum and ascending colon*, which are dilated, with a thickened wall, and contain focal to large areas of mucosal necrosis and ulceration, frequently covered by a pseudodiphtheritic membrane. The contents are yellow and mucoid. Hemorrhagic foci may be visible in the bladder mucosa.

Microscopically, the lesions in the upper alimentary tract are typical areas of necrosis and erosion of squamous epithelial cells. The epithelial cells at the periphery of necrotic areas are swollen and vacuolated and these may contain *herpesviral inclusions*. There is marked inflammatory reaction in the underlying lamina propria. The abomasal lesions consist of acute foci of mucosal necrosis. Inclusions are particularly evident in this area. Lesions in the cecum and colon are more extensive and consist of large areas of mucosal ulceration and necrosis, which may involve the entire thickness of the wall. The submucosa is edematous and markedly infiltrated by inflammatory cells. The mesenteric nodes are edematous and germinal centers are depleted of lymphoid cells. Focal areas of necrosis with a mild inflammatory cell reaction may also be present in liver, urinary bladder, and kidney.

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Other herpesviruses

Canid herpesvirus 1 causes systemic disease of neonatal puppies characterized by foci of necrosis and hemorrhage in a wide variety of organs, especially the lungs and renal cortices (see section on Viral infections of the pregnant uterus, Vol. 3, Female genital system). Focal areas of necrosis may occur in the intestine as part of the systemic syndrome.

Felid herpesvirus 1 (Feline viral rhinotracheitis virus) causes oral lesions (see section on Inflammation of the oral cavity, above). Viruses antigenically related to *Felid herpesvirus 1* have been isolated from dogs with diarrhea, but descriptions of lesions are not available.

Natural infections with ***Suid herpesvirus 1*** (SuHV-1; Pseudorabies virus, Aujeszky's disease virus) often result in necrotizing tonsillitis. Experimental infection of pigs with SuHV-1 may cause necrotizing enteritis of the distal small intestine. The enteric lesions are characterized by focal areas of necrosis of the cryptal mucosa, muscularis mucosae, and tunica muscularis. Immunohistochemically, antigen is documented in the dome area, the lymphoid follicles of Peyer's patches, and ganglion cells of Meissner's and Auerbach's plexuses.

Necrotizing enterocolitis in adult horses due to ***Equid herpesvirus 1*** has been reported rarely. At necropsy, there are multiple areas of hemorrhage, necrosis, and ulceration, some several centimeters in diameter, of the mucosa in both small and large intestine. Microscopically, these lesions consist of erosions and ulcerations of the mucosa, and necrosis of cryptal epithelial cells in adjacent areas. Cryptal epithelial cells and some proprial mononuclear cells may have acido- and amphophilic nuclear inclusions.

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Adenoviral enteritis

The adenoviruses that have been associated with enteric infections in humans, cattle, swine, horses, sheep, and dogs belong to the Adenoviridae, genera *Mastadenovirus* and *Atadenovirus*. Serological surveys show that widely divergent serotypes occur both within and between host species and their distribution is worldwide. All serotypes are morphologically similar; the virus consists of a nonenveloped icosahedral capsid, 70–80 nm in diameter, which has 252 capsomeres. Virus neutralization tests are used to distinguish serotypes. Adenoviral infection of cells causes *intranuclear inclusions*. Adenoviruses are relatively heat-resistant and can survive for several days at room temperature. Most adenoviruses are transmitted by feces, aerosols, or possibly fomites, to susceptible, usually suckling or recently weaned, animals. Infected animals may remain carriers for weeks.

Adenoviruses are highly host-specific. Infections in both humans and animals appear, in general, to be subclinical, and disease seems to occur more commonly in immunologically compromised individuals. Most infections are systemic; certain strains have a tropism for the respiratory tract, and others for the alimentary tract, vascular endothelial cells, or hepatocytes. Their enteric manifestations will be considered here.

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Bovine adenoviruses

There are 10 serotypes of *Bovine adenovirus* (BAdV) in the genera *Mastadenovirus* and *Atadenovirus*. Infection has been mainly associated with keratoconjunctivitis and respiratory disease. Many strains have been isolated from normal cattle. Serotypes 3, 4, 7, and 10 have been associated with enteric disease. It appears that after an initial viremic stage, the virus localizes in the *endothelial cells* of vessels in a variety of organs, resulting in thrombosis with subsequent focal areas of ischemic necrosis.

Clinically, enteric infections with BAdV occur sporadically in 1–8-week-old calves and in feedlot animals. Affected animals have fever, and diarrhea that may contain blood, and some animals will die peracutely from dysentery. They are dehydrated and the mucous membranes of the muzzle and mouth are congested. Dry, encrusted exudate may cover the muzzle and there may be serous to mucopurulent ocular and nasal discharges.

Macroscopic lesions may be present in the forestomachs, abomasum, and intestine. Those in the forestomachs are characterized by irregular, raised, red-to-gray necrotic areas, 2–4 mm in diameter on the mucosa of both the dorsal and ventral sacs of the rumen. In some cases, the areas of necrosis coalesce to give rise to diffuse necrotizing rumenitis. Ulcers up to 1.5 cm in diameter may be located on the ruminal pillars and these may be visible through the serosa. Similar lesions may be evident in the omasum. The abomasal mucosal folds are edematous and congested, with focal necrosis and ulceration in the mucosa that, like those in the forestomachs, may be visible on the serosal surface.

The intestinal lesions vary from slight dilation and distension with excessive fluid to severe multifocal or diffuse necrosis, which may be covered by a pseudodiphtheritic membrane. In young calves, the lesions are most severe in the jejunum and ileum, especially over the Peyer's patches. In feedlot cattle, the lesions may be most prominent in the colon. The mucosa is dark red (Fig. 1.116) and there is marked edema of the mesocolon. The mesenteric lymph nodes are enlarged and edematous.

Microscopically, *large basophilic to amphophilic inclusions* completely or partially fill the nuclei of endothelium in the vessels of the lamina propria and submucosa of affected areas of the rumen, abomasum, and intestine. The endothelial cells are swollen and necrotic, and some veins and lymphatics contain thrombi. Foci of ischemic necrosis are evident in the overlying mucosa, and in more advanced lesions, the necrosis extends across the muscularis mucosae (Fig. 1.117A). Fibrinocellular exudate often covers the mucosal surface. Intestinal crypts are dilated, lined by flat epithelial cells, and usually contain necrotic debris (Fig. 1.117B). There is usually marked submucosal edema, congestion, and fibrinous exudation. Foci of necrosis are evident in the lymphoid follicles of the Peyer's patches, which are also depleted of lymphocytes.

Typical inclusions may also be found in endothelial cells of vessels and sinusoids of the adrenal glands, mesenteric lymph nodes, liver, spleen, glomeruli, and interstitial capillaries in the kidney, and in the mucosa of the urinary bladder.

Confirmation of enteric BAdV infection depends on the demonstration of the virus in tissue, through electron microscopy, in situ hybridization, or isolation of the virus in tissue cell cultures. The latter is often difficult because different serotypes and strains of

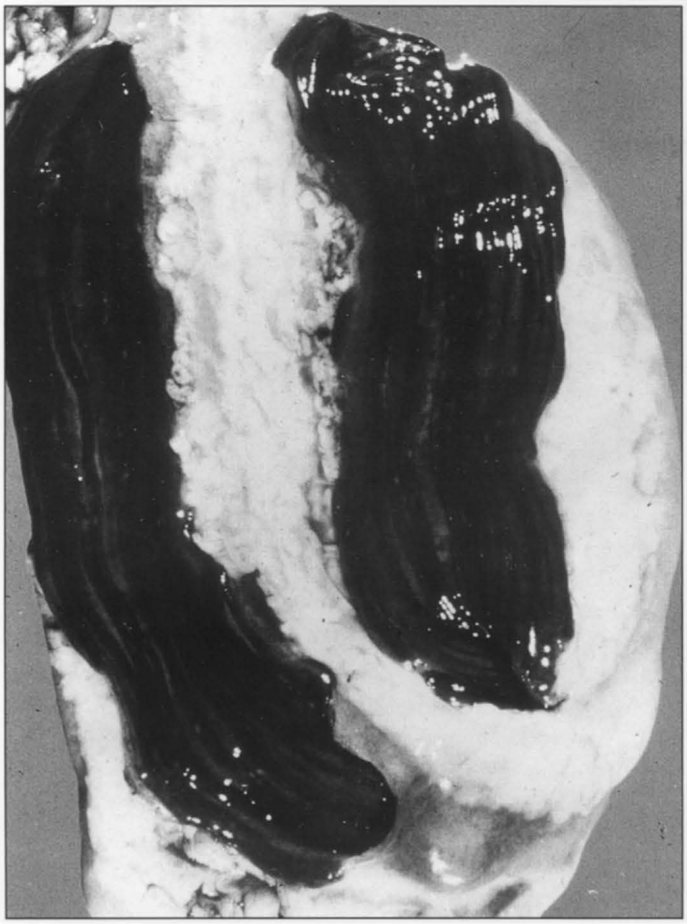

Figure 1.116 Congested and hemorrhagic colon caused by ***Bovine adenovirus*** infection.

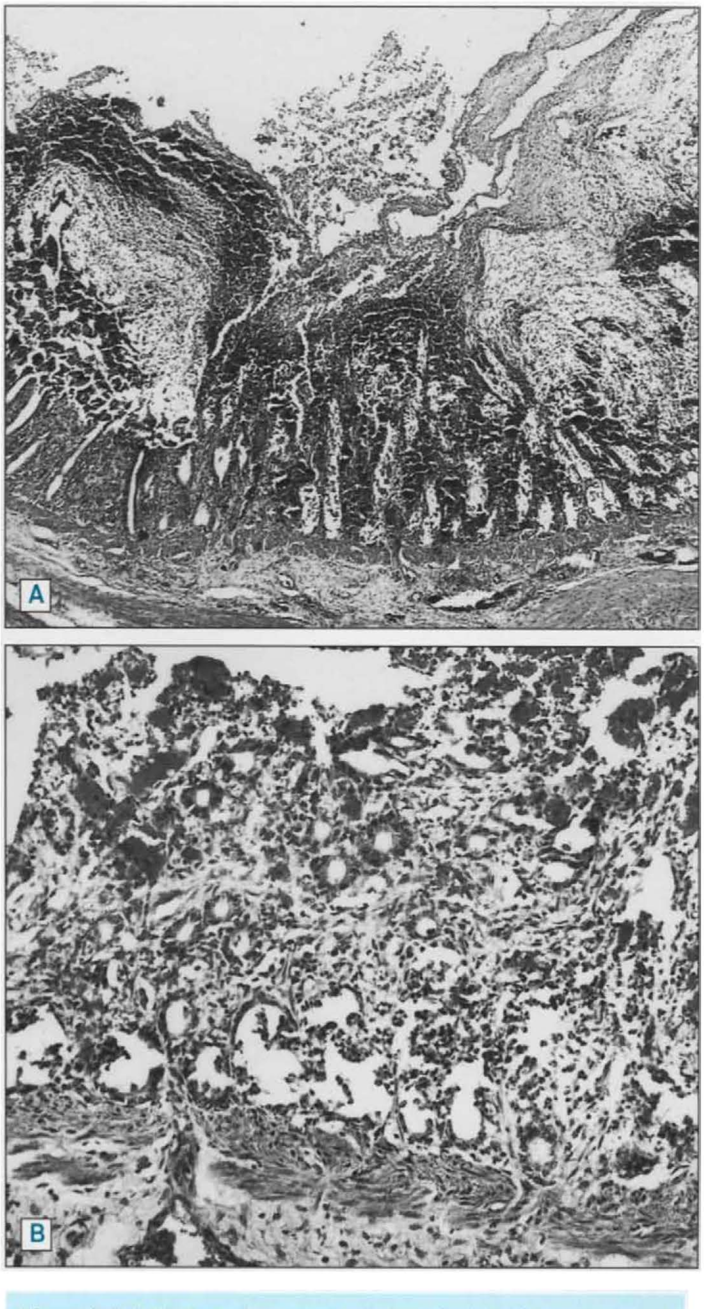

Figure 1.117 ***Bovine adenovirus*** infection. **A.** Infarctive necrosis and hemorrhage of the colon. **B.** Infarctive necrosis of crypt epithelium and hemorrhage in the colon.

the virus require specific tissue cell cultures and several blind passages may be required before cytopathic changes are evident.

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Porcine adenovirus

There are six serotypes of *Porcine adenovirus* (PAdV) which, according to serological surveys, are all common. Serotype 4 appears to be the most widely distributed strain of the virus in Europe and North America. Asymptomatic infections are most common in swine and PAdV may be isolated from feces of normal pigs; PAdVs are actually of more interest as viral vaccine vectors than as pathogens.

The importance of adenoviruses as a cause of enteric disease in the field remains controversial. When disease occurs, the macroscopic lesions in the intestine consist of excessive yellow watery to pasty contents and moderate enlargement of the mesenteric lymph nodes, which cannot be differentiated from other causes of diarrhea in neonatal pigs.

In contrast to the situation in calves, in which inclusions are located in the nuclei of endothelial cells, *inclusions in pigs are in enterocytes* in the distal jejunum and ileum, where primary viral replication likely occurs. The infected nuclei are enlarged, round, and displaced to the apical portion of the cell. The villi may be short and blunt. There may be a moderate mononuclear cell reaction in the lamina propria. Inclusions are also found in the squamous epithelial cells of the tonsils.

Ultrastructurally, infected nuclei of enterocytes are round and swollen and contain numerous typical adenoviral particles (Fig. 1.118). Affected enterocytes are cuboidal and the apical portion protrudes slightly into the lumen. The cell membrane and microvilli are irregular and the terminal web is absent. The rough endoplasmic reticulum shows local distension with formation of large multivesicular bodies. Eventually, there is complete loss of microvilli, and the cell membrane ruptures with the release of cell contents and virus particles into the gut lumen.

The significance of adenoviral inclusions in enterocytes must be interpreted with caution. A survey in Canada revealed that 4.4% of 5-day to 24-week-old pigs had adenoviral inclusions in enterocytes, mainly in the ileum. More than 50% of the pigs had diarrhea; however, other enteropathogens were found in most of these animals.

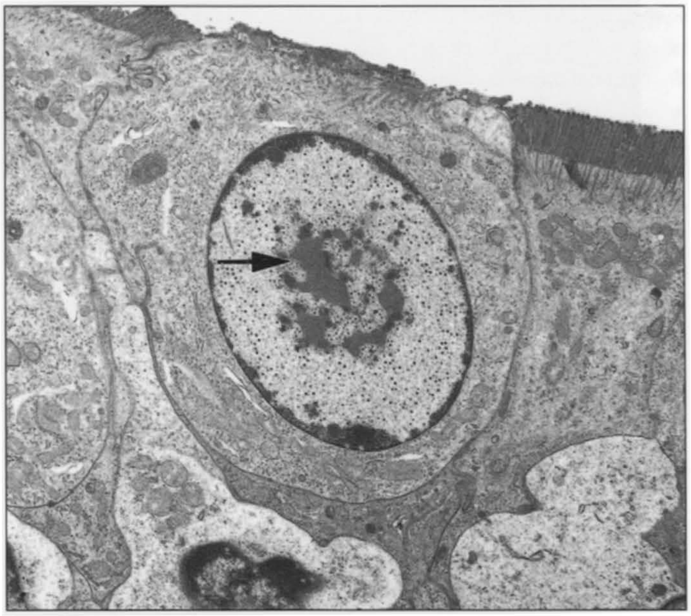

Figure 1.118 ***Porcine adenovirus***-infected cell in epithelium of dome over Peyer's patch; note inclusion (arrow). (Courtesy of DM Hoover, SE Sanford.)

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Equine adenovirus

Two serotypes of *Equine adenovirus* (EAdV) have been identified in horses. EAdV-1 has a worldwide distribution. Subclinical infections are common. Clinical disease occurs mainly as an upper respiratory infection in foals less than 3 months of age. The infection is particularly important in Arabian foals with combined immunodeficiency (CID), in which intestinal involvement is common. EAdV is capable of replication in the intestinal epithelium and produces duodenal villus atrophy after experimental infection.

Another adenovirus, proposed to be a prototype of EAdV-2, has been isolated in Australia from foals with diarrhea. *Rotavirus* was also identified in the feces of these foals. A serological survey showed that 77% of adult horses in the area had neutralizing antibodies to this particular serotype.

An unidentified alimentary tract adenoviral infection has been reported in an Arabian foal that did not have lesions of CID. The foal had diarrhea and progressive weight loss over a 2-month period. The macroscopic lesions consisted of ulcers in the distal esophagus and nonglandular mucosa of the stomach. The intestine contained soft to semifluid ingesta. Histologically, there was necrosis and ulceration of the esophageal and gastric squamous mucosa. Typical adenoviral inclusions were found at all levels of the small intestine. These were most commonly located in the villus epithelial cells, less often in the crypts, and only occasionally in the submucosal glands. There was focal-to-diffuse villus atrophy through the small intestine.

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Adenoviruses in other species

More than seven serotypes of *Ovine adenovirus* (OAdV) have been isolated from **sheep**. Serotypes 1, 2, and 3 have been recovered from feces of normal sheep, and lambs with enteritis and pneumoenteritis. Experimental inoculation of specific-pathogen-free lambs with OAdV-4 did not cause disease but the virus was re-isolated from feces and nasal secretions for several days postinfection. However, occasionally, there are reports of enteritis associated with abundant adenoviral inclusions in lambs and kids. In the former, inclusions were found predominantly in the lamina propria whereas in the latter they were mostly epithelial.

Goat adenovirus (GAdV) serotypes 1 and 2 can cause enteritis and diarrhea in **goat** kids; GAdV-1 is a serotype of *Ovine adenovirus D,* which also includes serotype 7 and isolate 287.

Two distinct but serologically related adenoviruses have been isolated from **dogs**. *Canine adenovirus 1* (CAdV-1) infection is usually subclinical, but it may cause infectious hepatitis, and diarrhea may be present in these cases. The virus has a particular tropism for hepatocytes and endothelial cells. The serosal hemorrhages in the gastrointestinal tract and possibly the diarrhea may be related to vascular damage in the serosa and mucosa respectively (see Vol. 2, Liver and biliary system). CAdV-2 is usually associated with upper respiratory infections in dogs (see Vol. 2, Respiratory system). Viruses serologically similar to CAdV-2 have been isolated from feces of diarrheic dogs. DNA fingerprinting of two of these isolates indicated that they are distinct from CAdV-2. It may be that the fecal isolates are due to swallowing of virus originating from upper respiratory tract infections.

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Enteric coronaviral infections

Coronaviruses cause disease affecting a number of organ systems in a variety of species. Among domestic mammals they mainly cause enteric infections, although coronaviruses are implicated in pneumonia in swine and cattle, and in feline infectious peritonitis.

Genus *Coronavirus* is found with the genus *Torovirus* in the family Coronaviridae. Coronaviruses have a single-stranded RNA genome. They are pleomorphic or roughly spherical and vary in size from about 70 to 200 nm in diameter, averaging ~100–130 nm. They have a phospholipid-bearing envelope, probably derived in part from host cell membrane. They gain their name from the characteristic "corona" of petal- or droplet-shaped radial surface projections ("peplomers") visible under the electron microscope in negatively stained preparations.

The coronaviruses infecting each species of host appear to be distinctive; some species are infected by more than one type of coronavirus. There are antigenic relationships among viruses from various hosts, and experimental cross-infection will occur between some host species, usually without pathologic consequences. The species *Transmissible gastroenteritis virus*, *Feline coronavirus* (with two biological types: Feline infectious peritonitis virus, and Feline enteric coronavirus), and *Canine coronavirus* are in antigenic group 1. *Bovine coronavirus* is in Group 2, antigenically related to *Porcine hemagglutinating encephalomyelitis virus*, *Mouse hepatitis virus*, and *Rat coronavirus*, among others. Persistent infections can occur.

Viral replication in the intestinal epithelium by coronaviruses is similar in all the species studied. Coronavirus infects and replicates in the apical cytoplasm of absorptive enterocytes on the tips and sides of intestinal villi. Virions are probably taken up by the apical border of the cell, by fusion with the plasmalemma. Replication and maturation appear to involve budding of virions from the cytosol through the membrane and into the lumen of vacuoles or cisternae in the smooth endoplasmic reticulum, where they accumulate. Virions are found in tubules of the Golgi apparatus. They may exit via that route from infected cells, by exocytosis at the apical cell membrane, or on the lateral cell surface, since viral particles are often seen lined up between microvilli or in the basolateral intercellular space between infected cells. Virus may also be released by lysis of infected cells. Coronaviruses will also infect some mesenchymal cells in villi and probably mesenteric lymph node.

Changes in the infected cell occur by about 12–24 hours after infection. Mitochondria in virus-infected cells swell, cisternae of smooth and rough endoplasmic reticulum dilate, the cytoplasm of infected cells loses its electron density, and cells lose their columnar profile. The terminal web is fragmented; microvilli swell and become irregular, perhaps in association with blebbing of the apical membrane. Damaged epithelium may lyse in situ, releasing virus retained in cytoplasmic vacuoles, or it may exfoliate into the lumen. Profuse diarrhea usually begins about the time that early cytologic changes are becoming apparent, but before there is extensive epithelial exfoliation.

Exfoliation of damaged epithelium may be massive over a relatively short period of time, leading to the development of *villus atrophy*, the severity of which largely reflects the degree of initial viral damage. Villi may appear fused along their sides or tips, and during the exfoliative phase some villi with denuded tips may be present. The enterocytes present on villi shortly after the initial exfoliative episode are mainly poorly differentiated low columnar, cuboidal, or squamous cells, with stubby irregular microvilli. Within 2–3 days, villi begin to regenerate and the epithelium becomes progressively more columnar, though still lacking a well-developed brush border and its complement of enzymes. Defective fat absorption is reflected in the accumulation of lipid droplets in the cytoplasm of enterocytes on villi. This is particularly marked over the period of about 2–5 days after experimental inoculation.

With progressive epithelial regeneration from the crypts, the *villus fusion*, which may be the result of adhesion of temporarily denuded lamina propria of adjacent villi, regresses. Separation begins along the basal margins of the adhesions and progresses toward the tips of the villi. There may be focal acute inflammation in the lamina propria of temporarily denuded villi, and a mild mononuclear infiltrate in the stroma of collapsed villi. Though several cycles of viral replication may occur, poorly differentiated enterocytes appear relatively refractory to infection, and the virus titer falls, presumably as local immune mechanisms also come into play. Hyperplasia of epithelium in crypts usually results in eventual resolution of the villus atrophy, restoring normal function.

The diarrhea that occurs is a result of electrolyte and nutrient malabsorption, with some contribution by secretion by crypt cells, and probably by poorly differentiated surface epithelium in the reparative phase. Mechanisms of diarrhea in villus atrophy are discussed in the section on the Pathophysiology of enteric disease. Remission of signs occurs within about 4–6 days as regeneration of villi occurs, providing the animal survives the dehydration, electrolyte depletion, and acidosis brought about by diarrhea.

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Swine coronaviruses

Three coronaviruses cause gastrointestinal signs in swine. *Porcine hemagglutinating encephalomyelitis virus*, in group 2 species of genus *Coronavirus*, causes vomiting and wasting disease in suckling piglets; this is a condition mainly mediated by infection of the central and peripheral nervous system (see Vol. 1, Nervous system). *Transmissible gastroenteritis virus* (TGEV) and *Porcine epidemic diarrhea virus* (coronavirus 777), both in group 1 species of genus *Coronavirus*, cause syndromes of acute diarrheal disease in all age groups, and chronic diarrhea and runting in weaned pigs. In some areas, coronaviruses, especially TGEV, are the major cause of diarrhea in neonatal swine.

Transmissible gastroenteritis (TGE) may affect swine of any age, causing vomiting, severe diarrhea, and, in piglets, high mortality. The disease is recognized throughout most of the world.

The epizootiology of TGE depends on the overall immune status of the herd and of the various age groups within the herd. Introduction of TGEV into a naive herd results in rapid spread of disease with high morbidity affecting all age groups. Sows and older pigs will show transient inappetence, possibly diarrhea and perhaps vomiting. Signs may be more severe in sows exposed to high virus challenge from infected baby pigs. Agalactia may occur in recently farrowed sows, perhaps related to TGEV infection of the mammary gland. *Suckling piglets develop severe diarrhea*, and mortality may approach 100% in piglets under 10–14 days old. Older pigs usually develop less severe signs and have lower mortality. In herds with enzootic infection, high piglet mortality may occur in the offspring of recently introduced naive sows, and diarrhea with lower mortality may occur in piglets over about 2–3 weeks of age as milk intake and concomitant lactogenic immunity wane. Infected pigs in the late suckling or weanling age group may runt. TGE is more prevalent in the winter, perhaps because the virus is not resistant to summer environmental conditions of warmth and sunlight. Baby pigs that are chilled also seem less able to survive the effects of infection.

The severity of disease in baby pigs is partly related to their inability to withstand dehydration, due to their small size, and to their susceptibility to hypoglycemia. Probably as significant is the differentiation, and low rate of turnover, of small intestinal epithelium in the neonate. The surface epithelium is mature and has an extensive vesicular network in the apical cytoplasm associated with uptake of macromolecules and colostrum during the first day or two after birth. Crypts are short and relatively inactive. The population of epithelium susceptible to infection on each villus is therefore large, and the capacity to regenerate new enterocytes is small. By about 3 weeks of age, epithelium is actively proliferative. Virus production by infected enterocytes in older pigs seems less efficient, and replacement of cells lost to infection is more rapid, contributing to the relative resistance seen in swine over about 3 weeks of age.

Piglets with TGE have the nonspecific gross appearance at necropsy of undifferentiated neonatal diarrhea. The stomach may contain a milk curd or bile-stained fluid. The small bowel is flaccid and contains yellow frothy fluid with flecks of mucus; chyle is not usually evident in mesenteric lymphatics since there is fat malabsorption.

The microscopic lesions are those of villus atrophy due to exfoliation of surface enterocytes (Fig. 1.119), the severity of which is a function of the age of the pig and the stage of the disease. In young piglets, the lesions are most severe about the time of the onset of diarrhea. In later phases or in older pigs there may be subtotal to moderate atrophy, and the mucosa may be lined by cuboidal to low columnar epithelium, with irregular nuclear polarity and an indistinct brush border. Severe atrophy is readily recognized at necropsy of neonatal piglets, by examination of the mucosa under a dissecting microscope. Lesions are most common in the middle and lower small intestine, and villi in the duodenum are usually tall and cylindrical. Lesions may be patchy, and several areas of lower small intestine must be examined before atrophy is considered not to be present. In animals beyond the neonatal age group, atrophy may not be so severe and readily recognized under the dissecting microscope, and the contrast with the normally shorter villi in the duodenum of older pigs is not as marked. Histologic assessment of the gut is essential.

Porcine respiratory coronavirus (PRCoV) is genetically and antigenically extremely close to enteric TGEV. It cross-reacts serologically,

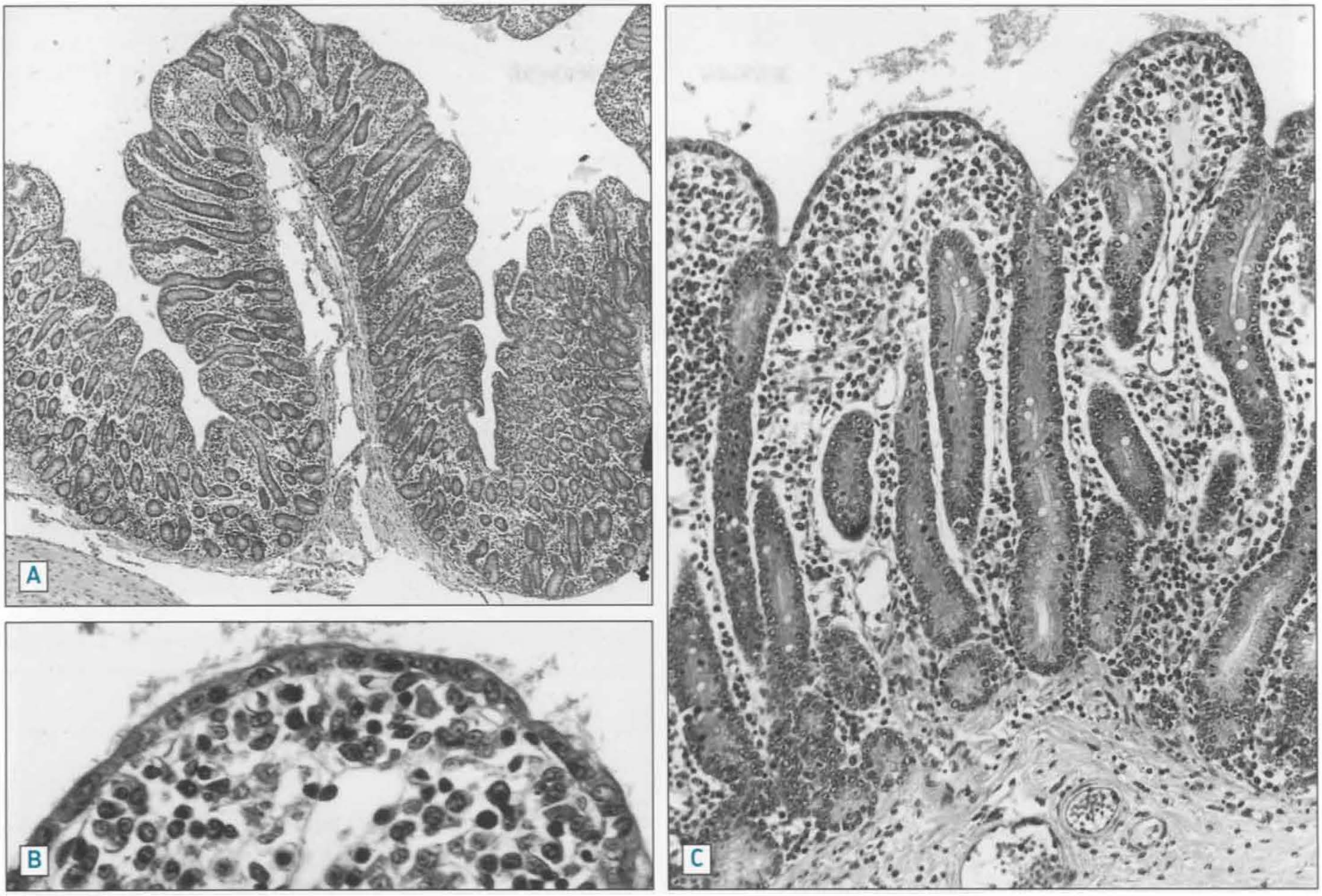

Figure 1.119 Transmissible gastroenteritis in a pig. **A.** Atrophy of villi in small intestine. **B.** Severely attenuated enterocytes on surface of an atrophic villus. **C.** Atrophy of villi, with compensatory hypertrophy of crypts of Lieberkühn. Surface epithelium is cuboidal or flattened.

and vaccinated sows successfully induce passive immunity against enteric infections. PRCoV is spread by inhalation, and infects lining cells of the upper respiratory tract. Mild bronchointerstitial pneumonia results from experimental infection, and the agent has been associated with outbreaks of respiratory disease.

Porcine epidemic diarrhea virus (PEDV), antigenically distinct from TGEV, is reported from England, continental Europe, China, and Taiwan. It causes disease that is essentially similar to TGE in epidemiology, pathogenesis, and lesions, but is milder. In addition to infection of epithelium low on villi and occasionally in small-bowel crypts, PEDV may also cause mild exfoliative lesions in colonic crypts. It is differentiated from TGEV by its distinct viral antigenicity and genome.

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Bovine coronavirus

In **neonatal calves,** *Bovine coronavirus* (BCoV) infection is a common cause of diarrhea, either alone or in combination with other agents, particularly *Rotavirus* and *Cryptosporidium*. The disease may be severe in combination with BVDV infection. BCoV is capable of infecting absorptive epithelium in the full length of the small intestine, and in the large bowel. Viral antigen is also found in macrophages in the lamina propria of villi and in mesenteric lymph nodes. In field infections, microscopic lesions are found most consistently in the lower small intestine and colon. Calves with BCoV infection usually develop mild depression, but continue to drink milk despite developing profuse diarrhea. With progressive dehydration, acidosis, and hyperkalemia, the animals become weak and lethargic; death ensues as a result of hypovolemia, hypoglycemia, and potassium cardiotoxicosis. Diarrhea in survivors resolves in 5–6 days.

At autopsy, affected animals have the nonspecific lesions of undifferentiated neonatal calf diarrhea. Rarely, mild fibrinonecrotic typhlocolitis is recognized at necropsy in calves with coronaviral infection. Mesenteric lymph nodes may be somewhat enlarged and wet.

The microscopic lesions of coronaviral infection in calves vary with the severity and duration of the infection; *villus atrophy in combination with mild colitis is typical* (Fig. 1.120). In the calf *small intestine*, villus atrophy is rarely as severe as that seen in neonatal swine with TGE. Rather, villi are moderately shortened, or have subtotal atrophy with stumpy, club-shaped, or pointed tips, and villus fusion may be common. In the early phase of the clinical disease, villi are often pointed and covered by cuboidal to squamous epithelium. Exfoliation of epithelium and microerosion may be evident. Later, the epithelium is cuboidal to low columnar, basophilic, with irregular nuclear polarity and an indistinct brush border. Cryptal epithelium is hyperplastic. The lamina propria may contain a moderate infiltrate of mainly mononuclear inflammatory cells, some of which may have pyknotic or karyorrhectic nuclei. In the early stages of infection, necrosis of cells in mesenteric lymph nodes is associated with viral replication. Peyer's patches in animals examined after 4–5 days of clinical illness often appear involuted, and are dominated by histiocytic cells. Whether this is the result of viral activity or the effect of endogenous glucocorticoids is unclear.

In the *colon* during the early phase of infection, surface epithelium may be exfoliating, flattened, and squamous or eroded in patchy areas; some colonic glands lined by flattened epithelium will contain exfoliated cells and necrotic debris. A moderate mixed inflammatory

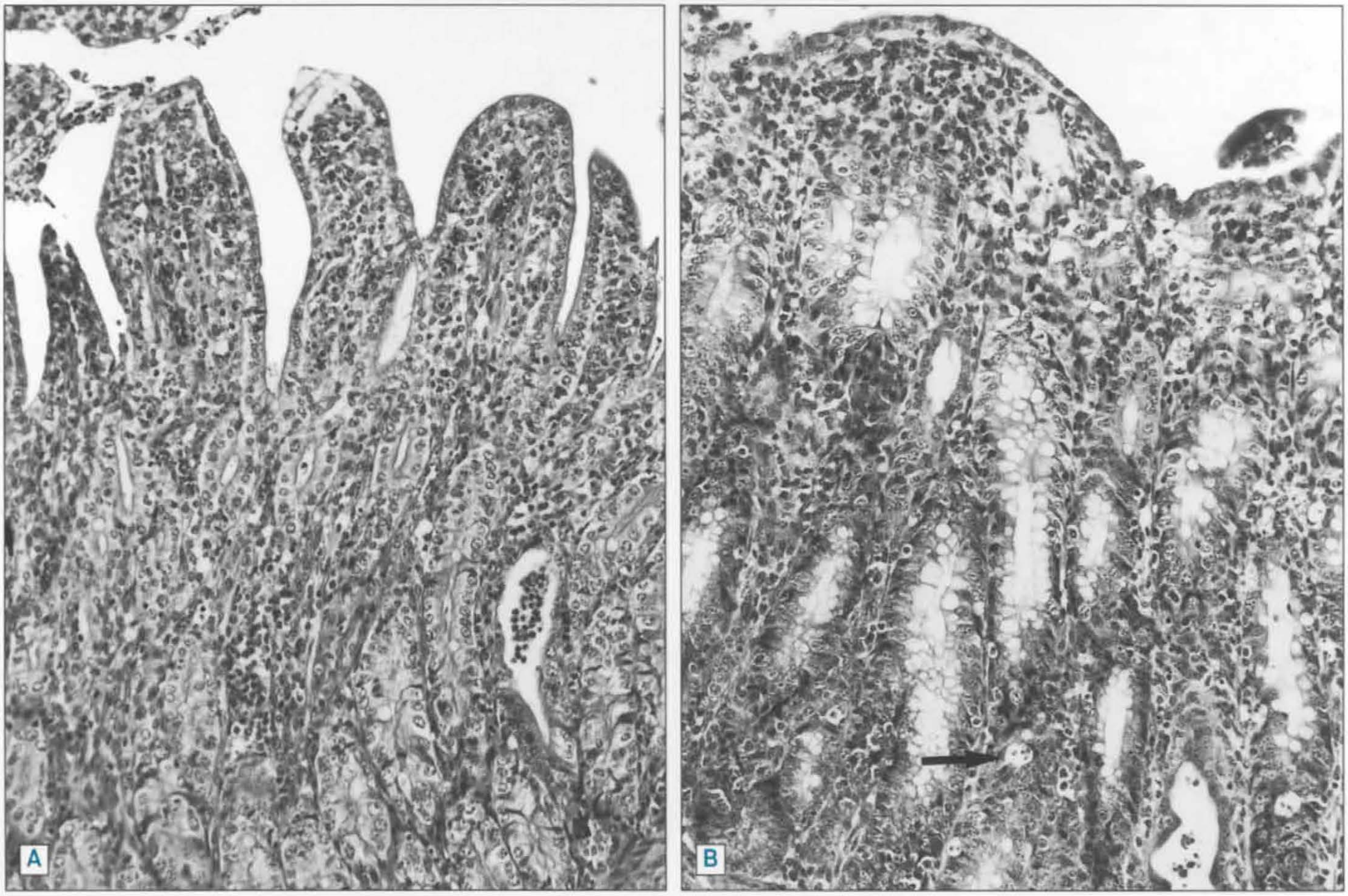

Figure 1.120 ***Bovine coronavirus* infection**. **A.** Blunt, fused villi with cuboidal surface epithelium in small intestine. **B.** Attenuation of surface epithelium and necrosis of gland epithelium (arrow), in colon. (Courtesy of M Morin.)

reaction is present in the lamina propria, and neutrophils may be in damaged glands or effusing into the lumen through superficial microerosions. Later in infection, some dilated debris-filled colonic glands will remain, but other glands will be lined by hyperplastic epithelium, and the surface epithelium will be restored to a cuboidal or low columnar cell type. Goblet cells are usually relatively uncommon. Colonic lesions may be recognizable in tissues from animals submitted dead, though postmortem change has obscured changes in the small intestine.

Live calves in the early stages of clinical disease are the best subjects for confirmation of an etiologic diagnosis. In calves becoming ill under 4–5 days of age, enterotoxigenic *Escherichia coli* is the main alternative diagnosis. *Rotavirus*, *Cryptosporidium*, and combined infections must be considered in calves 5–15 days of age. Infectious bovine rhinotracheitis, salmonellosis, and bovine viral diarrhea must also be considered. Both salmonellosis and bovine viral diarrhea may be associated with depletion of Peyer's patches and colitis that can be confused with that of coronaviral infection; neither is common in the strictly neonatal age group (under 7–14 days of age).

Respiratory tract infection also occurs in calves and feeders infected with BCoV. The virus replicates in the epithelium of the nasal turbinates and tracheobronchial tree, and *respiratory infection may precede, be concurrent with, or follow enteric infection.* Mild nasal discharge, cough, and increased respiratory rate are associated. Respiratory infections may play a role in maintaining the virus within a herd, and significant, but poorly characterized, pneumonia has been reported in some experimentally infected calves. In addition, coronaviral infection may predispose to subsequent respiratory bacterial infections or contribute to more severe respiratory disease as part of the shipping fever syndrome. Virus may be identified in tissue or nasal secretions by immunofluorescence or immunohistochemistry.

Winter dysentery is a syndrome in adult cattle that has been associated with BCoV in a number of areas around the world. Animals develop blood-tinged diarrhea, nasolacrimal discharge or cough, anorexia, and drop in milk production. Mortality is rare, but may occur. The colon of affected animals has linear congestion and hemorrhage along the crests of mucosal folds. Coronaviruses are commonly demonstrated in the feces of cattle with winter dysentery; seroconversions occur, and seroprevalence increases in affected herds; and coronavirus antigen is found in the colonic glands of affected animals, in which there is necrosis and exfoliation of epithelial cells. Certain management practices, notably housing animals in stanchions and use of equipment that handles both manure and feed, have been associated with the development of winter dysentery.

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Canine coronavirus

Canine coronavirus (CCV) is widely prevalent in the dog population. Although dogs of all ages appear to be susceptible to infection by CCV, the condition is probably most important as an *uncommon, transient, generally nonfatal, diarrhea in puppies*. Fatal infections have been reported in pups previously infected with parvovirus.

Viral replication occurs in the enterocytes of the small intestine, and in experimental infections in neonatal puppies, the lesion resembles the *villus atrophy* associated with coronaviral infection in other species. Diarrhea begins as early as 1 day after inoculation and in most animals by 4 days. Onset of signs coincides with the development of moderate villus atrophy and fusion. Enterocytes on villi become cuboidal, contain lipid vacuoles, and have an indistinct brush border. Lesions are most consistent and severe in the ileum. Resolution of villus atrophy within 7–10 days is associated with remission of signs.

Colonic infection by CCV was not demonstrated by immunofluorescence in experimental animals, though mild colonic lesions were described, including loss of sulfomucins from goblet cells and some epithelial shedding. However, in a report of lesions due to spontaneous CCV infection, colonic infection and lesions were demonstrated. There was watery content in the lumen of the small and large intestine, and in the cecum and colon fibrin mixed with some blood was evident. Mesenteric lymph nodes were enlarged and edematous. Villus atrophy in the jejunum was inconsistent, but there was necrotic debris in many glands in the cecum and colon. Virus-infected cells were exfoliating into the lumen.

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Feline and other enteric coronaviruses

Our understanding of the enteric implications of coronaviral infections of **cats** is still incomplete. It appears that *Feline enteric coronavirus* (FECV) can establish persistent infection in the intestine which, in rare cases, may be clinically apparent. Infection is very common. Diarrhea, when it occurs, is usually mild or moderate, perhaps with some blood, and kittens are most susceptible. Viral antigen is in cells on the tips of villi, and mild villus atrophy has been illustrated. Feline infectious peritonitis (see section on Peritonitis in cats, below), of far greater clinical significance, is due to viruses that arise by mutation from endemic FECV.

Coronaviruses have been recovered from the feces of **sheep** with transient diarrhea, and they have been associated with severe villus atrophy in several spontaneous outbreaks of diarrhea. No experimental confirmation of the pathogenicity of coronavirus in sheep is available.

Coronaviral infection may also be associated with diarrhea in **foals**, but again, experimental confirmation of pathogenicity is lacking.

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Rotaviral infections

Members of the genus *Rotavirus*, in the family Reoviridae, infect the gastrointestinal tract of most mammals and birds. Rotaviruses are classified according to antigenic properties with group, subgroup, and serotype categories. Segment 6 of the *Rotavirus* genome codes for the VP6 intermediate capsid protein, which is used to classify the virus into seven serogroups, A–G. *Group A rotaviruses (RV-A) are the most common* and infect all species of domestic animals, as well as humans, laboratory animals, and wildlife. RV-A can be subdivided into G and P serotypes based on VP7 and VP4 external capsid proteins respectively; there are 14 G serotypes and 20 P serotypes. The serotypes isolated most commonly from piglets with diarrhea are P[7],G5, P[6],G4, P[7],G3, and P[7],G11. Individual rotavirus serotypes have a surprisingly wide range of host susceptibility. Nongroup A rotaviruses infect pigs and ruminants, among domestic animals.

The ability to infect cells, and the serotype specificity of rotaviruses, are conferred by elements of the outer capsid layer. The viruses are probably generally host-specific, with little significant zoonotic potential. However, if epidemiologic circumstances are favorable, cross-species transmission may occur.

Rotaviruses infect the absorptive enterocytes and occasionally goblet cells on the tips and sides of the distal half or two-thirds of villi in the small intestine. Rotaviruses infect cells on the apical half (ruminants) or the entire villus (pigs), mainly in the jejunum and ileum. Virus production and the pathogenesis of infection are similar in all species studied. Rotaviruses adhere to cell receptors (e.g., integrins, sialic acid), and inner capsid components are internalized into the cell. Granular "viroplasm" containing incomplete virions is seen in the apical cytoplasm of infected cells, and virions acquire their complete capsid after budding into dilated cisternae of endoplasmic reticulum, where they accumulate. Elongate tubular structures are found in the nuclei and rough endoplasmic reticulum of some infected cells.

Virus-infected cells are most prevalent 18–24 hours after experimental infection, and they tend to diminish in number rapidly, so that by 3–4 days after infection few cells containing viral antigen are present. Infected enterocytes lose cytoplasmic electron density, and mitochondria swell, as does the cell generally. Swollen rarified cells and syncytia may occur in the enterocytes at the villus tips, but cells are fragile and will shed readily, particularly if autolysis intervenes. Syncytial cell formation has been recognized in porcine, bovine, and laboratory animal infections with rotavirus. Microvilli become irregular and somewhat stunted, and there may be some blebbing of membranes. Infected cells exfoliate into the intestinal lumen, and virus is released by lysis of damaged epithelium prior to or after exfoliation.

The *pathogenesis of diarrhea* with *Rotavirus* involves three mechanisms. First, malabsorption occurs secondary to destruction of enterocytes. Second, a vasoactive agent is released from infected epithelial cells and causes villus ischemia and activation of the enteric nervous system. Third, rotaviruses are capable of producing a nonstructural protein, NSP4, which acts as a *secretory enterotoxin*. This is the first viral pathogen known to produce a toxin.

Exfoliation of infected epithelium over a relatively short period of time results in villus atrophy. The mucosal surface is covered by cuboidal, poorly differentiated epithelium that has an ill-defined microvillus border and that may contain lipid droplets in the cytoplasm. Diarrhea is probably mediated by electrolyte and nutrient malabsorption, perhaps exacerbated by the effect of cryptal secretion. It begins about the time of early viral cytopathology 20–24 hours after infection, and may persist for a variable period, from a few hours to a week or more. Regeneration of the mucosa by epithelium emerging from crypts, and differentiating on reformed villi, is associated with remission of signs in animals surviving the effects of diarrhea.

Rotaviruses are widespread, if not ubiquitous, among populations of most species, and they are relatively resistant to the external environment. Protection against infection in neonates is apparently largely conferred by the presence of *lactogenic immunity*. Many individuals in a population probably undergo inapparent infection. Disease is seen in the various species when viral contamination of the environment is heavy, perhaps as a result of intensive husbandry practices, and lactogenic immunity is waning or absent. Though rotaviral infection is usually associated with younger age groups, and viral receptors on cells diminish with age in some species, naive older animals may become infected, sometimes with the development of diarrhea.

Cattle rotaviral infection

Rotaviral infection is mainly implicated in *diarrhea of neonatal beef and dairy calves*, both suckled and artificially reared, though there are reports of its association with diarrhea in adult cattle. Diarrhea may be produced in calves by rotaviral infection alone, but the condition is usually considered to be relatively mild or transient in comparison with that induced by enterotoxigenic *Escherichia coli* or *Bovine coronavirus*. Combinations of agents, including *Rotavirus*, are frequently involved in outbreaks of diarrhea in neonatal calves. *Rotavirus* may be implicated in animals developing signs at any time over the period up to about 2–3 weeks of age, and it is more commonly encountered in animals over 4–5 days of age. Rotaviral diarrhea is most severe in calves that have slower enterocyte regeneration times.

The gross lesions of rotaviral infection are the nonspecific findings of undifferentiated neonatal diarrhea in calves, described previously. *Microscopic lesions in the small intestine cannot be differentiated from those of coronaviral infection.* They may vary somewhat depending on the severity of the initial viral damage and the stage of evolution of the sequelae. Blunt club-shaped villi, mild or moderate villus atrophy, and perhaps villus fusion may be present (Fig. 1.121). Villi are covered by low columnar, cuboidal, or flattened surface epithelium with a poorly defined brush border. There is usually a moderate proprial infiltrate of mononuclear cells and eosinophils or neutrophils, and hypertrophic crypts may be evident. The distribution of lesions may vary between animals and perhaps with time after infection within an individual animal, since the onset of maximal viral damage may not occur synchronously throughout the full length of the intestine. Lesions and viral antigen should always be sought in the distal small intestine, and preferably at several sites along its length. *Rotavirus* does not cause gross or microscopic lesions in the colon, in contrast to coronavirus.

Swine rotaviral infection

Rotavirus infection is widespread and enzootic in most swine herds, and subclinical infection of piglets is common. It assumes particular importance as a cause of diarrhea in pigs with reduced lactogenic immunity, either as a result of early weaning or following normal weaning. High environmental levels of virus may result in disease in piglets suckling the sow, but in these circumstances the signs are usually relatively mild. *Rotavirus* may be a cause of "3-week," "white," or postweaning scours in piglets from 2 to 8 weeks of age.

The signs may resemble those of transmissible gastroenteritis (TGE), although *Rotavirus* infection is considered to be less severe. Vomition is less commonly encountered than with TGE, but depression, diarrhea, and dehydration are usual. The character of the

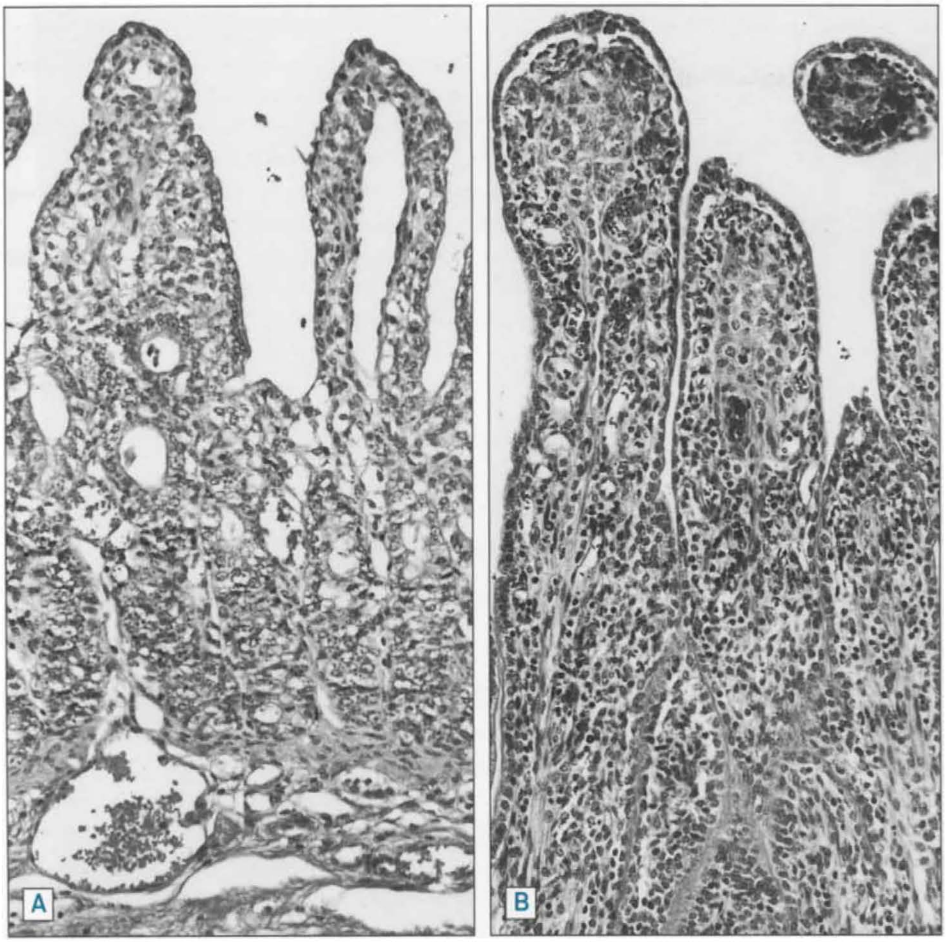

Figure 1.121 Bovine ***Rotavirus*** infection. **A.** Stumpy villi with severely attenuated surface epithelium. **B.** Club-shaped villi with cuboidal or flattened epithelium. (Courtesy of M Morin.)

feces varies with the diet. Steatorrhea occurs in white scours of suckling piglets. *Rotavirus* infection in swine may be associated with other causes of diarrhea, including *E. coli*, coccidiosis, adenoviral infection, and *Strongyloides*.

The gross and microscopic lesions and pathogenesis of rotavirus infection in pigs resemble those of TGE (Fig. 1.122). As in TGE, severity of lesions seems inversely related to age.

Rotaviral infection in other species

Neonatal lambs have proved a useful model for the demonstration of the importance of lactogenic immunity in preventing disease due to *Rotavirus*. *Rotavirus* may cause diarrhea in neonatal lambs alone or in combination with enterotoxigenic *E. coli* and *Cryptosporidium*. The pathogenesis and lesions of *Rotavirus* infection in lambs are like those caused in other species, with the exception that viral infection of the colon may occur.

In **foals** under 3–4 months of age, diarrhea may be associated with *Rotavirus* infection, though mortality is rare. Outbreaks have been reported in many areas of the world. The natural and experimental disease resembles that seen in other species, with significant viral infection limited to enterocytes in the small intestine, where villus atrophy occurs.

In young **puppies**, especially those under 1–2 weeks of age, diarrhea, occasionally fatal, may be caused by *Rotavirus* infection. In experimentally infected pups, green fluid content filled the lower small bowel and colon, and moderate villus atrophy was induced by exfoliation of epithelium from the distal half of villi.

Rotavirus has also been associated with diarrhea in **kittens**, although rotavirus can also be isolated from asymptomatically infected kittens.

Rotavirus infection should be sought in cases of diarrhea in young animals of any species and it should be particularly suspected in animals with villus atrophy in the small intestine. Rotavirus is part of the syndrome of undifferentiated neonatal diarrhea in any species.

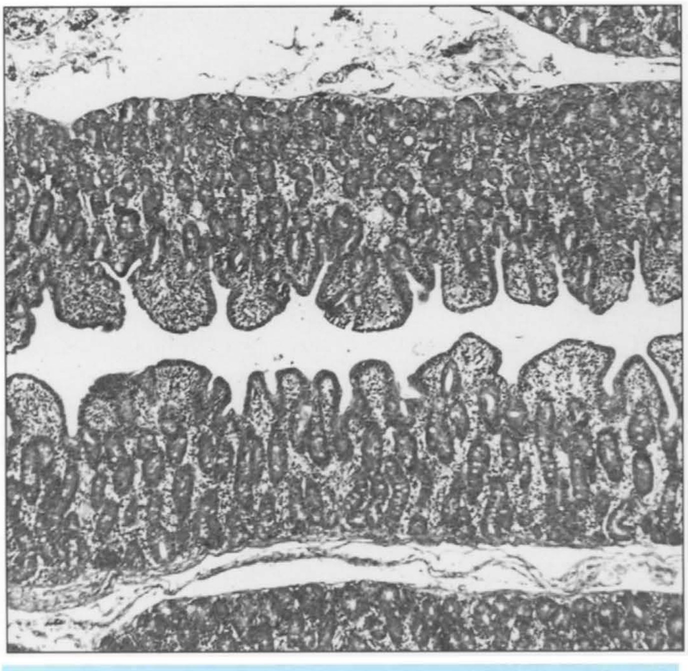

Figure 1.122 Villus atrophy, caused by ***Rotavirus*** infection, in the small intestine of a 3-week-old piglet.

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Parvoviral enteritis

The Parvoviridae are small nonenveloped viral particles about 18–26 nm in diameter, with icosahedral symmetry and a short single-stranded DNA genome. They replicate, and produce inclusion bodies, in the nucleus of infected cells. Members of the genus *Parvovirus* infect many species of laboratory and domestic animals. Among syndromes associated with parvovirus infection are: disease in cats, dogs, and mink (distinct from *Aleutian mink disease virus*) dominated clinically by enteritis; diarrhea in neonatal calves; and reproductive wastage in swine.

Feline panleukopenia virus (FPV), *Mink enteritis virus* (MEV) and *Canine parvovirus 2* (CPV-2), the agent causing parvoviral enteritis in dogs, are considered host range variants or strains of the feline parvovirus subgroup within the genus *Parvovirus*. These viruses are biologically distinct, varying in their hemagglutination characteristics, in vitro host cell ranges, infectivity, and virulence in experimentally inoculated hosts. There are subtle antigenic differences among them, detected by monoclonal antibodies; these, and differences in host specificity, are conferred by variations in only very small segments of the viral genome. Based on the nucleotide sequence in the gene for capsid proteins VP1/VP2, FPV and MEV are very closely related to each other, and somewhat less related to CPV-2. Two new antigenic types have evolved from CPV-2 – CPV-2a and CPV-2b – and are able to replicate in cats. Another variant, CPV-2c, has been isolated from leopard cats. The host ranges of FPV and CPV are determined by receptor binding, particularly to the transferrin receptor TfR. *Canine parvovirus 2* is distinct from *Canine minute virus* (CMV, or *Canine parvovirus 1*) that also has enteric lesions associated with infection.

Though autonomous parvoviruses may infect cells at any phase of the cell cycle, replication is dependent on cellular mechanisms only functional during nucleoprotein synthesis prior to mitosis. Hence, *the effects of parvoviral infection are greatest in tissues with a high mitotic rate*. These may include a variety of tissues during organogenesis in the fetus and neonate. In older animals, the proliferative elements of the enteric epithelium, hematopoietic and lymphoid tissue are particularly susceptible. At the time of virus assembly, *large basophilic or amphophilic Feulgen-positive nuclear inclusions* may be found in infected cells, especially in Bouin's fixed tissues. Parvovirus is demonstrated in these inclusions by electron microscopy. The chromatin in inclusion-bearing nuclei is usually clumped at the nuclear membrane. Inclusions are most prevalent late in the incubation period, prior to extensive exfoliation or lysis of infected cells. Hence, they are not commonly encountered in animals submitted for autopsy after a period of clinical illness culminating in death. Large nucleoli, seen in proliferative cells encountered in the intestine of parvovirus-infected animals, should not be confused with intranuclear viral inclusions.

The *pathogenesis* of FPV and of CPV-2 infection is sufficiently similar for them to be considered together here, followed by separate discussions of the specific diseases. Oronasal exposure results in uptake of virus by epithelium over tonsils and Peyer's patches. Infection of draining lymphoid tissue is indicated by isolation of virus from mesenteric lymph nodes 1–2 days after experimental inoculation. Release of virus into lymph, and dissemination of infected lymphoblasts from these sites, may result in infection of other central and peripheral lymphoid tissues, including thymus, spleen, lymph nodes, and Peyer's patches, 3–4 days after infection. Lymphocytolysis in these tissues releases virus, reinforcing cell-free viremia. Viremia is terminated when neutralizing antibody appears in circulation about 5–7 days after infection. Moderate pyrexia occurs at about this time.

Infection of the gastrointestinal epithelium is a secondary event, following dissemination of virus by circulating lymphocytes and cell-free viremia. Peyer's patches are consistently infected at all levels of the intestine, and epithelium in crypts of Lieberkühn over or adjacent to Peyer's patches usually becomes infected a day or so later. Infection of gastrointestinal epithelium at other sites in the gut is less consistent, but is usually more severe in the lower small intestine. It may be the result of virus free in circulation, or carried by infected lymphocytes homing to the mucosa. Maximal infection of cryptal epithelium occurs during the period about 5–9 days after infection.

The occurrence and severity of enteric signs are determined by the degree and extent of damage to epithelium in intestinal crypts. This seems to be a function of two main factors. The first is the availability of virus, which is influenced by the rate of proliferation of lymphocytes, and therefore their susceptibility to virus replication and lysis. The second factor influencing the degree of epithelial damage is the rate of proliferation in the progenitor compartment in crypts of Lieberkühn. If many cells are entering mitosis, large numbers will support virus replication and subsequently lyse. Destruction of cells in the crypts of Lieberkühn, if severe enough, ultimately results in focal or widespread villus atrophy and perhaps mucosal erosion or ulceration. The recognition, evolution, and sequelae of radiomimetic insult to the intestine, such as that caused by parvovirus, are described elsewhere (see section on Epithelial renewal in health and disease, above).

Regeneration of cryptal epithelium and partial or complete restoration of mucosal architecture will occur, if undamaged stem cells persist in most affected crypts, and the animal survives the acute phase of clinical illness. In some survivors, focal villus atrophy is associated with persistent dilated crypts containing cellular debris, and with local "drop-out" of crypts completely destroyed by infection. In rare animals recovered from acute disease, chronic malabsorption and protein-losing enteropathy are associated with persistent areas of ulceration caused by more extensive loss of crypts and collapse of the mucosa.

The low rate of replication of intestinal epithelium in germfree cats explains failure to produce significant intestinal lesions and clinical panleukopenia in experimentally infected animals. In spontaneous cases, the lower prevalence of parvoviral lesions in the colon and stomach, in comparison with the small intestine, reflects the relatively lower rate of epithelial proliferation in those tissues. The consistency of epithelial lesions in the mucosa over Peyer's patches probably results from high local concentrations of virus derived from infected lymphocytes in the dome and follicle. This may be coupled with local stimulation of epithelial turnover by cytokines released by T lymphocytes in the vicinity. Variations in the rate of epithelial proliferation related to age, starvation, and refeeding, or concomitant parasitic, bacterial, or viral infections, may also influence the susceptibility of crypt epithelium to infection, and therefore affect the extent and severity of intestinal lesions and signs.

Diarrhea in parvoviral infections is mainly the result of reduced functional absorptive surface in the small intestine. Effusion of tissue fluids and blood from a mucosa at least focally denuded of epithelium probably also contributes to diarrhea. Dehydration and electrolyte depletion are the result of reduced fluid intake, enteric malabsorption, effusion of tissue fluid, and, in some animals, vomition. Hypoproteinemia is common, and anemia may occur due to enteric blood loss; both are exacerbated by rehydration. Anemia reflects hemorrhage into the gut.

Proliferating cells in the *bone marrow* are also infected during viremia. Lysis of many infected cells is reflected in hypocellularity of the marrow caused by depletion of myeloid and erythroid elements, particularly the former. Megakaryocytes may also be lost, but seem the least sensitive cell population in the marrow. The number of neutrophils in circulation drops quickly in severely affected animals. This is due to failure of recruitment from the damaged marrow, and peripheral consumption, especially in the intestine. Transient neutropenia, of about 2–3 days' duration, occurs consistently in cats, and less commonly in dogs. In surviving animals, regeneration of depleted myeloid elements from remaining stem cells restores the circulating population of granulocytes within a few days. Neutrophilia with left shift may occur during recovery.

Lymphopenia, relative or absolute, results from *viral lymphocytolysis* in all infected lymphoid tissue. Relative lymphopenia is more consistently observed in dogs than neutropenia. When lymphopenia and neutropenia occur together, *the combined leukopenia may be profound in both dogs and cats*. In dogs surviving the lymphopenic phase, circulating lymphocytes return to normal numbers within 2–5 days, as regenerative hyperplasia occurs in lymphoid tissue throughout the body. Lymphocyte numbers increase rapidly, sometimes producing lymphocytosis in recovering dogs. However, there may be transient immunosuppression in gnotobiotic pups subclinically infected with CPV-2. Transient depression of T-cell response to mitogens occurs in cats a week after experimental infection with FPV. But immunosuppression by these agents appears to be of little clinical significance.

Most infected cats and dogs do not develop clinical disease. When it occurs, signs usually begin during the late viremic phase, about 5–7 days after infection. Severe enteric damage is the major cause of mortality. Shedding of infective virus in feces begins about 3–5 days after infection, when Peyer's patches and cryptal epithelium first become infected. Virus shedding persists until coproantibody appears to neutralize virus entering the gut, about 6–9 days after infection. Virus-infected cells may still be detected in crypts and Peyer's patches at this time, and virus complexed with antibody may be found in feces or intestinal content by direct electron microscopy. However, attempts to demonstrate virus in tissues or feces after several days of clinical disease, or at death, are often thwarted by the fact that virus is neutralized by antibody present in tissue fluids. Persistent or sporadic shedding of virus by recovered animals may be the result of virus replication in cells entering mitosis days or weeks after they were infected during the viremic phase.

Infection of the fetus during late prenatal life by FPV causes *anomalies of the central nervous system*, mainly hypoplasia of the cerebellum; anomalies of the central nervous system have not been reported in puppies with CVP-2, although there is mounting evidence from polymerase chain reaction studies that central nervous system lesions in puppies can be induced by fetal CVP-2 infections. Infection of proliferating cardiac myocytes in young puppies with CPV-2 results in nonsuppurative myocarditis and sequelae of acute or chronic heart failure (see Vol. 3, Cardiovascular system), but this is rarely seen in populations with a high prevalence of maternal immunity. A tentative association has been made between infection of kittens with FPV and myocarditis, as well as subsequent cardiomyopathy.

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Feline panleukopenia

Feline panleukopenia virus (FPV) infects all members of the Felidae, as well as mink, raccoons, and some other members of the Procyonidae. FPV is ubiquitous in environments frequented by cats, and infection is common, though generally subclinical. The disease panleukopenia (infectious feline enteritis, feline distemper) usually occurs in young animals exposed after decay of passively acquired maternal antibody, but it may occur in naive cats of any age. Clinical signs of several days' duration, including pyrexia, depression, inappetence, vomition, diarrhea, dehydration, and perhaps anemia may be evident in the history. However, many cases, particularly poorly observed animals or those prone to wander, may be presented as "sudden death." The pathogenesis of panleukopenia has been considered above. Lesions of the central nervous system in kittens are considered in Vol. 1, Nervous system.

At **necropsy**, external evidence of diarrhea may be present, the eyes may be sunken, and the skin is usually inelastic, with a tacky subcutis reflecting dehydration. Rehydrated animals may have edema, hydrothorax, and ascites due to hypoproteinemia. There is pallor of mucous membranes and internal tissues in anemic animals. Gross lesions of internal organs most consistently involve the thymus and the intestine. The thymus is markedly involuted and reduced in mass in young kittens. Enteric lesions may be subtle and easily overlooked. Hence *it is mandatory that intestine be examined microscopically despite the apparent absence of gross change.*

The intestinal serosa may appear dry and nonreflective, with an opaque ground-glass appearance. Uncommonly in cats, there may be petechiae or more extensive hemorrhage in the subserosa, muscularis, or submucosa of the intestinal wall. The small bowel may be segmentally dilated and can acquire a hose-like turgidity in places, perhaps due to submucosal edema. However, turgidity is difficult to assess in the intestine of the cat. The content is usually foul-smelling, scant, and watery, and yellow-gray at all levels of the intestine. The mucosa may be glistening gray or pink, with petechiae, perhaps covered by fine strands of fibrin (Fig. 1.123). Patchy diphtheritic lesions may be present, especially over Peyer's patches in the ileum. Flecks of fibrin and sometimes casts may be in the content in the lumen. Formed feces are not evident in the colon. Lymph nodes may be prominent at the root of the mesentery. Gross lesions elsewhere in the carcass are usually restricted to pulmonary congestion and edema in some animals, and pale gelatinous marrow in normally active hemopoietic sites.

Microscopic lesions are consistently found in the intestinal tract in fatal cases, and are usual in lymphoid organs and in bone marrow. The *intestinal lesions* vary with the severity and duration of the disease. Lesions may be patchy, and several levels of gut should be examined, preferably including ileum and, if possible, Peyer's patch. During the late incubation period and early phase of clinical disease, crypt-lining epithelium is infected. Intranuclear inclusions may be found, and damaged epithelium containing inclusions exfoliates into the lumen of crypts. Crypts are dilated and lined by cuboidal or more severely attenuated cells. The lamina propria between crypts contains numerous neutrophils and eosinophils at this time, and some emigrate into the lumen of crypts, where they join the epithelial debris.

Subsequently, severely damaged crypts may be lined by extremely flattened cells, and by scattered large bizarre cells with swollen nuclei and prominent nucleoli (Fig. 1.124A). Enterocytes covering villi are not affected. But as they progress off the villus, they are replaced by a few cuboidal, squamous, or bizarre epithelial cells, so that villi in affected areas undergo progressive collapse. If cryptal damage is severe and widespread, the mucosa becomes thin and eroded or ulcerated, with effusion of tissue fluids, fibrin, and erythrocytes. Inflammatory cells are usually sparse in the gut of such animals, and superficial masses of bacteria may be present, occasionally accompanied by locally invasive fungal hyphae. In less severely affected animals with disease of longer duration, corresponding to about 8–10 days after infection, scattered focal drop-out of crypts, or focal mucosal collapse and erosion or ulceration, may be evident. In these animals, remaining crypts recovering from milder viral damage show regenerative epithelial hyperplasia (Fig. 1.124B). Mucosal lesions are often most marked in the vicinity of Peyer's patches.

Lesions in the *colon* generally resemble those found in the small bowel, though they are often less severe or more patchy in distribution. Colonic lesions are present in about half of fatal cases of panleukopenia. Gastric lesions resulting from damage to mitotic epithelium are relatively uncommon in cats. They are recognized by flattening of basophilic cells lining the narrowed isthmus of gastric fundic glands, with some reduction in number of parietal cells in the upper portion of the neck of glands.

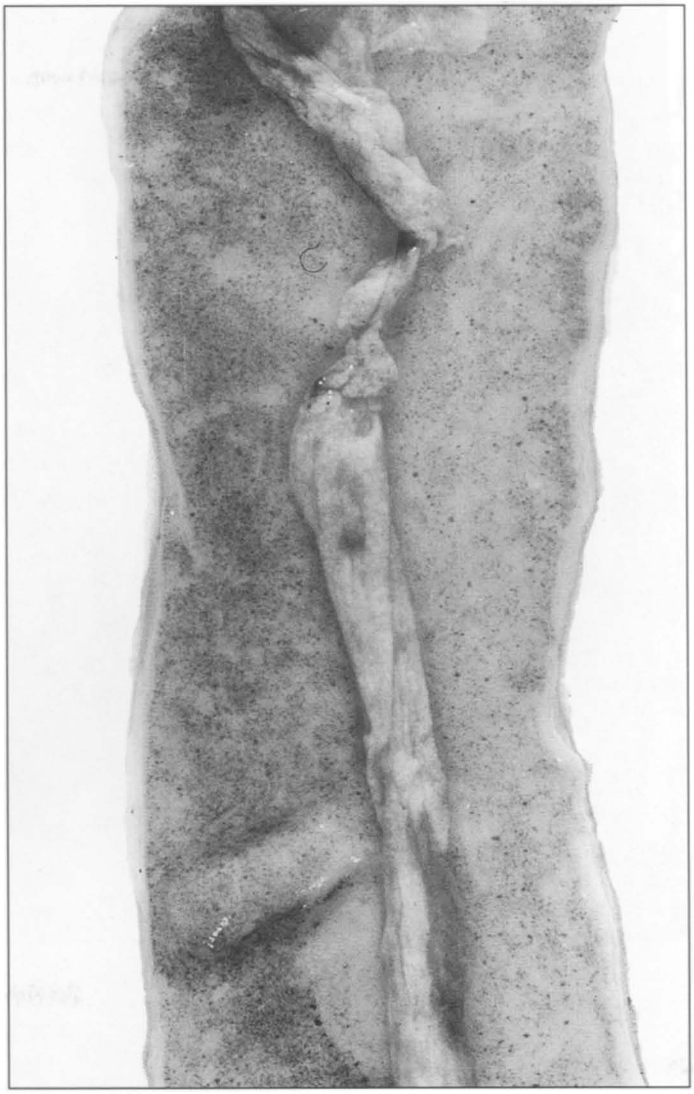

Figure 1.123 Petechiae and fibrin cast on mucosa of small intestine in **feline panleukopenia**.

Lesions of *lymphoid organs* during the early phase of the disease consist of lymphocytolysis in follicles and paracortical tissue in lymph nodes, in the thymic cortex and splenic white pulp, and in gut-associated lymphoid tissue. Lymphoid necrosis has been associated with induced apoptosis of virus-infected lymphocytes. Lymphocytes are markedly depleted in affected tissue and large histiocytes are prominent, often containing the fragmented remnants of nuclear debris. Follicular hyalinosis, the presence of amorphous eosinophilic material in the center of depleted follicles, may be seen. Erythrophagocytosis by sinus histiocytes may occur in lymph nodes, especially those draining the gut. Severely depleted Peyer's patches may be difficult to recognize microscopically. Later in the course of clinical disease, corresponding to the period beyond about 7–8 days after infection, prominent regenerative lymphoid hyperplasia may be found.

In severely affected animals at the nadir of the leukopenia, virtually all proliferating elements in the *bone marrow* may be depleted.

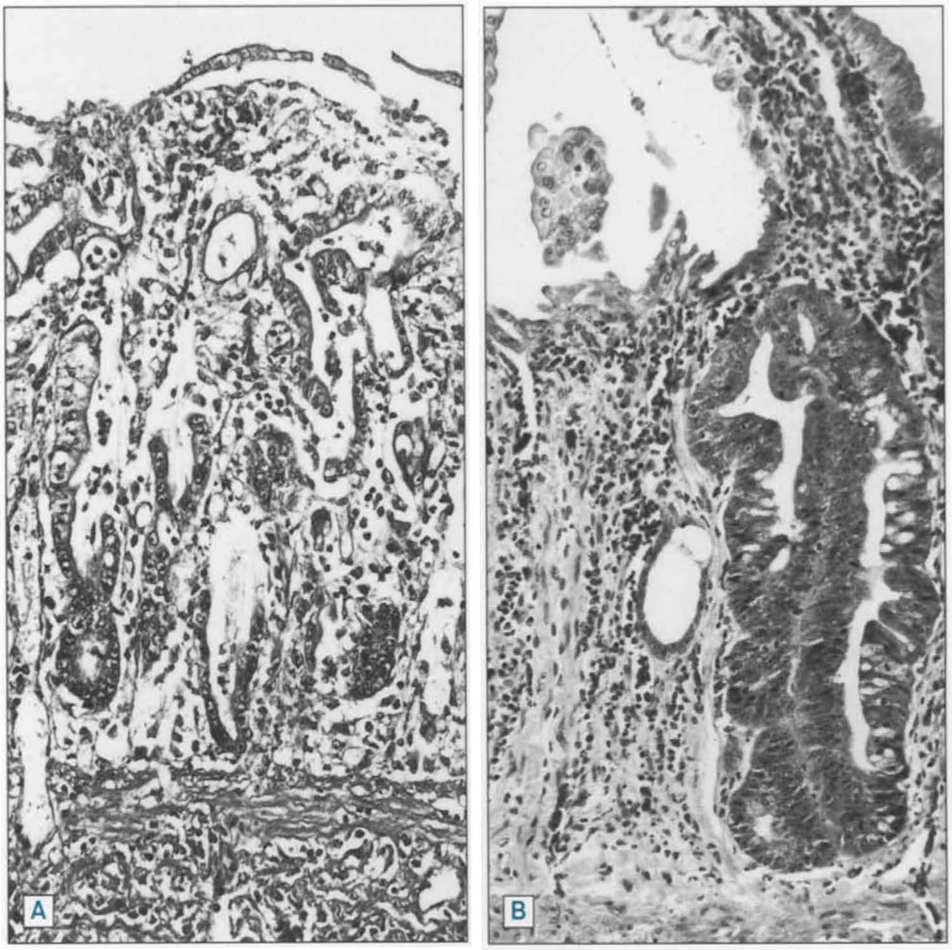

Figure 1.124 **A.** Severe atrophy of villi associated with damage to crypts of Lieberkühn, caused by ***Feline panleukopenia virus*** infection, in a cat. Attenuation of surface epithelium and depletion of proprial inflammatory infiltrate. **B.** Loss of crypts and collapse of proprial stroma; gland is lined by hyperplastic epithelium in a regenerating state in ***Mink enteritis virus*** infection 12 days after inoculation.

The extremely hypocellular, moderately congested marrow is only populated by scattered stem cells. Milder lesions mainly affect the neutrophil series, generally sparing megakaryocytes and the committed erythroid elements. During the later phases of the disease, marked hyperplasia of stem cells, and eventually of amplifier populations in the various cell lines, is evident.

In the *liver*, dissociation and rounding up of hepatocytes, and perhaps some periacinar atrophy and congestion, may be evident. This is probably associated with dehydration and anemia. Pancreatic acinar atrophy is also common, reflecting inappetence. The lung may be congested and edematous. In leukopenic animals, few white cells are seen in circulation in any organ.

A **diagnosis** of feline panleukopenia may be made on the basis of the characteristic microscopic intestinal lesions, in association with evidence of involution or regenerative hyperplasia of lymphoid and hematopoietic tissues. *Inclusion bodies* may be sought in these tissues, but are usually present in significant numbers only during the late incubation and early clinical period. Application of immunohistochemical techniques may identify viral antigen in tissue as late as 8–10 days after infection. Cryptal necrosis is also reported in the intestines of some cats with *Feline leukemia virus* infection, which must be differentiated.

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Canine parvovirus 2 infection

Canine parvovirus 2 (CPV-2) resulted from mutation of a closely related virus, likely an FPV-like virus from wild carnivores, such as foxes. It appeared spontaneously and virtually simultaneously in populations of dogs on several continents in 1978, and rapidly spread worldwide. Retrospective serologic studies suggest that it was circulating unnoticed in western Europe by 1976. In addition to domestic dogs, several species of wild canids, including coyotes, gray wolves, and raccoon dogs, are susceptible to infection.

Enteric disease due to this virus was epizootic for several years in naive populations of dogs, affecting animals of all ages. As the prevalence of antibody due to natural infection and vaccination increased, the problem subsided to one of an enzootic disease. It now affects those animals with reduced levels of passively acquired maternal immunity, or scattered naive individuals.

During the epizootic period, nonsuppurative viral myocarditis due to CPV-2 was prevalent in the offspring of naive bitches unable to protect pups with maternal antibody during the first 15 days of life, when replicating myocardial cells are susceptible to parvoviral

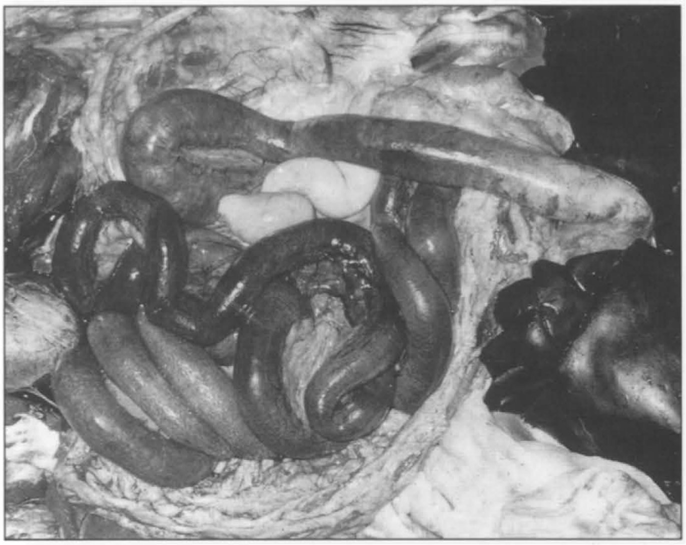

Figure 1.125 Segmental subserosal hemorrhage and mild fibrinous exudation on intestinal serosa in ***Canine parvovirus 2*** infection.

damage. Myocardial disease in pups due to CPV-2 is now fairly uncommon, as most bitches have antibody. Enteric and myocardial disease rarely occur together in the same individual or cohort of animals. Occasional cases of generalized parvoviral infection have been reported in susceptible neonates. Necrosis and inclusion bodies are found in organs such as kidney, liver, lung, heart, gut, and vascular endothelium. They are presumably related to mitotic activity during organogenesis.

Dogs with typical disease due to CPV-2 become anorectic and lethargic and may vomit and develop diarrhea, perhaps in association with transient moderate pyrexia. Relative or absolute lymphopenia or leukopenia of 1–2 days' duration may occur. Diarrhea may be mucoid or liquid, sometimes bloody, and is malodorous. After a period of 2–3 days, dogs either succumb to the effects of dehydration, hypoproteinemia, and anemia, or begin to recover.

Gross findings at autopsy of fatal cases are those of dehydration, accompanied by enteric lesions characteristic of the disease. There is often segmental or widespread subserosal intestinal hemorrhage, which may extend into the muscularis and submucosa. The serosa frequently appears granular due to superficial fibrinous effusion (Fig. 1.125). Peyer's patches may be evident from the serosal and mucosal aspects as deep red oval areas several centimeters long. The intestinal contents may be mucoid or fluid; sometimes they look like tomato soup, due to hemorrhage. The mucosa is usually deeply congested and glistening, or covered by patchy fibrinous exudate. Severe mucosal lesions may be widespread or segmental, and their distribution is irregular; thus tissues from several levels of the small intestine should be selected for microscopic examination. Gross changes in the colon are similar, but less common. The stomach may have a congested mucosa and contain scant bloody or bile-stained fluid. Mesenteric lymph nodes may be enlarged, congested, and wet, or be reduced in size. Thymic atrophy is consistently present in young animals, and the organ may be so reduced in size as to be difficult to find. The lungs often appear congested, and have a rubbery texture.

The **microscopic lesions** in stomach, small intestine (Fig. 1.126), colon, lymphoid tissue, and bone marrow due to CPV-2 infection do not differ significantly from those described above in cats with panleukopenia. Gastric lesions are perhaps more frequently encountered in dogs with parvoviral infection. Small intestinal lesions are invariably severe in fatal cases. The colon is involved in a minority of animals. Pulmonary lesions such as alveolar septal thickening by mononuclear cells, congestion, and effusion of edema fluid and fibrin into the lumina of alveoli may be related to terminal gram-negative sepsis and endotoxemia, which is common in fatal cases. Periacinar atrophy and congestion in the liver are attributable to anemia, hypovolemia, and shock, and prominent Kupffer cells probably reflect endotoxemia. Some studies have shown that viral inclusions may occur in tongue epithelium cells as well. Although these inclusions are nuclear, they often appear to be in the cytoplasm (pseudocytoplasmic). A case of erythema multiforme as a result of CPV-2 infection of keratinocytes has been described in a dog with concurrent parvoviral enteritis; viral inclusions were present in oral and skin epithelial cells.

The **diagnosis** of parvoviral enteritis in dogs follows the principles described for that of panleukopenia in cats. The disease must be differentiated from *Canine coronavirus* infection, which is very rarely fatal, and from canine intestinal hemorrhage syndrome, shock gut, intoxication with heavy metals or warfarin, infectious canine hepatitis, and other causes of hemorrhagic diathesis. Involution of gut-associated lymphoid tissue and cryptal necrosis caused by parvovirus must be differentiated from similar lesions occasionally seen in canine distemper.

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Canine minute virus

Canine minute virus (CMV, Minute virus of canines, *Canine parvovirus 1*) is most closely related to *Bovine parvovirus*, and is distinct from members of the feline panleukopenia parvovirus subgroup. Serologic prevalence in the USA is approximately 50% in adult dogs. The virus is capable of transplacental transmission to the fetus, and exposure of pregnant bitches was associated with fetal resorption, or birth of dead or weak pups. CMV causes enteric or respiratory symptoms in puppies less than 3 weeks of age. Large intranuclear viral inclusion bodies are evident in enterocytes. Interstitial pneumonia and myocarditis are variably present in naturally infected puppies, with death occurring sporadically.

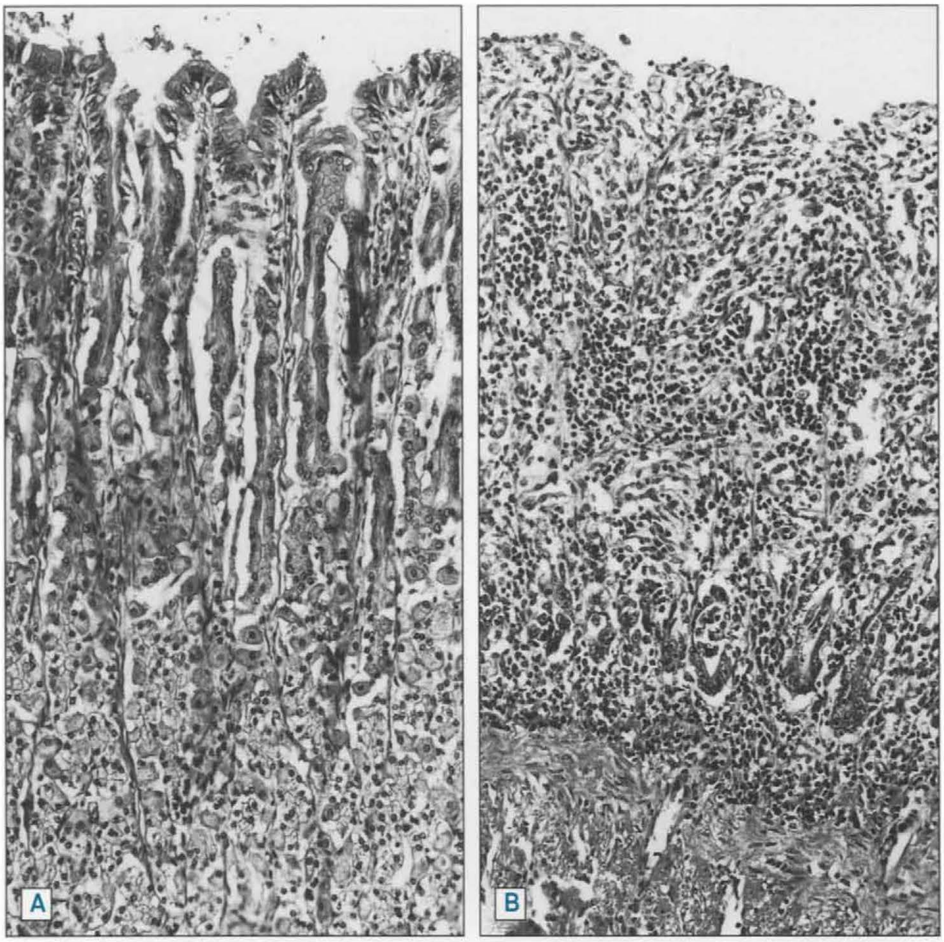

Figure 1.126 ***Canine parvovirus 2*** infection in a dog. **A.** Attenuation of epithelium lining isthmus and upper neck of fundic gastric glands. **B.** Loss of crypts of Lieberkühn and collapse of proprial stroma in small intestine. Remnants of crypt lining epithelium persist deep in lamina propria.

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Bovine parvovirus infection

The antigenically distinct *Bovine parvovirus* (BPV) has been recognized for many years and occurs widely in cattle populations on all continents. A single serotype is known. It has been isolated from the feces of normal and recently diarrheic calves as well as from conjunctiva, and from aborted fetuses. *The status of BPV as an enteric pathogen is unclear.* Virus shedding is not always associated with diarrhea, and it may be part of a mixed infection in diarrheic animals. Serological prevalence of antibodies to BPV is high, with 83% of cattle and 100% of herds being positive over 2 years of testing in one study. It is rarely diagnosed as a cause of death, and unless sought specifically by culture, direct electron microscopy, or molecular probe, would be missed as a cause of clinical diarrhea. Its significance may be greatest in neonatal calves and animals exposed while passive maternal antibody levels are waning, or in animals in the postweaning period.

The pathogenesis of infection with BPV resembles that in carnivores. Initial viral replication following oral inoculation is in tonsils and gut, with spread to systemic lymphoid tissues, resulting in transient lymphopenia. Viral antigen has been identified in the nuclei of epithelium in intestinal crypts and in cells in thymus, lymph nodes, adrenal glands, and heart muscle. Transient lymphocytolysis in infected tissues, and exfoliation of epithelium in crypts of the small and large intestine, with moderate villus atrophy, depletion of colonic goblets, and mixed inflammatory cell infiltration of the mucosa, have been seen experimentally. Intranuclear inclusions are present at the time when lesions are prevalent. Gross lesions other than abnormally fluid content in the gut are subtle, or absent. Intravenous inoculation of BPV into young calves causes severe watery diarrhea and prostration. Milder diarrhea occurs in calves infected orally. The severity of the disease may be potentiated by concurrent infection with other enteric pathogens, or other factors that may increase intestinal epithelial proliferation.

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Bacterial diseases of the alimentary tract

Virulence of bacterial pathogens

Evolutionary processes for bacterial survival, persistence, and proliferation are controlled by virulence genes and are subject to complex mechanisms of regulation of expression. Similarly, evolutionary processes for resisting the effects of bacteria on the host are determined by genetic factors and equally complex regulatory processes in the host. *Bacterial virulence can be resolved into five components*: (1) attachment; (2) colonization or entry into the host; (3) evasion of host defense; (4) multiplication and/or spread within the host, and damage to the host, by direct virulence attributes, or by stimulation of an immunoinflammatory response; and (5) transmission to other susceptible animals. The interplay between host and pathogen has been extensively studied for a number of important enteric bacterial pathogens, including *Salmonella* and *Escherichia coli*. Genes encoding a range of virulence characteristics in pathogenic bacteria, including adhesion factors, toxins, proteolytic enzymes, and other agents that promote tissue invasion, are often clustered in discrete regions of the genome known as *pathogenicity islands*. These appear to be sites of relative instability, and are thought to facilitate the horizontal transfer of virulence factors between bacteria, and their continued evolution. Similarities in the regulatory mechanisms for pathogenicity islands of important enteric pathogens, including *Salmonella*, *Shigella*, *Vibrio*, *Yersinia* and *E. coli*, are providing new insights into the reasons why strains of many genera of bacteria vary greatly in their host range and ability to cause disease.

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Escherichia coli

Escherichia coli has several virulence attributes that result in disease in animals. Principally, these promote **colonization or adhesion** to the mucosa; they cause **metabolic dysfunction or death of enterocytes**; they affect the **local or systemic vasculature**; or they promote **invasion and septicemia**. Disease syndromes caused by *E. coli* in domestic animals can be related to the combinations of virulence attributes expressed. Many terms and acronyms have been applied to the mechanisms of action of *E. coli*, with some becoming obsolete and others applying mainly to *E. coli* infections of laboratory animals and humans, rather than to domestic animals.

"**Enterotoxigenic**" *E. coli* (ETEC) cause secretory small-bowel diarrhea stimulated by enterotoxins produced by *E. coli* colonizing the mucosa of the small intestine. This condition is an important, common cause of diarrhea in neonatal animals of many species, and in postweaning pigs.

"**Enteropathogenic**" *E. coli* (EPEC) in humans may colonize the mucosa of the intestine by a mechanism involving adhesion-effacement ("**enteroadherent**" *E. coli* – EAEC, or "**attaching-effacing**" *E. coli* – AEEC). Some do not produce recognized toxins, but are associated with villus atrophy; they are an uncommon cause of disease in domestic animals.

Other strains of *E. coli*, many, but not all, of which are attaching-effacing, in addition secrete *cytotoxins* (Shiga toxins = verotoxins) that have an effect locally or systemically. Depending on the manifestation of this effect, such *E. coli* have been categorized as "**Shiga toxin-producing**" (STEC) = "**verotoxin-producing**" (VTEC), or "**enterohemorrhagic**" (EHEC). EHEC are a serious cause of foodborne illness in humans and have been incriminated as a cause of hemorrhagic enterocolitis in calves under a month of age.

Shigatoxigenic infections in swine, which are not attaching-effacing, are associated with some outbreaks of postweaning *E. coli* enteritis and also cause edema disease of weaned pigs, which is a systemic toxemia.

"**Enteroinvasive**" *E. coli* (EIEC) can be internalized by surface enterocytes and subsequently disseminate through the body to become septicemic. While EIEC are poorly documented in domestic animals, **septicemic colibacillosis** is a common manifestation of *E. coli* infection caused by strains adapted to avoid specific or innate systemic defense mechanisms, often in compromised hosts. The intestine is not necessarily the portal of entry and there may not be alimentary disease. The signs of *E. coli* septicemia are mainly referable to bacteremia, endotoxemia, and the effect of bacterial localization in a variety of tissue spaces throughout the body.

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Enterotoxigenic colibacillosis

Enterotoxigenic colibacillosis caused by enterotoxigenic *E. coli* (ETEC) is one of the major forms of diarrhea in neonatal pigs, calves, and lambs, as well as in humans.

Two major attributes confer virulence upon these strains of *E. coli*. These are the ability to colonize the intestine, and the capacity to produce toxins that stimulate secretion of electrolyte and water by the intestinal mucosa. *Colonization and enterotoxin production must occur together for disease to ensue*. The diarrhea produced by ETEC is accompanied by relatively minor microscopic evidence of inflammation, and by little or no architectural change in the mucosa. As a result, overt enteritis is usually not evident at autopsy, and the disease is part of the syndrome of undifferentiated diarrhea of neonatal animals.

Intestinal colonization results from the adhesion of *E. coli* to the surface of enterocytes on villi in the small intestine, and proliferation there (Fig. 1.127A). By adhering to the mucosa, bacteria are able to resist the normal peristaltic clearance mechanisms. Large numbers of organisms, of the order of over 10^7 per gram of mucosa, or 20–30 per enterocyte, cover the surface of villi. The ability to attach to enterocytes is conferred upon ETEC by pili, and may be enhanced by the presence of a capsule.

Fimbriae, or **pili** (also known as **colonization factor antigens – CFA**, with specific names in transition to a system of "F" numbers) are rod-like or filamentous projections from the cell wall of *E. coli* that attach to specific glycoconjugate receptors on the surface of enterocytes (Fig. 1.127B). They are distinct from type 1 fimbriae, which do not promote colonization of the gut. Fimbriae are polymers of protein (pilin) subunits, which are coded by plasmid (F4 (K88), F5 (K99), F18; some F6 (987P)) or chromosomal (F6 (987P), F17, F41) DNA. They are antigenically distinct, permitting recognition by specific antibody.

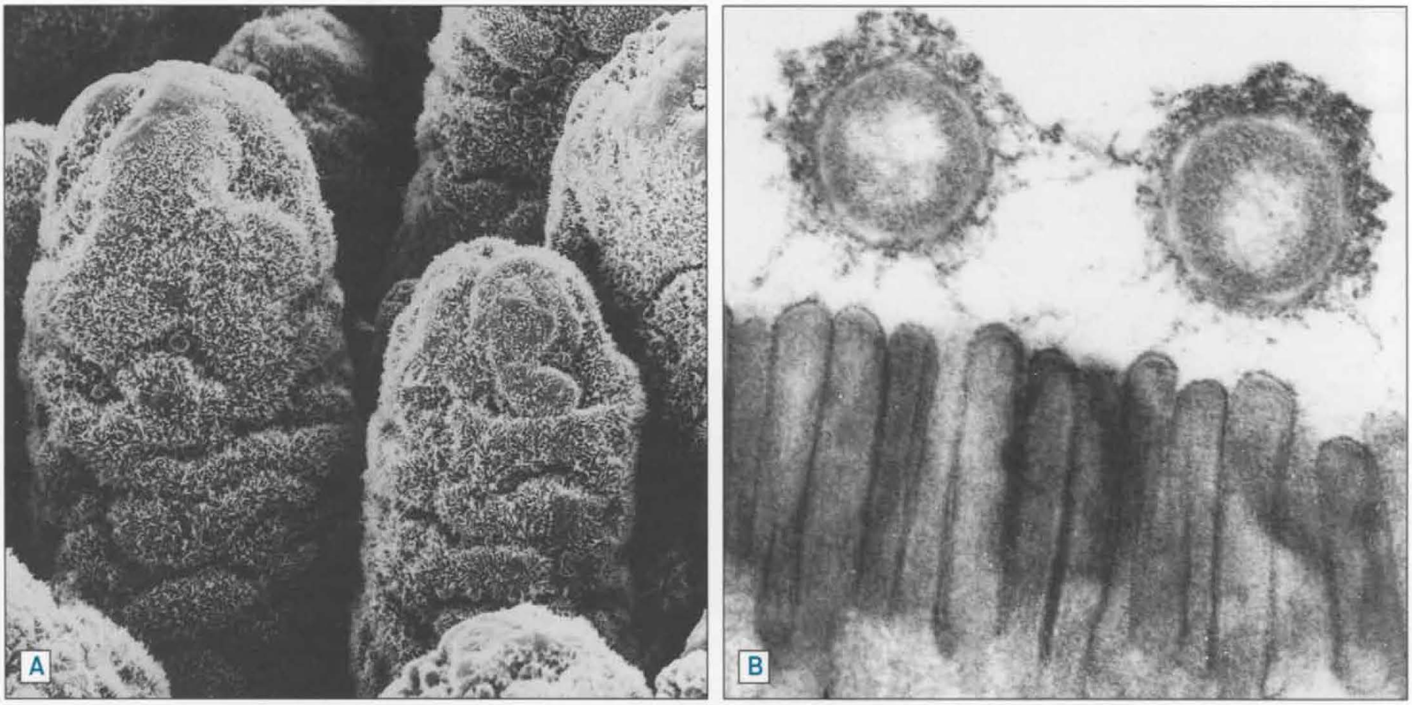
Fimbrial adhesins include **F5** (K99) and **F41** in strains affecting calves, lambs, and pigs; **F42, F165, F17, F18** in calves and pigs; and **F4** (K88), **F6** (987P), **F18** in pigs. Combinations of adhesins may be expressed by the same strain of ETEC; typically, F41 is expressed by strains also expressing F5, and seems to be of minor importance. Bacteria possessing F4 colonize the entire small bowel, whereas those with F5, F6, and F41 mainly adhere in the jejunum and ileum.

Susceptibility to bacterial fimbrial adhesins, especially F5 and F6, appears to be somewhat age-related; the ability of fimbria-bearing *E. coli* to colonize the small intestine is greatest in animals only a few days old. F5 receptors on enterocytes decline in availability with age, while receptors for F6 are shed into the lumen in older pigs, facilitating clearance of bacteria from the mucosa, and interfering with colonization. F18 receptors are not found in neonatal pigs, but are produced with increasing age to weaning. Stimulation of maternal immunity to fimbrial antigens causes secretion of lactogenic antibody, which combines with adhesins in the gut lumen, preventing colonization of the gut of suckling animals.

A nonfimbrial plasmid-encoded *adhesin involved in diffuse adherence* (**AIDA**) of *E. coli* to enterocytes in humans also occurs in strains from pigs associated with edema disease and postweaning diarrhea, often in combination with F18.

Enterotoxigenic strains of *E. coli* produce two classes of plasmid-encoded proteins – heat-labile toxin (LT) and heat-stable toxin (ST) – which act locally in the intestine to alter secretion and absorption of electrolyte and water by enterocytes.

Heat-labile toxin is a large immunogenic plasmid-encoded molecule, with two subgroups (LTI and LTII) and comprised of a small A subunit with two fragments (A1 and A2), which links A1 to a large pentamer of five B subunits. LTI is antigenically similar to cholera toxin, while LTII toxins have B subunits that differ from LTI. The B subunits bind to ganglioside receptors on the enterocyte surface; the toxin complex then dissociates, and the A1 subunit is internalized into the cell. It operates via an adenylate cyclase pathway to cause chloride secretion by enterocytes, sodium, and water following osmotically from the mucosa. Co-transport of sodium chloride by enterocytes, and associated water uptake, is probably also shut down at the same time. LT may also promote mucosal secretion by stimulation of local prostaglandin production, the enteric nervous system, and cytokine activation. LT has a latent period prior to the development of secretion, but the effects on the cell are irreversible.



Heat-stable toxin is classified as STa and STb based on biological properties, and is plasmid-encoded. STa causes an increase in cyclic guanosine monophosphate, which inhibits Na/Cl co-transport and therefore water absorption by surface enterocytes, while in crypt epithelium it promotes Cl^- and water secretion. STb, mainly produced by ETEC associated with pigs, may cause secretion by stimulation of prostaglandin E_2 and 5-hydroxytryptamine production. In pigs, STb can cause exfoliation of surface enterocytes, resulting in mild atrophy of villi.

Enterotoxigenic colibacillosis is among the commonest causes of diarrhea in **neonatal pigs**, from a few hours to about a week of age. Commonly, serogroups O8, O45, O138, O141, O147, O149, and O157, expressing F4, are involved in enterotoxigenic colibacillosis in piglets, though the prevalence of F4-bearing strains may be declining due to vaccination of sows. Less commonly, F5, F6, and F41 pilus adhesins are involved. STb is the most common toxin produced by porcine ETEC; when LT is found, it is in association with STb, which may be encoded on the same plasmid. STa also occurs in strains of ETEC in swine, alone, or in combination with other enterotoxins.

At necropsy, enterotoxigenic colibacillosis cannot be readily separated from the other common causes of undifferentiated neonatal diarrhea without laboratory assistance. Generally there is dehydration, usually with evidence of diarrhea, or a history of its occurrence in the herd. Other than the presence of characteristic fluid content in the flaccid small and large bowel, usually with clotted milk still in the stomach, the internal findings are unremarkable.

In contrast to the viruses and *Isospora*, *ETEC usually does not cause significant villus atrophy* (Fig. 1.128A). Small clumps, or a continuous layer, of bacteria may be found on the surface of enterocytes on villi in mucosal tissue sections, most consistently in ileum (Fig. 1.128B). Some neutrophils may be present in the proprial core of villi, and transmigrating the epithelium into the lumen.

The involvement of ETEC expressing F4 in postweaning diarrhea of pigs over 3 weeks of age, and distinct from postweaning colibacillosis caused by VTEC, discussed below, may be related to colonization of intestine in weaned pigs in which *Rotavirus* infection, changes in diet, or villus atrophy associated with hypersensitivity to dietary protein constituents, provide adhesin-bearing *E. coli* with a competitive advantage. It causes diarrhea for up to a week or so, with ill-thrift, but is uncommonly fatal, though a syndrome probably caused by endotoxic shock, similar to that associated with shigatoxigenic stains associated with postweaning colibacillosis, described below, may occur.

In **calves**, many cases of undifferentiated neonatal diarrhea are accounted for by enterotoxigenic colibacillosis, usually involving strains of serogroups O8, O9, O20, O64, O101 with fimbrial adhesins F5 and F41, and producing STa. Infection is typically restricted to the first 2–3 days of life, probably due to the loss of receptors for F5 in older calves.

ETEC must be differentiated from the other major causes of undifferentiated diarrhea in neonatal calves – *Bovine coronavirus* (BCoV), *Coronavirus*, *Rotavirus*, and *Cryptosporidium*, which typically dominate in calves older than a few days of age. However, ETEC is not uncommonly found in combination with BCoV or *Rotavirus* infection.

The gross findings in calves with enterotoxigenic colibacillosis are the nonspecific appearance of diarrhea and dehydration. The infection is differentiated in tissue sections from the other infectious causes of this syndrome by the *absence of severe villus atrophy* (Fig. 1.129) and by the presence of *bacteria on the surfaces of villi* in the distal small intestine. As in piglets, application of a variety of presumptive or specific tests for the presence of ETEC in the intestine confirms the diagnosis (Fig. 1.130).

Enterotoxigenic colibacillosis is a significant problem in **lambs** in some areas. The serotypes involved, pathogenesis, and diagnosis of the condition are similar to those in calves. Synergism with *Rotavirus* infection may occur.

There are several reports of ETEC isolated from **foals** with diarrhea. The organisms have pili, probably F41, and secrete LT or STa. However, their capacity to produce disease in foals is unproven. Diarrhea has not ensued in foals inoculated with F4-bearing *E. coli*,

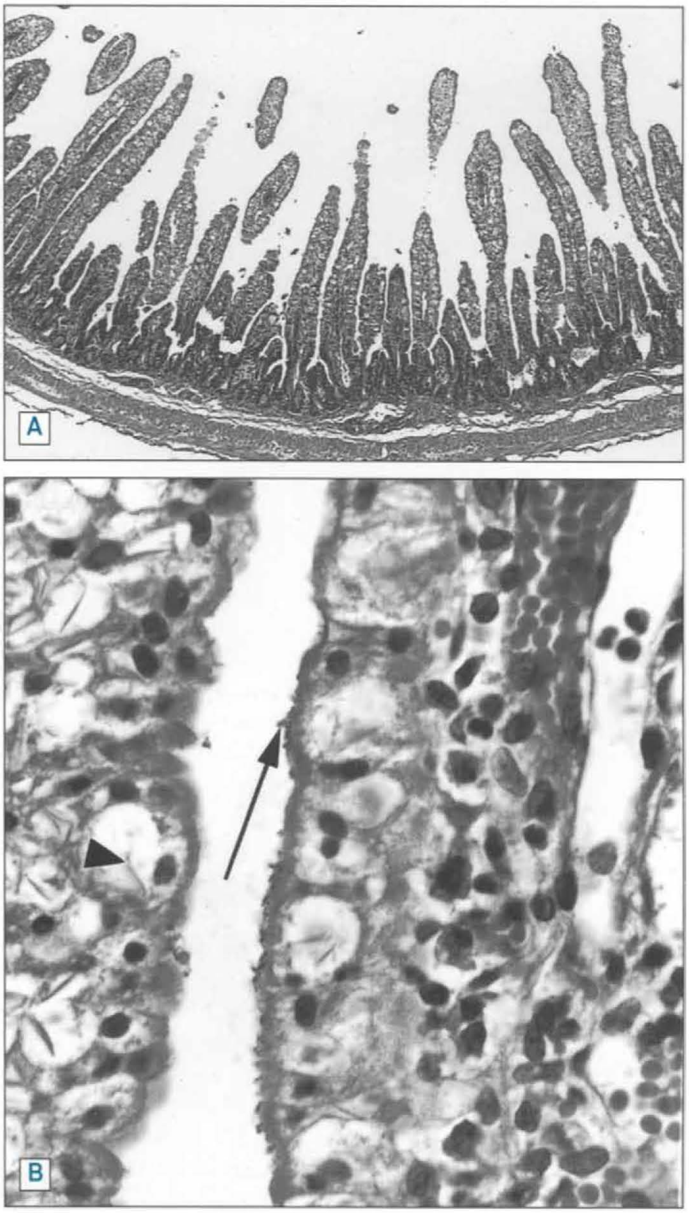


Figure 1.128 Enterotoxic colibacillosis in a piglet. **A.** Villi are tall and crypts are short, as is expected in a 2-3-day-old animal. **B.** Bacteria are present on surface of enterocytes (arrow). Cytoplasmic vacuoles containing eosinophilic spicules (arrowhead) are normal in the ileal mucosa of young piglets.

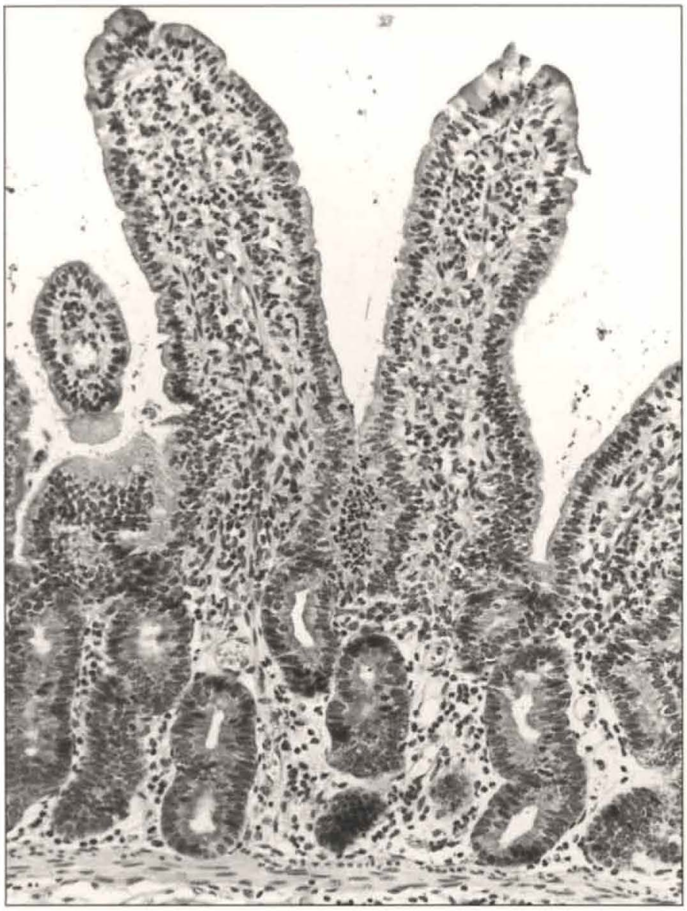


Figure 1.129 Enterotoxic colibacillosis in a calf. Mild neutrophil infiltrate in lamina propria and between base of villi. Atrophy of villi not evident, surface epithelium normal. (Courtesy of JJ Hadad, CL Gyles.)

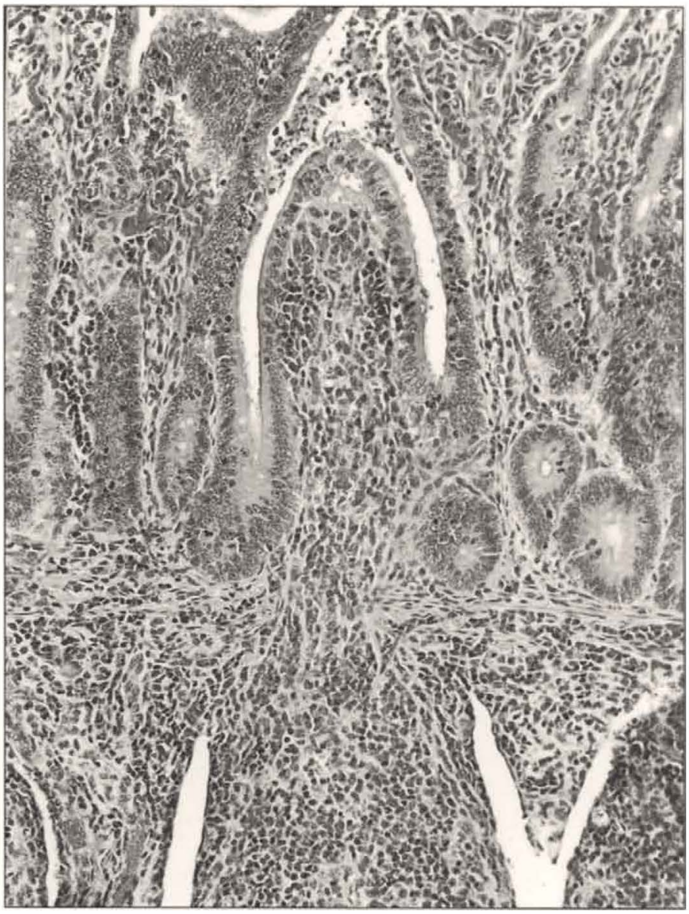


Figure 1.130 Enterotoxic colibacillosis in a calf. Neutrophil effusion into lumen over dome of Peyer's patch. (Courtesy of J Bellamy.)

despite the presence of F4 receptors on enterocytes, and it seems that ETEC has little significance in this species.

Strains of *E. coli* have been associated with diarrhea in neonates of **other species** of animals, especially young dogs, where they mainly produce STa. Generally the enterotoxigenicity and other attributes of virulence have not been well described in strains from other species.

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Enteropathogenic colibacillosis

Enteropathogenic *E. coli* (EPEC) are those that cause direct damage to the mucosa, through a *characteristic mechanism of attachment to, and effacement of, epithelium.* These **attaching-effacing *E. coli*** (AEEC) are more common in humans than in animals, where they are most important in pigs, dogs, and rabbits, though they have also been isolated from cats. Control of attaching-effacing activity resides in the **locus for enterocyte effacement** (LEE), a chromosomal pathogenicity island. EPEC have a complicated and sequential relationship with host cells. Long polar fimbriae may mediate initial bacterial interaction with the enterocyte. Secretion of bacterial proteins ensues, including **intimin**, which is an adhesin. A second protein, the **translocated intimin receptor**, is transported via a type III secretion system into the enterocyte cytoplasm, emerging on the cell membrane as the intimin receptor. In response to translocated EPEC proteins, the cell's cytoskeleton is reorganized, resulting in formation of cupped *pedestal-like structures* beneath the attached bacteria, and the subsequent loss of microvilli (Figs 1.119, 1.120, 1.131, and 1.132). Paracellular permeability increases as tight junctions between enterocytes loosen, and neutrophils migrate between cells into the lumen.

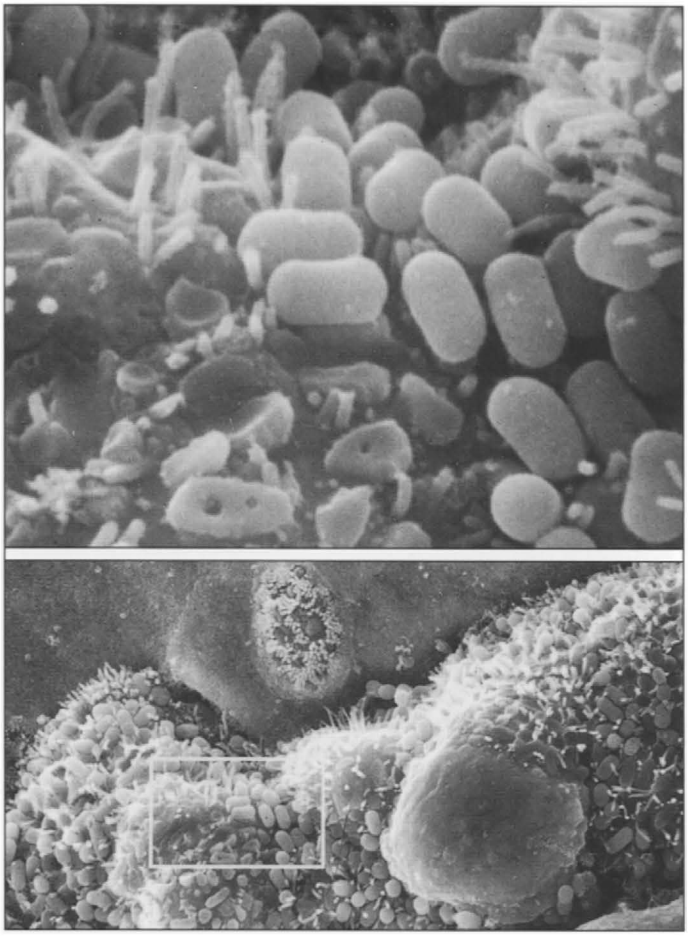

Figure 1.131 Scanning electron micrograph of the colon of a calf with **enterohemorrhagic *Escherichia coli* infection**. Lower: note irregularity of microvilli on cells infected by attaching-effacing *E. coli*, in comparison with microvilli on uninfected cells in background. Outline indicates field illustrated in upper photo. Adherent bacteria are on pedestals projecting from the surface of enterocytes. Occasional bacteria have been lost artifactually, exposing underlying "mushroom-like" pedestals. (Courtesy of M Schoonderwoerd, R Clarke.)

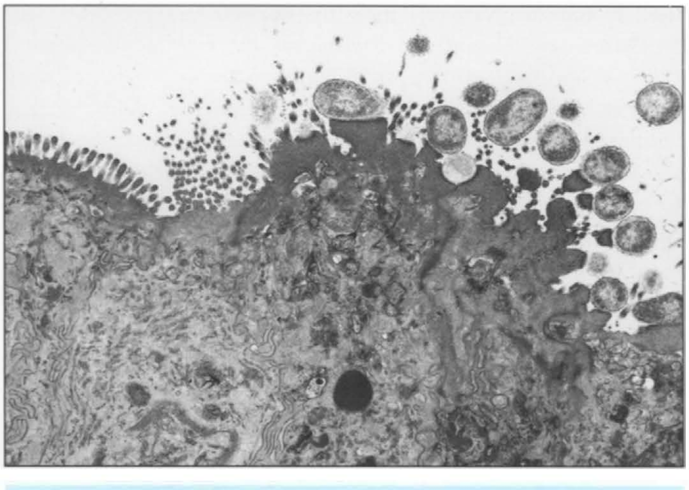

Figure 1.132 Transmission electron micrograph of enterocytes in the colon of a calf infected with enterohemorrhagic *E. coli*. **Enterocyte attaching-effacing *E. coli*** are on pedestals projecting from surface of infected cells. Microvilli are irregular and effaced on infected cells. Normal cell (left). (Reprinted with permission from Schoonderwoerd M, et al. Can J Vet Res 1988;52:484–487.)

In animals and humans, some strains of AEEC are pathogenic despite failure to secrete enterotoxins or cytotoxins. A heavy layer of plump coccobacilli may be found over the luminal aspect of enterocytes on villi throughout the small intestine, and on the surface of the large intestine. *The degree of diarrhea seems related to the extent of bacterial colonization, which is most consistent in lower small intestine and large bowel.* Enterocytes to which bacteria are adherent round up or contract, and exfoliate from the mucosa singly or in clumps, resulting in mild to severe atrophy of villi in the small bowel, and attenuation of surface cells, or microerosions, in the large intestine. Fusion of villi may occur in small intestine, and goblet-cell numbers are depleted in both large and small bowel. There is moderate mucosal congestion, and local infiltration by neutrophils.

Diarrhea is presumably related to maldigestion and malabsorption of nutrients and electrolytes in small intestine, perhaps with the additive effect of increased mucosal permeability, overloading the colon, the absorptive ability of which is also compromised by damage to surface cells. *Microscopic diagnosis is based on recognition of bacteria on the mucosal surface.*

In **pigs**, EPEC belonging to serogroups O45 and O103, infecting small and large intestine, are responsible for some cases of postweaning diarrhea. In **dogs**, and occasionally in cats, EPEC have been associated with diarrhea, often as a component of co-infections with viral or protozoal agents. Microscopic lesions characteristic of AEEC are typically found in the jejunum and ileum, less commonly in the colon, and, in dogs, sometimes in the stomach.

A distinct subset of EPEC is the **Shiga toxin-producing *E. coli*** (STEC), also known as **enterohemorrhagic *E. coli*** (EHEC). In addition to their ability to attach and efface, these strains produce cytotoxic Shiga toxins (Stx1, and its homologue Stx2 with its variants, c, d, e, f). Shigatoxin 1 is structurally identical to the Shiga toxin produced by *Shigella dysenteriae,* which has a profound cytopathic effect. Due to their effect on Vero cells in culture, these *E. coli* are also referred to as **verotoxin-producing *E. coli*** (VTEC). Shiga toxins, encoded in the genome of bacteriophages, are composed of an A subunit that has enzymatic activity and a B subunit that binds the toxin to the glycolipid receptor globotriaosylceramide (Gb3) on the cell surface. Once endocytosed and transferred via the Golgi apparatus to the rough endoplasmic reticulum, the toxin inhibits protein synthesis, which may be lethal to the target cell, and via separate mechanisms may induce apoptosis. The presence or absence of Gb3 on cell surfaces is a major determinant of the distribution of tissue susceptibility to Shiga toxins, which mainly affect intestinal epithelium and vascular endothelium. Some EHEC also produce hemolysins, which may assist survival in the gut by increasing iron availability. Acid tolerance may also promote colonization efficiency by enhancing survival in the stomach.

The EHEC strains produce disease predominantly in humans, although involvement of domestic animals has been highlighted in the public health arena due to the tendency for cattle and some other species to carry the organism asymptomatically, adherent to epithelium over lymphoid follicles in the rectal mucosa. The most widely recognized EHEC serotype is **O157:H7**, a major pathogen in humans, although over 200 other STEC serotypes have been identified. In addition to Shiga toxin production, virulence is attributable to attaching and effacing capability, encoded on the LEE.

In **calves under 4 weeks of age** (generally over 3 days of age, and most commonly in the second week of life), strains of EHEC (O5:NM, O8:H9, O26:H11, O103:H2, O111:NM, O111:H8, and O111:H11) have been associated with a syndrome of *erosive fibrinohemorrhagic enterocolitis*, with the development of dysentery. Fever is not characteristic, and animals may remain bright until the effects of dehydration and blood loss supervene. Death may occur within several days of onset of illness, but some cases will recover in 7–10 days.

At necropsy, gross lesions are usually confined to the spiral colon and rectum, though the ileum and cecum are occasionally involved with mild fibrinous or fibrinohemorrhagic enteritis/typhlitis. In the colon, changes vary from mild patchy congestion of the mucosa to marked mucosal reddening, with adherent mucus, necrotic debris, and blood; colonic contents are fluid and frequently blood-tinged (Fig. 1.133). There may be congestion of the margins of mucosal folds in the rectum, or overt fibrinohemorrhagic proctitis. Mesenteric lymph nodes are often enlarged, especially along the ileum, and occasionally there are lesions (arthritis, serositis) suggesting septicemia.

Microscopically, *in affected small intestine the profile of villi is ragged or markedly scalloped, and they are blunted, moderately atrophic, or fused.* Epithelial cells on villi in small bowel, and on the colonic surface, where lesions are most severe, are short, rounded up, and in some cases exfoliating singly or in small clumps, causing focal microerosions. Cells in some areas may be markedly attenuated. The microvillus border is indistinct, and covered by a heavy layer of prominent gram-negative coccobacilli (Fig. 1.134). Lesions in large bowel may extend down into glands, which may be dilated, lined by flattened epithelium, and filled with sloughed epithelium and leukocytes. In the small intestine, foci of bacterial adhesion may be patchy, on the sides of the upper third of villi, with extensive surrounding areas of normal epithelium. Crypts in areas of atrophic small intestine may be elongate, with numerous mitotic figures. In severely affected bowel, the mucosa and submucosa are congested, edematous, and occasional microvascular thrombi may be present. Sloughed enterocytes, erythrocytes, neutrophils, fibrin, and bacteria are in the lumen.

In **dogs**, STEC have been associated with dysentery, and in some dogs, hemolytic uremic syndrome and cutaneous edema and ulceration. In Greyhounds, the syndrome involving this triad has been termed *cutaneous and renal glomerular vasculopathy*, and has been attributed to consumption of beef contaminated with O157:H7 *E. coli*, and other STEC. Renal and cutaneous lesions are attributable to vascular damage caused by Shiga toxin (see Vol. 2, Urinary system; Vol. 1, Skin and appendages).

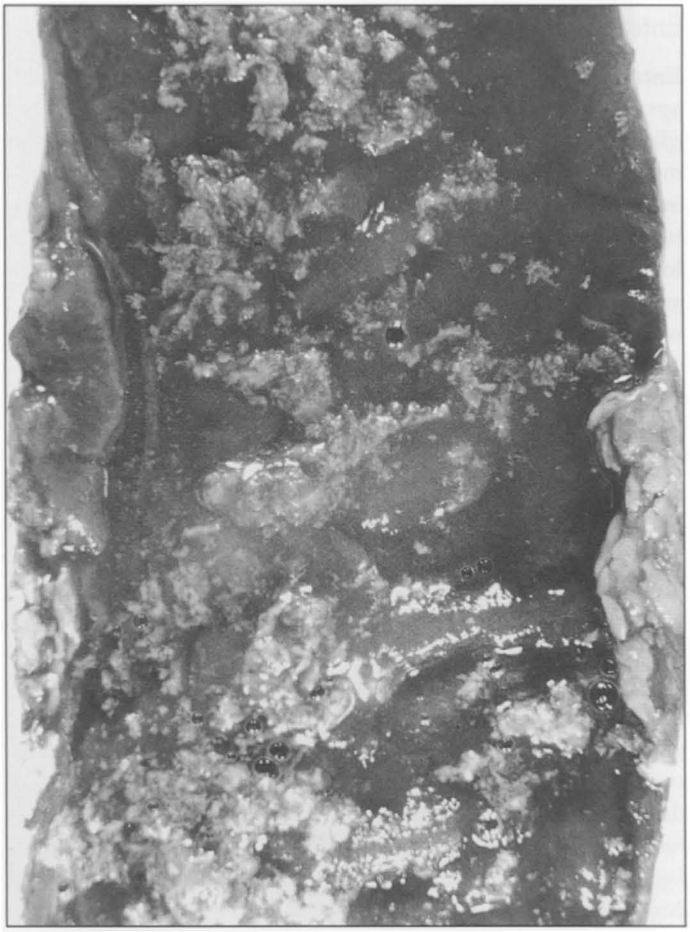

Figure 1.133 Fibrinohemorrhagic enteritis in the ileum of a calf with **enterohemorrhagic *E. coli* infection**. (Courtesy of M Schoonderwoerd, R Clarke.)

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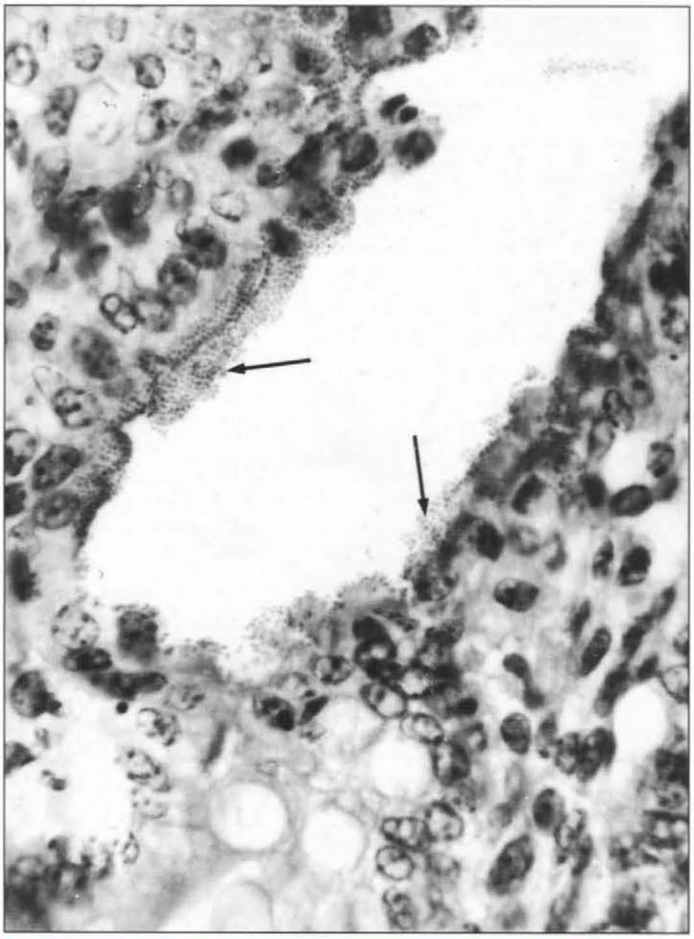

Figure 1.134 Adherent bacteria (arrows) on surface of enterocytes in the colon of a calf with **enterohemorrhagic *E. coli* infection**. (Courtesy of M Schoonderwoerd, R Clarke.)

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Edema disease and postweaning *E. coli* enteritis

Edema disease *is a distinct syndrome in pigs, characterized by sudden death, or the development of nervous signs, associated with enteric colonization by STEC*, especially serotypes O138, O139, and O141. The disease occurs most commonly in pigs within a few weeks after weaning, or after other change in feeding or management. It often occurs in association with outbreaks of postweaning *E. coli* enteritis. Rare reports exist of edema disease in suckling and mature animals. The disease may be sporadic or occur as an outbreak, usually affecting the best animals in a group, and mortality often approaches 100% of affected animals. Edema disease and postweaning *E. coli* enteritis have apparently declined in prevalence in parts of North America, perhaps with the use of concentrate rations based largely on soybeans and corn, rather than other grains.

Bacterial colonization of the gut is mediated by F18ab fimbriae. Susceptibility of pigs is genetic and related to the presence of receptors for the fimbriae. A Shiga toxin (Stx2e) producing vascular injury and edema has been incriminated in the pathogenesis of edema disease, and vaccination with Stx2e toxoid almost entirely prevents edema disease.

Some strains of *E. coli* that cause edema disease also produce secretory enterotoxin. Diarrhea is not a usual concomitant of edema disease in individual animals. Significant gross or microscopic lesions in the intestinal mucosa do not occur in edema disease, which appears to be a *classical enterotoxemia*, the active principle being absorbed from the gut and acting at a distant site. However, the means by which the toxin enters the circulation is unknown.

Experimentally, the target of Stx2e, like other Shiga toxins, is *vascular endothelium*, particularly of small arteries and arterioles. Preferentially affected organs include spinal cord, cerebellum, eyelid, and colon. However, a study to determine preferential binding

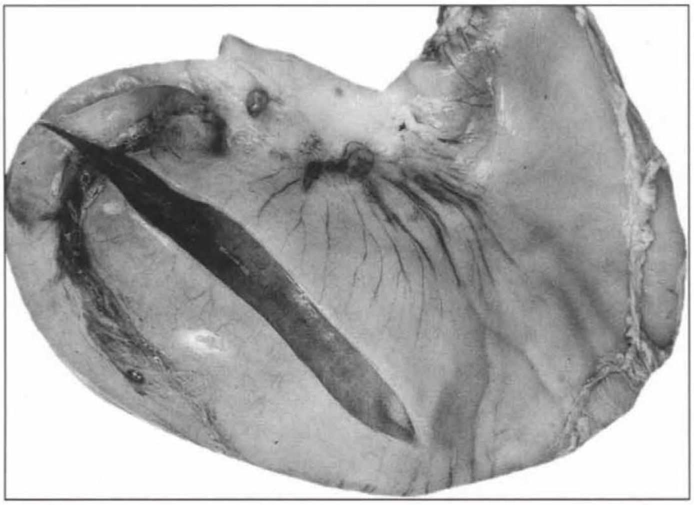

Figure 1.135 Edema of stomach wall in **edema disease** in a pig.

sites for Stx2e found receptors on a variety of tissues, not just the aforementioned. Stx2e causes *angiopathy*, which, in its early stages in experimental intoxication, is recognized by swelling of endothelial cells and mild intramural and perivascular hemorrhage. Pyknosis and karyorrhexis of smooth-muscle nuclei, often accompanied by fibrinoid degeneration or hyaline change in the tunica media, may be seen in subacute spontaneous cases. Proliferative mesenchymal elements are found in the tunica media and tunica adventitia in more advanced cases. However, inflammation is not at any stage a prominent component of the angiopathy, nor of the associated edema in most sites, and thrombosis of vessels is rarely encountered. Edema is probably due to vessel damage during the early stages of the angiopathy. The lesions are distinct from those expected with endotoxemia.

Swine with **edema disease** may die without premonitory signs. Others may have anorexia, or, more characteristically, show nervous signs, usually of less than a day's duration. An unsteady staggering gait, knuckling, ataxia, prostration and tremors, convulsions, and paddling occur. A hoarse squeal, the hoarseness attributed to laryngeal edema and dyspnea, may also be noted clinically.

At necropsy, **gross lesions** in acute deaths may be subtle or absent. Typically, *edema is variably present in one or more sites*. However, it may be mild and must be carefully sought, especially by "slipping" the suspected area over subjacent tissue. Subcutaneous edema may be present in the frontal area and over the snout, in the eyelids, and in the submandibular, ventral abdominal, and inguinal areas. Internally, there may be some hydropericardium, and serous pleural and peritoneal effusion, perhaps accompanied by mild or moderate pulmonary edema. More commonly, the serous surfaces merely appear glistening and wet. Edema of the mesocolon, of the submucosa of the cardiac glandular area of the stomach over the greater curvature, and of mesenteric lymph nodes is most consistently found. The gastric submucosal edema should be sought by carefully cutting through the muscularis to the submucosa. The edema fluid is clear, and slightly gelatinous (Fig. 1.135). It is rarely blood-tinged, and overt hemorrhage is usually not present in uncomplicated edema disease. The stomach is often full of feed, but the small intestine is relatively empty and the mucosa is grossly normal. The colon may contain somewhat inspissated feces.

In swine dying after a more prolonged clinical course, gross edema is often not present, though enlargement of mesenteric lymph nodes is present in a large proportion of cases. A few pigs may show foci of yellow malacia, usually bilaterally symmetrical, in the brainstem at various levels from basal ganglia to medulla.

Microscopically, edema in the sites of predilection mentioned above is the main lesion in swine dying acutely. It is generally devoid of much protein and contains few erythrocytes and inflammatory cells. A proportion of animals will also have meningeal edema and distended Virchow–Robin spaces in the brain. Vascular lesions may not be well developed in pigs dying suddenly. When present they usually consist of edema, hemorrhage, myocyte necrosis, and hyaline degeneration in the tunica media. Angiopathy is more consistently found in cases of longer standing. Affected vessels may be found in any tissue in the carcass. Brain edema and focal encephalomalacia in the brainstem are associated with the presence of lesions in cerebral vessels; necrosis may be a sequel to edema and ischemia. "Cerebrospinal angiopathy of swine" is probably a manifestation of edema disease.

A **diagnosis** of edema disease is based on nervous signs or sudden death in growing pigs, in association with typical gross and microscopic lesions, when they are present. In acute cases, heavy growth of hemolytic *E. coli* of one of the serotypes known to produce Stx2e is essential.

Edema disease must be differentiated from enteritis and endotoxemia due to *E. coli* in postweaning pigs; from mulberry heart disease in animals dying suddenly; and from salt poisoning, *Salmonella* meningoencephalitis, and other infectious encephalitides, in animals with nervous signs.

Postweaning *E. coli* enteritis (coliform enteritis of weaned pigs) typically occurs during the first week or two following weaning, or after some other change in feed or management. Postweaning diarrhea may be caused by classical enterotoxigenic F4 (K88) *E. coli*, but it is often associated with hemolytic *E. coli* of the same serotypes primarily implicated in edema disease, as well as serotype O149. The two diseases often occur in the same population of pigs, though usually affecting different animals. Typically, postweaning colibacillosis is a disease of high morbidity and variable mortality, with loss of condition in pigs suffering prolonged illness. Diarrhea is usually yellow and fluid, and stains the perineum. Deaths that occur may or may not follow a prior episode of diarrhea, and often appear to be related to endotoxemia.

In fatal cases, there may be blue-red discoloration of the skin and evidence of dehydration. Deep red *gastric venous infarcts* are present in almost all cases (Fig. 1.136). The small intestine is flaccid. The mucosa may be normal in color and the content creamy. In other animals the mucosa of the distal small intestine will be congested and the contents watery and perhaps blood-tinged or brown with flecks of yellow mucus or fibrin (Fig. 1.137). Cecal and colonic lesions are usually mild, but there may be some congestion and fibrinous exudate in the proximal large bowel. Mesenteric lymph nodes may be somewhat enlarged, congested, and juicy. Other organs are usually unremarkable grossly.

The **pathogenesis** of postweaning *E. coli* enteritis due to non-F4 *E. coli* is poorly understood, and the microscopic pathology is not well described. In swine with diarrhea, *E. coli* may be attached to the surface of villi by F18ac fimbriae. Atrophy of villi does not seem to be evident, and diarrhea is presumed to be mediated by enterotoxins. Mortality in animals with prolonged diarrhea and few gross intestinal

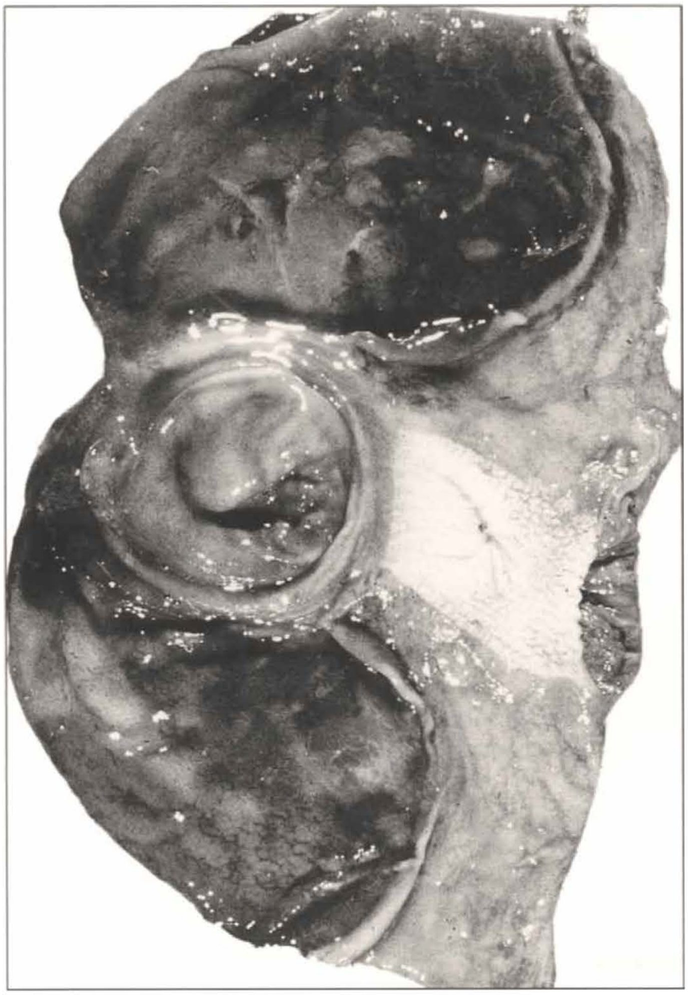

Figure 1.136 Deep red areas of venous infarction in the gastric mucosa in **postweaning colibacillosis** in a pig.

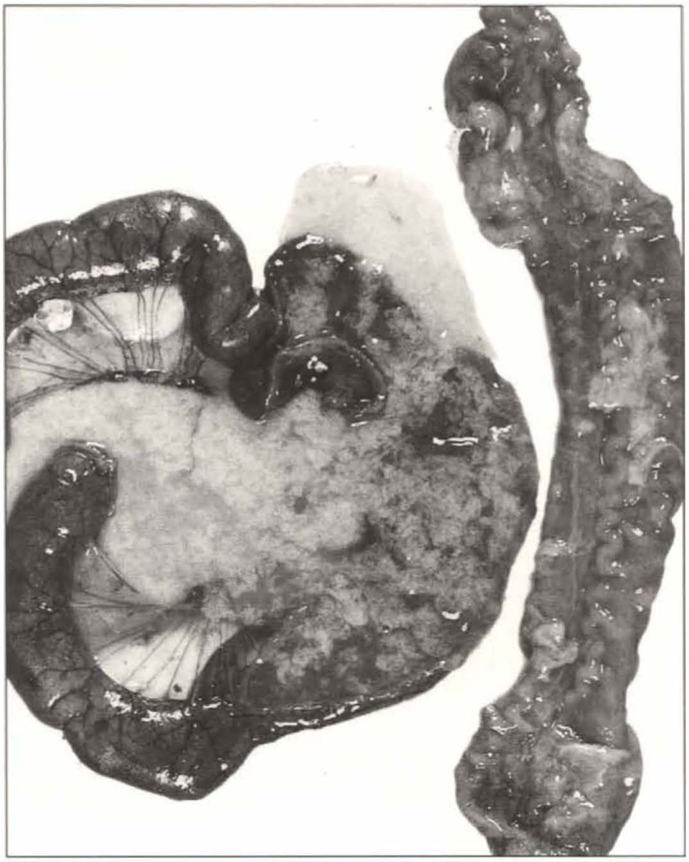

Figure 1.137 Acute catarrhal enteritis, with congested, flaccid small intestine in **postweaning colibacillosis** in a pig.

or extraintestinal lesions may be ascribed to dehydration. In animals dying of more acute disease, there is local microvascular thrombosis in sections of congested mucosa (Fig. 1.138), and the gross and microscopic lesions in other organs, especially those related to gastric mucosal and submucosal thrombosis and venous infarction, are suggestive of endotoxemia. Hemolytic *E. coli* of the implicated strains are consistently isolated in virtually pure culture from the lower small intestine and colon. However, they are present in the spleen and liver in only a few cases, suggesting terminal bacteremia.

The factors predisposing to the massive colonization of hemolytic *E. coli* are unclear. Loss of lactogenic immunity, a favorable environment for proliferation of bacterial strains with specific nutrient requirements, and promotion of epithelial colonization by the effects of antecedent *Rotavirus* infection, have been variously implicated.

A **diagnosis** of postweaning colibacillosis is suggested by the gross lesions in animals dying acutely or subacutely, and it is confirmed by culture and serotyping of associated strains of *E. coli*. The fatal disease must be differentiated from edema disease, proliferative hemorrhagic enteropathy, salmonellosis, and swine dysentery. Postweaning diarrhea due to uncomplicated *Rotavirus* infection, transmissible gastroenteritis, or associated with attaching-effacing O45:K "E65" *E. coli*, is usually nonfatal.

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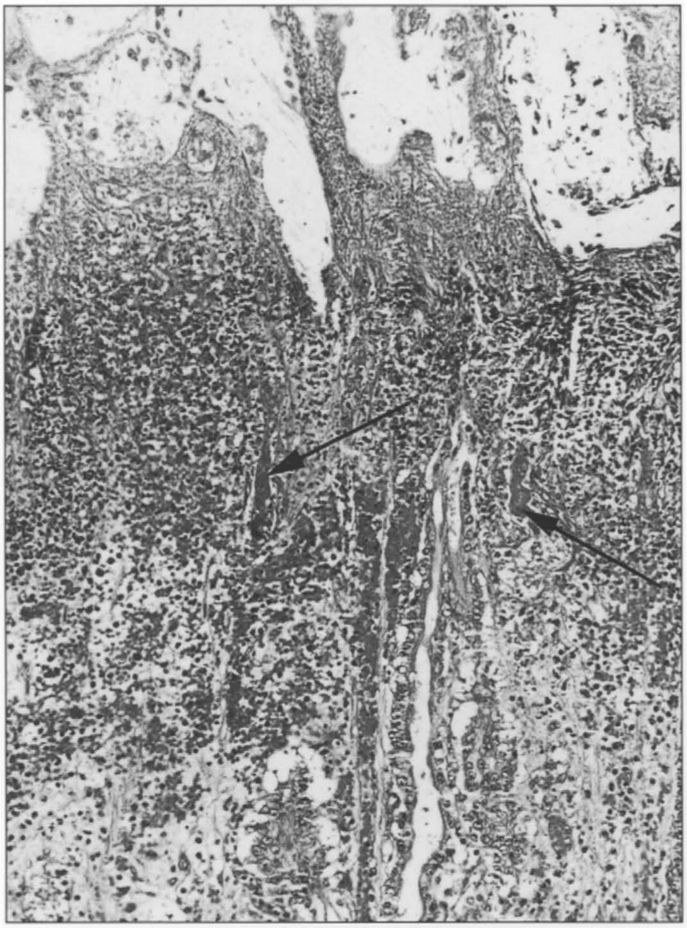

Figure 1.138 Thrombosis of venules (arrows) and necrosis of the superficial gastric mucosa in venous infarction in **postweaning colibacillosis** in a pig.

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Enteroinvasive *E. coli*

Strains of *E. coli* are recognized, infecting humans and certain other species, which have the capacity to invade or to be internalized by surface enterocytes of the small and large intestine, in which they multiply. In this sense they resemble *Shigella* in primates, and *Salmonella*. The enteroinvasiveness of *Shigella* and some strains of *E. coli* appears to be correlated with the presence of a high-molecular-weight plasmid coding for outer-membrane proteins involved in invasion. Multiplication of the organism within epithelial cells results in *local erosion and ulceration*, associated with acute inflammation in the mucosa.

Among domestic animals, *enteroinvasive colibacillosis has only been confirmed experimentally in neonatal swine*, using a strain of O101 *E. coli*. Spontaneous enteritis that appears to be due to enteroinvasive *E. coli* is rarely encountered in piglets up to weaning and in calves under 2 weeks of age. Diarrhea in experimentally infected piglets is described as gray-yellow, watery, and containing small clots. The gross findings may not be remarkable, or the intestine may appear congested in comparison with that in most diarrheic piglets. In spontaneous cases suspected of being due to enteroinvasive *E. coli*, the gastric fundus may also be congested, and this correlates with the presence of venous infarction visible microscopically. Experimental enteroinvasive colibacillosis in piglets causes villus atrophy that is comparable in severity to that induced by the common viruses of neonates. Enterocytes appear cuboidal or flattened and some are seen lysing. The lamina propria is edematous; capillaries are congested and infiltrated by neutrophils and other inflammatory cells. In spontaneous cases, thrombi may be evident in proprial capillaries and submucosal lymphatics. Neutrophils and tissue fluid effuse into the lumen between villi through epithelial discontinuities. Similar microthrombosis, proprial inflammation, enterocyte destruction, and effusion may be found in the cecum and colon. Intracellular organisms of O serogroup 101 were demonstrated by immunoperoxidase staining in the experimental study, but are not generally recognized in spontaneous cases suspected to be due to enteroinvasive *E. coli*. Edema and neutrophil accumulation in sinusoids of mesenteric lymph nodes are present. Experimental enteroinvasive colibacillosis in piglets has been associated with malabsorption and protein loss into the gut, presumably due to villus atrophy and effusive enteritis, respectively.

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Septicemic colibacillosis

Generalized systemic infection with *E. coli* occurs commonly in *calves*, and less commonly or sporadically, especially among young animals of the other domestic species. Predisposition to infection is a prerequisite for *E. coli* septicemia. This usually results from reduced transfer or absorption of maternal colostral immunoglobulin, or from intercurrent disease or debilitation. But certain strains of *E. coli*, especially O8, O9, O15, O26, O35, O45, O78, O86, O101, O117, and O137 in calves and lambs, and O115 in pigs and calves, are particularly associated with septicemia, and may possess characteristics that enhance their ability to invade and proliferate systemically in compromised animals.

Among factors conferring virulence upon these strains are plasmids coding for colicin V (Col V). Col V plasmids carry genes coding for aerobactin, a bacterial hydroxamate siderophore permitting survival in low-iron extracellular environments; outer-membrane proteins resisting bactericidal effects of serum, such as complement activation; and hydrophobic properties that impede phagocytosis, conferred by a capsule. Some produce cytolethal distending toxin, or fimbriae which impede phagocytosis. Endotoxin released by dying bacteria causes the vascular damage and shock associated with *E. coli* septicemia.

The portal of entry of *E. coli* causing septicemia probably varies somewhat. The navel in the neonate, the upper respiratory tract and possibly the tonsil, and the intestine are likely sites. In calves, adhesins such as P, F17, AfaE-VIII, and CS31A may promote enteric colonization and invasion. Enteritis is not a necessary, or even common, concomitant of colisepticemia in animals.

Colisepticemia is most commonly a disease of neonates, and may vary from peracute septicemia and endotoxemia resulting in sudden death, to subacute or chronic disease in which signs are related to sites of bacterial localization, especially in the meninges, joints, and eyes.

The lesions associated with colisepticemia in young animals of any species, especially calves, lambs, and foals, may vary from subtle to obvious. Mortality in hypogammaglobulinemic neonates may occur acutely with little in the way of abnormal gross findings. These may be limited to mildly congested or blue-red, slightly rubbery lungs, and a firm spleen, perhaps with evidence of omphalitis. *Microscopic changes* in the lungs include thickening of alveolar septa by mononuclear cells and neutrophils, and effusion of lightly fibrinous exudate and a few neutrophils into alveoli. There may be a corona of neutrophils around white pulp in the spleen, and neutrophils may be present in abnormal numbers in circulation in many organs, including lung and hepatic sinusoids. Kupffer cells may also be prominent in sinusoids in the liver. Fibrin thrombi may be evident in pulmonary capillaries, glomeruli, and hepatic sinusoids. Some calves will develop acute interstitial nephritis with foci of neutrophil accumulation, which with time evolve into "white-spotted kidney" in surviving animals.

More severe acute cases will show evidence of serosal hemorrhage, with perhaps some serosanguineous pericardial fluid. The lungs may be deep red-blue, rubbery, and fail to collapse. Interlobular septa may be slightly separated by edema, and froth or fluid may be present in the major airways. Meningeal vessels may be congested, and the meninges wet. The abomasum or stomach may have focal superficial ulcers, or more extensive deep red areas of venous infarction. There may be evidence of diarrhea and dehydration, with congestion of the small intestine. Microscopic lesions resemble those previously described, with more severe congestion, thrombosis, and edema in lungs, and perhaps other tissues. In cases not examined for some time after death, clumps of small bacilli may be seen in vessels throughout the body. The vascular permeability, thrombosis, and hemorrhage reflect endotoxemia and its sequelae.

Subacute cases may develop localized infection on serous surfaces, in the joints and meninges. Fibrinous peritonitis, pleuritis, and pericarditis, fibrinopurulent arthritis and meningitis, and hypopyon are commonly found, alone or in various combinations. Affected animals may have a history of lameness ascribable to arthritis, nervous signs due to meningitis, or general debilitation. Microscopic examination reveals the lesions already described in animals with active systemic disease, with the addition of extensive congestion and edema of inflamed serous surfaces, associated with an acute fibrinous inflammatory exudate.

In *lambs*, congestion and edema of the mucosa of turbinates and sinuses, perhaps with mucopurulent to hemorrhagic sinusitis, have been described. Fibrinous polyserositis and arthritis are sporadic manifestations of *E. coli* septicemia in growing or adult swine, and must be differentiated from the more significant *Haemophilus, Mycoplasma,* and streptococcal infections causing these lesions. Colisepticemia is a sporadic cause of mortality in litters of young puppies.

Diagnosis of colisepticemia is based on the isolation of *E. coli* in large numbers from more than one parenchymatous organ or other internal site, other than mesenteric lymph node (preferably liver, spleen, lung, or kidney), or from a site of serosal localization, in conjunction with compatible gross and/or microscopic lesions.

"**Watery mouth**," a syndrome characterized by drooling, depression, loss of appetite, and abomasal and abdominal distension, is associated with *E. coli* infection/bacteremia in lambs under 3 days of age in the UK. At necropsy, affected lambs are in poor condition. They may have unclotted milk and mucinous fluid in the distended abomasum; there is gas in the abomasum and intestine, and meconium retention is common. It is hypothesized that *E. coli* colonize the bowel, and in some manner cause loss of motility and functional obstruction. Fluid and gas accumulate in the abomasum. Bacteremia/ septicemia is terminal.

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Salmonellosis

The **taxonomy of *Salmonella*** is confusing and has recently been modified, based on molecular genetic analysis. The genus *Salmonella* is now considered to be comprised of two species, ***S. bongori*** and ***S. enterica***. There are six subspecies of *S. enterica* (*enterica, salamae, arizonae, diarizonae, indica,* and *houtenae*) and many (>2200) antigenically distinct serotypes or serovars. About 60% of *Salmonella* serotypes belong to *S. enterica enterica*, and occur in birds and mammals. Members of *S. e. enterica* are the predominant cause of salmonellosis in humans and domestic animals, but fewer than 50 of these serotypes have been isolated from mammals or birds with any frequency worldwide. The remainder of *S. enterica* and *S. bongori* serotypes are found in ectothermic animals or in the environment. In conventional terminology, the serotypes have been treated as species, but in the new terminology, the names of serotypes are capitalized, but not italicized (e.g., *S. enterica* Typhimurium when first used, followed later by *S.* Typhimurium). They are usually named on the basis of the locality in which the serotype was first isolated or identified, or on their host association and the clinical syndrome they may produce. Identification of isolates at the subserotype level, by phage typing, plasmid profile analysis, or other molecular techniques, is desirable when there is evidence of zoonotic transmission, or when epidemiologic tracing is necessary.

The **clinical and pathologic syndromes of salmonellosis** typically vary from localized enterocolitis to septicemia; abortion may also occur, with or without obvious systemic disease. While some serotypes are strongly host-adapted, others have a very wide host range. Highly host-adapted serotypes, such as *S.* Typhi (humans), *S.* Dublin (cattle), and *S.* Choleraesuis (swine), tend to produce severe systemic disease in adult, as well as juvenile animals, whereas serotypes with a broad host range, e.g., *S.* Typhimurium, tend to affect predominantly young animals in most species, and mainly cause enterocolitis, though septicemia may occur. There may be overlap between the two forms of disease, and if the animal survives, a carrier state of variable duration usually follows.

Asymptomatic ***Salmonella* carriage** may be common, depending on the species, and transmission can occur directly, or indirectly, by contamination of feed, water, or the environment from which the organism is ingested or inhaled. Stressors that compromise immune competence or disrupt the enteric bacterial ecosystem are often implicated in salmonellosis, and disease is usually more common and severe in young animals. The more common "*stressors*" associated with salmonellosis in domestic animals include transportation, starvation, changes in the ration, overcrowding, pregnancy, parturition, exertion, anesthesia, surgery, intercurrent disease, immunosuppressive drugs, and oral treatment with antibiotics and anthelmintics. Consequent changes in the anaerobic bacterial ecosystem that alter the volatile fatty acid composition of the enteric environment are permissive of *Salmonella* colonization.

There are many examples of enhanced susceptibility to salmonellosis associated with intercurrent disease. The best known is that between the *Classical swine fever virus* and *S.* Choleraesuis, an association so close as to have caused early pathologists to disregard the bacterium as a significant pathogen. The disease in adult cattle is usually sporadic, and often there are predisposing conditions, such as parturient paresis, ketosis, mastitis, and parasitic infestations. The stress of anesthesia and surgery may account in part for the serious outbreaks of salmonellosis that occur in hospitalized animals, especially horses, at veterinary schools.

The **pathogenesis of salmonellosis** may be divided into several stages: *entry* of the bacteria into the host and attainment of the primary site of infection, usually the enterocyte; attachment to the surface (*colonization*); and *invasion* of enterocytes.

For infection to take place, *Salmonella* must be present in sufficient numbers; generally a minimal infective oral dose of 10^7–10^9 organisms is needed to infect large domestic animals. After ingestion, the *Salmonella* must overcome nonspecific resistance factors, including the bactericidal effects of salivary enzymes, and the acid pH of the gastric environment. Mucus and lysozymes in the glycocalyx, peristalsis, and constant sloughing of enterocytes may interfere with attachment. Those organisms that survive the nonspecific resistance factors may colonize and invade enterocytes.

Invading *Salmonella* in some species enter the mucosa through M cells in the Peyer's patches, and host specificity of some *Salmonella* serotypes may be associated in part with specific receptor sites on these cells. *Salmonella* have been demonstrated in the Peyer's patches as early as 6 hours postinoculation. When bacteria invade through M cells, smaller numbers of *Salmonella* may enter through enterocytes in other areas of the small intestine. However, the M cell is not the main site of attachment in some circumstances, for instance in *S.* Typhimurium infections of calves and pigs.

In salmonellosis characterized primarily by enterocolitis, the organisms do not usually disseminate beyond the mucosa and the mesenteric lymph nodes, and the ensuing inflammation remains confined to the intestine. In those cases where bacteremia ensues, the organisms must be able to survive and replicate in macrophages and disseminate to other systemic sites, e.g., liver, lung, joints, meninges, or placenta and fetus.

The ability to attach, invade, and penetrate enterocytes is crucial to virulence, and the first step in the development of salmonellosis. A number of known virulence factors contribute to the pathogenesis of salmonellosis, including motility, pili, or fimbriae, effector proteins modifying the metabolism or causing death of host cells, and lipopolysaccharides. The information for such virulence attributes is often encoded in chromosomes in clusters of genes known as *Salmonella* pathogenicity islands (SPI).

Invasion of enterocytes, especially those in the ileum, occurs within 12 hours of oral infection. Ability to invade cells is dependent on a type III protein secretion system encoded in SPI-1. Protein targets of the secretion system are translocated into host cells, where they facilitate bacterial invasion by causing changes in the cytosol and ruffling of the cell membrane.

Motility, associated with the presence of flagella, is characteristic of many *Salmonella* serovars. Bacterial motility is generally not considered to be an important virulence determinant. However, it may enhance the movement of bacteria through the glycocalyx and facilitate attachment to specific receptor sites on enterocytes.

Fimbriae (pilus adhesins) encoded in chromosomes and on virulence plasmids are present on salmonellae, and they may play a role in colonization of the gut. Adherence of *Salmonella* to intestinal epithelial cells takes place in two stages. The first step is reversible, since the organisms can be easily washed off. Weak ionic and nonionic interactions between bacterial and host cell membrane surfaces are thought to be the binding forces responsible for this attachment. The second stage, referred to as "receptor-mediated endocytosis," is irreversible. It occurs after a lag period and it is characterized by degeneration of the microvilli on the epithelial cells, "ruffling" of the cell membrane, and macropinocytosis, resulting in the formation of membrane-bound vacuoles (endosomes) containing *Salmonella*.

The ultrastructural changes of *Salmonella* infection in the intestine were first described in experimental infections of guinea pigs. Large numbers of organisms are present in the lumen, on the surface of the brush border, and in enterocytes. There is an increase in the number of neutrophils in the gut lumen and within intercellular spaces, and some of these contain bacteria. Degeneration of microvilli, characterized by loss of filamentous cores, is associated with close adherence of bacteria. Other changes consist of elongation, swelling, budding and fusion of microvilli, and loss of the terminal web.

The organisms usually invade the cells through the brush border; however, they may also enter the mucosa through the intercellular junctional complex. In the cytoplasm, the bacteria are located within membrane-bound vacuoles, which may also contain remnants of microvilli and cytoplasmic debris. Most organisms remain intact and multiply during their transcellular migration in endosomes. Often, many bacteria are present in a single enterocyte during the early stages of infection, but cellular damage is mild and transient. The *Salmonella*–receptor complex dissociates as a result of the acidification of the endosomal content, allowing the receptor site to return to the apical plasma membrane and repeat the processes of endocytosis. After 24 hours, most bacteria are located within membrane-bound vacuoles in macrophages in the lamina propria. Many organisms are evident in the lumina of crypts, but invasion of cryptal epithelial cells evidently does not take place.

The **lipopolysaccharide** (LPS) moiety of *Salmonella* with smooth cell walls consists of an *O-specific side chain, a core portion, and a lipid A portion*. Most *Salmonella* isolated from animals have smooth cell walls, which influences virulence in several ways. These strains are more invasive, and are more successful at avoiding phagocytosis, and lysis in phagolysosomes after invasion, than are "rough" counterparts with incomplete LPS. LPSs reduce the susceptibility of the organisms to the host's cationic proteins; they stimulate local prostaglandin synthesis; and they prevent the activation and deposition of complement on the bacterial surface.

The main function of LPS may be to facilitate survival in the intestinal mucosa and eventual entry into deeper tissues. The involvement of LPS in invasion apparently varies among *Salmonella* serotypes, since some strains of *S*. Typhimurium do not require intact LPS to invade epithelial cells in vitro. On the other hand, more host-specific *Salmonella* serotypes, such as *S*. Typhi and *S*. Choleraesuis, require intact LPS or O-side chains. The lipid A portion of LPS is responsible for the endotoxin-mediated effects of *Salmonella* infection that are seen in systemic disease. Septicemia (endotoxemia) typically causes fever, leukopenia, hemoconcentration, lactic acidosis, coagulopathies, hypotension, and death.

Diarrhea in salmonellosis is not mediated by enterotoxins such as those involved in cholera and *E. coli* infections. Rather, *effector proteins* associated with SPI-1 induce secretory diarrhea by blocking chloride channel closure, while others attract neutrophils, and induce apoptosis of enterocytes. Proteins encoded in SPI-5 also promote neutrophil recruitment and electrolyte secretion. Mucosal inflammation leads to the accumulation of a number of mediators, including prostaglandin E_2, capable of causing hypersecretion of chloride by enterocytes, and consequent passive osmotic movement of water into the lumen. Loss of enterocytes, dying as a sequel to *Salmonella* invasion and neutrophil-induced tissue injury, results in a reduction in absorptive surface area, and causes defects in mucosal integrity, through which the protein- and neutrophil-rich exudate leaking from permeable vessels effuses.

Thus diarrhea is an outcome of active secretion of electrolyte, malabsorption due to reduced mucosal surface area and enterocyte competence, and inflammatory exudation, which may contain sufficient fibrinogen to form a pseudomembrane over the affected surface. The volume of fluid originating in lesions in the small intestine may overwhelm the capacity of the colon to compensate; as often as not in salmonellosis, the large intestinal mucosa is also involved, further compounding the compromise to electrolyte and water homeostasis in the gut.

Thrombosis of mucosal venules is common in *Salmonell*a enteritis, and may contribute to loss of mucosal viability. Such lesions may be due in part to the large amounts of **endotoxin** absorbed through the damaged mucosa, or released locally.

Enteritis in salmonellosis is thus characterized by fibrinous or fibrinohemorrhagic exudates over denuded small and large intestinal mucosae, directly mediated by the apoptosis and necrosis induced by invading bacteria, and by the necrotizing effects of local neutrophil activity and microvascular thrombosis.

The **systemic outcome of an infection with *Salmonella*** is determined by the genetic virulence determinants of the invading organism and the ensuing innate, humoral, and cell-mediated immune response of the host. *Salmonella* are considered to be *facultative intracellular pathogens*, and invading strains must have the ability to survive and replicate within macrophages in order to cause bacteremia or septicemia. This capacity is conferred by components coded in SPI-2, perhaps largely through inhibition of NADPH oxidase-mediated oxidative killing of *Salmonella* in cytoplasmic vacuoles, and in some species by factors encoded in SPI-2 and SPI-3. The virulence of several serotypes commonly associated with systemic infections in animals, including *S*. Typhimurium, *S*. Dublin, and *S*. Choleraesuis, is enhanced by intracellular survival in macrophages mediated by attributes encoded on virulence plasmids.

Salmonella taken up by resident macrophages elicit a *major immune response* in the host. There is considerable controversy about the roles played by cell-mediated and humoral immunity in the pathogenesis of salmonellosis, but *Salmonella* infection results in the release of cytokines by specifically stimulated T lymphocytes. They activate macrophages that phagocytose the organisms, and in such a circumstance, cell-mediated immunity is of paramount importance.

Once *Salmonella* bacteria have crossed the mucosa, they may enter the bloodstream via the lymphatics, perhaps carried in macrophages, and cause septicemia or transient bacteremia. Or they may remain indefinitely in the gut-associated lymphoid tissues and mesenteric lymph nodes. Increased susceptibility to salmonellosis in animals with intercurrent disease, or subjected to stress, may be related to relaxation of cell-mediated immunity to the organism. Septicemia may be of variable duration and severity but, as a rule, it is rapidly fatal in young animals. If, however, there is transient bacteremia, the organisms are removed by fixed macrophages, especially those of the spleen, liver, and bone marrow. They may continue to proliferate in such extravascular locations and subsequently may cause another bacteremic phase that may result in fatal septicemia or secondary localization in other tissues.

The carrier state is important in the epidemiology of the disease. Whether *Salmonella* can maintain themselves in the intestinal lumen is not clear; to some extent, at least, fecal shedding is likely to depend on intermittent seeding from the bile, or from macrophages in the lamina propria and gut-associated lymphoid tissue. The duration of the carrier state may be prolonged, or animals may rid themselves of the infection, probably by means of cell-mediated immunity. The carrier state is an unstable one, for it appears that if the carrier is subjected to some stress or debilitating disease it may succumb to disease; this often seems to occur in adult cattle. The carrier animal is a potential threat to any other animal that it contacts, either directly, or through the medium of its excreta, or byproducts such as bone or meat meal.

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Salmonellosis in swine

Many serotypes of *Salmonella* have been isolated from swine, and with poultry and cattle they form an *important reservoir of the organism*. The bacteria are carried in the lamina propria of the intestine, but also in the regional lymph nodes of the alimentary tract, so that carrier animals may not excrete the organism in the feces.

Three syndromes are associated with *Salmonella* infections in swine. (1) *Septicemic salmonellosis* is usually associated with the host-adapted *S.* Choleraesuis var. *kunzendorf*, although enteric lesions may be present with this serovar. Sporadic infections with *S.* Dublin have also been associated with septicemia in nursing pigs. (2) *Salmonella* Typhimurium most commonly causes *acute or chronic enterocolitis*, including necrotizing proctitis which may lead to rectal stricture. (3) *Salmonella* Typhisuis infection is characterized by *ulcerative enterocolitis*, as well as caseous tonsillitis and lymphadenitis.

***Salmonella* Choleraesuis** was once thought to be the cause of classical swine fever (formerly hog cholera) because gross lesions of septicemic salmonellosis and acute classical swine fever are similar. The latter disease is often complicated by *S.* Choleraesuis, the bacterium being recovered from 10–50% of pigs with classical swine fever.

The major clinical manifestations of *S.* Choleraesuis infection are *septicemia and enteritis*; they usually occur separately. Septicemia is more common. Oral inoculation of *S.* Choleraesuis initially results in septicemia and acute enterocolitis, followed in some cases by large necrotic and ulcerative lesions ("*button ulcers*") in the colonic mucosa. Enteritis is not necessarily chronic, or even clinically evident. Interstitial pneumonia and multifocal hepatic necrosis are the most consistent systemic lesions. In Europe, infection has also been associated with the development of fulminant fibrinous pneumonia. Immunohistochemical techniques reveal the preferential location of *S.* Choleraesuis in the colon and surface of ileal M cells in Peyer's patches. The invasive capability of this serovar is indicated by the presence of large numbers of organisms in proprial macrophages and regional lymph nodes.

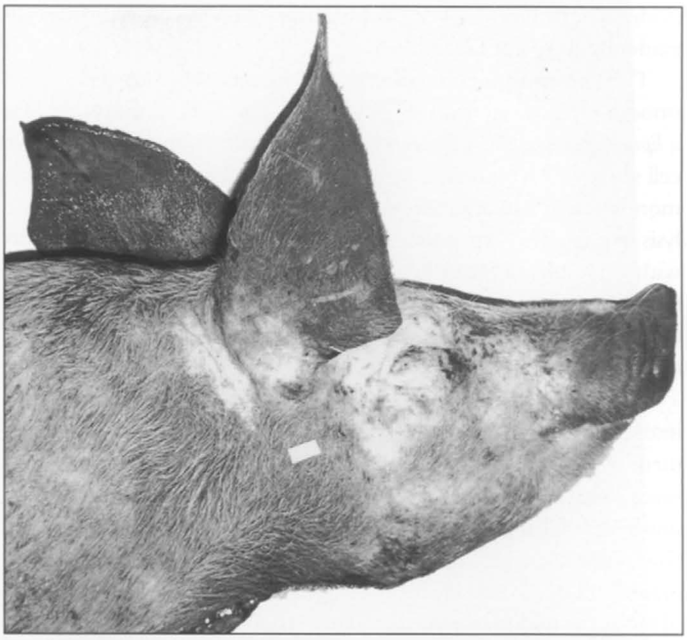

Figure 1.139 Note blue-red congestion of skin of ears, snout, and neck due to microvascular thrombosis caused by endotoxemia in **septicemic salmonellosis** in a pig.

Salmonellosis that is clinically septicemic is usually fatal. Death may occur quickly without observed illness, or after a course of a week or more. There is a high fever; characteristic but not pathognomonic blue discoloration of the skin, especially of the tail, snout, and ears (Fig. 1.139); caudal weakness; dyspnea that often leads to misdiagnosis of primary pneumonia; and sometimes terminal convulsions. Sows may abort during the septicemic phase of infection. Pigs recovered from this phase may have dry gangrene of the ears and tail, caudal paralysis, blindness, and diphtheritic enteritis. The *chronic or enteric form* may develop from the acute, but is usually insidious from the onset. It is characterized by loose yellow feces containing flakes of fibrin, progressive emaciation and debility, and eventual death. Some recover but fail to thrive, often partly owing to chronic bronchopneumonia.

At **autopsy**, there is blue or purple discoloration of the *skin*, which may be very intense about the head and ears. There may be superficial ischemic necrosis of the ears. Typically there are petechial hemorrhages in many organs and tissues. The lymph nodes are almost invariably hemorrhagic. The visceral nodes are more frequently and obviously involved than the peripheral ones, with the exception of those of the throat, which are usually hemorrhagic. The mesenteric lymph nodes are greatly enlarged, and they may be speckled with hemorrhages.

There may be hemorrhages, petechial or as small discrete blebs, on the *laryngeal mucosa* (Fig. 1.140). The *lungs* do not collapse because there is frothy fluid in the respiratory passages. They may be pale blue or purple. Beneath the visceral pleura there are small dark foci of hemorrhage. The lungs are wet and there is fluid in the interlobular tissue. The changes are best appreciated in the caudal lobes, because the cranial lobes are often the seat of acute lobular pneumonia. These pulmonary changes, attributable in part to endotoxin, account for the respiratory signs observed clinically. The *pneumonia* is interstitial due to endotoxemia and embolic organisms. The lobar cranioventral pneumonia may be due to ascending *Salmonella* alveolitis and bronchiolitis. Occasionally, the injury to the alveolar septa by *Salmonella* results

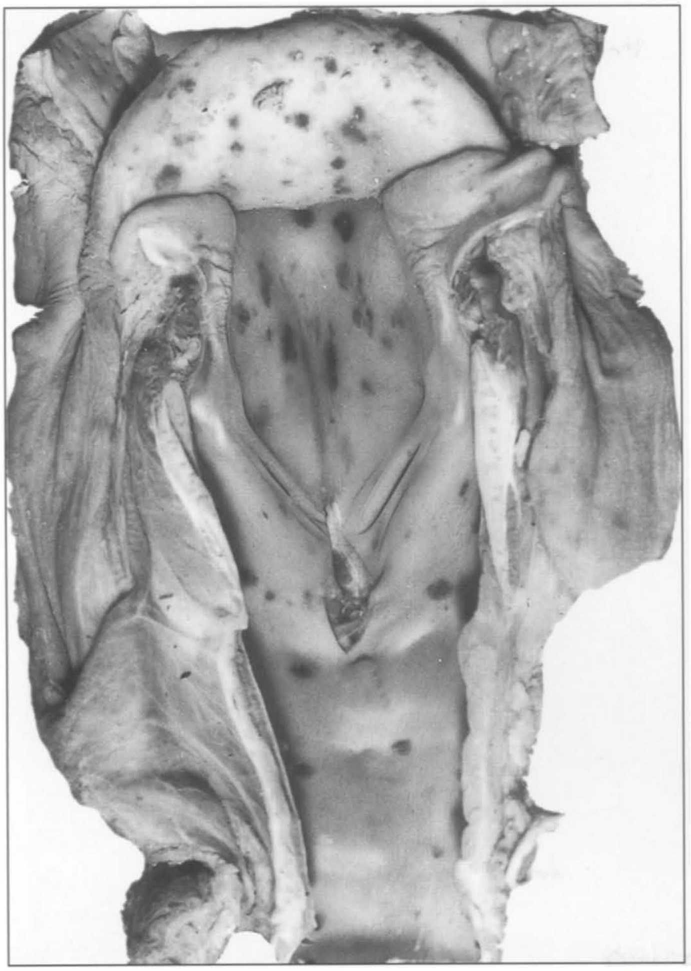

Figure 1.140 Laryngeal hemorrhages in **porcine salmonellosis**.

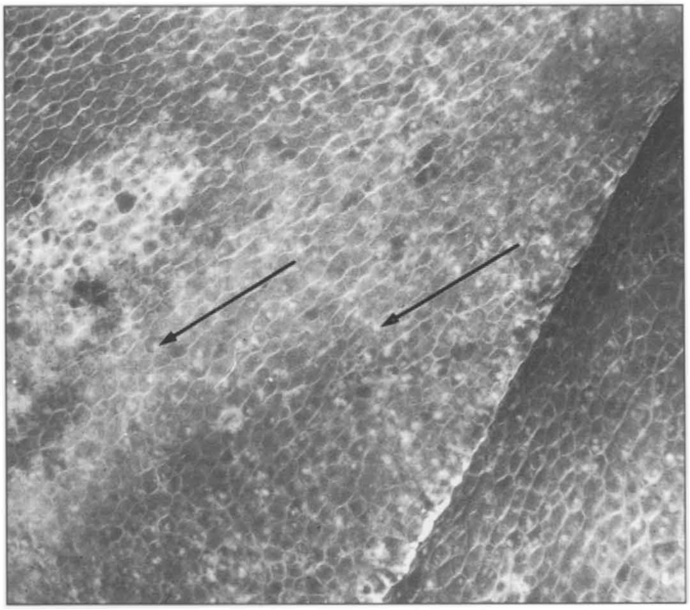

Figure 1.141 Paratyphoid nodules (arrows) in liver, in *Salmonella* septicemia in a pig.

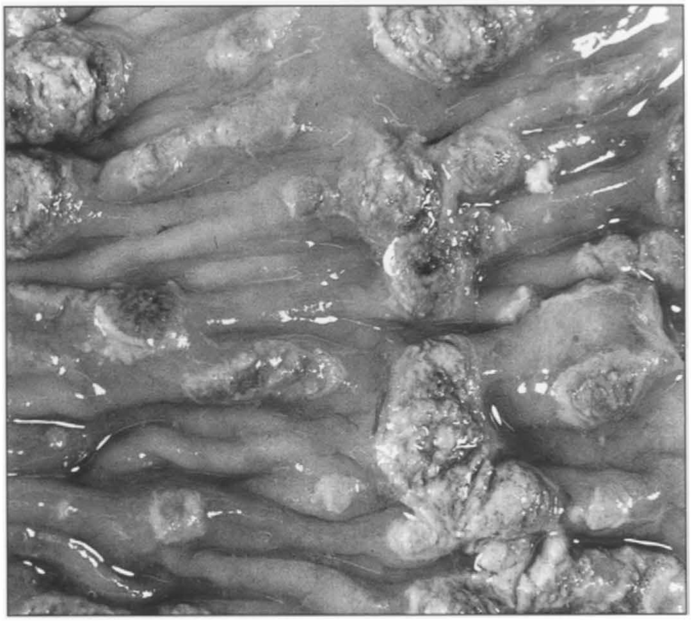

Figure 1.142 Button ulcers in colon in porcine salmonellosis.

in extensive fibrinous pneumonia of the caudal lobes. The cardiac serosae often bear petechiae, and in some more virulent infections there is fibrinohemorrhagic pericarditis with scant fluid exudation.

The *spleen is enlarged*, deep blue, firm with sharp edges; little blood oozes from the cut surface. There may be petechiae on the capsule, but the marginal infarcts of classical swine fever are not present. Other causes of splenomegaly, such as erysipelas, other septicemias, and African swine fever, must be differentiated.

The *liver* is usually congested, and focal hemorrhages may be visible in the capsule. In some cases the hemorrhages are very large, involving up to half of the central area in a lobule. They may be scattered at random throughout the liver or grouped, often at the edge of a lobe. In some, there are tiny yellow foci of necrosis, referred to as "*paratyphoid nodules*" (Fig. 1.141). Pinpoint hemorrhages are consistently present in the renal cortex. There may be only a few in each kidney or they may be so numerous as to cause the "turkey egg" appearance.

The *stomach* shows the intense red-black color of the severe congestion and venous infarction common to endotoxemia in pigs. If the animal survives a week or more, the superficial necrotic layer of the affected gastric mucosa sloughs. There may be no lesions in the *intestine*. There may be catarrhal enteritis or, more frequently, the enteritis is hemorrhagic, increasing in severity lower in the tract and terminating in a hemorrhagic ileitis. The mucosae of the colon and cecum may be normal but, if the course is prolonged, there is hyperemia, fibrinohemorrhagic inflammation, or button ulcers (Fig. 1.142).

Petechial hemorrhages may occur in the *meninges and brain*, but there is no gross inflammation. Localization sometimes occurs in *synovial membranes*, producing polysynovitis and sometimes **polyarthritis**. It is more usual to have an increase in the volume of fluid with red velvety hypertrophy of the synovial villi. The gross features described are usually not all present in any one case.

The **histologic changes** that occur in internal organs in acute disease are mainly associated with *endothelial damage due to endotoxin*, and *focal localization of bacteria*. The discoloration of the skin is initially due to intense dilation, congestion, and thrombosis of capillaries and venules in the dermal papillae. There is activation and necrosis of the endothelial cells in affected vessels. The renal lesions vary but principally affect the glomeruli. In some there is diffuse glomerulitis, and this is associated with mild nephrosis and hyaline casts. In others, the glomerulitis is exudative and hemorrhagic and in these a great many capillary loops contain hyaline thrombi. Embolic bacterial colonies are occasionally seen in the glomerular and intertubular capillaries. Fibrin thrombi may also be found in the afferent arterioles and interlobular arteries.

The pulmonary lesions are also characterized by thrombosis and vasculitis and a largely mononuclear cellular response in alveolar septa. There is flooding of the alveoli by edema fluid and moderate numbers of alveolar macrophages. This is the usual histologic picture; the extremes are acute fibrinous inflammation or a few scattered parenchymal hemorrhages.

In the liver, the *paratyphoid nodules* may be found in all transitional stages from foci of nonspecific necrosis to reactive granulomas. Typically there are few neutrophils, and whether the nodules are necrotic or reactive depends on their duration. The initial change is focal coagulative necrosis. About the margin, the macrophages accumulate and form small histiocytic granulomas which expand and displace the surrounding parenchymal cords.

In the spleen there are some scattered hemorrhages, but the overall histologic impression is of increased histiocytes with a scattering of neutrophils. The follicles are small and rather inactive. Very small foci of necrosis, containing many bacteria, may be sparse or relatively numerous, and these develop a reactive macrophage response and form the typical paratyphoid nodules.

Meningoencephalomyelitis occurs in a proportion of cases of septicemic salmonellosis. The lesion is fundamentally a vasculitis. There may be petechiae in the meninges but, microscopically, there is infiltration of large mononuclear cells in the pia-arachnoid and concentrated about the veins. The organism is relatively fastidious and cultures from postmortem samples may not be uniformly positive.

***Salmonella* Typhimurium** infection in swine produces a syndrome that differs from *S.* Choleraesuis in a number of ways. Clinically, the disease occurs in *feeder pigs* and is characterized by fever, inanition, and yellow watery diarrhea that may contain blood and mucus, especially in the later stages. The diarrhea may be chronic and intermittent. There is high morbidity but low mortality. Most pigs recover but may remain *carriers* for variable periods of time, and some may develop rectal stricture. The organism persists in tonsils, lower intestinal tract, submandibular, and ileocolic lymph nodes.

The pathogenesis and morphology of the enteric lesions differ from those described for *S.* Choleraesuis enteritis. The lesions with *S.* Typhimurium infection are mainly confined to the colon, cecum, and rectum, with minor involvement of the distal small intestine. There is acute enterocolitis with formation of a pseudodiphtheritic membrane on the mucosal surface (Fig. 1.143). Button ulcers are not associated with this or other nonhost-adapted serovars. Systemic dissemination and septicemia are rare.

Rectal stricture is thought to be a *sequel in most cases to ulcerative proctitis of ischemic origin,* caused by *S.* Typhimurium. It is characterized clinically by marked progressive distension of the abdomen, loss of appetite, emaciation, and soft feces. At autopsy, there is marked dilation of the colon, which is caused by narrowing of the rectum, 1–10 cm cranial to the anus (Fig. 1.144). The stricture is usually <1.0 cm in diameter and varies in length from 0.5 to 20 cm. There is marked fibrous thickening of the rectal wall, which may contain microabscesses. The dilation of the colon, proximal to the stricture, may consist of a well-demarcated widened area several centimeters long and wide. The colonic mucosa in this area is usually

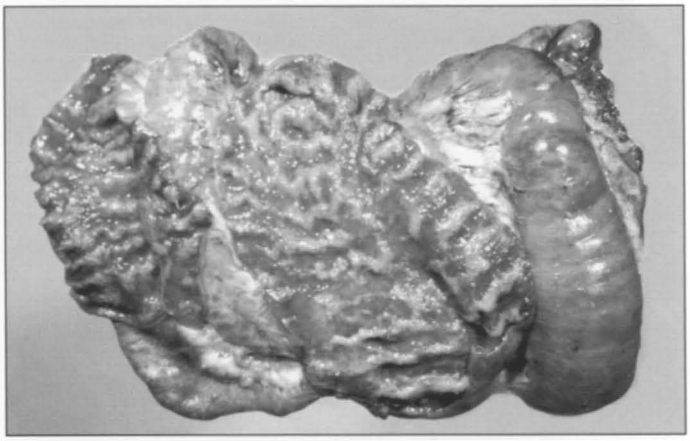

Figure 1.143 Fibrinous colitis in a pig infected with *Salmonella* Typhimurium.

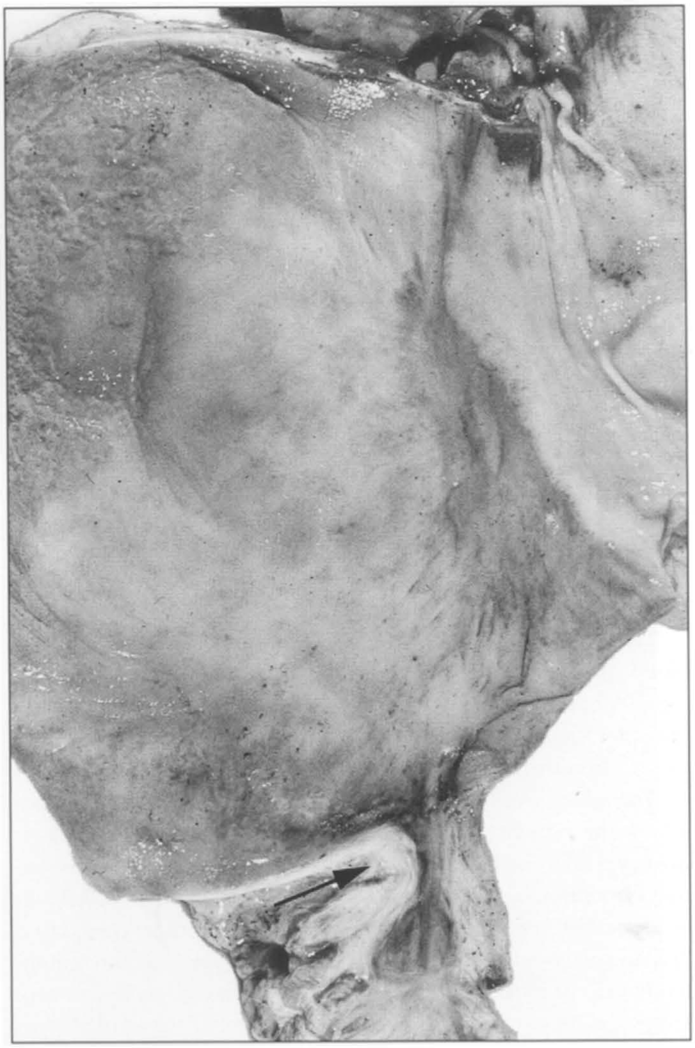

Figure 1.144 Rectal stricture in porcine salmonellosis. Opened colon is massively dilated proximal to stricture in rectum (arrow).

ulcerated and may be covered by fibrinous exudate. In some cases, there is more gradual but sometimes massive dilation of the entire colon and cecum, which are full of digesta, with ulceration of the mucosa just proximal to the stricture. The mucosa is always excessively corrugated; this is mainly the result of marked thickening of the internal muscularis. Anastomoses of the small intestine and/or colon to the dilated portion of the descending colon may occur. Localized chronic peritonitis is often associated with the dilated segments of the colon.

The stricture is located in an area of rectum that has a relatively poor blood supply, namely the junction of the circulatory fields of the caudal mesenteric and pudendal arteries. Ulcerative proctitis is consistently found in swine with typhlocolitis due to *S.* Typhimurium infection. Granulation of such lesions probably leads to cicatrization and stricture. The location, the persistent nature of this lesion in some pigs, and its limited capacity to heal are probably related to the restricted blood supply of the affected area.

***Salmonella* Typhisuis** infection is an *uncommon* condition in pigs. The disease, called *paratyphoid* in Europe, is now known to cause disease in pigs in the Americas and Asia. It is a progressive disease of 2–4-month-old pigs that is clinically characterized by intermittent diarrhea, emaciation, and frequently, massive enlargement of the neck region, the latter associated with caseous palatine tonsillitis, cervical lymphadenitis, and parotid sialoadenitis. There is also circular or button-like, to confluent, ulceration of the mucosa of the ileum, cecum, colon, and rectum. Other less frequent findings are caseous lymphadenitis of the mesenteric lymph nodes, interstitial pneumonia, hepatitis, and pericarditis.

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Salmonellosis in horses

The most common serovar in horses in most areas is ***S.* Typhimurium**, and its prevalence is increasing, especially that of multidrug-resistant definitive phage type 104 (DT104). Other serovars are usually associated with sporadic cases of disease. *Many horses are Salmonella carriers, and when they are stressed, diarrhea follows.* Abortion of pregnant mares has been associated with *S.* Abortusequi. *Salmonella* Infantum has been associated with disseminated infection localizing in a variety of tissues, including muscle, in a horse.

Treatment with antibiotics, especially orally, increases the risk of salmonellosis. Resistance to certain antibiotics is associated with the presence of *resistance (R) plasmids* that may be transferred to other bacteria, of the same or different species, by conjugation or transduction. Antibiotic-resistant *Salmonella* may not respond to treatment, and antimicrobial therapy may increase the potential for infection and disease, due to the suppression of the normal flora. Antibiotic-resistant strains have been associated with outbreaks of salmonellosis at veterinary teaching hospitals.

Salmonellosis in horses may be manifested clinically as *peracute* (usually septicemic), *acute*, and *chronic* forms, and as an *asymptomatic carrier state.*

The septicemic form occurs most commonly in foals 1–6 months of age. These animals are usually with their dams at pasture and predisposing factors are unclear. The infection in foals tends to be fatal. Affected animals are lethargic and develop severe diarrhea, often with characteristic green color, which may contain casts and blood. They are febrile and waste rapidly, to die in 2–3 days. Some survive for a week or more and these may develop signs of pneumonia, osteitis, polyarthritis, and meningoencephalitis.

The primarily enteric forms of the disease are more likely to occur in older horses. Most of the predisposing factors mentioned earlier apply to horses. *Salmonellosis is an occupational hazard of horses*, since most are exposed to long periods of transport, and to exertion due to overwork or excessive training.

Clinically, the acute disease is characterized by diarrhea and fever for a period of 1–2 weeks, followed by recovery or death. The chronic form persists for weeks or months. Affected horses pass soft, unformed manure that resembles cow feces. They lose their appetite, with subsequent progressive loss of weight and condition. In later stages, they become dehydrated and emaciated.

The gross lesions are those of enteritis and/or septicemia; the former are most consistently found at autopsy. As a rule, the longer the course, the lower in the intestine does one find the most severe lesions.

Acute septicemic cases show small hemorrhages on the serous or mucosal membranes. The visceral lymph nodes are always enlarged, juicy, and often hemorrhagic. Marked pulmonary congestion and edema, and renal cortical pallor and medullary congestion may occur. The main lesions are in the stomach and intestines. In **peracute or septicemic cases**, there is intense hyperemia of the gastric mucosa, probably venous infarction, with some edema and scattered hemorrhage. The small intestine may be congested with a mucous or hemorrhagic exudate. In **acute cases**, there is diffuse and intense fibrinohemorrhagic inflammation of the cecum and colon overshadowing any lesions in the upper intestine, and leading rapidly to superficial necrosis of the mucosa and a gray-red pseudomembrane (Fig. 1.145A). In **chronic salmonellosis**, enteric lesions may be few or subtle. Some animals have extensive or patchy fibrinous or ulcerative lesions of the cecum and colon. In others, raised circumscribed lesions about 2–3 cm in diameter may be evident, with a gelatinous submucosa and ulcerated mucosa. Some such lesions are more fibrinous, and resemble button ulcers (Fig. 1.145B).

Histological alterations of significance are usually limited to the intestine. However, in septicemic animals lesions typical of endotoxemia are present in lung, liver, kidney, spleen, and adrenal. There may be acute ileocecocolic lymphadenitis, and inflammation in sites of localization, such as growth plates in long bones, and the meninges. Depending on the duration of the enteritis, hemorrhage, necrosis, or diphtheresis may predominate, but the infiltrating leukocytes are largely mononuclear. The superficial coagulative necrosis of the mucosa may extend over large areas. A layer of fibrinocellular exudate may cover the necrotic mucosa. Fibrin thrombi are frequently present in the capillaries or venules of the lamina propria (Fig. 1.145C). There is usually marked congestion of submucosal vessels, which is accompanied by considerable edema.

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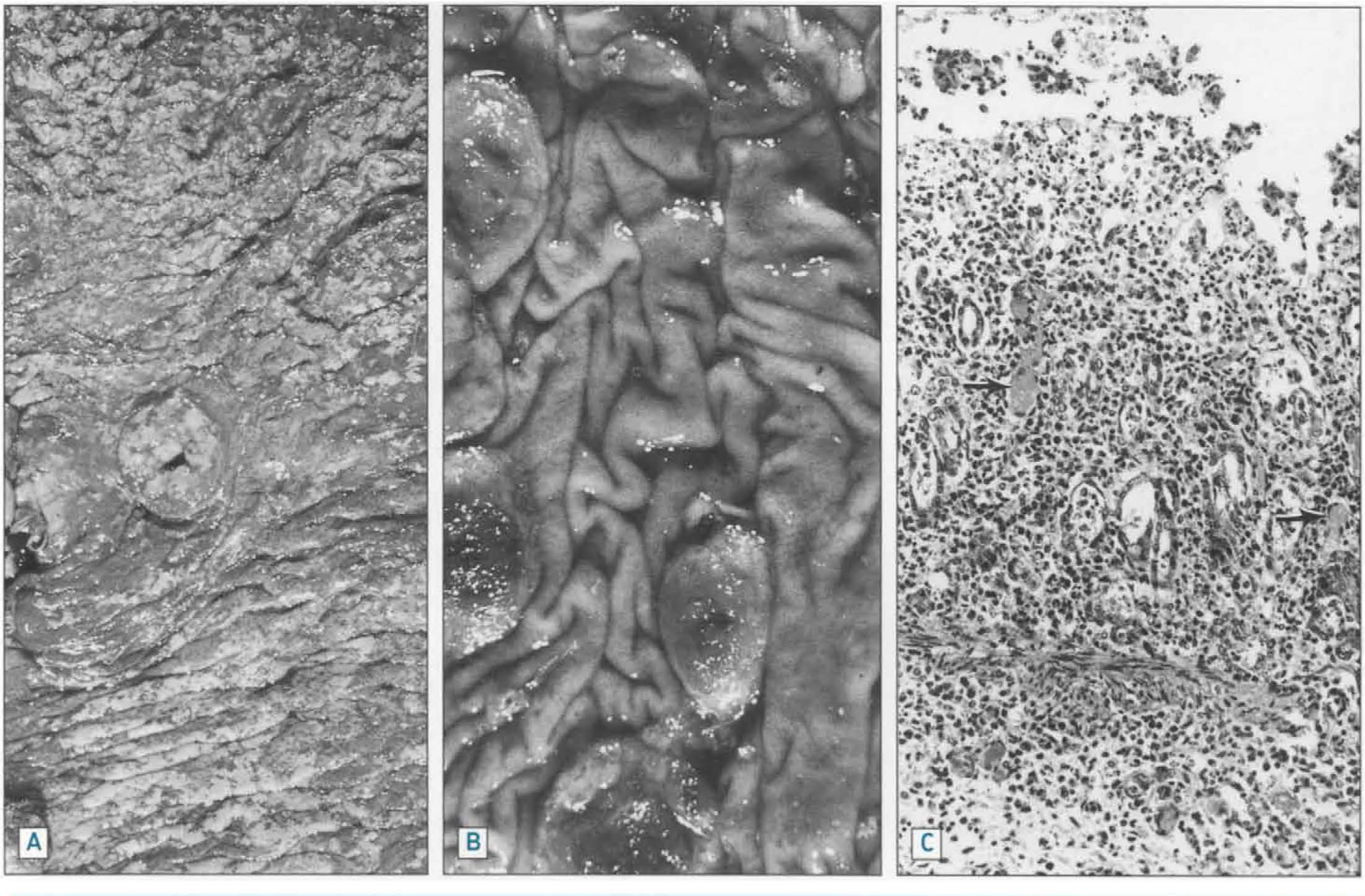

Figure 1.145 Salmonellosis in a foal. *Salmonella* Typhimurium. **A.** Focal and coalescent ulceration and diphtheresis involving ileocecal valve and mucosa. **B.** Nodular ulcerative lesions in colon in chronic salmonellosis. **C.** Superficial necrosis and effusion from colonic mucosa; several thrombosed vessels are in the propria (arrows).

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Salmonellosis in cattle

The serotypes usually incriminated are **S. Typhimurium** and **S. Dublin**; both are distributed worldwide. Wherever S. Dublin is found, it tends to be adapted to cattle and to occur in epizootics, whereas other serotypes usually cause more sporadic disease.

It is unusual to find salmonellosis in **calves** less than a week of age, in contrast to enteric and septicemic colibacillosis, which usually affect very young animals. *In calves, salmonellosis is a febrile disease typified by dejection, dehydration, and usually diarrhea.* Diarrhea is not always present, but when it is, the feces are yellow or gray, and have a very unpleasant odor. In older calves, there may be blood and mucus in the feces. In less acute cases, there may be delayed evidence of localization in the lung and synovial structures. Morbidity and mortality may be considerable, especially in calves that are confined, such as in vealer operations. Experimental infections in calves indicate that survival is inversely related to the numbers of *Salmonella* in the inoculum, and directly to the age of the calves.

The general appearance at **autopsy** of a calf with salmonellosis may resemble one with septicemic colibacillosis. However, *enlargement of mesenteric lymph nodes and gross enteric lesions* are generally observed in salmonellosis. There is moderately severe gastrointestinal inflammation, acute swelling, and hemorrhage of the visceral lymph nodes, and some petechiation of serous membranes. The enteritis may be catarrhal, but sometimes it is hemorrhagic or more commonly causes exudation of yellow fibrin (Figs 1.146 and 1.147). The mucosa overlying the lymphoid tissues may become necrotic and slough. In animals with fibrinous enteritis, the bowel wall is somewhat turgid and the serosa may have a ground-glass appearance. There is often diffuse, but perhaps mild, fibrinous peritonitis.

The intestinal lesions are usually most severe in the ileum, especially during the early stages of the disease. With time the jejunum and colon become involved but the duodenum remains relatively normal. The regional distribution of the lesions may, in part, be related to differences in the level of bacterial colonization of the mucosa. Twelve hours after oral infection of calves with S. Typhimurium, the numbers of bacteria are generally lower in the abomasum and duodenum than in the lower intestinal tract, while they are relatively constant from the jejunum through to the rectum.

The early **microscopic lesions** in the small intestine consist of a thin layer of fibrinocellular exudate on the surface of short and blunt villi (Fig. 1.148). This is followed by extensive necrosis and ulceration of the mucosa, with fibrin and neutrophils exuding from the ulcerated areas into the lumen (Figs 1.149 and 1.150). The lamina propria may be moderately infiltrated by mononuclear inflammatory cells. Fibrin thrombi are often evident in proprial capillaries and venules. There is also marked submucosal edema and the centers of lymphoid follicles in the Peyer's patches are completely involuted. Similar erosion, ulceration, and fibrinous effusion occur in the proximal large bowel.

Scanning electron microscopy of small intestine shows large numbers of bacteria on a tattered mucosal surface. Clusters of enterocytes slough off short villi (Fig. 1.151). Strands of fibrin emerge from the mucosal defects and cover the mucosa. Ultrastructurally, the lesions are similar to those described originally in guinea pigs, except that there is more damage to epithelium in calves experimentally infected with S. Typhimurium.

Characteristic changes usually occur in the *liver and spleen*, but may be absent in peracute septicemic cases. There is often fibrinous cholecystitis. In acute cases, the spleen is enlarged and pulpy as a result of congestion, but this is soon replaced by acute splenitis, present as miliary, tiny foci of necrosis or as reactive nodules. The liver is often pale with many minute *paratyphoid nodules*. In the spleen, macrophage reaction is sometimes diffuse. "Paratyphoid" granulomas may also be found microscopically in the kidney, lymph nodes, and bone marrow. These probably represent a cell-mediated immune response to embolic bacteria. In those animals that survive the acute phase of the disease, the inflammatory changes in lymphoid tissues progress

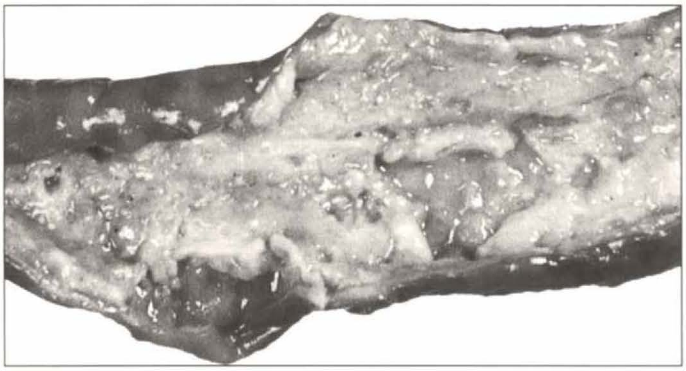

Figure 1.146 Diphtheritic enteritis caused by *Salmonella* Typhimurium infection in a calf.

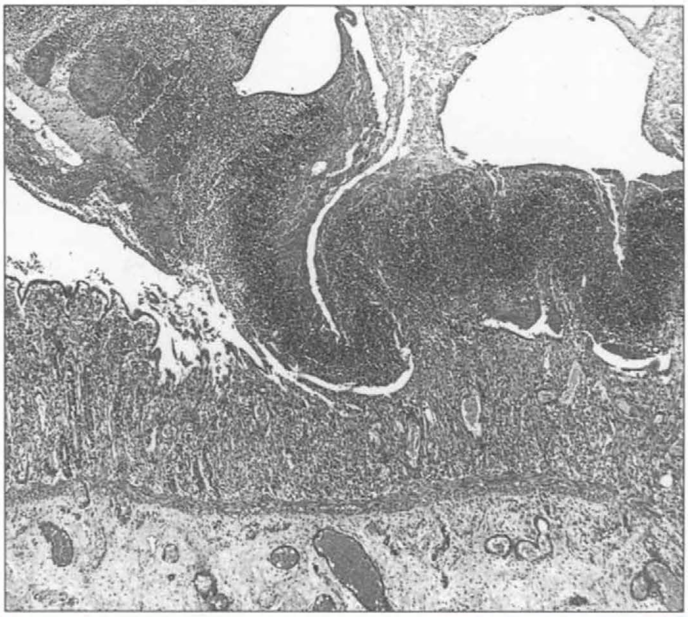

Figure 1.147 Diphtheritic membrane on the surface of the ileum in bovine salmonellosis. Exudate arises from eroded mucosa in which crypts of Lieberkühn are sparse or absent.

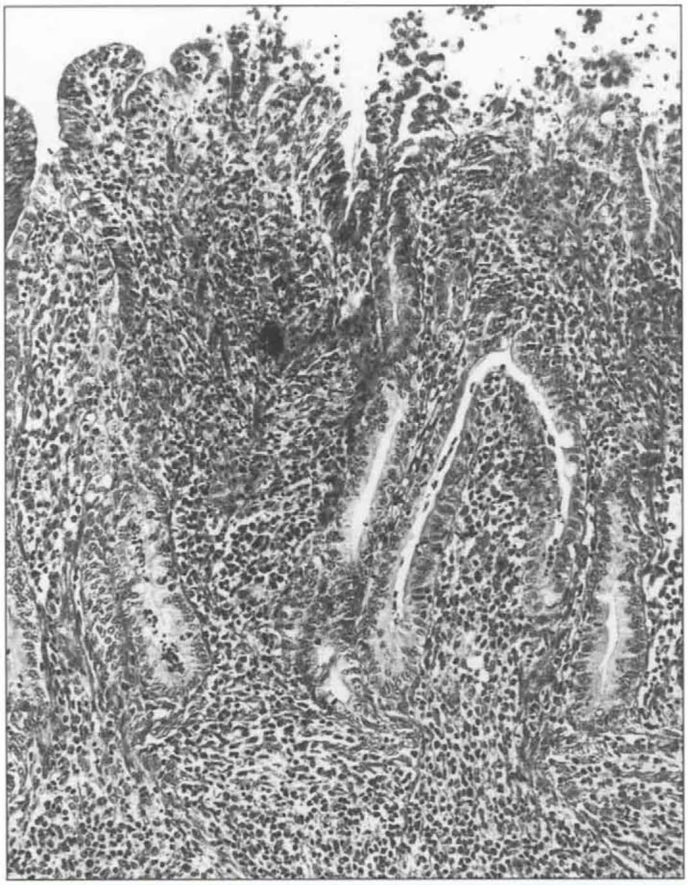

Figure 1.148 Atrophy of villi, exfoliation of surface epithelium, and effusion of neutrophils in ileum of a calf 12 hours after inoculation with **S. Typhimurium**. (Courtesy of R Clarke, CL Gyles.)

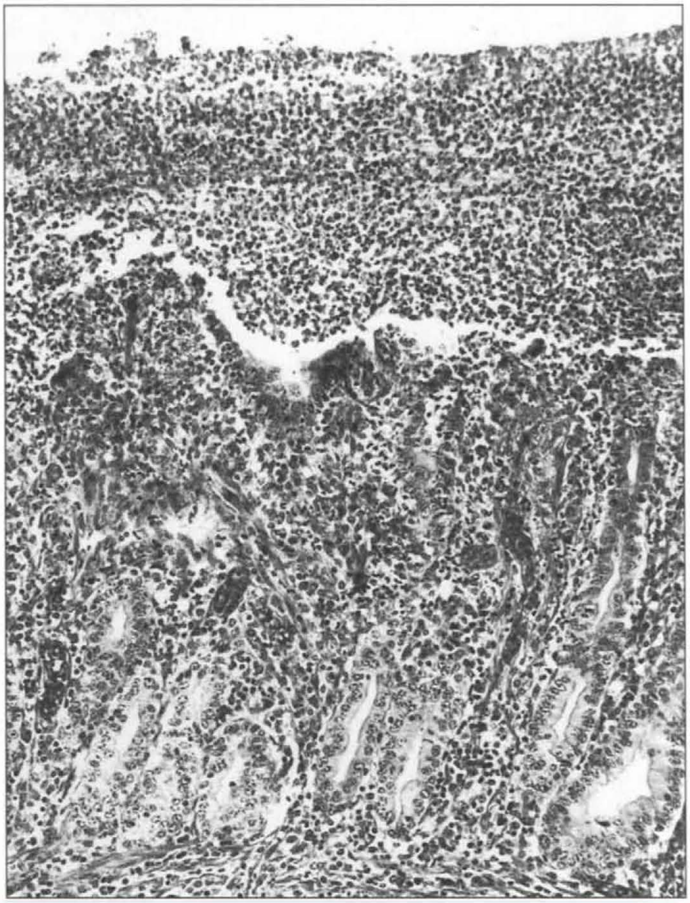

Figure 1.149 Atrophy of villi, erosion, and effusion of neutrophils and fibrin into the lumen of the small intestine of a calf. Some thrombosis of proprial vessels 36 hours after inoculation with **S. Typhimurium**. (Courtesy of R Clarke, CL Gyles.)

to an immunologic response, characterized by a diffuse reaction of medium-sized and large lymphocytes in the follicles, and plasma cells in the sinusoids. There may be marked cortical atrophy of the thymus. In calves with acute septicemia, pulmonary congestion and edema are visible at necropsy, with interstitial thickening of pulmonary alveolar septa by mononuclear cells in tissue section. There may be thrombosis of septal capillaries, and some effusion of edema fluid and macrophages into alveolar spaces.

In subacute salmonellosis of calves, there may be cranial bronchopneumonia, usually with adhesions and abscessation. Purulent exudate is in synovial cavities, and the organism is recoverable in pure culture from such affected joints and tendon sheaths. It may be mixed with *Arcanobacterium pyogenes* and *Pasteurella* in the lungs.

Salmonellosis in **adult cattle** may occur in *outbreaks* as it does in calves, but more often it is sporadic, and it may cause *chronic diarrhea and loss of condition*. The source of infection is usually the carrier animal. Other sources, such as feed containing protein of animal origin, or bone meal, should be considered when the disease is caused by an uncommon serovar. *Abortions* are most common with *S.* Dublin, but may occur with any serovar. In some herds, this may be the only clinical evidence of infection, although other animals often excrete the offending serovar in the feces. The *carrier state* of *S.* Dublin infection in adult cattle may persist for years, sometimes for life, in contrast to infections with other serovars, which rarely persist for more than 18 months. Dairy cows may persistently shed *Salmonella*, especially *S.* Dublin, in milk and cause infections in humans who drink raw milk. The morbid changes in adult cattle correspond to those in calves except that there is more pleural hemorrhage and the enteritis may be more hemorrhagic and fibrinous. The histologic changes in the liver and other organs are the same as those seen in calves.

Of serious concern for both cattle and humans is the emergence of a new multidrug-resistant strain of *S.* Typhimurium. This new strain, known as *Salmonella* Typhimurium definitive phage type 104 (DT104), was first seen in the UK in the late 1980s. It has now spread worldwide and in some parts of the USA is the leading cause of bovine salmonellosis. The potential for this strain to emerge as a major foodborne pathogen has caused great consternation among both agricultural and public health communities.

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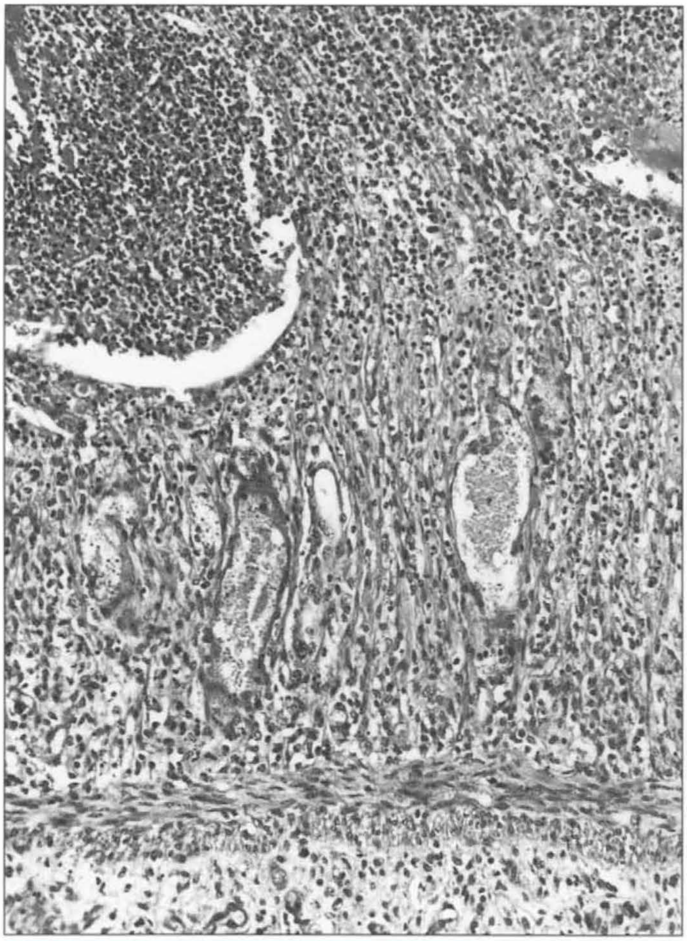

Figure 1.150 Eroded ileal mucosa, largely devoid of crypts of Lieberkühn, fibrin, and neutrophils in lumen, forming diphtheritic membrane, in a calf with **salmonellosis**.

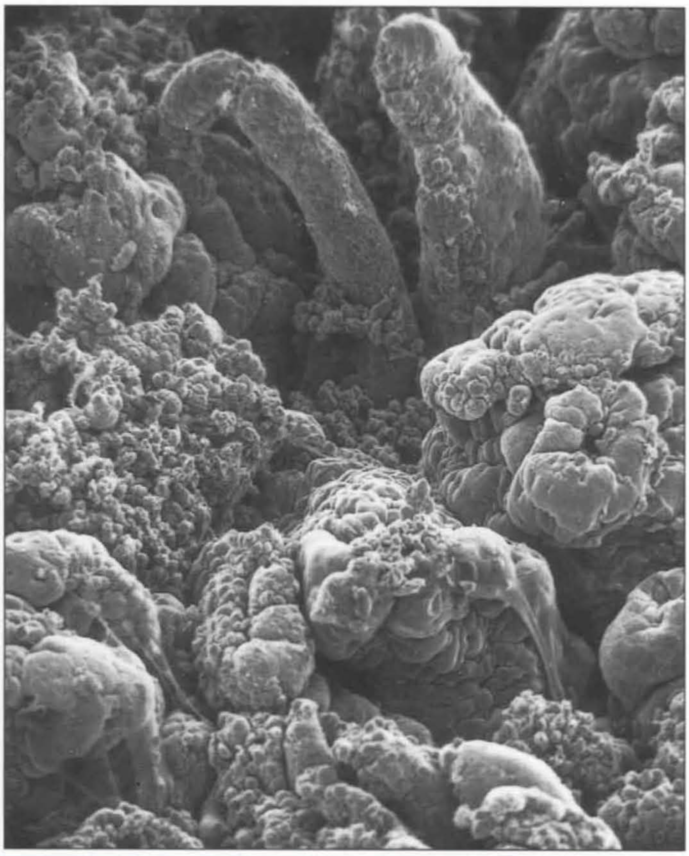

Figure 1.151 Scanning electron micrograph of the ileum of a calf 12 hours postinoculation with **S. Typhimurium**. Villi are atrophic, and rounded cells are exfoliated from surface. (Courtesy of R Clarke, CL Gyles.)

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Salmonellosis in sheep

As well as abortion caused by *S.* Abortus-ovis, abortion and neonatal death may follow infection of pregnant ewes by any species of *Salmonella*. While the prevalence of *S.* Abortus-ovis in the UK seems to be waning, *S.* Montevideo, on the other hand, has been associated with abortions in several flocks in the British Isles.

Salmonellosis is not a common disease in sheep, but outbreaks are always severe and may cause very heavy losses. Predisposing influences are necessary, and these are usually provided by circumstances that enforce congregation. Deprivation of food and water for 2–3 days may be sufficient and, coupled with fatigue, is the usual predisposing factor when sheep are transported or confined in holding yards. Deaths usually continue for 7–10 days after debilitating circumstances have been remedied.

The serovars usually found in sheep are ***S.* Typhimurium**, ***S.* Arizonae**, and ***S.* Enteritidis**. *Salmonella* Dublin is increasing in prevalence in the UK and the midwestern states of the USA. Experimental inoculation of sheep with *S.* Arizonae produces infection but rarely disease. Under natural conditions this host-adapted organism is frequently considered to be an infection secondary to some other disease, or an incidental finding in apparently healthy animals. Most serovars produce the same sort of disease, which closely resembles that seen in cattle both clinically and at autopsy. The major findings are fibrinohemorrhagic enteritis, and septicemia.

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Salmonellosis in carnivores

Salmonella can often be recovered from apparently healthy dogs and cats. However, primary disease rarely occurs. *Salmonella* has been recovered in high frequency from normal sled dogs lacking clinical disease. Raw meat fed to dogs has been shown to have a high incidence of *Salmonella* contamination. In dogs and cats, nosocomial infections are sometimes associated with hospitalization and antibiotic therapy. In dogs, salmonellosis may be secondary to canine distemper. It can cause bronchopneumonia, acute hemorrhagic gastroenteritis, swelling of the spleen and mesenteric lymph nodes, serosal hemorrhages, and foci of necrosis in the liver and other organs. Septicemia in puppies has been associated with *S.* Dublin infection. Salmonellosis has been reported in dogs with lymphosarcoma, shortly after the initiation of chemotherapy. The immunosuppressive effect of the treatment, or depressed cell-mediated immunity, probably predisposes to the development of disease.

Various serovars have been isolated from cats and most of these appear to cause subclinical infections. However, salmonellosis may be a problem in catteries and hospitals, affecting animals that are subjected to stressful conditions. Immunosuppression associated with *Feline leukemia virus, Feline immunodeficiency virus, Salmonella*-contaminated panleukopenia vaccine, or other intercurrent diseases is thought to predispose to salmonellosis in cats. *S.* Typhimurium is most commonly associated with such outbreaks. The disease is characterized by gastroenteritis and septicemia or a more chronic, nonspecific febrile illness, with neutrophilia and left shift. Conjunctivitis and abortions have also been associated with *Salmonella* infection in cats.

Because of their close association with humans, especially children and the aged, dogs and cats that are carriers are a potential source of zoonotic infection.

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Yersiniosis

Yersinia enterocolitica and *Y. pseudotuberculosis* are gram-negative organisms that, in domestic animals, cause *enterocolitis, mesenteric lymphadenitis*, and, less commonly, *septicemia*. There are sporadic reports of placentitis with abortion or perinatal mortality, epididymitis-orchitis, and mastitis in sheep and goats, and of abortion and pneumonia in cattle. *Y. pestis*, the cause of plague in animals and humans, is not considered here.

The *epidemiology* of yersiniosis is complex and poorly understood. These organisms may be shed in the feces by asymptomatic animals in the herd or flock, and by other species, such as rodents and birds, in the environment. The organisms can survive and grow in the environment at low temperatures, and in cool weather environmental contamination by *Yersinia* spp. may be considerable, resulting in significant oral challenge. Disease may in part be due to compromise of cell-mediated immunity, permitting establishment of invading organisms, or recrudescence of latent infection. Often, outbreaks occur under stressful circumstances, such as poor weather, flooding, after transport, during the breeding season, or in animals on a poor plane of nutrition.

Subsequent to ingestion, *the organisms invade through the intestinal epithelium or M cells* overlying Peyer's patches and attain the lamina propria or submucosal lymphoid follicles. Enormous recruitment of neutrophils and ensuing destruction of cytoarchitecture of the Peyer's patch and overlying epithelium result in formation of suppurative foci in place of follicles in Peyer's patches, and microabscesses in the lamina propria of the small or large intestine if invasion occurs elsewhere. *Yersinia* disseminates via lymphatics and hepatic portal venous drainage to mesenteric lymph nodes or to liver and the systemic circulation.

A number of *virulence factors* are recognized; all are related to the ability to invade cells or to evade the inflammatory response. These include expression of an adhesin termed invasin, which binds to beta-1 integrins expressed on the surface of intestinal M cells, promoting uptake of *Yersinia* by these and normally nonphagocytic cells, and causing epithelial cells to produce cytokines chemotactic for inflammatory phagocytes. A plasmid-encoded type III secretion system translocates effector proteins termed "yops" from bacteria into host cells. These enable invasive *Yersinia* to remain extracellular, evading phagocytosis and killing by neutrophils and macrophages by a variety of mechanisms. Although *Yersinia* reside extracellularly as microcolonies in suppurative foci in the lamina propria and lymph nodes, at least some appear to reside intracellularly, since a T-cell-mediated immune response is required to clear infection. Giant cells wall off foci of infection in subacute to chronic lesions, hence the specific name *pseudotuberculosis*.

Disease may be gradual in onset, subtle and chronic, producing a syndrome of diarrhea and ill-thrift, in cattle, sheep, and goats. Mild diarrhea with low mortality has been reported in Australia in weaned pigs with *Y. pseudotuberculosis*. More fulminant disease, characterized by severe, sometimes hemorrhagic, diarrhea, systemic infection, and prostration, may occur in cattle, some species of deer, especially chital and red deer, water buffalo, and exotic ungulates. Yersiniosis is an apparently uncommon cause of diarrhea, and occasionally fatal enterocolitis, mesenteric lymphadenitis, and systemic infection in carnivores. *Yersinia* also causes sporadic pneumonia and septicemia in foals.

Yersiniosis has been described worldwide, as a cause of disease in sheep, cattle, goats, deer, and pigs. The lesions of *Y. pseudotuberculosis* and *Y. enterocolitica* cannot be differentiated reliably grossly or microscopically.

In all species, **gross lesions** in clinically subacute to chronic yersiniosis may be mild. They are usually limited to abnormally fluid intestinal content, with congestion, edema, roughening, and perhaps small foci of pallor, focal hemorrhages, erosion, or mild ulceration and fibrin effusion. Raised nodules up to 5 mm in diameter, with depressed centers, may be evident in affected large bowel. Mesenteric lymph nodes are enlarged, congested, and edematous, perhaps with foci of necrosis. There may be mild fibrinous cholecystitis, and pale foci of necrosis scattered in the liver.

The infection is characterized **histologically** by *masses of gram-negative coccobacilli forming microcolonies*, in the lamina propria of villi and around the necks of crypts in the distal half of the small intestine, in Peyer's patches, and in the superficial mucosa of the large intestine. Intense local infiltrates of inflammatory cells, predominantly neutrophils, accumulate to form *microabscesses* up to about 300 μm in diameter around the bacteria, and effuse into the lumen through microerosions on the mucosal surface or in crypts. Small crypt abscesses may be present. In small intestine, there may be moderate atrophy of villi and hyperplasia of crypts, associated with increased infiltrates of chronic inflammatory cells. Microabscesses, or pyogranulomas surrounded by macrophages or giant cells, and sometimes containing bacterial microcolonies, may be present in the subcapsular and medullary sinuses of mesenteric lymph nodes.

In fulminant *Yersinia* infection in all species, there is *fibrinous or fibrinohemorrhagic enterocolitis*, with heavy local mucosal colonization by masses of coccobacilli, and marked neutrophil infiltration. Peyer's patches may be particularly involved, with grossly visible foci or confluent masses of caseous necrotic debris, as may be found in draining mesenteric lymph nodes, which are enlarged. There may be serosal hemorrhages on the gut, fibrinous peritonitis, and pleuritis, and foci of necrosis may also be present in the liver, lungs, and occasionally other parenchymatous organs; the characteristic microcolonies of coccobacilli are usually evident in them.

Caseous mesenteric lymphadenitis, with mature pyogranulomas containing microcolonies of bacteria, surrounded by neutrophils and giant cells, may occasionally be found as an incidental lesion, or in animals with *Yersinia* abscesses in other organs.

Yersiniosis is **diagnosed** in tissue section by finding characteristic microcolonies of coccobacilli in microabscesses (Fig. 1.152), and is confirmed by bacterial isolation. Microscopic lesions may not be detected in the intestinal mucosa of some clinically affected animals from which isolates are made, perhaps because lesions are patchy. Since *Yersinia* spp. are psychrophiles, cold enrichment and culture at temperatures below 37°C are used in their isolation.

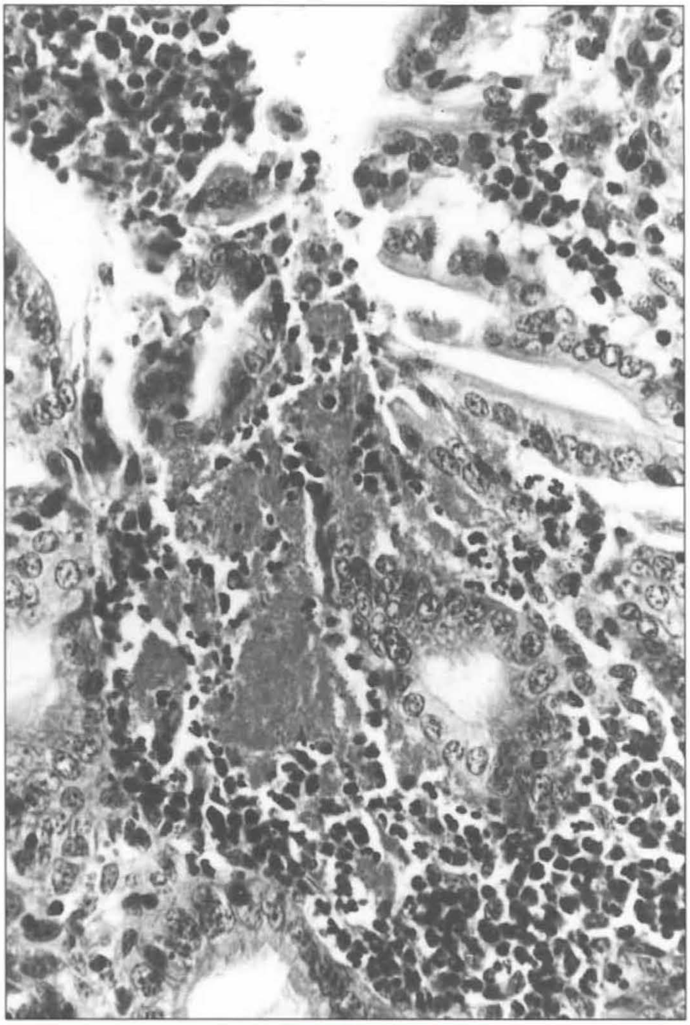

Figure 1.152 Microabscess around colony of ***Yersinia*** in lamina propria of the colon in a sheep. Note effusion of neutrophils through mucosal microerosion into lumen. (Courtesy of R Mason, Tasmanian Dept Primary Industry.)

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Lawsonia intracellularis infection

Lawsonia intracellularis infects a variety of species, including swine, horses, donkeys, deer, hamsters, guinea pigs, mice, rabbits, foxes, dogs, ferrets, several species of nonhuman primates, ostrich, and emus. In most, it causes a *characteristic proliferative lesion of cryptal epithelium in distal small intestine and/or large bowel*, associated with a diarrheal syndrome, ill-thrift, and, in emus, rectal prolapse.

L. intracellularis is a gram-negative, curved or S-shaped rod, that is microaerophilic and nonflagellated. It is an *obligate intracellular organism*; hence, cultivation requires the use of tissue culture.

Lawsonia is prevalent in swine worldwide, and it is in this species that infection is most important. Once infected, pigs shed the organism for weeks.

Formerly referred to as *intestinal adenomatosis complex*, and characterized by a number of syndromes (*porcine intestinal adenomatosis, necrotic enteritis, regional ileitis, and proliferative hemorrhagic enteropathy*), the conditions in swine now known to be caused by *L. intracellularis* are lumped as **porcine proliferative enteropathy** (PPE). The multiplicity of names reflects the fact that the etiology of these syndromes eluded definition for decades. PPE was associated with *Campylobacter mucosalis* or *C. hyointestinalis*, until it was finally reproduced by a newly discovered bacterium, "ileal symbiont intracellularis," the taxonomy of which was formalized as *Lawsonia intracellularis* in 1995. Although proliferative enteropathy due to *L. intracellularis is primarily a disease of swine*, it has been reported uncommonly in a number of other species, the most important among domestic animals being the *horse*, in which it mainly affects animals 3 months to 2 years of age.

Disease occurs most commonly in feeder pigs. However, piglets as young as 3 weeks of age, and adults, may have lesions of PPE. Clinical effects may vary from subtle subclinical disease with a mild decrease in growth rate to diarrhea and unthriftiness. Animals with extensive lesions may have anorexia, intermittent or persistent diarrhea, and severe weight loss. Death may follow a period of diarrhea and progressive cachexia, or it may occasionally occur as a result of perforation of an ulcerated intestine, or through peracute hemorrhage. Mortality may be very high.

The **pathogenesis** is dependent on undefined interactions with other bacteria in the gut, because gnotobiotic pigs inoculated with *L. intracellularis* fail to develop disease, whereas conventional pigs are quite susceptible. The pathogenicity of *L. intracellularis* is related to its active uptake by epithelial cells, in which they replicate, and cause to become hyperplastic. It has been theorized that it disrupts intestinal cell differentiation, but specific virulence attributes of *L. intracellularis* are poorly understood, as are molecular details of the agent–host cell interaction. In PPE, infection of cells lining mucosal glands may occur initially in the vicinity of Peyer's patches and mucosal lymphoid aggregates in the ileocecocolic region. In some experimental studies, lesions were first seen here, and in mildly affected spontaneous cases, lesions sometimes seem associated with these structures preferentially. Later, cells in cecal and colonic crypts become infected. *L. intracellularis* organisms internalized within membrane-bound vesicles are released into the apical cytoplasm of glandular epithelium where they lie free and replicate. Cell division is required for bacterial replication, which may explain its tissue tropism. Bacteria are passed on to daughter epithelial cells and exit via extrusion from the cytoplasm of enterocytes on villi or between crypt openings.

Infected epithelium is transformed to a population of highly mitotic cells, and goblet cells disappear. Glands are lined by crowded, dysplastic pseudostratified columnar epithelium, with basophilic cytoplasm (Fig. 1.153A). Nuclei may be open and vesicular with prominent nucleoli, or laterally compressed. The cytologic and biochemical characteristics of these cells suggest that they are poorly differentiated. Infected glands become elongate, dilated, and branching, causing thickening of the mucosa. Isolated plaques of affected mucosa may project above adjacent tissue. The use of the term "*adenomatosis*" to describe such a change is obvious. Hypertrophic glands sometimes protrude into lymphoid tissue in the submucosa. Occasionally, microscopic foci of adenomatous epithelium may be found in submucosal lymphatics or the regional lymph node.

Villi in infected small intestine undergo progressive atrophy, so that they may be entirely absent in well-established lesions. However, the proliferative lesion in the crypts seems primary, and not a hyperplastic response to increased epithelial exfoliation. Adenomatous areas merge sharply with adjacent normal mucosa. Masses of *L. intracellularis* are readily recognized in silver-stained tissue sections, as *curved rods*, infecting especially the *apical cytoplasm of cells in adenomatous glands* (Fig. 1.153B). These organisms have also been identified ultrastructurally in degenerate cells and macrophages in the lamina propria. Proprial and submucosal inflammation in areas of uncomplicated adenomatosis is not marked.

In the least complicated forms of the disease, **lesions** *are always found in the terminal portion of the ileum*, extending proximally from the ileocecal-colic orifice for usually less than a meter, though sometimes they can be found more cranially. In a proportion of cases, they occur in the cecum and proximal third of the spiral colon, but not without ileal involvement. In mild cases, which are likely to be subclinical, only a few ridge or plaque-like thickened areas project above the remainder of the mucosa. However, more typical widespread lesions cause the thickened mucosa to form irregular longitudinal or transverse folds or ridges. The surface may be intact, but commonly, small foci of fibrin exudation or necrosis may be evident.

Thickening of the adenomatous mucosa, and perhaps some edema of the submucosa, is reflected in accentuation of the normal reticular pattern on the serosa of the ileum (Fig. 1.154A). This results in a *cerebriform or gyrate pattern of projections and depressions on the serosal aspect of the intestine which is readily recognized, and virtually pathognomonic for this condition.* Mucosal lesions in the large intestine often form thickened plaque-like or almost polypoid masses, which may be confluent in

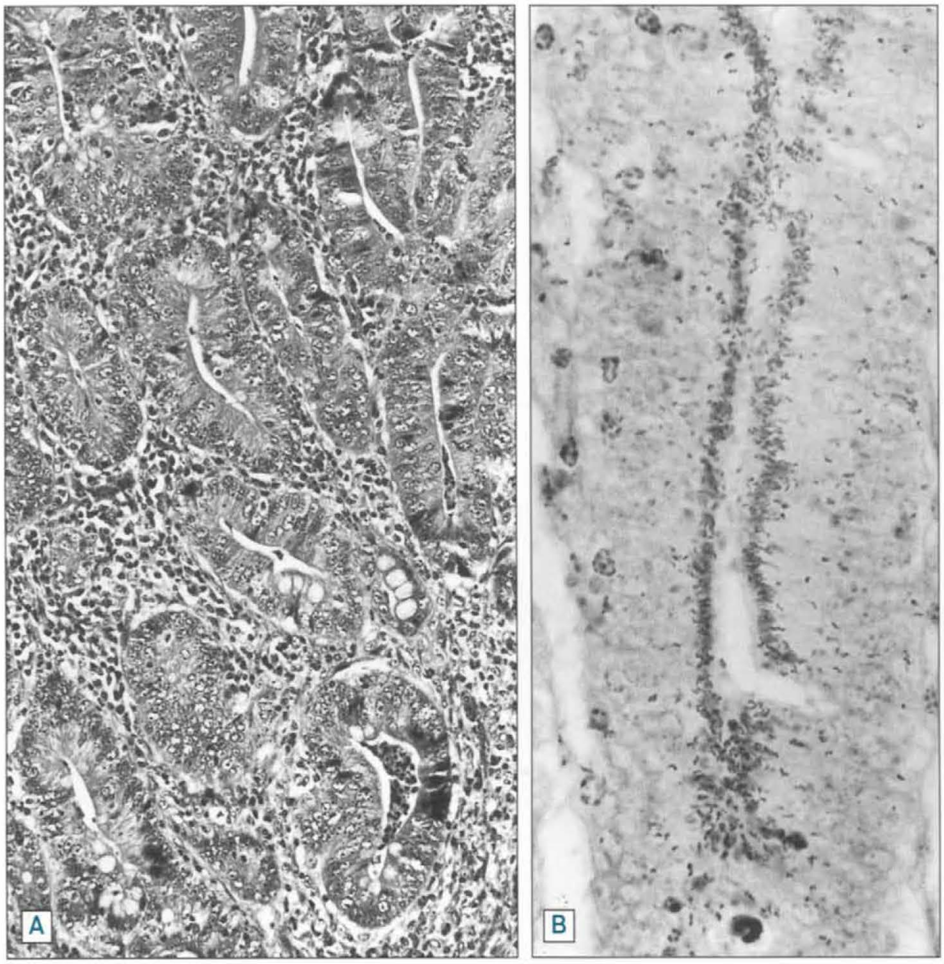

Figure 1.153 Porcine proliferative enteropathy. A. Adenomatous change in glands in the lamina propria. **B.** Masses of silver-stained ***Lawsonia* organisms** in cytoplasm of hyperplastic epithelium lining intestinal glands.

some areas, and are often eroded, with fibrin exudation (Fig. 1.154B). Serosal folds may be evident on extensively affected large intestine. The ileocolic lymph nodes are enlarged and hyperplastic.

Coagulative necrosis of adenomatous mucosa commonly occurs and is referred to as **necrotic enteritis**. It may be partly the result of pathogenic anaerobic large-bowel flora, colonizing the affected terminal ileum and large intestine. There may be effusion of fibrin from superficial lesions, and a pseudodiphtheritic membrane or luminal fibrin cast may be present. Caseous yellow-brown or blood-tinged necrotic mucosa may be found focally or widely in the distal ileum and proximal large intestine. The cerebriform pattern of serosal folding is evident in such cases (Fig. 1.155). While necrotic enteritis may be a sequel to other enterocolitides in swine, *adenomatosis is the most common primary lesion.*

Microscopically, coagulative necrosis of the mucosa may be focal and superficial, with local effusion of neutrophils and fibrin into the lumen, and an acute inflammatory infiltrate at the margin of the necrotic tissue. Frequently, necrosis extends to involve most the thickness of the mucosa, sometimes penetrating to the submucosa. A few islands of viable adenomatous crypts or glands may be left deep among the necrotic debris. Masses of bacteria, presumably fecal anaerobes, are found superficially in the necrotic tissue. With time, granulation tissue develops in ulcerated areas. Tissue in the upper ileum at the proximal margin of the zone of mucosal necrosis should be examined for adenomatosis since, in severe cases of necrotic enteritis, no remnants of such mucosa may persist elsewhere.

As the distal ileum experiences bouts of proliferation, necrosis, and ulceration, such granulation of ulcerated gut may result in progressive stricture of the lumen. This is the syndrome of PPE known as **regional ileitis**. There is often hypertrophy of the external muscle layer. Idiopathic ileal muscular hypertrophy also occurs in swine, apparently independent of antecedent PPE.

Acute or subacute intestinal hemorrhage and anemia occur in PPE, and were considered a distinct syndrome, **proliferative hemorrhagic enteropathy**, within the porcine intestinal adenomatosis complex. Animals may exsanguinate so quickly as to die without passing blood. Others pass dark-red feces for several days. This syndrome is more common in young adults, rather than growing pigs. It is usually sporadic, or of relatively low morbidity, but up to half the clinically recognized cases may die.

Animals dead of this *massive intestinal hemorrhage* are pale, and the perianal area may be smeared with blood. The typical cerebriform pattern is evident on the external surface of the distal ileum, which is thickened and turgid (Fig. 1.156A). Fluid blood or a loose or firm fibrin and clotted blood may be present in the ileum (Fig. 1.156B), and the contents of the cecum and colon may contain dark bloody digesta and feces. The mucosa of the affected ileum usually resembles that in uncomplicated PPE, and overt points of hemorrhage or ulceration are rarely discernible grossly. Rather the animals appear to suffer widespread diapedesis from the mucosa.

In tissue sections from animals dead with the hemorrhagic syndrome, there are extensive erosion and necrosis of proliferative

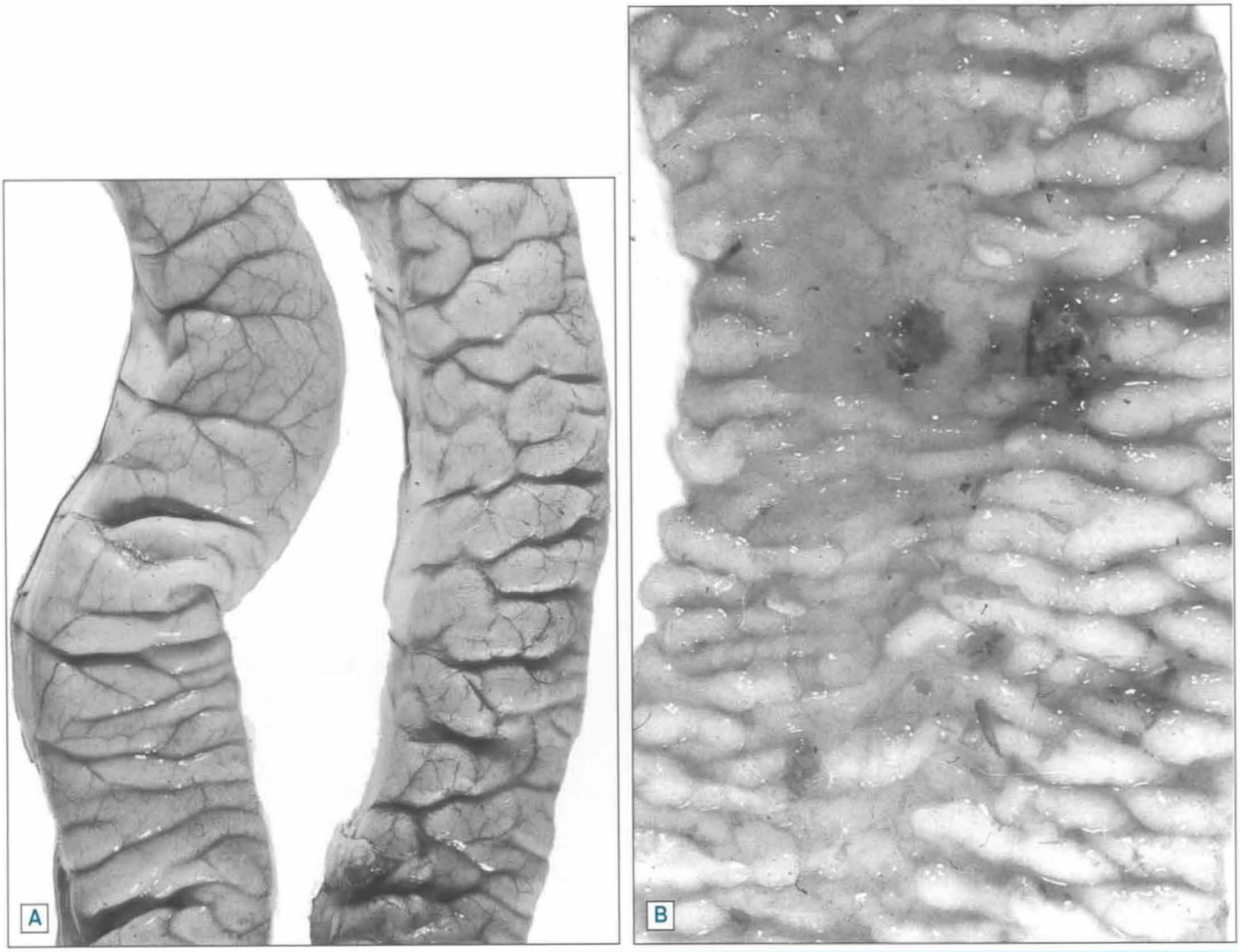

Figure 1.154 Porcine proliferative enteropathy. A. Exaggerated **reticular pattern of folds** on serosal aspect of the ileum. **B.** Raised nodular or **ridge-like areas of thickened mucosa** in the ileum, resulting from hypertrophy of glands.

epithelium in the superficial mucosa (Fig. 1.157). An acute inflammatory infiltrate is present in the upper lamina propria, small vessels are thrombosed, and heavy effusion of neutrophils on to the mucosal surface and into lumina of glands is evident. Fibrin and hemorrhage emanating from superficial mucosal vessels are in the intestinal lumen. More extensive coagulative necrosis of the mucosa is occasionally associated with this hemorrhagic clinical entity of PPE.

In **other species**, the lesions and pathogenesis of **proliferative enteritis** seem similar to those in PPE. In all species, including swine, *diarrhea is probably related to loss of functional mucosal surface area in distal small intestine and large bowel, while ill-thrift or wasting syndromes are attributable to protein-losing enteropathy*. Clinical signs and lesions associated with hypoproteinemia predominate in young horses, and diarrhea is an inconsistent finding, perhaps related to the large absorptive capacity of the equine colon. In horses, adenomatosis may involve a considerable amount of the small intestine.

A presumptive **diagnosis** of PPE in swine, and of proliferative enteritis in other species, can be based on typical gross and histologic lesions, accompanied by Warthin–Starry staining to visualize the intracellular bacteria. Confirmation can be obtained by immunohistochemistry using a specific antibody for *L. intracellularis* or by specific polymerase chain reaction.

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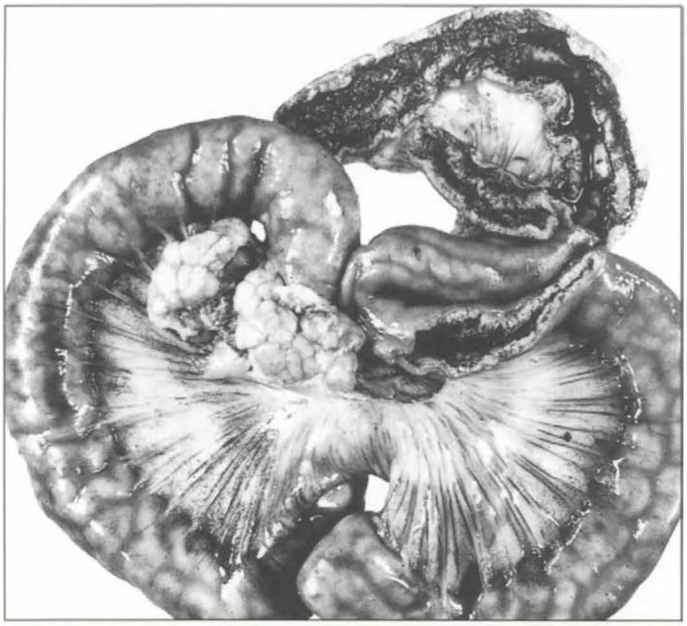
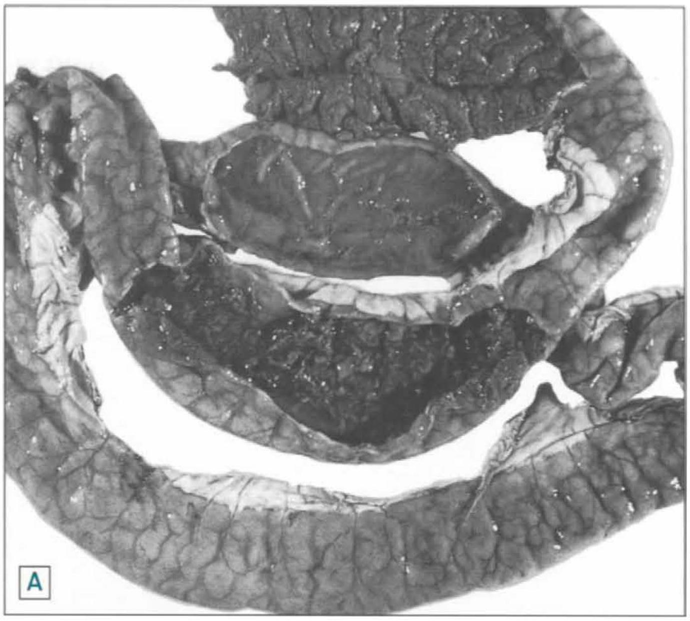


Figure 1.155 Necrotic enteritis, thick ileal wall, enlarged lymph nodes, and opaque mesentery, in **porcine proliferative enteropathy**.



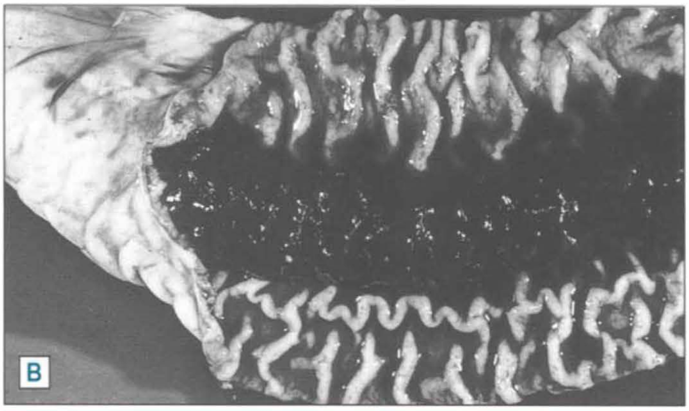


Figure 1.156 Proliferative hemorrhagic enteropathy form of porcine proliferative enteropathy. **A.** Folded necrotic mucosa, and fibrinohemorrhagic exudate in terminal ileum. **B.** Hemorrhage and blood clot on the nodular folded mucosa of the terminal ileum.

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Enteritis associated with Campylobacter spp.

Campylobacter jejuni and ***C. coli*** are common causes of diarrhea in humans, and *C. jejuni*, at least, is also capable of causing enteritis in animals. *C. jejuni* penetrates the surface mucus layer and adheres to the epithelium. Virulent strains enter cells by directed endocytosis caused by the products of a type III secretion system translocated from the bacteria into the cytoplasm of the cell. They often attain the lamina propria, inhabiting inflammatory cells there, from which organisms ultimately reach the systemic circulation. Many human infections are acquired by drinking raw milk, or from other animal foodstuffs, especially poultry products. *Chickens* are common asymptomatic shedders of *C. jejuni*; *C. coli* comes more commonly from *swine*.

C. jejuni has been associated with diarrhea characterized by the presence of blood and mucus in some dogs, despite the fact that it can often be isolated from a high proportion of asymptomatic animals. It has also been isolated from dogs with parvoviral enteritis and other viral infections; pre-existing infections may predispose to the development of pathologic effects of *C. jejuni*. The role of *Campylobacter* as a significant primary pathogen in dogs has been demonstrated in a few cases. These usually involve communal housing situations and often young dogs. *C. jejuni* can be implicated as the cause of lymphoplasmacytic inflammation centered on ileum and colon by ruling out other known etiologies, and demonstrating the organism in large numbers in association with the lesion. *Mild to moderate enteritis and colitis* has been described in naturally infected dogs, while in

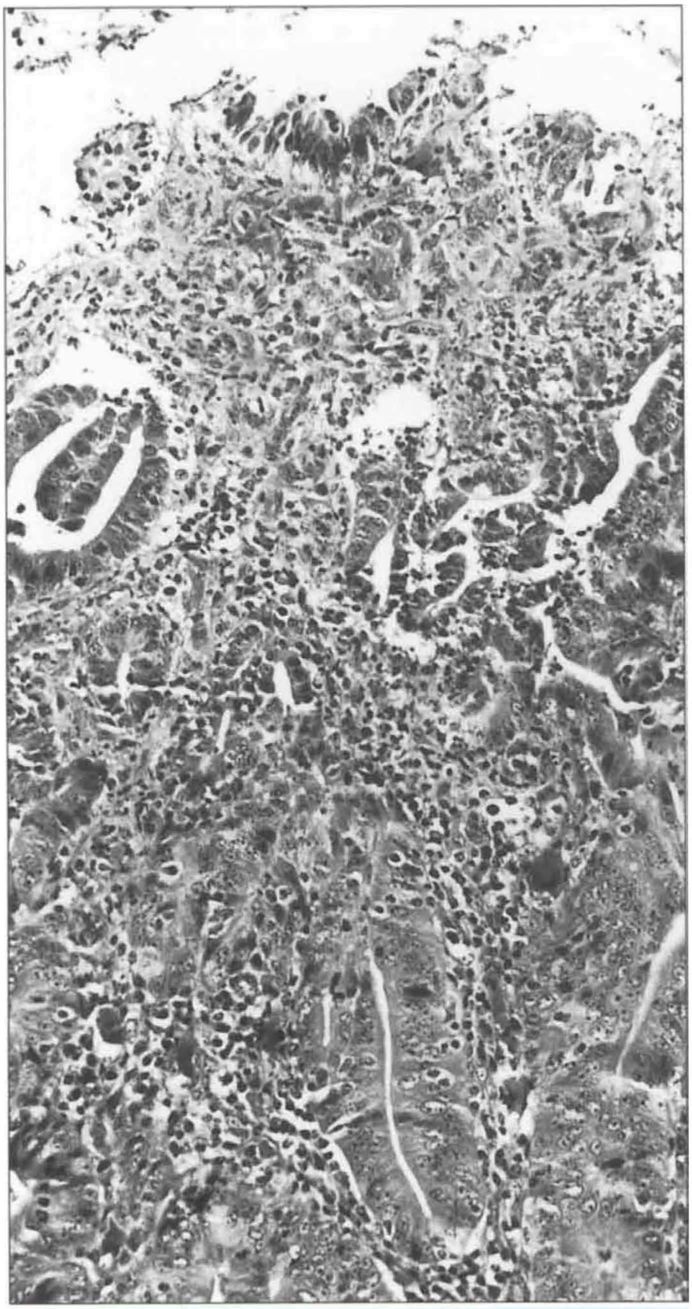

Figure 1.157 Proliferative hemorrhagic enteropathy, with superficial necrosis of mucosa associated with thrombosis of small vessels, hemorrhage, and effusion of fibrin and neutrophils, in porcine intestinal adenomatosis complex.

experimentally infected gnotobiotic and conventional dogs, lesions are limited to mild mucosal colitis. Erosive colitis has been associated with *C. jejuni* infection in mink, and was reproduced experimentally. Cats are merely asymptomatic carriers of *C. jejuni*.

"Weaner colitis" in sheep is a diarrhea of high morbidity and low mortality, reported from southeastern Australia, and associated with an unidentified *Campylobacter* species, not *C. jejuni*. At necropsy, there is abnormally fluid colonic content, but mucosal lesions are not seen; chronically affected animals may have edema and loss of body condition suggestive of enteric protein loss. Microscopically, there is erosive typhlocolitis. Goblet cells are depleted in number, and multifocal erosion, or occasionally, ulceration, of the mucosa is evident. Consistently, a layer of bacteria is present covering surface epithelial cells and in crypts. The disease has been reproduced by inoculation of the thermophilic catalase-negative *Campylobacter*-like organism, which is isolated from spontaneous cases on modified mycoplasma agar base medium.

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Spirochetal colitis in swine

Swine dysentery, caused by ***Brachyspira*** (formerly *Serpulina, Treponema*) ***hyodysenteriae***, is a well-recognized production-limiting disease of swine worldwide, characterized by *large-bowel diarrhea* with mucus, blood, or fibrin in the feces. It is now recognized that at least one other spirochete, ***B. pilosicoli***, can cause colitis in feeder swine, in a disease referred to as *porcine colonic spirochetosis*.

Swine dysentery occurs as a highly infectious disease, mainly of weaned pigs. As long ago as 1924, swine dysentery was known to be experimentally transmissible by dosing young pigs with colonic contents from affected pigs. *B. hyodysenteriae* is a gram-negative, anaerobic but oxygen-tolerant, *spirochete*, 6–9 μm long and 0.4 μm in diameter. It produces strong beta hemolysis on blood agar plates. The organism is motile, moving in serpentine fashion; it is loosely coiled and has 7–13 periplasmic flagella. Originally thought to have a very narrow host range, *B. hyodysenteriae* has also been associated with necrotizing typhlocolitis in rheas.

Swine dysentery can be reproduced by feeding pure cultures of *B. hyodysenteriae* to specific-pathogen-free and conventionally reared swine. Experimental reproduction of the disease in gnotobiotic pigs requires the presence of anaerobic bacteria indigenous to the normal colon, along with *B. hyodysenteriae*. There is apparently a *synergistic action between the spirochete and other anaerobes*, mainly *Bacteroides* and *Fusobacterium*. Several studies have documented the importance of a diet low in fiber and high in rapidly fermentable carbohydrates for the successful establishment and pathogenicity of *B. hyodysenteriae*.

The **pathogenesis** of swine dysentery is still incompletely understood. The only virulence factor consistently associated with

B. hyodysenteriae is the production of hemolysin, and how or whether it contributes to disease is unclear. Other potential virulence attributes await definitive correlation with severity of disease.

B. hyodysenteriae colonizes the mucus on the mucosal surface, in the lumen of colonic glands, and in goblet cells. Lesions, including exfoliation of surface epithelium, are associated with the presence of *large numbers of spirochetes and other anaerobic bacteria on the mucosa.* By in situ hybridization, large numbers of *B. hyodysenteriae* can be seen colonizing the mucus layer, the surface epithelium, and the crypts. Their association with epithelium appears random. Attachment of spirochetes to host cells has not been observed in vivo, and invasion is not essential for epithelial loss to occur. When spirochetes invade surface epithelial cells, they appear to do so through lateral membranes, and do not attach to and penetrate the luminal membrane. *Brachyspira* do not usually invade beyond the epithelial cells. The factors responsible for local necrosis or exfoliation of superficial epithelium in *B. hyodysenteriae* infection are unknown. However, the suite of associated lesions – hyperplasia of the proliferative compartment in crypts; goblet-cell hyperplasia; premature exfoliation of surface epithelium between crypt openings; and an associated mixed mucosal inflammatory cell infiltrate – is reminiscent of the changes in cell-mediated villus atrophy in the small intestine, raising the possibility that the lesion is at least in part immune-medicated.

The result is *mucosal colitis,* characterized by superficial erosion, with hyperplasia of cells in colonic glands, hypersecretion of mucus, and a mixed inflammatory infiltrate in the lamina propria. Thrombosis of capillaries and venules in the superficial areas of the colonic mucosa and in the gastric fundic mucosa (gastric venous infarction) is probably due to absorption, through the damaged mucosa, of endotoxin released by gram-negative bacteria.

The diarrhea in swine dysentery is due to *malabsorption of fluids and electrolytes in the colon.* This presumably results from damage to the superficial colonic epithelium. The normal colon of the pig has tremendous absorptive capacity. Interference with colonic absorption results in severe diarrhea and dehydration. Active fluid secretion by the colon, associated with bacterial enterotoxins, does not occur in swine dysentery. Fluid and electrolyte transport are normal in the small intestine.

There is usually an introduction of pigs, presumably *carriers,* into a herd prior to an outbreak. Once established in a herd, the infection tends to remain *enzootic,* and although treatment can effect a rapid clinical amelioration, it may not be curative, and relapses at greater or lesser intervals can occur. Apparently infection is not followed by a substantial immunity, although individual carrier pigs are resistant to further challenge with *B. hyodysenteriae* after recovery from disease. The morbidity may reach 90% and mortality 30%.

The disease occurs in pigs of all ages over ~2–3 weeks old, but particularly in pigs 8–14 weeks of age. Once initiated, it spreads rapidly by pen contact. The disease is initially febrile, but, with the onset of diarrhea, fever tends to subside. The initial diarrheic feces are thin, semisolid, and without blood or mucus; it is usually only after 1–2 days of diarrhea that blood and mucus appear in the feces. Some pigs die peracutely without showing diarrhea, and many that show diarrhea do not have dysentery, but pass feces which contain much mucus.

Grossly, pigs that die of swine dysentery are usually gaunt with a contracted abdomen; the eyes are sunken; and there may be blue discoloration of the abdominal skin. Associated lesions may include pericardial serous effusion, and intense congestion of the gastric mucosa due to venous infarction. The intestinal lesions, especially in young pigs dying acutely, and those that have been treated, can be easily overlooked because the mucosal colitis may be mild, patchy, and often more catarrhal than fibrinous.

In typical cases, dehydration gives a semiopaque ground-glass appearance to the serosa, and the wall of the cecum and colon is thickened. The colonic content in these cases is usually scant, and porridge-like dirty gray to red-brown and greasy in appearance. The mucosa, with patchy foci of light fibrin exudation, has the velvety thickening of catarrhal secretion (Fig. 1.158A). The most severe lesions approach those of salmonellosis in the extent and severity of fibrin effusion. The production of mucus in swine dysentery becomes copious in many chronic cases due to *remarkable goblet-cell hyperplasia.*

The earliest **microscopic lesions** are characterized by discrete areas of epithelial erosion on the superficial mucosa. Thin layers of fibrinocellular exudate cover the eroded areas. In more advanced cases, these areas become more diffuse but remain merely erosive, and exudation is more copious (Fig. 1.158B). There may be minor bleeding from small vessels in eroded mucosa. Fibrin thrombi are evident in the capillaries and venules of the superficial lamina propria. There is usually some edema of the lamina propria, submucosa, and serosa. Initially mucus is expelled from the basilar portions of the crypts (Fig. 1.159A). In concert with the increased turnover of epithelial cells associated with the superficial erosion, there is hyperplasia of cells deeper in the glands. The crypts are elongated, lined by proliferative basophilic epithelial cells that have large nuclei, and few differentiated goblet cells (Fig. 1.159B). Often, crypts subsequently become dilated and contain necrotic debris. Others have marked goblet-cell hyperplasia, and copious mucus production.

Porcine intestinal spirochetosis is caused by ***Brachyspira* (*Serpulina*) *pilosicoli*** (formerly *Anguillina coli*), which differs from *B. hyodysenteriae* in that it is only weakly beta-hemolytic and is capable of hippurate hydrolysis. *B. pilosicoli* has a wide host range, having been isolated from a number of other species of animals with lesions of intestinal spirochetosis, and it may be zoonotic.

Porcine intestinal spirochetosis has been seen in most major swine-producing areas of the world. The disease is characterized by *generally transient watery to mucoid diarrhea without blood.* Depression of weight gain is a significant clinical finding. Grossly, there may be mesocolonic edema, and the large intestine has abundant watery contents, with variable degrees of mucosal erosion. In more severe cases there may be a diphtheritic membrane.

Virulence attributes of *B. pilosicoli* are poorly defined, but motility and chemotaxis for mucus may be important. The organism is capable of polar attachment by one end of the organism to the apical membrane of enterocytes, resulting in displacement or effacement of microvilli. Histologically, spirochetes can often be seen as a dark fringe or "false brush border" on the luminal surface early in infection (Fig. 1.160). The organism can invade paracellularly, especially at the extrusion zone between colonic crypt units, attaining the lamina propria, where it may be phagocytosed by macrophages; ultimately, in some cases, it reaches draining lymph nodes. A mixed inflammatory infiltrate, including lymphocytes and macrophages, is present in the lamina propria between crypts. Later in infection, when epithelial attachment is rare, the organism persists in crypt lumens and mucus in goblet cells, and goblet-cell hyperplasia is evident, along with inflammatory cells and cellular debris in gland

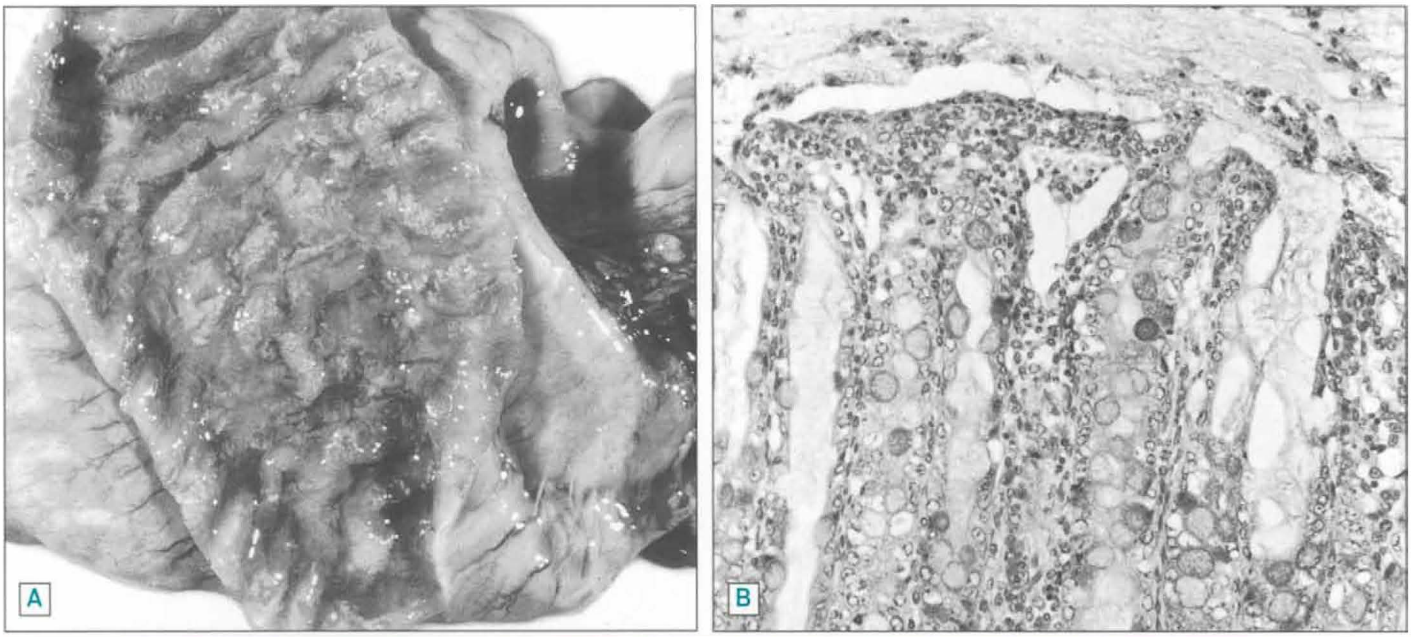

Figure 1.158 Swine dysentery. **A.** Patchy fibrinocatarrhal exudate on the colonic mucosa. **B.** Flattened and exfoliating epithelium on mucosal surface, and edema of superficial lamina propria. Mucus in glands and on surface, mixed with neutrophils and exfoliated epithelium.

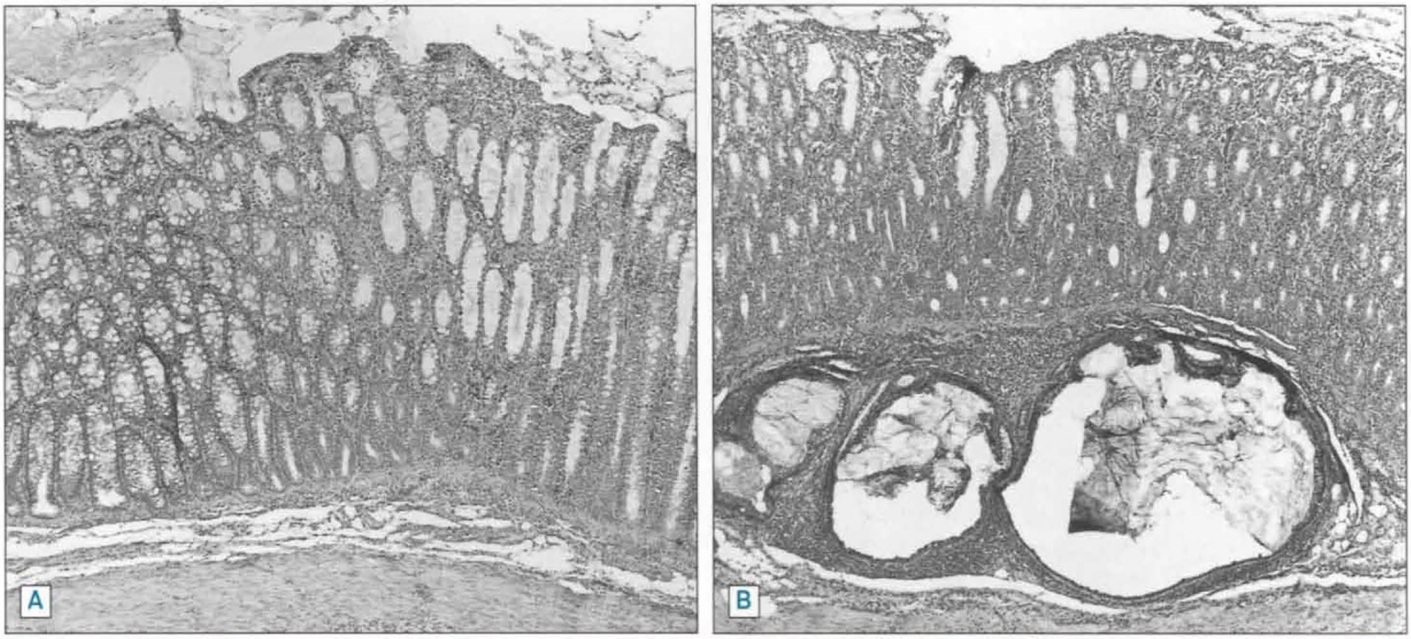

Figure 1.159 Swine dysentery. **A.** Hyperplastic glands with few goblet cells adjacent to mucosa with normal density of goblet cells. Copious mucus on surface. **B.** Hyperplastic glandular lining virtually devoid of goblet cells; "colitis cystica profunda," or herniation of mucous glands into submucosal lymphoid tissue.

lumens. The diarrhea and ill-thrift characteristic of infection may be related to loss of absorptive function, secondary to disruption of the brush border of enterocytes, and increased exfoliation of poorly differentiated cells at intercrypt extrusion zones, perhaps with enteric loss of plasma protein.

In **dogs, colonic spirochetosis** with mucosal colitis has also been described, in association with *B. pilosicoli* and perhaps with other *Brachyspira* spp., but a causal relationship has not been established experimentally.

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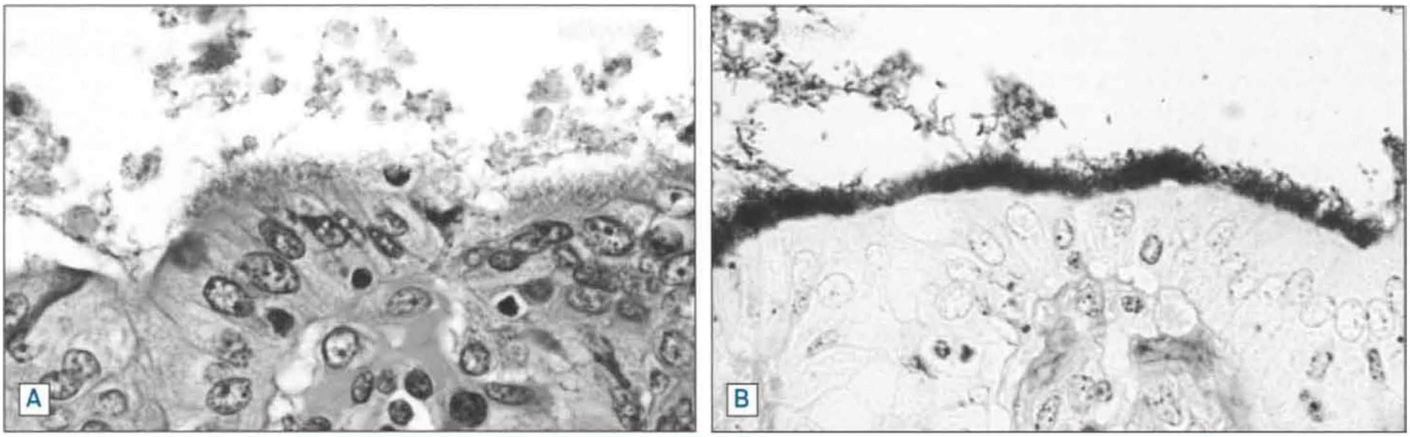

Figure 1.160 Porcine intestinal spirochetosis. A. Fringe of ***Brachyspira pilosicoli*** embedded in the apical border of colonic surface epithelial cells. Remnants of exfoliated epithelium are in the lumen (HE). **B.** *Brachyspira pilosicoli* demonstrated with Warthin-Starry stain.

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Diseases associated with enteric clostridial infections

Most of the important enteric clostridial diseases occur in herbivores and are caused by one of the five toxigenic types of ***Clostridium perfringens***. Enteritis in dogs is associated with *C. perfringens* and ***C. difficile***, and the latter agent is implicated in fibrinous enteritis, especially in horses, neonatal pigs, and dogs. ***C. piliforme*** (formerly *Bacillus piliformis*) causes Tyzzer's disease, characterized by enteritis and colitis, usually with multifocal necrotic hepatitis and myocarditis, in many animal species. ***C. chauvoei*** may affect the tongue and the smooth muscle of the lower alimentary tract, causing blackleg-like myositis (see Vol. 1, Muscle and tendon), while ***C. septicum*** causes clostridial abomasitis (braxy) in sheep and calves, discussed in the section on Stomach and abosmasum, above. ***C. botulinum*** causes toxicoinfectious botulism in horses, and by ingestion of toxin, botulism in cattle (see Vol. 1, Nervous system).

Table 1.2 Relationships among the five types of *Clostridium perfringens* A–E and the four major exotoxins (alpha, beta, epsilon, and iota)

	Toxin			
C. perfringens	Alpha	Beta	Epsilon	Iota
Type A	++	–	–	–
B	+	++	+	–
C	+	++	–	–
D	+	–	++	–
E	+	–	–	++

++ = significant amount of toxin, + = small amount, – = none detected.

There are five types of *C. perfringens*, designated *A–E*, which are differentiated on the basis of their production of the *four major antigenic exotoxins*, which, along with an enterotoxin, are the most significant virulence attributes of *C. perfringens* in the gut. *The major exotoxins are alpha, beta, epsilon, and iota*; the relationships among the five types and the four toxins are illustrated in Table 1.2.

Eight minor toxins are produced by *C. perfringens*, and some of these may be useful in identification of types and in division of types A, B, and C into varieties. Genetic evaluation of clostridial species may ultimately also provide more specific classification. There is not always a clear distinction among the different types of *C. perfringens*. Some strains lose their ability to produce one or more of their toxins when stored or cultured, and this complicates the identification of isolates and the assessment of their significance in disease outbreaks.

The **alpha toxin** is a lecithinase that acts on cell membranes, producing hemolysis or necrosis of cells. The **beta toxin** is a pore-forming toxin that induces a variety of neurologic effects; it appears to have a paralyzing effect on the intestine. The **epsilon toxin** is produced during active growth, as an inactive prototoxin that is activated by enzymic digestion. In culture, the appropriate enzymes (the minor toxins kappa and lambda) may be produced by the organism; in the intestine, trypsin is an effective activator. The **iota toxin** is also elaborated as a prototoxin and activated by proteolytic enzymes either in culture (lambda toxin) or in the intestine; it increases capillary permeability. **Kappa toxin** is a collagenase, and **lambda** a nonspecific proteinase. An additional toxin, **beta$_2$**, which, despite its name, is not related to beta toxin, has been implicated in enteric disease in swine and in typhlocolitis in horses caused by *C. perfringens* types A and C. Its pathogenicity is uncertain, but it may at least act as a virulence marker. Other minor toxins include **mu**, a hyaluronidase, and **delta**, a hemolysin.

Clostridial diseases of the intestine are often called **enterotoxemias**. Disease produced by *C. perfringens* type D, whose epsilon exotoxin is elaborated in the intestine but exerts its important effects on distant organs such as brain and kidney, is a true enterotoxemia. The hemolytic disease attributed to type A is also an enterotoxemia, but *in general the other types produce local intestinal lesions*. The production by some types of *C. perfringens*, especially type A, of an **enterotoxin**, distinct from the classical exotoxins, is potentially confusing. This enterotoxin is only elaborated by sporulating cells and is released upon lysis of vegetative cells. It is produced by all strains, but is not effective in type E. This enterotoxin is not involved in the pathogenesis of enterotoxemia ("*pulpy kidney disease*") caused by type D. Most significant in food poisoning by type A strains in humans, it has also been associated with antibiotic treatment-related diarrhea and infantile diarrhea. It is activated by proteolysis and alters plasma membrane permeability of the mammalian cell.

The **pathogenesis** of enteric infection with *C. perfringens* and *C. difficile* requires a *change in the enteric microenvironment* favorable to massive expansion of luminal populations of clostridia. Such changes may include a change in feed, abnormally nutrient-rich digesta, antibiotic therapy, altered pancreatic exocrine function or trypsin inhibitors, reduced motility, and primary infections with agents such as *Canine parvovirus*, or coccidia in piglets and chickens. These clostridia produce disease in thee general ways: (1) *local necrotizing effects of toxin* on the mucosa, causing hemorrhagic, fibrinous, or necrotic enteritis; (2) *secretory effects of locally acting enterotoxin*, causing diarrhea and minor mucosal lesions; or (3) *systemic absorption of (entero) toxin*, with effects at sites distant from the gut.

Alpha toxin produced by *C. perfringens* type A and beta toxin produced by types B and C probably account for the *severe mucosal necrosis and/or hemorrhage* evident in these infections. Bacteria alone are not pathogenic; exotoxins are required to induce disease. Beta toxin is trypsin-labile, and circumstances such as low enzyme levels in young animals, very high levels of toxin, or trypsin inhibitors could be important. Sows' colostrum contains a trypsin inhibitor, but it is not known if colostrum in other species possesses this factor. "Pig bel," a necrotizing jejunitis of humans in New Guinea that is probably caused by the beta toxin, has been causally related to consumption of heat-stable trypsin inhibitors in sweet potatoes. Naturally occurring protease inhibitors in soybeans appear to have a similar effect in guinea pigs. Intraduodenal inoculation of *C. perfringens* type C in combination with soybean flour produces acute fatal hemorrhagic enterotoxemia in lambs.

C. difficile produces two exotoxins, A, which is an enterotoxin, and B, which is a cytotoxin. Tissue damage is probably due to the effects of both toxins, which glycosylate and inactivate Ras GTPases, disabling signaling pathways in the cell. As well, they glycosylate Rho, which regulates the actin cytoskeleton; it condenses, tight junctions open, cells round up, and undergo apoptosis. They also cause release of proinflammatory mediators, attracting neutrophils, and activate secretion stimulated by the enteric nervous system. Hence, disease is characterized by fluid intestinal content, with patchy areas of colonic epithelial necrosis, through which neutrophils exude into the lumen, producing a so-called "volcano" lesion. Although not all isolates of *C. difficile* are toxigenic, over 20 toxinotypes or ribotypes have been described, based on sequence variations in the genes for the A and B toxin molecules, while 14 serotypes are recognized. Molecular epidemiologic investigation may permit associations of toxinotype with virulence.

C. piliforme, the cause of Tyzzer's disease, as an obligate intracellular agent infects epithelial cells, causing necrotizing and inflammatory lesions. The pathogenesis of Tyzzer's disease is not clearly dependent on toxin production, though some strains do produce cytotoxic proteins.

Diagnosis of disease due to the toxin-producing clostridia is dependent on demonstration of toxin or enterotoxin in gut content or feces of affected animals, by the most specific test available. While the presence of large numbers of a particular type of *C. perfringens* is suggestive of involvement with disease, this organism is commonly present in the gut in a variety of circumstances, where it cannot be implicated as an etiologic agent.

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Clostridium perfringens type A

C. perfringens type A is the *most common of the five types* and is the only one associated with the microflora of both soil and intestinal tract; it may be part of the normal intestinal microflora of domestic livestock species. Its major toxin is the *alpha toxin*; it also produces beta$_2$ toxin and the enterotoxin.

This is one of several clostridia that produce *gas gangrene* in humans and animals. The production of gas gangrene in wound and puerperal infections is probably a composite effect of the major and minor toxins elaborated by the organism. The necrotizing and hemolytic activity of the alpha toxin is assisted by the collagenase and hyaluronidase that disrupt connective tissues and permit the infection to spread. It is also associated with *alimentary syndromes* in domestic animals, including enteritis in foals; enterocolitis in horses; necrotizing enterocolitis in neonatal piglets; enterotoxemia and hemorrhagic enteritis in lambs and neonatal calves; diarrhea, and hemorrhagic enteritis in dogs; as well as necrotic enteritis in chickens. Type A has been suspected of causing disease in cattle but proof is often lacking. Calves inoculated intraruminally with *C. perfringens* type A developed anorexia, depression, bloat, and diarrhea and some of these calves died. Lesions included variable degrees of abomasitis and abomasal ulcers, discussed more fully with gastritis.

In **foals** infected with *C. perfringens* type A, lesions have been more localized to small intestine, the mucosa of which was dark purple. Microscopic lesions were confined to the gut, mainly the small intestine, and were characterized by marked diffuse necrosis of the mucosa. The remnants of necrotic villi were covered by large numbers of gram-positive rods consistent with clostridia. There was also marked hyperemia and hemorrhage of the lamina propria, submucosa and subserosa without a significant leukocytic reaction. Intravenous inoculation of *C. perfringens* type A in ponies resulted in acute colic and hemorrhagic gastroenterocolitis. The syndrome of adult equine clostridial colitis is covered below, in the section on Other clostridial diseases.

C. perfringens type A has been associated with white scours in suckling **pigs** and diarrhea in feeder pigs, although this association is not commonly recognized. The pigs had necrotizing enterocolitis, villus atrophy, and serositis, and enterotoxigenic strains may be associated with the syndrome.

A very rare disease of **calves** and **lambs** characterized by *acute intravascular hemolysis* is also associated with type A infections. Affected animals may be found dead or moribund, and jaundice and hemoglobinuria may be evident clinically. At autopsy, icterus, anemia and other changes of severe, acute, intravascular hemolysis are prominent. Severe diarrhea may occur in calves but enteric lesions are likely to be obscured by rapid autolysis. This hemolytic disease must be distinguished from other causes of acute intravascular hemolysis such as leptospirosis, bacillary hemoglobinuria caused by *C. novyi* type D (*haemolyticum*), and chronic copper poisoning. Presumably the hemolytic effect of the exotoxin is responsible for the intravascular hemolysis. Given that type A strains are commonly found in the intestines of ruminants, and that alpha toxin given intravenously is destroyed rapidly, it is apparent that there must be complex pathogenetic requirements for the development of this disease. The pathogenesis may be somewhat analogous to that of enterotoxemia caused by type D, which is discussed below.

Strains of *C. perfringens* type A, some determined to be enterotoxin-secreting, have been associated with diarrhea, sometimes bloody, in dogs. **Hemorrhagic canine gastroenteritis** (canine gastrointestinal hemorrhage syndrome) is a sporadic, peracute, hemorrhagic gastroenteritis, associated in some cases with *C. perfringens* type A, though in other cases the type involved has not been identified. Dogs with the peracute hemorrhagic disease are often found dead lying in a pool of bloody excreta; sometimes hemorrhagic diarrhea is noted prior to death. Autopsy reveals hemorrhagic enteritis and colitis (Fig. 1.161), and sometimes hemorrhagic gastritis is present. Colonic lesions tend to be more severe. Microscopically there is hemorrhagic necrosis of the gastrointestinal mucosa, which extends from the luminal surface into the mucosa. Numerous clostridia may line the necrotic intestinal structures or be distributed through the detritus, but they do not invade the intact tissue (Fig. 1.162). Recurrent diarrhea, sometimes bloody, has been associated with enterotoxin-secreting type A strains. Multiple serotypes of clostridia have been associated with nosocomial, usually nonfatal, cases of diarrhea in dogs. *C. perfringens* enterotoxin has been demonstrated twice as frequently in hospitalized dogs with diarrhea compared to controls without diarrhea.

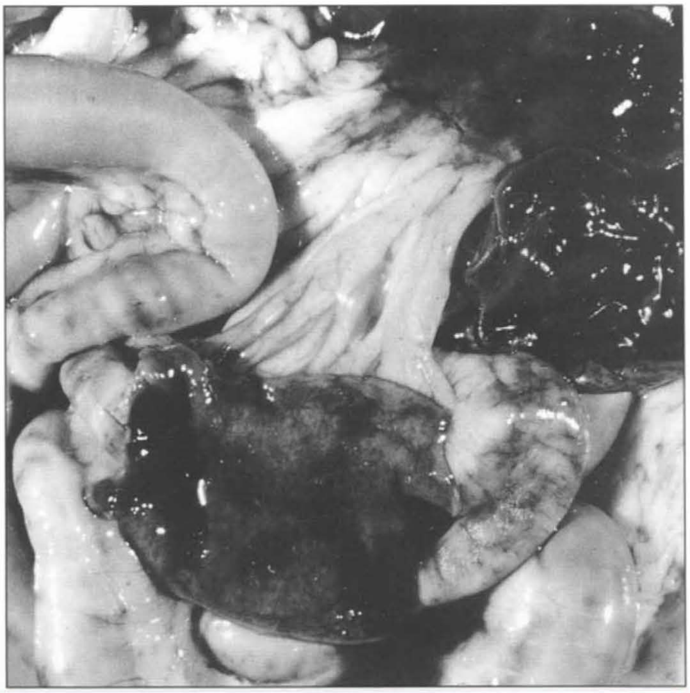

Figure 1.161 Hemorrhagic enteritis in a dog with **canine gastrointestinal hemorrhage syndrome**, associated with *Clostridium perfringens*.

Hemorrhagic bowel syndrome has been described in mainly *dairy cattle* in the USA. Affected cattle become acutely ill, with bloating, gut stasis, and melena. The lesions relate to necrohemorrhagic enteritis with extensive intraluminal hemorrhages and the lumen may contain large amounts of clotted blood (Fig. 1.163). The condition is associated with very high mortality. *C. perfringens* is present in large numbers in the feces from affected animals, and there may be an association with *C. perfringens* type A, though whether there is a causal relationship is uncertain.

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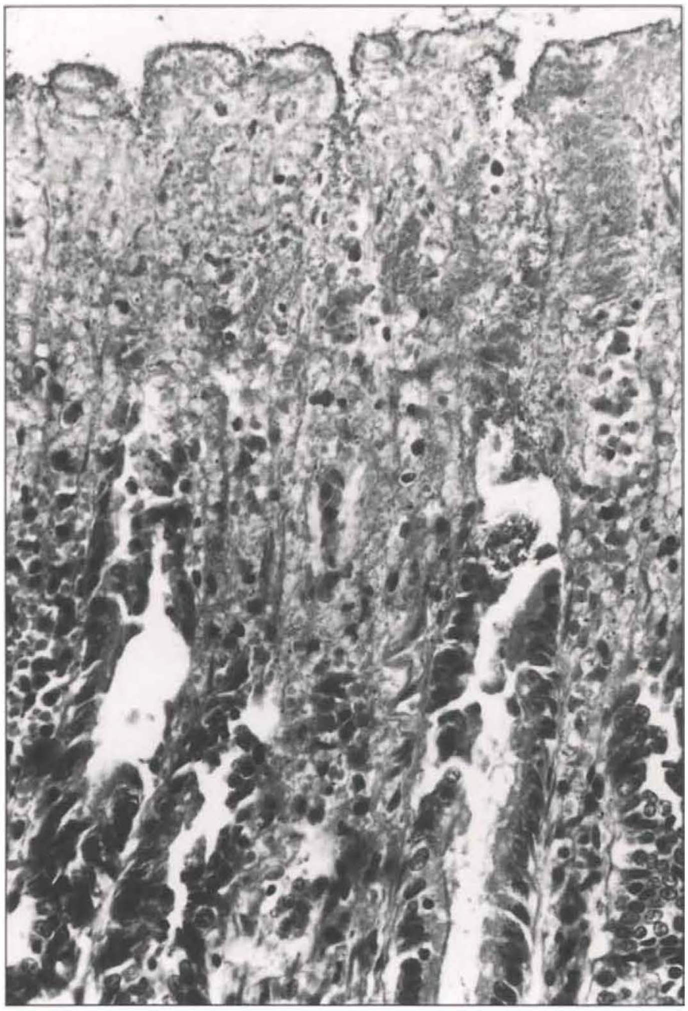

Figure 1.162 Coagulative necrosis and hemorrhage in the superficial mucosa of the colon in **canine gastrointestinal hemorrhage syndrome**. The mucosal surface is highlighted by a dark rim of clostridia.

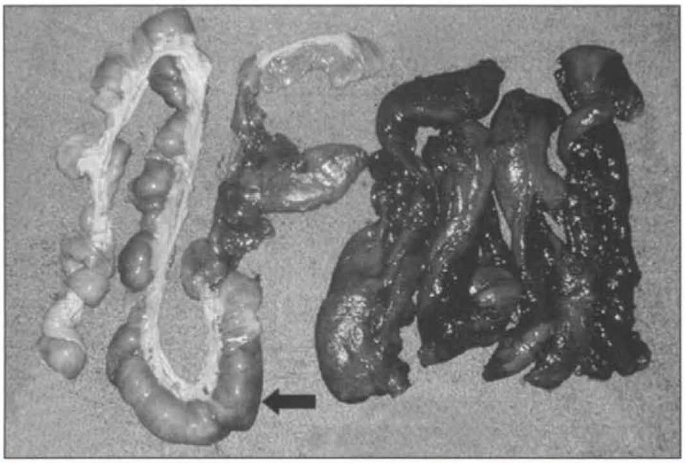

Figure 1.163 Bovine hemorrhagic bowel syndrome, associated with *Clostridium perfringens* type A. Unopened loop of jejunum containing clotted blood (arrow), and opened intestine with blood clots (right).

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Clostridium perfringens type B

Clostridium perfringens type B is reported from Europe, South Africa, and the Middle East, but not from North America and Australasia. It causes "*lamb dysentery*," usually in lambs up to ~10–14 days of age, dysentery in calves of approximately the same age, and dysentery in foals within the first few days of life.

In **lambs**, death may occur without premonitory signs, but there is usually abdominal pain, especially when animals are forced to rise, and passage of semifluid dark feces mixed or coated with blood. The abdomen is often tympanitic. A more chronic form in older lambs, which among other diseases is known as "pine" in England, is characterized by unthriftiness and depression, reluctance to suckle, and a peculiar stretching when the animal rises; such cases are reputed to respond well to specific antiserum.

Typical lesions are usually present, although in exceptional peracute cases they may be indistinct. The first impression on opening the abdominal cavity is that there is mesenteric torsion, a not uncommon accident in young lambs. The characteristic lesion is *extensive hemorrhagic enteritis*. The peritoneal cavity often contains a small amount of serous or blood-stained fluid.

In cases with more severe and deeply penetrating mucosal ulcerations, there may be overlying peritonitis with red fibrin strands on the local mesentery and intestinal adhesions. On the mucosal surface, they are irregular but well defined by a sharp margin and rim of intense hyperemia, and they contain a yellow necrotic deposit; they may coalesce to form extensive areas of necrosis. Usually the intestinal contents are blood-stained and may appear to be composed of pure blood, but in lambs that live for 3–4 days, there may be little or no hemorrhage evident. Histologically the wall of the intestine is hemorrhagic, and the areas of necrosis extend deeply into the mucous membrane, in some cases penetrating to the external muscle layers and serosa. There are large numbers of typical bacilli in the necrotic tissue, but few inflammatory cells.

The lesions present in other organs are those of *severe toxemia*. The liver is usually pale and friable, but may be congested. The spleen is normal or slightly enlarged and pulpy. The kidneys may be enlarged, edematous, pale, and soft from toxic degeneration. The pericardial sac contains abundant clear gelatinous fluid, the myocardium is pale and soft, and hemorrhages beneath the serous membranes of the heart are almost constant. The lungs are often slightly congested and very edematous.

The disease in **calves** caused by type B *C. perfringens* closely resembles that in lambs, usually affecting sucklings less than 10 days of age, with a course of 2–4 days characterized by prostration and dysentery. Older calves up to 10 weeks of age are sometimes affected. It appears that calves are more likely to recover, albeit slowly, than are lambs. The intestinal lesion is acute hemorrhagic enteritis with extensive mucosal necrosis and patchy diphtheritic membrane formations, especially in the ileum.

Although both "lamb dysentery" and "hemorrhagic enterotoxemia" occur in pastured animals, they are most serious in animals that are confined.

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Clostridium perfringens type C

Clostridium perfringens type C is present worldwide, and causes disease in adult sheep and goats, feeder cattle, and in neonatal lambs, calves, foals, and pigs.

In adult **sheep**, *C. perfringens* type C causes "*struck*," a disease of pastured animals that has a mortality rate of 5–15% in some areas. The disease in adult **goats** is probably similar in most respects to that in sheep. Death usually occurs suddenly with terminal convulsive episodes, but some animals, with infections not so peracute, stand in a straining position that probably indicates acute abdominal pain. In adult sheep, diarrhea or convulsions do not occur.

At necropsy, the peritoneal cavity contains up to 3 liters of clear, pale-yellow fluid that clots on exposure to air and that becomes stained with hemoglobin if necropsy is delayed. The small intestine is intensely hyperemic, either in patches or along most of its length, and in the zones of hyperemia there may be ulcers that may attain several centimeters in size. Ulcers are usually present, mostly in the jejunum, and are surrounded by a zone of hyperemia with a deep red base, although in some the necrotic material is dark green and adherent. The large intestine is normal. The primary intestinal lesion is superficial mucosal necrosis that advances more deeply, with a peripheral leukocytic reaction, congestion, and hemorrhage. The organisms are limited to the necrotic tissue.

The disease in feedlot **cattle** is similar to "struck." Animals are found either dead or moribund, and congestion and hemorrhage of the gastrointestinal tract are prominent. The jejunal and ileal content are bloody with fibrin clots and necrotic debris. Excessive straw-colored pleural and pericardial fluid and petechiation of epicardium and endocardium are present. Autolysis and postmortem bloat occur rapidly, and differentiation from ruminal tympany and other clostridial diseases is necessary.

The diseases caused by type C in **lambs**, **calves**, **pigs**, and **foals** are very similar and will be discussed together. Affected animals are usually young sucklings, which contract the disease within the first few days of life, often within the first 12 hours if they have been confined. Foals and most clinically affected lambs die, but in calves and pigs, subacute disease may occur in which there is diarrhea and unthriftiness.

Often, affected animals are found dead. Sick lambs may shiver, show abdominal pain, abdominal distension, dysentery, and prostration, and die in 12 hours or less. Sick calves show abdominal pain, some show diarrhea of sudden onset, and death is preceded by spasmodic convulsions.

Similar lesions occur at autopsy in all species, but may be less severe in lambs. In lambs, the intestinal changes vary from catarrh to acute hemorrhagic enteritis with mucosal necrosis that, like lamb dysentery, suggests strangulation. *The most prominent changes occur in the jejunum and ileum*, the lumen of which may contain *free blood*, which forms a clotted cast in fresh cadavers. Sometimes there is merely acute hyperemia of a segment of jejunum with edema of the wall, scant creamy intestinal content, and a few small ulcerations of the mucosa. The peritoneal cavity contains a small quantity of serous blood-stained fluid, and the local mesentery and peritoneum are often mildly inflamed, hyperemic, and bear red strands of fibrin. The mesenteric nodes are enlarged, wet, and congested. There is usually excess pericardial fluid and pulmonary interstitial edema. Ecchymoses on the serous membranes are nearly constant, and in a few cadavers all tissues, but especially the meninges and brain, are liberally sprinkled with small hemorrhages. These are sites of *bacterial embolism*, due to a massive terminal bacteremia by *C. perfringens*.

C. perfringens type C causes *hemorrhagic enteritis* of suckling **piglets** in many parts of the world. Rarely, epizootics occur in 2–4-week-old pigs and in weaned pigs. The disease occurs as epizootics in affected herds and regions, and may then remain enzootic. Poor hygienic conditions, overcrowding, and antibiotic treatment are thought to be predisposing factors in some outbreaks. Affected animals pass blood-stained feces in the terminal stages and there is marked hyperemia of the anus just prior to death. The predominant lesions occur in the small intestine, especially the jejunum, but the cecum and spiral colon are often involved, and occasionally lesions are confined to the large intestine. Lesions are similar in all areas, and in acute cases consist of *intestinal and mesenteric hyperemia, extensive necrosis of the intestinal mucosa, and blood-staining of the contents* (Fig. 1.164). There may be emphysema of the intestinal wall, which becomes fragile. Mesenteric lymph nodes are red, and sanguineous peritoneal and pleural fluid is present. Fibrinous intestinal adhesions may develop.

Microscopically, the necrotic process extends deeply and sometimes penetrates the muscularis mucosae. Numerous typical bacilli line up along the margin of involved villi and inhabit the necrotic tissue. Older pigs may not show intestinal hemorrhage but do have mucosal necrosis, and peritoneal and pericardial effusion.

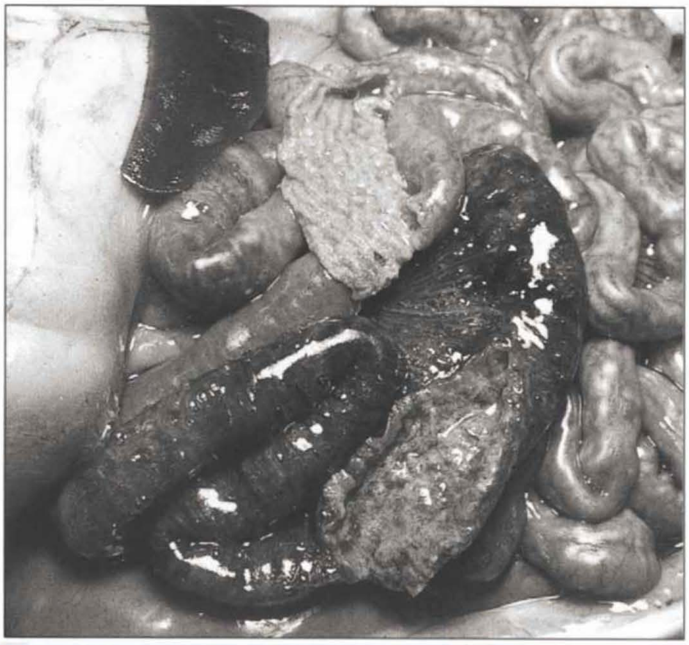

Figure 1.164 Necrotizing enteritis in a piglet, caused by ***Clostridium perfringens* type C** infection. (Courtesy of M. Bergeland.)

The disease due to *C. perfringens* type C in **foals** has been reported from the USA, Canada, and Australia. It usually occurs in foals <4 days of age. Typical clinical signs include weakness, yellow to brown watery diarrhea or dysentery, colic, and dehydration. Affected foals usually die in <24 hours. Macroscopic lesions are those of *acute hemorrhagic necrotizing enteritis*, usually in the distal two-thirds of the small intestine, although in some cases most of the small and large intestine may be affected. The microscopic lesions are similar to those described previously in the other species. In foals, the lesions must be differentiated from those seen with other clostridia.

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Clostridium perfringens type D

Enterotoxemia (*"pulpy kidney" disease, "overeating" disease*) caused by the toxins of *Clostridium perfringens* type D is an important disease of *sheep and goats* with a worldwide distribution. It occurs occasionally in calves, and very rarely in horses. *Focal symmetrical encephalomalacia* (FSE) of sheep is caused by the epsilon toxin of type D. Epsilon toxin binds to receptors on endothelial cells, especially in the brain and renal tubular epithelial cells, resulting in changes described below.

In most **lambs** and **calves** with type D enterotoxemia, the course is peracute and the animal is found dead. Lambs and calves may die in a few minutes in convulsions, and calves often bawl as from severe pain. Animals that survive longer may show drooling, rapid breathing, hyperesthesia, straining, opisthotonos, and terminal coma or convulsions. In adult sheep, in which the clinical course may be several days, diarrhea with the passage of dark semifluid feces is common. In sheep, subacute cases may occur and be followed by recovery. In some such cases, neurological signs may develop. These include blindness, ataxia, head pressing, and caudal paraparesis; the lesions of FSE are present in the brains of such cases. On other occasions these lesions are not preceded by signs of enterotoxemia. In **goats**, signs of enterotoxemia similar to those in sheep and lambs may be seen, but chronic enterotoxemia characterized by abdominal distension and pain, depression, and dark-green diarrhea may persist for several days to weeks. Nervous signs do not occur.

Lambs fed large amounts of grain or concentrate are highly susceptible – thus the synonym "overeating disease." The manner in which overeating leads to clostridial enterotoxemia is complex. Cultures of *C. perfringens* type D given orally are largely destroyed in the rumen and abomasum. The few organisms that reach the intestine proliferate rapidly and produce toxin, but when the numbers are no longer reinforced by escapees from the stomachs, they are rapidly cleared from the intestine by peristalsis.

The critical factor is almost certainly the *presence of starch in the small intestine*, providing a suitable substrate for these saccharolytic bacteria, and they proliferate to immense numbers – perhaps more than 10^9 organisms per gram of intestinal contents – and produce correspondingly large amounts of toxin. When the animal is suddenly provided with excessive quantities of food, or food of a different type, there is a delay before the ruminal flora can adapt. In this period, undigested or partially digested food may escape into the intestine, and if starch is there, as it is with overeating on grain, *C. perfringens* type D is likely to take advantage of it. The epsilon prototoxin is activated by digestive enzymes, especially the combination of trypsin and chymotrypsin.

A high concentration of epsilon toxin facilitates its own absorption from the intestine, probably in part by increasing the permeability of the mucosa. Necrosis of epithelium and moderate atrophy of villi are evident in some animals with type D enterotoxemia, and it is reasonable to assume that epithelial damage precedes the facilitated absorption.

Probably the disease develops in the same way in the calf as in the sheep. The virtual confinement of the disease to calves that are overfed suggests that this is the case. The acute disease in goats likely has a similar pathogenesis but the chronic disease, with lesions confined to the intestine, appears to be caused by local effects of type D toxins.

In lambs dead of acute enterotoxemia, the carcass is usually well nourished. In those with a course of 1–2 days, there is often evidence of a dark scour about the rump. Putrefactive changes occur rapidly. Often there is excessive straw-colored pericardial fluid that clots on exposure to air and congestion and edema of the lungs that may be severe enough to produce froth in all the respiratory passages; and hemorrhage beneath the endocardium of the left ventricle. There may be hemorrhages beneath other serous membranes (Fig. 1.165) such as the epicardium, and blotchy hemorrhages beneath the parietal peritoneum are characteristic. Sometimes the liver is congested and the spleen enlarged and pulpy. There is no gastrointestinal inflammation visible at necropsy. Short lengths of the small intestine are distended with gas, and are hyperemic.

Epsilon toxin binds to receptors on distal renal tubular epithelial cells and causes selective degeneration of distal tubules in a variety of species. In experimental cases and natural cases in lambs examined immediately after death, there may be medullary congestion, hemorrhage, and tubular degeneration (Fig. 1.166). However, rapid autolysis or delayed necropsy can also produce "pulpy kidney." The renal "lesions" can be useful diagnostic aids, as are the hyperglycemia and glucosuria associated with the toxemia, if they can be detected.

In adult sheep, the lesions are the same as those in lambs, but are more consistent and more advanced, with the exception of renal autolysis, which occurs less rapidly, and less commonly progresses to the stage of "pulpiness."

Brain lesions occur in lambs with subacute enterotoxemia and these are sufficiently consistent to be of diagnostic significance. They

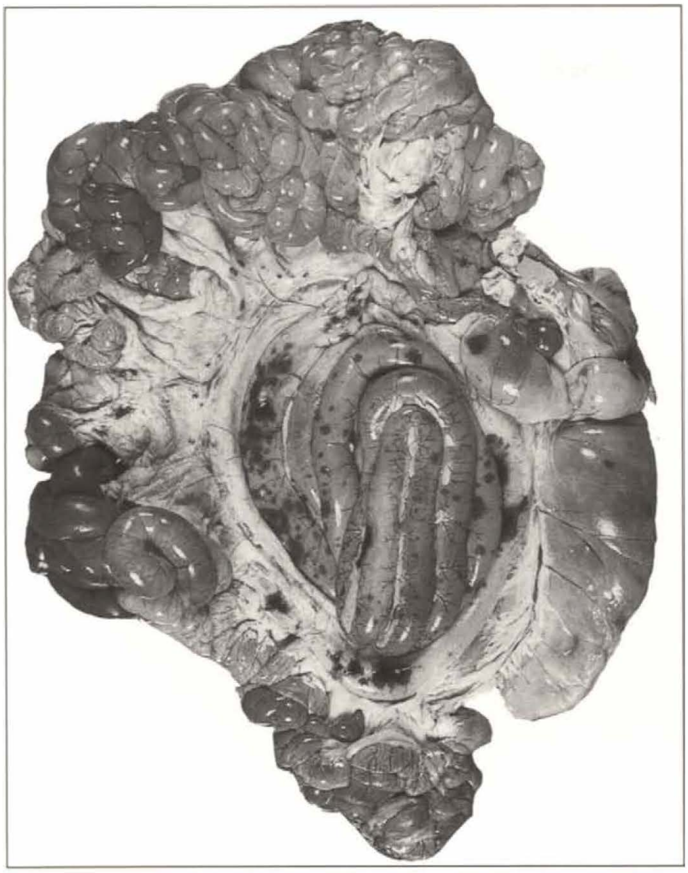

Figure 1.165 Peritoneal hemorrhages in a sheep with ***Clostridium perfringens* type D** enterotoxemia.

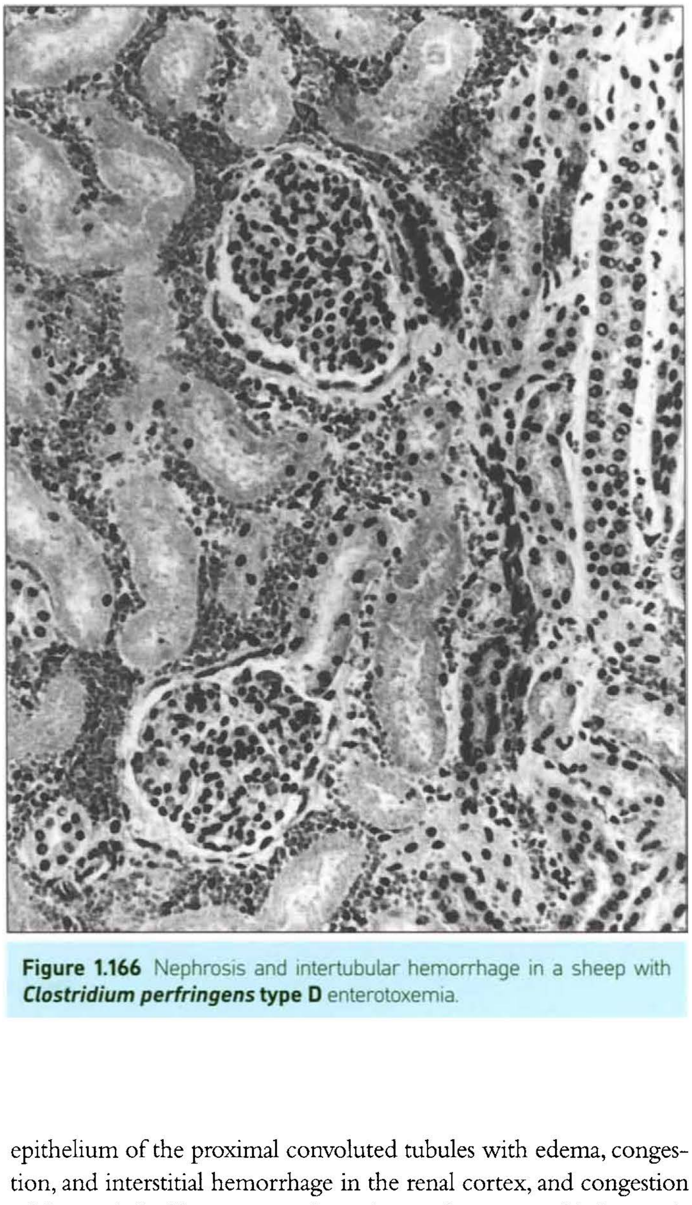

Figure 1.166 Nephrosis and intertubular hemorrhage in a sheep with ***Clostridium perfringens* type D** enterotoxemia.

develop in two patterns; each is bilaterally symmetric, hence the term **focal symmetrical encephalomalacia**. The commonest pattern involves the basal ganglia, internal capsule, dorsolateral thalamus, and substantia nigra; there are some minor variations of the pattern, but the lesions are always of the same type (Fig. 1.167). The second pattern affects the white matter of the frontal gyri, sparing only the communicating U fibers. The lesion begins with edema and the leakage of plasma and then red cells from the venules and capillaries in the affected areas. The altered permeability of the vessels is diffuse throughout the brain, sparing only heavily myelinated tracts such as the optic tracts and corpus callosum. This is well demonstrated by vital staining with trypan blue. The least change visible by light microscopy is the accumulation of protein droplets around small venules. Electron microscopically, *severe damage to vascular endothelium* is apparent and there is swelling of protoplasmic astrocytes. The foot processes around blood vessels and the processes around neurons are most severely swollen. Edema and hemorrhage lead to malacia in the affected areas.

Lesions in calves dying of enterotoxemia caused by *C. perfringens* type D may resemble those in lambs. Affected calves are usually 1–3 months of age. Splenic swelling is more common in calves than in lambs, and rapid autolysis of the kidney is not a prominent finding, as it is in lambs. However, subcapsular congestion and hemorrhage occur (Fig. 1.168), and sometimes a black clot of blood up to 1.0 cm thick forms around the kidney.

The *histologic changes* in enterotoxemia include, in addition to the brain lesions described above: mild degeneration and necrosis of the epithelium of the proximal convoluted tubules with edema, congestion, and interstitial hemorrhage in the renal cortex, and congestion of the medulla (these are autolytic changes but are useful diagnostically); superficial desquamation in the intestine with congestion, and numerous typical bacilli in the contents; congestion and hemorrhage of the spleen with disruption of reticulum; subepicardial hemorrhage and degeneration in the Purkinje network; and proteinaceous edema fluid in the lungs. All of these changes are secondary to endothelial damage produced by the epsilon toxin.

Type D enterotoxemia may be seen in both adult **goats** and kids. Four forms of the disease are recognized in goats: *peracute, acute, chronic, and subclinical.* The peracute disease is similar to that seen in lambs, and usually manifests as sudden deaths. The acute form is characterized by diarrhea and severe abdominal discomfort. Affected animals either recover or die within 2–4 days after the onset of clinical signs. The chronic form of the disease may last for a few days or weeks. Weight loss and diarrhea are its main clinical features. The principal macroscopic lesions in the acute and chronic disease are mild to severe hyperemia of the mucosa, especially of the distal small intestine, cecum, and spiral colon. The affected areas may be covered by a thin layer of fibrin. The intestinal contents are olive-green to red and mucoid. The mesenteric lymph nodes are enlarged and edematous. Hydropericardium, pulmonary edema, and "pulpy" kidneys may be seen, but are inconsistent, and brain lesions are absent.

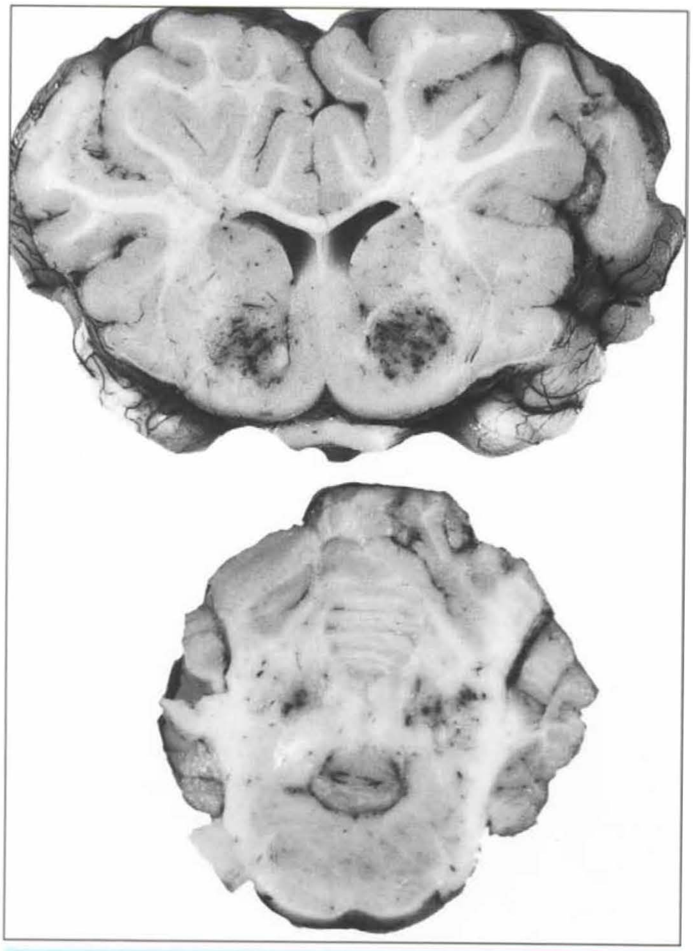

Figure 1.167 Focal symmetric encephalomalacia; hemorrhages and softening in internal capsules and cerebellar white matter in a sheep with *Clostridium perfringens* type D enterotoxemia.

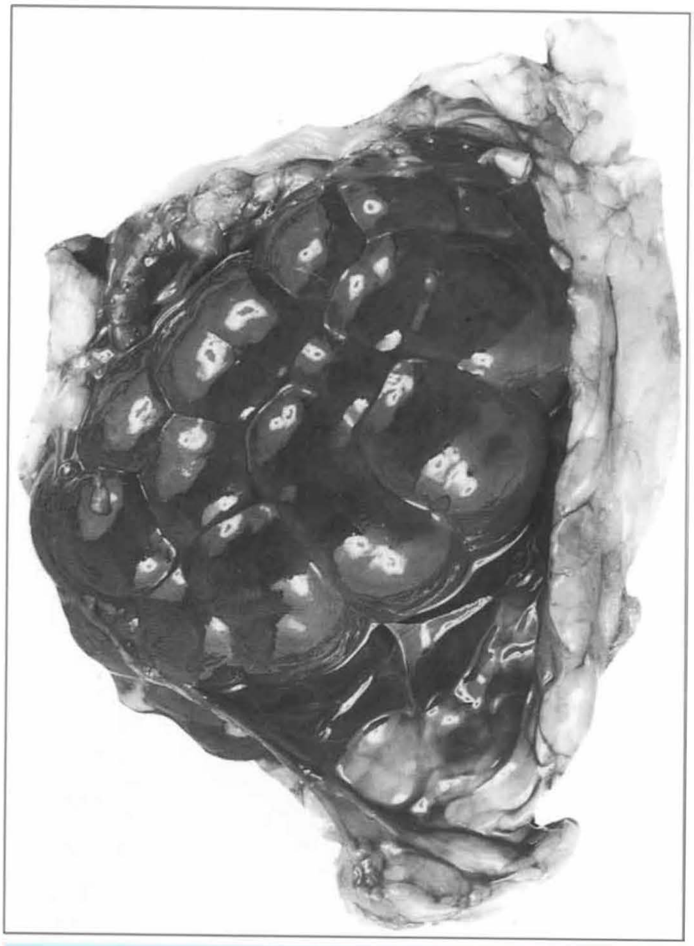

Figure 1.168 Renal cortical hemorrhage in a calf with *Clostridium perfringens* type D enterotoxemia.

In natural cases, the microscopic lesions in the small intestine vary from a mild pleocellular leukocytic reaction in the lamina propria to a mild fibrinous, or, rarely, hemorrhagic enteritis. In the latter, the tips of the villi are necrotic, eroded, and covered by fibrinocellular exudates or blood. Villus atrophy may follow. The leukocytic reaction in the lamina propria extends into the edematous submucosa. The proprial and submucosal vessels are congested. Essentially similar lesions occur in the large intestine, bearing in mind the anatomic differences. There is lymphocytolysis in the centers of the lymphoid follicles of the mesenteric lymph nodes. Lesions in the lungs and kidneys may be similar to, but are less consistent than, those seen in lambs. Cerebral edema may be present, but FSE has not been reported in goats with enterotoxemia, except after experimentally induced disease.

Experimental intraduodenal inoculation of whole cultures of *C. perfringens* type D in kids mainly results in colitis of the spiral colon. The reasons for the different manifestations of enterotoxemia in sheep and goats are unknown.

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Clostridium perfringens type E

Clostridium perfringens type E can cause intestinal disease in calves. Calves die acutely and have a congested ulcerated abomasum, and

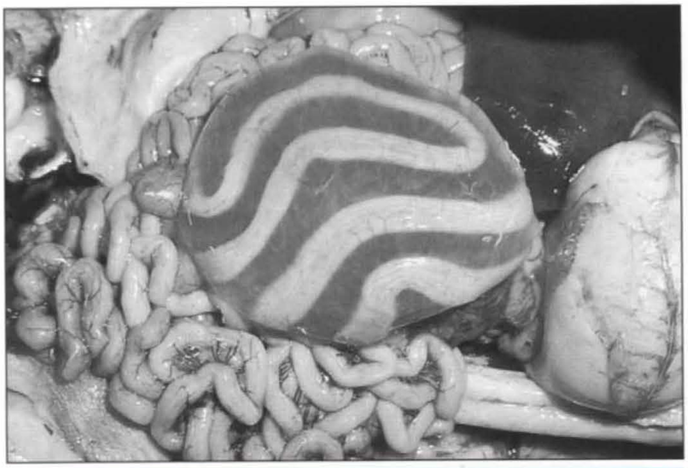

Figure 1.169 Marked edema of the mesocolon in a neonatal piglet infected with ***Clostridium difficile***. (Courtesy of AP Loretti.)

hemorrhagic enteritis that occurs segmentally along the small intestine. Mesenteric nodes are enlarged and red, and pericardial effusion and serosal hemorrhages may be present.

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Other clostridial diseases

Clostridium difficile is a gram-positive to gram-variable inhabitant of the soil and gut. This organism causes pseudomembranous colitis in humans, often, but not necessarily, with a history of antibiotic treatment. Its role in enteric disease of animals is just emerging, and perhaps with the exception of swine, evidence in most species is associative, rather than conclusively causal. Definition of its diagnostic significance is dependent on identification of the A and B toxins in gut content or feces by tests with high sensitivity and specificity.

C. difficile is increasingly being recognized as a cause of diarrhea due to *fibrinous colitis* in **neonatal pigs**, under about a week of age, and the disease has been reproduced experimentally using pure cultures of the organism. Piglets have diarrhea, dyspnea, scrotal edema, and mild abdominal distension. Hydrothorax and characteristic, though not pathognomonic, *edema of the mesocolon* (Fig. 1.169) are evident grossly, usually in association with patchy to extensive fibrinous typhlocolitis, with yellow pasty to fluid content and feces. Microscopically, there is exfoliation and attenuation of surface enterocytes, and patchy focal erosions on the colonic mucosa, through which fibrin and neutrophils exude, producing the "volcano" lesions. Occasionally deeper necrosis of the mucosa and colonic wall may occur.

C. difficile and its cytotoxin have been demonstrated in feces of **dogs** with diarrhea. Descriptions of lesions are not available, however, since there are no necropsies or biopsies reported. However, the organism is frequently shed in feces of normal puppies and their bitches, which makes its significance difficult to interpret.

In **horses**, *C. difficile* may be incriminated in diarrheal syndromes, including *"proximal enteritis"* and *hemorrhagic enteritis* in foals, and *colitis* in horses of all ages. Diarrhea and colic have been reproduced in foals using inocula of *C. difficile* spores and vegetative cells.

Equine intestinal clostridiosis is still undergoing definition; many cases probably fall into the syndrome formerly described as "colitis X," the etiology of which has been undefined. The syndrome is one of *severe acute-to-peracute colitis*, and it is probably related to dysbacteriosis and clostridial overgrowth, instigated by antibiotic therapy, stress, or changes in feeding regimens.

The clostridia most commonly incriminated in this syndrome include beta$_2$ toxin-secreting *C. perfringens* type A and *C. difficile*. There is a positive association between the disease and the presence of *C. perfringens* enterotoxin and/or *C. difficile* toxin A. Lesions are usually confined to the large bowel. There is extensive hemorrhage and edema in the mucosa, submucosa, and mesocolon. Microscopically, full-thickness necrosis of the mucosa is associated with thrombosis of small vessels; variable numbers of bacilli may be visible in section, but the lesion is not readily differentiated from other forms of severe acute colitis, for instance salmonellosis.

Tyzzer's disease is caused by the obligate intracellular bacterium ***Clostridium piliforme***. It is an uncommon affliction of many species of mammals, among them horses, cats, and dogs, though there seem to be bacterial strain differences that determine host susceptibility. Affected animals are often very young, or appear to be immunocompromised in some way.

While the lesions are not limited to the intestinal tract, animals become infected initially through the epithelium of the ileum, cecum, and colon, where lesions may vary from subtle catarrhal to fibrinohemorrhagic. Bacilli can be demonstrated using silver stains in enterocytes, often forming characteristic "pick-up-sticks" arrays. Ultimately, in most cases they disseminate elsewhere in the body, especially to liver and myocardium, where they cause acute-to-subacute necrotic lesions. The disease is discussed more fully in Vol. 2, Liver and biliary system.

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Paratuberculosis (Johne's disease)

Johne's disease is caused by ***Mycobacterium avium* subsp. *paratuberculosis*** infection. The etiologic agent of Johne's disease has been reduced to subspecies status within *M. avium* on the basis of the high (>90%) DNA homology among typical paratuberculosis strains and type strains of *M. a. avium*. The Johne's disease agent, which in culture is slow-growing, and dependent on mycobactin as a source of iron, possesses some unique cultural and biochemical traits. Genetically, a distinct difference from *M. a. avium* is the presence of the insertion sequence, **IS900**, of which *M. a. paratuberculosis* has 15–20 copies per organism.

The virulence attributes of *M. a. paratuberculosis* are poorly understood, but presumably reside in *resistance to killing in macrophages*, through inhibition of the conversion of phagosomes to phagolysosomes. The organisms proliferate in cytoplasmic vacuoles, and transmit to adjacent macrophages, expanding the population of infected cells and recruiting elements of the humoral and cell-mediated arms of the immune system to the site. Diffuse foci of granulomatous inflammation accumulate, reflected in a distinct profile of local cytokine expression. Infected macrophages traffic via lymphatics to the draining lymph nodes and via the portal venous drainage to the liver, ultimately gaining the central circulation.

The disease is most common in *domestic ruminants*, but infections by *M. a. paratuberculosis* can also be produced in pigs, and spontaneous disease occurs in a number of free-ranging and captive wild ruminants, camelids, and, rarely, in equids and captive primates. Numerous species of wild mammals, including lagomorphs, rodents, and carnivores, and several species of wild birds, are naturally infected, though not necessarily diseased. The infection can be transmitted experimentally to mice, hamsters, guinea pigs, rabbits, and macaques. A debate exists as to the role of *M. a. paratuberculosis* in the genesis of *Crohn's disease in humans*, a chronic granulomatous enteritis. *M. a. paratuberculosis* has been found in many of these cases, as detected by polymerase chain reaction of reactive fragments, but it is still undecided whether the association is causal or coincidental.

The **epidemiology and pathogenesis** of Johne's disease are best understood in cattle, and are assumed to be similar in sheep and goats. Infection is systemic, and organisms may be present in milk, semen, and urine, and may cross the placenta. However, exposure is mainly by ingestion of organisms shed in the feces. This may explain higher prevalence in dairy as opposed to beef animals. Susceptibility to infection is greatest in the first 30 days of life, although clinical disease does not usually develop in cattle until 2–5 years of age. This long incubation period has been termed "the iceberg effect" because, in any infected herd, although few animals may be showing clinical signs of Johne's disease, a much greater number are silently infected. Adults may become infected, but are less likely to develop the disease, and often recover from the infection. In addition, evidence is accumulating that the epidemiology is influenced by the soil and pasture type, presumably because these factors can affect the survival and proliferation of organisms in the environment.

Bacteria are taken up by M cells of the dome epithelium over lymphoid follicles and transported to macrophages in Peyer's patches. The immaturity of macrophages in younger animals, combined with the greater volume of organized intestinal lymphoid tissue, may help to explain the age susceptibility of infection. *The major lesions of Johne's disease are usually confined to the ileum, large intestine, and draining lymph nodes*. However, the infection is generalized, because in both clinical and subclinical cases, the organism can be cultured from a variety of parenchymatous organs and widely distributed lymph nodes. In fulminating infections, there is *bacteremia*, in blood or in infected phagocytes.

The incubation period of Johne's disease is protracted and irregular. Some carriers, in which bacteria persist in the mucosa and draining lymph nodes, may be infected for life without showing signs. The relationship between immune events and stages of the disease is speculative. Cell-mediated immunity clearly plays a role in the development of mucosal lesions and the onset of clinical disease. Exacerbations of clinical disease are often associated with parturition, a low nutritional plane, heavy milk yield, and intercurrent disease.

The pathogenesis of Johne's disease is related to the *granulomatous immunoinflammatory response in the lamina propria in the small intestine*, and the associated villus atrophy that develops. Malabsorption in the ileum, and filtration secretion from inflamed mucosa, overloads the capacity of the colon to resorb electrolyte and fluid; the function of the colon itself may be compromised by mycobacterial infection. There is malabsorption of amino acids, and enteric loss of plasma proteins, causing reduced productive efficiency, and when negative nitrogen balance occurs, a decline in body condition, and ultimate emaciation. Hypoproteinemia, when it develops, will further promote filtration secretion.

Clinically affected cattle are usually 2 years of age or older. The typical manifestation of Johne's disease is *profuse diarrhea passed effortlessly*. Clinical signs may be intermittent, with long intervening periods of remission. *Emaciation is progressive and ultimately fatal*, but the appetite is often retained and animals remain bright until the terminal stages.

Grossly, advanced cases of Johne's disease have marked loss of muscle mass and serous atrophy of fat depots, intermandibular edema, and fluid effusion in the body cavities. Plaques of intimal fibrosis and mineralization may be evident in the thoracic aorta. Specific gross lesions occur in the intestine and regional lymph nodes. The *mesenteric nodes, particularly the ileocecal, are always enlarged*, sometimes remarkably so, pale, and edematous, especially in the medulla. *Lymphangitis* is common, and the lymphatic vessels can often be traced as thickened cords from the intestinal serosa through the mesentery to the mesenteric nodes (Fig. 1.170). Often lymphangitis is the only recognizable gross change, and is specific enough to justify a presumptive diagnosis of Johne's disease at necropsy. The intestinal serosa often has a slight granular and diffusely opaque appearance because of subserosal edema and cellularity.

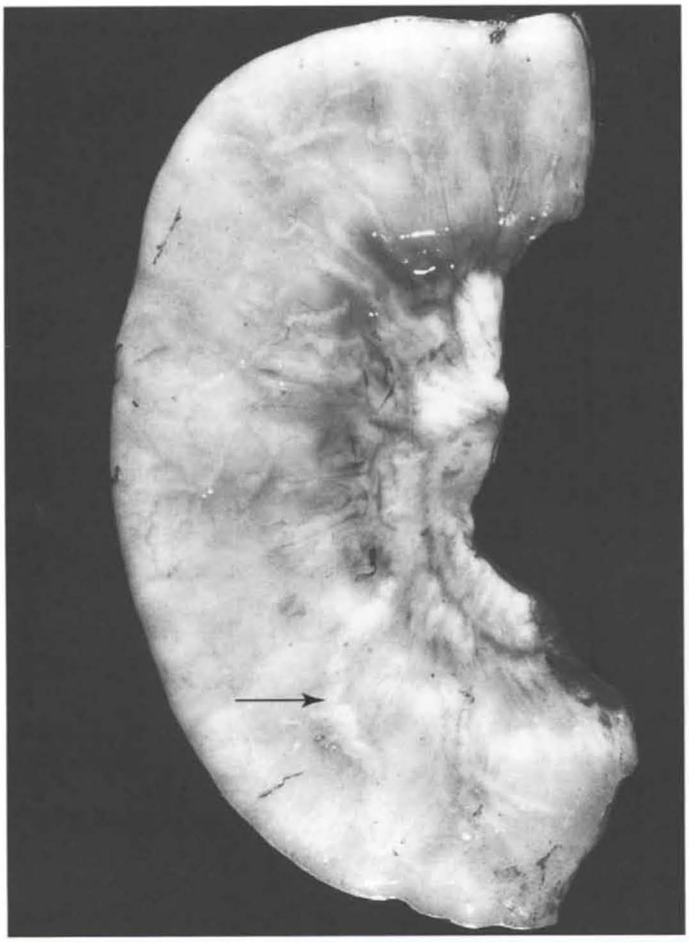

Figure 1.170 Serosal edema and **lymphangitis** (arrow) in a sheep with Johne's disease.

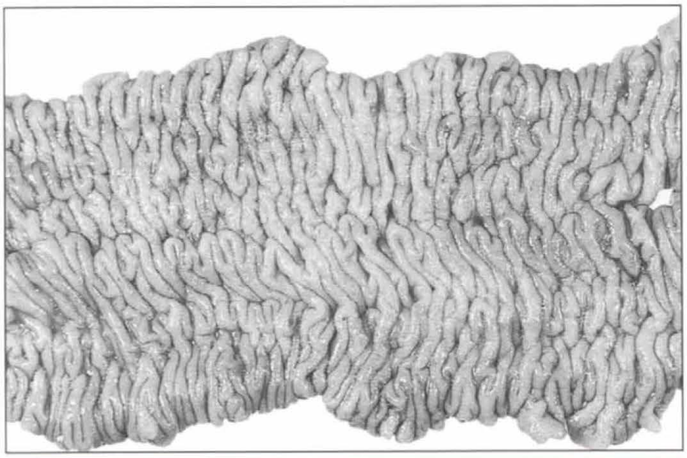

Figure 1.171 Thickened mucosal folds in the jejunum of a cow with **Johne's disease**.

Mucosal lesions may occur from the duodenum to the rectum; they may be segmental or continuous. They are usually best developed in the *lower ileum* (Fig. 1.171), and in the upper large intestine. The ileocecal valve is frequently described as enlarged, and this area is considered by some to be the earliest and most consistently affected, but abnormalities in this vicinity may not be notable.

The classical intestinal change is *diffuse thickening of the mucosa*, which is folded into transverse rugae, the crests of which may be congested. When well developed, the mucosal folds cannot be smoothed out by stretching. This lesion is due to accumulation of chronic inflammatory cells and edema in the mucosa and submucosa.

When gross lesions are well developed, the characteristic **microscopic change**, *transmural granulomatous enteritis*, is obvious. But in cattle in which gross changes are minimal or absent, the microscopic abnormalities are more subtle. In these the lamina propria is diffusely infiltrated with lymphocytes and plasma cells, and a large number of eosinophils. There may be very few macrophages (Fig. 1.172A), and the most characteristic change is an infiltrate of lymphocytes and plasma cells in the submucosa, and associated with the submucosal and mesenteric lymphatics.

In more clear-cut cases, villi are moderately to markedly atrophic, and macrophages are focally or diffusely distributed, in the villi, or deeper in the lamina propria, as part of an increased chronic inflammatory cell infiltrate. Giant cells may be present. The inflammatory infiltrate may abnormally separate and displace crypts, which are elongate, with hyperplastic epithelium. Crypts may be distended with mucus and exfoliated cells, probably due to compression and obstruction of their mouths by edema and inflammatory cells (Fig. 1.172B). *Masses of epithelioid macrophages may accumulate in the submucosa.* Foci of necrosis may occur within these aggregates of macrophages (Fig. 1.173), but in cattle, caseation and mineralization are extremely rare.

Lymphangitis is one of the most consistent changes. Initially the lymphatics are surrounded by lymphocytes and plasma cells and many contain plugs of epithelioid cells in the lumen. Granulomas may form in the wall and project into the lumen. These nodules may undergo some central necrosis.

Granulomatous lymphadenitis occurs in mesenteric lymph nodes in advanced cases. In the early stages, there is histiocytosis of the subcapsular sinus. Ultimately, nodular or diffuse infiltrates of epithelioid macrophages and giant cells may replace much of the cortex, and infiltrate the medullary sinusoids.

Of the other organs and tissues from which the bacilli may be isolated in cattle, lesions have only been described in the liver, hepatic lymph nodes, and, very rarely, the kidney and lungs. These are characteristically *focal granulomas*. They are most common in the liver, where foci of epithelioid cells and lymphocytes are found in the triads and scattered throughout the parenchyma. These lesions usually contain *demonstrable bacilli*, if macrophages are evident.

In sheep and goats, Johne's disease mainly occurs in adults and is characterized by *chronic wasting*; there may be breaks in the wool in sheep, and submandibular edema due to hypoproteinemia. The feces are often normal; they may be soft and unpelleted, but overt diarrhea is unusual, except intermittently in the terminal stages, perhaps because of the innately greater efficiency of electrolyte and fluid absorption in the colon of these species. In **farmed deer**, Johne's disease is clinically similar, but there are reports of disease in animals well under a year old.

In sheep, goats, and deer, enteric gross lesions are often mild, with little obvious thickening, and no transverse ridges; they are easily missed at necropsy. In sheep and goats, the bowel is occasionally quite remarkably thickened, and the lymphatics may be knotted as well as corded, the knots being focal granulomatous accumulations of epithelioid cells and lymphocytes, with relatively few organisms. Others may have an intestinal inflammatory infiltrate that is more lepromatous in

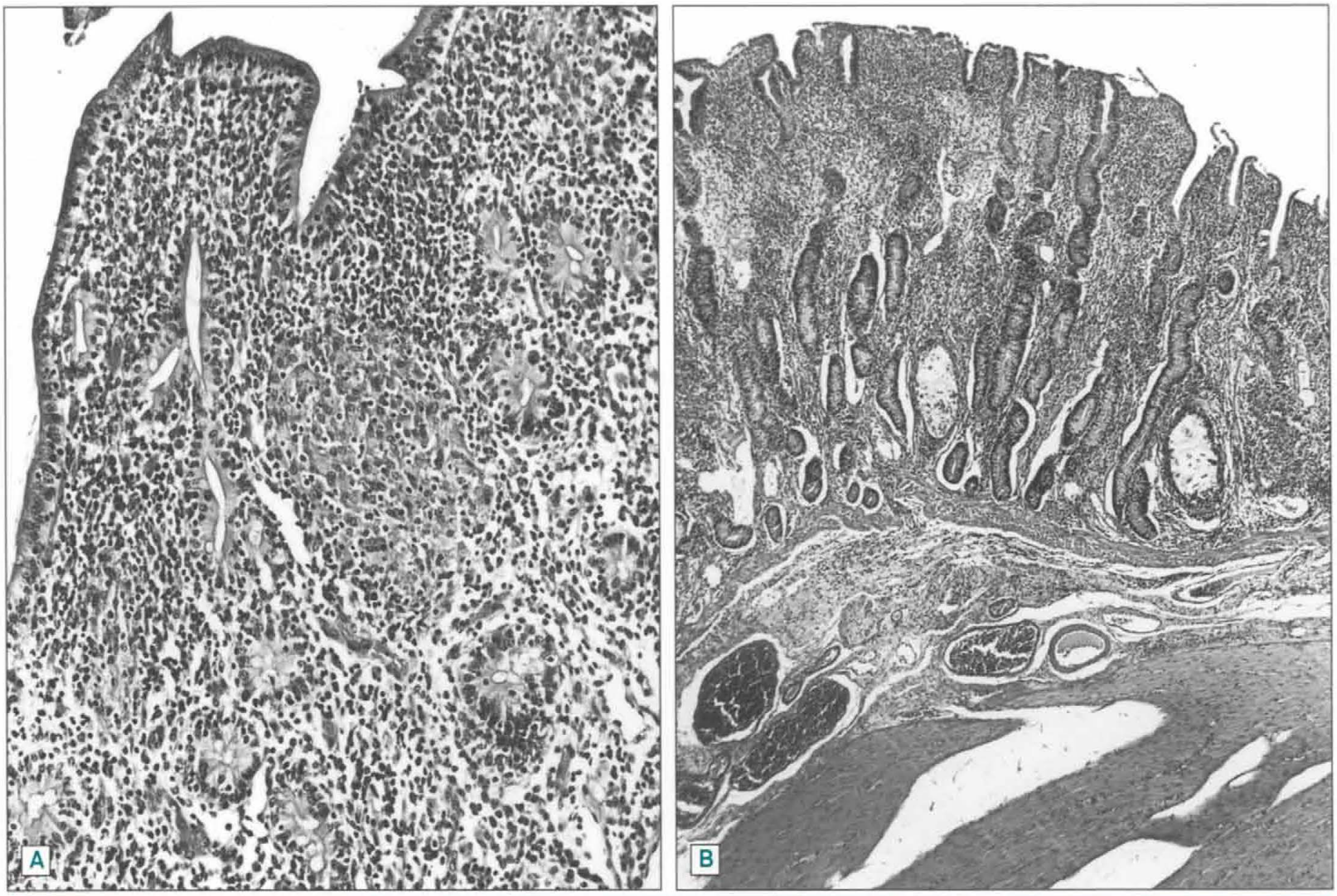

Figure 1.172 Johne's disease in a cow. **A.** Aggregate of **macrophages** in hypercellular lamina propria. **B.** Blunt **atrophic ileal villi**, and hyperplastic, and occasionally cystic, crypts. Edema of lamina propria, submucosa, and muscularis. Note heavy **inflammatory infiltrate** in lamina propria.

nature, with abundant organisms. In small ruminants, there may be lymphadenopathy affecting internal lymph nodes in particular, and variable lesions in the walls of blood vessels, meninges, liver, and spleen, all related to irregular lymphogranulomatous inflammatory foci. This latter lepromatous appearance is associated with negative intradermal skin test responses to johnin.

Goats, and some sheep, develop foci of tubercle-like caseation, often with mineralization, in the mucosa, submucosa, serosa, and lymphatics. Some may be grossly visible as white foci 1–4 mm in diameter, with modest surrounding fibrosis. Tubercles in the mucosa and lymph nodes of goats and deer may also mineralize (Fig. 1.174), and be large enough to replace much of the node. In sheep infected with the pigmented strain of the organism, the mucosa and lymph nodes may be orange. Scattered lymph nodes elsewhere in the body, and liver, lung, spleen, and other organs, may contain focal granulomatous lesions in sheep and goats. Some may mineralize. Lesions resembling those of lepromatous leprosy, with lymphocytic neuritis, have been described in the intestine of sheep, and axonal degeneration of the brachial and sciatic nerves has been reported in goats. Amyloidosis involving glomeruli, and occasionally other tissues, has also been reported in goats.

The organism is usually readily demonstrable in macrophages and giant cells in the lesions when appropriately stained by acid-fast techniques. However, in some clinical cases, especially the paucibacillary form in sheep, an extensive search may have to be made for individual macrophages or giant cells bearing a few acid-fast bacilli. Johne's disease in sheep, and especially in goats and deer, may resemble tuberculosis, on account of caseation and mineralization in granulomatous foci, and for such cases, and in any other situation where there is uncertainty about the acid-fast organism involved, positive identification of the etiologic agent is necessary. Antibodies with well-defined specificity for *M. a. paratuberculosis* have allowed the development of immunohistochemical tests, but these are not helpful when there are very few organisms. Polymerase chain reaction techniques are useful for confirming the diagnosis in individual cases. For isolation, the ileocecal lymph node, and affected segments of gut, are candidate sites for culture to confirm a diagnosis. Isolation may be difficult to accomplish, especially from sheep.

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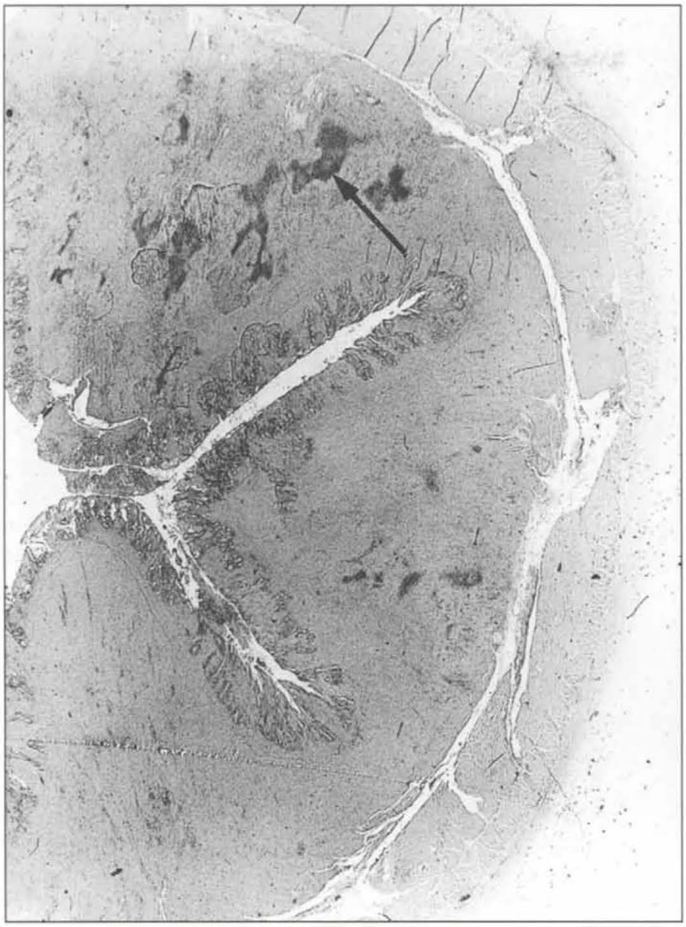

Figure 1.173 Hemisection of ileum showing **diffuse infiltration of cells** and small areas of **necrosis** (arrow) in **Johne's disease**.

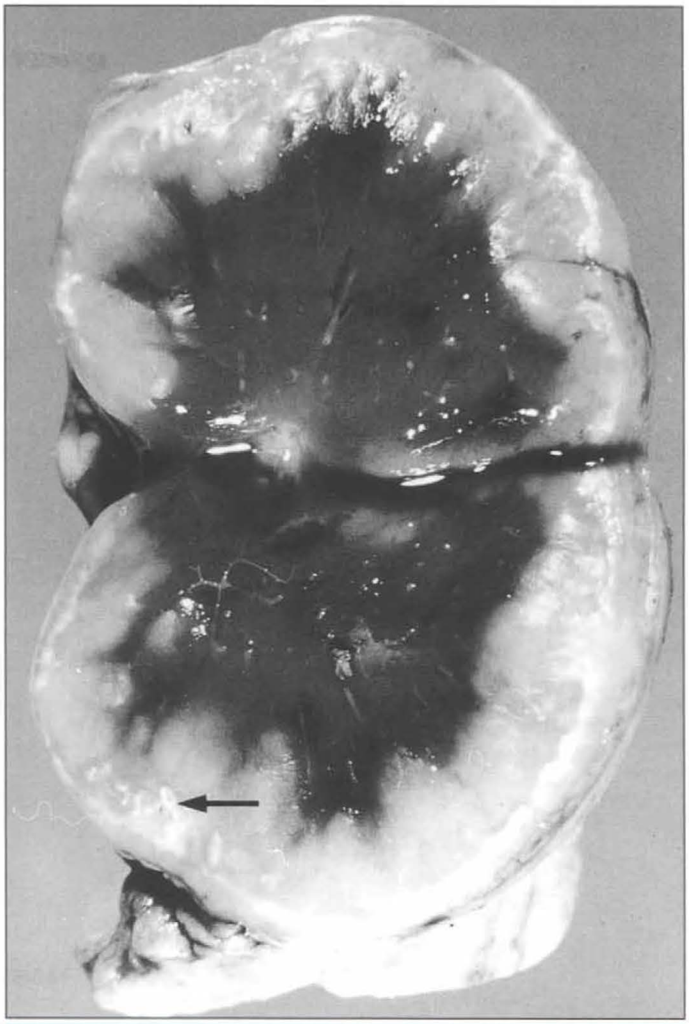

Figure 1.174 Ileocecal lymph node in a goat with **Johne's disease**. **Hyperplasia of lymphocytes** in the cortex. White foci of **mineralized caseous necrosis** in cortex (arrow).

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Rhodococcus equi enterocolitis of foals

Rhodococcus equi is an intracellular pathogen found in soil and as part of the normal intestinal flora of horses. There are both virulent and avirulent forms, with various *virulence factors* proposed, including capsular polysaccharide, cholesterol oxidase, cell wall mycolic acids, and a plasmid-encoded surface-expressed protein VapA. On farms where disease due to *R. equi* is endemic, there is a much higher proportion of virulent forms. Virulence factors may not be necessary for production of disease in an immunocompromised host. *R. equi* is usually associated with *suppurative bronchopneumonia of foals*. About half the pneumonic foals also have *ulcerative colitis*, and in some foals intestinal lesions alone occur.

The development of intestinal lesions appears to be dose-related, in that reproduction of the disease requires repeated oral infection. In natural disease, continual exposure to bacteria in swallowed respiratory exudate is probably an important source of infection in those animals with pneumonia.

Gross lesions may occur throughout the small and large intestines, but are usually most severe over Peyer's patches in small intestine, and in the cecum, large colon and related lymph nodes (Fig. 1.175A). Mucosal lesions consist of irregular ulcers up to 1–2 cm in diameter, often covered by purulent or necrotic debris (Fig. 1.175B). Edema of the wall of the gut may be severe. Mesenteric or colonic lymph nodes are often massively enlarged by edema and by caseous or purulent foci that may obliterate the structure of the node. Occasionally, massively enlarged abscessed lymph nodes are found without evidence of concurrent enteritis.

Microscopically, infection seems to occur by penetration of the specialized epithelium over Peyer's patches or intestinal lymphoid follicles. An initial neutrophilic response occurs and erosions of the epithelium develop subsequently. Macrophages and neutrophils accumulate in the lamina propria. The macrophages contain aggregates of *R. equi* but do not destroy them. Later, necrosis of lymphoid follicles occurs, and deep ulcers develop that contain masses of neutrophils, macrophages, and multinuclear giant cells. Pyogranulomatous lymphangitis and mesenteric lymphadenitis characterize the chronic enteric disease.

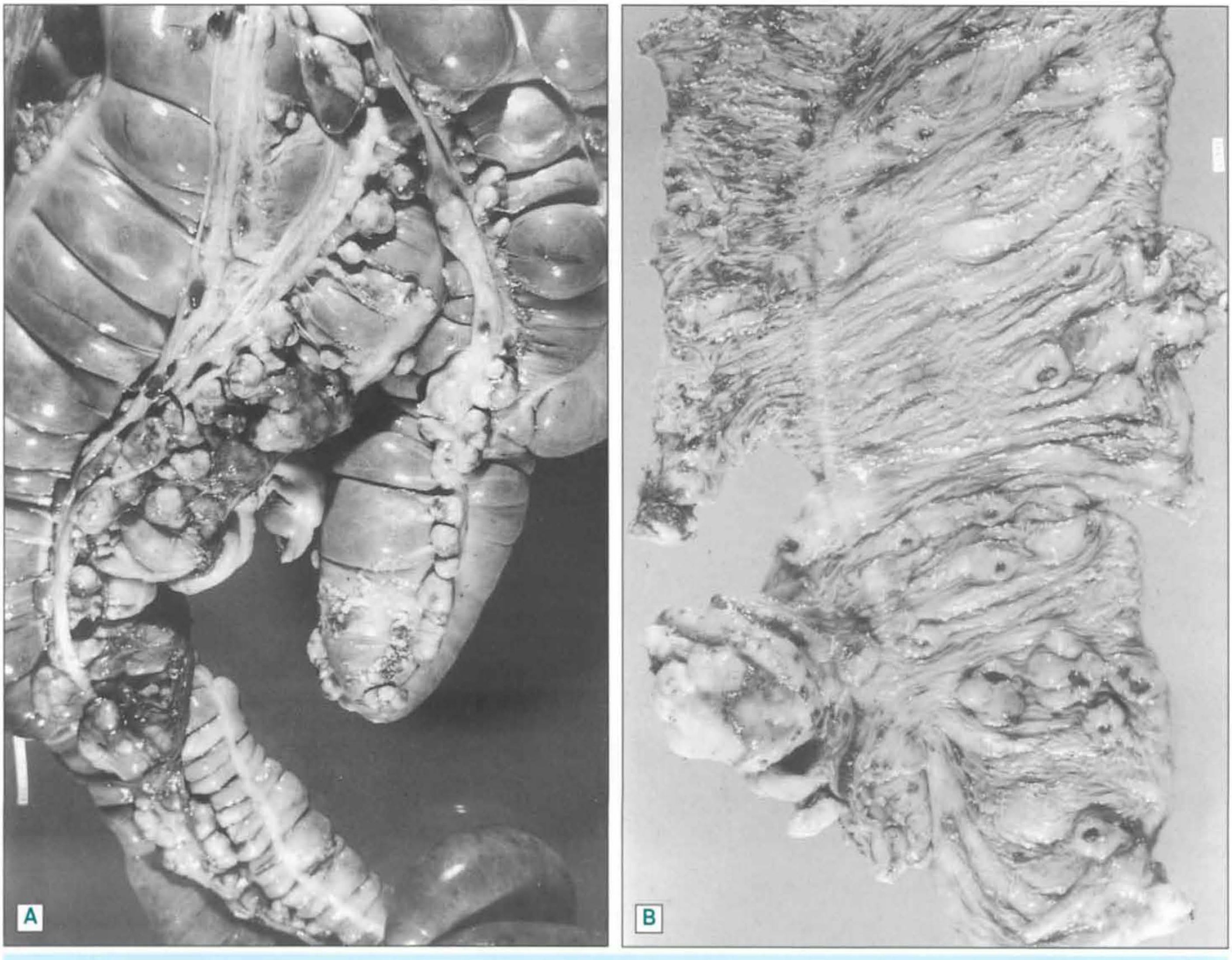

Figure 1.175 ***Rhodococcus equi* infection** in a foal. **A.** Enlarged suppurative cecal and colic lymph nodes. **B.** Craterous ulcerated lesions on colonic mucosa.

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Enterococcus sp. enteritis

Small gram-positive cocci identified as *Enterococcus durans*, found in the environment and the gut of various species, have occasionally been associated with diarrhea in suckling pigs, puppies, foals, calves, and suckling rats. The organisms from rats have been described, on genetic grounds, as *E. ratti*, whereas those from piglets have been described as *E. villorum* or *E. porcini*, which may be synonyms.

These bacteria adhere to the microvillus surface of enterocytes by fine filamentous pili. In tissue section, they form a layer of small cocci crowded on the entire surface of epithelial cells, from the tips to the base of villi. There may be mild-to-moderate villus atrophy and some desquamating enterocytes. Malabsorption associated with reduced brush border enzyme activity may explain diarrhea. Although in spontaneous cases in piglets, *Enterococcus* is frequently associated with other pathogens, the organism isolated from foals produced diarrhea when inoculated alone into gnotobiotic pigs.

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Bacteroides fragilis-associated diarrhea

Bacteroides fragilis is a nonspore-forming obligate anaerobe that is part of the normal enteric flora. Some enterotoxin-secreting strains have been associated with diarrhea in piglets, calves, lambs, foals, and humans. This enterotoxin is a *protease*, and probably damages the zonula adherens at the tight junction between enterocytes. Enterotoxigenic strains or cell-free culture filtrates cause secretion in ligated lamb or calf intestinal loops, and bacterial inocula cause diarrhea when administered orally to gnotobiotic piglets.

Bacteria do not adhere to the surface. Surface enterocytes round up and exfoliate, with villus attenuation and crypt elongation and hyperplasia. Infiltration of neutrophils is common. Damage may be seen in both small and large intestines. Ultrastructurally, affected cells lose their intercellular interdigitations, microvilli are shortened or absent, and the terminal web is disrupted.

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Anaerobiospirillum ileocolitis

In **cats**, ileocolitis has been associated with small spiral gram-negative bacteria, probably of the genus ***Anaerobiospirillum***. Cats may be asymptomatic, lethargic, and anorexic, or have vomiting and diarrhea. Microscopically, exfoliated epithelial cells and neutrophils are in dilated crypts in the ileum and colon, and bacteria stained with silver can be found in the lumen of crypts, in goblet cells, and sometimes in the lamina propria. Septicemia may occur, and renal failure has been associated.

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Enteritis due to Chlamydiaceae

Members of the family Chlamydiaceae are *obligate intracellular parasites*. Two genera are recognized on the basis of molecular genetics: *Chlamydia* and *Chlamydophila*. Among the three species in the former genus, only ***Chlamydia suis*** in pigs is associated with enteritis, while only one of the six species in the latter genus, ***Chlamydophila pecorum***, is associated with enteritis, in calves. Other syndromes associated with Chlamydiaceae in domestic animals include respiratory disease, polyarthritis, conjunctivitis, abortion, and encephalomyelitis, discussed in appropriate chapters elsewhere in these volumes.

The intestinal tract is the natural habitat for *C. pecorum*. Most infections are probably inapparent, but the intestine may be an important portal of entry in the development of systemic infections leading to hepatitis, arthritis, encephalitis, and pneumonia in ruminants. Enteritis may accompany or presage these diseases, and occasionally *C. pecorum* causes severe enteric disease in calves.

Following oral infection, *C. pecorum* infects mainly the enterocytes on the tips of ileal villi. These cells are in the G_1 phase of the cell cycle, which is required by *Chlamydophila* for multiplication. *C. pecorum* also infects other cells, including goblet cells, enterochromaffin cells, and macrophages, and the latter cells may transport the organisms systemically prior to being destroyed by them.

C. pecorum adsorbs to the brush border of enterocytes and enters the cell by pinocytosis. Following multiplication of organisms in the supranuclear region, the cells degenerate. *Chlamydophila* are released into the gut lumen, and into the lamina propria, where they infect endothelial cells of lacteals, whence they are released and become systemic.

Gastrointestinal disease caused by *Chlamydophila* is usually a problem of **calves** less than 10 days old, but it may affect older calves, and can produce recurrent diarrhea. Watery diarrhea, dehydration, and death are often accompanied by lesions, though not necessarily signs, of hepatitis, interstitial pneumonia, and arthritis. Gross lesions may occur in the abomasum and throughout the intestinal tract but are most consistent and severe in the *terminal ileum*. Mucosal edema, congestion, and petechiae, sometimes with ulceration, are usually observed. Serosal hemorrhages and focal peritonitis may occur. Histologically, *Chlamydophila* in inclusions may be demonstrable with Giemsa, Macchiavello, or immunoperoxidase staining. Central lacteals and capillaries are dilated, and neutrophils and monocytes infiltrate the lamina propria. Occasionally, granulomatous inflammation occurs in the intestinal submucosa and extends into the mesentery and to the serosa, producing the peritonitis observed grossly. Crypts in the small and large intestine may be dilated, lined by flattened epithelium, and contain inflammatory exudate.

C. suis has been recognized in the intestinal mucosa of **swine**, with approximately equal frequency in diarrheic and nondiarrheic animals. After experimental inoculation of *C. suis* into gnotobiotic piglets, there was necrosis and exfoliation of enterocytes on the apical half of villi, resulting in mild-to-severe villus atrophy in the distal jejunum and ileum, with moderate diarrhea. Lymphangitis and perilymphangitis were also evident in affected gut. In weanling pigs, similar lesions were induced, but no diarrhea.

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Potomac horse fever

This condition, also described as *equine monocytic ehrlichiosis* and *equine ehrlichial colitis*, was first defined clinically in 1979, and is characterized by fever, leukopenia, depression, loss of appetite, and diarrhea. It is caused by ***Neorickettsia*** (formerly *Ehrlichia*) ***risticii***, a member of the order Rickettsiales, which are *obligate intracellular bacterial pathogens*. *Neorickettsia* spp. replicate within the phagosome in the host cell, and use trematodes as hosts. Potomac horse fever typically occurs in the summer. It was first described in the Potomac river valley, and is associated with other river valleys in the northeastern USA. The condition is not commonly diagnosed as a cause of death elsewhere, though antibody has been reported from horses in many parts of North America, and in Europe. *N. risticii* in trematode metacercariae found in aquatic insects are probably ingested accidentally while drinking. The definitive hosts of trematode reservoirs are insectivorous bats, which themselves become infected with *N. risticii*.

The disease may be highly variable. Many infected horses seem not to get sick. Others develop severe colic, subcutaneous edema, laminitis, and shock: mortality can be up to 30% in untreated cases. Abortions of pregnant mares have been attributed to *N. risticii* infection.

The incubation period in experimental infections is about 9–14 days, and diarrhea begins 1–3 days after the onset of fever. Not all experimentally infected animals develop disease.

At **necropsy** of spontaneous cases, small vesicles are reported in the oral cavity, and epicardial hemorrhages and pulmonary congestion and hemorrhage, compatible with endotoxemia, are described. These are not reported in experimental cases; nor is laminitis. The lesions of the gastrointestinal tract are the most significant, in both spontaneous and experimental cases. In some animals there may be focal or more extensive erosions in the gastric mucosa, perhaps with some overlying fibrinous exudate. Lesions in the small intestine are generally limited to segmental areas of mucosal congestion or hyperemia, with occasional focal ulcers or hemorrhage, and are much less consistent and severe than those in the *cecum and colon*. The content of the large bowel is abnormally fluid, and may have a brown or red-brown color, and foul odor. In the cecum and colon, there may be patches of hyperemia 5–10 cm in diameter, aggregates of small ulcers a few millimeters in diameter, and petechial hemorrhages. Sometimes the mucosa of the entire cecum is widely hyperemic. Ulcers and petechial hemorrhage are more severe and consistent in the right dorsal colon. The small colon is usually unaffected grossly.

Microscopic lesions are most consistent in the large intestine; similar changes may be evident in small bowel. In areas of gross hyperemia, there is marked congestion and superficial hemorrhage in the mucosa. Associated with these lesions are superficial epithelial necrosis, erosion, and fibrin effusion. The mucosal surface is denuded, or perhaps covered by fibrinocellular exudate, and epithelium in the upper half of crypts is attenuated. Deeper parts of crypts are dilated and may contain necrotic epithelium and inflammatory cells. An abnormally intense mixed inflammatory cell population is in the lamina propria, and sometimes, the submucosa. Lymphoid tissue in the gut, mesenteric lymph nodes, and spleen is moderately involuted, compatible with the effects of the stress of systemic illness.

Organisms are not evident in hematoxylin and eosin-stained tissue. They are visible, in colon, and less consistently, in cecum, small colon, and small intestine, with *modified Steiner silver stain*. They appear as small clusters of 10–15 fine brown dots, under 1 μm in diameter, in the apical cytoplasm of epithelial cells deep in crypts, or as more numerous, smaller black structures distributed in the cytoplasm of macrophages in the periglandular lamina propria, or in a few glandular epithelial cells. Ultrastructurally, small dense elementary bodies may be found, alone or in small clusters in vacuoles in the cytoplasm of macrophages, mast cells, and crypt epithelium, or as the morula – aggregates of larger, more open organisms, in the same locations. *N. risticii* can be identified in feces or peripheral blood buffy coat by polymerase chain reaction, providing a more sensitive and specific means of diagnosis.

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Mycotic diseases of the gastrointestinal tract

Intestinal phycomycosis and aspergillosis

Mycotic invasion of the wall of the gastrointestinal tract is a common sequel to many diseases and lesions affecting the mucosa, and it may be the precursor to systemic infection. It is generally agreed that one or more of heavy fungal challenge, disruption of the normal flora, a primary local lesion, or lowered host resistance, is required for establishment of mycotic disease in the gut. Spores are probably carried across the mucosa by macrophages in the normal course of events, and only if phagocyte function is compromised, permitting germination, will they establish in the deeper tissues or become disseminated. Neutrophil function seems important in prevention of establishment of mycoses, whereas T-cell-mediated macrophage activity is involved in resolution of lesions.

Organisms associated with alimentary tract mycoses are *Aspergillus*; zygomycetes of the family Mucoraceae, including the genera *Absidia*, *Mucor*, and *Rhizopus*; the aquatic oomycete *Pythium*; and entomophthoracetes such as *Basidiobolus* and *Conidiobolus*. *Candida* spp. may also invade the wall of the alimentary canal; that is considered separately, below, as are enteric manifestations of histoplasmosis.

Aspergillus has relatively uniform narrow (3–6 μm) hyphae with relatively numerous septa; it typically displays acute angled dichotomous branching. **Mucoraceous fungi** are characterized in their invasive mycelial form by broad (6–25 μm), coarse irregular hyphae, with infrequent septation and random branching, sometimes surrounded by an eosinophilic sleeve in tissue sections. ***Pythium* spp.** have relatively narrow (up to 9–10 μm), thick-walled hyphae, with almost parallel walls and occasional septa; they branch at about right angles. In **entomophthoromycosis**, hyphae may vary from 5 to 25 μm in diameter, with thin irregularly parallel walls, infrequent septa, and rare random branching. They are characteristically surrounded by a wide sheath or sleeve of eosinophilic material in tissue section.

Lesions occur anywhere in the gastrointestinal tract, including the forestomachs of ruminants, and in the mesenteric lymph nodes. Clinical signs may be specifically related to the location of lesions (vomition, bloody diarrhea), or be nonspecific (malaise, weight loss), or be absent. Three types of lesion are produced: (1) *hemorrhagic and infarctive*; (2) *caseating*; *and* (3) *granulomatous*.

Mucoraceous fungi and ***Aspergillus*** typically cause *hemorrhagic and infarctive lesions*. Cases in cattle are seen following grain overload, mastitis, "downer cow syndrome," parturition, and subsequent to neonatal infectious bovine rhinotracheitis infection. Mucoraceous fungi frequently complicate Peyer's patch necrosis in cattle with mucosal disease, and are seen along the margins of the abomasal folds in calves with bacterial septicemia, producing mycotic abomasitis. These fungi can be found at any level of the gastrointestinal tract; however, omasum followed by rumen and reticulum are the most common sites. *Aspergillus* tends to be most common in the abomasum. These fungi have a propensity to invade mucosal and submucosal veins, producing *thrombosis and venous infarction*. Characteristically there is mucosal to full-thickness necrosis of the wall, which grossly is edematous and red-black due to venous stasis and hemorrhage. Often there is a relatively mild inflammatory response to the fungi. Spread to the liver and more distant organs via the portal and systemic circulations is not uncommon.

Mycotic ileitis and colitis in cats may occasionally be a sequel to panleukopenia. Intestinal lesions caused by the fungi (often *Aspergillus*) may be hemorrhagic and necrotizing with a prominent cellular response, but sometimes are small, localized, and difficult to find. In the latter cases, lesions of panleukopenia in the intestine and multifocal mycotic emboli in the lung suggest the pathogenesis. The lung appears to be the favored site of dissemination in cats. Mycotic enteritis with dissemination is a rare sequel to *Canine parvovirus 2* enteritis.

The gastrointestinal tract is probably a common portal of entry for many sporadic, disseminated zygomycoses, and aspergillosis, in animals. The presence of fungal hyphae in mesenteric lymph node granulomas of many clinically normal cattle indicates that, contrary to general impressions, *invasion by these agents across the intestinal mucosa does not lead invariably to systemic disease*. Fungi may produce a localized granulomatous lesion in specialized lymphoid tissue of the Peyer's patch or may be carried to the regional lymph node, while the mucosal lesion, if any, heals. In the lymph nodes, a granulomatous response, with giant cells that contain hyphal fragments, often develops; asteroid bodies may form around *Aspergillus* spp. Usually the granulomatous lesions produce little or moderate enlargement of lymph nodes, but sometimes a massive, caseating lymphadenitis results, with adhesions to adjacent structures.

Pythiosis (*oomycosis*), caused by the aquatic oomycete *Pythium insidiosum*, occurs most commonly in tropical and subtropical areas, but on occasion can be found in more temperate climates. It is best recognized as a cause of *cutaneous lesions in horses* (see Vol. 1, Skin and appendages). However, it causes enteric disease in dogs, and, less commonly, horses. There is segmental thickening and ulceration of the stomach and small intestine, with transmural granulomatous inflammation, and sometimes granulomatous peritonitis, with adhesions of the omentum. Obstruction may occur. Lymphovascular channels on the intestinal serosa are thickened, and the mesenteric lymph nodes are greatly enlarged and frequently embedded in a granulomatous mass. Small firm or caseous yellow foci, known as "*leeches*" or "*kunkers*," may be embedded in the firm fibrotic reactive tissue. Segments of bowel may be infarcted. Granulomatous inflammation is evident in the mucosa, and especially the submucosa; it extends along lymphatics transmurally. Granulomas, with a local mixed inflammatory infiltrate, often including eosinophils, may have liquefactive or caseous necrotic centers. Characteristic hyphae are difficult to see with hematoxylin and eosin; they are best exposed, in the areas of necrosis or centers of granulomas, by *silver stains*. Similar lesions are reported in horses with intestinal obstruction.

Entomophthoromycosis involving the gastrointestinal tract has been reported in a few dogs. Ulcerative stomatitis, gastritis, and enteritis are reported, with induration of involved tissues by a granulomatous inflammatory reaction containing the typical hyphae. In horses, entomophthoromycosis due to *Conidiobolus* may involve the lips and pharynx, as well as the nostrils and nasal mucosa.

Definitive **diagnosis** of mycotic lesions requires culture, which may be difficult, and identification of the isolate, which is frequently a specialist activity. If possible, granulomatous lesions of the gut should always be cultured for fungi as well as bacteria, in order to increase the frequency of specific diagnosis. Mycotic lymphadenitis must be differentiated from a mycobacterial, actinomycotic, or nocardial lesion. A presumptive diagnosis may be based on morphologic characteristics of organisms in tissue sections. Immunochemical procedures using specific antibody may add to the confidence of a morphological diagnosis.

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Candidiasis

Candida spp. are normal inhabitants of the alimentary tract of animals, existing as *budding yeasts* in association with mucosal surfaces. When there are changes in the mucosae, particularly squamous mucosae, or in the mucosal flora, the yeasts may become invasive; *branching, filamentous pseudohyphae and hyphae largely replace the yeast forms*. Only a few of the almost 200 *Candida* species cause candidiasis; in animals the most important are ***Candida albicans*** and ***C. tropicalis***.

Changes in the mucosal flora usually result from antimicrobial therapy that reduces the numbers of anaerobic bacteria and allows proliferation of *Candida* spp. Environmental and social stress, and treatment with anticancer and anti-inflammatory agents, may also predispose to candidiasis.

Candida spp., especially *C. albicans*, are capable of adhesion to epithelium, and this is important in virulence. Hypha formation is favored by carbohydrates such as sucrose, or polysaccharides that are less readily fermentable than glucose. Glucose is necessary for keratinolysis by the fungus. An endotoxin released during reproduction and death of *Candida* organisms may cause local damage, permit deeper penetration into squamous epithelium, and perhaps promote systemic dissemination.

Candida spp. are occasionally opportunistic invaders of mucosal lesions anywhere in the alimentary tract, but other fungi are more likely to take advantage of this kind of lesion, particularly in older animals. *Candidiasis is mainly a disease of keratinized epithelium in young animals, especially pigs, calves, and foals*. Accumulation of keratin due to anorexia probably contributes to the extensiveness of lesions in all species by increasing the substrate available to the fungus. Systemic infection appears to be associated with activation of virulence factor genes of *Candida* that facilitate dissemination and colonization. Adequate myeloperoxidase activity of leukocytes is important in prevention of dissemination of *Candida* spp. Foci of necrosis with a mainly neutrophilic infiltrate, and containing masses of proliferating organisms, are found in organs such as the spleen, liver, kidney, and heart.

In **pigs**, *Candida* spp. often invade the parakeratotic material that accumulates on the *gastric squamous mucosa*. Apparently these infections are innocuous. "*Thrush*" is candidiasis of the oral cavity; it is occasionally seen in young pigs, especially those raised on artificial diets, or in pigs with intercurrent disease. Lesions may be confined to the tongue, hard palate, or pharynx, but often involve the esophagus and gastric squamous mucosa as well. Rarely the glandular stomach is involved. Grossly the lesions are yellow-white, smooth, or wrinkled plaques more or less covering the mucosa. Histologically, the epithelium is spongy and contains yeasts and abundant hyphae and pockets of neutrophils and bacteria beneath the cornified layer. Congestion of vessels and a few inflammatory cells are present in the mucosal propria. Desquamation of the epithelium may produce small ulcers.

In **calves**, candidiasis occurs following prolonged antimicrobial therapy and in association with rumen putrefaction. Lesions are most often seen in the *ventral sac of the rumen*, but may involve the omasum and reticulum and occasionally the abomasum. Grossly the lesions resemble those of "thrush" in pigs, but the keratin layer tends to be thicker, less diffuse, and light gray. In the omasum, the leaves may be stuck together by the mass of fungus-riddled keratin. *C. glabrata* in the abomasum has been implicated as a cause of diarrhea in calves, especially during winter months. Disseminated candidiasis occurs more often in calves than in pigs, probably because of the relatively prolonged survival of calves with alimentary lesions. Candidiasis in calves must be differentiated from alimentary herpesviral infections.

Gastroesophageal candidiasis in **foals** involves the squamous epithelium and is associated with *ulceration adjacent to the margo plicatus*. Colic and anorexia are seen, and are probably related to the development of the ulcers, which may perforate, causing peritonitis.

In tissues, the presence of oval or round blastospores about 3–6 μm in diameter, which may be budding, mixed with pseudohyphae comprised of chains of elongate yeast-like cells, or with tubular septate hyphae, permits a provisional identification of *Candida* spp. Silver or periodic acid–Schiff stain enhances the organisms in section.

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Intestinal histoplasmosis

Histoplasma capsulatum is a *soil organism* of worldwide distribution. The disease, histoplasmosis, is endemic in certain areas, such as the Mississippi and Ohio river valleys of the USA, and in parts of southern Ontario and the Ottawa–St. Lawrence river valleys in Canada. There are sporadic cases elsewhere. It is important in humans and dogs, and occasionally occurs in other species. Infection generally occurs via *inhalation of spores*, and if lesions occur, they are usually confined to the lungs. Dissemination, with hepatic, splenic, and sometimes gastrointestinal lesions, develops in some dogs. It is associated with heavy exposure, young age, and perhaps some degree of host immunocompromise. Infection can also be produced by *ingestion*, and the rare examples of disease confined to the gastrointestinal tract may develop in this manner. Intestinal infection by ingestion of infected sputum is also possible.

Disseminated histoplasmosis is a disease predominantly of young dogs that are usually presented with weight loss, generalized lymphadenopathy, and often diarrhea with blood, and tenesmus. *Intestinal histoplasmosis* is reported as part of disseminated disease in cats, and as an isolated lesion in a horse.

At postmortem there may be hemorrhagic enteritis involving small and large intestine, or granulomatous thickening of the mucosa and intestinal wall with ulceration (Fig. 1.176), or no apparent lesions. Mesenteric lymph nodes are often markedly enlarged. Histologically, lesions, characteristically *transmural granulomatous inflammation*, may occur in stomach and small or large intestine. The nonulcerated areas of the mucosa contain focal to diffuse infiltrations of macrophages laden with *H. capsulatum* organisms within cytoplasmic vacuoles. The mucosa may be grossly thickened by the infiltrate, causing necrosis and ulceration. The cellular reaction may extend through the muscularis to the serosa. Macrophages filled with organisms are particularly prominent in the lymphoid tissue of the gut and the mesenteric nodes. *Periodic acid–Schiff stain* highlights the organisms in tissues. Microscopic diagnosis of gastrointestinal histoplasmosis is not difficult, but grossly the disease must be distinguished from intestinal lymphoma and in the colon, from colitis of other types. Histoplasmosis is discussed in detail in Vol. 3, Hematopoietic system.

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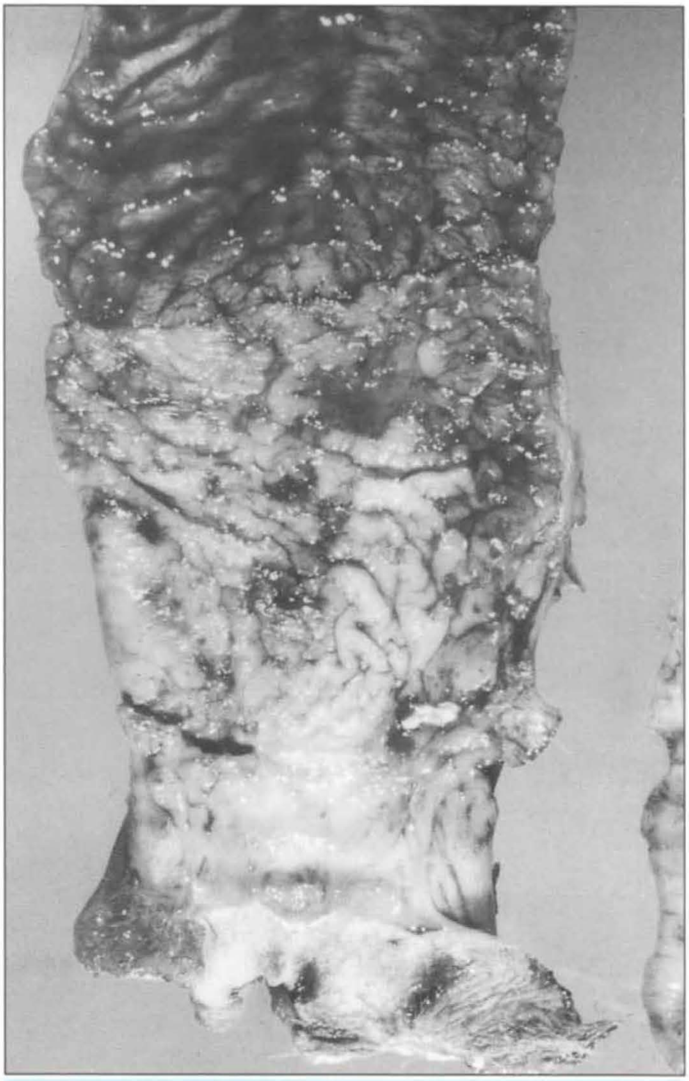

Figure 1.176 Ulcerative colitis in a dog with **histoplasmosis**.

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Protothecal enterocolitis

Prototheca spp. are *colorless algae* closely related to *Chlorella*. They are ubiquitous in raw and treated sewage and in water, and are found in feces, plant sap, and slime flux of trees. Two species, ***P. zopfii*** and ***P. wickerhamii*** cause disease in animals with both occurring occasionally in the same animal.

Lesions caused by *Prototheca* spp. include *cutaneous* infections of cats and human, *mastitis* in cows, and *disseminated* infections in cattle and dogs. The intestine and the eye are the most commonly involved sites in protothecosis of dogs.

Factors predisposing to the development of intestinal protothecosis are poorly understood. Skin infections are thought to result from traumatic inoculation, and it is possible that in the alimentary tract *Prototheca* is an opportunistic invader of existing mucosal lesions. Cattle, horses, and wild pigs pass *Prototheca* in the feces without apparent clinical disease. The chronicity of the disease and the mild host response are not consistent with a virulent infection. In dogs, Collies seem overrepresented among the cases reported, suggesting breed-related susceptibility or immune compromise.

Chronic, intractable, bloody diarrhea, or passage of blood-stained feces, is a frequent presenting sign, with progressive weight loss. *Hemorrhagic and ulcerative colitis* is a prominent enteric lesion, but changes may also develop in the small intestine. Mesenteric lymph nodes may be enlarged.

The *mild host response to infection* is characteristic of protothecosis; usually only a few lymphocytes and monocytes are present. In early lesions, the extracellular organisms are scattered in the lamina propria, but later they fill the lamina propria and are often packed in cords in the connective tissue of the submucosa. Lacteals are distended, while lymphatics and the sinuses of draining lymph nodes are filled with organisms.

Prototheca in tissue sections range from 5 μm spheres to 9 × 12 μm ovoids, with a refractile capsule, and are positive with *periodic acid–Schiff and silver stains*. The presence of endosporulation with formation of 2–20 sporangiospores within a single sporangium characterizes *Prototheca* spp. and *Chlorella* spp. *Chlorella* contain periodic acid–Schiff-positive cytoplasmic starch granules that are PAS-negative following diastase digestion; *Prototheca* do not contain these granules. Differentiation of the genera by a fluorescent antibody test using formalin-fixed material is also possible.

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Gastrointestinal helminthosis

The diagnosis of disease due to gastrointestinal helminths must be made with knowledge of their pathogenic potential and the mechanisms by which it is expressed. *Parasites are much more common than the diseases they cause*, and "**helminthiasis**," *the state of infection*, must be clearly differentiated from "**helminthosis**," *the state of disease*.

Gastrointestinal helminths fall into *five categories*, according to pathogenesis of disease:

1. The first group **resides free in the lumen of the intestine**, competing with the host for nutrients in the gut content. They are generally of low pathogenicity, except for rare massive infections, and are not likely to be lethal, except by obstruction. Some of these worms, in sufficient numbers, may cause subclinical disease such as inefficient growth, or clinical disease in the form of ill-thrift; others are essentially nonpathogenic. The ascarids, adult small strongyles (cyathostomes) of horses, and tapeworms such as *Moniezia* and *Taenia* spp., fall into this group, as may *Physaloptera* in the stomach of carnivores.
2. A second group of helminths, all nematodes, primarily cause **blood loss**. These worms feed on the mucosa, causing bleeding, or they actively suck blood. Anemia, hypoproteinemia, and their sequelae cause production loss, clinical disease, and death. *Haemonchus* in the abomasum, and in the intestine the hookworms of carnivores and ruminants, the large strongyles of horses, and *Oesophagostomum radiatum* in cattle are the main examples.
3. The third group, composed of nematodes and some flukes, mainly causes **protein-losing gastroenteropathy**, usually associated with inappetence and diarrhea. In the abomasum, *Ostertagia* and *Trichostrongylus axei* cause mucous metaplasia and hyperplasia of gastric glands, achlorhydria, and diarrhea. In the small intestine, *Cooperia*, *Nematodirus*, *Strongyloides*, *Trichostrongylus*, and larval paramphistomes in sheep and cattle cause villus atrophy. This may cause malabsorption of nutrients, electrolytes, and water. But probably more important is the loss of endogenous protein into the gut, mainly related to chronic mucosal inflammation. In heavy infestations with *Trichuris* spp., erosion results in loss of absorptive function, and effusion of tissue fluids, or, in severe cases, hemorrhagic exudate.
4. The fourth group causes **physical trauma to the intestinal wall** by burrowing into or inciting inflammatory foci in the submucosa or deeper layers. In the stomach, various species of spirurids embed in the mucosa, or establish in cystic spaces in the submucosa. In the intestine, acanthocephala cause local ulceration by their thorny hold-fast organ; larval stages of equine cyathostomes and *Oesophagostomum* spp. become encapsulated in the submucosa. Protein loss may occur from ulcerated areas, or when larvae emerge from the submucosa. The potential exists for perforation of the stomach or bowel, or for complications due to sepsis of submucosal nodules. Adhesion of inflamed serosal surfaces associated with nodules or perforations may impair motility.
5. Finally, some intestinal helminths, among them a few in the categories above, have **effects at sites distant from the gut**. This is usually the result of migrating larval stages of the worm, either in definitive or intermediate hosts. Larval *Habronema*, ascarids, hookworms, and equine strongyles may cause lesions in a variety of extraintestinal sites in the definitive host. Larval ascarids and taeniid metacestodes may cause lesions or signs due to migration in nonenteric locations in accidental or intermediate hosts.

A **diagnosis of helminthosis** should be reserved for cases where, ideally, three criteria are met: (1) the helminth is present, in numbers consistent with disease; (2) the lesions (if any) typically caused by the agent, are evident; and (3) there is a syndrome compatible with the pathogenic mechanisms known to be associated with the worm.

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Abomasal and gastric helminthosis

Ostertagiosis

A complex of related genera and species of trichostrongylid nematodes, including *Ostertagia*, parasitize the *abomasum of ruminants*. The nomenclature of these worms is in a state of flux; for the sake of simplicity, the disease that they cause will be termed ostertagiosis. *Ostertagiosis is probably the most important parasitism in grazing sheep and cattle in temperate climatic zones throughout the world*. It causes subclinical loss in production, and clinical disease characterized by diarrhea, wasting, and, in many cases, death. ***Ostertagia ostertagi*** and the associated *O. lyrata* infect cattle. Sheep and goats are infected by ***Ostertagia*** (*Teladorsagia*) ***circumcincta***. Some cross-infection by these genera occurs between sheep and cattle, but is of minor significance. Other species of *Ostertagia* and related genera, including *Marshallagia*, *Spiculopteragia*, and *Camelostrongylus*, infect wild ruminants, including farmed deer; some may also parasitize the abomasum of cattle, sheep, and goats. Their behavior in general resembles that of *Ostertagia* and *Teladorsagia*.

The life cycle is direct. Third-stage larvae exsheath in the rumen and enter glands in the abomasum, where they undergo two molts. Normally, early fifth-stage larvae emerge to mature on the mucosal surface, beginning 8–12 days after infection in *O. circumcincta* infections in sheep, and about 17–21 days after *O. ostertagi* infection in cattle. However, a proportion of larvae ingested may persist in glands in a hypobiotic state at the early fourth stage, only to resume development and emerge at a future time, perhaps many months hence. The prepatent period is about 3 weeks.

During the course of larval development, the normal architecture of the gastric mucosa is altered by *interstitial inflammation*, and *mucous metaplasia and hyperplasia of the epithelium lining glands*. In sheep infected with *O. circumcincta*, mucous metaplasia and hyperplasia occur in infected and surrounding glands early in infection, reaching a peak about the time of emergence of larvae on to the mucosal surface. In cattle with *O. ostertagi* (Fig. 1.177A), only glands infected with larvae undergo significant mucous change until about the time larvae leave the glands for the surface of the mucosa (Fig. 1.177B). Mucous change then becomes more widespread, involving uninfected glands in the vicinity of those that contained larvae.

In both species, affected glands are lined by mucous neck cells that proliferate, displacing parietal cells. Affected areas of mucosa thicken. In infected glands, in many cases the lining is flattened adjacent to worms (Fig. 1.177C), but is composed of tall columnar mucous cells elsewhere in the gland. The undifferentiated mucous cells lining uninfected glands also eventually differentiate into tall columnar mucous cells. If infection is not heavy, lesions are limited to a radius of a few millimeters around infected or previously infected glands. These form raised nodular pale areas in the mucosa, often with a slightly depressed center. Confluence of these lesions in heavily infected animals leads to the development of *widespread areas of irregularly thickened mucosa with a convoluted surface pattern*, likened to "*Morocco leather*" (Fig. 1.178).

Mucous metaplasia and hyperplasia are accompanied by a mixed population of inflammatory cells in the lamina propria. Lymphocytes, plasma cells, eosinophils, and a few neutrophils are present between glands in the infected abomasum, and globule leukocytes are common in gland epithelium. There may be edema of the lamina propria associated with permeability of proprial vessels. Lymphoid response in local lymph nodes has been characterized as primarily B-cell-oriented, which is a surprising reaction to a nematode parasite.

Mucosal lesions lead to achlorhydria, elevation of plasma pepsinogen levels, and local vascular permeability with loss of plasma protein. Widespread replacement of parietal cells by mucous neck cells results in progressive and massive decline in hydrogen ion secretion, with severe cases having a pH of up to 7 or more. This increased abomasal pH results in elevated levels of gastrin in the circulation. The permeability of the mucosa is also increased, which is reflected in backdiffusion of pepsinogen from the lumen of glands to the propria, and ultimately to the circulation. Intercellular junctions between poorly differentiated mucous neck cells are also permeable to plasma protein in tissue fluids, emanating from the leaky small vessels in the inflamed lamina propria. Significant loss of protein occurs into the lumen of the abomasum.

The cardinal signs of ostertagiosis in sheep and cattle are *loss of appetite, diarrhea, and wasting*. Plasma protein loss into the gastrointestinal tract, in combination with reduced feed intake, seems largely responsible for the weight loss and hypoproteinemia that occur in clinical ostertagiosis, and for loss in productive efficiency that occurs in subclinical disease.

Clinical ostertagiosis occurs under two sets of circumstances. The first, **"type I" disease**, is seen in lambs or calves at pasture during or shortly after a period of high availability of *infective larvae*. It is due to the direct development, from ingested larvae, of large

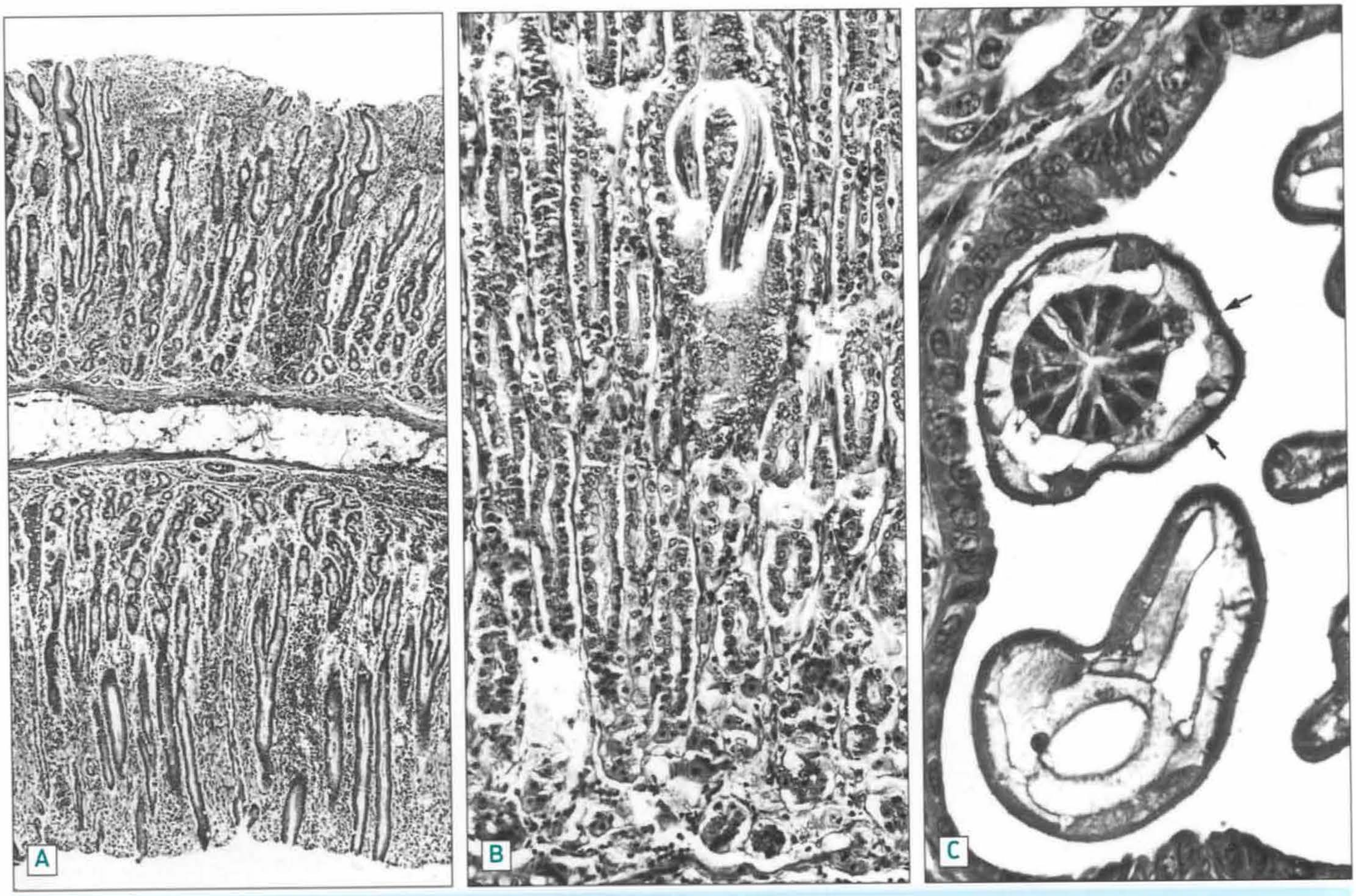

Figure 1.177 Ostertagiosis in the bovine abomasum. **A.** Mucous metaplasia and hyperplasia thickening fundic mucosa on abomasal fold. Moderate inflammatory infiltrate in propria between glands. **B.** Mucous metaplasia and hyperplasia deep in fundic mucosa. Larva in section in gland. **C.** *Ostertagia* in dilated gland lined by cuboidal mucous neck cells. Longitudinal cuticular ridges are visible as fine projections on the nematode (arrows).

numbers of adult worms, over a relatively short period of time. In contrast, **"type II" disease** is due to the synchronous maturation and emergence of large numbers of *hypobiotic larvae* from the mucosa, and it occurs when intake of larvae is likely low or nonexistent. It may occur in yearlings during the winter in the northern hemisphere, or during the dry summer period in Mediterranean climates. Heifers about the time of parturition may succumb, and this syndrome is also occasionally seen in animals experiencing environmental stress of any type.

The **diagnosis** of ostertagiosis is indicated at autopsy by an *abnormally elevated abomasal pH* (>*4.5*), in association with *typical gross lesions* on the mucosa. The adult worms are brown and thread-like, up to 1.5 cm long, but very difficult to see on the mucosa with the unaided eye. *Abomasal contents and washings* should be quantitatively examined for the presence of emergent or adult *Ostertagia* and other nematodes. A *portion of the mucosa should be digested* to permit recovery and quantitation of pre-emergent stages. Significant worm burdens in sheep are in the range of 10 000–50 000 or more. In cattle >40 000–50 000 adult worms may be present, and in outbreaks of type II disease hundreds of thousands of hypobiotic larvae are often detected in the abomasal mucosa. Typically, there is widespread mucous metaplasia and hyperplasia in dilated glands in sections of abomasum. *Ostertagia* are recognized in sections, on the mucosal surface or in glands, by the presence of prominent longitudinal cuticular ridges (synlophe) that project from the surface of worms cut transversely. In some cases, the worm burden may have been lost through attrition or recent treatment, and the diagnosis must be presumptive, based on the characteristic mucosal lesions.

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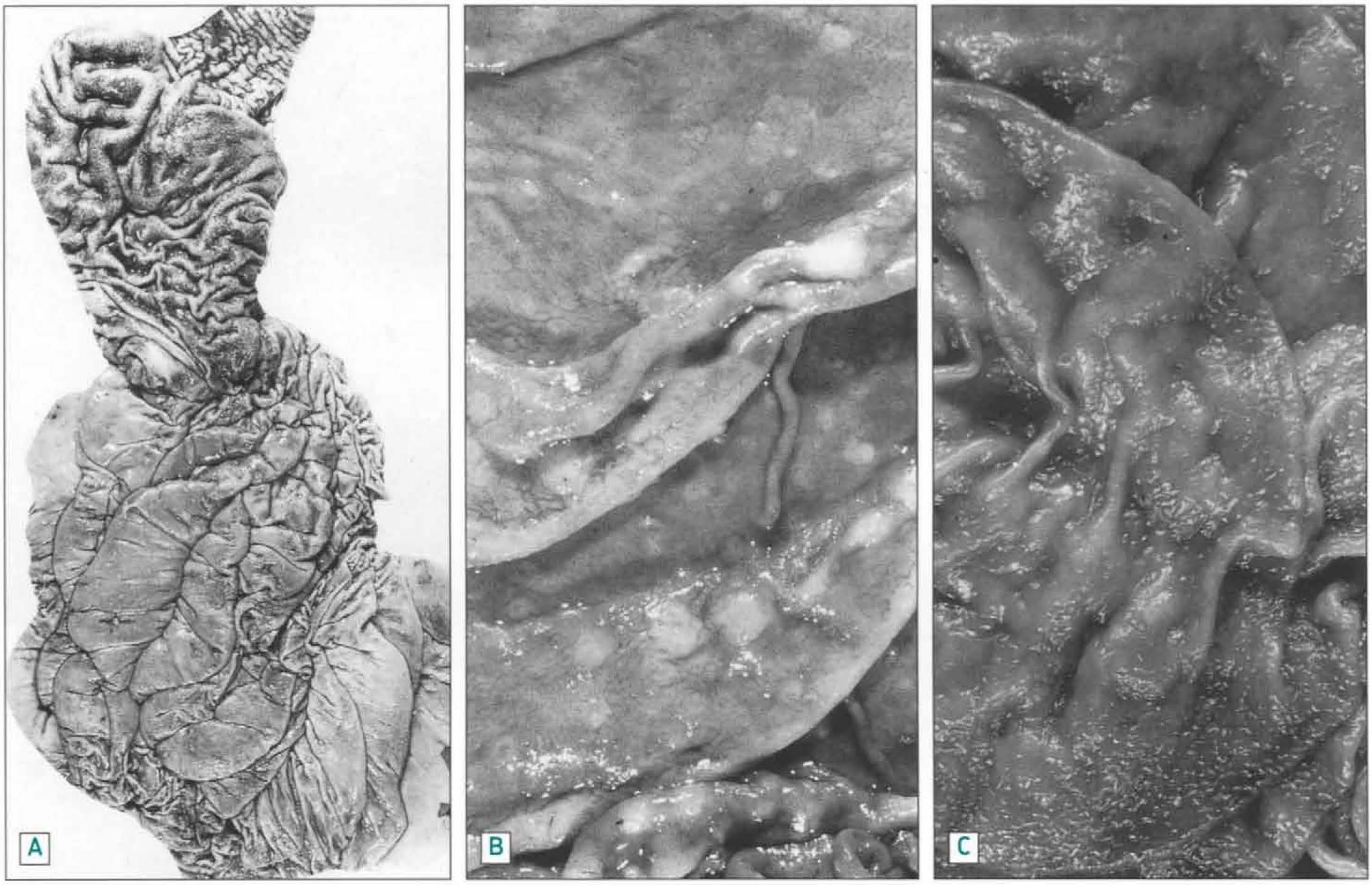

Figure 1.178 Ostertagiosis in abomasum. **A.** Acute edematous gastritis in a cow. **B.** Individual nodules and some confluent lesions in a sheep. **C.** Confluent thickening of hyperplastic glandular mucosa in a sheep.

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Hemonchosis

Hemonchosis is a common and severe disease in some parts of the world. *Haemonchus* species require a period of minimum warmth and moisture for larval development on pasture. As a result, they tend to be *most important in tropical or temperate climates with hot wet summers*. ***Haemonchus contortus*** infects mainly sheep and goats, whereas ***H. placei*** occurs mainly in cattle. Though *H. contortus* and *H. placei* will infect the heterologous host, the host–parasite relationship appears to be less well adapted, and the species do appear to be genetically distinct. ***Mecistocirrus digitatus*** causes disease very similar to hemonchosis in cattle and sheep in Southeast Asia and Central America.

By exploitation of hypobiosis or retardation of larvae, populations of *H. contortus* are able to persist in the abomasum of the host through periods of climatic adversity, such as excessive cold or dryness. Disease can be expected in animals, especially females, experiencing the synchronous "*spring rise*" or periparturient development and maturation of previously hypobiotic larvae, and in young animals heavily stocked at pasture during periods of optimal larval development and availability.

Haemonchus, commonly called the *large stomach worm, or barber pole worm*, is ~2 cm long. Females give the species its common name by their red color, against which the white ovaries and uterus stand out. The male is a little shorter and uniform deep red. These worms are equipped with a buccal tooth or lancet, and *fourth-stage and adult worms suck blood*. Ingested third-stage larvae enter glands in the abomasum, where they molt to the fourth stage and persist as hypobiotic larvae, or from which they emerge as late fourth-stage larvae to continue development in the lumen. The prepatent period for *H. contortus* in sheep is ~15 days; for *H. placei* in cattle is ~26–28 days; and for *M. digitatus* is ~61–79 days.

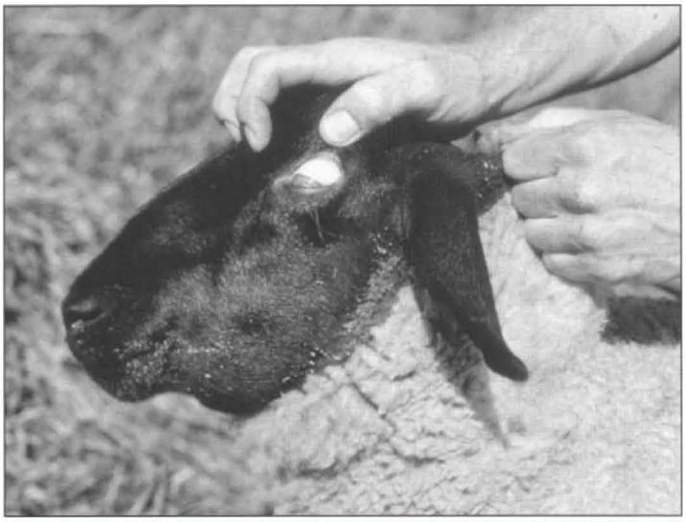

Figure 1.179 Severe **submandibular edema** ("bottle-jaw"), and **anemia**, manifest as conjunctival pallor, in ***Haemonchus contortus* infection** in a sheep.

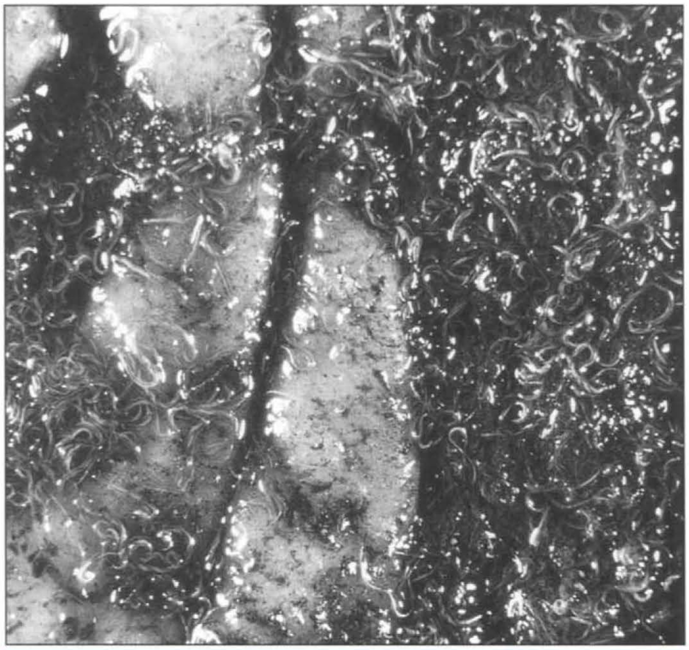

Figure 1.180 ***Haemonchus contortus*** in acid-hematin-tinged abomasal content in a sheep.

Hemonchosis may occur as *peracute or acute disease*, resulting from the maturation or intake of large numbers of larvae. It may cause more insidious *chronic disease*, if worm burdens are lower. The pathogenicity of *Haemonchus* infection, whatever its manifestation, is the result of blood-sucking activity, which causes anemia and hypoproteinemia.

Individual *Haemonchus* worms in sheep cause the loss of about 0.05 mL of blood per day. Of the order of a tenth to a quarter of the erythrocyte volume may be lost per day by heavily infected lambs; the plasma loss is concomitant and may be several hundreds of milliliters. The potential for the *rapid onset of profound anemia and hypoproteinemia* in heavily infected animals is obvious. Such animals succumb quickly, some even before the maturation of the worm burden. Less heavily infected animals may be able to withstand the anemia and hypoproteinemia for a period of time. They compensate by expanding erythropoiesis two- to threefold, and increasing hepatic synthesis of plasma protein. However, they are unable to compensate adequately for the enteric iron loss, despite intestinal reabsorption of a proportion of the excess, and they ultimately succumb some weeks later to *iron-loss anemia*, when iron reserves are depleted. Low-level infections may contribute to subclinical loss of production or ill-thrift through chronic enteric protein and iron loss. Low protein rations compound the effect of infection.

The clinical syndrome may vary somewhat. Some animals are found dead, without the owner observing illness. Others lack exercise tolerance, fall when driven, or are reluctant to stand or move, so weak are they from anemia. Edema of dependent portions, especially the submandibular area or head in grazing animals, is often observed (Fig. 1.179). In primary hemonchosis, there is no diarrhea; diarrhea may occur if intercurrent infection with large numbers of other gastrointestinal helminths occurs.

The **postmortem** appearance of animals with hemonchosis is dominated by the extreme pallor of anemia, apparent on the conjunctiva and throughout the internal tissues. The liver is pale and friable. There is usually edema of subcutaneous tissues and mesenteries, with hydrothorax, hydropericardium and ascites, reflecting the severe hypoproteinemia. The abomasal content is usually fluid, and dark red-brown, due to the presence of blood (Fig. 1.180). The abomasal rugae may be edematous due to hypoproteinemia, and focal areas of hemorrhage are evident over the surface. In animals that are not decomposing, the worms will be evident to the naked eye: if alive, writhing on the mucosal surface; if dead, less obvious and free in the content.

In clinically affected sheep and goats, usually about 1000–12 000 worms are found. The severity of the disease is a function of the number of worms on one hand, and to some extent, the size of the animal on the other. In lambs, 2000–3000 worms is a heavy burden, while in adult sheep and goats, 8000–10 000 are associated with fatal infection.

A high egg count is usually found on fecal flotation, since *Haemonchus* is a prolific egg-layer. However, in peracute prepatent infections, no eggs will be present. In recently treated animals, no worms may be present, and the diagnosis may have to be presumptive. On the other hand, treated animals returned to contaminated pasture may succumb to reinfection within 2–3 weeks. A serologic test targeting a somatic antigen has been developed.

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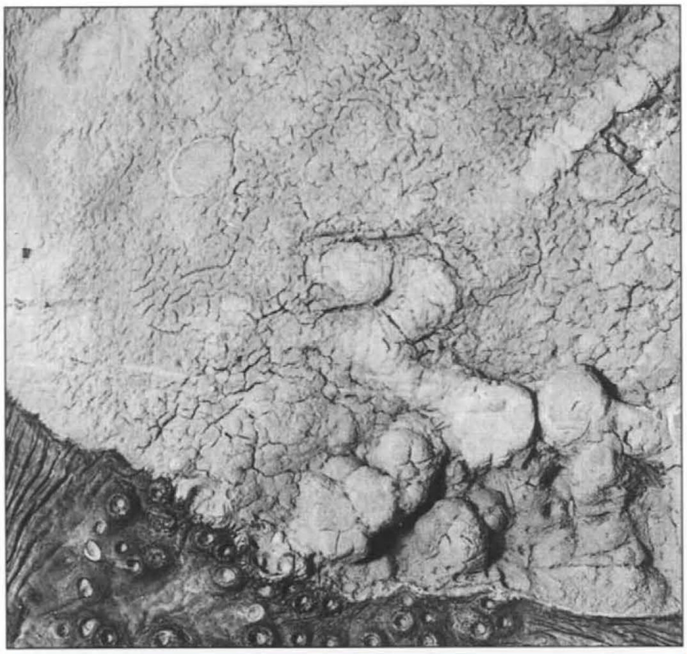

Figure 1.181 Hypertrophic gastritis in the fundic mucosa of a horse with **trichostrongylosis**. (Courtesy of NO Christensen and Skand Vet.)

Trichostrongylus axei infection

Trichostrongylus axei infects the *abomasum of cattle, sheep, and goats*, and the *stomach of horses*. It has a direct life cycle: third-stage infective larvae enter tunnels in the epithelium of the foveolae and neck of gastric glands in both fundic and pyloric areas. The worms live throughout their life *partly embedded in intraepithelial tunnels* at about this level of the mucosa. They molt to the fourth stage about a week after being ingested and to the fifth stage by ~2 weeks after infection. The prepatent period is ~3 weeks in calves and sheep, and about 25 days in horses.

Infections with *T. axei* are usually part of a *mixed gastrointestinal helminthosis*. However, in all hosts this species alone is capable of inducing disease, if present in sufficient numbers. After a period of several weeks, *mucous metaplasia and hyperplasia are seen in glands in infected areas of the mucosa*. In severely affected animals, flattening of surface epithelium with desquamation, or erosion of the mucosa, develops, accompanied by effusion of neutrophils, eosinophils, and tissue fluid. Fibroplasia may occur in the superficial propria in eroded areas.

In light infestations there may be no changes visible in the abomasum other than congestion of the mucosa. The gross lesions present in heavy *T. axei* infections reflect the hypertrophy of glands, and superficial erosion. *Circular or irregular raised white plaques of thickened infected mucosa* are present, often with a thick layer of mucus. Erosions or shallow ulcers may be present. In severe infections the entire mucosa appears edematous and congested.

Infection in horses is uncommon and is usually related to sharing pasture with sheep or cattle. In chronically infected horses, white raised plaques (Fig. 1.181) or nodular areas of mucosa are present, covered by tenacious mucus and surrounded by a zone of congestion. Mucosal lesions may be confluent in heavily infected animals, and erosions and superficial ulceration may be encountered. Infection may extend into the proximal duodenum, where polypoid masses of hypertrophic glandular mucosa are occasionally observed. Plasma pepsinogen levels may be elevated.

Achlorhydria develops in heavily infected sheep and cattle, associated with *diarrhea*, particularly in the latter species. Dehydration may prove severe in scouring calves. Plasma pepsinogen and gastrin levels increase, and hypoproteinemia and *wasting* occur. This suggests that the mucous metaplasia in the glands is associated with increased permeability and that plasma protein loss occurs into the gastrointestinal tract.

Though *T. axei* is not common as a primary cause of disease in any species, it should be sought at autopsy of animals with signs of wasting and perhaps diarrhea. The typical gross lesions in the stomach are distinctive in horses. In ruminants, they must be differentiated from those due to *Ostertagia*, with which animals may be intercurrently infected. The worms are very fine, and gastric washes or digestion are required to recover them quantitatively. The *distinctive intraepithelial location* of *T. axei* in section differentiates it from other nematodes inhabiting the abomasum of ruminants and the stomach of horses.

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Gastric parasitism in horses

The commonest parasites of the equine stomach are *larvae of bot flies* of the genus ***Gasterophilus***. Though they are not helminths, it is convenient to consider them here. There are six species of the genus, the common ones being ***G. intestinalis***, ***G. nasalis***, and ***G. haemorrhoidalis***, and the uncommon ones being *G. pecorum*, *G. nigricornis*, and *G. inermis*. The ova are deposited on the ends of the coat hairs in the face, intermandibular region, or on the lower body and legs. The eggs hatch spontaneously, or when stimulated by licking. The first-stage larvae penetrate the oral mucosa, molt, emerge, and migrate down the alimentary canal.

Gasterophilus intestinalis usually wander about in tunnels in the superficial mucosa of the cheeks, tongue, or gums for 3–4 weeks before moving to periodontal pockets containing purulent exudate in the gingival sulcus on the lingual aspect of molars, especially in the upper arcade. Here they molt before moving on to the base of the tongue, and to the stomach. This is the most common species, and in the stomach it attaches itself to the squamous mucosa of the cardia to complete its subsequent molts. ***G. nasalis*** first invade the gums, where they may be associated with pockets of purulent exudate in the interdental spaces, then pass to the stomach and settle on the pyloric mucosa and in the first ampulla of the duodenum. Members of any of these species may occasionally be found attached to the pharynx and esophagus but, except for *G. pecorum* that congregates in the pharynx

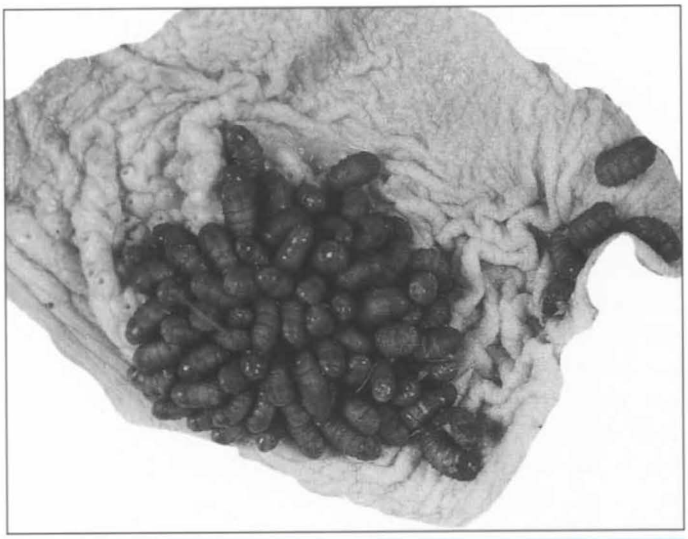

Figure 1.182 ***Gasterophilus* larvae** on gastric mucosa of a horse.

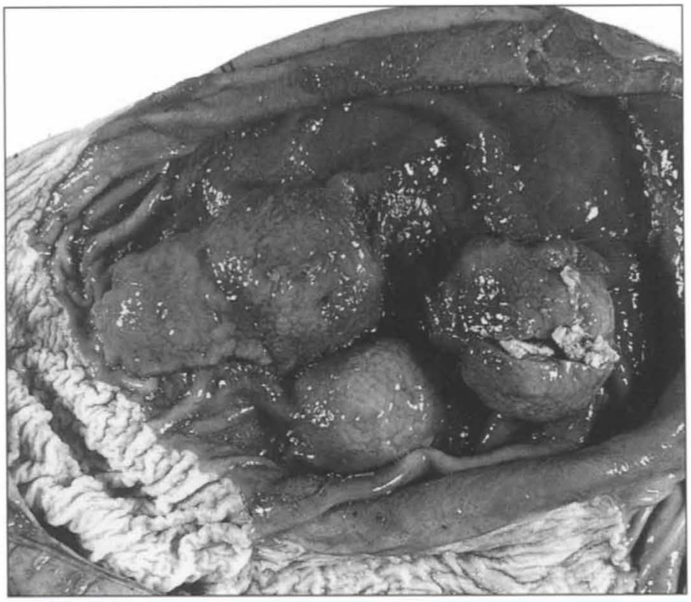

Figure 1.183 Nodules containing ***Draschia megastoma*** in the submucosa of glandular mucosa, near margo plicatus. Purulent content in sectioned nodule.

and causes pharyngitis, these preliminary migrations are uneventful for the host. In the summer following the deposition of the ova, the larvae leave the stomach and pass out in the feces to pupate. Those of *G. pecorum* and *G. haemorrhoidalis* may attach themselves for a short while to the wall of the rectum.

It is generally assumed that the larvae of *Gasterophilus* have little effect on their host. The larvae fasten themselves to the mucosa by chitinous oral hooks and they bore into the mucosa (Fig. 1.182). They apparently subsist on blood, exudate, and detritus, producing focal erosions and ulcerations at the point of contact. These defects in the cardia are surrounded by a narrow rim of hyperplastic squamous epithelium. Usually the number of epithelial defects exceeds the number of larvae, suggesting that they move about on the mucosa.

Severe infestations produce a dense pock-marked appearance of the pars esophagea, with chronic inflammatory thickening. Ulcers may occur in the glandular mucosa and, rarely, a large proportion of the affected pyloric mucosa may be lost. Healing occurs when the larvae migrate on, but may be complicated by secondary bacterial infection. Histologically, the ulcers penetrate the submucosa, which is chronically inflamed. The deep layers of eroded epithelium and the epithelial margins of ulcers in the squamous mucosa become hyperplastic and develop rete pegs. There seems to be no relationship between bot infestations and the development of gastric ulcers in the pars esophagea.

The **spirurid nematodes *Draschia megastoma*, *Habronema majus***, and ***H. muscae*** are also parasitic in the stomach of horses. The adult worms are 1–2 cm in length. The latter two species lie on the mucosal surface and are probably insignificant except possibly for a few erosions and mild gastritis. *D. megastoma* burrows into the submucosa to produce large tumor-like nodules (Fig. 1.183).

H. majus mainly uses *Stomoxys calcitrans* as its intermediate host and the other two species use various muscid flies. The *Habronema* larvae in the feces are swallowed by maggots of the appropriate intermediate host and persist through pupation and maturation of the fly. They leave the host fly via the proboscis when it seeks moisture, for instance, on the lips. Larvae deposited on or in cutaneous wounds, or in the eye, invade the skin or conjunctiva and provoke an intense local reaction, which becomes granulomatous and densely infiltrated with eosinophils (see Vol. 1, Skin and appendages). Occasionally *Draschia* and *Habronema* larvae may be found in the brain, or in the lungs, where they may become encapsulated and mineralize.

The only one of concern in the stomach is *D. megastoma*, which burrows into the submucosa of the fundus, usually within a few centimeters of the margo plicatus. Within the submucosa, the worms provoke a surrounding granulomatous reaction that contains them in a central core of necrotic and cellular detritus, with abundant eosinophils. These lesions form protrusions up to ~5 cm in diameter, with a small fistulous opening to the lumen. The nodules generally produce no clinical disturbance, though they have been considered to lead rarely to abscessation, adhesions of the stomach to the spleen, or perforation when infected with pyogenic bacteria.

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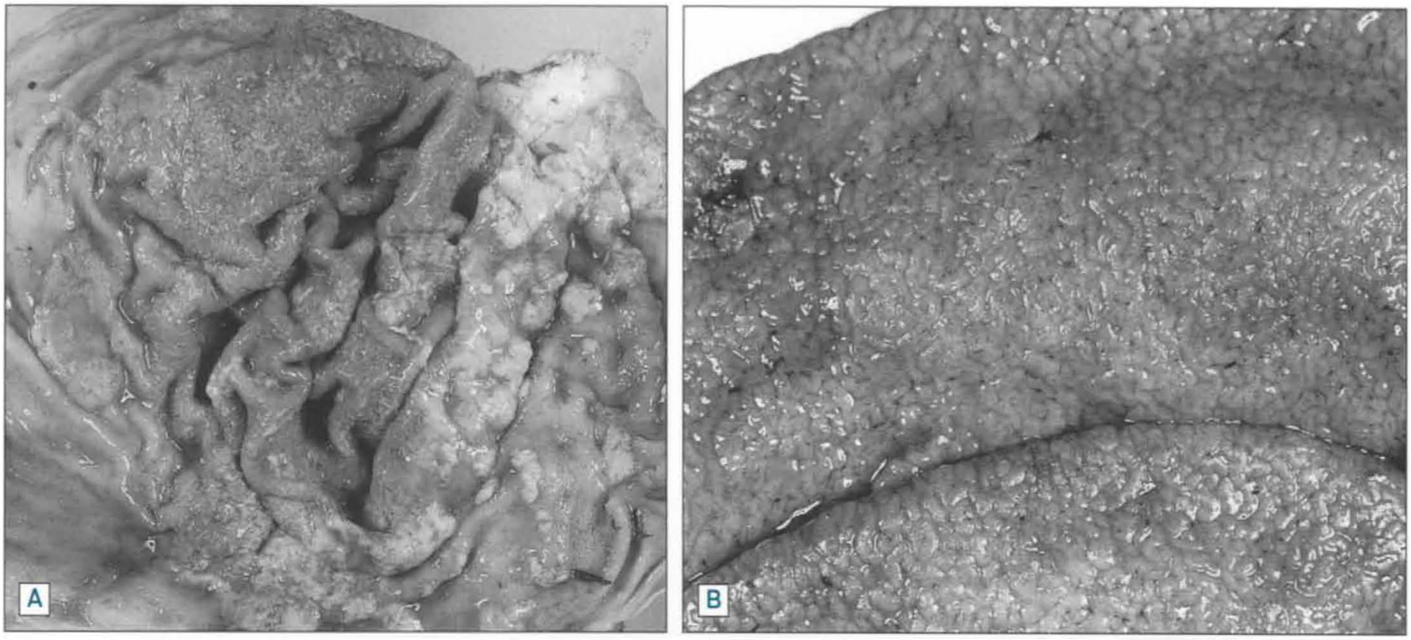
Gastric parasitism in swine

Gastric parasitism is not of great clinical or pathologic importance in swine and is rare in pigs reared in modern total confinement systems.



Ascaris suum, normally inhabiting the small intestine, may migrate or reflux to the stomach after death. *Hyostrongylus rubidus* is probably the most significant parasite of the stomach of swine; it and the various spirurids are more common in pigs allowed to forage. *Ollulanus tricuspis* is reported in pigs. It is more commonly encountered in cats, and is discussed with gastric parasitism in dogs and cats.

Hyostrongylus rubidus is a trichostrongylid nematode with a typical life cycle. Third-stage larvae enter glands in the stomach, especially in the fundic region, where they develop and molt twice. Pre-adult and adult worms emerge on to the gastric mucosa ~18–20 days after ingestion. The lesions produced by *Hyostrongylus* resemble those caused by *Ostertagia* in ruminants. There is *mucous metaplasia and hyperplasia of the lining of infected and neighboring glands*, and dilation of infected glands. The lamina propria in infected mucosa is edematous and infiltrated by lymphocytes, plasma cells, and eosinophils, and lymphoid follicles develop deep in the mucosa. Neutrophils and eosinophils may transmigrate the epithelium into dilated glands, the lining of which may become quite attenuated. There may be extensive erosions.

During the course of development of the worms, the mucous metaplasia and hyperplasia cause the formation of pale nodules in the vicinity of infected glands (Fig. 1.184A). In heavy infections these may become confluent, causing the development of an irregularly thickened convoluted mucosa (Fig. 1.184B), most notable in the fundic area and along the lesser curvature. Adult worms are fine, red, and thread-like in the gastric mucus; they are difficult to see with the naked eye.

Experimental infections of moderate degree do not produce obvious clinical signs or loss of production. However, loss of plasma protein has been documented in heavy *Hyostrongylus* infections. Inappetence, diarrhea, and reduced weight gains and feed efficiency also occur in these circumstances. In the field, hyostrongylosis is mainly associated with the "thin-sow syndrome," in which it seems probable that it may interact with nutritional and metabolic factors.

Spirurid nematodes parasitizing the porcine stomach include *Physocephalus sexalatus*, *Ascarops strongylina*, *A. dentate*, and *Simondsia paradoxa*. *Physocephalus* and *Ascarops* utilize dung beetles as intermediate hosts. ***Ascarops*** and ***Physocephalus*** are common in many parts of the world in swine with access to grazing. Large numbers of worms are required to cause ill-thrift. Worms in affected pigs may be free in the lumen or partly embedded in the mucosa, which may be congested and edematous, or eroded and ulcerated with fibrinous exudate on the surface. There may be chronic interstitial inflammation and fibrosis in the mucosa. ***Simondsia*** is found in swine in Europe, Asia, and Australia. The caudal portion of the female worm is globular, and is embedded in palpable nodules up to 6–8 mm in diameter in the gastric mucosa. *Gnathostoma doloresi* causes gastric ulcers and granulomas in pigs in eastern Asia. *G. hispidum* may cause lesions in the liver, and submucosal nodules in the gastric wall of pigs, similar to those produced by *G. spinigerum* in carnivores.

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Gastric parasitism in dogs and cats

Parasites are uncommonly encountered in the stomach of dogs and cats at necropsy and most are incidental findings, or postmortem migrants from the intestine.

Gnathostoma spinigerum occurs in the stomach of dogs and cats, and of a variety of nondomestic carnivores. It is more common in areas with warm climates. The life cycle of this spirurid nematode involves *Cyclops* as an aquatic invertebrate intermediate host, and a variety of fish, amphibian, or reptiles as second intermediate hosts. Ingested third-stage larvae may migrate in the liver, leaving tracks of necrotic debris, which eventually heal by fibrosis. In heavy infections, lesions associated with larval migration may be found elsewhere in the abdominal and pleural cavities, and in the skin. Adults are found in groups of up to 10 in nodules in the gastric submucosa. Nodules are up to ~5 cm in diameter, and open into the gastric lumen. Portions of nematodes may protrude through this opening. The worms lie in a pool of blood-tinged purulent exudate in the lumen of the nodule, the wall of which is comprised of granulation tissue and reactive fibrous stroma. Focal granulomas may center on nematode ova trapped in the connective tissue. Infection with *Gnathostoma* has been considered significant. Illness and death may be associated with disturbance of motility, chronic vomition, and occasional rupture of verminous nodules on to the gastric serosa, leading to peritonitis.

A number of species of ***Physaloptera***, including *P. praeputialis* (cat), *P. rara* (dogs and wild Canidae and Felidae) and *P. canis* (dog) are found in the stomach of dogs and cats. These nematodes utilize arthropod intermediate hosts and probably some vertebrate transport hosts. The adult worms, which may be mistaken for small ascarids, are found in the stomach, where they may be free in the lumen. More commonly they are attached as individuals or in small clusters to the gastric mucosa. Ulcers may be formed, and the cranial end of the worm may be embedded in the submucosa. Hyaline periodic acid–Schiff-positive material surrounds the cranial end of some worms, perhaps anchoring them in the tissue. These nematodes are not highly pathogenic, though heavy burdens may have the potential to cause significant gastric damage and chronic vomition.

Cylicospirura felineus and members of the genus ***Cyathospirura*** may be found in the stomach of domestic and wild felids. *Cylicospirura* are usually found in the submucosal nodules, similar to those formed by *Gnathostoma*, while *Cyathospirura* is usually found free in the lumen, or sometimes associated with *Cylicospirura* in gastric nodules. The pathogenicity of these species is poorly defined, but is likely low.

Ollulanus tricuspis is a small trichostrongyle, ~1 mm long, which inhabits the stomach of cats and swine. It is viviparous, and third-stage larvae developing in the uterus of the female are transmitted in vomitus. As a result, infection is usually not detected by fecal examination, and infection with this species may go unnoticed. In some parts of the world it is common, particularly in cat colonies and cats that roam. Clinical signs and gross lesions due to *O. tricuspis* are uncommon. *Vomition, anorexia, and weight loss* are the signs most frequently associated with infection. The worms lie beneath the mucus on the surface of the stomach, or partly in gastric glands. Infection is associated with increased numbers of lymphoid follicles deep in the gastric mucosa, increased interstitial connective tissue in the mucosa, and numerous globule leukocytes in the gastric epithelium. Heavy infection results in mucous metaplasia and hyperplasia of gastric glands, causing the surface of the stomach to be thrown into thickened convoluted folds, grossly resembling idiopathic hypertrophic gastritis of dogs. Gastric glands are often separated by the heavy reactive fibrous stroma in the mucosa. In gastric biopsies, this suite of microscopic changes in the mucosa should be recognized as characteristic of *Ollulanus* infection, even if worms are not present. *Ollulanus* are characterized in section by the numerous longitudinal cuticular ridges (synlophe) recognized as projections on the surface of sectioned worms.

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Intestinal helminth infections

Strongyloides infection

Strongyloides spp. parasitize all species of domestic animals considered here. Ruminants are infected by *S. papillosus*; horses by *S. westeri*; swine mainly by *S. ransomi*; dogs by *S. stercoralis*; and cats by *S. felis*, *S. planiceps* (= *S. catti*), and *S. stercoralis* in the small intestine, and by *S. tumefaciens* in the colon. The parasitic worms are parthenogenetic females, which produce larvae capable of direct infection of the host, or of developing into a free-living generation of males and females. The offspring of the free-living generation then adopt a parasitic existence.

Infection by third-stage larvae takes place by skin penetration, or to a lesser extent by ingestion and probably subsequent penetration of the gastrointestinal mucosa. Larvae attain the bloodstream, and in young animals, break out into pulmonary alveoli. They migrate to the large airways, whence they are carried up the mucociliary escalator to be swallowed, and establish in the small intestine.

Typically infecting the proximal small intestine of all species, *Strongyloides larvae establish in tunnels in the epithelium about the base of villi or in upper crypts*, and they persist in that location (Fig. 1.185). Adult worms are small, only 2–6 mm long, depending on species. In sufficient numbers, they cause villus atrophy, associated with a mixed, but mainly mononuclear inflammatory cell infiltrate into the lamina propria. Cryptal epithelium is hyperplastic. Villi are stumpy or there is subtotal villus atrophy. Surface epithelium is usually low columnar to cuboidal, with an indistinct brush border; it may be squamous, or in some cases, eroded. The nematodes are usually found in tunnels in the surface epithelium, not beneath the basal lamina. Embryonated or larvating ova may be retained in epithelial tunnels, and help to distinguish this nematode in tissue section from *Trichostrongylus*, in hosts in which both species occur.

Strongyloides ransomi is responsible for *diarrhea in suckling piglets* in some parts of the world. Minor local hemorrhage occurs as larvae migrate through the lungs, and thickening of alveolar septa is reported, associated with scattered aggregates of lymphocytes and plasma cells. In the duodenum, villus atrophy is associated with local malabsorption

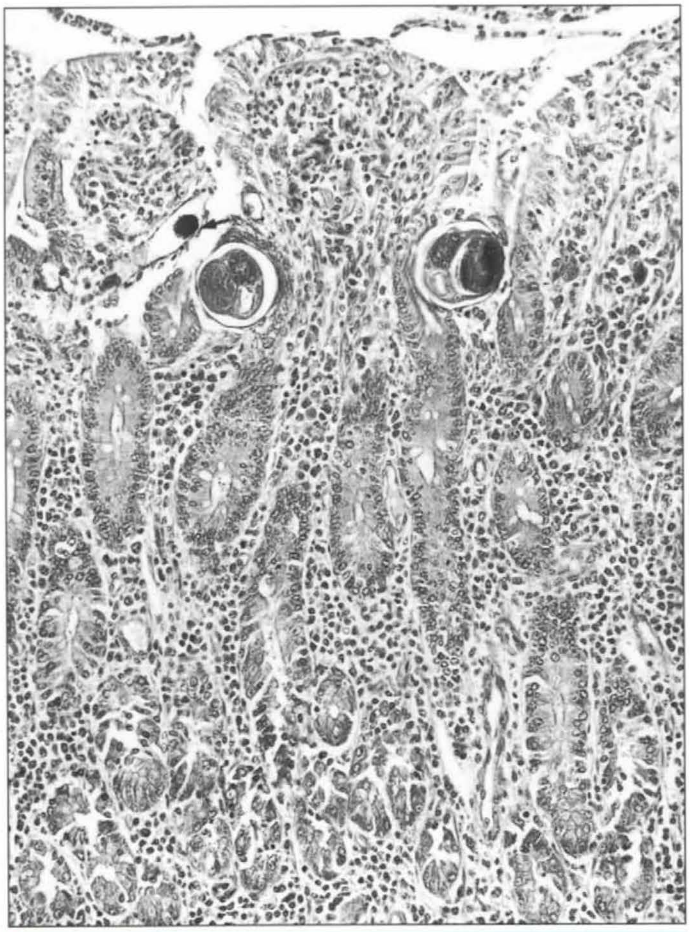

Figure 1.185 ***Strongyloides westeri*** in tunnels at base of a moderately atrophic villus in the intestine of a foal with diarrhea. An ovum is in a tunnel on an adjacent villus (arrow).

of amino acids, and with protein loss into the gut. Diarrhea occurs, presumably the result of malabsorption. Debilitation is the product of anorexia, protein loss into the gut, and nutrient malabsorption. Specific gross lesions other than those associated with diarrhea may be absent. Moderate to severe clinical disease in 3-month-old pigs is associated with 20 000–70 000 worms, most in the cranial 30–40% of the small intestine. Worms are evident in mucosal scrapings at autopsy.

S. westeri commonly infects *foals*. It is associated with diarrhea, occasionally fatal, in some animals under 4–5 months of age (Fig. 1.185). It is claimed that skin lesions by larval penetration may permit entry of *Rhodococcus equi*, which causes lymphadenitis. Millions of larvae are necessary to cause fatal infections experimentally.

S. papillosus may cause diarrhea and, in occasional overwhelming infections, death, in *suckling ruminants*, or young animals being artificially reared. There is also a syndrome of sudden death due to cardiac failure that is associated with heavy infections of *S. papillosus*, the pathogenesis of which is unclear.

S. stercoralis infections are most commonly fatal in *puppies* up to 2–3 months old, often from kennel environments. Affected dogs are wasted and dehydrated with evidence of diarrhea, perhaps blood-tinged, but the intestine may only be congested or unremarkable at autopsy. Severe villus atrophy and heavy mononuclear interstitial infiltrates are evident in the duodenum of affected dogs. Occasionally larvae may be present in granulomas in the lamina propria and submucosa. Focal interstitial pneumonia may be associated with pulmonary migration. There are numerous reports of *S. stercoralis* infecting humans, especially in situations of immunocompromise.

S. felis may cause mild focal granulomatous or eosinophilic interstitial pneumonia in response to lung migration of larvae. In some cases there is local adenomatous hyperplasia of the crypts of Lieberkühn in the vicinity of worms in the small intestine, but no more general lesions. Diarrhea is uncommon.

S. tumefaciens in cats has rarely been associated with chronic diarrhea. It differs from the other species discussed above, in being associated with the formation in the colon of submucosal nodules of proliferative glands, in which the worms are found. It is uncertain whether this is a specific lesion induced by infection, or merely herniation of infected colonic glands into space left by involution of a submucosal lymphoid follicle.

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Intestinal trichostrongylosis

Members of the genus *Trichostrongylus* parasitize the *proximal small intestine of ruminants* the world over. They cause significant subclinical inefficiency in production, or clinical disease characterized by *diarrhea, ill-thrift and, in some cases, death*. The most important species infecting sheep and goats are ***T. colubriformis***, ***T. vitrinus***, and ***T. rugatus***; others include *T. longispicularis*, *T. falculatus*, *T. capricola*, and *T. probolurus*. *T. colubriformis* and *T. longispicularis* also parasitize cattle. Though some *T. axei* may be found in the duodenum of cattle and sheep, this species is primarily parasitic in the abomasum.

Trichostrongylosis is most important in zones with a cool climate at some time of the year, but without extreme winters. It is a very important problem in many sheep-grazing areas of New Zealand, Australia, South Africa, South America, and the UK.

The life cycle is direct. Ingested third-stage larvae exsheath in the acid abomasal environment and establish preferentially in the proximal 5–6 meters of the small intestine in sheep. A small proportion of the population settles in the abomasal antral mucosa near the pylorus. The larvae in the intestine enter tunnels above the basal lamina, between enterocytes, mainly at the base of villi, and they persist throughout their life at least partially embedded in the epithelium. Usually, infecting larvae all develop over ~2 weeks into adult worms, with a prepatent period of ~16–18 days.

Experimental infections of all *Trichostrongylus* species studied indicate that the lesions and pathogenesis of disease caused by them are similar, though *T. vitrinus* seems more pathogenic than *T. colubriformis* and *T. rugatus*. Villus atrophy occurs in areas of intestine heavily populated by the worms, and the severity of the lesion within individual animals is correlated with the local density of the worms. The mechanism by which the atrophy occurs has not been investigated. However, in experimental infections, hyperplasia of cryptal epithelium seems to precede the onset of villus atrophy, which may be T-cell mediated.

The established lesion is characterized microscopically by *villus atrophy* that may vary considerably in severity (Fig. 1.186), in association with elongate, dilated, often straight crypts containing many mitotic cells. Goblet cells may be numerous in crypts in some infected animals. In animals with subtotal villus atrophy, the surface epithelium may vary from tall columnar relatively normal-appearing cells, to more domed or cuboidal epithelium lacking a well-defined brush border. Ultrastructurally, such epithelium appears poorly differentiated, containing numerous polyribosomes in the cytoplasm, and with stumpy, sparse, and irregularly oriented microvilli. In animals with subtotal villus atrophy, exfoliating rounded enterocytes are seen, as are focal and probably transient "leaks" of neutrophils and tissue fluids through the epithelial surface. In animals with more severe atrophy, the surface epithelium is flattened between the openings of crypts, and erosions of the mucosa may be evident, from which inflammatory cells and tissue fluid effuse.

The lamina propria in the affected area of intestine is populated by a moderately heavy mixed inflammatory cell population. Lymphocytes and plasma cells are prominent between crypts, with an admixture of eosinophils. Globule leukocytes may be present in the epithelium of crypts and occasionally villi, but this is often not marked in severely affected mucosa. The presence of globule leukocytes is associated with a positive immune response. In the superficial lamina propria, neutrophils often accumulate beneath the epithelium, and in areas of erosion or previous erosion there may be a thin transversely oriented layer of connective tissue. Abnormal permeability of the endothelium of capillaries and venules in heavily infected mucosa has

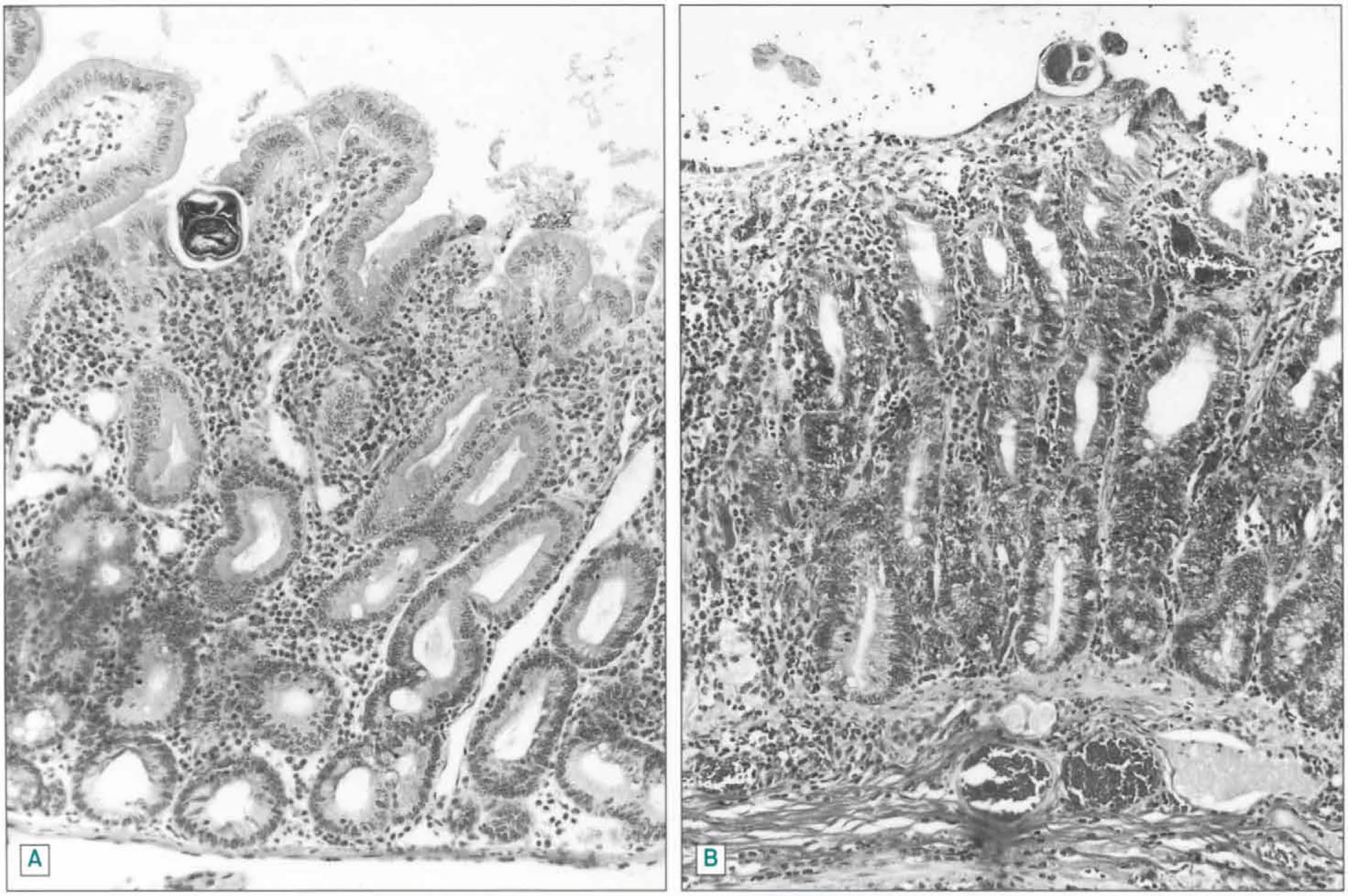

Figure 1.186 Intestinal trichostrongylosis in a sheep. **A.** Subtotal villus atrophy, caused by ***Trichostrongylus colubriformis***. Exfoliation of enterocytes, focal effusion of tissue fluid, dilated crypts, and mononuclear cells in lamina propria. **B.** Severe villus atrophy. Surface epithelium is attenuated or eroded, and crypts are hyperplastic. Nematode in tunnel in surface epithelium.

been demonstrated, and edema of the lamina propria may be evident in these areas.

The disease is marked clinically by depression, inappetence, which may be mild or profound, and by diarrhea and wasting. The cause of the inappetence is unclear. The pathogenesis of the diarrhea is uncertain. It is associated with the period when inappetence and gastric dysfunction occur. Though local malabsorption of nutrients, and presumably electrolytes and water, occurs in the duodenum, it seems unlikely that the absorptive capacity of the remaining small intestine and large bowel would be overwhelmed.

Weight loss or reduced productive efficiency is not related to nutrient malabsorption, since net absorption of nutrients over the length of the small intestine does not seem to be severely affected. Rather, the interaction of reduced feed consumption with increased loss of endogenous nitrogen into the gut seems to be responsible. There is considerable effusion of plasma protein into the intestine of infected animals, and this, coupled with exfoliation of epithelium that appears to be turning over at an increased rate, is the source of *protein loss*. In trichostrongylosis, compensation for increased catabolism of plasma protein and mucosal epithelial protein is at the expense of anabolic processes elsewhere in the body. Wool and muscle growth are hindered. Osteoporosis may occur.

Animals succumbing to trichostrongylosis are usually cachectic and dehydrated. Dark-green scoured feces will be on the skin or wool of the escutcheon or breech. There may be serous atrophy of internal fat depots, and marked atrophy of skeletal muscle. The subcutis is tacky. There may be edema of the mesentery and perhaps serous effusion into the body cavities, associated with hypoproteinemia, if dehydration is not severe. Mesenteric lymph nodes are enlarged and wet. The intestines are flaccid and the small bowel contains thin fluid green content, which in the duodenum may appear somewhat mucoid. The large intestine may contain similar fluid or pasty green, foul-smelling feces.

The mucosa of the duodenum in the freshly killed animal may be glistening and pink, but in spontaneous mortalities with superimposed postmortem change, it will be unremarkable. The proximal third of the small intestine (~5–7 meters) contains the bulk of the population of *Trichostrongylus*. A worm count on the small bowel usually reveals 15 000–80 000 *Trichostrongylus* in severe clinical infections. Subclinical or mild disease may be associated with fewer worms. The *diagnosis* is based on recovery of substantial populations of *Trichostrongylus* spp. in association with the clinicopathologic syndrome and villus atrophy. Mixed infections with other genera are common.

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Nematodirus and *Cooperia* infection

Nematodirus species infect the *proximal third of the small intestine of ruminants*. The most important species are *N. helvetianus*, which infects cattle; *N. spathiger*, *N. filicollis*, and *N. abnormalis*, which infect sheep, goats, and cattle; and *N. battus*, a parasite mainly of sheep, which will cause disease in calves.

The life cycle is direct. However, hatching of infective larvae of *N. battus* and *N. filicollis* from the egg is delayed. Eggs of *N. battus* deposited on the ground in one year hatch the next spring, following a period of conditioning by cold over winter. The epizootiologic pattern is that of infection of a susceptible lamb crop during one year by larvae produced by the previous year's lambs. Under these conditions, with many infective larvae, parasitic enteritis dominated by *Nematodirus* may occur. The larvae of *N. spathiger* and *N. helvetinus* are not delayed in hatching, and their epizootiologic pattern resembles that of *Trichostrongylus* spp. in grazing animals. They often form part of a mixed population of worms in parasitic gastroenteritis in grazing lambs and calves. The disease may occur in yarded calves as well as animals at pasture.

Third-stage larvae enter the deeper layers of the mucosa, perhaps entering crypts. Larvae emerge at the fourth or fifth stage to take up residence coiled among the villi, with their caudal ends protruding toward the lumen. They do not normally penetrate the epithelium.

The presence of large numbers of some *Nematodirus* species is associated with the development of villus atrophy, which is usually moderate in comparison with that induced by *Strongyloides* or *Trichostrongylus*. Villi are stumpy, bifurcate, perhaps fused, or ridge-like surface alterations that may replace the normal villus structures. Crypts may appear elongate and dilated.

Surface enterocytes may be domed, with loss of the prominent brush border, and irregular nuclear polarity. Ultrastructurally, such cells appear poorly differentiated and have irregular, deformed microvilli. Biochemical studies reveal reduction in levels of mucosal alkaline phosphatase and disaccharidases, which correlate with the severity of diarrhea in affected sheep.

The pathogenesis of the villus atrophy has not been determined, but it may be related to the development of an immune response to the nematodes in the lumen. A moderate mixed inflammatory response with substantial numbers of lymphocytes, plasma cells, and eosinophils is evident in the lamina propria. The presence of such an infiltrate, and moderate shortening of villi associated with poorly differentiated surface enterocytes, is consistent with the postulated induction of villus atrophy by cell-mediated immune activity in the lamina propria.

Lambs and calves with nematodirosis develop *severe dark-green diarrhea*, which stains the escutcheon or the breech of lambs. Affected animals may become inappetent, scour, and waste for several weeks before recovering, or they may die acutely. Disease is presumably mainly related to malabsorption and loss of appetite. At necropsy, other than the changes associated with dehydration and cachexia, findings are limited to fluid mucoid content in the upper small intestine, and soft or fluid feces in the colon. The mucosa of the duodenum is usually unremarkable or perhaps hyperemic with excess mucus on the surface. Clinical disease is associated with populations of ~10 000–50 000 or more *Nematodirus*.

Cooperia infect the *upper small intestine of ruminants*. The important species include *C. curticei*, mainly in sheep and goats, and *C. pectinata*, *C. punctata*, and *C. oncophora*, mainly in cattle. The latter is regarded as the least pathogenic of the three. Though both sheep and cattle may suffer from mixed burdens of helminths containing or dominated by populations of *Cooperia*, this species seems to be more significant in cattle, especially in cool temperate regions.

Cooperia has a typical trichostrongylid life cycle, but larvae do have the capacity to undergo hypobiosis to carry the population through periods of regular climatic adversity. The normal prepatent period is ~16–20 days. Like *Nematodirus*, *Cooperia* do not tunnel in the epithelium, but rather brace or coil themselves among villi to maintain their place in the intestine. In light infections, the worms are concentrated in the proximal third of the small intestine. Heavier infections, perhaps because they are associated with villus atrophy and therefore loss of the substrate against which to brace, are more evenly distributed down the intestine.

Heavy burdens of *Cooperia* in calves, >70 000–80 000 nematodes, may be associated with *inappetence, reduced weight gain, or weight loss and diarrhea*, with protein-losing enteropathy in experimental infections. The associated atrophy of villi is concomitant with reductions in the brush-border enzymes. The syndrome is typical of intestinal helminthosis and the diagnosis is confirmed by finding large numbers of the fine, coiled *Cooperia* in the small intestine.

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Hookworm infection

Members of the Ancylostomatidae infect dogs, cats, ruminants, and swine. Hookworms of the genus *Globocephalus* appear to be of little significance in swine. In dogs, *Ancylostoma caninum*, *A. braziliense*, and *A. ceylanicum* occur. The former is most common in tropical, subtropical, and warm temperate zones of Africa, Australia, Asia, and North America, where adequate humidity for larval development occurs. *A. braziliense* occurs in dogs and cats in the tropics and subtropics, while *A. ceylanicum* is found in both species in Sri Lanka and Southeast Asia. *Uncinaria stenocephala* occurs in dogs in cool temperate regions of Europe and North America. *A. tubaeformae* only occurs in the cat.

***Ancylostoma* spp.** are capable of infecting the host by four routes: (1) *orally*, with direct development to adult worms in the intestine; (2) by *skin penetration*, resulting in movement through the bloodstream to the lungs, and thence via the trachea to the pharynx and gut; (3) by the *lactogenic* transmission of third-stage larvae mobilized from dormancy in the skeletal muscle of parturient bitches; and (4) in occasional instances, by prenatal *transplacental* transmission of mobilized larvae. The latter route of transmission does not apparently occur in *A. braziliense* infection. Some larvae of *A. caninum* may become arrested at the third stage in the intestine, to resume development at a later time.

Ancylostoma spp. all usually *inhabit the small intestine*, where they move about the surface, several times a day attaching to feed, then moving on. They penetrate deeply into the mucosa, sometimes to, or through, the muscularis mucosae, taking a plug of tissue into the large buccal capsule. Tissue is lacerated by prominent teeth, and anticoagulant is released, permitting persistent blood flow. Blood-sucking activity begins when larvae enter the adult stage, in *A. caninum* ~8 days after infection. Blood loss is maximal while worms are attaining maturity between 12 and 16 days after infection, and then again during the peak period of egg production after ~3.5 weeks of infection. The prepatent period for *A. caninum* is ~15 days.

Ancylostomosis is the result of persistent blood loss, resulting in anemia and hypoproteinemia. There is considerable variation in the blood-sucking activity, and therefore the pathogenicity, of the members of the genus. *A. caninum* consumes in the range of 0.01–0.2 mL of blood per worm per day. Pups several months old with populations of the order of 300–400 worms may lose 10–30% of their blood volume per day, depending on body weight. *A. tubaeformae* in cats is a significant blood sucker. Experimentally, about 200 worms may cause anemia, weight loss, and mortality in 1.5 kg cats.

The anemia in ancylostomosis is at first normochromic and normocytic. Small size, poor iron reserves, and the low level of iron in bitch's milk make suckling pups with ancylostomosis susceptible to rapid development of the microcytic hypochromic anemia characteristic of iron deficiency.

Acute fatal ancylostomosis occurs most commonly in pups only 2–3 weeks of age, infected via the bitch's milk. Heavy infections acquired by this route may result in death from anemia and hypoproteinemia within a few days of initiation of blood-sucking activity, and before eggs are present in the feces. Anemia may also lead to mortality of pups after a course of longer duration. Percutaneous infection results in disease in older dogs held in runs or kennels under conditions of moisture and temperature conducive to larval development on the ground. Dermatitis due to larval penetration may be observed between the toes or on ventral contact surfaces of the body. Ancylostomosis in older dogs is usually typified by anemia, lack of exercise tolerance, weakness, and emaciation. Feces may be diarrheic, dark red or black, and are often mucoid. The major effect of ancylostomosis is due to increased loss of erythrocytes, iron, and plasma protein.

Animals dying of ancylostomosis are characteristically *extremely pale*. There is often glistening edema of subcutaneous tissues and mesenteries, and serous effusion into body cavities, attributable to hypoproteinemia. In chronic infections, cachexia may be evident. If recent exposure to heavy percutaneous infection has occurred, there may be dermatitis, and numerous focal hemorrhages scattered in the pulmonary parenchyma, reflecting disruption of vessels by larvae breaking out into alveoli. The intestinal content throughout the entire length is mucoid and deep red, from the erythrocytes voided into it by the worms (Fig. 1.187). The latter are visible, ~1–1.5 cm long, translucent, gray or red, depending on when they last consumed blood, dispersed over the mucosa, sometimes into the large intestine. They are often attached to the mucosa, and pinpoint red sites of recent feeding activity may be scattered over the intestinal surface. In a young pup, as few as 20–50 worms may be present in fatal infections, and they may be sufficiently sparsely scattered as to be overlooked, if not sought.

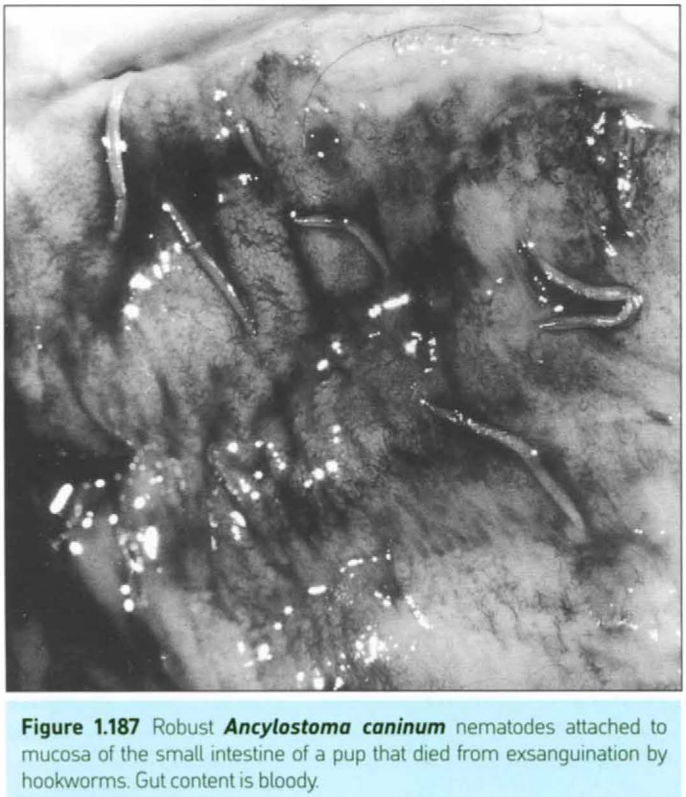

Figure 1.187 Robust ***Ancylostoma caninum*** nematodes attached to mucosa of the small intestine of a pup that died from exsanguination by hookworms. Gut content is bloody.

Uncinaria stenocephala mainly infects by the oral route; percutaneous infection is not efficient, though dermatitis may result; and prenatal and lactogenic transmission appear not to occur. This species sucks little blood and is much less pathogenic than *A. caninum*. However, heavy infections with this species, usually arising in contaminated communal kennel environments, may cause clinical disease and occasional mortality in pups. *Nonspecific lethargy, inappetence, and ill-thrift* are signs of infection, perhaps with diarrhea; anemia does not occur. Disease is associated with burdens of over ~1000 worms. The intestinal mucosa appears thickened, with scattered focal hemorrhages at sites of attachment.

The presence of large numbers of worms is associated with moderate atrophy and thickening of villi. The surface epithelium is irregular. Focal aggregates of mononuclear cells and some neutrophils are in the vicinity of the cranial end of worms embedded deep in the mucosa, a plug of tissue within their buccal cavity. Disease due to *U. stenocephala* may be related to villus atrophy, with protein loss into the gut, and perhaps malabsorption. Hypoproteinemia may be evident, but anemia does not occur. A similar syndrome may be associated with heavy infections of *A. braziliense*, in which hypoproteinemia also occurs.

All of these hookworms, but most especially *A. braziliense,* are associated with the development of cutaneous larva migrans in humans, as larvae can penetrate intact skin. *A. caninum* has been shown to cause eosinophilic enteritis in humans as well.

The **hookworms of ruminants** include: in cattle, *Bunostomum phlebotomum*, and in India and Indonesia, *Agriostomum vryburgi*; in sheep, *B. trigonocephalum* and, in India and Southeast Asia, Africa, and South America, *Gaigeria pachyscelis*. The life cycle of these nematodes is typical of hookworms. *Bunostomum* third-stage larvae infect by the oral or percutaneous routes, while *Gaigeria* only infect across the skin. Eggs and larval stages on the ground are extremely susceptible to desiccation, and hookworm disease in ruminants is most common in tropical or subtropical areas during wet seasons. However, stabled animals in cooler temperate areas may suffer disease resulting from larvae invading the skin from contaminated bedding. Following skin penetration, the usual pattern is seen, with migration of larvae to the lungs, where they molt to the fourth stage, and subsequently pass up the trachea to the digestive tract. Larvae taken in by ingestion spend some time in the deep mucosa of the intestine before emerging to mature in the lumen of the small intestine. The prepatent period of *Bunostomum* is long, ~7–8 weeks. *Gaigeria* larvae migrate via the lungs, and worms begin to lay eggs ~10 weeks after infection.

Both *Bunostomum* and *Gaigeria* cause *hemorrhagic anemia and hypoproteinemia*, especially in animals under a year of age. These species often occur with mixed gastrointestinal helminth burdens, and their effects are at least additive to those of the other worms. As few as 20–30 *Gaigeria* will cause anemia and hypoproteinemia in lambs and kids, though several times that number may be more usual in fatal cases. The size of the animal, the status of its iron reserves, and the plane of nutrition, especially the level of protein, likely influence the pathogenicity of these species.

At autopsy, the lesions expected in anemia and hypoproteinemia are evident. *Bunostomum* are often found in the lower half of the small intestine, while *Gaigeria* tends to be concentrated high in the duodenum. Blood and bite marks may be evident on the mucosa in the infected areas of intestine, but hemorrhage may be occult. The relatively low numbers of worms associated with disease, and their peculiar distribution, dictate that the entire gut be examined and flushed, and a careful search be made for these species in suspect cases.

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Oesophagostomum and *Chabertia* infection

Members of the genus ***Oesophagostomum*** infect *sheep, cattle, and swine*. They form *inflammatory nodules in the wall of the intestine*, incited by histotropic larval stages. *Ill-thrift*, *diarrhea*, and, in cattle, *anemia*, can be induced by adult populations in the lumen of the colon.

In **sheep**, two species, *O. columbianum* and *O. venulosum*, are probably most significant; the former is considerably more pathogenic and is particularly important in warm temperate to tropical areas. Third-stage *O. columbianum* penetrate deeply into the lamina propria, or sometimes to the submucosa, mainly in the small intestine, where they normally spend ~1 week. They molt, emerge, and mature in the colon. However, a proportion of fourth-stage larvae enter a second histotropic phase in nodules in the colonic submucosa. Adult worms in the colon may be pathogenic for lambs. Burdens of only a few hundred *O. columbianum* are associated with anorexia, mucoid feces or diarrhea, and ill-thrift.

At necropsy of animals with clinical oesophagostomosis, the carcass is emaciated, the mesenteric lymph nodes are enlarged, and the colonic mucosa is thickened, congested, and covered by a layer of mucus in which the worms are scattered. There is hyperplasia of goblet cells, and the lamina propria contains a heavy mixed inflammatory infiltrate with eosinophils and many immune-active cells. Globule leukocytes are in the epithelium of glands. Nodules caused by histotropic fourth-stage larvae, mainly in the large intestine, are ~0.5–1 cm in diameter and are comprised of a central caseous or mineralized core surrounded by a thin fibrous encapsulating stroma. Microscopically, the nematode (or its remnants) is present among a mass of necrotic debris in which eosinophils are prominent. Giant cells and macrophages may surround the necrotic material. Similar nodules may be found in liver, lungs, mesentery, and mesenteric lymph nodes. Those in the deeper layers of the gut project from the serosal surface, hence the name *"pimply gut."* They may cause adhesion to adjacent loops of gut or to other organs, and rarely may incite intussusception or peritonitis. However, in most cases, nodules are incidental findings at necropsy. *O. venulosum* is a much less significant parasite. It seldom causes significant nodule formation; when it does, the nodules are small and mainly in the cecum and colon.

In **cattle**, two species, *O. radiatum* and *O. venulosum*, occur, the former being the significant parasite. The life cycle is similar to that of *O. columbianum*. The disease caused by *O. radiatum* is characterized by *loss of appetite, reduced productive efficiency, anemia, hypoproteinemia, and diarrhea*. Anemia results from hemorrhage at sites of larval emergence, and from mucosal erosions and discontinuities in the gland lining, associated with maturing and adult populations of worms. Considerable exudation of tissue fluids and plasma protein from colonic lesions, in addition to that due to hemorrhage, contributes to the hypoproteinemia and gastrointestinal protein loss. Reduced growth, or loss in condition, is mainly the product of the interaction between protein effusion into the gut and inappetence. Diarrhea presumably results in part from loss of colonic absorptive capacity.

Oesophagostomosis may be fatal in calves. Animals may be pale from anemia, and edematous from hypoproteinemia. Cases of some duration will be cachectic. Colonic lymph nodes are enlarged. The mucosa of the colon is grossly thickened and folded by edema and increased mixed inflammatory cell infiltrates, including many immune-active cells, in the lamina propria. Colonic submucosal lymphoid follicles are large and active. Pathogenic worm burdens in calves are in the range of ~1000–10 000 *O. radiatum*. Although repeated exposure to infective larvae may result in the accumulation of large numbers of fourth-stage worms in nodules, formation of nodules has little pathogenic significance in cattle.

In **swine**, *O. dentatum*, *O. quadrispinulatum*, and several other species occur in the large intestine; the two mentioned are most widespread. The life cycle is typical of the genus. Third-stage larvae enter the wall of the cecum and colon, where they encyst and molt to the fourth stage, emerging ~1 week later to mature in the lumen. There is some indication that inhibitors of eicosanoid metabolism (indomethacin, acetylsalicylic acid) will suppress migration of these third-stage larvae. The larvae initially lie about the level of the base of the mucosa. They incite a reaction which causes local loss of the muscularis mucosae, so that the nodule formed involves both mucosa and submucosa, and the larvae ultimately reside in the submucosa. The nodules are grossly ~1–20 mm in diameter, umbilicate, and may contain yellow or black cheesy exudate in the center. Once the larvae molt and begin to move to the lumen, an intense influx of eosinophils and neutrophils occurs into the nodules, and a focus of necrotic debris and fibrin lies over the evacuated nodule.

Mucosal and submucosal edema causes thickening of the wall of the large bowel, and contraction of the cecum. Mucosal crypts elongate with increasing worm burdens. Lesions resolve following emergence of larvae.

Oesophagostomosis in swine is a *mild, usually subclinical disease*. Occasional diarrhea, depression in weight gain, and inefficiency of feed conversion may occur, especially during the period of emergence of larvae and maturation of worms in the lumen of the large intestine. Burdens of ~3000–20 000 adult worms are associated with subclinical disease experimentally. The nematodes are ~1–2 cm long, white, and are present in mucus on the surface of the gut, or in luminal content. Massive repeated challenge will cause severe typhlocolitis, but this seems to be purely an experimental phenomenon.

Chabertia ovina, a robust worm ~1–2 cm long, inhabits the *colon of sheep, goats, and cattle*. It is particularly a problem in cooler climatic zones, mainly in sheep. Phylogenetic analysis based on ribosomal DNA sequence data indicates that *C. ovina* is clustered within the Oesophagostominae. The life cycle of *Chabertia* resembles that of *Oesophagostomum*; third-stage larvae encyst in the wall of the small intestine, then emerge to mature in the cecum and colon. Disease in sheep is associated with the presence of mature worms in the colon. Feces are soft, mucoid, and perhaps blood-flecked, and ill-thrift may occur. The adults penetrate to the muscularis mucosae and take a plug of mucosa into the buccal capsule; minor hemorrhage may be related to physical trauma to the mucosa. More significant is loss of plasma protein from the mucosa, associated with numerous focal sites of trauma, and with widespread areas of mononuclear infiltration in the mucosa and submucosa. There is also hyperplasia of goblet cells. Grossly the lesions are characterized by edema of all layers of the wall of infected parts of the colon, and enlargement of colonic lymph nodes. Worms are generally concentrated in the proximal portion of the spiral colon, and the area that they inhabit may have numerous hemorrhagic foci corresponding to sites of former attachment. Pathogenic burdens may be as few as 150 worms and the species must be sought in its usual site of predilection or be missed.

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Equine strongylosis

Members of the family Strongylidae are common nematode parasites of the cecum and colon in horses, usually present as mixed infections. The subfamily Strongylinae, or **large strongyles**, includes the important genus *Strongylus* and the less significant genera *Triodontophorus*, *Oesophagodontus*, and *Craterostomum*. Members of this group are *plug feeders or blood suckers*, and *Strongylus* spp. undergo extensive extraintestinal migrations. The subfamily Cyathostominae, or **small strongyles**, includes eight genera of nematodes. Adults of this group *feed mainly on intestinal contents*, and are of little pathogenic significance. However, emergence of *histotropic larval stages* from the gut wall may cause disease.

Large strongyles

Strongylus vulgaris is relatively common, and has been considered the *most significant nematode parasite in horses*. However, infection levels have considerably decreased with the advent of improved anthelmintics. Larval forms cause *endoarteritis* in the mesenteric circulation, resulting in colic and infarction of the large bowel, while the adults cause *anemia and ill-thrift*. Infective third-stage larvae, ingested from pasture, penetrate the mucosa of the small and large intestine, and molt to the fourth stage. They enter the lumina of small arterioles, up which they migrate, on or under the intima, to reach the cranial mesenteric artery within 3 weeks. Three or 4 months later, after molting in that location to the fifth stage, the immature adults return down the mesenteric arteries to the wall of the cecum or colon, where they encapsulate in the subserosa, forming nodules ~5–8 mm in diameter. Returning larvae in nodules are surrounded by necrotic debris, neutrophils, some eosinophils, and macrophages, and the adjacent arteriole may be thrombosed. They eventually break into the lumen of the large bowel, especially cecum and right ventral colon, where they mature in another 1–2 months, ~6–7 months after infection. Some larvae may become trapped and encapsulated in arterioles in the mesentery on their way back to the gut, and remain there to die eventually.

Endoarteritis associated with migration and establishment of larvae in the cranial mesenteric artery and its branches is discussed in Vol. 3, Cardiovascular system, as are the consequences of aberrant migration in the aorta and other arteries. Syndromes associated with aberrant migration include *cerebrospinal nematodiasis* and *aortic-iliac thrombosis*. Lesions of the cranial mesenteric artery and of the cecal and colic arteries may lead to *colic* as a result of reduced perfusion or thromboembolism, or perhaps due to impingement upon autonomic ganglia in the vicinity of the arterial root at the aorta. Though many older horses are infected with adult worms, or have arterial lesions, the complications of colic and infarction caused by this parasite are most common in young horses. An *acute syndrome*, characterized by pyrexia, anorexia, depression and weight loss, diarrhea or constipation, colic, and infarction of intestine occurs in foals infected with large numbers of larvae, but not often in animals previously exposed to infection.

S. edentatus is also common and has a life cycle characterized by extensive larval migration. Third-stage larvae enter the intestinal wall and pass in the portal system to the liver where they incite inflammatory foci. Here they molt to the fourth stage and, ~30 days after infection, begin migrating through the hepatic parenchyma. The foci of inflammatory reaction in the liver are probably related to antigens

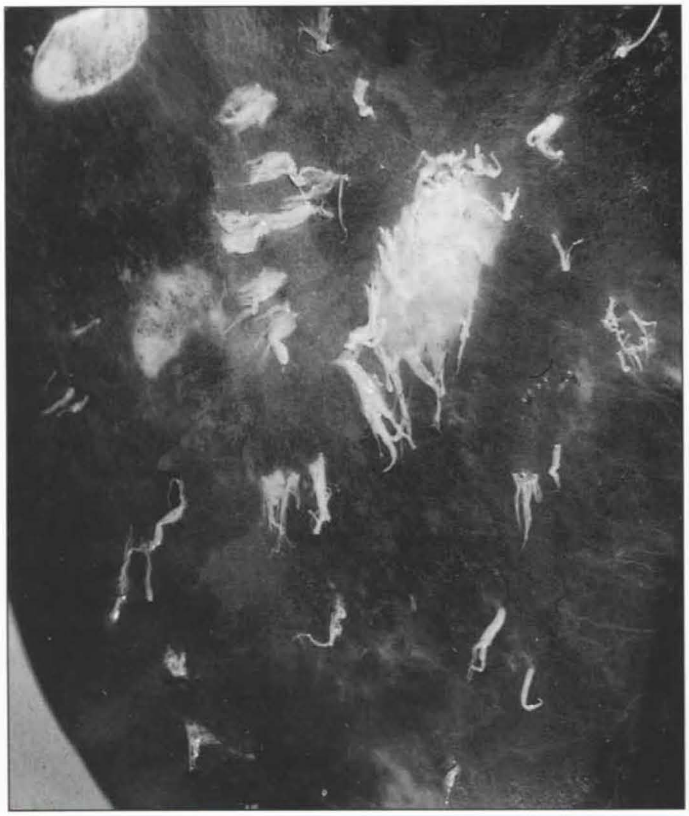

Figure 1.188 Fibrous tags and capsular scars, associated with migration of larval ***Strongylus edentatus***, on the diaphragmatic surface of a horse liver.

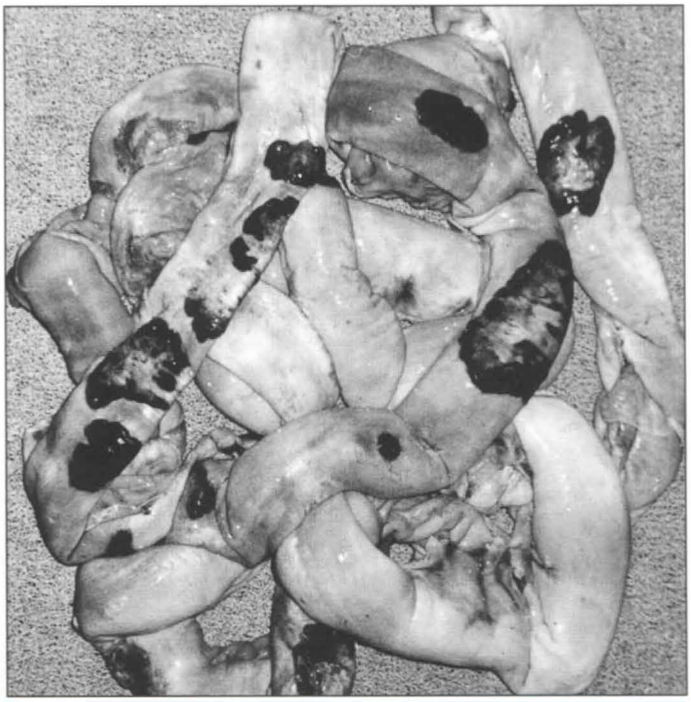

Figure 1.189 Hemomelasma ilei, large subserosal plaques of resolving hemorrhage on the small intestine of a horse, associated with damage caused by migrating larvae of **large strongyles**.

released by migrating and trapped larvae. They consist of a core of necrotic eosinophils, with a surrounding fibrous capsule, a mixture of neutrophils, eosinophils, and mononuclear cells, or recent necrotic foci or tracks infiltrated by neutrophils and a few eosinophils.

By 8–10 weeks after infection, larvae are migrating from the liver via the hepatic ligaments. Hemorrhagic tracks may be produced in the hepatic parenchyma. *Parenchymal scars and tags of fibrous tissue on the hepatic capsule*, especially the diaphragmatic surface (Fig. 1.188), commonly found at autopsy, are the legacy of migrating *S. edentatus*. Those migrating in the hepatorenal ligament enter the retroperitoneal tissue of the flank, where they may be encountered, often associated with local hemorrhage. Larvae in aberrant locations in the omentum, hepatic ligaments, and diaphragm may become encapsulated in eosinophilic granulomas and destroyed. Omental adhesions may also be a sequel to aberrant larval migration. In the flank, larvae persist for several months, molting to the fifth stage before returning from the right flank via the cecal ligament to the cecum and origin of the colon. Here they form nodules and edematous or hemorrhagic plaques in the wall of the gut, eventually perforating to the lumen, where they mature and begin to lay eggs ~10–12 months after infection. Lesions associated with the larval migration of *S. edentatus* are usually incidental findings at autopsy.

S. equinus is less prevalent and abundant than the other two members of the genus. Exsheathed third-stage larvae penetrate to the deeper layers of the wall of the ileum, cecum, and colon, molt to the fourth stage and produce hemorrhagic subserosal nodules, before moving to the liver through the peritoneal cavity. They migrate in the hepatic parenchyma for 6–7 weeks, then leave the liver, probably via the hepatic ligaments, to the pancreas and peritoneal cavity, where they molt to the fifth stage ~4 months after infection. They regain the lumen of the cecum and especially right ventral colon, by an unknown route, probably by direct penetration from the peritoneal cavity or pancreas. Pancreatic damage is usually mild and is mainly manifested by slight periductal infiltration of eosinophils.

Hemomelasma ilei is the term applied to slightly elevated *subserosal hemorrhagic plaques*, up to 1–2 × 3–4 cm in size, usually found along the antimesenteric border of the distal small intestine, or rarely on the large bowel (Fig. 1.189). They are associated with trauma by migrating larvae of *S. edentatus* in particular, but may be caused by larvae of any of the *Strongylus* spp. These lesions are comprised of edema, hemorrhage, and a mixed population of leukocytes, with macrophages ingesting erythrocytes prominent in evolving lesions. Occasionally a fragment of nematode or cuticle, or a migratory track, may be found in section. The lesion may be an incidental finding, and is probably a rare cause of clinical signs of colic.

Adults of all species in the Strongylinae are plug feeders and blood suckers. In sufficient numbers they may cause ill-thrift and anemia, as the result of active hematophagia and blood loss from recent sites of feeding activity. Increased albumin catabolism causing accelerated turnover of the plasma pool, and reduced red cell survival, have been demonstrated in horses infested with relatively low numbers (<100) of *S. vulgaris*. *Triodontophorus tenuicollis*, the most important species of that genus, tends to attach to the mucosa in clusters, usually in the right dorsal colon, causing local congestion and ulceration. *Triodontophorus* may be associated with significant blood loss.

Cyathostomins The **small strongyles**, or cyathostomins, are *essentially nonpathogenic as adults*, despite the fact that tens or many hundreds of thousands may be in the content of the large bowel.

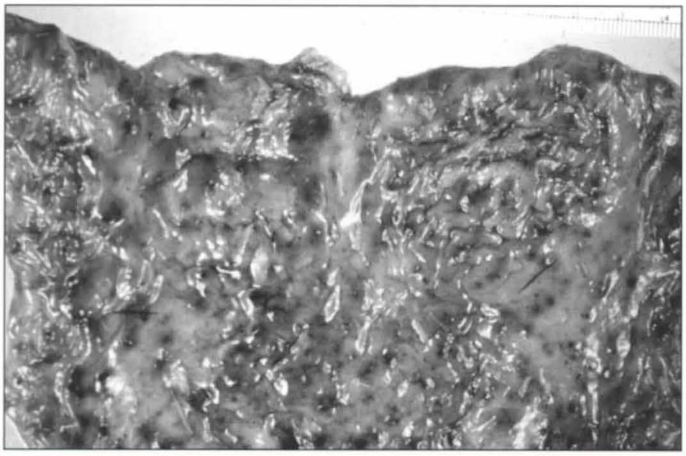

Figure 1.190 Equine colonic mucosa studded with 2–5 mm diameter nodules formed by **larval cyathostomins**, some of which are emerging, others of which have been released by incising nodules. Larvae are red due to nematode, not host, hemoglobin.

The larval stages migrate into the deep mucosa or submucosa of the large bowel (mainly cecum and ventral colon) to molt and develop, before emerging to the lumen to molt again and mature. Adults are mainly found in the dorsal and ventral colon. The third- or fourth-stage larvae may undergo hypobiosis or retarded development, persisting in nodules in the gut wall, only to mature sporadically, or perhaps more synchronously, as the adult population in the lumen turns over or is lost. Emergence of larvae causes rupture of the muscularis mucosae and intense local eosinophilia and edema, followed by infiltration of neutrophils and macrophages.

Mucosal nodules are up to only a few millimeters in diameter, slightly raised red or black, and may be umbilicate. Incision reveals a small translucent gray or red larval nematode. In heavy infections, the mucosa of the cecum and colon may be diffusely pocked by such nodules.

Disease attributable to larval cyathostomins usually occurs in heavily infected horses at the time of turnover of the adult population. It is due to *emergence of large numbers of hypobiotic larvae* over a short period, somewhat analogous to type II ostertagiosis in cattle. This occurs in the late winter, spring, and early summer in northern temperate climates. Cyathostominosis is a disease of horses over a year of age, and little resistance is apparent to repeated infection. Animals develop a syndrome characterized by *diarrhea, ill-thrift, and hypoalbuminemia*. In animals examined at this time, numerous nodules, containing immature cyathostomes or recently ruptured, are present in the mucosa of the cecum and colon (Fig. 1.190). The mucosa and submucosa are edematous and the mucosa congested and many recently emerged fourth-stage or early fifth-stage larvae may be in the gut content. Microscopically, there are numerous encysted cyathostomin larvae within the mucosa.

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Ascarid infection

Members of the family Ascarididae are *common and important parasites of swine, horses, dogs, cats, water buffalo, and, to a lesser extent, cattle.* They do not normally occur in sheep and goats. Their importance is related to incidental and sometimes significant lesions caused by *larvae during migration* in the tissues of definitive and accidental hosts, and to the effects of *adult worms* in the small intestine of the definitive host.

Ascaris suum is a large parasite, usually found in the *upper half of the small intestine of swine*; females measure up to 40 cm long. The life cycle is direct. Infective larvae, present in the resistant egg, are released in the intestine and penetrate the mucosa to be carried in the portal blood to the liver. They then pass to the lungs in the blood, and break out of capillaries into alveoli. Third-stage larvae may be found in liver and lung 3–5 days after infection. Larvae move up the respiratory tree to the pharynx, where they are swallowed, arriving in the intestine about a week after infection. Worms mature in the intestine, and begin to lay eggs ~2 months after infection. Small doses of eggs more commonly give rise to patent infections than do large doses. This probably results from excessive loss of migrating larvae due to resistance incited by the antigenic mass of the heavier infections.

Larval migration induces lesions in the liver and lungs (Fig. 1.191). Infections heavy enough to cause clinical signs are rare in swine reared under conditions of good hygiene and husbandry. However, respiratory signs characterized by dyspnea (commonly termed "thumps") may occur in piglets if large numbers of larvae migrate through the lungs. Gross lesions in pigs, associated with pulmonary migration of ascarids, are largely limited to numerous focal hemorrhages scattered over and through the pulmonary parenchyma.

Microscopically there is eosinophilic bronchiolitis. Bronchioles are surrounded by macrophages and eosinophils, and the bronchiolar mucosa is thrown into small folds, the epithelium frequently disorganized or perhaps eroded. The bronchiolar wall is infiltrated by

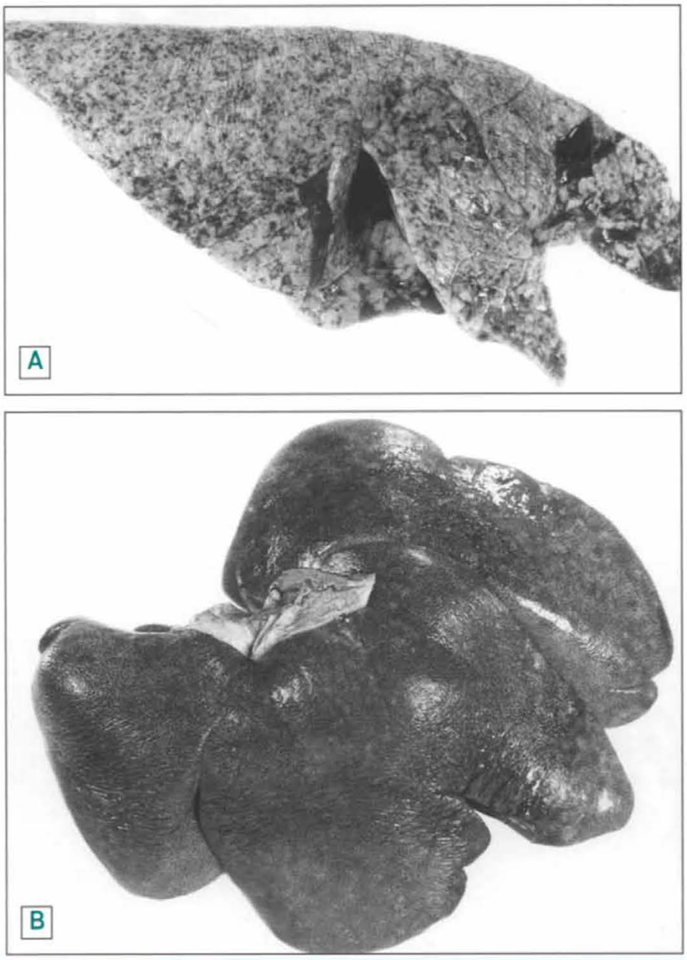

Figure 1.191 Effects of migrating ***Ascaris suum*** larvae in a pig. **A.** Multifocal parenchymal hemorrhages in the lung. **B.** Interstitial hepatitis.

eosinophils that are also present, with necrotic debris, in the lumen. There may also be an eosinophilic and granulomatous vasculitis.

Larvae are usually readily found in section. They may be present in alveoli, alveolar ducts, bronchioles, or bronchi, perhaps surrounded by eosinophils. In more chronic cases, larvae in tissue are in eosinophilic granulomas. The worms may be dead in cases of some standing, and are only recognized as an eosinophilic remnant or some bits of cuticle. Like all larval ascarids of mammals, *A. suum* in the lungs have *lateral alae visible in section.*

Lesions in the liver due to migrating *A. suum*, though not causing clinical disease, do result in considerable economic loss from condemnation at meat inspection. At first exposure to larvae, the lesions are related to mechanical damage caused by the worms, subsequent repair, and hypersensitivity reactions to excretory and secretory products of the larvae. Initially, hemorrhagic tracks are present near portal areas and throughout lobules. They are visible through the capsule as pinpoint red areas, perhaps slightly depressed and surrounded by a narrow pale zone. These lesions collapse and heal by fibrosis, causing scarring that involves most intensely the adjacent portal tracts. However, fibrosis extends diffusely through more distant tracts, emphasizing lobular outlines. There is a heavy eosinophil infiltrate in fibrotic septa, which becomes most obvious beginning ~10–14 days after infection. Granulomatous foci containing giant cells, macrophages, and eosinophils may center on the remnants of larvae trapped and destroyed in the liver.

The inflammatory infiltrates in livers of animals exposed to larval ascarids may become severe and diffuse, and this is reflected in the gross appearance of the liver, which has extensive "*milk spots*," and prominent definition of lobules. The liver is firm, and heavy scars may become confluent, obliterating some lobules and extending out to exaggerate interlobular septa throughout the liver. Where pigs are raised intensively, it is now rare to encounter extreme fibrosis of the liver associated with ascarid migration.

The pathogenicity of adult ascarids in the intestine is poorly defined. Heavy infections may obstruct the gut, being visible as rope-like masses throughout the intestinal wall. Ascarids may occasionally pass to the stomach and be vomited or migrate up the pancreatic or bile ducts. Rarely, intestinal perforation occurs. The presence of ~80–100 worms in 3-month-old swine fed low protein rations may depress feed intake and the efficiency of feed conversion. *A. lumbricoides* in humans interferes with carbohydrate, fat, and protein absorption, and *A. suum* probably has a similar influence. The effects of infection seem to be most apparent in animals on diets marginal in energy, and in quantity and quality of protein.

A. suum also infects animals other than swine. In sheep, and occasionally cattle, immature ascarids may be found in the intestine. Dyspnea and coughing associated with eosinophilic pneumonia, and focal eosinophilic hepatitis, may occur in lambs exposed to *A. suis*; mortality may occur, rarely. Liver lesions in lambs are usually too small to be significant at meat inspection.

In **calves** exposed to yards contaminated by pig feces containing *Ascaris* eggs, *severe acute interstitial pneumonia* may occur. Signs of dyspnea, tachypnea, coughing, and increased expiratory effort are usually first seen ~7–10 days after exposure, when large numbers of larvae are present in the lungs. Deaths may ensue over the following few days, and the lungs are moderately consolidated, light pink to deep red, with alveolar and interstitial emphysema and interlobular edema. Microscopically, there is thickening of alveolar septa, and effusion of fibrin, proteinaceous edema fluid, and macrophages into alveoli. Hemorrhage into alveoli may also occur. Larvae are present in alveoli and bronchioles and provoke acute bronchiolitis. Neutrophils are found around larvae in bronchioles; eosinophils may be present but are not prominent in animals dying acutely. In addition to being usually observed readily in tissue sections, larvae may be recovered from the airways by washing with saline, or from minced lung in saline or digestion fluid, by use of a Baermann apparatus. Tens of thousands to millions of larvae may be present in the lungs of fatal cases.

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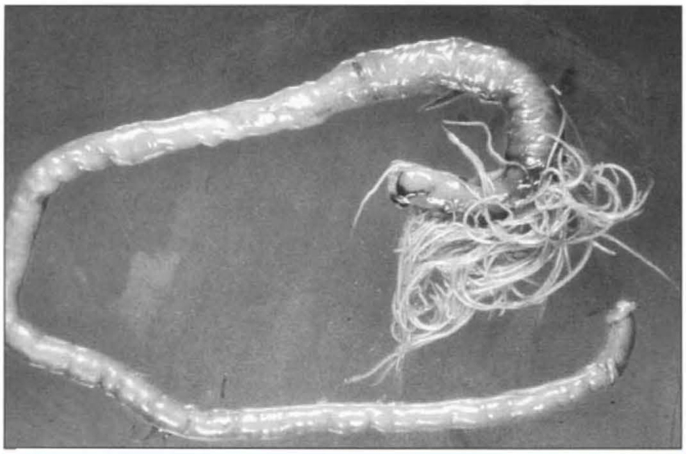

Figure 1.192 A heavy burden of ***Parascaris equorum*** impacted in and obstructing small intestine in a foal.

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Parascaris equorum is the *ascarid of horses*. It is widespread and common in young horses; it may contribute to ill-thrift and occasionally causes death by obstruction. *P. equorum* is a large nematode, females being up to 50 cm long. The life cycle resembles that of *A. suum*. Similarly, *hepatic and pulmonary lesions are associated with larval migration*, and coughing may occur at the time larvae are in the lungs, if infections are heavy. The prepatent period is ~10–15 weeks. The lesions in the lungs of foals with migrating *Parascaris* larvae, ~2 weeks after infection, are like those described in swine with *Ascaris*. Animals with resolving pulmonary lesions develop subpleural nodular accumulations of lymphocytes up to 1 cm in diameter, and there may be lymphocytic cuffing of pulmonary vessels.

It is possible to establish heavy infections of *P. equorum* in the intestines of foals a few months old, but not in yearlings, where larvae appear to be killed during hepatopulmonary migration. However, in heavily infected foals, many worms are lost from the intestine prior to patency, suggesting the possibility of an effect of crowding on the population of growing worms. A heavy burden of ascarids in the intestine may reduce weight gains in growing foals. Inappetence occurs but increased plasma protein catabolism or loss into the gut does not. Reduced weight gain may be due to decreased protein intake. Ascarid infection may reduce rate of intestinal transit. Heavy burdens can be associated with obstruction, intussusception or, rarely, perforation of the intestine (Fig. 1.192).

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The **ascarids of small animals** are *Toxascaris leonina*, infecting both cats and dogs, and *Toxocara canis* and *T. cati*, infecting the dog and cat respectively. All occur in the *small intestine*, mainly in young animals.

Toxascaris leonina has a life cycle that may be direct, but can involve a paratenic host. In the definitive host, larvae ingested in infective ova enter the wall of the gut, where they remain for several weeks, molting to the fourth stage and emerging to the intestinal lumen to molt again and mature. The prepatent period is ~10–11 weeks.

Toxocara canis has a complex life cycle. Puppies may be infected by ingestion of larvated ova, in which case larvae follow the pathway of hepatopulmonary movement in the bloodstream, and tracheal migration to the pharynx and gut, though some reach other tissues in the circulation. In older dogs most larvae ingested in eggs are disseminated in the circulation to various tissues, where they encyst, rather than undergoing development and tracheal migration. In the pregnant bitch, these larvae are mobilized, crossing the placenta to infect the fetus after day 42 of gestation. In the fetus, they remain in the liver, passing to the lungs after birth. Transmammary transmission of mobilized second-stage larvae also occurs, infecting the neonate via the colostrum. In a wide variety of species, infective larvae are disseminated hematogenously to many organs, where they settle, mainly in muscle. In some abnormal hosts, including humans, a syndrome termed "*visceral larva migrans*" is described, characterized by eosinophilia, general malaise, and perhaps signs related to granulomatous reactions to larvae in the eye, liver, lungs, and brain. Larvae in paratenic hosts eaten by young dogs undergo tracheal migration before maturing in the gut.

T. cati may infect cats directly from the larvated egg, via paratenic hosts, or, in kittens, by the transmammary route from the postparturient queen. Prenatal infection apparently does not occur. Larvae hatching from eggs migrate via the liver, lungs, and trachea, while those taken in from milk or prey do not. Following tracheal migration or ingestion in milk or prey, larvae enter the gastric wall, while fourth-stage larvae are found in the gastric contents, and the wall and lumen of the small intestine. *T. cati* will cause visceral larva migrans in humans, and if ingested as immature worms, will mature in the intestine.

Focal hemorrhages may be found in the lungs of puppies with migrating *T. canis* larvae. Larval *T. canis* are occasionally found in or associated with eosinophilic granulomas in the tissues of pups and older dogs. Inflammatory foci are most commonly seen grossly in the *kidney*, as white elevated spots 1–2 mm in diameter in the cortex beneath the capsule. They may be encountered in section in any organ, and are comprised of a small focus of macrophages, lymphocytes, and plasma cells, with a few eosinophils, and possibly containing a larva. Larvae may be destroyed in such foci, which heal by scarring. Considering the large numbers of larvae that must move through the tissues of dogs, and in many cases be sequestered there, relatively few are encountered incidentally, free or encapsulated in granulomas. Granulomas incited by *T. canis* larvae may be found in the *eye* on ophthalmoscopic examination, and retinitis, postinflammatory retinopathy, and blindness associated with *T. canis* larvae confirm that *ocular larva migrans* is a significant entity in dogs. Occult lesions similar to those occurring in dogs may be found in the tissues of cats infected with *T. canis*, though ocular larva migrans has not been recognized.

T. cati developing in the mucosa of the stomach and intestine may provoke a mild granulomatous response comprised of lymphocytes and a few macrophages about the coiled larva. Larvae free of such a response are also found in the mucosa and submucosa.

Heavy infections of ascarids in puppies and kittens, usually those reared in unhygienic communal environments, may result in *ill-thrift*. The most significant effects are those caused in the stomach and intestine by maturing *T. canis* in young puppies infected prenatally. The animals may develop weakness, lethargy, and vomition that is occasionally fatal. At autopsy the animal appears poorly grown for its age, pot-bellied, and cachectic, and masses of maturing worms are present in the intestine (Fig. 1.193) and perhaps stomach. Sometimes up to 20% of the body weight of young puppies may be accounted for by the worm burden. *T. cati* may be associated with clinical disease, but usually not death, in kittens up to several months of age. Medial hypertrophy of the pulmonary artery in cats has been associated with *T. cati* and *T. canis* infection; whether it is causal is unclear. Disease is rarely attributed to *T. leonina*.

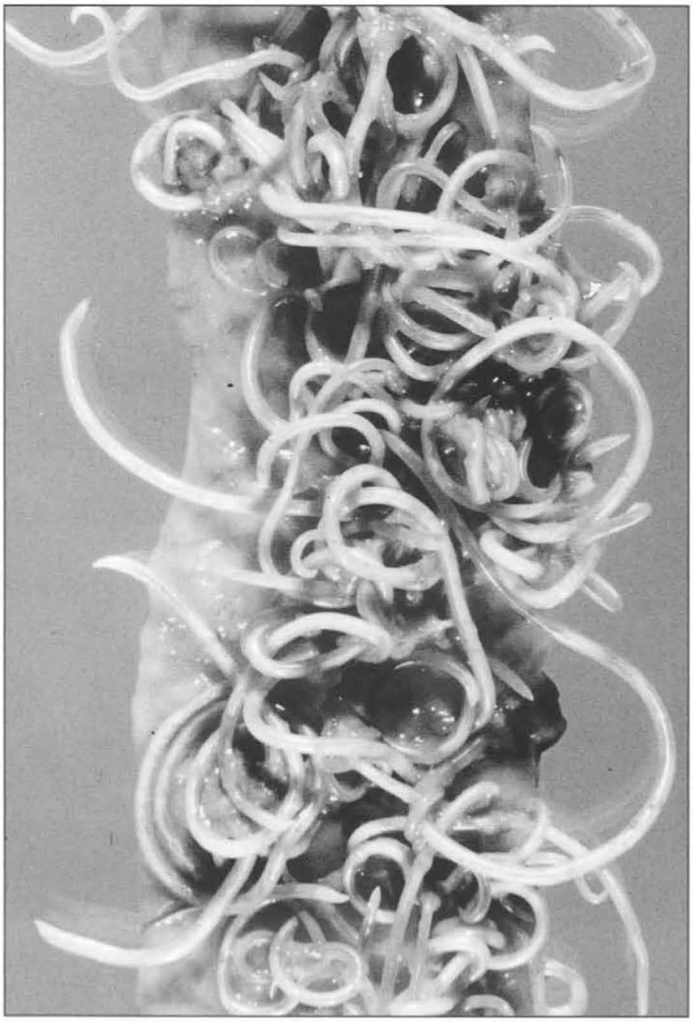

Figure 1.193 Tangled mass of ***Toxocara canis*** in the small intestine of a pup.

Mature *T. cati* are up to ~10 cm long; *T. canis* are up to ~18 cm long. In freshly dead animals, they are often coiled like a spiraled spring. They may maintain their place in the intestine by bracing against the gut wall in this way. The mechanism by which adults of these ascarids in the intestinal lumen impair growth has not been investigated. Ascarids occasionally enter the bile or pancreatic ducts, and many perforate those structures or the intestine.

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Toxocara (*Neoascaris*) ***vitulorum*** infects the *small intestine of young calves of domestic cattle*, mainly in the tropics and subtropics, and it is especially significant in water buffalo.

The life cycle involves transmammary transmission of third-stage larvae mobilized from the tissues of the dam within a few days of parturition. The larvae attain the liver of the calf and undergo tracheal migration. Patency occurs within ~1 month of birth, but worms are expelled within a short time, and by ~2–3 months of age, none are present.

Signs of infection include foul-smelling *diarrhea and ill-thrift*. Immature and mature worms both contribute to the signs. Heavily infected calves may die in an emaciated state, with burdens of up to 400–500 worms as much as 30 cm long in the intestine. Occasionally, migration up the bile duct or perforation of the gut may occur.

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Trichuris infection

Trichuris species, the **whipworms**, are so called because of their long thin cranial end and shorter stouter caudal portion. They inhabit the *cecum*, and occasionally the *colon*, of all the domestic animals considered here, except the horse. The host–parasite relationships include: in dogs – *T. vulpis*; in cats – *T. campanula* and *T. serrata*; in swine – *T. suis*; in sheep and goats – *T. ovis, T. globulosa, T. skrjabini*; in cattle – *T. discolor* and, less commonly, *T. ovis* and *T. globulosa*.

The life cycle is direct. Larvated ova are resistant to climatic insult, and persist in contaminated environments for several years. Ingestion of larvated eggs leads to release of third-stage larvae, which enter the mucosa of the proximal small intestine for up to 7–10 days, before returning to the lumen and passing on to the cecum, where they establish their adult existence. The prepatent period varies from ~6–7 weeks in the case of *T. suis* to 11–12 weeks for *T. vulpis*. In rare instances, disease may occur during the prepatent period, in which case ova will not be in the feces.

In all species, the cranial end of the worm is embedded at least partially in tunnels within the surface epithelium (Fig. 1.194), but not normally breaching the basal lamina. Light infections apparently cause little morphologic alteration in the mucosa and no disease. While *Trichuris* ingest blood, disease associated with them is not usually related to this activity. A protease produced by *T. globulosa* has been shown to promote degradation of mucosal tissue.

Heavy infection with *Trichuris* is associated with *severe and often hemorrhagic typhlitis or typhlocolitis in all species*. In the **dog**, large populations of worms overflow their normal habitat and infect the mucosa of the ascending, and often more distal, colon, sometimes extending to the rectum. The signs are chronic diarrhea or dysentery, perhaps with some weight loss. The blood and foul odor of the feces are due to hemorrhage and effusion of tissue fluid from the eroded mucosal surface. The mucosa is thickened, red, and edematous. The colonic content is fluid or porridge-like, and brown, tinged pink, or red. Masses of tangled worms are visible on the mucosa (Fig. 1.195). Microscopically the mucosa is widely eroded, or mildly ulcerated, and effusion of inflammatory exudate and blood is evident. Glandular epithelium is hyperplastic. Occasionally, *T. vulpis* infection may be associated with local or regional transmural lesions, with granulomatous foci and fibroplasia in deeper layers of the mucosa. Sometimes ova or worms are in these aberrant locations.

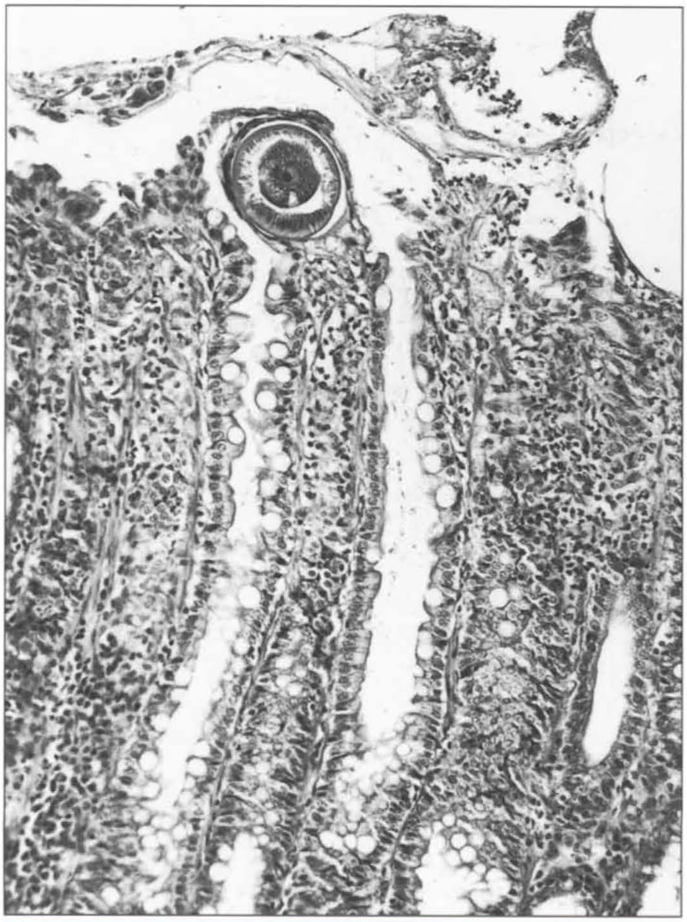

Figure 1.194 Mild erosive colitis in a dog with ***Trichuris vulpis*** infection. Cranial end of nematode is in tunnel in surface epithelium. There is exfoliation of epithelium, and effusion of neutrophils and fibrin from the surface.

Trichuris suis infection in **swine**, if heavy enough, may cause *mucohemorrhagic typhlocolitis* that is associated with anorexia, diarrhea, or dysentery, dehydration, ill-thrift and, in some cases, death. The disease is most common in animals exposed to dirt yards contaminated with infective *Trichuris* ova. The lesion is one of mucosal colitis, resembling that described in the dog. There is thickening of the mucosa with mucus hypersecretion from hypertrophic glands, coupled with erosion of, and effusion from, the mucosal surface. Lesions are more severe in swine with conventional gut flora than in those reared germfree, or free of known enteric pathogens. *T. suis* may suppress mucosal immunity to resident bacteria.

The large bowel in swine with *Trichuris* is thickened and congested, possibly with focal hemorrhages. The surface is glistening with mucus, perhaps with some fibrin exudation. The gross appearance may resemble that found in swine dysentery and the microscopic lesions are similar. However, closer examination reveals the presence

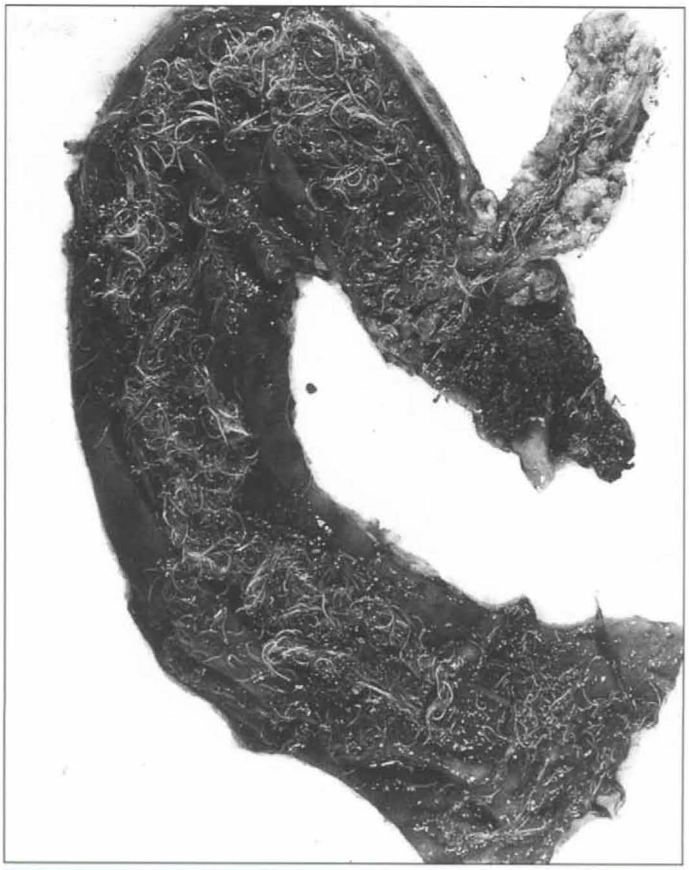

Figure 1.195 Hemorrhagic typhlocolitis in a dog caused by ***Trichuris vulpis***.

of the nematodes over the mucosa. Usually the thicker caudal end of the worms is noted. They may resemble at first glance *Oesophagostomum*, and only on more careful observation is the elongate thread-like cranial end seen.

The signs of the disease appear to be referable to loss of colonic absorptive function, and probably partly due to effusion of protein into the lumen. Erythrocyte loss is a minor component of the pathogenesis.

Trichurosis in **sheep** and **cattle** resembles that described in swine. The disease usually occurs in animals that are concentrated in areas contaminated by ova, and, in one reported case, immunocompromise due to persistent *Bovine viral diarrhea virus* may have predisposed. Affected animals develop chronic diarrhea with brown feces or dysentery, and loss of condition. At autopsy, the lesions are those of cachexia and hypoproteinemia, associated with mucohemorrhagic typhlitis or typhlocolitis.

A **diagnosis** of trichurosis in all species is usually readily made at autopsy. The worms have a characteristic morphology and are usually easily seen on the inflamed mucosal surface. In section, the thin cranial end of the nematodes, embedded in tunnels in the surface epithelium, contains the stichosome esophagus typical of members of the Trichuroidea, and a single bacillary band. The ova may be seen in the body of worms, in the gut lumen, or occasionally in tissue. They are barrel-shaped, have a thick wall and plugs at both poles of the egg. *Capillaria* spp. and their ova may be similar in tissue section, but are not expected in the cecum and colon.

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Cestode infection

Adult tapeworms inhabit the gastrointestinal tract, or the ducts of the liver and pancreas, where they are generally of minor significance. They are flattened, segmented colonies of sequentially maturing hermaphroditic reproductive units, or *proglottids*, forming an elongate strobila a few millimeters to many meters long. The Eucestoda are attached to the host by a specialized hold-fast organ, or *scolex*, which usually has four suckers, and perhaps a rostellum, sometimes armed with hooks. The Cotyloda may have elongate muscular grooves (bothridia) on the scolex. Cestodes lack an alimentary tract and absorb nutrients through the specialized absorptive surface or tegument of the proglottids.

Carnivores may be infected by tapeworms that use certain prey species as intermediate hosts. Metacestodes, or larvae, of members of the Taeniidae use as intermediate hosts some species of domestic animals and, accidentally, humans. They may cause disease, result in economic loss due to condemnation of tissues or organs at meat inspection, or have zoonotic significance.

Adult cestodes in tissue section are flattened, with internal organs in a loose parenchymatous matrix, often contain calcareous corpuscles in the outer region, and lack tubular digestive structures. They are segmented and the scolex may be encountered at the cranial end, attached to the intestine.

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Ruminants In ruminants, the more common and widely distributed intestinal tapeworms are *Moniezia expansa*, *M. benedeni*, and *Thysaniezia* (*Helictometra*) *giardi*. *Stilesia globipunctata* is found in the small intestine of sheep and goats in Europe, Asia, and Africa, whereas *S. hepatica* occurs in the bile ducts of ruminants in Africa and Asia. *Thysanosoma actinioides* occurs in the small intestine, pancreatic and bile ducts of ruminants in North and South America. *Avitellina* spp. occur in the small intestine of sheep and other ruminants in parts of Europe and Asia. The intermediate hosts of these tapeworms are oribatid mites or psocids (book lice).

Heavy infestations of the small intestine by *Moniezia*, *Thysaniezia*, and *Avitellina* are associated by some with diarrhea and ill-thrift in young lambs and calves. However, the evidence suggests that *Moniezia* is harmless; no effect on production or clinical signs can confidently be assigned to it.

The scolex of *Stilesia globipunctata* may be embedded in mucosal nodules 6–10 mm in diameter in the upper small intestine, with the thread-like strobila streaming into the lumen. There is a chronic inflammatory reaction around the scolex, which is deep in the mucosa, plugs of tissue being grasped by the suckers. Glands in the vicinity are hyperplastic, causing the nodules. The presence of up to 100 of these nodules has been associated with wasting, edema, and diarrhea.

Stilesia hepatica and *Thysanosoma actinioides* may cause mild fibrosis and ectasia of the bile ducts. Worms are often concentrated in the segmented sac-like dilations in the duct. These worms cause economic loss through condemnation of infected livers at meat inspection, and in areas where infection is common, this cost may be very significant.

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Horses The cestodes found in horses are *Anoplocephala perfoliata*, which colonizes the proximal cecum, especially at the ileocecal junction, and *A. magna* and *Paranoplocephala mamillana* in the small intestine and occasionally the stomach. The latter worm is small, under 5 cm in length, and is rarely associated with disease or lesions. *A. magna* tends to live in the lower small intestine, where it can attain a length of up to 80 cm, and a width of 2.5 cm. All use oribatid mites as intermediate hosts.

Anoplocephala perfoliata is more commonly associated with lesions, and occasionally with mortality. The risk of spasmodic colic increases with infection intensity. In areas of concentrated mucosal attachment by clusters of up to several hundred of this stumpy species, especially at the ileocecal orifice, erosion and ulceration of the mucosa occur. The depressed surface is often covered by fibrinous exudate, perhaps with some hemorrhage, or a local verrucous granulating mass may project into the lumen. *Ileal impaction* has been strongly associated with *A. perfoliata*. Partial obstruction of the ileocecal orifice may occur rarely, but no clear relationship is established between infection with *A. perfoliata* and the development of ileal muscular hypertrophy. *Ileocecal and cecocecal intussusception*, and, occasionally, perforation of the intestine or cecal rupture, have also been associated with infection by this tapeworm.

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Carnivores Dogs may be parasitized by ***Diphyllobothrium*** spp., as may be humans, cats, swine, and many other fish-eating mammals. The adults can be large, attaining lengths of up to 12–15 meters in humans, though those in animals tend to be shorter. The worm is ~2 cm across, and marked centrally by the uterus containing dark eggs, which are operculate. Intermediate stages occur in copepods and fish. The adult worm matures in the intestine of piscivorous mammals. Infection by *Diphyllobothrium* spp. is rarely, if ever, associated with clinical disease in animals.

Spirometra species are, like *Diphyllobothrium*, members of the subclass Cotyloda, and their life cycle is similar. The taxonomy of the genus is difficult, but among recognized species are *S. mansonoides*, infecting dogs, cats, and raccoons in North and South America, *S. mansoni* in dogs and cats in East Asia and South America, and *S. erinacei* found in cats and dogs in Australia and the Far East. Prospective hosts must have the opportunity for predation, since they are infected by the *plerocercoid* or "*sparganum*" found in the body cavity of the second intermediate host, usually an amphibian or reptile, or in another transport host.

Spargana can also occur in carnivores, swine, or even humans, if the procercoid in the first intermediate host, the *Cyclops*, is ingested, usually while drinking. Spargana are white, ribbon-like, but otherwise structureless worms up to several centimeters long. They may be found free or encysted in a thin, noninflammatory fibrous capsule, in the peritoneal cavity and intermuscular or subcutaneous tissue. A chronic inflammatory reaction may occur about dead spargana. The adult worms are nonpathogenic. Plerocercoids are of significance in humans, where they migrate mainly in subcutaneous tissues.

Mesocestoides spp. occasionally infect dogs, as well as other mammals and some birds, in North America, Europe, Asia, and Africa. These members of the subclass Eucestoda have a life cycle involving an insect or mite, and a vertebrate as second intermediate host. In the latter, infective *tetrathyridia*, ~1–2 cm long, flat, narrow, and bearing an invaginated scolex with four suckers, are found in the body cavities, liver, and lung. Tetrathyridia have the capacity for asexual multiplication, resulting in massive infections of intermediate hosts such as amphibia and reptiles, as well as dogs, cats, and other mammals. In the intestine of definitive hosts, *Mesocestoides* adults may also replicate asexually, and heavy infections may occur as a result of this, or from the consumption of large numbers of tetrathyridia in an intermediate host. Animals infected with intestinal *Mesocestoides* may develop diarrhea. Tetrathyridia replicating in the intestine of the dog may also penetrate the gut wall, and proliferate in the peritoneal cavity.

Tetrathyridia in the abdominal cavity of dogs and cats may cause peritoneal effusion (*parasitic ascites*), perhaps with the development of pyogranulomatous peritonitis and adhesions. Tetrathyridia 1–2 mm in diameter are scattered in the thick creamy exudate, along with similarly-sized "white bodies," comprised of necrotic parasite and host cellular debris. Mild infections may be discovered incidentally at necropsy. *Mesocestoides* infection of the abdominal cavity must be differentiated from peritoneal infections by cysticerci of several *Taenia* spp., which occur very rarely in carnivores.

Dipylidium caninum occurs in the dog, cat, fox, and, occasionally, children. It is ubiquitous. The narrow worms, up to 0.5 meters long, have distinctive cucumber-seed-like segments, and are often encountered incidentally in the small intestine at autopsy. They are of no pathologic significance. Cysticercoids develop in fleas and perhaps in the dog louse *Trichodectes canis*. Infection in the normal definitive hosts, or in accidental ones such as humans, is by ingestion of fleas containing cysticercoids.

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Taeniid tapeworms

Taeniid cestodes are the most important tapeworms in domestic animals, not because of the effects of the adult worm in the carnivorous definitive host, but rather due to the *metacestodes*, or larval forms, in intermediate hosts. Single oncospheres hatch from the egg in the upper small intestine, penetrate the epithelium, and are carried in the portal blood to the liver. Some species of metacestodes migrate in the liver, eventually to enter the peritoneal cavity. Others persist to develop in the liver, while still others pass on to the heart, lungs, and systemic circulation, establishing in muscle or a variety of other sites and tissues. Metacestodes may occasionally be found in organs other than the site of predilection.

Taeniid metacestodes assume four basic forms. The **cysticercus** is a fluid-filled, thin-walled, but muscular cyst, into which the scolex and neck of a single larval tapeworm are invaginated. The **strobilicercus** is a modification of this theme; late in larval development the scolex evaginates and is connected to the terminal bladder by a segmented strobila, so that it resembles a tapeworm, several centimeters long. The **coenurus** is a single or loculated fluid-filled cyst, in which up to several hundred nodular invaginated scolices are present in clusters on the inner wall. Each scolex is capable of developing into a single adult cestode in the intestine of the definitive host. The **hydatid cyst** is a uni- or multilocular structure, on the inner germinal membrane of which brood capsules develop. Within the brood capsules, invaginated protoscolices form. Brood capsules may float free in the cyst fluid, where they are termed "*hydatid sand.*" Internal daughter cysts can develop. Release of brood capsules or protoscolices into tissues, as a result of rupture of the hydatid cyst, may lead to development of new cysts. The alveolar hydatid cyst proliferates by budding externally.

Taenia taeniaeformis infects the intestine of domestic cats and some wild felids, and the strobilicercus, ***Cysticercus fasciolaris***, is found in the liver of small rodents. Formation of fibrosarcomas has been associated with chronic inflammation due to *C. fasciolaris*. The adults are up to 60 cm long, have no neck, and caudal segments are somewhat bell-shaped, so that this species is readily differentiated from the other cestodes found in the feline small intestine. Usually, only a few worms are present in the cat, and they are of no consequence.

T. pisiformis is common in the small intestine in dogs and some wild canids, which prey on rabbits and hares. ***C. pisiformis*** migrates in the liver of the intermediate host, causing hemorrhagic tracks that are infiltrated by a mixed inflammatory reaction, and ultimately heal by scarring. The pea-size cysticerci encyst in a thin, noninflammatory fibrous capsule on the mesentery or omentum, or on the ligaments of the bladder. Occasionally, cysticerci persist beneath the hepatic capsule. Burdens of up to 20–30 worms, sometimes more, may be present in the intestine of the dog.

T. hydatigena infects the dog, and the metacestode, ***C. tenuicollis***, the long-necked bladder worm, or false hydatid, is found in the peritoneal cavity of sheep, cattle, and swine, and occasionally other species. Immature cysticerci in the liver migrate through the parenchyma for several weeks as they develop, before emerging to encyst on the peritoneum anywhere in the abdominal cavity. The immature cysticerci are less than 1 cm long, ovoid, and translucent. They cause tortuous hemorrhagic tracks similar to those produced by immature liver flukes, and if large numbers are present, they may cause a syndrome of depression and icterus identical to acute fascioliasis.

Heavily infected livers, with 4000–5000 actively migrating cysticerci, are mottled due to the subcapsular and parenchymal hemorrhagic foci and tracks. Cysticerci up to 6–8 mm long may be present beneath or breaching the capsule by about 3 weeks after infection. Rarely, animals may exsanguinate into the abdominal cavity, or the hepatic necrosis may predispose to the development of black disease or bacillary hemoglobinuria.

Cysticerci trapped in the liver may persist in a fibrous capsule, or be destroyed in a cystic eosinophilic granuloma that may mineralize; this is common on the diaphragmatic surface where the falciform ligament is attached. Usually, the intensity of infection is low, and a few, but occasionally scores of cysticerci – delicate translucent fluctuant fluid-filled cysts up to 5 cm or more in diameter – are contained in individual thin, noninflammatory fibrous capsules scattered on the peritoneal serosa. When a cyst degenerates, it is destroyed by a granulomatous reaction and the fibrotic mass may mineralize. Hepatic migration by *C. tenuicollis* may, at any stage, cause condemnation of lamb and pig liver at meat inspection.

T. ovis infects the intestine of the dog, while the metacestode, ***C. ovis***, is in the muscle of sheep, where it causes cysticercosis, or "*sheep measles.*" Cysticercosis of muscle caused by *C. ovis*; by *C. bovis* in cattle; and by *C. cellulosae* in swine and other species, including dogs, is considered in Vol. 1, Muscle and tendon. The adult stages of the latter two cysticerci, *T. saginata* and *T. solium* respectively, occur in the small intestine of humans.

T. multiceps occurs in the intestine of dogs and wild canids but the metacestode, ***Coenurus cerebralis***, develops in the brain and spinal cord of sheep and other ungulates, and, rarely, in humans. In the goat, coenuri may also occur in other organs, beneath the skin and intramuscularly. The migration of small metacestodes in the central nervous system may cause tortuous red or yellow-gray tracks in the brain due to traumatic hemorrhage and malacia, and nervous signs or death may occur at this stage. More commonly, signs of central nervous system disease, termed "sturdy" or "gid," do not develop until coenuri, up to 4–5 cm in diameter, have developed more fully, 4–8 months after infection. Cysts may be present at any level and depth in the brain and spinal cord, and projecting into the cerebral ventricles, but they are most common near the surface of the parietal cortex in the cerebrum. They cause increased intracranial pressure, hydrocephalus, necrosis of adjacent brain, and sometimes lysis, perhaps extending to perforation, of the overlying cranial bone. Coenuri developing in the spinal cord may cause paresis or paralysis.

T. serialis infects dogs and foxes throughout the world. The larval coenurus is found in the subcutaneous and intermuscular connective tissue of lagomorphs. Cerebral coenurosis has been reported in cats.

Histologically, cysticerci and coenuri are recognized as generally cystic structures with an eosinophilic outer layer or tegument, which may appear fibrillar or almost ciliate on the outermost surface. Beneath the tegumental cells a less cellular area, which may contain calcareous corpuscles, gives way to a web-like, lightly cellular matrix, and the central open fluid-filled portion of the cyst. No internal organs are seen. Muscular inverted scolices, with suckers, and (in all but *C. bovis*) hooks on the rostellum, may be encountered in favorable sections, extending into the center of the metacestode. Size and shape of hooks may assist in a specific diagnosis, if they are fully developed. Immature migrating metacestodes lack organized scolices. Other sources should be consulted for details on the taxonomy and specific identification of adult and larval taeniid tapeworms.

Echinococcus spp. tapeworms occur in the small intestine of a number of species of carnivores, predominantly canids. In enzootic areas, the distinctive metacestodes, or *hydatid cysts*, are commonly found in normal or accidental intermediate hosts. Humans may accidentally become infected with the metacestode, and echinococcosis or hydatidosis is a significant public health problem where carnivores shedding *Echinococcus* eggs come in close contact with humans.

The species are *E. granulosus, E. multilocularis, E. oligarthus*, and *E. vogeli*. The latter two involve sylvatic cycles in Central and South America, with felids and canids as definitive hosts respectively, and rodents as intermediate hosts in which polycystic hydatidosis occurs; *E. vogeli* may infect humans. The other two species may use domestic animals as definitive hosts, and will be considered further here.

E. granulosus uses the dog and some other canids as the definitive host. The most widespread strain or genotype uses a *sheep–dog cycle*, and has been disseminated wherever there is pastoral husbandry of sheep. It is significant as a potential zoonosis in many parts of Eurasia and the Mediterranean region, some parts of the UK, North America, South America, continental Australia, and Africa. Eradication has been accomplished, or virtually so, in Iceland, New Zealand, and Tasmania. Other cycles affecting domestic animals include horse–; cattle–; camel–; pig–; water buffalo–; goat–, and human–dog. Sylvatic cycles include: in Eurasia and North America, cervid–wolf; in Argentina, hare–fox; in Sri Lanka, deer–jackal; in Australia, macropod–dingo. Not all cycles represent different genotypes.

In the small intestine of the definitive host, protoscolices evaginate and establish between villi and in the crypts of Lieberkühn. The scolex distends the crypt and the epithelium is gripped by the suckers and occasionally eroded, but there is little or no inflammatory response. The worms that develop are short, usually less than 6–7 mm long. They commonly have only 3–5 proglottids, the caudal gravid one making up almost half the length of the worm. Burdens of *E. granulosus* are often heavy, no doubt due to the large numbers of protoscolices ingested at a meal containing one or more hydatid cysts. The heavily infected intestine is carpeted by the tiny white blunt projections, partially obscured between the villi; the worms may resemble lymphangiectasia. Enteric signs are not normally encountered in dogs with intestinal hydatid tapeworms.

Penetration of oncospheres released from eggs in the intestine of the intermediate host takes them into the subepithelial capillaries, or perhaps the lacteal. The majority probably migrate via the liver, some carrying on to the lungs and general circulation. However, those gaining the lacteal may bypass the liver, entering the vena cava with the lymph, and either are filtered out in the pulmonary circulation or are disseminated. *Hydatid cysts occur most commonly in the liver and lung*, with some strain and host species variation in the relative prevalence in these organs. In sheep they may be more common in lungs, while in cattle and horses, the liver is the usual site of establishment. Less commonly in domestic animals, the brain, heart, bone, and subcutaneous tissue may be sites of development of hydatid cysts. A single cyst, or up to several hundreds, may be present, displacing tissue in infected organs. Disease is rarely attributed to hydatidosis in animals, even in those heavily infected. However, strategic location of one or more cysts may lead to heart failure, bloat or central nervous signs. Condemnation of infected organs at meat inspection causes economic loss.

Hydatid cysts are usually spherical, turgid, and fluid-filled. They usually measure 5–10 cm in diameter in domestic animals; rarely, cysts in animals may be larger, but in humans hydatid cysts can become huge. On the other hand, some fertile cysts in equine livers may be as small as 2–3 mm across. The lining of fertile cysts is studded with small granular *brood capsules*, which contain protoscolices; and "hydatid sand," comprised of free brood capsules and protoscolices, is in the fluid. The lining of sterile cysts is smooth. Though the potential exists for development of internal daughter cysts, and rare exogenous budding by herniated cysts, most hydatid cysts in domestic animals are unilocular. However, they may be irregular or distorted in shape due to the variable resistance of parenchyma and portal tracts or bronchi and by the profiles of bone or other resistant tissues.

Microscopically, immature hydatid cysts are surrounded by an infiltrate of mixed inflammatory cells, including giant cells and eosinophils. As they develop, a layer of granulation tissue, which may contain round cells and eosinophils, invests the cyst, and this evolves so that the inner portion of the fibrous capsule is comprised of mature collagenous connective tissue that is relatively acellular. Within this, and in close apposition, is the acellular lamellar hyaline outer layer of the hydatid cyst wall, comprised of a polysaccharide–protein complex, which, with time, may become hundreds of micrometers thick. The cyst is lined by the thin syncytial germinal layer from which the brood capsules form on fine pedicles. If the cyst is ruptured and protoscolices are released into tissue, secondary cysts may form from them.

Hydatid cysts may degenerate. The inner structures collapse, and the mass becomes caseous and may mineralize. Degenerate hydatid cysts grossly may resemble tuberculous lesions or metastatic squamous cell carcinoma.

E. multilocularis has a holarctic distribution, the adults occurring mainly in foxes, and the metacestodes in small rodents, especially voles and lemmings. Dogs and cats may also become infected with the worms in enzootic areas. Though the parasite is principally arctic, the cycle is found in the northern prairie area of North America and in eastern and central Europe, and is moving progressively southward. The mature cestodes in the intestine are similar to, but smaller than, *E. granulosus*. In the intermediate host, the metacestode mainly infects the liver, forming a cystic structure with internal brood capsules and protoscolices; but it is capable of external budding. As a result, racemose proliferative masses of metacestodes infiltrate infected livers. They may metastasize via the bloodstream to the lungs or bone, or implant in the peritoneal cavity. The inflammatory reaction to alveolar hydatids is comprised of macrophages, perhaps giant cells, lymphocytes, and plasma cells in an encapsulating fibrous stroma. The metacestodes are rarely found in domestic animals, but may infect humans who ingest eggs shed by infected carnivores.

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Intestinal fluke infection

Trematode infections of the intestine of domestic animals are, on the whole, uncommon. Dogs and cats in many parts of the world may be infected with *Alaria* spp., the second intermediate hosts for which are frogs or other amphibia. *Heterophyes heterophyes, Metagonimus yokagawi, Echinochasmus perfoliatus*, and *Phagicola longa* may infect dogs and cats fed fish that contain metacercariae. The former two occur in the Mediterranean area and the Far East; the latter in Eurasia. *Cryptocotyle* spp., most commonly parasitic in piscivorous birds, may also be found in dogs, cats, and mink fed infected marine fish.

Enteritis is attributed to ***Alaria, Echinochasmus,* and *Cryptocotyle*.** The flukes attach to the mucosa by suckers, and perhaps cause their effects by local irritation, erosion, and ulceration that large numbers of them may induce. Vomiting and ill-thrift have been associated with infection. The production of excessive intestinal mucus and hemorrhagic enteritis have been associated with intestinal fluke infection in small animals. The flukes involved are small, less than 4–5 mm long, and must be sought carefully at autopsy.

Nanophyetus salmincola occurs in the small intestine of dogs, cats, and humans, and in various fish-eating wild mammals and birds in the northwestern USA; Vancouver Island, Canada; and eastern Siberia. Its distribution is determined by that of the snails that are the first intermediate hosts. The second intermediate hosts are fish, especially members of the family Salmonidae. The adult flukes inhabit the small intestine, where they penetrate and attach to the mucosa. Large numbers may cause mucoid or hemorrhagic enteritis. *N. salmincola* transmits *Neorickettsia helminthoeca*, which causes "*salmon poisoning disease*," to canids consuming raw salmon infected with metacercariae. The disease is thought to be restricted to North America. However, similar organisms have been recognized in dogs with lesions compatible with salmon poisoning disease in southern Brazil.

Salmon poisoning disease has an incubation period of about 5–7 days, and is characterized clinically by pyrexia, anorexia, depression, weakness, and weight loss. There may be serous nasal discharge and mucopurulent conjunctivitis. Diarrhea with tenesmus develops; feces are scant yellow and mucoid or watery, often with some blood. The condition is usually fatal; if untreated, only 5–10% of infected dogs recover. They are immune to reinfection.

At necropsy, lesions are most consistently found in the lymphoid tissues. There is *generalized enlargement of lymph nodes*, especially in the abdominal cavity. Enlarged tonsils are everted from their fossae. The thymus is often increased in size in young dogs and the spleen may be swollen and congested. Peyer's patches and other intestinal lymphoid aggregates are elevated above the mucosal surface, and there may be petechial hemorrhages on the mucosa. Lymphoid tissue near the ileocecocolic valve may ulcerate and bleed. Ileocolic intussusception occurs in many cases. The liver of foxes becomes friable, and may rupture, causing hemorrhage into the peritoneal cavity. Focal hemorrhages may be seen in the mucosa of the bladder, and subpleural hemorrhages up to 2 cm in diameter usually occur.

Microscopic changes in lymph nodes include depletion of lymphocytes, focal necrosis with neutrophilic infiltrates, and increased numbers of histiocytes in the cortex and medulla. Similar changes may occur in the thymus, and splenic follicles may undergo necrosis. *Elementary bodies* of the *Neorickettsia* may be demonstrated in the cytoplasm of macrophages in lymphoid tissue, and in other visceral organs, by use of Giemsa or Macchiavello stains, or immunohistochemistry. In the small intestine, the flukes may be present embedded deep in the mucosa, though usually little reaction to them is present.

Lesions of the central nervous system occur in most cases. Leptomeninges may be somewhat opaque, but the lesions are best recognized microscopically. They are composed of macrophage accumulations in the leptomeninges and Virchow–Robin spaces, and focal gliosis in the parenchyma. Meningeal reaction is perhaps most consistent over the cerebellum, and is comprised of mild or moderate perivascular or more diffuse accumulations of histiocytes. Similar cells may cuff small and medium-sized vessels throughout the parenchyma. Focal gliosis is relatively sparsely distributed but seems most common in the brainstem. Elementary bodies are also demonstrable in macrophages in the central nervous system, and the diagnosis is usually made on the basis of this finding in macrophages in lymphoid tissue and/or brain. The organisms can be isolated and grown on primary canine monocyte cultures and in several other cell culture systems, but this is not a routine procedure.

Paramphistome infections in ruminants may cause significant intestinal disease. The adults, of the genera *Paramphistomum, Cotylophoron, Calicophoron, Ceylonocotyle, Gastrothylax, Fischoederius,* and *Carmyerius*, occur in the forestomachs of ruminants in various areas around the world. Infection is most common in warm-temperate to tropical areas. In the rumen, the red pear-shaped adult flukes, with their characteristic cranial and caudal suckers, are considered innocuous, though some papillae may become atrophic and slough (see Fig. 1.36).

When ingested, metacercariae encysted on herbage give rise to immature flukes that inhabit the duodenum, where massive infections may cause severe enteritis. In cattle, water buffalo, and American bison, the species incriminated in disease include *P. cervi*, *P. microbothrium, P. explanatum, Calicophoron calicophorum,* and various species of *Cotylophoron, Gastrothylax,* and *Fischoederius*. In sheep and goats, *P. microbothrium, P. ichikawai, P. cervi, P. explanatum, G. crumenifer, Cotylophoron cotylophorum,* and *F. cobboldi* have been associated with disease. The species involved vary with the host and geographic area.

After ~3–5 weeks in the small intestine, the worms normally migrate forward, through the abomasum, to establish and mature in the reticulorumen. However, if massive infection occurs, growth in the small intestine is retarded, and flukes may persist for months in the duodenum, prolonging the course of disease.

Calves and lambs with intestinal paramphistomosis are depressed and inappetent. Fetid *diarrhea* usually develops within several weeks of infection, and may contain immature flukes. Hypoproteinemia is reflected in submandibular edema in some animals and anemia is reported to occur occasionally. Morbidity and mortality can be substantial, and survivors may suffer considerable loss in condition.

The carcass may be in good or cachectic condition, depending on the duration of the disease, and there may be edema of subcutaneous tissues, abomasal folds, and mesentery, and fluid in the body cavities, due to hypoproteinemia. The proximal small intestine appears congested externally, and immature paramphistomes, deeply penetrating the intestinal wall, may be visible through the serosa. Occasionally, they will perforate the gut and be found free in the abdominal cavity. The mucosal surface of the duodenum is edematous, thickened, corrugated, and covered with mucus. Many immature pink or brown paramphistomes, a few millimeters long, are scattered over the surface and embedded in the mucosa. Most larval paramphistomes are in the first 3 meters of small intestine. In advanced infections, some may be present migrating forward on the abomasal mucosa, or already in the forestomachs.

In section, small larval paramphistomes are found deep in the lamina propria, occasionally in the submucosa, and sometimes in Brunner's glands. Larger immature forms are attached to the surface of the mucosa by a plug of tissue taken into the acetabulum. There is atrophy of villi, elongation of crypts, and possibly erosion or ulceration of the mucosa in heavily infected areas. Heavy infections are associated with fibroplasia. Protein loss into the gut, coupled with loss of appetite, seems to be the most important pathophysiologic consequence.

The other fluke occurring in the intestine of ruminants is *Skjrabinotrema ovis*, associated with catarrhal enteritis in sheep in Eurasia.

In **swine**, the paramphistomes *Gastrodiscoides* and *Gastrodiscus* may be found in the colon, where they are of little significance. *Fasciolopsis buski* and *Artyfechinosomum malayanum* may infect the small intestine of swine, as well as humans. They are of little importance in pigs other than as reservoirs for human infection.

In **horses** in Africa and India, the paramphistomes *Gastrodiscus aegyptiacus* and *Pseudodiscus colinsi* occur in the large bowel. Larvae of the former species have been associated with severe colitis in horses, but they are generally nonpathogenic.

Intestinal schistosomiasis, due mainly to *Schistosoma* spp. in ruminants, and *Heterobilharzia* in dogs, may cause *protein-losing enteropathy*, perhaps associated with granulomatous enteritis in response to deposition of ova in mucosal venules (see Vol. 3, Cardiovascular system). Treatment may precipitate hepatic lesions consisting of granuloma formation around dead parasites as well as periportal fibrosis.

Flukes in tissue section are generally somewhat flattened or globose, with a loose mesenchymal parenchyma in which the internal structures are suspended. The cuticle is eosinophilic, and may be spiny. Muscular oral and acetabular suckers and pharynx may be encountered in sections. Ceca are usually present, and elements of the male and female reproductive systems in these typically hermaphroditic adult worms (excepting the schistosomes) may be seen. The uterus may contain ova with a tan-yellow or brown shell, perhaps with an operculum, and ova are often seen in the intestinal lumen or in tissue. The developing miracidium may be present in ova. Schistosomes are recognized by their intravascular location and sexual dimorphism, the leaf-like male perhaps enveloping the slender cylindrical female within the gynecophoric canal, in section.

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Acanthocephalan infections

The Acanthocephala are a phylum of parasitic animals that have an elongate sac-like body, no internal alimentary canal, and use as the hold-fast a spiny protrusible proboscis. The life cycle typically involves obligate development in an intermediate host, usually an arthropod, and perhaps the utilization of a paratenic host to facilitate transmission. The acanthocephala of concern in domestic animals are in the genera *Macracanthorhynchus* and *Oncicola.*

Macracanthorhynchus hirudinaceus is the *thorny-headed worm of swine*, infecting the small intestine. The life cycle involves dung beetles or other Scarabaeidae, and foraging or rooting swine are prone to infection. Males are ~10 cm long, and the females up to 30–40 cm long, slightly pink, curved, and tapering caudally. The proboscis has ~6 rows of hooks, and is used to penetrate deeply into the intestinal wall. It incites a local granulomatous nodule called a "*strawberry mark*," with a purulent focus about the embedded proboscis. The proboscis may penetrate the muscularis, and the nodules around the proboscis, ~1 cm in diameter, may be visible on the serosal surface of the gut as gray or yellow suppurative foci, surrounded by a halo of hyperemic tissue. They occasionally perforate, causing peritonitis. Severely infected pigs may suffer ill-thrift and perhaps anemia, probably related partly to the potential for plasma protein loss and hemorrhage from numerous focal ulcerative lesions.

M. catalinum and *M. ingens* are smaller but similar thorny-headed worms that inhabit the intestine of a variety of wild carnivores, and occasionally the dog.

Oncicola canis occurs in the small intestine of wild carnivores, and occasionally the dog and cat. It rarely causes disease. Intermediate hosts are presumably arthropods, with insectivorous vertebrates acting as paratenic hosts. Up to several hundred worms, 0.5–1.5 cm long and dark gray, may infest the small intestine; usually infections are light. The proboscis is embedded to the subserosal level, and a focal nodular lesion develops about it.

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Protistan infections

Coccidiosis

The coccidia are members of the protistan phylum Apicomplexa, intracellular parasites characterized at some stage of the life cycle by a typical "apical complex" of organelles at one end of the organism. Members of the subclass Coccidiasina, which will be considered together under coccidiosis, all have a similar basic life cycle. It begins with infection of a cell, often, but not always, in the intestinal mucosa, by a **sporozoite** released from a **sporocyst** in the lumen of the gut. One or more cycles of asexual division, termed **schizogony or merogony**, follow, and the **merozoites** produced infect other cells, forming another generation of **meronts**, or transforming to sexual stages, termed **gamonts**. Gamonts subsequently develop into nonmotile female **macrogametes**, and motile male forms or **microgametes**. A nonmotile zygote produced by union of micro- and macrogametes forms an **oocyst**. **Sporogony**, production of sporocysts containing infectious sporozoites within the oocyst, may occur in the host, or, more commonly, after the resistant oocysts are passed in feces.

Members of the genus *Eimeria* and *Isospora* are **homoxenous**, with sexual and asexual development taking place in a single host. *Cystoisospora* (former *Isospora* spp. in carnivores) and the genera *Toxoplasma, Sarcocystis, Hammondia, Besnoitia, Frenkelia, Neospora*, and *Caryospora* are all **heteroxenous**, in which case asexual stages occur in an intermediate host. The heteroxenous genera exploit natural prey–predator relationships. In general, sexual development takes place in the intestinal mucosa of a predator, while at least one generation of asexual replication, often several, occurs in the tissues of one or more species of prey.

The *endogenous stages of coccidia are all intracellular*, except, temporarily, the merozoite and microgamete. Mature developmental stages are usually readily recognized; immature forms may not be easily identifiable. **Trophozoites**, small undifferentiated, rounded, basophilic forms with a single nucleus, usually within a parasitophorous vacuole in the host cell, are found at three stages of the life cycle. They occur following invasion by the infective sporozoite, prior to merogony; following invasion by a merozoite, prior to a subsequent generation of merogony; and following invasion by a merozoite, prior to differentiation into a recognizable gamont. Developing meronts are multinucleate. Merogony may involve "**endopolygeny**," multiple fission or apparent "budding" of merozoites from the periphery of the meront or from infoldings of it. A single residual body, surrounded by slightly curved, fusiform, or banana-shaped uninucleate merozoites, or many spherical clusters of merozoites with a central residuum, may be present. A second form of replication, termed "**endodyogeny**," occurs in meronts of many of the heteroxenous coccidia. Two daughter organisms develop within a mother organism, which is destroyed when they are released. The location of a meront, and the number of merozoites it contains, vary with the species and the generation of merogony. A very few, or up to tens or hundreds of thousands of merozoites may be released from a single meront.

Microgamonts mature in two steps. The first involves enlargement of the gamont and proliferation of nuclei. During the second phase, the microgametes differentiate about the periphery of the gamont, which may become deeply folded or fissured by invaginations. Immature microgametocytes during these stages may resemble developing schizonts. However, *fully differentiated microgametes* differ from merozoites in being small, densely basophilic, and comma-shaped, with 2–3 flagella. They may be present in swirling masses, perhaps with some residual bodies, in mature microgametocytes. Macrogametes, the female stage, have a large nucleus with a prominent nucleolus, and with time they usually enlarge to contain refractile eosinophilic "plastic granules" or wall-forming bodies, which give rise to the layers of the oocyst wall. *Mature macrogametes* typically have prominent wall-forming bodies, and contain clear or periodic acid–Schiff-positive amylopectin granules, and a large nucleus and nucleolus.

Fertilization by the microgamete leads to development of the zygote, and subsequent formation of the oocyst wall. The contained *sporont* is spherical, with nucleus and nucleolus, and amylopectin granules in the cytoplasm. Sporulation usually occurs outside the host, but in *Sarcocystis* and *Frenkelia* it occurs in the tissue of the definitive host; in *Caryospora*, sporulated oocysts develop in tissues of the prey host. Sporozoites are enclosed within sporocysts, which in turn are contained by the oocyst wall. Oocysts of most coccidia, or sporocysts of *Sarcocystis* and *Frenkelia*, are passed in the feces.

Coccidia of domestic animals are relatively host-, organ-, and tissue-specific. Asexual stages of *Toxoplasma* and *Neospora* are the obvious exception to this generalization. Species of *Eimeria, Isospora,* and *Cystoisospora* rarely occur in more than one genus of definitive host. Similar coccidia occurring in related genera of hosts, when tested, usually prove incapable of cross-infection.

The economic cost of coccidiosis in the food–animal species is considerable, in terms of mortality, morbidity, subclinical disease, and the cost of prevention and treatment. It is even more so in chickens. In dogs and cats, coccidiosis is a minor problem.

Virulence reflects a number of factors. Among these are the location and type of cell infected by various stages of the organism, the function of infected cells, and the degree of host reaction stimulated by infection.

The effects of infection on the host cell are several, and vary somewhat with the infecting species. Infected cells may be functionally compromised. They may hypertrophy; nuclei may enlarge or a considerable amount of cytoplasm may be displaced; and the outer membrane of infected cells may be highly modified, perhaps to facilitate metabolic exchange. The intercellular relationships may be affected. The rate of movement of infected epithelial cells up villi is altered.

Immunoinflammatory reactions may be incited by coccidial infection. In experimental systems, resistance to coccidial infection is thymus-dependent, and is largely mediated by T-cell-promoted intracellular killing directed mainly against asexual stages in the life cycle.

In mammals, acute inflammatory reactions in intestinal coccidiosis are most commonly associated with heavy infection and destruction of cells by the sexual stages and oocysts, rather than in response to asexual stages. In toxoplasmosis and neosporosis, necrosis and focal acute or chronic inflammatory reactions may be incited by actively replicating asexual stages in many organs. A syndrome characterized by hemorrhage occurs in some species infected with asexual stages of *Sarcocystis*, about the time that merogony occurs in vascular endothelium.

The effects of *intestinal coccidiosis* in mammals vary with the host–parasite system. They are mainly related to malabsorption induced by *villus atrophy*, or to anemia, hypoproteinemia, and dehydration due to exudative enteritis and colitis caused by *epithelial erosion and ulceration.* A *neurotoxin* has been associated with the development of nervous disorders in cattle with coccidiosis. Many species of coccidia appear to have little pathogenic effect under normal circumstances.

Coccidiosis is typically a disease of *intensively managed animals.* It is especially important in naive young animals exposed to a high level of infection. This is predisposed by high contamination rates associated with crowding, yarding, or high stocking rates on pasture. A damp substrate promotes oocyst sporulation and survival, and practices such as feeding on the ground, or the natural propensity of young animals to nibble or perhaps indulge in coprophagy, may promote infection. Although infections may not proceed to patency, chronic ingestion of oocysts may cause an intestinal immune response, villus atrophy, and perhaps ill-thrift, in some situations. Immune reactions may only halt development of, but not kill, endogenous asexual stages. Epizootiologic evidence suggests that under some circumstances there may be relaxation of resistance and resumption of development of the organisms, ultimately expressed in disease. This seems the likely explanation for outbreaks of bovine coccidiosis occurring during midwinter in freezing climates, or in post-parturient stabled dairy cattle.

Coccidiosis caused by members of the genera *Eimeria* and *Isospora* in the various species will be considered further here. The heteroxenous organisms, including *Cystoisospora, Toxoplasma, Neospora,* and *Sarcocystis*, will be considered subsequently, as will *Cryptosporidium*.

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Coccidiosis in cattle

Over a dozen species of *Eimeria* parasitize cattle; of these, *Eimeria zuernii* and *E. bovis* are potentially highly pathogenic, while several others, notably *E. ellipsoidalis, E. alabamensis*, and *E. auburnensis*, may cause diarrhea, but probably not death. *Coccidial infection is common*, and it usually comprises several species. Almost half the calves and yearlings in confinement operations shed oocysts, with calves shedding high numbers, while a much smaller proportion of cows shed low numbers of oocysts.

Disease occurs mainly in calves or weaned feeder cattle under ~1 year of age, when one or both of the potentially pathogenic species produce heavy infection. It may occur in animals at pasture or on range, concentrated at water holes, but is most common in animals in feedlots or yards where the level of sanitation is not high. The stress of shipping, cold weather, or intercurrent disease may be associated with outbreaks, which can occur in midwinter when oocyst transmission is expected to be poor. *Bovine parvovirus* infections have been associated with outbreaks of coccidiosis in a dry environment in northern Australia. Reactivation of latent schizonts in tissue may explain coccidiosis in stressed animals, or at a time when transmission is unlikely.

Coccidiosis is characterized by *diarrhea that may progress to dysentery* with mucus, and tenesmus, perhaps causing rectal prolapse. Animals dehydrate, and become hyponatremic and perhaps anemic. Morbidity may be high, but mortality is usually low. The duration of severe disease is ~3–10 days, after which most cases recover, since infection is essentially self-limiting. Some animals develop concurrent nervous signs, including tremors, nystagmus, opisthotonos, and convulsions, and many of these die within a few days.

The signs in bovine coccidiosis due to *E. zuernii* and *E. bovis* occur when the epithelium in the glands of the cecum and colon is infected by second-generation schizonts and gametocytes. In heavily infected animals, disease, and perhaps death, can occur before many oocysts are passed in the feces. The life cycles of both agents are similar, two schizogonous generations preceding gametogony. The first-generation schizont of ***E. bovis*** infects hypertrophic endothelial cells in lacteals on the upper part of villi in the *lower small intestine*, several meters proximal to the ileocecal valve. These schizonts may be large, up to ~300 μm in diameter, and are visible to the naked eye as *pinpoint white nodular foci in the mucosa.* They contain tens of thousands of merozoites, but are invested by only a narrow rim of mononuclear inflammatory cells, unless they degenerate, when a marked local mixed reaction develops, including neutrophils and macrophages. Merozoites released from these schizonts ~14–18 days after infection enter cells deep in cecal and colonic glands. In heavy infections, crypts of Lieberkühn in the terminal ileum may also be infected. Here they produce small second-generation schizonts, which in turn release merozoites, infecting other cells in the gland. Gametogony may begin as early as 15 days after infection, and oocyst production peaks ~19–21 days after infection.

The first-generation schizonts of ***E. zuernii*** may be about the same size as those of *E. bovis*. However, they are most common in the *terminal meter of the ileum* and are located in the lamina propria below the crypt–villus junction, often deep near the muscularis mucosae, rather than in the endothelium of the lacteal. Hence, they are not so readily visible grossly as those of *E. bovis*. The second-generation schizonts and gamonts of *E. zuernii* also occur in glands of the cecum and colon, but not the terminal ileum. The merozoites tend to be somewhat longer (up to 15 μm) and schizonts more numerous and of greater diameter (~14 μm) than those of *E. bovis*. The timing of the development of *E. zuernii* infection is similar to that of *E. bovis*. First-generation schizonts of *E. bovis* occasionally reach the mesenteric lymph node, where they may mature, with no significance.

Animals dying of coccidiosis have fecal staining of the hindquarters, and may be somewhat cachectic and anemic. The gross enteric lesions in severe cases are those of *fibrinohemorrhagic typhlocolitis*, which may extend to the rectum; if *E. bovis* is involved, the terminal ileum may be affected (Fig. 1.196) and perhaps a few schizonts will be visible in ileal villi. The contents of the large bowel are usually abnormally fluid, and may vary from brown to black to overtly bloody, possibly with flecks of mucus or fibrin. The mucosa is edematous, with exaggerated longitudinal and perhaps transverse folds, which may be congested. Submucosal edema is also marked. Fibrin strands or a patchy diphtheritic membrane may be present on the mucosa (Fig. 1.197), and fibrin casts can form. In milder cases, lesions are limited to congestion and edema of the mucosa.

In animals dying at the peak of infection, *virtually all cells lining cecal and colonic glands in many areas are infected by small schizonts, gamonts, or developing oocysts*. Cells infected by *E. bovis* tend to dissociate and project into the lumen of the gland. As cells are disrupted and oocysts are released into the lumen of glands, the remaining glandular epithelium becomes extremely attenuated, or the gland collapses (Fig. 1.198A).

Concurrently, the surface epithelium becomes squamous, or the *mucosa is eroded*, and effusion of fibrin, neutrophils, and hemorrhage occurs from dilated, congested superficial vessels. Oocysts released into the lumen of the colon may be seen in the exudate (Fig. 1.198B). At the same time, the mucosa begins to collapse, and the lamina propria is infiltrated by neutrophils, eosinophils, lymphocytes, macrophages, and plasma cells (Fig. 1.198C). Oocysts trapped in denuded glands in the collapsed mucosa may be surrounded by small giant cells.

If destruction is widespread, and the animal survives sufficiently long, the *mucosa may ulcerate* to the level of the muscularis mucosae, and begin to granulate. In areas where the lesion is patchy, glands that have been relatively spared may become lined with hyperplastic

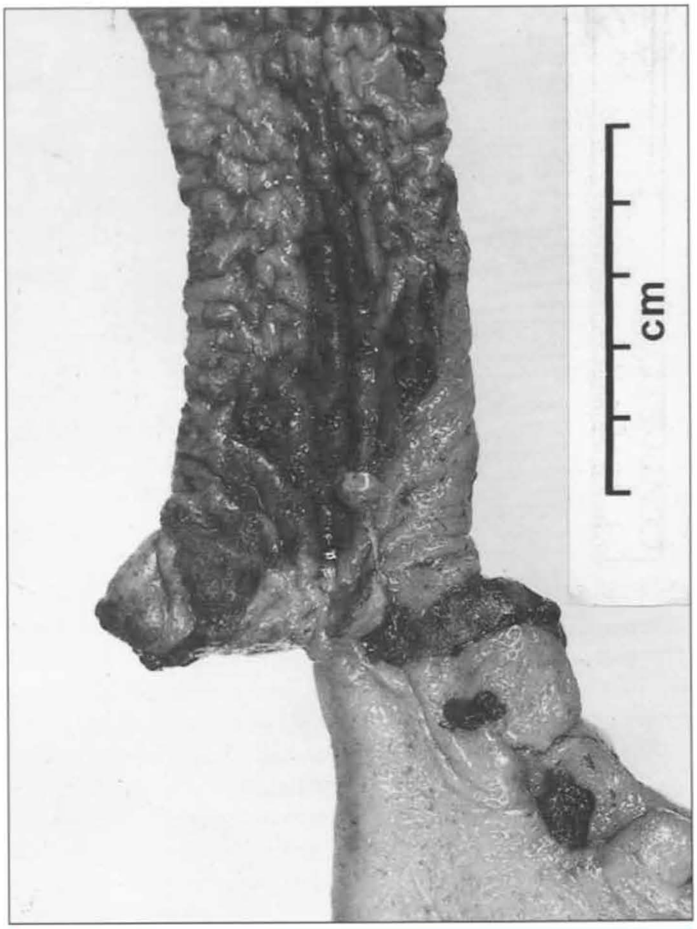

Figure 1.196 Acute enteritis, with mucosal thickening, large and minute ulcerations, and hemorrhages, in **bovine coccidiosis**.

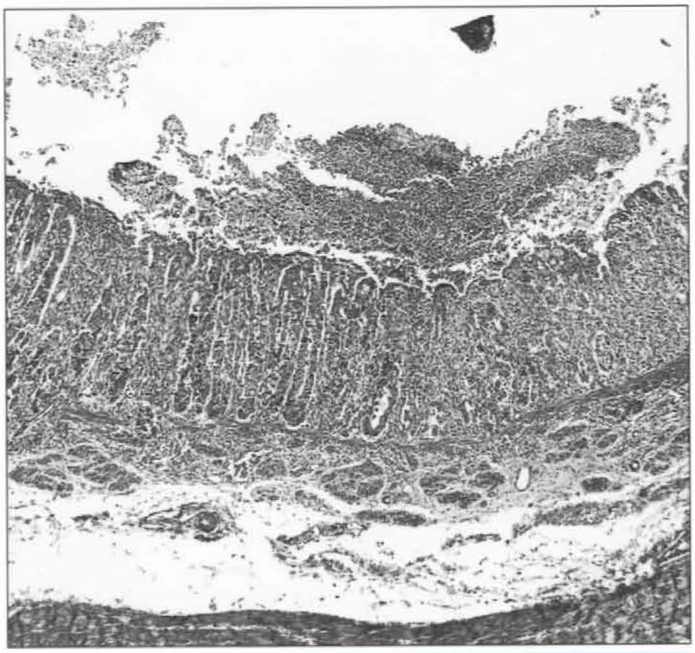

Figure 1.197 Damaged colonic glands and inflammatory exudate, in **bovine coccidiosis**.

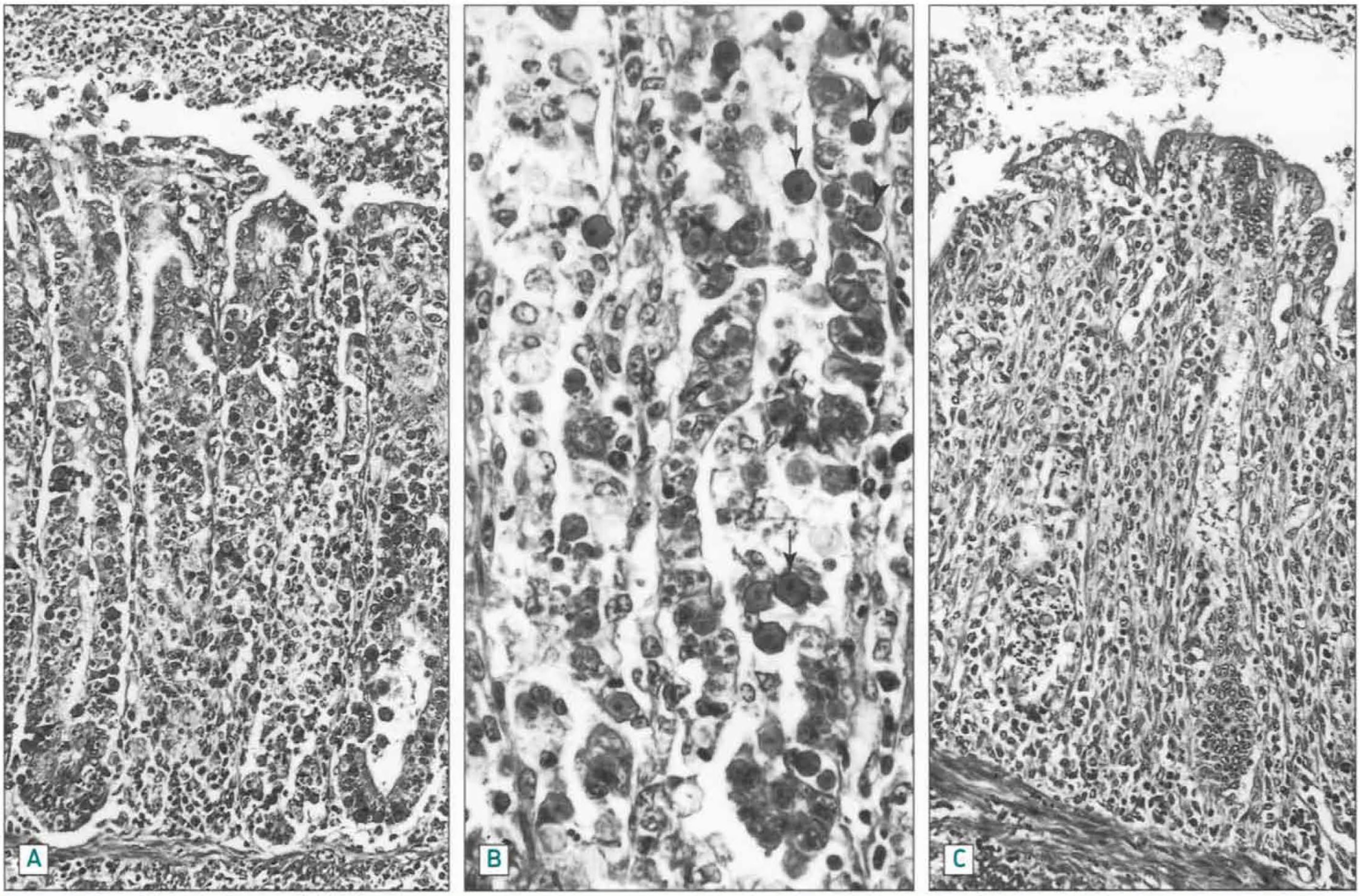

Figure 1.198 Bovine coccidiosis. A. Heavy infection and destruction of colonic glands by gamonts. Exudate covers mucosa. **B.** Destruction of colonic glands by developing gamonts (arrowheads). Oocysts are in the lumen of some glands (arrows). **C.** Destruction of colonic glands. Only a few glands remain in the mucosa.

epithelium, making an attempt to regenerate the mucosa. Flattened epithelial cells spread from these glands across the denuded surface, beneath the diphtheritic exudate. A few crenated oocysts in small giant cells in the stromal remnants of the mucosa may be all the evidence of coccidiosis found in lesions in animals surviving for 7–10 days.

Malabsorption due to mucosal damage in the cecum and colon, and inflammatory effusion and hemorrhage, explain the enteric signs of coccidiosis. The nervous signs in bovine coccidiosis are not associated with recognized lesions in the brain; they have been related to a neurotoxin found in the blood of affected animals.

The gross lesions of coccidiosis in cattle must be differentiated from those in salmonellosis, bovine viral diarrhea, rinderpest, malignant catarrhal fever, and bovine adenoviral infection, all of which may cause typhlocolitis. Coccidiosis can often be simply confirmed at autopsy by finding large numbers of developing stages in mucosal scrapings. Oocysts of *E. bovis* are ovoid, smooth, and ~28 × 21 μm; those of *E. zuernii* are subspherical to ovoid, smooth and ~18 × 15 μm.

While other coccidia are unlikely to be the primary cause of diarrhea or death in cattle, several have distinctive endogenous stages that may be recognized in tissue section. ***Eimeria auburnensis*** has a giant first-generation schizont that may be confused with those of *E. bovis* and *E. zuernii*. However, they are present usually 6–12 meters cranial to the ileocecal valve and form in the epithelium deep in crypts of Lieberkühn, though this may not be apparent due to plane of section, or following their migration into the lamina propria. Second-generation schizonts and gamonts of *E. auburnensis* develop in the lamina propria in the ileum, small schizonts in villi, and gamonts in the deeper lamina propria. Microgametocytes may be several hundred micrometers across. Oocysts are ~38 × 23 μm.

The other bovine coccidium with gamonts apparently developing in the lamina propria is ***E. bukidnonensis***. Oocysts of this species are large, ~48 × 35 μm and thick-walled, with a micropyle, and have been found in the lamina propria. ***E. alabamensis*** develops in vacuoles within the nucleus of epithelial cells in small intestine and, in heavy infections, the large bowel. Both schizonts and gamonts may be found together within the same nucleus. Gamonts of ***E. kosti*** have been described in the epithelium deep in the abomasal glands. None of these organisms is particularly pathogenic.

Eimeria bareillyi is associated with clinical coccidiosis in water buffalo calves. The serosal vessels in the distal half of the small intestine are congested, and the lumen of the lower small bowel contains creamy or yellow fluid content in which some mucus, fibrin, or blood may be present. Focal to coalescent pale raised plaques or polypoid masses may be present on the mucosa, or the surface may appear granular and necrotic, with petechial hemorrhages. The gross changes are caused by hypertrophy of crypts and villi, upon which virtually every cell is infected with developing gamonts or oocysts. *E. bareillyi* will not cross-transmit to domestic cattle, though *E. ellipsoidalis* and *E. zuernii* of bubaline origin will. *E. zuernii* is pathogenic in water buffalo.

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Coccidiosis in sheep and goats

Coccidial infection is universal in sheep and goats, and coccidiosis can be a significant problem in the young of both species. The etiology of coccidiosis in these species is complicated by the morphologic similarity of the coccidia infecting sheep and goats. Assumptions on the potential for cross-infection of coccidia between sheep and goats, and of the species found in each host, have been revised as new taxonomic and biologic information has come to light.

At present, about a dozen species of coccidia are found in each of sheep and goats. Of these, three (*Eimeria pallida, E. caprovina, E. punctata*) may occur in both sheep and goats, though the validity of *E. punctata* as a species is questioned. Eight species pairs of *Eimeria* occur, in which the coccidia look and behave similarly in sheep and goats, but do not cross-infect. Listing the sheep-adapted species of each pair first, these are: *E. ahsata–E. christenseni*; *E. ovinoidalis–E. ninakohlyakimovae*; *E. bakuensis* (=*ovina*)–*E. arloingi*; *E. granulosa–E. jolchijevi*; *E. crandallis–E. hirci*; *E. faurei–E. apsheronica*; *E. parva–E. alijevi*; *E. intricata–E. kocharli*. Two species are unique to sheep, *E. weybridgensis* (formerly *E. arloingi* "B"), and *E. marsica*; in goats, one species, *E. caprina*, is unique. In addition, giant schizonts of an unknown coccidian, termed *Globidium gilruthi*, are seen incidentally as pinpoint white foci in the abomasum of sheep and goats. The taxonomic confusion has been carried over into descriptions of the natural or experimental disease, since many infections were of mixed species, resulted from inocula of poorly defined species of coccidia, or occurred under circumstances where the oocysts associated were not described. However, while the taxonomic picture has changed, the syndromes associated with coccidiosis in sheep and goats have not.

Coccidiosis in these species is a *disease of young animals*. Under conditions of intensive pastoral husbandry or confinement, lambs and kids are exposed to oocysts of many species of coccidia within the first few days of life. Weaned lambs, presumably exposed to only light infections while at range, are also prone to coccidiosis when brought into feedlots. In young suckled animals and those in feedlots, exposed to large numbers of oocysts, signs may occur before oocysts are passed. Suckling lambs, ~5–8 weeks old, reared at pasture at relatively heavy stocking rates, may also develop signs, and occasionally die. Under these conditions, the disease needs to be differentiated from gastrointestinal helminthosis, which may be concurrent.

Outbreaks of coccidiosis in confined lambs and kids are usually acute and characterized by moderate morbidity and low mortality; there is green or yellow watery *diarrhea*, occasionally with blood or mucus. Yarded and grazing animals may also suffer weight loss, or subclinical ill-thrift. Signs are usually associated with lesions in the lower small intestine, caused by *E. ahsata* and *E. bakuensis* in lambs, and their analogues in goats, *E. christenseni* and *E. arloingi*, or with typhlocolitis, caused by *E. ovinoidalis* in sheep, and *E. ninakohlyakimovae* in goats. Some pathogenicity is also ascribed to *E. faurei, E. intricata, E. parva*, and *E. crandallis* in sheep, and presumably to their analogues in goats. Infections may be mixed, and gross and microscopic lesions may reflect this.

E. ovinoidalis in sheep and ***E. ninakohlyakimovae*** in goats presumably have similar endogenous development. In the sheep, giant schizonts up to 300 µm in diameter develop in cells deep in the lamina propria, in the terminal ileum. They release merozoites that enter epithelium in the glands of the cecum and colon, and perhaps distal ileum. Here small second-generation schizonts evolve, and other cells in glands in the same area subsequently become infected by the gametocytes.

These species are considered highly pathogenic and *E. ovinoidalis* is often associated with disease in feedlot lambs. Lesions other than those related to diarrhea, dehydration, and hypoproteinemia are limited to the terminal ileum, and especially the cecum and proximal colon, and are associated with second-generation schizogony and gametogony. Affected areas of gut are edematous and thickened. The most significant microscopic lesions are those in the cecum and colon, which resemble those in cattle due to *E. bovis* and *E. zuernii*. ***E. caprina*** in goats also seems to have pathogenic potential. Like *E. ninakohlyakimovae*, it causes typhlocolitis; the small intestine is not involved.

E. christenseni and *E. arloingi* in goats and their analogues, *E. ahsata* and *E. bakuensis* in sheep, are also associated with serious disease. They seem to have somewhat similar developmental cycles and lesions, though interpretation of the literature is clouded by confusion among these species. Many cases of coccidiosis in lambs attributed to *E. bakuensis* (as *E. arloingi*) may in fact have been due to *E. ahsata*, since the unsporulated oocysts, though of differing sizes, can be confused.

E. christenseni has a developmental cycle that involves giant schizonts up to nearly 300 µm across in the endothelium of the lacteal in villi in the middle small intestine. In heavy infections, every cell in a number of contiguous crypt–villus units may be infected. Though there may be an acute local reaction around ruptured primary schizonts, clinical disease is associated with the subsequent stages of development, diarrhea occurring during the late prepatent and patent periods. Affected intestine may be congested and edematous. Numerous pale white or yellow foci from a few millimeters to up to a centimeter in diameter, often visible from the serosa, are present as slightly raised plaques on the mucosa of the small bowel. These foci are areas of intense infection of cryptal and villus epithelium by gamonts and developing oocysts, and have been dubbed "*oocyst patches.*" There may be some hemorrhage into the intestine but the feces are rarely bloody.

E. arloingi undergoes a development similar to that of *E. christenseni* (Fig. 1.199) and causes similar gross and microscopic lesions in goats, with minor differences. First-generation schizonts are most numerous in the lacteals of villi in the lower jejunum, gamonts are mainly above the host cell nucleus, and the associated grossly visible plaques in the mucosa (Fig. 1.200) may tend to be more distal in the small intestine, and occasionally involve the large bowel. ***E. ahsata*** and ***E. bakuensis*** in sheep are similar.

Nodular polypoid structures, sometimes pedunculate, and about 0.3–1.5 cm in diameter, are encountered in the small intestinal mucosa of sheep and goats, usually as an incidental finding. These masses are comprised of hypertrophic crypt–villus units, in which virtually every epithelial cell is infected by mainly gametocytic stages of coccidia, which, in sheep, are probably *E. bakuensis* and *E. ahsahta.* Adjacent mucosa appears normal and is uninfected. The term "pseudoadenomatous" has been used to describe these polypoid lesions, and the oocyst patches or plaques discussed above in coccidia-infected sheep and goats (Fig. 1.200B). The infected epithelial cells appear somewhat hypertrophic, with eosinophilic cytoplasm and prominent brush borders. Often these coccidia-infected cells do not slough rapidly postmortem, in contrast to their uninfected fellows.

Why masses of infected cells apparently persist in chronically infected animals without clinical disease is unclear. However, the plaques and polyps may be the result of mitogenic stimuli from progamonts, the immature stages in crypt epithelium, which appear to divide by binary fission in synchrony with the infected host cell.

Coccidiosis may also cause ill-thrift and diarrhea in suckling or weanling lambs 5–6 weeks old heavily stocked on pasture. In the UK, ***E. crandallis***, which develops largely in the ileum, and ***E. ovinoidalis*** are mainly associated with this syndrome. ***E. weybridgensis*** (*E. arloingi* "B"), which infects most of the length of the small intestine, may also contribute. The only gross lesion in affected lambs is congestion and thickening of the mucosa of the lower small intestine.

Under some circumstances, probably sudden exposure to large doses of oocysts, ***E. crandallis***, at least, causes *villus atrophy* in infected areas of intestine. Giant first-generation schizonts develop in crypt cells that after infection migrate into the lamina propria. As the infection progresses, villi become stumpy or disappear, and in small bowel

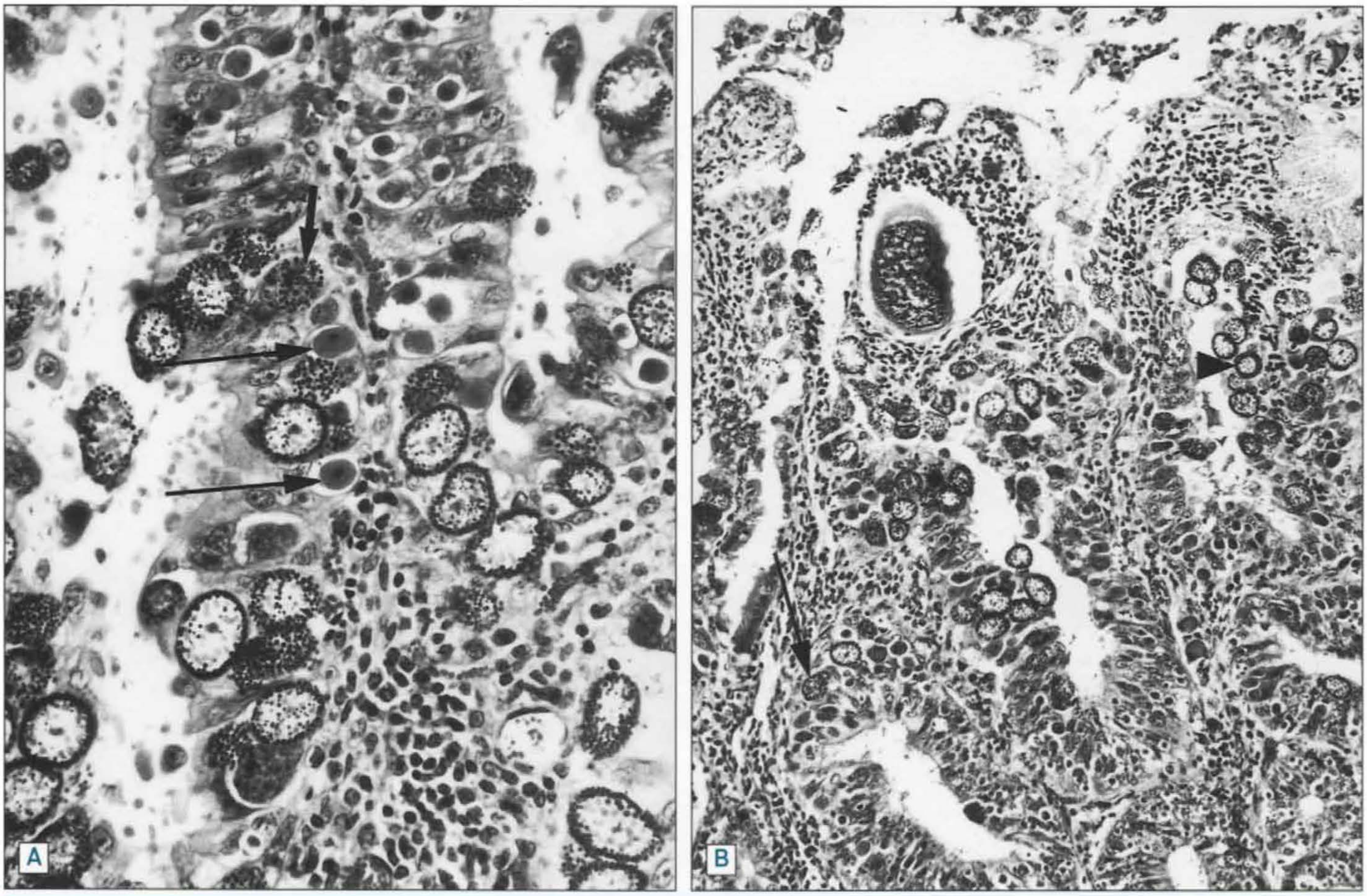

Figure 1.199 Coccidiosis (*Eimeria arloingi*) in a goat. **A.** Undifferentiated gamonts (long arrows), macrogametocytes (short arrow), and microgametocytes infect epithelial cells. **B.** Large schizont in lacteal in the ileum. Gamonts (arrow) and developing oocysts (arrowhead) in epithelium of crypts and villi.

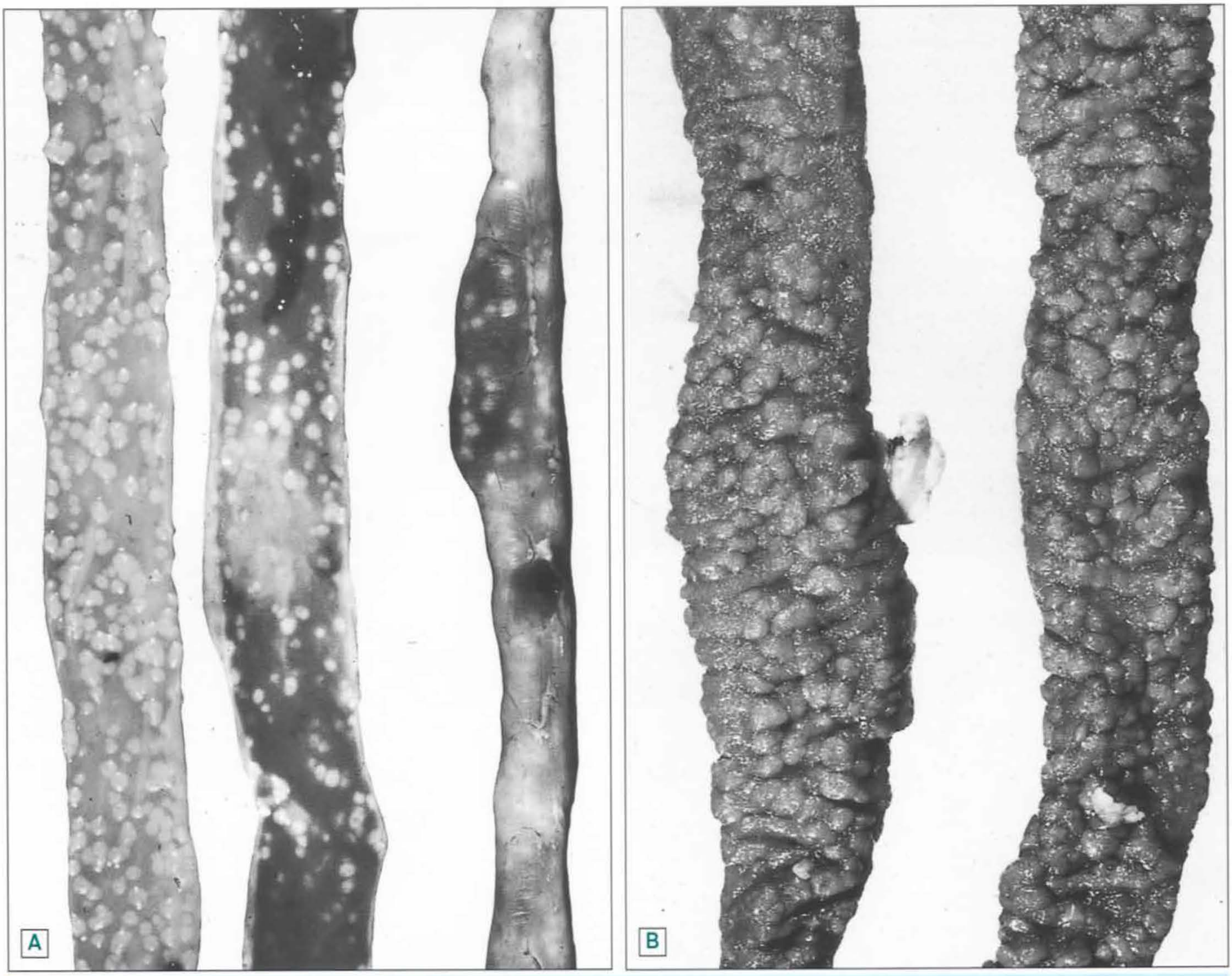

Figure 1.200 Coccidiosis (*Eimeria arloingi*) in a goat. **A.** White nodules on mucosa are visible from serosa (right). Hemorrhage in lumen. (Courtesy of PA Taylor.) **B.** Chronic coccidiosis showing mucosal hypertrophy.

and cecum, crypts are straight, hypertrophic, and contain proliferative epithelium. Asexual or, more commonly, sexual stages of coccidia are present in epithelium on the surface of the mucosa. In hyperplastic crypts, epithelial cells are infected by progamonts, which seem to be dividing in synchrony with host cells. Masses of macrophages may invest and invade the base of infected crypts, and apoptosis of infected and uninfected cells may occur, resulting in attenuation of surviving crypt epithelium. In heavy infections, there may also be thickening of the cecal mucosa by hyperplastic coccidia-infected cells. Occasionally, areas of small intestine and cecum, in which there has been severe damage to crypts, may become eroded.

Such lesions, if widespread, may cause malabsorption or perhaps protein-losing enteropathy. It is unclear whether atrophy of villi is the result of excess loss of epithelium directly due to the effects of coccidial infection, or whether it is mediated by an immune response.

E. apsheronica in the goat has minor pathogenic potential. Giant schizonts develop in the lamina propria of villi throughout the small intestine and in the cecum: second-generation schizonts are in the epithelium on villi in the small intestine, and in the cecum, but not the colon. Gametocytes have the same distribution. Pale foci in the mucosa, where gametocytes are concentrated, and focal areas of erosion and hemorrhage may occur in heavily infected animals.

Large schizonts are often encountered incidentally in submucosal lymphatics, or in the subcortical or medullary sinusoids of mesenteric lymph nodes in sheep and goats. Sometimes they may be visible grossly in these locations as pinpoint white foci. Occasionally, coccidial gametocytes or oocysts may also develop in intestinal lymphoid aggregates and mesenteric lymph nodes, where they may provoke a mild granulomatous reaction. Stages in lymph nodes probably result from establishment of sporozoites or primary merozoites swept from the lacteal into the lymphatic drainage early in infection. Development in such sites is not uncommon, but aberrant and likely dead-end. The species involved appear mainly to be those considered above, with a giant primary schizont developing in the lacteal.

In coccidiosis, *oocysts are usually numerous in feces*, but this is neither constant in, nor necessarily indicative of, disease. Mucosal scrapings or tissue sections of mucosa containing large numbers of asexual and gametogenous coccidial forms, in association with diarrhea, and perhaps some hemorrhage into the intestine, support the diagnosis, in the absence of other syndromes such as gastrointestinal helminthosis.

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Coccidia in horses

The only coccidium of horses reported with any frequency is ***Eimeria leuckarti***, which is found in horses and donkeys the world over. In one survey of foals in Germany, it was found in 100%. It may also occur in older animals. Its reputation for pathogenicity rests largely on the *distinctive large gamonts* found by pathologists in the lamina propria of the small intestine in animals dead of obscure enteric disease. However, implication of *E. leuckarti* in the disease process is rarely, if ever, convincing, and it is encountered incidentally in the intestine of horses dead of other clearly defined conditions. Furthermore, heavy experimental inoculations, producing many gamonts in the gut and heavy oocyst passage, have failed to elicit clinical signs.

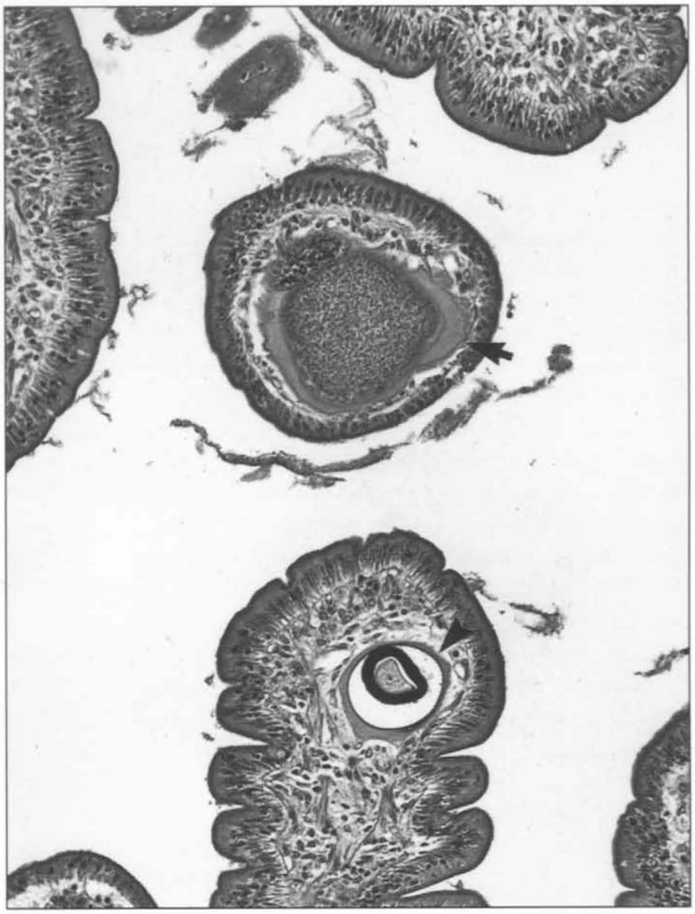
Figure 1.201 Microgametocyte (arrow) and developing oocyst (arrowhead) of ***Eimeria leuckarti*** in lamina propria, in a horse.

The stages present in the lamina propria of villi are giant microgametocytes and macrogametes, developing in markedly hypertrophic host cells, probably of epithelial origin (Fig. 1.201). The microgametocytes are up to ~250 μm in diameter, and when mature they contain swirling masses of microgametes. Immature microgametocytes very much resemble some of the giant schizonts of other species of coccidia, and have frequently been referred to as such; this stimulated the application of the term *Globidium* to the organism. However, the only schizont containing merozoites that has been recognized in horses was very small (12.5 μm in diameter), and in the epithelium of the ileum. The macrogametes have distinctive large eosinophilic or Schiff-positive granules that may be individual or confluent. The host cells are markedly hypertrophic with a fibrillar periphery, and the enlarged nucleus forms a crescent along one side of the parasitophorous vacuole. There is no inflammatory response to the gamonts, and only a mild reaction to degenerate stages in the lamina propria.

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Coccidiosis in swine

At least 8–10 species of *Eimeria* are thought to occur in swine, along with a single species of *Isospora*. The latter, ***Isospora suis***, is the most important; it causes *porcine neonatal coccidiosis*, a disease of piglets from ~5–6 days to ~2–3 weeks of age. This disease is recognized in the USA, Canada, the UK, and western Europe; it also occurs in Australia, and probably wherever swine are reared intensively. The condition is most severe in herds where continuous farrowing and total confinement are practiced, and some laboratories report a prevalence of 10–50% among scouring baby pigs. Rapid sporulation (12 hours) and short prepatent period (5 days) promote rapid build-up of infection in a farrowing house.

Porcine neonatal coccidiosis has high morbidity, and usually low but variable mortality. It causes yellow watery diarrhea, dehydration, loss of condition, and death, or at least a temporary check in growth. Some animals may runt severely. Illness usually begins at ~7–10 days of age. Piglets continue to nurse, but may vomit clotted milk. At autopsy, many piglets have the typical appearance of undifferentiated neonatal diarrhea, with no specific gross findings in the gastrointestinal tract other than fluid yellow content. However, the intestine in some animals with coccidiosis may look turgid, rather than flaccid, and in a minority of animals a fibrinous or fibrinonecrotic exudate is present in the lower portion of the small intestine. Occasionally, casts will form.

I. suis replicates in the epithelium on the *distal third of villi* (Fig. 1.202), mainly in the *jejunum and ileum*, though infected cells may be found in the duodenum and colon in a few animals. Piglets usually become infected within the first day or two of life, perhaps by ingestion of the sow's feces. Merogony occurs in vacuoles in the cytoplasm, usually beneath the nucleus of the host cell. Infection of host cells is maximal 4–5 days after infection, and by 5 days, gametogony is evident. Thick- and thin-walled sporulated oocysts have been observed in feces of infected pigs, but either form may cause disease. The onset of lesions and clinical signs corresponds with this period of heavy infection of cells, which undergo lysis. Villi may become markedly atrophic. The surface epithelium that remains is cuboidal to squamous, and infected epithelial cells may be seen degenerating or exfoliating (Fig. 1.203). Erosions may develop at the tips of villi (Fig. 1.204). In the remnant of the villus, neutrophil infiltration, a moderate increase in round cells and eosinophilic proteinaceous material, perhaps collagen, may be present in the lamina propria. Effusion of neutrophils and fibrin from the eroded tips of villi contributes to the fibrinonecrotic membrane seen in some animals, and ulceration can occur. Gram-positive bacilli are often present in the exudate. In animals surviving for a few days, the cryptal epithelium may be markedly hyperplastic.

The severity of the lesions is a function of the size of the inoculum and the age of the pigs. Heavier inocula, within limits, produce more cellular damage and villus atrophy; fibrinonecrotic enteritis indicates ingestion of a large dose of oocysts. However, severe lesions may not be associated with heavy shedding of oocysts, since relatively few gamonts are able to develop in the reduced population of epithelial cells remaining on villi. The severity of lesions and signs is much greater in piglets a few days old in comparison with those 2 weeks of age. This partly relates to the lower rate of replication of epithelium in the crypts of young piglets, and therefore the development of more severe villus atrophy. The smaller size of young piglets also makes them more susceptible to the effects of malabsorption and diarrhea. Animals previously exposed to *I. suis* have relatively strong resistance to challenge.

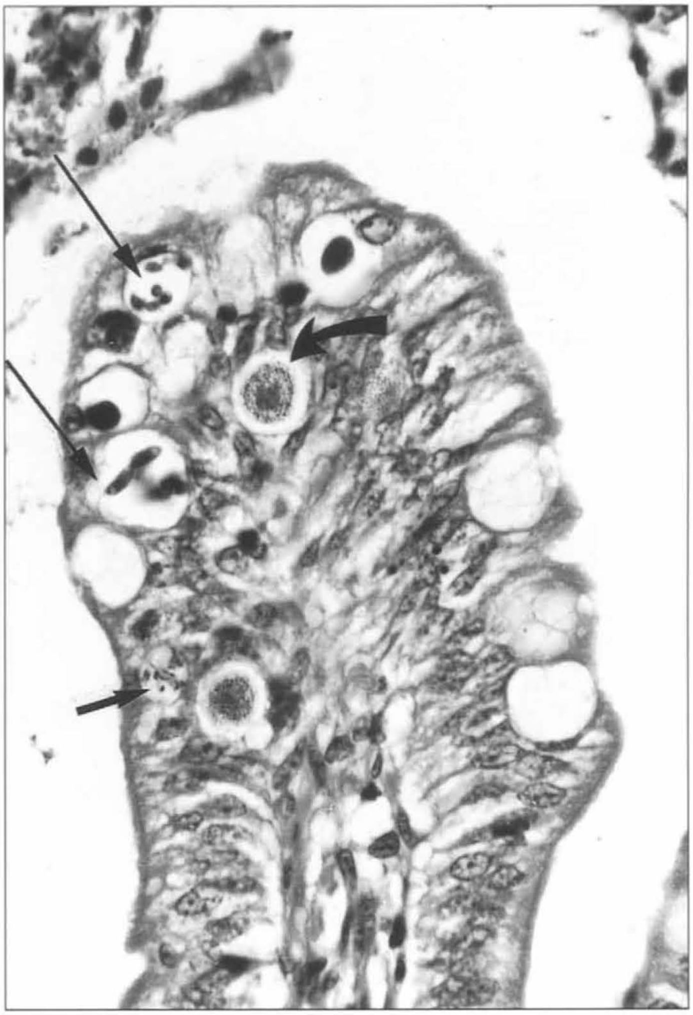

Figure 1.202 Meronts containing merozoites (long arrows), developing oocysts (curved arrow), and a microgametocyte (short arrow) in epithelial cells, in a pig with ***Isospora suis*** infection.

A **diagnosis** of coccidiosis must be considered in scouring neonatal piglets, and is strongly suggested by the presence of *fibrinonecrotic enteritis in the distal small bowel*. Atrophy of villi may be recognized at autopsy using a hand lens or stereomicroscope, or in tissue section. Asexual or sexual stages may be found in smears of mucosal scrapings. The distinctive binucleate type I meronts and pairs of large (12–18 μm in smears, 8–13 μm in sections) type I merozoites may be found in jejunal mucosa in the early phase of diarrheal disease. Multinucleate type II meronts and numerous small type II merozoites are the predominant stage during the clinical phase of disease. In section, these form clusters of 2–16 organisms like bunches of bananas, perhaps with a small residual body, in the parasitophorous vacuole in the enterocyte.

Macro- and microgamonts are present in moderate numbers by day 5 of infection, and a few oocysts may also be seen. Microgametocytes are ~9–16 μm in diameter, and are multinucleate. Oocysts in tissue sections are oval, ~15 × 12 μm, while those

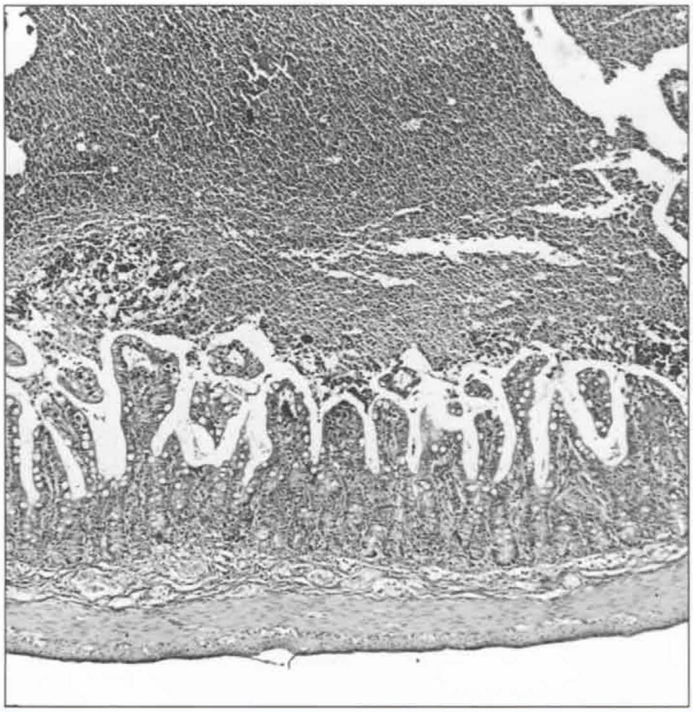

Figure 1.203 Blunting and atrophy of villi in a pig with ***Isospora suis*** infection. Lumen contains massive numbers of exfoliated epithelial cells, inflammatory cells, and coccidial stages, which appear grossly as exudate.

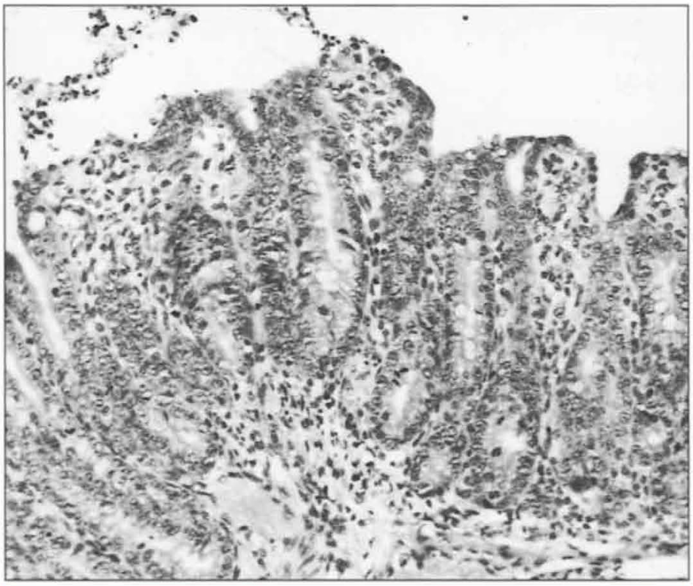

Figure 1.204 ***Isospora suis*** infection. Surface epithelium is severely attenuated, and there is erosion and effusion at tips of villi.

in smears are ~18 × 16 μm. Coccidial stages may be difficult to find in animals that have been ill for several days. Oocysts may not be found in feces, because the infection is not yet patent, the patent period has passed, or the lesions are very severe, reducing the number of oocysts produced.

Coccidiosis in older swine is due to several *Eimeria* species, and is uncommon but speciation can be accomplished. It typically occurs in animals with access to yards or pasture contaminated with oocysts. Weaners and growing pigs are affected. The species considered potentially pathogenic include ***E. scabra, E. debliecki***, and ***E. spinosa***. It is difficult to produce disease in experimentally inoculated pigs; *E. scabra* is probably the most pathogenic. Coccidiosis in swine due to *Eimeria* spp. is usually sporadic, or affects a few pigs in a group. Typically it causes diarrhea of a few days' duration, loss of appetite, and perhaps transient ill-thrift, or, in severe cases, emaciation. Occasionally animals die.

Lesions are usually limited to the lower small intestine, which may be congested or hemorrhagic, though overt blood is rarely found in the feces. Large numbers of schizonts, gamonts, and developing oocysts are in epithelial cells on villi and sometimes in crypts. Atrophy of villi, or erosion and local hemorrhage or inflammatory effusion may be evident, the lamina propria is edematous, and desquamated epithelium and oocysts are in the lumen of the gut. Rarely, heavily infected animals may have lesions in the large intestine. The species involved are diagnosed on the basis of the morphology of oocysts in feces or mucosal scrapings.

Coccidial gamonts and oocysts of a species resembling *E. debliecki* have been found infecting epithelium on the papilliform mucosa of cystic bile ducts in porcine liver. This is probably an aberrant site of development.

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Heteroxenous apicomplexan protists

Cystoisospora, Toxoplasma, Neospora, Hammondia, Sarcocystis, Besnoitia, and ***Frenkelia*** comprise this group of protists. All of these heteroxenous members of the Apicomplexa are known to *use carnivores as definitive hosts*, and have one or more generations of merogony in the tissues of various species of prey. *Frenkelia*, which some would place in the genus *Sarcocystis*, as far as is known utilizes only raptorial birds as definitive hosts, and small rodents as intermediate hosts. It will not be considered further.

Coccidiosis in dogs and cats

Although several species of *Eimeria* have been reported from dogs and cats, their status as genuine parasites of these hosts is in doubt. The significant coccidia of dogs and cats are members of the genus *Cystoisospora*, considered here, and of the genera *Toxoplasma, Sarcocystis, Hammondia, Besnoitia,* and *Neospora*, dealt with subsequently. *Caryospora* spp. may occasionally produce dermal coccidiosis in immunosuppressed dogs.

***Cystoisospora* spp.** are characterized by oocysts that are passed unsporulated in feces, and which, when sporulated, have two sporocysts lacking a Stieda body, each with four sporozoites. Following ingestion of sporulated oocysts of heteroxenous species, transport hosts, usually prey species such as mice and other small rodents, but sometimes other hosts, are infected by large sporozoite-like "hypnozoites" in phagocytic cells in lymph nodes and other tissues. These, when ingested by the predator, resume development in the intestine, and lead to asexual and sexual development in the definitive host. Heteroxenous passage is not obligatory, and sporulated oocysts are also directly infective to the definitive host.

In **dogs**, four species of *Cystoisospora* are recognized. Meronts of ***C. canis*** develop in the subepithelial lamina propria of the villi in the distal small intestine and, to a lesser extent, in large bowel. Gamonts occur beneath and within the epithelium of the ileum and large intestine, and the oocyst is the largest among *Cystoisospora* spp. of dogs, being ~38 × 30 μm. The other three are members of the "*C. ohioensis* complex." ***C. ohioensis*** develops exclusively in epithelial cells, mainly in the distal portions of villi along the length of the small bowel, especially in the ileum, and occasionally in the large bowel. It may be the most pathogenic species in dogs. The oocysts of *C. burrowsi*, *C. ohioensis,* and *C. neorivolta* are similar. Original literature should be consulted for details that will permit differentiation of these species in tissue. Endogenous stages of ***C. burrowsi*** occur in epithelial cells, and in the lamina propria of the tips of villi in the distal two-thirds of the small intestine. ***C. neorivolta*** mainly develops in proprial cells beneath the epithelium in the tips of villi in the distal half of the small intestine, and rarely in the cecum and colon. Occasional stages may be in the epithelium. *C. canis* and *C. ohioensis* are known to be heteroxenous. Meronts of an unknown coccidian, probably a *Cystoisospora* sp., have been found in the intrahepatic bile ducts of a dog, associated with severe suppurative cholangiohepatitis.

In **cats**, two *Cystoisospora* spp. occur. Meronts and gamonts of ***C. felis*** develop in epithelium of villi in the small intestine, and occasionally in epithelium in the large bowel. The oocyst is large, ~43 × 33 μm. ***C. rivolta*** also develops in epithelium on villi and in crypts and glands in the small and large intestine. Oocysts are ovoid, ~25 × 23 μm. Subepithelial schizonts and gamonts of an unknown coccidian, possibly a *Cystoisospora* species, have been associated with fatal enteritis in a cat.

Coccidiosis in the dog and cat is largely a clinical entity, *usually nonfatal.* The lesions of coccidiosis in small animals are poorly defined, and care must be taken not to ascribe disease to these organisms simply on the basis of the presence of endogenous stages in the mucosa of animals dead of enteric disease. Rotavirus and coronavirus might be expected to produce similar signs. However genuine cases of fatal coccidiosis do occur, though few are recorded in the literature. Affected animals are young, and usually from environments such as pet shops, animal shelters, or kennels in which standards of sanitation may not be high. There is a history of diarrhea of several days' duration, and the animal is dehydrated. Other than mild hyperemia of the mucosa and excessively fluid content of the small intestine and colon, gross lesions in the gut may not be evident. Microscopically, there may be moderate atrophy of villi, with attenuation of surface enterocytes, and perhaps effusion of acute inflammatory exudate from the tips of some eroded villi. Asexual and sexual stages of coccidia will be evident in moderate to large numbers in the epithelium or lamina propria of villi. In some cases, the large bowel may be infected, with exfoliation of surface epithelium, and accumulation of necrotic debris in some dilated glands.

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Toxoplasmosis

Toxoplasma gondii uses members of the Felidae as definitive hosts. It is optionally heteroxenous; cats may be infected directly by ingestion of oocysts, but probably most commonly by ingestion of asexual stages in the tissues of prey species. Cats excrete oocysts 3–10 days after ingesting *bradyzoites*, approximately 13 days after ingesting tachyzoites, and 18 days after ingesting oocysts. The latter occurs with very poor efficiency. Intermediate hosts are infected by oocysts shed in the feces of cats, or by a variety of other routes considered below. Five stages of asexual development are recognized in the intestinal epithelium of cats infected with tissue cysts from intermediate hosts. The gametocytes also develop in epithelium on villi, especially in the *ileum*. In heavy infections, exfoliation of infected epithelium from villi is associated with the development of *villus atrophy*, and occasional spontaneous cases of diarrhea in kittens seem to be caused by *Toxoplasma*-induced atrophy of villi and malabsorption.

In intermediate hosts, and in cats, extraintestinal asexual development occurs in a variety of organs and tissues. Rapidly dividing forms (*tachyzoites*) may by endodyogeny proliferate in cells in many sites for an indefinite number of generations, and are the stage associated with acute toxoplasmosis in cats and other species. Eventually, tachyzoites induce the formation of a cyst wall in a host cell, and divide slowly, forming bradyzoites, which reside in quiescent tissue cysts.

T. gondii, with *Neospora caninum*, is unique among protists in its ability to parasitize a wide range of hosts and tissues. *It is one of the most ubiquitous of organisms*; experimentally, essentially all homeothermic animals can be infected, and natural infections occur in birds, nonhuman primates, rodents, insectivores, herbivores, and carnivores, including domestic species and humans. Serologic surveys indicate that infection is widespread in most species of domestic animals; however, except for abortions in sheep and goats, overt disease is sporadic and rare.

Transmission may occur by a number of different routes. The shedding of oocysts in the feces of cats and wild Felidae has been mentioned earlier. Transplacental infection occurs commonly in sheep and goats and sporadically in swine and humans. Carnivorous animals and humans may become infected by ingesting oocysts from cats, or more commonly from cysts containing bradyzoites in tissues of infected animals, implying that the cycle of infection can be maintained by means of facultative homoxenous transmission, without a definitive host.

Systemic toxoplasmosis occurs most often in young animals, especially immunologically immature neonates and in immunocompromised hosts. Low levels of γ-interferon and the associated inability to activate macrophages are predisposing factors for systemic toxoplasmosis. In dogs, canine distemper, ehrlichiosis, and lymphosarcoma are commonly concomitant with toxoplasmosis. The infection in juveniles may be acquired pre- or postnatally. After ingestion, *Toxoplasma* organisms penetrate the intestinal mucosa. In cats, the enterointestinal cycle and systemic infection occur almost simultaneously. In other animals the tachyzoites are the first stage of infection, after invasion of the lamina propria by sporozoites released from the oocyst, or by bradyzoites released from the tissue cyst digested from food in the intestine.

Dissemination of *Toxoplasma* occurs in lymphocytes, macrophages, granulocytes, and as free forms in plasma. From the intestine the organism may follow two routes. It may spread via the lymphocytes to the regional nodes and from there in the lymph to the bloodstream, or it may pass in the portal circulation to the liver and from there to the systemic circulation. Further dissemination occurs to a wide variety of organs. Tachyzoites actively invade or are phagocytosed by host cells and are surrounded in a parasitophorous vacuole formed of host cell membrane. Tachyzoites proliferate, destroying the host cell, and cell-to-cell transmission may occur within infected organs.

Focal necrosis is common, and appears to be directly related to the rapid replication of tachyzoites. The outcome of infection is determined by a number of factors, including the number and strain of *Toxoplasma* in the infecting dose, and the species, age, and immune status of the host. Lesions in visceral organs are usually evident within 1–2 weeks after oral infection. Variable numbers of tachyzoites are usually found in the vicinity of the necrotic areas.

Specific immunity develops within a few days after infection; the cell-mediated arm is most significant in toxoplasmosis, mediated in large part by interleukin-12. This reduces the severity of infection but usually does not terminate it. Immune animals develop a *chronic or dormant form* of *Toxoplasma* infection that is characterized by the formation of *cysts*, containing bradyzoites. These are mainly located in the brain, skeletal muscle, and myocardium. Cysts may form as early as 1–2 weeks after infection and they may persist for months, possibly years. Intracellular encystment protects the bradyzoites from both cellular and humoral immune mechanisms. *Inflammation is usually not associated with cysts.* When the level of resistance drops below a critical level, for example, due to treatment with immunosuppressive drugs, intercurrent disease, or other factors that depress immunity, particularly decreased levels of γ-interferon, *a chronic infection may become reactivated.* The cysts rupture and cause severe local inflammation. Released bradyzoites rarely survive to infect other cells.

The **clinical signs** of toxoplasmosis vary considerably, depending on the organs affected. The most consistent signs reported are fever, lethargy, anorexia, ocular and nasal discharges, and respiratory distress. Neurological signs include incoordination, circling, tremors, opisthotonos, convulsions, and paresis. Paresis is often associated with radiculitis and myositis. In the dog, signs may coexist with those of canine distemper and are not sufficiently distinctive to allow ready differentiation.

Systemic toxoplasmosis has been reported in most species of domestic animals. The hallmarks are *interstitial pneumonia, focal hepatic necrosis, lymphadenitis, myocarditis, and nonsuppurative meningoencephalitis.* Pulmonary lesions are probably most consistently found, followed by central nervous system lesions. The lesions in the various organs are morphologically similar in most species, varying mainly in degree.

Macroscopic lesions in the lung vary from irregular gray foci of necrosis on the pleural surface to hemorrhagic pneumonia with confluent involvement of the ventral portions. Careful examination of the liver usually reveals either areas of focal necrosis or irregular mottling, and edema of the gallbladder. The spleen is enlarged, as are lymph nodes, which are wet and often red. Pleural, pericardial, and peritoneal effusions occur irregularly. Pale areas may be evident in the myocardium and skeletal muscle. Occasionally, the pancreas is the most severely affected organ, in which case an acute hemorrhagic reaction may involve the entire organ. Yellow, small, superficial intestinal ulcers with a hyperemic border have been reported in piglets. Large pale areas of necrosis may be present in the renal cortices, mainly in goats and kittens.

Microscopically, the early pulmonary lesions are characterized by *diffuse interstitial pneumonia*; the alveolar septa are thickened by a predominantly mononuclear inflammatory cell reaction with a few neutrophils and eosinophils. Macrophages and fibrinous exudate fill the alveoli. Foci of necrosis involving the alveolar septa, bronchiolar epithelial cells, and blood vessels are scattered throughout the lobules. These lesions are soon followed by regenerative changes that are characterized by hyperplasia and hypertrophy of alveolar lining cells, mainly type II pneumocytes: so-called *epithelialization of alveoli.* In some areas this may be so marked as to give the affected areas an adenomatous appearance. Tachyzoites are usually evident in alveolar macrophages and may also be found in bronchiolar epithelial cells and the walls of blood vessels.

In the *liver*, irregular foci of coagulative necrosis are scattered at random throughout the lobules. There is usually little evidence of inflammation associated with the necrotic areas. Variable numbers of tachyzoites may be present in hepatocytes and Kupffer cells, usually at the periphery of the lesions, but often at some distance. If the pancreas is involved, there is extensive peripancreatic fat necrosis, with areas of coagulative necrosis in parenchyma. Numerous tachyzoites are usually evident in both ductal and acinar cells.

Lesions in *lymph nodes* are often associated with infection in the corresponding organ. They are characterized by irregular areas of coagulative necrosis, mainly in the cortex. A moderate inflammatory reaction may be evident at the periphery of the necrotic areas. There may be necrosis and depletion of lymphocytes in the follicles. In more chronic cases, the changes are those of nonspecific hyperplasia of lymphoid cells in cortical and paracortical areas, with a large macrophage population in the medullary sinusoids. Tachyzoites may be seen in phagocytic cells in sinusoids. Similar lesions may occur in the spleen. Necrotic areas are mainly located in the red pulp in this organ.

In the *heart and skeletal muscle*, foci of necrosis and mononuclear cell inflammation may be part of toxoplasmosis. There is often some difficulty in distinguishing between tachyzoites and mineralization of mitochondria in myocytes but, at some distance from areas of acute reaction, inert cysts can usually be identified in healthy fibers.

Brain lesions may vary in appearance. In the most fulminating cases cerebral lesions may be relatively inconspicuous. They consist of nonsuppurative meningoencephalitis with multifocal areas of necrosis and often malacia. There is swelling of endothelial cells, necrosis of vessel walls, and vasculitis. There may be marked perivascular edema and hyperplasia of perithelial cells. Tachyzoites and occasionally cysts may be found in vessel walls and in necrotic areas in both gray and white matter at all levels of the brain. If survival is prolonged, residual cerebral lesions consist of *microglial nodules* along with more extensive hyperplasia of perithelial cells and perivascular fibrosis that tends to make the vessels very obvious. At this stage tachyzoites are rare, and cysts 30 μm in diameter with a wall of amorphous acidophilic material ~0.5 μm thick, located in areas away from the lesions, may be the only form seen. Spinal cord lesions resemble those seen in the brain.

Systemic toxoplasmosis is reported from *cats*, but is certainly seen less commonly in this species than in some others. Lesions are similar to those described for other species. Chronic granulomatous toxoplasmosis may involve the intestine in older cats and produce annular areas of thickening. The mucosa overlying the granulomas may be ulcerated.

The finding of tachyzoites and/or cysts in association with areas of coagulative necrosis in one or more organs is highly suggestive of toxoplasmosis. Often the *Toxoplasma* organisms are difficult to distinguish within the necrotic foci, and immunohistochemical techniques have proved very useful in highlighting their presence. Serological tests are of limited value in the diagnosis of disease associated with *T. gondii* infection.

The placental and fetal lesions associated with *Toxoplasma* infection and abortion are described in Vol. 3, Female genital system, and ocular lesions in Vol. 1, Eye and ear.

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Neosporosis

Neospora caninum causes disease in *dogs and ruminants* over much of the world. *Dogs and coyotes are definitive hosts*; cattle, water buffalo, and white-tailed deer can act as intermediate hosts. *Neospora* has been associated with systemic and central nervous system disease in dogs, and with abortion and central nervous system disease in neonatal ruminants. Tachyzoites undergoing endodyogeny, and cysts containing bradyzoites, are found in the tissues of affected animals. Transplacental transmission occurs in ruminants and dogs. Infection may be maintained in lines of cattle in this manner, while some subclinically infected bitches have given birth to successive litters of pups that became affected within the first few months of life. Transmission may also occur by ingestion of infected tissue, as in toxoplasmosis.

Dogs of all ages may be affected, but disease seems most characteristic as *encephalomyelitis, polyradiculoneuritis, and polymyositis* in puppies over ~5 weeks of age, and perhaps involving several animals in a litter. Ascending paralysis, muscle contraction causing hyperextension of the limbs, cervical weakness, and dysphagia may progress to death, or animals will stabilize with caudal paralysis. In adult dogs, there are signs of widespread involvement of the central nervous system, and disseminated disease may be evident, with polymyositis, myocarditis, and dermatitis associated with parasite infection. Though disease may be precipitated or exacerbated by glucocorticoid administration, *Neospora* is regarded as a *primary pathogen*. Circulating levels of γ-interferon may be related to immune resistance.

In acute systemic infections there may be hepatic enlargement with coalescing areas of pallor, related to widespread necrosis of hepatocytes; streaky pallor of muscles due to myonecrosis, mineralization, and nonsuppurative myositis; and pulmonary congestion and edema, due to subacute alveolitis. Tachyzoites are common in affected tissues.

Nonsuppurative encephalomyelitis is associated with the presence of tachyzoites and tissue cysts in neurons and neuropil; the degree of necrosis, gliosis, neovascularization, and demyelination presumably depends to some extent on the duration of the lesion. Retinitis is also reported in association with *Neospora*, as is pyogranulomatous ulcerative dermatitis, occasionally.

In **ruminant abortion**, *Neospora* may be associated with *necrotizing placentitis*, and with *myositis* and *nonsuppurative encephalomyelitis* of the fetus after about 90 days' gestation. While a well-documented etiologic agent of abortion in cattle, the extent of natural *Neospora*-induced abortions in sheep and goats is unknown. Experimentally, sheep and pigs experience placental infection and necrotizing encephalitis in the fetuses. *N. caninum* has also been reported from a case of equine protozoal myelitis.

Tachyzoites are approximately ovoid, ~5–7 μm long, and are found in small groups or large clusters, free in the cytoplasm or in parasitophorous vacuoles in many types of cells throughout the body. Tissue cysts are found in only the brain and spinal cord. They are spherical or slightly elongate, up to ~110 μm in greatest dimension. The cyst wall is ~1–4 μm thick, usually greater than the width of the bradyzoites, which are slender (1.5 × 7 μm), slightly curved, with an obvious nucleus; they stain weakly periodic acid–Schiff-positive.

Neospora must be distinguished from *Toxoplasma* in all species, and from *Sarcocystis* in aborted fetuses. *Neospora* tachyzoites resemble those of *Toxoplasma* in tissue section. Ultrastructurally, *Neospora* tachyzoites have over 11 rhoptries, whereas there are few in *Toxoplasma*. *Toxoplasma* is always found in a membrane-bound vacuole in the cytoplasm; *Neospora* tachyzoites are often not within a parasitophorous vacuole. *Neospora* tissue cysts are relatively uncommonly encountered, especially in acute cases. They are distinguished from *Toxoplasma* by the thicker wall (thinner than 0.5 μm in *Toxoplasma*). Perhaps the most common method of distinction is by immunohistochemistry using specific antibodies, while polymerase chain reaction is also useful. *Sarcocystis* meronts divide by endopolygony in endothelium in domestic animals; they are not in a parasitophorous vacuole; and merozoites lack rhoptries. Sarcocysts in muscle cells are within a parasitophorous vacuole; they have a distinct wall; and they are usually subdivided internally by septa.

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Hammondia infection

Hammondia spp. are obligatorily heteroxenous organisms, with the cat (***H. hammondi***) and dog (***H. heydorni***) as definitive hosts. They have also been known as *Toxoplasma hammondi* and *Isospora bahiensis*, respectively. *Toxoplasma*-like oocysts are shed in the feces of the definitive host and are infectious to intermediate hosts, mammals and birds (*H. hammondi*), and ruminants (*H. heydorni*). Here, bradyzoites develop in cysts in striated muscle, which are infective when ingested by the carnivore. Disease is not associated with infection of intermediate hosts; diarrhea may occur in heavily infected dogs.

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Sarcocystis infection

Sarcocystis is comprised of about 200 obligatorily heteroxenous species. Inconspicuous sexual stages occur in the epithelium at the tips of villi in the small intestine, and oocysts sporulate in the subepithelial lamina propria, producing two sporocysts within a thin oocyst wall. Sporocysts containing four sporozoites are shed in feces. These are infective to intermediate hosts, in which three generations of merogony occur in vascular endothelium, and a final cyst containing first *metrocytes* (mother cells), which produce *merozoites* (bradyzoites), is formed in myocytes and occasionally other cells. Ingestion of tissue cysts containing bradyzoites initiates gametogony in the definitive host. There is apparently no resistance to the development of gamonts, and no disease is associated with them in the definitive host.

Many species of *Sarcocystis* are recognized, based on prey–predator cycles. Sporogony of a given species usually occurs in only one or a few genera of carnivores. The number of species capable of acting as intermediate hosts may be narrow or wide, depending on the species of *Sarcocystis*.

Sarcocystis cysts in ovine and, occasionally, bovine muscle may be grossly visible, causing losses at meat inspection. *Eosinophilic myositis* can result from granulomas due to *Sarcocystis* infection of muscle. However, studies have shown that unaffected muscle often harbors more of these organisms than the inflamed portions. *Sarcocystis* infection in cattle (***S. cruzi***), sheep (***S. tenella***), and swine (***S. miescheriana***), and, experimentally, in goats (***S. capracanis***), may cause acute fatal disease characterized by anemia and widespread hemorrhage, which is associated with clotting disorders. As the disease progresses, cattle may develop inappetence, weight loss, reduced milk yield, hyperexcitability, hair loss, and in some animals, nervous signs. Ill-thrift associated with *Sarcocystis* infection may also occur in other species. Both syndromes are initiated during the endothelial phase of the infection. As well, abortion occurs during this phase in some species. Abortion associated with the acute disease is the result of the systemic illness, and the fetus is usually not infected. However, in cattle, some abortions, seen in otherwise clinically normal animals, are associated with meronts of *Sarcocystis* in the placenta and in vascular endothelium of the fetus, especially in the brain, and with nonsuppurative encephalitis. Encephalitis is occasionally associated with *Sarcocystis* infection in sheep, and in horses ***S. neurona*** is the cause of protozoal myeloencephalitis. Details of these syndromes are discussed in Vol. 1, Muscle and tendon; Vol. 3, Female genital system; and Vol. 1, Nervous system.

A *Sarcocystis*-like agent has also been implicated in mortality of Rottweiler dogs with hepatitis, encephalitis, and dermatitis.

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Besnoitia infection

Besnoitia spp. are also obligatorily heteroxenous, but the definitive host for many species has not been identified. Some stages of merogony and gametogony occur in the intestine of the only definitive host yet known, the cat, where they are not known to be pathogenic. Oocysts are shed unsporulated, and resemble those of *Toxoplasma* and *Hammondia* when sporulated. Meronts in the intermediate host develop in mesenchymal cells, probably fibroblasts, which become massively hypertrophic, forming cysts containing many clusters of merozoites (bradyzoites) in the host cell cytoplasm. Among domestic animals, cysts of *B. besnoiti* may assume some significance in the skin of cattle and goats (see The Skin and Appendages, Volume 1, Chapter 5), and *Besnoitia* cysts have been reported in association with laryngeal polyps in a horse.

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Cryptosporidiosis

Cryptosporidium is a small apicomplexan protist, found on the surface of epithelium in the gastrointestinal, biliary, and respiratory tracts of mammals, birds, reptiles, and fish. Respiratory infection is most significant in birds, and disease in mammals is generally enteric.

Although its taxonomic position in relation to other apicomplexan protists, and its species nomenclature, are in a state of flux, on molecular genetic grounds about 15 morphologically similar species of *Cryptosporidium* are recognized. Five of these occur in domestic animals: (1) ***C. parvum***, small, and initially described in the mouse intestine, but parasitic in cattle, other ruminants, and humans; (2) ***C. andersoni*** in cattle; (3) ***C. suis*** in pigs; (4) ***C. felis*** in cats; and (5) ***C. canis*** in dogs. *C. parvum* is zoonotic, as are *C. suis*, *C. felis*, and *C. canis* to a lesser extent, and disease in humans has been associated with contamination of water sources, food and milk products, as well as close contact with infected animals. However, humans also have a primate-adapted species – ***C. hominis***, and it is probably responsible for the majority of outbreaks of cryptosporidiosis not associated with direct animal contact.

All three stages of the *Cryptosporidium* life cycle – merogony, gametogony, and sporogony – occur extracytoplasmically in a vacuole within the apical region of epithelial cells, protruding above the cell surface (Fig. 1.205). The prepatent period of *C. parvum* in calves is ~7 days and infections usually persist for weeks or, if the animal is immunocompromised, perhaps months. Type I merozoites recycle in a new host cell to produce another meront generation, while type II merozoites differentiate to gamonts. Thin-walled oocysts excyst in the gut of the same host, resulting in autoinfection by the sporozoites released, while thick-walled oocysts are excreted to the external environment and are responsible for transmission to another host. Multiple generations of merogony, and autoinfection by excystment

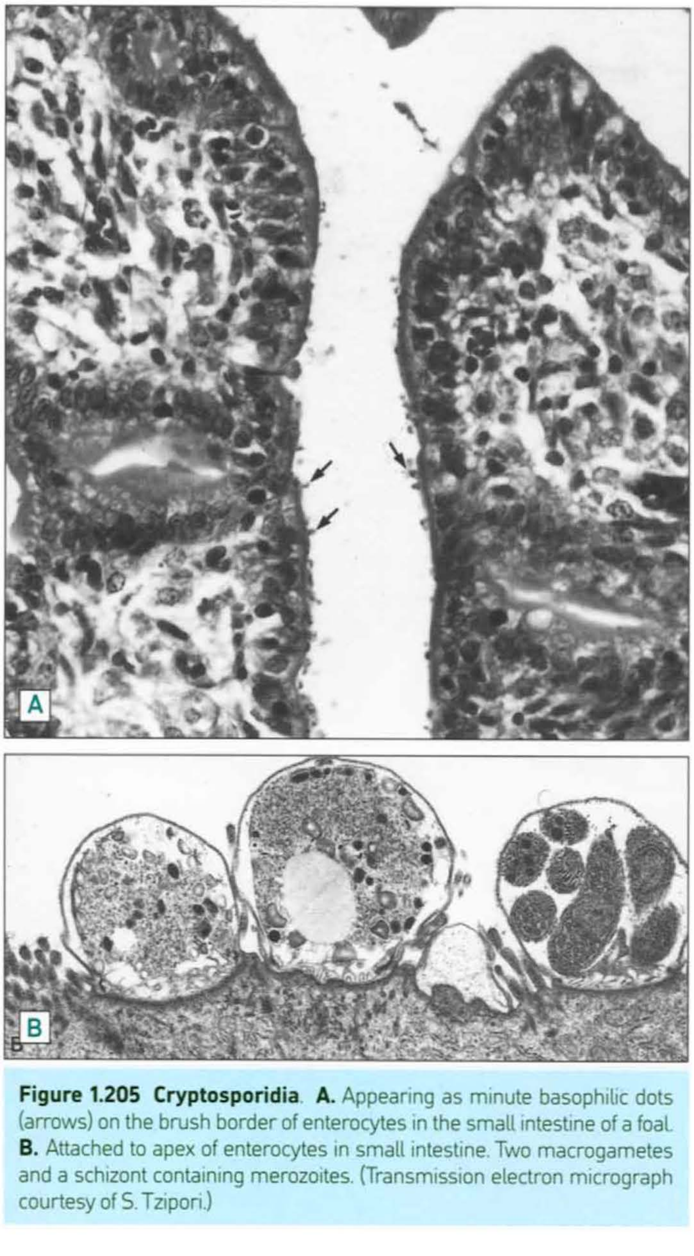

Figure 1.205 Cryptosporidia. A. Appearing as minute basophilic dots (arrows) on the brush border of enterocytes in the small intestine of a foal. **B.** Attached to apex of enterocytes in small intestine. Two macrogametes and a schizont containing merozoites. (Transmission electron micrograph courtesy of S. Tzipori.)

of thin-walled oocysts, result in a large biotic potential and promote heavy colonization of the gut.

The organisms are enclosed within a parasitophorous vacuole formed by apposition of two unit membranes of the host cell, probably caused by inversion of a microvillus by the infecting sporozoite or merozoite. A specialized "feeder" organelle is present at the attachment zone in the base of the vacuole, between the infecting organism and the cytoplasm of the host cell. Developmental stages are small, in most cases ~2–6 μm in diameter. Undifferentiated meronts and gamonts are recognized as small basophilic trophozoites. Mature schizonts contain small falciform merozoites. Macrogamonts are ~5 μm in diameter, and contain small granules. Oocysts in tissue sections are often collapsed into a crescent shape. The various stages may be recognized in wax- or plastic-embedded sections under the light microscope, but are best studied with the electron microscope. Oocysts containing four sporozoites may be demonstrated by fecal flotation, or in fecal smears stained with Giemsa, by a modified Ziehl–Neelsen technique, or with Auramine O or fluorescein-labeled antibody and examined with ultraviolet light.

Cryptosporidia are found in many circumstances, and in some species infection appears to be asymptomatic. Neonates are particularly susceptible to intestinal infections, and this is especially so among ruminants (calves, lambs, kids, red deer calves) infected with *C. parvum*. Diarrhea, anorexia, and depression in calves usually occur between ~1 and 4 weeks of age, and in lambs about 5–14 days old. However, naive calves up to 3 months of age are susceptible to infection and may develop diarrhea.

Cryptosporidial infections are mainly eliminated by cell-mediated immune phenomena, but the humoral arm also contributes. Immunosuppression is contributory to, but not essential for, the development of disease. Heavy infections are reported in Arabian foals with combined immunodeficiency, and cryptosporidiosis has occurred in cats with *Feline leukemia virus* infection, and in dogs with canine distemper. In immunocompromised individuals, organisms may be present at any level of the gastrointestinal tract, from esophagus to colon. Liver, gallbladder, pancreas, and their ducts may also be involved, as may the respiratory tract. Cryptosporidia frequently occur concurrently with enterotoxigenic *Escherichia coli*, rotaviral, or coronaviral infection in neonatal ruminants, but can be primary pathogens.

In all species, *intestinal cryptosporidiosis is associated with villus atrophy of variable severity*, characterized by blunting and some fusion of villi, and by hypertrophy of crypts of Lieberkühn (Fig. 1.206). Surface epithelium is usually cuboidal, rounded, or low columnar, and sometimes exfoliating or forming irregular projections at tips of villi. *Large numbers of cryptosporidia are usually visible in the microvillus border of cells on the villi* (Fig. 1.205A), and not in crypts of Lieberkühn, although occasionally, the reverse is true. Organisms typically are most heavily distributed in the distal half of the small intestine, especially in ileum, though occasionally cryptosporidia may occur in the cecum and colon. Mild proprial infiltrates of neutrophils and mixed mononuclear cells are present, probably attracted by proinflammatory cytokines released from infected epithelium. An increase in intraepithelial T lymphocytes has been documented in the intestine of infected calves.

Diarrhea in cryptosporidiosis is mainly attributable to malabsorption associated with villus atrophy and a population of immature enterocytes; and perhaps to the occupation of a large proportion of the surface area of absorptive cells by the organisms. Release of inflammatory mediators, principally prostaglandins, may stimulate mucosal secretion, and an increase in epithelial cell permeability to macromolecules has been demonstrated in vitro.

Cryptosporidium parvum is most significant in **calves**, as a cause of undifferentiated neonatal diarrhea, in which it must be differentiated particularly from coronaviral and rotaviral infection. Frequently it is concurrent with other agents causing this syndrome; it tends to be most prevalent in animals ~2 weeks old. A similar situation occurs in lambs, though disease does not appear to be as common, or well recognized, in that species. It is a sporadic or minor cause of sometimes fatal diarrhea in other species of ruminants.

Though cryptosporidiosis can be induced experimentally in **piglets**, it is a very rare cause of spontaneous disease in swine. It is only occasionally associated with disease of carnivores, and then often in probably immunocompromised animals. Though infection of **foals** is not uncommon, *Cryptosporidium* has been associated with disease mainly in animals with combined immunodeficiency,

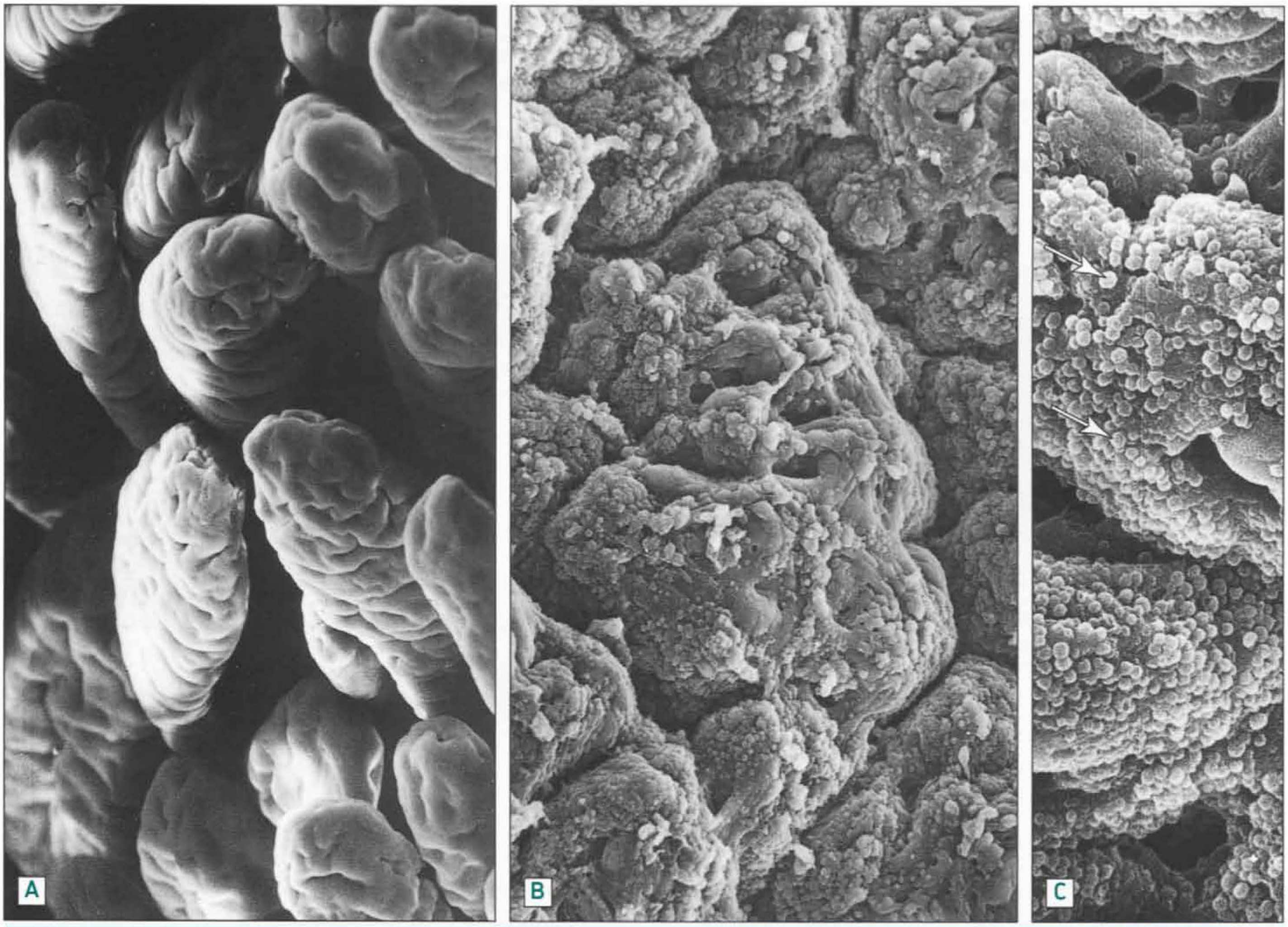

Figure 1.206 Cryptosporidia: scanning electron micrographs. **A.** Normal villi. **B:** Villus atrophy associated with cryptosporidiosis. Cryptosporidia are visible as minute spheres on mucosal surface. **C.** Detail of (**B**) showing cryptosporidia (arrows). (Courtesy of S. Tzipori.)

complicated by adenoviral infection. Disease has not occurred in successful experimental infections in foals, and the role of cryptosporidia in the etiology of neonatal diarrhea in foals is poorly defined, although occasional outbreaks attributable to *C. parvum* may occur.

The diagnosis is based on the presence of large numbers of cryptosporidia in sections of freshly fixed lower small intestine, preferably in association with villus atrophy. Examination of smears of ileal mucosa stained with Giemsa may allow a rapid diagnosis, or permit a diagnosis on tissue from an animal dead for some hours.

C. andersoni, in the *abomasum of weaned calves and older cattle*, is not associated with diarrhea, but plasma pepsinogen levels rise, and weight gains of some growing animals may be adversely affected. There is mucous metaplasia/hyperplasia in the fundic glands, which are dilated, with attenuation of the lining epithelium, on which cryptosporidia are numerous. Infections have been associated with decreased milk production.

Rarely, cryptosporidia are seen in gastric biopsies from **cats**, sometimes associated with mild gastritis. There is some indication that concurrent infection with *Helicobacter felis* will precipitate disease due to cryptosporidia.

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Amebiasis

Entamoeba histolytica is the cause of amebiasis in humans, non-human primates, and, rarely, in other species, including dogs and cattle; cats are susceptible to experimental infection. Infection in dogs is sporadic, probably acquired by exposure to cysts in feces from infected humans. Dogs tend not to pass encysted amebae; hence it has been suggested that they present little public health hazard, and are unlikely to support spread from dog to dog. However, under some circumstances, cysts may be shed, and fecal material containing motile trophozoites has been used to transmit infection orally to other dogs.

Amebae are usually nonpathogenic inhabitants of the lumen of the large bowel, but sometimes they cause colitis. The diet and immune status of the host, and virulence attributes of various strains of the organism, seem to influence pathogenicity. Adhesion to mucus by specific lectins, enzymatic degradation of mucus, and lectin-mediated adherence of amebae to host epithelium, are essential steps leading to tissue damage. Cysteine proteinases produced by *E. histolytica* contribute to epithelial cell damage and to induction of inflammation, both of which are involved in initiation of mucosal lesions. Cytolysis is induced by in-contact amebae.

Amebiasis in dogs is associated with diarrheic or mucoid feces, perhaps with some blood, or with dysentery. *Erosive mucosal colitis or ulcerative colitis* occurs in dogs with amebiasis, and disease seems more common or severe in animals with concomitant *Trichuris* or *Ancylostoma* infection.

Early lesions in human amebiasis seem to be diffuse acute mucosal colitis, with focal erosions or ulcerations. Amebae, though scarce, may be found in mucus on the colonic surface, but are most numerous in the fibrinocellular exudate over erosions or superficial ulcers. Ulcers advance as an area of necrosis and predominantly neutrophilic infiltrate, causing loss of glands, and extending for the full depth of the mucosa. Initial lesions consist of tiny erosions of the surface epithelium that evolve to deeper, more extensive ulcers. Established ulcerative amebic colitis classically has a flask-shaped ulcer, the narrow neck through the mucosa, and the broad base in the submucosa. There amebae, and necrosis, expand laterally, apparently less constrained by the architecture of the tissue. Intestinal perforations and intramural abscesses may form.

Amebae may be present, commonly in small clusters, in necrotic debris or in adjacent viable tissue, frequently not involved in an inflammatory reaction. Amebae in tissue, often surrounded by a clear halo, may be spherical or irregular, with extended pseudopodia, and are ~6–50 μm in diameter. The nucleus has a central dense karyosome and peripheral chromatin clumps. The cytoplasm may appear foamy, can contain remnants of erythrocytes in phagolysosomes, and contains glycogen, which makes the cytoplasm periodic acid–Schiff-positive. The lesions of established amebiasis in the colon of dogs resemble those in humans; the early lesions may as well.

Although dissemination of amebae, with abscessation in other organs, especially liver, lung, and brain, is a relatively common complication in humans, it seems rare in dogs. One such case occurred in an animal with canine distemper.

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Giardia and other flagellates

Giardia spp. are flagellate protists that inhabit the small intestine of a wide range of vertebrates. The taxonomy of the genus is difficult. It appears that four or five morphologically distinct "species" exist, each with a relatively wide host range within amphibians, birds, rodents, and other mammals. ***Giardia duodenalis*** (= *G. lamblia*) infection is common in humans, and it also occurs in a wide array of mammals. Molecular genetic investigations have identified at least seven genotypes within *G. duodenalis*, several of which (assemblages A, B) infect humans. Hoofed animals are infected by assemblages A and E, the former being potentially zoonotic, dogs by assemblage C, and cats by assemblage F. *Giardia* infection has been associated with disease, with various degrees of credibility, in most of these hosts.

Giardia trophozoites are pyriform in outline, ~10–20 μm long by 5–15 μm wide and 2–4 μm thick, and convex on the dorsal surface. The concave ventral surface is modified by the presence of a

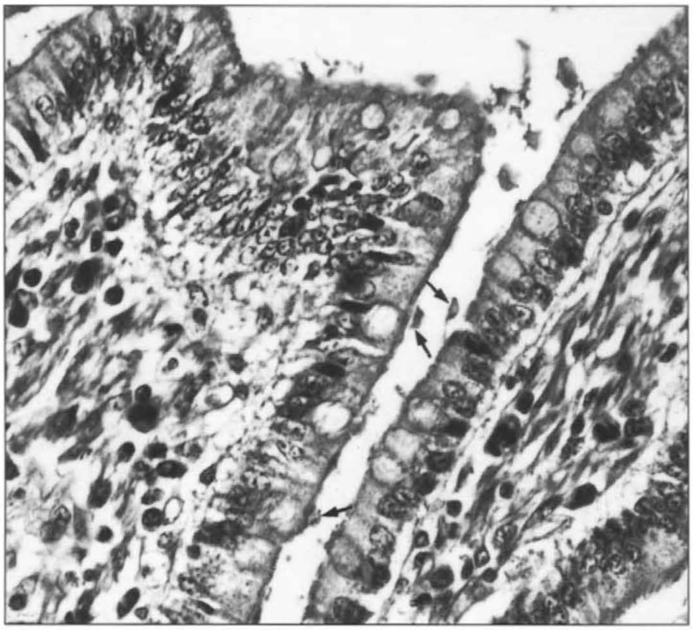

Figure 1.207 ***Giardia lamblia*** (arrows) applied to brush border of enterocytes on villi of a dog.

disk that functions in attachment. Nutrient absorption seems to occur through the dorsal surface. A pair of nuclei, two axonemes, two medial bodies, and four pairs of flagella are present, and the organisms *multiply by binary fission* in the gut lumen. They apply their ventral aspect to the microvillus surface of enterocytes (Fig. 1.207), usually between villi, in folds on the villus surface, or occasionally in crypts of Lieberkühn. *Giardia* has been demonstrated in the mucosa, but this is an unusual and probably aberrant location. Relatively resistant oval cysts are passed in the feces, and transmission is by the fecal–oral route.

The significance of *Giardia* as a pathogen in humans and other species has been controversial, since *asymptomatic infection is the rule*. However, under some circumstances *Giardia* may cause disease. How the host–parasite relationship is modified, and the pathogenesis of the disease, are still unclear.

In *young dogs and cats*, in which giardiosis is most important, though still uncommon, the main sign is intermittent or chronic *diarrhea*, which may persist for several months. The stool is soft, pale, mucoid, and greasy. Though appetite is not usually impaired, there may be a reduced growth rate or weight loss, suggesting malabsorption. A poor hair coat is attributed to deficiency of fat-soluble vitamins. Among animals other than dogs and cats, *Giardia* seems most convincingly to be associated with *enteric signs in neonatal calves*, which may pass soft mucoid feces, and have a reduced growth rate.

Gastrointestinal dysfunction has not been extensively documented in domestic animals. However, in a variety of experimental systems, deficiencies in microvillus-associated digestive enzymes, electrolyte and glucose malabsorption, and microvillus shortening or injury have been documented, though not consistently. Some humans with *Giardia* infection have malabsorption of D-xylose and vitamin B_{12}, with steatorrhea and hypocarotinemia. Excess fecal fat has been found in infected cats, but was not demonstrated in experimentally infected rats. Also, D-xylose malabsorption was not demonstrated in a dog with giardiosis.

Selective deficiencies in some brush border enzymes occur in humans with giardiosis, and in *Giardia*-infected calves. Possibly these are related to the direct effects of *Giardia* on microvilli, which may be deformed adjacent to adherent organisms, or diffusely shortened. *Giardia* may also inhibit the activity of pancreatic lipase, causing fat malabsorption. However, bacterial overgrowth of the small intestine may occur with *Giardia* infection, and associated bile salt deconjugation could explain steatorrhea in giardiosis.

Several mechanisms have been proposed to explain these findings. Although villus atrophy may occur in humans with giardiosis, this mainly occurs in a subgroup of patients with hypogammaglobulinemia. Marked histologic abnormality is not found in many cases of giardiosis in humans, and this also seems to be true for dogs, cats, and calves. In experimental murine giardiosis, infection is associated with hypertrophy of crypts and increased production of cells, combined with an increased rate of movement of enterocytes along villi, with an increased crypt:villus ratio. Deficiencies in brush border form and function may be attributable to incomplete differentiation of enterocytes in this circumstance, or perhaps to damage mediated by mucosal T cells. Intraepithelial lymphocytes are common in infected intestine, and altered epithelial kinetics may be related to cell-mediated immune reactions in the mucosa. Atrophy of villi has been associated with restoration of cell-mediated immune competence in *Giardia*-infected athymic mice, reinforcing the notion that immune phenomena may be involved in the pathogenesis of giardiosis.

Giardiosis is usually *diagnosed clinically* on the basis of typical cysts in fecal flotations, or trophozoites in intestinal aspirates or fecal smears, coupled with remission of clinical signs following therapy, and an inability to identify other potential causes of the signs. A diagnosis is sometimes based on findings in biopsies of small intestine, or at autopsy.

In all species, morphologic changes in the mucosa are not well defined in spontaneous cases of giardiosis. The mucosa may appear normal but there may be equivocal blunting of villi, perhaps associated with a moderate infiltrate of mononuclear cells into the core of the villus, or a heavy population of intraepithelial lymphocytes. *Giardia* should be sought in animals with malabsorption syndromes. They lie between villi, and are usually evident as crescent shapes, applied by their concave surface to the brush border of epithelial cells. In favorable sections through the level of the nuclei, they may appear to have a pair of "eyes." Trophozoites oriented along the plane of section may look as they do in smears, the paired nuclei giving the organism a "face-like" appearance. An abnormal number of bacteria, suggestive of overgrowth, may be present in the mucus and content in the vicinity, in symptomatic animals. A diagnosis of giardiosis should always be reserved for those cases in which no other explanation for the syndrome can be identified. Giardiosis has been associated with colitis in dogs but the association is not clearly causal.

Trichomonads, small flagellate protists that reproduce by binary fission, and are transmitted directly between hosts, are sometimes encountered in the feces of horses, cattle, dogs, and cats with diarrhea. Only in the latter species is there a causal association with disease.

Tritrichomonas foetus is associated with *persistent large-bowel diarrhea*, refractory to treatment, in **cats** under a year of age, and the syndrome has been reproduced experimentally. The microscopic lesions are typical of chronic mucosal colitis, most severe in areas colonized by the organisms. The diagnosis is confirmed in section by detection of pyriform or crescent-shaped organisms, about 5 × 7 µm in size, with a faint nucleus and eosinophilic cytoplasm, applied to the surface epithelium, or in the lumen of colonic glands, usually in large numbers. However, the organisms are present in only a little over half the sections examined from infected cats, and multiple samples may be necessary in order to have a high probability of detecting them. In some cases, trichomonads appear to disrupt the epithelium, attaining the subepithelial lamina propria around crypts, or they are associated with ulceration and foci of necrosis and pyogranulomatous inflammation that are distributed transmurally in affected areas of colon, and in draining lymph nodes.

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Balantidium

Balantidium is a large oval protist ~50–60 µm or more long, and ~25–45 µm wide, with a macronucleus and micronucleus, and covered by many cilia arrayed in rows. ***B. coli*** occurs in the large bowel of swine, humans, and nonhuman primates. It is very common in pigs, and many infected humans live in close contact with swine. It has also been reported from several dogs with access to swine yards, as a complication of trichurosis.

Balantidium is normally present as a *commensal* in the lumen of the cecum and colon, but is capable of *opportunistic invasion of tissues injured by other diseases*. On rare occasions, it may be a primary pathogen. In swine, where the organisms are most commonly encountered by veterinary pathologists, *Balantidium* may be found at the leading edge of the crateriform necrotizing or ulcerative lesions of the large intestine that develop secondary to intestinal adenomatosis (Fig. 1.208), swine dysentery, or perhaps salmonellosis. *Balantidium* is recognized in tissue by large size, ovoid shape, the dense curved or kidney-shaped macronucleus, and the presence of cilia (which may be accentuated by silver stains) in rows on the surface.

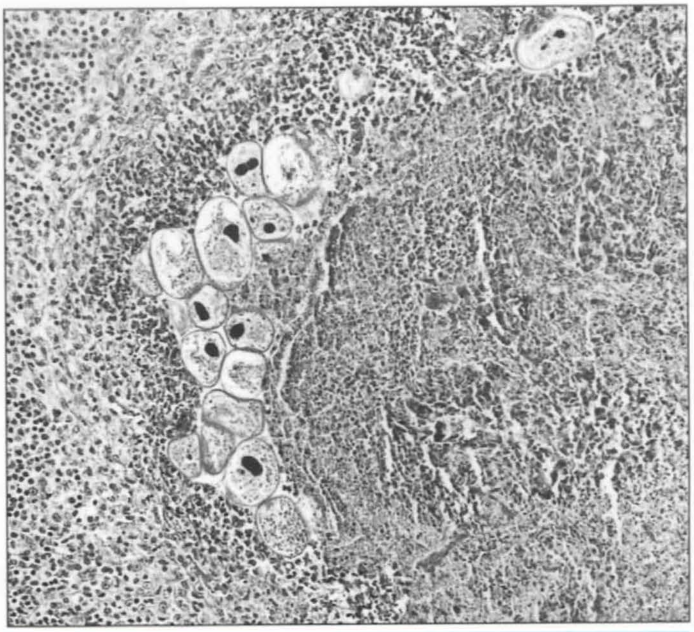

Figure 1.208 ***Balantidium coli*** in ulcerated colon of pig with intestinal adenomatosis and necrotic enteritis.

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PERITONEUM AND RETROPERITONEUM

General considerations

The peritoneum lines the abdominal cavity. Peritoneal diseases are commonly secondary to processes in the organs covered by peritoneum or arising in the retroperitoneum. The peritoneal cavity is incompletely divided into compartments by the mesentery, omentum, and ligaments covered by the peritoneum. Its surface area is greater than that of the skin. *The normal peritoneum is a smooth, shiny membrane that is semipermeable to the movement of water and small solute molecules. There is normally just enough fluid present in the cavity to keep it moist.* Peritoneal fluid is clear and watery, but in neonatal pigs and lambs, fluid may contain strands of mucinous coagulum lying on the intact serosal surfaces of abdominal viscera.

Normal peritoneal fluid is in osmotic equilibrium with plasma, but does not contain fibrinogen or other high-molecular-weight proteins, and generally does not clot, except in pigs. The peritoneal cavity has a dynamic circulation driven by intestinal motility and respiratory excursions, which can disperse fluid (and particles) completely throughout the abdominal and pelvic cavities within minutes to hours.

During organogenesis, the coelomic cavity develops and becomes partitioned into pleural, pericardial, and peritoneal cavities. The primary mesentery that develops supports the alimentary tube in the body cavity. Mesentery attached to the dorsal body wall persists as the mesentery in the adult but the mesentery attached to the ventral body wall degenerates, leaving a single abdominal cavity with persisting structures remaining as the gastrohepatic omentum, the falciform ligament, and in horses the nephrosplenic ligament. Sometimes these ligaments are involved in bowel entrapments. Defects in the peritoneal lining, either congenital or acquired, can also lead to entrapments of viscera.

The peritoneum consists of a single serosal lining layer of **mesothelial cells** on a basement membrane and a submesothelial layer of connective tissue, which is inapparent in some places such as over the liver, and abundant in other places such as the mesenteries. The mesothelial cells vary from squamous to low cuboidal with single small round nucleus. Cells bear surface microvilli, more abundant on the visceral than on the fixed parietal surface. Microvilli may have a role in retaining secreted, hyaluronate-based lubricants. Tight junctions and desmosomes are discontinuous in the surface layer, allowing for diffusion across the membrane. Hydraulic forces generally favor net movement of fluid from the vessels in the retroperitoneum into the peritoneal cavity. Mesothelial cells are fragile and will slough after exposure to such mild insults as exposure to air, physiological saline, intestinal dilation, and transient ischemia. However, cells regenerate quickly and, within a few hours, rounded primitive mesenchymal cells differentiate to mesothelium and cover defects in the surface.

Mesothelial cells have high fibrinolytic activity, which normally protects against formation of adhesions. If they are damaged and inflammation is initiated, fibrin effusion and inflammatory cell emigration from subserosal stroma may be rapid and profuse. Transforming growth factor-β is central in these events.

Both parietal and visceral peritoneum are continuously involved in transport of fluid in both directions. Normal fluid contains only traces of protein and secreted macromolecules, such as chondroitin sulfate, that reduce permeability. The electronegativity of the endothelium relative to mesothelium inhibits transfer of plasma proteins from capillaries into the cavity. The net hydraulic forces favor movement of fluid into the cavity. *Several pathways contribute to the drainage of peritoneal fluid.* Direct transfer through stomata in the membrane to subserosal lymphatics is the main mechanism, dominated by transfer into lymphatics of the ventral diaphragm, and thence via the sternal lymph nodes to the right lymphatic duct, or via mediastinal lymph nodes to the thoracic duct. Respiratory diaphragmatic movements assist transfer through the diaphragm to ventral mediastinal lymphatics. Less is taken up through the omentum and abdominal viscera, and drains via visceral lymphatics and lymph nodes to the thoracic duct. There is a lesser contribution to drainage by the pelvic serosa. Transfer of peritoneal fluid through and between mesothelial cells occurs but is minor.

The basement membrane is absent in minute foci, so-called **peritoneal "milk spots,"** pale semitransparent tiny foci that allow transfer of particulate matter from the peritoneal cavity to lymphatics in the mesentery and omentum. Although generally recognized as omental structures, such foci may also be functional on the parietal membranes of peritoneal, pericardial, and pleural cavities and in the mediastinum. *They are nests of macrophages mixed with lesser numbers of lymphocytes* and responsible, in part, for the removal of effete macromolecules and foreign particles from the peritoneal cavity. The presence of adhesion molecules and active cell proliferation in omental milky spots demonstrates that these are active sites for leukocyte migration and supply of peritoneal leukocytes.

The connective tissue that underlies the mesothelium has an ordinary mix of connective tissue cells and may not easily be distinguished from the retroperitoneum, but the cells appear to have alternative potentials in reaction to injury or surface abrasion and may replace differentiated mesothelial cells of the surface.

The **retroperitoneum** is the areolar and adipose connective tissue immediately beneath the peritoneal lining of the abdominal cavity. The concept of retroperitoneum properly includes the lymphatics and draining lymph nodes in addition to connective tissues, blood vessels, nerves, and the fixed and migratory mononuclear cell populations, most significant along the dorsum from the diaphragm to the anus. Its volume is small, except when adipose tissue accumulates, commonly in the dorsal retroperitoneum, around the kidneys, pelvic cavity, omentum, and mesenteries. With emaciation, fat stores of the retroperitoneum undergo *serous atrophy*, similar to those deposits in the subcutaneous tissues, the thorax, and the marrow cavities (Fig. 1.209).

There appear to be no absolute barriers to movement of fluid or exudates within the retroperitoneal space of dogs; it may travel in the fascia around the dorsal and ventral aspects of the sublumbar muscles, to gain access to the fascial planes of the abdominal wall. Suppurative processes of the retroperitoneum of dogs may drain at the flank, by passing along the fascial planes of the abdominal and the iliopsoas muscles, to emerge in the lumbodorsal triangle, cranioventral to the tuber coxae.

Antemortem and postmortem effusions and discoloration in the peritoneal cavity should be distinguished. Fluid accumulates in the peritoneal cavity after death, and this becomes stained with hemoglobin as soon as erythrocytes in the serosal vessels lyse. Such

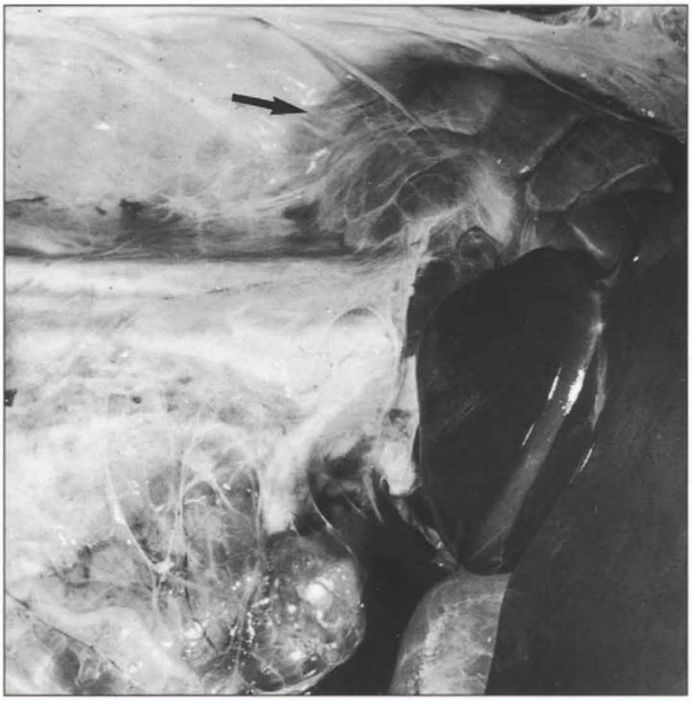

Figure 1.209 Serous atrophy of fat in a calf, resulting from protein-energy malnutrition. Note dark liver (right) and lack of normal perirenal (arrow) and retroperitoneal fat.

fluid does not clot and similar fluid is often also present in other serous cavities. Diffusion of bile pigments through the wall of the gallbladder, the bile ducts, or the duodenum will also stain adjacent viscera.

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Anomalies

Congenital abnormalities affecting the peritoneal membranes are associated most frequently with retention of effete embryonic structures or defective partitioning of the coelomic cavity.

A **persistent vitelline or omphalomesenteric duct** *may form a fibrous ligament between the intestine or Meckel's diverticulum and the umbilicus.* The remnant may be partial, not reaching the umbilicus, and may be attached to the mesentery or to a loop of intestine. These structures may become involved in herniation and obstruction or strangulation of the intestine. Persisting ducts may become cystic.

A **mesodiverticular band** is the result of a **persistent vitelline artery**. The band is a fold of mesentery, occasionally carrying a patent vitelline artery in its free edge, that extends from the cranial mesenteric artery, or from a spot partway down the mesenteric veil, to the antimesenteric side of the intestine (to the site of Meckel's diverticulum). The pocket formed between this fold and the normal mesentery *may entrap intestine*; defects may develop in it which permit strangulation of intestinal loops. Occasionally, double (left and right) mesodiverticular bands are present. Rarely, fibrous cords of mesenteric tissue may be found that do not appear to be part of embryonic remnants of vitelline structures.

The **falciform ligament**, which in its free margin may contain the remnant of the umbilical vein as the *round ligament*, varies in size among species and individuals; it is typically largest in young animals. There is potential for entrapment or strangulation of bowel, if a large persistent falciform ligament is perforated.

Partitioning of the coelom to separate the thoracic cavity from the developing gastrointestinal tract begins with the formation of the *septum transversum* from the ventral body wall, eventually forming the ventral part of the diaphragm. The dorsal part of the diaphragm is provided by downgrowth from the dorsal body wall of the paired *pleuroperitoneal and pleuropericardial folds*.

Congenital pleuroperitoneal diaphragmatic hernias are most commonly observed in dogs, but are occasionally observed in herd animals. *They usually involve a defect in the left dorsal quadrant of the diaphragm, presumably from failure of the left pleuroperitoneal fold to fuse with the septum transversum;* the reason for the particular susceptibility for the left side is unknown. The lesion in some breeds of dogs may have an autosomal recessive mode of inheritance. Some congenital pleuroperitoneal defects in small animals may be more extensive, to the point that virtually the entire diaphragm is missing. The margins of the diaphragmatic defect are smooth, and a large mass of abdominal viscera, often including stomach, spleen, liver, small intestine, and omentum, may pass into the thoracic cavity through the opening. Most small animals born with these defects probably die at, or shortly after, birth. In large animals, the lesions may be clinically silent; this is particularly so when only a small nubbin of liver is present in the hernia. The others produce respiratory difficulties, abdominal pain, and bloating if incarceration of herniated viscera occurs.

Peritoneopericardial diaphragmatic hernias *are triangular and ventral, and presumably result from abnormal development of the septum transversum.* They are more common than pleuroperitoneal hernias in small animals, perhaps because the animals live longer. They are sometimes associated with cardiac anomalies, malformations of the sternum and costochondral junctions, or umbilical hernias. Although various portions of the liver, spleen, omentum, and small intestine may herniate into the pericardial sac, these lesions are often clinically silent.

External hernias are the result of abnormal openings in the abdominal wall, which permit passage of the abdominal contents. They may be congenital or acquired. Congenital defects resulting in hernias are of several kinds. They include abnormally increased size of normal opening such as the inguinal canal; persistence of fetal openings as in umbilical hernias; and defects in closure of the abdominal cavity, as in schistosomus reflexus and diaphragmatic hernias.

Reactions of peritoneum to injury

Even minor injuries to peritoneum cause rapid loss of mesothelial cells and exposure of the subserosal tissue. The denuded area is rapidly covered by a deposit of fibrin that includes neutrophils and macrophages. If the cause is removed and the injury is minor, the surface is soon regenerated by flat cells similar to normal mesothelium. Reconstitution of the surface may be delayed if the subsurface tissues are damaged. It appears that the restoration of the surface may not be provided by proliferation and migration of the adjacent mesothelial cells; the experimental evidence favors an origin from multipotent mesenchymal cells lying in the immediate subserosa close to the basement membrane. Unlike other epithelial-like surfaces, in which wounds heal only from the edges, mesothelial healing occurs diffusely across the denuded surface.

Metaplasia of mesothelium is frequently seen in histological specimens. Simple metaplasia also occurs where alterations to surface contours of organs produce clefts or valleys in which fluid movement may slow, and in cases of hydroperitoneum. In these cases, mesothelial cells become cuboidal, columnar, or, if persistent, may produce nodules of hyperplastic mesothelial cells.

Papillary hyperplasia of mesothelium is frequent in some chronic forms of peritonitis, such as those due to actinomycotic infections in dogs and cats, and in organization of subserosal hemorrhages in spleen and intestine.

Traumatic lesions of the abdomen and peritoneum

Physical trauma to the abdomen is common. Among the *sequelae* are hemorrhage, peritoneal sepsis, uremia due to the escape of urine into the abdomen, and dysfunction of traumatized organs.

Blunt trauma to the abdomen, if sufficiently forceful, will result in contusion of abdominal viscera; avulsion of organs from supporting mesenteries or ligaments, and from their vascular supply; and perhaps laceration of the capsule of solid organs such as the liver, spleen, and kidney. Hollow organs, such as the stomach, gallbladder, and urinary bladder, may rupture, releasing their contents into the abdominal cavity. The momentary increase in intra-abdominal pressure resulting from such trauma may cause acquired hernias, forcing viscera through natural apertures such as the inguinal canal, weak points such as the perineum, or through lacerations in the diaphragm, or in the abdominal wall, with eventration.

In abdominal trauma, contusions and perforation or laceration may be evident on the underside of the skin, and in subcutaneous tissues, often in apposition with internal lesions. Animals with a lacerated liver or spleen may be pale from internal hemorrhage. Contusion or laceration of the kidney results in subcapsular, retroperitoneal, or peritoneal hemorrhage. The spleen is contracted in exsanguinated animals. The source of hemorrhage may be subtle slits or crevasses in the capsule of the involved organ, or the laceration may be obvious. Portions of spleen may implant and persist ectopically elsewhere in the abdomen (splenosis), and may be encountered as an incidental finding. Urine in the abdominal cavity, if not suspected, may be mistaken for ascitic fluid. Lacerations of the urinary bladder may be small and difficult to detect, particularly because the bladder contracts as urine is lost through the laceration. If the pregnant uterus is ruptured, fetuses may be free in the abdomen. They will die and cause peritonitis if the dam survives and they are not removed.

Endogenous forces may also result in herniation. The additional weight of intestinal contents in pregnancy, especially when complicated by an event such as hydrops amnios, may cause ventral hernia. Straining to defecate, or at parturition, may also cause herniation; the former is associated with perineal hernias in dogs; the latter, with acquired diaphragmatic hernias in horses. Tympany of the large bowel in horses, or the forestomachs in ruminants, may also cause defects in the abdominal wall or diaphragm. Antemortem lesions must be differentiated from postmortem tears due to bloating of viscera. *The presence of hemorrhage, fibrin deposits, and acute inflammation in torn muscle, or strangulation of herniated gut, is evidence for an antemortem condition.*

Acquired diaphragmatic hernia is usually a sequel to trauma. The diaphragm is weaker than the abdominal wall. If, at the moment of impact to the abdomen, the glottis is open, a very-high-pressure differential may be generated between the abdominal and thoracic sides of the diaphragm, which is relieved by laceration.

In small animals, the diaphragmatic muscle, rather than the tendinous part, tends to tear, but otherwise the location and orientation of the lesion are functions of events at the moment of impact. Almost any of the abdominal viscera may herniate into the thorax through the defect, but liver and small bowel do so most commonly. The lesion may be clinically silent for a considerable period, sometimes years, but sooner or later results in respiratory difficulty, hydrothorax, ascites, chylothorax, gastric tympany, and intestinal obstruction. At surgery or necropsy, acute diaphragmatic laceration is obvious. If chronic, the margin of laceration is usually thickened by fibroplasia and smooth, but rarely there may be adhesion to the viscera. Differentiation of such lesions from congenital hernias is based on the age, clinical history, and any evidence of scarring or adhesions at the margin of the diaphragmatic defect.

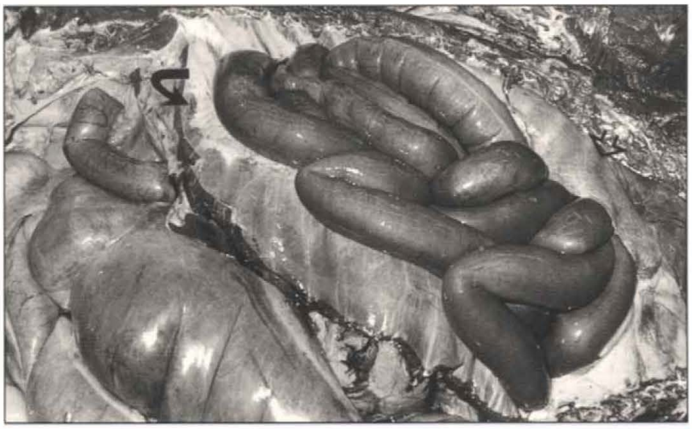

Figure 1.210 Acquired diaphragmatic hernia in a horse. Loops of small intestine have passed through the diaphragmatic laceration into the thoracic cavity, compressing lung (open arrow). Diaphragm (curved arrow).

In horses, acquired lesions usually involve the area where the tendinous portion meets the pars costalis, whereas postmortem laceration of the diaphragm in the horse is most common at the ventral midline, in the xiphoid area (Fig. 1.210). Most horses with acquired diaphragmatic hernias develop abdominal pain, rather than respiratory signs.

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Abnormal contents in the peritoneal cavity

Foreign material is commonly found in the peritoneal cavity. Materials of endogenous origin, such as ingesta or urine, indicate an abnormality of another organ. Rupture of the urinary bladder can result in **uroperitoneum**.

Ingesta is frequently found in the peritoneal cavity of horses and cattle, seldom in swine, sheep, and goats, and rarely in dogs and cats. The site of perforation or rupture in the stomach or intestine is usually easy to find, especially when the animal dies before there is time for peritonitis to develop. Once peritonitis has developed and the intestines and mesenteries become adhered together, the primary site of perforation may be very difficult to detect.

Ingesta may leak from any devitalized segment of small or large bowel, as a consequence of impaction with pressure necrosis; or tympany and rupture; or perforation from sharp objects; or ruptured stomach or perforated abomasal ulcer. Cecal rupture in cattle following overload or impaction usually occurs on the medial side of the cecum. **Rectal perforation** will also cause contamination of the abdomen with feces, and these lesions may be hard to find. Rectal perforation is most commonly secondary to *accidental iatrogenic injury*. Most tears occur about 25–30 cm from the anus, in the peritoneal portion of the rectum, on the dorsal aspect. Tears that perforate the muscularis have the potential for contamination of the peritoneal cavity. The less severe of these have an intact serosa, with formation of a subserosal diverticulum, or a fistula into the potential space of the mesorectum. These may result in perineal abscessation or fistulation, pelvic cellulitis, or rectal diverticula, among other complications. Often these eventually perforate the mesorectum, causing gross fecal contamination of the peritoneum.

Pneumoperitoneum commonly arises from perforation of the abdominal wall due to traumatic incidents, from concurrent pneumothorax and diaphragmatic tears, or from leakage of gas and liquid contents from perforations of the gastrointestinal tract. Less commonly, pneumoperitoneum can develop secondary to rupture of the urinary bladder or from the tubular reproductive tract in females.

Postmortem rupture of a viscus must be differentiated from an antemortem lesion. The rumen, stomach, or large bowel of putrefied, bloated carcasses may rupture. Autolysis of the abomasum may release gastric contents in calves and lambs fed milk replacers. The margins of the defect in the viscus are not hemorrhagic, and the peritoneal surfaces are not inflamed.

Hemoperitoneum *is the presence of blood in the peritoneal cavity*. The amount present at death is not necessarily an indication of the volume of bleeding during life, because the blood may be removed quite rapidly via diaphragmatic lymphatics. Blood in the cavity may remain fluid or be partially clotted.

Hemoperitoneum is seen most commonly in the dog and cat as a result of traumatic injury to the liver, spleen, and kidney, and in dogs is also associated with hemangiosarcomas of liver and spleen. Manual efforts at artificial resuscitation, if vigorous, may rupture the liver. Splenic hemangiosarcomas, which bleed into the abdomen, are common in dogs past middle age. Enlarged, tense livers and spleens because of infiltrating leukemic cells, fat, or amyloid are predisposed to rupture. Fine fissures in the capsule of the liver, and hemorrhage, may occur in infectious canine hepatitis.

In several species, *anticoagulant rodenticides*, such as warfarin, may cause hemorrhage that results in unclotted blood in the abdomen. Calves born of cows that have been fed moldy sweet-clover hay bleed from the umbilical vessels into the peritoneal cavity, as well as elsewhere. Manual ablation of a corpus luteum is a source of hemorrhage in cattle. In cattle and horses, laceration of the uterus or rupture of a uterine artery at parturition can result in fatal hemorrhage. In horses, hemoperitoneum may be due to hemorrhage from granulosa-thecal cell tumor of the ovary, and in all species any friable intra-abdominal or retroperitoneal neoplasm may occasionally rupture and bleed if traumatized.

Hemorrhage on or beneath the peritoneum, without free blood in the cavity, may occur in acute bacterial toxemias and in other conditions that interfere with vascular integrity or hemostasis. *Peritoneal hemorrhage must be differentiated from hemorrhagic peritonitis*, which is an important lesion in some diseases. Subserosal hemorrhage and *hemomelasma ilei* occasionally occur on the intestine of the horse, associated with trauma to vessels by migrating strongyles. Rarely, subserosal hematomas may cause intestinal obstruction. Hemorrhage into an omental or mesenteric cyst can cause sudden abdominal enlargement without free blood in the abdomen.

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Ascites

Ascites, or hydroperitoneum, is the presence of excess fluid, usually a modified transudate, in the peritoneal cavity. Ascitic fluid is generally watery, clear, or straw-colored, and contains a few leukocytes but may contain numerous desquamated mesothelial cells. The serosal lining is normal and glistening but may become slightly opaque as a result of subserosal edema.

Reduced removal of fluid from the peritoneal cavity *is mainly due to obstruction of the primary route of lymphatic drainage through the diaphragm*. The limited area for direct-to-lymphatic absorption and the small size of the stomata on the diaphragmatic and pelvic serosa explain the ease and rapidity with which peritoneal drainage can be blocked.

Obstruction of diaphragmatic lymphatics as a cause of ascites is illustrated in peritoneal carcinomatosis. Carcinomas may implant extensively on the diaphragm in the region of the lymphatic stomata. Neoplastic cells that are carried to the cranial abdomen by the normal peritoneal fluid circulation can obstruct lymphatic drainage, and may also produce fluid.

Ascites may also develop if there is obstruction to sternal lymphatic flow cranial to the diaphragm. This may occur with sclerosing lymphadenitis or space-occupying lesions in the ventral or cranial mediastinum, and is common in lymphosarcoma of adult cattle.

Overproduction of peritoneal lymph *is mainly related to altered hydrostatic pressure gradients in the hepatic and portal circulation*. The exception is rare **chylous ascites**, which results when the cisterna chyli leak fluid, which has a milky appearance due to the presence of chylomicron fat.

The balance of oncotic and hydrostatic pressure depends on the relative retention of large molecules within the circulation. Vascular injury in the portal circulation allows increased permeability to plasma protein and substantially favors transudation. Small amounts of ascitic fluid may be generated under these circumstances in a variety of systemic illnesses, such as the clostridial intoxications, endotoxemia, acute uremic syndromes in ruminants and pigs, and in the exudative diathesis of animals deficient in vitamin E.

Ascites resulting from overproduction of fluid is usually an expression of hepatic lymphedema. Increased prehepatic portal venous pressure alone does not usually lead to ascites; if present, such fluid is low protein, being derived from intestinal and mesenteric interstitial fluid. Acute portal vein obstruction causes intestinal infarction and death, but not ascites. Slowly developing portal hypertension of prehepatic, hepatic, or hepatic venous origin may cause transient ascites, which resolves with the development of collateral portal–postcaval venous shunts within a few weeks. However, portal hypertension of cardiac origin does not result in portocaval shunts, because the elevation in central venous pressure permits no pressure gradient between the portal and postcaval systems.

The sine qua non of hepatic ascites is that there be increased resistance in the intrahepatic or posthepatic circulation, with edema of the liver. The exception is the increase in portal blood flow caused by hepatic arteriovenous fistulas or anastomoses; in this circumstance, hydrostatic pressure at the level of the hepatic sinusoids is elevated by the arterialization of the portal flow.

The usual conditions causing increased hepatic or posthepatic resistance to blood flow are fibrosis of the liver and congestive heart failure. In diffuse fibrosis, intrahepatic resistance may be compounded by the development of arteriovenous anastomoses in the fibrous septa around nodules; these arterialize the hepatic portal circulation, further elevating hydrostatic pressure.

Additional causes of increased hepatic resistance to blood flow include: primary neoplasms of the liver, especially cholangiocellular carcinomas, which tend to be diffuse and infiltrative; secondary tumors, especially lymphosarcomas, which widely infiltrate the liver; extensive infestation with hydatid cysts; and chronic biliary trematodiasis, or other causes of chronic cholangiohepatitis and portal fibrosis. Tumors or abscesses in or compressing the hepatic vein as it leaves the liver, or obstructions in the caudal vena cava cranial to the entry of the hepatic vein, will cause posthepatic obstruction.

When the liver is congested, there is increased flow of hepatic lymph, which has a high concentration of protein. The bulk of hepatic lymph comes from the space of Disse, which is separated from the sinusoidal lumen only by a fenestrated endothelium that is freely permeable to plasma constituents, including large protein molecules. Hence, the formation of hepatic lymph is not regulated by plasma oncotic pressure, but is sensitive to small changes in hydrostatic pressure in the sinusoids. This accounts for the frequency with which ascites is associated with those diseases that cause increased central and hepatic venous pressure, or increased intrahepatic resistance to blood flow.

If the capacity of the hepatic lymphatics is exceeded, then lymph, high in protein, oozes from the hepatic capsule, presumably from the extensive lymphatic plexus there, and it may spill from the efferent lymphatics that pass from the porta hepatis to the cisterna chyli. In hepatic ascites, these efferent lymphatics become very large, numerous, and thick-walled.

The fluid that enters the peritoneal cavity is continually in flux. *Gross ascites will only develop when the normal high capacity to drain fluid from the abdomen is exceeded by the rate of production, and this requires an increase in renal retention of sodium and water.* The initiating factors causing excess of total body salt and water are uncertain but there are identified contributing factors. These include increase of adrenal medullary hormones, increased sympathetic output leading to reduced natriuresis, and activation of the renin–angiotensin system. Renal factors, including reduced excretion of water and increased reabsorption of sodium, are important in perpetuating the condition. The resultant expansion of plasma volume permits the development of edema and ascites in congestive heart failure, and ascites in hepatic disease. It also further increases hydrostatic pressure in the hepatic sinusoids, driving lymph production to the point that ascitic fluid accumulates. Secondarily, reduced effective vascular volume, associated with sequestration of fluid in ascites, expansion of the splanchnic circulation, and other systemic circulatory events, also causes sodium retention, via the renin–angiotensin pathway, with aldosterone secretion, and by parallel mechanisms. In heart failure, activation of the renin–angiotensin system is expected, due to reduced effective vascular volume.

Hypoproteinemia due to reduced hepatic synthesis may occur in severe liver disease. Normally, oncotic forces are subsidiary to hydrostatic forces at the level of the sinusoid in affecting the formation of lymph. However, in diffuse fibrosis, once advanced capillarization of sinusoids occurs, the free permeability of the hepatic vascular system to the flow of large molecules is reduced. In this circumstance, reduced plasma oncotic pressure due to hypoalbuminemia will promote continued hepatic lymph formation and ascites; it will also promote fluid flux to the peritoneum across the splanchnic capillary bed.

Hypoproteinemia of nonhepatic origin is most commonly associated with protein-losing enteropathy, such as Johne's disease, or with protein-losing nephropathy, as in glomerular amyloidosis or other glomerulopathy. Severe hypoproteinemia will reduce plasma oncotic pressure, promoting edema and permitting transudate to accumulate in serous spaces, including, but not exclusively, in the peritoneal cavity (Fig. 1.211).

Effusion of fluid, sometimes massive, in the peritoneal cavity, thorax, and ventral body wall is part of the postmortem picture in sheep and cattle that die of *urethral obstruction*, and the fluid may have a distinct uriniferous odor. The pathogenesis of this fluid accumulation is uncertain. Although in a few cases there is a rupture of the lower

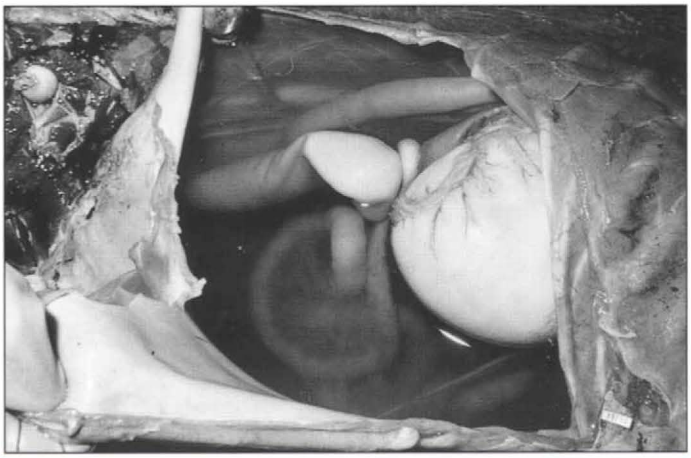

Figure 1.211 Ascites in a dog with profound hypoproteinemia due to enteric protein loss from lymphangiectasia.

urinary tract that might permit leakage, in many cases a rupture cannot be demonstrated. Renal uremia, especially that caused by acute toxic nephrosis, may also be accompanied by massive effusions. It is usually the case with ascites of urinary tract disease that the mesenteries and retroperitoneum also become saturated.

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Abdominal fat necrosis

Necrosis of mesenteric or other abdominal or retroperitoneal fat is a common finding at necropsy. The pathogenesis of all cases is poorly understood but there are a number of known causes.

In **pancreatic necrosis,** enzymatic necrosis of fat is always seen, and peripancreatic necrosis of fat may be the initial morphological change. In acute pancreatic necrosis, discrete foci or confluent masses of white necrotic adipose tissue are surrounded by a zone of intense hyperemia with fibrin deposited on the surface. Such lesions may be limited to the peripancreatic fat or be distributed throughout the abdominal cavity. Free droplets of fat can be found floating in the abdominal fluid. Microscopically, the acute lesion is made up of necrotic fat cells containing acidophilic, opaque, amorphous, or lacy substances, or basophilic fibrillar or granular mineralized material. The necrosis is attributed to release of proteolytic and lipolytic enzymes from necrotic acinar pancreas. Degeneration of neutrophils may contribute to the process, as may lipase released from the necrotic adipocytes.

Widespread or isolated **focal necrosis of abdominal fat** is frequently found in sheep, and sometimes in other animals. The necrotic fat is usually only seen at the chronic stage in the form of white small, dry, firm, chalky, or gritty plaques; a flat white color distinguishes the plaque from surrounding fat. There is usually no grossly apparent inflammatory reaction and, microscopically, the lesions resemble the chronic lesion described above with occasional macrophages containing large lipid vacuoles and necrotic debris encapsulated by connective tissue. The pathogenesis of this form of necrosis is unexplained.

Massive fat necrosis in cattle is reported most frequently in Channel Island breeds and occurs in excessively fat cattle. Both sexes are affected, but probably not clinically before the second year of life. The disease has several significant features. Although it is often seen as an incidental finding, it can be fatal, mainly by intestinal obstruction or by complications such as compression of the ureters. There may also be obstruction of the pelvic canal. The hard lumps of fat may be confused with fetal structures, lymphoid tumors, or other masses on abdominal palpation. Clinical signs are most commonly due to *intestinal obstruction* (Fig. 1.212).

The necrosis may occur in any portion of the omental, mesenteric, and retroperitoneal fat, and it is sometimes seen in intermuscular and subcutaneous fat, as well. In the early stage, there is acute inflammation, and the hard necrotic masses are surrounded by a zone of hyperemia; the overlying peritoneum may be necrotic, perhaps with adhesions to adjacent viscera. The masses may vary from small nodules to large solid masses that are encapsulated by fibrous tissue, and which on cut section are firm, dry, and caseous, or sometimes moist, oily, and deep yellow. The surfaces of such masses may be molded to the contours of adjacent organs.

Because of the unusual bulk of the necrotic tissue, and because necrotic fat is sometimes found in abnormal locations, such as under the serosa of the intestine, the condition has been called *lipomatosis*, but the lesions are not hyperplastic or neoplastic.

On microscopic examination the tissue resembles a mixture of acute and chronic fat necrosis with a few neutrophils, lymphocytes, and plasma cells, and many macrophages and giant cells, with some interstitial fibrosis. Elongate clefts and mineral may be present in macrophages and giant cells.

The pathogenesis of this diffuse *lipogranulomatosis* in cattle is not clear, but there is evidence of a dietary cause, somewhat similar to that of steatitis as it occurs in horses, pigs, and other species. Captive deer grazed on endophyte-infested tall fescue pastures may develop lipogranulomatosis with extensive abdominal fat necrosis; the pathogenesis is unclear.

Massive necrosis of abdominal fat is reported in a cat subsequent to trauma. Nodular necrosis of abdominal fat can be an incidental finding in aged dogs and cats.

Steatitis (*yellow-fat disease*) occurs in many species affecting the abdominal and peritoneal fat, along with other adipose tissue. It is associated with diets high in polyunsaturated fat and low in tocopherols, allowing oxidation of fatty acids. Peroxidation of susceptible lipids and membranes creates free radicals that provoke the

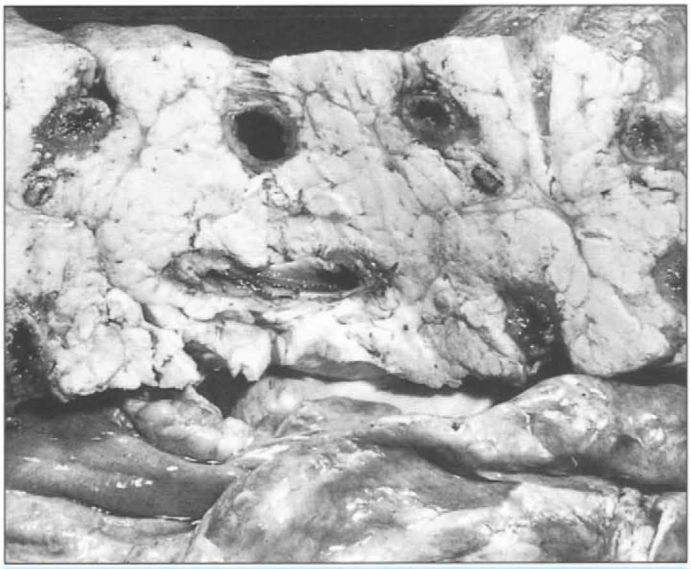

Figure 1.212 Mass of fat enclosing intestinal loops in **abdominal fat necrosis** in a cow.

characteristic inflammatory reaction in response to formation of irritant soaps and the formation of cholesterol deposits and ceroid-lipofuscin (see section on Panniculitis in Vol. 1, Skin and appendages).

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Peritonitis

Damage to the serosa of the peritoneal lining associated with inflammation, or **peritonitis**, is very common in the large domestic animals, and less common in dogs and cats. *Peritonitis may be classified as primary or secondary; as acute or chronic; as local or diffuse; as septic or non-septic; and on the basis of the type of exudate, which may be serofibrinous, fibrinopurulent, purulent, hemorrhagic, or granulomatous.* Differences in distribution, type of exudate, and duration of the lesion can be more or less anticipated from the source and the cause. Most cases of peritonitis in domestic animals are secondary; that is, they arise as complications of other events in the abdomen, but primary serositis in systemic infections is of much importance for agricultural livestock. *The causes of peritonitis are numerous and varied*, so only some of the more common and important of them are considered here.

Chemical peritonitis may be induced by a variety of agents. The intraperitoneal instillation of a number of therapeutic agents causes a mild, and usually inconsequential, peritonitis. However, lavage of the abdomen has been associated with serosal damage and formation of adhesions. *Talc*, formerly used as surgical glove powder, and starch, now used for that purpose, may provoke granulomatous peritonitis. Characteristic starch granules may be recognized in phagocytes within granulomas, forming a Maltese cross in polarized light, and with positive periodic acid–Schiff staining. *Barium sulfate* that accidentally spills into the abdomen during contrast studies is highly irritating, causing a potentially fatal hemorrhagic peritonitis, often complicated by intercurrent sepsis. Survivors will have extensive adhesions, and granulomas in which barium is seen in macrophages and giant cells.

The most devastating forms of chemical peritonitis are endogenous, and are caused by *bile and pancreatic enzymes*. The peritonitis caused by bile is intense and, if complicated by sepsis, may be rapidly fatal. There is very little exudate unless the leakage is minor and infected, and then it may become purulent. **Biliary peritonitis** is readily recognized by the typical yellow-green staining. The **peritonitis of pancreatic necrosis** is also acute and is common in dogs. The reaction about the pancreas, particularly its head, is liquefactive and purulent, and the exudate causes the lesser omentum to adhere to the pancreas and the adjacent liver and other organs. This local peritoneal reaction resolves completely if the animal survives, and only very minor adhesions or slight puckering of the mesentery persist, with fibrosis in the pancreas. The peritoneal exudate is usually scant, but is distinctive because, as well as pus, it contains white droplets of fats and soaps released from the adipose tissue by the pancreatic enzymes. Leakage of chyle from intestinal lymphatics often leads to tortuous lymphangitis and granulomatous peritonitis.

Bacterial peritonitis may occur if bacteria reach the peritoneum by direct implantation. This is usually as a result of perforating lesions (Fig. 1.213), from a contaminated "external" surface, either a hollow viscus in the gastrointestinal or urogenital tract, or from the skin. It is most commonly associated with peritoneal contamination by ingesta. In females, the abdominal cavity is open to the exterior through the reproductive tract, and infection may enter the abdomen through the reproductive tract. Endometritis caused by tuberculosis or brucellosis in cows, and brucellosis in sows, may reach the peritoneum through the fallopian tubes. Cystitis and rupture of the bladder may also result in peritonitis.

Bacterial peritonitis is also common as an extension from inflammation localized in an abdominal viscus or the umbilicus, or as a typical part of the syndrome in a number of specific bacteremic diseases. Acute serofibrinous peritonitis may occur by extension through the wall of a gangrenous intestine or uterus prior to rupture; death from toxemia may intervene before rupture occurs. Secondary peritonitis occasionally results by extension from retroperitoneal infection, or in ruminants from omental bursitis. In those cases in which peritonitis develops by direct extension, there is little difficulty in ascertaining its origin, even when the process becomes diffuse.

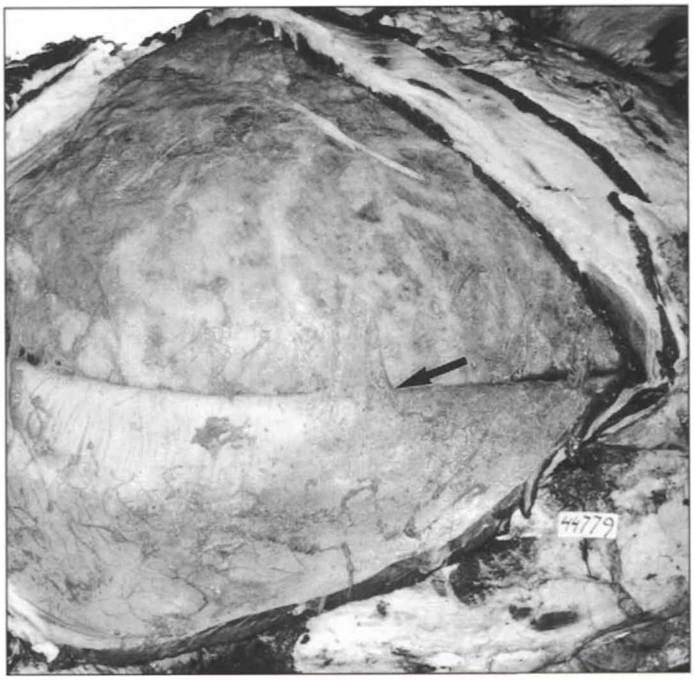

Figure 1.213 Acute diffuse fibrinous peritonitis in a calf as a consequence of perforating abomasal ulcer. A tenacious sheet of fibrin is present between the omentum and the abdominal wall (arrow).

Consequences of peritonitis

The consequences of peritonitis extend from insignificant to catastrophic. Acute generalized peritonitis is a catastrophic sequel in many local diseases of the abdominal cavity, but it may be a relatively insignificant secondary event in generalized infections, though useful to pathologists as part of the suite of lesions used in diagnosis. Obviously, peritonitis does not significantly affect the outcome of an *Escherichia coli* septicemia, or of clostridial infections such as blackleg or braxy.

Within the first few hours of generalized peritonitis, there may be intestinal hypermotility. However, *paralytic ileus*, mediated by autonomic innervation, soon supervenes. The development of ileus has the advantage that exudates are no longer distributed by intestinal movements, but has the disadvantage that fibrinous adhesions develop between loops of intestine, which may, if the animal recovers, produce fibrous adhesions and their sequelae.

The systemic effects of generalized peritonitis are largely related to detrimental effects on cardiovascular function, circulatory homeostasis, and acid–base balance. These are partly the result of sequestration of fluid and plasma proteins in the peritoneal exudate; sequestration of fluid and electrolyte in amotile gut; and loss of intestinal absorptive function. Endotoxin, and exotoxins such as those produced by clostridia, may be absorbed into the circulation, directly through the peritoneum, or via the lymphatic drainage. These cause increased vascular permeability, shock, and other detrimental cardiovascular effects. Death from toxemia may result before obvious peritonitis develops. Sepsis may spread via the lymphatics to the pleura and the sternal or mediastinal nodes, or it will attain the general circulation, causing septicemia.

Not all cases of generalized peritonitis are immediately fatal. Depending on the nature of the exudate, the lesions may resolve completely, be converted to diffuse adhesions, or persist in some localized areas as chronic active or adhesive peritonitis, sometimes with abscessation.

Peritoneal insult, with the initiation of inflammation by mediators released from local mast cells and other mesenchymal elements, results in massive outpouring of fibrinogen and chemotaxis of neutrophils. Tissue thromboplastin polymerizes fibrin. Damage to, or loss of, the peritoneal lining may diminish the fibrinolytic activity of mesothelium and its capacity to produce plasminogen activator; hence, normal fibrinolytic mechanisms are inhibited. Fibrin adheres to the denuded subserosal stroma, which is edematous, congested, or hemorrhagic. The lesion heals by fibroplasia and neovascularization, which extend from the subserosa into the fibrin framework. Mesothelium differentiates from mesenchymal elements in the subserosa. Hence, reconstitution of the mesothelium is not affected by the size of the defect. In uncomplicated situations, peritoneal lesions are debrided by phagocytes; fibroplasia and collagen deposition occur within days, and mesothelial reconstitution is accomplished by 5–8 days after the insult.

If the bridges of fibrin between damaged serosal surfaces persist beyond 3–4 days after the original insult, usually related to failure of fibrinolysis, they become organized by connective tissue, and **fibrous adhesions** form. Contraction of connective tissue as it matures, and in response to tension, may result in close apposition and adhesion of adjacent structures, or stenosis if fibrous bands surround or compress a viscus. The effect is impaired motility, obstruction, or circulatory embarrassment. Fibrous adhesions may remodel and occasionally will separate, but often persist as scars on the serosa.

Formation of adhesions seems to be promoted by ischemia, increasing severity of tissue necrosis, foreign material such as sutures on the serosa, and by sepsis, probably all acting to increase and prolong the inflammatory response. The duration of peritonitis may be a significant question in cases such as iatrogenic rectal perforation, and its assessment requires careful gross and microscopic examination of the serosal surface and the adherent exudates. The proliferation and degree of differentiation of fibroblasts in the exudate and in the edge of any wound, and the presence and degree of differentiation of regenerating mesothelial cells, will give some indication of the age of the lesion. The thickness of granulation tissue over the old serosa may give an indication of the duration of chronic peritonitis.

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Peritonitis in horses

Diffuse peritonitis in horses is usually acute and fatal; this is perhaps associated in part with the small omentum, and a poor capacity to wall off contaminated areas. In most cases, clinical peritonitis is caused by rupture or perforation of the stomach or intestine with spillage of contents. Copious purulent peritonitis may be seen in *Rhodococcus equi* infection of foals, which may also cause purulent mesenteric lymphadenitis. Intestinal infection of foals by *Actinobacillus equuli* causes fibrinous mesenteric lymphangitis and peritonitis. *Clostridium perfringens* in foals, and anthrax in older horses, causes acute hemorrhagic enteritis with peritonitis. Peritoneal lavage may cause severe diffuse chemical peritonitis. Seminoperitoneum, due to laceration of the vagina at breeding, is an unusual cause of acute diffuse nonseptic peritonitis; sperm are present in the cytoplasm of neutrophils. Chronic diffuse peritonitis is virtually never recorded in horses, other than rarely with nocardiosis.

Acute or chronic local peritonitis does occasionally occur from surgical or castration wounds with adhesion of colon to inner inguinal area; from other penetration from the skin; and from streptococcal abscesses in the mesentery. It may be secondary to local verminous lesions such as from suppurative gastritis in habronemiasis, or from perforating *Gasterophilus* or *Anoplocephala*. Migrating *Strongylus equinus* or *S. edentatus* larvae cause retroperitoneal lesions in the flank, perirenal fat, and diaphragm; perihepatitis with fibrous tags on the liver capsule; and chronic diffuse thickening and inflammation in the mesentery, omentum, and hepatorenal ligament, with occasional caseous nodules. Large strongyles returning to the gut may cause focal peritoneal lesions on the ileum, cecum, and colon, and migrations of large strongyles in the wall of the small intestine are associated with brown-black subserosal plaques known as **hemomelasma ilei**.

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Peritonitis in cattle

Acute diffuse fibrinopurulent peritonitis is common in cattle, and is usually the result of perforation of a viscus, especially the reticulum or uterus. Both may also result in local acute, and then chronic, peritonitis with adhesions. **Traumatic reticuloperitonitis** ("hardware disease") may be part of, or evolve to include, septic reticulopericarditis if the offending foreign body advances far enough. Disordered motility of the forestomachs, manifest clinically as "vagus indigestion," may follow scarring that interferes with esophageal function.

Where tuberculosis and brucellosis remain endemic, spread of infection from the uterine tube will produce granulomatous disease in the ovarian bursa and on adjacent serosa; the lesions of these two diseases are not readily distinguished.

Cattle seem to have a relatively high capacity to localize and wall off septic foci within the peritoneal cavity. Abscesses may develop from such foci. Sometimes these may become quite large; they usually contain creamy pus, from which mixed bacterial flora is most commonly isolated. Perforation of the abomasum or intestine is more likely to give diffuse fibrinous or fibrinohemorrhagic peritonitis, but occasionally will cause local chronic peritonitis and abscessation.

Extension of infection from the umbilicus of the neonate produces fibrinopurulent peritonitis, which is not localized, but is most severe along the ventral abdominal wall, or adjacent to associated liver abscesses, or along the urachus to the bladder. Fibrinous peritonitis may be an expression of *polyserositis* in neonatal calves with septicemic colibacillosis and neonatal streptococcal infection. Serofibrinous peritonitis, sometimes with very copious effusion (and with similar lesions on other serous membranes) is typical of sporadic bovine encephalomyelitis. Diffuse fibrinohemorrhagic peritonitis with prominent brick-red discoloration occurs in most cases of clostridial hemoglobinuria, and in some cases of blackleg and septicemic pasteurellosis; a more localized peritonitis of this type occurs in clostridial enteritis of calves caused by *C. perfringens* type B and type C and in braxy.

Tuberculosis causes white nodular granulomas up to several centimeters in diameter; actinobacillosis, although rather rare, produces the usual heavily scarified granulomas, especially about the peritoneum of the forestomachs.

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Peritonitis in sheep and goats

Peritonitis of specific cause is uncommon in **sheep**, except for the very local reaction that accompanies penetration of the intestine by the larvae of *Oesophagostomum columbianum,* and with sporadic cases of ovine pseudotuberculosis wherein caseous lesions in internal organs rupture. The uterus is probably the most common site in adults from which infection spreads to the peritoneum, usually subsequent to postpartum septic metritis. The peritonitis is fibrinopurulent and bloody. Serofibrinous peritonitis is a potential feature of any disease caused by *Mycoplasma,* and outbreaks of peritonitis in lamb flocks are frequently ascribed to coliform infection.

Mycoplasma mycoides may cause acute fibrinous peritonitis in **goats**, although acute death from septicemia, or arthritis and mastitis are more common. Paratuberculosis (Johne's disease) frequently produces nodular granulomatous lymphangitis in the mesentery and sometimes caseous or mineralized lymphadenitis.

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Peritonitis in swine

A few filmy strands of mucin with appearances of fibrin frequently overlie the intestine and the borders of the mesentery in many acute infectious diseases of swine, and in conditions that result in vascular damage, such as edema disease and vitamin E/selenium-responsive conditions; this does not qualify as peritonitis. Peritonitis is recognized as part of the pathological picture in several defined infectious syndromes in pigs, but changes in management, and emergence of porcine reproductive and respiratory disease and porcine postweaning multisystemic wasting syndrome as important entities have altered the patterns of expression of some infections.

Diffuse fibrinopurulent peritonitis is common in pigs (Fig. 1.214); the intestines may be so matted that they cannot be dissected. *Arcanobacterium pyogenes, Escherichia coli,* or a miscellany of organisms are frequently present in these, and in some the inflammatory process can be traced to castration wounds. In such cases, the peritonitis may be localized to the inguinal and pelvic regions; adhesions and death from intestinal obstruction may occur. Occasionally, *A. pyogenes* produces discrete encapsulated abscesses as profuse implants on both visceral and parietal peritoneum.

Serofibrinous peritonitis, with fibrinous arthritis and exudation on other serous surfaces, is indicative of Glasser's disease, caused by *Haemophilus parasuis. Mycoplasma hyorhinis* and possibly other mycoplasmas frequently complicate respiratory mycoplasmosis and produce serofibrinous peritonitis that becomes fibrous, with adhesions to a thickened serous membrane; this disease is to be distinguished from Glasser's disease, and from septicemias due to *Streptococcus suis* type 2. Small, firm, lemon-yellow nodules and flattened disks of inspissated fibrin are often found free in the peritoneal cavity in chronic *Mycoplasma* infections. In intestinal anthrax in swine, there is acute gelatinous hemorrhagic peritonitis typically localized to the mesentery between the intestine and the mesenteric node, the

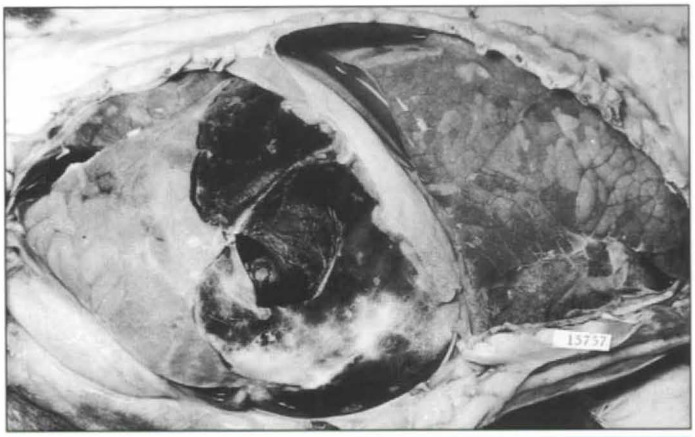

Figure 1.214 Fibrinous polyserositis in a pig with *Escherichia coli* septicemia.

distribution of the lesion being due to the lymphatic spread of the infection from the intestine. Tuberculous peritonitis in swine is localized and characterized by adhesions to the spleen. Rectal strictures in swine cause marked dilation of the colon and cecum. The serosa is frequently thickened, white, and covered with fibrin tags, similar to that of infectious serositis. Peritonitis is also common in pigs with gastric ulcers that perforate, but many pigs will die from catastrophic gastric hemorrhage prior to ulcers perforating the peritoneum.

Stephanurus dentatus larvae cause subserosal focal hepatitis and a mild reaction with edema in the perirenal fat and retroperitoneal tissue, and sometimes in the mesentery and local lymph nodes, as they move to the kidney.

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Peritonitis in dogs

Fibrinohemorrhagic peritonitis, usually slight and easily overlooked, is common in infectious canine hepatitis and toxoplasmosis. The peritoneum, especially that covering the intestine, is gray and granular like ground glass and there are a few red strands of fibrin, most of them about the liver. There is edema of the intestinal subserosa and frequent petechiae or larger hemorrhages. In parvoviral infection and toxoplasmosis, similar lesions may be present on the serosa of affected segments of intestine.

Suppurative peritonitis is uncommon in dogs; it has been observed in puppies as an extension from umbilical and hepatic abscesses caused by streptococci. Septic peritonitis involving a variety of agents, often including *E. coli* and anaerobes, may follow surgical contamination of the abdomen; a penetrating wound or perforation of the gut; rupture of the urinary bladder; or rupture of pancreatic, hepatic, or prostatic abscesses. Putrid peritonitis occurs when the uterus ruptures, either as a result of pyometra or septic metritis with fetal putrefaction.

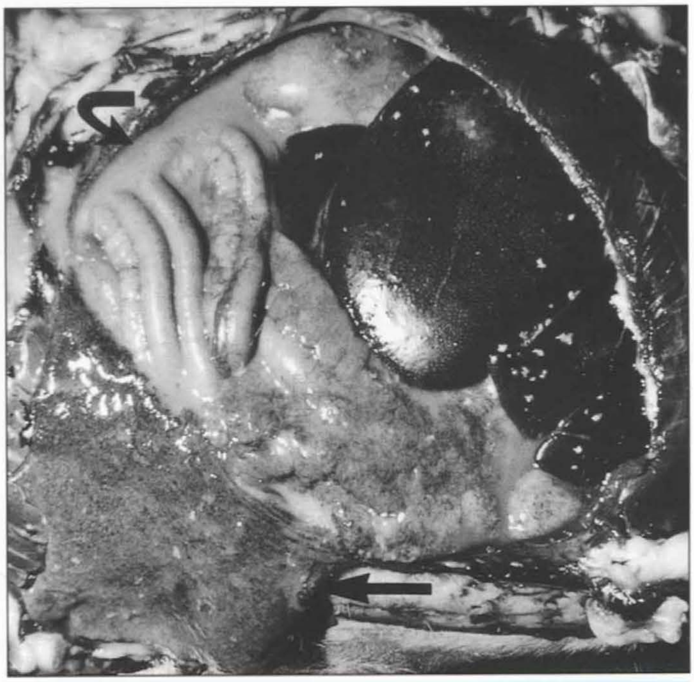

Figure 1.215 Nocardial peritonitis in a dog. The peritoneum of the reflected abdominal wall (arrow) and the omentum is granular due to adherent microcolonies of *Nocardia*. Note excess, cloudy peritoneal fluid (curved arrow).

A distinctive pyogranulomatous peritonitis occurs in dogs and cats. There is copious cellular exudate, red-brown and mushy (Fig. 1.215). The color is from blood derived from proliferation of thin-walled capillaries on serous surfaces (Fig. 1.216A), which are thickened, red, and edematous. Small yellow granules, free or attached to the peritoneum, should be seen (Fig. 1.216B). *Actinomyces* spp., most commonly *A. viscosus*, are mainly responsible for the gross lesions, often with small yellow sulfur granules free in the exudate, or in granulomas. The granulomas may be large and adherent, involving the omentum, mesenteries, and affected mesenteric lymph nodes (Fig. 1.217). *Nocardia asteroides* may be responsible for similar disease.

Chronic pyogranulomatous or granulomatous peritonitis occurs in the rare cases of eumycotic mycetoma and phycomycosis that involve the abdominal cavity. There is usually thickening and fibrosis of the serosa of affected segments of gut, with marked formation of adhesions, and the development of firm granulomatous masses on the gut wall and in the mesenteries and omentum. *Mycobacterium microti* (llama-type), the vole bacillus, is reported as a rare cause of severe diffuse granulomatous peritonitis.

Sclerosing encapsulating peritonitis, a characteristic pathologic syndrome, occurs rarely in the dog. A thick layer of progressively maturing granulation tissue lines the abdominal cavity, or parts of it, and covers or encapsulates the organs that are involved. The effect is to produce one or more thick-walled cystic spaces, enclosing some or most of the abdominal cavity and its organs. Within the space is a large volume of clear or serosanguineous fluid, with perhaps a few strands of more-or-less organized fibrin. The involved organs are often atrophic or misshapen due to the thick fibrous capsule on their surface.

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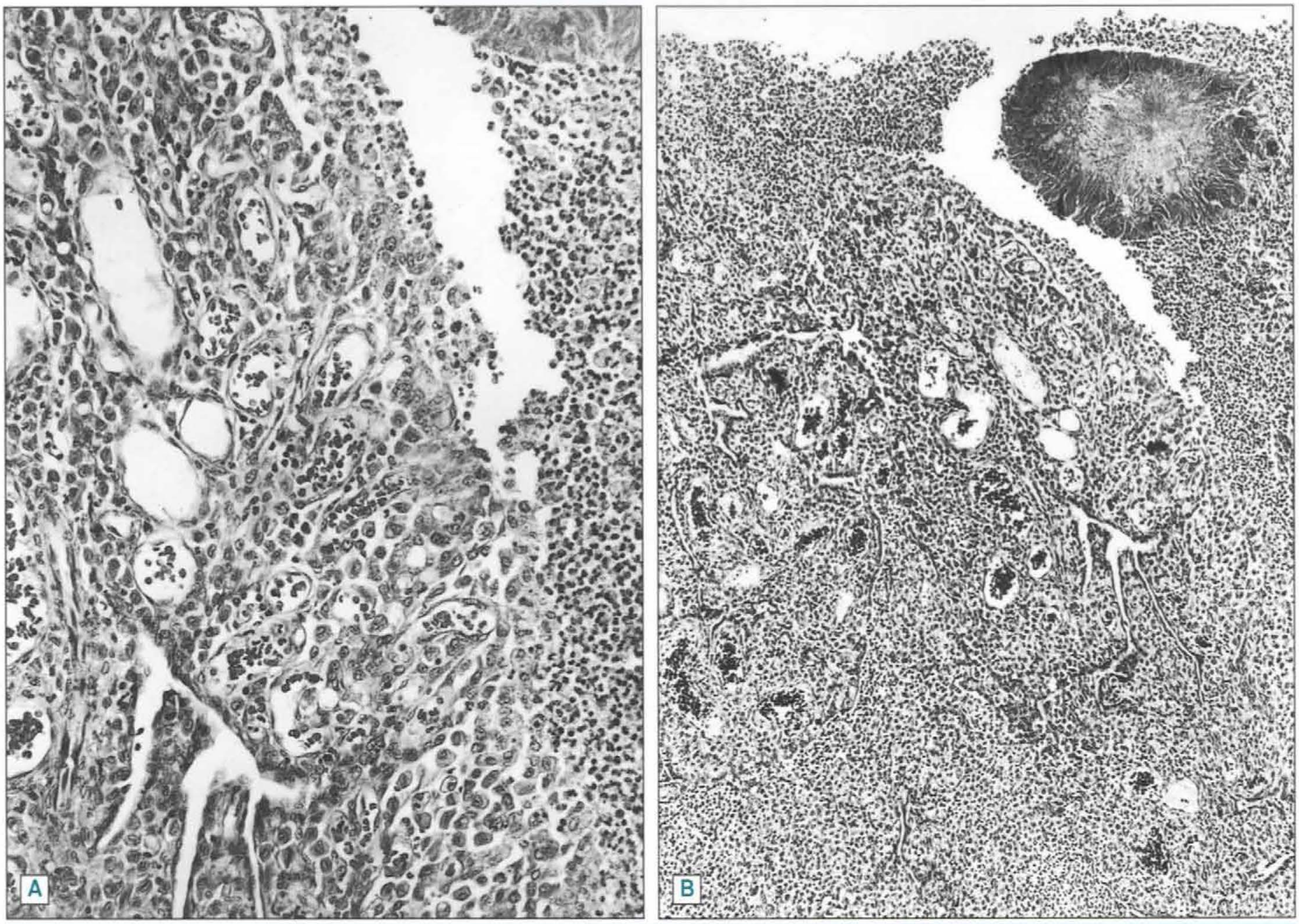

Figure 1.216 Nocardial peritonitis in a dog. **A.** Thin-walled capillaries on peritoneal surface. **B.** Pyogranulomatous inflammation. Bacterial colony (top right) is surrounded by neutrophils.

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Peritonitis in cats

Foul-smelling peritonitis occurs when the uterus ruptures due to pyometra or fetal putrefaction. Peritonitis also occurs from penetrating wounds or by extension from retroperitoneal tissues, and occasionally septic peritonitis is caused by anaerobes such as those associated with cat-bite abscesses. Actinomycotic peritonitis similar in appearance to the disease in dogs may complicate *Feline leukemia virus* infection in cats and the other myeloproliferative diseases.

Feline infectious peritonitis (FIP). The group 1 species in genus *Coronavirus*, family Coronaviridae, includes *Feline coronavirus* (FCoV), and its two biological types **Feline infectious peritonitis virus** (FIPV) and Feline enteric coronavirus (FECV), as well as *Transmissible gastroenteritis virus* of swine and *Canine coronavirus.* FCoVs can also be divided into types I and II based on cell culture cytopathic effect and other features; both FIPV and FECV strains are represented among FCoV types I and II. In domestic and wild felids, the various FCoV strains have a spectrum of virulence, from asymptomatic enteric infection and healthy lifelong carrier status, through symptomatic enteric infection (see the section on Enteric coronaviral infections), to virulent systemic infection, which is expressed as FIP. A small minority of cats resist infection, probably on a genetic basis.

FIPV appears to have evolved as a deletion mutation of FECV. Antibodies produced against FIPV cannot be distinguished readily from those stimulated by the other FCoVs. Genetic analysis of virus in catteries or closed colonies indicates that the viruses form a clade, but that individual cats may harbor a distinct quasispecies. In comparison to FECV, FIPV strains have less tropism for the gut. *FIPV replicates in macrophages*, which disseminate the virus to many parts of the body, and this is central to their virulence.

FCoVs are ubiquitous in cats, but the disease FIP is sporadic, and has a low prevalence. *Most cats do not develop disease when exposed to FIPV, and those cats that do develop the disease are usually under 2 years of age*. Resistance to FIPV infection is cell-mediated, and systemic clinical disease only occurs if the cell-mediated response is ineffective. The cytokine response appears to be important in the response to FPV infection; immunity against FIPV may be associated with low tumor

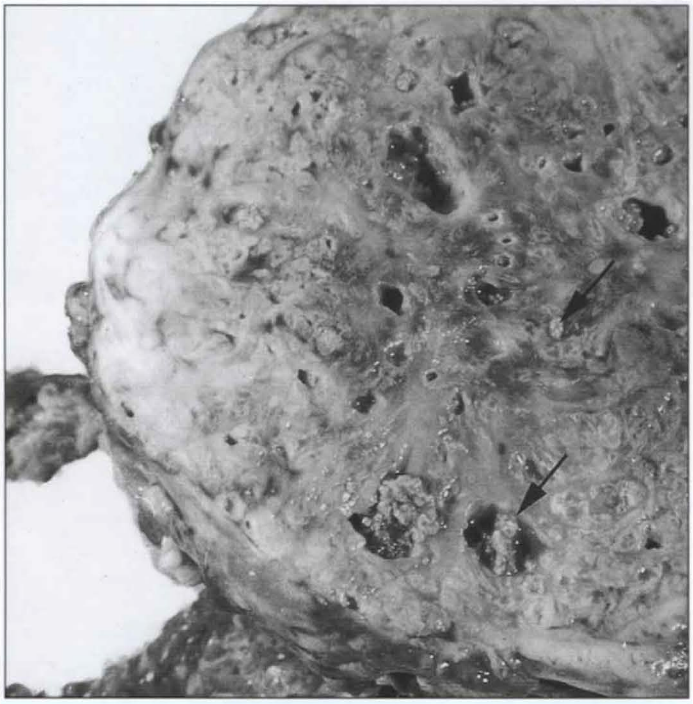

Figure 1.217 ***Actinomyces*** **peritonitis** in a dog. Granulomatous mass involving the mesentery and omentum; "sulfur granules" shell out of pyogranulomatous foci on the cut surface (arrows).

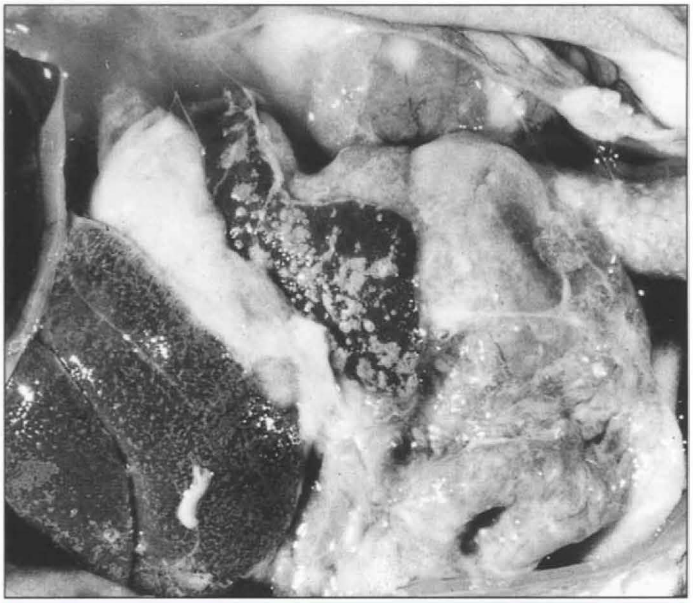

Figure 1.218 Feline infectious peritonitis. Exudative lesion with fibrin on mesentery and viscera, and granulomas in liver, spleen, kidney, and wall of large and small intestine.

necrosis factor-α/high interferon-γ mRNA responses, whereas high tumor necrosis factor-α/low interferon-γ mRNA responses favor disease. Cats that recover from FIP have humoral immune responses and immune complexes that are demonstrable in blood, but, although clearance of virus occurs, persistence may extend for several months with fecal shedding. Cats that do not clear FIPV develop either the dry or wet clinical forms of the disease depending on whether ineffective cell-mediated or humoral immunity dominates the clinical disease. Though often described as distinct entities, *the wet and dry forms of FIP are the extremes of a continuum of syndromes, with* ***vasculitis*** *and* ***pyogranulomatous inflammation*** *as the hallmarks.* Effusive disease is more common than the dry form.

Enteric infection may produce mild, subclinical blunting and fusion of villi. Incidence of the disease is not higher in cats infected with *Feline leukemia virus* or *Feline immunodeficiency virus*; however FCoV replicates 10–100-fold more in cats infected with FIV, thus enhancing the probability of spontaneous mutations.

Type III and type IV immune reactions occur in FIPV-infected cats with clinical disease. Arthus reactions occur in the walls of small venules and arterioles, especially in the serosal surfaces and parenchymatous organs, such as liver and kidney. *The cellular infiltrates around vessels are often characterized as pyogranulomatous, with mixed populations of neutrophils, macrophages, and lymphocytes.* Endothelial damage also results in disseminated intravascular coagulation in the terminal stages of the disease, with thrombocytopenia and increased circulating fibrin split products.

Cats with the effusive form of FIP may develop abdominal distension. Pleural effusion is present in ~25% of cases and may cause dyspnea. Cardiac tamponade due to pericardial effusion is rare. Ocular and central nervous signs are rare. The cats are hypergammaglobulinemic and may have leukocytosis and neutrophilia. Most go on to die; very few recover after passing through a phase of noneffusive disease.

Cats with noneffusive FIP have a chronic disease of insidious onset and frequently develop signs specific to organs severely affected by vascular lesions. These may include ocular disease; central nervous disorders such as ataxia, paraparesis, head tilt; specific nerve palsies, nystagmus, and behavioral changes; renal failure; and hepatic or pancreatic insufficiency. An uncommon intestinal manifestation may be seen as a protracted period of vomiting and diarrhea with a palpable mass usually at or about the ileocecocolic junction.

Peritonitis is present in most animals, although marked effusion is not found in those with the dry form. Up to 1 liter of abdominal exudate may be present in cats with effusive FIP. The fluid is usually viscous, clear, and pale to deep yellow, although it may be flocculent and contain strands of fibrin. The serosal surfaces may be covered with fibrin, giving them a granular appearance. Fibrin is frequently prominent over the visceral peritoneum (Fig. 1.218), and fragile adhesions may be present. There are white foci of necrosis or raised plaques or nodular cellular infiltrations on the serosa and extending into the organs or wall of the intestine. These vary in size from a few millimeters to a centimeter in diameter. The omentum may be contracted into a mass in the cranial abdomen, and adherent to itself and other abdominal surfaces. Mesenteries may be thickened and opaque.

The kidneys may be enlarged and nodular, with few or many, small to large, white, firm nodules protruding from the cortex (Fig. 1.219). Hepatitis and pancreatitis of variable degree may also be present, characterized by small, white foci of inflammation. The tunica vaginalis may be affected, resulting in periorchitis in intact males. Fibrin is usually less prominent in the thorax, but firm white foci may be present under the pleura, and the lungs may be dark and rubbery. Hydropericardium and epicarditis occur less frequently. Abdominal and thoracic lymph nodes may be enlarged and have a lobulated pattern on cut surface.

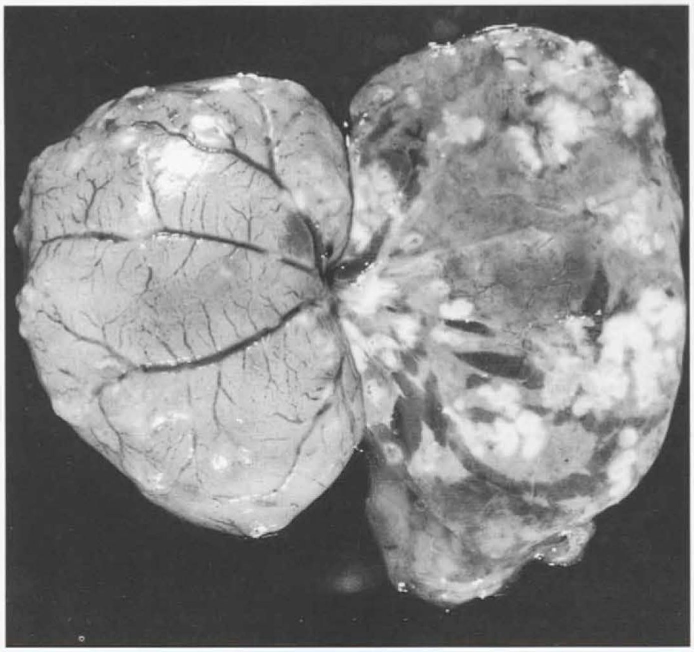

Figure 1.219 Feline infectious peritonitis. Focal pyogranulomatous lesions involving renal cortex and reflected capsule.

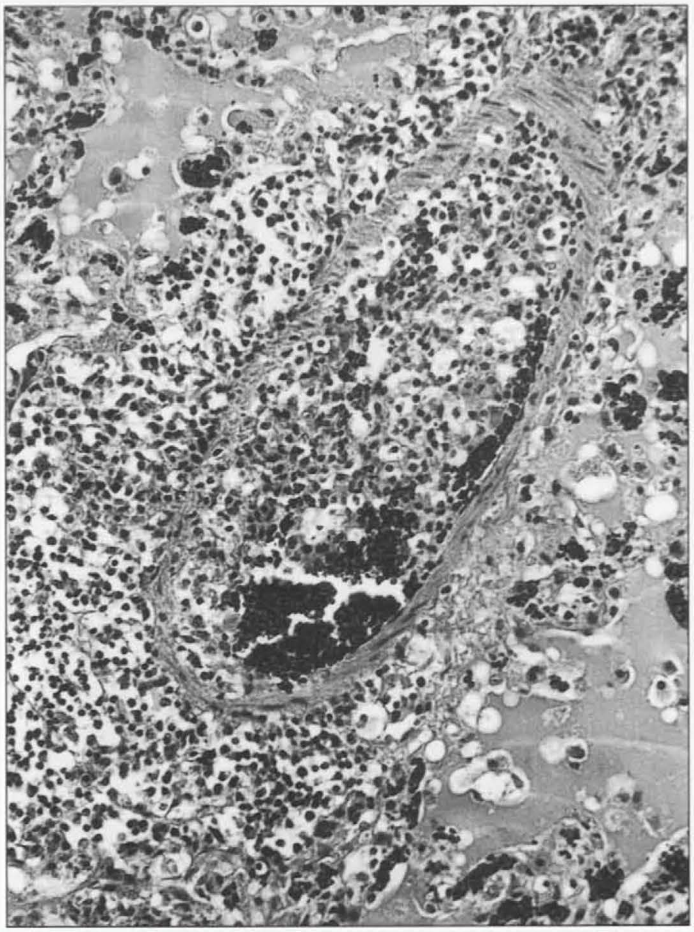

Figure 1.220 Feline infectious peritonitis. Vasculitis and perivasculitis in a lung with severe pulmonary edema.

In cats with noneffusive FIP, there may be inflammatory foci in the abdominal or thoracic organs as described above, or lesions may be restricted to the eyes and nervous system. Diffuse uveitis or chorioretinitis may progress to panophthalmitis; fibrin is often present in the anterior chamber. Lesions in the central nervous system can involve the leptomeninges, spinal cord, or brain, but usually are visible grossly only in the leptomeninges as thickening or white streaks. Occasionally, hydrocephalus or syringomyelia may result from ependymitis. The isolated intestinal lesion is marked thickening of the affected segment by nodular, firm, white tissue extending through the wall of the intestine with adhesion to enlarged lymph nodes.

The characteristic microscopic lesion is generalized vasculitis and perivasculitis, especially of venules (Fig. 1.220), with a focal mixed inflammatory reaction. This lesion occurs in the serous membranes, in the connective tissue of the parenchymatous organs, the eye, and the meninges. Neutrophils, lymphocytes, plasma cells, and macrophages accumulate in and around affected vessels. The endothelium swells, and medial necrosis may be evident, with narrowed vascular lumina; thrombosis may occur. The proportion of neutrophils in the reaction varies, and some lesions may be comprised mainly of a mixture of lymphoid and histiocytic cells. Fibroplasia is variable; occasionally, adventitial fibrosis occurs with little cellular infiltrate. The vascular lesion results in the serofibrinous and cellular exudate on the serosal surfaces, and the nodules visible on the surfaces and deeper in solid organs. The small random necrotic foci that are found in the parenchyma of the liver may be due in some cases to thrombophlebitis and infarction.

The microscopic changes on the omentum, mesentery, and serosal tissues vary in severity. Mild changes are proliferation of mesothelial cells, slight fibrin exudate with fibroblast proliferation, and scattered neutrophils and mononuclear cells. Severe changes result in a thick layer of fibrin adherent to the serosa, with necrosis and/or hypertrophy of mesothelium. Large numbers of neutrophils, mononuclear cells, and necrotic debris may be embedded in the fibrin. The vasculitis may extend from the serosa into the intestine, affecting the muscularis, myenteric ganglia, the submucosa, and the mucosa, which may be segmentally infarcted.

Lesions in various organs are caused by the vascular damage that occurs in the capsule and stromal connective tissue. They may be found throughout the body. Subcapsular infiltrates occur particularly in the liver, lung, and pancreas, and perivasculitis can develop deep in the parenchyma, especially in the kidney. In addition to focal lung lesions, there may be diffuse interstitial pneumonia, sometimes most severe close to the visceral pleura. Similarly, severe focal or generalized lymphoplasmacytic interstitial nephritis may develop. In the spleen and lymph nodes, there is histiocytosis, and either depletion or hyperplasia of lymphoid follicles. Lymphoid depletion may be a result of apoptosis of noninfected lymphocytes.

Cellular infiltrations in the spinal or cerebral meninges, the choroid plexuses, ependyma, and perivascular spaces tend to be more mononuclear and diffuse, with only occasional focal perivasculitis. Degenerative and necrotic lesions in the parenchyma of the central nervous system appear to be related to the vasculitis. The ependyma may be visibly roughened and develop reactive syncytia of lining cells. Ocular lesions are common, but usually subclinical (see Vol. 1, Eye and ear). *Effusive FIP must be differentiated from bacterial peritonitis in particular, but noneffusive forms may be similar to manifestations of lymphosarcoma, steatitis, mycotic infections, and toxoplasmosis.* With thorough necropsy, the constellation of lesions is usually sufficiently distinctive to allow a gross diagnosis with a high degree of accuracy. Testing by immunofluorescence or enzyme-linked immunosorbent assay may be useful in supporting a diagnosis of FIP, but *histopathology remains the only conclusive means of diagnosis of FIP.*

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Parasitic diseases of the peritoneum

Most of the parasites found in the peritoneal cavity are there in the normal course of migrations to another site, or as an accident. Only a few larval and adult helminths use the abdominal cavity as their normal habitat.

Cysticerci (*Cysticercus tenuicollis* in ruminants; *C. pisiformis* in lagomorphs) may be found on the peritoneum during their normal development; they are nonpathogenic, and excite virtually no tissue response, beyond a thin, bland fibrous capsule. Rarely, cysticerci have been encountered in the abdomen of carnivores, which are abnormal hosts. Spargana, elongate larval forms of *Spirometra* spp., may encyst in a bland fibrous capsule in the peritoneal cavity of carnivores and swine. Tetrathyridia, the larvae of the tapeworm *Mesocestoides*, may proliferate extensively in the abdominal cavity of carnivores, where they cause a characteristic pyogranulomatous peritonitis, or "parasitic ascites."

Fasciola hepatica larvae can cause acute and chronic peritonitis in cattle and sheep; the inflammation involves the parietal peritoneum and sometimes the visceral peritoneum, especially that of liver, spleen, and omentum. The changes may consist of many tags of fibrin or more diffuse thickening, and the young flukes may be found in the inflammatory lesions both on and beneath the peritoneum.

Dioctophyma renale seems not as well adapted to canids as to mustelids. In dogs, worms may not complete their migration from the duodenum to the right kidney. As a result, they sometimes initiate chronic perihepatitis in which reactions to characteristic ova, if not the worm or its remnants, may be found.

Stephanurus dentatus, in the course of its migrations through the liver and peritoneal cavity to the kidneys in pigs, may cause local hemorrhage, peritonitis, and perihepatitis.

Strongylus edentatus and ***S. equinus*** pass through the liver, and the ligaments and lumen of the peritoneal cavity, in their migration. Fibrous tags on the liver, particularly the diaphragmatic aspect, are sequelae of *S. edentatus* migration. The larvae of both species may be found in the retroperitoneal tissues of the dorsal abdomen in horses, and in the mesenteries and omentum, where they sometimes incite eosinophilic granulomas.

Ascarids of all species may occasionally cause obstruction and rupture of the small intestine or bile duct, so that they may be found in the abdomen as a terminal event.

Some parasites use the peritoneal cavity as their final habitat. ***Setaria* spp.**, onchocercid filarioid nematodes, inhabit the peritoneal cavity of many domestic and wild ungulates such as horses, cattle, buffalo, camels, sheep, goats, swine, deer, and antelope. They are commonly found at autopsy in cattle and horses in endemic areas. Some species of *Setaria* have a cosmopolitan distribution and may be found in several species of wild and domestic ungulates (*S. equina* in equids; *S. labiatopapillosa* in cattle, buffalo, and perhaps deer and antelope), while others are restricted geographically (*S. digitata*, Asia; *S. marshalli*, Asia) perhaps by the distribution of intermediate hosts.

Adult *Setaria* do not usually cause significant peritoneal lesions in their normal host. There are rare reports of occlusion of the uterine tube by *S. labiatopapillosa* in cattle. As well, adult *S. labiatopapillosa* in the peritoneal cavity, or in the vaginal space around the testis, may incite granulomatous peritonitis or periorchitis if they die; remnants of the dead nematode may be detected in sections of the granulomatous reaction. Adult *S. digitata* may occasionally be found in abnormal locations, such as the heart, lungs, and mesenteric lymph nodes, where they incite eosinophilic granulomas. The larval form of *S. digitata* can produce mild adhesive peritonitis and granulomas in the retroperitoneum and bladder of cattle. The larvae of *S. equina, S. digitata*, and perhaps others that normally spend part of their time in the central nervous system, may occasionally penetrate the neural parenchyma and cause lesions. The sheathed microfilariae deposited by adult females in the peritoneal cavity are found in the blood. The intermediate host may be a mosquito, or for some *Setaria* spp., biting flies (*Haematobia* and *Stomoxys* spp). The microfilariae develop into infective larvae in 2–3 weeks. They are released from the feeding arthropod and enter the final host.

Setaria digitata is normally found as an adult in the peritoneal cavity of cattle and buffalo in Asia, but its larvae can cause *cerebrospinal nematodiasis* in aberrant hosts (see Vol. 1, Nervous system). *Setaria* species that may occur normally in cervids have been reported to cause central nervous system lesions in deer; however, *Elaphostrongylus* larvae produce similar signs and lesions, and differentiation may be difficult.

S. digitata larvae may invade the eye of horses, via the optic nerve, as do the microfilariae of *S. equina*. Here they may cause endophthalmitis.

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Miscellaneous lesions of the peritoneum

Cysts of the peritoneum are rather common but insignificant. Those associated with genital adnexa are described with those systems. Cysticerci have been mentioned; *Echinococcus granulosus* may develop cysts on the peritoneum following the rupture of a mature hydatid into the abdomen. Small cysts, sometimes multiple, which are observed in the omentum, may be either inclusion cysts or sites of local lymphatic ectasia; they are inert.

The normal squamous mesothelial cells of the serosa may undergo **metaplasia** to a cuboidal or columnar type resembling epithelium. Such metaplasia is probably the mildest response of the peritoneum to irritation but may also be a response to estrogen. Inflammatory metaplasia leading to ossification may occur in peritoneal scars, especially in swine. It may also be found in the mesenteries and the dorsal retroperitoneum without obvious antecedent change, although ossification may occur following fat necrosis as well. Ossified areas are discoid, of variable size and shape, and are usually found in adipose tissue.

Neoplastic diseases of the peritoneum

Primary tumors of the peritoneum may arise from the serosa itself, from the subserous connective tissues, and from various differentiated tissues such as nerve sheaths. Tumors arising from the serosa are called **mesotheliomas**, sometimes termed "malignant." The qualification is without meaning, as virtually all can spread by implantation rather than metastasis, the latter being much less common.

Mesotheliomas are rare. They occur with greatest frequency in cattle and dogs but are occasionally reported in horses, cats, pigs, and other species. Interest in mesotheliomas has increased since the association between asbestos fiber and mesothelioma was discovered in humans. This association has not been confirmed in animals, though ferruginous bodies, suggestive of asbestos exposure, have been found in the lungs of some urban dogs with mesothelioma, and an association has been made between mesothelioma in dogs and exposure of owners to asbestos. *Many fiber types other than asbestos are capable of causing mesotheliomas and this ability seems to be related to fiber size and solubility*. In domestic animals, mesothelioma is notable because it occurs most frequently as a *congenital neoplasm* in fetal or young cattle (Fig. 1.221).

Mesotheliomas arise from the cells of the serous linings of pericardial, pleural, and peritoneal cavities, and they may involve all three locations simultaneously. They are typically pleural in pigs. They usually appear as *multiple firm, sessile, or pedunculated nodules*, from a few millimeters to 6–10 cm in diameter: *as villus projections*, on a thickened mesentery or serosal surface; or as *fibrous or sclerosing forms*, which are more plaque-like. The tumor is frequently associated with a milky or blood-tinged effusion, and in sclerosing tumors in which adhesions occasionally occur, the lesion might resemble chronic granulomatous peritonitis. Ascites is usually present as the result of blocked lymphatics.

Mesotheliomas of the pleura, pericardium, or peritoneum may assume a variety of histologic patterns. However, they usually appear *predominantly papillary*, and resemble adenocarcinoma, *or as spindle cells* resembling fibrosarcoma.

The most common tumor is a solid mass made up of single layers of dark, plump cuboidal, columnar or rounded, epithelioid cells with a distinct border and abundant pink cytoplasm, over proliferating fibrocellular stroma. Mitotic figures are typically not numerous. Some tumors have atypical cells with marked anisokaryosis and prominent nucleoli, or large multinucleate cells. The mesothelial cells form loops and festoons in a papillary pattern, or line cystic spaces and tubular structures. There may be a mucinous matrix in this acinar pattern. These mesotheliomas, which resemble adenocarcinoma, can mimic implantation of a true carcinoma so completely that adequate differentiation may rest on very careful examination for, and exclusion of, a primary focus of carcinoma. Especially challenging are those carcinomas that spread in subserosal lymphatics. Histochemistry and immunohistochemistry have not been successful in uniquely identifying malignant mesothelial cells in domestic animals.

Mesotheliomas that are predominantly fibrous may resemble fibrosarcomas. In the sclerosing forms, there may be a thick fibrous serosa with adhesions, and large anaplastic cuboidal cells may be found in clusters or lining cystic spaces within the fibrous tissue.

Lipomas are the most frequently encountered tumors of the peritoneal interstitium. These benign tumors are well known in horses, in which they usually originate in the mesenteries. They may reach enormous size, but their significance is that they tend to become pedunculated and may cause acute strangulation obstruction when the pedicle winds about a loop of intestine. In section, the core of some of these tumors is friable and necrotic, probably from ischemia; only the superficial centimeter or so remains viable, perhaps nourished by diffusion from the peritoneal environment (Fig. 1.222). In the dog, lipomas arise in the omentum, rather than the mesenteries, and settle on the abdominal floor. They may become very large, but tend not to be pedunculated. They do not, therefore, cause acute distress and, although they may appear malignant histologically, metastases are unusual. They too may develop a pseudocapsule and central area of necrosis.

Other tumors of the subserosal connective tissues, including myxomas, fibromas, and their malignant counterparts, are rare, although fibrosarcomas are observed in dogs, and an omental fibrosarcoma has been reported in a horse. Neurofibromatosis of cattle may involve the abdominal nerves and plexuses, and ganglioneuromas are also observed in this species. Extramedullary pheochromocytomas are observed in cattle and dogs, and nonchromaffin paragangliomas occur in dogs, the latter usually in association with similar tumors elsewhere.

Secondary tumors of the peritoneum are not common, but may occur in any abdominal neoplasia. These mainly arise by direct implantation, rather than by lymphogenous or hematogenous metastasis. Carcinomas are much more common than sarcomas.

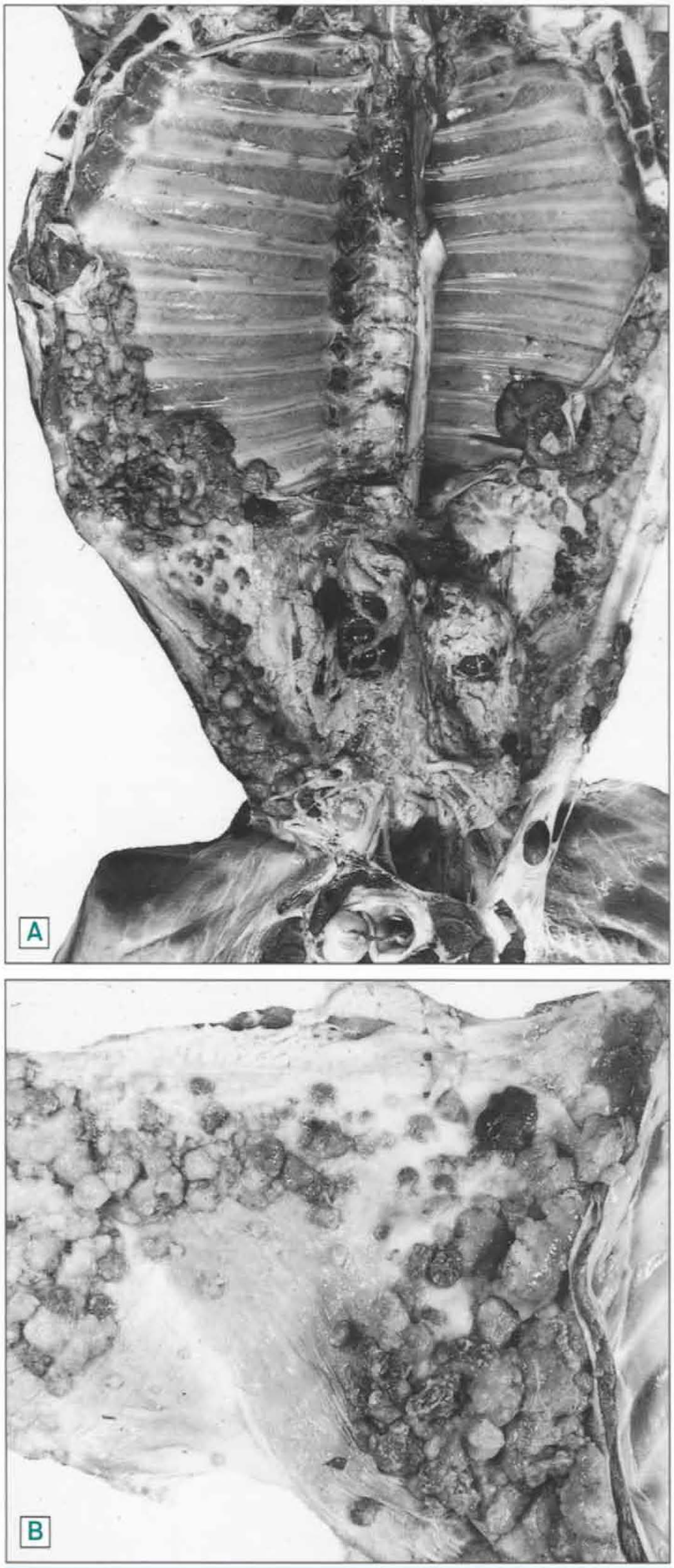

Figure 1.221 Congenital mesothelioma in a calf. **A.** Tumor nodules are confined to peritoneal cavity. **B.** Closer view of (**A**) showing nodules on peritoneal surface.

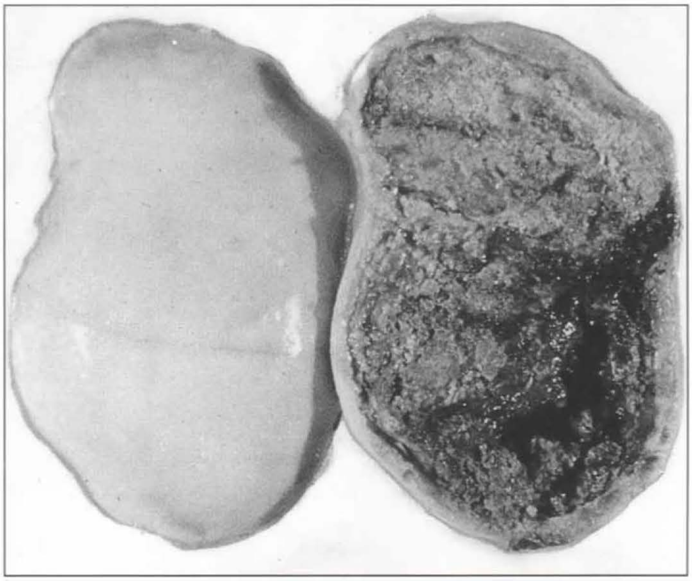

Figure 1.222 Pedunculated lipoma (transected) from the mesentery of a horse. Note necrotic core and thin capsule of viable tissue.

They may be very scirrhous and, when accompanied by ascites, may resemble chronic peritonitis; a relative or complete absence of adhesions is often a helpful distinguishing feature at necropsy. There are obviously many possibilities for the origin of secondary tumors; several common types are listed below.

Ovarian carcinomas are a cause of ascites, and may be difficult to differentiate from mesothelioma. Implants of ovarian carcinoma tend to be papillary. Bile duct carcinomas and pancreatic adenocarcinomas tend to be scirrhous, as do intestinal adenocarcinomas in cattle and sheep. Squamous cell carcinomas of the equine stomach form rather discrete implants that may resemble nodules of mesothelioma, or granulomas; they may be differentiated enough to be recognizably keratinized on gross inspection. Transitional cell tumors developing in cattle with enzootic hematuria implant locally on the pelvic surfaces; implants from rectal adenocarcinoma in dogs also tend to be confined to the pelvic peritoneum. Malignant melanomas of perineal origin in horses produce pigmented plaques on the peritoneum, or in the mesenteries, as may occasional metastatic melanomas in dogs. Pale raised plaques may arise on the peritoneum from implants of prostatic carcinoma in dogs.

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Diseases of the retroperitoneum

Problems in the retroperitoneum may originate in the stroma of the space itself, from organs in this space, or may extend from adjacent organs.

Retroperitonitis may arise from sepsis involving the pelvic or abdominal urogenital tract, or the mesenteric root; from penetrating wounds; from rectal tears in horses; and from migrating grass awns or other foreign bodies. Frequently, it will evolve to form a fluctuant abscess or draining fistula in the flank, based on the anatomic path of least resistance for advancing phlegmon in the soft tissue of the region. The lesion is usually a poorly encapsulated sinus or abscess, with a wall of granulation tissue and containing liquid pus. It may extend to involve adjacent vertebrae in periostitis or osteomyelitis. A foreign body may be encountered on exploration, or the lesion may be traced back to a primary septic focus in the abdomen, perhaps a renal or perirenal abscess; an ovarian stump with a remnant of unresorbed suture; or an abscess or pyogranuloma in the root of the mesentery. Usually, a mixed flora will be isolated. If *Actinomyces* spp. are implicated, there may be granulomatous lesions with sulfur granules in the abdomen or flank.

Nonneoplastic retroperitoneal masses include hematomas, or accumulations of urine, following trauma to the caudal abdominal or pelvic area. Hemorrhage may dissect widely in this area, and the origin may be difficult to detect. Rupture of retroperitoneal hematomas may be fatal. Retroperitoneal hemorrhage in newborn calves is often an indication of fracture of the spinal column due to rotation during assisted calving. Sublumbar hemorrhage is frequent in male lambs castrated by traction of the testis. Renal and perirenal cysts, and pseudocysts or capsular cysts, may also be encountered, usually incidentally.

Neoplasms of the retroperitoneal space include lipomas, fibrosarcomas, lymphosarcomas, osteosarcomas, and nonchromaffin paragangliomas, as well as primary renal adenocarcinomas and adrenal tumors. Occasional highly malignant adenocarcinomas of obscure origin occur in the dorsal retroperitoneum of dogs.

Metastatic tumors to the region include those commonly involving the sublumbar lymph nodes, such as lymphosarcoma, and transitional cell, prostatic, colonic, perianal gland, and anal gland malignancies. Metastatic involvement of the sublumbar chain of lymph nodes frequently stimulates periosteal reaction on the ventral aspect of adjacent vertebral bodies. Occasionally, tumors metastatic to the region will lyse vertebral bodies or transverse processes, and they may occasionally invade the spinal canal. Lytic and osteophytic reactions must be distinguished from the effects of osteomyelitis.

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2 Liver and biliary system

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GENERAL CONSIDERATIONS

The functional importance of the liver has long been recognized. Among ancient civilizations, the liver was regarded as both the seat of life and the mirror of heaven. Hepatoscopy, literally "looking in the liver" of sacrificial animals, was a widespread practice in ancient Babylonia and later in Rome. The detailed study of the liver for cosmological and divinatory purposes is perhaps best illustrated by the Etruscan liver of Piacenza, a bronze instructive model of a sheep's liver dating from the second century BC.

The liver plays a central role in processing dietary carbohydrates, lipids, amino acids, and vitamins; in the synthesis and turnover of most plasma proteins; and in the detoxification and biliary excretion of endogenous wastes and xenobiotic compounds. The liver also functions as an important organ of the innate immune system, integrated into the complex system of defense against foreign macromolecules. As such, hepatic disorders have far-reaching consequences, given the dependence of other organs on the metabolic function of the liver.

Structure and function

The number and shape of the liver lobes of the major domestic mammals vary among species. The typical shape enables the liver mass readily to enlarge or shrink as it adapts to functional demands. The **portal vein** supplies approximately 75% of total afferent blood flow, bringing venous blood from the forestomachs, glandular stomach, intestines, spleen, and pancreas. This portal flow is important for the rapid clearance of nutrients, xenobiotics, microorganisms, and potentially immunogenic materials that enter the circulation from the gastrointestinal tract. The liver is also supplied with oxygenated arterial blood via the **hepatic artery**, a branch of the celiac artery arising from the abdominal aorta. The hepatic artery contributes the remaining 25–30% of afferent blood flow, entering the liver at the hilus and branching in parallel with the portal veins. Most hepatic arterioles disperse into a peribiliary capillary plexus before entering the sinusoids, lowering pressure and preventing reversal of portal venous inflow. Portal and arterial blood eventually mix in the low-pressure hepatic sinusoids.

Hepatic sinusoids are cavernous microvessels lined by specialized endothelial cells, Kupffer cells, and pit cells that provide a suite of innate defenses and housekeeping functions. Hepatic sinusoids differ from vascular structures elsewhere in that they lack a typical basement membrane, and are supported by a specialized, discontinuous or loose extracellular matrix. Hepatic **sinusoidal endothelial cells** are fenestrated, and these 1000 Å diameter sieve-like pores control fluid, solute, and particulate interchange between blood and the perisinusoidal space, regulated by the action of the cellular cytoskeleton. Sinusoidal endothelial cells are actively pinocytotic and internalize and degrade various endogenous glycoproteins, glycosaminoglycans, and immune complexes. **Kupffer cells** are fixed macrophages attached to the inner sinusoidal wall in direct contact with blood moving at a relatively slow velocity. This arrangement facilitates phagocytic removal of particulates, especially bacteria that enter the portal blood via the lower alimentary tract. Kupffer cells also participate in the regulation of inflammatory and repair responses by secretion of various cytokines into the circulation and perisinusoidal space. **Pit cells** are large granular lymphocytes with natural killer activity that adhere to the sinusoidal endothelium where they are also well situated to participate in various innate immune defenses, for example targeting infected cells that enter the liver via the blood.

The sinusoids are separated from the adjacent hepatocellular plates by an extracellular space known as the **space of Disse** that contains **hepatic stellate cells** (also termed *lipocytes* or *Ito cells*), reticulin fibers, and nerves. The space of Disse is not readily visible by light microscopy unless there is fluid retention, such as can occur with impediment to venous outflow. While hepatic sinusoids in the normal liver lack a conventional basement membrane, the perisinusoidal space contains a low-density extracellular matrix consisting of collagen type IV, laminin, fibronectin, and minor amounts of collagen types I, III, V, and VI, nonfibrillar collagen XVIII, tenascin, and various proteoglycans. A conventional extracellular matrix composed of fibrillar collagen types I, III, V, and fibronectin is found in the external capsule (**Glisson's capsule**), septa, and around portal triads and central veins. "**Reticulin fibers**" refer to the components of the extracellular matrix that are stainable by silver impregnation techniques, consisting mainly of collagen type III with attached fibronectin and other glycoproteins. The fenestrated sinusoidal endothelium, coupled with the loose subendothelial matrix, allows for exchange of various macromolecules between hepatocytes and the sinusoidal blood. After hepatic injury, a denser, less permeable matrix resembling a true basement membrane may form and sinusoidal endothelial cells may lose their fenestrae (so-called "**capillarization" of sinusoids**), reducing uptake and secretion of plasma proteins and other metabolically important substances.

The **terminal hepatic venules** ("*central veins*") collect the outflow blood from the sinusoids. These venules converge into the larger **hepatic veins** that empty into the caudal vena cava. In most species, increased pressure in the vena cava during right-sided heart failure or hepatic vein thrombosis causes passive congestion and distension of the hepatic veins and sinusoids. However, the large and small hepatic veins in dogs and pigs have more prominent smooth-muscle valves that reduce the influence of central venous pressure on the sinusoids. Fluids from the perisinusoidal space drain into **lymphatics** in the extracellular connective tissue spaces of the liver capsule and the portal tracts. These flow out the portal hilus to the hepatic lymph nodes and eventually enter the thoracic duct. In some species such as the dog there are also lymphatics around the larger hepatic veins; these cross the diaphragm into the mediastinum.

The vasculature of the liver parenchyma defines its functional microanatomy, but *debate continues as to what best represents the hepatic structural–functional unit.* Mammalian hepatocytes are organized in plate-like monolayer arrays among the sinusoids. In section, these plates appear as "cords," but, in three dimensions, *plates, sinusoids, and tracts anastomose in a complex pattern.* Arrangements of hepatocytes are referred to as either **acini** or **lobules**.

- The **hepatic acinus** of Rappaport is a functional subunit divided into *zones* in relation to blood supply:
  - **zone 1** hepatocytes are closest to the oxygen- and nutrient-rich arterial and portal inflow.
  - **zone 3** (**periacinar**) hepatocytes are nearest the outflow (terminal hepatic venule).
  - **zone 2** is the transitional mid-zone.
- The **classical hepatic lobule** is a six-sided anatomic arrangement of hepatocytes centered on the terminal hepatic venule, also termed the "central vein" in this context. Peripherally, lobules are outlined by fibrovascular septa extending from the portal tracts. In the pig liver, septa form obvious lobular perimeters, but in most mammalian species the lobules are less pronounced because connective tissue is more restricted to portal tracts. The terms **periportal** and **centrilobular** are mainly used for pathological conditions that are centered on either the *portal tracts* or the *central veins* of the classical lobule.

The functional activity of hepatocytes is determined by their microenvironment, and virtually all liver functions have a zonal gradient. Zone 1 hepatocytes, exposed to the blood with the highest concentration of oxygen, insulin, glucagon, and amino acids, are the principal site of gluconeogenesis, protein synthesis, aerobic metabolism, urea cycle, lipid and cholesterol metabolism. In zone 3, glycolysis, lipogenesis, and the major biotransformation functions are more active, including the expression of most cytochromes P450, glucuronyl transferases, glutathione S-transferases and other biotransformation/detoxification enzymes. *Zone 3 hepatocytes are therefore more susceptible to hypoxic injury as well as injury by toxic substances that are metabolically activated by cytochromes P450.* By comparison, *hepatocytes in zone 1 are more susceptible to direct-acting toxicants*, such as ingested metal salts, due to their proximity to the vascular inflow. Under the influences of various inducers, the patterns of enzyme expression can extend beyond the resting limits. Zonal variation is not restricted to parenchymal cells, but is also apparent in the structure and function of sinusoidal endothelial cells, Kupffer cells, perisinusoidal stellate cells, and the composition of the matrix in the space of Disse.

The **portal tract**, or *portal triad*, is a well-defined structure *containing at least one small arterial branch, a portal vein branch, and a bile duct*, surrounded by connective tissue composed primarily of type I collagen. Due to the pattern of progressive branching of the portal tract system, individual tracts exhibit a range of sizes and shapes, from round to triangular or branching. In larger portal tracts, lymphatic channels and autonomic nerve fibers may be seen. The **bile duct** system is a branching outflow that ultimately enters the proximal duodenum. Most species, with the exception of the horse and rat, have a bile storage diverticulum (**gallbladder**). Cats occasionally have divided or bipartite gallbladders, and the bile duct joins the pancreatic duct before entry into the duodenum. Intrahepatic bile ducts range in size from the larger septal or trabecular ducts (internal diameter of more than 100 μm in humans) to the smaller interlobular ducts, and tend to be adjacent to a hepatic artery branch of approximately the same size. **Bile ductules** are smaller yet (lumen size of less than 20 μm) and are located at the periphery of portal tracts. Bile ducts are lined by cuboidal to low columnar **bile duct epithelial cells**, subtended by a periodic acid–Schiff (PAS)-positive basement membrane.

Cells of the liver

Hepatocytes (referred to as **parenchymal cells**) constitute about 70–80% of the liver mass. However, more than 50% of liver DNA is found in smaller **nonparenchymal cells** (bile duct epithelium, hepatic stellate cells, sinusoidal endothelium, Kupffer cells) and itinerant cells (such as leukocytes). The hepatocyte is a polygonal epithelial cell, arranged in single-cell-thick anastomosing plates, separated by hepatic sinusoids. Each hepatocyte is therefore exposed to sinusoidal blood on two sides. A discontinuous line of hepatocytes, termed the **limiting plate**, is found at the interface with the collagenous extracellular matrix of the portal tract. Normal hepatocytes have abundant eosinophilic cytoplasm and most have a single, round, centrally placed nucleus with finely dispersed chromatin and at least one nucleolus. Some binucleate hepatocytes are present normally in mammals and can become more numerous in response to various stimuli and injuries that induce or affect regeneration.

Hepatocytes are metabolically highly active cells, containing an array of organelles including smooth and rough endoplasmic reticulum, mitochondria, lysosomes, peroxisomes, Golgi complexes, and transport vesicles. A greater proportion of the genome is expressed in the normal liver than has been observed in any other tissue, an indication that brief surveys of liver functions are necessarily oversimplified. However, those constituents that are most abundant have most influence on the microscopic appearance of the liver. The normal hepatocyte contains abundant glycogen that can be demonstrated by PAS staining, as well as variable amounts of stored triglycerides and various proteins such as ferritin, an iron-binding protein. The cytoplasm of zone 3 hepatocytes may also contain uniform, golden-brown granules of **lipofuscin**. This so-called "wear-and-tear" pigment becomes more prominent with age, and progressively accumulates in midzonal and periportal hepatocytes. Hepatocytes have a cytoskeleton composed of microtubules, microfilaments, and intermediate filaments. Microtubules are found throughout the cytoplasm, and are involved in the movement of secreted proteins into the extracellular perisinusoidal space; accordingly, microtubule inhibitors such as colchicine and vinca alkaloids may reduce hepatic protein secretion. Microfilaments, composed of actin and myosin, are concentrated around the bile canaliculus, where they are involved in canalicular peristalsis and bile secretion; microfilament inhibitors result in cholestasis. Intermediate filaments (predominantly cytokeratins 8 and 18) form an irregular meshwork extending from the plasma membrane to the perinuclear zone, and are responsible for the spatial organization of the hepatocyte.

There are three morphologically and functionally distinct surfaces of the hepatocyte plasma membrane:

1. The **sinusoidal domain** has irregular microvilli that project into the space of Disse, increasing hepatocyte surface area and functioning in exchange of substances with the blood. Numerous membrane receptors for glycoproteins, asialoglycoproteins, peptides, hormones, growth factors, immunoglobulin A, and other endocytotic or signaling ligands are concentrated at the sinusoidal pole, on the surface or in trans-Golgi and endocytotic vesicles. In addition, transmembrane proteins involved in plasma exchange of small ionic substances with the sinusoidal plasma, and transmembrane proteins responsible for matrix recognition, are concentrated on the sinusoidal surface.
2. The **intercellular domain** is specialized for adhesion via junctional complexes, including desmosomes, tight junctions, and intermediate junctions, as well as for intercellular communication via gap junctions. Within the intercellular domain is the **bile canaliculus**, the beginning of the bile drainage system of the liver. *The canaliculus is an intercellular space between two adjacent hepatocytes, isolated by tight junctions.*
3. The **bile canalicular domain** contains various adenosine triphosphate-dependent carriers that export many products, including leukotrienes, bile salts, xenobiotics, and their metabolites into the bile.

The **canal of Hering** connects the bile canaliculus to the portal bile ductules. The epithelial cells in this transition zone between hepatocytes and bile ducts have considerable regenerative potential and can give rise to proliferating bile ductules seen in various forms of liver injury. This compartment is also believed to contain the greatest number of pluripotential stem cells that can differentiate into other liver cell lineages. Differentiated hepatocytes have a low rate of mitosis but most can replicate to maintain functional liver mass; however, with aging and in some disease conditions, the replicative potential of hepatocytes declines. During development and some chronic liver diseases, *undifferentiated ductal precursor cells known as* "**oval cells**" can emerge from the terminal bile duct or canals of Hering. Oval cells have been considered to be pluripotential **hepatic stem cells**, capable of generating new hepatocytes, although they are more likely to differentiate into bile duct epithelium. Bone marrow-derived pluripotential stem cells also appear to have the ability to differentiate into hepatocytes.

Although derived from common embryologic progenitor cells, **bile duct epithelial cells** differ from hepatocytes in both phenotype and function. They contain a strongly developed network of intermediate filaments, including cytokeratins 7 and 19. They also express marked heterogeneity along the anatomical course of the biliary system. Functionally, bile duct epithelial cells modify the composition of bile and secrete various substances into the bile. They express γ-glutamyltranspeptidase that removes glutamic acid from glutathione conjugates. Intrahepatic cholangiocytes express the sodium-dependent glucose transporter and aquaporins responsible for glucose and water uptake, as in the renal proximal tubule.

Kupffer cells *are specialized fixed-tissue macrophages located in sinusoidal lumens*, mainly at branch points. They are proficient phagocytes of apoptotic and necrotic cells, particulates, and microorganisms, and the liver is a major "filtering organ" for clearance of bacteria from the blood. There are species differences in the efficiency of this process, which is more effective in carnivores than in ruminants. Kupffer cells can kill bacteria, and can respond to various biologically active bacterial components, including lipopolysaccharides, lipoteichoic acids, and peptidoglycans. Activated Kupffer cells secrete tumor necrosis factor-α and other cytokines, and nitric oxide; these contribute to peripheral vasodilation and hypotension in systemic inflammatory response syndromes initiated by bacterial components. Other secreted cytokines such as interleukin-1 and -6 mediate the acute-phase response and some aspects of the immune and liver regenerative responses. These cytokine responses of Kupffer cells are also believed to be important in regulating the extent of adaptive immune response or tolerance to potentially antigenic macromolecules that can reach the liver through the portal blood.

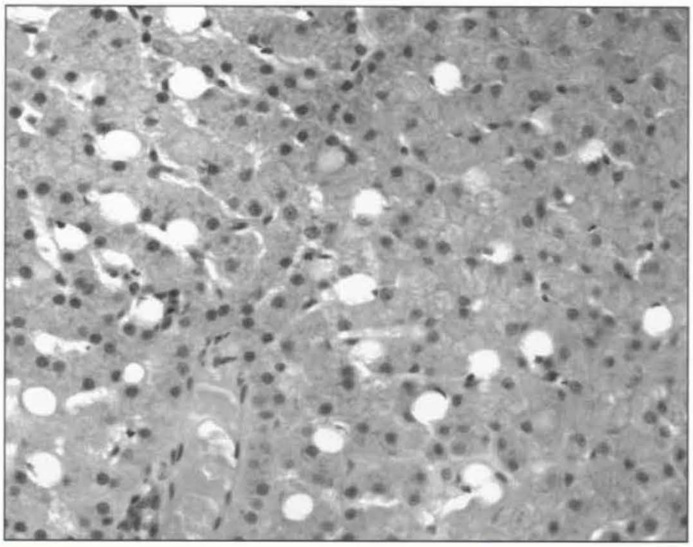

Figure 2.1 Perisinusoidal **hepatic stellate cells** (Ito cells) in a cat.

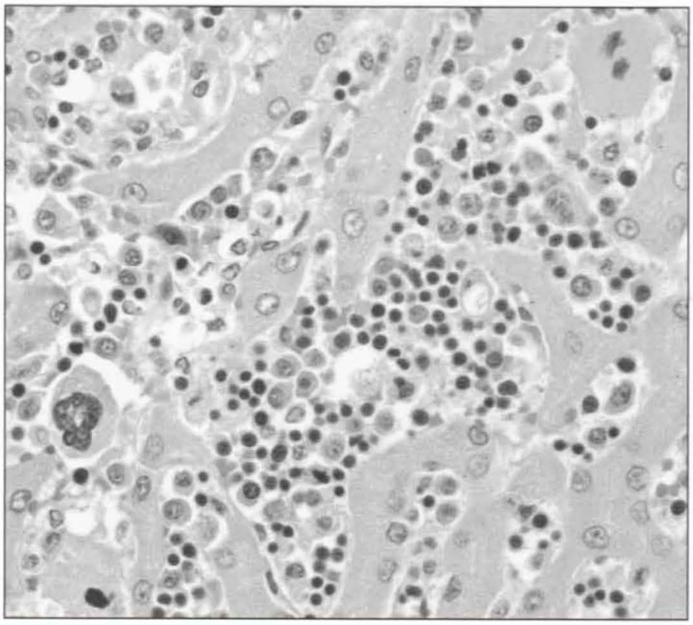

Figure 2.2 Hepatic **extramedullary hematopoiesis** in a dog.

Perisinusoidal **hepatic stellate cells** (also know as *lipocytes, Ito cells, or fat-storing cells*) envelop sinusoidal endothelial cells where they produce and maintain the extracellular matrix that supports the sinusoidal endothelium. They also store lipid droplets that are rich in **vitamin A**, and can become greatly distended with lipid in carnivores on some diets (Fig. 2.1). Stellate cells also function in the control of microvascular tone and regulate sinusoidal blood flow. Cytokines, such as transforming growth factor-β released in response to tissue injury, stimulate stellate cells to redifferentiate to myofibroblast-like cells that express desmin and smooth-muscle actin. Activated stellate cells increase the deposition of extracellular matrix, transforming sinusoidal endothelium to a less permeable capillary form and reducing transfer of macromolecules between the hepatocytes and the blood. In severe chronic injury leading to cirrhosis, the increased expression of contractile cytoskeleton within activated stellate cells can further restrict blood flow.

Hematopoiesis in fetal life of mammals occurs mainly in the perisinusoidal compartment of the liver sinusoids. Postnatally, hepatic hematopoiesis declines but can return as *extramedullary hematopoiesis* in conditions of increased demand (Fig. 2.2). The degree of hepatic extramedullary hematopoiesis in larger species can have diagnostic significance, but in rodents and other small animals, it can be an incidental observation.

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DEVELOPMENTAL ANOMALIES

Congenital hepatic cysts

Congenital cysts of the liver occur in all species, and are of diverse origin. Their number, size, and degree of loculation are quite variable. Cyst walls are composed of connective tissue lined by flattened or cuboidal epithelium. The content is clear and serous.

Serous cysts are occasionally found attached to the capsule on the diaphragmatic surface in calves, lambs, and foals. These cysts are usually small and multiple, but some are isolated and very large (Fig. 2.3). Their origin is not known, but it is variously postulated that they are serosal inclusion cysts, part of congenital polycystic biliary anomalies, or of endodermal origin. They do not contain bile. The declining incidence of these anomalies with age suggests that a large proportion of them involute in the early postnatal period. *Serous cysts should be differentiated from acquired cysts, parasitic cysts, and biliary cystadenomas.*

Intrahepatic congenital cysts *are thought to derive from embryonic bile ducts*, which develop from bipotential liver progenitor cells in contact with the mesenchyme of the portal vein, forming the ductal plate. Failure of remodeling of the ductal plate into mature tubular ducts results in so-called *ductal plate malformations*. **Congenital fibrocystic diseases**, characterized by dilation of segments of intrahepatic bile ducts and variable degrees of fibrosis, likely represent ductal plate malformations at various levels of the biliary tree.

Congenital cystic lesions involving the hepatic biliary system and renal tubules have been reported in juvenile dogs, cats, pigs, goats, and Swiss Freiberger foals, and have been compared to congenital

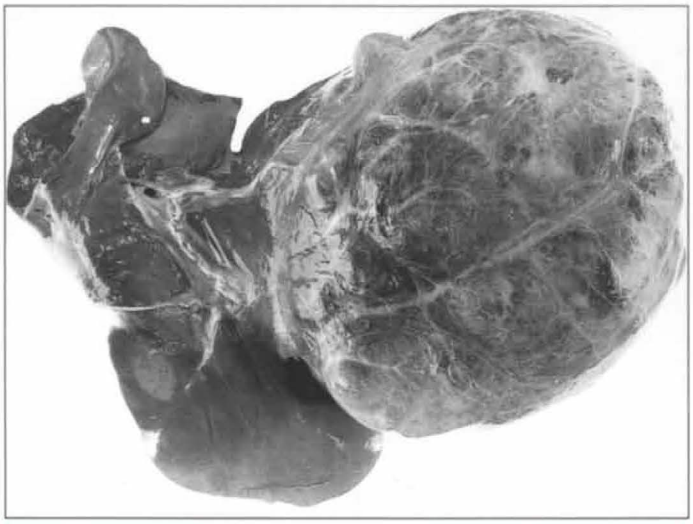

Figure 2.3 Hepatic **serous cyst** in a sheep.

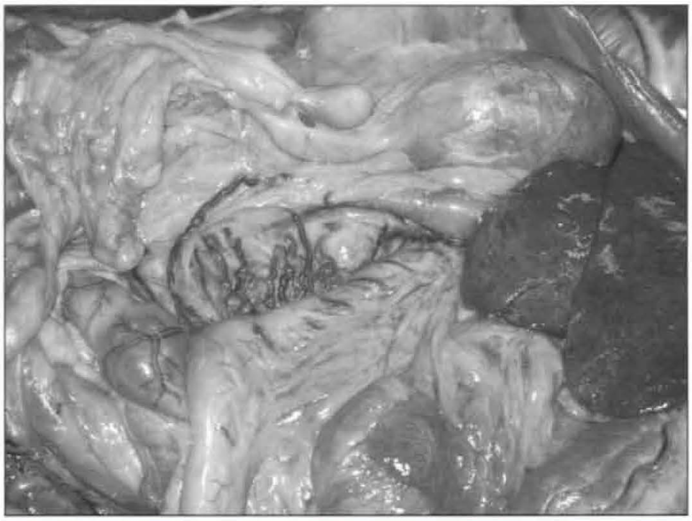

Figure 2.4 Multiple acquired **portosystemic vascular shunts** in a dog with chronic liver disease.

fibropolycystic disorders of humans. Affected livers may be enlarged enough to cause abdominal distension. The parenchyma is expanded by irregularly shaped, branching, cystic biliary structures, with cystic dilation of internal and external biliary tracts. Variable degrees of periportal fibrosis may be present. The common bile duct is patent, and may be dilated and tortuous. Polycystic renal anomalies may also be present, and cysts may also be found in pancreas or other organs. Animals may die from progressive renal insufficiency, and/or from hepatic dysfunction and portal hypertension associated with hepatic fibrosis. Hepatic fibrosis and cysts are present in a significant proportion of cats with *polycystic kidney disease*, inherited in Persian cats as an autosomal dominant trait, and similar to the adult form of polycystic kidney disease in humans. Polycystic kidney and liver disease reported in related West Highland White Terrier litters resembles the autosomal recessive polycystic kidney disease of children. An inherited basis for the disease has been proposed but not proven for dogs (Cairn and West Highland White Terriers) and Swiss Freiberger horses; however the exact mode of inheritance is unknown in the majority of cases in domestic animals. *Congenital hepatic fibrosis*, resembling a similarly named disease in humans related to autosomal recessive polycystic kidney disease, has been reported in a neonatal calf, accompanied by cyst formation in the kidney and lung. Macroscopic congenital dilation of the larger intra- and extrahepatic bile ducts and diffuse cystic kidney disease, resembling Caroli's disease in humans, has been reported in dogs.

Biliary atresia

Anomalies of the extrahepatic biliary system include *absence of the gallbladder*, and *absence or atresia of one or more ducts*. Biliary atresia has been reported in lambs, calves, foals, a cat, a dog, and a pig. In carnivores, bile duct atresia may lead not only to jaundice, but also to vitamin D-deficiency rickets, due to inability to absorb fat-soluble vitamins. Congenital atresia may be associated with defects in the developmental morphogenesis of bile ducts, or in utero vascular, inflammatory, or toxic insults to the biliary tree that culminate in the obliteration of the lumen. Most cases are sporadic, but an outbreak of idiopathic congenital atresia and dysplasia of the biliary tract has been reported in lambs and calves in Australia.

Congenital vascular anomalies

These include abnormal anastomotic connections between the hepatic artery and the portal vein, portosystemic shunts between the portal vein and other systemic veins, and primary hypoplasia of the portal vein. *Large extrahepatic* **congenital** *portosystemic shunts are readily distinguished from shunts that are* **acquired** *during portal hypertension; the latter are typically multiple, thin-walled, and tortuous collateral venous connections between the portal vein or its tributaries and caudal vena cava* (Fig. 2.4). While multiple acquired shunts do not develop in the presence of congenital portosystemic shunts, they can arise with other congenital abnormalities, such as arteriovenous fistulae or hypoplasia or dysplasia of portal veins, as a consequence of portal hypertension. Acquired shunts resulting from portal hypertension secondary to liver injury and repair are discussed in the section on Vascular factors in hepatic injury and circulatory disorders, below.

Congenital intrahepatic arteriovenous fistulae have been reported in dogs and cats. These are *arterioportal fistulae*, representing congenital communications between branches of the hepatic artery and the portal vein. Mixing of higher-pressure arterial blood with venous blood results in retrograde flow into the portal vein, arterialization of the portal circulation, and development of portal hypertension, with the opening of vestigial, low-resistance collateral extrahepatic portosystemic communications (acquired extrahepatic shunts). The fistulae may be macroscopic or microscopic, are typically multiple, and may involve one or more lobes of the liver. The hepatic parenchyma of affected lobes may be atrophied, with dilated, tortuous, pulsatile vessels visible on the capsular surface. Histopathological findings include hyperplasia and anastomoses of arterioles and venules (Fig. 2.5). Affected vessels have irregularly thickened walls with intimal hyperplasia consisting of smooth-muscle proliferation and deposition of elastin fibers, focal subintimal fibromuscular proliferation, and smooth-muscle hyperplasia of the tunica media. Degenerative changes characterized by deposition of mucinous

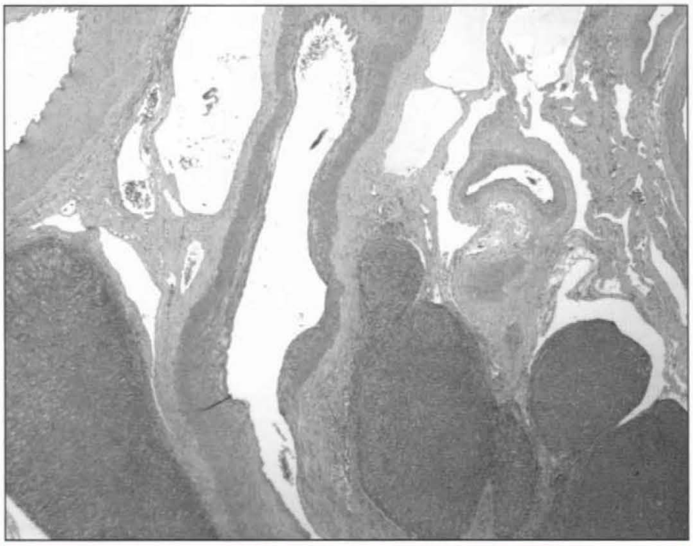

Figure 2.5 Congenital **intrahepatic arterioportal fistulae** with thick-walled anastomosing vessels and atrophy of adjacent parenchyma in a dog.

material and mineral in the intima and media of arterioles, as well as thrombosis and recanalization of portal veins, are also observed. Adjacent hepatic parenchyma may be atrophic, with periportal fibrosis, and bile duct hyperplasia, arteriolar proliferation, and relative collapse of portal vein branches within portal tracts. Arteriovenous fistulae may also be acquired, developing subsequent to abdominal trauma, rupture of hepatic artery aneurysms, and secondary to hepatic vein obstruction or cirrhosis with extreme portal hypertension.

Congenital portosystemic vascular anomalies *are typically single anomalous vessels that directly connect the portal venous system with the systemic venous circulation.* They occur in dogs and cats, and, rarely, in pigs, foals, and calves. These vascular anomalies may be either intrahepatic or extrahepatic in location. The most common **intrahepatic shunt**, located in the left hepatic division, is a *persistent patent fetal ductus venosus.* Central and right divisional intrahepatic shunts have also been described in dogs and cats. The major types of **extrahepatic shunts** include direct shunting from the portal vein or major tributary (typically left gastric or splenic veins, less commonly the gastroduodenal or mesenteric veins) to the caudal vena cava (*portocaval shunt*) or to the azygos vein (*portoazygos shunt*), or connection of the portal vein to the caudal vena cava which itself shunts to the azygos vein. Extrahepatic portosystemic shunts may have hypoplasia of the portal vein distal to the origin of the shunt. Large-breed dogs typically have large intrahepatic shunts, usually a patent ductus venosus, but sometimes other large intrahepatic communications. Small-breed dogs and cats usually have single large extrahepatic shunts between the portal vein and vena cava or azygos vein. An inherited basis is suspected for several breeds, including Irish Wolfhounds, Maltese, Yorkshire Terriers, and Australian cattle dogs.

Affected dogs are usually presented as adolescents with failure to thrive or with the neurobehavioral manifestations of hepatic encephalopathy (see section on Hepatic dysfunction, below). Often there is a clinical history of depression, convulsions, and other nervous signs that are exacerbated by a high-protein diet, and may be alleviated by dietary control. Because there is no cause of portal hypertension, these dogs do not usually develop ascites.

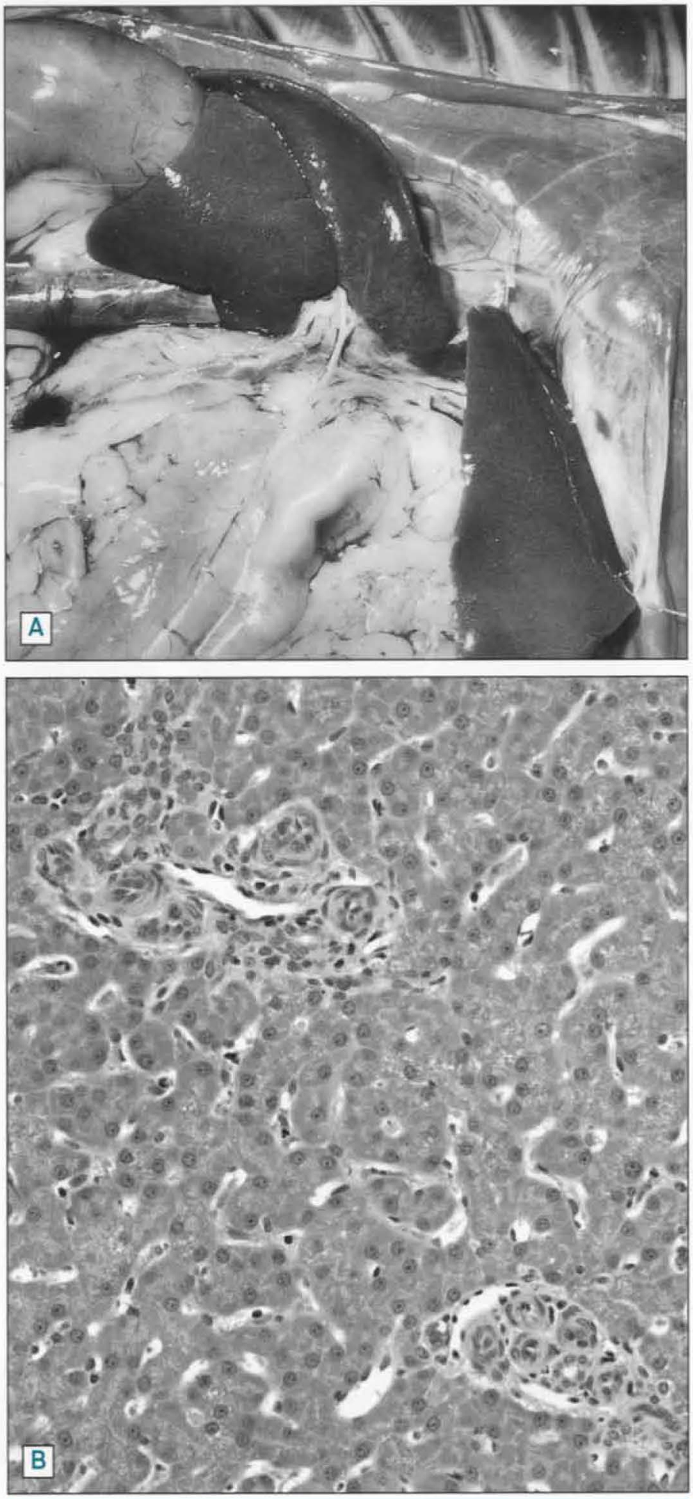

Figure 2.6 Congenital portocaval shunt. A. Small liver in a dog with persistent ductus venosus. **B.** Histology of the liver of a dog with an **extrahepatic congenital portocaval shunt**. Closely spaced portal triads contain multiple sections of hepatic arterioles, and lack discernable portal veins.

The liver that has been bypassed by a congenital shunt is hypoplastic (Fig. 2.6A), largely because of diversion of hepatotrophic factors, such as insulin, glucagons, and epidermal growth factor, that originate in the intestine and pancreas. These livers may be smooth-surfaced with normal color and texture. Histologically, hepatocytes and acini are small with close spacing of portal triads. Larger portal veins may appear collapsed and empty of circulating blood elements; portal veins in smaller triads may be small, collapsed, or indistinguishable. Hepatic arterioles are often more prominent, and may be multiple and tortuous (Fig. 2.6B), related to increased arterial perfusion. Numbers of juvenile-appearing vascular structures within triads may also appear increased. Dilated vascular structures devoid of blood, presumably small- and large-caliber lymphatics, are often prominent in the periphery of some portal triads. Dilated lymphatics may also be present in the connective tissue surrounding hepatic veins. Hepatic veins may have prominent smooth muscle. There may be increased deposition of fibrous connective tissue surrounding portal triads and hepatic veins. Multiple small lipogranulomatous foci with hemosiderin in Kupffer cells are typically present throughout the liver, especially in animals that have survived for some time.

Primary hypoplasia of the portal vein has been reported in dogs, affecting either the extrahepatic or intrahepatic portal vein, or both. Intrahepatic portal vein hypoplasia has been suggested to be the underlying lesion in conditions previously described as *hepatoportal fibrosis* and *idiopathic noncirrhotic portal hypertension* in some young dogs. *Primary portal vein hypoplasia is accompanied by portal hypertension and the development of multiple collateral portosystemic shunts.* Histologically, there is hypoplasia or absence of portal vein radicles, and secondary arteriolar proliferation. Moderate to marked portal fibrosis may also be present. These changes represent sequelae to underperfusion and thus can be indistinguishable from what occurs in conjunction with congenital portosystemic shunts.

A congenital **hepatoportal microvascular dysplasia** without accompanying macroscopic portosystemic shunts has been reported in a kindred of Cairn Terriers, and more recently in Yorkshire Terriers, Maltese, and other small and toy dog breeds. These dogs have elevated serum bile acids suggestive of a portosystemic vascular anomaly, but lack identifiable intrahepatic or extrahepatic shunting vessels. Histologically, cases resemble the changes reported with macroscopic portosystemic vascular anomalies, although the changes may be evident in only some of the liver lobes. Histologic abnormalities include small, poorly formed, or juvenile portal structures; increased numbers of arteriolar sections in portal triads; randomly distributed, small-caliber or juvenile-like vessels throughout the parenchyma; central venous mural hypertrophy with smooth-muscle proliferation, with or without fibrosis; dilation of pericentral vascular spaces, and endothelial cell hyperplasia in portal areas. Lipogranulomas containing hemosiderin are also typically present. In some Cairn Terriers, abnormal apposition of portal tracts with small hepatic venules has been observed, with no intervening hepatocellular parenchyma. These venules also exhibited irregular mural smooth-muscle thickening, and communicated with central veins. However, serial sections failed to reveal direct interconnections between hepatic venules and portal veins. *Unlike macroscopic portosystemic vascular shunts, portal hypertension may develop in severe cases of microvascular dysplasia, with secondary or acquired portosystemic shunting and ascites.* Microscopic capillary abnormalities at the level of the hepatic lobule, with intrahepatic sinusoidal or postsinusoidal shunting, have been postulated. More recently, this disorder has been viewed as part of the disease spectrum of portal vein hypoplasia.

Macroscopic and microscopic portosystemic vascular anomalies may potentially co-occur in dogs, as evidenced by a lack of resolution of histologic changes in additional liver biopsies subsequent to macroscopic shunt ligation. It has been suggested that some animals with portosystemic vascular anomalies have some degree of microvascular anatomic malformation, and it may be that these may represent varying expressions of a more general portal vascular malformation.

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DISPLACEMENT, TORSION, AND RUPTURE

The position of the liver should be observed as soon as the abdomen is opened at necropsy. *Most* **displacements** *are caudal, so that the margins of the liver extend behind the costal arch.* Caudal displacements are the result of hepatic enlargement, or caudal displacement of the diaphragm secondary to pleural effusion or other space-occupying lesion in the thorax. Congenital or acquired displacements associated with ventral and diaphragmatic hernias are common. Usually only one lobe goes into the thorax with other viscera; lobar blood supply may not be compromised, but the affected lobe is usually severely congested and may rupture, or, given time, become indurated.

Liver lobe torsion has been reported in swine, dogs, a cat, and a horse. The left lateral lobe appears to be predisposed, due to its mobility, large size, and relative separation from other lobes. *The resultant infarction can cause death due to shock or hemorrhage.* The lobe may contain clostridial spores that are likely to germinate in the necrotic tissue, which will undergo putrefaction and be dry and crepitant compared to the other lobes.

Rupture *of the liver occurs commonly as the result of trauma, because the organ is fragile relative to its mass.* It is quite common for fatal liver rupture to be produced by the sudden accelerations and pressures of vehicle collisions, without much evidence of trauma to other parts of the body. Large tears may be obvious in the liver capsule and hepatic parenchyma after trauma. However, in some cases, anastomosing linear patterns of fine shallow capsular fissures, concealed in part by clotted blood, can be found. Moderate blood loss from capsular ruptures is followed by rapid coagulation, then fibrinolysis of most of the free clot. The liquid blood is then rapidly returned to the circulation by transdiaphragmatic lymphatic absorption, and, a day or so after the trauma, all that is seen is a delicate tracery of capsular tears that have been sealed by residual thrombus, with a little associated pale necrotic parenchyma. Liver rupture is often clinically occult, since quite large ruptures may not disturb liver function unless severe enough to cause rapid exsanguination, or unless the biliary tract is involved. Rupture of major bile ducts leads to yellow-stained *bile peritonitis*, which may remain sterile and become chronic, or may be fatal, particularly if infected by enterohepatic circulation of bacteria such as clostridia.

The liver is more likely to rupture after trauma in young animals. Fatal ruptures occur in foals during parturition, sometimes concurrently with costal fractures, and in the smaller species subject to energetic emergency resuscitation. Diffuse hepatic conditions with enlarged friable parenchyma (e.g., acute hepatitis, amyloidosis, severe congestion, severe lipidosis, and infiltrating neoplasms) are more likely to rupture, sometimes spontaneously, and the clinical consequences relate to the extent of hemorrhage. Parasites that penetrate the capsule cause numerous small ruptures but seldom lead to significant hemorrhage.

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HEPATOCELLULAR ADAPTATIONS AND INTRACELLULAR ACCUMULATION

The liver must be highly adaptable to balance function with changing demand. Increases in the size of hepatocytes (*hypertrophy*) and their numbers (*hyperplasia*) collectively bring a larger population of hepatocytes into service. Such adaptations in hepatic mass and function result from alterations in the expression of many genes. These responses are more evident in smaller species, notably in laboratory rodents that have a very pronounced liver growth response after exposure to various xenobiotics. The liver can also adapt to reduced demand by a combination of *cellular atrophy* and *apoptosis*. Removal of surplus hepatocytes involves local phagocytosis of membrane blebs and apoptotic fragments, with degradation in phagolysosomes of neighboring hepatocytes and Kupffer cells. Hepatocytes can be lost by apoptosis in substantial numbers with minimal elevation in serum enzymes such as alanine aminotransferase.

Hepatocellular atrophy

Hepatic mass readily adapts to metabolic demands, and *the liver can undergo marked atrophy due to catabolism during illness and/or starvation without much evidence of impaired hepatic function.* During prolonged starvation, some hepatocytes are removed by apoptosis without replacement, but most of the atrophy is explained by loss of cytoplasmic mass. Atrophic livers, as seen, for example, in old grazing herbivores with poor teeth, are dark and small, and the capsule may appear too large for the organ, showing fine wrinkles on handling. These livers may even appear to be firmer than normal due to condensation of normal stroma. Histologically, portal triads and hepatic venules are closer together, and lobules contain increased numbers of smaller hepatocytes with scanty cytoplasm (Fig. 2.7).

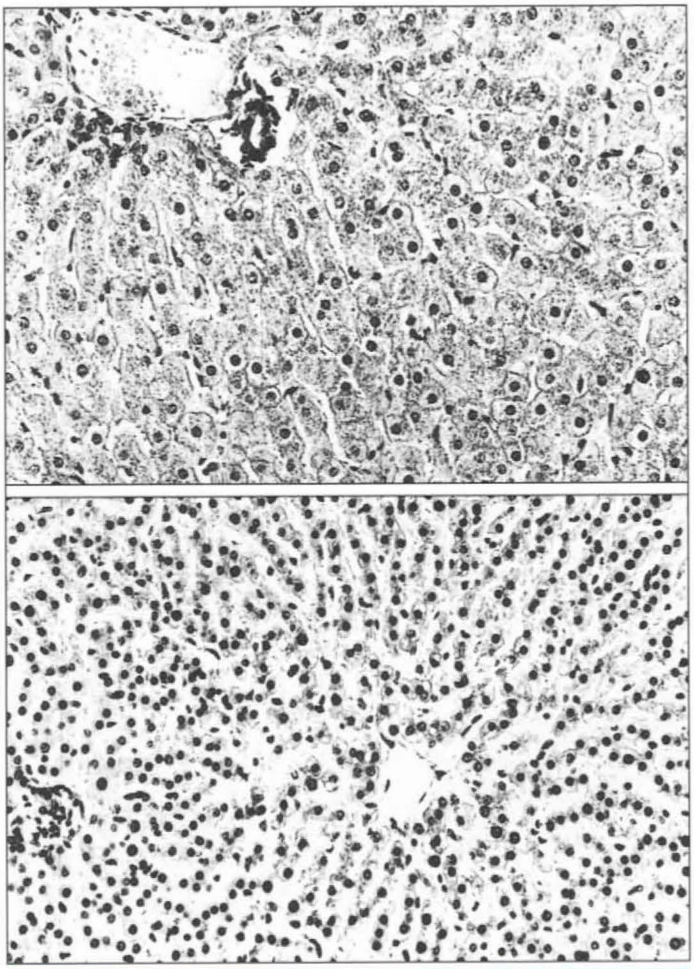

Figure 2.7 Normal (top) and **atrophic liver** (bottom) from a starved sheep, at same magnification.

Hepatic atrophy can also result from impaired replication of hepatocytes. Most adult hepatocytes are replicatively competent, although mitotic figures are infrequent because healthy hepatocytes have a relatively long lifespan of several months. Trophic factors supplied via the portal circulation regulate replication and mass of the liver. Atrophy of part of the liver may be a response to pressure or to impairment of blood or bile flow. The histologic features of this atrophy are similar to those of starvation atrophy. However, the functional consequences of focal hepatic atrophy are minor because the remaining liver can adapt by *compensatory hypertrophy*, a process that involves replication and enlargement of hepatocytes. Local pressure atrophy occurs adjacent to space-occupying lesions in the liver or as a result of chronic pressures from neighboring organs, such as distended rumen in the ox. *Right hepatic lobe atrophy*, reported in horses, has been suggested to result from long-term compression from abnormal distension of the right dorsal colon and base of the cecum. Chronic diffuse diseases of the biliary tract, such as sporidesmin poisoning and fascioliasis, are likely to cause atrophy of the left lobe in ruminants, probably as a result of the greater difficulty in maintaining adequate biliary drainage from this lobe, whose bile ducts are longer than those of the right in these species. The atrophy of biliary obstruction is complicated by some degree of superimposed inflammation and fibrosis.

Hepatocellular mass can be rapidly lost by autophagocytosis and apoptosis (see section on Patterns of cell death in the liver, below). **Autophagocytosis** is the aggregation of cytoplasmic organelles that are subsequently transferred to autolysosomes, whereas **apoptosis** results in fragmentation and phagocytosis by neighboring cells. Autolysosomes lack fragments of DNA seen in lysosomes that contain nuclear portions of apoptotic cells; however, many apoptotic bodies composed of cytoplasm alone are indistinguishable. In hepatocytes, large, histologically obvious inclusions associated with atrophy have been referred to as *cytosegresomes, Councilman bodies, or acidophilic bodies*. These are spherical, refractile, eosinophilic structures seen in liver cells that have been sublethally injured by various insults, including hypoxia, intoxications, malnutrition, nutrient deficiencies, and some viral infections. Note that hepatocytes during autolysis can imbibe plasma that forms similar round but less eosinophilic, non-membrane-bound cytoplasmic inclusions. The long-term sequel to increased cell turnover in this manner is atrophy accompanied by *hepatocellular residual bodies* containing oxidized lipid debris known as *lipofuscin*. Residual bodies are slowly lost by degradation or by extrusion (exocytosis) into the canaliculi or the extracellular space, where they are taken up by Kupffer cells, and accumulate in hemosiderin- and lipid-enriched tertiary lysosomes. The amounts of residual debris in hepatocytes and hemosiderin in Kupffer cells are indicators of cumulative cell turnover due to aging, liver injury, or both.

Hepatocellular hypertrophy

As the liver adapts to increased functional demand, it enlarges, partly by hypertrophy of individual hepatocytes, and partly by their replication. These complex responses are still poorly understood, although many trophic factors that act on hepatocyte surface and nuclear receptors are involved. *Exposure to various xenobiotics can induce the expression of many genes, resulting in increased volume of hepatocyte cytoplasm*, especially components of the smooth endoplasmic reticulum (SER). Agents that elicit this response act via nuclear receptors such as the arylhydrocarbon-activated receptor, the constitutive androstane receptor, or the pregnane X receptor. Phenobarbital, for example, is a potent inducer of the various enzyme systems of the SER, including several cytochromes P450. The amount of induced synthesis of SER is greatest in periacinar hepatocytes, where it is observed as loose aggregates of pale eosinophilic material, often intermixed with glycogen, that may distend the cell or displace the nucleus and other organelles to the periphery (Fig. 2.8). The response can be seen within a few days after exposure to various drugs and other xenobiotic compounds. Accordingly, the liver becomes grossly enlarged and darker brown. This induction is reversible, and after discontinuation of exposure to the inducing agent, the additional SER is removed by autophagocytosis and many hepatocytes undergo apoptosis. While these changes are considered physiological adaptations, there are potential adverse sequelae; accordingly, this response has considerable toxicological significance, and will be dealt with further in the section on Toxic hepatic disease (see below).

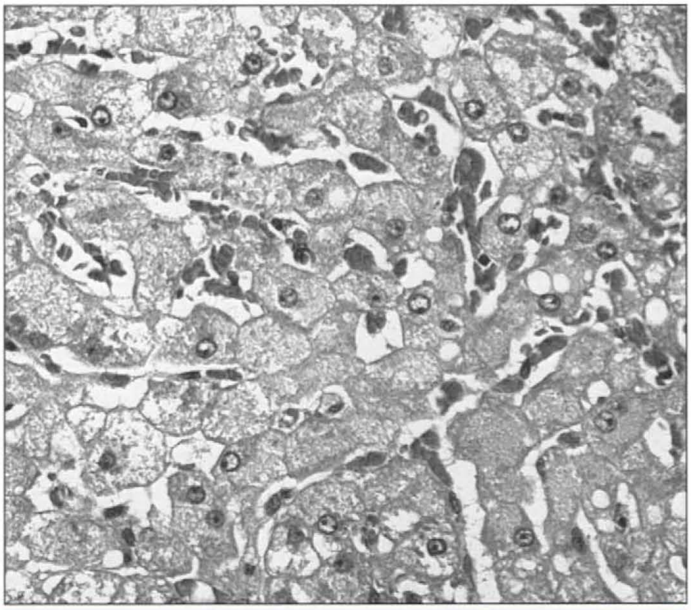

Figure 2.8 Smooth endoplasmic reticulum induction secondary to chronic phenobarbital administration in a dog.

Polyploidy and multinucleation

Most mature mammalian hepatocytes are tetraploid or octaploid, whereas many immature and replicating hepatocytes are diploid. The relative proportion of these varies among species, with a shift towards increasing polyploidy with increasing age. Polyploidy is more common in rodents and is believed to result from asynchrony of cell division in which binucleated diploid cells undergo a second round of DNA replication, giving rise to two tetraploid daughter cells. Impaired replication can also increase numbers of polyploid cells. The term **megalocytosis** was first used in the description of the changes of liver cell cytoplasm and nucleus that occur in pyrrolizidine alkaloid poisoning (Fig. 2.9). This form of megalocytosis has some specific features and is described in the section on Chronic hepatotoxicity, below. Impaired regeneration, atrophy and >4N polyploidy can also

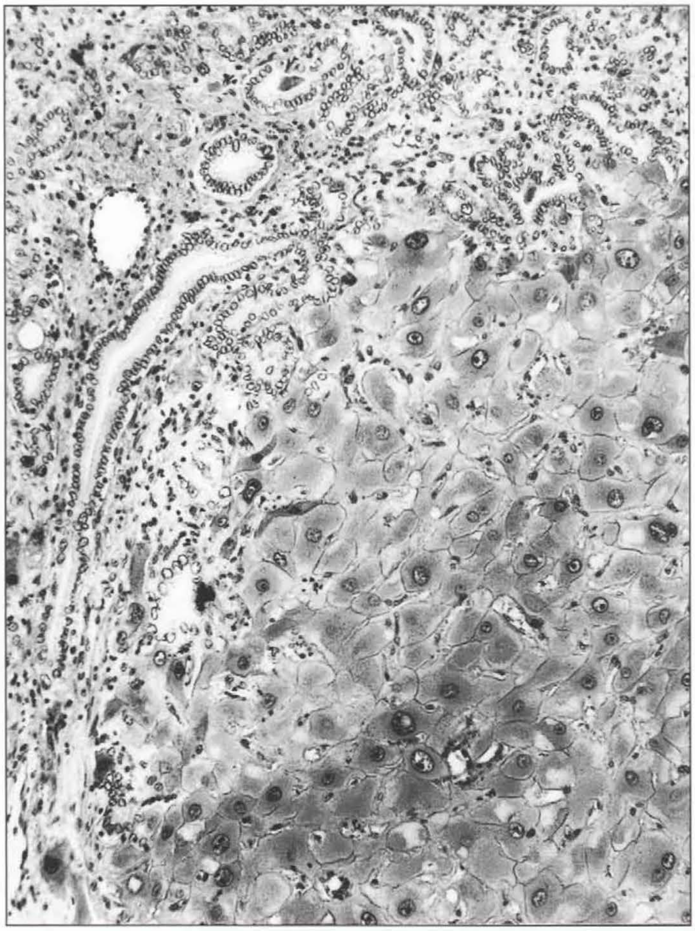

Figure 2.9 Megalocytosis and bile duct proliferation in *Crotalaria* poisoning in a horse.

be produced by other DNA-damaging agents such as aflatoxins. A feature of these patterns of atrophy with polyploidy is the persistence of larger replicatively impaired polyploid hepatocytes amid regenerating smaller diploid hepatocytes, hepatocellular nodules, and hyperplastic bile ductules.

Most hepatocytes are mononuclear but a variable proportion are binucleated, especially in young or regenerating livers of small animals. Multinucleation of nonneoplastic hepatocytes is a rare phenomenon in domestic mammals, and its diagnostic or pathogenetic significance is usually unclear. Multinucleation can result from incomplete cell division or cell fusion, but this distinction is difficult to determine. Hepatocytes can fuse during severe steatosis, but the degree of multinucleation in fatty livers is hard to discern because the plasma membrane perimeters of fatty hepatocytes are ill defined. Syncytial multinucleation of hepatocytes has been observed in various degenerative and regenerative conditions. In protoporphyria of Limousin cattle, small clusters of hepatocytes contain 4–10 or more closely packed nuclei but it is not clear whether this represents fusion or multiple nuclear divisions. Syncytial hepatocytes are a characteristic of postinfantile giant cell hepatitis of children, and similar hepatocyte multinucleation can be observed in association with some forms of hepatitis in cats, foals, and piglets. Multinucleated hepatocytes have been described in young cats with neoplasms (thymic lymphomas), and in cats with experimental dioxin poisoning.

Intranuclear inclusions

In addition to the various nuclear inclusions associated with some viral infections, *three types of inclusions may be found in hepatocyte nuclei*:

1. Spherical, apparently hollow globules within the body of the nucleus are *membrane-bound entrapped nuclear membrane invaginations* that ultrastructurally contain cytoplasmic components such as glycogen and mitochondria. These inclusions are infrequent in otherwise normal livers but are more often seen in chronically injured livers, especially in chronic pyrrolizidine alkaloid poisoning, in which polyploid nuclei are more likely to indent and invaginate.
2. Eosinophilic block-like intranuclear inclusions with a regular crystal lattice ("*brick inclusions*") are common in hepatocytes and renal proximal tubular epithelium. They are more numerous in old animals, particularly dogs. Their composition and pathogenesis are still unknown, but they evidently have little effect on the health of the cells in which they occur, even when they are large enough to distort the nucleus. They do not contain heavy metals and can be distinguished from the acid-fast, noncrystalline intranuclear inclusions observed in renal epithelial cells and occasionally in hepatocytes in lead poisoning.
3. *Lead inclusions* consist of a lead–protein complex and have a characteristic furry electron-dense ultrastructure.

Pigmentation

Congenital melanosis occurs in calves and occasionally in lambs and swine. The melanin deposits may be numerous, and vary in size from flecks to irregular, blue-black areas 2 cm or more in diameter. The melanin is confined to the capsule and the stroma. These deposits are sharply defined in young animals, but become more diffuse and fade with age.

A condition known as **acquired melanosis** is the massive accumulation of black pigment (not melanin) in hepatocytes and Kupffer cells of mature sheep and, less frequently, cattle after prolonged grazing on extensive unimproved pastures in inland eastern Australia, the Falkland Islands, and Scandinavia. The condition in Norway has also been described as *hepatic lipofuscinosis*. The color of the affected livers ranges from a dull gray to uniform black, and there is usually a prominent acinar pattern. In severe cases, there is also pigmentation of the hepatic lymph nodes, lungs, and renal cortex. Histologically, the pigment is present as granules in lysosomes in periportal and midzonal hepatocytes and macrophages of the liver (Fig. 2.10), the proximal tubular epithelium of the kidneys, and in alveolar and interstitial macrophages in the lung. There is no evidence of liver dysfunction, even in the blackest livers. The source of this pigment is not known, but the epidemiologic features of its occurrence indicate that it is derived from a component of the diet that is sequestered within lysosomes.

Lipofuscin *is the term given to small, golden, granular cytoplasmic deposits derived from the lipid component of membranous organelles*, more obvious in cells near the periphery of the acinus. Lipofuscin accumulates in hepatocellular lysosomes and indicates senility, atrophy, or increased turnover of membrane lipids. The pigment is particularly common in the liver of old cats. **Ceroid** is a yellow pigment similar to lipofuscin and is associated with *peroxidation of fat deposits*.

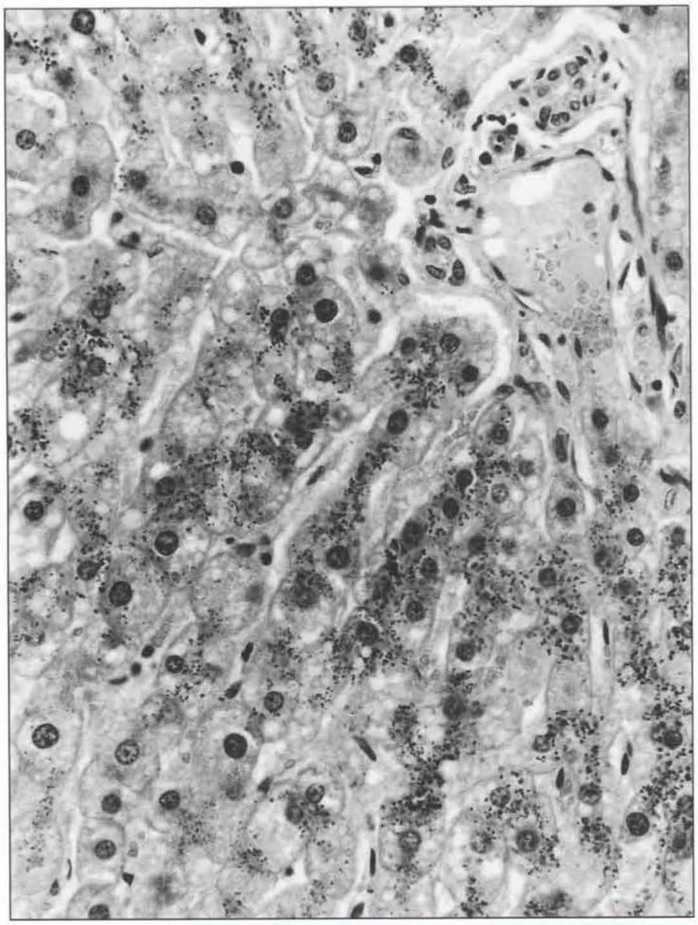

Figure 2.10 Lysosomal pigment in periportal hepatocytes in acquired melanosis in a sheep.

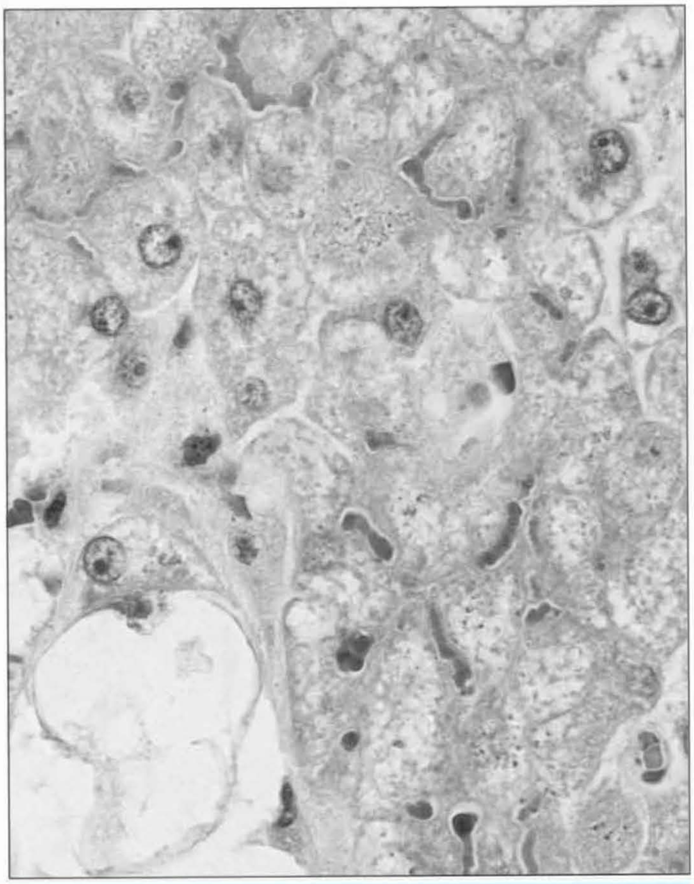

Figure 2.11 Bile plugs distending bile canaliculi (with concurrent hepatocyte hydropic degeneration secondary to corticosteroid therapy) in immune hemolytic anemia in a dog.

Black pigment also accumulates in hepatocellular lysosomes of mutant Corriedale sheep with hyperbilirubinemia. These sheep have a condition resembling the human Dubin–Johnson syndrome, in which there is mutation in the canalicular multispecific organic anion transporter. This suggests that retention of the pigment might be a sequel to a hepatic excretory defect.

In **congenital erythrocytic protoporphyria** (ferrochelatase deficiency) of Limousin and Blonde d'Aquitaine cattle, a dark golden-brown lipofuscin-like lysosomal material is present in portal areas, in Kupffer cells, sinusoidal endothelial cells, and heavily concentrated in the cytoplasm of hepatocytes.

Hemosiderin deposits are seldom sufficient to give gross discoloration, but if so, the color is dark brown. The pigment is detected microscopically as yellow or brown crystals chiefly in the Kupffer cells, although small amounts may be found in hepatic cells. The ferric iron component of this pigment can be demonstrated by *staining with Prussian blue*, otherwise it can easily be confused with lipofuscin. Diffuse hemosiderosis occurs quite commonly in all species, and its presence is usually *suggestive of excess hemolytic activity* relative to the rate of reutilization of iron. Thus it is seen in hemolytic anemias, anemia of copper deficiency, in cachexia, and after blood transfusions. It may be seen in the periacinar zones in severe chronic passive congestion of the liver. Localized hemosiderosis occurs in areas of hemorrhage. *Most hemosiderin deposits are punctate Prussian blue staining aggregates in Kupffer cells* but can also be seen as finer particles in hepatocytes. Hemosiderin is normally present in the liver in the early neonatal period, when fetal hemoglobin is being replaced by mature hemoglobin.

Hemosiderin should be distinguished from **hematin**, which is produced by the action of formic acid on hemoglobin following a prolonged postmortem interval, and is usually regarded as a *histological artifact*. Hematin is also an iron-containing pigment, but the iron is in the reduced ferrous state and does not stain with ferricyanide. It takes the form of crystalline brown deposits that are birefringent under polarized light, mainly within hemoglobin-rich areas such as blood vessels. Hematin is darker than hemosiderin and occurs in irregular clumps, often extracellularly. Hematin may, however, be found in Kupffer cells and macrophages in small amounts.

Bile pigmentation may impart an olive-green color to the liver in diffuse or segmental obstructive biliary disease or intrahepatic cholestasis. Histologically, conjugated bile pigments may distend bile canaliculi, visible microscopically as green-yellow stellate lakes between the hepatocytes (Fig. 2.11). In this case, the identity of the pigment is obvious, but when it is present in granular form in hepatocyte or Kupffer cell cytoplasm, it may easily be confused with hemosiderin and hematin. Death of individual hepatocytes releases the canalicular plugs into the space of Disse and the sinusoids.

Hemochromatosis is common in humans and some birds but rare in domestic mammals. A form resembling heritable hemochromatosis has been reported in Salers cattle. Affected animals have a

30–100-fold increase in liver iron content, with hepatomegaly, hemosiderin accumulation in hepatocytes, and periportal bridging fibrosis with nodular regeneration. Hemosiderin also accumulates in Kupffer cells, lymph nodes, pancreas, spleen, and other organs.

Hemochromatosis resembling the secondary form of the disease in humans has been observed in sheep and cattle exposed to *high levels of iron in pasture and water.* The liver is enlarged and brown with diffuse fine nodularity, and the hepatic and adjacent lymph nodes are also darkened. Large amounts of iron are present in the hepatic parenchyma, the biliary epithelium, and the cortex of lymph nodes, and lesser amounts are present in the broad fibrous septa. The iron is stored predominantly in lysosomes. Brown discoloration of bone marrow resembles the osseous pigmentation of porphyria. The pathogenesis of nutritional siderosis is unknown. Hemochromatosis has also been described in horses, with animals displaying signs of liver failure and neurologic impairment. The microscopic lesions in the livers of these animals are similar to those for other species.

Brown crystalline deposits of **2,8-dihydroxyadenine** have been described in hepatocytes and in other tissues in slaughtered cattle with no evidence of other disease. These accumulations are strongly birefringent under polarized light, and are seen in the cytoplasm of hepatocytes and macrophages of hepatic lymph nodes and as extracellular deposits in portal stroma and renal tubular lumens. Grossly, the portal stroma stood out as a green network, and affected lymph nodes were enlarged and the medullary sinusoids were distended with green pasty material. The crystals were identified as 2,8-dihydroxyadenine by a panel of crystallographic methods and mass spectrometry, and it was suggested that a deficiency of adenine phosphoribosyltransferase might be responsible; however, this has not yet been confirmed.

Pigments of parasitic origin are particularly associated with flukes. Heavy deposits of *black iron–porphyrin compound* are formed around the cysts and migratory pathways of *Fascioloides magna.* Lesser amounts of similar pigment are deposited about bile ducts infested by *Fasciola hepatica* (see section on Helminthic infections, below). The presence of this pigment in the hilar nodes should suggest otherwise inapparent infestations by flukes. In schistosomiasis, the liver may be gray owing to the accumulation of black pigment in Kupffer cells.

Vacuolation

The term "**vacuolar hepatopathy**" *has been used to denote multifocal or diffuse zonal hepatocellular cytoplasmic vacuolation* before a more specific term can be applied, for example, before contents of vacuoles are identified. Specific forms of hepatocyte vacuolar change include hydropic swelling, glycogenosis, and lipidosis or steatosis. **Hydropic degeneration** is an early cytoplasmic ballooning seen after various toxic and metabolic insults, hypoxia, and cholestasis. Water and sodium ion influx expands membranous compartments of mitochondria, lysosomes, and endoplasmic reticulum. The term **feathery degeneration** is applied to the type of hydropic change that occurs in hepatocytes in which there has been prolonged cholestasis. The cells are swollen and vacuolated and crisscrossed by a fine protoplasmic network that is brown with bile pigments.

Hepatic glycogenosis or **glucocorticoid hepatopathy**, often referred to as **steroid-induced hepatopathy**, is probably the most severe example of hepatocellular vacuolar change in dogs. *It involves glycogen accumulation induced by hyperadrenocorticism, due to either functional adrenocortical or pituitary tumors, or to treatment with glucocorticoids* (Fig. 2.12). The liver is enlarged and pale tan. Hepatocytes are swollen, and the cytoplasm appears as a fine, feathery reticulum that encloses multiple spaces with poorly demarcated edges. Vacuolar change may range from mild to severe, with swelling of hepatocytes from 2 to 10 times normal size, and displacement of the nucleus and organelles to the cellular periphery. Early changes are midzonal in distribution, but the zonal distribution may be very variable and may become diffuse in long-standing cases or with higher doses. Single-cell drop-out, multifocal small aggregates of neutrophils, and scattered small foci of necrosis are also commonly observed, but few hepatocytes are lost in this manner. The ill-defined vacuolar boundaries in steroid hepatopathy are readily distinguishable from spherical lipid vacuoles in hepatic steatosis. *The pathogenesis of this condition is uncertain*, since glycogen storage alone does not fully explain the influx of fluid, and possible perturbations in hepatocellular ion channels or aquaporins have not been assessed. The amount of glycogen remaining in affected cells is widely variable, a function of the original glycogen concentration, recent catabolism, and postmortem dissolution. Glycogen content can best be demonstrated after rapid fixation in alcohol, followed by staining with periodic acid–Schiff, and can be confirmed as glycogen by its sensitivity to digestion with diastase. Livers affected by glucocorticoid hepatopathy maintain normal hepatocyte functions, but the condition can be confirmed by the induction and *serum increase of glucocorticoid-inducible alkaline phosphatase* with minor or negligible increases in alanine aminotransferase.

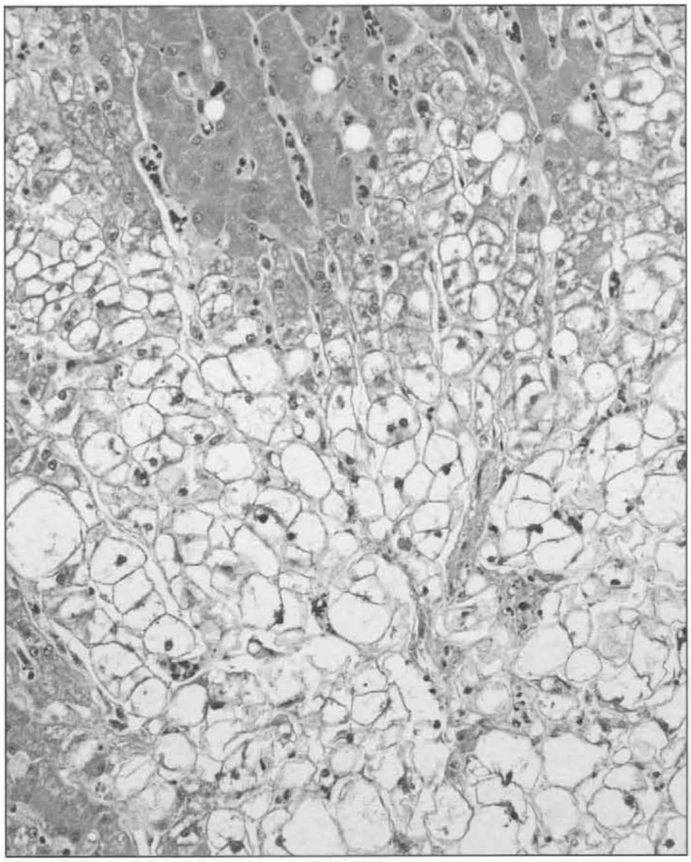

Figure 2.12 Severe **hydropic degeneration** associated with glucocorticoid administration in a dog.

Loss-of-function mutations in hepatic and renal glucose-6-phosphatase resulting in increased hepatic glycogen storage have been reported in littermate Maltese puppies. These dogs have severely debilitating problems in maintaining their blood glucose because dephosphorylation of glucose-6-phosphate is a key step in both glycogenolysis and gluconeogenesis. The condition is typified by severe hepatocellular glycogenosis resembling glucose-6-phosphatase deficiency in humans (von Gierke's disease or **glycogen storage disease type Ia**). Livers are markedly enlarged, pale, and have diffuse vacuolation of hepatocytes with large amounts of glycogen and small amounts of lipid. Renal tubular epithelium is also vacuolated. **Type II** and **type III glycogenosis** have also been reported in dogs, the latter with hepatic glycogen storage.

Characteristic hepatocellular vacuolation is also described in dogs with nodular livers presented clinically with superficial necrolytic dermatitis collectively known as *hepatocutaneous syndrome* (see below).

Hepatic lipidosis

Hepatic lipidosis *is the term most often used to describe fatty livers of animals*, whereas in humans, the term *steatosis* is more commonly used. Both refer to the *visible accumulation of triglycerides (triacylglycerols) as round globules in the cytoplasm of hepatocytes.* The threshold for application of these terms is vague because triglyceride storage and transport are normal hepatic functions, but they are appropriate when the amounts are greater than would normally be seen. Hepatic lipidosis can be physiological or pathological. Increased mobilization of triglycerides during *late pregnancy or heavy lactation in ruminants* is associated with hepatic lipidosis. The lipid represents increased transit of triglycerides in an otherwise healthy liver, so there is little diagnostic significance in mild degrees of hepatic lipidosis in lactating cows. In some of these animals, severe energy deficiency can also lead to clinical ketosis with metabolic acidosis. In addition, severe lipidosis occurs in high-producing diary cows fed diets where either the mix of available fatty acids is incorrect or lipids are oxidized and rancid.

Hepatic lipidosis is common in injured hepatocytes because the normal high throughput of fatty acids and triglycerides can be readily impeded at various points in the complex pathway of hepatic lipid metabolism and secretion of very-low-density lipoproteins (VLDL). Hepatocytes obtain some fatty acids from albumin and other carrier proteins in the portal blood, but most is hydrolyzed by sinusoidal endothelial hepatic lipase from triglyceride in chylomicrons or VLDL in plasma. Very-long-chain fatty acids are initially oxidized by acyl-coenzyme A oxidase in peroxisomes to shorter acyl-coenzyme As that can be transported via a carnitine-dependent process into mitochondria for further oxidation. While some fatty acid is utilized for energy in hepatic mitochondria, most from dietary or adipose sources is converted to triglycerides that are further processed into lipoproteins in the hepatocyte endoplastic reticulum and actively secreted into the plasma as VLDL. Endothelial lipoprotein lipase in muscle and adipose tissue hydrolyzes triglycerides in the VLDL, and the fatty acids released are utilized locally by beta-oxidation in mitochondria of muscle or re-esterified into triglyceride in adipocytes. The depleted VLDL, referred to as low-density lipoproteins, are returned by receptor-mediated endocytosis into hepatocytes where their constituents are catabolized and recycled.

The synthesis and export of VLDL in hepatocytes are energy- and resource-dependent, so any disturbance of the supply of apoproteins, phospholipids, cholesterol, or adenosine triphosphate, or physical disruption of the organelles involved in synthesis, assembly, and secretion has the potential to inhibit lipoprotein synthesis or secretion. Incoming fatty acids can continue, allowing triglyceride globules to accumulate in the hepatocyte cytoplasm. In some species, damage to peroxisomes reduces the initial peroxisomal oxidation step for catabolism of long-chain fatty acids and also upregulates various genes regulated by peroxisome-proliferator activated receptor-α. Lipoproteins in the endoplasmic reticulum, Golgi, and membrane-bound secretory vesicles can also accumulate if there is selective damage to the distal secretory apparatus. Damage by various toxic and hypoxic insults will not lead to triglyceride accumulation in hepatocytes if supplies of mobile triglycerides from adipose tissue or the diet are low.

The microscopic appearance of triglyceride globules in hepatocytes ranges from small discrete microvesicles to large coalescing macrovesicles (Fig. 2.13). Small droplets of fat, usually in a periportal and juxtasinusoidal position, can normally be found in the liver. The amount of triglyceride present in the earlier stages of degeneration while globules are small is usually much more than can be appreciated microscopically. Increased mobilization and acute injury tend to produce microvesicular lipidosis in which numerous small and discrete micellar lipid globules are distributed around the central nucleus. VLDL within secretory vesicles and endoplasmic reticulum can be retained but cannot coalesce. This pattern can also occur in some toxic hepatopathies. Acute lipidosis with predominantly microvesicular accumulation tends to result in a modestly enlarged pale liver without much change in texture.

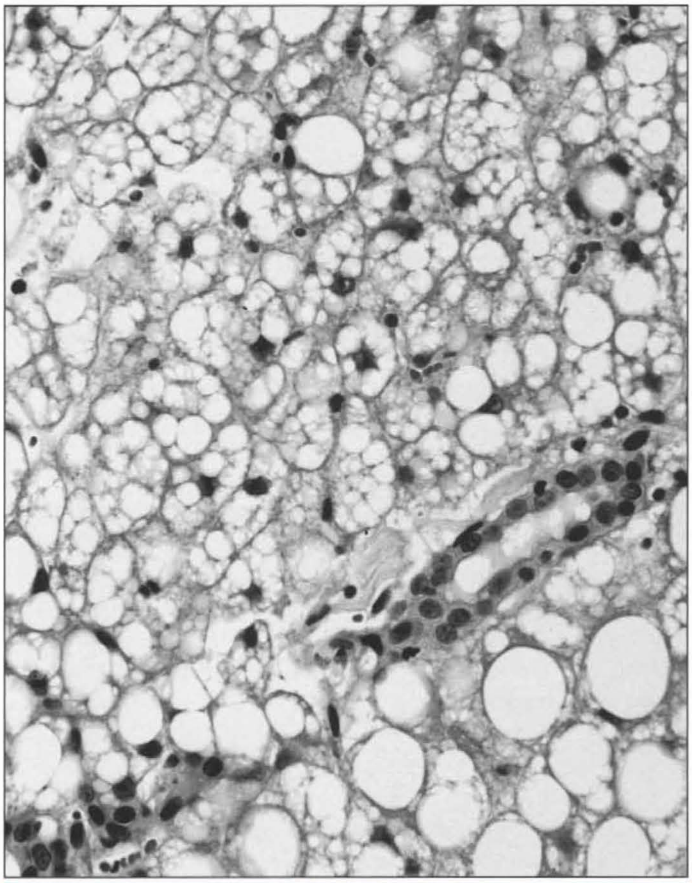

Figure 2.13 Microvesicular and macrovesicular **hepatic lipidosis** in a cat.

In some more protracted toxic injuries, lipid globules tend to coalesce into large central macrovesicles that displace the nucleus. The pathogenesis of the larger globules is not well understood, but it involves some alteration of the globule–cytoplasm interface that normally prevents coalescence of the small micellar globules. These fatty livers tend to be more yellow, enlarged, and the texture is more friable. Each hepatocyte usually contains one large globule (macrovesicle) that alters the contour of the cell and displaces the nucleus. The sinusoids are compressed and appear underperfused, and the tissue at low magnification resembles adipose tissue. In severe degeneration, the liver is moderately or greatly enlarged, with a uniform light yellow color. The edges are rounded, and the surface is smooth. The cut surface has a diffuse greasy appearance, or a red and yellow lobular pattern if there is also hepatic congestion or zonal necrosis (Fig. 2.14). *In severe diffuse hepatic lipidosis, the parenchyma is less dense and portions will float in water or fixative.*

Assessment of the functional significance of lipidosis depends on the differentiation of physiological lipidosis due to increased mobilization by otherwise normal hepatocytes from pathological changes that represent some degenerative change in hepatocytes. However, *fat accumulation is a sensitive response to hepatocellular injury and can occur in the absence of other obvious alterations in hepatic structure or function.* The triglyceride globules themselves are not harmful to hepatocytes, so the amount of fat present is more an indicator of the duration of insult and triglyceride supply than of the severity of hepatic injury. Lipidosis is usually reversible, although a liver that has been fatty for some time is more likely to have concurrent damage, including fibrosis, pigment accumulation, and nodular hyperplasia, mostly attributable to ongoing peroxidative damage, cell turnover, and activation of stellate cells. Fatty livers are more vulnerable to a wide range of toxic and nutritional insults, so other necrogenic insults and responses can be concurrent. Increased levels of fatty acids in the liver increase the oxidative stress and contribute to membrane lipid peroxidation, reduced hepatocyte lifespan, and some local repair responses. Some chronic changes commonly seen in the *livers of old dogs* can be associated with fatty liver; these include lipogranulomas, fatty cysts, ceroid accumulation, and, rarely, mineralizing focal fibrous reactions to accumulations of cholesterol.

When lipid accumulates in large amounts, there is a tendency for *groups of the fat-laden cells to rupture or fuse and eventually form a multinucleate rim about a foamy mass of lipid.* This epithelial structure is known as a **fatty cyst**, as is the next stage, which occurs when released lipid is picked up by macrophages that form foamy aggregations in sinusoids, in the stroma of portal triads, and in hepatic venules (Fig. 2.15). Subsequent peroxidation of the less saturated fatty acids and covalent modification and polymerization of oxidized lipids form a complex of lysosomal residues known collectively as **ceroid**. This material is only slightly soluble in lipid solvents and is periodic acid–Schiff-positive, variably acid-fast, and autofluorescent. In histologic sections, ceroid pigment appears as colorless or yellow

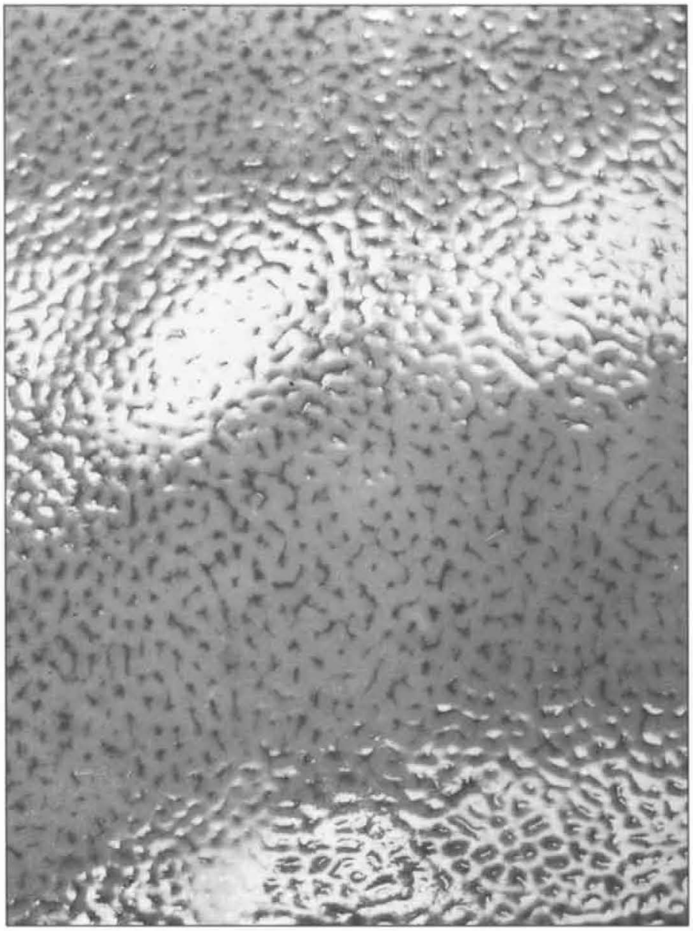

Figure 2.14 Severe fatty liver with periacinar necrosis resulting from acute anemia in a fat goat.

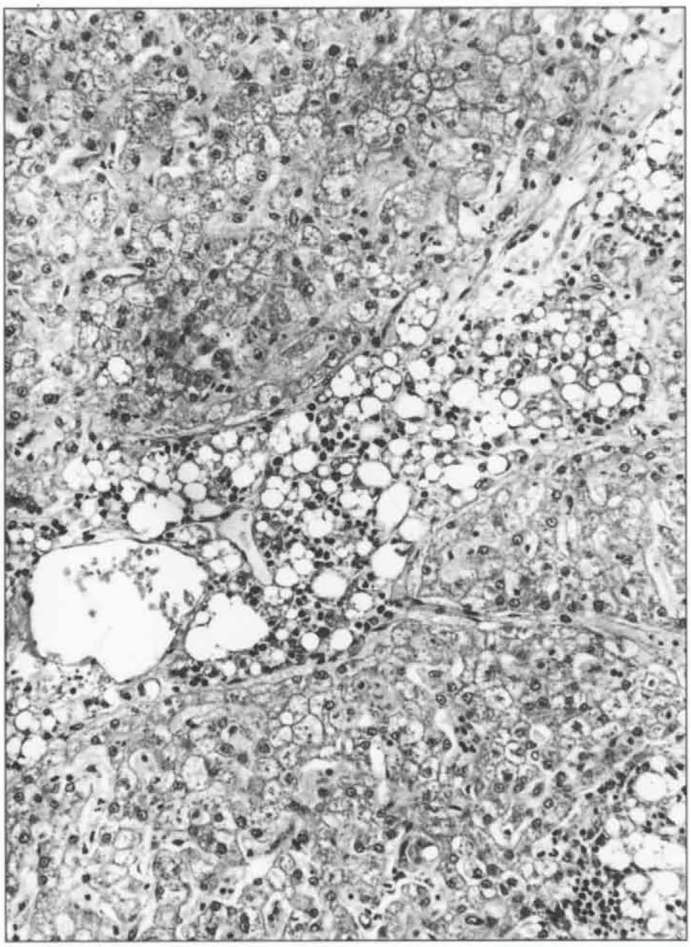

Figure 2.15 Accumulations of lipid-filled macrophages, some of which are coalescing into **"fatty cysts"** in hepatic stroma in a dog.

irregular fragments associated with the lipid globules in macrophages and, to a lesser extent, hepatocytes and portal lymph nodes that become slightly enlarged, yellow-green, and rather oily on section. If the hepatocellular lipidosis subsequently resolves, the fatty cysts with ceroid residue remain. These may be larger than, but otherwise not readily distinguished from, the more common hepatic **lipogranulomas**. These are discrete perisinusoidal clusters of macrophages and stellate cells, containing cytoplasmic lipid vacuoles, lipofuscin, and hemosiderin, that accumulate and congregate with age and hepatocellular turnover.

Occasionally in the liver of old dogs there can be found sharply defined, fibrous, stony, hard masses, usually close to the surface, sometimes as much as 3–4 cm in diameter. These masses are usually sufficiently mineralized to show up distinctly on clinical radiographs. The mineral appears to be deposited on a matrix of degenerate collagen, laid down about perivascular foci of foamy macrophages and accumulations of cholesterol. The fibrous tissue may be laid down in response to the continued presence of fat, ceroid, or cholesterol, but no reason is apparent for the strictly localized distribution of the reaction. There are no recognizable hepatocytes in these lesions.

Physiologic fatty liver *occurs in late pregnancy and heavy lactation, particularly in ruminants.* Obvious lipidosis is also seen in neonates, especially in those species whose milk is relatively rich in fat. These livers are fatty enough to be pale to the naked eye. The high rate of mobilization of triglycerides is mainly responsible for fatty liver in lactating ruminants. However, the condition is a concern because mobilization also increases when the dietary energy intake is insufficient relative to the production demands that are greatest in early lactation of high-producing cows. Insufficient dietary intake by an animal with adequate fat reserves depletes hepatocellular glycogen and initiates a heavy demand for triglycerides from adipose tissue. The liver depends primarily on fatty acid oxidation for its own considerable energy needs. It must also synthesize a large amount of protein and phospholipids for lipoprotein export to other tissues, and this can be rate-limiting, resulting in accumulation of triglyceride in the cytoplasm. In starvation, the reduced availability of protein and lipotrope cofactors such as choline can exacerbate the bottleneck. The liver is the main supplier of glucose for the brain and milk saccharides, and it adapts by converting fatty acid metabolites to glucose (gluconeogenesis) and ketones.

Acute **ketosis** of lactating dairy cows with intake insufficiency or secondary to abomasal displacement is usually associated with fatty liver with a predominantly diffuse microvesicular pattern. *Cows are more tolerant of ketosis associated with lactation than are pregnant ewes that can die from starvation-induced* **pregnancy toxemia** *and ketoacidosis.* In cows, hepatic lipidosis is predominantly periacinar, whereas it is most severe in the periportal zone in pregnancy toxemia of sheep. The hepatic changes reflect increased mobilization of triglycerides from adipose tissue rather than hepatic disease per se. In cows and ewes with increased mobilization of triglycerides, there may be indistinct foci of white discoloration of abdominal fat that tend to be obscured when adipose tissue solidifies postmortem.

Fatty liver of **diabetes** *occurs when insulin is deficient or inactive due to lack of functioning receptors.* Reduced insulin-dependent glucose uptake by cells leads to accelerated lipolysis from adipose tissue in much the same way as when energy intake is limiting. The liver is thus presented with a large load of fatty acids, and the rate by which this moves through the liver can be impeded in various ways. Insulin deficiency alone will produce fatty liver. Some cases in carnivores are also complicated by concurrent exocrine pancreatic insufficiency, so protein malabsorption can be a contributing influence on diabetic hepatic lipidosis. The periacinar hepatocytes usually show the greatest degree of lipidosis, but in advanced long-standing diabetes, the change is often diffuse and marked.

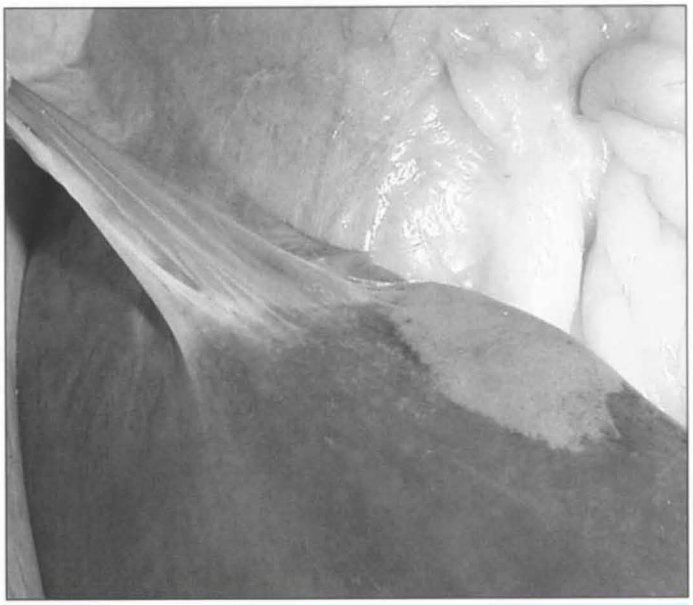

Figure 2.16 Subcapsular focal fatty change (**"tension lipidosis"**) associated with capsular ligamentous attachment in an ox. (Courtesy of AP Loretti.)

Lipoprotein synthesis and transport are dependent on oxidative metabolism, so **hypoxia** of hepatocytes leads to triglyceride accumulation. The two most common causes of hepatocellular hypoxia are *anemia* and reduced sinusoidal perfusion in *passive venous congestion.* In these situations, hepatic lipidosis is most severe in the periacinar zone provided that the adipose and dietary supply of triglyceride is sufficient. Hepatic lipidosis can be grossly visible as a yellow zonal pattern in chronic passive congestion of the bovine liver, but is less obvious in carnivores with passive hepatic congestion.

Local hypoxia is probably the basis for another example of fatty liver. Small, sharply demarcated patches of intense fatty infiltration are often seen in bovine livers at or adjacent to sites of capsular fibrous adhesions – so-called "**tension lipidosis**" (Fig. 2.16). These patches are neither swollen nor shrunken, usually extend less than 1 cm into the parenchyma, and are of the same consistency as normal liver. The acinar structure of these lesions is undisturbed, but the hepatocytes therein show pronounced lipidosis, presumably related to interference with local perfusion caused by tensions transmitted to the parenchyma by the adhesion.

Fatty liver due to **intoxication** is common. There are several stages of the cycle of hepatic lipid metabolism that can be affected selectively by various toxins to produce fatty liver. For example, it is possible experimentally to cause triglyceride accumulation by interfering with mitochondrial fatty acid oxidation with sublethal doses of cyanide, or by inhibiting apolipoprotein synthesis by administration of orotic acid. Most toxins that cause fatty liver in naturally occurring situations, however, also produce a greater or lesser degree of hepatocellular necrosis. Fatty liver occurring as a manifestation of toxic hepatic disease will be further discussed in the section on

Toxic hepatic disease, below, but the generalization may be made here that most important veterinary hepatic intoxications cause widespread membrane damage and/or disturbance of protein synthesis. These cause lipid accumulation in the hepatocyte by interfering with lipoprotein synthesis and export, as well as with fatty acid oxidation. *Lipidosis requires time and a negative energy balance to develop, so it is more likely to occur in toxicoses with a longer clinical course.* However, if adipose reserves are depleted, there is less lipid available to accumulate in the liver.

While fatty liver in domestic animals is more frequently associated with generalized interferences with energy metabolism, there are some specific **nutritional deficiencies** that will produce fatty liver. These have usually been defined under experimental conditions. *Choline deficiency*, in conjunction with deficiency of other lipotropic factors such as *L-methionine* and *vitamin* B_{12}, rapidly produces fatty liver, largely as a result of reduced synthesis of phosphatidylcholine, a component of secreted lipoproteins. Fatty liver in experimental choline deficiency involves lipid peroxidation and increased hepatocellular turnover, leading to cirrhosis and neoplasia. It is unlikely that primary choline deficiency occurs in domestic animals, but other lipotrope deficiencies have been reported.

Ovine white-liver disease, first described in lambs in New Zealand and now known to occur in southern Australia, the UK, and continental Europe, and in goats, is a syndrome of ill-thrift, anorexia, mild normocytic normochromic anemia, occasionally with photosensitization and icterus. The condition is associated with low liver cobalt levels and low plasma concentrations of vitamin B_{12}, and the disease has been shown to be *cobalt- and vitamin* B_{12}*-responsive.* Lambs up to 1 year of age are more commonly affected than ewes, and pastures are likely to be adequate at the times of peak incidence in late spring and early summer.

In the early stages, the liver changes consist of vacuolar accumulation of triglyceride in hepatocytes, usually most severe in the periacinar zones. In addition, ceroid pigment is present in all cases, early in hepatocytes and later also in sinusoidal cells and macrophages. The fatty change may be very severe in the early stage, the liver being grossly swollen. A moderate degree of bile ductular proliferation is also a consistent feature (Fig. 2.17), and the epithelium of the smaller ductules in the triads is dysplastic. Spongy degeneration of cerebral white matter, typical of the hyperammonemia of hepatic failure, is present in some cases.

Experimental feeding of a diet low in cobalt to sheep resulted in reduced growth rate, anorexia, lacrimation, alopecia, and emaciation, and marked reduction in plasma and liver vitamin B_{12} concentrations. At necropsy, livers were pale, swollen, and fatty, with severe fatty degeneration characterized by accumulation of lipid droplets and lipofuscin in hepatocytes, dissociation and hepatocyte necrosis evident on histology, characteristic of spontaneous cases of ovine white-liver disease. The disease can be produced in cobalt-deficient sheep fed diets high in propionate precursors, which may help explain the explosive nature of outbreaks on lush pasture.

Hepatic lesions similar to lipotrope-deficient forms of experimental nutritional cirrhosis have been reported in sheep, goats, cattle, deer, and pronghorn antelope from Texas, New Mexico, and northeastern Mexico. *Hard yellow-liver disease, or* **hepatic fatty cirrhosis**, *is a progressive, chronic disease characterized by weight loss and hepatic encephalopathy.* The disease typically appears in years following above-average winter rains, followed by drought conditions in the summer months. Grossly visible liver lesions in sheep begin in the subcapsular hepatic parenchyma along the porta hepatis as pale yellow, firm areas, spreading peripherally to involve approximately 80% of the liver in the final stages of the disease. Microscopic changes include accumulation of fine cytoplasmic lipid droplets in periacinar hepatocytes, later involving the entire lobule, with rupture and formation of fatty cysts. Periacinar fibrosis accompanies the ruptured fatty cysts, progressing to widespread bridging periacinar fibrosis, with islands of regenerating hepatocytes. Kupffer cells and macrophages in regional lymph nodes, spleen, and lung contain abundant ceroid. The etiology of this condition is unknown, although unidentified hepatotoxins, possibly altering lipoprotein synthesis and secretion, combined with nutritional stress, have been postulated.

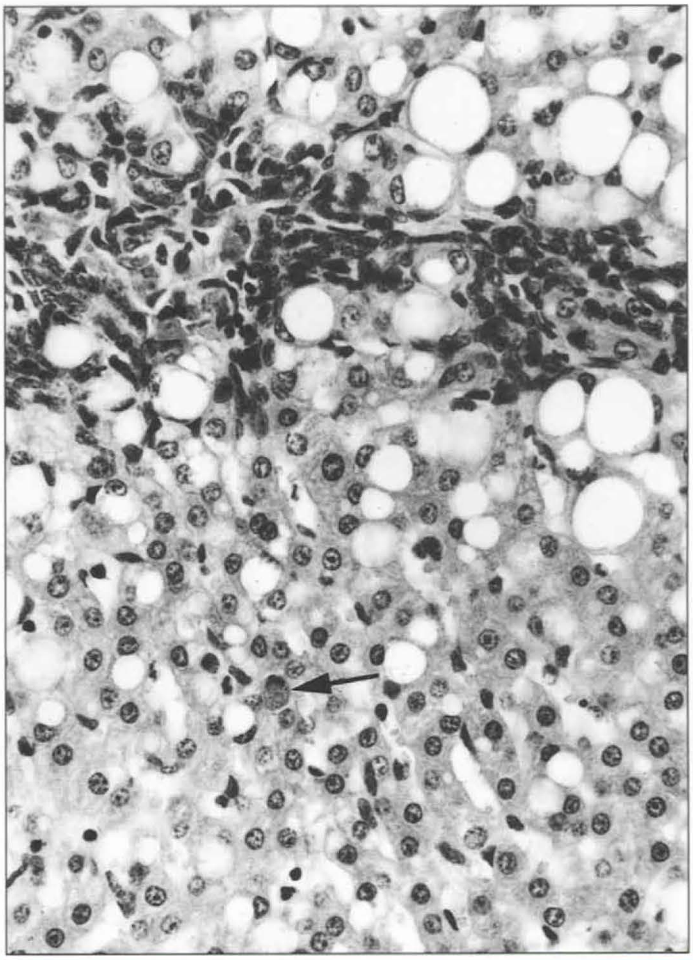

Figure 2.17 Fatty change and cholangiolar proliferation in **ovine white liver disease**. Note ceroid in sinusoidal macrophage (arrow). (Courtesy of S.McOrist.)

Equine hyperlipemia *is almost exclusively a disease of ponies*, and among these the Shetland breed predominates. The disease has also been reported in American miniature horses and donkeys. The disease is usually fatal after about a week. Pregnant or lactating mares are most likely to develop the disease, particularly if they are excessively fat and have recently suffered reduced feed intake due to onset of parturition, conditions such as laminitis or parasitism, or other causes of stress. The clinical course is marked by somnolence, complete anorexia, and colic, progressing to mania in some cases, although most simply become progressively more depressed. Some ponies develop ventral subcutaneous edema and most develop moderate diarrhea. All show marked increase in plasma triglyceride concentration, predominantly VLDL, but all other lipid fractions are elevated, and the lipemia is sufficient to impart a striking milkiness to the serum and blood. Metabolic acidosis is a consistent feature in animals that die.

The liver at necropsy is severely fatty and may have ruptured; the lipidosis also extends to heart and skeletal muscle, kidney, and adrenal cortex. *The hepatic lipidosis is remarkable only by its severity*; there may be some focal hepatocellular necrosis, and there is consistent prolongation of bromosulfophthalein retention times and elevation of serum alkaline phosphatase levels. Evidence of disseminated intravascular coagulation is seen as serosal hemorrhages and microscopic thrombi in various organs, and even gross infarction of myocardium and kidney. *Small lipid emboli* may be detected in frozen sections of lung, myocardium, and brain in these animals; their relationship to the microthrombosis is uncertain.

The pathogenesis of this disease is obscure. Since the excess lipid in liver and blood is in the form of triglyceride, the implication is that the liver is capable of esterifying fatty acid mobilized from depot fat. The triglyceride thus formed is presumably then exported to the plasma as VLDL until the plasma transport mechanisms are saturated, at which stage lipids begin to accumulate in the hepatocytes. Another possibility is that there is an inability on the part of all tissues other than the liver to utilize fatty acids from VLDL at the normal rate, while triglyceride synthesis from fatty acids continues in the liver.

It has been proposed that an underlying cause of pony hyperlipemia is a *comparative resistance to insulin* in susceptible animals, and that this is compounded in stressful episodes by *increased levels of circulating cortisol.* Various steroid hormones, including glucocorticoids, have been shown to interfere with insulin action, and hyperlipemic ponies often have elevated plasma insulin levels, which suggests reduced function of insulin receptors. However, plasma ketones are much less consistently elevated; this suggests that there is not much evidence of the increased ketogenesis that one might expect in insulin resistance.

Hepatic lipidosis is common in companion animals. Dogs eating diets deficient in vitamin E may develop severe hepatic lipidosis, but there are many circumstances in companion animals where the exact cause for individual cases of lipidosis cannot be determined.

The syndrome of **feline hepatic lipidosis** *most commonly occurs in obese, nutritionally stressed female cats, presented with vomiting, anorexia, weakness and weight loss, jaundice, and hepatomegaly* (Fig. 2.18). Neurobehavioral signs indicative of hepatic encephalopathy, other than drooling and depression, are rarely reported. Affected cats generally have hyperbilirubinemia, and a significant increase in serum alkaline phosphatase activity, in the face of normal or modest increases in γ-glutamyltranspeptidase activity. Untreated, the mortality rate is high. The liver has diffuse, macrovesicular or microvesicular lipidosis, by definition affecting greater than 50% of hepatocytes. Focal or zonal lipid accumulation in less than 50% of the parenchyma is considered more likely to be physiologic, or associated with other systemic abnormalities, rather than idiopathic feline hepatic lipidosis. Bile pigment accumulates in canaliculi or Kupffer cells and can be confused with lipofuscin and ceroid.

The pathogenesis of hepatocellular triglyceride accumulation in this disease is obscure, and likely multifactorial, involving increased mobilization and uptake of nonesterified fatty acids by the liver, alterations in formation and release of VLDL, and impaired oxidation of fatty acids within hepatocytes. Ultrastructural studies have demonstrated decreased numbers and abnormal morphology of hepatic peroxisomes as well as mitochondria, both of which are important in the oxidization of fatty acids, but whether these changes are significant or simply adaptive responses is unknown. Starvation may reduce the availability of proteins, choline, and other precursors necessary for lipoprotein synthesis.

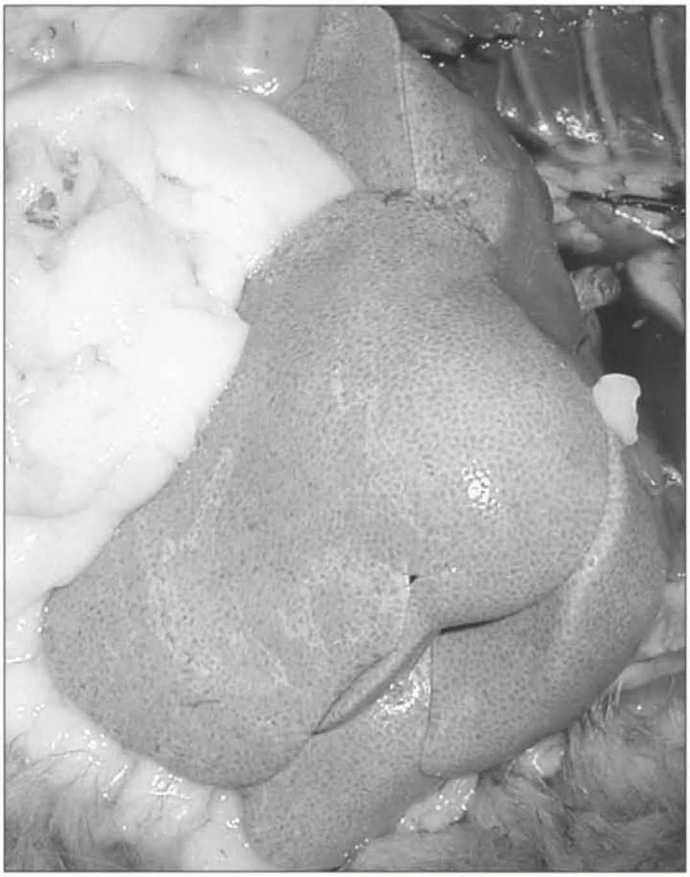

Figure 2.18 Hepatomegaly due to **hepatic lipidosis** in a cat. (Courtesy of AP Loretti.)

Severe hepatic lipidosis can also develop in cats *concurrent with or secondary to other major medical problems* such as diabetes mellitus, which alter metabolism of fat. Acute pancreatitis appears to be an important predisposing disease to secondary hepatic lipidosis in cats – an association with clinical significance as it has a poorer prognosis than uncomplicated idiopathic hepatic lipidosis. Secondary hepatic lipidosis has also been reported with concurrent inflammatory liver disease such as cholangiohepatitis, renal disease, small intestinal disease, neoplasia, and hyperthyroidism.

Familial hyperlipoproteinemia has been described in cats, associated with *congenital lipoprotein lipase deficiency.* The condition is characterized by lipid and ceroid accumulation in liver, spleen, lymph nodes, kidney and adrenal glands, multifocal xanthomas, and focal arterial degenerative changes. An autosomal recessive mode of inheritance is suspected. **Idiopathic hyperlipoproteinemia** has also been reported in Miniature Schnauzer dogs, although the metabolic defect has not been identified. Affected dogs have fasting lipemia,

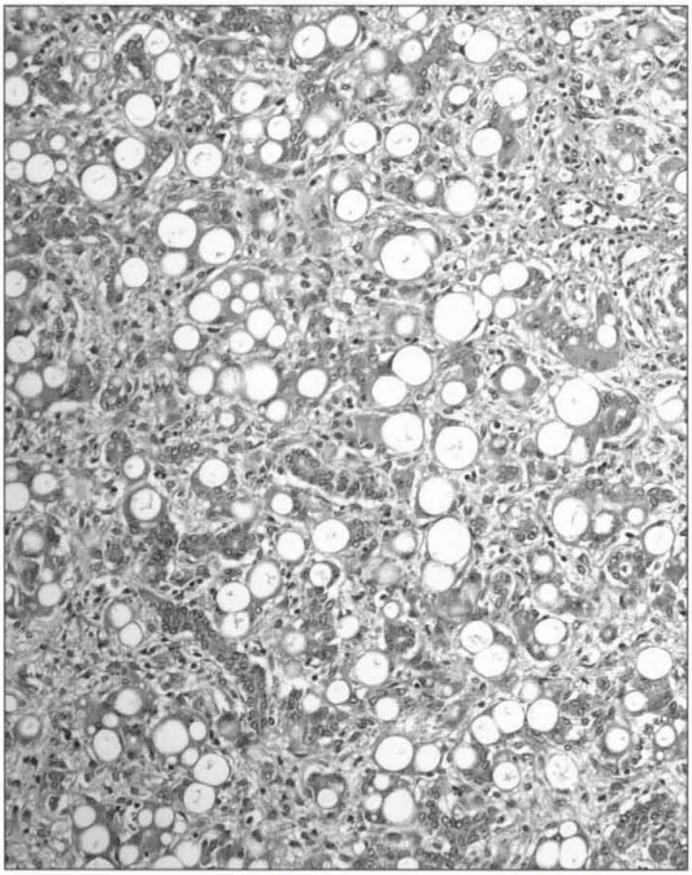

Figure 2.19 Hepatic lipidosis, portal fibrosis, and bile duct hyperplasia due to **hepatic lipodystrophy** in a Galloway calf. (Courtesy of MJ Hazlett.)

hyperchylomicronemia, and elevated plasma VLDL. Affected animals may develop severe vacuolar hepatopathy associated with both glycogen and triglyceride accumulation, with eventual stromal collapse and regenerative nodule formation.

An incompletely characterized condition known as **hepatic lipodystrophy** has been recognized in pedigree Galloway calves since 1965. Calves initially appear normal but develop lethargy, tremors, and opisthotonos, and die by 5 months of age. On postmortem examination, affected calves have an enlarged, pale, mottled liver. Histologically, there is marked hepatic lipidosis with portal fibrosis and bile duct hyperplasia (Fig. 2.19). Vacuolar changes in the white matter of the brain are consistent with hepatic encephalopathy. A metabolic defect has been proposed.

Lysosomal storage diseases

In common with other tissues in animals with heritable deficiency of specific lysosomal enzymes, liver cells may accumulate substrates normally catabolized by the missing enzyme. These lysosomal stores can be less obvious in the liver than in other tissues such as the central nervous system, and although they are unlikely to affect hepatic function, they can sometimes be recognized in liver biopsies. However, *hydropic and fatty changes in hepatocytes can obscure or lead to misidentification of lysosomal storage vacuoles.* Kupffer cells and bile duct epithelium may be more severely affected than hepatocytes, which have additional catabolic and excretory pathways, including the ability for lysosomal exocytosis into the bile canaliculi. In animals with ceroid lipofuscinosis, lysosomal storage is minimal in hepatocytes compared to that in the brain. Examples of storage disorders associated with hepatomegaly and abnormal hepatic or Kupffer cell lysosomal inclusions include *GM_1 gangliosidosis* (beta-1-galactosidase deficiency) in cats, *mucopolysaccharidosis type I* (alpha-L-iduronidase deficiency) in dogs (Fig. 2.20), and *α-mannosidosis* in cats. These and others are discussed in more detail in Vol. 1, Nervous system.

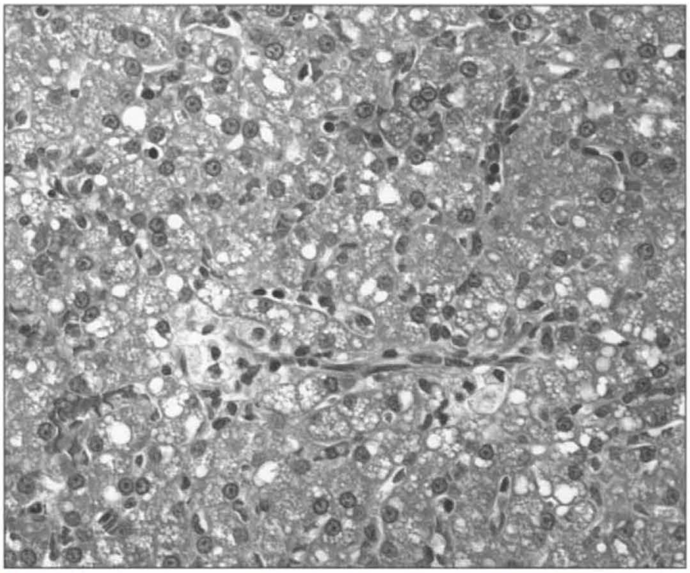

Figure 2.20 Vacuolated hepatocytes and Kupffer cells due to lysosomal accumulation of glycosaminoglycans in **mucopolysaccharidosis type I** (alpha-L-iduronidase deficiency) in a dog.

Toxic inhibition of lysosomal hydrolases can cause acquired lysosomal storage disease. For example, *swainsonine* in various plants inhibits α-mannosidase. Some *amphophilic drugs* can partition into the lysosome and inhibit normal catabolism of membrane phospholipids. This can appear as a systemic phospholipidosis in which Kupffer cells and hepatocytes can be affected. While this is a well-recognized side-effect of some drugs in experimental exposures, it is unlikely to occur with regular therapeutic regimens.

Amyloidosis

In most species, *hepatic amyloidosis is usually part of generalized amyloidosis.* **Systemic amyloidosis** of domestic animals is typically associated with overproduction of amyloid A (AA), an amino-terminal fragment of serum amyloid A, a highly inducible acute-phase protein in most species. In humans, AA amyloidosis occurs either as a familial trait (familial Mediterranean fever), or secondary to a sustained acute-phase reaction in chronic inflammatory or neoplastic diseases. Amyloid infiltration of the liver occurs sporadically in cattle, horses, dogs, and cats as a secondary response to chronic disease or tissue-destructive process. In horses, hepatic amyloidosis occurs chiefly as a result of chronic inflammation and has been well recognized in horses used for the production of hyperimmune serum. Horses may develop icterus and other signs of hepatic failure, but cattle die first of the primary disease or from uremia resulting from concurrent renal amyloidosis. Dogs and cats typically develop signs of renal dysfunction, although cats may be presented with spontaneous hepatic rupture. **Familial AA amyloidosis** is recognized in Chinese Shar-Pei dogs. The condition resembles familial Mediterranean fever in humans, and is characterized by febrile episodes, swollen hock syndrome, and the development of renal and sometimes hepatic amyloidosis. Familial AA amyloidosis is also recognized in Abyssinian cats, and suspected in Siamese and Oriental cats.

Affected livers in horses are pale, enlarged with rounded edges, friable, and prone to fracture (Fig. 2.21); in cattle, affected livers may be firm. Amyloid is deposited first in the parenchyma about the portal tracts and appears gray and waxy. Amyloid is deposited in the perisinusoidal space between the sinusoidal lining and hepatocytes (Fig. 2.22) and is sometimes found in the walls of the afferent vessels. The surrounded hepatocellular cords atrophy. On hematoxylin and eosin staining, amyloid appears as *homogeneous eosinophilic amorphous extracellular material*. Staining with Congo red results in apple-green birefringence when viewed with polarized light. Thioflavine T stain produces yellow-green fluorescent staining of amyloid viewed under ultraviolet light. Affected livers are predisposed to rupture and bleed.

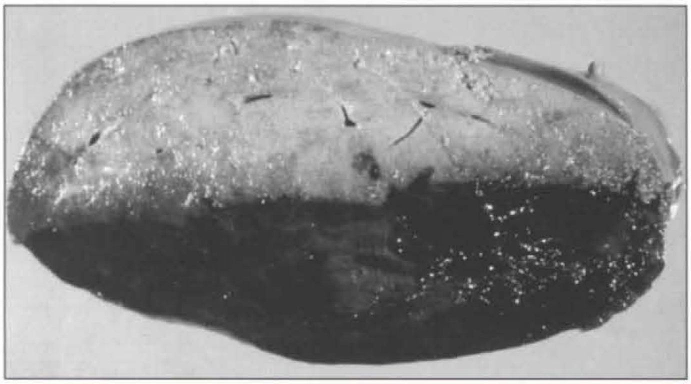

Figure 2.21 Section of large, pale liver with massive subcapsular hemorrhage in **amyloidosis** in a horse.

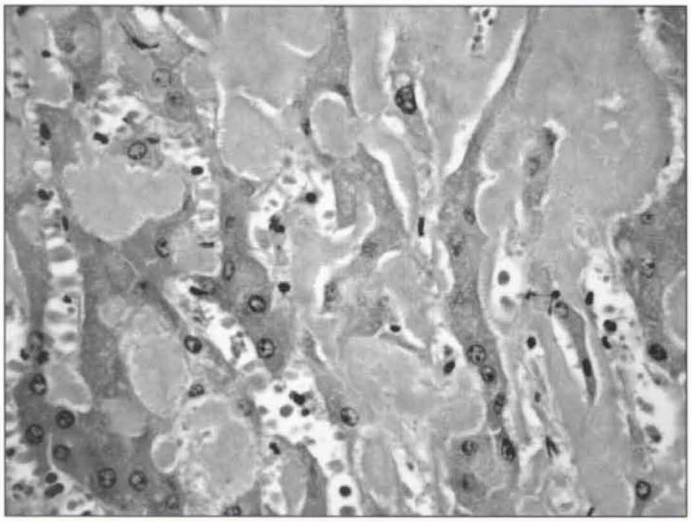

Figure 2.22 **Amyloid** in the space of Disse compressing the hepatocellular plates in a horse.

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PATTERNS OF CELL DEATH IN THE LIVER

All cells in the liver can die after lethal injury, but most studies of cell death in vitro and in experimental and spontaneous liver disease deal with hepatocellular death. Typical **necrosis** and **apoptosis** of hepatocytes (as described below) can be differentiated by morphologic and other features in routine sections of a diseased liver. **Necrosis** *is generally regarded as a somewhat passive but more severe injury to cell membrane integrity or other vital functions*, leading to enzyme leakage, inflammation, and tissue repair. By comparison, **apoptosis** *is a form of programmed cell death* that permits removal of cell debris without such leakage or inflammation. However, it is becoming evident that there is *considerable overlap between the two main forms of cell death*, and some patterns of cell death, including "**single-cell necrosis**" cannot be neatly assigned to either. There is increasing evidence of nonapoptotic patterns of programmed cell death, for example during epithelial exfoliation and in death of large nonreplicating cells, e.g., neurons, that involve some of the effectors and events responsible for apoptosis, but the affected cells do not fragment into smaller apoptotic bodies that can be efficiently cleared by phagocytosis.

Types of cell death

In routine diagnostic settings, the terms necrosis, apoptosis, and single-cell necrosis can be based on descriptive criteria recognized in routine hematoxylin and eosin-stained sections of liver (Fig. 2.23). There have been attempts to refine the terminology and criteria for cell death in the liver, but this is still hindered by our lack of understanding of different cell death pathways and responses of hepatocytes in various diseases. Some recommend the use of the term necrosis for all death responses in hepatocytes, with further qualification by descriptors such as apoptotic, oncotic (cell swelling), and single-cell necrosis. This approach can be useful in experimental studies in which the appropriate cytologic cell death markers can be applied to justify the descriptor.

One might expect that the different types of cell death might be informative in relation to causes and pathogenesis. However, *many insults can initiate either necrosis or apoptosis of hepatocytes*; these include hypoxia, reactive oxygen metabolites, hepatotoxic chemicals, viral infections, bacterial toxins, and inflammation. Some of the molecular events are common to both. Mitochondrial damage and several other activation responses are common to apoptosis and necrosis, and programmed cell death responses can be activated before they are overwhelmed by more intense injury responsible for necrosis. Susceptibility therefore depends on many factors, including the level and duration of insults, replication status, and integrity of the various homeostatic and cytoprotective functions. For a detailed review of these complex death pathways as they apply to the liver, readers are referred to the bibliography.

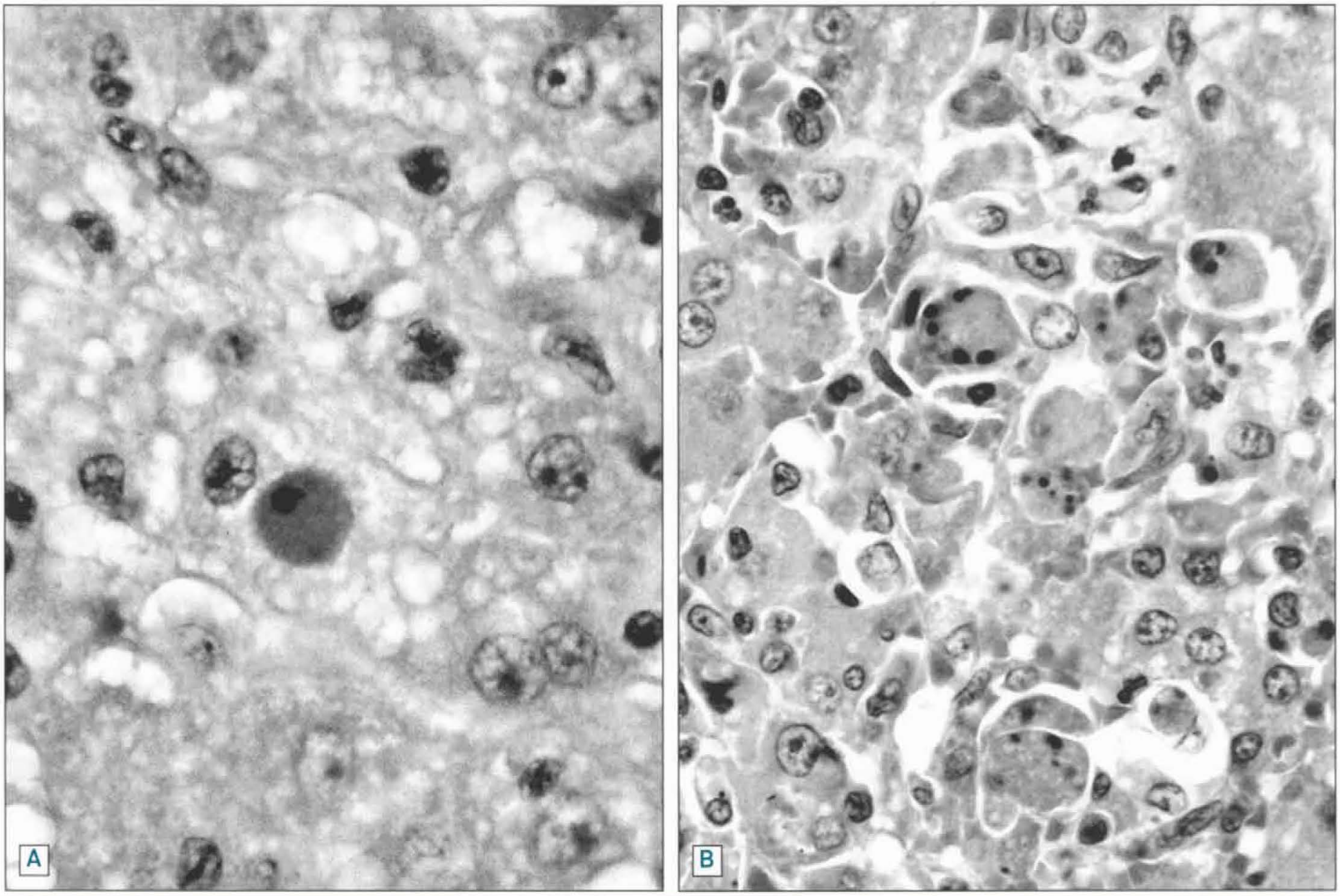

Figure 2.23 **A. Apoptotic hepatocellular fragment** engulfed by an adjacent hepatocyte, without a cellular inflammatory reaction. **B.** Multiple hepatocytes undergoing **"single-cell necrosis"** in association with an inflammatory response. The dead cells exhibiting karyorrhexis are intact and extracellular.

Apoptosis

Hepatocytes can undergo typical **apoptosis** (Fig. 2.23A), *with nuclear and cell fragmentation into smaller apoptotic bodies bound by intact plasma membrane that are rapidly phagocytosed and degraded by neighboring hepatocytes or Kupffer cells.* The rapid disappearance of these fragments means that very few in a section can indicate a rapid rate of hepatocellular death and turnover. Rapid phagocytic removal of apoptotic hepatocyte fragments typically minimizes secondary inflammation, although in some instances the capacity to remove dead cells may be exceeded. Apoptotic fragmentation occurs most obviously in lethally injured replicating cells, elicited by insults that damage DNA and activate cell cycle checkpoints. However, most adult hepatocytes are not replicating and remain in the G_0 phase of the cell cycle, so apoptosis is more likely to be observed in livers that are immature, regenerating, or hyperplastic. Apoptosis can also be observed during acute atrophy and during hepatic involution after adaptive hypertrophy.

Apoptosis can be initiated by extrinsic or intrinsic events. *Extrinsic mechanisms* can be activated via cell surface death receptors by cytokines such as the fas ligand and tumor necrosis factor-α. *Intrinsic mechanisms* involve either DNA damage with activation of p53 and p21, or mitochondrial injury with release of proapoptotic factors. Activators of the latter pathway include oxidative stress, redox changes, covalent binding, and lipid peroxidation, all of which are also necrogens for the liver. These increase mitochondrial membrane permeability, either through direct membrane damage, or more commonly by opening of regulated transmembrane pores formed by *bax* and other members of the *bcl-2* family. Cytochrome C and other intermembrane mitochondrial factors form an apoptosomal complex that activates the distal caspase pathways that execute many of the cell fragmentation events.

Both extrinsic and intrinsic pathways lead in turn to disruption of the cytoskeleton and nucleus primarily through the activation and direct action of caspases 3, 6, and 7. *An important feature of apoptosis is the requirement for adenosine triphosphate (ATP) to initiate the execution phase.* If the degree of mitochondrial damage is sufficient to exhaust ATP stores, membrane ion homeostasis deteriorates and leads to a mechanism of cell death more consistent with necrosis. For example, while mild to moderate oxidative stress may initiate intrinsic pathways of apoptotic cell death, processes leading to marked oxidative stress typically cause cell death by necrosis due not only to the severity of mitochondrial damage, but also to direct inhibition of the proapoptotic caspase cascade.

Injury to the endoplasmic reticulum (ER) leads to "*ER stress*" that can also activate the distal caspase pathway by a separate mechanism. Hepatocytes are also susceptible to an intrinsic mechanism of apoptosis that is active after removal of hepatotrophic influences, including those that function via cell adhesion or via the constitutive androstane receptor, and other nuclear receptors.

Phagocytosis of apoptotic fragments follows flipping of phosphatidylserine from the inner leaflet of the plasma membrane to the outer leaf, where it can then be recognized by the phosphatidylserine scavenger receptor. Various assays can be used to detect portions of these pathways in hepatocytes; caspase-cleaved cytokeratin 18 can be recognized by antibodies to an internal domain, while chromatin fragmented by a calcium-activated endonuclease generates double-strand breaks that can be tagged by the TUNEL technique. However, there are still few diagnostic situations in which these indicators have been shown to be useful. Increased numbers of apoptotic hepatocytes can be more readily assessed with a suitable cytological marker. *In routine sections, a previous increase in apoptosis can be suggested by increased amounts of lysosomal debris and pigment in Kupffer cells, but this is not specific for apoptosis.*

Necrosis

Necrosis *is morphologically recognizable by initial cell swelling and subsequent loss of plasma membrane integrity, leading to cell lysis. Release of cellular contents typically elicits a secondary inflammatory response and provides the serum enzymes that are useful clinically to detect liver necrosis.* However, these sequelae can also occur after hepatocyte apoptosis, either because the capacity for phagocytic removal of dead cells is exceeded, or because the insult worsens such that necrosis may supervene.

Necrosis is often a consequence of profound loss of mitochondrial function involving the opening of the membrane permeability transition pore, a megachannel composed of inner- and outer-membrane proteins. This results in mitochondrial depolarization and ATP depletion. Much of the ATP in hepatocytes is used for membrane homeostasis of Na^+, K^+, and Ca^{2+} ions. ATP depletion or physical damage to membranes of the cell surface, mitochondria, and ER leads to loss of ion homeostasis, altered cellular volume regulation, and increased intracellular calcium ion concentrations. Activation of calcium-dependent endonucleases, proteases (e.g., calpains), and phospholipases is responsible for the terminal events in necrosis. Some authors prefer the term "*oncosis*" to emphasize the cell swelling response in necrosis to contrast with cell shrinkage in apoptosis.

The term **coagulative necrosis** *may be applied to groups or zones of intact but dead hepatocytes that have shrunken slightly, stain intensely with eosin, and may have visible but distorted nuclei* (Fig. 2.24). These cells may also be dehydrated but, unlike apoptosis, the removal of water is not an active process and the affected cells do not undergo spontaneous fragmentation. It seems that coagulative necrosis, which is often seen in acute hepatotoxicity, is the *result of sudden and catastrophic denaturation of cytosolic protein*, which imparts a rather dense, rigid texture to the dead cells, somewhat preserving their shape. *The term* **lytic necrosis** *has been used for areas of necrosis in which the hepatocytes are disintegrating, usually in the presence of infiltrating phagocytes, especially neutrophils* (Fig. 2.25). This is consistent with the later stages of postnecrotic inflammation that follow necrosis rather than apoptosis.

Single-cell necrosis

Hepatocytes sometimes die in a spotty or random manner in a form of cell death referred to as "**single-cell necrosis**" (Fig. 2.23B). The Councilman body in viral hepatitis is a classical example in this process. Hepatocytes remain intact but shrink into a single rounded detached cell that is more slowly removed by macrophages, preceded by a few neutrophils. Single-cell necrosis has some features of apoptosis, but the pattern of nuclear and cytoplasmic degeneration and the inflammatory consequences are more consistent with those that follow necrosis. Intact hepatocytes are too large to be phagocytosed so they are more likely to leak cytoplasmic enzymes and elicit an inflammatory response. Single-cell necrosis of hepatocytes often occurs in livers in which more typical apoptotic fragments are also present,

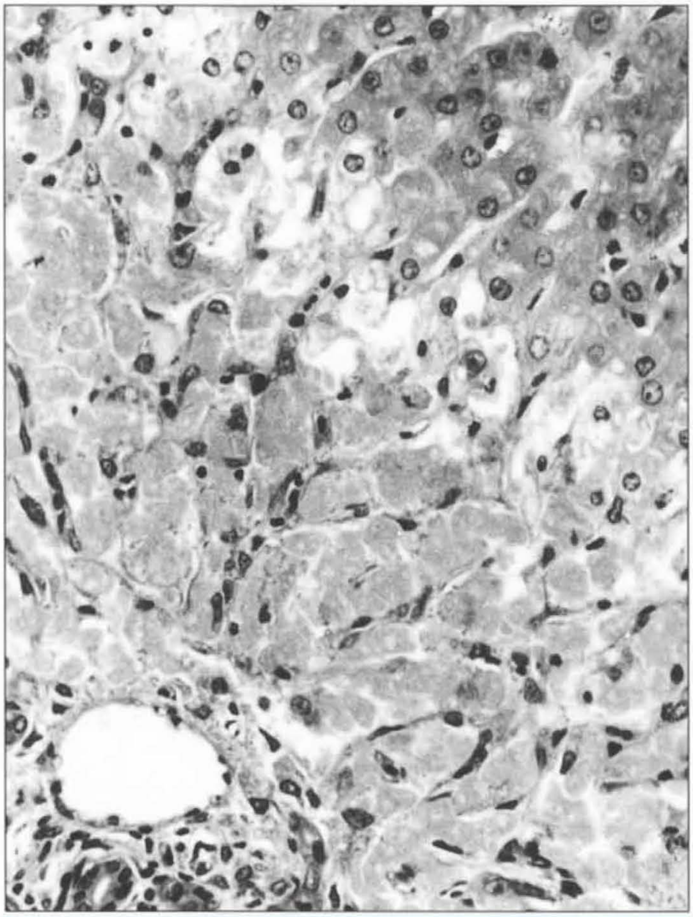

Figure 2.24 Coagulative necrosis of periportal hepatocytes due to ngaione poisoning in a sheep. (Courtesy of AA Seawright.)

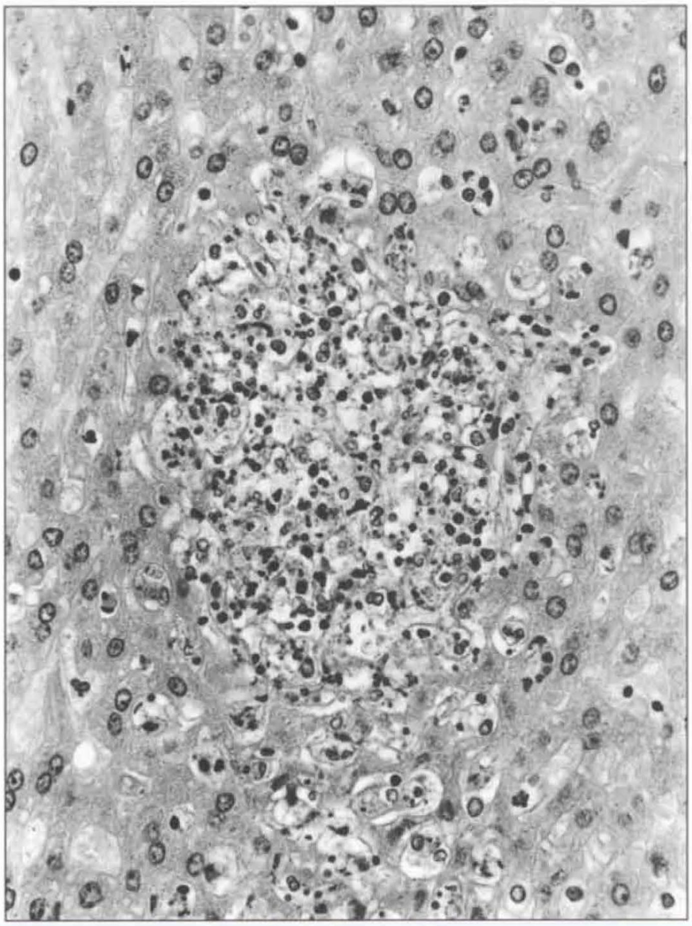

Figure 2.25 Focal inflammation and **lytic necrosis** in salmonellosis.

but in some conditions, individual necrotic cells are observed with the expected neutrophil and phagocyte response, indistinguishable from those in focal or zonal necrosis. It remains to be seen if non-apoptotic programmed cell death is responsible for some forms of single-cell necrosis.

Loss of cell anchorage can activate a form of programmed cell death related to apoptosis that is referred to as **anoikis**. Detached cells can die intact, as in exfoliation, or undergo typical fragmentation into apoptotic bodies. Hepatocyte survival in vitro depends on their integrins that adhere to the extracellular matrix, but it is still unknown how loss of these contributes to cell death of hepatocytes in the intact liver.

Tissue patterns of cell death

Focal necrosis

Focal necrosis is very common in necropsy material. The lesions are microscopic or barely visible to the naked eye and are usually numerous. Their designation as focal depends on their size and on a random distribution relative to the acini. There may be a tendency for focal necrosis to occur nearer to the axial portal vessels than to the periphery of the circulatory fields, and to be concentrated in some acinar agglomerates rather than others.

Focal necrosis occurs in many infections, parasitic migrations, and instances of biliary obstruction, and in these the designation **focal hepatitis** will often be more appropriate, since most are attended by some degree of focal inflammation. The infectious causes may be viral, such as *Equid herpesvirus 1* in the fetus (Fig. 2.26), or bacterial. Many septicemic bacterial infections consistently produce focal hepatic lesions; examples are salmonellosis, tularemia, pseudotuberculosis, listeriosis in the fetus and newborn, and *Mannheimia haemolytica* septicemia in lambs. The focal necrosis may be the outcome of a Kupffer cell reaction, as in salmonellosis, or of bacterial embolism. The cause can usually be determined by histologic examination.

In cattle, focal necrosis in few or many visible foci is common at necropsy and is common enough to be important at slaughter; it is responsible for the descriptive appellation "*sawdust liver.*" The pathogenesis is not known and probably varies, but it may be caused by organisms from the gut that reach the liver in the portal blood. *The lesion is not specific and consists of focal parenchymal necrosis with disruption of reticulin fibers and infiltration of neutrophils and lymphocytes* (Fig. 2.27); frank suppuration does not occur. This lesion is said to be more frequent in livers from feedlot-fattened cattle.

Focal necrosis in biliary obstruction follows rupture of distended canaliculi or smaller cholangioles, with the formation of small bile lakes. The yellow pigment is readily visible microscopically and provokes small granulomas with giant cells.

Focal necrosis is of very little functional significance for the liver, even when numerous. They heal with some scarring, but this too probably disappears in time. They are of diagnostic importance in some diseases

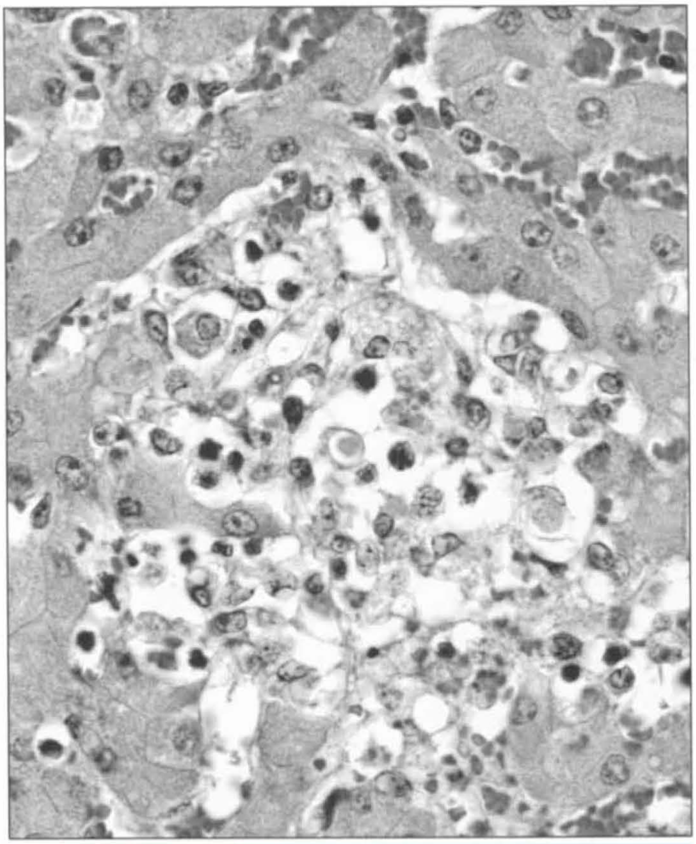

Figure 2.26 Focal necrosis due to *Equid herpesvirus 1* in a foal.

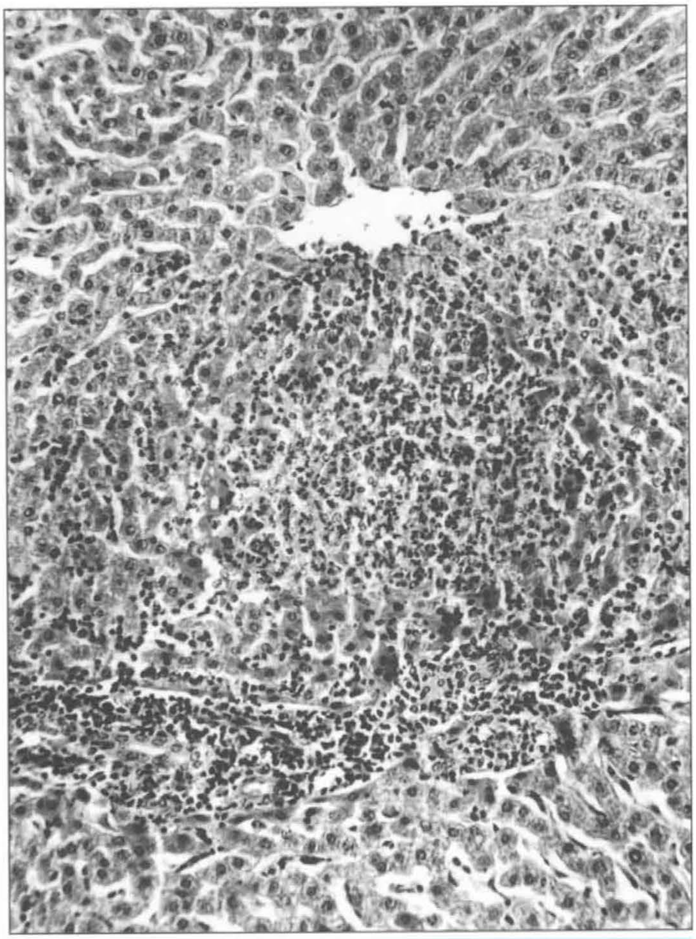

Figure 2.27 Focal hepatitis – the so-called **"sawdust liver"** of cattle.

such as salmonellosis, and as indicators of possible bacteremia for meat inspectors.

Zonal necrosis

While various terms are used, the simplest way to visualize and designate zonal patterns of necrosis is by the numerical zonation of the liver, as proposed by Rappaport. This can become awkward when necrosis extends from hepatocytes into the adjacent portal or central vascular tracts. In these circumstances, the terms *periportal or periacinar descriptors* might be more useful.

Zone 3 necrosis (periacinar necrosis) *is the most common form of zonal necrosis in domestic animals.* The hepatocytes in the periacinar zone are particularly vulnerable to necrosis, in part because they are farthest from incoming arterial and portal venous blood bearing oxygen and essential nutrients. They also contain the greatest concentration of cytochromes P450 that activate various exogenous compounds into reactive metabolites capable of injuring or killing hepatocytes (see section on Toxic hepatic disease, below).

Severe viral infections, such as *Canine adenovirus 1* and *Rift Valley fever virus*, can produce periacinar necrosis, and the reasons for the increased susceptibility of the hepatocytes of this zone in these diseases are not established. Plausible explanations include zonal expression of entry receptors used by viruses to infect hepatocytes, or ischemia-related hepatocellular swelling and sinusoidal damage that reduce effective perfusion of the periacinar hepatocytes.

Periacinar degeneration and necrosis are seen commonly in animals that have died rather slowly. It is assumed that, in the agonal period, the hepatocytes in this zone are disproportionately affected by tissue hypoxia as a result of the failing circulation. This necrosis is more extensive if the animal is anemic. Periacinar necrosis is also seen in passive venous congestion of the liver.

In the liver with periacinar necrosis, there is usually a prominent zonal pattern, which takes the form of a fine, regular, pallid network of surviving, often fatty hepatocytes in the periportal zone, which stands up above the red, collapsed areas adjacent to the hepatic venules (Fig. 2.28). The zonal necrotic insult frequently affects the sinusoidal endothelium, allowing erythrocytes to enter the perisinusoidal space and contribute to the redness of the necrotic zones. Necrotic cells that are removed can be replaced by stagnant blood, at least in the acute phase. However, this red-on-yellow zonal pattern is difficult to interpret grossly because hepatic lipidosis may also have a zonal distribution without having significant necrosis.

Periacinar coagulative necrosis gave rise to the time-honored designation "**centrilobular necrosis**" based on the classical hepatic lobule. However, vascular influences on the susceptibility to necrosis mean that segments of the lobule can be differentially affected. Frequently, the areas of necrosis are joined to one another, thus cutting the conventional lobules into segments, and at the same time outlining the periphery of the circulatory fields of the hepatic acini (Fig. 2.29). Some of these areas of necrosis extend up to larger portal triads, because the periphery of some acini may lie against the larger portal tracts. Often, the hepatocytes between the necrotic

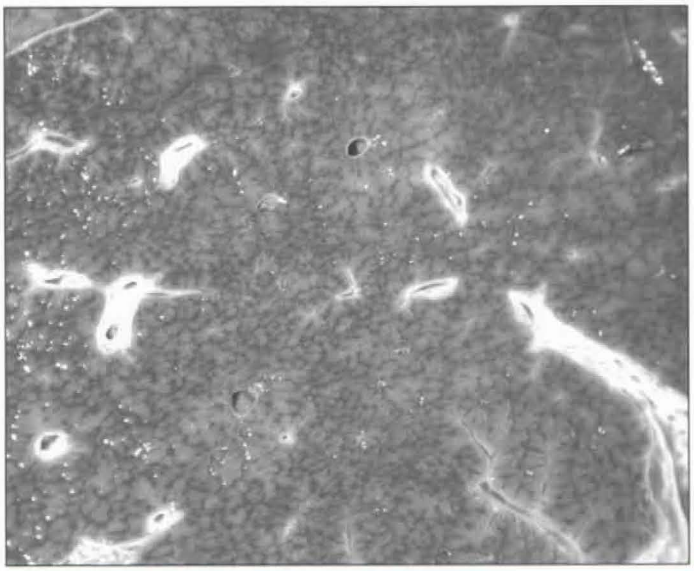

Figure 2.28 Chronic passive congestion (**"nutmeg liver"**) in an ox.

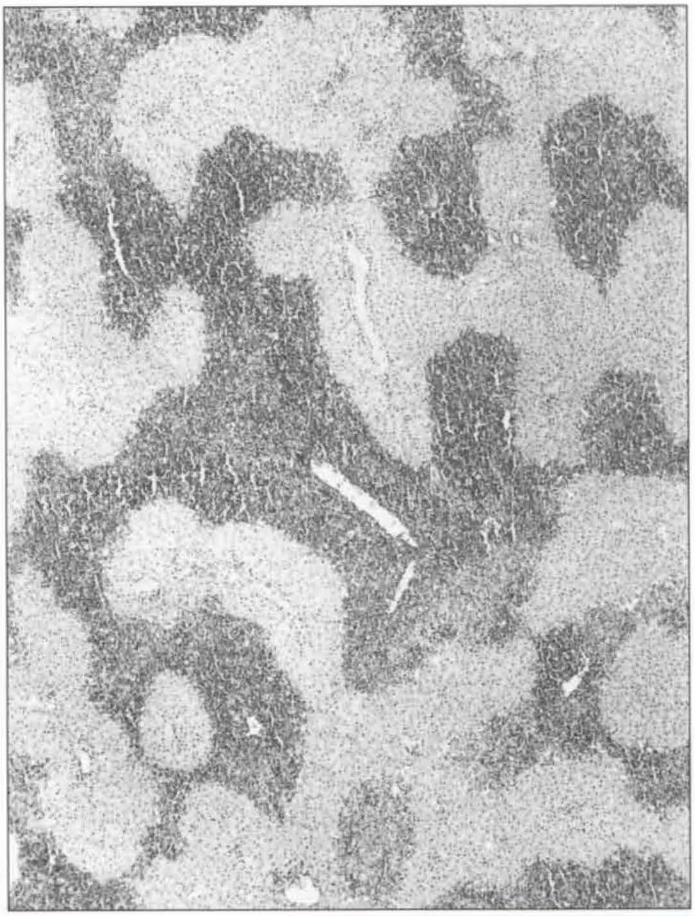

Figure 2.29 Acini outlined by **periacinar hemorrhagic necrosis** due to *Cestrum* poisoning in a sheep.

and more normal zones show hydropic degeneration or fatty change (Fig. 2.30). However, not all acini in a classical lobule may be equally affected, so the terms zone 3 or periacinar necrosis are preferable.

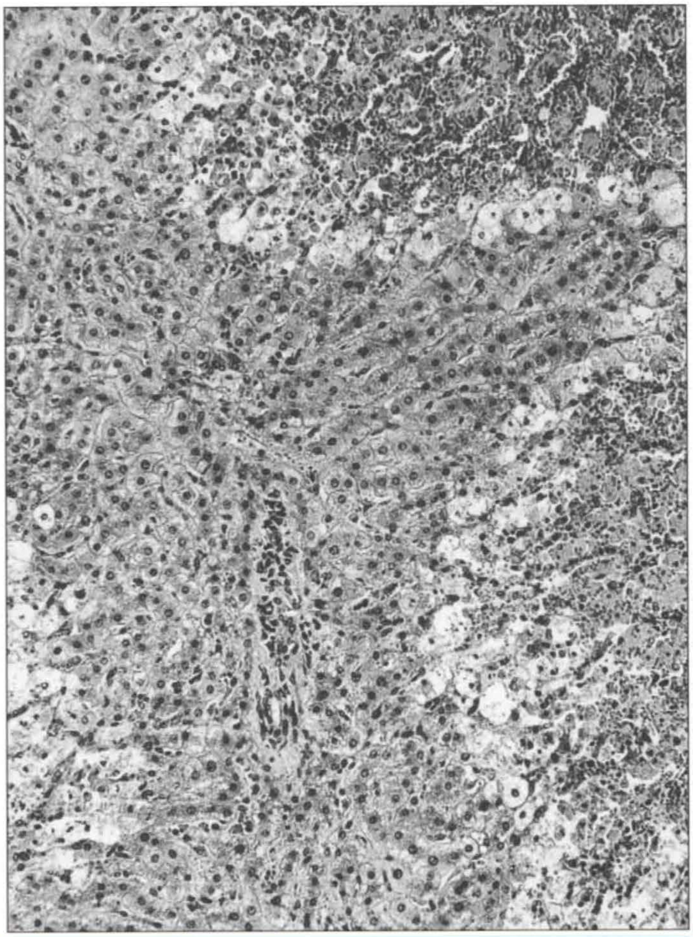

Figure 2.30 Acute **periacinar necrosis** in *Cestrum parqui* poisoning in an ox. Necrotic zone is bordered by hydropic hepatocytes.

If the insult is of short duration, quite extensive periacinar necrosis may be followed by phagocytic infiltration and hepatocellular proliferation and complete restoration of normal structure and function within a few days. Severe periacinar necrosis may be followed shortly by proliferation of cholangiolar and bile duct epithelia that also respond to the regenerative stimulus. With restitution of the normal complement of hepatocytes, the proliferative response in the biliary tract subsides unless the original insult is continuous or repeated.

Some intoxications can produce selective **zone 2 (midzonal) necrosis**, affecting only a narrow, sharply defined band of hepatocytes (Fig. 2.31). It may be more diffuse within the acinus, so that periportal or periacinar degeneration may be superimposed on the more severe midzonal lesion.

Zone 1 (periportal) necrosis (see Fig. 2.24) *is also an uncommon lesion*, perhaps more often seen than midzonal necrosis, and can be caused by direct-acting hepatotoxins. It is usual to find in the same liver areas that show one or more of the patterns of zonal necrosis.

The various forms of zonal necrosis cannot reliably be distinguished from one another grossly, but one may expect to see in periportal necrosis a reversal of the pattern seen in periacinar necrosis; that is, in periportal necrosis the surviving hepatocytes about the hepatic venules may appear as pale, raised islands within a regular network of red, collapsed periportal tissue. Careful scrutiny may reveal the smallest hepatic venules at the center of the pale islands.

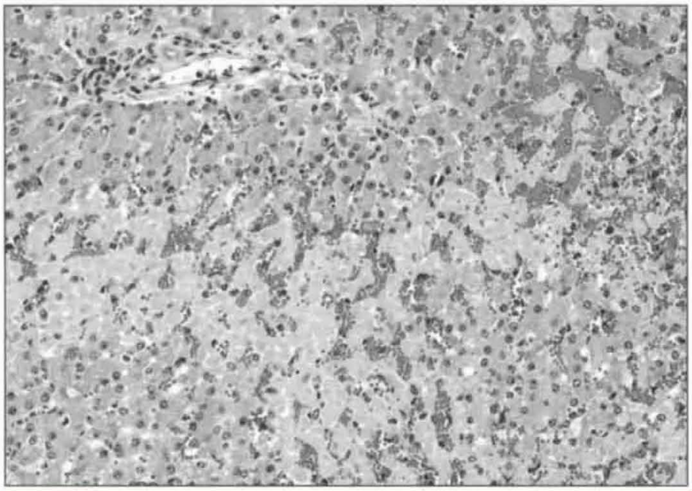

Figure 2.31 Acute **midzonal necrosis** in a foal.

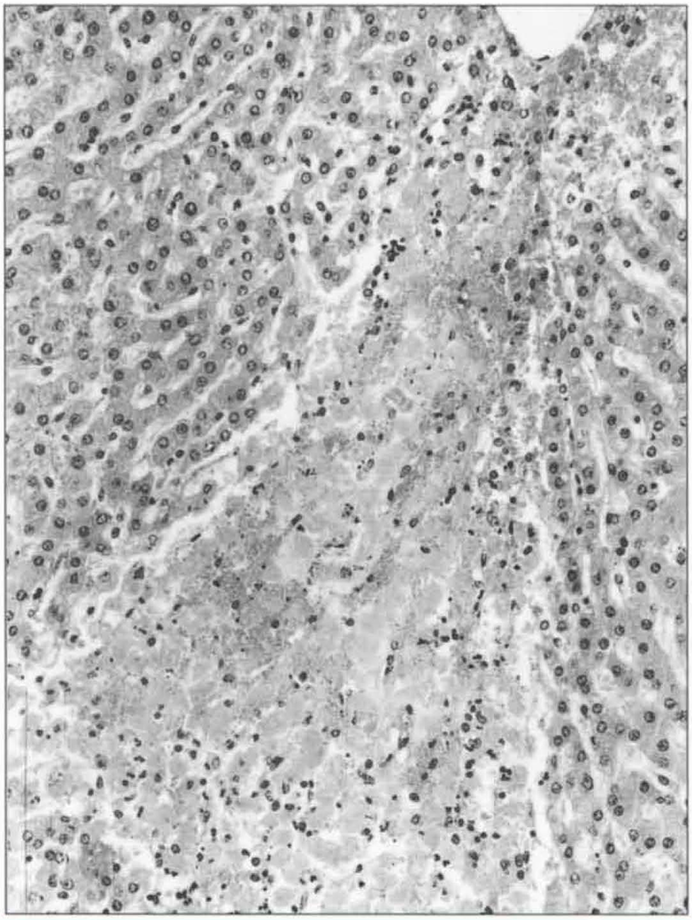

Figure 2.32 **Paracentral necrosis** in ngaione poisoning in a sheep.

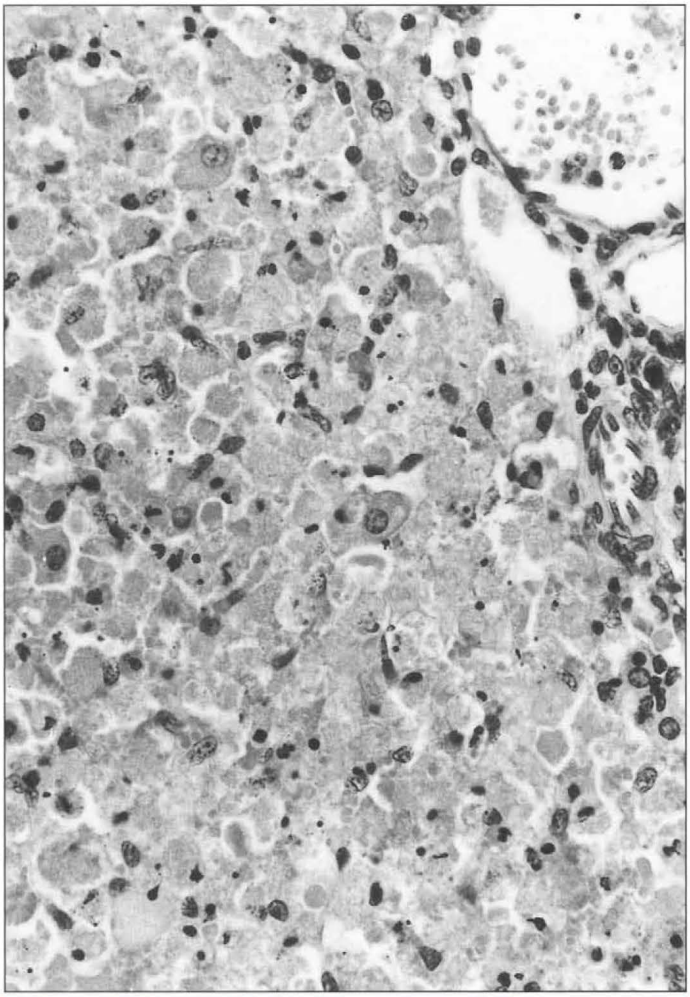

Figure 2.33 **Massive necrosis** with destruction of the periportal limiting plate due to algal poisoning in a sheep. (Courtesy of ARB Jackson.)

Paracentral necrosis, *a form of coagulative necrosis, occurs when an isolated complete hepatic acinus dies and is viewed in transverse section.* It is possibly an *ischemic lesion* or *infarct* produced by an occlusion of a terminal portal venule, such as may occur in disseminated intravascular coagulation. Its appearance in certain of the acute hepatotoxicities (Fig. 2.32) probably represents the death of a single complete acinus as a result of local vascular insufficiency, although, theoretically, high local microsomal enzyme activity or local deficiency of hepatocellular protective factors may play a part. Occlusion and rupture of a bile ductule or cholangiole are other potential causes of paracentral necrosis.

Massive necrosis

Massive necrosis refers to necrosis of entire hepatic acini, not necessarily necrosis of the liver as a whole. By accepted definition, *every cell in the affected acinus is dead, including the hepatocytes of the limiting plate* (Fig. 2.33). Without surviving parenchyma to support regeneration, affected acini collapse, so that portal areas and hepatic venules are approximated and the intervening stroma is condensed. Such a liver must regenerate from surviving hepatocytes in other, less severely affected lobules. The distribution of massive hepatic necrosis often relates to the distribution of larger vessels. Collapse, condensation, and subsequent scarring are characteristic, the end result being known as **postnecrotic scarring**. The liver is typically not uniformly involved. Large areas of parenchyma remain intact and these enlarge amid scarring areas during compensatory regeneration.

A liver that is the seat of massive necrosis may be of normal size or smaller. Fine red threads of fibrin may be present on the surface, especially in the grooves between the lobes. There is a surface mosaic appearance of red, gray, or yellow areas intermingled with areas of dark red. The gray or yellow areas of parenchyma, representing surviving tissue, form irregular, coalescing patches that may be less than 1.0 cm in diameter. *The intermingled red areas represent areas of necrosis, hemorrhage, and collapse, and these are depressed a few millimeters below the surface.* In the healing stage, the depressed areas of hemorrhage and necrosis are condensed, shrunken, and scarified so that the surface of the liver is traversed by fine or heavy scars that separate large nodules of regenerative hyperplasia. Further acute episodes may be superimposed so that the presented lesion may be a mixture of acute massive necrosis and postnecrotic scarring.

Hepatosis dietetica *of swine is an increasingly uncommon syndrome of massive hepatic necrosis in association with its immediate or late effects, namely "yellow-fat disease," degeneration of skeletal and cardiac muscle, serous effusions, ulceration of the squamous mucosa of the stomach, and fibrinoid necrosis of arterioles.* These lesions may occur alone or in any combination, although all seldom occur in one animal. They are known to be of nutritional origin, and the fact that the various lesions can occur separately indicates the complexity of the pathogenesis. Experimental observations have revealed the *need for concurrent deficiencies of sulfur-containing amino acids, tocopherols, and trace amounts of selenium if hepatic necrosis is to develop.* Selenium protects efficiently against the hepatic necrosis and massive effusions, and tocopherols are probably protective against other lesions that occur as part of the syndrome. The pathogenesis is incompletely understood, but is in part related to the generation of free radicals, exacerbated by deficiency of free radical scavengers such as vitamin E, and selenium, protective against reactive oxygen radicals through its role in glutathione peroxidase and some other selenoproteins. Hepatosis dietetica occurs in rapidly growing pigs fed diets largely of grain and containing protein supplements lacking in either quality or quantity. There is some evidence that in pigs that are nutritionally predisposed, a cold, damp environment or some other stress may precipitate the disease. Death usually occurs without signs of illness or after a short period of dullness. Melena, dyspnea, weakness, and trembling may be observed in some cases. Jaundice is indicative of a relapsing course.

Affected pigs are usually in good condition. The carcass may be anemic if ulceration of the gastric mucosa has occurred, and, in these cases, free and digested blood may be found in the stomach and intestine. Jaundice is not common, but *yellow staining of adipose tissues (yellow-fat disease)* is. In relapsing cases, hemorrhagic diathesis may occur, manifested mainly by hemorrhage into and about joints. Protein-rich fluid collects in the serous cavities in small volume. Fine strands of fibrin are present in the peritoneal cavity.

Pulmonary edema accompanies myocardial lesions that consist of intramural and subendocardial hemorrhages with focal areas of hyaline degeneration. The changes in the liver dominate the autopsy findings. The massive hepatic necrosis is of the typical appearance described earlier (Fig. 2.34), and in a number of cases, both acute and chronic lesions are found. The sites of severest injury are the dorsal parts on the diaphragmatic surface. The right lobe may escape and later undergo marked hypertrophy. The gallbladder is often edematous.

The histologic changes that occur in this syndrome are described elsewhere with the particular organs involved. Additionally, fibrinoid degeneration of small arteries occurs in some cases. The arterial degeneration may occur in any organ or in most organs but is relatively common in only the small vessels of the mesentery, gut, and heart (see mulberry heart disease, in Vol. 3, Cardiovascular system).

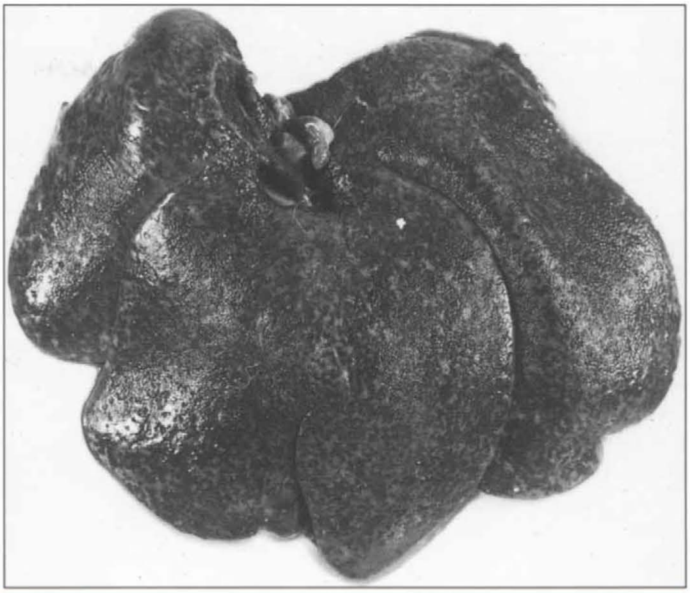

Figure 2.34 Massive necrosis with early postnecrotic collapse in **hepatosis dietetica** in a pig.

Piecemeal necrosis

Models of *immune-mediated hepatocyte necrosis* have emerged from studies of human viral hepatitis and some forms of drug-induced chronic hepatitis. *This pattern of necrosis amid sites of more active inflammation is sometimes referred to as piecemeal necrosis.* The mechanisms may involve either direct damage to hepatocytes by the uptake of antigen–antibody complexes, or cooperation between macrophages and T lymphocytes. These may cause cell-mediated destruction of hepatocytes that have taken up these complexes or, perhaps, native antigen or virus. Whatever the agency, the mode of cell removal in this sort of injury often takes the form of *single-cell necrosis or apoptosis*, which may be directly triggered by immunologically competent cells. This type of liver injury, in which *inflammation characteristically disrupts the limiting plate,* giving an irregular appearance to the periportal zone, is discussed further in the section on Chronic hepatitis, below.

Necrosis of sinusoidal lining cells

When hepatocytes are being destroyed by the elaboration of toxic molecules within their cytoplasm, it is to be expected that the *sinusoidal lining cells may also suffer should the products of these biotransformations spill into the space of Disse.* Erythrocytes increase in the perisinusoidal space and, when endothelial damage is severe, the regions of necrosis are hemorrhagic. Necrosis of sinusoidal endothelial cells is seen very early in the course of hepatotoxicities, such as acute acetaminophen and ngaione poisoning. Microcystin-LR is a highly toxic cyclic heptapeptide produced by *Microcystis aeruginosa*, an aquatic cyanobacterium (blue-green alga). This toxin is selectively injurious to hepatic sinusoidal endothelial cells by inhibiting protein phosphatases. When the cytoskeleton becomes hyperphosphorylated, endothelial cells undergo apoptosis, leading to hemorrhagic necrosis. *Sinusoidal phagocytes are sometimes vulnerable to necrosis* by virtue of their role in clearing the portal blood of particulate or colloidal material; should these particles be toxic or infectious, Kupffer cell necrosis may occur alone, but more usually there is damage to surrounding hepatocytes as well.

Necrosis of bile duct epithelium

It is unusual for the bile duct epithelium to be singled out by specific necrogenic insults, but this is seen in intoxication by *sporidesmin* (see section on Toxic hepatic disease, below). Usually there is accompanying portal inflammation. Some experimental toxicants such as α-naphthylisothiocyanate are necrogenic to bile duct epithelia and elicit a local inflammatory cholangitis. Idiosyncratic drug-induced cholestasis can lead to acute cholangiolar injury as well as chronic cholestasis with damage and loss of bile ducts (*vanishing bile duct syndrome*).

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RESPONSES OF THE LIVER TO INJURY

Hepatocellular replication

Hepatocytes replicate normally during embryogenesis, growth, and maintenance replacement. However, in various disease conditions, notably during adaptive hypertrophy or loss of functioning liver mass, replication or "regeneration" of hepatocytes and nonparenchymal cells increases. The liver can grow or shrink to meet metabolic demands after injury or in response to situations of excess functional mass, such as after lactation or weight loss. The remarkable ability of the liver to regenerate was recognized in ancient Greek mythology. The Titan Prometheus offended Zeus by giving mankind the gift of fire, and for punishment was chained to a rock on Mount Caucasus, where, every evening, an eagle devoured his liver. Overnight, his liver grew back, perpetuating his torture until his rescue by Heracles.

Hepatic mass is tightly regulated by control of mitosis and apoptosis, and similar mechanisms appear to control hepatic growth across species, although time courses may differ. *As much as 70% of the normal liver can be removed surgically without clinical insufficiency, and in the course of a few weeks it is back to normal size, although not its original shape.* Such regrowth of the liver is commonly termed "*regeneration;*" however, *new lobules and lobes cannot regenerate*; rather, *there is diffuse replication of surviving hepatocytes on the existing stromal matrix.* In situations in which replication cannot quickly satisfy the regenerative stimulus, nodular regeneration can arise from areas or cells that are more responsive.

Mature hepatocytes are usually in the postmitotic or G_0 resting stage of the cell cycle, but, under the influences of growth factors such as hepatocyte growth factor and interleukin-6, they can enter the cell cycle and replicate. The proportion that can do this varies with species and age, but in young animals in which most hepatocytes can replicate, only one or two rounds of replication are required to restore hepatic mass. Thus, increased numbers of mitotic hepatocytes are most numerous in the first few days after loss of liver mass. Enlargement of hepatic mass can also occur in part by increased production of cytoplasmic components or by the inhibition of apoptosis, in effect an extension of hepatocyte lifespan.

The initiation, progression, and cessation of this growth response are regulated on many levels, with built-in redundancy of signaling. Experimental studies in normal rodent liver have shown that, while hepatocytes in their normal state turn over very slowly, they retain replicative capacity that declines with age. Replication can be elicited by the action of various growth factors on hepatocytes that have been primed to respond. Priming can involve proteolysis of the extracellular matrix of the liver, and activation of Kupffer cells by tumor necrosis factor-α. These secrete interleukin-6, that renders hepatocytes competent to respond to other growth factors, particularly hepatocyte growth factor, transforming growth factor-α, and epidermal growth factor, facilitated by the co-mitogens insulin and epinephrine. Hepatocyte growth factor is produced by stellate cells and binds to the extracellular matrix, from which it is released during proteolysis. Cessation of liver growth when mass is restored is regulated by multiple pathways that are less well understood. Increased expression and responsiveness to growth inhibitors such as transforming growth factor-β and activin A induce extracellular matrix and suppress hepatocyte replication; these are downregulated during liver regeneration and increase as replication subsides.

Other cells in the hepatic lineage can respond to a protracted stimulus to regenerate by proliferating and differentiating into hepatocytes. These include **ductal precursor cells**, a bipolar (common ductal-hepatocyte) determined stem cell, represented in the adult liver by small "**oval cells**" in the terminal bile ductules (canals of Hering), and a putative multipotent **periductular liver progenitor cell** derived from circulating bone marrow stem cells.

These concepts of regeneration do not apply neatly to regeneration in livers with various age- or disease-related conditions. *Affected areas of liver require a supply of arterial and portal blood and free drainage of bile*, and these can be less than ideal. Also, some insults impair replicative competence of hepatocytes and nonparenchymal cells and this impedes and distorts regeneration. After massive necrosis or partial hepatectomy, surviving cells everywhere in the liver could contribute to the replacement of liver mass. After focal necrosis, proliferation is more restricted to adjacent differentiated cells. Accelerated single-cell necrosis, such as occurs in chronic copper poisoning in sheep, is compensated by replication of hepatocytes of seemingly random distribution. In the common periacinar necrosis, surviving hepatocytes, especially those in the periportal zone, can, after a delay of 24 hours or so, rapidly replace the lost cells. In periportal and midzonal necrosis, regeneration is provided by surviving cells in other zones.

Regeneration in livers during sustained or repetitive hepatocellular injury by toxic and idiopathic insults is often nodular rather than diffuse. Under these conditions, the liver tends to become smaller, atrophic, and the nodularity may be impressed on the liver by constricting bands of fibrous tissue. The toxins most active in this regard are not lytic liver necrogens but rather DNA-damaging toxicants such as pyrrolizidine alkaloids, phomopsin, aflatoxin, and various carcinogens with alkylating properties. All of these impair hepatocellular replication, and over time and the loss of hepatocytes through attrition or necrosis, *nodules emerge and enlarge as clones of hepatocytes with induced resistance to toxic injury.* The size of the nodules is a reflection of their opportunity to contribute to hepatic mass. If there is concurrent necrosis and inflammation, the degree of fibroplasia will be greater. These nodular atrophic livers are also characterized by bile duct hyperplasia, as oval cells and cholangioles are also able to respond to the mitogenic stimuli. If the liver eventually reaches the mass required, some of these induced populations can shrink by apoptosis or redifferentiation into more normal hepatocytes and ducts.

Compensatory liver atrophy, for example during cachexia, results in a discolored smaller firmer liver, whereas *acute atrophy* arising from diffuse cell death results in symmetrical flaccidity. In these conditions, the organ usually maintains its original shape. However, in chronic atrophy arising from hepatocellular loss or impaired regeneration, the organ becomes smaller and firmer as hepatocytes are lost from the condensing stroma. *Under a prevailing stimulus to regenerate, some altered hepatocytes escape inhibition and flourish as nodules*, resulting in a multinodular atrophic liver with most of the gross features of postnecrotic cirrhosis. Experimentally, various toxic insults that inhibit normal hepatocellular regeneration eventually cause liver atrophy with nodules. These nodules are composed of hepatocytes that more efficiently detoxify, excrete, and otherwise resist chemical insults, for example by excluding iron. However, hepatotoxins such as aflatoxin and pyrrolizidine alkaloids that inhibit proliferation also cause necrosis, so postnecrotic scarring and atrophy can be concurrent. In these conditions, some of the nodules originate from atypically differentiated cells generated by mutagenic insults, and some are clusters of regenerating normal hepatocytes.

Some of the common foci of atypical hepatocytes and hyperplastic nodules that develop spontaneously in canine livers may also have a survival advantage in the face of necrosis or atrophy, and give rise to the larger nodules that flourish in atrophic livers. Livers that are small and nodular (Fig. 2.35A, B), but lack the postnecrotic fibrovascular bridging characteristic of cirrhosis, can be reliably described as **hepatic atrophy with nodules**, pending further qualification according to necroinflammatory, mitoinhibitory, or combined pathogenesis. The nodular livers of some dogs presented with hepatocutaneous syndrome may be one example of this process (see section on Hepatocutaneous syndrome, below).

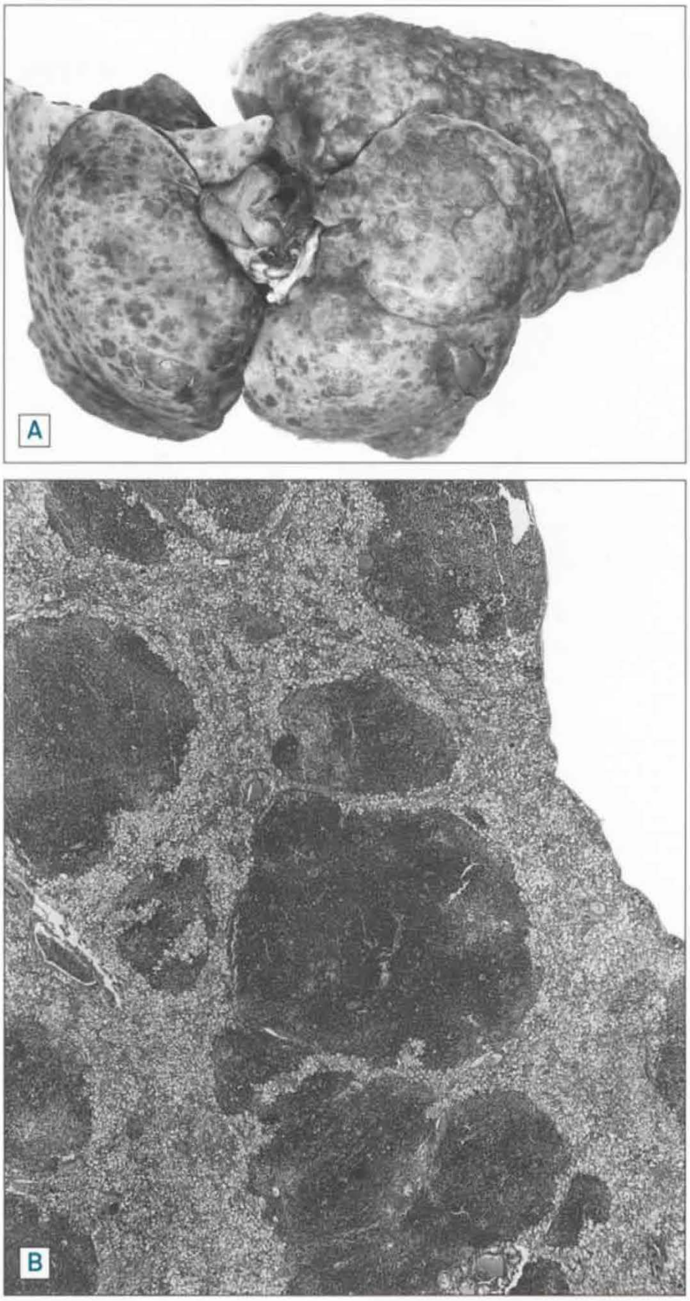

Figure 2.35 **A. Fatty liver** with nodules in a dog. **B.** Histology of **(A)** demonstrating **diffuse lipidosis with nonfatty nodules** in a dog.

Bile duct hyperplasia

Biliary hyperplasia is a characteristic reaction of the liver to particular types of insult, but much has still to be learned regarding the proliferative potential of these structures and the metabolic stimuli that provoke them. Ductular proliferation, with the formation of new, irregular, and tortuous channels in the portal and periportal region, is commonly associated with *bile duct obstructions* (Fig. 2.36). It may also develop secondary to *local portal inflammation and fibrosis*. In some situations, proliferation of ducts is a byproduct of sustained ineffective stimulus for the liver to regenerate (see above).

Bile duct proliferation can occur quite independently of changes in the parenchyma, particularly when an irritant stimulus is centered on the triads. Such pure bile duct proliferation may be a typical response to toxins, such as α-naphthylisothiocyanate in experimental models, or to physical biliary obstruction before infection complicates the picture. Injury to bile duct epithelium rather than hepatocytes is seen with paraquat intoxication and with exposure to the mycotoxin sporidesmin. There is also evidence for heterogeneity in the response of small and large cholangiocytes to different forms of hepatic injury or toxins. When the insults are removed the excess of bile ducts may disappear completely. The most extravagant expression of hyperplasia in the large ducts occurs in hepatic coccidiosis and some forms of hepatic distomiasis.

The originating cells of bile duct hyperplasia are thought to be *the hepatic bipolar stem cells, or oval cells*. Studies on isolated cells suggest that these intercalated cells are pluripotent and may differentiate into hepatocytes or cholangiolar epithelium. In experimental carcinogenesis or spontaneous hepatic disease in animals, cholangiolar

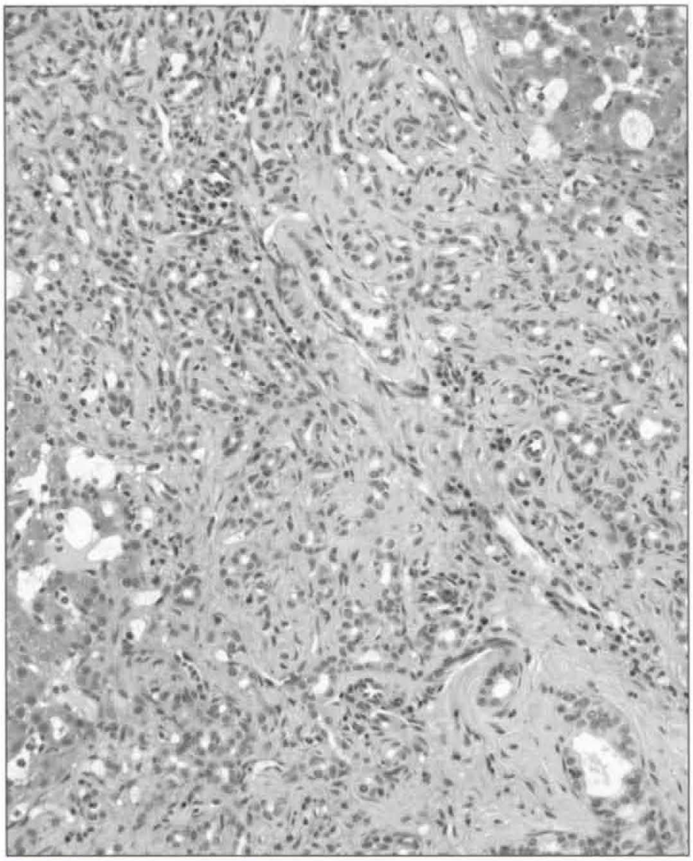

Figure 2.36 Portal fibrosis and bile duct hyperplasia secondary to bile duct obstruction in a horse.

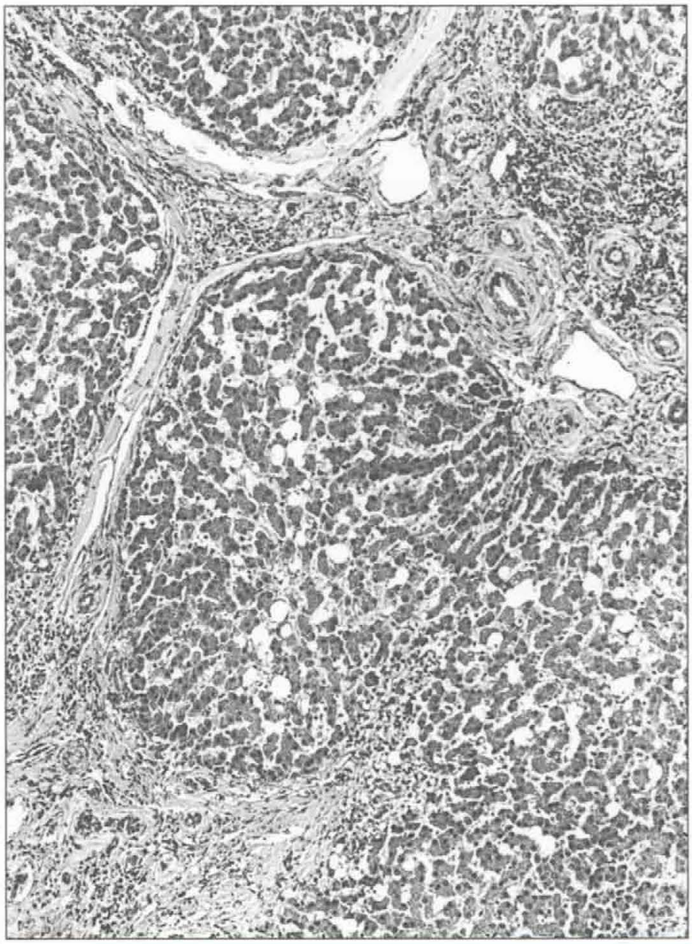

Figure 2.37 Biliary fibrosis secondary to chronic cholangitis in a dog.

hyperplasia may be an attempt to regenerate parenchyma when the parenchymal cells themselves have lost this capacity. Illustrative examples from natural disease are provided by the toxicoses of phomopsin, pyrrolizidine alkaloids, and aflatoxin, and by equine serum hepatitis and ovine white-liver disease.

Hepatic fibrosis

Fibrosis, the collagenous repair stage of necroinflammatory liver diseases, is a complicated spectrum of reactions that increase the deposition of extracellular matrix in the different portal, central, and perisinusoidal compartments, according to the distribution of the injury. The normal liver contains conventional connective tissue containing fibrillar collagen types I and III, predominantly within the connective tissue surrounding portal tracts and around terminal hepatic veins. Type IV collagen, mainly expressed by stellate cells, forms a delicate interstitial reticulin framework in the perisinusoidal space of Disse, between hepatocytes and sinusoidal endothelial cells. In diffuse interstitial hepatic fibrosis, accumulating types I and III collagen and other components of the extracellular matrix, including proteoglycans, fibronectin, and hyaluronan, are increased in the space of Disse. This increase in extracellular matrix reflects both increased expression of extracellular matrix components and reduced activity of matrix metalloproteases. On type I collagen, sinusoidal endothelia tend to lose fenestrae. This converts hepatic sinusoids from fenestrated endothelial channels that freely exchange proteins and other solutes between plasma and hepatocytes, to *higher-resistance, faster-flow tubular channels with limited solute exchange*. These changes, termed **capillarization** of sinusoids, are a common local response to liver injury, but must be extensive before they compromise the overall functional performance of the liver.

The major source of interstitial collagen production is the fat-storing perisinusoidal hepatic stellate cells. After some forms of liver injury, these cells proliferate and become activated, losing vitamin A storage and undergoing a major phenotypic transformation into smooth-muscle actin-expressing myofibroblast-like cells. The stimuli for this activation may include inflammation with release of proinflammatory cytokines such as tumor necrosis factor and interleukin-1, release of transforming growth factor-β and reactive oxygen species from injured hepatocytes and Kupffer cells, disruption of the extracellular matrix or direct stimulation of stellate cells by toxins. Once activated, hepatic stellate cells continue to produce a number of cytokines and growth factors capable of paracrine and autocrine stimulation, as well as tissue inhibitors of metalloproteinases, that perpetuate the process of fibrosis.

Hepatic fibrosis is potentially reversible. If the underlying insult is effectively treated or stopped, apoptosis of hepatic stellate cells and reduced expression of metalloproteinase inhibitors slow the production of extracellular matrix and allow degradation of immature collagen by matrix metalloproteinases to proceed. The balance is tipped in favor of progressive fibrosis when the insult continues to act, and

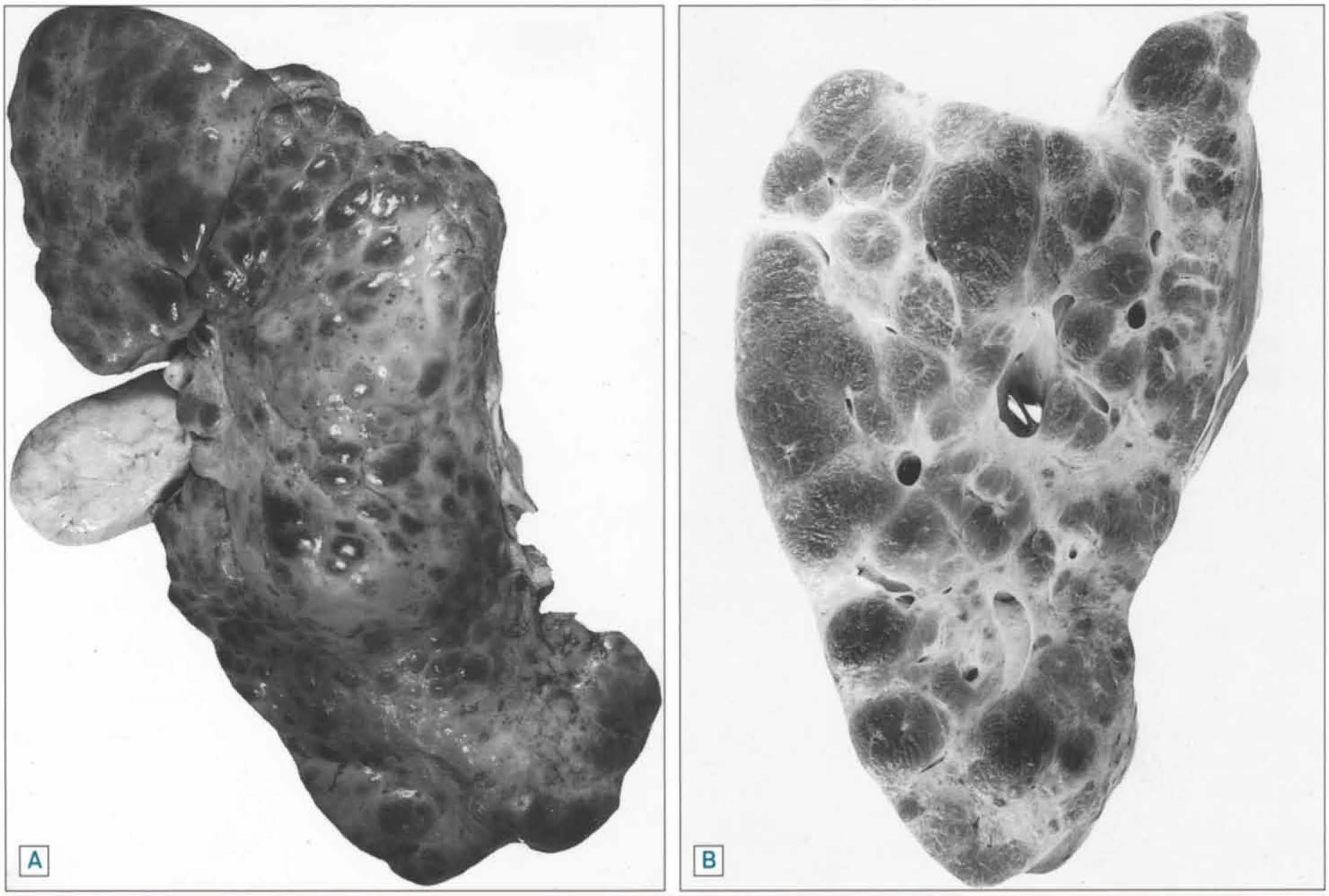

Figure 2.38 Postnecrotic scarring in an ox. **A.** Capsular surface. **B.** Cut surface.

collagen maturation and cross-linking potentially reduce its susceptibility to enzymatic degradation, or when the initial damage is so severe that the scar that results is extensive enough to exacerbate damage to the parenchyma by progressive sclerosis that impedes circulation.

The *distribution of fibrosis* in the liver reflects the pathogenesis of the necroinflammatory disease responsible:

- In the event of *inflammation in the portal triads*, the fibrosis remains largely confined to these areas and is termed **biliary fibrosis** (Fig. 2.37).
- **Postnecrotic scarring** *occurs after massive necrosis*, where large areas of parenchyma are destroyed. The reticulin network collapses and condenses, and portal areas converge, resulting in broad, irregular bands of scar tissue with variable irregular areas of parenchymal regeneration interspersed (Fig. 2.38A, B).
- **Diffuse hepatic fibrosis** is the outcome of *chronic parenchymal injury* such as prolonged inflammation or multiple episodes of zonal necrosis. The fibrosis throughout the lobules bridges connective tissue tracts in portal areas and hepatic venules to produce pseudolobulation, in which small areas of parenchyma are separated by a pattern of fibrosis on the scale of true lobules. This pattern of fibrosis can lead to capillarization of sinusoids, or intrahepatic bypass of portal and arterial blood, both of which can reduce the influences of portal growth factors on liver regeneration. These and accompanying malnutrition and hypoxia are important in the genesis of the hepatocellular atrophy that is almost always concomitant with diffuse hepatic fibrosis.
- Postnecrotic fibrosis that develops around hepatic venules is termed **periacinar fibrosis**. This is the most common pattern *following zonal necrosis in hypoxic and toxic injury*. Good examples are seen in animals with prolonged passive venous congestion of the liver (Fig. 2.39A, B), especially when this is due to extracardiac sources of increased venous pressure, rather than to congestive heart failure. Otherwise, this pattern of fibrosis is a response to toxic injury; poisoning by pyrrolizidine alkaloids may cause it in ruminants, and extraordinary development of periacinar fibrosis may follow accidental exposure to nitrosamines in several species.

Fibrosis as a postnecroinflammatory response is mechanistically distinct from simple condensation of stromal elements within atrophic segments of the liver, although often the two processes are concurrent.

Cirrhosis

The term cirrhosis is most correctly used for the end-stage of diffuse hepatic disease characterized by nodular regeneration with fibrovascular bridging scars in which there are intrahepatic portal-hepatic vascular shunts. Cirrhosis involves disruption of the architecture of the entire liver, a reflection

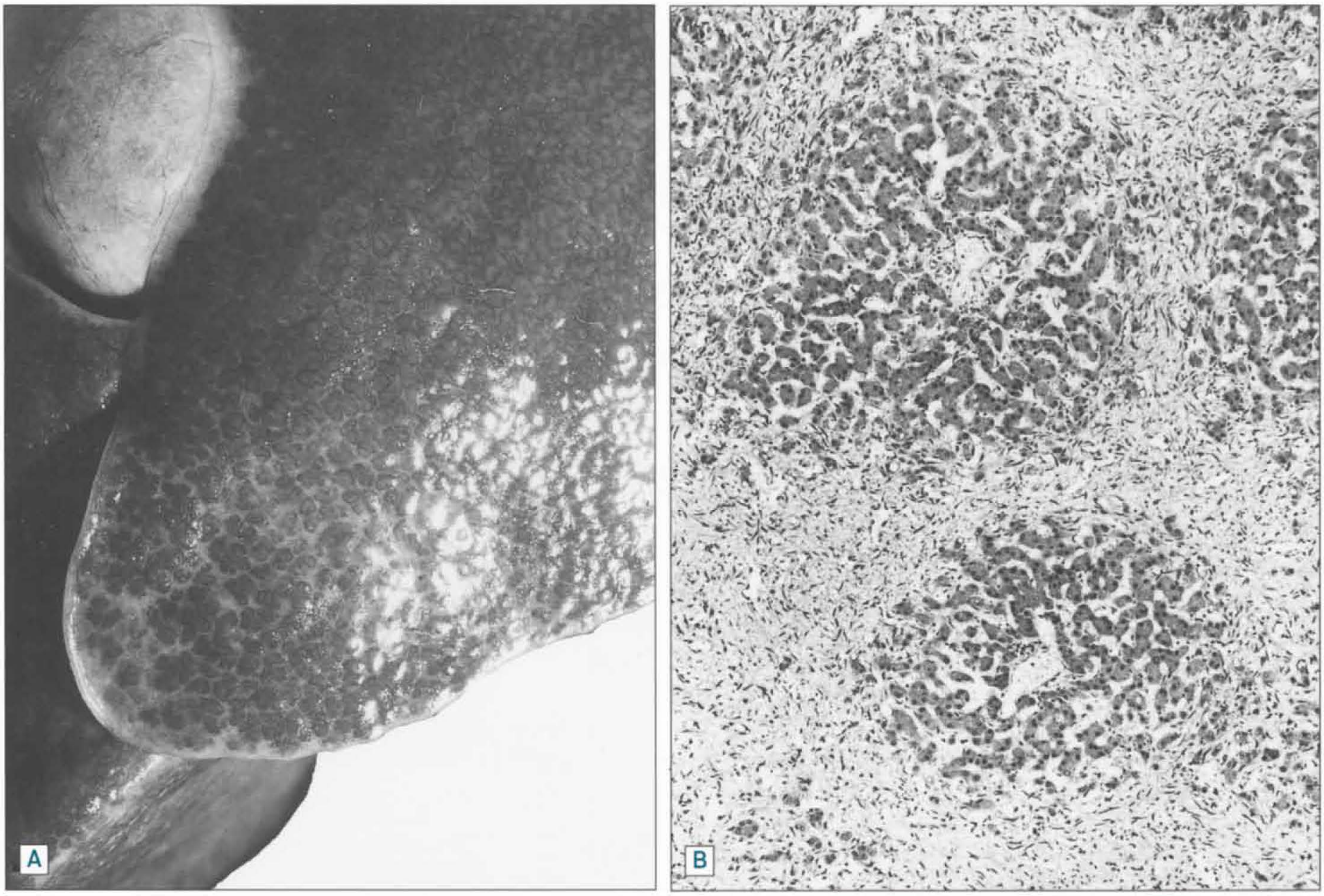

Figure 2.39 A. Periacinar fibrosis (cardiac fibrosis), most severe at the periphery of the lobe in chronic passive congestion in a dog with chronic heartworm disease. **B.** Section of (**A**).

of diffuse parenchymal injury, and subsequent fibrosis. *Note that not all nodular livers are cirrhotic* (see section on Hepatocellular replication, above). Clinically, *cirrhosis results in hepatic insufficiency*, mainly in the form of ascites and hypoproteinemia rather than excretory dysfunction and icterus, although in advanced cases all manifestations of fatal liver failure can occur. The *hallmarks of cirrhosis* are:

1. *Bridging fibrous septa*, ranging from delicate bands to broad scars that replace multiple adjacent lobules. These fibrous septa contain vascular channels, originating from sinusoids in collapsed parenchyma or angiogenesis that allow a considerable portion of blood to bypass hepatocytes. These microvascular channels in fibrous septa resist flow more than normal sinusoids and contribute to portal hypertension.
2. *Impaired exchange* between hepatocytes and sinusoidal blood, due to the increase in perisinusoidal extracellular matrix.
3. *Parenchymal nodules*, created by the regenerative attempts of entrapped hepatocytes, and varying in size from less than 3 mm in diameter (micronodular) to several centimeters in diameter (macronodular). These nodules are composed of trabeculae that are typically two or more cells thick, with relative reduction of sinusoidal space. Some expanding nodules may compress the vessels within fibrous septa, contributing to portal hypertension.
4. *Ongoing damage and reorganization* of the hepatic connective tissue, sometimes with areas of portal and arterial thrombosis and segmental ischemia.

Cirrhosis is usually the end-result of several pathogenetic processes, namely cell death (necrosis or apoptosis) and active inflammation with chronic fibrosis. Cirrhosis is not a synonym for chronic hepatic fibrosis, although some insults that cause chronic diffuse fibrosis in the liver can lead to cirrhosis. There is emerging evidence to suggest that hepatic fibrosis is potentially reversible, whereas the vascular changes in cirrhosis are generally regarded as irreversible. The nodular pattern of regeneration in cirrhosis is due to expanding islands of surviving parenchyma entrapped between bands of scar tissue. At the point of liver failure, these regenerative attempts are insufficient to restore function, but the reasons for this are poorly understood. Plausible contributors to this impairment are: altered circulation through the bridging scar tissue; portal hypertension and shunting; deposition of extracellular matrix in the perisinusoidal space; reduced supply of portal growth factors; inhibition of proliferation of extracellular matrix; and toxic inhibition of hepatocellular proliferation. The characteristic fibrovascular septa that bridge portal and central vascular tracts are readily observed in liver sections, but the portal-hepatic shunting therein can be difficult to identify. Clinical evidence for portosystemic shunting, such as impaired ammonia or bile acid clearance, can reflect acquired portocaval shunts that often develop in chronic hepatic fibrosis.

Acquired portosystemic shunting

Any chronic liver disease that causes sufficient fibrosis or atrophy to restrict portal blood flow significantly has the potential to cause the development of collateral portosystemic shunts. These are described below in the section on Vascular factors in hepatic injury and circulatory disorders.

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HEPATIC DYSFUNCTION

Hepatic failure is a syndrome that results from inadequate hepatic function. This may be the result of sudden, massive hepatic destruction, or, more frequently, the end-point of progressive liver damage. Failure can be manifest as inadequacy of one or more of the major functions of the liver, especially energy metabolism, detoxification and excretion, bile production, and protein secretion. Liver alterations are often recognized by serum biochemistry, imaging, or liver biopsy, but severe clinical dysfunction or failure is uncommon. The liver is an organ with very large functional capacity and regenerative potential, and many of the plasma proteins produced by the liver are supplied in abundance, so many insults do not cause clinically significant liver dysfunction. Signs of insufficiency do not develop until the reserves in production, supply, and recovery are exhausted, by which time the lesions may be advanced and irreversible. *The central role of the liver in many systemic functions means that liver disease can be manifest as lesions or dysfunctions elsewhere, for example in the brain, skin, blood, and gut.* Also, disease that selectively affects the parenchymal, circulatory, or ductular components can present different clinical syndromes.

Cholestasis and jaundice

- **Cholestasis** is a general term for impedance of bile output from the liver.
- **Jaundice (icterus)** is the discoloration of tissues and body fluids by an excess of bile pigments. *Jaundice can have prehepatic or cholestatic origins.*
- **Prehepatic jaundice** is usually related to overproduction of bilirubin from heme catabolism in hemolytic disease.
- **Cholestatic jaundice** can be hepatic or posthepatic:
  - In *hepatic jaundice*, there may be impaired uptake, metabolism, secretion, and transport of bile pigments within the liver.
  - In *posthepatic jaundice*, cholestasis is related to obstruction of bile flow at the level of the major bile ducts or gallbladder.

Hepatic causes of jaundice can be conveniently subdivided into: (1) failure of hepatocytes to take up or conjugate bilirubin; (2) impaired excretion and transport of conjugated bilirubin in canaliculi or ducts within the liver; and (3) impaired function of hepatocellular adenosine triphosphate-binding cassette transporters and aquaporins that drive the osmotic flow of bile. The bile secretion pathway has multiple steps that can be a target of a particular cholestatic mechanism. These include impaired uptake of unconjugated bilirubin, reduced production of conjugated bilirubin, slow canalicular transport, leakage from the duct system, and physical obstructions at various levels within and beyond the liver. However, the injurious effects of cholestasis mean that it is common for these prehepatic, hepatic, and posthepatic causes to be combined to various degrees in any case of jaundice. In hemolytic disease, for example, the large amount of bilirubin presented to the liver for excretion may overload both the hepatocellular bile uptake and bilirubin conjugation mechanisms, as well as the intracellular and canalicular transport process. In addition, the anemia usually associated with severe hemolytic disease will compromise hepatocellular function and further hamper bilirubin excretion. Hepatocellular accumulation of bile salts in obstructive jaundice interferes with bile conjugation and transport by the smooth endoplasmic reticulum. For these reasons, *the ratio of conjugated to unconjugated bilirubin in hyperbilirubinemia is not a reliable indicator of pathogenesis in most cases of jaundice.*

Bilirubin is conjugated by uridine diphosphate-glucuronyl transferase in the endoplasmic reticulum of hepatocytes. From there it is transported into the bile by the ATP-dependent canalicular multispecific organic anion transporter (cMOAT), a broad-spectrum transporter of many conjugates and bile salts. Hepatocellular membrane damage or ATP depletion reduces this output. Experimentally, there is a direct relationship between bile flow and the secretion of bile salts, glutathione, and bicarbonate by various energy-dependent transporters. Bile salts undergo enterohepatic recycling, involving apical bile salt transport in enterocytes, hepatic bile salt transport at the sinusoidal pole, and bile salt export pumps at the canaliculi. Canaliculi also express various other transporters, including P-glycoprotein (multidrug resistance protein 1 or mdr1) and cMOAT.

Severe diffuse liver necrosis obviously impairs bilirubin conjugation at various levels, but *the severity of jaundice depends on the amount of liver affected, the chronicity, the supply of heme for catabolism, and nonhepatic routes of bilirubin excretion.* Focal liver injury can have substantial local cholestasis but jaundice does not occur because bilirubin can be cleared by unaffected parts of the liver. Similarly, segmental duct obstructions that spare some parts of the liver can lead to cholestasis but not jaundice. Rarely, cholestasis and its indicators can occur in the absence of primary hepatocellular damage and other signs of liver failure. This can occur in toxicity by *Lantana camara* in ruminants and as an idiosyncratic reaction to some drugs.

Intrahepatic cholestasis can also occur through disruption of the structural integrity of the canaliculi, as discussed later for *L. camara* toxicity. Poisons such as phalloidin and cytochalasin that disrupt the polymerization cycle of pericanalicular actin microfilaments are cholestatic because these contractile filaments are required for propelling bile along the canaliculi. Some of the enzymes involved in bile production, alkaline phosphatase and γ-glutamyl transpeptidase, cMOAT, and P-glycoprotein are inducible under the regulation of various nuclear receptors. Cholestasis is a common effect of sepsis, and lipopolysaccharide reduces expression of cMOAT and other transporters. Bile flow also slows during anorexia by mechanisms that are not yet clear. In cats with prolonged anorexia, bile pigments can be observed as canalicular casts in liver sections; these likely reflect secretion of conjugated bilirubin without osmotic flow.

Some of the various transporters and conjugating enzymes involved in bilirubin excretion can be genetically defective. *Congenital hyperbilirubinemia in mutant Southdown sheep* is due to impaired hepatic uptake of unconjugated bilirubin. These animals have few liver lesions, but eventually develop chronic renal disease, the reason for which is not clear. Unconjugated bilirubin levels in the plasma are consistently elevated, but sufficient excretion takes place to prevent them from becoming icteric. They become photosensitized, indicating that excretion of phylloerythrin is less efficient than that of bilirubin.

Hyperbilirubinemia in mutant Corriedale sheep is a defect in excretion of conjugated bilirubin similar to congenital defects in cMOAT in other species. There is also elevation of plasma bilirubin (just over half of which is conjugated), but there is no obvious jaundice. Nevertheless, phylloerythrin excretion in these Corriedales is also sufficiently impaired to produce photosensitization. There is impaired excretion of other conjugated metabolites, and there is pigmentation of the liver by polymerized residues of retained catecholamine metabolites. This pigment, resembling lipofuscin, accumulates in lysosomes in the pericanalicular cytoplasm.

Extrahepatic biliary obstruction initially causes increased pressure in bile ducts with dilation and stasis of content in the smaller radicles in the portal triads. Shortly, however, the parenchymal changes are the same as in intrahepatic cholestasis. Brown bile pigment is present in canaliculi and hepatocyte cytoplasm. The canaliculi are distended and sometimes loculated. The bile plugs are homogeneous. In the hepatocyte cytoplasm, initially in periacinar zones and later in all zones, the pigment is present in large, irregular lysosomes. In long-standing cholestasis, such as may occur with extrahepatic biliary obstruction, cholangioles become hyperplastic and some hepatocytes become hydropic with a reticulated appearance to the cytoplasm and are coarsely impregnated with pigment. Foci of parenchymal necrosis release small lakes of bile that can become surrounded by macrophages and giant cells.

The recognition of jaundice at necropsy sometimes involves differentiation of bile staining of tissues from the yellow staining caused by accumulation of carotenoid pigments. These latter are limited to fat depots and are to be expected in certain species such as horses; sometimes there are breed influences, as seen in the yellow fat of Channel Island breeds of dairy cattle. The yellow discoloration of fat depots of older cats is less well understood; in animals fed ox liver, carotenoids may again be responsible; in others there may be some contribution by ceroid-type pigments. Distinction of the fatty pigments from bile depends on the absence of the former from pale, nonfatty tissues such as periosteum and dermal collagen.

Photosensitization

Photosensitization is the term applied to inflammation of skin (usually unpigmented) due to the action of ultraviolet light of wavelengths 290–400 nm on photodynamic compounds that have become bound to dermal cells. These compounds may have been deposited unchanged in the skin after ingestion, when the normal liver is incapable of excreting the native compound. This is known as **primary photosensitization** and is seen, for example, after ingestion of hypericin in St. John's wort (*Hypericum perforatum*). Photodynamic agents may also be produced by aberrant endogenous metabolism. This can occur, for example, in congenital erythropoietic protoporphyria due to ferrochelatase deficiency in Limousin and Blonde d'Aquitaine calves.

Hepatogenous photosensitization is an almost invariable accompaniment of cholestasis of more than a few days' duration in herbivores that are kept in sunlight and that have been eating green feed. Phylloerythrins are green photoactive catabolites of plant porphyrins (mainly chlorophyll) that are generated by the alimentary microflora of herbivores. Some phylloerythrin is absorbed and normally excreted in the bile by the transporters that eliminate bilirubin. Cholestasis in herbivores can increase retention of phylloerythrin in the blood, and it can result in photosensitive dermatitis of unpigmented areas of skin exposed to sunlight for several days. It is possible, however, for mild photosensitization to appear in the absence of gross or microscopic evidence of cholestasis in animals grazing alfalfa, *Paspalum*, pangola, or *Panicum* grasses; however, it is unclear if these are related to phylloerythrin or other photoactive products of these forages. Absence of serum biochemical evidence for cholestasis or liver damage is used to differentiate primary from secondary photosensitization in these circumstances.

If no hepatic changes can be discerned in photosensitized animals, the possibility of primary photosensitization must be considered, but hepatogenous photosensitization cannot be excluded unless adequate liver function tests are performed.

Hepatic encephalopathy

The neurologic manifestations of hepatic failure are variable and nonspecific; they range from dullness, through complete unawareness and compulsive aimless movement, to mania and generalized convulsions. There is considerable variation in the clinical signs of hepatic encephalopathy between different species. Sheep rarely show more than dullness and central blindness, with perhaps some compulsive chewing movements and tremor. The picture in cattle is similar, but mania and aggression may also be seen, while frenzy is more often recorded in horses. The closer clinical observation accorded to cats and dogs may reveal more subtle behavioral changes, and inappetance and vomiting are commonly reported in carnivores with portosystemic shunts. *In animals with acute hepatic disease, hepatic encephalopathy is a serious sign usually indicating imminent death.* However, in animals with portosystemic shunting or deficiency of a urea cycle enzyme, neurologic signs can be intermittent for many months, and may disappear after appropriate dietary modification.

Ammonia toxicity is responsible for the major part of the clinical signs and the brain lesions. The ammonia is largely of dietary origin, derived from protein and urea by microflora in the large bowel and from hepatic deamination of amino acids. Ammonia is normally removed in the first pass of portal blood through the liver, wherein it is incorporated with carbon dioxide into carbamoyl phosphate that enters the urea cycle. In shunting or hepatic failure, ammonia that bypasses this outcome accumulates in the general circulation and crosses the blood–brain barrier, leading to encephalopathy. Astrocytes are the site of ammonia detoxification in the brain, and eliminate ammonia by the synthesis of glutamine through amidation of glutamate by the enzyme glutamine synthetase. Elevated blood ammonia is postulated to increase the accumulation of glutamine in astrocytes, resulting in osmotic stress and astrocyte swelling, the consequences of which include altered gene expression, changes in multiple neurotransmitter systems, and brain edema. In addition, ammonia can be directly neurotoxic, altering neurotransmission, and potentially contributing to cerebral energy failure through inhibition of α-ketoglutarate dehydrogenase, a rate-limiting enzyme in the tricarboxylic acid cycle. Numerous studies have also implicated alterations in inhibitory and excitatory neurotransmitters, including γ-aminobutyric acid, endogenous benzodiazepines, and serotonin, as well as their receptors. While infusion of ammonia or hyperammonemia due to deficiency of urea cycle enzymes reproduces similar neurologic signs and vacuolar lesions, other noxious substances in the alimentary tract are also believed to contribute to hepatic encephalopathy resulting from liver failure. These include *variably toxic amines, captans, thiols, and short-chain fatty acids* that are normally removed from the portal blood in one passage through the liver after production in the large bowel. In cases of complete liver failure, hypoglycemic convulsions may result from failure of glucose synthesis.

The microscopic lesions of hepatic encephalopathy are subtle and variable, likely because necropsy examinations of the brain are performed at various time points of chronicity and clinical severity. There are also significant differences in the microscopic lesions between species. *The hallmark of hepatic encephalopathy is Alzheimer type II astrocytosis, in which astrocytes are enlarged with swollen nuclei, margination of chromatin and prominent nucleoli.* Other common changes are microcavitation or spongiosis of myelinated tracts of the cerebrum (internal capsule), thalamus, hypothalamus, and cerebellum, although this is not a consistent finding in cats, ruminants, or horses (see Vol. 1, Nervous system). These changes can be observed best in animals with chronic liver disease and portosystemic shunts; in animals with acute liver failure, the brain lesions can be minimal. In addition, similar changes in the brain can be observed with renal disease, including the formation of Alzheimer type II cells.

Hemorrhage and liver failure

Hemorrhagic diathesis characterized by widespread ecchymoses and petechiae can occur when the liver is the site of rapid consumption of plasma-clotting factors. The liver is the source of plasma proteins involved in the clotting cascade, but these are normally supplied in substantial excess, and are further inducible as part of the acute-phase response to inflammation. Thus, *coagulopathy with hemorrhage is most likely to occur when there is acute liver necrosis*, for example, in acute canine hepatitis or acetaminophen toxicity. Under these conditions, there is significant intrahepatic consumption of clotting factors at sites of endothelial necrosis in the damaged liver. While the damaged liver also fails to resupply the clotting factors, this is probably a minor contributing influence. *In more chronic liver diseases with hypoproteinemia, coagulation tests may be prolonged but hemorrhagic diathesis is unlikely*, unless there is an additional demand for hemostasis, for example during the trauma of surgery. Consumption of clotting factors can also occur in septic diseases that affect the liver and other tissues.

Nephropathy

Acute liver failure may be accompanied by oliguria and biochemical indications of renal failure. The kidneys are large, moist, and stained by bile pigments that are present histologically in tubular epithelium and luminal casts. The renal component is referred to as **biliary nephrosis**. The mechanisms are not known but may represent a perfusion deficit with shunting of blood from cortex to medulla. **Hepatorenal syndrome** is a complication of advanced cirrhosis in humans, characterized by renal failure in which there are no intrinsic renal morphologic or functional causes. The pathogenesis of hepatorenal syndrome is related to *deterioration in effective arterial blood volume due to splanchnic arterial vasodilation and reduced venous return and cardiac output.* Intense compensatory vasoconstriction of the renal circulation results in decreased glomerular filtration and resultant renal failure.

Edema and ascites

Ascites (retention of excess low-protein peritoneal fluid) is a feature of chronic liver failure, but is more frequently associated with systemic venous congestion (e.g., right-side heart failure) or hypoproteinemia secondary to protein-losing renal or alimentary tract conditions. Retention of peritoneal fluid can also sometimes result from mechanical obstructions to mesenteric and peritoneal lymphatics by inflammatory or neoplastic lesions. Peritoneal fluids with a higher protein and cell content that accumulate in various abdominal inflammatory conditions are considered exudative inflammatory conditions (see section on peritonitis, in Vol. 2, Alimentary system).

Reduced synthesis of albumin and globulins by the failing liver can reduce vascular oncotic pressure, but edema resulting from this mechanism is generalized. The pathogenesis of ascites in the absence of systemic edema in chronic liver failure is more complex. *It is likely that ascites in advanced liver disease is influenced by both mechanical and dynamic influences on blood flow through a damaged liver.* For example, fibrosis and nodular expansion can impede blood flow. There are also dynamic influences of stellate cell contraction and hepatic vein smooth muscle that affect portal venous pressure. Ascites is often observed in severe portal hypertension, for example in arterioportal fistulae, but the increase in pressure per se might not be directly responsible for fluid retention. In some experimental models, portal hypertension induced by portal stricture does not cause ascites, and clinical portal hypertension is not necessarily associated with ascites. Also, passive venous congestion of the spleen and viscera are not obvious features of portal hypertension. Lymphatic drainage through the mesenteric lymph nodes and thoracic duct remains available for removal of peritoneal fluids in most examples of liver disease with ascites. These various findings suggest that *ascites might result from other hemodynamic dysfunctions that affect splanchnic arterial blood* *flow.* Various factors that regulate splanchnic blood flow, including histamine, nitric oxide, endothelins, atrial natriuretic peptide, calcitonin gene-regulated peptide, tumor necrosis factor-α, substance P, and vasoactive intestinal peptide are produced locally in the splanchnic circulation and are normally cleared by the liver. Increased generation or impaired clearance by a failing liver could contribute to increases in splanchnic arteriolar blood flow, increased hydrostatic pressure in the splanchnic capillaries, overproduction of splanchnic lymph, increased portal pressure, sodium retention, and increased cardiac output. Also, some vasoactive mediators induce contraction of stellate cells and intrahepatic vascular smooth muscle, so impaired clearance might dynamically affect hepatic perfusion. Others could restrict venous outflow through established portosystemic collateral veins. In humans, there is evidence that autonomic regulation of splanchnic blood flow is also dysregulated in patients with ascites.

Hypoglobulinemias

The liver is a major part of the innate immune system, clearing microorganisms and immune complexes from the circulation, and supplying various plasma proteins that are important in host defense, including plasma antiproteinases that limit activity of some microbial toxins or endogenous proteinases activated during coagulation, complement activation, inflammation, and tissue repair. The liver also produces various effector molecules of the innate immune system, including complement proteins, antimicrobial lectins, and mineral transport proteins that restrict iron and copper from pathogens. In spite of the importance of this spectrum of innate immune functions, *increased susceptibility to infection and inflammatory disease is not generally recognized as a sequel to liver failure.*

Hepatocutaneous syndrome

An *idiopathic vacuolar hepatopathy with parenchymal collapse and nodular regeneration* has been reported in dogs and cats that have been presented clinically with **superficial necrolytic dermatitis**. Some cases have been considered under the clinical term "*hepatocutaneous syndrome.*" The skin disease resembles necrolytic migratory erythema in humans, a well-defined paraneoplastic syndrome typically associated with hyperglucagonemia secondary to glucagon-secreting pancreatic neoplasia, but also reported in individuals with hepatitis, cirrhosis, celiac disease, chronic malabsorption, and inflammatory bowel disease. *In dogs, superficial necrolytic dermatitis is most commonly associated with liver disease*, including severe vacuolar hepatopathy, idiopathic hepatocellular collapse, and hepatopathy secondary to anticonvulsant drug administration, and more rarely with pancreatic adenocarcinoma and gastric carcinoma.

Clinical presentation is typically because of the dermatitis; skin lesions include erythema, crusting, exudation, ulceration, and alopecia, involving footpads, periocular, perioral, anogenital regions, and pressure points. The dermatologic lesions are described more fully in Vol. 1, Skin and appendages. Affected dogs have depressed plasma amino acid concentrations, inconsistent elevations of plasma glucagon levels, and may also become diabetic. The liver of affected dogs is usually grossly nodular, resembling cirrhosis (Fig. 2.40); however, histologically, there is typically moderate to severe vacuolation of hepatocytes, with parenchymal collapse accompanied by nodular regeneration. Inflammation, necrosis, and fibrosis are not usually prominent. Although some studies report fibrosis typical of cirrhosis, more characteristically there is a network of reticulin and fine collagen fibers representing the remnants of collapsed hepatic lobules, with proliferation of bile ductules. The vacuolated hepatocytes stain with oil red O for lipids. *The hepatic lesions have been suggested to support an underlying metabolic, hormonal, or toxic etiology.* In humans, persistent hyperglucagonemia stimulates prolonged gluconeogenesis, resulting in secondary hypoaminoacidemia. Although hepatic insufficiency can increase glucagon levels in animal models, in dogs, the link between liver disease and hypoaminoacidemia remains unclear, although a hypermetabolic state with exaggerated amino acid catabolism has been suggested. Other biologically active molecules that are normally cleared by the normal liver or generated by a damaged liver should also be considered.

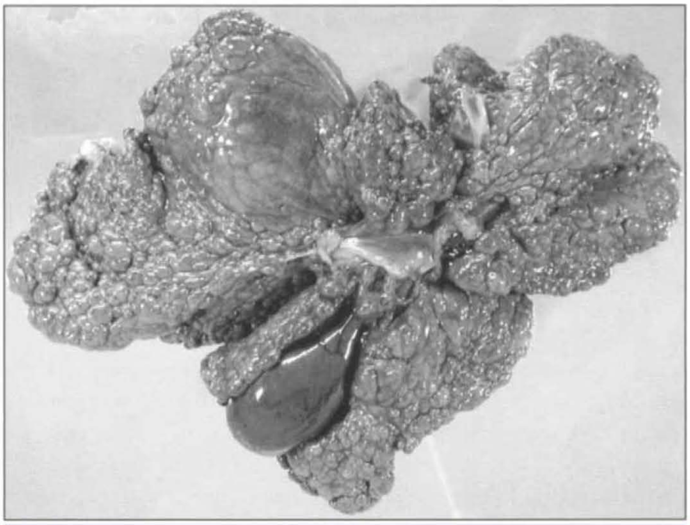

Figure 2.40 Nodular liver: **hepatocutaneous syndrome** in a dog. (Courtesy of JL Caswell.)

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POSTMORTEM AND AGONAL CHANGES IN LIVER

The liver, rich in nutrients for bacteria and freely exposed to agonal invaders from the intestine, *undergoes postmortem decomposition very rapidly*. Gas bubbles generated by anaerobic saprophytes form first in the hepatic blood vessels, but soon suffuse large portions of the organ. The vessels and adjacent parenchyma are stained by hemoglobin. The substance of the organ becomes soft and clay-like, and the formation of putrefactive gases may make it foamy. On the capsular surface, irregular, pale foci are visible; they resemble infarcts or fatty areas but can be observed to increase in size and, microscopically, are without cellular reaction. Bacilli are present in large numbers in such foci. Green-black pigmentation of the capsule and superficial parenchyma occurs where the liver is in contact with gut. The lobes surrounding the gallbladder are stained brown with bile.

There is much microscopic structural change in the liver approaching and immediately following death. In general, the confusing autolytic changes mainly affect the regions around the hepatic venules. Indeed, cytologic criteria of necrosis in periacinar necrosis are more reliably found in animals that have been allowed to die or that are killed in extremis. Shrinkage of liver cells and disappearance of many with widening of periacinar sinusoids are seen after death with hepatic congestion. *Dissociation of liver cells* may be complete, with every cell in every cord separated and free from adjacent cells so architectural patterns are lost. The early expression of this change affects periacinar cells, which become detached, rounded in contour, condensed, and hyperchromatic. The dissociation is particularly seen in feline panleukopenia and leptospirosis, but it is related somewhat to postmortem change.

VASCULAR FACTORS IN HEPATIC INJURY AND CIRCULATORY DISORDERS

Hepatic artery

The hepatic artery brings approximately one-third of the afferent hepatic blood supply; this enters a peribiliary capillary plexus in the portal triads, before anastomosis with the portal veins and entry into the sinusoids. Anomalous origin of the arterial supply is common. Hepatic arterial occlusions occur rather commonly in animals but usually involve small intrahepatic branches and are of little consequence. Large segments of the liver may be necrotic in cats as a result of thrombosis of the aorta and hepatic artery. Verminous arteritis may occlude the hepatic artery in horses. The extent of necrosis depends on how completely the obstruction excludes collateral circulation and also on the oxygen tension of the portal blood. Ischemic areas of liver can be sequestered, but in some instances bacteria such as clostridia and other anaerobes can flourish and release potent toxins with systemic effects.

Portal vein

The portal vein drains the splanchnic viscera and normally contributes two-thirds of hepatic blood flow. Portal blood flow in some animals is streamlined, such that blood from the stomach and duodenum flows preferentially to the left lobes, whereas blood from the jejunum and ileum flows to the right lobes. Streamlining may account for the different regional distributions sometimes observed with metastatic tumors and infections. The umbilical vein usually delivers to the left lobe, so hematogenous umbilical infections tend to localize in the left lobe.

The liver cannot regulate portal venous flow, so hepatic blood flow is largely dependent on the portal venous supply balanced by arterial supply controlled by the sympathetic nervous system. If portal blood flow increases, the hepatic arterial supply is reduced, or vice versa. Hepatic blood thus flows evenly through the sinusoids under a very-low-pressure gradient. The liver can receive approximately 25% of the cardiac output, event though it represents about 2.5% of body mass. Approximately 25% of the weight of the liver in situ is blood. Reduction in the volume of hepatic flow leads to atrophy of deprived segments of liver.

Obstruction of the portal vein, if sudden and complete, produces a condition akin to strangulation of the gut, and death occurs quickly without significant hepatic change. Obstruction of a large branch of the portal vein in cattle, sheep, and cats leads to acute ischemia of a wedge of tissue in which necrosis may be zonal or massive. Obstruction to many small portal radicles is common, with necrosis of many acini. It is evident that if a collateral supply develops and oxygenation remains adequate, obstruction of portal radicles will have no immediate effect on the hepatic parenchyma, save perhaps to make it more sensitive to toxic injury. There is, however, a long-term effect, probably nutritional. The parenchyma in the affected lobe, deprived of hepatotrophic factors, loses much of its regenerative power and atrophies fairly rapidly, allowing condensation and scarification of the stromal tissues.

Acute increase in pressure in the portal vein may occur in any severe episode of widespread acute hepatic necrosis; the cause appears to be simple obstruction of the sinusoidal flow by thrombosis and actual sinusoidal disruption. In such animals there is severe acute congestion of the liver, slight ascites, free fibrin accumulations in the abdomen (not the firm capsular adhesions seen in passive congestion), and distended portal lymphatics.

Obstruction of the extrahepatic portal vein is quite uncommon. External compression may occur due to adjacent abscesses or neoplasms. **Portal vein thrombosis** may be caused by damage to the portal vein by local inflammatory processes, or be associated with states of hypercoagulability or retrograde intravascular growth of hepatic neoplasms. In dogs, thrombosis of the portal vein has been associated with distant neoplasia, immune-mediated hemolytic anemia, protein-losing nephropathy and enteropathy, pancreatitis, peritonitis, and corticosteroid administration. Portal vein obstructions of slow development are expected to lead to portal hypertension and its consequences. Atresia of the portal vein can be demonstrated in some cases of extrahepatic congenital portosystemic shunting, but this is not associated with portal hypertension. Primary hypoplasia of the portal vein is discussed in the section on Developmental anomalies, above.

Obstruction to intrahepatic portal vessels is a consequence of progressive fibrosing lesions of primary hepatic disease centered on the portal triads. The small portal vessels may be obliterated in the proliferative portal lesions and new connections may be established, including small functional arteriovenous communications. Regenerative hepatic nodules and neoplasms may deform and compress portal vessels in some locations.

Portal hypertension is caused by sustained impedance to portal flow anywhere along the path from the portal vein to the right heart.

- **Prehepatic** portal hypertension is associated with occlusion of the portal vein by thrombi, stenosis, extraluminal compression, or as a result of congenital portal vein hypoplasia.
- **Intrahepatic** sites of increased vascular resistance may be further divided into:
  - *presinusoidal* (including intrahepatic portal vein hypoplasia, intrahepatic arterioportal fistulae, periportal neoplastic or inflammatory infiltrates, and periportal fibrosis);
  - *sinusoidal* (cirrhosis or long-term inflammatory disease with fibrosis and capillarization perisinusoidal, neoplastic infiltrations); and
  - *postsinusoidal* (perivenular fibrosis, veno-occlusive disease).
- **Posthepatic** hypertension is associated with increased resistance to blood flow in the major hepatic veins, caudal vena cava or right heart, and includes obstruction of the hepatic veins, neoplastic occlusion (e.g., pheochromocytoma or aortic body tumors), and kinking of the caudal vena cava, associated with abdominal trauma. The complications of portal hypertension include acquired portosystemic shunts, congestive splenomegaly, and ascites.

Efferent hepatic vessels

Efferent flow begins in the sinusoids and passes through terminal hepatic venules and larger hepatic veins to the vena cava. In the sinusoidal network, flow is interconnected in many directions. However, in normal conditions lobule outflow is closely matched to inflow. In dogs, hepatic veins have substantial smooth-muscle sphincters that regulate outflow, but these are less obvious in other species. Splanchnic engorgement during anesthesia and anaphylaxis is an indication that these smooth muscles control canine hepatic outflow in a dynamic manner. There are likely to be important differences in the structure and function of outflow veins in humans due to the postural influences on hepatic venous pressure.

The amount and arrangement of conventional connective tissue around the terminal hepatic veins and larger hepatic veins influence the patterns of fibrosis observed in various patterns of periacinar injury. Hypoxia from anemia, heart failure, or shock can cause periacinar necrosis, as can many toxic agents that are preferentially injurious to zone 3 hepatocytes. Necrosis at this level elicits a tissue repair response involving the fibrous connective tissue along the terminal hepatic venules. Tissue fibrosis can increase the resistance of the hepatic parenchyma and slow local outflow. This can redirect sinusoidal blood to alternative less-affected venules, unless the necrosis is extensive. Within the sinusoids, flow may become quicker in sinusoids that are capillarized by the deposition of extracellular matrix in the perisinusoidal space, but the net flow of blood through such fibrotic liver is reduced. **Hepatic veno-occlusive disease** *is the obliteration of small intrahepatic veins associated with damage to the sinusoidal endothelium, accumulation of red cells and fibrin in the subintimal space, and subsequent subendothelial fibrosis.* This pattern of postnecrotic fibrosis can contribute to the development of portal hypertension. Veno-occlusive disease is a feature of pyrrolizidine toxicosis in humans ingesting these alkaloids in so-called bush tea. In domestic animals, occlusive changes in terminal venules are notable in poisoning by ragwort (*Senecio jacobea*) in cattle and also in dogs treated experimentally with the pyrrolizidine alkaloid monocrotaline. Veno-occlusive disease has also been reported as a consequence of prolonged chemotherapy, radiotherapy, and bone marrow transplantation in humans, and similarly in dogs treated with irradiation or busulphan. Idiopathic veno-occlusive disease with perivenular fibrosis around central and sublobular veins, causing Budd–Chiari-like syndrome (see below), has also been reported as a rare occurrence in the dog and cat. Total or subtotal obliteration of central hepatic or sublobular veins by subintimal accumulation of collagen and fibrous tissue has also been reported in captive snow leopards and cheetahs.

Obstruction at the level of the large hepatic veins can occasionally occur due to suppurative phlebitis, hepatic abscesses, neoplasms, or other physical obstacles that impinge on the hepatic veins. Similar space-occupying lesions in or around the vena cava in the diaphragm or mediastinum can also affect hepatic outflow, along with systemic venous return. However, *by far the most common cause of diffuse hepatic congestion is cardiac failure.*

Passive venous congestion of the liver denotes elevation of pressure in the hepatic veins and venules relative to the pressure in the portal venules. This may be due to congestive heart failure as a result of congenital or acquired cardiac disease or pericardial disease, or, much less frequently, to partial obstruction of the larger hepatic veins or caudal vena cava by abscess, neoplasm, thrombus, or severe heartworm disease (dirofilariasis). Passive congestion is also seen when part of the liver is incarcerated as a result of diaphragmatic hernia, or when a lobe is subjected to chronic partial torsion.

Acute passive congestion occurs when the liver is suddenly engorged with blood as a result of anaphylaxis, shock, or other acute insult. In the early stages, the liver is swollen, dark, and bloody on section, and there is little accentuation of the acinar pattern. There is usually excessive blood-tinged abdominal fluid, because the congested liver elaborates lymph at an increased rate; this may be seen exuding from the capsule during life and distending hilar and gall-bladder lymphatics in fresh carcasses. Because this lymph is rich in most clotting factors, it tends to clot on the liver capsule, and the lobes may be stuck together by fibrin, which is often blood-tinged.

The microscopic picture in acute passive congestion is one of fairly uniform sinusoidal engorgement, with accompanying distension of lymphatics in the stroma of hepatic veins, portal triads, and capsule. There follows rapidly fatty change, trabecular atrophy, and necrosis of periacinar hepatocytes with retention of the perisinusoidal reticulum framework. Erythrocytes tend to move into the perisinusoidal spaces left by the lost hepatocytes, and may be seen trapped there when blood drains from sinusoids and veins in freshly fixed sections. The intrahepatic network of lymphatics at this stage becomes very distended and may form extensive cavernous channels about hepatic veins and venules, in portal triads, and just beneath the capsule. Distension of the space of Disse may be seen, but rarely, if ever, in tissue well fixed soon after death, and is best regarded as a postmortem artifact. By comparison, perisinusoidal edema is more obvious in hepatic congestion associated with shock or inflammatory conditions.

In **chronic passive congestion**, the amounts of fibrous connective tissue in the liver increase in various patterns that differ among species. The capsular surface becomes thicker and more opaque, and can develop a finely nodular texture with the formation of capsular plaques. In dogs and cats, the edges of the central lobes become rounded, whereas the margins of lateral and caudate lobes are sharpened by peripheral atrophy and fibrosis. If the cause of the congestion is still present, there is usually copious ascites at this stage. In species in which fibrosis is more pronounced, a chronically congested liver has a distinct reticulated acinar pattern, often more obvious beneath the capsule than in deeper parenchyma. This pattern is known as "**nutmeg liver**" and is *due to the contrast of red periacinar zones of congestion with loss of hepatocytes, among pale swollen periportal parenchyma composed of viable hepatocytes that are fatty* (see Fig. 2.28). This classical pattern of chronic passive congestion is most obvious in ruminants and horses. The "nutmeg" pattern can be mimicked by some forms of toxic periacinar necrosis or fatty change but should always be distinguishable from them by the presence of fibrous plaques in Glisson's capsule in the passively congested liver. The pale lobular pattern is less obvious in carnivores and should not be expected in animals that have insufficient adipose reserves for mobilization. Over longer periods, *periacinar fibrosis links hepatic venules with one another and with the larger portal triads in a pattern known as* **cardiac fibrosis** (Fig. 2.39B).

Inflammatory changes in the outflow veins are sometimes observed. Acute inflammation and thrombosis of sublobular veins are typical of acute salmonellosis in many species but these changes are terminal and not associated with hepatic dysfunction. Inflammatory cell infiltrates are occasionally observed in the larger hepatic veins, and have been described in dogs with parvoviral myocarditis. Muscular hypertrophy of walls of hepatic veins has been described in dogs with arteriovenous fistulae. Fibrous remodeling of these veins has been associated with nitrosamine intoxication in domestic animals.

The term **Budd–Chiari syndrome** is used to describe the clinical features associated with hepatic venous outflow obstruction caused by thrombosis of the main hepatic veins in humans. In veterinary medicine, the term **Budd–Chiari-like syndrome** has been applied more broadly to encompass the clinical signs and pathologic changes that develop in response to various mechanical causes of postsinusoidal obstruction of hepatic venous flow, resulting in development of *hepatomegaly, postsinusoidal portal hypertension, high-protein ascites, and acquired portosystemic collateral shunting*. The syndrome has been infrequently reported in dogs and cats. Causes include obstruction of flow due to tumors (e.g., intraluminal leiomyosarcoma, adrenal pheochromocytoma with extensive invasion into the caudal vena cava in the dog) or abscesses in the liver or caudal vena cava, hepatic venous or venal caval thrombosis, congenital malformation (fibrous web), kink or acquired stricture occluding the lumen of the vena cava or hepatic veins (intrahepatic postsinusoidal venous obstruction), or cardiac malformations impairing right atrial function (cor triatrium dexter, neoplasia). Histologic changes include distension of the hepatic veins and perivenous sinusoidal congestion, which, with chronicity, leads to perivenous fibrosis typical of chronic passive congestion.

Thrombosis of the caudal vena cava has been described in detail in cattle, where rupture of a hepatic abscess into the caudal vena cava is the most common etiology. Sequelae include pulmonary emboli, endoarteritis, multifocal pulmonary abscessation, and chronic suppurative bronchopneumonia. **Hepatic vein thrombosis**, associated in humans with polycythemia vera, pregnancy, and the postpartum state, oral contraceptive use and intra-abdominal cancers, is a rare occurrence in dogs.

Acquired portosystemic shunts

Congenital shunts have been described in the section on Developmental anomalies, above. *Acquired portosystemic shunts within and external to the liver can develop in association with various chronic liver diseases that lead to portal hypertension.* These shunts can be multiple, small, and may be difficult to identify postmortem. They are easily destroyed by routine dissection, so they must be noted before removal of the abdominal viscera. Acquired shunts can be associated with evidence of portal hypertension, such as distension of the portal veins and ascites, but when shunts are multiple and well established, portal hypertension is somewhat relieved. *It is important to distinguish these varicose dilations that develop in response to portal hypertension from true congenital portosystemic shunts.* Acquired shunts arise in vestigial nonfunctional portosystemic communications that dilate and become functional in response to portal hypertension. They tend to develop between mesenteric veins and the caudal vena cava, right renal vein, or gonadal veins, and are multiple, taking the form of a plexus of tortuous, thin-walled vessels (see Fig. 2.4). Esophageal shunts and varicosities are common in humans with cirrhosis, but are much less important in domestic animals. This may be related to postural differences such that shunts are more likely to enter the vena cava where central venous pressure is least. In dogs, acquired shunts are most numerous along the caudal mesentery.

Telangiectasis

Telangiectasis is cavernous ectasia of groups of sinusoids that occurs in all species, but is particularly common in cattle. The lesion is not functionally significant. Telangiectases occur throughout the liver as dark-red areas, irregular in shape but well circumscribed, and ranging from pinpoints to many centimeters in size. Sectioned or capsular surfaces are depressed after death, and on cutting they appear as cavities from which the blood drains to reveal a delicate network of residual stroma and strands of atrophic hepatocytes. The pathogenesis of telangiectasis remains uncertain; however, an ultrastructural study of pretelangiectasis (sinusoidal dilation) and telangiectasis (blood-filled cavities) in Holstein–Friesian steers demonstrated *sinusoidal barrier alterations*, leading to sinusoidal capillarization, with progressive formation of a true basement membrane and perisinusoidal fibrosis. A toxic or metabolic etiology has been suggested.

Telangiectasis in livers is quite common in older cats. As with the bovine lesion, there is no evidence clinically of related liver dysfunction. In cats, the cavities are rather more frequent in the subcapsular zone (Fig. 2.41) and rarely exceed 2–3 mm in size. There are often other senile changes in these livers, such as chronic fatty change, nodular hyperplasia, and chronic cholangiohepatitis, but there is no evidence that these changes have any causal relationship to the telangiectasis.

Peliosis hepatis

Peliosis hepatis is a term used to designate a hepatic vascular disorder characterized by cystic, blood-filled spaces in the liver. The histologic lesion is characterized by multiple, dilated, blood-filled spaces surrounded by fibromyxoid stroma containing inflammatory cells and dilated capillaries. The blood-filled cystic spaces may be partially lined by endothelium; some may merge directly with surrounding hepatic

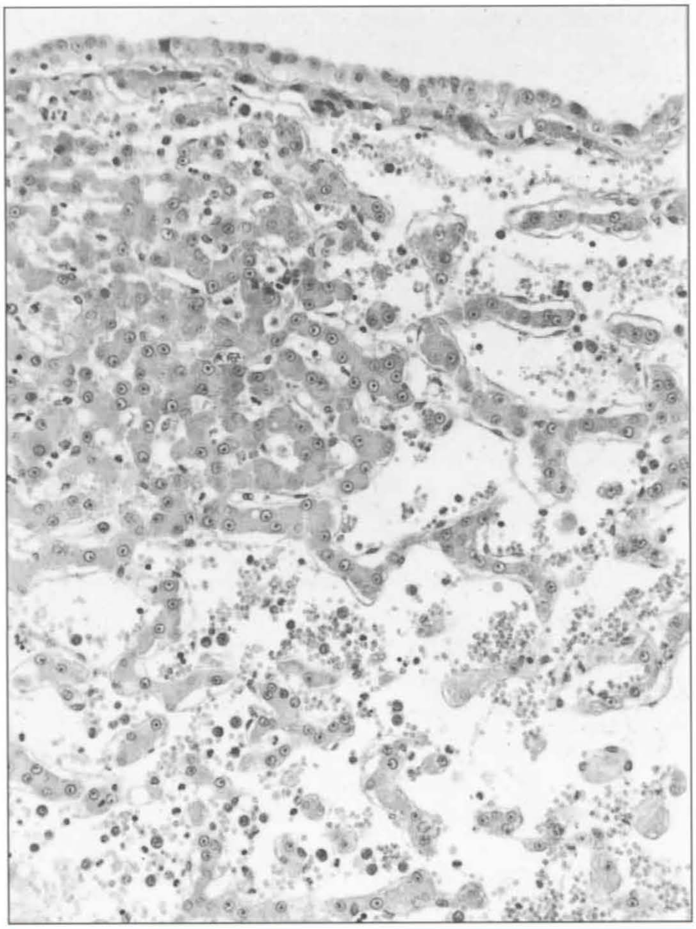

Figure 2.41 Subcapsular **telangiectasis** in a cat.

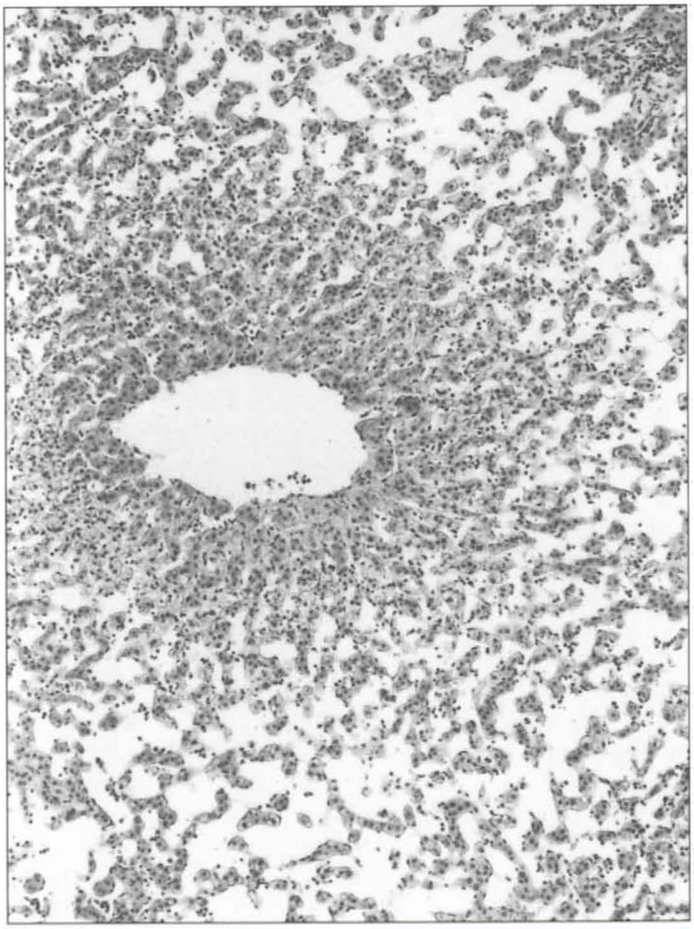

Figure 2.42 Periportal sinusoidal dilation due to chronic *Pimelea* poisoning in an ox.

sinusoids. These lesions were originally described in human patients with tuberculosis. More recently, it is described as an idiosyncratic association with administration of various drugs, including anabolic and contraceptive steroids. Infectious peliosis hepatis caused by *Bartonella henselae* or *B. quintana* infection has also been described in immunosuppressed human patients. *Peliosis hepatis has been reported in cattle, dogs, and cats, but the pathogenesis is unknown. B. henselae* DNA has been identified by polymerase chain reaction from the liver of a single canine case. Bovine hepatic telangiectasis and human peliosis hepatis have been suggested to share a similar pathogenesis. Primary alteration of the sinusoidal barrier, with increased deposition of basement membrane components in the perisinusoidal region and fibrosis, may alter oxygen and substrate exchange between hepatocytes and the blood, leading to hemodynamic imbalance, hepatocyte atrophy, and eventually to sinusoidal disruption.

A specific condition termed "*peliosis*" develops in cattle poisoned by plants of *Pimelea* spp. This form *begins as diffuse periportal sinusoidal dilation* (Fig. 2.42). Since these changes are also found in these animals in the spleen and in other organs with sinusoidal microcirculation, it seems that the lesions may be adaptive to progressive and dramatic increases in total blood volume. In the late stages of the intoxication by *Pimelea*, the liver may resemble a huge, blood-filled sponge. The animals eventually die of a combination of hemodilutional anemia and circulatory failure.

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INFLAMMATORY DISEASES OF THE LIVER AND BILIARY TRACT

The inflammatory response in the liver is unusual for three main reasons. First, *hepatic microvasculature is structurally and functionally different from tissues with capillary vasculature.* Microvascular permeability to plasma proteins, a hallmark of acute inflammation in most tissues, is a normal property of the fenestrated sinusoidal endothelium of the liver. Thus, edema is not a prominent feature of acute parenchymal inflammation, although it can be observed in the capsule, biliary tracts, and gallbladder. Microvascular blood flow in hepatic sinusoids is also less responsive to the actions of various vasoactive mediators that alter blood flow in most other acutely inflamed tissues. Second, *hepatic Kupffer cells have some distinctive properties that enable them to deal with many potentially inflammatory insults in the portal circulation.* While they appear to have a higher threshold for activation, they can release large amounts of potent mediators such as nitric oxide, interleukin-6, and tumor necrosis factor-α that can influence cell populations in the liver and elsewhere. Third, *the liver has central regulatory influences on many proinflammatory insults and inflammatory mediators.* The liver produces most plasma proteins with anti-inflammatory functions, and is also the site of degradation of most soluble plasma proteins. Kupffer cells are the main site of clearance of immune complexes from the circulation, and they have an important role in the development of immunotolerance to potential antigenic substances absorbed from the intestine.

These unusual aspects of the inflammatory response in the liver can make it more difficult to differentiate some degenerative and inflammatory conditions in this organ. Ongoing cell death and repair can appear inflammatory and acute leukocyte responses can cause necrosis and apoptosis in the liver. *The term* "**necroinflammatory**" *is convenient when the underlying pathogenetic mechanisms of necrosis and inflammation are unknown.* Areas of focal hepatitis are commonly found in the liver, particularly in older animals. These usually reflect previous areas of focal infection or necrosis that are of little functional significance because the liver has so much functional reserve and regenerative capacity.

Increased numbers of leukocytes (**sinusoidal leukocytosis**) are observed in hepatic sinusoids in many acute or subacute bacteremias. These changes can be diagnostically useful but they do not constitute evidence of hepatitis unless there is obvious infiltration of granulocytes, monocytes, or lymphocytes into the perisinusoidal space. **Extramedullary hematopoiesis** can also appear as focal aggregates of myeloid cells in the perisinusoidal compartment. These changes (sometimes termed *myeloid metaplasia*) can be distinguished from inflammatory infiltrates by the presence of immature myeloid cells. Occasionally, hematopoietic cells from the splenic red pulp can be artifactually extruded into the portal vasculature and appear in the liver, usually as nucleated cell aggregates in the larger portal veins.

Infectious agents capable of causing hepatitis include viruses, bacteria, fungi, and helminths. Autoimmune and idiosyncratic drug responses are also observed in frequently, but often the etiology of acute or chronic hepatitis cannot be determined.

Acute hepatitis

The liver is subject to infectious and degenerative insults that elicit inflammatory responses in various patterns, for which the general term hepatitis is appropriate. **Cholangiohepatitis** *applies to hepatic inflammation centered on the biliary tract and extending into adjacent hepatic parenchyma.* The term **hepatitis** *is used for focal or diffuse hepatic conditions that are either caused by infectious agents or characterized by a leukocytic infiltrative inflammatory response, irrespective of the cause.* This definition allows us to include viral infections that are hepatotropic, even though the lesions are mainly characterized by hepatocellular necrosis or apoptosis rather than by the inflammatory response to the agent. Demonstration of sufficient amounts of injurious infectious agents within the lesions by inclusion bodies, antigens, or nucleic acids are important in making this diagnosis. The term hepatitis is also used for responses to some hepatic toxicants, metals, or drug metabolic idiosyncrasies in which there is a prominent leukocytic response to damaged cells. However, in responses in which single necrotic hepatocytes elicit a mild neutrophilic or histiocytic response, the term hepatitis is less appropriate because the pattern of injury is primarily degenerative.

Leukocyte infiltrates in diffuse hepatitis tend to accumulate mainly in the vicinity of the portal tracts, around major bile ducts, or sometimes in the capsule and around the central veins. Small but important numbers of neutrophils and mononuclear cells, including lymphocytes, are usually seen in the perisinusoidal space and among hepatocytes, and are often focally concentrated in sites of necrosis. Edema is an unusual feature of acute hepatitis, but is seen in severe injury, for example in infectious canine hepatitis. Grossly, hepatic edema is most obvious in the gallbladder, large extrahepatic bile ducts, hepatic lymph nodes, and sometimes in the capsule. Microscopically, edema is also evident in the portal triads and sometimes by an increase in the perisinusoidal space.

Kupffer cells are key participants in the acute inflammatory responses in the liver. They can enlarge and accumulate vacuoles and lysosomal debris during regular phagocytic removal of microorganisms, cell debris, and extravascular erythrocytes. They can also be activated to secretory histiocytes that release various cytokines and other mediators that induce hypertrophic or proliferative responses of hepatocytes, stellate cells, and endothelium. Activated Kupffer cells are larger and more prominent or numerous in sections, their nuclei are larger and vesicular, and their cytoplasm is basophilic and may contain vacuoles or ingested particulate matter. In overwhelming infections, many of the Kupffer cells and adjacent sinusoidal endothelial cells and hepatocytes undergo necrosis.

Patterns and character of inflammation in hepatitis vary according to causative agent, severity and stage of disease, the route of entry into the liver, and pathogenesis of liver injury. Some viral pathogens such as *Canine adenovirus 1* can cause acute and diffuse hepatitis, with widespread hepatocellular necrosis, mixed leukocyte infiltrates, sinusoidal congestion, and edema. By comparison, most infectious causes of hepatitis, for example toxoplasmosis, various herpesviruses, and various bacteria, produce a more patchy pattern of inflammation with focally intense leukocyte and Kupffer cell responses in the vicinity of areas of necrosis. The distribution, character, and chronicity of these focal lesions are important diagnostically, but they typically do not damage enough functional hepatic parenchyma, ducts, or vasculature to produce systemic signs of liver failure. Foci of hepatitis with necrosis are common incidental findings, and these are assumed to reflect localized responses to bacteria that arrive via the portal system. Occasionally such focal necroinflammatory lesions are large and numerous, for example in cattle with rumenitis.

Chronic hepatitis

Chronic liver disease in domestic animals has historically been classified into several different entities based on morphologic criteria. In human medicine, classification of chronic hepatitis has been simplified, and morphologic divisions such as chronic active hepatitis and chronic persistent hepatitis, originally defined in specific clinical contexts, have been abandoned due to problems of evolving definitions and application, and lack of correspondence with prognosis. The single designation "**chronic hepatitis**" is now used for *chronic necroinflammatory disease lasting more than 6 months*, further modified by specifying the etiology, type, and severity of inflammation and degree and distribution of necrosis (activity or grade), and the degree of fibrosis (chronicity or stage). Systems for *grading and staging chronic hepatitis* have been developed (Tables 2.1 and 2.2). More detailed *histological activity index* scoring systems have also been developed. In human medicine, chronic hepatitis is usually the result of chronic infection with hepatotropic viruses, and, less commonly, autoimmune, drug-induced or associated with inherited metabolic diseases such as Wilson disease. In veterinary medicine, chronic liver disease may develop following chronic bile duct obstruction, infection with hepatotropic infectious agents, familial or hereditary metabolic diseases, or may be toxic, drug-induced, or possibly autoimmune in origin. However, *the majority of chronic liver disease is idiopathic*, reflecting deficiencies in our current level of understanding of the etiological, pathophysiological, and clinical implications of the patterns of inflammation and necrosis seen in domestic animals.

Regardless of the etiology, *initial acute liver damage will not progress to fibrosis or cirrhosis unless the inflammation and damage are protracted*, for example, by ongoing hepatocellular injury mediated by immunological mechanisms, including antibody- and lymphocyte-mediated cytotoxicity, or ongoing oxidative damage. Clinical signs are nonspecific in the early stages, but as the disease progresses to involve more of the liver and impair regeneration, icterus, ascites, and hepatic encephalopathy may develop as typical correlates with hepatic insufficiency.

Chronic hepatitis of humans has several characteristic lesions. These are also evident in animals, although not to the same degree or frequency as described in the various forms of viral, immune-mediated, and idiosyncratic hepatitis in humans. The first is **periportal interface hepatitis**, sometimes referred to as "*piecemeal necrosis*" (Fig. 2.43). *This necroinflammatory change initially destroys the limiting plate of periportal hepatocytes, and may continue to erode into the hepatic parenchyma, expanding the portal areas.* Portal inflammation is variable in intensity, and includes infiltration by lymphocytes and plasma cells. Bridging necrosis, with tracts of necrosis dissecting across the hepatic lobule between portal triads or between portal areas and central veins, may also develop. Degenerative changes affecting hepatocytes in areas of interface hepatitis include cell swelling and apoptosis. Bile duct degeneration, multifocal necrosis, and hepatocellular regeneration in the form of increased numbers of binucleated and trinucleated cells and mitotic figures, may also be seen. Deposition of collagen and basement membrane material in the space of Disse leads to capillarization

Table 2.1 Grading the degree of disease activity in chronic hepatitis

	Lesions and degree of injury			
Category	Portal area inflammation	Periportal (piecemeal) necrosis	Nonzonal necrosis	Bridging and/or multiacinar necrosis
Mild	Mild, patchy	Absent or mild	Mild	Absent
Moderate	Moderate	Moderate	Moderate	Absent
Marked	Marked	Marked	Marked	Absent
Very marked	Marked	Marked	Marked	Present

Adapted from Ishak KG. Pathologic features of chronic hepatitis. Am J Clin Pathol 2000;113:40–55.

Table 2.2 Staging the progression of chronic hepatitis

	Component lesions		
Category	Fibrous expansion of portal areas	Bridging fibrosis (portal to portal, portal to central, central to central)	Bridging with nodules (cirrhosis)
Mild	Absent or mild	Absent	Absent
Moderate	Moderate	Absent (occasional)	Absent
Marked	Marked	Marked	Absent (occasional nodule)
Very marked	Marked	Marked	Present

Adapted from Ishak KG. Pathologic features of chronic hepatitis. Am J Clin Pathol 2000;113:40–55.

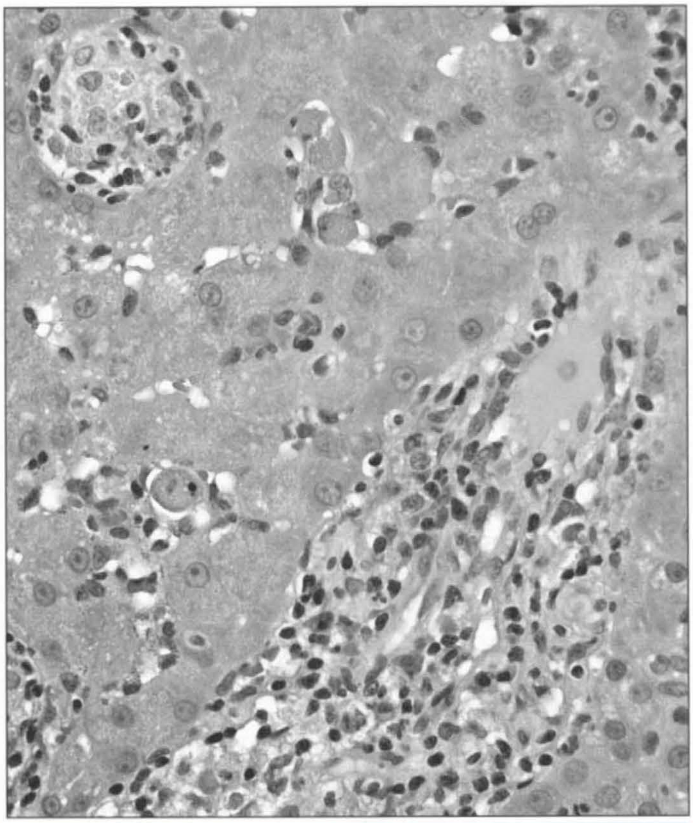

Figure 2.43 Periportal interface hepatitis in chronic hepatitis in a dog.

of hepatic sinusoids. Single or small groups of hepatocytes may be isolated and entrapped in expanded portal areas. Fibrosis may progress to bridge portal tracts, culminating in the development of cirrhosis.

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Chronic hepatitis in dogs

Chronic hepatitis is a relatively common diagnosis in dogs. While infectious etiologies, and drug- and toxin-induced chronic hepatic disease have been described, *the majority remains idiopathic*, although some appear to be breed-associated or familial. *Breeds of dogs* reported to be at increased risk for chronic hepatitis include the Bedlington Terrier, West Highland White Terrier, Doberman Pinscher, American and English Cocker Spaniel, Skye Terrier, Labrador Retriever, and Standard Poodle. Apart from the hereditary hepatic copper accumulation in Bedlington Terriers, the etiology of the breed-related disorders remains unclear.

The pathophysiologic mechanisms of chronic hepatitis in dogs are poorly understood. Given the importance of autoimmune hepatitis in human medicine, several studies have evaluated the role of immune mechanisms in dogs. Peripheral blood mononuclear cell proliferation in response to liver membrane antigens was higher in dogs with chronic hepatitis, supporting the hypothesis of an immune-mediated process, although whether this is primary or secondary to hepatocyte destruction is not known. An immunohistochemical study of the inflammatory infiltrate in 16 dogs with chronic hepatitis identified CD3+ lymphocytes as the most numerous lymphoid cells in liver sections from affected animals. Degenerate hepatocytes were occasionally surrounded by these cells, and necrosis was positively correlated with the numbers of CD3+ lymphocytes, suggesting a role for an immune-mediated process in chronic hepatitis. The number of α-smooth-muscle actin-positive cells (periductular myofibroblasts and activated perisinusoidal stellate cells) in portal triads and fibrous septa was positively correlated with the stage of fibrosis, lending support to the role of these cells in fibrogenesis in the canine liver. Upregulation of major histocompatibility complex class II antigen expression in hepatocytes has been demonstrated in Doberman hepatitis in association with lymphocyte infiltration, and it has been suggested that hepatocytes presenting a putative major histocompatibility complex class II molecule-associated autoantigen could be targets for T-cell-mediated immune attack. However, a primary autoimmune pathogenesis has yet to be demonstrated.

Liver disease associated with increased hepatic **copper** *occurs in various breeds of dogs that develop acute hepatic necrosis, subacute hepatitis, chronic hepatitis, and cirrhosis.* Copper is an essential metal cofactor for cuproenzymes, acting as a redox catalyst for a number of cellular oxidases. However, interaction with cellular hydrogen peroxide can yield highly reactive hydroxyl radicals. Hepatic copper accumulation may result from a primary metabolic defect in hepatic copper metabolism, or possibly as a secondary event resulting from abnormal hepatic function, cholestasis, and altered biliary excretion, a mechanism described in the human literature but not conclusively demonstrated in dogs. *A hereditary, autosomal recessive copper-associated hepatopathy associated with impaired biliary copper excretion and progressive accumulation of copper within hepatocytes has been well documented in Bedlington Terriers, and postulated in other breeds, including West Highland White Terriers and Skye Terriers.* The chromosomal locus of the gene in affected Bedlingtons has been mapped and a mutation in the *MURR1* gene identified, while other defects in the canine homologs of human genes for the copper-transporting ATPase, *ATP7B*, defective in Wilson disease in humans, and *ATOX1*, the copper chaperone delivering copper to *ATP7B*, have been excluded as possible candidates. While its function is unknown, the protein Murr1 has been shown to interact with the copper transporter ATP7B, and may be a copper chaperone involved in directing copper to biliary excretion. The human *MURR1* gene is also polymorphic and one mutation is believed to contribute to the pathogenesis of Wilson disease in a minority of human patients.

The role of copper in the pathogenesis of hepatic disease is less clear in breeds of dogs other than the Bedlington Terrier. Elevated hepatic copper (>400 μg/g dry weight) has been reported in clinically normal dogs, as well as in diseased liver from other breeds. Liver disease with concurrent copper accumulation has been reported in the Doberman Pinscher, West Highland White Terrier, Skye Terrier, Dalmatian, Keeshond, and Poodle, as well as others. *Chronic cholestasis, hepatitis, and cirrhosis of any etiology could increase liver copper concentrations;* secondary copper accumulation occurs in the majority of human patients with primary biliary cirrhosis, prolonged extrahepatic bile duct obstruction, or chronic liver disease. When the hepatic concentration of copper surpasses 400 μg/g dry weight, the excess begins to accumulate within lysosomes in all breeds of dogs, first demonstrable in the hepatocytes of zone 3, later in zone 2, and finally in zone 1 at the highest copper concentrations, a distribution that differs from that of other copper-loaded animal species. At levels exceeding 400 μg/g dry weight, copper-loaded lysosomes become demonstrable by the histochemical stains, rubeanic acid and rhodanine, allowing a qualitative assessment of copper storage. Regardless of the reasons for copper accumulation, lysosomal copper can exceed a threshold or be released when hepatocytes die, and thereby contribute to the development of hepatitis. *Hepatic pathology does not typically occur at concentrations less than 2000 μg/g dry weight*, although higher values can still be found in some dogs with normal liver histology.

Bedlington Terriers *are the only breed to date shown to accumulate copper continuously throughout life,* and hepatic copper concentrations in these animals may be very high; as much as 12 000 μg/g dry weight has been recorded, and levels higher than 5000 μg/g are common. Affected Bedlingtons are usually presented with signs of progressive liver failure, including ill-thrift, wasting, ascites, and signs of encephalopathy. An acute form may occur in some dogs, with acute hepatic necrosis and release of copper into the systemic circulation, where it provokes a hemolytic crisis and rapidly developing anemia and icterus. The most characteristic feature of the histology is the presence of numerous golden-brown refractile granules in hepatocytes, and darker pigment in Kupffer cells. The hepatocellular granules are copper-engorged lysosomes, which preferentially

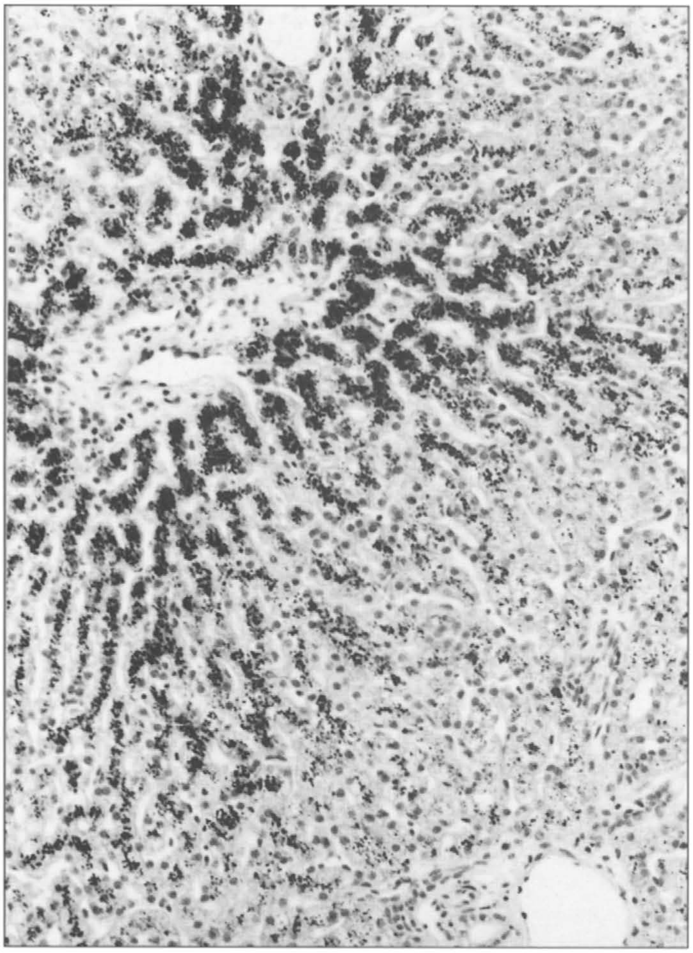

Figure 2.44 Section of liver from Bedlington Terrier, stained with rhodanine for copper to demonstrate the predominantly zone 3 distribution of **lysosomal copper**.

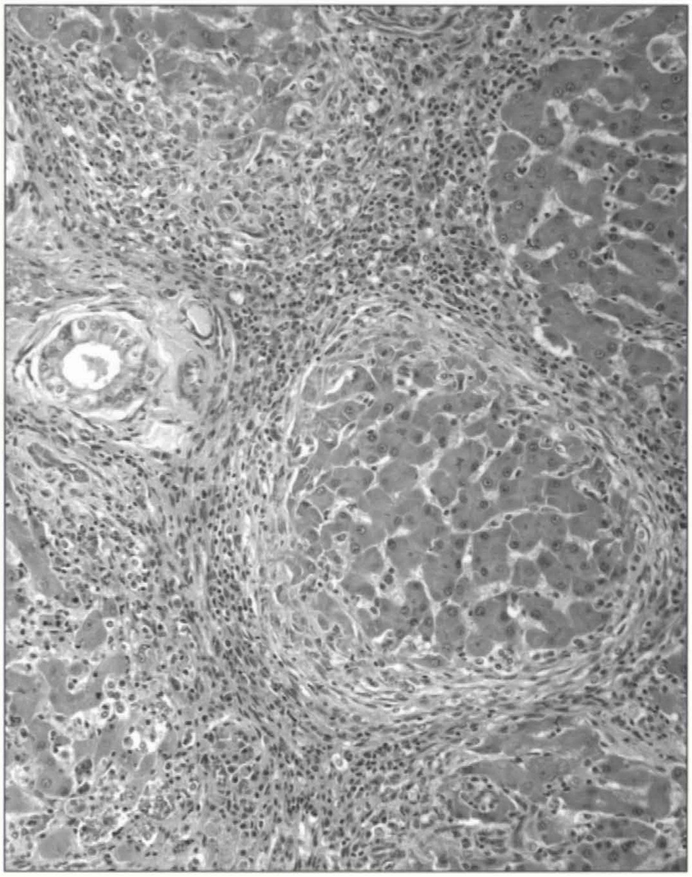

Figure 2.45 Periportal interface hepatitis with mixed inflammatory infiltrates and bridging portal fibrosis in a Doberman Pinscher dog.

accumulate in the cytoplasm of zone 3 hepatocytes. Evidence of hepatitis generally does not develop until levels exceed 2000 μg/g. The initial histologic lesion is multifocal centrilobular hepatitis, with foci of macrophages, lymphocytes, plasma cells, and neutrophils among the copper-laden hepatocytes in zone 3 (Fig. 2.44). Apoptotic hepatocytes, some containing copper granules, appear at the periphery of some foci. Copper levels exceeding 3000 μg/g dry weight result in widespread massive necrosis in some dogs. Survivors may progress to develop postnecrotic cirrhosis. Grossly, the livers in later stages are fibrotic, pale, and finely nodular.

Chronic hepatitis is well documented in **Doberman Pinschers**. The disease is more common in middle-aged female dogs. Histologic changes in the livers of dogs exhibiting clinical signs of advanced hepatic disease include piecemeal necrosis of periportal zone 1 hepatocytes, with a mixed inflammatory cell infiltrate, as well as necrosis of zone 3 hepatocytes with bridging necrosis crossing the lobule. Various degrees of portal fibrosis develop, with bile duct proliferation, bridging fibrosis, and development of cirrhosis in the most severely affected dogs (Fig. 2.45). Accumulation of copper in periportal zone 1 hepatocytes, particularly in areas of degeneration and necrosis, is prominent, although central hepatocytes also have some copper-positive granules (Fig. 2.46). Intrahepatocyte bile pigment accumulation and intracanalicular bile stasis are present in periportal zones, along with iron accumulation in Kupffer cells and macrophages.

Two recent studies, however, document that piecemeal necrosis and marked periportal inflammation are not prominent lesions in early stages of hepatic disease in Dobermans. One retrospective histologic study of the lesions in the livers of 35 Dobermans in the early precirrhotic stages of the disease found a consistently different pattern, with progressive fibrosis, inflammation, and hepatocyte loss beginning in zone 3, affecting hepatocytes around the terminal hepatic vein branches. The earliest changes were inflammation, predominantly small lymphocytes, plasma cells, and individual or clusters of pigmented macrophages often containing iron or copper, collagen deposition around the small hepatic vein branches, and apoptosis of scattered hepatocytes in zone 3. Copper-containing cells were exclusively in zone 3 hepatocytes, contrary to previously published reports. As the disease progressed, collagen deposition increased around the hepatic veins. In some specimens, thin fibrous septa radiated from the hepatic vein branches. In a few dogs, lymphoplasmacytic inflammation was mild and restricted to the connective tissue surrounding smaller branches of the hepatic vein. In the majority of cases, inflammation was distributed equally to the connective tissue around hepatic veins and the connective tissue of the portal tracts. In the most severe cases, infiltrating inflammatory cells were also present in the parenchyma among the hepatocytes. The sinusoids adjacent to the areas of fibrosis were converted to endothelial-lined, thin-walled vessels. Histologic changes were the same regardless of hepatic copper concentration, which appeared to be incidental to the disease progression.

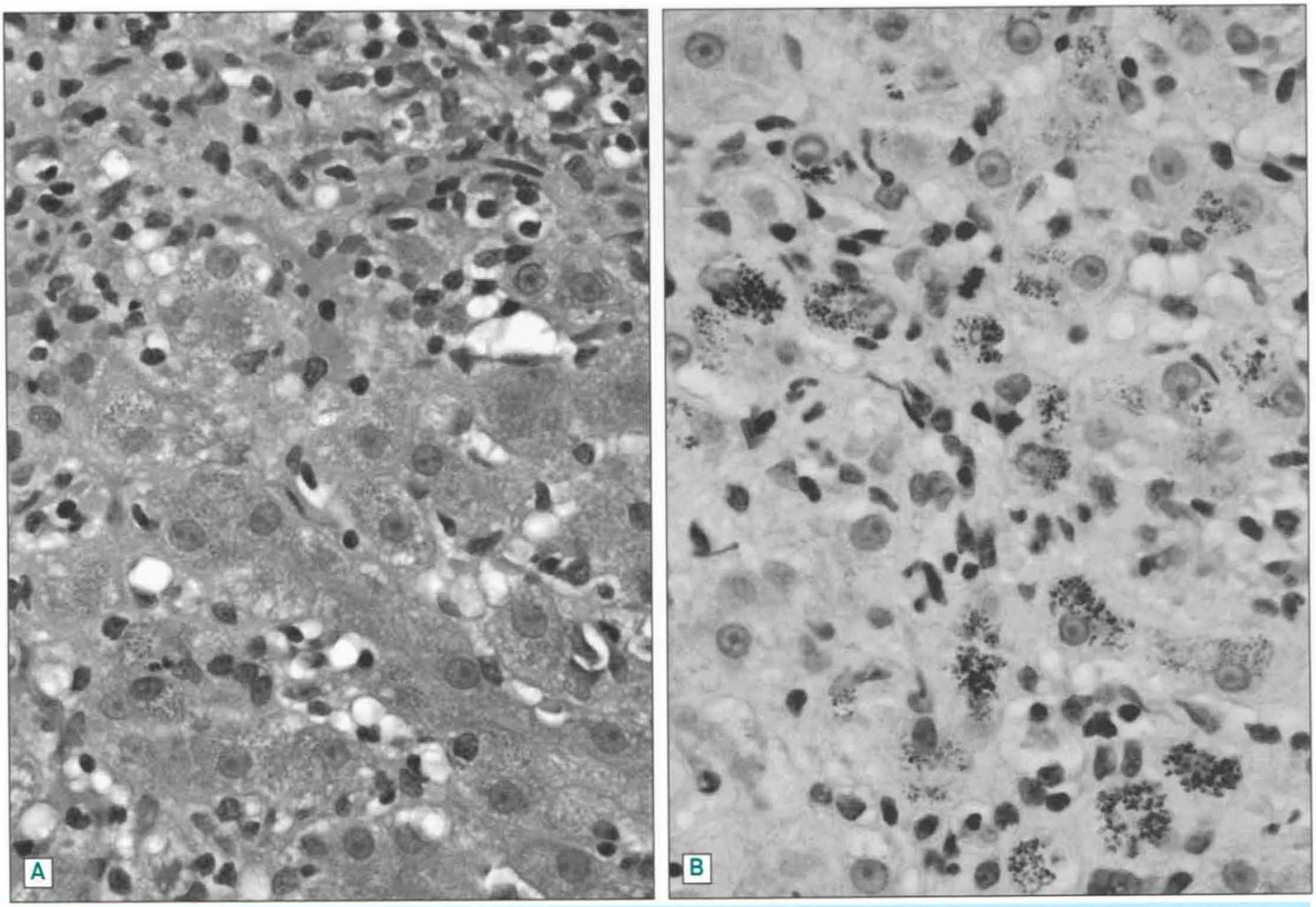

Figure 2.46 **A.** Periportal inflammation and hepatocellular **copper** accumulation in a Doberman Pinscher dog. **B.** Liver in **(A)** stained for **copper** with rhodanine.

The second study of the histologic hepatic lesions in 18 Dobermans suffering from subclinical hepatitis reported that the most significant changes in biopsy samples were multifocal clusters of inflammatory cells in the parenchyma, and portal inflammation. Inflammatory cells were mainly lymphocytes, but neutrophils and macrophages also appeared. Numbers of plasma cells were low. Periportal piecemeal necrosis was usually mild or moderate, and the limiting plate surrounding the portal area was never markedly ruptured. Three dogs had minimal bridging necrosis. Expansion of the portal area, fibrosis, and bile duct proliferation were uncommon, and, if present, mild. Every subclinically affected dog had excessive copper staining in its liver. Five dogs later euthanized for reasons other than liver disease showed progression of hepatic lesions, with at least moderate piecemeal necrosis, and moderate to marked portal expansion. Four dogs were later euthanized because of progressive hepatic disease. All had moderate to marked piecemeal and bridging necrosis, moderate to marked portal expansion, bile duct proliferation, and fibrosis.

Elevated concentrations of hepatic copper have been reported in many, but not all, Doberman Pinschers with chronic hepatitis. *The significance of increased hepatic copper concentration in this breed remains controversial.* It has been reported that clinically normal Doberman Pinschers (with no evidence of liver disease on liver biopsy) are one of several breeds that have higher hepatic copper concentrations than what is considered normal, with a mean hepatic copper concentration of 413 ± 298 μg/g and a range of 140–1500 μg/g. In one report of clinically affected Dobermans, the mean concentration was approximately 2000 μg/g dry weight, sequestered in zone 1 (periportal) hepatocytes. A study of 23 affected Dobermans reported one dog with normal hepatic copper concentration (250 μg/g), 12 dogs with copper levels <2000 μg/g (range from 650 to 1900 μg/g), and 10 dogs with copper levels >2000 μg/g (the highest 4700 μg/g). A study of Dobermans with early precirrhotic hepatitis reported elevated hepatic copper concentrations in 30 of 35 dogs. Of the 22 affected dogs whose liver copper was measured by atomic absorption analysis, 10 dogs had hepatic copper concentrations ≥2000 μg/g dry weight, 3 of which had copper >3000 μg/g, and 12 dogs had copper concentrations ranging from 650 to 1900 μg/g dry weight. In these dogs, the copper-laden hepatocytes were found in zone 3, adjacent to the hepatic vein. The copper accumulation was suggested to be neither the cause nor the effect, but occurred antecedent to the disease, and was incidental to disease progression. However, a recent investigation into the possibility of impaired copper excretion in affected Doberman Pinschers reported that 5 dogs with elevated liver copper and persistent subclinical hepatitis but without demonstrable cholestasis had comparable rates of plasma copper clearance to control dogs, but reduced rates of biliary excretion of ^{64}Cu. This provides additional support for the view that copper accumulation contributes to chronic hepatitis in Dobermans, and is more likely a cause than a consequence of liver damage in this breed.

West Highland White Terriers *are at increased risk of developing chronic hepatitis and cirrhosis.* There is evidence to support *familial* hepatic copper accumulation in some West Highland White Terriers, although the mode of inheritance is not completely understood. Hepatic copper accumulates in zone 3 hepatocytes, up to about 8 months of age, with concentrations rarely exceeding 2000 μg/g dry weight. Clinical illness directly attributable to copper hepatotoxicity (concentrations >2000 μg/g dry weight) in West Highland White Terriers is, however, apparently uncommon. *Idiopathic chronic hepatitis progressing to cirrhosis does also occur in this breed,* and may be distinguished on the basis of a different zonal location and morphology of the inflammatory lesions. In the idiopathic disease, inflammatory foci are smaller, composed of a single apoptotic hepatocyte or fragments of cells accompanied by a few lymphocytes and plasma cells, and are commonly localized to zone 1, or may be random in distribution. In dogs with copper toxicosis, foci of inflammation and necrosis were larger, always found around the central vein among copper-laden hepatocytes, and were composed of debris-filled macrophages, lymphocytes, plasma cells, and scattered neutrophils, with occasional apoptotic hepatocytes around the periphery. *Distinguishing between copper toxicosis and idiopathic chronic hepatic disease may be difficult in cirrhotic livers,* which, irrespective of the underlying cause, may have reduced copper burdens due to connective tissue displacement of hepatic parenchyma, and typically lower concentrations of copper in regenerative nodules.

Chronic hepatitis been reported in genetically related **Skye Terriers**, accompanied by modest and somewhat inconsistent hepatic copper accumulation. Lesions ranged from hepatocellular degeneration and necrosis with mild inflammation in zone 3, to chronic hepatitis and cirrhosis with marked intracanalicular cholestasis. Hepatic copper concentrations ranged from 800 to 2200 μg/g dry weight, and copper-containing hepatocytes were found predominantly in zone 3. The etiology is unknown, although a defect in bile secretion has been suggested.

Chronic liver disease associated with elevated hepatic copper concentrations has been reported in **Dalmatians**. A range of necroinflammatory changes has been reported, including multifocal, piecemeal, centrilobular to massive hepatic necrosis, and cirrhosis, although, in one study of 10 dogs, various degrees of piecemeal necrosis and bridging fibrosis were the most common histologic change, with either primarily lymphocytic or neutrophilic inflammatory infiltrates. Morphologic or biochemical evidence of cholestatic liver disease was not prominent. Hepatic copper concentrations ranged from 745 to 8390 μg/g dry weight (mean 3197 μg/g) in one report of 10 dogs, aged 2–10 years, with a variable zonal distribution. Three previous cases in young Dalmatians reported hepatic copper concentrations of 7940 μg/g dry weight, 1916 μg/g wet weight, and 2356 μg/g wet weight, and two of these reports describe diffuse positive staining for copper in all hepatocytes, with the strongest staining observed in centrilobular hepatocytes.

Chronic hepatitis has been reported in **Cocker Spaniels**, characterized by a brief clinical illness with ascites, weight loss, and other signs typical of hepatic disease. Affected dogs develop chronic hepatitis and cirrhosis, and, at necropsy, livers are typically small and firm, with multiple small regenerative nodules. Histologically, there is moderate to severe portal hepatitis, with inflammatory infiltrates of predominantly lymphocytes, plasma cells, and fewer neutrophils, and variable degrees of portal fibrosis and bridging fibrosis. Piecemeal necrosis and limiting plate destruction have been reported. Biliary hyperplasia is a common feature. Hepatic copper staining is variable, and hepatic copper accumulation does not appear to be a consistent feature. α_1-antitrypsin deficiency *has been suggested to play a role in chronic hepatitis in some dog breeds, including English Cocker Spaniels,* although whether this is an epiphenomenon or cause of chronic liver disease has not yet been proven. α_1-antitrypsin is a member of the serine proteinase inhibitor (serpin) superfamily, and is a potent inhibitor of neutrophil elastase. Hereditary α_1-antitrypsin deficiency in humans is associated with mutations that perturb the protein's tertiary structure and promote polymerization. Accumulation of altered forms of α_1-antitrypsin in the endoplasmic reticulum of hepatocytes, with the formation of inclusion bodies and subsequent cell death has been associated in humans with neonatal hepatitis, juvenile cirrhosis, and adult hepatocellular carcinoma.

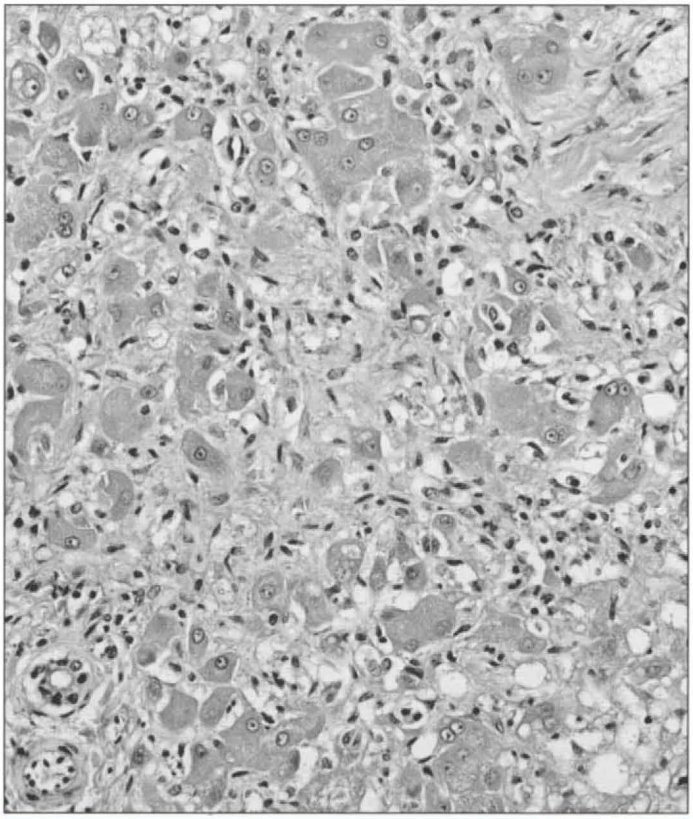

Figure 2.47 Lobular dissecting hepatitis with diffuse fine interstitial fibrosis isolating hepatocytes in a juvenile Golden Retriever dog.

Noninflammatory **idiopathic hepatic fibrosis** *has been reported in young dogs,* typically <2 years of age, and is associated with chronic hepatic failure and portal hypertension. Histologically, there are various patterns of hepatic fibrosis, including diffuse pericellular (reticulofibrosis), periportal, and central perivenous (periacinar) fibrosis. Diffuse pericellular fibrosis is characterized by intralobular reticulin deposition that surrounds individual hepatocytes, and alters normal hepatic architecture (lobular dissecting hepatitis). The periportal pattern (hepatoportal fibrosis) has been discussed previously, and has been associated in some cases with congenital hypoplasia of the portal vein. Central perivenous fibrosis resembles hepatic veno-occlusive disease; however, there is no evidence of intimal damage, and the fibrous tissue is deposited peripheral to the intimal lining of the terminal hepatic veins.

Lobular dissecting hepatitis, *associated with predominantly sinusoidal inflammation and fibrosis, has been described in young dogs with ascites and acquired portosystemic shunts.* The liver is usually small, pale with a predominantly smooth surface, and occasional hyperplastic nodules. The histologic lesion is characterized by dissection of lobular parenchyma by reticulin and fine collagen fibers into individual and small groups of hepatocytes, accompanied by a mixed inflammatory infiltrate of lymphocytes, plasma cells, and lesser numbers of neutrophils and macrophages (Fig. 2.47). Activated fibroblastic cells, likely hepatic stellate cells, may also be prominent along sinusoids. Hepatocytes form rosettes or pseudoductular structures, and regenerative nodules may be present. Portal inflammation and periportal fibrosis are not conspicuous features of this disease. The etiology of this disorder remains unknown.

Granulomatous hepatitis *has been reported in dogs with various systemic parasitic, mycotic, and bacterial infections.* However, granulomatous hepatitis with signs of clinical liver disease, including hepatomegaly, icterus, ascites, and elevated hepatic enzymes, has also been reported in dogs with intestinal lymphangiectasia and inflammatory bowel disease, lymphosarcoma, and histiocytosis. The histologic lesion consists of multifocal infiltrates of histiocytes, often associated with lymphocytes and plasma cells, neutrophils, or eosinophils.

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Miscellaneous inflammatory liver disease

Idiopathic acute hepatic disease, *also known as* **equine serum hepatitis** *or* **Theiler's disease**, *is a common cause of acute hepatic failure in horses.* The disease was originally observed in horses passively immunized against African horse sickness and later in horses passively immunized against anthrax, tetanus, and equine encephalomyelitis. Various injectable biologics of equine origin have been associated with the disease, including *Clostridium perfringens* toxoids, tetanus antitoxin, and equine herpesviral vaccines prepared from equine fetal tissue and pregnant mare serum. However, while such an association holds for the majority of cases, there are many, usually sporadic, cases in horses that have not received any injections.

While the disease remains idiopathic, *a viral cause that is serum-transmissible, similar to hepatitis B in humans, is proposed*. The incubation period of 42–60 days, sometimes up to 90 days, is similar to some other viral causes of hepatitis. The occurrence of equine serum hepatitis among inoculated animals is variable and attempts to reproduce the disease by experimental transmission have rarely been successful. It is unknown how many exposed horses get subclinical disease, because the condition is seldom diagnosed before the onset of hepatic failure. However, some horses do recover after transient illness with jaundice, and some can survive with residual neurologic problems.

The onset of the typical clinical syndrome is sudden, with death occurring in 6–24 hours. Clinically, there is jaundice, hyperexcitability, often with mania, continuous walking and pushing, apparent blindness, and ataxia. Death occurs suddenly without a period of prostration.

At autopsy, icterus is present, with moderate ascites; the spleen is normal or congested, and there may be petechial hemorrhages on serous membranes and renal cortices, and some congestion of the intestine with hemorrhage into its lumen. Grossly, the liver usually appears to be of normal size, although it may be slightly enlarged or atrophic and flabby due to the acute loss of many hepatocytes. The liver may be stained by bile pigments, and its surface mottled with a few strands of fresh fibrin. The mottling is more evident on the cut surface, which appears as if severely congested and sometimes fatty (Fig. 2.48A).

Microscopically, the hepatic lesion is considerably older than the clinical course would suggest (Fig. 2.48B). *There may be a few surviving swollen and vacuolated periportal hepatocytes or sometimes complete depletion of parenchymal cells in the section.* In less affected regions in animals with adequate dietary or adipose reserves to mobilize, there is severe macrovesicular fatty change in most remaining hepatocytes. Acute necrosis is typically not seen, and there is no significant hemorrhage. Variable numbers of apoptotic hepatocytes are expected. In the periphery of the acini, severely ballooned cells undergo dissolution to leave scattered fatty cysts, but most of them disappear to leave either sinusoids that are dilated and filled with blood or a condensed and distorted reticulin framework. Extensive deposits of bile pigments are present in Kupffer cells and hepatocytes. Leukocytes, including lymphocytes, plasma cells, histiocytes, and a few neutrophils, may infiltrate diffusely, but not in large numbers. There is diffuse but very slight fibroplasia, especially in the portal units. In some livers, there are small irregular clusters of proliferating ductular cells in the portal areas, resembling the regenerating cholangiolar or oval cells that replicate in atrophic livers.

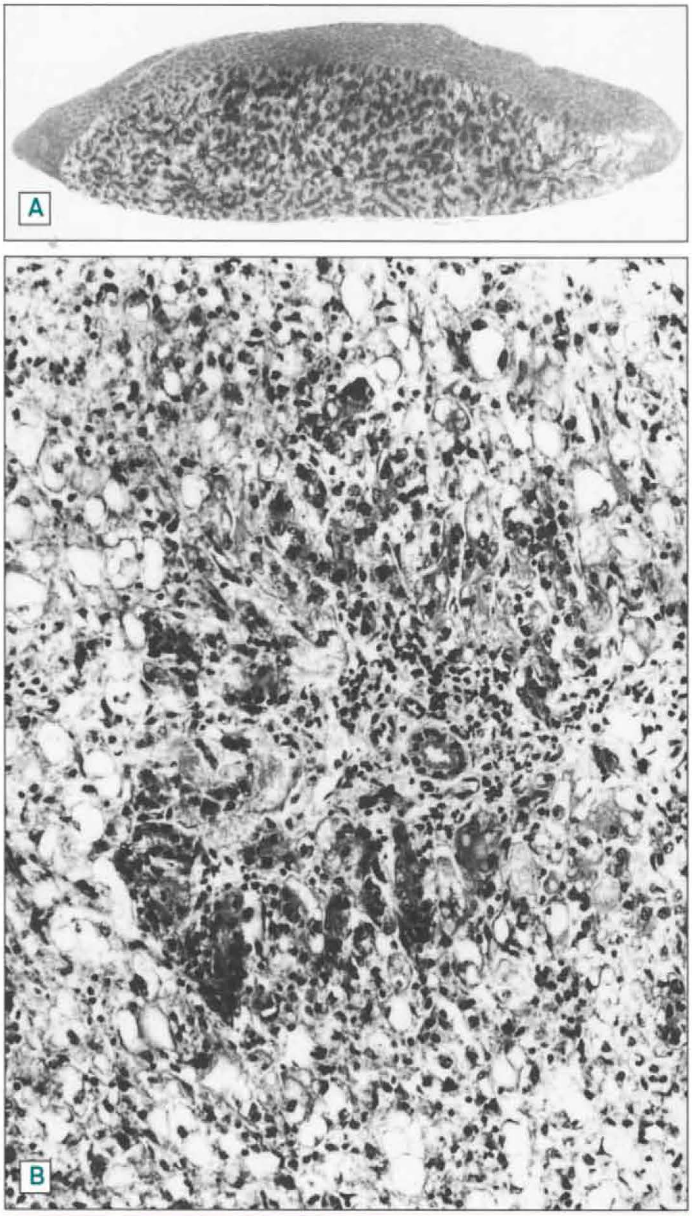

Figure 2.48 Equine serum hepatitis. A. The reticular pattern in this slice of liver suggests **zonal necrosis**. **B.** Section of the liver in **(A)** to show extensive periacinar degenerative changes, cellular infiltration, and periportal survival zone.

Focal or diffuse **fetal hepatitis** *occurs in a number of intrauterine fetal infections.* Whether caused by bacteria or viruses, focal necrosis is often present and bacteria or viral inclusions can often be identified. Frequently, however, the lesions are subtle and diffuse and without necrosis, and the inflammatory process may be difficult to distinguish from hematopoiesis. There is inflammatory edema of the connective tissues of the portal triads with distension of lymphatics there and beneath Glisson's capsule. A preponderance of mature granulocytes in the portal triads and in the adventitia of hepatic veins helps to distinguish inflammatory infiltrates from hematopoietic foci, which are predominantly in sinusoids.

Giant cell hepatitis *is an uncommon lesion in animals, but is recorded in cats, calves, and foals.* There is evidence for maternal leptospirosis in some cases in foals. Two cases are reported in young cats with concurrent thymic lymphomas. The liver is deeply bile-stained. Histologically, the acinar structure is effaced and the blood vessels engorged. Hepatocytes are large and syncytial and may contain 10 or more nuclei. The pale or ballooned cytoplasm contains bile pigments, and cytoplasmic invaginations into hepatocyte nuclei are common. Inflammatory cells are not conspicuous. Liver parenchymal giant cell transformation occurs in humans in a wide variety of congenital and neonatal liver disorders, including bile duct obstruction associated with biliary atresia, viral and bacterial infections, some metabolic disorders such as galactosemia, some cases of Down syndrome and other genetic disorders, as well as in idiopathic neonatal hepatitis, a cholestatic condition of undetermined cause. While giant cells were originally suggested to be a marker of infantile obstructive cholangiopathy, their association with a wide range of disorders supports an *alternative conclusion that giant cell formation represents a nonspecific reaction of the infant's hepatocytes to various types of injury.*

Hypertrophic hepatic cirrhosis in calves is described from Germany and occasionally observed elsewhere. Death may occur in liver failure within a few days to weeks of birth or the disease may be discovered at slaughter for veal. The liver is moderately enlarged with rounded borders, very firm but smooth on the surface, and gray. Histologically, there is some biliary hyperplasia but the lesion is dominated by diffuse fibrosis infiltrated in the early stages by mononuclear inflammatory cells. No cause has been identified. Similar lesions of **congenital hepatic fibrosis** may be found in unborn or aborted calves.

Systemic granulomatous disease has been reported in cattle grazing *hairy vetch (Vicia villosa).* The disease is characterized clinically by dermatitis, pruritus, diarrhea, wasting, and high mortality. Histologic lesions include infiltration of skin and internal organs, including portal areas of the liver, by monocytes, lymphocytes, plasma cells, eosinophils, and multinucleated giant cells. The pathogenesis is unknown, although the inflammatory reaction has characteristics of a type IV hypersensitivity reaction. Alternatively, vetch lectin may act to activate T lymphocytes directly to initiate the cellular response.

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Inflammatory diseases of the biliary tract

Inflammation of the gallbladder is termed **cholecystitis**. *Inflammation of the large bile ducts is* **cholangitis**, *whereas inflammation of the smallest intrahepatic bile ductules is termed* **cholangiolitis**. Cholangiolitis is uncommon in animals, and occurs mainly in conjunction with inflammation of larger ducts. Destructive cholangiolitis has been observed in dogs, and is associated with adverse drug reactions in humans.

Cholecystitis

Cholecystitis is uncommon, and may occur alone if the neck of the gallbladder is obstructed. More typically, it is associated with concurrent cholelithiasis. *Cholecystitis is thought to be due to reflux of intestinal bacteria into the gallbladder via the bile ducts, or to hematogenous entry of bacteria from the adjacent hepatic circulation.* Aerobic gram-negative bacteria are the most frequent isolates from canine cases, although occasionally anaerobic bacteria such as clostridia have been cultured. *Campylobacter jejuni* has been isolated from two dogs with bacteremia and cholecystitis. Canine cholecystitis has been associated with various disorders, including diabetes mellitus, severe enteritis, biliary stasis, septicemia, as well as with the use of immunosuppressive drugs, each of which may promote bacterial colonization of the gallbladder. In the acute lesion, histologic changes include neutrophilic inflammatory infiltrates in the wall and lumen of the gallbladder, with focal erosion or ulceration, and edema. More chronic stages develop typical mixed inflammatory infiltrates, with fibrosis. Occasionally, the infiltrate may be predominantly lymphoplasmacytic, with formation of lymphoid follicles within the mucosa.

Gallbladder infarction characterized by transmural coagulative necrosis of the gallbladder wall with intravascular fibrin thrombi has been reported in dogs. Predisposing factors have not been identified; however, the lack of significant concurrent inflammatory response suggests that this is not simply a sequela to underlying cholecystitis.

Cholangitis/cholangiohepatitis

Although pure cholangitis does occur, involvement of the periportal hepatic parenchyma by extension of inflammation from the ducts is almost inevitable, and such lesions can quite accurately be regarded as cholangiohepatitis.

Bile contains various antimicrobial factors, including beta defensins and bile acids that are inhospitable to most bacteria, except for some species with particular capsule adaptations. Bacterial cholangiohepatitis is usually caused by common opportunists of enteric origin, such as coliforms and streptococci. Some bacteremic organisms, including some *Salmonella*, can be cultured from the bile, but the mechanism by which they get there is unknown. Salmonellosis is a distinctive cause of fibrinous cholecystitis in cattle, especially calves.

The *pathogenesis of bacterial cholangiohepatitis* depends on various predisposing conditions, similar to those involved in the pathogenesis of pyelonephritis. These include infection by bacteria that reach the ducts hematogenously, facilitated by localization in the peribiliary plexus or by extension from sinusoidal Kupffer cells or foci of necrosis. Descending cholangitis is occasionally observed in cattle with suppurative hepatitis with extension from abscesses directly into the ducts or via the portal lymphatics. Cholangiohepatitis of this origin may be restricted in its distribution to biliary fields, but in some cases it does become quite diffuse in the biliary system. Alternatively, bacteria can extend up the ducts from the intestine, facilitated by bile stasis due to mechanical or functional obstructions. The inflammatory and proliferative responses in cholangitis further interfere with bile flow and exacerbate the ductular spread of bacterial infections once they are established.

The course and pathologic changes in cholangiohepatitis vary greatly, from fulminating suppurative infection to persistent mild inflammation that over a period of months or years leads to hepatic fibrosis of biliary distribution. *Severe suppurative cholangiohepatitis* may follow a short course to death, the effects being those of the infection itself, which may become septicemic, rather than of hepatic injury. At autopsy, the liver is swollen, soft, and pale, and its architecture is blurred. Few or many suppurative foci may be visible beneath the capsule and on the cut surface (Fig. 2.49). They are small, sometimes miliary in distribution, and not encapsulated. Lesions in other organs may be those of septicemia and jaundice. Microscopically, the larger

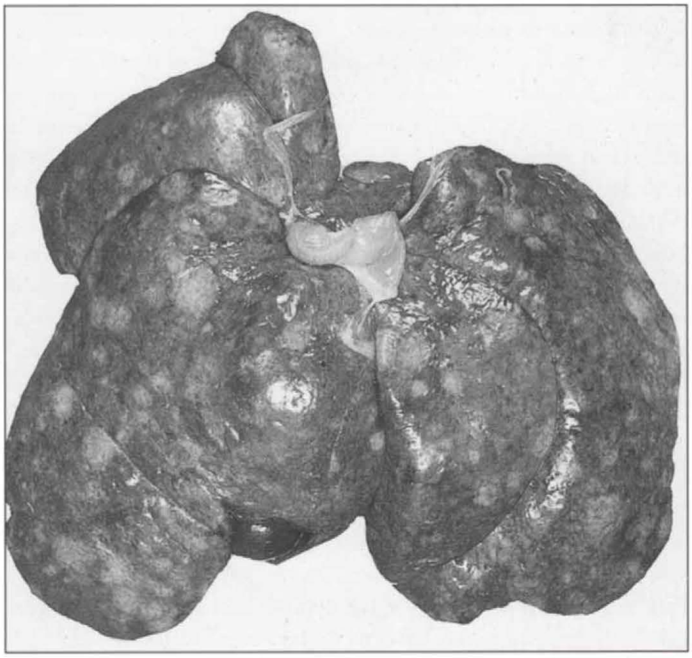

Figure 2.49 Acute **cholangiohepatitis** in a dog.

ducts contain purulent exudate, and the smaller ones are disintegrated. Dense masses of neutrophils, liquefied or not, are present in the portal triads and infiltrate the degenerate parenchyma.

In *subacute and chronic cholangiohepatitis*, the inflammation is more proliferative than exudative. The liver is enlarged and may be of normal shape, or distorted owing to irregular areas of atrophy and regenerative hyperplasia. Its surface may be smooth or finely granular; the capsule is thickened and may bear fibrous villi or be adherent to adjacent viscera. Within areas of duct obstruction, retention of bile pigments can be found in the regions served by the occluded ducts, but systemic icterus (or photosensitization in herbivores) is unlikely unless a very large amount of liver is affected. On the cut surface, the enlarged portal tracts are easily visible and accentuate the architecture of the organ. Eventually, the new fibrous tissue replaces the parenchyma, and in chronic diffuse cases in which the original infection persists, continuous fibroplasia may produce hepatic enlargement, the organ becoming huge, gray, and gristly. This spectrum of liver changes is typical of alsike clover poisoning ("big-liver disease") in horses (discussed in the section on Toxic hepatic disease, below). Alternatively, the chronic fibrosis may occur in wedge-shaped areas oriented to a small bile duct. The enlarged interlobular ducts may be readily visible and frequently contain plugs of inspissated secretion and debris. This lesion may be referred to as *biliary infarction.*

Microscopically, the reaction remains centered on portal tracts. These are expanded in subacute cases by infiltration of leukocytes and macrophages and the proliferation of small ducts and, in chronic cases, chiefly by organizing fibrous tissue and proliferating bile ducts (see Fig. 2.37). Encroachment on the parenchyma is minimal but inevitable. Continued degeneration of the periportal parenchyma is probably an additional stimulus to local fibroplasia, which, as well as thickening the smallest portal triads, extends along their length and links up with neighboring triads, thus subdividing the acini into segments. The hepatic venules and sublobular veins are involved. Regenerative nodules are not a prominent feature of cholangiohepatitis unless large areas of parenchyma have been destroyed, in which case the least damaged lobes are expanded by coarse nodules.

Suppurative or neutrophilic cholangiohepatitis *of ill-defined pathogenesis occurs in mature cats and less commonly in dogs.* The acute stage is characterized by edema and neutrophilic portal infiltrates, inflammation and degeneration of bile ducts with neutrophils in ductular lumens and emigrating through the ductular epithelium, infiltration of inflammatory cells into hepatic lobules and periportal hepatocellular necrosis. Mixed cholangiohepatitis, with neutrophils, lymphocytes, and plasma cells infiltrating portal areas accompanied by bile duct proliferation, biliary epithelial degeneration, and various degrees of periportal to bridging fibrosis, may represent the subacute stage (Fig. 2.50), while concentric periportal fibrosis and pseudolobule formation may represent the most chronic stage of the disease. Suppurative cholangiohepatitis is more prevalent in cats 11–15 years of age, and often occurs in conjunction with other disorders, including acute extrahepatic bile duct obstruction, pancreatitis, or inflammatory bowel disease. *This condition has been suggested to be the result of acute inflammation in the biliary tree associated with ascending bacterial infection.* In the cat, the biliary and pancreatic ducts have a common entry to the duodenum, and simultaneous infectious inflammation of these systems is common. *Escherichia coli* is the most common bacterial isolate; however, *Bacteroides*, hemolytic *Streptococcus*, and clostridia have also been reported.

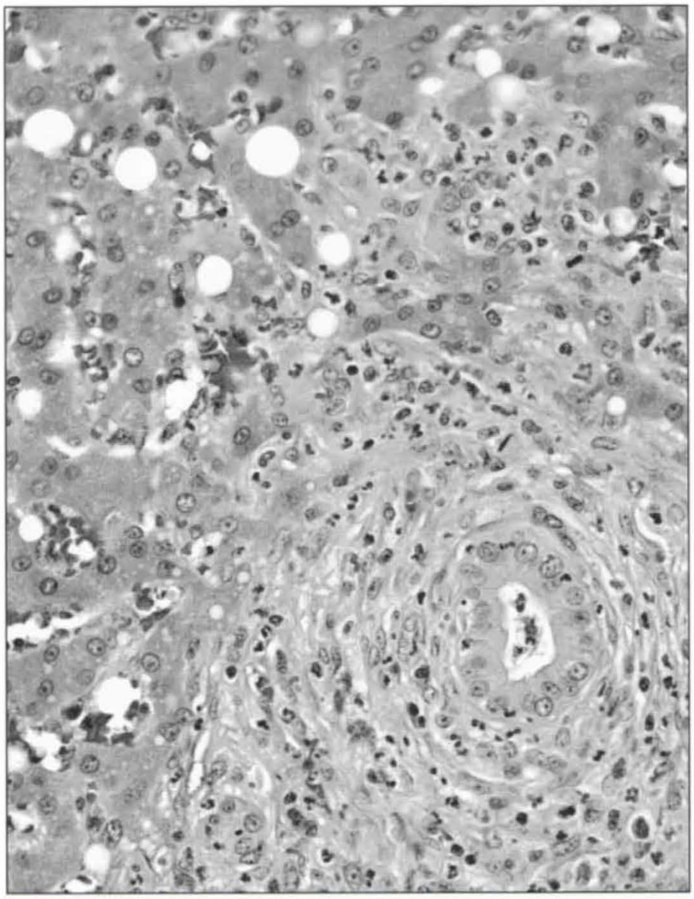

Figure 2.50 Bile duct inflammation with portal edema, mixed inflammation, and early fibrosis in a case of **suppurative cholangiohepatitis** in a dog.

An apparently distinct condition of cats, **lymphocytic portal hepatitis** *is characterized by lymphocytic or lymphoplasmacytic infiltrates confined to the portal area, with no evidence of periportal necrosis or invasion of adjacent hepatic lobules* (Fig. 2.51A). Bile duct proliferation is present, but there is no evidence of bile duct epithelial degeneration or cholangitis. Periportal fibrosis is typically present, but there is no bridging between portal areas and central veins. The condition is seen most often in cats over 15 years of age, and is slowly progressive, but does not result in pseudolobule formation. Lymphocytic portal hepatitis differs from descriptions of **feline progressive lymphocytic cholangitis/cholangiohepatitis**, which has been suggested by some authors to be part of the chronic phase of neutrophilic cholangiohepatitis, as described above (Fig. 2.51B). However, progressive lymphocytic cholangitis/cholangiohepatitis has been reported predominantly in younger cats, is not apparently associated with concurrent intestinal or pancreatic disease, and is postulated to have an immune-mediated pathogenesis. This condition is typified by lymphocytic inflammation of portal tracts, with infiltration of bile ducts, extension into periportal hepatic parenchyma, bile duct proliferation accompanied by small foci of neutrophil and macrophage infiltration, and fibrosis.

Biliary tract obstruction

Cholelithiasis *(gallstone formation) is seldom observed in animals.* The choleliths usually form in the gallbladder and are composed of a

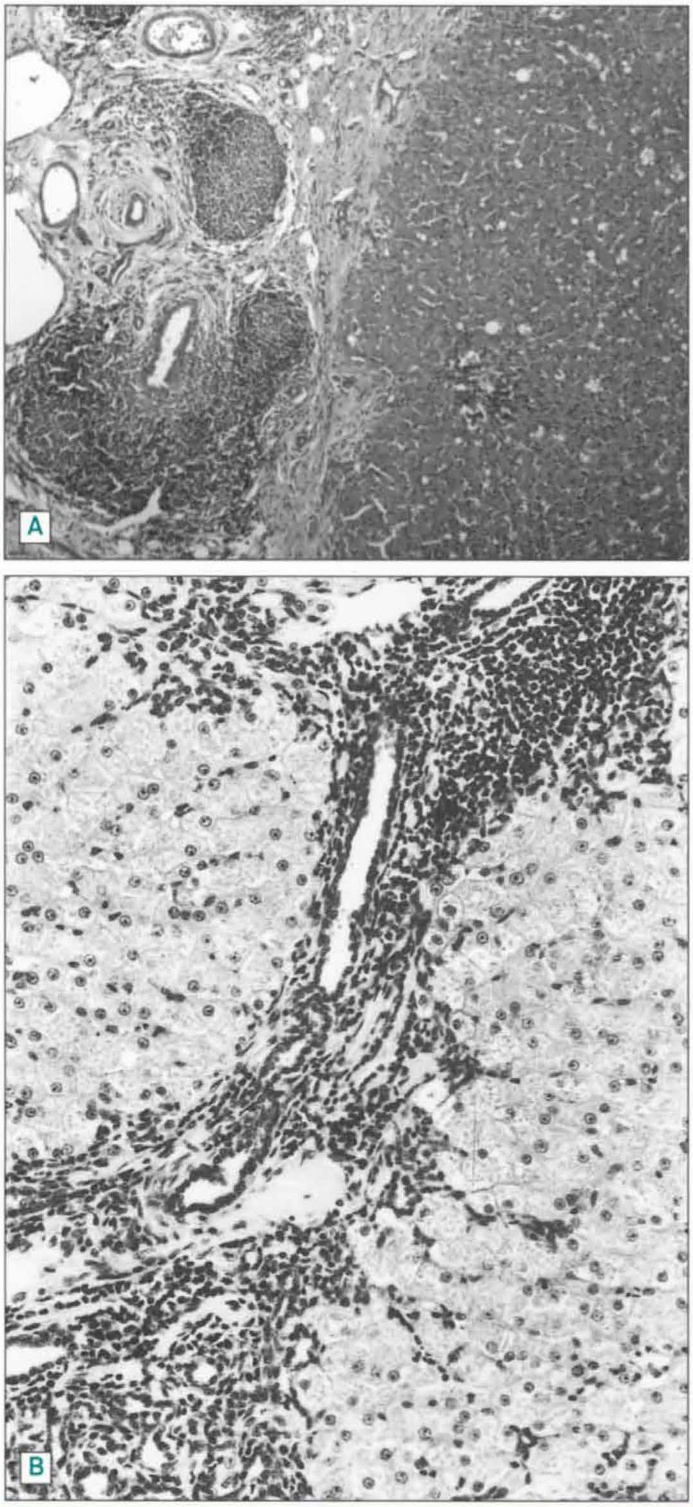

Figure 2.51 A. Lymphocytic portal hepatitis in a cat. **B. Lymphocytic cholangiohepatitis** in a cat.

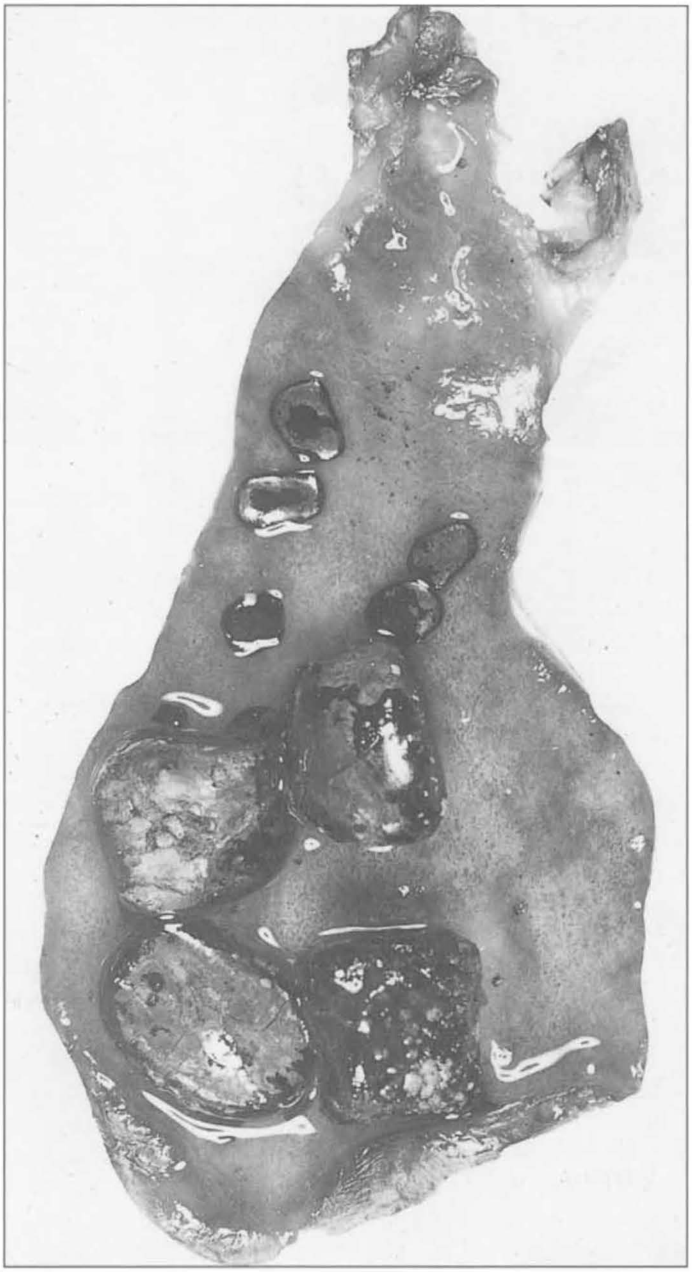

Figure 2.52 Gallstones in the opened gallbladder of a dog.

mixture of cholesterols, bile pigments, salts of bile acids, calcium salts, and a proteinaceous matrix. Choleliths of mixed composition are yellow-black or green-black and are friable. *Pigment stones*, composed of calcium bilirubinate, and *cholesterol stones* have also been reported in dogs. There may be hundreds of small stones or a few large ones (Fig. 2.52). The large stones are usually faceted. The origin of these mixed gallstones is uncertain, but their development is probably secondary to chronic mild cholecystitis and related to disturbances of the resorptive activities of the gallbladder, whereby the bile salts are removed faster than the stone-forming compounds. Gallstones are usually asymptomatic. Occasionally, they lodge in and obstruct bile ducts and cause jaundice. The larger stones may cause pressure necrosis and ulceration of the mucosa, local dilations of the bile ducts, and saccular diverticula of the gallbladder. Calculi seldom form in the ducts, although calcareous deposits often do so in fascioliasis of cattle. Calcium bilirubinate calculi have been reported in the bile ducts of horses (Fig. 2.53), associated with intermittent jaundice.

Occasionally, particles of solid ingesta may find their way into the gallbladder; sand has been seen in sheep, and seeds in pigs.

Biliary obstruction is rarely due to impacted gallstones. Usually it is *due to cholangitis or cholecystitis*, the obstruction being produced

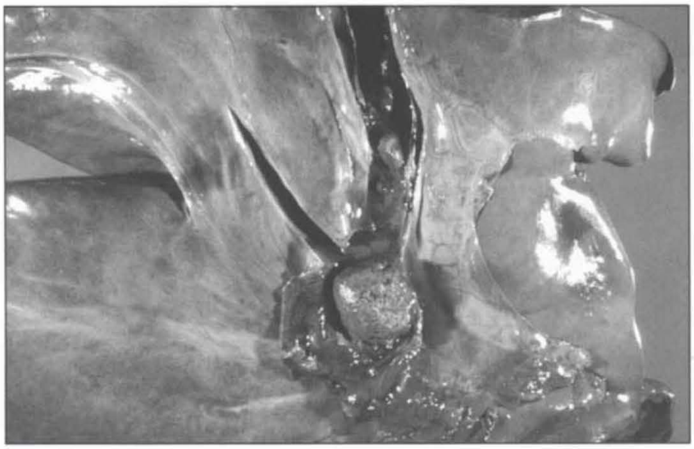

Figure 2.53 Cholelith obstructing the bile duct in a horse.

by masses of detritus and biliary constituents, parasites, or cicatricial stenosis of the ducts. Adult ascarids may cause mechanical obstruction. Inspissated bile-stained friable plugs are occasionally responsible for obstructions in segments of the liver in horses. Tumors of the pancreas and duodenum, and tumors and abscesses of the hilus of the liver and portal nodes, may cause compression stenosis of the ducts. Edematous swelling of the papilla in enteritis may also be of significance. Biliary obstruction by abnormal intraluminal mucoid secretion (*gallbladder mucocele*) has been reported in dogs (see section on Ectopic, metaplastic, and hyperplastic lesions, below).

The consequences of biliary obstruction depend on the site and duration of the obstruction. When the main duct is involved, there is jaundice. When one of the hepatic ducts is involved, there is no jaundice, and depending on the efficiency of biliary collaterals, there may be no pigmentation of the obstructed segments of liver. Increases in serum γ-glutamyltranspeptidase and alkaline phosphatase usually occur when a sufficiently large amount of the duct system is affected. The ducts undergo progressive cylindric dilation, which may be extreme. The smallest interlobular ducts and the cholangioles proliferate. There is inflammation in the walls of the ducts and the portal triads, and this is probably due in part to chemical irritation by bile acids but is largely due to secondary bacterial infections. These infections may be acute and purulent, or low-grade; in these cases, bacteria may not be easily cultured. The cholangiohepatitis that almost inevitably follows has been described earlier.

Inflammatory stenoses of larger ducts may recanalize via mucosal glands, which can proliferate and link to form a tortuous detour around the obstruction.

Rupture of the biliary tract or the gallbladder causes steady leakage of bile into the peritoneal cavity, the omentum being unable to seal even small defects. The bile salts are very irritating and may cause *acute chemical peritonitis*. The peritoneal effusion that follows may remain sterile; more often it is infected by enteric bacteria, and severe diffuse peritonitis ensues. This may be rapidly fatal, particularly if clostridia are involved. Most perforations of the biliary tract are traumatic in origin.

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INFECTIOUS DISORDERS OF THE LIVER

Viral infections

Various systemic viral diseases may affect the liver. Adenoviral infections of lambs, calves, and goat kids can cause multifocal hepatic necrosis and cholangitis, in addition to pneumonia. Lymphohistiocytic hepatitis with single-cell necrosis of hepatocytes and perilobular fibrosis is a frequent histologic lesion in cases of postweaning multisystemic wasting syndrome in pigs, associated with *Porcine circovirus* type 2 infection. Neonatal *Canid herpesvirus 1* infection in puppies causes disseminated focal necrosis and hemorrhages in parenchymal organs, including the liver, with formation of amphophilic intranuclear inclusion bodies in epithelial cells of kidney, lung, and liver. Similar microfoci of hepatic necrosis sometimes occur in aborted or newborn foals with congenital *Equid herpesvirus 1* infections (see Fig. 2.26). *Feline coronavirus* infection can cause granulomatous hepatitis in some infected cats, as part of feline infectious peritonitis.

The viral diseases included below are those in which the liver is the major target organ for viruses that cause substantial hepatic disease that sometimes culminates in hepatic failure. Unlike humans, in which several pathogenic hepatitis viruses from various families are well described, the major domestic mammals are vulnerable to few viruses that target hepatocytes. Some hepadnaviruses have been found in ducks, woodchucks, and herons. Closely related human and swine genotypes of *Hepatitis E virus* (HEV – Caliciviridae) can replicate in porcine hepatocytes wherein antigens and viral nucleic acids can be detected. There is evidence for fecal–oral transmission of HEV in pigs, and zoonotic transmission, but experimental HEV infection has not been reported as a primary cause of hepatitis in pigs.

Infectious canine hepatitis

Canine adenovirus 1 (CAdV-1) infection can cause infectious canine hepatitis, a severe liver disease in dogs and other canids. Vaccination has made the disease rare in many countries in which it was endemic.

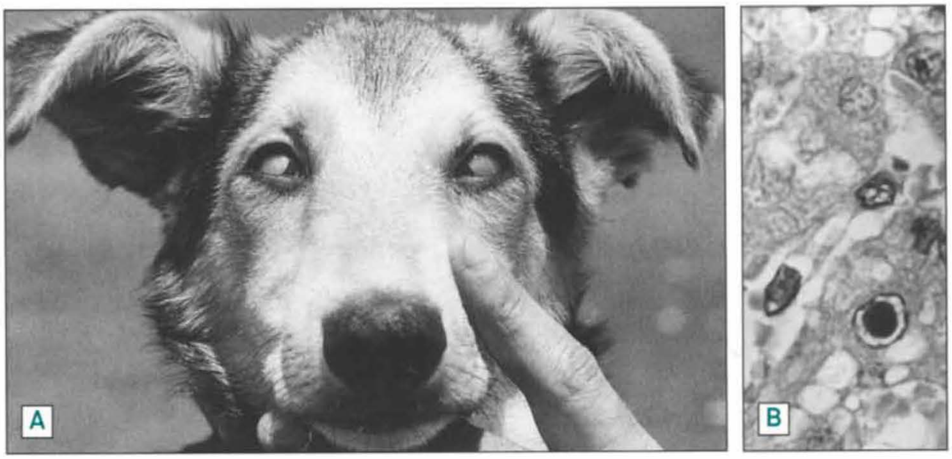

Figure 2.54 Infectious canine hepatitis (*Canine adenovirus 1* infection). **A. Corneal edema** in convalescent stage. **B. Intranuclear inclusion body** in hepatocyte.

Deaths from infectious canine hepatitis are usually sporadic, although small outbreaks can occur among young dogs in kennels. Fatalities seldom occur among dogs more than 2 years of age. In areas where the disease is not controlled by vaccination, it is probable that most dogs in the general population contact CAdV-1 in the first 2 years of life and suffer either inapparent infection or mild febrile illness with pharyngitis and tonsillitis.

In more severe cases, there is vomiting, melena, high fever, and abdominal pain. There may be petechiae on the gums; the mucous membranes are blanched, and only occasionally are they slightly jaundiced. Nervous signs of nonspecific character occur in a few cases. There is also a peracute form of the disease in which the animal is found dead without signs of illness, or after an illness of only a few hours. In convalescence, there may be a unilateral or bilateral opacity of the cornea caused by edema, which disappears spontaneously (Fig. 2.54A).

Canine adenovirus 1 has special tropism for endothelium, mesothelium, and hepatic parenchyma, and it is injury to these that is responsible for the pathologic features of edema, hemorrhage (which is predominantly serosal), and hepatic necrosis. The histologic specificity of the lesions depends on the demonstration of *large, solid intranuclear inclusion bodies in endothelium or hepatic parenchyma* (Fig. 2.54B). Inclusions are occasionally observed in other differentiated cells but always have the same morphologic and tinctorial features, being deeply acidophilic with a blue tint.

The morbid picture of spontaneously fatal cases is usually distinct enough to allow a diagnosis to be made grossly at necropsy. Superficial lymph nodes are edematous, slightly congested, and often hemorrhagic. Blotchy hemorrhages may be present on the serous membranes (Fig. 2.55A), and there is usually a small quantity of fluid, clear or blood-stained, in the abdomen. Hemorrhages on the serosa of the cranial surface of the stomach are usually linear, the so-called *paintbrush* type. Jaundice, if present, is slight. The mesenteries are slightly moist, and the serosa of the small intestine has a ground-glass appearance. The liver is slightly enlarged, with sharp edges, and is turgid and friable, sometimes congested, with a fine, uniform, yellow mottling. Red strands of fibrin can be found on its capsule, especially between the lobes. In the majority of cases, the wall of the gallbladder is edematous (Fig. 2.55B); when edema is mild, it may be detected only in the attachments of the gallbladder. In cases in which the gallbladder is edematous, it may also be darkened by intramural hemorrhages.

Gross lesions in other organs are inconstant. Small hemorrhagic infarcts may be found in the renal cortices of young puppies. Hemorrhages may occur in the lungs, and occasionally there are irregular areas of hemorrhagic consolidation in the caudal lobes. Hemorrhages in the brain occur in a small percentage of cases. These are capillary and venular hemorrhages best appreciated when darkened by formalin, and then, depending on their concentration, the affected portions of brain appear gray or dark brown. Microscopic hemorrhages occur in any part of the brain, but when numerous enough to be grossly visible, they are confined to the midbrain and brainstem, avoiding the cerebral cortex and cerebellum. Hemorrhagic necrosis of medullary and endosteal elements occurs in the metaphyses of long bones in young dogs, and the hemorrhages are readily visible through the thin cortex of the distal ends of the ribs.

At low magnification, the histologic changes in the liver are quite reminiscent of the zonal necrosis of acute hepatotoxicities. *There is an as yet unexplained susceptibility of the periacinar parenchyma to necrosis in this disease.* Close to the portal triads, the hepatocytes may be near normal in appearance, except for loss of basophilia and the presence of a scattering of inclusion bodies. In spontaneously fatal cases, most of the parenchyma of the peripheral and central portions of the acini is dead, the hepatocytes having undergone granular acidophilic coagulative necrosis, and in some of these, ghosts of inclusion bodies may be detectable. The margin between necrotic parenchyma and viable tissue is usually quite sharp, although in the viable tissue there are many individual hepatocytes undergoing apoptosis, most of them without inclusion bodies. Fatty changes are common but not constant. The dead cells do not remain long, so the sinusoids become dilated and filled with blood. The reticulin framework remains intact, an observation in keeping with the fact that, *in recovered cases, restitution of the liver is complete.* Massive necrosis with collapse does not occur. As is typical of severe periacinar necrosis, the necrotic zones, initially eccentric areas about hepatic venules, extend and link up to isolate portal units. Intranuclear inclusions can be found in Kupffer cells in variable numbers. Many of the Kupffer cells are dead, others are proliferating, and others are actively phagocytic in the removal of debris. Leukocytic reactions in the liver are mild and are directed against the necrotic tissue; mononuclear cells are present, but neutrophils, many degenerating, predominate. There is some collection of bile pigment, but it is moderate, in keeping with the short course of the disease.

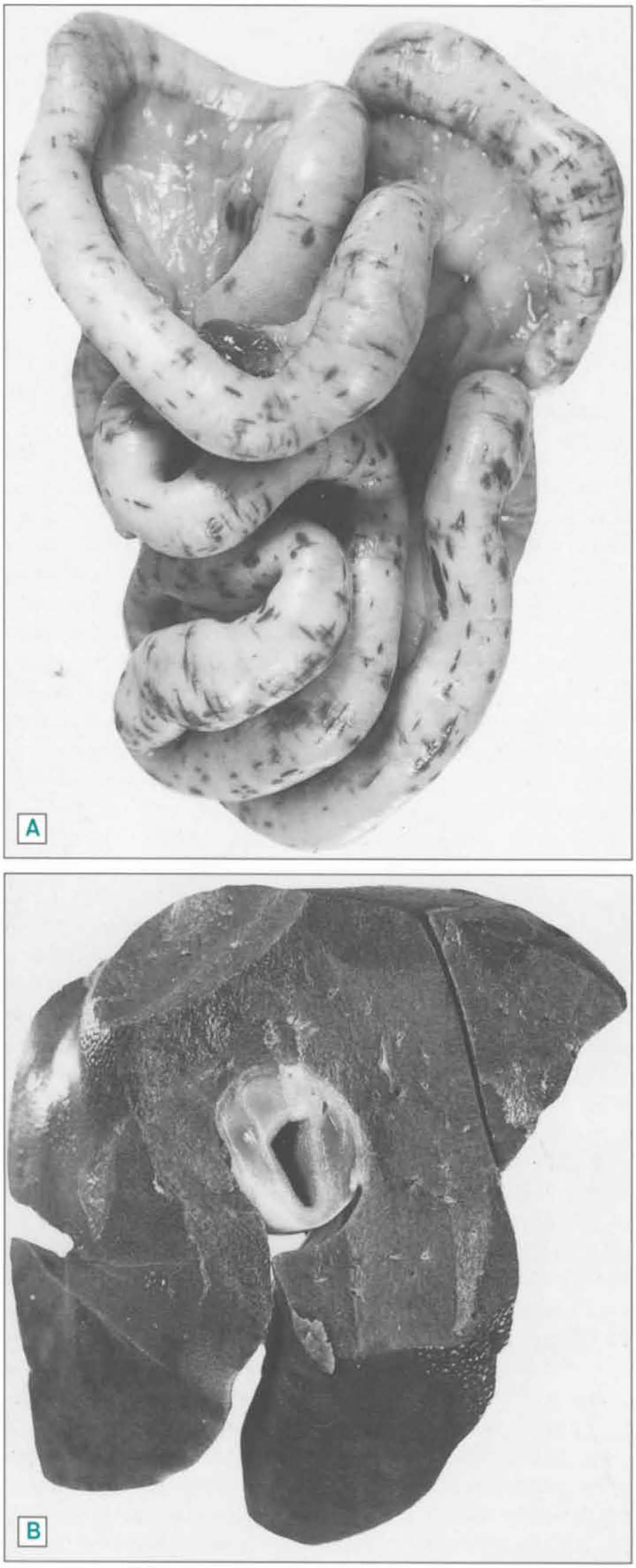

Figure 2.55 Infectious canine hepatitis. **A. Serosal hemorrhages** over the intestines. **B.** Severe **edema of the gallbladder wall**.

Microscopic lesions in other organs are largely due to injury to endothelium. Inclusion bodies in endothelial cells can be difficult to find and are looked for with most profit in renal glomeruli, where endothelium is concentrated. Occasionally, they are found in the epithelium of collecting tubules. When areas of hemorrhagic consolidation of the lungs are present, there is hemorrhage, edema, and fibrin formation in the alveoli, and in these consolidated areas, inclusions are often common in alveolar capillaries and even in dying cells of the bronchial epithelium. Changes in the brain are essentially secondary to vascular injury and may be absent. Hemorrhages, if present, are from capillaries and small venules, and inclusions in endothelial nuclei can usually be found in vessels that have bled. Other endothelial and adventitial cells are hyperplastic and mixed with a few lymphocytes. Small foci of softening or demyelination may be present in relation to the hemorrhages.

Lymphoreticular tissues are congested, and inclusions may be found in the primitive reticulum cells of follicles, in the red pulp of the spleen, and in macrophages anywhere.

The detailed pathogenesis of infectious canine hepatitis has yet to be worked out. Many infections appear to be clinically silent, and other dogs recover after mild febrile disease with tonsillitis. The fulminating pattern of clinical disease and convalescent phenomena needs further examination. Some sudden deaths in this disease are associated with midbrain hemorrhage, and others occur with, at most, slight structural evidence of liver injury. Following oral exposure, which is probably the natural route of infection, viral multiplication occurs in the tonsils and leads to tonsillitis. The tonsillitis is sometimes quite severe and may be fatal with extensive clear edema of the throat and larynx. Fever accompanies the tonsillitis and apparently precedes the viremic phase, which is of short duration and accompanied by severe leukopenia. Hepatic necrosis develops at about day 7 of experimental infection. The sequence of developments in the liver is not clear, but it is possible that viral proliferation occurs first in Kupffer cells. In surviving animals, hepatic regeneration occurs rapidly, and there do not appear to be any significant residual lesions. Small foci of hepatocellular necrosis involving one to several liver cells may still be present at 2 weeks, and foci of proliferated Kupffer cells may be detectable for another week or two, but progressive hepatic injury does not occur in the natural disease. Progressive hepatic injury does not seem to follow the acute phase of the natural disease, despite the fact that adenoviral antigen may be demonstrated immunohistochemically in Kupffer cells in dogs with various forms of chronic hepatitis, and chronic hepatitis has been produced by experimental adenoviral infection in dogs after certain immunological manipulations. This is consistent with failure to demonstrate virus in the liver after about day 10 of experimental infection. A review of 45 dogs with chronic hepatitis or cirrhosis failed to reveal CAdV-1 by either polymerase chain reaction or immunohistochemistry, although the possibility that the virus initiates hepatic damage by provoking self-perpetuating hepatitis could not be excluded. Focal interstitial nephritis occurs commonly, and the cellular infiltrates are persistent but not functionally significant. They consist of interstitial lymphocytic accumulations, especially about the corticomedullary junction and in the loose stroma of the pelvis.

Corneal edema is a late development (see Fig. 2.54A). It may occur by day 7 of infection, but is usually delayed to between 14 and 21 days. Viral antigen can be detected in these eyes by fluorescent techniques, but not in the corneal structures. Inflammatory edema is present in iris, ciliary apparatus, and corneal propria, and inflammatory cells are abundant in the filtration angle and iris. The infiltrates are principally plasma cells, and there is evidence that the ocular lesion is a hypersensitivity reaction to viral antigen.

Originally it was assumed that the widespread tendency to hemorrhage in this disease was due to leakage from damaged vascular endothelium, coupled with an inability on the part of the damaged liver to replace clotting factors. While these effects play a role, it is now known that *the exhaustion of clotting factors is in large part due to their accelerated consumption, as the widespread endothelial damage is a potent initiator of the clotting cascade.*

Wesselsbron disease

This disease is caused by *Wesselsbron virus*, an arthropod-borne flavivirus that, according to serological surveys, is widespread in Africa in various species of animals and birds. Various *Aedes* mosquitoes are the vectors. Humans are also susceptible to clinical and inapparent infection. *The virus produces outbreaks of abortion and perinatal death in sheep.* Susceptible adults rarely show clinical signs but may have a biphasic febrile response to infection; other clinical signs, when present, are of hepatitis and jaundice.

The lesions in lambs dying within 12 hours of birth consist mainly of widespread petechiae and gastrointestinal hemorrhage; longer survival allows jaundice to develop, and the liver becomes orange-yellow, enlarged, friable, and patchily congested. The bile in the gallbladder becomes thick and dark in some cases, but this may be due more to hemorrhage into the gallbladder than to hemolysis. Lymph nodes are rather constantly enlarged, congested, and edematous.

The most characteristic histologic changes are seen in the liver. There are randomly scattered foci of necrosis, with apoptosis and proliferation of sinusoidal lining cells (Fig. 2.56). Mononuclear cells and pigment-filled macrophages accumulate in the portal stroma as well as in the sinusoids. In a variable proportion of cases, *hepatocyte nuclei may contain eosinophilic, irregular inclusions.* These are not accompanied by as much margination of nuclear chromatin as that associated with conventional viral inclusions, and their significance is obscure. Wesselsbron viral antigen can be demonstrated in necrotic acidophilic and degenerating hepatocytes and rarely in the inclusions. In jaundiced animals there may be considerable canalicular cholestasis; whether or not this is the result of hemolysis does not appear to have been determined. Hepatocellular proliferation is apparent in the less acute cases. Lymphoid follicles in lymph nodes and spleen show pronounced lymphocyte necrosis and stimulation of lymphoblasts.

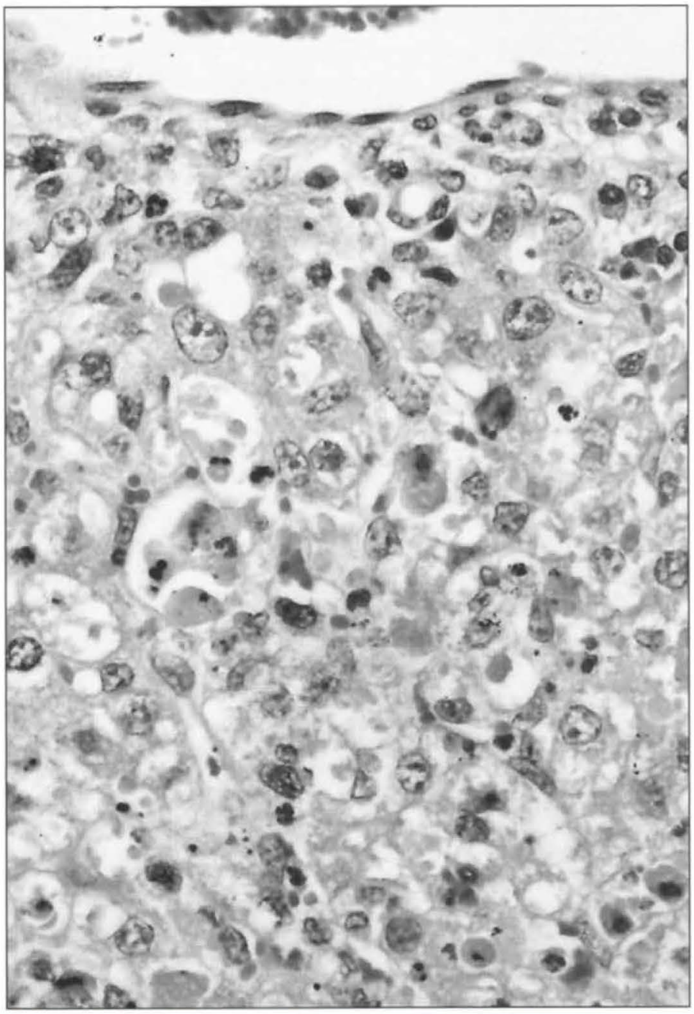

Figure 2.56 Focal necrosis and apoptosis in Wesselsbron disease in a lamb. (Courtesy of S Youssef.)

Rift Valley fever

This is an *arthropod-borne viral infection of ruminants and humans*, in many respects similar to Wesselsbron disease. *Rift Valley fever virus* (RVFV) is, however, responsible for greater losses than the former infection. Morbidity and mortality may occur in adult sheep; death sometimes occurs in adult cattle, but it is chiefly a disease of the young, causing *heavy mortality among lambs, kids, and calves and abortion in ewes, does, and cows.* The infection in enzootic form is widespread in eastern and southern Africa but it has, in plague-like proportions, extended to West Africa, Egypt, and the Arabian peninsula. RVFV is a member of the *Phlebovirus* genus, one of the five genera in the family Bunyaviridae. It is transmitted by many species of mosquito of the genera *Culex* and *Aedes,* in which transovarial passage can occur. Mosquitoes, once infected, remain so and in them the virus is not pathogenic. High levels of viremia occur in sheep and cattle and are maintained for up to 5 days. During epizootics, the virus may be spread by fomites, aerosols, and mechanically by other biting insects.

In endemic situations, the disease in adults is usually mild, but in epidemics in sheep and goats, severe illness occurs with fever, vomiting, mucopurulent nasal discharge, and dysentery. The mortality rate is then very high in lambs and up to 50% in adults. The disease in cattle is less severe but pregnant animals abort and the mortality rate in calves may reach 30%.

As in Wesselsbron disease, *the gross postmortem picture is dominated by widespread hemorrhage, ranging from serosal petechiae to severe gastrointestinal bleeding.* The liver in the acute cases in neonatal lambs is similar to that in cases of Wesselsbron disease, being yellow, swollen, soft, and patchily congested or hemorrhagic. In older animals and in less acute cases, however, the liver tends to be darker and show scattered pale foci of necrosis 1–2 mm in diameter. There may be fibrinous perihepatitis, edema of the gallbladder wall, and moderate, blood-tinged ascites. Experimental infection of calves produced encephalomyelitis in an animal that survived the initial viremic stage.

Within 12 hours of experimental infection of lambs, there are *randomly distributed foci of hepatocellular necrosis in the liver.* These foci include knots of inflammatory cells and prominent apoptotic bodies and initially involve about half a dozen hepatocytes (Fig. 2.57). Within a few hours, however, these primary foci enlarge and may become almost confluent. In the meantime, the remaining parenchyma may rapidly undergo necrosis that spares only a small rim of periportal hepatocytes. In naturally infected calves, the primary foci of necrosis undergo lysis more rapidly than the surrounding parenchyma; these foci thus have a striking "washed-out" appearance. Where the expanding foci of necrosis include portal triads, there may follow fibrinous vasculitis and thrombosis. Fibrin deposition in sinusoids is common, and so is mineralization of necrotic hepatocytes. Cholestasis is apparent in sections but is not a prominent feature.

Eosinophilic intranuclear inclusion bodies, often elongated, are sometimes seen in degenerate hepatocytes; there is associated nuclear vesiculation and chromatin margination. There is necrosis in germinal centers of lymphoid follicles in lymph nodes and spleen similar to that seen in Wesselsbron disease. Renal glomerular hypercellularity and necrosis have been described in the experimental disease. By immunohistochemistry, RVFV antigen can be detected in focal areas in cytoplasm of degenerating and necrotic hepatocytes but not in nonparenchymal cells.

Ultrastructural studies have shown condensation of degenerate hepatocytes, abundant apoptosis, and the presence of mummified, membrane-bound fragments of hepatocellular cytoplasm, but sinusoidal lining cells are not notably damaged; the Kupffer cells instead participate in the uptake of the dying hepatocytes. There is abundant fibrin in sinusoids in the vicinity of the primary foci of necrosis and also within hepatocytes and macrophages. *The intranuclear inclusions are composed not of recognizable viral particles but of filaments.* Virus is occasionally discernible in the cytoplasm, associated with tubular membranes.

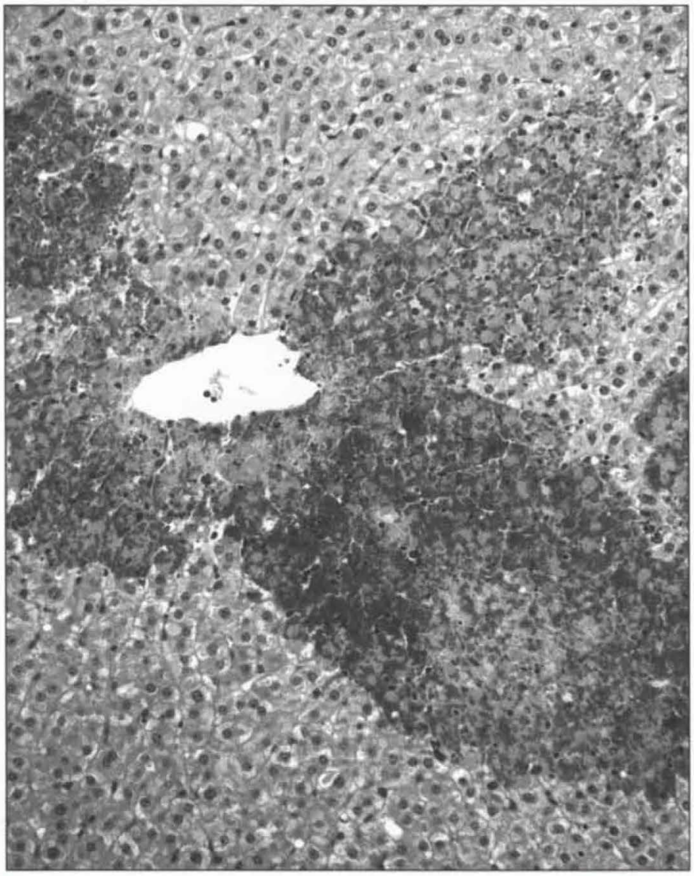

Figure 2.57 Focal necrosis with hemorrhage in Rift Valley fever in a lamb. (Courtesy of S Youssef.)

It seems that the primary infection of the liver produces the primary necrotic foci, from which more virus spreads to damage neighboring parenchyma; the virus clearly has a marked preference for hepatocytes over other tissues. The hemorrhagic component of the syndrome is probably related to consumption of clotting factors; there is no direct evidence for the endothelial damage seen in infectious canine hepatitis.

There has been some confusion in early reports from Africa of liver diseases of ruminants. Some cases of phyto- and mycotoxicosis and of chronic copper poisoning have probably been ascribed to viral infection, and vice versa. It is also understandable that Rift Valley fever and Wesselsbron disease may have been mistaken for one another, but these two diseases may be distinguished by careful pathologic examination as well as by immunodetection or isolation of the viruses. *In summary, Rift Valley fever is characterized by obvious focal hepatic necrosis, on which is superimposed an almost massive periacinar and midzonal necrosis; cholestasis is not as prominent as it is in Wesselsbron disease. The liver lesions in the latter disease consist of smaller, randomly distributed foci of hepatocellular necrosis, more active reaction by the sinusoidal lining cells, and more obvious cholestasis.*

Canine acidophil-cell hepatitis

This is a transmissible hepatitis of dogs described only in the UK and Ireland. It has been transmitted experimentally to normal dogs and rats by parenteral injections of liver and serum from affected animals. The naturally occurring disease takes the form of *chronic progressive liver damage with eventual liver failure,* attended clinically by ascites, signs of hepatic encephalopathy, and causing shrinkage and nodular distortion of the liver. *The most characteristic histologic feature of affected livers is the presence of shrunken, acidophilic hepatocytes that are concentrated initially in the limiting plates adjacent to the portal triads.* There is little or no inflammation associated with affected cells, but portal triads eventually become linked by fibrous trabeculae. Experimentally infected dogs usually remain clinically normal, but regular serum sampling reveals intermittent elevations in alanine aminotransferase levels. The characteristic acidophilic hepatocytes tend not to be so obvious in some experimentally transmitted cases, which raises the possibility that this virus may be involved in otherwise unexplained cases of chronic canine liver disease.

No infectious agent has been isolated from the original cases of canine acidophil cell hepatitis. Recent efforts to reproduce the condition by transmission of material from various spontaneous cases of canine hepatitis have had limited success. Transient hepatitis has been observed in one recipient. However, no agent has been found and polymerase chain reaction screening of livers has been negative for hepadnaviruses, *Canine adenovirus 1, Hepatitis E virus, Hepatitis C virus,* and *Canine parvovirus.*

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Bacterial infections

Bacterial hepatitis is common, but, with a few important exceptions, is usually focally distributed and of little clinical significance. Bacteria may gain entrance to the liver in various ways: by direct implantation, for example by foreign-body penetration from the reticulum; by invasion of the capsule from an adjacent focus of suppurative peritonitis; hematogenously via the hepatic artery or portal and umbilical veins; or via the bile ducts.

Excepting peracute septicemias, *there are few specific bacterial infections that have a sustained or repeated bacteremic phase without producing hepatic lesions.* There are, in addition, many cases of nonspecific bacteremia, especially portal in origin, in which focal hepatitis occurs. Because their differential diagnosis is of some importance, it is probably useful to list here those specific bacterial diseases in which focal hepatitis is expected or characteristic, but not constant. The specific infections may occur as fetal or perinatal infections. The list includes *Listeria monocytogenes* in fetal and neonatal lambs, calves, foals, and piglets; *Campylobacter fetus* in fetal and neonatal lambs; *Actinobacillus equuli* in foals; *A. suis* in pigs; *Yersinia pseudotuberculosis* in lambs and occasionally in dogs and cats; *Francisella* (*Yersinia*) *tularensis* in lambs and cats; *Mannheimia haemolytica* and *Histophilus somni* (*Haemophilus agni*) in lambs; *Salmonella* spp. in all hosts (see Fig. 2.25), *Clostridium piliforme* (Tyzzer's disease) in foals and dogs; *Nocardia asteroides* in dogs; and the mycobacteria in all hosts.

Hepatic abscess

Hepatic abscesses, quite apart from the lesions of the specific infections just given, *are common*, especially in cattle. They may arise by direct implantation of a foreign body from the reticulum or by direct invasion of the capsule from a suppurative lesion of traumatic reticulitis and may be single or multiple, but in either case they are often preferentially distributed to the left lobe. They may be hematogenous from portal emboli, or by direct extension of omphalophlebitis. Arteriogenic abscesses via the hepatic artery may occur in pyemias but are quite uncommon.

Omphalogenic abscesses are more common in calves than in other species but occur in all. The bacterial flora is frequently mixed, but *Arcanobacterium* (formerly *Actinomyces* or *Corynebacterium*) *pyogenes*, *Fusobacterium necrophorum*, streptococci, and staphylococci usually predominate. Hepatic abscesses are not inevitable sequelae to omphalitis or even to omphalophlebitis, but they do not develop from navel infections in the absence of omphalophlebitis. As there is no flow of blood in these vessels after birth, involvement of the liver is by direct growth along the physiologic thrombus. Omphalophlebitis can be quite severe without extension to the liver. Hepatic abscesses of omphalogenic origin are often restricted to the left lobe (Fig. 2.58), but they may be restricted to the right or be diffuse in their distribution.

Hepatic abscesses are also common and of much economic importance in feedlot cattle. They are usually found at slaughter but, when numerous, may be fatal after a few days of vague digestive illness. Their pathogenesis and character are discussed with rumenitis, to which they are a sequel (see Vol. 2, Alimentary system). Liver abscesses in feedlot sheep likely have a similar pathogenesis, with *F. necrophorum* as the primary isolate. A second category includes parasitic granulomas populated by various opportunistic bacteria.

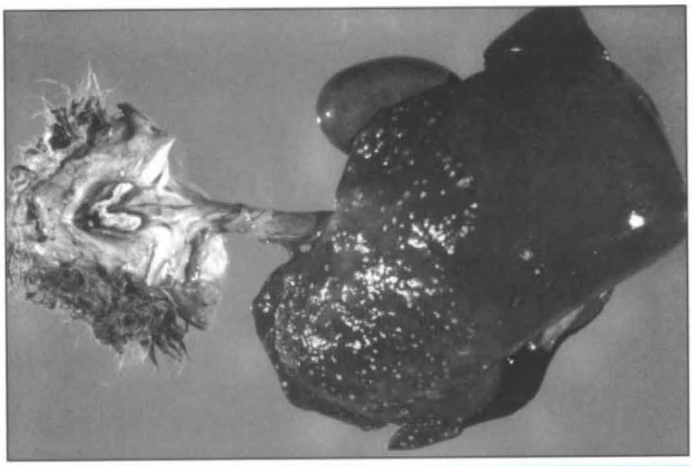

Figure 2.58 Omphalophlebitis with **miliary metastatic abscesses** in the left lobe of the liver in a calf.

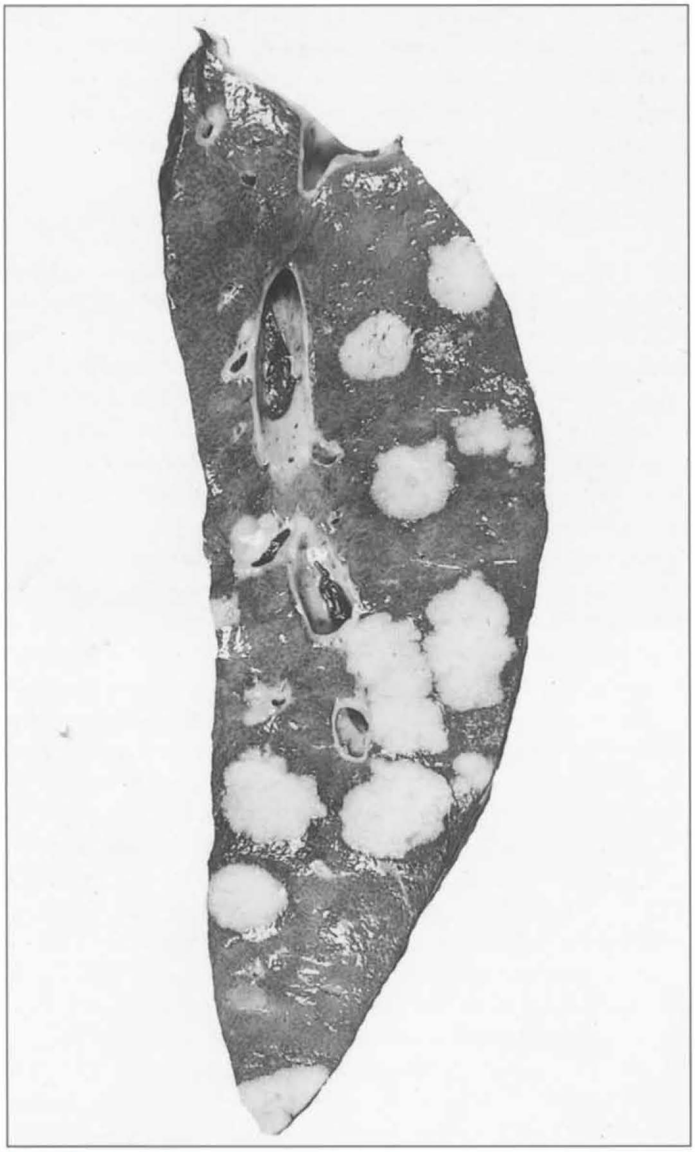

Figure 2.59 Early **hepatic necrobacillosis** in an ox (*Fusobacterium necrophorum* infection). Pale areas of coagulative necrosis are bordered by acute inflammation.

Hepatic abscesses of biliary origin occur in all animals. They are perhaps most frequent in pigs in which ascarids have migrated into the bile ducts. Cholangitic abscesses in horses, dogs, and cats are usually caused by enterobacteria as part of a fulminating ascending cholangiohepatitis that is fatal after a short course.

The *sequelae* of hepatic abscessation are variable. Usually, they are insignificant and asymptomatic. Sterilization of the focus with either resorption and complete healing or encapsulation is common. Those near the surface of the liver regularly produce fibrinous and then fibrous inflammation of the capsule and adhesion to adjacent viscera. They seldom perforate the capsule but do commonly break into hepatic veins to produce any one or a combination of thrombophlebitis of the vena cava, endocarditis, or pulmonary abscesses or embolism. Acute extension of a hepatic abscess into the major hepatic vein can lead to pulmonary embolism that can be acutely fatal. In adults, death may occur if the hepatic abscesses are multiple and fresh, and especially if they are necrobacillary in origin; death is probably the result of toxemia. Generalization is common, especially from omphalogenic abscesses of young animals.

Hepatic necrobacillosis

Occasionally, *F. necrophorum* infection of the liver is observed following *omphalophlebitis in lambs and calves*, or as a complication of *rumenitis in adult cattle.* In feedlot cattle, both *F. necrophorum* subsp. *necrophorum* (biotype A) and subsp. *funduliforme* (biotype B) have been isolated. The hepatic lesions are multiple and typical of necrobacillary infection, being slightly elevated, rounded, dry areas of coagulative necrosis, sometimes a few centimeters in diameter (Fig. 2.59) and surrounded by a zone of intense hyperemia. Affected neonates seldom live long enough for the necrotic foci to liquefy and assume the appearance of ordinary abscesses, but this may be seen in adult cattle. *The histologic appearance of the foci in the stage of coagulative necrosis is quite characteristic.* The necrotic amorphous central area is bordered by a zone of wholesale destruction of leukocytes, whose nuclear chromatin is dissipated in a finely divided form, and among which the filamentous fusobacteria are mostly concentrated. Outside this zone there is severe hyperemia and hemorrhage, and thrombosis of local vessels is common. The lesion in neonatal lambs is to be distinguished from that caused by *Campylobacter fetus*.

The pathogenic mechanisms of *F. necrophorum* involve various toxins, especially a high-molecular-weight leukotoxin specifically toxic to ruminant neutrophils. This unique toxin activates neutrophils and induces their apoptosis, consistent with the remarkable abscess-inducing propensity of *F. necrophorum* in ruminants. However, other toxins, including collagenolytic activity, are also implicated as virulence factors. Mixed infections are frequent, and synergism between *F. necrophorum* and other pathogens may also play a role in the pathogenesis of liver necrosis and abscessation.

Necrotic hepatitis (black disease)

Organisms of the genus *Clostridium* are notably circuitous in their means of producing disease. This is true of *C. novyi*; *type B strains produce potent exotoxins and are the cause of black disease* (*infectious necrotic hepatitis*). The alpha toxin of *C. novyi* belongs with the large clostridial cytotoxins produced by *C. difficile* and *C. sordelli.* These toxins enter cells by receptor-mediated endocytosis and inhibit ras and rho guanosine triphosphatases by glycosylation. The beta toxin is a necrotizing and hemolytic phospholipase C (lecithinase). Black disease occurs in nonimmune animals when these exotoxins are released by *C. novyi* within an anaerobic focus in the liver. These anaerobic sites, which provide a suitable environment for germination of *C. novyi* spores, are most commonly a result of migrating helminths.

C. novyi is widely distributed in soil, and the spores are continually ingested by grazing animals in areas where black disease occurs. Some spores cross the mucous membranes, probably in phagocytes, and remain as latent infections in histiocytic cells, mainly in the liver, spleen, and bone marrow. The duration of latency in tissue is not known, but it can be many months. In endemic areas, many healthy sheep, cattle, and dogs harbor latent infections in their livers. *Black disease is principally an acutely fatal disease of sheep in regions where the inciting helminths are endemic.* The disease is most commonly initiated by migrating larvae of the common liver fluke *Fasciola hepatica.*

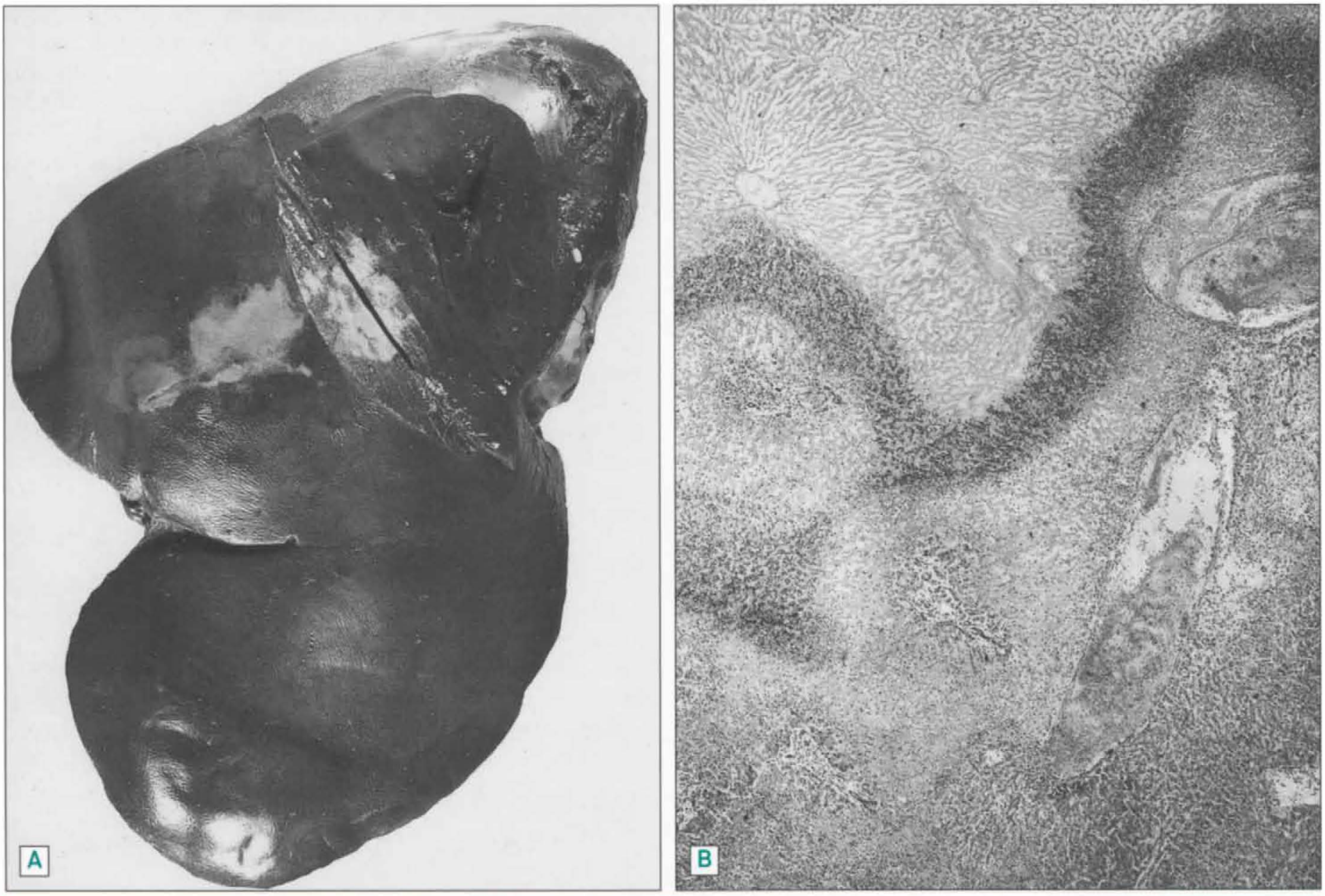

Figure 2.60 A. Infectious necrotic hepatitis ("black disease") in a sheep. Note irregular pale area of necrosis in right lobe. **B.** Section of (**A**). Vascular thrombosis and an area of **coagulative necrosis** isolated by a zone of acute inflammatory infiltrate.

In Bessarabia and France, it is endemically related to the distribution of *Dicrocoelium dendriticum*, the lancet liver fluke. Sporadic cases may be related to *Cysticercus tenuicollis* infection or may be idiopathic.

Deaths in sheep from black disease occur rapidly and usually without warning signs. Illness, if observed, is brief and characterized by reluctance to move, drowsiness, rapid respiration, and quiet subsidence. Affected animals are usually in good nutritional condition. Postmortem decomposition occurs rapidly. *The name of the disease is derived from the appearance of flayed skins, the dark coloration being caused by an unusual degree of subcutaneous venous congestion.* Frequently there is edema of the sternal subcutis, and airways contain stable foam. Serous cavities contain an abundance of fluid that clots on exposure to air. The fluid is usually straw-colored, but that in the abdomen may be tinged with blood. The volume of fluid in the abdomen and thorax may vary from about 50 mL to 1.5 L. The pericardial sac is distended with similar fluid in amounts up to ~300 mL. Subendocardial hemorrhages in the left ventricle are almost constant. Patchy areas of congestion and hemorrhage may be present in the pyloric part of the abomasum and in the small intestine.

The typical and diagnostic lesions occur in the liver and are always present. They are usually clearly evident on the capsular surface, the diaphragmatic surface especially, but the organ may have to be sliced carefully to find them. The liver will be the seat of either the acute traumatic hemorrhagic lesions of acute fascioliasis, or the cholangiohepatitis of the chronic disease, or both. *The lesion of black disease, and occasionally there are several, is a yellow-white area of necrosis 2–3 cm in diameter, surrounded by a broad zone of intense hyperemia, roughly circular in outline, and extending hemispherically into the substance of the organ* (Fig. 2.60). There may be a coagulum of fibrin on the capsular surface overlying the necrotic area. Occasionally, the essential lesions are rectilinear in shape or very irregular. The lesions appear homogeneous on the cut surface, but some contain poorly defined centers of soft or cheesy material.

The histologic evolution of the hepatic lesions begins with the necrotic and hemorrhagic tracts caused by wandering immature flukes. These are sinuous tunnels ~0.5 cm in diameter that contain blood, necrotic hepatic cells, and the leukocytes, chiefly eosinophils, attracted by the flukes. About the tunnels is a narrow zone of coagulative necrosis, also produced by the flukes. As usual, the necrotic tissue is demarcated by a thin zone of scavenger cells, chiefly neutrophils. If latent spores are present in the necrotic areas, they quickly vegetate and are visible in sections as large, gram-positive bacilli. In nonimmune animals, the vegetative organisms elaborate exotoxins that cause necrosis of the surrounding tissue, including the eosinophils of the fluke tunnel. As the area of necrosis expands, the bacterial proliferation keeps pace so that bacilli can be found in all parts of the necrotic focus but not in the surrounding viable tissue. *Usually bacteria are concentrated at the advancing margin of the lesion*, just inside a zone of infiltrated neutrophils. At about the time of death and immediately afterward, the bacilli scatter in the liver and to other organs.

Bacillary hemoglobinuria

Bacillary hemoglobinuria is a counterpart of black disease. The cause is *Clostridium haemolyticum*, which is closely related to *C. novyi*. Both species produce the beta toxin, a necrotizing and hemolytic lecithinase (phospholipase C). The pathogenesis of the two diseases is comparable, as both depend on a focus of hepatic injury within which latent spores can germinate. *Bacillary hemoglobinuria as an endemic disease exists only in areas where Fasciola hepatica abounds, and it is probable that flukes are the primary cause of the initiating lesion.* The disease does occur sporadically where there are no flukes and may be prompted by other parasites or other diverse focal lesions, which are smudged out in the expanding areas of necrosis. There is scant information on the ecology of the organism, but it is clear that it has its own environmental requirements, and the disease will not persist in areas where these requirements are not met. The spores will remain in the livers of cattle for several months after removal from pastures where the disease is endemic. Spores may persist in the bones of cadavers for 2 years. Spores of this and other sporulating anaerobes can frequently be demonstrated in the liver, where they are probably retained in Kupffer cells.

Bacillary hemoglobinuria occurs in *cattle and sheep*. It is characterized clinically by *intravascular hemolysis with anemia and hemoglobinuria*, but, perhaps reflecting variety in exotoxins between strains of the organism, hemolysis may not be a feature. The essential lesion is hepatic and similar to that of black disease but is much larger and usually single. It has been described as an infarct secondary to portal thrombosis, and although this may occur in isolated cases, it is scarcely a creditable pathogenesis for a disease of endemic occurrence. Thrombosis does occur in the affected areas but can be a result rather than a cause of the initial lesion and is found more frequently in the hepatic venules than in branches of the portal vein. There is severe anemia, the kidneys are speckled red or brown by hemoglobin, and the urine is of port-wine color. Peritoneal vessels are injected, and in some cases there is severe, dry, fibrinohemorrhagic peritonitis.

Clostridium piliforme infection

Clostridium piliforme (formerly *Bacillus piliformis*) infection has been known for a long time as *Tyzzer's disease*, a cause of severe losses in laboratory rodents; however, it has also been reported in *foals, calves, dogs, and cats*. Although the disease is probably initiated by an intestinal infection, lesions in the gut are less specific and constant than those in the liver, which consist of *focal hepatitis and necrosis*.

Affected foals usually die between the ages of 1 and 4 weeks; often they are found dead after a short illness. The liver shows pale foci up to a few millimeters across; these are represented microscopically by randomly distributed foci of coagulative necrosis with moderate neutrophilic infiltrate. This lesion is not diagnostic in itself; its specificity depends on the presence of the causal organism in hepatocytes in the periphery of the necrotic zones. At present, *C. piliforme* can only be isolated with difficulty on artificial media, so diagnosis is usually based on demonstration of the large, long bacilli in the cytoplasm of degenerate and also otherwise apparently normal hepatocytes at the periphery of the necrotic zones. The organisms are gram-negative and are best delineated with silver impregnation techniques such as that of Warthin–Starry, but they may be seen with routine stains such as Giemsa, particularly when the material is fresh. Differentiation from other organisms including postmortem saprophytes can be achieved by immunohistochemistry or immunofluorescence. *The bacilli tend to lie in sheaves or bundles* (Fig. 2.61). There may also be colitis sufficiently severe to cause diarrhea, but not as severe as that seen in rabbits with this disease.

Only a few cases of Tyzzer's disease have been reported in dogs and cats. The liver lesions are essentially the same as those in foals and rodents, and there is also enteritis or enterocolitis. Immunodeficiency predisposes to the disease because it occurs sporadically in dogs that have undergone immunosuppressive or anticancer therapy. Such cases may be complicated by concurrent viral, mycotic, or protozoal infections.

Leptospirosis

Leptospirosis is associated with acute jaundice and cholestatic hepatic disease and renal failure in dogs, and is discussed in more detail in Vol. 2, Urinary system. The hepatic lesions described following acute experimental infection of dogs with *Leptospira kirschneri* serovar *grippotyphosa* include mixed perivascular periportal infiltrates of neutrophils,

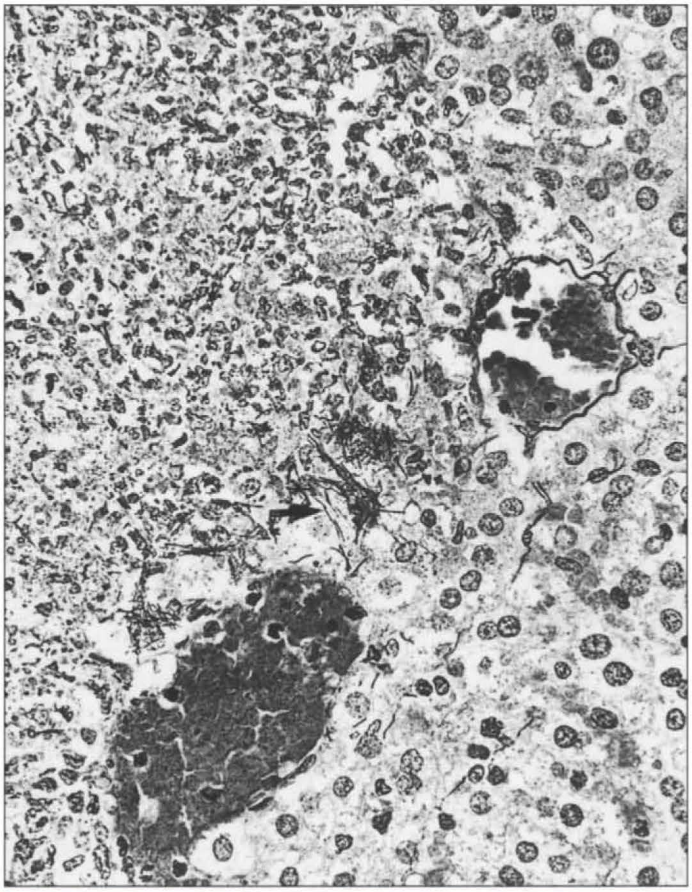

Figure 2.61 Focal hepatitis in *Clostridium piliforme* (*Bacillus piliformis*) infection (**Tyzzer's disease**). Bacilli in bundles in hepatocytes at margin of lesion (arrow), with Warthin–Starry stain.

lymphocytes, and plasma cells, with mild hepatic lipidosis, dissociation of hepatocytes and intracanalicular bile plugs evident by day 12 postinfection, along with increased hepatocellular mitotic activity. *Clinical icterus has been attributed to cholestasis due to dissociation of hepatocytes*. In a retrospective study of dogs with supportive clinical signs and microscopic agglutination test titers of ≥320 for one or more serovars tested including *autumnalis*, *bratislava*, *canicola*, *grippotyphosa*, *icterohaemorrhagiae*, and *pomona*, histologic lesions in the liver were subtle, with sinusoidal neutrophil margination, Kupffer cell hypertrophy, and low levels of hepatocellular single-cell necrosis and mitoses. Some livers had diffuse interstitial lymphocytic hepatitis, with mitotic figures, anisokaryosis, binucleation, and some degree of lobular collapse. Chronic hepatitis has also been experimentally produced by leptospiral infection in dogs; however, clinical cases are rarely documented. Sixteen juvenile Beagle dogs from a single breeding colony vaccinated for *L. interrogans* serogroups *canicola* and *icterohaemorrhagiae* developed chronic hepatitis. Leptospires were identified within bile canaliculi by special stains, electron microscopy, and immunohistochemical techniques. Significant antibody titers were not identified in 6 dogs from which leptospires were isolated. However, serological survey of kennelmates demonstrated high titers to serogroup *australis*.

Other bacteria

A single case of clinical disease associated with ***Helicobacter canis*** has been reported in a 2-month-old puppy with peracute disease causing weakness and vomiting prior to death. Multiple coalescing yellow foci in the liver up to 1.5 cm in diameter consisted of hepatocellular coagulative necrosis with infiltrating mononuclear cells and neutrophils. Spiral bacteria were visualized by Warthin–Starry silver stains in area of necrosis, within bile canaliculi and occasionally in bile duct lumens. This organism has previously been identified in the blood of diarrheic children, and in the feces of 4% of dogs in an epidemiologic study examining the incidence of *Campylobacter*-like organisms in 1000 dogs.

Bartonella spp. infection has been associated with a wide variety of granulomatous syndromes and peliosis hepatis in humans, often in association with immunosuppression; however, the extent to which infection induces disease in dogs is currently unknown. *Bartonella henselae* has been identified in liver tissue by polymerase chain reaction from a single case of peliosis hepatis in a Golden Retriever and granulomatous hepatitis in a Basset Hound, while *B. clarridgeiae* DNA was identified in liver tissue from a Doberman Pinscher with histologic lesions of Doberman hepatopathy.

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Helminthic infections

Various helminths, cestodes, nematodes, trematodes, and even the degenerate arachnid *Linguatula serrata* produce inflammation of liver and bile ducts. Those parasites that have the biliary system as their final habitat will be discussed in detail below. The others produce hepatic lesions in the course of their natural or accidental migrations, and are discussed under the organ (for most of them, the gut) that is their final habitat. It is useful however to describe briefly here the lesions produced by larvae in transit.

The initial lesion produced by wandering larvae is traumatic. Sinuous tunnels permeate the parenchyma and often breach the capsule. In the tunnels there are free red cells, degenerating hepatocytes, and leukocytes, chiefly eosinophils, which react to the parasites. Bordering the tunnel is a narrow zone of coagulative necrosis of parenchyma with infiltrated neutrophils at its margin. Eosinophils also infiltrate the portal triads. The necrotic parasitic tracts heal by scarification, and the fibroblastic tissue, infiltrated with eosinophils, is eventually incorporated into the portal units (interstitial hepatitis). Most larvae escape from the liver but some eventually become encapsulated in the liver in abscesses containing numerous eosinophils. The abscesses may caseate and come to resemble tubercles, and eventually many are heavily mineralized to form permanent pearly nodules. In sheep, the most common cause of this type of hepatitis (aside from liver fluke) is ***Cysticercus tenuicollis*** in its wandering phase. Lambs may die of severe hemorrhagic hepatitis (Fig. 2.62) caused by very heavy infections of this parasite, and in pigs, an aberrant host, *C. tenuicollis*, can produce a very intense inflammatory reaction.

In pigs, larvae of ***Ascaris suum*** and ***Stephanurus dentatus*** produce similar but distinct patterns of focal interstitial hepatitis. The ascarids produce their distinctive accentuation of the stroma ("*milk spots*") (Fig. 2.63) when quite small larvae are immobilized by the host's inflammatory reaction; thus the foci are relatively small. The fibrotic lesion produced by *S. dentatus* larvae, on the other hand, is less focal and more in the nature of a track, and there are usually small, inflamed, capsular craters where the larvae have emerged from the liver to migrate to their preferred perirenal site. There will be obvious portal phlebitis at the hepatic hilus when infection by *S. dentatus*

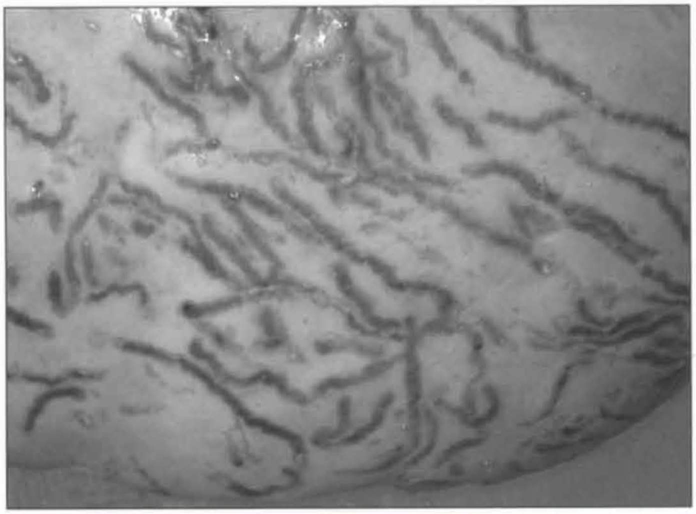

Figure 2.62 Hemorrhagic subcapsular migration tracks caused by *Cysticercus tenuicollis* in a sheep.

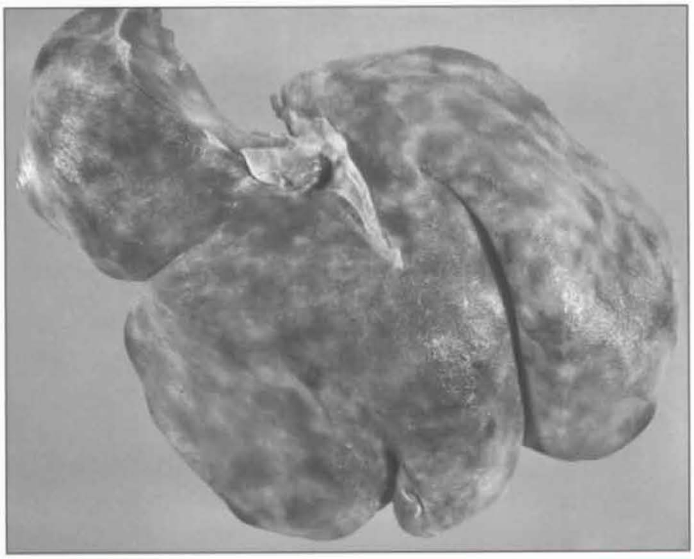

Figure 2.63 Multifocal interstitial hepatitis ("milk-spot liver") caused by *Ascaris suum* migration in a pig.

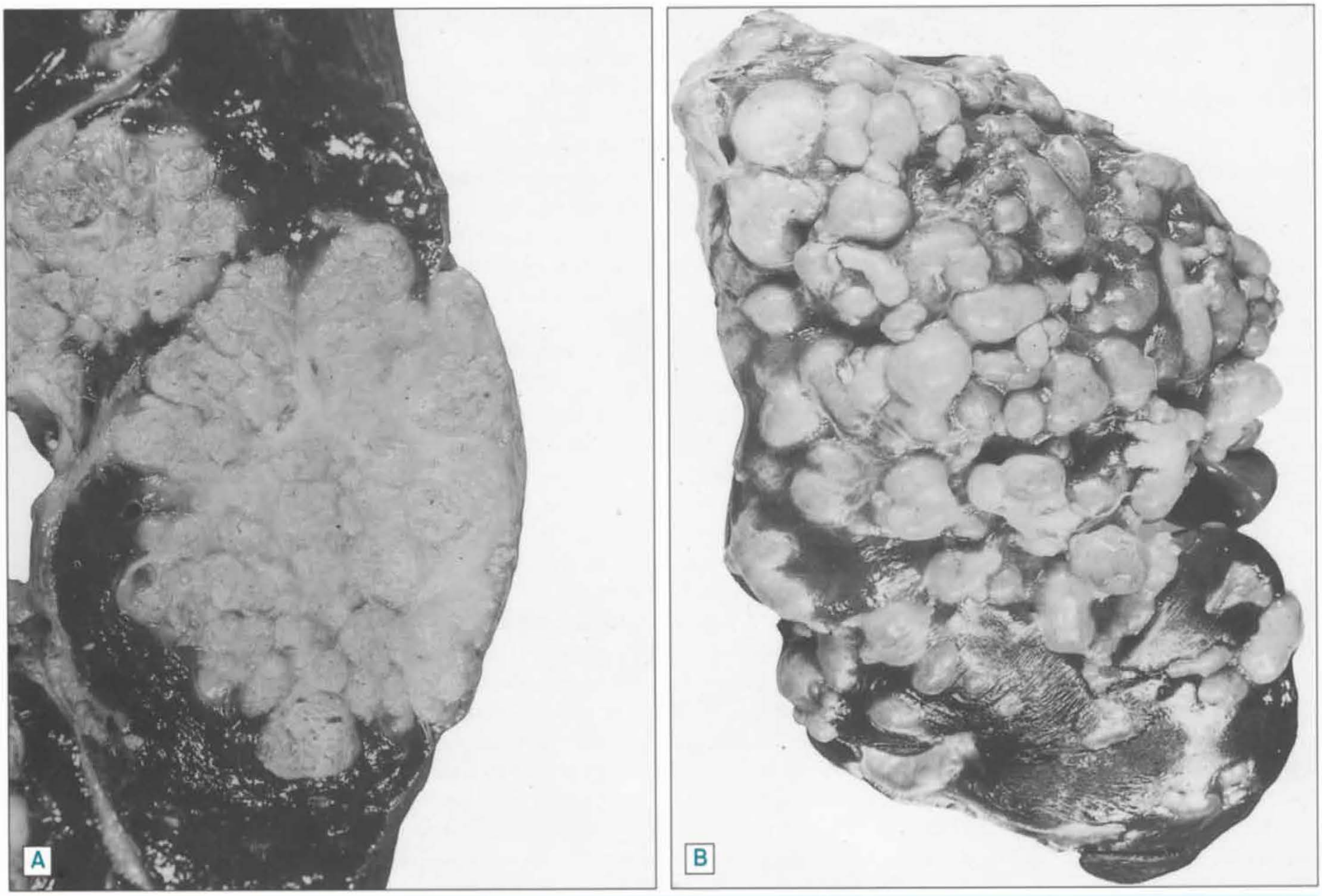

Figure 2.64 A. Degenerate *Echinococcus granulosus* resulting in mineralized membranous debris and fibrosis in an ox. **B.** Severe **hydatid liver disease** (*Echinococcus granulosus* infestation) in a sheep.

has been by the oral route, and in these livers the parenchymal lesion is more severe in this vicinity.

Migration tracks left by *larval strongyles* are common under the liver capsule in young horses and are probably related to the dense, discrete fibrous tags that are almost universally found on the diaphragmatic surface of the liver of mature horses. The range of strongyle species capable of causing these lesions has not been defined. There are other and more devious means by which parasites produce hepatic

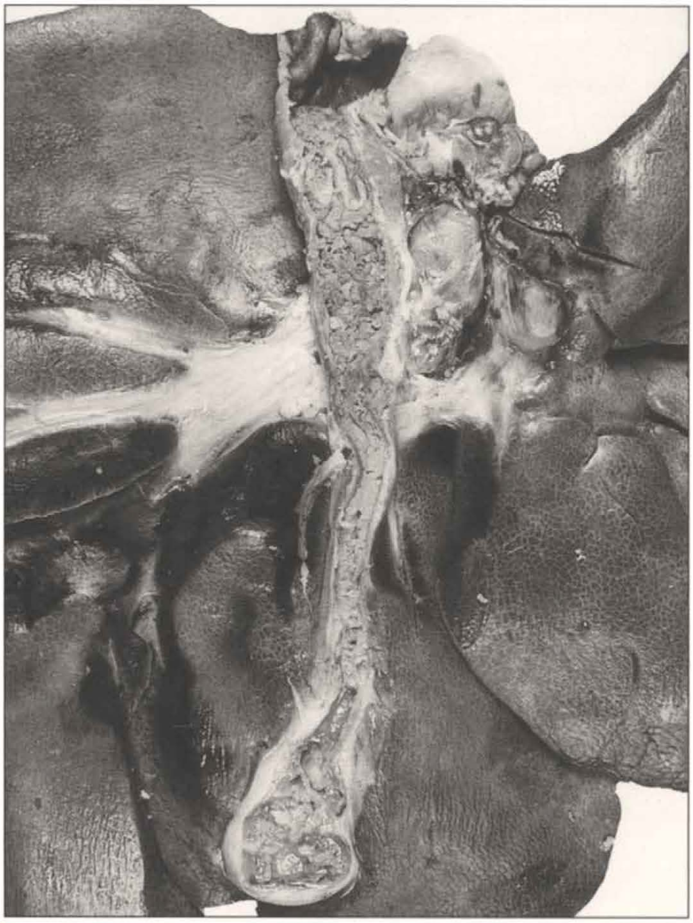

Figure 2.65 Cholangitis and cholecystitis secondary to *Ascaris suum* invasion in a pig.

lesions; the hydatid intermediate stages of *Echinococcus* encyst in the liver and may destroy much of it (Fig. 2.64); the larvae of *Ascaris suum* in cattle add to the usual insult by causing portal phlebitis and small areas of infarction; the adults of *Ascaris* in all species, but especially in pigs, may migrate into the bile ducts (Fig. 2.65); and the eggs of schistosomes enter in the portal blood to lodge in the intrahepatic portal vessels and provoke granulomatous inflammation.

Cestodes

Stilesia hepatica and ***Thysanosoma actinioides***, the "fringed tapeworm," are the only cestodes that inhabit the bile ducts. They are parasites of ruminants, *Stilesia* occurring in Africa, *Thysanosoma* in North and South America. The life cycles of the parasites are not completely known but probably involve oribatid mites as intermediate hosts. *T. actinioides* may also be found in the pancreatic ducts and small intestine. Usually, the infestations are light but, even when heavy, are not of much significance. Very heavy infestations by *S. hepatica* occur without signs of illness, though the bile ducts may be nearly occluded, slightly thickened, and dilated. Saccular dilations of the ducts may occur and be filled with worms. The fringed tapeworm is perhaps more pathogenic, and unthriftiness may accompany heavy infestations.

Echinococcus granulosus hydatid cysts occur most commonly in the liver of ruminants in endemic areas but have been reported as incidental necropsy findings in the liver of horses. The cysticerci and hydatids, which in the intermediate stages invade the liver, are discussed further in Vol. 2, Alimentary system.

Nematodes

Calodium hepaticum (Capillaria hepatica) is the one nematode that in the adult phase inhabits the liver. It is a slender worm, morphologically resembling the whipworms, and it lives in the parenchyma rather than in the bile ducts. The usual hosts of the adult stage are rodents, but sporadic infestations are observed in dogs. These worms are *not highly pathogenic.* The adults provoke some traumatic hepatitis, and the eggs, which are deposited in clusters, provoke the development of localized granulomas. The eggs are readily recognized by their ovoid shape and polar caps. The granulomas can be seen through the capsule or in the substance of the liver as yellow streaks or patches. The eggs cannot escape from the liver unless a predator eats them. Predators, however, act only as transport hosts, and the ingested eggs are passed in the feces. Larvae develop in the eggs only in the external environment, and the cycle is completed when a suitable host eats the mature larvae in the eggs.

Trematodes

Various trematodes (flukes) are parasitic in the livers of animals. They belong to the families Fasciolidae (*Fasciola hepatica*, *F. gigantica*, *Fascioloides magna)*, Dicrocoeliidae (*Dicrocoelium dendriticum*, *D. hospes*, *Platynosomum concinnum)*, and Opisthorchiidae (*Opisthorchis tenuicollis*, *O. sinensis*, *Pseudamphistomum truncatum*). The diseases produced are known collectively as **distomiasis**.

Fasciola hepatica*, the common liver fluke of sheep and cattle, is the most widespread and important of the group.* Patent infestations can develop in other wild and domestic animals and in humans. These flukes are leaf-shaped and ~2.5 cm long in sheep and slightly larger in cattle. *They are found in the bile ducts.* Being hermaphroditic, only one fluke is necessary to establish a patent infestation, and each adult may produce 20 000 eggs per day. The longevity of the adult flukes is amazing and is potentially as great as or greater than that of the host; they have been known to survive for 11 years, and it seems that they can produce eggs all this time. The eggs are eliminated in bile, and on pasture, in conditions of suitable warmth and moistness, hatch a larva (miracidium) in ~9 days. If the environmental temperature is low, the incubation period may be delayed for some months. The miracidium can survive only in moisture. It is actively motile and penetrates the tissues of the intermediate host, which is an aquatic snail. Different snails serve this purpose in different countries, but all of them belong to the genus *Lymnaea.*

Each *miracidium*, on penetrating a snail, develops into a mother *sporocyst* that reproduces, probably parthenogenetically, giving rise to a small number of the second generation, the *redia.* Each redia gives birth to either redia or *cercariae*, or to the two successively. Cercariae, the larval stage of the third (sexual) generation, first appear 1–2 months after the miracidium penetrates. Cercariae continue to escape daily for the life of the snail, but even so, total cercarial production is only 500–1000. They actively escape from the snail and are attracted to green plants, where they encyst and become infective metacercariae in 1 day. These can remain infective for 1 month in summer

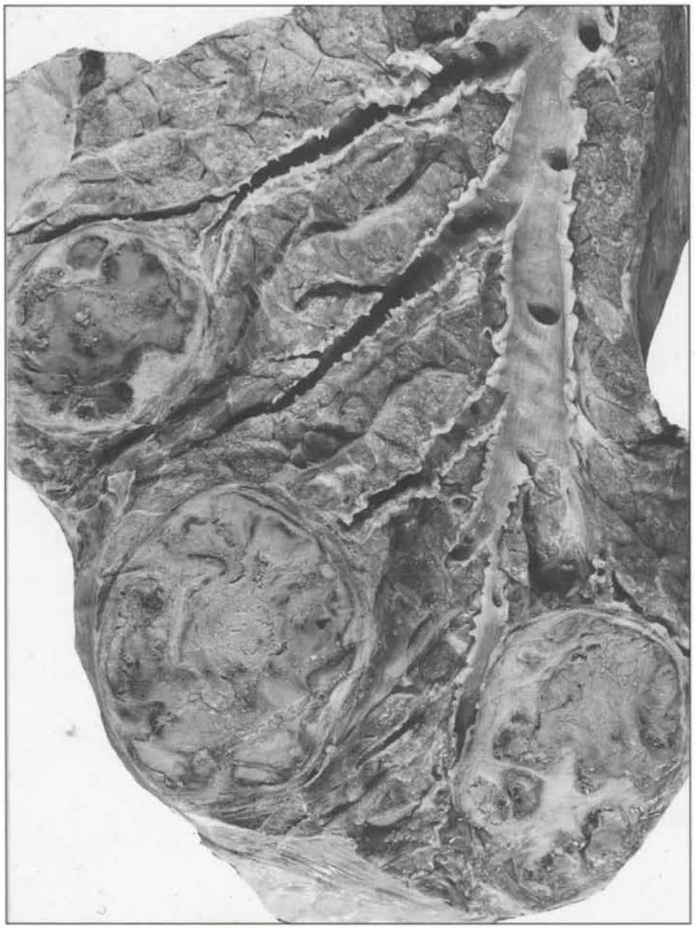

Figure 2.66 Three **caseous abscesses** at the ends of bronchi due to aberrant migration of flukes in an ox.

and up to 3 months in winter. The developmental events from egg to this stage take 1–2 months under favorable conditions.

Infestation occurs by ingestion. Excystment occurs in the duodenum. The young flukes penetrate the intestinal wall and cross the peritoneal cavity, attaching here and there to suck blood and penetrate the liver through its capsule; a few no doubt pass in the portal vessels or migrate up the bile duct. They wander in the liver for a month or more before settling down in the bile ducts to mature, which they do in 2–3 months. Some may, by accident, enter the hepatic veins and systemic circulation to lodge in unusual sites; intrauterine infestations are on record. *Lesions caused by aberrant flukes are quite common in bovine lung.* They consist of resilient nodules just under the pleura of the peripheral parts of the lung. They range in diameter from ~1 to many centimeters and consist of thinly encapsulated abscesses situated at the ends of bronchi (Fig. 2.66). The content is slightly mucoid, unevenly coagulated brown fluid; in some lesions the reaction is predominantly caseous. The location of the lesion suggests that it begins as a peripheral bronchiectasis that later becomes sealed off. The fluke persists in the debris, but is small and hard to find.

The essential lesions produced by *F. hepatica* occur in the liver and may be described, first, as those produced by the migratory larvae, and second as those produced by the mature flukes in the bile ducts; the two often intermingle. There is the further incidence of peritonitis, which is produced by the young flukes on their way to the liver and, perhaps also, by some that break out through the capsule.

Usually there is no obvious reaction to the passage of young flukes through the intestinal wall and across the peritoneal cavity, except for small hemorrhagic foci on the peritoneum, where the flukes have been temporarily attached. Few or many parasites may be found in any ascitic fluid and attached to the peritoneum of the diaphragm and the mesenteries. When the infestations are heavy and repeated, such as may be observed in sheep, cattle, and swine, *peritonitis* occurs. The young flukes at this stage are <1.0 mm long. The peritonitis may be acute and exudative or chronic and proliferative. It is usually concentrated on the hepatic capsule (Fig. 2.67), especially its visceral surface, but may be restricted to the parietal peritoneum or to the visceral peritoneum, including the mesenteries of the gut. In acute cases, there are fibrinohemorrhagic deposits on the serous surfaces, and in chronic cases there may be fibrous tags, with adhesions or a more or less diffuse thickening by connective tissue. Many young flukes can be found microscopically in the fibrinous deposits, and in the diffuse peritoneal thickenings there are tortuous migration tunnels containing blood, debris, and the young parasites (Fig. 2.68). In cases with involvement of the visceral peritoneum, young flukes can be found in enlarged mesenteric lymph nodes.

The acute lesions in the liver caused by the wandering flukes are basically traumatic, but there is an element of coagulative necrosis, which is possibly related to toxic excretions of the flukes. The migratory pathways are tortuous tunnels that appear on cross-section as hemorrhagic foci 2–3 mm in diameter. If a tunnel is followed, a young fluke less than 1.0 mm long can be found at the end. When the infestation is heavy, the liver may appear to be permeated by dark hemorrhagic streaks and foci. Older tunnels from which the debris has been cleared may appear as light yellow streaks due to infiltration of eosinophils (Fig. 2.67). Microscopically, fresh tunnels are filled with blood and degenerate hepatocytes and are soon infiltrated by eosinophils. Later, histiocytes and giant cells arrange themselves about the debris and remove it, and healing occurs by granulation tissue, which is rich in lymphocytes and eosinophils. In light infestations the scars may disappear, but in heavy infestations they may fuse with each other and with portal areas to produce moderate irregular fibrosis. There may, as yet, be no change in the bile ducts. Probably, *most of the young flukes reach the bile ducts, but some do not and become encysted in the parenchyma.* One or more flukes may be present in each cyst, which consists of a connective-tissue capsule and a dirty brown content of blood, detritus, and excrement from flukes. The cysts ultimately caseate and may mineralize or are obliterated by fibrous tissue. These cysts are most frequent on the visceral surface, where they cause bulging of the capsule.

Heavy infestations by immature flukes may cause death in the stage of acute hepatitis. Such an outcome is not common, but occurs in sheep. It is estimated that 10 000 metacercariae ingested over a short period are necessary to produce acute death in sheep. Death may occur suddenly or after a few days of fever, lassitude, inappetance, and abdominal tenderness. This is also the stage of the parasitism in which *black disease* occurs (see above).

Mature flukes are present in the larger bile ducts and cause cholangiohepatitis. The relative importance of different factors in their pathogenicity is not known, but they cause mechanical irritation by the action of their suckers and scales, cause obstruction of the ducts with some degree of biliary retention, predispose to bacterial infections,

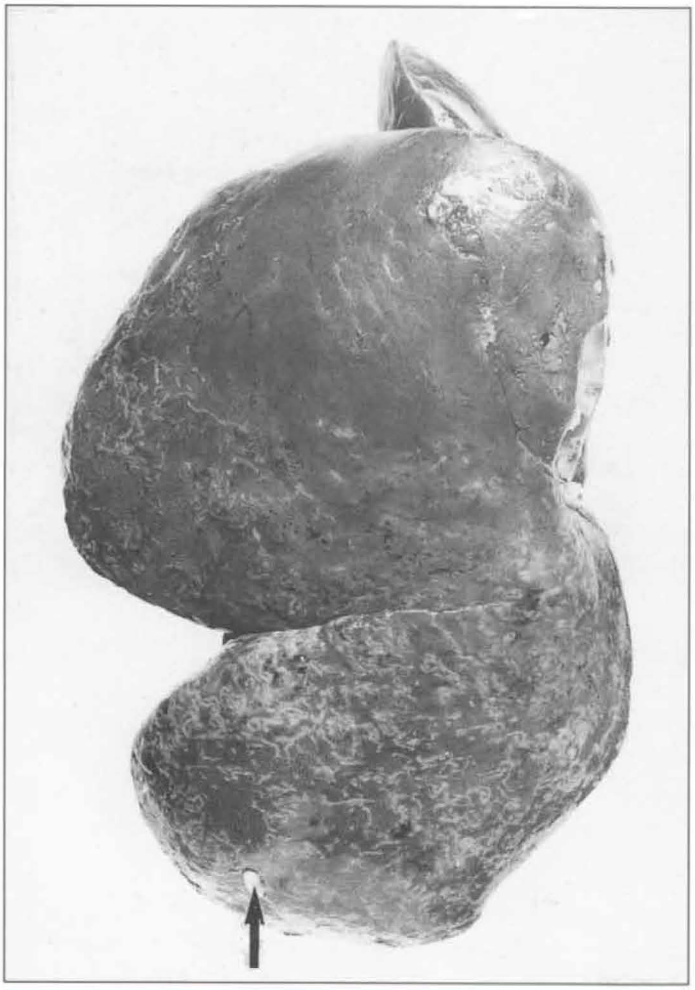

Figure 2.67 Tracks of immature ***Fasciola hepatica* flukes** in acute infestation, concentrated in left lobe of a sheep. Degenerate cysticercus (arrow).

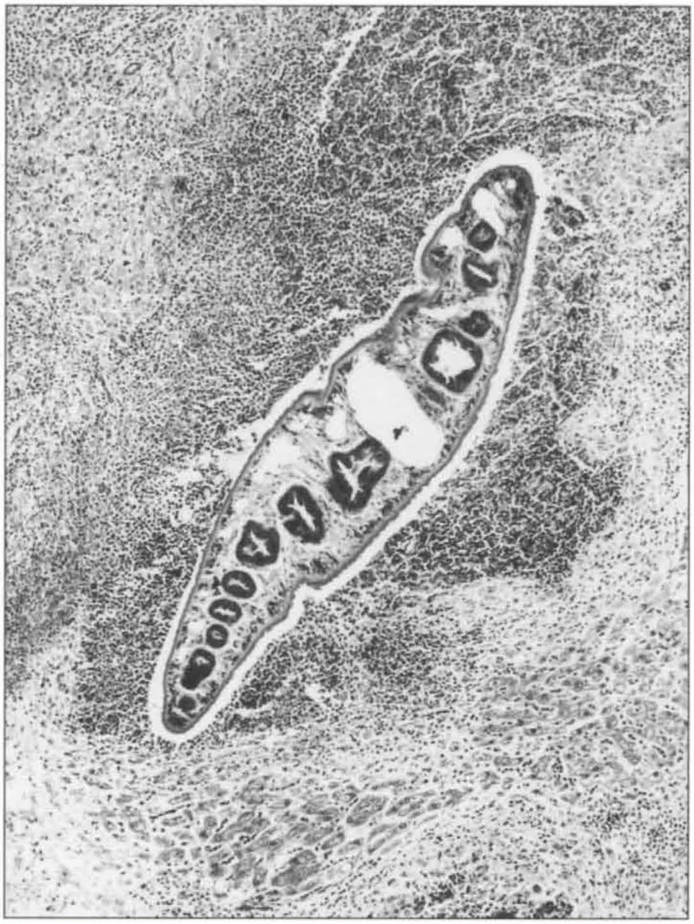

Figure 2.68 Immature fluke, accompanied by acute inflammation in a sheep.

suck blood, and probably produce toxic and irritative metabolic excretions.

The *biliary changes* occur in all lobes but are usually most severe in the left, and the right may be moderately hypertrophied (Fig. 2.69A). From the hilus, *the bile ducts on the visceral surface stand out as white, firm, branching cords* that in extreme instances may be 2 cm in diameter and allow detectable fluctuation over extended segments or in localized areas of ectasia. This dilation of the ducts in sheep, swine, and horses is largely mechanical and is due to *distension by masses of flukes and bile*. It is permitted by the relative paucity of new connective-tissue formation in the walls of the ducts in these species; this in turn is probably related to the rather mild catarrhal type of inflammation in the lumina of the ducts. In *cattle*, desquamative and ulcerative lesions in the large bile ducts are more severe than in other species, and there is a correspondingly greater proliferation of granulation tissue in and about the walls of the ducts. The walls of the ducts in cattle are, in consequence, much thickened, and the lumen is irregularly stenotic and dilated and lined largely by granulation tissue. This contributes the typical "*pipe stem*" appearance to the ducts in cattle; the connective tissue may be, in addition, mineralized, sometimes so heavily that it cannot be cut with a knife. The bile ducts contain dirty dark-brown fluid of a mucinous or tough consistency, formed from degenerate floccular bile, pus, desquamated cells and detritus, clumps of flukes, and small masses of eggs in dark-brown granular aggregates.

Although the lesions are most obvious in ducts large enough to contain the flukes, there is, with time and severe or repeated infestations, *progressive inflammation in the smaller portal units* due to direct irritation by the flukes, superimposed infections, and biliary stasis. The course of events is as described earlier for subacute and chronic cholangiohepatitis. The proliferating connective tissue and bile ductules in individual portal areas extend to join each other and the scars left over from the migratory phase, so that inflammatory fibrosis may obliterate parenchyma in many foci. In such livers, the left lobe, which is the one most severely affected, may be atrophied, indurated, and irregular (Fig. 2.69B).

The development of cholangiohepatitis of the degree described depends on long-standing or heavy infestations. Lesions of lesser severity, or those less fully developed, are associated with light infestations of short duration. They may then be recognized only by local dilations of the ducts, or even these may not be readily apparent. In such mild infestations, the fact of past or present parasitism may only be suggested by the detection of characteristic black iron-porphyrin pigments, grossly visible in the hilar nodes. It also contributes to the character of the biliary contents.

Chronic debility with vague digestive disturbances is common in chronic fascioliasis, and deaths are common among sheep. Clinically and at postmortem there are, in addition to the essential lesions,

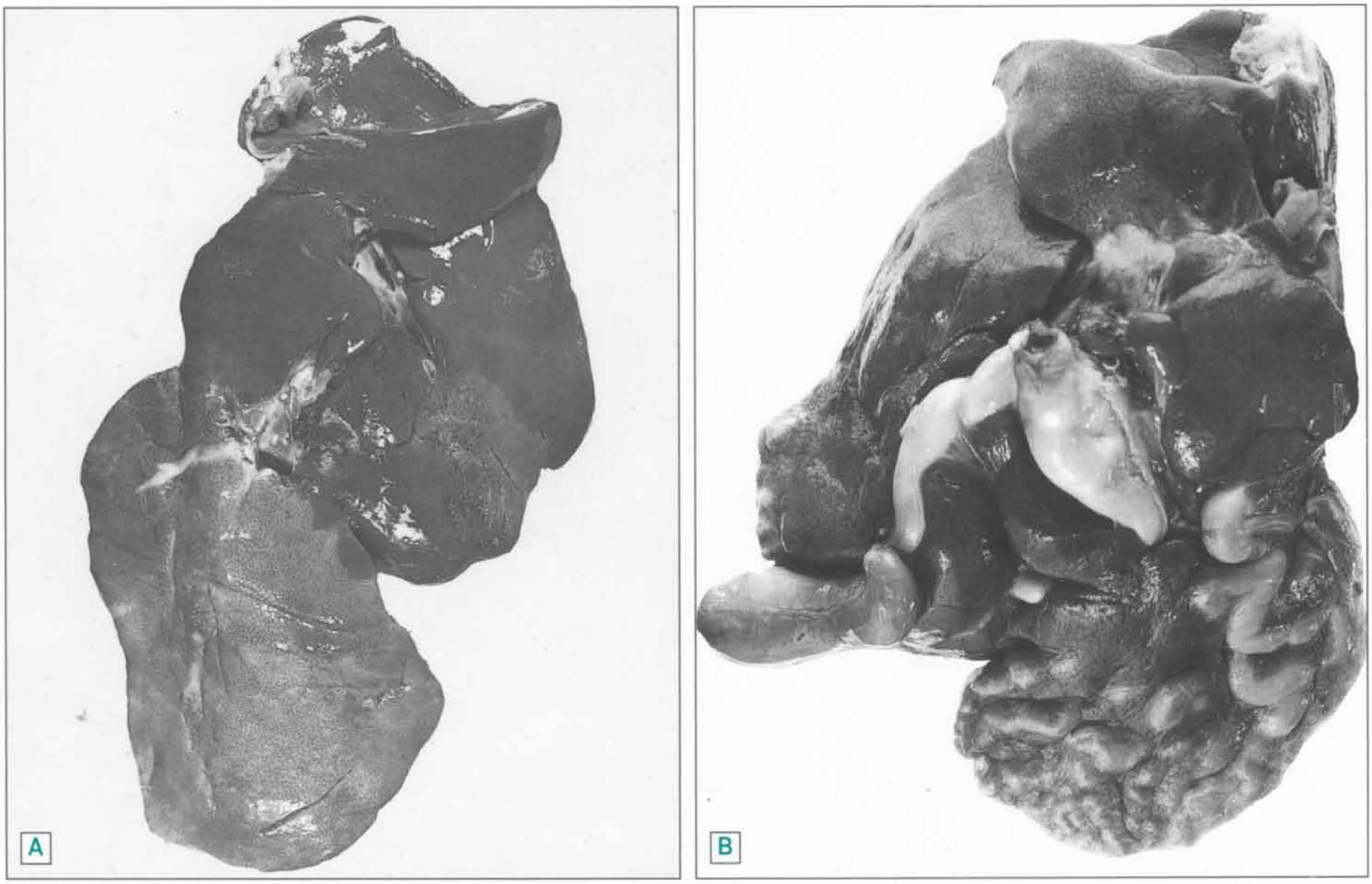

Figure 2.69 Fascioliasis in sheep. **A.** Subacute *Fasciola hepatica* infestation, causing early **cholangiohepatitis and cholestasis** in left lobe. **B.** Bile duct ectasia, atrophy of left lobe, hypertrophy of right lobe resulting from **chronic fascioliasis**.

more or less severe anemia, moderate anasarca, and cachexia. Jaundice is seldom seen.

Fasciola gigantica displaces *F. hepatica* as the common liver fluke in many parts of Africa and in nearby countries, southeast Asia, and the Hawaiian islands. It is two or three times as large as *F. hepatica*, but its life cycle and pathogenicity are comparable.

Fascioloides magna *is the large liver fluke of North America.* It is a parasite of ruminants and lives in the hepatic parenchyma, not in the bile ducts, although in tolerant hosts, Cervidae, the cysts in which it localizes communicate with the bile ducts to provide an exit for ova and excrement. The life cycle of this parasite generally parallels that of *Fasciola hepatica*. The young flukes are very destructive as they wander in the liver. In cattle, they wander briefly, producing large necrotic tunnels before becoming encysted. The cysts, enclosed by connective tissue, do not communicate with bile ducts but form permanent enclosures for the flukes, their excreta, and ova. *The cysts, which may be 2–5 cm in diameter, are remarkable for the large deposits of jet black, sooty iron-porphyrin pigment they contain* (Fig. 2.70), and except for the flukes and soft contents, they superficially resemble heavily pigmented melanotic tumors. Commonly, these flukes pass from the liver to the lungs of cattle, to produce lesions of similar character. In sheep, this parasite wanders continuously in the liver, producing black, tortuous tracts, which may be 2 cm in diameter, and extensive parenchymal destruction. Even a few flukes may kill a sheep.

The **dicrocoelid flukes** inhabit both biliary and pancreatic ducts. *Eurytrema pancreaticum* prefers the pancreas (and is described with that organ) but in heavy infestations can be found in the bile ducts. *Dicrocoelium* and *Platynosomum* prefer the bile ducts. These are small, narrow flukes 0.5–1.0 cm long and may easily be mistaken for small masses of inspissated bile pigment. They are not highly pathogenic, and even in heavy infestations there may be no signs of the toxemia observed in infestations by *F. hepatica*. These flukes may occur as mixed infestations.

Platynosomum fastosum is a parasite of cats in North America and the Amazonian regions of South America. The life cycle involves snails and lizards and presumably an arthropod. The infested livers are enlarged, friable, and may be bile-stained. There is catarrhal inflammation of biliary passages, but it is not severe, and the walls may not be much thickened. The ducts are dilated and easily visible. There are vague digestive disturbances, and heavy infestations may cause complete anorexia and death.

Dicrocoelium hospes is found in cattle in countries south of the Sahara. Little is known of it, but it is presumed to be comparable in all respects to the better-known *D. dendriticum* (the lancet fluke or small liver fluke), which is common in Europe and Asia and sparsely distributed in the Americas and North Africa. *Dicrocoelium* spp. are found in dry lowland or mountain pastures, whereas *Fasciola* spp. occur in wetter habitats, so the prevalence of *Dicrocoelium* is increasing

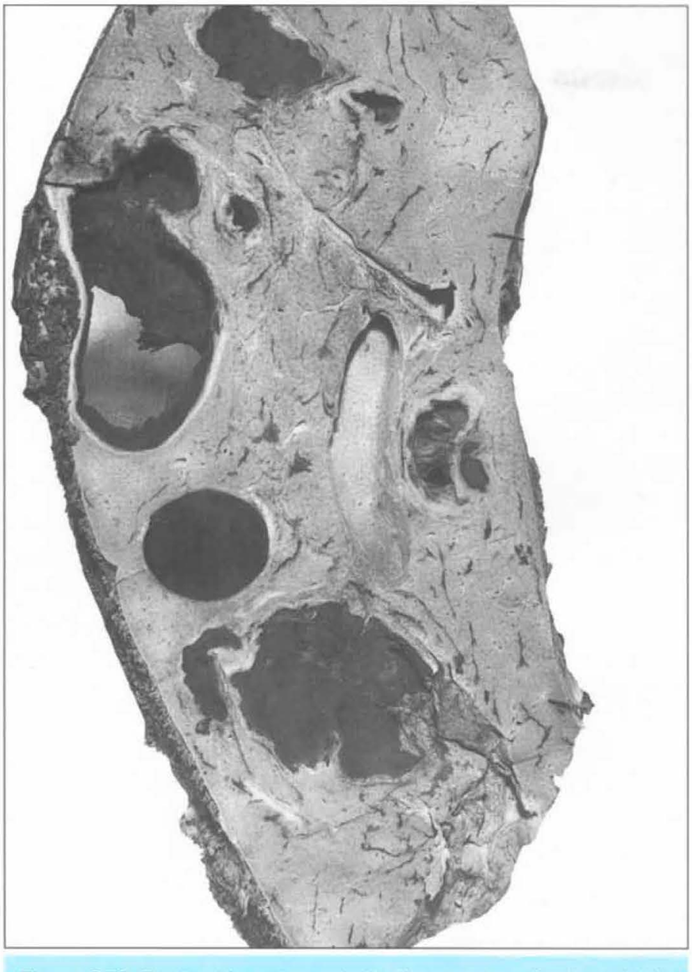

Figure 2.70 Destructive pigmented lesions produced by *Fascioloides magna* in an ox.

with desertification. This fluke is no more fastidious in its choice of final hosts than many other species of fluke, and, depending on opportunity, it can infest all domestic species, with the possible exception of cats. It is, however, of most importance as a parasite of sheep and cattle, in which it inhabits the bile ducts. Other domestic species and rodents are important as reservoirs.

The life cycle of ***Dicrocoelium dendriticum*** differs in some details from that of *F. hepatica*. The eggs are embryonated when laid and do not hatch until swallowed by one of the many genera of land snails that are the first intermediate hosts. In the snails, the mother sporocyst produces a second generation of daughter sporocysts, which in turn produce cercariae. The cercariae leave the snail in damp weather and are expelled from the snail's lung, clumped together in slime balls. The slime balls are not infective until the cercariae are swallowed by, and encyst in, the ant *Formica fusca*; other ants may be involved in different countries. The cycle is completed when the definitive hosts swallow the ants. The route of migration of the larvae from the gut to the liver is probably via the bile ducts from the duodenum.

The pathologic changes in the liver produced by *D. dendriticum* are those of cholangiohepatitis that is less severe than that produced by *Fasciola hepatica*. The severity and diffuseness of the hepatic lesion are determined by the number of lancet flukes present, and they may be in the thousands. The flukes and their eggs darken the dilated ducts. Even in early infestations, there may be some scarring of the organ at its periphery. In heavy infestations of long standing, there is extensive biliary fibrosis, producing an organ that is indurated, scarred, and lumpy and that at the margins may bear areas that are shrunken and completely sclerotic. The histologic changes are the same as those in fascioliasis, with perhaps more remarkable hyperplasia of the mucous glands of the large ducts.

The **opisthorchid flukes** are parasites in the bile ducts of carnivores. They may also occur in swine and humans, and one species, *Opisthorchis sinensis* (*Clonorchis sinensis*), is an important human parasite. There is some uncertainty regarding the proper classification of these flukes, and some of those given below may not be valid species. The life cycles, where known, include mollusks as the first intermediate hosts and freshwater fish as the second.

***Metorchis conjunctus** is the common liver fluke of cats and dogs in North America* and is important as a parasite of sled dogs in the Canadian Northwest Territories. The first intermediate host is the snail *Amnicola limosa porosa*, and the second is the common suckerfish *Catostomus commersonii*. The cercariae actively burrow into the musculature of the fish to encyst and become infective. The immature flukes crawl into the bile ducts from the duodenum and mature in ~28 days. Infestations may persist for more than 5 years. *Metorchis albidis* has been described in a dog from Alaska, *Parametorchis complexus* in cats in the USA, and *Amphimerus pseudofelineus* in cats and coyotes in the USA and Panama; the life cycles are not known but are presumed to include fish. *Metorchis bilis* has been reported in red foxes and occasionally in cats from Germany.

***Opisthorchis felineus** is the lanceolate fluke of the bile ducts of cats, dogs, and foxes in Europe and Russia.* It is particularly common in eastern Europe and Siberia and is more sparse in other areas. *Metorchis bilis* infects red foxes. *Opisthorchis sinensis* is well documented as the Oriental or Chinese liver fluke; it is endemic in Japan, Korea, southern China, and southeast Asia. There are additional species of *Opisthorchis* in humans and animals, but they are less well known than the species cited. The first intermediate hosts for the miracidia of *O. tenuicollis* and *O. sinensis* are snails of the genus *Bithynia*, and several genera of cyprinid fishes can act as second intermediate hosts.

Pseudamphistomum truncatum occurs in carnivores and humans sporadically in Europe and Asia. Its life cycle is as for *Opisthorchis*.

The opisthorchid flukes, so far as known, resemble *Dicrocoelium* in migrating up the bile ducts to their habitat. This may be the reason that they are more numerous in the left than in the right lobes of the liver. They can probably live in the liver for as long as the host lives. The pathologic effects are comparable to those of *D. dendriticum*. Light infestations may be asymptomatic, and heavy infestations may cause jaundice, chronic cholangiohepatitis, and severe biliary fibrosis. Both in humans and animals, adenomatous and carcinomatous changes of the biliary glands have occurred in association with these parasites; the association is probably more than coincidental.

Protozoal infections

Protozoal hepatitis is due mainly to infection with *Toxoplasma*, *Neospora*, and *Leishmania*, described elsewhere. Granulomatous hepatitis has been described in dogs associated with systemic infections by *Hepatozoon canis*.

Hepatic coccidiosis, expressed as acute cholangiohepatitis similar to that in mink and rabbits, has been observed in isolated cases in the goat, calf, and dog. These infections are usually considered aberrant, and often coincide with intestinal coccidiosis. Coccidial meronts and in some cases gamonts are present within the cytoplasm of biliary epithelium. The organisms are presently unclassified.

Fungal infections

Fungal infections of the forestomachs with hematogenous dissemination to the liver occur occasionally in cattle and sheep, usually as a complication of rumenitis. Lesions in the liver are typically hemorrhagic infarcts initially or granulomatous with chronicity, and are associated with infection by *Aspergillus fumigatus* and various members of the class Zygomycetes.

Granulomatous hepatitis associated with disseminated fungal or algal infections has also been reported in dogs. The species involved include *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Sporothrix schenckii*, *Aspergillus* sp., and *Prototheca* sp.

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TOXIC HEPATIC DISEASE

Role of hepatic biotransformation

An understanding of hepatic biotransformation is essential for an appreciation of the hepatotoxic potential of foreign compounds. Lipid-soluble compounds, including endogenous metabolites as well as nonpolar foreign compounds (known as **xenobiotics**, and *including plant or fungal-derived secondary metabolites consumed in food, environmental chemicals, and drugs*) must be converted to water-soluble products to permit elimination from the body in urine and bile. The enzymatic machinery responsible for this biotransformation is most active in the liver. The liver is strategically located to intercept ingested foreign compounds, and it is likely that *hepatic biotransformation enzymes* co-evolved in part to deal with naturally occurring toxic and lipid-soluble metabolites produced by plants that formed part of the diet. While these enzyme systems were formerly referred to as drug-metabolizing enzymes, or liver detoxification systems, recent research has underscored the importance of these systems in metabolism of endogenous compounds, including arachidonic acid, eicosanoids, cholesterol, biosynthesis of bile acids, and synthesis and metabolism of steroids and vitamin D.

Enzymatic biotransformation in the liver consists of two phases. *Phase I reactions* typically involve the addition of reactive polar groups through *oxidation* and, less frequently, reduction and hydrolysis reactions. In *phase II*, the metabolic product of phase I (or the original compound if it possesses a polar group that permits metabolism) undergoes *conjugation*, typically with water, glutathione, sulfate, glucuronate, or other groups. The conjugate is typically less toxic, and more water-soluble than the parent compound.

Phase I reactions are mainly performed by members of the *cytochrome P450 superfamily*, the so-called *mixed-function oxidase system*, which are integral parts of the smooth endoplasmic reticulum. The oxidative reactions of phase I are a double-edged sword, and while they are important in providing reactive sites for further conjugative detoxification reactions, in the process they can yield transient reactive intermediates, including epoxides and free radicals, which can potentially bind to and damage adjacent macromolecules, resulting in hepatotoxicity. *Toxic hepatic injury depends on the balance between production of reactive metabolites and their detoxification by conjugation and other protective reactions.* The cytochrome P450s can be found in all parts of the hepatic acinus, but the hepatocytes of zone 3 have a higher content than those of zone 1, which apparently accounts for the *zone 3 predominance of injury produced by compounds metabolized by this system*, including carbon tetrachloride and acetaminophen. In contrast, *hepatocytes in zone 1 are more susceptible to direct-acting toxicants such as metal salts*, due to their proximity to incoming portal and arterial vascular flow.

Phase II reactions are generally *detoxification reactions*, although there are exceptions. The enzymes of phase II are predominantly located in the cytosol, and include various transferases capable of conjugating molecules containing suitable polar groups with endogenous substances. One important conjugation pathway is with reduced glutathione, mediated by the glutathione *S*-transferases. Active metabolites of acetaminophen and various other agents are detoxified by conjugation with glutathione, and depletion of glutathione reserves plays a critical role in the development of toxicity. Hepatic glutathione is also important in the removal of free radicals and reactive oxygen species through the action of glutathione peroxidase.

Efforts to elucidate mechanisms for hepatic injury are complicated by the presence of multiple alternative pathways for metabolism of compounds, the presence of many different isoforms of various enzyme families, particularly the cytochromes P450, and the increasing numbers of genetic polymorphisms identified within individual alleles coding for these isoforms. Many genes for cytochromes P450 and various phase II conjugation enzymes have regulatory sequences that include various transcriptional response elements that respond to nuclear receptors. These receptors resemble steroid receptors and include the arylhydrocarbon hydroxylase receptor, peroxisome proliferator-activated receptors, the constitutive androstane receptor, and the pregnane X receptor. These receptors are activated by various xenobiotics and endogenous ligands so the overall biotransformation phenotype depends on both genetic background, and the environmental, dietary, and medical experiences that can influence expression of these genes. The nuclear receptor genes themselves also vary among species, and there are species differences and gene polymorphisms that affect the receptors or the expressed genes. Many factors can therefore modify metabolism, including sex, age, diet, previous or concurrent exposure to environmental compounds, or the effects of intercurrent disease, particularly those that alter hepatic functional mass. These complexities explain some of the differential susceptibility among species and individuals to drug-induced and other toxic hepatic injury.

Classification of hepatotoxins and mechanisms of toxicity

Hepatotoxic agents have typically been classified according to their mechanism of injury, and by the morphologic type of injury they cause. Hepatotoxins have either *intrinsic toxicity*, or they may injure only particularly susceptible hosts as an *idiosyncratic reaction* (discussed further in the section on Adverse drug reactions, below). Intrinsic toxins may be *direct-acting*, or *indirect-acting*, if they are metabolized to reactive metabolites.

Acute hepatotoxic injury may be **cytotoxic**, resulting from damage to the structural or functional components of hepatocytes that maintain cellular integrity, or **cholestatic**, resulting from interference with the various components essential for bile flow. *Acute cytotoxic injury is often manifest as steatosis (lipidosis)*, a result of impairment of movement of triglycerides through the liver, interference with VLDL synthesis or transport, or impaired hepatic consumption of fatty acids by mitochondrial oxidation. *It may also be manifest as necrosis or apoptosis.* Acute hepatic injury can injure elements of the structure or function of the biliary tree, resulting in defects in bile transport.

The histologic changes in acute toxic hepatic injury are rather stereotyped. They range from apoptosis, through confluent coagulative and zonal necrosis, to massive hemorrhagic destruction that includes sinusoidal lining cells. The histology of these acute intoxications is usually characterized by *severe periacinar necrosis*. Rarely, the pattern of necrosis may be periportal or midzonal (the characteristics of these injuries are described above in the section on Patterns of cell death in the liver). Depending on the nutritional status of the animal, there may be variably severe fatty or hydropic change in hepatocytes adjacent to the necrotic zones. The necrotic cells may accumulate calcium. Variations on the general process of hepatocellular necrosis have little diagnostic or pathogenetic specificity. In sublethally injured cells there may be spectacular but nonspecific clumping of smooth endoplasmic reticulum, particularly in the periacinar zones.

The *clinical and gross characteristics of fatal acute intoxications* that destroy liver parenchyma are rather consistent, regardless of the origin of the toxin. The animal dies after a brief period of dullness, anorexia, colic, and various neurologic disturbances, including convulsions; these are attributed to hepatic encephalopathy. Postmortem examination reveals a slight excess of clear, yellow abdominal fluid, which contains sufficient fibrinogen to form a loose, nonadherent clot. The appearance of the liver depends on the severity and stage of the injury. Severe acute toxicity that destroys endothelium typically results in a hemorrhagic zonal pattern, in which case the liver may be deep red-purple and obviously swollen and turgid. In less severe injury without hemorrhage, the liver tends to be lighter brown due to a combination of edema (exclusion of sinusoidal blood), destruction of cytochrome pigments, and accumulation of bile pigments and/or fat. If the animal survives for several days, the liver develops a typical zonal yellow fatty change as triglyceride accumulates in sublethally injured hepatocytes.

In acute fatal hepatotoxicities there is almost always widespread hemorrhage resulting from lack of coagulation factors. Petechiae and ecchymoses are seen most consistently on serous membranes, especially on the epi- and endocardium and abdominal viscera. Diffuse hemorrhage into the gut, particularly the duodenum in ruminants, is also common, as are hemorrhages into the wall of the gallbladder. Hemorrhages are largely due to excessive consumption of clotting factors and platelets within the areas of necrosis in the liver, although the concurrent failure of the damaged liver to replace those coagulation factors undoubtedly becomes important when these factors are consumed. Gross lesions such as icterus and photosensitization that reflect failure of biotransformation or excretion of endogenous materials develop too slowly to be a feature of acutely fatal hepatotoxicities. However, the rate of accumulation of bilirubin increases when hemorrhage occurs in the necrotic liver or as a consequence of coagulopathy.

Chronic hepatotoxic injury *may manifest in many patterns*, as previously described. These include chronic necroinflammatory hepatitis, steatosis, cirrhosis, atrophy with nodules, hepatic vein thrombosis, veno-occlusive disease, peliosis hepatis, cholangitis, cholangiofibrosis, and carcinogenesis. In contrast to the acute intoxications, chronic hepatotoxicities are more likely to present a mix of these responses and this can provide more diagnostic specificity. For example, agents that impair hepatocellular regeneration might not be potent necrogens but tend to produce hepatic apoptosis and atrophy, fibrosis of various patterns, compensatory bile duct hyperplasia, nodular regeneration, some degree of cholestasis, and frequently megalocytosis (polyploidy). Such hepatotoxins are potential carcinogens because they favor the selective growth of hepatocellular nodules that are resistant to mitoinhibitory effects.

Clinical signs of chronic hepatotoxicity are usually problems resulting from inadequate detoxification and excretion; these include jaundice, photosensitization, and hepatic encephalopathy. Most of the toxins responsible for chronic hepatotoxicity may produce acute nonspecific zonal or massive necrosis if experimentally administered at dose rates higher than those to which animals are likely to be exposed in the field, although such acute toxicity is occasionally observed in field cases of the diseases.

The range of substances that can cause hepatotoxicity is so broad that it encompasses virtually all categories of natural and synthetic chemicals. These include metals (iron, copper), drugs (e.g., acetaminophen), plant components (phytotoxins), fungal metabolites (mycotoxins), bacterial products (e.g., cyanobacterial microcystin-LR) and various industrial products (especially aromatic solvents). Many drugs are also hepatotoxic in idiosyncratically sensitive, overdosed, or accidentally poisoned animals, as in humans. Differences in individual susceptibility to hepatotoxic responses likely occur for all classes of chemicals that are metabolized in the liver.

Hepatotoxic plants occur in botanical families as diverse as the relatively primitive Cycadaceae through the Compositae and Solanaceae. The evolutionary relationships between herbivores and toxic plants are complex; in some situations, consumption of plants by herbivores has competitive or neutral advantage for the plant, especially those that are lush and prolific, so toxicity is counterproductive or unnecessary. However, in arid or semiarid habitats, plants must put up more resistance to herbivores that could obliterate them. Indigenous herbivores are typically reluctant to do this, and are either resistant or reluctant to graze some plants. Plants sometimes protect critical parts at particular stages of growth. For example, *Xanthium pungens* (Noogoora burr) concentrates its toxin in the cotyledons. The seeds are rarely eaten, even by cattle, but intoxications can occur when this plant is eaten shortly after germination. Phytotoxic liver disease is therefore more frequently encountered in animals grazing pastures at particular times of the year, with limited choice or supply, or when hungry animals are introduced to plants for which their natural or induced resistance is low. The patterns of liver disease resulting from consumption of toxic plants is for the most part regular in comparison with what is seen with exposure to other classes of hepatotoxins. An exhaustive review of the toxicity of all such plants would be repetitive. However, some plants that do produce more distinctive patterns of liver lesions will be discussed in more detail.

In the following sections, toxic hepatic disease will be separated into drug- or pharmaceutical-induced hepatotoxicity (so-called adverse drug reactions), and hepatotoxicity associated with exposure to plant or environmental toxins, including metals. The latter have been somewhat arbitrarily divided into acute and chronic, and while it is recognized that the difference between acute and chronic hepatotoxicity is often simply a matter of dose rate, it is convenient to categorize the sources according to the syndrome of liver damage which they most commonly produce. Various plants and moldy feeds are hepatotoxic and some phytotoxins (e.g., pyrrolizidine alkaloids) and mycotoxins (e.g., aflatoxins) are notorious hepatotoxins and hepatocarcinogens. Many phytotoxins and mycotoxins target other organ systems or have physiological rather than pathological effects. Some of those that cause diagnostic lesions are discussed under the respective target tissues elsewhere. Generally, these areas of toxicology are better accessed in comprehensive references on phytotoxins or mycotoxins.

Adverse drug reactions: drug-induced hepatic disease

Hepatotoxicity is an important adverse drug reaction in humans, and while hepatotoxic drug reactions have been recognized in dogs and cats, the true prevalence of adverse hepatic drug reactions in domestic animals is unknown. The vulnerability of the liver to drug-induced injury relates to several factors. The liver is exposed to high concentrations of orally administered drugs due to its location as the recipient of portal venous blood flow. The uptake of exogenous and endogenous compounds from the portal circulation is facilitated by a number of basolaterally located, sodium-independent solute transporters known as *organic anion transporting polypeptides*. Foreign compounds may be concentrated in the liver to various degrees by mechanisms that remain obscure, but which are related to special binding proteins of hepatocytes, and attachment to enzymatic sites where metabolic conversions occur. The most important factor, however, is the liver's role as the primary site of biotransformation for many therapeutic agents, resulting in exposure to high concentrations of metabolites. These water-soluble metabolites are excreted in the bile via members of the ATP-binding cassette superfamily of transport proteins located on the apical canalicular membrane; some may be reabsorbed in the enterohepatic circulation. In addition, many drug administration regimens may achieve relatively high millimolar concentrations, resulting in depletion of conjugating cofactors such as glutathione, which can reach the lower limits required for other cytoprotective roles.

Adverse drug reactions in general can be classified as intrinsic or idiosyncratic, and these categories apply well to the types of drug-induced hepatotoxicity that are observed in humans and animals. **Intrinsic adverse drug reactions** *are considered to be dose-related, predictable, reproducible in experimental animals, and the underlying mechanisms are typically known*. Intrinsic hepatotoxic drugs can cause liver injury in overdose situations in most normal recipients but are also capable of inducing similar liver damage at lower doses in individuals with genetic or acquired abnormalities in drug metabolism. The majority of intrinsic hepatotoxicants are converted to *reactive metabolites*, or co-generate lipoperoxidative free radicals. By comparison, **idiosyncratic adverse drug reactions** *are typically less dose-related and more unpredictable and occur in only a small proportion of exposed individuals.* The mechanism of idiosyncratic hepatotoxicity is often not known, but it reflects an unusual susceptibility of individual recipients to effects that are unrelated to the drug's therapeutic action or overdose toxicity. The basis for the idiosyncracy may be acquired, for example, a result of previous or concurrent exposure to other agents that induce drug-metabolizing pathways, or genetic, a result of enzyme polymorphisms. There is accumulating evidence of considerable genetic diversity (polymorphisms) in hepatic drug metabolism and immune responsiveness among individual humans and domestic animals, and these may explain many unusual responses to drugs. *Idiosyncratic adverse drug reactions can be further classified as metabolic or immunologic.* **Metabolic idiosyncracies** involve excessive generation of a regular toxic metabolite, or altered metabolism to unusual hepatotoxic metabolites. This variation could be qualitative, with the production of a toxic metabolite not normally produced, or quantitative, with overproduction of a normally minor hepatotoxic metabolite. **Immunologic idiosyncracies** involve immune-mediated hypersensitivity responses to the drug metabolites that covalently bind to liver proteins and initiate a hapten allergy, with further liver injury resulting from the ensuing specific immune response or upregulation of components of the innate immune system.

The morphological forms of hepatic injury produced by chemical agents are varied. Acute toxic injury to the liver can be cytotoxic (hepatocellular), cholestatic, or mixed. *Cytotoxic hepatocellular injury* results in hepatic degeneration, zonal necrosis, focal and nonzonal necrosis (apoptosis), or lipidosis, accompanied by the clinicopathological features of acute hepatic injury. In general, necrosis produced by intrinsic hepatotoxins is zonal, whereas that produced as an idiosyncratic reaction to a drug is usually nonzonal, and can be diffuse, focal, or massive. *Cholestatic injury* is a reflection of failure of bile excretion associated with canalicular injury or other alteration of bile secretion, and presents with the features of obstructive jaundice. The histologic manifestation consists of bile casts in canalicular spaces, with variable parenchymal injury. *Mixed toxic insults* display the features of both hepatocellular and obstructive injury. Chronic toxic injury to the liver may be manifest as chronic hepatitis, with fibrosis progressing to cirrhosis, vascular injury such as veno-occlusive disease, and neoplasia.

The analgesic **acetaminophen** represents a well-characterized example of an intrinsic hepatotoxin with species differences in metabolism and associated susceptibility to oxidative and hepatic injury. Dogs and cats can tolerate doses within therapeutic levels; however, higher doses may saturate the glucuronidation and sulfation detoxification pathways, resulting in increased formation of the reactive benzoquinone-imine metabolites via a third cytochrome P450-mediated pathway. These can be scavenged by glutathione conjugation; however, massive doses can cause lethal acute hepatic failure, associated with overproduction of reactive metabolites, and depletion of hepatic and erythrocyte glutathione. Clinical toxicity occurs at lower doses and is more severe in cats because they express fewer hepatic isoforms of glucuronyltransferase and are unable to accelerate excretion of excess metabolites through glucuronide conjugation. This is compounded by a propensity to hemoglobin oxidation, resulting in methemoglobinemia. Toxicity results when reduced glutathione levels drop below a threshold level, resulting in marked oxidative stress, and allowing reactive metabolites to bind covalently to cellular macromolecules.

Acute hepatic disease has been attributable to adverse reactions to administration of a wide variety of therapeutic drugs, particularly in companion animals. Submassive to massive hepatic necrosis and cholestatic hepatitis have been reported in dogs as an idiosyncratic reaction to **trimethoprim-sulfonamide** combination therapy. Severe lobular to massive hepatic necrosis may occur in cats associated with repeated oral administration of **diazepam** at recommended doses. Severe periacinar hepatic necrosis has been associated with use of the anthelmintic **mebendazole** in dogs. Hepatotoxicity has been reported in 4 dogs treated with **amiodarone**, a class III antiarrhythmic agent. This is one of the more commonly reported adverse effects of amiodarone in humans, related to the drug's effect on lipid metabolism. Diffuse and massive hepatocellular injury characterized by hepatocellular vacuolar change, lytic necrosis, apoptosis and bridging necrosis, with mild secondary inflammation and cholestasis, has been reported in dogs administered therapeutic dosages of **carprofen**, a nonsteroidal anti-inflammatory drug used in the treatment of degenerative joint disease and management of acute pain. Administration of the anabolic steroid, **stanozolol**, has also been associated with elevated alanine aminotransferase, coagulopathy, and development of hepatic lipidosis with cholestasis in cats. Interestingly, adult beef cattle testing positive for stanozolol in urine also had hepatic changes, including cholestasis, periportal fibrosis and inflammation, and focal necrosis, although the changes could not conclusively be attributed to anabolic steroid administration.

Other pharmacologic agents associated with acute hepatic injury include **thiacetarsemide**, **methoxyflurane**, **halothane**, **oil of pennyroyal**, and inadvertent subcutaneous injection of intranasal *Bordetella bronchiseptica* – canine parainfluenza vaccine in dogs, **methimazole**, **glipizide**, and the photodynamic therapy agent **aluminum phthalocyanine tetrasulphonate** in cats.

The anticonvulsant drugs **primidone**, **phenytoin**, and **phenobarbital** have been associated with the development of *chronic hepatic disease and cirrhosis in dogs*. These drugs, used either alone or in combination, can cause biochemical and clinical signs of hepatic dysfunction in up to 14% of dogs treated for more than 6 months, but only a small percentage of cases progress to cirrhosis and hepatic failure. The most consistent histologic finding in the majority of dogs treated with phenobarbital is *proliferation of the hepatocellular smooth endoplasmic reticulum due to induction of microsomal enzymes*, including various subfamilies of cytochrome P450. This results in hepatocellular swelling with fine diffuse granularity, a so-called "ground-glass" appearance to hepatocyte cytoplasm. This histologic change is an adaptive response, reflected clinically by increases in serum concentrations of alkaline phosphatase, alanine aminotransferase, and γ-glutamyltranspeptidase, but is not indicative of hepatocellular injury. Actual hepatotoxicity may represent an idiosyncratic reaction in a small percentage of treated dogs, although the possibility of dose-dependent intrinsic hepatotoxicity with long-term treatment has not been ruled out. It is also possible that the enzyme induction associated with anticonvulsant therapy may alter the ability of the liver to detoxify other nonspecified compounds that could be the effectors of liver damage. Certainly, enzyme induction may alter the pharmacokinetics of other co-administered drugs. *Chronic hepatitis associated with anticonvulsant therapy is characterized by bridging portal fibrosis, biliary hyperplasia, nodular regeneration, and mild inflammatory cell infiltrates.* A separate syndrome of *cholestatic hepatotoxic injury with jaundice* has also been described in dogs receiving high doses of phenytoin in combination with primidone or phenobarbital. This is characterized by intrahepatic cholestasis, with hepatocellular swelling, vacuolation, and small multifocal areas of hepatocellular necrosis, and has been suggested to represent a metabolic disturbance rather than direct cytotoxic hepatocellular injury. *Superficial necrolytic dermatitis* associated with typical hepatic pathology of parenchymal collapse, vacuolation, and nodular regeneration has also been reported as a separate syndrome in dogs with a history of chronic phenobarbital therapy.

Acute and chronic hepatic disease, characterized by periportal hepatitis, periportal fibrosis, and biliary hyperplasia have been reported in dogs treated with **oxibendazole-diethylcarbamazine** combination therapy for prevention of hookworm and heartworm. Chronic hepatic disease has also been reported following administration of **mibolerone**, **methotrexate** and **CCNU** (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) in dogs, **ketaconazole** in dogs and cats, and **megestrol acetate** and **griseofulvin** in cats.

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Acute hepatotoxicity: plant-derived and environmental toxins

Cyanobacteria (blue-green algae)

These primitive highly toxic microorganisms can flourish as a seasonal bloom on lakes and ponds that have accumulated phosphates and nitrates as runoff from fertilized soils. Outbreaks of poisoning are not common, but they occur in many countries and may be responsible for heavy mortality among mammals or birds that drink from affected bodies of water. Most well-documented cases have involved ***Microcystis aeruginosa***, which may poison stock after the bloom has been piled by wind against the shores of expanses of water. Other toxic species are included in the genera *Anabaena* and *Aphanizomenon*. Some deaths are too sudden to be due to liver damage and are probably the result of the "*fast-death factor*" that has been found in some blooms. The syndrome is one of collapse and prostration, with hyperesthesia that may be manifest as convulsions and death within a few minutes; there are no specific lesions in this form of the toxicosis.

The most well-understood cyanobacterial hepatotoxin is *microcystin-LR, a highly potent cyclic heptapeptide protein phosphatase inhibitor*. Ruminants are most commonly poisoned but poisoning has been reported in horses, sheep, dogs, and domestic poultry. The toxin is released when the microorganisms disintegrate, which may occur spontaneously in bodies of water or after the application of copper sulfate for algae control, or in the rumen or stomach after ingestion. The toxin is taken into hepatocytes by membrane carriers and causes cell damage by inhibiting cytoplasmic protein phosphatase 1 and 2A. This leads to disorganization of hepatocyte and endothelial cytoskeletal actin filaments, and disruption of their shape and integrity, leading to necrosis, apoptosis, and perisinusoidal hemorrhage. The distribution of the necrosis is usually periacinar to massive (see Fig. 2.33). The pattern may vary within the individual liver and from case to case. In subacute intoxications, the liver is severely fatty and necrosis is limited to individual hepatocytes or small groups, rather than being zonal. Phagolysosomes and bile pigments accumulate in the cytoplasm and there is slight biliary proliferation and fibrosis.

Amatoxins

Amatoxins are potent hepatotoxins found in several mushroom genera, including *Amanita, Galerina*, and *Lepiota*. The genus ***Amanita*** contains several species, including *A. phalloides, A. verna, A. virosa*, and *A. ocreata*, which are considered extremely toxic. These mushrooms are mycorrhizal with various species of deciduous and coniferous trees, and may be found in urban, suburban, and rural areas. The amatoxins are *bicyclic octapeptides*, ingestion of which is responsible for gastroenteritis, hypoglycemia, and fulminant liver failure in humans, dogs, and other animals. Amatoxins inhibit nuclear RNA polymerase II, thus interfering with transcription and inhibiting protein synthesis, resulting in cell death. Dogs dying after ingestion of *Amanita* spp. have massive hepatocellular necrosis with focal areas of hemorrhage. Surviving periportal hepatocytes had vesicular nuclei with loss or fragmentation of nucleoli, consistent with ultrastructural reports of chromatin condensation, dissolution of the nucleolus, and decline in nucleolar RNA content.

Cycadales

Members of this order have been responsible for chronic hepatotoxicity and neurotoxicity in cattle, but acute hepatotoxicity has been reported in sheep that have eaten the seeds or young leaves of species of Zamiaceae. The toxin responsible is *methylazoxymethanol, which is the aglycone of various nontoxic glycosides, including cycasin and macrozamin*, in these plants. The toxin is split from the glycoside in the gut, and its hepatotoxicity is the result of further metabolism in hepatic microsomes; the pattern of necrosis is thus periacinar. The metabolites of the aglycone are apparently *potent alkylating agents*, and the chronic liver lesions reflect this; there is megalocytosis (which is not as persistent as that of pyrrolizidine alkaloid poisoning), nuclear hyperchromasia, cholestasis, fatty change, and various degrees of diffuse fibrosis. Cytosegresome formation and cytoplasmic invaginations into nuclei are apparently not a prominent feature. There is fairly consistent nephrosis.

Cycads also produce a *neurotoxic amino acid, beta-N-methylamino-L-alanine* (BMAA), reportedly of cyanobacterial origin. BMAA is excitotoxic, activating neurotransmission mediated by glutamate receptors, which in excess can lead to cytotoxic influx of calcium ions. Chronic cycad poisoning of cattle causes a chronic nervous disorder characterized by a progressive proprioceptive deficit. This is due to axonopathy in upper spinocerebellar and lower corticospinal tracts. The axonopathy, morphologically subtle at first, may eventually progress to frank Wallerian degeneration.

Solanaceae

Toxic species of the genus ***Cestrum*** (jessamine) include *Cestrum diurnum*, a cause of enzootic calcinosis in cattle attributed to active vitamin D analogs; the other known toxic species all produce similar hepatic disease. Speciation within the genus is uncertain, partly due to hybridization; however, the species named as hepatotoxic are *C. parqui, C. laevigatum*, and *C. aurantiacum*.

Cestrum spp. cause acute hepatotoxicity in the field in South America, southern and central Africa, and Australia. Cattle are more frequently poisoned than other species, but sheep and goats are susceptible, and fowl may be if they eat the fruit. The young leaves and unripened berries are the most toxic parts of the plant. In acute hepatotoxicity, there is marked periacinar and midzonal coagulative necrosis and hemorrhage (see Figs 2.29 and 2.30). There are no records of chronic liver disease caused by this plant, and photosensitization is rarely seen. The toxin is water-soluble and has been identified as an *atractyloside* (see below).

Compositae/Asteraceae

Xanthium pungens (Noogoora burr) in Australia *and X. cavanillesii*, (the cocklebur) in Brazil, the USA, and South Africa, have been reported to be hepatotoxic while in the seedling stage because the toxins are concentrated in the cotyledons. The burrs are also toxic, and although usually too coarse to be grazed, they can be consumed if ground into feeds. Cattle, swine, and sheep are susceptible, and toxicosis typically occurs following a period of feed scarcity, after flooding or rain has allowed germination. The clinical signs and lesions are not specific, being those described for acute hepatotoxins in general. The toxic principle is a *diterpenoid glycoside, carboxyatractyloside. Wedelia glauca* also contains atractyloside and causes acute hepatotoxicity in cattle and sheep in Uruguay and Argentina. The condition has been reproduced experimentally in sheep and cattle and rats.

Atractyloside was first identified as the hepatotoxic principle in the Mediterranean thistle, *Atractylis gummifera*, a woody plant that grows in southern Europe and North Africa. These toxins inhibit exchange of ATP from the mitochondria with adenosine diphosphate (ADP) in the cytosol, a process essential for oxidative phosphorylation. Atractylosides inhibit the ADP/ATP carriers (AACs), including the form expressed in the liver. Carboxyatractyloside blocks exchange by binding to a cationic functional domain of bovine AAC1. Lack of ATP and mitochondrial pore leakage lead to apoptosis and necrosis, with ion pump failure, lipid peroxidation, and glutathione depletion. The extent of necrosis depends on dosage, but in lethal poisoning with hepatic failure there is typically midzonal vacuolation and periacinar necrosis, but it can be panlobular.

Other plants produce similar lesions but the toxins have not been identified. *Helichrysum blandowskianum* is hepatotoxic to cattle and sheep and has caused sudden deaths with periacinar necrosis in southern Australia. The condition has been reproduced experimentally. High mortality with acute periacinar liver necrosis has been reported in cattle grazing sprouting plants of *Vernonia rubricaulis* in Brazil. Similar lesions were reproduced with 3 g/kg of sprouting plants. Various species of Asteraceae in South Africa, including *Asaemia axillaris, Athanasia trifurcata, Lasiospermum bipinnatum, Hertia pallens*, and *Pteronia pallens*, have been associated with field outbreaks of acute hepatotoxicity in grazing sheep or cattle. Similar liver lesions have been reproduced experimentally. Experimental intoxications by all of these species have in some animals produced midzonal as well as periacinar necrosis. *Asaemia axillaris, Athanasia trifurcata*, and *Lasiospermum bipinnatum* are also associated with more chronic liver toxicity with photosensitivity.

Ulmaceae

Trema tomentosa (*T. aspera*), the poison peach, has caused severe losses in cattle in Australia. Similar disease has been reported in goats ingesting *Trema micrantha* in Brazil, and hepatotoxicity has been reproduced in rabbits. The syndrome is acute, there is no photosensitization, and mildly intoxicated animals may recover completely. The toxic principle is a glycoside, designated *trematoxin*. The pattern of necrosis is consistently periacinar and is identical in appearance to that of *Cestrum* and *Xanthium* poisoning.

The gross and microscopic pathology of experimental poisoning by *Trema, Xanthium pungens*, and *Cestrum parqui* has been shown to be identical in all morphologic respects in the same group of sheep.

Myoporaceae

Hepatotoxic species so far incriminated are *Myoporum deserti, M. acuminatum, M. insulare*, and *M. tetrandum* of Australia, and *M. laetum* in New Zealand, southern Brazil, and Uruguay. The toxic oils are contained in the leaves and branchlets, but within the species there is variation in the chemical characters and toxicity of the oils; not all strains of toxic species are toxic. There is some delay between ingestion and absorption of the *furanosesquiterpenoid oils*, the best known of which is *ngaione*, which are responsible for intoxication, so 24–48 hours may elapse before signs of toxicity appear. Some animals live long enough to become photosensitized; others may die much more rapidly, with pulmonary edema. The edema appears to be a direct effect of the toxin after metabolism by alveolar lining cells.

Livers from intoxicated sheep may show a striking, broad pattern of variable congestion and even infarction, which is superimposed on the more regular acinar pattern of periportal necrosis. Sections of these livers reveal acute fibrinoid necrosis of portal vessels, which suggests that the coarser lesions may have a vascular basis.

Halogenated hydrocarbons

Various halogenated hydrocarbons, such as *carbon tetrachloride* (used for many years as a fasciolicide), *bromobenzene, hexachloroethane, tetrachloroethylene*, and *chloroform*, are activated by cytochromes P450 to hepatotoxic or nephrotoxic metabolites. They have similar hepatotoxic properties but these agents are little used now, so few animals encounter them in toxic doses. However, some of these chemicals have been used experimentally to investigate the mechanisms of hepatotoxicity so they warrant some consideration here. Carbon tetrachloride is metabolized to trihalomethane, a necrogenic free radical metabolite, and reactive oxygen radicals are concurrently generated. Periacinar hepatic necrosis with midzonal hydropic change due to membrane peroxidation occurs within 30 hours of experimental exposures but lethal toxicity occurs later, when steatosis and the early stages of tissue repair responses are manifest.

Phosphorus

White phosphorus has historically been used for vermin control, and may be present in some incendiary devices such as fireworks. As a rodenticide, it is mixed with fat in order to promote absorption, and much of the dose is transported to the liver shortly after ingestion. A small amount may be lost by vomition, for elemental phosphorus is directly irritant to the gastrointestinal tract. The mechanism of phosphorus hepatotoxicity is uncertain; it is apparent that metabolism to a toxic intermediate is not necessary, but there is some dispute on the involvement of lipoperoxidation in the hepatocellular injury. There is evidence that protein synthesis is impaired early and that this is responsible for the lipid accumulation that is so prominent a feature. A few hours after ingestion of phosphorus there is severe colic and vomition. If the animal survives this acute phase there may be apparent recovery for a few days, followed by jaundice and other signs of liver failure, and death by about the fifth day. At necropsy there is severe icterus and fatty liver, the latter sometimes being predominantly periportal in distribution. Hepatocellular necrosis is not often a prominent feature histologically, notwithstanding the evidence of liver failure. Fatty change is also seen in the myocardium and distal nephrons.

Iron

Iron–dextran complexes have been widely used in the prevention and treatment of anemia in suckling swine. Very occasionally, *severe losses may occur in animals with marginal vitamin E-selenium deficiency*; in these cases there is, apparently, iron-catalyzed lipoperoxidation in hepatocytes and muscle. The result of this is sudden massive hepatic necrosis similar in many respects to that of hepatosis dietetica. Large amounts of potassium escape into the circulation from the liver and muscle, and sudden death may result from the cardiotoxicity of this ion. At necropsy, there is staining of subcutaneous tissues and lymph nodes near the site of injection, and there are lesions in the liver or skeletal muscles. The liver is of normal size and of normal color or pale, depending on whether or not the animal is anemic. The presence of underlying necrosis may be indicated only by the numerous small or large hemorrhages present on the capsular and cut surface (Fig. 2.71). Insoluble iron compounds with the staining reactions of hemosiderin are found in mesenchymal cells in many tissues, the largest amounts being in macrophages of the local lymph nodes and in Kupffer cells.

Death in piglets from hepatic necrosis occurs about 10 hours after administration but saccharated iron may, in the same circumstances as above, produce acute widespread muscle necrosis at about 24 hours rather than hepatic necrosis. The myocardium is not affected.

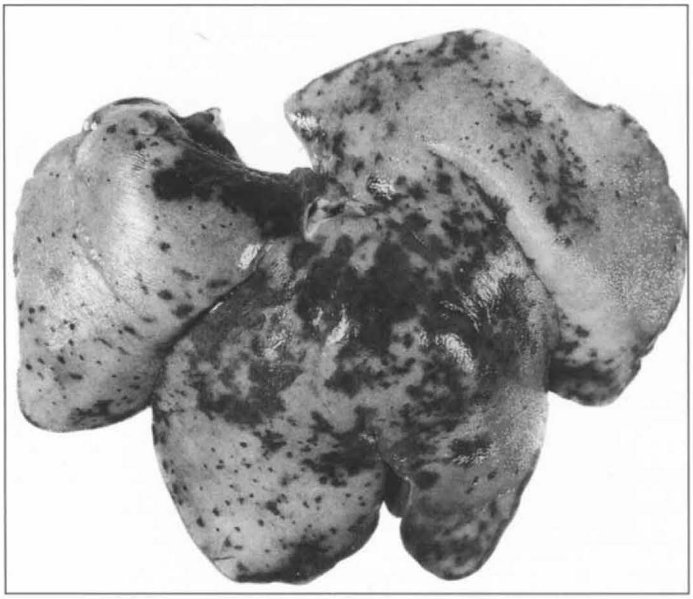

Figure 2.71 Early massive necrosis with random hemorrhage and pallor due to **iron dextran poisoning** in a pig.

Acute hepatotoxicity is reported in young *foals* due to administration of a proprietary paste of iron and yeast products, given as a dietary supplement within a few hours of birth. Not all foals so treated became sick, but those that were developed severe acute periacinar hepatocellular necrosis, resembling the disease in piglets.

Acute iron intoxication has also been reported in young *cattle* administered injectable hematinics containing elemental iron, and rarely in adult *horses* administered oral vitamin supplements containing ferrous fumarate or ferrous sulfate. Oversupplementation, low vitamin E or selenium concentrations, or concurrent disease may have contributed to these cases. Consumption of *elemental iron* produces a periportal or panlobular pattern of necrosis.

Sawfly larvae

This acute hepatotoxicity of cattle and, to a lesser extent, sheep, is of particular interest in that the toxin is present in an insect, the larva of the sawfly *Lophyrotoma interruptus* or *L. zonalis* (Pergidae). This larva is parasitic on the leaves of the tree *Eucalyptus melanophloia*, and heavy infestations may occur in parts of northeastern Australia. *L. zonalis* has been introduced to Florida to control the spread of *Melaleuca quinquenervi*. A similar intoxication by the blue-black birch sawfly, *Arge pullata*, from birch trees has been recognized in sheep and goats in Denmark. In Uruguay, a similar problem has been reported in cattle and sheep that consume the South American sawfly *Perreyia flavipes*. The production of such similar intoxications from unrelated substrates suggests that this class of insect manufactures the hepatotoxin de novo. On completion of feeding, masses of larvae sometimes accumulate at the base of the tree where they may die and decompose. Cattle in particular find these masses attractive, perhaps as a result of nutritional stress. The hepatotoxins produced by *Lophyrotoma* include *lophyrotomin*, a free acid form that causes periportal hemorrhagic necrosis, and *pergidin*, which causes periacinar coagulative necrosis. Pergidin, the major toxic constituent of the South American sawfly, is a heptapeptide containing several D amino acids.

Chronic hepatotoxicity: plant-derived and environmental toxins

Aflatoxin

The **aflatoxins** are a group of *bisfuranocoumarin compounds* produced as metabolites mainly by *Aspergillus flavus*, *A. parasiticus*, and *Penicillium puberulum*. The metabolites are designated by spectral qualities, and the major ones are B_1, B_2, G_1, and G_2. Many others may be produced in minor amounts in fungal colonies or as metabolic products of the major toxins in animals. *The most significant and best studied of the aflatoxins is B_1* because of its relative abundance and its potency as a hepatotoxin.

Strains of ***Aspergillus*** differ in the varieties and amounts of individual toxins produced, indicating that the biosynthesis of the toxins is genetically determined. The production of toxins also varies under different conditions of fungal growth, being influenced by the quality of the substrate, temperature, relative humidity, and moisture content of the substrate, and microbial competition. Thus the toxicity of moldy feedstuffs is impossible to assess without measurement of toxin production. Aflatoxins can be produced on growing crops in the field, but much greater levels are likely to accumulate in *stored or unharvested mature grains*, particularly if they are damaged by moisture. Various feeds other than grains, ranging from legume stubbles to bread, may be the substrate in outbreaks of aflatoxicosis.

Aflatoxins are metabolized by the hepatic mixed-function oxidase system to various toxic and nontoxic metabolites, the proportions of which vary with the species and age of animal involved. The most potent of these is the 8,9-epoxide metabolite of aflatoxin B_1; this binds to adenine in nucleic acids in sensitive species that lack adequate glutathione *S*-transferase-mediated resistance. Sheep, adult cattle, and rats are quite resistant to the toxin, whereas dogs, pigs, calves, mice, and ducklings are sensitive and may be fatally intoxicated by a dose rate of less than 1.0 mg/kg body weight.

Prolonged exposure to low concentrations of the toxin may merely produce reduced growth rates and moderate enlargement of the liver without any significant hepatic signs. The enlargement may be partly due to hypertrophy of hepatocellular smooth endoplasmic reticulum and some degree of fatty change. As the level of aflatoxin in the ration increases (in young pigs, e.g., to 1.0 mg/kg ration), the liver may show all or none of the following changes: pallor, enlargement, bile staining, increased firmness due to fibrogenesis, and fine nodular regenerative hyperplasia. There may also be edema of the gallbladder and bile-tinged ascites in more severe cases. Even under experimental conditions, some individuals may show minimal liver lesions, while their fellows, under the same levels of exposure, die of liver failure. Histologically, affected livers show obvious increase in size of some hepatocytes and their nuclei (megalocytosis) with focal necrosis or apoptosis. Bile ductules proliferate early, and reticulin and collagen deposition occurs throughout the acinus according to no distinct pattern. Fatty change in affected livers is variable in extent and occurrence, and bile pigments accumulate in canaliculi and hepatocytes in more severely affected livers. Minor degrees of megalocytosis may be seen in proximal tubular epithelium in the kidney. *The changes produced resemble those of pyrrolizidine alkaloid toxicosis.* This can be attributed to the fact that aflatoxin and pyrrolizidine alkaloids *inhibit hepatocellular regeneration* such that nodules and ductules regenerate as the liver becomes atrophic. These are also *genotoxic* and *carcinogenic* so they might be involved in the occurrence of liver cancers in animals, as they are in humans.

At higher dose rates of aflatoxin, most periacinar hepatocytes disappear and are replaced by a mixture of inflammatory cells, fibroblasts, and primitive vascular channels. The liver may be much smaller than normal, particularly in young animals, due presumably to mitotic inhibition, and focal hepatocellular necrosis is more obvious or may be supplanted by zonal (periacinar) necrosis. Fatty change in these livers may be severe and uniformly distributed.

Acute, fulminating liver necrosis is sometimes seen in dogs that eat moldy bread, dog food, or garbage, which may contain very high concentrations of the toxin. Younger animals are much more susceptible and may die within a few hours; the gross postmortem picture is dominated by widespread hemorrhage and massive hepatic necrosis.

Fumonisin

Fumonisin B_1, a mycotoxin elaborated by certain strains of ***Fusarium moniliforme*** in infected corn, induces hepatocellular carcinomas in laboratory rats, and leukoencephalomalacia in horses. Hepatic toxicity has also been reported in horses, sheep, and baboons. Severe pulmonary edema and hydrothorax have been reported in swine fed contaminated corn screenings. More recent studies have shown fumonisin B_1 also to be a *primary hepatotoxin in pigs*. Experimental feeding trials with pigs have shown dose-related differences in pathology. Pigs intubated with a minimum of 16 mg fumonisin B_1/kg body weight per day developed interlobular edema, variable hydrothorax, and pulmonary edema, while pigs intubated with 8 mg/kg per day for 7–8 days, or fed diets containing 200 mg fumonisin B_1/kg of feed for 21 days, developed marked icterus. Histologic changes included hepatocellular necrosis without a zonal distribution, depletion of centrilobular hepatocytes, lobular disarray, and megalocytosis characterized by large numbers of swollen hepatocytes with abundant granular eosinophilic cytoplasm and occasional large nuclei, randomly interspersed with small angular hepatocytes and scattered necrotic cells. These pigs showed no evidence of pathologic changes in the lungs, whereas pigs given higher doses showed *hepatic necrosis in addition to pulmonary edema.* The liver changes appear to be reversible upon cessation of exposure. Gilts fed low levels of fumonisin B_1 for 90 days developed hepatic nodular hyperplasia, along with hyper- and parakeratosis and hyperplasia of the distal esophageal mucosa. Fumonisins are inhibitors of sphingosine and ceramide synthetase, and the subsequent inhibition of sphingolipid biosynthesis may be involved in the toxic hepatic effects.

Phomopsin

There are two distinct manifestations of toxicity associated with *Lupinus* spp.; discussed here is the condition formerly known as *"lupinosis," which is a true mycotoxic liver disease.* The teratogenic and neurotoxic effects of some of the isoquinoline alkaloids from the plants themselves are discussed in Vol. 1, Bones and joints and Vol. 1, Nervous system.

The fungus ***Phomopsis leptostromiformis*** is parasitic on green *Lupinus* plants, but it becomes saprophytic after the host dies. Phomopsins (A or B) are produced if the lupin stubble is moistened, and such stubbles may remain toxic for months. Severe acute liver damage has been described in sheep on very toxic stubbles in Western Australia, but in many of these cases it has been difficult to separate the toxicity of the lupins from that of copper, which in this area is often concentrated in ovine livers (see section on Copper, below).

The usual syndrome of phomopsin poisoning is subacute to chronic. Inappetance occurs soon after experimental administration of the toxin is begun; liver damage is clinically inapparent for several days, although there is early hydropic change and accelerated cell death among hepatocytes. Increased mitotic activity is soon apparent, although by this time the liver has become smaller. The mitotic activity is in fact largely ineffectual, as close examination reveals that many mitotic figures are abnormal (Fig. 2.72A). There is either clumping or dispersal of chromatin, and *there appears to be mitotic arrest at late metaphase.* Remaining hepatocytes swell, their cytoplasm becomes granular and their nuclei vesicular. With progression, hepatic fibrosis occurs, predominantly diffuse in distribution, and by this stage there is usually clinical icterus and anorexia. There will be variably severe fatty change, dependent to a large degree on the fat reserves of the animal (Fig. 2.72B). There is also accumulation of complex pigment in macrophages in portal stroma and about hepatic venules;

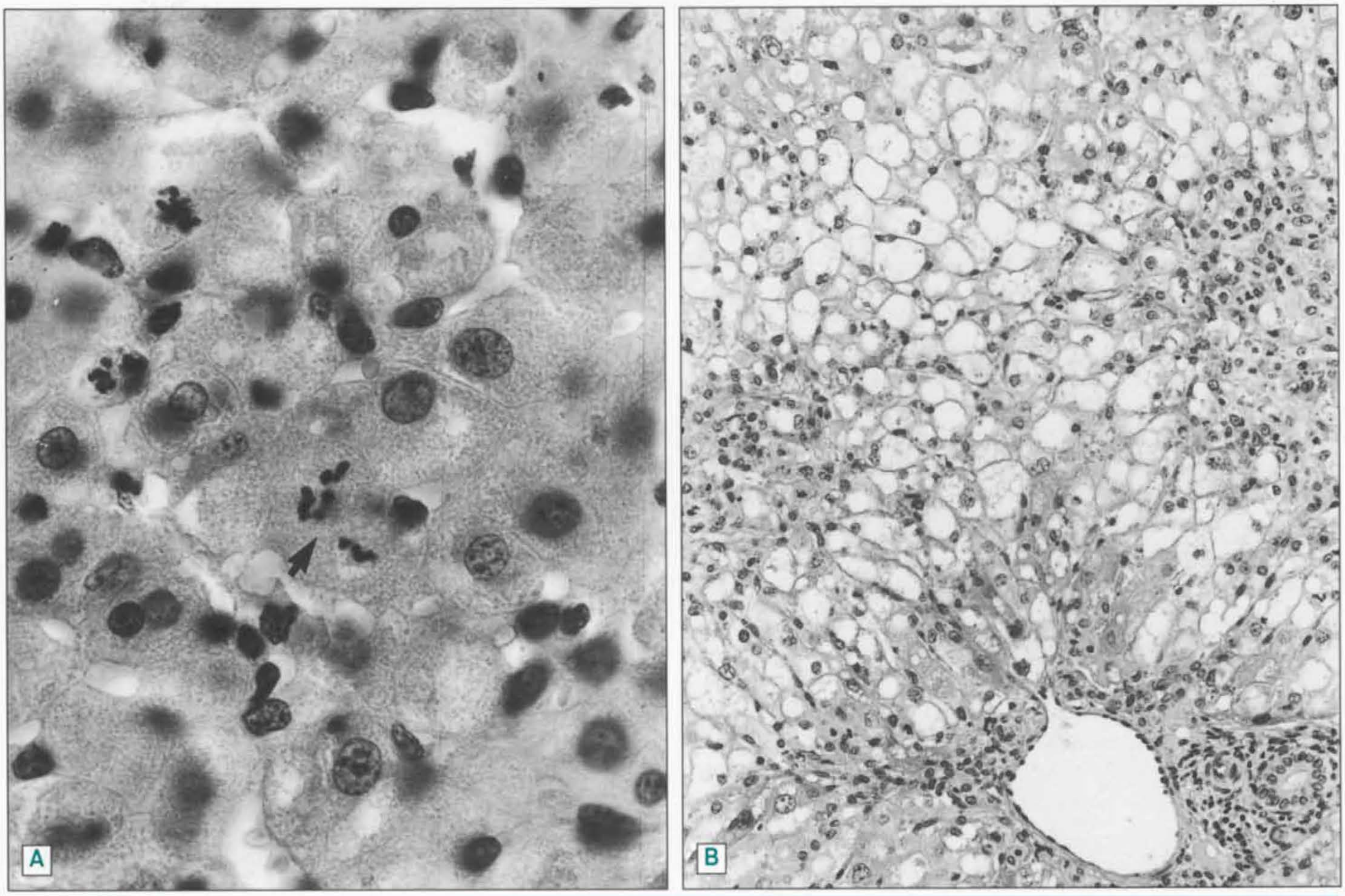

Figure 2.72 Phomopsin poisoning (lupinosis) in sheep. **A.** Numerous, and **abnormal, mitotic figures** (arrow) in subacute phomopsin poisoning. **B.** Zonal fatty change, periacinar collapse and periportal megalocytosis. (Courtesy of JG Allen.)

this granular material contains lipofuscin, ferric iron, and copper at least. Bile duct proliferation is also a prominent feature of the chronic disease.

The liver continues to shrink, presumably as a result of continued mitotic inhibition and progressive fibrosis. The organ is small, tough, and has a finely granular surface and texture. It is pale gray-orange but usually retains its shape. In naturally occurring cases, however, discontinuous intake of the toxin may produce a liver grossly distorted by asymmetric nodular regeneration and fibrosis. The atrophic changes are most severe in the left lobe.

Photosensitization occurs in phomopsin-poisoned sheep; it may be severe if the animals have access to green feed while under the influence of the toxin. Lupinosis in sheep has also been experimentally observed to induce mild skeletal myopathy, resembling nutritional myopathy.

Phomopsin poisoning in cattle causes most losses when the animals are lactating or heavily pregnant; in these animals the syndrome is essentially one of ketosis, to which such cows would be predisposed by the anorexia that is an obvious clinical feature of this intoxication. Pregnant sheep are less likely to have access to toxic lupin roughage during late gestation, otherwise ketosis triggered by phomopsin would be expected just as often as in cattle.

Chronic hepatic fibrosis with fine, nodular regeneration may occur infrequently in cattle due to chronic phomopsin poisoning. Similar liver changes may also be seen in horses, in which there may also be hemolytic anemia of unknown pathogenesis.

Sporidesmin

The mycotoxin *sporidesmin* is produced by the fungus ***Pithomyces chartarum***; the most important substrate is dead ryegrass (*Lolium perenne*) that has been moistened in warm weather. Intoxication causes *chronic liver damage and severe hepatogenous photosensitivity* ("*facial eczema*") and is a serious cause of loss of sheep and, to a lesser extent, cattle on the North Island of New Zealand. Sporadic and subclinical intoxication occurs irregularly on the South Island and in southern Australia and South Africa. Sporidesmin intake in conjunction with ingestion of *Tribulus terrestris* causes another hepatogenous photosensitivity (*geeldikkop*); this is similar to but distinct from facial eczema, and is described below.

Sporidesmin is concentrated in the fungal spores, and the toxigenicity of pasture is related to the density of the spores in it. Sporidesmin is not specifically hepatotoxic. Administration of the toxin does produce rapid disorganization of hepatic cell organelles and triglyceride accumulation, but these are mild and nonspecific changes. If administered in suitable dosage, the toxin causes permeability alterations in many tissues and will, for example, produce corneal edema on local application. *The hepatobiliary lesions are due* *to the excretion of unconjugated sporidesmin in bile and its concentration there.* Sporidesmin is also excreted in urine, and if the dose is high enough, edema and mucosal hemorrhage occur in the bladder. The hepatic lesion is due to irritation of mesenchymal tissues in the portal triads and surrounding the bile ducts. If the concentration of sporidesmin is high enough, the biliary epithelium undergoes necrosis, and diffusion of toxin produces irritative lesions and necrosis in the adjacent blood vessels.

The liver in acute forms of the disease is enlarged, with rounded edges, and is finely mottled and discolored yellow-green by retained bile pigments, although the discoloration may be blotchy. There is mild edema and congestion of the wall of the gallbladder, which may be distended with bile of normal quality or with mucin (white bile). The extrahepatic ducts are thickened and prominent, and there is edema of the adventitia. The ductal changes may extend to the papilla of Vater and can be traced by the naked eye deeply into the parenchyma. In more chronic cases, alterations of size and pigmentation of the liver are variable. Pale areas of capsular thickening, which may be elevated or depressed, are visible. On cut surfaces they extend deeply as wedge-shaped areas in which biliary fibrosis has produced an exaggerated acinar pattern, and the parenchyma is pale and atrophic; these areas are related to occluded bile ducts and are sometimes referred to as *biliary infarcts.*

The liver is firm and cuts with increased resistance. The intrahepatic ducts are conspicuous. There is irregular stenosis of their lumens, some are occluded by cellular debris and inspissated bile or mucin, and in some, cicatrization of the new fibrous tissue causes complete atresia. Occlusion of the ducts causes the parenchyma served by them to undergo atrophy, necrosis, and fibrosis. The livers of animals that have survived an attack of cholangiohepatitis of this genesis are distorted in shape and size by large nodules of regeneration and persistent areas of atrophy and fibrosis. The atrophy and fibrosis may affect either lobe, but usually the left most severely (Fig. 2.73).

Histologically, the changes are those of an *acute cholangitis or cholangiohepatitis* to which there is a minimum of leukocytic reaction. There is extensive necrosis of the lining of the larger intrahepatic ducts and the extrahepatic ducts, the epithelium being cast off as debris mixed with a few leukocytes. There is edema of the adventitia of the ducts, with active fibroplasia and scarring. Inflammatory cells are present, but not in large numbers, and they are chiefly lymphocytes and histiocytes. Injury to the smaller radicles of the bile ducts is less severe, but fibrosis and collagenization are active. The portal tracts are enlarged by fibrous tissue and by the active generation of new bile ducts that follows.

In more severe intoxications, there may be *coagulative necrosis of blood vessel walls in the portal triads*; when this is incomplete, the most damaged segment of the vessel tends to be that adjacent to the nearest injured bile duct. Both arteries and veins may be affected, and it is possible that the so-called bile infarcts are related to vascular insufficiency as well as to impaired bile drainage. Changes in the hepatic parenchyma are minimal and secondary to those in the portal triads. In acute cases, there may be extensive pigmentation of hepatocytes and Kupffer cells by bile pigments, but this is irregular in distribution in the liver. Inspissated bile can be found in the bile ducts and canaliculi. There is some necrosis of hepatocytes adjacent to inflamed portal areas, and other areas of necrosis, focal in type and distribution and probably the result of biliary obstruction, may be numerous.

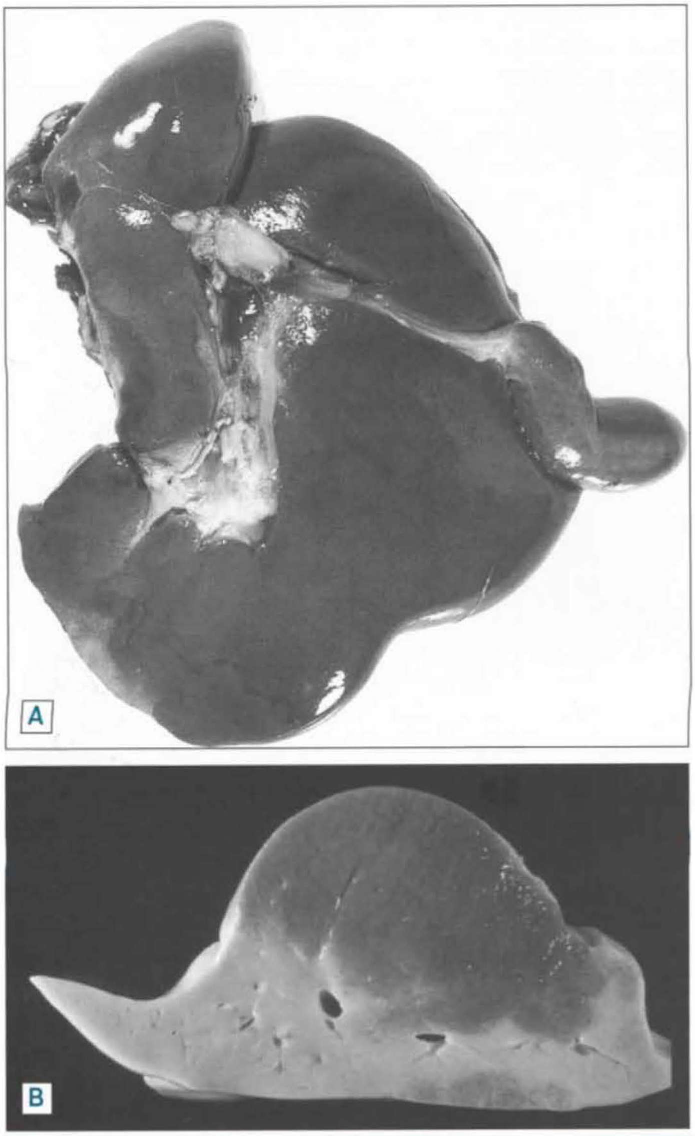

Figure 2.73 Chronic sporidesmin poisoning ("facial eczema") in sheep. **A.** Atrophy of left lobe with hypertrophy of right and caudate lobes. (Courtesy of WJ Hartley.) **B.** Cross section showing marked atrophy and fibrosis of left lobe, and hypertrophy of the right lobe. (Courtesy of KG Thompson.)

Other morbid alterations include great enlargement of the adrenals produced by cortical hypertrophy; sclerotic intimal plaques in the arteries, veins, and lymphatics in the hilus of the liver; and a tendency for the newly formed bile ductules to recanalize occluded ducts.

Pyrrolizidine alkaloids

Pyrrolizidine alkaloids have been identified from over 6000 plants of three families – the Asteraceae (Compositae), Leguminosae (Fabaceae), and Boraginaceae. The main genera responsible for plant toxicoses in domestic mammals are ***Senecio, Crotalaria, Heliotropium, Cynoglossum, Amsinckia, Echium***, and ***Trichodesma***, and are widely distributed around the world. More than 600 pyrrolizidine alkaloids have been identified chemically, and most of the toxic plant species contain more than one of the alkaloids. So far, only a small proportion of the known alkaloids have well-characterized toxicity; these are all esters of one of three amino alcohol bases (necines) or acids (necic acids). The *retronecine group* includes *monocrotaline, retrorsine, retronecine, ridelliine, senecionine, and jacobine*, and the *heliotridine group* includes *lasiocarpine and heliotrine.* These toxins must be metabolized to more reactive forms for toxicity. Cytochromes P450 mediate the N-oxidation of the esters or necine bases. They can also mediate the two-step hydroxylation of the necine bases at the C3 and C8 positions; this is followed by spontaneous dehydration to the *highly reactive dehydropyrrolizidine (DHP) derivatives (pyrrolic esters).* The toxic DHP metabolites are electrophilic and can bind covalently to proteins and nucleic acids at guanine and adenine residues. The DNA-binding activity is responsible for their genotoxicity; the DHP-derived DNA adducts are a common pathway for carcinogenic activity of pyrrolizidine alkaloids. The DHP metabolites can be detoxified by glutathione conjugation by glutathione *S*-transferases. Ester linkages at the C7 and C9 positions can be hydrolyzed by carboxylesterases to generate the necine or acid moieties; this is generally considered to be a detoxification pathway.

The toxicity of a pyrrolizidine alkaloid containing plant depends on many factors. For an individual pyrrolizidine alkaloid, toxicity depends on the amount of alkaloid that can be converted to reactive metabolites, the rate of cytochrome P450-mediated generation of the DHP, and the efficiency of detoxification by glutathione conjugation. Toxicity also depends on the species exposed, age, and sex of the animal intoxicated, the metabolic activity and the mitotic stage of its target cells. Grazing animals are more likely to consume plants that contain pyrrolizidine alkaloids, but since the toxins are produced by the plants to deter herbivores, it is unusual for an animal to consume large amounts. Ruminants, especially sheep and goats, are much less susceptible than pigs, in part because the toxins can be degraded in the rumen. However, sheep can graze out stands of *Senecio* that would be lethal to cattle. The seeds are also toxic and the small seeds of *Amsinckia* may, depending on harvesting technique, heavily contaminate other harvested grains used in prepared pig and poultry feeds.

Most pyrrolizidine alkaloids are hepatotoxic because they are metabolically activated to DHPs in the liver. However, monocrotaline is also bioactivated in the lung and is therefore pneumotoxic. There are three common expressions of pyrrolizidine poisoning. First, *acute periacinar zonal necrosis* occurs in animals ingesting large amounts of these alkaloids. Because the plants responsible are unpalatable, naturally occurring outbreaks of acute poisoning are generally restricted to circumstances where animals face starvation, such as grazing pastures affected by prolonged drought. Ingested dosages in grazing circumstances are usually too low for acute effects; however, acute hemorrhagic periacinar necrosis has been described after experimental exposures to high doses of pyrrolizidine alkaloids. The periacinar pattern of zone 3 necrosis produced is similar to that caused by many other hepatotoxins that are bioactivated by hepatic cytochromes P450. Second, phasic (usually seasonal) repetitive exposure to these alkaloids leads to *hepatic atrophy with formation of regenerative nodules. This is the most common expression of field exposure to pyrrolizidine alkaloids and affected livers show a characteristic pattern of hepatocellular polyploidy known as* **megalocytosis** (Figs 2.9 and 2.74). Third, prolonged exposure exclusively to *Heliotropium* induces *firm, fibrotic atrophic livers without nodular regeneration.* If seasonal exposure to these toxic alkaloids declines, then a remarkable degree of recovery is possible.

Pyrrolizidine alkaloids inhibit DNA synthesis and mitosis in hepatocytes, but some are able to replicate their DNA without undergoing mitosis, resulting in greatly enlarged hepatocytes with large convoluted polyploid nuclei. Some enlarged nuclei have cytoplasmic invaginations that can become entrapped as intranuclear inclusions. However, many hepatocytes in an affected liver do not become megalocytic. Those that are completely inhibited do not replicate DNA at all, whereas those that are more resistant can replicate more normally and give rise to nodular populations of smaller, more normal hepatocytes. Inhibited hepatocytes and megalocytes are long-lived but eventually many undergo apoptosis.

In *chronic pyrrolizidine alkaloid poisoning*, the liver can become atrophic as hepatocytes are lost faster than they can be replaced. The atrophy can be compensated to various degrees by megalocytosis and regeneration of small, less inhibited hepatocytes that proliferate in a nodular pattern. Concurrently, there is *usually proliferation of bile duct epithelial cells in the portal triads.* This is largely explained by the propensity of cholangiolar epithelia to proliferate in response to the regenerative stimuli that prevail when liver mass is inadequate. *There may also be some periportal fibroplasia that varies with species and exposure; typically it is minimal in sheep, moderate in horses, and may be marked in cattle* (Fig. 2.75). In **cattle**, the fibrous tissue can infiltrate along the sinusoids to dissect lobules, separate individual cells, and link up with the walls of efferent veins. In acute poisoning, in which there is

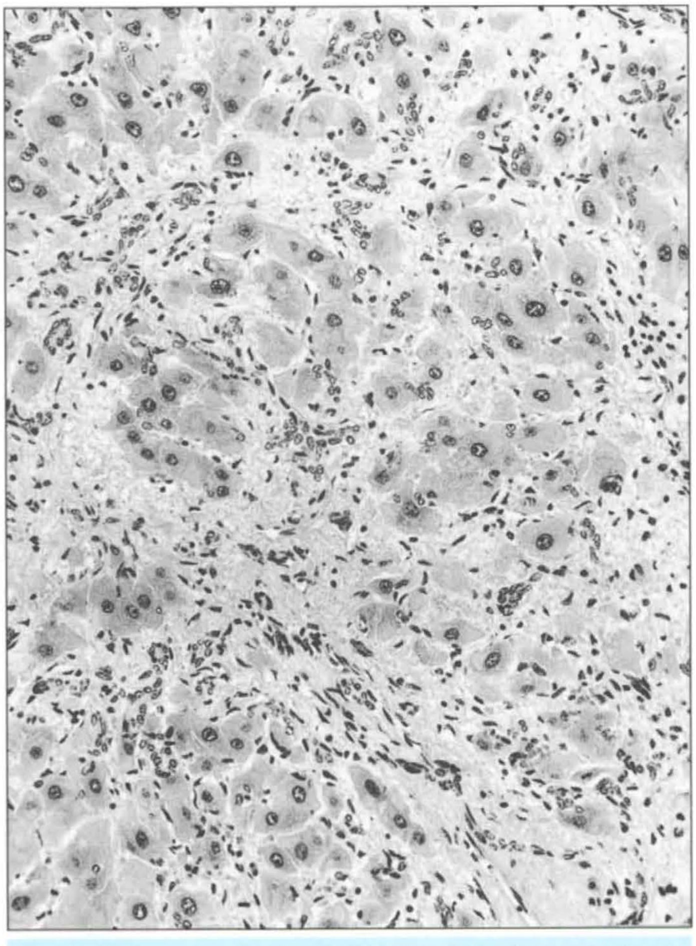

Figure 2.74 Megalocytosis and diffuse fibrosis in ***Crotalaria* poisoning** in an ox. Fibrous septa have subdivided the acini.

periacinar necrosis, fibrosis can also develop in a "veno-occlusive" pattern around and sometimes obliterating the hepatic venules. This pattern may occur in some field cases in cattle, but is more commonly observed in humans exposed to pyrrolizidine alkaloids in herbal preparations or "bush teas" and it can be produced experimentally after sublethal acute experimental hepatotoxicity. In fatal cases in cattle, the livers are very tough and nodular (Fig. 2.76), and the nodules have variably efficient biliary drainage and cytochrome expression, so there may be a very striking color pattern, ranging from fatty yellow through green and brown. These fibrotic livers can result in portal hypertension with ascites, severe mesenteric edema, and diarrhea. Excretory insufficiency with moderate jaundice and photosensitization can also occur.

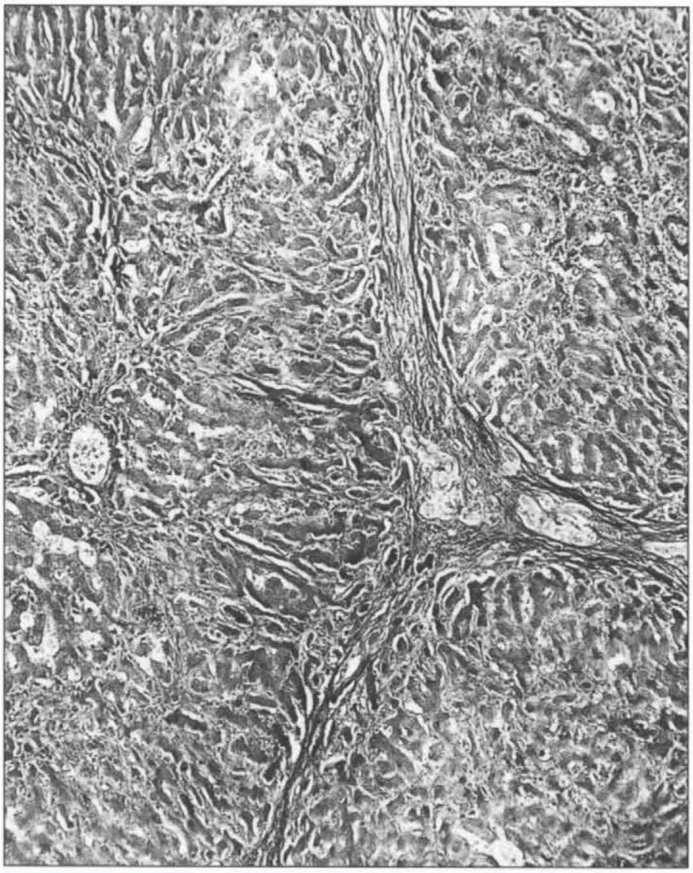

Figure 2.75 Diffuse fibrosis of liver in chronic ***Senecio* poisoning** in an ox.

The disease in **sheep** is always protracted as a consequence of the relative resistance of this species; indeed, clinical signs may not be seen until after a second season of exposure. The plants most often implicated are *Heliotropium europaeum* and *Echium plantagineum*. The shape of these failed livers is normal, but they are small, gray-yellow, fairly smooth, and toughened by condensation of normal stroma rather than by fibroplasia. If the liver copper content was high before intoxication, toxicity can culminate in copper release and an episode of intravascular hemolysis. In this case, the carcass will be intensely jaundiced, and the kidneys are stained with methemoglobin and bilirubin. The relationship of chronic copper poisoning to pyrrolizidine alkaloid poisoning is discussed below.

Horses are susceptible to both acute and chronic toxicosis, and liver failure produced in horses by these alkaloids is similar to that seen in cattle. Horses are more likely to manifest signs of hepatic encephalopathy with head-pressing and compulsive walking; in some places these nervous signs give rise to colloquial names such as "walkabout" and "walking disease." Administration of a high dose of *Cynoglossum officinale* resulted in severe liver disease within 7 days after dosing, with elevated serum enzymes, altered bile acid metabolism, and extensive hepatocellular necrosis with minimal periportal fibrosis and biliary hyperplasia, and little or no megalocytosis. Edema and infarction of the cecum and colon were also present at necropsy. Administration of a low dose to horses for 14 days resulted

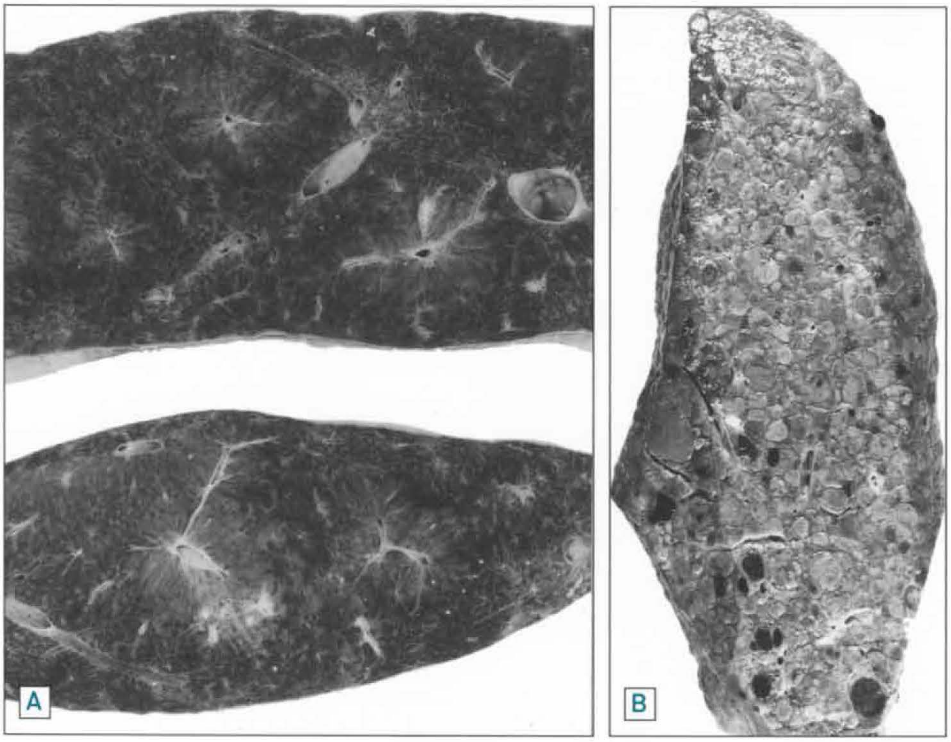

Figure 2.76 Pyrrolizidine alkaloid toxicity. A. Diffuse fibrosis, mimicking "nutmeg" liver of chronic congestion, in ***Senecio* poisoning** in a calf. **B.** Fibrosis and nodular regeneration, with some nodules stained by bile, due to ***Heliotropium* poisoning**, in a bovine liver.

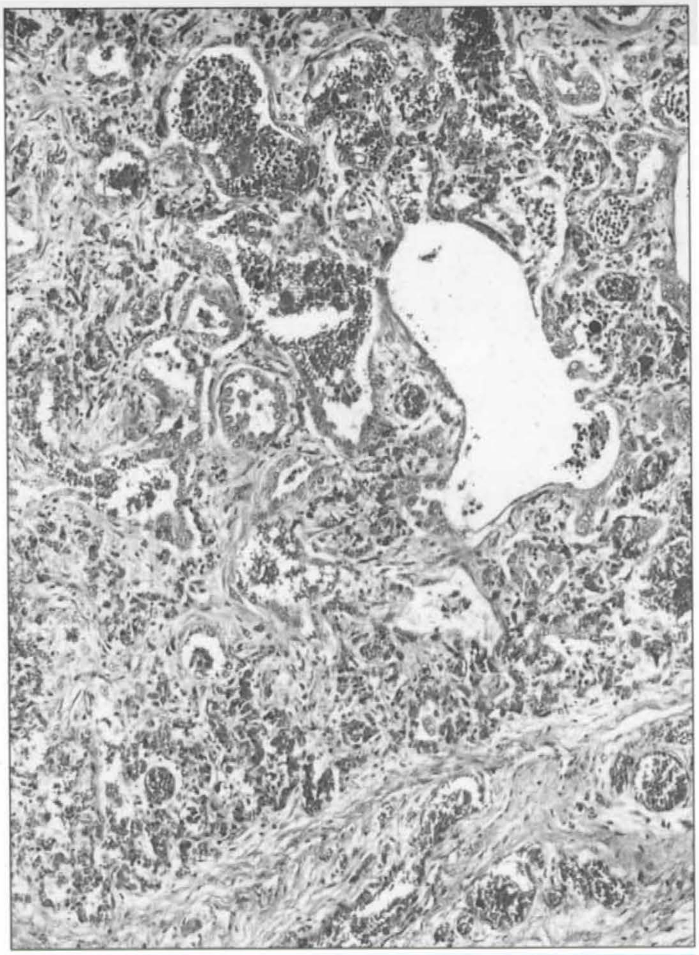

Figure 2.77 Pulmonary congestion and hemorrhage, septal fibrosis, and epithelial metaplasia due to ***Senecio* poisoning** in a pig.

in transient clinical depression and weight loss, transient elevations of serum enzymes and bile acids, and minimal periportal hepatocellular necrosis with fibrosis, developing extensive megalocytosis by week 14. The megalocytosis became the most prominent change 6 months after exposure.

The metabolites of pyrrolizidine alkaloids affect tissues other than the liver; death in some instances may be due to renal damage and in others to pulmonary vascular and interstitial lesions. Variation in the source of the toxin and in the species of target animals accounts for the differences in susceptibility of the different tissues. Alkaloids from *Crotalaria* affect the widest range of tissues in most animals; most notably, monocrotaline causes diffuse lung injury, leading to pulmonary edema progressing to fibrosis. Respiratory difficulty has been described in horses eating *C. dura*, and *C. crispata* produces similar lesions. Sheep develop pulmonary signs after eating *C. globifera* and *C. dura*, and pigs after eating *Senecio jacobaea*. The experimental feeding of *C. spectabilis* to rats or the injection of monocrotaline, extracted from the plant, produces progressive pulmonary disease, pulmonary hypertension, and cor pulmonale, with necrotizing vasculitis of the pulmonary arterioles. Emphysema occurs in pigs and is an outstanding feature of the pulmonary disease of horses. The essential reactive lesion is diffuse fibrosis of alveolar and interlobular septa, with patchy epithelialization occurring more slowly (Fig. 2.77).

Lantana camara

Lantana camara is an attractive ornamental shrub that grows readily in tropical and subtropical habitats. It contains various *toxic pentacyclic triterpenes*, the most abundant of which are lantadene A, B, and C. Other potentially toxic constituents have also been identified, but lantadene A appears to be the most toxic; at high doses experimentally it causes severe acute zonal necrosis of the liver. However, *L. camara* poisoning in grazing animals is *subacute or chronic cholestasis, characterized by severe icterus and photosensitization*, most commonly seen in cattle, but rarely in sheep and goats. The latter species are quite susceptible to the toxin, but are less likely to eat the plant.

Photosensitization is usually severe after 2 days, but jaundice is more severe in chronic cases. Heavily intoxicated cattle can die within 2 days, but most fatal cases run a course of about 2 weeks. Ruminal stasis and anorexia appear early and the large bowel contains dark, dry feces. The rumen is a reservoir of toxin that remains active and can perpetuate the intoxication after access to the plant is curtailed. The liver is enlarged, pale, and stained yellow, orange, or green-gray by bile pigment. The gallbladder is greatly distended with pale, sometimes slightly mucoid, bile.

The severity of the liver changes may be much less than expected from the intensity of the icterus and photosensitization. *The most consistent histologic finding in the liver is hepatocellular enlargement and fine cytoplasmic vacuolation, together with some degree of bile accumulation in canaliculi, hepatocyte cytoplasm, and Kupffer cells.* The canalicular cholestasis is usually more severe in the periacinar zones, while the cytoplasmic vacuolation is often more pronounced in the periportal hepatocytes. There is usually some bile duct proliferation, and in some cases there will be a high incidence of periportal apoptosis, focal coagulative necrosis, or hepatocellular dissociation.

Electron microscopy reveals an apparent increase in volume of smooth endoplasmic reticulum and a quite characteristic form of collapse of many bile canaliculi. Other canaliculi are distended and have damaged microvilli. Since much of the bilirubin that accumulates in the plasma of such animals is conjugated, it seems that the cholestasis is due in large measure to direct interference with canalicular transport of bile. The mechanisms of cholestasis have not been determined but damage to the contractile pericanalicular cytoskeleton or its associated cell adhesion molecules required for canalicular integrity are plausible targets (see section on Cholestasis and jaundice, above).

Animals are usually polyuric due to nephrosis, resulting in severe dehydration in part related to a disinclination to drink. The kidneys are slightly enlarged and wet on section, and, especially in the more chronic cases, the cortex is pale and the medulla hyperemic. The renal lesion is nonspecific nephrosis, ranging in severity from mild vacuolar change to patchy tubular necrosis and extensive tubular cast formation. The role of the hyperbilirubinemia in the production of the renal damage has not been assessed. Severe myocardial necrosis can be produced in sheep by *Lantana* poisoning, and may be responsible for the early deaths in cattle.

Tribulosis and related toxicoses

Consumption of ***Tribulus terrestris***, particularly by the grazing of young wilted plants or sometimes hay, causes cholangitis and photosensitization in sheep. This plant has caused enormous loss of sheep in

South Africa, where the toxicosis is known as **geeldikkop** ("yellow bighead," due to icterus and marked edema of ears and face). The disease has been reproduced by oral administration of crude extracts of *steroidal saponins* of *T. terrestris*, but which of the various saponins in the plant are responsible for the disease is unknown. The disease has also been reproduced experimentally by co-administration of sporidesmin and *T. terrestris*, but the liver lesions are histologically distinct from those produced by sporidesmin alone (facial eczema).

In geeldikkop, the most characteristic gross finding is the presence of a white, semifluid accumulation of fine, crystalline material that can be expressed from the cystic duct and larger intrahepatic ducts. The gallbladder mucosa is also partly covered with a fine, crystalline deposit. The gross lesions of geeldikkop are similar to those of facial eczema in that there is generalized icterus, and the liver is discolored by bile pigment and either slightly swollen or distorted, according to the duration of the disease. In poisoning by sporidesmin alone, however, there is more obvious edema and fibrosis of bile ducts, and bile infarcts in the parenchyma are more common. The acute lesion consists of swelling and feathery vacuolation of hepatocytes, and marked hyperplasia of Kupffer cells that may show similar cytoplasmic changes. In these acute cases, the presence of acicular crystals may be very difficult to detect (Fig. 2.78A). *With more chronic intoxication, the most consistent histologic abnormality is the presence in bile ducts of various amounts of crystalline material* (Fig. 2.78B). The crystals are fine, flat, and are deposited in affected ducts, and, less commonly, in hepatocytes themselves and in renal tubules. There may be associated bile ductular proliferation in severe cases, but often the degree of histological hepatocellular damage is mild compared to the severity of the photosensitization. The cholestasis is not solely due to mechanical obstruction by the crystals, since photosensitization can occur in such outbreaks in animals whose livers show very little cholangitis and contain very few crystals. The severity of peribiliary fibrosis is more variable and probably depends on the relative contribution of sporidesmin to the intoxication. There is some hepatocellular degeneration and apoptosis, and there is fairly uniform swelling of cytoplasm. Bile pigment accumulates in Kupffer cells and hepatocyte cytoplasm, but not to any marked extent in canaliculi. Focal necrosis of the gallbladder mucosa is often present. The severity of the hepatic lesion increases with the duration of exposure.

The biliary crystals from sheep with geeldikkop have been determined to be composed principally of *calcium salts of steroidal sapogenins* present in *T. terrestris*. Plant saponins are metabolized in the rumen and liver to episapogenin glucuronides, which in the presence of calcium may precipitate, forming the characteristic biliary crystals. Whether biliary occlusion by these steroidal plant saponins is the sole cause of the liver damage has yet to be determined. Other unidentified plant components may also play a role. *Hepatocellular damage likely also plays a role in phylloerythrin retention.* It has also been suggested that the toxins responsible may act primarily on the membranes of the bile canaliculi, in a manner similar to that of *Lantana* poisoning.

Crystal-associated cholangiohepatopathy is not specific for *Tribulus* intoxication. Similar changes occur in the hepatogenous photosensitivity disease, **alveld**, in sheep grazing pastures in Norway, the British Isles, and in the Faeroe Islands containing *Narthecium ossifragum*. Similar disease has been reported in ruminants intoxicated by *Agave lecheguilla, Nolena texana*, and the pasture species *Brachiaria decumbens* (signal grass) in Brazil and the *Panicum*, especially *P. coloratum*, kleingrass. In those cases studied (*T. terrestris, P. dichotomiflorum, P. schinzii, Narthecium ossifragum*), the characteristic crystalloid material deposited in the bile is principally composed of glucuronides derived from the metabolism of steroidal saponins present in the ingested plants. Foamy macrophages are also present in mesenteric and hepatic lymph nodes, and are also found in healthy cattle and sheep grazing *B. decumbens* in Brazil. With the exception of *Nolena texana*, steroidal saponins have been demonstrated in all of these

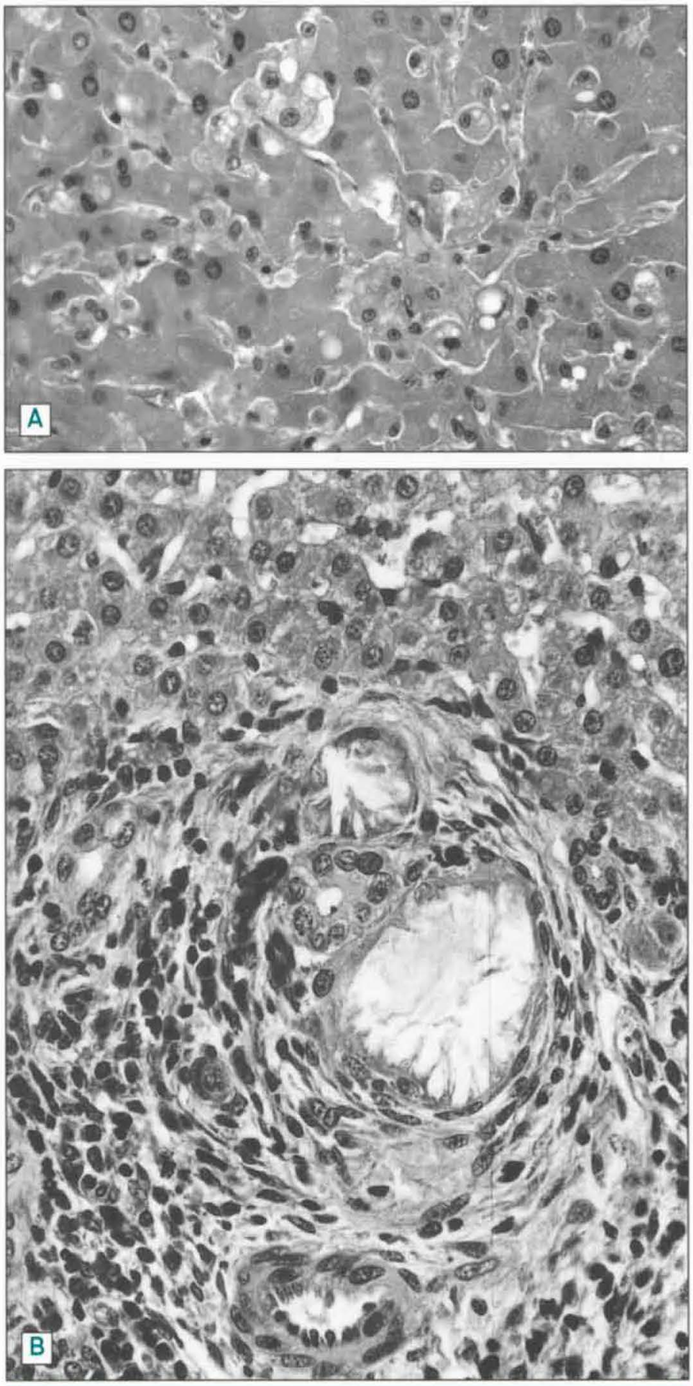

Figure 2.78 **A.** Hepatocyte swelling, feathery vacuolation and degeneration, with marked Kupffer cell hyperplasia – an early lesion of **geeldikkop** in a sheep. (Courtesy of RF Slocombe.) **B.** Chronic cholangitis and plugging of bile duct by lipophilic crystalloid material, in photosensitivity disease of sheep grazing **pangola grass**. Similar to reaction seen in geeldikkop.

plants. As with *T. terrestris*, extracts of *B. decumbens* containing steroidal saponins produced typical lesions of cholangitis with crystal deposition when orally administered to lambs, with *Pithomyces chartarum* spore counts below detectable levels. Similarly, analysis of samples of *B. decumbens* and *P. dichotomiflorum* on which cattle and goats had recently been photosensitized showed only low levels of *P. chartarum* spores, and all isolated obtained failed to produce sporidesmin. Despite this, it is also apparent that neither *Tribulus* nor *Panicum* stands are at all times dangerous. Levels of saponins have been shown to vary greatly within a species from site to site, and with the age of the plant. Additionally, the role of sporadic environmental conditions such as wilting, which might concentrate the saponins or other unidentified toxins, or the synergistic involvement of endophytic fungi, remains to be determined.

Other plants reported occasionally to produce unexpected photosensitivity include *Digitaria, Cooperia pedunculata, Nidorella foetida,* and *Chloris*, and such valuable pasture genera as *Medicago, Trifolium,* and *Avena*.

Nitrosamines

Epizootics of poisoning by **dimethylnitrosamine** occurred in Norwegian cattle, sheep, and fur-bearing animals, from 1957 to 1962. The toxin was present in herring meal and was thought to be a *reaction product of trimethylamine and other lower amines with sodium nitrite*, added as a preservative, the reacting amines being products of decomposition. Animals consumed several pounds of the toxic meal each day before becoming ill. *This intoxication has little importance in livestock now that it is easily prevented.*

At necropsy, there was moderate anasarca and signs of hemorrhagic diathesis. Livers affected acutely were enlarged and firm, with mottled discoloration and sometimes a nutmeg appearance. In chronic intoxication, the liver was small, granular, and very firm. A number of cases recovered after prolonged convalescence, and in these there was atrophy and fibrosis of the left and caudate lobes, and the right lobe was hyperplastic and hemispheric. Histologically, in acute cases, there was widespread hemorrhagic necrosis of periacinar distribution and an unusual degree of intimal and subendothelial reaction in sublobular and hepatic veins. The chronic lesion was dominated by extensive fibrosis of periacinar distribution, with obliterative changes in many central and sublobular veins. Sporadic cases of portal hypertension in Greyhound dogs have been recognized where hepatic veno-occlusive disease is the predominant lesion. In these cases, a history of feeding meat treated with large amounts of nitrites or sulfites in an attempt to preserve the color of fresh meat is common, but a causal relationship has not been established.

The *hepatocellular changes are not specific* for dimethylnitrosamine or other hepatotoxic nitrosamines. Nitrosamines are metabolized in the liver and other tissues to reactive alkyl groups that bind covalently to various macromolecules, especially guanine in nucleic acids. Hepatotoxicity develops more slowly than in response to toxicants that induce membrane peroxidation, as many hepatocytes with DNA damage undergo apoptosis when stimulated to replicate. There is some megalocytosis, nuclear vesiculation and nucleolar prominence, cytoplasmic intranuclear inclusions, and variable fatty change and cytoplasmic bile accumulation. The typical chronic effects of chronic experimental nitrosamine hepatotoxicity, such as hepatocellular fibrosis, nodules, and neoplasms, have not been evident in the accidental poisonings. Dimethylnitrosamine-induced hepatotoxicosis in dogs has been proposed as a model of toxin-induced progressive hepatic disease in this species.

Indospicine

Legumes of the genus ***Indigofera*** have for some years been known to contain the toxic amino acid indospicine (6-amidino-2-hexanoic acid), which is a structural analogue of arginine, and which was shown in early experimental work to be hepatotoxic for rats and other species. The dose rates necessary to cause chronic liver injury in these species were, however, quite high, and field cases of intoxications were only seen in cattle grazing *I. spicata*, which has the highest naturally occurring concentrations of indospicine. More recently, in Australia, a serious outbreak of fatal liver disease occurred in dogs that had been fed meat from horses that had been grazing *I. linnaei*, a plant native to the arid zones of Australia that has been known to produce in horses a chronic neurological disorder known as "*Birdsville horse disease*." The disease in horses is not associated with liver damage, although these animals do accumulate indospicine in most tissues, including muscle.

Dogs fed indospicine-contaminated meat for several weeks may develop *progressive liver damage*, which begins as vacuolation of a narrow band of periacinar hepatocytes, followed shortly by accumulation of mononuclear inflammatory cells in this zone and in the stoma of the hepatic venules. Progression of the lesion is marked by piecemeal necrosis of a widening zone of periacinar hepatocytes, disorganization, vacuolation due to hydropic and fatty change, and accumulation of ceroid pigment in macrophages (Fig. 2.79). Moderate periacinar fibrosis is seen in later stages, as well as pronounced canalicular cholestasis: by this stage, affected animals begin to show icterus, inappetance, and depression. Bile ductular proliferation is not a marked feature. Death is attended by the usual signs of hepatoencephalopathy and tendency to bleed spontaneously. There is no evidence of direct neurological damage as is seen in horses.

This intoxication is remarkable by virtue of its unpredictability. Experimental intoxication by pure indospicine has validly been shown to reproduce the naturally occurring intoxication caused by horsemeat feeding; however, liver failure can be produced by either means in only a small proportion of dogs so exposed. On the other hand, milder degrees of liver damage are reliably produced by both methods. Thus it seems an idiosyncratic response is superimposed upon a more consistent effect of the toxin; the nature of the accompanying inflammatory response suggests that the former may be immunologically mediated. This has yet to be established, as has the proposal that the mechanism of the intoxication is due to competitive inhibition of arginine.

Trifolium hybridum (alsike clover)

Alsike clover is a legume that grows well on wet clay soils in North America, where it is sometimes responsible for outbreaks or single cases of *cholangiohepatitis in horses* that consume it as pasture or hay. *The toxic principle has not been isolated*, but it likely is in highest concentrations in the flowering stage used for hay. However, there are evidently contributing factors because, while alsike clover is widespread in cultivation, *toxicity is rare*. Toxicity usually occurs when alsike clover is a major component of pastures or hay, but some horses are believed to graze it selectively in a mixed pasture.

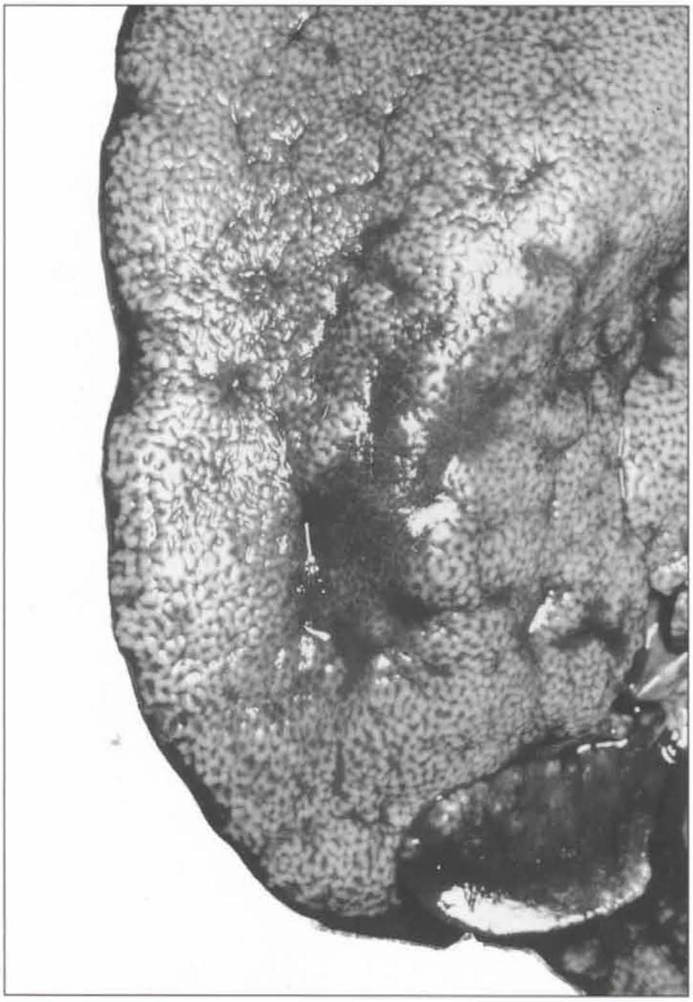

Figure 2.79 Acinar fatty and hydropic change, alternating with post-necrotic collapse, due to **indospicine poisoning** in a dog.

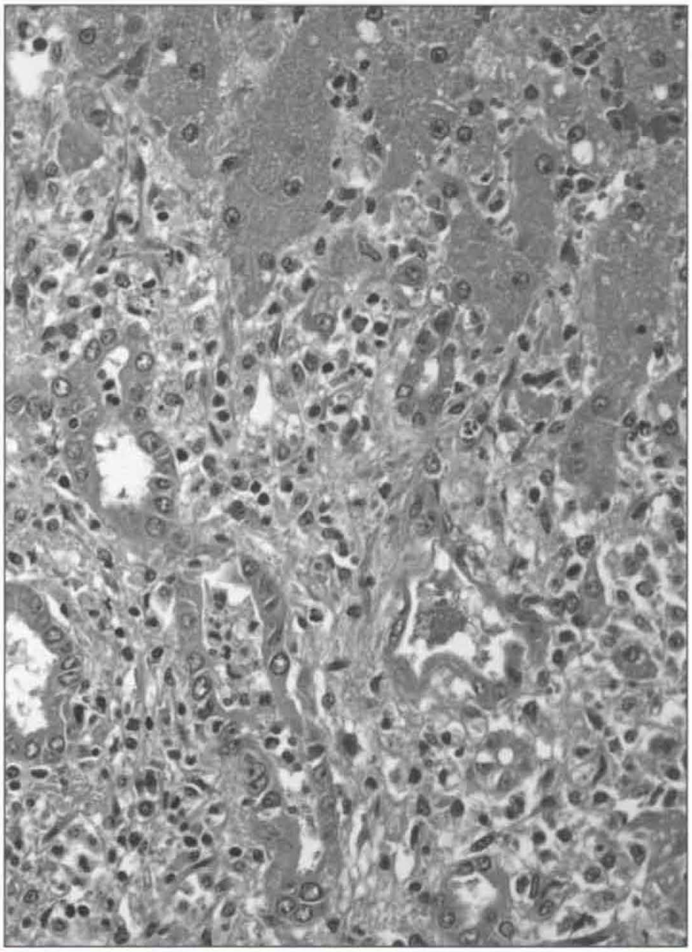

Figure 2.80 Chronic cholangiohepatitis due to **alsike clover poisoning** in a horse.

Clinical signs of poisoning initially include mild colic, ill-thrift, and anorexia, with increasing occurrence of signs of hepatotoxicity with cholestasis. In some there can be icterus and photodynamic dermatitis. Exposure to the plant can occur for a year or more before signs of hepatic insufficiency develop, but cholestatic liver disease can be detected biochemically before horses become ill. More severely affected horses display neurological disturbances, either excitement or mania in irregular episodes, or long periods of extreme dullness, anorexia, apparent blindness, forced wandering, head pushing, and yawning. Clinical problems related to hypoproteinemia such as coagulopathy or ascites are not a feature of this disease, probably because most of the hepatocytes are not directly affected.

The lesions of alsike clover poisoning are those of *diffuse subacute to chronic cholangiohepatitis*, with the major lesions within and adjacent to all intrahepatic biliary tracts. The liver is typically enlarged, sometimes greatly so, pale, and tough or rubbery in consistency. The surface is smooth, but its appearance is mottled. The mottling is clear on the cut surface and is caused by bands of gray fibrous tissue, distinctly visible to the naked eye, surrounding and compressing each lobule. Near the margins of the liver and in some other areas, scar tissue may completely replace the parenchyma. Areas of parenchymal atrophy may occur upstream of some occluded ducts. Microscopically, there is pronounced proliferation of fibrous tissue in and around the portal tracts associated with hyperplasia of well-formed bile ducts (Fig. 2.80). Some areas have prominent neutrophil infiltration into ducts but in many areas this does not occur. In the early stages, the proliferation of bile ducts is often greater than the proliferation of the fibrous tissue, but later the proportions are reversed. The proliferating tissue extends slowly to connect adjacent portal triads, circumscribing areas that correspond to conventional lobules. The fibrous tissue does not permeate along the sinusoids, and there is gradual and uniform constriction of the parenchyma.

This distribution of liver lesions suggests that the harmful factor in alsike clover is excreted into the bile in a form that damages the ducts. However the cholangiohepatitis is similar to that observed in areas of the liver that are drained by chronically obstructed bile ducts, so it seems likely some of the later changes are secondary to cholestasis. Agents that interfere with bile excretion have the potential to interfere with elimination of various potentially injurious substances. Thus it is possible that alsike clover ingestion might increase toxicity of other factors in the forage.

Copper

The heavy-metal copper is an essential trace element, facilitating electron transfer reactions by a number of key enzymes with critical roles in connective tissue cross-linking (lysyl oxidase), antioxidant defense (superoxide dismutase), cellular respiration (cytochrome C oxidase), and catecholamine biosynthesis (dopamine β hydroxylase). However, *copper's redox properties are also responsible for its toxicity at excess concentrations, through mediation of free radical production resulting in direct oxidation of cellular components.* The liver is the major organ involved in the regulation of copper levels, and homeostasis is maintained by the balance of dietary intake and copper excretion via the bile. *Hepatic copper toxicosis can result from a primary metabolic defect in hepatic copper metabolism, altered hepatic biliary excretion of copper, or from excess dietary intake of the element.*

There is significant variation in species susceptibility to copper toxicosis. Sheep as a species are most prone to copper poisoning, due to reduced biliary excretion of copper. Some breeds are more susceptible than others, reflecting differences in the efficiency of intestinal absorption of copper, rather than differences in efficiency of biliary excretion. Altered biliary excretion of copper in sheep does not appear to be associated with alteration in the structure or expression of the gene for ATP7B, the copper transporting P-type adenosine triphosphatase that is defective in Wilson disease, the human autosomal recessive disorder of hepatic copper metabolism, although functional analysis of the protein has yet to be performed.

Copper-associated hepatopathy has been reported as an *autosomal recessive disorder in Bedlington Terriers*, and as a *familial hepatopathy in West Highland White Terriers and Skye Terriers* (see the section on Chronic hepatitis in dogs, above). A single case of a hepatopathy associated with excess hepatic copper concentrations (4074 μg/g) has been reported in a Siamese cat. In dogs and sheep, toxic amounts of copper can accumulate in the liver while dietary copper levels are not excessive by standards for other species. Copper poisoning does occur in cattle and pigs, but in these species it is due to abnormally high intake of the element. Acute copper poisoning can occur following either the ingestion or injection of excess copper, and *animals deficient in vitamin E appear especially susceptible to acute copper poisoning.*

Chronic copper toxicosis in sheep occurs as a result of the presence of three environmental factors acting alone or in concert. First, *excessive copper intake* may occur as a result of contamination of water (naturally occurring or through the use of copper piping or fixtures), pasture or prepared feed; the latter is difficult to avoid when feed mills are preparing rations for different species and is probably partly responsible for the observation that housed sheep are more prone to copper poisoning than animals at pasture. Second, increased copper accumulation occurs as a result of increased availability of dietary copper; this happens when *dietary levels of molybdenum are unusually low.* Molybdenum, in the presence of sufficient sulfate, forms insoluble complexes with copper in the gut and liver, making the copper biologically inert. Subterranean clover growing on calcareous soils in southern Australia may be relatively deficient in molybdenum, and in these areas British breeds of sheep are known to be more susceptible than Merinos to chronic copper poisoning. *Other hepatotoxins* constitute the third environmental factor that predisposes sheep to outbreaks of chronic copper poisoning. The most important of these are pyrrolizidine alkaloids (from *Heliotropium* or *Echium*) in eastern Australia, and phomopsin from lupins in western Australia and, possibly, South Africa.

The basis for chronic copper poisoning in sheep is the peculiar avidity of the liver for copper, coupled with the very limited rate at which this species can excrete the element in the bile. After intraportal injection of a copper isotope, practically all the radioactivity is removed during the first passage through the liver. Most of the copper is sequestered in hepatocellular lysosomes, where it does little damage at concentrations of up to 200–300 μg/g dry weight. As the concentration rises, there is presumably more interaction between other cell components and the copper. There is some evidence that lysosomal membranes lose integrity and allow copper and lysosomal hydrolases to damage the rest of the cytoplasm. By the time the liver copper concentration has reached 300 μg/g or more, there is a histologically apparent increase in hepatocellular turnover, with single hepatocytes undergoing apoptosis within a dense knot of neutrophils. At still higher copper levels, the apoptotic rate increases, while all cells become swollen and their nuclei vesicular. The mitotic rate increases, presumably to keep pace with the accelerated loss of hepatocytes, and large macrophages appear in the sinusoids and stromal spaces about the vessels (Fig. 2.81). These cells contain eosinophilic or somewhat brown, granular debris, which consists of copper-containing lipofuscins.

Sheep with liver copper concentrations in excess of 1000 μg/g may be clinically and hematologically normal, so long as the increasing mitotic rate produces enough new hepatocytes to take up the copper released by dying cells. At this stage, however, there will be elevated levels of liver-specific enzymes in the plasma. If the rate of hepatocellular loss exceeds the capacity of the liver to phagocytose

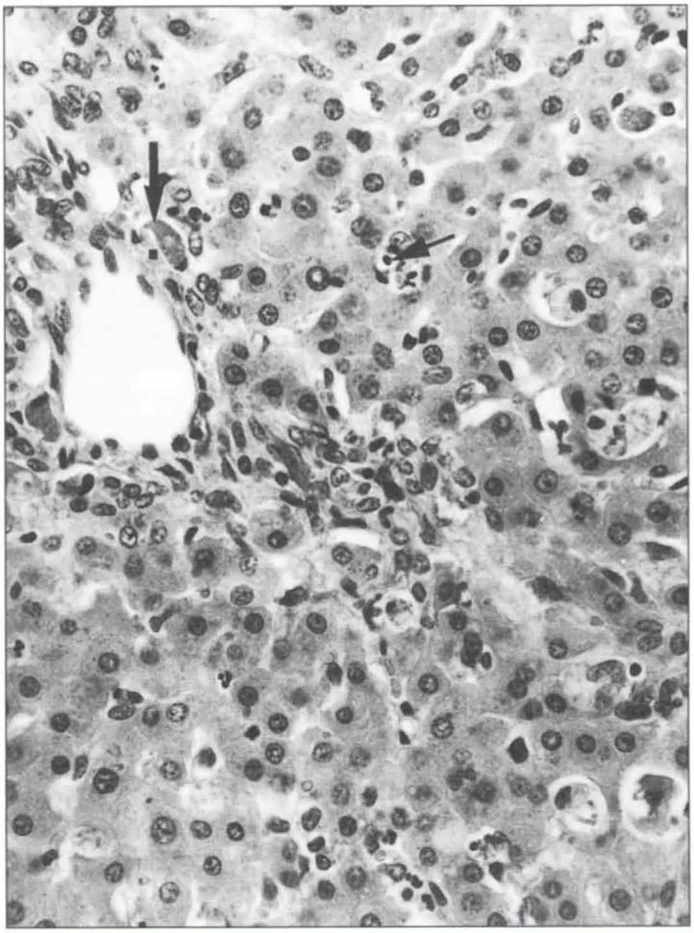

Figure 2.81 Apoptosis (smaller arrow), focal leukocyte aggregation, and pigmented macrophages in sinusoids and stroma (larger arrow) in the liver of a sheep in the prehemolytic phase of **chronic copper poisoning**.

and sequester cell debris quickly, the plasma copper levels can rise to levels that are high enough to damage circulating erythrocytes, and intravascular hemolysis ensues. *The effect of the hemolysis and anemia on the liver is to accelerate the rate of hepatocellular necrosis; thus copper enters the circulation at an increasing rate and acute copper toxic crisis is manifest.* The lethal clinical syndrome is then one of *paroxysmal intravascular hemolysis and liver failure*, in which a sheep may pass from apparent good health to death within ~6 hours. Stresses, such as brief starvation, may also precipitate the crisis in susceptible sheep but the mechanisms involved are unknown. During the hemolytic crisis, some of the copper is lost from the disintegrating liver; some passes into the urine, and kidney copper concentration rises to 1000 μg/g or more. *Blood or kidney copper levels* therefore give a truer indication of a prior hemolytic crisis due to chronic copper poisoning than does elevation of liver copper alone.

The gross lesions of fatal chronic copper poisoning are those of acute copper toxicity. The carcass is discolored by marked icterus, superimposed on which is the red color imparted by free hemoglobin. Often there is a brown hue as well, because a proportion of the hemoglobin is oxidized to methemoglobin. The spleen is engorged, dark, and soft. The kidneys are deep red-brown to black and the urine deep red, as a result of hemoglobinuria with oxidation to methemoglobin, and concurrent icterus. The liver is often slightly soft and swollen, and deep orange, but if the condition arises after long-term liver injury, atrophy and fibrosis may be evident. Other changes such as liver necrosis from various causes, including hypoxia due to anemia, heart failure, and shock, complicate the spectrum of liver lesions.

A breed of sheep from the Hebridean island of North Ronaldsay has apparently adapted to a seaweed diet low in both copper and molybdenum but rich in zinc. *Zinc is also capable of interfering with copper uptake*, and these sheep, while avoiding copper deficiency, are exquisitely susceptible to chronic copper poisoning when transferred to normal pasture. Hepatic disease associated with copper toxicity in this breed appears to differ morphologically from other domesticated sheep breeds. Copper accumulation begins in periportal hepatocytes, accompanied by a mixed inflammatory infiltrate and cholangiolar proliferation. Characteristic pericellular fibrosis, initially confined to the portal tracts and periportal zones, later extends to diffuse fibrosis and cirrhosis, associated ultrastructurally with numerous hepatic stellate cells. North Ronaldsay sheep have been proposed as a possible animal model for human non-Wilsonian hepatic copper toxicosis and cirrhosis of infancy, and for investigation of copper-associated hepatic fibrogenesis.

The events described in sheep also occur in **chronic copper poisoning in pigs and cattle**, and acute intravascular hemolysis may be seen, especially in calves. Usually, however, there is less of the acute terminal chain reaction in these species, and there is more evidence of chronic liver damage with extensive portal fibrosis and biliary hyperplasia within the triads.

Acute copper poisoning *is most often seen in ruminants after accidental administration of single large doses of copper*, by either the oral or parenteral routes. Iatrogenic copper toxicosis has been induced by administering copper oxide boluses to neonatal calves, and by injection with copper disodium edetate in weanling calves. Copper toxicosis has also been reported in veal calves fed milk replacer supplemented with various copper-containing hematinics. The liver lesion varies with chronicity of exposure, from nonspecific acute periacinar necrosis, to cholangiohepatitis with periportal fibrosis. Intravascular hemolysis may occur if plasma copper levels are sufficiently elevated. Acute gastroenteritis may be produced by oral dosage and may also be seen after sufficiently large parenteral doses of copper.

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HYPERPLASTIC AND NEOPLASTIC LESIONS OF THE LIVER AND BILE DUCTS

In recent years, much has been learned about the multistep and multifactorial pathogenesis of neoplasia in the liver. This is the benefit of several circumstances that increased the need to understand responses of the liver to carcinogenic insults. Foremost is that *liver cancers are the most common fatal carcinoma in humans*, largely because of their association with endemic hepatitis B virus in Asia and Africa. Second, many xenobiotics have been found to increase the occurrence of liver neoplasms in laboratory rodents, increasing the concern that environmental, foodborne and medicinal hepatotoxins might be avoidable cancer risks. Third, the liver provides an exceptional model in which to observe and characterize the metabolic, cellular, and mutational events underlying the various stages of cancer development.

The available literature on liver neoplasms is immense and beyond the scope of this section, which will deal more with the usual conditions encountered by biopsy or necropsy of domestic mammals. Remarkably, *the occurrence of fatal liver malignancies is rather uncommon in aged dogs and cats*; this suggests that they are either less exposed, or they are more resistant to the etiological agents responsible for liver neoplasms in humans. Nonetheless, *atypical hyperplastic hepatocellular foci and nodules are common in older dogs*, and are increasingly encountered in the imaging and laparoscopic investigation of hepatic diseases in dogs. The reason why such lesions in dogs evidently do not progress to malignancies at the frequencies observed in humans, rodents, and fish remains intriguing and obscure.

Ectopic, metaplastic, and hyperplastic lesions

Ectopic and metaplastic lesions in the gallbladder have been reported. Ectopic tissue includes hepatocyte nodules attached to or within the wall, and pancreatic islands, which may include islets of Langerhans. Gastric ectopia, readily distinguishable by the presence of chief and parietal cells, may form plaques or large polyps. Ectopia of cardiac or pyloric mucosa is mimicked by metaplastic changes.

Hyperplastic nodules of hepatocytes are common in old dogs, but rare in other species. The lesions in dogs do not have a breed or sex predisposition, but their incidence increases sharply with age. Hyperplastic nodules typically develop in livers of normal mass, whereas **regenerative nodules** arise as a result of compensatory hyperplasia of surviving hepatocytes in a background of hepatic injury, atrophy, and fibrosis.

Hyperplastic nodules are usually multiple and randomly distributed throughout the lobes. The grossly visible nodules are spherical, well circumscribed, varying in size from 2 mm to 3 cm or more, and may bulge from the capsular surface. They may be sharply distinct from the surrounding parenchyma due to color differences, either lighter than the surrounding liver due to increased lipid or glycogen content or reduced expression of cytochromes within hepatocytes comprising the nodule, or darker because the sinusoidal vessels are distended with blood. Some nodules are the same color as the surrounding tissue and can only be identified by examination of a washed or blotted slice under a strong light.

The larger hyperplastic nodules grow expansively; they do not induce a fibrous capsule, but they can compress the surrounding parenchyma. The hepatocytes making up the focal hyperplasias are often phenotypically different from those in adjacent parenchyma. Old canine livers often have microscopic focal hyperplasia of similarly altered hepatocytes that can be distinguished from the surrounding parenchyma, but they evidently grow more slowly and do not compress their boundaries. These can be regarded as examples of the numerous atypical focal hyperplasias that arise as altered foci, nodules, plaques, or polyps in various tissues of old dogs. In more prominent paler nodules, hepatocytes can be enlarged and vacuolated with lipid or glycogen accumulation (Fig. 2.82). Mitotic figures are uncommon. The sinusoids of the nodules are often dilated and foci of hematopoiesis are occasionally observed. Necrosis and hemorrhage in hyperplastic nodules are rare.

The significance of hyperplastic nodules in old dogs is usually negligible. After acute liver necrosis, these atypical hepatocytes within preexisting hyperplastic foci and nodules may have phenotypic alterations that confer a survival advantage, and can survive and flourish as larger nodules amid regions of necrosis, so in these instances they can be beneficial. While hyperplastic nodules rarely progress to lesions with detrimental impact, they are often found during liver imaging, laparotomy, or biopsy and need to be differentiated from other focal lesions of more significance. Because they are atypically differentiated, they are the potential source of secreted products of clinical or diagnostic significance but their potential in this regard is unexplored. *The hallmark of the hyperplastic nodule, in contrast to the hepatic adenoma and hepatocellular carcinoma, is that it largely retains normal liver architecture, including a modified lobular structure with recognizable central veins and portal triads.* However this feature may be difficult to appreciate in small biopsies. The biological distinction between atypical hyperplasia and neoplasia is sometimes equivocal; in the latter,

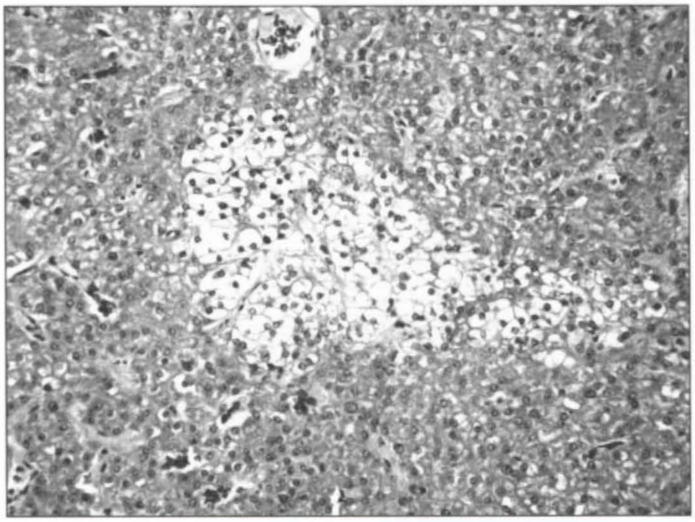

Figure 2.82 Vacuolated hepatocytes in a small **altered focus** in an old dog.

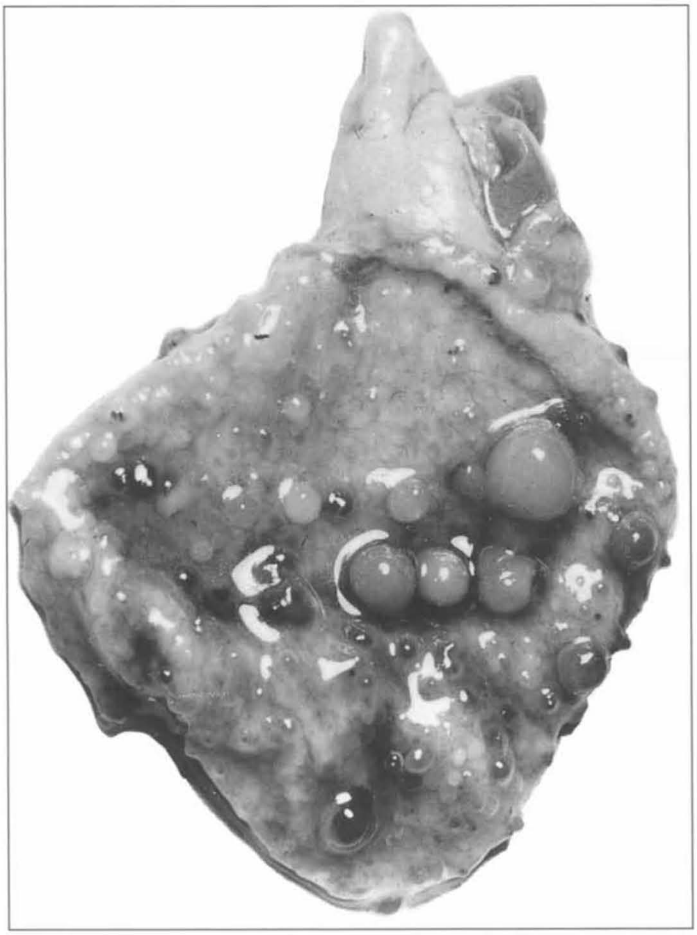

Figure 2.83 **Cystic mucosal hyperplasia** of the gallbladder in a dog.

continued growth that is independent of promoting influences is responsible for enlargement, and the designation of adenoma should apply to larger lesions that are enlarging.

Cystic mucinous hyperplasia *of the mucus-producing glands in the mucosa of the gallbladder* has been reported as an incidental lesion in dogs and sheep. These cystic hyperplastic nodules are often numerous, may be sessile or polypoid, and contain mucin (Fig. 2.83).

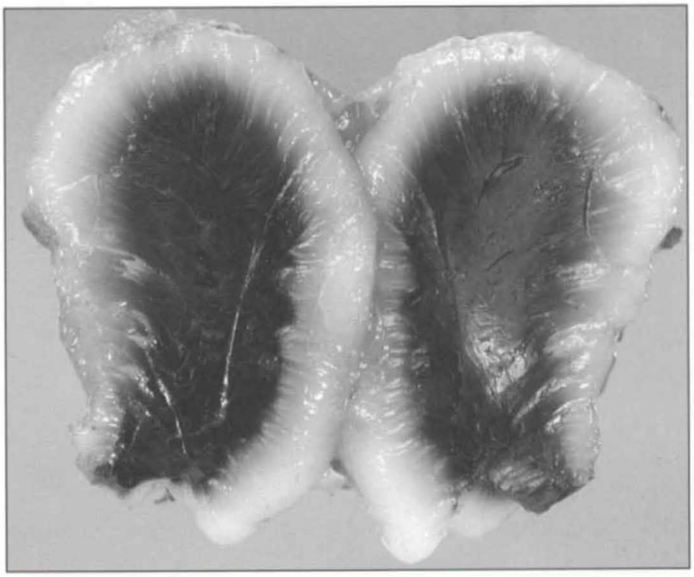

Figure 2.84 **Gallbladder mucocele** in a dog. (Courtesy of AR Vince.)

Dilation of the gallbladder with accumulated mucoid secretion (**gallbladder mucocele**) has been reported in dogs. Histologically, affected gallbladders display hyperplasia of mucus-secreting mucosal glands, as well as formation of mucus-filled cysts. Severely affected gallbladders may be distended with abnormal semisolid accumulations of mucus, and may undergo ischemic necrosis and rupture (Fig. 2.84). Bile-laden mucus may also extend into the cystic, hepatic, and common bile ducts, resulting in variable degrees of extrahepatic biliary obstruction. The etiology of this condition is unknown, and may be related to decreased gallbladder motility, bile stasis, and altered bile composition and viscosity.

Mucosal hyperplasia in the large bile ducts is frequently observed in the long-standing mild cholangiohepatitis of fluke infestation. The hyperplasia is of microscopic dimensions, but in some instances it appears histologically to be atypical. Localized, polypoid foci of cystic hyperplasia are specific changes in cattle poisoned by highly chlorinated naphthalene.

Hepatocellular tumors

Epithelial neoplasms of the liver and bile ducts include tumors of either hepatocellular or cholangiocellular origin, and have been reported much more commonly in dogs and cats than in cattle, horses, sheep, or pigs. *Hepatocellular neoplasms are reportedly more common in dogs, whereas cholangiocellular tumors predominate in cats.* Unlike the situation in humans, there are no clear associations in domestic animals between hepatobiliary tumors and viruses, chemical carcinogens, mycotoxins, or drugs such as synthetic steroids. With the exception of the occasional association of liver tumors with certain flukes or tapeworm larvae, and the rare adenoma seen in the bovine liver with chronic pyrrolizidine poisoning, *hepatobiliary tumors in domestic animals are not obvious successors to antecedent liver disease.*

Hepatocellular adenomas (sometimes termed *hepatomas*) are usually single and they may be 15 cm or more in diameter. The smaller ones project as smooth nodules, but some of the larger specimens are lobate, and some pedunculated (Fig. 2.85). They are soft

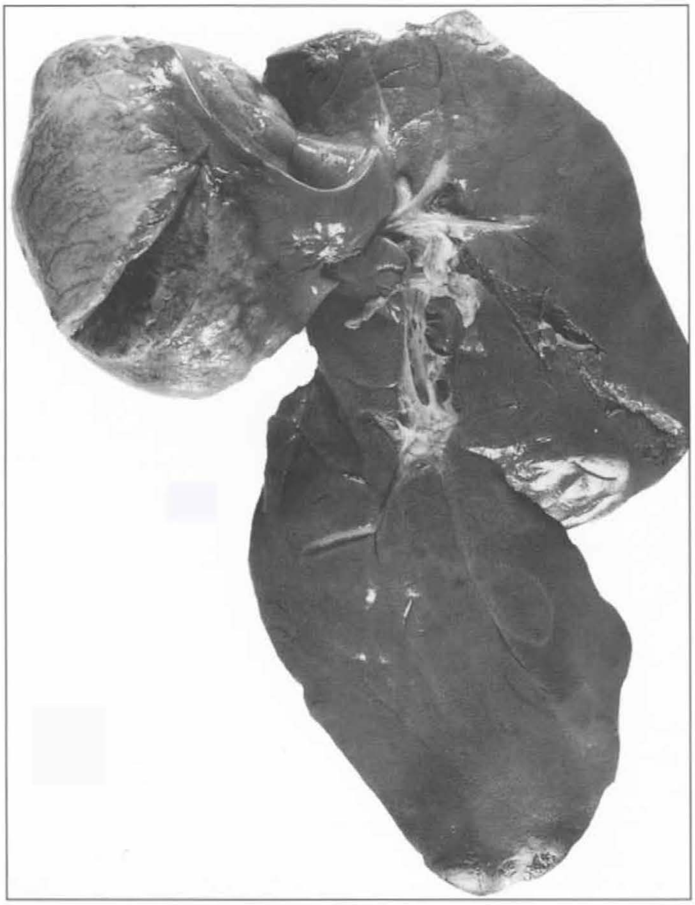

Figure 2.85 Pedunculate **hepatocellular adenoma** in the caudate lobe of the liver in a sheep.

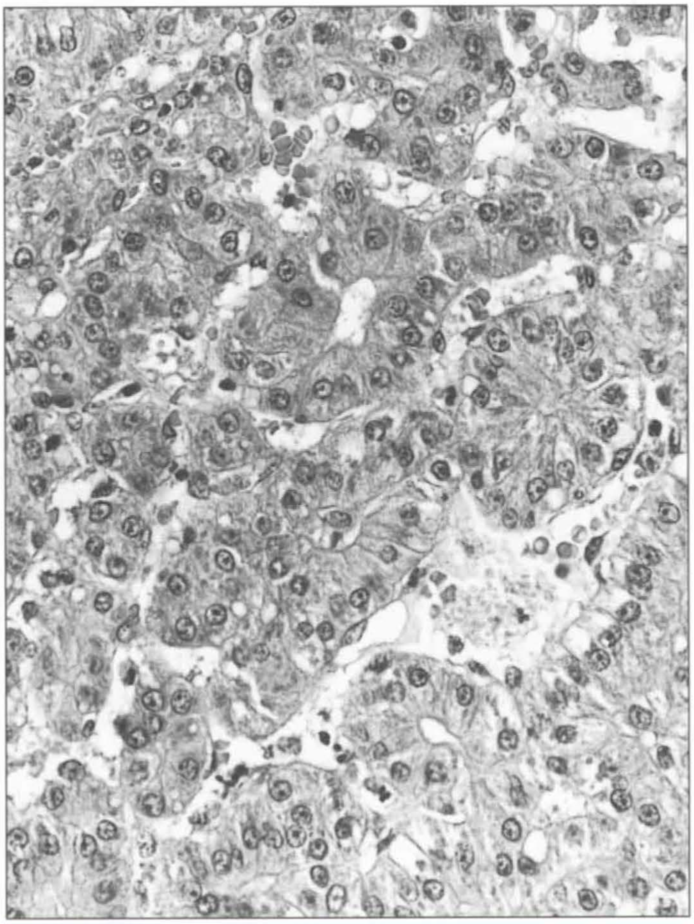

Figure 2.86 **Hepatocellular adenoma** in a dog.

and light brown or yellow; some are pigmented by bile, the remainder of the liver not being pigmented. Demarcation is fairly sharp, and while they compress adjacent parenchyma, they are not typically encapsulated. Histologically, the cells do not differ markedly from normal hepatocytes or hyperplastic nodules, and often contain lipid, glycogen, or occasionally protein secretory vacuoles. *Cells are arranged in cords or trabeculae, but typical acinar structures and vascular architecture are absent* (Fig. 2.86). *Adenomas usually lack central veins and hepatic venules, multiple portal tracts, bile ducts, or cholangioles.* Bile pigments may be present. In sheep, cattle, and dogs, hematopoietic foci may sometimes be found in the adenomas.

Hepatocellular carcinomas are uncommon, and may be single and massive, nodular or diffuse. Several features suggest malignancy, including the absence of pedunculation or clear demarcation and the presence of varied coloration of the cut surface produced by hemorrhage, necrosis, fatty change, and bile pigmentation. Invasion of portal vessels, sometimes clearly evident grossly, is decisive. Some of these tumors in cattle are scirrhous, hard, and white. Carcinomas occasionally penetrate the capsule to implant on the peritoneum. Hematogenous metastases occur first in the lungs, and some appear to arrive in masses to impact in relatively large vessels.

Free venous invasion is typical of the hepatocellular tumors and intravascular spread may extend to the large hepatic veins and vena cava. Retrograde growth in the portal veins may extend to the spleen and the stomach and the resulting portal hypertension and varicose venous shunts assist further spread in portal fields. Spontaneous rupture is common and may cause fatal hemoperitoneum.

Hepatocellular carcinomas may be quite variable in histologic appearance, and multiple patterns may occur within a single neoplasm. *Trabecular carcinomas are the most common histologic pattern.* Neoplastic cells resemble normal liver, and grow in plates or trabeculae that vary from few to many cells thick. Necrosis may be present, and sinusoids may be dilated. Other patterns include *adenoid carcinomas*, characterized by acinar arrangement of neoplastic hepatocytes within scant connective tissue stroma, and *solid carcinomas*, characterized by solid sheets of poorly differentiated, often pleomorphic cells that do not form sinusoids (Fig. 2.87). A feature of some hepatocellular tumors is the presence of giant cells, quite conspicuous by virtue of a large nucleus, multilobed nuclei, or multiple nuclei. Mitotic figures are more frequent in carcinomas than adenomas, but may not be a prominent feature of well-differentiated carcinomas.

Hepatoblastomas, rare benign tumors putatively originating from pluripotent small-cell precursors, have been reported in sheep, dogs, horses, and a bull. Hepatoblastomas in humans are neoplasms of infancy and childhood. Cases in domestic species have been reported in both young and adult animals.

Hepatic carcinoids, presumably arising from neuroendocrine cells, have been reported in both dogs and cats. These are aggressive neoplasms, with frequent intrahepatic spread, and metastasis to local lymph nodes and peritoneum. Like other neuroendocrine tumors,

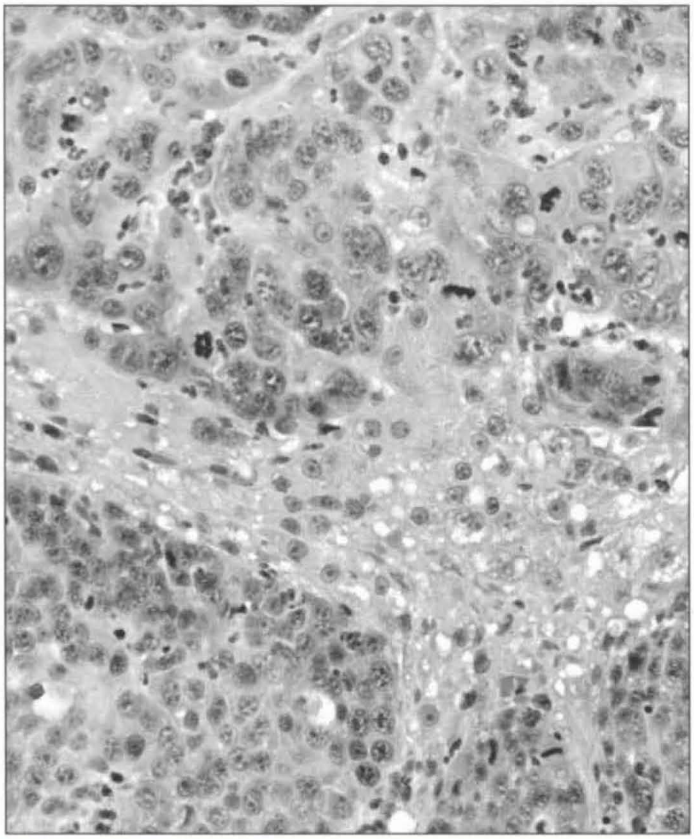

Figure 2.87 Invasive solid **hepatocellular carcinoma** in a dog.

hepatic carcinoids typically form nests of cells separated by a fine fibrovascular stroma. The cells are oval to spindle-shaped, and may form a rosette or pseudolobular pattern. Mitotic figures are usually frequent. Argyrophilic cytoplasmic granules may be detected by silver impregnation stains; more precise identification of carcinoids may require immunohistochemical stains for neurosecretory products such as glucagon or serotonin.

Cholangiocellular tumors

Cholangiomas (cholangiocellular adenomas) are mainly seen in old dogs and cats. Cholangiomas typically arise within the hepatic parenchyma. Extrahepatic cholangiomas are rare, with the exception of gallbladder adenomas reported in cattle in abattoir surveys. The smaller specimens may be solid on the cut surface and white, but the large specimens, and many of the small ones, are cystic, containing a clear watery to viscous fluid. Their volume may be greater than that of the lobe they occupy. The solid specimens are composed of a large number of tubules lined by a single layer of cuboidal biliary epithelium. The stroma varies in amount. There is no sign of active invasion of the parenchyma. **Cystic cholangiomas (biliary cystadenomas)**, a subtype commonly seen in cats, are multilocular and lined by atypical bile duct epithelia that may be flattened by pressure, or in some areas papillary. The septal stroma is collagenous.

Cholangiocarcinomas (cholangiocellular carcinomas) are reported in dogs, cats, sheep, cattle, horses, and goats. These tumors may be massive or multinodular (Fig. 2.88). In dogs and cats, the tumors are almost always multiple or diffuse. The multiple nodules of tumor might represent intrahepatic lymphogenous metastases, but the possibility of multicentric origin must be entertained. Hematogenous metastases are unusual, but metastases to the regional nodes are common. In cats especially, there is a tendency to invade Glisson's capsule and implant on the peritoneum.

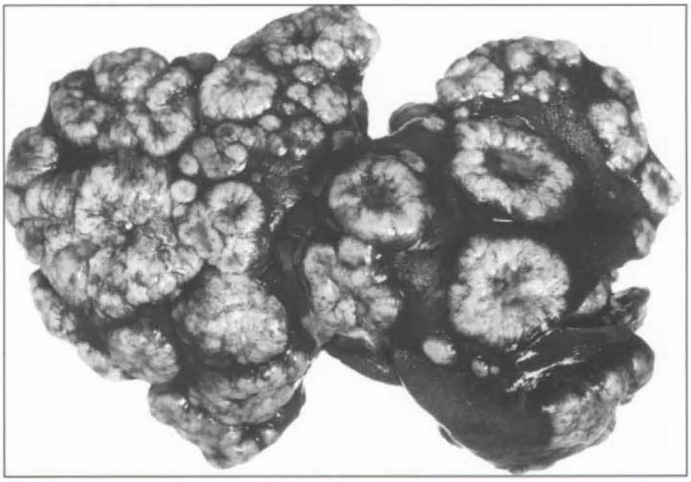

Figure 2.88 Multiple umbilicated foci of **cholangiocarcinoma** in a cat.

Affected livers are usually otherwise normal, with no suggestion as to an underlying cause. In humans, the relatively high incidence of bile duct carcinoma in some regions is related to chronic infestation with opisthorcid flukes, especially *Opisthorchis viverrini* and *Clonorchis sinensis*. Flukes and bile duct tumors have been observed in carnivores. The biliary change usually associated with these flukes is hyperplastic or adenomatoid proliferation of the epithelium and submucosal glands with goblet-cell (intestinal) metaplasia. There is minimal inflammatory change in the absence of superimposed bacterial infection.

Cholangiocellular tumors can usually be distinguished from the hepatocellular variety by their multiplicity, firmness, white color produced by more or less abundant stroma, and the typical umbilicate appearance of those that involve the capsule. The central depressed area can be due to necrosis or cavitation associated with insufficient neovascularization of the central parts of the tumor nodules. Even multiple nodules may not cause much enlargement of the liver, but the diffuse variety may cause great enlargement, although with retention of shape, and severe bile pigmentation.

Microscopically, cholangiocellular carcinoma is usually distinctly adenocarcinomatous, producing ductules and acini, and sometimes papillary formations. The cells are cuboidal or columnar, with a small amount of clear or slightly granular cytoplasm. The nuclei are small and fairly uniform, and nucleoli are not prominent. Mitotic figures are often abundant. The tubules do not contain bile, but in well-differentiated specimens may contain mucin. The epithelial components are separated by fibrous connective tissue stroma, which may have pronounced collagen deposition, the so-called *scirrhous response* that gives the tumor a firm texture. *Cholangiocarcinomas have a highly invasive growth pattern* (Fig. 2.89), and frequently metastasize to hepatic lymph nodes, lungs, and peritoneal cavity.

Primary intrahepatic cholangiocellular carcinoma can be difficult, and often impossible, to distinguish from metastatic adenocarcinomas, especially those of pancreatic origin. Mucus secretion and intrasinusoidal permeation

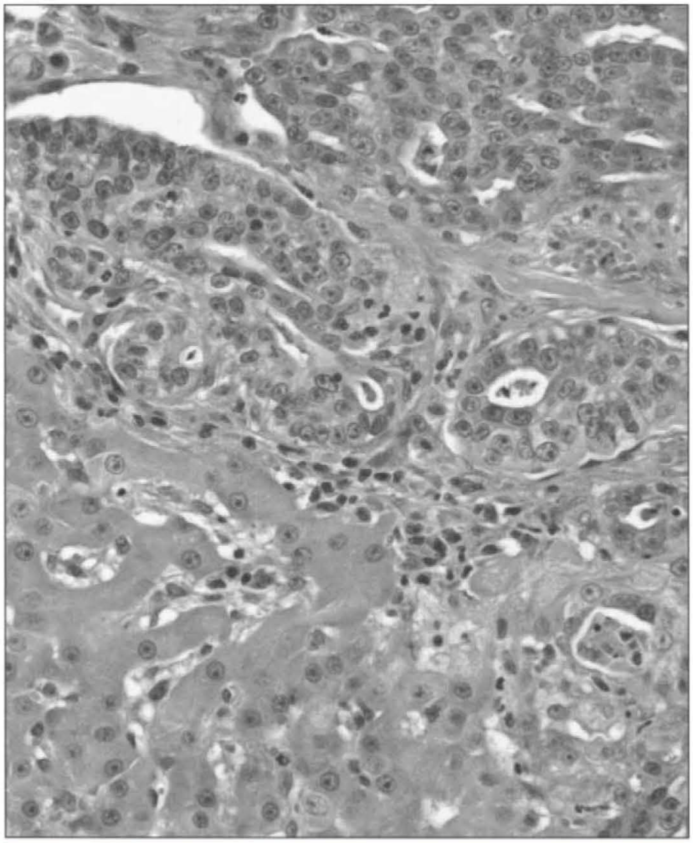

Figure 2.89 Invasive **cholangiocarcinoma** in a cat.

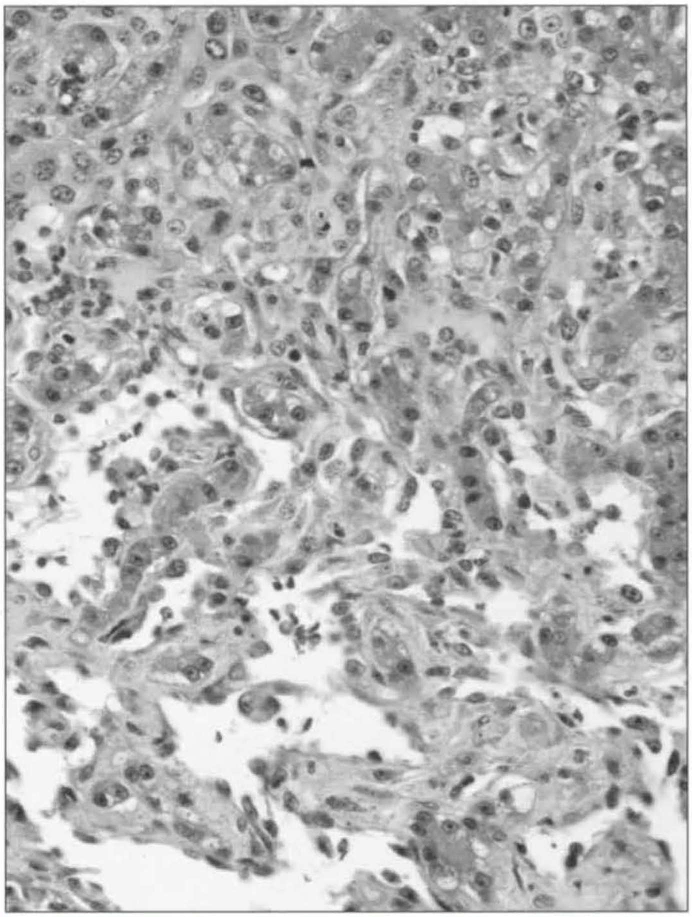

Figure 2.90 Neoplastic endothelial cells in this **hemangiosarcoma** are infiltrating hepatic sinusoids in a dog.

are more typical of biliary origin. Distinction from hepatocellular carcinoma of adenoid pattern may be assisted by demonstration of mucin, abundant mitotic figures, and presence of a prominent connective tissue stroma.

Extrahepatic bile duct adenocarcinoma has been reported in cats, as have adenocarcinoma of the gallbladder, and, more rarely, extrahepatic bile duct neuroendocrine carcinoma, and adenocarcinoma of the hepatopancreatic ampulla. Intramural mineralization of the gallbladder (so-called "porcelain gallbladder" in humans) has been reported in a dog, associated with an adenocarcinoma of the gallbladder neck.

Mesodermal tumors

Primary mesenchymal tumors of the liver are quite uncommon. Benign tumors of smooth muscle (**leiomyomas**) are occasionally observed in the gallbladder of dogs and oxen. **Leiomyosarcomas** and **fibrosarcomas** have been reported in the liver of cats and dogs; a single case of botryoid-type embryonal rhabdomyosarcoma has been reported in a cat. Hepatic **hemangiomas** have been reported in dogs and a pig. Primary **hemangiosarcomas** are seen in dogs, cats, and cattle; hemangiosarcoma of the liver should always be considered metastatic until proven otherwise by diligent search. Some of these tumors are solitary, large, and gray-white, with scattered hemorrhagic areas, and others are ill defined and cavernous. The latter may rupture into the peritoneal cavity to produce severe hemorrhage. Microscopically, it may be impossible to find malignant cells in or lining cavernous areas. At the margins of the tumor, there is a distinctive pattern or growth in which small, solid nodules of malignant cells may be found, or these cells can be found forming capillary structures or invading along preexisting sinusoids. The latter phenomenon is particularly characteristic (Fig. 2.90). As the cells invade along the sinusoids, perhaps in single file, they initially produce little distortion of the hepatic cords. Behind them the sinusoids are spread widely apart, and individual hepatocytes or portions of cords are isolated and appear to be floating freely, surrounded by a thin layer of connective tissue and neoplastic cells.

The **myelolipoma** is an unusual tumor that develops in the livers of domestic cats and wild felidae. The tumor develops as multiple growths in one or more lobes, varying from 0.5 to 5 cm in diameter; if they project above the surface of the liver, they are irregularly nodular. Myelolipomas are friable, and yellow to orange in color due to their high fat content. The neoplasm is composed of normal-appearing, mature adipocytes with a variable admixture of myeloid cells, including both mature and immature cells of the granulocytic, erythrocytic, and megakaryocytic series. In captive wild cats, similar lesions have been seen in the spleen. These were judged to be separate developments of the same process. Metastasis to other organs has not been reported.

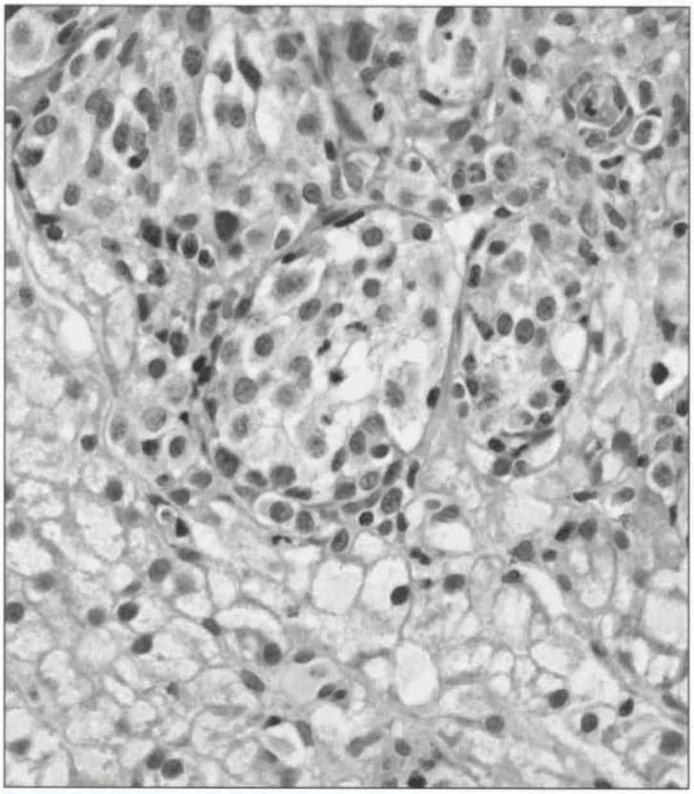

Figure 2.91 Histiocytic sarcoma in the liver in a dog.

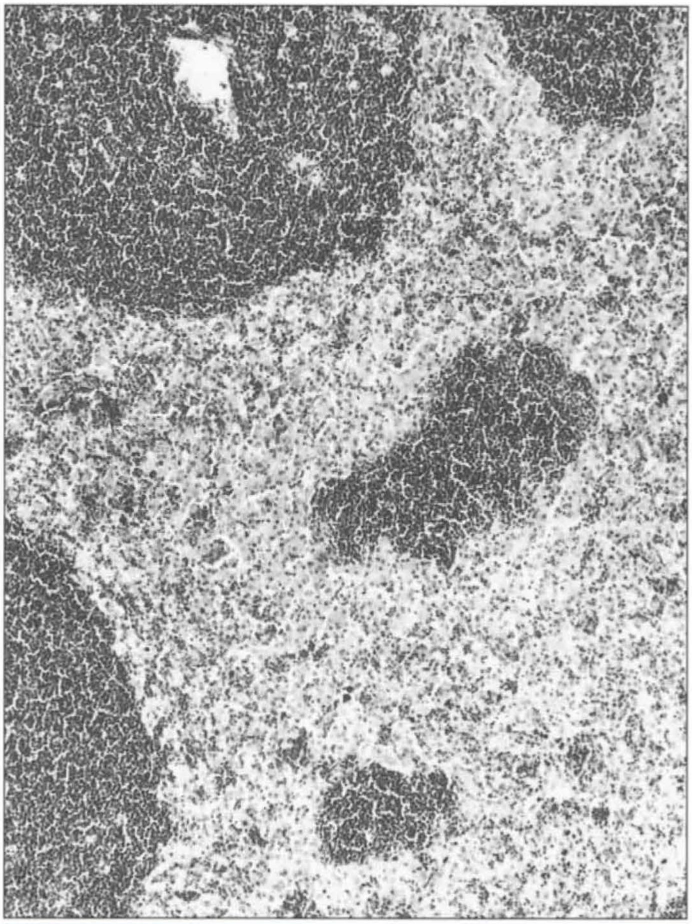

Figure 2.92 The malignant infiltrate in this **lymphoma** is heaviest in the stroma of triads and hepatic venules in a dog.

Metastatic neoplasms

The liver is particularly "fertile soil" that is readily accessible to most metastatic malignancies of the abdominal cavity. Metastatic solid neoplasms may be multiple but are usually not numerous and usually do not elicit clinical or biochemical evidence of hepatic injury or dysfunction. Malignancies arriving via the portal vein, such as pancreatic or gastric carcinoma or hemangiosarcomas, may practically replace the liver before producing clinical signs, one of which may be icterus caused either by extrahepatic bile duct obstruction, or by intrahepatic cholestasis, or both. Some of the carcinomas (e.g., thyroid, mammary), melanoma, and sarcomas from more remote sites also metastasize to the liver via the lungs and hepatic artery, and some (e.g., ovarian carcinoma or mesothelioma) implant on to the capsular surface from within the peritoneal cavity.

The hepatic perisinusoidal and periportal compartments are hospitable to various hematopoietic neoplastic cell types. Accordingly, disseminating lymphomas, malignant histiocytosis, myeloid and erythroid leukemias, and mast cell tumors often localize in the liver along hepatic sinusoids. Some lymphomas, histiocytic sarcomas (Fig. 2.91), and mast cell tumors also produce solid sarcoma masses in the liver. *Lymphoma in the liver is common* (Fig. 2.92), especially when the spleen is involved, and occasionally, the liver appears to be the major or primary site affected. Preferential involvement of the liver, spleen and bone marrow is characteristic of $\gamma\delta$ T-cell lymphomas in dogs. Diffuse infiltration of the liver in myeloproliferative disorders and mast-cell leukemia may cause extreme enlargement of the organ; the infiltrates localize preferentially in and around the sinusoids. Hepatic infiltration may be in discrete nodules 2 cm or more in size, but it is usually diffuse in the connective tissues of the portal triads.

Melanomas and hemangiosarcomas have characteristic gross features in the liver, but most metastatic tumors cannot be distinguished by their gross appearance. Sarcomas do tend to form a few large, smooth-surfaced nodules, and carcinomas do tend to form more nodules and to be umbilicate when in contact with Glisson's capsule. Hemangiosarcomas come from the spleen, usually, and may virtually replace the liver with small, blood-filled caverns. Their microscopic appearance is the same as that of the primary tumors (see above).

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3 Pancreas

Jennifer A. Charles

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EXOCRINE PANCREAS

General considerations

The pancreas is a composite organ consisting of exocrine acinar lobules and endocrine cells. The endocrine cells are especially concentrated in the islets of Langerhans but are also dispersed as individualized cells. Interdependency between the exocrine and endocrine components is expressed anatomically in a *portal circulation* in which afferent blood flow follows a glomeruloid path through the islets before supplying the adjacent acinar tissue. This vascular arrangement enables different islet cell types to modulate the functions of each other and also the exocrine tissue.

Pathological processes of the pancreas, with the exception of acute pancreatic necrosis that may manifest as an acute abdominal crisis, are usually revealed as metabolic disturbances, maldigestion, or cholestasis. There are large reserves of endocrine and exocrine function, and *metabolic and digestive disturbances do not manifest until a large proportion of the organ or of the islets of Langerhans is lost.* Pancreatic disease that does not provoke abdominal pain or compromise biliary drainage can remain clinically silent for prolonged periods.

The pancreas develops from two separate diverticula of the endodermal lining of the portion of the embryonic gut destined to become the duodenum. Pancreatic growth is initiated at sites where the endodermal epithelium contacts endothelium of the vitelline veins and the fusing dorsal aortae. Following endothelial contact, dorsal and ventral buds begin to evaginate. Arising dorsally from the cranial duodenum, the dorsal diverticulum ultimately gives rise to the left lobe of the pancreas and the accessory pancreatic duct. The ventral diverticulum develops from the main stem of the hepatic diverticulum and gives rise to the right lobe of the pancreas and the pancreatic duct. Under the inductive influence of mesenchyme into which it grows, the proliferating epithelium arborizes to form the exocrine ductal system. Epidermal growth factor (EGF) promotes this ductal development. Secretory acinar cells arise directly as diverticula from the sides of the smaller terminal branches of developing ductal epithelium very early in organogenesis. The endocrine tissue is also of endodermal origin and its differentiation precedes that of the exocrine elements. Endocrine differentiation is promoted by transforming growth factor-β_1 (TGF-β_1) and by contact with vascular endothelium.

The early embryonic pancreas widely expresses the homeobox gene *pancreatic duodenal homeobox factor-1* (*PDX1*). This gene plays an essential role in pancreatic development. Its targeted mutagenesis in mice leads to arrest of pancreatic development at the bud stage, although insulin- and glucagon-positive cells still differentiate. In adult life, expression of this gene is almost exclusively restricted to β cells of the islets in which it is believed to act as a key transcription factor for insulin. The basic helix–loop–helix gene, *p48*, is thought to be responsible for committing pluripotent endodermal cells to exocrine pancreatic differentiation.

Rotation of the hepatic and the ventral pancreatic diverticula results in apposition and partial fusion of the dorsal and ventral pancreatic anlagen. The duct systems of the two pancreatic anlagen also fuse to produce an anastomotic network, although this process may fail so that pancreatic development is incomplete.

There are differences between and within species as to which of the embryonic ducts serves as the main conduit in the developed pancreas. The *accessory pancreatic duct*, which enters the descending duodenum at the minor duodenal papilla, does not persist in small ruminants and in the majority of cats; it is the major duct in the dog, the lesser duct in the horse, and the only duct in the pig and ox. The *pancreatic duct* is the only duct in small ruminants and most cats and is the main duct in the horse; in dogs, it is the lesser duct and is occasionally absent. It opens into the cranial duodenum at the major duodenal papilla, with or immediately adjacent to the bile duct.

The mature ductal system is in two compartments: the *main collecting and interlobular ducts* lined by columnar mucus-secreting epithelium, and the *intralobular ducts* to which the acinar and endocrine cells are ontogenetically related. The latter relationships may explain the mixture of cell types that may be observed in pancreatic adenocarcinomas. Some of the intralobular ductules (which are synonymous with intercalated ducts) may terminate in a single spheroidal acinus. However, in humans and dogs and probably other species, the intralobular ductules tend to form anastomotic loops in which multiple acini may drain via branches of the same ductule. This three-dimensional arrangement helps to explain some of the lesional patterns seen in the exocrine parenchyma in chronic ductal obstruction. There may also be continuities between acinar cells of adjacent acini. Acinar cells are electrically coupled through tight junctions to form functional units that are larger than single acini; this arrangement is often reflected in pathological processes of hyperplasia and atrophy in which the target unit may be a collection of acini.

The lobules of the pancreas are separated by fine connective tissue septa that extend from the capsule. In general, each lobule receives blood from a single arterial branch and much of the circulation to the acinar parenchyma is supplied by a portal system of capillaries that emerge from the sinusoids of the islets. In species other than the horse, exocrine tissue distant from islets is perfused directly from arterial branches. The acinar portal circulation allows the islet hormones to exert trophic or inhibitory effects on acinar cells. *Insulin, in particular, and pancreatic polypeptide are trophic for acinar tissue; somatostatin and glucagon are inhibitory.*

The pancreas receives extrinsic *innervation* from the sympathetic and parasympathetic nervous systems and axons of enteric neurons also project to it. An intrinsic neural component is also present, with ganglia being randomly scattered throughout the parenchyma. These ganglia are thought to operate as *pacemakers* controlling endocrine and exocrine secretion. Pancreatic neurons synthesize, store, and release several biologically active substances, including acetylcholine, norepinephrine, encephalins, nitric oxide, vasoactive intestinal peptide, gastrin-releasing peptide, galanin, and neuropeptide Y. Alterations to the neural network are known to occur in humans and cats with chronic pancreatitis and dogs with pancreatic acinar atrophy, possibly as a compensatory mechanism to support secretory function or to stimulate tissue repair. How the various neurotransmitters control the pancreatic microcirculation and modulate the function of the exocrine and endocrine cells in health and disease is incompletely understood.

The major function of the exocrine pancreas is the synthesis and secretion of **digestive enzymes**. Acinar cells secrete trypsinogens, chymotrypsinogens, proelastases, procollagenase, procolipase, prophospholipase A_2, and procarboxypeptidases as inactive proenzymes, whilst amylase and lipase are secreted in their active forms. The proenzymes and enzymes are packaged within acinar cells into membrane-bound zymogen granules. Stimulation of exocrine secretion is mediated via vagal innervation and by release of secretagogues by endocrine cells of the duodenal mucosa. Entry of gastric acid and fatty acids into the duodenum causes local release of secretin which in turn stimulates secretion of water and bicarbonate by pancreatic ductal epithelium, particularly by the centroacinar cells that line the initial part of the intralobular ductules. The bicarbonate contributes to neutralization of gastric acid in the duodenum. However, this is not essential for maintaining a neutral pH for optimal activity of the digestive enzymes as the duodenal mucosa itself has an enormous capacity to secrete bicarbonate and absorb hydrogen ions. The presence of undigested lipid and amino acid peptides in the duodenal lumen promotes release of *cholecystokinin* (CCK) by mucosal endocrine cells; this secretagogue promotes rapid discharge of digestive enzymes from zymogen granules into the ducts. CCK also has a direct, rapid, and substantial trophic effect on acinar cells, as does its analogue cerulein; this effect is normally inhibited by trypsin. The observation in the 1940s of pancreatic hypertrophy in animals fed diets rich in soybeans was explained by the presence of a trypsin inhibitor in the feed.

Within the duodenum, exocrine secretions also inhibit bacterial proliferation, exert a trophic effect on the mucosa, and contribute to the normal degradation of exposed mucosal brush border enzymes. The exocrine pancreas also produces *intrinsic factor*, which is essential for the absorption of cobalamin (vitamin B_{12}) in the ileum. In addition, the organ plays an important role in *zinc homeostasis*, accumulating zinc absorbed from the intestines and secreting it into pancreatic juice. This function is partly mediated by the zinc-binding protein, metallothionein, which is present in acinar cells and exocrine secretions.

Several mechanisms protect the pancreas from autodigestion. From the earliest stages of synthesis, digestive proenzymes and enzymes are segregated into the lumen of the rough endoplasmic reticulum of the acinar cells. This sequestration is maintained during processing in the Golgi apparatus, where packaging into zymogen granules takes place. Enzymatic cleavage of an activation peptide from trypsinogen is usually initiated in the duodenal lumen by enterokinase secreted by duodenal mucosal cells. The activated trypsin cleaves activation peptides from the other digestive proenzymes. Muscular sphincters in the pancreatic ducts prevent reflux of activated enzymes from the duodenal lumen. If normal acinar secretion is inhibited, lysosomal hydrolases within acinar cells are capable of degrading zymogen granules. Protease inhibitors are also important defences against autodigestion. Acinar cells synthesize pancreatic secretory trypsin inhibitor and α_1-protease inhibitor (α_1-PI) (which is synonymous with α_1-antitrypsin). These inhibitors are released with the zymogen granules into the ducts. α_1-PI is also present in circulation and in intercellular spaces and acts as a readily diffusible, transient inhibitor of serine proteases. Also in circulation are α_1-macroglobulin and α_2-macroglobulin, which irreversibly bind serine and other proteases prior to removal by macrophages.

The exocrine pancreas is a labile organ. It synthesizes much more protein on a weight-for-weight basis than does any other tissue and consumes a correspondingly large amount of precursor substrate. The response of the exocrine pancreas to changes in nutrient intake is rapid, and adaptation to new diets can produce dramatic alterations in the composition of pancreatic juice. *Secretion of proteases* is a reflection, in part, of dietary protein levels; *amylase secretion* is influenced by the level of dietary carbohydrate and by plasma levels of cortisol and insulin. The dietary influence on *lipase secretion* is less clear but secretion is to some extent dependent on dietary protein levels. Acinar cell hypertrophy and hyperplasia leading to organ enlargement occur in response to diets rich in protein and energy. When these substrates are withdrawn, the organ reverts to normal mass via autophagy and apoptosis. If dietary protein and energy become suboptimal, acinar cells and the exocrine organ atrophy.

Maternal malnutrition during gestation and lactation may retard maturation of the exocrine pancreas of the offspring. Paradoxically, the enzyme content of the pancreas may increase in the offspring; whether this increase enhances the ability of the offspring to resist malnutrition remains unclear.

The exocrine pancreas is normally functionally immature at birth, and synthesis of some digestive enzymes may not commence until several weeks postnatally. Enzymatic activity of milk and proximal intestinal secretions compensate for this insufficiency in suckling animals. The weight of the pancreas increases rapidly during the immediate postnatal period, chiefly because of an increase in the number rather than size of acinar cells. Colostrum, which contains growth-promoting factors such as epidermal growth factor (EGF) and insulin-like growth factors, plays a role in this early organ growth. Release of endogenous gut hormones such as gastrin that have trophic effects on the pancreas may also contribute. Glucocorticoids promote maturation of the acinar cells, and the enzyme activity of the organ increases with age in the perinatal period. At weaning, dietary and hormonal changes combine to cause abrupt increases in pancreatic enzyme synthesis and secretion. In animals weaned early, the surge in circulating glucocorticoids is considered to be more important than the dietary changes in triggering the alterations to enzyme output.

In aged humans, the weight of the pancreas declines and there may be intralobular fibrosis, degranulation and atrophy of acinar cells, and ductal epithelial hyperplasia. These involutionary changes are associated with decreases in the volume of pancreatic secretions and in their content of bicarbonate and digestive enzymes. The responsiveness of the human pancreas to such secretagogues as secretin and CCK also diminishes with advancing age. Altered exocrine pancreatic structure and function are likely in aged domestic animals but are yet to be described.

Acinar, ductal, and islet cells are all capable of **regeneration**. Rapid regeneration of exocrine parenchyma may follow acinar destruction. Slower regeneration of both exocrine and endocrine elements follows partial surgical ablation. Complete restitution of exocrine parenchyma by mitotic division and hypertrophy of viable acinar cells may follow minor cell loss. Extensive or persistent parenchymal injury may provoke proliferation of ductular epithelium and connective tissues. *The success of pancreatic repair following injury is influenced by the activity of various peptide growth factors, by the integrity of the blood supply and excretory ducts, and by competitive fibrogenesis.*

Ductular epithelium has the potential to differentiate towards acinar cells, islet cells, and even hepatocytes. Known stimuli of mitotic division of ductular epithelial cells include EGF, keratinocyte growth factor, and TGF-α.

Stellate cells akin to those of the liver have been identified in the normal pancreas in a periacinar location and appear to be the major mediators of pancreatic fibrogenesis. In the quiescent state, pancreatic stellate cells express desmin but not α-smooth-muscle actin (α-SMA). Following activation by injury, these cells acquire a myofibroblastic phenotype and express α-SMA. They proliferate in response to platelet-derived growth factor (PDGF), and, in response to TGF-β, synthesize fibrillar collagens, including collagen I. Macrophages may release both PDGF and TGF-β, but injured acinar cells adjacent to sites of fibroplasia appear to be a significant paracrine source of TGF-β. Activated stellate cells are themselves capable of producing TGF-β_1. They also express matrix metalloproteinases (MMPs), which are capable of degrading extracellular matrix components, and tissue inhibitors of these. As TGF-β_1 reduces the expression of MMP-3 and MMP-9, pancreatic fibrosis may be a consequence of both increased collagen synthesis and reduced collagen degradation.

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Developmental anomalies of the exocrine pancreas

With a few important exceptions, anomalies of the exocrine pancreas and its ducts are usually of little clinical importance.

Agenesis or **aplasia** (complete absence) of the pancreas may be associated with more generalized and severe malformations incompatible with survival.

Pancreatic hypoplasia *occurs sporadically in calves.* The defect is in the exocrine tissue; the islets are normal in number and morphology. The hypoplastic organ is small, pale, and loosely textured. Its margins are poorly defined, but the parenchyma is centered on a normal duct system. Microscopically, acinar tissue is present as small scattered clusters of cells in glandular array. Some of the cells may appear well differentiated and contain zymogen granules, but most are small, basophilic, and of indifferent type. Affected calves have clinical signs of exocrine pancreatic insufficiency. Pancreatic hypoplasia was originally described in dogs, but the condition is now regarded as an atrophic rather than hypoplastic disorder.

Variations in the disposition of the ducts *are common in dogs.* Most dogs have two separate ducts opening into the duodenum, with interductal anastomoses within the pancreas, and with the accessory pancreatic duct the major conduit. A small percentage of dogs may have only an accessory duct entering the duodenum, and some may have three functional openings into the intestine.

Connection of the pancreas to the spleen by a band of pancreatic tissue has been described as a congenital anomaly in a piglet. An **annular pancreas**, *characterized by a thin flat ring of normal pancreatic tissue completely encircling the duodenum*, has also been observed in dogs and piglets; the annulus may cause duodenal stenosis. This anomaly probably reflects failure of normal embryonic rotation of the duodenal anlagen.

Congenital stenosis or **cystic dilation of a pancreatic duct** is occasionally reported in domestic animals, and *congenital intrapancreatic cysts* lined by squamous or low cuboidal epithelium are occasionally seen in lambs. Cystic dilation of intra- and interlobular pancreatic ducts accompanied by polycystic kidneys and cystic bile ducts is reported in piglets and goat kids. A primary inherited defect in epithelial cell growth and differentiation is suspected. Affected animals typically develop renal failure.

Saccular ductal distension to form a structure resembling a gallbladder and termed a **pancreatic bladder** occurs rarely in cats. Such cysts may be congenital or acquired. They are usually asymptomatic, but large cysts may compress the bile duct to cause jaundice. *True congenital duplication of the gallbladder* with the accessory organ arising from the ventral pancreatic bud has also been observed in cats.

Ectopic or **accessory pancreatic tissue** is rarely identified. In dogs, small pancreatic nodules may be found in the submucosa or muscularis of the stomach or intestine (especially in the duodenum), the mesentery, gallbladder, spleen, or liver. Ectopic pancreatic tissue has been described in the spleen in cats. The ectopic rests are of normal morphology, although islets are not always present. The rests are presumed to be functional but a ductal outlet may not be demonstrable. Ectopia may result from dislocation of portions of the duodenal buds during development, by persistence of an anlage that would normally regress, or by activation of pancreatic transcription factors within pluripotential endodermal epithelium. In humans, ectopic pancreatic tissue is increasingly recognized in association with abnormal gut peristalsis, with gut mucosal inflammation or ulceration, and as a site of neoplastic development. No such association has been made in domestic animals.

Intrapancreatic hepatocytes are occasionally observed as an incidental finding, especially in neonates. Hepatocytes may also appear in the adult pancreas during attempted regeneration after massive lobular injury. According to experimental data, the origin of the hepatocytes can vary. Transdifferentiation of acinar cells can occur directly, without intermediate cell division, and can be induced *in vitro* by administration of synthetic glucocorticoids. Alternatively, the hepatocytes may be derived from pancreatic ductular and periductular interstitial cells capable of multidirectional differentiation. Such pancreatic stem cell equivalents may be akin to the oval cells of the liver. The hepatocytes may be found as individualized or clustered cells within the exocrine parenchyma, ductal epithelium, or even islets.

Pacinian corpuscles are normally prominent in the interlobular connective tissues of the pancreas of the *cat*, and may be grossly visible as discrete nodules 1–3 mm in diameter.

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Regressive changes of the exocrine pancreas

Degeneration, necrosis, and atrophy

The exocrine pancreas is a labile organ that is susceptible to many adverse influences. Degeneration or necrosis of individual acinar cells may occur in various local and systemic disorders, including febrile states, viral infections, intoxications, hypovolemic or septic shock, nutritional deficiencies and cachectic disorders, and with prolonged fasting. Degeneration of acinar cells is always a component of exocrine pancreatic atrophy, and is also expected when inflammatory, necrotizing, or neoplastic processes involve the pancreas.

Pancreatic degeneration should not be confused with postmortem autolysis. **Autolysis** is rapid and is accelerated by high temperatures and by rough handling of the organ at necropsy, with cell rupture and release of digestive enzymes. The color of the organ may grossly deepen to a dark red-brown or green as autolysis progresses. Postmortem hemorrhage into the interstitium of the body of the pancreas is frequently seen in dogs and horses. Patchy autolysis is common in histological material, with the affected areas appearing blanched, tending to resist eosin, and staining slate-gray with hematoxylin. Autolytic dissociation of exocrine cells and nuclear pyknosis may develop by 4 hours after death.

Apoptosis is accompanied by little or no inflammation. *Apoptosis is responsible for cell deletion during embryonic development, normal involution, pathological atrophy, and during regression of hyperplastic organs.* It can also mediate cell loss following mild cellular injury. Apoptosis often occurs in the pancreas but is infrequently described. It is a distinctive change ultrastructurally but can be easily confused with cell necrosis at the light microscopic level. Apoptosis has been observed as the principal means of acinar cell loss in zinc toxicity in ducklings, and is also reported in experimental duct ligation, after lipopolysaccharide administration, and in pancreatic involution following hypertrophy.

Acinar cells that have undergone degeneration are usually smaller than normal. The nucleus moves centrally and there is variable loss of zymogen granules. In fatty degeneration, the cytoplasm may contain single large clear vacuoles or numerous small vacuoles. In many conditions, degenerate and necrotic acinar cells are interspersed. Necrotic cells shrink and are often surrounded by narrow clear halos. The cytoplasm may be vacuolated or hypereosinophilic, and the nucleus appears pyknotic or karyorrhectic.

Multifocal pancreatic degeneration and necrosis *involving small clusters of acinar cells is a common microscopic finding in systemic infections with epitheliotropic viruses.* Associated inflammation is often minimal. In most cases, the lesions are incidental to the course of the disease unless release of digestive enzymes magnifies the process or significant injury is also sustained by islet cells. Agents responsible include adenoviruses in several species (Fig. 3.1), *Canine distemper virus* and *Canine parvovirus*, *Felid herpesvirus 1*, *Foot-and-mouth disease virus*, *Encephalomyocarditis virus*, *African swine fever virus*, and virulent strains of *Classical swine fever virus*. Low-virulence strains of *Classical swine fever virus* can be responsible in congenitally infected piglets. In both classical swine fever and African swine fever, necrosis of islets may be more pronounced than that of acinar cells. Multifocal degeneration and necrosis may also be seen in many intoxications, including trichothecene mycotoxicosis of pigs due to consumption of T-2 toxin, deoxynivalenol (vomitoxin), or diacetoxyscirpenol (anguidine) produced by *Fusarium* species. These mycotoxins may also cause pancreatic interstitial edema, hyperplasia of ductular epithelium, and necrosis of islet cells.

Acinar degeneration, necrosis, and resultant exocrine pancreatic atrophy may be produced experimentally in rodents by administration of ethionine or excess arginine, methionine, or phenylalanine. In some of these experimental models, massive necrosis of the pancreas may ensue and there may be necrosis of peripancreatic and more distant adipose tissue. Experimental selenium toxicity (particularly organic selenium) has produced pancreatic edema, hemorrhage, and necrosis in pigs. Intoxication by *Cassia occidentalis* may also cause necrosis of acinar cells in pigs.

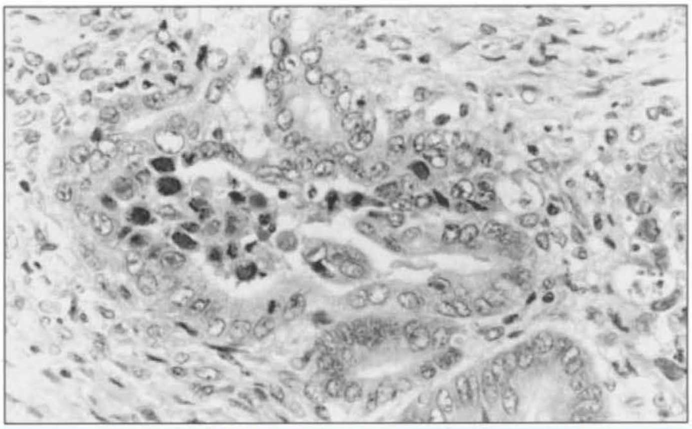

Figure 3.1 Intranuclear adenoviral inclusion bodies in the pancreatic ductal epithelium of a foal with combined immunodeficiency.

Zinc toxicity *causes striking pancreatic lesions in several species.* Pancreatic damage has been produced experimentally in cats, sheep, calves, chickens, and ducklings and has been observed in naturally occurring intoxications of caged birds, dogs, sheep, and calves and in piglets receiving total parenteral nutrition. Zinc toxicity may cause mild nephrosis in several species, bone and joint lesions resembling osteochondrosis in pigs, Heinz body hemolytic anemia in dogs, and necrotizing abomasitis and duodenitis in ruminants, but the pancreas bears the brunt of the injury in subacute and chronic exposure and is often the only organ in which lesions are found.

Toxicity may result from ingestion of zinc from zinc-plated or galvanized metal surfaces or recently minted pennies, from topical use of zinc oxide creams and lotions, from formulation errors in feed or milk replacers, or from prophylactic or therapeutic use of zinc salts for conditions such as facial eczema in sheep and cattle.

There are qualitative differences amongst species in the pancreatic lesions but, in all, *degeneration and necrosis of exocrine elements dominate, with exocrine atrophy and fibrosis developing in chronicity.* The islets are usually unaffected. In chronic stages, atrophic acinar cells without discernible zymogen granules may form dilated ductule-like structures embedded in scar tissue (Fig. 3.2). These structures are sometimes referred to as *tubular complexes* and are often mistaken for hyperplastic ductules. Some tubular complexes may be lined by attenuated ductular rather than acinar epithelium; these are thought to be a consequence of dilation or other modification of pre-existing intercalated ducts.

In piglets, the acinar cells appear to be targeted and undergo necrosis and progressive atrophy, with ultimate development of severe interstitial fibrosis. Acinar cells are also targeted in ducklings, but are deleted by apoptosis.

In sheep and, to a lesser extent, cattle, ductal epithelium appears to be the target of early necrosis. Inflammation, acinar degeneration and necrosis, and interstitial fibrosis are largely centered on the damaged ducts. In many respects, the lesions in ruminants resemble those of pancreatic duct obstruction and may in part result from obstruction

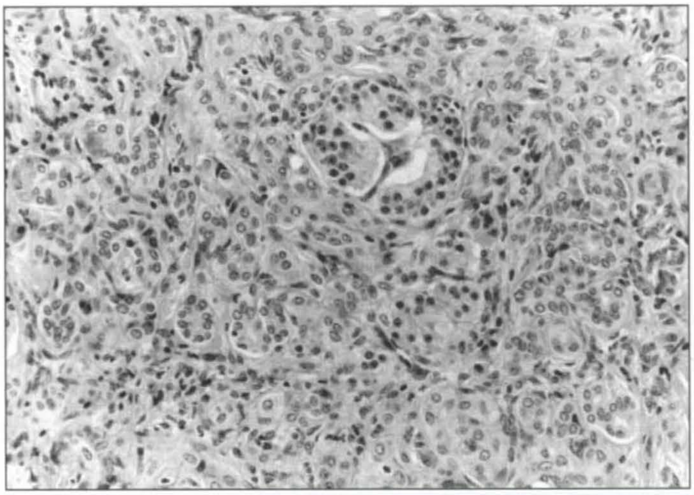

Figure 3.2 Tubular complexes (ductule-like structures) at the periphery of a lobule in a cow with **chronic zinc toxicity**.

of the ducts by debris and inflammation. However, the lesions of zinc toxicity tend to develop at a slower rate and there is less uniform involvement of lobules than is the case in duct obstruction.

Lipomatosis of the pancreas occurs occasionally in cats, pigs, and cattle, usually as part of generalized obesity but sometimes restricted to the pancreas. Adipose tissue accumulates in the interstitium and disperses the parenchyma to create a false microscopic impression of diminished exocrine mass with fatty replacement. Although there may be some pressure atrophy of the parenchyma, *lipomatosis is not functionally significant.*

Pancreatic lipofuscinosis is occasionally seen in *dogs deficient in vitamin E.* Accumulation of lipofuscin causes khaki to brown discoloration of the pancreas and of *intestinal smooth muscle* grossly; the urinary bladder and mesenteric lymph nodes may also be affected. The golden-brown pigment granules in the basal cytoplasm of pancreatic acinar cells and intestinal myocytes are periodic acid–Schiff-positive, sudanophilic, and weakly acid-fast. Microscopic pigment accumulation may occur in other tissues, including the pigmented epithelium of the retina.

Vacuolation of exocrine acinar, ductal epithelial and islet cells due to accumulation of substrates in lysosomes can be seen in *storage disorders* such as α- and β-mannosidosis and galactosialidosis. Vacuolation of ductal epithelium due to glycogen accumulation is a feature of *diabetes mellitus.*

Exocrine pancreatic atrophy is common but is often overlooked. It may be primary or secondary to other disorders of the pancreas or its ducts. Juvenile pancreatic atrophy of primary type is a common syndrome in dogs and is discussed separately below.

In **primary atrophy**, the organ is diffusely and uniformly affected. A decrease in mass is usually the only gross abnormality discernible, although fatty infiltration may occasionally be noted. Microscopic changes are often subtle.

Acinar cells undergo atrophy if dietary protein or energy levels are suboptimal. Diffuse atrophy of the organ is common in domestic animals in starvation, prolonged anorexia, cachexia, protein-caloric deficiency, and maldigestive and malabsorptive syndromes. In protracted starvation, the pancreas may be reduced to less than 10% of normal mass. Other tissues, particularly the liver, may also atrophy in these conditions. Diffuse pancreatic atrophy may also result from deficiencies of essential amino acids, vitamin A or trace elements such as zinc, copper, and selenium (Fig. 3.3).

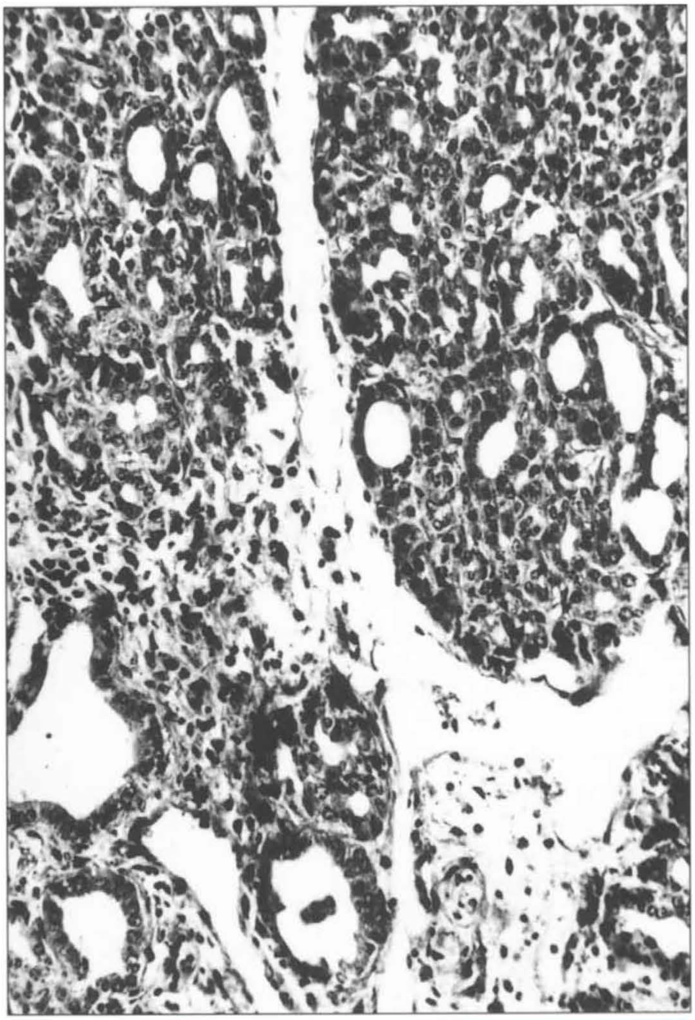

Figure 3.3 Atrophy of acinar parenchyma and dilation of ducts in **vitamin A deficiency** in a calf.

Acinar atrophy may be a consequence of altered intracellular metabolism or the cells may be indirectly affected by changes to the interstitium. The molecular pathogenesis undoubtedly differs according to the cause. In atrophy of copper deficiency, for example, experimental evidence suggests that the initial alteration may involve the basement membranes of acinar cells and the adjacent interstitium, leading to acinar cell dissociation. Irrespective of the cause, *acinar atrophy is characterized microscopically by progressive depletion of zymogen granules, cell shrinkage, and cytoplasmic basophilia* (Fig. 3.4A, B). Cytoplasmic vacuolation may be prominent. Ultrastructurally, autophagic vacuoles develop rapidly within acinar cells undergoing atrophy and residual bodies are freely extruded. Apoptosis may be widespread. The cells lose their polarity and dissociate so that the glandular arrangement is lost, with the small, darkly staining cells arranged into diffuse sheets. The reduction in number and size of acinar cells increases the prominence of the ductular system and of the interstitial connective tissue.

Secondary pancreatic atrophy is the result of local processes, particularly duct obstruction but also interstitial fibrosis, chronic

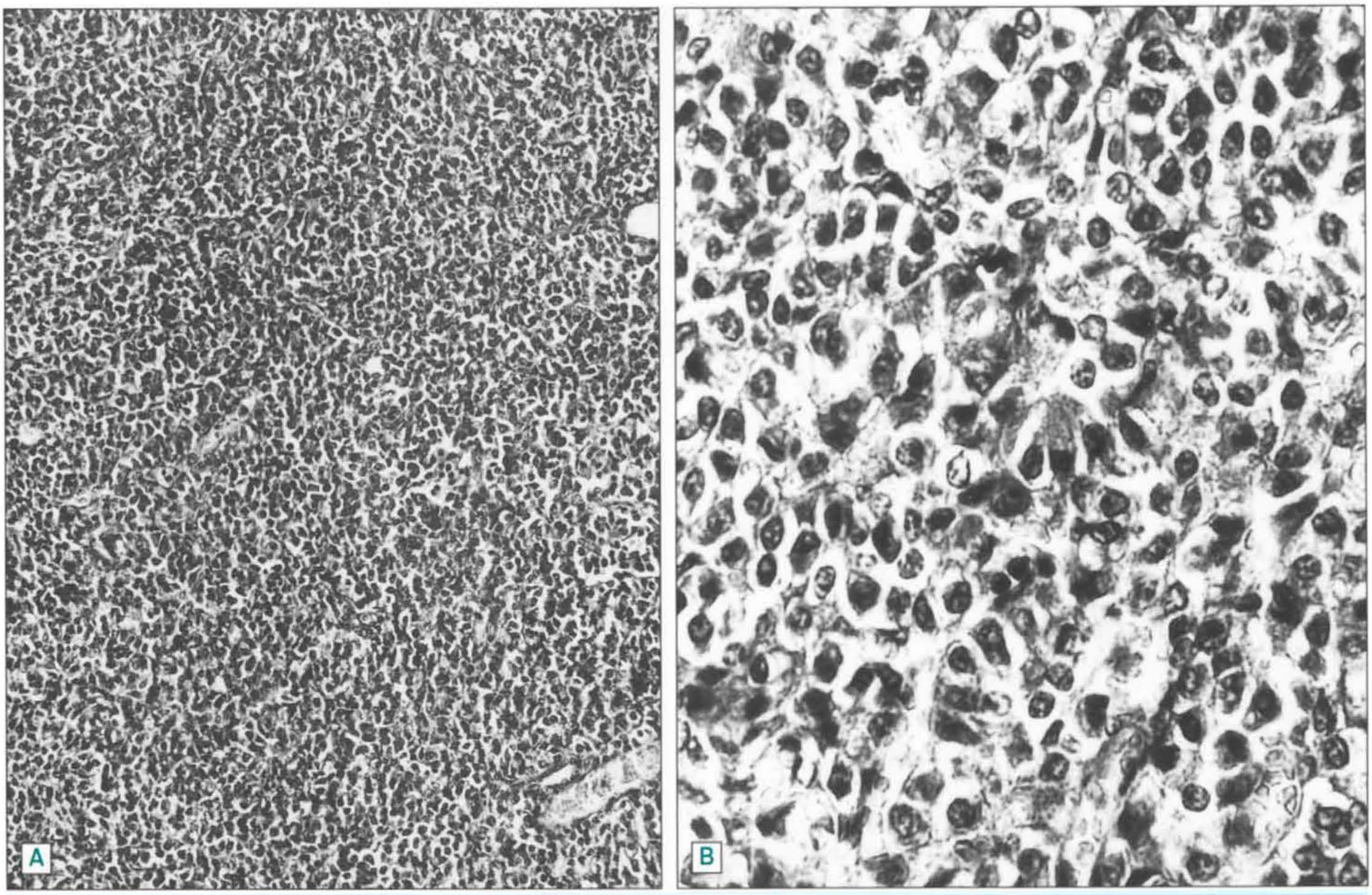

Figure 3.4 Severe chronic **nutritional atrophy** of the exocrine pancreas in a pig. **A.** Low-power view. **B.** Detail of (**A**) demonstrating loss of cell polarity and zymogen granules and cytoplasmic vacuolation.

inflammation, or neoplasia. Atrophy in these conditions is of nonuniform distribution. Affected areas are reduced in size, misshapen, coarsely nodular, and firm. Histologically, the atrophic areas contain few small acinar cells and consist largely of fibrofatty connective tissue in which islets, ducts, nerves, and blood vessels are condensed.

Obstruction of the pancreatic ducts may result from external compression by neoplasia or chronic inflammation and associated fibrosis or from luminal occlusion by parasites, inflammatory exudate, scar tissue, or pancreatoliths. Experimental ligation of the ducts causes ductal ectasia (with rupture of intercellular junctions between the lining epithelial cells), rapid atrophy, degeneration and loss of acinar cells by apoptosis and necrosis, mild interstitial edema and inflammation, and mild periductal fibroplasia (Fig. 3.5A, B). A later development is proliferation of ductular epithelium, particularly in the smallest ductules, and this may give rise to new ductules, acinar cells, or islet cells. Hyperplastic ductules need to be distinguished from tubular complexes formed by atrophy and dilation of acini or pre-existent ductules.

In experimental duct obstruction, discharge of digestive enzymes by acinar cells ceases despite continued synthesis, and the digestive enzyme concentration within the pancreas increases. Ultrastructurally, zymogen-containing organelles at the apices of the acinar cells enlarge and lysosomal enzymes become redistributed into them. The organelles then become more fragile and susceptible to rupture. It is postulated that duct obstruction may interfere both with zymogen exocytosis and with events in lysosomal enzyme transport from the Golgi complex to lysosomes so that lysosomal enzymes are diverted into secretory pathways. Such co-localization of lysosomal hydrolases and digestive enzymes is also a phenomenon of several diet- and secretagogue-induced models of acute pancreatic necrosis. Lysosomal cathepsin B can activate trypsinogen. Co-localization can therefore result in intracellular release of activated digestive enzymes and thereby mediate acinar cell loss. However, ductal obstruction alone is insufficient to provoke acute pancreatic necrosis.

Because endocrine tissue is spared from atrophy following temporary duct obstruction, duct ligation was the method experimentally employed initially to isolate insulin from the pancreas. However, more intense interstitial inflammation and fibrosis develop in spontaneous duct obstructions (other than developmental stenoses) than in experimental surgical models. Exocrine atrophy due to spontaneous ductal obstruction is therefore often compounded by ischemia and pressure atrophy induced by scarring, and the islets may also sustain injury.

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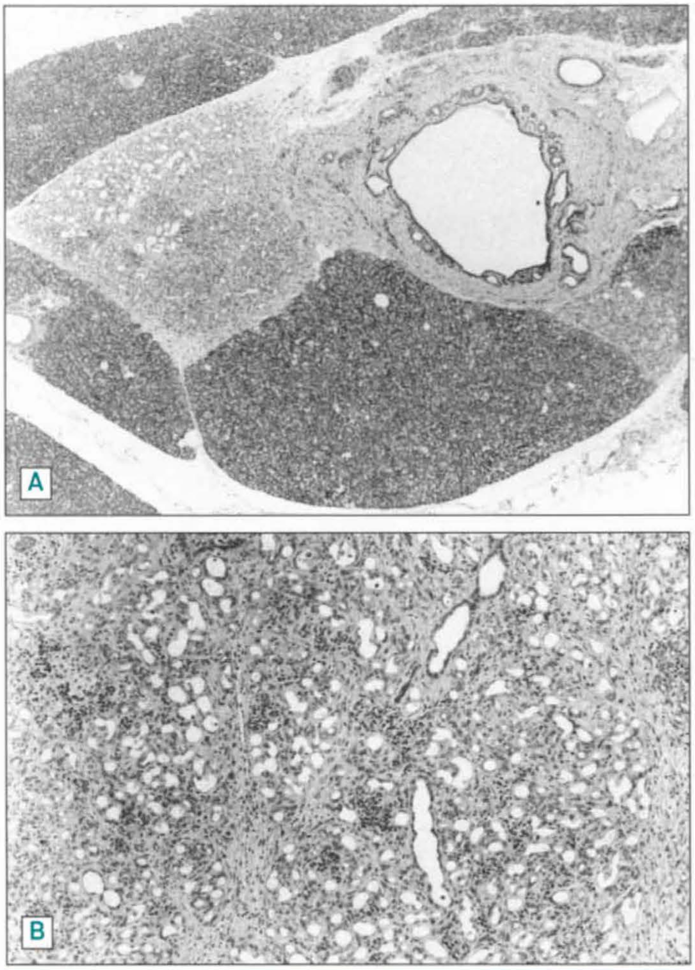

Figure 3.5 A. Severe chronic **lobular atrophy and ductal dilation** following obstruction of a duct in a dog. **B.** Detail of **(A)** showing **severe parenchymal atrophy**, condensation of connective tissue and ducts, ductal dilation, and lymphocytic interstitial inflammation.

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Pancreatic acinar atrophy in dogs

Pancreatic acinar atrophy of juvenile onset is an important condition in dogs, but until recently was designated erroneously as "pancreatic hypoplasia." It is most commonly diagnosed in German Shepherds, in which an autosomal recessive pattern of inheritance has been demonstrated. A familial predisposition has also been noted in Rough-Coated Collies and is suspected in English Setters. The condition is reported sporadically in other breeds. In both the German Shepherd and the Collie, the prevalence of the condition has been estimated to be approximately 1%.

Atrophy of the exocrine parenchyma is preceded by intense but patchy infiltration by T lymphocytes in association with degeneration, atrophy, apoptosis, and necrosis of acinar cells. Cytotoxic CD8+-positive cells predominate over CD4+-positive cells in the pancreas as the disease progresses. The lymphocytes may be found both between and within acinar cells, and intraepithelial lymphocytes may also be observed within the ductal epithelium. Lymphoid follicles may form in the parenchyma, and plasma cells, macrophages, and eosinophils may also infiltrate. Plasma cells are more commonly observed in more advanced areas of parenchymal destruction. Reactive fibroplasia is not a significant feature of the process. This subclinical stage of the disease has been termed **atrophic lymphocytic pancreatitis**. *The nature of the lesion suggests that the condition may be an autoimmune cell-mediated process directed against acinar cells*; there is currently no evidence to incriminate autoantibodies in the pathogenesis.

Clinical signs of *exocrine pancreatic insufficiency* do not appear usually until affected dogs are 6–12 months of age. However, the natural course of the disease is highly variable. The subclinical phase may last years and, in some dogs, insufficiency may never manifest. In a significant proportion of dogs, the clinical onset may be immediately preceded by concurrent illness or by a change in the normal household environment or diet.

By the time clinical signs emerge, exocrine atrophy is advanced and there is usually little remaining evidence of the preceding degenerative and inflammatory events. At necropsy of clinically affected dogs, the intestines are distended by bulky ingesta. The peritoneal cavity is devoid of fat and the clearness of the mesenteries allows the flimsy tissue of the atrophic pancreas to be recognized (Fig. 3.6). The main ducts and their larger tributaries can be readily

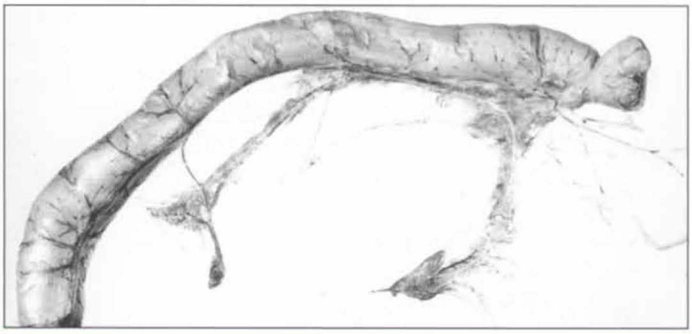

Figure 3.6 Condensation of exocrine and endocrine tissue around the normal duct system in advanced **juvenile pancreatic acinar atrophy** in a dog.

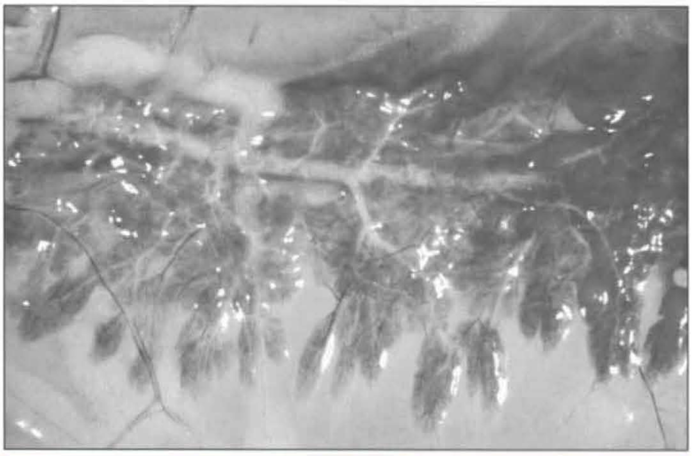

Figure 3.7 Residual parenchyma is condensed about prominent ducts in advanced **juvenile pancreatic acinar atrophy** in a dog.

discerned (Fig. 3.7) and many of the smaller ducts are recognizable grossly with the aid of transillumination. The ducts are of normal size, length, and configuration. Surrounding them is a thin pink veil of residual acinar parenchyma. Microscopically, the pancreatic lobules are small and composed of small acinar cells that stain darkly and do not assume a glandular arrangement. Tubular complexes may be identifiable within the atrophic lobules. Occasional isolated lobules of normal appearance may be present. There is increased prominence of the connective tissues, ducts, islets, blood vessels, and nerves of the pancreas due to condensation (Fig. 3.8). Mild interstitial and periductal fibrosis may be present, but fatty replacement of the organ is more common.

The islets are usually histologically normal. However, haphazard distribution of various islet cell types, hyperplasia of pancreatic polypeptide and delta islet cells, and emergence of a profusion of nerve fibers immunoreactive for enkephalins and vasoactive intestinal peptide within islets have been noted in dogs have with pancreatic acinar atrophy. The significance of these observations is uncertain.

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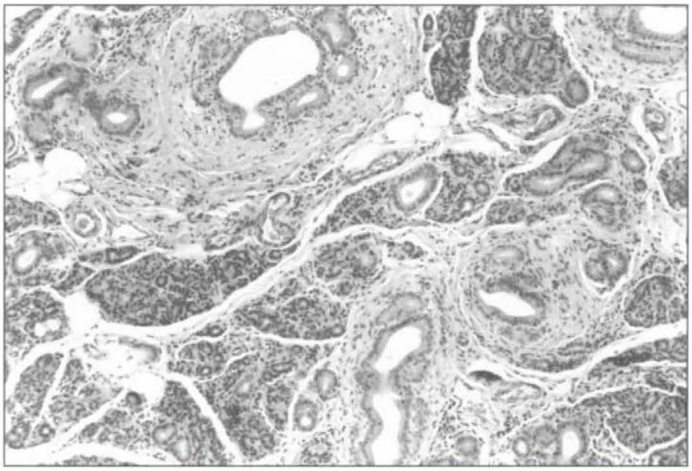

Figure 3.8 Microscopic appearance of **juvenile pancreatic acinar atrophy**.

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Exocrine pancreatic insufficiency

Progressive loss of exocrine parenchyma ultimately leads to maldigestion of nutrients. The considerable functional reserve of the pancreas, however, delays the clinical onset of exocrine pancreatic insufficiency (EPI) until at least 85–90% of secretory capacity has been lost. Salivary, gastric, and intestinal mucosal enzymes may also assist in digestion in earlier stages of pancreatic disease, as may bile salt-activated lipase in milk in suckling animals.

EPI is most commonly diagnosed in dogs but occurs sporadically in other species. Juvenile pancreatic acinar atrophy is the most common *cause* in the dog, but the condition can also result from pancreatic necrosis, chronic pancreatitis, or exocrine pancreatic neoplasia. In cats, the most common cause of EPI is chronic pancreatitis. EPI referable to pancreatic hypoplasia is occasionally observed in calves. Infrequent causes of EPI include gastric hyperacidity leading to reduced activity of pancreatic enzymes (especially lipase) within the duodenum, and exocrine pancreatic atrophy referable to chronic ductal obstruction, or severe and protracted protein malnutrition. In any species, EPI may be accompanied by diabetes mellitus if the underlying pathological process has destroyed islets as well as exocrine parenchyma.

The most common clinical signs of EPI are diarrhea and chronic weight loss despite a normal to voracious appetite. The hair coat may be of poor quality and marked muscle atrophy may be apparent in some animals. Severely cachectic animals may be weak but, unless there is concurrent diabetes mellitus, animals with EPI are usually bright and alert. Some may display pica or coprophagia, and affected cats may vomit or be anorexic. Flatulence and marked borborygmus are common. The feces are commonly pale, soft, voluminous, and malodorous but may occasionally be watery or even of normal appearance.

Steatorrhea is not invariably present, but can lead to greasy soiling of hair of the tail and perineum, especially in cats. There may be a pot-bellied appearance due to bulky intestinal contents, and affected dogs are at increased risk of intestinal accidents, especially mesenteric torsion. Hepatic lipidosis may also develop in dogs and cats with EPI.

Malassimilation of nutrients in EPI is not simply due to failure of intraluminal digestion by pancreatic enzymes. Pancreatic proteases normally cleave proenzyme forms of small intestinal microvillar membrane enzymes and also inactivate or degrade exposed brush border enzymes. In several species, EPI is associated with increased activity of the jejunal brush border enzymes, sucrase, and maltase, and an increase in high-molecular-weight microvillar membrane proteins, which are thought to be intestinal proenzymes. These alterations may impair the intestinal phase of digestion and absorption. In spontaneous EPI, increased activity of enterocyte lysosomal degradative enzymes occurs, possibly to compensate for the above changes. Protein synthesis by the jejunal mucosa is also reduced in dogs with EPI. The mechanism is unclear but malnutrition and decreases in luminal trophic factors (such as pancreatic secretions and products of digestion) and humoral trophic factors (such as insulin, glucagon, and gastrin) may be implicated. Intestinal transport mechanisms for monosaccharides, disaccharides, fatty acids, and amino acids are also disturbed in EPI to contribute to malabsorption. These functional changes in the intestinal mucosa are not usually associated with histological lesions. However, dietary sensitivity leading to inflammatory bowel disease is also a potential consequence of EPI as there is increased intestinal mucosal exposure to antigenic macromolecules due to the deficiency of pancreatic proteases.

Small intestinal bacterial overgrowth (SIBO) *is a syndrome of bacterial proliferation in the upper small intestinal lumen associated with chronic or recurrent small-bowel diarrhea.* It commonly complicates EPI in dogs, especially German Shepherds, but has yet to be reported in cats with EPI. In SIBO, the feces are often foul-smelling and watery, and diarrhea is occasionally accompanied by vomiting. Alterations in the duodenal/jejunal microflora in EPI are chiefly a consequence of the presence of excess luminal substrates, but loss of the antibacterial properties of pancreatic secretions and abnormalities in intestinal immunity or motility may also contribute. The clinical signs reflect enterocyte damage caused by bacterial generation of alcohols, hydroxylation of fatty acids, bacteria-induced deconjugation of bile salts, and other mechanisms.

The effects of bacterial overgrowth depend on the type of bacteria and probably the duration of overgrowth. Pure bacterial cultures may be obtained from the duodenum of affected animals, but mixed infections are more typical, with *Escherichia coli*, enterococci, and anaerobes most commonly involved; clostridia predominate occasionally. When obligate anaerobes such as *Clostridium* or *Bacteroides* species are present in large numbers, the activities of many intestinal brush border enzymes may decrease and, in some cases, there may be villus atrophy. Proteases, glycosidases, or other products released by obligate anaerobes may inactivate or cleave the exposed brush border enzymes and this cannot be offset by increased protein synthesis. Lactase appears to be particularly susceptible. The intestinal microflora may also indirectly impair digestion and absorption by competing for nutrients or by decreasing the luminal concentration of bile salts.

Because adherence of bacteria to the brush borders of villus enterocytes is not an essential feature of SIBO, *small intestinal biopsies often reveal minimal lesions and are seldom diagnostic.* Quantitative culture of duodenal juice is currently regarded as the most accurate means of confirming SIBO, with counts of greater than 10^5 colony-forming units/mL generally being regarded as significant in dogs; however, higher counts can be found in clinically healthy animals.

A marked decrease in the serum cobalamin (vitamin B_{12}) concentration occurs in many dogs and most cats with EPI. The greater prevalence in cats may reflect the almost exclusive pancreatic origin of intrinsic factor in this species. Cobalamin deficiency can contribute in turn to nutrient malabsorption by promoting villus atrophy and it may also induce anorexia. The serum folate concentration is often increased in dogs with EPI, as a consequence of SIBO and increased bacterial synthesis of the vitamin. In cats with EPI, the serum folate level is often decreased, presumably reflecting concurrent proximal small intestinal disease, and occasionally a coagulopathy may develop as a consequence of malabsorption of fat-soluble vitamin K. Malabsorption of vitamins A, D, and especially E may also occur in EPI.

Confirmation of a diagnosis of EPI in cats and dogs is most accurately achieved via a species-specific assay of serum trypsin-like immunoreactivity. In other species, microscopic examination of fresh feces for undigested fat, starch, or muscle fibers and tests of fecal proteolytic activity can be utilized.

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Acute pancreatic necrosis

Acute pancreatic necrosis is a common disease of dogs. A comparable condition occurs in humans and is occasionally seen in cats and rarely in horses and swine. The condition may manifest as an *acute and potentially life-threatening syndrome* or as a *chronic relapsing syndrome* that may culminate in exocrine pancreatic insufficiency and diabetes mellitus.

The **terminology** of the disease has been a source of confusion in the literature, with the condition being variably described as "pancreatic necrosis," "necrotizing pancreatitis," or simply "pancreatitis."

Clinicians tend to use the term pancreatitis to describe both this condition and also diseases in which inflammation is centered on the ducts and interstitial tissues, often qualifying only on the basis of whether clinical disease is acute or chronic. This is understandable because clinical manifestations of these disorders overlap substantially and distinction is often impossible without biopsy of the organ. However, these disorders have different etiologies, pathogenesis, and histologic characteristics. *The condition seen commonly in dogs is designated in these volumes as acute pancreatic necrosis because this term emphasizes the basic necrotizing nature of the process.* Pancreatic conditions in which inflammation rather than necrosis is the predominant process are discussed separately in the section on Pancreatitis, below.

The key event in the **pathogenesis** *of acute Pancreatic necrosis is activation of trypsinogen to trypsin within acinar cells.* Trypsin is then capable of activating the potent arsenal of pancreatic proenzymes to cause autodigestion of the organ. In experimental models of pancreatic necrosis that involve dietary manipulation, pancreatic hyperstimulation, or ductal obstruction, intracellular activation of trypsin follows fusion of zymogen granules with lysosomes. These structures are normally strictly segregated within acinar cells. Following co-localization, the trypsinogen activation peptide can be cleaved by lysosomal proteases such as cathepsin B. Pancreatic secretory trypsin inhibitor in zymogen granules is ineffective at inhibition at the acidic pH of the lysosome. In secretagogue hyperstimulation models of pancreatic necrosis, co-localization of zymogen granules and lysosomes is associated with a sustained rise in the cytosolic ionized calcium level and altered intracellular calcium signaling. Sustained elevation of intracellular ionized calcium also occurs with hypoxia, hypercalcemia, hyperlipidemia, and ductal hypertension, suggesting that this may be a common pathway by which various conditions initiate pancreatic necrosis.

The earliest lesions of acute pancreatic necrosis in most dogs are perilobular, with necrosis and reactive inflammation concentrated at the periphery of affected lobules and with more or less extensive involvement of adjacent adipose and other connective tissues. The duct system and centrilobular parenchyma are intact at this stage of the disease. Because the periphery of the pancreatic lobule is also the periphery of the circulatory field, hypoperfusion and possibly reperfusion may be important in the pathogenesis of necrosis and could account for its occasional onset after prolonged hypotension, following abdominal surgery or in conjunction with gastric dilation-volvulus.

Local release of the activated proteolytic and lipolytic enzymes induces necrosis, with liberated lipases initiating hydrolysis of peripancreatic fat. Activated elastase and phospholipase A appear to be particularly important in expanding the area of pancreatic necrosis. Trypsin, phospholipase A, elastase, lipase, and colipase damage the walls of local blood vessels to induce vasoactive amine release, increased vascular permeability, edema, hemorrhage, and thrombosis; enzymatic tissue necrosis may therefore be compounded by *superimposed ischemic necrosis.* There is local activation of the complement cascade and release of cytokines such as tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, and platelet-activating factor (PAF). Some of these molecules promote chemotaxis of leukocytes into the area. The leukocytes amplify the pancreatic damage via the generation of oxygen-derived free radicals and additional cytokines. Products of inflammation may also directly activate digestive proenzymes. Cytokines, nitric oxide, and activated digestive enzymes are released into the circulation. Consumption of circulating protease inhibitors, such as α_1-protease inhibitor and particularly α_2-macroglobulin, permits systemic activation of the kinin, coagulation, fibrinolytic, and complement systems by proteolytic enzymes. The potential consequences include a systemic inflammatory response, hypotension, hypovolemic shock, disseminated intravascular coagulation (DIC), multiple organ failure, and death. These systemic consequences are not a feature of the clinically mild forms of the disease.

Release of activated enzymes, vasoactive peptides, inflammatory mediators, and embolic debris into the systemic circulation may lead to multifocal hepatocellular necrosis, pulmonary edema and acute interstitial pneumonia, myocardial injury, and development of cardiac arrhythmias. Acute renal failure may result from microvascular thrombosis in DIC or from nephrosis induced by hypotension. Other significant complications include portal phlebothrombosis, pulmonary thromboembolism, physical obstruction of the extrahepatic bile duct or duodenum and intestinal ileus. Glucagon released from the necrotic pancreas stimulates calcitonin release from thyroid C cells so that hypocalcemic tetany may also develop. Excess circulating free fatty acids may also depress serum calcium levels, and dystrophic mineralization of necrotic foci may contribute in a minor fashion to the hypocalcemia.

Mortality rates of 27–42% are reported in dogs with acute pancreatic necrosis, although most dogs with mild disease clinically recover within a few days. In humans with the condition, the extent of pancreatic necrosis and the presence of secondary bacterial infection of necrotic tissue are the most important factors determining the occurrence of multiple organ failure and subsequent mortality. Infection is more likely to occur in human patients with necrosis involving more than 50% of the organ. Although the extent of necrosis may well influence the outcome in dogs, secondary infection of the organ occurs rarely in this species. The greater prevalence of sepsis in humans reflects the fact that reflux of duodenal contents up the pancreatic duct is an important event in initiating pancreatic necrosis in that species. Factors that have been associated with mortality in affected dogs have included obesity, concurrent diabetes mellitus, hyperadrenocorticism, hypothyroidism, epilepsy, and prior gastrointestinal disease.

Although most dogs clinically recover from acute pancreatic necrosis, it is doubtful whether complete resolution of the pathologic process ever occurs. Rather, if the animal survives the initial episode, the necrotizing process smolders continuously and often asymptomatically until there is almost complete destruction of the pancreas. Recurrence of clinical signs of acute necrosis may occur intermittently throughout this period, and clinicians often refer to this pattern of disease as "chronic relapsing pancreatitis." There is seldom any difficulty in finding microscopic areas of acute necrosis in chronically affected organs. Whether the apparently relentless course is due to persistence of the primary pathogenetic mechanism or to a self-perpetuating property of the lesion is not known. The initial lesion is often localized to one portion of the organ, whereas the smoldering foci are often multiple, of random distribution, affect the periphery of the organ remnants, and tend to avoid any surviving parenchyma in the original focus, the latter being identifiable by fibrosis and atrophy. Clinical signs of exocrine and endocrine insufficiency may develop as a consequence of relentless destruction of the organ. *Pancreatic necrosis is a common cause of diabetes mellitus in dogs*, as the necrotizing process does not spare the islets.

Middle-aged to older dogs that are overweight or obese are at increased **risk** of acute pancreatic necrosis. Miniature Schnauzers, Yorkshire and Silky Terriers, nonsporting breeds, and perhaps Miniature Poodles are predisposed, and female dogs are more likely to be affected than males. There is often a history of recent dietary indiscretion, especially consumption of a large fatty meal, and experimentally the condition is more severe in dogs fed a high-fat, low-protein diet than in those fed a low-fat diet. These observations and the predisposition of Miniature Schnauzers with idiopathic hyperlipidemia to the disease may be a consequence of the elevating effect of hyperlipidemia on cytosolic ionized calcium levels in acinar cells. Alternatively, high-fat diets may promote excessive release of CCK and hyperstimulation of the exocrine pancreas. Dogs with hyperadrenocorticism, hypothyroidism, hypercalcemia, or uremia may also be at increased risk of acute pancreatic necrosis. Abdominal trauma may be the trigger in some animals and the condition has also been associated with the use of such drugs as azathioprine. In many cases, an inciting cause is not identified.

Mild cases of acute pancreatic necrosis in dogs may be clinically silent. *The most common* **clinical signs** *in more severe cases are anorexia, vomiting, depression, weakness, abdominal pain, and dehydration.* Diarrhea, fever, or jaundice may also be present. A cranial abdominal mass may be palpable and some dogs have mild ascites. Less often, affected dogs are presented in shock, DIC, or respiratory distress or with cardiac arrhythmia.

Cats *occasionally develop acute pancreatic necrosis*, but the condition is difficult to diagnose ante mortem and to distinguish clinically from the more common condition of interstitial pancreatitis. Older domestic shorthaired cats are most commonly affected and there is no sex predisposition. Affected cats are not usually overweight and some may be presented with weight loss. The condition has been reported following abdominal trauma (including that associated with falling from a great height) and in cats with lipodystrophy, acute hypercalcemia, ductal reflux of duodenal contents, and organophosphate poisoning. The most common clinical signs in affected cats are nonspecific and comprise anorexia, lethargy, and depression. Dehydration, tachypnea, and hypothermia are more commonly seen than vomiting, abdominal pain, or jaundice, and a palpable abdominal mass or fever is detectable in only a minority. Many affected cats are hyperglycemic and glucosuric and some may have ketonuria, hypocalcemia, and hypokalemia. Serious complications of acute pancreatic necrosis in cats are as described in dogs. Severe hepatic lipidosis may also develop in affected cats.

Grossly, in fatal cases of acute pancreatic necrosis, a small volume of turbid serosanguineous fluid containing free lipid droplets is usually present in the peritoneal cavity. Petechial and ecchymotic hemorrhages may be present in the pancreas and adjacent omentum and mesenteries but extensive hemorrhage is not usually a feature. Numerous small white chalky areas of fat necrosis, each with an intensely hyperemic border, are present adjacent to the pancreas and in the mesentery. In some cases, foci of necrotic fat are widely distributed throughout the peritoneal cavity and may be detectable as far away as the ventral mediastinum, to which digestive enzymes have been conveyed by lymphatics. The entire pancreas may be edematous, swollen, and soft or edema may be confined to localized areas. The necrotizing process may be confined to the central pancreas or one wing. Fibrin strands pass from the affected surface of the pancreas to the omentum, mesenteries, and the visceral surface of the liver. The cut surface of the pancreas has a variegated appearance due to merging of white areas of fat necrosis and gray-yellow areas of parenchymal necrosis; one or other appearance may predominate (Fig. 3.9). The texture is unusually greasy. Areas of parenchymal necrosis are soft and may liquefy.

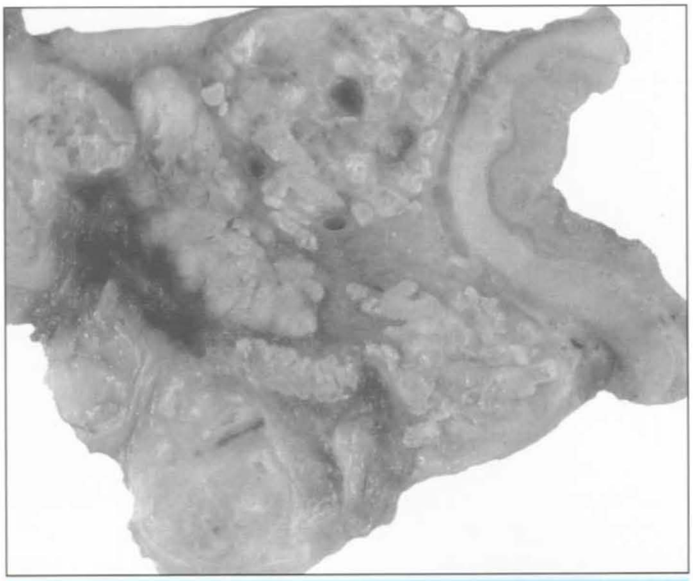

Figure 3.9 Cut surface of the pancreas after **repeated episodes of acute pancreatic necrosis** in a dog. Note the variegated appearance of the pancreas due to hemorrhage, necrosis, dystrophic mineralization, and fibrosis. Duodenal wall is at right.

Histologically, there is necrosis of peripancreatic adipose tissue and pancreatic parenchyma, edematous separation of the interstitium, mural necrosis, and thrombosis of blood vessels and reactive inflammation (Fig. 3.10A, B). Infiltrating leukocytes, chiefly neutrophils and macrophages, congregate at the boundary of necrotic and viable tissue. The necrotic fat saponifies and may undergo mild dystrophic mineralization. The initial parenchymal lesions at the periphery of the lobules are composed of small foci of coagulative necrosis in which the acinar cells become shrunken and acidophilic. These foci rapidly expand and liquefaction of the necrotic tissue leaves large lakes of debris. The collagenous stroma resists digestion for some time. Much fibrin may be precipitated in the edematous interstitium, and capillaries at the margins of necrotic areas may be occluded by fibrin thrombi. Phlebothrombosis, which may be associated with phlebitis or mural necrosis, may be apparent both adjacent to and distant from the parenchymal lesions; similar changes may occur in the small arteries but these vessels are less susceptible to injury. Widespread microvascular thrombosis may be apparent in multiple organs if DIC developed prior to death.

Infrequently, inflammatory masses such as abscesses, pseudocysts, or phlegmon may develop in the pancreas in the aftermath of acute necrosis. These lesions are discussed below in the section on Pancreatitis.

The duration of the disease influences the gross appearance of the pancreas. In chronic phases, the organ may be irregular in conformation and knobby or reduced to a few distorted lobules adjacent to where the ducts enter the duodenum (Fig. 3.11). In some cases, the remnants of the organ are too small to be visibly appreciated but they may still be palpable in areas indicated by a slight puckering of the mesentery. Scar tissue is usually not extensive and adhesions are

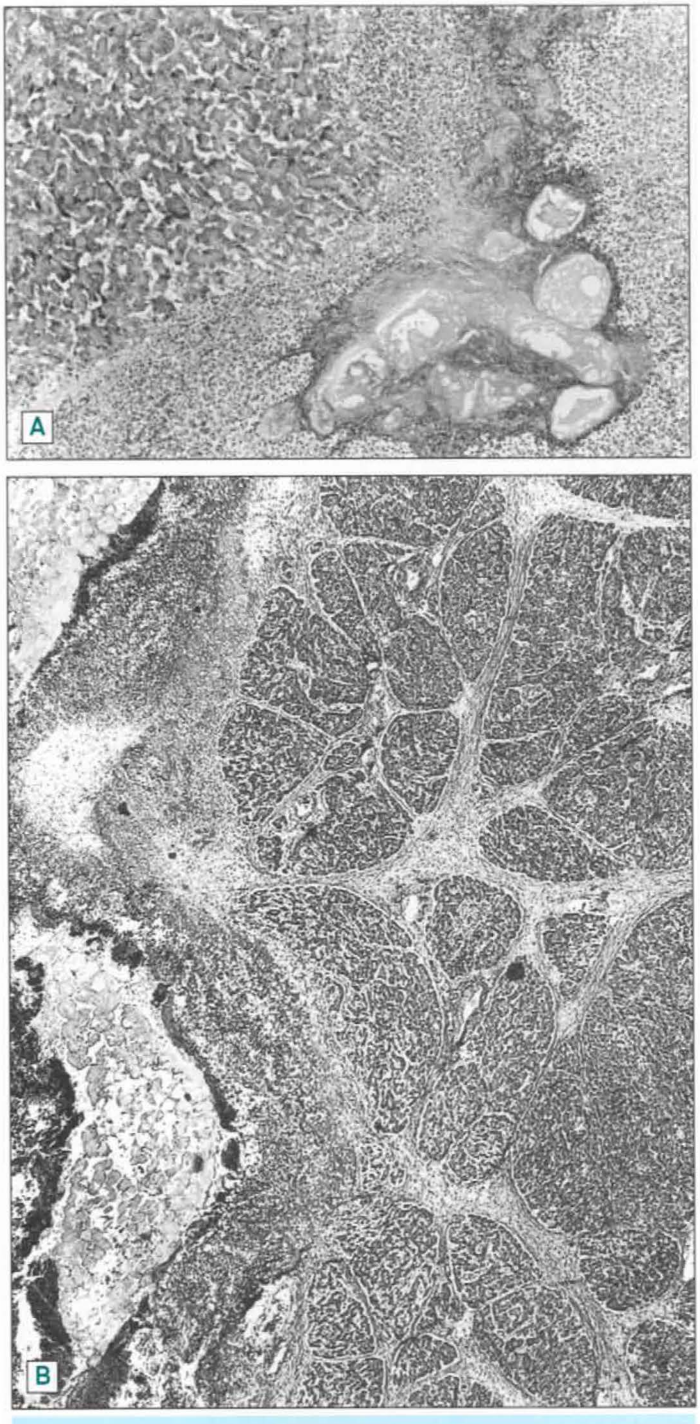

Figure 3.10 Acute pancreatic necrosis in a dog. **A.** Intense necrosis and inflammation is centered on peripancreatic fat (right), with relative sparing of exocrine parenchyma (left). **B.** Acute necrosis of the pancreas (left) with evidence of chronic postnecrotic interstitial scarring (right).

absent or minor. Microscopically, a few small rounded lobules remain and these are compressed and atrophic. Interstitial tissue is increased but this is probably due as much to condensation of stroma as to fibrosis. Blood vessels and nerves are also condensed into the small area of the pancreatic remnant. Islets often cannot be identified. Lesions of diabetes mellitus or intestinal abnormalities typical of EPI may also be present in advanced cases.

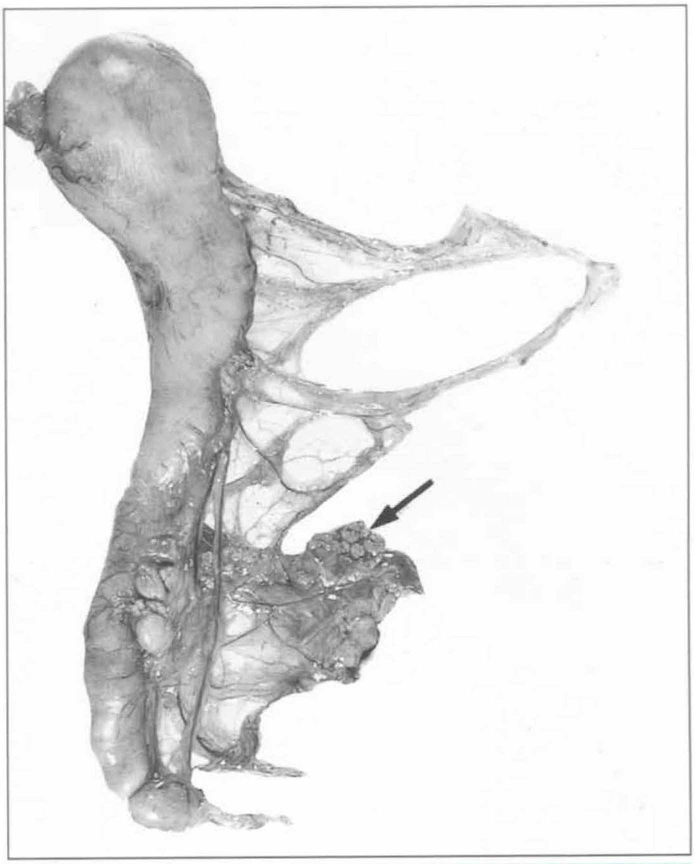

Figure 3.11 The pancreas is reduced to a nodular remnant (arrow) due to **postnecrotic scarring** in a dog.

A minority of dogs with pancreatic necrosis may develop multifocal necrotizing panniculitis and comparable subcutaneous lesions have been experimentally induced in cats by ligation of the pancreatic ducts. Release of activated enzymes, particularly phospholipase A, lipase, amylase, and trypsin, into the systemic circulation has been incriminated in the pathogenesis. In humans, the tendency to develop panniculitis in association with pancreatic necrosis is associated with a decrease in the serum concentration of α_1-PI or α_2-macroglobulin. A comparable association has yet to be demonstrated in small animals.

Acute hemorrhagic pancreatic necrosis, in which the necrotizing process is initially of centrilobular and periductal distribution, is a common subtype of acute pancreatic necrosis in humans, with approximately 80% of cases being associated with cholelithiasis or alcohol abuse. A similar lesion is occasionally observed in dogs. Predisposing factors in dogs are uncertain but the condition has been described in dogs envenomated by scorpions, following therapeutic use of asparaginase, and in association with necrotizing arteritis referable to visceral leishmaniasis. It is likely that in many affected dogs the initiating event is reflux of activated digestive enzymes and bile salts from the duodenum into the main pancreatic duct to cause ductal necrosis. Reflux may be encouraged by increased luminal pressures during severe vomiting. The initial periductal parenchymal lesions rapidly enlarge and may become confluent to involve most of the pancreas. The organ is initially edematous and hyperemic, and the parenchyma becomes friable and hemorrhagic. The extensive hemorrhage may obscure the parenchyma and necrotic foci in adjacent adipose tissue. Apart from the intensity of accompanying hemorrhage, this stage may be histologically indistinguishable from the more common pattern of acute pancreatic necrosis that commences at the periphery of the organ.

Life-threatening complications develop more commonly in the hemorrhagic form of pancreatic necrosis in dogs. In cases initiated by reflux of duodenal contents, enteric organisms may colonize the necrotic tissue and may cause abscessation.

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Pancreatitis

Hematogenous infections may produce small, randomly distributed foci of pancreatic inflammation with necrosis of small clusters of acinar cells. **Multifocal pancreatitis** is commonly seen in systemic infections with epitheliotropic viruses, but inflammation is usually less prominent than degeneration and necrosis. These conditions have been discussed in the section on Degeneration, necrosis, and atrophy, above. Bacterial embolism to the pancreas is not

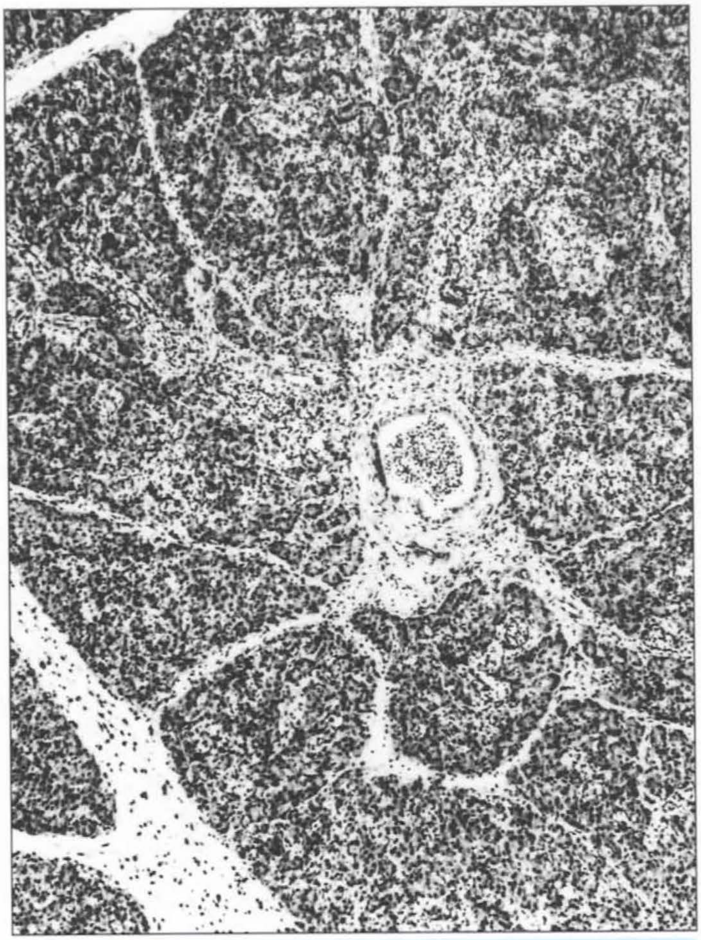

Figure 3.12 Inflammation is centered on a duct in **acute interstitial pancreatitis** in a cat.

uncommon in cats and dogs with septic peritonitis, but does not influence the outcome.

Acute interstitial pancreatitis is common in systemic toxoplasmosis, especially in cats (Fig. 3.12). Grossly, the organ is slightly swollen by diffuse interlobular edema, or there may be hemorrhage and necrosis with comparable lesions in the duodenum.

Chronic interstitial pancreatitis is the pattern of inflammation that is usually seen in domestic animals. It is seldom of clinical significance but may occasionally be responsible for pancreatic insufficiency. The lesion is not uncommon in cats, is occasionally observed in horses, and is rare in other species. *Chronic interstitial pancreatitis usually arises by extension of an inflammatory process that commences in the ducts.* The cause is usually an ascending infection involving intestinal bacterial flora, but may also be caused by intraductal trematodes. Pancreatic ductal malformations, intraductal parasites, duodenal disease, high luminal pressures in the duodenum arising from vomiting, and various miscellaneous causes may predispose to ascending infection. In horses, chronic interstitial pancreatitis may be provoked by the larvae of *Strongylus equinus* or, less commonly, *S. edentatus* that pass part of their developmental cycle in and about the pancreas. In cats, in which the pancreatic and bile ducts fuse before entering the duodenum, ascending cholangitis usually coexists with interstitial pancreatitis. Cholangitis may also develop in affected horses. Clinical signs referable to cholangitis may overshadow those of pancreatic involvement.

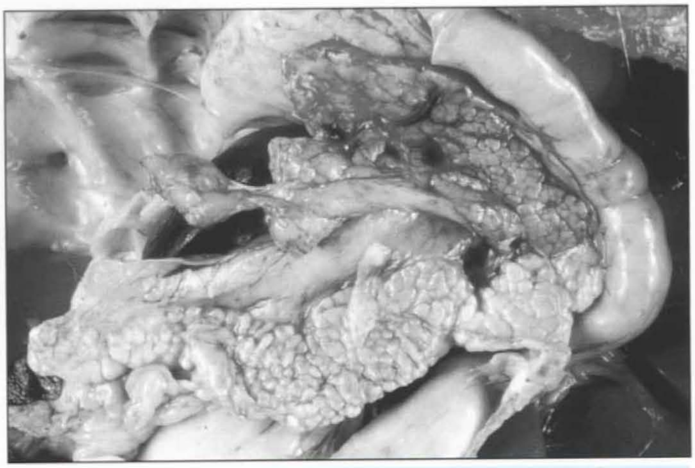

Figure 3.13 Exaggerated lobulation of the pancreas due to diffuse interstitial fibrosis in **chronic interstitial pancreatitis** in a cat.

In chronic interstitial pancreatitis, the organ may be enlarged or reduced in size. In horses, the tendency is for enlargement and the organ may be replaced by a tough mass of scar tissue with adhesions to adjacent structures. Flattened remnants of pancreatic tissue may be visible near the surface of the mass. Incision reveals tortuous and eccentrically dilated ducts that may contain purulent exudate or a large volume of slightly opaque mucus.

In cats, the pancreas is usually reduced in size and is firm, gray, and multinodular (Fig. 3.13). Clear retention cysts are often visible through the capsule and particularly on the cut surface. Fibrosis may not be recognizable on gross inspection, but there are exceptional cases in which the whole organ is converted into a shrunken, distorted, fibrous remnant. Histologically, the ducts contain catarrhal exudate and are enveloped by dense fibrosis (Fig. 3.14). Localized ductal stenoses and cystic dilations may occur. The epithelium of the ducts is hyperplastic and there may be squamous metaplasia. Fibrous tissue extends from around the ducts to the interlobular stroma and subdivides many of the lobules (Fig. 3.15). The interstitial tissues are permeated by leukocytes, chiefly lymphocytes and plasma cells. Acinar parenchyma atrophies as a consequence of fibrosis and ductal obstruction, but the islets are usually well preserved.

Inflammatory masses involving the pancreas and peripancreatic tissues are well-documented sequelae of acute pancreatitis in humans, but there are few reports of pancreatic phlegmon, abscess, or pseudocyst development in domestic animals. The masses can provoke clinical signs indistinguishable from those of pancreatitis and pancreatic necrosis.

A **pancreatic phlegmon** *is a solid mass of indurated pancreas and adjacent tissues that results from inflammation, edema, and necrosis.* A phlegmon may develop within a few days of onset of acute pancreatic necrosis and typically resolves spontaneously within 2–3 weeks, after the necrotizing process subsides. Necrosis may cause some cavitation of a phlegmon, but a fluid-filled pocket does not usually form.

Pseudocysts *are intra- or peripancreatic, fluctuant pockets of pancreatic enzyme secretions, necrotic debris, inflammatory exudates, and blood.* They are more common than abscesses as sequelae of acute or chronic

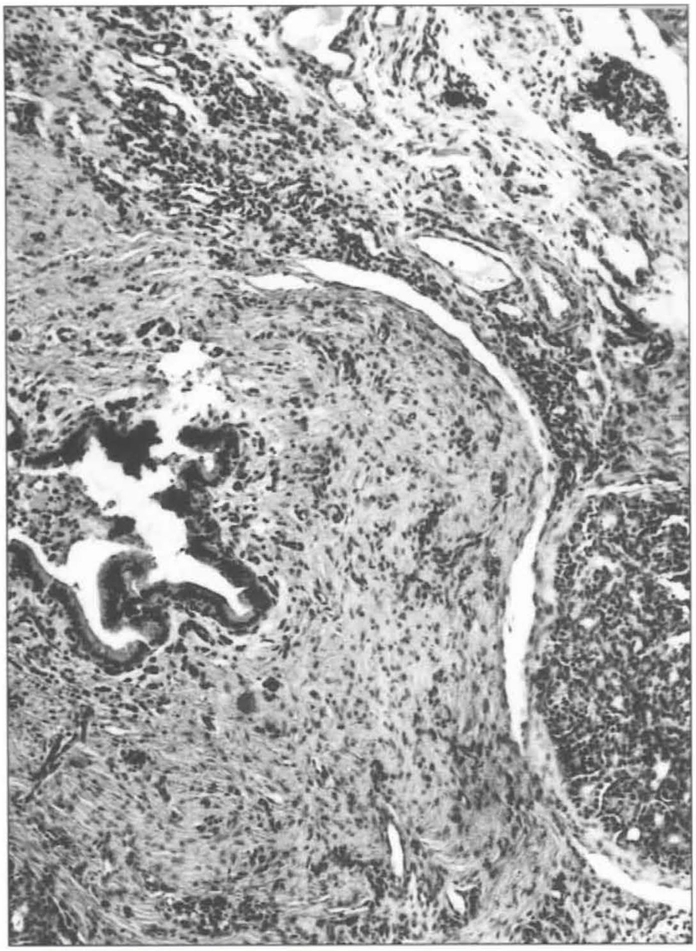

Figure 3.14 Severe periductal fibrosis in **chronic interstitial pancreatitis** in a cat.

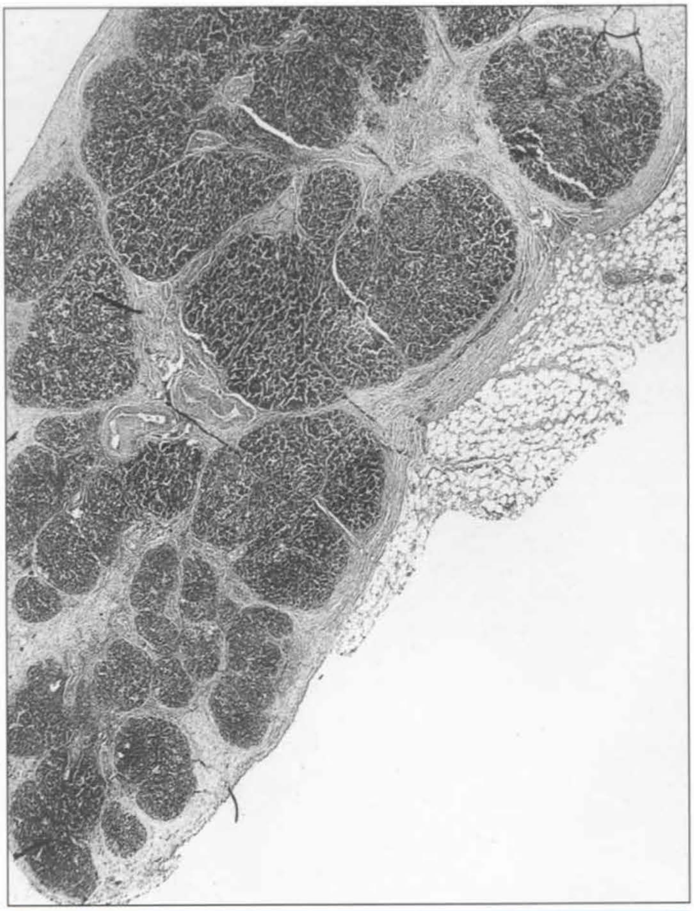

Figure 3.15 Marked interlobular fibrosis in **chronic interstitial pancreatitis** in a cat.

pancreatic necrosis. Rarely, they may result from trauma or neoplasia. Pseudocysts may develop within days to weeks of onset of pancreatic necrosis. Disruption of pancreatic ducts by inflammation or necrosis and duct occlusion contribute to their development. Pseudocysts are usually solitary and unilocular and may reach 10 cm in diameter. The wall may be thin or thick and formed by granulation tissue that matures to fibrous scar tissue. Unlike true cysts, there is no epithelial lining. Chronic inflammation may be intense in the fibrous capsule; hemosiderin, cholesterol crystals, and mineral deposits may be incorporated into the capsule. Small pseudocysts may spontaneously resolve over several weeks. Large, thick-walled, or mineralized cysts tend to persist or enlarge. Complications include compression of the extrahepatic bile duct, rupture causing severe hemorrhage or generalized peritonitis, and secondary infection leading to abscessation.

Pancreatic abscesses *are pockets of purulent exudate and necrotic debris within the pancreas or adjacent tissues.* Sterile abscesses may form within areas of intense liquefactive necrosis of parenchyma. Larvae of *Stephanurus dentatus*, migrating from the liver across the peritoneum to the perirenal area in pigs, may encyst in the pancreas and provoke abscessation. Septic abscesses may result from secondary infection of necrotic parenchyma or, less often, a pseudocyst. They can also arise by direct extension from a neighboring focus of infection, as they occasionally do from septic peritonitis and from perforated esophagogastric ulcers in pigs. Suppurative lesions of lymphohematogenous origin are rare. Enteric bacteria are usually isolated from abscesses in humans and are probably derived from the biliary tree or from adjacent inflamed or devitalized bowel. However, almost all reported pancreatic abscesses in dogs have been sterile. Some abscesses may track cranially as far as the mediastinum or caudally in the retroperitoneum to the pelvic inlet. Some may contain chyle as a result of lymphatic leakage, and gas bubbles may occasionally be present in septic abscesses. *Abscessation can be a serious and potentially fatal complication*, with death a result of rupture with severe hemorrhage or bacteremia; spontaneous resolution is rare.

Systemic **granulomatous infections** may involve the pancreas, but lesions are usually microscopic and less important than those in other viscera. Chronic sclerosing and granulomatous pancreatitis due to zygomycotic fungi has been reported in dogs, and pyogranulomatous pancreatitis occurs in feline infectious peritonitis. Disseminated cryptococcosis may occasionally involve the pancreas of cats, but is usually only associated with mild pyogranulomatous inflammation. Destructive granulomatous and eosinophilic pancreatitis can be a feature of multisystemic eosinophilic epitheliotropic syndrome of horses (Fig. 3.16); a proportion of these cases are examples of enteropathy-associated lymphoma.

In **pancreatolithiasis**, *concretions form within the pancreatic duct system* (Fig. 3.17). The calculi are occasionally detected in cattle at slaughter, being slightly more common in cattle over 4 years of age, but are rare in other species. The calculi are usually hard, white,

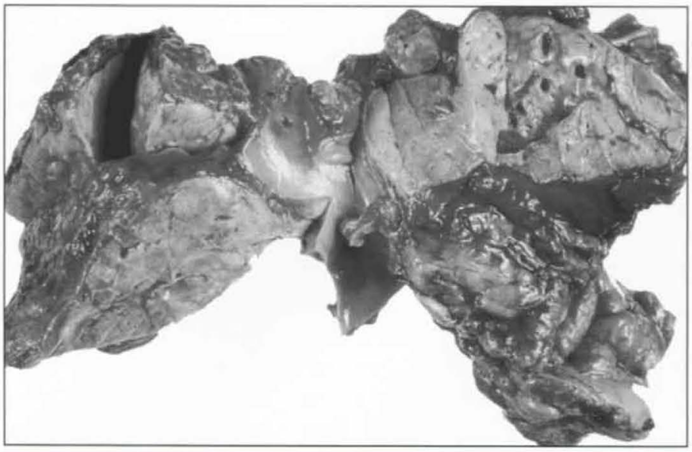

Figure 3.16 Chronic eosinophilic **interstitial pancreatitis** in a horse.

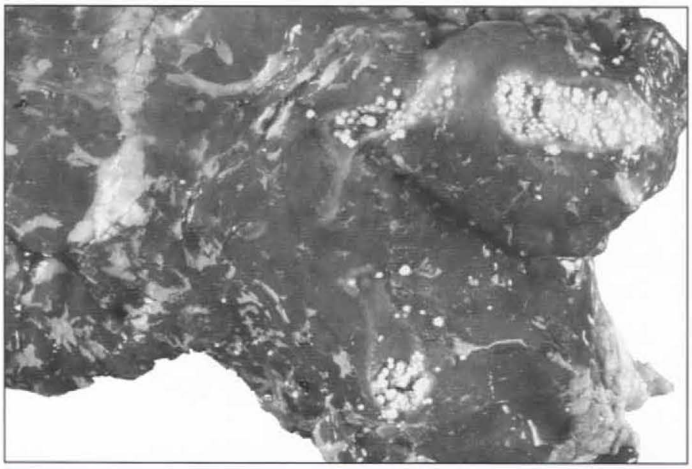

Figure 3.17 Pancreatolithiasis in a cow.

numerous, and small, often resembling sand grains, and are chiefly composed of carbonates and phosphates of calcium and magnesium. The calculi are associated with and may be a consequence of ductal inflammation, including that provoked by flukes. Affected ducts may be irregularly stenotic or dilated and there may be cystic dysplasia of the mucosal glands. The calculi are typically incidental findings at necropsy and are not a significant cause of complete duct obstruction. Obstructive pancreatolithiasis with concurrent pancreatitis has been reported in a cat.

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Parasitic diseases of the pancreas

The pancreatic lesions of systemic toxoplasmosis and of migrating larvae of *Strongylus equinus, S. edentatus,* and *Stephanurus dentatus* have been described in the preceding section.

Various **metazoan parasites** may be found within the pancreatic ducts of domestic animals. Their significance depends on the extent to which they occlude the ducts, either by direct physical obstruction or by provoking inflammation and periductal fibrosis. Ascarids may invade the ducts from the intestine in pigs and dogs. *Thysanosoma actinioides*, the fringed tapeworm, may spill from the bile ducts of ruminants into the pancreatic ducts and small intestine. In heavy infestations in herbivores and carnivores, trematodes such as *Dicrocoelium dendriticum, Opisthorchis tenuicollis, O. felineus, O. viverrini, Clonorchis sinensis, Platynosomum fastosum, Metorchis conjunctus*, and *Amphimerus pseudofelineus* may be found in both the pancreatic and bile ducts. These parasites are described in Vol. 2, Liver and biliary system.

Dicrocoelid flukes of the genus ***Eurytrema*** may inhabit both the pancreatic and biliary ducts but prefer the former. *Eurytrema pancreaticum* is the most important species and is a common parasite of the pancreatic and bile ducts of ruminants and other herbivores throughout Asia and South America and is also reported in Madagascar and Russia. *E. coelomaticum* is common in Brazilian cattle and is reported in ruminants in China. *E. tonkinese* is found in cattle in Vietnam. Snails act as the first and grasshoppers the second intermediate hosts. The flukes are presumed to gain access to the pancreatic ducts directly from the duodenum following ingestion of an intermediate host.

Other species of *Eurytrema* are found in carnivores, wild herbivores and birds. Pigs may harbor *Eurytrema* flukes of either herbivore or carnivore origin. *E. procyonis* is a common parasite of the pancreatic ducts of raccoons in many parts of the USA, and has also been detected in red and gray foxes and domestic cats. In areas in which the fluke occurs, up to 14% of cats may be infected. The common garden snail, *Mesodon thyroidus*, and grasshoppers act as intermediate hosts. *E. procyonis* is infrequently reported in the bile ducts and gallbladder of cats.

Intraductal flukes may provoke only mild luminal and periductal inflammation, hyperplasia of the ductal epithelium, and periductal fibrosis. In other animals, however, there may be pronounced cording and dilation of the ducts and chronic interstitial pancreatitis, leading to near total loss of exocrine parenchyma and severe replacement fibrosis. Duct obstruction is thought largely to mediate the periductal fibrosis and the progressive atrophy of exocrine elements. Irritation of the ductal mucosa by the flukes and their ova and inflammation in response to dead flukes contribute to the progressive scarring.

E. pancreaticum preferentially targets the left pancreatic lobe in ruminants. Infestation of cattle with *E. pancreaticum* may cause progressive wasting; emaciation, recumbency, and death have been reported in heavily infested sheep. Although islets are preserved in most cases, progressive destruction culminating in diabetes mellitus has been reported in sheep. Glucosuria has also been noted in raccoons harboring *E. procyonis*. In cats, *E. procyonis* is found in the small- and medium-sized ducts of both pancreatic lobes, but the tail of the pancreas is usually more severely affected than the head. Duct obstruction in cats is thought to be responsible for the significant reduction in the volume of pancreatic secretions and in the content of digestive enzymes and bicarbonate. With heavy infestations, the major pancreatic duct may be greatly dilated and much of the organ is shrunken, pale, and fibrotic. However, most cats are asymptomatic and diagnosis is by identification of typical dicrocoelid eggs with a single operculum in the feces.

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Hyperplastic and neoplastic lesions of the exocrine pancreas

Hyperplasia of ductular epithelium is occasionally seen, often in an environment of chronic pancreatitis, exocrine parenchymal atrophy, and interstitial fibrosis, or within foci of nodular hyperplasia of the exocrine parenchyma. It is also reported as an aging phenomenon, at least in humans. The hyperplastic lining epithelium may protrude as papillae into the duct lumina and the affected ducts may be dilated.

Nodular hyperplasia of the exocrine parenchyma *is common in older dogs, cats, and cattle*, but is of no clinical significance. In most cases, there is no evidence of a preceding pancreatic insult, so it is unlikely that the nodules reflect regenerative effort. Solitary foci are unusual, with *multiple small nodules scattered throughout the organ being typical* (Fig. 3.18). Individual lobules may contain multiple hyperplastic nodules; complete transformation of a lobule into a hyperplastic nodule is uncommon. The nodules are of variable size but do not usually exceed the size of normal lobules. Their gray-white color and firm consistency contrast grossly with the color and texture of normal pancreas. Where superficially positioned, the smooth-surfaced nodules barely project.

Histologically, there is a mosaic of normal and hyperplastic tissue within affected lobules (Fig. 3.19). Only the exocrine tissue is involved. *The hyperplastic foci are not encapsulated and usually do not compress the adjacent parenchyma, features used to distinguish a solitary hyperplastic lesion from an exocrine pancreatic adenoma.* The cells within each nodule tend to be uniform, with at least some attempt at acinar formation. They may closely resemble their normal counterparts except for altered staining intensity and cell size. The cells may be larger than normal, with expanded, brightly acidophilic cytoplasm, or they may be smaller and paler than normal, forming low cuboidal acini. Cells may be vacuolated and, in some nodules, arranged as irregular sheets or small clusters without an obvious lumen.

Exocrine pancreatic adenomas *are rare*, and some of those reported in older dogs, cats, and cattle may have been hyperplastic nodules. Adenomas are usually solitary and small. They are usually incidental findings at necropsy and their rarity suggests that they do

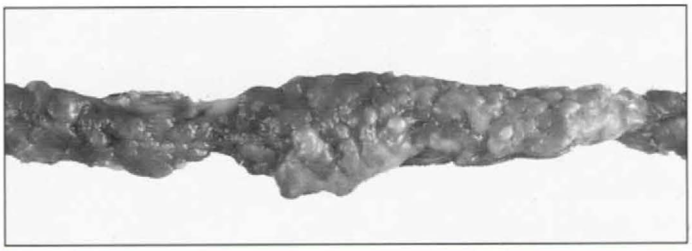

Figure 3.18 Nodular hyperplasia of the exocrine parenchyma in a cat.

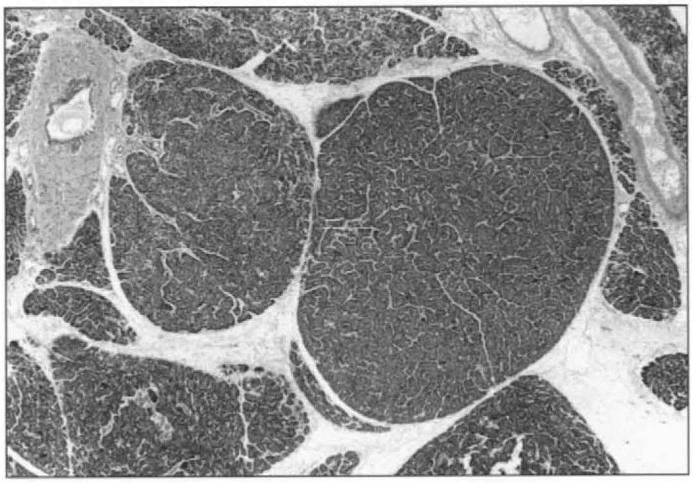

Figure 3.19 Nodular hyperplasia of the exocrine parenchyma in a cat.

not give rise to pancreatic adenocarcinoma. Adenomas may protrude from the surface of the pancreas, and grossly they more closely resemble normal parenchyma than do hyperplastic nodules. *Expansive growth causes compression of adjacent parenchyma from which the tumor is usually separated by a thin fibrous capsule.* The histological pattern of an adenoma may be predominantly tubular or rarely acinar. In the tubular type (which is thought to be derived from ductal epithelium), small or large cystic spaces are lined by cuboidal or columnar epithelial cells and may contain mucin. Mitoses are rare.

Exocrine pancreatic adenocarcinoma *is uncommon in all domestic animals.* Most cases are observed in dogs and, less commonly, in cats. A few cases have been reported in cattle and horses, and the tumor appears to occur rarely in pigs and sheep. The malignancy is largely one of older age but some affected dogs have been only 3 years of age. Female dogs may be at slightly greater risk than males. Whether the Airedale Terrier is genuinely predisposed remains unconfirmed.

The incidence of pancreatic adenocarcinoma in humans has increased over recent decades. Smoking, consumption of meat- and fat-rich diets, alcohol and methylxanthine compounds such as coffee, and exposure to radiation and to chemical carcinogens, including *N*-nitroso compounds produced by intestinal bacteria, are implicated in tumor development in humans. There is also a weak link between chronic pancreatitis and pancreatic malignancy. *Nitrosamines* are potent pancreatic acinar carcinogens in hamsters, mice, and rats, with exposure leading progressively through enzyme-altered foci and hyperplastic and dysplastic nodules to malignancy. Chemicals or other factors inducing spontaneous pancreatic adenocarcinoma in dogs and cats are yet to be identified. *Chronic pancreatitis* was speculated to have been a contributory factor in the development of pancreatic adenocarcinoma at a relatively young age in two gray Collies with cyclic hematopoiesis, but a genetic predisposition could not be excluded.

The most common clinical signs in dogs and cats with this neoplasm are nonspecific: anorexia, weight loss, depression, lethargy, and vomiting. There may be abdominal pain or a palpable abdominal mass. Ascites may develop if there is extensive peritoneal implantation. Occasionally, there may be signs of diabetes mellitus or exocrine pancreatic insufficiency due to extensive neoplastic destruction of the pancreas or complete obstruction of the pancreatic ducts. There is a tendency for these tumors to arise centrally within the gland or in the duodenal wing; these may provoke early clinical signs of biliary obstruction. Rarely, the first clinical signs reflect distant metastasis. Multifocal necrotizing panniculitis with mineralization of necrotic adipocytes has been observed in a few dogs with pancreatic adenocarcinoma. A rare syndrome of *paraneoplastic alopecia* affecting the ventrum, legs, and usually the face has been observed in cats with pancreatic adenocarcinoma. Pseudohyperparathyroidism leading to hypercalcemia has been reported in a dog with pancreatic adenocarcinoma.

Pancreatic adenocarcinoma may appear as a solitary, discrete, circumscribed mass or there may be multiple masses throughout the organ. The neoplasm is usually firmer than the adjacent pancreas. Some carcinomas are highly scirrhous and cannot be grossly distinguished from postinflammatory scarring (Fig. 3.20). The cut surface of the neoplasm is typically heterogeneous and yellow to gray-white; areas of necrosis, hemorrhage, and mineralization may be obvious. Necrotic foci are often found in adipose tissue within and adjacent to primary and metastatic nodules and there may be adhesions of the pancreas to adjacent tissues. Some tumors may contain mucin-filled cysts.

Three main morphological patterns of pancreatic adenocarcinoma are recognized histologically: *small tubular (the most common form), large tubular, and acinar.* In the *tubular forms*, glandular structures resembling pancreatic ducts are formed by mucus-secreting cuboidal or columnar cells (Fig. 3.21). Some tumors may contain localized

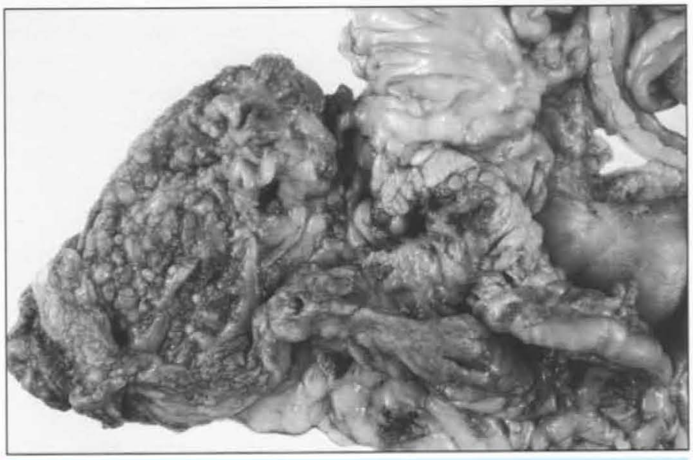

Figure 3.20 Pancreatic adenocarcinoma in a dog. The mass is scirrhous with extensive implantation on the gastroduodenal mesenteries and omentum.

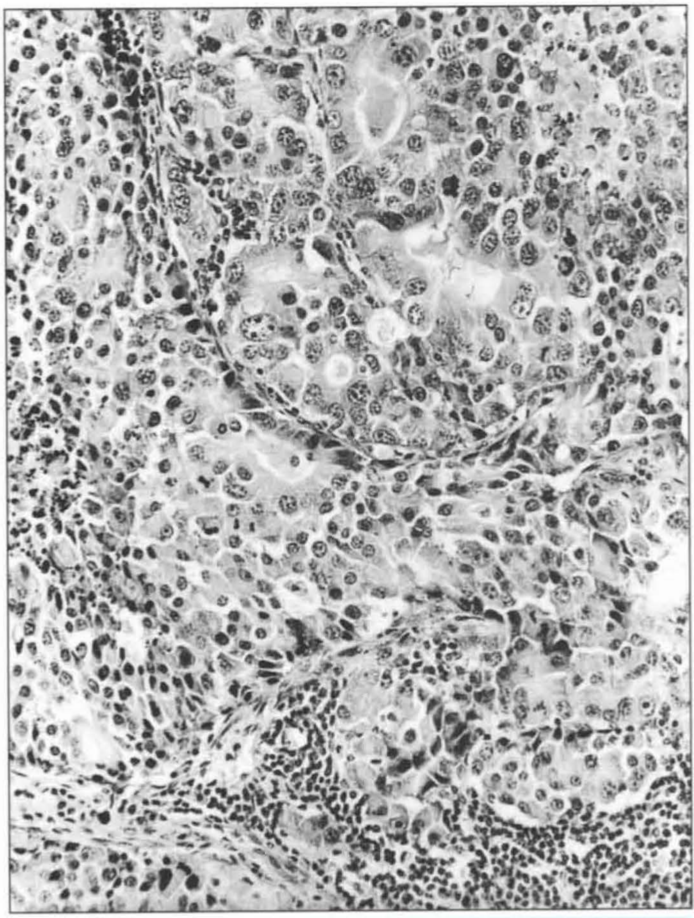

Figure 3.21 Pancreatic adenocarcinoma in a dog. Invasive neoplastic cells are forming tubular arrays and display karyomegaly and abnormal mitoses; lymphocytes are accumulating at the tumor margins.

ductular arrangements of well-differentiated cells that may be difficult to distinguish from the ductular response to incomplete obstruction. Well-differentiated *acinar tumors* are composed of small cells arranged into acini mimicking normal parenchyma.

The *histogenesis* of pancreatic adenocarcinoma has not been clarified in domestic animals. Traditionally, the tubular forms have been considered to be derived from ductal epithelium whilst tumor cells containing brightly eosinophilic zymogen granules have been interpreted to be of acinar origin. This distinction is often arbitrary and the histological pattern may vary considerably within a single tumor. Moreover, pluripotent ductular epithelium or endodermal stem cells could give rise to both tubular and acinar forms.

Undifferentiated carcinomas that do not form acinar or ductular structures are also seen. Some of these may be entirely composed of sheets of small anaplastic cells. Poorly differentiated tumors usually have a higher mitotic index and a more pronounced fibrous stroma.

The tendency of pancreatic adenocarcinomas to form tubules or acini usually permits their distinction from islet cell tumors. Detection of eosinophilic granules in tumor cells permits distinction of pancreatic adenocarcinoma from invasive or metastatic adenocarcinoma of gastrointestinal or biliary origin. Electron microscopy or immunohistochemical analysis for secretory products may be needed to clarify the origin of poorly differentiated tumors.

Pancreatic adenocarcinoma usually behaves aggressively and metastases are often established by the time of diagnosis. Local invasion into the wall of the duodenum is common and there may be obstruction of pancreatic or bile ducts. Neoplastic invasion of blood vessels and lymphatics may be obvious histologically in the pancreas, with tumor cells commonly tracking in the perineural lymphatics. Metastasis is most commonly to the liver, lungs, and local lymph nodes, but may also involve the spleen, kidneys, and diaphragm, and rarely the skin. Peritoneal implantation is common and there is also a propensity to extend along visceral nerves to the dorsal ganglia. Metastatic behavior cannot be predicted by the histological pattern as well-differentiated tumors can metastasize widely. The large tubular form is, however, purported to metastasize less readily than the other forms.

Only a single case of **hepatopancreatic ampullary carcinoma** has been recorded in domestic animals; the tumor arose from the junction of the common bile duct and main pancreatic duct within the wall of the duodenum in a cat. Such tumors may cause obstruction of either or both ducts. The neoplasm is discussed in Vol. 2, Liver and biliary system.

Nonepithelial neoplasms rarely arise in the pancreas. Those reported include fibrosarcoma, hemangiosarcoma, liposarcoma, neurofibroma, and neurofibrosarcoma. **Metastasis to the pancreas** from malignancies arising elsewhere appears to be rare but the paucity of reports may reflect lack of systematic examination and sampling of the organ at necropsy. The organ may become involved in hematopoietic malignancies and disseminated lymphoma. Neoplasms arising in the bile ducts, stomach, or duodenum, including intestinal lymphoma, may directly invade the pancreas.

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ENDOCRINE PANCREAS

General considerations

The endocrine cells of the pancreas are chiefly aggregated within the islets of Langerhans, which are scattered throughout the exocrine parenchyma. There are four principal types of endocrine cells.

- **β cells** secrete *insulin* and an insulin-antagonist, *islet amyloid polypeptide* (IAPP or amylin), comprise 60–70% of all islet cells, and are present within all islets.
- **α cells**, which secrete *glucagon*, are the next most numerous cells.
- **δ cells** are present in small numbers in all islets and secrete *somatostatin* and, in some species, IAPP.
- **PP (or F) cells** secrete *pancreatic polypeptide* (PP); these cells are particularly numerous in sheep.

α cells in dogs and both α and β cells in pigs contain dopamine and serotonin. In cats, both α and PP cells may synthesize serotonin. Other cell types that may be present in islets of some species include *enterochromaffin (EC) cells* (that secrete serotonin), *D_1 cells* (that secrete vasoactive intestinal peptide, VIP), *P cells* (that secrete bombesin and are only reported in fetal islets), and *G cells* (that secrete gastrin in fetal islets). Insulin-secreting cells are confined to the pancreas, but the other islet cell types have functional counterparts in the gastrointestinal tract.

In most mammalian species, β cells are clustered at the center of islets. However, in the horse, islets of the left and intermediate lobes of the pancreas typically have α cells at their core, with β cells and a few δ and PP cells at their periphery. In cats, β cells are also largely situated at the periphery of the islets. Ultrastructurally, the secretory granules of the various cell types are distinct, but islet cells are most accurately distinguished by immunohistochemical techniques that label specific hormone products (Fig. 3.22). β cells can also be readily highlighted by histochemical stains such as aldehyde fuchsin.

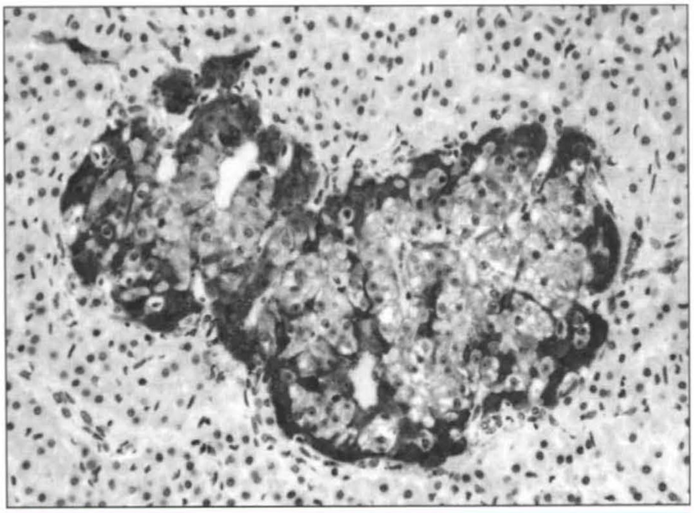

Figure 3.22 Positive immunoperoxidase staining of **insulin-secreting islet cells** in a horse. Note that the distribution of insulin-secreting cells within the islet is not random.

Secretory granules of all islet cells stain positively with chromogranin A and neuron-specific enolase.

Insulin *has both metabolic and mitogenic effects on target tissues.* The insulin receptor, which has tyrosine kinase activity, is widely distributed throughout the body, but the main target cells are hepatocytes, striated myocytes, fibroblasts, and adipocytes. *The principal function of insulin is to increase the rate of transfer of glucose into target cells to form intracellular glycogen*, thereby lowering the blood glucose concentration. It also enhances glucose oxidation and formation of ATP. Hyperglycemia causes immediate release of stored insulin from β cells, whilst sustained hyperglycemia promotes synthesis of the hormone. Insulin also increases the transfer of other monosaccharides, some amino acids, fatty acids, potassium, and magnesium into target cells. It directs amino acids into protein synthesis rather than gluconeogenesis, and promotes uptake of fatty acids by adipocytes to form storage triglycerides. *Insulin is a major anabolic hormone.* In concert with insulin-like growth factors (IGF-1, IGF-2), it promotes DNA and RNA synthesis and stimulates growth and differentiation of cells. Indeed, insulin and IGFs can bind to and activate their heterologous receptors. Growth retardation has been observed in mouse pups genetically rendered insulinopenic throughout development. The effects of insulin on cell growth and differentiation afford one explanation for the commencement of insulin synthesis so early in embryonic development, despite insulin not being required for glycemic control in the fetus.

IAPP is stored in the same secretory vesicles and is co-secreted with insulin in response to glucose and other secretagogues. The amount of IAPP secreted in response to glucose stimulation is usually approximately 1% of the amount of insulin released on a molar basis. However, expression of the two peptides is at least in part independently regulated, and β cells are also heterogeneous in their content of IAPP and insulin and in their response to glucose signals, so that this molar proportion may alter. The ratio is reduced during fasting. The ratio is increased in obesity and glucose intolerance, which has been incriminated in the pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus.

The *biological actions of IAPP* are incompletely understood, but experimental evidence supports a role in glucose metabolism, calcium homeostasis, and control of blood pressure and appetite. At least some of its effects may be mediated via binding to cell receptors for calcitonin gene-related peptide, with which it shares approximately 45% amino acid sequence homology. IAPP inhibits basal and glucose-stimulated insulin secretion by β cells; it also inhibits basal and insulin-stimulated glycogen synthesis in skeletal muscle and promotes muscle glycogenolysis. Hepatic uptake of lactate released from skeletal myocytes as a result of glycogenolysis stimulates gluconeogenesis and boosts the blood glucose concentration. In promoting hepatic gluconeogenesis, IAPP acts synergistically with glucagon but via a different mechanism. IAPP also induces hypocalcemia by inhibiting osteoclast-mediated bone resorption. IAPP shares substantial sequence homology with the hypotensive peptide, adrenomedullin, and, when administered intravenously, provokes tachycardia and lowers systemic blood pressure via vasodilation. IAPP also acts as a satiety hormone on the hypothalamus to suppress appetite.

Glucagon *is released from α cells in response to hypoglycemia.* It acts to boost the blood glucose concentration by stimulating hepatic glycogenolysis and gluconeogenesis. It also stimulates lipolysis. **Somatostatin** has potent inhibitory effects on the secretion of insulin, glucagons, and PP but its role within the islet remains largely enigmatic. Conversely, insulin appears to have a major regulatory effect on somatostatin secretion by δ cells. Hypertrophy and hyperplasia of δ cells have been demonstrated in insulinopenic humans and animals. Very little is known about the function of **PP** apart from its capacity to inhibit exocrine pancreatic secretion and intestinal motility, and to stimulate secretion of gastric and intestinal enzymes. **VIP** induces glycogenolysis and hyperglycemia and stimulates gastrointestinal fluid secretion.

Islets are *innervated* by sympathetic, parasympathetic, and peptidergic nerves. Activation of the sympathetic nervous system in hypoglycemic states stimulates glucagon release from α cells and epinephrine release from the adrenal medulla to boost blood glucose.

Islets are *richly vascularized* and may receive up to 10% of pancreatic arterial blood flow. Small arterioles penetrate the β cell-rich core of the islets and then form a glomeruloid network of fenestrated capillaries that drain into collecting venules either within or external to the islets. This portal arrangement permits high concentrations of insulin to be carried to endocrine cells situated in the islet mantle and exposes acinar cells to hormones.

β cells are polarized, with one domain facing an arterial capillary and another a venous capillary. Numerous microvilli project from the β cells into canaliculi that lie between the lateral surfaces of contiguous cells. Interstitial fluid is thought to flow through the canaliculi in an arterial to venous direction, with glucose uptake by β cells occurring by means of transporters (particularly glucose transporter-2) on the microvilli. Glucose sensing by the β cell is via the products of glucose catabolism. It is therefore dependent upon the rate of intracellular glycolysis and especially upon the rate-limiting glycolytic step that is catalyzed by glucokinase.

Electrical activity of β cells is synchronized within individual islets, possibly via intercellular gap junctions. An increase in these gap junctions is associated with enhanced insulin secretion. There also appears to be synchronization of activity of endocrine cells in all islets, permitting periodic oscillations in serum insulin and glucagon concentrations; such synchrony may reflect intrinsic neural coordination. Nevertheless, the population of β cells appears to be heterogeneous in function. Thresholds for glucose stimulation of insulin secretion and synthesis vary amongst β cells and there also appear to be differences in the response of islets in different regions of the pancreas and of β cells within individual islets to glucose stimuli.

Interactions *among different types of islet cells are complex and incompletely understood.* An increase in the blood glucose concentration normally simultaneously stimulates insulin secretion by β cells and suppresses glucagon secretion by α cells. Suppression of the α cells is thought to be largely a consequence of the high concentration of insulin carried by the portal vascular system from the β-cell core of the islets to α cells in the islet mantle. Somatostatin release by δ cells may also contribute to inhibition of glucagon release by adjacent α cells. Studies in dogs, rats, and primates reveal that islet hormones carried via the portal system have a paracrine effect on downstream islet cells, in a sequence from β to α to δ cells. α and δ cells are therefore influenced by insulin carried centrifugally by the portal system, but β cells are not influenced by glucagon or somatostatin, and α cells are not influenced by somatostatin via the portal blood. Although glucagon at pharmacological doses stimulates insulin secretion, there is no convincing evidence that glucagon plays a significant physiological role in regulating β-cell function. Somatostatin, in contrast, acts physiologically to inhibit insulin release from β cells. δ cells are of dendritic shape, and granule-containing projections of these cells lie adjacent to α, β, and PP cells. This arrangement may afford an anatomic basis for paracrine-inhibitory effects of somatostatin on the latter cells. Glucose appears to be the chief stimulus for somatostatin release by δ cells. Gradual somatostatin release from endocrine cells in the proximal intestine during the late phases of nutrient absorption may also act to slow postprandial insulin release, thereby permitting a smooth return of the blood glucose concentration to a fasting level.

β cells have receptors for numerous peptides other than insulin, glucagon, and somatostatin. These include arginine vasopressin, oxytocin, prolactin, placental lactogen, growth hormone (GH), glucocorticoids, estrogens, progesterones, cholecystokinin (CCK), secretin, gastrin, galanin, nerve growth factor, glucose-dependent insulinotropic peptide (GIP), and glucagon-like peptide 1 (GLP-1). Gastrin, CCK, and secretin are known to stimulate insulin release from β cells, but the physiological roles played by many of the other peptides in the islets remain to be established.

The insulin response to ingested glucose is greater than that to intravenously administered glucose. The augmented response to ingested glucose is largely attributable to insulinotropic effects of gastrointestinal hormones released during nutrient absorption. These so-called *incretins* are responsible for rapid insulin release soon after a meal and prior to a major increase in the blood glucose concentration. Both GIP and GLP-1 have been identified as significant incretins with additive insulinotropic effects. GLP-1 also appears indirectly to inhibit glucagon release and to stimulate somatostatin release, whereas GIP appears to be capable of stimulating glucagon release. GIP is chiefly synthesized by K cells in the mucosa of the duodenum and proximal jejunum and released in response to ingested carbohydrates and lipids. GLP-1 is produced by L cells in the mucosa of the distal small intestine and colon. These incretins are thought to play a critical role in glucose tolerance. A sluggish early insulin response to ingested carbohydrate has been demonstrated in humans with glucose intolerance, and the insulinotropic effect of GIP is lost in patients with type 2 diabetes mellitus. Whether the impaired incretin effect in diabetes reflects decreased sensitivity of β cells to GIP, decreased endogenous GLP-1 activity, or a combination of both is uncertain.

In **pregnancy**, there are substantial changes in maternal metabolism to meet increasing fetal metabolic demands. Increased caloric intake, enhanced insulin secretion, peripheral insulin resistance, and increased lipid metabolism in the dam result in sparing of glucose and amino acids for utilization by the fetus whilst lipids are used as an alternative maternal fuel. Crucial to these changes in maternal metabolism during pregnancy, at least in rodents and humans, are *hypertrophy and hyperplasia of β cells within existing islets and enhancement of their secretory response to glucose.* These adaptations, particularly the lowering of the threshold for secretion and, to a lesser extent, mitotic division of β cells, permit maternal islets to maintain elevated insulin secretion in the face of normoglycemia. Failure of these adaptive processes leads to *gestational diabetes mellitus.* In normal pregnancy, there is also decreased glucose tolerance, which reflects peripheral insulin resistance with a reduced rate of glucose clearance. Placental lactogens appear to be responsible for the upregulation of β-cell function in rats and humans during pregnancy; in humans, prolactin may also play a significant role. Progesterone may be responsible for counteracting the stimulatory effects of lactogenic hormones in the latter stages of pregnancy when insulin secretion returns towards normal levels.

β cells also have receptors for 1,25-dihydroxycholecalciferol and the latter is thought to modulate insulin secretion. Many in vivo studies have demonstrated that vitamin D administration improves insulin secretion in vitamin D-deficient animals but, rather than a direct effect of the vitamin, this may be but a consequence of restoration of normocalcemia and removal of the inhibitory effect of high levels of parathormone on insulin secretion. Moreover, some studies have demonstrated an inhibitory rather than stimulatory role of 1,25-dihydroxycholecalciferol on both β-cell growth and insulin secretion in vitamin D-deficient rodents.

The hypothesis that the endocrine pancreas is of neural crest (ectodermal) origin has been refuted by multiple studies. *Islets are now understood to develop from primitive endodermal epithelium of the embryonic foregut and their differentiation precedes that of exocrine elements.* Experimental studies indicate that endocrine differentiation is dependent upon interactions of endodermal epithelium with vascular endothelium, and that these interactions may be promoted by expression of vascular endothelial growth factor by the endodermal cells. Insulin expression first appears at sites where endoderm of the dorsal pancreatic diverticulum contacts endothelium of the portal vein. Although islet cells typically first appear as individual cells associated with the ducts, their development is not dependent on prior formation of the ducts. The four major islet cell phenotypes are derived from a common stem progenitor cell and, during early maturation, there may be transient stages of expression of multiple islet hormones and co-expression of enzymes involved in catecholamine synthesis.

Several *regulatory genes* expressed in endocrine cells of the embryonic pancreas have been identified. They include the homeodomain genes, *PDX1*, *Hlxb9*, *Nkx6.1*, *Isl-1*, *Pax4*, and *Pax6*, and the basic helix–loop–helix genes, *BETA2 (NeuroD)* and *neurogenin3 (ngn3)*. *ngn3* appears to act as a genetic switch that specifies differentiation of pluripotent endodermal cells into endocrine cells. Mice that are homozygous for a null allele of *ngn3* lack all endocrine cell types and die of diabetes mellitus within a few days of birth. Conversely, overexpression of *ngn3* results in premature differentiation of progenitor cells into endocrine cells at the expense of exocrine pancreatic development. The other regulatory genes appear to function in controlling proliferation, differentiation, and survival of precursors of the four major islet cell types once *ngn3*-induced commitment to an endocrine phenotype has occurred.

Insulin production by β cells is not essential for normal endocrine pancreatic organogenesis. However, insulinopenia throughout embryonic and fetal development in mice results in the emergence of hyperplastic islets, suggesting that insulin may function as a negative inhibitor of islet cell growth in utero.

Factors implicated in **growth and maturation of islet cells** include GH, prolactin, TGF-α, gastrin, and nerve growth factor (NGF). TGF-α and gastrin act in concert to induce the differentiation of ductular precursor cells into β cells. GH and prolactin act together to promote expression of receptors for NGF on β cells. In response to GH, both fetal and adult β cells release IGF-1, which in turn stimulates β-cell replication.

Islet cells may constitute up to 10% of the total cells of the early embryonic pancreas, and the first to form may co-express insulin and glucagon. Initially, the islet cells are individualized but later they appear in small nonvascularized clusters. Formation of typical islets by aggregation of cells occurs at a relatively late stage of gestation and vascularization of the islets occurs independently of that of the exocrine parenchyma. Individualized and clustered islet cells may persist, especially at the periphery of lobules. Islet cells lying outside acinar tissue in association with ducts may give rise to tumors in adult animals.

Differing rates of contribution of the dorsal and ventral duct anlagen may account for regional variations in the density of islets and for the variety of cell types present within islets. The distribution of β and δ cells is relatively constant throughout the pancreas in cats, dogs, cattle, and horses, but the density of α and PP cells is variable. In these species, islets rich in α cells are derived from the dorsal pancreatic primordium and its accessory pancreatic duct and are therefore concentrated in the left pancreatic lobe. Islets rich in PP cells are derived from the ventral primordium and its pancreatic duct and are concentrated in the right lobe. Islets rich in both cell types may be found in the body of the pancreas.

Glucose does not stimulate insulin synthesis and release by fetal β cells, although they are capable of responding to other insulin secretagogues. Glucose sensitivity of β cells is only acquired after birth, even though both glucokinase and glucose transporter-2 are both expressed in fetal β cells. Insulin gene expression increases between birth and weaning, and regulation of insulin secretion in response to glucose matures after weaning.

Two morphological types of islets can be identified in ruminants in the late fetal and neonatal periods. *Small islets* (which correspond to conventional islets of other species) are 25–200 μm in diameter and are embedded in exocrine parenchyma. *Large islets* (which diminish in size with age and are rarely observed in adulthood) are 100–1600 μm in diameter, lie in interlobular connective tissue, and chiefly consist of β cells. Endocrine cells of the large islets of fetal calves in the third trimester of pregnancy co-express insulin and galanin, with a surge in galanin synthesis occurring shortly before parturition and reaching a peak by day 5 postnatally. During the same period, there is a marked increase in the number of galanin-releasing nerve fibers innervating the exocrine acini and ducts. The significance of galanin production in the perinatal period in cattle is uncertain, but this peptide is known to inhibit insulin secretion and to have an inhibitory effect on smooth-muscle contractility, gastrointestinal motility, and release of several gastroenteric neurotransmitters and hormones.

Islets have been estimated to comprise 20–30% of fetal pancreatic mass. Formation of new endocrine cells and islets normally ceases in mice after weaning, but the mechanism responsible for this cessation is unknown. Postnatal expansion of the exocrine parenchyma results in a gradual proportional decrease in islets to less than 5% of organ mass by adulthood despite an increase in the volume of islets and the size of individual islet cells.

Islet cells in adults are capable of mitotic division. Replication of β cells can be experimentally stimulated by exposure to glucose, D-mannose, essential amino acids, glucocorticoids, gastrin, CCK, GH, prolactin, IGF-1 and IGF-2, platelet-derived growth factor (PDGF), epidermal growth factor (EDF) and indeed by insulin itself via stimulation of the IGF-1 receptor. The response to amino acids is important in the fetus, in which islets lack sensitivity to glucose, but is less prominent in the adult. Notwithstanding these observations, most β cells have been shown in postnatal rats to have entered an irreversible G_0 phase of the cell cycle and only a small fraction of the population is capable of mitotic division. This fraction declines from approximately 10% in the perinatal period to less than 3% in early adulthood. An alternative to division of pre-existent islet cells is replacement of islet cells by stem cells.

Various hypotheses exist regarding the location of *islet stem cells* in adult animals. Some researchers have proposed that these cells reside in the ductal epithelium, the interstitium, or within islets or even within the bone marrow or spleen. Others have suggested that replacement of β cells in adult animals may occur via transdifferentiation of acinar cells or islet cells expressing hormones other than insulin. Irrespective of their location, facultative stem cells are postulated to proliferate following pancreatic injury, differentiate into β cells, and thence migrate to replenish existing islets or cluster to form new islets. Budding of acinar and islet cells from hyperplastic ductules has been observed histologically in many experimental models in a variety of mammalian species, with expression of *PDX1* occurring concomitantly with the β-cell neogenesis. Following experimental surgical removal of 90% of the pancreas in rats, the mitotic index of pre-existent islet cells increases, but only for a few weeks. Sprouting of ductules occurs from the cut surface of the organ and new islet and acinar cells may be generated from these hyperplastic foci. Similarly, ductular hyperplasia with budding of new islet cells can be experimentally induced by provoking chronic inflammation within the pancreas. Indeed, studies in rodents have indicated that *the entire pancreatic mass of β cells can be replaced every 30 days by processes of programmed cell death and formation of new islet cells from progenitor cells located in the ductal epithelium.* Toxic ablation of β cells in rodents by administration of alloxan or streptozotocin is usually associated with permanent cell loss. Lack of significant cell replacement in the latter model may reflect destruction of both stem cells and mature β cells. Nevertheless, during the period of partial recovery from streptozotocin-induced injury in mice, there is evidence of expression of *PDX1* in δ cells and subsequent transformation of these cells into β cells. The capacity for transformation of ductal or non-β islet cells into insulin-secreting islet cells is not surprising, given that any cell, if altered to allow transcription of the insulin gene, can produce proinsulin. In fact, processing, packaging, and regulated secretion of insulin are possible in endocrine cells that have been so altered.

Notwithstanding these observations that support the concept of islet stem cells in adult animals, a study that used a genetic cell lineage tracing technique demonstrated no stem cell replacement of β cells in adult mice, even following substantial pancreatectomy. Rather, new β cells appeared to be replaced entirely by mitotic division of pre-existent β cells. One explanation that could reconcile this finding with previous observations is that there may be unipotent pancreatic endocrine stem cells in which the insulin gene is transcriptionally active so that these stem cells are indistinguishable from differentiated β cells.

Reduction in pancreatic insulin content with **age** is recognized in rats and mice. However, although smaller islets may lose their ability to respond rapidly to glucose, this appears to be compensated by a normal insulin response from larger islets. Experimental data in rodents indicate that release of insulin, glucagon, and somatostatin is maintained throughout the lifespan. Alterations in endocrine pancreatic structure and function with advancing age are not well documented in domestic animals.

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Developmental anomalies of the islets

Aplasia and **hypoplasia of islets** are reported in dogs as causes of insulin-dependent diabetes mellitus, often manifesting in the first few months of life. *In aplasia, no islets are identifiable.* As islets are irregularly distributed in the normal pancreas, many parts of the organ must be examined microscopically to establish the diagnosis. *Hypoplasia of β cells is predominantly identified in purebred dogs.* It is inherited as an autosomal recessive trait in Keeshonds and a similar disorder has been reported in a Chow Chow pup and as a probable inherited disorder in Golden Retrievers. In the Keeshond, β cells are absent from islets but individual β cells remain scattered throughout the acinar parenchyma. In other cases of islet hypoplasia, a few small islets may be present. The exocrine pancreas shows moderate atrophy consistent with withdrawal of trophic influences but is otherwise normal.

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Degenerative lesions of the islets

Atrophy, necrosis, amyloidosis, and sclerosis

Atrophy of the islets may occur as a result of fibrosis in chronic interstitial pancreatitis, but diabetes is seldom a complication. Atrophy may also be observed in insular amyloidosis.

Vacuolar degeneration of β and sometimes α cells due to glycogen accumulation is a common finding in diabetes mellitus.

Necrosis of the islets occurs in *acute pancreatic necrosis*. Their progressive destruction in this condition is a common cause of diabetes mellitus in dogs. Insular insufficiency may also arise from extensive neoplastic destruction of the pancreas. Although several viruses can replicate within the pancreas and provoke insulitis and necrosis of islet cells, most are not known to provoke clinically significant islet injury. The exceptions are *Bovine viral diarrhea virus* and *Foot-and-mouth disease virus*, both of which may lead to type 1 (insulin-dependent) diabetes mellitus in cattle.

Amyloidosis of the islets occurs in humans, other primates, raccoons, and domestic and wild cats and is a *characteristic lesion of type 2 (non-insulin-dependent) diabetes mellitus* in more than 90% of affected humans and cynomolgus macaques and in more than 80% of affected cats. Insular amyloid may also be detected in clinically healthy cats, with the amount deposited increasing with age, but the prevalence is significantly lower in age-matched nondiabetic animals than in diabetics. The amyloid is a useful morphological marker of disturbed β-cell function and, when detected in normoglycemic cats, is indicative of a potential prediabetic state. Islet amyloidosis has not been reported in diabetic dogs, but may be found in islet cell tumors in this species.

Although the extent of amyloid deposition in diabetes is not directly related to the severity of impairment of glucose tolerance, the amyloid may contribute to dysfunction and degeneration of islet cells by acting as a diffusion barrier. Although the deposits are typically extracellular, amyloid may form intracellularly within β cells as a consequence of intracellular digestion (crinophagia). Such deposits are directly toxic; they disrupt cell membranes and may induce apoptosis or necrosis of β cells.

Islet amyloidosis is a localized primary form of amyloidosis, in which the amyloid deposits are restricted to the islets. The major constituent of insular amyloid is islet amyloid polypeptide (IAPP) produced by β cells. In the species in which insular amyloidosis occurs, IAPP has a central amyloidogenic amino acid sequence (residues 20–29), and a high local concentration of the hormone favors the formation of amyloid fibrils. Increased IAPP secretion occurs in obesity and has been demonstrated in normoglycemic cats with impaired glucose tolerance. However, studies in transgenic mice overexpressing human IAPP indicate that overproduction of the hormone is insufficient alone to induce amyloid formation. An accompanying defect in β-cell synthesis, processing, transport, secretion, or degradation of IAPP may also be necessary. It is postulated that, in insulin resistance, constant overstimulation of insulin and IAPP secretion may lead to IAPP fibrillogenesis via the depletion of factors required to process and secrete IAPP. Alternatively, fibril formation may be promoted by an increase in β-cell synthesis and secretion of IAPP relative to insulin. There is experimental evidence that an increase in the IAPP-to-insulin ratio may occur in response to hyperglycemia, hypertriglyceridemia, and to corticosteroid excess. Insulin itself appears to inhibit IAPP amyloidogenesis.

In **cats**, amyloid deposition occurs particularly in peripheral parts of the islets, in the immediate vicinity of β cells. Extracellular fibril formation is initiated in close association with the β-cell membranes. A perivascular orientation of amyloid around islet capillaries may be obvious in some cases. In cats with advanced diabetes, most islets are affected and may be remarkably enlarged. Most of the islet space is occupied by amyloid and islet cell numbers are severely depleted (Fig. 3.23). Unlike secondary systemic amyloid derived from serum amyloid A, the affinity of insular amyloid for Congo red dye and its birefringence characteristics on polarization are retained after exposure to potassium permanganate and dilute sulfuric acid.

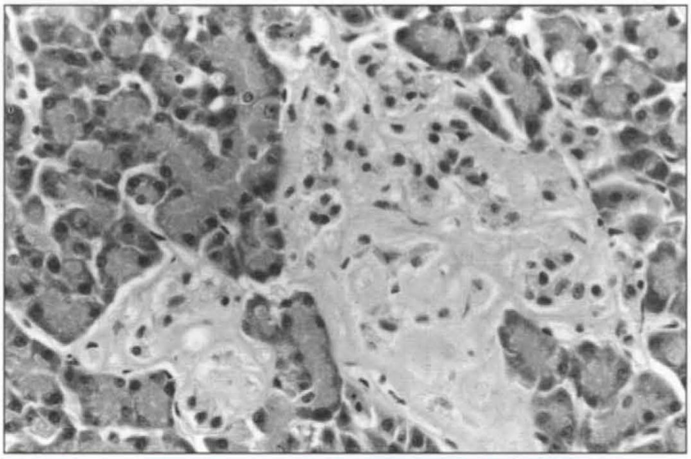

Figure 3.23 Amyloidosis of islets of Langerhans in diabetes mellitus in a cat.

In **dogs** with diabetes mellitus that is not the result of pancreatic necrosis, various degrees of *sclerosis of the islets* may occur. Mild sclerosis is, however, occasionally observed in aged nondiabetic dogs, so its diagnostic significance is difficult to assess. The sclerotic process may be focal or diffuse within affected islets and the collagen deposits commonly appear hyalinized. Mild fibrosis and focal mineralization have also been observed in conjunction with lymphocytic insulitis in **cattle** with type 1 diabetes mellitus.

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Diabetes mellitus

Diabetes mellitus is a common metabolic disorder of dogs and cats, but is rarely seen in cattle, small ruminants, pigs, or horses.

The clinical syndrome of diabetes is caused by failure of the β cells to provide sufficient insulin to meet the metabolic demands of the body. The insulin deficiency may be absolute or relative, and a consequence of inadequate synthesis and release by β cells, decreased sensitivity of target organs to insulin, antagonism of insulin by other hormones or drugs, or a combination of these mechanisms. Although the etiopathogenesis of diabetes mellitus may vary, all cases share some common metabolic, clinical, and pathologic features.

The most common *clinical signs* of uncomplicated (nonketoacidotic) diabetes mellitus are weight loss despite a normal to increased appetite, polydipsia, and polyuria. *A relative or absolute deficiency of insulin leads to a generalized catabolic state.* Persistent hyperglycemia reflects a combination of decreased glucose entry into cells, diminished glucose oxidation, increased glycogenolysis, and increased gluconeogenesis from amino acid sources. Diversion of amino acids from protein synthesis into gluconeogenesis contributes to muscle atrophy and weight loss. Hyperlipidemia results from increased lipolysis and diminished uptake of free fatty acids by adipocytes. The hepatocytes are presented with a large excess of mobilized fatty acids that cannot be adequately processed and exported as lipoproteins because of reduced glucose availability and a shortage of ATP. Hepatic lipidosis results and there may be palpable hepatomegaly. Hyperglycemia increases the filtered load of glucose in primary urine to a level that exceeds the renal tubular threshold for resorption, leading in turn to glucosuria, osmotic diuresis, polyuria, and compensatory polydipsia. Dehydration may develop if water intake does not match urinary losses. Electrolyte disturbances, particularly hypokalemia, largely reflect solute loss in urine. Glucosuria represents a significant caloric loss that contributes to weight loss. Polyphagia despite persistent hyperglycemia reflects decreased entry of glucose into neurons of the hypothalamic satiety center.

Diabetes mellitus is of insidious onset. The normal pancreas contains a substantial reserve of β cells, and insulin-secreting capacity may have to be reduced to 20% of normal before the catabolic processes become dominant. The anabolic effects of insulin are exerted on many tissues and through numerous metabolic steps, not all of which are deranged equally or simultaneously as the insulin deficiency develops. *Impaired glucose tolerance develops early in the condition, and is a feature that is common to all types of diabetes mellitus.*

Classification of diabetes mellitus

The classification of diabetes mellitus into its various subtypes continues to evolve as new knowledge is gained. The reference classification is that used for the human condition, although it is not directly applicable to domestic animals.

In the human classification, **type 1 diabetes mellitus** *is generally consistent with juvenile-onset and insulin-dependent diabetes mellitus.* Patients with type 1 diabetes have a *severe absolute deficiency of insulin* due to autoimmune destruction of β cells. They have negligible insulin response to secretagogues such as glucose and are dependent upon insulin therapy for survival.

Type 2 diabetes mellitus *is generally consistent with adult-onset and non-insulin-dependent diabetes mellitus.* However, some patients may develop this form of diabetes when young and insulin dependence may emerge over time. Type 2 disease is characterized by both *impaired insulin action (insulin resistance)* and *inadequate insulin secretion* to meet metabolic demands. The capacity of β cells to secrete insulin and the number of β cells decline as the disease advances. Depending on the stage of the disease and the functional capacity and number of β cells present, affected patients may be hypo- or hyperinsulinemic. Islet amyloidosis develops in the majority of patients.

Complex genetic traits and environmental influences influence the occurrence of both type 1 and type 2 diabetes in humans.

A third category (previously termed type 3 or secondary diabetes mellitus, but now referred to in the reference classification as **specific types of diabetes**) includes all cases of diabetes mellitus arising as a consequence of insulin antagonism or nonspecific destruction of islets in pancreatic inflammatory, necrotizing, or neoplastic processes.

Glucose toxicity and lipotoxicity

Irrespective of the mechanism underlying diabetes mellitus, once persistent hyperglycemia develops, insulin secretion by β cells becomes suppressed. In both type 1 and overt type 2 diabetes, there is profound unresponsiveness of β cells to changes in blood glucose concentration. This is thought to be due to the deleterious effects of chronic hyperglycemia on β cells, a phenomenon known as *glucose toxicity.*

Glucose normally stimulates transcription of the insulin gene by causing phosphorylation of *PDX1* to enhance binding of the latter to the insulin gene. Glucotoxicity of β cells following prolonged hyperglycemia is mediated by downregulation of glucose transporters on the membranes of β cells and hence downregulation of expression of both the insulin and *PDX1* genes. As well as impairing β-cell secretion of insulin, the persistent hyperglycemia contributes to induction of peripheral insulin resistance.

Glucose toxicity is initially reversible if glycemic control is improved (termed *glucose desensitization*). If hyperglycemia persists for more than 2 weeks, however, it leads to glycogen accumulation in islet cells and cell death. The susceptibility of β cells to glucose toxicity is thought to be greater if β cells are hyperfunctioning, as they may well be if β-cell loss has preceded the onset of hyperglycemia.

Persistent increases in serum fatty acids may also suppress insulin secretion by β cells in a phenomenon termed *lipotoxicity*. Lipotoxicity and glucotoxicity may suppress insulin secretion via a common pathway, as both are associated with an increase in the intracellular concentration of long-chain acyl-coenzyme A molecules.

Secretion of other islet hormones in diabetes mellitus

Impaired responsiveness of α and δ cells to glucose also occurs in diabetes mellitus. In type 1 diabetes, there is a relative or absolute increase in the serum glucagon concentration. This contributes to hyperglycemia by promoting hepatic glycogenolysis. It may also promote development of ketoacidosis by stimulating hepatic oxidation of fatty acids. The glucagon concentration also fails to rise in response to episodes of insulin-induced hypoglycemia. Glucagon responses to hyper- and hypoglycemia are also impaired in type 2 diabetes. One hypothesis is that deficient intraislet secretion of insulin is responsible for both failure of suppression of α-cell secretion in the face of hyperglycemia and failure of glucagon release in the face of hypoglycemia. Whether or not chronic hyperglycemia has a direct deleterious effect on α cells is unknown. Cats with type 2 diabetes have been shown to have mean fasting and glucose-stimulated serum glucagon levels that are significantly higher than those of normoglycemic cats.

Type 1 diabetes mellitus in humans and cattle

Although the initiating triggers are often not identifiable, the absolute hypoinsulinemia of type 1 diabetic humans is a direct result of depletion of β cells by selective autoimmune destruction. Genetic linkages confer high and low levels of susceptibility to this type of diabetes and an association with certain human lymphocyte antigen (HLA) haplotypes has been confirmed. The complex phenotype that is highly susceptible is characterized by poor β-cell defense mechanisms, efficient antigen presentation, and dysregulated cytokine production.

Exposure of genetically susceptible individuals to foreign antigens structurally similar to components of β cells is thought to be an important mechanism by which a cell-mediated immune response becomes directed against β cells. Viruses are the most likely candidate sources of foreign protein and several viruses of humans (group B Coxsackie, mumps, Epstein–Barr and rubella viruses, cytomegalovirus, influenza virus, herpesvirus, and enteric rotavirus) are suspected to play a role in the pathogenesis of type 1 diabetes in genetically predisposed individuals. Group B Coxsackie viruses and *Encephalomyocarditis virus* have also been incriminated in the development of type 1 diabetes in mice. The concept of molecular mimicry being important in triggering autoimmune attack has been supported by the finding that there is substantial sequence and epitope homology between Coxsackie B viral proteins and glutamic acid decarboxylase (GAD); GAD is the enzyme responsible for the synthesis of GABA and is expressed at high levels in β cells. Sequence homology between bovine milk casein and p69 protein of islet cells has also been demonstrated, lending support to the hypothesis that consumption of bovine milk by human infants may potentially lead to autoimmune islet attack.

β-cell apoptosis in type 1 diabetes appears to be largely mediated by perforin released by infiltrating cytotoxic CD8+ T lymphocytes. However, there is evidence in spontaneous animal models of diabetes and in prediabetic humans that the period of lymphocytic insulitis is preceded by a prolonged phase during which local synthesis and secretion of cytokines may contribute to β-cell dysfunction and/or loss. Intracellular proteins derived from any dying β cells may be taken up by islet dendritic cells. The latter respond by synthesizing and releasing cytokines that may damage additional cells.

β cells appear to be particularly susceptible to injury by cytokines and free radicals. IL-1, interferon-γ (IFN-γ), and TNF-α can all interfere with normal β-cell function and integrity via induction of free radicals. β-cell proteins damaged by free radicals may be presented to the immune system in a more antigenic form so that a self-perpetuating cycle of islet injury may be initiated. When acting in concert, IFN-γ and TNF-α almost completely abolish glucose-stimulated insulin secretion. The latter two cytokines can independently upregulate expression of class I major histocompatibility complex (MHC) molecules on β cells and, in combination, they can induce the de novo expression of class II MHC molecules on β cells. Increased expression of MHC molecules in turn promotes homing of autoreactive lymphocytes to the pancreas; homing may also be promoted by IL-2. IFN-γ may also activate quiescent autoreactive T lymphocytes so that they become responsive to antigens encountered in their vicinity. β-cell destruction may also involve Fas ligand, either directly or via generation of cytotoxic molecules such as nitric oxide. Although differentiation of helper T lymphocytes becomes shifted in type 1 diabetes towards the helper T lymphocyte type 1 (Th1) pathway, Th2 pathway cytokines such as IL-10 may also play a role in β-cell destruction via effects on the local vasculature and by promoting local release of other cytokines.

The immune destruction of β cells in humans proceeds slowly and years may elapse between the triggering event and the onset of clinical diabetes. In most patients, this period is characterized by the presence of circulating autoantibodies that serve as predictive markers of the disease. Autoantibodies may be directed against β-cell membrane or cytosolic antigens or even insulin, but appear to reflect an epiphenomenon rather than being directly implicated in the pathogenesis of islet destruction. During the asymptomatic phase, some islets will have lost all β cells, some will contain degenerating islet cells and lymphocytic infiltrates, some will be normal, and others may be hyperplastic. Ultimately, all β cells are lost and the inflammatory infiltrates disappear.

The islets of humans in advanced stages of type 1 diabetes are characterized by marked depletion of β cells, normal δ cell numbers, and normal to moderately reduced numbers of α cells. Such patients have reached a permanent state of insulin dependence.

Diabetes mellitus in cattle appears to be of comparable pathogenesis to type 1 diabetes in humans. Both *Bovine viral diarrhea virus* in persistent infections and *Foot-and-mouth disease virus* are capable of provoking autoimmune lymphocytic insulitis, leading to insulin-dependent diabetes in young cattle.

A comparable pathogenesis may occur sporadically in other species and may explain the occasional observation of lymphocytic insulitis and the presence of circulating antibodies directed against β-cell components and/or insulin in the absence of prior insulin therapy. However, there is little evidence to suggest that autoimmune islet destruction is a significant mechanism responsible for diabetes in cats or dogs.

Type 2 diabetes in humans and cats

Type 2 diabetes accounts for approximately 90% of cases of diabetes in humans and is a polygenic disease in which there is dysregulation of insulin secretion and insulin resistance. Not all the genes involved are known, but specific HLA haplotypes have been implicated. Insulin resistance is largely genetically determined in humans and is common in indigenous populations in which the incidence of diabetes is high. Chronic malnutrition may contribute to the high prevalence of disease in these populations. Experimental studies in rats have demonstrated that fetal or neonatal protein malnutrition may predispose to impaired glucose tolerance in adulthood and to gestational diabetes in pregnant females; moreover, the offspring of affected females are themselves at risk of developing type 2 diabetes by adulthood. Factors such as obesity, reduced physical activity, high dietary intake of fat and processed carbohydrates, inadequate dietary fiber, and stress greatly influence the expression of the genetic abnormality in humans by promoting insulin resistance.

All humans with type 2 diabetes have both insulin resistance and inadequate insulin secretion, but the relative severity of these two defects is variable. During the preclinical phase of impaired glucose tolerance, patients have a characteristic pattern of insulin secretion in response to glucose: the first-phase secretory response is attenuated, and the second phase is delayed and exaggerated. In later stages of the disease, the second phase of the insulin secretory response is lost.

The major defect in β-cell function in type 2 diabetes appears to be in glucorecognition, such that there is a marked decrease in the capacity of extracellular glucose to stimulate insulin release. This is thought to result from reduced synthesis of the glucose transporter in the β-cell membrane. Insulin release postprandially is normally initiated by GLP-1 acting in synergy with glucose. GLP-1 also inhibits glucagon release from α cells. In type 2 diabetes, augmentation of insulin secretion by GLP-1 is reduced despite increased secretion of this incretin. This is thought to be a consequence of rapid desensitization of the GLP-1 receptor in response to an increased GLP-1 concentration. When persistent hyperglycemia develops, the response of the β cells to glucose stimuli is further diminished by glucose toxicity so that the diabetic state becomes self-perpetuating.

Obesity is an important risk factor for the development of type 2 diabetes as it exacerbates any inherited predisposition to insulin resistance and leads to impaired glucose tolerance. Obesity causes internalization of insulin receptors on target cells, reduced insulin receptor affinity, and postreceptor defects in oxidative and nonoxidative glucose metabolism. However, obesity alone does not explain the occurrence of disease as most obese humans are normoglycemic and diabetes develops in only a small proportion.

Skeletal muscle is the most important site of insulin resistance, although the liver and other tissues are also involved. The major defect responsible for resistance in type 2 diabetes is at the level of postreceptor processing of insulin in muscle and leads to reduced glycogen synthesis within myocytes. IAPP is implicated as it antagonizes insulin-stimulated uptake into myocytes and also directly inhibits glycogen synthetase. IAPP promotes breakdown of muscle glycogen and this leads to release of lactate, which is then utilized in hepatic gluconeogenesis.

Some of the other risk factors for type 2 diabetes, such as *physical inactivity*, also contribute to insulin resistance, as does chronic hyperglycemia.

In the early stages of the disease, basal hyperinsulinemia and an exaggerated response to glucose may be detectable and these are considered to be compensatory responses to the insulin resistance. The combination of peripheral insulin resistance and defective insulin secretion promotes persistence of hyperglycemia. As overt diabetes emerges and progresses, insulin secretion by β cells declines and there is usually also progressive islet amyloidosis. Insulin independence is expected in the earlier stages of the disease, but in later stages insulin dependence may develop.

A small proportion of type 2 human diabetics are monogenic, with inactivating mutations of genes critical for normal β-cell function. Heterozygote carriers of the mutant genes develop early-onset diabetes (*termed mature-onset diabetes of the young, or MODY*). Four MODY genes have been identified. The first encodes glucokinase, the rate-limiting enzyme for glucose metabolism in β cells. The other three genes encode transcription factors required in the regulation of insulin gene transcription: *PDX1* and *hepatocyte nuclear factors 1α (HNF1α)* and *4α (HNF4α)*. The clinical characteristics of **juvenile-onset diabetes mellitus of dogs** closely resemble those of MODY in humans.

Most cases of **diabetes in cats** bear a close clinical and pathologic resemblance to human type 2 diabetes, although there is a more frequent occurrence of ketoacidosis and insulin dependence in cats than in humans. Most diabetic cats are insulin-dependent by the time of diagnosis but, in earlier stages of the disease, have an attenuated first-phase insulin secretory response to glucose and an exaggerated second-phase response. Fasting hyperinsulinemia may be demonstrable in some cats and glucose stimulation may induce an increase in the serum insulin concentration.

In both cats and humans with type 2 diabetes, there is both progressive loss of β-cell function and progressive loss of β cells from islets. Reduced numbers of both β and α cells have been demonstrated in diabetic cats, with the δ-cell fraction remaining normal. This is comparable to findings in many humans with type 2 diabetes, although some patients may have α-cell hyperplasia.

Mechanisms incriminated in the gradual decline in function and number of β cells are glucose toxicity, lipotoxicity, and islet amyloidosis. The marked decrease in insulin secretion that develops as persistent hyperglycemia and overt diabetes emerge is largely referable to glucose toxicity. Islet amyloidosis is detectable in most diabetic humans and cats but is not a prerequisite of type 2 diabetes. The mechanisms by which amyloidosis contributes to altered function and elimination of islet cells have been discussed in Degenerative lesions of the islets, above.

IAPP is deeply incriminated in the pathogenesis of type 2 diabetes in humans and cats, not only because of its amyloidogenic properties but also because of its effect on insulin action in target tissues. Normally, the ratio between the plasma IAPP and insulin concentrations is relatively constant. However, an absolute or relative increase in the IAPP concentration develops in impaired glucose tolerance once fasting hyperglycemia develops and is also present in the early stages of overt type 2 diabetes. As IAPP inhibits glucose-stimulated insulin secretion by β cells and increases hepatic gluconeogenesis, it can contribute to hyperglycemia. In skeletal muscle, IAPP is responsible for insulin resistance by inhibiting glucose uptake and by promoting glycogenolysis. As adipose tissue is insensitive to the action of IAPP, it has been postulated that increased plasma concentration of IAPP could also promote a shift of glucose mobilized from muscle glycogen into adipocyte fat to cause obesity as a secondary event. In later stages of type 2 diabetes, the plasma concentration of IAPP becomes subnormal as a result of β-cell degeneration and loss.

The concept of glucose toxicity is of enormous clinical importance in cats with type 2 diabetes as early therapeutic intervention to reduce the magnitude of hyperglycemia may preserve β-cell function and even allow remission of the diabetic state. With early intervention and adequate glycemic control, remission is possible in 20–40% of cats presented with diabetes. Resolution of these so-called *transient diabetes cases* occurs most commonly within 1–3 months of commencing insulin therapy.

Diabetes mellitus chiefly affects *older cats*, with the highest incidence in those 8 years of age or older. The *Burmese breed* appears to be overrepresented amongst diabetic cats in Australia, but breed predispositions have not been identified elsewhere. Male cats, particularly *castrated males*, are more commonly affected than females.

Obesity significantly increases the risk of developing glucose intolerance and clinical disease in cats. Physical inactivity and consumption of commercial feeds rich in highly digestible carbohydrate may contribute to obesity. Obese cats are insulin-resistant and chronic hypersecretion of insulin to combat this may ultimately exhaust the β cells. Reduction in tissue sensitivity to insulin of as much as 52% has been demonstrated in obese cats. This is probably the major mechanism by which glucose intolerance develops but reduced glucose effectiveness (the ability of glucose itself to promote its own uptake by cells at basal insulin levels) is also a feature of weight gain in cats and may contribute. Cats that are lean because of underlying low tissue sensitivity to insulin are particularly at risk of developing glucose intolerance if they gain weight, especially if they have fasting hyperinsulinemia. The latter observation is consistent with the theory that prolonged secretion of excess insulin ultimately exhausts the β cells. It remains to be seen whether low insulin sensitivity in lean cats is genetically determined, as it is in humans.

Several factors may explain why male cats are at greater risk of diabetes than females. First, males are predisposed to develop obesity and, when obese, tend to have a significantly greater mass of adipose tissue than do affected females. The greater the fat mass, the less effective is insulin in lowering the blood glucose concentration. Second, lean male cats appear to have significantly lower tissue sensitivity to insulin than do lean females and the sensitivity declines further following weight gain. Obese male cats also appear more likely to develop fasting hyperinsulinemia than obese female cats.

Diabetes mellitus in dogs

The typical age range for clinical diabetes in dogs is *4–14 years*, with a peak incidence at 7–9 years of age. *Females* are twice as likely as males to be affected and *obesity* increases the risk because of induction of insulin resistance and impaired glucose tolerance. Breeds that are commonly affected include the Miniature and Toy Poodle, Beagle, Dachshund, Standard and Miniature Schnauzer, Miniature Pinscher, Samoyed, Spitz, Lhasa Apso, Bichon Frise and Australian, Fox, Yorkshire, and Cairn Terriers. A genetic basis for the disease has been demonstrated in Keeshonds and is suspected in Golden Retrievers; in these breeds, clinical onset may be at a few months of age and is referable to islet hypoplasia.

The etiopathogenesis of diabetes is uncertain for a large proportion of canine cases. In many dogs, diabetes is referable to *nonspecific islet destruction* by pancreatic necrosis or to *insulin antagonism* arising from concurrent illnesses such as hyperadrenocorticism. Inherited forms of diabetes are rare. Autoimmune destruction of β cells may be responsible in a minority of dogs.

Almost all diabetic dogs are insulin-dependent by the time of diagnosis. Non-insulin-dependent diabetes is rare and most cases are identified in severely obese dogs. A honeymoon period has been observed in some dogs with insulin-dependent diabetes soon after diagnosis; at this stage, glycemic control can be achieved with small doses of insulin. However, the required dose of insulin usually increases over the following 3–6 months as residual functioning β cells are destroyed.

The clinical course of diabetes mellitus in dogs has many similarities to that of type 1 diabetes in humans in that *most affected animals have an absolute insulin deficiency at the time of diagnosis, the loss of β-cell function is permanent, and lifelong insulin therapy is mandatory.*

Unlike the case in cats, *transient diabetes is uncommon in dogs.* Diabetes may be reversed by ovariohysterectomy of bitches with insulin antagonism by progesterone in pseudopregnancy. Very early therapeutic intervention in other insulin-antagonistic disorders such as hyperadrenocorticism may also be successful in reversing the diabetes.

Diabetes mellitus secondary to pancreatic disease

Diabetes mellitus referable to nonspecific islet destruction is most often seen in dogs. Acute pancreatic necrosis is a common cause of insulin-dependent diabetes mellitus in adult dogs. Diabetes develops in approximately 15% of dogs that have the chronic relapsing pattern of the pancreatic necrosis and there may be concurrent exocrine pancreatic insufficiency. Extensive organ destruction by pancreatic adenocarcinoma may also provoke diabetes mellitus in older cats and dogs. Chronic pancreatitis is occasionally responsible in cats and horses, but in most cases of pancreatitis there is insufficient β-cell destruction to provoke diabetes.

Secondary insulin antagonism, diabetic ketoacidosis, and nonketotic hyperosmolar diabetes

Antagonism of insulin by diabetogenic hormones or drugs is an important mechanism responsible for diabetes mellitus in small animals. *Diabetogenic hormones include glucocorticoids, glucagon, epinephrine, progesterone, thyroxine, and growth hormone (GH).* The antagonism is exerted in peripheral tissues and may lead to sustained hyperglycemia that may ultimately exhaust the β cells. Affected animals are initially non-insulin-dependent, and early clinical intervention to control the underlying disease may allow resolution of the diabetes if an adequate population of functional β cells still remains. However, animals with insulin antagonism are likely to have had some reduction in β-cell number prior to the onset of overt diabetes and are prone to have clinical recurrence of diabetes if insulin antagonism recurs for any reason thereafter. Failure to intervene early in insulin antagonism commonly results in transition from non-insulin-dependent diabetes mellitus to a permanent insulin-dependent diabetes mellitus state.

Endogenous progesterone is diabetogenic in the bitch and persistent corpora luteal action in *pseudopregnancy* may initiate diabetes, with clinical signs usually emerging 3–4 weeks post estrus. Pyometra sometimes coexists with the diabetes. Antagonism of insulin by progesterone and synthetic progestagens in dogs is indirect via stimulation of release of GH from the pituitary; the excess GH inhibits insulin receptor activity and intracellular responses to insulin. Ovariohysterectomy may resolve the diabetes.

Increased progesterone concentration can also result in onset or exacerbation of diabetes during *pregnancy*. Hyperglycemia in pregnant diabetic bitches enhances the secretion of fetal GH so that pups may be large at term. Hyperplasia of β cells and neonatal hypoglycemic seizures have been observed in offspring of bitches in which diabetes was poorly controlled during pregnancy.

Synthetic progestagens such as medroxyprogesterone acetate and megestrol acetate can also induce diabetes in dogs. Synthetic progestagens are directly diabetogenic in cats by virtue of their glucocorticoid-like activity.

Estrogen reduces the sensitivity of insulin receptors. Exacerbation of diabetes at estrus has been observed in dogs.

Insulin antagonism by excess endogenous cortisol or administered corticosteroids is common in dogs with *hyperadrenocorticism*. It is the most consistent expression of that condition in cats, being largely responsible for the major clinical signs of polyuria, polydipsia, and polyphagia.

Functional *pituitary acidophil tumors* secreting excess GH may cause diabetes mellitus via insulin antagonism in cats and dogs. In horses with large *adrenocorticotropic hormone-secreting adenomas of the pars intermedia*, glucose intolerance associated with hyperinsulinemia may develop but it is not referable to hormonal antagonism of insulin. Downregulation of insulin receptors on target cells is thought to develop in response to chronic polyphagia that in turn is referable to hypothalamic compression by the adenoma rather than to diabetes or significant hypercortisolemia.

Thyroxine and triiodothyronine are thought to accelerate insulin degradation and clearance to stimulate gluconeogenesis and glycogenolysis. Concurrent hyperthyroidism may therefore exacerbate diabetes mellitus.

Insulin antagonism may also occur in small animals with glucagon-secreting islet cell tumors and with pheochromocytomas producing excess catecholamines. β-blockers and diuretic drugs such as furosemide and thiazides have been reported to trigger diabetes in dogs. Diuretics may induce hypokalemia that impairs both glucose uptake by peripheral tissues and insulin secretion. Diuretics also promote hyperglycemia by stimulating glycogenolysis in the liver and skeletal muscle.

If diabetic animals develop a concurrent stressful illness, increased circulating levels of diabetogenic stress hormones such as catecholamines, cortisol, glucagon, or GH may induce insulin antagonism and exacerbate the hyperglycemia. This greatly increases the risk of development of **diabetic ketoacidosis** (DKA).

DKA is most commonly observed in dogs and cats in which diabetes mellitus has gone unrecognized and untreated. It may also develop in treated patients that are receiving an inadequate insulin dose, especially if another stressful illness is superimposed. As the discrepancy between available insulin and the metabolic demand for it widens, the stimulus for lipolysis increases so that there is increased entry of free fatty acids into the liver. In the absence of adequate glucose, hepatocellular metabolism shifts from triglyceride synthesis to fatty acid oxidation. β-hydroxybutyric and acetoacetic acids and acetone are generated from excess acetyl-coenzyme A and dissociate to provoke ketoacidosis. Ketone bodies in circulation compound the hyperosmolality attributable to hyperglycemia whilst those in urine contribute to osmotic diuresis.

Once ketogenesis commences, severe illness usually manifests within 7 days. DKA is characterized by severe hyperglycemia, a metabolic titration acidosis, ketonemia, ketonuria, severe hyperosmolality, osmotic fluid shifts from intracellular to extracellular compartments, exacerbated osmotic diuresis with loss of sodium, potassium, magnesium, and glucose, and profound dehydration and hypovolemia. Hypovolemic shock will exacerbate the acidosis by inducing lactic acid release from hypoxic tissues. Clinical signs include lethargy, anorexia, depression, dehydration, vomiting, tachypnea, weakness, and abdominal pain. If ketoacidosis is severe, there may be a strong odor of acetone and dyspnea with slow deep breathing (Kussmaul's respiration). Cats may become recumbent and moribund. Death may result from hypovolemia, hyperviscosity of blood, severe acidosis, hypokalemia, thromboembolism, and/or renal failure.

Nonketotic hyperosmolar diabetes is also a life-threatening complication but occurs much less frequently than DKA in cats and dogs, notwithstanding that many sick diabetic cats are hyperosmolal. The syndrome is characterized by extreme hyperglycemia and hyperosmolality, severe dehydration due to profound osmotic diuresis, and central nervous system depression in the absence of ketoacidosis. Clinical signs reflecting the cerebral effects of progressive hyperosmolality may include restlessness, ataxia, nystagmus, mental confusion, depression, delirium, seizures, hyperthermia, semicoma, or coma. Death may result from respiratory failure. In humans, this form of diabetes is usually seen in aged type 2 diabetics with concurrent illness. Low levels of endogenous insulin in these patients are sufficient to maintain antilipolytic and antiketogenic effects but are insufficient to allow adequate glucose uptake by tissues.

Lesions of diabetes mellitus

The lesions of diabetes mellitus in cats and dogs are comparable. *Apart from emaciation, and possibly dehydration, the principal postmortem finding is diffuse hepatic lipidosis.* The pancreas may appear normal or reveal the lesions of postnecrotic scarring, neoplasia, or chronic pancreatitis. Hypertriglyceridemia may be indicated by lipemia.

Microscopic lesions can usually, but not always, be found in the pancreas. In addition to the insular changes described earlier, there may be *vacuolation of islet cells and of epithelial cells lining the smaller ducts* (Fig. 3.24). Vacuolation reflects accumulation of glycogen and is a specific lesion of diabetes mellitus but is thought to be present only in severe and rapidly developing cases. There is usually severe diffuse macrovesicular hepatic lipidosis and often biliary epithelial vacuolation due to glycogen accumulation (Fig. 3.25). In dogs, glycogen may also accumulate within the nuclei of hepatocytes. *Glycogen nephrosis* is a specific lesion of diabetes. The glycogen is chiefly deposited in tubular epithelium of the loops of Henle and the distal convoluted tubules and mainly in the nephrons of the inner cortex. There is also fatty degeneration of the epithelium of the proximal renal tubules (Fig. 3.26). Lipid emboli are occasionally detectable in glomerular capillaries.

In chronic diabetes, *diffuse or nodular glomerulosclerosis* may develop. Periodic acid–Schiff-positive basement membrane material accumulates to cause hyaline thickening of the capillary basement membranes and sclerosis of lobules in the glomerular tufts. Diffuse thickening of the mesangium also develops and may lead to glomerular obliteration. There may be tubular evidence of proteinuria referable to increased glomerular permeability. Basement membrane thickening may also be apparent in Bowman's capsule and convoluted tubules as well as in capillaries in other organs.

A majority of human diabetics succumb to secondary complications involving the heart, kidneys, vasculature, and nervous system. Some of these complications are a consequence of protracted hyperglycemia and the formation of *advanced glycosylation end-products*. These irreversible products accumulate on long-lived proteins such as those of the lens, renal glomeruli, collagen, and peripheral nerve myelin, and they may lead to significant structural and functional alterations to these proteins. Vascular injury is responsible for many of the complications of diabetes in humans.

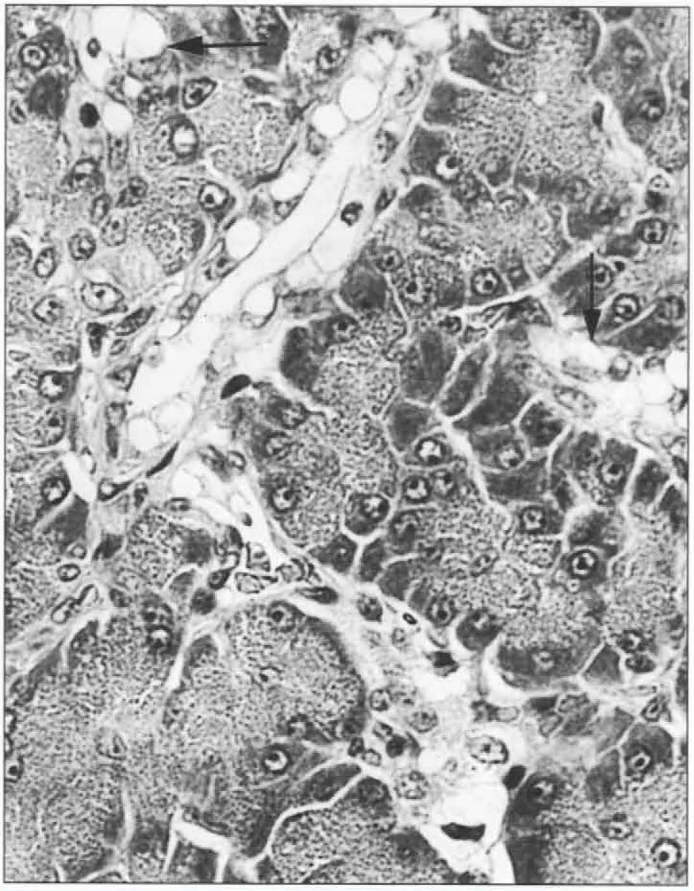

Figure 3.24 Hydropic degeneration of islet cells (arrows) and ductal epithelium due to glycogen accumulation in diabetes mellitus in a dog.

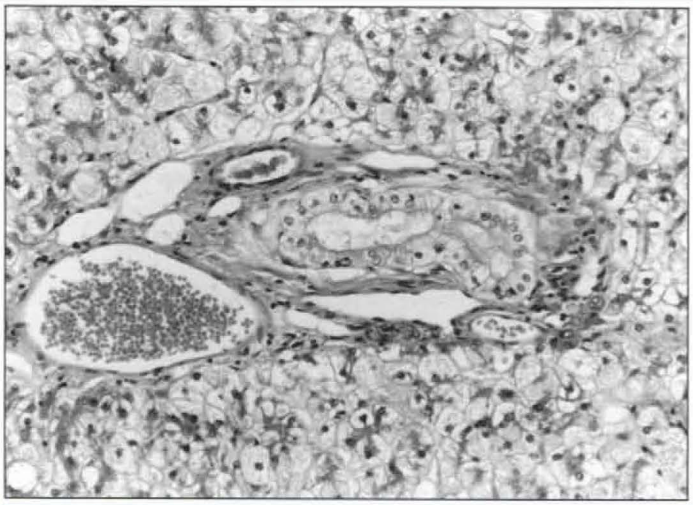

Figure 3.25 Severe hepatic lipidosis with **hydropic degeneration** of bile duct epithelium due to glycogen storage in diabetes mellitus in a dog.

Most of the complications that are so important in human diabetes are rarely seen in domestic animals, probably because of the shorter duration of the disease in animals. *Glomerulosclerosis* is usually only seen in cats and dogs with chronic diabetes of several years' duration. *Diabetic retinopathy* is diagnosed infrequently in dogs and only manifests 5 years or more after onset of diabetes. The retinal vascular changes comprise venous dilation and tortuosity, saccular capillary microaneurysms, and focal hemorrhage, and are most severe at the posterior pole. These nonproliferative lesions in dogs resemble early stages of human diabetic retinopathy. Diabetic retinopathy has been experimentally induced in cats.

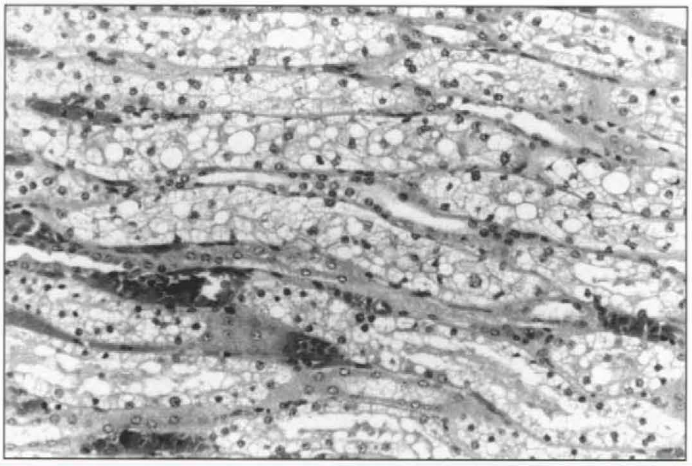

Figure 3.26 Fatty degeneration of proximal renal tubules in diabetes mellitus in a dog.

Cataract formation is an early and common complication of diabetes in the dog and may be the presenting sign. Dogs with poorly controlled diabetes and erratic fluctuations in blood glucose concentration appear to be particularly at risk of developing cataracts. Cataracts can develop rapidly and are irreversible. Cataracts are observed infrequently in diabetic cats, suggesting that their lens metabolism differs from that of dogs or that the lens capsule is less permeable to glucose.

Peripheral demyelinating neuropathy is a well-recognized complication of diabetes in cats but occurs very infrequently in dogs. It usually involves the distal limbs symmetrically and approximately 8% of diabetic cats have a characteristic plantigrade stance. There may be progressive paraparesis, pelvic limb hyporeflexia, proprioceptive deficits, and atrophy of distal hindlimb musculature. Thoracic limb involvement is rare. Autonomic and cranial nerve involvement is common in human diabetes, but is reportedly rare in dogs. The peripheral neuropathy involves axonal degeneration and segmental demyelination and remyelination in distal parts of motor and sensory nerves. The pathogenesis is uncertain but probably multifactorial. Possible mechanisms include depletion of intracellular myoinositol and decreased Na-K ATPase (both of which could impair nerve conduction), nonenzymatic glycosylation of tubulin (which could impair axonal transport), glycosylation of myelin (leading to impaired conduction and macrophage uptake), and altered fatty acid metabolism (with impaired synthesis of cell membranes and myelin). The neuropathy is reversible if glycemic control is achieved soon after it manifests, but not in protracted cases.

As is the case in human diabetes, *secondary infections are common in diabetic cats and dogs.* Most involve the respiratory and urinary tracts and skin. Emphysematous cystitis is occasionally seen in diabetic animals with lower urinary tract infections involving glucose-fermenting bacteria such as *Escherichia coli*, *Proteus* sp., and *Aerobacter aerogenes*. Abnormal neutrophil function has been demonstrated in diabetes, including defective chemotaxis, impaired adhesion, and decreased phagocytic and intracellular killing capacity. Glucose binding to complement C3 may also cause diminished opsonization of microorganisms. In poorly controlled diabetes, lymphocyte proliferative capacity in response to mitogens is also decreased, and there is reduced expression of T and B surface markers.

Diabetes mellitus has also been associated with the development of *superficial necrolytic dermatitis* and, rarely, vitiligo in dogs, a skin fragility syndrome in cats, and cutaneous xanthogranulomas in both species. These disorders are discussed in Vol. 1, Skin and appendages.

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Hyperplasia of the islets and nesidioblastosis

The total number of islet cells in health represents the balance between cell division or neogenesis and cell loss via apoptosis or necrosis.

Subtle proliferative responses of the islets may be difficult to detect microscopically due to regional variation in islet density within the pancreas and because of alterations in islet size that may occur with aging. *Diffuse hyperplasia of islets is rarely appreciated in domestic animals but is probably underdiagnosed.* It has been described in humans and other primates, mice, rats, hamsters, and horses. The enlarged islets appear hypercellular and may compress the adjacent exocrine parenchyma. Islets may comprise more than 40% of the affected pancreatic lobules and most of the hyperplastic cells are insulin-secreting.

The cause of islet hyperplasia is uncertain but it may represent a *compensatory phenomenon* following pancreatic injury with partial loss of islets or in conditions of decreased synthetic function of islets or antagonism of their hormone products. In many cases, islet

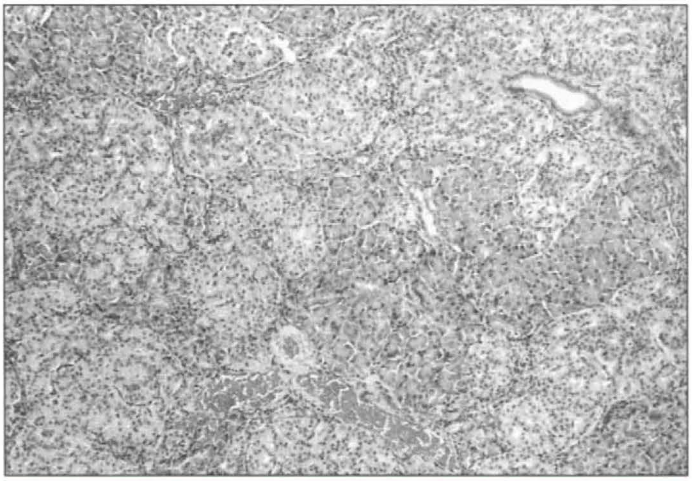

Figure 3.27 Nesidioblastosis (ductular and islet cell proliferation) in a dog.

hyperplasia is accompanied by evidence of β-cell degeneration or islet hyperemia. In humans, islet hyperplasia is seen in hyperinsulinemic hypoglycemia of infants, in infants born of diabetic mothers, in chronic pancreatitis, in Zollinger–Ellison syndrome, and in multiple endocrine neoplasia. In rats and humans, hyperplastic islets have been reported in pancreatic fibrosis. Hyperplasia is also documented in aged rats and horses, in sedentary obese rats, and in hamsters treated with corticosteroids.

Nesidioblastosis *is the term used to describe combined ductular and islet cell proliferation* (Fig. 3.27A, B). Newly formed islet cells appear to bud from proliferating epithelium of small intralobular ductules and thence migrate and organize into discrete islets. Nesidioblastosis may coexist with islet hyperplasia. Nesidioblastosis is typical of *hyperinsulinemic hypoglycemia* in humans. It can be experimentally induced in rodents by pancreatic injury with partial loss of islet cells, partial pancreatectomy, and partial pancreatic duct obstruction. Nitrosamine administration to hamsters induces nesidioblastosis early in pancreatic carcinogenesis, although the islet tissue is eventually replaced by exocrine neoplastic tissue. Nesidioblastosis has also been described in aged rats and horses, in dogs and humans with islet cell neoplasia, and in dogs with type 1 diabetes mellitus.

Neogenesis of islets from ductules is thought to be mediated by growth factors produced within the pancreas and acting locally. As induction of islet neogenesis may be a means to combat absolute or relative insulin deficiency in diabetes mellitus, intensive research is currently focused on delineating the roles of such trophic factors as ilotropin, regeneration protein, insulin-like growth factors (IGFs), and neurotrophins.

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Neoplasia of the islets

Pancreatic islet cell tumors are uncommon in domestic animals. Most have been described in dogs and ferrets. They may be benign or malignant and, although usually solitary, may be multiple. In cattle, islet cell tumors may coexist with pheochromocytomas.

Grossly, islet cell tumors are characterized by their firm consistency, sharp demarcation, homogeneity, and distinctive pale gray-purple color. Circumscription and even encapsulation are not reliable indicators of benign behavior as solitary and circumscribed carcinomas may have metastases in adjacent lymph nodes and commonly the liver.

Microscopically, both adenomas and carcinomas form architectural patterns that are highly reminiscent of normal islets. The cells form trabecular, pseudoacinar, or glandular arrays or solid nests separated by a delicate fibrovascular stroma (Fig. 3.28A, B). Ductular structures may be identifiable within both adenomas and carcinomas, and small nests of exocrine acinar cells may be found within adenomas, particularly towards the periphery. Stromal deposits of *amyloid*, derived from IAPP, may be present in insulin-secreting tumors. Most islet cell tumors stain positively with chromogranin A and neuron-specific enolase.

Both adenomas and carcinomas may be associated with syndromes of hormonal hypersecretion, although some tumors are clinically silent. The release of hormones is typically inappropriate and not subject to normal physiological controls, although most tumors are not completely autonomous. Numerous peptide hormones have been identified within islet cell tumors in humans, and more than one hormone can usually be demonstrated by immunohistochemistry within the majority of such tumors in dogs, ferrets, and cattle. The variety of peptide-producing cells present in carcinomas is usually reiterated in metastases. Notwithstanding the presence of multiple hormones, *clinical signs primarily reflect excess or inappropriate secretion of only one hormone.* Clinical syndromes referable to production of insulin, gastrin, glucagon, PP, and somatostatin have been documented in domestic animals; syndromes referable to secretion of VIP, secretin, IAPP, or adrenocorticotropic hormone are yet to be identified.

Insulinoma

Most functional islet cell tumors described in domestic animals have contained a large proportion of β cells and been associated with a *syndrome of hyperinsulinism, episodic hypoglycemia, and neurologic signs.* Most reported cases have involved *middle-aged to older dogs*, with Boxers, Fox Terriers, German Shepherds, Labradors, Golden Retrievers, Irish Setters, Weimaraners, Collies, German Shorthaired Pointers, and Standard Poodles being overrepresented. Insulin-secreting tumors are also common in *ferrets*, especially males, and may coexist with estrogen-secreting tumors of the adrenal cortex. They are very infrequently observed in older cattle, cats, and horses.

Clinical signs reflect both inadequate glucose supply to the central nervous system and increased catecholamine secretion by the adrenal medulla in response to hypoglycemia. *The most common signs are episodic seizures, weakness, and collapse* that, with ataxia, depression,

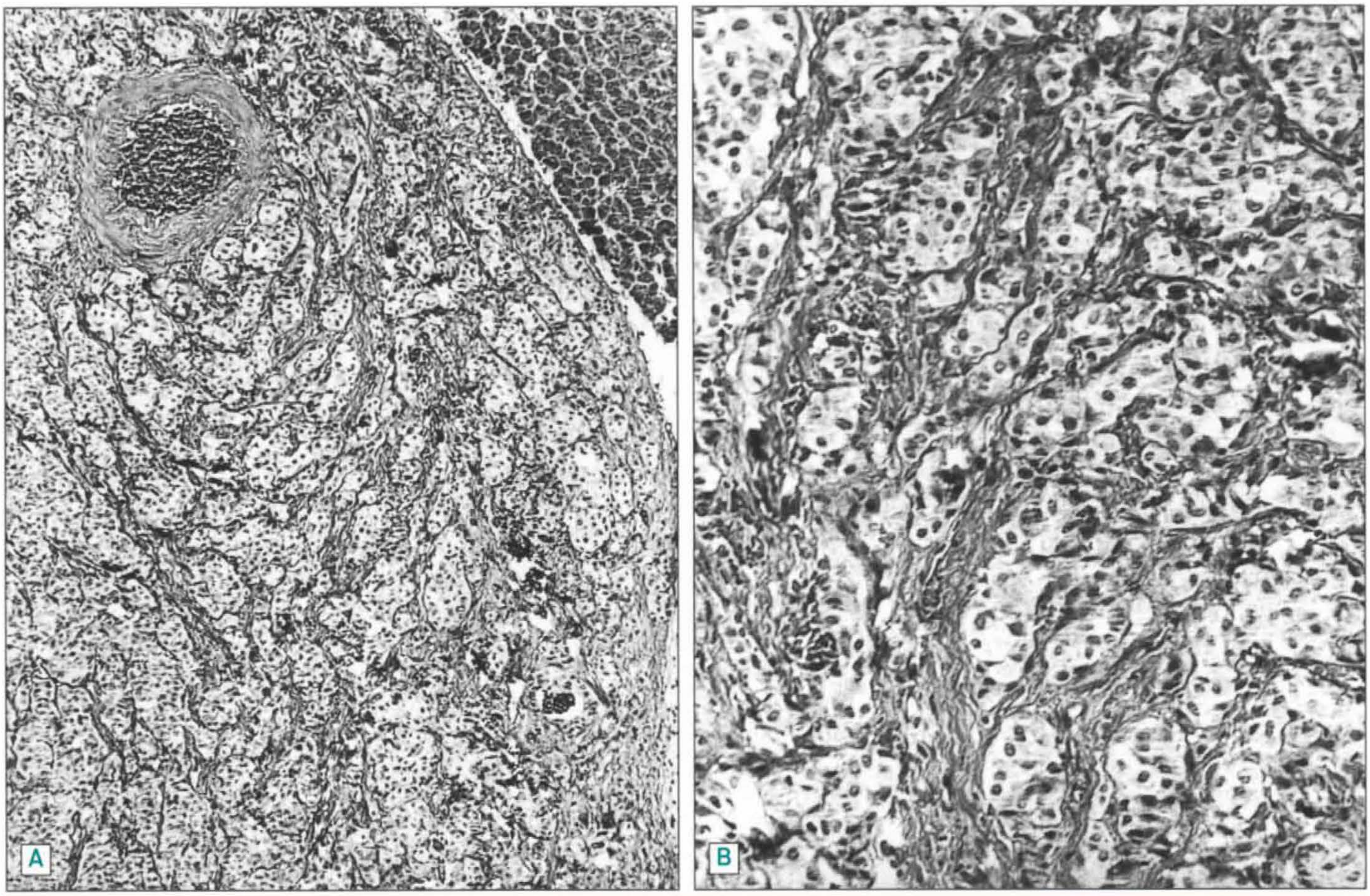

Figure 3.28 Pancreatic islet cell tumor. **A.** Low-power view of the tumor compressing acinar parenchyma. **B.** Detail of (**A**).

and mental confusion, reflect *neuroglycopenia* and are rapidly alleviated by administration of glucose. Signs of sympathetic nervous system stimulation include trembling, muscle fasciculations, restlessness, hunger, and behavioral changes and often precede signs of neuroglycopenia. Ptyalism is a common sign in affected ferrets. The severity of clinical signs depends upon the duration and severity of hypoglycemia and the rate at which the blood glucose concentration falls. When fasting hypoglycemia is recurrent or persistent, adaptation permits tolerance of extremely low blood glucose levels without obvious clinical signs.

Clinical episodes are often triggered by fasting, exercise, excitement, or eating. In fasted animals, insulin secretion by the tumor exacerbates the decline in the blood glucose level. Most dogs with insulinomas will become hypoglycemic after a fast of 8 hours or less. Feeding often stimulates excessive insulin secretion by the tumor to result in hypoglycemia 2–6 hours postprandially. Exercised or excited animals with insulinomas have increased glucose utilization by all tissues because of abnormal availability of insulin and this is exacerbated by impaired hepatic glycogenolysis and reduced capacity for hepatic gluconeogenesis.

The frequency, intensity, and duration of episodes increase progressively over time. However, the episodes are characteristically of brief duration because release of counterregulatory hormones such as catecholamines, glucagon, and cortisol act to restore the blood glucose concentration above the critical threshold. If these compensatory mechanisms are inadequate, syncope, coma, or, more commonly, seizures develop. Seizures may stimulate further counterregulatory hormone release to boost the glucose level so that the seizures usually last only seconds to a few minutes. Rarely, repeated episodes of severe and prolonged hypoglycemia may cause irreversible cerebrocortical necrosis.

Uncommon manifestations of insulinomas in dogs include weight gain, due to the anabolic effects of insulin, and persistent insomnia. In a minority of affected animals, peripheral polyneuropathy develops and is characterized by demyelination, remyelination, and, to a lesser extent, axonal degeneration. Although the neuropathy may have a metabolic pathogenesis, it is considered more likely to result from an immune-mediated process due to common antigens being shared by tumor cells and components of peripheral nerves.

Most insulinomas are small, measuring 1 cm or less in diameter, so that a thorough search for a suspected tumor is necessary. Intraoperative identification may be facilitated by intravenous administration of methylene blue that becomes concentrated in the endocrine pancreas. Unlike the case in humans, *most insulinomas in domestic animals are malignant*, with locally invasive growth and early metastasis to regional lymph nodes and liver. Carcinomas tend to be larger than adenomas and may have a multilobular appearance and contain foci of hemorrhage and necrosis. Their aggressive behavior is belied by their usually well-differentiated histological appearance, although the component cells may be less uniform in size and shape than in adenomas. Demonstration of vascular or lymphatic invasion, the most important criterion of malignancy, may require examination of multiple sections. Adenomas are slow-growing, sharply delineated, and encapsulated, and are amenable to surgical excision.

Gastrinoma

Gastrin-secreting islet cell tumors are rare, but have been described in *dogs* and *cats*. Gastrin is not normally produced in mature pancreatic islets but rather by cells of the pyloric antral and proximal duodenal mucosa. Immunohistochemistry is needed to confirm a diagnosis of a pancreatic islet cell gastrinoma.

The clinical syndrome caused by hypergastrinemia is known as the **Zollinger–Ellison syndrome**. Excess gastrin causes gastric acid hypersecretion, antral mucosal hyperplasia, and gastroduodenal ulceration. There may also be reflux esophagitis and/or jejunal ulceration. Affected dogs and cats are presented with clinical signs of anorexia, vomiting, hematemesis, intermittent diarrhea (particularly with steatorrhea), and severe weight loss. Steatorrhea results from inactivation of pancreatic lipase and precipitation of bile salts at a low duodenal pH. Melena, abdominal pain, and polydipsia may also be observed, and affected animals may be anemic. Hyperplasia of thyroid C cells has been observed in a few dogs with a gastrinoma and may reflect stimulation of calcitonin release by gastrin, but this does not appear to be of clinical significance.

An elevated serum gastrin concentration may support a diagnosis of gastrinoma, especially if measurement of the pH of gastric fluid confirms hyperacidity, but provocative testing with secretagogues such as calcium or secretin may be necessary to demonstrate gastrin hypersecretion.

Gastrin-secreting tumors may be solitary or multiple and are usually of firm consistency, with often a prominent hyalinized stroma microscopically. Even when grossly circumscribed, there is usually infiltrative growth at their periphery. Metastasis to regional lymph nodes and liver is common and in most cases has occurred by the time of diagnosis so that the *prognosis is usually guarded to poor*.

Glucagonoma

A syndrome of *mild nonketoacidotic diabetes mellitus, diffuse vacuolar hepatopathy, hypoaminoacidemia, and superficial necrolytic dermatitis* has been recognized in humans in association with islet cell tumors that secrete excess glucagon. A similar syndrome, accompanied by small intestinal diarrhea, has been described in a few *dogs* with islet cell carcinomas secreting glucagon. Characteristic lesions of erythema, hyperkeratotic crusting, and erosion affect the footpads, mucocutaneous junctions, ears, and frictional and pressure points. *Diabetes mellitus results from the insulin-antagonistic effects of glucagon in stimulating hepatic gluconeogenesis and glycogenolysis*. Diarrhea is thought to be attributable to glucagon causing reduced absorption or stimulating net secretion of water and electrolytes by the small intestinal mucosa. The cutaneous lesions are induced by amino acid deficiency, with protein depletion of the epidermis. Theories as to why such a deficiency develops in an animal with a glucagonoma include amino acid utilization in gluconeogenesis and consumption of amino acids, especially tryptophan, in the synthesis of glucagon itself. The cutaneous lesions are not, however, pathognomonic of a glucagonoma. Indeed, most reported cases of superficial necrolytic dermatitis in dogs have been associated with severe vacuolar hepatopathy of obscure cause, with no pancreatic tumor detected. (Superficial necrolytic dermatitis is discussed in detail in Vol. 1, Skin and appendages).

PPoma

A clinical syndrome resembling Zollinger–Ellison syndrome has been observed in a single *dog* with an islet cell tumor secreting both insulin and pancreatic polypeptide (PP). Clinical signs included chronic vomiting, anorexia, and weight loss and were associated with duodenal ulceration and hypertrophic gastritis. PP secretion may have been responsible as this hormone decreases pancreatic bicarbonate secretion and is also a weak stimulant of gastric acid secretion.

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4

Urinary system

M. Grant Maxie and Shelley J. Newman

Kidney

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Kidney

GENERAL CONSIDERATIONS

The kidney is the central organ involved in the maintenance of a constant extracellular environment in the body. The vital homeostatic functions performed by the kidney include excretion of waste products, maintenance of normal concentrations of salt and water in the body, regulation of acid–base balance, and production of a variety of hormones (erythropoietin, renin, prostaglandins), including metabolism of vitamin D to its active form, 1,25 dihydroxycholecalciferol ($1{,}25(OH)_2D_3$). *The essential requirements for normal renal function are: adequate perfusion with blood (pressure greater than 60 mmHg), adequate functional renal tissue, and normal elimination of urine from the urinary tract.*

Failure of the urinary mechanism occurs if there is inadequate perfusion (**prerenal failure**), inadequate processing (**renal failure**), or inadequate discharge (**postrenal failure**). The outcome so far as the composition of body fluids is concerned is always approximately the same; there is imbalance of salt and water, and of acids and bases, and there is retention of wastes. *The most commonly used index of failure is the amount of urea that is retained.* Urea itself is rather harmless, but if other urinary functions are sufficiently disturbed, a fairly consistent set of clinical signs emerges and the syndrome is called **uremia.**

Water and salt are, quantitatively and in an evolutionary sense, the most important constituents of body fluids. The assumption during evolution of a terrestrial existence and homeothermy by mammals imposed a need to conserve water and salt and simultaneously excrete large quantities of metabolites. Homeothermy, with sustained high body temperatures, imposed a sustained high metabolic rate, a need for control of the peripheral circulation, and structural modifications to the integument. Carbon dioxide is very soluble in water, and highly diffusible, and responsibility for its excretion has been transferred from the integument of aquatic and amphibian ancestors to the respiratory system of mammals; as the end-product of fat and carbohydrate metabolism, carbon dioxide presents no problem of elimination to the kidneys. However, nitrogenous wastes, and a multitude of other organic compounds and inorganic substances, impose a substantial load on the renal mechanism. Mammals excrete urea, which is highly soluble, diffusible, and osmotically active; excretion of wastes and conservation of water in mammals require concentrating mechanisms capable of raising the osmotic pressure of urine above that of blood. The extracellular fluid and plasma of the body are dialyzed through the kidney many times in a day, producing copious amounts of primary urine, of which almost all is reabsorbed. Although this system effectively rids the body of wastes, it requires a large expenditure of energy and a dependable renal blood supply.

Renal blood flow is normally high, up to 25% of the cardiac output. The kidneys are not given to reactive hyperemia, although blood flow may be increased by the action of pyrogens in febrile states. Pathologists are particularly concerned with the causes and consequences of reduced renal perfusion. Peripheral and splanchnic blood flow may be sacrificed in the event of crisis, but renal blood flow is only diminished in order to preserve circulation to the prime priorities of cardiac output, the heart and brain. Renal oxygen consumption, relatively small in relation to renal blood flow, is high relative to that of other tissues and is equal to about 10% of whole-body consumption. Most of the oxygen consumed by the kidney is expended in the resorption of essential solutes, a predominantly cortical function, and hence the consequences of reduced perfusion are largely borne by the cortex.

A unique feature of renal blood flow is that it varies little over a wide range of arterial pressures, thus ensuring a stable *glomerular filtration rate (GFR).* The kidneys possess intrinsic mechanisms by which they vary their vascular resistance in order to maintain constant glomerular filtration. Renal blood flow, upon which the function of nephrons depends, is normally directed predominantly toward the outer *cortical nephrons* rather than to the inner cortical, or *juxtamedullary nephrons*, but may be redistributed between the two areas. Reduction in perfusion is a common occurrence in the kidney, affects primarily the cortex, and may lead to atrophy of tubular epithelium and tubular dilation, to necrosis of tubular epithelium, or to more or less extensive necrosis of the renal cortex. Blood flow to juxtamedullary nephrons is maintained more effectively than is flow to outer cortical nephrons, and blood may be directed from the outer cortex to the inner cortex under conditions of reduced renal blood flow. Preservation of blood flow to juxtamedullary nephrons serves to maintain function of the nephrons that are able to conserve water, and hence aid in maintaining circulating blood volume, and that are able to concentrate urine, and hence to eliminate waste products economically.

The *glomerulus* produces ultrafiltrate as the net result of the difference between the high systemic blood hydrostatic pressure and the low proximal tubular hydrostatic pressure. Urinary ultrafiltrate is isosmotic with plasma and there is essentially zero colloid osmotic pressure in ultrafiltrate to favor diffusion. The glomerular basement membrane is semipermeable, and dissolved substances and particles in suspension move across the glomerular capillaries at rates determined by size, shape, and electrical charge. Malfunction of the glomerulus may be due to inadequate glomerular blood flow and/or structural alterations that decrease or increase its permeability. The outcome of glomerular injury is influenced by the limited capacity in the glomerulus for disposal of deposits, the capillary obstruction which results from swelling of endothelial and mesangial cells, the production of basement membrane-like material by injured mesangial cells, and permeability changes in basement membranes.

The principal function of the kidney is the regulation of salt and water balance; this requires a system of monitors and feedbacks in order to achieve fine regulation. The *renin–angiotensin system* regulates sodium balance, and the *hypothalamus and antidiuretic hormone* control osmotic and volume regulation. The structural basis of the renin–angiotensin system is provided by the *juxtaglomerular apparatus*; to maintain *glomerulotubular balance*, the GFR is adjusted locally and continually to changes in the chloride concentration of tubular fluid. Generally, and over the longer term, the renin–angiotensin system stimulates the adrenal glands to produce *aldosterone* to assist in the retention of sodium and therefore of water.

The formation of primary urine requires that substances from plasma must pass freely across the glomerular membrane and into the tubules. Once within the tubules the substances are, in a strict sense, outside the body. Many of the filtered substances are retrieved by a system of *selective reabsorption* that requires special machinery and energy. The function may be defective, allowing essential substances to be wasted in the urine. This occurs when enzymes are genetically deficient, or cortical epithelial cells are injured, or, because plasma levels of filterable substances are unusually high or the glomerulus is unusually permeable, the quantity of the material to be reabsorbed exceeds the transport capacity of the epithelial cells. Myriad tubular functions are carried out sequentially, and the structure of the tubule varies along its length corresponding to the function to be performed; at least 12 functionally and anatomically distinct segments are described.

Terrestrial mammals have a need to conserve water while eliminating high loads of waste, and a need therefore to concentrate urine above the tonicity of plasma. This function is provided by the *loop of Henle*; juxtamedullary nephrons have long loops and cortical nephrons have short loops. Some species have predominantly long loops and some predominantly short loops; those animals with a preponderance of long loops have greater concentrating ability. Although the deep or juxtamedullary nephrons are the first to develop and are active while centrifugal development of the rest of the cortex is under way, neonatal nephrons have immature functional capacity and a relative inability to conserve sodium. Neonatal pigs, in particular, are thus very susceptible to dehydration. In calves, in contrast to most other neonates, renal functional capacity approaches that of adult cattle within 2–3 days of birth.

According to the "**intact nephron**" or "adaptive nephron" hypotheses, nephron function is an all-or-none phenomenon. In progressive renal disease, the remaining nephrons respond by hyperplasia and hypertrophy, since new nephrons cannot be formed following renal maturation. Glomerular filtration and tubular function then increase concomitantly, and glomerulotubular balance and homeostasis are maintained. *All of the renal components are interdependent, and if one component is irreversibly damaged, function of the other components is impaired*: glomerular disease can cause decreased peritubular perfusion and tubular atrophy; tubulointerstitial disease can cause glomerular obsolescence. *There is a tendency for all forms of chronic renal disease to destroy all four components of the kidney, resulting in chronic renal failure and shrunken scarred* **end-stage kidneys**; differentiation of the initiating cause may be impossible. Interstitial inflammation and fibrosis may be primary events, but they also commonly accompany primary diseases of the glomeruli and tubules. In the past, chronic generalized renal disease in dogs was frequently ascribed to be the result of interstitial nephritis, and leptospirosis was espoused as the most common cause. Because of vaccination, canine leptospirosis is no longer prominent, and chronic renal disease in dogs, as it now occurs, is the end-result of a wide variety of causes.

Anatomy

The *renal collecting system* is derived from the ureteral bud (metanephric duct), a diverticulum of the mesonephric (Wolffian) duct, and consists of *the ureter, pelvis, calyces, and collecting ducts*. Nephrons develop from the metanephric blastema and attach to the growing ends (ampullae) of the collecting system. The *uriniferous tubule* consists of the nephron and the collecting tubule. The renal calyces are the cup-shaped recesses of the pelvis that enclose conical masses of medullary pyramids. The apex of a pyramid is referred to as a *papilla*, and its tip is fenestrated by collecting ducts (area cribrosa). The fornix is the uppermost blind end of a calyx or pelvis.

The kidneys of domestic animals can be classified as **unipyramidal** (unilobar) or **multipyramidal** (multilobar). *Cats, dogs, small ruminants, and horses have unipyramidal kidneys*. In cats, one lobe is present, and papillary ducts open into a calyx on a single renal papilla. In dogs, small ruminants, and horses, there is complete or partial fusion of several lobes and a single crest-like papilla (*renal crest*). *Pigs have multipyramidal kidneys* in which there are several distinct renal lobes, pyramids, and their respective papillae. Extensions of renal cortex between the pyramids are known as the *renal columns of Bertin*. Simple papillae occur in central pyramids, and compound papillae in pyramids at the renal poles; this is of pathogenetic significance because compound papillae are more susceptible to ascending infection. *The kidneys of cattle are also multipyramidal*, but have distinct external lobation, with each lobe having one pyramid. The renal calyces of cattle join directly to form the ureter without forming a pelvis. Note the difference

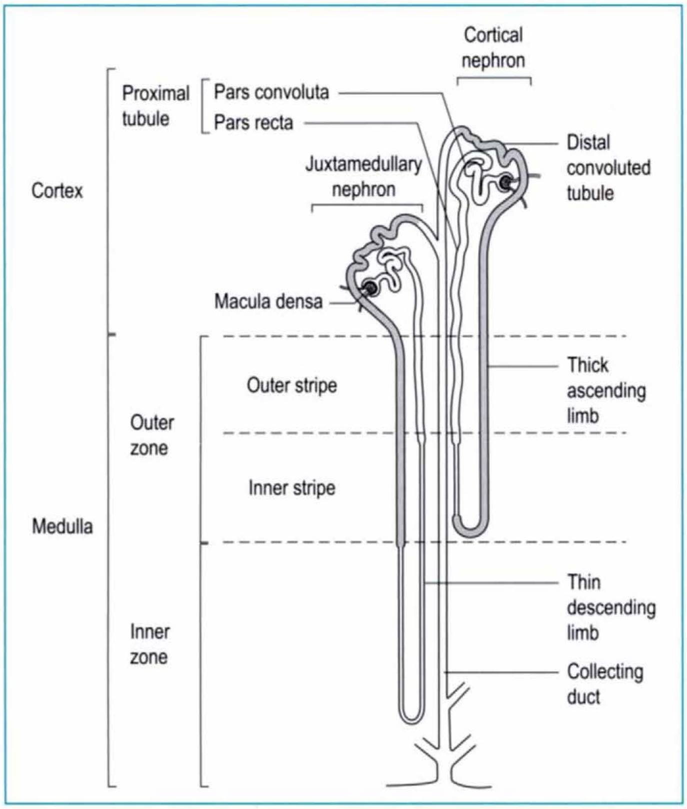

Figure 4.1 Diagram showing components of **cortical and juxtamedullary nephrons** and their location in zones of the kidney.

between a renal lobe and a renal lobule. A *renal lobule* consists of a medullary ray and its associated nephrons. *Medullary rays* are seen histologically as lighter-staining linear areas in the cortex, and consist of collecting tubules, thick ascending limbs, and the terminal straight portions of the proximal tubules. Interlobular arteries course between lobules.

On sagittal section of a kidney, *subdivisions of cortex and medulla* may be distinguished, particularly in dogs and sheep. The *cortex* has a darker outer zone, and a paler inner zone, which in mature dogs often has prominent pale streaks due to the presence of fat in collecting ducts. Outer and inner zones may be seen in the *medulla* of canine kidneys, and the outer zone may be further subdivided into outer and inner bands (stripes), due to the presence of the thick segments of the descending and ascending limbs of the loop of Henle, respectively. The inner zone of the medulla (papilla) contains the thin segments of the loop of Henle (Fig. 4.1). Mucous glands are large and prominent in the medulla of equine kidneys, and mucus and crystals are normally present in the equine renal pelvis.

The functional unit of the kidney is the **nephron**, which consists of the *renal corpuscle, proximal tubule, loop of Henle, and distal tubule.* The glomerulus and Bowman's capsule comprise the renal corpuscle. The following approximate numbers of nephrons are present in each kidney: human: 1 000 000; dog: 400 000; cat: 200 000. The number of nephrons is fixed at birth in most mammals, although nephrogenesis may continue for several weeks after birth in animals with a short gestation period, such as dogs, cats, and pigs.

Renal diseases may primarily affect one of four subdivisions of the kidney: blood vessels, glomeruli, tubules, and interstitium.

Vascular supply

Although the kidneys constitute only about 0.5% of body weight, *they receive 20–25% of cardiac output.* In multilobed kidneys, the *renal artery* divides in the pelvic region to form *interlobar arteries* that run in the renal columns between lobes up to the corticomedullary junction, where they branch to form *arcuate arteries.* These arteries run along the corticomedullary junction parallel to the capsule and terminate by radiating *interlobular arteries* into the cortex. Glomerular *afferent arterioles* are given off by the interlobular arteries, and give rise to several lobules of capillary loops within the glomerulus. The capillaries later rejoin to form the glomerular *efferent arteriole* and then divide again to form a peritubular capillary plexus. The efferent arterioles of the juxtamedullary nephrons branch to form *descending vasa recta* (straight vessels), which enter the medulla. The ascending vasa recta reform from the medullary capillary plexus and form a *countercurrent exchange system* with their closely associated descending vasa recta. The glomerular capillary tufts are perfused at high pressure that favors filtration; the capillary bed rising from the efferent arterioles is low pressure, favoring reabsorption.

Because the renal artery and its branches are *end-arteries,* occlusion of any branch leads to infarction. Interference with glomerular capillary flow markedly alters peritubular blood flow, especially in the medulla. The medulla is particularly sensitive to ischemia because of its relative avascularity and the low hematocrit in medullary capillaries.

Intrarenal blood flow is finely controlled by complex mechanisms, which are still being elucidated. The **juxtaglomerular apparatus** consists of the *afferent and efferent arterioles* adjacent to their respective glomerulus, the *macula densa* of the corresponding distal convoluted tubule, and a collection of cells (*lacis or nongranular cells*) at the glomerular hilus between the arterioles. Myoepithelial cells (juxtaglomerular or granular cells) of the afferent arteriolar wall contain *renin.* When afferent arteriolar blood pressure, and hence GFR, decreases, an intrinsic afferent arteriolar myogenic response and **tubuloglomerular feedback** (a negative-feedback loop) are activated. The tubular fluid chloride concentration is decreased at the macula densa, causing dilation of the afferent arteriole and constriction of the efferent arteriole to increase the GFR, as follows. Renin is released and this proteolytic enzyme cleaves angiotensinogen, an α_2 globulin of plasma, to form angiotensin I. Angiotensin I is subsequently cleaved by angiotensin I-converting enzyme to form *angiotensin II,* one of the most potent pressor agents known, and angiotensin III. Angiotensin III and, to a lesser extent, angiotensin II also initiate adrenocortical synthesis of *aldosterone,* which aids, over the longer term, in increasing afferent arteriolar perfusion by stimulating resorption of sodium from the distal convoluted tubules and collecting ducts, resulting in water retention and expansion of the extracellular fluid volume. Both angiotensin II and III induce prostaglandin synthesis. Angiotensin II also stimulates the secretion of *antidiuretic hormone* (ADH, vasopressin). The above events, in concert with angiotensin-mediated efferent arteriolar constriction, are sensed by the macula densa, and the tubuloglomerular feedback mechanism detailed above is reversed, leading to afferent arteriolar constriction, decreased GFR, and hence restoration of **glomerulotubular balance.**

The above mechanisms of blood pressure sensing and tubuloglomerular feedback result in maintenance of glomerular filtration pressure within fine limits over a broad range of systemic arterial blood pressure. The renin system is intimately interrelated with adenosine, atrial natriuretic peptide, endothelin, vasopressin, the renal kallikrein-kinin system, renal sympathetic nerves, and prostaglandins to *autoregulate intrarenal blood flow and modulate tubular salt and water transport.* The contribution of these factors to control of renal blood flow varies between normal and abnormal states; for example, *prostaglandins* are apparently of little significance in maintaining intrarenal blood flow under normal circumstances, but become important during periods of hypotension. *Endothelin-1*, which is released from endothelium by a range of perturbations, is a potent vasoconstrictor of afferent and efferent arterioles and a constrictor of mesangial cells; its action is countered by the vasodilatory effects of nitric oxide and prostacyclin. *Atrial natriuretic peptide* is released from the cardiac atria in response to volume overload and causes natriuresis and diuresis through vascular and other actions (endocrine effect). *Urodilatin*, another natriuretic peptide, is produced by distal convoluted tubule cells, and causes diuresis by local inhibition of sodium and water resorption (autocrine-paracrine effect).

The *renal venous system* begins with formation of venules from the peritubular vasa recta, and then closely parallels the arterial system. Veins in the outer cortex drain into stellate veins, which in cats are present on the capsular surface and are prominent, and thence into interlobular veins. Veins within the kidney have very thin walls and are susceptible to compression.

Lymphatics occur in the renal cortex and medulla. One set of lymphatics drains the cortical and medullary interstitium and follows the pattern of the vascular system; another set of lymphatics drains the capsular area. Lymphatic flow increases after urinary obstruction and in interstitial disease.

Glomerulus

The glomerulus is a vascular-epithelial structure designed for the ultrafiltration of plasma. It develops embryologically by the invagination of a capillary-rich mesenchymal mass into the Bowman's space, an epithelium-lined sac. The visceral epithelium covers the glomerular capillaries to become an essential part of the filtration membrane, and the parietal epithelium, or Bowman's capsule, lines the urinary space that receives glomerular ultrafiltrate. The arterioles enter and leave the glomerulus at the *vascular pole*, and urine enters the proximal tubule at the *urinary pole* of the glomerulus. The *glomerular filtration membrane* consists of three layers: (1) capillary *endothelium* containing fenestrae of 50–100 nm diameter; (2) *glomerular basement membrane (GBM)*, which is 100–300 nm thick and consists of a central electron-dense lamina densa and peripheral electron-lucent layers, the lamina rara interna and externa; and (3) *visceral epithelial cells (podocytes)*. The podocytes have complex interdigitating trabeculae whose *foot processes (pedicels)* are embedded in the lamina rara externa of the GBM. The pedicels are separated by 25–50 nm wide *filtration slits*, which are bridged by thin *slit diaphragms* with pores of 6–9 nm diameter. GBM is produced continuously by the podocytes, and is continuous below these cells, but does not completely encircle the glomerular capillaries; endothelial cells and mesangium are in direct contact. GBM is a complex porous meshwork composed of collagen (primarily type IV), laminin, polyanionic proteoglycans (mainly heparan sulfate), fibronectin, entactin, and other glycoproteins.

A large volume of glomerular ultrafiltrate is formed as a product of the high hydrostatic pressure of arteriolar blood, and the selective permeability of the filtration membrane. The entire plasma volume is filtered about 100 times per day. The glomerulus is highly permeable to water and small solutes, but virtually excludes albumin (molecular weight ~70 000, radius 3.6 nm) and higher-molecular-weight plasma proteins from the filtrate. The GBM is a *size-dependent barrier* to filtration, prohibiting passage of particles with radius greater than 3.5 nm. The filtration membrane also excludes particles from filtration on the basis of *charge*. Thus, anionic molecules such as albumin are repelled by virtue of the presence of negatively charged sialoglycoproteins in the lamina rara interna and externa and the negatively charged cell coats of the endothelial and epithelial cells. Obviously then, *changes in porosity or charge of the filtration membrane alter glomerular permeability and can lead to proteinuria, a hallmark of glomerular damage.* A small amount of albumin normally passes through the filter but is rapidly resorbed in the proximal tubules. The glomeruli of neonates are permeable to colostral protein for a few days.

The **mesangium** is the central region of the glomerulus that forms a supporting framework about which the glomerular capillaries ramify. *The mesangial matrix is basement-membrane-like periodic acid–Schiff (PAS)-positive glycoprotein, and the mesangial cells are phagocytic, contractile cells, which are derived from vascular smooth-muscle cells.* These cells function in phagocytic removal of deposited macromolecules, removal of GBM, and may modulate intraglomerular blood flow; mesangial cells both respond to and produce a variety of cytokines. Mesangial cell hyperplasia and increased mesangial matrix (mesangial sclerosis) are common changes in glomerular disease.

Tubules

The kidney functions by producing a large volume of protein-free glomerular filtrate from which is resorbed the constituents that the body needs. Thus, about 99% of the sodium chloride and water filtered are resorbed. This system effectively rids the body of wastes that are filtered by the glomeruli and not resorbed by the tubules. *Structure of a tubule segment is correlated with its function.* Thus, cells of the proximal convoluted tubule have a well-developed brush border and numerous mitochondria. Energy for the sodium pump in this actively resorptive area is provided by mitochondrial oxidative phosphorylation; this area is, therefore, especially vulnerable to ischemia. Also, toxins are frequently resorbed by or secreted by the proximal tubule, causing chemical injury.

About 90% of the hydrogen ion excretion by the kidney occurs in the proximal tubule. The proximal tubule actively resorbs large quantities of sodium and chloride, and water passively follows, hence 60–80% of the glomerular ultrafiltrate that enters the proximal tubule is resorbed isotonically. *Tubules and peritubular capillaries are in close apposition to allow rapid removal of resorbed sodium chloride and water.* Sodium is co-transported with glucose and amino acids. Hydrogen ions are accompanied by sodium bicarbonate as counterion. Proximal tubules are not responsive to aldosterone or vasopressin. In addition to salt and water, proximal tubules resorb glucose, amino acids, calcium, phosphate, uric acid, proteins, and potassium. Many substances, e.g., glucose, amino acids, water-soluble vitamins, are "threshold substances" which are almost completely resorbed until a certain concentration is reached in the glomerular filtrate, at which concentration the transport maximum is exceeded and the substance appears in the urine. Overloading of the resorptive capacity of the proximal tubules in conditions such as diabetes mellitus also overwhelms the resorptive capacity of distal tubules and leads to **osmotic diuresis**. Interference with the production, release, or action of ADH on the distal nephron and collecting ducts, as occurs in pituitary or renal diabetes insipidus, results in obligatory **water diuresis** and excretion of hypo-osmotic urine and hence polyuria. Proximal tubules secrete hydrogen ions, organic acids, *p*-aminohippurate, penicillin, and some iodinated radiopaque materials.

The long loops of Henle of the juxtamedullary nephrons penetrate deep into the medulla and assist in making the *renal medulla the only hypertonic tissue in the body*. Urine concentrating ability is directly proportional to the length of the loop of Henle; desert-dwelling rodents have very long loops of Henle, whereas baby pigs have short loops and are thus very susceptible to dehydration. The loops of Henle serve as *countercurrent multipliers* and the capillaries as simple *countercurrent exchangers*, with the flow in each occurring in opposite directions. The source of the solute gradient that becomes greater towards the tip at the papilla is the loop of Henle (active process). The gradient is preserved by the vasa recta countercurrent exchanger (passive process). Sodium chloride is pumped (chloride active, sodium passive) from the ascending limb of the loop of Henle into the interstitium, in turn drawing water from the descending limb, and progressively increasing the solute concentration in the descending limb. The ascending limb is impermeable to water, thus the tubular fluid becomes dilute and may be hypotonic. More salt may be resorbed from the collecting ducts, further diluting the fluid. Water diuresis occurs when a state of water excess exists and ADH is not released from the neurohypophysis. If *antidiuresis* is required, ADH is released and renders the epithelium of the collecting tubule highly permeable to water, reducing the volume of tubular fluid entering the hypertonic medulla. This remaining fluid is then concentrated to 3–4 times the blood osmolality (280 mosmol/L) during passage to the tip of the papilla. The water resorbed in the medulla is passively transported into the ascending vasa recta and removed. The solute concentration of the medulla is about one-half due to sodium, and one-half due to urea. During antidiuresis, ADH renders the cortical portions of the collecting ducts permeable to water but not urea, thus increasing the urea concentration of the luminal fluid. ADH increases the permeability of the medullary portions of the collecting ducts to urea and water; urea then diffuses from collecting tubule to interstitium until luminal urine and interstitial concentrations are equal. Tubules are not functionally mature in the kidneys of most neonates, thus they cannot generate hypotonic urine and hence conserve solute in the medulla; inability to produce a medullary solute gradient limits their capacity to concentrate urine.

There is little, if any, further modification of urine once it leaves the tip of the papilla. However, urea may be conserved by moving across the renal papillary epithelium from the pelvic urine. Either osmotic diuresis or water diuresis may result in **medullary solute washout** due to increased tubular flow rates and hence decreased efficiency of the countercurrent multiplier, the loop of Henle. The quantities of urea and sodium chloride resorbed are decreased due to decreased tubular fluid transit time. Hence, the medullary solute gradient decreases, as does urine concentrating ability. The same mechanism is operative in chronic renal disease in which there are high tubular flow rates in the small number of hypertrophic tubules present.

Although body buffers and pulmonary control of carbon dioxide excretion are the first line of defense in protecting the pH of the extracellular fluids, *it is the action of the kidneys that ultimately corrects acid–base balance*. The kidneys excrete the excess alkali or acid responsible for the disturbance. Thus, the kidneys correct metabolic alkalosis by excreting alkaline urine containing the excess bicarbonate. The kidneys correct metabolic acidosis by increasing resorption of filtered bicarbonate, excreting titratable acids (secrete hydrogen ions), and by producing ammonia.

Interstitium

The interstitium normally contains peritubular capillaries and a few fibroblasts, and interstitial tissue is usually only obvious around interlobar, arcuate, and interlobular arteries. *Expansion of the cortical interstitium is abnormal*, and may occur due to edema, cellular infiltration, or fibrosis. The glycosaminoglycan content of the medullary interstitium increases with age and ischemia. Specialized interstitial cells produce prostaglandins, particularly PGE_2 and $PGF_{2\alpha}$.

Examination of the kidney

Gross examination

The systematic gross inspection of a kidney entails observation of its *size, shape, color, and consistency*. The kidneys are usually equal in size and about three vertebrae long. Renal enlargement may occur due to addition of blood, edema fluid, fat, urine in the pelvis or tubules, or swollen or hypertrophic nephrons. Acute inflammation causes renal enlargement due to addition of fluid and cells, whereas chronic inflammation causes scarring and loss of substance. The kidneys are usually bean- or horseshoe-shaped, although the bovine kidney is lobated externally. Focal lesions, such as those caused by infarction or pyelonephritis, may markedly distort the renal outlines, whereas more generalized diseases such as glomerulonephritis do not. The normal renal color is brown-red, except in mature cats, in which the cortices are yellow due to their high fat content. The fat content in cats is apparently hormonally determined; pregnant females and sexually inactive old males have the most fat, pseudopregnant females and castrated males have somewhat less, estrous females and sexually active males have moderate amounts, and anestrous females have little or no renal fat. Kidneys autolyze fairly rapidly after death, particularly in fat animals in a warm environment. Pulpy kidneys occur due to clostridial enterotoxemia in sheep, and may be difficult to distinguish from advanced autolysis.

The urinary system may be maintained intact during examination, or the kidneys may be removed from the carcass. The kidney should not be severed from the ureter until the absence of hydronephrosis has been established. Each kidney is cut in the sagittal plane and the cut surface is examined. The *normal cortex-to-medulla ratio* in this plane is about 1:2 or 1:3. *The cortex normally accounts for 80% of the renal mass*, a fact which is better appreciated in a coronal, than in a sagittal, section. Diffuse diseases of the kidney usually respect the integrity of the medulla. Pyelonephritis is peripelvic with involvement of the medulla. A pale cut surface suggests the deposition of fat or acute early nephrosis; if the surface is pale and wet, there is edema and diffuse tubular dilation. Focal glomerular lesions do not cause glomerular prominence, but diffuse lesions, such as amyloidosis or diffuse glomerulitis, very often do. *Removal of the renal capsule is essential for examination of the outer surface of the cortex.* The capsule should strip easily and leave a smooth surface underneath. Tearing of the cortex may indicate fibrous adhesions of scarring, except in the horse, which normally has trabeculae attached to the capsule.

Histologic examination

The kidney must be trimmed so that a section from capsule to papilla is available. The section should first be examined subgrossly for evidence of localized lesions, e.g., infarcts, abscesses, granulomas, pyelitis, mineralization, neoplasm, scars, medullary loss. The section is then scanned at low power and lesions are noted. Glomeruli are normally distributed randomly throughout the cortex with approximately 3–4 present per 40× field. Tubules should be tightly packed with little intervening connective tissue. Goat and sheep glomeruli are more cellular than those of most species. Horses have the largest glomeruli of domestic animals (about 200 μm diameter) and the only glomeruli that are visible grossly. Glomeruli in neonatal kidneys are small and hyperchromatic, particularly in the outer cortex. Nephrons may be seen in the "S" stage of development in the outer cortex of fetuses and in neonates with ongoing centrifugal renal development, such as dogs, cats, and pigs.

After scanning the section, the four basic elements of the kidney should be systematically examined, namely glomeruli, tubules, interstitial tissue, and blood vessels, to determine which structure is primarily injured.

Reactions of **glomeruli** to injury consist basically of combinations of cellular proliferation, leukocytic infiltration, thickening of the basement membrane, and hyalinization or sclerosis. Epithelium of the proximal tubule may be present in the glomerular urinary space in acute nephrosis or, more commonly, due to squeezing of the kidney during gross examination, especially if the kidney is autolytic; this change is termed infraglomerular reflux. Hyaline casts in tubules usually indicate increased glomerular permeability.

Tubules, especially proximal convoluted tubules, are the kidney elements most susceptible to ischemia and many toxins; degenerative changes are nonspecific in character and usually not diagnostic of the causative condition. The proximal convoluted tubule is the longest cortical part of the nephron, and hence proximal tubules comprise the bulk of the cortex. Proximal tubules are more eosinophilic than the other tubules and have a brush border that may be seen in rapidly fixed preparations and which is well demonstrated by the PAS stain. Delay in fixation leads to sloughing of apical cytoplasm into tubular lumina (potocytosis). Cells of the distal tubule do not have a brush border, are flatter, and more numerous in cross-section of the tubule than are those of the proximal tubule, and the tubular lumina are larger.

Cortical tubules and capillaries are closely associated in order to facilitate fluid resorption, and **connective tissue** is normally visible in the cortex only near arteries. Hence cortical fibrosis is a significant finding, especially in an animal with renal insufficiency. Fibrosis may cause tubular obstruction and hence create retention cysts proximally.

Blood vessel changes may occur in various systemic states as in other organs, e.g., arteriolosclerosis in diabetes mellitus, arteritis in malignant catarrhal fever.

The **renal medulla** should be closely examined because this is the site of urine concentration and the accumulation of protein casts. Tubules and ducts in the medulla are normally separated by more connective tissue and ground substance than those in the cortex. Tubulointerstitial mineralization is common in renal disease and may be seen along with cortical mineralization in cases of hypercalcemic nephropathy. The papilla should be carefully examined for evidence of papillitis, an early lesion in pyelonephritis, for amyloid, and for papillary necrosis. Inclusion bodies are often prominent in pelvic transitional epithelium in canine distemper; distemper inclusions must be differentiated from nonspecific epithelial inclusions.

Renal biopsy

Percutaneous needle biopsy of kidneys is useful to evaluate renal disease in domestic animals, and *is of particular use in cases of acute glomerulonephritis, nephrotic syndrome, asymptomatic proteinuria, and acute renal failure.* It is usually possible to obtain 10–20 glomeruli using a TruCut or a Franklin–Silverman biopsy needle in an adult dog; a minimum of ~5 glomeruli is necessary to characterize a glomerular lesion. The main hazard of the technique is arterial puncture and hemorrhage, which on rare occasions is fatal.

In addition to the usual hematoxylin and eosin staining of paraffin sections of kidney, several other techniques are useful:

1. The PAS *stain*, which stains glomerular and tubular basement membranes and mesangial matrix. Thin sections (3 μm) must be used. The PAS-methenamine silver technique is an improvement of this stain.
2. *Silver impregnation* of basement membranes and of leptospires.
3. *Other special stains for fibrin, amyloid, and lipids.* Microscopic resolution of glomerular lesions is markedly improved by the use of 1 μm thick methacrylate-embedded sections.
4. *Immunofluorescence* studies for localization of immunoglobulins, complement, fibrin-related compounds, and foreign antigens, especially in glomeruli.
5. *Immunoperoxidase* techniques, for localization of a wide variety of antigens.
6. *Electron microscopy*, particularly for characterization of glomerular basement membrane deposits.

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Renal disease, renal failure, and uremia

Renal disease, which encompasses any deviation from normal renal structure or function, is usually subclinical. *Severe renal disease may lead to renal failure*, which is typically divided into acute and chronic forms. **Acute renal** *disease* is characterized by rapid onset of oliguria or anuria and azotemia; it may result from acute glomerular or interstitial injury or from acute tubular necrosis, and is often reversible. **Chronic renal** *failure.* is the end-result of many chronic renal diseases, is usually irreversible, and is characterized by prolonged duration of signs of uremia.

Clarification of terminology is in order:

- **Uremia** literally means urine in the blood. It is a *clinical syndrome of renal failure*, caused by biochemical disturbances, and is often accompanied by extrarenal lesions.
- **Azotemia**, which is sometimes incorrectly used synonymously, is a biochemical abnormality characterized by *elevation of blood urea and creatinine*, but without obligatory clinical manifestations of renal disease. Azotemia may be of renal or of extrarenal origin.
- **Prerenal azotemia** results from renal hypoperfusion, due to conditions such as congestive heart failure, shock, or hemorrhage.
- **Postrenal azotemia** occurs due to urinary obstruction.

The evolution from normal renal function to uremia over the course of progressive renal disease occurs through *four overlapping stages*:

1. In the stage of **diminished renal reserve**, the GFR is about 50% of normal, and the animal is asymptomatic but is susceptible to additional renal insults.
2. At the stage of **renal insufficiency**, GFR is 20–50% of normal; the animal is azotemic and becomes polyuric.
3. In the stage of **renal failure**, GFR is only 20–25% of normal, the kidneys cannot maintain homeostasis, and uremia ensues, with its attendant gastrointestinal, cardiovascular, respiratory, and skeletal complications.
4. In **end-stage renal disease**, GFR is less than 5% of normal and the animal is in the terminal stages of uremia.

The biochemical disturbances of uremia reflect changes in the normal renal functions directed to regulation of fluid volume, regulation of electrolyte and acid–base balance, excretion of waste products, and metabolism of hormones. The clinical signs may be related to the renal disease itself, as with pyuria or renal pain; to the effects of reduced renal function, as with metabolic acidosis or dehydration; or to the compensatory responses to renal dysfunction, as with hyperparathyroidism.

Interference with **fluid volume regulation** may result in either dehydration or anasarca. *Dehydration* due to reduced renal concentrating ability may be related to lesions of the renal medulla and juxtamedullary nephrons. Vomition, and sometimes diarrhea, often exacerbates the dehydration. *Anasarca*, caused by reduction in the volume of glomerular filtrate in diffuse renal disease or by activation of the renin–angiotensin system, occurs infrequently. A more common cause of edema is hypoproteinemia due to loss of protein through injured glomeruli.

Disturbances in **electrolyte balance** include excesses and deficits of plasma sodium, potassium, and calcium. The handling of these substances by the kidney is complex, even in health; in disease it is often paradoxical and involves disordered tubular function, compensatory mechanisms, and endocrine imbalances. Excesses of plasma sodium, potassium, and calcium contribute to anasarca, cardiotoxicity, and hypercalcemic nephropathy respectively, whereas deficits may cause dehydration, muscular weakness, tetany, and osteodystrophy.

Several aspects of the uremic syndrome contribute to **acid–base imbalance** and metabolic acidosis. Compensatory hyperventilation may occur. The main factors leading to acidosis in uremia are a reduced capacity of distal and collecting tubules to produce ammonia, increased retention of hydrogen ions, and increased utilization, with impaired reabsorption, of bicarbonate ions. The term **uremic acidosis** encompasses simultaneous azotemia, anion retention, and acidosis; **renal tubular acidosis** (discussed below in the section on Specific tubular dysfunctions) describes acidosis without retention of urea or anions.

Failure to excrete metabolic wastes is the basis for tests of renal function; *elevated blood levels of urea and creatinine indicate reduced glomerular filtration*. However, azotemia occurs only after the loss of 75% or more of the GFR, and is thus an insensitive indicator of renal disease. Tests of concentrating ability, such as the water deprivation test, are also of limited usefulness because concentrating ability is not affected until at least two-thirds of the renal mass has become nonfunctional.

Disturbances in endocrine function are important causes of signs and lesions in uremic animals. Retention of phosphate, due to reduced glomerular filtration, causes depression of ionized calcium, deficiency of $1,25(OH)_2D_3$, increased synthesis and secretion of parathyroid hormone (PTH), and development of secondary hyperparathyroidism (see section on Fibrous osteodystrophy, Vol. 1, Bones and joints). Reduced renal catabolism of PTH, and end-organ resistance to PTH, may contribute to hyperparathyroidism. Most uremic animals have hyperphosphatemia, and normocalcemia or hypercalcemia. Sometimes hypercalcemia and hypophosphatemia occur in uremic dogs, possibly because of lack of feedback inhibition of PTH release. Similar changes occur in some uremic horses and may be related to decreased renal excretion of calcium.

Nonregenerative **anemia** in uremic animals is of multifactorial pathogenesis and may result from a combination of factors, including decreased renal production of erythropoietin and possibly inhibitory effects of increased serum PTH concentrations (see Vol. 3, Hematopoietic system). A bleeding disorder associated with a platelet factor 3 abnormality may exacerbate the anemia. Renal metabolism of vitamin D is sometimes impaired in animals with diffuse renal disease, and may add an element of osteomalacia to renal osteodystrophy. Abnormal glucose metabolism occasionally occurs in uremic dogs, and may be a result of peripheral resistance to insulin-mediated uptake of glucose or to reduced breakdown of insulin by diseased kidneys.

As well as the metabolic disturbances attributable to particular biochemical derangements, there is in uremic animals a nonspecific effect of "**uremic toxins.**" This is probably due to the combined action of toxic products of protein catabolism and the various chemical and endocrine imbalances described above. These toxins include molecules of low, medium and large molecular weight. Low-molecular-weight molecules (<500 Da) include glomerulopressin, guanidines (e.g., creatine, creatinine), methylamines, myoinositol, oxalate, phenylacetylglutamine, phosphate, polyamines, pseudouridine, purines (e.g., uric acid), pyrimidines (e.g., thymine, uridine), trihalomethanes, and urea. "Middle molecules" range from 500 to 5000 Da, and include α- and β-chains of fibrinogen. Large molecules (>5000 Da) include various peptides and cytokines, such as parathyroid hormone, β_2-microglobulin, granulocyte-inhibitory proteins, degranulation-inhibiting proteins, and chemotaxis-inhibiting protein. These various toxins are thought to be responsible for the profound malaise that characterizes the uremic syndrome.

The *cause of death in uremia* varies from case to case. Metabolic acidosis, hyperkalemia, or hypocalcemia may be severe enough to be fatal.

Gross lesions in uremia may be divided into those that caused uremia and those that resulted from it. The former are usually intrarenal, but may not be in prerenal and postrenal azotemia. Lesions that are the result of uremia are mainly, but not exclusively, extrarenal.

Most forms of prerenal azotemia have a common basis of reduced renal blood flow and glomerular filtration. This occurs in a variety of circumstances, including severe dehydration, massive hemorrhage, especially into the upper intestinal tract, congestive heart failure, and shock of any cause. Reduced renal flow in these conditions is part of an adaptational mechanism that diverts blood to vital organs such as brain and heart. When the diversion is severe or prolonged, intrarenal mechanisms that channel renal flow away from cortical nephrons to juxtamedullary nephrons may produce patchy or diffuse cortical ischemia and necrosis (see section on Renal cortical necrosis and acute tubular necrosis, below). In such cases, if the animal survives, prerenal azotemia may be superseded by uremia of renal origin originating in cortical necrosis.

Postrenal azotemia is always due to obstruction to the outflow of urine, and hence is oliguric or anuric. The causes are those which, if intermittent or incomplete, may lead to hydronephrosis or, if sudden and complete, may lead to rupture or leakage of the lower urinary tract.

A form of extrarenal uremia, distinct from those mentioned, occurs in *newborn animals*, especially pigs, and is characterized by very high levels of blood urea. The kidneys of newborn pigs, dogs, and cats are functionally "immature," and incapable of producing hypertonic urine. Normally this is of no consequence, because milk provides enough fluid to excrete, in hypotonic urine, the small amount of waste produced. However, when these newborn animals are anorectic, they lack both nutrients and fluid, and begin to catabolize tissue proteins and purines. Being unable to excrete the excess solute from protein and purine breakdown, their blood urea and uric acid reach very high levels. Since anorexia is usually associated with fever, vomition, or diarrhea, fluid loss is very rapid. In pigs, the excess solute is deposited in the inner medulla as streaks of light-yellow urate precipitates (Fig. 4.2), which disappear during histologic processing. Pigs are apparently unique among mammals in that they do not reabsorb urates from glomerular filtrate; this accounts for their concentration in the medulla. It is not clear whether baby pigs also have an "immature" liver that fails to convert uric acid to allantoin.

The **nonrenal lesions of uremia** occur inconstantly and unpredictably, though they tend to be seen most often in dogs, especially those with chronic rather than acute renal failure. Many animals dying with uremia are cachectic. This is probably caused by the anorexia, vomition, and diarrhea which often occur, and which necessitate body tissue catabolism to supply energy. Besides this

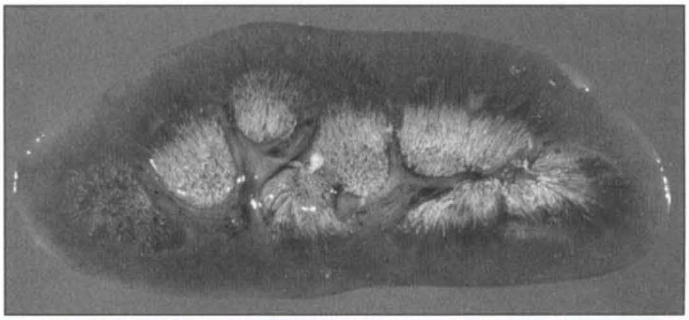

Figure 4.2 Urate calculi in renal medulla of a 3-day-old dehydrated piglet.

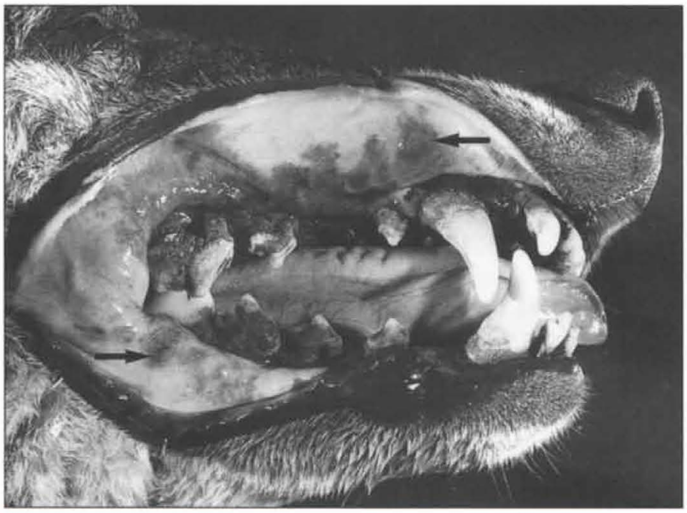

Figure 4.3 Ulcers of buccal mucosa (arrows) in a dog with **uremia**.

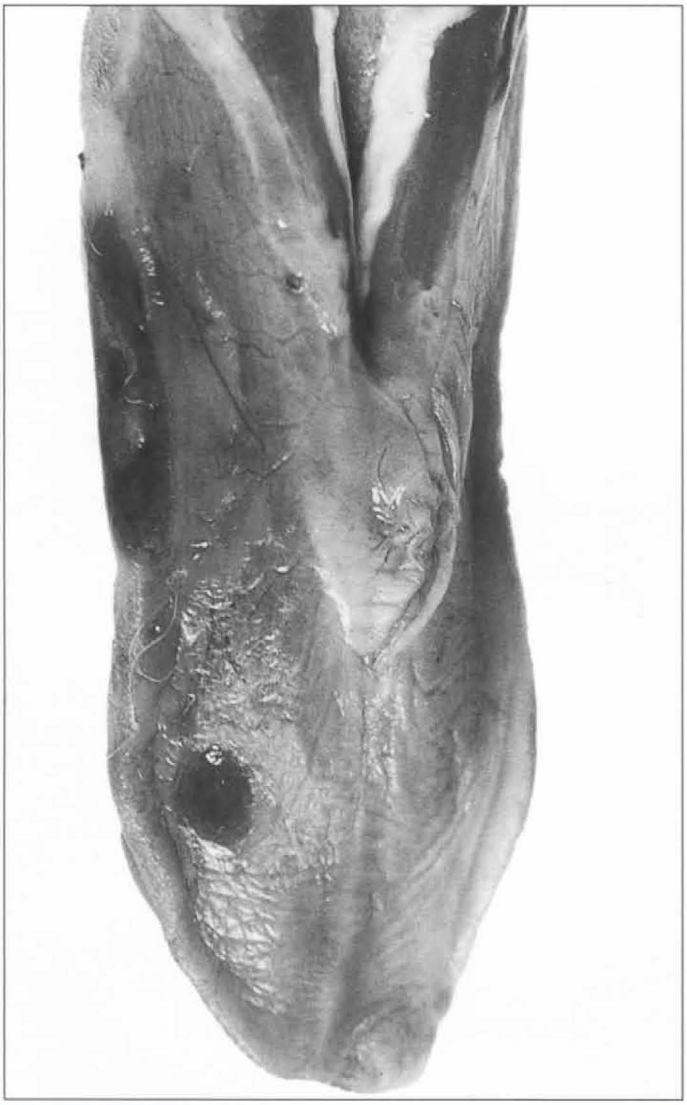

Figure 4.4 Ulcers on lateral margin and ventral surface of tongue in a dog with **uremia**.

general lack of condition, several distinctive lesions may develop in the gastrointestinal, cardiovascular, respiratory, and skeletal systems (see also these systems).

Ulcerative, necrotic stomatitis occurs in dogs and cats, and there is usually a foul-smelling brown film coating the tongue and buccal mucosa (Figs 4.3 and 4.4). Like the gastrointestinal changes, oral lesions are more common in chronic than in acute uremia. The pathogenesis of the ulcers is not always clear, but some are associated with fibrinoid necrosis of arterioles and some are related to bacterial production of ammonia from urea in the saliva. Large areas of the *gastric mucosa* are often swollen, suffused with red-black blood, and partly ulcerated. This lesion, often called "gastritis," is initially noninflammatory, though opportunist bacteria may infect the ulcerated mucosa. Mucosal infarction occurs secondary to arteriolar necrosis. *Mineralization of the middle and deep zones of the gastric mucosa is common* (Fig. 4.5). Necrosis and mineralization of the muscular coats are sometimes present. Intestinal lesions resemble those in the stomach, but they are less frequent, less severe, and without mineralization. Gastrointestinal lesions probably account for much of the vomition, diarrhea, and melena of uremic dogs. Intestinal intussusceptions sometimes develop in dogs with gastrointestinal lesions. Cats may have gastrointestinal lesions similar to dogs but, in uremic cattle, colitis is more common and the stomach and proximal intestine are edematous. The liver shows inconstant degenerative changes, and there may be acinar dilation of the pancreas with inspissation of secretion. The hyperamylasemia and hyperlipasemia that occur in some uremic dogs are apparently the result of concurrent pancreatitis rather than due to reduced renal clearance of the enzymes.

Arterial lesions found in animals with acute renal insufficiency (*arterial hyaline degeneration*) or with chronic renal failure (*hyperplastic arteriolosclerosis*) are discussed elsewhere (Vol. 3, Cardiovascular system). Lesions of various organs, such as uremic gastric infarction, may result from arterial degeneration and thrombosis. Arteriolar lesions in the myocardium sometimes cause ischemia and degeneration. Arterial degeneration is perhaps most frequent in acute uremia due to tubular necrosis. In chronic uremia, the left ventricle is often hypertrophied and dilated. *Hypertension* is common in canine nephritis, and ventricular hypertrophy may be initiated or exaggerated by the hypertension. Lesions in the circulatory system are unusual, except in the dog, but arterial degeneration may occur in the intestines

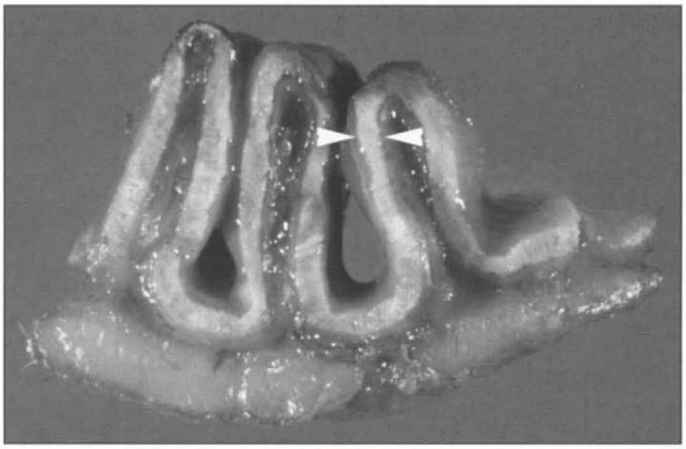

Figure 4.5 Marked **gastric mucosal mineralization** (layer between arrow heads) in a uremic dog.

in cattle with acute nephrosis and contribute to the colonic lesions of acute uremic syndromes in this species. In cattle with urethral calculi, pericardial effusion may be part of the anasarca. In dogs, hydropericardium with dull granulation of the pericardium may occur. Edematous distension of retroperitoneal tissue, expressed particularly as *perirenal edema*, occurs in pigs and cattle; the underlying renal lesion is usually acute tubular necrosis caused by ochratoxin or *Amaranthus retroflexus* in pigs, and oak poisoning in cattle (see section on Nephrotoxic tubular necrosis, below).

Most animals dying in uremia develop *terminal pulmonary edema*. The mechanism is obscure and the edema is not always associated with significant pulmonary congestion; increased permeability of alveolar capillaries is the most likely pathogenesis. In a few animals, acute pneumonia develops terminally. It may be associated with aspiration of gastric content, and its fulminant nature is possibly related to the immunosuppression that develops in uremia. Pulmonary mineralization occurs in chronically uremic dogs. Mineral is deposited in the walls of the alveolar ducts and pulmonary arterioles. Dull granulations of the visceral pleura may be present over the cranial lobes. Occasionally in uremia, spectacular pulmonary lesions develop. They may be visible in radiographs as lines of increased density spreading out from the hilus and these features are those of interstitial edema. At autopsy, the lung is edematous and resilient and the alveolar spaces contain much fibrin in the edema fluid. Leukocytes are present but may be a response to accidental superimposed infection. Mineralization is extensive, with impregnation particularly on reticulin of alveolar walls that are widened (Fig. 4.6). The lesion is referred to as uremic lung or *uremic pneumonitis*; it is not common and when it occurs it may be patchy.

Perhaps the most constant lesion in the dog is *mineralization beneath the parietal pleura* in the cranial intercostal spaces (Fig. 4.7). It is preceded by necrosis of the subpleural connective tissue with extension to intercostal muscle and overlying pleura. Once mineralization has occurred and the pleura is repaired, the lesion appears as gray-yellow thickenings, horizontally wrinkled. The cranial intercostal spaces are involved first; when deposition is extensive many more spaces may be affected.

The pathogenesis of diffuse tissue mineralization in uremia is not clear. There are at least two types of mineral deposit, and serum concentrations of calcium, magnesium, phosphate, and carbonate probably determine the type of calcium phosphate compound that is formed. When calcium is higher than magnesium, apatites are formed, whereas the opposite relationship favors deposition of amorphous calcium phosphate. The regularity with which certain tissues and organs are mineralized is no doubt related to local characteristics such as tissue glycosaminoglycans, local pH, and cellular factors.

The effects of chronic uremia on the *skeleton* are discussed in the section on Fibrous osteodystrophy, Vol. 1, Bones and joints. *Enlarged parathyroids* are common in dogs with chronic renal failure; osseous lesions are less so.

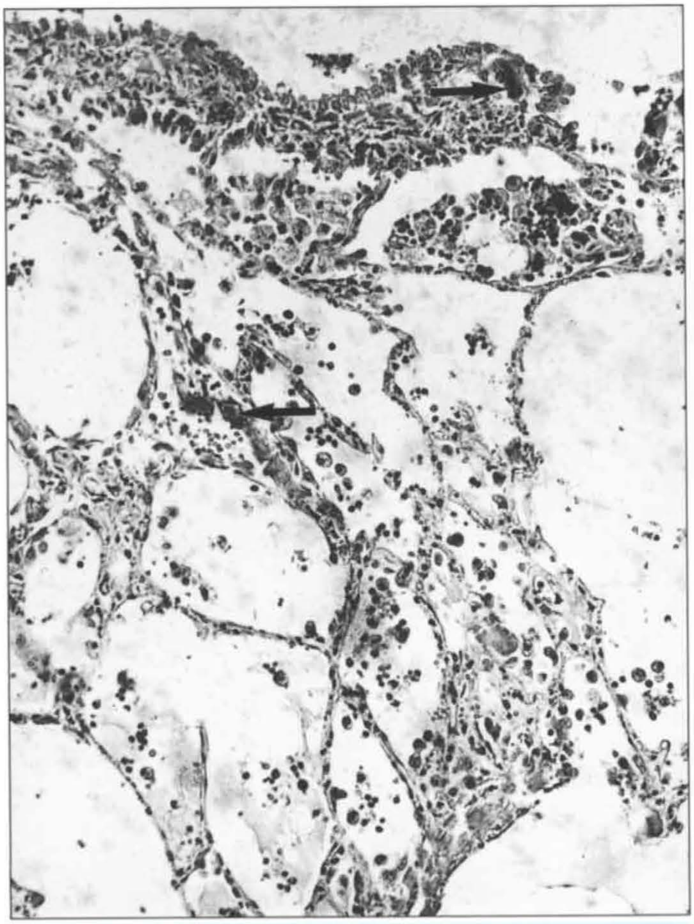

Figure 4.6 Mineralization of alveolar and bronchiolar wall (arrows), mild edema, and intra-alveolar cellular exudate in a dog with uremia.

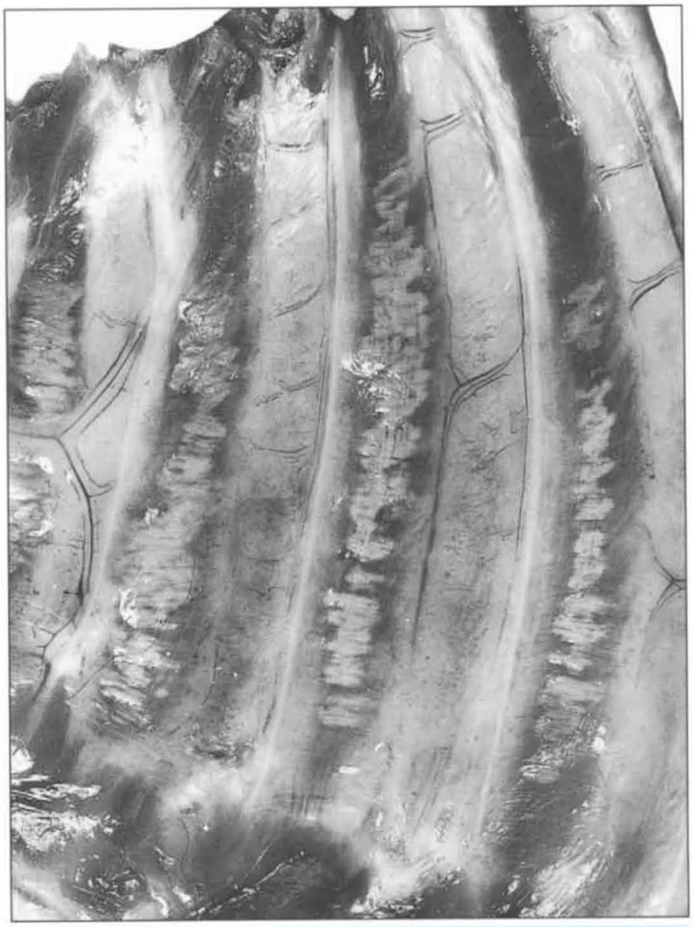

Figure 4.7 Necrosis and mineralization beneath intercostal pleura between cranial ribs in a uremic dog.

Uremic encephalopathy is an uncommon complication of uremia in domestic animals. It has been reported in dogs, ruminants, and horses, and is expressed as white-matter spongiform degeneration that may be accompanied by reactive astrogliosis.

The **renal lesions of uremia** are varied but, if the syndrome is chronic, certain common changes tend to occur. *The end-result is a fibrosed, mineralized kidney with sclerosed glomeruli, and sometimes areas of hyperplastic and hypertrophic tubules.* Often this can only be diagnosed as "**end-stage kidney**." Severe mineralization occurs late in the course of renal failure and may diffusely involve glomerular and tubular basement membranes, or be deposited at the corticomedullary junction. In the latter case, temporary ischemia with reflow involving the straight part of the proximal tubule, which is at the periphery of the postglomerular circulation, may be responsible. According to the "precipitation-calcification hypothesis," phosphorus absorbed from the intestines in excess of the excretory capacity of nephrons will induce precipitation of calcium phosphate microcrystals in tubular lumina, the interstitium, and renal capillaries. Dietary phosphate restriction may inhibit renal mineralization and its attendant inflammation, scarring, and loss of nephrons. Interstitial fibrosis and glomerulosclerosis are slowly progressive lesions that are common in the end-stages of many renal diseases. *Progressive interstitial fibrosis is thought to be the final common pathway to chronic renal failure.* Hyperplasia and hypertrophy of tubules are inconstant changes that probably precede the onset of renal failure. Azotemia develops when glomerular filtration is reduced to 75% of normal. Until this time, adaptive changes in intact nephrons maintain renal function at an adequate level as other nephrons are incapacitated. The glomerulus is probably the limiting factor in this compensatory mechanism, since it has a relatively limited ability to increase its function. It seems unlikely that uremia provides a suitable environment to permit or encourage compensatory hyperplasia, and it is likely that enlargement of nephrons is an early response to a reduction in renal mass rather than an adaptation to uremia.

Regardless of the initiating cause, **chronic renal failure tends to be progressive**. Progression occurs because of persistence of the primary disease problem and/or the addition of other renal insults or complications, such as urinary tract infection, systemic hypertension, and intrarenal deposition of mineral. As well, *endogenous factors* that often develop as compensatory mechanisms may contribute to perpetuation of renal failure. These factors include glomerular capillary hypertension and hyperfiltration, renal hypertrophy, increased renal oxygen consumption, and increased renal ammoniagenesis. Surviving nephrons may also be injured as a result of altered phosphate metabolism, altered lipid composition, and by increased activity of the coagulation system. High dietary protein intake may increase renal blood flow and GFR, possibly through the actions of mediators such as glucagon, growth hormone, prostaglandins, the renin–angiotensin system, biogenic amines, and a hepatic-derived renal vasodilator known as glomerulopressin. Although contentious, restricting protein intake may minimize renal hemodynamic changes and slow the progression of chronic renal failure. Glomerular hyperperfusion can lead to glomerulosclerosis and proteinuria. Compensatory hyperfunction of renal tubules in a failing kidney can lead to increased renal ammoniagenesis, increased renal ammonia concentration, activation of the third component of complement, and complement-mediated inflammation and injury; such tubulointerstitial lesions may be progressive.

Also to be considered when assessing renal disease in a mature or aged animal are **normal aging changes** that occur in the absence of any specific renal insult; these "background" changes may lead to decreased renal reserve, but usually not to renal failure. In aged dogs, for example, *renal weight is reduced* by 20–30% due to decreased size and number of nephrons. In humans, GFR decreases with age because the renal fraction of cardiac output decreases and because of intrarenal vascular changes such as coalescence of glomerular capillaries in juxtamedullary glomeruli and atrophy of arterioles of cortical glomeruli. Glomerular mesangial volume increases, proximal tubule volume and length decrease, and interstitial connective tissue increases. Changes in the ground substance of medullary connective tissue can reduce medullary hyperosmolality and hence renal concentrating ability. Aged animals with purely senescent renal changes have *decreased compensatory abilities*, such as hypertrophy, and are hence very susceptible to renal insults. Proteinuria may be taken as an indicator of increased glomerular permeability, but there is no sensitive and specific test for quantifying renal aging at present.

Renal transplantation is available in a number of centers as a therapeutic option for dogs and cats with end-stage renal disease. Various immunosuppressive protocols are used to prevent postoperative rejection of the transplanted kidney, particularly necessary when the donor is unrelated to the recipient. Surgical complications may be noted at necropsy, e.g., avulsion of the renal vein, torsion of the vascular pedicle. The various stages of rejection of renal allografts have been extensively categorized in human pathology, and can be followed by serial renal biopsy.

- *Antibody-mediated rejection* due to pre-existing antidonor lymphocytotoxic antibody may be hyperacute or acclerated acute.
- *Acute/active rejection* consists of various combinations of interstitial inflammation, tubulitis, intimal arteritis, and transmural arteritis.
- *Chronic/sclerosing allograft nephropathy* consists of various grades of interstitial fibrosis, tubular atrophy, and tubular loss; accompanying transplant glomerulopathy is primarily defined by the presence of "double contours" in affected glomeruli, caused by interposition of mesangium in glomerular basement membranes.

Although a canine or feline renal transplant may enjoy long-term (months to years) success, the pre-existing condition, e.g., systemic hypertension, may of course persist and eventually lead to failure of the transplanted kidney. *Complications* of immunosuppressive therapy in allograft recipients include a spectrum of infectious processes, including a wide range of bacteria and mycoplasmas, yeast/fungi (*Candida, Penicillium*), and protozoa (*Toxoplasma, Encephalitozoon, Pneumocystis, Giardia*). In general, the uninephrectomized kidney donor survives well, and its remaining kidney hypertrophies to meet the body's needs.

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Juvenile, familial, and hereditary nephropathies

Juvenile nephropathy is a broad all-encompassing term for noninflammatory, degenerative, or developmental, chronic renal disease of obscure pathogenesis in *young animals*, and is relatively common in inbred lines of animals, particularly dogs and, less so, cats.

Familial nephropathy is a more restrictive term applied to renal disease observed in *families of animals*, particularly dogs. Familial nephropathy occurs in many breeds, including: Alaskan Malamute, Beagle, Chow Chow, Doberman Pinscher, Keeshond, Lhasa Apso, Miniature Schnauzer, Newfoundland, Norwegian Elkhound, Rottweiler, Shar-Pei, Shih Tzu, Soft-Coated Wheaten Terrier, and Standard Poodle.

Hereditary nephropathy *is the definitive term used once the inheritance of the nephropathy has been determined*. Hereditary nephropathies have been investigated and documented in an expanding number of breeds, including Bernese Mountain Dog, Brittany Spaniel, Bull Terrier, Cairn Terrier, English Cocker Spaniel, German Shepherd, Samoyed, West Highland White Terrier, Abyssinian cat. This category will continue to expand as inheritance of disease entities is defined; classifications will also be further refined as the pathogeneses of somewhat obscure familial and juvenile diseases are more fully characterized. For example, the familial renal disease of juvenile Samoyed dogs has now been well characterized as an X-linked hereditary glomerulopathy caused by mutations in the *COL4A5* gene, which encodes the alpha 5(IV) collagen chain. Given greater knowledge of heritability, breed societies are able to undertake control programs.

Examples of specific familial and/or hereditary renal diseases abound, and will be discussed below under their respective sections of developmental defects, glomerular disease, tubular disease, and renal neoplasia. *In general, the canine familial renal diseases are characterized by renal failure, mostly in immature or young adult dogs, which is not associated with primary renal inflammation.* For most breeds, only terminal clinical signs and end-stage lesions are described – inheritance, pathogenesis, and early morphologic changes are not reported. The age of onset of renal failure varies from a few weeks to several years but, in most cases, is 4–18 months. This wide age range and the lack of morphologic specificity hinder the definition of these diseases since there is a danger that any noninflammatory renal lesion in a dog of an appropriate breed will be diagnosed as "familial."

It is probable that some of the chronic interstitial nephritides formerly attributed to leptospirosis were familial renal diseases. The possibility that many of these diseases are examples of renal dysplasia seems unlikely since primitive ducts are not present. Glomeruli with the appearance of immaturity must be interpreted cautiously, particularly when they are found in areas of scarring.

Many of the canine familial renal lesions primarily involve either glomeruli or interstitium around glomeruli. Interstitial lesions are fibrous, and may be radial streaks or generalized. The fibrotic segments often contain apparently immature glomeruli and this pattern is found frequently, but not exclusively, in Lhasa Apso and Shih Tzu dogs; the radial conformation of these scars is suggestive of an ischemic origin. Such "asynchronous differentiation" of nephrons may be the result of acquired scarring and mechanical inhibition of maturation. Whether familial renal diseases are expressions of dysplasia is a moot point. The sine qua non of dysplasia (primitive ducts, cartilage nodules – see section on Renal dysplasia, below) is rarely met in affected kidneys; expanding the definition of dysplasia to include the presence of immature glomeruli greatly, and perhaps illegitimately, expands the number of affected cases of juvenile renal disease characterized as being "dysplastic." Until etiopathogenesis of these syndromes is better defined, they might better be termed "nephropathies."

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ANOMALIES OF DEVELOPMENT

The embryology of mammalian kidneys involves the sequential development of three successive but overlapping structures: the *pronephros*, *mesonephros*, and *metanephros*. The first two become vestigial, but act as inducers of the definitive kidney, the metanephros. Pronephric tubules, arising in the intermediate mesoderm of the cervical region, form the pronephric duct by fusion and extension of their caudal ends. This duct opens into the cloaca and, although the pronephric tubules are not functional in mammalian embryos, the utilization of their duct by the mesonephric tubules gives them potential significance in the genesis of renal anomalies. Mesonephric tubules develop from thoracic mesoderm caudad to the pronephros. The mesonephros is functional in mammalian embryos but degenerates before birth. In males, some of the caudal tubules persist as efferent ducts of the epididymis, and the duct itself is utilized as the vas deferens. In females, cystic remnants of mesonephric tubules in the mesovarium may form the epoophoron and paroophoron, and remnants of the duct are known as Gartner's duct.

The formation of **the metanephros**, *the definitive kidney*, begins with the development of a *ureteral bud* from the mesonephric duct immediately cranial to its junction with the cloaca. The bud, accompanied by vessels and nerves, grows into a mass of mesenchymal cells, the *metanephric blastema*, and it is on the interaction of these two structures that normal renal and ureteral development depend. As the bud grows into the blastema it makes a specific number of successive, dichotomous divisions. The tubes formed by early divisions of the ureteral bud dilate and become the pelvis and calyces of the kidney. Tubes formed by later divisions develop into collecting ducts, and the last divisions give rise to collecting tubules. As the blind end of each collecting tubule, the ampulla, grows into the metanephric blastema, it induces compact masses of cells to form about it. These masses soon cavitate, become S-shaped, and unite with the side of the ampulla, which continues to advance and divide.

The cavitated cell masses develop into *nephrons*. Connection of the lumens of the nephron and the collecting tubule occurs very soon after the cell mass cavitates. Glomerular development involves the formation of a lateral invagination in the S-shaped mass by mesenchymal cells that differentiate into endothelial and mesangial cells and become linked with the renal vasculature.

The complicated formation of the kidney provides for many patterns of malformation. The interaction of ureteral bud and metanephric blastema involves mutual inductions, and malformations of renal tissue are often accompanied by ureteral anomalies. Ectopic budding of the ureter from the mesonephric duct appears to be the primary event that precedes many congenital anomalies of the kidney and urinary tract. Ectopia results in hypoplastic kidney, ectopia of the ureterovesical orifice, urinary outflow obstruction, and/or reflux. The genes involved in navigating ureteric budding to the correct site often also regulate later developmental events of the kidney and urinary tract. Candidate genes have been proposed for a wide range of urinary developmental defects in humans and laboratory rodents.

The incidence of urinary tract anomalies is not known for most species. Survey results from large numbers of lambs suggest that anomalies occur much more often than is commonly believed.

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Abnormalities in the amount of renal tissue

Lack of renal tissue may be complete (*agenesis*) or partial (*hypoplasia*).

Renal agenesis may be caused by developmental failure of the pronephros, mesonephros, or ureteral bud, by absence or complete unresponsiveness of the metanephric blastema, or by complete degeneration of the metanephric blastema. Partial degeneration, or partial responsiveness of the blastema to the inductive influences of the ureteral bud, probably produces renal dysplasia. The presence of even a fragment of recognizable metanephric tissue necessitates this diagnosis.

Agenesis may be unilateral or bilateral. As is the case with all renal anomalies, agenesis may be associated with other urogenital deformities. Unilateral renal agenesis is compatible with normal life if the other kidney is normal; however, contralateral dysplasia, or even hypoplasia, may be present, in which case renal failure ultimately develops. The ureter may be absent, or malformed with a blind end that terminates in connective tissue at the renal site. Bilateral agenesis is inconsistent with postnatal life. Renal agenesis occurs infrequently in all species, except when there is a *familial incidence*, as in some Beagles, Shetland Sheepdogs, and Doberman Pinschers, and in Large White pigs. Bilateral agenesis may account for some stillbirths, but this can only be assessed by careful examination of fetuses.

Hypoplastic kidneys are small, but most abnormally small kidneys, even in young animals, are not hypoplastic. The limited size of hypoplastic kidneys is associated with *a reduced number of histologically normal nephrons, lobules, and calyces*. Renal hypoplasia is a quantitative defect caused by reduced mass of metanephric blastema or by incomplete induction of nephron formation by the ureteral bud. When the amount of blastema is normal, but there is malfunction of the ureteral bud, renal dysplasia probably develops. Most of the small kidneys diagnosed as hypoplastic are probably dysplastic or scarred. The term "cortical hypoplasia" should be avoided since it is inconsistent with established concepts of renal embryology and anatomy. *Renal hypoplasia is rare*. It may be unilateral or bilateral. When unilateral, contralateral hypertrophy is expected.

Bilateral hypoplasia probably always leads to renal failure, and this often complicates or precludes the diagnosis because of the secondary changes that develop. Several forms of renal hypoplasia occur in humans but, because of the confusion of terminology, the variations and incidence of the condition in animals cannot be assessed. Kidneys suspected of being hypoplastic should be weighed along with their mate and examined for evidence of dysplasia and hypertrophy. In humans, in the absence of acquired disease, a decrease in size of one kidney by more than 50% or reduction of total renal mass by more than one-third is taken as evidence of hypoplasia.

Details regarding **excess renal tissue** are not well documented. *Duplication of ureters and kidneys* occurs in cattle, pigs, and dogs, but it is not clear whether total renal mass is increased.

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Anomalies of renal position, form, and orientation

The kidneys develop in the pelvis and migrate to their sublumbar location, meanwhile rotating so that the ureter attains its normal orientation. During this movement, the blood supply shifts from the iliac arteries to the aorta. Various disruptions of this procedure may occur. Vitamin A deficiency in sows may cause anomalies such as those described below.

Malposition of the kidneys (*renal ectopia*), observed more frequently in swine than in dogs or cats, is usually caudal with the kidney in the pelvic or inguinal position. One or both kidneys may be displaced. The kidneys may be normal or abnormally small. The renal arteries arise close to the bifurcation of the aorta or from the iliacs. The ureter is short but may be kinked and thereby predisposed to hydronephrosis and pyelonephritis, or may empty into the genital tract, causing urinary incontinence. *Crossed renal ectopia* refers to malposition of a kidney that has crossed the midline; it may fuse with its contralateral partner.

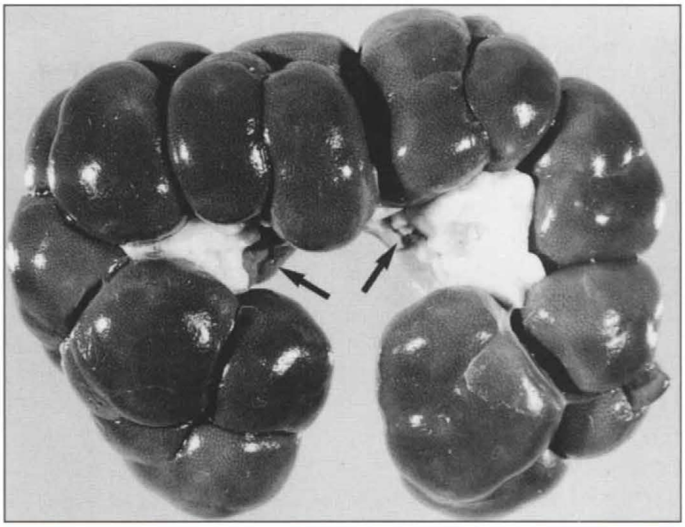

Figure 4.8 Horseshoe kidney in a calf. Ureters have been severed (arrows).

Fusion of the kidneys may occur in utero. *Horseshoe kidney*, seen in all species, results from fusion of the cranial or caudal poles of the kidneys (Fig. 4.8). The fusion may involve only a small portion of the capsule or parenchyma or be sufficient to produce a common pelvis. The ureters are not involved and their disposition depends on whether the cranial or caudal poles are fused. Such kidneys function normally.

Fetal lobulations, normal in embryos and cattle, may persist owing to failure of fusion of individual renal segments. They are not of pathologic significance.

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Renal dysplasia

Renal dysplasia is disorganized development of renal parenchyma due to anomalous differentiation. If normal development of the collecting duct system ("renal branching morphogenesis") is disrupted, then renal dysplasia can result. Transcription factors, growth factors, and cell surface signaling peptides are critical in regulation of renal branching morphogenesis. Renal lesions may be gross or microscopic. Renal dysplasia is *usually congenital*, but in cats, dogs, and pigs, which have an active subcapsular nephrogenic zone at birth, dysplasias may be caused by disease in the early neonatal period until differentiation of the nephrogenic tissue is completed. The quantitative changes that continue until maturity are not susceptible to dysplastic influences.

Dysplastic changes may be obscured by secondary compensatory, degenerative, and inflammatory changes.

The **causes** of renal dysplasia are ill defined, but many are likely *hereditary*. Cases of familial renal disease in several *dog* breeds often have dysplastic features. An autosomal dominant form of cystic renal dysplasia has been reported in *Suffolk sheep*. Some of a range of renal lesions reported in *Japanese black (Wagyu) cattle* have a dysplastic component, particularly the condition of multicystic renal dysplasia. Renal tubular dysplasia in this breed is due to deletion of the paracellin-1 gene from chromosome 1, and it may be the antecedent of chronic interstitial nephritis also reported in this breed. Most *human* cases of renal dysplasia are associated with intrauterine *ureteral obstruction,* and some cases in foals, pigs, and calves probably have the same cause; rapid growth and the increased activity of the intrarenal renin–angiotensin system in the fetus may cause greater susceptibility to permanent renal injury. Renal dysplasia in kittens is caused by fetal infection with *Feline panleukopenia virus*, in puppies by neonatal infection by *Canine herpesvirus*, and in calves is associated with fetal infection by *Bovine viral diarrhea virus*. Other teratogenic agents can undoubtedly produce renal dysplasia in these and other species. Renal dysplasia in pigs has been attributed to *hypovitaminosis A*; formation of the trigonal wedge and proper insertion of the ureteral buds into the bladder, and hence proper renal development, are mediated by vitamin A and proto-oncogene Ret signaling pathways.

There is considerable variation in the appearance of those dysplastic kidneys that are grossly abnormal. Most of them are small, which accounts for their frequent misdiagnosis as hypoplastic. They are usually misshapen and fibrosed with thick-walled cysts and dilated tortuous ureters (Fig. 4.9). One or both kidneys may be affected. If the lesion is unilateral, the ipsilateral ureter should be examined for anomalous valves, diverticula, or atresia. If both kidneys are involved, scrutiny of the bladder and lower urinary tract is indicated. Some dysplastic kidneys may be only slightly irregular in contour (Fig. 4.10) or may appear normal, in which case microscopic examination is required for diagnosis. Dysplasias caused by teratogenic viruses are usually included in this group. Considerable emphasis has been placed on the size of renal arteries in dysplasias, but changes should be interpreted with caution since degenerative lesions in the renal parenchyma may be accompanied by vascular atrophy. Extrarenal anomalies, such as imperforate anus, may occur in association with renal dysplasia.

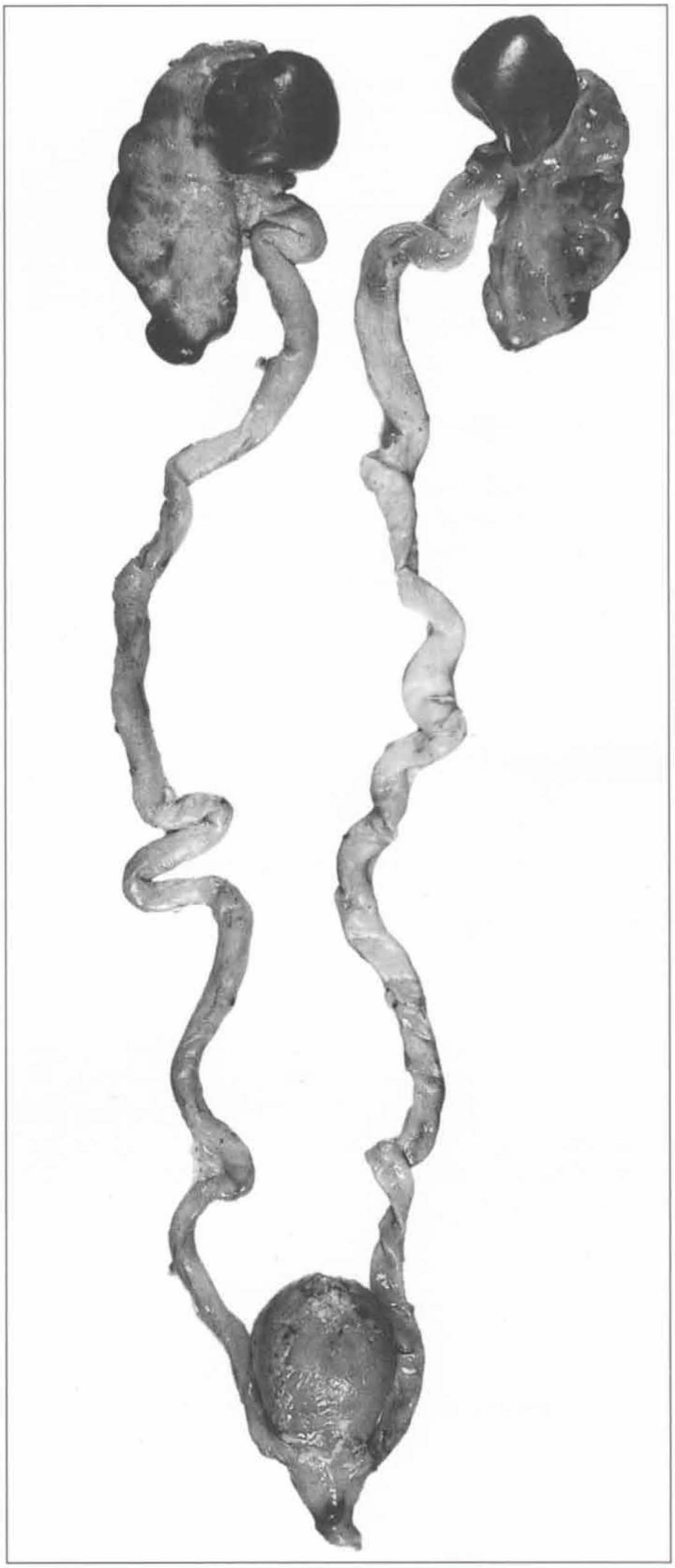

Figure 4.9 Renal dysplasia in a pig. Dilated ureters lead to fibrotic malformed kidneys.

The **microscopic criteria** of renal dysplasia are the presence of structures inappropriate to the stage of development of the animal, or the development of structures that are clearly anomalous. Among the former are *areas of undifferentiated mesenchyme* in cortex or medulla (Fig. 4.11), and *groups of immature glomeruli* (small glomeruli with peripheral nuclei and inapparent capillaries) in the cortex of adolescent or adult animals. Anomalous structures include *collecting tubules ending blindly* in cortical connective tissue, *atypical tubular epithelium*, and *primitive (metanephric) ducts* lined by cuboidal or columnar epithelium and surrounded by concentric layers of mesenchyme (Fig. 4.12). *Dysontogenic (cartilaginous or osseous) metaplasia*, which occurs in some dysplastic human kidneys, is rarely present in dysplastic kidneys in domestic animals. This absence is probably a function of the stage of development at which metanephric injury occurs. Cartilage nodules imply injury to uncommitted mesenchyme, that is, to nephrogenic cord before its interaction with the ureteral bud. Other anomalous structures clearly are nephrogenic, and represent improper interaction of ureteral bud and mesenchyme. There is some support for the opinion that only primitive ducts and cartilage nodules are prima facie evidence of dysplasia, on the grounds that all other lesions may be produced by acquired renal disease. Some of the more prominent

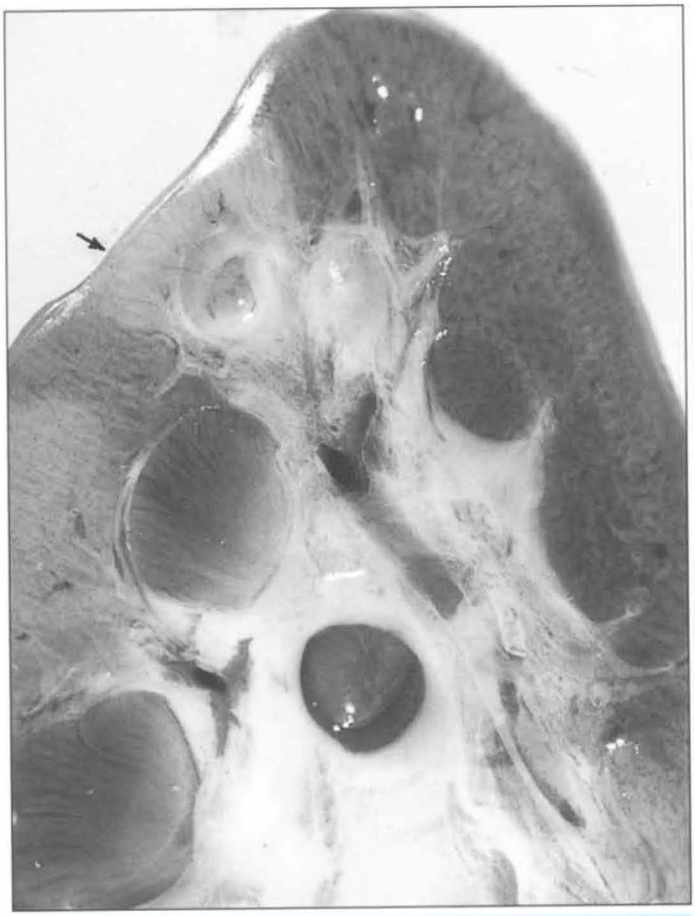

Figure 4.10 **Focal renal dysplasia** (arrow) in a pig.

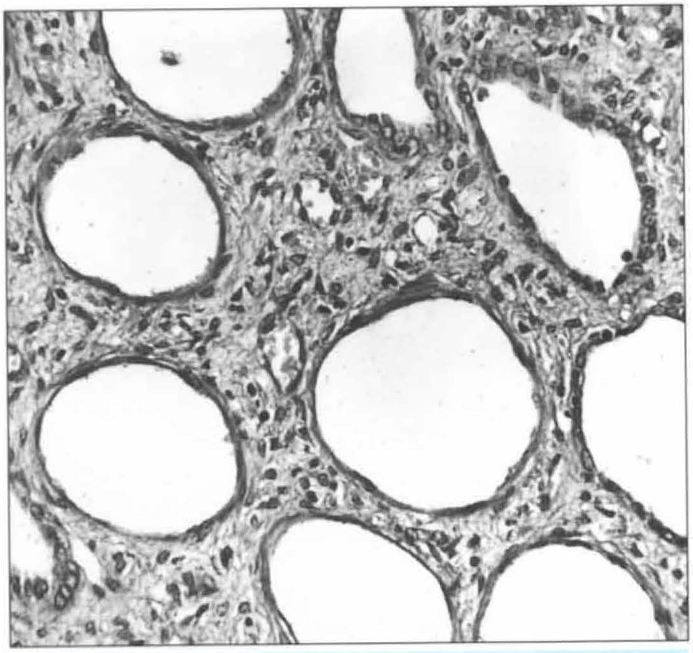

Figure 4.11 Loose intertubular mesenchyme and dilated medullary tubules in **renal dysplasia** in a calf.

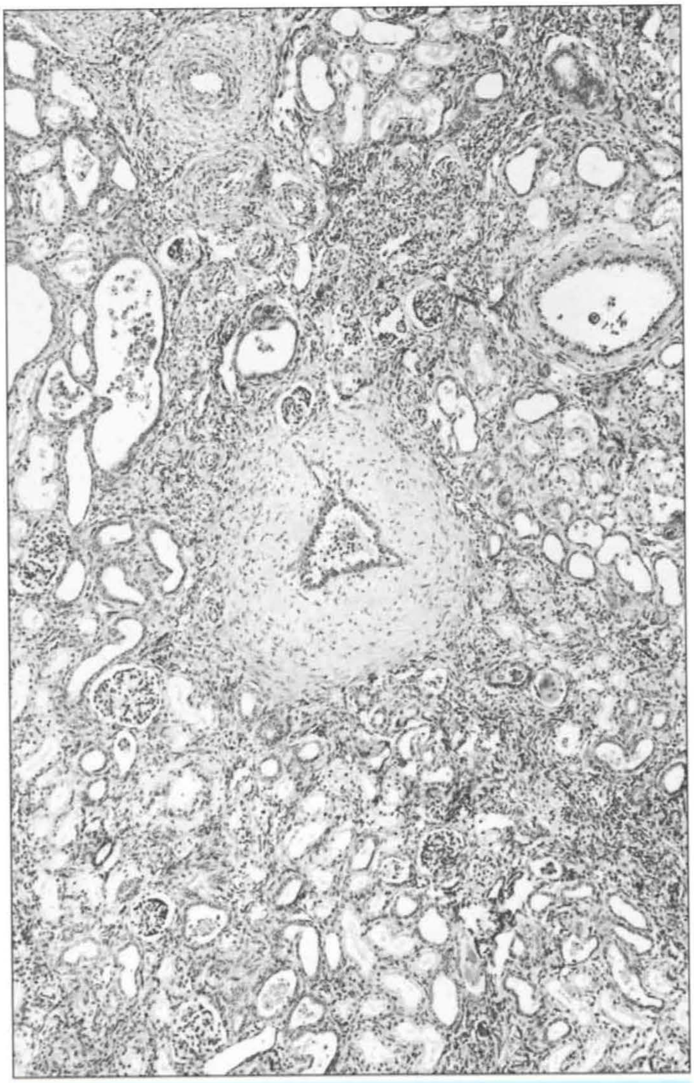

Figure 4.12 **Renal dysplasia** in a calf. Thick-walled primitive duct in renal cortex is lined by cuboidal epithelium. (Courtesy of S Groom.)

gross lesions of dysplastic kidneys, such as fibrous cysts, dense medullary fibrosis, and fibrous wedges extending from pelvis to cortex, are probably regressive changes due to obstruction or infarction. Because *ureteral anomalies* are often concomitant, dysplastic kidneys are abnormally susceptible to pyelonephritis. Otherwise there is little or no evidence of infection in the renal parenchyma. The dark nuclei of improperly differentiated nephrogenic tissue should not be misinterpreted as lymphocytes.

Renal dysplasia with gross changes usually causes *uremia*. When lesions are bilateral, this is explicable in terms of reduced functional renal mass. With unilateral dysplasia, systemic hypertension induced by constriction of arteries in the affected kidney may be responsible.

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Renal cysts

Cystic diseases of the kidney include various conditions characterized by one or more grossly visible cystic cavities in the renal parenchyma. No satisfactory classification of renal cysts exists, but an acceptable compromise would be based on mode of inheritance, or lack thereof, the presence of lesions in other organs, and the clinical course in affected animals.

Cysts can arise during organogenesis, and may be associated with histologic criteria of renal dysplasia. In humans, most dysplastic kidneys are cystic and most cystic diseases of childhood are dysplasias. Cysts can arise in nephrons and collecting tubules after the end of nephrogenesis. Examples are provided by steroid-induced cysts in various species, and atypical cysts in patients undergoing long-term hemodialysis. *Cysts can develop in any part of the nephron, including the glomerular space, or in the collecting system.* In *glomerulocystic disease,* they only develop in Bowman's space, but this exclusivity is exceptional. There is no evidence that cysts are caused by failure of nephrons to unite with the collecting system. Analyses of their content indicate that *they are part of functional nephrons,* and that their activity is consistent with their location in the nephron.

Three **mechanisms**, which are not mutually exclusive, may lead to the formation of renal cysts:

1. Renal cysts may be caused by *obstructive lesions* – examples are the acquired retention cysts of chronic renal disease, some dysplastic cysts, and possibly those of glomerulocystic disease.
2. A fundamental change, of unknown origin, may occur in the *tubular basement membrane* and result in formation of saccular or fusiform dilations of the tubules.
3. Disordered growth of tubular epithelial cells may lead to *focal hyperplastic lesions* and cyst formation.

Mutation of polycystic kidney disease (PKD) genes in humans may alter production of *polycystin* proteins, which are important in cell–cell and cell–matrix interactions; altered tubular epithelial growth and differentiation may lead to cyst formation. Enhanced renal cystogenesis occurs in humans with primary aldosteronism or primary renal potassium wasting, perhaps as a consequence of chronic hypokalemia and tubular obstruction by proliferating tubular epithelial cells. Renal cysts are dynamic structures and their growth may be modified by pharmacologic means.

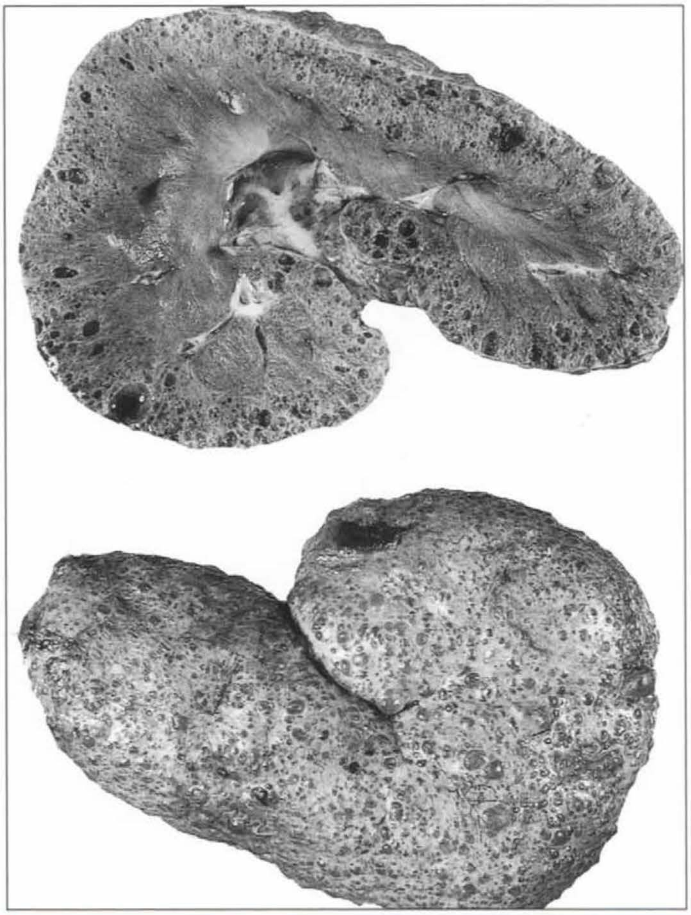

Figure 4.13 Polycystic kidney from an 8-year-old horse.

Many *chemicals,* such as long-acting corticosteroids, diphenylamine, polychlorinated biphenyls, 5,6,7,8-tetrahydrocarbazole-3-acetic acid, alloxan, diphenylthiazole, and nordihydroguaiaretic acid, cause renal cysts in experimental animals. Corticosteroids induce hypokalemia, and cyst formation is prevented by injections of some potassium salts, but in general the mechanisms of cyst development are not known. It seems possible that some of the therapeutic, prophylactic, and pollutant chemicals to which animals are exposed could be responsible for sporadic cases of renal cysts.

Renal cysts vary in size from the barely visible (Fig. 4.13) to structures that exceed that of the organ itself (Fig. 4.14). Most lie within these limits, and although they are more numerous in the cortex than medulla, this may simply reflect the relative volumes of the two regions. The cyst wall is clear or opaque depending on the amount of surrounding connective tissue. The content is watery. Cysts are lined by flattened or cuboidal epithelium that grossly is smooth and shiny. A few cysts are more or less divided by thin trabeculae but most are unilocular and roughly spherical, ovoid, or fusiform.

Simple renal cysts occur in all species but are most common in pigs and calves. There are different patterns of occurrence in pigs, but it is not clear whether similar patterns exist in other animals. The usual

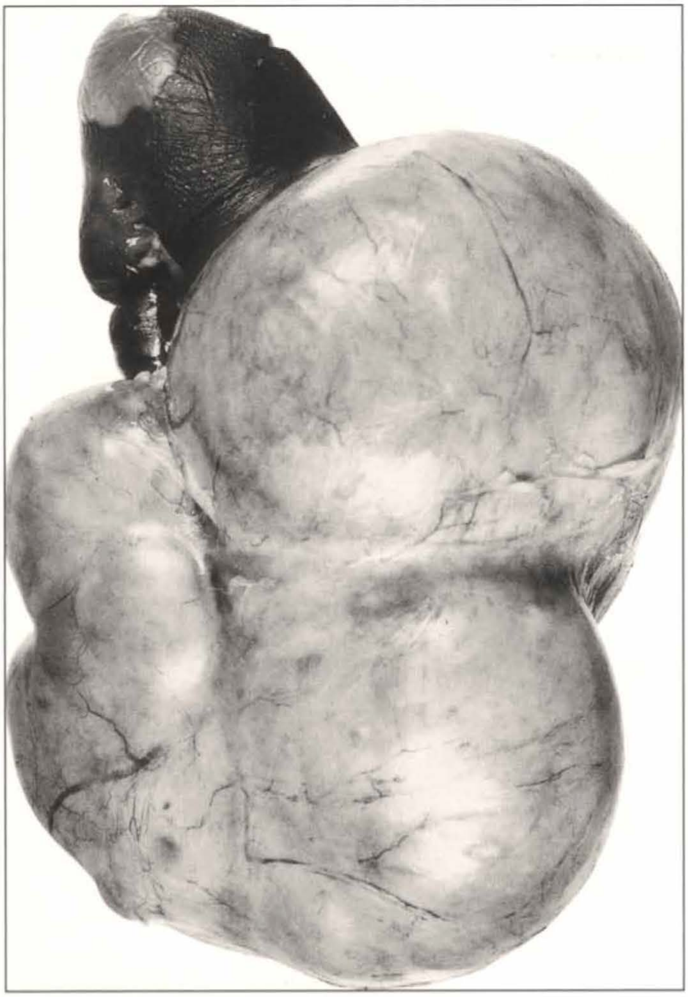

Figure 4.14 Renal cysts involving both poles of the kidney of a pig.

finding in pigs is one or a few unilocular cortical cysts, about 1–2 cm across, that bulge from the renal surface or are exposed when the kidney is sliced. They are usually bilateral, and are incidental findings in young pigs. Affected kidneys are discarded in abattoirs. Although usually regarded as sporadic occurrences, these lesions may be examples of a cystic renal disease that is inherited as an autosomal dominant trait. Polygenic inheritance may determine the number of cysts in animals with the dominant gene. In this condition, few cysts are present at birth but they gradually increase in number, and there may be 80–90 by 1 year of age. Cysts occur in different parts of the kidney and nephron. Signs of renal failure are not seen, but the condition has similarities to a human cystic disease in which renal failure develops in adults – a similar course in mature swine is not inconceivable.

Occasionally areas containing many small cysts occur in one lobe of a bovine kidney or one pole of an equine kidney. They are not significant clinically.

Polycystic kidney disease (PKD) occurs in two major forms:

1. *Autosomal dominant* polycystic kidney disease (ADPKD), in Bull Terriers and Persian cats, is similar to the *adult-onset* form of PKD in humans, which is linked to defects in genes *PKD1* and *PKD2*. Renal cysts develop bilaterally, are of proximal or distal tubular origin, grow progressively over time, and lead to chronic tubulointerstitial nephritis and renal failure in adult life. ADPKD in Persian cats, also linked to a *PKD1* gene defect, is commonly accompanied by hepatic cysts and/or hepatic fibrosis, and pancreatic cysts.
2. *Autosomal recessive* PKD (ARPKD), described in West Highland White and Cairn Terriers, Persian cats, and sheep, is similar to the *childhood* form of PKD in humans. ARPKD in West Highland White Terriers may be accompanied by hepatic biliary cysts.

Congenital PKD, of unknown inheritance, occurs in pigs, lambs, calves, goat kids, puppies, kittens, and foals and can also be associated with cystic bile ducts, bile duct proliferation, and sometimes pancreatic cysts. This form of PKD in domestic animals is manifest by stillbirths or death in renal failure during the first few weeks of life. Grossly the kidneys are large and pale, and contain numerous 1–5 mm cysts that involve both cortex and medulla. Bile duct cysts range from barely visible up to 3 cm across, and the gallbladder and biliary system are often distended with bile that discolors the liver. PKD has also been reported as a sporadic event in adult horses.

Uremic medullary cystic disease complex is a group of progressive renal disorders of children that leads to chronic renal failure as a result of formation of corticomedullary cysts, cortical tubular atrophy, and interstitial fibrosis. A similar syndrome has been identified in a Shetland Sheepdog.

Glomerulocystic disease is occasionally seen in stillborn foals and Collie dogs. Cysts, barely visible, are only present in Bowman's spaces, perhaps as a consequence of periglomerular fibrosis and hence stenosis of the glomerulotubular junction. Their significance in foals is not known. In affected dogs, uremia develops.

Acquired cysts of the kidney do develop when tubules are obstructed by scar tissue. They are multiple and small, rarely exceeding 1.0 cm in diameter. Most are located in convoluted tubules and Bowman's spaces. Hyperplastic collecting tubules are sometimes grossly visible as elongated cysts in the medulla of dogs with renal failure. These acquired cysts are distinguishable from primary cysts in that they occur in kidneys with extensive scarring.

Perinephric pseudocysts occasionally develop uni- or bilaterally as a collection of fluid, which may be urine, blood, lymph, or transudate, in the space between the renal capsule and the renal reflection of the peritoneum; *the space is not lined by epithelium* and is thus a pseudocyst rather than a true cyst. Potential causes include trauma, ureteral or lymphatic obstruction, venous congestion, and hypertension. Affected cats are usually old and have concomitant chronic renal disease.

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CIRCULATORY DISTURBANCES AND DISEASES OF THE BLOOD VESSELS

Renal hyperemia

Active hyperemia is seen in acute nephritis but especially in the acute septicemias and bacterial intoxications. The kidney is swollen and uniformly dark, although in some cases the hyperemia may be largely restricted to the medulla. Microscopically, all vessels, especially capillaries, are filled with blood. Very acute congestion with intertubular hemorrhages occurs in clostridial enterotoxemia of lambs and calves.

Passive hyperemia (congestion) follows the usual principles. Affected kidneys are enlarged and dark and the capsular vessels are injected. On section, the corticomedullary junctional zone is dark and prominent and there is engorgement of visible tributaries.

Renal hemorrhages

Hemorrhages are especially common in the renal cortex in a variety of bacteremias and viremias and sometimes in healthy slaughtered animals. Petechiae are very common in piglets dead of any cause. Many or few pinpoint hemorrhages occur beneath the capsule in classical swine fever (hog cholera). African swine fever, and porcine salmonellosis (Fig. 4.15). In porcine erysipelas, the hemorrhages tend to be larger and more irregular in size and shape. Severe hemorrhage in the wall of the renal pelvis and the medulla sometimes occurs in classical swine fever, in other acute infections of swine, and in the hemorrhagic diatheses; the hemorrhage occurs from the congested medullary vessels. Extensive subcapsular hemorrhage is not uncommon in clostridial enterotoxemia of calves; it produces a black cast molded to the shape of the cortex.

Renal infarction

Infarcts of the kidney are common lesions of localized coagulative necrosis produced by embolic or thrombotic occlusion of the renal artery or of one of its branches. The sequelae depend on whether the obstructing material is septic or bland and on the size and number of the vessels obstructed (Fig. 4.16). Bland thrombi produce typical infarcts; septic thrombi produce abscesses that may heal, sequestrate, or discharge into the pelvis. Thrombosis of a trunk of a renal artery will produce total or subtotal necrosis of the kidney, the extent of the

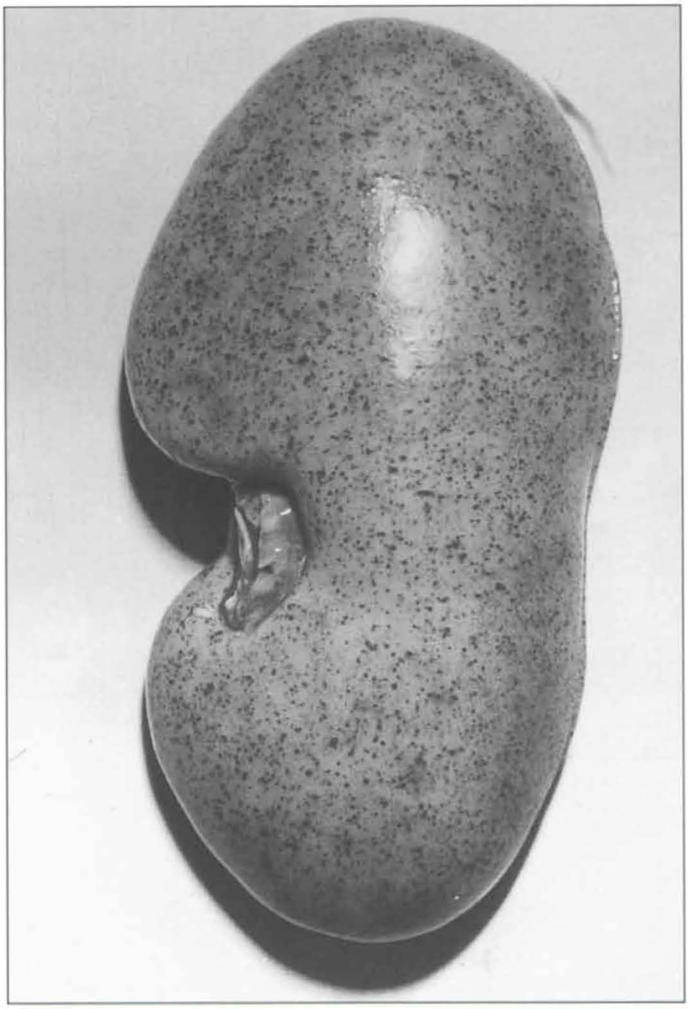

Figure 4.15 Renal cortical petechiae in **African swine fever**. (Courtesy of C Brown.)

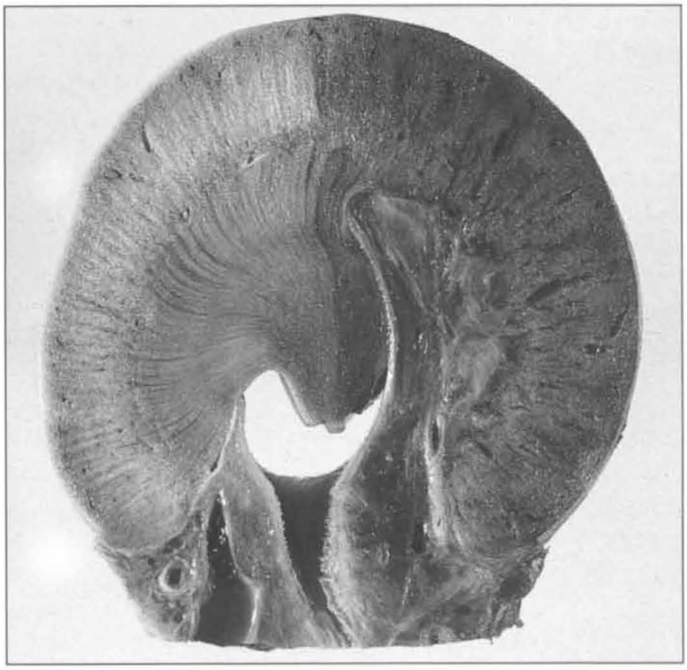

Figure 4.16 **Infarction** of half kidney following ligation of one of two renal arteries in a dog. (Courtesy of BP Wilcock.)

latter depending on the presence and efficiency of parahilar and capsular collaterals. If an arcuate artery is obstructed, there is necrosis of a wedge of both cortex and medulla; if an interlobular vessel is involved, infarction is limited to the cortex. The ease and the frequency with which the kidneys are infarcted result from their vascular architecture being of the "end-artery" type and the large volume of blood that continually traverses them.

Soon after total obstruction of a vessel, the related wedge of tissue is swollen and intensely cyanotic and it is congested by the blood that oozes into the vessels from collaterals. There is no sharp line between the infarcted zone and the adjacent normal tissue because in the narrow boundary zone there is an outer part in which blood continues to ooze slowly and an inner part that is more or less well served by diffusion from the viable tissue. In the outer part of the marginal zone, the red cells survive and circulation may be re-established, but this zone persists for the first 2–3 days; it is usually referred to, apparently erroneously, as the zone of reactive hyperemia. The limit of useful diffusion determines the actual limit of the infarct, and it is here that dehemoglobinization begins, neutrophils accumulate, and the area of total necrosis begins. The dehemoglobinization begins from the periphery at about 24 hours and may be complete in 2–3 days, the infarcted area then being white (Fig. 4.17A). Before decoloration begins, the area that will be affected is outlined by a thin but distinct white line of leukocytes.

The sequence of degenerative changes in the infarcted tissue reflects the specialization and sensitivity of the various structures. At the outer margins, only a few proximal tubules show epithelial necrosis. More centrally, every proximal tubule is dead and inside the zone of diffusion everything is dead (Fig. 4.17B). In the peripheral dead zone there may in a week or so be some revascularization along preformed channels. The necrotic zone is progressively replaced by fibrous tissue, and healed infarcts persist as pale gray-white scars, wedge-shaped and much depressed below the surface. The scars may be difficult or impossible to distinguish grossly from focal healed pyelonephritis.

Minute emboli that lodge in the glomerular or intertubular capillaries may produce small infarcts that are not detectable macroscopically. Because of the small size of such infarcts, there may be adequate diffusion across the infarcted zone so that leukocytes do not accumulate, epithelial necrosis is minimal and soon repaired, and circulation is re-established. Commonly, infarcts of various ages in a kidney indicate recurrent embolic episodes.

Primary vascular disease of the kidneys in animals is of little significance. Renal arteriosclerosis is not rare as an incidental finding in cattle, and arteritis is occasionally observed as "polyarteritis nodosa" or in systemic disease such as malignant catarrhal fever. A variety of degenerative proliferative arterial changes occur in chronic diffuse inflammatory disease, but they are probably secondary, although

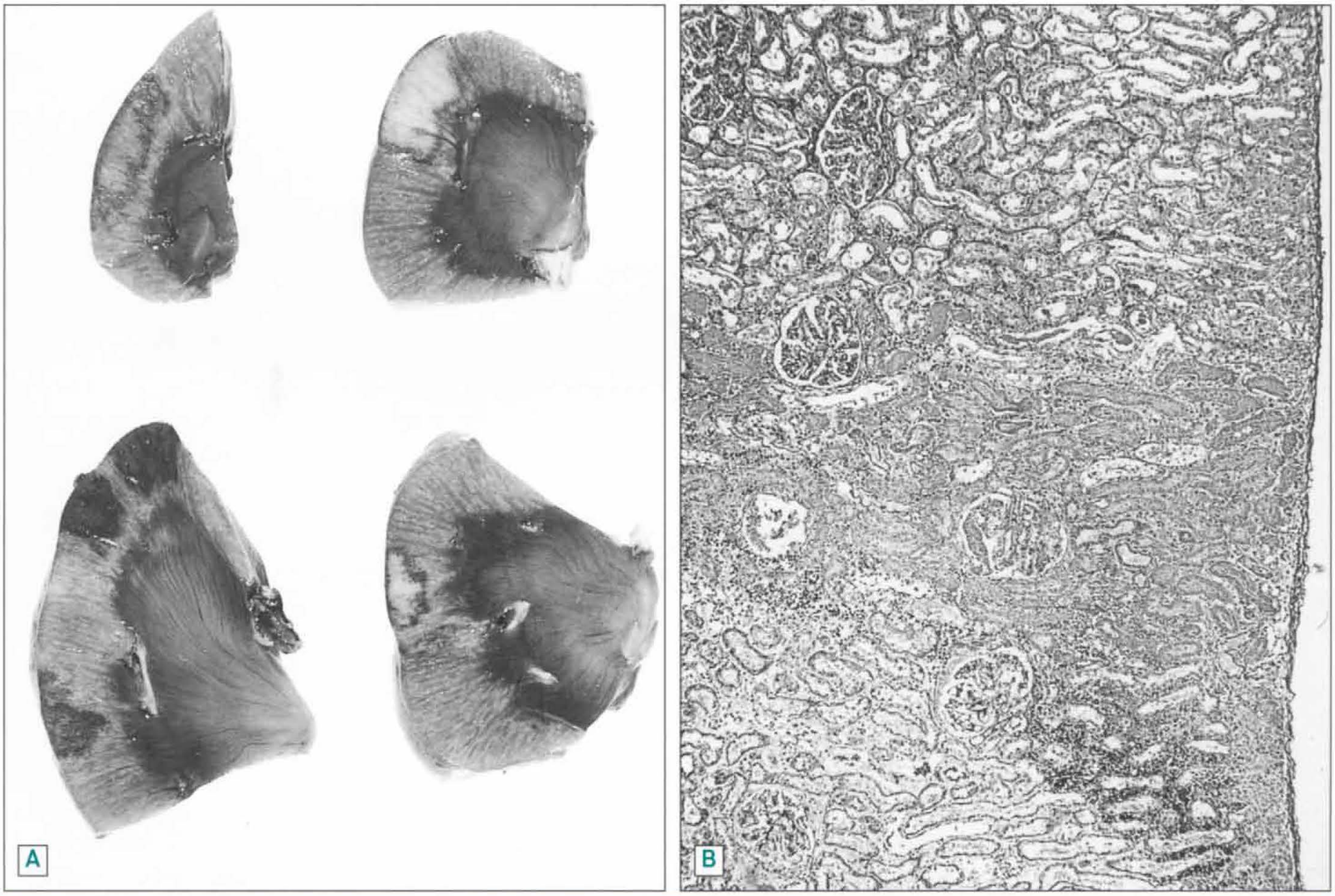

Figure 4.17 A. Renal infarcts in a cow. The dark (hemorrhagic) infarcts are about 1 day old; those with dehemoglobinized centers are 2-3 days old. **B. Renal infarct** in a sheep.

exaggerated by the *hypertension* that is expected to develop. Hypertension is common in canine and feline renal failure, and can lead to further deterioration in renal function by causing additional glomerular and tubular damage. Familial *renal telangiectasia* is an oddity that occurs in Pembroke Welsh Corgis and results in hematuria.

Renal cortical necrosis and acute tubular necrosis

Acute tubular necrosis and renal cortical necrosis are grouped together here for purposes of discussion; acute tubular necrosis is also discussed later in the section on Diseases of tubules. These lesions occur infrequently in animals, but may, if severe, cause acute renal failure and death. *Either variety of renal necrosis is usually a manifestation of hypoperfusion, or "shock,"* which may be classified as cardiogenic, hypovolemic, septic, or neurogenic. Outbreaks of renal cortical necrosis of unknown cause have occurred in kennels of dogs. Renal cortical necrosis and acute tubular necrosis occur in cattle in a variety of endotoxemic conditions, such as mastitis or metritis, and in gastrointestinal diseases, such as severe enteritis and grain overload. In horses, this often provides the fatal outcome to "azoturia." Bilateral cortical necrosis is a rare complication of esophagogastric ulceration in swine, and apparently results from hemorrhagic shock.

In **acute tubular necrosis**, there is patchy necrosis of segments of both proximal and distal tubules with involvement also of the basement membranes; necrotic tubular epithelial cells become casts, which cause tubular blockage and oliguria. Other contributors to oliguria are tubular backleak through disrupted tubular basement membranes, compression of medullary tubules by blood vessels dilated with trapped red cells, reflex constriction of afferent arterioles, and decreased glomerular membrane permeability. In **renal cortical necrosis**, the whole or part of both cortices is involved and there is destruction of both tubules and glomeruli. It appears probable that these lesions represent differences in degree and that they result from patchy or complete renal ischemia.

The usual balance between the renin–angiotensin and the eicosanoid systems that maintains fine regulation of intrarenal blood flow is disrupted during ischemia. During hypotension, perfusion of outer cortical nephrons is reduced while perfusion of inner cortical nephrons is maintained, that is, *intrarenal blood flow is redistributed toward the inner cortex and medulla.* This reaction occurs because the vasoconstrictive effects of angiotensin II and adrenergic stimulation are unopposed in the outer cortex, while in the inner cortex, prostaglandins modulate vasoconstriction; PGE_2 is produced in the medulla in response to ischemia, travels in tubular fluid to the area of the juxtaglomerular apparatus, and has a local vasodilatory effect on afferent arterioles of juxtamedullary nephrons.

The duration of ischemia is of obvious importance for the pathogenesis of necrosis. Complete ischemia of less than 2 hours' duration can be expected to be followed by *good reflow* if cardiac output and blood pressure are restored to normal, whereas total ischemia of longer duration may be followed by patchy reflow or complete failure of reflow in the cortex, medulla, or both. Reflow is inhibited primarily because of vascular congestion by red cells swollen by plasma water uptake, and perhaps also by ischemia-induced swelling of endothelial cells of glomeruli, vasa recta, and peritubular capillaries, and by swelling of parenchymal cells. This *"no-reflow" phenomenon* may make an important contribution to renal ischemia and acute renal failure. Severe ischemic injury may permanently alter renal capillary density, resulting in decreased urinary concentrating ability and predisposing to interstitial fibrosis. This progression may be mediated through local synthesis of *angiostatin*, a proteolytic cleavage product of plasminogen that inhibits angiogenesis, promotes endothelial cell apoptosis, and disrupts the integrity of capillaries.

Another mechanism by which renal cortical necrosis occurs is via the generalized Shwartzman reaction, an example of *disseminated intravascular coagulation*, which is often due to gram-negative endotoxemia. Endothelial injury in glomerular and peritubular capillaries leads to microthrombosis and hemorrhagic renal cortical necrosis. This severe degree of renal damage is usually rapidly fatal but minimal lesions of this pathogenesis are common in a number of bacteremic diseases and visible as petechial or larger hemorrhages in the cortex.

The cellular destruction that occurs in ischemic renal injury begins with a decreased ability to produce adenosine triphosphate and hence an increase in membrane permeability that allows influx of calcium into the cell. Excess free cytosolic calcium activates phospholipases that further increase membrane permeability and lead to membrane disruption and generation of toxic lipid byproducts. Increased intracellular calcium also interferes with mitochondrial respiration and causes increased production of free radicals that further injure cell membranes and mitochondria. Unfortunately, reperfusion can be deleterious because reoxygenation increases the production of free radicals.

The **gross appearance** of the kidneys, other than those with hemorrhagic renal necrosis, varies considerably from case to case; it is probably determined by the severity, distribution, and duration of the ischemia and the quality of the reflow. In acute tubular necrosis, the cortices are finely mottled or flecked by small yellow foci of necrosis. In renal cortical necrosis, the cortices may be totally affected (Fig. 4.18) or the injury may be patchy (Fig. 4.19); pigs tend to develop a "turkey egg" pattern marked by hemorrhagic glomeruli, but die before marked necrosis is evident, whereas cattle sometimes develop a distinctive patchy cortical necrosis (Fig. 4.20). The reaction is the same as for infarction given above. A narrow subcapsular rim of viable tissue may remain. The affected areas of cortex are pale, almost white, slightly swollen, and stop sharply at the corticomedullary junction. The irregular areas of cortical infarction may be outlined by hemorrhage. The medulla may be normal, but in some cases there is severe congestion of the inner stripe of the outer medulla or of the whole of the medulla so that it is swollen and resembles a blood clot.

The **histologic appearance** of a kidney with acute tubular necrosis includes irregular necrosis of the proximal tubules, often with disruption of the tubular basement membranes. Hyaline and granular casts may be present, particularly in distal tubules and collecting ducts. There may be interstitial edema as a result of tubular leakage. Preferential damage occurs to the pars recta and thick ascending limb, primarily because of their location in the poorly perfused outer medulla. If the animal survives the ischemic episode, evidence of regeneration may be seen in ~1 week, namely tubules lined by flattened epithelial cells with hyperchromatic nuclei and occasional mitoses. In cases of severe ischemia and renal cortical necrosis, various patterns of infarction may be seen, with glomeruli and vessels, as well as tubules, being necrotic. Microthrombi may be

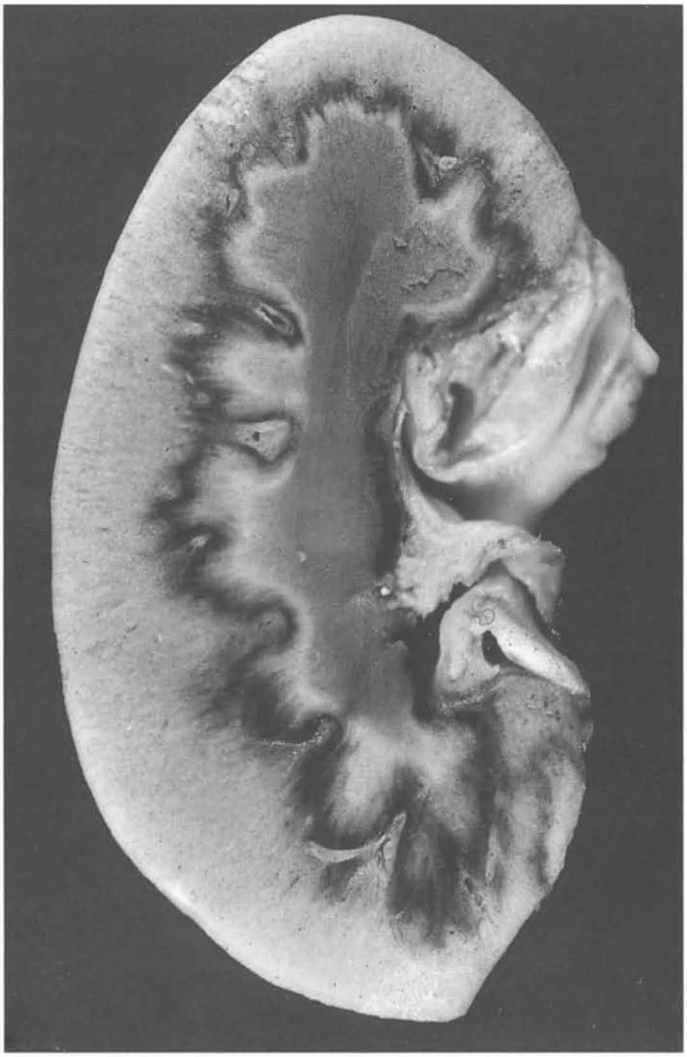

Figure 4.18 Renal cortical necrosis in a pig. The pale cortex is delineated by a hemorrhagic zone, which emphasizes the vascular supply.

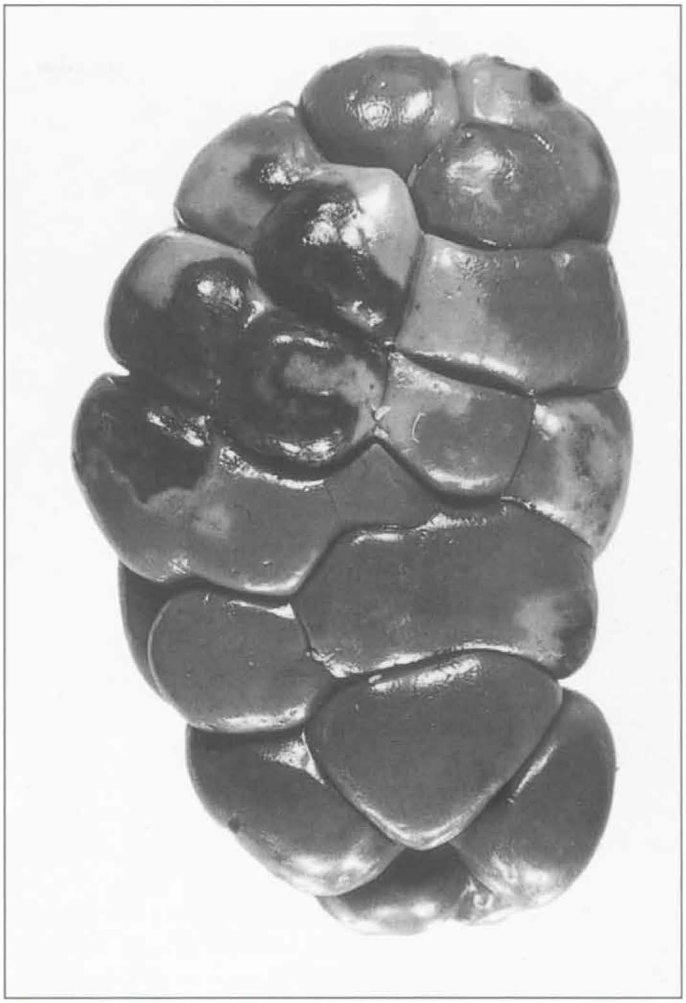

Figure 4.19 Renal cortical necrosis in a cow. Note recent hemorrhagic and older dehemoglobinized areas of infarction.

seen in capillaries, and hemorrhage may be present in glomeruli. The medulla is usually preserved.

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Renal medullary necrosis

Under certain circumstances, medullary necrosis is the primary manifestation of renal injury. As noted above, when renal hypotension occurs, cortical necrosis usually predominates due to redistribution of arterial flow to juxtamedullary nephrons. However, medullary vessels are damaged by greater than 2 hours of ischemia and there may hence be failure of both medullary and cortical reflow after temporary ischemia, and both medullary and cortical necrosis result. In the case of venous occlusion, the elevated intrarenal blood pressure maintains the patency of lower-resistance cortical vessels but not of

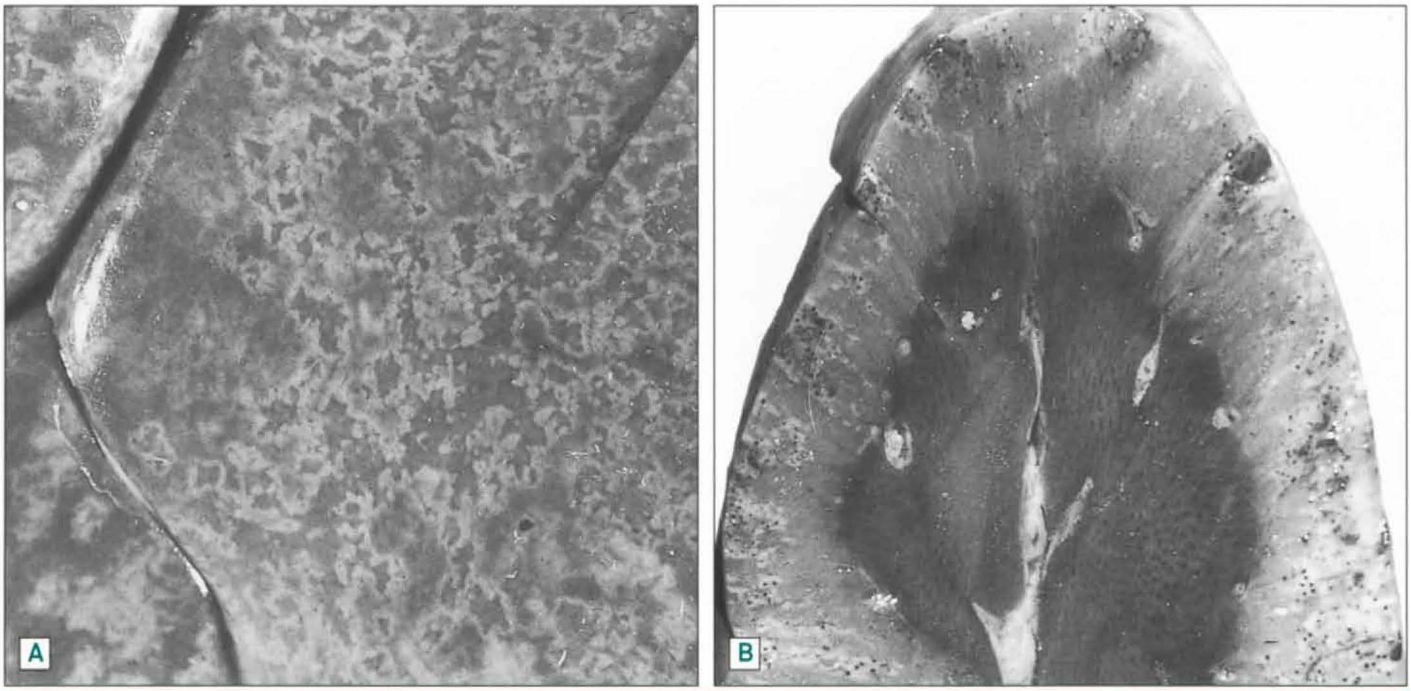

Figure 4.20 A. Capsular surface of kidney demonstrating **renal cortical necrosis** associated with metritis and mastitis in a cow. **B.** Cut surface of kidney shown in (**A**). Note predominance of lesions in the outer cortex.

higher-resistance medullary vessels, and hence medullary infarction predominates.

Toxic and hypoxic insults to the kidney are synergistic, and several protective mechanisms must be incapacitated to produce medullary injury. Prostaglandins are important autoregulators of renal perfusion. *Nonsteroidal anti-inflammatory drugs* (NSAIDs), such as aspirin, phenacetin, phenylbutazone, flunixin meglumine, and ibuprofen, inhibit cyclooxygenase, resulting in decreased production of PGE_2 and loss of its vasodilatory effect on arterioles of juxtamedullary nephrons. As well, NSAIDs may be directly toxic to renal medullary interstitial cells. Papillary necrosis is characteristic of "**analgesic nephropathy**" in humans, and also occurs in animals, especially seen as renal crest necrosis in dehydrated horses treated with phenylbutazone (Fig. 4.21). Chronic interstitial fibrosis is an associated lesion in affected humans; cortical atrophy follows obstruction of collecting ducts.

Dehydration is involved in the pathogenesis of papillary necrosis in racing Greyhounds. Papillary necrosis occurs in lambs and calves that are dehydrated when treated with phenothiazine, and the necrosis is again apparently due to *ischemia*. Accidental ingestion of a combination of monensin and roxarsone has caused renal medullary necrosis in normally hydrated dogs and pups. Diabetes mellitus is not the important pathogenetic factor in animals that it appears to be in humans.

Urinary obstruction and *pyelonephritis* are common causes of papillary necrosis in animals. Fulminating pyelonephritic infections may produce necrosis of the papilla with scant inflammatory reaction in the early stages. The long thin-walled vessels supplying the medulla are easily occluded by compression, which could be caused by edema of the papillary interstitium alone. Compression of vessels is probably the mechanism by which *amyloidosis* causes papillary necrosis in cats. Deposition of amyloid mainly in the renal medulla, with or without

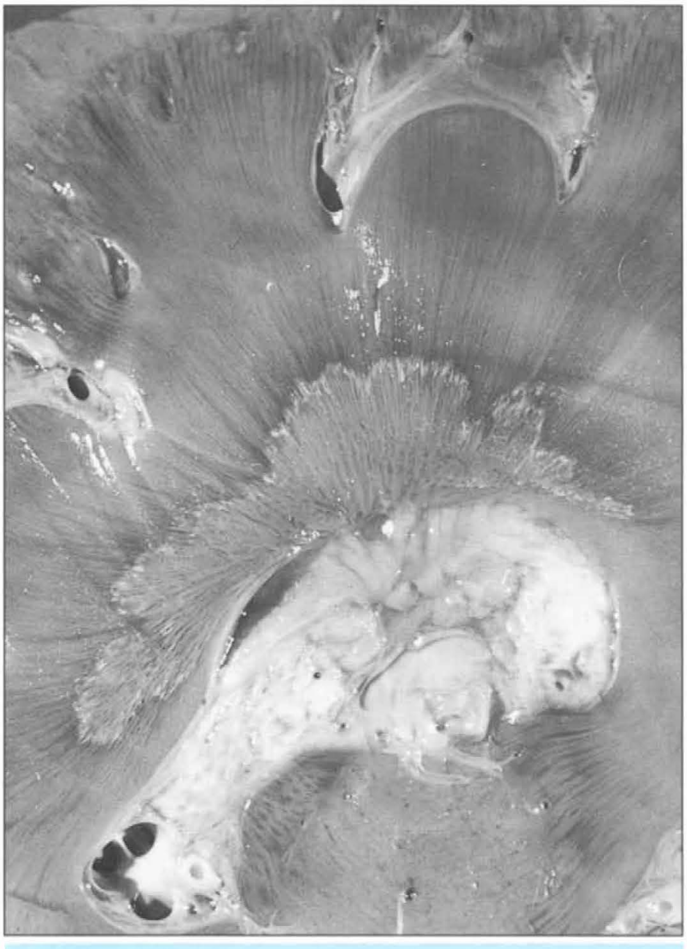

Figure 4.21 Renal papillary necrosis in a horse. Lesion developed following intestinal resection and treatment with nonsteroidal anti-inflammatory agents.

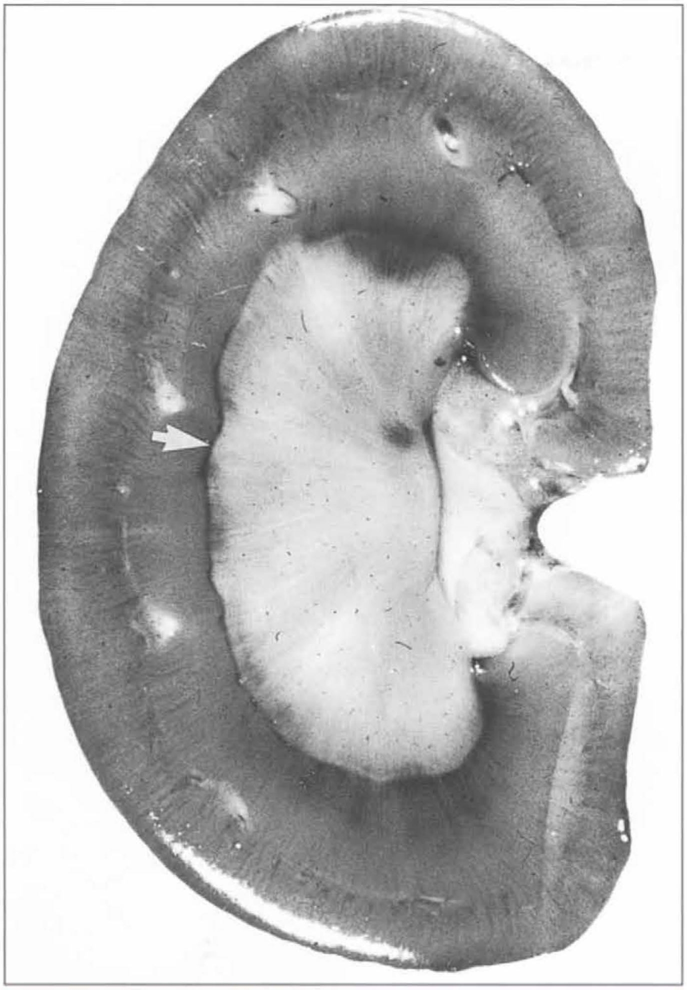

Figure 4.22 Renal medullary necrosis in a dog. The necrotic inner zone of medulla is outlined by a thin line of hemorrhage (arrow).

little concomitant glomerular deposition, occurs often in cats and occasionally in cattle (see section on Amyloidosis, below).

The *gross lesions* of medullary necrosis vary greatly in their extent and stage of development (Figs 4.21 and 4.22). Acute papillary infarction may be an incidental finding in an animal dead of other causes, such as dehydration and electrolyte imbalances in neonatal diarrhea. Massive medullary infarction would no doubt be part of acute renal failure leading to death. In an animal that survives an episode of medullary necrosis, medullary scarring occurs, the papilla may slough, and secondary cortical scarring is seen. The sloughed papilla may remain in the pelvis and may become mineralized. *Microscopic lesions* cover the usual range of necrosis and scarring. *Sequelae* to medullary necrosis are based on the loss of the ability to concentrate urine, and include chronic renal failure and uremia.

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Hydronephrosis

Hydronephrosis is dilation of the renal pelvis and calyces associated with progressive atrophy and cystic enlargement of the kidney. The cause is some form of *urinary obstruction*, which may be complete or incomplete, existing at any level from the urethra to the renal pelvis. The obstruction may be caused by anomalous development of the lower urinary passages or it may be acquired. *Acquired causes* include urinary calculi in any location, prostatic enlargement in the dog, cystitis especially if it is hemorrhagic, compression of the ureters by surrounding inflammatory or neoplastic tissue, displacement of the bladder in perineal hernias, and acquired urethral strictures. Depending upon the site of obstruction, hydronephrosis may be unilateral or bilateral, and there may be some degree of hydroureter and dilation of the bladder.

The pathogenesis of hydronephrosis is based on the persistence of glomerular filtration in the presence of urinary obstruction, plus the development of ischemic lesions. Even with sudden complete obstruction, glomerular filtration continues since filtrate diffuses into the renal interstitium and perirenal spaces, where it is drained by lymphatics and veins. However, continued filtration creates increased pressure throughout the nephrons, collecting ducts, calyces, and pelvis, and shearing forces develop between the compressible parenchyma and the resistant connective tissues of the trabeculae. Pressure atrophy and apoptosis of tubular epithelium occur, and hence tubular function and concentrating ability diminish. As well, blood vessels are compressed, particularly hilar veins and inner medullary vessels, leading to *papillary ischemia and necrosis*. An interstitial inflammatory response, triggered by activated tubular epithelial cells and leukocytes, releases a range of vasoactive factors, growth factors, and cytokines, leading to progressive interstitial fibrosis. Glomerular filtration progressively decreases due to intrarenal vasoconstriction, and nephrons atrophy and are replaced by scar tissue.

The degree of development of hydronephrosis depends on whether or not it is bilateral, the completeness of the obstruction, and on other complications of obstruction. The development of an extensive degree of hydronephrosis requires that it be unilateral (Fig. 4.23). Bilateral obstruction, which includes obstruction localized to the bladder or urethra, results in early death from uremia. Unilateral obstruction will produce the greatest degree of hydronephrosis, especially if the obstruction is incomplete or intermittent, because glomerular filtration will be little suppressed, and such kidneys may be massively enlarged. If an obstruction is removed within about 1 week, renal function returns. After about 3 weeks of complete obstruction, or several months of incomplete obstruction, irreversible renal damage occurs. If hydronephrosis is unilateral, the remaining

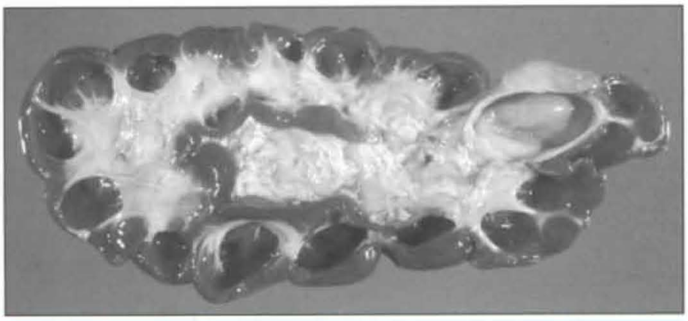

Figure 4.23 Hydronephrosis in a cow. Chronic lesion shows loss of medulla and cortical atrophy.

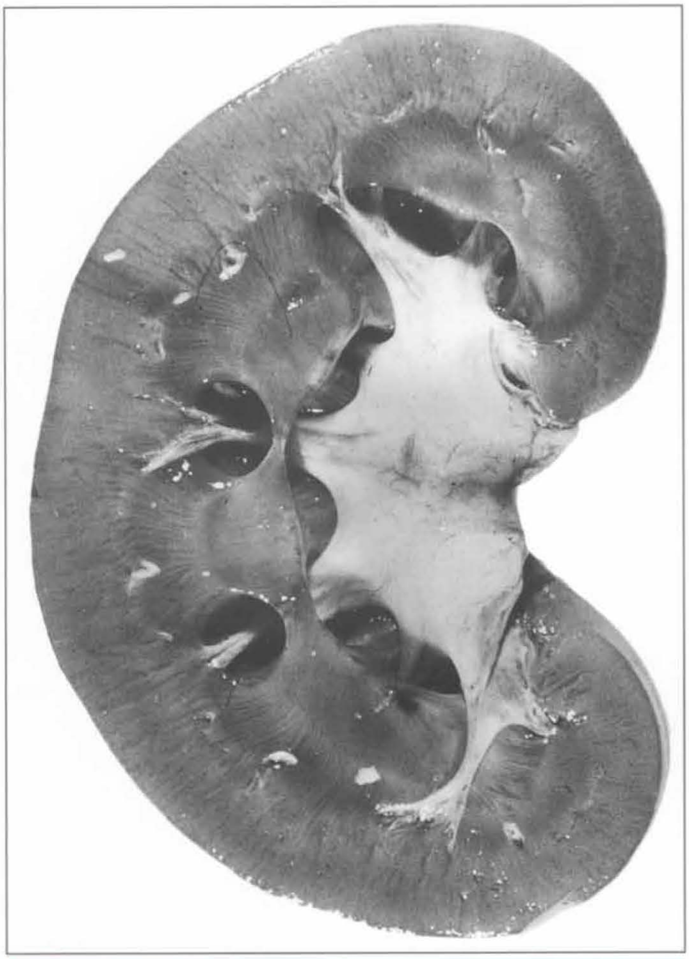

Figure 4.24 Early **hydronephrosis** in a sheep, showing swollen kidney and dilation of calyces.

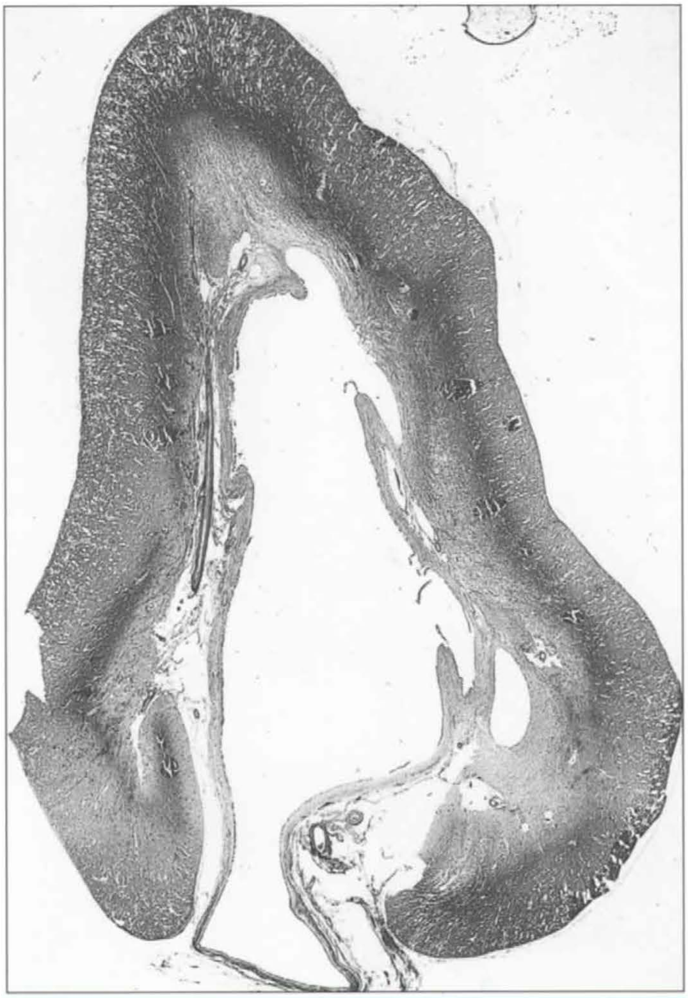

Figure 4.25 Early **hydronephrosis** in a pig, with dilated pelvis and cortical tubules. There is a discontinuous zone of congestion at the corticomedullary junction.

kidney, if normal, compensates adequately. Urinary stasis predisposes to infection, and hence pyelonephritis may be superimposed upon hydronephrosis or vice versa.

Early *gross changes* consist of progressive dilation of the pelvis and calyces with blunting of the apices of the pyramids (Figs 4.24 and 4.25). Eventually these may become excavated to form multilocular cysts communicating with the pelvis and separated by an intricate series of ridges that represent original septa (Fig. 4.23). In advanced cases, the kidney may be transformed into a thin-walled sac with only a thin shell of atrophic cortical parenchyma.

The *microscopic changes* begin with dilation of the proximal convoluted tubules and shortly there is also dilation of the distal and straight segments. The latter persists, with atrophy of the epithelium, but the dilation of the proximal tubules subsides; these portions then atrophy, become separated, and are replaced by light diffuse cortical fibrosis (Fig. 4.26). Much of the tubular atrophy may be through apoptosis. The glomeruli persist for a long time, flattened and spread apart. Various degrees of ischemia up to infarction may develop patchily in the cortex if the obstruction is sudden and complete; the infarcts are venous in origin. There is progressive destruction of the pyramids by liquefaction necrosis that spares the pelvic epithelium and tissue in a narrow zone immediately beneath. The necrotic tissue, to which there is no reaction, is liquefied and removed, and the pyramids are gradually destroyed.

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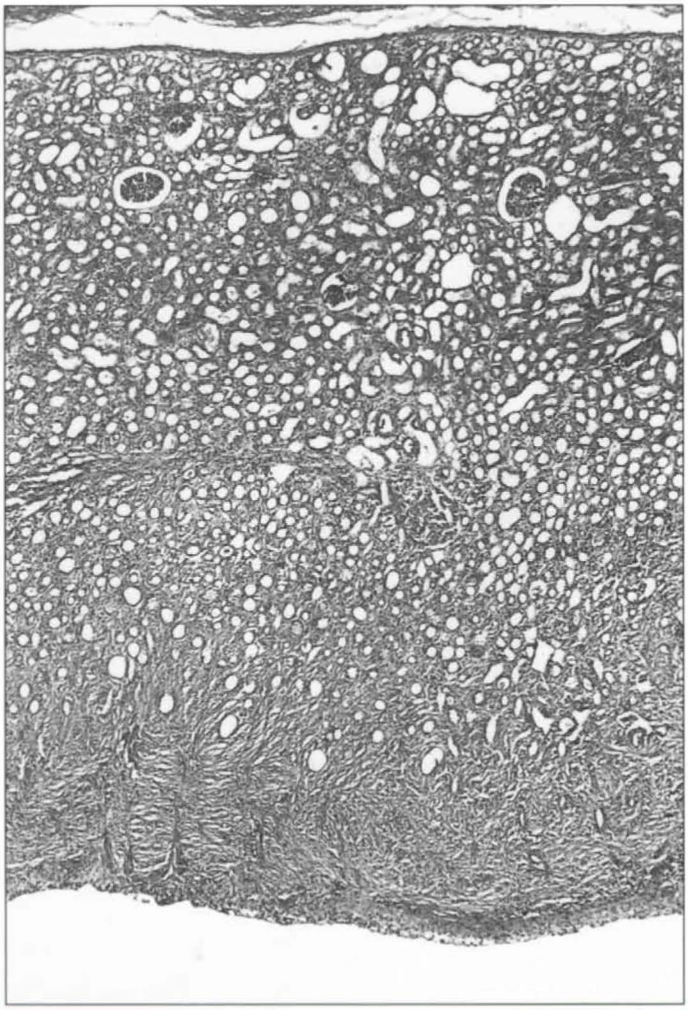

Figure 4.26 Section through cortex and medulla of kidney with **chronic hydronephrosis**. Note fibrosis of medulla (below) and dilation of tubules.

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GLOMERULAR DISEASE

Glomerular disease is of importance because, first, interference with glomerular blood flow, as well as decreasing the formation of ultrafiltrate, *impairs peritubular perfusion* and hence may cause loss of the entire nephron, and second, glomerular permeability may be altered, leading particularly to *proteinuria*.

- The terms *glomerulonephritis* and *glomerulonephropathy* are used interchangeably, and imply that secondary tubulointerstitial and vascular changes accompany the primary glomerular disease.
- The term *glomerulitis* is used when inflammation is restricted to glomeruli, as may occur in acute septicemias.
- *Glomerulopathy* refers to glomerular disease without cellular inflammatory components.

Glomerulonephritis, which is usually of immune origin, is a common form of renal disease in domestic animals, and is a common antecedent of end-stage kidneys and renal failure, particularly in dogs and cats.

Glomeruli may also be significantly affected in a variety of *systemic diseases*, such as **amyloidosis**, which is discussed below, systemic lupus erythematosus, diabetes mellitus, bacterial endocarditis, and in various forms of vasculitis.

The *clinical presentations* of any renal disease are of limited variety, and clinicopathologic findings in animals with glomerular disease usually have no specificity. Thus, hematuria, proteinuria, oliguria, hyposthenuria, and azotemia occur in glomerular and other renal diseases; *only proteinuria, occurring in the absence of urinary tract inflammation, is particularly indicative of glomerular damage*, namely increased glomerular permeability. Marked proteinuria, as occurs in chronic glomerulonephritis and in amyloidosis, can lead to development of the **nephrotic syndrome**, which is a clinical syndrome characterized by hypoalbuminemia, generalized edema, and hypercholesterolemia; edema is less common in dogs than in humans with the nephrotic syndrome. The edema is the result of decreased plasma colloid osmotic pressure, stimulation of the renin–angiotensin–aldosterone system, and release of antidiuretic hormone in response to hypovolemia. The hepatic response to hypoproteinemia is apparently a generalized increase in production of proteins, including lipoproteins, leading to hyperlipoproteinemia and hypercholesterolemia. Proteinuria due to glomerular disease may be *highly selective* or *poorly selective*, that is, albumin or albumin plus globulins respectively appear in the urine. In summary, *glomerulonephritis can be expressed clinically as acute or chronic renal failure, or as the nephrotic syndrome.*

The following terms are generally accepted for the description of glomerular disease, and are often appended to the histologic diagnosis:

generalized – involves all glomeruli to some extent
focal – involves only some glomeruli
diffuse (global) – involves the whole glomerulus
segmental (local) – involves only part of the glomerulus
mesangial – affects primarily the mesangial area

Classification of glomerulonephritis in humans is relatively advanced and complex, based on extensive clinicopathologic correlations, clinical outcomes and responses to therapy. The following simple classification system is currently in use for glomerulonephritis in domestic animals:

membranous – basement membrane thickening predominates
proliferative (mesangioproliferative) – cellular proliferation predominates (Fig. 4.27)
membranoproliferative (mesangiocapillary) – both changes are present (Fig. 4.28)
glomerulosclerosis – a combination of increased mesangial matrix and obliteration of capillary lumina; progressive hyalinization sometimes results in *glomerular obsolescence*, in which the glomerulus is a shrunken, eosinophilic, hypocellular mass (Fig. 4.29).

Lesion development may be at different stages in glomeruli of the same kidney, and the type of glomerular reaction may not be uniform among glomeruli.

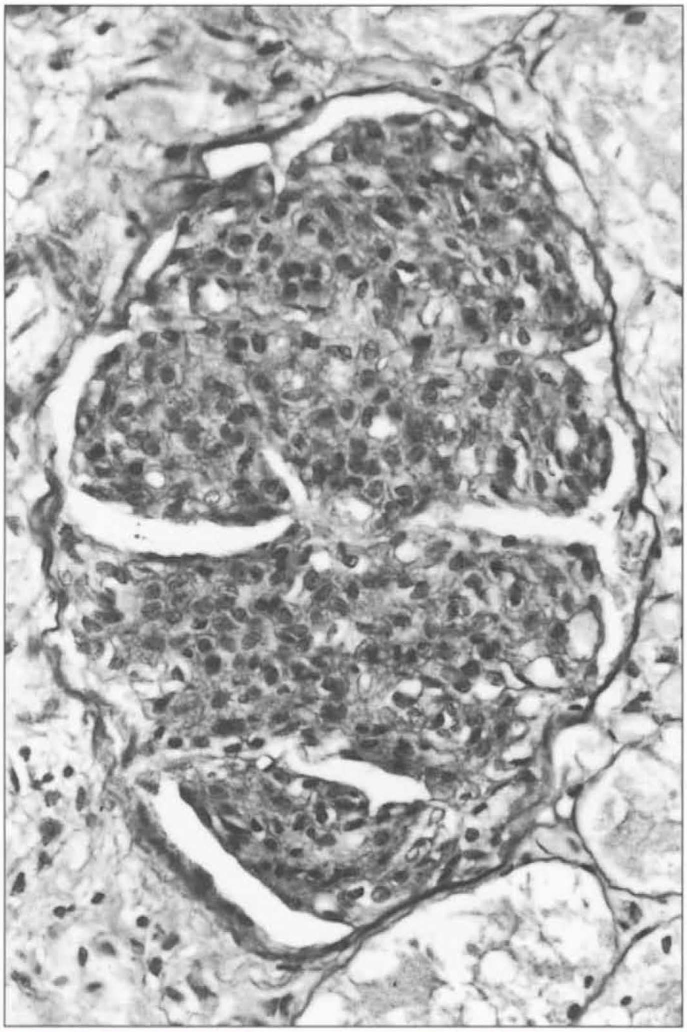

Figure 4.27 Proliferative glomerulonephritis in a 12-week-old Doberman Pinscher.

Histologic changes in glomerulonephritis

Glomeruli commonly exhibit a spectrum of histologic changes. In view of the large renal reserve, it is important to correlate clinical and histologic findings in order to determine the clinical importance of glomerular changes. For example, in sheep and goats, glomerulonephritis is often generalized and well developed histologically, but is usually of little functional significance. Conversely, histologic glomerular changes may be slight but clinical disease may be marked, as occurs in **minimal-change disease**, wherein a mild ultrastructural lesion (fusion of foot processes) is accompanied by marked proteinuria; this lesion has been produced experimentally in dogs by infection with *Ehrlichia canis*.

Glomeruli may be nonspecifically involved in renal reactions such as renal cortical necrosis, discussed above, and may be secondarily involved in tubulointerstitial diseases such as pyelonephritis. Septic emboli frequently lodge in the glomerular and peritubular capillary beds and cause focal glomerulitis and focal interstitial nephritis in diseases such as porcine erysipelas and in actinobacillosis of foals (Fig. 4.30A). Generalized glomerulitis, not necessarily associated with other renal change, is seen in acute septic disease and is characterized by increased glomerular cellularity involving either or both epithelial and endothelial cells. Inclusions are common in glomerular endothelium in infectious canine hepatitis (Fig. 4.30B).

Basic inflammatory reactions of exudation, necrosis, and thrombosis occur in glomeruli as elsewhere but some changes are typically glomerular.

Cellularity of the glomerular tuft may be increased by proliferation of endothelial, epithelial, or mesangial cells. This assessment is usually subjective. Proliferation of endothelial versus mesangial cells may be difficult to distinguish, and the term *endocapillary glomerulonephritis* is then applied. Mesangial cells in glomeruli of dogs usually occur singly or in pairs, hence at least three mesangial cells must be in close proximity before the term *mesangial hyperplasia* is applied. In response to fibrin exudation into the urinary space in severe glomerular damage, monocytes invade, parietal epithelial cells proliferate, and *glomerular crescents* are formed. Some of the cells, possibly monocytes, undergo metaplasia to fibroblasts and produce collagen. In humans, crescents are most commonly seen in cases of rapidly progressive extracapillary glomerulonephritis, but they are only indicative of severe glomerular damage, and not pathognomonic of any one disease.

Neutrophils and monocytes may accumulate within glomerular capillaries and infiltrate the glomeruli, and may accompany cellular proliferation in glomerulonephritis.

The **swelling of foot processes** and their subsequent retraction is a reversible reaction of the visceral epithelial cells, and apparently occurs in response to basement membrane damage and protein leakage. Podocytes do not replicate postnatally. Severe injury can cause loss of podocytes and exposure of "bare" GBM, which can adhere to parietal epithelial cells leading to fusion of part of the tuft with the interstitium and passage of filtrate inside the *adhesion* ("synechia") towards the interstitium.

Glomerular capillary walls may be thickened in routine hematoxylin and eosin-stained sections due to endothelial or epithelial swelling and/or **thickening of the GBM**. The GBM itself is not visible unless special stains, such as PAS or PAS-methenamine silver, are employed on thin (1–3 μm) sections. Electron microscopy is required to characterize properly the morphology of the thickening, which may be regular, as occurs in diabetic glomerulosclerosis, or irregular, as with deposition of electron-dense material in *subendothelial, intramembranous, or subepithelial* (epimembranous) locations. *These electron-dense deposits are usually immune complexes.* Thickened peripheral capillary walls are particularly prominent in cases of membranous glomerulonephritis, and are referred to as "*wire loops*;" subepithelial immune complexes (*humps*) may become separated by projections (*spikes*) of basement membrane, and eventually surrounded by and incorporated within the membrane (Fig. 4.31). This marked thickening of the GBM is somewhat paradoxically associated with marked proteinuria, probably as a result of changes in the charge and pore size of the GBM. In membranoproliferative glomerulonephritis, thickening of the GBM may be due to infiltration and splitting by mesangial processes and matrix, and is seen with silver stains as a characteristic *double-contoured glomerular basement membrane* or "*tram-tracks,*" a change that is also termed "*reduplication.*" According to the World Health Organization classification of human glomerular diseases, membranoproliferative glomerulonephritis can be subdivided into *type I* (subendothelial deposits), *type II* ("dense deposit disease," intramembranous deposits), and *type III* (both subendothelial and subepithelial deposits, with spike formation).

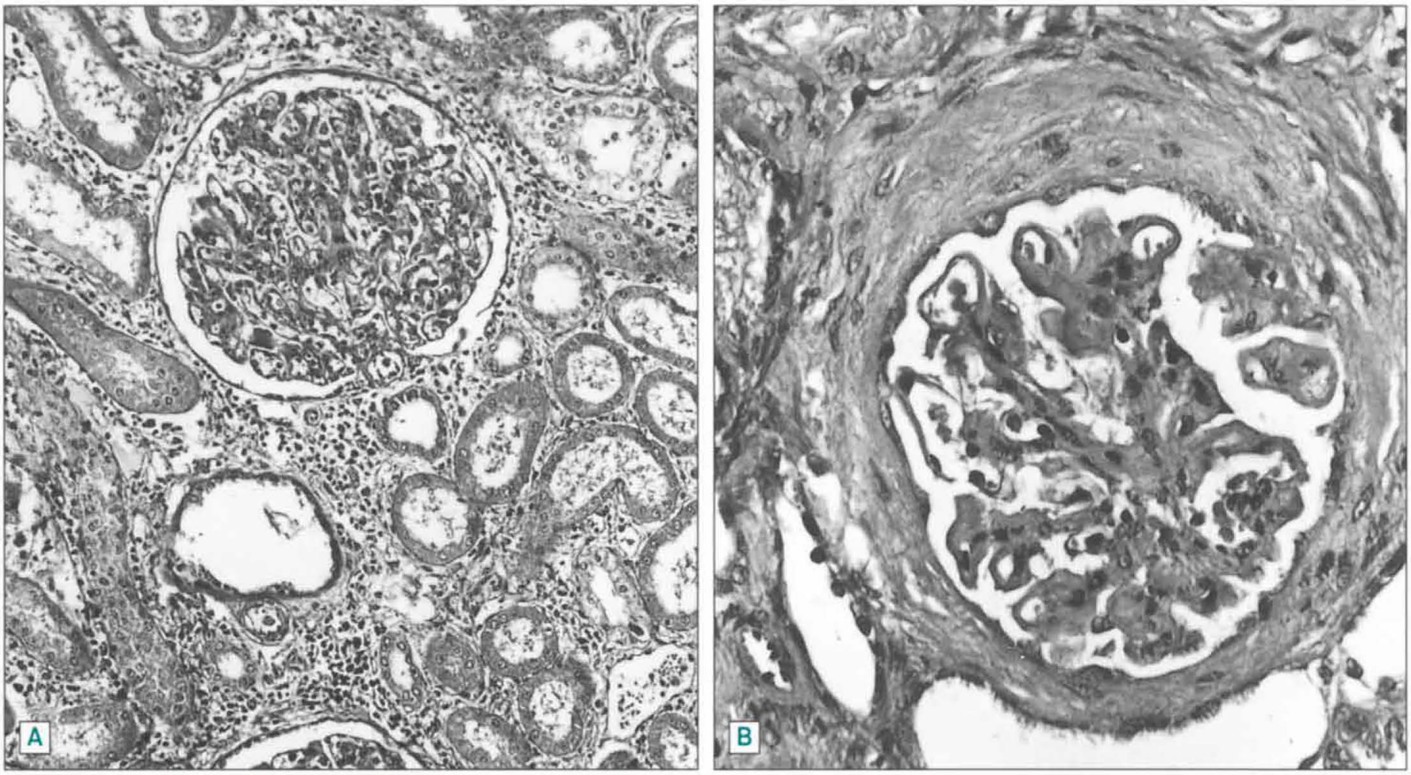

Figure 4.28 A. Membranoproliferative glomerulonephritis and interstitial nephritis in a horse with equine infectious anemia. **B. Membranoproliferative glomerulonephritis** in a dog. Note the marked thickening of Bowman's capsule.

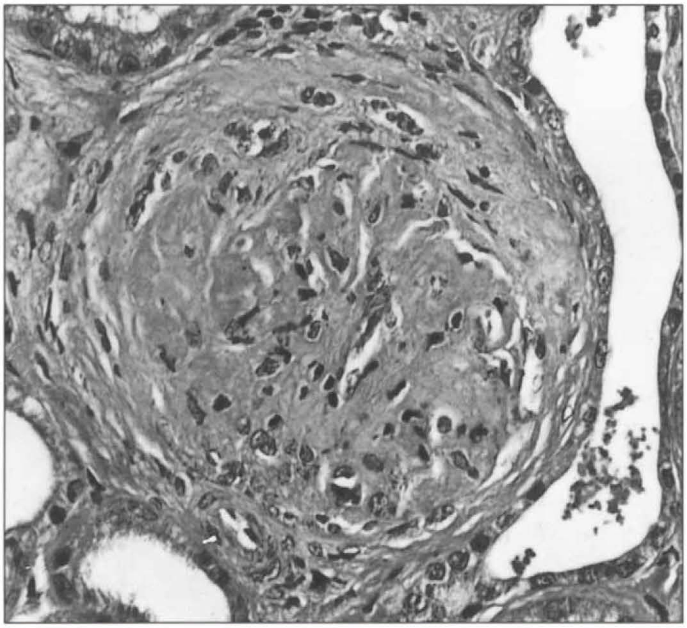

Figure 4.29 Obsolescent glomerulus in chronic glomerulonephritis in a dog.

As seen by light microscopy, **hyalinization**, or accumulation of homogeneous, eosinophilic, PAS-positive, basement membrane-like material, is common in the mesangial areas in glomerulonephritis, especially in chronic cases. This mesangial sclerosis may be reversible but, if irreversible and progressive, may lead to obsolescence of the glomerulus. Hyaline material is commonly deposited in the mesangium in *diabetes mellitus* and *amyloidosis*; in diabetes mellitus, diffuse (Fig. 4.32) or occasionally nodular hyaline deposits (Kimmelstiel–Wilson nodules) may be seen in glomeruli.

Renal glomerular fibrosis, characterized by the primary deposition of collagen type III in glomeruli, has been reported in dogs, pigs, and a cat (only collagen type IV is normally present in glomeruli). Proteinuria and renal failure result from mesangial sclerosis and capillary collapse. This variant of glomerulosclerosis resembles the human condition collagenofibrotic glomerulonephropathy.

Thickening of Bowman's capsule may occur due to various combinations of hyperplasia of parietal epithelial cells in crescents, invasion by monocytes, thickening of the basement membrane, and periglomerular fibrosis. These changes may be particularly marked in glomerular ischemia due to vascular occlusion.

Glomerular tuft atrophy may occur subsequent to scarring, which causes tubular constriction, inhibits or stops tubular fluid flow, and causes dilation of Bowman's capsule and secondary atrophy of the glomerular tuft (*"glomerulocystic" change*).

Nonglomerular histologic changes in glomerulonephritis include *tubular proteinuria*, which is prominent in membranous glomerulonephritis but is most marked in glomerular amyloidosis. Proteinuria can damage and activate tubular epithelial cells, causing release of proinflammatory cytokines and growth factors that induce *interstitial fibrosis*. Indeed, the amount of tubulointerstitial fibrosis often correlates closely with the degree of chronic renal dysfunction in glomerulopathies. Histologic changes may also be of *ischemic origin* due to decreased glomerular, and hence efferent arteriolar and peritubular, blood flow, and may result from acute and chronic interstitial *inflammation*. Hence, *tubular atrophy, interstitial fibrosis, and scarring occur in advanced lesions*, eventually producing the nonspecific histologic picture of *end-stage kidney*. Tubulointerstitial and glomerular damage may be coincident and due to the same mechanism rather than either one being primary.

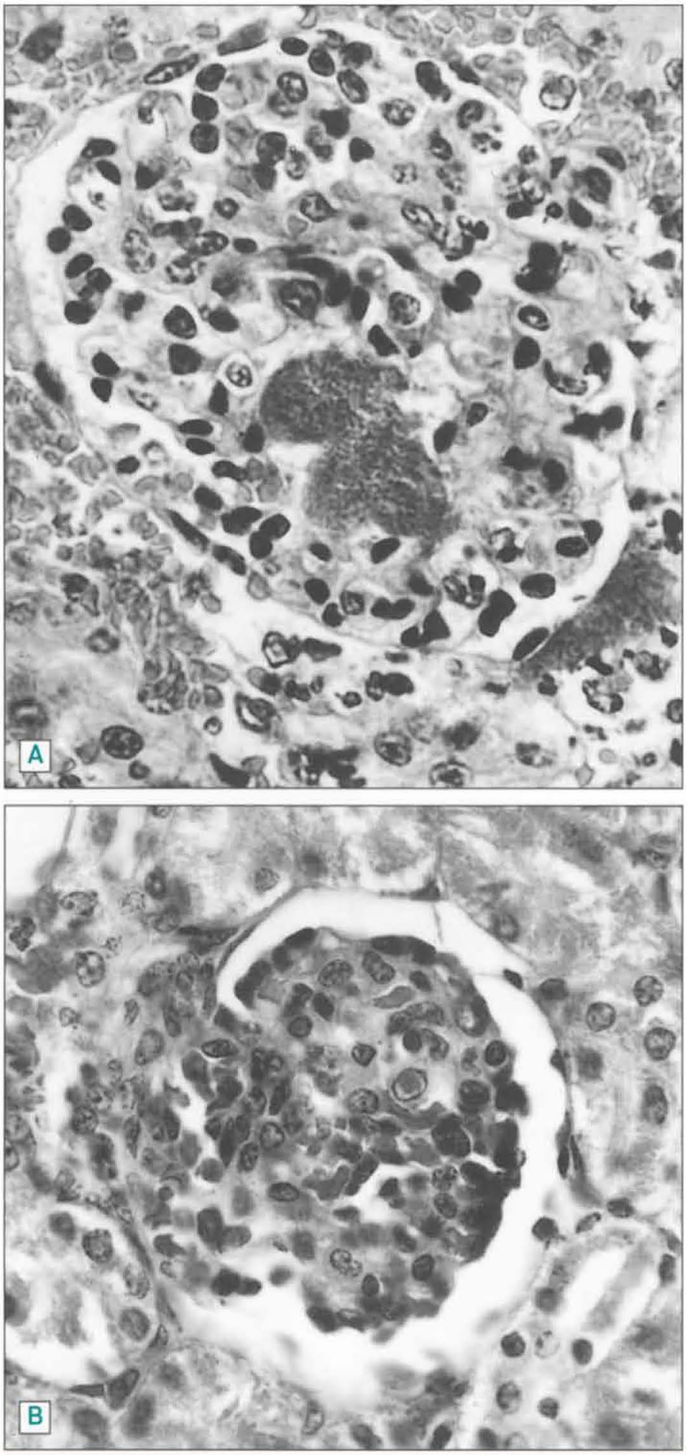

Figure 4.30 A. Embolic nephritis in a foal. Colonies of *Actinobacillus equuli* are present in glomerular and intertubular capillaries. **B. Intranuclear inclusion body** of *Canine adenovirus 1* (infectious canine hepatitis virus) in a glomerular endothelial cell.

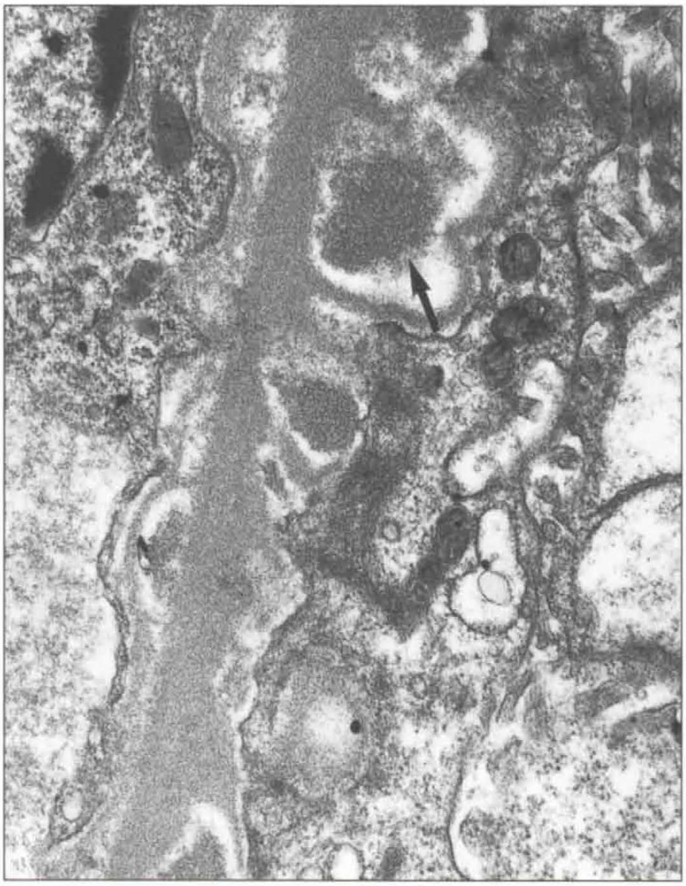

Figure 4.31 Membranous glomerulonephritis in a dog. Dense deposits are on the subepithelial side of the basement membrane: some are separated by spikes of membrane, others completely surrounded (arrow). (Courtesy of B Horney.)

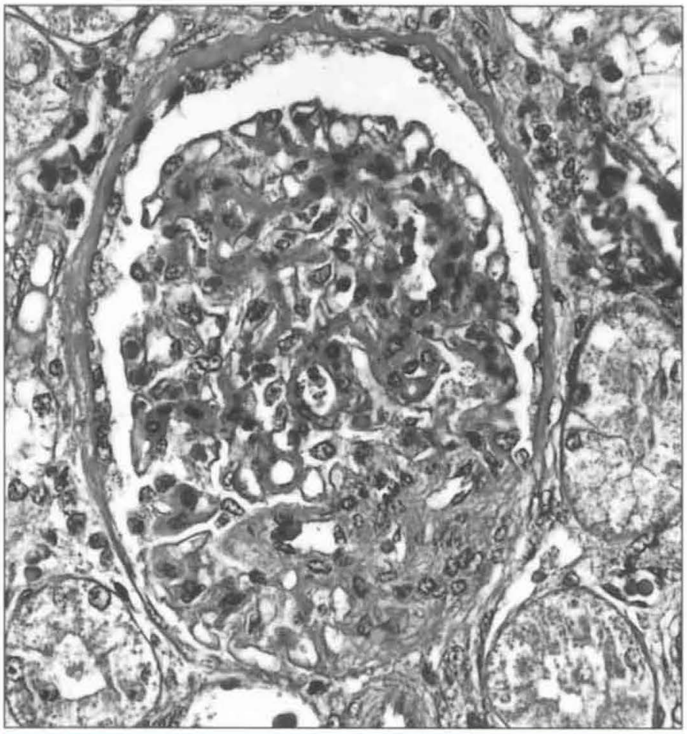

Figure 4.32 Glomerulosclerosis and **glycogen nephrosis** in diabetes mellitus in a dog.

Pathogenesis of generalized glomerulonephritis

Glomerulonephritis may result from the deposition in glomeruli of circulating immune complexes unrelated to glomerular components, from formation in situ of antibodies against GBM, or from activation of the alternative pathway of complement. Some types of glomerulonephritis are of unknown pathogenesis. By far the most commonly identified pathogenesis of glomerulonephritis in domestic animals is immune-complex deposition.

In **immune-complex glomerulonephritis**, circulating nonglomerular antigen–antibody complexes localize in glomeruli and are visible by immunofluorescence or electron microscopy as granules within, or on either side of, the GBM. Causative antigens may be exogenous, e.g., serum sickness, or endogenous, e.g., nucleic acid in systemic lupus erythematosus. Immune-complex deposition may cause *acute or chronic, membranous or proliferative lesions*. The classical example of immune-complex glomerulonephritis is acute, or "single-shot," serum sickness. When a large quantity of foreign protein is injected intravenously into an experimental animal, immune complexes occur in glomeruli in a *characteristic "lumpy-bumpy" pattern, as seen by immunofluorescence* (Fig. 4.33). By electron microscopy, the complexes are seen as irregular electron-dense deposits in a subendothelial or subepithelial location or within the mesangium. The immune complexes usually contain complement as well as antigen and antibody. In general, it has been believed that immune-complex deposition, and hence glomerular damage, occurs during the period of equivalence of antigen and antibody concentrations or during slight antigen excess. An alternative view is that antigens capable of penetrating the basement membrane localize, or are "planted," in a subepithelial position, and then bind antibody of low avidity. This antigen localization may be charge-dependent. As well as taking part in the usual immune-complex glomerulonephritis, dirofilarial antigens, for example, can apparently be deposited directly in basement membrane, inducing formation in situ of immune complexes, and producing linear fluorescence, which is usually a characteristic of anti-GBM disease (Fig. 4.34). Candidates for "planted" antigens that may localize in glomeruli and result in the formation in situ of immune complexes include viral, bacterial, and parasitic products, drugs, DNA, and large aggregated proteins, e.g., aggregated immunoglobulin G (IgG). The relative importance of circulating soluble immune complexes versus in situ formation of complexes in causing

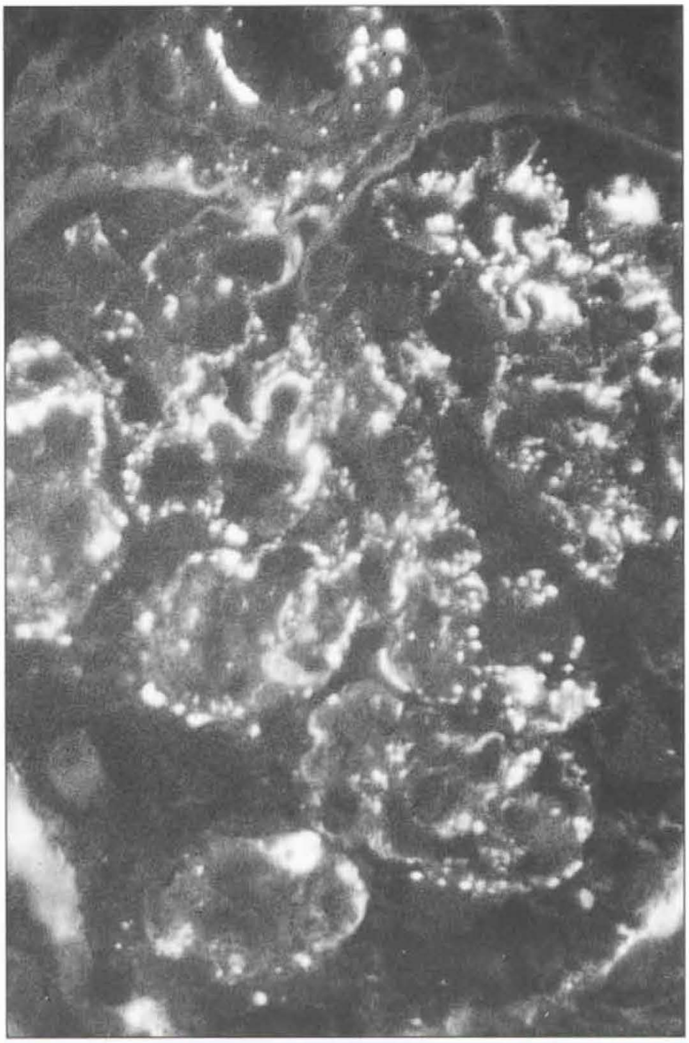

Figure 4.33 C3 granular ("lumpy-bumpy") immunofluorescence in postinfectious glomerulonephritis. (Courtesy of BN Wilkie.)

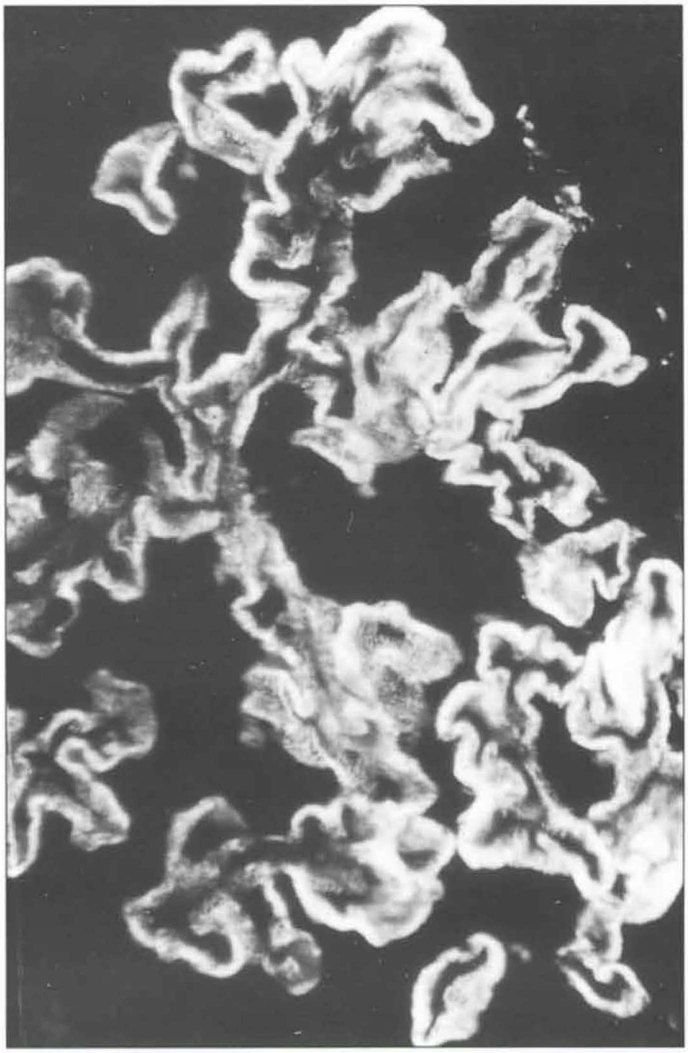

Figure 4.34 Immunoglobulin G linear immunofluorescence in membranous glomerulonephritis. (Courtesy of BN Wilkie.)

immune-complex glomerulonephritis is not resolved. Another modification of the classical soluble immune-complex disease model is that insoluble or poorly soluble complexes play a major role in subendothelial and mesangial deposit diseases.

Chronic serum sickness occurs when repeated small doses of foreign protein are given to an animal, and circulating immune complexes are continually present. This condition of continued antigenemia occurs during various microbial and parasitic infections, such as *Feline leukemia virus* infection and canine dirofilariasis, as well as during continued release of endogenous antigens, such as nucleoprotein in systemic lupus erythematosus or tumor-specific or tumor-associated antigens.

It is not completely straightforward to ascribe the cause of glomerulonephritis to immune complexes since the presence of immunoglobulins and complement in a lesion does not necessarily indicate that they are responsible for the lesion; C3, C1q, and IgM are sticky molecules that may adhere to previously injured tissue. As well, the significance of circulating soluble immune complexes is controversial, since they may be present in the absence of glomerulonephritis and, even if present, they may be more markers for the disease than actual causative agents, since *glomerular damage may be caused by various combinations of insoluble, soluble, and in situ-formed immune complexes.* To go one step further, some investigators dispute the primary role of immune complexes in the pathogenesis of glomerulonephritis and suggest that glomeruli that have suffered primary damage by other agents, such as hydrocarbons, are then susceptible to secondary immunologic damage.

The reasons for *localization of immune complexes* in various glomerular sites, namely subendothelial, intramembranous, subepithelial, or mesangial, are complex. Localization may be affected by the size, shape, charge, and chemical composition of the complexes. Thus, large complexes tend to localize in the mesangium and cause mesangiopathic glomerulonephritis, whereas small complexes (or antigens) penetrate the glomerular loop to produce the membranous or proliferative form. Antibody avidity may affect localization; complexes containing high-avidity antibody localize in the mesangium, while those with low-avidity antibody may localize subepithelially. Penetration of the basement membrane by complexes may be aided by products of inflammation, such as IgE-mediated release of histamine and serotonin from platelets and basophils, which is induced by antigen. The location of deposits may change with time; subepithelial deposits (humps) separated by spikes of GBM may become surrounded by GBM and hence become intramembranous deposits.

Modification of glomerular immune complexes occurs; they may be eliminated or may enlarge. Complexes may be eliminated by solubilization by excess antigen, phagocytosis by neutrophils, macrophages, or mesangial cells, passage through mesangial channels and egress at the vascular pole, excretion by mesangial cells through epithelial cells into the urinary space, degradation within the mesangial matrix, extracellular degradation by proteases, or by solubilization by complement. Thus, for example, removal of the source of persistent antigenemia in pyometra of dogs by ovariohysterectomy results in resolution of glomerulonephritis and cessation of proteinuria. Conversely, complexes may enlarge due to combination with various bloodborne reactants, such as small amounts of antigen, free antibody, immune complexes of the same or different specificity, complement components, or antibodies against immunoglobulins or complement components (immunoconglutinin or C3 nephritic factor).

In **anti-GBM glomerulonephritis**, *antibodies are formed against intrinsic GBM antigens*, resulting in a *linear pattern of immunofluorescence* reflecting the uniform distribution of immunoglobulins and complement along the GBM. There is a notable lack of dense or other deposits on electron microscopic examination. This lesion was the first recognized immune-mediated nephritis, and was induced in rats by injection of antirat-kidney antibodies obtained from rabbits or ducks immunized with rat kidney tissue; this experimental model is also referred to as *Masugi or nephrotoxic nephritis.* Although arbitrarily separated, *anti-GBM glomerulonephritis and immune-complex glomerulonephritis are not separate conditions but actually two poles of a spectrum of immune-mediated glomerular disease.* In fact, the typical linear immunofluorescence pattern of anti-GBM glomerulonephritis is gradually converted to the granular pattern typical of immune-complex glomerulonephritis with time, as linearly arrayed immune complexes become reorganized into dense aggregates in subepithelial and subendothelial locations.

Anti-GBM glomerulonephritis occurs as a component of Goodpasture's syndrome in humans, but occurs much less frequently than does immune-complex glomerulonephritis. In domestic animals, anti-GBM disease is apparently rare, having been reported in a horse and suspected, but not proved, in several dogs.

While the above descriptions of immune-complex glomerulonephritis and anti-GBM disease are conceptually useful, the mechanisms operative in clinical cases are often less well defined and more complex. Thus, in the well-recognized acute poststreptococcal disease in humans, factors implicated in glomerular injury include immune-complex deposition, damage by antibodies that cross-react with streptococci and GBM, activation of the alternative complement pathway, and cell-mediated reactivity to altered GBM.

Mechanisms of glomerular injury

Several mechanisms result in glomerular injury once immune complexes are formed or deposited in glomeruli in either immune-complex glomerulonephritis or in anti-GBM disease. The best-established mechanism is that of *complement fixation with resultant chemotaxis of neutrophils.* Complement components C3a, C5a, and C567 attract neutrophils, which, in the process of ingesting complexes, release lysosomal enzymes, arachidonic acid metabolites and oxygen-derived free radicals, and hence cause GBM damage. This is the *complement-leukocyte-dependent mechanism.* The terminal membrane attack complex of complement, C5b-9, can damage glomeruli independent of neutrophils through damage of epithelial, endothelial, and mesangial cells, with release of oxidants, cytokines, and other mediators. Complement fragments cause release of histamine from mast cells, and hence cause increased capillary permeability, which may be important in allowing deposition of further immune complexes in the capillary wall.

Chemokines (*chemo*tactic cyto*kines*) play an important role in transmigration of leukocytes into sites of inflammation. Produced by glomerular, mesangial, tubular, and interstitial cells, these locally active molecules are produced in response to simulation by agents such as proinflammatory cytokines, immune complexes, complement activation, and hypoxia, perhaps through the production of free radicals. Once chemokines reach the endothelial surface, they activate circulating leukocytes bearing the respective surface receptors. Local attachment of leukocytes occurs through interaction of leukocyte integrin with adhesion molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, on endothelial, mesangial, and interstitial cells. *Leukocytes migrate along the chemokine gradient to the site of injury.* Activated leukocytes will initiate their effector functions, such as respiratory burst, immune complex phagocytosis, release of hydrolases, removal of matrix, cell debris, and apoptotic cells. Activated leukocytes will also release additional mediators, chemokines, and cytokines, in an amplification loop that will recruit more leukocytes. A late influx of T cells can then be generated by production of T-cell-specific chemokines.

It is paradoxical that, while complement participates in glomerular injury, it also is capable of solubilizing immune complexes and accelerating their removal. Hence, hereditary hypocomplementemia, as occurs in Finnish Landrace lambs, may contribute to persistence of immune complexes and facilitate damage.

Interaction of complement fragments with platelets can initiate coagulation, thrombosis, and fibrinolysis; Hageman factor links the complement, coagulation, and kinin-forming systems. Fibrin and its degradation products are often present in glomeruli in glomerulonephritis, and fibrinogen that leaks into the urinary space is a stimulus to monocyte infiltration, proliferation of parietal epithelial cells, and crescent formation.

Monocytes play a role in glomerular damage also, especially through their interaction with mesangial cells. Monocytes may be beneficial in removing immune complexes, but may cause enzymatic damage, as do neutrophils. There is evidence that they transform to cells of fibroblastic type in glomerular crescents.

The central role of *mesangial cells* in production of the cellular lesions of glomerulonephritis is increasingly being recognized. Mesangial cells can be stimulated to produce inflammatory mediators, including oxygen free radicals, interleukin-1, arachidonic acid metabolites, and a variety of growth factors, and may initiate glomerulonephritis in the absence of inflammatory cells. Macrophages and other immune cells can also release a variety of mediators, such as interleukin-1, beta-endorphin, tumor necrosis factor, and platelet-derived growth factor (PDGF), which promote growth of mesangial cells and hence increased production of mesangial matrix. Proliferating mesangial cells release autocoids, such as interleukin-1 and PDGF, producing an amplifying loop of inflammation. The growth-promoting factors are antagonized by suppressive factors such as transforming growth factor-β. Immunomodulatory peptides released by proliferating mesangial cells stimulate replication and activation of macrophages. These activated macrophages amplify the inflammatory lesion and also phagocytize immune complexes.

Cell-mediated hypersensitivity reactions occur in some humans with progressive glomerulonephritis, and may help cause glomerular damage.

Cytotoxic antibodies directed against glomerular antigens may cause direct cell injury without formation of deposits. These antibodies may be directed against mesangial cell antigens, endothelial cell surface proteins, or visceral epithelial cell glycoproteins.

In addition to the immunologic causes of glomerulonephritis discussed above, a number of *nonimmunologic causes of glomerular injury* have been invoked. These include increased glomerular capillary pressure, coagulation in response to endothelial injury, serum lipid abnormalities, and glomerular hypertrophy.

Morphology of glomerulonephritis

Acute glomerulonephritis may not significantly alter the gross appearance of the kidney, which may be slightly or markedly enlarged, pale, soft, and edematous. The glomeruli may be visible as fine red dots. Petechial hemorrhages may be visible if bleeding has occurred from the inflamed glomeruli. In **subacute glomerulonephritis**, the kidney is enlarged, perhaps greatly, and pale with a smooth surface and nonadherent capsule (there may be numerous cortical petechiae indicating recurrent acute episodes). The capsule is tense and the cut surface bulges, and the cortex is wide and yellow-gray, which demarcates it from a normal-colored medulla. This subacute phase is anatomically and developmentally arbitrary and grades into the **chronic** phase in which the kidney is shrunken and contracted with generalized fine granularity of the capsular surface (Fig. 4.35). The capsule may be adherent. On cut surface, the cortex is rather uniformly narrowed and corticomedullary markings are obscured. Fine cysts may be present, developed from obstructed tubules. This stage when contraction is severe is grossly indistinguishable from chronic interstitial nephritis. It can usually be distinguished from the end-result of pyelonephritis, which tends to produce more irregular contraction and scarring, often with intervening areas of normal parenchyma.

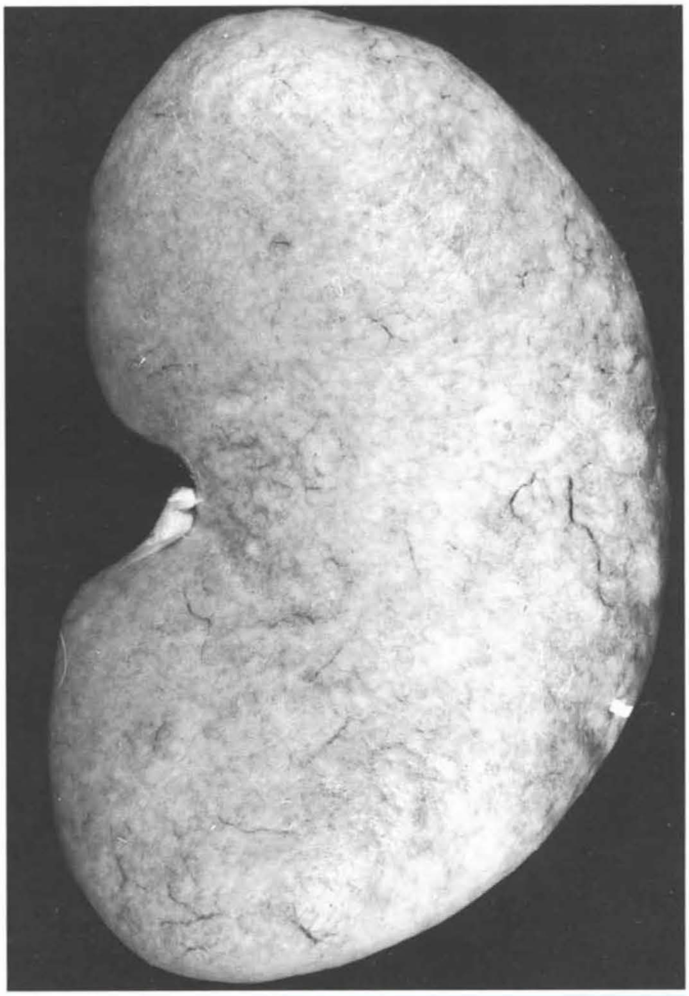

Figure 4.35 Pale granular kidney of **subacute-chronic glomerulonephritis** in a dog.

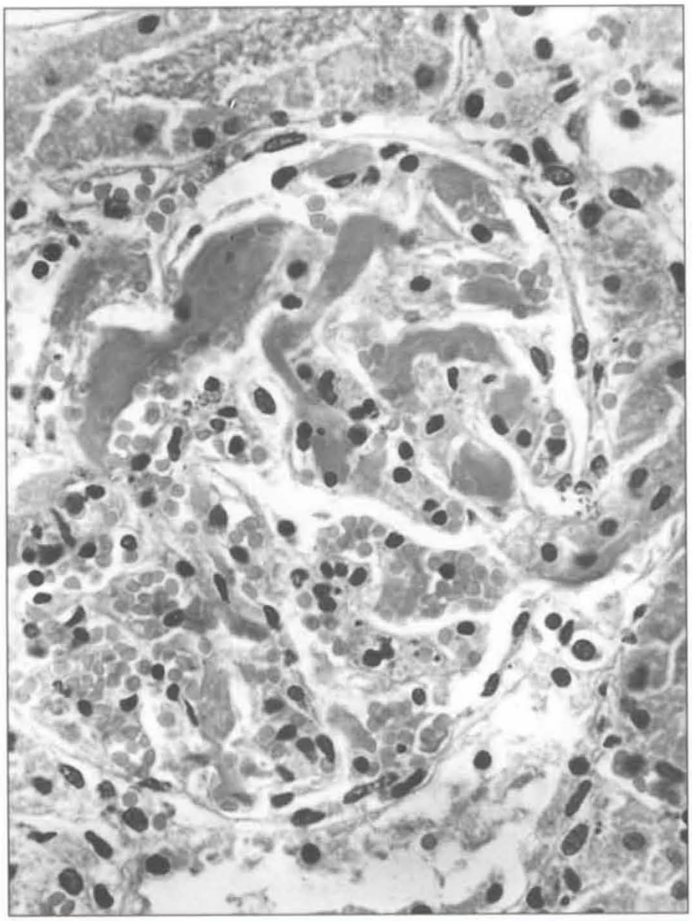

Figure 4.36 Fibrinoid thrombi in glomerular capillaries of a calf with colibacillosis.

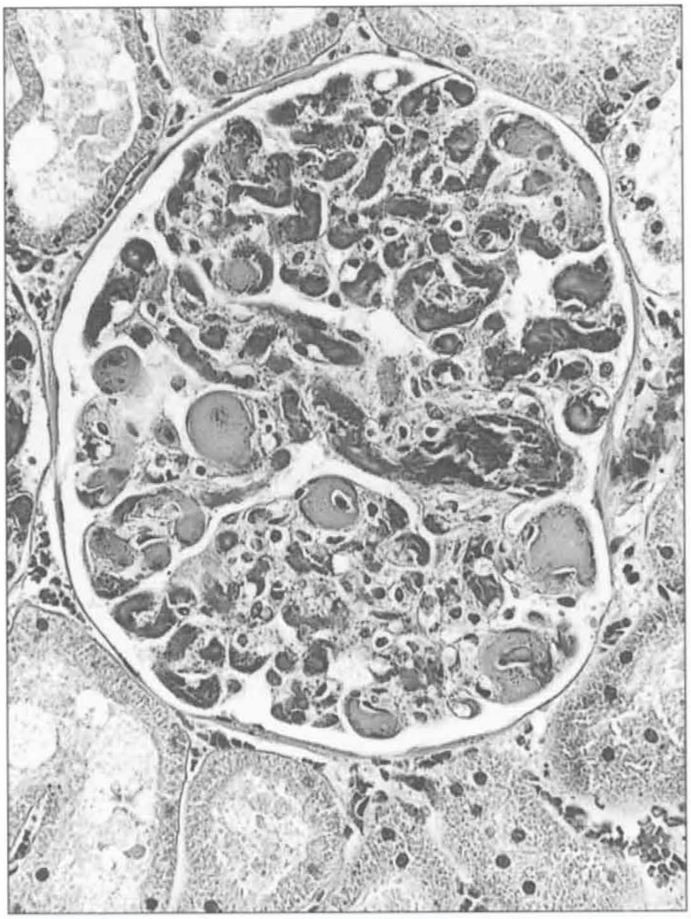

Figure 4.37 Pale-staining glomerular **hyaline thrombi** in an adult male fallow deer, as part of disseminated intravascular coagulation that occurred in epizootic hemorrhagic disease. (Courtesy of F Leighton.)

The **histologic features of the acute phase** are those of exudative inflammation. Initially hyperemic, the glomeruli soon become ischemic as a result of edematous thickening of the capillary walls and swelling of endothelial and epithelial cells. Neutrophils marginate in the capillaries and with the swollen and proliferated native cells of the glomerulus give a distinct impression of hypercellularity. The tuft swells and occupies most of the capsular space; any remaining space may contain migrated leukocytes, precipitated protein, or extravasated erythrocytes. Occasionally, fibrin thrombi form in the capillaries and cause focal necrosis and hemorrhage into the capsular space (Figs 4.36 and 4.37). This form of hemorrhagic glomerulonephritis with the formation of fibrin thrombi is the usual picture seen in swine with petechial hemorrhages as a gross manifestation. Concomitantly, interstitial edema develops. The tubular epithelium may contain hyaline droplets; casts of protein, erythrocytes, and leukocytes form in the urine.

Although functional changes vary greatly, some can be anticipated. The swelling and resultant ischemia of the glomeruli reduce filtration so that there is oliguria. The concentrating capacity of the tubules is unimpaired, as yet, so the urine is of high specific gravity. It also contains protein, and hyaline, granular and red-cell casts.

In the **subacute** phase, either mesangial hyperplasia or glomerular crescent formation may predominate. Fatty degeneration of the tubular epithelium may occur in this stage, as may hyaline droplet formation or necrosis. Casts of protein, leukocytes, and necrotic epithelial cells are present in the tubules.

In the **chronic** phase, fibrous scarring of glomeruli occurs. There may be a reduction in the apparent number of glomeruli as sclerotic glomeruli blend with surrounding scar tissue. If the original proliferative phase was mesangial, scarification may obliterate the tufts, transforming them to large or small masses of collagen and preserving the capsular space. If the proliferation was initially epithelial, fibrosis may completely obliterate the capsular space but, since the two forms usually coexist, obliteration of both glomerulus and capsule is usual. Although all glomeruli are usually involved, the degree varies somewhat so that many retain some function. The interstitial reaction initiated during the edematous exudative phase develops prominently with fibrosis and lymphocytic infiltration. Large numbers of tubules undergo disuse, ischemic or pressure atrophy, and are replaced by scar tissue, and the fibrosis becomes slowly self-perpetuating. Tubules that remain connected to functioning glomeruli may become dilated and develop epithelial hypertrophy and hyperplasia; these are in part responsible for the fine granularity of the surface and streakiness of the cut surface.

In chronic glomerulonephritis at the stage of decompensated renal failure, there is an increased volume of urine with a fixed, low specific gravity. Albuminuria may be only slight and casts may be absent. Death occurs in uremia. Acute glomerulonephritis does not inevitably lead to fatal chronic glomerulonephritis. In fact, healed mild glomerular lesions with full renal function are much more common than debilitating or fatal chronic generalized glomerulonephritis. The only evidence of previous injury may be mild mesangial sclerosis and the presence of occasional small adhesions between peripheral capillary loops and the parietal epithelium.

Once the glomerular filtration rate has decreased to 30–50% of normal, progression to end-stage renal failure tends to be inexorable. This sequence is thought to occur partly because of continuation of the primary glomerular condition, partly because of the addition of complicating factors such as hypertension, and perhaps partly because of adaptive changes in glomeruli in a failing kidney. These adaptive changes include hypertrophy and glomerular capillary hypertension, with resulting epithelial and endothelial injury and proteinuria. Mesangial cells respond by hyperplasia and by production of extracellular matrix; combined with intraglomerular coagulation; this response leads to glomerulosclerosis. A proposed common determinant of glomerulosclerosis is a sustained increase in glomerular permeability to macromolecules. In any case, these further reductions of renal mass contribute to a vicious cycle of continuing glomerulosclerosis.

Prevalence of glomerulonephritis

The frequency of diagnosis of glomerulonephritis in domestic animals has increased dramatically, mostly due to increased awareness and understanding of glomerulonephritis by clinicians and pathologists. There may also be a real increase in prevalence caused by poorly understood factors such as the increased use of modified live virus vaccines that may result in persistent antigenemia and predispose to immune-complex disease. Immune complexes commonly circulate throughout life, but few individuals develop significant lesions, so various factors such as genetic susceptibility or defective immune or other mechanisms may be operative in affected animals. Many associations of infectious and other diseases with glomerulonephritis have been identified (Table 4.1). In essence, *any infection of low pathogenicity that is able to produce persistent antigenemia has the potential to cause immune-complex disease.* Most of the animal glomerulonephritides characterized to date are of immune-complex origin and of the *membranoproliferative type.* The morphology of the glomerular lesion is of little assistance in identifying its cause however, since many agents cause the same type of lesion, and conversely one agent can produce a spectrum of glomerular changes. Most cases in animals are idiopathic, and hence may be referred to as *primary;* those occurring in association with other diseases or in which glomerular lesions contain known antigens are referred to as *secondary.*

Associations of particular types of glomerulonephritis with specific clinical presentations, clinical course, and outcome are rare in veterinary medicine but common in human medicine. An exception is *membranous glomerulonephritis, which is often associated with the nephrotic syndrome. Most other forms of glomerulonephritis are associated with chronic renal failure.*

The prevalence and importance of glomerulonephritis vary with species. In general, mild glomerular lesions (hypercellularity and occasional glomerular adhesions) are common, chronic lesions leading to renal failure are less common, and the acute disease is rare.

Table 4.1 Causes of immune-mediated glomerulonephritis in domestic animals

Viral	*African swine fever virus* *Aleutian mink disease virus* *Bovine viral diarrhea virus* *Canine adenovirus 1* (infectious canine hepatitis) *Classical swine fever virus* *Equine infectious anemia virus* *Feline immunodeficiency virus* *Feline infectious peritonitis virus* *Feline leukemia virus*
Bacterial	*Borrelia burgdorferi* Canine pyometra *Campylobacter fetus* Chronic pancreatitis Subacute valvular endocarditis
Protozoal	African trypanosomiasis Canine leishmaniasis Coccidiosis *Encephalitozoon cuniculi*
Helminths	*Dirofilaria immitis*
Neoplasms	Various
Autoimmune	Antiglomerular basement membrane disease Immune-mediated hemolytic anemia Polyarteritis Systemic lupus erythematosus
Hereditary	Canine familial renal disease Hypocomplementemia in Finnish Landrace lambs

Dogs

Glomerulonephritis is a common finding in dogs and is a leading cause of renal failure; it is usually membranoproliferative. In canine *pyometra,* tubulointerstitial lesions appear to be of more significance than glomerulonephritis, which may be present as an age-related change rather than as a product of pyometra. Focal segmental glomerulosclerosis is reported in a colony of Maltese Beagle dogs with *glucose-6-phosphatase deficiency* (glycogen storage disease Ia); hepatomegaly and renomegaly occur because of vacuolation of hepatocytes and proximal tubular epithelial cells respectively. A unique form of rapidly progressive and fatal renal disease putatively associated with infection with *Borrelia burgdorferi* is characterized by membranoproliferative glomerulonephritis, diffuse tubular necrosis with regeneration, interstitial nephritis, and has been noted in young adult Labrador and Golden Retriever dogs.

Some of the *familial glomerular diseases* of dogs have been well characterized and will be discussed now.

Samoyed hereditary glomerulopathy

Samoyed hereditary glomerulopathy occurs in both sexes but is much more common and severe, and has an earlier onset and more rapid course, in males than in females. Affected males develop proteinuria and wasting at 2–3 months of age, azotemia after 5 months, renal failure after 7 months, and die by 15 months of age. The disease is inherited as *an X-linked dominant trait*; severe disease occurs in about 50% of male offspring of carrier dams. A mutation in exon 35 of the *COL4A5* gene, the gene encoding the α5 chain of type IV collagen, results in a 90% reduction in the amount of α5 chain in GBMs, and probably leads to inadequate cross-linking of collagen type IV in the GBM. Normal glomerular development is arrested in affected dogs; there is failure of the switch from the α1/α2 to α3/α4/α5 network of type IV collagen, which is essential for normal glomerular structure and function. Glomerular lesions consist of *membranoproliferative glomerulonephropathy, progressing to glomerulosclerosis*; fibrin may be present in the urinary space. As well, there may be thickening of basement membranes of Bowman's capsules and tubules, periglomerular and interstitial fibrosis, and mild interstitial infiltration by mononuclear inflammatory cells. The pathognomonic transmission electron microscopic change, which precedes light microscopic changes, is *multilaminar splitting of the lamina densa of the GBM* (Fig. 4.38); electron-dense particles are occasionally present between the split layers. Scanning electron microscopy reveals effacement of foot processes and the presence of microvilli and globular projections on podocytes. Proteinuria is initially selective (primarily albumin) but becomes nonselective as the GBM lesion becomes more severe.

Samoyed hereditary glomerulopathy closely resembles *X-linked human hereditary nephritis* in its clinical, genetic, ultrastructural, and immunohistological features. The nerve deafness and ocular abnormalities that occur in *Alport syndrome*, a form of human hereditary nephritis, are not features of the disease in Samoyeds.

An X-linked dominant hereditary nephritis has also been characterized and reported in a family of *mixed-breed dogs*; labeling of α3-α6(IV) collagen chains was absent from GBMs in affected dogs.

Bull Terrier hereditary nephritis

A model for autosomal dominant human Alport syndrome, Bull Terrier hereditary nephritis is an *autosomal dominant* syndrome with either juvenile or adult onset seen with proteinuria and progressive azotemia. Kidneys have marked loss of nephrons, thickened glomerular and tubular basement membranes, thickened Bowman's capsules with glomerular adhesions, marked interstitial fibrosis at the corticomedullary junction, tubular dilation, and focal interstitial nephritis. Glomeruli become sclerotic and Bowman's spaces are commonly dilated. Ultrastructurally, GBM's are lamellated and often have intramembranous dense deposits. Foot processes are commonly effaced and mesangial matrix is expanded. Collagen chains α3(IV), α4(IV), and α5(IV) are expressed in the GBMs of affected dogs.

English Cocker Spaniel hereditary nephritis

A naturally occurring model for autosomal recessive human Alport syndrome, English Cocker Spaniel hereditary nephritis is a progressive *autosomal recessive* disorder of basement membrane (type IV) collagen. Affected dogs develop proteinuria and juvenile-onset chronic renal failure. Histologic lesions begin in a radial pattern that eventually becomes diffuse throughout the cortex. Lesions begin as segmental thickening of GBMs and mesangial expansion, and progress through segmental glomerular fibrosis to complete glomerulosclerosis. Periglomerular fibrosis and tubulointerstitial nephritis also develop. Ultrastructurally, typical GBM thickening and multilamellation occur. Type IV collagen chains are expressed in GBMs as follows: α1–α2(IV) are increased, α3(IV) and α4(IV) chains are absent, α5(IV) chains are markedly decreased, whereas α6(IV) chains are present.

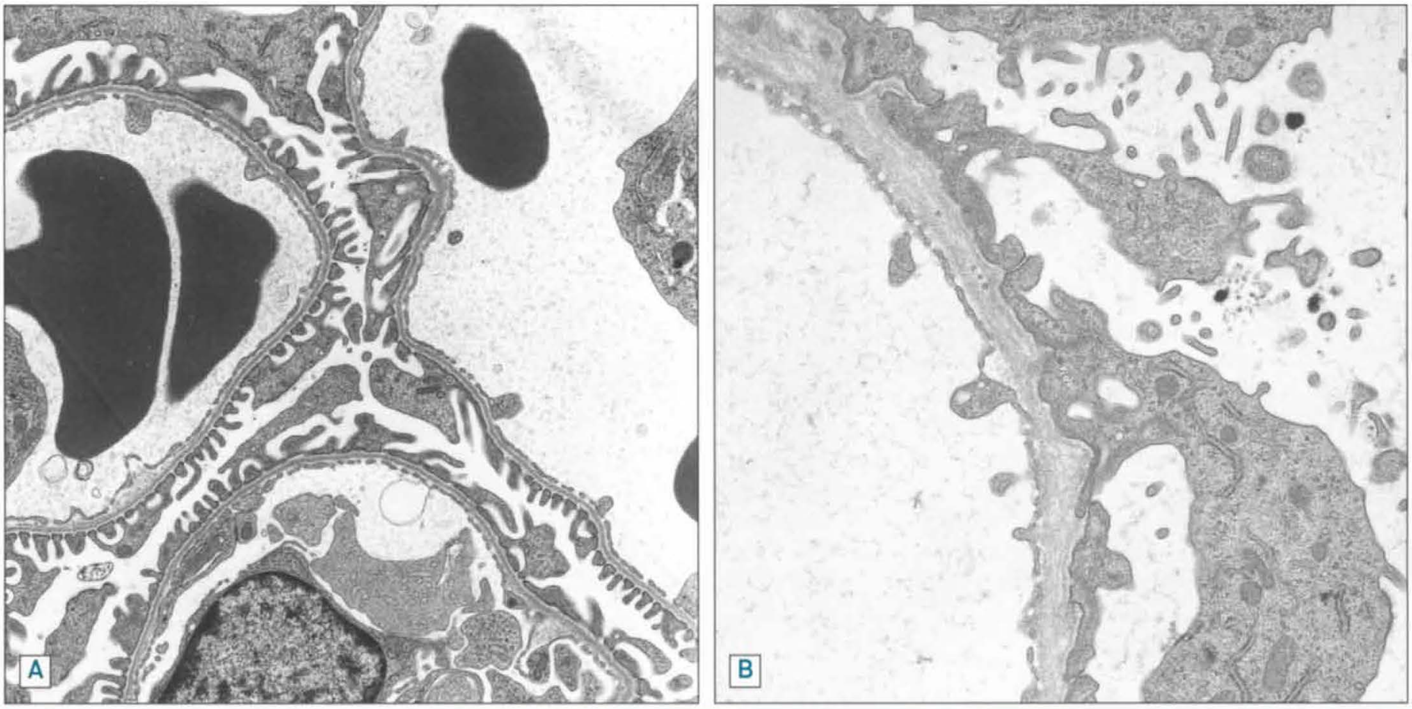

Figure 4.38 A. Normal glomerulus showing epithelial (podocyte) foot processes, fenestrated endothelium and trilaminar basement membrane. **B. Samoyed hereditary glomerulopathy**, 4-month-old male. Note multilaminar, irregular splitting of basement membrane. (Courtesy of B Horney.)

Familial glomerulonephropathy in Bernese Mountain Dogs

This condition is inherited as an *autosomal recessive*, and is expressed as proteinuria and renal insufficiency in young adults. It is characterized as a membranoproliferative glomerulonephritis with concomitant tubulointerstitial nephritis; GBMs are reduplicated, there is mesangial interposition in GBMs, and immune-complex-type subendothelial deposits are detectable. IgM and complement component C3 are deposited in glomeruli.

Familial glomerulonephropathies in other dog breeds

Familial glomerulonephropathies have been reported in other dog breeds but are less well characterized. There is a familial tendency in **Doberman Pinschers** to develop proteinuria and renal failure; the precise mode of inheritance is unknown. Grossly, the kidneys are light brown, slightly small, and have diffuse, fine, subcapsular pits that appear as radial streaks on cut surface. There may be fine white stippling of the subcapsular surface due to the protein-filled tubules and lipid in epithelial cells in the subacute stage of the disease (Fig. 4.39). The histologic renal lesion is membranoproliferative glomerulonephritis with various degrees of tubulointerstitial disease; the glomerular lesion begins as focal segmental mesangial thickening and accentuation of glomerular lobulation (Fig. 4.27). Ultrastructurally, GBMs display two distinct lesions: the lamina densa may be lamellated and contain intramembranous electron-dense deposits, or there may be diffuse attenuation of the lamina densa with thickening of the GBM by collagen fibers. These ultrastructural lesions resemble those in Samoyeds and humans with hereditary nephropathy.

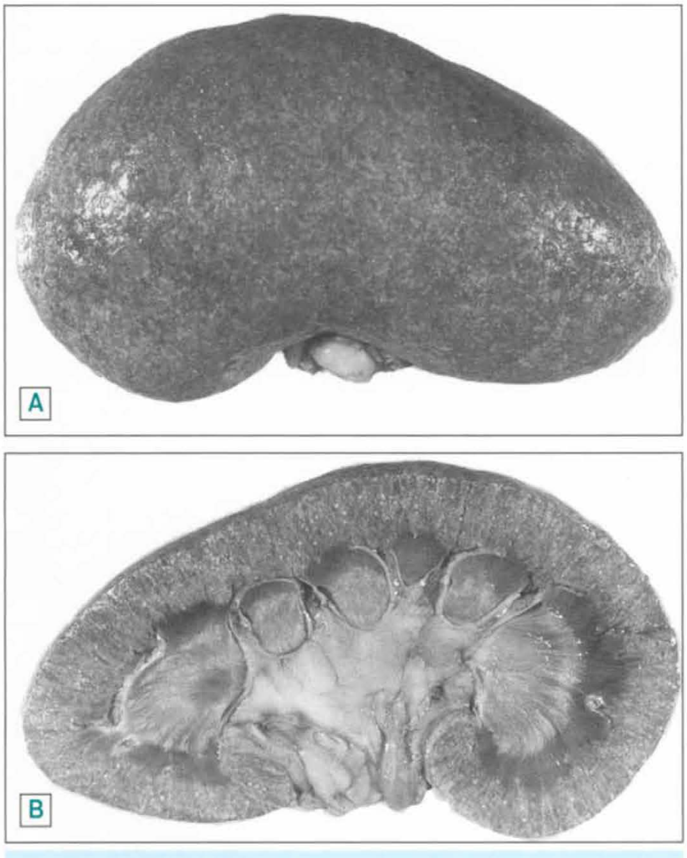

Figure 4.39 Familial renal disease in a **Doberman Pinscher**. **A.** White stippling due to protein-filled tubules and lipid in tubular epithelium. **B.** Cut surface of kidney shown in (**A**).

Familial glomerulonephropathy is reported in a litter of **Beagles**; the multilamellated GBM lesion resembles that seen in Samoyeds and Cocker Spaniels.

Inherited deficiency of the third component of complement in **Brittany Spaniel** dogs leads to the development of membranoproliferative glomerulonephritis.

Glomerulosclerosis in juvenile **Newfoundland** dogs appears to be familial, but of unknown inheritance; proteinuria and renal failure ensue. *Glomerulofibrosis* results from subendothelial and mesangial deposition of collagen fibrils and an increase of mesangial matrix.

The protein-losing nephropathy in **Soft-Coated Wheaten Terriers** is familial but of unknown, but possibly autosomal recessive, inheritance. Affected dogs have chronic glomerulonephritis/glomerulosclerosis and may have features of renal dysplasia.

Familial renal diseases of uncertain inheritance occur in **Lhasa Apsos** and **Shih Tzus, Standard Poodles, Shar-Peis, Old English Sheepdogs, Miniature Schnauzers, Rottweilers**, and **Chow Chows**. They share nonspecific lesions in their end-stage kidneys – radial scarring of cortex and medulla, cystic glomerular atrophy, presence of immature glomeruli, and loss of nephrons. Glomerular lesions may be secondary to primary interstitial lesions.

Familial renal disease in **Norwegian Elkhound** dogs is seen as rapidly progressive renal failure in juvenile dogs; the mode of inheritance is unknown. Histologic changes begin as *periglomerular fibrosis* with hypertrophy and hyperplasia of parietal epithelium and progress to more or less diffuse fibrosis of cortex and medulla and progressive loss of glomeruli. In contrast to the hereditary glomerulopathies discussed above, the lesion in Norwegian Elkhounds involves glomeruli, but is *primarily a noninflammatory tubulointerstitial nephropathy*.

Cats

Glomerulonephritis is common in cats, is predominantly *membranous*, and is a major cause of the nephrotic syndrome and/or renal failure. Type III membranoproliferative glomerulonephritis has been reported in a cat.

Horses

Glomerulonephritis is fairly common in horses, but renal failure is rare. Membranoproliferative glomerulonephritis often occurs in horses with equine infectious anemia (Fig. 4.28A), and the renal lesions may be important. *Streptococcus equi* and herpesviral infections are suggested as causes of glomerulonephritis in horses.

Swine

Acute fatal glomerulonephritis occurs sporadically in swine, but is of little economic importance. Deposition of immune complexes containing IgG and C3 is common in the mesangium of normal slaughter swine, but the mesangioproliferative glomerulonephritis is not of clinical significance. The *porcine dermatitis and nephropathy syndrome* (PDNS) in feeder pigs is associated with *Porcine circovirus-2* (PCV-2) infection and/or the antibody response to PCV-2. The systemic necrotizing vasculitis in PDNS appears to be immune-mediated, and is accompanied in the kidney by exudative glomerulonephritis and interstitial nephritis. *Hyaline droplets*, of unknown pathogenesis and significance, form in the glomerular mesangium of some pigs. An autosomal recessive *hereditary deficiency of factor H*, a complement inhibitory protein, caused lethal membranoproliferative glomerulonephritis type II (dense deposit disease) in Norwegian Yorkshire pigs; factor H deficiency led to massive glomerular deposition of complement, intramembranous dense deposits, and mesangial hypercellularity.

Ruminants

Immunologic evidence of glomerulonephritis is common in ruminants, but clinical disease is not. Glomeruli of sheep and goats are often hypercellular and have membranous changes, but the changes appear to have little clinical significance. An interesting exception is the *membranoproliferative glomerulonephritis of* **Finnish Landrace sheep**, which is present at birth, and is characterized by recessive inheritance of a deficiency of the complement component C3; in affected lambs, blood levels of C3 are about 5% of normal. This congenital deficiency contributes to the development of membranoproliferative (mesangiocapillary) glomerulonephritis, probably due to impaired complement-mediated solubilization of immune complexes in glomeruli. Affected lambs are clinically normal at birth, but die at 1–3 months of age due to renal failure. At autopsy, the kidneys are enlarged, and have pale cortices and glomeruli that are grossly visible as red spots. The glomerular lesion is characterized by mesangial proliferation, capillary wall thickening, and often by the formation of glomerular crescents. Subendothelial electron-dense deposits are present and consist of C3, smaller amounts of IgM and IgA and, with prolonged survival, progressively larger amounts of IgG; this pattern resembles type I membranoproliferative glomerulonephritis of humans. Glomerular changes begin in the lambs in utero and develop progressively after birth. Choroid plexus lesions also occur due to immune-complex deposition, and lead to encephalopathy.

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Amyloidosis

Amyloidosis is a group of disorders in which *amyloid, an eosinophilic, homogeneous, hyaline, proteinaceous material,* is deposited in the walls of small blood vessels and extracellularly in a variety of sites, particularly in renal glomeruli. Because all amyloid fibrils have a β-pleated sheet structure, the term *β-fibrilloses* is an alternative descriptor of amyloid deposits and amyloidosis. Deposition of amyloid causes pressure atrophy of adjacent cells, and, depending on the organs involved, can lead to chronic renal failure, nephrotic syndrome, thrombosis, hepatic failure, spontaneous hepatic hemorrhage and rupture, arthritis, or diabetes mellitus. Amyloidosis is seen in a number of presentations:

- **Reactive systemic amyloidosis** (secondary, or AA) is the most common form of amyloidosis in domestic animals (dogs, cattle, horses, cats; rare in swine and goats). **AA amyloid** is derived from *serum protein AA* (apoSAA; serum amyloid-associated), an acute-phase reactant apoprotein product of hepatocytes predominantly. ApoSAA is produced in excess as a result of chronic antigenic stimulation, such as occurs in persistent infectious, inflammatory or neoplastic conditions.
- **Immunoglobulin-derived amyloidosis** (primary, or AL) is the most common form in humans, but it is uncommon in domestic animals (dog, horse, cat). **AL amyloid** is produced from *immunoglobulin light chains* in plasma cell dyscrasias as a product of monoclonal B-cell proliferation; either λ or κ light chains may predominate in this dysproteinemia.
- **Familial** amyloidosis occurs in a number of species and breeds. Systemic AA amyloidosis occurs in Beagles, Shar-Peis (likely autosomal recessive), gray Collies, English Foxhounds, Abyssinian cats (likely autosomal dominant with incomplete penetrance), and Siamese and Oriental cats. Systemic κAL amyloidosis has been described in a Holstein cow with bovine leukocyte adhesion deficiency.
- **Apolipoprotein AI** (apoAI)-derived amyloidosis affects the pulmonary vessels of old dogs.
- **Islet amyloid polypeptide** (IAPP)-derived amyloidosis is common in the pancreatic islets of cats with non-insulin-dependent diabetes mellitus.

Both AA and AL amyloidosis can occur in either systemic or localized forms. The *cause* of amyloid fibril formation and deposition is obscure, but the *nidus theory* postulates that amyloid fibrils serve as templates for fibril growth and as scaffolding for fibril polymerization; amyloid-enhancing factor may be involved. Macrophages appear to be important in the conversion of precursor proteins to amyloid fibrils. Always associated with amyloid are extracellular matrix components, including amyloid P component (a glycoprotein of the pentraxin family), glycosaminoglycans, and proteoglycans.

Most cases of amyloidosis in domestic animals are of the reactive systemic type. Deposits of AA amyloid may be found in many organs and may be concentrated in one or other of them, such as the liver or spleen, but *the kidney is the organ most commonly involved in amyloidosis*. Localization of amyloid is usually *glomerular* in most breeds of dogs, but *medullary* localization predominates often in Shar-Pei dogs, in cats, and occasionally in cattle. Systemic amyloidosis involving the liver can lead to fatal spontaneous liver hemorrhage.

Amyloidosis is most common in older **dogs** and is usually idiopathic, although some cases do occur in association with chronic suppurative and granulomatous lesions in other tissues. Glomerulopathy and amyloidosis occur in dogs with *Hepatozoon americanum* infection in the southern USA, and in dogs with *Ehrlichia canis* infections. Dogs with glomerular amyloidosis develop progressive renal insufficiency and proteinuria that may be sufficiently severe to cause the *nephrotic syndrome*. Dogs with medullary amyloidosis without glomerular involvement, e.g., the Shar-Pei, may have little or no proteinuria. Amyloidosis is less common in **cats** than dogs and is primarily medullary; hence marked proteinuria is an uncommon finding. In **cattle**, glomerular amyloidosis can cause severe proteinuria; medullary amyloidosis is reported as a common subclinical disease. A chronic suppurative or tissue-destructive process is occasionally demonstrable in affected cattle. Amyloidosis occurs systemically (AA amyloid) in **horses** used for antiserum production, as well as in localized nodules (AL amyloid) in the skin and the upper respiratory system. **Sheep and goats** can develop glomerular and medullary amyloidosis in the course of chronic inflammatory processes. **Pigs** rarely develop amyloidosis.

With small deposits of amyloid, the **gross** appearance of the kidney may be normal except for a slight increase in size and a peculiar translucence of the glomeruli. The characteristic renal change is one of paleness, enlargement, and increase in consistency. In cattle, the kidneys may be very large. The capsule strips smoothly to reveal a cortical surface with a finely stippled appearance due to numerous fine yellow spots that are glomeruli, and gray points of translucence that are dilated tubules. On cut surface, the cortex is widened and presents the same appearance as the capsular surface, rather like pumice stone. *Affected glomeruli stain brown-red when exposed to an iodine solution; subsequent exposure to dilute sulfuric acid changes the color to purple* (Fig. 4.40).

Histologically, amyloid is first deposited in the mesangial area and in the subendothelium of glomerular capillaries. Nodules of amyloid gradually develop until the glomeruli, when uniformly involved, are enlarged and converted to homogeneous spheres with loss of endothelial and epithelial nuclei (Fig. 4.41). Similarly, amyloid is deposited in tubular basement membranes in cortex and medulla, and eventually broad cuffs of amyloid appear around the tubules. The physical presence of amyloid causes ischemia and pressure atrophy of nephrons, and resultant scarring. The tubules contain a striking number of pink hyaline casts of protein and are dilated, sometimes to such an extent that normal renal structure is not recognized ("thyroidization") (Fig. 4.42). Urinalysis reveals a high level of protein, and the loss of protein from this source may cause hypoproteinemic edema. The amyloidotic kidney, especially when medullary amyloidosis predominates, may have various of the features of end-stage renal disease, including interstitial fibrosis and lymphoplasmacytic infiltration, tubular atrophy, tubular dilation, mineralization, intratubular deposition of oxalate crystals, glomerular atrophy, and glomerulosclerosis.

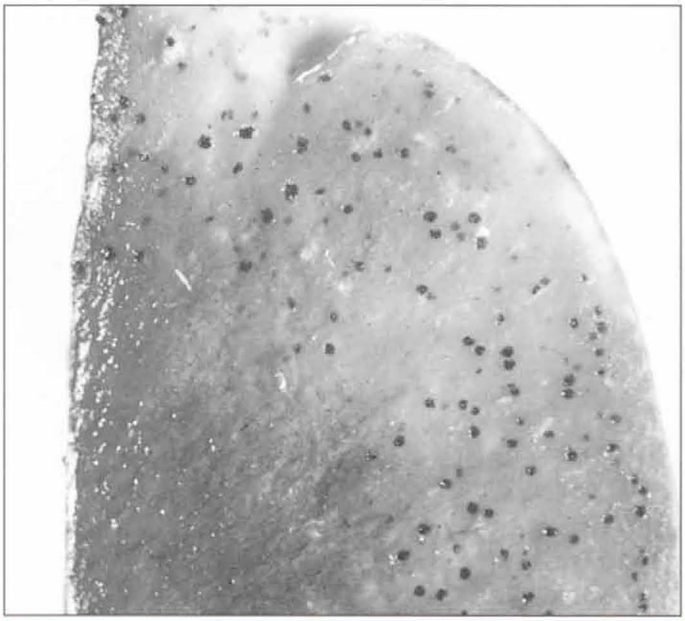

Figure 4.40 Amyloidosis in a cow. Glomeruli are prominent after exposure to iodine, followed by dilute sulfuric acid.

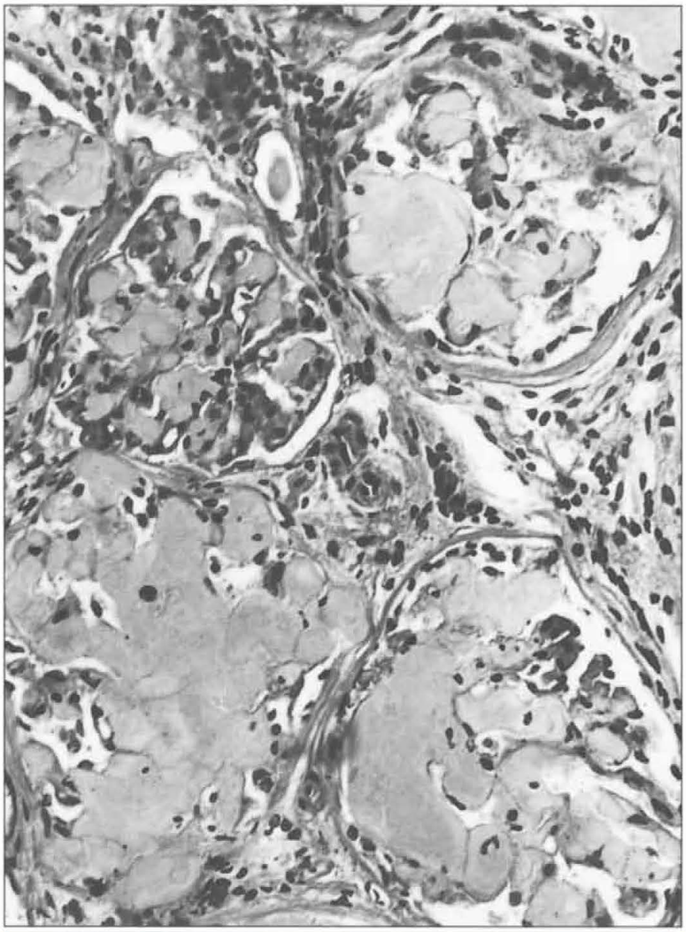

Figure 4.41 Amyloidosis in a dog. Severe lesions in glomeruli have caused atrophy of tubules and aggregation of tufts.

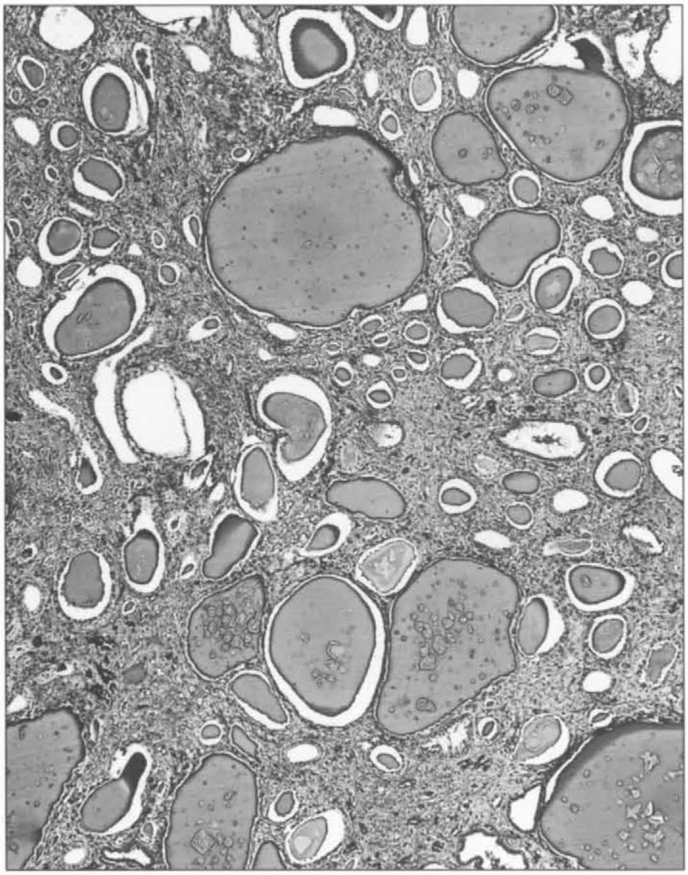

Figure 4.42 Marked proteinuric **"thyroidization"** in medulla of a sow with glomerulosclerosis. (Courtesy of D Johnson.)

Thrombosis of the pulmonary arteries or renal veins is occasionally a prominent finding in dogs with the nephrotic syndrome due to amyloidosis; they are in a *hypercoagulable state* due to stimulation of production of acute-phase proteins, such as fibrinogen, while simultaneously losing low-molecular-weight anticoagulants, such as antithrombin III, due to increased glomerular permeability.

The **cat** (including Abyssinians, Siamese, domestic shorthairs, and captive tigers and cheetahs) differs somewhat from other species, in that amyloid is deposited mainly in the papilla and outer medulla with relative sparing of the glomeruli. The kidneys are very firm, shrunken, and coarsely nodular. The papilla, in cats dying in renal failure, is necrotic, or excavated in some that survive. The nodularity is due to scarring extending from medulla to capsular surface; medullary interstitial fibrosis may also be diffuse. The capillary and tubular basement membranes in the medulla are thick and hyaline, and this is presumed to produce capillary occlusion with papillary necrosis on the one hand, and occlusion of collecting ducts with obstructive atrophy and fibrosis in the medullary rays on the other. The prevalence of amyloidosis in cats is much increased on diets providing excess vitamin A (see Vol. 1, Bones and joints).

Amyloid is nonspecifically eosinophilic in routine sections and weakly birefringent in polarized light. *The histologic diagnosis is thus usually confirmed with special stains.* Amyloid is stained a light orange-red with *Congo red*, and then exhibits green birefringence in polarized light; elimination of the Congo red staining affinity of amyloid by oxidation of tissue sections in potassium permanganate indicates that the amyloid is of the AA type. Retention of congophilia after pretreatment with potassium permanganate indicates the presence of AL amyloid; the potassium permanganate reaction may be unreliable in cats. Amyloid exhibits a bright yellow fluorescence after staining with *thioflavine-T*, which may be required in cats due to poor staining of amyloid with Congo red in this species. The above staining patterns of amyloid are due to its characteristic beta-pleated pattern, which also confers on it resistance to proteolysis and insolubility, and hence its resistance to removal from tissues. Specific immunohistochemistry can be useful in identifying amyloid and in distinguishing amyloid types. The characteristic nonbranching 7–10 nm diameter fibrils of amyloid may be seen by electron microscopy. The β-fibrils have a distinctive X-ray diffraction pattern. Definitive characterization of amyloid rests on isolation of the amyloid and amino acid sequencing; the amino acid sequences of feline SAA and AA amyloid vary slightly between Abyssinian and Siamese cats.

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Glomerular lipidosis

Large *foam cells*, which by appropriate techniques are seen to contain sudanophilic droplets, may be found in one or more lobules of glomerular tufts in many dogs (Fig. 4.43). In paraffin preparations, the cells are closely packed and finely vacuolated and the cell boundaries are distinct. These large cells appear to arise from mesangial cells. This lesion is not associated with glomerulonephritis and has no functional significance.

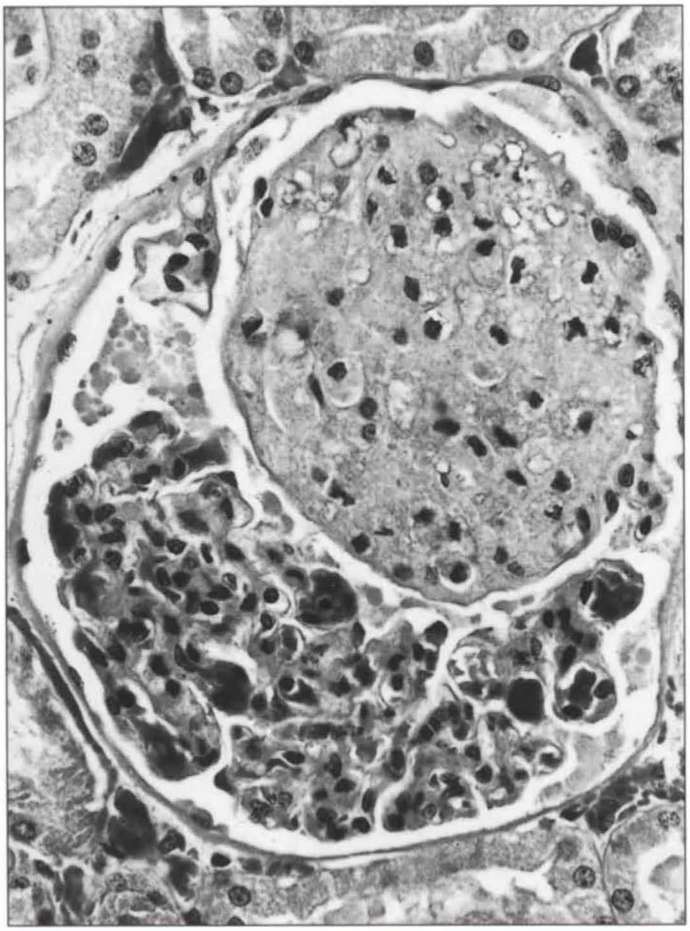

Figure 4.43 Glomerular lipidosis in a dog.

Lipid embolization of renal arterioles and glomerular capillaries occurs occasionally in dogs with diabetes mellitus or following trauma. The lipid in these cases is intravascular rather than within foam cells.

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DISEASES OF TUBULES

Diseases of tubules are primarily reflected in morphologic changes in lining epithelial cells, although specific defects of function due to enzyme deletions may not be visible histologically. Degenerations are best regarded as homeostatic disturbances of intracellular functions that may become balanced at new levels or be sufficiently severe that the cell or part of it dies. As noted above, *the tubules and interstitium are intimately associated, and damage to one affects the other*, thus, many disorders are termed *tubulointerstitial diseases* and are discussed later. Regeneration of tubular epithelium can occur (Fig. 4.44) but postnatal development of new nephrons is limited to a short period of time, depending upon species. The response of the kidney to destruction of tubules is limited to compensatory hypertrophy of remaining nephrons. Thus, the tubules remaining in damaged kidneys are often large and dilated.

Degeneration and swelling of tubular cells cause the kidney to enlarge and bulge on cut surface. *Hydropic degeneration* occurs due to damage to mitochondrial membranes and formation of vacuoles, and is potentially reversible. *Necrotic tubular cells* are eosinophilic, have pyknotic nuclei, and slough into the tubular lumen where they form cellular or coarsely granular casts (Figs 4.44–4.46). Tubules filled with *proteinaceous fluid* usually indicate that there is increased glomerular permeability in that nephron; extreme proteinuria may indicate the presence of the nephrotic syndrome clinically. *Tamm–Horsfall mucoprotein*, which is produced in the ascending limb of the loop of Henle and the distal tubules, and/or autolytic tubular epithelial cytoplasm may appear as a granular pink substance in many cortical tubules, but this is not a lesion. *Hyaline droplets*, which are pink homogeneous globules of protein, appear in the cytoplasm of proximal tubular cells in nephrons with increased glomerular permeability. The hyaline droplets are lysosomes swollen with protein that is undergoing proteolysis and will be returned to the circulation as amino acids. Protein reabsorption by proximal tubules is a physiological process; hyaline droplets indicate that this mechanism is saturated.

Fatty degeneration is difficult to evaluate in swine, dogs, and cats, which normally have considerable quantities of fat in the renal epithelium; infiltration fat accumulates in the loop of Henle in the outer medulla in starved animals. In fatty degeneration, the fat is usually found in the cells of the convoluted tubules.

Thickening of the basement membrane is seen in a variety of situations involving chronic tubular damage and is usually associated with atrophic tubular epithelium. In renal *amyloidosis*, amyloid is deposited on the tubular basement membrane, as well as in glomeruli. Disruption of the tubular basement membrane occurs in ischemic necrosis and frequently in focal renal lesions, and may allow herniation of tubular epithelial cells, with some of these cells persisting as interstitial foam cells.

Acute tubular necrosis

Acute tubular necrosis, or *nephrosis*, is a reversible condition in which tubular degeneration is the primary process, and it is an important cause of **acute renal failure.** "Nephrosis" is an imprecise term applied to noninflammatory renal disease, particularly tubular degeneration; *acute tubular necrosis* is a more accurate descriptor of the changes to be discussed here. Affected animals are *oliguric or anuric* and die within a few days unless given appropriate therapy. *The principal causes of acute tubular necrosis are ischemia and nephrotoxins.* Another major cause of acute renal failure is postrenal, namely complete urinary outflow obstruction. Severe acute glomerulonephritis can also cause acute renal failure, but is uncommon in domestic animals. The renal tubules, and particularly the proximal straight tubule and the medullary thick ascending limb, are metabolically very active and are hence the renal components that are most susceptible to ischemia or nephrotoxins. "Lower nephron nephrosis" was a term applied in the past to acute tubular necrosis, but is inaccurate and no longer in use.

Ischemic or **tubulorrhectic acute tubular necrosis** follows a period of hypotension (shock) that causes marked renal ischemia. *Prolonged renal ischemia causes renal cortical necrosis*, that is, all cortical

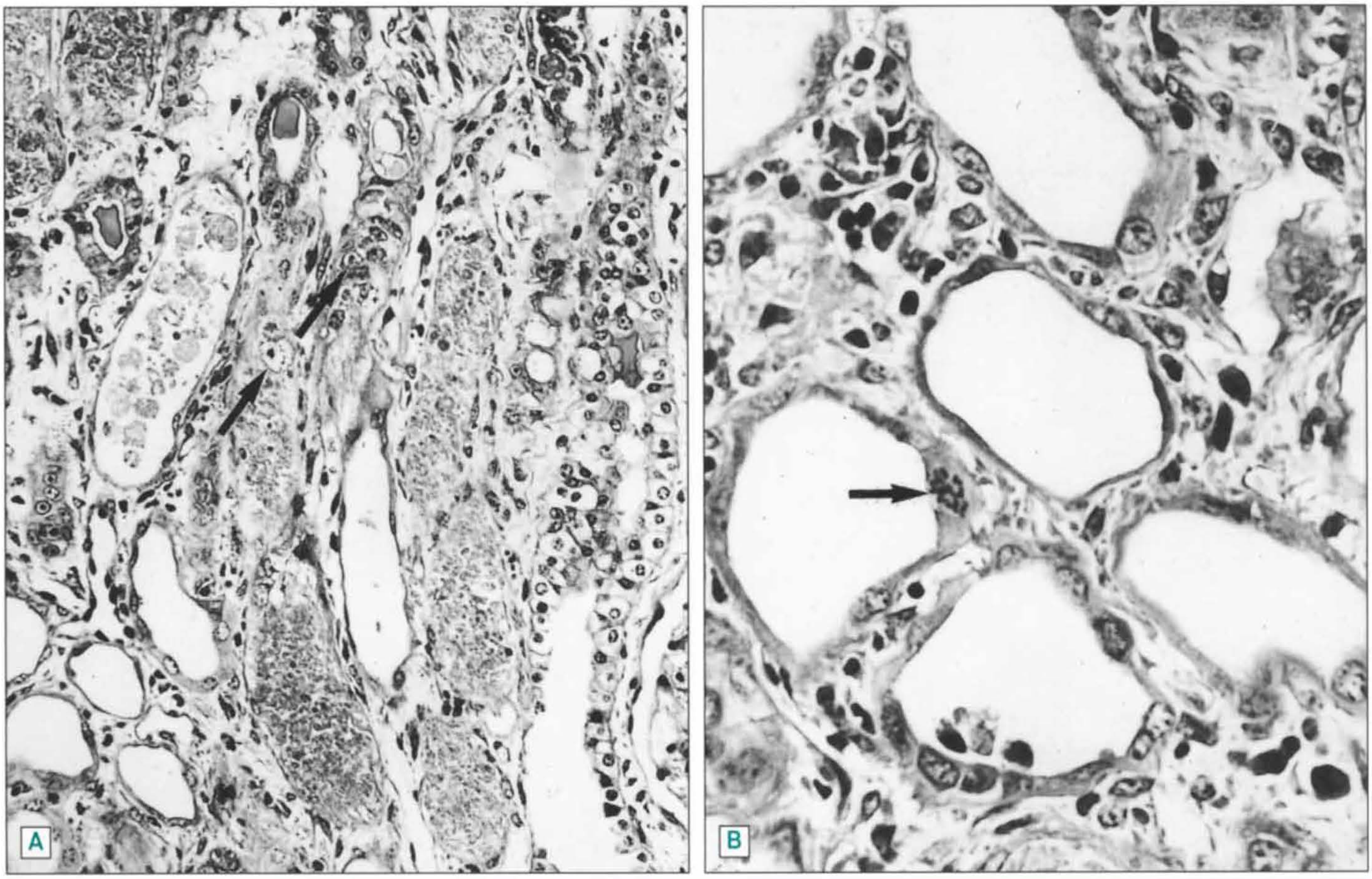

Figure 4.44 Nephrosis due to *Amaranthus retroflexus* toxicity in a calf. **A.** Granular and hyaline casts. Tubules lined by flattened epithelium. Regenerating epithelial cells in tubules (arrows). **B.** Mitotic figure in regenerating tubule (arrow).

structures are affected (discussed above). Massive hemolysis is a cause of acute tubular necrosis and produces an ischemic pattern known as *hemoglobinuric nephrosis*. Neither hemoglobin nor myoglobin are primary nephrotoxins, but they contribute to renal failure produced by other causes, such as hypotension. *Ischemic acute tubular necrosis is characterized histologically by focal necrosis along nephrons, particularly of the proximal tubules, and distal tubules to some extent, plus disruption of tubular basement membranes (tubulorrhexis) and occlusion of lumina by casts.* Eosinophilic hyaline and granular casts commonly occur in the distal tubules and collecting ducts and consist of Tamm–Horsfall mucoproteins, degenerate epithelial cells, hemoglobin, myoglobin, and other plasma proteins. Interstitial edema and accumulation of leukocytes in dilated vasa recta are common findings, and they may predate the occurrence of overt acute tubular necrosis. Glomeruli are usually normal. Vessel dilator, one of the atrial natriuretic peptides, stimulates regeneration of the brush borders of proximal tubules and may ameliorate acute tubular necrosis. After about 1 week, *epithelial regeneration* may be seen as tubules lined by flattened epithelium with hyperchromatic nuclei and mitoses (Fig. 4.44). The renewed cells are smaller than normal initially and may appear close-packed on the basement membrane. Their relationship with each other is also abnormal and they frequently tend to pile up in small clusters that eventually disappear. If the initial injury is mild and/or supportive therapy is adequate, *recovery* of architecture may be complete within about 2–3 weeks.

Nephrotoxic acute tubular necrosis, or exogenous toxic nephrosis, is contrasted here with ischemic acute tubular necrosis, and specific examples are given below. Renal tubules, and especially proximal tubules, are particularly susceptible to a wide variety of toxic agents as a consequence of their great metabolic activity and their exposure to agents in the large volume of ultrafiltrate that they resorb in the process of urine formation. Their enzyme systems are thus exposed to, and inactivated by, agents such as heavy metals, which bind to sulfhydryl groups. *Nephrotoxic acute tubular necrosis is usually characterized histologically by extensive necrosis of predominantly proximal tubules, but with preservation of tubular basement membranes* (Fig. 4.45). These two features distinguish toxic from ischemic acute tubular necrosis, in which necrosis is patchy and disrupts basement membranes. Preservation of tubular basement membrane is necessary to provide the framework for epithelial regeneration, and hence ischemic damage to tubules often has a worse prognosis for the kidney and the animal than does toxic damage. Ischemia often complicates toxic acute tubular necrosis because the swelling of tubular epithelial cells caused by the toxin impairs intrarenal blood flow.

The *pathogenesis* of acute renal failure and oliguria in either ischemic or toxic acute tubular necrosis remains controversial. Obstruction of tubular flow by cellular debris and casts and by interstitial edema appears to be an important factor (Fig. 4.46). Other proposed mechanisms include preglomerular vasoconstriction, possibly

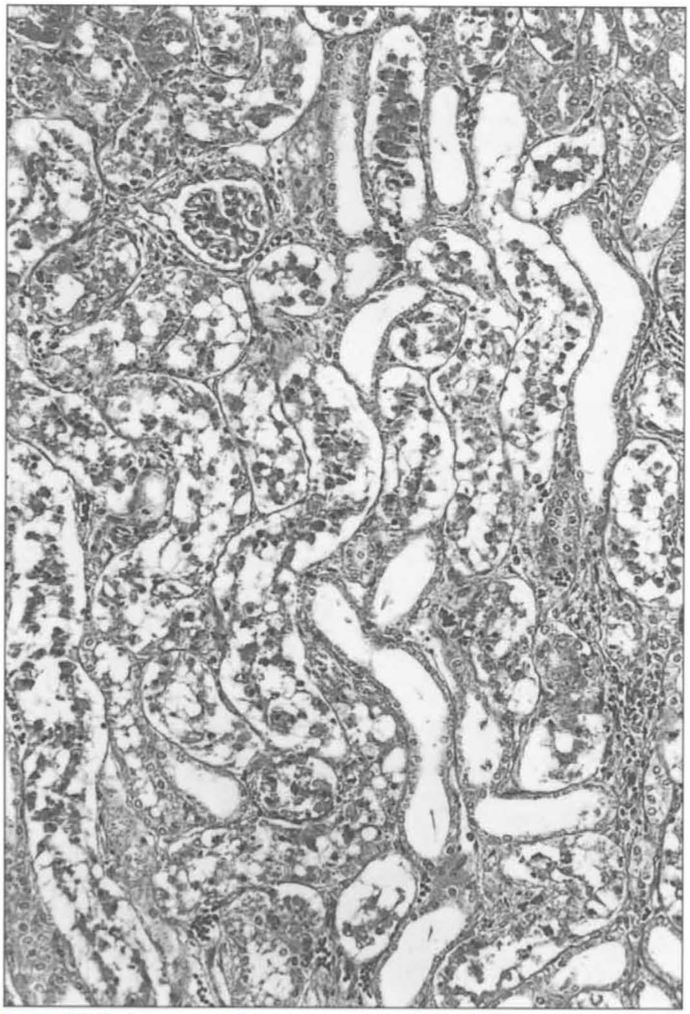

Figure 4.45 Acute tubular necrosis due to mercuric chloride poisoning. Necrosis, fragmentation, and shedding of tubular epithelium.

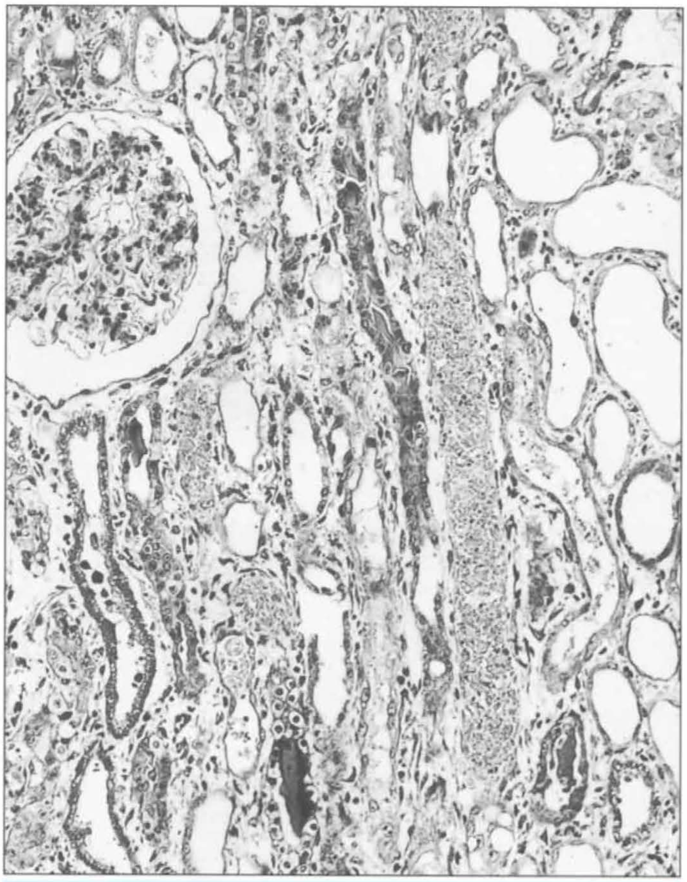

Figure 4.46 Subacute nephrosis due to *Amaranthus retroflexus* toxicity in a calf. Note granular and hyaline casts, dilation of Bowman's spaces, interstitial edema, and tubules lined by flattened epithelium.

due to activation of the renin–angiotensin system; leakage of tubular fluid into the interstitium (tubular backleak); and impaired glomerular permeability or vascular reactivity. Disruptions of the actin cytoskeleton of proximal tubular cells and breakdown of apical membrane microvilli occur due to ischemia. Redistribution of tubular cell membrane proteins causes loss of cell polarity, abnormal ion transport across tubular cells, and hence increased delivery of sodium to the distal tubule; the resulting tubuloglomerular feedback leads to vasoconstriction. *Oliguria* is the result of various combinations of these factors. At the oliguric stage of acute renal failure, hyperkalemia is a life-threatening event. If the animal survives the oliguric phase, diuresis occurs and electrolyte imbalances, such as hypokalemia, may contribute to death. As tubular regeneration proceeds, azotemia resolves and tubular function slowly returns.

Nephrotoxic tubular necrosis

Numerous toxic substances can cause acute tubular necrosis in domestic animals (Table 4.2). Some of these agents are no longer important as nephrotoxins; for example, organomercurials were commonly used as fungicides on seed grains that were occasionally inadvertently fed to animals and humans with disastrous results. This use of mercury has been banned. Similarly, highly chlorinated naphthalenes, which cause hyperkeratosis and nephrosis in cattle, have been excluded from the farm environment. Sulfonamides were formerly important as nephrotoxins, but newer formulations are more soluble and less toxic. Some newly introduced agents have nephrotoxicity as a side-effect, e.g., aminoglycosides. Numerous additional antibiotics that are toxic to humans, e.g., penicillin, semisynthetic penicillins, and polymixins, may also prove to be toxic to domestic animals. The toxicity of many of the exogenous agents is exacerbated by various systemic states, such as dehydration or shock, which concomitantly impair renal function in the affected animal. Discussion of specific examples of nephrotoxins follows.

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Table 4.2 Agents that are nephrotoxic in domestic animals

Exogenous	Animal venoms Antimicrobials Aminoglycosides (neomycin, kanamycin, gentamicin, streptomycin, tobramycin, amikacin) Amphotericin B Cephalosporins Polymixins Sulfonamides (sulfapyridine, sulfathiazole, sulfadiazine) Tetracyclines Antineoplastic agents (cisplatin, doxorubicin, methotrexate) Cantharidin (blister beetle) Chlorinated hydrocarbons *Clostridium perfringens* type D, epsilon toxin (pulpy kidney) Contrast media Ethylene glycol Menadione (vitamin K_3) Metals (arsenic, bismuth, cadmium, lead, mercury, thallium) Methoxyflurane Monensin Mycotoxins – citrinin, ochratoxin A Paraquat Plants *Amaranthus retroflexus* (pigweed) *Isotropis* *Lantana camara* Oxalates – various plants *Quercus* spp. (oak) – tannins *Terminalia oblongata* (yellow-wood) Sodium fluoride (superphosphate fertilizer)
Endogenous	Bile Hemoglobin Myoglobin

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Aminoglycosides

These antibiotics are widely used against gram-negative infections, and include, in decreasing order of nephrotoxicity, **neomycin, kanamycin, gentamicin, streptomycin, tobramycin**, and **amikacin**. *Aminoglycosides are obligate nephrotoxins*; in cats, aminoglycosides are ototoxic as well as nephrotoxic. Foals are particularly prone to nephrotoxicosis. Aminoglycosides are not metabolized but are eliminated from the body primarily by glomerular filtration; they selectively accumulate in, and cause damage to, proximal tubules. The *tubulotoxic effects* of aminoglycosides include damage and loss of the brush border, and formation of cytosegrosomes and myeloid bodies; this overloading of lysosomes with phospholipids (lysosomal phospholipidosis) results from aminoglycoside-induced inhibition of phospholipases. Lysosomal dysfunction and/or leakage may lead to tubular cell necrosis. Damage is dose-related and is enhanced by pre-existing renal impairment. Toxicity is manifest clinically by inability to concentrate urine, polyuria, enzymuria, proteinuria, hematuria, cylindruria, and azotemia. Acute renal failure may ensue. Aminoglycoside nephrotoxicity is reversible; recovery from the nephrosis may occur even in the face of continued therapy because the regenerating cells have increased resistance to aminoglycoside toxicity.

Tetracyclines

Tetracyclines cause several syndromes of renal disease in humans: progressive azotemia due to their antianabolic effect, a reversible Fanconi syndrome due to the use of outdated tetracycline containing degradation products, and reversible nephrogenic diabetes insipidus. An overdose of oxytetracycline can produce acute tubular necrosis and renal failure in dogs. Tetracycline administration has been reported to cause acute nephrosis and death in calves due to the presence of tetracycline degradation products; high doses of oxytetracycline can cause acute nephrosis in treated cattle. *Use of tetracyclines is contraindicated in animals in renal failure*; tetracyclines are excreted from the body primarily by the kidneys, thus renal dysfunction leads to increased serum drug concentrations and enhanced nephrotoxic potential. Doxycycline, a newer semisynthetic tetracycline, is not reported to be nephrotoxic.

Sulfonamides

Severe nephropathy may follow the ingestion of excessive doses of sulfonamides, especially if treated animals are dehydrated. Toxicity was much more common previously when only less-soluble forms of the drug were available, e.g., sulfapyridine, sulfathiazole, and sulfadiazine. *Crystalline nephropathy is now rare as the newer shorter-acting*

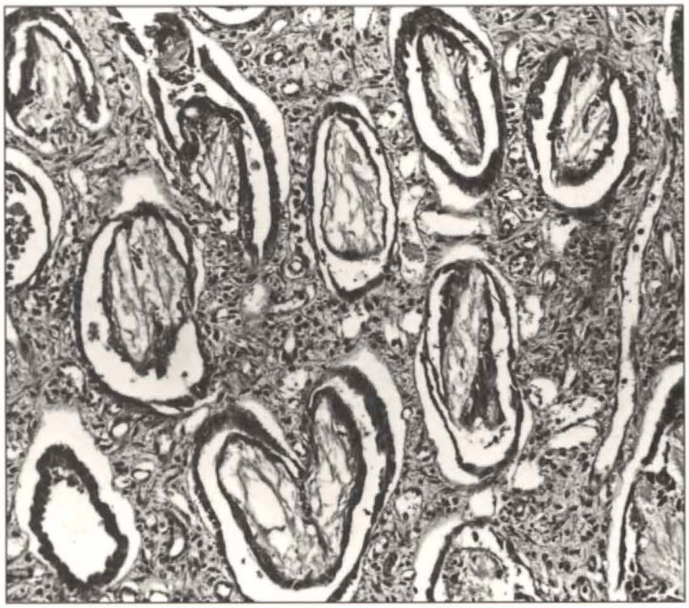

Figure 4.47 Sulfonamide nephrosis in a calf. Sulfa crystals (removed in processing) have been covered by epithelium from collecting tubules.

sulfonamides have greater solubility. Affected kidneys are slightly enlarged and congested, and the sulfonamide crystals are grossly visible in the medulla, pelvis, and in some cases even in heavy deposits in the bladder. The deposits are yellow, and form pale radial lines in the medulla. Crystals are not observed in section as they are dissolved during processing. The epithelium of the proximal convoluted tubules and of Bowman's capsule undergoes severe hydropic degeneration. There is little evidence of necrosis in the distal and collecting tubules, but epithelial proliferation and swelling are prominent and the tubules become densely populated with large dark-staining cells. Some papillary formations into the lumen may even be present, and commonly in the collecting tubules there is more or less complete duplication of the epithelium, the inner epithelium enclosing a faintly basophilic hyaline precipitate (Fig. 4.47). There is a diffuse but mild interstitial reaction about the corticomedullary junction. It appears that the *renal lesions are due to both local toxic and mechanical (tubulo-obstructive) effects* and that hypersensitivity does not play a role in animals, as it apparently does in humans.

Amphotericin

Amphotericin B is an antifungal agent, a polyene antibiotic, whose most important toxic effect is renal dysfunction. It causes decreased renal blood flow and glomerular filtration due to *renal vasoconstriction*, and is also *directly toxic* to renal tubular epithelial cells. Necrosis of proximal and distal tubules occurs, and there is mineralization of intratubular casts. Nephrotoxicity of amphotericin B has been reduced by complexing the drug with lipids or entrapping it in liposomes.

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Ethylene glycol

Dogs and cats are commonly poisoned by ingestion of ethylene glycol. The seasonal incidence of this poisoning coincides with the changing of engine antifreeze solutions in the spring and autumn. Cattle are also occasionally poisoned. Ethylene glycol, which is present in a 95% concentration in antifreeze solutions, has a sweet taste and is usually voluntarily ingested, especially by young male dogs. Cats are more susceptible, but less commonly affected, than dogs; the minimum lethal dose is 1.5 mL/kg for cats and 6.6 mL/kg for dogs.

Ethylene glycol, which itself is of low toxicity, is rapidly absorbed from the gastrointestinal tract, and most is excreted unchanged in the urine. A small percentage is oxidized by alcohol dehydrogenase in the liver to glycoaldehyde, which is in turn oxidized to glycolic acid, glyoxylate, and finally oxalate. *Glycoaldehyde and glyoxylate are the primary nephrotoxic metabolites*; they act by causing adenosine triphosphate depletion and membrane phospholipid and enzyme destruction. Other end-products of metabolism are lactic acid, hippuric acid, and carbon dioxide.

Depression, ataxia, and osmotic diuresis develop within a few hours after ingestion of ethylene glycol. Although oxalate crystals are deposited around cerebral vessels and in perivascular spaces (Fig. 4.48), nervous signs are due to the effect of aldehydes and possibly to the severe metabolic acidosis that develops as a result of accumulation of lactic acid, glycolate, and glyoxylate. Over the next 12 hours, pulmonary edema, tachypnea, and tachycardia occur. If the animal survives for 1–3 days after ingestion, acute renal failure develops, primarily due to renal tubulotoxic damage. Severe renal edema impairs intrarenal blood flow and contributes to nephrosis and renal failure. Soluble calcium oxalates in the blood precipitate in the ultrafiltrate of the renal tubules as the pH of the fluid decreases. Calcium oxalate crystals may be found in tubular lumina (Fig. 4.49), in tubular cells, and in the interstitium; they are light yellow, arranged in sheaves, rosettes, or prisms, and are birefringent with polarized light. Tubular lesions, which are most severe in proximal tubules, range from hydropic degeneration to necrosis to regeneration.

In animals surviving the acute toxic insult, calcium oxalate crystals are thought to be of importance in causing renal failure. *Large numbers of crystals in tubules are virtually pathognomonic of ethylene glycol poisoning*; a few crystals may be seen with chronic tubular obstruction. Occasional calcium oxalate crystals are normally seen in urine

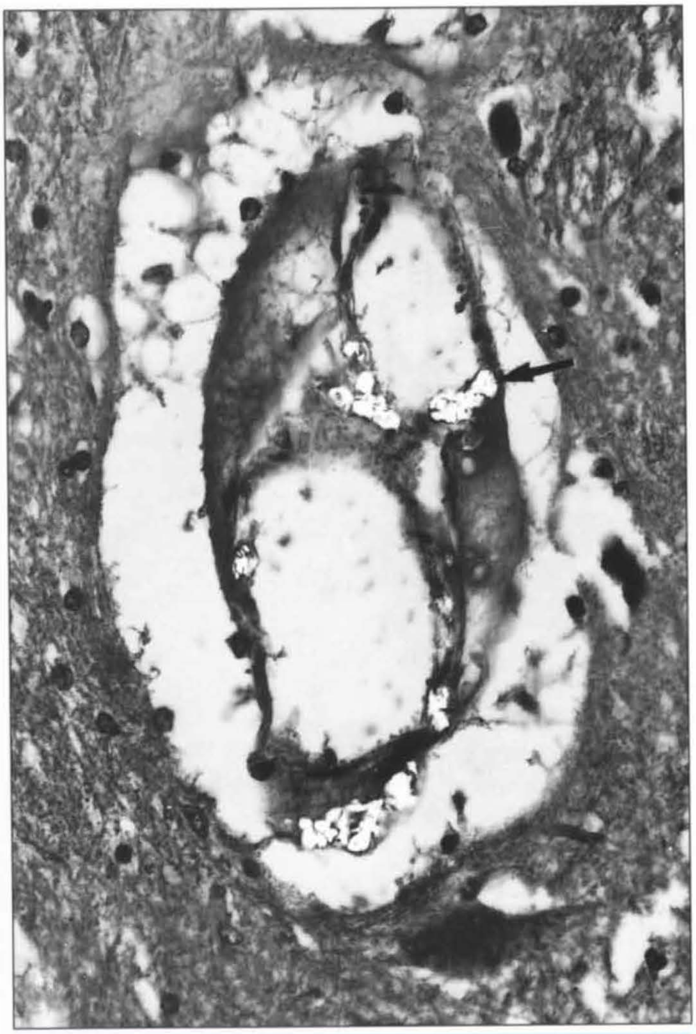

Figure 4.48 Oxalate encephalitis in a dog, caused by ethylene glycol poisoning. Partially crossed polarization filters. (Courtesy of RW Wilson.)

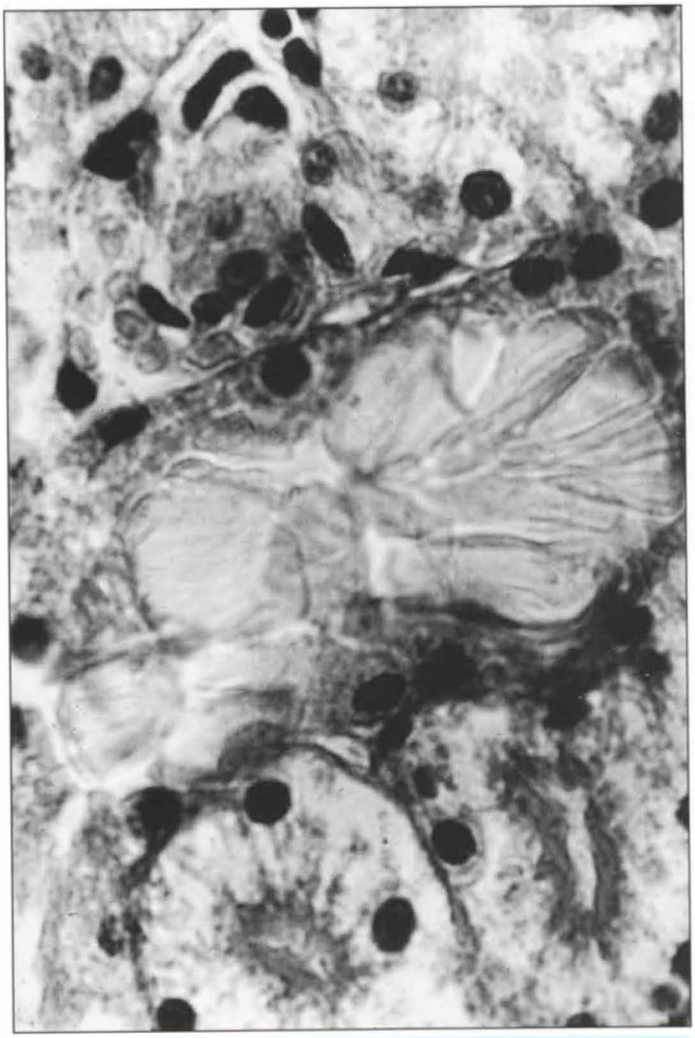

Figure 4.49 Oxalate crystals in proximal tubule in a dog.

sediment of dogs; large numbers of these crystals are highly suggestive of poisoning. Hypocalcemia due to the formation of crystals is usually mild in dogs. Animals that survive the acute exposure toxicity may have renal interstitial fibrosis. Few crystals may be left in the tubules; they tend to be removed in the weeks following their deposition. The diagnosis may be confirmed by detection of ethylene glycol in stomach content or blood by gas chromatography early in the toxicosis, or by detection of glycolic acid in urine, serum, or ocular fluid by mass spectrometry later in the toxicosis.

Oxalate

Plants are the usual source of oxalate poisoning in sheep and cattle. **Plants** that may contain toxic amounts of oxalate are *Halogeton glomeratus*, halogeton; *Sarcobatus vermiculatus*, greasewood; *Rheum rhaponticum*, the common garden rhubarb; *Oxalis cernua*, soursob; *Rumex* spp., sorrel, dock. Plants of lesser importance are *Portulacca oleracea, Trianthema portulacastrum,* and *Threlkeldia proceriflora,* as well as some cultivated species such as mangels and sugar beet. Young plants may contain the equivalent of 7% or more of potassium oxalate; the amount decreases with maturity and drying of the plant. The above-listed plants are only eaten in unusual circumstances and not readily.

Species of **grasses** in the genera *Cenchrus, Panicum,* and *Setaria,* which are widely cultivated in tropical and subtropical areas and which accumulate large amounts of oxalate, have been associated with renal oxalosis in cattle and sheep and with skeletal disease in horses, the latter due to conditioned calcium deficiency (see Vol. 1, Bones and joints).

The **fungi** *Aspergillus niger* and *A. flavus* can produce large quantities of oxalates on feedstuffs. Large doses of **ascorbic acid** (vitamin C) have caused oxalate nephrotoxicosis in humans and in a goat; ascorbic acid is a metabolic precursor of oxalate. **Primary hyperoxaluria**, a rare inherited metabolic condition, occurs in humans, cats (inbred colony of domestic shorthairs), dogs (family of Tibetan spaniels (Shih Tzus)), and in Beefmaster cattle (likely autosomal recessive). **Pyridoxine (vitamin B_6)** deficiency and **methoxyflurane** anesthesia can also cause renal oxalosis.

Mortality rates of 10% may occur in sheep when, as frequently happens in some areas, these animals graze on almost pure stands of halogeton or of soursob. It is more usual, however, for fatalities to be sporadic. Under natural conditions, sheep may ingest up to 75 g of oxalate a day; *the rumen degrades the salt efficiently by metabolism of oxalate to bicarbonate and carbonates.* Depending on the diet before exposure to oxalate-containing plants and, therefore, on the microbial composition of the rumen, some variation is to be expected in the ability of ruminal contents to degrade the salt. Cattle are less commonly affected under range conditions than are sheep, but cattle and sheep are equally susceptible to experimental poisoning. Horses are resistant to oxalate-induced nephrosis and succumb to acute gastroenteritis only after receiving unnaturally large amounts of the chemical; they may develop osteodystrophia fibrosa with prolonged exposure.

Chelation of calcium by unmetabolized oxalate in the ingesta contributes to hypocalcemia. Following absorption, oxalates combine with calcium to form insoluble calcium oxalate; hypocalcemic tetany may result. Calcium oxalate may crystallize in vessel lumens or walls, causing vascular necrosis and hemorrhage, or in renal tubules, causing tubular obstruction and acute renal failure. The nephrotoxicity of oxalates involves more than mechanical obstruction, and may be due in part to intracellular chelation of calcium and magnesium and hence interference with oxidative phosphorylation. Clinically, weakness, prostration, and death may follow within 12 hours of ingestion of oxalate-containing plants.

Oxalates are produced endogenously in the degradation of glycine, an important constituent amino acid of collagen and elastin. Uptake of normal dietary oxalate may increase in a variety of enteric diseases (enteric oxalosis). *Oxalosis can be prominent in the kidneys of aborted bovine fetuses*, and may reflect maternal intake of oxalate-containing plants or moldy feed. A few oxalate crystals can frequently be found in scarred tubules in any species; these crystals are usually without significance.

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Mycotoxins

Aspergillus and *Penicillium* spp. produce a number of nephrotoxic mycotoxins, namely **ochratoxins, citrinin, fumonisin, oxalate**, and **viridicatumtoxin**, which can contaminate feed grains. *Ochratoxin A (OTA)* is of most significance in disease; the toxicity of OTA is enhanced by penicillic acid, which is co-produced by *Penicillium ochraceus*. In pigs, OTA and citrinin produce proximal tubular degeneration and atrophy, cortical interstitial fibrosis, and glomerular hyalinosis; the renal insufficiency produced is usually subclinical. Acute renal disease occurs very rarely, and is manifested by severe perirenal edema resembling that produced by redroot pigweed (*Amaranthus retroflexus*, see below). Ochratoxicosis also increases the susceptibility of pigs to secondary bacterial infections. Ochratoxin A has been suggested as the cause of endemic (Balkan) nephropathy in humans. Ochratoxins are normally degraded in the rumen; thus, toxicity is unlikely to occur in ruminants.

Moldy feed also produces mycotoxic nephropathy in horses. Citrinin produces tubular degeneration in swine, horses, and sheep. *Fumonisins*, mycotoxins produced by *Fusarium* fungi, alter sphingolipid metabolism and cause leukoencephalomalacia in horses and pulmonary edema in pigs; they are hepatotoxic in all species. Fumonisins are nephrotoxic in sheep and cattle; renal lesions include vacuolar change, apoptosis, karyomegaly, and tubulo-obstructive changes in proximal tubules.

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Amaranthus

Ingestion of redroot pigweed, *Amaranthus retroflexus*, causes perirenal edema and acute renal failure due to toxic nephrosis in swine and cattle, and uncommonly in horses. The nephrotoxic principle of *A. retroflexus* has not yet been identified; the plants often contain high levels of nitrate and oxalate, but neither nitrate nor oxalate poisoning usually occurs and does not produce perirenal edema. Phenolic compounds have been identified in the leaves of *Amaranthus*, and may be of similar importance to those in *Quercus* spp. (oak, see below). Lush growth of pigweed occurs in early summer and this plant often dominates the weed growth in disused lots and yards to which animals are moved when other pasture is depleted. Weakness, recumbency, and often death follow 5–10 days after grazing begins.

Grossly, there is marked perirenal edema, which may be blood-stained and may be accompanied by edema of mesenteries, bowel wall, and ventral abdominal wall, with moderate ascites and hydrothorax. The kidneys are pale but not usually enlarged. Histologically, there is degeneration and coagulative necrosis of both proximal and distal tubules (Figs 4.44 and 4.46); there may also be mild glomerular epithelial injury and hypercellularity. Tubules contain granular casts. The perirenal edema seen in acute cases is apparently due to tubular backleak, with subsequent lymphatic drainage and leakage into perirenal connective tissue. The probable cause of death is heart failure due to hyperkalemia. Survivors may develop renal interstitial fibrosis and tubular dilation (Fig. 4.50).

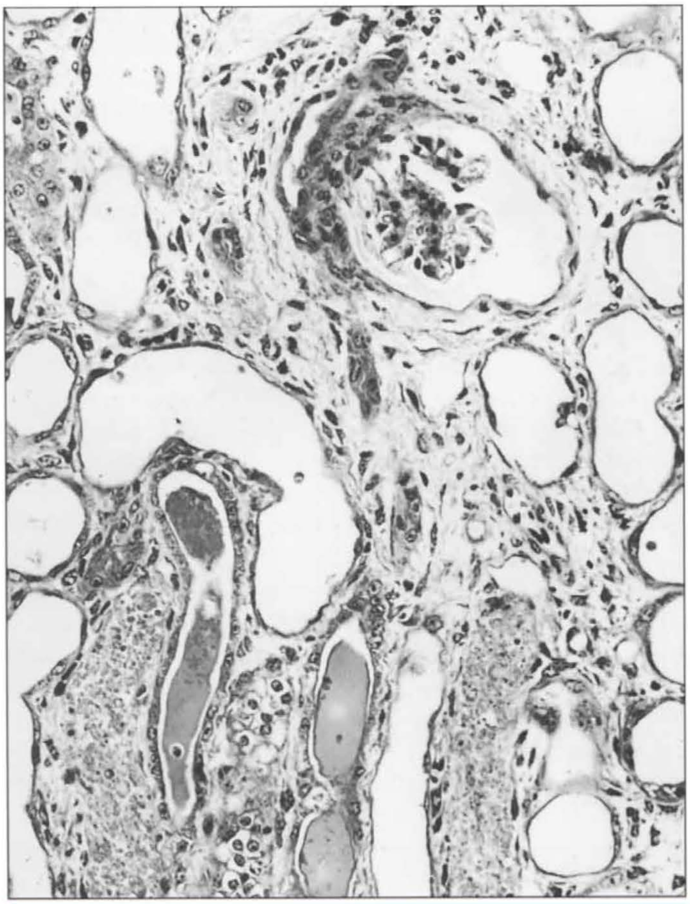

Figure 4.50 Fibrosis beginning around glomeruli and between tubules following tubular necrosis due to **Amaranthus retroflexus**.

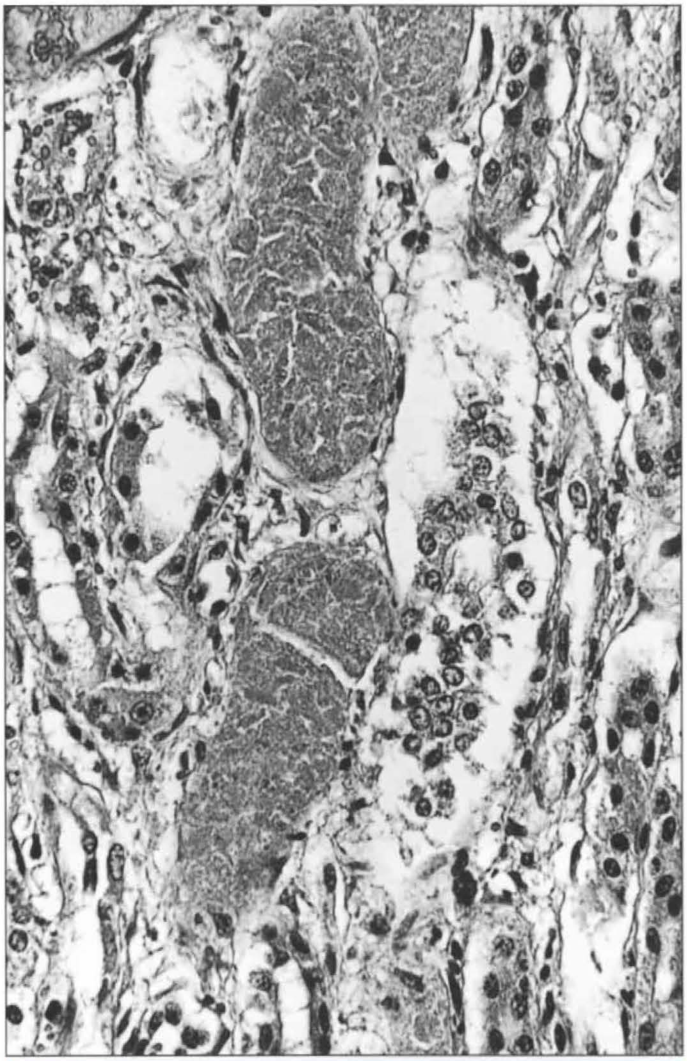

Figure 4.51 Nephrosis due to oak poisoning of a 6-month-old Hereford calf. Note necrotic tubule adjacent to more normal tubules. (Courtesy of R Werdin.)

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Other plant toxicoses

Poisoning of ruminants and occasionally of horses by *blossoms, buds, leaves, stems, and acorns* of **oak shrubs and trees (*Quercus* spp.)** occurs wherever these grow, and in areas where the acorns are accepted as a dietary staple in late autumn and early winter. There are many species and varieties of oaks but not all are palatable. They are all potentially poisonous by virtue of the *tannins* they contain, but, in small quantities, they are eaten with impunity. Ingestion of large amounts of the material can cause hydrothorax, ascites, marked perirenal edema, alimentary tract ulceration, and acute tubular necrosis and can result in large death losses. The toxic substances are *gallotannins*; they are hydrolyzed to tannic acid, gallic acid, and pyrogallol, which appear to be the active toxic metabolites. Binding of tannins to endothelial cells results in *endothelial damage* and accounts for perirenal edema and fluid leakage into body cavities. The alimentary lesions are due to the strong ability of oak tannins to bind to peptide linkages and precipitate protein; this astringent effect of tannins is used in leather tanning. The gastrointestinal and mesenteric edema and ulcerative enterocolitis that develop in poisoned horses are also partly the product of disseminated intravascular coagulation.

In acute oak poisoning, there is marked perirenal edema and hemorrhage, the kidneys are swollen and pale and, in cattle, are rather uniformly sprinkled with cortical hemorrhages of 2–3 mm diameter. The glomeruli are ischemic but otherwise normal except for dilation of the urinary space after several days. There may be microscopic hematuria. Necrosis of the epithelium of the proximal tubules can be complete, to produce dense homogeneous casts within the basement membranes. In less severe injury, adjacent groups of tubules may vary considerably in the extent of degeneration (Fig. 4.51). Some animals may recover but, in others, the renal lesion progresses with shrinkage and diffuse fibrosis and scattered collections of mononuclear cells. *The completeness of the necrosis in groups of tubules with intratubular hemorrhage distinguishes the nephrosis of acute oak poisoning from that of most other causes.*

Poisoning by the **yellow wood tree, *Terminalia oblongata***, in Australia is reputed to produce lesions similar to those of oak poisoning. Yellow wood produces at least two toxic factors: punicalagin, a tannin that is hepatotoxic, and terminalin, a condensed tannin that is nephrotoxic. In acute yellow wood poisoning of cattle and sheep, coagulative periacinar hepatic necrosis predominates, accompanied in cattle by acute renal tubular necrosis. Subacute and chronic intoxications are characterized by renal fibrosis and atrophy.

Several species of the genus ***Isotropis*** are toxic to ruminants. In both cattle and sheep, reference is made to abomasitis and enteritis, petechial hemorrhages throughout the gastrointestinal tract, perirenal edema, and accumulation of fluid in the body cavities and subcutis. The renal lesions are dominated by necrosis of proximal tubular epithelium. In many acute poisonings, there is abundant proteinaceous fluid in Bowman's space.

Various members of the **lileaceae family** of plants can cause acute tubular necrosis in animal species, for example, *Narthecium ossifragum* (bog asphodel) in ruminants, several lilies, and their hybrids (*Lilium* spp.) in cats.

Grapes and raisins contain an unknown toxic principle that causes proximal renal tubular degeneration and/or necrosis in dogs.

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Specific tubular dysfunctions

Renal tubular dysfunction may occur secondary to other conditions; for example, glucosuria occurs when the tubular transport maximum for glucose is exceeded in diabetes mellitus and acute enterotoxemia, and heavy-metal toxicity causes glucosuria and aminoaciduria due to tubular degeneration. The primary tubular transport defects identified in domestic animals are *hyperuricosuria in Dalmatian dogs, essential cystinuria, the syndrome of multiple resorptive defects in Basenji dogs, renal tubular acidosis*, and *primary renal glucosuria*. Hyperuricosuria and cystinuria are of importance because they predispose to urolithiasis, and are discussed in that section.

Basenjis and several other breeds of dogs may develop a *proximal renal tubular disorder* similar to the "**Fanconi syndrome**" in humans; the condition is hereditary in Basenjis. The syndrome in dogs is characterized by polyuria, polydipsia, hyposthenuria, glucosuria, normoglycemia, hyperphosphaturia, proteinuria, and aminoaciduria. The aminoaciduria may be generalized or limited to cystinuria. Affected dogs have impaired renal tubular reabsorption of glucose, phosphate, sodium, potassium, uric acid, and amino acids, possibly due to alterations in membrane transport proteins. Polyuria results from the glucosuria and natriuresis. The syndrome develops in adult dogs and is usually slowly progressive. Dehydration and acidosis result in renal papillary necrosis and death due to renal failure. Histologic renal changes are nonspecific, and include interstitial fibrosis and tubular atrophy. Affected dogs often have marked karyomegaly in occasional tubular cells. Ultrastructural abnormalities have not been noted in tubular cells. A congenital Fanconi syndrome in Border Terriers was associated with rickets, likely as the result of hyperphosphaturia and decreased hydroxylation of 25-$(OH)_2D_3$. Acquired Fanconi syndrome has been reported in dogs in association with gentamicin nephrotoxicity, ethylene glycol nephrotoxicity, and primary hypoparathyroidism. A presumptive case of Fanconi syndrome with marked growth retardation has been reported in a young Simmental bull.

The renal tubular acidosis that occurs as part of the Fanconi syndrome is the most common example in dogs of **proximal, or type II, renal tubular acidosis**, in which proximal tubules fail to reabsorb filtered bicarbonate. Proximal renal tubular acidosis is usually a self-limiting disease that is not evident clinically. **Distal, or type I, renal tubular acidosis** occurs in dogs, cats, and horses, is caused by defective excretion of hydrogen ions by distal tubules, and produces hyperchloremic metabolic acidosis that is more severe than that in the proximal form of renal tubular acidosis. Untreated distal renal tubular acidosis can result in osteomalacia, nephrocalcinosis, nephrolithiasis, and renal failure. Transient renal tubular acidosis is a feature of amphotericin B nephrotoxicity.

Primary renal glucosuria may occur as a singular transport abnormality without other defects. This defect is an inherited disorder in Norwegian Elkhounds.

The mannosidoses, hereditary **lysosomal storage diseases,** can cause vacuolation of neurons, renal tubular epithelial and other cells in humans, cattle, goats, and cats; similar lesions are caused by some plant intoxications, e.g., *Swainsona, Astragalus, Oxytropis*. Salers calves affected with *β-mannosidosis* primarily have a variety of neurologic deficits but also have greatly enlarged kidneys that are characterized by marked vacuolation of the cytoplasm of proximal tubular epithelial cells (Fig. 4.52); this lysosomal storage disease is inherited as an autosomal recessive trait. Although the renal lesion is histologically dramatic, renal dysfunction is not reported (see Vol. 1, Nervous system).

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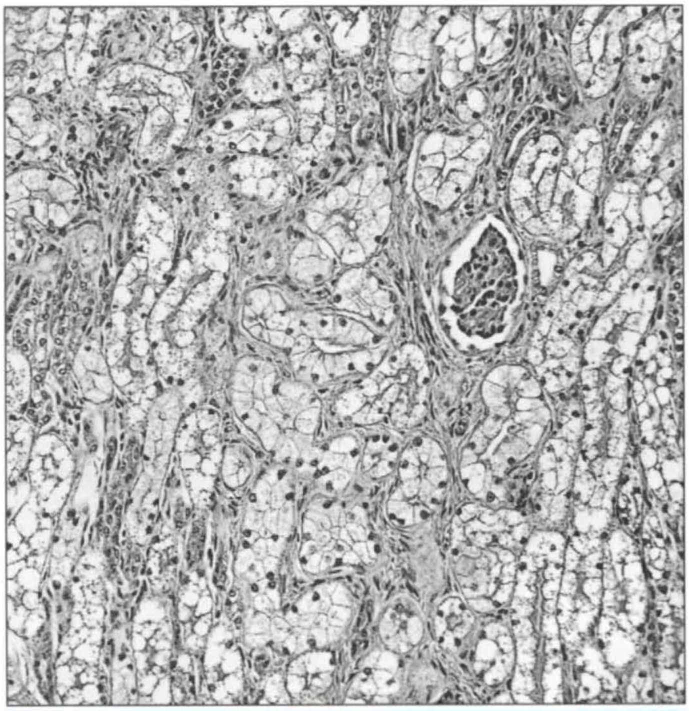

Figure 4.52 Beta-mannosidosis in a newborn term Salers calf. Note marked vacuolation of renal tubular epithelial cells. (Courtesy of L Bryan.)

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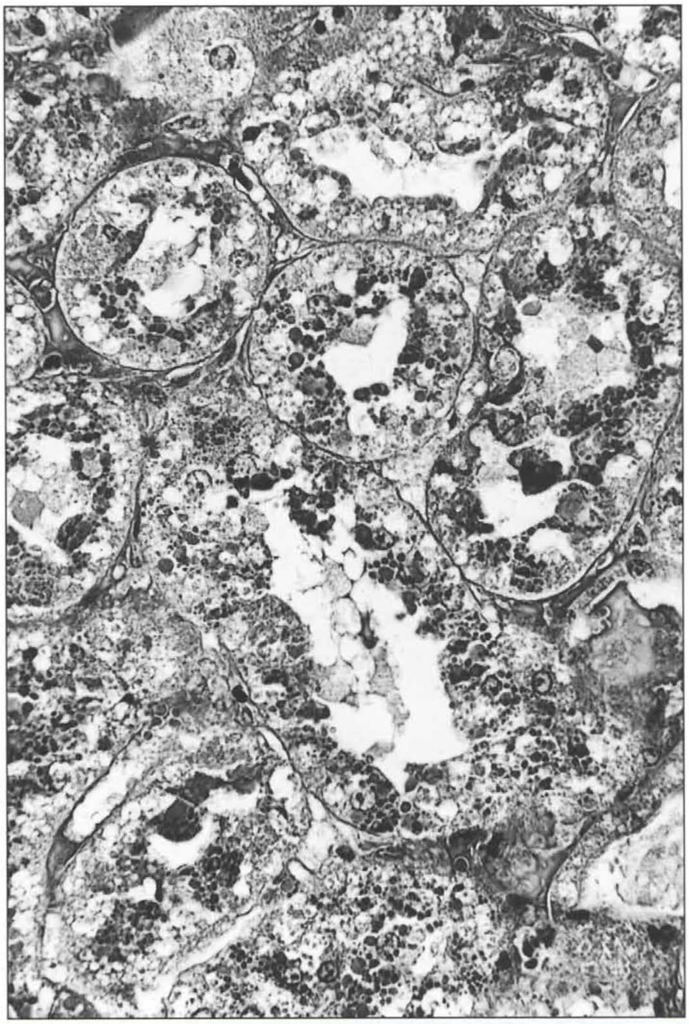

Figure 4.53 Renal tubular hyaline droplets in a sheep, as a consequence of hemolysis caused by chronic copper poisoning. (Courtesy of L Bryan.)

Pigmentary changes

Following acute hemolytic crises of any cause, *the kidneys may be very dark, almost black*, as a consequence of concentrated **hemoglobin**. Initially, the discoloration is uniform, but shortly most of the hemoglobin is dispersed, except from scattered clusters of nephrons in which it persists for many days to produce diffuse brown speckling of the cortices. The color alone provides a good distinction between this pigmentary change and the red or blue speckling that occurs in acute hemorrhagic glomerulitis. It is particularly characteristic of the hemolytic crisis of chronic copper poisoning in sheep. Microscopically, the hemoglobin appears as fine red granules in the epithelial cells of the tubules (Fig. 4.53) and as red casts in the lower reaches of the nephron, especially Henle's loop and the collecting tubules. The same histological picture occurs following incompatible blood transfusions and in paralytic myoglobinuria of horses, in which disease the pigment is **myoglobin**. Nutritional myopathy of young animals does not produce this renal lesion because the myoglobin content of their muscle is very low. Hemoglobin and myoglobin pigmentation persist after hemoglobin or myoglobin are no longer detectable grossly in urine samples. As noted above, *neither hemoglobin nor myoglobin is a primary nephrotoxin*, but they contribute to renal failure produced by other causes, such as hypotension. Tubular obstruction by myoglobin casts can contribute to acute renal failure in horses with severe rhabdomyolysis.

Hemosiderosis occurs in the course of chronic hemolytic anemia and as residue from acute hemoglobinuric episodes. The pigment is found in epithelial cells of proximal tubules, where it is produced by the degradation of resorbed hemoglobin, and it may be sufficient to produce a distinctive brown coloration of the cortex.

Lipofuscinosis of the kidneys of adult cattle consists of the *deposition of brown iron-free pigments* with staining characteristics of lipofuscin (Fig. 4.54A). The pigmentation also affects striated muscles, giving them a dark-brown appearance. On the cut surface of the kidney, the pigmentation occurs in radial dark lines in the cortex but spares the medulla. Microscopically, it is present as fine, brown granules in the epithelial cells of the convoluted tubules. An *environmental lipofuscinosis* of the liver, kidney, and other organs is discussed in Vol. 2, Liver and biliary system. Mild brown discoloration of renal cortex, liver, and lymph nodes, and yellow-brown discoloration of gray matter of brain and spinal cord occur in sheep with the *acquired lipofuscin storage disease* caused by poisoning with the plant *Trachyandra divaricata*.

Cloisonné kidney is a nonclinical pigmentary condition in goats. The renal cortices are uniformly brown or black, due to thickening and *brown pigmentation of basement membranes* restricted to the convoluted portions of the proximal tubules (Fig. 4.54B). The basement membrane thickening is due to the deposition of *ferritin and*

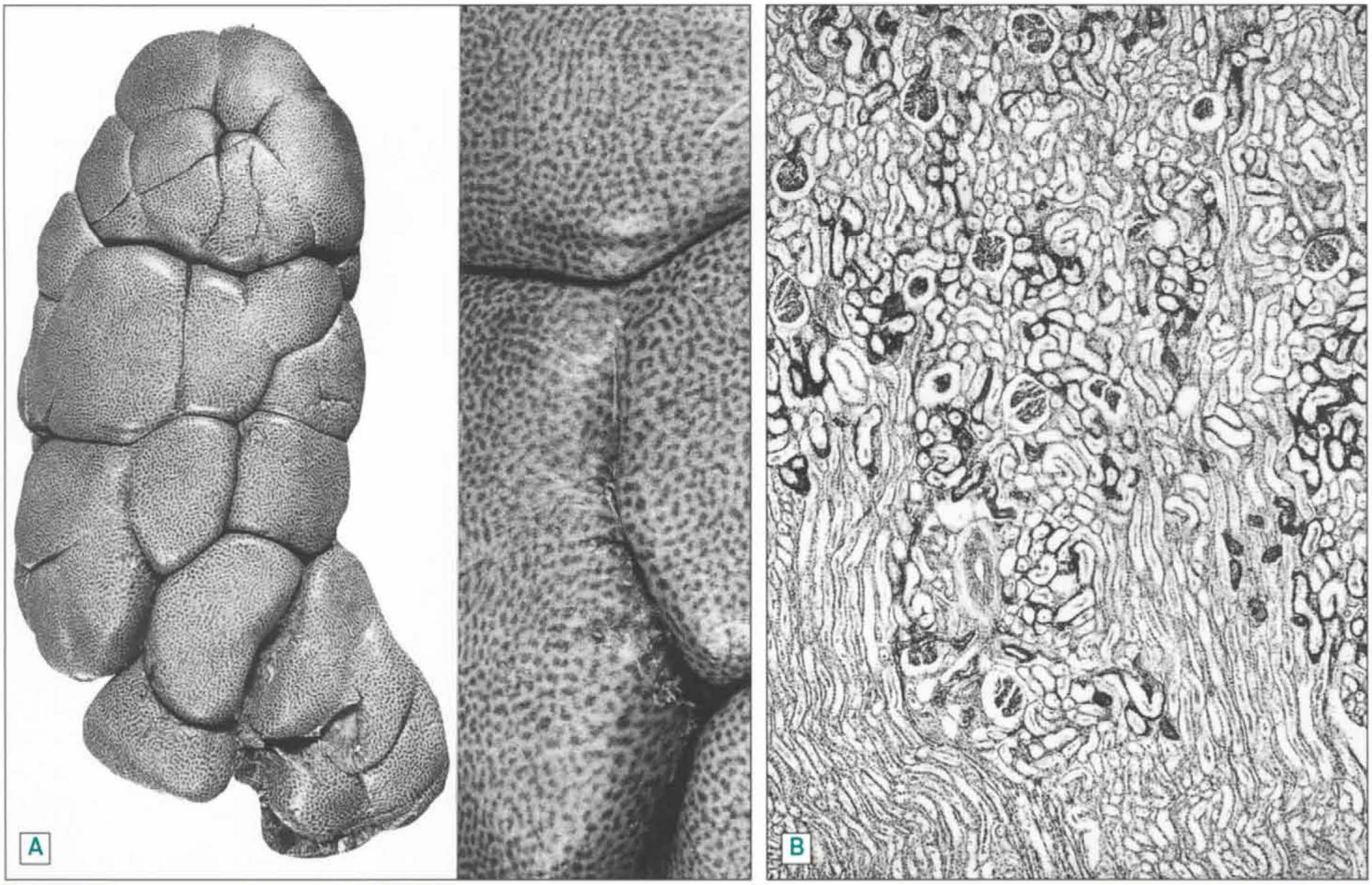

Figure 4.54 A. Lipofuscinosis in a bovine kidney. Inset: close-up of subcapsular surface. **B. Cloisonné kidney** from a goat. (Courtesy of A Zubaidy.)

hemosiderin, which is presumably the result of repeated episodes of intravascular hemolysis. A similar renal discoloration and basement membrane thickening has been reported in a horse, but was found to be due to lipofuscinosis.

In congenital **porphyria** of cattle, swine, and cats, *brown pigmentation* affects the cortex. Histologically, the pigment is present in the tubular epithelium and the interstitial tissue. The pigment is excreted in the urine that, if allowed to stand in light, develops a port-wine color due to photic activation of porphyrins. The urine and tissues fluoresce blue-green in ultraviolet light.

A *green-yellow pigmentation* of swollen kidneys is common in **icterus** of hepatic origin and less notable in hemolytic icterus unless there is concomitant hepatic injury. It is described below in the section on Hepatorenal syndromes. Olive-green coloration of the renal cortex is common in newborn lambs, calves, and foals. The pigment is bilirubin and its presence is probably due to immaturity of hepatic conjugating mechanisms.

Light green to green-yellow discoloration of liver, and hepatic, renal, and other lymph nodes is occasionally observed in cattle at slaughter. Histologically, numerous brown acicular crystals are present in the cytoplasm of hepatocytes, macrophages, and renal tubular epithelial cells. Crystals may be present in renal tubules, and renal calculi are rarely present. The crystals are **2,8-dihydroxyadenine**, a metabolite of the purine adenine. The crystal deposition and pigmentation are of little significance, and the cause of this condition is unknown.

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Hepatorenal syndromes

The term **hepatorenal syndrome** is applied to *functional renal failure that occurs in human patients with cirrhosis and ascites*; an exact parallel has not yet been identified in domestic animals. Marked renal hypoperfusion results from renal vasoconstriction, which may be a manifestation of underfilling of the arterial circulation that occurs subsequent to splanchnic vasodilation. Cirrhosis leads to an imbalance of vasodilators, e.g., nitric oxide, and vasoconstrictors, e.g., endothelin. Although the kidneys themselves have minimal pathologic changes, prognosis is grave.

Hypotension may result from the vasodepressor, cardiodepressor, and diuretic effects of cholemia; thus, the uremia in jaundiced patients may be of prerenal origin. Concentrations of bilirubin and bile acids may be greatly elevated in the blood, especially in obstructive jaundice, and the pigment accumulates in tubular epithelial cells, which are frequently swollen and hydropic ("**cholemic nephrosis**"); the actual toxicity of bilirubin and bile salts is still under debate. Some nephrotoxins are also (or mainly) hepatotoxic, e.g., carbon tetrachloride and *Lantana camara*.

Pseudohepatorenal syndromes include conditions in which liver and kidneys are both affected, but in which renal disease is not secondary to hepatic disease; included are a wide variety of infectious, circulatory, genetic, and other systemic disorders.

Glycogen nephrosis

In diabetes mellitus in dogs and cats, glycogen is deposited in the tubular epithelium, producing marked vacuolation of the epithelium in the outer medulla and inner cortex. Glycogen can be readily demonstrated by appropriate techniques. The deposition occurs in the ascending limb, disappears following insulin administration, and has no effect on renal function. Often in diabetes mellitus of long duration there is hyaline thickening of the afferent arteriole, which may extend to the capillary basement membranes of the glomerulus.

Nephrogenic diabetes insipidus

This condition has been reported in dogs and foals with polyuria, polydipsia, and hyposthenuria. Affected animals are unresponsive to water deprivation, to exogenous administration of antidiuretic hormone (vasopressin), and to infusion of hypertonic saline. The basis of the defect is a *lack of responsiveness of the cells of the distal tubules and collecting ducts to the hormone*. The defect may be *congenital* (possible X-linked disorder in two foals), or *acquired* as the result of tubulointerstitial diseases such as pyelonephritis or hypercalcemic nephropathy, or due to the effect of drugs such as tetracycline.

Hypokalemic nephropathy

Chronic potassium depletion, caused by diarrhea, adrenal overactivity, and some renal diseases, can result in impaired urine concentration and polyuria. *Coarse vacuolation of the proximal convoluted tubules* is due to reversible dilation of intercellular spaces. Chronic potassium depletion can lead to lymphoplasmacytic tubulointerstitial nephritis and interstitial fibrosis, perhaps due to increased renal ammoniagenesis. Vacuolar degeneration of proximal tubular cells has been reported in ewes deliberately given 11–17 times the recommended dose of *thiabendazole*. Many of the ewes developed hypokalemia, hypoproteinemia, and uremia before death; potassium loss was thought to occur through the kidneys. In a possible case of hypokalemic nephropathy in a dog, *chronic vomition* due to a gastric foreign body was thought to be the cause of potassium depletion. *Corticosteroids* produce hypokalemia and reversible vacuolar degeneration of tubular cells. Hypokalemia may be related to the tubular dilation commonly seen in baby pigs with *diarrhea*, and is related to experimental production of renal cysts.

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Miscellaneous tubular conditions

In addition to the standard patterns of parenchymal cell degeneration, there are several miscellaneous histologic changes that may be found in renal epithelium. *Some of the* **pyrrolizidine alkaloids** *cause megalocytosis in the proximal tubules*. The lesion is similar in type to that in hepatocytes but much less conspicuous and not observed to cause functional disturbance. Some pyrrolizidine alkaloid-containing plants, particularly *Crotalaria* spp., cause significant glomerular megalocytosis and sclerosis in pigs (see Vol. 2, Liver and biliary system).

Occasional nuclei in tubules of old dogs may be polyploid. *In the proximal tubules, eosinophilic, crystalline, intranuclear inclusions* ("**brick inclusions**") *are commonly encountered in old dogs.* Similar inclusions occur in the liver, their source is unknown and, although they resemble the inclusions produced in experimental animals by heavy metals such as bismuth, *they do not contain heavy metals*. In cells in the same location, *subacute* **lead poisoning** *produces amorphous acid-fast inclusion material in nuclei that are large, pale, and vesicular.*

Large, eosinophilic, intranuclear inclusions occur in hepatocytes and renal collecting duct epithelial cells in goats. The pathogenesis of this **nuclear glycogenosis** is unknown, and it does not appear to be of functional significance. Nuclear hyperglycogenation occurs in humans with type I glycogen storage disease.

Eosinophilic intracytoplasmic inclusion bodies have been reported in the renal collecting duct epithelial cells of dogs. The inclusions consisted of **iridovirus** particles, and the infection appeared not to be significant.

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TUBULOINTERSTITIAL DISEASES

This term comprises *diseases that involve primarily the interstitium and tubules*, and acknowledges that inflammatory and degenerative diseases of the interstitium almost always impair tubular function. Hence, **interstitial nephritis**, which may be acute or chronic, focal or generalized, suppurative or nonsuppurative, and **pyelonephritis** are classified as tubulointerstitial diseases. There is obviously overlap with the previous category of acute tubular necrosis, since animals that survive acute tubular necrosis often develop interstitial inflammation and fibrosis. Also included as tubulointerstitial disease may be nephropathy due to any of hypercalcemia, hypokalemia, analgesics, and oxalates, as well as immunologically mediated tubulointerstitial disease. In humans, and likely also in domestic animals, *tubulointerstitial nephritis can be caused by a vast array of agents*, including infections, toxins, immunologic disorders, chemicals, and therapeutic drugs (Fig. 4.55).

Interstitial inflammation and fibrosis and tubular atrophy and degeneration are predominant histologic features of these diseases. Interstitial nephritis is usually hematogenous and part of systemic disease, or it may occur secondary to glomerular disease; pyelonephritis is usually urogenous. Both interstitial nephritis and pyelonephritis are usually due to infectious agents, although agents are often not identified, especially in chronic cases. Acute renal injury, including glomerular injury, leads to release of a wide array of cytokines and growth factors from tubular epithelial cells and peritubular capillaries, causing interstitial inflammation and ongoing tubulointerstitial damage. Monocytes are recruited, interstitial fibroblasts are activated,

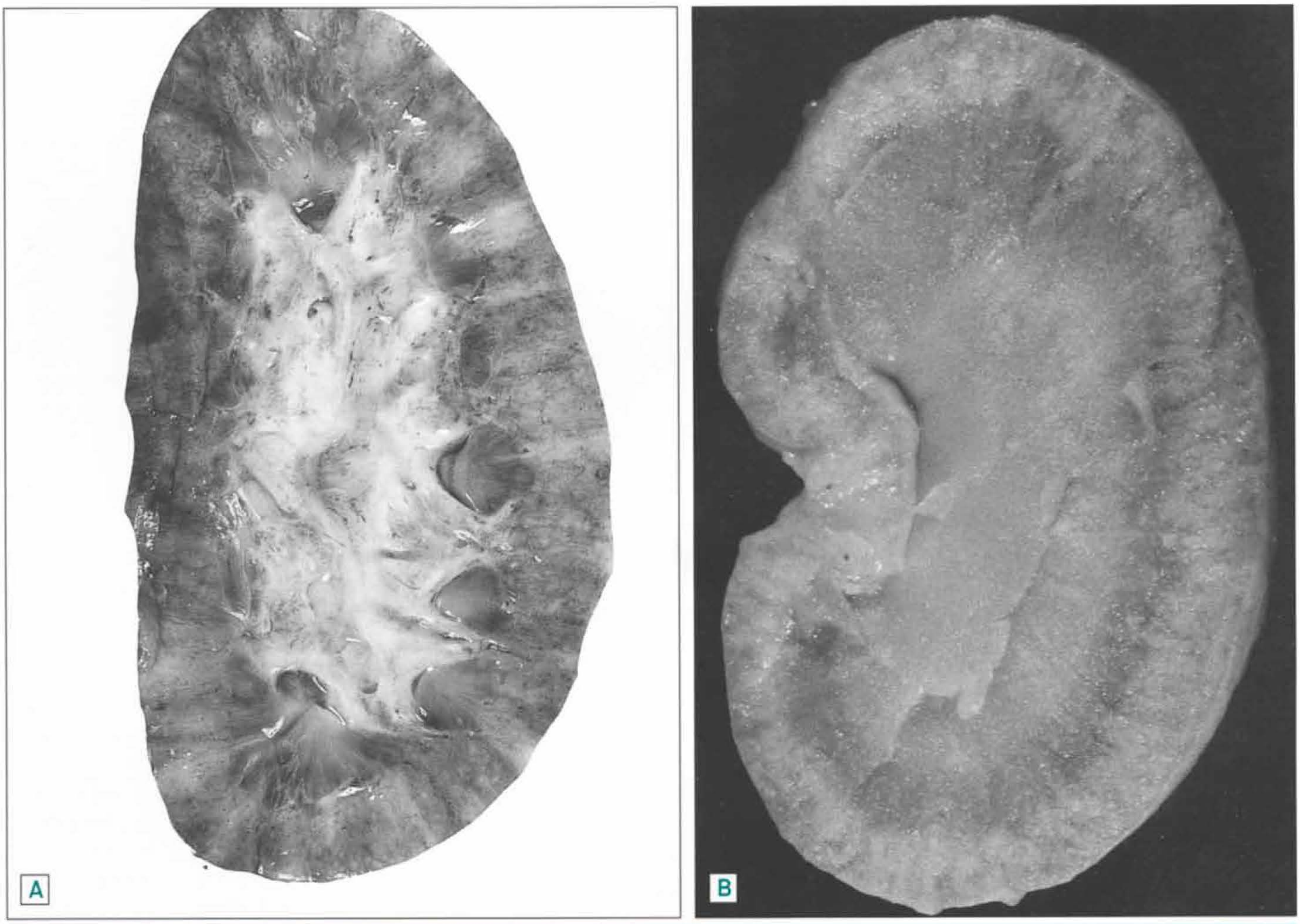

Figure 4.55 A. Acute interstitial nephritis in a pig. **B.** Chronic renal fibrosis and mineralization (**"end-stage kidney"**) in a Shih Tzu dog.

interstitial myofibroblasts appear, and fibrosis ensues. Primary glomerulopathy can incite the above sequence of events through the action of filtered proteins on tubular epithelial cells. Injury to glomeruli and vessels may of course also occur secondarily in tubulointerstitial disease because of the interdependence of renal structures. In any case, *the process of destructive fibrosis and loss of nephrons underlies progressive renal disease*.

The hallmark of glomerular disease is persistent proteinuria, whereas tubulointerstitial diseases are more likely to demonstrate defects of concentrating ability or specific tubular defects of resorption or secretion. However, the endpoint of both classes of renal disease and the usual clinical presentation is decompensated renal failure with isosthenuria and uremia.

Immunologically mediated tubulointerstitial disease has been identified in humans, and rarely in domestic animals. Hypersensitivity reactions occur to a variety of drugs, e.g., methicillin. Tubular immune-complex disease occurs in some patients with lupus nephritis and glomerulonephritis, indicating that autoantibodies may cross-react with glomerular and tubular basement membranes; antitubular basement membrane autoantibody has been identified in a dog.

Nonsuppurative interstitial nephritis

Focal inflammation with slight scarring and accumulations of lymphohistiocytic cells is common in kidneys. The causes are seldom known and probably not specific; some lesions may be primarily inflammatory and some may be foci of antigen persistence in scars of other origins.

As a form of nephritis, this may be acute or chronic, and multifocal or generalized, probably depending upon the intensity of the insult and the efficiency of the host's responses. **Acute interstitial nephritis** *is characterized by acute clinical onset, and histologically by interstitial edema, leukocytic infiltration, and focal tubular necrosis*. In **chronic interstitial nephritis**, *there is mononuclear cell infiltration, interstitial fibrosis, and generalized tubular atrophy*. Many infectious agents are capable of causing nonsuppurative interstitial nephritis, and some are discussed here, but agents are often not identified in naturally occurring cases, especially in the chronic stages.

Interstitial nephritis is common in dogs and cats, both as a primary disease and secondary to glomerular diseases. *Leptospira* serovar *canicola* has commonly been associated with acute generalized interstitial nephritis in dogs, although its importance has declined because of the efficacy of vaccination; serovar *icterohaemorrhagiae* and *Canine adenovirus 1* (infectious canine hepatitis) are also commonly involved. According to serologic evidence, leptospirosis is common in domestic animals and, in severe cases, interstitial nephritis often occurs. In survivors of acute infections, inflammatory cells gradually disappear, marked interstitial fibrosis and loss of nephrons cause contracture of the kidney, and chronic interstitial nephritis results. Diffuse interstitial nephritis is less common in large domestic animals than in dogs; the usual end-result of leptospirosis in cattle and swine is multifocal interstitial nephritis. Leptospirosis is discussed in detail below.

Encephalitozoon cuniculi, an obligate intracellular microsporidian parasite, causes diffuse nonsuppurative to granulomatous interstitial nephritis and granulomatous encephalitis in immature dogs. The renal lesion is characterized by heavy, almost pure, interstitial infiltrates of plasma cells. The gram-positive organisms occur in tubular epithelial cells, tubular lumina, and in vessel walls. The disease is discussed in Vol. 1, Nervous system.

The best-known form of multifocal nonsuppurative interstitial nephritis is the "*white-spotted kidney*" of calves. This is common and appears to be of little significance because it is largely an incidental finding in young calves and is probably obliterated with advancing age. The cause is usually undetermined but is most likely the result of bacteremia; *Escherichia coli* can occasionally be recovered from the lesions. *Salmonella* and *Brucella* are other suggested causes. Affected kidneys contain multiple small white nodules of up to 1 cm diameter throughout the cortex. The larger nodules may bulge from the capsular surface of the kidney and adhere to the capsule (Fig. 4.56A). Histologically, the initial lesion is a microabscess, which is soon replaced by numerous lymphocytes and occasional plasma cells and macrophages. Progressive fibrosis results in healing by scar tissue. The inflammation and scarring may cause tubular obstruction and/or atrophy (Fig. 4.56B).

Multifocal interstitial nephritis also occurs in cattle in the course of *malignant catarrhal fever, theileriosis, lumpy-skin disease*, and as a fatal *autosomal recessive disorder* in Japanese Black cattle (Wagyu); in sheep, in *sheep-pox*; and in the horse, in *equine infectious anemia*. The renal lesions do not contribute significantly to the course of these diseases but are of some diagnostic importance; their gross and histological features are given elsewhere. Multifocal pyogranulomatous lesions are a rather consistent finding in the kidneys of cats with *feline infectious peritonitis*. *Canine herpesvirus* produces severe necrotizing nephritis as part of the systemic disease in puppies (see Vol. 3, Female genital system). Other causes of nonsuppurative interstitial nephritis in dogs include *Leishmania* spp., *Borrelia burgdorferi, Hepatozoon canis*, and *Canine adenovirus 1*.

Cattle and horses grazing *vetch* (*Vicia* spp.) can develop dermatitis, diarrhea, and ill-thrift and develop lesions in skin, kidney, and other internal organs, characterized by multifocal eosinophilic granulomatous inflammation. Similar lymphocytic to lymphogranulomatous lesions are reported in cattle fed *citrus pulp*.

Suppurative interstitial nephritis

Bacterial infection of the kidneys may be either **hematogenous** (Fig. 4.57), and cause embolic suppurative nephritis, or **urogenous**, and cause pyelonephritis. Pyelonephritis means inflammation of both the pelvis and renal parenchyma, and, while pelvic inflammation may result from septic renal foci (Fig. 4.58), most cases of pyelonephritis arise from ascending urinary infections.

Adenoviral infection of sheep is most significant as a cause of pneumonia, but also causes mild, multifocal, suppurative interstitial nephritis, with characteristic large, basophilic, intranuclear inclusion bodies in endothelial and/or interstitial cells.

Embolic suppurative nephritis

This is analogous to abscess formation in any organ, and occurs when any of a wide variety of bacteria is seeded in the kidneys in the course of bacteremia or septic thromboembolism. Bacteria alone or in small clumps, and small septic emboli, lodge mainly in glomerular and peritubular capillaries (Figs 4.30A and 4.59), and may produce multiple small abscesses or fewer large ones. Larger emboli lodge in afferent vessels and produce septic infarcts that may be unilateral.

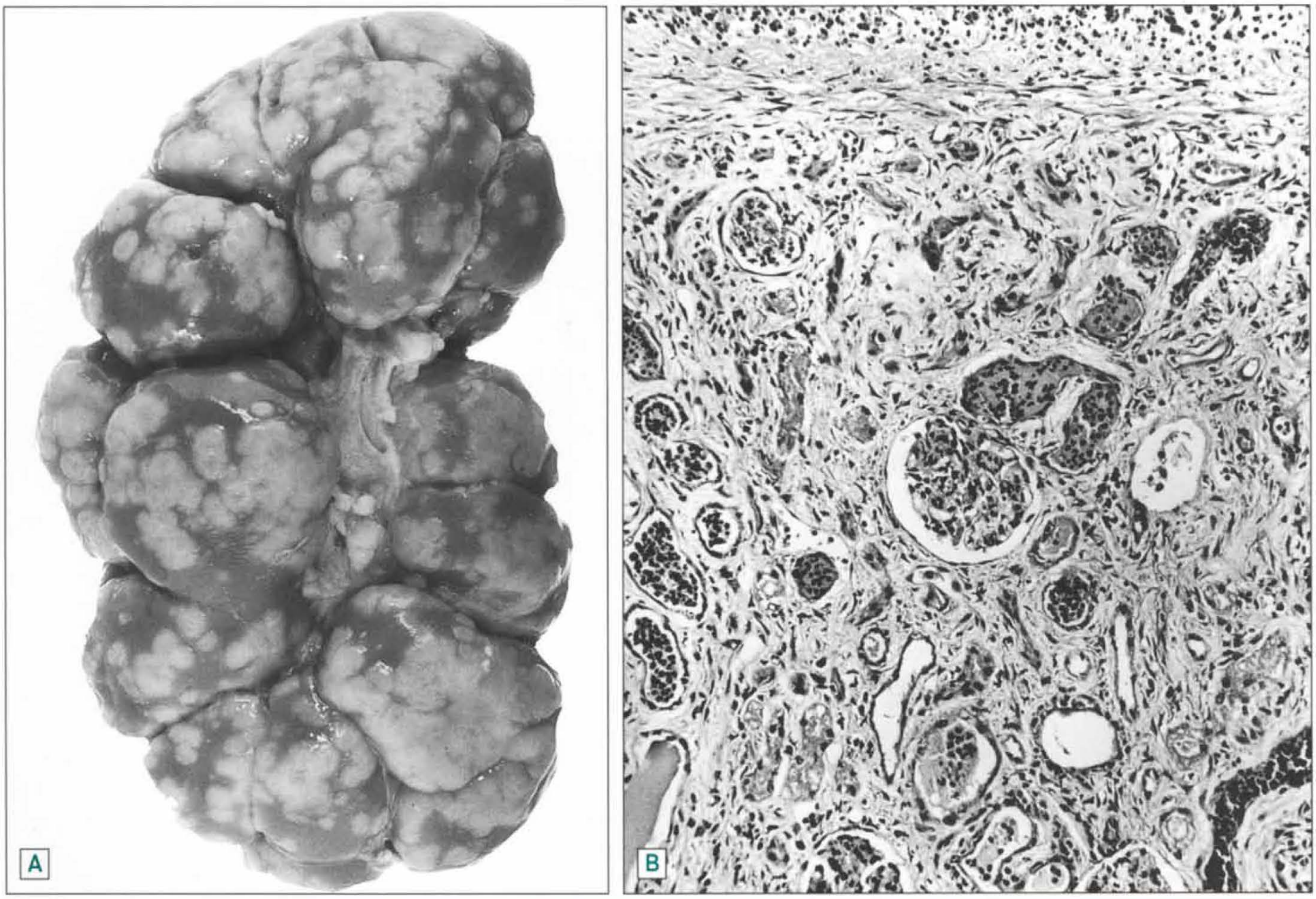

Figure 4.56 A. "White-spotted kidney" from a calf. **B.** Section of cortex in (**A**) showing interstitial reaction.

Many bacteria undoubtedly pass through the glomerular capillary walls into the tubules, where they are probably harmless unless there is stasis of urine. Developed abscesses are generally cortical rather than medullary, but in bacteremia caused by gram-negative enterobacteria, microscopic suppurative foci may be scattered in the medulla. If healing of the suppurative lesions occurs, it is by scar formation. The healed lesions of isolated abscesses are occasionally seen, but extensive renal scarring as a sequel to multiple abscessation is rare as the animal is likely to die of the primary disease or from renal insufficiency early in the course of the disease.

In *horses*, the most common cause of embolic suppurative nephritis is *Actinobacillus equuli,* which is acquired in utero, during parturition, or shortly after birth; it is probably an umbilical infection (Fig. 4.60). Death may occur due to fulminating septicemia. In foals that survive for several days, typical microabscessation is seen in the kidneys and other organs, and polyarthritis is present. The abscesses are usually green-yellow foci of up to 3 mm diameter that contain a small droplet of pus. The most common cause of embolic nephritis in *swine* is probably *Erysipelothrix rhusiopathiae.* Embolic glomerulonephritis may be seen grossly as glomerular hemorrhages; microabscesses form in the interstitium. In adult *cattle*, most cases are caused by *Arcanobacterium pyogenes* from valvular endocarditis, the septic emboli often producing large, randomly distributed abscesses and infarcts. In *sheep and goats*, renal abscessation caused by *Corynebacterium pseudotuberculosis* is common.

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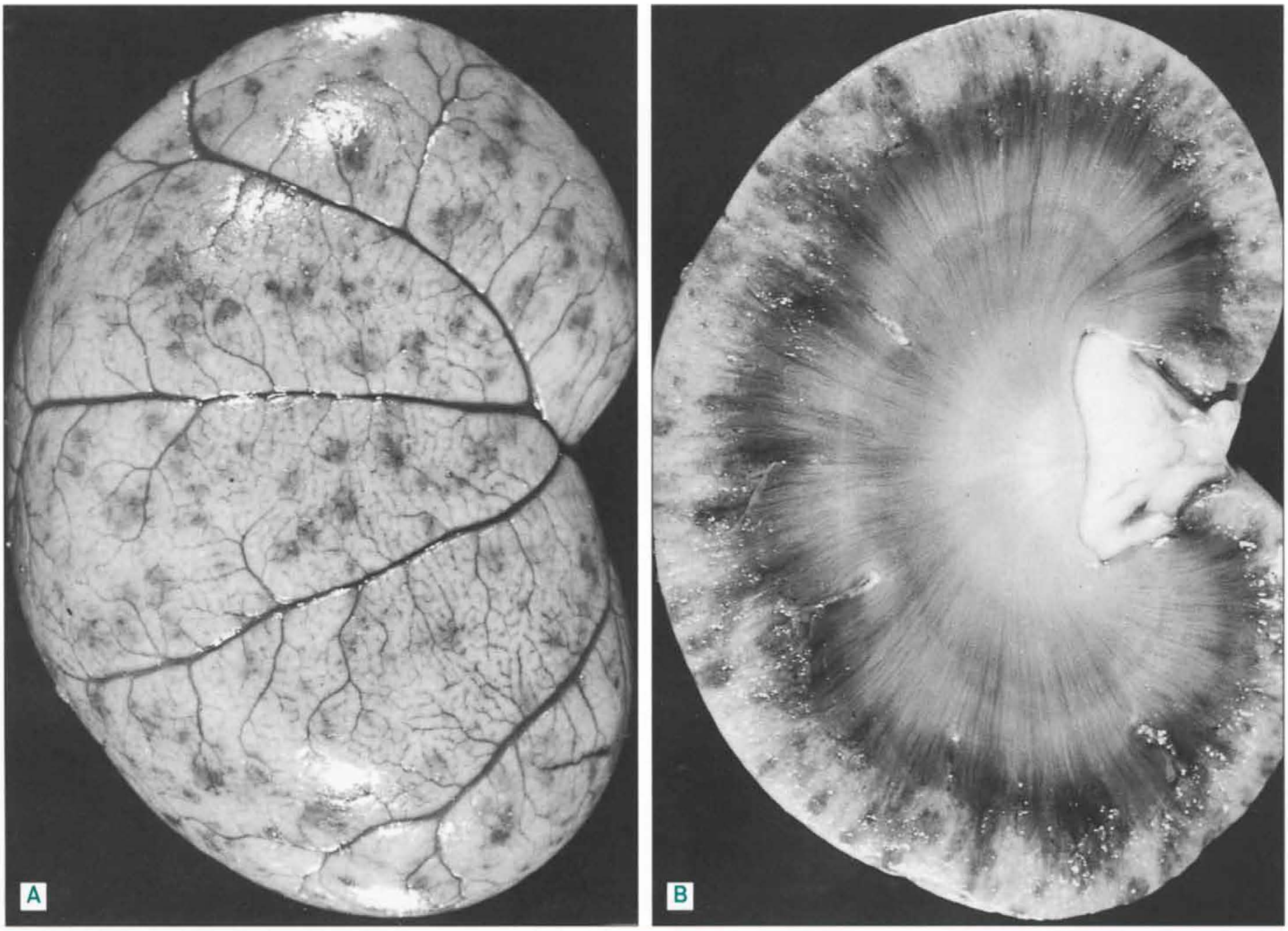

Figure 4.57 A. Multifocal embolic nephritis in a cat with *Pasteurella multocida* bacteremia. **B.** Cut surface of kidney in (**A**).

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Leptospirosis

John F. Prescott

Leptospirosis is an important, largely hidden, complex, neglected, and intriguing spirochetal infection of animals and humans caused by serovars of *Leptospira interrogans* (sensu lato). *It is particularly important as a cause of abortion and stillbirth in farm animals but also causes loss through acute disease (septicemia, hepatitis, nephritis, meningitis) in these and other animals.* Many aspects of leptospirosis are poorly understood because of difficulties in diagnosis, the changing pattern of disease because of vaccination and antibiotic use, and the complexities of host–leptospiral relationships. Although generalizations are helpful, leptospirosis may best be understood in terms of the individual relationship between specific serovars and particular host species rather than as a single disease with a common epidemiology, host response, and approach to control.

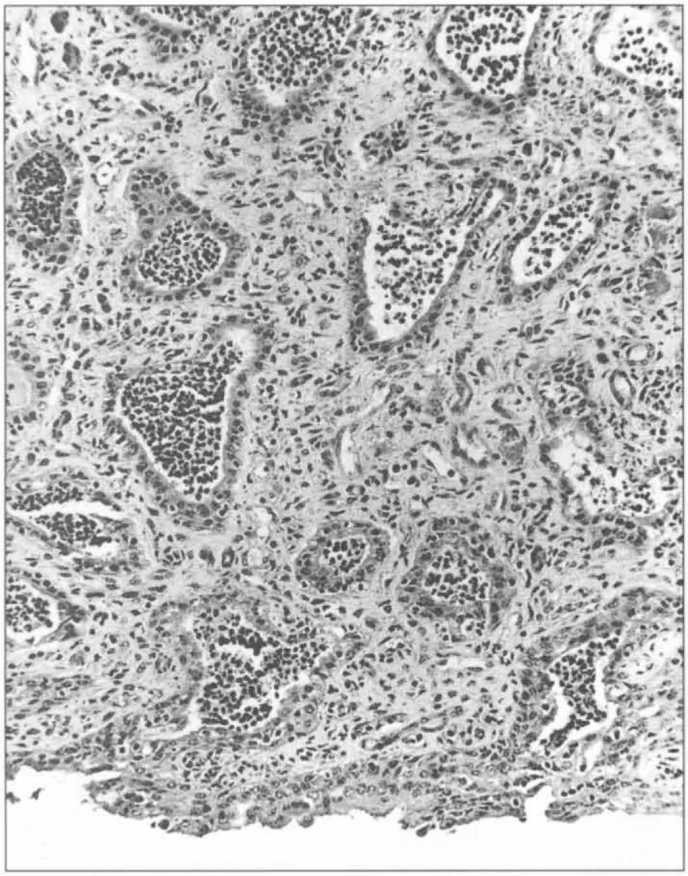

Figure 4.58 Descending pyelonephritis in "white-spotted kidney" from a calf.

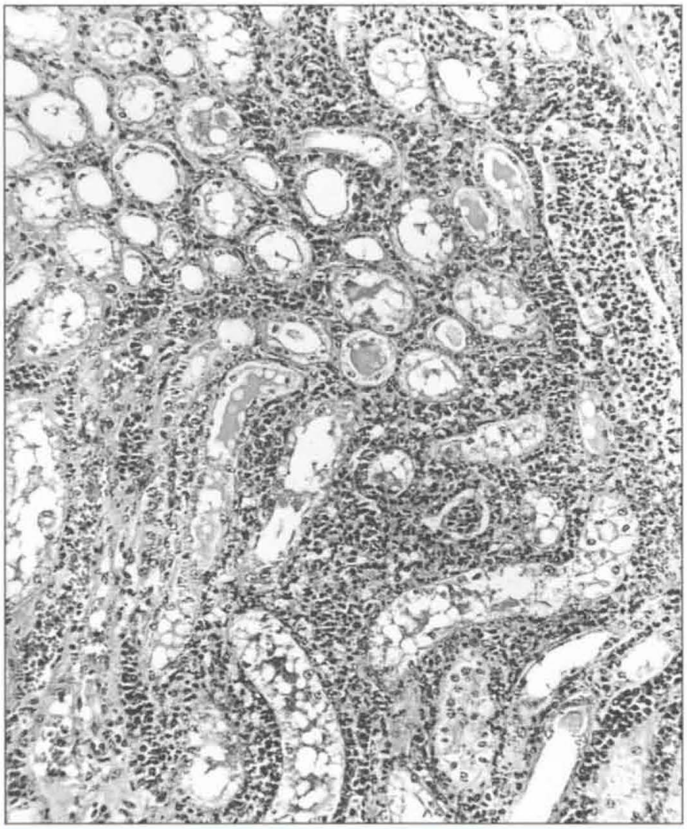

Figure 4.59 Hematogenous interstitial nephritis in a dog with *Klebsiella pneumoniae* bacteremia.

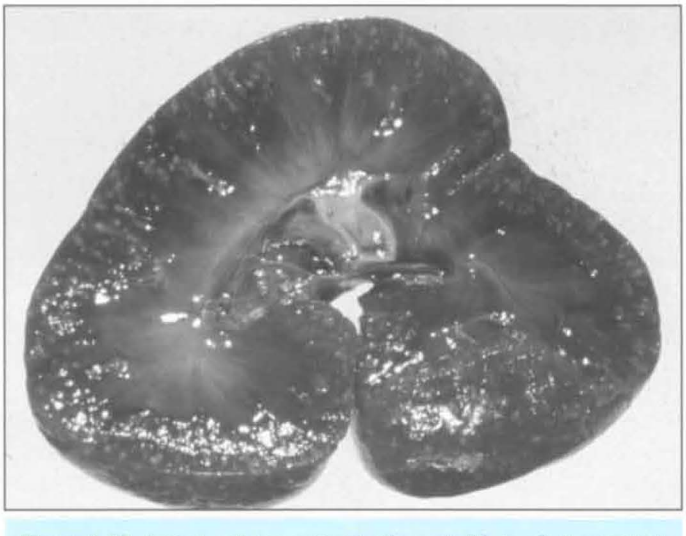

Figure 4.60 *Actinobacillus equuli* **embolic nephritis** in a 2-day-old foal.

Leptospirosis can be caused by any of the 180 serovars belonging to the 19 serogroups of what used to be recognized as one species, *Leptospira interrogans* (sensu lato), but which now contains 10 species of pathogenic *Leptospira*. Unfortunately, neither serogroup nor serovar reliably predicts the species of *Leptospira*, since the same serogroup (i.e., shares some common antigenic determinants) or serovar (i.e., antigenically indistinguishable isolate) may occur within two or more species. Thus, some genetically unrelated leptospires, belonging to one of the 10 species in what used to be described as *L. interrogans*, may be antigenically identical. For example, serovar *hardjo* is found within *L. borgpetersenii*, *L. interrogans*, and *L. meyeri*. *The use of serotyping is increasingly being replaced by restriction enzyme analysis of chromosomal DNA to identify "genotypes."* Thus, using the same example, genotyping has divided serovar *hardjo* into two distinct types, hardjo-bovis and hardjoprajitno, which correspond with the species (*L. borgpetersenii*, *L. interrogans*, respectively) to which these types belong and which differ in both geographic distribution and in virulence. Restriction enzyme analysis has also assigned most North American serovar *pomona* isolates to genotype kennewicki, and further identified pig-associated and cattle-associated subtypes. The scheme used in this section is to use the species and serovar name (e.g., *Leptospira borgpetersenii* serovar *hardjo*) with genetic type where relevant. The current taxonomy of *Leptospira* is confusing, because some leptospirologists are naturally reluctant to abandon the serological scheme, but is described well by Levett (2001).

Although many serovars are recognized globally, only a limited number are usually endemic to a particular region (Table 4.3). Geographic differences in the distribution of serovars are marked, but the true incidence and prevalence of leptospirosis are largely undetermined for most countries and regions. Serological surveys tend to be flawed because antigens chosen may not represent serovars present in the country, because serological prevalence does not necessarily indicate disease significance, because sampling is often done on the basis of convenience rather than in a carefully designed manner, and because titers designated as "significant" (usually ≥1:100 in the

Table 4.3 Common leptospiral serovars of disease significance in domestic animals

Serovar	Maintenance host	Distribution	Species disease significance
autumnalis	Rodents	Global, tropics especially	+ ?Dogs
bratislava	Pigs, horses (dogs?)	Global	++++ Pigs +++ Horses, dogs
canicola	Dogs	Global	+++ Dogs + All species
grippotyphosa	Raccoon, skunk, small rodents	Global	++ Dogs + All species
hardjo type hardjo-bovis	Cattle, sheep	Global	++ Cattle, sheep
type hardjoprajitno	Cattle	Global? (some exceptions)	+++ Cattle, sheep
icterohaemorrhagiae	Rats	Global? (some exceptions)	+++ Dogs, humans
pomona type kennewicki	Pigs	Global? (some exceptions)	++++ Pigs +++ Cattle, all species
tarassovi	Wildlife, cattle, pigs	Europe, Australasia	+++ Pigs

Table 4.4 General distinctions between maintenance and incidental hosts in leptospiral infection

Characteristic	Maintenance host	Incidental host
Susceptibility to infection by the serovar	++++[a]	++
Endemic transmission within host species	++++	+
Pathogenicity for the host	++	+++
Type of disease caused	Chronic, reproductive loss especially, nephritis	Acute or chronic
Persistence in kidney	++++	+
Persistence in genital tract	+++ (*bratislava, hardjo*)	–
Microscopic agglutinating antibody response	Low titer, sometimes nil	High titer
Efficacy of vaccination in controlling losses	± to ++++	++++
Examples:	Pig, *bratislava, pomona*; Dog, *canicola*	Pigs and cattle, *canicola, grippotyphosa*; Cattle, *pomona*

[a] *Arbitrary scale for comparison.*

microscopic agglutination test, MAT) may underestimate the true seroprevalence of some host-adapted serovars.

Each serovar is adapted to and may cause disease, in particular "maintenance" hosts, although they may cause disease in any other species, the "incidental" hosts. The general distinctions between these types of host are shown in Table 4.4. For some serovars and hosts, there may also be "intermediate" forms between the two major categories of host–parasite relations, because the phenomenon of host adaptation appears to be an ongoing characteristic of pathogenic leptospires.

The natural reservoir of pathogenic leptospires is the proximal convoluted tubules of the kidney, and in certain maintenance hosts the genital tract. In maintenance hosts particularly, transmission may be direct, through urine splashing, in postabortion discharges, venereally, through milk, or transplacentally in congenitally affected animals. Infection of incidental hosts is more commonly indirect, through environmental contamination by the urine of carrier animals. Close contact of maintenance hosts aids spread but, especially for incidental hosts, environmental conditions determine the success of indirect transmission. Optimal conditions for survival of leptospires are moist, warm (optimal 28°C), and neutral or mildly alkaline conditions ("flood fever," "swamp fever"). Under ideal conditions, leptospires may survive weeks or months in waterlogged soil ("mud fever") or stagnant water. Under adverse conditions, survival is measured in minutes. *Leptospirosis thus occurs especially in the autumn in temperate climates ("fall fever"), and in the winter in tropical climates,* seasons that also often coincide with the greatest population sizes of wildlife shedders.

Leptospires penetrate exposed mucosal surfaces or water-softened skin and disseminate throughout the body, in a leptospiremic phase lasting up to 7 days, but multiply especially in the liver, kidneys, lungs, placenta, udder, and cerebrospinal fluid. The development of agglutinating and opsonizing antibody ~6 days after infection clears the organisms from sites other than those that immunoglobulins penetrate poorly (proximal convoluted tubules of the kidney, cerebrospinal fluid, vitreous humor of the eye), and, for certain serovars, the genital tract (oviducts, uterus, vagina in females, testes, epididymes, prostate, and seminal vesicles in males) of maintenance hosts.

Most leptospiral infections are supposedly subclinical, particularly in nonpregnant and nonlactating animals, only detected by the presence of antibody or of *minor lesions of interstitial nephritis at slaughter.*

Infection may also cause *acute or subacute systemic disease* (nephritis, hepatitis, endotoxemia, sometimes hemoglobinuria) during the leptospiremic phase or, after leptospiremia has ceased, *chronic disease* in the form of abortion or stillbirth, infertility, or recurrent uveitis.

Acute and often severe disease may occur in the leptospiremic phase, particularly in young animals. *Jaundice is a common manifestation of acute disease* and occurs because of hemolysis due to hemolysin production, and because of hepatocellular injury of both toxic and ischemic origin. The clinical disease is characterized by fever, jaundice, hemolytic anemia, hemoglobinuria, pulmonary congestion, and occasionally meningitis. Capillary injury caused by inflammatory cytokine release occurs in acute disease. Anemia is initially the result of *hemolysins* but may later be due to *antibodies* causing an intravascular hemolytic anemia by reaction with leptospiral products coating red blood cells. *Postsepticemic localization of leptospires in the kidneys is associated with focal or diffuse interstitial nephritis and with acute, transient tubular degeneration.*

Renal failure is a clinically recognized consequence of infection with *canicola* and other serovars in dogs but is not often described in other animals, even though interstitial nephritis, especially in pigs, can be extensive. *Interstitial nephritis can be caused by all serovars.* Leptospires reach the kidney hematogenously and migrate randomly, persist briefly in the interstitial spaces, and enter tubules at all levels of the nephron. Once antibody develops, the leptospires localize to the proximal convoluted tubules, where they may multiply. The physiological changes to glomerular filtrate lower in the nephron, and in formed urine, damage leptospires. The *interstitial phase* is accompanied by marked vascular alterations that produce hyperemia, edema, and endothelial swelling. Tubular epithelial degeneration is likely the combined result of hypoxia due to hypovolemia and red cell loss, of poorly described bacterial toxins including lipooligosaccharides and sphingomyelinases, of free hemoglobin, and of the interstitial inflammatory reaction to leptospires. Leptospiral antigen is demonstrable by immunoperoxidase staining in epithelial cell phagosomes, and is the counterpart of minute, round, black spherical bodies seen with silver stains. Tubular degeneration is well established by 2 weeks and is accompanied by *interstitial infiltration of plasma cells and lymphocytes*, which may be focal or diffuse. Leptospiral antigen is present in peritubular macrophages. The renal lesions may be insignificant, or may cause chronic debility or death in uremia.

Abortion, stillbirth, or the birth of congenitally infected young may follow weeks or months after maternal leptospiremia and is the most important form of the disease in ruminants and pigs. Serovar *bratislava* may also cause infertility in swine, as may serovar *hardjo* infection in cattle. Horses are particularly likely to develop *recurrent uveitis* ("periodic ophthalmia") following serovar *pomona* infection, but uveitis can develop in other species following leptospirosis.

Leptospires are delicate, slender spirochetes (6–20 × 0.1 μm), with hooked ends like question marks, which led to the name *L. interrogans*. They do not stain with the usual aniline bacterial dyes but may be stained by Giemsa or, in tissue, by silver-impregnation techniques of Levaditi or Warthin–Starry. Dark-field microscopy is generally used in routine laboratory study. *Demonstration of leptospires by dark-field microscopy of fluids, or silver staining of tissue sections, are insensitive methods that give both false-negative and false-positive results*, the latter being a ready trap for the inexperienced. *Immunofluorescence* of urine, or of homogenates of tissues such as fetal lung and kidney or of placenta, is an excellent diagnostic technique, almost equivalent in sensitivity to isolation in experienced laboratories. It is a method of choice in the diagnosis of leptospirosis. Difficulties may be experienced with serovar *bratislava* because of the small size of these organisms. *Polymerase chain reaction (PCR)-based diagnosis* will increasingly challenge immunofluorescence as a diagnostic method of choice. A number of PCR procedures are available which generally are more reliable for urine than for tissues. Leptospires die readily in tissues or body fluids unless kept at 4°C. Results may be improved if tissues are submitted to laboratories in leptospiral transport medium.

Culture is expensive and often time-consuming, and usually restricted to highly specialized laboratories. *The microscopic agglutination test (MAT)*, which is serovar-specific and to some extent serogroup-specific, has been a mainstay of diagnostic approaches to leptospirosis and is often made by comparison of acute and convalescent titers, since it is important to rule out "background" infection in making a serological diagnosis. In the diagnosis of abortion, the MAT suffers sometimes from the low titers that may occur with host-adapted serovars in their maintenance host, and also from difficulties in interpretation of titers. These may fail to rise between acute and convalescent because abortion may follow many weeks after infection in a maintenance host. In fetuses, MAT on fetal fluids is often a useful diagnostic approach, but dilutions should start at 1:10. The diagnostic interpretation of MAT titers is discussed by Bolin and Prescott (1999).

Cattle

Species and serovars of major importance are *L. borgpetersenii* serovar *hardjo* type hardjo-bovis and *pomona* type kennewicki in North America, and, in Europe, *L. interrogans* serovar *hardjo* type hardjoprajitno and *L. borgpetersenii* serovar *hardjo* type hardjo-bovis. There is serologic evidence of infection with other serovars, including *canicola*, *grippotyphosa*, and *icterohaemorrhagiae*, among others, but these serovars are not often implicated in disease, although geographic variations will occur. Serovar *hardjo* has become increasingly recognized with a concomitant, apparent decline in importance of serovar *pomona*. Cattle and sheep are reservoir hosts for *hardjo*. Serovar *hardjo* type hardjoprajitno, which is recognized in Europe but not in North America or Australia and New Zealand, appears to be more virulent than type hardjo-bovis. In Northern Ireland, where serovar *hardjo* type hardjoprajitno occurs, *hardjo* was recognized in one study as responsible for nearly half of all abortions. This type was isolated from most aborted or stillborn fetuses, whereas type hardjo-bovis was mainly isolated from the kidney and genital tract of carrier cows. In one large study in Ontario, Canada, where only hardjo-bovis rather than hardjoprajitno is thought to occur, serovar *hardjo* was identified in about 6% of abortions; no serovar *pomona* was recognized.

The most severe (but uncommon) manifestation of acute infection occurs in calves infected with incidental serovars, especially *pomona*. Hemoglobinuria is usually the first sign noticed and may be transient or last 2–3 days. In fatal cases, it gives the urine a port-wine color. Hematuria from hemorrhage into renal tubules may give rise to blood clots in the urinary tract. There is fever, anemia, icterus, dyspnea because of pulmonary congestion, and occasionally meningitis. High levels of albumin and bilirubin are present in the urine. In cows, agalactia with small quantities of blood-tinged milk, which may be yellow-stained and thickened like colostrum, is also typical. Abortion may occur during the acute phase or several weeks later during convalescence. The fetus is frequently decomposed, indicating death some time before abortion.

The most common form of the disease is a less severe, "subacute" form characterized in dairy cows by a 2–10-day drop in milk production with transient pyrexia. In this "milk drop syndrome" or "flabby udder mastitis" form, the milk has the consistency of colostrum, with thick clots and yellow staining, and the udder has a soft texture. This form is commonly associated with serovar *hardjo* type hardjoprajitno, but may be caused by all serovars.

The chronic form of the disease, most commonly associated with serovars *hardjo* and *pomona*, is fetal infection in pregnant cows, seen as abortion, stillbirth, or the birth of premature and weak infected calves. Abortion and stillbirth is often the only manifestation of infection, but may be related to an episode of illness up to 6 weeks (*pomona*) or 12 weeks (*hardjo*) earlier. Serovar *hardjo* type hardjoprajitno appears to be more virulent, and to cause more abortion, than *hardjo* type hardjo-bovis.

The hematologic changes are mild in cases not characterized by hemoglobinuria, and severe in those that are. Animals with *acute intravascular hemolysis* will have moderate to marked anemia, but hemoglobin levels may be erroneously high because of the measurement of free hemoglobin associated with hemoglobinemia.

The postmortem appearance of an animal that dies of acute leptospirosis is characterized by mild icterus and severe anemia. Hemorrhages may be absent, or ecchymoses may be numerous on serous membranes and in the subcutis. The lungs are pale, edematous, and expanded, and fluid, stained by bilirubin, widens the septa. The liver is enlarged, friable, anemic, and bile-stained. It may contain hemorrhages and small zones of necrosis about the central veins, but necrotic foci are not usually seen grossly. Hemoglobinuria is to be expected but may not be present. The kidneys are swollen; during a hemolytic crisis they are dark, but later the pigmented foci become restricted to small groups of tubules and have an appearance suggestive of numerous small hemorrhages. Still later, the kidneys show more or less numerous small gray foci of interstitial reaction that are rather indistinct and more numerous in the cortex than in the medulla (Fig. 4.61). It is doubtful whether cattle die of chronic interstitial nephritis caused by leptospires, but in animals that have recovered from the acute diseases, foci of renal inflammation are the only morphologic traces remaining. "White-spotted" kidneys in neonatal calves may be the result of congenital leptospirosis caused by serovar *hardjo*, which is probably common, but this speculation has not been examined.

The histologic changes in bovine leptospirosis are neither prominent nor specific. Edema of the lungs is apparent, and in scattered groups of alveoli and septal lymphatics, there are thin laces of fibrin. Zonal necrosis occurs in the liver; it is usually periacinar and typically a change of severe anemic anoxia. Kupffer cells are hyperplastic and contain excessive amounts of hemosiderin, and there is diffuse but mild cellular infiltration in the portal triads. In the uncommon infections caused by *icterohaemorrhagiae*, necrosis in the liver and dissociation of the cords of hepatic cells are more likely to occur than in infections caused by the other serovars. If the survival period in the acute hemolytic disease is sufficiently long, the biliary canaliculi become distended with bile.

In the acutely fatal disease, there are often marked degenerative changes in the epithelium of the cortical renal tubules, the changes varying in severity from hydropic swelling to necrosis and desquamation. The desquamated epithelium produces, in the tubules, granular and cellular casts in addition to the hyaline ones of albuminuria and hemoglobinuria and those due to direct bleeding into the tubules. Pigmentation by intracellular hemoglobin and biliary pigments does not become fully evident until 2 days, and the tubules, which are then strikingly involved, occur in clusters. There is also edema of the kidneys, with distension of the interstitial tissues with fluid, and in this there is mild but diffuse infiltration of plasma cells and lymphocytes. *In the acute disease, the organisms can often be demonstrated by appropriate stains in the liver,* in which they are partly intracellular, *and in the kidneys,* in which they occur in the tubular epithelium and frequently as clusters in the tubular lumen (Fig. 4.62).

In recovery from the acute disease and in due course in subclinical illness, the organisms localize in *microcolonies in the kidneys.* The microcolonies are in intratubular clumps; it is exceptional to find organisms in the interstitial tissue. The organisms are shed in the urine. *Renal localization is associated with more or less widespread focal interstitial nephritis* (Fig. 4.63). The nonsuppurative inflammatory reaction is virtually confined to the cortex; lymphocytes and plasma cells predominate. The reaction subsides very slowly, and inflammatory cells decrease in number as the focal lesions scarify. An odd feature of the active lesions is the atypical regenerative pattern of tubular epithelium within the areas of inflammation; the regenerating cells produce a few bizarre syncytia as well as a few giant cells morphologically similar to those of a foreign-body reaction (Fig. 4.63B).

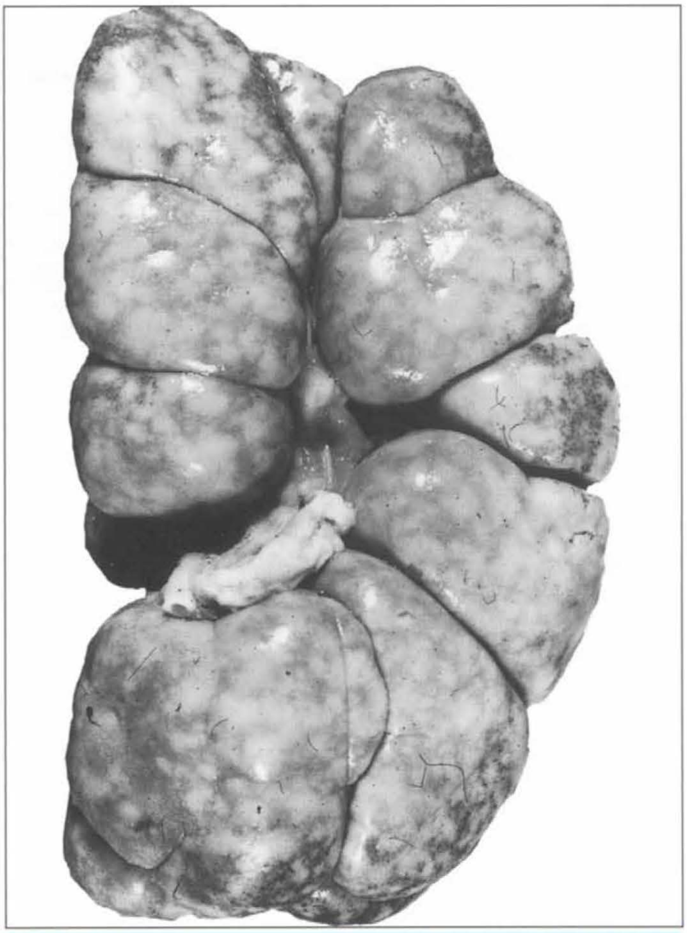

Figure 4.61 Interstitial nephritis caused by *Leptospira* serovar *pomona*, bovine kidney.

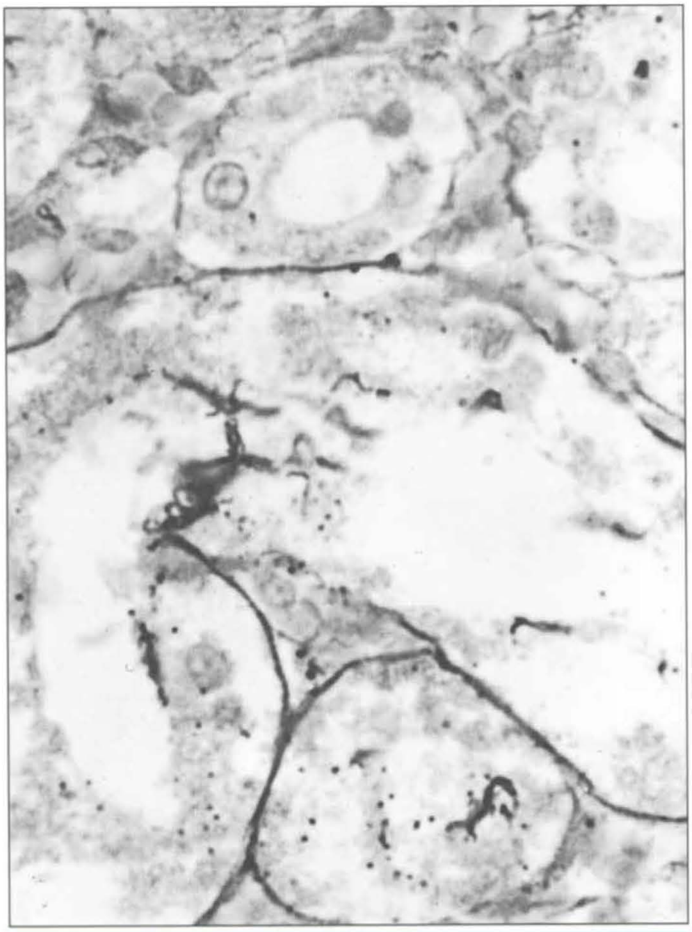

Figure 4.62 Intratubular leptospires in a bovine kidney; silver stain.

Aborted fetuses show no specific changes, although the organism can often be demonstrated in fetal tissues (see Vol. 3, Female genital system). The fetuses are sometimes edematous, but the edema is not specific, and in many cases there is advanced autolysis or putrefaction by the time the fetus is aborted. The placenta may show mild microscopic changes of placentitis; there is a tendency for it to be unduly retained.

Sheep, goats, and deer

Sheep, goats, and deer may be less susceptible than cattle to clinical leptospirosis. The major serovar in sheep is *hardjo*, which is maintained in a maintenance cycle independent of cattle. Other serovars may also cause disease. Acute disease in lambs resembles that described above in calves. *Hardjo* infection of ewes may cause late-term abortion, stillbirth, and weak lambs, and agalactia in recently lambed ewes. Other serovars may sometimes cause abortion. Leptospirosis in goats is not often described, but clinical and pathological changes are similar to those described for cattle. Fatal nephritis of farmed young red deer due to serovar *pomona* has been described in New Zealand. Kidneys were 2–3 times normal size and showed histologic changes of severe chronic active nephritis, characterized by extensive, segmental inflammation of cortical interstitial tissue.

Dogs

Dogs are susceptible to infection with a number of serovars, but those best recognized to cause severe disease are *canicola* and *icterohaemorrhagiae*. *Canicola* is host-maintained in the dog, but widespread use of vaccination has virtually eradicated this infection from dogs in many countries. *Icterohaemorrhagiae* is generally acquired from rats, and other serovars (*autumnalis*, *bratislava*, *pomona*) from their respective hosts (Table 4.3). Serological evidence of infection with serovar *bratislava* is increasingly recognized and this organism may cause nephritis and abortion in dogs; it has been suggested that this serovar is host-adapted to the dog, in addition to the pig and possibly the horse. Clinical infections with serovars *canicola* and *icterohaemorrhagiae* are now usually rare because of vaccination in dogs, but infection with other serovars may be underreported. In recent years, leptospirosis in urban dogs has resurged in northeastern North America and elsewhere, associated with *grippotyphosa*, *pomona*, possibly *autumnalis*, and rarely other serovars acquired from raccoons, from skunks, and to a lesser extent other wildlife found in suburban and urban environments. Clinically normal dogs can shed pathogenic leptospires in urine, which may pose a zoonotic risk; serologic testing is a poor predictor of urinary shedding. Current *canicola*- and *icterohaemorrhagiae*-based vaccines provide only serovar-specific protection.

It is not possible to separate, on the basis of clinical evidence, acute infections caused by different serovars, although, as a generalization, *icterohaemorrhagiae* exerts its effect more specifically on the liver, *canicola* on the kidney, and other serovars in a less severe way on both organs. *Icterohaemorrhagiae* is associated with severe icterus, and *canicola* with severe renal injury, but the syndromes shown by these and other serovars overlap.

Hyperacute infection, which is usually one of puppies and usually caused by *icterohaemorrhagiae*, is acute in onset, with the course of a fulminating septicemia; death occurs in a few hours to 2–3 days. There is fever, dehydration, hypersensitivity, and a marked tendency to hemorrhage, providing for hematemesis, melena, epistaxis, and petechiae on the mucous membranes. In the *acute disease*, in which icterus is a prominent feature, the onset may be sudden, with the development of icterus delayed, or the onset may be insidious, with icterus as the first abnormality observed. There is frequently an oculonasal discharge, which may suggest distemper, especially in younger animals. Body temperature is usually only mildly elevated at the time of clinical evaluation.

Widespread hemorrhages characterize both the hyperacute and the acute disease, although icterus is usually absent in the former. The most consistent gross lesions occur in the kidneys, and in acute cases consist of *subcapsular hemorrhages with multifocal ecchymotic and petechial hemorrhages throughout cortical areas but most prominent in subcapsular regions*. With chronicity, these foci become pale and the capsule is increasingly adherent. Residual lesions in dogs given viable *canicola* and killed 100 days later consisted of symmetrically enlarged kidneys with nodular and wedge-shaped lesions 4–8 mm in diameter at the corticomedullary junction. Lesions have been described in the liver of dogs with spontaneous infections of *grippotyphosa* that vary from gross enlargement, with focal lesions, sclerosis, ascites, and atrophy, to fibrosis and nodular hyperplasia.

In animals dying acutely, focal necrosis is frequent in the liver but may not be present. *The characteristic, but not specific, change is dissociation of the cells of the parenchymal cords.* The dissociated cells become discrete

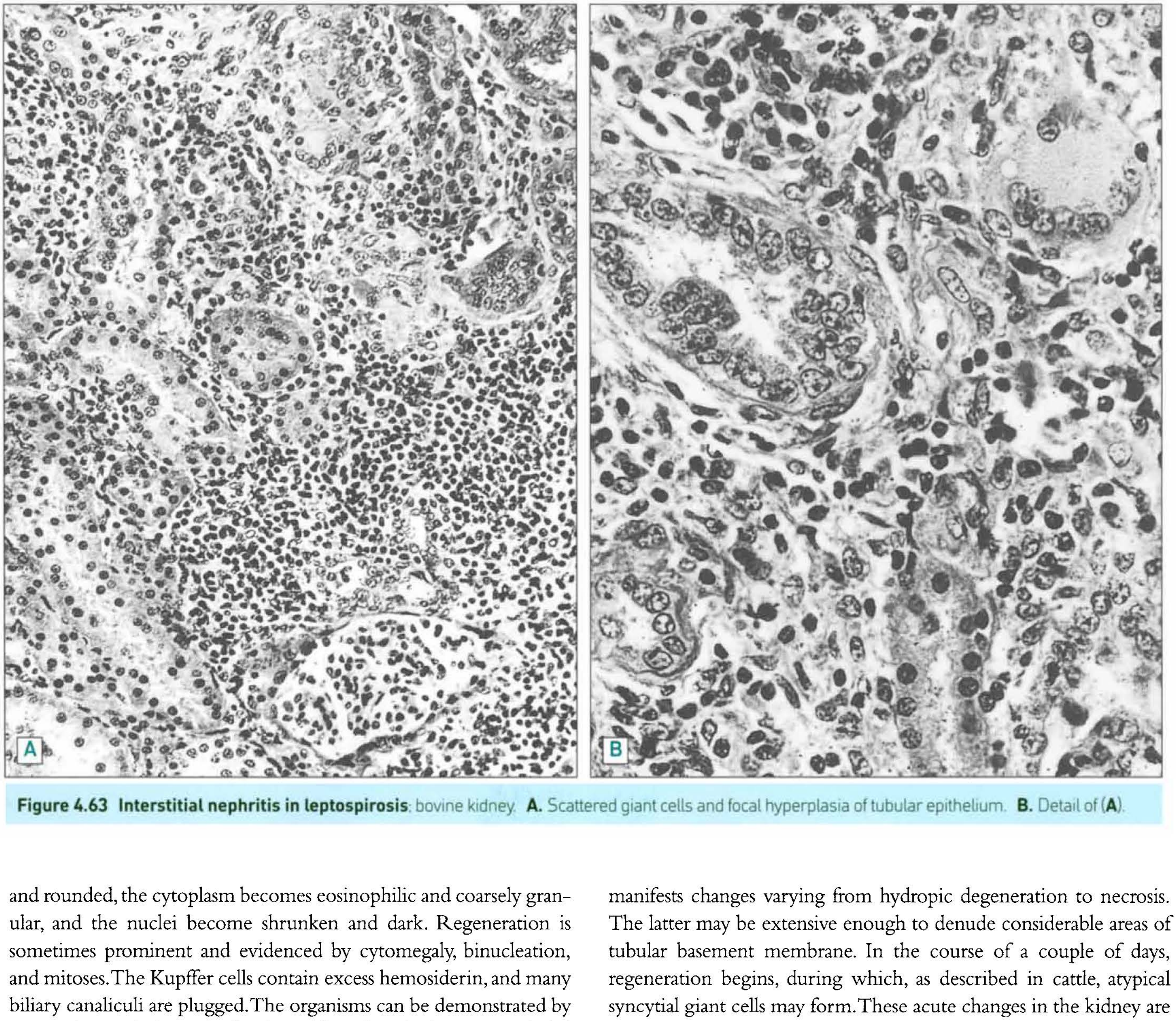

Figure 4.63 Interstitial nephritis in leptospirosis; bovine kidney. **A.** Scattered giant cells and focal hyperplasia of tubular epithelium. **B.** Detail of (**A**).

and rounded, the cytoplasm becomes eosinophilic and coarsely granular, and the nuclei become shrunken and dark. Regeneration is sometimes prominent and evidenced by cytomegaly, binucleation, and mitoses. The Kupffer cells contain excess hemosiderin, and many biliary canaliculi are plugged. The organisms can be demonstrated by appropriate techniques in the sinusoids and in the hepatic epithelial cells. Serovar *grippotyphosa* may cause chronic active hepatitis, with peribiliary fibrosis, and lobular disarray, with irregular fibrosis.

In dogs that survive the acute septicemic, icteric type of disease, or fail to manifest a septicemic phase at all, the clinical and pathological emphasis shifts from the liver to the kidneys. The resultant syndrome is the usual way in which infections by *canicola* and the incidental serovars (*grippotyphosa, pomona*) are manifest. The signs are those of *renal insufficiency* and vary in their severity and rapidity of progression; death may supervene rather rapidly from the renal failure of acute diffuse nephritis, but it also appears that initially inapparent infections, particularly with *canicola*, may set in motion a train of events in the kidneys that, after a long time, possibly many years, results in the renal failure of chronic interstitial nephritis. In these, *the renal syndromes*, the lesion caused directly by the leptospires is *acute diffuse interstitial nephritis*, from which recovery may occur to a focal pattern, or which may progress to *subacute and chronic interstitial nephritis*.

In the acute phase, the renal changes are severe but largely characterized by degeneration rather than by reaction. The brunt of the injury is borne by the convoluted tubules, the epithelium of which manifests changes varying from hydropic degeneration to necrosis. The latter may be extensive enough to denude considerable areas of tubular basement membrane. In the course of a couple of days, regeneration begins, during which, as described in cattle, atypical syncytial giant cells may form. These acute changes in the kidney are accompanied by interstitial edema and a diffuse but sparse infiltration by leukocytes, principally lymphocytes and plasma cells. Leptospires are demonstrable in the tubular epithelium and in the lumina of tubules, frequently in clusters. Early in the renal phase, the organisms may be found in any part of the cortex, but later they are more restricted in their distribution and are then mainly to be found in the more superficial, subcapsular areas. With chronicity, the interstitial cellular exudate is decreased and fibrosis increases, with mild diffuse and moderate focal interstitial sclerosis and thickened Bowman's capsule and basement membranes with focal periglomerular fibrosis. There is increased cellularity of the renal pelvis, and the fibrosis extends into the papillae. The lymph nodes and spleen may be enlarged and edematous or hemorrhagic, and microscopically there is depletion of lymphocytes and increase of sinusoidal reticular cells. Erythrophagocytosis is prominent.

Swine

Although any leptospiral serovar may cause disease in pigs, until recently the most important serovar recognized in North America was serovar *pomona* (type kennewicki), a subtype of which is host-adapted to swine. In Australia and some European countries, *tarassovi* (formerly *hyos*), another pig-adapted serovar, causes losses as serious as those of *pomona*. More recently, a new species, *Leptospira fainei*, has been isolated from pigs with reproductive problems in Australia, but its broad significance remains to be determined. Understanding of leptospirosis in swine is changing rapidly with the recognition of the widespread and dominant prevalence of antibodies to the Australis serogroup and the isolation of *bratislava* and *muenchen* from aborted and stillborn pigs. The role and importance of Australis serogroup leptospires are only partially understood, in part because of the difficulty of isolating these leptospires. Incidental infections with serovars *canicola, grippotyphosa, hardjo*, and other serovars have been locally important. Antibody to *icterohaemorrhagiae* is widespread, but the serovar is of rare disease significance.

Only a small proportion of infected animals develop clinical illness, usually an unrecognized transient episode of mild fever, anorexia, and depression. Hemoglobinuria and icterus may occur rarely in piglets.

The principal aspect of the disease in pigs, apart from transmissibility to other species, *is abortion and the birth of small litters of weak piglets*, which may reach outbreak proportions. This is particularly liable to occur if pregnant sows with no acquired immunity are exposed during the second month of pregnancy. Abortion is associated with heavy leptospiral infection of the fetus.

The usual pattern of leptospirosis in pregnant sows, caused by serovars other than those of the Australis serogroup, is for the litter to be delivered 1–3 weeks prematurely, with some of the fetuses dead and some born alive, only to die shortly afterward. The organism can usually be recovered from the fresher ones, some of which in each litter are expected to have lesions. Not all aborted fetuses in the litter will yield leptospires. Straw-colored pleural effusion, somewhat viscid, may be accompanied by effusion of lesser volumes in the other serous cavities. Petechial hemorrhages may be present in the pulmonary pleura, epicardium, renal cortex, and peripelvic tissue, and occasionally elsewhere. The liver and spleen are swollen and dark, and tan foci of necrosis, 2–5 mm in size, in the liver, especially near the margins, are characteristic when present.

Histologic changes are also inconstant, but part or all of the picture is usually seen in one or more of the piglets, especially in those dying at or very soon after birth. Hepatitis is acute, with infiltrated neutrophils and lymphocytes in portal areas and surrounding the foci of coagulative necrosis (Fig. 4.64A). Focal myocarditis with coagulative necrosis and mononuclear infiltration is less frequent than infiltration beneath epicardium and endocardium (Fig. 4.64B). These latter infiltrations, also of mononuclear cells, are light but quite diffuse. In addition to many minor foci of interstitial reaction in the kidney, large, fairly circumscribed infiltrates of mononuclear cells are present in the peripelvic parenchyma, sometimes involving

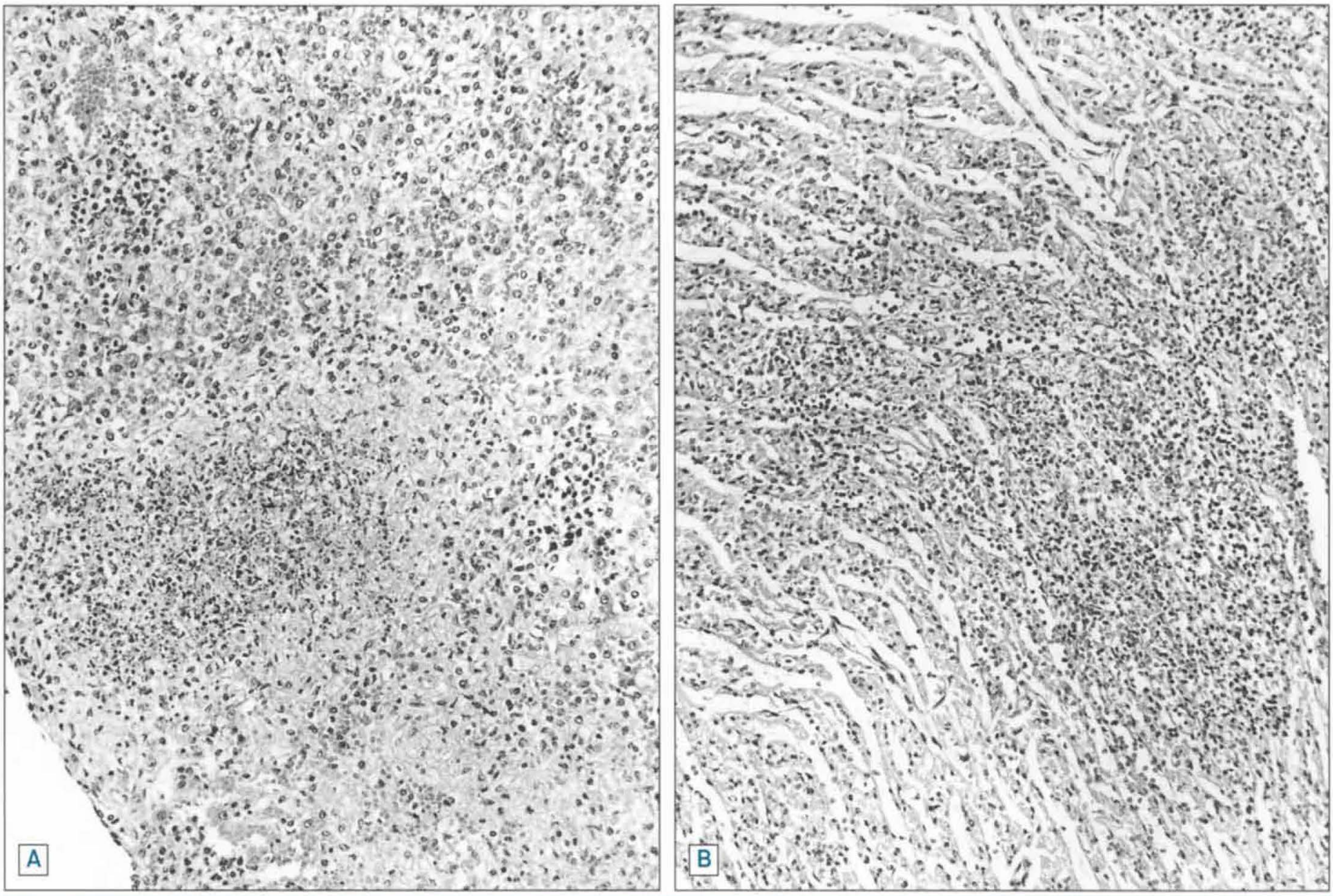

Figure 4.64 Leptospirosis in a porcine neonate. **A. Hepatitis** with subcapsular necrosis. **B.** Interstitial lymphocytic **myocarditis**.

the papilla (Fig. 4.65A) and encroaching on the parahilar cortex. The medullary tissues swarm with organisms (Fig. 4.65B).

The lesions as described are not specific for *pomona* infections. They have also been observed in *tarassovi* and can be anticipated with some other serovars.

Infection with serovars belonging to the Australis serogroup produce subtler losses than those described for *pomona* or other serovars. The lesions have not been described. In Northern Ireland and the USA, late-term abortion and stillbirth have been reported, characterized by the birth of live, dying, and dead piglets within a litter. Late-term abortion is less characteristic than production of small litters of viable piglets, accompanied by stillborn piglets and by piglets that die shortly after birth. Diagnosis is problematic because of the extreme difficulty of isolation of serovars *bratislava* and *muenchen*, but approaches include fetal and maternal serology, immunofluorescence of fetal lung and kidney homogenates, and in future PCR-based diagnosis.

A distinct *infertility ("repeat breeder") syndrome* associated with Australis serogroup infection has been described in Northern Ireland. Disease is most noticeable in sows bred to infected boars for the first time or when susceptible animals are introduced into infected herds. Serovars *bratislava* and *muenchen* are commonly isolated from the genital tracts of sows and boars in infected herds. Venereal transmission is thought to be common.

The majority of instances of chronic interstitial nephritis in pigs, lesions of which are often observed at slaughter, are leptospiral in origin, particularly associated with serovar *pomona*.

Horses

There is widespread serological evidence of leptospiral infection in horses but acute disease is apparently rare. Clinical features include fever, anorexia, depression, and icterus in the acute disease, and abortion, premature foaling, and chronic uveitis ("periodic ophthalmia") in the chronic disease. Improved diagnostic techniques have confirmed the importance of leptospirosis in horses. It is now recognized that horses are maintenance hosts for *bratislava*, that leptospires are involved in fatal hepatic and renal disease in foals, that leptospires may be more important in abortion than previously thought, and that leptospirosis continues to cause chronic uveitis in horses.

Leptospires of a variety of serovars have been isolated or identified by immunofluorescence in Northern Ireland in neonatal foals or adults with fatal icterus. Different serovars, including *bratislava, canicola, grippotyphosa, hardjo, icterohaemorrhagiae* but particularly *pomona*, may also cause subacute disease characterized by fever, but inconstantly by icterus, in adults. Although of generally minor significance per se, these infections have important chronic sequelae in the form of abortion or chronic uveitis. In North America, serovars in leptospiral

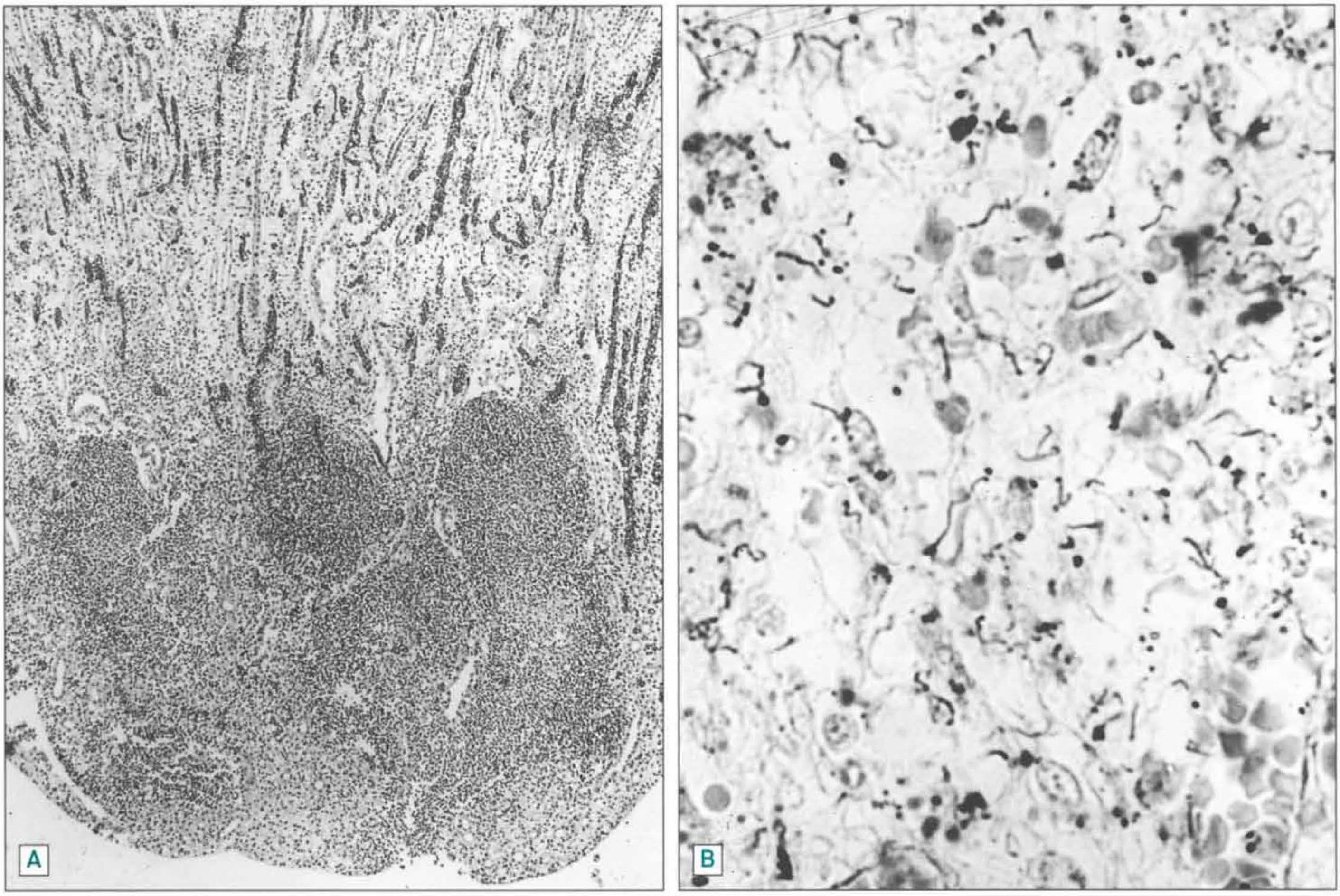

Figure 4.65 Leptospirosis in a porcine neonate. **A.** Renal **papillitis**. **B. Organisms** in foci of papillitis.

abortion are, in order of importance, *pomona, grippotyphosa*, and *bratislava*, the first two being incidental infections. Abortion therefore tends to be seasonal (late fall and early winter). *Giant cell hepatitis in aborted fetuses* has been associated with leptospiral infection; the lesions were characterized by dissociation of hepatocytes, disruption of hepatic cords, and numerous large, multinucleated hepatocytes. Recurrent uveitis may develop several months after leptospiral infection, particularly with serovar *pomona*. Antibody in the aqueous humor of the eye may considerably exceed serum antibody and assist in the diagnosis in such cases. Use of PCR and of isolation techniques has shown the presence of leptospiral DNA or organisms in a high proportion of these cases.

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Pyelonephritis

Pyelonephritis is inflammation of the pelvis and renal parenchyma, usually resulting from infection ascending from the lower urinary tract; the hematogenous route of infection is very uncommon. It is characterized by inflammation, necrosis, and eventually deformity of the calyces, in association with areas of tubulointerstitial inflammation and necrosis, and is hence distinguishable from other forms of nephritis. It is usually accompanied by ureteritis and cystitis. In *acute pyelonephritis*, cellular infiltration and necrosis predominate, with pelvic and medullary involvement more severe and advanced than involvement of the cortex. In *chronic pyelonephritis*, fibrosis replaces inflammation. In both cases, the disease process is asymmetrical within the kidney, and chronic pyelonephritis may produce very irregular contracture of the kidneys with pelvic deformities. **Pyonephrosis** is the term applied to severe suppuration of the kidney in the presence of complete or nearly complete ureteral obstruction; the infected hydronephrotic kidney is converted to a sac of pus. Suppuration may extend through the renal capsule during the course of pyelonephritis to produce a **perinephric abscess**.

The pathogenesis of pyelonephritis begins with establishment of infection in the lower urinary tract; urinary tract infection in general is dealt with in the section on Cystitis, below. Organisms involved in urinary tract infection are usually endogenous bacteria of the bowel and skin, such as *Escherichia coli*, staphylococci, streptococci, *Enterobacter, Proteus*, and *Pseudomonas*, and more specific urinary pathogens, such as *Corynebacterium renale, C. cystitidis*, and *C. pilosum* in cattle, and *Actinobaculum (Eubacterium) suis* in pigs. Mycoplasmas are rarely involved in cattle. Infection is often mixed, and while *C. renale* may be present in bovine pyelonephritis, and is an obligate parasite of urinary mucosae, various enteric pathogens may be of more pathogenetic importance. Fungal infection is a rare cause of pyelonephritis in dogs.

Virulence of bacteria in the urinary tract, as elsewhere, is enhanced by the presence of pili on their surface. Pili assist adhesion of *C. renale* to urinary epithelium; this process is pH-dependent. The type of pili expressed by the bacteria may also be important later in the course of pyelonephritis; type 1 fimbriate *Escherichia coli* induce greater activation of neutrophils and hence more renal scarring than do nonfimbriate or P-fimbriate *E. coli*.

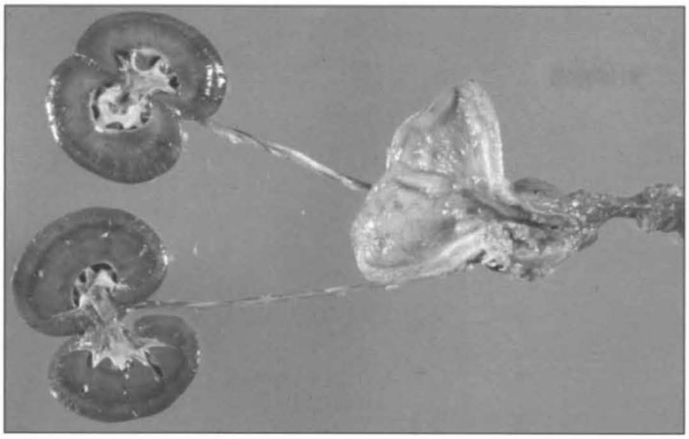

Figure 4.66 Prostatitis, hydronephrosis, and **pyelonephritis** in an 8-year-old male dog.

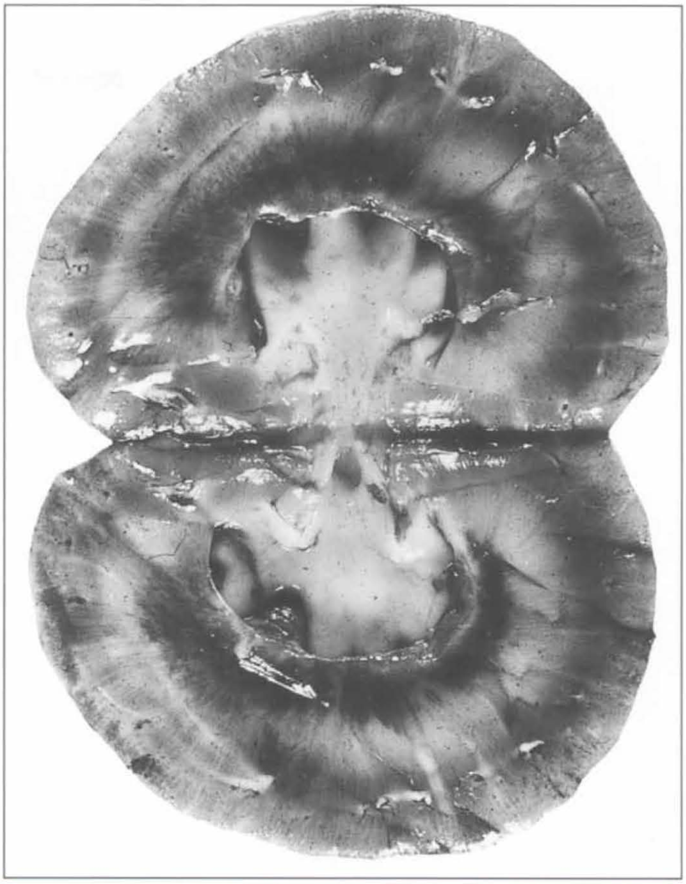

Figure 4.67 Acute pyelonephritis in a dog. There is ulceration of the renal crest and hemorrhage in medulla; pale streaks extend to the cortical surface.

One of the *urinary tract defenses* against bacterial infection and colonization is shedding of mature epithelial cells with attached bacteria. Normal voiding of urine, plus immune and other mechanisms, usually maintains sterility of the bladder, but once bacteria enter the bladder, e.g., via catheterization, they grow well in urine of low osmolality or alkaline pH. *Stasis of urine* is an important predisposing factor in the pathogenesis of cystitis and of pyelonephritis. Urinary obstruction may be caused by ureteral anomalies in young animals, kinked ureters in pigs, pregnancy, urolithiasis, and prostatic hypertrophy (Fig. 4.66). *Females are predisposed to urinary tract infection* because of their short urethras, urethral trauma, and possibly due to hormonal effects. Urethral catheterization can carry bacteria into the bladder. Clinically, urinary infection is indicated by bloody or cloudy urine, with pyuria and bacteriuria.

Once infection is established in the bladder, probably the most significant mechanism in causing renal infection is **vesicoureteral reflux**. This retrograde flow of urine up the ureters and into the kidney (*intrarenal reflux*) during micturition may carry bacteria as far as the urinary space of glomeruli. Reflux may occur during micturition, especially if there is urinary obstruction, or as a result of external compression of the bladder, as occurs during manual compression of the bladder in dogs and cats for collection of urine samples. Swine have a long intravesical ureter – 5 mm at birth, 36 mm at maturity – that should impede reflux in mature animals. Vesicoureteral reflux is very common in puppies, and is a function of the short intravesical length of the ureters and hence an easily overcome vesicoureteral valve; reflux decreases with age and the development of greater intravesical length of the ureter and its more oblique entry through the bladder wall. Vesicoureteral reflux of sterile urine does little renal damage; the ureteral muscular layers may hypertrophy. Cystitis may alter normal ureteral peristalsis, perhaps causing reversed peristaltic waves. Hence, persistent renal infection may result from vesicoureteral reflux, and possibly from reversed peristalsis, in animals with bladder infection and thus contribute to chronic active pyelonephritis. Progression of pyelonephritis probably depends upon persistence of bacterial infection, or at least of bacterial antigens.

For a number of reasons, *the medulla is the part of the kidney most susceptible to infection*. It is relatively hypoxic because of the low hematocrit in vasa recta; hypertonicity depresses the phagocytic activity of leukocytes, and ammonia may interfere with activation of complement. In pigs, the renal poles are more susceptible to infection because the collecting ducts of their compound papillae do not collapse as readily as do those of the simple papillae of the central lobes, when exposed to the increased pelvic pressure of vesicoureteral reflux; hence, intrarenal reflux is more common at the poles. Invasion of the kidney from the pelvis probably progresses by way of the collecting ducts, as is suggested by the early development of lines of suppuration along the straight tubules and the presence of bacterial colonies in the tubules, and by direct invasion across the degenerate eroded pelvic epithelium.

Pyelonephritis is often bilateral, but not necessarily symmetrical. *Acute disease is seen most commonly in sows, and chronic pyelonephritis in cows and dogs*. A general description will be given of acute and chronic pyelonephritis before species differences are noted in more detail. **Acute disease** characteristically begins with necrosis and inflammation of papilla or renal crest ("necrotizing papillitis") in an irregular pattern (Fig. 4.67). Bacteria may be abundant in the collecting tubules (Fig. 4.68). Associated wedge-shaped areas of parenchyma are swollen, dark-red, and firm. Hyperemia subsides and *suppurative tubulointerstitial nephritis and tubular necrosis* then predominate in these radially distributed wedges. Tubules are obliterated by the inflammation, tubular obstruction and dilation occur, and glomeruli, although initially resistant, may be obliterated. Leukocytic casts are present in tubules, and hence may be found on urinalysis. As the process becomes **chronic**, mononuclear cells replace neutrophils, and fibrosis proceeds and

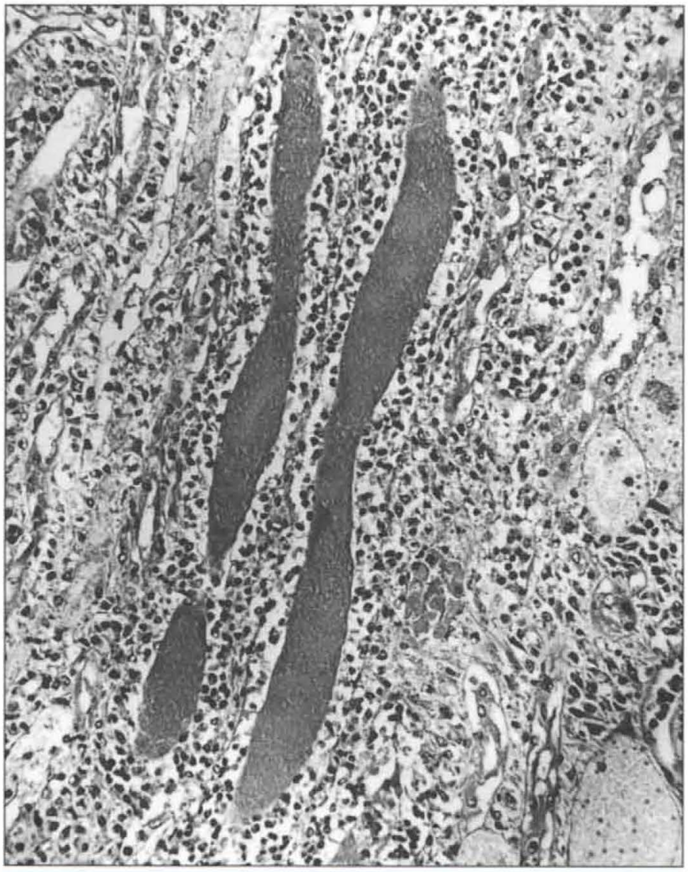

Figure 4.68 Acute ascending pyelonephritis in a cow. Bacterial colonies are present in collecting tubules, with suppuration.

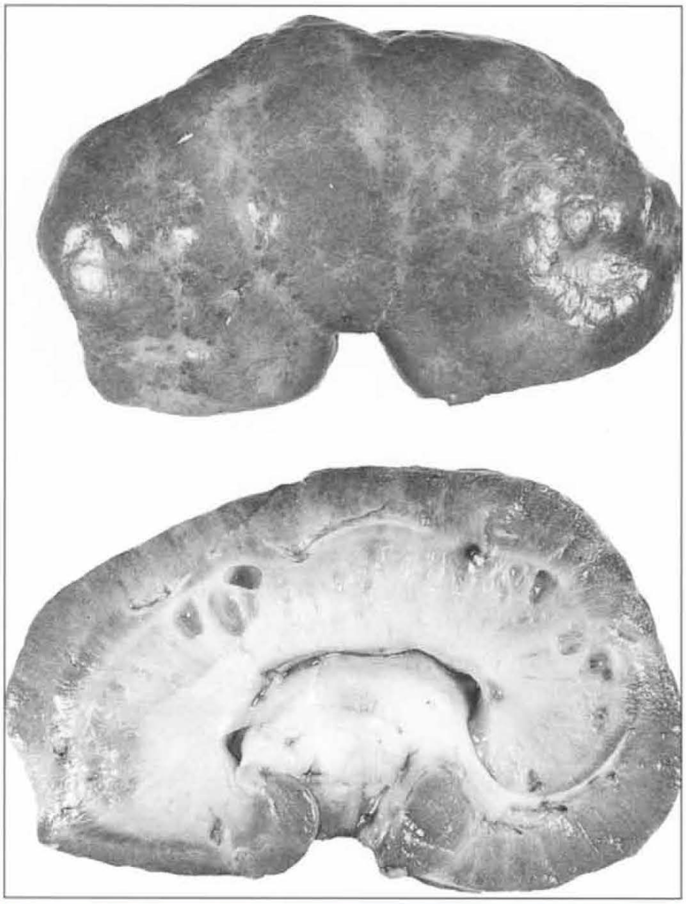

Figure 4.69 Chronic pyelonephritis in a dog. Ulceration of renal crest and deep irregular scars that are most prominent at the poles.

eventually predominates. Contraction of the scars and loss of renal substance result in a wide variety of patterns of renal scarring, usually with deep cortical depressions. The *scars in pyelonephritis* extend from capsule to pelvis and are distinguished from those of other nephritides and infarcts by the associated fibrosis and deformities of the renal papilla and dilation of calyces and pelvis. The pelvis often contains exudate and debris. The papillary defects may be small and difficult to detect in mild cases. More or less total involvement of the kidney may produce a firm pale shrunken kidney with an irregular surface (Fig. 4.69) that may be difficult to differentiate from other end-stage kidneys resulting from ischemic lesions.

In **dogs** and **cats**, acute pyelonephritis is not often detected, but scars attributed to chronic pyelonephritis are common. In dogs, accumulation of colloid-like material in tubules and glomerular capsules dilated due to scarring may produce a thyroid-like histologic appearance ("thyroidization") (Fig. 4.70). Calculi may form in the pelvis on the nidi provided by cellular debris.

In **swine**, acute pyelonephritis is seen occasionally, but the chronic disease is rare. Shortening of the intravesical portion of the ureter and widening of the ureteric orifices, as occurs in sows as a consequence of cystitis, may facilitate vesicoureteral reflux and development of pyelonephritis. *Acute pyelonephritis occurs in sows postpartum or 3–4 weeks postbreeding*; young males are occasionally affected, and some of these have urinary tract anomalies. Blood-stained urine or discharge may be seen, but it is not unusual for prostration and death to occur in 12 hours or so. Severe cystitis and

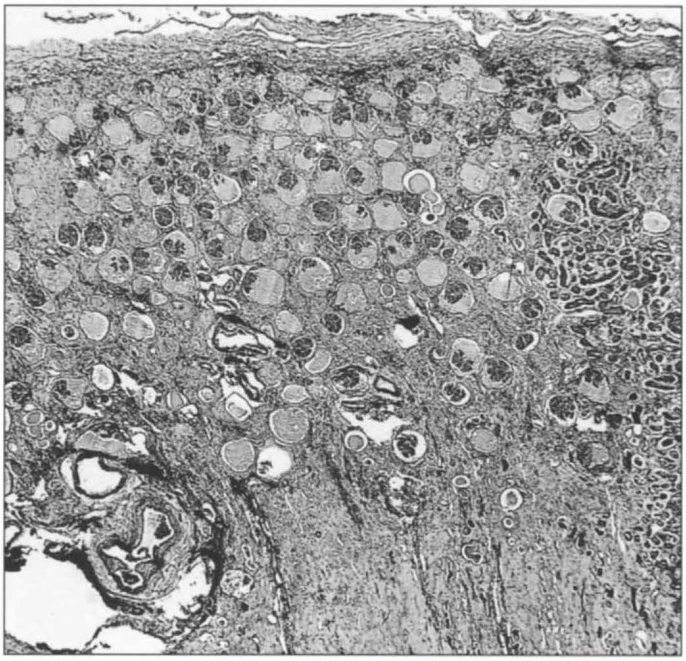

Figure 4.70 Cortical and medullary scarring in **pyelonephritis** in a dog. Note inflammatory cells at corticomedullary junction and beneath capsule.

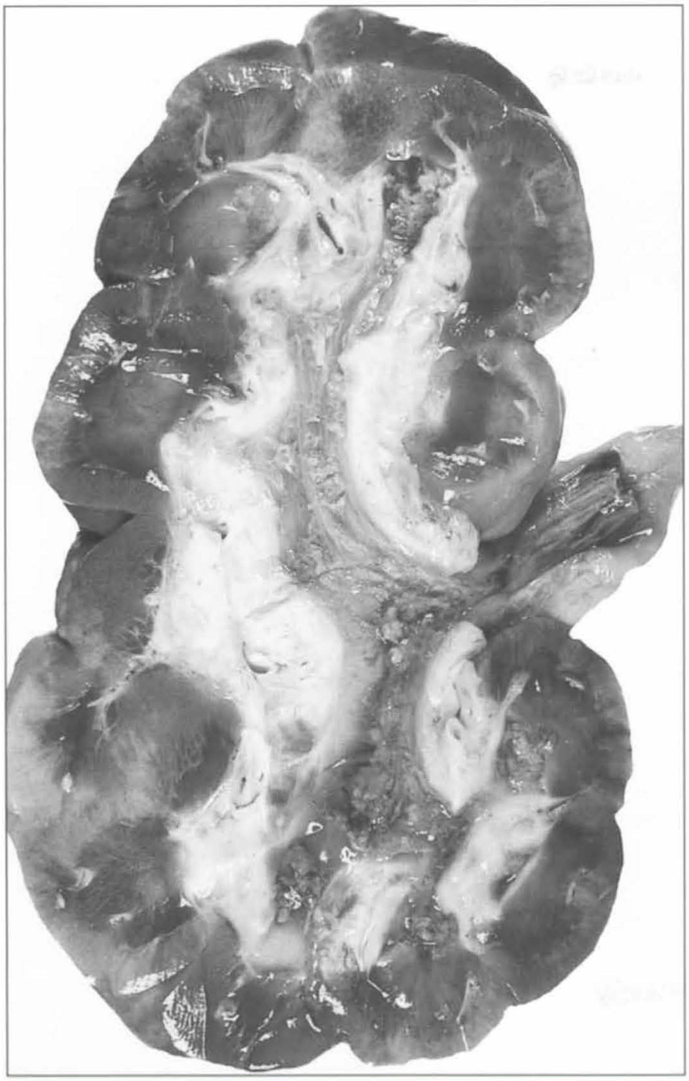

Figure 4.71 Acute pyelonephritis in a cow. Note casts and detritus in calyces and ureters, erosion of papillae, and irregular interstitial inflammation of parenchyma.

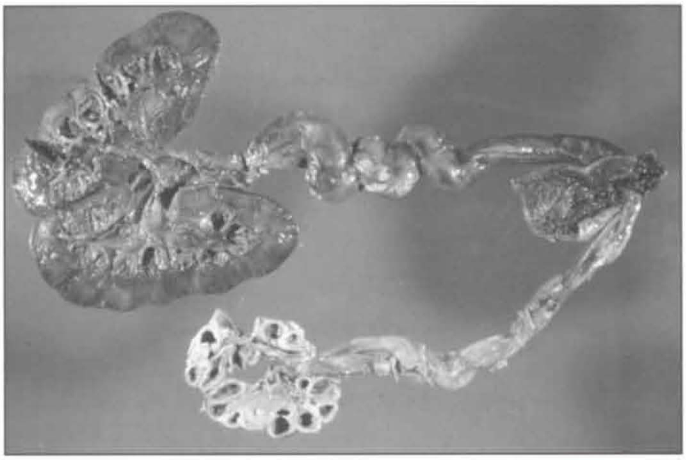

Figure 4.72 Ulcerative cystitis, dilated ureters (pyoureter), scarred shrunken kidney (below), and chronic-active **pyelonephritis** (above) in a cow.

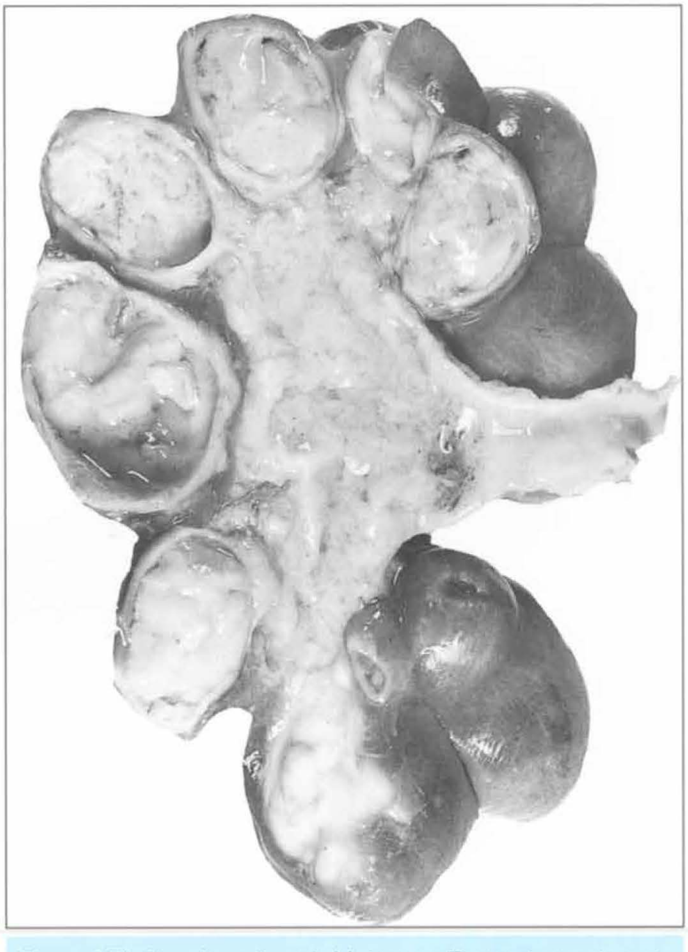

Figure 4.73 Chronic pyelonephritis in a cow. The renal parenchyma is scarred and atrophic, and exudate fills the distended, thickened calyces.

ureteritis are usually present with yellow-brown or bloody mucoid exudate. The renal poles are preferentially involved, and the infection may be fulminant and erupt through the renal capsule to produce retrorenal hemorrhage and inflammation. Less severe examples of tubulointerstitial nephritis also occur, and are seen as pale areas of cellular infiltration involving wedges or entire lobes.

In **cattle**, chronic pyelonephritis is a significant sporadic disease in cows, but acute pyelonephritis (Fig. 4.71) is usually only an incidental finding. Tubulointerstitial nephritis in affected cows may be minimal, and slowly progressive, suppurative destructive papillitis may predominate. The medulla of each lobe is fairly uniformly destroyed (Fig. 4.72). Eventually the cortex remains as a narrow capsule surrounding large amounts of pus in the calyces (Fig. 4.73). Alternatively, and perhaps more commonly, the pattern of radially distributed tubulointerstitial nephritis develops as described above. Enlarged tan lobes are granular due to interstitial inflammation, and interstitial fibrosis may become extensive (Fig. 4.74). Rupture of the kidney occurs in males with obstructive urolithiasis after development of fulminant pyelonephritis, but rupture is less common in cattle than in swine. Pyelonephritis is uncommon in **sheep**.

Pyelonephritis is also uncommon in **horses**, but may occur for the usual pathogenetic reasons; it can cause renal hemorrhage that may first become evident because of hematuria.

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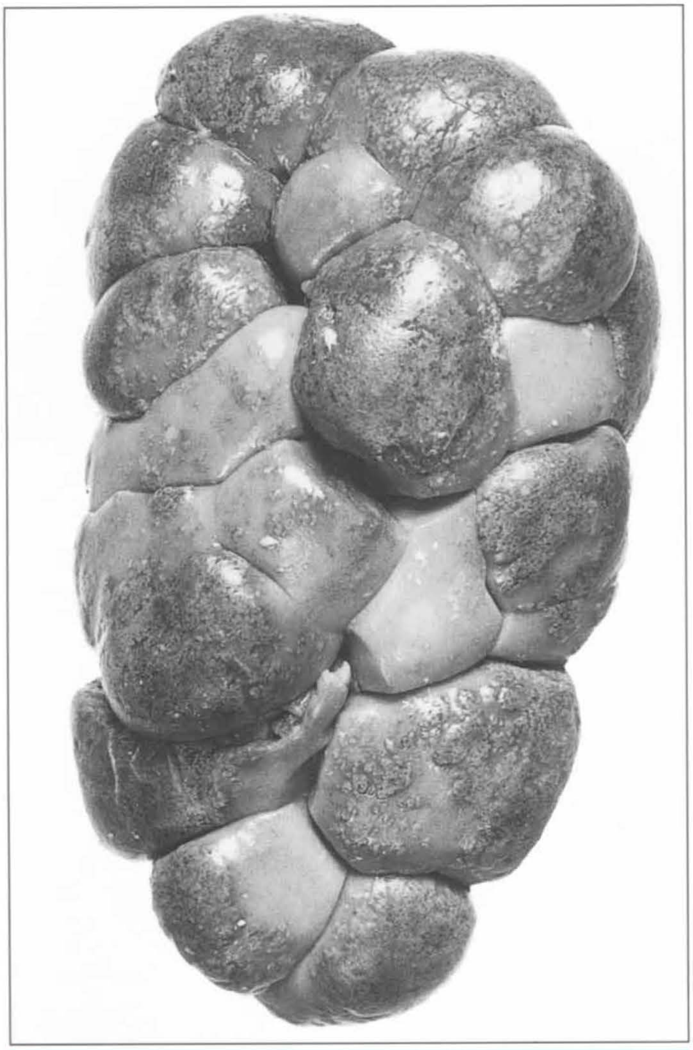

Figure 4.74 Chronic pyelonephritis in a cow. Scarred lobes alternate with lobes showing multifocal suppuration.

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Hypercalcemic nephropathy

Hypercalcemia occurs often in dogs and cats, and may be sufficiently severe to cause renal failure (hypercalcemic nephropathy).

- The *leading cause is the hypercalcemia of malignancy, or pseudohyperparathyroidism*, a paraneoplastic syndrome. A nonendocrine tumor, usually lymphosarcoma or adenocarcinoma of the apocrine glands of the anal sac, stimulates bone resorption via parathyriod hormone-related protein (PTHrp), a peptide that resembles parathyroid hormone (see Vol. 3, Endocrine glands and Vol. 3, Hematopoietic system). Hypercalcemia of malignancy is less commonly caused by osteolytic neoplasms.
- Poisoning by *rodenticides containing vitamin D* (0.075% cholecalciferol) has become an important cause of hypervitaminosis D and hypercalcemia in dogs and cats; the same syndrome follows ingestion of *human antipsoriatic preparations* that contain synthetic vitamin D analogues, e.g., calcipotriol, tacalcitol. Diagnosis of cholecalciferol rodenticide poisoning may be aided by determination of the renal $25(OH)D_3$ concentration; as well, the renal calcium to phosphorus ratio is <0.1 in normal dogs, but 0.4–0.9 in cholecalciferol-poisoned dogs, and >2.5 in dogs dead of ethylene glycol poisoning.
- *Other less common causes* of hypercalcemia are primary hyperparathyroidism, chronic renal failure with secondary hyperparathyroidism, and hypoadrenocorticism.
- Hypercalcemia resulting in nephrocalcinosis and mineralization of other soft tissues is well known in grazing animals. Leading examples of *plants containing vitamin D_3* sterol are *Solanum malacoxylon, Cestrum diurnum, Trisetum flavescens*, and *Medicago sativa.*

Hypercalcemia results in inactivation of adenyl cyclase, and hence decreased cyclic adenosine monophosphate formation; sodium transport is impaired in the ascending limb of the loop of Henle, the distal tubule, and collecting ducts. Natriuresis results. As well, hypercalcemia interferes with antidiuretic hormone receptors in the collecting ducts, resulting in renal diabetes insipidus. The resultant polyuria and compensatory polydipsia are reversible if the primary cause of the hypercalcemia is removed. If hypercalcemia persists, *progressive renal mineralization* occurs, beginning with tubular basement membranes and epithelium, particularly in the outer zone of the medulla (Fig. 4.75) and eventually involving the interstitium, vessels, and glomeruli. Tubular epithelial mineralization and cast formation cause tubular obstruction, and eventually loss of nephrons.

Other less significant examples of renal mineralization also occur. The deposition of calcium salts in the form of clumps of granules

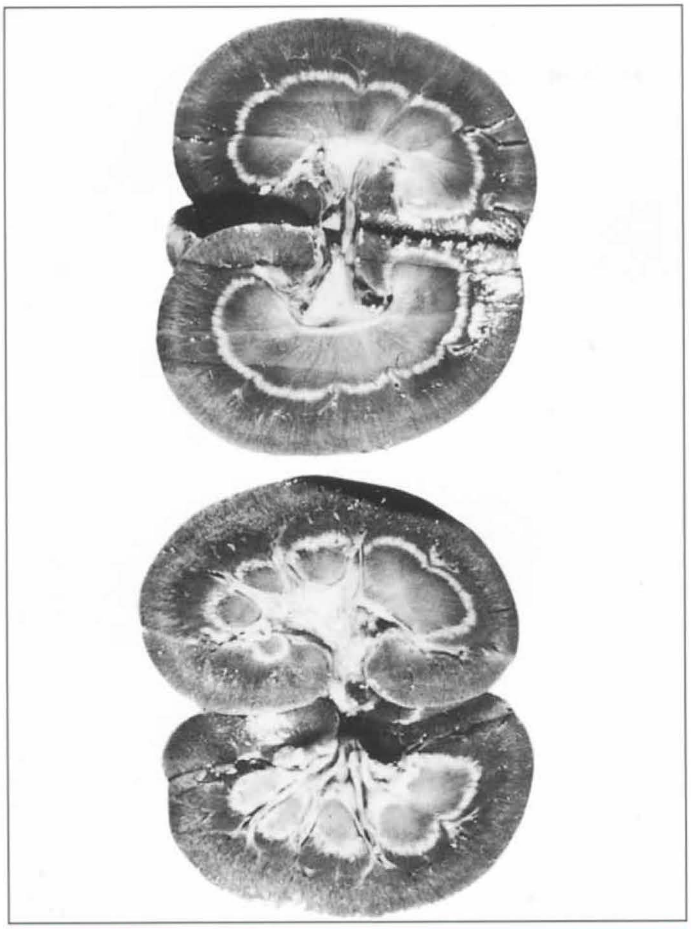

Figure 4.75 Hypercalcemic nephropathy in a dog.

in the lumen and lining of collecting tubules and in the adjacent interstitium is rather common. They are associated with hypomagnesemia in some species; they are not significant.

Mineralization is very common in dogs, most unusual in other species, and occurs very quickly in casts, in dead and degenerate epithelium, and in injured basement membranes. In uremia, it may also involve the glomeruli and small blood vessels. Renal mineralization is a frequent result of secondary hyperparathyroidism induced by *chronic renal insufficiency* and coexists with similar deposits in the lungs, gastric mucosa, and other organs (see section on Renal disease, renal failure, and uremia, above). Dogs fed a high-phosphorus diet similarly develop diffuse renal mineralization.

Miscellaneous interstitial lesions

Extramedullary hematopoiesis occurs in the kidneys of dogs under a variety of circumstances, all of which probably have a common denominator of bone-marrow depression or injury. When the kidneys are sites of hematopoiesis, it is usual that the liver, lymph nodes, spleen, adrenals, and lungs are also. The most pronounced hematopoiesis occurs in canine pyometra.

Bone occasionally develops in association with urinary tract tissue, for example, following urinary tract surgery or in hydronephrotic kidneys. Transitional epithelium stimulates transformation of mesenchymal cells to osteoblasts.

Renal telangiectasis occurs in cattle, dogs, cats, mink, and ferrets; it is familial in Pembroke Welsh Corgis. It is a rare cause of hematuria in dogs.

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Parasitic lesions in the kidneys

Toxocara canis

The most common parasitic lesion in the kidneys is the focal scar of reaction to the larvae of *Toxocara canis* in canine kidneys (Fig. 4.76A). These small granulomas, 2–3 mm in diameter, are found on the surface and cross-section of the cortex. In the early stages of development, the lesions are gray-yellow and have soft centers; later they become firm and white and the superficial ones cause dimpling of the cortex. *Each granuloma surrounds an entrapped larva* (Fig. 4.76B), which is usually hard to find in sections. The granuloma is largely composed of epithelioid cells and lymphocytes with an occasional eosinophil. Healing of the lesions occurs after the death and removal of the larvae; the residual scars are typical and consist of dense concentrically arranged fibrous tissue. Similar lesions are produced in calves by the migratory larvae of *T. cati* and *T. canis* acquired by fecal contamination of feed, and by *T. (Neoascaris) vitulorum,* especially in buffaloes. In cats, *T. canis* can produce disseminated granulomatous disease marked by the presence of large numbers of eosinophils in granulomas. Occasionally, ascarid larvae are found in other sites occupying granulomas similar to those in the canine kidney (Fig. 4.77).

Stephanurus dentatus

Stephanurus dentatus is the *kidney worm of swine.* It is widely distributed in tropical and subtropical countries, and the prevalence in grazing swine in such areas can be very high. *The worms encyst in perirenal fat and adjacent tissues, the cysts communicating with the renal pelvis.*

The life cycle of *S. dentatus* can be direct or, experimentally, involve earthworms as transport hosts. The latter mechanism has not been shown to occur naturally. Eggs are passed in the urine of the pig, sometimes in immense numbers, and the larvae hatch in 2–3 days. The infective stages are vulnerable to sunlight and drying. Infection may occur by penetration through the skin or by ingestion, and prenatal

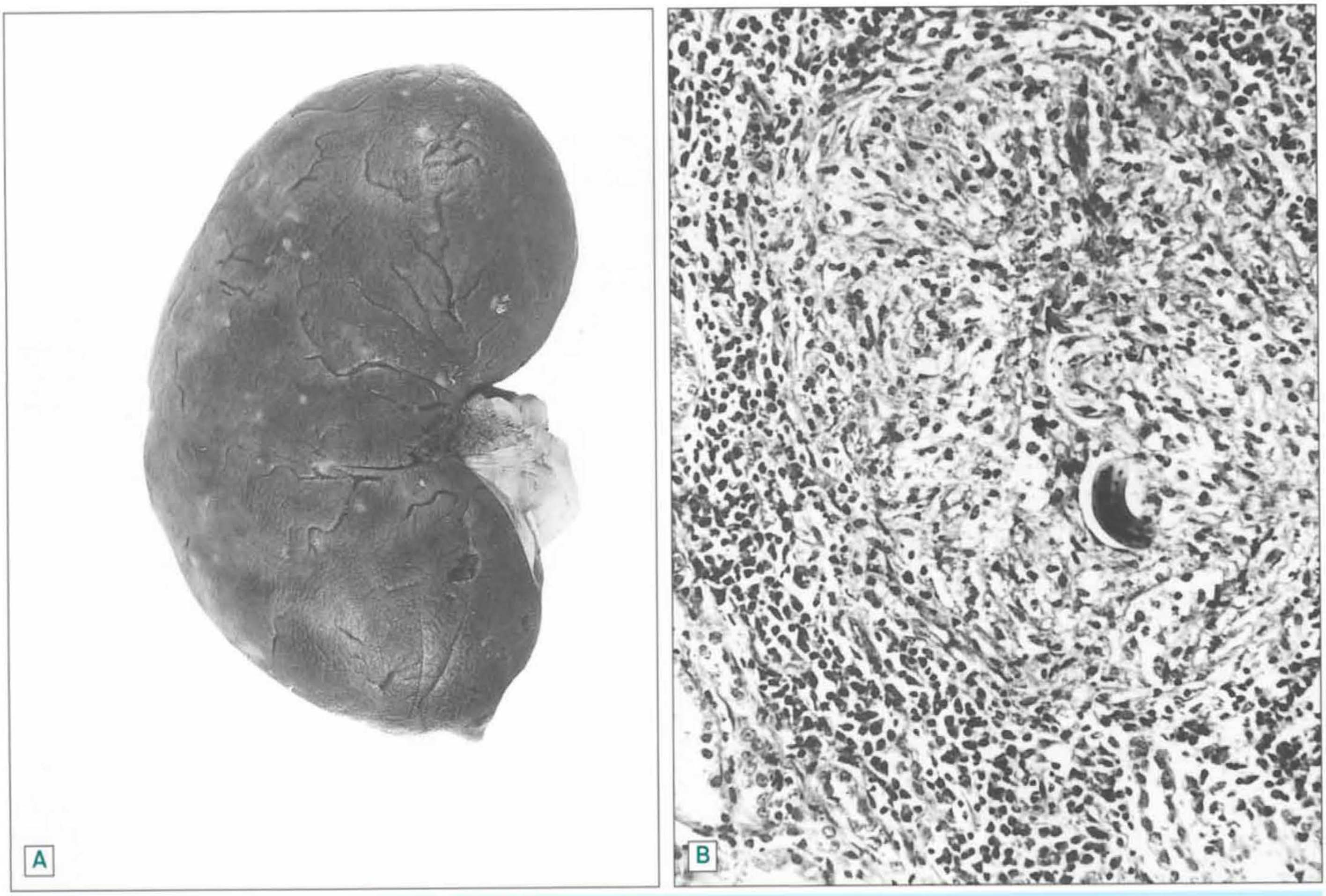

Figure 4.76 A. Cortical granulomas caused by *Toxocara canis* in a canine kidney. **B.** Section from (**A**) showing larva in granuloma.

infections occur. Following oral infection, third-stage larvae migrate from the small and large intestine via the portal circulation and mesenteric lymphatics to the liver. A few migrate across the peritoneal cavity. After skin infection, most larvae migrate to the lungs and reach the intestines following tracheal migration. Deaths due to peritonitis and intestinal intussusception occur in some pigs 20–30 days after heavy infections and are associated with larval migration from the mesenteric nodes to the liver. The infective larvae migrate in the tissues of the pig, especially in the liver, where they may stay for several months. The hepatic migrations provoke considerable injury and severe interstitial hepatitis with lesions of the same character but much more severe than those produced by the larvae of *Ascaris suum*. *S. dentatus* also produces portal phlebitis with thrombosis in some pigs (see Vol. 2, Liver and biliary system). The liver is usually enlarged and may be very hard. It is pale and the lobulation is remarkably emphasized by the perilobular fibrosis that develops. Many lobules are obliterated by contracting and proliferating scar tissue in the portal areas, and all lobules are more or less reduced in size in the diffuse hepatitis of heavy or prolonged larval infestation.

Many larvae are destroyed in the liver, being encapsulated in small abscesses that are eventually obliterated. *From the liver, the larvae migrate across the peritoneal cavity to the perirenal region.* Many become encysted in abscesses in adjacent tissue, especially the pancreas, and it is not unusual for some to invade the vertebral canal and cause paraplegia. The definitive site is the tissue around the renal pelvis and ureter wherein the adults encyst (Fig. 4.78). Occasionally, the cysts may be found in the kidney itself. The cysts communicate with the lumen of the ureter, allowing escape of eggs. Developmental stages in the definitive host take a long time, and patency may not be established for 9 months or more. Once patency is established, the mature females may lay eggs for 3 years or longer.

Dioctophyma renale

Dioctophyma renale is the *giant kidney worm*, the largest of parasitic nematodes. It has a worldwide distribution but its incidence is unknown. The worm is red and cylindrical, and in dogs the females measure 20–100 cm long and 4–12 mm in diameter. Males are 14–45 cm long and 4–6 mm in diameter. *D. renale* is usually found in dogs, mink, cats, and other fish-eating mammals, but is recorded in the pig, ox, and horse. The definitive hosts are wild fish-eating carnivores, mink in particular, in which the worms are smaller and usually located in the kidney.

The life cycle involves two aquatic intermediate hosts. Eggs passed in the urine are very resistant to the external environment and may survive for 2–5 years. Embryonation requires 1–7 months depending on the climatic conditions. The embryonated eggs are ingested by the intermediate hosts, which are aquatic oligochaetes ("mud worms;" *Lumbriculus variegatus*), and develop through first and second larval stages. Third- and fourth-stage larvae can either

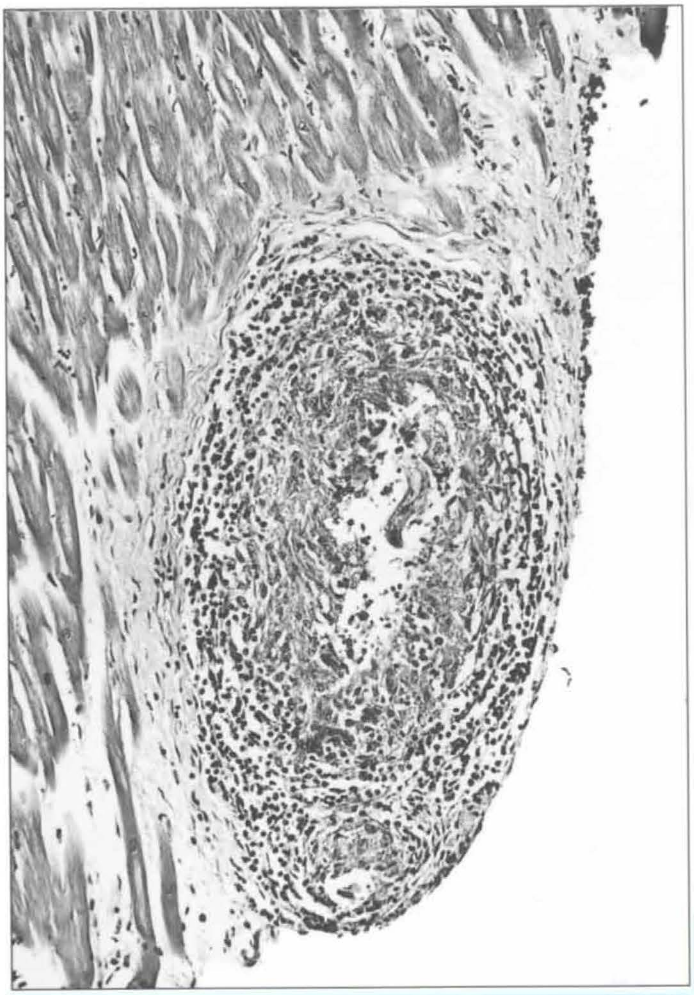

Figure 4.77 Myocardial granuloma containing an **ascarid larva**, from a dog. (Courtesy of GW Thomson.)

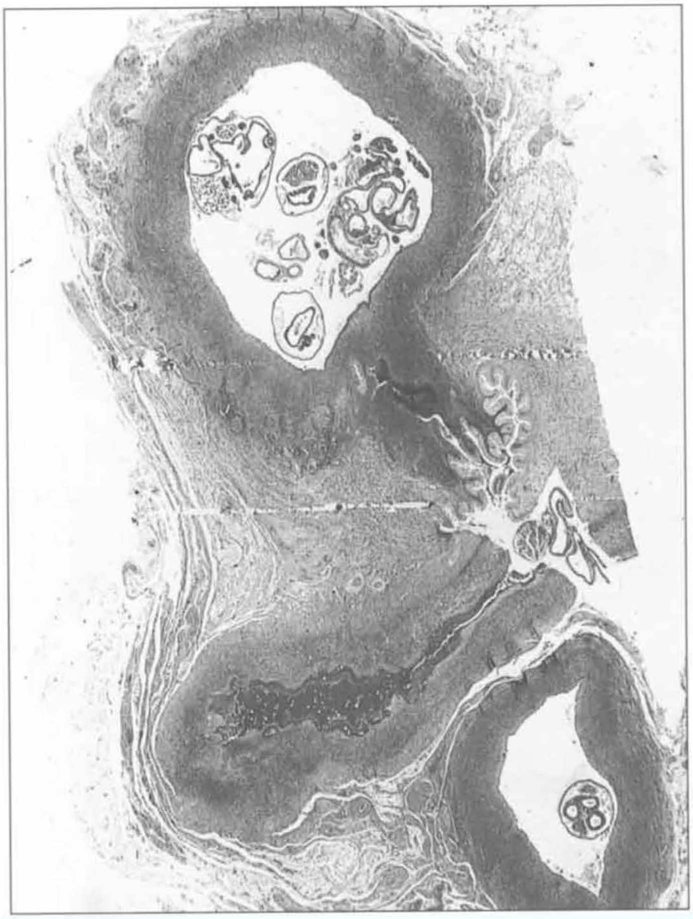

Figure 4.78 Encysted **Stephanurus dentatus** in hilus of a porcine kidney.

develop directly in the worm, or within fish or frog intermediate hosts, e.g., the northern black bullhead (*Ictalurus melas*), green frog (*Rana clamitans*). Following ingestion by mammals, infective third- or fourth-stage larvae penetrate the gut wall and migrate across the peritoneal cavity to the kidney. The life cycle from egg to adult requires 3.5–6 months, but may take 2 years.

The adult worms live in the renal pelvis but may encyst in a body cavity, the uterus, mammary gland, or bladder. The adults are very destructive, causing initially hemorrhagic pyelitis that shortly becomes suppurative, and the parenchyma is progressively destroyed until only the tunic contains the worm and exudate (Fig. 4.79).

Dogs, the domestic species usually affected by *D. renale*, are regarded as abnormal hosts because usually only one or a very small number of worms is present, and worms of both sexes are found in only about one-third of infections. *Intrarenal parasites in dogs and mink are more common in the right kidney than the left*, but in ~60% of infected dogs, parasites are in the peritoneal cavity only. In the peritoneal cavity, *D. renale* often entwines a lobe of the liver and may cause erosion of the hepatic capsule with hemoperitoneum, or produce infarction and rupture. On occasion they rear up to startle surgeons engaged in exploratory laparotomy. Simultaneous renal and peritoneal infections occur in ~15% of dogs. Thus, most canine infections are not patent.

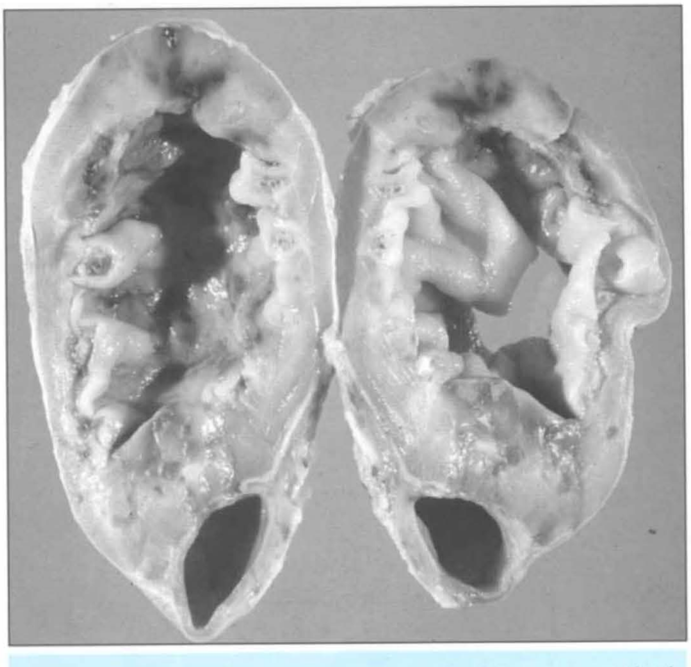

Figure 4.79 Marked destruction of renal parenchyma as a result of **Dioctophyma renale** infection.

Pearsonema (Capillaria) plica

Pearsonema (Capillaria) plica may be found in the lumen of the renal pelvis, ureter, or urinary bladder of dogs, foxes, and smaller carnivores. Although widely distributed, it is not a common parasite. The life cycle is not clearly known but is probably indirect, earthworms being intermediate hosts. Ingestion of earthworms from infected premises causes patent infections in 61–68 days. Pathologic effects are not usually attributed to *P. plica* infection but hematuria and dysuria are occasionally produced; worms embedded in bladder, ureter, or pelvis may invoke mild submucosal inflammation.

Other species of *Pearsonema* occur in urinary bladder of other animals – *P. mucronata* in mink and *P. feliscati* in the cat; the latter may be the same as *P. plica*. Light infestations are common but harmless; the rostral end of the worm embedded in the surface layer of epithelium provoking at most a light cellular infiltration of the lamina propria.

Klossiella equi

Klossiella equi is a *sporozoan parasite of the kidney of the horse and its relatives,* including the zebra, donkey, and burro. It is apparently *quite rare and harmless.* The life cycle is not fully known. It is thought that, following infection with sporocysts from the environment, sporozoites are released and enter the circulation. One schizont generation develops in glomerular endothelium and another in proximal tubular epithelium. Sporogony occurs in the epithelium of the thick limb of Henle's loop and sporocysts are passed in the urine (Fig. 4.80). Heavy infections can result in rupture of tubules and lymphoplasmacytic interstitial nephritis.

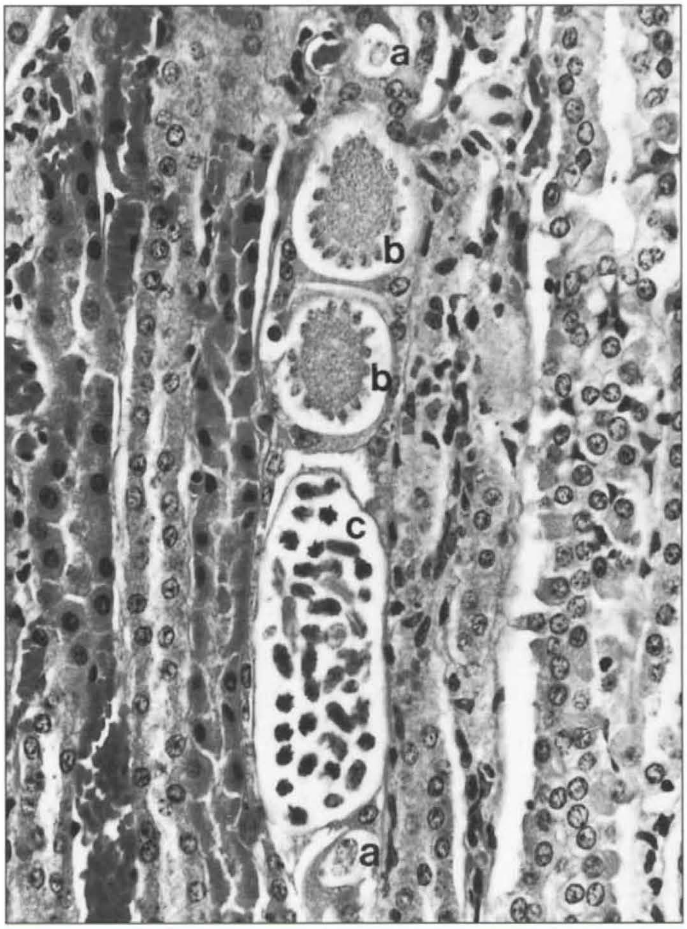

Figure 4.80 ***Klossiella equi*** in an equine kidney. Within tubular epithelial cells are gamonts (a), sporonts (b), and sporocysts (c).

Granulomas may be found in the renal pelvis in *schistosomiasis* of cattle and sheep (see Vol. 3, Cardiovascular system) and larvae of *Setaria digitata* may produce granulomas in the bladder of cattle in Asia (see Vol. 2, Alimentary system).

Halicephalobus gingivalis

Halicephalobus gingivalis (formerly *Micronema deletrix*), a saprophagous nematode that produces granulomatous masses in the nasal cavity of horses and is occasionally responsible for cerebral vasculitis and hemorrhagic necrosis, also localizes in the kidney. *Renal infections are characterized by multifocal to coalescing granulomas* containing numerous larval and adult rhabditiform nematodes plus occasional embryonated eggs; the cream-colored renal masses may resemble neoplasms macroscopically.

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RENAL NEOPLASIA

Primary renal tumors are uncommon. One abattoir survey in the UK found 8.5, 0.9, and 4.3 cases per million animals in cattle, sheep, and pigs respectively. Renal tumors were found in ~0.15% of horses in two large surveys. Primary renal tumors comprise about 1% of all canine neoplasms and 1.5–2.5% of feline neoplasms. In one large retrospective study of primary canine renal neoplasms, most were of epithelial origin (85.2%), demonstrated metastatic disease, and had an associated poor prognosis (mean survival 6.8 months).

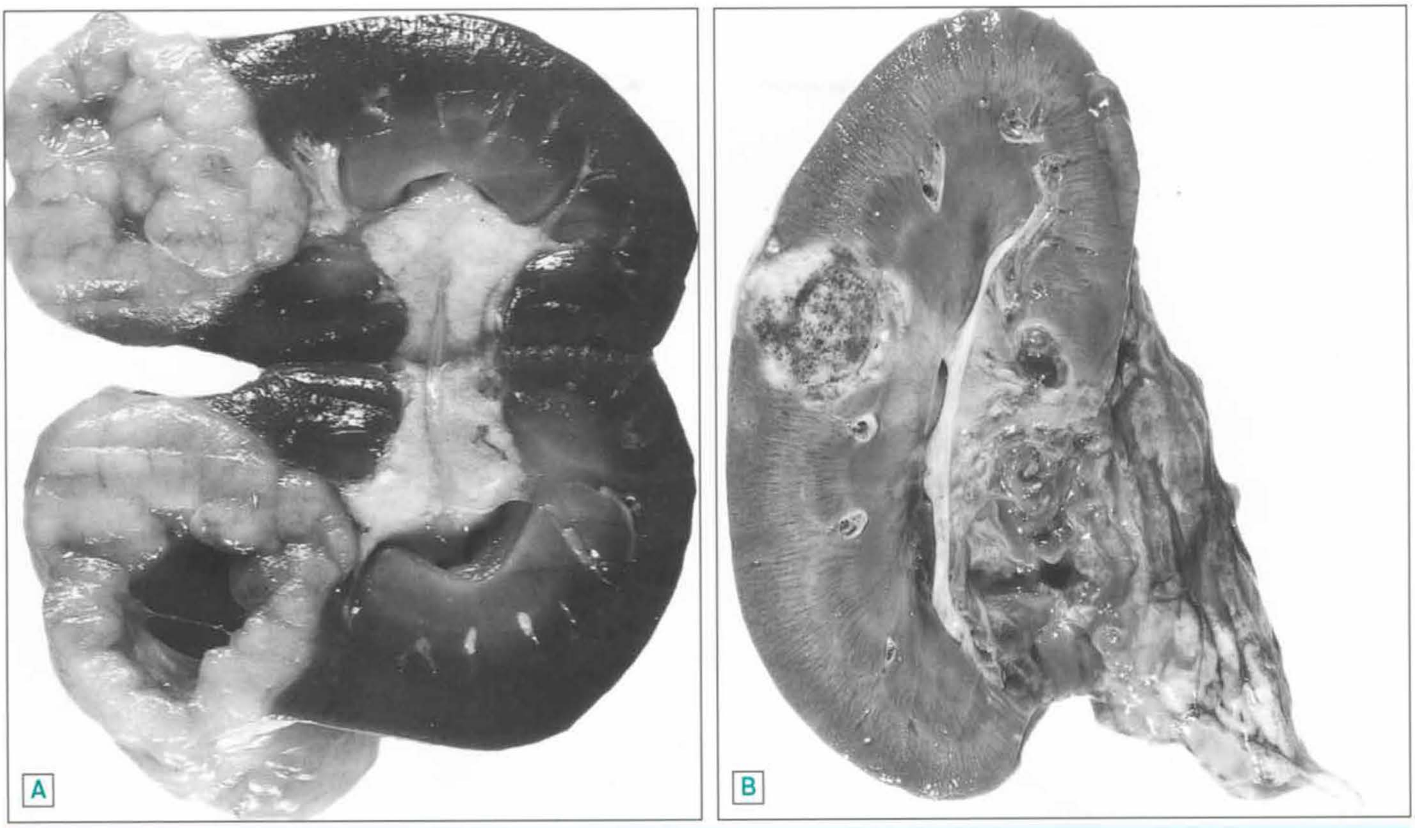

Figure 4.81 **A. Renal carcinoma** showing cystic degeneration, from a sheep. **B. Renal carcinoma** from a dog, with extension to pelvis and pelvic structures.

The vast majority of renal neoplasia is metastatic to the kidney, by hematogenous or lymphatic spread or by direct extension.

Renal adenoma

Renal adenomas are rare; they are said to occur more often in cattle and horses than in other species. In dogs, they comprise about 15% of primary renal epithelial tumors. *Adenomas are usually incidental autopsy findings.* Renal adenomas arise from epithelium of the proximal convoluted tubules. Grossly, they tend to be *solitary nodules* less than 2 cm across but occasionally are larger. They grow expansively. Microscopically, the tumor cells are cuboidal with moderate to abundant acidophilic cytoplasm. They form solid sheets, or papilliform or tubular structures, and stromal tissue is scant. Tumors with mixed architectural patterns occur. Histologic differentiation of adenoma and renal carcinoma is sometimes impossible; a few "adenomas" may be small, well-differentiated carcinomas. In cattle, adenocarcinomas are virtually always microscopically multiple, in contrast to the solitary nature of adenomas in this species.

Renal carcinoma

Carcinomas are the most common primary renal tumors of dogs, cattle, and horses. There are only sporadic reports in cats, sheep, and pigs. These tumors occur in mature and old animals; thus, their incidence is relatively low in some species. The average age of affected dogs is ~8 years, although this tumor has been reported in dogs less than 2 years of age. Males are affected about twice as often as bitches. This is in contrast to the overwhelming higher occurrence in cows, likely because of a preponderance of females in the aged cattle population. Common presenting signs are hematuria, palpable abdominal mass, and weight loss. Polycythemia associated with excessive erythropoietin production is very rarely seen in animals, but paraneoplastic erythrocytosis occurs in 1–5% of humans with renal adenocarcinoma. Polycythemia typically resolves after removal of the tumor. Hypertrophic osteopathy may be seen in cases of renal carcinoma with pulmonary metastases. Disseminated intravascular coagulation, extreme neutrophilic leukocytosis, leukemoid blood response, and bone infarcts are additional rare canine renal adenocarcinoma-associated paraneoplastic syndromes.

Grossly, renal carcinomas are spherical or ovoid masses, typically located in one pole of the kidney (Fig. 4.81A). Usually they are well demarcated from the remainder of the kidney, which is atrophic and compressed. Often the tumor is much larger than the original size of the host kidney but still has a discrete border. The tumor is usually gray or light yellow, often with darker areas of necrosis and hemorrhage. Invasion of the renal pelvis, ureter, renal vein, and hilar lymphatics may be visible (Fig. 4.81B).

In humans, five types of renal carcinoma are recognized based on cell morphology and origin, including clear cell, chromophilic, chromophobic, oncocytic, and collecting-duct variants (Bellini's duct tumors). Immunohistochemistry for lectins can be useful for distinguishing origin because these are site-specific within the various levels of the nephron. *Histologically*, there are a variety of cell types and architectures found in the animal forms (Fig. 4.82) but, if examined carefully, small or large areas composed of renal "clear cells" are found most commonly. These cells have vacuolated cytoplasm and only the basal nucleus and cytoplasmic outline are visible. The

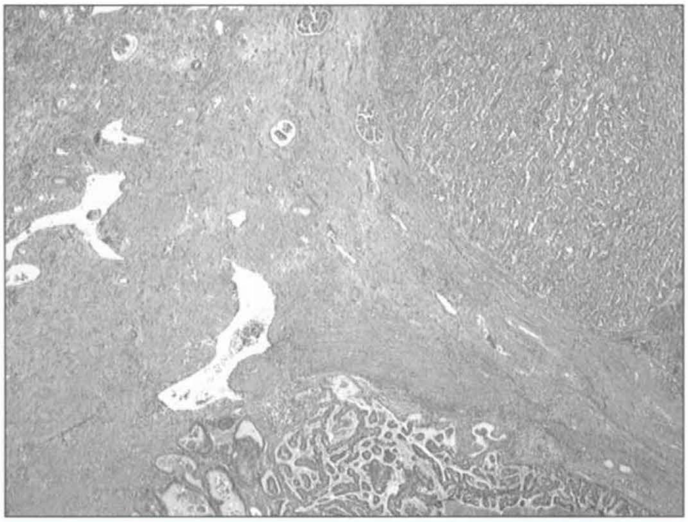

Figure 4.82 Renal carcinoma, consisting of sheets, tubules, and papillary structures, invading and compressing renal cortical tissue.

vacuoles contain fat. The presence of clear cells, even in metastases, should suggest renal carcinoma but is not pathognomonic – similar cells occur in certain endocrine tumors and other carcinomas. In other areas, the cells are cuboidal with denser acidophilic or basophilic cytoplasm. They may be arranged in sheets, papillary or tubular structures, and occasionally they line small cystic spaces. All of these patterns may occur in a single tumor, and apparently there is no prognostic value associated with any pattern. The stroma of renal carcinomas is scant but highly vascularized, which predisposes to the extensive necrosis often seen grossly.

Renal carcinomas tend to grow expansively, but satellite nodules often develop from local permeation. Invasion of the renal vein is always possible but may not result in metastases. Peritoneal implantation sometimes occurs. Usually by the time a dog is presented for examination, widespread metastases are present, especially in lungs and liver, but also in brain, heart, and skin. Such behavior is not invariable, and in some cases unilateral nephrectomy is curative. In contrast, in **cattle** there is a low rate of systemic metastasis but bilateral renal involvement is common. This may be explained by multiple de novo development and less by intrarenal metastasis. Additional characteristic findings in bovine renal cell carcinomas are corpora amylacea, proteinaceous secretions, and hemosiderin. There is a report of clear cell renal carcinoma in a **horse** manifesting initially as an aggressive metastatic oral tumor. The oncocytic form of renal carcinoma has been reported infrequently in rats and dogs.

Renal carcinomas are rarely diagnosed in **cats**, but typically occur unilaterally, in older cats (mean 9 years), with no sex or breed predilection and only sporadic metastases. Bilateral de novo development has also been documented.

Middle-aged and older German Shepherd **Dogs** with generalized *nodular dermatofibrosis* concurrently also have **renal cystadenocarcinomas** or cystadenomas, which are usually bilateral (Fig. 4.83); the carcinomas will occasionally metastasize to regional lymph nodes, peritoneum, liver, spleen, lung, and bone. Affected bitches also often have multiple uterine leiomyomas; the syndrome may be the result of a paraneoplastic process in which renal tumor-derived growth factors stimulate accumulation of fibrous tissue in various sites. The syndrome is apparently *inherited in an autosomal dominant mode in German Shepherd Dogs*, and caused by a tumor suppressor gene located on chromosome 5. Because cases of nodular dermatofibrosis with various degrees of cystic renal pathology have been reported in a variety of dog breeds, some postulate that there is concurrent initiation of fibrosis in both the skin and the kidney, the latter leading to outflow obstruction of renal tubules with expansion and eventual cyst formation. There may be progression through a continuum of lesions, including renal epithelial cyst, cystic adenomatous hyperplasia, renal cystadenoma, and cystadenocarcinoma, with purebred dogs perhaps being prone to more rapid transformation to malignancy. Thus, all dog breeds are potentially at risk.

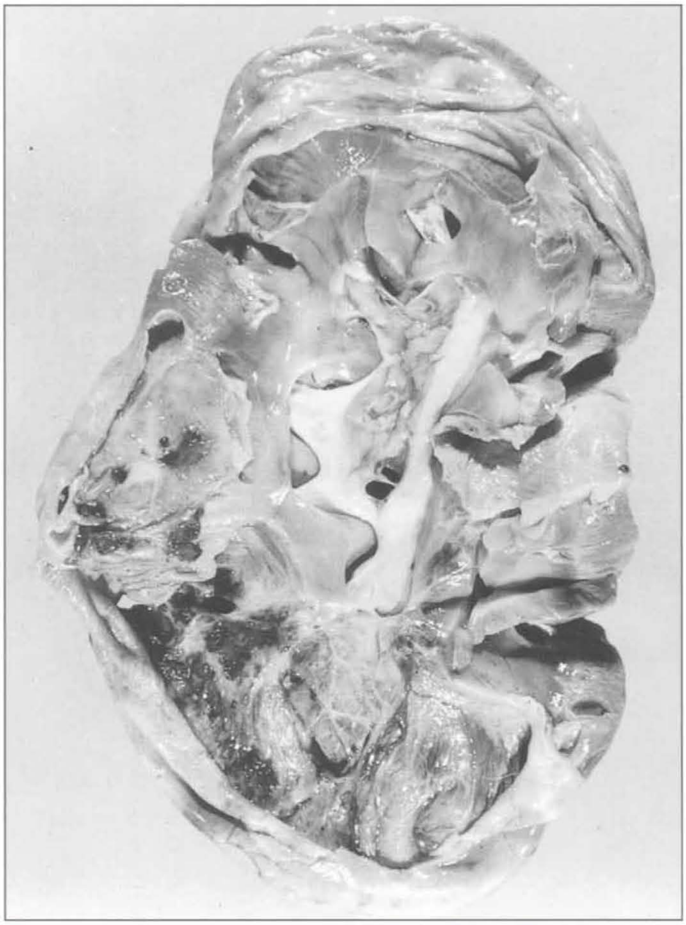

Figure 4.83 Renal cystadenocarcinoma from a German Shepherd Dog.

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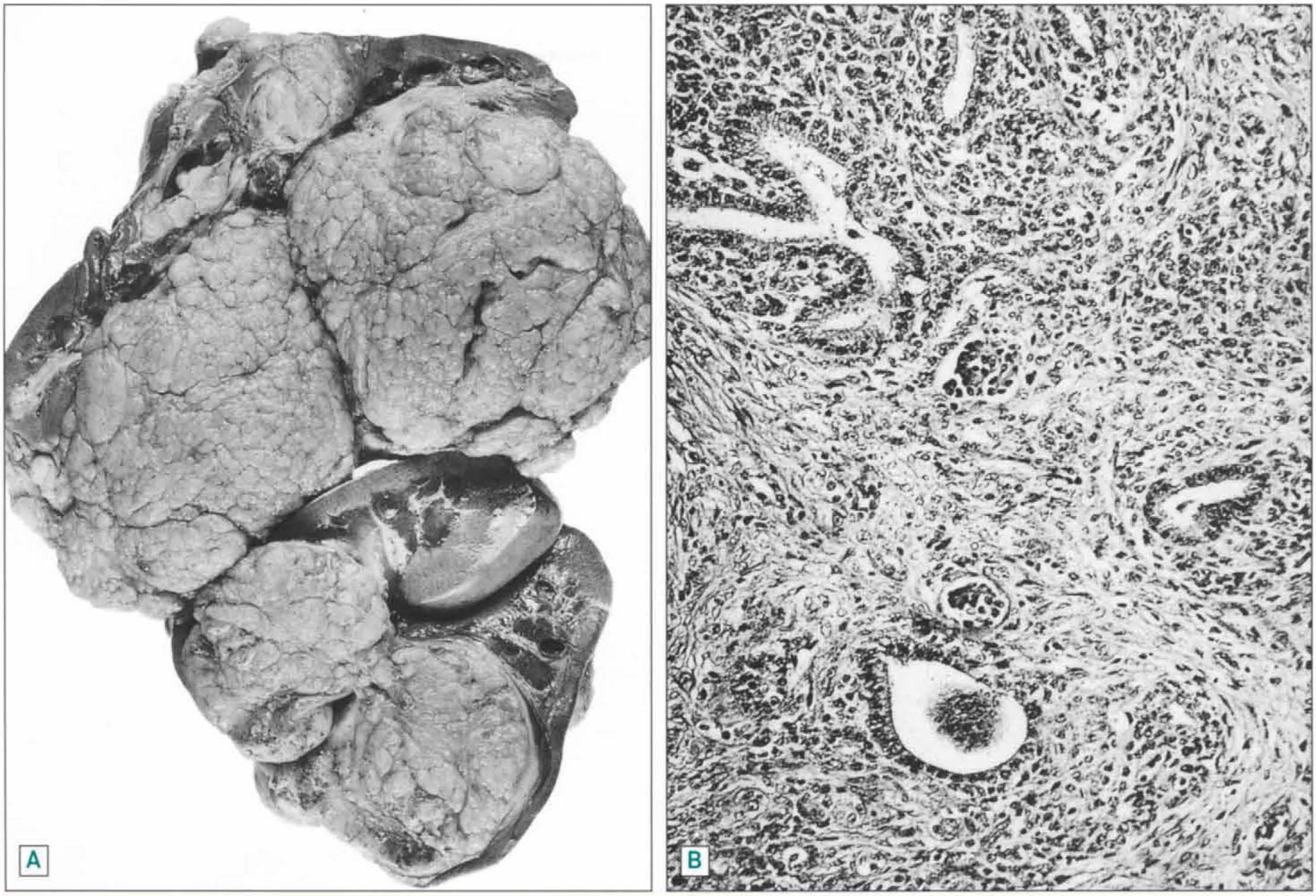

Figure 4.84 A. Bilateral nephroblastomas from a pig. Lobulated tumors distort and replace renal parenchyma. **B. Nephroblastoma** from a pig. Note tubular and glomerular structures.

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Nephroblastoma

Nephroblastoma (embryonal nephroma, Wilms' tumor) is the most common primary renal tumor of pigs and chickens. Abattoir surveys of pigs in the UK found 3.5 per million swine slaughtered, and in the USA 43.5 per million, with a frequency of 197 cases per million in one area. Nephroblastomas occur far less often in calves and in dogs, and are very uncommon but reported in sheep, horses, and cats. They are usually seen in young animals and sometimes in fetuses, but also in mature sows and are more common in adult dogs than in pups. In rats, various carcinogens, including dimethylnitrosamine, can induce formation of nephroblastomas amongst other tumors.

Nephroblastomas are *true embryonal tumors* that arise in primitive nephrogenic blastema and in foci of renal dysplasia. The presence of tissues not typically associated with the kidney, such as cartilage and skeletal muscle, indicates an origin in pluripotential mesenchyme of the metanephron. These tumors establish the important principle that all component tissues of the kidney arise from a common blastema and that this neoplasm may represent a form of arrested development. Finding nephrogenic rests of nodular renal blastema in dogs suggests this species shares a similar pathogenesis and histogenesis to that seen in Wilms' tumors in humans.

Grossly, nephroblastomas may attain a huge size and cause abdominal enlargement. They are often multiple in the affected kidney, growing expansively and compressing the adjacent parenchyma, and they are encapsulated. They are usually unilateral but a few are bilateral (Fig. 4.84A) and these sometimes unite across the midline to form a single large mass. Widespread metastases to lung and liver occur in over half the canine cases, but are rare in pigs and calves.

Rarely these tumors are seen as primary extramedullary and intradural spinal canal masses between T10 and L2. German Shepherds and male dogs seem to be overrepresented with the spinal form, which may occur in the absence of renal involvement. There is a report of spinal cord nephroblastoma with suspected intraspinal metastasis. The cut surface of nephroblastomas is variegated or lobulated and the larger ones have extensive areas of hemorrhagic necrosis. The characteristic cut surface reveals a myxomatous soft, gray-white or tan tissue, which feels spongy.

Histologically, the characteristic features are primitive glomeruli with primitive Bowman's spaces, abortive tubules, and a loose spindle-cell stroma (Fig. 4.84B) that may show some differentiation to a variety of mesenchymal tissues, including striated muscle, collagen, cartilage, bone, and adipose tissue. The mesenchymal components may predominate over epithelial elements in certain tumors, especially in ruminants. When all three components (blastemal, epithelial, and stromal) exist in equal proportions, these forms are referred to as *triphasic (mixed) nephroblastomas*. Commercially available antibody to the human Wilms' tumor gene product C-19 successfully stains canine nephroblastomas. These tumors are also typically glial-fibrillary-acidic-protein-negative and variably vimentin- and cytokeratin-positive. Tubular and glomerular differentiation indicate a good prognosis; anaplasia and a sarcomatous stroma are associated with metastasis and a poor prognosis. Rarely, polycythemia is seen as an associated paraneoplastic syndrome.

Other tumors

Transitional cell papilloma and **carcinoma** of the renal pelvis are very rare tumors, which occur in the dog, cow, pig, and horse. Squamous and glandular metaplasia develop in the carcinomas. Primary pelvic **squamous cell carcinoma** is extremely uncommon, but in dogs they have been noted in association with pelvic calculi. Transitional cell tumors are discussed in the section on the Urinary bladder, below.

Primary mesenchymal tumors of the kidney occur but may be diagnosed only after very careful examination to exclude a primary focus in some other tissue. The prognosis associated with renal mesenchymal neoplasms is usually grave. Fibrous and vascular tumors are the most common types. **Hemangioma** is the most frequently encountered benign renal neoplasm in dogs; hematuria is the most consistent accompanying sign. An intracapsular **angiomyolipoma** (mesenchymal hamartoma) has been reported in the kidney of a cat. A case of primary feline renal **hemangiosarcoma** has been reported. Renal **interstitial cell tumors** occur in the cortex and medulla of old dogs and arise from cells distinct from interstitial fibroblasts. **Benign cortical fibromas** also occur in older dogs. A vimentin-positive tumor termed a **congenital mesoblastic nephroma** has been reported in a dog; the tumor was composed of fibromatous and myxomatous areas. Other rare primary renal tumors include neurofibroma, malignant fibrous histiocytoma, and oncocytoma. **Renal oncocytomas** are rare, usually unilateral, benign tumors composed of oncocytes, which are large polygonal cells with bright eosinophilic granular cytoplasm. Periodic acid–Schiff stain accentuates the cytoplasmic granules, which on electron microscopy are mitochondria. The cells are typically arranged as compact nests, cords, or tubules. The histogenesis is unclear but it is thought that these tumors originate from intercalated cells of the collecting ducts; the cells express cytoplasmic keratin.

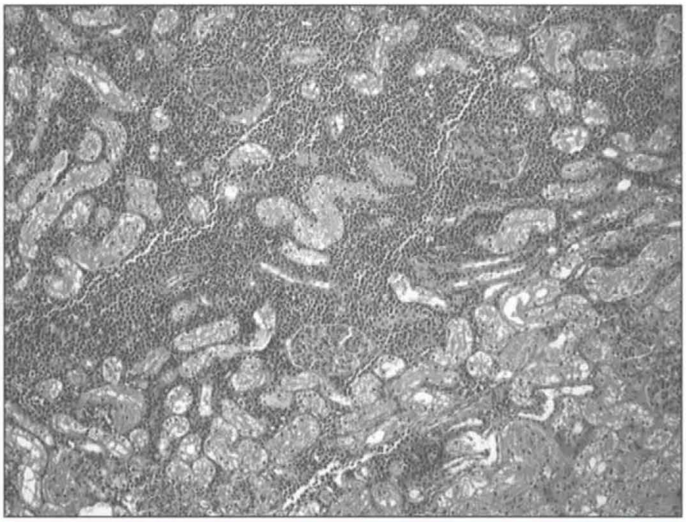

Figure 4.85 Diffuse infiltration and disruption of renal parenchyma by **lymphosarcoma**.

Metastatic tumors are common in the kidneys, and disseminated neoplasms of any type are likely to localize there, typically with bilateral involvement of the cortices. Many such metastases are of microscopic size and of hematogenous origin. Retrograde lymphatic invasion along the renal lymphatics may occur from carcinomas in adjacent organs.

In dogs, primary **pulmonary adenocarcinoma** with renal metastases may be difficult or impossible to distinguish from primary renal carcinoma with pulmonary metastases, since their microscopic appearance is quite similar.

Renal involvement in **lymphosarcoma** is common in those species in which the neoplasm is common. This is the most common renal neoplasm in cats, but is more often a metastatic rather than a primary lesion. The involvement may be diffuse or nodular. When the nodular lesions are grossly visible, they are numerous, poorly defined, fatty in appearance and project hemispherically above the surface. The capsule is not adherent. When lymphomatous involvement is diffuse (Fig. 4.85), the organ is enlarged and has a uniform white fatty appearance. The differentiation of lymphomatous metastases from interstitial nephritis often requires microscopic examination and, indeed, metastases may sometimes only be discovered, especially in cats, by microscopic examination. Polycystic kidney disease has been found concurrently with renal lymphosarcoma in a cat. Peripelvic and periureteral infiltrates, which cause hydronephrosis, are common in cattle.

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Lower urinary tract

GENERAL CONSIDERATIONS

The lower urinary tract consists of **ureters, urinary bladder**, and **urethra**. The ureters and bladder, and also the renal pelvis, are lined by transitional (nonkeratinizing) stratified epithelium – the **urothelium**. The ureters are of uniform diameter throughout, and usually course directly to the bladder, although they may be tortuous and dilated distally in baby pigs. The ureters enter the bladder wall obliquely and are covered by a mucosal flap. Histologically, the ureteral mucosa is present in longitudinal folds; there are poorly defined internal and external longitudinal muscle layers, a prominent middle circular layer, and either adventitia or peritoneal serosa. Thus, peritonitis may involve the ureters and interfere with their peristalsis. Ureters and the renal pelvis of horses have simple branched tubuloalveolar mucous glands in the propria-submucosa. Histologically, the bladder is an expanded ureter, lined by stratified transitional epithelium ranging from 3 to 14 cells thick depending on the species and degree of distension. Superficial cells, which contain tubulovesicular compartments, supply additional membrane to allow for bladder distension. The basolateral plasma membrane of the superficial cells and the plasma membrane of the intermediate layer of transitional cells interdigitate and are attached by desmosomes that provide structural stability and elasticity during distension and relaxation. Lymphoid nodules are commonly found in the lamina propria of all domestic animals. Theliolymphocytes are common in ruminants. Eosinophilic intracytoplasmic inclusion bodies of canine distemper seen in urinary bladder epithelial cells must be differentiated from similar nonspecific inclusions.

The function of the ureter is to propel urine from the kidney to the bladder by peristalsis. In this it is assisted by the renal pelvis. Ureteral peristalsis is controlled by one or more pacemakers, which are located in the recesses of the renal pelvis, and by the activity of the ureteropelvic junction, which determines whether a pacemaker stimulus initiates a peristaltic wave. The regulation of nerve impulses for smooth-muscle contraction is mediated by tachykinin activation of neurokinin-2 receptors. At low urine production rates, peristalsis may occur with every fifth pacemaker stimulus, but during diuresis, every stimulus initiates a peristaltic wave. The ureters pass obliquely through the muscular wall of the bladder at the ureterovesical junction. *The segment of ureter in the bladder wall, the intravesical ureter, forms the basis of the* **vesicoureteral valve**, *which prevents reflux of urine from bladder to ureter.* When the length of the intravesical ureter is short, as is often the case in puppies, reflux frequently occurs. Thus, the angle of the ureteral entry and the thickness of the bladder wall influence the competence of the valve. The urinary bladder stores urine and, in concert with the urethra, expels it. During continence, the bladder is relatively flaccid and the urethra acts as a valve. During micturition, contraction of the *detrusor muscle* – the urinary bladder musculature – pumps urine through the relaxed urethra. Sphincter mechanism incompetence or detrusor instability can lead to incontinence, seen most commonly in bitches.

Embryologically, the ureters are formed by buds from the mesonephric ducts, which develop craniad to their entrance into the cloaca. The cloaca is divided into a dorsal rectum and ventral urogenital sinus by the urorectal fold in such a way that the urogenital sinus is continuous with the allantois. The allantois originates as an evagination of the hindgut. At this stage of development, the mesonephric duct and ureteral bud form a Y shape – one arm of the Y is the ureteral bud and the other arm plus the stem of the Y are formed by the mesonephric duct. As the allantois and urogenital sinus develop, the stem of the Y is absorbed and the mesonephric duct and ureteral bud enter the urogenital sinus independently. In females, the entire urethra, plus the vaginal vestibule, is derived from the urogenital sinus, but in males only the prostatic urethra is so derived. The penile urethra forms by closure of a groove, the urethral groove, on the caudal face of the penis. The bladder, which is formed from the cranial part of the urogenital sinus and the caudal part of the allantoic diverticulum, communicates with the allantois via the urachus, which forms from the cranial part of the intraembryonic allantoic diverticulum. The communication is severed at birth and the urachus closes but remains as the umbilical ligament of the bladder.

Most ailments of the lower urinary tract are associated with obstruction and infection, which are often concomitant. Unlike the gastrointestinal tract, which has a normal microbial flora throughout its length, only the most distal part of the male urethra, and the female vagina, normally host microorganisms. The operation of sphincter-like mechanisms in the urethra and vesicoureteral valves, and the intermittent pulsatile flow of urine from the kidneys and bladder, normally prevents the movement of organisms higher up the tract. The susceptibility of the urinary bladder of the female to severe infections is undoubtedly related to its short distensible urethra and its proximity to the external environment and especially the rectal flora. On the other hand, the anatomy of male urethras, with their flexures, ossa, and appendages, make them prone to obstruction, particularly by calculi. Specific factors concerned in the establishment and maintenance of infections in the urinary bladder and their spread to the kidney are discussed in the sections on Cystitis and Pyelonephritis. The causes and effects of obstruction are considered in the sections on Urolithiasis and Hydronephrosis.

The **urothelium** of the renal pelvis, ureter, and perhaps the trigone of the bladder originate from mesoderm, whereas in the rest of the bladder it has an endodermal origin. Thus, urothelium responds to chronic irritation from infections, calculi, and excreted chemicals by proliferation and/or metaplasia, both of which are regarded as premalignant changes. Downwards proliferation of urothelium, a common reactive change, results in groups of proliferating cells isolated in the submucosa, known as **Brunn nests**. If the center of the nest undergoes liquefaction, *cystitis cystica, ureteritis cystica,* or *pyelitis cystica* results. *Pyelitis, ureteritis, or cystitis glandularis* develops if the epithelium lining the cyst undergoes mucous metaplasia. Metaplasia of squamous or mucous types is often superimposed on predominantly proliferative lesions. The male urethra has a thin lining of transitional epithelium; that of the female is similar but has stratified squamous epithelium at its termination. Most of the adenomas and adenocarcinomas of the lower urinary tract develop in areas of mucous metaplasia. A few may arise in submucosal glands or mesonephric and urachal remnants. Urothelium that is not exposed to urine has a tendency to undergo metaplasia to an intestinal-type epithelium – such is the fate of some cystic urachal remnants, which are normally lined by transitional epithelium.

Urine may be obtained for urinalysis or culture by cystocentesis at autopsy. The bladder may be dilated in downer animals, even in states of dehydration, due either to the absence of the correct posture needed for urination or to decreased medullary tonicity due to hypoproteinemia (low urea production) that has led to polyuria. Dog bladders are often constricted at necropsy and hence have a thick wall. If the bladder can be dilated by pulling it between the fingers, the thickening is not pathologic. Horse urine normally contains mucus and crystals.

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ANOMALIES OF THE LOWER URINARY TRACT

Ureters

Agenesis of the ureters is due to failure of the ureteral bud to form, and may be unilateral or bilateral. In dogs, it is often accompanied by renal agenesis (see section on Anomalies of development, above). **Duplication** of a ureter is caused by the formation of two ureteral diverticula from the mesonephric duct. The caudal ureter drains the caudal part of the kidney and the cranial one drains the cranial part. Usually the caudal ureter empties normally while the other is ectopic. Duplication is rare but occurs in dogs and pigs (Fig. 4.86). **Ureteral dysplasia** occurs in association with renal dysplasia. **Ureteral valves** are occasionally seen in dogs.

Ectopic ureter *is the most important ureteral anomaly*. Rather than terminating at the trigone of the bladder, the affected ureter may empty into the vas deferens, vesicular gland, or urethra of the male, or the bladder neck, urethra, or vagina of the female. In bitches, the ectopic ureter usually terminates in the vagina or urethra, whereas in cats urethral termination is most common. There are two possible causes: either the ureteral bud arises too far craniad to be incorporated into the urogenital sinus, or the differential growth of the sinus is abnormal and the ureter fails to migrate to its usual location. Ectopic ureters occasionally empty into the rectum because of anomalous cloacal division by the urorectal fold. Very rarely they empty into the cervix, uterus, or uterine tube, possibly as a result of aberrant origin from the paramesonephric (Müllerian) duct. Even more rarely, an ectopic ureter may be blind-ended cranially in cases with accompanying renal agenesis.

Ectopic ureter is most common in dogs and is diagnosed up to 20 times more frequently in females than in males. However, this sex difference may be apparent or exaggerated since affected females are usually incontinent from birth, whereas affected males may not be. Termination of the ectopic ureter proximal to the external urethral sphincter in males leads to retrograde filling of the bladder rather than to incontinence. Ectopia can be unilateral or bilateral and is often associated with other urinary tract abnormalities, including bladder agenesis or hypoplasia, renal agenesis or hypoplasia, ureteral or bladder duplication, and branching of the terminal ureter. In the USA, certain dog breeds have a high risk for the defect, including the Siberian Husky, Bulldog, Newfoundland, Labrador Retriever, West Highland White Terrier, Fox Terrier, and Miniature and Toy Poodles. The breed predilection in the UK includes the Golden

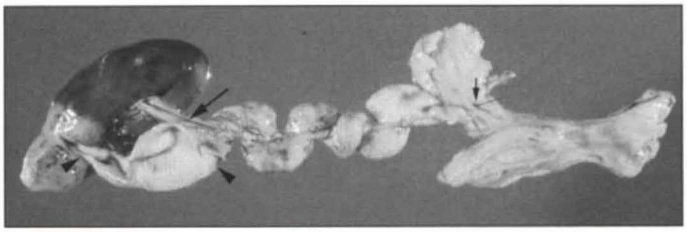

Figure 4.86 Renal dysplasia and **ureteral duplication** in a pig. The ureter draining the dysplastic cranial part of the kidney (between arrowheads) is markedly dilated and tortuous; the ureter draining the caudal part (between arrows) is traced by a wire from kidney to bladder trigone.

Retriever, Labrador Retriever, Skye Terrier, Border Terrier, Briard, Bulldog, and the Griffon. The defect is familial in Siberian Huskies and Labrador Retrievers. Ectopic ureter also occurs in White Shorthorn bulls and tends to involve the region of the seminal vesicles. It is a rare anomaly in horses.

A **ureterocele** is a congenital abnormality that is defined as *a focal submucosal dilation of the distal ureter,* especially involving that portion that lies within the urinary bladder wall. It may be associated with normal anatomic emptying (orthotopic or intravesical ureterocele), or an ectopic location (ectopic ureterocele). A proposed grading scheme is as follows: grade 1 has no concurrent ureteral or renal disease, grade 2 has unilateral hydroureter, hydronephrosis, or chronic renal disease, and grade 3 is similar to grade 2 but with bilateral hydroureter and a consistently worse prognosis.

Ureteral anomalies often predispose to hydronephrosis/hydroureter and urinary tract infection that may culminate in pyelonephritis. **Ureteral diverticula** result from transitional cell hyperplasia, mucinous metaplasia, and submucosal urothelial proliferation with the formation of small cysts. Neoplastic transformation may occur more readily in such sites.

Urinary bladder

Duplication of the urinary bladder occurs in dogs and causes dysuria, incontinence, and, less frequently, abdominal distension and cryptorchidism. The extra bladder originates dorsally between the urinary tract and uterus or rectum. Cystic remnants of the urorectal fold may be responsible for this defect.

Patent or **pervious urachus** is the *most common malformation of the urinary bladder* and is seen more often in foals than in other animals. Animals with this defect dribble urine from the umbilicus because the urachal lumen fails to close and remains as an open channel between the apex of the bladder and the umbilicus; a patent urachus is susceptible to infection and abscessation. Rupture of the urachus causes uroperitoneum. The condition must be differentiated from perinatal rupture of the bladder. Occasionally, urachal obliteration is partial, and rests of epithelium remain intact to develop into **urachal cysts** at any point between the umbilicus and the bladder. These cysts may become quite large but usually are small and multiple and are attached to the midline of the bladder. Occasionally they adhere to the intestinal serosa. The urachus is normally lined by transitional epithelium, but metaplasia to squamous or mucus-secreting columnar epithelium is common in urachal cysts and sinuses. Urachal remnants in the bladder wall may give origin to neoplasms.

Diverticula of the bladder may be primary or acquired secondary to partial obstruction to the outflow of urine, or as the result of pressure changes exerted during normal contractions. Diverticula are usually seen at the vertex, where they represent incomplete closure of the urachus (vesicourachal diverticulum) with an area of discontinuity in the muscle but also occur less commonly in the trigone region in association with a malformation of the bladder muscle wall. Contraction of the bladder tends to distend the diverticulum, but rupture does not occur. Additionally, stasis of urine in the diverticulum eventually leads to persistent infection and inflammation. Calculi may form in the diverticulum. If the distal urachus remains patent to the exterior through the umbilical opening, a **urachal sinus** results.

Urethra

Urethral **agenesis**, **duplicated** urethra, **ectopic** urethra, and **imperforate** urethra occur rarely in dogs. Recently, the first case of bovine urethral duplication was reported in a Swiss Braunvieh heifer with other urogenital malformations, as is often the case. This animal had cytogenetic confirmation of reciprocal autosomal translocation between chromosomes 20 and 22, suggesting that genetic anomalies could be responsible for this condition. Hypospadias is described with male genitalia. *The most common urethral anomaly is* **urethrorectal** or **rectovaginal fistula**, which is caused by incomplete division of the cloaca into rectum and urogenital sinus by the urorectal fold. In males, the communication involves the pelvic urethra and affected dogs urinate from the rectum. In females, the opening is in the vagina and may be associated with imperforate anus. These defects are reported in dogs, cats, pigs, rabbits, alpacas, and foals, often as one of several congenital anomalies. These changes usually predispose to urogenital tract infections but sometimes are incidental autopsy findings. An uncommon occurrence of this lesion as a single anomaly has been documented in a mature gelding.

In male ruminants and swine, a **urethral recess** is normally present near the ischial arch and is not anomalous, but is an impediment to catheterization. Dilation of the urethral recess can cause midline perineal swelling in ruminants. A case of **congenital megalourethra** in a calf was characterized by dilation of the penile urethra, with a poor urine stream and a lower urinary tract infection.

Urethral atresia and **urethral hypoplasia** have been noted in newborn freemartin intersex calves with uroperitoneum following rupture of the urethra or urinary bladder. **Urethral strictures** have been documented in intersex ruminants, a llama, and a Nubian goat.

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Acquired anatomic variations

Displacements of ureters and urethra are sometimes caused by local inflammatory and neoplastic swelling. Their main significance to the urinary system is related to obstruction of urine flow. Ureteral and urethral displacements may also occur with variations of position of the bladder. **Torsion** of the bladder is uncommon; it may be partial or complete about the long axis of the organ. Presence of part of the bladder within the pelvis in dogs, so-called "*pelvic bladder,*" is thought to be a normal variation, dependent on the degree of distension of the bladder, and is not a cause of incontinence.

Dorsal **retroflexion** occurs in male dogs with tenesmus in response to prostatic enlargement or constipation. The normal position is assumed following emptying. A more serious form occurs with prolapse of the vagina in cows, sows, and bitches and occasionally in perineal hernia of older male dogs. In these cases, the bladder may be present in the hernial sac and, because of the retroflexion, there is obstructive kinking of the neck of the bladder and sometimes of the urethra and ureters. If the ureters are patent, the accumulation of urine in the bladder contributes to the size of the hernia. Hydronephrosis or rupture of the bladder may occur if the condition is not corrected.

Eversion of the bladder (*invagination into and through the urethra*) occurs in females and is permitted by the short, wide urethra in this sex. The bladder may arrive in the vagina. This can occur in any of the larger species and is perhaps most common in mares. Eversion is predisposed to by circumstances in which increased intra-abdominal pressure or straining occurs, often after parturition. Hypocalcemia in periparturient dairy cows may contribute by resulting in decreased bladder tone prior to eversion. The everted bladder may allow for herniation of intestine. Rarely, *umbilical eversion* of the bladder can occur in foals that experience traumatic tearing of the umbilical cord and urachus during birthing. Eversion of the bladder is to be distinguished from **prolapse** of the bladder; in eversion, the mucosal surface protrudes from the vulva, whereas *in prolapse the bladder has been displaced through a rent in the vagina and the serosal surface appears.*

Hydroureter, or *dilation of a ureter*, may be due to obstruction by calculi, neoplasms, or inflammatory debris, or due to accidental ligation during surgery, e.g., ovariohysterectomy, or following post-surgical scarring, and will lead to hydronephrosis. Dilation without physical obstruction occurs in association with peritonitis and may be due to loss of muscle tone. Dilation of ureters is often present in neonatal pigs with enteric infections. Congenital hydroureter and hydronephrosis often occur in piglets in association with epitheliogenesis imperfecta.

Ureters may **rupture** as a consequence of blunt physical trauma and only rarely in association with parturition. Ureters may also be accidentally **transected** during surgery, e.g., ovariohysterectomy. Leakage of urine from a traumatized ureter is the most common cause of a **urinoma** – a retroperitoneal accumulation of urine sometimes referred to as a *paraureteral or uriniferous pseudocyst* due to its fibrous connective tissue wall and absence of an epithelial lining. Urethral obstruction and urine leakage from a rent in the renal capsule are also documented causes. **Perinephric pseudocysts** resemble urinomas but surround the kidney and are thought to be associated with chronic renal failure in cats. Foals experience **ureteral tears** that are thought to be the result of blunt nonpenetrating trauma. The common bilateral nature suggests a possible congenital predisposition in this species.

Dilation of the bladder may be of local obstructive or neuroparalytic origin. The wall is thin and almost transparent, and the distended organ may extend almost to the liver. Brief periods of distension may allow quick return of normal contractibility, but severe or prolonged distension may result in loss of tone, which is not restored before bacterial complications terminate the condition. The *causes of obstruction* include calculi, prostatic enlargement in dogs, accumulated inflammatory detritus or blood clots in the urethra, urethral strictures, and tumors of the neck of the bladder or urethra. Detrusor atony of the bladder may follow prolonged dystocia in the dog.

Neurogenic disorders of micturition may be the result of abnormalities of the sphincter or of the bladder and may result from failure to store urine, failure to void urine, or a combination of both. Neurogenic distension follows spinal injury with loss of tonic parasympathetic outflow from the sacral plexus. Spinal myelitis, as in canine distemper and rabies, may also cause paralysis of the bladder. Perhaps it most commonly follows herniation of intervertebral disks in the dog. Cystitis and, less commonly, fundic necrosis secondary to compression of vessels within the distended urinary bladder wall, are the usual complications. In horses in particular, bladder paralysis and distension can lead to sabulous urolithiasis. Descriptions of *detrusor-urethral dyssynergia* in dogs are sparse and most have been documented in young and middle-aged large-breed dogs. This condition resembles urinary obstruction by uroliths but it is a functional disorder of the voiding phase of micturition. This condition occurs with involuntary contraction of the external urethral sphincter in the postprostatic urethra or with contraction of the smooth muscle of the neck of the bladder or prostatic urethra (internal sphincter). Typically, these animals have dysuria with interrupted spurting of urine and a large residual urine volume.

Sphincter mechanism incompetence *is the most common cause of urinary incontinence in middle-aged, spayed bitches*, but the prevalence is also higher in animals with body weights over 30 kg, docked breeds (Doberman Pinschers, Old English Sheepdogs, and Rottweilers, as well as in Weimaraners and Setters). In females, an intrapelvic bladder neck, a short urethra, reduced urethral tone and neutering before first estrus predispose. Restoration of normal anatomic location of urethra/urinary bladder by surgical procedures is often curative. Bladder neck position (i.e., intrapelvic), breed, prostate size, and castration status are significant risk factors for incontinence in male dogs. Congenital urethral sphincter mechanism incompetence in cats has been associated with genitourinary dysplasia (urethral hypoplasia and vaginal and/or uterine hypoplasia/aplasia).

Distension of the bladder occurs when calves fed indigestible milk-replacer starve to death. They are usually recumbent for several hours before death but this is probably due to weakness rather than nervous disease. Renal concentrating ability is depressed by starvation because of lack of urea and a partial insensitivity to antidiuretic hormone and mineralocorticoids. The bladder distension is possibly caused by overproduction of dilute urine.

Hypertrophy of the bladder is fairly common in dogs and less so in other species. It is a response to long-standing partial obstruction to the outflow of urine.

Rupture of the bladder (cystorrhexis) often occurs following urethral obstruction, such as occurs in urolithiasis, but rarely following pelvic trauma or dystocia; traumatic rupture of the bladder can occur during parturition in mares and cows. The interval between obstruction and rupture depends somewhat on the competence of the vesicoureteral valve and whether hydronephrosis develops. Rupture of the bladder occurs in newborn foals, with an incidence of 0.2–2.5%, affecting either the dorsal or ventral aspect of the viscus; males are most often affected. Some ruptures are congenital and most are probably caused by birth trauma. Twists in the amniotic portion of the umbilical cord may compress the urachus, causing distension of both bladder and urachus and predisposing to rupture. Only rarely is the urinary bladder rupture secondary to atrophy of circular smooth-muscle fibers in the dorsal bladder wall.

Urine in the peritoneal cavity, or **uroperitoneum**, follows leakage from the kidneys, ureter, bladder, or urethra. Diagnosis of uroperitoneum is supported by the finding of peritoneal-to-serum ratios for potassium, phosphate, and creatinine greater than 2:1. Calcium carbonate crystals are occasionally seen in the peritoneal fluid. Uroperitoneum has been seen increasingly in foals in association with positive sepsis scores (45%) in one recent study, and was a negative prognostic indicator. *Escherichia coli*, *Actinobacillus equuli* and, less commonly, *Clostridium perfringens* are implicated in associated sepsis or urachal infections. Abdominal trauma is the most common cause related to urine leakage from the bladder in dogs and cats; catheterization and bladder expression are the most common causes of urethral leakage in cats. Urinary calculi and obstruction by transitional cell carcinoma are also causes.

Although uncommon in domestic swine, **urethral polyps** have been reported in Vietnamese pot-bellied pigs. It seems most likely that these lesions arise secondary to trauma to the mucosal folds of the urethral recess associated with repeated catheterization. **Urethral caruncles** have been described in humans and dogs and form as a result of chronic inflammation of the urethral mucosa. Glandular structures are intermixed with a granulomatous infiltrate, often producing an obstructive mass lesion.

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CIRCULATORY DISTURBANCES

Hemorrhages *are the most important and most common indicator of circulatory disturbances.* In the ureters and urethra, they are associated with obstructive calculi, and ureteral hemorrhage is part of acute ascending infections. Urethral hematomas following pelvic trauma may predispose to urinary bladder rupture. In the bladder, hemorrhages are typically located in the propria mucosa and may occur in any septicemia. Small hemorrhages with the shape of tiny hematomas are common and considered diagnostically significant in classical swine fever, African swine fever, porcine salmonellosis, and equine purpura hemorrhagica. Larger hemorrhages are present in bracken fern poisoning of cattle. Hemorrhage occurs with acute cystitis and neoplastic diseases, and hemorrhage with hematoma formation is seen following rupture of the bladder.

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UROLITHIASIS

Urolithiasis is the presence of calculi (uroliths or stones) in the urinary passages. Calculi are grossly visible aggregations of precipitated urinary solutes, urinary proteins, and proteinaceous debris; minerals predominate in **calculi**, whereas matrix usually predominates in **urethral plugs**. *Calculi typically have a central nidus, surrounded by concentric laminae ("stone"), an outer shell, and surface crystals.* Many calculi are hard spheres or ovoids with a small amount of organic matrix impregnated with inorganic salts. *Urethral plugs are masses of sandy sludge with a much higher organic component* whose form is largely determined by the shape of the cavity they fill. Even densely mineralized calculi of the same type may have quite a different appearance depending on whether they are located in renal pelvis or urinary bladder. Many calculi contain significant quantities of "contaminants" such as calcium oxalates in "silica" calculi; a few are relatively pure.

The diseases caused by uroliths are among the most important urinary tract problems of domesticated animals. Several factors are important in predisposing to calculus formation and several are important in precipitating disease. These are not the same for all conditions. Obviously, calculogenic material must occur in urine in quantities sufficient to be precipitated. Sometimes this concentration is achieved because a substance is metabolized in an unusual way, as is uric acid in Dalmatian dogs; or it may be processed abnormally by the kidney, as is cystine in cystine stone-formers; or abnormally high levels of a substance in the diet, such as silicic acid in native pastures, may produce potentially dangerous urinary levels. Regardless of the type of calculus, certain factors are more or less important; these are **urinary pH**, in terms of its optimum for solute precipitation, and **reduced water intake**, in relation to the degree of urine concentration. Other predisposing conditions include **infection** (see section on Struvite calculi, below), obstruction, structural abnormalities, foreign bodies, and drug-induced changes in urine composition, e.g., by sulfonamides. A foreign body, such as a suture, grass awn, catheter, or needle, can act as a nidus for urolith formation. Deficiency of vitamin A is frequently suggested as a factor predisposing to urolithiasis, but the evidence is equivocal; it may contribute in exceptional circumstances by producing metaplastic changes in the urinary epithelium.

Urine is often supersaturated with respect to the components of stone-forming salts, and this **supersaturation** is the essential precursor to initiation of urolith formation (nucleation). Supersaturation may be in the **unstable** region where spontaneous precipitation occurs (homogeneous nucleation, the precipitation-crystallization theory of urolith initiation), or in the **metastable** range where precipitation occurs by epitaxy or heterogeneous nucleation (one type of crystal grows on the surface of another type). Although formerly it was thought that urinary proteins such as uromucoid, which make up 5–20% or more of some calculi, were pre-eminent initiators of crystal formation in the metastable range (*the matrix-nucleation theory of urolith initiation*), it is now believed that in many cases either co-precipitation of proteins and minerals occurs or that proteins are adsorbed on to formed crystals. It is possible that crystals of one salt, for which urine is supersaturated in the unstable range, cause epitactic induction of crystals of another salt, for which supersaturation is metastable. Crystals are much more common in urine than are calculi. Even though equine urine, for example, is normally supersaturated with calcium carbonate and crystalluria is normal, horses experience a low prevalence of calculi. The factors that promote crystal growth and crystal aggregation or, more importantly, prevent them in some animals, are poorly understood. Experimentally, high levels of urinary inorganic pyrophosphate and magnesium are important inhibitors of calcium phosphate and calcium oxalate crystallization, and pyrophosphate also inhibits aggregation of calcium phosphate crystals. Certain urinary macromolecules, probably glycosaminoglycans, are also strong inhibitors of crystal aggregation in experimental systems. Deficiency of inhibitors of crystallization may be important in calcium oxalate and calcium phosphate calculogenesis (*crystallization-inhibition theory of urolith initiation*). It is not known why calculi stay in the renal pelvis and urinary bladder until they are large enough to cause disease.

The important types of urinary calculi are given below according to species (Table 4.5). Although one mineral may predominate in a urolith, *many uroliths are of mixed composition*. Overlap in the gross appearance of uroliths usually precludes specific gross diagnosis of mineral type. The division indicated in Table 4.5 is arbitrary. Obviously, silica calculi are only important where ruminants are pastured, and clover stones are only important where subterranean

Table 4.5 Composition and importance of urinary calculi

Species	Common types	Uncommon types
Dog	Struvite Oxalate Purines (urate, uric acid, xanthine)	Silica Cystine Calcium phosphate
Cat	Struvite Oxalate	Urate Cystine
Ox	Silica Struvite Carbonate	Xanthine
Sheep	Silica Struvite Oxalate "Clover stones" Carbonate	Xanthine
Horse	Carbonate	
Pig		Urate

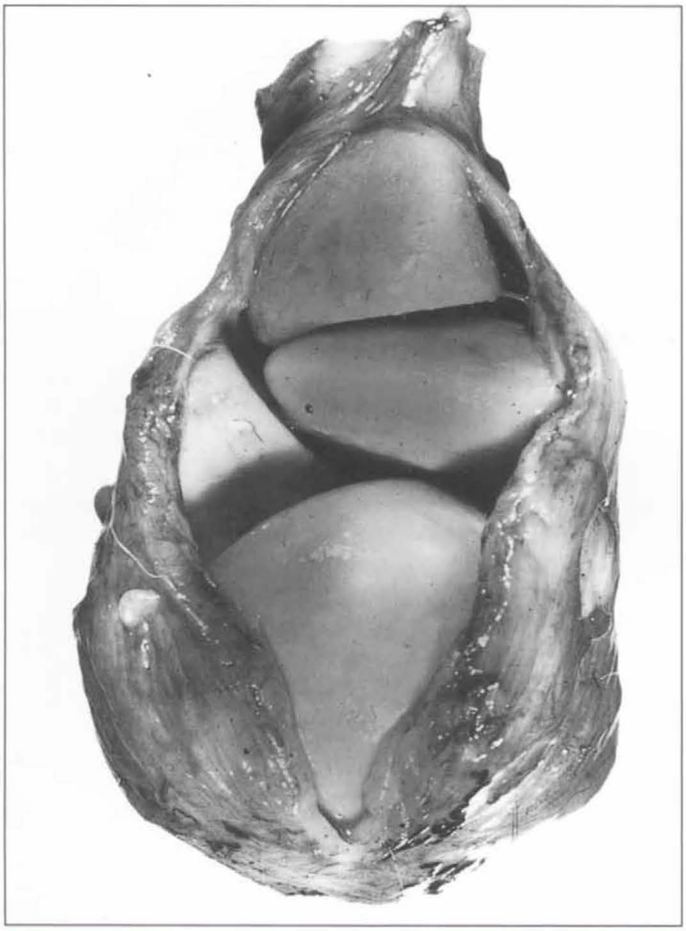

Figure 4.87 Faceted **calculi** filling the urinary bladder of a dog.

clover grows. *In general, calculi are important in cattle, sheep, dogs, and cats, less important in horses, and unimportant in pigs.* In *pigs*, uroliths are occasionally found in the renal pelvis of old animals and, more often, in the pelvis of dehydrated sucklings (see Fig. 4.2; and see section on Renal disease, renal failure, and uremia, above). In *horses*, they occur sometimes as single or several, spherical or faceted carbonate stones in the bladder; urethral obstructions are rare. In *dogs*, several breeds are predisposed to formation of calculi, namely Dachshunds, Dalmatians, Cocker Spaniels, Pekingese, Basset Hounds, Poodles, Schnauzers, and small terriers.

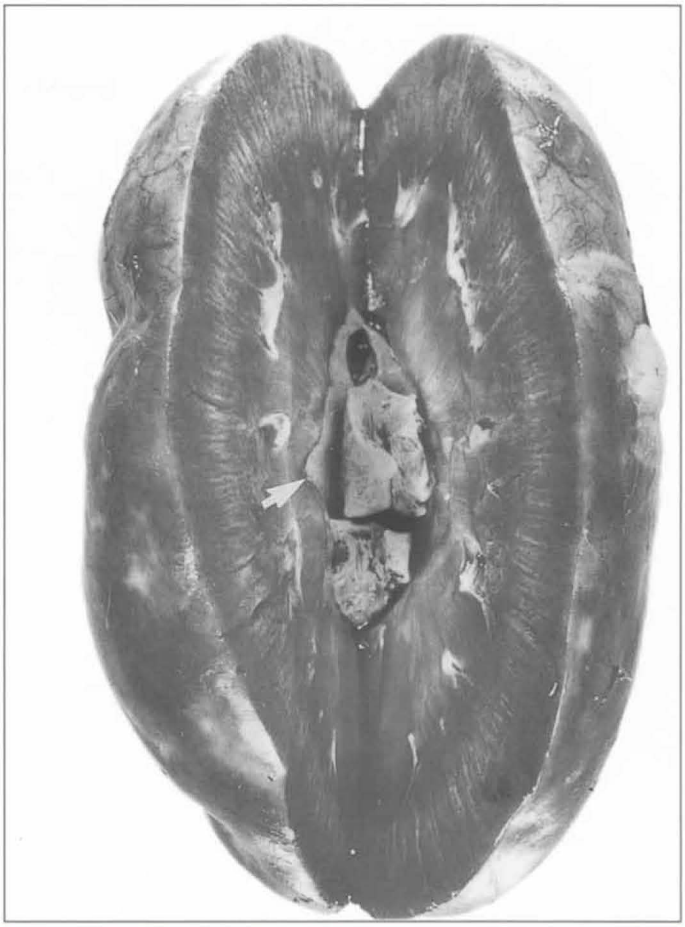

Figure 4.88 Calculus in the renal pelvis of a dog. The renal crest is ulcerated (arrow), and the cortex is irregularly scarred.

Calculi may form in any part of the urinary duct system, from the renal pelvis to the urethra. Some uroliths clearly originate in the lower urinary tract, but the point for development of most is not known (Fig. 4.87). In experimental urolithiasis produced by oxalates or calcium phosphate in laboratory animals, the calculi, initially microscopic, form in the collecting tubules and encrust on the renal papilla. They may grow large enough to make voidance impossible (Fig. 4.88). It is not known whether this is a general phenomenon. Tubular microlithiasis may simply represent crystallization in the highly concentrated urine of the medulla, or, alternatively, it may indicate the production there of an abnormal or excessive matricial substance. Obstructive nephroliths and ureteroliths occasionally develop in horses and cause secondary chronic tubulointerstitial nephritis; the suggested pathogenesis is renal medullary crest necrosis resulting from the use of nonsteroidal anti-inflammatory drugs in dehydrated horses, with subsequent mineralization of the sloughed necrotic renal crest material. Nephroliths are uncommon forms of uroliths in most species (1–4% of canine uroliths).

Small calculi may be voided in the urine, but *impaction in the urethra is common in males.* The common sites of urethral impaction are

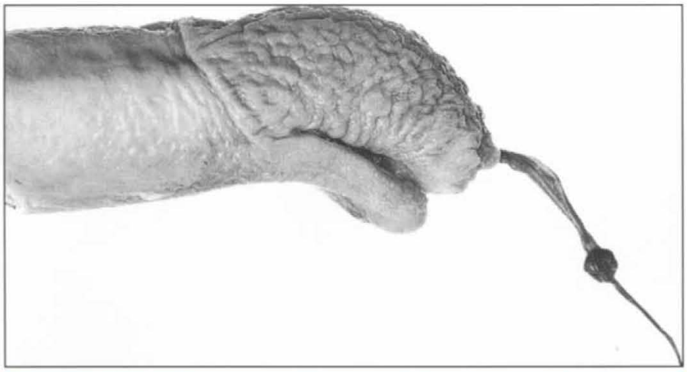

Figure 4.89 Calculus impacted in vermiform appendage of a ram.

the ischial arch, the sigmoid flexure of ruminants, the vermiform appendage of rams (Fig. 4.89), the proximal end of the os penis in dogs, and anywhere along the urethra of male cats. At the point of impaction, there is local pressure necrosis with ulceration of the mucosa and, because the urinary stasis favors bacterial growth, acute hemorrhagic urethritis develops and often ascends to the bladder and even to the kidney. Hydronephrosis is not a prominent development with urethral calculi, because rupture of the urethra with leakage of urine into the surrounding tissues, often associated with infection and acute cellulitis, terminates the condition fairly quickly.

Because of the development of numerous stone analysis centers, large numbers of companion animal uroliths have been studied and knowledge is expanding on the prevalence of mineral composition, breed predilections, geographical region prevalence, and the association with urinary tract infections.

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Silica calculi

In **ruminants** these calculi are hard, white to dark-brown, radiopaque, often laminated, and up to 1 cm across. In the bladder of ruminants they are spherical, ovoid, or mulberry-shaped and have smooth surfaces, but in the kidney they are angular and irregular, having the shape of the minor calyces where they are located almost exclusively.

"Pure" silica stones contain about 75% silica as silica dioxide. Mixed calculi contain some calcium oxalate or carbonate. Silica calculi contain about 20% organic matter. Most have a friable core, which is high in amorphous silica and low in organic matter. The core is surrounded by a layer of organic matter, which separates it from the outer concentric laminations, which are high in silica.

Silica calculi are very common in pastured ruminants and are a major cause of urinary tract obstruction. They occur with increasing prevalence in dogs and rarely in horses. Silica calculi are present in over 50% of steers on native (unimproved) range in western Canada; fewer than 5% develop urethral obstruction. The singularity of adjacent laminae in the calculi is consistent with intermittent deposition as urine composition changes. Certain grasses contain 4–5% or more of silica; the level increases through the growing season. Most of it is relatively insoluble but that in the cell sap is relatively soluble, unpolymerized silicic acid. Rumen fluid becomes saturated with respect to silicic acid. After absorption, some is returned to the gut in digestive secretions; less than 1% of dietary silica is excreted in urine and up to 60% is resorbed from the filtrate. However, when urine production is very low, either because of the nature of the diet or because of high insensible fluid losses in hot climates, the concentration of silicic acid in urine may reach five times the saturation level. Even so, precipitation from solution requires other substances, probably proteins of renal or serum origin, in the urine. Calculus formation is reduced to subclinical levels by adding salt to the ration, thereby ensuring high water consumption. Acid-forming effects of the diet and a reduction in the dietary calcium-to-phosphorus ratio also reduce silica urolith formation.

Silica calculi in **dogs** are primarily detected in males, are located in the bladder and urethra, and often cause urinary obstruction. Male German Shepherd Dogs and Old English Sheepdogs are at increased risk for formation of these calculi. Unlike cystic silica calculi in ruminants, bladder stones in dogs have very irregular shapes with surface protrusions; many silica uroliths have a "jackstone" configuration. Silica uroliths in dogs may be multi-layered or single-layered, and may contain calcium oxalate and struvite. Large quantities of plant-derived ingredients, particularly corn gluten feed, rice hulls, and soybean hulls, in dry dog food are implicated as risk factors for development of silica uroliths. Some cases are associated with lower urinary tract infections, often with *Staphylococcus* species. Silica uroliths are likely less soluble in acid than in alkaline urine.

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Struvite calculi

Struvite stones are white or gray, radiopaque, chalky, usually smooth, and easily broken. They may be pure struvite but usually contain other compounds such as calcium phosphate (which may form a shell around a struvite), ammonium urate, oxalate, or carbonate. They may be single and large, or numerous and sand-like. Struvite is *magnesium ammonium phosphate hexahydrate* ($MgNH_4PO_4.6H_2O$); formerly it was called "triple phosphate," a misnomer.

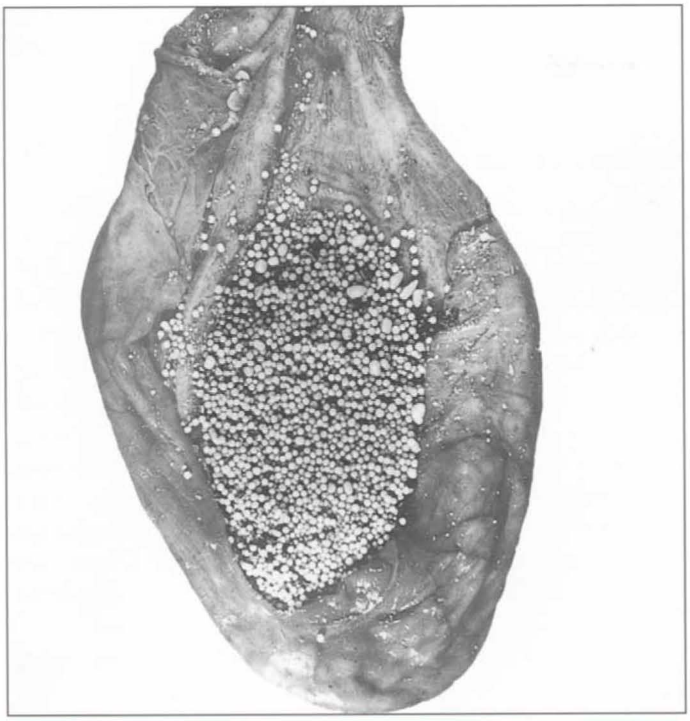

Figure 4.90 Struvite calculi in urinary bladder of a cow.

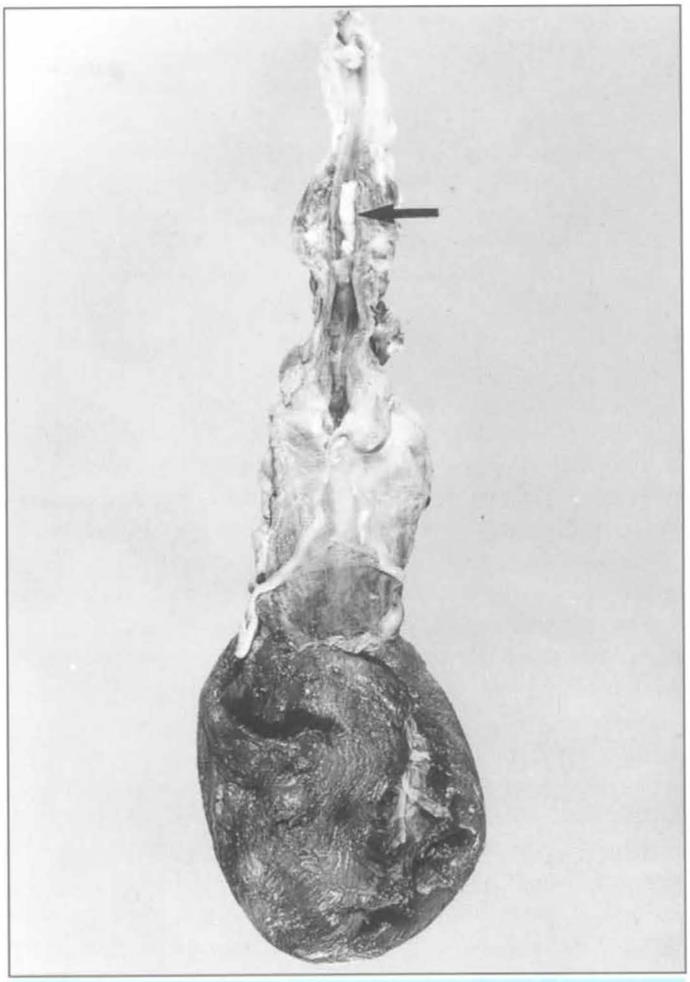

Figure 4.91 Plug of struvite crystals in urethra (arrow) and hemorrhagic cystitis in a cat with feline lower urinary tract disease.

Struvite calculi are important in dogs, cats, and ruminants (Fig. 4.90). In **dogs**, they are the most common types; females are particularly susceptible, perhaps because they develop bladder infections more often than males. Struvite calculi are often single, rapidly forming masses which mold to the shape of the cavity they occupy. They are called *infection calculi* in recognition of their common association with infection. Bacterial ureases from staphylococci and *Proteus* induce supersaturation of urine with struvite by increasing urine pH and ammonium ions. Alkaline urine decreases struvite solubility and increases ionization of trivalent phosphate, both of which favor calculus formation. Factors other than urease production, which are associated with infection, are probably important in the genesis of struvite stones. A high incidence of struvite calculi in Miniature Schnauzers may be related to a familial susceptibility to urinary tract infections. The reason for *sterile* struvite urolithiasis in three related English Cocker Spaniels and in an inbred line of Beagles is not known, but familial risk factors and dietary factors are postulated. The somewhat refractory response of struvite calculi to medical or dietary manipulations in dogs is thought to be due to the presence of associated hydroxyl apatite and concentric laminations, which have a low porosity.

In **cats**, most cases of the heterogeneous group of disorders referred to as *feline lower urinary tract disease* (FLUTD) are idiopathic, and are discussed below in the section on *Cystitis*. One manifestation of FLUTD is urolithiasis, in which discrete calculi develop in the urinary bladder of young to middle-aged cats (increased risk for cats between 4 and 10 years of age). Additional risk factors are associated with Russian Blue, Himalayan, or Persian breed, and castrated male or spayed female status. Intact females had a reduced risk. Struvite crystalluria is often seen in cats with and without calculi; the reasons for aggregation of crystals into sterile calculi are obscure. The prevalence of struvite uroliths and struvite urethral plugs has declined since the mid-1980s when cat foods were reformulated; there has been a concomitant increase in calcium oxalate urolithiasis in cats. Formation of struvite uroliths can be induced in previously normal cats fed calculogenic diets containing 0.15–1.0% dry weight magnesium. Primary pores in struvite calculi are an important feature and may allow dietary and medicinal manipulations successfully to dissolve these uroliths. Coagulase-positive staphylococci and other bacteria may be cultured from the urine or calculi of some affected cats, and the formation of infection-induced struvite calculi is similar to that mentioned above for dogs; these calculi are much less common than are sterile struvite uroliths.

Of considerably more importance than discrete calculi are the amorphous accumulations of protein, cellular debris, and struvite crystals that form sabulous (matrix-crystalline) urethral plugs in male cats, with castrated males being at a higher risk and spayed females at the lowest risk. Animals between 2 and 7 years of age are predisposed. This condition has been known as "feline urologic syndrome," a term no longer favored; it results from concomitant occurrence of urinary tract inflammation and the presence of various types of urine crystals. It is characterized by dysuria, hematuria, and urethral obstruction. If unrelieved, the obstruction can lead to bladder distension, hemorrhagic cystitis, azotemia, and death (Fig. 4.91). *The obstructive material, which becomes molded to the shape of the urethra in male cats, may be either* *struvite "sand" or rubber-like protein matrix, or a mixture of the two*; the matrix contains Tamm–Horsfall mucoprotein, albumin, globulins, cells, and cellular debris. The inflammatory component of plugs may be the product of infection with viruses, bacteria, or fungi. Various viruses, including feline cell-associated herpesvirus (a strain of *Bovine herpesvirus 4*), feline syncytia-forming virus, and *Feline calicivirus*, have been implicated as potential urinary pathogens in cats. There is no concrete evidence for *Mycoplasma* or *Ureaplasma* being urinary pathogens in FLUTD. Addition of magnesium and phosphate to the diet causes disease in some, presumably predisposed, cats and, conversely, reduction of dietary magnesium reduces the incidence. Alkaline urine pH is likely of more significance in the formation of struvite crystals than is magnesium intake. The apparent increased incidence during cold winter months may be due to decreased fluid consumption or increased intervals between urinations. The incidence of urethral plugs has decreased as more cats have been fed magnesium-restricted/pH-controlling diets (total mineral <6% dry matter (DM), Mg 0.05–0.10% DM, Mg <20 mg/100 kcal dose equivalent, maintain an average resting urine pH of 6.5 or less and an average postprandial peak pH not to exceed 7.0).

In **ruminants**, struvite calculi usually occur in feedlot cattle or sheep on high grain rations and obstruction may develop in up to 10% of steers. As in cats, *calculi usually form a gritty sludge with a high proportion of matrix*. Inhibition of urethral growth by early castration predisposes to obstruction, and increased water consumption tends to prevent obstruction. Animals with crystalluria often have crystals adhering to preputial hairs. Diets high in phosphate can cause a very high incidence of calculi in sheep; a calcium-to-phosphorus ratio of 1:2 or wider appears to be the critical factor, but the form in which they are fed and the balance of other constituents such as magnesium, sodium, and potassium are probably also important. Additional potassium tends to promote phosphate urolithiasis. Magnesium deficiency leads to renal mineralization and tubular microlithiasis, at least in laboratory species, and both sodium and magnesium are competitive with calcium, and increase the solubility of calcium salts in urine. There may also be a genetic effect on urolithiasis in sheep; urolithiasis is more likely to develop in sheep that excrete phosphorus mainly in urine than in those that excrete phosphorus mainly in feces.

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Oxalate calculi

Oxalate calculi are hard, heavy, white, or yellow, and typically covered with jagged spines, though some are smooth. They tend to be large and solitary in the bladder.

Oxalate calculi occur as the calcium oxalates, *whewellite (calcium oxalate monohydrate)* and *weddellite (calcium oxalate dihydrate)*. Their development is not well understood, but obviously *hypercalciuria and hyperoxaluria are involved*. There are several causes of hypercalciuria (see section on Hypercalcemic nephropathy, above). Oxalic acid is synthesized from glyoxylic and ascorbic acid and may be ingested in certain foods. Hyperuricosuria may be involved in oxalate precipitation, since sodium hydrogen urate may act as a heterogeneous nucleator. Dietary magnesium and citrate inhibit the formation of calcium oxalate uroliths by forming soluble complexes with oxalate and calcium respectively.

Oxalate (and silica) calculi may be important in **sheep** grazing grain stubble but the source of the oxalate is not known. Oxalate-containing plants are not apparently a source since oxalate is metabolized in the rumen; nonetheless, occasional exceptions to this general rule do seem to occur. Feeding a low-calcium diet (0.3% Ca) has produced oxalate urolithiasis in steers, possibly because of increased bone resorption resulting in increased plasma concentrations of hydroxyproline, an oxalate precursor. High magnesium intakes inhibit formation of oxalate calculi while low levels induce formation in some species.

In **dogs**, oxalate calculi are second to struvite calculi in prevalence and are of increasing importance, but little is known of their origins. Calcium oxalate and calcium phosphate (hydroxyapatite or calcium apatite) calculi occur in dogs with primary hyperparathyroidism, hypercalcemia, hyperadrenocorticism, or following exogenous steroid administration. Canine dietary factors such as high protein, fat, Ca, P, Mg, Na, K, Cl, and use of canned foods are thought to decrease the risk of development of these stones. Males are more frequently affected than females, and they are seen more commonly in older animals. The Miniature Schnauzer, Bichon Frise, Lhasa Apso, Yorkshire Terrier, Shih Tzu, and Miniature Poodle are breeds at increased risk. In Miniature Schnauzers predisposed to development of calcium oxalate uroliths, hypercalciuria, decreased urinary oxalate, normal urinary citrate, increased brushite urinary relative supersaturation, lower urine volume, and elevated excreted uric acid levels are consistent findings.

The prevalence of oxalate uroliths in **cats** has increased markedly from the early 1980s through the 1990s. This increase has been accompanied by a marked decline in the prevalence of struvite uroliths. The underlying cause for oxalate uroliths in cats is usually unknown, but likely related to diet. Several dietary factors can contribute to calciuria, including high animal-source protein, low magnesium, high sodium chloride, and diets formulated to acidify urine. Risk factors also include use of a single brand of cat food without additional supplementation, and maintaining cats in an indoor environment only. Persian, Himalayan, and several other breeds are at increased risk; cats with calcium oxalate uroliths tend to be older than cats with struvite uroliths. Additionally, male and neutered cats are at higher risk for the development of oxalate calculi. Oxalate uroliths in cats have been associated with parathyroid neoplasia, as in dogs.

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Uric acid and urate calculi

These *purine calculi* are usually multiple, hard, concentrically laminated, brown-green, and moderately radiodense. In the bladder, they are frequently spherical and less than 5 mm across. Most contain ammonium urate with some uric acid and phosphate; in others, sodium urate is the predominant salt.

Urate stones are most common in dogs, especially Dalmatians, but also occur in pigs (see section on Renal disease, renal failure, and uremia, above) and rarely in cats. Neonatal piglets in a negative energy balance produce increased purine catabolites, which predispose to urate urolithiasis. Dalmatians excrete high levels of uric acid in their urine. This is due to defective hepatocellular uptake of uric acid, which results in incomplete conversion of uric acid to allantoin, a more soluble product of purine metabolism; this defect is an inherited autosomal recessive trait. Hepatic uricase levels are normal. Additionally, the defective transport system involves renal tubules and prevents reabsorption of uric acid from glomerular filtrate. Active tubular secretion also contributes. Predisposing factors for urolith formation include hyperuricemia, hyperammonemia, hyperuricosuria, hyperammonuria, aciduria, and a genetic predisposition. Male Dalmatians are predisposed. Other dog breeds with a higher incidence of urate urolithiasis include English Bulldogs, Miniature Schnauzers, Shih Tzus, and Yorkshire Terriers. Dogs with portosystemic shunts have ammonium biurate crystals in their urine, and may have urate-containing calculi in kidneys and bladder.

Urates exist in supersaturated urine as lyophobic colloids, which are flocculated (coalesced and precipitated) by high levels of ammonium ion and, to a lesser extent, by low pH. Urea-splitting organisms may be important in the development of urate calculi since production of ammonium ion favors calculus formation. Also, although higher pH inhibits precipitation of uric acid, it favors precipitation of ammonium urate, as well as phosphates, which are often found in urate calculi.

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Xanthine calculi

Xanthine stones are yellow to brown-red, often concentrically laminated, friable, and irregularly shaped. They are radiolucent. *Xanthine is a metabolite of purines*, which seldom appears in urine because it is normally degraded by xanthine oxidase to uric acid.

Xanthine calculi occur occasionally in dogs and are reported in sheep, calves, and a cat. A high incidence in sheep was circumstantially related to deficiency of molybdenum in unimproved pasture; molybdenum is a component of xanthine oxidase. Several cases in calves in Japan were also associated with deficiency of xanthine oxidase. Xanthine precipitates in acid urine. Calculi usually form in the collecting ducts and calyces of the kidney, and may cause hydronephrosis.

Two forms exist in dogs, as in humans. The *primary form* is inherited as an autosomal recessive, and is due to an inborn enzyme defect in xanthine oxidase. This hepatic enzyme catalyzes two sequential steps in degradation of purines, namely conversion of hypoxanthine to xanthine, and then xanthine to uric acid. This form has been noted most often in Dachshunds and recently in a family of Cavalier King Charles Spaniels. The *secondary form* (iatrogenic) is the more common in dogs, especially Dalmatians, and is usually the result of previous treatment with allopurinol, which binds to and inhibits the action of xanthine oxidase.

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Cystine calculi

Cystine calculi are small and irregular, soft and friable, waxy, and light yellow to red-brown, turning to green on exposure to daylight. Many cystine calculi consist of pure cystine; others may also contain calcium oxalate, struvite, brushite (calcium hydrogen phosphate dihydrate), and complex urates.

Cystine stones occur in dogs and rarely in cats. They comprise about 1–3% of canine calculi in North America, but 20–30% in some European centers, being second to struvite calculi in incidence. Cystinuria occurs in both males and females, but cystine calculi and urinary obstruction occur almost exclusively in males. They are primarily found in the urinary bladder, but nephroliths are common in Newfoundlands. Other predisposed breeds include Dachshunds, Bulldogs, Mastiffs, Bassett Hounds, and Tibetan Spaniels. Although blood cystine levels are normal, an *inborn error of metabolism in affected dogs* results in high levels of urinary cystine because of defective proximal tubular reabsorption from glomerular filtrate. Many dogs with cystinuria also have high levels of other amino acids in their urine, but these are more soluble than cystine. Cystine precipitates in acid urine, but factors other than urinary pH are probably important in the genesis of cystine stones since dogs with crystalluria do not always form them. The incidence of recurrent urolithiasis is enhanced in those dogs with higher excretions of urinary cystine. The mode of inheritance of cytinuria is unknown in most breeds of dogs, but it is transmitted as a simple autosomal recessive trait in Newfoundlands. In some dogs the severity of cystinuria may decrease with age.

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Clover stones

Sheep grazing estrogenic pastures, particularly subterranean clover, or those *injected or implanted with estrogens*, may have an incidence of fatal urinary obstruction as high as 10%. There are probably three *separate developmental patterns* and, in each, the obstructing material is soft or pulpy and scantily mineralized. Probably the most common pattern is urethral obstruction by desquamated cells and secretions of accessory glands originating in the pelvic urethra under the influence of estrogen. The second type, the so-called "clover stone," is usually found in the renal pelvis as a yellow, soft material, which eventually leads to fibrosis and shrinkage of the kidney. It affects both sexes equally. These calculi contain benzocoumarins, which may be metabolites of phytoestrogens. Third, sudden and serious mortalities may occur in male sheep grazing subterranean clover during its period of rapid maturation. The urethral process becomes impacted with soft white paste consisting mainly of calcium carbonate and an unidentified organic material probably related to isoflavones.

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Other types of calculi

Several other types of calculi develop in animals; they may be important locally or simply be curiosities. **Tetracycline**, **sulfonamide**, and **barium** stones, iatrogenic and rare, fall into the latter category. Other chemical compounds are more common but often constitute a minor part of certain uroliths. Stones with a high **carbonate** content are associated with very alkaline urines and are seen in ruminants consuming high oxalate plants or clover-dominated pastures.

In horses, calcium carbonate crystals are seen commonly in normal urine. Calculi are much less frequent, but are most commonly composed of **calcium carbonate**, usually in the crystalline form of calcite, and substituted vaterite (the Ca of $CaCO_3$ is replaced in various amounts by Mg, K, and Mn); weddellite (calcium oxalate dihydrate) may also be present. Alkaline pH and dietary factors are implicated in their formation.

Fewer than 3% of canine stone patients have uroliths composed of **calcium phosphate**. Factors that decrease calcium phosphate solubility and hence predispose to urolith formation include: alkaline urine, hypercalciuria (hyperparathyroidism and other hypercalcemic disorders), reduced concentrations of crystallization inhibitors (inorganic pyrophosphates, citrate, magnesium ions, nephrocalcin), or increased concentrations of crystallization promoters (epitactic induction by calcium oxalate and monosodium urate crystals).

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INFLAMMATION OF THE LOWER URINARY TRACT

Inflammation of the lower urinary tract centers on involvement of the urinary bladder, i.e., **cystitis**. **Ureteritis** is rare in the absence of cystitis, and clinical **urethritis** in animals is usually associated with obstruction by a calculus from the bladder. Under normal circumstances, the bladder is resistant to infection, and bacteria are quickly eliminated by the normal flow of normal urine. *Predisposition to urinary tract infection* (UTI) occurs when there is stagnation of urine due to obstruction, incomplete voiding at micturition, or urothelial trauma. *Other risk factors for UTI* include catheterization, vaginoscopy, urinary incontinence, vaginitis, or administration of antibiotics or corticosteroids within the last 60 days. Of itself, normal voiding is not sufficient to prevent or eliminate bladder infection. Indeed, complete voidance does not occur but residual urine is rapidly diluted or added to by continuing excretion. *Defense mechanisms* in the bladder and urethra, which prevent bacterial adhesion to mucosal surfaces, are essential if bacteria are to be removed by urine flow. Tamm–Horsfall mucoprotein, produced in the kidneys, may bind to bacterial adhesins and prevent adherence. Local production of immunoglobulin A and a surface glycosaminoglycan layer is probably important in preventing attachment of organisms to the normal urothelium, and immunoglobulin G may have similar activity in specific UTIs. Urinary oligosaccharides may be able to detach adherent bacteria. Voiding of sloughed urothelial cells with attached bacteria aids their clearance. Incomplete voiding at micturition may be a result of diverticula of the urinary bladder or vesicoureteral reflux. The presence of residual urine can maintain a bladder infection, allowing organisms to take advantage of any opportunity to invade the urothelium.

Unlike human urine, which tends to be a good medium for bacterial growth, animal urines, especially those of dogs and cats, usually have antibacterial activity. This activity is related to urine pH and particularly to urine osmolality. In general, the further the pH is from the optimum range of 6–7, the less likely it is to support bacterial growth. The antibacterial effect of acid urines is related to their concentration of undissociated organic acids. High concentrations of urea and other solutes increase urine osmolality and contribute significantly to its bacteriostatic effect.

In order to infect the urinary tract, uropathogens must compete with the normal bacterial flora of the distal urethra, vulva, or prepuce. *The usual causes of cystitis are* **bacteria** *from the urethra, the origin of which are almost always the rectal flora.* Cystitis is common in young animals with patent urachus, and the bacterial flora is mixed. When bacteria breach the surface defenses of the urothelium and attach to the epithelial cells, the cells are desquamated through a rapid apoptosis-like mechanism involving caspase activation and host DNA fragmentation. When the urothelium is penetrated, clearance is prevented and neutrophils and macrophages in the submucosa respond. Leukocytes in urine are not usually phagocytic.

A variety of bacteria may be involved in bladder infections (see section on Pyelonephritis, above) and these include in all hosts *Escherichia coli, Proteus vulgaris*, streptococci, and staphylococci. In a large compilation of canine urinary tract infection culture data, the six most common isolates included, in decreasing prevalence: *E. coli* 44.1%, *Staphylococcus* spp. 11.6%, *Proteus* spp. 9.3%, *Klebsiella* spp. 9.1%, *Enterococcus* spp. 8.0%, and *Streptococcus* spp. 5.4%. Infection with a single organism was seen in >72 % of infections, and there was a predisposition for females. The *Corynebacterium renale* group (*C. renale, C. pilosum, C. cystitidis)* is important in cows, and less so in other species, where it is usually part of a mixed infection. *C. urealyticum* can cause an "encrusted cystitis" in dogs because of the deposition of struvite (magnesium ammonium phosphate) along the bladder mucosa secondary to ammonia release by these urease-producing bacteria. *Actinobaculum (Eubacterium) suis* is the primary cause of cystitis and pyelonephritis in swine and a leading cause of death in sows; *A. suis* infections typically produce macrohematuria and alkaline urine.

Mycoplasmas are uncommon causes of UTI in dogs and cattle. Urogenital infections causing prostatitis, orchitis, nephritis, and cystitis may occur in canine **blastomycosis**. *Aspergillus, Candida*, and *Nocardia* are unusual causes of cystitis in dogs and cats. *Candida* infections can be seen more commonly with predisposing factors such as diabetes mellitus, prolonged antibiotic or glucocorticoid usage, aciduria, indwelling catheters, or an immunocompromised state. *Borrelia burgdorferi* has been detected by nested flagellin PCR in the urine of dogs with cystitis or prostatitis, suggesting that urinary signs can be associated with Lyme disease in dogs.

Bacterial pathogens of the urinary tract, such as *Escherichia coli*, can express a formidable array of *virulence factors*, including fimbriae or pili adhesins (P fimbriae, type 1 fimbriae), nonfimbrial adhesins, aerobactin (an iron chelator), hemolysin, capsular polysaccharide (K antigen), and anticomplementary serum resistance. The most urovirulent strains often express multiple virulence factors simultaneously to produce a synergistic or additive effect. Certain virulence factors, such as adhesins, specifically favor the development of pyelonephritis, while others favor cystitis. In humans, *E. coli* with P fimbriae bind to renal pelvic urothelium and are thought to be responsible for pyelonephritis. Type 1 fimbriae (pili) are of most importance in bladder colonization. Type 1 pilus tips interact with membrane glycoproteins known as uroplakins. Attachment results in exfoliation of bladder epithelial cells as part of an innate host defense system. In contrast, gram-positive bacteria utilize extracellular polysaccharides for adhesion.

Cystitis

Cystitis does occur without obvious predispositions of the types described above. There is a higher incidence in females, which is probably associated with the shorter urethra. Pathological urine may be a better medium for bacterial growth than normal urine. Although the glucosuria of diabetes mellitus promotes bacterial growth, the influence of other reducing substances and of even slight levels of proteinuria may be significant in the development of cystitis with this disease. Decreased leukocyte efficiency may also be involved. Diabetic animals with associated Cushing's disease, pyometra, or progesterone-somatotrophin-induced diabetes mellitus are at increased risk for developing urinary tract infections with *E. coli. Emphysematous cystitis* develops in some dogs and cats with diabetes mellitus, and is thought to be a result of fermentation of sugar by glucose-fermenting bacteria. Emphysematous cystitis is less commonly detected in nondiabetic animals (Fig. 4.92). Hormone-induced changes as occur in hyperestrogenism may also affect the functional integrity of the urethral and vesicular epithelium, and the role of hormones in the production of glycosaminoglycans in the urogenital tract may also change the susceptibility to UTIs. During estrus in sows, estrogen causes the urine pH to rise, producing an alkaline environment suitable for the growth of *Eubacterium suis.*

As well as the opportunistic bacterial UTIs that develop in all species, there are a few diseases of which inflammation of the lower urinary tract is often a part. Hemorrhagic cystitis sometimes occurs in *malignant catarrhal fever* in cattle and deer, and occasionally is the dominant gross feature in the disease. Linear granulomas occur in the renal pelvis, ureter, and bladder of cattle with *Schistosoma mattheei* infections. Cystitis in horses and cattle (but not sheep) grazing *Sorghum* sp. is associated with ataxia caused by degenerative encephalomyelopathy; the bladder lesions are almost certainly

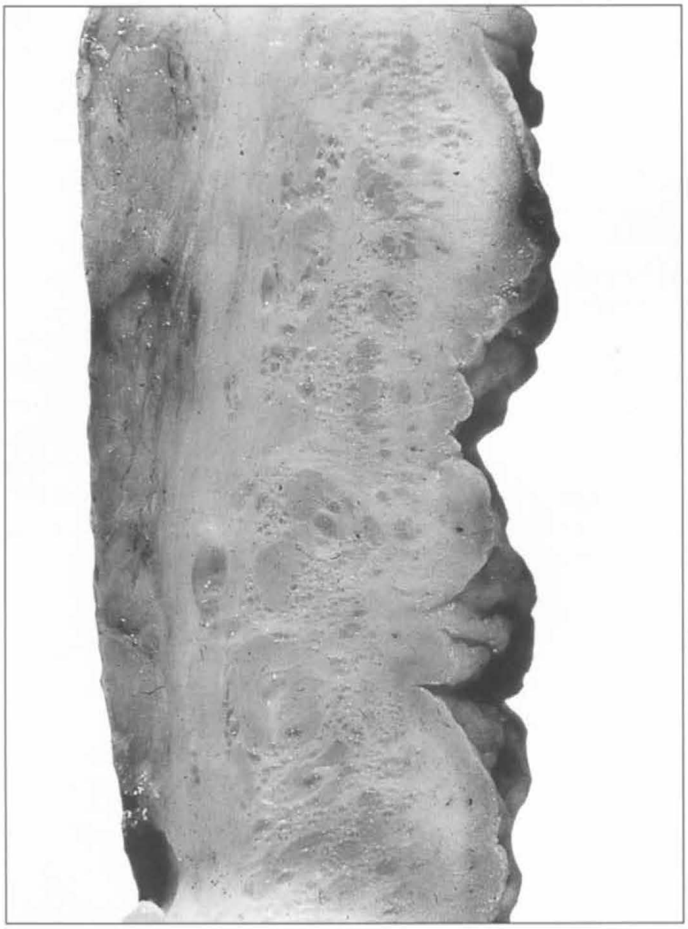

Figure 4.92 Emphysematous cystitis in a dog. Not associated with diabetes mellitus.

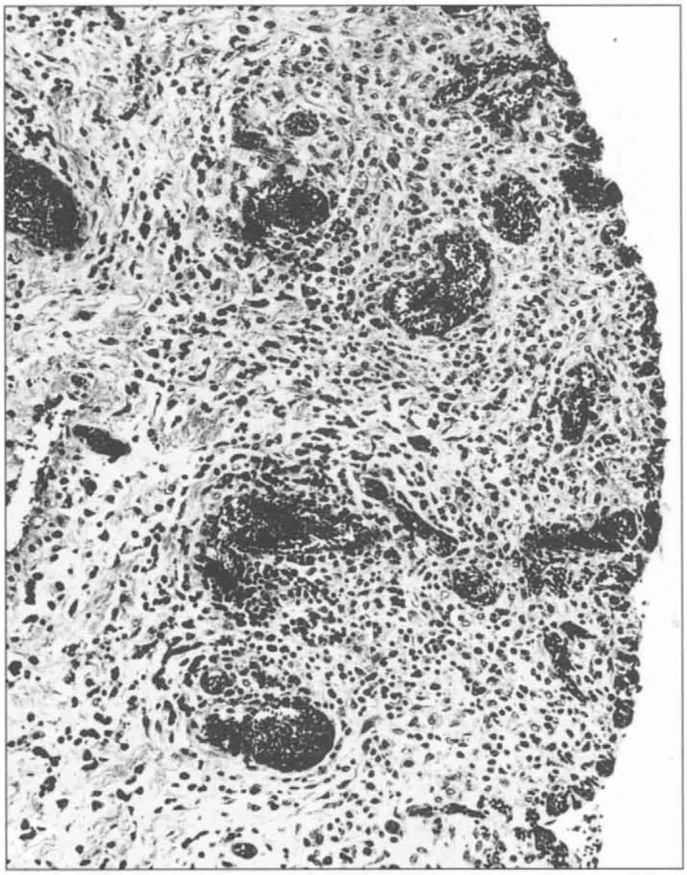

Figure 4.93 Acute hemorrhagic cystitis with complete loss of epithelium, bovine.

neurogenic in origin. The cause of a similar *epizootic cystitis of horses* in Australia is not known.

Lower urinary tract disease (LUTD) in cats may result from UTI of viral, bacterial, or fungal origin; uroliths; urethral plugs; congenital or acquired anatomic defects of the bladder or urethra; or iatrogenic causes. Abyssinian cats, cats greater than 10 years of age, and spayed females are predisposed to bacterial UTI. Novel feline caliciviruses have been isolated from LUTD cases; further studies are required to determine if these are uropathogens and/or causative agents. Many cases of LUTD remain noninfectious and idiopathic (iLUTD). Feline iLUTD bears considerable resemblance to the somewhat obscure condition in humans called "interstitial cystitis." **Feline interstitial cystitis** is characterized by chronic irritative voiding signs (dysuria, hematuria, pollakiuria, and/or inappropriate urination), sterile and cytologically negative urine, and cystoscopically visible submucosal petechial hemorrhages ("glomerulations"). This common feline condition may result from decreased urinary excretion of glycosaminoglycans, increased bladder permeability, and neurogenic inflammation. Increased sympathetic activity is confirmed in feline interstitial cystitis cases and this affects bladder function locally. Nonspecific histologic changes in the bladders of affected cats include submucosal edema, dilated submucosal vessels with margination of neutrophils, and submucosal hemorrhage; there may also be increased numbers of submucosal mast cells. Cats between 4 and 10 years of age are predisposed.

Sterile hemorrhagic cystitis may occur in dogs and cats treated for neoplastic or immunologic diseases with *cyclophosphamide*. Activated metabolites of the drug cause mucosal ulceration, hemorrhage, and edema. Signs of cystitis occasionally follow an 8-week course of therapy but have also been noted within 24 hours of administration of the first therapeutic dose. Concurrent treatment with other drugs, degree of diuresis, and pre-existing cystitis may influence the prevalence of cyclophosphamide-induced lesions. Fibrosis and mineralization of the bladder may result in persistent hematuria and incontinence. Transitional cell carcinoma may develop in the bladder of dogs in association with prolonged cyclophosphamide therapy.

Eosinophilic cystitis is an uncommon form of cystitis seen in older dogs with a history of urolithiasis and characterized by the predominance of eosinophils in a proliferative fibroblastic mass.

Cystitis is differentiated into acute and chronic forms, but there is considerable overlap in both the lesions and the causes. In simple **acute** catarrhal inflammation, there is moderate hyperemia and submucosal edema and the surface is covered with a layer of tenacious catarrhal exudate. The urine is cloudy. Histologically, there is degeneration and desquamation of the epithelium and prominent leukocytic infiltration. Submucosal vessels are dilated and cuffed by leukocytes. In somewhat more severe grades of inflammation, leukocytes may infiltrate all layers of the bladder wall, and hemorrhage from the dilated vessels may be severe enough to produce large clots in the bladder. These hemorrhagic complications are common in cystitis (Fig. 4.93) following urethral obstruction, especially in cats and cattle. When the

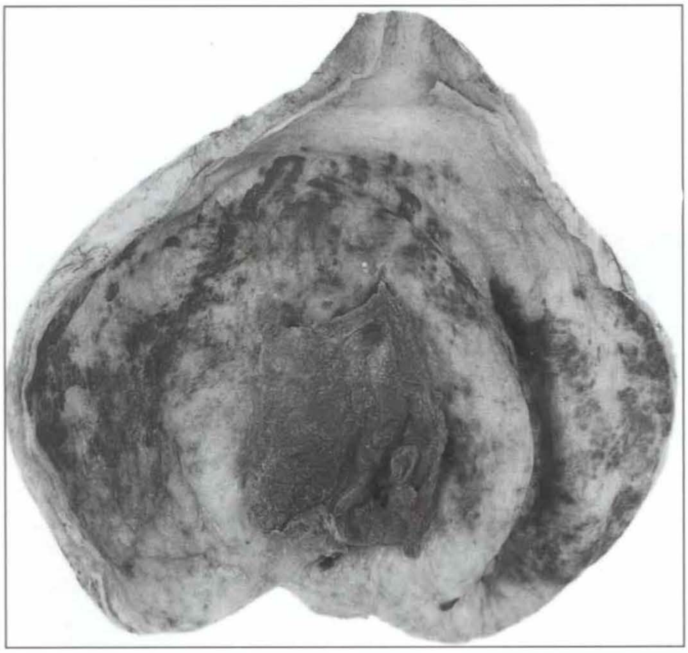

Figure 4.94 Acute fibrinous cystitis in a dog.

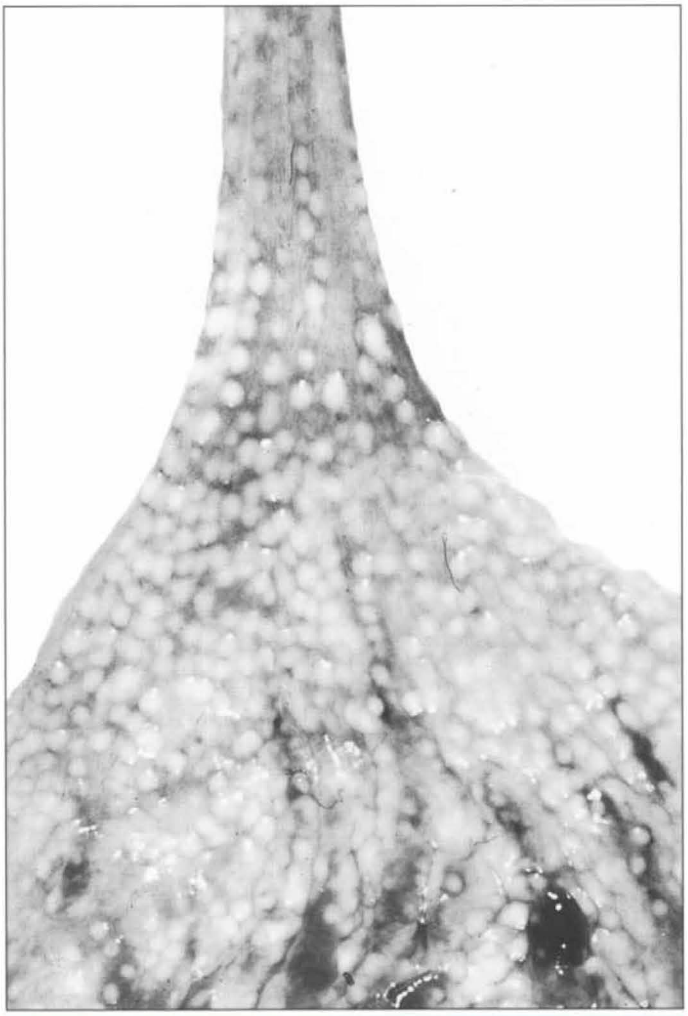

Figure 4.95 Follicular cystitis in a dog.

inflammatory process is severe, the cystitis may be of superficial fibrinous or deep diptheritic type (Fig. 4.94). In both, there is a thick, dirty-yellow friable surface encrustation, which may peel with difficulty. A large portion of the mucosa may become necrotic in addition to deeper layers of the bladder wall. Ulcerations may penetrate the wall to the serosa or predispose to rupture.

Chronic cystitis may also take a number of anatomic forms. The simplest occurs in association with vesical calculi. The mucosa is irregularly reddened and usually thickened. There is some epithelial desquamation and the submucosa is heavily infiltrated with mononuclear inflammatory cells; there are few neutrophils. In addition, there is often connective tissue thickening of the submucosa and hypertrophy of the muscularis.

There are also some special anatomic forms of chronic cystitis. In **follicular cystitis**, which is common in dogs, the mucosa is studded with gray-white nodules about 1 mm across (Fig. 4.95) which may be confluent or surrounded by a zone of hyperemia. Histologically, the nodules are aggregates of proliferating lymphocytic cells. These are immediately beneath the epithelium, which may be normal or ulcerated.

Chronic polypoid cystitis is common in any species (Fig. 4.96). In this, the mucosa is thrown into many folds or villus-like sessile projections. The polyps are covered by epithelium over a core of proliferated connective tissue densely infiltrated with mononuclear leukocytes. The polyps often undergo mucoid degeneration in cattle, or the epithelium may undergo metaplasia to a mucus-secreting, glandular type, often resembling colonic epithelium. Such polyps may break down and cause intermittent hematuria. Obstructive uropathy is another sequela. Biopsy is required to differentiate them from neoplasms.

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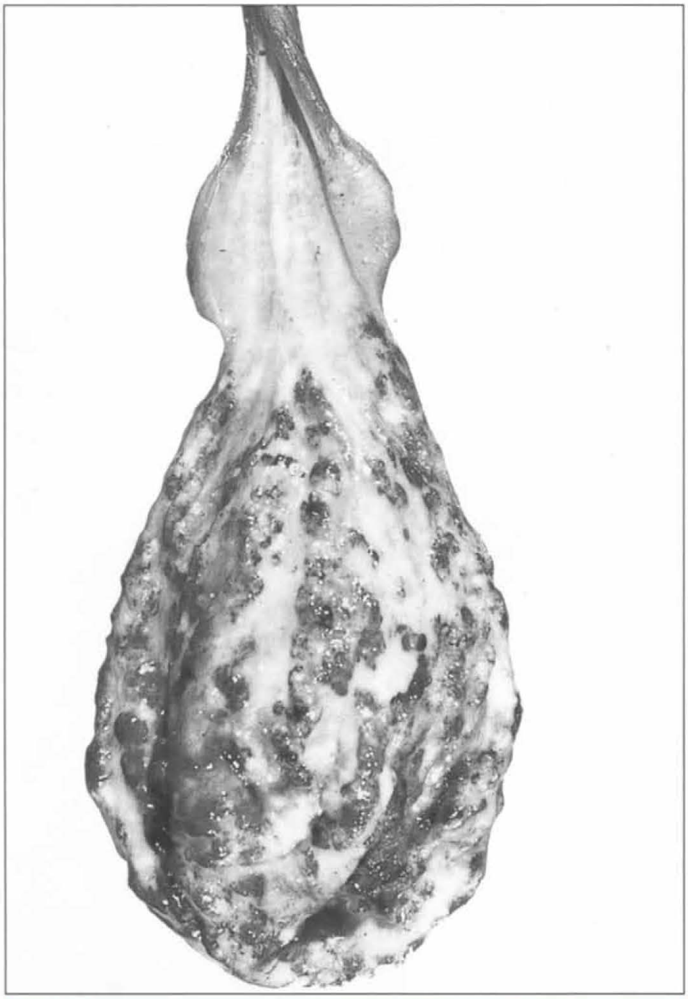

Figure 4.96 Polypoid cystitis in a dog.

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Enzootic hematuria

Enzootic hematuria is a syndrome in mature cattle characterized by persistent hematuria and anemia, and is associated with hemorrhages or neoplasms in the lower urinary tract. In over 90% of cases, the hematuria originates from tumors of the urinary bladder. Outbreaks of the disease are reported in sheep.

Enzootic hematuria occurs on all continents but is restricted to particular locations. In endemic areas, up to 90% of adult cattle may be affected. *The syndrome is attributed to chronic ingestion of* **bracken fern** and is reproducible experimentally. The extent and persistence with which toxic ferns are grazed probably influences the incidence of bladder lesions. There are two subspecies of bracken fern: *Pteridium aquilinum* subsp. *aquilinum*, containing eight varieties, and *P. aquilinum* subsp. *caudatum*, containing four varieties, and it is not known whether all varieties are toxic. *P. revolutum*, a species of bracken fern common in south Asia, and *P. esculentum*, a bracken fern of Australia, also produce enzootic hematuria. In areas where bracken does not grow, other ferns, such as *Cheilanthes sieberi* (mulga or rock fern from Australia), are capable of producing enzootic hematuria.

Bracken fern is one of the most common plants on the planet, and is said to be the only plant that causes naturally occurring tumors in animals. *It contains several toxic substances,* including a thiaminase, a variety of carcinogens (quercetin, shikimic acid, prunasin, ptaquiloside (braxin C), ptaquiloside Z, aquilide A, and others), and a "bleeding factor" of unknown structure. Following administration of ptaquiloside to guinea pigs, hemorrhagic cystitis results, suggesting that this is one of the toxic principles in bracken fern hematuria. There is a strong link between bracken fern and Bovine papillomavirus 2 (BPV-2) in the development of bladder neoplasms in animals with enzootic hematuria; the relationship of oncogenic viruses to bracken is discussed in Vol. 2, Alimentary system.

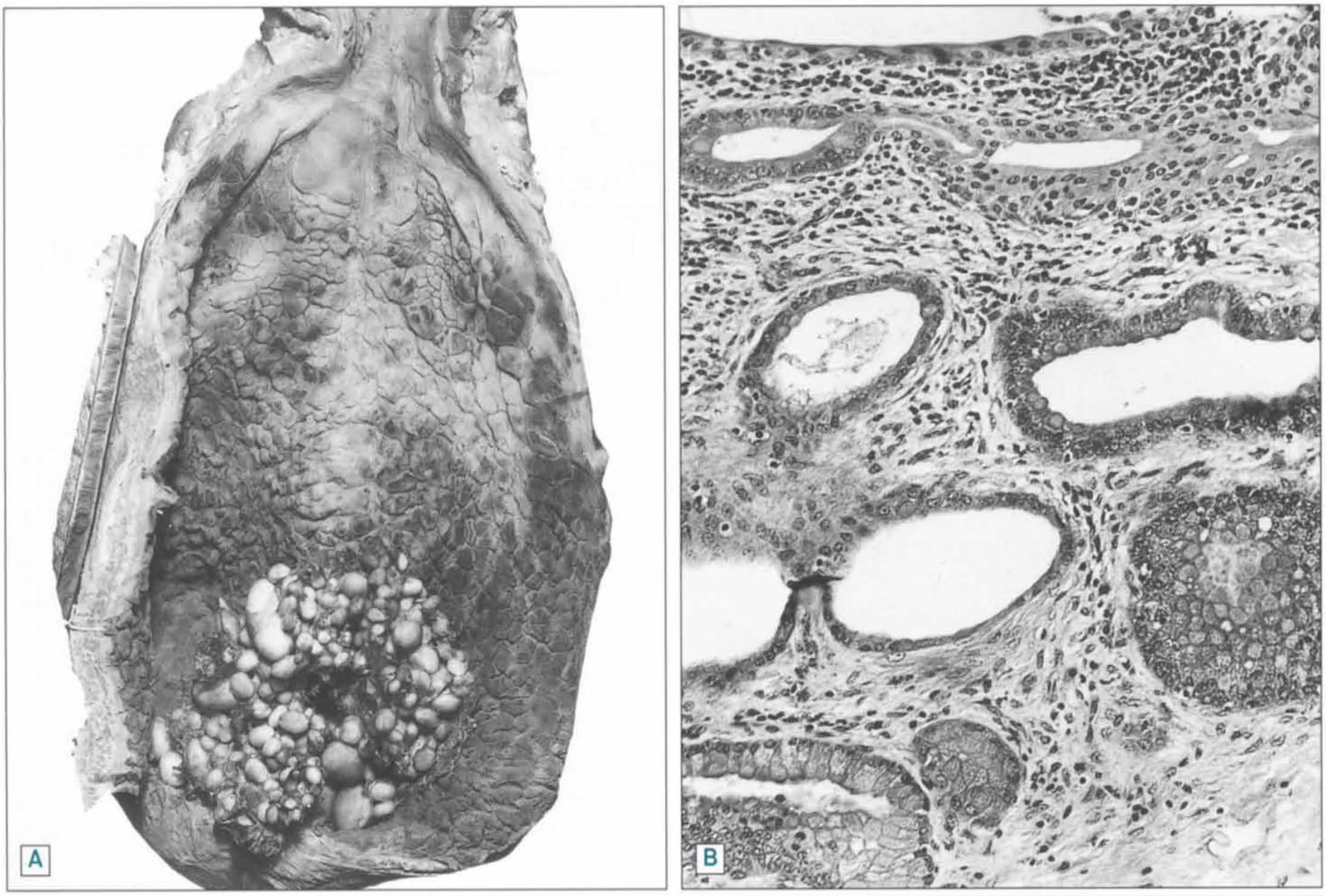

Figure 4.97 Enzootic hematuria in a cow. **A.** Dark **angiomatoid lesions** and confluent **nodular tumors** involving most of the bladder mucosa. **B. Mucous adenocarcinoma** of urinary bladder. There is ulceration and inflammation of the bladder surface (above, right).

Cattle fed low levels of bracken fern develop microscopic, followed by macroscopic, hematuria. Microhematuria is usually associated with petechial, ecchymotic, or suffusive hemorrhages in the urothelium of the renal calyces, pelvis, ureter, and bladder. These lesions appear to be a manifestation of the hemorrhagic syndrome characteristic of acute bracken fern poisoning (see Vol. 3, Hematopoietic system). In some cases, microscopic hematuria occurs before gross lesions are visible. Diffuse or patchy areas of pink discoloration develop in the bladder mucosa and, microscopically, ectasia and engorgement of capillaries are present. These altered vessels are prone to hemorrhage into the bladder wall or lumen and nodular hemangiomatous lesions develop in affected areas. In a few animals, macroscopic hematuria is solely associated with these non-neoplastic changes, but it is usually caused by the development of tumors, which ulcerate and bleed into the lumen (Fig. 4.97). Occasionally tumors also develop in the renal pelvis and ureter, and hepatic hemangiomas accompany bladder tumors in a few animals. Most neoplasms are located on the ventral and lateral walls of the bladder, where constant contact with urine is provided.

Several types of epithelial and mesenchymal neoplasms may develop, including transitional cell and squamous cell carcinoma, papilloma, adenoma, hemangioma, hemangiosarcoma, leiomyosarcoma, fibroma, and fibrosarcoma. Multiple tumors of more than one type may be present, and in over 50% of affected cattle mixed epithelial–mesenchymal neoplasms develop. *Papillomas, fibromas, and hemangiomas with carcinomas are the most common types.* Malignant types may invade locally and about 10% of epithelial malignancies metastasize to iliac nodes or lungs. Chronic cystitis usually accompanies the neoplastic changes. The gross and microscopic appearance of the inflammatory and neoplastic lesions is conventional; Brunn nests may develop in the mucosa. Epithelial neoplasms appear to develop from the hyperplastic and metaplastic (squamous and mucous) changes in the urothelium that often accompany the vascular lesions described above. A pagetoid variant of urothelial carcinoma in situ has been reported in a cow that also had enzootic hematuria and concurrent BPV-2 infection; fragile histidine triad (Fhit) protein, expressed by the tumor suppressor gene *FHIT*, was found to be absent in some of the pagetoid cells, correlating with late-stage neoplastic progression. Uroplakins are urothelial cell differentiation products that form a major portion of the asymmetric unit membrane and are more consistently expressed in the superficial transitional epithelium. A decrease has been noted in superficial positive immunohistochemical uroplakin staining in increasingly malignant urothelial tumors in cattle with chronic enzootic hematuria. Although not necessarily of use in prognosticating tumors, staining for uroplakins may assist the identification of metastatic urothelial cell clusters and further characterize transitional cell differentiation in neoplasia.

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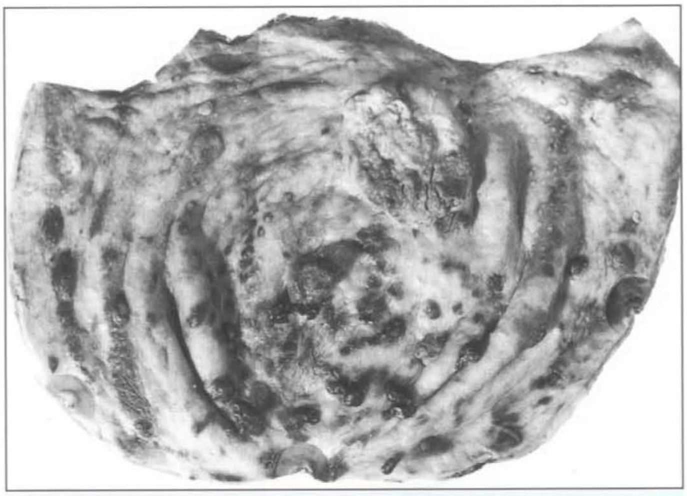

Figure 4.98 Chronic polypoid cystitis with multiple sessile and papillary areas of **carcinoma**.

NEOPLASMS OF THE LOWER URINARY TRACT

Neoplasia of the lower urinary tract is uncommon but most often occurs in dogs, cats, and cattle (Figs 4.97A and 4.98) (see section on Enzootic hematuria, above). There are few data for other animals, thus the following discussion concerns mainly these species; almost all bovine tumors are part of enzootic hematuria. Tumors of the urinary bladder account for fewer than 1% of all canine neoplasms, and a lower prevalence in cats. They occur somewhat more often in female than male dogs in some studies, but in other studies they are more frequent in male dogs, as they are in human males. The Scottish Terrier, Shetland Sheepdog, Beagle, and Collie seem to be at greater risk than other breeds. The greater susceptibility of the urinary bladder than of other parts of the tract to neoplasia may be due to more prolonged exposure of the bladder mucosa to urinary carcinogens. *In general, urinary bladder tumors are slow-growing and late to metastasize.* With the exception of rhabdomyosarcoma, neoplasia of the lower urinary tract usually occurs in old animals.

Bladder neoplasia may be caused by a variety of *industrial chemicals* (2-naphthylamine, benzidine), tryptophan metabolites (ortho-aminophenol), chronic irritation, foreign bodies (sutures), viruses, bracken fern, and cyclophosphamide. The greater concentration of *tryptophan* metabolites in dog urine than in cat urine may account for the higher prevalence of bladder tumors in dogs. *Grading of urinary tract neoplasms according to tumor invasiveness, lymph node involvement, and presence of metastases (the TNM system)* can assist with prognostication and selection of therapy; the use of biological markers (antigens, tumor products) shows promise of improving clinical staging of urinary tumors in human medicine.

Secondary tumors are rare; those of the bladder comprise ~5% of tumors of this organ. These originate in pelvic organs or as peritoneal implants.

Epithelial tumors

Epithelial tumors comprise ~80% of the lower urinary tract neoplasms. They occur as adenomas, papillomas, and carcinomas, and most of them develop in the bladder of old animals.

Adenomas are rare in all species; they originate from areas of mucous metaplasia of the urothelium and may be single or multiple with a papilliform or pedunculated appearance. Microscopically, they form glandular structures, some of which contain mucin. **Papillomas** constitute ~17% of primary tumors of the urinary bladder. They tend to be multiple and may be pedunculated or sessile, occasionally involving most of the mucosa. They are covered by well-differentiated transitional epithelium, which is demarcated by basement membrane from a delicate supporting stroma. Squamous metaplasia of the epithelium may develop. Papillomas are susceptible to superficial necrosis, which results in hematuria. In dogs, some papillomas undergo malignant transformation to form transitional-cell (TCC) or adenocarcinomas. *Papillary hyperplasia*, to be differentiated from the much rarer papilloma, occurs in the urinary bladder of cattle and can cause urinary obstruction and hydronephrosis.

Carcinomas of the lower urinary tract are of four histologic types: (1) TCC; (2) squamous cell; (3) adenocarcinoma; and (4) undifferentiated carcinoma. Together they make up ~60% of primary bladder tumors in dogs and cats; the majority of these are TCCs. Carcinomas may be solitary or multiple and usually do not reach a large size before they cause hematuria or death from urinary complications. Epithelial metaplasia and squamous and glandular hyperplasia are often present in the urothelium adjacent to epithelial neoplasms; Brunn nests are most often found in association with adenocarcinomas.

TCCs may be papillary, polypoid or sessile. Occasionally they are not visible on the vesical mucosa, even though the bladder wall is infiltrated diffusely; the tumors may be present in any part of the bladder, but are often in the bladder neck or trigone. Tumors originating in the prostatic urethra are easily overlooked at gross necropsy. Features of tumors that tend to be localized include papillary architecture, in situ tumor, low tumor grade, and a strong lymphoid response. Metastatic disease is seen with infiltrative and nonpapillary growths, increasing tumor grade, deeper invasion, vascular invasion, and peritumoral fibrosis (Fig. 4.99). Occasionally, transitional cells can induce fibroblasts from the desmoplastic areas surrounding metastases to produce heterotopic bone. A few TCCs contain areas of squamous metaplasia. *About 50% of TCCs metastasize*, sometimes in a rampant or unpredictable manner. The usual pattern is for late metastasis to regional lymph nodes and lungs, but

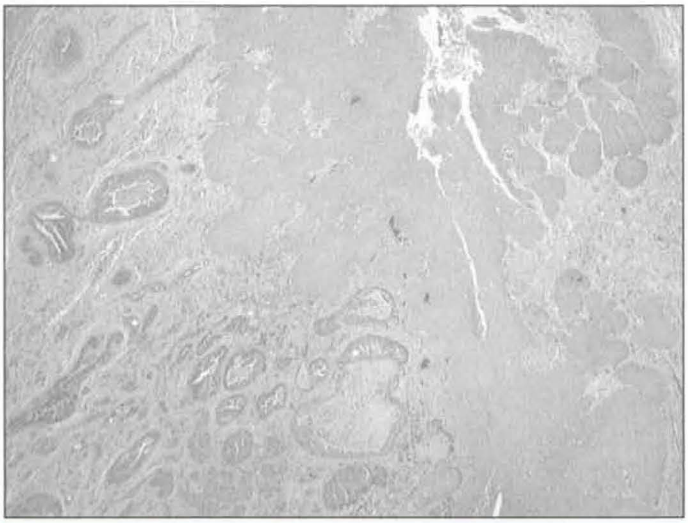

Figure 4.99 Transitional-cell carcinoma from the ureter of a dog, present transmurally and inducing marked desmoplasia.

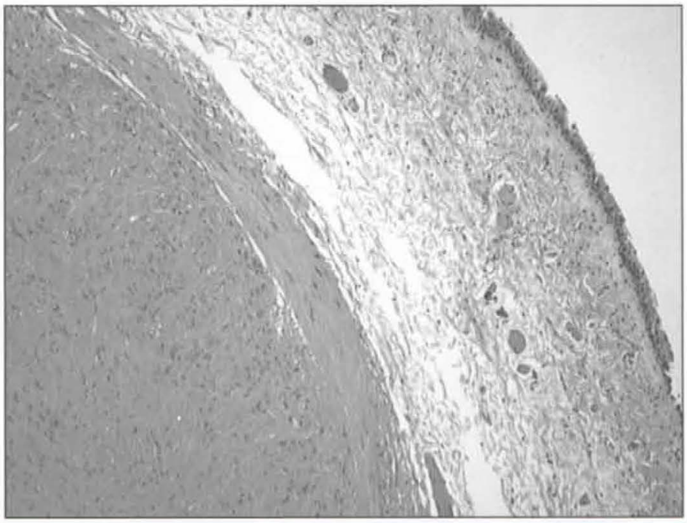

Figure 4.100 Leiomyoma, growing expansively in the wall of the urinary baldder of a dog.

peritoneal implantation or retrograde lymphatic spread to the soft tissue and bones of the hindlimbs or vertebrae is common. Occasionally solitary metastasis to bone occurs. Immunohistochemically, canine TCCs often stain for antigens similar to tumor-associated glycoprotein 72 (TAG-72), a high-molecular-mass glycoprotein used in the diagnosis of carcinomas originating in numerous human tissues. Recently, uroplakin III has been determined to be a specific and sensitive marked for canine transitional cell neoplasms. The presence of uroplakin III was not noted to correlate with tumor grade. Expression of cyclooxygenase-2 in neoplastic urinary bladder epithelium, but not in normal epithelium, suggests this may be a useful marker for neoplastic transformation and growth and can be targeted for therapeutic effects. *In order to assist clinical management of TCC, pathology descriptions should include tumor architecture, tumor grade, and depth of invasion based on sampling of multiple sites.*

Squamous cell carcinomas and **adenocarcinomas** are usually nonpapillary infiltrative growths, which are grossly nodular or sessile and often ulcerated. They develop in areas of squamous or mucous metaplasia. Histologically they are "pure," without transitional-cell areas (Fig. 4.97B). Squamous cell carcinomas and adenocarcinomas occur in dogs, cattle, and cats, and are the most common urinary bladder neoplasm in horses. Squamous cell carcinomas occur most often in bitches in the urethra, the distal two-thirds of which is lined by stratified squamous epithelium. Apparently these are less likely to metastasize than are transitional-cell tumors. **Undifferentiated carcinomas** are those very rare primary neoplasms that do not conform to one of the histologic types mentioned above.

Mesenchymal tumors

Mesenchymal tumors comprise fewer than 20% of tumors of the lower urinary tract. Neoplasms causing enzootic hematuria in cattle are about 10% mesenchymal and 55% mixed, with most of the nonepithelial tumors in these mixtures being hemangiomas. A few vascular tumors also occur in the bladder and about the urethra of dogs, but most mesenchymal tumors in dogs are leiomyomas or fibromas. **Leiomyomas** originate in the smooth-muscle coats of the urinary bladder (Fig. 4.100) and form well-defined projecting spherical white nodules. The nodules may be multiple and seem to have a predilection for the neck of the bladder, where they may interfere with urinary outflow. **Leiomyosarcomas** are very rare and generally do not metastasize; immunohistochemical staining for smooth-muscle actin and desmin are expected. **Fibromas** probably arise from subepithelial connective tissue, are usually solitary, and have a typical gross and microscopic appearance. **Fibrosarcomas** are rare; they are likely to metastasize, often widely.

Rhabdomyosarcoma is a rare tumor in any location (see Vol. 1, Muscle and tendon). **Botryoid** (shaped like a bunch of grapes) **rhabdomyosarcoma** occurs in the urinary bladder and occasionally the urethra of young dogs; large breeds, particularly the St. Bernard, seem to be overrepresented. This tumor has been reported in a Maltese bitch, two Poodles, and a filly. The youthfulness of the affected animals (less than 2 years old in most cases) raises the possibility that these tumors arise in rests of embryonic myoblasts. Grossly the tumors usually occur at the trigone and project into the bladder as botryoid masses. They infiltrate the wall and may metastasize, but are usually identified because of urinary obstruction before this occurs. This tumor has been associated with hypertrophic osteopathy in a dog. Microscopically, there is usually a mixture of fusiform and pleomorphic cells with some strap cells and multinucleated cells. Cytoplasmic cross-striations are sometimes present (Fig. 4.101). Immunoperoxidase staining of the intermediate filament desmin may aid in the diagnosis of this tumor.

Involvement of the urinary bladder in primary **lymphosarcoma** has been reported only rarely in domestic animals; primary canine epitheliotropic T-cell lymphoma involving the urinary bladder is even more rare.

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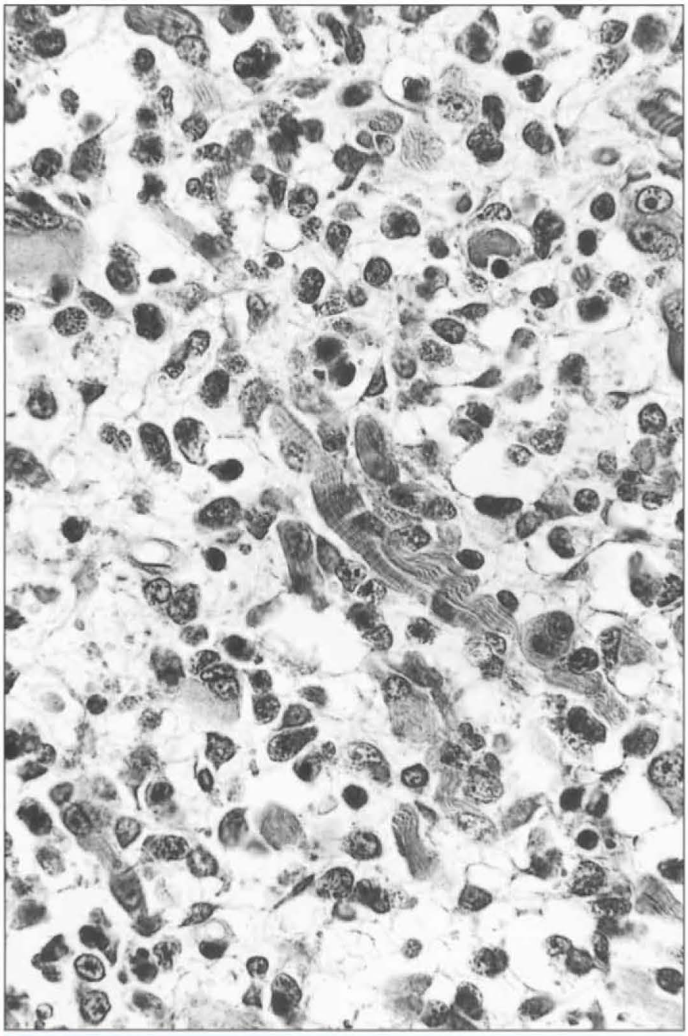

Figure 4.101 Botryoid rhabdomyosarcoma, with a striated strap cell, in the urinary bladder of a 2-year-old English Setter dog. (Courtesy of M Ayroud.)

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5

Respiratory system

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GENERAL CONSIDERATIONS

Of all the organ systems, *the respiratory tract may be unique in its vulnerability to injurious agents*. The involuntary nature of ventilation, necessitated by aerobic respiration and the need for oxygen, actively pulls large volumes of air into the lungs (estimated at 5400 L/day in dogs and 11 400 L/day in horses). Along with this air there are a variety of potentially injurious materials. In addition, the flow of the entire cardiac output passing through the extensive and delicate vascular bed of the gas exchange region of the lungs provides a separate avenue for the delivery of potentially harmful substances to the respiratory tract. The dual exposure routes are particularly important in determining the expression of disease in the lung, with *distinct patterns of lesions depending upon whether the route of entry is airborne (aerogenous) or vascular (hematogenous)*. The array of potentially injurious agents includes airborne microorganisms, toxic particulates, and gases in ambient air, and a wide variety of infectious agents and extrinsic or intrinsic toxins delivered via the pulmonary circulation. The patterns of pulmonary disease, as well as mechanisms to prevent respiratory injury, are covered later in this chapter.

In order to understand the response of the respiratory system to injury, it is necessary to review the general anatomic features of the respiratory system and biology of the major cellular constituents therein. There has been a rapid increase in our understanding of the complexity of the biology of the lung, elucidating, for example, the role of the recruitment of bone marrow-derived stem cells during lung injury. While many of the features to be discussed are common to other taxa, our overview is based upon the *mammalian respiratory tract*; excellent reviews covering the respiratory biology of birds, reptiles, amphibians, and fish are available in the literature.

The upper airway

The **nasal airways**, except for the external nares, are noncollapsible structures encased in bone. The passages for air contain *osseous and cartilaginous turbinates* (conchae), and are divided by a cartilaginous *nasal septum*. The respiratory turbinates subdivide the nasal cavity into distinct air passages, which direct airflow into the lower respiratory tract and to sites of olfaction. The presence of respiratory turbinates is restricted to mammals and birds, and is assumed to be important in the evolution of endothermy and the high levels of oxygen consumption associated with metabolism in these species; similar structures are not present within ectothermic animals.

Functionally, the *respiratory turbinates are important in warming and humidifying the inspired air* to prevent desiccation of the lower respiratory tract, and in reclaiming moisture and heat from the saturated vapor leaving the lungs. The nasal submucosa is richly supplied with a complex vascular plexus, nerves, and submucosal glands. In addition to the air-warming functions, most species have vascular shunts between the respiratory turbinates and the brain vasculature, suggesting a role in *cooling the brain* during periods of intense activity in mammals and birds. Stimulation of the parasympathetic innervation in the nasal cavity leads to increased vascular permeability and submucosal gland secretion, which occlude the nasal airways and increase resistance to airflow. This is countered by the sympathetic nerve fibers, which favor vasoconstriction and decreased vascular permeability.

The mucosa of the nasal cavity has four distinct epithelial types: (1) *stratified squamous;* (2) *transitional;* (3) *ciliated respiratory; and* (4) *olfactory*. The relative distribution and extent of each of these cell types vary among species. Stratified squamous epithelium is confined to the nasal vestibule at the entrance to the nasal cavity. Separating the squamous epithelium from the more caudal respiratory epithelium is a zone of nonciliated cuboidal to columnar cells, the transitional epithelium. Most of the epithelium lining the nasal mucosa is comprised of respiratory epithelium. This is a complex epithelium that includes pseudostratified ciliated, mucous, nonciliated columnar, cuboidal, and basal cells. *Olfactory epithelium is located in the caudal and caudodorsal nasal cavity*, lining the osseous and cartilaginous ethmoturbinates. This epithelium is the most phylogenetically conserved of the nasal epithelium, reflecting the ancient origins of olfaction. The epithelium is composed of olfactory sensory cells, sustentacular cells, and basal cells. *Olfactory epithelium is rich in cytochrome P450-dependent monooxygenase enzyme activity* and, therefore, is capable of bioactivating a variety of chemicals into potentially toxic intermediate forms. Hence, olfactory epithelium is susceptible to many of the chemicals toxic to the nonciliated bronchiolar epithelial cells and type II pneumocytes in the lung, as has been reported after exposure to 3-methylindole in horses.

The **nasopharynx** is lined by ciliated pseudostratified epithelium with zones of stratified squamous epithelium. *Lymphoid nodules* are abundant throughout the submucosa, and are one of the components of Waldeyer's ring, a collection of lymphoid tissues that encircle the oropharynx and nasopharynx. *The auditory (eustachian) tubes* extend from the nasopharynx to the middle ears. In horses, diverticula in each auditory tube form the *guttural pouches*, which are located dorsocaudal to the caudal pharynx. The function of the guttural pouches is not completely understood, but they may play a role in cooling blood destined for the brain during periods of intense activity and respiration. This respiratory-based cooling mechanism is facilitated by the anatomy of the internal carotid artery as it courses in a crease along the caudal wall up to the dorsal surface of the pouches. As with the pharynx and auditory tube, the mucosa of the guttural pouches is lined by pseudostratified ciliated epithelium.

The **larynx** is comprised of a complex of cartilage plates lined by stratified squamous epithelium as well as pseudostratified ciliated respiratory epithelium. The larynx is at the boundary between the respiratory system and the oropharynx, and communicates directly with the oropharynx. The cartilage of the larynx is important in vocalization in animals, and also resists deformation and obstruction from ingesta or external pressure. There is considerable variability in the anatomy of the larynx between domestic species, reflecting both interspecies variability as well as changes brought about through selective breeding; this is particularly true for the brachycephalic breeds of dogs, where the anatomy of the upper airways is associated with significant clinical disease.

The **trachea** extends from the terminus of the larynx to the *carina*, the branch point for the primary bronchi leading into the right and left lungs. With its relatively simple gross anatomy, and seemingly simple function serving as the conduit for air traveling to and from the lungs, the trachea is often overlooked in routine pathology examinations.

Organization of the lung

The organization and subdivision of the lower respiratory tract arise as a function of the development of the organ, and vary considerably by species (lung development is reviewed later in the chapter). Many of these gross features are important in the pathogenesis of disease and the susceptibility of individual species to a variety of insults, including infectious agents and inhaled toxins and particulate matter. Additionally, proper usage of terminology is important in the gross description of lesions at the time of necropsy.

The divisions of the lungs, from largest to smallest, include lobes, bronchopulmonary segment, and terminal acinus. In all of the domestic species, the lung is divided into a right and left lung, each of which is served by a single **primary bronchus** (mainstem bronchi) arising at the tracheal bifurcation. Each of the two lungs is further subdivided into individual lobes. The **lung lobe** represents the largest subdivision of the right and left lungs. *The lobe is defined as the gas exchange region ventilated through the* **lobar (secondary) bronchus**. The numbers and distribution of lung lobes vary by species. All domestic species except the horse have two lobes in the left lung (cranial and caudal) and four lobes in the right lung (cranial, middle, caudal, and accessory); the horse lacks a right middle lung lobe. The cranial lobe of the left lung is subdivided into a cranial and caudal part in all species except the horse, and the right cranial lobe of domestic ruminants is similarly divided into a cranial and caudal part. Interestingly, the lobar bronchus (**tracheal bronchus**) of the right cranial lung lobe of ruminants and pigs arises from the trachea before the primary bronchi bifurcate from the trachea to the right and left lungs. This location proximal to the primary bronchi predisposes the right cranial lung lobe to inhalation of foreign materials, especially liquids, as well as accumulation of inflammatory exudates from the more distal lung fields as they are cleared by the mucociliary apparatus. It is therefore not uncommon to find this lung lobe extensively involved in instances of inhalation pneumonia or with severe bacterial bronchopneumonia.

Depending upon the species, further subdivisions into individual **lobules** by connective tissue septa may or may not be grossly visible; when present, these interlobular septa are contiguous with the surface pleura. *In humans, pigs, and cattle, lobulation is highly developed, with individual lobules readily seen on the pleural surface of the lung. Equine, ovine, and caprine lungs are intermediate in lobulation; canine and feline lung are essentially devoid of septation and lobule formation.* A **bronchopulmonary segment** is the area served by a tertiary bronchus that arises from the lobar (secondary) bronchi. Well-developed interlobular septa that nearly completely surround individual bronchopulmonary segments, as in the pig and cow, limit collateral ventilation as well as the movement of cells and molecules between adjacent segments. Functionally, this is important in these species in limiting the extension of inflammation between lobules. Well-developed connective tissue septa can effectively sequester inflammation in affected lobules, leading to the characteristic gross pathology especially common in bacterial bronchopneumonia, with severely affected lobules adjacent to less affected lung lobules. The lack of collateral ventilation is also largely responsible for the common, incidental, interlobular emphysema found in cattle lungs at the time of necropsy. This emphysema occurs antemortem with preterminal labored breathing, and reflects the accumulation and tracking of pulmonary gasses along the path of least resistance within the septa, rather than extending through the septa to adjacent lobules.

Organizationally, the next smallest discernible functional unit within the lung distal to the bronchopulmonary segment is the **terminal acinus**, *which is comprised of one terminal bronchiole and its associated gas exchange region of the lung.* The anatomy of the terminal acinus varies among domestic species, especially the length between the terminal bronchiole and the alveoli. This junction is comprised of respiratory bronchioles and/or alveolar ducts; depending upon the species, it may be entirely one or the other, or a combination thereof. **Respiratory bronchioles** are bronchioles with variable lengths of their architecture interrupted by periodic alveolar outpocketings before transitioning into the gas exchange region. **Alveolar ducts**, in contrast, are cylindrical airways, circumferentially surrounded by alveoli, and invested with spiral smooth muscle. The anatomy of these distal airways is of more than just academic interest; this junction with the gas exchange region is a common site for initiation of viral and bacterial infections, for injury from insoluble toxic gases, and for the deposition of small particulate matter such as asbestos fibers.

Vascular supply to the lung

The vascular supply to the lung is dual, arriving through both the pulmonary and bronchial circulation. This unique system of perfusing the lung with blood has important implications in the pathogenesis of pulmonary disease. The *pulmonary arterial circulation* receives the entire output of the right ventricle and is characterized as a high-flow, low-pressure system; in contrast the *bronchial arterial circulation*, being a part of the systemic arterial blood vasculature, is a low-flow, high-pressure system. The bronchial circulation supplies nutrition to the large bronchi and blood vessels, the pleura, and much of the interstitium associated with these structures. The bronchial arteries can arise from a variety of sites, including the aorta, intercostal arteries, and subclavian arteries. The pulmonary circulation nourishes the alveolar parenchyma and distal bronchioles and, in the alveolar capillary bed, is the site of gas exchange. A *dual venous drainage* is also maintained: *pulmonary veins* drain the capillaries served by the pulmonary arteries, and *azygous veins* drain those supplied by the bronchial arteries.

The morphology of the two vascular systems in most species is readily apparent. Muscular pulmonary arteries have an internal and external elastic lamina, whereas the external elastic lamina is indistinct or absent in bronchial arteries. There is much variability in the histomorphology of the pulmonary arteries in the domestic species. At the pulmonary trunk, the arteries of most species have considerable numbers of elastic fibers interspersed with the smooth muscle. The more distal arteries and arterioles do not have elastic fibers. The smaller branches of pulmonary arteries vary considerably between species in their morphology, especially the thickness of the tunica media. *Cats, pigs, and cattle have the thickest pulmonary arteries of the domestic species*, and in domestic cats the medial hypertrophy/hyperplasia can be quite pronounced, though usually incidental. Because of the presence of smooth muscle in the small pulmonary arterioles of many domestic species, care needs to be taken in diagnosing pulmonary arterial hypertension based simply upon arteriolar histology. Pulmonary veins follow the bronchial tree in most domestic species, and have a thin fibrous wall in all of the species except for cattle and pigs, where there is interspersed smooth muscle.

While the primary function of the pulmonary vascular system is oxygenation of venous blood for delivery to the systemic arterial circulatory system, the arterial and venous systems have other important functions. Of particular importance is the *proper maintenance of tissue fluid balance within the lung*. Under normal circumstances there is a fine balance between fluid movement into the alveolar lumen, and reuptake into the interstitium and alveolar capillaries to prevent interstitial and alveolar edema. As with other tissues, vascular hydrostatic pressure and interstitial colloid oncotic pressure favor filtration of fluid into the pulmonary interstitium, while plasma colloidal oncotic pressure and interstitial hydrostatic pressure keep fluid from moving into the alveolar space. The capillary endothelial cells play a variety of roles in this balancing act. Normal pulmonary capillary endothelial cells transport albumin into the alveolar interstitium through caveolae-dependent transcytosis to maintain the correct interstitial oncotic pressure. Under inflammatory conditions, cytokines such as tumor necrosis factor-α induce the formation of intercellular gaps between endothelial cells, allowing less restrictive movement of albumin and other macromolecules into the interstitium, and subsequently into the alveolar lumen, where it forms the eosinophilic alveolar edema seen histologically.

The pulmonary vasculature is a dynamic tissue, capable of remarkable physiologic and morphologic adaptations given the proper stimuli. Remodeling of the architecture of the pulmonary vasculature can occur under a variety of disease states, and the relative contributions of both the pulmonary and bronchial systems vary with the disease in question. *Angiogenesis* (formation of new blood vessels from pre-existing vasculature) is primarily a feature of the bronchial circulation; the pulmonary arterial system has limited angiogenic capacity. Angiogenesis is particularly important in primary lung neoplasms, where the continued nutrition, and therefore survival, of the tumor is dependent upon this process. Both the pulmonary arterial and bronchial circulation can play a role in providing nutrition to pulmonary metastatic neoplasms. Angiogenesis, as a response to inflammation within the respiratory tract, can be a prominent feature of remodeled airways; such newly formed blood vessels are prominent within the lamina propria and submucosa in chronic bronchitis, and may be increased in asthmatic airways. Experimentally, *Mycoplasma pulmonis* infection in mice leads to increased numbers of blood vessels within the trachea. Regardless of the inciting stimuli, these newly formed vessels are prone to protein leakage and can contribute to local edema formation, and even hemoptysis.

Cellular architecture and cell biology of the lung

Before considering select cellular constituents of the lower respiratory tract, it is important to be mindful of the functional interdependence of the cellular constituents of the respiratory system in relation to organ development, homeostasis, and response to injury. This interdependence has its origins in the embryologic development of the lung, specifically the close association of the endodermal and mesodermal components (see section on Lung development and growth, below). In the adult lung, this anatomic and functional relationship has been termed the *epithelial–mesenchymal trophic unit*. Conceptually, this unit considers the epithelial and mesenchymal components in the respiratory tract together; disparate tissue constituents, such as airway epithelial cells, smooth-muscle cells, extracellular matrix proteins, and inflammatory cells communicate and direct the organ response during development, health, and disease. The relationship between these various components likely extends the length of the lower respiratory tract, and possibly the upper respiratory tract, determining whether there is restoration of the injured tissue or replacement with permanently remodeled tissue, with consequent changes in organ function.

In all domestic species, the **trachea and bronchi** *are surrounded by rings of hyaline cartilage*. These rings resist physical deformation, thereby helping to maintain the patency of the upper airways. In spite of their greater diameter, relative to the bronchioles, approximately 80% of the resistance to pulmonary airflow resides within the first 4–7 generations of the conducting airways. Partly because of this, any process that results in significant narrowing of the bronchi can result in clinically detectable changes at an earlier stage than a similar process involving the bronchioles.

Bronchioles, in contrast to bronchi, *have no cartilage in their walls to prevent airway collapse*. Bronchiolar airway patency is dependent on the attachment of alveolar septa to the thin connective tissue layer in the bronchiolar wall. The one exception to this rule is marine mammals, both cetaceans and pinnepeds, which possess well-developed cartilage rings around even the smallest bronchiole that prevent airway collapse in the face of supra-atmospheric pressures found at depth within the oceans. In terrestrial mammals, the radially arranged alveolar septa are tethered to and pull on the bronchiolar wall to maximize the luminal diameter during peak lung volume during inspiration. During expiration, as the forces from the interalveolar septa decrease, the bronchiolar lumen decreases in diameter. Because of their smaller diameter, collapsibility and thin-walled structure, bronchioles are much more susceptible to disease processes occurring in the surrounding alveolar parenchyma than are bronchi, and they individually have a higher resistance to airflow than individual bronchi. In spite of this, there is less resistance to airflow in the distal airways because of the higher number of bronchioles. Therefore, a large percentage of bronchioles must be affected before clinical evidence of disease is detected (e.g., hypoxemia due to ventilation/perfusion abnormalities or dyspnea due to small-airway obstruction).

Histologically, there is great cellular diversity within the mucosal epithelium of the conducting airways, the cell populations changing depending upon the species and airway generation under consideration. This complexity is difficult to appreciate in routine two-dimensional histologic sections. The trachea, bronchi, and proximal bronchioles are lined by pseudostratified epithelium composed of a mix of ciliated, mucous, and nonciliated cells. In general, the numbers of ciliated and mucous cells decrease within the distal airways, with a greater proportion of nonciliated (Clara) cells present within the bronchioles. These distribution factors can play an important role in the region-specific susceptibility of the airway epithelium to specific forms of injury, especially infectious agents, particulate matter, and toxins (either inhaled or within the systemic circulation).

Proximal generations of bronchioles are lined by epithelium that is shorter but otherwise indistinguishable from the pseudostratified epithelium found in the distal bronchi. More distally, the small bronchioles are lined by a simple columnar to cuboidal epithelial lining, composed almost entirely of ciliated cells and nonciliated bronchiolar (Clara) cells; in many species, Clara cells are the predominant cell type.

While the principal function of the ciliated cell – movement of airway-lining fluid – is well known, the biology of the **Clara cell** is not as well appreciated. This nonciliated nonmucus-secreting cell functions as a *progenitor cell* for epithelial repair within the airway mucosa, as it has the capacity to divide and differentiate into ciliated cells or other nonciliated cells. In addition, Clara cells of many species have abundant smooth endoplasmic reticulum within the apical cytoplasm, which possesses high concentrations of cytochrome P450-monooxygenase enzymes. Indeed, on a per-cell basis, Clara cells of some species have greater metabolic capacity than even hepatocytes. Therefore, *Clara cells are extremely sensitive to a variety of xenobiotics and natural toxins*; metabolism of these compounds can generate intermediate metabolites that form adducts with macromolecules within the cell, and lead to Clara cell injury. Finally, Clara cell secretory protein (CC10), secreted by these cells in response to adrenergic stimuli, may dampen the inflammatory response by inhibition of phospholipase A_2.

Smooth-muscle cells are located around the circumference of the bronchi and bronchioles, and extend to the ostia of alveolar outpocketings in the respiratory bronchioles, and into the alveolar ducts of some species. Historically, the *airway smooth-muscle cell* (ASMC) was considered to be a relatively static cell population, contributing little to the structure of the remodeling lung beyond its ability to undergo hypertrophy in response to chronic airway inflammation as in asthma and chronic bronchitis. Their importance was primarily seen as the transducer of bronchoconstriction and bronchodilation. Smooth-muscle cells can undergo hyperplasia in response to paracrine signals arising from the airway epithelium in inflammatory diseases, and this doubtless contributes to the accentuation of smooth muscle found histologically in chronic bronchitis and asthma.

There is considerable evidence that smooth-muscle cells are important members of the epithelial–mesenchymal trophic unit. *They produce a wide array of cytokines, chemokines, growth factors, and extracellular matrix metalloproteases*, allowing them to modify the interstitial matrix directly around smooth-muscle bundles. *ASMCs also secrete matrix proteins*, including fibronectin, elastin, and a variety of laminins and collagens. The matrix produced by ASMC can then modulate the proliferation, migration, and apoptosis of smooth-muscle cells. Transforming growth factor-β is a potent profibrogenic cytokine produced by ASMC; in bovine trachealis smooth-muscle cells, plasmin can stimulate release of active TGF-β from the cells, which in turn results in collagen upregulation by the ASMC. This paradigm may partly explain the increased extracellular matrix found around smooth-muscle bundles in chronically inflamed airways.

The **alveolar parenchyma** is the site of the only essential function of the lung, that being *gas exchange to support cellular aerobic respiration*. Regardless of the media for gas exchange (water or air), the essential design of the gas exchange membrane in animals is remarkably similar, consisting of an epithelial layer exposed to the exchange media, in close apposition to blood-filled capillaries. This relatively simple arrangement belies the complexity of the biology of the region. In mammals, there are five major cell types associated with the alveolar parenchyma: (1) *type I and type II pneumocytes*, (2) *capillary endothelial cells*; (3) *interstitial fibroblasts*; and (4) *alveolar macrophages*.

Type I pneumocytes *are large, flat, squamous epithelial cells that line ~93% of the alveolar surface*. Type I pneumocytes are terminally differentiated, thus they do not contribute to the resolution of alveolar injury through regeneration of new epithelial cells. They are particularly vulnerable to oxidant injury in part because of their large membrane surface area and low levels of important antioxidants, especially reduced glutathione. Irreversible injury to type I cells is quickly followed by sloughing of the cells from the alveolar basement membrane, which triggers a rapid regenerative response orchestrated by the type II pneumocytes. This description of type I cells suggests a passive role in the lung, facilitating gas exchange across the plasma membrane, and poised for injury and loss following any number of insults. While this is true, they also play a critical part in maintaining normal lung fluid composition, providing a structural basis for preventing movement of fluid into the alveolus, and clearing fluid and protein from the alveolus during pulmonary edema.

Type II pneumocytes *are the primary cuboidal cell lining the alveolus*; the brush cell or type III pneumocyte is also cuboidal, but much less numerous. Histologically, they are found most commonly at the intersection of two alveolar septa, the so-called "corners" of the alveoli. The ultrastructure of the type II pneumocyte is similar among mammals, with apical microvilli, and characteristic cytoplasmic osmiophilic lamellar inclusions, which are the site of surfactant storage. The primary functions of type II pneumocytes are to *synthesize pulmonary surfactant*, to serve as *progenitor cells* for replacement and turnover of alveolar epithelium, and to *metabolize xenobiotics*.

Pulmonary surfactant (name derived from its behavior as a *surface active agent*) is a complex mixture of phospholipids and small amounts of protein synthesized by type II epithelial cells and bronchiolar Clara cells. Surfactant is approximately 80% phospholipids, 10% neutral lipids, and 10% surfactant proteins (SP-A, SP-B, SP-C, SP-D), and is found at the air–liquid interface along the epithelial surfaces of both the conducting airways and alveoli. Interestingly, there are differences in the composition and presumed function of surfactant in these two locations. The main function of surfactant in the alveoli is to *lower surface tension in the alveolar space during expiration to prevent alveolar collapse during respiration*. Beyond its role in regulating alveolar surface tension, the surfactant phospholipids and proteins also have important immunoregulatory and pulmonary defense functions (see section on Lung defenses, below).

The proliferative capacity of type II pneumocytes is important in alveolar development and postnatal lung growth; the lungs of most species are not fully developed, undergoing continued maturation after birth (see section on Lung development and growth, below). Type II cells can rapidly proliferate to repopulate denuded basement membrane following injury to type I pneumocytes. This process follows a general pattern, consisting of rapid migration to cover the basement membrane, followed by proliferation, and re-differentiation to the dominant alveolar epithelium. The process is dependent upon the ability of the type II pneumocyte to modify the extracellular matrix to facilitate this process. Type II cells synthesize a variety of matrix components, including fibronectin, type IV collagen, and proteoglycans, as well as matrix-degrading enzymes such as metalloproteases.

The **brush cell**, sometimes known as the *type III pneumocyte*, is a poorly understood epithelial cell of the respiratory tract. Although known as the type III pneumocyte in the lung, their distribution is not restricted to the alveolus, and similar cells are also found in the nasal cavity, trachea, and conducting airways, as well as within the gastrointestinal tract and gallbladder. The function of brush cells is not completely understood, but most evidence suggests that they *act as chemoreceptors*. In the alveolus, they may sense hypoxia and regulate capillary perfusion accordingly. The presence of brush cells has been documented within the respiratory tract of rats and humans, and it is assumed that similar cells are present in domestic animals.

Fibroblasts are found within the alveolar wall associated with a network of normal extracellular matrix proteins, including collagen and elastin. This network is not uniformly distributed within the membrane; this leads to regions of the wall that are "thick" and "thin," with gas exchange only occurring where this network is absent. The fibroblasts of the lung are heterogeneous in terms of morphology, functionality, and expression of surface markers. This heterogeneity is particularly well documented in experimental and human cases of pulmonary fibrosis. The surface glycoprotein Thy-1 is an example of a marker of fibroblast heterogeneity in the lung. The presence or absence of Thy-1 on lung fibroblasts is associated with variation in fibroblast adhesion to extracellular matrix, production of cytokines, and ability to produce extracellular matrix proteins including collagen. The array of matrix proteins potentially produced by lung fibroblasts is extensive, and includes collagen types I, III, IV, V, and VI, elastin, laminin, fibronectin, glycosaminoglycans, and proteoglycans.

Pulmonary macrophages are an important class of lung cells, and are found in a variety of compartments within the organ. Five separate populations of macrophages in the lung have been recognized: (1) *alveolar macrophages*; (2) *interstitial macrophages*; (3) *pulmonary intravascular macrophages*; (4) *dendritic cells*; and (5) *pleural macrophages*. As with other organ systems, pulmonary macrophages are *derived from circulating monocytes*; their further differentiation is partly determined by the specific tissue microenvironment within which they reside.

Alveolar macrophages are integral to maintaining pulmonary homeostasis and the sterility of the alveolar parenchyma. The role that pulmonary macrophages play in pulmonary defense is discussed subsequently (see section on Lung defenses, below). Less appreciated is the role of alveolar macrophages in preventing the accumulation of native proteins, especially surfactant, within the alveolus. *Alveolar proteinosis*, a condition of alveolar accumulation of surfactant components, can be caused by local deficiency of granulocyte–macrophage colony-stimulating factor; this impairs the ability of the alveolar macrophage to clear surfactant, leading to its accumulation in the alveolus.

Alveolar macrophages function as *important regulatory cells* in the lung, controlling inflammatory, immune, and repair processes through the release of a wide array of cytokines and other regulatory molecules. Inflammation and immunity are promoted by macrophage synthesis and release of cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α and interferon-γ, as well as by release of inflammatory mediators that include leukotriene B_4, C_4, platelet-activating factor, and thromboxane A_2. Repair processes are generally promoted or otherwise regulated by release of cytokines that include transforming growth factor-β and -α, fibroblast growth factor, insulin-like growth factor, and platelet-derived growth factor.

Dendritic cells (DC) are *ubiquitous immunoregulatory cells*, found in all tissues. Derived from bone marrow, the DC are motile cells involved in surveillance for antigens at the epithelial surfaces of the respiratory tract. Their location extends from the mucosa of the nasal cavity, to the epithelia of the conducting airways, the pulmonary interstitium, and pleura. Various subpopulations of DC can be distinguished by means of morphologic characteristics, primary location, and unique surface marker proteins.

Morphologically, DC have numerous long processes, and function as antigen-presenting cells, priming naive T cells and driving the cells into T-helper type 1 (Th-1) or Th-2 effector cells. The ability of DC to present antigen to T cells requires that the DC themselves mature; this occurs within regional lymph nodes under the influence of appropriate proinflammatory signals. The numbers of DC can increase markedly in the respiratory tract under the proper stimuli, and they play an important role in allergic airway disease and asthma.

Interstitial macrophages are the least well-understood class of pulmonary macrophages. Because of their location within the interstitium of the conducting airways and alveolar parenchyma, they are more difficult to isolate and characterize than other macrophage populations. Interstitial macrophages may be a precursor cell for alveolar macrophages, but they also have unique functions. In mice, interstitial macrophages have less phagocytic activity than alveolar macrophages, and instead are primarily immunomodulatory cells, expressing more major histocompatibility complex class II molecules and producing more cytokines, such as IL-1 and IL-6, than the alveolar macrophage.

Pulmonary intravascular macrophages (PIMs) are unique mononuclear phagocytes found within the alveolar capillaries of select species, including cattle, sheep, goats, horses, pigs, cats, and cetaceans. PIMs may be recruited into the lungs of rats and humans during inflammatory lung diseases. PIMs form membrane-adhesive complexes with the capillary endothelium, which *keeps them localized to the vascular bed*. These cells are highly phagocytic and play an important role in the clearance of circulating bacteria and particulates in the pulmonary circulation. PIMs release an array of proinflammatory mediators following interaction with particulates and bacterial endotoxin. Because of their proinflammatory properties, they can contribute to the lung injury coincident with acute lung inflammation. Depletion of PIMs can attenuate lung injury following experimental bacterial infections or exposure to endotoxin.

Stems cells and the lung

The biology of stem cells, and their place in maintaining tissue integrity in health and disease, are recognized. Stem cells are considered to have nearly unlimited self-renewal and are divided into embryonic and adult stem cells. *Embryonic stem cells* reside within the inner cell mass of blastocysts and are pluripotent, capable of generating cells representative of all three embryologic germ layers (endoderm, mesoderm, and ectoderm). *Adult stem cells*, on the other hand, are multipotent, and capable of differentiating into a limited range of site-appropriate cell lines. Unipotent stem cells can only contribute to a single cell line; the differentiation of type II pneumocytes into type I pneumocytes is an example. Adult stem cells are found within fully differentiated tissues, and are involved in maintaining tissue homeostasis, replacing cell loss from apoptosis, and participating in tissue repair after injury.

In the lower respiratory system, there appear to be reservoirs of adult stem cells in each of the major anatomic localities; thus, in the trachea, cells with stem cell characteristics have been identified within the submucosal glands and with a Clara-like cell associated with neuroepithelial bodies. The basal cell of the trachea and bronchi is important in maintaining the attachment of the respiratory epithelium to the basal lamina, and there is evidence that they are a central stem cell involved in replenishing the epithelium of the proximal airways following injury. Clara cells are the primary stem cell of the bronchioles, and can readily replace both themselves and ciliated cells. Interestingly, when Clara cells are selectively removed from the airways using the Clara cell-specific toxicant naphthalene, there is morphologic evidence of the ciliated cell participating in reconstitution of the airway epithelium. The ciliated cell is traditionally considered to be terminally differentiated, so this information suggests that there is more plasticity to the airway epithelium than previously considered.

Finally, brief mention should be made of colonization of the lung with *bone marrow-derived stem cells.* A growing body of literature suggests that circulating stem cells can engraft within the lung and differentiate into both epithelial and mesenchymal cells. This process has been particularly well documented in experimental models of lung injury, and has important implications in understanding the pathogenesis of lung disease, and potential novel therapeutic avenues.

Lung defenses

The necessary function of gas exchange requires that the lung remain exposed to the environment. Thus, the sterility of the lung is continuously challenged by immense quantities of microorganisms and foreign material in inhaled air, and by opportunistic pathogens within droplets that are aspirated from the nonsterile upper respiratory tract. Despite this constant challenge, the lung must remain sterile or at least minimally contaminated, because excessive numbers of bacteria induce inflammation, which impedes gas exchange in the lung. Additionally, because of the tremendous blood flow through the lung, contamination of the lung by bacteria carries a significant risk of bacteremia. Thus, *the lung requires a multi-layered system of defense against infectious agents.* The objective is to maintain the sterility of the lung without inducing inflammation, as inflammation is detrimental to gas exchange. This is accomplished by the following:

- The *mucus* that lines the airways entraps particles and microbes, and is propelled by coughing or by ciliary beating to the pharynx, where it is swallowed.
- *Antibody and innate defense proteins* kill microbes directly, prevent them from colonizing mucosal surfaces, and/or opsonize the microbes to make them more easily ingested by phagocytes.
- *Alveolar macrophages* and *newly recruited neutrophils* that recognize foreign invaders, particularly in the presence of opsonins, engulf and kill these microbes without inducing inflammation.

If the infection cannot be contained by these mechanisms, inflammation is triggered in an attempt to control the threat. In these situations, alveolar macrophages and airway or alveolar epithelial cells produce cytokines and other mediators that recruit neutrophils and more monocytes–macrophages to the site of invasion. Inflammatory mediators also induce the production of antibacterial proteins by epithelial cells of the airway surface and submucosal glands. But this response has the potential to do harm: *inflammatory exudates impair gas exchange, leukocyte-derived enzymes and oxygen radicals cause injury to lung tissue, and repair processes may result in organization of alveolar exudates or fibrosis of alveolar septa that permanently decreases lung compliance and thickens the blood–gas barrier.* The significance of these sequelae depends on the extent of lung involvement. The tremendous reserve capacity of the lung means that many animals have localized areas of pneumonia that are of no clinical significance, yet mild changes present throughout the lung can seriously compromise pulmonary function.

The inspired air may carry a variety of particulates, including infectious agents, allergens, and inert particles. *The site of* **particle deposition** *in the respiratory tract depends on their size, density, and charge.* Particles larger than about 10 μm diameter are almost completely removed in the nasal cavity, mainly by impaction at sites in the nasal turbinates where the airflow changes direction. Particles that are 3–10 μm diameter are more likely to impact the mucosa of the trachea and bronchi, where they can be cleared by the mucociliary apparatus. In general, particles less than 5 μm have the greatest potential to reach the deep lung (terminal bronchioles and alveoli), although this is most common with smaller particles (1–2 μm). The velocity of airflow declines precipitously in the terminal airways because the total cross-sectional area increases; as a result, particle deposition in the bronchioles and alveoli results from sedimentation, diffusion, and electrostatic charge.

Coughing and mucociliary clearance *are the major mechanisms for clearing of particles that become entrapped in the airways.* Mucociliary clearance of particles from distal airways requires 2–6 hours, and the presence of innate defense proteins within the airway mucus, described in more detail below, probably serves to limit bacterial growth during this time. Mucociliary clearance may be as rapid as 10 mm/min in the trachea, but is much less rapid in the bronchioles. The airways are covered by a *lower periciliary liquid layer* (formerly known as the sol or hypophase) and a superficial mucous layer. The mucous layer contains abundant glycoproteins that are sticky and tend to trap a wide variety of particles that impact on the airway surfaces. The quality of the periciliary liquid layer is also critical for clearance of entrapped particulates, because the low viscosity of this fluid permits the cilia of the epithelial cells to beat effectively. Additionally, clearance of sputum from the larger airways by coughing requires that the periciliary liquid layer be fluid and abundant, allowing the superficial mucous layer to be easily propelled. Finally, effective ciliary function is necessary for mucociliary clearance, and requires coordinated unidirectional ciliary beating at an adequate frequency of around 15 beats per second. The regulation of this system is not well known. However, shear stress on the epithelium both increases the ciliary beating frequency and promotes epithelial secretion to maintain the quality of the liquid and mucous layer. Nitric oxide, cyclic adenosine monophosphate, and cyclic guanosine monophosphate are other regulators of ciliary beat frequency. When entrapped particles reach the nasopharynx, they have the opportunity to interact with the well-developed lymphoid tissue in the tonsils and nasopharyngeal mucosa before reaching the pharynx and being swallowed. Although this clearance of inhaled particles is normally beneficial, it is a method for the spread of agents such as *Mycobacterium bovis* and *Rhodococcus equi*, and is important in the migration of helminth eggs and larvae. Mucociliary clearance may be impaired by infection of the airway epithelium with viruses, mycoplasmas, or *Bordetella*, exposure to cold air or ammonia, squamous metaplasia due to chronic bacterial infection or toxin exposure (cigarette smoke and environmental pollutants being the best-characterized examples), inherited anomalies of ciliary structure or function, or abnormal mucus production in human cystic fibrosis.

The mucus covering the airway mucosa contains a rich spectrum of **antimicrobial factors**, which operate by several distinct mechanisms. Some cause *direct injury to pathogens*, such as the β-defensins, anionic antimicrobial peptides, cathelicidins, lactoferrin, lysozyme, the complement membrane attack complex, and lactoperoxidase. *Lactoferrin binds iron* and makes it unavailable for use by many bacteria, while neutrophil gelatinase-associated lipocalin – secreted by airway and alveolar epithelial cells in addition to neutrophils – binds bacterial siderophores that allow pathogens to scavenge ferric iron. Other components, including immunoglobulin A and polysaccharides within the mucus, *block the attachment of bacteria to mucosal surfaces*. Finally, *infectious agents may be opsonized* by immunoglobulin G, the complement component C3b, and surfactant proteins A and D. Two families of antimicrobial proteins, defensins and collectins, deserve particular mention. The *defensins* are a family of 3–5 kDa, cationic, arginine-rich peptides that are present in neutrophil granules, intestinal Paneth cells, and epithelial cells of the trachea and bronchi. Some defensins are constitutively expressed, while others are rapidly induced by proinflammatory cytokines, lipopolysaccharide, and other stimuli that activate nuclear factor-κB. The defensins are able to kill bacteria, fungi, and enveloped viruses by forming pores within microbial membranes that are rich in anionic phospholipids, and may also have roles in leukocyte chemotaxis and wound healing. The *collectins* are a family of calcium-dependent carbohydrate-binding lectin proteins that include the surfactant proteins A and D. Both of these proteins are produced by type II pneumocytes, and SP-A is also secreted by Clara cells in the bronchioles. These pulmonary collectins agglutinate and opsonize bacteria, viruses, and fungi, and also maintain surfactant homeostasis, have antioxidant activity, and bind lipopolysaccharide to regulate inflammatory responses.

Alveolar macrophages *are critical for defending the lung against particles that are deposited in alveoli, and also play a key role in recycling and removal of surfactant.* Their population is maintained by ongoing recruitment of blood monocytes. Alveolar macrophages are decorated by a variety of cell surface receptors that permit recognition of particles opsonized by antibody, complement, or collectins. In addition, CD14, Toll-like receptors, mannose receptors, and the scavenger receptor permit responses against bacteria that have not been previously encountered by the host. Alveolar macrophages phagocytose most opsonized particles within 2–4 hours, although the ingestion of inert particles such as carbon and silicates is much slower. Alveolar macrophages kill bacteria using reactive oxygen and nitrogen species and enzymes within their lysosomes. In addition, these cells are highly effective in promoting an inflammatory response by *secreting cytokines and chemotactic cytokines* that recruit and activate neutrophils and macrophages. These newly recruited phagocytes are important in the clearance of more severe bacterial infections of the lung. Alveolar macrophages are integral to the immune response by presenting antigen, secreting cytokines that modulate the response, and serving as effector cells in delayed hypersensitivity reactions. However, compared to other macrophage populations, alveolar macrophages are relatively poor antigen-presenting cells. The actual physical removal of particulates from alveoli is inefficient, in contrast to their removal when deposited on the mucociliary blanket. Most particles phagocytosed by macrophages are either inactivated or sequestered. Alveolar macrophages are mainly cleared through the bronchioles on the mucociliary blanket. As the particulate load increases, as occurs in the pneumoconioses, some particles penetrate into the pulmonary interstitium by endocytosis across the type I pneumocytes, where they enter the lymph and are phagocytosed by interstitial macrophages. Particle-laden macrophages often cluster around bronchioles and blood vessels, and some eventually find their way to the local lymph nodes. In addition to the alveolar and interstitial macrophages, *intravascular macrophages* are present in the lung of cats, horses, ruminants, and pigs. These cells serve to remove infectious agents and other particles from the blood, and thus serve an analogous role to Kupffer cells in the liver and macrophages in splenic sinusoids.

Pulmonary immune responses are initiated when inhaled antigens are taken up by *dendritic cells*, which inhabit the airway epithelium and migrate to bronchial lymph nodes where the antigen is presented to lymphocytes. Although *bronchus-associated lymphoid tissue* is often visible histologically, this structure is not present in germ-free animals of most species and may represent a consequence of inflammation and antigenic stimulation. Finally, *T lymphocytes and natural killer cells* constantly survey the pulmonary milieu, and are probably most useful in defense against viruses, intracellular bacterial pathogens, and fungi.

Thus, the lung is protected from inhaled bacteria by the filtering effect of the nasal cavity, innate and acquired humoral defenses, mucociliary clearance in the airways, alveolar macrophages, recruited neutrophils, and T lymphocytes and natural killer cells. These defenses can be considered in multiple layers, where most inhaled particles are removed in the nasal cavity or neutralized by innate defense proteins in the airway mucus prior to being cleared by the mucociliary apparatus. The small bronchioles seem to be a site of particular vulnerability, being poorly served by either mucociliary clearance or alveolar macrophages. Pathogens that reach the alveoli may be phagocytosed and killed by alveolar macrophages, particularly if the particles have been opsonized by complement or the surfactant proteins A and D, or by antibody if a previous encounter with the agent has resulted in an immune response. Still greater threats invoke a suppurative inflammatory response, which may be essential to clear bacteria from the lung but also carry the risk of compromised lung function and damage to the pulmonary tissue.

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NASAL CAVITY AND SINUSES

General considerations

The responses of the respiratory tract to injury, and the resulting patterns of disease, are largely determined by the structural and functional complexity of the system. Most of the diseases of the respiratory system are caused by agents arriving by either the airborne (aerogenous) or bloodborne (hematogenous) routes, each with its own special pathogenetic considerations.

The respiratory system is under constant assault from potentially injurious agents; these include airborne microorganisms, oropharyngeal flora, toxic particulates and gases in ambient air, and a wide array of infectious agents and extrinsic or intrinsic toxins delivered via the pulmonary circulation. Pulmonary defense mechanisms are remarkably effective under most circumstances in preventing disease agents from entering or remaining in the lung and in neutralizing these agents if they penetrate initial barriers.

The *upper respiratory tract* comprises the air-conducting structures from the external nares to the trachea at the level of the thoracic inlet, including the specialized system of paranasal sinuses and the auditory (eustachian) tubes, and the guttural pouches of horses. The anatomy of the upper respiratory tract is described above. Functionally, this system conducts and conditions the air moving into and out of the lower respiratory tract during respiration. The upper airways, by virtue of their position within the respiratory tract, are constantly exposed to materials from the external environment; they play an important role in surveying the inspired air for pathogens and are exposed to a variety of potentially toxic particulates and gases. The anatomy of the upper airways is also important in the generation of species-specific vocalization in animals, and abnormal vocal sounds can be an important clinical indicator of diseases involving the upper airways.

Development of the upper airways

The development of the upper airways is complex and poorly understood in domestic animals, compared to the considerable information known in species commonly used in biomedical research. The development of the upper airways is closely tied to that of the osseous and soft tissues of the cranium, as well as the upper gastrointestinal tract. In mammals, the development of the face, including the nasal cavity, involves coordination of the development of five distinct facial primordia. The structures of the head and neck arise from the pharyngeal arches, with contributions from the ectoderm, endoderm, mesoderm, and neural crest cells. Induced or inborn errors in this complex process can result in abnormalities in the nasal cavity. Neural crest cells are particularly sensitive to potential teratogens, and may mediate many of the craniofacial defects arising from such teratogen exposure. As with the lower respiratory tract, the development of the airways of the upper respiratory tract is tightly regulated by a complex of genes, including members of the homeobox family; alterations in members of this family can result in malformation of the face, including the nasal cavity. The nasal cavity continues to develop after birth; in particular the postnatal development of the paranasal sinuses plays an important role in the development of the remaining upper airways as well as the nonrespiratory facial structures. Selective breeding for particular craniofacial features, particularly in dogs and cats, has resulted in divergent "normal" nasal anatomy, some of which can adversely affect airflow.

Congenital anomalies

Congenital anomalies of the nasal cavity and sinuses are rare but occur in all species. They are usually part of more extensive craniofacial defects accompanied by various malformations of the mouth and eyes. Animals with absent, underdeveloped, or severely distorted nasal regions are usually stillborn or die immediately after birth because of the severity of the craniofacial defects.

Choanal atresia, *in which one or usually both of the communications between the nasal cavity and nasopharynx fails to form*, occurs commonly in foals, with additional reports in dogs, sheep, and llamas. It is usually the result of persistence of the choanal membrane, which can result in partial or complete obstruction of airflow and exercise intolerance. Descriptions of the histology of the persistent tissue are limited, with pseudostratified columnar epithelium and chronic inflammation present in the resected tissue from a dog with choanal atresia. In addition to membranous blockage of the choanae, persistent osseous tissue is reported in humans with choanal atresia.

Not all developmentally related defects of the nasal cavity manifest themselves at birth. A variety of localized defects that can affect the nasal region can take time to become apparent. Defects of tooth germ origin are especially likely to be of this type. *Paranasal sinus cysts* in foals or young adult horses are cystic bony nodules filling the maxillary or frontal sinuses. The masses can distort the profile of the maxillary bone sufficiently to cause obstruction of the ipsilateral nasal passage, destruction of the nasal turbinates, distortion of the teeth, and deviation of the nasal septum. *Cystic nasal conchae* are congenital lesions that cause progressive nasal obstruction with inspiratory and expiratory noise in cattle. They are bilateral or unilateral, smooth-surfaced, bony expansions of the ventral nasal conchae, containing a mucus-filled cavity lined by epithelium. *Epidermal inclusion cysts* are described in the nasal diverticulum (false nostril) of horses.

Nasal amyloidosis

Deposits of amyloid sometimes occur in the nasal submucosa of horses. The deposition is not part of generalized amyloidosis, although there might be concurrent cutaneous amyloidosis, particularly of head, neck, and cranial thorax. The nasal vestibule and rostral portions of the septum and turbinates are mostly involved, but the deposits may extend to the larynx. The amyloid may be in nodules of various sizes or deposited diffusely. The resulting stenosis can be severe enough to cause signs of nasal obstruction. The amyloid deposits have a smooth surface and the usual waxy sheen of amyloid on cut surface. The amyloid is deposited in the walls of submucosal vessels and the basement membrane of mucosal glands as well as in the connective tissue. Macrophages and lymphocytes are usually intermingled with the interstitial amyloid deposits, and giant cells are found adjacent to nodular deposits. There may be severe ulceration of the mucosa, especially overlying large nodular masses.

Nasal amyloidosis in horses is of the *AL type*, composed of immunoglobulin light chains or light-chain fragments. AL amyloidosis is idiopathic or associated with myeloma in humans, but this association has rarely been made in horses. In other species, there are few reports of amyloid deposition in the nasal cavity. Nasal septal amyloidosis is seen as an aging change in some strains of laboratory mice. Because immunoglobulin components are integral to the formation of localized deposits of amyloid, the disease may represent a plasma cell dyscrasia, even if overt increases in plasma cell numbers are not apparent histologically. Indeed, amyloid deposition is seen periodically in extramedullary plasmacytomas, and may comprise the majority of these neoplasms, with few of the neoplastic cells apparent amongst the amyloid. This should be considered as a possibility in any animal with extensive amyloid deposition, regardless of location.

Circulatory disturbances

The ability of the nasal mucosa to regulate the temperature of inspired and expired air is facilitated by the network of blood vessels within the mucosa and submucosa. Immediately beneath the epithelium there is a complex plexus of blood vessels, in addition to highly distensible venous sinuses within the submucosa. Nasal congestion occurs when this process pools blood within the venous sinuses; the engorged vasculature causes physical obstruction to airflow. *The generous vascular endowment of the nasal vasculature, in addition to its functional activity, leads to frequent nasal hyperemia.* Congestion of the nasal mucosa is a common, nonspecific finding during postmortem examination of animals. Active hyperemia is part of the acute stage of inflammation. In the nasal and bronchial airways, plasma readily exudes from postcapillary venules into the lamina propria. While the exudation of plasma may have adverse consequences, it may play an important defensive role by delivering antibody and innate defense proteins to the nasal and bronchial mucosa.

Epistaxis *refers to hemorrhage from the nose, without regard to the source of the bleeding.* The hemorrhage may arise from the nasopharynx or from deeper within the respiratory tract. Epistaxis due to hemorrhage from the nasolacrimal duct in horses has been associated with bacterial infections of the distal lacrimal system. Examples of epistaxis originating in the lungs include exercise-induced pulmonary hemorrhage in horses, and rupture of embolic pulmonary abscesses in cattle. *Blood-stained foam* is a frequent nonspecific finding present in, and issuing from, the nose of cadavers. This finding is indicative of nasal and/or pulmonary microvascular rupture associated with terminal congestion, edema, and hemorrhage.

Epistaxis originating within the nasal region is most commonly caused by trauma, inflammation, or neoplasia. It may also be part of any of the hemorrhagic diatheses (see Vol. 3, Hematopoietic system), such as those causing thrombocytopenia. Epistaxis has been reported as a manifestation of purpura hemorrhagica, in horses that had the more characteristic findings of subcutaneous edema and mucosal hemorrhages. In most inflammatory hemorrhage, the extravasation is initially submucosal. Rhinitis with hemorrhage into the nasal lumen is a result of mucosal ulceration extending into the underlying vascular bed, as occurs in mycotic infections of the nasal mucosa or guttural pouches, or with neoplasia. Rarely, nasal hemorrhage may be the result of hypertension or vascular aneurysms.

Immunology of the upper respiratory tract

Before one considers inflammation and inflammatory diseases of the upper respiratory tract, it is important to develop an understanding of some of the basic features of the immune system of the nasal airways. As with other mucous membranes, the upper-airway mucosa has an indigenous immune system, the *nasal-associated lymphoid tissue* (NALT). NALT is considered to be a component of the broader mucosal-associated lymphoid system present in a variety of mucous membranes.

Waldeyer's ring is a collection of lymphoid tissues that encircles the oropharynx and nasopharynx of animals. The degree of organization of this lymphoid tissue varies by species. In addition, there are lymphoid follicles and loose aggregates of mononuclear cells within the lamina propria. These immune tissues induce a local immune response, and also stimulate a systemic immune reaction to nasally delivered antigens.

The organization of NALT is similar to mucosal lymphoid tissues at other sites. Specialized follicle-associated epithelium, with microfold (M) cells, covers the follicles and transfers antigen to the underlying lymphoid follicles. Within the follicles there are T-cell- and B-cell-rich regions, as well as dendritic cells and macrophages involved in antigen presentation. The formation of NALT is a postnatal event that is at least partly regulated by acquisition of commensal microbes in the nasopharynx. This resident microbial flora is often established by specific adherence of bacterial adhesins to carbohydrates on epithelial cells.

In addition to the role that nasopharyngeal commensals play in the formation of NALT, they can prevent colonization of the mucosa by more virulent organisms and modulate the immune response during infection. Experimentally, exposure of germ-free rats to the common rodent pathogen *Mycoplasma pulmonis* resulted in an exuberant immune response within the nasal mucosa when compared to conventional rats exposed to the same organism. Injury to the nasal mucosal surface can allow infection of the underlying tissues by certain of the normal flora or, more importantly, affect surface binding sites so that adherence and colonization by pathogenic microorganisms can occur. Finally, disruption of the normal nasopharyngeal flora by prolonged antibiotic therapy may result in fungal or opportunistic bacterial infections of the nasal cavity, further illustrating the role of these commensals in mucosal defense.

Rhinitis

Rhinitis, inflammation involving the nasal cavity, can be localized or a manifestation of systemic disease. Causes of nasal inflammation include viral, fungal, bacterial, and parasitic infections, allergens, inhalation of irritant gases, and particulate dusts (Table 5.1). Most cases of significant acute rhinitis begin with serous exudation, which change in the course of the disease to catarrhal and then purulent inflammation. Pseudomembranous, ulcerative, or hemorrhagic rhinitis are signs of severe damage. Chronic rhinitis is most commonly manifested by proliferative changes involving the mucosa and NALT.

During the initial **serous** stages of rhinitis, regardless of the inciting conditions, the mucosa is swollen and gray to red depending on the degree of hyperemia. Histologically, the epithelial cells are normal or show hydropic degeneration and loss of cilia. Goblet cells and submucosal glands are hyperactive. The secretion is a thin, clear seromucin, which contains a few leukocytes and epithelial cells. The underlying lamina propria is edematous and sparsely infiltrated by inflammatory cells. The swelling of the mucous membrane tends to cause mild respiratory discomfort and the familiar sneezing and snuffling.

Within hours or a few days, serous rhinitis is modified partly by changes in glandular secretion and partly by bacterial infection. The hyperemia, edema, and swelling are then aggravated and the discharge becomes *mucous, catarrhal, or purulent* because of the emigration of large numbers of leukocytes and desquamation of epithelial cells. Regenerative hyperplasia of surviving epithelium can occur, but in purulent rhinitis extensive ulceration may be evident.

Chronic rhinitis may cause progressive fibrosis of the lamina propria with atrophy of the glands, and atrophy with focal *squamous metaplasia of nasal epithelium*. With **pseudomembranous rhinitis**, fibrinous membranes can be peeled off without leaving gross underlying defects. In contrast, the deeper **fibrinonecrotic (diphtheritic) membrane** adheres firmly to the underlying tissue, leaves a raw ulcerated surface when removed, and is caused by bacteria such as *Fusobacterium necrophorum* or some viral infections. **Granulomatous rhinitis** causes diffuse or nodular lesions, and may be caused by fungi, mycobacteria, foreign material, or *Bartonella* sp. **Nasal/nasopharyngeal stenosis** or cicatrix formation occurs in cats and horses, probably as a result of previous erosive rhinitis with fibrous organization of exudates. Irregular webs of scar tissue may occlude the nasal passages, resulting in upper respiratory tract noise but only minimal discharge.

In subacute to chronic rhinitis, diffuse or localized polypoid thickenings of the mucosa develop (Fig. 5.1). The **nasal polyps** are initially sessile but can, when larger, become pedunculated. The ease with which the nasal lamina propria becomes engorged and edematous, plus the tendency of protuberances into the nasal meatus to compromise venous and lymphatic drainage because of constriction in their basal regions, are probable factors in the persistence or progression of inflammatory polyps. They occur occasionally in horses and cats, less commonly in other species. Polyps are soft, pink-gray, irregularly nodular, pedunculated or sessile masses. They have a chronically inflamed, edematous, myxomatous core, covered by variously hyperplastic, metaplastic, or ulcerated epithelium. Chronic polyps can become more fibrous, with organization and maturation of the collagenous stroma.

Idiopathic lymphoplasmacytic rhinitis is an important condition in the dog, and to a lesser extent in the cat. In dogs, the clinical disease may be either unilateral or bilateral, although histologic lesions are usually bilateral. Grossly, there is increased mucus production, mucosal inflammation, and turbinate destruction. Histologically,

Table 5.1 Major causes of nasal and sinus disease in domestic animals

Dogs
- Foreign body
- Tooth root infection
- Mycotic rhinitis
- Idiopathic lymphoplasmacytic rhinitis
- Allergic rhinitis
- Ciliary dyskinesia
- Adenocarcinoma
- Olfactory neuroblastoma
- Other considerations
    1. *Bordetella bronchiseptica*
    2. *Canine adenovirus 2*
    3. *Canine distemper virus*
    4. *Canine parainfluenza virus*
    5. *Linguatula serrata*
    6. *Pneumonyssoides caninum*
    7. *Rhinosporidium seeberi*

Cats
- *Felid herpesvirus 1*
- *Feline calicivirus*
- *Cryptococcus*
- Foreign body
- Nasopharyngeal stenosis
- Nasopharyngeal polyp
- Lymphoma
- Adenocarcinoma
- Other considerations
    1. *Bordetella bronchiseptica*
    2. *Chlamydophila* sp.
    3. *Mycoplasma* spp.
    4. Mycotic rhinitis
    5. *Rhinosporidium*

Horses
- *Equid herpesvirus*
- *Equine influenza virus*
- *Equine rhinitis virus*
- *Streptococcus equi*
- *Streptococcus zooepidemicus*
- Bacterial sinusitis
- Ethmoid hematoma
- Sinonasal polyps
- Ethmoid and sinus cysts
- Squamous cell carcinoma
- Maxillary bone tumors (fibrous dysplasia, ossifying fibroma, osteoma, fibrosarcoma)
- Other considerations
    1. Tooth root infection
    2. Nasal amyloidosis
    3. Glanders
    4. Mycotic rhinitis or sinusitis
    5. Phycomycosis
    6. *Schistosoma* spp.

Cattle
- *Bovine herpesvirus 1*
- *Bovine parainfluenza virus 3*
- Mycotic nasal granuloma
- Allergic rhinitis
- Other considerations
    1. *Limnatis* spp.
    2. *Mammomonogamus* spp.

Sheep and goats
- *Salmonella arizonae*
- *Oestrus ovis*
- Enzootic nasal tumor

Swine
- Inclusion body rhinitis
- Atrophic rhinitis

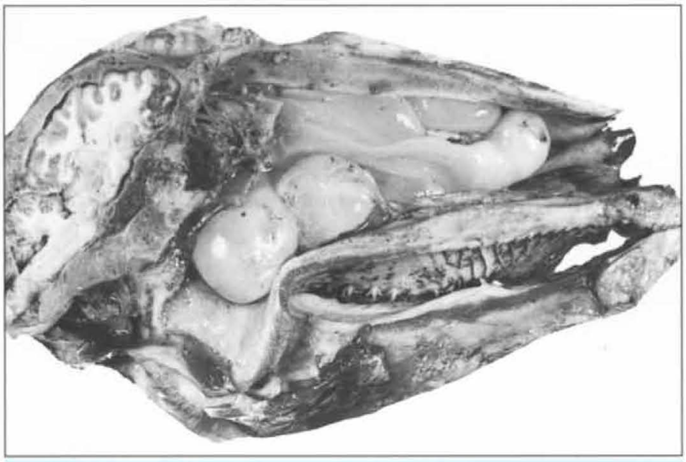

Figure 5.1 Myxomatous polyps in chronic rhinitis in a sheep.

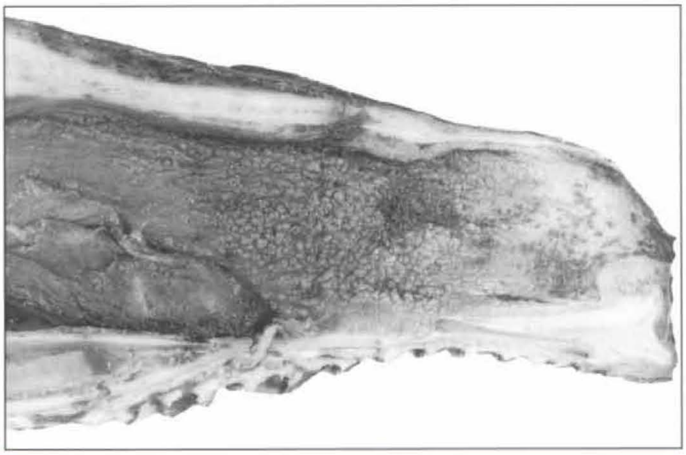

Figure 5.2 Hyperplastic mucosa of rostral nasal septum in **nasal granuloma** in a cow.

lymphocytes and plasma cells are the predominant inflammatory cell components. Chronic inflammation may lead to diffuse or polypoid thickening of the nasal mucosa and obstruction of nasal passages (Fig. 5.1). The fibrotic stroma is heavily infiltrated by lymphocytes and plasma cells, and the surface epithelium may be hyperplastic, eroded, or undergo squamous metaplasia. The cause of this disease remains unknown and the pathogenesis remains to be elucidated. It seems reasonable to consider that following an unknown initial insult there develops a self-propagating cycle that involves dysregulation of the local immune system, resulting in mucosal damage and further infection by normally nonpathogenic commensal flora and self-sustaining inflammation. Nevertheless, lymphoplasmacytic inflammation is a common reaction of the nasal mucosa to injury, and occult causes such as foreign body, bacterial or fungal infection, or neoplasia must be ruled out.

Allergic rhinitis is observed sporadically in dogs, cats, and horses. The disease in *dogs* is diagnosed on the basis of oculonasal discharge, sneezing, nose rubbing, head shaking, and perhaps epistaxis, and the *presence of eosinophils in nasal exudate.* There is no definitive information on either the pathological or immunological basis of the condition. *Cattle* and occasionally *sheep* develop a seasonal rhinitis that in its clinicopathological features is consistent with an allergic pathogenesis. Some evidence has been provided that affected cattle are allergic to pollen antigens. A familial predisposition has been reported. It occurs chiefly in the summertime when the pastures are in bloom. Affected animals have nasal discharge, lacrimation, sneezing, and evidence of nasal itching. The nasal mucosa is pale and thick from edema fluid, and mucosal erosions may be visible in the rostral nares. Eosinophils and mucus are a prominent component of the exudate. Histologically, *the surviving nasal epithelium is hyperplastic or eroded and is infiltrated by eosinophils.* The glandular epithelium can be hypertrophied and mucus is produced in excess. In more severe cases, in which there is extensive superficial diphtheresis, many of the small mucosal vessels show fibrinoid necrosis.

Nasal "granuloma" *is generally considered to be a more chronic form of allergic rhinitis.* The affected mucosa is mainly in the caudal portion of the nasal vestibule and the rostral region of the nasal septum and ventral meatus. In long-standing cases, the lesions extend further caudally to involve the caudal nasal cavity and even the larynx and proximal trachea. Grossly, *the hyperplastic epithelium is granular or has multiple nodular projections covered by intact epithelium* (Fig. 5.2). Histologically, the nodules typically consist of hyperplastic epithelium with areas of squamous metaplasia, covering a superficial edematous lamina propria with a central core of inflamed granulation tissue. Goblet-cell hyperplasia is more pronounced in the ducts of the nasal glands, at the lateral boundaries of the nodules. Eosinophils infiltrate the superficial lamina propria and epithelium, and mast cells are increased in number. Vascular proliferation, fibroplasias, and accumulation of mostly lymphocytes and plasma cells in the cores of nodules are features of chronicity. It is believed that the condition is due to an immediate (type I) hypersensitivity to plant pollens or fungal spores. Because there appears to be a familial predisposition in Jersey cattle and to a limited extent in other cattle, the existence of susceptible "atopic" animals has been proposed. The condition is therefore sometimes referred to as *atopic rhinitis.*

Mycotic nasal granulomas in cattle are less common. They also occur mostly in the rostral portion of the nasal cavity but *tend to be larger polypoid masses.* They frequently have yellow-green cores associated with massive eosinophil accumulation. Histologically, components of the lesions are similar to those previously described for the chronic allergic "granulomas," but hyphae and chlamydospores of fungi surrounded by macrophages, giant cells, and eosinophils are present. Various fungi, normally saprophytic on plants, have been isolated. It is tempting to speculate that the mycotic granulomas represent the small proportion of the more nonspecific allergic granulomas in which the causative allergen is able to vegetate. Mycotic rhinitis in canids is discussed in the section on Infectious respiratory diseases of dogs, below.

Sinusitis

Inflammation of the paranasal sinuses often goes undetected unless it has caused facial deformity or a fistula in the overlying skin. Sinusitis is of most significance in the horse because of the size and complexity of its paranasal sinuses and the compounding effects of limited drainage and tendency for periodontitis to extend to sinusitis. Sinusitis is very common in sheep as a response to larvae of *Oestrus ovis.* It also follows penetration of infection in dehorning wounds, fractures, and periodontitis. Seromucinous sinusitis may occur in viral infections of the upper respiratory tract, but it is not usually detected in routine diagnostic pathology samples. In acute catarrhal or purulent rhinitis, the mucosal swelling tends to occlude the orifices of the sinuses and impair drainage. The secretions and exudates then accumulate and render chronic purulent sinusitis almost inevitable. The histologic features of sinusitis are similar to those of rhinitis. *The accumulation of seromucinous secretion is referred to as* **mucocele**, and *the accumulation of purulent exudate is referred to as* **empyema** *of the sinus*. It is also less likely to drain and resolve spontaneously and therefore more likely to cause epithelial atrophy and metaplasia, and distort the bony walls of the sinuses by pressure or osteomyelitis.

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Nonneoplastic proliferative disorders of the nasal cavity and sinuses

Polypoid thickening of the nasal mucosa can occur in cases of chronic rhinitis and sinusitis. In these chronic inflammatory lesions, mucosal and submucosal hyperplasia may become florid enough to form a discernible mass within the nasal cavity or sinuses (see Fig. 5.1). While this change is a nonspecific remodeling response in the nasal cavity, there are two specific types of polyps in domestic species whose clinical features and pathology are distinctive.

Progressive ethmoid hematomas (PEH, hemorrhagic nasal polyp) **of horses** *arise from the ethmoid turbinate region of the equine nasal cavity*. These masses usually occur in older horses, and may be more common in the Thoroughbred and Arabian breeds. PEH are unilateral hemorrhagic growths, which arise from the submucosa of the ethmoid turbinates. The cause and pathogenesis of PEH remain to be determined, although it is assumed that it represents an aberrant vasoproliferative response to submucosal hemorrhage. The lesions are progressive and may continue to enlarge until the cylindrical mass extends to the external nares. Grossly, PEH is a mottled, hemorrhagic fibrovascular mass that can fill the ipsilateral nasal meatus, or occasionally grow into the maxillary sinus. Histologically, PEH mostly consists of organizing hemorrhages of various ages with extensive siderosis and mineralization of connective tissue fibers. The inflammatory response is variable, but multinucleate giant cells are often reported within the stroma. The surface is often covered by attenuated epithelium with underlying glands, or squamous metaplasia may occur. Ulceration of the surface epithelium is common.

Nasopharyngeal polyps of cats *are nonneoplastic inflammatory masses arising within the middle ear or auditory tube.* Clinical disease is seen primarily in young cats 1–3 years of age. The signs of disease are dependent upon the location of the polyp. Extension of the mass into the pharynx is associated with dyspnea, dysphagia, and gagging; involvement of the nasal cavity may cause sneezing, nasal discharge, and protrusion through the nares; while involvement of the middle ear may result in ataxia, Horner's syndrome, and/or facial nerve paralysis. When the polyps extend into the external ear canal they are known as **aural polyps**. The cause and pathogenesis of the disease are not known, although the appearance of the lesions suggests progressive fibroproliferation in response to localized chronic inflammation. Histologically, feline nasopharyngeal polyps consist of a loose fibrovascular core covered by ciliated respiratory or squamous epithelium. Even when the surface is completely covered by squamous epithelium, ciliated cells are usually visible within nests of epithelial cells buried in the stroma. The surface of the masses is frequently ulcerated, and the stroma is infiltrated by a mixed chronic inflammatory infiltrate of lymphocytes, plasma cells, and macrophages; ulceration results in significant numbers of neutrophils infiltrating the stroma. Recurrence of the polyps is relatively common when removed by simple traction, if the base of the mass is not excised.

Neoplasms of the nasal cavity and sinuses

While *primary neoplastic disease of the nasal cavity and sinuses of domestic animals is not common*, examination of nasal biopsy specimens to rule out such disease is common. Primary nasal neoplasia is most frequent in the dog and cat, with fewer reports in horses. Endemic, retroviral-induced ethmoidal tumors in sheep and goats are an important disease in specific geographic regions and are described with the infectious diseases of these species. There is no apparent relationship between the frequency of nasal tumors and breed-specific craniofacial anatomy in dogs. Interestingly, there is some epidemiologic evidence of a link between exposure to environmental tobacco smoke and coal-burning and kerosene heat sources, and the development of nasal neoplasms in dogs.

Origin from the nasal cavity is usual in dogs and cats, whereas in horses tumors of the paranasal sinuses are most common. Because of the inflexible osseous encasement, even benign growths can be associated with significant clinical disease. In general, *most neoplasms of the nasal cavity and sinuses, regardless of species, are carcinomas*, followed in decreasing frequency by sarcomas of cartilage, fibrous tissue, or bone. Regardless of histogenesis, malignant nasal neoplasms tend to be soft, pale, and fleshy-to-friable masses. The neoplasms invade and destroy adjacent structures, including the brain, but metastasis is uncommon (Fig. 5.3).

A variety of other neoplasms are reported in the nasal cavity of domestic species. These may be primary to the nasal cavity or part of systemic neoplastic disease. Primary lymphoma of the nasal cavity is the most common of these neoplasms, likely arising from the NALT. Other neoplasms reported in the nasal cavity include transmissible venereal tumor, mast cell tumor, and histiocytic sarcoma.

Squamous cell carcinomas predominate in the cat and horse, though they may be seen in any of the domestic species. In the cat, most originate from the nasal vestibule, while in the horse the maxillary sinus is a common site. The latter may arise from the epithelium of dental alveoli. Histologically, squamous cell carcinomas in the nasal cavity and sinuses share the typical features of the neoplasm seen elsewhere in the body, although keratinization is not as prominent.

Transitional carcinomas resemble the transitional (nonciliated) nasal epithelium. These neoplasms are most common in dogs. Transitional carcinomas are so called because they assume the stratified cuboidal appearance of transitional epithelium. Because of their characteristic appearance, they are sometimes referred to as *respiratory epithelial carcinomas* and, in the literature on human nasal tumors, they are often classified as *nonkeratinizing squamous cell tumors*. Transitional carcinomas typically consist of thick stratified layers of mostly cuboidal cells with rounded nuclei, indistinct cell borders, and a distinct basement membrane beneath the layer of neoplastic cells. Large transitional carcinomas have complex infolding or pleating of the thick epithelial bands separated by delicate fibrovascular septa. Microcysts are sometimes present within the epithelial layers, and must be distinguished from the acini seen in adenocarcinomas.

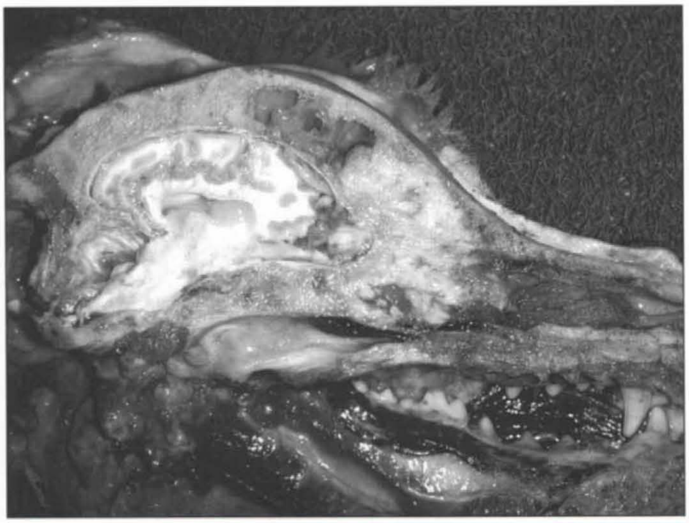

Figure 5.3 Nasal adenocarcinoma in the ethmoid region has invaded frontal sinus and through cribriform plate into the brain in a dog.

The transitional nasal epithelium in the dog is endowed with a complement of cytochrome P450 monooxygenase enzymes within the smooth endoplasmic reticulum, which may play a role in metabolizing inhaled and circulating xenobiotics in the canine nose. Metabolism of a variety of xenobiotics into reactive intermediate molecules is a well-documented mechanism of neoplastic transformation in other organs, and may play a role in canine transitional epithelial nasal carcinogenesis. This is supported by the finding that the expression of cyclooxygenase-2 is increased in the neoplastic epithelial cells.

Nasal adenocarcinomas are common in dogs and cats. They often form papillary fronds and glandular acini (Fig. 5.4). The acini are formed by a single layer of cuboidal to tall columnar cells with a basement membrane, unlike the microcysts of the transitional tumor, which are within a thick layer of tumor cells. Mucin-filled acini are a fairly common feature of adenocarcinomas.

Adenoid cystic carcinomas are rare tumors with a multilobular organization and at least some lobules with a characteristic striking cribriform pattern. These tumors possibly arise from salivary gland tissue in the soft palate rather than from nasal epithelial structures. **Adenosquamous (mucoepidermoid) carcinomas** have mucin-filled acinar components and foci with squamous differentiation. Both glandular and squamous elements must exhibit criteria for malignancy.

Undifferentiated carcinomas are mostly solid tumors in which there are large packets or nodules of round to polygonal cells with no discernible pattern. This diagnosis is usually made after examination of multiple samples fails to reveal regions with a pattern resembling the more differentiated types of carcinoma discussed above. Special methods are needed to separate these tumors from others such

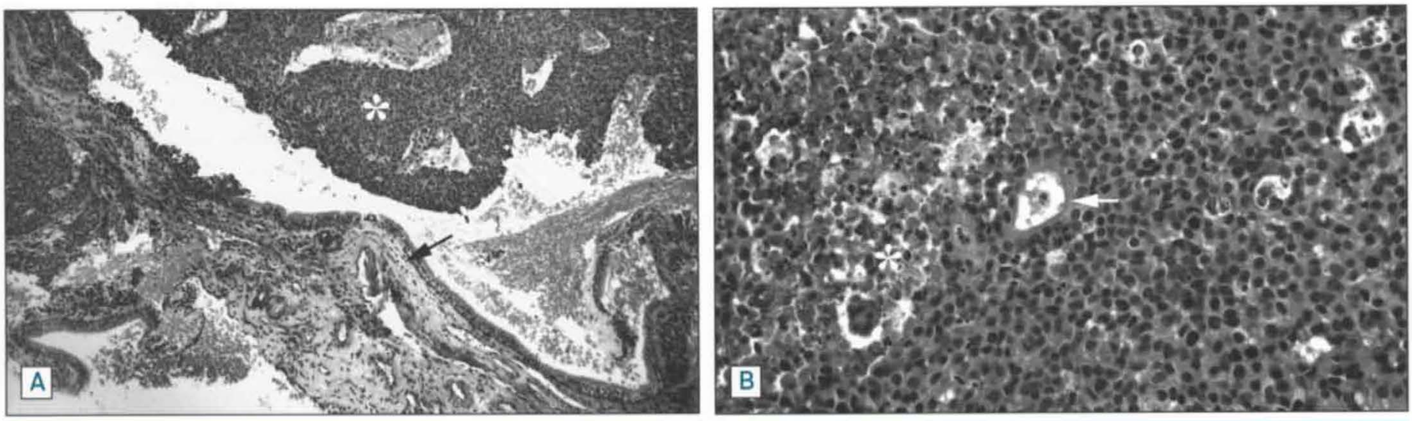

Figure 5.4 Nasal adenocarcinoma, in a dog with a chronic unilateral hemorrhagic nasal discharge; previous biopsies revealed only lymphoplasmacytic rhinitis. **A.** A densely cellular neoplasm (asterisk) contrasts with the normal adjacent nasal mucosa (arrow). **B.** The angular neoplastic cells have a predominantly solid arrangement, with occasional acini (arrow) and areas of necrosis (asterisk).

as olfactory neuroblastoma, amelanotic malignant melanoma, lymphoma, and poorly differentiated mast cell tumor. Carcinomas with mixed phenotypic expression are fairly common, especially if multiple sections are examined; for example, transitional carcinomas sometimes have scattered foci of squamous metaplasia or adenocarcinomatous regions.

Olfactory neuroblastoma (esthesioneuroblastoma, esthesioneuroepithelioma) is a rare neoplasm arising in the ethmoturbinate region of the caudal nasal cavity. Penetration through the cribriform plate and into the cerebral cortex is commonly observed. Most of the reported tumors have occurred in cats and dogs. It is unclear whether they arise from one or more of olfactory neuroepithelial cells, residual neural crest cells, or local components of the dispersed neuroendocrine system. The tumors are highly cellular and consist of a uniform population of round to elongated cells with moderately dense nuclei and small amounts of cytoplasm. *An important diagnostic feature is the presence of palisading around vessels and rosette formation*. In the absence of these features, the olfactory neuroblastoma is not distinguishable on routine examination from lymphoma or undifferentiated carcinoma. Definitive diagnosis may be facilitated by ultrastructural and immunohistochemical examinations. Limited investigation has suggested the presence of *Feline leukemia virus* in feline olfactory neuroblastomas, but it is unknown whether the association is causal.

Neuroendocrine carcinoma of the nasopharynx is formed by a *packeted* arrangement of rounded epithelial cells with indistinct borders, which express synaptophysin and neuron-specific enolase. These tumors may have invasive and metastatic behavior. **Paranasal meningioma** occurs in dogs and horses, and in dogs appears similar to intracranial meningiomas but has a more invasive behavior. **Enzootic nasal tumors** of retroviral etiology are discussed below in the section on Infectious respiratory diseases of sheep and goats. **Nasomaxillary tumors of young horses**, including fibrosarcoma, ossifying fibroma, and fibrous dysplasia, are described elsewhere (see Vol. 1, Bones and joints). Other tumors of the nasal cavity include **papilloma**, **adenoma**, **lymphoma**, **mast cell tumor**, **melanoma**, **transmissible venereal tumor**, and a variety of **sarcomas**.

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PHARYNX, LARYNX, AND TRACHEA

Pharynx

The pharynx, as the site where the respiratory and alimentary systems conjoin, is exposed to both inhaled and ingested materials, some of which may have deleterious effects on the animal. Additionally, the complexity of pharyngeal organogenesis, at the junction between craniofacial development and the respiratory and alimentary systems, predisposes the region to involvement in a variety of congenital malformations. Many of these malformations arise as unintended consequences of selective breeding for desirable craniofacial features in domestic species, especially brachycephalic dogs and cats.

In **pigs**, a *caudal diverticulum of the pharynx lies immediately dorsal to the esophagus*. In young pigs, plant awns and similar foreign materials occasionally lodge in this diverticulum, causing pharyngitis and cellulitis that may lead to dysphagia and death from starvation. Iatrogenic pharyngitis, even with perforation of the caudodorsal wall of the pharynx, may occur in **cattle** during incorrect oral administration of boluses or esophageal intubation.

In young **horses**, it is not uncommon to find prominent lymphoid follicles within the pharyngeal mucosa, and in most instances this should be considered an incidental finding as these follicles are part of the normal pharyngeal mucosal immune system in the horse. **Chronic pharyngitis with lymphoid hyperplasia** is a syndrome in young racehorses that is often detected endoscopically in Thoroughbreds <5 years of age. Polypoid projections of the pharyngeal mucosa may occur in association with the hyperplastic lymphoid follicles. The cause of this syndrome is not known, though it is assumed to represent persistent, excessive local immune stimulation.

The *guttural pouches* of Equidae are ventral diverticula of the auditory tubes. They tend to become involved in inflammatory processes in analogous fashion to the paranasal sinuses. Complications differ, however, because severe guttural pouch inflammation can extend to involve nearby cranial nerves (VII, IX, X, XI, XII), vessels and the cranial sympathetic trunk, or even spread to adjacent bones, middle ear, brain, or atlanto-occipital joint. Suppurative inflammation leading to empyema occurs mostly after upper respiratory infections, particularly with *Streptococcus equi* or other streptococci. Fibrinous or fibrinonecrotic (diphtheritic) inflammation is usually associated with fungal infection, generally *Aspergillus* spp., and hence is commonly referred to as *guttural pouch mycosis*. Fibrinonecrotic inflammation is highly suggestive of, but not pathognomonic for, fungal infection. Because the fibrinonecrotic inflammation extends deeply, and fungi frequently invade vessels and other structures, severe complications are much more likely to follow than from guttural pouch empyema. Examples are thrombosis, aneurysm formation, and rupture of the internal carotid artery with epistaxis, ischemic lesions or osteitis, and fusion of stylohyoid and petrous temporal bones. Guttural pouch hemorrhage may also result from fracture of the basisphenoid bone. A less common condition is *guttural pouch tympany*. This is mostly seen in young animals, and the accumulation of air is presumed to be due to valvular action of the nasopharyngeal orifice of the eustachian tube. *Tumors of the guttural pouches* are rare, but when encountered are most likely to be squamous cell carcinomas. Pharyngeal tumors are discussed with neoplasia of the mouth (see Vol. 2, Alimentary system).

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Larynx and trachea

Congenital anomalies

Congenital anomalies of the larynx are rare, while those of the trachea are relatively common in some domestic species. In dogs, and to a lesser extent cats, selective breeding for brachycephalic features has led to upper-airway obstruction that can cause clinically important respiratory signs. This spectrum of clinical disease in dogs is known as the **brachycephalic airway syndrome**. This syndrome is of lesser importance to pathologists, as tissues are not usually submitted for analysis. Insufficient pharyngeal space for the soft palate in these breeds (elongation of the soft palate) is the most significant anatomical feature contributing to the brachycephalic airway syndrome. Other common laryngeal diseases in the dog include *eversion of laryngeal saccules* and *laryngeal collapse*.

Tracheal hypoplasia is found primarily in brachycephalic canine breeds, and may or may not be accompanied by additional respiratory or cardiac disease. The hypoplasia of the trachea results in a reduction in the luminal diameter of the entire trachea; *the cartilage rings are typically entire circles*, rather than the C-shaped rings of normal trachea. There is a dearth of information regarding the histopathologic features of hypoplastic trachea vis-à-vis normal trachea in affected dogs.

Tracheal collapse, as a clinical entity, refers to *dorsoventral narrowing of the trachea* resulting in coughing and exercise intolerance. The disease is most common in middle-aged miniature breeds of dogs. The cartilage rings are abnormally shaped and form shallow arcs, and the dorsal trachealis muscle (tracheal membrane) is widened and flaccid. The membrane is thin in uncomplicated cases but becomes thickened when there is superimposed chronic inflammation. Earlier descriptions of the histologic and histochemical features associated with tracheal collapse describe foci of hypocellularity of the hyaline cartilage and areas where cartilage is replaced by fibrous and/or fibrocartilaginous tissue; reduced concentrations of chondroitin sulfate and calcium are associated with the reduction in density of chondrocytes. It remains to be determined if these changes are causative or the result of biomechanical changes associated with the tracheal collapse. Investigations into the neural network of the canine trachea reveal a multilayered nervous system that has components throughout the wall of the trachea, including within and around the trachealis muscle; this system might play a role in the physiologic changes associated with tracheal collapse.

Malformations of the cross-sectional shape of the trachea can be found as an incidental finding in nearly any species at the time of necropsy. They are most common in the dog and horse. Tracheal trauma causing fracture of the cartilage may lead to a variety of tracheal ring abnormalities. Compressive inflammatory lesions or neoplasms can also lead to focal tracheal compression with alterations in the tracheal shape.

In the **horse**, *subepiglottic cysts* are believed to arise from thyroglossal duct remnants. *Entrapment of the epiglottis* below the aryepiglottic fold is usually associated with congenital hypoplasia or acquired shortening or distortion of the epiglottis. A short epiglottis also predisposes to dorsal displacement of the soft palate and these conditions sometimes occur together. *Persistent frenulum of the epiglottis* has been reported in newborn foals, predisposing to oronasal reflux of milk after nursing and aspiration pneumonia. The persistent frenulum of the epiglottis leads to dorsal displacement of the soft palate, which can be assessed endoscopically.

Laryngeal paralysis

In domestic species, laryngeal paralysis is most commonly reported in dogs and horses. Unilateral or bilateral paralysis of the larynx is the most common cause of abnormal respiratory noise (*roaring*) in horses, while the condition in dogs is typically bilateral and results in abnormal barking and exercise intolerance. In **horses**, *the condition is almost always a left-sided hemiplegia due to idiopathic degeneration of the left recurrent laryngeal nerve*. The resulting denervation atrophy affects the intrinsic laryngeal muscles supplied by this nerve, but the atrophy is not uniform; the laryngeal adductor muscles, especially the lateral cricoarytenoid muscle, are affected first, followed by the abductor muscles. Grossly, the affected muscles are pale and atrophic, and the cricoarytenoid muscle may be reduced to only the fascial remnants. The cricothyroid muscle, which is supplied by the cranial laryngeal nerve, is the only intrinsic muscle not affected. The dorsal cricoarytenoid muscle is the main abductor of the larynx through its insertion on the muscular process of the arytenoid cartilage and, hence, is largely responsible for laryngeal opening during respiration. When denervation atrophy and dysfunction affect the dorsal cricoarytenoid muscle, the left arytenoid cartilage sags into the lumen and obstructs airflow during inspiration, particularly during intense exercise.

Microscopic examination of affected nerve fibers reveals *severe loss of myelinated fibers in middle and distal portions of the left recurrent laryngeal nerve*. Ultrastructural features indicate progressive loss of fibers in the left recurrent nerve accompanied by chronic demyelination, remyelination, and abortive regenerative attempts. Similar but milder changes can be detected ultrastructurally in the distal right recurrent nerve. The reasons for the axonal disease are still disputed. The axons in the left recurrent laryngeal nerve are much longer than those in the right recurrent nerve and this presumably makes them more susceptible to damage. The extent to which damage is caused by traumatic interruption of axoplasmic flow, neuritis by extension from guttural pouch disease, vitamin deficiency, or neurotoxins has still to be established. Idiopathic laryngeal paralysis – the most common form encountered – is typically unilateral, whereas bilateral paralysis is more typical of cases caused by hepatic encephalopathy and general anesthesia.

In **dogs**, laryngeal paralysis occurs most commonly in older, large or giant breed, males. The disease is usually bilateral. While the cause is often not identified, the relationship between recurrent laryngeal axonopathy and denervation atrophy of the laryngeal muscles is usually as described in horses. In dogs, unlike horses, laryngeal paralysis may be a single manifestation of generalized neuromuscular disease. Some breeds of dogs are predisposed to developing laryngeal paralysis, including Bouvier des Flandres and mixed Husky–Malamute puppies, where it is associated with apparent hereditary degeneration of the nucleus ambiguus; Rottweilers, as a manifestation of a polyneuropathy; and white-coated German Shepherd Dogs. Occasionally, laryngeal paralysis occurs secondary to hypothyroidism or following general anesthesia. In dogs, laryngeal paralysis may predispose to aspiration pneumonia.

Circulatory disturbances

As in the other regions of the upper airways, the mucosa of the larynx is often congested at the time of necropsy, and mucosal petechiae may be present; these findings are rarely of any significance. Laryngeal hemorrhages affecting the mucous membrane on the dorsal surface of the epiglottis occur in septicemia. They are of some diagnostic significance in salmonellosis of swine and classical swine fever.

Laryngeal edema may be part of a local or systemic inflammatory process, and can be caused by infectious laryngitis (see section on Infectious diseases of the respiratory system, below), inhalation of irritant materials, local trauma, anaphylaxis, or hyperthermia. The amount of edema varies, but is most severe in the region of the epiglottis, the aryepiglottic folds, and the ventricles.

Tracheal edema and hemorrhage syndrome of feedlot cattle, in which the lumen is partially obstructed by severe mucosal and submucosal edema of the dorsal region of the distal half of the trachea (Fig. 5.5), occasionally causes death by asphyxiation in feedlot cattle. The loud inspiratory noise made by severely affected animals has given rise to the clinical term "honker syndrome." The cause of this syndrome is not known, but the increased prevalence during hot weather and the frequent presence of concurrent bronchopneumonia suggest that hyperpnea with negative intratracheal pressures during inspiration may cause mechanical injury to the tracheal mucosa.

Laryngitis and tracheitis

The location of the larynx and trachea is such that frequently they become inflamed as part of inflammatory diseases of either the upper or lower parts of the respiratory tract. Tracheitis frequently accompanies bronchitis and bronchopneumonia. Laryngitis can, however, occur without wider involvement of the respiratory tract. Laryngitis can occur as a part of *oral necrobacillosis* (calf diphtheria) caused by *Fusobacterium necrophorum* in calves and swine or it may occur without lesions elsewhere (Fig. 5.6). *Laryngeal ulcers* occur in feedlot cattle with bronchopneumonia, and probably represent mucosal damage by the repeated trauma of laryngeal closure in dyspneic animals. *Laryngeal infarcts* with ulceration may overlie lesions of septic phlebitis in calves with *Histophilus somni* septicemia and pneumonia.

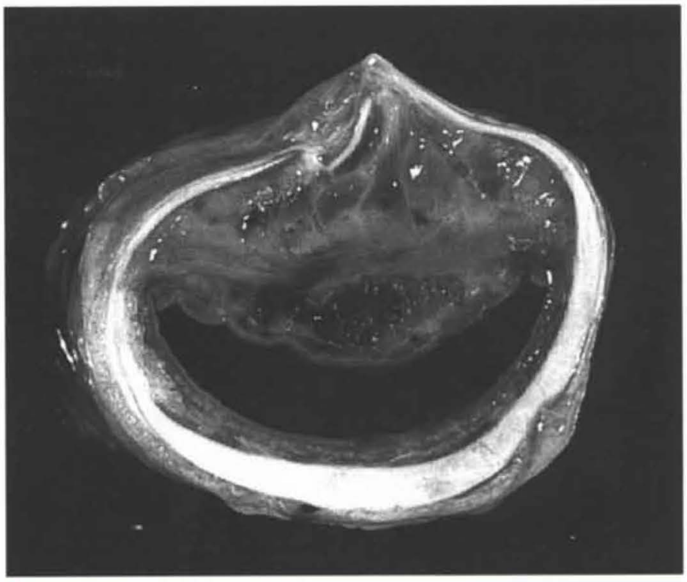

Figure 5.5 Tracheal edema and hemorrhage syndrome in a feedlot calf. Segmental thickening of the tracheal mucosa partially obstructs the lumen. (Courtesy of EG Clark.)

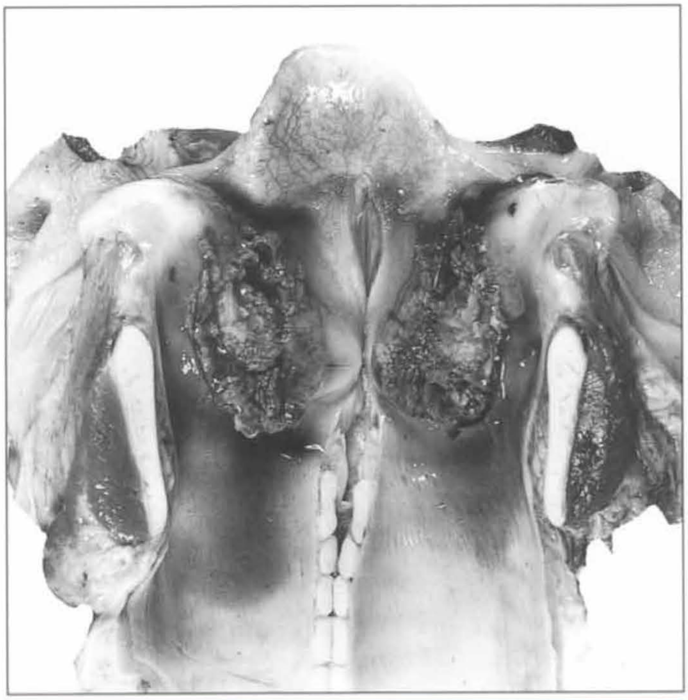

Figure 5.6 Necrotic laryngitis in a calf.

Laryngeal chondritis (*arytenoid chondropathy*) occurs in young horses, as well as sheep and calves. It is characterized by ulceration of mucosa at the rostral margin of the arytenoid cartilage, immediately caudal to the attachment of the vocal fold. The lesions are variable and may consist of a granuloma, purulent tract, or noninflammatory necrosis of the cartilage. Inflamed lesions may result in laryngeal edema that obstructs airflow. The pathogenesis is not known, but may involve mucosal trauma as the larynx closes forcefully in rapidly breathing animals.

Small foci of *mineralization*, often with accompanying granulomatous inflammation, occur in the lamina propria of the dorsal trachea and ventral turbinates of adult pigs. The cause is not known. Foci of chronic polypoid tracheitis are occasionally observed in dogs and cats. The thickening may be sufficient to cause significant stenosis and dyspnea. The cause is unknown but the various pathogenetic factors involved are probably similar to those responsible for nasal polyps. *Squamous metaplasia* of tracheal epithelium is a feature of vitamin A deficiency and severe iodide toxicosis. *Parasitic diseases* of the larynx and trachea, discussed later in the chapter, include *Mammomonogamus laryngeus* in the larynx of cattle, and *Eucoleus aerophilus, Oslerus osleri*, and *Spirocerca lupi* in the trachea of dogs.

Neoplastic diseases of larynx and trachea

Primary neoplasms of the larynx and trachea are rare in domestic species. Papillomas and squamous cell carcinomas are the most common epithelial tumor; papillomas involving the larynx are usually part of widespread papillomatosis, as may be seen in immune-compromised animals. **Tracheal chondroma and osteochondroma** is an uncommon condition of young dogs, and may represent a dysplastic cartilaginous mass rather than a true neoplasm. It forms an expansile mass of disorganized cartilage, with ossification of the central core of some lesions. Rare tumors reported to affect the larynx or trachea include adenocarcinoma, rhabdomyoma, rhabdomyosarcoma, chondroma, osteochondroma, chondrosarcoma, osteosarcoma, leiomyosarcoma, and mast cell tumor. Mucosal involvement of the larynx and trachea in lymphosarcoma or malignant mast cell tumor is an uncommon occurrence in cats and dogs; deformation or invasion of the larynx by adjacent neoplasms in the thyroid glands or lymph nodes may occur.

Laryngeal rhabdomyoma (formerly known as *oncocytoma* or *granular cell tumor*) occurs in young dogs as a solitary raised nodule in or close to the lateral ventricle of the larynx. It consists of lobular masses of angular pleomorphic cells with abundant, deeply eosinophilic, granular, or foamy cytoplasm. Diagnosis using only light microscopy is difficult and the designation as rhabdomyoma depends on demonstrating *myosin and muscle-specific actin* using immunohistochemistry. Ultrastructurally, there are numerous mitochondria and intermitochondrial glycogen granules.

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LUNGS

Lung development and growth

The lungs develop early in gestation, forming from a ventral diverticulum in the foregut endoderm. The six stages of lung development and growth are the embryonic, pseudoglandular, canalicular, saccular, alveolar, and vascular maturation stages. Important development continues postnatally in most mammalian species.

During the **embryonic** stage of lung growth (30–50 days of gestation in a bovine fetus), the primordial trachea and right and left primary bronchi develop, growing into the surrounding mesenchyme. The growth of the conducting airways occurs primarily in the **pseudoglandular** stage; in most species the airways develop through a series of asymmetric dichotomous branching. Differentiation of conducting airway epithelial cells begins proximally and extends distally at this stage, with development of ciliated cells, mucous cells, basal cells, and bronchial cartilage. At the end of the pseudoglandular period, all branches of conducting airways have developed and are embedded in mesenchymal stroma. Distal airway branches are lined by cuboidal to columnar epithelial cells that contain abundant cytoplasmic glycogen. During the **canalicular** stage of growth (120–180 days of gestation), vascular "canalization" of the interstitium occurs, with capillaries growing in apposition to the airway epithelium to form the developing air–blood interface. Also during the canalicular stage, there is development of the type II pneumocytes and type I pneumocytes within the newly formed terminal airspaces. Fetal lung development to the **saccular** stage (180–240 days' gestation in the bovine fetus) is important for survival outside of the uterus. Lung volume and gas exchange surface increase markedly, and there is further reduction in mesenchyme between airspaces. The terminal branches of the conducting airways extend through the nascent parenchyma, and conditions are set for alveolar development. Small accumulations of elastic fibers, the site of secondary alveolar septal development, are deposited during this stage. During the **alveolar** stage of lung growth (240–260 days), true alveoli form by ingrowth of septa from intersaccular crests at the sites of elastic fiber deposition. These secondary septa have a doubled capillary layer separated by mesenchymal tissue. The acquisition of a mature air–blood interface, with a single capillary layer, occurs in the final stage of lung development, the stage of **microvascular maturation**. Microvascular maturation occurs after birth. The end-result is a reduction of the interstitium and maturation of the interalveolar wall. Further subdivision of alveolar septa results in continued increases in alveoli and alveolar surface area during a period of rapid postnatal growth. Airways increase in diameter and length through coordinated growth as overall lung volume and weight increase.

Movement of fetal lung fluid is important in directing normal lung development. Fetal respiration and spontaneous contraction of fetal conducting airways facilitate the movement of the fluid. Interestingly, the contraction of fetal airways will occur in lung explants without central neurologic control because of the phasic contraction of the airway smooth muscle. Neural innervation of the developing lung occurs early in development, arising from neural tissue migrating through the fetal mesenchyme. Studies in the fetal pig show that both developing nerves and ganglia are present within the fetal bronchial tree as early as the pseudoglandular stage of development. This cholinergic neural innervation coordinates the fetal airway contractions mentioned above.

The coordination of intrauterine and extrauterine lung development and growth is complex and influenced by both local tissue factors as well as influences originating beyond the developing lung. Local epithelial–mesenchymal interactions, outlined above, are necessary for directing the distal expansion of the lungs; indeed, the formation of the diverticula from the foregut endoderm, branching morphogenesis, and cell differentiation are specifically coordinated by the adjacent lung-specific mesenchyme. In each stage of lung development, a number of diffusible mediators and transcription factors direct organogenesis. Examples include the fibroblast growth factor family, transforming growth factor-β, and the sonic hedgehog-signaling pathway.

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Congenital anomalies

Congenital anomalies of the lung are rare and may reflect abnormal development of the lung bud, pulmonary circulation, or both. Major malformations such as pulmonary agenesis are incompatible with life and are often accompanied by malformations in other organs.

- **Malformations of the lung bud** include pulmonary agenesis, hypoplasia, or duplication; atresia or abnormal positioning of bronchi that makes them susceptible to chronic infection, congenital lobar emphysema, bronchogenic cysts in the lung or mediastinum, congenital cystic adenomatoid malformation, epidermoid cysts, and pulmonary or cartilaginous hamartomas.
- **Vascular anomalies** include atresia of a branch of the pulmonary artery, anomalous pulmonary venous drainage, and pulmonary arteriovenous malformation.
- **Anomalies of both components** include hamartoma and accessory lung (bronchopulmonary sequestration); and the *hypogenetic, or scimitar, lung syndrome*, which is characterized by unilateral pulmonary hypoplasia, atresia or hypoplasia of the pulmonary and systemic arterial supply, and anomalous venous drainage into the vena cava.

Pulmonary hypoplasia *is defined as reduced lung weight, and hypoplastic lungs usually also have reduced numbers of alveoli*. In contrast, the airways generally develop normally. Pulmonary hypoplasia (Fig. 5.7) may accompany malformations in other organs. Its development is closely linked to the presence of lung liquid that is secreted by the pulmonary epithelium, fills the airspaces, and is required for growth of the lung mass. *Pulmonary hyperplasia* is the result of overdistension of the lung, resulting from tracheal obstruction or laryngeal paralysis. In contrast, pulmonary hypoplasia ensues if the lung volume is not maintained. Pulmonary hypoplasia is caused by conditions that compress the lung, including congenital diaphragmatic hernia,

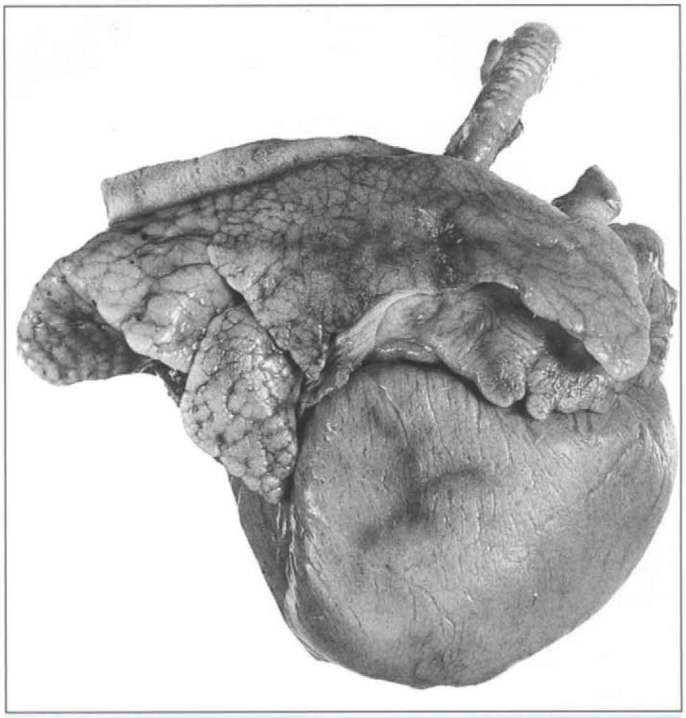

Figure 5.7 Hypoplastic lung in a calf.

intrathoracic masses, or pleural effusions; by oligohydramnios, in which the reduced uterine volume causes abnormal fetal positioning that compresses the chest and lungs; by impaired fetal breathing movements from abnormalities of the fetal nervous or musculoskeletal systems; by tracheal fistula; and perhaps by reduced fluid secretion as a result of fetal hypoxemia or high levels of epinephrine.

Adenomatoid malformation, also known as *bronchial hypoplasia or adenomatoid hamartoma*, is characterized by one or more intrapulmonary masses that are swollen, spongy or cystic, fluid- or air-filled, and lobulated. The masses are formed by adenoma-like proliferation of bronchioles that form cysts varying from microscopic to several centimeters in diameter, which are lined by pseudostratified columnar epithelium. The bronchi are hypoplastic and lack cartilage and smooth muscle in their walls. Alveoli may be normal, or airway obstruction may result in air trapping and enlargement of the alveoli (congenital lobar emphysema).

Pulmonary hamartomas *are tumor-like masses of pulmonary tissue within the lung.* They are often supplied by anomalous branches of the aorta and drained by pulmonary veins, but lack communication with the bronchi or with the pulmonary arteries. Histologically, the development of bronchi, bronchioles, alveoli, and blood vessels is variable; those formed by alveolar tissue without bronchioles have been termed microcystic hamartomas or polyalveolar lobe.

Accessory lungs *are masses of pulmonary tissue completely separated from the lung and are found in the pleural space, mediastinum, or abdominal cavity.* They may contain bronchi that connect to the upper alimentary system (congenital bronchopulmonary foregut malformation), are supplied by systemic but not pulmonary arteries, and have a pleural covering separate from the lung. The microscopic features include bronchial hypoplasia, dilation of bronchioles, and variable development of alveoli.

In **anomalous pulmonary venous drainage**, the pulmonary veins drain into the right rather than the left atrium. Completely (total) or partially anomalous forms differ in whether all of the pulmonary veins are abnormal or not.

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Lung lobe torsion

Lung lobe torsion most often affects the right middle or left cranial lung lobe of dogs, presumably because of the narrow waist at the hilus. Deep-chested breeds – Afghan Hounds in particular – form the bulk of the cases. Other species and other lobes are occasionally affected. Predisposing causes are not apparent in most cases, but a minority of affected dogs have underlying causes such as neoplasia, pneumonia, or atelectasis of the affected lobe, pleural effusion, congenital dysplasia or hypoplasia of the bronchial cartilage, previous thoracic surgery, or pneumothorax. The clinical signs are variable and include respiratory distress, coughing, and hemoptysis. The affected lobe is twisted at the hilus and deeply congested. Pleural effusion is consistently present and may be transudative, chylous, exudative, or bloody. Histologic lesions vary from simple congestion in acute or partial torsions, to diffuse alveolar damage with fibrinous alveolar exudates, to pulmonary infarction with coagulative necrosis of all tissue elements.

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Atelectasis

Atelectasis *refers to incomplete expansion of the lung.* Atelectatic lung is homogeneously dark-red and sunken relative to aerated lung, and the texture is fleshy or firmer and nonspongy relative to normal lung. Microscopically, simple atelectasis appears as slightly congested alveolar walls lying in close apposition with slit-like residual lumina having sharp angular ends (Fig. 5.8). Atelectasis is a common artifact in immersion-fixed lung tissue, so that each alveolar septum falsely appears to have two or more lines of erythrocyte-filled capillaries. Atelectatic alveoli often contain scant edema fluid and excess alveolar macrophages. The edema may result from hypoxic damage, hypoxic vasoconstriction, or reduced surfactant activity.

In **congenital atelectasis**, the lungs appear as in the fetus but are dark red-blue because of dilation of alveolar capillaries. They are of fleshy consistency and do not float. Alveoli are partially distended with fluid and epithelial cells are rounded. Sloughed epithelial cells (squames) from the oronasal regions and amniotic fluid are usually present in the alveolar fluid, possibly with bright yellow particles of meconium. Small numbers of "squames" can be present in the lungs of normal term fetuses but large numbers, especially if meconium is also present, indicate aspiration of amniotic fluid during the exaggerated respiratory movements of the asphyxiated fetus in utero. Patchy congenital atelectasis due to incomplete expansion is usually due to weak respiratory movements caused by asphyxia or damage to respiratory centers in the brainstem. In the neonatal period, it is often not possible to distinguish atelectasis of incomplete expansion and acquired atelectasis of briefly aerated lung.

Acquired atelectasis and **alveolar collapse** are used synonymously. **Obstructive atelectasis** *is caused by complete airway obstruction.*

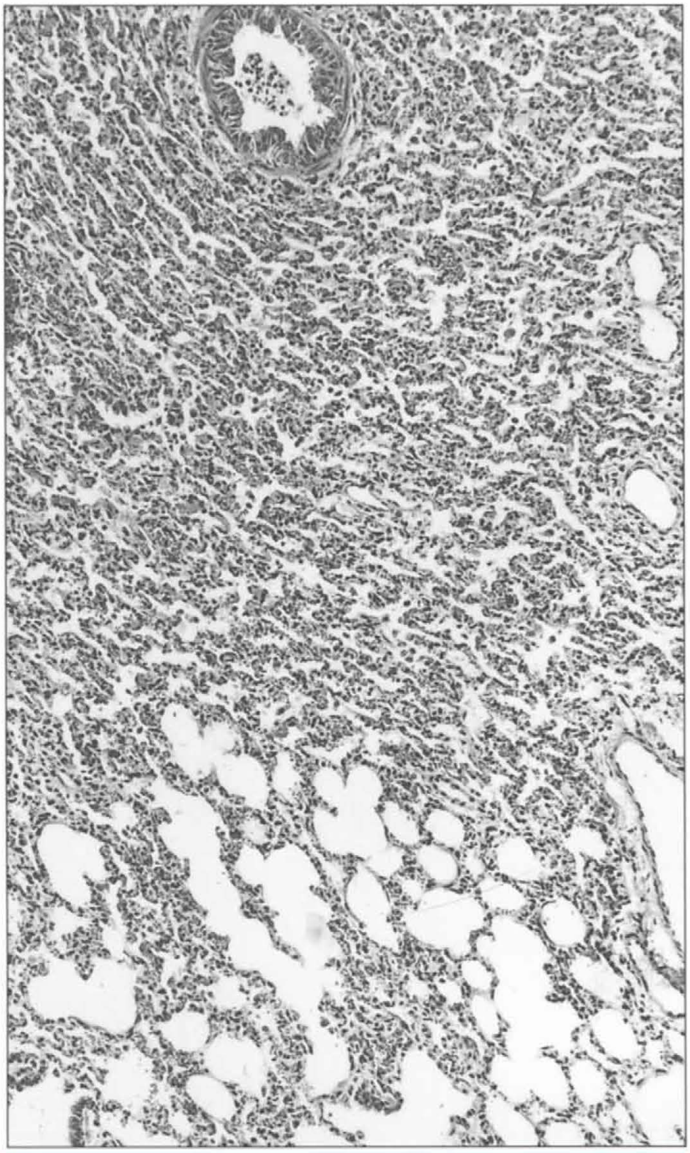

Figure 5.8 Atelectasis of the lung in a lamb.

Whether atelectasis follows obstruction depends on the size of airway obstructed and the degree of collateral ventilation. Complete blockage of lobar or segmental bronchi is necessary for atelectasis in the dog and cat where collateral ventilation is extensive. Blockage of small bronchi or even bronchioles can result in atelectasis in bovine lungs where there is insignificant collateral ventilation. Atelectasis is more likely to develop in dependent lung regions where alveoli are smallest and airways most easily compressed. Evidence of bronchial obstruction by exudate, parasites, aspirated foreign material, granulomas, or tumors can often be seen grossly, and the atelectatic lesions often follow a lobular pattern.

Compressive atelectasis *is caused by pleural or intrapulmonary space-occupying lesions.* Examples are hydrothorax, hemothorax, exudative pleuritis, and mediastinal and pulmonary tumors. In large animals, the atelectasis caused by pleural effusions often occurs below a sharply demarcated fluid line. Abdominal distension, as in severe ascites and ruminal tympany, may cause partial atelectasis, typically in the cranial regions where ventilatory movements are most easily compromised by intra-abdominal pressure. Large animals with prolonged recumbency, such as anesthesia, may develop atelectasis of the down side. The contributing factors are shallow amplitude of respiration causing impaired ventilation of dependent lung, gradual loss of surfactant activity, and pooling of secretions in the lower airways.

Massive atelectasis is mostly seen as a *sequel to pneumothorax.* What appears to be total atelectasis is seen in animals that die while breathing 80–100% oxygen during anesthesia or intensive care. Because of the speed with which the oxygen is resorbed into the tissues and the lack of nonabsorbable gas (especially nitrogen, as is present within atmospheric air), *the lungs are usually completely devoid of gas by the time they are examined after death.* Grossly, the entire lung field is markedly reduced in size; because of this, the heart will look especially prominent when the pluck is examined.

Pulmonary emphysema

Emphysema in its widest sense refers to tissue expansion by air or other gas. In the lung there are two major forms:

1. **Alveolar (vesicular) emphysema** refers to abnormal and permanent enlargement of alveoli, accompanied by destruction of their walls and an absence of obvious fibrosis. Emphysema thus differs from hyperinflation or overdistension of the alveoli, which is a reversible lesion.
2. **Interstitial emphysema** is the presence of air within interlobular, subpleural, and other major interstitial zones of the lung. *"Emphysema," unless otherwise qualified, should only be used for alveolar emphysema.*

Emphysematous lungs are grossly voluminous, pale, and puffy. When the lesion is diffuse, the lungs fill the thoracic cavity and may bear imprints of the ribs. The enlarged airspaces are often visible as small vesicles, and in severe cases coalescence of airspaces can produce large air-filled bullae. Emphysematous bullae occasionally rupture to cause fatal pneumothorax. Ruptured bullae are notoriously inconspicuous, and their detection is facilitated by submerging the lung in water or saline before inflating it with air, or by filling the trachea with saline to detect pleural leaks.

Enlargement and coalescence of airspaces are apparent histologically, and are most reliably assessed on lungs that have been infused with fixative to a volume approximating the in vivo state. Scanning electron microscopy, which dramatically reveals the moth-eaten appearance (Fig. 5.9), is best for visualization of early lesions. The emphysema may affect the terminal bronchioles and adjacent alveoli (*centrilobular or centriacinar emphysema*), or more uniformly involve the entire lobule (*panlobular or panacinar*). Less important forms are *paraseptal emphysema*, which affects distal alveoli bordering interlobular septa or pleura, and *irregular or paracicatricial emphysema*, which results from distortion of airspaces by adjacent contracted scar tissue.

Current knowledge of the **pathogenesis of emphysema** is based on investigations of the human disease and of animal models. Emphysema is an important condition in humans, where it frequently coexists with chronic obstructive pulmonary disease attributable to cigarette smoking and is the result of an imbalance between proteases and antiproteases. *Neutrophil-derived serine proteases – particularly elastase – and matrix metalloproteinases from a variety of sources are the likely culprits*, and their concentrations are enhanced by the neutrophil and macrophage activation induced by chronic bronchitis. Although

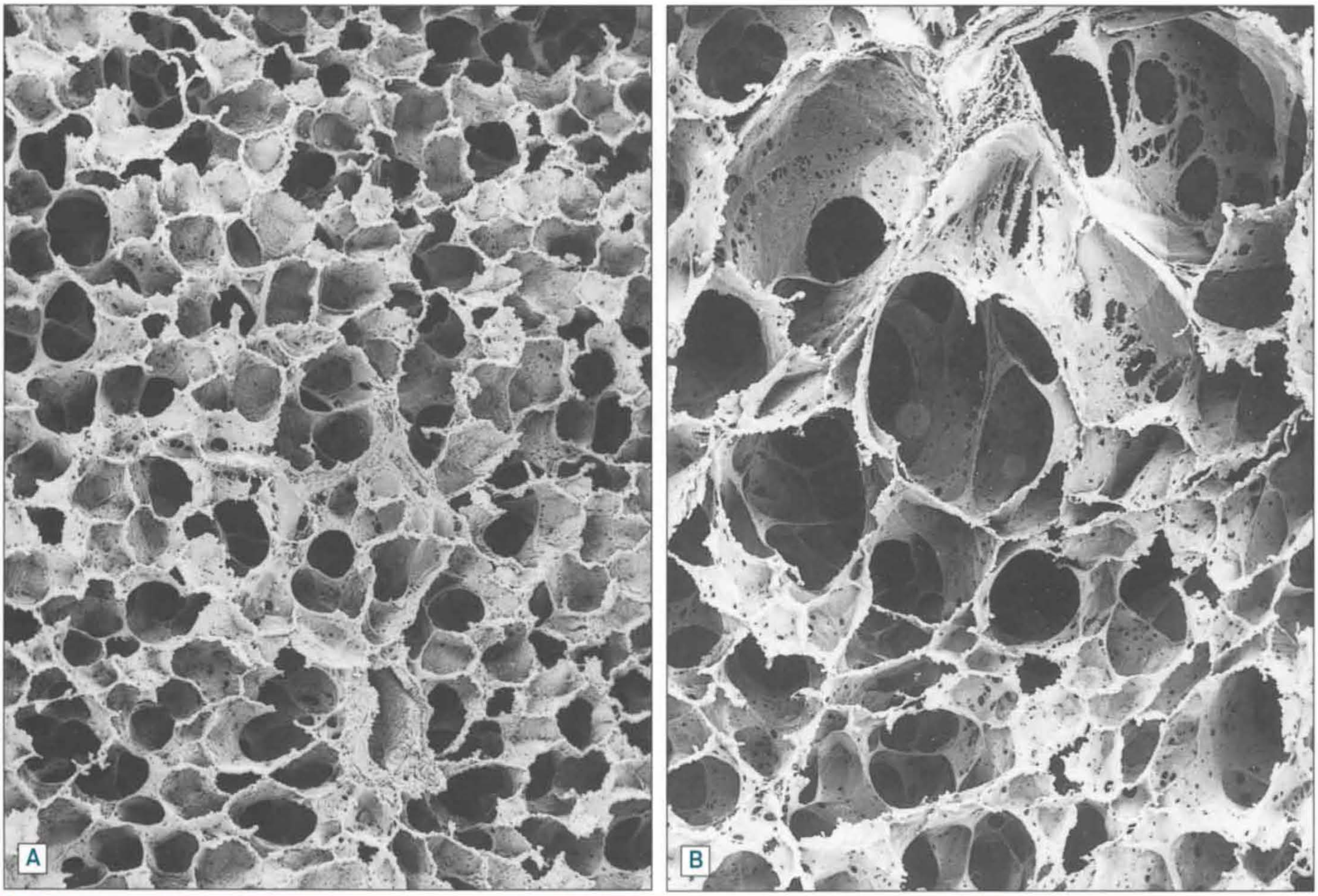

Figure 5.9 Scanning electron micrographs. **A. Normal** equine lung. **B. Emphysematous lung**. Note alveolar enlargement and alveolar wall destruction.

antiproteases – α_1-antitrypsin, secretory leukoprotease inhibitor, and tissue inhibitors of matrix metalloproteinases – protect normal lung from proteolytic damage, their function may be reduced by genetic deficiency or by oxidative stress from cigarette smoke and the resulting inflammation. Unchecked proteases degrade elastin and other matrix proteins in the interstitium, and the weakened alveolar septa are vulnerable to stress-induced failure with the tensions generated by each ventilatory cycle.

The emphysematous lung is dysfunctional. The loss of alveolar septa reduces the alveolar surface area; although the affected portion of lung may be larger than normal, gas exchange is reduced. Loss of elastic recoil caused by degradation of elastin further compromises lung function. These factors make hyperpnea necessary to maintain lung function, which may exacerbate the disease by placing additional ventilatory stresses on the damaged lung.

Emphysema is of much less clinical significance in animals. It occurs in horses with heaves. Lobar emphysema may be congenital (usually caused by hypoplasia of bronchial cartilage) or acquired (due to bronchial mucous plugs and lung lobe torsion). **Congenital lobar emphysema** is rare in dogs, and usually results from aplasia or hypoplasia of bronchial cartilage. The right middle or cranial lung lobes are greatly enlarged by alveolar and/or bullous emphysema, sometimes with bronchiectasis. Pneumomediastinum, subcutaneous edema, or pneumothorax may occur. Histologically, alveoli are expanded and the alveolar septa are thickened by fibrous tissue. Affected dogs typically have slowly progressive dyspnea and coughing, and surgical excision is usually curative. **Multifocal bullous emphysema** also occurs in dogs, and may have extensive alveolar hemorrhage with alveolar and interstitial fibrosis.

Interstitial emphysema is distinguished from alveolar emphysema by the presence of air in the connective tissues and lymphatics of the lung, including the interlobular septa, beneath the pleura, and around vessels and airways (Fig. 5.10). Positive-pressure ventilation with excessively high tidal volume is an occasional cause in all species. Interstitial emphysema without alveolar emphysema occurs frequently in cattle, presumably because of their well-developed interlobular septa and lack of collateral ventilation of the alveoli. It is an incidental finding in cattle at slaughter, and is common in recumbent ("downer") cows with periparturient paresis or toxic mastitis, where it may develop as cows forcibly exhale or grunt against a closed glottis. Thus, *the finding of interstitial emphysema in cattle does not necessarily imply primary lung disease.* Dramatic interstitial emphysema occurs in cattle with interstitial pneumonia caused by 3-methylindole toxicity, other toxicants, or pneumonia caused by *Bovine respiratory syncytial virus*. Interstitial emphysema in such cases may result from abnormally high intra-alveolar pressures caused by increased expiratory effort or bronchiolar obstruction, or perhaps from altered permeability or strength of the barrier

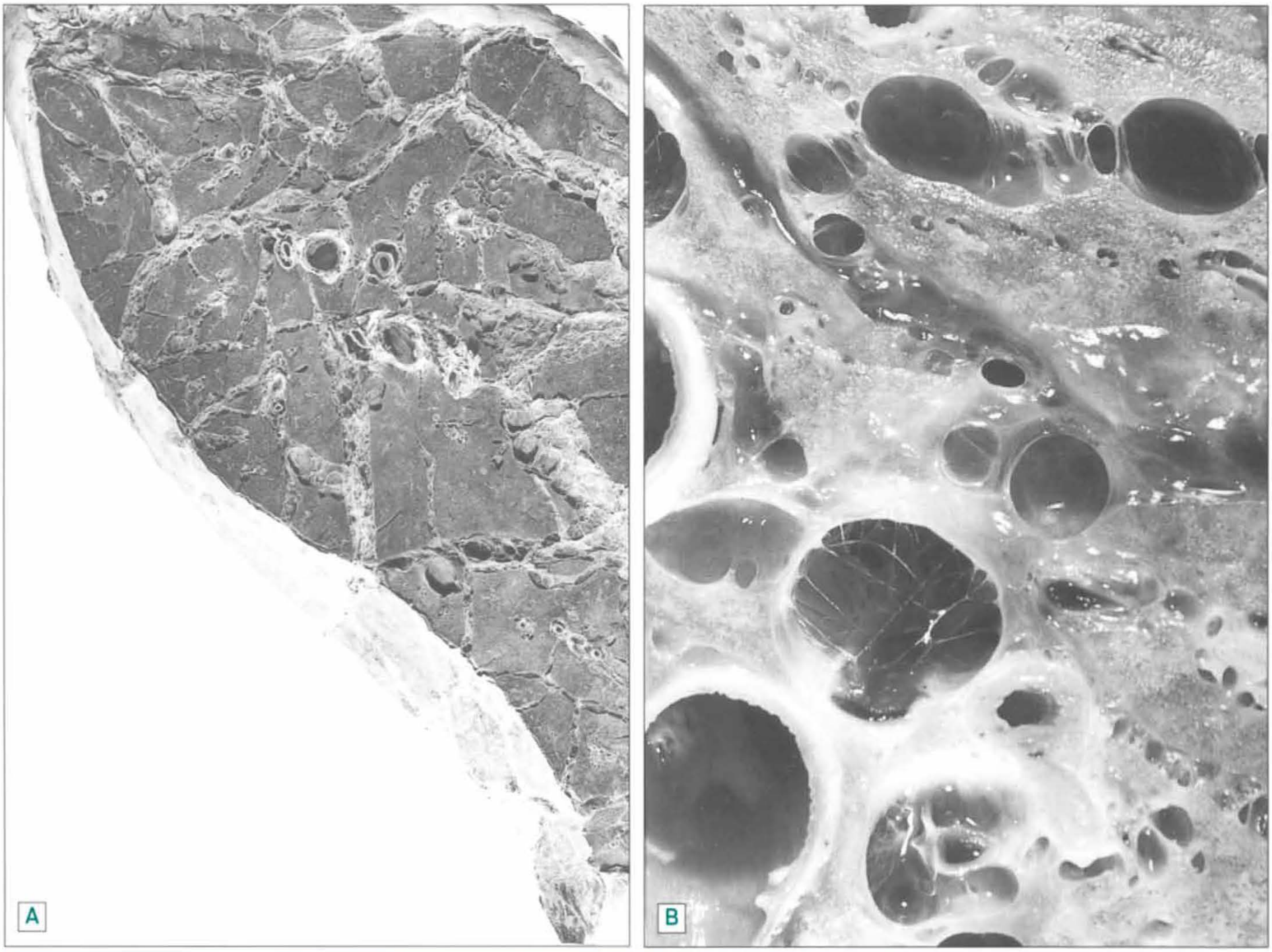

Figure 5.10 A, B. Interstitial emphysema in a calf, secondary to acute interstitial pneumonia. Note bubbles of air in interlobular septa.

between airspaces and interstitium. In animals surviving sufficient length of time with severe interstitial emphysema, the air can extend along lymphatics to the bronchial and mediastinal lymph nodes or along fascial planes of the mediastinum to beneath the skin of the back.

Overdistension of alveoli is a commonly encountered finding at necropsy, although this does not qualify as emphysema unless there is destruction of alveolar septa. These lesions mostly result from airway obstruction or spasm, with air trapping in alveoli and failure of the lung to deflate normally. Distended alveoli can be found at necropsy in the apices and along the sharp ventral border of the lungs of dogs, cats, and horses (Fig. 5.11), and along the margin of a consolidated lung.

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Circulatory disturbances of the lung

Given the fact that all blood must pass through the lungs, and that perfusion is of such critical importance to pulmonary gas exchange, it is not surprising that the lung is a common target for a variety of circulatory disturbances. Active **hyperemia** is part of the acute inflammatory response and is a feature of acute pulmonary injury

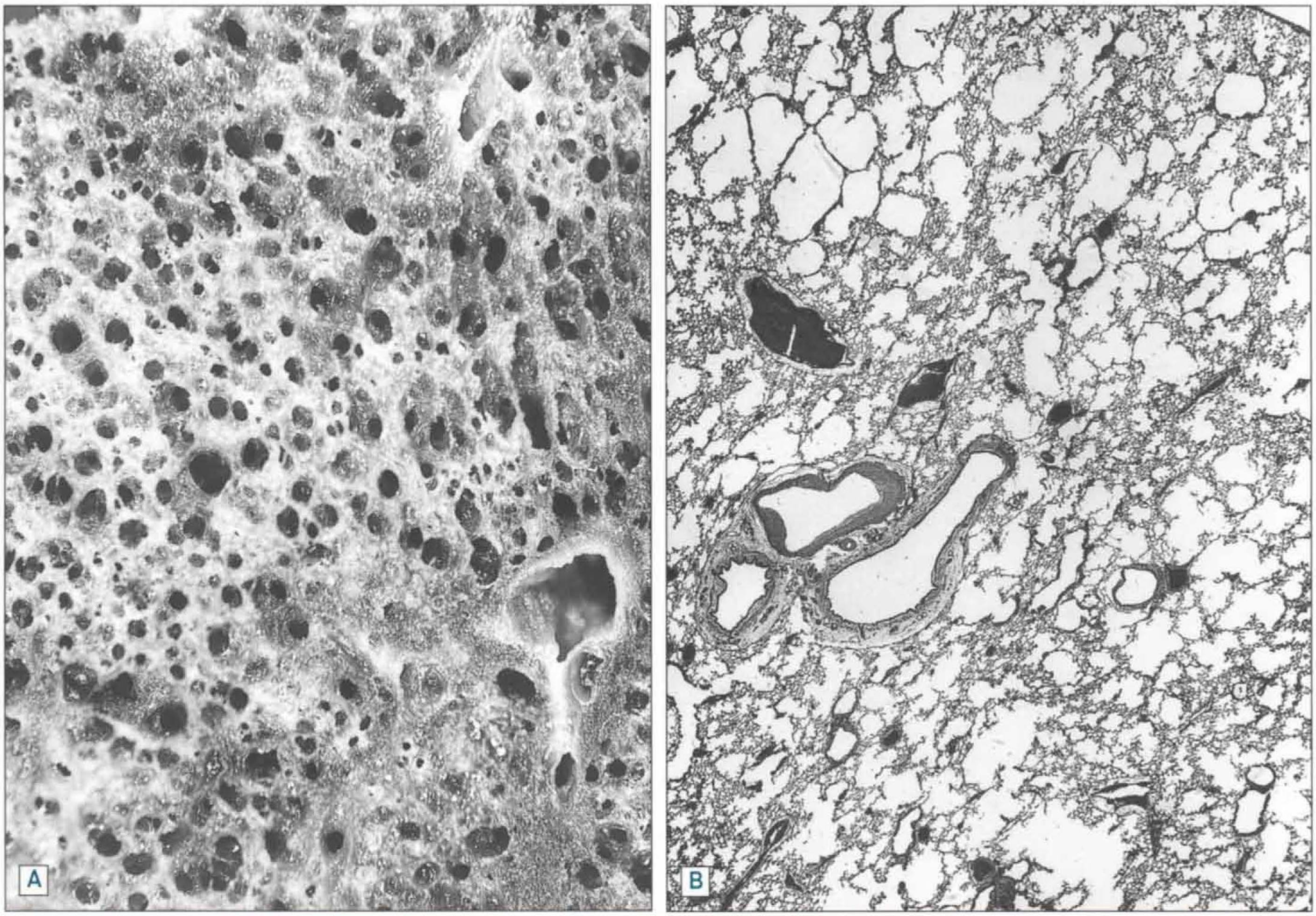

Figure 5.11 Emphysema in the lung of a dog. **A.** Subgross. **B.** Histology.

of many types. Pulmonary **congestion** is most commonly caused by left heart failure and inflammation. However, since hypostatic or diffuse pulmonary congestion is such a common incidental finding at necropsy, it should be accorded little diagnostic significance unless accompanied by edema or firmness of the tissue. *The color of lung tissue is often deceptive; increased texture is a more reliable indicator of disease.*

Histologically, the presence of *hemosiderin-laden macrophages* (**"heart failure" cells)**, which are best detected with Perls's stain for iron, or *erythrophagocytosis* are useful indicators of chronic congestive heart failure or of chronic pulmonary hemorrhage (Fig. 5.12), but their absence does not rule out acute heart failure. Additional findings in chronic heart failure include: hypertrophy of the tunica media of small pulmonary vessels due to pulmonary hypertension, vascular adventitial edema, alveolar interstitial fibrosis that often forms concentric rings around small blood vessels, or rarely diffuse alveolar damage with protein-rich edema.

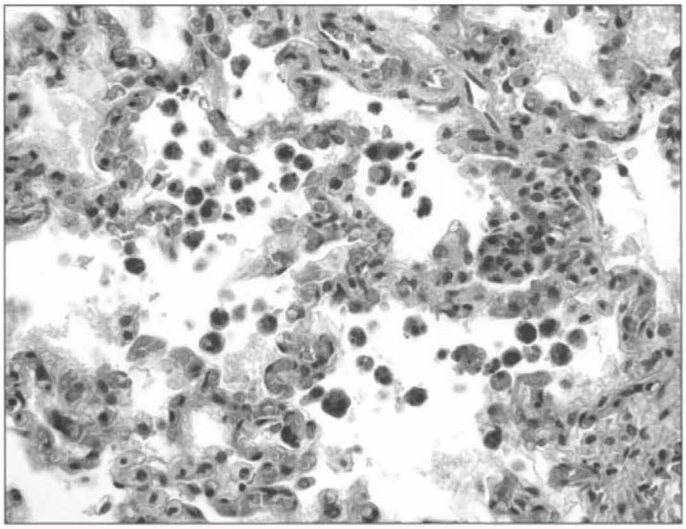

Figure 5.12 Chronic pulmonary congestion due to left heart failure in a dog. Alveoli contain increased numbers of macrophages, with hemosiderin in their cytoplasm (**heart failure cells**).

Pulmonary edema

Pulmonary edema is a frequent complication of many diseases and is therefore one of the most commonly encountered pulmonary abnormalities. If severe, pulmonary edema has a catastrophic effect on lung function by reducing pulmonary compliance, blocking ventilation of the alveoli, obstructing gas exchange across the alveolar septa, and reducing the surface area of the air–liquid interface in the alveoli. In addition, proteins present in the edema fluid interfere with surfactant function, further reducing compliance and contributing to pulmonary dysfunction. Edema of the lung is, in many respects, similar to edema of other tissues, and is governed by the permeability of the vascular wall and by Starling forces – the balance of hydrostatic and osmotic pressures between the intravascular and interstitial compartments. Distinctive aspects in the lung include the importance of type I pneumocytes as barriers to fluid movement, the role of type II pneumocytes and Clara cells in active transport of water from the alveolus, the effect of surface tension on fluid movement, and fluid exchanges between the alveolar and bronchiolar airspaces and the pulmonary interstitium. It is useful to consider separately the means by which the alveoli are kept dry, and the ways in which excessive fluid is drained from the alveoli.

The alveoli are kept dry by several related mechanisms. First, the tight junctions between alveolar type I pneumocytes are relatively impermeable to fluid and proteins, and confer 92% of the barrier to albumin flux across the blood–air barrier. In contrast, the capillary endothelium is a less effective barrier to movement of fluid and small molecules. Complementing this barrier function are Na^+-K^+-ATPase channels (sodium pumps) on the basolateral surface and passive sodium channels on the apical surface of type II pneumocytes. In addition, the surface tension in airways may draw fluid out of alveoli into distal bronchioles, where fluid resorption by Clara cells is driven by a similar bevy of ion channels. The net effect is actively to transport sodium out of the distal airspaces into the interstitium and to force water along the same pathway by osmosis, thus resulting in a slow but steady flow of liquid from the alveoli to the interstitium. Drainage of interstitial fluid from the interstitium to the lymphatics is facilitated by negative pressures in the lymphatic vessels. The rate at which fluid is cleared from the distal airspaces varies by species, being slowest in dogs, intermediate in sheep, and fastest in rodents and rabbits.

Active transport of alveolar fluid is enhanced by catecholamines (through β_2-adrenergic receptors) and glucocorticoids; this is probably important in clearance of fluid from the lungs of neonates, and is also used to advantage in the therapy of pulmonary edema. Conversely, these active transport mechanisms are impaired by hypoxia (as a result of high altitude), reactive oxygen and nitrogen species (as a result of inflammation), halogenated anesthetics, oleic acid, and perhaps lidocaine, suggesting that these factors – in addition to damage to type II pneumocytes or Clara cells – are additional contributors to development of pulmonary edema.

Causes of pulmonary edema *include elevated hydrostatic pressure, increased permeability of the alveolar wall, and several miscellaneous causes* (Table 5.2). *Elevated hydrostatic pressure* is commonly caused by elevated left atrial pressure in left-sided heart failure (*cardiogenic edema*), or less often from hypervolemia due to excessive fluid administration. In these situations, fluid loss into the alveoli may occur from either the alveolar septa or the peribronchiolar or perivascular connective tissue.

Table 5.2 Causes of, and contributors to, pulmonary edema

Increased venous hydrostatic pressure
- Left heart failure
- Increased blood volume
- Pulmonary venous occlusion

Increased permeability of the alveolar barrier
- Pneumonia
- Diffuse alveolar damage
- Endotoxemia/septicemia

Impairment of active transport of fluid from distal airspaces
- Damage to type II pneumocytes or Clara cells
- Hypoxia (high altitude)
- Oxygen and nitrogen radicals (increased by inflammation)
- Halogenated anesthetics
- Possibly lidocaine
- Malnutrition
- High alveolar protein content

Reduced oncotic pressure
- Hypoproteinemia (uncommon)

Lymphatic obstruction
- Neoplastic emboli in lymphatics or lymph nodes (rare)

Neurogenic pulmonary edema, secondary to brain trauma

Acute upper-airway obstruction
- Strangulation, hanging

Hypoglycemia

Increased permeability of the blood–alveolar barrier (noncardiogenic edema) is the second major mechanism of pulmonary edema, and is typified by a more rapid onset of high-protein edema. This form of edema develops in diffuse alveolar damage (discussed below in the section on interstitial and bronchointerstitial pneumonia), endotoxemia, anaphylaxis, and within the lesions of bacterial pneumonia. In the case of diffuse alveolar damage, edema may result from more minor injury than is needed to cause formation of hyaline membranes. Fumonisin toxicity in swine and *African horsesickness virus* and *Hendra virus* infection in horses are specific causes of spectacular pulmonary edema.

Several *additional causes of pulmonary edema* are uncommonly encountered in veterinary pathology. Hypoproteinemia from glomerular, intestinal, or liver disease may cause pulmonary edema, but edema in other tissues is usually more prominent. Obstruction of lymphatic vessels or lymph nodes by neoplastic cells is a rare cause of pulmonary edema. Increased intracranial pressure from trauma to the central nervous system causes "*neurogenic pulmonary edema*" by obscure means, probably related to both catecholamine-induced pulmonary hypertension and increased vascular permeability. Hypoxia due to high altitude induces pulmonary edema, an effect of both impaired alveolar active sodium transport and pulmonary hypertension. Acute upper-airway obstruction from strangulation or hanging causes pulmonary edema, probably from negative intrathoracic pressures or by similar mechanisms to neurogenic edema.

On **gross examination**, edematous lungs are wet, heavy, and do not completely collapse when the thorax is opened, and fluid oozes from the cut surface. The ratio of lung weight to body weight (or to heart weight in ruminants) is a useful measure of pulmonary edema. Edema is prominent in the pleura and the pulmonary interstitium, and may form shallow pools in the hilus of the lung or the mediastinum. In cattle and swine, the interlobular septa are obviously distended by clear fluid (Fig. 5.13). Foam often fills the trachea and bronchi and flows from the nostrils, although this is a common incidental finding in horses and sheep dying of a variety of causes. The thoracic cavity may contain excess fluid.

Histologically, edema fluid is acidophilic, homogeneous, or faintly granular material filling alveoli, except for occasional discrete holes that represent trapped air bubbles. The same material is often present in interstitial tissue and lymphatics around vessels and airways and in interlobular septa and subpleural zones in those species where these are well developed. Chronic edema is accompanied by a diffuse increase in the number of alveolar macrophages, and in heart failure these may contain phagocytosed erythrocytes or hemosiderin.

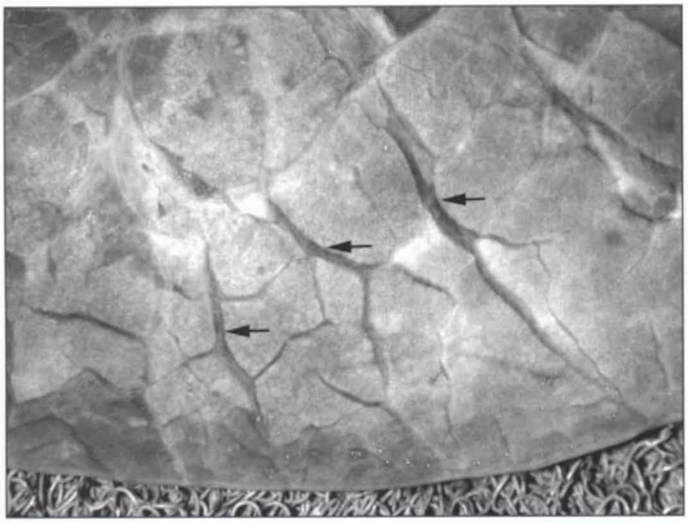

Figure 5.13 Pulmonary edema secondary to heart failure in a pig. Fluid in interlobular septa (arrows) accentuates the lobular pattern.

Table 5.3 Causes of pulmonary thrombosis and embolism

Pulmonary thrombosis

- Glomerulonephritis and glomerular amyloidosis
- Immune-mediated hemolytic anemia
- Hyperadrenocorticism or corticosteroid therapy
- *Dirofilaria immitis, Angiostrongylus vasorum*
- Disseminated intravascular coagulation: sepsis, neoplasia, pancreatitis, peritonitis, burns, massive trauma, bacterial pneumonia
- Heart disease
- Atherosclerosis, pulmonary vasculitis

Sources of emboli

- Valvular endocarditis
- Jugular thrombosis associated with catheterization
- Hepatic abscess with thrombosis of the hepatic artery (cattle)
- Thrombosis of uterine and pelvic veins (cattle)
- Thrombi in deep veins of the legs, in recumbent (downer) cows

Histopathology is neither sensitive nor specific for detection of pulmonary edema: the protein in edema fluid can leach from sections during processing and be quite inconspicuous; conversely, pink material often fills the alveoli in autolysed carcasses or those euthanized with barbiturates. Therefore, gross examination is usually a more accurate indicator of the presence and severity of edema.

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Thrombosis, embolism, and infarction

Pulmonary thrombosis causes acute dyspnea, with open-mouth breathing and air hunger in some cases, and is often rapidly progressive and fatal. The underlying causes include Virchow's triad of increased coagulability of the blood, damage to endothelial cells or the vessel wall, or stagnation of venous blood flow (see Vol. 3, Cardiovascular system). The major causes are listed in Table 5.3. Thrombi that develop in the lung are usually microscopic, whereas grossly visible thrombi generally arise by embolism from a distant site. Microvascular thrombi may dissolve rapidly after death if the fibrinolytic system has been activated, as is expected in disseminated intravascular coagulation.

The lungs are strategically situated to catch **emboli** carried in venous blood, and the most common sources are listed in Table 5.3. *The significance and sequelae of pulmonary thrombosis and embolism depend on the extent of vascular obstruction, the rapidity of its development, and the presence of sepsis.* Minor thrombi or emboli may be inconsequential and not cause infarction, because the lung is supplied by both pulmonary and bronchial arteries and has extensive collateral channels. Pleural effusion is often noted, even when infarcts are absent. Emboli may cause alveolar hemorrhage without apparent necrosis of the lung tissue. Ischemia and infarction are more likely if thrombosis or embolism occurs at the periphery of the lung (rather than affecting the large central vessels), or if the bronchial or systemic circulation is also impaired, for example by concurrent heart failure. When they occur, infarcts form wedge-shaped, raised, firm, red-blue, hemorrhagic lesions at the periphery of the lung lobes, and fibrinous exudate may give the pleural surface a dull granular appearance. Emboli that obstruct large branches of the pulmonary artery may cause sudden death due to cor pulmonale and acute right heart failure; right ventricular dilation may or may not be present. Large thrombi are best detected by dissection of the major branches of the pulmonary arteries, as these functionally catastrophic lesions may be easily missed if only cross-sections of lung are examined.

Septic embolism with massive numbers of bacteria, as occurs when an abscess ruptures into a vein, induces acute pulmonary edema or interstitial pneumonia. Septic emboli that are less fulminant result in multifocal infarcts, abscesses, or chronic suppurative embolic pneumonia.

Fat embolism is rarely important in animals. The fat can originate from bone marrow at sites of fracture and from severe hepatic lipidosis when the hepatocytes rupture. The emboli lodge in alveolar capillaries and produce sausage-shaped distensions that are empty in routine paraffin sections. **Megakaryocytes** are frequently found in pulmonary capillaries, particularly in dogs. A small number of megakaryocytes derived from bone marrow are present in circulating blood and lodge in the lungs, where they continue to produce platelets. This is a normal occurrence, but may be accentuated when there is compensatory extramedullary hematopoiesis in spleen and liver. **Emboli of brain tissue** may be encountered in cattle when captive bolt guns are used with excessive vigor and induce marked increases in intracranial pressures.

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Pulmonary hemorrhage

Pulmonary hemorrhages vary from petechiation to massive filling of large regions by blood. It is usually possible to distinguish hemorrhage from congestion based on the gross appearance: *hemorrhages are usually multifocal or patchy, whereas congestion is diffuse within the affected region of the lung*. Pulmonary hemorrhages occur frequently in the hemorrhagic diatheses, septicemias, disseminated intravascular coagulation, and severe congestion. They can also be caused by vasculitis, infarction, ruptured aneurysms, trauma, hemangiosarcoma, tumors that have undergone necrosis, or drug reactions. Aspiration of blood is frequent at slaughter, and has a characteristic pattern of multiple, small, bright-red foci with feathery or indistinct borders.

Abscesses *that erode large blood vessels may cause massive hemorrhage*. Affected animals may develop hemoptysis or be found dead with blood flowing from the nares, and expectorated blood is often detected in the stomach. They are most common in cattle in which liver abscesses erode the hepatic vein to cause pulmonary thromboembolism and lung abscesses.

Exercise-induced pulmonary hemorrhage *is common in racing horses*. Pulmonary hemorrhage can be detected in many racing horses following strenuous exertion, although epistaxis – blood at the nostrils – is apparent in only 1–10%. Examination of Perls' Prussian blue-stained preparations of alveolar macrophages reveals iron/hemosiderin in nearly all racing horses, suggesting that mild hemorrhage occurs in most or all racing horses during strenuous exercise. The frequency of exercise-induced hemorrhage increases with age and severity of exertion. The hemorrhage is probably the result of the marked elevations in pulmonary arterial and capillary pressures that occur during strenuous exercise, leading to stress-induced failure of capillary integrity. Bronchopulmonary anastomoses or uneven ventilation of neighboring lung lobules may further promote hemorrhage, but definitive confirmation of their importance is lacking.

The hemorrhage occurs in the dorsocaudal portions of both caudal lobes, and appears as red, brown, or gray discoloration of the lung or as patchy, blue-brown subpleural foci. Histologically, the main features in affected regions are hemosiderin-laden macrophages in airspaces and in the interstitium, alveolar septal fibrosis, and mild bronchiolitis and bronchiolar fibrosis. These changes are the result of intra-alveolar hemorrhage rather than the cause, and can be created by intrapulmonary injection of autologous blood. Increased prominence of bronchial arteries and thickening of the tunica media of interstitial arterioles are also noted in some cases. Massive pulmonary hemorrhage is on occasion the only detectable lesion in horses that die during exercise; hemosiderin-laden alveolar macrophages in such cases are an indicator of previous episodes of sublethal pulmonary hemorrhage.

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Table 5.4 Causes of pulmonary arterial hypertension

Idiopathic (primary) pulmonary arterial hypertension

Left-to-right shunts
- Ventricular septal defect
- Patent ductus arteriosus

Increased resistance to blood flow through the lungs
- Left-sided heart failure causing pulmonary venous hypertension: mitral insufficiency or stenosis, subaortic stenosis, cardiomyopathy
- Hypoxic vasoconstriction of pulmonary arterioles: high altitude (brisket disease), bronchiolitis obliterans, chronic bronchitis or bronchiolitis (equine heaves)
- Pulmonary thromboemboli (including *Dirofilaria immitis*)
- Pulmonary arterial disease: pulmonary vasculitis, arteriosclerosis
- Chronic interstitial pneumonia with fibrosis and occlusion of pulmonary vessels

Pulmonary vasculitis, pulmonary hypertension

Pulmonary vasculitis is uncommon in domestic animals. *Septic pulmonary vasculitis* is caused by septicemia or showering of the lung with bacterial emboli, and results in numerous necrotic neutrophils in vessel walls and often thrombi occluding the lumen. Other infectious causes include *Feline infectious peritonitis virus*; *Dirofilaria immitis* in dogs; acute *Bovine viral diarrhea virus* infection, malignant catarrhal fever, *Histophilus somni* and *Aspergillus* sp. in cattle; purpura hemorrhagica, *Equine arteritis virus*, *Equid herpesvirus 1*, *African horse sickness virus*, and *Hendra virus* in horses; *Porcine reproductive and respiratory syndrome virus* (PRRSV), *Porcine circovirus* type 2, and *Nipah virus* in swine. Idiopathic suppurative or nonsuppurative vasculitis is occasionally encountered but poorly documented in dogs, and may result from immune-complex deposition or other immune-mediated vascular injury.

Pulmonary arterial hypertension is diagnosed by direct measurement of pressures in the live animal, but histologic lesions are highly suggestive. The causes of pulmonary arterial hypertension are listed in Table 5.4. Cardiac or vascular anomalies that cause *shunting of blood* from the high-pressure systemic circulation into the low-pressure pulmonary arteries induce pulmonary hypertension, which may eventually increase pulmonary vascular resistance to the extent that the flow reverses from right to left. *Left-sided congestive heart failure* provokes left atrial and pulmonary venous hypertension, which may progress to pulmonary arterial hypertension. Finally, *hypoxic pulmonary vasoconstriction* (constriction of the arterioles supplying alveoli experiencing low oxygen tension) or *thromboembolism* of the pulmonary vessels will increase resistance and induce hypertension. Other causes include sporadic primary pulmonary hypertension in humans, familial forms caused by mutations in the

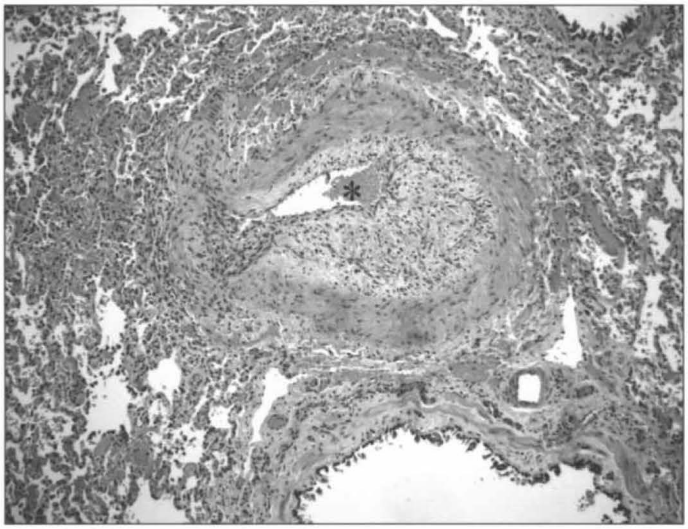

Figure 5.14 Pulmonary hypertension secondary to embolism in a dog. Pulmonary artery with thickening of the tunica intima and media, and a small lumen (asterisk).

gene encoding bone morphogenetic protein receptor 2, monocrotaline (a pyrrolizidine alkaloid in the seeds of *Crotalaria spectabilis*) toxicity, and adverse drug reaction. There is marked individual variation in severity of pulmonary hypertension in animals with known inciting causes, and genetic influences and perhaps other factors are important determinants of the course of disease. **Pulmonary venous hypertension** is expected in congestive left-sided heart failure, and is an important cause of pulmonary edema.

Acute pulmonary arterial hypertension causes endothelial degeneration, fibrinoid necrosis, and vasculitis. This lesion is reported during clinical crises in animals with patent ductus arteriosus, implying that it either causes or results from acute exacerbations of pulmonary hypertension. *Chronic remodeling of the pulmonary arterioles is a more common manifestation*, with thickening of the tunica intima by endothelial cell proliferation and fibrosis, hypertrophy of the tunica media, and more variable fibrosis or edema of the adventitia (Fig. 5.14). Small arterioles of the caliber that normally have a thin tunica muscularis may become muscularized. A *spectrum of morphologic variants* are described in human cases: concentric, eccentric, or obliterative intimal proliferation; thrombosis and recanalization of the lumen; and plexiform lesions. The latter consist of a web-like plexus of vascular lumens, each surrounded by concentric rings of endothelial cells and matrix, which extends through the vessel wall, and is thought to represent chronic attempts to repair pressure-induced aneurysms. In cattle, smooth-muscle hyperplasia may also be noted around bronchioles and in alveolar septa. These lesions are to be differentiated from the common finding of medial hypertrophy of medium-sized pulmonary arteries in cats, which is not associated with either clinical disease or pulmonary hypertension.

Vasoconstriction of pulmonary arterioles in cases of pulmonary hypertension is associated with increased levels of the vasoconstrictors thromboxane A_2 and endothelin-1, and reduced levels of the vasodilators prostacyclin and nitric oxide. Vascular endothelial growth factor and angiopoietin-1 are thought to mediate the remodeling of arterioles that result in the histologically apparent lesions in cases of pulmonary hypertension.

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Pulmonary neoplasia

Primary pulmonary tumors are encountered most often in dogs and cats, and are rare in other species. Epithelial neoplasms are the most common category of neoplasms encountered in lung specimens. Because the vascular bed of the lung is a frequent site for metastatic neoplasms, it is essential to differentiate nonpulmonary metastases from intrapulmonary metastasis from primary lung neoplasms. Distinguishing between these two processes is particularly challenging when dealing with widespread epithelial neoplastic disease in the lung. The histologic features should be evaluated in light of the gross pathology findings. *Features supporting the diagnosis of a primary lung neoplasm include the presence of a single large mass along with any smaller metastases, and histologic demonstration of ciliated cells or a bronchioloalveolar pattern.* Since pulmonary carcinomas often form intrapulmonary metastases, the presence of tumor emboli within vessels is not helpful in distinguishing primary from metastatic tumors. In dogs, immunohistochemical detection of thyroid transcription factor-1 appears to be useful for distinguishing pulmonary or thyroid carcinomas from other tumors that have metastasized to the lung.

Because malignant lung tumors are often widespread within the lung at the time of diagnosis, it is often difficult to identify a specific site of origin. As a rule, hilar or central neoplasms are more likely of bronchial origin, while peripheral tumors are more likely to have arisen from distal bronchiolar or alveolar epithelium. Further confounding attempts to categorize lung carcinomas neatly are the variety of morphologic and immunohistochemical characteristics found within regions of some lung neoplasms. For example, adenocarcinomas with a predominantly bronchioloalveolar pattern of differentiation may also contain areas of papillary or tubular arrangement of the cells. Similarly, squamous cell carcinoma and adenosquamous carcinoma reflect a squamous pattern of differentiation, likely from foci of previous squamous metaplasia within airways, and do not imply an origin from squamous epithelium. With this in mind, the nomenclature of the neoplasms discussed below is based upon, and reflects, the predominant cellular phenotype or pattern of differentiation (Table 5.5).

Papillary adenomas are located in the bronchi or in the lung parenchyma, and are characterized by a papillary pattern of growth of well-differentiated epithelial cells that contain mucus in the cytoplasm. These are uncommon neoplasms that are described in cattle

Table 5.5 Classification of epithelial lung tumors in domestic mammals

Benign epithelial lung tumors

- Papilloma
- Adenoma: papillary or bronchioloalveolar patterns

Malignant epithelial lung tumors

- Bronchial gland carcinoma
- Squamous cell carcinoma
- Adenocarcinoma: papillary, acinar, solid, or mixed patterns; or bronchioloalveolar carcinoma
- Adenosquamous carcinoma
- Large-cell carcinoma
- Small-cell carcinoma
- Neuroendocrine tumor
- Pulmonary blastoma
- Combined carcinoma
- Carcinosarcoma

Based on Dungworth DL, et al. Histological Classification of Tumors of the Respiratory System of Domestic Animals. 2nd series. vol. VI. Washington, DC: Armed Forces Institute of Pathology. 1999.

and cats. **Bronchioloalveolar adenoma** is a relatively common incidental finding in the lungs of older dogs; reports in other species are infrequent. Grossly, these neoplasms are usually identified within the subpleural parenchyma as well-demarcated pale moderately firm masses. On cut section the surface may appear multicystic. Histologically, these cystic spaces correspond to alveolus-like structures lined by well-differentiated cuboidal or columnar epithelial cells that show no anisokaryosis and rare mitotic figures. The term bronchioloalveolar implies that the neoplastic cells differentiate to resemble Clara cells or type II pneumocytes and the alveolar architecture is retained, and implies an origin in the periphery of the lung rather than the bronchi. In some cases, epithelial cells form papillary protrusions that resemble papillary adenoma, but mucus production is not expected.

Pulmonary carcinomas *are the most common pulmonary neoplasms in dogs, cats, and cows*, and include two major groups: adenocarcinomas and bronchioloalveolar carcinomas. Adenocarcinomas may be subdivided into four major patterns: papillary, acinar, solid, and mixed. For practical purposes, only the papillary and acinar forms are commonly seen; purely solid carcinomas of the lung are rare and will not be discussed here. Bronchioloalveolar carcinomas are considered as a separate entity subsequently.

Dogs with pulmonary papillary/acinar adenocarcinoma typically exhibit coughing, dyspnea, or lethargy. The location in dogs is often peripheral rather than hilar, with no obvious origin from the bronchi. Thus, the term bronchogenic adenocarcinoma is not favored for these neoplasms. Thus, the term bronchogenic adenocarcinoma is not favored for these neoplasms. Adenocarcinomas arising from bronchi have a growth pattern similar to the papillary and acinar adenocarcinomas, and are difficult to distinguish unless there is clear association with the large conducting airways. Papillary/acinar adenocarcinomas form an irregularly shaped, usually poorly demarcated mass that is most often located in the caudal lung. Intrapulmonary metastases are common, and may be grossly apparent, although numerous smaller metastases and neoplastic emboli are often detected histologically. The angular neoplastic epithelial cells form papillae or acini, or, rarely, solid sheets, and extensive areas of necrosis are frequent. Mucus production is inconsistently demonstrated by PAS staining. Alveoli at the periphery of the tumor often contain neoplastic cells and/or increased numbers of macrophages. Indicators of a poor prognosis include clinical signs of respiratory disease and involvement of regional lymph nodes; other features that are of more limited prognostic significance are the identification of histologic patterns other than papillary, higher histologic grades, and multiple tumors. *Paraneoplastic syndromes* associated with pulmonary adenocarcinomas include hypertrophic osteopathy, hypercalcemia, fever, and secretion of adrenocorticotropic hormone.

Pulmonary adenocarcinoma is a relatively common neoplasm of older **cats**. The tumors may be single, multiple, or lobar. Affected cats may develop progressive dyspnea and coughing due to expansion of the tumor, intrapulmonary metastasis, or pleural invasion and implantation causing pleural effusion. However, many affected cats exhibit no evidence of respiratory disease, and the clinical signs result from metastasis to other viscera, brain, muscle, eye, bone, or skin. *A peculiar feature of this tumor is the propensity to cause lameness by metastasizing to the digits*, especially to the dermis of the skin on the dorsum of P3 and beneath the footpad epidermis. Even highly malignant feline pulmonary adenocarcinomas may appear histologically well differentiated, and well-formed ciliated neoplastic cells are occasionally discovered on careful inspection of the primary tumor or the metastases. Occasionally, the epithelial metaplasia associated with *feline idiopathic pulmonary fibrosis* (IPF) is mistaken for an adenocarcinoma or bronchioloalveolar carcinoma. Unlike true epithelial neoplasms, which have features consistent with their clonal origin, feline IPF is a heterogeneous lesion with evidence of smooth-muscle proliferation, fibrosis, and honeycomb lung formation. Conversely, cats with feline IPF can have coincident primary lung carcinomas, so it is important for the pathologist to inspect the pulmonary parenchyma away from the lung neoplasm to identify changes typical of feline IPF.

Bronchioloalveolar carcinoma is less common in dogs and cats than papillary/acinar adenocarcinoma. *This pattern is characteristic of jaagsiekte* (ovine pulmonary adenomatosis/carcinomatosis), the retroviral lung tumor discussed below in the section on Infectious respiratory diseases of sheep and goats. In dogs, they form subpleural masses, which by definition are formed by alveolar structures lined by cuboidal or columnar epithelium (Fig. 5.15). Mucus secretion is not expected, but some neoplasms have accumulations of eosinophilic secretion within the lumen of the tumor. The cell of origin is assumed to be the terminal bronchiolar nonciliated (Clara) cell or type II pneumocytes. Neoplastic cells with Clara cell-like differentiation contain Clara cell secretory protein-10, detectable by immunohistochemistry, and dense-core secretory granules (the storage site of CC-10) are detectable using electron microscopy. Cells that differentiate to type II pneumocytes express surfactant protein C and may have surface microvilli and lamellar bodies, both features of type II pneumocytes but not Clara cells. Some bronchioloalveolar carcinomas may have areas with a papillary growth pattern; this finding may blur the distinction between this tumor and pulmonary adenocarcinoma. This malignancy is differentiated from bronchioloalveolar adenoma by infiltrative growth extending beyond the alveoli immediately adjacent to the tumor, variable forms of differentiation within the tumor, cellular atypia, and presence of mitotic figures. The behavior in cats is similar to that described for other forms of pulmonary adenocarcinoma.

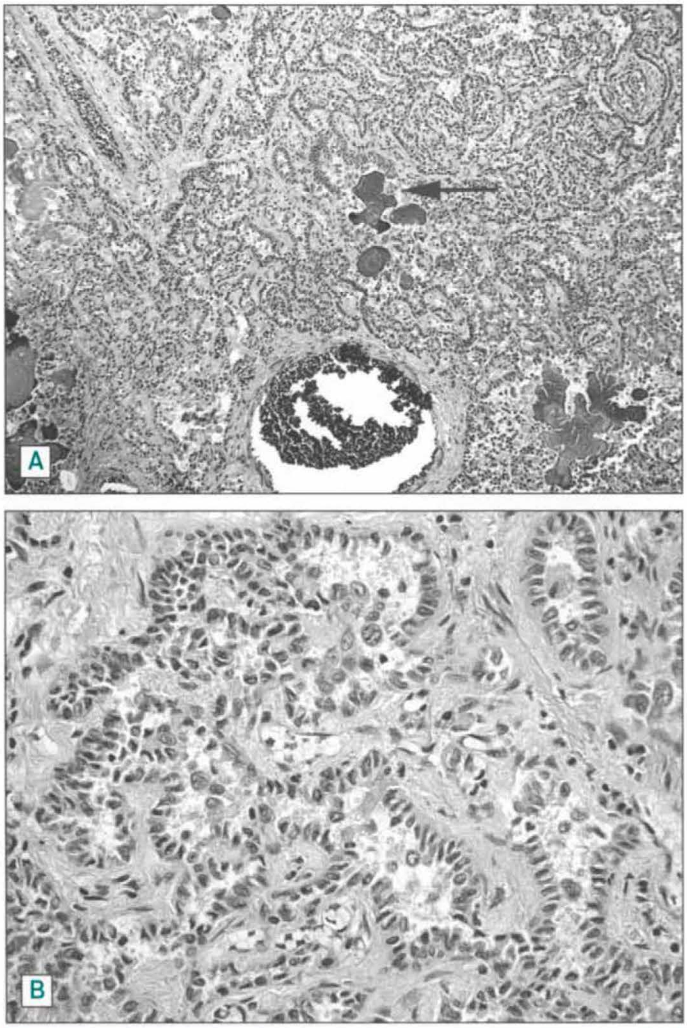

Figure 5.15 Bronchioloalveolar carcinoma in a dog. Low- and high-power photomicrographs of alveolus-like spaces lined by cuboidal epithelium. Several alveoli contain eosinophilic concretions of surfactant-like material (arrow in **A**).

Bronchial gland carcinoma is a rare low-grade malignancy that shows clear evidence of arising from the wall of a bronchus. The neoplastic cells form acini comprised of well-differentiated epithelial cells with prominent mucus production. **Squamous cell carcinoma** is common in the lung, and is histologically similar to that in other tissues. **Adenosquamous carcinoma** forms both acinar/tubular and squamous patterns of differentiation, and has a particularly aggressive growth habit. **Large-cell carcinoma** is a rare and anaplastic variant of pulmonary carcinoma, in which the individual neoplastic cells are large, with abundant cytoplasm; the cells tend to cluster around bronchioles and efface the lung tissue. Histiocytic sarcoma and large-cell neuroendocrine tumor are important differential diagnoses in suspected cases of large-cell carcinoma. **Carcinosarcoma** is a rare primary pulmonary tumor with both adenocarcinomatous and sarcomatous components, reported in a cat.

Neuroendocrine tumors in humans are classified as low-grade carcinoids, intermediate-grade atypical carcinoids, high-grade small-cell carcinoma, and high-grade large-cell neuroendocrine carcinoma. All are rare in domestic animals, and often affect the young. *Pulmonary neuroendocrine tumor is the preferred term because the validity of subclassifying these tumors in animals is unknown.* **Carcinoids** in humans are thought to arise from bronchial neuroendocrine tissue and exhibit the characteristic features of neuroendocrine tumors: arrangement in nests, palisades, or pseudorosettes; expression of chromogranin and synaptophysin; and the presence of dense-core granules visible by electron microscopy. Expression of neuron-specific enolase is considered nonspecific, and is found in some nonneuroendocrine carcinomas in humans. The few cases of pulmonary neoplasms in dogs that resemble carcinoids have developed adjacent to a bronchus, and have a neuroendocrine pattern of nests or ribbons of cells separated by delicate vascularized stroma. The cells are uniform, round to polygonal, with abundant pale eosinophilic cytoplasm and relatively small nuclei. Ultrastructural examination reveals many small, dense, secretory granules in the cytoplasm. They express neuron-specific enolase, but expression of other, more specific markers used for identifying neuroendocrine differentiation is poorly characterized. In human neuroendocrine tumors, the features indicative of a higher grade (i.e., atypical rather than typical carcinoid) are mitotic rate greater than 2 per 10 high-power fields, and coagulative necrosis of the tumor. In the few cases of neuroendocrine tumors reported in dogs, increasing tumor grade correlates with progression from well-demarcated nodules to infiltrative or metastatic neoplasms. **Small-cell carcinoma** is a rare and poorly characterized neuroendocrine tumor described in dogs and cows. It has a hilar location, and consists of loosely arranged packets of small cells (approximately 10 μm diameter) separated by a thin stroma. The neoplastic cells have scant cytoplasm, and may be round (resembling lymphocytes), fusiform ("oat cells"), or polygonal. The immunohistochemical staining patterns in domestic animals are not characterized.

Pulmonary blastoma, rarely described in adult cows, is a multinodular lesion that implants in the pleural cavity, metastasizes to lymph node, and invades extrapulmonary structures. Blastoma consists, by definition, of *malignant epithelial and mesenchymal components*, although there is much variation in the relative amount of each component between tumors and within different areas of the same neoplasm. The cuboidal epithelial cells are arranged in nests or branching tubules. The mesenchymal component appears embryonal, with loose matrix separating spindle-shaped, stellate, or angular cells with vesicular or hyperchromatic nuclei.

Granular cell tumor *is the most common primary lung tumor in horses, and is thought to originate from Schwann cells in the peribronchial tissue.* It is often discovered as an incidental finding in older horses, but can cause coughing or, rarely, dyspnea. Metastasis is not described. Grossly, the mass is white to beige, usually multinodular and unilateral. Granular cell tumors are associated with large bronchi, and often bulge into the bronchial lumen to cause variable degrees of obstruction. Histologically, the neoplasm is comprised of sheets or lobular aggregates of large, round, or angular cells with abundant cytoplasm containing innumerable tiny acidophilic granules (Fig. 5.16). There is minimal variation in cell size, and few mitotic figures are found. The granules will usually stain positively with Luxol fast blue and more variably with periodic acid–Schiff. Immunohistochemical markers for pulmonary granular cell tumors in horses are widely expressed in a variety of other cell types, and so are not pathognomonic for this neoplasm; S100 and vimentin are consistently present, with inconsistent expression of neuron-specific enolase and glial fibrillary acidic protein (GFAP).

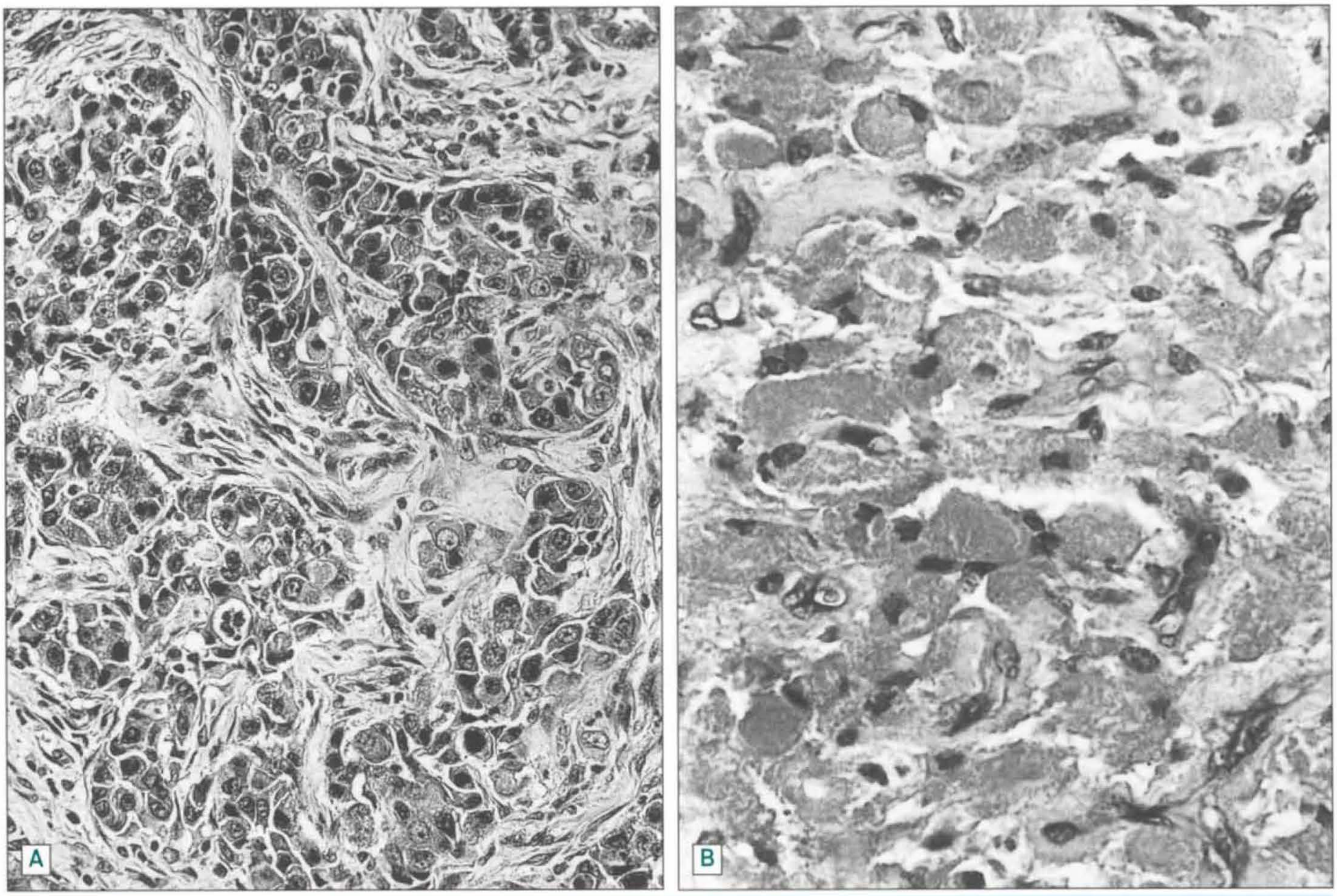

Figure 5.16 Granular cell tumor of the lung of a horse. The neoplastic cells are arranged in nests (**A**) and have abundant granular eosinophilic cytoplasm (**B**).

Lymphomatoid granulomatosis is a very poorly understood disease in veterinary and human medicine. Reports of this condition are not common, but occur mostly in dogs, and rarely in cats. While the disease is discussed here as a pulmonary neoplasm, there is considerable debate as to whether the analogous condition in humans represents a neoplasm or a non-neoplastic lymphoproliferative disorder. No data are available in veterinary medicine addressing the clonality of the lymphoid cells, so the biology of this disease remains enigmatic at this point. Most cases affect 4–9-year-old dogs, and cases as young as 9 weeks are recorded; this relatively young age of onset substantiates the doubts as to whether this is a neoplastic disorder. The pulmonary masses of lymphomatoid granulomatosis are usually multiple and found within the caudal lobes. They are poorly demarcated, and pale, or can occur as a diffuse infiltrate within the affected lobes. Lymphomatoid granulomatosis frequently involves the bronchial lymph nodes, and occasionally similar infiltrates are found within the liver and other viscera. Histologically, *lymphomatoid granulomatosis is an angioinvasive, destructive, pleocellular infiltrate.* The morphology of the infiltrating cells varies; the putative neoplastic cells are round, with distinct borders. They are highly variable in size and appearance and often have a high mitotic rate. Large histiocytic and plasmacytoid forms predominate, binucleate cells may be found, and eosinophils, small lymphocytes, and plasma cells may be numerous. *Invasion of the pleomorphic cells into the walls of blood vessels is the characteristic feature of this disease* (Figs 5.17 and 5.18). Infarction with coagulative necrosis and fibrosis is frequently present. The process may affect local lymph nodes, but it is the progressive involvement of the lung that is the cause of death in most cases. Although the neoplastic cells in dogs appear to be lymphocytes, and variable expression of CD3 has been reported in the atypical cells of canine lymphomatoid granulomatosis, immunophenotypic characterization of the pulmonary infiltrate in dogs diagnosed with this condition is incomplete. In human medicine, there is a growing body of evidence that lymphomatoid granulomatosis is associated with Epstein–Barr virus infection; no information is available pertaining to the role that viral infections play in the canine disease. The differential diagnosis includes eosinophilic granulomatosis, anaplastic carcinoma, and pulmonary round cell tumors such as histiocytic sarcoma, lymphoma, and mast cell tumor.

Histiocytic sarcoma *(malignant histiocytosis) is a malignant neoplasm derived from myeloid dendritic cells* (see Vol. 3, Hematopoietic system). It affects the lung and/or thoracic lymph nodes, usually with concurrent involvement of other organs. The infiltrate may replace an entire lung lobe, or form multifocal infiltrates throughout the lung. The morphology varies from individualized round cells with scant to abundant eosinophilic cytoplasm, to spindle-shaped or dendritic cells with indistinct borders. The nuclei are round or cleaved with marked anisokaryosis. Most cases have significant numbers of multinucleate cells, lymphocytes, and plasma cells intermingled with the neoplastic cells. Immunohistochemical

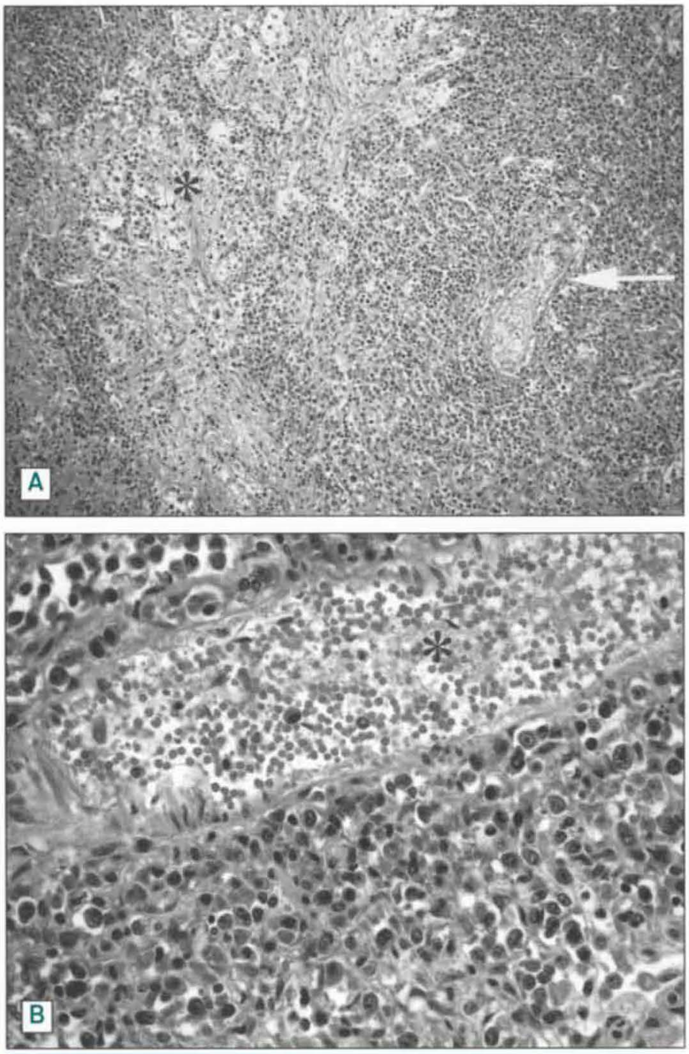

Figure 5.17 Lymphomatoid granulomatosis in a dog. **A.** Neoplastic round cells invade vessel walls (arrow), with areas of ischemic necrosis and fibrosis (asterisk). **B.** Higher magnification of a blood vessel (asterisk in lumen) infiltrated by neoplastic cells.

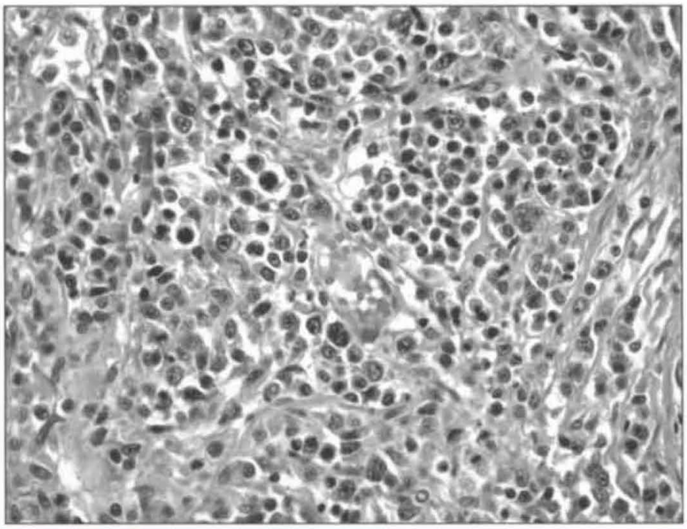

Figure 5.18 Lymphomatoid granulomatosis in a dog. Pleomorphic round cells arranged around a small blood vessel.

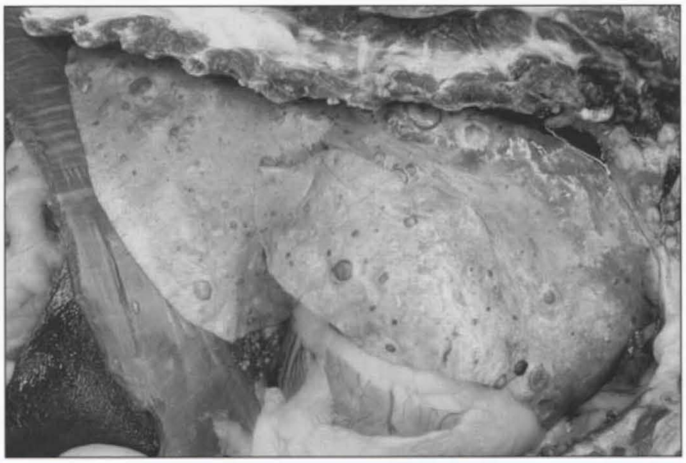

Figure 5.19 Pulmonary metastases of **malignant melanoma** in a dog.

identification is best performed on frozen sections, where the cells are CD1+, CD11c+, major histocompatibility complex class II+, and intercellular adhesion molecule (ICAM)-1+; demonstration of a CD18+, CD3–, CD79a– phenotype in formalin-fixed specimens provides tentative confirmation of the diagnosis.

Metastatic tumors commonly arise within the lung because of the organ's rich capillary and lymphatic network. As mentioned above, it is often difficult to distinguish metastatic disease arriving from a distant, nonpulmonary primary neoplasm from intrapulmonary spread of a primary lung neoplasm. This distinction is the most difficult with aggressive carcinomas. An important part of the diagnosis of a primary lung tumor is thorough examination of the body to exclude possible nonpulmonary sites of primary neoplasia. *The most easily recognizable pattern of metastatic tumors is of multiple, similarly sized nodules throughout the pulmonary parenchyma* (Fig. 5.19); *primary lung tumors usually form one or a few large tumors, sometimes with smaller intrapulmonary metastases.* Metastatic carcinomas may form acini, solid sheets, lepidic growth along pre-existing alveolar septa, and/or clusters within blood vessels. Some of the most common include lymphoma in all species; uterine adenocarcinoma in cows and sows; malignant melanoma in horses; and osteosarcoma, hemangiosarcoma, oral melanoma, mast cell tumor, transitional cell carcinoma, and adenocarcinomas of mammary, biliary, pancreatic, intestinal, anal sac, or thyroid origin in dogs and cats.

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Anatomic patterns of lung injury

The pulmonary inflammatory response varies according to the nature of the causative agents, their distribution (particularly the route by which they reach the lung), and their persistence. Pulmonary diseases can be classified in several ways:

- *Morphologic pattern*, according to initial site of involvement and the pattern of spread of the lesion: bronchopneumonia, interstitial pneumonia, bronchitis/bronchiolitis, bronchointerstitial pneumonia, and embolic pneumonia.
- *Histologic character*: fibrinous, suppurative, granulomatous, necrotizing, proliferative, fibrosing.
- *Etiology*: viral, bacterial, parasitic, toxic, allergic.
- *Duration*: acute, subacute, chronic.
- *Functional abnormality*: obstructive versus restrictive.
- *Epidemiologic patterns*: shipping-fever pneumonia, enzootic pneumonia.

The approach described here emphasizes morphologic patterns of pneumonia, for two reasons. First, gross and histologic examination is usually sufficient to classify a condition by this scheme, even if the etiology cannot be identified. Second, knowledge of the pattern of pneumonia provides important clues as to the probable etiology, route of exposure to the causative agent, pathogenesis of the lesions, effect on pulmonary function, and the sequelae and complications. Emphasizing the similarities between specific diseases, by grouping them based on morphologic patterns, often enhances our ability to recognize the sometimes subtle differences among these conditions.

Bronchial necrosis and inflammation

Lesions that specifically target the airways include those that cause epithelial necrosis and those that induce airway inflammation; a combination of these patterns is frequently, but not always, present. Diseases may affect the bronchi, bronchioles, or both; and the diagnosis of bronchointerstitial pneumonia, described later, is appropriate when both airway and alveolar epithelial cells are damaged. *The major consequences of these diseases are coughing, airway obstruction, and impairment of lung defenses.* The flow of air can be obstructed by bronchoconstriction resulting from contraction of airway smooth muscle, by leukocytes or mucus within the lumen of the airway, and by thickening of the airway wall by edema and leukocytes. The result is failure of alveolar ventilation that may cause hypoxemia and hypercapnea, and reflex vasoconstriction that reduces perfusion of hypoventilated areas of lung. Pulmonary compliance is reduced because of the increased pressure needed to ventilate the alveoli. Clinically important airway obstruction is particularly notable with bronchiolar disease, where it manifests as expiratory dyspnea because the airway obstruction is exacerbated from the slight collapse that occurs during exhalation.

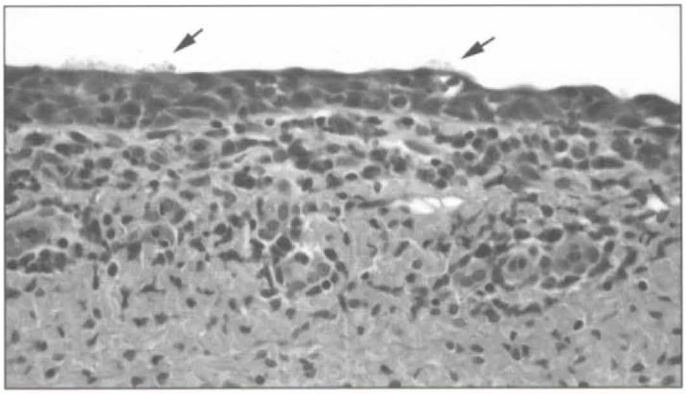

Figure 5.20 ***Nipah virus*** infection in a pig. Squamous metaplasia of bronchial epithelium, with two remaining islands of ciliated cells (arrows).

Bronchitis is relatively common in domestic animals but receives inadequate attention by pathologists because it is not usually fatal. Bronchitis and/or bronchial necrosis may be caused by viral or bacterial infection, parasitism, allergic disease, or exposure to irritants or toxins.

Morphologic manifestations of acute bronchitis include the same range of inflammation described for upper airways. The exudates may be catarrhal, mucopurulent, fibrinous, fibrinopurulent, or purulent. Ciliated epithelial cells are most sensitive to a wide array of injurious agents and are often the first to undergo necrosis and slough. *Catarrhal bronchitis* represents a relatively mild reaction in which the irritation induces both a mild neutrophil-dominated inflammatory response and secretion of mucus by goblet cells and bronchial mucosal glands. In *purulent bronchitis*, the exudate is yellow or white and viscid. *Fibrinonecrotic bronchitis* resulting from viral or occasionally mycotic infection is characterized by areas of epithelial necrosis with loosely adherent fibrinous exudate. In such cases, gentle removal of the exudate reveals a granular lesion of epithelial necrosis, which differentiates the lesion from the expectorated mucus that is often present in the large airways of animals with chronic bronchopneumonia. Animals with aspiration pneumonia often have extensive necrosis of the bronchial epithelium, with green-brown foul-smelling exudate.

Bronchial necrosis can resolve by epithelial regeneration if the offending stimulus is removed or neutralized. More severe or prolonged injury to epithelium results in fibrosis of the lamina propria, as well as accumulation of lymphocytes, macrophages, and plasma cells. Epithelial hyperplasia, mucous hyperplasia, and/or squamous metaplasia may also become prominent following mucosal injury of any cause (Fig. 5.20). Chronic infection with damage to the airway wall may result in bronchiectasis, discussed below. Fibrous polyps are a rare response to bronchial mucosal injury.

Common airway diseases in domestic animals include acute tracheobronchitis ("kennel cough") of dogs (discussed in the section on Infectious respiratory diseases of dogs, below), chronic bronchitis of dogs, feline asthma, heaves in horses, and *Dictyocaulus* spp. in cattle, sheep, goats, and horses. Bronchitis also results from inhalation of bacteria from the upper respiratory tract, and rarely when the focal lesions of tuberculosis or caseous lymphadenitis erode into a large airway. Bronchitis causes coughing that may be paroxysmal, and airway obstruction manifests in wheezing. However, it is not expected to be fatal unless the lesion induces widespread bronchoconstriction, obstructs a large airway, or leads to secondary bronchopneumonia as a result of aspiration of infected material or impaired mucociliary clearance.

Chronic bronchitis in dogs

Chronic bronchitis is a common condition in older, small and medium-sized dogs, and manifests as chronic productive or nonproductive cough, exercise intolerance, expiratory or inspiratory wheezes, reduced expiratory airflow, and hypoxemia. The etiology in individual cases is rarely identified, and the lesions are probably self-perpetuating. Proposed causes include dry air, airborne pollutants including sulfur dioxide and particulates, second-hand tobacco smoke, chronic gingivitis with aspiration of debris, immune-mediated inflammation, and viral infection. Opportunistic bacterial pathogens are occasionally isolated, but their significance as a primary cause is questionable; similarly, viral and bacterial infections are well recognized to induce acute exacerbations in human patients with chronic bronchitis in which infection is not the primary cause. Many dogs with chronic bronchitis also have chronic left heart failure, suggesting that cardiogenic pulmonary edema may result in chronic coughing that generates airway inflammation and mucus hypersecretion. Chronic exposure to sulfur dioxide or cigarette smoke are canine models of bronchitis, and cause similar histologic lesions to those in the natural disease.

The major gross finding is excessive mucus or mucopurulent exudate in the trachea and bronchi. The bronchial mucosa in severe cases is hyperemic, thickened, edematous, and granular rather than glistening, and lymphoid nodules or hyperplastic polyps may project into the lumen. Microscopically, the bronchial mucosa is edematous and contains many lymphocytes, plasma cells, and occasional macrophages and neutrophils (Fig. 5.21). Eosinophils are present in some cases but not in others. There is hyperplasia and hypertrophy of the mucosal glands, hyperplasia of goblet cells, and variable hyperplasia, ulceration, or squamous metaplasia of the surface epithelium. Intraluminal mucus is commonly mixed with abundant neutrophils. Many cases of uncomplicated bronchitis persist for years, but *sequelae* in severe cases include bronchiectasis; bronchopneumonia; or alveolar atelectasis leading to pulmonary hypertension, cor pulmonale, and medial hypertrophy of pulmonary arteries. Collapsing trachea and chronic bronchitis are frequent companions: chronic inflammation is thought to progress to malacia of the tracheobronchial cartilage in some cases and contribute to development of tracheal collapse. Emphysema is not an important sequel to chronic bronchitis in dogs, in contrast to chronic obstructive pulmonary disease in humans.

Eosinophilic bronchitis is common in dogs. Most cases are probably allergic; these are rarely fatal, and the histologic features of mild cases are not well described because they often respond favorably to corticosteroid therapy. The nematodes *Crenosoma vulpis, Eucoleus aerophilus* (*Capillaria aerophila),* and *Oslerus osleri* are additional causes of eosinophilic airway disease.

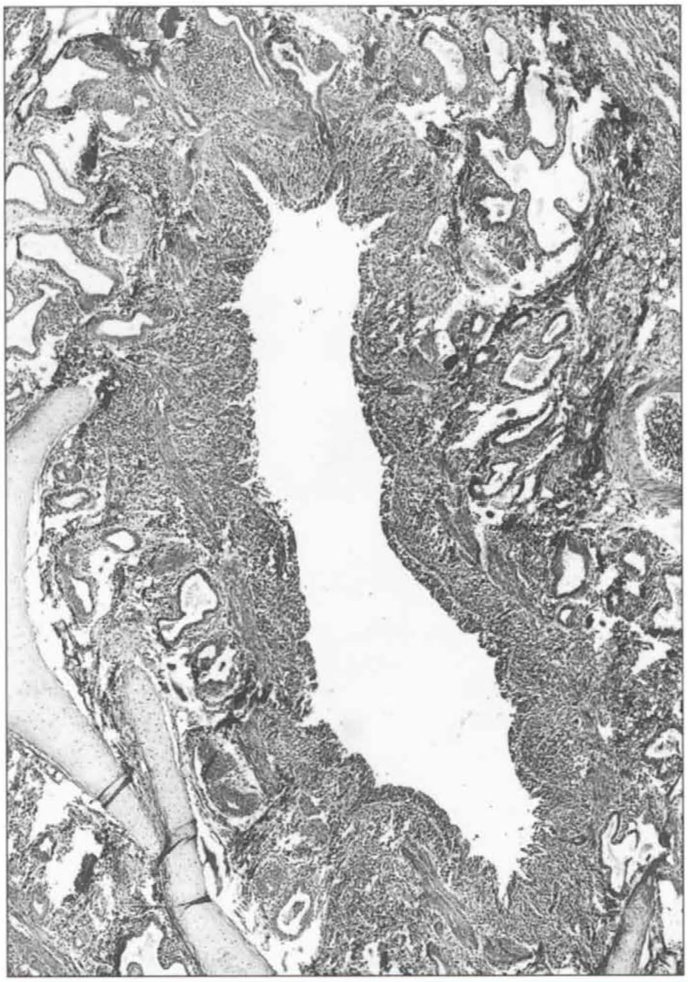

Figure 5.21 Chronic bronchitis in a dog.

Eosinophilic bronchopneumopathy is a rare condition of young dogs, and Siberian Huskies are overrepresented. It causes chronic gagging, cough, dyspnea, nasal discharge, and variable blood eosinophilia and neutrophilia. The diffuse lung lesions are not completely described, but include chronic eosinophilic bronchitis with epithelial hyperplasia, ulceration, or squamous metaplasia. Bronchiectasis is a reported complication in some cases. The disease shares several features with eosinophilic pulmonary granulomatosis, which is discussed below.

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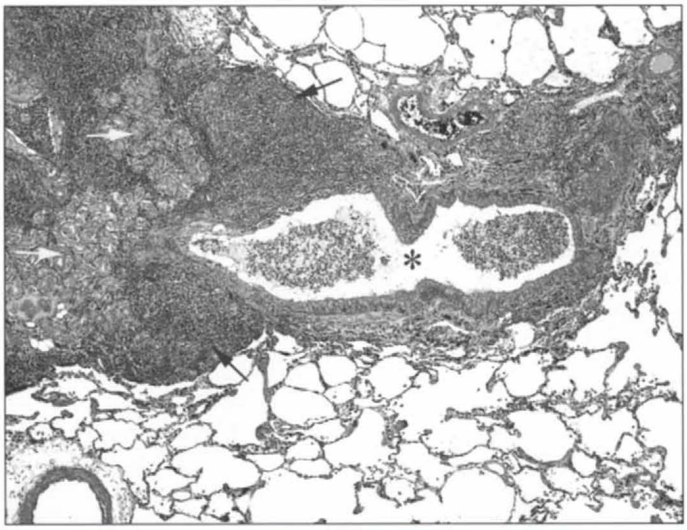

Figure 5.22 Feline asthma. Lesions target the bronchi, with eosinophils in the lumen (asterisk), follicular lymphoid hyperplasia surrounding bronchi (black arrows), and hyperplasia of bronchial glands (white arrows).

Acute and chronic bronchitis in cats

Feline asthma *is relatively common, and is defined by the clinical findings of reversible airway obstruction with inflammation.* Most cases have an acute onset or recurrent bouts of dyspnea or wheezing that respond to treatment with bronchodilators and corticosteroids, and are *mainly the result of bronchoconstriction due to airway smooth-muscle hyperreactivity.* Thickening of the bronchial wall may be noted radiographically, and eosinophils are usually numerous in bronchoalveolar lavage fluid. Neutrophils or macrophages predominate in bronchoalveolar lavage fluid in some cases that are otherwise typical of feline asthma. Other cats have chronic coughing or wheezing, although chronic bronchitis is a more accurate, albeit nonspecific, diagnosis in these cases if it cannot be demonstrated that the signs are readily reversible. Cats parenterally sensitized then challenged with Bermuda grass allergens develop allergen-specific immunoglobulin E (IgE), IgG, and IgA; airway hyperreactivity; and the following histologic lesions in the bronchi: narrowing of the lumen, smooth-muscle hyperplasia, infiltrates of eosinophils in the wall and lumen, hypertrophy and hyperplasia of goblet cells and mucosal glands, and erosion of the surface epithelium. The histologic findings in natural cases are not as well characterized because mortality is uncommon. In addition to smooth-muscle hyperplasia and eosinophil infiltration, lymphoplasmacytic infiltrates with lymphoid follicles may develop in the adventitia of the bronchi, and bronchioles may contain similar lesions (Fig. 5.22).

Chronic bronchitis in cats may be a manifestation of ongoing low-grade allergic reactions, although these do not fulfill the criteria of acute onset of reversible airway obstruction, as is necessary for the diagnosis of asthma. The role of infectious agents, including *Mycoplasma* spp., as causes of chronic bronchitis in cats is controversial. Bronchial parasites are rare in cats.

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Bronchiectasis

Bronchiectasis is defined as permanent dilation of bronchi, as a result of chronic bronchial obstruction and infection. Although most cases are a complication of bronchopneumonia or bacterial bronchitis, bronchiectasis may result from focal bronchial obstruction by an inhaled foreign body, granuloma, tumor, or broncholith. It rarely occurs as a congenital malformation, and immune-mediated bronchitis may lead to bronchiectasis in humans and animals. The lesion is most common in **cattle**, where it is almost always a sequel to bronchitis that occurs within the cranioventral lesions of bacterial bronchopneumonia. Bronchiectasis is relatively common in cattle with chronic bronchopneumonia, because complete lobular septation and lack of collateral ventilation both impair resolution of bronchopneumonia and lead to more extensive atelectasis because of airway blockage. Bronchiectasis is common in **dogs**, and Cocker Spaniels and Miniature Poodles may be overrepresented. Many have chronic cough, but it is uncertain whether bronchiectasis is the initial cause of the cough or a complication of underlying chronic bronchitis. The lesions commonly affect the right cranial or middle lung lobes, and are usually the result of bacterial infection or occasionally of tumors or bronchial foreign bodies. Bronchiectasis is common in dogs with ciliary dyskinesia. The condition in **cats** usually affects the caudal or middle lobes, and is often a sequel to chronic bronchitis, bronchopneumonia, or pulmonary neoplasia. Endogenous lipid pneumonia and emphysema are concurrent findings in some cases. Bronchiectasis infrequently develops in **pigs, sheep**, and **goats** with severe parasitic bronchitis, and in **horses** with chronic heaves. There are three anatomic forms of bronchiectasis: (1) *cylindrical bronchiectasis* causes relatively uniform dilation of the bronchus; (2) *saccular bronchiectasis* is probably a more advanced stage, where there are circumscribed or fusiform cyst-like lesions at the end of progressively dilated airways; and (3) *varicose bronchiectasis* – rarely identified in animals – forms focal constrictions along dilated airways. The smaller distal bronchi are usually affected by bronchiectasis, and a similar lesion may involve the bronchioles.

Obstruction of bronchi by purulent exudate is the key to development of bronchiectasis, and the resulting destruction of bronchial walls leads to their permanent dilation. In these situations, neutrophil-derived proteases and oxygen radicals damage the bronchial epithelium and connective tissue. This results not only in weakening of the bronchial wall, but also permanent failure of mucociliary clearance that perpetuates the infection and pooling of exudates in the lumen. Exudates in more distal airways and alveoli cause airway obstruction and atelectasis; as a result, the weakened larger airways are pulled outward with each inspiration and thus dilate over time.

The dilated bronchi are filled with viscid yellow-green creamy exudate, which may eventually form caseous or inspissated masses in the lumen. The intervening parenchyma is atelectatic and often fibrotic. The bronchi may be so dilated as to be visible from the pleural surface, but are best appreciated when sectioned transversely

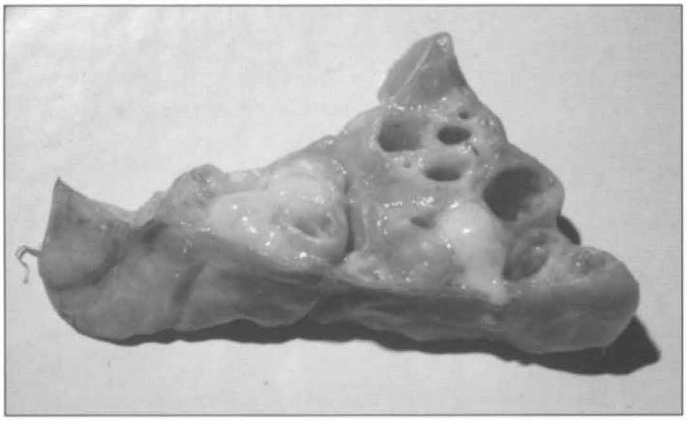

Figure 5.23 Bronchiectasis in a dog. The lung contains many greatly dilated bronchi filled with purulent exudate.

(Figs 5.23 and 5.24). *They may be confused with abscesses, but the clues to their origin include their linear and regular rather than haphazard distribution, the finding of remnants of cartilage, and their eventual continuity with recognizable bronchi.* In severe cases, the dilated bronchi give a honeycombed or cystic appearance to the lobe. Microscopically, the lumen is filled with mucus, cellular debris, leukocytes, and occasionally blood. The epithelium often shows some combination of attenuation, ulceration, mucous or squamous metaplasia, and hyperplasia. The mucosa and submucosa are densely infiltrated with all types of leukocytes and are often obscured by granulation tissue, and this destructive and fibrotic lesion may extend to the deeper tissues and destroy cartilage and submucosal glands; *complete destruction of the bronchial wall leads to formation of a lung abscess.* Normal bronchi are dynamic structures that actively regulate airflow and defend the lung through mucociliary clearance; those crippled by

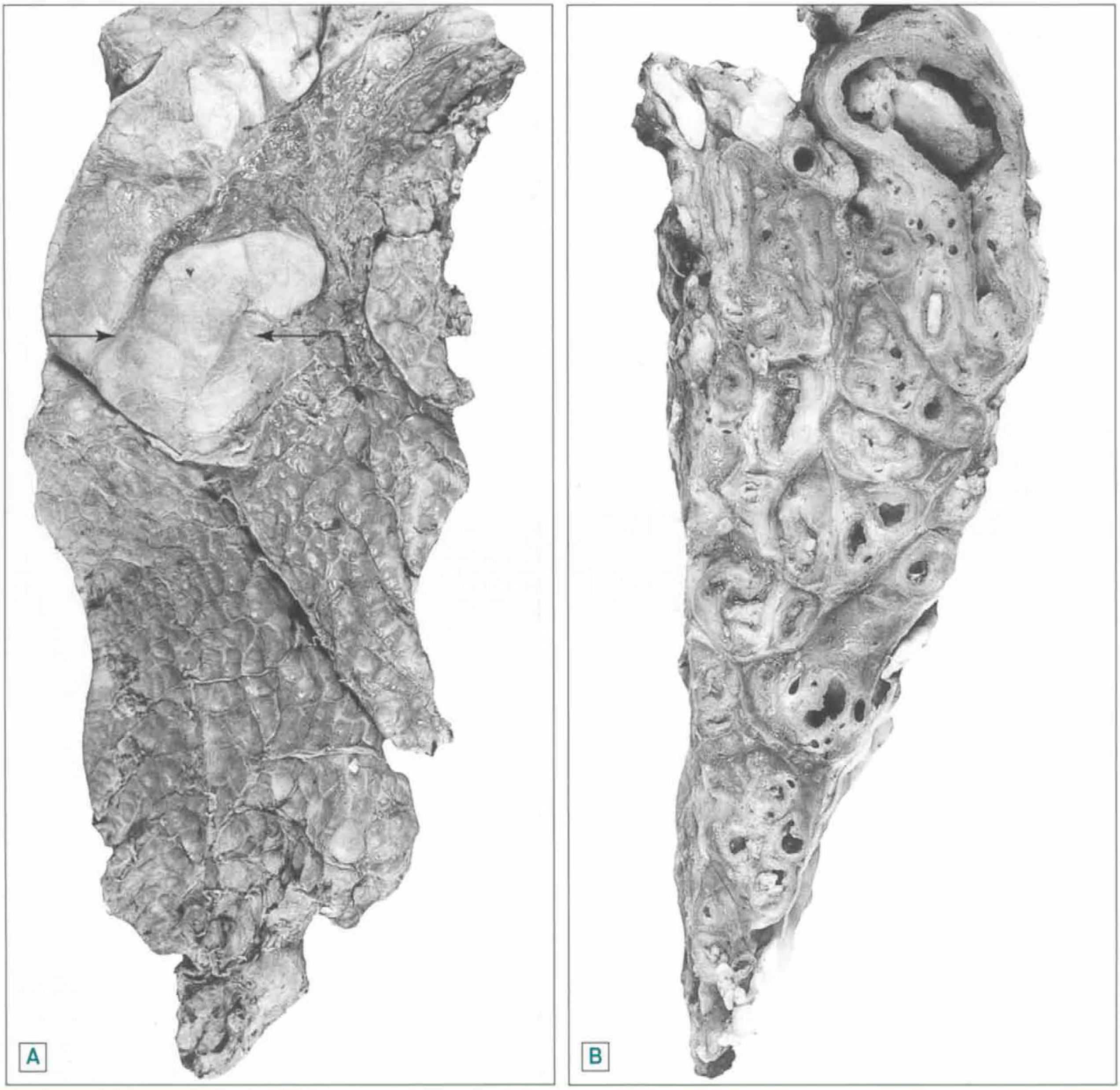

Figure 5.24 Bronchiectasis in a calf. **A.** Nodular lesions visible on the pleural surface correspond to (**B**) dilated bronchi filled with exudate. There is air trapping in the adjacent lobules (arrows in **A**).

bronchiectasis are forever dilated and unable to clear their burden of harmful pus and pathogens.

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Primary ciliary dyskinesia

Cilia are complex structures: the "9 + 2" or "9 + 0" arrangement of microtubular doublets forms the basic structure, inner and outer dynein arms are the motors that forces the cilium to bend, and nexin links and radial arms provide support. These structures contain hundreds of proteins, so it is not surprising that *primary ciliary dyskinesia is a diverse collection of disorders* involving mucus-propelling cilia in the nasal cavity and bronchi, sperm flagella, nodal cilia in the developing embryo, water-propelling cilia in the ependyma and epididymis, and cilia in the middle ear and retina. Primary ciliary dyskinesia or *immotile cilia syndrome* is an inherited condition reported infrequently in many dog breeds, which manifests as recurrent or persistent rhinitis and sinusitis, bronchiectasis, bacterial pneumonia, and/or male infertility due to reduced spermatozoal motility with abnormal tails. About half of affected humans and dogs have *Kartagener's syndrome*, defined as the combination of sinusitis, bronchiectasis, and situs inversus. The latter represents left–right reversal of the thoracic and/or abdominal viscera due to dysfunction of cilia in the embryological node. Otitis media or hydrocephalus may occasionally be present.

The *diagnosis* of ciliary dyskinesia is based on abnormal ciliary motility in short-term cell cultures, in vivo imaging studies to measure movement of mucus, and/or detection of ultrastructural abnormalities in cilia. Since bacterial toxins or adhesion of *Bordetella* or perhaps *Mycoplasma* cause ciliary dysfunction, detection of dysfunction in cilia from more than one tissue is necessary. Ultrastructural studies are also useful in differentiating primary from acquired ciliary dyskinesia. Absence or reduced number of inner and/or outer dynein arms, of the radial spokes that extend from these arms, and/or of the central microtubules are common ultrastructural lesions in primary ciliary dyskinesia, and other primary and secondary changes are described. The ultrastructural diagnosis depends heavily on the quality of the preparation, and some dogs with the syndrome do not have ultrastructural changes in cilia.

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Bronchiolar necrosis and inflammation

Bronchiolitis and/or bronchiolar necrosis are *caused by* viral infection, inhalation of toxic gases, toxins that are metabolized by cytochrome P450 in nonciliated Clara cells, hypersensitivity reactions, or inflammatory reactions to inhaled irritants (Fig. 5.25). Autoimmune disorders, drug reactions, and reactions to mineral dusts are further causes of bronchiolar injury in humans. In addition, bronchiolar lesions are frequently an extension of bronchitis, bronchiectasis, or bacterial bronchopneumonia. The designation of *bronchointerstitial pneumonia* is appropriate when necrosis targets both the bronchiolar and the alveolar epithelium, as occurs particularly in viral infections and toxic injury.

Airway obstruction occurs more readily in bronchioles than in bronchi, because their lack of cartilage rings and small luminal size readily permits collapse and occlusion by exudate. According to Poiseuille's law of frictional resistance, resistance to airflow varies with the fourth power of the radius, so narrowing of the airway by half will increase airway resistance 16-fold. Thus, if diffuse in its distribution, even minor airway obstruction by bronchoconstriction, intraluminal exudates or mucus, or edema of the wall has profound effects on the work of breathing and on alveolar ventilation. These functional changes manifest as forced expiratory efforts, hypoxemia, and hypercapnea in the later stages. *Complete airway obstruction causes atelectasis, while partial obstruction leads to air trapping and overdistension of alveoli.* Both of these situations lead either to shunting of nonoxygenated blood past the perfused but hypoventilated alveoli, or arteriolar constriction that reduces perfusion of these hypoventilated alveoli but causes pulmonary hypertension if the airway obstruction is widespread.

Bronchiolitis obliterans (also known as *bronchiolitis fibrosa obliterans, obliterative bronchiolitis,* or *organizing bronchiolitis)* is a sequel to

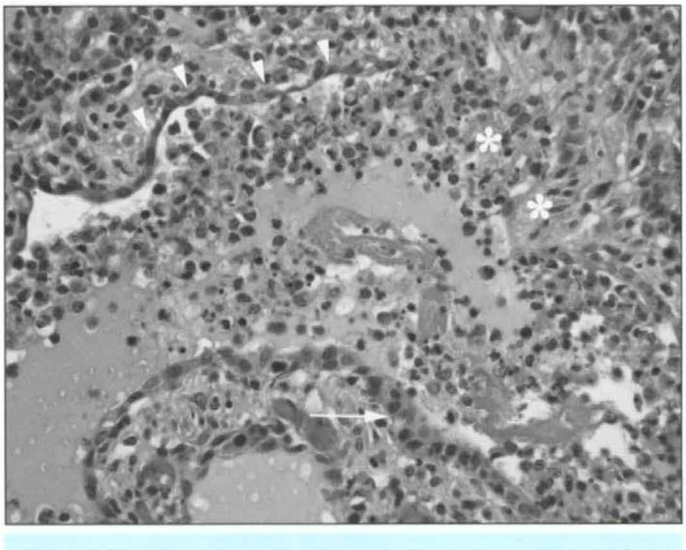

Figure 5.25 ***Nipah virus*** infection in a pig. A bronchiole is filled with fibrin and necrotic cells. The epithelium varies from eroded (asterisks), to attenuated (arrowheads), to normal (arrow).

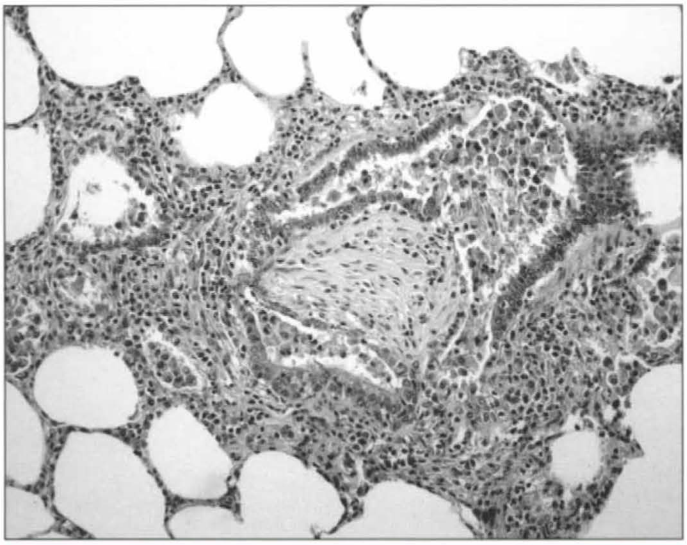

Figure 5.26 Bronchiolitis obliterans in a calf. The lumen of the bronchiole is occluded by a mass of fibrous tissue covered by cuboidal epithelium.

chronic bronchiolar damage. The term is used here to describe the presence of fibrous polyps occluding the bronchiolar lumen; bronchiolitis obliterans is occasionally employed elsewhere to describe acute lesions in which intraluminal aggregates of neutrophils obstruct airflow. The lesion is distinct from **constrictive bronchiolitis**, a lesion not commonly identified in animals, in which fibrosis of the bronchiolar wall causes external compression that reduces the diameter of the lumen.

Bronchiolitis obliterans is wound healing gone awry. Any agent that severely damages the bronchiolar epithelium – viral infection, neutrophil-mediated injury due to bacterial pneumonia, toxic gases, toxins metabolized by Clara cells, lungworms, and rejection of transplanted lung – may lead to fibrin formation in the bronchiolar lumen. As in any wound, fibrinous exudates that are not rapidly removed heal by fibroblast infiltration, neovascularization, and sliding and proliferation of epithelial cells across their surface. Organization of exudate into granulation tissue can take place in as little as 7–10 days after injury, and regeneration of epithelium over its surface can occur in the same time period. Although this repair proceeds with the best intentions, *fibrous polyps occluding the airway may have disastrous and permanent effects on airflow and alveolar ventilation.*

Bronchiolitis obliterans is commonly identified within lesions of chronic bronchopneumonia in cattle, as a consequence of viral or neutrophil-mediated epithelial damage. Calves with a prior history of viral pneumonia occasionally develop right heart failure with right ventricular dilation, subcutaneous edema, hepatomegaly, and ascites. The gross lung lesions in such calves may be minor, but histologic examination reveals epithelium-covered fibrous polyps that occlude the lumen of most bronchioles (Fig. 5.26), as well as medial hypertrophy of pulmonary arterioles. The latter is a histologic indicator that widespread alveolar hypoventilation has incited pulmonary hypertension, which has in turn caused cor pulmonale with right heart failure.

Scattered subpleural foci of **bronchioloalveolar hyperplasia** are a common incidental finding in dogs. The lesions can be difficult to distinguish from early bronchioloalveolar carcinoma, but the hyperplastic lesions are usually multifocal, line terminal bronchioles, do not enlarge to form masses, are usually associated with fibrosis and lymphocytic inflammation of the adjacent tissue, and have no features of cellular atypia.

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Heaves in horses

Heaves is a common condition of mature horses. *The term chronic obstructive pulmonary disease is inappropriate for the equine disease*, as the condition differs from this human condition and is more similar to human asthma. *Heaves is a nonseptic, inflammatory, obstructive airway disease of adult horses characterized by airway hyperresponsiveness and reversible airway obstruction due to bronchospasm.* Other mechanisms of airway obstruction include accumulation of mucus and neutrophils in bronchiolar lumina and thickening of the bronchiolar wall. It is usually induced or exacerbated by exposure to organic dusts (particularly dusty, poor-quality hay) that may contain allergens, endotoxins, fungi, actinomycetes, and particulate irritants.

Although heaves is undoubtedly an inflammatory disease, it remains controversial whether allergy (IgE- and mast cell-mediated type I hypersensitivity) is important to the pathogenesis. Evidence supporting a mast cell-dependent or allergic basis includes elevated levels of IgE and histamine in bronchoalveolar lavage fluid, and enhanced histamine release from pulmonary mast cells of horses with heaves. However, skin test results do not correlate with airway reactivity, and bronchoalveolar lavage cells in horses with heaves express both interleukin-4 (IL-4) and interferon-γ (in addition to tumor necrosis factor-α, IL-1β, and IL-8) mRNA, suggesting that a pure T-helper-2 immune response is not characteristic of this disease. Challenge with molds, mold extracts, or endotoxin-containing dusts has induced airway neutrophilia and obstruction in heave-susceptible but not normal horses, implying that all of these factors may contribute to the airway responses.

The disease occurs in stabled horses in northern regions and as a summer pasture-associated form in warm wet climates. *Affected horses have chronic cough, increased expiratory effort, wheezing, variable nasal discharge, and exercise intolerance.* Increased pulmonary resistance and reduced dynamic compliance are typical functional changes, and the bronchioles are hyperresponsive to agonists. The signs wax and wane depending on exposure to dusts, and a seasonal effect is common, but many cases are slowly progressive over the course of months or years.

The lesions specifically target the small bronchioles, and alveolar changes are usually minor. Bronchioles contain mucus, neutrophils, and sloughed epithelial cells in their lumens; increased numbers of goblet cells in the epithelium with degeneration and sloughing of Clara cells; and lymphocytes, plasma cells, and mast cells in the lamina propria and adventitia (Fig. 5.27). Eosinophils are usually infrequent, but are numerous in some cases; their presence perhaps reflects a different stage of the disease. Peribronchiolar fibrosis and bronchial epithelial hyperplasia are inconsistent. The smooth muscle may be hypertrophied as a result of chronic bronchoconstriction, but muscular

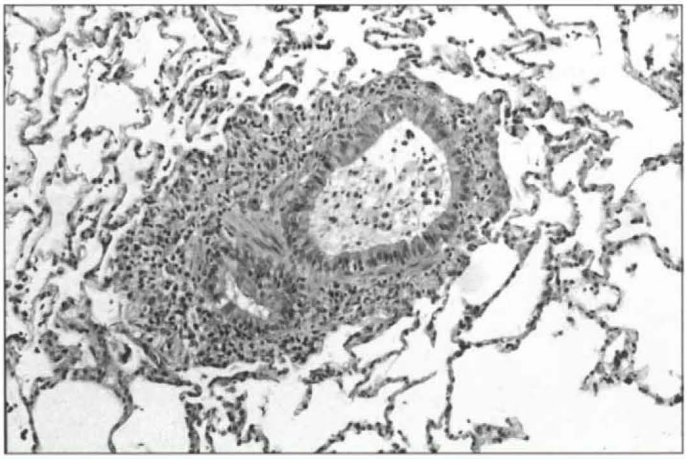

Figure 5.27 Heaves in a horse. The alveoli are normal, but the bronchiole is filled with mucus and neutrophils, and surrounded by lymphocytes.

hypertrophy cannot be readily assessed histologically because the bronchospasm that occurs at death or when tissues are biopsied induces artifactual thickening of the tunica muscularis. Mucus spills into the adjacent alveoli in severe cases, and overinflation of alveoli may result from trapping of air distal to the obstructed bronchioles, but true emphysema with destruction of alveolar septa is not expected. The number of mast cells in the bronchiolar wall and neutrophils in the lumen are positively correlated with clinical severity, although histologic lesions do not correlate well with clinical severity or prognosis.

Inflammatory airway disease is a term used to describe a heaves-like condition in younger horses. It occurs in 2–4-year-old horses, and results in a similar pattern of chronic, episodic, nonseptic bronchiolitis leading to cough, excessive airway mucus production, and/or poor racing performance. Bronchoalveolar lavage cytology reveals neutrophilia in some cases, eosinophilia in others, and some with increased numbers of mast cells. The histologic features are not described. Prior viral infection and exposure to airborne environmental allergens, dusts, and/or endotoxins are proposed causes. Whether it is a different disease or an early stage of heaves is uncertain.

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Bronchopneumonia

The hallmarks of bronchopneumonia are an exudative lesion originating at the bronchiolar–alveolar junction, and an airborne route of entry of the causative agent. Bronchopneumonia is caused by bacterial or fungal infection.

The lesions most often affect the cranioventral regions of the lungs, with neutrophils and sometimes fibrin or macrophages filling the bronchioles and alveoli.

The bronchiolar–alveolar junction is the major site of deposition of 0.5–3.0 μm diameter particles, yet it seems to be particularly vulnerable to bacterial infection. In particular, the terminal bronchioles receive limited protection from the mucociliary clearance that is more active in the larger airways, or from the alveolar macrophages that protect the more distal airspaces. This situation is worsened by the fact that clearance of large volumes of debris from the alveoli requires that this material transits the narrow lumen of the bronchiole, so inflammation of the bronchiole readily obstructs clearance of bacteria and exudates from the alveoli, especially in species that lack collateral alveolar ventilation.

The characteristic cranioventral distribution of bacterial bronchopneumonia in animals is probably largely explained by gravitational influences, which result in both increased deposition of inhaled particles and pooling of aspirated secretions in these regions. There is evidence that *intravenous* administration of *Mannheimia haemolytica* also causes pneumonia in cranioventral regions, lending some support to the idea that defenses might be less effective in this area of the lung.

Causes and predisposing factors

Bronchopneumonia is in most cases caused by opportunistic bacterial pathogens (Table 5.6), and development of disease requires increased exposure of the lung to bacteria, impairment of the pulmonary defenses, or both. *Aspiration pneumonia* is an obvious situation where pulmonary challenge with massive numbers of bacteria overcomes the lung defenses. Common conditions predisposing to aspiration pneumonia in animals include force-feeding or concurrent gastric intubation, vomiting and regurgitation, anesthesia, cleft palate, and neurologic disease causing laryngeal dysfunction. Similarly, in cattle, *stress* leads to increased numbers of *Mannheimia haemolytica* bacteria colonizing the nasopharynx, which presumably results in increased number of bacteria within inhaled droplets. The *causes of impaired lung defenses* have received more attention, and include viral and mycoplasmal infection, exposure to cold, toxic gases, and inherited conditions. Agents or conditions that impair

Table 5.6 Major causes of bacterial bronchopneumonia in domestic animal species

Cattle and sheep	Swine	Horses	Dogs and cats
Mannheimia haemolytica *Histophilus somni* *Pasteurella multocida*	*Actinobacillus pleuropneumoniae* *Pasteurella multocida* *Streptococcus suis* *Haemophilus parasuis* *Actinobacillus suis* *Bordetella bronchiseptica*	*Streptococcus* spp. *Rhodococcus equi* *Actinobacillus* sp. *Klebsiella* sp.	*Bordetella bronchiseptica* *Pasteurella* spp. *Streptococcus* spp. *Staphylococcus* sp. *Escherichia coli* Anaerobic bacteria

mucociliary clearance include exposure to cold, *Bovine herpesvirus 1*, *Bovine respiratory syncytial virus*, *Bovine parainfluenza virus 3*, *Mycoplasma hyopneumoniae*, a variety of toxic gases, and ciliary dyskinesia. Alveolar macrophage function is reduced by *Bovine herpesvirus 1*, *Porcine reproductive and respiratory syndrome virus*, stress, aflatoxin, and T-2 mycotoxin. Conditions that reduce the number or function of circulating neutrophils may also predispose to opportunistic bacterial pneumonia, including parvoviral enteritis, acute *Bovine viral diarrhea virus* infection, chemotherapy, stress, acidosis, uremia, and several mycotoxins.

Morphology

The typical gross appearance of bronchopneumonia is irregular consolidation in cranioventral regions, and most cranioventral lung lesions in domestic animals represent bronchopneumonia. There are, however, several exceptions to this rule. First, some viral infections, including *Bovine respiratory syncytial virus*, typically induce a cranioventral pattern of bronchointerstitial pneumonia that can easily be mistaken for bronchopneumonia. Second, the lesions of bronchopneumonia in swine caused by *Actinobacillus pleuropneumoniae* and *Actinobacillus suis* usually affect the middle or caudal lung lobes. Third, while the lesions of bronchopneumonia in dogs and cats may be cranioventral, it is not unusual to find a patchy distribution throughout the lung. *Consolidated lung is best detected by its firm texture on palpation.* These lesions may be dark red, gray-pink, or gray depending on the age and nature of the process, but a color change without altered texture may simply represent patchy areas of congestion or hemorrhage rather than bronchopneumonia. The cut surface is usually edematous, and catarrhal or purulent material can be expressed from small airways in subacute cases. In contrast, exudate is not apparent in cases of fulminant fibrinous bronchopneumonia because leukocytes are enmeshed in a tangle of fibrin, and the affected lung is discolored, dry, or edematous, and cuts crisply.

The lesions of bronchopneumonia may be lobular or lobar.

- **Lobular bronchopneumonia** is generally less severe and more slowly progressive, and *the angular outlines of individual lobules are visible at the margin of affected and normal lung tissue* (Fig. 5.28). The lobular pattern is most apparent in cattle and swine, which have well-developed interlobular septa.
- **Lobular pneumonia** *is characterized by consolidation of the entire pulmonary lobe* (Fig. 5.29). This is usually a fulminating bronchopneumonia, and typical causes are *M. haemolytica* in cattle, *Actinobacillus pleuropneumoniae* in swine, and aspiration pneumonia. In many such cases, the pulmonary parenchymal lesions are accompanied by *pleuritis*, which may vary from a dull granular appearance to a spectacular coating of fibrin with large pockets of edema.

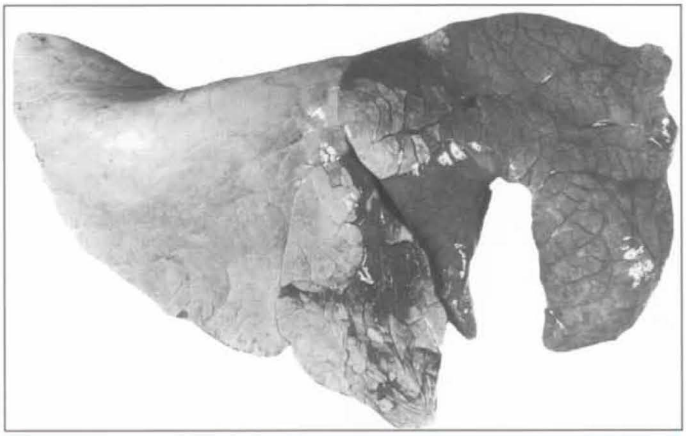

Figure 5.28 Acute lobular bronchopneumonia in a calf. The border between affected and unaffected lung follows a lobular pattern. Lobulation is emphasized in dark areas of consolidation by interlobular edema.

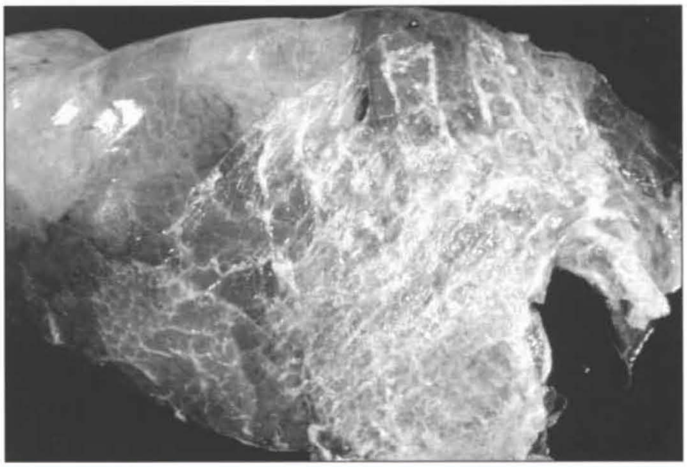

Figure 5.29 *Mannheimia haemolytica* pneumonia in a calf. The cranioventral lung is dark red and hard, and covered by a veil of fibrin.

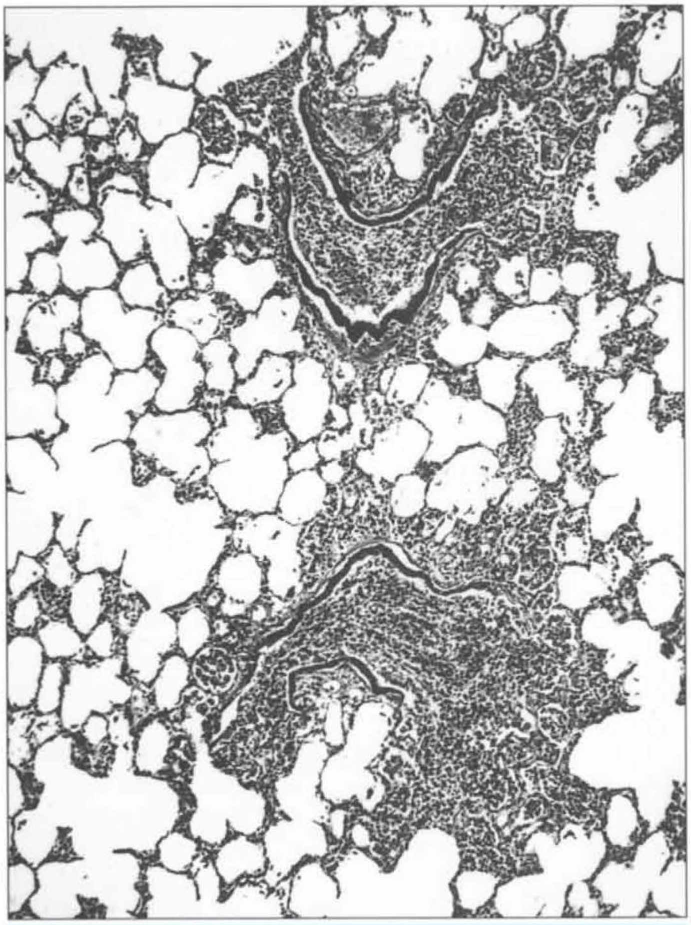

Figure 5.30 Initial lesion of **bronchopneumonia** at bronchioloalveolar junction.

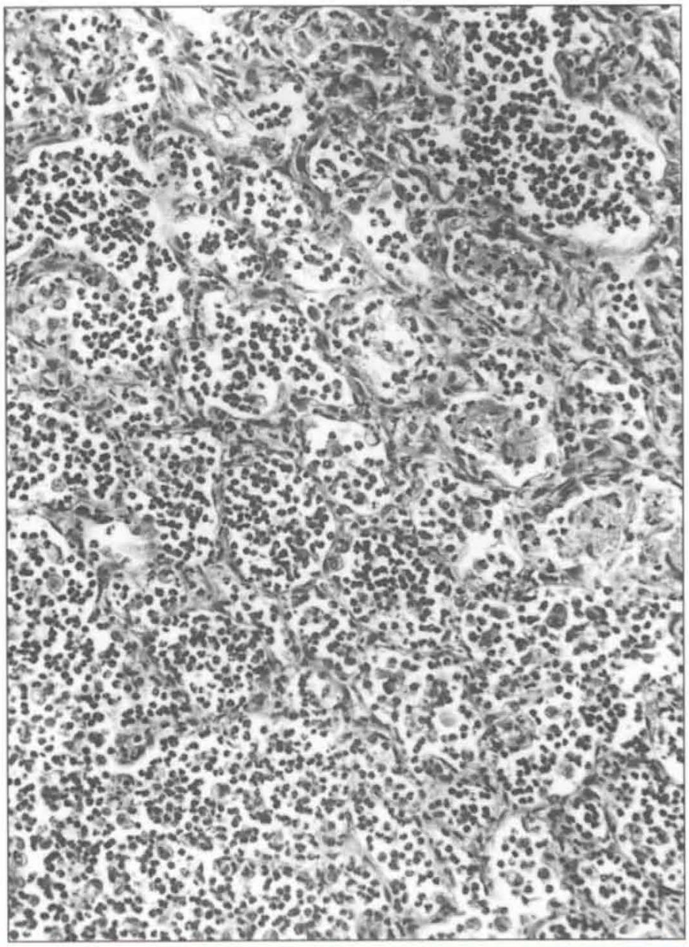

Figure 5.31 Subacute suppurative bronchopneumonia in a pig. Neutrophils fill alveoli.

Histologically, the nidus of inflammation in bronchopneumonia is in the bronchiolar–alveolar junction. In early bronchopneumonia, bronchioles and adjacent alveoli are filled with neutrophils and sometimes cell debris, mucus, fibrin, and macrophages, and the wall of the bronchiole may be edematous and infiltrated by neutrophils (Fig. 5.30). By the time the bronchopneumonia is clinically apparent, the exudate has usually filled the bronchioles and alveoli, and it is often impossible to appreciate that the lesion is centered on the terminal airways (Fig. 5.31). In mild cases the bronchiolar epithelium is usually normal; extensive bronchiolar necrosis suggests an underlying viral infection. However, bronchiolar necrosis can develop during experimental infection with *M. haemolytica* and probably with other bacteria, and is thought to represent neutrophil-mediated damage to the epithelium. Suppurative or lymphoplasmacytic inflammation may be visible in the bronchi. The alveoli in mild or early lesions are atelectatic and edematous, but this is obscured by the suppurative infiltrate in severe lesions.

Resolution and sequelae

Resolution of bronchopneumonia is possible if the infectious agent is destroyed by the immune response or antimicrobial therapy. Neutrophils rapidly undergo apoptosis, and fibrin may be removed by plasmin and/or phagocytosis by macrophages. Macrophages and extracellular debris are mostly cleared through the airways with the aid of coughing and collateral ventilation. If there is minimal damage to the alveolar septa and blood vessels, catarrhal or mild purulent bronchopneumonia can begin to resolve within 7–10 days and return to normal within 3–4 weeks. In ruminants and swine, the lack of collateral ventilation impedes clearance of alveolar exudates, and these species have a greater propensity to develop chronic suppurative bronchopneumonia.

Death from fulminant lobar bronchopneumonia may occur with only 20–40% of the lung affected. In such cases, the cause of death is not respiratory failure but rather *toxemia*, and these animals are profoundly depressed and have grossly apparent petechiation of serosal surfaces and muscles throughout the body. The extent of lung involvement in animals with chronic bronchopneumonia – up to 80% of the lung in some calves with chronic *Mycoplasma bovis* pneumonia – highlights the tremendous reserve capacity of the lung that must be consumed before bronchopneumonia causes death by simple filling of alveoli with exudate.

Chronic suppurative bronchopneumonia *develops if the infection remains active.* In many such cases, the lesions are colonized by secondary pathogens – such as *Arcanobacterium pyogenes* in cattle – and the primary cause can no longer be identified. Chronic bronchopneumonia may manifest simply as filling of bronchioles and alveoli with neutrophils, due to persistent bacterial infection and ongoing recruitment of neutrophils. In other cases, the chronic infection results in *pulmonary fibrosis, bronchiectasis, abscessation, or sequestration.* Extensive fibrinous exudate or injury to alveolar septa may heal by fibroplasia, which may either cause thickening of alveolar septa or locally extensive areas of fibrosis. Organization of fibrin-containing pleural exudate often produces *pleural adhesions. Abscesses* may develop within areas of chronic bacterial infection, and occasionally erode blood vessels to cause fatal hemorrhage.

A **sequestrum** is *a mass of necrotic lung parenchyma, separated from viable lung tissue by purulent exudate, and usually encased in a fibrous capsule.* Sequestra commonly develop from the infarcted lung tissue in contagious bovine pleuropneumonia, and in pneumonia caused by *Mannheimia haemolytica* in cattle or *Actinobacillus pleuropneumoniae* in swine. Expansion of the caseous lesions associated with *Mycoplasma bovis* appears grossly similar. The lesions vary in size from several centimeters to large masses that occupy the entire side of the lung, and are firm, gray or red, and may be friable or have a foul odor. Histologically, the sequestered tissue retains the ghost-like remnants of the alveolar architecture, but all of the cells are necrotic. The surrounding fluid exudate is key to sequestering the nonviable lung tissue, because macrophages cannot effectively swim across the exudate to remove the necrotic tissue. As a result, *sequestra are permanent and nonfunctional, and act as a nidus of persistent bacterial infection.* The term "pulmonary sequestration" describes a quite different condition in human pathology, where a congenitally anomalous mass of pulmonary tissue, which may be separate from or contiguous with the lung itself, is not connected to the bronchial tree and is perfused by an anomalous vascular supply.

Aspiration pneumonia

Aspiration pneumonia refers to pneumonia caused by aspiration of foreign material, often in liquid form, reaching the lungs through the airways. This distinguishes it from most bronchopneumonias that are caused by inhalation of tiny particles. The response to the aspirated material depends on three factors: (1) the nature of the material; (2) the bacteria that are carried with it; and (3) the distribution of the material in the lungs.

Widespread distribution of inhaled milk or combination of milk and gruel is occasionally observed in *pail-fed calves.* The course of the disease in these cases can be as short as 1 day. The gross appearance is not characteristic. The lungs remain inflated; they are hyperemic and small amounts of exudate can be expressed from the small airways. Histologically, there is acute bronchiolitis with various degrees of acute alveolitis. Lipid droplets, and sometimes plant material, can be seen in the lesions. Aspiration of ruminal contents can produce a similar picture in recumbent cattle, but in these cases the aspirated material is usually obvious, and there is often hemorrhagic tracheobronchitis.

When the distribution of foreign material is more localized, discrete foreign-body granulomas, bronchopneumonia, lobar pneumonia, or gangrene of the lungs occurs. Cattle and lambs frequently aspirate inflammatory exudate from necrotic laryngitis. Lambs with nutritional myopathy affecting the muscles of deglutition aspirate milk and plant material, including whole grain. Pigs in dry, dusty environments and fed dry finely particulate food can aspirate starch granules and particles of plants from the feed. *Any cause of dysphagia or regurgitation, including pharyngeal paralysis and megaesophagus, is likely to lead to aspiration pneumonia.* It is also a hazard of anesthesia. Aspiration of vomitus and medications occurs in all species. The aspiration of vomitus in a simple-stomached animal is often rapidly disastrous and death can occur from laryngeal spasm or acute pulmonary edema before there is time for much inflammation to develop. The possibility of aspirated material being responsible must always be considered in any case of fulminating lobar or bronchopneumonia, especially if the history suggests one of the predisposing conditions just mentioned, or if the lesions are unilateral and necrotizing. Careful search will usually reveal evidence of foreign material, but this is not the case when the material is largely or entirely liquid.

Interstitial and bronchointerstitial pneumonia

Interstitial pneumonia *(or interstitial lung disease) is a broad term that describes damage to, or inflammation involving, the alveolar or interlobular septa, which represent the interstitium of the lung.* This contrasts with *bronchitis and bronchiolitis* that involve the airways, and *bronchopneumonia* which forms exudate in the airspaces of the alveoli and distal airways. The terminology used to classify the interstitial pneumonias suffers from a lack of consensus in veterinary pathology. We use the term interstitial pneumonia to define a broad range of conditions affecting the alveolar and interlobular septa. There are many histologic patterns of interstitial pneumonia identified in human pathology, including diffuse alveolar damage, usual interstitial pneumonia, nonspecific interstitial pneumonia, organizing pneumonia, respiratory bronchiolitis, desquamative interstitial pneumonia, lymphoid interstitial pneumonia, and granulomatous interstitial pneumonia; yet the relevance of most of these terms to diseases of domestic animals has not been defined. The term "*atypical pneumonia*" has been used in the past to describe interstitial pneumonias; we suggest that its use be abandoned because it is also used for unusual forms of bronchopneumonia.

Diffuse alveolar damage

The most commonly identified form of interstitial pneumonia is diffuse alveolar damage, which represents diffuse injury to type I pneumocytes or endothelial cells in the alveolar septa, and results in formation of hyaline membranes, proliferation of type II pneumocytes, and interstitial fibrosis. Note that other authors define interstitial pneumonia synonymously with diffuse alveolar damage. *Acute respiratory distress syndrome* is a clinically defined condition with an acute onset of bilateral pulmonary disease characterized by hypoxemia but no evidence of left atrial hypertension, with diffuse alveolar damage as the histologic lesion in many cases.

Gross lesions *are widely distributed throughout the lungs, often with greater involvement of dorsocaudal regions* (Fig. 5.32). This pattern is in sharp contrast to the cranioventral distribution of lesions in most cases of bronchopneumonia. Histologically, most causes of diffuse alveolar damage follow a stereotyped pattern of response, with an acute exudative phase, subacute proliferative phase, and chronic fibrosing phase. Because of the similar histologic appearance of this response, irrespective of the etiology, identifying the specific cause in a particular case is often based on clinical investigations and identifying lesions in other organs.

In the **acute exudative phase**, the alveolar septa are congested and the alveoli contain protein-rich edema fluid with delicate

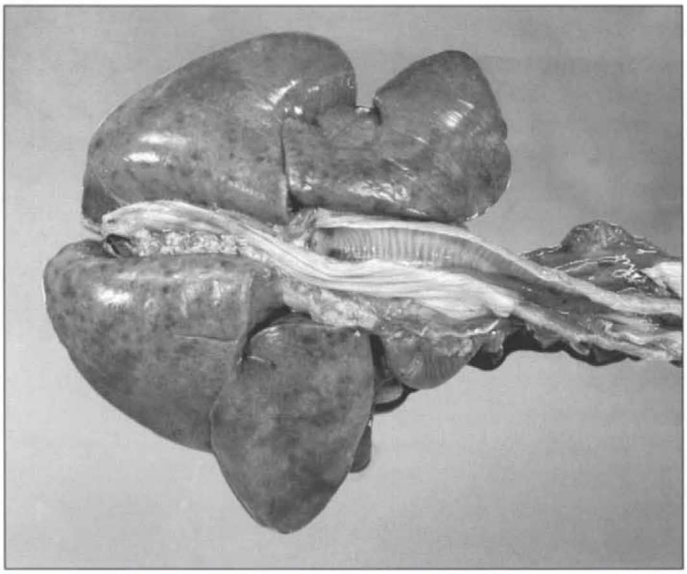

Figure 5.32 Diffuse alveolar damage in a dog. The lung is diffusely rubbery and fails to collapse. Multifocal hemorrhages are present.

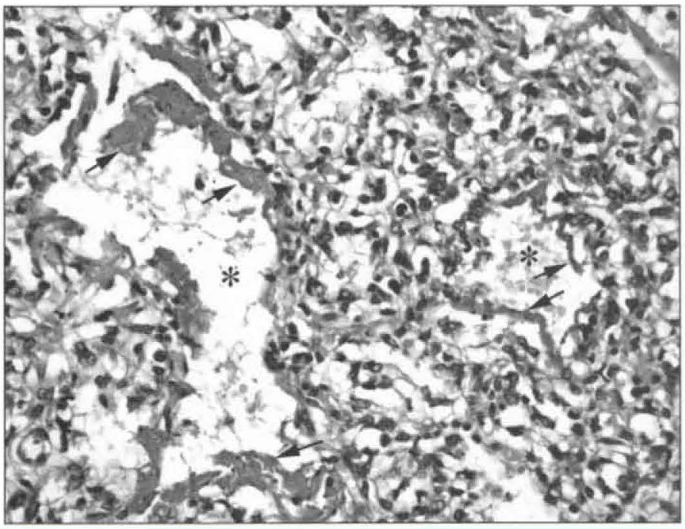

Figure 5.33 Diffuse alveolar damage in a neonatal puppy. The alveoli (asterisks) are lined by hyaline membranes (arrows), and the interstitium is thickened and hypercellular due to immaturity.

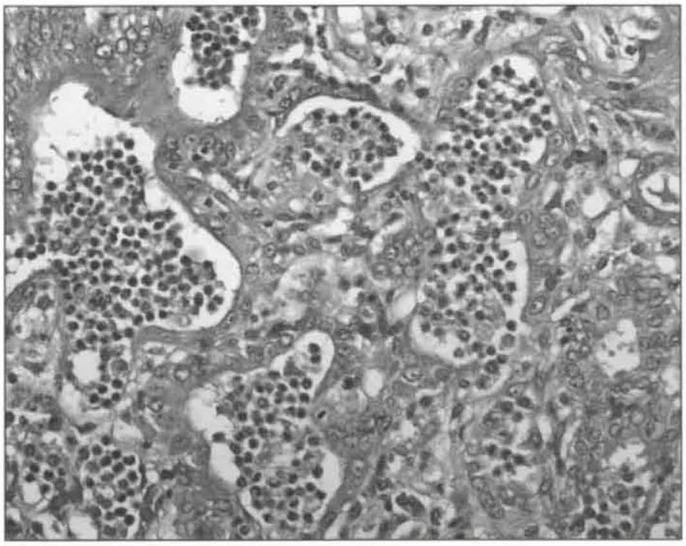

Figure 5.34 Porcine reproductive and respiratory syndrome. Alveoli are completely lined by cuboidal type II pneumocytes, and contain neutrophils and macrophages in the lumen.

interlacing fibrin strands and variable numbers of neutrophils and macrophages. However, *the characteristic lesion is the presence of hyaline membranes,* which are aggregates of fibrin, other serum proteins, and cell debris. These appear as linear masses of discrete, densely eosinophilic material lining the junction between the alveolus and the alveolar septa (Fig. 5.33). Since type I pneumocytes are 10 times less permeable than endothelial cells, injury to type I pneumocytes causes loss of interstitial fluid into the alveolus. Alveolar type II pneumocytes (and possibly type I pneumocytes) reabsorb alveolar fluid by active transport, so injury to these cells further promotes alveolar edema. Although type II pneumocytes cover only 10% of the alveolar surface, they are critical in production and recycling of surfactant lipids and proteins, and their damage leads to abnormal intra-alveolar surface tensions that may further damage the epithelium.

Type II pneumocytes *repair the alveolar epithelium by spreading along the alveolar surface, proliferating to repopulate the epithelium, secreting new basement membrane components, and differentiating into membranous type I pneumocytes.* Type II pneumocytes rapidly spread to cover the denuded alveolar surface prior to undergoing mitosis, but this feature is not recognized in histologic sections. Single type II pneumocytes are impossible to distinguish on hematoxylin and eosin-stained sections from alveolar macrophages; however, type II pneumocytes are more easily recognized when they form a single layer of cuboidal cells lining the alveolus (Fig. 5.34), and cytokeratin immunohistochemistry is useful to detect or confirm subtle lesions. Proliferation of type II pneumocytes is initially observed at 2–3 days and is extensive by 6 days after insult; it represents a dysfunctional stage of epithelial repair because these cuboidal cells block effective gas exchange. Type II pneumocytes are the major source of basement membrane if this structure has been previously damaged. If the injurious stimulus has been removed, then the type II cells differentiate into type I pneumocytes, and surplus cells undergo apoptosis. The cuboidal type II cells may no longer be visible histologically by 5–7 days after a mild single injurious stimulus, but may remain for prolonged periods if the injurious stimulus persists or in the presence of interstitial fibrosis. In some cases, the remarkable proliferation of type II pneumocytes may be mistaken for carcinoma.

Interstitial fibrosis develops in two ways. Fibrinous alveolar exudates may be invaded by fibroblasts and organized into fibrous tissue that resembles granulation tissue in a skin wound; this fibrous tissue is later incorporated into the alveolar wall and covered by type II pneumocytes. Alternatively, fibrous tissue may develop within the alveolar septum itself as a consequence of repetitive, persistent, or severe damage to epithelial or endothelial cells, or by persistent inflammation in the alveolar septum. Fibroblasts can appear within alveoli by 3–5 days after severe fibrinous exudation, collagen fibers can be detected histologically by 5–7 days, and fibrosis may be well developed by 14 days (Fig. 5.35). Myofibroblasts or smooth-muscle cells are a prominent feature of some interstitial pneumonias, such as maedi.

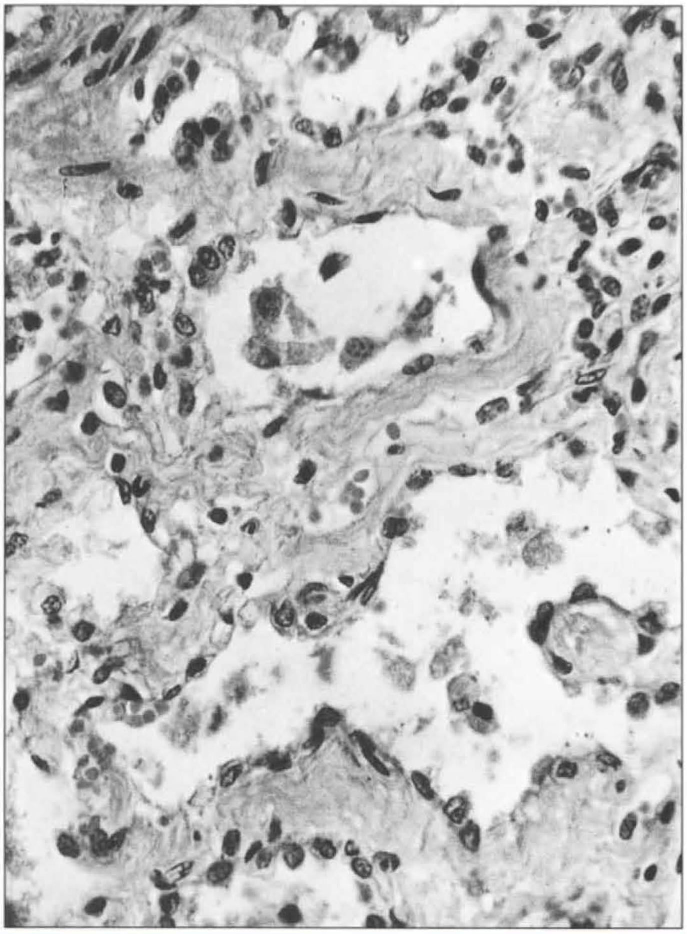

Figure 5.35 Fibrosis of alveolar septa and persistence of type II pneumocytes, as a **sequel to diffuse alveolar damage** in an ox.

The intimate encounters shared by alveolar epithelial cells, fibroblasts, and macrophages are key to understanding the chronic sequelae of diffuse alveolar damage. Normal type II pneumocytes limit fibroblast proliferation and the development of fibrosis; conversely, repetitively injured type II pneumocytes and macrophages secrete transforming growth factor-β and other growth factors that stimulate interstitial fibrosis. On the other hand, growth factors secreted by fibroblasts and macrophages promote type II pneumocyte proliferation and may preclude their differentiation to type I pneumocytes, and this possibly accounts for the observation that fibrotic alveolar septa are often lined by cuboidal epithelial cells. Alveolar fibrosis is not necessarily permanent, as fibrous tissue is remodeled and may be removed by matrix metalloproteases. However, since this process occurs over many months, and because the cause of chronic injury to the alveolar septa is often not identified or eliminated, *the finding of marked interstitial fibrosis confers a guarded prognosis*.

Diffuse alveolar damage causes hypoxemia that is refractory to oxygen supplementation, the difference between alveolar and arterial oxygen tension resulting from the barrier to diffusion of oxygen across the alveolar wall. Carbon dioxide diffuses more readily than oxygen, and hypercapnea is an inconsistent finding. Pulmonary compliance is reduced, which increases the work of breathing and may reduce the functional lung volume. Cardiac function is expected to be normal.

Table 5.7 Causes of acute or chronic diffuse alveolar damage, a form of interstitial pneumonia

- Pulmonary infections: many viruses, *Toxoplasma gondii*, *Feline infectious peritonitis virus*, ascarid larval migration, *Dictyocaulus*
- Thermal injury: inhalation of steam or smoke in barn or house fires
- Acid-induced injury from aspiration of sterile vomitus in monogastric animals
- Toxic gases: nitrogen dioxide, sulfur dioxide, chlorine, 100% oxygen, ammonia, phosgene, ozone
- Ingested toxins: paraquat, kerosene, 3-methylindole, ipomeanol, perilla mint ketone, *Brassica*, Crofton weed
- Septicemia and endotoxemia, disseminated intravascular coagulation, possibly shock
- Massive trauma, strangulation, near drowning, pulmonary contusion
- Ischemic lung injury: lung lobe torsion, reperfusion injury
- Chronic left heart failure
- Pancreatitis, uremia, parvoviral enteritis, irradiation
- Surfactant dysfunction: prematurity (hyaline membrane disease), inherited defects in surfactant proteins B or C
- Ventilator-induced lung injury
- Adverse drug reactions
- Acute hypersensitivity pneumonitis

The causes of diffuse alveolar damage are legion (Table 5.7), and most cause direct injury to alveolar epithelial cells or capillary endothelial cells in the alveolar septa. Many **infectious agents** infect type I pneumocytes, including respiratory syncytial viruses, parainfluenza viruses, herpesviruses, *Feline calicivirus*, adenoviruses, and *Toxoplasma gondii*. Many viruses also infect airway epithelial cells and cause bronchiolar necrosis; this pattern of damage to bronchioles and alveoli represents bronchointerstitial pneumonia, and is discussed below.

Noninfectious causes of direct injury to type I pneumocytes include *thermal and chemical injury* from inhalation of steam or smoke, as occurs in barn or house fires, *acid-induced injury* from aspiration of sterile vomitus in monogastric animals (associated with vomiting, anesthesia, or seizures), and inhalation of *toxic gases* such as chlorine, nitrogen dioxide, sulfur dioxide, phosgene gas, and high concentrations of oxygen. Other xenobiotics must be *metabolized* to reactive intermediates that damage the alveolar epithelium. These include 3-methylindole, ipomeanol, perilla mint, and Crofton weed, and are considered in the section on Toxic lung injury, p. 573.

Ventilator-induced lung injury occurs when high tidal volumes cause injury to the alveolar septa and/or the small bronchioles. The most intuitive mechanism is overdistension of alveoli with damage to the septa, leading to interlobular emphysema, pneumomediastinum, or pneumothorax. Less obvious is the repetitive trauma suffered by alveoli and small bronchioles as they collapse at the end of expiration and are subjected to the high pressures required for reinflation. This mechanical strain and shear stress damages the type I pneumocytes and endothelial cells, and thus results in alveolar edema, formation of hyaline membranes, and infiltration of neutrophils in the alveoli or terminal bronchioles. Finally, ventilator-induced injury may result in neutrophil influx and increased levels of proinflammatory cytokines in the alveolar lining fluid, and this inflammatory response may be stimulated by abnormal stretch or strain pressures on macrophages or epithelial cells. The histologic lesions may include hyaline membranes, bronchiolar necrosis, and/or neutrophil infiltration into alveoli, but it is usually impossible to determine if these lesions are caused by mechanical ventilation or by pre-existing lung

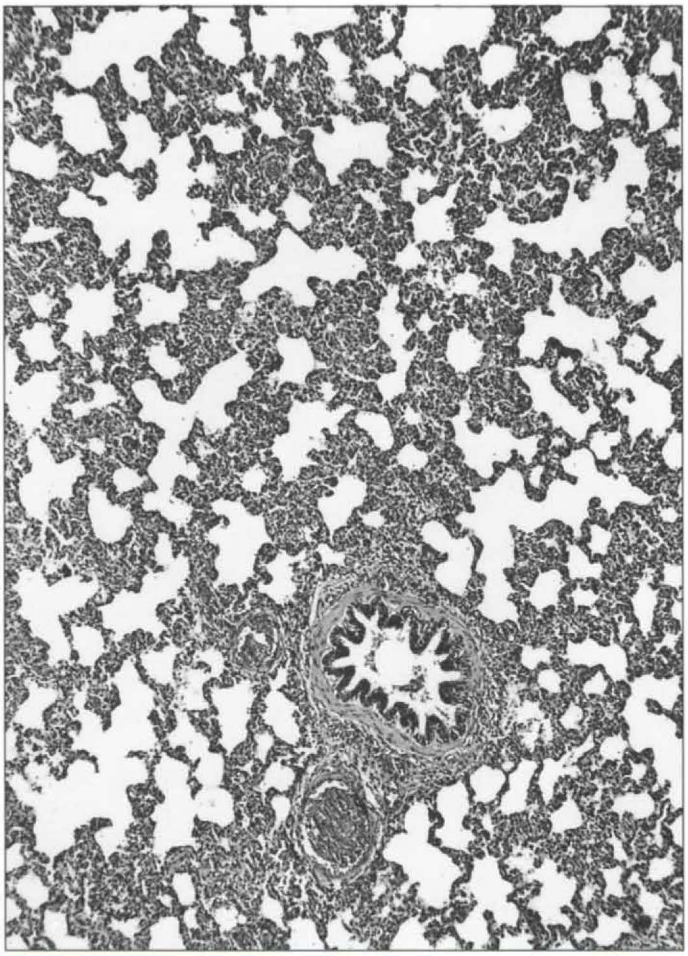

Figure 5.36 Acute interstitial pneumonia in septicemic salmonellosis in a pig. Thickening of alveolar walls by leukocytes.

disease. Pneumothorax, if present, is more suggestive of ventilator-induced injury, if the clinical history is supportive.

Septicemia and endotoxemia *are well-recognized causes of interstitial lung lesions.* Sepsis induces an inflammatory cascade that affects the function of endothelial cells in the alveolar septa, by cytokine-induced retraction of endothelial cells leading to increased permeability, neutrophil-mediated damage to the endothelium or matrix proteins, and/or by promoting capillary thrombosis. Sepsis also causes surfactant dysfunction; in addition to direct injury to surfactant-producing type II pneumocytes, the serum proteins that flood the alveoli inhibit surfactant function. This in turn provokes atelectasis and abnormal surface tensions in the alveoli that physically damage the type I pneumocytes.

Septicemic lung injury may manifest as a pulmonary interstitial reaction, in which the alveolar septa appear hypercellular due to hypertrophy of pulmonary intravascular macrophages and/or aggregation of neutrophils (Fig. 5.36). With more severe disease, there are diffuse or patchy areas within the lung of marked congestion and serofibrinous exudation into the alveoli. Although the alveoli contain loosely fibrillar deposits of fibrin, hyaline membranes and type II pneumocyte proliferation are variably identified in septicemic lung injury. Thrombi may be identified in small venules and capillaries, but this is an infrequent and inconsistent finding in animals dying of septicemia.

Additional causes of diffuse alveolar damage *include massive trauma, shock, disseminated intravascular coagulation, pancreatitis, and uremia.* Although these associations are well established, the exact mechanisms are poorly described. Neutrophil-induced lung injury contributes to diffuse alveolar damage in several experimental models, but the importance of this effect in natural disease is controversial.

Bronchointerstitial pneumonia

Bronchointerstitial pneumonia is characterized by the presence of both bronchiolar necrosis and diffuse alveolar damage, and is thus a manifestation of injury to both the bronchiolar and the alveolar epithelium. Bronchointerstitial pneumonia is commonly caused by aerogenous viral infections that infect both of these cell types, or by toxins that are metabolized by both Clara cells in the bronchioles and type II pneumocytes in the alveoli. This pattern is not to be confused with bronchopneumonia, in which the bronchioles and alveoli are filled with leukocytes as a result of bacterial infection, but necrosis of the bronchiolar or alveolar epithelium is not usually present.

Other forms of interstitial pneumonia

Several forms of pneumonia encountered in veterinary pathology clearly target the alveolar or interlobular septa, but do not follow the pattern of diffuse alveolar damage. Some examples include the following:

- **Granulomatous pneumonia**, caused by mycobacteria, fungi, *Actinobacillus*, *Actinomyces*, *Nocardia*. Miscellaneous causes of granulomatous pneumonia include silicosis, intravenous injection of Freund's complete adjuvant or immunostimulants containing mycobacterial cell wall extracts, and porcine dermatosis vegetans.
- **Maedi**, in which there are perivascular and peribronchiolar cuffs of lymphocytes, and hyperplasia of smooth muscle in the alveolar septa, but usually minimal type II pneumocyte proliferation.
- **Porcine reproductive and respiratory syndrome**, characterized by thickening of alveolar septa with mononuclear cells, and variable hyperplasia and necrosis of pulmonary macrophages. Type II pneumocyte proliferation may be present, but is usually not prominent.
- **Metastrongylid nematode infections**, such as *Muellerius* and *Aelurostrongylus*, in which the nematodes inhabit the alveoli and terminal bronchioles, and incite a chronic inflammatory response, smooth-muscle hyperplasia, and occasionally fibrosis of the alveolar septa.
- **Pneumoconiosis**.

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Specific noninfectious interstitial pneumonias

Anaphylaxis and hypersensitivity pneumonitis

Anaphylaxis and anaphylactoid reaction affect the lungs in cattle, resulting in bronchoconstriction, apnea, pulmonary edema with blood-stained froth in the airways, emphysema, pulmonary hypertension, and systemic hypotension. Bronchoconstriction without marked alveolar changes is more typical of anaphylaxis in cats and horses. The lungs are congested and contain alveolar and interlobular edema, the trachea and bronchi contain froth, and emphysema may result from the marked dyspnea. Congestion or edema of the larynx is prominent and potentially fatal in some cases.

Hypersensitivity pneumonitis (extrinsic allergic alveolitis) in animals occurs mainly in adult cattle reared in confinement and rarely in horses, and often affects multiple animals in the group. It results from chronic inhalation of spores of thermophilic actinomycetes (especially *Saccharopolyspora rectivirgula*, formerly *Micropolyspora faeni*) in moldy hay, leading to inflammatory reactions that occur through several mechanisms concurrently. The bacteria directly activate complement by the alternative pathway and there is solid evidence for immune-complex (type III) reactions, even though the morphology suggests a delayed (type IV) hypersensitivity reaction. In cattle, tiny gray foci may be grossly visible throughout the lung. Lymphoplasmacytic or granulomatous infiltrates are consistently present in the alveolar septa and often around the bronchi and bronchioles, and multinucleate macrophages are occasionally seen. The more characteristic lesions, present during acute exacerbations of disease, are noncaseating granulomas in the alveoli that are accompanied by edema and infiltration of eosinophils, neutrophils, and lymphocytes. Proliferation of alveolar type II pneumocytes, fibrosis of the alveolar septa and peribronchiolar tissue, and bronchiolitis obliterans may be noted in chronic cases. Hyaline membranes are uncommon but may occur following acute challenge.

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Eosinophilic interstitial lung diseases

The eosinophilic interstitial pneumonias are a compilation of pulmonary diseases united by the presence of eosinophils in the alveoli or alveolar septa. They generally correspond to the clinical term **pulmonary infiltrates with eosinophilia**, defined by blood eosinophilia and radiographic evidence of generalized pulmonary infiltrates. The best described are those caused by parasites, which are discussed more fully in later sections but include both adult parasites residing in the lung and migrating larva. Less well characterized are those forms that probably represent hypersensitivity reactions. Those primarily affecting the airways (including eosinophilic bronchopneumopathy) are discussed above. **Eosinophilic pulmonary granulomatosis** is a rare condition in dogs, characterized by chronic cough, variable dyspnea, and blood eosinophilia. The lungs contain nodular necrotizing lesions formed by eosinophils, epithelioid macrophages, and fibrosis, with type II pneumocyte proliferation and eosinophil infiltrates in the surrounding alveoli. Many dogs with eosinophilic pulmonary granulomatosis have heartworm disease, although microfilariae are not detected in the granulomas. Other cases are clearly not infected with *Dirofilaria*, and may be hypersensitivity reactions. **Less severe eosinophilic pneumonias** are poorly described in dogs and cats, in part because the steroid-responsiveness of many cases precludes their examination at necropsy. The alveoli contain eosinophils, lymphocytes, and macrophages, and the degree of fibrosis is variable. **Other causes** of eosinophilic lung disease in dogs and cats include eosinophilic bronchitis and eosinophilic bronchopneumopathy (discussed above), angiotropic T-cell lymphoma (lymphomatoid granulomatosis), eosinophil infiltrates associated with carcinomas and lymphomas, other parasitisms, and fungal infections. Drug reactions cause eosinophil-rich lesions in humans, but are not described in dogs and cats.

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Acute interstitial pneumonia in feedlot cattle

Interstitial pneumonia commonly causes an acute onset of dyspnea and open-mouth breathing in feedlot beef cattle, particularly those approaching slaughter weights, and is most prevalent in the summer and autumn. Heifers are disproportionately affected in many reports, and the case fatality rate is high. The gross lesions are often variegated due to a lobular distribution, and are most prominent in the dorsocaudal lung. The red, rubbery, edematous, heavy lungs fail to collapse when the chest is opened, and interlobular and subpleural emphysema are frequent. Many cases have concurrent gross lesions of acute or chronic bacterial bronchopneumonia in the cranioventral lung. *Hyaline membranes* are the characteristic histologic feature of the acute lesions, and *type II pneumocyte proliferation* is often present. More variable lesions include bronchiolitis obliterans, infiltration of neutrophils and eosinophils, and patchy alveolar hemorrhages that possibly arise from emphysematous rupture of alveolar septa.

The cause is uncertain. Epidemiologic evidence suggests 3-methylindole, melengestrol acetate (fed to feedlot heifers to suppress estrus), dusty environment, hot weather, and concurrent bacterial infection as possible causes or contributing factors. Although *Bovine respiratory syncytial virus* occasionally causes acute bronchointerstitial pneumonia soon after arrival in feedlots, it seems not to cause diffuse interstitial pneumonia late in the feeding period. Some more chronic cases, in which eosinophils and granulomas are present, may represent a hypersensitivity reaction.

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Interstitial and bronchointerstitial pneumonia in foals

Bronchointerstitial pneumonia in 1–4-month-old foals has been recognized as a sporadic disease for many years, yet the etiology remains mysterious. Affected foals may be found dead without premonitory signs or develop acute onset of severe dyspnea, tachypnea, and pyrexia, with profound hypoxemia and variable hypercapnea. Case fatality rates are high and the foals respond poorly to therapy. It remains possible that there is not a single etiology for this disease, but that the reaction pattern may result from any of several infectious and noninfectious insults.

At necropsy, lesions are present throughout the lungs in a diffuse or lobular pattern. The lungs are firm, heavy, edematous, reddened, and fail to collapse. Concurrent lesions of *Rhodococcus equi* pneumonia are present in up to 40% of cases, with cranioventral bronchopneumonia or scattered foci of liquefactive pyogranulomatous pneumonia. *Histologic lesions* reflect the bronchointerstitial pneumonia, and include necrosis of the epithelium lining terminal bronchioles, alveolar hyaline membranes, exudation of fibrin and few neutrophils in alveoli, and syncytial cells in the bronchi or alveoli. The prevalence of syncytial cells and the extent of bronchiolar versus alveolar lesions are variable, supporting the hypothesis that more than one etiology may induce this pattern of reaction.

A viral etiology seems likely, as the age of affected foals correlates with waning maternal passive immunity. A respiratory syncytial virus and *Equid herpesvirus 2* (EHV-2) have been identified in some cases using polymerase chain reaction, indirect immunofluorescence, and enzyme-linked immunosorbent assay antigen detection tests for respiratory syncytial virus; or virus isolation and indirect immunofluorescence tests for EHV-2. However, attempts to identify viral agents in the vast majority of cases have been unsuccessful. Other proposed causes include an aberrant response to *R. equi* or other bacterial pathogens, a complication of hyperthermia resulting from bacterial pneumonia or treatment with erythromycin, endotoxemia, and xenobiotics including 3-methylindole, pyrrolizidine alkaloids, and pentachlorophenol that are metabolized by Clara cells and type II pneumocytes.

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Equine multinodular pulmonary fibrosis

A progressive fibrotic lung disease occurs in adult horses. The lesions are restricted to the alveolar parenchyma, and form *distinctive, grossly apparent nodules of pulmonary fibrosis*. These lesions can be tumor-like, with discrete borders separating the edge of the fibrotic nodule from the unaffected alveolar parenchyma (Fig. 5.37A). The second gross manifestation is of multiple coalescing foci of fibrosis. In each instance, the nodules of fibrosis involve all of the lung lobes. Histologically, the foci of fibrosis follow the septal walls, which are expanded by abundant mature collagen; the lumens of the airspaces frequently contain a mixed inflammatory infiltrate of macrophages and neutrophils. Small numbers of macrophages within the infiltrate are hypertrophic and there are large eosinophilic intranuclear viral inclusion bodies within these cells (Fig. 5.37B). Preliminary analysis of affected tissues has identified consistent presence of a γ-herpesvirus, similar to *Equid herpesvirus 5*. The association of the virus with the fibrotic lung disease remains only an association, and its role in causation is not clear.

Interstitial pneumonia in dogs

Diffuse alveolar damage manifesting clinically as **acute respiratory distress syndrome** is occasionally encountered in dogs. Affected animals develop acute onset of dyspnea with tachypnea, and variable coughing and lethargy, which is usually rapidly progressive and fatal within 3 days. Findings considered secondary to dyspnea include pneumothorax and subcutaneous emphysema, and gastroesophageal intussusception. At necropsy, the lungs are diffusely firm with a liver-like texture, edematous and heavy, and mottled. *Hyaline membranes* are a nearly consistent feature, with proliferation of type II pneumocytes in the less fulminant cases. The predisposing causes include septicemia, bacterial pneumonia in other areas of lung, aspiration of sterile gastric acid, massive trauma, shock, and strangulation. Many cases are idiopathic. Similar findings may be identified in lung lobes that have undergone torsion, but are restricted to the affected lobe.

A syndrome of interstitial fibrosis is described in 6–12-year-old **West Highland White Terrier dogs** with chronic dyspnea, exercise intolerance, or coughing. Histologic lesions include deposition of collagenous matrix in the alveolar septa and interstitial infiltrates of lymphocytes, plasma cells, and macrophages; severely affected cases have proliferation of alveolar type II pneumocytes. The interstitial fibrosis may be diffuse, regional, or multifocal in distribution, and is quite variable in severity. Mildly affected cases have discontinuous deposition of matrix in alveolar septa that is most obvious when it forms concentric rings around alveolar capillaries.

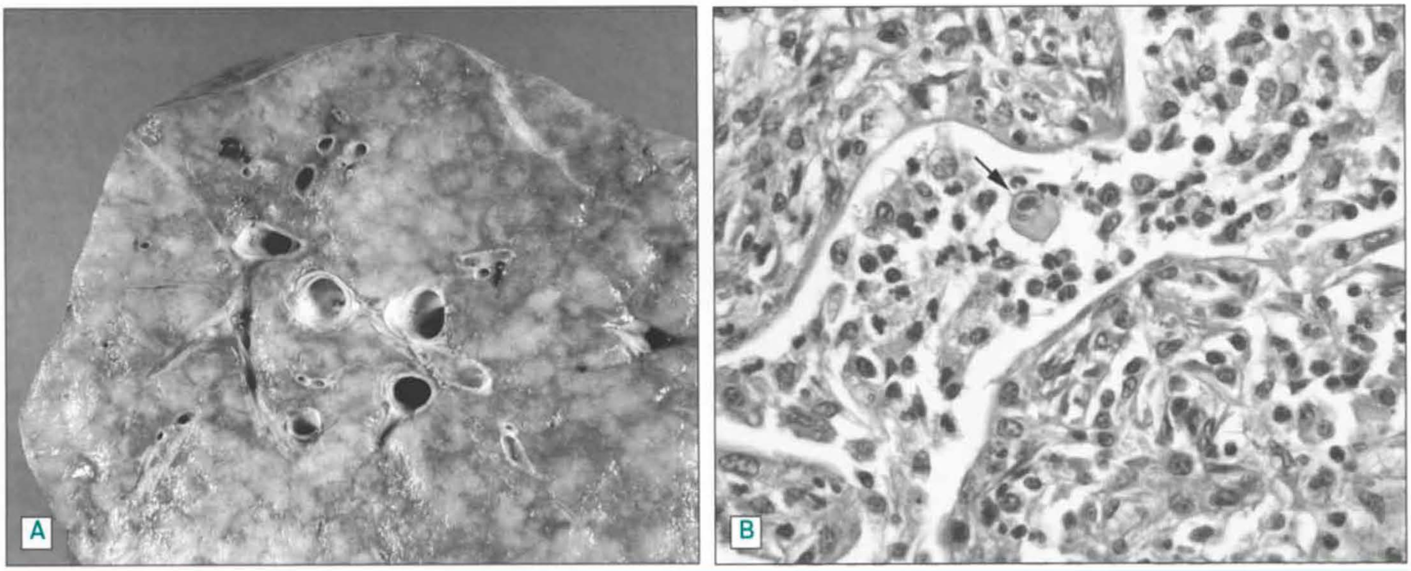

Figure 5.37 Equine multinodular pulmonary fibrosis. A. The entirety of the lung parenchyma contains pale nodules of fibrosis separated by darker regions of less affected lung. **B.** Within the remnant airspaces are neutrophils and macrophages, with small numbers of large macrophages containing prominent intranuclear viral inclusion bodies (arrow).

An apparently inherited condition characterized by diffuse alveolar damage is reported in 4–10-month-old **Dalmatian dogs**. Affected dogs have a short course of tachypnea, increased respiratory noise, and dyspnea. The lungs are diffusely wet, heavy, firm, and purple-red with scattered hemorrhages. The microscopic findings include a patchy distribution of edema, infiltration of lymphocytes and plasma cells, hyaline membranes and type II pneumocyte proliferation, metaplasia and atypia of the bronchiolar epithelium, and focal fibrosis.

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Idiopathic pulmonary fibrosis in cats

Chronic interstitial lung disease is uncommon but not rare in cats, and is usually seen as *chronic progressive tachypnea, respiratory distress, and cough in middle-aged to older cats*. The disease has been likened to idiopathic pulmonary fibrosis (cryptogenic fibrosing alveolitis) in humans. Lesions are often present throughout the lung, but tend to be patchy or multinodular rather than diffuse. The histologic lesions include multifocal or irregular areas in which alveolar septa are remarkably thickened by smooth muscle, fibrosis, and focal clusters of fibroblasts. Alveoli are lined by prominent cuboidal or columnar epithelial cells, which often contain mucus or occasionally undergo squamous metaplasia (Fig. 5.38). There is variation in the relative prominence of the epithelial proliferation and matrix deposition. Interstitial infiltration of lymphocytes is a variable finding, and concurrent bronchioloalveolar carcinomas are identified in some affected cats.

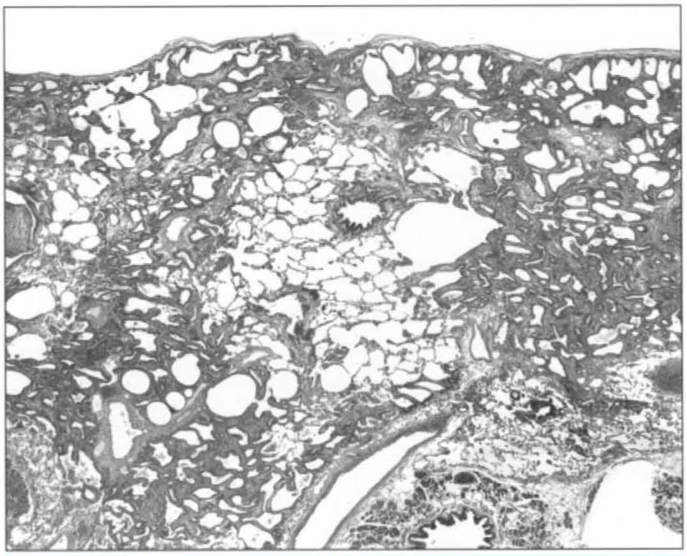

Figure 5.38 Feline idiopathic pulmonary fibrosis. Lung remodeling is patchy, with fibrosis and honeycomb lung interspersed with relatively unaffected lung. Note the extensive subpleural involvement.

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Neonatal respiratory distress syndrome

Interstitial and bronchointerstitial pneumonia is common in neonatal foals and is occasionally encountered in other species. In foals, the causes include *Equid herpesvirus 1*, septicemia, hyaline membrane disease, and meconium aspiration. Other causes of respiratory disease in neonates include bronchopneumonia secondary to placentitis or aspiration of ingesta, and persistent pulmonary hypertension.

The **biology of pulmonary surfactant** *is central to the pathogenesis of hyaline membrane disease.* Surfactant is composed of dipalmitylphospatidyl-choline (DPPC, which is responsible for much of the surface tension-lowering function), other lipids, and the surfactant proteins (SP) A–D. Surfactant lipids are synthesized in type II pneumocytes and some are also produced by Clara cells, initially stored in lamellar bodies, then secreted on to the alveolar surface, where they arrange in a functional surface-active form known as *tubular myelin* that adsorbs to the alveolar air–liquid interface. The biology of the system is quite dynamic: with each breath, some of the tubular myelin is converted to a less functional vesicular form that is recycled by type II pneumocytes or degraded by alveolar macrophages. SP-A, SP-B, and SP-C are important for secretion and recycling of the surfactant lipid, formation of tubular myelin, and the proper insertion of these lipids into the alveolar surface film; SP-A and SP-D also function in innate defense of the lung.

Neonatal hyaline membrane disease is well recognized in premature or full-term foals, and is occasionally seen in premature puppies, calves, lambs, and piglets. The pathogenesis in domestic species is assumed to result from a *failure of the immature type II pneumocytes to secrete functional surfactant*, leading to elevations in surface tension that cause alveoli and small bronchioles to collapse with each exhalation. The tensions and shear stresses during reinflation of these collapsed airspaces injure type I pneumocytes and Clara cells. Abnormalities of surfactant have been documented in premature calves with hyaline membrane disease, but have not been adequately investigated in other species of domestic animals. Fetal hypothyroidism and possibly hypoadrenocorticism are causes of the condition in piglets, because thyroid hormone is necessary for maturation of type II pneumocytes. Other contributing factors in all species are fetal asphyxia, aspiration of amniotic fluid, reduction in pulmonary arteriolar blood flow, and inhibition of surfactant by fibrinogen, other serum constituents in edema fluid, or by components in aspirated amniotic fluid.

Affected foals have respiratory distress from the time of birth, an expiratory grunt or "bark," hypoxemia, and in some cases heart failure and/or convulsions and opisthotonos. The lungs are diffusely atelectatic, plum red, rubbery in texture, and sink or become partially submerged in formalin. Histologically, in addition to the thickened hypercellular alveolar septa expected in immature lung, hyaline membranes line the collapsed alveoli and sometimes the small bronchioles. Alveoli are edematous, and cellular debris is occasionally noted. **Bronchopulmonary dysplasia**, a chronic complication of prematurity, mechanical ventilation, and oxygen therapy in human infants, is characterized by alveolar hypoplasia, thickening of alveolar septa by type II pneumocyte proliferation and fibrosis, bronchiolar damage, and medial hypertrophy of pulmonary arteries; this complication is rare in domestic animals.

Meconium aspiration syndrome is described in human infants, neonatal calves, and rarely in other species. Affected lungs contain variable but usually low amounts of amorphous yellow-orange meconium, keratin, and/or squamous epithelial cells, associated with atelectasis, hemorrhage, and a mild but diffuse alveolar infiltrate of neutrophils, macrophages, and occasional multinucleate cells. Recent investigations of human neonatal mortalities have *questioned whether aspiration of meconium is a cause of the condition, or merely a frequent concurrent finding*. These studies suggest that chronic asphyxia due to placental insufficiency, placentitis causing neonatal sepsis, or acute asphyxia due to prolonged parturition may cause or at least exacerbate the pulmonary lesions and pulmonary hypertension that are characteristic of meconium aspiration syndrome.

Familial forms of neonatal respiratory distress are occasionally encountered but not documented in domestic species. Interstitial pneumonia caused by mutation of the surfactant protein genes are described in humans and rodents, and their manifestations vary between genotypes and species: SP-A deficiency is associated with bronchopulmonary dysplasia and/or increased susceptibility to bacterial infection; SP-B mutations result in alveolar proteinosis, failure of formation of tubular myelin, and/or diffuse alveolar damage; SP-C mutations in humans cause fibrosis of the alveolar septa with infiltration of mononuclear cells, similar to that seen in idiopathic pulmonary fibrosis; and SP-D-deficient mice develop alveolar lipidosis with type II pneumocyte proliferation. A familial form of neonatal respiratory distress is described in pigs, and may result from pulmonary dysmaturity due to congenital hypothyroidism. Affected piglets have diffuse alveolar damage with hyaline membranes and bronchiolar necrosis, and features suggesting hypothyroidism, including mildly prolonged gestation, fine hair coat, generalized edema, and thyroid follicular hyperplasia with lack of colloid.

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Pneumoconiosis

Pneumoconiosis – lung disease ensuing from inhalation and retention of inorganic dusts – is uncommon in animals because they lack occupational exposures that are the basis for most human cases. In general, inorganic dusts are engulfed by and persist within macrophages, which secrete mediators that induce the *characteristic fibrosis and granulomatous inflammation*. Mild pulmonary **anthracosis** is a common incidental finding in city-dwelling animals or those cohabiting with cigarette smokers, in which carbon particles accrue within macrophages adjacent to airway bifurcations. **Asbestosis** is characterized by *asbestos bodies* – linear masses with globose ends formed of asbestos encrusted with ferritin – within granulomatous and fibrosing lung lesions. Sheep are used as an experimental model of the disease, but natural cases are rare. Asbestos-associated mesothelioma is rarely suggested in domestic animals.

Silicate pneumoconiosis in horses and, rarely, dogs is the only reported clinically important pneumoconiosis in animals, and occurs in certain geographic areas containing crystalline forms of free silica in the soil. Particles that are 0.5–5 μm in size are most likely to be retained in the distal airspaces, and filamentous particles are more likely to cause disease than amorphous ones. The clinical signs included weight loss, exercise intolerance, and dyspnea. Miliary lesions are distributed throughout the lung, and consist of extensive areas of fibrosis containing multifocal granulomatous infiltrates, often with necrosis and mineralization in the centers. Birefringent, eosinophilic, or brown crystals are indistinctly visible in the cytoplasm of the macrophages, or sometimes also free in the tissue, and are best detected by electron microscopy and identified by X-ray spectroscopy.

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Lipid pneumonia and alveolar filling disorders

Lipid pneumonia *is a special form of aspiration pneumonia in which droplets of oil are aspirated into the lung.* Spectacular flooding of the lungs occurs when mineral oil is drenched through a stomach tube accidentally placed into the lung of horses or cattle with gastrointestinal disease, or more variable amounts may be aspirated if the oil is carelessly given per os. Cases in cats result from administration of laxatives containing mineral oil. Oily droplets may be noticed in the trachea and bronchi, or when sections of lung are immersed in formalin. The reaction is typically dominated by macrophages, but the appearance depends on the nature of the oil. In general, *vegetable oils* such as olive oil are not irritating and they are eventually resorbed with little reaction or fibrosis. *Oils of animal origin* are irritants and provoke early exudation of serofibrinous fluid and leukocytes. This is later replaced by foamy macrophages and giant cells that fill the alveoli; the alveolar septa are thickened by mononuclear cells and fibrosis. The oil is ultimately resorbed. The purest cellular response occurs to *mineral oil*, which is the usual offender in animals. Mineral oil can be identified by its permanence and by its failure to stain with osmic acid. The lipid is both extracellular and intracellular. Lipid-laden macrophages tend to fill the alveoli, and in time, they accumulate in the lymphatics that surround the bronchi and blood vessels. Fibrosis of alveolar septa and proliferation of alveolar type II epithelial cells are conspicuous, and the foamy macrophages tend to be incorporated into the alveolar septa by extension of the fibroplasia. Unless complicated by secondary bacterial infection, the lesions have a characteristic yellowish, homogeneous, or finely mottled appearance. The bronchial lymph nodes occasionally contain droplets of oil. *Pneumonias caused by aspiration of exogenous lipid must be differentiated from the so-called endogenous lipid pneumonias described below.* The most important distinguishing feature is that, in lipid aspiration pneumonias, there are extracellular globules of lipid. In paraffin-embedded sections, these appear as clear spherical spaces with distinct borders formed by the compressed cytoplasm of macrophages and giant cells.

Alveolar filling disorders *are a group of conditions characterized by accumulation of abnormal material within alveoli.* They are usually incidental findings but are occasionally responsible for clinical disease. In general, material may accumulate in alveoli because its clearance is impeded by obstructed airways, it is produced in excess, or its removal is impaired. These conditions are often overlapping, and include alveolar histiocytosis, endogenous lipid pneumonia, alveolar proteinosis, alveolar phospholipidosis, pulmonary hyalinosis, and alveolar microlithiasis.

Alveolar histiocytosis and **endogenous lipid pneumonia** (foam-cell pneumonia, cholesterol pneumonia) *are opposite ends of a spectrum characterized by focal accumulations of foamy macrophages in alveoli.* The condition is mostly encountered in laboratory rodents and mustelids. Endogenous lipid pneumonia is infrequently reported in cats, and minor lesions of alveolar histiocytosis are common in dogs. Many cases are idiopathic, but some are sequelae to obstruction of airways by exudates, bronchoconstriction, tumors, or anomalous bronchi, with the result that lipids such as surfactant accumulate within alveoli. Drug-induced alveolar phospholipidosis in laboratory rodents is a well-described sequel to administration of a group of cationic amphophilic drugs. Lipid storage diseases and injury to type II pneumocytes by particulates and irritant gases are additional causes described in humans. The lesions should be distinguished from the foamy macrophages encountered in *Pneumocystis*, *Histoplasma*, *Leishmania*, or environmental mycobacterial infections. Grossly, the lungs have irregularly distributed, often subpleural, yellow-white, firm foci that appear as sharply defined flecks or bulging nodules that may exceed 1 cm diameter (Fig. 5.39). Histologically, *lesions of alveolar histiocytosis consist of alveoli filled with foamy macrophages*, with only small amounts of interstitial fibrosis and accumulation of lymphocytes and plasma cells. The lesion is considered *endogenous lipid pneumonia* if lipids can be demonstrated within the macrophages by oil red O or Sudan black stains on frozen sections. In severe cases, there are intracellular and extracellular cholesterol crystals (but no extracellular lipid droplets), more severe interstitial fibrosis, accumulation of neutrophils and mononuclear cells, and type II pneumocyte proliferation. The large cholesterol crystals stimulate development of giant cells and intra-alveolar fibroplasia.

Alveolar proteinosis *(lipoproteinosis) is a rare but clinically significant disorder characterized by accumulation within alveoli of acellular eosinophilic material consisting of surfactant proteins and phospholipids.* The material is strongly periodic acid–Schiff (PAS)-positive and diastase-resistant, and ultrastructurally consists of lamellar and tubular myelin-like arrays. Alveoli may be lined by type II pneumocytes, but inflammation and fibrosis are usually minimal. The accumulation of surfactant lipids and proteins may result when they are produced in excess by type II pneumocytes, as occurs in rabbits and rats following inhalation of silica dust, surfactant protein (SP)-B deficient humans, or SP-D knockout mice. Alternatively, impaired degradation of these lipids and proteins by alveolar macrophages induces a similar lesion, as occurs in granulocyte–macrophage colony-stimulating factor

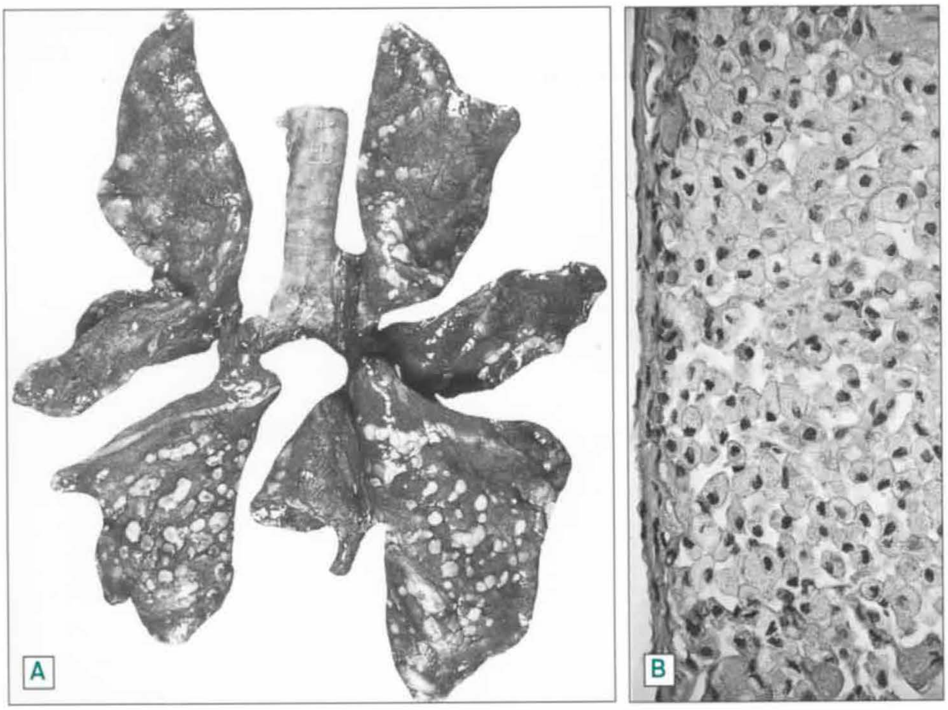

Figure 5.39 Endogenous lipid pneumonia in a cat. **A.** Multiple discrete pale subpleural nodules, consisting of (**B**) accumulation of foamy macrophages within alveoli.

(GM-CSF) knockout mice, humans with autoantibodies to GM-CSF, interleukin-10-induced inhibition of GM-CSF function, or inherited absence of the receptor. A similar lesion occurs in goats with pneumonia caused by *Caprine arthritis encephalitis virus*.

Pulmonary hyalinosis, *which consists of multifocal accumulations of macrophages and giant cells containing hyaline or laminated material*, is seen as an incidental finding in the lungs of old dogs. Histologically, the cytoplasm of macrophages and giant cells is greatly distended by amorphous or laminated, amphophilic, strongly PAS-positive material. This finding is in contrast to the extracellular deposits seen in pulmonary alveolar microlithiasis, discussed in the next section.

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Pulmonary mineralization and alveolar microlithiasis

Metastatic mineralization of the pulmonary interstitium is a feature of uremic pneumonopathy in dogs with chronic renal failure, and similar lesions may result from hypercalcemia, hyperphosphatemia, or alkalosis. Mineralization occurs within the smooth muscle and connective tissue fibers in alveolar septa, pulmonary veins, and the walls of bronchioles. There are often no gross lesions, or the lung may be gritty and porous. Alveoli are edematous and contain increased numbers of macrophages, but other evidence of inflammation is minimal in dogs. Alveolar septa and pulmonary veins, like the gastric mucosa, may be predisposed to mineralization because of the alkaline microenvironment resulting from carbon dioxide excretion. Mineral deposits in the lung undergo *ossification* with time.

Pulmonary alveolar microlithiasis is characterized by *laminated and mineralized PAS-positive mineralized concretions* that form extracellular deposits in the alveoli and alveolar septa, and may become ossified. This condition is rarely reported in animals, but may be associated with clinical signs of respiratory disease if the lesions are extensive. An analogous condition in humans has an apparently familial basis in some cases, while others are sequelae to heart failure. Minor lesions of *focal ossification of the pulmonary interstitium* are occasionally encountered as incidental findings in dogs and cattle.

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Toxic lung injury

The lung is the target of toxic injury by several mechanisms: inhalation of gases or fumes that are directly toxic to epithelial or endothelial cells, ingestion or inhalation of toxins that are metabolized to reactive intermediates mainly by Clara cells or type II pneumocytes, hypersensitivity reactions, effects of inhaled persistent fibers such as asbestos or fiberglass, and xenobiotic-induced carcinogenicity.

Severe lung injury from **inhalation of toxic gases** is rarely encountered in domestic animals because they do not have the occupational exposures that are usually responsible in humans. The anatomic location of injury by inhaled toxins depends on the dose, chemical reactivity, aqueous solubility, and particle size. Water-soluble gases such as sulfur dioxide and ammonia usually cause upper respiratory injury, whereas less soluble gases such as nitrogen dioxide are more widely distributed and may cause bronchiolar or alveolar injury. Poisoning of cattle, pigs, and chickens by nitrogen dioxide (silo gas) may cause massive mortality with lesions of pulmonary edema and congestion, necrosis of bronchial and bronchiolar epithelium, and alveolar hemorrhage and/or fibrin exudation. Acute pulmonary injury caused by **smoke inhalation** is seen in animals trapped in burning buildings. When asphyxiation is not immediate, the combined chemical and heat effects of smoke can cause widespread epithelial necrosis and exudation, and death within a few days.

Exposure to 85–100% **oxygen** damages capillary endothelium and type I pneumocytes, and causes serofibrinous exudation in alveoli or diffuse alveolar damage. In intensive care patients, it may be impossible to determine if diffuse alveolar damage was caused by the underlying disease, or hyperoxic or ventilator-induced lung injury. *Reactive oxygen species* (superoxide, hydroxyl radicals, and hydrogen peroxide) are currently favored as the active metabolites, and these cause cellular injury by lipid peroxidation, protein oxidation, and DNA strand breaks. Neutrophil-dependent lung injury and dysfunction of surfactant are additional proposed mechanisms of hyperoxic lung injury.

The toxicity of other compounds depends on their **conversion to reactive intermediate metabolites by phase I enzymes**, including the family of toxin-specific cytochrome P450 (CYP) isozymes. The CYP isozymes have a species-dependent distribution in nasal tissue, Clara cells, type II pneumocytes, and to a lesser extent in ciliated epithelial cells, type I pneumocytes, endothelial cells, and pulmonary macrophages. The reactive intermediates are further detoxified by phase II enzymes, including glutathione-S-transferases and glucuronyltransferases, to form water-soluble metabolites that are excreted. However, toxicity may develop when the phase II systems are inadequate (e.g., when glutathione is depleted or glutathione-S-transferase activity is impaired or absent) or overwhelmed by high concentrations of the reactive metabolite. In these situations, the electrophilic metabolite binds to essential proteins or nuclei acids, or leads to formation of oxygen radicals that damage cell membrane lipids. Thus, *the cellular susceptibility to toxic injury depends not only on cell-specific expression of the appropriate cytochrome P450 isozymes, but also on the adequacy of the antioxidants (glutathione, vitamin E, superoxide dismutase, and others) and phase II enzymes that protect the cell from injury.* Inherited polymorphisms in CYP genes and induction of CYP by glucocorticoids or other drugs may also influence the susceptibility of individual animals to pulmonary toxins.

3-Methylindole toxicity *causes acute bovine pulmonary emphysema and edema, or fog fever, in cattle.* L-tryptophan in grazed pastures is metabolized in the rumen (or the large colon of nonruminants) to 3-methylindole, and this is converted primarily by CYP to an electrophilic intermediate that alkylates cellular macromolecules, resulting in lipid peroxidation and membrane damage. The early ultrastructural changes are necrosis of nonciliated and ciliated bronchiolar epithelial cells and type I pneumocytes, and transient swelling of endothelial cells. Although type II pneumocytes have high CYP activity, the high levels of glutathione and phase II enzymes are thought to spare these cells from injury. The disease affects adult cattle in the autumn, within 4–10 days after they are moved from dry to lush pastures; younger animals are resistant to the toxic effects of 3-methylindole. Affected animals have acute onset of dyspnea with expiratory effort, tachypnea, open-mouth breathing, and froth in the mouth. The trachea is filled with foam, and the lungs contain striking alveolar and interlobular edema, resulting in heavy wet lungs that fail to collapse. Interlobular or bullous emphysema is prominent in the caudal lobes in less fulminant cases. Histologically, hyaline membranes line the edematous alveoli and alveolar ducts, and type II pneumocyte proliferation is extensive in the subacute stages. The alveolar septa are distended by edema, and may contain eosinophils and/or neutrophils in natural cases. Although bronchiolar lesions are often absent in natural cases, bronchiolar necrosis is described following intentional administration of 3-methylindole. The *differential diagnoses* for this histologic picture includes other toxins listed below; anaphylaxis, which causes pulmonary edema, congestion, emphysema, and rarely eosinophil infiltration, but usually not hyaline membranes; extrinsic allergic alveolitis, in which lymphocytes and granulomas form within alveolar septa; viral pneumonia, where bronchiolar and alveolar lesions are usually both present and syncytia may occur; and acute infection with *Ascaris suum* or *Dictyocaulus viviparus* larvae, which generally occur in younger calves and have many eosinophils with at least a few histologically visible larvae. In contrast to the predominantly alveolar damage in ruminants, experimental 3-methylindole toxicity in horses causes necrosis of Clara cells in the bronchioles, probably reflecting the species-dependent distribution of cytochrome P450.

4-Ipomeanol from moldy sweet potatoes induces a comparable condition in calves, of diffuse alveolar damage with or without bronchiolar injury following CYP-induced formation of a reactive intermediate. **Perilla ketone** toxicity (from perilla mint, *Perilla frutescens*) in cattle and horses follows a similar pattern. Other toxicants causing diffuse alveolar damage in cattle include stinkwood (*Zieria arborescens*, a small tree in southeastern Australia), moldy garden beans, *Brassica* (turnip tops or kale), and rape. **Carbolic dips** are reported to cause proliferative interstitial pneumonia in sheep. Pulmonary lesions are also produced in horses, pigs, sheep, and cattle by **pyrrolizidine alkaloids** (*Crotalaria, Trichodesma, Senecio*); the resulting vascular lesions are described in the section on Pulmonary vasculitis, pulmonary hypertension, above. **Crofton weed** (*Eupatorium adenophorum*) is a poisonous plant that produces chronic interstitial pneumonia in horses. Ingestion of the flowering plant causes multifocal or diffuse chronic interstitial pneumonia in which proliferation of alveolar epithelial cells and fibroplasia are the prominent features.

Paraquat *is a herbicide that causes acute diffuse alveolar damage*, and is most often encountered in dogs and cats. Paraquat undergoes reduction-oxidation (redox) cycling in alveolar type II pneumocytes, which depletes the cell of NADPH and glutathione and results in uncontrolled generation of superoxide anion. Cases of malicious poisoning are more likely to cause fulminating pulmonary edema and hemorrhage because of the high dosage, whereas in accidental poisonings there is more often time for hyperplasia of alveolar type II cells and fibroplasia to be superimposed on the earlier exudative changes. In the acute intoxication with survival up to 2–3 days, the lungs are heavy, dark, and rubbery, and the alveolar spaces are filled with fluid and blood with much fluid in the hilar connective tissue. Thin hyaline membranes are present in alveolar ducts. With longer survival, profuse fibroplasia and type II pneumocyte proliferation thicken the alveolar septa. Extrapulmonary lesions are often present and include patchy necrosis of the adrenal zona glomerulosa and of renal tubular epithelium. Poisoning by the rodenticide α-naphthylthiourea (ANTU) also causes respiratory distress but it causes pulmonary edema and pleural effusion without the tendency to epithelial hyperplasia and fibroplasia.

Drug reactions are important causes of acute or chronic pulmonary injury in humans, manifesting as pulmonary edema, asthma-like disease, bronchiolar necrosis, diffuse alveolar damage, eosinophilic pneumonia, pulmonary vascular disease with hemorrhage, or pleural effusion. Pulmonary drug reactions are poorly described and apparently rare in domestic animals.

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Embolic pneumonia

Pulmonary abscesses usually arise either from chronic bronchopneumonia or from septic emboli lodging in the pulmonary vessels (Fig. 5.40). A cranioventral location and associated bronchiectasis are evidence of origin from bronchopneumonia. Multiple, widely distributed abscesses usually indicate hematogenous origin and are often associated with an obvious source of septic emboli elsewhere in the body, such as endocarditis or hepatic abscesses with phlebitis of the hepatic vein in cattle. The occurrence of abscesses in other tissues supports a hematogenous route of infection. *Other causes* of pulmonary abscess include aerogenous fungal infections that often have a disseminated pattern, aspirated foreign bodies such as plant awns, direct traumatic penetration of the lung, and specific hematogenous infections such as caseous lymphadenitis and melioidosis. It is often impossible to determine the pathogenesis of isolated abscesses, if lesions are not identified in other tissues. Abscesses may erode through the pleura to cause empyema, through blood vessels to cause massive blood loss, or into a bronchus to cause fatal airway obstruction or fulminant suppurative bronchopneumonia.

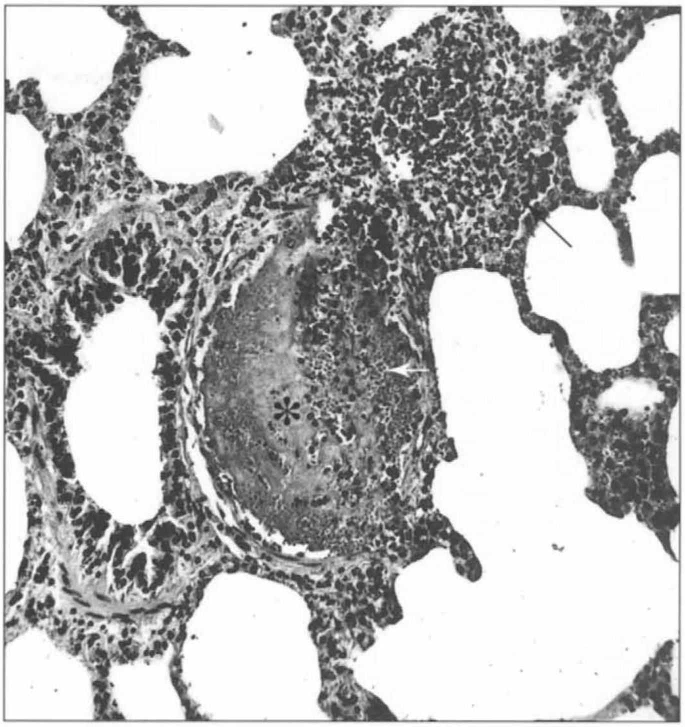

Figure 5.40 Suppurative embolic pneumonia in an ox. Thrombosis (asterisk) of a pulmonary artery with bacteria (white arrow) and neutrophil infiltration (black arrow) of the vessel wall and surrounding alveoli.

PLEURA

The relatively simple anatomy of the pleura belies the underlying complexity of the tissue. Mesothelial cells form a thin continuous layer and produce an underlying basal lamina. Although the thoracic mesothelial lining is contiguous, it is subdivided into *parietal pleura*, covering thoracic wall, diaphragm, and mediastinum; and *visceral pleura*, covering the lungs.

Mesothelial cells are flat cells with surface microvilli on their apex; tight junctions present between the cells have an important role in maintaining proper thoracic fluid balance. Mesothelial cells sample materials within the thoracic cavity using both pinocytosis to take in pleural fluid as well as phagocytosis to engulf particles such as bacteria. The cells participate in inflammation through production of cytokines and deposition of extracellular matrix; mesothelial cells are capable of producing large amounts of collagen and other extracellular matrix proteins. Both procoagulant and anticoagulant proteins are produced by the mesothelium, and an imbalance in these proteins, especially plasminogen-activator inhibitor, can promote pleural fibrin deposition.

Mesothelial cells normally have little baseline mitotic activity, and their ability to replace themselves following injury is not well understood. In response to pleural injury, mesothelial cells become cuboidal, and during long-standing stimulation often form villus projections into the pleural cavity covering a core of mesothelial-derived extracellular matrix and small capillaries.

The pleura has limited local defenses. *Kampmeier's foci*, found within the parietal pleura of the costal surface and mediastinum, are comprised of aggregates of lymphocytes and macrophages and are covered by a layer of modified mesothelial cells that are assumed to function similarly to mucosal-associated lymphoid tissues in the surveillance of the pleural fluid. Grossly, these aggregates may be seen as small white foci within the costal parietal pleura.

Pleural fluid is present in very small amounts (1–2 mL) in health. It provides for mechanical coupling between the lung and the chest wall in the negative-pressure environment of the thoracic cavity. Additionally, it acts as a lubricant between the lung and thoracic wall during respiration. Pleural fluid enters the thoracic cavity through stomata in the costal parietal pleura, following a hydrostatic gradient, and is pulled into the pleural space with the recoil of the lung during respiration. Pleural fluid is removed through visceral and parietal pleura through stoma, and to a lesser extent by transcellular means through mesothelial cells. **Pleural effusion** *occurs when filtration of fluid exceeds reabsorption*. Less commonly, pleuroperitoneal migration of fluid may result in fluid accumulation in the thoracic cavity through microscopic defects in the diaphragm when ascites is present.

Congenital anomalies of the pleura and mediastinum are of little significance. *Congenital cysts of branchial pouch origin* are most often encountered in brachycephalic dogs. They are found in the cranial mediastinum, often in close association with thymic tissues, and are lined by a single layer of cuboidal epithelium. *Bronchogenic cysts* are occasionally identified in the mediastinum. **Degenerative** changes in the pleura occur in uremia in dogs, with mineralization of elastin and collagen fibers in the parietal pleura of the intercostal spaces. *Pleural cysts* lined by keratinizing squamous epithelium are described in horses, but their cause is uncertain. Tetrathyridia of the cestode *Mesocestoides* sp. cause cystic lesions on the pleural surface.

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Pneumothorax

Pneumothorax *refers to the presence of air or gas in the pleural cavities, and results in atelectasis because negative intrapleural pressure cannot be maintained*. In normal animals, the diaphragm moves caudally and an in-rush of air is heard when the diaphragm is pierced at necropsy; these features are absent in cases of pneumothorax. In small animals, where detection of this diaphragmatic movement and in-rush of air in normal animals may be challenging, pneumothorax can be detected by opening the chest under water and observing the escape of air bubbles.

Pneumothorax can be spontaneous or traumatic. *Spontaneous pneumothorax* is most commonly caused by rupture of emphysematous bullae. Other causes include rupture of a cavitated abscess or pyogranuloma that communicates with an airway, or of a parasitic cyst in paragonimiasis. *Traumatic pneumothorax* is usually the result of accidental puncture of the thoracic wall and/or visceral pleura. Traumatic pneumothorax can also be a complication of cardiac resuscitation or biopsy of the lung. Air that tracks through the pulmonary interstitium to the mediastinum (*pneumomediastinum*) does not usually escape into the pleural cavities unless there is traumatic rupture of the mediastinum.

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Noninflammatory pleural effusions

Hydrothorax *is the accumulation in the pleural space of transudate, which is clear, watery, colorless, or light yellow, and has a low protein and cell content*. Chronic hydrothorax causes pleural opacity because of reactive hyperplasia of mesothelial cells, which produce fibrous thickening of the underlying pleural connective tissue. Hydrothorax is caused by increased venous pressure, lymphatic obstruction, and rarely hypoproteinemia; or by extension from a peritoneal effusion. It is particularly common in cats with cardiomyopathy, and occasionally develops from congestive heart failure in other species. Hydrothorax is also a feature of specific diseases such as "mulberry heart disease" in swine, black disease in sheep, African horsesickness, and ANTU poisoning.

Chylothorax *is the accumulation in the pleural space of lymph, which appears milky and has a high triglyceride and lymphocyte content* (Fig. 5.41). The diagnosis is confirmed by measurement of a higher triglyceride concentration in the fluid than in serum, or a reduced cholesterol-to-triglyceride ratio. Chylothorax is seen in cats and occasionally dogs, and *most cases are idiopathic*. Recognized causes include cardiomyopathy and right-sided heart failure, where increased central venous pressure prevents emptying of lymph from the thoracic duct into the vena cava; thoracic masses such as lymphoma, thymoma, or granulomas that cause thoracic duct obstruction; or infrequently dirofilariasis, lung lobe torsion, diaphragmatic hernia, or congenital anomalies in Afghan Hounds. Traumatic rupture of the thoracic duct is a well-known but exceedingly rare cause. *Chylous effusion causes atelectasis and stimulates noninflammatory pleural fibrosis*. Repeated removal of the effusion may induce dehydration, electrolyte disturbance, depletion of lipids and fat-soluble vitamins, hypoproteinemia, and lymphopenia.

Hemothorax *is the presence of blood in the pleural cavity*. It is most often the result of traumatic rupture of blood vessels, but it can also be caused by coagulopathies such as anticoagulant rodenticide toxicity, rupture of highly vascularized tumors such as hemangiosarcoma, lung lobe torsion, or erosion of the wall of a vessel by an inflammatory or neoplastic process. Chronic hydrothorax or chylothorax may lead to the development of well-vascularized papillae on the pleura, and rupture of these may result in a blood-stained effusion.

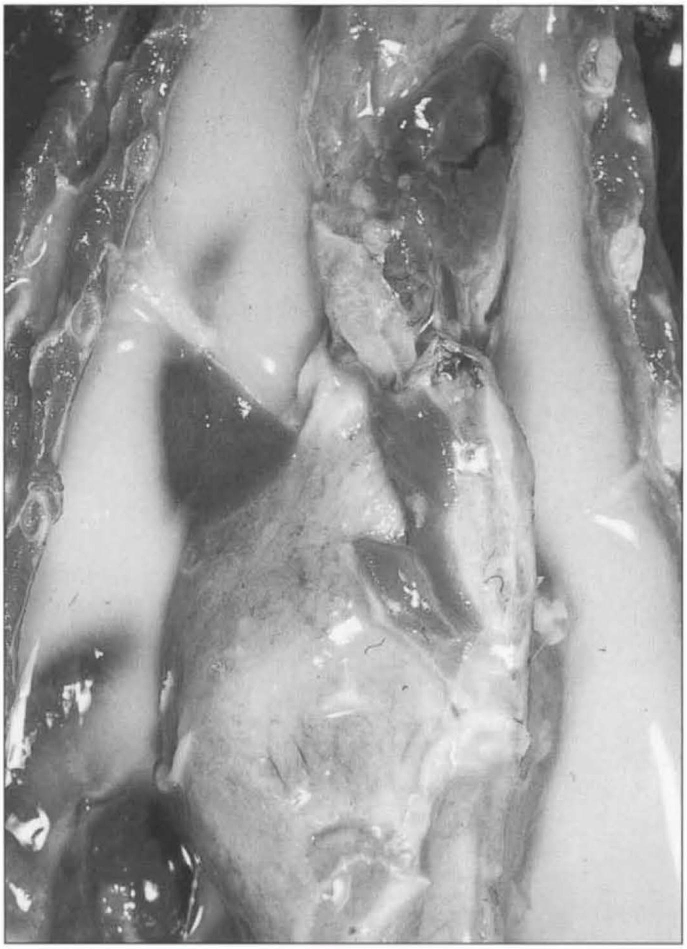

Figure 5.41 Chylothorax in a cat.

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Pleuritis

Infectious agents most often reach the pleura from the blood or from lesions of bronchopneumonia, aspiration pneumonia, or abscesses in the underlying lung. Other routes of infection include perforation of the chest wall, penetrating foreign bodies from the esophagus or reticulum, perforating esophageal ulcers, lymphatic permeation from the peritoneal cavity, or direct extension from a mediastinal abscess. *Pleural defenses against microorganisms are much weaker than those of the lung*, and even a few organisms reaching the pleural surfaces are apt to have serious consequences. The appearance varies depending on the species affected, cause, magnitude of infection, and chronicity. Fibrinous exudates often form loosely adherent strands or veils of elastic material on the pleural surface. In more florid fibrinous lesions, the prosector is greeted by voluminous serofibrinous exudate when the chest is opened, and lakes of watery clear yellow fluid alternate with pockets of turbid and foul-smelling exudate. Creamy white suppurative exudate fills the pleural space in cases of **pyothorax** or **thoracic empyema**. Chronic lesions readily form adhesions between the parietal and visceral pleura, yet the functional impact of adhesions on lung function is usually minimal.

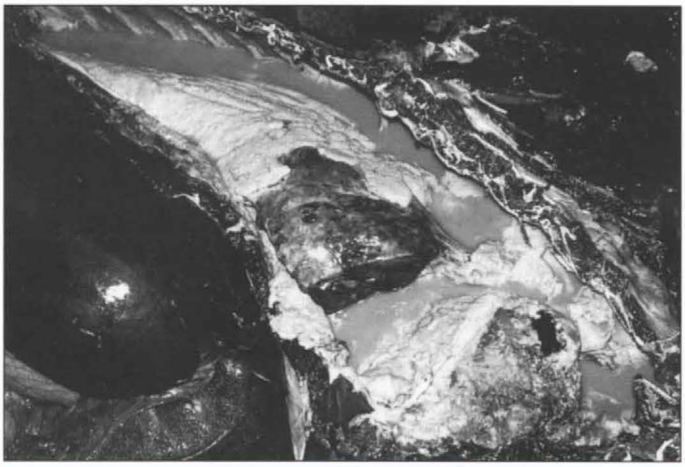

Figure 5.42 Severe fibrinopurulent pleuritis in a horse.

Pleuritis in **swine** may be an extension from obvious lesions of bronchopneumonia caused by *Actinobacillus pleuropneumoniae* or *A. suis*. *Streptococcus suis*, *Haemophilus parasuis*, and *Mycoplasma hyorhinis* are common causes of septicemia in swine, and the fibrinous or fibrinopurulent pleuritis caused by these agents is usually accompanied by exudates on other serosal surfaces, joints, or meninges. The causes in **cattle** include bronchopneumonia, direct extension from lesions of traumatic reticuloperitonitis, and septicemic *Histophilus somni* infection.

Pleuritis in mature **horses** often occurs with a recent history of travel, and is described later in the section on infectious diseases of the respiratory system (Fig. 5.42). The pleural exudate is unilateral or bilateral, and usually so florid that it masks the causative underlying lung lesion; but nearly all cases have either a focal area of consolidated lung that suggests aspiration pneumonia, or a bilateral lesion of necrotizing bronchopneumonia. *Mycoplasma felis* is an uncommon cause of pleuritis in horses without underlying lung lesions. Pleuritis occurs in neonatal foals with septicemia caused by *Actinobacillus equuli* or other gram-negative bacteria.

In **dogs**, pyothorax occurs mostly in hunting dogs or those with access to rural environments. The lesions are bilateral or occasionally unilateral, and pneumonia is absent or minor. The exudate is typically bloody and opaque, resembling tomato soup, or is more rarely creamy or darkly serofibrinous (Fig. 5.43). The pleural surfaces are usually thickened and velvety, red or gray-yellow, and fibrotic. *Actinomyces, Nocardia,* and *Bacteroides* spp. are the most frequently recovered organisms, and these are commonly associated with the presence of yellow "sulfur" granules amid the pus and a characteristic pyogranulomatous pleuritis and mediastinitis. Mixed infections are common, however, and a variety of other organisms can be present. Migrating grass awns or florets, usually impossible to find in the copious pleural exudate, are thought to be the source of infection in most cases.

In **cats**, feline infectious peritonitis is a common cause of pleuritis, and multifocal pyogranulomas are visible on the pleural surface. Pyothorax is fairly common in cats (Fig. 5.44), and *Pasteurella*

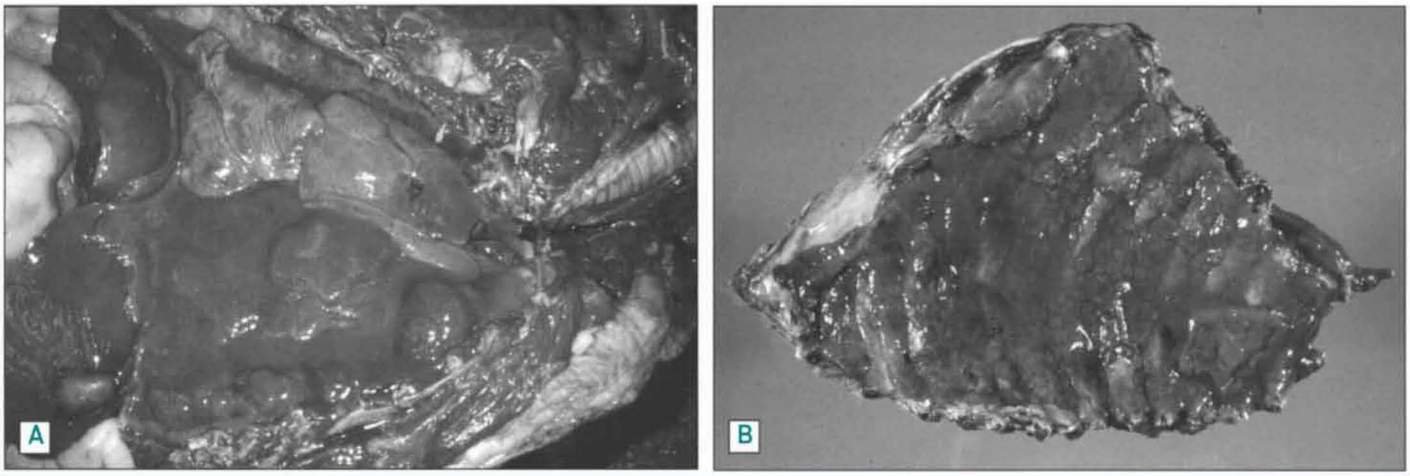

Figure 5.43 ***Nocardia* pleuritis** in a dog. **A.** The pleural cavity contains abundant thick red exudate. **B.** The parietal pleura is thickened and covered with exudate.

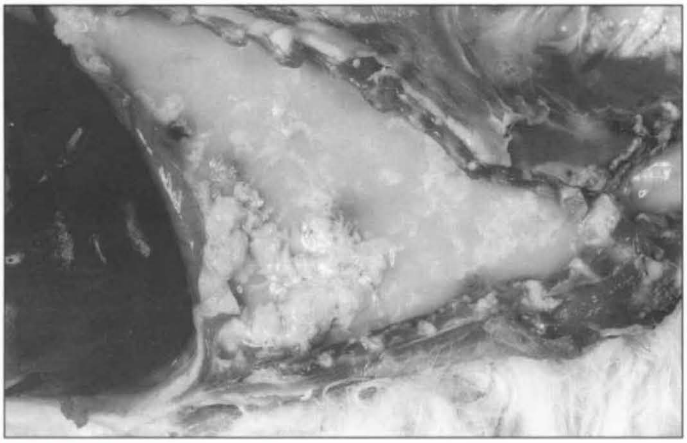

Figure 5.44 **Pyothorax** in a cat.

multocida, various gram-negative enteric bacteria, streptococci, and staphylococci are commonly isolated, often in mixed infection. Although most cases are idiopathic, penetrating bite wounds, migrating awns, extension from bacterial pneumonia, or penetration of a foreign body from the esophagus are likely causes.

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Neoplastic conditions of the pleura

Mesothelioma arises from the pleura, as well as from the pericardium or peritoneum. Histologically, *epithelioid, sarcomatous, and biphasic patterns* are described. The epithelioid cells form nests, ribbons, acini, or papillary protrusions that resemble carcinoma, and the mesenchymal component may resemble fibrosarcoma. *Immunohistochemical detection of both vimentin and cytokeratin, and detection of acid mucins by Alcian blue stain, are useful in distinguishing mesothelioma from other epithelial or nonepithelial neoplasms.* Detection of the calcium-binding protein calretinin is used as a marker of mesothelioma in humans; its expression in a mesothelioma in a horse is described, but the reliability of this test in animals is unconfirmed. Pleural mesothelioma spreads and implants within the pleural cavity to cause persistent thoracic effusion, and often invades the underlying tissue or reaches the abdominal cavity via lymphatics.

In humans, the pleura is a primary site of pathology associated with inhalation exposure to asbestos fibers. While asbestos-induced mesothelioma is the best-known manifestation, benign pleural lesions include pleural effusion and focal or diffuse pleural fibrosis. *Ferruginous bodies*, which are asbestos fibers coated by ferritin and amorphous protein, have been detected with increased frequency in the lungs of dogs with mesothelioma, but further studies are needed to determine if this association is repeatable and causal.

Metastatic neoplasms localizing to the pleura are uncommon. Transpleural dissemination of carcinomas and sarcomas may occur by extension from the lungs, chest wall, or mediastinum, and carcinomas from the abdominal cavity can reach the pleura by pleuroperitoneal migration of fluid through the diaphragm. A fragment of hyaluron, a high-molecular-weight extracellular protein produced by mesothelial cells, has been shown to interact with CD44 receptors on neoplastic cells to effect pleura-specific colonization by carcinomas.

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INFECTIOUS DISEASES OF THE RESPIRATORY SYSTEM

Infectious respiratory diseases of swine

Viral diseases

Porcine reproductive and respiratory syndrome

Porcine reproductive and respiratory syndrome (PRRS) was first identified in the late 1980s, and is now worldwide in distribution, with the probable exception of Australia and Sweden. It is an *important cause of reproductive failure and interstitial pneumonia*, and a predisposing factor for bacterial pneumonia and septicemia. The disease varies greatly in severity and clinicopathologic presentation depending on the strain of the *Porcine reproductive and respiratory syndrome virus* (PRRSV), the age of pigs affected, the level and distribution of immunity within the herd, and the presence of other pathogens.

On initial exposure, infection spreads slowly through the naive herd and causes a variety of clinical presentations: anorexia and lethargy of all age groups; reproductive failure characterized by late term abortion, stillbirth, mummified fetuses, and weak-born piglets; and interstitial pneumonia causing fatal hyperpnea, dyspnea, and lethargy in suckling pigs infected in utero or in the neonatal period, in weaned pigs, and to a lesser extent in grower-finisher pigs. "Sow abortion and mortality syndrome" describes a devastating disease resulting from PRRSV infection of naive herds, in which up to half of the sows may abort and sow mortality may reach 10%.

Viral infection often persists and circulates within the herd indefinitely, the result of prolonged viral shedding from individual animals and continuous entry of naive animals. The disease in endemically infected herds is highly variable: many herds have stable infections in which virus continues to circulate but does not cause clinical disease; others have endemic respiratory disease or a failure to thrive in nursery and grower-finisher pigs, or a PRRSV-induced increase in susceptibility to bacterial pneumonia and septicemia; and some herds have continuing losses due to abortion in gilts or dyspnea in neonatal or weaned pigs.

The PRRS virus is an enveloped, 50–65 nm diameter, positive-sense, single-stranded RNA virus in the family Arteriviridae. The viral genome includes 8 open reading frames (ORFs): ORF 1a and 1b encode proteins necessary for replication and transcription, ORFs 2–4 encode structural glycoproteins of uncertain function, ORF 5 encodes an envelope glycoprotein, ORF 6 encodes the integral membrane protein M, and ORF 7 encodes the nucleocapsid protein N. Genomic variability is an important feature of PRRSV, and may be attributed to the high mutation rate of RNA polymerase and perhaps recombination between viral strains. As a result, PRRSV isolates vary in terms of the severity of respiratory disease, reactivity in antigen- and nucleic acid-based diagnostic assays, and possibly in patterns of lesions in other organs, including brain and heart. These differences in virulence have not been shown to correlate with simple alterations in specific genes; rather, the virulence of particular strains is apparently determined by a combined effect of many genetic differences.

The pathogenicity of modified live vaccine strains of PRRSV is controversial. Vaccine strains of the virus can infect in-contact animals and persist in herds, but do not commonly cause clinical disease. However, as these vaccine strains circulate in swine herds, *mutation to a less attenuated form of the virus may result in disease caused by vaccine-derived strains*. Extra-label intranasal administration of modified live PRRSV vaccine has induced clinical signs and lesions typical of PRRS.

The common mode of *transmission* of PRRSV is direct nasal, oral, or coital contact with saliva, oropharyngeal mucus, urine, semen, serum, mammary secretions, or perhaps feces of infected pigs. Transmission of PRRSV by artificial insemination has been documented, and transmission by contamination of pharmaceutical preparations or needles with infected serum is a potential risk. Transmission by fomites may occur, but because the enveloped virus is rapidly inactivated in most environmental conditions, disinfection procedures should be effective. Aerosol transmission is apparently of limited importance, but can occur at low frequency over short distances.

The pathogenesis of PRRS centers on infection of macrophages at the site of nasal, tonsillar, or pulmonary infection, extension to associated lymphoid tissue, rapid viremia, and infection of macrophages throughout the body. Following experimental intranasal challenge, viral antigen is expressed within 12 hours of infection by nasal and tonsillar macrophages and epithelium, pulmonary alveolar, intravascular, and interstitial macrophages, bronchiolar epithelium, and endothelial cells of pulmonary arterioles. Viremia occurs rapidly, in some cases within 12 hours of infection, and leads to widespread infection of monocytes and tissue macrophages. Viral antigen may be detected in myocardial macrophages and endothelium, follicular macrophages and dendritic cells in lymph node, interdigitating cells in the thymus, macrophages and dendritic cells in the spleen and Peyer's patches, and macrophages in hepatic sinusoids, adrenal gland, interstitium of the kidney, and choroid plexus.

Persistent infection is a key feature of PRRSV. Virus has been detected in serum for 3 weeks; in nasal mucosa, pulmonary macrophages, and spleen for 4 weeks; and in oropharyngeal mucosa for up to 22 weeks after infection. Animals remain infectious for in-contact naive pigs for at least 22 weeks after infection. Persistent infections are of epidemiologic importance in the maintenance of PRRSV infection in endemically infected herds, yet the reasons for failure of the immune response to clear the infection and the role of cell-mediated immunity in protection and viral clearance are unknown. Following experimental PRRSV infection, serum antibody detectable by ELISA and virus-neutralizing antibody are first detectable at 7–14 days and 9–28 days after infection, peak at 5 weeks and 10 weeks, and remain detectable for 46 weeks and over 1 year, respectively. Antibody responses are directed against all proteins encoded by ORFs 2–7; the products of ORF 3, 4, and 5 may represent protective epitopes.

Infection with PRRSV impairs host defenses, and increases susceptibility to infection with *Streptococcus suis*, *Haemophilus parasuis*, *Salmonella choleraesuis*, and other opportunistic pathogens. Experiments to investigate this effect have been conflicting, and the ability of PRRSV to predispose to other agents is probably dependent on

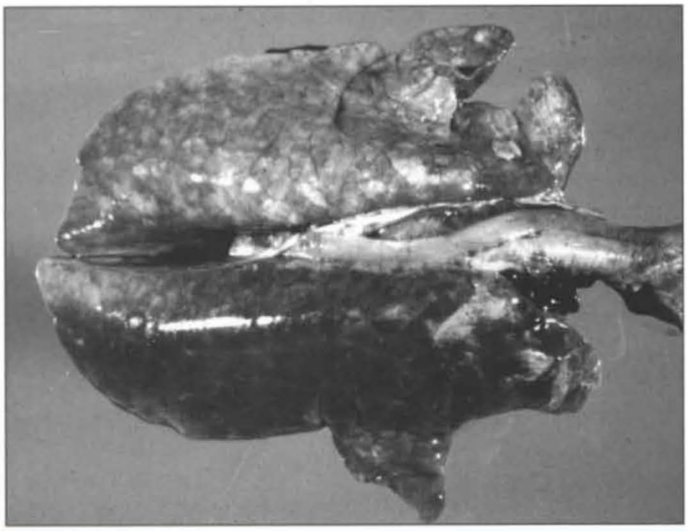

Figure 5.45 Porcine reproductive and respiratory syndrome. The lung is diffusely rubbery, fails to collapse, and has a lobular pattern of reddening.

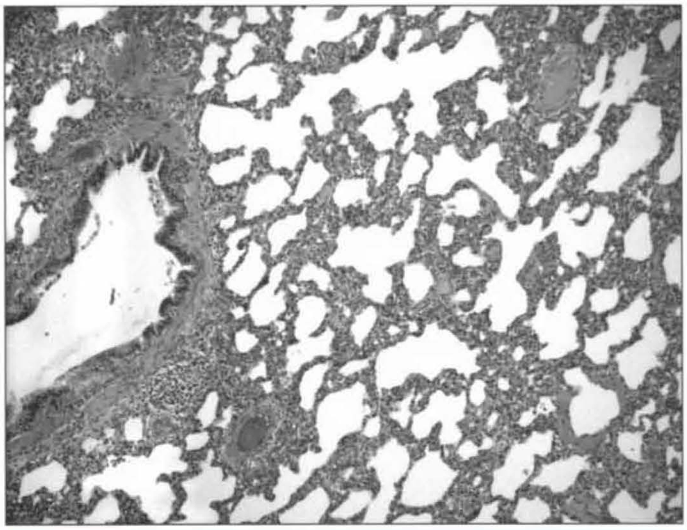

Figure 5.46 Porcine reproductive and respiratory syndrome. The alveolar septa are diffusely thickened by mononuclear cells. The bronchiole is normal.

the viral and bacterial strains examined and on the specific experimental conditions. The ability of PRRSV to infect and reduce the function of pulmonary intravascular macrophages is one mechanism whereby PRRSV predisposes to septicemia; a similar impairment of alveolar macrophage functions – including phagocytosis, oxidative burst, and cytokine secretion – may reduce lung defenses against bacterial bronchopneumonia. Subclinical or clinical infection with *Mycoplasma hyopneumoniae* exacerbates the clinical signs and lesions of PRRS, possibly because mycoplasma-induced proliferation and activation of macrophages enhance the replication or persistence of PRRSV. In contrast, infection with PRRSV has little effect on the severity of mycoplasmal lesions. This finding is of clinical importance, as control of *M. hyopneumoniae* is one strategy for minimizing the effect of PRRSV in endemically infected herds. The effect of PRRSV infection on immune responses to other pathogens has not been fully addressed, but a state of generalized immunosuppression is not a likely feature of PRRS.

Animals infected with PRRSV develop apoptosis of alveolar and pulmonary intravascular macrophages, and mononuclear cells in alveolar septa. Because apoptotic cells are more numerous than virus-infected cells, the mechanism of apoptosis may not represent a direct effect of viral infection. Similarly, although interstitial pneumonia is extensive in swine with PRRS, virus-infected cells are relatively infrequent, suggesting a role for host-derived factors such as cytokines in development of lesions.

The major **lesions** *of the postnatal form of PRRS include interstitial pneumonia, generalized lymphadenopathy, and lymphocytic infiltrates in multiple organs.* Gross lung lesions vary from undetectable to affecting the entire lung, and tend to be most striking in younger age groups. The lungs fail to collapse when the diaphragm is incised, occasionally retain impressions of the ribs, and have generalized, patchy, lobular, or diffuse distributions of lesions (Fig. 5.45). Affected areas of lung are discolored tan or red, and have a firm texture reminiscent of thymus, which contrasts with the crisp hard texture of bacterial pneumonia. A cut section reveals separation of lobules and oozing of edema fluid. In pigs that develop bacterial bronchopneumonia secondary to PRRS, lesions of cranioventral consolidation may be superimposed on the diffuse interstitial pneumonia. In these cases, the lesions of PRRS may be subtle, yet play a critical role in the development of pneumonia in the herd. Lymph nodes throughout the body – most notably the bronchial, mediastinal, cervical, and inguinal nodes – are enlarged, white or tan, solid, and rarely contain multiple clear cavitations on cut section. Periocular and subcutaneous edema, and mild serous effusions into body cavities, are variable findings.

Histologic *lung lesions are of interstitial pneumonia.* Alveolar septa are thickened due to infiltration of lymphocytes and macrophages (Fig. 5.46). This feature is easily obscured by atelectasis in routinely prepared samples, but can usually be confirmed by searching for areas of lung in which alveoli are not collapsed – where a single row of erythrocytes is visible in alveolar septal capillaries. Alveoli contain a cellular infiltrate of macrophages, lymphocytes, and fewer neutrophils, and scattered clusters of alveoli are filled with necrotic cells with pyknotic nuclei or karyorrhexis. *The latter is highly suggestive of PRRS, and probably represents clusters of apoptotic macrophages.* Type II pneumocytes are increased in number, but may or may not form a continuous layer of cuboidal epithelium lining the alveolus (Fig. 5.47). Bronchiolar epithelium is not affected by PRRSV, and a finding of bronchiolar necrosis should incite a search for an alternative or additional diagnosis.

Histologic examination of lymph nodes, tonsils, and spleens of pigs with PRRS may reveal *follicular and paracortical hyperplasia, and apoptosis of follicular lymphocytes.* Multinucleate cells, probably of histiocytic origin, may be present. Although this lesion is also common in *Porcine circovirus* type 2 (PCV2) infection, it has been described in PRRSV-infected pigs that have no evidence of PCV2 infection.

Perivascular infiltrates of lymphocytes, macrophages, and plasma cells occur in many organs in PRRS. Commonly affected sites include the nasal mucosa, heart, kidney, and brain. Severe neurologic disease occasionally occurs in association with other manifestations of PRRS, possibly related to the infecting viral strain, and lesions of the central nervous system include lymphocytic meningoencephalitis, lymphocytic perivascular cuffs, and focal gliosis. PRRSV may cause *vasculitis* in the lung and other organs, manifesting as necrosis

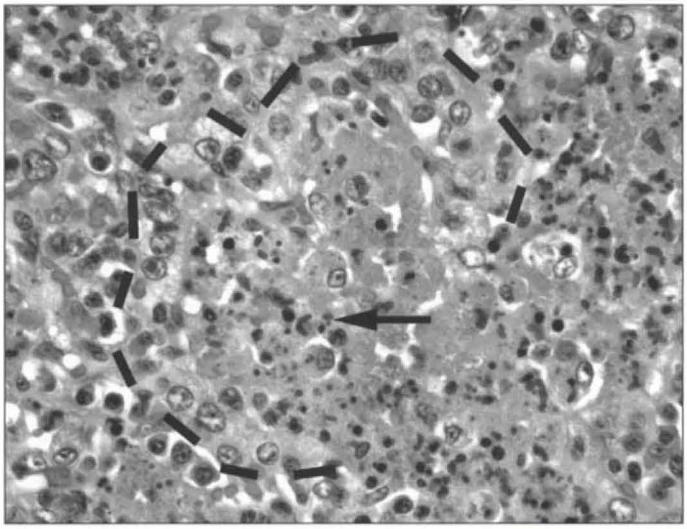

Figure 5.47 Porcine reproductive and respiratory syndrome. The alveolus is lined by cuboidal epithelial cells (outlined by dashed line), and filled with macrophages that often contain pyknotic nuclei or karyorrhexis (arrow).

of the walls of large and small blood vessels in addition to perivascular and mural infiltrates of lymphocytes and plasma cells.

Aborted fetuses usually lack gross or histologic lesions, and, when present, the lesions are often not specific for PRRSV. Infection may progress from one fetus to another, resulting in live, stillborn, and mummified fetuses in the same litter. Fetal lesions that suggest PRRS include segmental or diffuse hemorrhage of the umbilical cord due to fibrinoid necrosis and suppurative inflammation of the umbilical artery, mild interstitial pneumonia, pulmonary arteritis, lymphocytic myocarditis and encephalitis, and retroperitoneal and mesocolonic edema.

Diffusely firm lungs and histologic lesions of interstitial pneumonia are common findings at necropsy. In addition to PRRS, *a major differential diagnosis is septicemia.* Lesions of lymphadenopathy and mild proliferation of type II pneumocytes favor a diagnosis of PRRS, but differentiation requires ancillary testing. Porcine circovirus may cause a similar pattern of bronchointerstitial pneumonia, but granulomatous infiltrates and inclusion bodies are often present in lymph nodes and Peyer's patches. Proliferative and necrotizing pneumonia causes a similar alveolar lesion to PRRS, but bronchiolar necrosis is a feature of this disease that is absent in PRRS.

The arsenal of assays to confirm a **diagnosis** of PRRS in pathologic tissue specimens has recently expanded, and includes virus isolation, fluorescent antibody tests, immunohistochemistry, polymerase chain reaction (PCR), and in situ hybridization. Fluorescent antibody tests on frozen sections and immunohistochemistry on formalin-fixed paraffin-embedded sections of lung and tonsil are an economical approach to diagnosis, but because these assays are less sensitive than PCR, multiple sections should be examined. Virus isolation on chilled or frozen samples of lung, lymphoid tissues, serum, EDTA-treated blood, or pulmonary alveolar macrophages is a highly sensitive but laborious and slow method of diagnosis. Detection of viral nucleic acid by PCR is a rapid technique that is as sensitive as virus isolation and is becoming widely used in many laboratories. Serology is useful on a herd level to identify prior exposure to PRRSV in unvaccinated herds.

Results of diagnostic assays must be interpreted with caution in herds vaccinated with modified live viruses, as vaccine strains of PRRSV may circulate and persist in these herds and cause positive tests. An advantage of using PCR or virus isolation is the use of subsequent molecular analyses that assist in differentiating field and vaccine strains of virus in vaccinated herds. Identification of patterns of restriction fragment length polymorphisms (RFLP) in ORF 5 is commonly used to differentiate vaccine strains from some field strains; however, the RFLP pattern of vaccine strains may change to an intermediate pattern as they circulate in herds. A second strategy distinguishes vaccine and field strains by identifying glycine and arginine, respectively, in residue 151 of ORF 5.

Obtaining a diagnosis of PRRS in aborted fetuses can be frustrating, because lesions are usually absent or nonspecific and the agent often cannot be identified. Two techniques – the use of PCR assays on fetal lung and lymphoid tissue, and identification of antibody to PRRSV in precolostral sera – are currently recommended, but the low diagnostic rate remains a problem considering the importance of reproductive failure in swine herds.

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Swine influenza

Orthomyxoviruses are pleomorphic, spherical to filamentous, 80–120 nm viruses containing negative-sense single-stranded RNA. Transcription of viral genes occurs in the nucleus, viral proteins are produced in the cytoplasm, and virions bud from the plasma membrane. Three genera of orthomyxoviruses are described based on variation of the nucleocapsid proteins: (1) ***Influenza type A viruses*** cause influenza in swine, horses, humans, and other species; (2) *type B viruses* represent human isolates only; and (3) *type C viruses* are rare and cause mild or subclinical infections in humans and swine. Orthomyxoviruses readily develop genomic variants, due to *genetic drift* caused by point mutations and *genetic shift* due to recombination of genomic segments. *Identification of viral strains is based on antigenic variation of the 15 hemagglutinin (H) and nine neuraminidase (N) envelope glycoproteins*, which mediate virus entry into cells and release of virus from infected cells, respectively. Analysis of viral strains is of practical importance in epidemiologic tracking of outbreaks and changing disease patterns, and to ensure that vaccine strains are representative of those in the field to ensure protective immunity.

Influenza viruses are usually host-specific, and although several strains are known to cross species barriers, transmission rates are generally low and the foreign strains are probably not maintained in the population. Although of only moderate consequence for direct zoonotic potential, this phenomenon is important in the evolution of new viral strains. *Since swine possess cell-surface receptors for both avian and human viral strains, it is postulated that swine are hosts for reassortment among avian, porcine, and human strains of influenza viruses, with the potential for human pandemics due to the introduction of novel pathogenic strains into immunologically naive human populations.*

Influenza viruses are transmitted by aerosol or by contact with secretions. Infection is usually restricted to the respiratory tract, although myocarditis, myositis, and encephalitis – probably as a consequence of viremia – are uncommon complications in horses and humans.

The principal lesion of influenza is necrosis of bronchiolar and bronchial epithelium, which represents cell lysis due to viral infection. Acute histologic lesions include attenuation of airway epithelium (Fig. 5.48), while hyperplasia of bronchiolar epithelium begins as early as 4 days after infection. Similar to the lesions of other viral lung pathogens, lymphocytes and plasma cells infiltrate the bronchiolar mucosa and encircle bronchioles and blood vessels. Neutrophils invade the mucosa and exude into the airway lumen in primary viral infection, but are particularly numerous if there is secondary bacterial pneumonia. Alveoli may be edematous and contain increased numbers of macrophages and neutrophils, but hyaline membranes or type II pneumocytes are not typical features of influenza. Viral infection is transient: in swine influenza, viral antigen is maximally expressed at 24 hours and often absent by 72 hours after infection. In all species, viral injury to airway epithelium predisposes to bacterial pneumonia, presumably due to failure of mucociliary clearance of opportunistic pathogens.

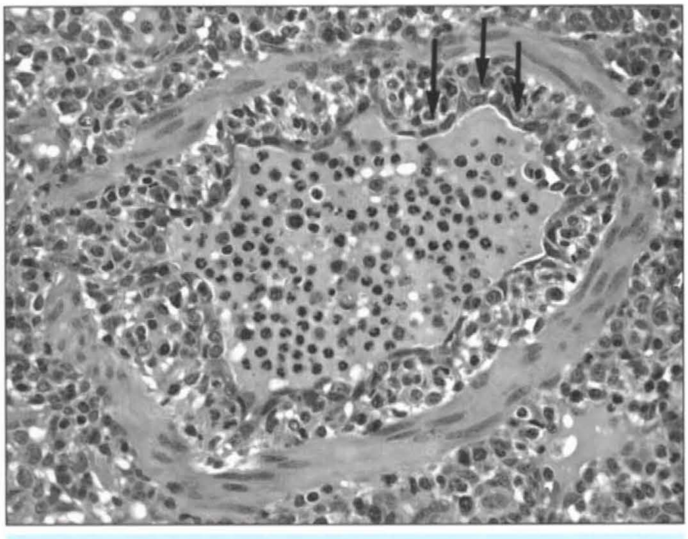

Figure 5.48 Swine influenza. The bronchiole contains neutrophils and is lined by markedly attenuated epithelium (arrows), and lymphocytes infiltrate the bronchiolar mucosa and surrounding tissue.

Swine influenza is an important form of respiratory disease, manifesting as rapidly spreading outbreaks of severe nonfatal disease or as endemic disease as part of the porcine respiratory disease complex. Disease outbreaks are most common in the winter in cool climates, or late summer in warm climates. Disease severity may be enhanced by stresses, climate change, PRRSV infection, or concurrent disease. Outbreaks of disease have a characteristic clinical presentation: the disease appears acutely and spreads rapidly to affect all ages of pigs, and despite severe clinical signs of coughing, fever, and stiffness, most pigs recover in 5–14 days and the mortality rate is low. An endemic form of disease is increasingly common, in which pigs develop bacterial bronchopneumonia secondary to subclinical infection with influenza virus. In this form of disease, the clinical picture is not specific for influenza and differentiation from other causes of porcine respiratory disease complex requires laboratory testing.

Identification of swine influenza virus (SIV) subtypes is important because there is limited cross-protection between subtypes; thus it is imperative that vaccines in current use are representative of circulating field strains. Subtype H1N1 is the common SIV in North America, and antigenic variants of this virus have been identified. H3N2 viruses have been detected more recently in North America. Avian-like H1N1 subtype as well as H3N2 subtypes are both isolated in Europe, and H1N2 viruses isolated in France and Japan probably represent genetic reassortment of these subtypes. SIV is transmitted by infected droplets, aerosol, or contact with infected secretions. After experimental infection, serum antibody titers are detectable in many pigs by 7 days and peak 2–3 weeks after infection, and mucosal immunoglobulin A (IgA) and IgG are maximal at 1 and 4 weeks after infection, respectively. Pigs shed virus for 5–7 days after infection, although viral antigen is often absent from lung as little as 72 hours after infection. Virus infection is probably maintained in swine populations by continuous infection of naive swine, as there is no evidence of a long-term carrier state or significant wildlife vector.

The **gross lesions** of swine influenza include a *cranioventral lobular distribution of atelectasis*, which is often sharply demarcated from normal lung tissue. Affected lobules are red, firm, and collapsed. Adjacent lobules are occasionally emphysematous. Generalized pulmonary edema may occur. Mediastinal and bronchial lymph nodes are enlarged and edematous, but usually not congested. The mucosa of the trachea and bronchi is often hyperemic and edematous. The development of secondary bacterial bronchopneumonia may obscure the gross lesions of swine influenza and cause red, firm, raised, lobular lesions in the cranioventral lung.

The **histologic lesions** reflect *necrosis of airway epithelium* and, to a lesser extent, alveolar epithelium, and include attenuation of bronchiolar epithelial cells, necrotic debris and neutrophils in bronchiolar lumens, atelectasis, and peribronchiolar, perivascular, and interstitial infiltrates of lymphocytes and plasma cells (Fig. 5.48). Vacuolation of bronchial and bronchiolar epithelial cells with loss of cilia occurs by 8 hours after experimental infection. By 24 hours, there is necrosis of bronchiolar and bronchial epithelial cells, and accumulation of sloughed necrotic cells in the lumen. Alveoli and airway lumens contain moderate numbers of neutrophils and edema fluid, and many alveoli are atelectatic. Mononuclear cell infiltration occurs by 48 hours after infection. Alveolar injury, manifesting as exudation of neutrophils and scant fibrin with thickening of alveolar septa, is described in experimental cases at 48 hours after infection, but is often inapparent or obscured by bacterial pneumonia in natural disease. Later lesions include hyperplasia of airway epithelium as regeneration occurs, and peribronchiolar and perivascular aggregation of lymphocytes and plasma cells as immune responses develop. Infection is, in most cases, restricted to the respiratory tract and associated lymph nodes, although viremia has been infrequently described in neonates.

The **diagnosis** is suggested by the characteristic clinical signs of severe, rapidly spreading disease with high morbidity and low mortality, and by the characteristic histologic lesions of bronchiolar necrosis. *Differential diagnoses* for the latter include *Porcine circovirus* type 2 infection, proliferative and necrotizing pneumonia, pseudorabies, *Nipah virus* infection, and airway injury due to inhaled toxic gases. The diagnosis is confirmed by inoculation of embryonated eggs, with subsequent identification of virus by detection of hemagglutinating activity. More rapid methods of obtaining a definitive diagnosis include immunohistochemistry on fixed tissues using monoclonal antibodies to conserved type A nucleoproteins, fluorescent antibody testing on frozen sections of affected lung, and polymerase chain reaction. Sample selection is critical for the diagnosis of swine influenza. Viral titers are highest 24 hours after infection, before gross lesions are obvious, and virus often cannot be identified by 72 hours after infection. Consequently, *selecting tissue from the cranioventral areas of the lung, from pigs early in the course of the disease, is critical for both histologic and virologic diagnosis.* Pigs with a fever are the most suitable subjects for diagnostic investigation. The transient nature of the infection may complicate an investigation of the role of swine influenza in herds with endemic respiratory disease. In these cases, herd-level serology may be useful, as most pigs develop hemagglutination inhibition titers of greater than 1:80 within a week of infection.

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Porcine circovirus and postweaning multisystemic wasting syndrome

Porcine circovirus (genus *Circovirus*, family Circoviridae) has recently emerged as a common pathogen of swine. *Porcine circovirus* type 1 (PCV1) has been recognized for many years as a nonpathogenic cell culture contaminant. In contrast, *Porcine circovirus* type 2 (PCV2) was first isolated in 1997 from pigs with a novel disease, *postweaning multisystemic wasting syndrome* (PMWS). *Serum antibody to PCV2 is widespread in swine herds, and PCV2 infection is common in herds with no clinical evidence of PMWS.*

PMWS was originally described in high-health-status herds, but many herds with PMWS are infected with PRRSV or other pathogens. PMWS is usually a slowly progressive disease within the herd, with most cases developing in 5–12-week-old pigs. Morbidity is typically 5–10% but may be up to 20%, and most affected animals die or are euthanized. Pigs with PMWS lose weight or gain poorly, and have a variable combination of tachypnea, respiratory distress, diarrhea, pallor, and jaundice. Although uncommon, the finding of jaundice in swine should suggest a diagnosis of PMWS.

Porcine circoviruses are 17 nm diameter, nonenveloped, and contain circular single-stranded DNA. The number of encoded genes or open reading frames is controversial, and the function of each gene product is not yet known. Although isolates of PCV2 share 96% homology, the nucleotide homology of PCV1 and PCV2 is less than 80%.

Porcine circoviral antigen and nucleic acid are most consistently present in the cytoplasm of monocytes, macrophages, and dendritic cells throughout the body, and less often in the nuclei and cytoplasm of epithelial cells in bronchioles, pulmonary alveoli, liver, renal tubules, stomach and intestine, and pancreas. Vascular smooth-muscle and endothelial cells occasionally express circoviral antigen. Transmission is poorly characterized.

Current evidence indicates that PCV2 is sufficient to cause PMWS, as experimental infection of gnotobiotic or cesarean-derived colostrum-deprived pigs induces mild clinical signs and lesions of PMWS in the absence of other demonstrable pathogens. However, co-infection with PCV2 and other pathogens, including parvovirus or PRRSV, results in more severe disease. Immunostimulation itself, either as a consequence of viral infection or vaccination, precipitates more severe disease. Thus, *activation of the immune response may encourage replication of PCV2, resulting in more severe manifestations of PMWS.*

The role of PCV2 in other swine diseases remains controversial, in part because the virus can be isolated from many healthy pigs. In particular, although many pigs with dermatitis-nephropathy syndrome, proliferative and necrotizing pneumonia, sow abortion and mortality syndrome, congenital hypomyelination, and abortion with fetal myocarditis have evidence of PCV2 infection, definitive proof of their causal association is lacking.

The spectrum of **gross lesions** present and their severity are highly variable. Swine with PMWS are often thin, pale, and may be jaundiced. Lymph nodes throughout the body – particularly the inguinal, mesenteric, and bronchial nodes – are enlarged, soft, and white or gray-tan, and *this lymphadenopathy is the most consistent feature of PMWS.* Lung lesions, which are common, consist of interstitial pneumonia with generalized firm or rubbery texture, failure to collapse, and mottled color. Cranioventral bronchopneumonia is a frequent concurrent lesion. The liver may be atrophic and discolored yellow-orange. Kidneys may contain patchy pallor or obvious white foci. Lesions resembling dermatitis-nephropathy syndrome may also occur, including swelling and edema of the kidneys, and cutaneous hemorrhages and necrosis.

The characteristic **histologic lesions** of PMWS in many tissues are *granulomatous infiltrates and lymphoid depletion, with formation of distinctive inclusion bodies within the cytoplasm of macrophages.* Inclusions are usually multiple within a single macrophage, 1–3 μm diameter, round, and basophilic (Fig. 5.49). Inclusion bodies are most numerous and obvious in lymphoid tissues, including lymph nodes, Peyer's patch, tonsil, splenic periarteriolar lymphoid sheaths, and thymus. In these tissues, there is depletion of lymphocytes from B-cell follicles, with striking infiltration of macrophages into follicles and, to a lesser extent, T-cell-rich paracortical areas. Multinucleate histiocytes may be present amid the granulomatous infiltrates.

Histologic examination of the lungs of pigs with PMWS reveals diffuse or patchy *granulomatous interstitial pneumonia.* Epithelioid macrophages, multinucleated macrophages, lymphocytes, and fewer eosinophils or neutrophils infiltrate alveolar septa and may fill the alveoli. Lymphocytes and macrophages often encircle bronchioles and blood vessels (Fig. 5.49). Bronchiolar necrosis is a variable finding, and may progress to bronchiolitis obliterans in chronically affected pigs.

Liver lesions are highly variable, progressing from mild aggregates of lymphocytes in portal tracts, to single-cell necrosis of hepatocytes, periacinar necrosis, or widespread hepatocellular loss with condensation of hepatic stroma. Perivascular infiltrates of lymphocytes and macrophages may be present in other organs, including kidney, myocardium, leptomeninges, pancreas, adrenal, stomach, and intestine. Vasculitis, with necrosis of tunica muscularis accompanied by lymphohistiocytic infiltrates, and exudative glomerulonephritis are less common lesions.

Infection with PCV2 is usually identified using *immunohistochemistry or polymerase chain reaction.* The virus may be isolated in PK15 cells or identified by in situ hybridization, but these find more use in research than as diagnostic techniques. Serologic assays, including indirect immunofluorescence and immunoperoxidase tests, have been developed, but the high prevalence of antibodies to PCV2 in healthy pigs limits the utility of this test in disease diagnosis.

It is critical to recognize that demonstrating PCV2 infection does not in itself indicate a diagnosis of PMWS. A **diagnosis** of PMWS is mainly based on the demonstration of consistent clinical findings and gross and histologic lesions, and is supported by the demonstration of active PCV2 infection by immunohistochemistry or polymerase chain reaction. PRRS is the major differential diagnosis for lesions of interstitial pneumonia and lymphadenopathy in pigs. The following

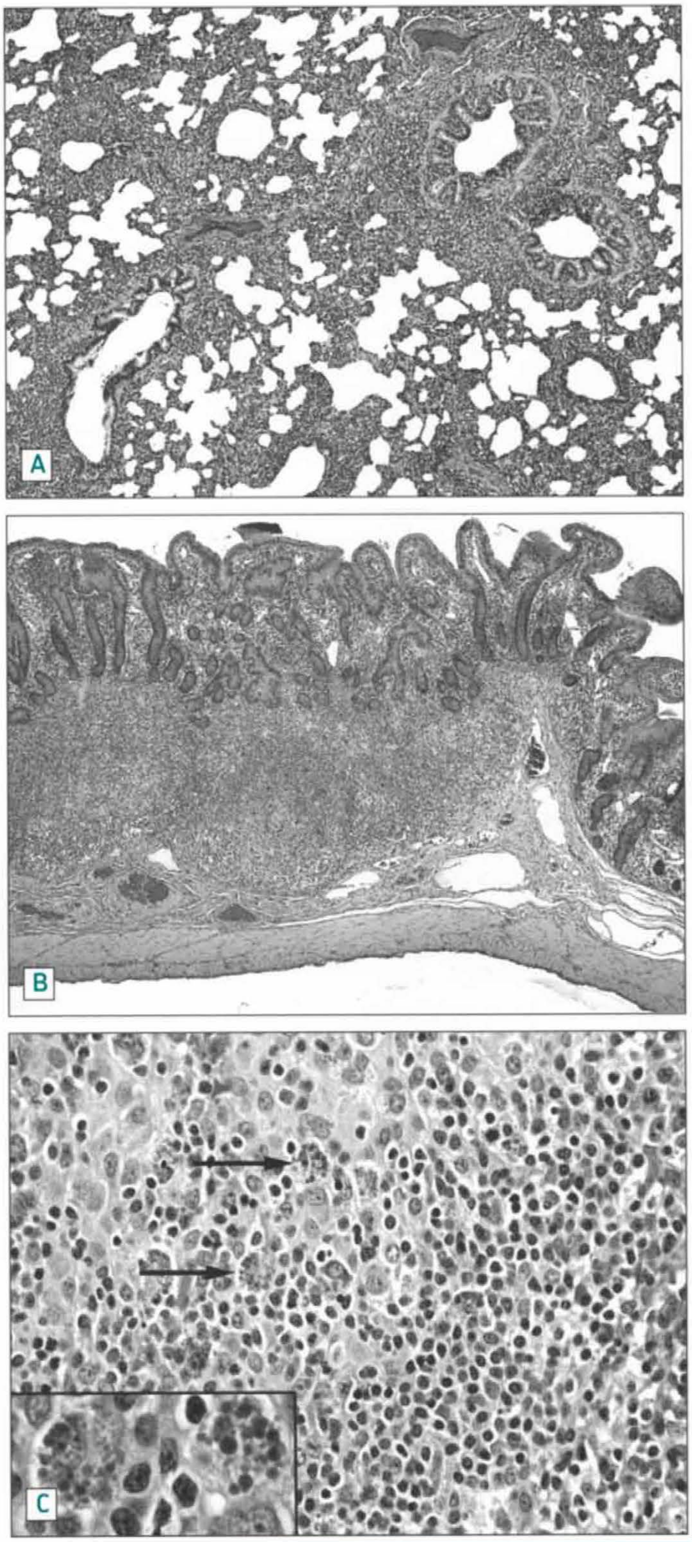

Figure 5.49 Postweaning multisystemic wasting syndrome in a pig. **A.** Lung, with diffusely thickened alveolar septa, and lymphocytes encircling bronchioles and blood vessels. **B.** Lymphoid depletion of Peyer's patch. **C.** Peyer's patch, with depletion of lymphocytes and infiltration of macrophages. Some macrophages contain numerous intracytoplasmic basophilic inclusion bodies (arrows, and inset).

are helpful differentiating features, but *the lesions of PMWS and PRRS may be difficult to distinguish, and many pigs are co-infected with PCV2 and PRRSV*:

- Multiple basophilic inclusion bodies in macrophages are a diagnostic feature of PCV2 infection, and support the diagnosis of PMWS.
- Bronchiolar necrosis is present in some cases of PMWS (and swine influenza), but is not a lesion of PRRS.
- Lung lesions of PMWS tend to be dominated by macrophages, whereas lesions in PRRS are more lymphocytic, although there is much overlap in the morphology of these diseases.
- Granulomatous infiltrates and multinucleate macrophages in lymphoid organs are more typical of PMWS than of PRRS, although both lesions may be present in PRRS.

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Proliferative and necrotizing pneumonia

Proliferative and necrotizing pneumonia is a severe acute disease of weaner and grower pigs. Affected animals have acute onset of severe dyspnea, tachypnea, and fever. Gross lesions usually affect the entire lung, but may be restricted to the cranial and middle lobes. Lung lesions include remarkably increased firmness, failure to collapse, reddening, and edema. Generalized lymphadenopathy is usually present. Histologically, alveoli are edematous, contain macrophages and abundant necrotic cellular debris, and are lined by cuboidal type II pneumocytes. Alveolar septa are thickened by mononuclear cell infiltration. Although many of these lesions may be present in severe cases of PRRS, *bronchiolar necrosis is often present in proliferative and necrotizing pneumonia but absent in PRRS.*

The understanding of the cause of this lesion is evolving. When the lesion was first reported in the early 1990s, it was associated with an H1N1 influenza virus that was genetically and antigenically distinct from common North American swine influenza virus isolates. Later publications associated proliferative and necrotizing pneumonia with PRRSV infection, H3N2 influenza virus and, more recently, with PCV2. PRRSV can be detected in nearly all cases, whereas other pathogens are less consistently identified. It remains to be clarified whether this lesion is caused by particular strains of PRRSV or by combined infection with several agents, or if it represents a pattern of reaction shared by more than one pathogen.

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Porcine respiratory coronavirus

Porcine respiratory coronavirus (PRCoV) is a minimally pathogenic virus that arose in the mid-1980s by deletion and point mutation of the *Transmissible gastroenteritis virus* (TGEV) genome. European and American isolates of PRCoV differ, and apparently developed independently from TGEV. Following experimental challenge, neonatal pigs develop viremia and respiratory infection, whereas in 5-week-old pigs, viral antigen is limited to the respiratory tract, particularly alveolar and bronchiolar epithelial cells and alveolar macrophages. Lesions of naturally infected pigs are not described. In experimental cases, there is necrosis of bronchiolar epithelium, mild proliferation of type II pneumocytes, mononuclear cell infiltrates in alveolar septa, and lymphocytes and macrophages in alveoli. Most field cases are subclinical, and the major importance is that *antibodies to PRCoV cross-react with those to TGEV, leading to false-positive serologic tests in swine herds that are expected to be free of TGEV.* Diagnosis at necropsy is based on isolation or indirect fluorescent antibody tests. Virus neutralization tests and some enzyme-linked immunosorbent assays (ELISAs) do not differentiate PRCoV from TGEV antibodies, but newer blocking ELISAs do differentiate these coronaviruses.

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Inclusion body rhinitis – porcine cytomegalovirus

Porcine cytomegalovirus (PCMV) infections are ubiquitous and occur throughout the world, but clinical disease is much less frequent. *Inclusion body rhinitis is typically an acute to subacute disease of 3–5-week-old suckling piglets.* Piglets exhibit fever, sneezing, catarrhal nasal exudate, shivering, and occasional dyspnea. Morbidity is high and mortality is low unless secondary bacterial infections develop. Systemic cytomegalovirus infections usually affect piglets less than 3 weeks of age. These may be found dead without premonitory signs, or exhibit sneezing, lethargy, and anorexia, subcutaneous edema of the jaw and tarsal joints, and dyspnea. Infection of naive pregnant sows induces mild lethargy, anorexia, and delivery of stillborn or weak piglets.

Inclusion body rhinitis is caused by PCMV, a β-herpesvirus. Piglets commonly shed the virus soon after weaning at 3 weeks of age, suggesting that infection is usually acquired by contact with nasal secretions of infected cohorts. Other pigs, particularly those that develop generalized disease, are probably infected from the sow in the neonatal period. The virus replicates in nasal submucosal and lacrimal glands. Viremia develops at 5–14 days after infection, depending on the age of the pig, and leads to infection of epithelial cells in renal tubules, liver, duodenum, and elsewhere. Pulmonary alveolar and splenic macrophages may be additional sites of viral replication. Virus is shed in nasal and ocular secretions, in the urine, and in vaginal secretions of sows.

Gross lesions in pigs infected with cytomegalovirus are usually only seen in piglets less than 3 weeks of age with generalized disease, and may include catarrhal rhinitis, hydrothorax, hydropericardium, pulmonary and subcutaneous edema, and renal petechiae. **Histologically**, *large 8–12 μm basophilic intranuclear inclusion bodies* are numerous in the epithelial cells of the nasal submucosal glands and their ducts (Fig. 5.50). Affected glands are not diffusely distributed but tend to occur in irregular clusters. As the inclusion forms, cytomegaly and karyomegaly develop. The nuclear membrane becomes indistinct and the inclusions appear as blue-gray smears, and the necrotic epithelium sloughs into the lumen. The developing immune response incites a lymphocytic infiltrate in the lamina propria, macrophages and lymphocytes cluster within degenerating glands, and there is squamous metaplasia of the surface epithelium.

Systemic lesions develop following viremia, with intranuclear inclusion bodies in epithelial cells of renal tubules and glomeruli; lacrimal, Harderian, and salivary glands; and less commonly in hepatocytes and sinusoidal lining cells, adrenal gland, esophageal submucosal glands, lymph nodes, spleen, renal medulla, lung, and elsewhere. *Inclusions are most numerous in epithelium but also occur in macrophages and endothelial cells*; this vascular disease accounts for the petechiation and edema. Inclusion bodies are often accompanied by cytomegaly, lymphocytic infiltrates, and occasionally focal necrosis. Focal gliosis with intranuclear inclusions in scattered glial cells occurs throughout the central nervous system. The viremic phase may last for 2–3 weeks and is followed by persistent infection in pulmonary macrophages.

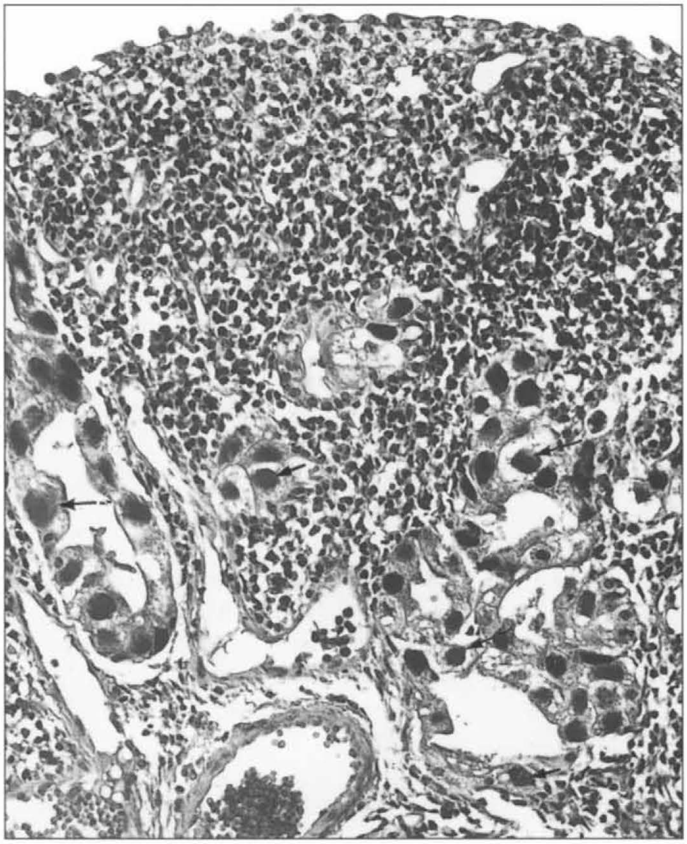

Figure 5.50 Inclusion body rhinitis in a pig. Large, basophilic intranuclear inclusions (arrows) in enlarged epithelial cells of the mucosal glands, with lymphocytic rhinitis.

Inclusion bodies, cytomegaly, and karyomegaly are pathognomonic when present. Adenovirus is the only viral agent likely to cause similarly large basophilic intranuclear inclusion bodies, but adenovirus does not induce cytomegaly and is usually restricted to intestinal epithelium in swine. The intranuclear inclusions of pseudorabies are eosinophilic, less obvious than those of cytomegalovirus, focal necrosis is more prominent, and lesions are most prominent in brain, respiratory tract, and lymphoid tissue. Confirmation of cytomegalovirus infection may be achieved by electron microscopy to search for herpesviral particles, or by virus isolation, immunofluorescence, or PCR on nasal scrapings, lung wash cells, or kidney homogenates.

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Pseudorabies

Pseudorabies (Aujeszky's disease) is described in more detail in Vol. 1, Nervous system, for neurologic signs predominate in most cases of pseudorabies in young swine. However, *certain strains of pseudorabies virus tend to cause respiratory disease*, particularly following aerosol exposure of older pigs. The respiratory form is typified by high morbidity and low mortality, unless secondary bacterial pneumonia develops. Affected pigs are depressed, febrile, and display episodes of sneezing, coughing, and dyspnea. Gross lesions in the upper respiratory tract are most common, and include necrotizing rhinitis and laryngotracheitis, with foci of necrosis in the tonsils. Lung lesions are less consistent, but patchy areas of reddening and consolidation may be scattered throughout the lung, or target the cranioventral lung. Histologically, *bronchointerstitial pneumonia* is characterized by necrosis and sloughing of bronchial and bronchiolar epithelium, with *frequent intranuclear eosinophilic or faintly basophilic inclusions* in the early stages of disease. Alveolar lesions include multifocal necrosis, sloughing of necrotic alveolar epithelium, fibrinous exudate in alveoli, hemorrhage, infrequent inclusion bodies, and infiltration of lymphocytes, macrophages, and fewer plasma cells and neutrophils. Experimentally, necrotizing lesions develop within 3–6 days of infection, infiltration of lymphocytes is prominent by days 5–8, and repair occurs during days 6–10.

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Nipah virus

Nipah virus emerged as an epidemic cause of nervous and respiratory disease in pigs and humans in Malaysia in 1998 and 1999 and in humans in Bangladesh in 2004. The disease also affects dogs and cats, and horses and goats develop an antibody response. Nipah and Hendra viruses are the lone members of the genus *Henipavirus* (family Paramyxoviridae). *Pteropus* fruit bats (flying foxes) are the reservoir and major source of infection; pigs are considered a major source of infection for humans and other pigs, while human-to-human transmission appears to be rare.

Gross lesions are nonspecific, but *the characteristic histologic findings are lymphocytic and/or fibrinoid vasculitis; lymphoid necrosis; syncytial cells affecting lymphoid tissues, endothelium, and perivascular cells; and intracytoplasmic eosinophilic inclusion bodies.* The lesions include lymphocytic meningitis and, to a lesser extent, encephalitis that is present throughout the brain but most severe in olfactory bulb; necrotizing, lymphocytic, and/or hyperplastic rhinitis, tracheitis, bronchitis, and bronchiolitis (Fig. 5.20); lymphohistiocytic bronchointerstitial pneumonia; necrosis of lymphoid tissues, particularly tonsil and submandibular and bronchial lymph node; and lymphocytic infiltrates in multiple organs.

Nipah virus is readily isolated from blood, tonsil, nasal mucosa, lung, and other lymphoid tissues. Viral antigen is demonstrated by immunohistochemistry in epithelial cells in tonsil, trachea, bronchioles, and alveoli; alveolar macrophages; meningeal connective tissue and possibly astrocytes; endothelium and muscularis of inflamed blood vessels; and areas of lymphoid necrosis.

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Bacterial diseases

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumoniae (APP) causes contagious pleuropneumonia, an important cause of severe, often fatal, pneumonia in growing pigs. The disease is most common in 6-week to 6-month-old hogs. The severity is highly variable, but case fatality rates of 20–80% are common in acute outbreaks. Clinical signs in severely affected swine include fever, lethargy, severe dyspnea, cyanosis, bloody discharge from the nose, and occasionally vomiting or diarrhea. Convalescent pigs with chronic pneumonia may fail to thrive and display exercise intolerance and coughing.

APP is a gram-negative coccobacillus of the family Pasteurellaceae, and is classified based on requirement for an exogenous source of nicotinamide adenine dinucleotide (NAD) – biovar 1 is NAD-dependent, biovar 2 is NAD-independent. Twelve serotypes are defined by antigenic variation in capsular polysaccharide and cell wall lipopolysaccharide. Serotypes 1, 5, and 7 are most common in North America, serotypes 2 and 9 are prevalent in Europe, and serotypes 1, 7, and 12 are frequent in Australia. Serotypes 1, 5, 9, and 11 secrete both Apx I and II toxins and tend to cause more severe disease than other serotypes. Immunity to heterologous serotypes is only partially protective.

APP benefits from a rich spectrum of *virulence factors.* Three cytotoxins, named Apx I, II, and III, are members of the RTX (repeats in toxin) family of secreted toxins, along with *Mannheimia haemolytica* leukotoxin and *Escherichia coli* hemolysin. A fourth Apx toxin gene expressed in vivo but not in vitro is of uncertain significance. The Apx toxins are potent inducers of cytolysis in porcine neutrophils, alveolar macrophages, erythrocytes, and epithelial cells. Low concentrations induce an oxidative burst in porcine neutrophils. These toxins appear critical to development of disease, as Apx toxin-deficient mutants have reduced virulence, which is restored when the toxin genes are reintroduced. Lipopolysaccharide induces macrophage activation and secretion of neutrophil chemoattractants, procoagulant activity, and complement activation, similar to that described for *M. haemolytica*. The capsule impairs phagocytosis by macrophages and may prevent complement activation. Isolates with thin capsules may be less pathogenic. APP produces superoxide dismutase, catalase, and hydroperoxide reductase, which may protect against oxidative killing by neutrophils and macrophages. Other virulence factors include fimbrial adhesins, outer membrane proteins, iron-binding proteins, metalloproteinase, and urease.

Infection is acquired by direct contact with infected pigs or by spread of aerosol droplets over short distances. The bacteria may be carried in the nasopharynx of apparently healthy animals, and these carriers are the principal method of introduction onto a naive farm. In contrast to *Pasteurella multocida* and *Bordetella bronchiseptica* in pigs and *M. haemolytica* of cattle, APP often causes disease in the absence of predisposing factors. Nevertheless, disease severity is enhanced by *Mycoplasma hyopneumoniae* or pseudorabies infections, and by factors that cause ciliary stasis. The interaction between PRRSV and APP infections is controversial.

Following inhalation, APP rapidly binds to the epithelium lining terminal bronchioles and alveoli. Neutrophil infiltration and alveolar exudate develop as early as 90 minutes after infection. Neutrophil recruitment is primarily due to secretion of neutrophil chemoattractants by macrophages in response to infection, for bacterial products themselves do not directly induce neutrophil chemotaxis. *Leukocyte necrosis is a prominent feature of the histologic lesions*, and is presumably mediated by the Apx cytotoxins.

Immunity is conferred by mucosal IgA and serum IgG antibody responses, particularly to the Apx cytotoxins. However, heterologous infections impart only partial immunity, and this effect is not dependent on patterns of Apx toxin expression, implying that antibody against other bacterial antigens also contributes to protection. Antibody against surface components such as capsular polysaccharide greatly enhances phagocytosis of APP by neutrophils and macrophages, and neutrophils but not macrophages effectively kill these opsonized bacteria in vitro. In endemically infected herds, maternal antibody declines over the first 12 weeks of life, and active immune responses contribute to increasing titers thereafter. In experimental infections, serum antibody titers are detectable at 2 weeks and maximal at about 5 weeks after infection.

Gross findings in pigs that die of contagious pleuropneumonia are typified by *fibrinosuppurative, hemorrhagic, and necrotizing lobar pneumonia or pleuropneumonia.* Identical gross lesions may be caused by *Actinobacillus suis* and, less frequently, by *Salmonella choleraesuis*; these are important differential diagnoses in minimal-disease herds expected to be free of APP. The lesions commonly affect the *middle or caudal lung lobes*, and may be unilateral or bilateral (Fig. 5.51). Lesions are deep red, firm to hard, protrude above the surrounding lung, and cut crisply. The cut surface often exhibits sharply demarcated, irregularly shaped, 1–10 cm foci of coagulative necrosis that are friable and pale. In peracute cases, interlobular septa are expanded by fibrin and edema, and fibrinous exudate on the pleural surface ranges in appearance from a haze resembling ground glass to mats of elastic fibrin. The bronchi may contain frothy or bloody fluid, and blood may ooze from the nostrils. Bronchial and mediastinal lymph nodes are enlarged, edematous, and congested. The pericardial and peritoneal cavities may contain scant serosanguineous fluid. Lesions in chronic survivors include fibrous pleural adhesions, sequestra that develop from large foci of coagulative necrosis, and locally extensive pulmonary fibrosis or abscessation. Acutely affected pigs occasionally develop hyaline thrombi and fibrinoid necrosis of glomerular capillaries, afferent arterioles, and interlobular renal arteries, perhaps mediated by endotoxemia.

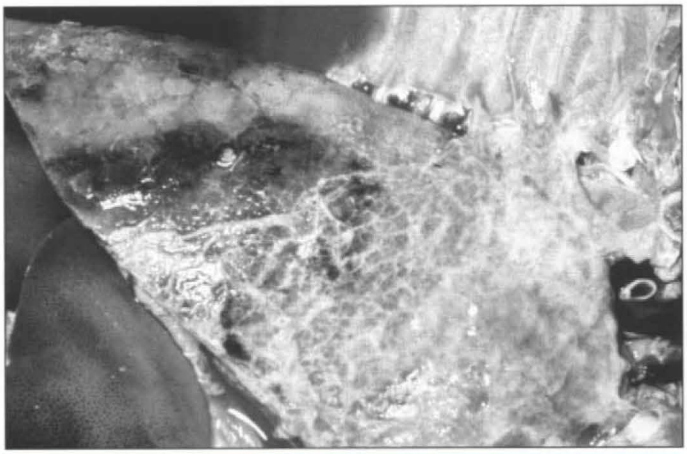

Figure 5.51 ***Actinobacillus pleuropneumoniae* pneumonia** in a pig. The cranial part of the caudal lung lobe is black and hard, and the pleura is covered by fibrin.

Microscopic investigation of these lesions reveals filling of alveoli and terminal bronchioles by fibrin, neutrophils, fewer macrophages, and many necrotic leukocytes that are probably neutrophils. The foci of necrosis, which are often centered on alveolar septa, are delineated by a basophilic band of intense neutrophil infiltration and extensive neutrophil necrosis. In the center of the necrotic areas, the exudate varies from protein-rich edema, to fibrin, to leukocytes. As in other areas of the lung, many of the leukocytes contain streaming, lightly basophilic, homogeneous chromatin debris similar to the "oat cells" of bovine pneumonic pasteurellosis. These neutrophils are in an apparent state of activation, and express tumor necrosis factor-α, IL-1, and IL-8 mRNA at high level.

The mucosa of bronchi and bronchioles may be invaded by neutrophils with fewer macrophages and neutrophils, and bronchiolar epithelium may be necrotic or sloughed. Nevertheless, a discovery of extensive bronchiolar necrosis should prompt a search for concurrent viral infection, such as influenza. Thrombi may develop in small venules and capillaries in the alveolar and interlobular septa, and fibrinoid vasculitis has been infrequently described. Lymphatics in the alveolar septa are distended by serofibrinous exudate with variable numbers of neutrophils. Extrapulmonary lesions are uncommon, but include renal glomerular thrombosis, renal vasculitis, or osteomyelitis.

Lesions of localized or locally extensive fibrinous and necrotizing pneumonia suggest APP, *A. suis* or, less commonly, *S. choleraesuis* as likely causes. *Definitive* **diagnosis** *depends on isolation of the agent, and microscopic identification of suppurative bronchopneumonia with neutrophil necrosis.* Isolation on blood agar requires a *Staphylococcus* streak or supplemented medium containing a source of nicotinamide adenine dinucleotide. Alternatively, bacteria in lung smears or primary cultures can be identified using antigen detection techniques such as ELISA or latex agglutination. Current polymerase chain reaction assays for APP cross-react with other *Actinobacillus* spp, and this assay is not commonly employed. Serologic identification of APP is particularly useful in the maintenance of minimal-disease herds; assays include the reference standard complement fixation test, which is recognized to have low sensitivity, and an ELISA that is useful but suffers from poor repeatability.

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Bronchopneumonia caused by miscellaneous bacterial pathogens

The porcine respiratory disease complex is an exemplar of multifactorial disease causation. Although bacterial pneumonia is the final cause of death in many cases of porcine respiratory disease, its development is highly dependent on host factors, environmental influences, and other infectious agents. Important contributing environmental factors include elevated levels of dust and ammonia, temperature fluctuations and, to a lesser extent, extremes of temperature and humidity. In temperate climates, the incidence of swine pneumonia is maximal in the autumn and early winter, in part caused by fluctuating temperatures and the impact of cold weather on barn ventilation and air quality. Immunity to respiratory pathogens is dependent on patterns of pig flow through production units, vaccination practices, and alterations in innate resistance due to social and environmental stresses, nutrition, and management practices. Against this background of host and environmental factors, interactions between multiple pathogens are commonly identified in swine pneumonia. Thus, *effective control of disease requires that diagnostic investigations identify not only the immediate causes of fatal disease, but also the underlying infectious and noninfectious factors.*

Porcine reproductive and respiratory syndrome virus, *Mycoplasma hyopneumoniae*, swine influenza virus, and *Porcine circovirus 2* may all cause primary lung disease. In addition, all of these agents impair the normal pulmonary defenses against inhaled bacterial pathogens by, among other mechanisms, impairing mucociliary clearance and suppressing alveolar macrophage function. Swine are commonly exposed to the **opportunistic pathogens** *Pasteurella multocida*, *Streptococcus suis*, *Bordetella bronchiseptica*, and *Haemophilus parasuis*, and failure of lung defenses due to viral or mycoplasmal infection, poor air quality, or stresses are probably key factors in development of disease caused by these bacterial pathogens.

P. multocida serotypes A and D are important causes of fatal pneumonia in weaner and grower-finisher pigs. The massive doses of *P. multocida* needed for experimental induction of disease suggest that the predisposing causes listed above are necessary components of the natural disease. *Bordetella* is particularly noteworthy as a cause of fatal pneumonia in piglets less than 3 weeks of age, but may also cause bronchopneumonia in older swine.

Gross lesions of bronchopneumonia, in which cranioventral areas of lung are swollen, consolidated, reddened, and sharply demarcated from more normal lung, are typical of infection with these opportunistic bacterial pathogens. In many, but not all, cases, mucopurulent exudate may be expressed from airways on cut section. Fibrinous pleuritis may be present, but the massive serofibrinous exudate typical of *Actinobacillus pleuropneumoniae* is not present. Bronchial lymph nodes are often enlarged and mottled red and white. The predominant **histologic findings** are numerous neutrophils and macrophages filling alveoli and bronchioles. Fibrin exudation may occur but is usually minor. These lesions contrast with those of *A. pleuropneumoniae* and *A. suis*, where fibrinous exudate in the lungs and on the pleural surface is often prominent, most of the neutrophils are necrotic and have streaming basophilic chromatin, and focally extensive coagulative necrosis is often present. In cases of bronchopneumonia the bronchiolar epithelium is generally normal or mildly hyperplastic, but bronchiolar necrosis is usually absent unless there is underlying SIV or PCV2 infection. *M. hyopneumoniae* also causes a cranioventral pattern of bronchopneumonia, but in contrast to the red swollen lung of bacterial bronchopneumonia, the acute lesions of mycoplasmosis are red and atelectatic, while the subacute lesions are gray to tan and only mildly swollen to firm. Cranioventral bronchopneumonia superimposed on generalized or patchy lesions of interstitial pneumonia suggests underlying PRRSV or PCV2 infection, or septicemia.

While bacterial bronchopneumonia may be the immediate cause of death, viral or mycoplasmal pathogens should be considered as underlying causes. Microbiologic testing is commonly employed to identify the spectrum of pathogens causing pneumonia, but the plethora of currently available tests does not diminish the importance of morphologic examination of the lung. Determining the patterns of gross and histologic lesions allows the pathologist to narrow the list of differential diagnoses to select a panel of ancillary tests efficiently, to provide a tentative diagnosis when rapid therapeutic intervention is required or when ancillary microbiologic tests are negative, and to evaluate critically the contribution of particular pathogens, many of which are commonly present in healthy swine, to the case being investigated.

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Atrophic rhinitis

Nonprogressive atrophic rhinitis (NPAR), caused by *Bordetella bronchiseptica* and other factors, causes mild transient sneezing and nasal discharge, and is *not usually of herd significance*. In contrast, **progressive atrophic rhinitis** (PAR) is caused by toxin-producing strains of *Pasteurella multocida*, often in concert with other bacterial pathogens, and is *a serious cause of production loss*. PAR affects pigs of at least 6–12 weeks of age, causing sneezing, mucopurulent nasal discharge, unilateral epistaxis, nasal deformity, failure to thrive, and

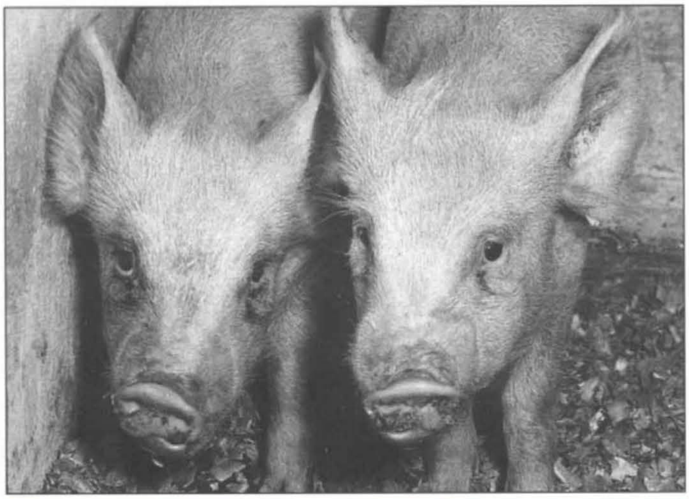

Figure 5.52 Atrophic rhinitis with deviation of the snouts of two pigs.

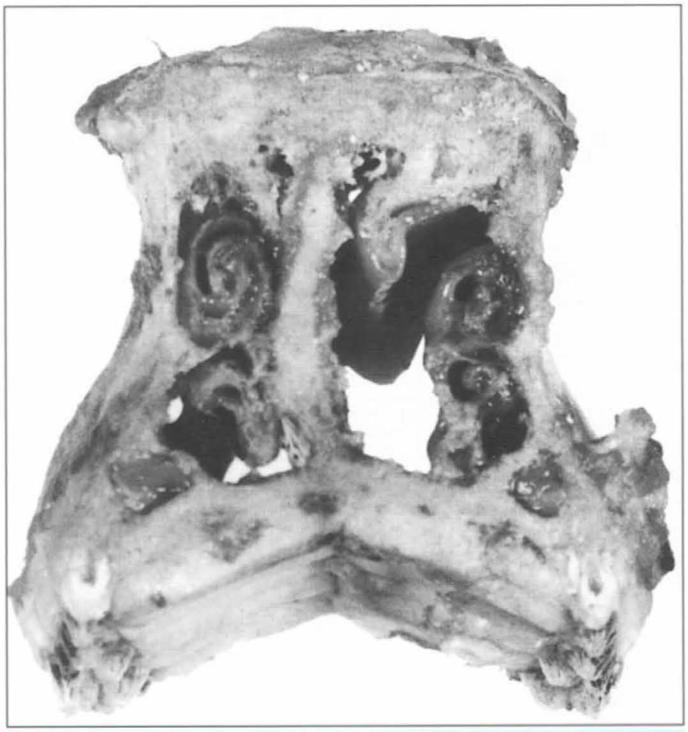

Figure 5.53 Atrophic rhinitis in a pig, with atrophy of turbinates and deviation of the nasal septum.

secondary bacterial bronchopneumonia. Freedom from PAR is a frequent requirement in minimal-disease herds, so *differentiation of PAR and NPAR is of substantial significance.* Toxigenic *P. multocida* infects rodents, cats, dogs, and ruminants, and may be of zoonotic concern.

Both NPAR and PAR are multifactorial conditions for which air quality and the presence of other pathogens influence the severity of disease. NPAR is primarily caused by strains of *B. bronchiseptica* that adhere to nasal cilia and tonsillar epithelium, and produce a toxin that causes mucosal edema, loss of cilia, and resorption of turbinate bone. Infection is acquired by inhalation of infected aerosol droplets.

Progressive atrophic rhinitis is caused by cytotoxin-producing strains of P. multocida type D or, less commonly, type A. Infection is acquired by inhalation following direct contact with infected pigs. Colonization of the nasal mucosa and tonsil is inefficient, but augmented by factors that harm the nasal mucosa, including *B. bronchiseptica* infection, ammonia, and dusts. The *cytotoxin*, also referred to as *dermonecrotic toxin*, is a secreted, heat-labile toxin encoded by the *tox*A gene. Intranasal or intramuscular administration of *P. multocida* cytotoxin has similar effects on the nasal turbinates, including epithelial hyperplasia, glandular atrophy, resorption of turbinate bone by osteoclasts, reduced formation of bone by osteoblasts, and fibroblast proliferation. The cytotoxin does not directly affect osteoclast function; rather, hyperplasia and increased function of these cells are apparently stimulated by soluble mediators secreted from nearby stromal cells in response to the cytotoxin.

Distortion of the snout is a prominent external finding in pigs with chronic atrophic rhinitis (Fig. 5.52). Brachygnathia superior occurs when the maxillary bones of the snout fail to grow. Lateral deviation toward the most severely affected side is common.

Atrophy and malformation of the nasal turbinates are the principal lesions of atrophic rhinitis, and are more severe in the ventral than the dorsal conchae. In cross-sections of the snout at the level of the first or second upper premolar teeth, the normally scroll-like turbinates are misshapen and form irregular or jumbled finger-like protrusions (Fig. 5.53). Turbinate atrophy may be partial or complete, leaving a hollow nasal cavity with an often-deformed nasal septum.

Histologic lesions in the nasal turbinates include *osteoclast hyperplasia, osteoclast-mediated resorption of bone, and replacement by fibrous tissue containing many plump fibroblasts.* Lymphocytes and fewer neutrophils infiltrate the nasal mucosa, and there may be squamous metaplasia of the ciliated epithelium.

The gross lesions of NPAR and PAR are qualitatively similar, although those of NPAR are generally less severe. Nevertheless, given the significance of a diagnosis of progressive atrophic rhinitis due to toxigenic *P. multocida*, definitive identification is usually warranted. *B. bronchiseptica* and *P. multocida* can be readily isolated from swabs of nasal mucosa or tonsil, but *definitive diagnosis of PAR depends on demonstrating P. multocida cytotoxin production using PCR or an ELISA.* Various necropsy-grading systems have been described to assess the severity of nasal turbinate atrophy. These grading systems are not intended to differentiate NPAR from PAR, but are useful in monitoring the herd response to management or therapeutic interventions.

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Mycoplasmal diseases

General features of mycoplasmas

Mycoplasmas, which are members of the class Mollicutes, are the smallest self-replicating organisms, lack a cell wall, and have a protein- and lipid-rich plasma membrane. They are obligate parasites, and several species lack the ability to synthesize required amino acids, cholesterol, and fatty acids. The success of culturing mycoplasmas in vitro is highly dependent on the particular species: some mycoplasmas grow so readily that they commonly contaminate cell cultures, yet many of the pathogenic mycoplasmas grow slowly, have frustratingly fastidious growth requirements, and are easily overgrown by other agents. Mycoplasmas have a relatively small genome, 580–1380 kb for the species examined to date, which contains many repetitive elements and a propensity for genomic rearrangement through homologous recombination. Many mycoplasmas have highly specific host and tissue tropism, and are commonly commensals on mucosal surfaces. Nevertheless, spread of infection through the blood with subsequent localization in joints or serosal surfaces is a feature of some mycoplasmoses. *Most mycoplasmal pathogens of the respiratory tract adhere to ciliated epithelium*, mediated in some species by a specialized attachment organelle. A few mycoplasmas invade host cells, but this has not been demonstrated for mycoplasmal pathogens of animals.

High-frequency variation of surface antigens allows several mycoplasmas to evade the humoral immune response by changing their surface lipoprotein antigens as antibodies against these structures are formed. The mechanisms by which *Mycoplasma* infections result in disease remain poorly characterized. Mycoplasmas have not been shown to secrete exotoxins. *Pathogenic mechanisms* of respiratory disease include ciliostasis due to membrane alterations, alteration of host prostaglandin synthesis, induction of apoptosis in lymphocytes or other host cells, and nonantigen-specific stimulation of immune responses as a consequence of superantigens in the mycoplasmal plasma membrane. The latter is the likely factor that induces the *prominent peribronchiolar lymphocytic inflammation that is so characteristic of many pulmonary mycoplasmoses.*

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Mycoplasma hyopneumoniae

Mycoplasma hyopneumoniae is the cause of *mycoplasmal pneumonia of swine*, also known as *enzootic pneumonia*. It is a common, chronic, usually nonfatal disease of young pigs. The disease may be endemic, or spread slowly but progressively through a facility over the course of weeks, and the morbidity rate may be as high as 70–100%. Although pigs as young as 5 weeks of age may develop disease, it is *most important in grower-finisher pigs* and may be a key component of fatal multifactorial pneumonia in 4–6-month-old hogs. Clinical expressions of the uncomplicated disease are coughing, unthriftiness, poor weight gain, and reduced feed conversion. Because there is usually low mortality associated with mycoplasmal pneumonia, the lesions are generally seen in slaughtered animals or those dying from other diseases. When deaths do occur, they are mainly due to superimposed bacterial infections.

Like other mycoplasmal pathogens, *M. hyopneumoniae* is a tiny bacterium that lacks a cell wall. The agent adheres to ciliated epithelium of the large airways and, in lower number, of the bronchioles, using 97 and 145 kDa outer-membrane proteins. Adherent organisms are closely associated with the cilia, and there is often extensive ciliary loss. In chronic infections, mycoplasmal antigen and nucleic acid are also present in alveolar and interstitial macrophages. The cell membranes of many mycoplasmas, presumably including *M. hyopneumoniae*, contain superantigens that induce polyclonal proliferation of lymphocytes and result in characteristic aggregates of lymphocytes around airways and blood vessels. Infection with *M. hyopneumoniae* is mainly transmitted by direct contact with nasal secretions from infected pigs, although aerosol transmission over short distances may occur. A common clinical scenario involves infection of a few young pigs by contact with an infected sow, followed by slow spread to in-contact pigs when groups are assembled in weaner or grower-finisher units.

Clinical disease is not an inevitable consequence of infection with *M. hyopneumoniae*. Rather, its development is highly dependent on other predisposing factors, including crowding, poor air quality, fluctuations in temperature or humidity, and the presence of other pathogens. Many infections are subclinical, and carrier animals are important sources of infection of naive herds. Primary *M. hyopneumoniae* infection is usually not fatal, but reduced mucociliary clearance and possibly impaired macrophage function may lead to fatal bacterial pneumonia caused by opportunistic pathogens such as *Pasteurella multocida, Streptococcus suis, Bordetella bronchiseptica*, or *Haemophilus* spp. The interaction of PRRSV with *M. hyopneumoniae* is particularly noteworthy: mycoplasmal infection exacerbates the severity and the duration of PRRS, although the effect of PRRSV infection on mycoplasmal pneumonia is apparently minor. As a result, new infections with *M. hyopneumoniae* may cause outbreaks of PRRS in herds with previously subclinical PRSSV infections.

The characteristic **gross feature** *of mycoplasmal pneumonia is red-tan-gray discoloration, collapse, and rubbery firmness affecting cranioventral regions of the lungs in a lobular pattern* (Fig. 5.54). Lesions often affect cranial and middle lung lobes, accessory lobe, and cranioventral portions of the caudal lobes. The mildest lesions resemble atelectasis, with a lobular pattern of reddening and collapse. More severe lesions evolve from dark red through gray-pink to more homogeneous gray as the lesion ages over the several-month course of disease. The rubbery or thymus-like texture of the lung contrasts with the more firm or

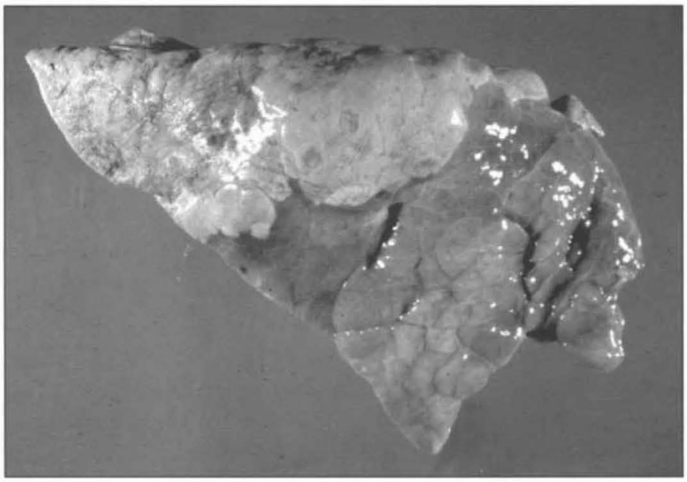

Figure 5.54 ***Mycoplasma hyopneumoniae* pneumonia** in a pig. The cranioventral lung (right) is dark, collapsed, and rubbery.

hard consistency of *Pasteurella* pneumonia, although this is often present concurrently. The cut surface of affected lung is edematous, and catarrhal exudate can be squeezed from bronchi. The bronchiolar orientation of the inflammation often results in a regular pattern of small gray nodules against a red background. *M. hyopneumoniae* rarely causes serofibrinous pleuritis or polyserositis, but this is more commonly due to *M. hyorhinis* or opportunistic bacteria. Pulmonary lymph nodes are enlarged and on cut surface are moist, usually bulging and sometimes hyperemic.

Histologically, subacute to chronic mycoplasmal pneumonia in swine is typified by lymphocytic infiltrates around airways, increased numbers of alveolar macrophages, and alveolar edema. In the fully developed mycoplasmal pneumonia, there is *extensive lymphoid hyperplasia* around bronchi, bronchioles, and their associated vessels; and lymphocytes infiltrate the lamina propria of the airway mucosa. The epithelium is often histologically normal or mildly hyperplastic. Although ciliary loss and exfoliation of ciliated epithelium may occur, this if difficult to detect by light microscopy. Goblet cells in the airway mucosa are increased in number and the bronchial submucosal glands are hyperplastic, reflecting the excessive mucus secretions seen grossly. The alveolar exudate consists predominantly of macrophages and protein-rich edema, but variable numbers of plasma cells, lymphocytes, and neutrophils are present. The alveolar septa are mildly thickened by lymphocytes and few plasma cells (Fig. 5.55).

Experimental studies of the pathogenesis of mycoplasmal pneumonia indicate that typical gross lesions do not occur until 2–4 weeks after infection. The rate of development of lesions is dependent on the dose and strain of *M. hyopneumoniae*, method of administration, and susceptibility of the pigs exposed. Young pigs naturally exposed to infectious aerosols soon after birth can develop lesions by the time they are 3–5 weeks of age. In the first week after experimental infection, neutrophils are present in airway lumens and, to a lesser extent, in alveoli. The numbers of neutrophils diminish and lymphoid cells increase over the subsequent several weeks to reach the fully developed stage of consolidation. After it has reached its peak some 5–6 weeks after infection, lesions of uncomplicated mycoplasmal pneumonia may persist or completely resolve within 2 months.

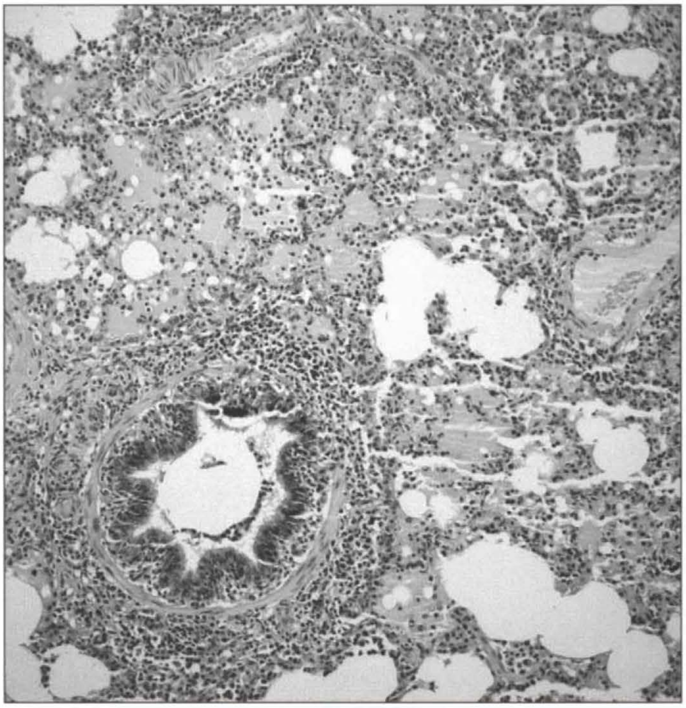

Figure 5.55 ***Mycoplasma hyopneumoniae* pneumonia** in a pig. The bronchiole is surrounded and invaded by lymphocytes, and the alveoli are edematous and contain increased numbers of macrophages.

The gross lesions of gray-tan discoloration and thymus-like texture are quite characteristic. However, many fatal cases are secondarily infected by *Pasteurella multocida* or other opportunists, resulting in red, firm or hard, cranioventral consolidation that appears identical to the bacterial pneumonia that complicates PRRSV, PCV2, or SIV. Similarly, the well-developed histologic lesions with thick peribronchial cuffs of lymphocytes are highly suggestive, yet lymphoplasmacytic peribronchiolitis – usually milder – is also often present in chronic PRRSV, SIV, or PCV2 pneumonia. In these cases, other histologic features can suggest a *tentative diagnosis*: *M. hyopneumoniae* also induces alveolar edema and increased numbers of plump alveolar macrophages, PRRSV results in diffuse lymphocytic infiltrates in alveolar septa, SIV and some PCV2 infections are accompanied in the acute stages by attenuation of bronchial epithelium, and granulomatous infiltrates in lymph nodes suggest PCV2 infection.

Establishing a definitive **diagnosis** in swine herds with pneumonia often requires laboratory support. *M. hyopneumoniae* is extremely fastidious in its growth requirements, and isolation in culture is not a useful method of diagnosis. More sensitive confirmation of a morphologic diagnosis can be obtained by identifying antigen in lesional lung tissue by immunohistochemistry or fluorescent antibody tests, or detection of *M. hyopneumoniae* DNA by polymerase chain reaction using lung tissue, nasal swabs, or transtracheal washes. Detection of serum antibody by ELISA is a useful method of confirming prior exposure, or validating the *M. hyopneumoniae*-free status of minimal disease herds.

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Fungal diseases

Pneumocystis carinii

Pneumocystis carinii is a sporadic cause of acute or chronic dyspnea, usually without fever, in pigs, foals, dogs, and other domestic animals. Outbreaks of respiratory disease in pigs have been associated with *Pneumocystis* infection, but it is doubtful that *Pneumocystis* was the primary cause of respiratory disease. *Clinical disease due to Pneumocystis is unlikely without underlying immunosuppression*; identified causes include corticosteroid or other immunosuppressive therapy; severe combined immunodeficiency in foals, sometimes in association with adenovirus or *Rhodococcus* infection; common variable immunodeficiency of Miniature Dachshunds, demodicosis, or canine distemper in dogs; and perhaps PRRSV or PCV2 infection in pigs.

Although previously considered a protozoan, molecular analyses have conclusively determined that *P. carinii* is a *fungus*, probably in the class Ascomycetes. Isolates from different host species should be considered distinct species or subspecies, and there is little evidence that the fungus is of zoonotic importance. The life cycle is based only on morphologic evaluation, but involves a 1–4 μm diameter, thin-walled, *uninucleate, trophic form* that replicates by binary fission; and a 5–8 μm diameter, *thick-walled, multinucleate cyst form* that develops eight intracystic bodies, which may be daughter forms that are products of sexual replication. The latter are released, attach to type I pneumocytes, and presumably develop into trophic forms. The cell wall is composed of mannose-rich polysaccharides and the major surface glycoprotein. This glycoprotein A is the immunodominant antigen and mediates binding of *Pneumocystis* to type I pneumocytes and macrophages, surfactant proteins, and fibronectin. Both macrophages and cell-mediated immune responses are essential for controlling *Pneumocystis*; hence, clinical disease implies that there has been impairment of macrophage function or cell-mediated immune responses. In addition to the space-occupying effect of the fungus, *Pneumocystis* may alter lung function by interfering with surfactant homeostasis.

Gross lesions of *Pneumocystis* pneumonia are diffuse or patchy, red to yellow-brown regions of rubbery firmness or consolidation. The characteristic histologic finding is *foamy or "honeycomb" material filling alveoli*, due to the presence of numerous intra- and extracellular, 8-μm-diameter, round or crescent-shaped, clear fungal bodies, each containing one or more 0.5-μm-diameter, lightly basophilic bodies (Fig. 5.56). Foamy macrophages are present and contain the organisms, but the presence of lymphocytes, plasma cells, type II pneumocytes, and interstitial fibrosis is variable. *The diagnosis is made by identifying the fungi in histologic sections.* They are easily overlooked on hematoxylin and eosin-stained sections, but the wall of the cyst forms stains with methenamine silver or PAS. *Pneumocystis* cannot be cultured using conventional techniques, but novel cell culture systems have recently been described.

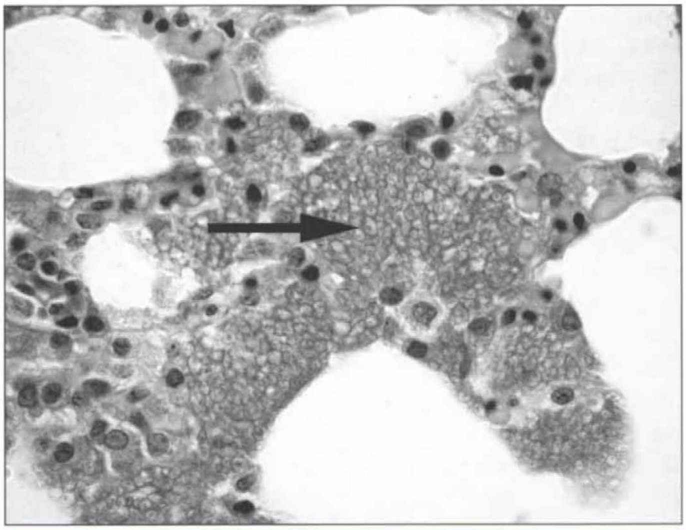

Figure 5.56 *Pneumocystis carinii* infection in a pig. The alveolus is filled with innumerable fungi, each with clear cytoplasm and a tiny dark nucleus (arrow).

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Parasitic diseases

Ascaris suum, because of its tremendous biotic potential, may migrate in huge numbers and occasionally be fatal. The lesion is acute, diffuse, eosinophilic, interstitial pneumonia associated with the presence of large numbers of larvae. Rare parasitisms of the respiratory tract of pigs include ***Paragonimus*** spp. and **hydatid disease**.

***Metastrongylus** apri (elongatus), M. pudendotectus*, and *M. salmi* are all parasitic in the bronchi and bronchioles of pigs. They are nematodes of the superfamily Metastrongyloidea, family Protostrongylidae. Because the intermediate host is the earthworm, pulmonary metastrongylosis is now rare in housed swine, but it is common in wild boar. They are believed to be responsible for the occasional transmission of swine influenza virus. The adult worms are white, thread-like, and 14–60 mm in length. In heavy infections, which are mostly in young pigs, they may be found in all lobes of the lung. When there are fewer worms, particularly as occurs in older animals, the worms may be restricted to airways along the caudoventral borders of the caudal lobes. In histologic sections, the adults have polymyarian coelomyarian musculature, cuboidal multinucleate intestinal epithelium with an indistinct microvillus layer, and thick-shelled eggs or embryonated eggs in the uterus. Eggs are laid in the bronchi and a few of them hatch there, but most hatch after passing to the exterior in the feces. First-stage larvae are inactive and are capable of prolonged survival in moist conditions. Their further development depends on ingestion by earthworms, which are the intermediate hosts. The larvae develop to the third, infective stage in about 10 days, and then remain quiescent unless the earthworm is eaten by a pig. The larvae may survive for as long as 18 months in the earthworms and, by that time, thousands may be accumulated by a single worm. Migration within the pig is through the lymphatics from the intestine to the lungs. Some larvae pass through the liver and produce focal hepatitis, as is more commonly caused by *Ascaris suum*.

Even with heavy adult infestations, gross lesions are inconspicuous. The worms live in the smallest airways, and on superficial examination of the lungs the presence of the parasites is frequently only indicated by gray-pink nodules 1–3 mm in diameter and by hyperinflated lobules along the caudoventral margins of the caudal lobes. Histologically, the lesions are similar to those produced by *Dictyocaulus* spp. The initial lesions are multiple foci of intense accumulations of eosinophils surrounding larvae in alveoli. Subsequently, when reproduction is active, an eosinophilic and granulomatous response occurs to the eggs and larvae in the alveoli. The prepatent period for *Metastrongylus* spp. is about 25 days, after which the rate of egg production rapidly reaches a peak and then subsides to a low level. At this later stage, the adults persist mainly in the bronchioles and small bronchi and provoke goblet-cell hyperplasia and exudation of mucus, eosinophils, neutrophils, and mononuclear cells. The bronchiolar epithelium is mildly hyperplastic, and eosinophils, lymphocytes, and plasma cells permeate the lamina propria.

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Infectious respiratory diseases of cattle

Viral diseases

General features of herpesviruses

Herpesviruses are 120–200 nm diameter, and composed of an icosahedral nucleocapsid surrounded by a proteinaceous tegument and an outer envelope. Herpesviruses have large genomes composed of double-stranded DNA. Viral infection of cells induces a highly regulated cascade of viral gene expression: preformed tegument proteins and the products of immediate-early and early genes regulate the host transcriptional machinery and viral gene expression, and late genes encode structural proteins of the virus. Viral glycoproteins often form peplomers that project from the surface of the envelope; in addition to being key targets of protective immune responses, these envelope glycoproteins mediate viral entry into target cells, cellular fusion to form syncytia, and spread of virus between cells without exposure of the virus to extracellular antibody. Some envelope glycoproteins act as complement C3b or immunoglobulin Fc receptors, and several herpesviruses encode chemokine or chemokine receptor homologues that modulate the immunoinflammatory response. Viral replication occurs in the nucleus, and virions acquire an envelope by budding through the nuclear membrane. Lysis of the cell releases the mature virions to the extracellular space.

Herpesviruses are classified into *α-*, *β-*, and *γ-herpesvirinae* subfamilies. *The* ***α-herpesviruses*** *are characterized by rapid growth in cell culture, lytic infection of cells, necrotizing lesions, the formation of syncytia in some instances, eosinophilic intranuclear inclusion bodies, latent infections, and narrow or broad host ranges.* Most α-herpesviruses establish latent infections in neurons of the trigeminal ganglia and other sensory ganglia, as well as other sites, including olfactory bulb, optic nerve, nasal turbinate, and cornea. During latent infections, gene expression is restricted to the so-called latency-associated transcripts, which have been utilized as markers of latent infection. Alpha-herpesviruses affecting the lung of domestic mammals include *Bovine herpesvirus 1* (infectious bovine rhinotracheitis), *Caprine herpesvirus 1; Suid herpesvirus 1* (pseudorabies), *Equid herpesviruses 1* and *4* (equine abortion, equine viral rhinopneumonitis), *Canid herpesvirus 1*, and *Felid herpesvirus 1* (feline viral rhinotracheitis).

The **β-herpesviruses**, including *Suid herpesvirus 2* (porcine cytomegalovirus) have a limited host range, and these cytomegaloviruses induce pathologic enlargement of infected cells and prominent basophilic intranuclear inclusion bodies. Replication of cytomegaloviruses is slower than that of α-herpesviruses, and disease is more chronic. As the immune response is generally effective, disease due to cytomegalovirus occurs principally in immunodeficient or immunosuppressed animals.

Infectious bovine rhinotracheitis

Infection with *Bovine herpesvirus-1* (BoHV-1, Infectious bovine rhinotracheitis virus) is widespread in cattle populations, but the disease infectious bovine rhinotracheitis (IBR) is uncommon in areas where vaccination is practiced. Although sporadic outbreaks of IBR still occur, the ability of BoHV-1 infection to impair lung defenses and predispose to bacterial pneumonia are probably of more importance to the beef industry. Feedlot calves usually develop IBR at least 3 weeks after entry to the feedlot, with an abrupt onset of fever, anorexia, tachypnea, mucopurulent nasal discharge, and dyspnea or open-mouth breathing. The nasal mucosa and muzzle are often hyperemic, suggesting the epithet "red nose," and white, loosely adherent plaques may peel from the surface. The mortality rate is low unless secondary bacterial pneumonia develops. Keratoconjunctivitis is common in calves with IBR, and affected cows or others in contact may abort. Vulvovaginitis and balanoposthitis are uncommon in calves with IBR. Calves that are infected in utero with BoHV-1 may be aborted, or may develop systemic disease in the neonatal or early suckling period up to about 1 month of age.

Affected calves are febrile, depressed, anorexic, and may display dyspnea or diarrhea.

Bovine α-herpesvirus infections cause infectious bovine rhinotracheitis, keratoconjunctivitis, bronchointerstitial pneumonia, abortion, encephalitis, systemic herpesvirus infection in young calves, and pustular vulvovaginitis or balanoposthitis. The factors that determine the manifestation are not fully understood, but include the following: route of infection (genital infection usually manifests as vulvovaginitis or balanoposthitis); strain of the virus (a distinct virus type, BoHV-5, is the usual cause of herpesviral encephalitis in calves); and degree of immunity (systemic herpesviral infection develops in young calves with low serum antibody titers to the virus).

BoHV-1 contains a large 136 kb genome that encodes 67 proteins. Ten envelope glycoproteins have been described. The glycoproteins gC and gD bind cell surface receptors, allowing virus entry into the cell by gB, gH, and gL. Fusion of cells, mediated by gB and gD, allows intercellular spread of virus and evasion of the immune response, and is the molecular basis of the epithelial syncytia that are occasionally noted histologically. The gC glycoprotein binds C3 component of complement and prevents complement activation, while gI and gE are Fc receptors for immunoglobulin that impair antibody-mediated neutralization of the virus.

BoHV-1 infects a wide range of animals, including cattle, sheep, goats, llamas, swine, water buffalo, mustelids, and rabbits, but *primary viral respiratory disease is only described in cattle.* Sources of BoHV-1 infection include clinically or subclinically infected cattle, and reactivation of latent infections. Wild ruminants may be subclinical reservoirs. Infection is transmitted by aerosol; direct contact with nasal, ocular, or vaginal secretions; or by indirect contact with fomites, semen, feed, or water. Systemic spread of the virus is a feature of the syndromes of systemic infection, abortion, and encephalitis. Viremia is not usually evident in cases of IBR, keratoconjunctivitis, vulvovaginitis, or balanoposthitis, although monocyte- or lymphocyte-associated viremia may occur at low level. In the latter conditions, virus spreads along mucosal epithelium, nerves, and lymphatics. *Latent infection of trigeminal or other sensory ganglia is common,* and probably follows axonal spread of infection rather than viremia. In latently infected neurons, viral DNA is present but, with the exception of a few "latency-associated transcripts," viral RNA or protein cannot be detected. Stress, glucocorticoid or epinephrine therapy, hyperthermia, or hypothermia trigger reactivation of the latent infection, and virus reaches the mucosal surface by retrograde axonal transport where mucosal replication leads to shedding of infectious virus. Reactivated infections are usually subclinical, but may act as a source of contagion to naive, in-contact animals.

Infection of the respiratory tract induces *clinical disease* after an incubation period of 2–6 days, and virus is shed in nasal secretions for at least 10–16 days. Clinical IBR is primarily an effect of lytic infection of nasal and airway epithelial cells. Functional abnormalities such as serotonin- or dopamine-induced bronchoconstriction may also contribute to clinical signs. Secondary bacterial pneumonia in calves with IBR is a consequence of the destruction of ciliated epithelium by lytic viral infection, and impairment of the ability of alveolar macrophages to phagocytose bacteria and secrete neutrophil chemotactic factors. These impairments of lung defenses are maximal at 4–5 days after viral infection.

Interferon-α and -β secretion is detectable within 5 hours of BoHV-1 infections, and peaks at 36–72 hours. These interferons mediate innate resistance to spread of the infection, in part by recruiting macrophages and natural killer cells to the sites of viral infection. Cell-mediated immune responses are detectable in the respiratory tract within 5 days of infection, and mediate lysis of virus-infected cells. Neutralizing IgG is first detected at 10 days after infection, and IgA responses develop later. Antibody prevents disease in calves exposed to the virus. In infected calves, antibody neutralizes free virus and may prevent mucosal shedding of virus following reactivation of latent infection. However, because the virus evades the humoral immune response by intracellular spread to adjacent cells, a combination of cellular and humoral immunity is probably necessary for recovery from infection.

Gross lesions of IBR are usually restricted to the *nasal cavity, larynx, and trachea.* Pustules erupt early after infection, but are fragile and rarely observed. Petechiae, a granular appearance of the mucosa, and serous exudate may be present in acute lesions. More commonly, there is intense hyperemia of the mucosal tissue, multifocal coalescing erosions with loosely adherent plaques of white debris, or diffuse ulceration covered by a mat of fibrinonecrotic and suppurative exudate forming a pseudodiphtheritic membrane (Fig. 5.57). When the exudate is peeled from the surface of the trachea or nasal cavity, dull granular eroded tissue remains. In contrast, in cases of bacterial pneumonia in which expectorated material accumulates on the tracheal or nasal mucosa, gentle removal of the exudate reveals a shiny intact mucosal surface. In severe cases, obstruction of the laryngeal or tracheal lumen by abundant exudate may prove fatal. Emphysema is a sequel to dyspnea in severely affected calves.

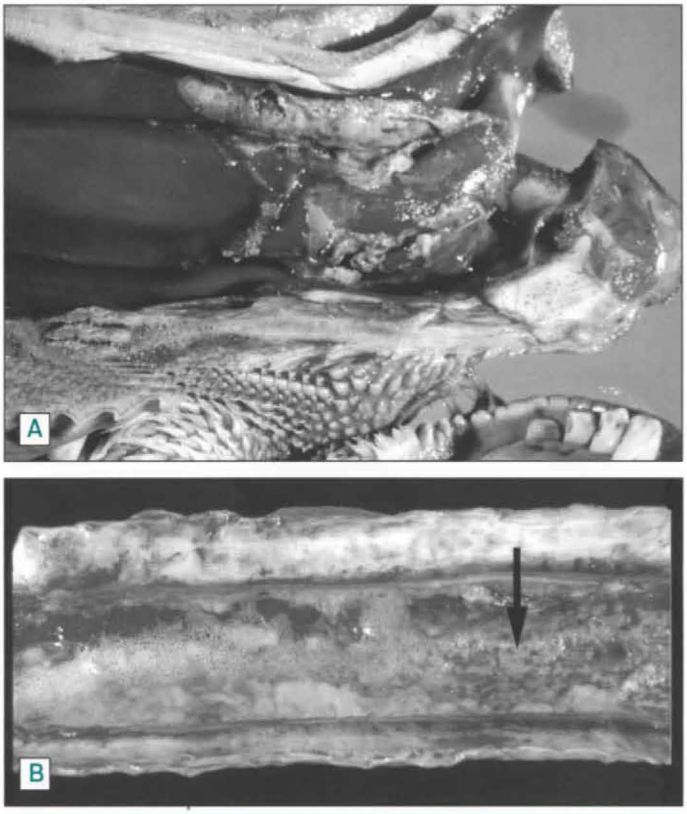

Figure 5.57 Infectious bovine rhinotracheitis. A. Fibrinous exudate adherent to the eroded mucosa of the rostral nasal cavity. **B.** Multifocal coalescing ulcers (arrow) of the tracheal mucosa.

The earliest **histologic lesions**, in the first 2 days after infection, include cytoplasmic vacuolation and pallor and nuclear pyknosis or karyolysis. Eosinophilic intranuclear inclusion bodies are present in the epithelium of the nasal turbinates, tracheal submucosal glands, and bronchi, but are rare in the tracheal surface epithelium. *Inclusions are absent in most diagnostic cases*, and the lesions at these later times include erosion or ulceration of nasal and tracheal mucosa, with necrosis and exfoliation of infected epithelial cells and exudation of neutrophils and fibrin. Neutrophils and mononuclear cells infiltrate the lamina propria. Mild perivascular infiltrates of lymphocytes may be detected in the trigeminal ganglion and brainstem.

Pulmonary lesions of primary viral pneumonia are uncommon, but *young calves may develop bronchointerstitial pneumonia without significant upper respiratory lesions*. There is erosion of bronchiolar epithelium and proliferation of type II pneumocytes. Epithelial syncytia may be numerous in alveoli, and eosinophilic intranuclear inclusion bodies are more common than is seen in IBR.

Systemic lesions of BoHV-1 infection in young calves include multiple, coalescing, 1–7 mm diameter, sharply demarcated foci of necrosis in the upper digestive tract, including the oral cavity, esophagus, and rumen. Necrotic foci are often covered by adherent debris and ingesta, which appears as caseous clumps of curdled milk. Multifocal, pinpoint to 1 mm diameter, white foci of necrosis are often present in the liver, and occasionally in kidney and spleen. The nasopharynx and larynx may contain erosive and exudative lesions, but tracheal and pulmonary lesions are not typical. Histologically, the foci of caseous necrosis affect the full thickness of the epithelium, and are accompanied by infiltration of neutrophils. Eosinophilic intranuclear inclusion bodies are usually apparent at the margins of the necrotic lesions. The injured mucosa may be secondarily infected with *Candida* sp. yeast. The lesions of herpesviral abortion are described in Vol. 3, Female genital system.

The gross appearance of IBR is highly suggestive of the causative agent. A casual observer may mistake loosely adherent expectorant for the tracheal ulceration of IBR; *Bovine parainfluenza virus 3* can cause nasal erosions, but aspiration or inhalation of chemical irritants, which is rarely encountered, is the only process likely to be confused with florid lesions of IBR. However, as subclinical BoHV-1 infection impairs pulmonary defenses, laboratory investigation to demonstrate this pathogen is often warranted in calves with bacterial pneumonia. The virus grows readily in cell culture and produces characteristic cytopathic effects. Thus, *virus isolation* from nasal swabs, nasal mucosa, trachea, or lung is an excellent means of definitive diagnosis, and has the advantage that novel strains may be identified and archived. Fluorescent antibody tests and immunohistochemistry are also useful for the diagnosis of BoHV-1 infection, and are advantageous when a rapid diagnosis is needed. A caveat is warranted when diagnosing BoHV-1 infection in the absence of characteristic lesions: *cattle with other diseases may experience stress-induced reactivation of latent herpesviral infections, leading to identification of the reactivated virus in any of these diagnostic assays.* Samples should be obtained early in the course of disease, as antibody in the exudate of subacute lesions may preclude isolation of the virus and block antigen–antibody interactions in antigen-based assays. Detection of seroconversion is useful to associate BoHV-1 infection with clinical disease.

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Bovine respiratory syncytial virus

Infection with *Bovine respiratory syncytial virus* (BRSV) is common in North American and European beef and dairy herds. Seroprevalence studies suggest that about half of range calves are exposed to BRSV, and most nonexposed calves seroconvert within a month of entering feedlots. The seroprevalence of BRSV in adult cattle varies from 40 to 95%. *BRSV is an important cause of both acute outbreaks of respiratory disease and "enzootic pneumonia"* in 2-week to 5-month-old dairy and beef calves, with a peak incidence at 1–3 months of age. BRSV certainly causes *fatal bronchointerstitial pneumonia* soon after arrival in beef feedlots, but the role of BRSV in acute interstitial pneumonia in the late feeding period is questionable. Finally, *BRSV predisposes to bacterial pneumonia* in feedlot beef cattle by impairing lung defenses, and occasionally causes respiratory disease in naive adult dairy cows.

The disease is most prevalent in the autumn or early winter. *Clinical signs* in calves and cows are similar and include high fever, coughing, tachypnea, and variable nasal discharge and conjunctivitis. Some animals develop dyspnea with open-mouth breathing and increased abdominal effort. Calves often maintain a reasonable appetite in the face of severe respiratory distress, in contrast to the consistent depression of cattle with bacterial pneumonia. A biphasic clinical course is not present in all cases; when present, it is characterized by transient pyrexia and mild respiratory disease, temporary improvement for days to weeks, then rapid onset of severe respiratory distress.

BRSV, a member of the genus *Pneumovirus* in the family Paramyxoviridae, is pleomorphic, spherical or irregularly shaped, enveloped, 80–450 nm diameter, and contains negative-sense single-stranded RNA. The viral genome encodes 10 proteins, including the G glycoprotein which mediates attachment of viral particles to host cells, the F protein which induces fusion of infected cells, matrix proteins (M, M2 or 22 K), nucleocapsid protein (N), phosphoprotein (P), polymerase (L), small hydrophobic protein (SH), and nonstructural proteins (NS1(1C) and NS2(1B)). Subgroups of BRSV have been defined by antigenic and nucleotide differences of the G and F proteins, but their clinical significance is uncertain.

An understanding of the **pathogenesis** of BRSV pneumonia has been hindered by the difficulty of establishing experimental infections that are representative of naturally occurring disease. In general, experimentally infected calves develop less severe clinical disease and less extensive lesions than natural cases, in part because in vitro passage of the virus in cell culture rapidly attenuates virulence. As a result, descriptions of the pathogenesis of experimental infections are not necessarily representative of natural disease. *Airborne spread, probably by aerosol, is the common route of infection.* In natural cases, viral infection is restricted to the respiratory tract, and viral antigen is most abundant in bronchiolar epithelium of the cranioventral areas of lung. Alveolar type II pneumocytes and macrophages express lesser amounts of viral antigen, and infrequent virus-infected cells are present in nasal, tracheal, and bronchial epithelium. The virus infects but does not replicate in lymphocytes in vitro. Following experimental infection of naive calves, viral shedding was first detected on the second day after infection, was maximal at about 4 days, and was absent by 8 days after infection. This transient infection is consistent with the difficulty experienced in demonstrating virus in naturally occurring subacute or chronic cases.

Interactions with other agents may contribute to the severity of disease. Experimental BRSV infections result in more severe disease if calves are also infected with *Bovine viral diarrhea virus* or exposed to 3-methylindole. BRSV is an important predisposing factor in the development of bacterial bronchopneumonia in cattle, because the virus impairs alveolar macrophage function and destroys ciliated epithelium.

Calves with BRSV develop specific serum and mucosal antibody responses; however, most antibody is directed to the F and N proteins and is nonneutralizing, and serum titers are poorly correlated with protection from disease. Similarly, passively acquired colostral antibodies do not prevent disease. Although virus-neutralizing antibody is produced during infection, the role of humoral immunity in protection from disease requires further investigation. As for many viral infections, CD8+ T-lymphocyte responses may be important in recovery of calves following BRSV infection.

The role of the immune response in enhancing disease is controversial. It has been proposed that vaccination with inactive virus promotes a T-helper-2 immune response in which antiviral IgE exacerbates the severity of disease. Further, the biphasic clinical course, noted in some but not all natural cases, has been interpreted to represent a worsening of the disease as an immune response develops. Such comparisons have been used as a model to study human RSV infection, where children vaccinated with a formalin-inactivated vaccine developed more severe and prolonged disease course than nonvaccinates. However, attempts to reproduce this phenomenon in cattle have given conflicting results, and vaccinated calves developed milder disease than nonvaccinates in several studies. Recent investigations suggest that vaccination with inactivated vaccines results in a more rapid onset of disease following viral challenge, but a similar or milder clinical course and lung lesions compared to nonvaccinates. Similarly, it has been proposed that virus- or immune complex-induced activation of complement may contribute to bronchoconstriction or pulmonary lesions in calves infected with BRSV, but the significance of complement activation in infected calves remains uncertain.

Gross lesions *in lungs of calves with naturally occurring BRSV pneumonia often differ in cranioventral and caudodorsal areas of lung.* The cranioventral lung is atelectatic, collapsed, deep red or mottled, and rubbery in texture. In contrast, the caudodorsal areas fail to collapse and are edematous, heavy, and firmer than normal (Fig. 5.58). *Variations in these gross lesions occur commonly*: first, occasional cases have a generalized rubbery texture and red discoloration, with no difference between cranial and caudal lung; second, calves that die in respiratory distress may develop marked subpleural and interlobular emphysema with formation of bullae; and third, the raised, consolidated, firm to hard lesions of bronchopneumonia may obscure the aforementioned viral lesions in cases with secondary bacterial infection. Apart from hypertrophy and edema of bronchial and mediastinal lymph nodes, lesions in other organs are usually not noted. Chronic cases of viral pneumonia with bronchiolitis obliterans, including those caused by BRSV, may die of heart failure due to pulmonary hypertension, as a result of hypoxic vasoconstriction. Lesions in these animals include hypertrophy of the right ventricle of the heart, subcutaneous edema, and ascites.

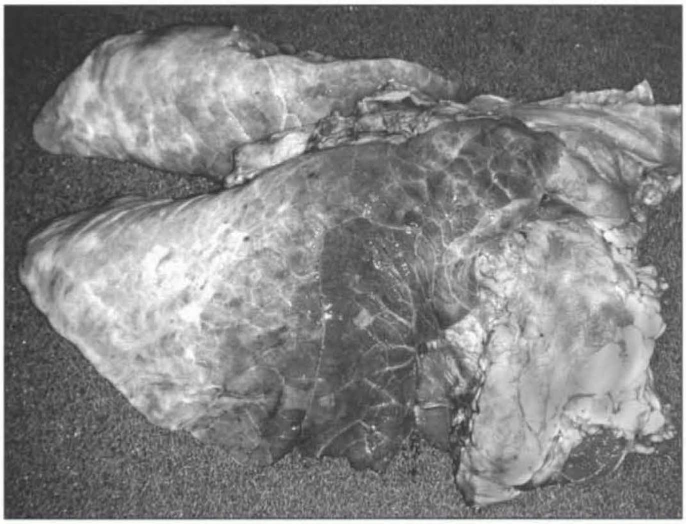

Figure 5.58 ***Bovine respiratory syncytial virus* infection** in a calf. The cranioventral lung is atelectatic and dark red; the caudodorsal lung is pale, emphysematous, and fails to collapse.

The **histologic hallmarks** of pneumonia caused by BRSV are *bronchointerstitial pneumonia, characterized by necrotizing bronchiolitis, formation of bronchiolar epithelial syncytia, and exudative or proliferative alveolitis.* The lesions and the presence of viral antigen are most obvious in the cranioventral areas of lung. In the acute lesions, from 1 to 8 days after infection, bronchioles are lined by flattened epithelium, bronchiolar lumens contain necrotic epithelial cells and modest numbers of neutrophils, and lymphocytes infiltrate around bronchioles (Fig. 5.59). Alveoli contain moderate numbers of neutrophils and macrophages, and alveolar septa may be mildly thickened by mononuclear cells, but hyaline membranes are infrequent. Syncytia are prominent at this stage, and appear as multinucleate cells closely associated with the bronchiolar epithelium, sloughed into bronchiolar lumens or, less commonly, in the alveoli. Alveolar syncytia may closely resemble the multinucleate macrophages that clear fibrin from alveoli in cases of fibrinous pneumonia; but the presence of bronchiolar syncytia is a more reliable indicator of viral infection. *Intracytoplasmic eosinophilic inclusion bodies* are occasionally present in syncytial cells and uncommonly in bronchiolar and alveolar epithelium.

The *subacute lesions* of BRSV infection – beginning at about 8 days after infection – represent *early repair of the above lesions following lymphocyte-mediated lysis of virus-infected cells.* The bronchiolar

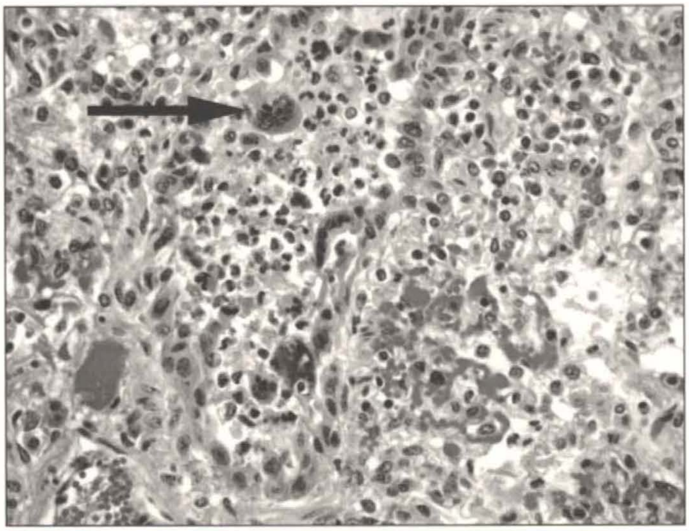

Figure 5.59 ***Bovine respiratory syncytial virus* infection** in a calf. The epithelium lining the bronchiole has a ragged appearance, and epithelial syncytia (arrow) are present. The lumen is filled with neutrophils and sloughed epithelial cells.

epithelium becomes hyperplastic, and there is disappearance of syncytial cells as viral antigen is cleared. Proliferation of type II pneumocytes appears as scattered tombstone-like cells or complete cuboidal epithelialization of the alveoli. Lymphocytes and plasma cells encircle bronchioles and blood vessels and thicken alveolar septa, and are most numerous 10 days after infection. Bronchiolitis obliterans may occur as early as 10 days after infection, and consists of plugs of fibrous tissue covered by epithelium that partially occlude bronchiolar lumens. Medial hypertrophy of pulmonary arterioles may be prominent in calves that develop pulmonary hypertension secondary to bronchiolitis obliterans.

The histologic lesions of necrotizing bronchiolitis with syncytial cells should be highly suggestive of BRSV infection, but two caveats should be considered. First, *Bovine parainfluenza virus 3* infection may also induce the formation of syncytia with intracytoplasmic inclusion bodies. Second, alveolar multinucleate macrophages are a common finding in fibrinous bronchopneumonia, and probably represent an attempt to remove the fibrinous alveolar exudate. This can present a diagnostic dilemma, since many calves with BRSV develop secondary bacterial pneumonia. Thus, if alveolar but not bronchiolar multinucleate cells are present, a diagnosis of BRSV infection is not indicated unless ancillary laboratory tests support the diagnosis.

Various assays are available to confirm BRSV infection. Lung tissue from the lesional cranioventral areas of lung are the most profitable sites to detect virus; viral antigen or nucleic acid is rarely demonstrated in caudodorsal lung tissue. Virus isolation is not recommended for routine diagnostic use, since the development of an immune response interferes with virus isolation and the virus is easily inactivated by transport conditions. Because the development of an antibody response may also interfere with assays that identify viral antigen, detection of viral nucleic acid using the *polymerase chain reaction* may be more effective for subacute or chronic cases. Nevertheless, *identification of viral antigen using fluorescent antibody tests, immunohistochemistry, or antigen detection ELISA are useful and cost-effective methods of diagnosing BRSV infection.*

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Bovine parainfluenza virus 3

Infection with *Bovine parainfluenza virus 3* (BPIV-3) is widespread in 2–8-month-old beef and dairy cattle, but *clinically significant disease due to primary BPIV-3 infection is rare.* Clinical findings in uncomplicated primary BPIV-3 infection include transient fever, nasal discharge, sporadic coughing, mild depression, and increased respiratory rate. The virus causes occasional outbreaks of nasal disease in mature cows, with nasal erosions, fibrinous casts, epistaxis, and submandibular edema. Seroconversion to BPIV-3 commonly occurs during periods of temperature fluctuations in the autumn, in association with shipping fever of feedlot calves and with enzootic pneumonia of dairy calves. The prevalence of positive serum titers to BPIV-3 varies from 2 to 67% on arrival in feedlots, but 23–91% of animals seroconvert within a month after arrival. There is a variable association between seroconversion to BPIV-3 and development of pneumonia, and BPIV-3 predisposes to bacterial bronchopneumonia in some, but not all, cases of shipping fever.

BPIV-3 is a member of the genus *Respirovirus* in the family Paramyxoviridae. Paramyxoviruses are pleomorphic, 150–200 nm diameter, and contain negative-sense single-stranded RNA. Structural proteins include hemagglutinin-neuraminidase glycoprotein (HN), fusion glycoprotein (F), high-molecular-weight protein (L), a phosphoprotein (P), nucleocapsid protein (NP), and matrix protein (M). The HN protein mediates viral attachment to and release from infected cells, and the F protein allows penetration and spread of the virus between infected cells. Both the HN and F proteins are necessary for formation of syncytia and pathogenicity of the virus. BPIV-3 causes subclinical, genetically stable infection of humans, and is of current interest as a vaccine vector for human parainfluenza viral antigens.

BPIV-3 infects ciliated and nonciliated epithelial cells of the upper and lower respiratory tract, alveolar macrophages, type II pneumocytes, and lymphocytes. The *virus reduces pulmonary defenses* by several mechanisms: injury to ciliated epithelium results in functional and structural impairment of mucociliary clearance, alveolar macrophages from infected calves have reduced capacity for phagocytosis and oxidative killing of bacteria, and infected alveolar macrophages induce contact-mediated suppression of lymphocyte responses.

The HN and F proteins are immunodominant antigens, and serum antibody titers to these antigens correlate with disease resistance. Serum antibody responses develop at 8 days after experimental infection and peak at about 12 days, and a strong anamnestic response occurs after later infections. Cytotoxic cell-mediated immune responses, which are probably important for clearance of virus-infected cells, are maximal at 6–9 days after infection.

The **gross lesions** in natural cases of primary BPIV-3 pneumonia are usually mild, and consist of a *cranioventral or generalized, lobular pattern of gray-red discoloration, firm, or rubbery texture, and mild swelling or atelectasis of lung tissue.* In calves that die in respiratory distress, there may be emphysema of the caudal lung lobes. *Bronchiolitis and mild bronchitis are the major* **histologic lesions** in natural cases of primary uncomplicated BPIV-3 pneumonia. Epithelial cells lining small bronchioles are rounded, occasionally vacuolated, and slough into the lumen, and the epithelial layer may be discontinuous, attenuated, or hyperplastic. In the acute stages, many bronchial, bronchiolar, and alveolar epithelial cells and alveolar macrophages contain prominent *eosinophilic intracytoplasmic inclusion bodies*. In experimentally infected calves, inclusions are most numerous from 2 to 4 days after infection, and less common from 5 to 12 days. *Epithelial syncytial cells may be present in alveoli*, but are fewer than in *Bovine respiratory syncytial virus* infection. Airways contain low numbers of neutrophils, and lymphocytes infiltrate the mildly edematous bronchiolar walls. Alveoli are atelectatic, edematous, and contain increased numbers of macrophages and neutrophils. Proliferation of alveolar type II pneumocytes and thickening of alveolar septa are described in experimental infections using high doses of virus, but are uncommon in natural cases. Fibroblasts and macrophages begin to organize the bronchiolar exudate at 7–12 days after experimental infections, and bronchiolitis obliterans may develop thereafter.

BPIV-3 infection should be considered in calves with necrotizing bronchiolitis. Confirmation of the **diagnosis** is based on *virus isolation or detection of viral antigen.* Virus isolation in cell culture from fresh or frozen lung tissue harvested from cranioventral areas of lung with lesions, with virus identification by hemadsorption of erythrocytes, is a useful method of diagnosis. Antigen detection tests are more rapid, and include a fluorescent antibody test on frozen lung tissue and immunohistochemistry on formalin-fixed lung tissue. These diagnostic methods are effective in acute cases, but may be unrewarding in the more common necropsy presentation of chronic bacterial pneumonia with concurrent bronchiolar necrosis or bronchiolitis obliterans. *Definitive diagnosis in these cases may be impossible*, because viral antigen may be cleared long before the bacterial pneumonia proves fatal. If acute and convalescent sera are available, then demonstration of a *rising antibody titer* using the hemagglutination inhibition assay supports the histologic diagnosis.

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Bovine coronavirus

Bovine coronavirus (BCoV) is an important cause of enteric disease in young calves, and the same strains occasionally induce respiratory disease in 2–16-week-old calves. Clinical signs include serous nasal discharge, sneezing, and coughing. The virus replicates primarily in the nasal and tracheal epithelium, and occasionally in the lung. Experimental oral infection of calves with a virulent pneumoenteric isolate resulted in diarrhea and dyspnea, and pulmonary lesions of interstitial pneumonia and hemorrhage. BCoV may be isolated using specific rectal tumor cell lines, or viral antigen may be demonstrated by immunofluorescence.

Many calves shed BCoV in their nasal secretions and/or seroconvert to BCoV in the first month after arrival in feedlots, but the association between seroconversion or virus shedding and increased risk of respiratory disease has been variable in the studies reported. Lesions of bronchiolitis and alveolitis with bronchiolar syncytia have been described in a feedlot calves with concurrent BCoV infection and bacterial pneumonia, but the contribution of other viruses such as *Bovine respiratory syncytial virus* to these lesions is uncertain.

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Bovine viral diarrhea virus

The contribution of Bovine viral diarrhea virus (BVDV) to pneumonia in cattle is a long-standing source of controversy. Virus can be isolated from the lung in primary (acute) infections and in persistently infected calves. Bacterial bronchopneumonia is often present in calves with natural or experimental primary BVDV infections, and BVDV infections are common during times of increased susceptibility to bacterial pneumonia. Although one must be cognizant of the role of other pathogens in such situations, *epidemiologic studies imply that BVDV-infected calves do indeed have a heightened susceptibility to bacterial pneumonia.* Mechanisms by which BVDV predisposes to bacterial pneumonia include reduced antimicrobial protein production by virus-infected airway epithelial cells, reduced function of alveolar macrophages, and virus-induced neutropenia. BVDV has also been shown to exacerbate *Bovine respiratory syncytial virus* and *Bovine herpesvirus 1* infections in experimental situations.

It has been proposed that some isolates of BVDV induce primary respiratory disease, independent of opportunistic bacterial pathogens. During primary BVDV infection, viral antigen is present in pulmonary alveolar macrophages and in airway epithelium. In addition, viral antigen may be demonstrated in the tunica muscularis of arterioles in the lung, heart, and occasionally in other tissues, often in association with lesions of lymphocytic arteritis. Calves or lambs challenged with certain isolates of BVDV develop fever and hyperpnea or, in other studies, nasal discharge and coughing. The lesions described are mild, consisting of peribronchiolar, perivascular, or interstitial aggregates of lymphocytes and macrophages, and/or mild suppurative exudate in bronchioles. However, other studies have found no lung lesions in calves infected with BVDV, and *the importance of BVDV as a cause of primary lung disease is probably minimal.*

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Bovine adenovirus

Bovine adenoviruses are a recognized cause of hemorrhagic colitis, but their role in respiratory disease is more controversial. Epitheliotropic bovine adenoviruses are occasionally isolated from feedlot calves with bacterial pneumonia, and many calves seroconvert to a range of *Bovine adenovirus* (BAdV) serotypes within 5 weeks of entering feedlots. Adenoviruses may predispose to bacterial pneumonia by injuring ciliated epithelium and, for some strains, by impairing alveolar macrophages. However, compared to the roles of stress and other viral infections, *adenoviruses are probably minor contributors to bacterial pneumonia in feedlot cattle.*

Calves experimentally infected with BAdV generally develop only mild respiratory disease; the severity of primary adenoviral pneumonia is probably dependent on both the strain of the BAdV and the immune status of the calf. Experimentally infected calves developed lobular or patchy areas of atelectasis and reddening. *Histologic lesions* include proliferation of bronchial and bronchiolar epithelium, lymphocytic infiltration of airway epithelium, formation of *large basophilic intranuclear inclusions* in airway epithelial cells, and exfoliation or necrosis of virus-infected cells. In well-developed cases, there is extensive bronchiolar necrosis, occlusion of bronchiolar lumens by sloughed epithelium, and inclusions in intact or sloughed epithelial cells. Thickening of alveolar septa by mononuclear cells may occur, but proliferation of type II pneumocytes is uncommon. Other BAdV strains cause no clinical signs and few lesions after experimental infection of calves, and there is much interest in developing these minimally pathogenic BAdV strains as DNA-based vaccine vectors for cattle.

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Bovine rhinovirus

Two serotypes of *Bovine rhinovirus* (BRV) are widespread in cattle populations. *BRV is generally believed to be of little importance as a cause of respiratory disease in cattle.* Virus isolation and serologic responses occasionally provide circumstantial evidence that it is involved in causing upper respiratory disease. Experimental infection has inconsistently induced bronchointerstitial pneumonia.

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Bacterial diseases

Bacterial bronchopneumonia due to Pasteurellaceae: *Mannheimia haemolytica*, *Histophilus somni*, and *Pasteurella multocida*

Bacterial bronchopneumonia is a common and economically important disease of cattle, particularly in temperate climates, and is commonly caused by bacteria of the family Pasteurellaceae. *The most important clinical entities are enzootic pneumonia of young calves and shipping fever of feedlot cattle,* but bacterial pneumonia may occur in any age group, often as a complication of viral infection, stress, or other impairment of lung defenses.

Shipping-fever pneumonia has long been considered the most economically important disease of beef cattle in temperate climates. Recent years have seen considerable advances in understanding of pathogenesis and preventive strategies, with the result that, although the disease remains prevalent, mortality rates are often lower than in the past. The *classic presentation* of shipping fever is of pneumonia affecting multiple calves, 3 days to 3 weeks after being shipped from their farm of origin to a feedlot. Affected animals exhibit acute onset of depression, pyrexia, anorexia, rapid and shallow respiration, and mucopurulent nasal discharge. Coughing is variably present and dyspnea develops in later stages. Most cattle respond rapidly to appropriate antibiotic therapy if given early in the course of disease, but relapses are common. Most reports of shipping fever describe 15–45% morbidity, 1–5% mortality, and case fatality rates of 5–10% depending on the rapidity with which treatment is initiated. *Risk factors* for shipping fever include those associated with: (1) increased exposure of naive calves to animals shedding viral pathogens, such as mixing of calves from various sources; (2) stress, such as weaning, transport, crowding, deprivation of feed and water, handling, vaccination, dehorning, and pregnancy checking; (3) metabolic acidosis, such as rapid introduction to grain or corn silage; and (4) exposure of calves to adverse environmental conditions, such as changes in temperature and humidity, cool weather in the autumn months, snow or rain, or dusty environment.

Bacterial pneumonia is also common in 1–4-month-old dairy and veal calves, and less common in suckling beef calves. Although commonly referred to as "*enzootic pneumonia*," cases may be sporadic, enzootic, or occur in outbreaks. Affected calves are febrile, hyperpneic, variably dyspneic, depressed, and anorexic. *Risk factors* include those that: (1) affect air quality, such as indoor housing, poor ventilation, high stocking density, high levels of airborne droplets or dust, and humid conditions; (2) reduce lung defenses, such as failure of passive transfer of immunoglobulin and high environmental ammonia concentrations; (3) expose calves to viral or mycoplasmal pathogens, such as housing calves and adults together; and (4) cause stress, such as concurrent diseases.

The Pasteurellaceae are gram-negative coccobacilli that are common causes of pneumonia or septicemia in domestic animals. *Many inhabit the upper respiratory tract of healthy animals,* but cause pneumonia when pulmonary defenses are impaired by viral infections, stress, temperature fluctuations, or noxious gases, or cause septicemia in naive animals or those with reduced systemic defenses. In general, the defense against many Pasteurellaceae depends on a combination of innate resistance mechanisms and acquired humoral immunity. Most members of the family are shrouded in a polysaccharide capsule that mediates resistance to phagocytosis by macrophages and neutrophils, and several species secrete RTX (repeats in toxin) toxins that induce necrosis of leukocytes. Endotoxin incites inflammation in response to infection with members of the Pasteurellaceae family. The most common pathogenic Pasteurellaceae of domestic animals include *Pasteurella multocida* of swine, ruminants, and cats; *P. trehalosi* of sheep, *Mannheimia haemolytica* of ruminants, *Actinobacillus pleuropneumoniae* and *A. suis* of swine, *A. equuli* of horses, *Histophilus somni* of cattle and sheep, and *Haemophilus parasuis* of swine.

Mannheimia haemolytica (formerly *Pasteurella haemolytica* biotype A): 12 serotypes of *M. haemolytica* are discerned based on soluble capsular polysaccharide antigens. Serotype names were maintained when the species *P. haemolytica* biotypes A and T were reclassified as *M. haemolytica* and *P. trehalosi*, respectively; as a result, serotypes 3, 4, 10, and 15 are represented only by *P. trehalosi*. Serotype 2, and, to a lesser extent, serotype 1, is a sporadic isolate from the nasopharynx of healthy calves. Serotype 1 is the most common isolate from pneumonic lung, but other serotypes in addition to many untypable isolates regularly cause bovine pneumonia.

Virulence factors of *M. haemolytica* include leukotoxin, lipopolysaccharide, capsular polysaccharide, transferrin-binding proteins A and B, O-sialoglycoprotease, neuraminidase, IgG1-specific protease, outer-membrane proteins, and fimbriae. All serotypes secrete *leukotoxin*, a member of the RTX family of bacterial toxins, during the log phase of growth. The gene products of *lkt*A and *lkt*C represent the active leukotoxin, while *lkt*B and *lkt*D are necessary for toxin secretion. At high concentrations, leukotoxin induces lysis of ruminant leukocytes and platelets, mediated by binding to CD18 on the leukocyte surface and formation of pores in the cell membrane. In contrast, at lower concentrations, leukotoxin activates leukocytes and platelets. Although leukotoxin clearly plays a role in the pathogenesis of pneumonia, disease does develop in calves vaccinated against leukotoxin and in those challenged with *M. haemolytica* constructs that do not secrete leukotoxin.

Lipopolysaccharide is a major component of the cell wall, and the presence of O-polysaccharide side chains confers a smooth phenotype to most serotypes. Lipopolysaccharide from *M. haemolytica* stimulates alveolar macrophages to secrete inflammatory mediators, including tumor necrosis factor-α, interleukin-1, and the chemokines IL-8, ENA, and GRO-α. Other effects of *M. haemolytica* lipopolysaccharide on alveolar macrophages include triggering of oxidative burst, an important bactericidal mechanism that may also lead to oxidative tissue injury; expression of tissue factor, a mediator of the thrombosis characteristic of the disease; and secretion of nitric oxide, which may augment lung defenses but is also a potent mediator of vasodilation and hypotension. Other effects of lipopolysaccharide include activation of the extrinsic coagulation pathway, and complement activation. The mannose-rich polysaccharide capsule confers resistance of *M. haemolytica* to phagocytosis by macrophages and neutrophils, and may also block complement-dependent killing. Neuraminidase enhances adhesion of bacteria to mucosal epithelium by cleaving sialic acid from the surface of epithelial cells, and may reduce mucus viscosity to prevent mucociliary clearance.

Low numbers of M. haemolytica colonize the nasopharynx of normal calves, and there are several potential explanations for the shift from a commensal to a pathogenic relationship. First, stress or exposure to cold results in greater numbers of nasopharyngeal bacteria, so inhalation of droplets containing increased numbers of bacteria may overwhelm pulmonary defenses. The mechanisms whereby stress or exposure to cold induces expansion of the bacterial population, and whether these factors also alter expression of bacterial virulence factors, have not been defined. Second, some studies indicate that stress and cold exposure induce a shift from serotype 2 to the more pathogenic serotype 1. Third, viral infections or stress impair lung defenses. The lung is normally exposed to low numbers of aspirated *M. haemolytica*, but a combination of mucociliary clearance, antimicrobial function of secreted mucosal proteins, and phagocytosis by alveolar macrophages controls this infection without inciting an inflammatory response. *Bovine herpesvirus 1* (BoHV-1), *Bovine respiratory syncytial virus* (BRSV), and *Bovine parainfluenza virus 3* infect ciliated epithelium and reduce mucociliary clearance, and BoHV-1 and BRSV infect and impair the function of alveolar macrophages. In addition, preliminary evidence suggests that BoHV-1 infection causes upregulation of CD18 on the surface of bovine neutrophils. Since CD18 is a receptor of *M. haemolytica* leukotoxin, neutrophils in BoHV-1-infected calves may be more susceptible to the effects of this pathogen. Viral infections may also impair secretion of antimicrobial proteins by airway or alveolar epithelium. The mechanisms whereby stress impairs innate pulmonary defenses are poorly defined. As a result of one or more of these processes, it is likely that exposure of the lung to increased numbers of virulent *M. haemolytica* coincides with a reduction in pulmonary defenses, and these alterations lead to increased colonization of the cranial lung lobes.

Clinical disease is principally a consequence of the host response to infection of the lung with *M. haemolytica*, rather than a direct effect of bacterial toxins on lung tissue. Massive exudation of fibrin into alveoli is a prominent feature of severe pneumonic pasteurellosis, and is mediated in part by a direct effect of lipopolysaccharide on endothelial cells. However, other mechanisms of vascular leakage also occur, such as exposure of endothelium to macrophage-derived proinflammatory cytokines such as tumor necrosis factor-α. Phagocytosis of *M. haemolytica* by alveolar macrophages is limited unless the bacteria are opsonized by antibody or complement; the significance of other opsonins, such as pulmonary or serum collectins, remains speculative. Alveolar macrophages which have ingested *M. haemolytica*, or been exposed to bacterial products, secrete an array of neutrophil chemoattractants, including interleukin-8, ENA, GRO, platelet-activating factor, and leukotriene B4. Neutrophils migrate to the alveoli and terminal bronchioles within 3 hours of experimental challenge with *M. haemolytica*. Neutrophil migration to alveoli employs a β_2 integrin-independent mechanism, whereas neutrophil recruitment to airways is dependent on interactions between β_2 integrin and ICAM-1. In contrast to most bacterial infections, in which neutrophils phagocytose and kill the offending bacteria, neutrophils recruited to the sites of established infection with *M. haemolytica* are probably lysed by the effects of leukotoxin. Thus, *this disease is characterized by massive recruitment of neutrophils that are ineffective in killing bacteria, but nonetheless exacerbate the severity of clinical disease*. Infiltration of neutrophils and additional macrophages into alveoli is harmful in several ways: the physical presence of these cells precludes normal ventilation and gas exchange; the secretion of oxygen radicals, nitric oxide derivatives, and proteinases secreted by leukocytes may injure pulmonary cells and the interstitial stroma; expression of tissue factor by activated alveolar or intravascular macrophages promotes fibrin formation in the alveolus and thrombosis of blood vessels in the alveolar septa; and both macrophages and neutrophils secrete chemoattractants that perpetuate the recruitment of additional leukocytes.

Focal areas of coagulative necrosis are a characteristic feature of infection with M. haemolytica. Some represent infarcts due to thrombosis of intralobular blood vessels, but bacterial toxins or leukocyte secretions might also cause direct lung injury. These foci of necrosis are encircled by a dense band of leukocytes, many of which are necrotic, and these leukocytes are in a state of intense activation featuring prominent gene expression of interleukin-8 and inducible nitric oxide synthetase. Profound depression and "toxemia" are constant features of acute infection with *M. haemolytica*; although the basis for this systemic illness is unknown, tumor necrosis factor-α in the plasma of some cases may be responsible.

Immune responses to *M. haemolytica* occur rapidly following exposure, with alveolar IgM detectable by 48 hours after experimental infection, and detectable serum IgM and IgG by 5 and 7 days after infection, respectively. Animals that recover from disease or are vaccinated with live bacteria are immune to subsequent challenge, and serum antibody titers to leukotoxin, outer-membrane proteins, and capsule all correlate with resistance to naturally occurring disease. However, it is likely that an immune response to several antigens is necessary for protection, for the presence of antibody to individual antigens – including leukotoxin, capsular polysaccharide, lipopolysaccharide, or outer membrane proteins – does not confer complete protection.

Histophilus somni (formerly *Haemophilus somnus*) benefits from a similarly broad array of virulence factors. Resistance to complement-mediated serum killing is mediated by proteins that bind the Fc component of immunoglobulin, making these Fc receptors unavailable for complement activation. Lipooligosaccharide has many proinflammatory effects, analogous to lipopolysaccharide in other gram-negative bacteria. The lipooligosaccharide of disease-associated, but not commensal, isolates of *H. somni* undergoes structural and antigenic variation during the course of an experimental infection, and this variation may serve as a mechanism of immune evasion and resistance to complement-mediated killing. Transferrin-binding proteins enable the bacteria to use transferrin as an iron source for growth. *Histophilus somni* binds to and induces apoptosis in endothelial cells in vitro, and although this effect may contribute to the vasculitis that is characteristic of *H. somni* septicemia, these processes are as yet poorly characterized. Immune-complex deposition is apparently not the cause of vasculitis. *H. somni* is considered a facultative intracellular pathogen as it survives within monocytes, yet both intra- and extracellular bacteria are numerous in acute pneumonia. Finally, *H. somni* impairs the phagocytic function of neutrophils and macrophages, and induces degeneration of macrophages and apoptosis of neutrophils.

In contrast to the advances made in understanding other ***Pasteurella multocida*** infections, notably atrophic rhinitis and fowl cholera, the pathogenesis of pneumonia due to *P. multocida* is poorly described. Capsular serotype A is most commonly isolated from cases of pneumonia. As for *M. haemolytica*, the bacteria are carried in the nasopharynx of many clinically normal calves. An antiphagocytic capsule and a neuraminidase enzyme are described, but their importance and role in disease are uncertain.

The **gross lesions** of acute fulminant "shipping-fever" pneumonia due to *M. haemolytica* or *H. somni* include *cranioventral lobar or lobular fibrinous bronchopneumonia, foci of coagulative necrosis, and variable fibrinous pleuritis*. Lesions are most severe in the cranial and middle lung lobes, but may also affect the caudal lung. Acute fulminant cases often exhibit lobar fibrinous pneumonia and pleuritis and may die with as little as 30% of the lung affected, due to systemic effects of the infection rather than failure of lung function. Lesions are often sharply demarcated from less affected areas of lung, are discolored red or gray, and the filling of alveoli with fibrin bestows a firm or hard texture to the affected lung (see Fig. 5.29).

The cut section of the lung is purple-red, firm or hard, and moist. It is often not possible to express exudate from the transected airway, for the leukocytes are enmeshed in polymerized fibrin. Sharply demarcated, irregularly shaped, 0.5–5 cm diameter, pale, dry foci of coagulative necrosis are frequent in fulminant cases of *M. haemolytica* or *H. somni* infection (Fig. 5.60). Bronchi may contain suppurative material or hemorrhage, or may be free of exudate. Interlobular septa are distended with fibrinous or serofibrinous exudate, and this lends a marbled appearance to the cut surface (Fig. 5.61). In the most severe cases, fibrin forms a mat or lattice over the pleural surface of affected lung lobes, and the pleural cavity may contain serofibrinous yellow fluid that clots when the chest is opened. Fibrinous pericarditis may accompany the pleuritis.

The lung lesions of acute *P. multocida* infection are in many respects similar, but lack the focal coagulative necrosis and abundant fibrinous exudate seen in *M. haemolytica* and *H. somni* infections. As a result, suppurative exudate oozes from small bronchi when a cut section is compressed, and purulent or catarrhal exudate may line the mucosal surface of the large airways. *Necrosis of laryngeal epithelium at the point of contact of the vocal folds is commonly identified in cattle with severe pneumonia*, due to mucosal trauma induced by abrupt laryngeal closure in a dyspneic animal. Infectious thrombotic meningoencephalitis and septicemia usually occur independently of bronchopneumonia, and calves with *H. somni* pneumonia do not commonly have encephalitis or myocarditis.

Sequelae are common in survivors of acute fulminant disease, and include *sequestra, bronchiectasis, abscesses, and fibrous pleural adhesions*. Sequestra consist of a necrotic core of firm but friable tissue,

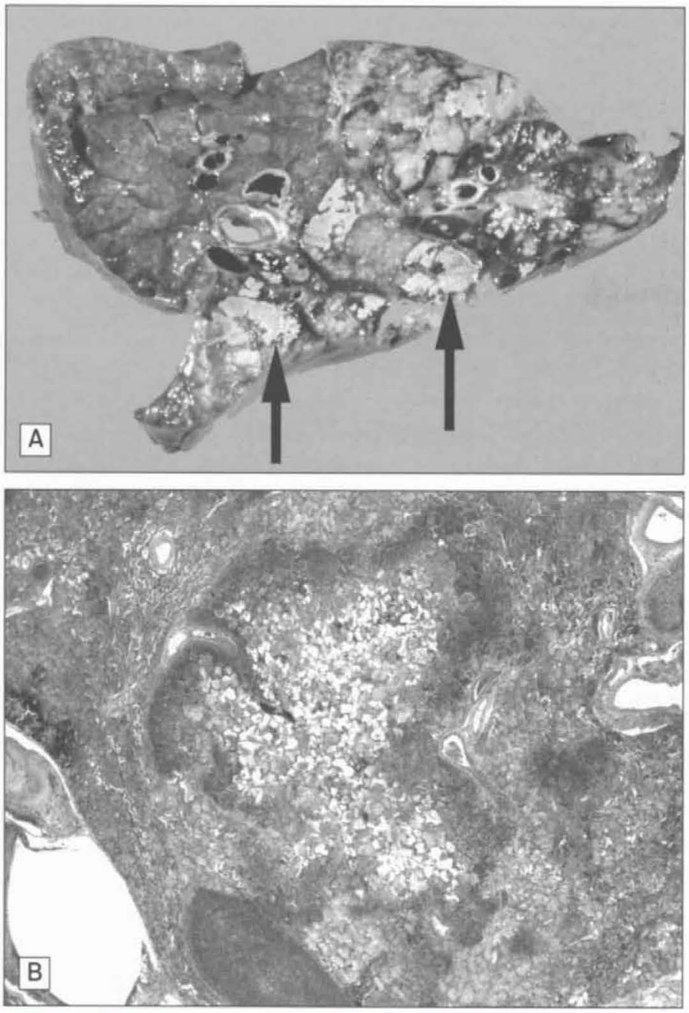

Figure 5.60 ***Mannheimia haemolytica* pneumonia** in a calf. **A.** Sharply demarcated, irregularly shaped foci of necrosis within the consolidated lung (arrows). **B.** Histologically, the foci of coagulative necrosis are delineated by a band of necrotic leukocytes.

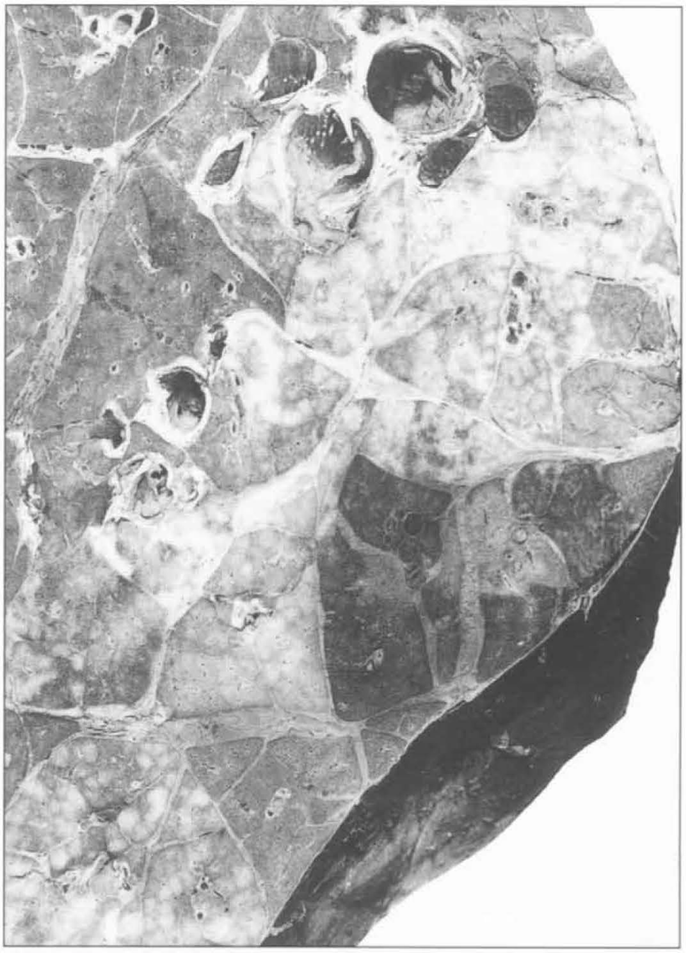

Figure 5.61 ***Mannheimia haemolytica* pneumonia** in a calf. Marbled pattern produced by differing degrees and stages of consolidation.

surrounded by fluid or caseous exudate, enveloped in a capsule of fibrous tissue. Bronchiectasis manifests as dilated airways that are filled with purulent exudate and thickened by fibrous tissue. The surrounding lung tissue is invariably atelectatic, and may be swollen due to persistent bronchopneumonia. Ectatic bronchi appear similar to abscesses, but bronchiectasis is typified by a regular distribution of purulent foci, the occasional presence of cartilage remnants on gross or histologic examination, and communication with a bronchus. Lesions of bronchiectasis and abscesses are often secondarily colonized by *Arcanobacterium pyogenes* or other opportunistic pathogens.

Subacute, less fulminant infection with Pasteurellaceae bacteria induces a lobular distribution of lesions, and most fatal cases have at least 60% of the lung affected. Compared to acute cases, subacute and chronic lesions may be pale red-tan or mottled red and gray, and the texture varies from hard to firm to rubbery. Mild cases with atelectasis and rubbery consolidation may be difficult to distinguish from lesions of viral pneumonia.

The **histologic lesions** of *acute fulminant "shipping-fever" pneumonia due to M. haemolytica or H. somni are characterized by fibrinous and suppurative bronchopneumonia with necrosis of leukocytes.* Alveoli and small bronchioles are filled with variable proportions of neutrophils and macrophages, fibrin, edema fluid, erythrocytes, necrotic cellular debris, and aggregates of bacteria. Necrosis of intra-alveolar leukocytes is a characteristic feature of infection with *M. haemolytica*, where leukotoxin is the likely cause of the neutrophil cell death. Leukocyte necrosis may also be present in *H. somni* infection, and this agent is capable of inducing apoptosis in neutrophils in vitro by uncharacterized mechanisms. The necrotic leukocytes are often lytic and exhibit a streaming pattern of pale basophilic chromatin, termed "*oat cells*" for their resemblance to grains of oats (Fig. 5.62). The nature of the necrotic leukocytes has long evaded definitive identification, but *it is likely that both neutrophils and macrophages are affected.* Larger airways may contain suppurative exudate, but neutrophil infiltration of the mucosa is often absent.

Necrosis and attenuation of bronchiolar epithelium, when present, should prompt a search for underlying viral infection. However, this lesion is present in some calves experimentally infected with *M. haemolytica* or *H. somni*, and may represent neutrophil-induced damage to the epithelium. Thrombi commonly occlude arterioles, venules, and alveolar septal capillaries. Interlobular septa are distended with serofibrinous exudate, and polymerized fibrin may occlude lymphatics in the interlobular septa. Sharply demarcated, irregularly shaped areas of coagulative necrosis are often apparent. In these, alveolar and bronchiolar lesions are as described above, but all cells within the lesions are necrotic, and karyolysis or karyorrhexis is prominent. A dense band of necrotic leukocytes encircles these foci of necrosis (Fig. 5.60), and thrombi occasionally fill blood vessels at the edge of or within the lesions. Suppurative phlebitis with fibrinoid necrosis may occur in pneumonia caused by *H. somni*, but is only common in acute fulminant cases.

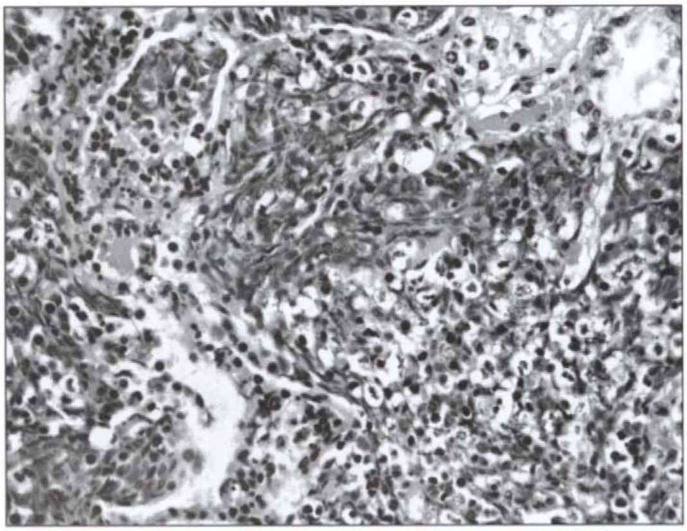

Figure 5.62 ***Mannheimia haemolytica*** **pneumonia** in a calf. Alveoli are filled with necrotic oat-shaped leukocytes with streaming chromatin.

Pulmonary changes in calves that survive an episode of fulminant shipping-fever pneumonia are poorly described in the literature. Macrophages and multinucleate histiocytic cells encompass the alveolar masses of polymerized fibrin; removal of fibrin by these macrophages and by fibrinolysis presumably occurs in some nonfatal cases. Otherwise, alveolar fibrin is infiltrated by fibroblasts and replaced by connective tissue, with two possible sequelae: incorporation of the re-epithelialized alveolar fibrous tissue into the alveolar septum to form a thickened alveolar septum and a "recanalized" alveolar lumen, or large expanses of pulmonary fibrosis. Chronic suppurative or lymphoplasmacytic bronchitis occurs regularly in calves with chronic pneumonia.

Less fulminant cases of *M. haemolytica* or *H. somni* infection, and the acute or subacute changes induced by *P. multocida*, tend to lack the fibrinous alveolar exudate, thrombosis, and interlobular fibrin that are typical of fulminant shipping-fever pneumonia. Rather, these lesions are dominated by exudation of neutrophils in alveoli and bronchioles, congestion of pulmonary vessels, but little other morphologic change in lung tissue. Whereas leukocyte necrosis is a constant feature of active *M. haemolytica* infection, lesions caused by *P. multocida* contain viable, nonnecrotic neutrophils.

The gross lesions of cranioventral bronchopneumonia are usually **diagnostic**. The swollen and hard nature of bacterial pneumonia aids in the differentiation from the atelectatic cranioventral lesions of *Bovine respiratory syncytial virus* pneumonia. A histologic finding of suppurative bronchopneumonia implies a diagnosis of bacterial pneumonia; the important challenge is to identify the underlying causes. Lesions of aspiration pneumonia may closely resemble bacterial pneumonia; however, necrosis of alveolar leukocytes is not expected, and focal or unilateral, necrotizing, and foul-smelling lesions containing histologically recognizable foreign material are more typical of aspiration. Differentiation of the foci of necrosis from those seen in *Mycoplasma bovis* pneumonia is discussed in the section on mycoplasmal disease.

The use of *aerobic and microaerophilic culture* to identify the specific etiology is commonly successful unless animals have been medicated with antimicrobial. Subacute or chronic cases may be colonized by opportunistic bacteria and the primary bacterial cause may no longer be present. Immunohistochemistry to identify *Mannheimia haemolytica* and *H. somni* has been described, and may be of use in antimicrobial-treated, autolysed, or archival tissues, or if fresh tissue is not available. Detection of serum antibody to *M. haemolytica* using a bacterial agglutination assay, leukotoxin neutralization assay, or leukotoxin ELISA is a common research tool, but finds limited use in field investigations.

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Hemorrhagic septicemia of cattle

Hemorrhagic septicemia of cattle is caused by Pasteurella multocida types B or E. This disease was originally recognized in western Europe in the 19th century as a severe epidemic in deer which later spread to cattle, wild and domestic pigs, and horses. Sheep, bison, camels, and buffalo may also be affected. *Hemorrhagic septicemia is now limited largely to tropical countries from Egypt to the Philippines, and in these regions is primarily a disease of buffalo.* Outbreaks in tropical climates occur particularly during the rainy season. In intervening periods, the organism is apparently maintained in the nasopharyngeal regions of carrier cattle or buffalo. The start of an outbreak depends on some stress disturbing the balance in a carrier animal. This results in extensive proliferation and dissemination of the organisms to susceptible contact animals.

Approximately 10% of animals survive subclinical infections and become immune, but once clinical signs are apparent, the mortality is almost 100%, even with appropriate antimicrobial therapy. This, together with the immense proliferation of organisms in the clinical disease, suggests that bacterial toxins (particularly endotoxin) are important in causing death.

Hemorrhagic septicemia is a peracute disease and many animals die without clinical signs. Less fulminant cases exhibit high fever and rapid prostration, with profuse drooling. The saliva and feces contain large numbers of bacteria. Petechial hemorrhages are present on the serous membranes and in various organs, especially the lungs and muscles. Severe endotoxemia may cause *acute, fibrinohemorrhagic interstitial pneumonia*. Lymph nodes are swollen and hemorrhagic, and there may be blood-stained fluid in the serous cavities. Acute gastroenteritis, which can be hemorrhagic, is often present. The spleen is not greatly enlarged, which is a point of differentiation from anthrax.

There is a *peracute edematous form of hemorrhagic septicemia*, in which edema of the throat is unusually pronounced. The whole head, tongue, brisket, or a limb may also be affected. The swellings are produced by copious, clotted, straw-colored exudate. Although these lesions are caused by hemorrhagic septicemia, death is caused by asphyxiation.

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Cilia-associated respiratory bacillus

Cilia-associated respiratory (CAR) bacillus is a recognized cause of chronic respiratory disease in rats, mice, and rabbits. The bacilli interdigitate between cilia and induce lymphocytic bronchitis and bronchiolitis, bronchiectasis, and lung abscesses. Morphologically similar bacteria colonize the tracheal epithelium of up to 83% of slaughter cattle and 46% of swine submitted for necropsy. The bacteria are of similar length and diameter as the cilia, but are visualized with Warthin–Starry silver stains (Fig. 5.63). In *calves*, there is an association between the presence of CAR bacilli and the presence of lymphoid follicles in the airway wall, but it remains unknown if this is a causal relationship. *Porcine* CAR bacilli are apparently nonpathogenic, for they are present in pigs with and without respiratory disease, and experimentally infected pigs have not developed clinical signs or lesions.

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Tuberculosis

Bovine tuberculosis, caused by Mycobacterium bovis, is a chronic disease characterized by caseating granulomas in lung, lymph nodes, and other organs. Control programs have minimized the occurrence of bovine tuberculosis in many developed countries. The disease is rare in Canada, the USA, and Australia, although infected wildlife reservoirs remain and cases continue to occur in domestic livestock. Most recent reports of bovine tuberculosis in the USA and Canada have occurred in farmed cervids or in localized geographic areas. In most of Europe, fewer than 0.4% of herds are infected, but the prevalence is higher in Ireland, Spain, and Italy. In contrast, bovine tuberculosis is endemic in New Zealand and many, but reportedly not all, countries in Africa, Asia, Central and South America; 10–35% of herds are infected in many of these endemic areas.

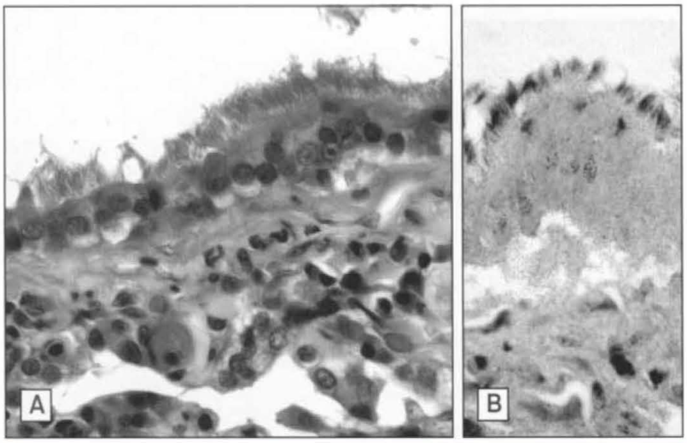

Figure 5.63 Cilia-associated respiratory (CAR) bacillus infection in a rat. **A.** The bacteria cause accentuation of the cilia on the apical surface of the bronchiolar epithelial cells. **B.** With Warthin–Starry silver stain, the bacteria stain black among the unstained cilia.

In many countries, the success of tuberculosis eradication schemes is complicated by *wildlife reservoirs*, which maintain infection and transmit disease to cattle. Probable wildlife reservoirs of *M. bovis* infection include European badgers (*Meles meles*) in Ireland and the UK, brush-tailed possum (*Trichosurus vulpecula* Kerr) in New Zealand, swamp buffalo (*Bubalus bubalis*) in Australian Northern Territory, Cape buffalo (*Syncerus caffer*) in Ruwenzori National Park in Uganda, bison (*Bison bison*) in Wood Buffalo National Park in western Canada, and various species of deer in the USA and New Zealand. In contrast, dead-end or spill-over hosts, including feral pigs, goats, wart hogs, cats, mustelids, and humans, may be infected with *M. bovis* but do not maintain infection in an area and rarely transmit infection to cattle.

Many mycobacteria persist in soil for prolonged periods, and the infectivity of *M. bovis* is likely maintained for several weeks in the environment. However, because oral infections require high doses of bacilli, the importance of environmental survival in the epidemiology of disease is likely limited.

The classical tubercle bacilli are *M. tuberculosis* (human), *M. bovis* (bovine), and *M. avium* (avian). Two other closely related species are *M. microti* from voles and *M. africanum*. Differing strains of *M. avium* are now commonly included with strains of the very closely related *M. intracellulare* as the *M. avium-intracellulare* complex. To avoid confusion surrounding the term **tuberculosis**, convention limits it to diseases caused by *M. tuberculosis* or *M. bovis*. Other conditions are referred to as **mycobacteriosis** or **atypical mycobacteriosis**.

Saprophytic mycobacteria are widespread in soil and water, on vegetation, and in mucous membranes of the oropharynx. These organisms typically cause disease in immunologically compromised hosts, and the manifestations are cervical lymphadenitis, pulmonary lesions similar to tuberculosis, or cutaneous lesions associated with local penetration of organisms through wounds. An example is *M. marinum*, which is abundant in pools in regions with temperate climates and causes tuberculous disease in fish and cutaneous ulcers in humans. This organism is grouped with the *photochromogens* (Runyon's group I) together with *M. kansasii*. The latter produces cervical lymphadenitis and pulmonary disease in humans, and has been isolated from cow's milk and from cattle in the USA and South Africa. *Scotochromogens* produce pigment without photoactivation (Runyon's group II) and include *M. scrofulaceum*, which is closely related to the *M. avium-intracellulare* complex. These organisms have been isolated from disease in cattle and tuberculoid lesions in dogs. Another member of the group, *M. aquae*, has been isolated from nodular lesions on the teats of cows. *Nonphotochromogens* (Runyon's group III) have organisms in the *M. avium-intracellulare* complex as the most important members. Some strains are virtually nonpathogenic and others produce generalized disease in calves and pigs.

Mycobacteria of Runyon's group IV grow rapidly at room temperature, are usually nonpigmented, and include many saprophytes.

Within this group, *M. smegmatis* is one of the organisms isolated from tubercle-like lesions in lymph nodes of swine and also causes bovine mastitis following its injection into the udder. Another member, *M. fortuitum*, was initially isolated from lesions in bovine lymph nodes and is an occasional cause of mastitis in cattle. Members of this group, particularly *M. fortuitum, M. chelonei*, and *M. phlei*, cause cutaneous mycobacteriosis in cats and dogs.

The three main species of tubercle bacilli, *M. tuberculosis, M. bovis*, and *M. avium*, occur most frequently in their respective hosts, but cross-infections do occur and various other species of animals are affected.

- **Bovine tuberculosis** refers mainly to disease in cattle caused by ***Mycobacterium bovis***, but the term is also used to describe the pathogenic effects of this agent in other hosts. The host range of *M. bovis* is broad, including cattle, deer, elk, bison, buffalo, goats, camels, llamas, swine, elephants, rhinoceros, dogs, foxes, cats, mink, badgers, and nonhuman and human primates. Natural disease is most common in cattle, cervids, humans, and swine.
- ***M. avium*** causes mycobacteriosis chiefly in birds and is occasionally found in cattle, swine, horses, sheep, and monkeys.
- ***M. tuberculosis*** is chiefly responsible for tuberculosis in humans, and occasionally infects pigs, captive monkeys, dogs, cats, cattle, and psittacine birds.

Human infections with *M. bovis* are well documented, but are much less common than *M. tuberculosis*. Immunosuppressed individuals, such as those with the acquired immunodeficiency syndrome (AIDS), are at particular risk. Ingestion of milk from cows with mammary tuberculosis is a major route of infection in humans, typically inducing cervical lymphadenitis or other nonpulmonary forms of disease. This pattern of disease is of great historical importance as the impetus for identification of the tubercle bacillus and the subsequent *pasteurization of milk*, but infection from raw milk continues to occur in regions where bovine tuberculosis is common. Transmission from cattle to humans by aerosol or by contamination of cutaneous wounds also occurs, most frequently in those in close contact with infected cattle.

Mycobacteria are nonmotile, nonspore-forming pleomorphic coccobacilli. They are gram-positive but almost unstainable by the simpler bacterial stains because of their high content of lipids. They are routinely stained with carbol-fuchsin, and then resist decoloration by inorganic acids. This property of acid-fastness of the stained bacilli depends on the amount and spatial arrangement of mycolic acids and their esters in the bacterial wall. They are also demonstrable by fluorescent dyes such as auramine. Sometimes, in cultures or in old lesions, the organisms have a beaded or granulated appearance. This beading is partly caused by the presence of lipid droplets within the bacteria and is an indication of an unfavorable environment for organisms in the postexponential growth phase.

In addition to the cell membrane and peptidoglycan layers found in other bacteria, *the mycobacterial cell wall contains a large hydrophobic layer of mycolic acids, which bestows hydrophobicity on the cell wall, conferring environmental and antimicrobial resistance.* The waxes and cell wall glycolipids are important inducers of the initial macrophage response and, together with peptidoglycan (muramyl dipeptide), are responsible for most of the adjuvant activity of mycobacteria that facilitates recruitment of antigen-presenting cells. Increased glycolipid content of mycobacterial cell walls, acid-fastness, and the amount of trehalose dimycolate (cord factor) in the cell wall are associated with increased virulence. Other glycolipids (mycosides) appear to form a barrier against lysosomal digestion and partly explain the ability of the organisms to survive after phagocytosis by macrophages. Intracellular survival is also facilitated by preventing fusion of phagosomes and lysosomes. The differing effectiveness of such mechanisms determines the relative ability of various mycobacteria to resist intracellular degradation.

Tuberculoproteins are the other major category of immunoreactive substances in mycobacteria. They provide most of the antigenic determinants, but the adjuvant activity of the lipids and polysaccharides in the mycobacterial cell wall is needed for an animal to produce an immunologic response to these tuberculoproteins. *Purified protein derivatives* (PPD) from mycobacteria are capable of eliciting delayed-type hypersensitivity once the animal is sensitized, however, and this is the basis of *tuberculin testing*. Both tuberculoproteins and the adjuvant lipids are present in infection, and the result is the development of both humoral and cell-mediated immune responses. Humoral antibodies can be demonstrated by serologic techniques, but do not participate in the development of lesions or in the production of immunity. Cell-mediated responses are responsible for both aspects of the disease.

The *method of transmission* influences the spectrum of lesions of bovine tuberculosis. Inhalation of droplet nuclei or dust particles containing *M. bovis* is the most common route of infection and leads to infection of the upper and lower airways. Oral infection requires a greater dose of bacilli than airborne infection to cause disease, and elicits lesions in the gut and associated lymph nodes. Transplacental transmission is a sequel of endometrial tuberculosis, and leads to fetal lesions in hepatic and portal lymph nodes. Less common routes of infection include percutaneous inoculation, genital transmission, and intramammary infusion of contaminated pharmaceutical preparations.

Important concepts in the pathogenesis of tuberculosis include the ability of mycobacteria to survive within macrophages, and the role of cellular immune responses in inciting granulomatous inflammation and enhancing the ability of macrophages to kill bacilli. Most animals that are infected with *M. bovis* do not develop clinical disease. The outcome of infection depends on bacterial factors, including the dose and virulence of infecting bacteria, but also on host factors, including the state of immune competence and heritable resistance to tuberculosis. Heritable variation in disease susceptibility has been identified in cattle and in deer. In humans and mice, mycobacterial resistance is partly related to allelic variation in the natural resistance-associated macrophage protein (Nramp) genes 1 and 2, which affect intracellular survival of pathogens by modifying lysosomal transport of divalent cations, including iron and manganese. Other forms of heritable resistance to *M. bovis* are unrelated to variation in Nramp genes.

There are several facets to immune-mediated control of tuberculosis, and most of this understanding is based on the study of *M. tuberculosis* infections in humans and laboratory animals. In the early phases of infection, bacilli are phagocytosed by macrophages and may be eliminated. Alternatively, infected macrophages may remain at the site of primary infection for prolonged periods before the disease progresses. An initial innate immune response develops at this site of primary infection, as macrophages secrete cytokines – particularly tumor necrosis factor-α and the C-C chemokines – that recruit additional macrophages and lymphocytes to the site.

Macrophages stimulated by exposure to mycobacteria secrete interleukin-12, which skews the immune response to favor secretion of interferon-γ and interleukin-2 by CD4+ T-helper-1 lymphocytes. These interferon-γ-producing T-helper lymphocytes signal the development of **cell-mediated immunity**, first detected at 14–28 days after infection by positive tuberculin skin test reactions. The arrival of these antigen-specific lymphocytes is crucial for host defense, activating macrophages and thus allowing them to overcome the block in phagosome maturation, and upregulate production of bactericidal products, including reactive nitrogen and oxygen intermediates and lysosomal enzymes, killing intracellular bacilli. Activated macrophages appear epithelioid, with abundant cytoplasm and indistinct cell borders, or form multinucleate giant cells.

The cytokines tumor necrosis factor-α and interferon-γ act synergistically to promote formation of the **tuberculoid granuloma**, a dynamic structure that prevents spread of infection to other sites in the lung, as well as animal-to-animal transmission, and represents a localized target for the immune response. Excessive tissue-damaging cell-mediated immune response, called **delayed-type hypersensitivity**, kills heavily infected macrophages, forming the caseous center of the granuloma. Matrix metalloproteinases released from activated macrophages are a likely cause of the characteristic cell necrosis and liquefaction of tissues in some lesions of tuberculosis. Bacilli within the caseous center do not multiply but may remain dormant as latent infections that may persist for years, until immunosuppression caused by diseases, drugs, hormones, or malnutrition, or other unidentified factors disturb the balance between host and agent, allowing proliferation of the pathogen and reactivation of the disease.

Whether the delayed-type hypersensitivity reaction is beneficial or harmful to the host depends on the circumstances. On the one hand, the reaction to relatively small numbers of bacilli causes accelerated tubercle formation that enhances the killing of the organisms, and helps prevent reinfection or dissemination from the initial site of infection. On the other hand, the delayed-type hypersensitivity response to large amounts of mycobacterial antigen causes extensive cell necrosis and tissue destruction, which is seriously detrimental. Liquefaction, brought about by hydrolytic enzymes of macrophages and possibly neutrophils, is the most harmful response. The bacilli multiply extracellularly in the liquefied material and are available in large numbers for dissemination through cavities, vessels, and airways. In summary, *the final determinants of the nature and intensity of lesions are the magnitude of the bacterial infection, the intensity and appropriateness of the immune response, and the modifying influences of the structure of the tissue involved.*

Gross or histologic lesions are absent in many cattle that react to tuberculin skin tests, and although *M. bovis* cannot be isolated from many of these cases, they are generally considered to be infected for the purposes of epidemiology and control. Thorough examination of lymph nodes throughout the body is necessary before declaring a carcass free of visible lesions; *only a single lesion is present in most cattle with gross lesions of tuberculosis.* The distribution of lesions of bovine tuberculosis depends on the mode of transmission. In most cases, gross lesions are restricted to the respiratory tract and associated lymphoid tissues. Tubercles or craterous ulcers in gut or mesenteric lymph nodes suggest an oral route of infection, or ingestion of infected sputum that has been coughed up from the lung. Generalized disease is less common but well described.

In **respiratory infections**, *lesions are most common in retropharyngeal, bronchial, and mediastinal lymph nodes*, and less frequent in mandibular and parotid lymph nodes and palatine tonsils. Lung lesions are detected in only 10–20% of cattle with gross lesions, and often affect the caudal lobes. *The classic gross lesion is the tubercle: a circumscribed, often encapsulated, 1–40 mm diameter, pale-yellow or white focus of granulomatous inflammation, often with caseous necrosis and/or mineralization* (Fig. 5.64). Larger lesions may contain liquefied or suppurative exudate and be mistaken for abscesses caused by pyogenic bacteria. Bacilli are released from expanding tubercles into the airways, and coughing up of infected sputum may spread the infection by ingestion to cause lesions in intestine or mesenteric lymph nodes, by adhesion to laryngeal or tracheal mucosa to incite ulcers or ulcerating tubercles, or by aspiration to seed secondary sites in the lung. Multiple, raised, yellow, 1–3 mm plaques on the mucosal surface of the nasal cavity and nasopharynx are early lesions following experimental intranasal infection. Erosion of pulmonary tubercles through the pleura may result in implantation of bacilli throughout the pleural cavity, with development of multiple granulomas on pleural surfaces (Fig. 5.65). Lymphatic spread has been suggested as an alternative route of pleural infection.

Generalized lesions are reported in about 1% of animals with gross lesions of tuberculosis, and probably result from hematogenous dissemination of bacilli following erosion of the wall of a blood vessel by an expanding tubercle. Embolic lesions are most common

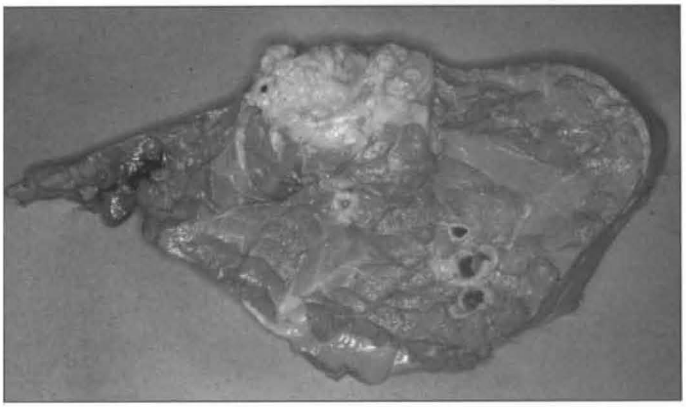

Figure 5.64 Tuberculosis in a cow. Pale bulging granuloma in the lung.

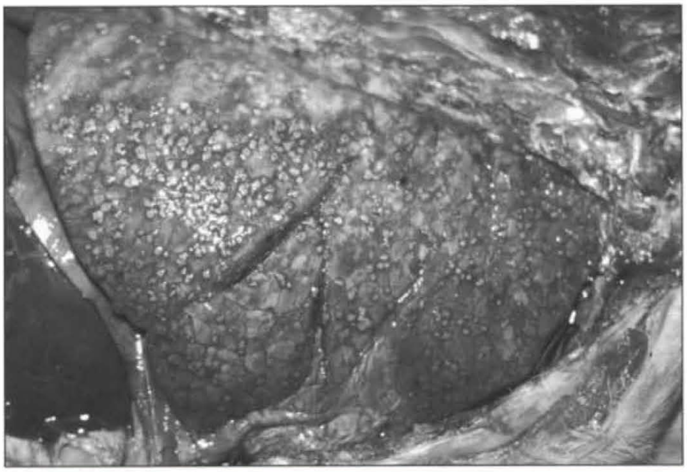

Figure 5.65 Tuberculosis in a cow. Granulomas disseminated throughout the pleura.

in lung, and may involve lymph nodes, bone, liver, kidney, mammary gland, uterus, pleura, peritoneum, pericardium, and meninges. Lesions are rare in salivary gland, pancreas, spleen, brain, myocardium, or muscle. In some instances, presumably following substantial release of bacilli into the blood, the presence of innumerable tiny white foci justifies the term "*miliary tuberculosis*." In contrast, other carcasses display generalized lesions that are variable in size and degree of caseation or fibrosis, and imply a less catastrophic but more prolonged bacteremia. Erosion through serosal or mucosal surfaces by expanding tubercles spreads the infection by implantation on pleural, peritoneal, pericardial, or meningeal surfaces or along the airways, intestine, or urinary tract.

Histologic features of the tubercle include: (1) a central coagulum of caseous necrosis, consisting of eosinophilic homogeneous material with scant nuclear debris and a variable degree of mineralization; (2) a mantle of macrophages and Langhans-type multinucleate giant cells; (3) a capsule containing lymphocytes, clusters of neutrophils in some cases, and a rim of collagenous connective tissue in chronic lesions; and (4) rare to numerous acid-fast bacteria within macrophages and giant cells of the mantle zone or extracellularly in the caseous core (Fig. 5.66). In experimentally infected cattle, the early lesions at 7 days after infection consisted of intra-alveolar clusters of macrophages, necrotic neutrophils, giant cells containing neutrophil remnants, and acid-fast bacteria. At 14 days after infection, corresponding to the onset of lymphocyte proliferative responses, tubercles contained central aggregates of neutrophils surrounded by epithelioid macrophages. In this study, necrosis in the center of the tubercles was noted at 21 days after infection, and the first mineralized lesions were at 35 days. The amount of fibrosis increases with time, and tends to be more prominent in individuals and species with greater resistance. The presence of neutrophils is highly variable, and their presence in large numbers is most common in cases with rapid multiplication of bacteria, numerous reactive lymphocytes, and easily distensible tissues.

In **cervids**, *M. bovis* causes a similar spectrum of lesions as in cattle, but several unique features are of note. As in cattle, lesions are most common in retropharyngeal lymph nodes, lungs, thoracic lymph nodes, and mesenteric lymph nodes. However, deer may develop superficial lymphadenitis and abscesses that drain to the skin surface. Disease progression may be more rapid in cervids than cattle; deer shed larger numbers of bacilli, and the disease may be more likely to remain subclinical. These features suggest that deer are less able to contain *M. bovis* infections than cattle, although limited evidence indicates they are not more susceptible to infection.

In elk (*Cervus elaphus* nelsoni), red deer (*C. elaphus* elaphus), and fallow deer (*Dama dama*), fibrosis and giant cells are less evident than in cattle, the presence of mineralization is more variable, and, importantly, *suppurative inflammation may be prominent*. Multifocal lesions of

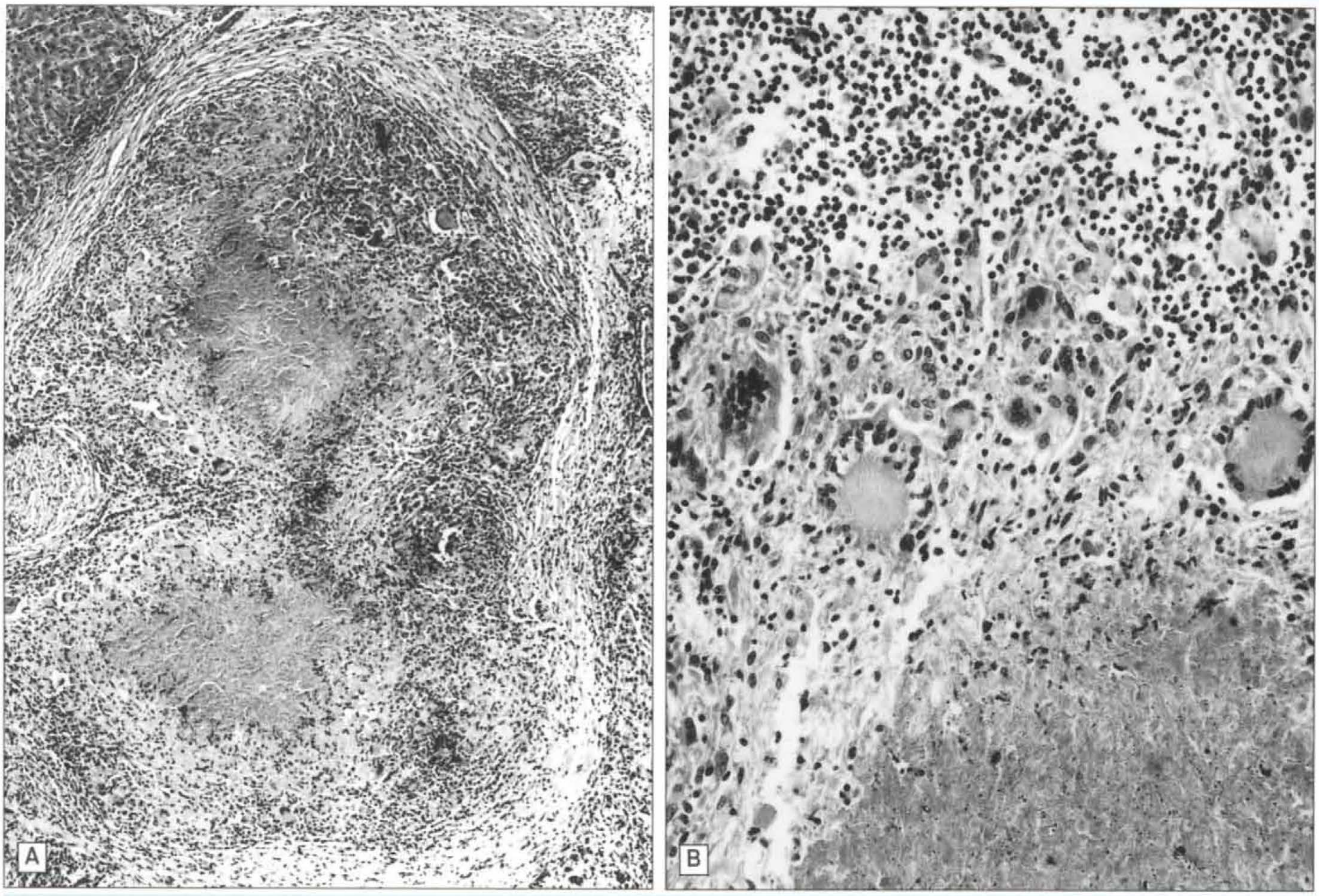

Figure 5.66 Tuberculosis. **A.** Caseous tubercles are encapsulated by fibrous tissue. **B.** Periphery of tubercle with giant cells and epithelioid cells bordering caseous necrosis.

caseous necrosis or suppurative inflammation in the lungs and lymph nodes of cervids are commonly caused by *Fusobacterium necrophorum* infection, and it has been recommended that these lesions be carefully examined for acid-fast bacteria as a routine procedure. Occasional cases of tuberculosis in these species may present concentrically laminated caseous abscesses reminiscent of caseous lymphadenitis in sheep. The lesions of tuberculosis in sika deer (*C. nippon*) are distinctive, consisting of nonencapsulated invasive accumulations of epithelioid macrophages and numerous irregularly shaped giant cells, few neutrophils, and minimal necrosis or mineralization.

Tuberculosis in **horses** is often *alimentary*, with lesions in *retropharyngeal and mesenteric lymph nodes and intestine*. Intestinal infection may cause localized ulcers or thickened mucosal lesions reminiscent of Johne's disease in cattle. Tubercles in horses are usually uniform, gray, smooth (lardaceous), with little caseation or mineralization but abundant fibrosis, and may resemble sarcomas. Tuberculosis in **small ruminants** is rare, but similar in most respects to the disease in cattle. Tuberculosis in **swine** is often *systemic*, and the morphology depends on the infecting pathogen. *M. bovis* produces caseous and mineralized tubercles similar to those that occur in cattle, and the lesions are often surrounded by a fibrous capsule. In the liver, there is a tendency for the caseous centers to liquefy. *M. avium* produces lesions that are proliferative in nature and consist of tuberculous granulation tissue resembling the lardaceous or sarcomatous lesions described in equine tuberculosis. The histologic appearance is of diffuse infiltration of macrophages, epithelioid cells, and Langhans' giant cells accompanied by extensive fibroplasia and numerous bacilli.

Tuberculosis in **dogs and cats** usually appears as *granulation tissue* in which macrophages are scattered at random and giant cells are rare. *Discrete tuberculous granulomas are uncommon*, and composed principally of epithelioid cells surrounded by narrow zones of fibrous tissue in which there are scattered small collections of lymphocytes and plasma cells. Necrosis is often present in the centers of larger granulomas. Giant cells are rare. The presence of central necrosis and fairly small numbers of acid-fast bacilli in lesions of cats helps to distinguish lesions of tuberculosis from those of feline leprosy. The primary foci in the lungs of dogs develop in most cases in the dorsal part of the caudal lobes. Dissemination within the lungs occurs quite rapidly and is predominantly intrabronchial with the production of tuberculous bronchitis and bronchiolitis rather than bronchopneumonia. Pleuritis or peritonitis often accompanies primary infections in the lungs or intestine, respectively, with diffuse or finely nodular pleural thickening by granulation tissue containing few macrophages and bacilli.

A thorough necropsy examination is the most sensitive method of detecting tuberculosis after death, as the likelihood of isolating M. bovis in culture is miniscule if gross lesions are not detected.

Gross lesions of focal caseous lesions in lymph nodes or lung, or histologic detection of granulomas, particularly with central areas of necrosis and/or mineralization, should prompt an extensive and thorough search for acid-fast bacilli. A finding of low numbers of bacilli is more suggestive of bovine tuberculosis than of mycobacteriosis caused by *M. avium*. However, *bacterial culture using egg-based or agar-based media enriched with serum or blood is the gold standard for definitive diagnosis of bovine tuberculosis*, particularly if the diagnosis is likely to have a serious impact. Specimens should be collected using sterile technique, to avoid contamination with environmental mycobacteria that may overgrow *M. bovis* in culture. Immunohistochemical identification of *M. bovis* has been described. This procedure is more sensitive than examining acid-fast-stained sections, in terms of number of animals detected, staining intensity, and ease of detection, but the antibodies in current use cross-react with other mycobacteria as well as some gram-positive bacteria and fungi.

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Mycoplasmal diseases

Contagious bovine pleuropneumonia

Contagious bovine pleuropneumonia (CBPP), an OIE (World Organization for Animal Health) List A disease, is an important barrier to global trade, and a production-limiting disease in Africa. The disease is present in much of Africa except the Mediterranean and southeast African countries, and has recently occurred in southern Europe, central and eastern Asia, and the Middle East. CBPP caused devastating epidemics in cattle populations in the 19th century, but *sporadic disease is now more common in endemically infected countries practicing vaccination*. When the agent is introduced into naive populations, the effects vary from insidiously spreading disease to spectacular mortality affecting up to 50% of the animals.

The clinical signs and mortality rates are highly variable, depending on the strain of the pathogen, the age of the animal (adults are more susceptible than young stock), and the presence of other diseases, crowding, or stressors. Peracute cases die without premonitory signs. Acutely ill animals are febrile, anorexic, and depressed, with profound loss of milk production, open-mouth rapid breathing, mucoid nasal discharge, and occasional coughing. Chronically affected animals may be clinically normal or have an intermittent cough and fever, yet shed the organism intermittently. Calves less than 6 months old commonly develop polyarthritis affecting carpal and tarsal joints, rather than pneumonia.

CBPP is caused by Mycoplasma mycoides subspecies mycoides small colony type, which is different from the large colony type that causes pleuritis and pneumonia in goats in many countries throughout the world. There is marked strain-dependent variation in virulence, and the pathogenicity apparently declines as in vivo passage of the organism occurs during an outbreak. Galactan in the mucus capsule is correlated with virulence, and induces necrosis, thrombosis, and inflammation. The host range includes cattle (*Bos taurus*), zebu (*B. indicus*), water buffalo (*Bubalus bubalis*), bison, and yak, although *cattle are most commonly affected by clinical disease. Mycoplasma mycoides* is an obligate pathogen that survives poorly in the environment, so transmission usually depends on close contact with animals shedding the bacteria. Transplacental infection may occur. *The respiratory tract is the site of primary infection*, and bacteremia develops secondarily. The incubation period varies from 5 to 200 days, but is typically 20–40 days.

Gross lesions are often unilateral and restricted to the *caudal lung lobes*. In the acute stages, there are mats of fibrin and fluid exudate covering the pleural surface. Interlobular septa are remarkably distended with serous fluid, and the lobules vary from normal to consolidated and red or yellow-gray. The pattern of interlobular edema and lobular consolidation lends a *characteristic marbled appearance* to the lung. Focal areas of necrosis increase in size over time, and eventually develop into sequestra. Sequestrum formation and fibrous thickening of the pleura with adhesions to the ribs are typical of chronic CBPP. Pericarditis, peritonitis, and polysynovitis occur infrequently in adult cattle (Fig. 5.67).

Histologic examination of bronchioles and alveoli reveals serofibrinous or suppurative exudates with necrosis of neutrophils, and multifocal areas of coagulative necrosis in the lung parenchyma. Interlobular septa are expanded by edema and fibrin, and interlobular lymphatics contain fibrin. Thrombosis of arteries, veins, and lymphatics is common, and nonsuppurative arteritis may be evident. *Vasculitis* is central to the development of the necrotic lesions and sequestration of lung tissue (Fig. 5.67).

The **differential diagnosis** includes *Mannheimia haemolytica* and *Mycoplasma bovis*. The unilateral lesions, prominent inflammation in interlobular septa, and frequent development of sequestra should suggest a diagnosis of CBPP, although the latter two features do occur in shipping fever due to *Mannheimia haemolytica*. The lesions of *Mycoplasma bovis* lack the extensive fibrinous exudates in interlobular septa and on the pleural surface seen in CBPP.

Definitive **diagnosis** is based on *culture on special media*, which may take several weeks. *Frozen samples of lesional lung, pleural fluids, and lymph nodes are optimal for isolation of the agent*. Immunohistochemistry and the polymerase chain reaction are more rapid methods of diagnosis. Various serological assays are useful for screening of herds, but not for individual diagnosis. Development of improved methods to detect subclinical carriers is an area of intensive research, as current serologic assays lack sensitivity.

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Mycoplasma bovis

The role of *Mycoplasma bovis* as a cause of mastitis and of infertility of dairy cows is discussed elsewhere. The two principal manifestations of *M. bovis* in the respiratory system are as a component of *enzootic pneumonia of young calves*, and *chronic pneumonia and polyarthritis in feedlot cattle*. The latter is an emerging disease in intensively raised feedlot cattle in North America and Europe. In contrast to the appearance of shipping fever, calves with lesions of *M. bovis* pneumonia or polyarthritis usually die or are euthanized in the second or third month after arrival. These calves have often had prolonged antimicrobial therapy for nonresponsive or relapsing respiratory disease, which is indistinguishable from shipping fever in the early stages. Calves may be lame and have swelling of one or more joints, and many calves exhibit both respiratory disease and lameness.

The prevalence of chronic pneumonia and polyarthritis is often low, although up to 30% of calves may be affected in exceptional cases. Nevertheless, since the diagnosis is only made on fatal cases, the true prevalence of the disease is unknown. In contrast, the prevalence of infection with *M. bovis* has been reported to vary from 20 to 70%, and most calves seroconvert to *M. bovis* during the first month in the feedlot.

M. bovis may be carried and shed in the secretions of the respiratory tract, the genital tract, and the mammary gland. Infection of the respiratory tract probably occurs by direct contact with nasal secretions, or perhaps by inhalation of infected droplets. Contact with genital secretions or ingestion of infected milk may cause infection in neonatal calves. Infection may remain confined to the respiratory tract, or extend from the lung to cause bacteremia with subsequent infection of joints and other tissues.

M. bovis is seemingly well equipped to evade the immune response. The bacterium induces apoptosis of bovine lymphocytes, suppresses the proliferative response of lymphocytes to mitogens, and impairs neutrophil activation. The bacterial surface is decorated with a family of highly immunogenic variable-surface lipoproteins, encoded by at least 13 *vsp* genes containing multiple repeat sequences, which undergo high-frequency phase variation (turning expression on and off) and size variation. *M. bovis* seems to alter surface lipoprotein expression in response to binding of specific antibody, suggesting that rapid changes in surface antigen expression is a mechanism of evading the humoral immune response, in a manner analogous to *Trypanosoma* sp. In support of this hypothesis, although antibody titers develop 2–3 weeks after experimental infections, the cellular and humoral immune responses are not protective.

Assessing the antimicrobial susceptibility patterns of *Mycoplasma* sp. is problematic, but several recent reports indicate that many isolates of *M. bovis* are resistant to antimicrobials commonly used in feedlot medicine, including florfenicol, tilmicosin, oxytetracycline, and spectinomycin. The pathogenetic implications of this antimicrobial resistance are uncertain. However, one theory suggests that *M. bovis* may be one of several pathogens infecting calves as they enter the feedlot, but the ability of this bacterium to resist antimicrobials

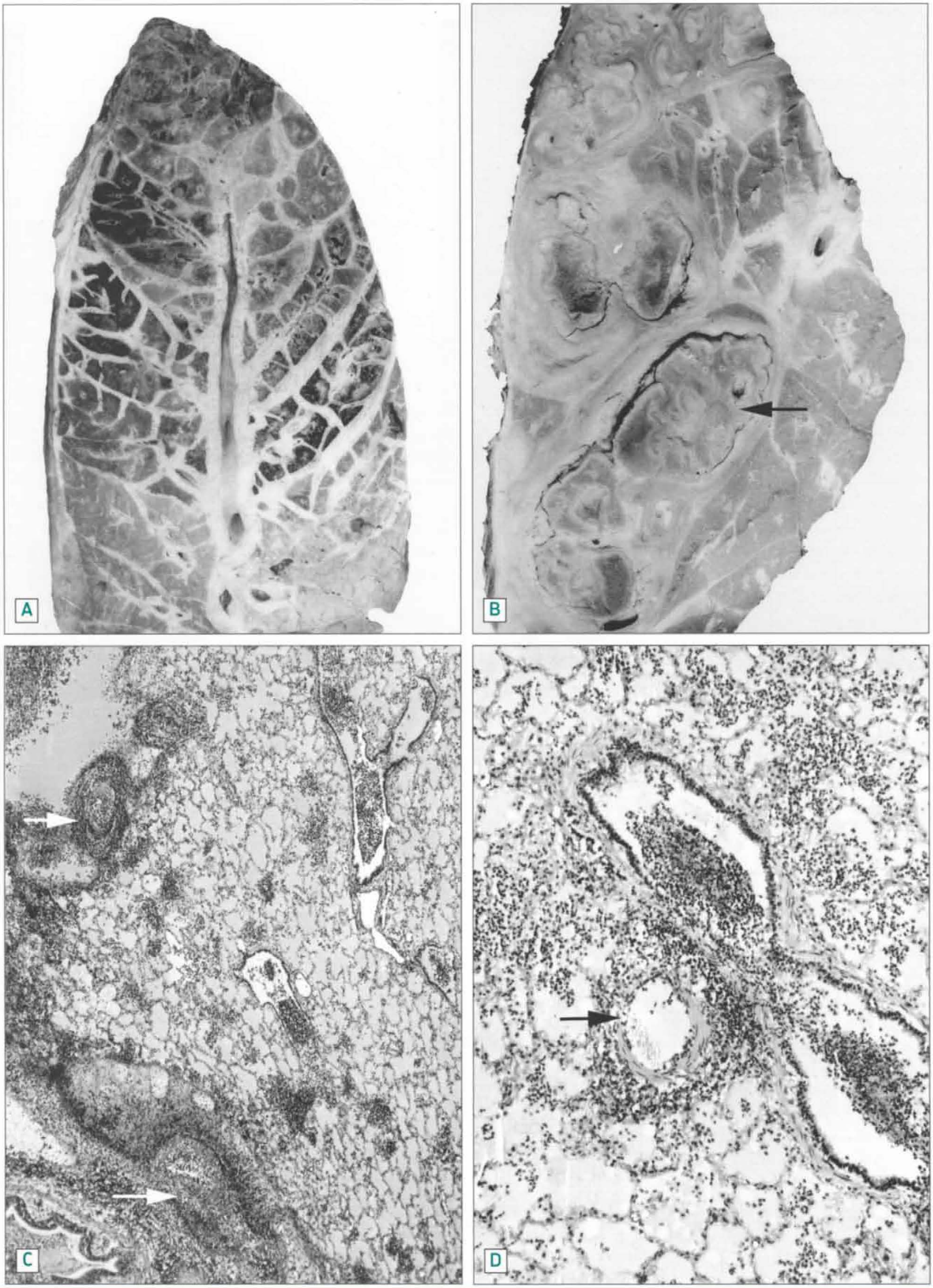

Figure 5.67 Contagious bovine pleuropneumonia. A. Distension of interlobular septa. **B.** Sequestra (arrow) with encapsulation of necrotic tissue. **C.** Acute fibrinonecrotic pneumonia with inflammation, arteritis, and necrosis in interlobular septa and peribronchial interstitium (arrows). **D.** Acute arteritis (arrow) affecting a peribronchial artery.

and evade the immune response leads to chronic disease that fails to respond to therapy.

Many calves with lesions of *M. bovis* pneumonia are also infected with *Mannheimia haemolytica*, and there is epidemiologic and morphologic evidence that the lesions of *Mycoplasma bovis* may evolve out of those of *Mannheimia haemolytica*. Most cases have concurrent infection with *Mycoplasma arginini*, suggesting possible synergy. The role of *M. bovis* in predisposing to bacterial pneumonia is uncertain, as *M. bovis* is reportedly less effective than *M. dispar* at impairing ciliary function.

The **gross appearance** of *M. bovis* pneumonia may be indistinguishable from suppurative bronchopneumonia of other causes: there is cranioventral reddening and consolidation, scant fibrin may cover pleural surfaces, and bronchial lymph nodes are enlarged and diffusely white. However, *the characteristic lesions are raised, white, sharply demarcated, friable foci of caseous necrosis within the consolidated areas of lung* (Figs 5.68 and 5.69). These foci of necrosis are often 2–10 mm in diameter, but occasionally enlarge to many centimeters diameter. The largest lesions may contain fluid pus rather than caseous exudate, presumably due to secondary or concurrent bacterial infection. These foci of necrosis form sequestra in some cases. Uncommonly, foci of necrosis and inflammation may be present in bronchial lymph nodes, and similar nodular masses are rarely encountered in the tracheal submucosa, along the dorsal tracheal ligament, or in other tissues.

Joint lesions are present in ~50% of calves with *M. bovis* pneumonia, and the lesions may vary considerably within the same animal. *Pneumonia is found in all calves with M. bovis arthritis.* Acute lesions consist of serofibrinous exudate within joint cavities and tendon sheaths, and the synovium is reddened and hyperplastic. Cartilage erosion is reported in experimental cases but is often absent in clinical material. Purulent or fibrinopurulent exudate is often florid in more established lesions. Foci of caseous necrosis, similar to those described in the lung, may be present in the synovium or joint capsule. Other joints have minimal lesions in the articular cavity, but extensive edema and foci of caseous necrosis in the tendon sheaths or the periarticular soft tissues.

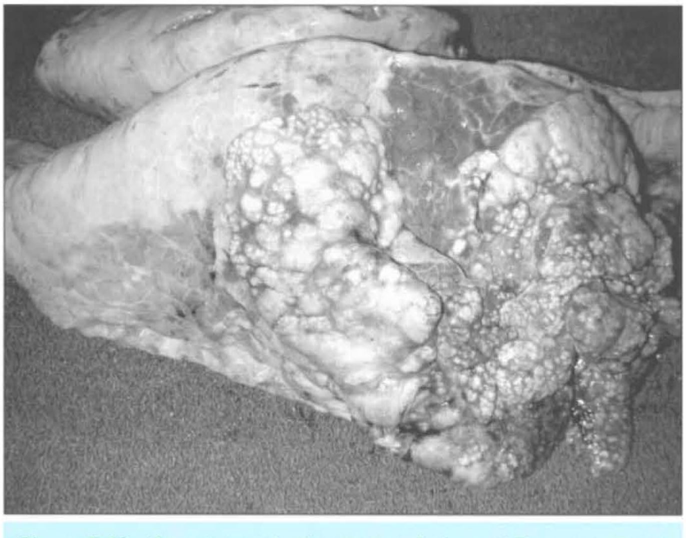

Figure 5.68 ***Mycoplasma bovis* pneumonia** in a calf. The cranioventral 60% of the lung is consolidated and contains innumerable white raised nodules.

Other manifestations of *M. bovis* infection include fibrinous, purulent, or caseating *otitis media* in young calves, which may be associated with feeding of mastitic milk; decubital abscesses; and a proposed association with *necrotizing myocarditis* of the papillary muscle that resembles *Histophilus somni* myocarditis.

The **histologic lesions** of suppurative bronchopneumonia are nonspecific, but the characteristic appearance of the foci of necrosis suggests a diagnosis of *M. bovis*. The lesions originate in small bronchioles, alveoli, and alveolar septa. The mildest, and probably earliest, lesion consists of suppurative exudate within small bronchioles, in which leukocytes have a characteristic appearance – they are necrotic but retain their cellular outlines, and have hypereosinophilic cytoplasm and inapparent or fragmented nuclei (Fig. 5.70). As the lesion progresses, there is erosion of bronchiolar epithelium, with expansion of the necrotic foci to incorporate peribronchiolar alveoli. Macrophages and lymphocytes infiltrate the margins, infrequent giant cells may be present, and immature fibrous tissue develops at the outermost edge of the lesion. Large lesions may contain coagulative necrosis in their centers, with retention of the alveolar and bronchiolar architecture of the dead parenchyma. Mineralization of the necrotic tissue is frequent. Lesions affecting bronchi may progress to bronchiectasis.

Using immunohistochemistry, mycoplasmal antigen is most abundant at the periphery of the foci of necrosis and within the bronchiolar exudate. Lesser amounts of antigen are present at the apical surfaces of bronchiolar and bronchial epithelial cells, and within alveolar macrophages and neutrophils. Foci of necrosis or lymphoplasmacytic inflammation may be present in the liver, kidney, or other viscera.

The gross and histologic appearance of the foci of necrosis is distinctive. The foci of necrosis caused by *Mannheimia haemolytica* or *H. somni* are irregular in shape, nonfriable, delineated by a white rim, retain

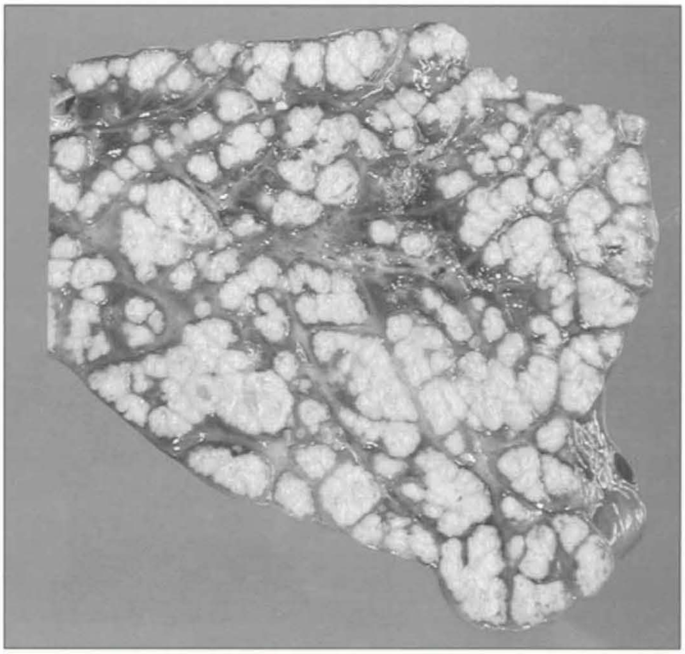

Figure 5.69 ***Mycoplasma bovis* pneumonia** in a calf. Consolidated lung contains coalescing white crumbly nodules of caseous necrosis, separated by atelectatic lung tissue.

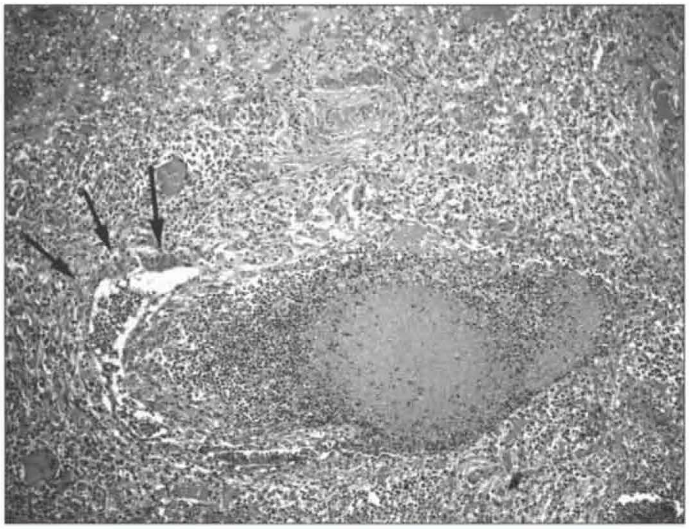

Figure 5.70 ***Mycoplasma bovis* pneumonia** in a calf. A bronchiole contains a few remnants of epithelium (arrows), and is filled with suppurative exudate containing a developing focus of caseous necrosis.

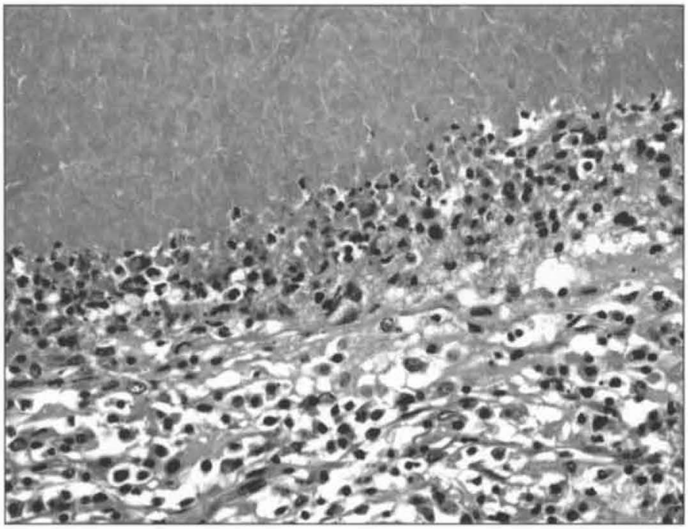

Figure 5.71 ***Mycoplasma bovis* pneumonia** in a calf. A well-developed focus of caseous necrosis containing an acidophilic coagulum (top), separated by a layer of necrotic neutrophils and macrophages from a fibrous capsule (bottom).

the histologically visible tissue architecture (coagulative necrosis), and contain streaming necrotic leukocytes. In contrast, the lesions of *Mycoplasma bovis* are usually circular and white throughout, and appear histologically as an eosinophilic coagulum exhibiting the ghost-like outlines of necrotic leukocytes (Fig. 5.71). Larger foci with fluid pus rather than crumbly content may be appropriately termed abscesses if a fibrous capsule can be demonstrated, and *M. bovis* may be isolated along with other bacterial pathogens such as *Arcanobacterium pyogenes*. Sequestra caused by *M. bovis* are indistinguishable from those caused by *Mannheimia haemolytica* or *H. somni*, unless typical foci of caseous necrosis are detected in the adjacent consolidated lung tissue.

Various techniques are available to confirm the tentative **diagnosis** of *Mycoplasma bovis*. It grows readily on *Mycoplasma* enrichment media, and is occasionally isolated on blood agar. However, many calves without apparent pneumonia are infected with *M. bovis*. Thus, *the diagnosis rests on identifying both infection with this agent and the presence of the characteristic lesions*. Immunohistochemistry is also advantageous for correlating the location of mycoplasmal antigen with the characteristic lesions. Seroconversion occurs in many healthy calves in the first month after entering feedlots, making this assay of dubious significance for diagnosis of individual cases.

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Miscellaneous *Mycoplasma* spp. of calves

Mycoplasma spp. are regularly isolated from the lungs of calves with pneumonia, but their importance in disease is often difficult to interpret. Only *M. bovis* is likely to cause severe primary disease. Infection with ***M. dispar, Ureaplasma diversum***, and perhaps ***M. bovirhinis*** causes bronchiolitis and may contribute to development of acute or chronic enzootic pneumonia in young calves by predisposing to bacterial pneumonia. Experimental infections with ***M. bovigenitalium, M. arginini***, *and* ***M. canis*** induce mild lesions and no clinical signs.

Mycoplasma spp. attach to ciliated epithelial cells, on and between microvilli and covering the base of the cilia. *The typical lesion is chronic catarrhal bronchitis and bronchiolitis with prominent lymphocytic cuffs around airways*. Affected lungs contain patchy, cranioventral, purple-red foci of atelectasis. When secondarily infected by opportunistic bacterial pathogens, more confluent, meaty consolidation may also occur. Microscopically, accumulations of neutrophils and mucus are present in airway lumens and there is increased prominence of goblet cells. Moderate infiltrates of lymphocytes and fewer plasma cells encircle the airways and accompanying blood vessels. Inflammation of alveoli adjacent to terminal bronchioles occurs in experimental infections in colostrum-deprived, specific-pathogen-free calves and can probably occur in heavy natural infection. Alveolar lesions are not specific, and include atelectasis, accumulation of neutrophils and alveolar macrophages with occasional lymphocytes and plasma cells, and mild hypercellularity of alveolar septa. As the infection becomes chronic, lymphocytic peribronchiolar cuffs thicken, develop germinal centers, and extend to the lamina propria of the bronchiole. At this stage, the epithelium may be hyperplastic with goblet-cell hyperplasia and hypertrophy of bronchial submucosal glands.

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Fungal diseases

Mortierellosis

Acute fatal mycotic pneumonia is a sequel to placental infection with *Mortierella wolfii, the most important cause of mycotic abortion of cattle in New Zealand*. This condition has rarely been reported elsewhere. Acute fibrinonecrotic pneumonia occurs in infected cows within a few days of abortion or parturition, due to extensive hematogenous dissemination of fungi when the placenta detaches. In the acute disease, the lung is diffusely firm, edematous, and red-yellow. Histologic lesions include serofibrinous and suppurative exudate in alveoli, with vascular necrosis, thrombosis, and coagulative ischemic necrosis of lung parenchyma. In the subacute and chronic disease, there is an embolic pattern of multifocal fibrinous and suppurative inflammation. Similar lesions may occur in the spleen. Fungal hyphae are present within the lesions, but may be inconspicuous in chronic lesions. The hyphae are periodic acid–Schiff-positive, nonseptate, branching, and vary from 2 to 12 μm in diameter.

Other fungi such as *Aspergillus*, *Mucor*, and *Rhizopus* are occasional opportunistic invaders of lung, and cause nodules of caseous necrosis or granulomatous inflammation.

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Parasitic diseases

Dictyocaulus viviparus

Dictyocaulus viviparus is a common and important cause of respiratory disease in cool, wet, seasonal climates. **Primary infections**, sometimes known as "husk," cause disease in calves during their first grazing season, and occasionally affect mature animals that have not had sufficient exposure to develop immunity. Although clinical disease may develop within 3 weeks of access to infected pastures, it is most common late in the grazing season when calves have been at pasture for 3–5 months. Affected herds usually have high disease prevalence, and mortality rates are variable depending on the degree of pasture contamination. A second manifestation, **reinfection syndrome**, occurs when partially immune adult cattle on endemically infected farms have access to pastures that are contaminated with large numbers of infective larvae. In these herds, there is a high prevalence of coughing, tachypnea, and depression about 2 weeks after exposure. Some animals develop fatal dyspnea without coughing.

D. viviparus, the only adult nematode known to infect the lung of cattle, is a member of the superfamily Trichostrongyloidea, family Dictyocaulidae. *Adult lungworms inhabit the large bronchi*. The eggs are embryonated when laid, and hatch rapidly. First-stage larvae are expelled from the lung by coughing, are swallowed, and then passed in the feces. Further development to infective third-stage larvae occurs on the ground over the next 5–7 days. Larval development is optimal in moist cool conditions; the larvae can develop at temperatures as low as 5°C, and their viability is prolonged at these temperatures. The third-stage larvae are ingested by cattle, penetrate the wall of the intestine, and migrate via the lymphatics to the mesenteric lymph nodes. In the lymph nodes, they molt to form fourth-stage larvae, and then go by way of lymph and blood to enter the lungs about 7 days after infection. Some larvae accidentally take the portal route and are destroyed in the liver, or rarely enter the systemic circulation to cause intrauterine infections in the fetus. The final molt to the fifth-stage larvae occurs in the bronchioles, and adults develop in the larger airways. Eggs are first detectable in feces at 21–30 days after infection; the infection is usually patent for another 1–3 months. However, a few egg-laying adults may persist in some animals over the winter months, and serve as a source of pasture contamination in the following grazing season.

The clinical and pathologic manifestations of *Dictyocaulus* infestation are dependent on the stage of infection, the level of immunity of the host, and the number of invading larvae. *The following description pertains to primary infections of naive animals*; reinfection of partially immune cattle is discussed subsequently. *Lesions differ in the prepatent period, the patent period, and the period of recovery*. During the **prepatent period**, from 7 to 25 days after infection, the principal lesions are eosinophilic bronchiolitis and/or alveolitis in response to the presence of larvae in the alveoli and bronchioles. Clinical signs include coughing and tachypnea, and calves infected with many larvae may die at this stage. Where the larvae emerge from pulmonary capillaries, they cause microscopic foci of necrosis and fibrin exudation, with an alveolar infiltrate of eosinophils and fewer neutrophils, macrophages, and giant cells. Mononuclear cells thicken the alveolar walls, and hyaline membranes or hyperplasia of alveolar type II epithelial cells may occur. The larvae, some of them dead, can be found in the alveoli. When the number of larvae is large, the foci of acute interstitial pneumonia may be visible grossly as lobular or smaller foci that are slightly depressed, purple, and widely distributed throughout the lungs.

By about the tenth day, many of the larvae have reached the terminal bronchioles. Frothy fluid is present in the bronchi and, in very heavy infestations, there is edema, atelectasis, and/or emphysema. Eosinophils invade the septal tissues in large numbers and pursue the larvae into the bronchioles. Most of the bronchioles contain plugs of exudate composed largely of eosinophils. Neutrophils, lymphocytes, and macrophages are present in smaller numbers in both the lumens and walls of the bronchioles. The early bronchiolar epithelial response is of degeneration and sloughing, but subsequently hyperplasia and metaplasia occur. At this stage, adult worms are not present in the airways, but larvae are detectable histologically or in a smear of exudate harvested from the smallest airways.

During the **patent period**, beginning 25 days after infection, the inflammatory response targets adult worms in the bronchi, and eggs and recently hatched first-stage larvae that have been aspirated into the alveoli. At this stage, calves may exhibit chronic coughing, increased respiratory rate, and weight loss, or develop severe dyspnea and death. Adult worms, which are slender, white, and up to 8 cm long, are most numerous in the dorsocaudal bronchi of the caudal lobes (Fig. 5.72). Infections may be missed if only the trachea and large cranial bronchi are examined, as *many infestations are restricted to the small branches of the caudal bronchi*. Patchy lobular atelectasis may be the only other gross finding in mild infestations. In more severe cases, there are wedge-shaped, red-gray, firm, depressed areas of consolidation at the caudal border of the caudal lung (Fig. 5.73). Examination of a cut section may reveal similar areas of consolidation in much of the pulmonary tissue surrounding larger bronchi.

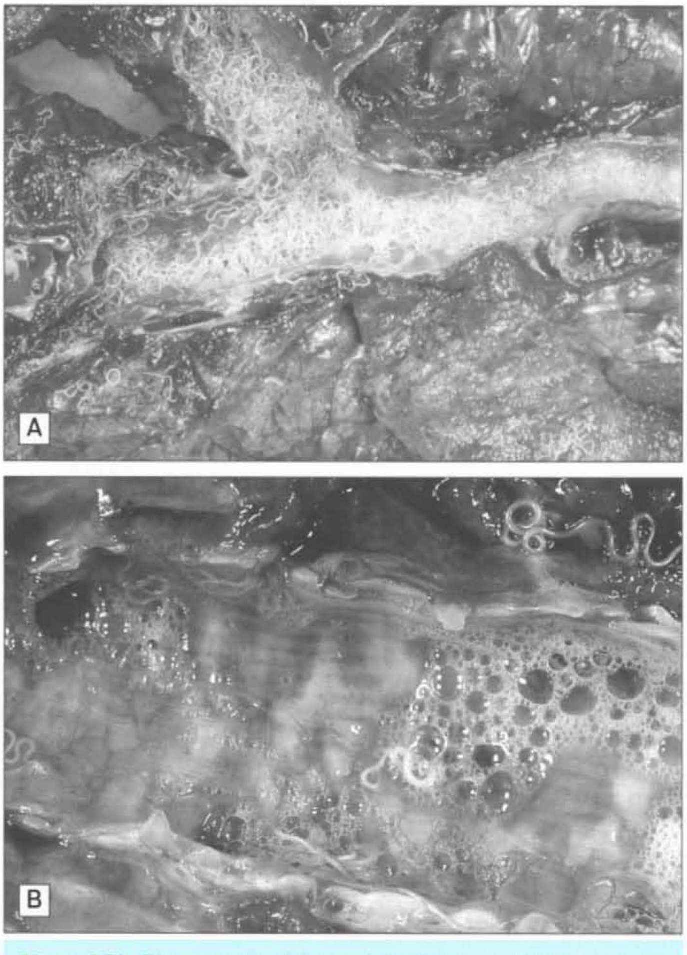

Figure 5.72 ***Dictyocaulus viviparus* infection** in a calf. Worms may be numerous (**A**) or few (**B**) within the frothy or catarrhal exudate in the lumen of caudal bronchi. (Courtesy of EG Clark.)

Histologic lesions in patent infections include eosinophilic catarrhal bronchitis, atelectasis, and eosinophilic granulomatous alveolitis. The bronchi and large bronchioles contain adult worms, eggs, and larvae mixed with mucus and numerous eosinophils. In histologic sections, adult worms have thick intestinal epithelium composed of a few multinucleated cells with indistinct microvilli, prominent lateral chords, and coelomyarian-polymyarian musculature, and the uterus may contain larvae or embryonated eggs. The airway mucosa contains infiltrates of eosinophils and mononuclear cells, the epithelium is hyperplastic and contains increased numbers of goblet cells, and the peribronchial lymphoid tissue is expanded. The accompanying alveolar lesions consist of atelectasis secondary to the obstructive bronchiolitis, and macrophages, giant cells, and lymphocytes encircling fragments of cuticle, aspirated eggs, or newly hatched larvae. It is complicated in some cases by bacterial bronchopneumonia. Alveolar septa are thickened by cellular infiltration, slight fibroplasia, and inconsistent proliferation of type II pneumocytes. At this stage, the infection may be diagnosed by gross observation of adult worms in the bronchi, examination of histologic sections of bronchi and alveoli, identification of all stages in wet mounts of airway mucus, and/or identifying larvae in feces by the Baermann technique.

During the **period of recovery**, *adult worms are eliminated.* However, gross lesions of consolidation may persist, and there is fibrosis of peribronchiolar alveoli with prominent lymphocytic cuffs around bronchi. Establishing a firm diagnosis at this stage may be impossible, as adults are not present and the feces no longer contain larvae. The distribution of lesions in the caudal lung helps to differentiate chronic parasitism from mycoplasmosis, which usually affects the cranioventral areas of lung. Acutely fatal exacerbations of the

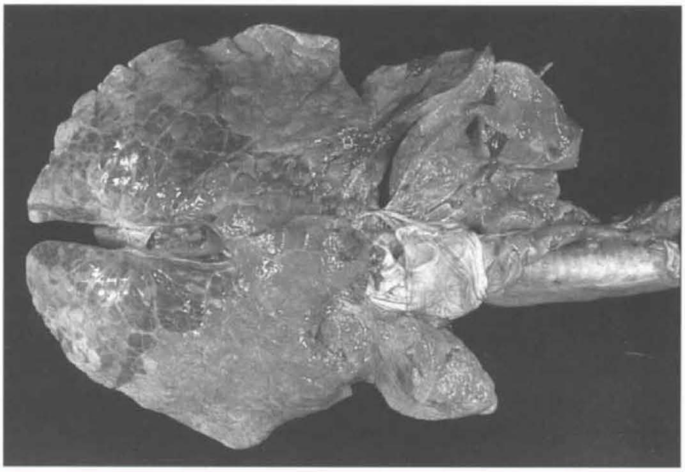

Figure 5.73 ***Dictyocaulus viviparus* infection** in a calf. Patchy areas of consolidation in the caudal lung lobes. (Courtesy of EG Clark.)

disease during the period of recovery, when larvae and adults are no longer present, have been described. These cases may be impossible to differentiate from toxic interstitial pneumonia, but acute exacerbation of a chronic illness is not typical of pulmonary toxicity.

Emphysema may be a prominent gross lesion in severe cases of *Dictyocaulus* infection, and is probably a consequence of forced expiration, due to severe dyspnea, in the presence of bronchiolar obstruction by exudate and bronchoconstriction. Cases in which emphysema is the major lesion at necropsy may be mistaken for acute interstitial pneumonia of toxic cause. This is particularly likely when the pulmonary damage is caused by massive invasion of larvae, and mature worms are not yet present for gross detection. Microscopic detection of larvae and immature worms usually provides the diagnosis.

The typical lesion in the **reinfection syndrome** – where partially immune adult cattle are infected with numerous larvae – are scattered, 2–4 mm diameter, gray nodules that often contain a green caseous center. The nodules represent dense accumulations of lymphocytes and plasma cells, encircling an eosinophilic and granulomatous response to a degenerating larva. As a result of this robust immunoinflammatory response, the larvae do not reach the large airways or develop into adults, and the infection does not usually achieve patency.

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Miscellaneous parasites

Ascaris suum larvae are an occasional cause of severe interstitial pneumonia in calves housed in barns where pigs were previously kept. In these situations, infective eggs are ingested, larvae hatch within 18 hours, and migrate to the lungs 5–13 days after infection. The lesions are of severe acute interstitial pneumonia, in which the lungs are diffusely rubbery, wet, and heavy, and interlobular septa are distended with clear fluid. Migration of larvae through the liver may cause white streaks. Histologically, there is alveolar edema and hyaline membranes or type II pneumocyte proliferation, and numerous eosinophils infiltrate alveolar septa and exude into the alveoli. Larvae are readily identified in cases that die about a week after infection, but a diligent search may be required in calves that survive for 2–3 weeks after infection. Larvae cannot be consistently recovered from lung digests. The histologic appearance of the larvae is characteristic: they have lateral alae, large lateral chords, and an intestine (the only internal organ present) composed of few uninucleate cells.

The trematodes ***Fasciola gigantica*** and ***F. hepatica*** occasionally invade the lungs accidentally from the liver. Since they are large parasites which wander extensively, a small number in the lungs can produce extensive cavitations. ***Schistosoma nasalis*** causes nasal granulomas in India. In cattle, lung is less common than liver as a site of hydatid cysts caused by ***Echinococcus granulosus***.

The **leeches** *Limnatis nilotica* and *L. africana* are taken in while drinking and attach to the mucosa of the pharynx and nasopharynx. They suck large quantities of blood, and their presence induces localized edema that leads to dyspnea or asphyxiation. The **nematodes** *Mammomonogamus* (*Syngamus*) *nasicola* and *M. ierei* are found in the nasal passages of ruminants in tropical countries, and *M. laryngeus* causes lymphocytic polypoid hyperplasia in the pharynx and larynx of cattle in tropical Asia and South America.

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Infectious respiratory diseases of sheep and goats

Viral diseases

Ovine respiratory syncytial and parainfluenza viruses

Many aspects of the pathogenesis, lesions, and role of these viruses in predisposing to bacterial pneumonia are similar to those described for the bovine viruses. Ruminant respiratory syncytial viruses have been divided into bovine and ovine subgroups based on nucleotide and antigenic differences in the G protein. Sheep are susceptible to infection and disease with both ovine and bovine subgroups, and ovine strains infect cattle and deer.

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Adenovirus

Adenoviral infections are common in sheep, but primary adenoviral pneumonia is rare. *Ovine adenovirus* (OAdV) serotype 6 exacerbates disease caused by experimentally inoculated *Mannheimia haemolytica*, and adenoviruses are occasionally isolated from outbreaks of bacterial pneumonia in lambs. These findings suggest that adenoviral infection may predispose to development of bacterial pneumonia in some groups of lambs. Lesions in experimentally infected lambs are similar to those described for calves. A noteworthy feature of the lesions caused by at least one strain of OAdV is the enlargement of both nucleus and cytoplasm of inclusion-bearing bronchiolar and alveolar epithelial cells, which could be mistaken for cytomegalovirus infection. Adenoviruses have been isolated from goats, but their role in causing disease appears minor.

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Peste des petits ruminants

Peste-des-petits-ruminants virus (PPRV) (see Vol. 2, Alimentary system) is closely related to *Rinderpest virus* in the genus *Morbillivirus*. Sheep and goats with PPR commonly have clinical signs and lesions affecting the respiratory system, in addition to enteric lesions that resemble those of rinderpest. The gross lesions usually affect the cranioventral lobes of the lung, which are firm, reddened, and may have fibrinous exudate on the pleural surface. Histologic lesions are of *bronchointerstitial pneumonia*, and resemble the lesions of canine distemper, another morbillivirus. There is necrosis and attenuation of epithelial cells lining the trachea, bronchi, and bronchioles; proliferation of alveolar type II pneumocytes; and formation of epithelial syncytia in the alveoli. Infected epithelial cells often contain eosinophilic inclusion bodies in the cytoplasm or the nucleus. As the virus is difficult to isolate in culture, definitive diagnosis depends on immunohistochemistry or serology.

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Lentiviral pneumonia: maedi-visna (ovine progressive pneumonia) and caprine arthritis-encephalitis viral pneumonia

Lentiviral infections are common in sheep and goats worldwide, with the exceptions of Iceland, where the disease was eradicated in 1965, and Australia and New Zealand, where ovine lentiviral disease has not been recorded. The seroprevalence varies greatly, for example from 1 to 70% in various areas of the USA. Most infected animals are not clinically ill, and infection may become widespread before diseased animals are noticed. *Lentiviruses cause slowly progressive pneumonia, encephalitis, arthritis, and mastitis; the various syndromes may occur independently or concurrently.* The descriptive terminology of the ovine disease is a colorful consequence of the history of the disease. The respiratory form in sheep, which is termed *maedi* ("dyspnea" in Icelandic) in much of the world and ovine progressive pneumonia in the USA, is the most common presentation, and manifests as inexorably progressive dyspnea, hyperpnea, and weight loss in sheep greater than 3 years of age. Other outcomes in sheep are less frequent and include encephalitis, arthritis, and mastitis. Encephalitis – *visna* ("fading away" in Icelandic) – was common in the original Icelandic description but is now a rare expression of the disease, and results in ataxia, trembling, and profound weight loss. Mastitis and arthritis are uncommon in sheep, and manifest as agalactia and a hard udder, or lameness and swollen joints (particularly carpal joints) respectively.

Caprine arthritis encephalitis virus (CAEV) causes two common clinical diseases. The first, *arthritis in adult goats*, results in acute or chronic lameness and swelling of carpal or tarsal joints. The second manifestation, *neurologic disease* in 2–4-month-old kids or less commonly in older animals, is characterized by progressive ataxia and weakness beginning in the hindlimbs. *Pneumonia*, which is less frequent than arthritis and neurologic disease, may occasionally be the major presenting complaint in kids or adults, or occur concurrently with arthritis or neurologic disease.

Maedi-visna, ovine progressive pneumonia, and CAE are caused by closely related lentiviruses in the family Retroviridae, genus *Lentivirus*; these share more homology with feline immunodeficiency virus than with other lentiviruses. The extreme genetic diversity created by replicative infidelity of the lentiviruses has blurred the concept of distinct viral species: although distinct viral clades can be identified, ovine and caprine isolates are represented in each, and French ovine lentivirus isolates have greater homology to CAEV than to *Visna/maedi virus* (VISNA). These findings suggest interspecies transmission; however, the identification of CAE but not maedi-visna in Australia implies that a species barrier exists for some strains. The comparative phylogeny of small ruminant lentiviruses remains an active area of research.

Small ruminant lentiviruses are enveloped, 100 nm diameter, and contain a dense nucleoid and a positive-sense single-stranded RNA genome. Viral genes include *gag*, which encodes group-specific nucleocapsid and matrix glycoproteins that are detected by antibody-based diagnostic tests; *pol*, which encodes reverse transcriptase and other enzymes; *env*, encoding the surface glycoprotein that mediates receptor binding and virus entry into cells, and is the target for neutralizing antibody; and the regulatory proteins encoded by *vif, rev, and tat*. Long terminal repeats that flank the encoding regions of the genome bind transcription factors and are necessary for replication.

Lentiviral diseases in sheep and goats are marked by their variability. Viral strains or quasispecies may differ in their virulence or their propensity to cause pulmonary, nervous, joint, or mammary disease. Disease manifestation is also influenced by host genetics, as certain breeds of sheep are more susceptible to disease than others. Finally, the local tissue microenvironment affects viral replication and development of disease; for example, proinflammatory cytokines generated in response to concurrent infection with other pathogens augment lentiviral replication and may hasten disease onset.

Transmission of small ruminant lentiviruses through colostrum or milk is the major method of disease spread. Inhalation of nasal secretions following prolonged close contact, as occurs in confined animals in the winter months, is a recognized mode of horizontal transmission. The virus is shed in semen, but infection by coitus or artificial insemination has not been documented. In utero transmission occurs rarely or never. Ovine lentivirus infects a variety of cell types, including choroid plexus epithelium, fibroblasts, endothelial cells, and monocytes. However, viral replication is restricted in these cells, and complete viral replication and assembly only occur in mature macrophages. Viral antigen is widespread, having been detected in lung, bone marrow, mammary gland, lymph node, spleen, synovium, brain, and spinal cord of sheep with maedi, and is most abundant in areas of lymphocytic inflammation. Similarly, viral nucleic acid is demonstrable in lung, liver, spleen, lymph nodes, brain, synovium, intestine, kidney, and thyroid gland of goats with CAE.

In contrast to primate and feline lentiviruses, *immunosuppression is not a feature of small ruminant lentiviral infection. Infection and shedding of the virus persist for life*, but the disease in sheep develops slowly and the incubation period usually exceeds 2 years. The mechanisms of persistent lentiviral infection are not clear, but may include proviral integration, lack of avidity of neutralizing antibody, or decoy viral antigens that are immunodominant but nonneutralizing. Integration of provirus into the host genome is a necessary feature for viral replication, occurs increasingly as the disease progresses, and may facilitate persistence of the virus. The development of serum neutralizing antibody responses during persistent infection is perplexing; the higher affinity of virus for macrophage receptors than for antibody has been suggested as a reason for failure of these antibodies to prevent cell-to-cell spread of infection in vivo.

The pathogenesis of the inflammatory infiltrates characteristic of small ruminant lentiviral infections requires further study. Infection of alveolar macrophages triggers production of proinflammatory cytokines that presumably directly or indirectly recruit and activate leukocytes in the area of infection, and may also incite fibrosis or smooth-muscle hyperplasia. Cytokine-dependent activation of macrophages stimulates lentiviral replication, which may in turn stimulate additional leukocyte recruitment. Whether lentiviral infection contributes directly to altered organ function, or whether this is an exclusive sequel to the immunoinflammatory response, remains enigmatic.

The lungs of **sheep** with maedi are remarkably heavy, pale gray or tan, and fail to collapse when the chest is opened. The lesions are generalized but most obvious in the caudal lobes, and vary from a diffusely firm or rubbery texture, to multiple coalescing gray and firm foci (Fig. 5.74). Mediastinal and bronchial lymph nodes are enlarged, white, and edematous. Lesions of cranioventral bronchopneumonia caused by *Pasteurella multocida* or *Arcanobacterium pyogenes* are commonly superimposed on the lesions of maedi. Lungworms – *Dictyocaulus* or *Protostrongylus* – are common in sheep with maedi, and an association between maedi and retroviral pulmonary adenomatosis has been described.

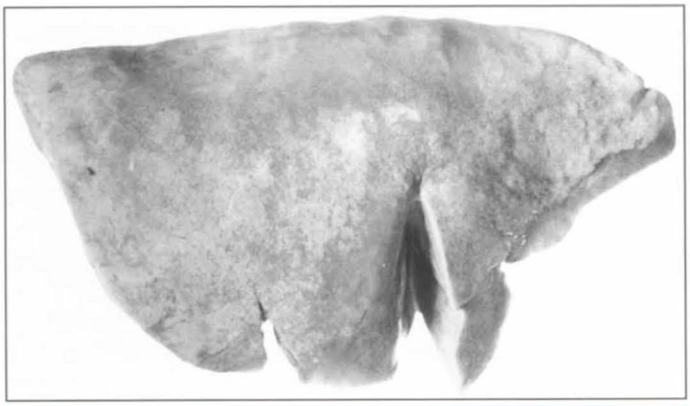

Figure 5.74 Maedi in a sheep. The lungs failed to collapse and have widespread gray-white mottled pallor.

The histologic pulmonary lesions are of interstitial pneumonia. Infiltrates of lymphocytes, plasma cells, and macrophages thicken alveolar septa and form cuffs around blood vessels and airways. The tendency for these cellular infiltrates to form *lymphoid nodules with germinal centers* is a characteristic feature of maedi. In addition to the cellular infiltrate, alveolar septa are thickened by *hypertrophy of smooth muscle* and by *mild interstitial fibrosis* (Fig. 5.75). Hyperplasia of type II pneumocytes is not prominent, in contrast to pulmonary adenomatosis and CAE viral pneumonia. Mild hyperplasia of bronchiolar epithelium is occasionally present.

Mastitis is an uncommon clinical presentation of VISNA infection, but is a frequent subclinical lesion. There is diffuse firmness and pallor of the mammary gland, and histologic lesions include follicular aggregates of lymphocytes, plasma cells, and macrophages in the mammary interstitium. Similarly, although clinical neurologic disease typical of visna is rare, histologic lesions of lymphocytic leukoencephalomyelitis, gliosis, demyelination, and meningitis are occasionally encountered in sheep with maedi, and tend to be most severe adjacent to the ependyma. Mesangial or proliferative glomerulonephritis is an uncommon lesion in VISNA-infected sheep.

The lung lesions of **goats** infected with CAEV are distinctive. The gross appearance is of multifocal coalescing pale firm foci throughout the lung. Histologically, these foci are abruptly demarcated from relatively unaffected areas of lung. In the lesional areas, alveoli are filled with densely eosinophilic fluid and lined by a continuous or patchy layer of cuboidal type II pneumocytes, and alveolar septa are thickened by lymphocytes.

Goats with *arthritis* due to CAEV commonly have unilateral or bilateral carpal lesions, but tarsal, fetlock, stifle, and atlanto-occipital joints may be affected. Histologic lesions include striking villus hyperplasia of the synovium, and synovial infiltrates of lymphocytes, plasma cells, and macrophages. Fibrosis, mineralization, and necrosis of synovium and joint capsule develop in chronically affected animals. The brain and mammary lesions of CAE are similar to those of sheep with maedi.

Serology has long been the mainstay of confirming a morphologic **diagnosis** of maedi, but is subject to several caveats. First, because the prevalence of infection is up to 90% in some geographic areas, serology may be more useful as a tool to detect infected flocks than to diagnose the cause of a respiratory disease problem. Second, although infection is lifelong, infected animals may become transiently seronegative, resulting in false-negative tests. Third, recently infected sheep may be seronegative, for development of an antibody titer may take several months. The agar gel immunodiffusion test is most commonly used, and has a sensitivity of 100% and specificity of 81% compared to the "gold standard" immunoprecipitation test. Recently developed enzyme-linked immunosorbent assays (ELISAs) have greater sensitivity, require less laboratory expertise, and may be more economical.

Assays that detect viral antigen or nucleic acid in tissues have been more recently developed for both maedi and CAE. Immunohistochemistry, using a panel of monoclonal antibodies to detect antigenic variants, has the advantage of associating the presence of viral antigen with the histologic lesion, but reagents are not yet widely available. In situ hybridization is a highly sensitive technique that also associates lesions with viral nucleic acid, but the cost and limited availability restrict its use as a diagnostic tool. Polymerase chain reaction (PCR) appears to be a promising method to detect proviral DNA or viral

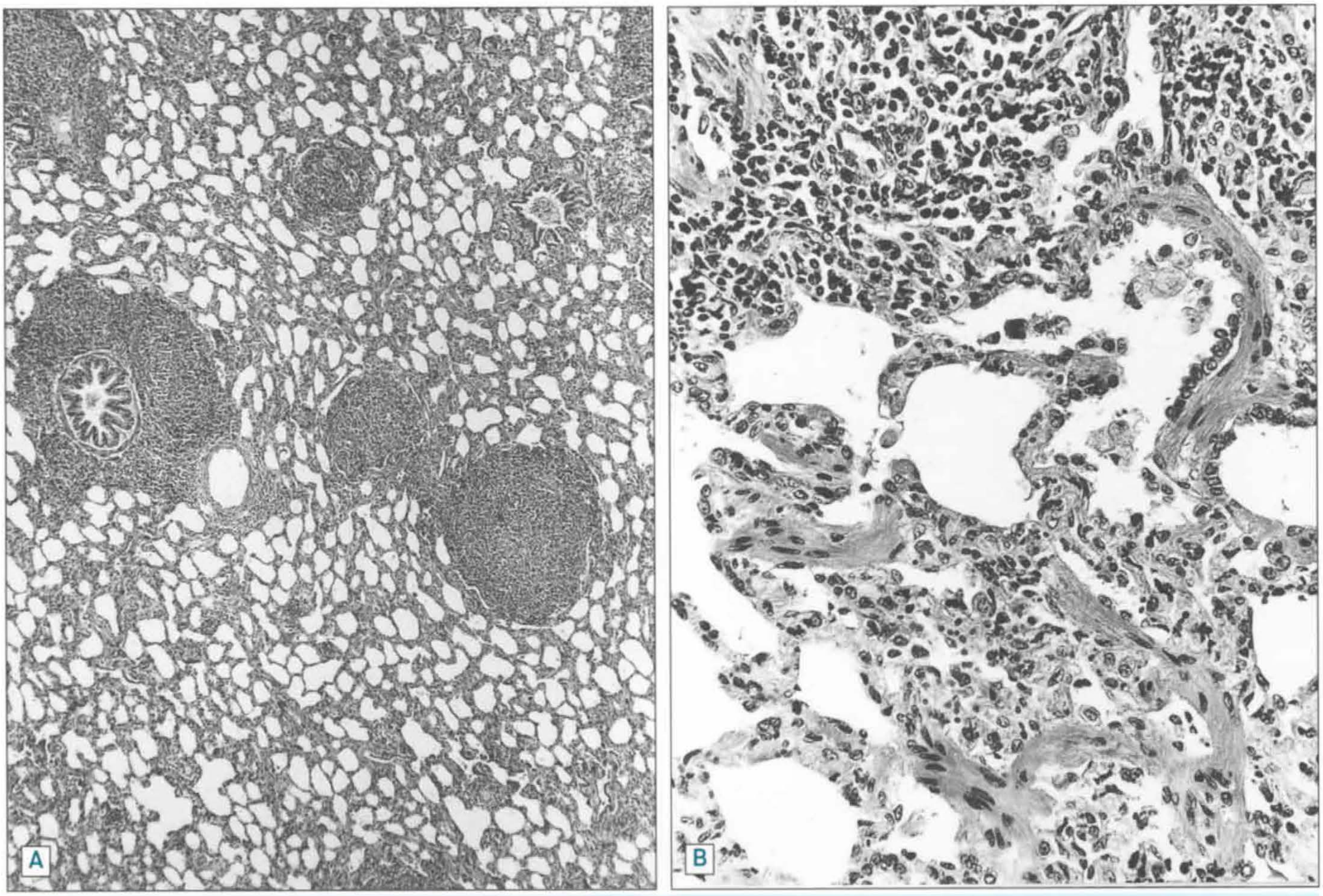

Figure 5.75 Maedi in a sheep. **A.** Prominent accumulations of lymphocytes around blood vessels and airways, with diffuse thickening of alveolar walls. **B.** Hyperplasia of smooth muscle of terminal bronchioles and alveolar septa.

RNA in blood mononuclear cells or lung tissue. Virus isolation from explant cultures of spleen, lung, or lymph nodes is as sensitive as current PCR assays, but is more costly and time-consuming.

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Enzootic nasal tumor of sheep

Viral adenomas and carcinomas of the nasal cavity of sheep and goats are geographically widespread, but absent from Australia and New Zealand, and there are regions with greater prevalence, including sheep in the USA, Canada, France, Germany, and Spain, and goats in India, Spain, and France. The disease may be enzootic, with multiple animals affected in certain flocks. Clinical disease typically arises in adult sheep and goats, but lambs as young as 6 months have been affected. Clinical signs are insidious, progressive, and may include stertor, inspiratory dyspnea, open-mouth breathing, nasal discharge, nasal deformity, and weight loss.

Enzootic nasal adenoma and carcinoma are associated with and probably caused by a betaretrovirus, named "Enzootic nasal tumor virus" (ENTV-1 from sheep, ENTV-2 from goats), which is closely related to, but distinct from Ovine pulmonary adenocarcinoma virus. The disease has been transmitted to neonatal goats using cell-free nasal fluids from affected goats or preparations of concentrated virus, with an incubation period of

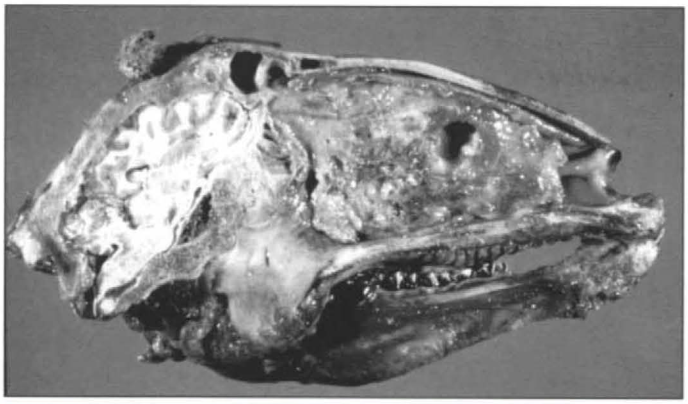

Figure 5.76 Enzootic nasal tumor in a sheep. A fleshy white tumor originating in the ethmoid region fills the nasal cavity.

12–24 months. The presence of multiple affected animals in some flocks suggests that the disease is contagious, presumably by contact with nasal secretions. The mechanisms whereby infection with ENTV leads to neoplasia have not been defined.

Tumors may be unilateral or bilateral and *usually originate from the ethmoid turbinates*, but fill much of the caudal nasal cavity before severe clinical signs develop (Fig. 5.76). In protracted unilateral cases, the entire nasal cavity is occluded by tumor, there is deviation of the nasal septum, and tumor may protrude from the nostril. Expansion into sinuses, contralateral nasal cavity, pharynx, or cranial vault may occur, but metastasis is rare. The neoplastic tissue is white, firm, and multinodular, or may contain brown-red areas of hemorrhage and necrosis.

The histologic appearance is of adenoma or low-grade adenocarcinoma. The cuboidal or pseudostratified nonciliated epithelial cells form orderly tubular, papillary, or acinar arrangements. Neoplastic cells have basal round nuclei and a variable mitotic rate. Mucus secretion is occasionally abundant, and the fibrovascular stroma is usually scant. Neoplastic tissue and adjacent nonneoplastic tissues often contain numerous lymphocytes, and glandular hyperplasia or polyps may be present.

The diagnosis is usually based on gross and histologic findings. Sporadic (nonviral) nasal tumors are apparently rare in small ruminants. Extracellular or budding retroviral particles may be demonstrated in neoplastic tissue by electron microscopy in 75–100% of cases. Other diagnostic methods include polymerase chain reaction and Western blot of neoplastic tissue.

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Ovine pulmonary adenocarcinoma (jaagsiekte)

Ovine pulmonary adenocarcinoma (OPA), also known as jaagsiekte or ovine pulmonary adenomatosis, is a contagious retroviral bronchioloalveolar carcinoma of sheep, and may rarely affect goats. The disease is common in South America, South Africa, and Scotland, where 5–20% of necropsy cases contain pulmonary tumors. OPA occurs regularly in the rest of Europe, Africa, and Asia, but is rare in North America and not reported in Australia. Mortality rates tend to increase gradually for several years following introduction of infection, and subsequently decline gradually. Clinical disease is most common in 2–4-year-old sheep, but 3-month-old lambs have also been affected. Clinical signs include progressive dyspnea, tachypnea, exercise intolerance, nasal discharge, coughing, and weight loss; fever and anorexia are unusual unless secondary infections occur. Drainage of lung fluids from the nose following elevation of the hindlimbs is a characteristic clinical sign seen in some sheep.

Ovine pulmonary adenocarcinoma virus (OPAV; Jaagsiekte sheep retrovirus), the agent of OPA, is a 100 nm diameter, enveloped, betaretrovirus. The following arguments support the role of OPAV as the etiology: OPAV is consistently present in neoplastic cells but not in nonneoplastic epithelium or stroma, the disease can be reproduced with cell-free OPAV-containing filtrates of tumor tissue, and the incubation period is dependent on the amount of retroviral reverse transcriptase in the experimental inoculum. However, defining the exact role of OPAV in disease development is impeded by the inability to isolate OPAV in cell culture without the use of tumor-derived cells, by the presence of closely related endogenous retroviral proviral sequences that are common in neoplastic and nonneoplastic tissue of affected and normal sheep, and by the occasional, albeit inconsistent, isolation of other viruses from tumors.

Many aspects of the pathogenesis of OPA remain nebulous. The disease is presumably acquired by direct contact with infected nasal secretions. The typical incubation period has been estimated to be 2 years, but is age- and dose-dependent; tumors may develop in 10–20 days if high doses of virus are administered experimentally to neonatal lambs. Disease is more common in certain breeds of sheep, but the reasons for this familial predisposition are unknown. Oncogenes have not been identified in OPAV, but the envelope protein is sufficient to induce similar tumors in immunodeficient mice, and is the likely mechanism of tumor formation in sheep. Acquired resistance to disease has been identified in endemically infected flocks, and this disease resistance has been attributed to immunotolerance as a result of expression of related endogenous retroviruses.

Lungs of sheep with OPA contain multiple, firm, raised, gray or white, moist masses in the cranial and middle lung lobes, with less extensive involvement of caudal lobes (Fig. 5.77). Affected lungs are heavy, up to three times their normal weight, and airways are filled with foamy secretion from the neoplastic cells. The tumors are expansile, do not commonly form intrapulmonary metastases, and metastases to regional lymph nodes are present in fewer than 10% of cases. Maedi and/or bacterial pneumonia are often present in sheep with OPA, and the diffuse lymphocytic interstitial pneumonia or cranioventral suppurative bronchopneumonia induced by these diseases may complicate the gross and histologic appearance of the tumors.

The histologic appearance is of a well-differentiated bronchioloalveolar carcinoma. Within the neoplastic masses, the alveolar architecture remains, but alveoli are lined by cuboidal or columnar neoplastic epithelial cells (Fig. 5.78). Proliferation of neoplastic cells results in papillary or acinar structures filling alveoli and obscuring their outline. Alveoli adjacent to the tumors are atelectatic and contain many macrophages, and interstitial fibrosis may be present in advanced cases. Neoplastic cells exhibit ultrastructural features of type II pneumocytes, including cytoplasmic lamellar bodies and surface microvilli; less commonly, the presence of dense core granules suggests a Clara cell phenotype.

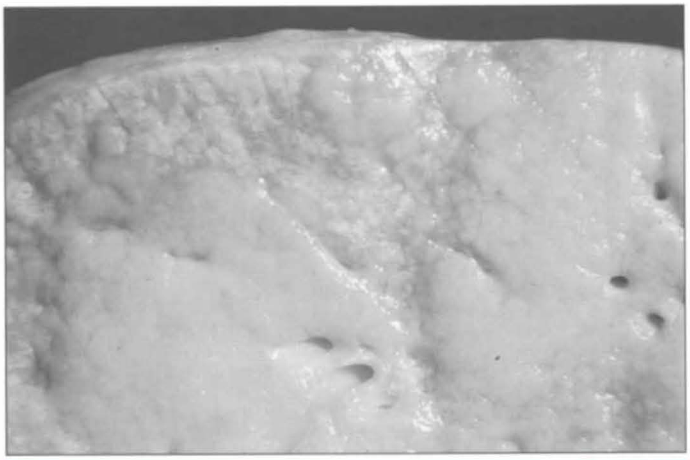

Figure 5.77 Ovine pulmonary adenocarcinoma (jaagsiekte) in a sheep. The lung contains irregularly sized, coalescing, bulging, white nodules.

The pulmonary lesions of OPA are characteristic, as spontaneous or noninfectious bronchioloalveolar carcinoma is rare in sheep. The disease may be confused with proliferative interstitial pneumonia, which may occasionally be multifocal rather than diffuse. Serologic tests are not sensitive, for antibody responses to the virus are minimal. A polymerase chain reaction assay appears promising as a method of confirming infection, and is able to differentiate OPAV from endogenous sheep retroviral sequences and *Enzootic nasal tumor virus*. Interpretation may be complicated by a high prevalence of infection in endemic areas. The assay would be of particular use in confirming the disease in geographic areas where jaagsiekte is rare, and in identification of infected sheep as a method of developing disease-free flocks.

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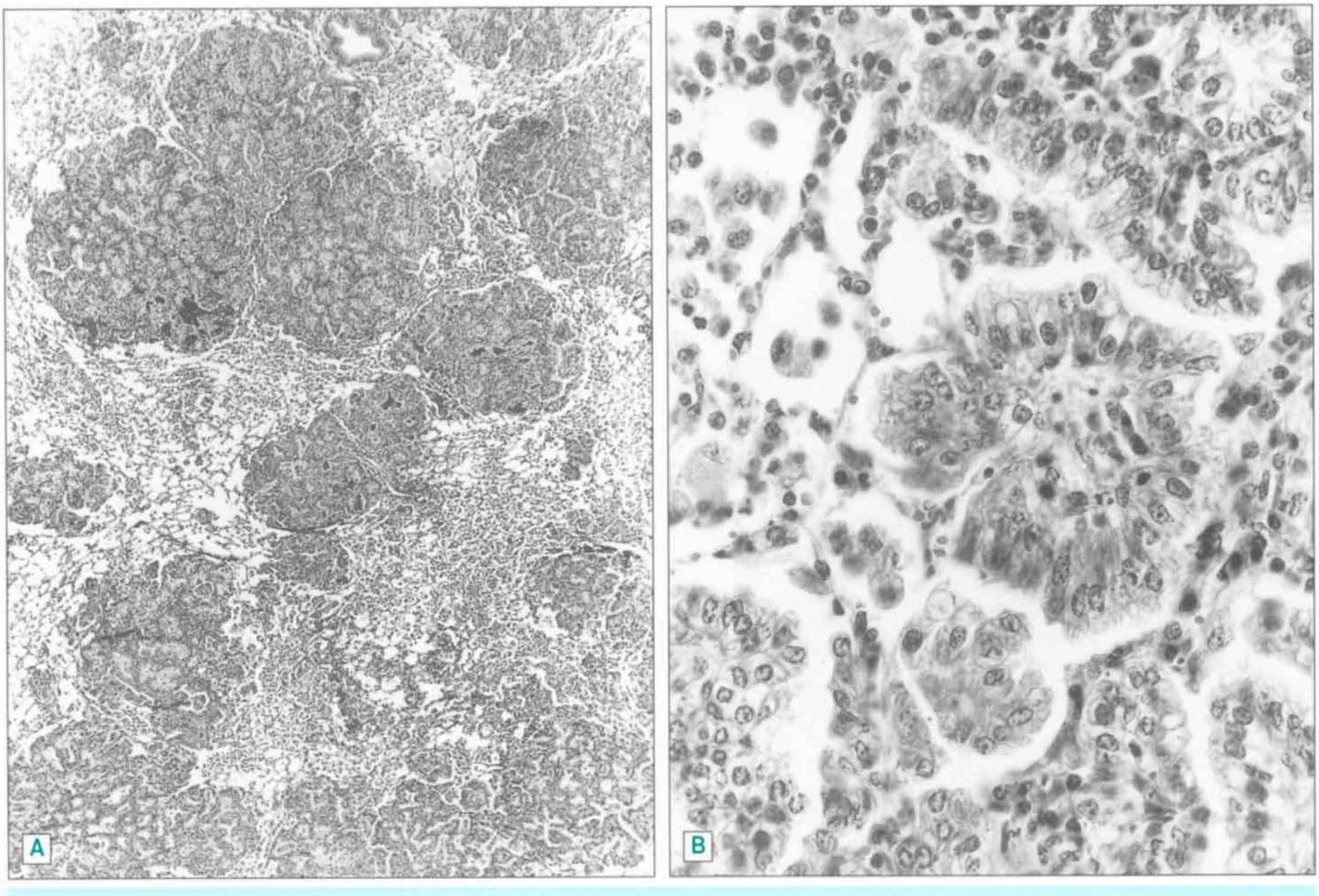

Figure 5.78 Ovine pulmonary adenocarcinoma. A. Multiple discrete tumor nodules with acinar and papillary patterns. **B.** Higher power showing columnar cells lining alveoli and forming papillae within the lumina. (Courtesy of K Perk and Adv Vet Sci 1982;26:267–287.)

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Bacterial pneumonia

Bacterial pneumonia in sheep is caused by *Mannheimia haemolytica*, *Pasteurella multocida*, or *Histophilus somni (Haemophilus agni)*. *M. haemolytica* can also cause septicemia in the absence of pneumonia in lambs less than 2 months of age. The causative agents, predisposing factors, and pathogenesis are in many respects similar to those described in cattle. Sudden climatic changes, gathering and handling, and predisposing infection with *Mycoplasma* spp., *Ovine* or *Bovine respiratory syncytial virus, Bovine parainfluenza virus 3*, or adenovirus are the most commonly recognized predisposing causes. The lesions are of hemorrhagic or fibrinonecrotic, lobar or lobular bronchopneumonia and serofibrinous pleuritis in acute cases, and fibrinopurulent bronchopneumonia leading to abscessation and fibrous pleural adhesions in subacute to chronic cases.

M. haemolytica causes sporadic cases and small outbreaks of acute pneumonia and pleuritis in goat kids. The disease usually occurs as a peracute disease in late autumn and winter. At necropsy, a sheet of yellow fibrin adheres loosely to the pleural surfaces. The cranial lobes of the lungs are firm and dark red with a transition to less-firm tissue with mottled gray-pink discoloration in the caudal lobes. Acute diffuse bronchopneumonia is characterized microscopically by neutrophils, protein-rich exudate, and numerous bacteria filling bronchioles and alveoli. There is usually little evidence of necrosis. In the caudal lobes, neutrophils fill the bronchioles and extend into the alveoli.

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Septicemic pasteurellosis

Septicemia caused by *Pasteurella trehalosi* (formerly *P. haemolytica*, biotype T) occurs mainly in weaned lambs during the fall months, but it can occur in other age groups and at other times of the year. Deaths, which seldom exceed 5% of the sheep at risk, usually follow within a few days of changes in pasture, feed, or other management practices.

Signs of illness are vague and the usual course is short, or lambs may be found dead with no opportunity to exhibit clinical signs. At necropsy, *petechial and ecchymotic hemorrhages* are usually present in subcutaneous tissues, particularly of the neck and thorax, in intermuscular fascia, and in the pleura, epicardium, and mesentery. Retropharyngeal and mesenteric lymph nodes are hemorrhagic and edematous. *Ulcerative lesions* covered by yellow plaques of fibrin and necrotic debris are common on the tongue, pharynx in the area of tonsillar crypts, larynx, and esophagus. The abomasal, intestinal and colonic mucosae occasionally have shallow ulcers (Fig. 5.79). The lungs are diffusely congested and edematous. *Multifocal hemorrhagic infarcts may be scattered throughout the lungs*, and the liver may contain pinpoint to 1 cm diameter yellow necrotic foci. There is occasionally inflammation of joints, pericardium, meninges, and choroid plexus, but these lesions are absent in peracute cases.

Microscopic examination of tissues reveals *widespread bacterial embolism*. Multifocal pulmonary lesions consist of masses of bacteria occluding capillaries, accompanied by hemorrhagic and fibrinous exudate into alveoli. A peripheral zone of necrotic leukocytes may be present. The pale hepatic foci consist of colonies of bacteria with a surrounding zone of ischemic necrosis. There may be thrombosis of the adjacent tributaries of the portal vein and small amounts of parenchymal necrosis, but the leukocyte response is generally slight. This is probably related to the short course of the disease and perhaps to the effects of bacterial toxin on leukocytes. Bacterial emboli are regularly found in the spleen and adrenals, occasionally in the kidney, but are rare in other organs. Masses of bacteria adhere to the surface of the pharyngeal ulcers and occlude underlying blood vessels and lymphatics, and probably represent the *principal site of bacterial proliferation and systemic invasion.*

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Melioidosis

Melioidosis is a systemic infectious disease affecting a wide range of animal species in tropical climates. The disease is endemic in southeast Asia and northern Australia, is probably common in the Indian subcontinent and the Caribbean, and also occurs in southern Africa and the Middle East. The prevalence is highest in wet seasons, and causes acute outbreaks or chronic endemic disease. Clinical signs are variable and reflect the range of organs affected, and case fatality rates are high. Melioidosis is most common in goats, sheep, pigs, and rodents, and is an emerging tropical disease of humans. Horses, deer, camels, and laboratory animals are less commonly affected, while dogs, cats, cattle, water buffalo, and fowl are resistant to the disease unless they are immunosuppressed.

Melioidosis is caused by *Burkholderia (Pseudomonas) pseudomallei*, a facultatively anaerobic gram-negative bipolar-staining bacillus. Infection is acquired from contaminated soil or water, by inhalation, ingestion, or inoculation of cutaneous wounds. *Acute disease*, most common in young animals, may initially affect the lungs with later spread to other organs, or be primarily septicemic from the site of inoculation. *Chronic disease* is the more frequent manifestation, in which abscesses in multiple organs cause chronic nonspecific illness or are an incidental finding of public health importance at slaughter.

Virulence factors of *B. pseudomallei* include the iron-scavenging protein malleobactin, secreted proteases that degrade host tissues, capsular polysaccharide that confers resistance to killing by phagocytes, lipopolysaccharides, and an endotoxin-derived polysaccharide that allows serum resistance. The bacterium survives within

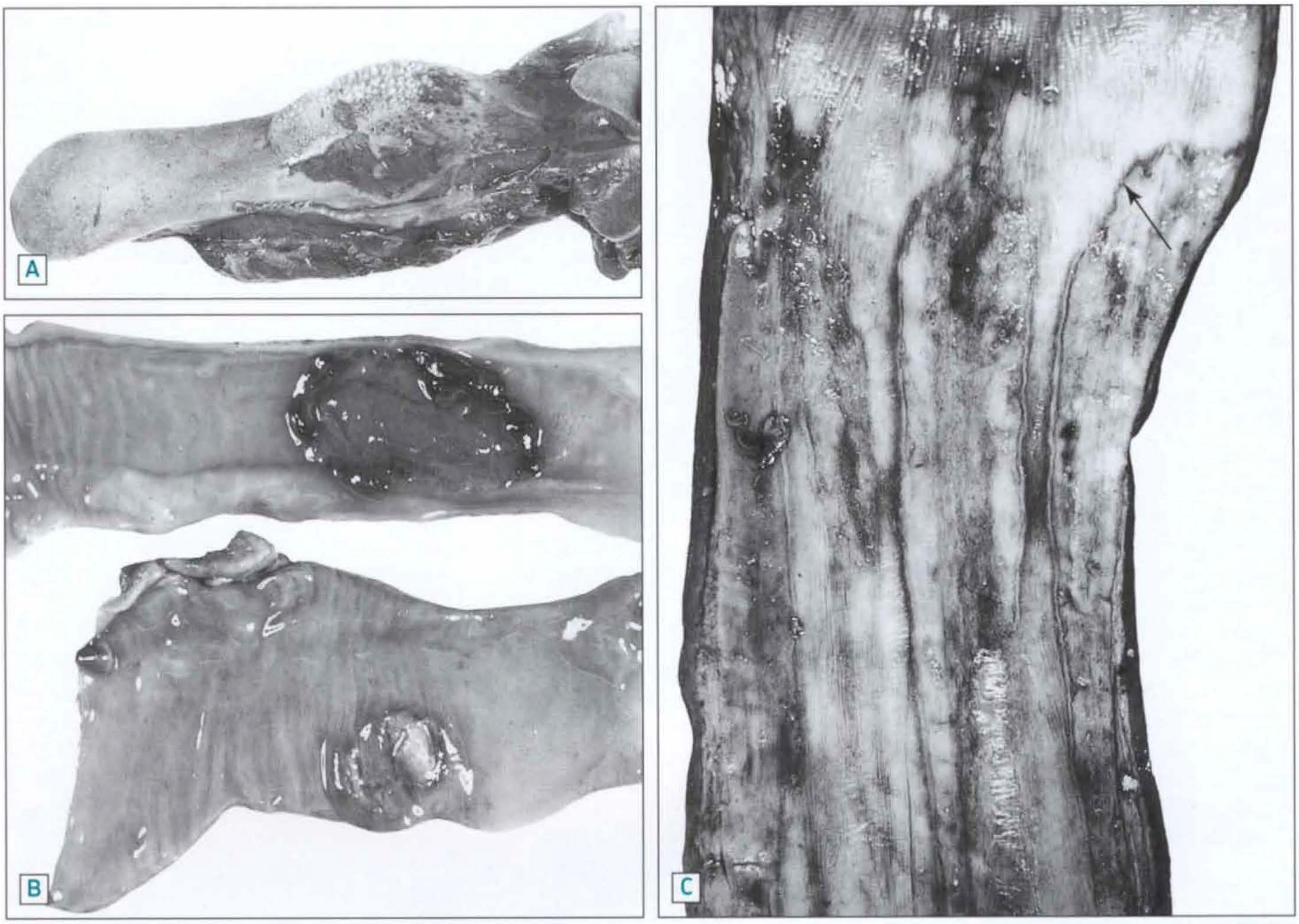

Figure 5.79 ***Pasteurella trehalosi* septicemia** in a lamb. **A.** Ulceration of tongue. **B.** Ulceration of intestine. **C.** Ulceration of esophagus. Only small areas of intact esophageal epithelium remain (arrow).

macrophage phagolysosomes and elicits a granulomatous and suppurative tissue reaction. The bacterial antigens that confer protective immunity are of current interest to human vaccine production; preliminary evidence suggests flagellar proteins and O-polysaccharides as promising targets of protective immune responses.

Melioidosis causes abscesses in multiple organs. The lungs are most consistently affected, with disseminated coalescing nodules, or locally extensive areas of consolidation. Abscesses also affect the spleen, liver, lymph nodes, kidney, or joints, and may occur in any visceral tissue (Fig. 5.80). Lesions are particularly widespread in goats, and may include mastitis or aortic aneurysm. Neurologic disease is most common in goats; gross lesions are rare, but multifocal aggregates of neutrophils and lymphocytic perivascular cuffs are present in the brainstem and spinal cord. Nodular lesions in the nasal mucosa may be mistaken for glanders. Endometritis and placentitis are important manifestations in cattle.

The abscesses of melioidosis are not distinctive in their appearance. *The chronic lesions are encapsulated nodular masses, up to 5 cm diameter, with creamy or caseous yellow centers.* Multifocal aggregates of neutrophils form within 12 hours of infection. Granulomas, present by 3 days, form nodular aggregates of epithelioid macrophages and lymphocytes, and these develop central areas of caseous necrosis and neutrophil infiltration.

The gross and histologic appearance of melioidosis is not pathognomonic. Caseous lymphadenitis, glanders, and abscesses caused by other bacteria may have a similar appearance, and the brain lesions may be mistaken for listeriosis. A tentative diagnosis may be based on identifying gram-negative bipolar-staining bacilli in sections or tissue smears, but *definitive diagnosis requires culture.*

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Chlamydial diseases

Intratracheal challenge of lambs with *Chlamydophila psittaci* induces mild infiltration of neutrophils and macrophages into alveoli and alveolar septa, and mild proliferative alveolitis. Lesions were most prominent adjacent to terminal bronchioles. Moderately sized cuffs of lymphocytes are present around bronchioles and small blood vessels at the height of the lesion. The chlamydiae are mostly destroyed during the acute phase of inflammation, which then subsides. Resolution can

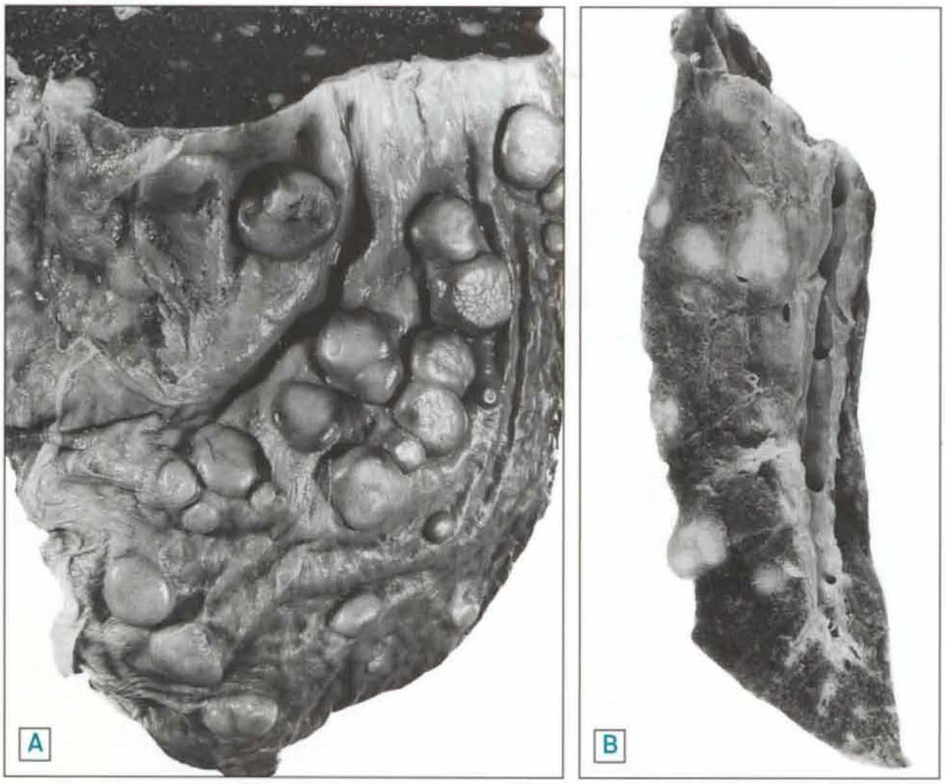

Figure 5.80 Melioidosis in a sheep. **A.** Splenic abscessation. **B.** Chronic pulmonary abscessation. (Courtesy of WT Hall and Queensland Department of Agriculture.)

be complete within 3–4 weeks of experimental infection. Chlamydiae may contribute to some cases of enzootic pneumonia in lambs and calves, in combination with other agents, but are probably of *limited importance as primary pulmonary pathogens.*

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Mycoplasmal diseases

Contagious caprine pleuropneumonia

Contagious caprine pleuropneumonia (CCPP) was first described in Africa in the late 1800s and remains an important disease in northern and western Africa, the Middle East, and the Indian subcontinent. The difficulty in isolating the causative agent in culture caused much confusion in the early literature, but CCPP is now defined as a specific disease of goats caused by *Mycoplasma capricolum* ssp. *capripneumoniae*. Although other mycoplasmas may cause similar lesions, these are not considered CCPP. Classical CCPP caused massive outbreaks with high morbidity and mortality in naive goats of all ages. *Currently, CCPP, an OIE list B disease, causes endemic sporadic disease in some countries and intermittent epizootics in others.* Affected goats are lethargic, dyspneic, and febrile, have bouts of coughing, but may continue to eat. Young goats may be lame due to polyarthritis.

Mycoplasma capricolum ssp. *capripneumoniae* (formerly *Mycoplasma* F38) is phylogenetically related to, but distinct from, *M. mycoides*. The virulence factors of this pathogen have not been well defined. Subclinical carriers are important sources of infection, and are the usual source of introduction of the disease into naive herds. Stress may trigger shedding of organisms from subclinically infected animals. Individual goats are infected by inhalation of droplets through close contact with coughing goats. The infection is restricted to the respiratory tract, and does not cause disease in species other than goats.

Pulmonary lesions are often unilateral but may be bilateral. The pleura is covered by a thick layer of fibrin, and serous fluid fills the pleural cavity. Lobular areas of consolidation and purple-gray discoloration are present in the lung, either diffusely or forming focal lesions, and these may contain extensive areas of necrosis or have a dry granular texture. Fibrinous pericarditis is common. Chronic cases develop focal nodules of necrosis and mineralization with a fibrous capsule, or lung abscesses if secondary bacterial infection develops. However, in contrast to contagious bovine pleuropneumonia, *sequestra and interlobular edema are not features of the caprine disease.* Histologically, the exudate in alveoli and interlobular septa varies from serofibrinous to fibrinopurulent. Thrombosis and vasculitis are often present. With time, the fibrinous exudates organize to form a mass of fibrous tissue.

Gross lesions are suggestive, but not diagnostic. Other mycoplasmas – particularly *M. mycoides* ssp. *mycoides* (large colony type), and *M. mycoides* ssp. *capri* – may cause epidemics with similar lung lesions. Features that suggest CCPP rather than other mycoplasmas include the absence of interlobular edema and inflammation, and the absence of mastitis, arthritis, or keratitis. Laboratory examination is required for definitive diagnosis.

M. capricolum ssp. *capripneumoniae* is an extremely difficult organism to propagate in culture, although it can often be recovered from the pleural fluid and consolidated lung of acutely ill animals. Improved isolation techniques are under development, and culture remains the gold standard for regulatory purposes. Detecting mycoplasmal nucleic acid using the polymerase chain reaction on samples of pleural fluid is a more rapid and sensitive method of diagnosis. A latex agglutination test is an effective method of serodiagnosis.

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Other mycoplasmal pneumonias

Many mycoplasmas cause disease in sheep and goats throughout the world, including *M. mycoides* ssp. *mycoides* large colony type (LC), *M. mycoides* ssp. *capri*, *M. capricolum* ssp. *capricolum*, *M. putrefaciens*, *M. agalactiae*, and *M. ovipneumoniae*. *M. capricolum* ssp. *capripneumoniae*, the cause of contagious caprine pleuropneumonia (CCPP), is discussed above. *M. agalactiae* causes contagious agalactia of sheep and goats, but may also induce respiratory disease. Minimally pathogenic mycoplasmas in small ruminants include *M. arginini*, *M. auris*, *M. cottewi*, and *M. yeatsii*. Transmission requires close contact with infected animals. Young animals are often infected by ingestion or inhalation of contaminated milk.

These agents cause a diverse spectrum of clinical signs and lesions. *Mastitis and agalactia are common in adults, and may be accompanied by arthritis or keratitis. Kids and lambs more commonly develop pneumonia or pleuropneumonia, fibrinopurulent polyarthritis, or septicemia with fibrinous polyserositis and interstitial pneumonia.* The lung lesions may resemble those described above for CCPP, but thickening of interlobular septa by edema and fibrin exudation is not typically present in CCPP. *M. mycoides* ssp. *mycoides* LC is particularly capable of causing pleuropneumonia, polyarthritis, and septicemia in goat kids and to a lesser extent in lambs; the worldwide distribution of this pathogen is in contrast to those of contagious caprine and bovine pleuropneumonia. *M. capricolum* principally causes fibrinopurulent polyarthritis in kids, but may also cause septicemia in kids and mastitis in does.

Mycoplasma infections contribute to some cases of *enzootic pneumonia in lambs and kids*. Although the strains of mycoplasma isolated from these animals may not be highly pathogenic, the additional contributions of stress, poor air quality, adverse weather conditions, respiratory syncytial or parainfluenza viral infections, and secondary bacterial pneumonia combine to cause disease. Consolidation and rubbery texture of the cranioventral lung are typical gross features. Histologic lesions of catarrhal bronchitis and bronchiolitis with prominent lymphocyte infiltration in and around small airways should suggest the presence of mycoplasmas, but *diagnosis requires culture of lesional lung tissue*.

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Parasitic diseases

Oestrus ovis

Oestrus ovis adult flies deposit first-stage larvae on the nares. The larvae molt twice as they *migrate through the nasal passages*. Larvae may become incarcerated in sinuses or recesses of turbinates as they grow, and eventually die there. Development in the nasal passages can take up to 10 months, although larvae deposited early in summer are able to mature in that season. The full-grown larvae are about 3 cm long, with black oral hooks, dorsal dark transverse bands, and ventral rows of small spines. Pupation occurs on the ground.

The larvae attach to the mucous membrane by their mouthparts. They produce mucosal defects at the points of attachment and, since the cuticle is spinous, cause irritation as they wander. An immune response against excreted or secreted larval antigens, and the ensuing eosinophil and mast cell response, may also contribute to nasal inflammation and irritation. Affected sheep develop catarrhal rhinitis and sinusitis. Apart from persistent annoyance and the debility that this may cause, *there are seldom untoward effects of the parasitism*. Rarely, larvae penetrate the cranial cavity, or secondary bacterial infections spread from the olfactory mucosa to the meninges. Mild infestations of *O. ovis* occur in goats and dogs, as well as conjunctival infections in humans.

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Muellerius capillaris

Muellerius capillaris is the most common and ubiquitous lungworm of sheep and goats (Table 5.8). There is usually no clinical evidence of respiratory disease in sheep, although experimental infections may cause hyperpnea, impaired pulmonary gas exchange, and reduced weight gains. Heavy infestations are purported to predispose to bacterial and viral infections of the lung.

Muellerius capillaris, also known as the *small lungworm of sheep and goats*, is a nematode of the superfamily Metastrongyloidea, family Protostrongylidae. Adults, which are 12–24 mm long and thread-like, live in nodular lesions in the alveoli and rarely in bronchioles, but are usually not visible at necropsy. Eggs are laid and rapidly hatch, and first-stage larvae are coughed up, swallowed, and passed in the feces. In

Table 5.8 Major lungworms of sheep and goats

Lungworm	Gross features	Histologic features
Muellerius capillaris	Pale nodules Worms are microscopic	Adults and larvae mainly in alveoli Alveolar fibrosis ± granulomatous inflammation
Cystocaulus ocreatus	Dark nodules Hair-like worms	Adults and larvae in alveoli and ***small*** bronchioles
Dictyocaulus filaria	Focal atelectasis or consolidation ± emphysema Thread-like worms in bronchi	Bronchi and ***large*** bronchioles contain adult worms. Lymphoplasmacytic or eosinophilic inflammation, and smooth muscle and epithelial hyperplasia
Protostrongylus rufescens and *Neostrongylus linearis*	Angular nodules Adults grossly visible in parenchyma and smallest bronchi	Adults in ***terminal*** bronchioles Granulomatous and/or eosinophilic bronchiolitis, with prominent peribronchiolar lymphofollicular cuffs

some cases, each nodule in the lung may contain adult worms of only one sex. The infection in such cases is sterile, and larvae will not be detected in feces. The intermediate hosts are various slugs and snails. The infective stage is reached after two molts in the intermediate host, and the life cycle is completed when sheep and goats swallow the intermediate hosts. The larvae migrate to the lungs, presumably via the lymphatics, and break out into the alveoli. As a consequence of this type of life cycle, infections are gradually acquired, and large worm burdens are seldom observed in animals <6 months of age. On the other hand, heavy infections are not common in old sheep and goats, as repeatedly infected animals become resistant.

The characteristic finding is multiple, 1–20 mm diameter (usually 2–4 mm), subpleural nodules in the dorsal regions of the caudal lobes (Fig. 5.81). However, they may occur anywhere in the lung, and occasionally in the regional lymph nodes. The nodules are soft and hemorrhagic in the acute stages, corresponding to histologic lesions of hemorrhage and eosinophil infiltration. However, most necropsy cases represent chronic infestations with raised gray-pink or yellow nodules that may mineralize.

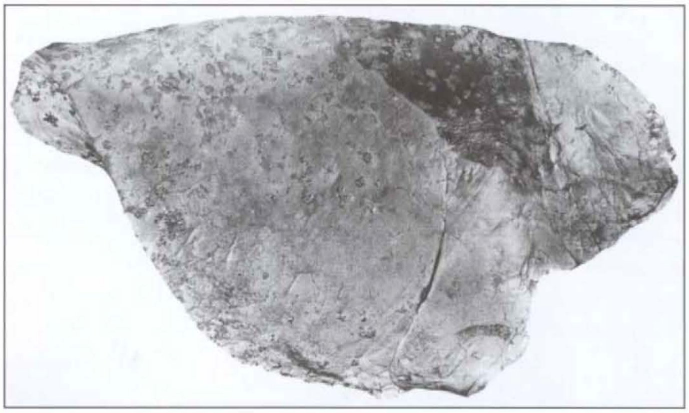

Figure 5.81 ***Muellerius capillaris* infection** in a sheep. Multifocal subpleural nodules of interstitial pneumonia, mainly in the dorsocaudal aspects of the lung.

The nodules are formed by masses of adult worms, embryonated eggs, and coiled larvae. The adults have polymyarian-coelomyarian musculature, the intestine is composed of few multinucleate cells with an indistinct brush border, and the uteri often contain embryonated eggs or larvae. The inflammatory response is often minimal, although alveolar septa may be thickened by fibrous tissue, lymphocytes, and smooth-muscle hyperplasia. In older animals or chronic infestations, presumably associated with developing resistance, the cellular reaction is more marked and targets the first-stage larvae and adults. Eosinophils aggregate around the larvae; the alveolar spaces contain macrophages, eosinophils, and giant cells. Larvae that escape into small bronchioles are enclosed in plugs of mucus and cellular debris. When larvae leave the nodules, the cellular reaction subsides, but thickening of the alveolar septa persists because of patchy or diffuse fibrosis. Adult worms incite a similar reaction of eosinophils, macrophages, and giant cells. *The cellular debris becomes mineralized, particularly when the worms die, and these mineralized nodules persist indefinitely as spherical masses of calcium salts surrounded by a fibrous capsule.*

The gross appearance of *Muellerius* infestation can closely resemble that of ***Cystocaulus***. However, the nodules of *Cystocaulus* are darker and may be larger than those of *Muellerius*, the adult worms may be grossly visible, and bronchial epithelial hyperplasia is more likely in *Cystocaulus* infestation. In ***Dictyocaulus*** and ***Protostrongylus*** infections, the adult worms are located in large bronchioles/bronchi and the terminal bronchioles, respectively.

Muellerius infestation in **goats** has been associated with severe diffuse interstitial pneumonia in the absence of nodular lesions. Histologic lesions included diffuse thickening of alveolar septa with mononuclear cells, and fibromuscular hyperplasia in alveolar septa; but the possibility that these lesions might have been caused by *Caprine arthritis encephalitis virus* or *Mycoplasma* infection cannot be ruled out.

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Protostrongylus rufescens

Protostrongylus rufescens is a nematode of the superfamily Metastrongyloidea, family Protostrongylidae that infects sheep, goats, and deer. The adults are 16–35 mm in length, red, and mainly inhabit the terminal bronchioles. Eggs hatch to first-stage larvae in the lung, and are passed in the feces. They enter intermediate hosts, which are various genera of terrestrial snails, by boring through the foot. Two molts occur in the snail and the infective third-stage larvae develop in 46–49 days. Sheep and goats eating the snails are infected by the larvae, which pass by way of the mesenteric lymphatics to the lungs.

Lesions consist of 2–4 cm diameter, angular, firm, tan, nodules distributed throughout the lungs. The nodules are usually soft, in contrast to the firm texture of *Muellerius* nodules. Cross-sections may reveal fine white worms exuding from the parenchyma or occasionally leading to a small bronchus. Histologically, an eosinophil inflammatory reaction targets adult worms, larvae, and eggs in the terminal bronchioles. Prominent lymphofollicular aggregates surround these airways. Smooth-muscle hyperplasia is not a feature of *Protostrongylus* infestation.

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Dictyocaulus filaria

Dictyocaulus filaria, the *large lungworm of sheep and goats*, is a slender, white, 3–10 cm long nematode that inhabits mainly the small bronchi. The life cycle and lesions are similar to those described for *D. viviparus*, which is described in detail with respiratory diseases of cattle. The lesions are localized, 3–4 cm diameter areas of atelectasis or consolidation that are most common in the dorsal aspects of the caudal lobes. Bronchi are prominently thickened by smooth muscle and cuffs of lymphocytes and granulocytes, and often contain adult thread-like worms. A granulomatous alveolar reaction to aspirated eggs may occur.

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Miscellaneous parasites

Neostrongylus linearis, a comparable nematode to *Protostrongylus rufescens*, is common in western Europe, the Middle East, and probably elsewhere. The adults are 5–15 mm long, and terrestrial gastropods are the intermediate hosts. The worms are contained within 1–4 mm diameter, red-violet or gray-pink nodules that are most numerous in the caudal lobes.

Cystocaulus ocreatus (*C. nigrescens*) reside in 5–20 mm diameter, dark, firm nodules in the dorsocaudal lung. Cross-sections may reveal brown-black hair-like worms, which move when the lung section is immersed in saline. The life cycle resembles that of *Muellerius*.

Mammomonogamus (*Syngamus*) ***nasicola*** and *M. laryngeus* are nematodes of the nasal cavity and larynx. ***Schistosoma nasalis*** causes nasal granuloma in India. ***Habronema*** spp. is a rare cause of fibrotic nodules adjacent to bronchioles. In sheep, the lung is a common site of hydatid cysts caused by ***Echinococcus granulosus***.

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Infectious respiratory diseases of horses

Viral diseases

Equine influenza

Equine influenza virus infection is widespread in most intensively managed horse populations, and causes outbreaks of disease when young naive horses are mingled at races and shows, particularly in the autumn and winter months. As well, epizootics of influenza occur when new antigenic variants of influenza viruses emerge. Serologic studies suggest that 19 and 24% of horses with upper respiratory disease seroconvert to **A/equi/1** and **A/equi/2** influenza viruses, respectively, in the 28 days after illness. As for influenza in swine and humans, *the disease in horses is usually characterized by high morbidity and low mortality, unless secondary bacterial pneumonia develops*. However, introduction of certain strains of A/equi/2 influenza virus into naive populations has caused outbreaks in which most horses became ill and up to 35% died. Most outbreaks spread rapidly through groups of horses, and are characterized by acute onset of fever of less than 4 days' duration, severe nonproductive cough, mucopurulent nasal discharge, lethargy, and anorexia. Dependent edema is an uncommon finding. Although most clinical signs resolve within 1–2 weeks, coughing may persist for weeks or months.

General features of orthomyxoviruses are described with swine influenza. H7N7 influenza virus, designated A/equi/1, was an important cause of equine respiratory disease in the 1950s, but has not been isolated since 1978. Nevertheless, the continued presence of antibody titers suggests that this virus continues to cause subclinical infections. **H3N8** subtypes, designated A/equi/2, were first isolated in 1963, and the propensity for antigen drift due to genomic mutation has resulted in periodic epizootics since that time.

Risk factors include close confinement, transportation, training, and mixing of animals. These factors probably increase the chance of contact between naive and subclinically infected animals, and reduce innate and acquired immunity. Infection is acquired by inhalation of infected aerosols. Viral replication is most extensive in epithelial cells of the upper respiratory tract and trachea, where infective viral particles bud from the plasma membrane within 2–4 days after infection. Influenza reduces mucociliary clearance both by impairing ciliary beating and by inducing necrosis of infected cells, and may also impair alveolar macrophage function; the ensuing bacterial pneumonia is responsible for many of the fatal cases. Influenza seems to favor the subsequent development of heaves, by increased reactivity of the airways to irritant stimuli and/or by increased antigen exposure in the lung as a consequence of reduced mucociliary clearance. Epithelial repair may take 3 weeks.

The surface hemagglutinin is the immunodominant antigen, and inactivated vaccines induce short-lived protection that correlates with induction of antibody to this antigen. Vaccinated horses may be infected and shed virus, but have a milder and shorter clinical course. There is no cross-protection between A/equi/1 and A/equi/2 subtypes, and incomplete cross-protection between strains of A/equi/2; consequently, it is essential to include currently circulating viral strains in vaccines. Horses that recover from natural infection have more prolonged resistance to disease, for at least 1 year, but in this case resistance is not closely correlated to antihemagglutinin antibody titers. The role of innate immunity and cellular immune responses in defense against equine influenza are uncertain.

Gross and histologic lesions of uncomplicated equine influenza have not been adequately described, as horses rarely die in the acute stages of disease. Occasional cases in foals have extensive pulmonary lesions, with necrosis of bronchiolar epithelium and infiltration of lymphocytes and neutrophils. Rarely, severe viral infection causes damage to brain, heart, gastrointestinal tract, kidney, and other parenchymal organs as well as severe ocular and pulmonary lesions.

In nonfatal cases of upper respiratory infection, **diagnosis** is based on demonstrating *seroconversion* and *virus isolation* from nasal swabs. Isolation of influenza virus is only reliable during the phase of pyrexia in the first day or two of illness. In the rare cases that are acutely fatal, virus may be demonstrated in lung by isolation or by immunohistochemistry.

Equid herpesvirus 4 and *Equine rhinitis A virus* (*Equine rhinovirus-1*) are the other major causes of upper respiratory infections in horses, in addition to *Equine arteritis virus*, *Equid herpesvirus 1*, adenovirus, and perhaps mycoplasmas. Serologic investigation and virus isolation are recommended methods of differentiating these pathogens. Bronchiolar necrosis is an uncommon lesion in horses; other causes include bronchointerstitial pneumonia of foals, and toxic injury to bronchiolar epithelium.

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Equid herpesvirus

Equid alpha-herpesviruses (EHV) cause upper respiratory infections, abortion, systemic disease of neonates, and neurologic disease. The latter three manifestations are described in more detail elsewhere. Systemic disease of yearling horses or adults is rare, but notable for the high mortality rates that may occur. Herpesvirus infections of the upper respiratory tract are most common in foals less than a year of age. Affected animals are febrile with serous or mucopurulent nasal discharge, but coughing may not be present. Infection is rarely fatal, unless secondary bacterial pneumonia develops.

Upper respiratory infections may be caused by EHV-1 or 4, although the latter is more common in some surveys. Viral replication occurs in the nasopharynx and associated lymphoid tissue, and the development of viremia is dependent on the strain of the virus and prior exposure of the host. Virus is shed from nasal secretions starting 2–10 days after infection. Viral shedding commonly occurs only during the phase of pyrexia, but is occasionally prolonged up to 3 weeks after infection.

The low mortality rate in the absence of secondary bacterial infection is fortunate from a clinical standpoint, but has resulted in a paucity of descriptions of the naturally occurring lesions of the disease. Serous or catarrhal rhinitis and conjunctivitis are obvious clinically, and suppurative exudate may form as bacterial infection establishes. Intranuclear inclusions are apparently rare in postnatal respiratory infections. Definitive **diagnosis** of EHV-1 or 4 infection of the upper respiratory tract depends on *isolation of virus* from nasal swabs from febrile animals, or demonstrating *seroconversion* in paired serum samples.

EHV-1 may cause systemic disease in live-born neonates. Lesions are similar to those in aborted foals, described in Vol. 3, Female genital system. These lesions include bronchointerstitial pneumonia, multifocal necrosis in liver, spleen, adrenal, and other tissues, and prominent intranuclear inclusion bodies. EHV-1 occasionally causes systemic disease in yearling or adult horses. Lesions include pulmonary or systemic necrotizing vasculitis, pulmonary edema and hemorrhage, lymphoid necrosis, and encephalomyelitis with vasculitis.

Recently, a gamma-herpesvirus has been associated with lesions of necrotizing bronchiolitis, increased numbers of macrophages and neutrophils in alveoli, interstitial fibrosis, and syncytial cells in alveoli. Confirmation of a causal relationship between the gamma-herpesvirus infection and the lesions of bronchointerstitial pneumonia is pending.

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Adenovirus

Equine adenovirus infection is widespread in horses. Adenoviral pneumonia is a well-described complication of inherited combined immunodeficiency in young Arabian foals, but the adenoviral disease has become rare as this genetic immunodeficiency becomes less common. At necropsy, there is a lobular or confluent pattern of atelectasis and/or consolidation of the cranioventral lung. In the early stage of severe infection, there is extensive necrosis and sloughing of bronchiolar epithelium. Later, bronchiolar epithelium is hyperplastic, and swollen superficial epithelial cells contain amphophilic intranuclear inclusion bodies. Occasionally, there are intranuclear inclusions in alveolar epithelial cells and increased numbers of macrophages and neutrophils. Secondary bacterial pneumonia or *Pneumocystis* pneumonia may also be present in these immunocompromised foals.

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Hendra virus

Hendra virus (HeV) was first described in 1994 in Australia, as a cause of fatal pulmonary disease of horses. The virus also causes disease in humans, cats, and guinea pigs. Subclinical infections in fruit bats (flying foxes) are likely sources of infection for other mammals. HeV and the related *Nipah virus* are members of the genus *Henipavirus*, family Paramyxoviridae. *HeV has tropism for endothelial cells, and many of the lesions arise from vascular injury.* Viral particles are detectable in the cytoplasm of endothelial cells. Gross lesions in horses include prominent *pulmonary edema and congestion* with dilation of subpleural lymphatic vessels, petechial hemorrhages, and abundant tracheal froth. The most prominent histologic lesion is serofibrinous fluid and increased numbers of macrophages in alveoli. Closer inspection may reveal subtle vascular lesions in the lung, heart, glomeruli, and stomach, consisting of thrombi in capillaries and/or necrotic debris and hemorrhage in the walls of arterioles. The presence of syncytial cells in small vessels is a unique but inconsistent lesion. The *differential diagnosis* for massive pulmonary edema includes African horse sickness, heart failure, and anaphylaxis.

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Bacterial diseases

Rhodococcus equi

Rhodococcus equi is an important cause of pneumonia and occasionally enteric disease in 2–4-month-old foals, and may affect animals up to 6 months of age. The prevalence of the disease is highly variable between farms, and probably reflects the level of contamination of the environment. Environmental factors favoring the disease include hot seasonal temperatures in temperate climates, neutral soil pH, and repetitive high-density stocking of dry paddocks. The clinical presentation may be either acute or insidiously progressive, although the infection and lesions are invariably chronic by the time clinical signs are noted. Affected foals usually develop disease in the summer, and are presented with pyrexia, tachypnea, dyspnea, cough, and mucopurulent nasal discharge. *Because of the chronic nature of the disease, case fatality rates are high in untreated foals.* Animals with the *colonic form* of disease may have diarrhea, weight loss, ascites due to hypoproteinemia, and occasionally colic.

Foals with bronchointerstitial pneumonia, described in detail elsewhere, frequently have concurrent *R. equi* infection, yet the relationship between these conditions remains obscure. Foals with *R. equi* pneumonia occasionally have histologic evidence of *Pneumocystis carinii* infection of the lung.

R. equi is a gram-positive facultative intracellular pathogen, distantly related to mycobacteria, which grows well in soil and in horse manure. *Pulmonary infection results from inhalation of soil-borne bacteria*, and inhalation of greater numbers of bacteria is facilitated by the presence of a dusty, contaminated environment. Enteric infections may result from ingestion of soil-contaminated feed, or swallowing expectorated material from the lungs. Experimental studies indicate that hematogenous spread from enteric lesions is not a common route of pulmonary infection.

The bacterium is engulfed by equine macrophages but survives this encounter by preventing phagosome–lysosome fusion. Nevertheless, macrophages are able to kill *R. equi* when the bacteria are opsonized or when the macrophages are activated by lymphocyte-derived cytokines. Infection elicits a pyogranulomatous inflammatory response, presumably due to cytokines secreted by infected macrophages or responding lymphocytes.

The *virulence* of *R. equi* varies between isolates, and correlates closely with the presence of an 85 or 90 kb virulence-associated plasmid. VapA is a 15–17 kDa protein encoded by the virulence-associated plasmid that is detectable in 88–98% of isolates from pneumonic lungs. Although experimental infection with plasmid-cured strains resulted in asymptomatic infection, recombinant expression of vapA by these strains did not cause reversion to virulence. Thus, although the virulence-associated plasmid seems necessary for the development of disease, plasmid-encoded proteins in addition to vapA, such as the recently described vapB and vapC, also contribute to virulence. Other virulence factors of *R. equi* include glycolipids containing long-chain mycolic acids, capsular polysaccharide, and the "equi factors" cholesterol oxidase and choline phosphohydrolase.

Immune responses to *R. equi* have been intensively investigated because of the interest in developing vaccines against this important disease of foals. The finding that passive immunization with hyperimmune plasma is effective in preventing disease indicates that humoral immunity is protective, probably because antibody-mediated opsonization augments phagocytosis and killing of *R. equi* by macrophages and neutrophils. The protective antigens of *R. equi* have not been clearly defined, but vapA is one likely candidate. In contrast to the role of humoral immunity in prevention of disease, T-helper-1 cellular immune responses are probably necessary for recovery from established infection. Both CD4+ and CD8+ T cells are important in clearing *R. equi* in mouse models of disease. CD4+ T cells produce interferon-γ, which activates macrophages to produce more bactericidal nitric oxide, oxygen radicals, and peroxynitrite, while CD8+ T cells induce lysis of infected macrophages.

The *pulmonary lesions* caused by *R. equi* are most constant in the cranioventral areas of lung, but most of the lung may be affected in severe cases. *The most consistent lesion is pyogranulomatous bronchopneumonia*, in which 1–10 cm, white-tan, firm, coalescing nodules occupy much of the lesional lung (Fig. 5.82). Intervening areas of lung are reddened and firmer than normal. With time, the centers of the pyogranulomas become friable and caseous or liquefying. These may be mistaken for abscesses, but they generally lack a fibrous capsule. Pleuritis is uncommon. Bronchial lymph nodes are enlarged and may contain focal caseous necrosis.

Intestinal lesions are present in half of the foals with lung lesions, and lesions are restricted to the intestine in about 5% of cases. The colon, cecum, and associated lymph nodes are most commonly affected, but small intestinal lesions may occur. Colonic lesions begin as focal mucosal ulcers that are centered on lymphoid follicles. The ulcer bed is reddened and covered with fibrin, and the borders of the ulcers are raised and pale due to pyogranulomatous infiltrates (Fig. 5.83). Pyogranulomas or foci of caseous necrosis may extend through the wall of the intestine. The colonic lymph nodes are often enlarged, pale, and contain necrotic foci. The colonic pyogranulomas enlarge over time, and may develop central areas of caseation or liquefaction. Large abdominal pyogranulomas with necrotic centers, which grossly resemble abscesses and cause intestinal adhesions, may be the sole lesion in some cases.

The earliest histologic lesion following *R. equi* infection is suppurative bronchopneumonia. By 4 days after infection, alveoli and bronchioles are filled with macrophages, neutrophils, and fewer lymphocytes and plasma cells. *Macrophages are numerous in the well-developed lesions, but neutrophils may also be plentiful. Gram-positive bacteria are present in the cytoplasm of plump uni- or multi-nucleate macrophages amid this exudate*, and are particularly numerous within degenerating macrophages that have cytoplasmic eosinophilia and nuclear pyknosis (Fig. 5.84). Areas of caseous necrosis, when present, usually contain cellular debris, many neutrophils and macrophages, fibrin, and edema.

In the intestine, the dome epithelium overlying lymphoid follicles is the primary route of infection. Macrophages underlying the dome epithelium contain intracytoplasmic bacilli, and neutrophils infiltrate the epithelium and exude into the lumen. With time the lymphoid follicles are invaded by macrophages and neutrophils, and central areas of caseous necrosis eventually extend to the mucosal surface. In the developed lesions of ulcerative colitis, ulcers are filled with fibrinonecrotic debris and encompassed by neutrophils and macrophages, many of which contain bacteria.

About one-third of affected foals have *polyarthritis*. This may cause severe lameness, suppurative synovitis, and positive joint cultures, due to hematogenous spread of infection. In contrast, other foals have milder, probably *immune-mediated polyarthritis*. These are characterized

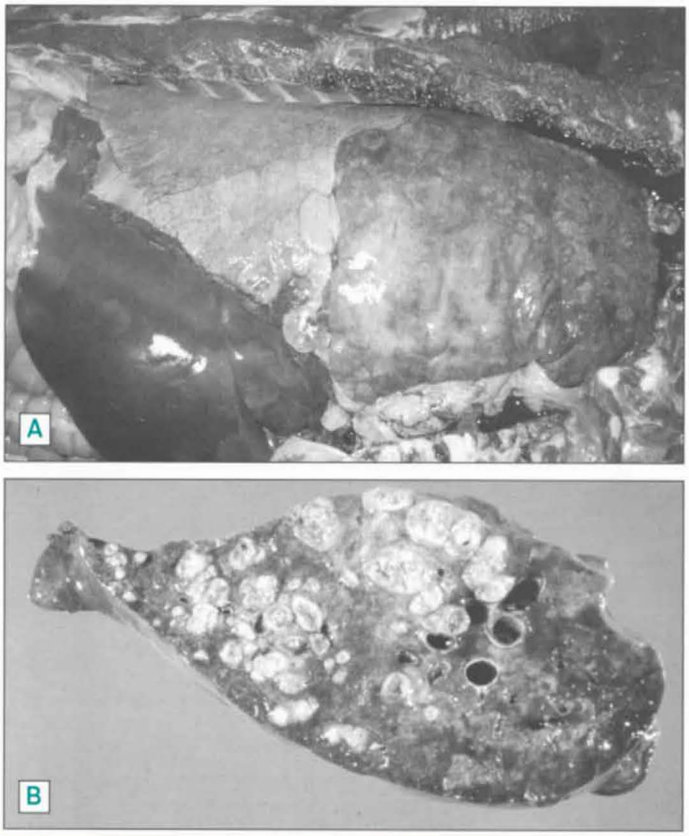

Figure 5.82 ***Rhodococcus equi* pneumonia** in a foal. **A.** The cranioventral lung is consolidated. **B.** The cut surface reveals multiple liquefying foci of pyogranulomatous inflammation.

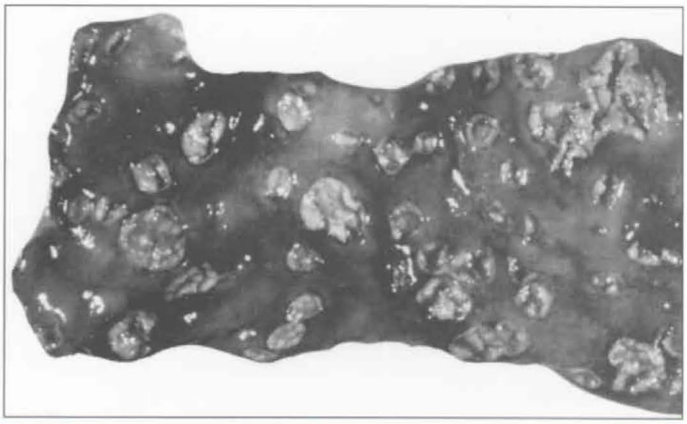

Figure 5.83 Multiple discrete ulcers in the colon of a foal with ***Rhodococcus equi* pneumonia**. (Courtesy of JA Johnson, et al., and Vet Pathol 1983:20:450–459.)

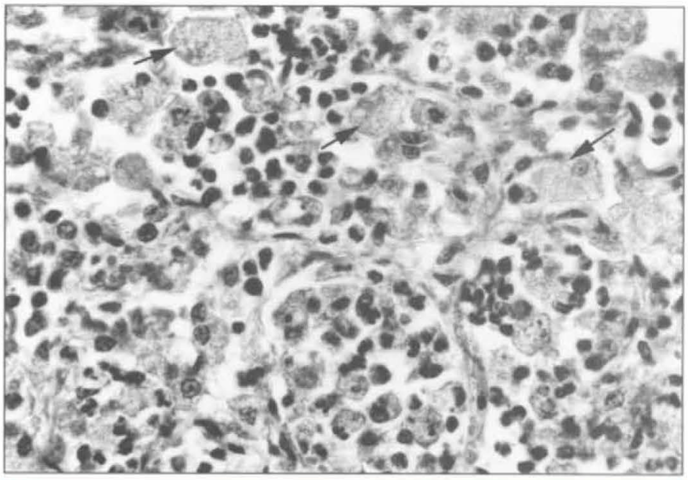

Figure 5.84 ***Rhodococcus equi* pneumonia** in a foal. Alveoli filled by macrophages, which contain large numbers of bacteria (arrows).

by sterile joints, lymphoplasmacytic synovitis, mononuclear cells in joint fluid, and immunoglobulin deposits in synovium.

More widespread dissemination of infection in foals can occasionally give rise to dermal abscesses, hepatic or splenic abscesses, osteomyelitis affecting the vertebrae or metaphyses of long bones, and hypopyon. *R. equi* may also cause ulcerative lymphangitis, and has been associated with metritis and abortion in mares, metritis in cows, pneumonia in calves, and tubercle-like lesions in lymph nodes of pigs and cattle.

Lesions of cranioventral pyogranulomatous pneumonia or multifocal ulcerative colitis in foals are highly suggestive of *R. equi* infection. In most cases, the *diagnosis is easily confirmed by culture*, or by identifying intracytoplasmic gram-positive bacilli in macrophages on impression smears or tissue sections. Idiopathic bronchointerstitial pneumonia or *Pneumocystis carinii* infection may be present in foals with *R. equi* pneumonia.

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Opportunistic bacterial pathogens in foals

Pneumonia caused by opportunistic bacterial pathogens is common in 1–8-month-old foals, and appears to be of similar pathogenesis as in other species. *Streptococcus zooepidemicus* is the most prevalent cause, with occasional cases resulting from infection with α-hemolytic streptococci, *Bordetella bronchiseptica*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Actinobacillus equuli*, and others. Pneumonia in neonates is commonly caused by bacteria that cause septicemia, and the two processes may be concurrent; *Escherichia coli*, *Streptococcus* spp., *A. equuli*, and *K. pneumoniae* are the most common isolates.

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Equine bacterial pneumonia and pleuropneumonia

Pleuropneumonia is common in 2–4-year-olds, and older horses may also be affected. Presenting signs include fever, depression, respiratory distress, and colic. Evidence of thoracic pain may be apparent at rest, with grunting respiration or abduction of the elbows, or be elicited by thoracic percussion. Horses with bacterial pneumonia develop fever, tachypnea, and coughing. Risk factors for the development of both pneumonia and pleuropneumonia include recent transportation, general anesthesia (probably leading to aspiration pneumonia), other stressful events, and viral infection.

Numerous opportunistic bacterial pathogens have been isolated from lesions of pleuropneumonia in horses, and *mixed infections are present in most cases*. Attempts to isolate bacteria may fail in cases that have been heavily treated with antibiotics. *Streptococcus equi* ssp. *zooepidemicus* is the most common isolate; others include *S. equi* ssp. *equi*, *Actinobacillus suis*-like bacteria, other streptococci, *Pasteurella* spp., *Bordetella bronchiseptica*, and *Escherichia coli*, *Bacteroides*, and *Fusobacterium*. *Mycoplasma felis* is an agent to be considered in the differential diagnosis of cases of pleuritis without pneumonia.

Identification of a unilateral distribution of lesions and a mixed infection with aerobic and anaerobic bacteria imply that *many cases result from aspiration pneumonia*. The equine trachea at the thoracic inlet often contains a pool of secretions contaminated by bacteria – the so-called "*tracheal puddle*." One frequent pathogenesis seems to involve transport of horses with the head held in an elevated position, thus predisposing to aspiration of these contaminated secretions into the lung.

The morbid anatomy of well-developed cases of pleuropneumonia is spectacular, and the lesions are often more chronic than suggested by the clinical history. Massive amounts of fibrin and malodorous serofibrinous exudate fill the pleural space, and may form cavitating masses encased in maturing fibrous tissue (see Fig. 5.42). Most cases of pleuritis in horses arise from underlying pulmonary lesions, although the lesions of pneumonia are overshadowed by the pleural exudate. Although often described as lung abscesses, the lung lesions are more usually localized areas of consolidation. Occasionally, pleuritis extends from bilateral lesions of bronchopneumonia, which are reddened, firm, and may contain crumbly necrotic debris. Localized areas of hemorrhagic infarction are frequently present in the consolidated lung, and careful inspection of the arteries may reveal the thrombi that caused the infarcts. Lesions in the lung are often unilateral; pleural exudate in these cases is also usually unilateral.

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Strangles and *Streptococcus equi*

Strangles is a common acute contagious disease of horses characterized by inflammation of the upper respiratory tract and abscessation in the regional lymph nodes. The disease commonly occurs in young horses following exposure to carriers or diseased horses. Clinical signs may include purulent nasal discharge, inappetence, fever, depression, unilateral or bilateral swelling of the throat region, stertor, and dysphagia.

Strangles is caused by *Streptococcus equi* ssp. *equi*, a Lancefield group C streptococcus that is an obligate parasite of the upper respiratory tract of equids. A range of virulence factors has been described for *S. equi*. The hyaluronic acid capsule is necessary for virulence, mediates binding of the bacterium to host cells, and confers resistance to phagocytosis. The M protein and a second cell wall protein bind fibrinogen and prevent deposition of complement factor C3b, thereby blocking recognition and phagocytosis by macrophages. Peptidoglycan activates complement by the alternative pathway, inciting recruitment of neutrophils to sites of infection. The fact that many of the recruited neutrophils rapidly undergo necrosis may be an effect of streptolysin-S and another cytotoxin.

S. equi in exudates can survive for many months in the external environment, and may be transmitted by fomites. The initial source of infection, however, is usually a carrier animal or one with active but not necessarily obvious clinical disease. The incubation period of strangles is 3–4 days, although it may be as short as 2 or as long as 15 days. The pathogenesis of the infection involves rapid transport of bacteria from the tonsil to local lymph nodes, and adhesion to nasopharyngeal epithelial cells. There is intense chemotaxis of neutrophils to the mucosa and regional lymph nodes, yet the organisms resist phagocytosis and neutrophil-mediated killing. Recovery from strangles confers immunity in about 75% of horses, associated with immunoglobulin A (IgA) and IgG antibody produced locally in the nasopharynx. Although antibodies to M protein confer partial immunity, other protective antigens are not yet fully defined.

The submandibular and retropharyngeal nodes are the first and usually the most severely affected. The swollen lymph nodes are initially firm, but this swelling becomes fluctuant as the suppurative exudate liquefies. The typical and favorable outcome of the lymphadenitis is for the abscesses to rupture on to the skin 1–3 weeks after onset of infection, releasing creamy yellow-white pus containing numerous infective bacteria. The nasal lesions are those of a purulent rhinitis but are otherwise nonspecific.

Most cases of strangles recover quickly unless the enlarging lymph nodes obstruct the upper respiratory tract. Twenty percent of clinically affected animals develop complications. These may involve extension of infection to adjacent structures, resulting in purulent sinusitis, guttural pouch empyema, periorbital abscessation, facial cellulitis, or local damage to cranial nerves, resulting in laryngeal paralysis (roaring), facial nerve paralysis, or Horner's syndrome. Horses with guttural pouch empyema may remain infected, intermittently shed bacteria, and be an important source of infection for at least 8 months after resolution of the acute disease.

More serious complications of pneumonia or pleuropneumonia, myocarditis, mesenteric lymph node abscesses, and purpura hemorrhagica are often fatal. Retropharyngeal abscesses may discharge into the pharynx, allowing pus to be aspirated into the lungs where localized areas of necrotizing pneumonia develop. Metastatic abscesses ("*bastard strangles*") occasionally form in the liver, kidneys, synovium, and brain, but are most common in mediastinal and mesenteric lymph nodes. Abscesses in these lymph nodes tend to be very large and, although rupture is unusual, the suppurative process can permeate to adjacent serous membranes and cause purulent pleuritis or peritonitis. Purpura haemorrhagica is a systemic leukocytoclastic vasculitis, and follows deposition of immune complexes of IgA or IgG with bacterial M protein in small blood vessels and glomeruli. Purpura hemorrhagica develops in 1–2% of cases, 2–4 weeks after the acute infection. There is edema of the head and limbs, petechial hemorrhages on mucosal and serosal surfaces and in muscles, and occasionally glomerulonephritis.

Because similar, but usually more mild, lesions may be caused by *S. zooepidemicus* or other bacteria, *definitive diagnosis relies on isolation of S. equi from purulent exudate.* A polymerase chain reaction (PCR) assay is described and is apparently more sensitive in detecting chronically infected horses.

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Glanders

Glanders is a disease of historic importance, and flourished especially among cavalry horses. It has disappeared from many countries, but still exists in Eastern Europe, Asia, and South America. It is caused by *Burkholderia (Pseudomonas) mallei* and typically affects horses. Disease may occur in carnivores, sheep, and goats, but not in cattle or pigs. Humans are susceptible, and glanders is of current interest as a potential bioterrorist threat. *B. mallei* is sensitive to the external environment, and infection is acquired directly or indirectly from excretions and discharges of affected animals. In the absence of definitive information, it is assumed that the organisms traverse the pharyngeal mucosa, and perhaps the intestinal mucosa, and are conveyed to the lungs, where lesions almost always occur. From there, hematogenous spread is believed to result in nasal, cutaneous, and lymph node lesions.

Glanders is characterized by nodular lesions in the lungs, and ulcerative and nodular lesions of the skin and respiratory mucosa. Lung lesions are present in all cases. There is a generalized distribution of pinpoint to 2 cm diameter *pyogranulomatous nodules throughout the lung*, often with central areas of liquefactive necrosis. Histologically, the nodules

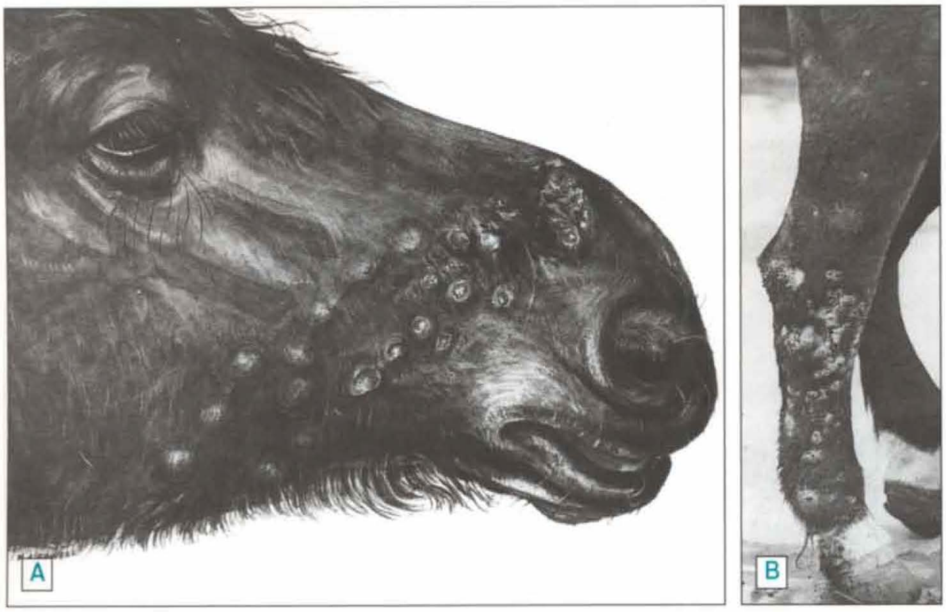

Figure 5.85 Glanders in a horse. **A.** Ulcers and farcy buds along facial lymphatics. **B.** Cutaneous glanders with nonulcerated buds. (Reproduced from Hunting W. Glanders. London: W Brown, 1908.)

are composed of a central core of neutrophils, often with necrosis of neutrophils and liquefaction of the tissue, and a peripheral rim of epithelioid macrophages and fibrosis. The relative proportions of neutrophils, macrophages, fibrosis, and mineralization are variable.

Nasal lesions are often unilateral, with copious, purulent, green-yellow exudate. Multiple small nodules in the submucosa each consist of an inner core of neutrophils and a periphery of macrophages. The core liquefies and the overlying mucosa may slough, leaving a crateriform ulcer that heals to form a white stellate scar. Similar pyogranulomatous ulcerative lesions line the pharynx, larynx, and trachea. Hematogenous metastases are common in the spleen and less common in other tissues. Enteric lesions are rare. *The cutaneous lesions of glanders are termed "farcy."* These consist of chains of nodules or ulcers that follow lymphatic vessels, and represent purulent lymphangitis with extensive leukocyte necrosis (Fig. 5.85).

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Mycoplasmal diseases

Although several mycoplasmas have been isolated from the respiratory tract of horses, particularly *Mycoplasma equirhinis* and *M. felis*, there have been no studies to determine whether they are capable of causing bronchiolitis or bronchointerstitial pneumonia. *M. felis* is an uncommon cause of fibrinous pleuritis without pneumonia, and lesions were reproduced by intrapleural inoculation of *M. felis*. *M. felis* has also been associated with outbreaks of lower respiratory tract disease in horses, based on seroconversion and isolation of the organism.

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Fungal diseases

Coccidioidomycosis, cryptococcosis, and pneumocystosis are described elsewhere in this chapter. **Pulmonary aspergillosis** is an uncommon disease of horses, and usually represents hematogenous spread of fungal hyphae resulting from colitis, as a consequence of both neutropenia and disruption of the mucosal barrier. Lesions are multifocal embolic pneumonia, often centered on pulmonary vessels, and include neutrophil and fibrin exudate in alveoli, hemorrhage, necrosis, and leukocytoclastic vasculitis. Septate branching hyphae are most common at the periphery of foci of necrosis.

Phycomycosis is a chronic nasal or cutaneous disease of horses and rarely of cattle. It is caused by *Conidiobolus coronatus* and *Basidiobolus haptosporus*, which are saprophytic fungi of the class *Zygomycetes*, and is limited to tropical and subtropical climates. The nasal lesions are most common in the nostril, and consist of ulcerating and cavitating granulomas containing coral-shaped granules. Histologic evaluation reveals eosinophils, neutrophils, macrophages, and giant cells. Fungal hyphae are numerous only in the granules and may be encased in brightly eosinophilic amorphous material (Splendore–Hoeppli phenomenon) that is grossly visible as granules in the necrotic areas of the mass. The hyphae of *C. coronatus* are nonpigmented, commonly septate, irregular in contour, 5–13 μm, and irregularly branched. Hyphae of *B. haptosporus* appear similar but are 5–20 μm diameter. *Pythium insidiosum* (an aquatic fungus-like oomycete pathogen) induces a similar lesion, and the hyphae are 2–7 μm diameter, thick-walled, and infrequently septate. The diagnosis is based on clinical and histologic findings; definitive diagnosis requires culture. The differential diagnoses for eosinophilic cutaneous or nasal masses in horses include habronemiasis and mast cell tumor.

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Parasitic diseases

Dictyocaulus arnfieldi is mainly a lungworm of donkeys, in which it survives for long periods with few clinical signs. *D. arnfieldi* is an occasional cause of chronic coughing in horses. Infection of adult horses often results in failure of the worm to develop to sexual maturity, and larvae cannot be detected in the feces of these animals. The worms can mature if horses or ponies are infected as young foals. The gross lesions are scattered, wedge-shaped foci of overinflation, mostly in caudal lobes. In the center of the lesions are *small bronchi packed with coiled adult worms*. Histologically, the worms are associated with goblet-cell hyperplasia and lymphocytic infiltrates. Adult worms cause relatively little luminal response, whereas first-stage larvae stimulate an intense mucopurulent reaction, or chronic catarrhal and eosinophilic bronchiolitis.

Parascaris equorum larval migration may cause *nodular lesions in the lung*. The acute lesions are distinctive by virtue of the mass of eosinophils present and the occasional presence of ascarid larvae in bronchioles or alveoli. However, the chronic nodules consist of aggregates of lymphoid tissue with fibrosis and mineralization, and need to be differentiated from residual lesions of small abscesses or granulomas.

Miscellaneous parasitisms include *Schistosoma nasalis*, a cause of nasal granuloma in India; hydatid cysts; and *Habronema* spp., a rare cause of fibrotic nodules adjacent to bronchioles.

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Infectious respiratory diseases of dogs

Viral diseases

Canine distemper

Canine distemper is now uncommon in many vaccinated dog populations but remains as a cause of periodic outbreaks, even in regions where vaccination is practiced, when naive groups of dogs are exposed to the virus. Canine distemper continues to be a frequent and serious disease in many parts of the world. *Canine distemper virus (CDV) infects a wide range of terrestrial carnivores*, including Canidae (wild and dometic dogs), Mustelidae (ferrets, mink), and Procyonidae (raccoons). Ferrets are particularly susceptible. Some species of seals are vulnerable to CDV infection, but distinct phocid morbilliviruses are also important causes of distemper-like disease. Neurologic disease is reported in javelinas, or collared peccaries. Large felids develop a fatal neurologic disease caused by a variant of CDV. A role for CDV has been proposed for hypertrophic osteodystrophy of dogs and Paget's disease of humans, but other investigations refute these hypotheses.

Clinical disease is most common at 12–16 weeks of age, as puppies with waning passive immunity are exposed to subclinically infected dogs. *The infection is systemic, and clinical signs are often referable to the respiratory, gastrointestinal, and nervous systems*. Ocular disease, pustular and/or hyperkeratotic cutaneous lesions, dental defects, and abortion are other manifestations. In some cases, clinical signs are primarily due to secondary infections that are a consequence of *virus-induced immunosuppression*, probably an effect of viral infection of lymphocytes and macrophages. Secondary diseases include *Bordetella*, adenovirus, and *Pneumocystis* infections of the lung; toxoplasmosis, Tyzzer's disease, sarcocystosis and encephalitozoonosis; and enteric infections with *Cryptosporidium* or attaching-and-effacing *Escherichia coli*.

The systemic form of the disease often begins as fever and conjunctivitis, with rapid progression to coughing, depression, anorexia, vomiting, and diarrhea. Affected dogs may die at this stage, fully recover, or develop neurologic disease 1–4 weeks later. Neurologic disease may also be the primary clinical manifestation, particularly in animals with partial immunity. Neurologic manifestations are quite variable depending on the anatomic location of the lesions, and include seizures, cerebellar or vestibular ataxia, paraparesis or paraplegia, and myoclonus.

CDV is in the genus *Morbillivirus,* family Paramyxoviridae. As for *Measles virus* and *Rinderpest virus*, which are closely related morbilliviruses, CDV is a large, 150–250 nm diameter, irregularly shaped virus composed of an outer lipoprotein envelope, an inner matrix, and a nucleocapsid containing single-strand negative-sense RNA. The envelope is studded with hemagglutinin glycoproteins, which mediate viral attachment to host cells; and fusion glycoproteins, which allow penetration of host cells and fusion of infected with uninfected cells.

Virus is shed in secretions of the respiratory tract, and to a lesser extent in other secretions. Infection is usually acquired by inhaling aerosols or by close contact with infected dogs. The virus infects macrophages of the upper respiratory tract or lungs, which convey it to local lymph nodes and tonsils during the first 24 hours. The virus replicates further in local lymphoid tissues, and by 2–5 days after exposure is present in lymphoid tissues throughout the body, including bone marrow, thymus, spleen, and intestinal lymphoid tissue.

Clinical signs of fever, depression, and anorexia develop about 5 days after infection. At this stage of viremia, virus particles are free in the plasma as well as within blood mononuclear cells. *Further development of the disease is highly dependent on the immune status of the host, the titer of antibodies to envelope glycoproteins, the age of the host, and the strain of virus.* Dogs with adequate humoral and cellular immunity are able to neutralize the virus and clear the infection by 14 days after infection, and may not shed virus from mucosal surfaces. In dogs with intermediate levels of cellular and humoral immunity, viremia may result in infection of mucosal epithelium and brain. Virus is shed in secretions but clinical signs attributable to epithelial infection may be minor or absent, although neurologic disease may develop in these partially immune dogs. Dogs that fail to mount an adequate immune response develop systemic infection of epithelial tissues that evokes clinical signs of respiratory and enteric disease, infection of the central nervous system, and shedding of virus in respiratory secretions, feces, and urine.

The **histologic lesions** of canine distemper are fairly specific when the disease is well developed, particularly if inclusion bodies are apparent. However, *lesions in mild cases are nonspecific*, particularly in dogs with clinical signs limited to the upper respiratory tract. *Inclusion bodies are most numerous at 10–14 days after infection*, but their numbers diminish rapidly by 5–6 weeks. *Inclusions are most obvious in brain and epithelial tissues*, and less easily identified in lymphoid tissues. Inclusion bodies can be found in the central nervous system before changes of encephalomyelitis are present, and they often persist in the neural tissue when they have disappeared from other sites. The inclusion bodies are eosinophilic and often intranuclear in nervous tissue but usually intracytoplasmic in other tissues.

Lymphopenia and lesions in lymphoid organs are regularly present. *Lymphoid lesions* may be inapparent on gross examination, or there may be either atrophy or edematous swelling. Thymic atrophy is particularly common. In the earliest lesions following experimental infection, there is depletion of lymphocytes in the cortical zone of the lymph nodes within 6 days of infection. By day 9, the lymph nodes and spleen contain lymphocytic necrosis and depletion, and infiltration of neutrophils. Syncytial cells may form in the nodes and these often contain inclusion bodies. Approximately 2 weeks after exposure, hyperplasia of histiocytic cells develops, but repopulation of the node by lymphocytes may be delayed for weeks or months. Thymic atrophy is due both to loss of cortical thymocytes as well as to great reduction in the medulla.

Respiratory tract lesions are common. Serous, catarrhal, or mucopurulent exudate covers the congested nasopharynx. The lungs are edematous, and secondary bronchopneumonia is often present, particularly in subacute or chronic cases. *The specific lesion of canine distemper is bronchointerstitial pneumonia*, which usually appears as patchy, generalized, red-tan, rubbery lesions beneath the pleura and at the margins of the lung. Generalized diffuse reddening with firm texture is a less common manifestation. Histologically, bronchioles contain scant suppurative exudate, there is patchy necrosis and attenuation of bronchiolar epithelium, and lymphocytes are present around bronchioles. *Inclusion bodies are often most obvious in the cytoplasm of bronchial and bronchiolar epithelial cells*. Alveoli contain protein-rich edema fluid, scant fibrin, mononuclear cells, and necrotic epithelial cells. Alveolar septa are thickened by mononuclear cells. Proliferation of type II pneumocytes, which occasionally contain cytoplasmic inclusions, may result in a complete cuboidal lining to scattered groups of alveoli (Fig. 5.86). Inclusion bodies are common in type II pneumocytes and alveolar macrophages, and *alveolar epithelial syncytial cells are a characteristic feature when present* (Fig. 5.87). Chronic lesions in subpleural and peribronchiolar alveoli include macrophage accumulation, type II pneumocyte proliferation, and alveolar septal fibrosis. Inclusion bodies tend to persist in the bronchiolar and alveolar epithelium longer than in other nonneural tissues.

Intracytoplasmic and rarely intranuclear inclusion bodies are regularly found within swollen transitional epithelial cells of the *urinary bladder and renal pelvis* in the acute systemic disease. Inclusion bodies, mild degenerative changes, and mononuclear cell infiltrates may be present in a variety of other epithelia, including the gastric surface epithelium, chief and parietal cells of the stomach, cholangiolar epithelium in the liver, pancreatic ductular epithelium, epididymis, and testis.

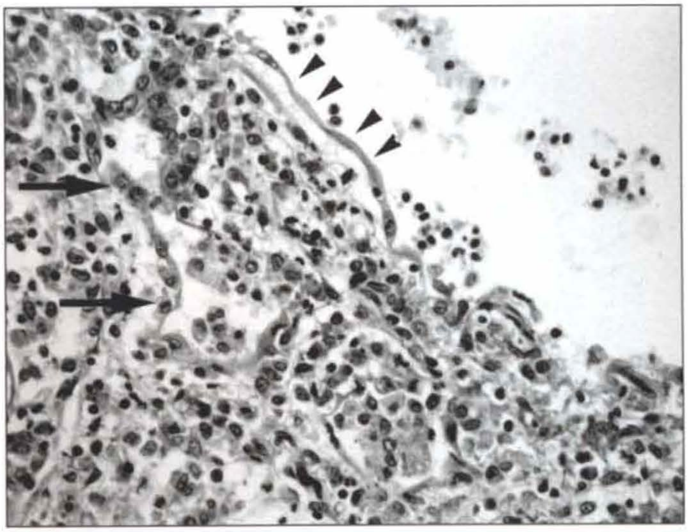

Figure 5.86 Canine distemper. Bronchointerstitial pneumonia, with a bronchiole (upper right) lined by attenuated epithelium (arrowheads), and alveoli lined by cuboidal type II pneumocytes (arrows).

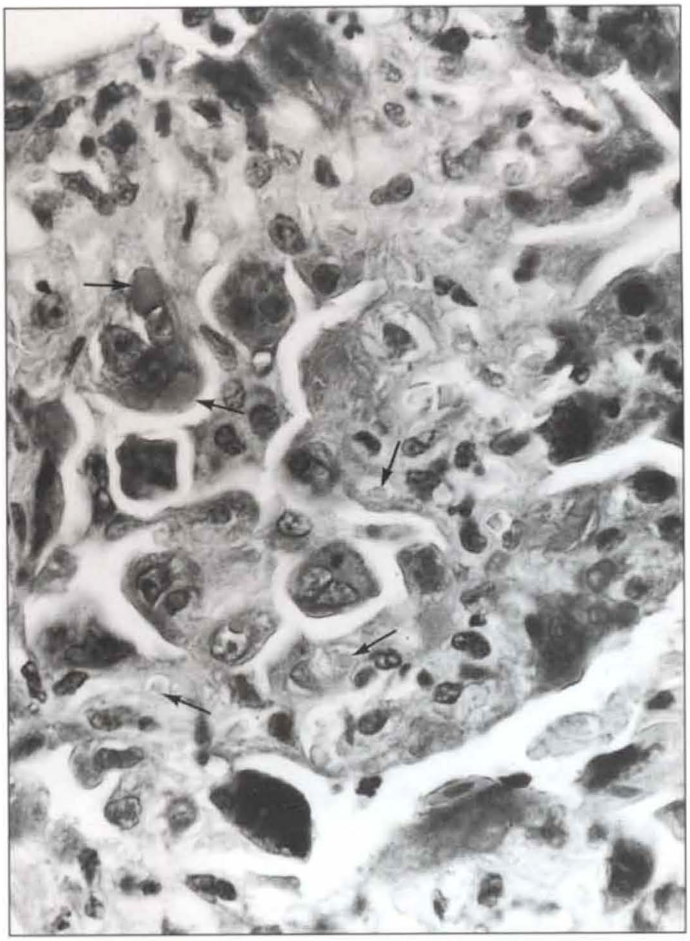

Figure 5.87 Canine distemper in a dog. Syncytia in alveoli with cytoplasmic inclusion bodies (arrows).

In the *central nervous system*, the virus appears first in perivascular astrocytes and macrophages, but infection of the choroid plexus epithelium occurs early, and the cerebrospinal fluid contains large amounts of virus. Lesions in the white matter and gray matter

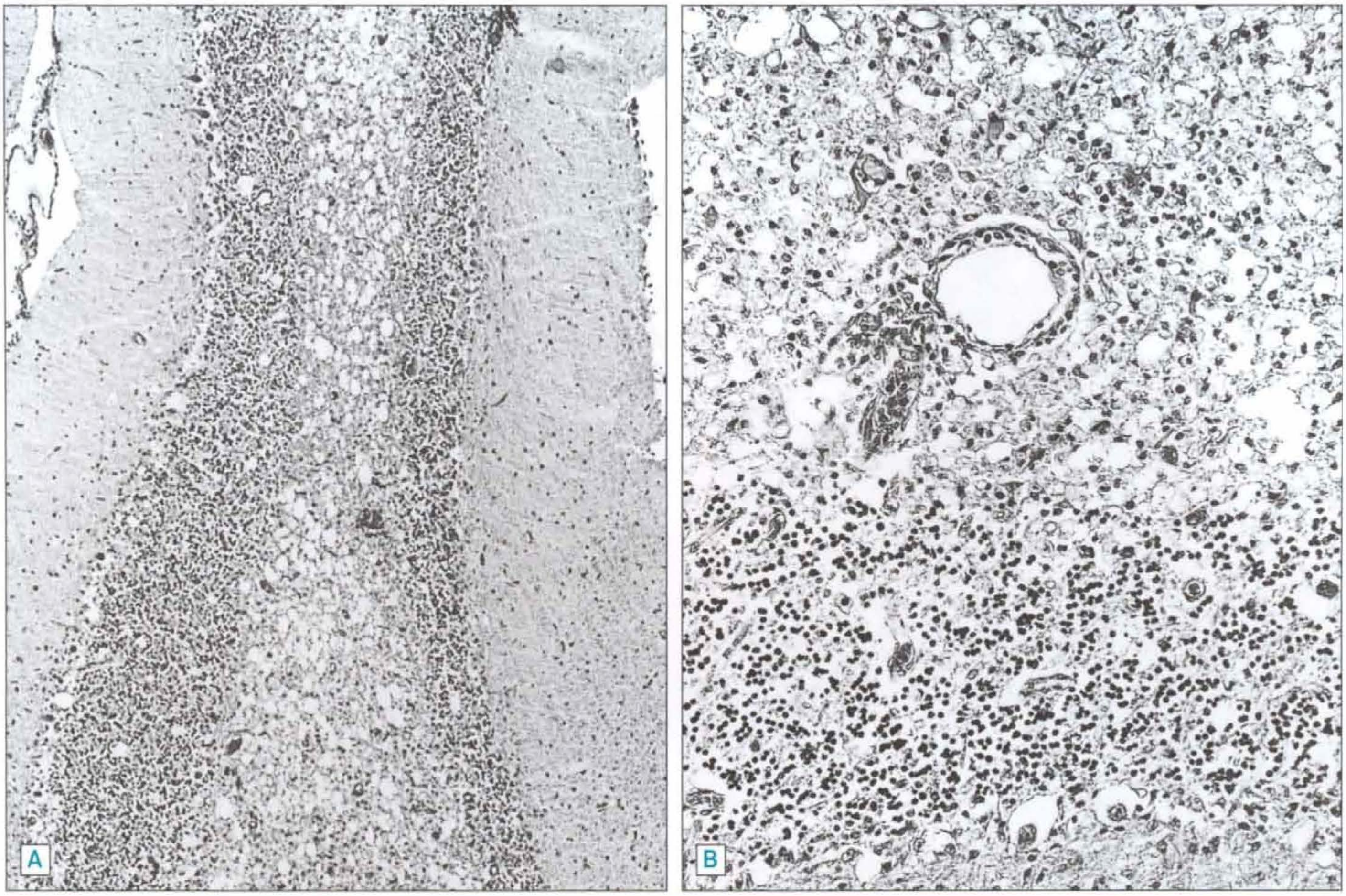

Figure 5.88 Canine distemper in a dog. **A. Demyelination** in cerebellar white matter. **B.** Perivascular cuffing and demyelination in cerebellar white matter.

differ histologically; these may occur concurrently or one may predominate. *Demyelination in white-matter tracts* is most severe in the cerebellum, rostral medullary velum, optic tracts, spinal cord, and surrounding the fourth ventricle, and probably arises from distribution of virus through the cerebrospinal fluid. The lesions are multifocal or patchy in distribution, with vacuolation of the neuropil, loss of myelin (particularly notable with Luxol fast blue stain), and, in the early stages, preservation of axons (Fig. 5.88). Astrocytes usually contain nuclear and less frequently cytoplasmic inclusions, and astrocyte-derived syncytia are present in a minority of cases. As the lesion progresses, gliosis and axonal degeneration may occur and mononuclear cells infiltrate the lesion in low numbers; however, the early lesion is noninflammatory. Animals that survive may be left with sclerotic astrocytic foci and myelin loss.

Gray-matter lesions, which are less frequent than lesions of white matter, often target the cortex of the cerebellum and cerebrum, brainstem, and spinal cord. In early stages, inclusion bodies are usually present in the nucleus or occasionally in the cytoplasm of neurons; these neurons undergo necrosis and mononuclear cells congregate around the dying neurons. With time, a nonsuppurative inflammatory response develops, and mononuclear cells aggregate around blood vessels and infiltrate the neuropil. Nonsuppurative meningitis is usually mild.

Old-dog encephalitis is a rare variant, possibly caused by infection with replication-defective virus. This syndrome is characterized by chronic progressive neurologic disease, widely distributed perivascular infiltrates of lymphocytes and plasma cells, and intranuclear inclusions in astrocytes and neurons. Viral antigen may be demonstrated by immunohistochemistry, but virus cannot be isolated from the brain. Canine distemper may occasionally result from administration of modified live CDV vaccines. Neurologic disease is the usual manifestation in these cases, and lesions in the gray matter include neuronal necrosis, intranuclear inclusions, and lymphocytic encephalitis.

Dental lesions follow infection of young animals with CDV. Necrosis and cystic degeneration of ameloblastic epithelium of the developing tooth, associated with syncytia and cytoplasmic inclusions, give rise to the *defective enamel* seen in animals that have recovered from infection. The defects vary from focal depressions to large, sharply demarcated areas of enamel hypoplasia.

Ocular lesions include conjunctivitis, keratitis, retinitis, and optic neuritis. Conjunctivitis is very common in the early stages of disease, and occasionally extends to the cornea to cause ulcerative keratitis. Retinal lesions, which are common following systemic infection, include intranuclear inclusions in ganglion cells and glia, degeneration of ganglion cells, photoreceptor loss, retinal edema, and perivascular cuffs of mononuclear cells. These lesions progress to neuronal loss, retinal scarring, and proliferation of retinal pigment epithelium in the chronic stages. Lesions in the optic nerve are inconstant, but papilledema may be observed in acute cases, and gliosis or demyelinating neuritis in chronic ones.

Cutaneous lesions of hyperkeratosis and parakeratosis affect the footpad and nose, and rarely the haired skin. The epidermis may contain syncytial cells, and nuclear and cytoplasmic inclusion bodies. Pustular dermatitis due to secondary pyoderma may be present. Experimentally infected dogs commonly develop *bone lesions*, with necrosis of osteoclasts and consequent persistence of the primary spongiosa. Pale streaks in the heart caused by multifocal *myocardial necrosis and mineralization* are described in association with canine distemper.

The histologic findings are characteristic if a spectrum of lesions is present and inclusion bodies are discovered. Rabies and poxviruses are the other causes of intracytoplasmic inclusions in dogs, but the clinical and pathologic findings in these diseases are usually distinct from canine distemper. Other viruses causing inclusion bodies in bronchiolar or airway epithelial cells include *Canine adenovirus 2*, *Canid herpesvirus 1*, and *Canine minute virus* (*Canine parvovirus 1*). Confirmation of the diagnosis benefits from a diversity of diagnostic options, including virus isolation, immunohistochemistry, indirect immunofluorescence, in situ hybridization, and the polymerase chain reaction.

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Infectious tracheobronchitis – kennel cough

Infectious tracheobronchitis, or kennel cough, is a common clinical entity that is usually encountered following mixing of dogs at kennels, pet stores, and dog shows. Affected dogs develop persistent, harsh, nonproductive or productive coughing, but are often not febrile or anorexic. Most cases recover spontaneously, although signs can persist for 3 weeks or longer. Bacterial pneumonia is a well-recognized but uncommon complication of kennel cough in dogs.

Tracheobronchitis in dogs may be caused by a variety of infectious agents, and more than one agent can often be isolated from individual cases. *There is emerging consensus that* ***Bordetella bronchiseptica*** *should be viewed as the primary agent of kennel cough*, but that *Canine parainfluenza virus 2* and *Canine adenovirus 2*, and to a lesser extent *Canine distemper virus* and *Mycoplasma* spp., have predisposing roles. However, *Bordetella* cannot be isolated in some outbreaks of kennel cough, suggesting that *primary viral tracheitis may occasionally be of primary clinical significance.*

B. bronchiseptica is a gram-negative coccobacillus that is commonly carried in the upper respiratory tract, but not the lungs, of healthy dogs and many other domestic animals. It is an opportunist that is associated with infectious tracheobronchitis in dogs, atrophic rhinitis in pigs, and suppurative bronchopneumonia in many species. There is considerable genetic diversity among strains of *B. bronchiseptica*, but the relation of genetic variation and virulence remains an area for exploration. The expression of virulence factors of *Bordetella* is dependent on environmental conditions, regulated by the *Bordetella virulence gene (bvg) operon*, such that virulence genes are repressed at 25°C or in the presence of sulfate or nicotinic acid, and activated at 37°C. After entering the host, a first wave of *bvg*-regulated genes is expressed, encoding the adhesive proteins filamentous hemagglutinin (FHA), pertactin, and fimbriae that allow *B. bronchiseptica* to attach to ciliated epithelial cells. Following attachment of bacteria to the mucosal surface, a second wave of *bvg*-regulated genes is expressed that mediate motility, iron scavenging, and urease and phosphatase activity.

Secreted toxins are important contributors to bordetellosis. The adenylate cyclase toxin (hemolysin) is an RTX (repeats in toxin) toxin, like *Mannheimia haemolytica* leukotoxin and the Apx toxins of *Actinobacillus pleuropneumoniae*. The RTX domain of the toxin forms pores in target cell membranes that permit transfer of the adenylate cyclase component. Entry of this toxin into leukocytes causes increased cyclic adenosine monophosphate production that impairs phagocytosis and oxidative burst. Other virulence factors that are not regulated by the *bvg* operon include a lipooligosaccharide with endotoxin activity and a soluble peptidoglycan-derived tracheal cytotoxin. The latter is not directly toxic to tracheal epithelium, but stimulates host cells to produce nitric oxide that in turn induces ciliostasis and apoptosis of ciliated epithelial cells.

B. bronchiseptica adheres to macrophages and neutrophils, probably by binding FHA to complement receptors on macrophages. Following adhesion, the bacteria are internalized and survive within macrophages without inciting an oxidative burst, and may induce apoptosis of these cells through secretion of the adenylate cyclase toxin. In addition, *Bordetella* is able to enter and survive within nonphagocytic cells, presumably affording protection from host defenses and a ready supply of nutrients.

Although mucosal antibody responses are detectable within 4 days of infection and the duration of clinical illness is usually only 2–3 weeks, *Bordetella* infections often persist for 2–3 months and infected dogs may remain a source of infection for others in the kennel. Immune responses to *B. bronchiseptica* confer partial protection against subsequent challenge. FHA and pertactin are immunodominant antigens, and mucosal antibody responses to these antigens are partially protective.

Canine parainfluenza virus 2 (CPIV-2), also known as parainfluenza type 2 or SV-5, is commonly isolated from dogs with kennel cough. Clinical signs develop in experimentally infected dogs 3–10 days after infection. Virus is shed in nasal secretions for about 8 days after infection. Lesions are those of mild tracheobronchitis and bronchiolitis, with epithelial necrosis, mixed cellular inflammatory infiltrates, and submucosal edema. The virus does not replicate in macrophages and does not induce significant bronchointerstitial pneumonia in immunocompetent dogs. However, some strains may cause clinically detectable tracheobronchitis without concurrent *Bordetella* infection. In addition, CPIV-2 predisposes to bacterial pneumonia by impairing mucociliary clearance, and increases the bronchoconstrictive response to agonists such as histamine. Infection has been shown to impair olfaction in the absence of nasal lesions, but the clinical significance of this intriguing phenomenon is unknown.

Gross lesions may be absent in dogs with kennel cough, or mucopurulent exudate may be noted in bronchi. Enlargement of tonsils and tracheobronchial lymph nodes is inconsistent. *Histologic lesions* are by definition restricted to the trachea and bronchi; involvement of terminal bronchioles and alveoli would prompt a diagnosis of bronchopneumonia. Neutrophils infiltrate the tracheobronchial mucosa within 24 hours of infection with *B. bronchiseptica*, and increase in intensity as the disease worsens. Bacteria may be faintly visible as clumps of amphophilic material amid the cilia at the epithelial surface. Necrosis of ciliated epithelium is uncommon, but may result from underlying viral infection, neutrophil-mediated epithelial injury in areas with extensive purulent exudate, and effects of secreted bacterial toxins. As the disease progresses, there is lymphoid hyperplasia around bronchi and in draining lymph nodes. Infection is restricted to the respiratory tract and associated lymph nodes. Sequelae to tracheobronchitis include bronchiectasis and suppurative bronchopneumonia, which probably develop when the secreted toxins of *Bordetella* cause impairment of mucociliary clearance and leukocyte function.

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Canine adenovirus 2

Canine adenovirus 2 (CAV-2) is serologically related to, but genetically distinct from, CAV-1, the cause of infectious canine hepatitis. The contribution of CAV-2 to infectious tracheobronchitis or kennel cough is discussed above. Naturally occurring adenoviral pneumonia is rare in dogs, and is usually a consequence of immunosuppression. Gross lesions may be cranioventral or disseminated, lobular or confluent, and consist of atelectasis, reddening, edema, and mild firmness. *Amphophilic intranuclear inclusions* are present in alveolar macrophages, with fewer in type II pneumocytes and airway epithelium. Bronchioles contain necrotic epithelial cells and suppurative exudate, and macrophages, neutrophils, and fibrin are present in alveoli. Peribronchiolar and interstitial infiltrates of lymphocytes occur but are not prominent.

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Canine herpesvirus

Although most commonly diagnosed as a systemic disease in neonatal puppies, *Canid herpesvirus 1* may occasionally be isolated from older dogs with *acute respiratory disease*. Aerosol challenge of 12-week-old dogs caused necrotizing rhinitis, bronchointerstitial pneumonia, and multifocal alveolar necrosis with infrequent eosinophilic intranuclear inclusion bodies. Intraperitoneal inoculation of neonatal puppies with the same viral strain caused necrosis and hemorrhages in many organs, supporting the contention that the manifestation of disease is dependent on the route of exposure and the age of the host. Herpesviruses are infrequently isolated from dogs with acute tracheobronchitis (kennel cough), either alone or with other infectious agents. The respiratory lesions are those to be expected from herpesviruses, namely necrotizing rhinotracheitis and possibly bronchopneumonia. Eosinophilic intranuclear inclusions can sometimes be found in epithelial cells in early lesions, particularly in nasal mucosa.

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Bacterial diseases

Pneumonia caused by opportunistic bacterial pathogens is moderately common in dogs. Because most of the pathogens involved are commonly isolated from healthy dogs, a diagnosis of bacterial pneumonia should prompt a search for predisposing causes. *Aspiration pneumonia* may result from megaesophagus, anesthesia, coma, or other causes of regurgitation or vomiting. Alternatively, bacterial pneumonia may arise as a consequence of *impaired respiratory defenses*. These include respiratory viral infections, particularly canine distemper, parainfluenza, or adenovirus-2; immunosuppressive therapy that induces immunosuppression or neutropenia; concurrent disease conditions that impair immune responses or neutrophil function, including uremia, hyperadrenocorticism, diabetes mellitus, parvoviral enteritis, systemic mycoses, or primary immunodeficiencies; ciliary dyskinesia; and environmental and social stresses.

Multiple bacterial pathogens are isolated from the lung of many dogs with bacterial pneumonia, as would be expected if the primary problem in these dogs is failure of lung defenses. *Pasteurella multocida, Bordetella bronchiseptica, Escherichia coli, Streptococcus* spp., *Moraxella* spp., *Staphylococcus intermedius, Klebsiella pneumoniae*, and *Acinetobacter* spp. are the most common isolates from transtracheal aspirates of dogs with pneumonia. *Bordetella* is discussed more fully under the heading of Infectious tracheobronchitis – kennel cough, above. Pneumonia caused by β-hemolytic group C and group G streptococci may develop into septicemia, or bacteremia leading to polyarthritis, meningitis, endocarditis, or hematogenous infection of other viscera. Some outbreaks of streptococcal pneumonia have no identifiable predisposing causes, suggesting that some highly virulent strains may be primary pathogens in dogs. *E. coli* seems to be most frequently associated with aspiration pneumonia; complications include bacteremia or disseminated intravascular coagulation.

The morphology of bacterial pneumonia in dogs does not usually suggest a specific infectious agent. Lesions may affect entire lobes or follow a lobular distribution. In contrast to the characteristic cranioventral distribution of bronchopneumonia in ruminants and swine, *bronchopneumonia in dogs may affect primarily the cranial or middle lobes, or present a patchy or diffuse distribution throughout the lung.* Acutely affected areas of lung are plum red, swollen, firm, and edematous. Fibrinous pleuritis is usually minimal. As the lesion ages, they become white or gray-brown, mottled, and granular. In cases of aspiration pneumonia, the lesions are often localized, foul-smelling, and necrotic. Histologically, neutrophils fill alveoli and bronchioles, but leukocyte necrosis and thrombosis are not typical features of canine pneumonia.

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Mycoplasmal diseases

Mycoplasmas that infect the respiratory tract of dogs include *Mycoplasma canis, M. cynos, M. bovigenitalium, M. spumans, M. edwardii, M. gatae, M. feliminutum*, and several unclassified species of *Mycoplasma, Ureaplasma*, and *Acholeplasma*. Mycoplasmas are present in the nasopharynx of most normal dogs, and can be isolated from tracheobronchial washes from about 30% of normal adult dogs and a similar percentage of those with pneumonia. Mycoplasmas are more likely to be isolated from young dogs with septic inflammation and colonization of airways by aerobic bacteria. However, it remains possible that mycoplasmas secondarily colonize airways that are injured by other pathogens, for many dogs with suppurative pneumonia are concurrently infected with mycoplasmas and other bacterial pathogens. Experimental infection with *M. cynos* did not result in clinical signs of disease, but did induce peribronchial infiltration of lymphocytes and plasma cells, exfoliation and hyperplasia of bronchiolar epithelium, and neutrophil exudation into bronchioles and alveoli. Experimental infection with *M. bovigenitalium* resulted in mild suppurative and lymphocytic bronchiolitis. Pathologic changes were not apparent in dogs challenged with *M. spumans, M. canis*, or *M. gatae*. Clinical signs were not apparent in any of the *Mycoplasma*-challenged dogs. Unclassified mycoplasmas have been associated with similar lesions and with chronic bronchitis and bronchiectasis in dogs. Thus, although *M. cynos* may induce mild pulmonary lesions, and although *Mycoplasma* sp. colonizes the lungs of many dogs with pneumonia, *the importance and prevalence of Mycoplasma sp. as a primary cause of lung disease in dogs are uncertain.*

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Fungal diseases

Mycotic rhinitis

Mycotic rhinitis is common in dogs and occasionally diagnosed in other species. Cases may be presented with variable combinations of chronic sneezing, stertor, unilateral or bilateral mucopurulent nasal discharge, nasal hemorrhage, and nasal pain. Distortion of nasal bones may occur, and extension of infection to adjacent structures may rarely induce exophthalmos or neurologic disease.

Aspergillus fumigatus is the usual cause of mycotic rhinitis in dogs, although other species of *Aspergillus* or *Penicillium* are occasional causes of otherwise similar disease. These fungi are common in the environment and reach the nasal cavity of most normal animals by inhalation. It is not known whether disease results from exposure to higher numbers of inhaled fungi or from suppression of the normal nasal defenses, but immunosuppression or other predisposing causes has only rarely been identified in dogs with nasal aspergillosis.

Lesions are often focal within the nasal cavity or paranasal sinuses, and consist of yellow, green, or black plaque-like mats or masses of fungal growth. The surrounding mucosa is hyperemic and edematous, and there is often purulent exudate, caseous debris, or hemorrhage throughout the nasal cavity. Destruction of turbinate bones is common, and remodeling of the nasal septum or nasal bones may occur. *Histologic diagnosis requires biopsy of the fungal plaques*, which may be very localized despite the presence of widespread exudate. If these plaques can be harvested by an aggressive rhinoscopist, the diagnosis is not usually challenging – they consist entirely of numerous fungal hyphae, which occasionally contain conidia. *Aspergillus* spp. hyphae are 5–7 μm diameter and have parallel sides, frequent septa, and branch dichotomously at 45°. Other histologic lesions may include ulcerative suppurative rhinitis and necrotizing vasculitis.

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Rhinosporidiosis

Rhinosporidiosis is a disease of humans and dogs, and rarely of other domestic mammals and fowl. The disease is endemic in wet environments of the Indian subcontinent, uncommon in North America and Africa, and rare in Europe. Molecular analyses suggest that the causative agent, ***Rhinosporidium seeberi***, is not a fungus, but is a member of the Mesomycetozoa or so-called "DRIP" clade of aquatic protistan parasites. The agent can only be cultured with specialized techniques. The pathogenesis is uncertain, but infection may result from exposure of nasal mucosa to contaminated water.

The typical lesion is a *single unilateral nasal polyp*, which is soft, pink, up to 3 cm diameter, and bleeds easily. Histologic investigation reveals loose fibrous tissue, granulomatous inflammation, and sporangia of *R. seeberi*. Juvenile sporangia are 15–75 μm diameter, unilamellar, and contain a single nucleus. Mature sporangia, which may be visible grossly as pinpoint white foci, are 100–400 μm diameter and contain numerous 5–10 μm diameter endospores. The large size of the sporangia and the presence of endospores might only be mistaken for *Coccidioides*, and finding such structures in a nasal polyp is considered diagnostic.

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Blastomycosis

Blastomycosis is an infectious noncontagious disease of dogs and humans, with occasional cases in cats, horses, and other species. The disease occurs primarily in North America, with occasional cases in Africa, Europe, Asia, and Central America. Endemically infected areas in North America include the Mississippi, Ohio, and St. Lawrence river basins, northern Ontario, and the mid-Atlantic States.

Blastomycosis is most common in young male sporting dogs that reside near water. The highly variable clinical presentation reflects the diversity of organs affected. Many dogs have insidious onset of weight loss, fever, coughing, and dyspnea due to pulmonary involvement. Ocular pain and blindness, nodular ulcerating skin lesions, and lameness are other common presenting signs.

Blastomyces dermatitidis is a dimorphic fungus. At environmental temperatures, it grows as a mycelial form and produces spores that are infectious to animals. The ecological source of the mycelial form is uncertain, but is associated with acidic sandy soils. *Recently disturbed soil* is a recognized factor in some outbreaks, and geographic clustering of cases is well documented. *At body temperatures, the fungus grows as a yeast form that is not contagious.* Although the potential exists for mycelial forms to grow in cooled tissue specimens, reports of infection acquired at necropsy are rare and have been caused by accidental penetrating wounds rather than inhalation.

Virulence factors of *B. dermatitidis* are poorly described. BAD-1 is a surface protein that mediates adhesion to host cells, may modulate the inflammatory response, and is an immunodominant antigen. Antibody responses to BAD-1 reduce disease severity, but are not completely protective. The cell wall polysaccharide α-glucan is associated with virulence and may protect against killing by macrophages.

Infection is usually acquired by inhalation of mycelial forms into terminal bronchioles, where they rapidly convert to the yeast form. Local inoculation may be the cause of the rare lesions that are restricted to the skin. Experimentally infected dogs develop a much higher prevalence of lesions than for other systemic mycoses, although in most the disease is mild and resolves without treatment. Subclinically infected dogs are apparently rare. Yeast forms proliferate in the lungs and disseminate via the blood and lymphatic vessels. Naturally infected animals may have suppression of humoral and cellular immune responses but, as for other systemic mycoses, this probably represents an effect rather than a cause of the disease. The mechanisms of this immunosuppression are not known. Whether immunosuppression is a prerequisite for disseminated disease is uncertain. T-helper-1 immune responses have been associated with protection in mouse models, and immune responses target cell wall components, including BAD-1.

The lung is the most consistently affected site. There is generalized multifocal distribution of coalescing gray-white nodules, which often range from 3 to 20 mm diameter (Fig. 5.89). Most pulmonary nodules are firm throughout, but some become abscesses or undergo central caseation and then may fistulate into a bronchus or on to the pleura. Mineralization is minimal or absent. Microscopically, the granulomas are formed by a mass of epithelioid macrophages usually accompanied by histiocytic giant cells and variable numbers of neutrophils, an outer layer of lymphocytes and less reactive macrophages, and often a peripheral rim of fibrous tissue. In some cases, there is extensive caseous necrosis in the centers of the granulomas, with merely a thin rim of macrophages. *Yeast bodies are quite variable in number and may be missed if only hematoxylin and eosin-stained sections are examined*; in cases treated with antifungal drugs or with partial immunity, a diligent search of sections stained by the periodic acid–Schiff reaction or with methenamine silver may be required to reveal the fungi. The yeast forms are 5–15 μm (or occasionally up to 30 μm) diameter, round, nonencapsulated, with a distinct wall about 1 μm thick, and granular protoplasm completely or partly filling the center (Fig. 5.90). The double contour of the wall that is well described in cytologic preparations or culture imprints is often inapparent histologically. Occasional yeast display *broad-based budding* of single daughter cells. Filamentous or pseudohyphal forms and conidia are infrequently found in tissues, and are usually accompanied by yeast forms.

Disseminated lesions are common in lymph nodes, eyes, skin and subcutaneous tissues, bones, and joints. Testes, prostate, brain, heart, liver, spleen, kidneys, intestines, and other organs are less commonly affected. The lesions are either granulomas with numerous epithelioid and giant cells, or pyogranulomatous foci with central accumulation and necrosis of neutrophils and macrophages.

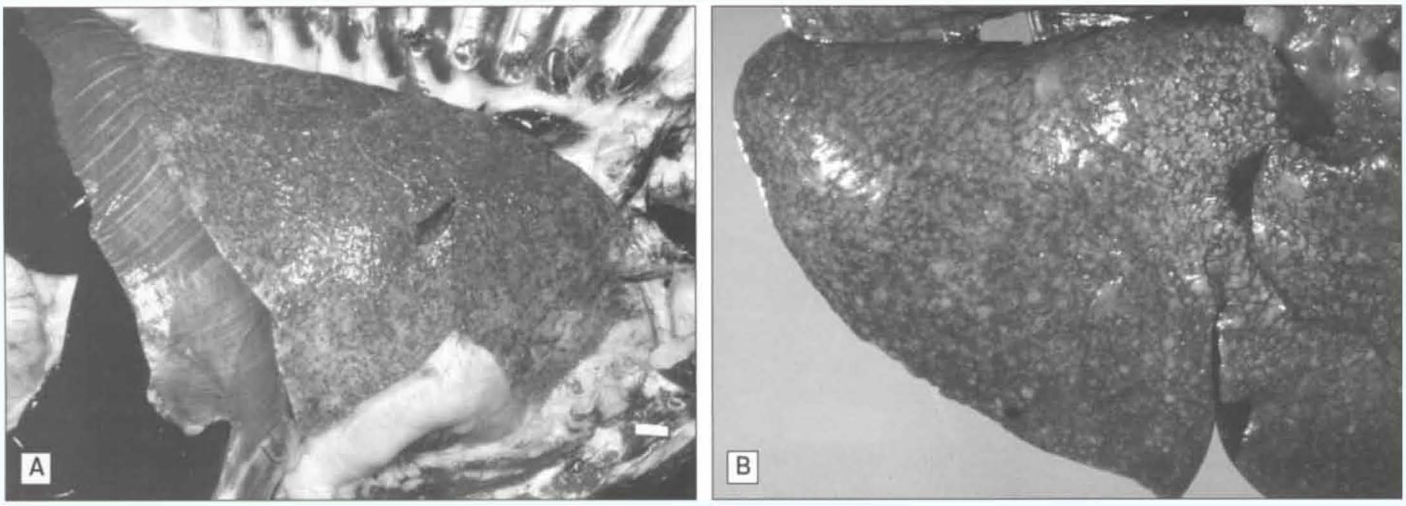

Figure 5.89 Blastomycosis in a dog. The lung contains a generalized pattern of coalescing white raised nodules.

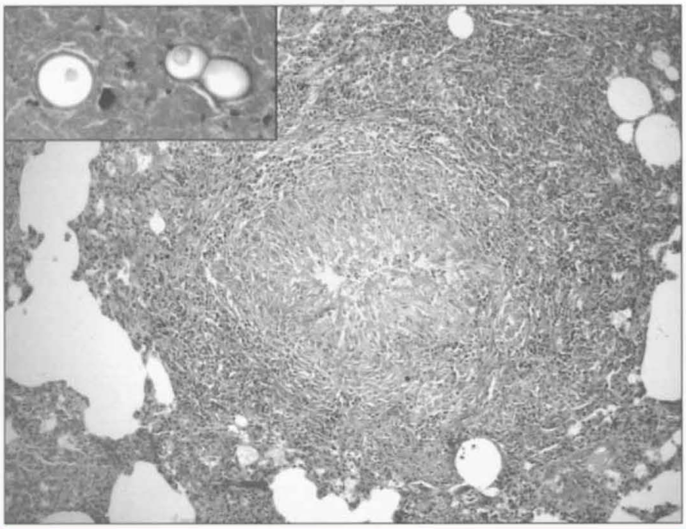

Figure 5.90 Blastomycosis in a dog. Granuloma in the lung with yeast in the center. Inset: Yeast with thick wall and broad-based budding.

Demonstration of the yeast bodies in tissue section or in cytologic preparations is the usual method of diagnosis. Blastomyces is readily differentiated from other fungi in culture, but safety precautions must be in place because infection of laboratory personnel by the cultured mycelia is well documented. Serologic tests are available, but false negatives are common.

The *differential diagnosis* for multinodular lesions in lung and other organs includes other systemic mycoses and metastatic neoplasia. Neoplastic nodules are often larger and more variable in size than the lesions of blastomycosis; examination of impression smears or histologic sections readily differentiates the two. Morphologic features are usually adequate to differentiate *Blastomyces* from other fungi: *Cryptococcus neoformans* usually has a thick capsule and only a mild inflammatory response, *Histoplasma capsulatum* at 2–4 μm is much smaller and resides in the cytoplasm of macrophages, and *Coccidioides immitis* at 20–200 μm is much larger and often contains endospores. Mutants of *Cryptococcus* that lack the characteristic thick capsule may closely resemble *Blastomyces* and incite a similar granulomatous response, but the presence of narrow-based budding differentiates them from *Blastomyces*, which divides by broad-based budding. *Blastomyces*, *Cryptococcus*, and *Histoplasma* are reliably differentiated in culture by the presence and morphology of mycelia, conidia, and yeast.

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Cryptococcosis

Cryptococcosis, also referred to as European blastomycosis, is a mycotic disease of worldwide distribution. *Cryptococcosis is the most common systemic mycotic disease of cats*, and also affects dogs, horses, cattle, humans, and many other species. Disease is sporadic and, as is usually true for the systemic mycoses, the infection is apparently *neither contagious nor zoonotic. Most cases have chronic nasal disease*, with sneezing and serous or mucopurulent discharge. Other common manifestations include ulcerating cutaneous nodules, neurologic disease, chorioretinitis or panophthalmitis, and pneumonia.

Cryptococcus neoformans is a basidiomycete yeast-like fungus. Unlike the dimorphic fungi *Blastomyces* and *Coccidioides*, the sexual mycelial phase of *Cryptococcus* does not occur under normal laboratory conditions. *C. neoformans* ssp. *neoformans*, the usual cause in temperate climates, is a saprophyte found in soil, pigeon or other avian guano, and decaying organic matter. *C. neoformans* ssp. *gatti* is found mainly in association with eucalyptus trees in tropical climates. Four serotypes are defined by antigenic variation of the capsule; serotypes A and D comprise *C. neoformans* ssp. *neoformans*, and serotypes B and C comprise *C. neoformans* ssp. *gatti*.

Most infected animals do not develop clinical disease. It is commonly assumed that, as in humans, clinical disease only develops if immune responses are impaired by malnutrition, corticosteroid therapy, or immunosuppressive lentiviral infection. However, predisposing causes of immunosuppression are not often discovered in naturally occurring cases in cats. Infection is mainly acquired by inhalation of basidiospores (yeast cells) from contaminated dust. Most cases develop nasal disease, and infection may progress by local spread to the brain, aspiration to the lung, or hematogenous spread to brain, eyes, lymph nodes, skin, and other organs. Occasional cases of cutaneous cryptococcosis are probably the result of local inoculation, and *cryptococcal mastitis* in cows is an ascending rather than hematogenous infection.

The major virulence factors of Cryptococcus are the capsule and the production of melanin. The thick capsule, composed of glucuronoxylomannan and other mannose-rich polysaccharides, impairs phagocytosis, activates complement, and may suppress T-cell responses. The role of the capsule in concealing the yeast from the immune response is highlighted by uncommon strains of *Cryptococcus* that lack a capsule; these are readily phagocytosed, incite a strong granulomatous response, and are generally minimally pathogenic. Most strains consistently produce a capsule in tissues, but its thickness is variable in cultures.

The ability to synthesize melanin when grown on specific substrates is associated with virulence, and attributed in part to the enzyme phenoloxidase (laccase). Melanin and/or phenoloxidase may scavenge oxygen radicals produced by activated macrophages, and modulate the host immunoinflammatory response. Other potential virulence factors include secretion of eicosanoids and mannose protein that modulate immune and inflammatory responses, and production of superoxide dismutase and laccase that augment resistance to oxidative killing.

Immunity to *Cryptococcus* is dependent on delayed-type hypersensitivity reactions, in which interferon-γ and other cytokines elicit and activate macrophages and perhaps neutrophils to kill the yeast using reactive nitrogen and oxygen intermediates. In addition, cytotoxic T-cell responses may directly limit viability or proliferation of this pathogen.

The lesions take the form of gelatinous masses, granulomas, or ulcerating nodules. Facial swelling is a common feature of cryptococcal rhinitis of cats. Infection may spread locally from the nasal cavity to involve the skin, oral mucosa, eyes, or brain, and occasionally there is wider dissemination to local lymph nodes, lung, and other viscera. Skin lesions are often nodular and ulcerative. Visceral lesions consist of multifocal discrete white gelatinous lesions. Gross lesions in the brain are often subtle, but may include gelatinous material in meninges.

The prominent histologic lesion is a mass of yeast, and the abundant non-staining capsular material lends a "soap bubble" appearance to the lesion (Fig. 5.91). In contrast to other mycotic infections, the granulomatous reaction is often quite minimal, presumably because the capsule masks the yeast from recognition by phagocytes. *C. neoformans* yeast bodies are 4–8 μm diameter, plus a capsule which varies from 1 to 30 μm thick (Fig. 5.92). Occasional yeast have single buds that are

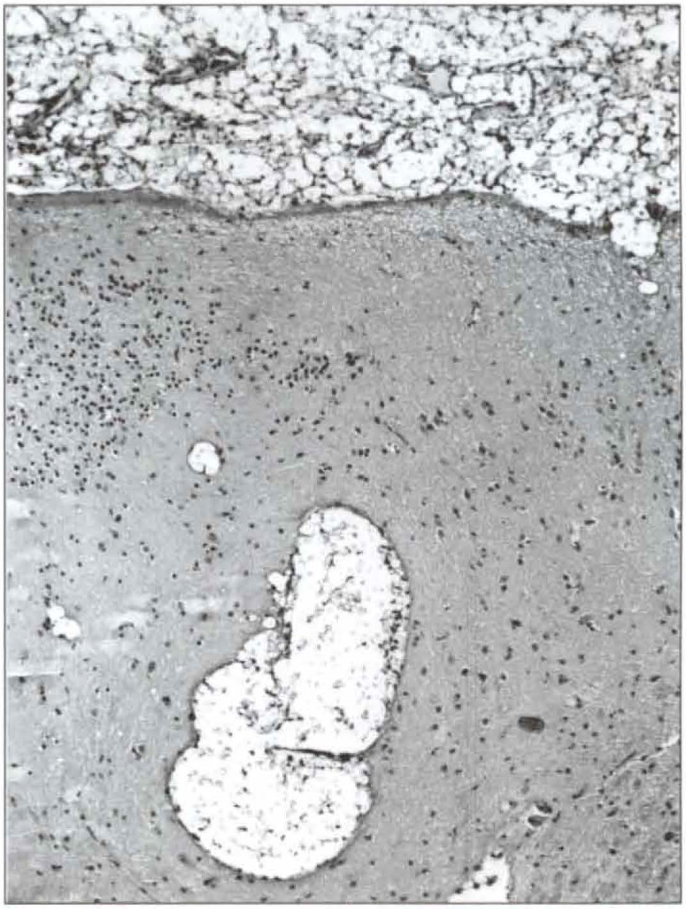

Figure 5.91 Cryptococcosis in a cat. Granulomatous meningitis with extension along Virchow-Robin space. Note the characteristic soap-bubble appearance.

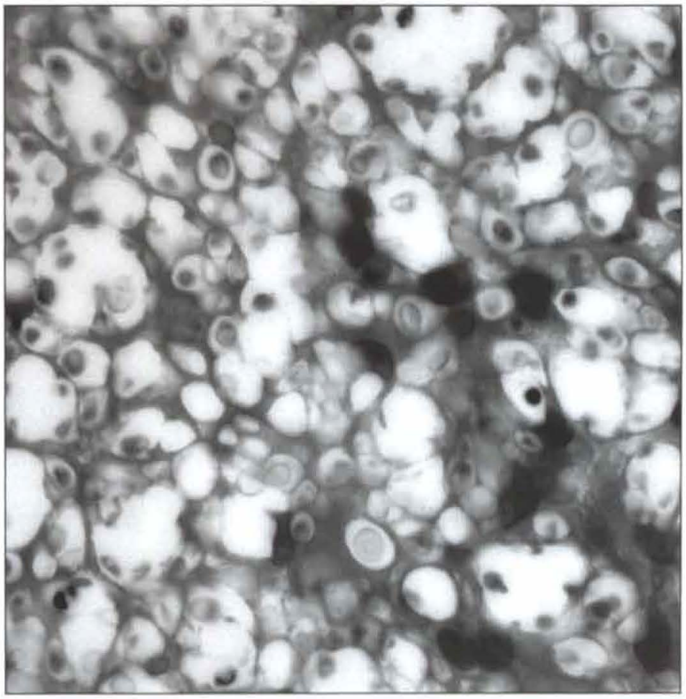

Figure 5.92 Cryptococcosis in a cat. The material in the lung is mainly formed by yeast with a thick capsule, and relatively few macrophages.

attached by a thin stalk; *this narrow-based budding differentiates Cryptococcus from Blastomyces.*

The *diagnosis* is usually based on identifying the yeast in histologic sections or cytologic smears. *The thick capsule is characteristic – C. neoformans* is the only pathogenic fungus with a capsule – and it can be easily identified with mucicarmine. In wet mounts, the capsule can be identified by negative staining with India ink. The yeast bodies stain with periodic acid–Schiff (PAS) or methenamine silver, and melanin production may be demonstrated with Masson–Fontana stain. Culture, which is required for definitive diagnosis, is easily performed on Sabouraud's agar. Detection of antibodies to capsular antigen in serum or cerebrospinal fluid is a useful method of clinical diagnosis.

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Coccidioidomycosis

Coccidioidomycosis is endemic in the semiarid Lower Sonoran life zone of the southwestern USA, northern Mexico, and parts of Central and South America. The disease affects humans and dogs, is less common in horses and cats, and may cause incidental pulmonary lesions in cattle and swine. Clinical manifestations in dogs include fever; chronic respiratory disease with coughing, weight loss, and eventual respiratory distress; visceral disease causing anorexia, weight loss, and malaise; draining cutaneous nodules; lameness resulting from osteomyelitis; ocular disease; and heart failure due to myocardial or pericardial lesions. Nodular skin disease is the most common presenting sign in cats. Clinical findings in horses include chronic weight loss, pulmonary disease, and lameness due to osteomyelitis.

Coccidioides immitis is a geophilic dimorphic fungus. The mycelial form survives well in dry hot conditions, grows after periods of intense rainfall in soil containing fecal or other organic matter, and releases arthroconidia that are widely disseminated in wind-blown dust after the soil desiccates. *Inhalation of airborne arthroconidia is the usual route of infection*; local inoculation occasionally causes a cutaneous lesion that does not usually progress to systemic infection. *Although most animals in endemic areas probably become infected during their life, relatively few develop disease.* Following deposition in the lung, the arthroconidia transform into the yeast form. Immature *spherules* are 10–20 μm diameter (Fig. 5.93); as they mature, the spherules (or

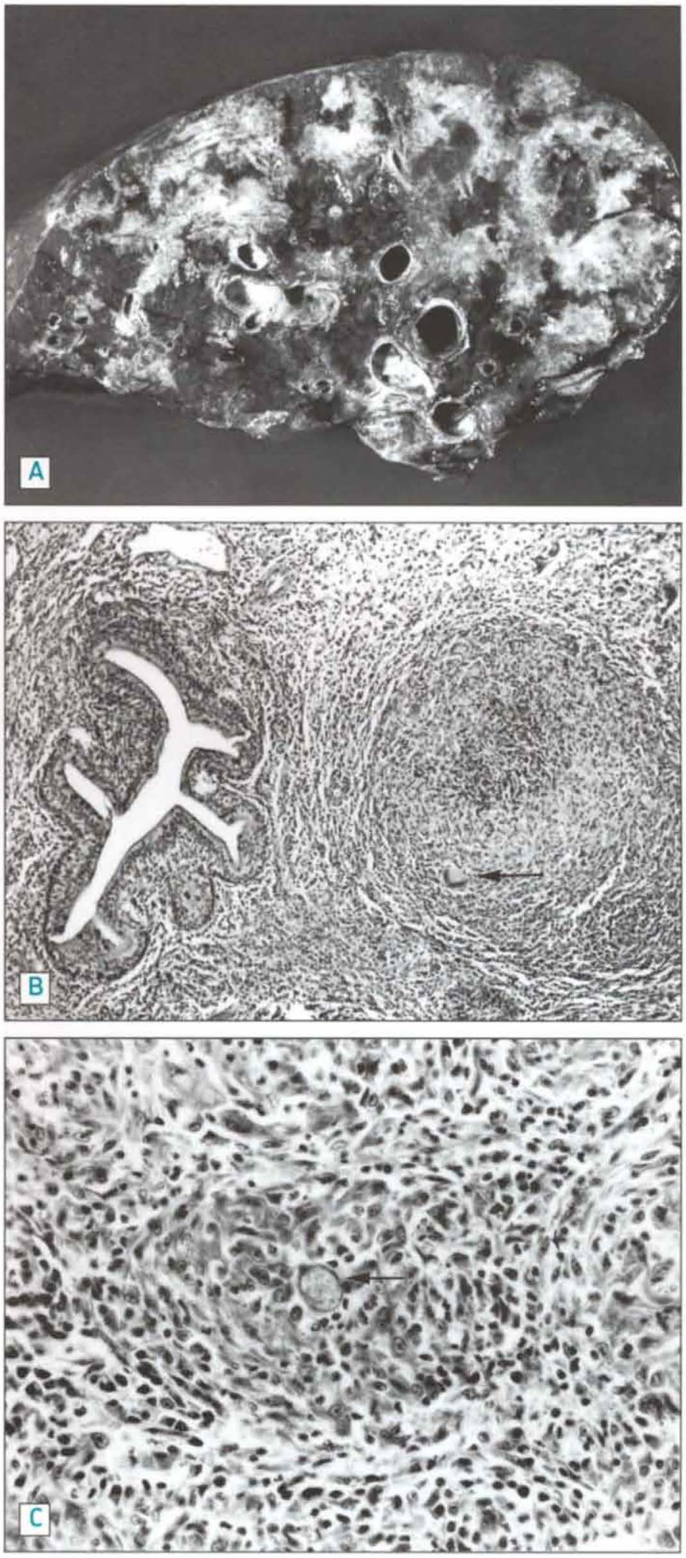

Figure 5.93 Pulmonary coccidioidomycosis in a horse. **A.** Chronic granulomatous interstitial pneumonia, with multiple coalescing foci of consolidation. **B.** Granuloma adjacent to a bronchiole, with a giant cell (arrow). **C.** Spherule (arrow) in the center of a small granuloma. (Courtesy of TE Dorr.)

sporangia) enlarge up to 200 μm diameter and develop numerous 2–5 μm *endospores*. Mature spherules rupture, and the released endospores form either new spherules in tissue or mycelia if released to the environment. Infection of necropsy personnel has been attributed to inhalation of aerosolized tissue endospores, although the paucity of such cases suggests the risk is limited.

Lesions may be limited to the *lungs*, where they vary from nodular to miliary. The *pyogranulomas or granulomas* are gray-white nodules that often contain a caseous or liquefying center (Fig. 5.93). Large nodules may be formed by a collection of small discrete granulomas separated by fibrous tissue. The initial reaction to the infectious forms – inhaled arthroconidia and endospores released from mature spherules – is primarily suppurative. The lesion forms a pyogranuloma or granuloma as it matures, with epithelioid macrophages, a few giant cells, lymphocytes, and neutrophils. Spherules may be numerous, but chronic cases with little neutrophil reaction may have only a few organisms engulfed by giant cells. In cattle, spherules are often enmeshed in eosinophilic material similar to the Splendore–Hoeppli phenomenon; this may reflect the higher level of resistance of this species.

Systemic lesions are present in a minority of infected animals, and result from hematogenous spread from the lung. Cases with systemic lesions that lack pulmonary involvement probably reflect resolution of the lung disease rather than an extrapulmonary route of infection. Tracheobronchial lymph nodes are often enlarged and reactive, but generalized lymphadenopathy is uncommon. Nodular lesions in the skin develop into draining nodules or fluctuating abscesses. Osteomyelitis often occurs late in the disease, with osteolytic granulomatous or cavitating masses surrounded by proliferating new bone tissue. Granulomatous lesions may be present in the pericardium or the heart and cause right-sided heart failure. Granulomas in the central nervous system are most common in cerebrum and midbrain. Ocular lesions include chorioretinitis, retinal detachment, anterior uveitis, and keratitis. Other organs affected include liver, spleen, kidney, and testes. Abortion and mastitis are described in horses.

The **diagnosis** is usually established by identifying the spherules in tissue sections. Spherules are most numerous in lesions with prominent suppuration. *The large size and endosporulation of the mature spherules are characteristic*, and can only be confused with *Rhinosporidium seeberi*. Culture is a useful method of definitive identification; safety precautions are essential because arthroconidia are easily detached from the mycelial form and are highly infectious for laboratory personnel. Serum antibodies to *C. immitis* can be detected in most cases, particularly in the acute stages of disease, and high titers suggest active disease.

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Adiaspiromycosis – *Emmonsia* spp.

Adiaspiromycosis is primarily a disease of wild rodents, but rarely affects domestic animals and humans. The causative agents, ***Emmonsia parva*** and ***E. crescens*** (formerly *Chrysosporium* spp.), are dimorphic fungi that are closely related to *Blastomyces*. Lesions are most common in the *lung* but occasionally involve local lymph nodes, and consist of *nodules of granulomatous inflammation*. The diagnosis is based on finding adiaspores – large, spherical, uninucleate conidia – in the nodules. The adiaspores of *E. parva* are often 10–20 μm diameter, while those of *E. crescens* may be up to 300 μm diameter. Both have a thick PAS-positive wall. Their large size and thick wall might be mistaken for *Coccidioides*, but endosporulation is not a feature of *Emmonsia*.

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Parasitic diseases

Pneumonyssoides caninum

Pneumonyssoides (Pneumonyssus) caninum is a parasitic mite of the nasal cavity and paranasal sinuses of dogs. The prevalence is ~20% in Scandinavian dogs, but it appears to be less common in other areas. The female adult mite is motile, oval, light yellow, and 1–1.5 mm long. The mites are presumably transmitted by direct contact. They are usually an incidental finding, but clinical signs include sneezing, head-shaking, and impaired olfaction. The mites induce catarrhal rhinitis and sinusitis with goblet-cell hyperplasia, hyperemia, and infiltration of neutrophils, eosinophils, and lymphocytes. *Pneumonyssoides* is, in addition to allergic rhinitis, a consideration in cases of eosinophil-rich inflammation in the nasal mucosa.

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Linguatula serrata – pentastomiasis

Linguatula serrata, a member of the phylum *Pentastomida*, is of wide geographic distribution. Adult pentastomes are large, long-lived, specialized hematophagous parasites of the respiratory tracts of reptiles, amphibians, and carnivorous mammals. They are related to, but distinct from, the arthropods. The adults of *L. serrata* are transversely striated and tongue-shaped (hence the name). Males are ~2 cm in length and females 1.0–1.2 cm. The definitive hosts are carnivores, but in aberrant parasitisms herbivores and humans may be host to the final stage. Herbivorous animals are the intermediate hosts. Carnivores are infected by eating the infected viscera of herbivores, and the nymphs migrate to the nasal passages, where they mature. The parasites may be found anywhere in the nasal cavity, and occasionally they find their way into the paranasal sinuses or pass via the eustachian tube to the inner ear. They lie on the surface of the nasal mucosa and induce nasal irritation and a catarrhal to lightly blood-stained exudate. The gravid females discharge a large number of eggs that are removed by sneezing. The larvae develop in the alimentary tract of the intermediate host and migrate to the mesenteric lymph nodes and other organs, where they encyst and develop into infective nymphs. The small cysts are common in mesenteric nodes of cattle and sheep in some countries, and contain brown fluid; older lesions may mineralize and resemble tubercles.

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Eucoleus aerophilus

Eucoleus aerophilus (Capillaria aerophila) is a trichurid nematode (order Enoplida, superfamily Trichuroidea, family Trichuridae) *that parasitizes the trachea and bronchi of wild canids, domestic dogs, and occasionally cats.* The worms are slender, 2–3 cm long, and embedded in the airway mucosa. Histologic examination reveals characteristic features: bacillary bands, which are segmental thickenings of the hypodermis; a stichosome, which is a deeply basophilic gland encircling the esophagus; and the possible presence of embryonated eggs. The eggs are laid in the airways, move with mucus to the pharynx, are swallowed and passed in the feces. The eggs are oval with bipolar plugs, and closely resemble those of *Trichuris vulpis* of the intestine or *Pearsonema* (*Capillaria*) plica of the urinary bladder. The larvae undergo initial development in the egg, and then progress to the infective stage within earthworms, which are a required intermediate host. Eggs also hatch in about 40 days under suitable environmental conditions, but the resulting larvae are apparently not infective. After ingestion of the earthworm, the larvae reach the lungs in ~1 week and are mature in the trachea in about 25 days. Most infestations of *E. aerophilus* are inapparent and provoke only mild catarrhal inflammation. Heavy infestations cause more severe irritation that may result in obstruction of the lumen of the airways. Chronic coughing and intermittent dyspnea may then be observed, and secondary bacterial bronchopneumonia may occur.

A related trichurid, *E. (Capillaria) bohmi*, is occasionally reported in the nasal cavity and sinuses of wild canids and rarely in domestic dogs.

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Oslerus osleri

Oslerus (*Filaroides*) *osleri* is of wide geographic distribution and is common in wild canids. The tracheal nodules are uncommonly encountered during bronchoscopy or necropsy of domestic dogs, and they rarely cause clinical signs of chronic coughing or dyspnea. Most infestations occur in dogs less than a year of age, and are acquired from the dam through grooming or regurgitative feeding.

O. osleri is a 5–15 mm long nematode of the superfamily Metastrongyloidea, family Filaroididae. The thin-walled embryonated eggs are coughed up and swallowed and many hatch before being passed as infective larvae in the feces. Unlike other metastrongyles, the Filaroididae do not require an intermediate host. The first-stage larvae of *O. osleri* are immediately infective, and pups are infected by ingestion of larvae in the saliva, tracheobronchial secretions, or feces of their dams. Larvae migrate from the gut through the blood to the lung. They develop into fifth-stage larvae by 5 weeks after infection, and the tracheal nodules are detectable at 10 weeks and well developed by 18 weeks. The caudal end of the gravid female protrudes through the epithelium, and the eggs are laid on to the tracheal surface.

The typical lesions are single or multiple, 1–10 mm diameter, firm, gray-pink, sessile or polypoid, submucosal nodules in the trachea and bronchi, often in the region of the tracheal bifurcation (Fig. 5.94). The larger masses are oval with the long axis parallel to that of the trachea. On careful inspection, coiled worms are visible through the intact overlying mucosa.

The nodules are formed by coiled adult or fifth-stage larval nematodes lying in tissue spaces in the lamina propria, and the nodules are often encircled by fibrous tissue (Fig. 5.94). The adults have coelomyarian musculature, a gut formed by few multinucleate cells with indistinct microvilli, and larvae or embryonated eggs within the uterus. The live worms provoke little reaction apart from a few lymphocytes and plasma cells. Dead worms incite a foreign-body reaction with neutrophils and giant cells. Immature worms, probably still migrating towards the trachea, may be found in the pulmonary lymphatics and occasionally in the alveoli without significant tissue reaction. The *diagnosis* is based on identifying the adults in histologic or crush preparations of the tracheal nodules, or discovering larvae in smears of tracheal mucus or Baermann preparations of feces.

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Crenosoma vulpis

Crenosoma vulpis is a common lungworm of foxes, but also occurs in other canids, including domestic dogs, perhaps in mustelids, but not in cats. It is a nematode of the superfamily Metastrongyloidea, family Crenosomatidae, and snails and slugs are intermediate hosts. *Adult worms reside in bronchioles and small bronchi.* After a prepatent period of 18–21 days, adults produce larvae that are passed in the feces for about 8 months. The usual gross lesions in dogs are gray consolidation of the dorsocaudal lung. Histologically, the lesions caused by adult worms are catarrhal, eosinophilic bronchitis and bronchiolitis. Aspirated larvae may induce a granulomatous reaction in alveoli.

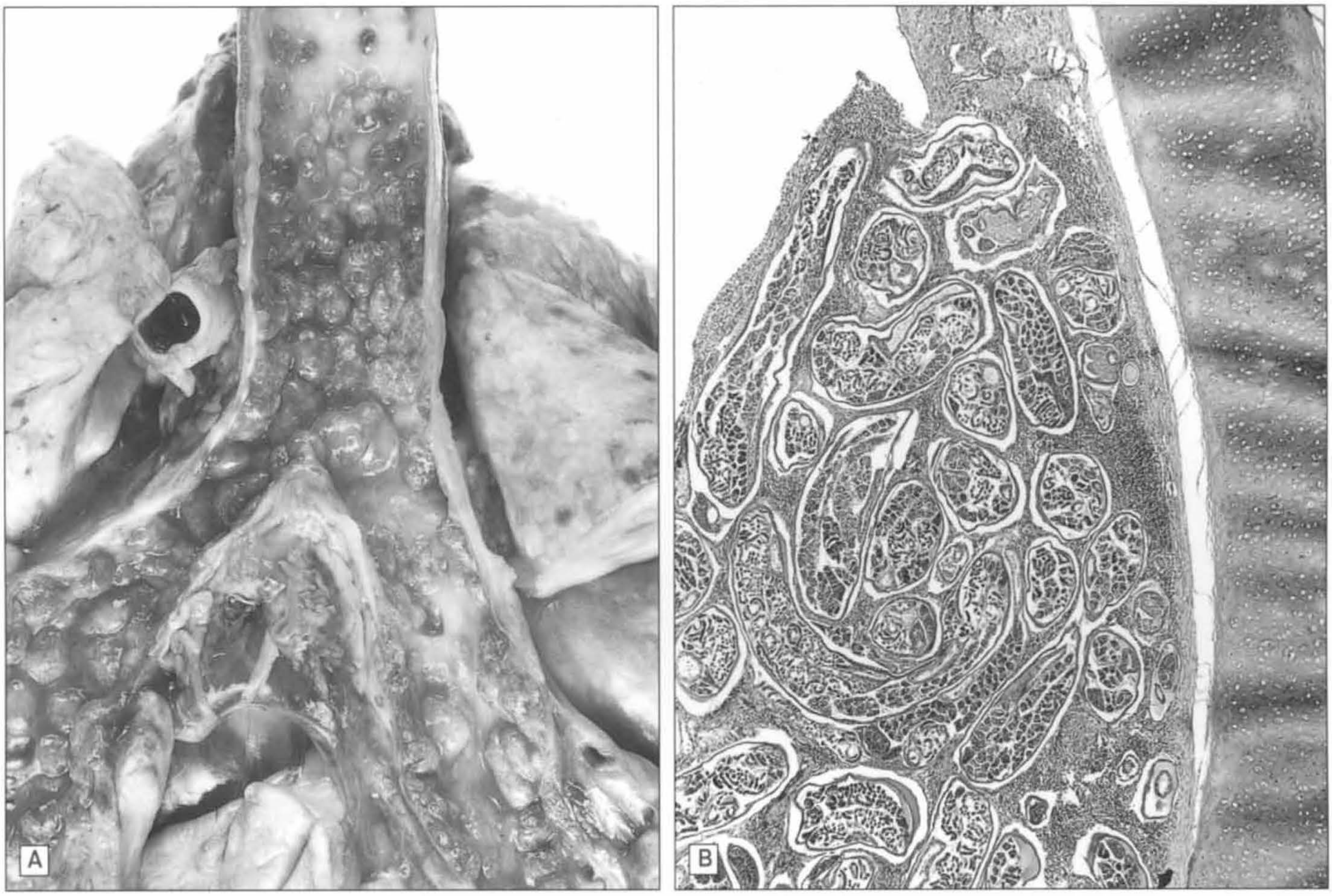

Figure 5.94 ***Oslerus osleri*** infection in a dog. **A.** Numerous nodules in the mucosa at the tracheal bifurcation. **B.** Histologic section of a nodule showing cross-sections of worms and mononuclear cell reaction.

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Filaroides hirthi

Infection is mostly reported in colonies of laboratory Beagles, but cases are described in pet dogs. Adult *Filaroides hirthi* are 6–10 mm in length and contain larvae or embryonated eggs in the uterus. *The adults live in alveoli and respiratory bronchioles.* Like other members of the Filaroididae, *F. hirthi* has a direct life cycle, and infective first-stage larvae are passed in the feces. Many infections are probably acquired from the dam.

In most cases the lesions are incidental at necropsy, with gray-tan or black-green, 1–5 mm diameter nodules scattered widely in subpleural regions of the lung. Nodules may have clear cystic centers, or be white and firm. The lesions in fatal cases, which may occur in immunosuppressed dogs, are of severe generalized diffuse or miliary *granulomatous pneumonia.* Histologically, there is little response to living adult worms, but a severe granulomatous response with many eosinophils occurs around dead or degenerating worms. Killing the worms with an anthelmintic may incite the severe response. Larvae stimulate an acute neutrophilic reaction. Foci of granulomatous interstitial pneumonia can often be found in which worm remnants may no longer be identified. The **diagnosis** is based on discovery of larvae in smears of bronchial exudate, or identifying the adults in histologic sections of lung. Definitive diagnosis requires extraction of intact adults from the lungs, as the histologic appearance is similar to *Andersonstrongylus milksi.*

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Andersonstrongylus milksi

Andersonstrongylus milksi (*Angiostrongylus milksi*, *Filaroides milksi*) is a metastrongylid nematode in the superfamily Metastrongyloidea, family Angiostrongylidae. The literature related to this parasite is confusing, because the diagnosis in many reports is exclusively based on histologic lesions. However, examination of intact worms, which are difficult to tease from the lung, is required to differentiate *A. milksi* and *Filaroides hirthi* definitively. A molluscan intermediate host has been proposed, but the life cycle is unknown. *Adults inhabit the bronchioles and the alveoli*, and the gross and histologic lesions are similar to those caused by *F. hirthi*. Larvae may also be found in the brain, abdominal viscera, and other organs.

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Angiostrongylus vasorum

Angiostrongylus vasorum, or French heartworm, occurs in endemic foci in parts of western Europe, and is also reported in Uganda, South America, Eastern Asia, and Canada (Newfoundland). Most cases develop *chronic heart failure*, but *acute interstitial pneumonia* occurs in heavily infected dogs. Uncommon manifestations include disseminated intravascular coagulation, uveitis in response to aberrant migration to the anterior chamber of the eye, and larval emboli in kidneys, brain, or other tissues.

A. vasorum is a nematode of the superfamily Metastrongyloidea, family Angiostrongylidae. *Adults inhabit the pulmonary arteries and right ventricle of dogs and foxes.* Eggs pass via the blood to the lungs, where the larvae hatch, penetrate into alveoli, are coughed up, and are passed in the feces. The prepatent period is 38–57 days. Snails and slugs serve as intermediate hosts. *Angiostrongylus* can be differentiated from *Dirofilaria* by examination of intact adults, or by histologic examination. *Angiostrongylus* has thin coelomyarian musculature, a large, strongyloid intestine composed of few multinucleate cells, and eggs in the uterus. The larvae in the lungs are wider and more developed than the microfilariae of *Dirofilaria*. In contrast, *Dirofilaria* has well-developed coelomyarian musculature, a smaller intestine, and a uterus containing microfilariae.

Lesions during the prepatent period are mild. *Adult, 14–21 mm long worms are present in the pulmonary arteries*, and the lungs contain a few 1–2 mm red nodules, consisting of aggregates of eosinophils and mononuclear cells. The females have a "barber-pole" appearance due to the helically arranged red gut and white ovaries. More severe lesions develop at the time of patency, including proliferative endoarteritis in response to the adult worms in the pulmonary arteries, and eosinophilic and granulomatous pneumonia as a consequence of embolized eggs and larvae. Arterial lesions include thrombosis, thickening of the tunica intima by fibromuscular tissue and numerous eosinophils, medial hypertrophy, and lymphoplasmacytic aggregates in the adventitia. Pulmonary lesions consist of red or golden-brown, nodular or confluent areas of hemorrhage, edema, and firmness at the periphery of the lung. Histologically, *coalescing granulomas* formed by macrophages, eosinophils, and giant cells are centered on parasite eggs and larvae in the lung. There is mild proliferation of type II pneumocytes, alveolar hemorrhage, hemosiderin-laden macrophages, and arteriolar thrombosis. Fibrosis and recanalization of arterial thrombi develop as the lesion ages. Similar granulomas are reported in brain, kidney, and other tissues.

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Infectious respiratory diseases of cats

Viral diseases

Feline viral rhinotracheitis and *Felid herpesvirus 1* infection

Felid herpesvirus 1 (FeHV-1) infection is widespread in most cat populations, is an important contributor to upper respiratory tract disease in cats, and is a common cause of conjunctivitis and keratitis. Clinical signs are most common in kittens but occur regularly in adult cats, perhaps due to recrudescence of latent infections. Morbidity may be high in naive populations of kittens, but mortality is generally low and most cats recover in 10–14 days. Clinical signs include fever, oculonasal discharge, sneezing, coughing, and anorexia. *Chronic rhinitis and sinusitis* may develop due to intermittent reactivation of latent infections, loss of respiratory defenses due to epithelial or turbinate damage, or failure of drainage of sites of secondary bacterial infection in the sinuses.

General features of this family are described in the section on Infectious bovine rhinotracheitis, above. FeHV-1, like other α-herpesviruses, *causes cytolytic infections of mucosal epithelial cells and establishment of latency in the trigeminal ganglion, optic nerve, olfactory bulb, and cornea.* Reactivation of latent infection is often of unknown cause, but may be triggered by corticosteroid therapy, stresses associated with environmental changes, or parturition. The virus replicates optimally at temperatures less than 37°C; thus, most infections are limited to the upper respiratory tract and conjunctiva.

FeHV-1 is transmitted by contact with infected nasal or ocular secretions, or by aerosol for a distance of about 1 meter. Transmission by fomites may occur, but the virus survives for less than 18 hours under most environmental conditions. The host range is limited to felids; occasional cases are reported in cheetahs. The incubation period is typically 2–4 days. In most cases, infection is restricted to the nasal mucosa, nasopharynx, sinuses, and tonsils, with lesser viral replication in the conjunctiva and upper trachea. Viremia may occur in neonates, but is not common in older kittens. It is likely that most cats that recover from the disease remain latently infected, and perhaps 20–40% of these cats intermittently shed infective virus during periods of stress, corticosteroid therapy, change in housing, or lactation.

The distribution of **gross lesions** corresponds to the predilection sites for viral replication – the epithelium of nasal passages, pharynx, soft palate, conjunctivae, tonsils, and, to a lesser extent, trachea. The initial serous inflammation becomes mucopurulent or fibrinous within a few days, and crusting is often present around the eyes and nares. Multifocal erosions of the nasal mucosa are covered by mucoid or mucopurulent exudate. The trachea may contain hemorrhage or fibrinous exudate. Tonsils are enlarged and contain petechiae or rare foci of necrosis. The regional lymph nodes are usually enlarged, reddened, and edematous. Ulceration of the tongue is rare and only in severely affected cats. This contrasts with the frequent finding of vesicular to ulcerative lesions on tongue, hard palate, or nostrils with *Feline calicivirus* infection. Ocular involvement is usually limited to purulent conjunctivitis, but it can progress to ulcerative keratitis.

Microscopically, *large eosinophilic intranuclear inclusion bodies* are present in many virus-infected cells during the period of active viral replication from 2 to 7 days after infection. They may be found in lesions from cats dying of the disease, but are rarely detected beyond 7 days after infection and cannot be relied upon for diagnosis. Infected cells undergo hydropic change with cytoplasmic swelling and pallor. There is loss of epithelial organization and the disrupted epithelium is soon eroded or ulcerated. An acute inflammatory reaction develops with exudation of fibrin and many neutrophils. Focal necrosis accompanied by acute inflammation may be found in tonsils and local lymph nodes. Necrosis and resorption of turbinate bones have been described in experimental infections, and may impair nasal clearance of opportunistic bacterial pathogens. Repair may be delayed for up to 17 days after infection, and the epithelial tissue may undergo squamous metaplasia as it repairs.

Pulmonary involvement is uncommon except in fatal cases. In fulminating cases of viral infection, there is widespread multifocal necrotizing bronchitis, bronchiolitis, and interstitial pneumonia, with extensive serofibrinous flooding of airspaces. Secondary bacterial bronchopneumonia is a more common complication of FeHV-1 infection than is primary viral pneumonia.

Systemic disease is uncommon in FeHV-1 infection, in contrast to the analogous infections of young calves and dogs. Ocular lesions are described elsewhere. Experimental infection of pregnant queens produces abortion and generalized neonatal disease, but this has been difficult to identify in natural outbreaks. Multifocal hepatic, pancreatic, and adrenocortical necrosis are the expected features of systemic disease. A syndrome of nasofacial ulcerative dermatitis and stomatitis with histologic lesions of eosinophilic inflammation and infrequent epithelial intranuclear inclusion bodies may be associated with corticosteroid therapy or crowding. Necrosis accompanied by inclusion bodies has also been found in sites of osteogenesis in a wide variety of bones of kittens after intravenous inoculation. Degeneration of olfactory nerve fibers and focal lymphocytic infiltration of the olfactory bulbs have occurred in experimentally infected, germ-free cats, but the extent of lesions in the brain has not been properly documented.

The major *differential diagnosis* for upper respiratory disease in cats is *Feline calicivirus* infection. Minor or uncommon contributors include *Chlamydophila felis*, *Bordetella bronchiseptica*, and *Mycoplasma* spp.

The finding of eosinophilic intranuclear inclusion bodies amid necrotizing lesions of respiratory epithelium is usually sufficient for **diagnosis**. If necessary, this diagnosis can be confirmed by *virus isolation*. Several other techniques are applicable to necropsy tissues, including immunohistochemistry, polymerase chain reaction (PCR), in situ hybridization, and indirect immunofluorescence assays. PCR assays may be more sensitive than virus isolation for detection of infection in vaccinated cats, presumably due to the presence of serum neutralizing antibodies.

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Feline calicivirus

Feline calicivirus (FCV) is a common cause of upper respiratory tract disease, and also causes oral ulcers, chronic stomatitis, pneumonia, and lameness. Infection with FCV is widespread, and 15–25% of cats are subclinical carriers. Morbidity is often high, particularly in kittens, but most cats recover from clinical disease. Clinical signs are variable and include serous or mucopurulent nasal and ocular discharge, *oral ulcers*, conjunctivitis, sneezing and coughing, anorexia, and fever. Outbreaks of rapidly progressive fatal disease have been described.

FCV is a nonenveloped, 35–40 nm diameter, positive-sense, single-stranded RNA virus in the genus *Vesivirus*, family Caliciviridae. Open reading frames 1, 2, and 3 encode a helicase, a protease, and polymerase; the single capsid protein; and an RNA-associated structural protein, respectively. The capsid protein is the target of protective immune responses, but hypervariable regions in this gene confer antigenic variability that allows viral persistence in the face of a developing immune response.

Transmission is by direct contact with infected oronasal secretions, and fomites are a potential source of infection. The clinical manifestation of the disease probably depends on the strain of the virus, the immune status of the host, and the route of infection. The incubation period varies from 2 to 14 days, and many cats recover in 7–14 days after onset of illness. However, *viral infection persists despite resolution of clinical signs*, and 25% of infected cats continue to shed virus for months or years. These cats are important sources of infective virus for naive cats or cats with immunity to other strains of calicivirus. Occasionally, these chronically infected cats develop chronic lymphoplasmacytic and/or ulcerative stomatitis that is refractory to therapy.

Although FCV may cause upper respiratory infections, *ulcerative stomatitis and interstitial pneumonia are the predominant manifestations.* The oral ulcers, which begin as vesicles that rapidly rupture, are most often present on the dorsal surface or lateral margins of the tongue and on the hard palate. Cutaneous ulcers may occur on the nares and the muzzle.

Caliciviral pneumonia is an uncommon but not rare occurrence. Pneumonia is more likely following aerosol rather than oronasal exposure, and specific strains or quasispecies of FCV may have tropism for the lungs. Gross lesions are irregularly distributed but often include the margins of the cranioventral lung. The virus causes lytic infection of type I pneumocytes, resulting in acute to subacute interstitial pneumonia. Hyaline membranes may be present, and the alveoli often contain serofibrinous exudate and neutrophils. As the lesion heals, type II pneumocytes proliferate and form a cuboidal lining of the alveoli, and alveolar septa are thickened by lymphocytes, plasma cells, and fibrous tissue in the later stages.

An epizootic of highly contagious, rapidly fatal, systemic FCV infection affecting both kittens and adult cats has been described. Affected cats had oral ulcers as well as alopecia and cutaneous ulcers on the limbs, footpads, and inguinal region. Subcutaneous tissues were edematous with foci of fat necrosis, and there was pancreatitis with peripancreatic fat necrosis, disseminated intravascular coagulation, intestinal crypt necrosis, single-cell necrosis of hepatocytes, and interstitial pneumonia.

It is often impossible to distinguish FCV infection from other causes of upper respiratory tract infection in cats, particularly *Felid herpesvirus 1 (FeHV-1)* and *Chlamydophila*. *The finding of oral ulcers is suggestive of calicivirus infection.* In contrast to the bronchointerstitial pneumonia caused by FeHV-1, airway lesions are usually not present in FCV infection. Toxoplasmosis, sepsis, and aspiration of gastric acid are additional causes of interstitial pneumonia in cats. The lesions are, in most cases, not etiologically specific and *definitive diagnosis requires laboratory support.* FCV can be isolated from early lesions. Immunohistochemistry and polymerase chain reaction are alternative methods of diagnosis.

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Bacterial diseases

Bacterial pneumonia is uncommon in cats. The most common isolates are *Pasteurella multocida* and *Bordetella bronchiseptica*. These agents are carried in the nasopharynx of most healthy cats, and development of pulmonary infection requires that the normal lung defenses be impaired or overwhelmed. In addition to infection with *Felid herpesvirus 1* or *Feline calicivirus*, the predisposing causes are analogous to those described for canine bacterial bronchopneumonia.

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Chlamydial diseases

Chlamydophila felis (formerly *Chlamydia psittaci*) is of most importance as a *cause of persistent conjunctivitis in cats.* It is a minor contributor to upper respiratory disease in cats and, despite the disease name "feline pneumonitis," does not apparently cause pulmonary infection or disease.

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Mycoplasmal diseases

Mycoplasma felis, M. gatae, M. arginini, and *Acholeplasma laidlawii* often colonize the upper respiratory passages of cats. Limited evidence suggests that mycoplasmas are probably not important primary causes of respiratory disease in cats. Mycoplasmas can be isolated from tracheobronchial washes of 20% of cats with pulmonary disease, but from few normal cats. As in dogs, the significance of this finding is complicated by the potential for mycoplasmas secondarily to colonize lungs that are diseased for other reasons. Kittens could be experimentally infected with *M. felis, M. gatae, and M. arginini*, but did not develop clinical signs of respiratory disease. Unclassified *Mycoplasma* spp. have been isolated from pulmonary abscesses and lesions of suppurative pleuritis in cats.

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Parasitic diseases

Toxoplasmosis

Toxoplasmosis is described more fully in Vol. 2, Alimentary system. Lung lesions are most commonly identified in kittens, and consist of *pinpoint white foci or diffuse interstitial pneumonia* (Fig. 5.95), either alone or as a component of multisystemic disease. Histologically,

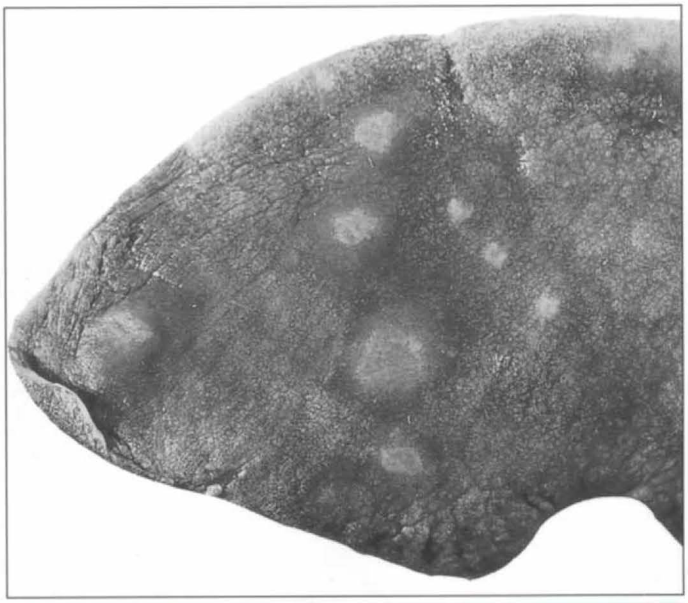

Figure 5.95 Toxoplasmosis in a cat. Multifocal necrotizing interstitial pneumonia.

there are multifocal to diffuse alveolar lesions, consisting of fibrin exudate or hyaline membranes, fibrinonecrotic debris in alveoli, proliferation of type II pneumocytes, and infiltration of neutrophils, mononuclear cells, and variable numbers of eosinophils. *Diagnosis* requires identification of cysts in the interstitium or in alveolar lining cells (Fig. 5.96).

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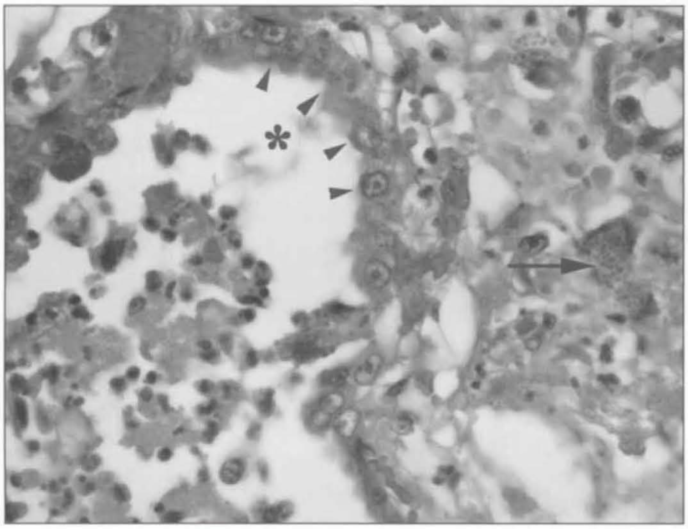

Figure 5.96 Toxoplasmosis in a cat. The alveolus (asterisk) is lined by cuboidal epithelium (arrowheads). A protozoal cyst is present in the interstitium (arrow).

Aelurostrongylus abstrusus

Aelurostrongylus abstrusus is a moderately common lungworm of cats. Many cats with nodular lung lesions display no clinical signs, but heavy infections cause chronic coughing or increased respiratory rate.

A. abstrusus is a nematode of the superfamily Metastrongyloidea, family Angiostrongylidae. The adults, which are slender and up to 1 cm long, live in the terminal and respiratory bronchioles. The eggs form nodular deposits in alveoli, where they embryonate and hatch to give first-stage larvae. The larvae reach the airways, are coughed up and swallowed, and are passed in the feces. Snails and slugs are intermediate hosts, while birds, rodents, frogs, and lizards are paratenic (transport) hosts. The life cycle can be completed if a cat eats either an intermediate host or a paratenic host. Infective larvae migrate to the lungs and reach maturity ~4–6 weeks after ingestion of the third-stage larvae.

Heavy infections in the prepatent period result in randomly distributed hemorrhages or white foci, which represent an eosinophilic and granulomatous reaction to the migrating larvae. *In the patent period, there are 1–10 mm diameter, firm, yellow, protruding nodules scattered throughout the lungs.* In severe infections, nodules may coalesce to form confluent areas of consolidation.

Microscopically, *the nodules are formed by masses of eggs and larvae in the alveoli and terminal bronchioles, with fewer adult worms* (Fig. 5.97). Eosinophils and neutrophils infiltrate the early lesions, but most cases are dominated by mononuclear cells and giant cells in the alveoli and around degenerating eggs and larvae. Alveoli are dilated and alveolar septa may be disrupted. Necrosis and mineralization seldom occur. Lymphocytic nodules form around vessels and airways, and there is hypertrophy and hyperplasia of the smooth muscle in the walls of the bronchioles and alveolar ducts. The presence of adult worms, eggs, or larvae in the bronchioles is associated with chronic catarrhal and eosinophilic bronchiolitis. In older lesions from which eggs and larvae have disappeared, alveoli remain epithelialized and septa are persistently thickened by fibrous tissue and smooth muscle.

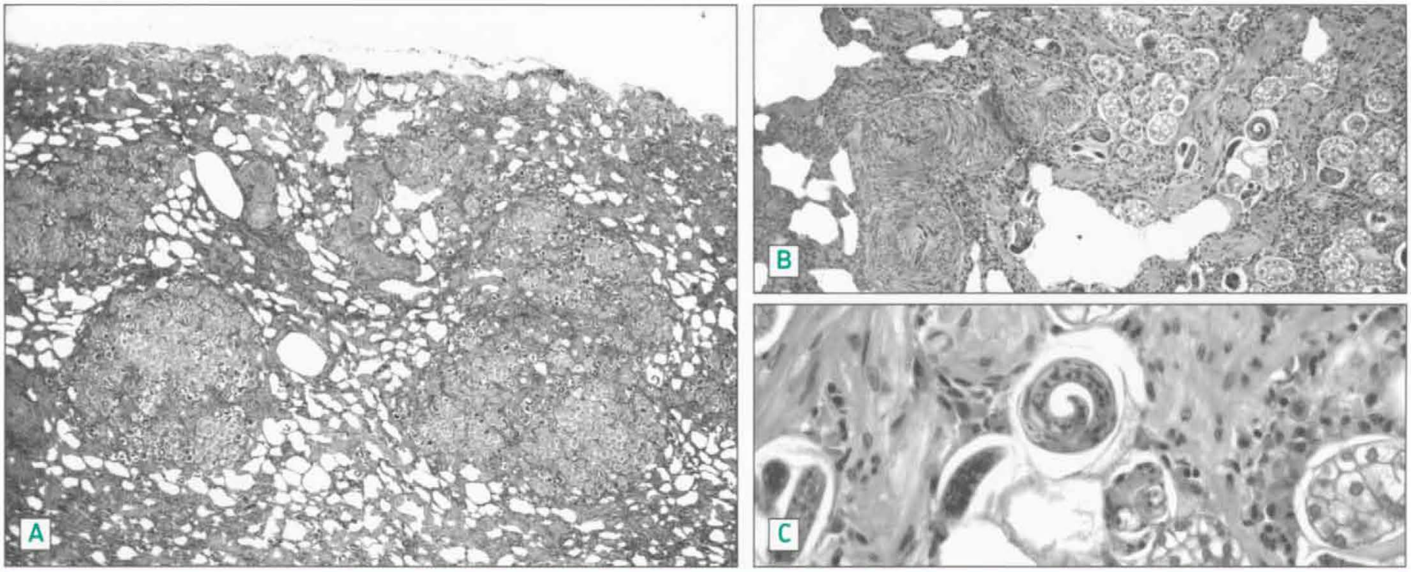

Figure 5.97 ***Aelurostrongylus abstrusus* infection** in a cat. **A.** The lung contains multiple subpleural nodules. **B.** Medial hypertrophy of pulmonary arteries and hyperplasia of smooth muscle in terminal bronchioles and alveolar septa. **C.** Nodules are filled with nematode adults, coiled larvae, and eggs.

Bronchial submucosal glands and smooth muscle in the media of small pulmonary arteries and arterioles may be quite prominent in cats infected with *Aelurostrongylus*, but these changes are also common in clinically healthy cats that have no evidence of parasitism.

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Paragonimus kellicotti

Of the *trematodes*, the only genus that has its final habitat in the lungs is *Paragonimus*. *Paragonimus kellicotti* occurs in the USA, and *P. westermanii* in the Far East. Mink and other fish-eating carnivores are the usual hosts of *P. kellicotti*, but it infects many other species. Among domestic animals, it is *most commonly found in cats* and occasionally in dogs. Clinical signs are usually absent in animals with paragonimiasis.

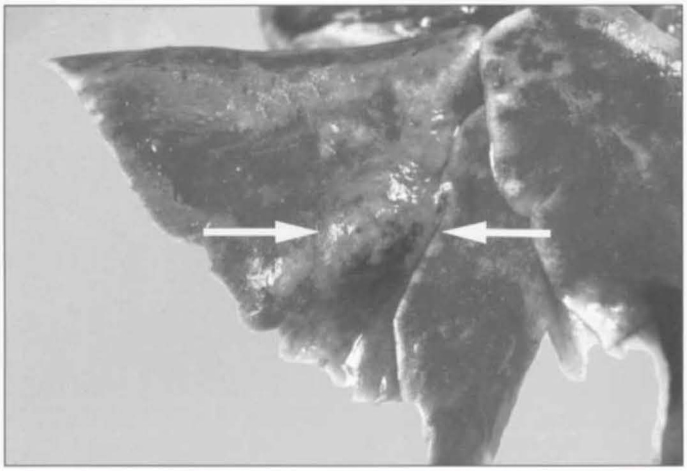

Figure 5.98 ***Paragonimus kellicotti*** infection in a cat. The caudal lung lobe contains a raised 1-cm-diameter nodule (arrows) containing an adult trematode.

The life cycle of the parasite is typical of the trematodes. The first intermediate hosts are small aquatic snails. The second intermediate host is a freshwater crab or crayfish. When the crayfish is eaten by the definitive host, the metacercariae are liberated in the intestine and migrate across the peritoneal and pleural cavities to the lungs. Their passage through the pleura is marked by multiple small hemorrhages and foci of eosinophilic and fibrinous pleuritis that heal as small umbilicated scars. Adult flukes are ovoid, red-brown, and up to 17 mm in length. They are often found in pairs in inflammatory cavitations in the pulmonary parenchyma and occasionally in the bronchi. The cavitations frequently communicate with bronchioles, permitting liberation of eggs into the airways, expulsion to the nasopharynx, and passage in the feces.

The cavitations, which are more common in the caudal lobes, are spherical, 1–3 cm diameter, soft, and dark red-brown (Fig. 5.98). The cavitations contain intense eosinophilic and granulomatous inflammation, hemorrhage with numerous hemosiderin-laden macrophages, a fibrous capsule, and adult flukes. The adults exhibit characteristic features of trematodes: the body is filled with loose parenchyma and there is no body cavity, paired ceca contain dark pigment, oral suckers are present in some sections, the tegument has surface spines, and vitellaria beneath the tegument contain eosinophilic globular yolk material. In patent infections, there are numerous 80–100 μm long, yellow-brown, operculate eggs, which persist as fractured shells in chronic lesions (Fig. 5.99). As cavitations mature and establish connections with bronchioles, they become partially lined by cuboidal epithelium to form *true cysts*. At this stage, bronchioles contain eggs and eosinophilic exudate, with hyperplasia of peribronchiolar glands and smooth muscle. Other lesions include chronic catarrhal eosinophilic bronchiolitis, granulomatous pleuritis, and pleural lymphangitis associated with the presence of eggs. Rupture of the cysts may rarely cause pneumothorax.

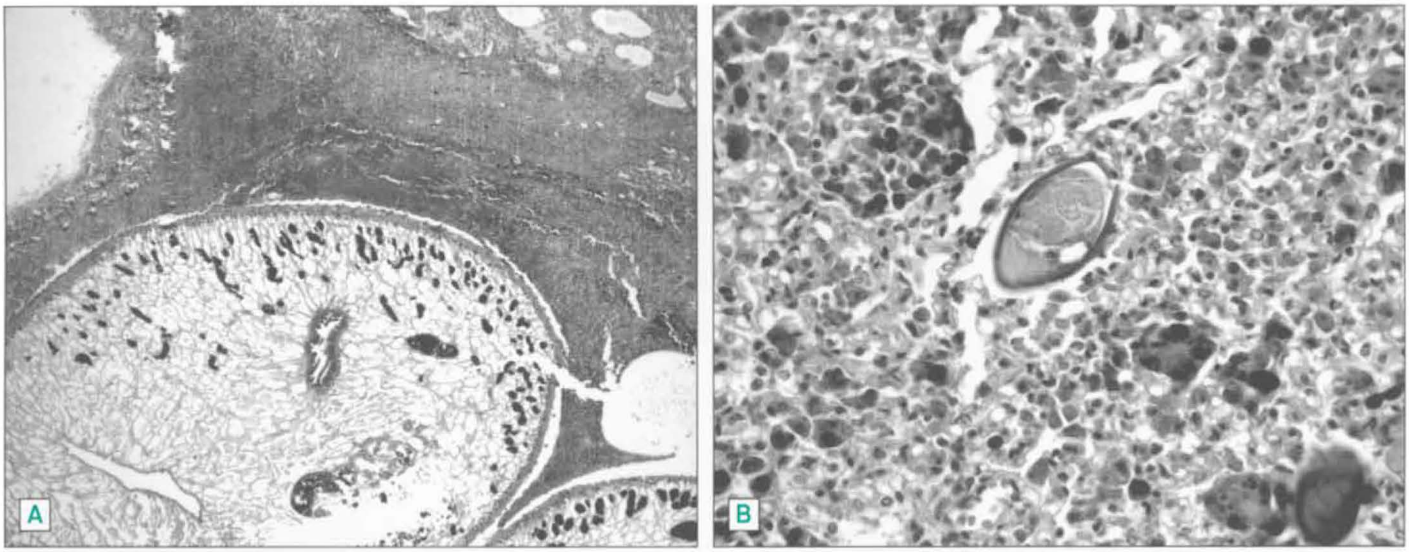

Figure 5.99 ***Paragonimus kellicotti*** **infection** in a cat. **A.** Adult trematode with adjacent atelectatic lung tissue. **B.** Operculate pigmented egg, with granulomatous inflammatory response.

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Miscellaneous parasites

Mammomonogamus (*Syngamus*) *ierei* is a common nematode causing lymphocytic laryngitis of cats in the Caribbean. *Eucoleus aerophilus (Capillaria aerophila)* is described in the section on Infectious respiratory diseases of dogs, above. *Oslerus (Anafilaroides) rostratus* is a viviparous parasite of cats that causes sinuous thickenings of the walls of large bronchi, formed by adult worms within cystic spaces. When the adults die, they provoke intense infiltration of neutrophils, with eventual mineralization and fibrosis.

Troglostrongylus spp., bronchial nematodes related to *Crenosoma*, are found near the Dead Sea. *Vogeloides felis* and *Metathelazia spp.* are spirurid nematodes that inhabit the bronchial wall and lung parenchyma, respectively. Ascarid larval migration is common in the lung of kittens and puppies, and may cause mild coughing.

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1

Cardiovascular system

M. Grant Maxie and Wayne F. Robinson

Diseases of the heart

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Diseases of the heart

The pathologic and functional changes observed in an organ or organ system are intimately related to the biology of the organ affected. We therefore first consider the normal form and function of the heart, and then its reaction to injury, under the anatomic units of the pericardium, endocardium, and myocardium.

NORMAL CARDIAC STRUCTURE AND FUNCTION

Gross anatomy

The heart is a **muscular pump** that sends oxygenated, nutrient-rich arterial blood throughout the body via the systemic circulation, and pumps de-oxygenated blood into the pulmonary circulation. Located within the mediastinum, the heart is enclosed in the fibroserous **pericardial sac**, which is lined by a serosal membrane and contains several milliliters of clear serous fluid that acts as a lubricant. **Heart weight** varies with species, age, sex, nutritional status, and fitness level of the animal, and averages about 1% of body weight in newborns, and decreases to 0.3–0.8% in juveniles and adults.

The heart consists of a right and left side; each side consists of an atrium and a ventricle. The ventricles function as two pumps in series. Venous blood from the body enters the **right atrium**, passes into the **right ventricle**, and is pumped through the pulmonary artery into the lungs to be oxygenated and to give up its carbon dioxide. Oxygenated blood returns via the pulmonary veins to the **left atrium**, enters the **left ventricle**, and is then pumped to the body via the aorta. Ventricular thickness varies greatly. Left ventricular free wall and interventricular septum are normally 2–4 times thicker than the right ventricular free wall. *An increase in myocardial mass is termed* **hypertrophy**; *an increase in chamber volume is termed* **dilation**. *An overall increase in cardiac size is termed* **cardiomegaly**.

The four cardiac valves are structured to allow unimpeded unidirectional blood flow, to prevent backflow, and to withstand high pressure. The **atrioventricular (AV) valves**, supported by tendinous cords (chordae tendineae) and papillary muscles of the ventricles, allow flow from the atria into the ventricles and prevent backflow into the atria. The right AV (RAV, tricuspid) valve has 3 valve cusps; the separation into 3 cusps may be difficult to discern. The left AV (LAV, bicuspid) valve consists of 2 cusps. The **pulmonic and aortic semilunar** (crescent moon-shaped) **valves** each have 3 cusps, and they allow flow into the pulmonary artery and aorta respectively and prevent backflow into the ventricles. The nodules (*nodules of Arantius*) in the center of the free edges of the semilunar valve cusps are normal structures. Valve cusps are normally thin and translucent. The free edges of the valve cusps (*cooptation region*) normally overlap during closure, and therefore fenestrations of the valve edges are usually insignificant. Normal valvular function depends on coordinated actions of the respective annulus and leaflets, and in the case of AV valves, the tendinous cords, papillary muscles, and ventricular walls.

The cardiac muscle and valves are supported at the base of the heart by the **cardiac skeleton**, *which consists of four fibrous rings, the fibrous triangle, and the fibrous or membranous part of the ventricular septum*. The fibrous triangle fills the space between the atrioventricular openings and the base of the aorta – it consists of dense fibrous connective tissue in pigs and cats, fibrocartilage in dogs, hyaline cartilage in horses, and bone (*os cordis*) in large ruminants.

The **blood supply to the heart** is primarily via two major coronary arteries. The *left and right coronary arteries* arise, respectively, behind the left and right cusps of the aortic valve at the base of the aorta. The left coronary artery gives rise to the left descending and the left circumflex coronary arteries. The epicardial coronary arteries give rise to the intramural arteries that penetrate the myocardium. Most coronary arterial blood flow occurs during ventricular diastole, when the coronary microcirculation is not compressed by myocardial contraction.

The **conduction system of the heart** consists of specialized conduction fibers that initiate and conduct an electrical impulse. The **sinus node** (sinoatrial node, SA node) is located subepicardially at the junction of the cranial vena cava and the right auricle. The impulse from this pacemaker causes atrial depolarization and contraction, and travels through internodal bundles to the **atrioventricular (AV) node** located in the interatrial septum just cranial to the coronary sinus. The impulse is delayed in the AV node before traveling via the **AV bundle** (bundle of His) to the **left and right bundle branches**, or crura, before terminating in **Purkinje fibers**. These modified myocardial cells ramify within the myocardium and transmit the depolarizing impulse to ventricular myocytes.

The components of the conduction system have different rates of diastolic depolarization, with the sinus node the most frequent and dominant. The frequency of depolarization of the sinus node is in turn modified by the autonomic nervous system. Atrial and ventricular myocytes do not normally exhibit the property of automaticity. When injured, however, the myocytes may repeatedly depolarize independent of a stimulus from the conduction system and may become dominant pacemakers. There are diseases that specifically affect the conduction system, but in domestic animals dysrhythmias are usually the result of disease involving the atrial and ventricular myocytes.

Histology

The cardiac wall has three layers:

1. the **epicardium**, the outermost layer;
2. the **myocardium**, the thick muscular middle layer; and
3. the **endocardium**, the innermost layer, which is continuous with the tunica intima of the great vessels entering and leaving the heart.

The **epicardium**, or visceral pericardium, consists of a thin layer of mesothelium resting on elastic fiber-rich connective tissue that merges with that of the myocardium. The epicardium is continuous with the **parietal pericardium**, which consists of an inner mesothelial layer and a thick layer of collagen and elastic fibers. The cavity between the visceral and parietal pericardium contains serous fluid that lubricates the surfaces and provides frictionless cardiac motion. Although the **pericardial sac** is not a vital organ, its proper function includes prevention of sudden cardiac dilation; assurance of equal end-diastolic transmural pressures throughout the ventricles; limitation of right ventricular stroke work; hydrostatic compensation for gravitational or inertial forces; reduction of friction; and maintenance of cardiac alignment and streamlined cardiac flow.

The **myocardium** consists of unique striated muscle cells – **cardiac myocytes** – embedded in a well-vascularized connective tissue framework. Individual myocytes are intimately joined at intercalated discs in order to function as a unit, and account for about 2/3 of the myocardial volume. Each myocyte consists of a single, central nucleus; mitochondria; contractile myofibrils composed of actin and myosin; sarcoplasmic reticulum that stores calcium needed for contraction; and the cell membrane (sarcolemma) and T tubules needed for impulse conduction. Myocytes may be binucleate in some species, e.g., dogs, and are commonly multinucleate in pigs (4–16 nuclei per cell). The actin and myosin filaments comprise contractile units called **sarcomeres**, which are demarcated by Z lines. Mitochondria occupy about 20–30% of the volume of cardiac myocytes versus 2% in skeletal muscle, reflective of the great dependence of cardiac muscle on aerobic metabolism. Sarcomere length varies from 1.6 to 2.2 μm; *ventricular dilation increases sarcomere length, which enhances contractility (Frank–Starling relationship)*. Atrial cardiac myocytes are typically smaller than ventricular cardiac myocytes; specific granules in atrial myocytes contain the hormone **atrial natriuretic factor** (ANF).

The cardiac **interstitium** contains blood vessels and fibroblasts in a diverse extracellular matrix that consists of collagens, proteoglycans, noncollagenous glycoproteins, growth factors and cytokines, and extracellular proteases. The collagen network of the heart is arranged into 3 interconnected regions: the collagenous weave of the *endomysium* around individual fibers, the *perimysium* around groups of fibers, and the *epimysium* around the whole muscle. This fibrillar collagenous network of the myocardium prevents over-stretching of myofibers, transmits myofiber-generated force to the chamber, and provides tensile strength and stiffness to the chamber. The collagenous struts that connect adjacent myofibers provide proper alignment during contraction. Struts that connect myocytes to capillaries help to maintain capillary patency during high intraventricular pressure.

As do all tissues, the myocardium has a limited set of *reactions to injury*, but the pattern and distribution of lesions may aid in arriving at a morphologic and etiologic diagnosis. The stage of irreversible damage to a myocyte, at least in ischemia, is determined by structural and functional changes in the mitochondria. Irreversible damage occurs after only 30 min of ischemia, whether or not flow is restored.

Only in neonatal hearts are cardiac myocytes able to *regenerate*. Once the neonatal period passes and a particular myocyte or group of myocytes is lost, there is no replacement. There is progressive scavenging of the necrotic remnants of the myocytes and replacement by fibrosis. Remaining myocytes do have the capacity for *compensatory hypertrophy*.

Myocardial injury may be functionally manifest as either irregularities in the rate or rhythm of impulse formation and conduction (dysrhythmias), or as depression in the force of myocardial contraction. **Dysrhythmias** are usually associated with acute, often focal myocardial injury. **Contractility disturbances** occur when there are either insufficient numbers of ventricular myocytes for effective contraction, or when there is ineffectual contraction of normal numbers of myocytes. Generalized, ineffective myocardial contraction is most commonly seen as a feature of dilated cardiomyopathies.

The **endocardium** lines the heart and consists of a monolayer of *endothelium* on a continuous basement membrane, covering the *inner subendothelial layer* of dense collagen, and the *outer subendothelial layer* composed of collagen, elastin, and blood and lymph vessels. The **atrioventricular (AV) valves** are endocardial infoldings with a central layer (*fibrosa*) of dense irregular connective tissue covered by layers of elastic fibers and, on the ventricular side, loose connective tissue (*spongiosa*). The central collagen of the AV valves is continuous with the dense collagen of the *chordae tendineae*, which are attached to the ventricular papillary muscles. The **aortic and pulmonic semilunar valves** consist of a *ventricularis* layer of collagen and radially aligned elastin on the ventricular side, a central *spongiosa* layer of water and glycosaminoglycans, and a *fibrosa* layer of collagen and elastin arranged in a circumferential direction on the great vessel side of the valves to resist back pressure of blood. Valve cusps are predominantly avascular.

Valvular abnormality from any cause can lead to disturbances of blood flow through the heart either by altering the normal unidirectional pattern of flow, or by impeding the chamber inflow or outflow. Alterations in hemodynamics reflect changes in systolic workloads characterized by changed pressure loading during contraction (*afterload*), or changed volume loading during diastole (*preload*). Most disorders have only a single preload or afterload change imposed. This encompasses all of the valvular disorders that cause either **insufficiency** (*failure to close*) or **stenosis** (*narrowing, failure to open*). Some of the congenital heart abnormalities, such as tetralogy of Fallot, have multiple preload and afterload effects. The general rules are:

1. valvular insufficiency increases the preload on the ventricle;
2. semilunar valvular stenosis, outflow tract stenosis, and hypertension increase the afterload on the ventricle
3. AV valvular stenoses and pericardial disorders decrease the preload on the ventricles.

The **coronary arteries** feed a dense capillary network that supplies the myocardium, endocardium, epicardium, cardiac skeleton, and bases of the cardiac valves. Blood collected by venules and veins is drained into the right atrium via the *coronary sinus*. Lymphatic capillaries draining the cardiac connective tissue, and are continuous with larger lymph vessels in the endocardium and epicardium. Sympathetic and parasympathetic innervation is extensive in the atria, and particularly around the SA and AV nodes.

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HEART FAILURE

Heart failure *is the end-point of a number of causes, rather than a specific disease*, and denotes a situation in which all compensatory mechanisms have been exhausted, and the heart is unable to meet the demands of the animal. The syndrome is characterized by *diminished cardiac output ("forward failure")*, or *damming back of blood in the venous system ("backward failure")*, or both. The heart can fail because of **impaired pump function** or because of **increased cardiac work demands** – both mechanisms may be operative in some cases. The heart can fail as a pump because of

1. decreased myocardial contractility, or loss or replacement of myofibers, or
2. decreased distensibility (*compliance*), or
3. dysrhythmia (abnormal heart rate and/or rhythm).

Increased cardiac work demands on one or both ventricles result from disturbed hemodynamics, in the form of sustained *pressure overload* (e.g., obstructed flow in aortic valvular stenosis) or *volume overload* (e.g., regurgitant flow in mitral valvular regurgitation).

Congestive heart failure *is characterized by vascular congestion and edema fluid within the interstitium and body cavities.* Not all cases of heart failure are of the congestive type. While in congestive heart failure the clinical manifestations are more or less constant, in **acute heart failure** there may be intermittent weakness and syncope caused by a substantial change in heart rate or rhythm resulting in a precipitous drop in cardiac output. The effect of acute heart failure is often sudden unexpected death, often with minimal lesions. **Circulatory failure, or shock**, *denotes a state of inadequate peripheral vascular perfusion* and is used to describe a state that may or may not be the result of heart failure. It is characterized by a drop in effective circulating blood volume. Common causes are acute internal or external hemorrhage, dehydration, or endotoxic shock. Shock can of course lead to acute heart failure.

Based on clinical manifestations, heart failure may be predominantly either left-sided failure or right-sided failure. **Left-sided failure** *results in left atrial dilation, pulmonary congestion and edema*, and clinical signs of dyspnea and cough. A prominent feature of chronic left-sided heart failure is the presence of hemosiderin-laden macrophages ("*heart failure cells*") in pulmonary alveoli, the result of diapedesis of red cells into the alveoli. **Right-sided failure** *results in excessive right atrial pressure and systemic venous congestion*, expressed as jugular distension, hepatic and splenic enlargement, ascites, and peripheral edema. **Cor pulmonale** *is defined as right heart failure secondary to pulmonary disease*, such as chronic obstructive pulmonary disease, dirofilariasis, or pulmonary thromboembolism. Because the cardiovascular system is closed, failure of one ventricle will ultimately lead to failure of the other, culminating in global or **biventricular failure**.

While there are many causes that lead to intermittent or permanent lowering of effective cardiac output, there is a limited set of responses to this by the animal. The major compensatory mechanisms include the **intrinsic cardiac responses** *of dilation and hypertrophy*, and the **systemic responses**, *which include increased heart rate and peripheral resistance, redistribution of blood flow, venular constriction, and increased blood volume.* In each case, the compensatory responses are at least temporarily beneficial and are directed toward increasing cardiac output to meet the metabolic needs of the animal. The range within which the compensatory mechanisms result in an increase in cardiac output is wide. Indeed, the increase may be up to five times the basal rate. As cardiac output falls below the requirements of the animal, signs of congestive heart failure appear.

These may be intermittent or prolonged, depending on the nature of the defect.

An untoward side effect of the systemic responses is increased capillary hydrostatic pressure that leads to the accumulation of **edema fluid.** This can involve the systemic or pulmonary veins. *Right-sided lesions*, such as right atrioventricular valvular insufficiency, pulmonic stenosis, or pulmonary hypertension, result in peripheral dependent edema [e.g., submandibular edema ("bottle-jaw"), brisket edema], ascites, hydrothorax, and hydropericardium. *Left-sided defects*, such as left atrioventricular or aortic valvular insufficiency cause pulmonary edema as the predominant finding.

Intrinsic cardiac responses in heart failure

Cardiac dilation

Dilation is a response to an increased workload in both physiologic and pathologic states. Increasing the end-diastolic volume, and hence stretching the myofibers, can increase the contractile force of the heart and increase the stroke volume and cardiac output. This is known as the **Frank–Starling relationship**, or heterometric autoregulation. Transient cardiac dilation is an acute response to increased demands, e.g., increased exercise. Continued stretch increases contractile force to a limit, after which increased stretch will result in a decrease in tension developed. The limit of stretch in most species appears to be a sarcomere length of 2.2–2.4 μm. Chronic dilation of a ventricle can occur through addition of sarcomeres and hence lengthening of myocytes.

Various disease conditions can cause an increased diastolic workload (preload) and hence dilation of the heart, such as arteriovenous shunts, and atrioventricular and semilunar valvular insufficiencies. *Acute volume overload of a chamber is expected to lead to physiologic dilation*, whereas chronic volume overload is one stimulus to the development of cardiac hypertrophy.

Cardiac hypertrophy

Cardiac hypertrophy is a reversible increase in the mass, but not the number, of myocardial cells. In general, **chronic pressure overload** *leads to myocardial hypertrophy*, whereas **chronic volume overload** *leads to combined ventricular dilation and hypertrophy*. Hyperplasia, or increase in the number of cells, is not an option as a myocardial response to workload given that the capacity of the myocyte to divide decreases rapidly prior to birth, and little mitotic activity is observed after the first few weeks of life. Cardiac hypertrophy is a compensatory response to an increase in mechanical work or to trophic signals, e.g., stimulation of β-adrenergic receptors in hyperthyroidism. The following discussion will be concerned only with the process of hypertrophy following a defined change in workload or stimulation. The presence of hypertrophy in the absence of an observable increase in workload will be considered to be primary and is discussed under the cardiomyopathies.

For hypertrophy to occur, there are requirements of **time, a healthy myocardium, and adequacy of nutrition of the myocardium.** The mass and size of the heart are increased through the actions of mechanical stimuli (stretch) and trophic stimuli (polypeptide growth factors; vasoactive agents such as angiotensin II and α-adrenergic agonists) that increase the rate of protein synthesis, the amount of protein in each cell, the size of myocytes, and the number of sarcomeres and mitochondria. The hypertrophic response is accompanied by selective up-regulation of several immediate early-response genes and embryonic forms of contractile and other proteins. The phenotype of the hypertrophic myocyte may be changed by this expression of embryonic genes, e.g., induction of atrial natriuretic factor occurs in ventricular myocytes, and late response genes such as β-myosin heavy chain and skeletal α-actin may be expressed (a switch from adult to fetal/neonatal forms). Other genes are also activated and selectively regulated in hypertrophy, including immediate early genes or proto-oncogenes that encode early regulatory factors (*c-jun*, *c-fos*, *egr-1*); growth factors (transforming factor-β, insulin-like growth factor, fibroblast growth factor), vasoactive agents (α-adrenergic agonists, endothelin-1, angiotensin II), and components involved in receptor-mediated signaling pathways, such as protein kinase C.

Physiologic hypertrophy of the myocardium in response to strenuous exercise is an extension of the normal growth process, and is usually without deleterious effect. However, *in pathologic states, hypertrophy is an adaptive response of limited benefit.* Hypertrophic myocytes have impaired intrinsic contractility and relaxation; impaired ventricular relaxation and compliance cause increased end-diastolic pressure and limited exercise performance. Once further muscle mass cannot meet the demands posed by increased workload, heart failure ensues. Degenerative changes occur in myofibers, including loss of myocardial contractile elements. Limitations to continued hypertrophy and the reasons for eventual myocardial failure include inadequacy of the vascular supply to the enlarged fibers, diminished oxidative capacity of mitochondria, altered protein synthesis and degradation, and cytoskeletal alterations. The capillary density in hypertrophic myocardium typically does not keep pace with myofiber size, intercapillary distances increase, and fibrous tissue is deposited in the interstitium ("*reactive interstitial fibrosis*"). Also, the altered isoforms of proteins produced by expression of fetal genes may be less functional than adult forms. Myocyte hypertrophy occurs only if increased protein synthesis exceeds the rate of degradation. Similarly, hypertrophy of the ventricle occurs only if growth of individual myocytes exceeds the apoptotic loss of myocytes; excessive apoptosis can contribute to failure of a hypertrophic heart. This postulate is supported by experimental work with receptor-mediated Gαq signaling of cultured rat cardiac myocytes (Gαq is the α subunit of the Gq family of G proteins, guanine nucleotide-binding proteins, which transduce signals) – moderate levels of Gq signaling stimulate cardiac hypertrophy, whereas high-level Gq activation results in cardiac myocyte apoptosis.

There are distinctive anatomic patterns of hypertrophy that accompany the increase in workload. **Concentric cardiac hypertrophy**, *that is, an increase in mass of the ventricle without accompanying increase in end-diastolic volume*, characterizes increased systolic loads (*increased afterloads*), such as aortic stenosis, pulmonic stenosis, and pulmonary hypertension in patent ductus arteriosus. There is often a decrease in the volume of the ventricular lumen. An increase in diastolic load (*increased preload*), typically produced by atrioventricular or semilunar valvular insufficiencies or by arteriovenous shunts, results in **eccentric cardiac hypertrophy**, *which is an increase in myocardial mass accompanied by increased end-diastolic volume (dilated chamber).* Because of dilation, the thickness of the involved ventricular wall is usually no more than normal and may be less.

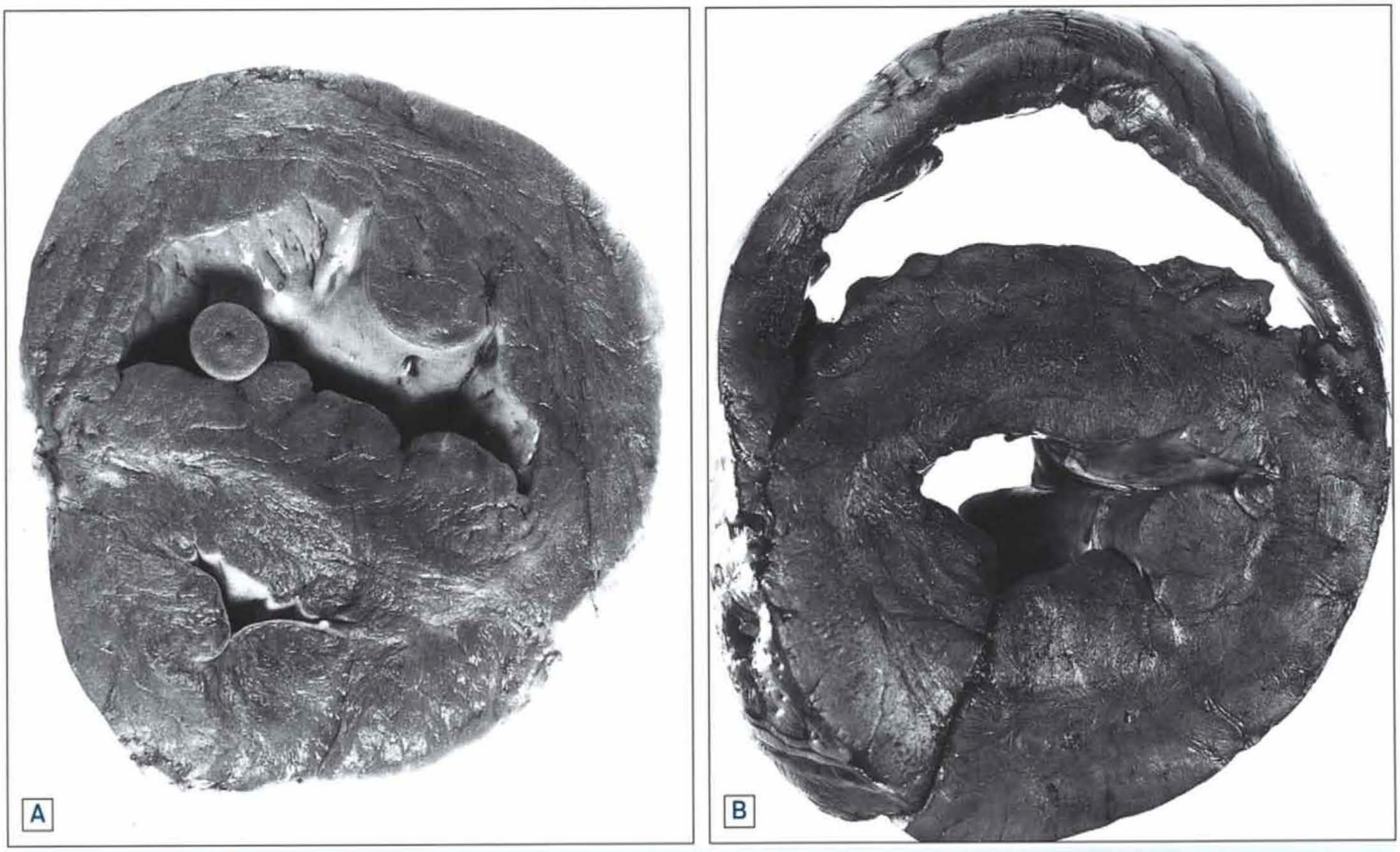

Figure 1.1 Myocardial hypertrophy. **A.** Cross-section of heart of a cow with chronic interstitial pneumonia. The **right ventricle** is greatly hypertrophied. Note marked increase in size of trabecula septomarginalis (moderator band). **B.** Cross section of heart of a dog with chronic glomerulonephritis. The **left ventricle** is hypertrophic and encroaching on the diastolic capacity of the right ventricle.

The gross appearance of the hypertrophic heart depends on the chamber affected and the nature of the insult. In general, hypertrophy of the right side of the heart makes the heart broader at its base; hypertrophy of the left side increases the organ length; bilateral hypertrophy produces a more rounded shape than normal.

In *concentric hypertrophy*, there is increased thickness of the wall of the affected chamber, and a marked increase in the size of the papillary muscles and the trabeculae carneae. Although the hypertrophy may emphasize one or other chamber, the whole heart is involved. When the right ventricle is involved, the moderator band (trabecula septomarginalis) may be much thickened (Fig. 1.1A). Extreme hypertrophy of one chamber may encroach on the diastolic capacity of its opposite number (Fig. 1.1B). Microscopically, the myocytes are enlarged, but the increase in the size of fibers is not uniform and is not always easy to discern on routine microscopy.

In *eccentric hypertrophy and dilation*, the heart tends to be globose, and even though the mass is increased, the wall is usually thin. The papillary muscles may also be attenuated.

In both types of hypertrophy and dilation, the endocardium may be diffusely opaque as a result of subendocardial fibrosis, and this alteration may be the best indication of dilation in the atria, in which dilation and hypertrophy can be difficult to assess.

An example of concentric cardiac hypertrophy occurs in cats with **hyperthyroidism** (thyrotoxicosis), a condition usually due to the presence of thyroid hyperplasia or adenoma. The thyroid glands in these cases are unilaterally or bilaterally enlarged, nodular, pink to dark-brown, and may contain cysts. Microscopically, the thyroids usually exhibit a mixture of hyperplastic areas, adenomatous nodules, and normal follicles (see also disorders of the thyroid gland in Vol. 3, Endocrine glands). The pathogenesis of ventricular hypertrophy in this disease is not clear, but may involve the direct action of thyroid hormones on myocardium, enhanced myocardial adrenergic receptor number or affinity, peripheral vasodilation, and work hypertrophy in response to increased peripheral tissue demands for oxygen and dissipation of heat. The hearts in most cases are symmetrically hypertrophied; however, some exhibit asymmetric hypertrophy. The left ventricular lumen is usually reduced in size. Affected myofibers are enlarged, but are not in disarray in the great majority of cases. The hypertrophy is reversible on return to euthyroidism.

Systemic responses in heart failure

The *extracardiac features of heart failure* stem from two basic pathophysiologic changes: **fluid accumulation** and **tissue or organ ischemia**. Depending on the cause of the heart failure, both effects may be present, but it is more usual for one to predominate.

Fluid accumulation results from the retention of sodium and water, which primarily involves the kidneys, and also involves atrial natriuretic factor released from the heart. The influence of the failing heart on the kidneys stems from its inability to supply them with an adequate flow of blood. Blood flow through different parts of the kidneys depends on the vasomotor tone of blood vessels within the parenchyma. It is considered that many, if not all, of the intrarenal blood flow changes in heart failure follow increased activity of the sympathetic nervous system.

The kidneys receive approximately 20% of the output of the left ventricle, almost all of which flows through the renal cortices. One of the earliest changes following a drop in cardiac output is redistribution of blood flow within the kidney. There is reduced flow through the outer renal cortex and increased flow within the outer renal medulla. This results in readjustment of the *filtration fraction*, which is the ratio of glomerular filtration rate (GFR) to renal blood flow. Contrary to expectations, there is a *less* than proportionate drop in GFR compared with renal blood flow resulting in an *increased* filtration fraction. As a consequence, proportionally more sodium moves through the glomerular filter, leading to proportionally more sodium being delivered into the proximal convoluted tubule. Because the rate of sodium resorption remains constant, a greater number of sodium ions are resorbed. Also, because of the increased filtration fraction, local plasma osmotic pressure in the efferent arteriole increases, causing greater resorption of sodium and water.

The alteration in renal blood flow in heart failure also increases the activity of the **renin–angiotensin–aldosterone** system, producing more sodium resorption from the distal convoluted tubule. There is also increased water-retaining activity by **antidiuretic hormone**.

A mechanism within the heart also regulates blood volume complementing the activity of aldosterone and the renin–angiotensin system. **Atrial natriuretic factor** (ANF), with natriuretic and diuretic properties, is present in granules in some of the atrial myocytes. If the atrial pressure is elevated or the atria are distended, ANF is released and causes natriuresis, vasodilation, suppression of the renin–angiotensin–aldosterone axis, and decreased arterial blood pressure. *In terms of homeostasis, ANF has effects opposite to those of aldosterone, thus providing a balance to fluid regulation.* Although plasma ANF is significantly increased in dogs with chronic left AV valvular insufficiency, it is not clear whether the metabolic effects of aldosterone or ANF predominate, but it would appear that the effects of aldosterone override those of ANF.

It should be noted that none of the hormones mentioned produce the edema of congestive heart failure if administered alone. In addition, once a new steady state has been reached, the hormonal state returns to relatively normal limits. Lastly, *the mechanisms that are brought into play are not exclusive to the syndrome of heart failure.* Any situation that leads to a drop in effective circulating blood volume will activate the sodium- and water-retaining mechanism. The fundamental difference between these states and congestive heart failure is that the total blood volume in heart failure is already more than adequate, but the *effective blood volume* is much diminished because of the poor cardiac output. The volume changes in heart failure should be viewed as an integrated response by the body to compensate for the inability of the heart to respond to the normal hemodynamic needs of the body.

The expansion of blood volume has both a beneficial and a detrimental effect. By increasing blood volume, venous return is enhanced and, in turn, cardiac output and tissue perfusion are improved. However, this is to the detriment of the balance between capillary hydrostatic pressure and plasma osmotic pressure. This leads to an increase in the amount of fluid in the interstitial spaces and body cavities.

Syndromes of circulatory failure

Circulatory failure, the term implying severe systemic consequences, falls into three general categories: cardiac syncope, peripheral circulatory failure, and congestive heart failure.

Cardiac syncope

Cardiac syncope is characterized clinically by profound changes in blood pressure and heart rate with bradycardia or tachycardia, either of which may result in inadequate output of blood. Both may occur in the presence or absence of organic heart disease.

In one form of cardiac syncope, hypersensitive or hyperactive reflexes, for which the vagus nerve is the efferent limb, may result in reflex inhibition of the heart, manifest as extreme bradycardia or as asystole. The sudden deaths that result from acute pleural irritation or the tracheal irritation of aspirated vomitus fall into this group, and obviously there may be no organic heart lesion.

In a second form of cardiac syncope, the heart rate is extremely rapid, and the cardiac output severely reduced. Such may occur in paroxysmal tachycardia, atrial flutter or fibrillation, and ventricular fibrillation.

Third, in organic heart disease with complete obstruction of impulse conduction from the atrium to the ventricle (complete heart block), syncope may occur if there is sufficient delay before the ventricle assumes an independent rhythm.

Finally, cardiac syncope may terminate a syndrome of congestive cardiac failure when the cardiac reserve is depleted and the heart cannot increase its output sufficiently to meet sudden increases in peripheral needs.

Peripheral circulatory failure

Peripheral circulatory failure is characterized by reduction in the effective circulating blood volume with insufficient venous return and reduced cardiac output. Acute hemorrhage and shock are examples of this form of circulatory failure.

Congestive heart failure

The combination of compensatory mechanisms, brought into play to maintain cardiac output, is in general successful. However, there is also the planting of the seeds of destruction. Both the local increase in venous hydrostatic pressure and the increased sodium and water retention by the kidneys tend to promote the development of interstitial edema. Depending on the inciting abnormality, it is usual for one side of the heart to fail before the other, but it must be remembered that the cardiovascular system is a closed circuit and that failure of one side will eventually embarrass the other.

Left-sided heart failure is ushered in by progressive dilation of the left ventricle and atrium, although this progression may be marked by exacerbations and remissions if hypertrophy is given time to develop. The major extracardiac manifestations of left-sided failure arise from the damming back of blood in the lungs and the diminution in cardiac output. The *pulmonary venous congestion* is transmitted back to the capillaries of the alveolar wall, and edema fluid accumulates in the interstitial tissue and the alveolar spaces. The consequent reduction in pulmonary vital capacity and impaired gaseous exchange of cardiogenic pulmonary edema result in hypoxic stimulation of the carotid sinus and medullary respiratory centers so that *reflex dyspnea* occurs. A *wheezing bronchial cough* is common and is presumed to be due to irritation of the respiratory mucosae by the edema fluid. Cyanosis may be present but is more often the rule in right ventricular failure.

At necropsy the **lungs** are usually of normal color, but may be light brown, and are heavy and wet. Stable, white froth is present in the airways, and fluid exudes from the cut surface. There is little evidence of the abundant fluid on microscopic examination, because of its low protein content. The alveoli contain erythrocytes and a scattering of macrophages, some of which contain hemosiderin. It may be necessary to use a differential stain for iron to confirm the presence of these so-called "*heart-failure cells*" or siderophages. They are more numerous in chronic disease, and hemosiderin within their cytoplasm may be sufficient to produce tawny discoloration of the lungs.

In **right-sided heart failure**, the major extracardiac manifestations depend on increased hydrostatic pressure in the systemic and portal venous systems, and the reduction of flow from the lungs to the left ventricle. Renal complications occur more frequently in right-sided than in left-sided heart failure, leading to increased blood volume, peripheral edema, and more marked azotemia.

There is some species difference in the **distribution of edema** in congestive heart failure. In ruminants and horses, *dependent subcutaneous edema* is expected; in the other species, excess subcutaneous fluid is scant or absent. In dogs, the predominant accumulation of fluid is in the *peritoneal cavity*. In cats, it is in the *thorax*.

Grossly the **liver** is enlarged and congested and has a "nutmeg" appearance on section due to *chronic passive congestion*. Microscopically the sinusoids are dilated, with atrophy of the parenchyma about the central veins. In more severe or acute cases, the parenchyma in this location may undergo degeneration or necrosis. It is exceptional for an animal with congestive failure to live long enough for severe fibrosis and nodularity to occur. Impaired hepatic function is not usually a significant part of the clinical course, although jaundice may be observed.

Congestion of the **stomach and intestines** is evident and this may impair their function, which is manifest usually as diarrhea. In horses, the subserosal lymphatics, particularly of the large bowel, are often readily discernible, dilated, and filled with edema fluid. The systemic and portal veins are distended and the **spleen** is enlarged and congested. However, this latter finding is masked if the animal in question has been euthanized using barbiturates.

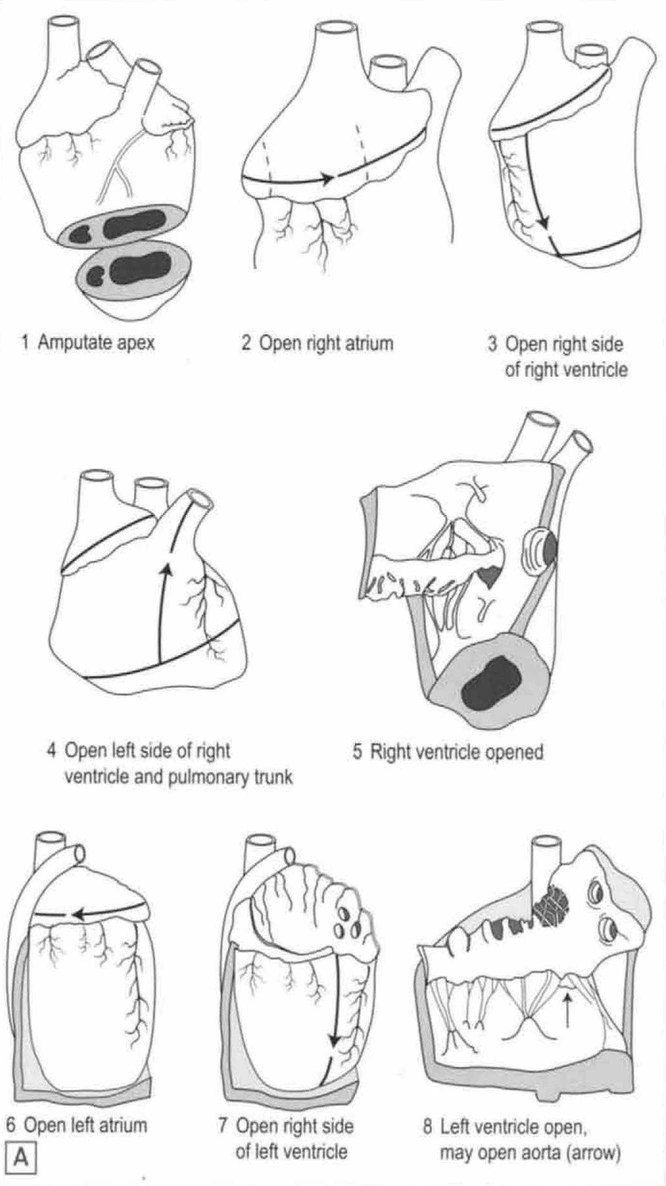
1 Amputate apex
2 Open right atrium
3 Open right side of right ventricle
4 Open left side of right ventricle and pulmonary trunk
5 Right ventricle opened
6 Open left atrium
7 Open right side of left ventricle
8 Left ventricle open, may open aorta (arrow)

Figure 1.2 A. Gross examination of the heart (see text for details). (1) Transect apex of heart and examine ventricular walls. (2) Open right atrium from caudal vena cava to the tip of the right atrial appendage. (3) Follow blood flow through right ventricle. (4) Cut right ventricular free wall adjacent to septum and out pulmonic valve. (5) Right ventricle and pulmonary trunk opened: moderator band transected. (6) Open left atrium. (7) Open left ventricle towards the apex. (8) Left ventricle opened and left atrioventricular valve exposed: aorta may be opened as indicated by arrow.

EXAMINATION OF THE HEART

In a **gross postmortem examination** of the heart, it is important to examine four major areas: pericardium, myocardium, mural and valvular endocardium, and the great vessels. A useful system is to follow the route of blood flow through the heart, that is, an inflow–outflow method of dissection (Fig. 1.2A). This technique may require modification in the case of cardiac anomalies. Examination of the heart in the planes used for echocardiographic examination could be beneficial.

- The initial examination of the heart and great vessels is best made with the organs in situ to assess abnormalities of size and position.
- Incise the pericardial sac and examine the pericardial fluid before the thoracic viscera are removed; note the volume and color of the fluid, the presence of fibrin, etc.
- Remove the thoracic viscera and examine the external surfaces of pericardium, epicardium, and great vessels.
- Open the abdominal aorta in situ to the iliac bifurcation and examine for thrombi and verminous arteritis; remove the aorta with the abdominal viscera in a horse to better examine the cranial mesenteric artery and its branches.

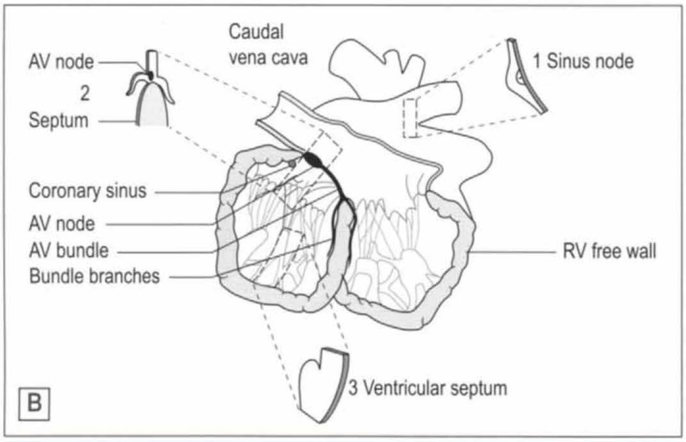

Figure 1.2 B. Tissue sampling from the heart. The cardiac conduction system can be assessed via **block 1**, in which the *sinus node* is located subepicardially in the terminal groove at the junction of cranial vena cava and right atrial appendage, and **block 2**, in which the *atrioventricular node* is located subendocardially on the right side of the interatrial septum, just cranial to the coronary sinus; serial sections through this block will reveal the AV node, the common bundle, and the origins of the bundle branches. A block through the *left ventricular free wall including papillary muscle*, or **block 3** through the ventricular septum, is the minimal representative sample to take from a grossly normal heart.

Table 1.1 Heart weight:body weight (HW/BW) ratios and left ventricular free wall plus septum:right ventricular free wall [(LV + S)/RV] ratios in necropsy accessions of cattle without significant cardiovascular disease

Age	HW/BW (%) (*n*)	(LV + S)/RV (*n*)
<7 days	0.91 (5)	1.69 (5)
7–30 days	0.90 (6)	2.13 (6)
3–365 days	0.48 (9)	2.78 (10)
>1 year	0.48 (11)	2.78 (15)

- The heart may be detached from the lungs if anomalies are absent, but it is usually better left attached to allow careful examination of vessels, e.g., pulmonary artery for thrombi.
- Examine the atria, ventricles, and coronary arteries externally, and confirm normal relationships.
- *When opening the heart, always inspect structures before cutting, and try to preserve a stenotic or dilated valve or vessel.*
- Transect the ventricles in their lower third perpendicular to the long axis of the heart, and check for hypertrophy and/or dilation, myodegeneration or necrosis, mineralization, fibrosis, and mural thrombosis. The apex may remain hinged to the remaining heart. Do not transect the apex if an apical ventricular septal defect is suspected.
- Open the *right atrium*, from the caudal vena cava to the tip of the atrial appendage. Note any thrombus, and check the tricuspid valve and the foramen ovale/fossa ovalis. Leave the cranial vena cava unopened, as a block of tissue to include the *sinus node* may be taken at the junction of the cranial vena cava and the right auricle. Examine the right atrioventricular valve (RAV, tricuspid valve) before cutting.
- Open the lateral side of the *right ventricle* adjacent to the ventricular septum. Examine the RAV and chordae tendineae. The septal leaflet of the RAV is normally thicker than the free leaflets. Transect the moderator band (trabecula septomarginalis).
- Cut through the rostral wall of the right ventricle, and open the *pulmonic valve* if there is no stenosis. Check the pulmonary arteries for thrombi, especially in animals with indwelling jugular catheters. Note the patency or closure of the ductus arteriosus (bear to the right when opening the pulmonary artery in neonates to avoid cutting through a patent ductus arteriosus and creating a false anomaly).
- Open the *left atrium* by cutting into the atrial appendage and then parallel to the ventricular groove. Examine the interatrial septum, foramen ovale/fossa ovalis, and the left atrioventricular valve (LAV, mitral valve).
- Open the *left ventricle* by cutting along the caudal border of the left ventricle adjacent to the ventricular septum, examining the LAV before cutting it. Chordae tendineae should be carefully examined before opening the LAV to ensure that pre-existing ruptures of the chordae are detected.
- Continue to follow the blood flow by cutting through the *aortic valve* and cutting along the aorta – note that the pulmonary artery will be transected by this action.
- The circumference of the four cardiac valves may be measured at this time with a flexible ruler. Normal LAV/RAV valve ratio for dogs is 0.66.

The right ventricle is responsible for systemic circulation in the fetus. Hence, in neonatal hearts, the wall thickness of the left and right ventricles is approximately equal; it is not until a month or more after birth that the mature proportions are attained. Ventricular wall thickness is poorly correlated with ventricular mass, and heart weights provide more valid information about ventricular hypertrophy than do thickness measurements, especially in cases of dilation and eccentric hypertrophy. *To accurately assess cardiac mass, the heart should be weighed and the weights compared to body weight.* After removing the pericardial sac and post-mortem blood clots, weigh the whole heart, right ventricular free wall, and left ventricular free wall plus septum.

- The ratio of normal heart weight to body weight (HW:BW) ranges from 0.5 to 1.0%, depending on age and species (Tables 1.1 and 1.2).
- The HW:BW ratio is higher in neonates than in adults; the more athletic species (horses, dogs) have higher HW:BW ratios; HW:BW ratio is increased by training or exercise; and males typically have greater HW:BW ratios than do females of the same species.
- The ratio of the weight of the left ventricle plus septum divided by the weight of right ventricular free wall [(LV +S)/RV] is 2.8–4.0 in mature animals; (LV +S)/RV >4.0 indicates *left ventricular hypertrophy*; (LV +S)/RV <2.8 is consistent with *right ventricular hypertrophy*.

The usual tissue block taken for routine **histology** is of left ventricular papillary muscle, as this area is one of the most susceptible to damage and most representative in cases of generalized disease. Detailed examination of the heart, including the conduction system, requires collection of tissue blocks (Fig. 1.2B) from the junction of the cranial vena cava and right auricle (sinus node), both atria, the interatrial septum/dorsum of the ventricular septum at

Table 1.2 Reference values for heart weight:body weight (HW/BW) ratio and ventricular ratio [(LV + S)/RV] in normal mature animals at necropsy

Species	n	HW/BW (%)		(LV + S)/RV	
		$\bar{x}$	$\bar{x} \pm 2$ SD	$\bar{x}$	$\bar{x} \pm 2$ SD
Dog	21	0.71	0.43–0.99	3.26	2.39–5.12
Horse	12	0.69	0.41–0.97	3.12	2.43–4.34
Cat	9	0.58	0.28–0.88	3.45	2.94–4.17
Cow	15	0.48	0.30–0.66	2.78	2.43–4.00
Goat	11	0.46	0.26–0.66	3.12	2.50–4.17
Sheep	8	0.41	0.17–0.65	3.33	2.63–4.54
Pig	8	0.40	0.32–0.48	2.94	2.38–3.84

HW, heart weight; BW, body weight; LV + S, left ventricular free wall + septum; RV, right ventricular free wall; $\bar{x}$, mean; SD, standard deviation.

the base of the heart just cranial to the coronary sinus (atrioventricular node), right ventricular free wall and AV valve, ventricular septum, left ventricular free wall/papillary muscle and AV valve, great vessels, plus any grossly evident lesions.

The selection of sections to study the **specialized conduction system** should include the sinus node, the atrioventricular node, the common bundle (bundle of His), left and right crura (bundle branches), and conducting fibers. The **sinus node** lies subepicardially in the terminal groove (sulcus terminalis) at the junction of the cranial vena cava and the right atrium. Sections should include either side of that site, to incorporate tissue in the sulcus terminalis region. The **atrioventricular node** is obtained by removing a block of tissue from the coronary sinus to the cranial edge of the septal leaflet of the right AV valve. The block includes interatrial septum and dorsal ventricular septum, and will then contain the atrioventricular node, which is located subendocardially on the right side of the interatrial septum, the common bundle, and the left and right crura. The specimen should be serially sectioned into samples 3 mm thick, and all samples should be processed.

In routine cases, there are no special requirements for fixation. Stains of particular use are hematoxylin and eosin, Masson's trichrome, phosphotungstic acid hematoxylin, Luxol fast blue, and Gomori's aldehyde fuchsin.

Note that *rigor mortis* begins earlier in myocardial than in skeletal musculature, and reaches greater development in the more powerful left ventricle. Rigor should completely express the blood from the left ventricle; rigor of the right ventricle is less efficient, and emptying is incomplete. The presence of some clotted blood in the right ventricle is normal, whereas if present in the left ventricle it is indicative of incomplete rigor and therefore perhaps of severe myocardial degeneration. The presence of unclotted blood in the left ventricle some hours after death is more difficult to interpret. Unclotted blood – the result of fibrinolysis – may flow back into the ventricle when rigor passes.

Blood usually clots slowly after death and permits erythrocytes to sediment. Where blood is present in volume, as in the heart and arterial trunks, this process of sedimentation and subsequent clotting leads to the formation of "currant jelly" and "*chicken fat*" *clots*, the former containing erythrocytes, and the latter largely devoid of them. "Chicken fat" clots are to be expected in horses, which normally have a rapid erythrocyte sedimentation rate, and are in relative excess in anemia. Postmortem **clots** are to be distinguished from **thrombi**; clots are not attached to the endocardium.

Biopsy of the heart in not commonly undertaken in vivo, but is possible via transvenous **endomyocardial biopsy**. Multiple biopsy samples of the right ventricular myocardium may be obtained by this means, and may be used, for example, to monitor the cardiotoxic effects of anthracycline therapy.

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CONGENITAL ABNORMALITIES OF THE HEART AND LARGE VESSELS

In the transition from fetal to neonatal life, substantial adjustments occur within the cardiovascular system: there are alterations in the pressures in cardiac chambers and great vessels, the pattern of blood flow, and the volume of blood flow. Because of these changes, the retention postnatally of fetal vascular communications, such as the ductus arteriosus, may place an excessive load on the heart in the postnatal period and beyond. There are also congenital heart defects, such as pulmonic stenosis, which compromise the fetus, the newborn, and the adult. It is typically only those defects that allow adequate in utero development and a reasonably successful perinatal life that are recognized; anomalies sufficiently severe to cause death in utero, or in the neonatal period, often are not.

As with most diseases, there is a spectrum of change. *The variation in severity of a particular lesion may be wide and will necessarily influence whether clinical signs are observed.* As such, there is a higher incidence of congenital heart disease than is recognized clinically. There is also a group of congenital heart diseases that do not produce clinical signs of heart failure, but which are manifested by upper alimentary dysfunction. Although little is known of the causes of many cardiac malformations in domestic mammals, there is no doubt that, especially in dogs, *some are genetically determined.* The demonstration that some congenital heart diseases are genetically determined arose from the observation that the incidence of defects was higher in purebred populations. It has been thought that both patent ductus arteriosus and conotruncal defects have a polygenic inheritance pattern, where the full expression of these diseases depends on the inheritance of a number of genes from different loci. However, studies on Keeshonds with conotruncal defects indicate that the inheritance pattern is most compatible with a single autosomal locus. The data suggest the presence of a mutant allele that is partially penetrant in heterozygotes and fully penetrant in homozygotes. Congenital subaortic stenosis in the Newfoundland dog is also genetically determined; it may either be polygenic or a single dominant gene that is variably expressed. Table 1.3 outlines the breed-specific predisposition to congenital heart disease in the dog.

There may be other as yet undefined factors that contribute to the development of congenital heart disease in domestic animals. In humans, cardiac and vascular anomalies are common features of several syndromes produced by chromosomal abnormalities and viral infections such as rubella. There is little evidence to suggest the presence of similar abnormalities or infections associated with congenital heart disease in domestic animals.

Table 1.3 Breed-specific predispositions to congenital heart disease in dogs

Defect	Breed
Patent ductus arteriosus	Bichon Frise, Chihuahua, Cocker Spaniel, Collie, English Springer Spaniel, German Shepherd, Keeshond, Kerry Blue Terrier, Maltese Terrier, Pomeranian, Poodle, Shetland Sheepdog, Yorkshire Terrier
Pulmonic stenosis	Airedale Terrier, Beagle, Chihuahua, English Bulldog, Fox Terrier, Mastiff, Miniature Schnauzer, Samoyed, Scottish Terrier, West Highland White Terrier
Subaortic stenosis	Boxer, English Bulldog, German Shepherd, German Shorthaired Pointer, Golden Retriever, Great Dane, Newfoundland, Rottweiler, Samoyed
Persistent right aortic arch	German Shepherd, Great Dane, Irish Setter
Tetralogy of Fallot	English Bulldog, Keeshond
Atrial septal defect	Doberman Pinscher, Samoyed
Ventricular septal defect	English Bulldog
Tricuspid insufficiency or dysplasia	German Shepherd, Golden Retriever, Great Dane, Labrador Retriever, Weimaraner
Mitral insufficiency or dysplasia	Bull Terrier, English Bulldog, Chihuahua, German Shepherd, Great Dane

The pattern and incidence of congenital cardiac disease varies with the species examined. In **dogs**, patent ductus arteriosus, pulmonic stenosis, and subaortic stenosis are common (Table 1.4). In **cattle**, atrial and ventricular septal defects and transpositions of the main vessels are most frequently diagnosed. Subaortic stenosis and endocardial cushion defects are the most frequent anomalies in **pigs**. In **cats**, endocardial cushion defects and congenital mitral insufficiency appear to be common. Congenital cardiovascular disease is quite uncommon in **horses**.

In cases of suspected cardiac abnormality, it is essential to examine the heart and large vessels in situ because relations are difficult to trace once the organ is removed. Some animals are born with hearts that, although of normal arrangement, are very small. In the majority of cases of cardiac abnormality, the anomaly is reflected in gross enlargement of the organ and in alteration of the size or disposition of the large vessels. Cardiac malformations are extremely variable, and their analysis can be perplexing if it is not remembered that they do follow fairly simple basic patterns. The recognition of the primary abnormality is an essential first step. This then assists in the recognition of secondary abnormalities, which develop as adjustments to allow blood to circulate through the heart. An understanding of the mechanics of abnormal development is necessarily based on an understanding of the normal development of the organ, for which reference should be made to a standard text of embryology.

There is no completely satisfactory system for classifying congenital heart defects, as defects may be complex or may overlap as

Table 1.4 Proportions (%) of canine congenital cardiac anomalies in four surveys

Anomaly[a]	Patterson (1953–65), $n = 248$	Mulvihill, Priester (1964–71), $n = 700$	Hunt et al. (1977–89), $n = 100$	Tidholm (1989–96), $n = 151$
Patent ductus arteriosus	30	36	34	11
AV valve dysplasia	4	9	18	15
Pulmonic stenosis	21	11	18	20
Aortic stenosis	15	6	10	35
Persistent right aortic arch	8	12	7	-
Ventricular septal defect	7	10[b]	7	12
Tetralogy of Fallot	4	-	3	0.6
Atrial septal defect	4	3	0	3
Other	7	13	3	3
Total (%)	100	100	100	100

[a] *~7–9% of dogs had more than one anomaly.*
[b] *Includes tetralogy of Fallot.*

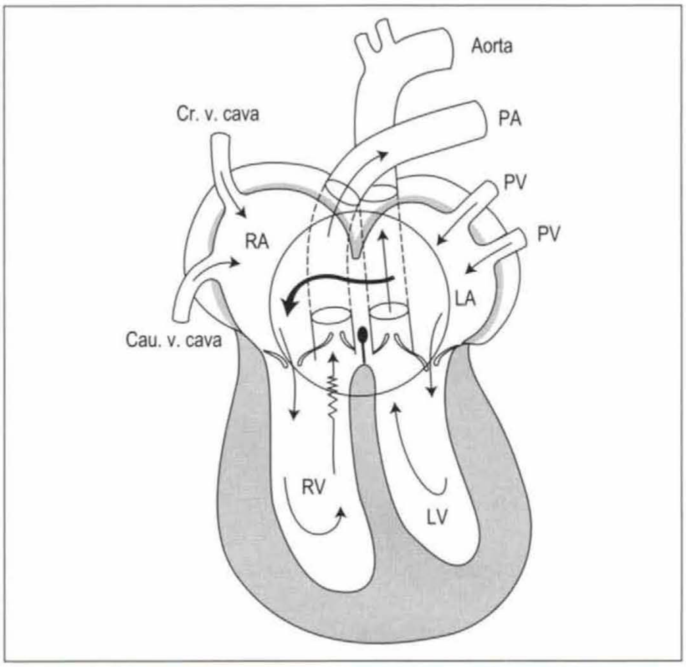

Figure 1.3 Atrial septal defect. Blood flows through the defect from the left atrium to the right atrium. The right ventricle dilates and hypertrophies under increased preload. A relative pulmonic stenosis is induced because of the increased volume in the right ventricle. Both atria also dilate following increased volume loads. Reverse shunts may occur with blood flowing from the right to the left atrium. (Figures 1.3, 1.5, 1.6, 1.7, 1.8, 1.10 from Robinson WF, Huxtable CRR, eds. Clinicopathologic Principles for Veterinary Medicine. Cambridge: Cambridge University Press, 1988, with permission. Abbreviations in these figures: LV, left ventricle; RV, right ventricle; LA, left atrium; RA, right atrium; PA, pulmonary artery; PV, pulmonary veins; Cr. v. cava, cranial vena cava; Cau. v. cava, caudal vena cava.)

part of a spectrum. Based on a combination of anatomical defects and functional effects, they may be classified as:

- malformations causing systemic to pulmonary (left-to-right) shunting,
- malformations of cardiac valves,
- transposition complexes,
- miscellaneous cardiac anomalies, and
- vascular anomalies.

Malformations causing systemic to pulmonary (left-to-right) shunting

In the development of the heart, there are three major arteriovenous communications: between the atria, the ventricles, and the great vessels. These connections are necessary for shunting of blood in the fetus. The atrial and ventricular septa close in utero; the foramen ovale and the ductus arteriosus close early in the neonatal period. Failure of closure results in *atrial septal defect, ventricular septal defect, or patent ductus arteriosus*, three of the more common congenital cardiac defects in domestic animals. Less common defects in this group include *atrioventricular septal defect, persistent truncus arteriosus, aorticopulmonary window*, and *anomalous pulmonary venous return*.

Atrial septal defect

In the fetus, separation of the left and right atria commences with the downgrowth of the *septum primum*, which grows toward the atrioventricular junction, where the developing endocardial cushions begin to form the atrioventricular valves and separate the ventricles. The septum fuses with the endocardial cushions, obliterating the *ostium primum*, and then begins to fenestrate in its middle. The fenestration is destined to become the *ostium secundum*. A second septum (*septum secundum*) develops downward and to the right of the septum primum. With its semilunar edge, the septum secundum and the remains of the septum primum form the **foramen ovale**. Within the left atrium, the septum primum forms the flap valve of the foramen ovale that allows blood flow from right atrium to left atrium in the fetus. In the majority of animals, anatomic closure of the foramen ovale follows functional closure postnatally. A *probe-patent foramen ovale* postnatally is not an atrial septal defect, for although the foramen ovale may not be anatomically closed, it is functionally closed (*valvular competent*) because left atrial pressure exceeds right atrial pressure.

An atrial septal defect can result from:

- defects of the septum between the right upper pulmonary veins and the cranial vena cava (**sinus venosus defect**),
- failure of fusion of septum primum with the endocardial cushions (**ostium primum defect**, low in the atrial septum adjacent to the atrioventricular valves, a form of atrioventricular septal defect), or
- an excessively large ostium secundum or inadequate development of the septum secundum (**ostium secundum defect**, in midseptum at the site of the fossa ovalis, the most common type).

The consequence of an atrial septal defect in the neonate is excessive flow from the left to right atrium, with resultant volume overload on the right ventricle and elevated central venous pressure (Fig. 1.3). In some cases, following the development of pulmonary hypertension, the flow through the defect is reversed, leading to cyanosis.

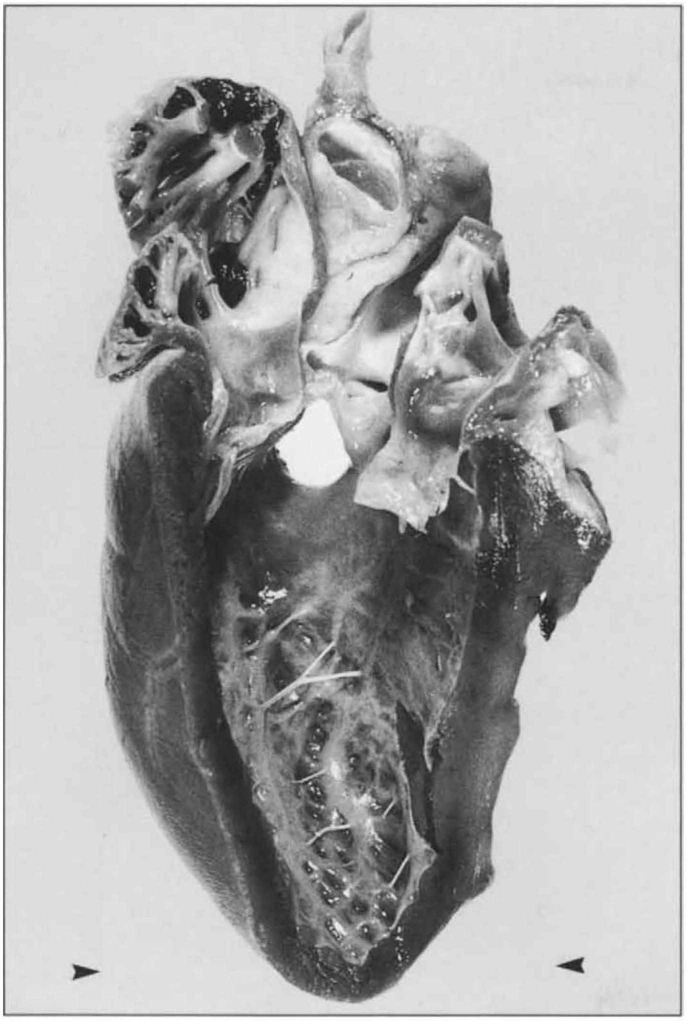

Figure 1.4 Ventricular septal defect in a goat. The defect is just below the aortic valve and the left ventricle is dilated. (5 cm between arrows.)

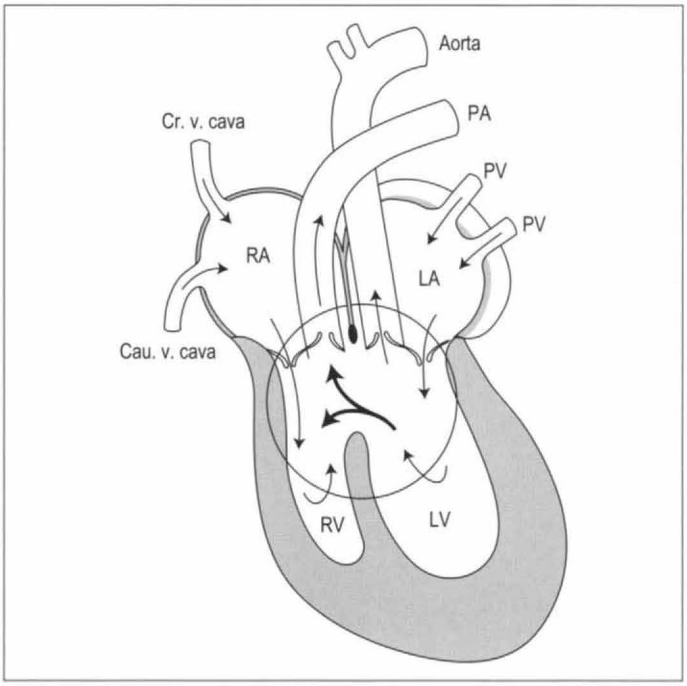

Figure 1.5 Ventricular septal defect. Both increased preload and afterload are imposed on the right ventricle by a VSD. The increased volume of blood returning from the pulmonary circuit also places increased preload on the left atrium and ventricle.

Atrioventricular (AV) septal defect

Also known as *endocardial cushion defects* or *atrioventricular canal defects*, these malformations arise from deficiency of the AV septum that separates the left ventricular inlet from the right atrium. The atrial septum primum must fuse with the endocardial cushions, which in turn contribute to the development of the atrial and ventricular septa, and to the medial leaflets of the left and right atrioventricular valves. Anomalous development may result in a range of anatomic defects, including *ostium primum defect (partial AV canal), ventricular septal defect with a cleft in the tricuspid valve*, or *common atrioventricular canal*. The common or complete AV septal defect consists of an ostium primum defect, a ventricular septal defect, and a common AV orifice.

Defects of the atrioventricular canal are among the most common defects in the pig. In the largest series examined, 40% of pigs with congenital heart disease had this defect. It is also a frequent finding in *cats*.

Ventricular septal defect

Ventricular septal defect (VSD) is one of the most common defects encountered in domestic animals. The separation of the left and right ventricles is completed by three parts of the embryonic heart: the muscular portion of the septum, the downward growth of the conotruncal ridges, and the membranous portion of the septum derived from the endocardial cushions. Defects can be related to defective development of any of the three parts. VSDs are usually single (Fig. 1.4), but may be multiple, and *most commonly involve the membranous septum*. This type of VSD is termed *paramembranous* or *perimembranous*, as they exceed the bounds of the membranous septum and involve a muscular defect at their periphery; they may also be referred to as subaortic or infracristal. Less common sites of VSD are *subpulmonary* (infundibular, conal, supracristal), *below the septal leaflet of the tricuspid valve*, or *in the muscular portion of the ventricular septum* towards the apex of the heart. While occurring most commonly as an isolated defect, VSD is also seen as part of a number of other defects, such as tetralogy of Fallot or persistent truncus arteriosus. There appears to be a high incidence of *spontaneous postnatal closure* of small VSDs in humans, and a similar phenomenon has been reported in dogs.

The presence of a VSD has no deleterious effect on the fetus because left and right ventricular pressures are equal and there is therefore little flow across the defect. *Postnatally, the effects of VSDs are related to the size of the defect and the level of pulmonic vascular resistance relative to the systemic resistance.* Pulmonary vascular resistance normally drops postnatally, leading to a *left-to-right shunt*. Left ventricular output is maintained by an increase in end-diastolic volume and augmentation of contractility by the Frank–Starling mechanism. Since right ventricular pressure equals left ventricular pressure, the right ventricle is confronted with a large systolic and diastolic load. *Both ventricles undergo hypertrophy*, the left being more obviously eccentric in nature (Figs 1.4 and 1.5).

Eventually, pulmonary hypertension can lead to shunt reversal (right-to-left), cyanosis, and death. The term **Eisenmenger complex** is applied to VSD cases in which, instead of the usual left-to-right shunt, flow has been reversed to a right-to-left shunt through the VSD as a consequence of severe pulmonary hypertension that is in turn due to high pulmonary vascular resistance. The more general term of **Eisenmenger syndrome** is applied to any anomalous circulatory communication (e.g., ASD, VSD, PDA) that leads to obliterative pulmonary vascular disease, with resulting reversal of the left-to-right shunt across the pulmonary-systemic communication.

Patent ductus arteriosus

Patent ductus arteriosus (PDA) is one of the more common defects, and it is recorded in all species. In the dog it has a polygenic inheritance pattern, and PDA may result from hypoplasia or asymmetry of ductal smooth muscle plus aorta-like elastic tissue in the ductus wall. The ductus develops from the sixth left branchial arch, and functions in the fetus to divert a major portion of blood from the pulmonary artery to the aorta. The flow from venous to arterial side is a consequence of the presence of the high vascular resistance of the fetal pulmonary bed. The structure of the normal ductus differs from the adjacent pulmonary artery or aorta. In contrast to the large elastic arteries, the media of the ductus has a dense layer of smooth muscle, which is responsive to a number of compounds. Among the most powerful are epinephrine, norepinephrine, and angiotensin; less effective ones include oxygen, acetylcholine, bradykinin, and indomethacin, a prostaglandin inhibitor. Prostaglandin E_2 keeps the ductus in a dilated state. Of the compounds mentioned, oxygen and acetylcholine are probably the most important in vivo in causing constriction.

With contraction of the medial smooth muscle, the ductus becomes functionally closed in the first few hours after birth. There are exceptions. The ductus in some foals may remain patent for up to 5 days, and a continuous murmur is detectable up to that time. A ductus arteriosus that remains patent beyond this time is considered to be abnormal. They are minimally responsive, or nonresponsive, to oxygen and other compounds that constrict the normal ductus.

Continued patency results in a number of sequelae that are dependent on the size of the PDA and the relationship between the pulmonary and systemic vascular resistance. In uncomplicated cases, the blood flow is from the aorta to the pulmonary artery *(left-to-right shunt)* during both systole and diastole. Depending upon the ensuing rise of pulmonary arterial pressure, there may be left-to-right flow only during systole. In severe cases, the shunt is reversed and flow from the pulmonary artery to the aorta *(right-to-left shunt)* occurs. If the ductus is of sufficient size, there is volume overload of the left ventricle and pressure overload of the right ventricle, resulting in compensatory hypertrophy of both ventricles. There is also left atrial dilation resulting from increased pulmonary blood flow (Fig. 1.6). The ascending aorta and pulmonary artery are dilated, probably resulting from a combination of turbulent flow and altered pressure relationships. The development of congestive heart failure with a large PDA is not only related to pulmonary vascular resistance, but also to the ability of the left ventricle to handle volume overload. In dogs where aorticopulmonary shunts were created experimentally, a communication of 3 mm or less in diameter may lead to the slow development of left ventricular hypertrophy, but it is otherwise well tolerated. A window of 5 mm in diameter may lead to pulmonary hypertension, with degenerative changes in pulmonary vessels and congestive heart failure.

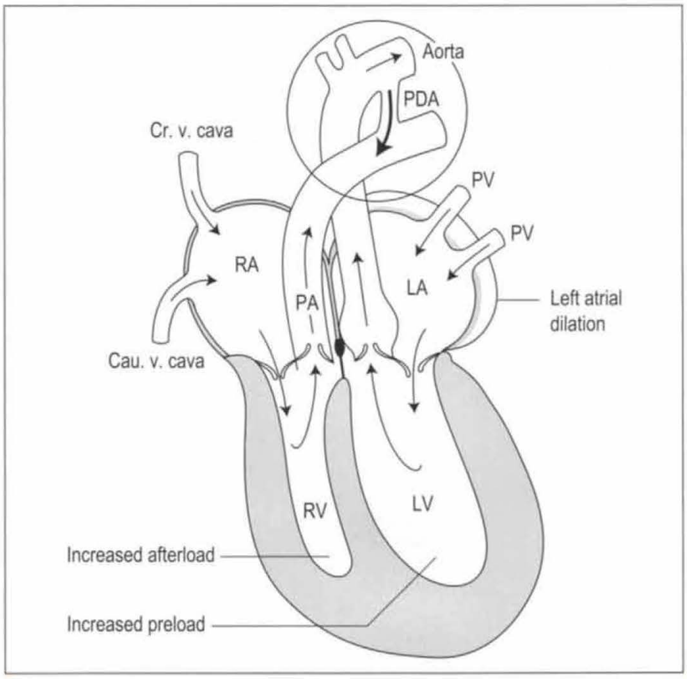

Figure 1.6 Patent ductus arteriosus. In most cases, blood flow through the PDA occurs during both systole and diastole from the aorta to the pulmonary artery. The right ventricle hypertrophies concentrically because of the increased afterload (increased pulmonary hypertension). The left atrium and ventricle hypertrophy eccentrically because of the increased preload (increased volume of blood returning from the pulmonary circuit). LV, left ventricle; RV, right ventricle; LA, left atrium; RA, right atrium; PA, pulmonary artery; PV, pulmonary veins; Cr.v.cava, cranial vena cava; Cau.v.cava, caudal vena cava.

As a consequence of turbulent flow, a PDA is predisposed to thrombosis, which is referred to as *endocarditis of the ductus*. The anatomic appearance of the PDA ranges from a *ductus diverticulum*, which is a blind funnel-shaped outpouching of the aorta at the site of the ductus, to a ductus that approaches the diameter of the aorta. The length of the patent ductus may also vary. In some cases there is virtually no ductus, but, instead, an opening between the aorta and pulmonary artery, which are closely approximated. Hypoplasia and asymmetry of ductus-specific smooth muscle and the presence of aorta-like elastic tissue in the ductus wall, as has been observed in some cases, may cause patency. In dogs, as the number of genes that determine its presence increases, the histologic appearance of the PDA more closely resembles the aorta, and the severity of the defect increases.

Malformation of semilunar or atrioventricular valves

Failure of adequate development of the semilunar valves usually results in valvular or subvalvular stenosis. Anomalous atrioventricular valves may be insufficient or stenotic or both.

Pulmonic stenosis

Pulmonic stenosis is a relatively common congenital anomaly in dogs, but an unusual finding in other domestic species. It is inherited in Beagles, and is suspected to be so in English Bulldogs, Bull Mastiffs, Chihuahuas, and terrier types. *The term pulmonic stenosis encompasses*

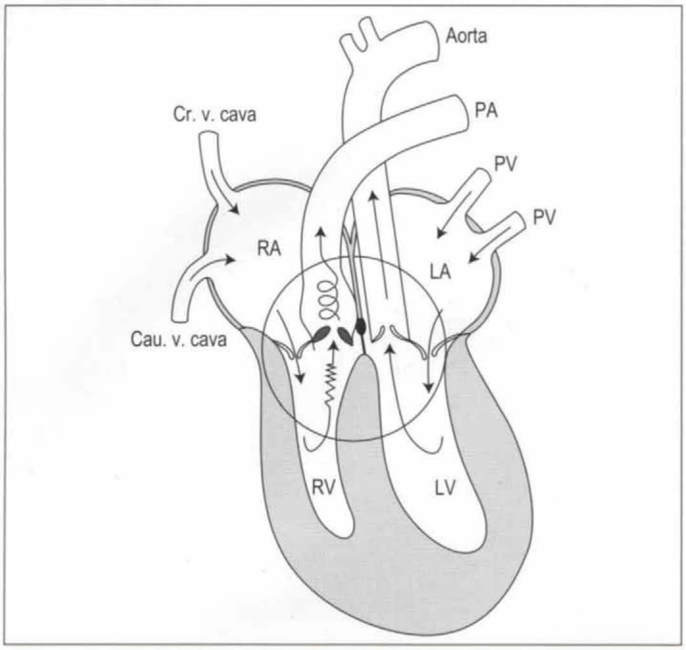

Figure 1.7 Pulmonic stenosis. The stenosis is almost always valvular and places increased afterload on the right ventricle, which undergoes concentric hypertrophy. There is also poststenotic dilation of the pulmonary artery.

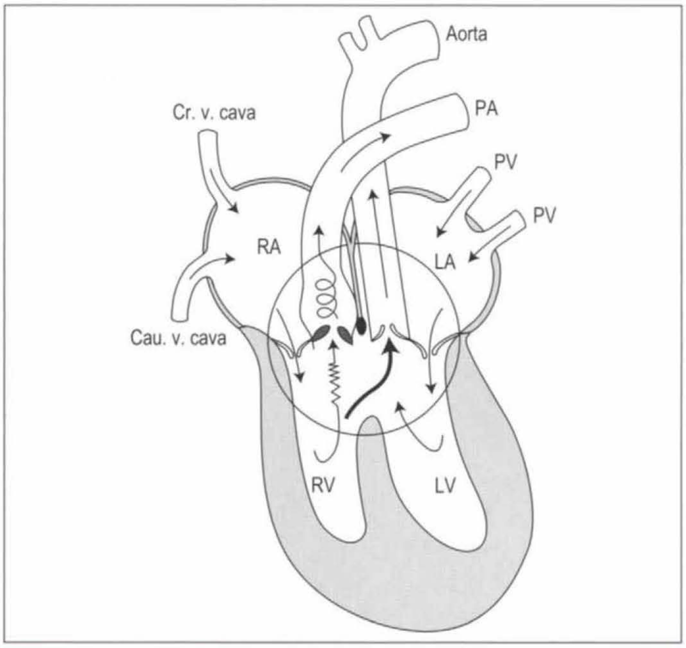

Figure 1.8 Tetralogy of Fallot. The severity of the pulmonic stenosis determines the predominant direction of blood flow. If severe, a proportion of the blood in the right ventricle flows into the aorta via the VSD. The right ventricle hypertrophies concentrically because of the increased afterload placed on it.

three anatomic variations: supravalvular, valvular, and subvalvular or infundibular stenosis. **Valvular stenosis**, the most common form in dogs, is probably due to disordered fusion of the valve cushions and their failure to hollow out properly. The valve, which is then more or less dome-shaped with an irregular central perforation, is surrounded by three recognizable sinuses of Valsalva that are small and irregular in form. **Subvalvular or infundibular stenosis** is produced by a ring of connective tissue that encircles the upper portion of the outflow tract of the right ventricle, or by hypertrophy of the crista supraventricularis muscle ridge. With each form, the pulmonary trunk is dilated and thin walled (Fig. 1.7). This is probably due to a combination of turbulent flow and a drop in pressure, creating a venturi effect in the pulmonary artery. *Concentric right ventricular hypertrophy* is always present because of the *increased afterload* placed on the right ventricle.

The primary lesion in **tetralogy of Fallot** is *obstruction to right ventricular outflow* either through pulmonic stenosis or infundibular stenosis that results from abnormal conotruncal partitioning. The other lesions in the tetrad are *ventricular septal defect, overriding aorta,* and *secondary right ventricular hypertrophy.* Tetralogy is one of the congenital heart diseases that invariably results in clinical signs. *Affected animals fatigue easily and are usually cyanotic and polycythemic.* The latter is a response to hypoxia. Growth rate is usually retarded. At least in Keeshonds, the condition is *inherited as a defect at a single autosomal locus* in which there is partial penetrance in heterozygotes and complete penetrance in homozygotes. Analysis of affected Keeshonds has revealed *various grades of malformation.* The spectrum of anomalies included: subclinical defects of the crista supraventricularis, ventricular septal defect, pulmonic stenosis with abnormal non-patent interventricular septum, and tetralogy of Fallot. Closure of the interventricular septum is contributed to by the conotruncal septum. The flow of blood from the right ventricle into the dextroposed aorta does not depend on the degree of overriding, but on the severity of the pulmonic stenosis (Fig. 1.8).

In English Bulldogs, the breed most commonly affected by pulmonic stenosis, the stenosis is frequently caused by a *circumpulmonary left coronary artery* that originates from a single right coronary artery. The cause of this syndrome appears to be malformation of the left aortic sinus of Valsalva and inversion of the proximal segment of the left main coronary artery.

Aortic and subaortic stenosis

Isolated congenital *aortic valvular stenosis* is distinctly uncommon. **Subvalvular aortic (subaortic) stenosis** is, in contrast, a common defect in pigs and is associated with what has been termed *endocardiosis of the left atrioventricular valve.* In one study of 321 pigs that had left atrioventricular valvular abnormalities (endocardiosis), 258 had accompanying subaortic stenosis. *Subaortic stenosis is among the more frequently encountered anomalies in dogs,* and it is often seen in association with left atrioventricular valvular disease. Dog breeds commonly affected include the German Shepherd Dog, Weimaraner, Golden Retriever, German Shorthaired Pointer, Saint Bernard, Newfoundland, and English Bull Terrier.

The *anatomic appearance of the subvalvular lesion* ranges from a number of small fibrous plaques on the endocardial surface of the interventricular septum, to a completely encircling fibrous band just below the aortic valve (Fig. 1.9A, B). There may also be involvement of the aortic valve. The subendocardial thickenings are composed of connective tissue in irregular arrangements, intermixed with abundant mucinous ground substance. The change is sometimes fibrocartilaginous in nature. Where there is an accompanying mitral valvular defect, it composed of a thickened, ridged and larger anterior leaflet, which forms part of the left ventricular outflow tract. The anterior leaflet forms about one-third of the cylinder that is the left

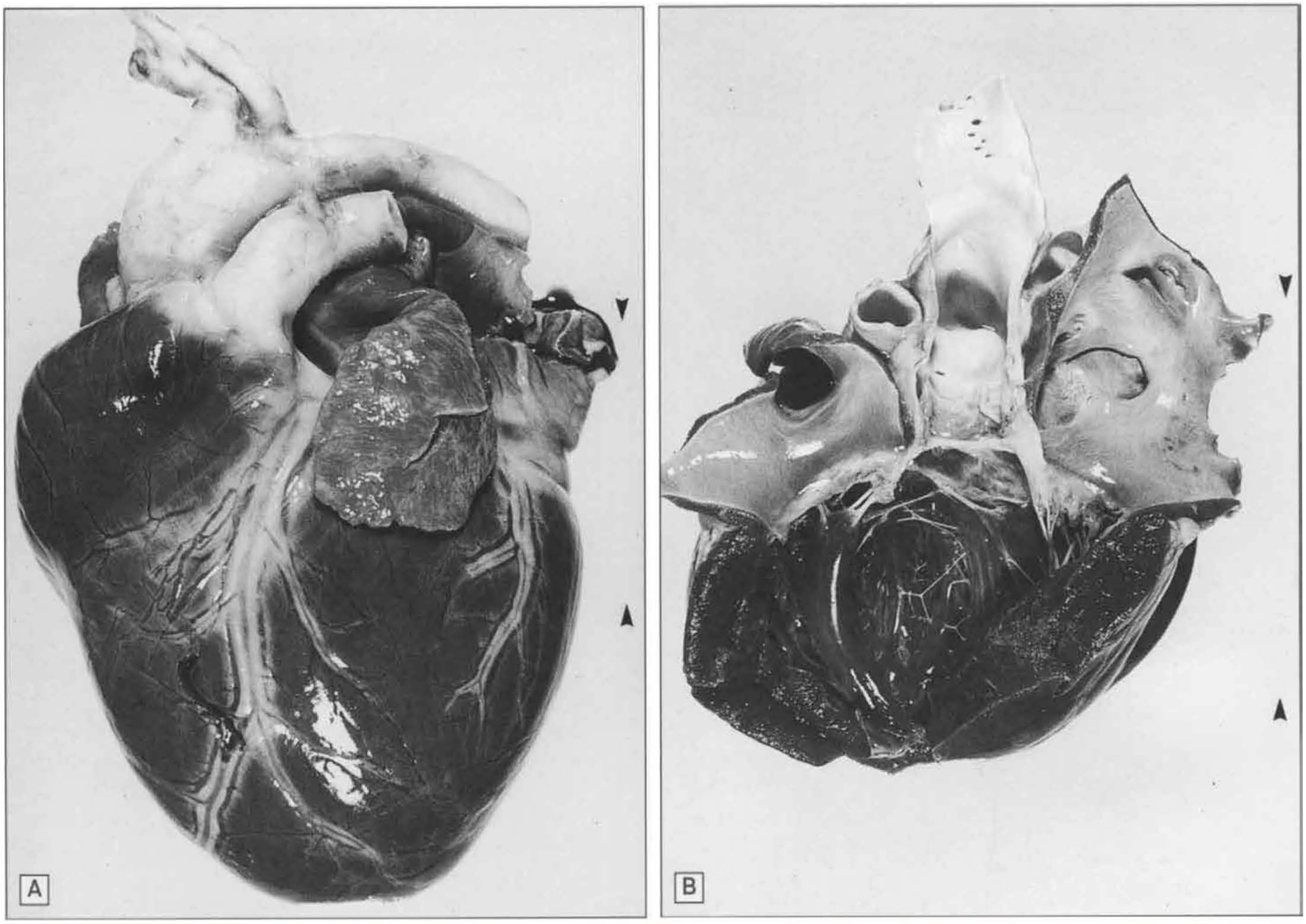

Figure 1.9 A. Subaortic stenosis in a dog. **A.** The left ventricle occupies most of the ventricular mass and the ascending aorta is dilated. (5 cm between arrows.) **B.** The opened left ventricle is concentrically hypertrophied and thick fibrous bands encircle the subaortic area. (10 cm between arrows.)

ventricular outflow tract and when abnormal completes the stenosing subaortic ring.

As with pulmonary outflow tract obstruction, *increased afterload on the left ventricle and turbulent post-stenotic flow produce compensatory concentric hypertrophy of the involved ventricle and post-stenotic dilation of the aorta* (Fig. 1.10). Intimal proliferation of collagen and deposition of glycosaminoglycans in the intramural coronary arteries is common. This may well explain the presence of multifocal myocardial necrosis and fibrosis, and the relatively common clinical finding of sudden death, presumably due to the generation of ventricular dysrhythmias following myocardial necrosis.

The *heritability* of this anomaly in dogs is either polygenic or involves a major dominant gene with modifiers. There is some question as to whether it is a true congenital lesion. In one experimental study, no dog exhibited the typical lesion before 25 days of age. There is also some evidence that the severity of the condition increases with age.

Dysplasia of the tricuspid valve

Dysplasia of the tricuspid valve appears to be one of the more common defects observed in the cat, but is uncommon in other domestic species. The anatomic characteristics of tricuspid dysplasia are focal or diffuse thickening of the leaflets, some of which may be absent, or short chordae tendineae and papillary muscles, and direct fusion of portions of the affected valve with the ventricular wall. In tricuspid dysplasia, *the valve is insufficient, the right atrium is enlarged, and the right ventricle is eccentrically hypertrophied.* The anomaly may be associated with malformation of the mitral valve complex or ventricular septal defect. In **Ebstein's anomaly** of the tricuspid valve, there is downward displacement of the basal portions of the valve into the right ventricle. The morphologically similar *canine tricuspid valve malformation* has been mapped to a susceptibility locus on chromosome 9 in several kindreds of purebred Labrador Retriever dogs. **Tricuspid atresia** – absence of the tricuspid orifice – is accompanied by an interatrial communication, right ventricular hypoplasia, and usually a VSD.

Mitral valvular insufficiency or stenosis

A mixed-frequency holosystolic murmur, with its point of maximum intensity on the left caudal sternal border, is indicative of mitral insufficiency. Commonly heard in old dogs with endocardiosis, it may also be associated with moderate to severe left-sided

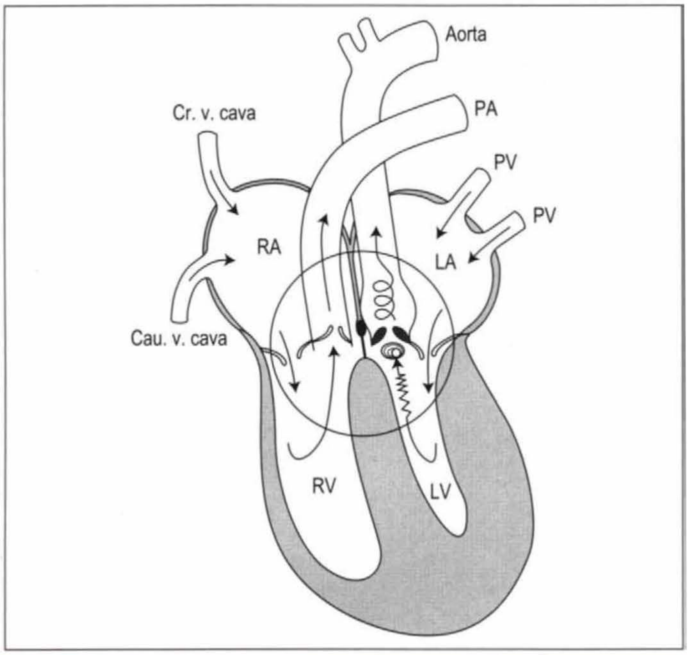

Figure 1.10 Aortic and subaortic stenosis. The left ventricle hypertrophies because of the increased afterload, and the aorta dilates distal to the stenosis.

failure in young dogs and cats. *Malformation of the mitral valve complex is probably the most common congenital cardiac anomaly in the cat.* The lesion has a relatively wide spectrum of severity, and *may result in either an insufficient or a stenotic valve.* Anatomically, there is an enlarged annulus, short thick leaflets, short thickened chordae tendineae, upward malposition of atrophic or hypertrophic papillary muscles, and enlargement of the left atrium and ventricle. There is also diffuse endocardial fibrosis. In dogs, the lesion has been most commonly seen in Great Danes, English Bull Terriers, and, to a lesser extent, German Shepherds.

Transposition complexes

Some of the more severe cardiac anomalies are a combination of defects of the aorta and pulmonary artery. Malposition or **transposition of arterial trunks** is a condition in which the aorta lies in relation to the pulmonary artery such that it receives blood from the right ventricle, the basic defect being *dextroposition of the aorta. There are four degrees, or types, of this anomaly:*

- *overriding aorta* – (the most common type), the aorta straddles the septum, which is defective, and receives blood from both ventricles, and the pulmonary artery leaves the right ventricle;
- *partial transposition* – both vessels leave the right ventricle;
- *overriding pulmonary artery* – the pulmonary artery straddles a defective ventricular septum and the aorta emerges from the right ventricle;
- *complete transposition* – the aorta emerges from the right ventricle and the pulmonary artery emerges from the left (Fig. 1.11).

It is usual in these transposition complexes for either the pulmonary artery or the aorta to be *hypoplastic.*

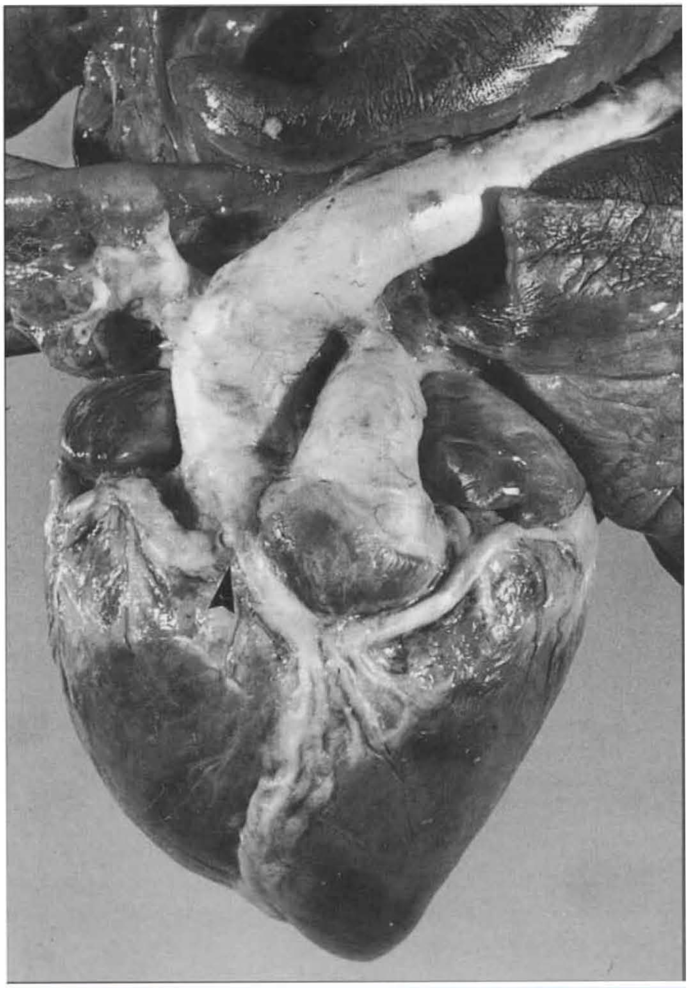

Figure 1.11 Complete transposition of aorta and pulmonary artery in a foal. Coronary arteries arise from a common trunk (arrowhead).

Miscellaneous cardiac anomalies

Congenital hematomas *(hemocysts) on the margins of the atrioventricular valves are common, especially in calves,* but appear to be of little consequence. These are usually blood-filled cysts lined by endothelium; they originate in the clefts normally present in the substance of the valves in intrauterine life. The cysts may measure up to 1.0 cm in diameter and be multiple. There is some question as to whether the cysts involute within a few months of birth, or whether they persist for a year or more, by which time the content is changed to serous fluid. A similar process, termed *valvular telangiectasis* in the septal cusp of the tricuspid valve in Beagles, has been reported to progress from telangiectasis through scarring and osseous metaplasia.

Primary endocardial fibroelastosis is characterized by diffuse endocardial thickening by collagen and elastic fibers, and left ventricular hypertrophy and dilation in the absence of any associated cardiac malformation. The fact that diffuse endocardial thickening occurs when any chamber of the heart remains dilated for a prolonged period has probably led to the confusion that exists about endocardial fibroelastosis. There have been reports in the literature describing the existence of this condition in a number of species, but the primary disease has only been well documented in cats and, to a lesser extent, dogs. The disease in affected Burmese kittens commences with localized endocardial lymphedema. This is detectable microscopically, but not grossly, at 1 day of age. Progressive deposition of endocardial collagen and elastin follows and allows macroscopic detection from 20 days of age. The left atrium is dilated, and the left ventricle is hypertrophied and dilated. The endocardial fibrosis progressively involves cardiac conducting fibers, which exhibit some degenerative change. Although not definitely established, there appears to be little doubt of the inherited nature of the disease.

False tendons are thin, often branching, structures that *traverse the cavity of the left ventricle* and are not connected to the valve cusps; they are common in domestic mammals. They are composed of cardiac muscle, blood vessels, fibrous tissue, and Purkinje cells. False tendons are of debatable significance, but may play a role in innocent murmurs and cardiac dysrhythmia.

Cor triatriatum (triple atria) is a rare lesion in cats and dogs resulting from failure of incorporation of the common pulmonary vein into the left atrium. A membrane separates the left atrium into two compartments, which can impede pulmonary drainage and lead to pulmonary edema. **Cor triatriatum dexter** (right atrium) can cause ascites.

Epithelial inclusions are found occasionally in ventricular myocardium in well-defined areas of discoloration or sponginess. The inclusions are present as acinar or ductular structures lined by a simple layer of cuboidal epithelial cells on a basement membrane. The inclusions are possibly of endodermal origin from the foregut and represent displacements arising very early in embryogenesis, when the heart rudiment is adjacent to the developing foregut.

Myocardial bridges are anatomical variants that are superficial myocardial bands that run across short segments of a coronary artery located epicardially in the dog, cat, goat, sheep, and human. The bridges appear to be of little functional significance.

Congenital absence of the pericardium occurs in dogs. Affected animals are usually asymptomatic.

Variations in the position of the heart are not cardiac malformations but, instead, malformations of adjacent structures:

- In **ectopia cordis**, the heart may lie in *extrathoracic, presternal, or intra-abdominal positions* (Fig. 1.12). Dislocations within the thorax are the result of asymmetric pressure, as for example, in congenital diaphragmatic hernia or pleural effusion.
- In **peritoneopericardial diaphragmatic hernia**, which occurs in dogs and cats, intestine and/or liver are present in the pericardial sac.
- **Dextrocardia** is a rare condition in dogs in which the apex of the heart, which normally points to the left (levocardia), points to the right. Dextrocardia may be part of *situs inversus*, in which the heart and other internal organs are transposed in a mirror-image fashion. *Kartagener's syndrome*, a rare condition in dogs, includes dextrocardia, sinusitis, and bronchiectasis, the last two features as a result of ciliary dyskinesia.

Vascular anomalies

Persistence of the right aortic arch is a well-known anomaly in dogs and has been observed in cattle. *It is due to persistence of the right fourth aortic arch instead of, as is normal, the left fourth aortic arch.* A right aorta descends to the right of the midline, arches over the origin of the right bronchus, and descends either to the left or right of the vertebral column. With the aorta in this position, the ductus arteriosus (ligamentum arteriosum), passing from the aorta to the pulmonary artery, *encloses the esophagus and compresses it against the trachea.* Obstruction of the esophagus at this point leads to dysphagia and, shortly, to dilation of the cervical portion of the esophagus (Fig. 1.13).

A **double aortic arch** is a variation of the foregoing in which *the left arch persists as well as the right.* Both arches arise from the ascending aorta. The right arch, which is usually the larger of the two, follows the course given above. It is joined above the esophagus by the small left arch. The significant end result is *constriction of the esophagus.*

Aorticopulmonary septal defect, or *window*, is a rare defect in dogs that results from incomplete division of the truncus arteriosus. Pulmonary hypertension develops, leading to right-to-left shunting of blood.

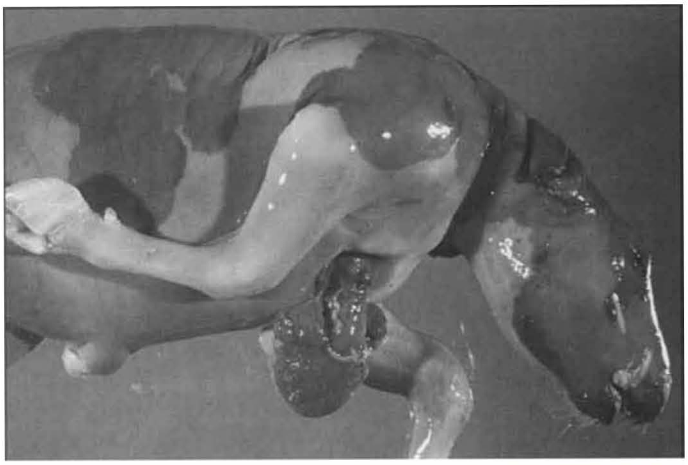

Figure 1.12 Ectopia cordis in a bovine fetus.

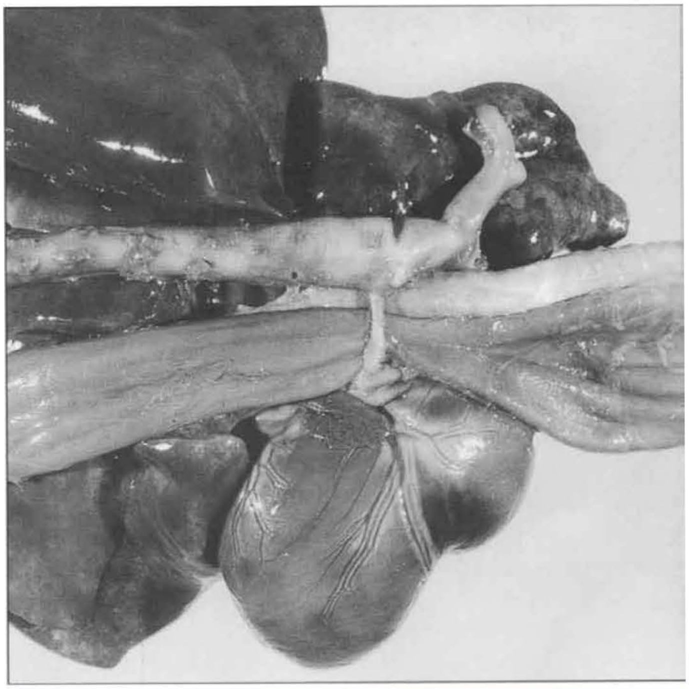

Figure 1.13 Persistent right aortic arch in a dog. The esophagus is constricted by aorta, trachea, and ligamentum arteriosus.

Coarctation of the aorta is another rare defect in dogs, and is seen as narrowing of the aorta adjacent to the ductus arteriosus/ligamentum arteriosum. Left ventricular pressure overload can result in left heart failure. Additional malformations of the aorta reported in dogs include *interruption of the aorta* and *tubular hypoplasia of the ascending aorta*.

Congenital aneurysm of the aorta or of the pulmonary artery, involves, for either vessel, the trunk and arch, but may not extend beyond the insertion of the ligamentum arteriosum. Aortic aneurysm may be associated with aneurysm of one or more of the aortic sinuses of Valsalva.

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PERICARDIAL DISEASE

Primary disease confined to the pericardium is rare, but the close anatomic relationship of the pericardium to the heart, lungs, and pleura sometimes results in the extension of disease processes from the latter organs to the pericardium. Somewhat paradoxically, the pericardium is not needed for normal life, but pericardial disease can be life threatening. The pericardial sac may be removed surgically for relief of pericardial constriction.

The pericardial cavity may communicate with the peritoneal cavity through clefts in the diaphragm; hence herniated intestine may be found surrounding the heart as a result of congenital *peritoneopericardial diaphragmatic hernia.* Partial or complete *congenital absence of the pericardial sac* is without clinical effect. *Pericardial cysts* are rare congenital anomalies that occur in one of the costophrenic angles.

Epicardial fibrous fronds occur on the atria of Beagle dogs as an occasional incidental finding at necropsy.

Noninflammatory lesions of the pericardium

Hydropericardium

The pericardial sac normally contains a very small quantity of clear, serous fluid. *Any excess in the volume of fluid is referred to as hydropericardium.* A small increase in volume occurs by transudation after death, and it is soon reddened by postmortem hemolysis.

Hydropericardium is often part of *generalized anasarca* and is thus seen in many cachectic illnesses, perhaps as the result of hypoalbuminemia, and in *congestive heart failure* (Fig. 1.14). It may also be the product of local events such as implanted metastases of *neoplasms* on the pericardium, and lymphomatous infiltrations of the myocardium; primary tumors about the base of the heart and in the cranial mediastinum are rather common causes of hydropericardium. Hydropericardium may also accompany congenital and acquired *peritoneopericardial hernias* and *uremia. Infectious processes* of the pleura, especially granulomatous inflammations, may cause irritation of the pericardium sufficient to provoke a sterile serous effusion; the infection may eventually extend to the pericardial sac. If drainage of the thoracic duct is disrupted, for example by a cardiac neoplasm, then the fluid may be chylous (*chylopericardium*).

In hydropericardium, the serosal surfaces remain smooth and glistening, but if the fluid persists for a long time, it may produce slight fibrous thickening and opacity of the pericardial and epicardial surfaces. It is usually about the base of the ventricles, and small villus proliferations of the serosa may rupture and result in a blood-stained effusion. Neoplastic involvement of the pericardium characteristically has this result.

The pericardial fluid is clear or light yellow, without floccules, and with low content of protein. Fluid rich in protein, including fibrinogen, is often found in acute toxemias of clostridial (gas-gangrene group) origin. Clotting occurs soon after exposure to air. In such cases, exudation is due to direct injury to the endothelium by circulating toxins. Fluid that is copious and gels on exposure also is characteristic of African horse sickness and of heartwater. In pigs with edema disease or mulberry heart disease, fibrin is often formed antemortem. The presence of protein, especially fibrin, in pericardial fluid is only an index of the degree of endothelial injury and increased permeability and is not a precise point of distinction between an exudate and a transudate. **Inflammatory exudates** can be differentiated from **transudates** on the basis of the higher content of protein and cells in exudates and histologic evidence of inflammation involving the pericardium and epicardium.

The volume of fluid that accumulates is quite variable and of lesser significance than the rate at which it accumulates. When fluid accumulates rapidly, the pericardium is put under tension, the heart is compressed, and venous blood pools in the splanchnic and systemic circulations due to impaired atrial and ventricular filling (**cardiac tamponade**). When the fluid accumulates slowly, there is time for stretching and adaptation of the pericardium so that very

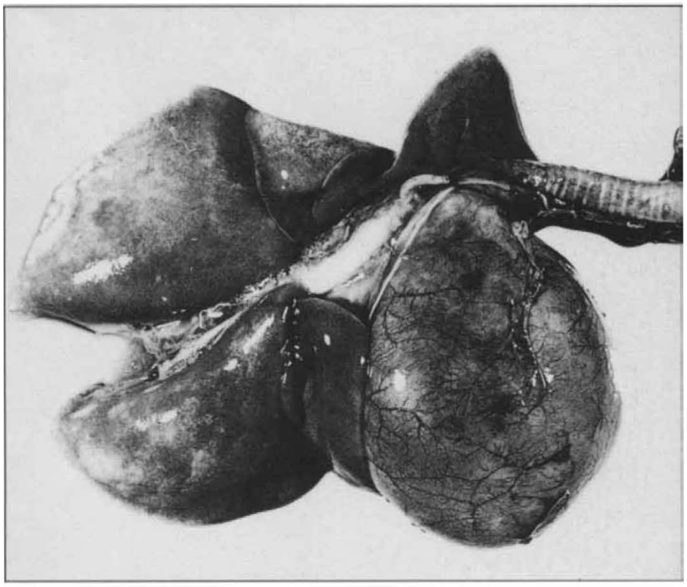

Figure 1.14 Hydropericardium in a cat. Pericardium is distended and pericardial vessels are injected.

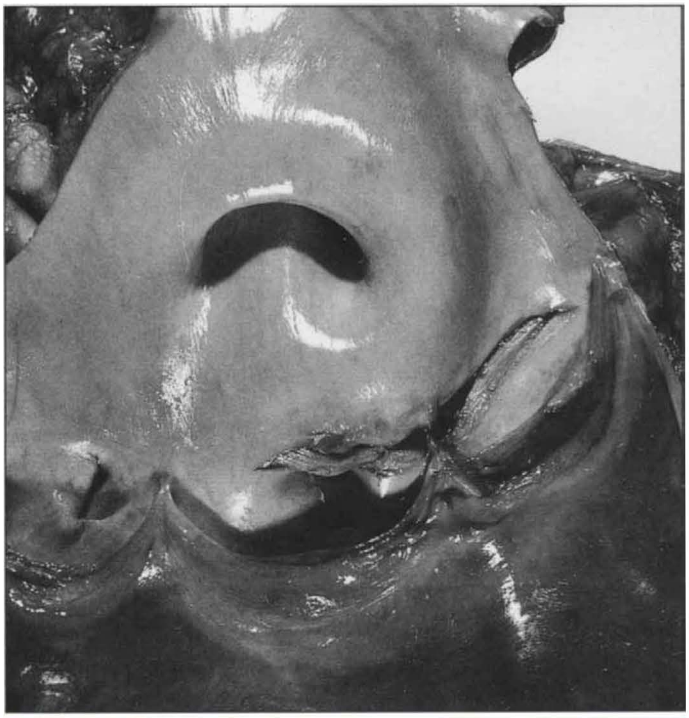

Figure 1.15 Aortic rupture, which caused hemopericardium and sudden death in a horse.

large amounts of fluid can accumulate before there is significant impairment of diastolic filling of the heart.

Hemopericardium

The term hemopericardium is limited to accumulations of pure blood in the pericardial cavity, and should not apply to mixtures of blood and serous fluid. If the blood is clotted, it can be assumed that the process is a true hemopericardium. Death usually supervenes rapidly. It is an unusual lesion, except following cardiac puncture or other direct trauma. It is seen in *horses* in which rupture of the intrapericardial aorta or atria occurs (Figs 1.15, 1.16). In *dogs*, hemopericardium occurs in atrial rupture in endocardiosis, hemorrhage from atrial hemangiosarcoma, in ulcerative atrial endocarditis of renal failure, and rarely, following rupture of a coronary artery or from hemorrhage from a heart base thymic remnant. In young, growing *swine*, rupture of the heart, particularly of an atrium, a coronary artery or the aorta, occurs on rare occasions. These deaths have not been examined specifically with deficiency of copper in mind, but attention is drawn to the remarkable cardiovascular lesions of experimental deficiency, which is discussed with diseases of the arteries.

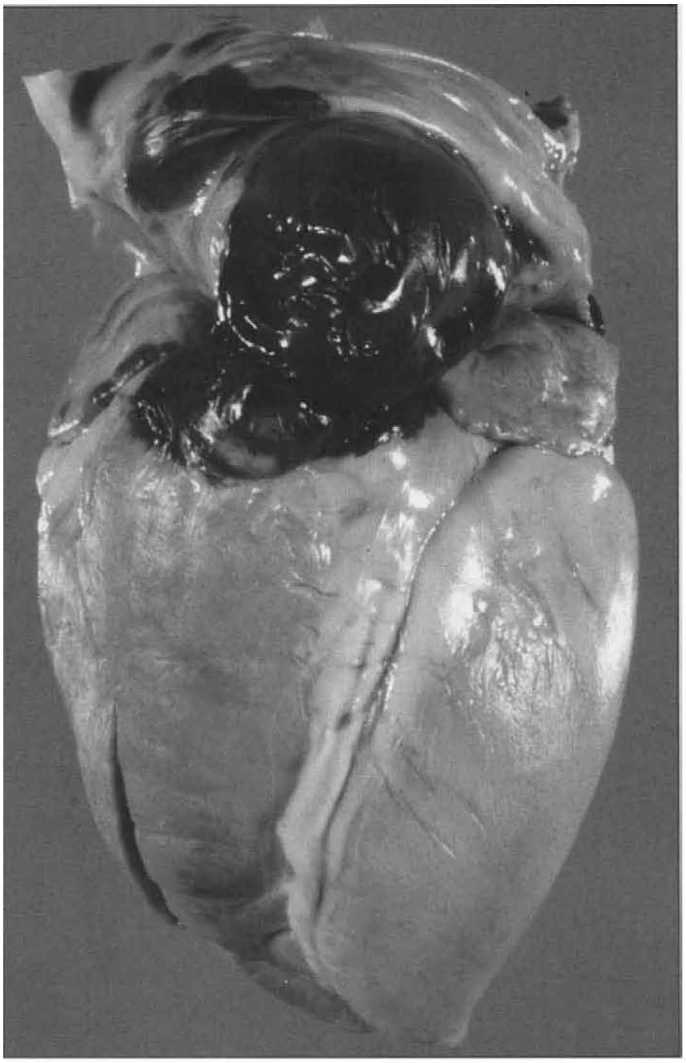

Figure 1.16 Hemorrhage at site of traumatic **right atrial tear** in a 4-day-old foal. Death resulted from hemopericardium and cardiac tamponade.

Idiopathic pericardial hemorrhagic effusion occurs predominantly in large-breed dogs, and is characterized by the presence of both clotted and unclotted blood in the pericardial sac without obvious cause. The clinical presentation is that of slowly developing right-sided heart failure, with some having accompanying left-sided heart failure. The heart sounds are muffled or inaudible on initial presentation with no evidence of dysrhythmias. However, some subsequently develop atrial fibrillation. The clinical course can be prolonged. The slow onset of clinical signs suggests either slow or intermittent bleeding allowing distension without tamponade. Indeed, some dogs may make a full clinical recovery following aspiration of the pericardial fluid.

Serous atrophy of pericardial fat

In cachexia of any cause, and paralleling fairly closely the reduction in body weight, there is progressive mobilization of depot fat, including that beneath the epicardium. As the lipid vacuoles are reduced in size, they are replaced by proteinaceous fluid, and with a concomitant increase in interstitial fluid, the depots are converted to gray, gelatinous masses that may be flecked by small white foci of fat necrosis (Fig. 1.17).

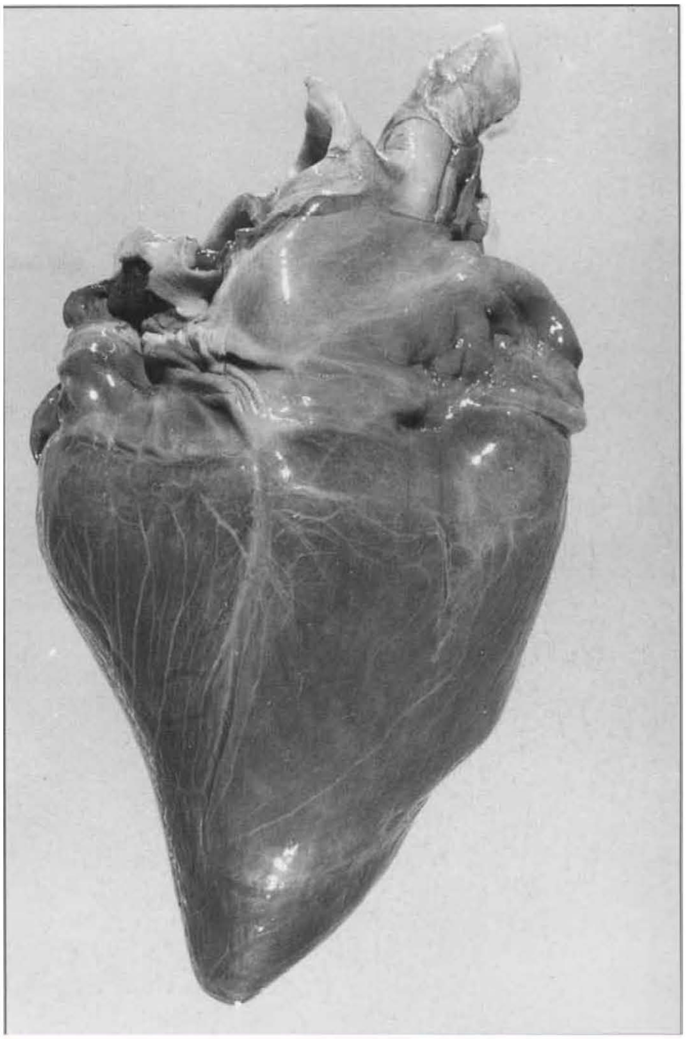

Figure 1.17 Serous atrophy of epicardial fat. The fat is gelatinous and translucent, and the myocardium is dark.

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Pericarditis

It is seldom possible by examination of the lesion itself to decide the cause of pericardial inflammation, although a decision on the cause is often possible when the total pathologic picture in the cadaver is noted. For this reason and for descriptive purposes, we employ a morphologic classification of pericarditis.

Fibrinous pericarditis *is usually the result of hematogenous microbial infections*, but it may arise by lymphatic permeation from an inflammatory process in adjacent tissue. In *cattle*, fibrinous pericarditis, with or without some hemorrhage, is commonly part of pasteurellosis, blackleg, sporadic bovine encephalomyelitis, contagious bovine pleuropneumonia, clostridial hemoglobinuria, and some of the neonatal coliform infections that enter via the umbilicus. In *swine*, it is frequently part of the morbid picture of Glasser's disease and pasteurellosis, is a common complication of porcine enzootic pneumonia (infection with *Mycoplasma hyopneumoniae* and other agents), and is occasionally observed in salmonellosis and in streptococcal infection of piglets. Fibrinous pericarditis in adult *sheep* is usually part of pasteurellosis; in lambs, it is usually part of pasteurellosis or caused by streptococci. In *horses*, *Mycoplasma felis* causes pericarditis and pleuritis; streptococcal pericarditis may coexist with polyarthritis. Fibrinous pericarditis in horses may also be associated with the mare reproductive loss syndrome. Pericarditis is rare in *cats*, but it is seen as part of feline infectious peritonitis.

In fibrinous pericarditis, there is seldom significant exudation of fluid, so distension of the pericardial sac is not to be expected. The exudation of fibrin usually begins about the base of the heart and extends from there to cover, more or less completely, both the pericardium and epicardium. The fibrin is gray-white but it may be flecked with blood, or yellow if a large number of leukocytes are added to the exudate. Except for small pools of serum or serous exudate, the pericardium and epicardium are in apposition. When the leaves are drawn apart, the exudate is drawn out into villus-like projections to give an appearance responsible for the names "cor villosum," "shaggy heart," and "bread-and-butter pericarditis" (Fig. 1.18A, B).

The completeness of resolution depends upon how quickly the fibrinous exudate can be removed, which is a function of the amount of fibrin, and how quickly the mesothelium can be regenerated, which is a function of the amount of mesothelium that has been destroyed. *Restorative processes compete with the processes of organization.* Within a week or so, there will be well-formed fibrous tissue in the deepest parts. Immediately superficial to this there will be young granulation tissue and then a stratum of fibroblasts mixed with leukocytes, and on the surface there remains the fibrin clot infiltrated with numerous leukocytes. If the course is prolonged, organization and scarring will result in focal or diffuse fibrous adhesions between the pericardial surfaces, with partial or complete obliteration of the sac.

The *residual lesions of fibrinous pericarditis* occur as more or less distinct variants. Focal, patchy thickenings of the epicardium without adhesions form minimal residue and are usually more distinct on the ventricles than on the atria, where they may be obscured by normal fat. The original scar tissue may undergo fatty metaplasia or be edematous. (The same lesion also occurs covering healed foci of superficial myocarditis.) Focal or diffuse adhesive pericarditis varies in extent from a few "violin strings" across the sac to complete obliteration of the sac. Inclusion cysts lined by mesothelium are often present in fibrous adhesions, and there tends to be excess fluid present in non-obliterated portions of the sac. The pericardium is separable from the epicardium by blunt dissection. As the connective tissue is not dense, there is usually no embarrassment of cardiac function.

Purulent pericarditis *almost invariably denotes the presence of pyogenic bacteria*, either as primary pathogens or as opportunists in fibrinous pericarditis. It occurs almost solely in cattle as a result of traumatic perforation by a foreign body originating in the reticulum (Fig. 1.19), but it is observed in cats and horses in association with empyema (pyothorax). Migrating grass awns ("foxtail," *Hordeum glaucum* and *H. leparinum*) have been identified as causes of suppurative pericarditis in dogs in the western USA.

The suppurative pericardial fluid may appear as thin, cloudy exudate, as frank, creamy pus, or as a mixture of pus and masses of fibrin. The color depends on the organisms present, but usually varies from yellow to green, being irregularly dirty gray when putrefactive bacteria are present. The exudate is usually foul smelling. The volume of exudate varies from a thin layer on the serosal surface to 4 L or more. In the early stages, it can be wiped or washed off to reveal the vascular injection and granularity of the membranes. Soon, the whole of the epicardium is covered with coagulum; the parietal layer of the pericardium is less severely affected, and separation of the two presents a shaggy appearance of the heart (cor villosum).

Microscopically, the subjacent tissues are densely infiltrated with leukocytes, the reaction extending more deeply than in fibrinous pericarditis, the formation of new blood vessels and connective tissue is prominent, and the overlying coagulated exudate is densely infiltrated with leukocytes.

Resolution probably never occurs. Because of the severity of the inflammatory reaction, *healing is by organization*, as is characteristic of all suppurative inflammations. Although the course of suppurative pericarditis may be prolonged, the persistence of the inciting agent, or more usually agents, prevents complete organization. **Constrictive pericarditis** resulting from progressive organization can be so severe and diffuse that it leads to impaired diastolic filling

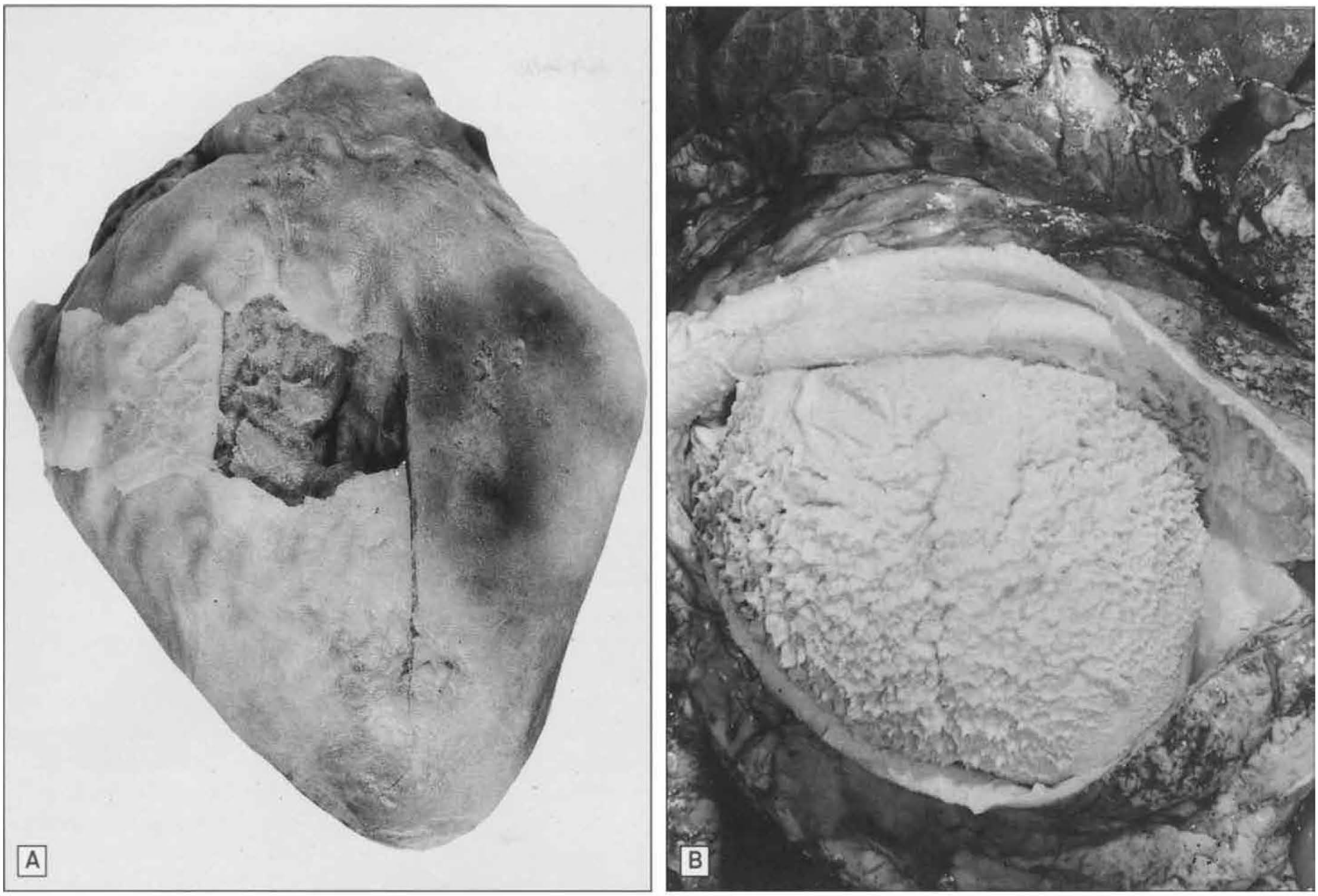

Figure 1.18 A. Fibrinous pericarditis in a pig. There is some blood staining of the exudate, which can be peeled off. **B. Chronic pericarditis** with "bread and butter" exudate in a cow. The reflected pericardium is thickened.

and the development of right-sided heart failure (Fig. 1.20). The adhesions cannot be broken down by blunt dissection and may be mineralized. They obliterate the pericardial cavity, but they may also extend beyond it to involve the mediastinum. The heart, which is confined by scar tissue, may be smaller than normal, or it may be greatly enlarged and hypertrophic, especially when there are also extrapericardial adhesions present. *There is always severe cardiac dysfunction, and death occurs from congestive heart failure.*

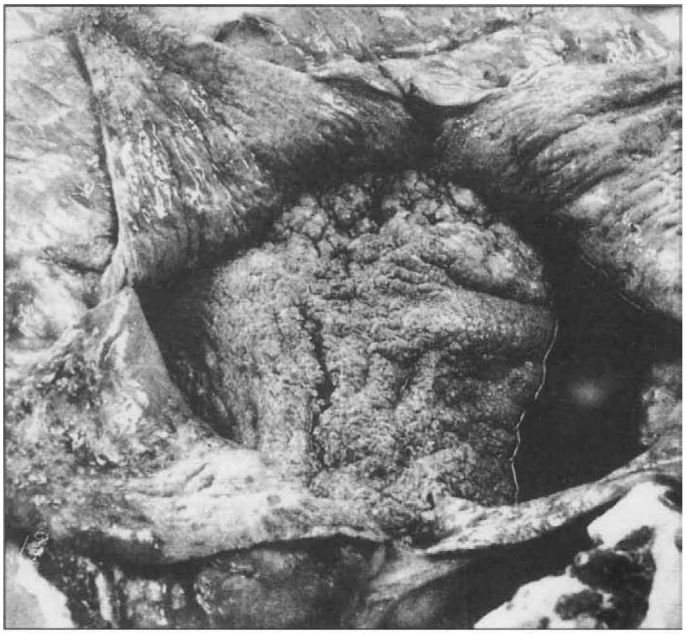

Figure 1.19 Putrid, traumatic pericarditis in a cow. The pericardium is reflected. The pericardial sac contains dirty liquid exudate, and the epicardium bears profuse granulation tissue. (Courtesy of P Olafson.)

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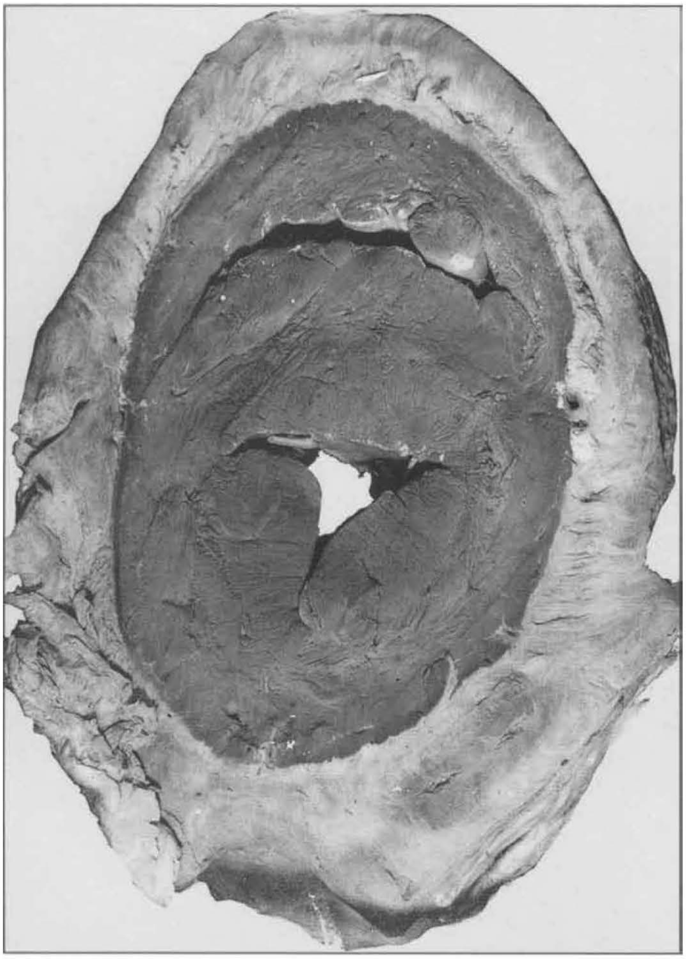

Figure 1.20 Constrictive pericarditis in a cow. Dense scar tissue has obliterated the pericardial sac and constricts the ventricles, the walls of which are hypertrophic.

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ENDOCARDIAL DISEASE

The most clinically significant endocardial lesions affect the heart valves rather than the mural endocardium. Valvular lesions may cause **stenosis**, or **valvular insufficiency**, or both. *Stenosis of a valve, or failure to open completely, impedes the forward flow of blood. Valvular insufficiency, regurgitation, or incompetence results in reversed flow of blood.* Valvular dysfunction may produce abnormal heart sounds that are detectable clinically as *murmurs.* The consequences of valvular disease depend upon the rapidity of onset, type, and duration of the lesion, and may be ameliorated by cardiac compensatory mechanisms. For example: congenital aortic or pulmonic valvular stenosis result in myocardial hypertrophy and *progressive heart failure*; rupture of chordae tendineae causes *acute heart failure*; myxomatous degeneration of the mitral valve in aging dogs causes valvular insufficiency and *congestive heart failure.*

The propensity of **thrombi** to form on the free margins of valves is well known, but the factors leading to it are not. It may be related to the continual movement and the resulting apposition of the surfaces of the free margins of the valves. There are also ill-defined factors, such as intercurrent disease or increased workload, which promote the development of thrombi. The lack of an internal blood supply to the valve may also be a contributing factor, and in common with other vascular structures, injured endothelial cells release inflammatory mediators, such as prostaglandins, and other substances, such as adenosine diphosphate, which are potent stimulators of platelet aggregation. Once the endothelium is removed, the exposed collagen also stimulates the aggregation of platelets. The presence of the initial thrombus leads to further clotting. In contrast to myocytes, endothelial cells have a great capacity for regeneration and for covering any breach in their continuity. If the original insult is removed, which without appropriate treatment is not often, the valve heals by fibrosis, but a shrunken, distorted, insufficient, or stenotic valve may remain.

Little is known of the process leading to the abnormal development of valves in the embryo, or the progressive accumulation of glycosaminoglycans in the degenerative disease, endocardiosis. The various theories are discussed under the appropriate heading. However, both lead to deficiencies of valve function.

Acute rupture of chordae tendineae can occur spontaneously or in association with chronic valvular degeneration or valvular endocarditis. As well, acute rupture of chordae tendineae or papillary muscles, and resultant acute valvular insufficiency and heart failure, and can be caused by blunt trauma to the chest, e.g., falls, hit by motor vehicle.

Degenerative lesions

Myxomatous valvular degeneration ("endocardiosis") in dogs

Myxomatous valvular degeneration, commonly termed *endocardiosis, is the most common cardiovascular lesion in dogs*, and is frequently encountered as an incidental finding at autopsy. Advanced endocardiosis of the left atrioventricular (AV) valve leads to *mitral insufficiency* and regurgitant flow into the left atrium, noted clinically as systolic murmurs, and culminates in left-sided heart failure. The shrunken, distorted AV valves are seen with greatest frequency in toy, small, and medium breeds of dogs, especially in males of breeds such as the Poodle, Pomeranian, Schnauzer, Chihuahua, Doberman Pinscher, Whippet, Fox Terrier, Boston Terrier, Cavalier King Charles Spaniel, Dachshund, and the English Cocker Spaniel. There are significant negative associations for the Labrador Retriever and the German Shepherd Dog. *The prevalence of the disease increases with age*, from 5% at less than 1 year of age to >75% at 16 years of age. Endocardiosis is also reported in the mitral valves of normal market-weight pigs, but appears to be inconsequential.

Endocardiosis affects chiefly the left AV valve (Fig. 1.21A, B). The right AV valve is less severely and less frequently affected. Only occasionally are the aortic and pulmonary semilunar cusps involved. The AV cusps are shortened and thickened. The thickening of the leaflet may be more or less uniform with a rounded edge, or prominent nodular thickenings may affect the free margin. *The valves are opaque and white, but the surface is smooth and glistening, without any evidence of*

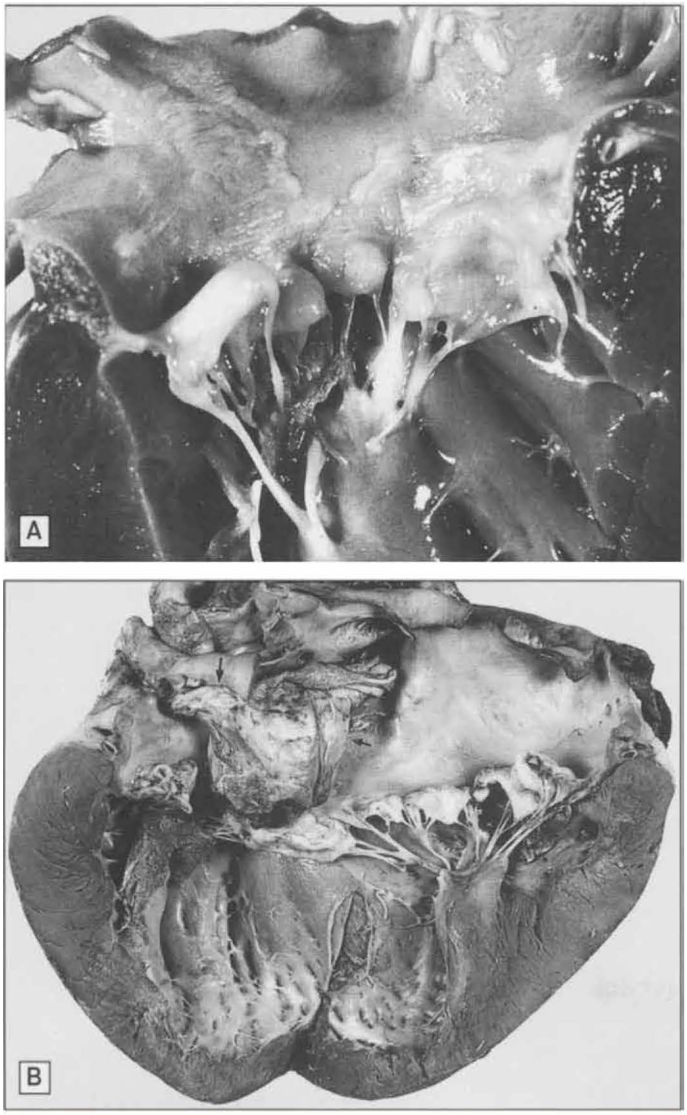

Figure 1.21 Endocardiosis. A. Thickened, distorted left atrioventricular valve with focal endocardial fibrosis ("jet lesions") in the left atrium of a dog. **B.** Endocardiosis of left AV valves and atrial thrombosis (arrows) in a dog.

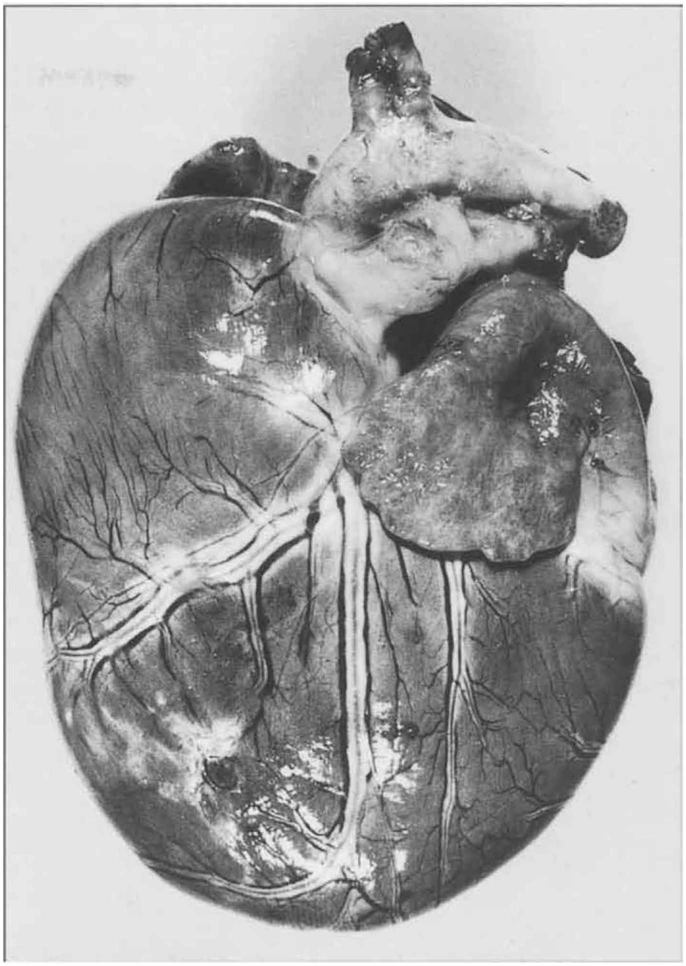

Figure 1.22 Dilated left ventricle and atrium with focal ventricular fibrosis in a dog, the result of **endocardiosis**.

inflammation. The chordae tendineae may also be thickened and occasionally are ruptured, allowing eversion of the leaflet into the atrium and leading to acute ventricular failure. The mitral annulus may be enlarged.

The least severe gross change consists of a few small, discrete nodules at the line of closure of the valve. This progresses to multiple larger nodules that tend to coalesce in the area of contact. There may be irregular areas of opacity in the proximal portions of the leaflet. The nodules may coalesce to form plaque-like deformities in the area of contact. The chordae tendineae are thickened at their points of insertion into the valve, and there are clearly defined areas of opacity on the basal portions of the leaflet. The most severe change is characterized by a gross distortion of the valve by gray-white nodules and elevated plaques. The cusps are contracted, thickened, and irregular. There may also be fixed upward displacement of the free margin of the valve. Enlargement and redundancy of the valve may be seen as doming or hooding of the body of the valve towards the atrium; this prolapse of the body of the redundant left AV valve into the atrium ("*mitral valve prolapse*") may be seen both echocardiographically and at necropsy. Chordae tendineae may be ruptured, in which case the free edge of the valve may prolapse as a *flail leaflet*. Care should be taken in the assessment of the valves, since both the left and right AV valve leaflets thicken with increasing age, and the free margin of the septal leaflet of the right AV valve is normally distinctly thicker than the free leaflets.

Changes secondary to AV valvular insufficiency are *dilation of the atria*, particularly the left atrium, and *dilation of the left ventricle* (Fig. 1.22). In a small majority of cases, the left ventricle is eccentrically hypertrophied. The left atrial and ventricular endocardium may be diffusely thickened by fibrosis following prolonged dilation. There may also be evidence of regurgitation in the form of focal elevated streaks and plaques of subendocardial fibrosis in the atria ("*jet lesions*"). Left atrial tears may also be seen in advanced cases, and these tears may lead to hemopericardium. Mural thrombi can form in the dilated left atrium; dislodgement of the thrombi can result in thromboembolism, e.g., of coronary arteries leading to myocardial infarction.

Microscopically, the earliest changes are present on the atrial side of the valves. There is proliferation of the endothelium, increased numbers of subendothelial fibroblasts and macrophages, and splitting and separation of elastic fibers between the atrialis and spongiosa. However, *it is the thickening of the spongiosa and degeneration of* *the fibrosa that are the most prominent features of endocardiosis.* The spongiosa is greatly thickened by the proliferation of loose fibroblastic tissue and the deposition of the proteoglycans, hyaluronic acid, and chondroitin sulfate. In a few cases, amyloid may be deposited. There is no increase in collagen or elastic tissue in the spongiosa. The changes in the fibrosa are also marked. Collagen bundles become swollen and hyalinized, fragment, and disappear. In advanced cases, only scattered remnants of the fibrosa remain. Similar changes are seen in chordae tendineae. Intramural coronary arteriosclerosis and focal myocardial necrosis and fibrosis are commonly seen in the left ventricular myocardium, especially the papillary muscle, if the ventricle is hypertrophic.

The cause of endocardiosis is not known, but is likely a genetically influenced degeneration of connective tissue. This suggestion is supported by the observation that the most frequently affected breeds are of the chondrodystrophoid type. Endocardiosis is *inherited* in Dachshunds and Cavalier King Charles Spaniels; a polygenic mode of inheritance is suggested. There is a striking similarity of endocardiosis to the *prolapsed mitral valve syndrome* of humans, in which there is deposition of glycosaminoglycans in the spongiosa secondary to an as yet undefined abnormality of collagen metabolism. Mitral valve prolapse in humans occurs in association with connective-tissue disorders such as Marfan's syndrome, Ehlers–Danlos syndrome, osteogenesis imperfecta, and a variety of muscular dystrophies.

Mitral regurgitation also occurs commonly in **horses**, and can result from endocardiosis, endocarditis, flail valve leaflets, rupture of chordae tendineae, and mitral valve prolapse. Clinical signs of exercise intolerance, poor performance, or congestive heart failure result. Dilation of the pulmonary artery may also ensue, indicating pulmonary hypertension, and may presage pulmonary artery rupture.

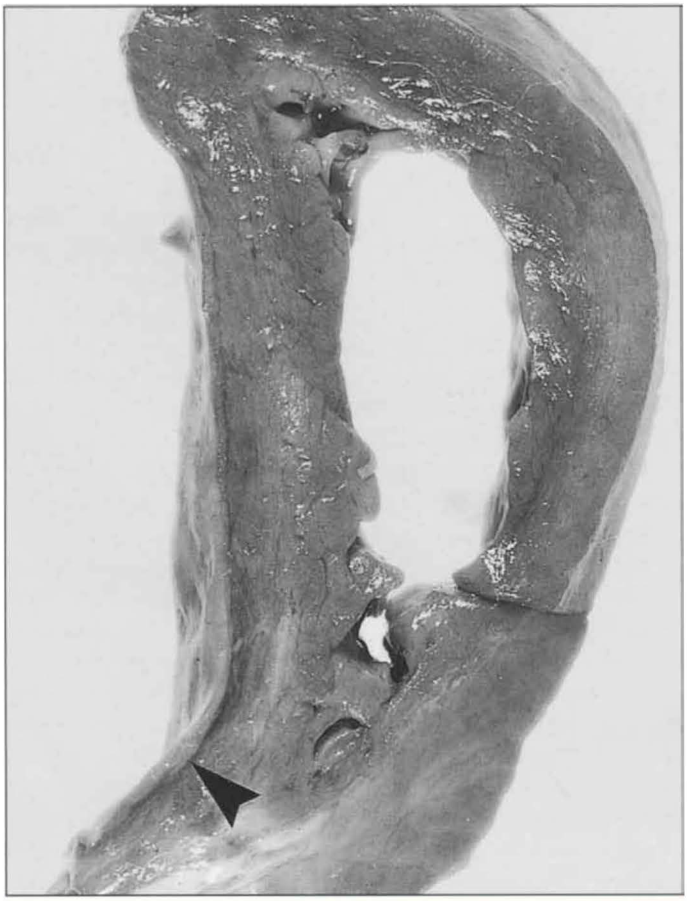

Figure 1.23 Endocardial fibroelastosis (arrowhead) in a calf.

Valvular cysts

Congenital hematomas (hemocysts) on the margins of the atrioventricular valves are common, especially in calves (see above: Miscellaneous cardiac anomalies). *Blood cysts* and *serous cysts* have been reported in the atrioventricular valves in 11% of the hearts in a series of 30 000 slaughter cattle, with a higher incidence in cows than calves. Blood-filled cysts were often present on both atrioventricular valves, while serous cysts occurred more frequently on the left atrioventricular valve. The findings in this study suggest that cysts do not regress with age, indeed their incidence and size may increase with age. The endothelium lining the blood-filled cysts was positive for factor VIII-related antigen. The cysts may represent ectasias of blood vessels and lymphatics.

Subendocardial fibrosis

Subendocardial fibrosis may be diffuse or focal, congenital or acquired (Figs. 1.23 and 1.24). The congenital lesions are discussed

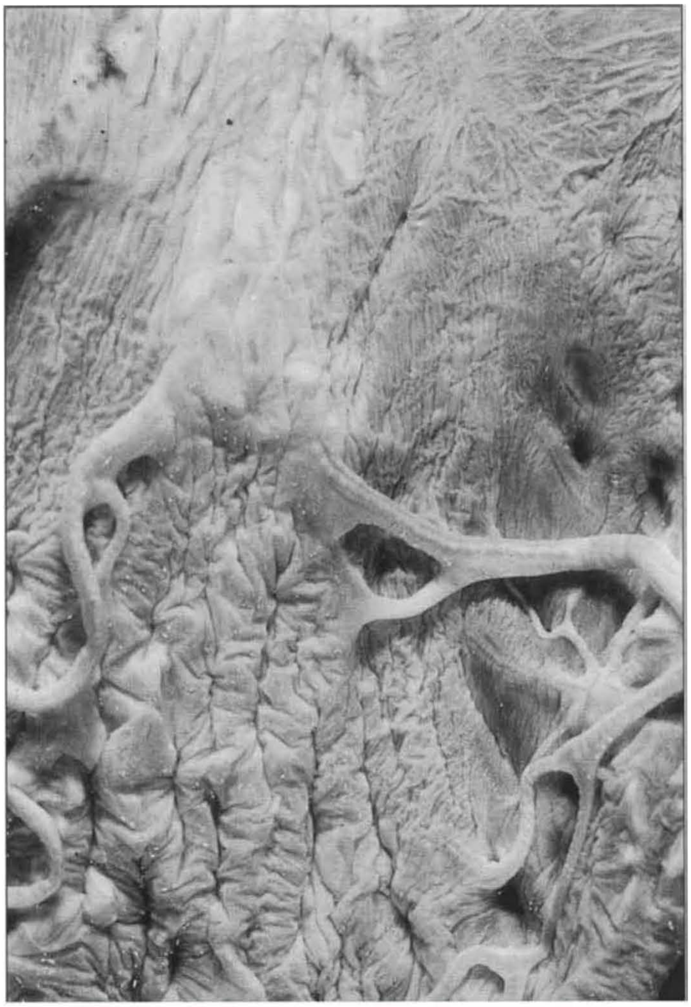

Figure 1.24 Endocardial fibrosis in a cow.

under Congenital abnormalities of the heart and large vessels. *Diffuse subendocardial fibrosis is seen whenever a ventricle or atrium is dilated for a prolonged period.* It is probably best exemplified by the dilated cardiomyopathy of large-breed dogs.

Subendocardial fibrosis in localized areas is observed chiefly in the atria and is regarded as a reaction of the endocardium to abnormal jets of blood or to turbulence following congenital or acquired valvular disorders (Figs. 1.22 and 1.25). They are loosely termed **jet lesions,** but are probably areas subject to increased static pressure in turbulent flow.

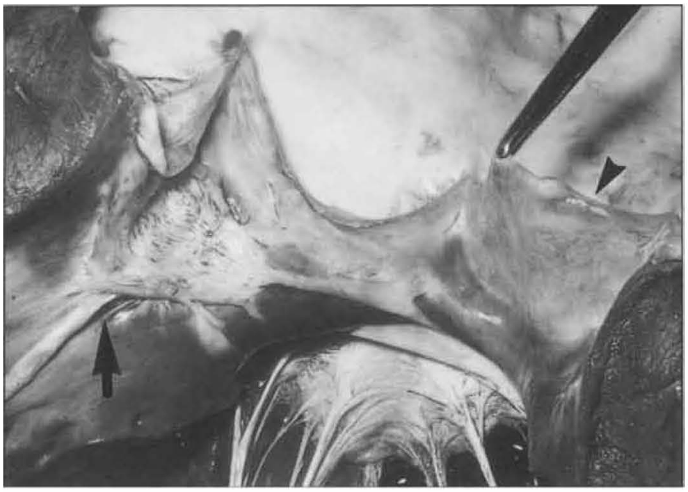

Figure 1.25 Aortic valve fenestrations (arrowhead) causing endocardial "jet lesion" (arrow) in the left ventricle of a horse.

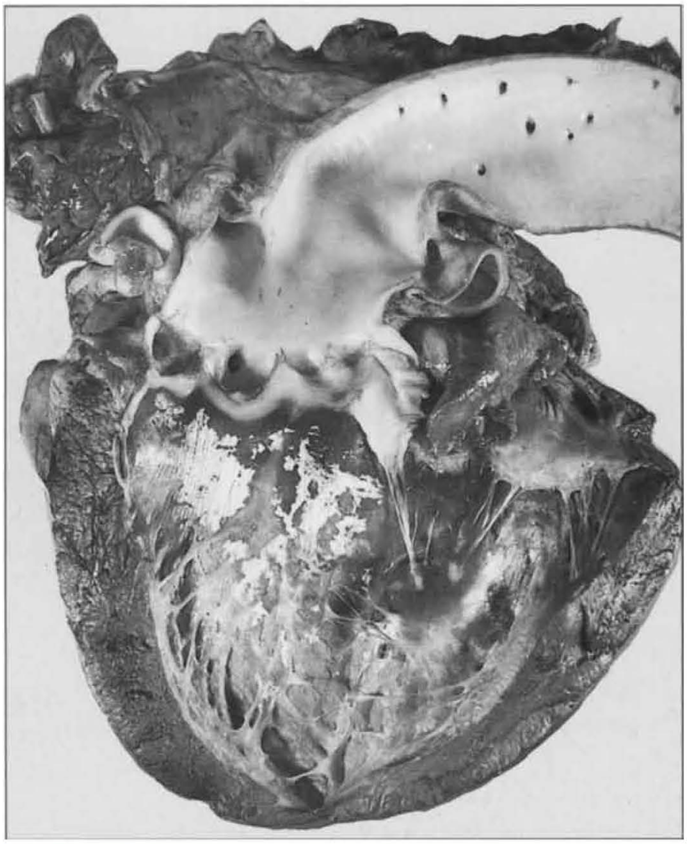

Figure 1.26 White subendocardial plaques of **mineralization** in the left ventricle of a cow.

Subendocardial mineralization

Subendocardial mineralization is associated with a variety of disorders. It occurs commonly in opaque plaques or as small grains in the left atrium, and occasionally in the trunk of the aorta in dogs that have recovered from ulcerative endocarditis of *renal insufficiency* (see below). Prominent white plaques of mineralization also occur beneath the endocardium of the right ventricle in *nutritional myopathy* of lambs. Subendocardial mineralization in the atria and left ventricle may accompany endocardial fibrosis when these cavities are acutely dilated, either as congenital or acquired defects, and in a variety of *prolonged debilitating diseases in cattle* (Fig. 1.26). Calcium and phosphorus are deposited in degenerate subendocardial muscle, but more commonly in fibroelastic tissue. The fibroelastic tissue is modified either by chronic disease or elevated serum levels of calcium and phosphorus, as in *vitamin D intoxication*, or following the ingestion of plants containing vitamin D analogs (see Vol. 3, Endocrine glands). Proprietary *rodenticides* based on calciferols cause this lesion in dogs.

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Endocarditis

Endocarditis is one of the more significant of the endocardial alterations. It is *usually bacterial* in cause, the exceptions being occasional *parasitic* or *mycotic* lesions. Any portion of the endocardium may become inflamed, but *the lesions are usually primary on the valves*, from which there may be some encroachment on the adjacent mural

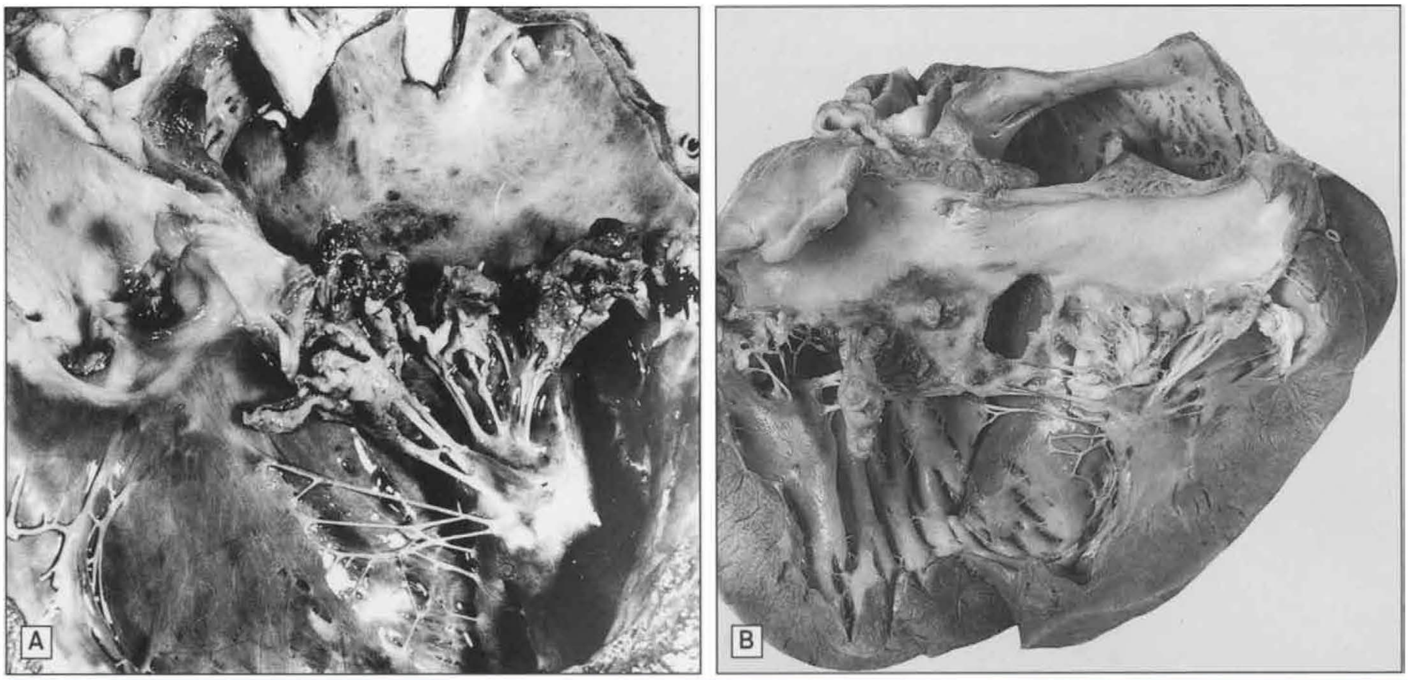

Figure 1.27 Valvular endocarditis. A. Caused by *Escherichia coli* infection in a dog. **B.** Valvular endocarditis with valvular rupture and extension to the ventricular wall in a dog.

endocardium (Fig. 1.27A, B). The mitral valve is most commonly affected in most species, followed by aortic, tricuspid, and pulmonic, except in cattle, in which the tricuspid valve is more commonly affected.

Many bacterial species are capable of causing acute **valvular endocarditis**, and the larger the number of cases examined, the wider the variety of pathogens recognized. In **cattle**, *Arcanobacterium pyogenes* is probably the most common pathogen, and a primary focus of infection can often be found in some other site, such as a traumatic peritoneal abscess, hepatic abscess, metritis, or mastitis. Streptococci of enteric origin are also of some importance in cattle, although the manner of their systemic invasion is not clear. *Erysipelothrix rhusiopathiae* has caused endocarditis in a variety of animals, most commonly in the **pig**; however, streptococci are more commonly isolated from cases of acute bacterial endocarditis of swine. **Horses** seldom develop bacterial endocarditis, but the lesion has been observed in association with *Streptococcus equi, Actinobacillus equuli, Escherichia coli, Pseudomonas aeruginosa*, and *Candida parapsilosis*. A number of cases in horses have originated from septic jugular vein thrombophlebitis. Minor outbreaks of endocarditis occur in **lambs** and are usually caused by *Streptococcus* spp., particularly enterococci; there may be associated polyarthritis. Endocarditis is seldom observed in **dogs**, but can be associated with a variety of organisms, especially *Streptococcus* spp., *Staphylococcus aureus*, and *Escherichia coli*, and less frequently with *Bartonella* spp., *Erysipelothrix rhusiopathiae, Erysipelothrix tonsillarum, Pseudomonas aeruginosa*, or *Pasteurella multocida* (Fig. 1.28). Endocarditis is unusual in **cats**, but has been caused by *Bartonella* spp. and *Streptococcus* spp.

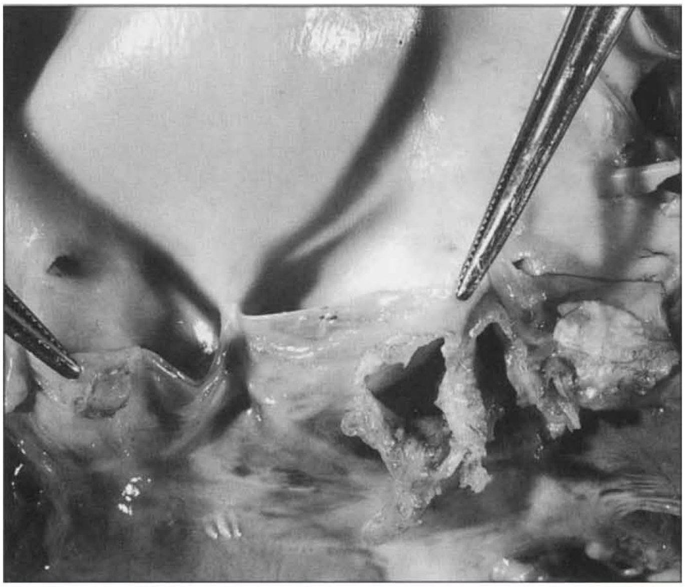

Figure 1.28 Aortic valvular endocarditis in a dog. The valve cusp is perforated.

The manner by which bacteria localize on a valve is not clear, and there is seldom detectable evidence of some separate underlying disease of the leaflet. In probably all cases of active thrombotic valvular endocarditis, bacteria are present in the lesion. Two factors that must be included in any concept of pathogenesis are

1. the tendency for lesions to occur at the *lines of apposition of the valve surfaces* exposed to the forward flow of blood, and
2. the necessity for *sustained or recurrent bacteremia.*

It is possible that simple debilitation of endothelial cells in recurrent bacteremia, aggravated locally on the valves by the trauma of apposition, may alter the adhesiveness of the leaflet endothelium sufficiently for bacterial adhesion to occur. It is of significance that valvular endocarditis can be produced in normal animals by a single intravenous injection of bacteria of suitable type and virulence; the incidence of endocarditis is increased if the hearts of such animals are subjected to increased workloads or the valves are traumatized during the experiments. There is also the possibility that bacterial endocarditis can begin within the valve during septicemia, as has been demonstrated by isolation of bacteria from the spongiosa of pig valves.

Particular strains of *Erysipelothrix rhusiopathiae* can selectively adhere to the valvular endothelium of porcine heart valves in vitro, with greatest numbers at the base of chordae tendineae. The phenomenon of *selective adherence* has been demonstrated for pathogens associated with other organ system disease, notably staphylococci and streptococci in mastitis and enterotoxigenic coliforms in colibacillosis. There is also an immunologic cross-reaction between *E. rhusiopathiae* antigens and valvular and myocardial antigens. Various *Streptococcus* spp. have been shown to adhere to exposed basement membrane of porcine valves.

The lesion of **acute bacterial endocarditis** is usually observed as an *irregular vegetation* on the affected valve. It is quite exceptional to find the earliest lesion, which consists of *irregular ulcerations* on the swollen leaflet. The composition of vegetations is similar to that of thrombi with, however, few platelets. *Numerous bacterial colonies are present in the vegetations*, almost always in pure populations. It is wise to make preliminary identification of the organisms by smears, because although they persist in colonies buried in the vegetations, they may not be cultivable. With the current techniques of molecular biology, such as PCR, it should be possible to identify at least the genus of the non-cultivable organisms embedded in the thrombus. The bacteria that initiate the lesion are enmeshed in the early layers of thrombi and are buried deeply as new layers are formed on the surface. With continued multiplication, many microscopic colonies are formed, and without appropriate antimicrobial treatment, it is their persistence in the protected environment that is the reason healed bacterial endocarditis is seldom seen. Even though the bacteria in the thrombi may lose their vitality, as indicated by lack of cultivability, they are persistently antigenic.

Grossly, the vegetations are yellow-red or yellow-gray and usually covered by a thin clot of blood, which can be easily peeled off. The surface of the vegetation is friable, and small vegetations can be easily removed to leave a granular, eroded surface on the valve. The ulcerations are especially common in the commissures and at the free margins of the valves, imparting to them a rough, serrated appearance. The primary valvular lesion frequently extends to the adjacent mural endocardium and, in the cases of aortic valvular endocarditis, to the adjacent aortic intima in the sinuses of Valsalva; in the latter eventuality, the lesion may involve an orifice of a coronary artery, predisposing to *myocardial embolism*. Endocarditis of the atrioventricular valves tends to spread along the chordae tendineae, causing some of them to rupture. Acute swelling and inflammatory change take place in the substance of the valve itself, often with necrosis, which obscures the line of partition between the valve and its surface vegetation.

Organization of the thrombus proceeds from the base by the usual process of granulation, which is likely to be impeded or destroyed by bacterial growth. Early scarification in the deepest layers separates the thrombus from the underlying myocardium and is frequently accompanied by mineralization.

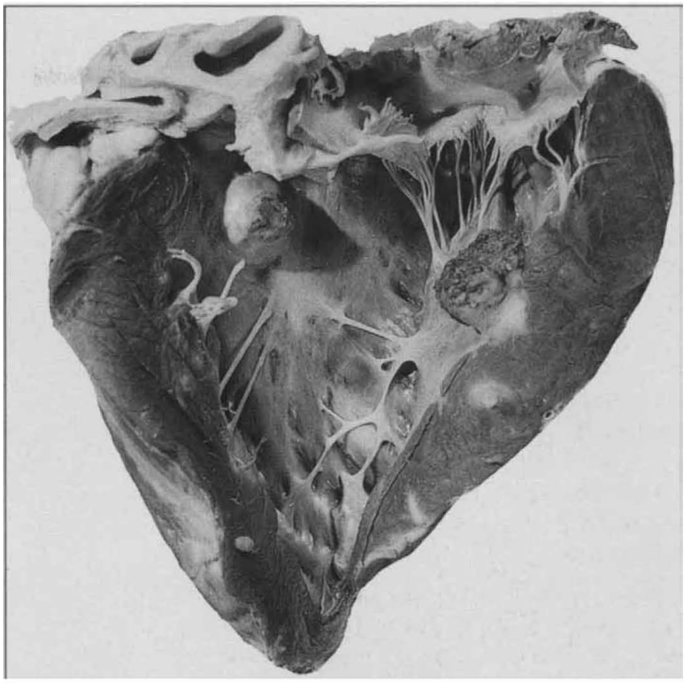

Figure 1.29 Myocardial abscessation and scarring. There is extension of inflammation to the mural endocardium with thrombus development.

Valvular endocarditis is commonly fatal, although resolution may be complete if lesions are only minor. Clinical signs in animals with endocarditis may only be detected late in the disease, with the most common being *pyrexia, lameness, and cardiac murmurs.* The frequency with which clinical signs are observed is dependent on the husbandry conditions under which the animal is kept. Other sequelae are those resulting from valvular damage or embolism. Portions of the vegetations may become detached and carried as **emboli**, to become impacted in the vessels of other organs. Such emboli may be either *bland*, that is, consist of thrombotic material only, or *septic*. Emboli arising in the right heart may produce pulmonary abscesses or pulmonary thrombosis. These latter do not produce pulmonary infarcts in the absence of preexisting pulmonary congestion. Emboli arising in the left heart produce their most obvious effects in the kidney and spleen as septic or aseptic infarcts and embolic glomerulitis, and in the myocardium as myocardial abscesses or interstitial myocarditis. Arthritis is commonly observed in association with endocarditis in the dog. Cerebral embolism is rare in animals. Unless the valvular lesions are very slight, there is, with healing, some degree of permanent damage, especially if the inflammation is recurrent. Shrinkage or adhesion of leaflets may cause narrowing of the orifice (stenosis), or insufficiency as a result of failure of the distorted valves to close the orifice. Stenosis and insufficiency may coexist. The usual outcome is *congestive heart failure.*

Mural endocarditis may be merely an extension to the walls of the cardiac chambers of a process originating on the valves. This is almost invariably true of endocarditis caused by *Arcanobacterium pyogenes*. Small foci of mural endocarditis may be found adjacent to foci of myocarditis, especially myocardial abscesses (Fig. 1.29). There are some additional occurrences of mural endocarditis worthy of mention.

Parasitic endocarditis caused by the larvae of *Strongylus vulgaris* occurs in horses acutely in the form of vegetative valvular endocarditis (Fig. 1.30), and chronically as caseous and calcareous nodules attached to the endocardium at various sites, including the apex of the left ventricle and protruding into the cavity. There is, in the same cases, parasitic aortitis of the bulb. With the advent of effective anthelmintics, parasitic endocarditis in horses is rare.

An acute form of **ulcerative mural endocarditis** occurs in cattle dying from *blackleg*, as red thrombotic masses attached to the free wall of the right ventricle and, less often and less extensively, on the right atrial and valvular endocardium.

Various unique **intracardiac fistulae or shunts** have been reported a sequelae to endocarditis in dogs, including: extension of aortic valvulitis into the right coronary artery and fistulation into the right atrium; perforation of the membranous interventricular septum leading to a left ventricular outflow tract–right atrial shunt (*Gerbode defect*) in a dog with severe mural endocarditis due to a *Bordetella*-like organism; and a fistula between the left ventricular outflow tract and the left atrium adjacent to the mitral valve annulus in a dog with *Staphylococcus intermedius* endocarditis.

One of the more common forms of mural endocarditis occurs in **renal failure** in dogs. The lesion is confined, within the heart, to the left atrium, but similar lesions occur in the large elastic arteries, being more frequent in the pulmonary and aortic trunks immediately proximal to the valves, and less frequent in the descending aorta and its major branches. (The vascular lesions associated with renal failure are discussed under Diseases of the vascular system.) The endocardial and major-arterial lesions are more common in acute than in chronic renal failure, insofar as these syndromes are distinguishable.

The *ulcerative atrial lesion*, which is identical to the arterial lesion, begins as a swelling of the interstitial spaces of the subendocardium or intima of the arteries, with deposition of glycosaminoglycans. Initially, the endothelium is intact, and when viewed grossly in this stage the endothelial surface is seen to be raised slightly, finely wrinkled, and slightly opaque but still shiny. The lesion may not progress farther than this but, instead, heals with some fibrosis, which leaves areas of uneven opacity. More usually, however, there is progression to necrosis involving the cellular elements as well as collagen, elastic, and reticulin fibers. The necrotic tissue ulcerates, and the margin is densely infiltrated by leukocytes. The ulcerations may perforate the wall of the atrium. Thrombus, in greater or lesser amounts, forms on the ulcerated surface. Heavy deposits of calcium salts often form in the degenerate tissue. If renal sufficiency is reestablished, the endocardial lesion may heal leaving irregular patches of fibrosis with an intact endothelium, often covering persistent white plaques of mineralization. Other extrarenal lesions of uremia are considered in Vol. 2, Urinary system.

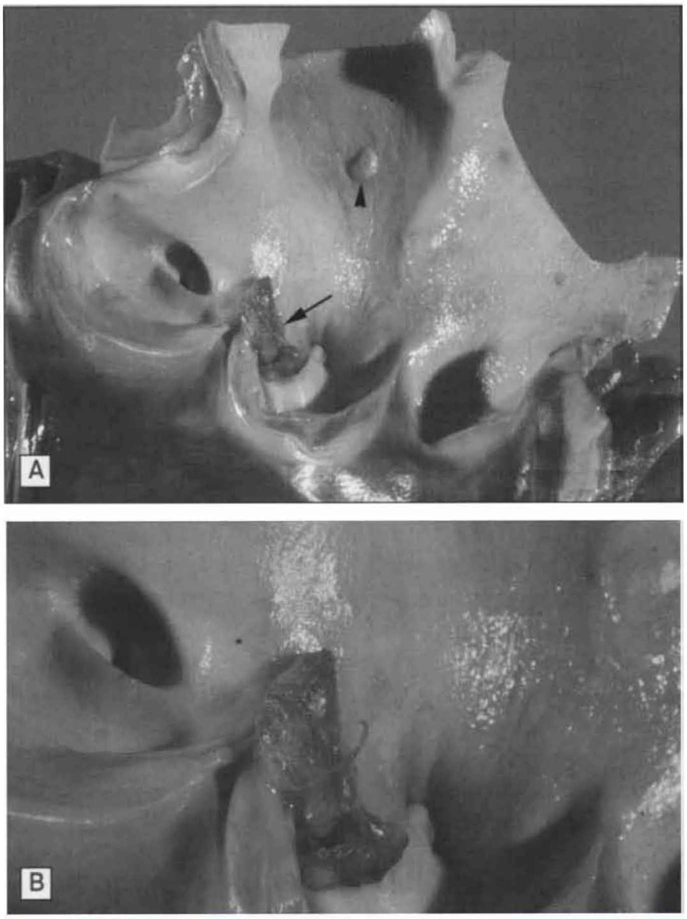

Figure 1.30 A. Vegetative aortic **valvular endocarditis** (arrow) and calcareous aortic intimal nodule (arrowhead) caused by *Strongylus vulgaris* infection in a horse. **B.** A strongyle larva was present in the aortic valvular vegetation.

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MYOCARDIAL DISEASE

Myocardial dysfunction can be the product of a wide variety of agents and conditions (Table 1.5). We will deal in this section with the range of myocardial disease, from the more-or-less specific myocardial conditions of *degeneration, necrosis, hemorrhage, and myocarditis, through idiopathic myocardial disease (cardiomyopathy). The reaction of myocardium to insults is limited*, and includes the usual expressions of degeneration, necrosis, inflammation, and healing. Acute changes, such as necrosis and hemorrhage, will be discussed first. Given time to adapt to an insult, a diseased heart may grossly take one of two conformations: dilated or hypertrophic. This classification is useful conceptually, but is somewhat arbitrary, and individual cases will often have features of more than one form, e.g., the heart in a cat with hyperthyroidism will hypertrophy, but it may eventually dilate and fail.

Hemorrhage of the heart and its membranes

Petechial and larger hemorrhages beneath the epicardium are so common in *horses* that die naturally that they are almost to be regarded as normal. The incidence of hemorrhages in sheep and cattle is somewhat lower; they are seldom seen in dogs and cats. *Subepicardial hemorrhages are common in instances of asphyxia or death in anoxia, and in many acute infectious fevers.* Larger ecchymotic hemorrhages that may involve most of the epicardium occur in the *hemorrhagic diatheses*.

Subendocardial hemorrhages have the same pathogenesis but are considerably less common. Ecchymotic hemorrhages beneath the endocardium of the left ventricle occur in instances of *acute cerebral injury* and are useful as a diagnostic indication of *clostridial enterotoxemia* in lambs and calves, being present in a considerable proportion of cases.

Small interstitial capillary hemorrhages *within the myocardium* accompany those within the subserosa. Deep myocardial hemorrhages that do not extend to the serosa are commonly present in pigs that die of *asphyxia*; they are distributed in the wall of the right ventricle in the vicinity of the descending coronary grooves. A remarkable degree of myocardial hemorrhage occurs in *mulberry heart disease of swine* (see below).

Table 1.5 Causes of myocardial dysfunction in domestic animals

Infectious	Viruses, chlamydia, rickettsia, bacteria, fungi, protozoa, helminths
Toxic	Ionophores (monensin, salinomycin, maduramicin), catecholamines, antineoplastics (cyclophosphamide, doxorubicin, daunorubicin), doxycycline, gossypol, saccharated iron, thallium, toxic plants (many), insects (blister beetle (*Epicauta*))
Metabolic	Hyperthyroidism, hypocalcemia
Nutritional deficiency	Vitamin E–selenium, taurine (cats), copper, thiamine
Genetic	Porcine stress syndrome
Neuromuscular	Muscular dystrophy
Storage disorders, other depositions	Glycogen storage disease, amyloidosis
Infiltrative	Neoplasia (lymphosarcoma, hemangiosarcoma)
Idiopathic	Cardiomyopathy

Myocardial degeneration

Cardiac muscle is structurally similar to skeletal muscle and is subject to the same anatomic types of degeneration. There is, however, a greater liability for cardiac muscle to undergo degeneration as a response to many nonspecific causes, probably related to its continuous activity. *Diffuse nonspecific myocardial degeneration* occurs secondarily in a variety of systemic diseases, especially infectious fevers, anemia, and toxemia.

Hydropic change, *or vacuolar degeneration*, of the myocardium is characterized grossly by a dull gray appearance and increased friability so that the myocardium is easily torn. On cut surface, the muscle is smoother than normal, the outlines of individual muscle bundles obscured. **Fatty change** – the appearance of lipid vacuoles in myocyte cytoplasm – is a more severe event than hydropic change, and may be recognized as uneven patches of pale yellow myocardium. The process of fatty change is not uniform, and it is sometimes possible to recognize, beneath the endocardium, alternating bundles of fibers or strips of myocardium more yellow than the remainder ("thrush-breast heart"). Both hydropic change and fatty change are reversible forms of cellular injury.

Atrophy *of the heart occurs in chronic wasting diseases and malnutrition*. In some cases, the atrophy is accompanied by dark pigmentation, and it is then called "*brown atrophy*." It should be recognized, however, that atrophy can occur without pigmentation, and pigmentation can occur without atrophy. Ruminants are principally affected by atrophy, brown or not, and rarely does the atrophic heart produce clinical disturbance. The epicardial fat may be gelatinous, the endocardium wrinkled, and myocardium brown and friable. Histologically, the muscle fibers are thin, the nuclei small and dark, and masses of brown pigment granules are present within secondary lysosomes at the nuclear poles. The pigment is *lipofuscin*, an oxidation product of unsaturated lipids.

Mineralization *occurs quite commonly in the myocardium*, and is to be expected whenever there is necrosis of fibers. Calcium salts are selectively deposited in the Purkinje network in organomercurial poisoning in cattle, in which the mineralization is preceded by hyaline necrosis of the Purkinje fibers and is followed by surrounding fibrosis.

Xanthosis (xanthomatosis) refers to the *abnormal brown pigmentation* of the myocardium and skeletal muscles seen in cattle, particularly mature Ayrshire cows (see Vol. 1, Muscle and tendon). The pigment is *lipofuscin*, and is present near the nuclear poles in cardiac myocytes. Microscopically, lipofuscin ("wear-and-tear pigment") is commonly seen at the nuclear poles of cardiac myocytes in aged dogs and cats.

Myocardial necrosis

Focal to massive myocardial necrosis, or residual scars thereof, is a common lesion in necropsy material. The causes are quite varied and part of a number of disease syndromes described elsewhere in these volumes.

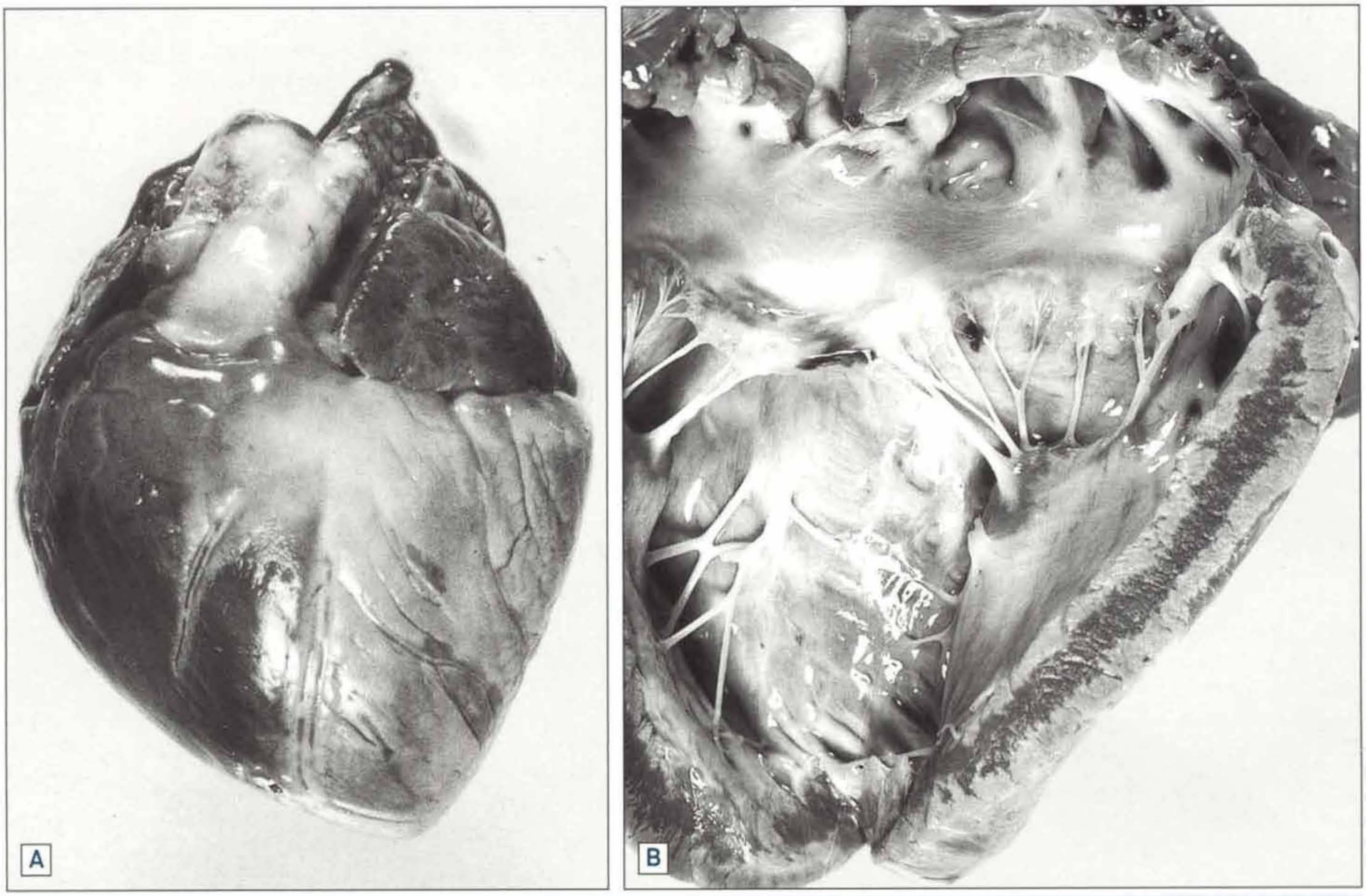

Figure 1.31 Nutritional myopathy in a calf. **A.** Marked pallor of left ventricle due to myodegeneration. **B.** Cut surface of left ventricle showing subepicardial and subendocardial myodegeneration.

In infectious disease, the distinction between myocardial necrosis and myocarditis with myofiber necrosis is somewhat arbitrary – inflammatory cells will invade necrotic myocardium; in acute myocarditis, inflammatory cells should be intimately associated with necrotic myocardial cells and adjacent myocytes may appear normal. Necrosis predominates in highly fatal foot-and-mouth disease in neonatal lambs, piglets, and calves, whereas the residual lesions in older cattle qualify as myocarditis with a significant inflammatory response. Fulminating infection by *Canine distemper virus* in young puppies is purely degenerative, whereas **parvoviral** and **herpesviral** infections in the same age group contain elements of an inflammatory response. Of the bacterial diseases, and excepting blackleg, *Histophilus somni* appears to be responsible for a residual syndrome of sudden death in cattle with myocardial necrosis or infarction, although in some cases myocardial abscesses may be present.

Severe, often fatal, myocardial necrosis is typically part of the important **vitamin E and selenium-responsive syndromes** of nutritional myopathy of lambs, calves (Fig. 1.31A, B), swine, and horses, and in mulberry heart disease of swine. It may also be seen as part of equine rhabdomyolysis and of capture myopathy and other exertional syndromes. (See Vol. 1, Muscle and tendon.)

Deficiency disease, additional to those that respond to vitamin E and selenium, may include myocardial necrosis. It occurs, although not invariably, in **thiamine deficiency** in carnivores, always as a single acute episode. Falling disease of cattle in Australia and Florida is a syndrome of sudden death believed to result from prolonged **copper deficiency**. Myocardial scars accompany acute lesions suggesting repetitive episodes and resembling the lesions of fluoroacetate poisoning in cattle eating the gigee plant, *Acacia georginae*. In cats, **taurine deficiency** causes myocardial failure.

Toxic myocardial degeneration is common, and although some examples are accompanied by degeneration of skeletal muscle they are described here. Injectable **saccharated iron** compounds, by virtue of the capacity of iron to generate free radicals in ferric/ferrous translations, cause fatal myocardial necrosis in piglets. **Monensin**, an ionophore that is widely used as a coccidiostat and growth promotant in ruminants, may contaminate compounded feeds for simple-stomached animals and is cardiotoxic for horses and pigs (see Vol. 1, Muscle and tendon for details of toxicity in other species). Also, ruminants fed dried poultry litter containing **maduramicin** or **salinomycin** develop severe myocardiocyte atrophy and interstitial fibrosis sufficient to result in clinical signs of congestive heart failure. Myocardial necrosis occurs in dogs ingesting rodenticides that contain **thallium** – these have been widely replaced by warfarin analogues and calciferols.

Galenia africana is a bushy plant that is toxic to sheep and goats in South Africa. The toxin has not been identified. Hepatotoxicity appears to develop first, advancing from dilation of central veins and sinusoids to centrilobular and bridging fibrosis, with areas of coagulation necrosis. Myocardial changes begin with myocyte hypertrophy and progress to patchy degeneration and necrosis of myofibers and light fibrosis.

Plants that contain **cardiac glycosides** of both the bufadienolide and cardenolide types are of worldwide distribution. Poisoning by **bufadienolide glycoside-containing plants** is the most important plant-associated poisoning of livestock in southern Africa, and may be acute or chronic. **Acute poisoning** is caused by plants of the family Iridaceae ("tulp"), e.g., *Homeria pallida* (the cape tulip), *H. miniata*, *Moraea polystachya*, *M. bipartita*; family Liliaceae ("slangkop"), e.g., *Urginea sanguinea*; and family Santalaceae, e.g., *Thesium lineatum* ("witstorm"). As well, several species of *Bryophyllum* that occur in southern and eastern Africa, and Australia, e.g., *B. tubiflorum* and *B. pinnatum*, contain bufadienolides (bryotoxins A, B, C, or bryophyllins). In acute poisoning, death occurs within a few hours of ingesting the plant. Affected animals show cardiovascular (tachycardia, arrhythmia, heart block), gastrointestinal, respiratory, and neuromuscular signs. Some cases may live for a few days and exhibit scattered foci of myocardial necrosis, but usually a diagnosis must be based on circumstantial evidence and the presence of plant parts in the ingesta. The cardiac glycosides are inhibitors of the sodium–potassium ATPase pump, which is responsible for maintaining membrane ion concentrations and the membrane potential of cardiac muscle fibers.

Chronic cardiac glycoside poisoning ("krimpsiekte") is a botulism-like, paretic condition of small stock that is caused by members of the family Crassulaceae ("plakkies") that contain cumulative bufadienolides, e.g., *Tylecodon wallichii*, *T. ventricosus*, *Cotyledon orbiculata*, *Kalanchoe lanceolata*.

Cardenolide cardiac glycoside-containing plants, e.g., *Nerium oleander* (common pink oleander), *Thevetia peruviana* (yellow oleander), of the family Apocyanaceae, are seldom eaten by livestock and are usually of little veterinary importance. However, when ingested, typically as plant clippings, their positive inotropic effects on the heart can cause tachycardia, arrhythmias, and sudden death. Long-term exposure to oleander can result in degeneration, fibrosis, and mononuclear cell infiltration of the myocardium. Oleandrin, the responsible cardenolide, can be detected in blood or urine by thin-layer chromatography or by high- performance liquid chromatography/mass spectrometry (LC/MS). Summer pheasant's eye (*Adonis aestivalis*), which contains cardenolides similar to oleander and foxglove (*Digitalis* spp.), causes endocardial and epicardial hemorrhage and mild multifocal myocardial necrosis; strophanthidin, the aglycone of several cardenolides in *Adonis*, can be detected by LC/MS/MS in gastrointestinal contents from affected horses.

Not all acute cardiotoxicity of botanical source can be ascribed to glycosides. Whereas chronic ***Phalaris*** toxicity does produce interesting changes in the central nervous system, the acute toxicity is of much greater economic importance as the cause of quick death in animals first exposed to grazing on this pasture species. The acute toxic factor in *Phalaris* appears to be a tryptamine alkaloid, which may interfere with the metabolism and action of serotonin and other catecholamines. The fast death factors of **lupins and algae** still need definition. In sheep, ***Lantana camara*** causes myocardial necrosis that might have an entirely different pathogenesis, possibly dependent on reduced myocardial perfusion as a result of chronic reduction in circulating blood volume. Poisoning by ***Cassia occidentalis*** (coffee senna), ***Karwinskia humboldtiana*** (coyotillo), and ***Cicuta douglassi*** (western water hemlock) causes muscular and myocardial necrosis in livestock. ***Vicia villosa*** (hairy vetch) poisoning involves many organs, including the heart. The lesions are unusual for a poisoning in that there is a substantial chronic eosinophilic granulomatous inflammatory response associated with the necrosis. Young ruminants (before the rumen has developed), pigs, and dogs are susceptible to the cardiotoxic effects of *gossypol*, a toxic alcohol, when **cottonseed meal** is incorporated in their feed as a protein supplement. ***Eupatorium rugosum*** (white snakeroot) causes myocardial and skeletal muscle damage through the action of *tremetol*, another toxic alcohol. High erucic acid **rapeseed oil** can cause myocardial degeneration and necrosis in a variety of species – this effect has been mitigated by the development of canola, which has low erucic acid content.

Ingestion of **locoweeds** (certain species of *Astragalus* and *Oxytropis*) increases the severity and rapidity of onset of high-mountain disease in cattle grazing at high altitudes. The locoweed toxin is swainsonine, an indolizidine alkaloid. Another indolizidine alkaloid, castanospermine, is found in the seeds of the Moreton Bay chestnut tree (*Castanospermum australe*); this toxic legume causes myocardial degeneration when eaten by horses or cattle.

Cantharidin, the toxic principle in **blister beetles** (*Epicauta* spp.), is thought to be the cause of gastric lesions, hemorrhagic cystitis, enterocolitis, and myocardial necrosis in horses that ingest the beetles in baled alfalfa hay.

Focal myocardial necrosis is also frequently observed as an incidental finding on microscopic examination in many diseases, and its immediate pathogenesis can seldom be ascertained, but *the lesions are thought to have an ischemic basis*. Ischemic myocardial necrosis occurs in thrombotic disease such as disseminated intravascular coagulation, in the microangiopathy of vitamin E/selenium deficiency, and in inflammatory vascular disease such as periarteritis nodosa. Poor myocardial perfusion may be the cause of the multifocal myocardial necrosis seen occasionally in hypocalcemic parturient dairy cows. The subendocardial necrosis seen following acute brain injury (the "*brain-heart syndrome*") may result from coronary arterial spasm and/or catecholamine excess. Another example of **catecholamine-induced myocardial necrosis** is that seen in cases of functional pheochromocytoma. The exact mechanism(s) of catecholamine toxicity is unknown, but may involve free radical damage of myocyte membranes.

Coronary embolism is a relatively common cause of focal myocardial necrosis, the emboli originating from vegetations of left-sided endocarditis; septic emboli may result in myocardial abscesses. In granulocytopenic diseases, bacterial embolism may result in multiple minute myocardial infarcts, the best examples being seen in bracken fern (*Pteridium aquilinum*) poisoning in cattle. Myocardial infarction may occur in polyarteritis (periarteritis nodosa and allied lesions).

Arteriosclerosis, although common in animals, is very seldom severe enough to be responsible for ischemia of the myocardium. In aged dogs, some degree of *hyalinosis of the intramural coronary arteries* is frequently seen (Fig. 1.32) and may be associated with, and presumably the cause of, myocardial infarcts. This condition is called **multifocal intramural myocardial infarction** (discussed with Degeneration of arteries). Hearts that are dilated or hypertrophic are susceptible to focal necrosis of apparently random distribution, although larger lesions are more obvious in the subendocardium of the left ventricle and papillary muscles.

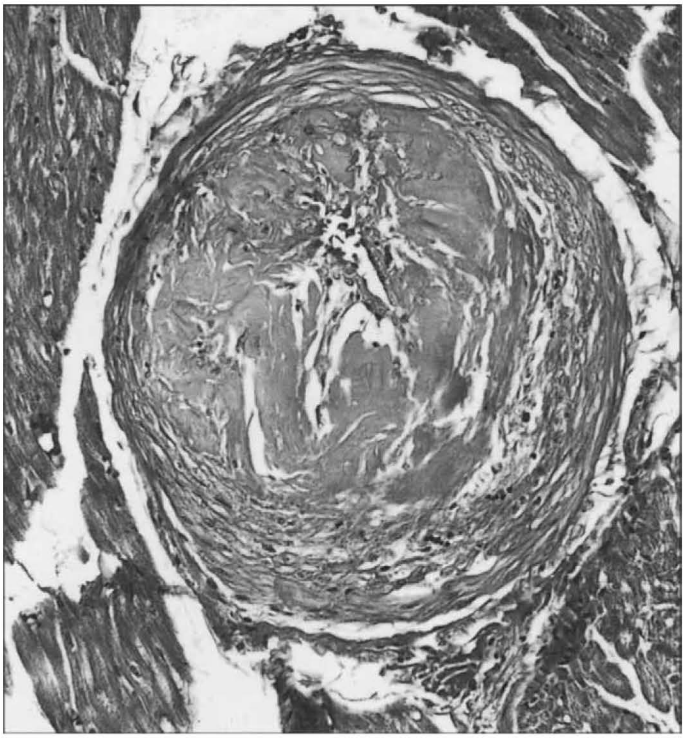

Figure 1.32 Marked **hyalinosis** of an intramural coronary artery.

The gross and microscopic appearance of myocardial necrosis is dependent on the interval between the initial insult and death. **Gross changes** of necrosis are not readily apparent until 12 hours after injury. By 18 to 24 hours, the affected area, in which calcium salts may be deposited, is paler and gray-brown. The overlying serous membrane is usually normal. *Myocardial degeneration, mineralization, and necrosis are most common in the subendocardial myocardium.* This area undergoes the greatest intramyocardial tension during systole and is the most likely to be ischemic. However, proliferative or thrombotic lesions completely occluding vessels in these areas are rarely observed even with careful technique. The common small foci of myocardial necrosis may not be visible from the serous surface, and perhaps may be recognizable only microscopically. The necrosis resulting from infarction will, if sufficiently large, involve the serosal surfaces and produce fibrinohemorrhagic thickening of the pericardium that may neatly overlie and indicate the infarcted area. In cases dying within 24 hours, the pericardial reaction may be the only indication grossly of infarction, the necrotic muscle at this stage not being clearly altered or merely pale. The necrotic area becomes more sharply defined by hyperemia by the second to fourth days. By the tenth day there is beginning replacement of the necrotic zone by fibrous ingrowth. Replacement fibrosis is well established by the end of the sixth week (Figs. 1.33 and 1.34). A substantial, often transmural loss of myocardium and replacement by fibrous tissue may lead to the development of aneurysms of the ventricular wall.

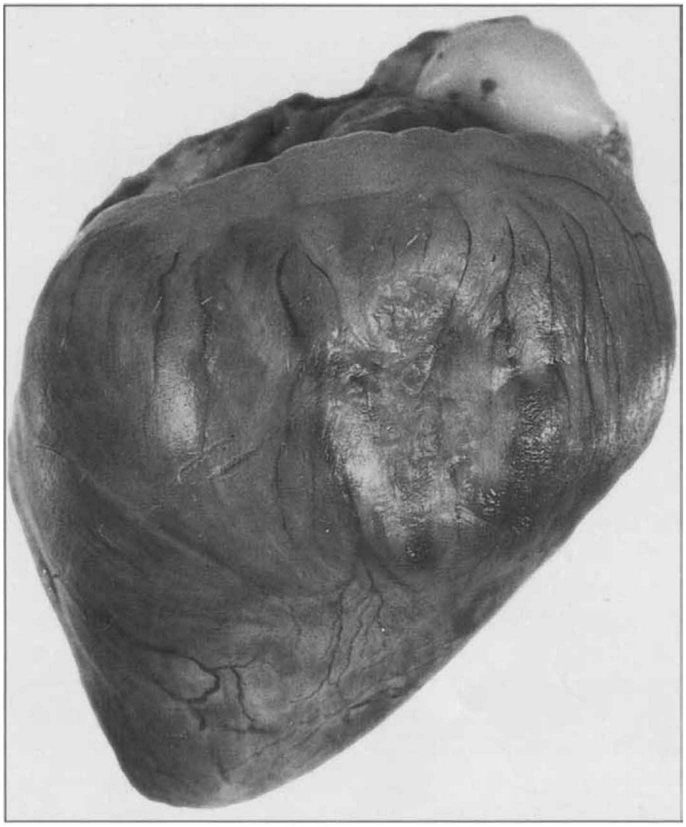

Figure 1.33 Healed, scarified **infarct** in wall of right ventricle of a dog.

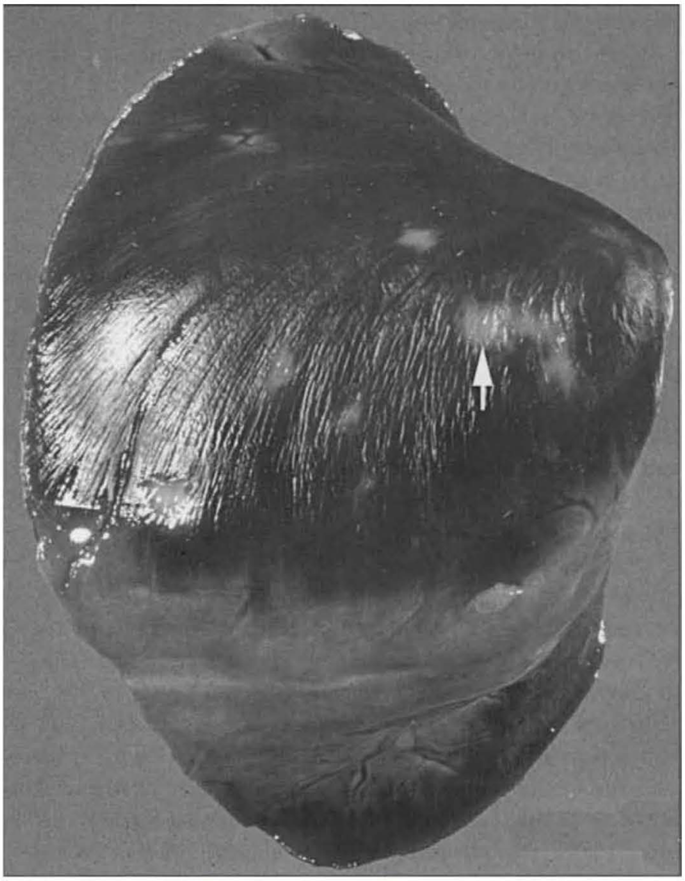

Figure 1.34 Myocardial scar near the apex of the left ventricle of the heart of a horse with vegetative aortic valvular endocarditis caused by *Strongylus vulgaris* infection.

Microscopically, lesions are not usually detectable for the first 6 to 12 hours. However, ultrastructural changes are observed within 1 hour. Recognition as early as 2 hours may be possible using special stains such as hematoxylin-basic fuchsin-picric acid. Necrosis becomes apparent by routine stains after 12 hours. Subsequently, neutrophils infiltrate the affected area and the nuclei of the myocytes become pyknotic. At this stage, macrophages are evident. Granulation tissue appears at the periphery of the lesion toward the end of the first week and usually predominates by the end of the sixth week.

There are variations in the microscopic appearance of myocyte necrosis and some attempt has been made to associate the differing microscopic patterns with particular causes, but these associations are not definitive. The first variant is **coagulative necrosis** characterized by cellular swelling, nuclear hyperchromasia, early loss of striations, and a "granular" appearance of the myocyte. This type of change is observed primarily in ischemic states. **Coagulative myocytolysis** is typified by the presence of thick, irregular, eosinophilic bands, with an accompanying loss of striations and lightly staining granular areas in myocytes. The bands are hypercontracted sarcomeres ("*contraction bands*") (Fig. 1.35) and the light granular areas are mitochondria. Evidence from experimental models suggests that the alteration is the result of the *excessive release of endogenous catecholamines*. However, this change may be observed in normal myocardium if it is fixed immediately after death. **Colliquative myocytolysis** appears as a loss of striations, and homogeneous, weakly eosinophilic cytoplasm. The ultrastructural appearance is characterized by intracellular edema, with disintegration of fibrils and other organelles. *Waviness of myocardial fibers* is claimed by some to be one of the earliest changes observed in myocardial infarction in humans. The affected fibers are not necrotic, but are thinner and undulating in appearance. It is thought that the waviness is due to irreversible stretching followed by compression of ischemic fibers by the surrounding viable myocardium. Attenuated wavy fibers ($<6\ \mu m$ diameter, wavy appearance) have been noted to be common in the myocardium of dogs with dilated cardiomyopathy.

Anichkov cells, also known as "*caterpillar cells*," have been observed in myocarditis and a variety of naturally occurring and experimentally induced myocardial necroses. They are also seen in rheumatic fever in humans in association with Aschoff bodies. Anichkov cells appear as large mononuclear cells in which the nuclear chromatin is present in an undulating wavy ribbon with slender processes radiating from it (Fig. 1.36). The origin of these cells is in dispute. Suggestions include a fibroblastic, pericytic, endothelial, or myocytic origin. Depending on the theory accepted, the function is either that of a macrophage or an abortive attempt at myocyte regeneration.

A few of the more interesting examples of myocardial degeneration and necrosis are described below.

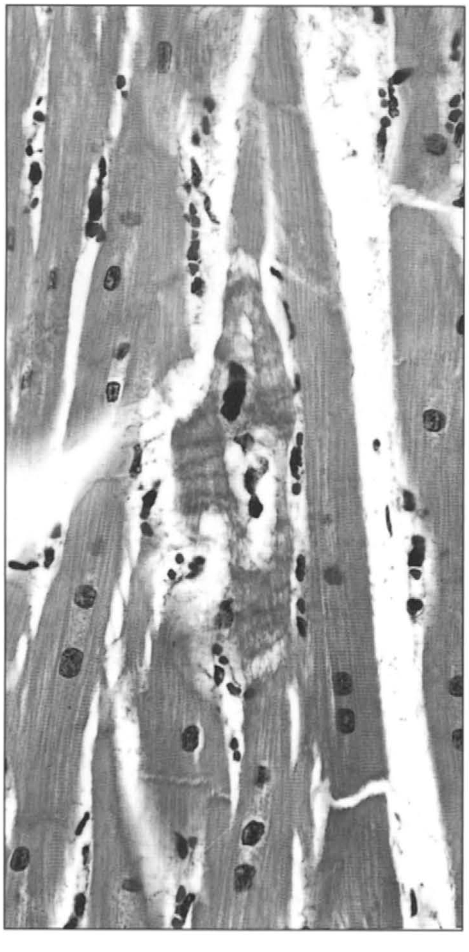

Figure 1.35 Hypercontracted sarcomeres in porcine stress syndrome. (Courtesy of AL Metz.)

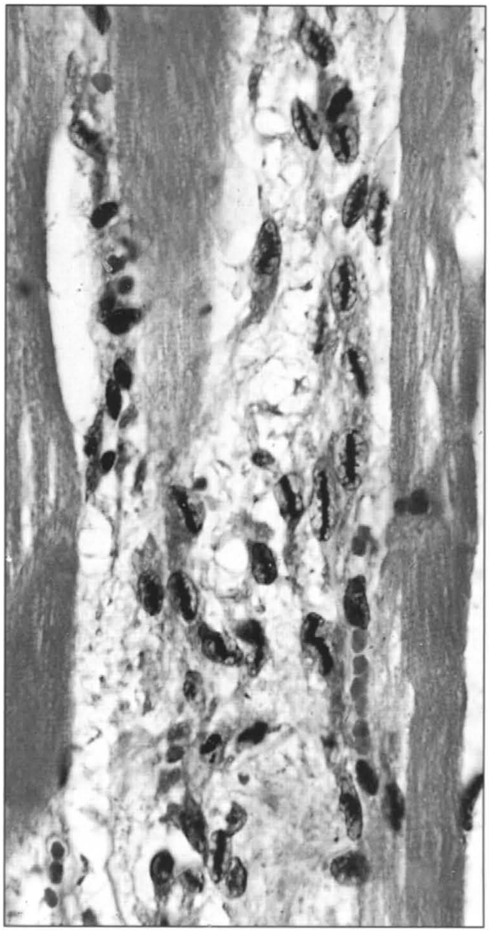

Figure 1.36 Anichkov cells ("caterpillar cells") in interstitium of sheep heart with multifocal myocardial degeneration.

Fluoroacetate poisoning

Sodium fluoroacetate (compound 1080) is used extensively in some parts of the world as a *rodenticide*. It is also highly toxic for most domestic mammals. The median lethal dose for most species is about 0.25–1.00 mg/kg of body weight. Fluoroacetate is not directly toxic, but becomes so when converted to fluoroacetyl-coenzyme A. This compound is then combined with oxaloacetic acid to form fluorocitrate. The citric acid cycle enzymes cis-aconitase and succinic dehydrogenase are inhibited by fluorocitrate, effectively inhibiting the production of adequate amounts of ATP. Accidental poisoning by fluoroacetate occurs either by direct ingestion of the poison, or indirectly, as when dogs eat poisoned rabbits.

Fluoroacetate of **plant origin** is responsible for sometimes devastatingly large episodes of poisoning of ruminants. Horses apparently are not affected, perhaps because they do not eat the responsible plants in adequate amounts and perhaps because, like humans, birds, and some native animals, they are considerably more resistant to the poison than most other species. Plants known to accumulate fluoroacetate in lethal quantities for ruminants are *Gastrolobium, Oxylobium*, and *Acacia georginae* in Australia, *Dichapetalum cymosum* (gibflaar) in South Africa, and *Palicourea marcgravii* in Brazil. Whether fluoroacetate has any role in the physiologic economy of the plants is not known, but in the case of *A. georginae*, there are differences in the development of toxicity in the species. In *D. cymosum*, there are seasonal differences, toxicity being greater in spring and autumn, and especially in young leaves. Young leaves, including sucker growth, and pods and seeds are more toxic than the mature leaves of *A. georginae*.

The syndromes of intoxication vary with the species affected but are either chiefly *neurologic*, as in dogs, which become extremely excited and convulsive, or chiefly *cardiac*, as in ruminants. Sheep may collapse suddenly and die within a few minutes. Those less severely affected may develop cardiorespiratory distress and weakness if driven, with death supervening shortly thereafter. Some showing these signs, if left undisturbed, may recover to appear normal until again forced to exercise. The syndrome in cattle is the same as in sheep, death occurring with cardiac failure, fibrillation, cyanosis, dyspnea, and terminal convulsions. Exercise, excitement, or a large drink of water may precipitate clinical signs.

The necropsy findings in ruminants are referable to *myocardial injury*. This may or may not be conspicuous, depending on the size of the dose and the opportunity for repeated episodes of poisoning. The concentration of fluoroacetate in *D. cymosum* is large, and histologic evidence of acute myocardial injury is seen. In *A. georginae* poisoning, both acute and chronic myocardial changes may be seen. There is some flabbiness of the myocardium, with prominent hemorrhages beneath the cardiac serosa. In acute cases there are irregular areas of myocardial pallor and mottling sometimes associated with older scars. There is multifocal myocytolysis or hyaline degeneration of the myocardial fibers, with intense infiltration of mononuclear cells. Loss of sarcoplasm leaves an open meshwork of reticulum and vessels, which eventually condenses and scar.

Gousiekte

Gousiekte ("quick" disease) is a plant poisoning of ruminants in southern Africa that is of great economic importance in that region. The disease is characterized by acute heart failure 5–8 weeks after the ingestion of certain rubiaceous plants. Clinical signs are seldom seen in natural cases; animals are usually found dead. A number of rubiaceous plants can cause the disease including *Pachystigma pygmaeum*, *P. thamnus*, *P. latifolium*, *Fadogia homblei*, *Pavetta harborii*, and *Pavetta schumanniana*. Pavetamine is the water-soluble, heat-stable toxin responsible for causing gousiekte.

Gross pathologic changes include generalized congestion, ascites, hydropericardium, hydrothorax and pulmonary edema. Ventricular dilation is an inconsistent feature. However, the ventricular walls are thinner and have a tough consistency. In a small proportion of cases the heart is macroscopically normal. Microscopically there is *focal-to-extensive myofiber loss with replacement fibrosis.* The surviving myofibrils may be atrophied, and groups of lymphocytes and macrophages may be present in areas of fibrosis.

Avocado poisoning

Sheep and goats may die following the *ingestion of fresh leaves* from some varieties of the avocado tree (*Persea americana*); the active principle is termed "*persin*." Animals may die suddenly after ingesting comparatively few leaves, whereas others may show few ill effects. In those that die, lesions consist of endocardial hemorrhage, especially of the papillary muscles; hydropericardium; pale, flabby heart; ascites; hepatic degeneration; and pale kidneys. Cardiac lesions are those of *myocyte necrosis* and congestion and hemorrhage of variable severity.

Consumption of avocado leaves also results in depression of milk production in lactating goats. The affected mammary glands are edematous and reddened with clots in the large ducts. The Guatemalan, but not the Mexican, variety of avocado induces these changes in the mammary gland.

Taurine deficiency in cats

Taurine-deficiency myocardial failure (TDMF) *in cats is a primary systolic myocardial disorder associated with taurine deficiency*; most cases of feline dilated cardiomyopathy are actually examples of TDMF. Decreased systolic function results in signs associated with decreased cardiac output giving rise to bilateral congestive heart failure. End-diastolic ventricular volume and pressure are increased, as are atrial volume and pressure.

Taurine (2-aminoethane sulfonic acid), an *essential amino acid for cats*, is abundant in fresh meat, but must be supplemented in commercial feline diets to prevent the development of TDMF, central retinal degeneration, and developmental abnormalities in kittens. Taurine is required in myocardium for the modulation of tissue calcium influx through cardiac calcium channels. Taurine supplementation will restore myocardial function to normal in most cats with low plasma taurine levels and echocardiographic evidence of cardiac chamber dilation and myocardial failure. Cats, and other mammals, have an obligatory need to conjugate bile acids with taurine or glycine; cats not only have a limited capacity to synthesize taurine, but are unable to utilize glycine for bile acid conjugation.

Cats are now unlikely to die of TDMF. In cats that do die, gross and histologic cardiac lesions are as described below for feline dilated cardiomyopathy.

Myocardial necrosis secondary to neural injury

The effect of the central nervous system on the myocardium is mediated by the autonomic nervous system, and in particular the sympathetic nervous system. The heart is richly innervated with autonomic fibers, and myocytes, particularly ventricular myocytes, have high concentrations of β receptors. Both the rate and inotropic state of the heart are stimulated by catecholamines. In a number of species, the continuous administration of *norepinephrine* for 1 or 2 weeks results in focal myocardial necrosis. Multifocal myocardial necrosis may develop in dogs with paroxysmal tachycardia due to *functional pheochromocytomas*. Also, experimentally induced *intracranial hemorrhage* produces focal myocardial necrosis, which can be prevented by prior administration of either reserpine, a catecholamine-depleting drug, or propanolol, which blocks β receptors.

Multifocal myocardial necrosis occurs in dogs, horses, cows, sheep, pigs, goats, and wildlife in association with neurologic disease of diverse origin (“**brain-heart syndrome**”), from external trauma to infectious disease. The primary lesion may involve the head, central nervous system, or major nerve plexuses. The microscopic appearance of the myocardial lesions is dependent on the time elapsed between insult and death. It varies from acute myocardial degeneration and necrosis to almost complete resolution by fibrosis. There are, no doubt, a number of cases with no gross or microscopic lesions where insufficient time elapsed between the onset of clinical signs and death. *The findings of multifocal myocardial necrosis on necropsy in any species should alert the pathologist to examine the central nervous system for abnormality.* Secondary multifocal myocardial necrosis also occurs in dogs with *gastric torsion*, and in dogs and cats with acute necrotizing *pancreatitis* and septic peritonitis.

Doxorubicin (Adriamycin) cardiotoxicity

Doxorubicin, an antineoplastic agent of the anthracycline antibiotic group, is used in the treatment of lymphosarcoma and other neoplasia in dogs; *cardiotoxicity is the major factor limiting its use.* Acute cardiotoxicity appears to be mediated by peroxidative injury or expression of cytokines. As well, binding of doxorubicin to nuclear and mitochondrial DNA causes blockage of DNA, RNA, and protein synthesis. Chronic doxorubicin cardiotoxicity, which may be due to decreased protein synthesis as well as altered divalent cation concentration, occurs in dogs given cumulative doses. Congestive heart failure occurs in such dogs; microscopic myocardial changes consist of myocytic vacuolar degeneration (“*adria cells*”), myocytolysis, myofibril atrophy, and fibrosis. The cardiotoxicity of doxorubicin is reduced when administered as a pegylated liposomal form.

Porcine stress syndrome

Porcine stress syndrome (PSS) is primarily a disease of skeletal muscle (see Vol. 1, Muscle and tendon); the PSS gene mutation is not expressed in myocardial muscle. *Death from PSS may occur as a result of peracute heart failure secondary to the metabolic and hormonal sequelae of PSS*, namely acidosis, hyperkalemia, hyperthermia, and *hypercatecholemia*. The latter may produce myocardial necrosis. Sudden death due to cardiac failure also occurs in sows, both with and without association with the PSS gene mutation.

Clinically, affected pigs exhibit muscular tremor, dyspnea, hyperthermia, and cutaneous blanching and erythema. Death quickly ensues. Typical necropsy findings include the rapid development of rigor, pulmonary edema, hydropericardium, and splanchnic congestion. In some, there is epicardial and endocardial hemorrhage with irregular areas of pallor in the left ventricular myocardium. The presence or absence of myocardial necrosis in porcine stress syndrome is a major difference between the European and North American descriptions of the disease. *Myocardial necrosis is a common finding in European cases.* When present, the necrotic areas are prominent in the inner third of the wall and papillary muscles. The microscopic appearance of the necrotic fibers is characterized by loss of the normal striation pattern, the presence of contraction bands, and fine granulation of the sarcoplasm (Figs. 1.35 and 1.37).

Mulberry heart disease of swine

The name “mulberry heart” is vaguely suggested by the *extensive hemorrhages on the surface of the heart* (Fig. 1.38). The disease occurs in pigs only, chiefly those 2–4 months of age and in excellent condition, but it has been observed in animals from 3 weeks to 4 years of age. Among old pigs the incidence is sporadic, but in young pigs it occurs in short, snappy outbreaks. In most cases, typically affected animals are *found dead*. The circumstances of sudden death combined with the gross and microscopic lesions strongly suggest that the affected animals die of acute heart failure following the development of ventricular dysrhythmias.

Raising weanling piglets on a diet deficient in selenium and vitamin E can regularly reproduce the disease. Such animals may develop disease

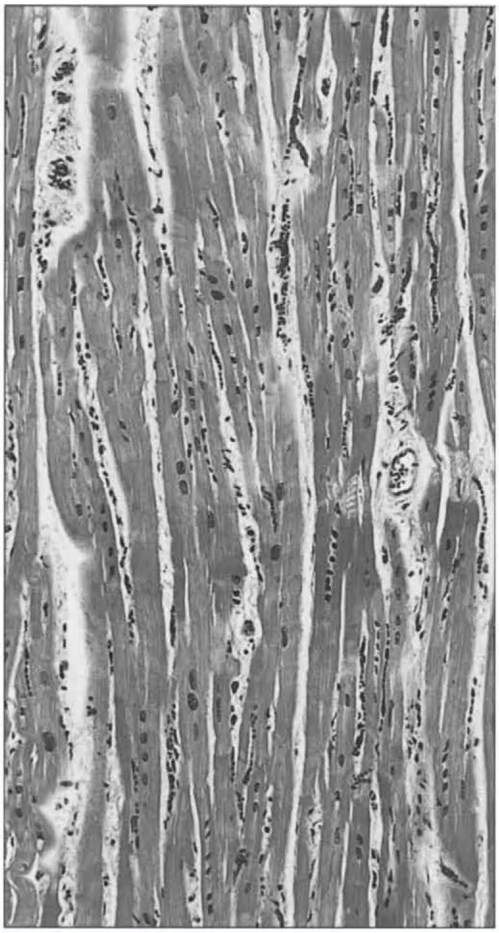

Figure 1.37 Acute myodegeneration, karyomegaly, and nuclear rowing in **porcine stress syndrome.** (Courtesy of AL Metz.)

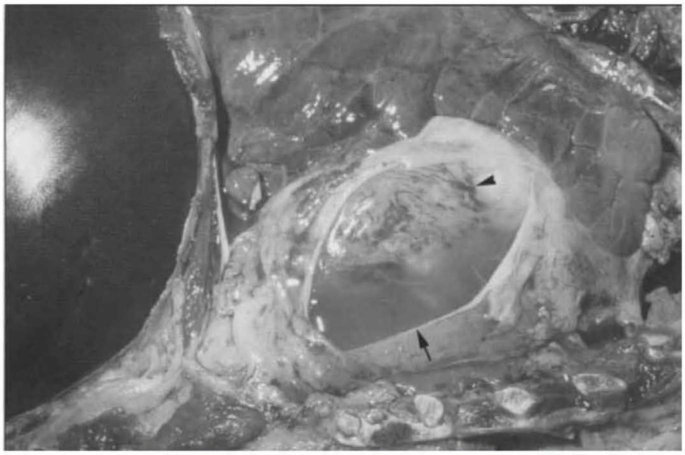

Figure 1.38 Distended pericardial sac and congested lung in **mulberry heart disease** in a feeder pig. Pericardial sac is opened to show epicardial hemorrhages (arrowhead) and pericardial effusion (arrow) containing fibrin strands.

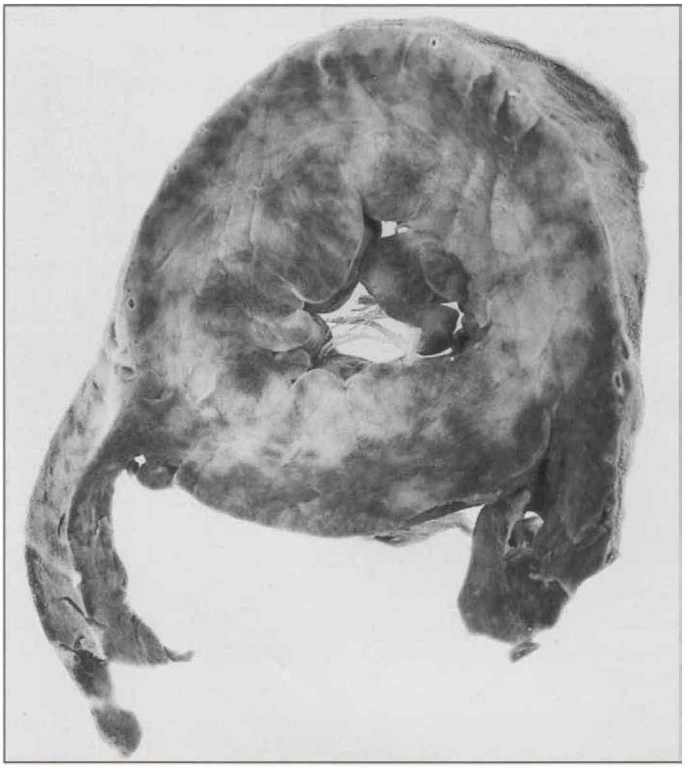

Figure 1.39 Cross-section of heart showing myocardial pallor and multifocal hemorrhage in **mulberry heart disease** in a pig.

within two weeks. As discussed in diseases of muscle (in Vol. 1, Muscle and tendon), selenium is an integral part of the membrane enzyme *glutathione peroxidase*, which reduces toxic lipid peroxides to hydroxy acids. *Vitamin E is an antioxidant* and acts synergistically with selenium to protect membranes from high concentrations of lipoperoxides. Field studies, particularly from the Scandinavian countries, have shown that while dietary selenium supplementation markedly reduces the incidence of hepatosis dietetica and nutritional myopathy, the prevalence of mulberry heart disease remains the same. It is also evident that tissue levels of selenium, particularly those in the heart and liver, are within the normal range. *It is now considered that vitamin E deficiency plays a central role in the development of mulberry heart disease.* However, there is not universal agreement on this. While some studies show lower tissue levels of vitamin E in affected pigs, others show no difference. Additionally, it has been suggested that the disease is the result of altered vitamin E metabolism and not a consequence of inadequate dietary vitamin E levels. It may be that mulberry heart disease is precipitated following a lack of bioavailability of vitamin E in tissues, or possibly a greater requirement for vitamin E under certain dietary circumstances, such as diets containing large amounts of unsaturated fat.

Animals dying of mulberry heart disease are always in *excellent body condition*. There may be slight cyanosis of the ears and ventral abdomen, and exophthalmos, the latter due to orbital and palpebral edema. The intermuscular connective tissues are usually wet, and there may be obvious edema, especially in the axillary and inguinal regions and near the xiphoid process. The skeletal muscles are normal.

The principal lesions occur in the thorax. Some 50 mL or more of heavy, straw-colored fluid that clots on exposure to air is invariably present in the pleural cavity. The lungs are edematous and slightly or severely congested, but not severely enough to account for the degree of edema. The pericardium is edematous and opaque and is always acutely distended with fluid transudate. Either heavy strands or a lace of fibrin float in the fluid (Fig. 1.38). The fibrin is not fixed to the serous surface and, from where it is in contact with the epicardium, it can easily be lifted off to reveal a clean, glistening, serous membrane. *Hemorrhages, linear and ecchymotic, are present beneath the epicardium*. They may be few or they may be extensive and involve the epicardium of all chambers, the myocardium, and beneath the endocardium of the papillary muscles and septum (Fig. 1.39). In some, multiple pale streaks and patches of necrosis extend from the epicardial surface into the myocardium. There may be similar lesions visible from the endocardial surface.

The abdominal cavity contains a small volume of clear transudate and some fine unattached strands of fibrin on the intestine. The stomach usually contains a mass of dry feed, and its fundic mucosa is diffusely congested. The small intestine is empty and dry, with serosal vessels congested. The liver is congested, sometimes markedly so, with edema of the wall of the gallbladder. In some animals that survive 24 hours or more, there is *bilaterally symmetric softening of the white matter* forming the cores of the cerebral gyri. The softenings are visible grossly as gray, translucent, depressed areas studded with tiny hemorrhages that are sometimes confluent (Fig. 1.40).

Microscopically in acute deaths, degeneration of myofibers may be minimal or absent. In these cases there is merely subserosal edema, with some plugging of lymphatics by fibrin, and interstitial hemorrhage. In others, which are probably less acute cases, there are *substantial areas of myocardial necrosis*. The extent of mineralization of the necrotic fibers varies, but never appears to be as severe as that seen in lambs or calves with nutritional myopathy.

The second prominent lesion, which is most probably unrelated to the myocardial necrosis, is observed in arterioles of many organs. The change is seen in the heart, kidneys, liver, stomach, intestine, mesentery, skeletal muscle, and skin. There appears to be gradation in the severity of change, from endothelial swelling with increased permeability, to necrosis of smooth muscle cells of the media. *The basic appearance includes the accumulation of fibrinoid in arteriolar walls, formation of hyaline thrombi, disruption of endothelium, and necrosis of smooth muscle cells.*

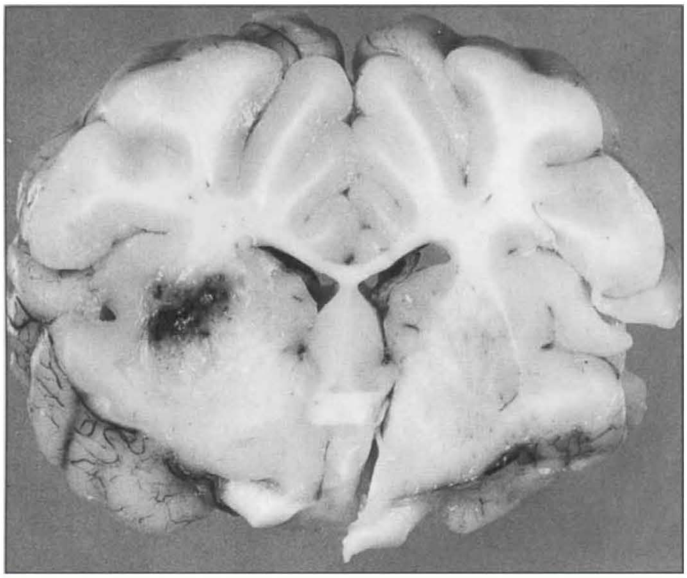

Figure 1.40 Cross-section of brain showing hemorrhage in region of internal capsule in **mulberry heart disease** in a pig.

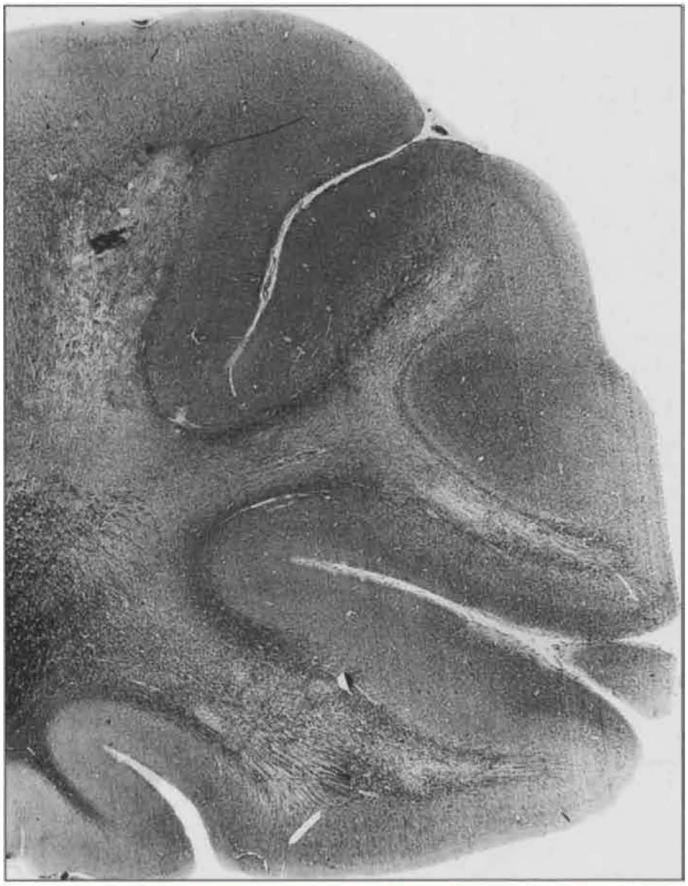

Figure 1.41 Liquefaction of white matter in the cerebrum, sparing corpus medullare and U fibers in **mulberry heart disease** in a pig.

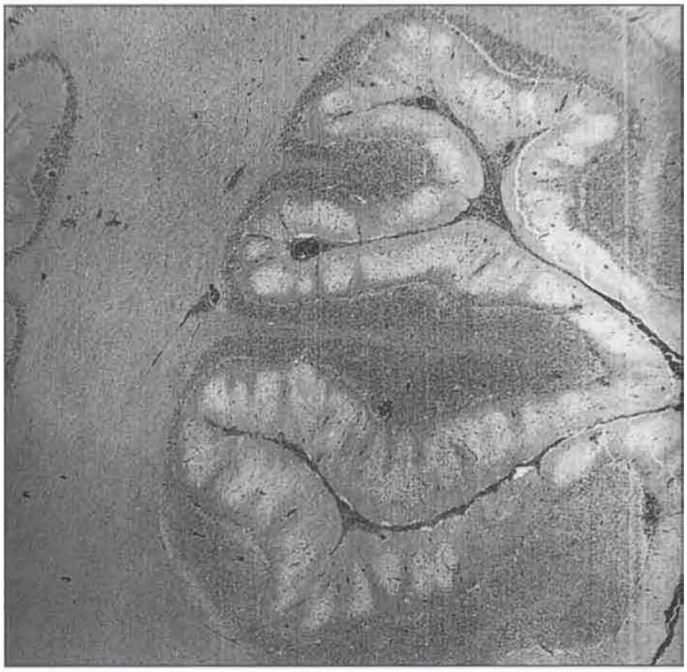

Figure 1.42 Numerous confluent areas of softening in the molecular layer of the cerebellum in **mulberry heart disease** in a pig.

Periodic acid-Schiff (PAS) staining of affected arterioles emphasizes the accumulation of fibrinoid. The lungs show congestion and edema only. There is congestion and edema of the liver, associated in some cases with central necrosis in the lobules. Proteinaceous fluid produces focal detachments of the retina and intercapillary edema of the glomerular tufts.

The development of the *cerebral lesions* can be correlated roughly with the duration of the clinical illness. In the majority of cases, where death occurs suddenly, there is no microscopic change in the brain, or merely some slight venous congestion and edema of the cortical white matter. In pigs that live for some hours, there is edematous separation of the fiber tracts of the white matter of the frontal cortex, with acute swelling and fragmentation of oligodendroglia. The overlying gray matter is edematous. In animals that survive for 24 hours or more, it is usual to find severe lysis of the white matter in the cerebrum, which is more or less extensive but usually avoids the internal capsule, corpus medullare, and association fibers (Fig. 1.41). The cores of the gyri of the frontal lobes are most consistently and severely involved, but in some cases all portions of the cerebrum are involved, and in these there may be similar lesions in the thalamus and brainstem. The vessels, venules especially, in the degenerate areas are severely injured and may be totally necrotic, although more frequently there is only endothelial and adventitial swelling and proliferation. Hyaline thrombi are formed in many vessels, and droplets of similar character form in the perivascular spaces. The overlying gray matter is edematous. The vessels are hypertrophic and cuffed by adventitial cells and a few eosinophils. In a few cases, degenerate foci are also present in the cerebellum; these involve the molecular layer with foci of softening (Fig. 1.42) or narrow sheets of hemorrhage.

The lesions in mulberry heart disease depend on the length of time pigs survive after the initial damage occurs. *Most pigs die from ventricular dysrhythmia*, and those that die immediately no doubt have substantial areas of myofiber death, but there has been insufficient time for the morphologic manifestations of the necrosis to appear. A period of 6–12 hours must elapse before myofiber necrosis can be reliably recognized. After that, the lesions become increasingly evident and progress through stages to replacement fibrosis of the necrotic areas if the pig survives.

In the experimentally induced disease, serum enzyme levels indicative of myocardial damage may be raised for up to 21 days before death. The extent of cardiac damage is related to the length of time the serum enzymes are raised or, alternatively, to a large increase over a shorter period. *The microangiopathy prominent in this disease probably occurs independently of the myofiber necrosis.* It is also nonspecific, as the same change is seen in many porcine viral infections and bacterial toxemias. It is, however, a useful indicator of the presence of mulberry heart disease.

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Myocarditis

Myocarditis, or inflammation of the myocardium, is a common lesion found in a wide variety of systemic diseases (Table 1.6), but it is rarely primary. It occurs hematogenously in many infectious diseases, and by direct extension from inflammatory lesions of the endocardium and pericardium. A number of specific parasitic infestations also produce myocarditis. Effectors of the immune and inflammatory responses in myocarditis can have numerous deleterious effects on the myocardium: cytolytic T lymphocytes can produce focal myocyte necrosis; activated lymphocytes and macrophages can release a host of inflammatory cytokines that can lead to altered myocyte metabolism, decreased contractility, and dysrhythmias, and to increased matrix and myocardial fibrosis. Death due to myocarditis may occur acutely due to dysrhythmia; inflammation is often focal and can be missed grossly. Non-fatal cases of myocarditis may progress to congestive heart failure, which ensues subsequent to myocyte loss and fibrosis.

Table 1.6 Causes of myocarditis in domestic animals

Viruses	E.g., *Canine herpesvirus, Canine parvovirus 2, Encephalomyocarditis virus, Equid herpesvirus 1, Foot-and-mouth disease virus, Murid herpesvirus* (murine cytomegalovirus), *Porcine circovirus 2* and/or *Porcine parvovirus* (in PMWS, postweaning multisystemic wasting syndrome), *Pseudorabies virus, West Nile virus*
Bacteria	E.g., *Actinobacillus equuli, Actinobacillus suis, Bartonella vinsonii, Borrelia burgdorferi* (Lyme disease), *Clostridium chauvoei, Histophilus somni, Leptospira* spp., *Streptococcus suis*
Rickettsia	E.g., *Ehrlichia risticii*
Protozoa	E.g., *Neospora caninum, Sarcocystis, Toxoplasma gondii, Trypanosoma* spp.
Helminths	E.g., *Trichinella spiralis*

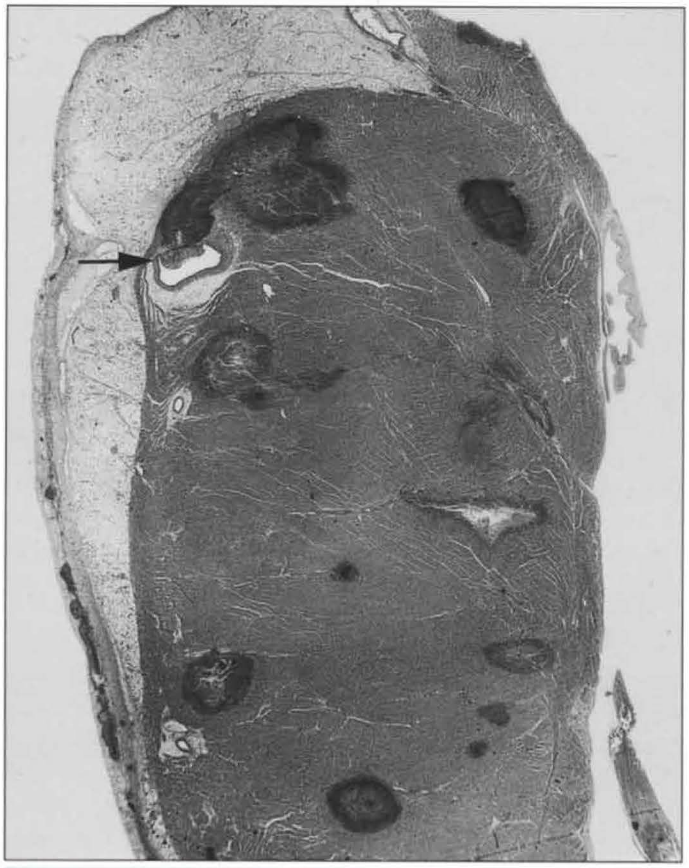

Figure 1.43 Embolic suppurative myocarditis, due to *Arcanobacterium pyogenes*, in a lamb. Multiple abscesses, one of which has encroached upon and caused thrombosis in a coronary artery (arrow).

Pyogenic bacteria, which may originate from any other suppurative focus in the body or, as in the case of *Listeria monocytogenes* and *Actinobacillus equuli*, produce foci of acute **suppurative myocarditis** that may develop into abscesses with inevitable destruction of the involved tissue (Fig. 1.43). In these, it is often possible to demonstrate bacterial emboli. *Clostridium chauvoei* may produce necrotizing myocarditis similar to the typical lesion in skeletal muscle. Tuberculous myocarditis is rare, and when present, the granulomatous reaction is typical of the species. Necrobacillary myocarditis occurs in cattle secondary to extensive necrobacillosis in other tissues; the suppurative reaction is typical for the organism. Other bacterial causes of suppurative myocarditis include *Clostridium piliforme* (*Bacillus piliformis*) and *Citrobacter koseri*. There is also extensive myocarditis in some systemic mycoses, such as *Aspergillus terreus* in the German Shepherd Dog.

Less specific **interstitial myocarditis** *occurs in many infectious diseases and is not detectable by the naked eye.* The basic pattern is usually the same, the reaction being centered on the interstitial and perivascular connective tissues, with edema and infiltration of lymphocytes, plasma cells, macrophages, and some eosinophils. Neutrophils are few. The changes in the myocardial fibers vary widely in extent and in the severity of degeneration. The cause may sometimes be apparent in the histologic nature of the lesion, as, for example, in malignant catarrhal fever of cattle, in which inflammation is centered on the blood vessels. In toxoplasmosis, the degeneration of myocytes predominates over the inflammatory reaction and the organism is identifiable. *In most cases of interstitial myocarditis, however, diagnosis of the cause can only be made by reference to the total pathologic picture.* Only infrequently can death be attributed to the myocardial lesions, and then only when considerable degeneration of the myocardial fibers has occurred, as in foot and mouth disease of young animals. Some other viral infections, such as canine parvovirus infection and encephalomyocarditis virus infection, are exceptions to this general principle.

Myocarditis is seen in **systemic viral infections**, such as canine distemper, canine herpesviral infection, and pseudorabies in piglets and dogs. The myocarditis caused by *Canine parvovirus-2* infection is discussed in Vol. 2, Alimentary system. *Porcine parvovirus* is a common cause of abortion, stillbirth, and mummification (see Vol. 3, Female genital system). However, porcine parvoviral DNA has been detected in suckling piglets by both PCR and to a lesser extent, by in situ hybridization in cases of non-suppurative myocarditis. Myocarditis is a feature of postweaning multisystemic wasting syndrome (PMWS) of weaned pigs – a syndrome experimentally reproduced by co-infection of pigs with *Porcine circovirus-2* and *Porcine parvovirus*. This lesion had formerly been ascribed to infection with *Porcine reproductive and respiratory syndrome virus*.

"**Eosinophilic myocarditis**" is so called because the *reaction to sarcocysts* is predominantly by eosinophils, which produce the typical, small, green to gray-green foci observed occasionally in cattle at routine meat inspection. This syndrome is not to be confused with the eosinophilic myocarditis present in cases of poisoning by ingestion of *hairy vetch* (*Vicia villosa*).

Encephalomyocarditis virus infection

There are three members of the genus *Cardiovirus* in the family Picornaviridae, and the type species is *Encephalomyocarditis virus* (EMCV). All species are antigenically related and by most tests are indistinguishable from each other. EMCV can infect humans and other mammals, principally swine and subhuman primates. *Rodents, in which it appears to be clinically inapparent and behaves as an enterovirus, may act as reservoir hosts*, but infection in laboratory rodents commonly produces fatal encephalitis or myocarditis. The source of infection in outbreaks of myocarditis in swine is thought to be through consumption of feed or water contaminated with virus from rats or other rodents. Ingestion of diseased rodent carcasses is also a likely source of infection. Experimental transmission of disease to swine has been accomplished by feeding the virus, but susceptible pigs kept in close contact with infected pigs did not develop disease.

Sudden death or death following a brief period of excitation and collapse characterizes the clinical disease in the peracute form in swine. In less severe cases, there may be fever, anorexia, and progressive paralysis. The mortality rate is variable. On necropsy there is hydrothorax, hydropericardium, ascites, and pulmonary congestion and edema. In severe cases, there is extreme pallor of the ventricles, with yellow-to-white foci 2–10 mm in diameter throughout the myocardium. In less severe cases, gross lesions are minimal to absent. *Microscopically, the dominant lesions are focal-to-diffuse myocardial necrosis.* Patchy mineralization of necrotic areas is evident, accompanied by a *mononuclear inflammatory reaction* that varies greatly in intensity. Survival allows fibrous scarring to develop. There is little evidence of encephalomyelitis. EMCV infection has also been associated with reproductive failure characterized by mummified fetuses and stillbirth, as well as failure of conception and early embryonic deaths. Fetuses that die toward the end of gestation have multifocal myocardial necrosis.

Experimentally infected swine may die between 2 and 11 days postinoculation. Virus is present in the feces for 7–9 days following oral administration and may be isolated from all organs, with the myocardium exhibiting the highest titer of virus. The virus titer falls rapidly and may not be found in cases that have been infected for 11 days or more. Experimental inoculation of mice regularly produces both encephalitis and myocarditis. Strains of EMCV may be adapted to produce either encephalitis or myocarditis.

Parasitic myocarditis

The parasites that tend to localize in the myocardium are the same as those that have an affinity for skeletal muscle. Parasites without such affinity may also be found in the myocardium in the course of their wanderings, especially if they are migrating in an abnormal host or in unusually large numbers in the normal host.

Of those parasites with an affinity for cardiac and skeletal muscle, the ubiquitous **sarcocysts** are the most common (see Vol. 1, Muscle and tendon). *The finding of myocardial sarcocysts is very common in ruminants.* This increases with the age of the animal and implies a previous acute sarcocystosis in which schizonts are formed in endothelial cells, usually with little effect. The sarcocysts may be found in the cardiac conducting fibers as well as myocardial fibers and normally appear to be harmless. The *multifocal eosinophilic granulomatous reaction* to degenerating ***Sarcocystis cruzi*** cysts may occur alone or in association with similar lesions of eosinophilic myositis in skeletal muscles. Degenerate cysts are occasionally visible within foci of myocarditis. The cyst content is highly toxic, but whether degeneration or rupture of the cyst is primary or is secondary to some other cause of focal myocardial degeneration is unknown.

A rather severe myocarditis in dogs caused by the protozoal parasite ***Neospora caninum*** *is seen in association with myositis and encephalomyelitis.* Affected dogs vary in age from 2 days to 7 years and may be presented with hindlimb paresis or ataxia, skeletal muscle atrophy and in some cases, sudden collapse attributable to dysrhythmias induced by the myocarditis. The differentiation of *Neospora caninum* from *Toxoplasma gondii* in paraffin-embedded tissue is best accomplished by the use of immunohistochemistry. Additionally, *Neospora caninum* is commonly associated with bovine abortion.

The various **cysticerci** of *Taenia ovis*, *T. saginata*, *T. solium*, and hydatid cysts are frequently found in the myocardium, which is one of their sites of predilection (Fig. 1.44A, B). These parasites are described with parasitic diseases of the intestine (in Vol. 2, Alimentary system), which is the habitat of their definitive stage.

Trichinella spiralis is described with diseases of muscle in Vol. 1, Muscle and tendon. However, it is worth noting here that the larvae of this parasite invade the myocardium, but are seldom found there, either because they continue their migration or are

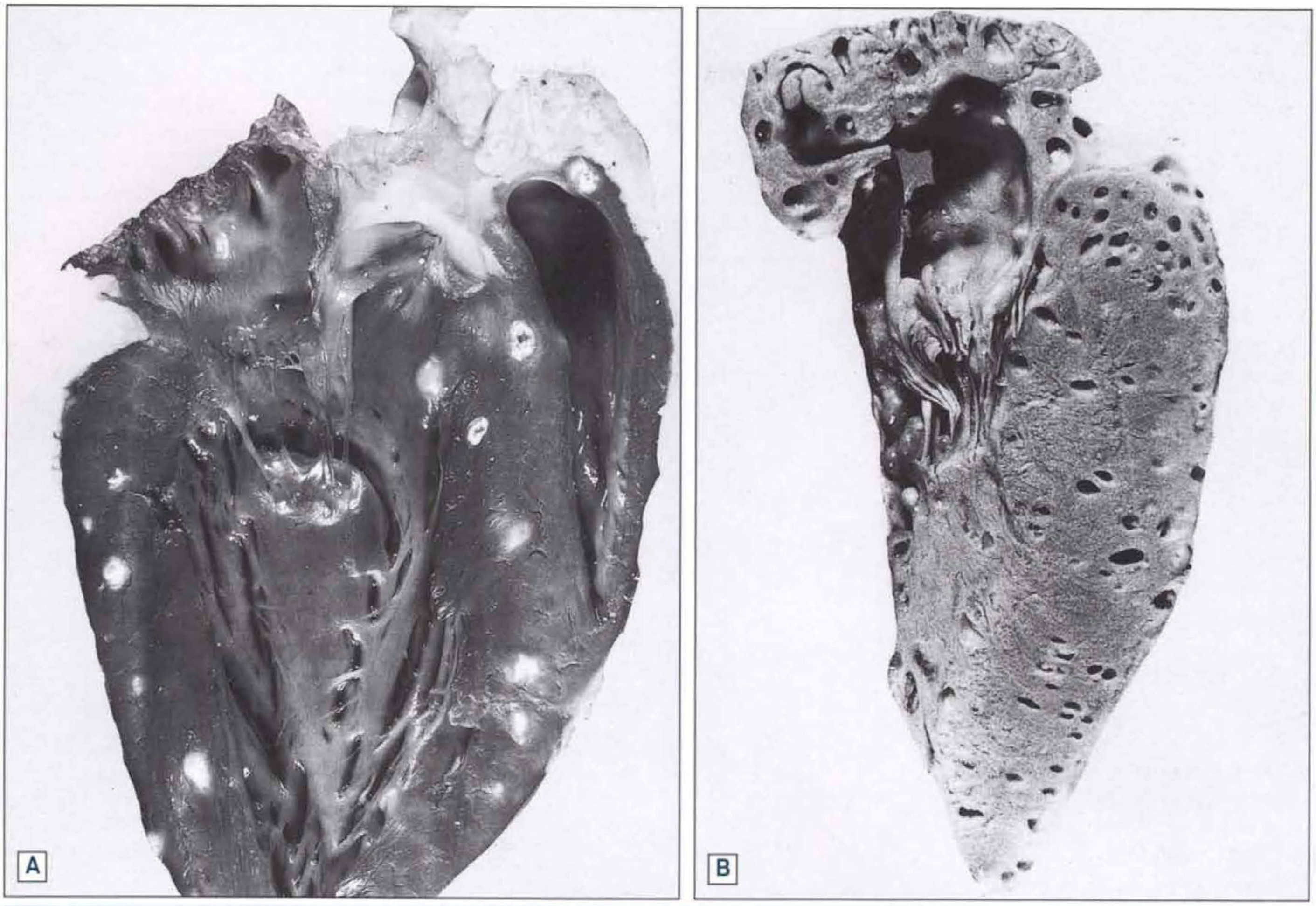

Figure 1.44 Parasitic myocarditis. **A.** Mineralized foci of degenerate ***Cysticercus ovis*** in a lamb. **B.** Cysts of ***Cysticercus cellulosae*** in the myocardium of a pig.

destroyed. The myocardial reaction is a *severe interstitial myocarditis*, with basophilic degeneration and necrosis of fibers in the areas of inflammation.

The *pyogranulomatous myocarditis* caused by ***Trypanosoma cruzi*** (Fig. 1.45) is described in Vol. 3, Hematopoietic system. In Africa, myocarditis can occur in dogs infected with *Trypanosoma brucei*, and in cattle infected with *T. congolense* or *T. vivax*.

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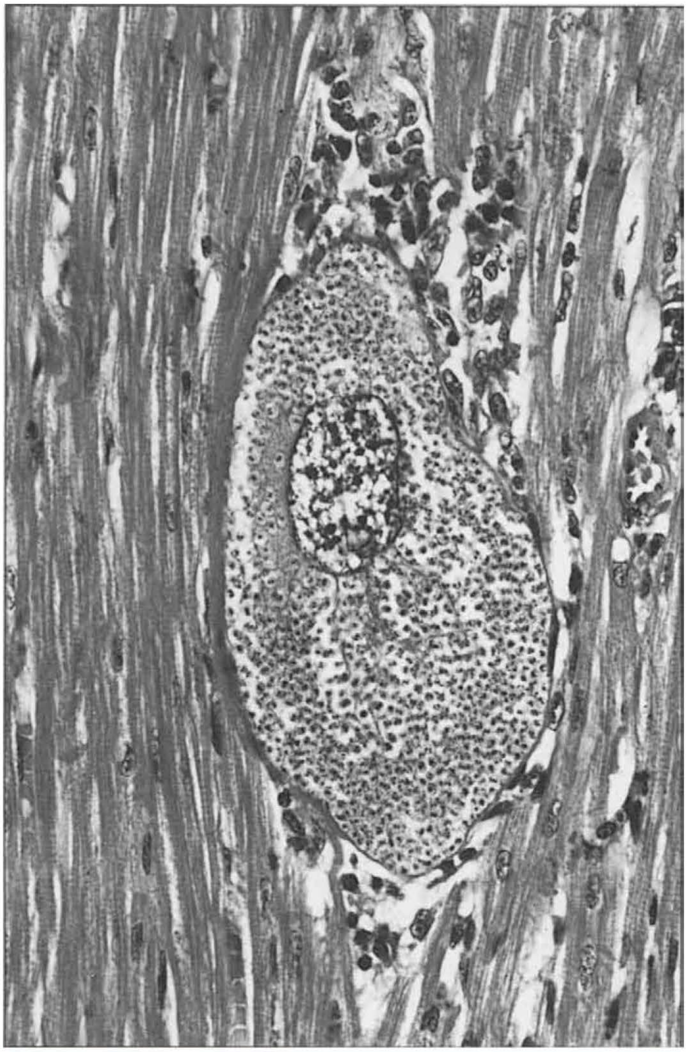

Figure 1.45 ***Trypanosoma cruzi*** in a dog. Amastigote-containing pseudocyst in myocardium, with a mild, mononuclear cell reaction.

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Cardiomyopathies

The term **cardiomyopathy** was originally coined for a group of myocardial diseases in humans that were of unknown or obscure cause. It remains essentially a diagnosis by exclusion. In humans, several criteria must be satisfied to establish the diagnosis. They are the absence of

1. significant coronary artery disease or anomaly,
2. vascular disease or anomaly,
3. systemic hypertension, past or present, and
4. shunts inside or outside the heart.

Features usually present are

1. cardiomegaly, due to generalized dilation or hypertrophy,
2. mural thrombosis, usually in the left ventricle, and
3. fibrosis or other lesions in the myocardium indicative of generalized myocardial involvement.

Of these features described for humans that must be absent, coronary arterial disease and systemic hypertension may be disregarded in domestic animals because of their comparative rarity. An additional exclusion, at least for cats, should be that of hyperthyroidism.

Since the original definition, the term cardiomyopathy has been used loosely to describe any abnormality of the myocardium for which an etiology cannot be defined. There has also been some attempt to classify cardiomyopathies into primary (obscure etiology) and secondary (known systemic or etiologic abnormalities) categories. Under such classifications, the list of diseases is seemingly endless. *It is therefore preferable to restrict use of cardiomyopathy to* **idiopathic primary myocardial disease**.

Progress has been made in the understanding of the cardiomyopathies, particularly with one of the forms of feline cardiomyopathy and in hypertrophic cardiomyopathy in people. *Most cases of dilated cardiomyopathy in cats are now recognized as taurine-deficiency myocardial failure (TDMF)*, which is described above with myocardial degeneration/necrosis. Mutations in a number of *genes* coding for contractile proteins promote the development of hypertrophic cardiomyopathy in people. There is also evidence that one of the cardiomyopathies in dogs, and a familial dilated cardiomyopathy in people, are caused by an absolute or metabolic deficiency of *carnitine*. These should properly be no longer regarded as cardiomyopathies because the cause is known. If the understanding of the etiology and pathogenesis of the cardiomyopathies continues to progress, then the term cardiomyopathy will become obsolete. We are, however, not at that stage, and will continue to use the term and discuss these diseases in terms of primary diseases of the cardiac muscle.

Cardiomyopathies generally fall into three forms: **(1) dilated (congestive)**, **(2) hypertrophic**, and **(3) restrictive**. As the clinical and pathologic patterns of the cardiomyopathies were first described in people, a brief discussion of the essential features in this species is warranted.

Dilated (congestive) **cardiomyopathy** (DCM) is the most common form in humans. There is no unifying genetic or metabolic basis for the hypocontracting heart of DCM. It probably represents a common expression of myocardial damage produced by a variety of as yet undefined myocardial insults. Known causes of morphologically similar cardiac chamber dilation and failure include myocarditis, alcohol abuse, cocaine abuse, cobalt toxicity, and genetic influences. *DCM is characterized by dysfunction of the systolic phase of the cardiac cycle*, whereby there is lowered force of contraction and increased ventricular end-diastolic volume. There is enlargement and dilation of both atria and ventricles. The ventricles are hypertrophied, but, because of the dilation, the hypertrophy is not as apparent as in the hypertrophic form of the disease. Microscopically, there is variation in myocyte size, myocyte degeneration and necrosis, and variably severe fibrosis. These changes are, however, disappointingly nonspecific.

Hypertrophic cardiomyopathy (HCM) *is a diastolic rather than a systolic ventricular disorder*. The hypertrophic ventricle is abnormally stiff (less compliant) resulting in impaired ventricular filling. It is inherited as an autosomal dominant characteristic in approximately 50% of human cases, within which there is a high prevalence of subclinical disease. The cause or causes of the remaining 50% of cases is not known. Sudden unexpected death is often the first manifestation of HCM. There is left, and sometimes right, ventricular hypertrophy, reduced size of the ventricular lumen, and dilation and often hypertrophy of the atria. The pattern and extent of the ventricular hypertrophy varies from localized hypertrophy of the ventricular septum (*asymmetric septal hypertrophy*), to the hypertrophy being symmetrical or even apical in nature. Microscopically, *the myocytes are hypertrophied, with many arranged in a whorled or disorganized pattern*. While the disordered myocyte arrangement is not unique to HCM, it is more severe. Fibrosis of variable severity accompanies the hypertrophic process.

Mutations in genes that code for proteins associated with the process of cardiac contraction have been identified in cases of HCM. Understanding of the molecular nature of HCM in people has progressed rapidly, providing insight into cardiomyopathies in domestic species. Genes involved in HCM include the cardiac troponin T gene (cTnT), the myosin heavy chain gene (MyHC), and the cardiac myosin-binding protein C gene (MyBPC3). The mutations in each gene appear not to be singular in nature; for example, at least 7 mutations have been identified in the MyBPC3 gene alone. The inherited form of HCM is therefore a genetically diverse disease, which presents as a common clinical and pathological syndrome. Interestingly, mutations of the cTnT gene can cause both DCM and HCM, and mutation in the same codon in the cardiac troponin I (cTnI) gene causes both HCM and RCM.

Restrictive cardiomyopathy (RCM) *is characterized by impaired ventricular filling due to reduced ventricular compliance, with unimpaired systolic function*. The ventricles are usually of normal size, but both atria are usually dilated. Reduced compliance of one or both ventricles may occur because of interstitial or endomyocardial fibrosis with or without eosinophilia of the ventricles. Restrictive cardiomyopathy may also result from the deposition of *amyloid* or occur in association with the disease *sarcoidosis*. These *infiltrative cardiomyopathies* are, strictly speaking, secondary cardiomyopathies.

The *endomyocardial form* of RCM is subdivided into two major forms: *Loeffler endomyocarditis* occurs predominantly in temperate climates; *endomyocardial fibrosis* is most common in equatorial Africa. Although the end-stage of both forms is pathologically indistinguishable, the natural history and clinical patterns are quite separable. The Loeffler (hypereosinophilic) variant principally affects males and is associated with hypereosinophilia, thromboembolism, and arteritis. In endomyocardial fibrosis, there is no male predominance, and it occurs in younger patients with no accompanying eosinophilia. At least for the Loeffler variant, endomyocardial fibrosis results from release of granules from infiltrating eosinophils in the earlier phase of the disease.

Cardiomyopathies occurring in domestic animals have been similarly classified and have been documented in cats, dogs, cattle, and pigs.

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Feline cardiomyopathies

Feline cardiomyopathies constitute a diverse group of morphologic and functional disorders, and are probably the most common and well-recognized cardiomyopathies in domestic animals. Echocardiography is the definitive test used clinically to differentiate and classify feline cardiomyopathies, which include, in decreasing order of prevalence, hypertrophic, restrictive, dilated, unclassified, and excessive left ventricular moderator band cardiomyopathies. Although feline cardiomyopathy was only recognized as an entity in the recent past, a common consequence of cardiomyopathy, *iliac thromboembolism*, was originally described more than 60 years ago. There is a wide range in the age of onset of clinical signs, from 7 months to 24 years. Presenting clinical signs include lethargy, anorexia, dyspnea, tachypnea, and occasionally abdominal distension. Murmurs, most often associated with mitral or tricuspid insufficiency, gallop rhythms, and dysrhythmias of various types are frequently encountered. Approximately one-third of cats with cardiomyopathy develop unilateral or bilateral thromboembolic hindlimb ischemia. The causes of feline cardiomyopathy remain obscure. A possible role for viral infection and inflammation in the pathogenesis of feline cardiomyopathy is suggested by the detection by PCR of *Feline panleukopenia virus* genome in cats with idiopathic hypertrophic, dilated, and restrictive cardiomyopathies and myocarditis.

Hypertrophic cardiomyopathy, the most common form of cardiomyopathy in cats, *is a diastolic disorder*, resulting from ventricular myocardial hypertrophy in the absence of an obvious cause. There is normal or near-normal systolic myocardial function, but diminished capacity to accept diastolic flow from the left atrium. Left ventricular volume is normal or decreased and the papillary muscles are usually greatly enlarged in relation to the volume of the ventricle. The left atrium is usually dilated. A concentrically hypertrophied heart also occurs in cats with *hyperthyroidism* and this should not be confused with hypertrophic cardiomyopathy (see Cardiac hypertrophy, above).

The pathologic features of HCM include an enlarged heart, with comparatively massive *symmetric concentric hypertrophy* of the ventricles in the majority of cases, but especially the left ventricle. The left ventricular lumen is often slit-like. Some cases have *asymmetric hypertrophy of the left ventricle*, with the ventricular septum thicker than the left ventricular free wall. Findings of lesser frequency include atrial thrombi (Fig. 1.46), focal areas of ventricular fibrosis, and fibrosis of the endocardium in the region of the left ventricular outflow tract. Microscopically, myofibers are hypertrophied and contain vesicular nuclei. *Myofiber disarray*, characterized by interweaving of myofibers in the left ventricular free wall and ventricular septum, may be observed. This may also be present in the right ventricle. *Diffuse interstitial fibrosis is also a consistent feature*, especially in the inner two-thirds of the left ventricular free wall. Scattered focal areas of dense replacement fibrosis are seen in a minority of cats. As indicated by increased plasma cTnI concentrations, cats with moderate-to-severe HCM have ongoing myocardial damage, which may be the stimulus for replacement fibrosis. Focal endocardial thickening by fibrosis also occurs commonly. Intramyocardial arteries may have medial hypertrophy, and scattered perivascular accumulations of lymphocytes and monocytes may be present.

Ultrastructurally, the hypertrophied myofibers have large pleomorphic nuclei with undulating surfaces and prominent nucleoli. Dense accumulations of Z-band material are also evident in myocytes, as is the presence of lipofuscin granules. While these changes are seen in normal cat myocytes, the intensity of the changes is greater in cardiomyopathic cases. The cause of feline HCM is unknown; it appears to be inherited as an autosomal dominant trait in Maine Coon cats and American Shorthairs, and is likely heritable in a variety of other breeds.

Restrictive cardiomyopathy is a less common and less well-defined form of cardiomyopathy and is characterized by *impaired diastolic ventricular filling*, which may be due to severe endomyocardial fibrosis. Systolic function is usually normal. Feline RCM has also been termed "*left ventricular endocardial fibrosis*" (LVEF). *Endomyocarditis* may be an antecedent to LVEF. Feline RCM occurs predominantly in older cats with clinical signs of left-sided or bilateral heart failure. Cardiac murmurs and dysrhythmias are common. Pathologic features include *severe endocardial thickening with mural thrombosis*. Left atrial enlargement is marked, the result of the restriction of ventricular filling, and a thrombus may be present. The left ventricle is thickened and the lumen volume may be significantly diminished. Microscopically, the myocardium and endocardium may be replaced by granulation tissue of various ages; adjacent myocardium may be infiltrated by histiocytes, lymphocytes, and plasma cells. *Bartonella* infection has been suggested as a possible cause of feline endomyocarditis. RCM has been reported in cats with hypereosinophilic syndrome.

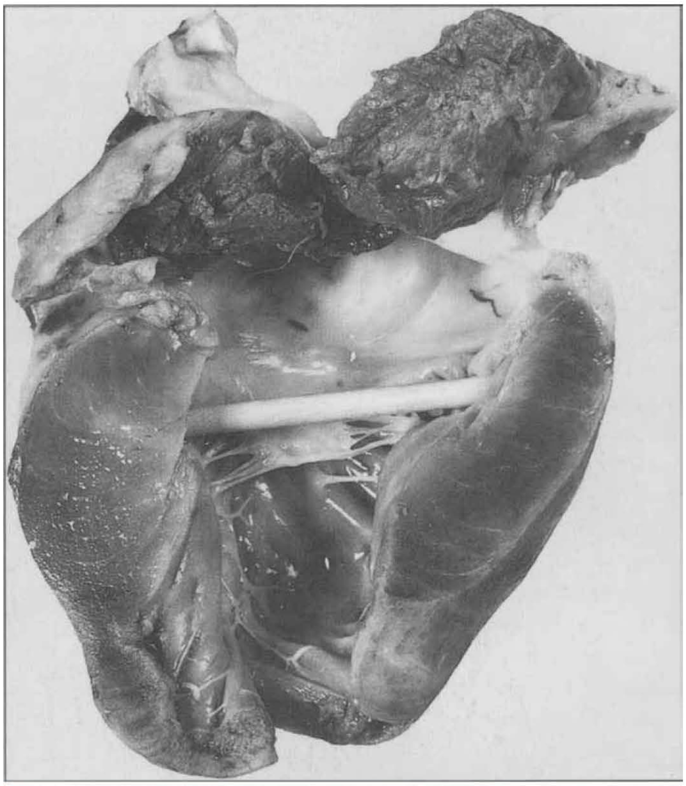

Figure 1.46 Thrombus in left atrium in a cat. The thrombus is red, recent, and attached in the auricle. Aortic embolism caused death.

Dilated cardiomyopathy has decreased markedly in prevalence since the discovery of *taurine-deficiency myocardial failure* in cats. On necropsy, all chambers of the heart are enlarged and the ventricles are dilated and flabby with thinned walls. The endocardium may be pale and slightly thickened by subendocardial fibrosis. The papillary muscles and trabeculae may be atrophied and appear small in relation to the ventricular volume. Often only minor lesions are found on microscopy. Myocardial fibers are moderately hypertrophied, accompanied by areas of mild diffuse interstitial fibrosis. There may also be focal areas of myofiber loss with replacement fibrosis. In some cases, there may be extensive areas of myocardial fibrosis. There is no significant disarray of myofibers. Ultrastructural examination contributes little to the understanding of the pathologic picture.

Excessive moderator bands (*false tendons*) in the left ventricle bridging the ventricular septum and free wall and entangling the papillary muscles are associated with heart failure and death in cats. Although not strictly a cardiomyopathy, it is considered under the heading of cardiomyopathies as the clinical syndrome occurs in mature cats. *In all probability, it is a congenital defect that is only manifest later in life.* Affected cats exhibit a range of clinical signs that are mostly referable to left-sided heart failure. They include lethargy, dyspnea, anorexia, pale mucous membranes, and cardiac murmurs. Radiographic findings include cardiomegaly, hydrothorax, and pulmonary edema. Various conduction disturbances also occur.

Gross changes are dominated by the presence of numerous pink-white bands spanning the left ventricular lumen and papillary muscles. Most commonly, the bands connect the cranial and caudal papillary muscles to the ventricular septum. In others, the bands insert between the ventricular septum and the left ventricular free wall. The left ventricle may be dilated or show increased wall thickness. In neither case is the heart weight significantly different from that of normal cats. The left atrium is enlarged, and pulmonary edema, pleural effusion, hepatic congestion, and aortic thromboembolism may be found. Microscopically, *the abnormal moderator bands consist of Purkinje cells and dense, mature collagen covered by endothelium.* Loose fibrous connective tissue is present between the endothelium and the dense collagen, with the variation in size of the moderator bands the result of more or less mature collagen. Ventricular alterations include myocyte atrophy or hypertrophy, focal fibrosis, and intramural coronary arterial luminal narrowing, intimal thickening, medial hyperplasia, and perivascular fibrosis.

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Canine cardiomyopathies

The most commonly recognized is **dilated cardiomyopathy** (DCM), especially in young to middle-aged giant and large-breed dogs, such as the St. Bernard, Irish Wolfhound, and Great Dane, but also afflicting an ever-growing list of other breeds, including Airedale Terriers, Boxers, Doberman Pinschers, English Cocker Spaniels, Newfoundlands, Portuguese Water Dogs, Presa Canario Dogs, and Standard Poodles.

The causes and pathogenesis of canine DCM are unknown. There is a *familial tendency* in the Doberman Pinscher, English Cocker Spaniel, Boxer, Great Dane, Irish Wolfhound, and Newfoundland; DCM may be an X-linked recessive trait in Great Danes. Infantile dilated cardiomyopathy is inherited in Portuguese Water Dogs as an autosomal recessive trait. There is a strong association between the presence of cardiomyopathy and a particular complement C4 phenotype (C4:4) in the English Cocker Spaniel. This is not suggested to be causal, but to indicate a genotype that is predisposed to the development of the disease. It has been suggested that elevated levels of metalloproteinases and neutrophil elastase in the myocardium of Dobermans with cardiomyopathy contribute to the pathogenesis of the disease. A deficiency in the mitochondrial respiratory chain has been demonstrated in Doberman Pinschers, evident by marked impairment of the mitochondrial production of ATP; it is not known whether this is a primary defect or a general manifestation of a failing heart. Nutritional deficiencies may be involved in the pathogenesis of DCM. *Carnitine deficiency* may play a role in some subtypes of cardiomyopathy, although the evidence is not strong. Low blood *taurine* concentrations have been found in dogs with DCM that had been fed certain lamb-rice commercial diets. The DCM in Portuguese Water Dogs can be reversed in some pups by oral taurine supplementation. *Hypothyroidism* has been noted as a reversible cause of myocardial failure in two Great Danes. There is support for the hypothesis that alternative splicing of *cTnT variants* may desynchronize myocardial contraction and contribute to the development of cardiomyopathy and heart failure. *It is likely that a defect or defects at the genomic level underlie DCM in several canine breeds.*

Canine DCM has the *clinical features* of a short history of cough, depression, dyspnea, weight loss, syncope, and polydipsia, or may occur without premonitory signs as sudden death. Clinical signs may be due predominantly to left-sided congestive heart failure and hence pulmonary edema, or may be due to biventricular failure and include ascites. Atrial fibrillation is the most common electrocardiographic abnormality; ventricular tachycardia and ventricular premature contractions are common in most Doberman Pinschers. There is cardiomegaly, with increased end-diastolic volume and poor contractile function. Soft systolic murmurs, indicative of mitral and tricuspid insufficiency, may be heard on auscultation. *The prognosis is poor,* with mean survival times of 6 to 12 months after the onset of treatment.

Necropsy findings in typically affected cases are those of *congestive heart failure.* All chambers, particularly the left ventricle, are markedly dilated and may be hypertrophied. The atrioventricular rings are dilated (Fig. 1.47), the endocardium may be opaque due to subendocardial fibrosis, and there may be atrial thrombosis. There may also be some evidence of infarction in distant organs. The finding, in some cases, of multifocal myocardial degeneration, necrosis, or fibrosis is expected, but unrewarding, with regard to possible causes.

Ultrastructural examination of myocardium from cases of dilated cardiomyopathy shows increases in intermyofibrillar spaces, lipofuscin granules, fat droplets and myelin figures, mitochondrial hyperplasia, disruption of myofibrils, and Z band thickening. These ultrastructural changes are not specific for cardiomyopathy, but are more severe than seen with other causes of congestive heart failure.

There appear to be *two histologically distinct forms of DCM*: (1) the "fatty infiltration-degenerative" type seen in boxers and Doberman pinschers, and (2) the "attenuated wavy fiber" type seen in many giant, large-, and medium-sized breeds, including some Boxers and Doberman Pinschers. This histologic classification of canine DCM may be superior to classification by breed-specific syndromes, since both forms may affect some breeds, such as Boxers and Doberman Pinschers. This postmortem classification does not however assist antemortem etiological diagnosis of DCM, which remains difficult if not impossible.

A variant of DCM is **arrhythmogenic right ventricular cardiomyopathy** (ARVC), a primary familial myocardial disease in humans and in *Boxer dogs* that is associated with cardiovascular morbidity and risk of sudden death. ARVC in Boxers is characterized by various events, alone or in combination, including ventricular arrhythmias of suspected right ventricular (RV) origin, syncope, heart failure, and/or sudden death. Right ventricular dilation or aneurysms are common. Histopathological findings include severe myocyte loss in the RV, with replacement by fatty or fibrofatty tissue. Focal fibrofatty lesions may also be present in both atria and in the left ventricle (LV). Myocarditis may be present in the RV and LV in dogs with sudden death and may be the source of arrhythmias. ARVC is an ion channel defect in humans, with mutations identified at the ryanodine receptor 2 gene and the desmoplakin gene. It is transmitted as an autosomal dominant trait in humans and Boxers.

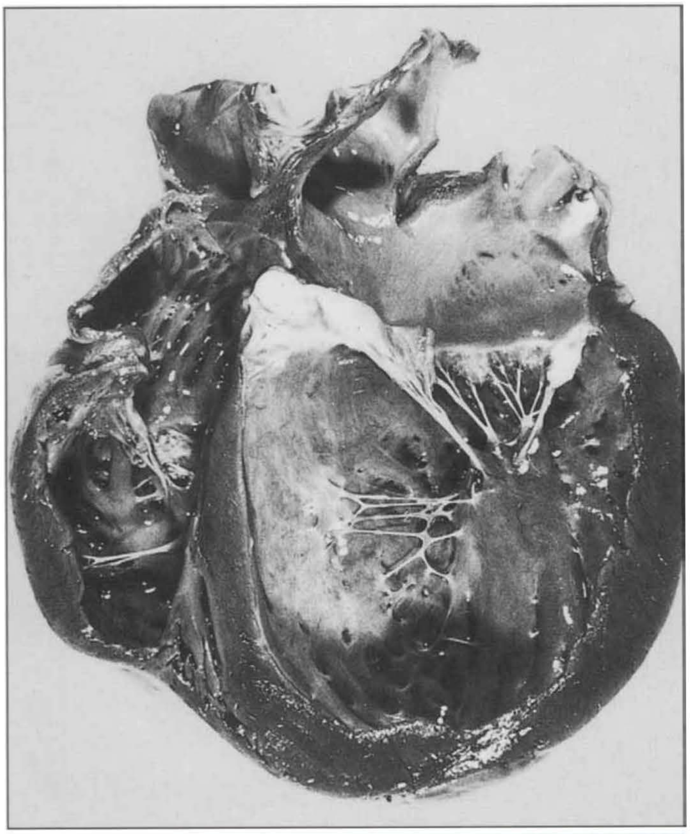

Figure 1.47 Dilated (congestive) **cardiomyopathy** in a dog. The left ventricle is dilated and the endocardium is fibrotic.

Hypertrophic cardiomyopathy is much less common than the dilated form. Associated clinical syndromes include sudden death, death during anesthesia, and congestive heart failure. Disproportionate thickening of the ventricular septum may cause the ratio of the interventricular wall thickness to left ventricular free wall thickness to exceed 1.2:1, and there may be histologic evidence of myofiber disarray in the ventricular septum.

In **canine X-linked muscular dystrophy** in golden retrievers, an animal model for Duchenne muscular dystrophy in humans, affected and carrier dogs develop *Duchenne cardiomyopathy* characterized by myocardial interstitial, especially subendocardial, fibrosis, leading to cardiac insufficiency.

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Bovine cardiomyopathies

Several syndromes of cardiomyopathy are recorded in cattle. **Dilated cardiomyopathy** *occurs in* **Holsteins** *in Canada, Australia, and Japan, and in Simmental-Red Holstein crossbreds in Switzerland.* Many of the cases emanate from common sires, and an autosomal recessive mode of inheritance has been suggested. Inappropriate ubiquination of proteins may play a role in pathogenesis. The cardiomyopathy occurs in young adult to mature animals, with the clinical signs particularly referable to right-sided heart failure including dependent subcutaneous edema, hydrothorax, ascites, and severe hepatic congestion. There is no indication of the presence of electrocardiographic abnormalities. The heart is enlarged with dilation of both ventricles. Microscopically there is a mixture of atrophied and hypertrophied myofibers with vacuolation, and there are islands of interstitial and replacement fibrosis, fatty replacement, and ongoing myofiber necrosis.

Dilated cardiomyopathy in Japanese black calves is considered to be inherited as an *autosomal recessive trait.* Sudden death or death after a short period of severe dyspnea occurs in calves younger than 30 days. Lesions found at necropsy include marked cardiac enlargement with left ventricular dilation, hydropericardium, hydrothorax, ascites, pulmonary edema, and hepatic and splenic congestion. Extensive myocardial degeneration and necrosis with fibrosis are present in the left ventricle, particularly affecting the papillary muscles. The right ventricle is similarly but less often involved. The clinical and pathologic pattern is reminiscent of that seen in Hereford calves with cardiomyopathy.

A cardiomyopathy associated with a **tightly curled ("wooly") haircoat** *in both polled and horned Herefords* has also been described. The cardiomyopathy is inherited as an *autosomal recessive trait.* Affected animals are less than 6 months of age and are usually found dead. In some cases detected before death, severe ventricular dysrhythmias are evident. Even in some calves that die at 1 week of age, there are severe lesions in the ventricles consisting of necrosis, mineralization and fibrosis of the myocardium (Fig. 1.48A, B).

Hypertrophic cardiomyopathy has been reported in Holsteins.

Bovine generalized glycogenosis type II (Pompe's disease), although not a primary cardiomyopathy, deserves mention. Most

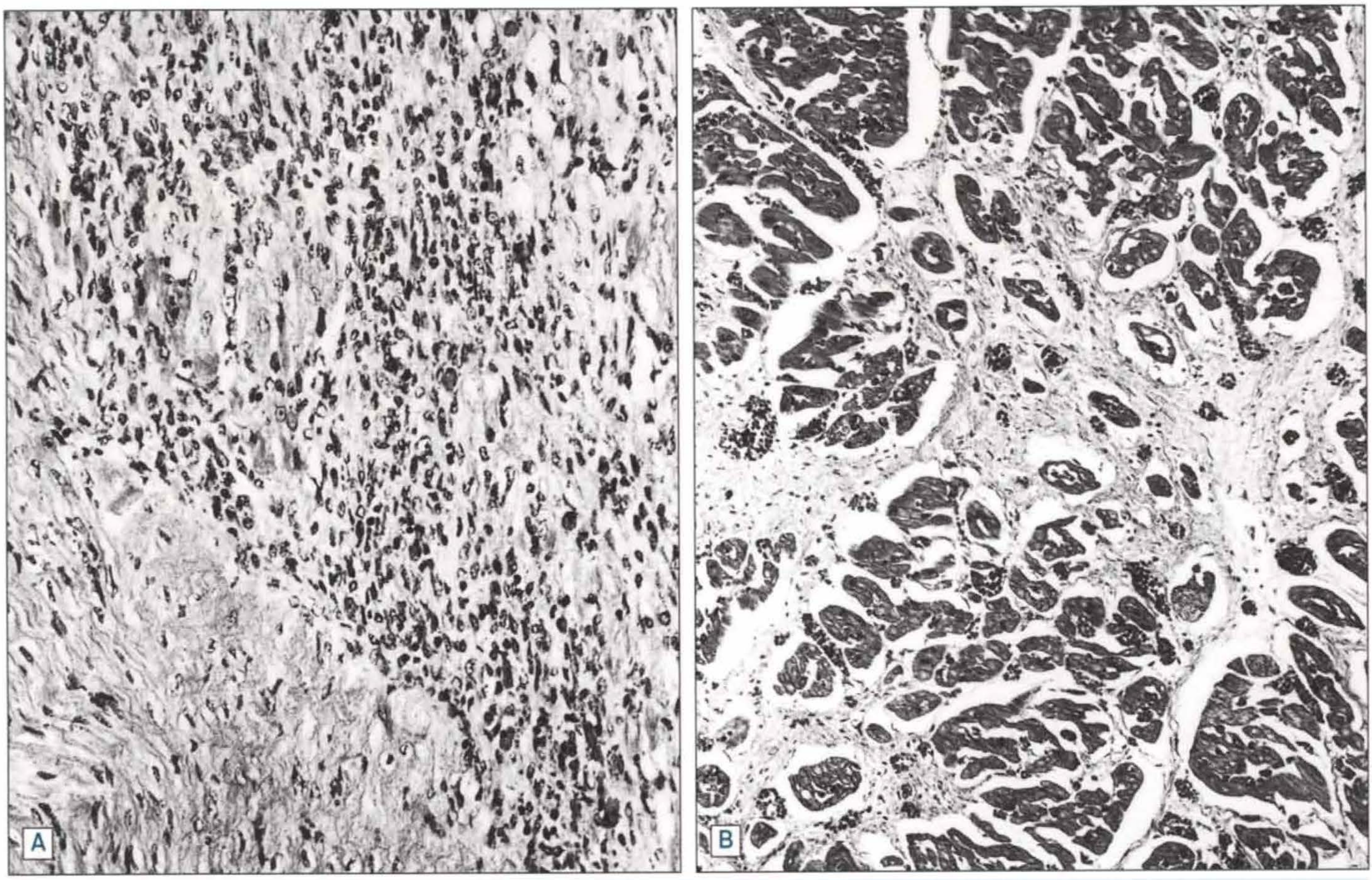

Figure 1.48 Cardiomyopathy in curly-coated poll Hereford calves. A. Degeneration and reparative fibrosis of myocardium, in a 2-week-old calf. **B.** Interstitial fibrosis of myocardium, in a 1-week-old calf.

affected animals have generalized muscle weakness. Occasional cases, however, exhibit clinical signs of left-sided heart failure, and in these the heart is eccentrically hypertrophied.

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DISEASES OF THE CONDUCTION SYSTEM

The clinical and electrocardiographic features of primary conduction system disturbances are well documented, but comparatively rare. The clinical signs are primarily intermittent collapse, and sometimes sudden death. Electrocardiography may show a variety of abnormalities, such as sinoatrial arrest, second- or third-degree atrioventricular block, atrioventricular dissociation, left or right bundle branch block, Wolff–Parkinson–White syndrome (rarely), and ventricular tachycardia. It should not be forgotten that *many if not most dysrhythmias are secondary to more or less extensive myocardial degeneration, necrosis, inflammation, or neoplasia*, particularly when in close proximity to the conduction system.

In contrast, *reports on the gross and microscopic examination of cases of primary conduction system disturbances are remarkably sparse*. This may be due in part to negative findings in some cases, or to time and effort required to adequately examine the conduction system. When an abnormality of the conduction system is suspected, tissue samples from the appropriate regions should be collected as part of the necropsy (see Examination of the heart, above). The use of special stains such as Masson's trichrome and PTAH assist in highlighting the presence of excessive fibrosis and the relative paucity of contractile elements in the cells of the conduction system, respectively. For purposes of comparison, it is advisable to have a reference set of blocks of the conduction system from normal animals.

Sudden unexpected death or episodes of viciousness have been associated with *atrioventricular nodal, or common bundle degeneration*, particularly in the *Doberman Pinscher*. Note that the presence of mineralized cartilage or osseous metaplasia in the central fibrous body in close proximity to conduction tissue is a normal finding in large breed dogs at all ages. The common bundle is fibrotic, degenerate, and in some cases replaced by fat. In most cases there is myointimal proliferation in arterioles in this region, possibly leading to ischemia of the common bundle. A similar phenomenon has been observed in other breeds, including the German Shepherd.

There is also a syndrome of sudden death in outwardly healthy, nonsyncopal *young German Shepherd Dogs* that is suspected to be hereditary in nature. Affected animals die unexpectedly during sleep or during rest following exercise. *Ventricular tachycardia* is the most common arrhythmia, the frequency of which increases during rest when the heart rate is slowest, and ends in ventricular fibrillation. The underlying defect is suggested to be regional paucity of sympathetic nerves in ventricular myocardium.

Hereditary stenosis of the common bundle has been observed in cases of syncope and sudden death in *Pug dogs*. Electrocardiographically, such dogs have intermittent sinus arrest and paroxysmal second-degree atrioventricular block. The sinus node, the atrioventricular node, and the bundle branches are normal. The paroxysmal atrioventricular block could be related to the lesion in the common bundle, but the sinus pauses are probably the result of excessive parasympathetic activity. Affected dogs are abnormally sensitive to small amounts of acetylcholine.

Deafness in the Dalmatian dog is associated with an abnormal blotchy coat color, and such animals may die suddenly. A proportion of affected dogs exhibit episodes of sinus pauses. In one that was available for necropsy, the right atrium was fibrotic and atrophied. This was accompanied by marked narrowing of the coronary arteries, including those of the sinus node. These clinical and pathologic findings are similar to those seen in congenital deafness in humans.

The presence of an atrophic and fibrotic atrium in the above case is the outstanding feature of the syndrome of **persistent atrial standstill** (atrioventricular muscular dystrophy), which occurs most commonly in *English Springer Spaniels* and has been described in *Siamese cats*. The atrium is grossly dilated and the walls are thin, transparent, and pink. Microscopically, there is myocyte atrophy, fibrosis, with a variable mononuclear cell presence. In some cases, atrophy of skeletal musculature is also evident. The syndrome is similar in many respects to a form of muscular dystrophy in people termed Emery-Dreifuss disease.

Sick sinus syndrome occurs in Miniature Schnauzers, West Highland White Terriers, and Dachshunds, and is familial in Miniature Schnauzers. Prolonged sinus pauses cause syncope.

Defects of the conduction system of **Alaskan sled dogs** that died suddenly during the Iditarod race included marked fibrosis or fatty infiltration of the sinus node, AV node, AV bundle, and bundle branches, and focal scarring of the left ventricle. These pathological changes may have led to fatal dysrhythmias.

Equine grass sickness (equine dysautonomia) is suspected to be a clinical form of botulism. The neurodegenerative changes noted in terminal parasympathetic ganglionic neurons in the heart, which include chromatolysis and vacuolation, may account for the tachycardia noted in this disease.

Ventricular preexcitation, a rare cause of weakness, syncope, or congestive heart failure in dogs and cats, can result when sinus node impulses bypass the normal conduction system via *accessory pathways* (bundle of Kent, James fibers, Mahaim fibers, or intranodal tract) to cause premature ventricular activation.

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NEOPLASMS OF THE HEART

Primary neoplastic disease of the heart in all domestic species is rare, with the possible exception of hemangiosarcoma in the right atrium of dogs (see Vascular neoplasms, this chapter). In contrast, *metastasis to the myocardium from primary tumors of other organs is relatively common.* Primary tumors of the aortic body (chemodectomas) and of ectopic thyroid and parathyroid may arise at the base of the heart in dogs (Fig. 1.49) (see Vol. 3, Endocrine glands).

Hemangiosarcoma, a tumor of high-grade malignancy, may either arise as a primary in the myocardium, or metastasize to the myocardium, most commonly from the spleen or the skin. Characteristics of the tumor are described under Vascular neoplasms.

Other primary cardiac tumors are rare, and include *rhabdomyoma, rhabdomyosarcoma, myxoma, myxosarcoma, chondrosarcoma, osteosarcoma, granular cell tumor, fibroma, fibrosarcoma, lipomas, pericardial mesothelioma, and undifferentiated sarcoma.*

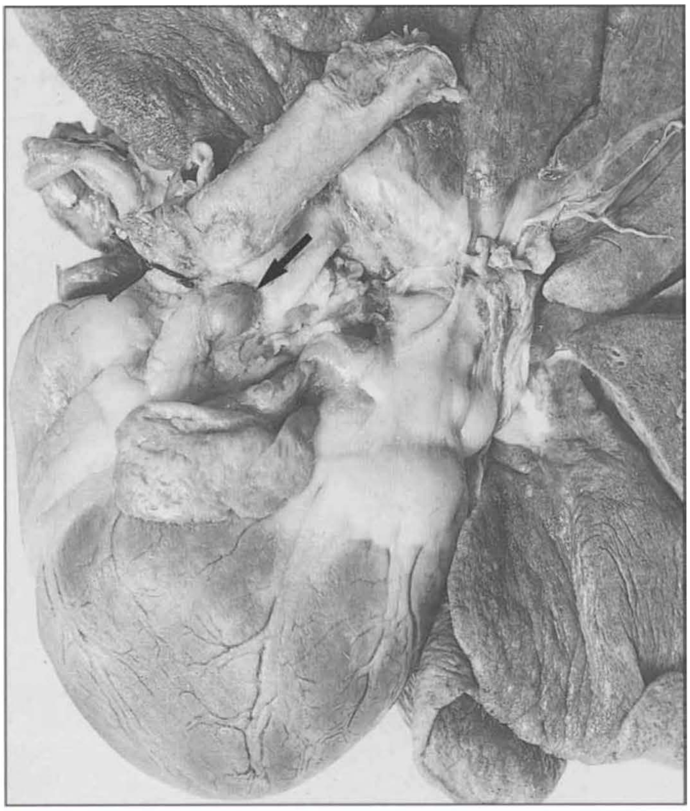

Figure 1.49 Cardioaortic chemoreceptor tumor in a dog. Tumor is within pericardial sac in the adventitia of the pulmonary artery (arrow).

Rhabdomyomas *are anomalous formations (hamartomas) of myocardial fibers seen as one or more circumscribed but nonencapsulated nodules within the myocardium.* It has been suggested that swine cardiac rhabdomyomas are not hamartomas but may represent congenital dysplasia of the perinatal cardiac myocytes with myofibrillar degeneration. Rhabdomyomas are rare in animals, but are most common in pigs, and have been observed in cattle, sheep, and dogs; a breed predisposition has been noted in red wattle pigs. Grossly, the nodules are gray and may be found anywhere in the heart. Those projecting into a chamber are very susceptible to hemorrhage and necrosis, and may become cystic (Fig. 1.50). Histologically, the cell type is predominantly mature myocardiocyte in type in a variant of rhabdomyoma, but cells in rhabdomyomas are more typically large, vacuolated, glycogen-rich, with scant myofibrils, and resemble Purkinje cells; cells with fine cytoplasmic strands extending from the nucleus to the peripheral cytoplasm are referred to as "spider cells." Fibrosis can be extensive, and the connective tissues may be impregnated with iron and calcium salts.

Myxomas are the most common cardiac tumor in adult humans, but are rare in domestic animals; they have been reported in the dog, cat, and cattle. They are believed to arise from multipotent vasoformative cells. They may originate from the endocardium of any of the four cardiac chambers or from the heart valves. Gross appearance is often multilobular, soft, and gelatinous. Histologically, myxomas are covered by endothelium on their surface, and are hypocellular, consisting of stellate or globular cells, blood vessels, and smooth muscle cells within an abundant myxoid matrix. Blood flow may be interrupted by these intracavitary space-occupying masses, and although they do not metastasize, they may generate emboli.

Primary cardiac **chondrosarcoma** occurs rarely and most probably arises from cartilaginous or like tissue that is present in the cardiac skeleton. Of the few that have been described, one arose in the right atrium with the others arising in the atrioventricular valves. As the tumors produce little cartilaginous matrix, a positive reaction to the presence of S100 protein is required to confirm the chondrocytic origin of the neoplastic cells.

A **granular cell tumor** or *granular cell myoblastoma* has been described in the right atrium of a dog. Granular cell tumors are rare in animals but have been reported to occur in a variety of organs including the lung, brain, meninges and tongue. The tumor has a distinctive light and electron microscopic appearance consisting of spindle-shaped to large polygonal cells containing prominent cytoplasmic granules within lysosomes. The cells are considered to be of neural origin as nerve-specific S100 protein is present within the cytoplasmic granules.

Neurofibromas (Schwannomas) *are either isolated or multiple benign neoplasms of peripheral nerves* (Fig. 1.51). Cattle are most frequently affected, with the most common sites being peripheral nerves, brachial plexus, autonomic ganglia, intercostal nerves, and cardiac nerves. The disease is usually detected only at slaughter. When the heart is involved, the tumors are single or multiple, round or nodular masses, either on the epicardial surface or within the myocardium. The microscopic appearance is of interwoven bundles or whorls of elongated cells (Fig. 1.52). There may be palisading of nuclei and variable amounts of collagen present. The principal cell is probably of Schwann or perineural origin.

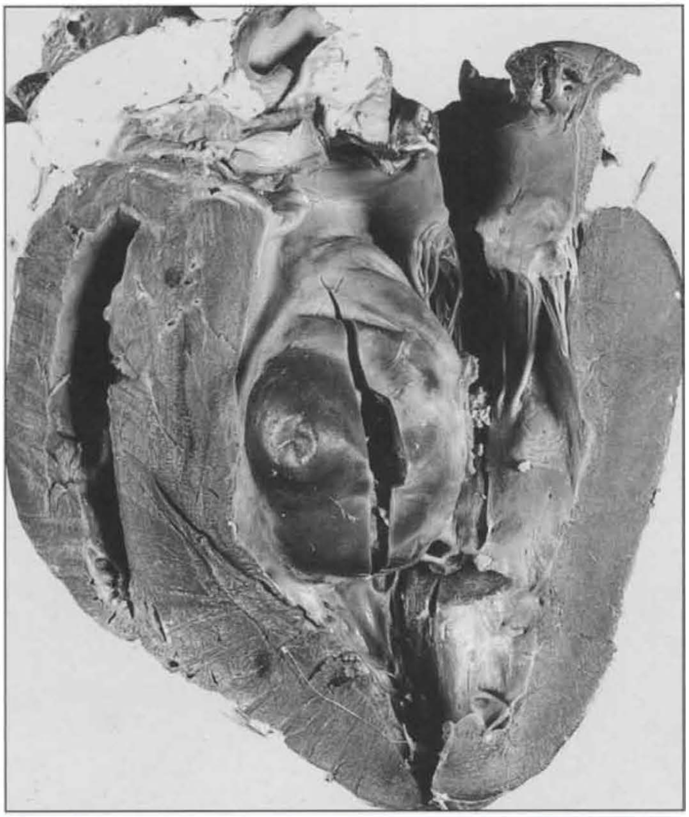

Figure 1.50 Cystic degeneration of a **rhabdomyoma** in the left ventricle, bovine.

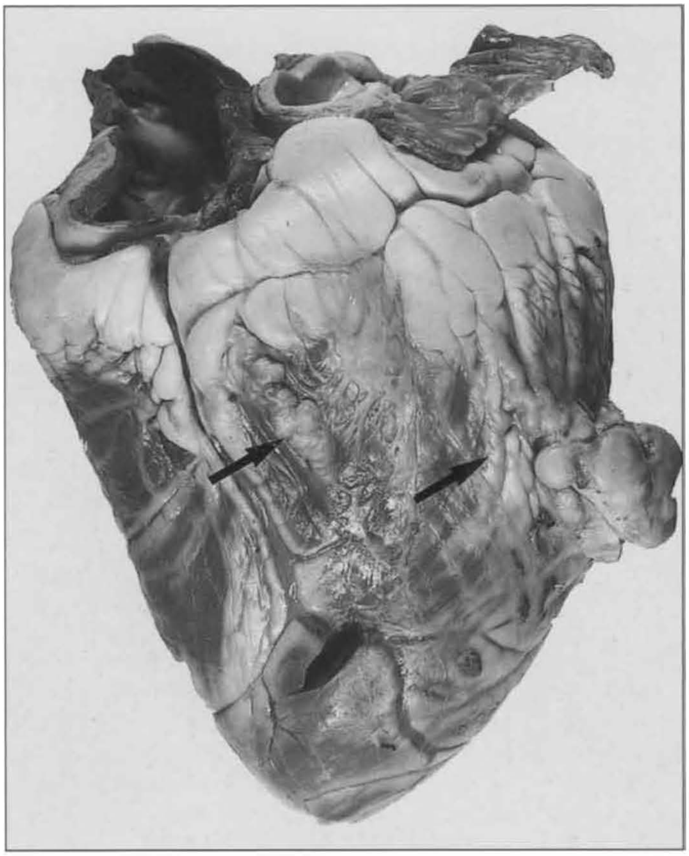

Figure 1.51 **Neurofibroma**, bovine. There are corded (arrows) and nodular enlargements of epicardial nerves.

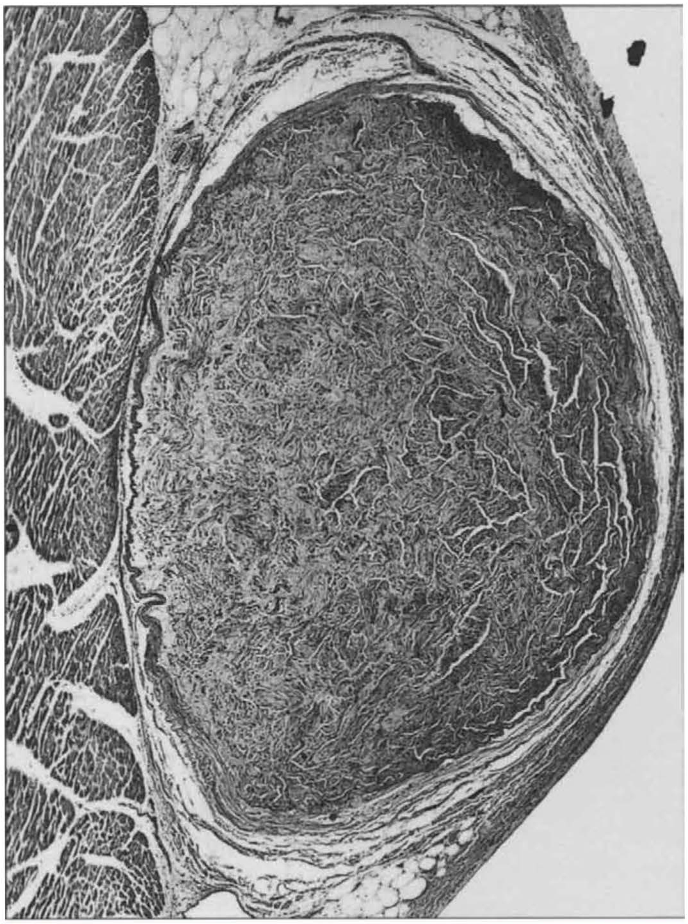

Figure 1.52 Section through nerve in Fig. 1.51.

Lymphoma *is the most common metastatic neoplasm involving the heart* (Fig. 1.53A, B), and occurs most commonly in the cow and the dog. It may be nodular or diffuse, although the distinction is arbitrary because there is overlap of the two forms. In the *nodular form*, there are foci, primarily in the atria (particularly the right atrium), which may be up to 5 cm or more in diameter. The nodules are covered by endothelium and are therefore smooth. When beneath the epicardium, they may be difficult to distinguish from epicardial fat. They are prone to central necrosis, and this resembles necrotic adipose tissue. On section, the wall of the atrium is more diffusely thickened, is moderately soft to cut, and has a pale appearance. If squeezed, milky fluid exudes from the cut surface. Globular masses like those beneath the epicardium are also found under the endocardium projecting into the cardiac cavities. In the *diffuse or infiltrating form*, the myocardium appears irregularly thickened and gray-white, with the ventricular walls being especially involved. These foci or areas of infiltration blend with normal myocardium and hence are difficult to distinguish from foci of myocardial degeneration or myocarditis. If they cause some thickening of the myocardium, such lesions are to be first regarded as lymphoma and indicate the need for careful search for primary lesions elsewhere in the body. Lymphoid neoplasms are considered in detail in Vol. 3, Hematopoietic system.

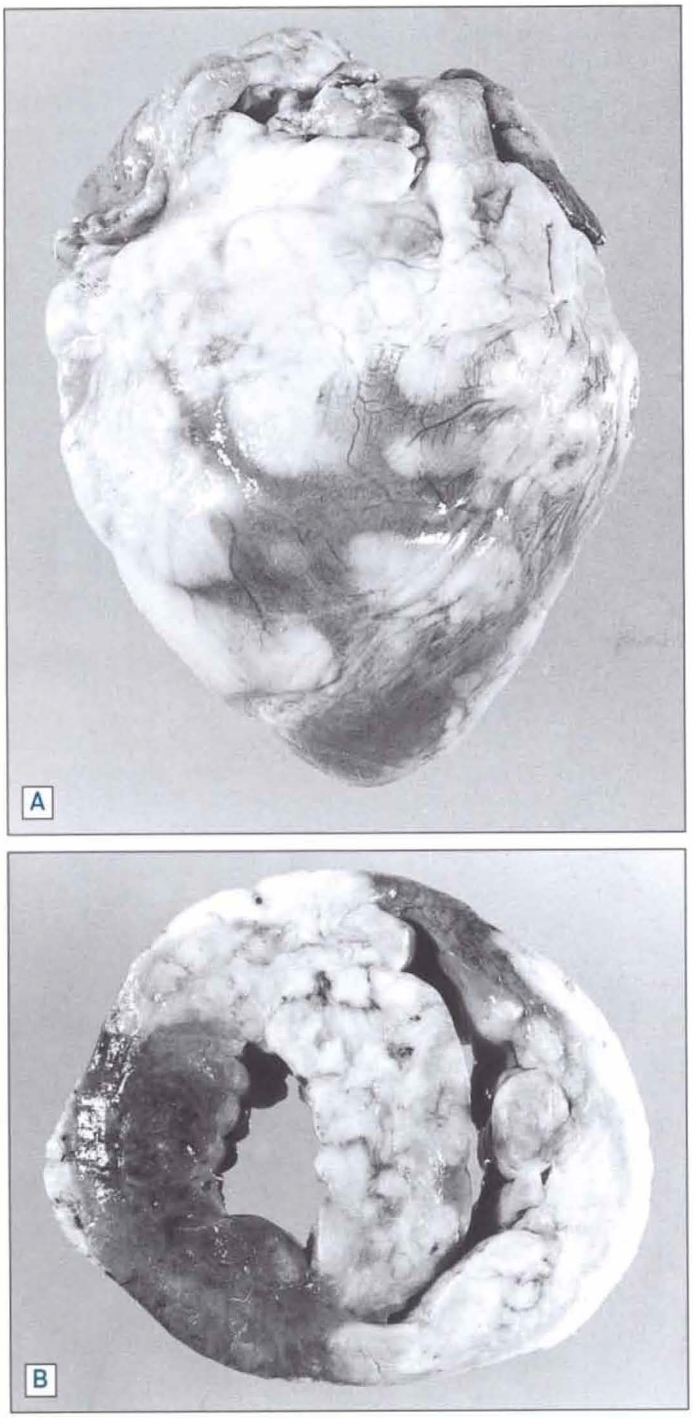

Figure 1.53 Lymphoma in the heart of a goat. **A.** Nodular and confluent lymphomatous infiltration of the myocardium. **B.** Cross section through the heart.

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Diseases of the vascular system

GENERAL CONSIDERATIONS

The vascular system may be arbitrarily divided into the arterial system, the microcirculation, the venous system, and the lymphatic system. Impaired function of the vascular system can be generalized, as occurs in shock, or may be more localized as when decreased supply of oxygenated arterial blood to tissue causes ischemia, or impaired venous drainage causes congestion, or decreased lymphatic drainage of tissue fluids causes lymphedema.

The **arterial system** is subdivided into *large elastic arteries, medium and small muscular arteries, and arterioles,* with gradual transitions between these divisions. Arterial vessels are characterized histologically by walls composed of three layers: the internal, middle, and external tunics; or *tunica interna* (intima), *tunica media* (media), and *tunica externa* (adventitia).

The **intima** consists of endothelium; subendothelial connective tissue, which contains collagen, elastin, proteoglycan (ground substance), fibroblasts, and smooth muscle cells; and the internal elastic membrane. The thickness of the intima decreases as the vessel size decreases, until the subendothelial layer eventually disappears. The internal elastic membrane is not readily distinguishable in elastic arteries because of its continuity with medial elastic tissue, but is very prominent in muscular arteries, and disappears at the level of the smaller arterioles.

The **media** is the thickest layer of the vessel wall, and it consists of concentric layers of smooth muscle cells and elastic fibers, and the external elastic membrane. The media consists of fenestrated elastic laminae in elastic arteries, with smooth muscle cells lying between laminae. Intercellular ground substance is especially prominent in the tunica media of elastic arteries in the horse. *The elastic laminae diminish and smooth muscle predominates as vessels become more distant from the heart.* The media of muscular arteries is up to 40 muscle cells thick, whereas the media of arterioles is 1–3 cells thick. Definitions of the upper limit of arteriolar diameter range from 300 μm if fixed by perfusion to 100 μm if allowed to contract, but there are no sharp distinctions between small arteries and large arterioles. The thickness of the media as seen in routine material is greatest in relation to the luminal diameter in small arteries and arterioles, and it is these vessels, particularly arterioles, which participate actively in blood pressure regulation and also bear the consequences of hypertension. Contraction of muscular arteries and arterioles upon an animal's death forces blood from the lumina, and causes longitudinal folding that appears as scalloping of the internal elastic membrane when seen in cross-section. The external elastic membrane is best defined in the large elastic arteries, and becomes indistinct in muscular arteries.

The **adventitia** consists of a feltwork of elastic and collagen fibers continuous with the surrounding connective tissue. The interlacing network of collagen fibers in the adventitia limits expansion of the elastic arteries. Elastic fibers decrease in the adventitia with decreasing vessel size. *Vasa vasorum* and *nervi vasorum* supply the adventitia and the outer half of the media. *The intima and inner half of the media are avascular* and dependent upon diffusion from the vascular lumen, and are thus more subject to degenerative changes than the outer media.

The **microcirculation** *is the exchange system in which gases, nutrients, and waste products are transferred between the blood and the extravascular tissues.* The microcirculation consists of vessels of less than 100 μm in diameter: namely, *arterioles, terminal arterioles (metarterioles, precapillary sphincter areas), capillaries, postcapillary venules, and venules.* This classification overlaps microcirculation and macrocirculation (arteries and veins), but is useful conceptually as will be seen in disorders such as disseminated intravascular coagulation that affect primarily the microcirculation. Arterioles open through precapillary sphincters or through metarterioles into capillaries in most tissues, or into sinusoids in the liver. Capillaries are low-pressure tubules, of about 8 μm in diameter, composed of endothelial cells surrounded by a basal lamina; the endothelial cells are connected by tight junctions that allow rapid passage of small molecules such as glucose. The basal lamina is a molecular sieve that prevents passage of larger protein molecules. Capillary endothelial cells possess few endoplasmic organelles, but often contain pinocytotic vesicles. *Capillaries* may be: *continuous,* the most common type; *fenestrated* (60 to 80 μm fenestrae closed by thin diaphragms), as in the gastrointestinal tract and endocrine glands; or *porous* (without diaphragms closing the pores) as in renal glomeruli. Fenestration of the endothelial cells allows increased permeability. During tissue healing, cords of endothelial cells grow into the tissues by mitosis, develop lumina, and become functional capillaries. Undifferentiated perivascular cells, or *pericytes,* are ensheathed by glycocalyx that is continuous with the capillary basal lamina. Mitosis of pericytes is easily stimulated and they may be transformed into fibroblasts or smooth muscle cells. *Sinusoids* are less uniform than capillaries and more permeable because there are large openings between the endothelial cells and a discontinuous basal lamina.

Veins are termed *small, medium, or large* or, alternatively, *venules, collecting veins, and great veins*; all have large lumina in relation to their wall thickness. Classification of veins by wall characteristics is difficult because the layers in their walls may be absent or difficult to distinguish. Venules of greater than 30 μm in diameter have an incomplete muscular media and thin adventitia. Venular endothelium is normally more permeable than that of capillaries and is also more sensitive to vasoactive amines, the action of which can cause leakage. In the lymph node paracortex, *postcapillary venules,* which are nonmuscular venules with prominent endothelium, are important sites of lymphocytic traffic. The media is of increasing thickness in medium and large veins, and the internal elastic lamina becomes more prominent. However, *the adventitia is the thickest layer in veins, whereas the media predominates in arteries.* Backflow of blood in veins is prevented by the presence of *semilunar valves,* which are invaginations of the intima into the venous lumen. Venous valves are not present in venae cavae or hepatic portal vein. Paucity of valves in veins of the head and face may contribute to incidental venous congestion in these areas.

Lymphatic capillaries originate in loose connective tissue, and consist of very permeable walls and endothelial cells that may lack, or have a discontinuous, basal lamina. In larger lymphatics, *valves* are present, the basal lamina is continuous, and walls consist of three ill-defined layers; an internal elastic lamina is usually absent. The thin-walled veins and lymphatics are more susceptible to compression and occlusion, are more often involved by inflammation in adjacent tissue, and are more liable to neoplastic invasion than are the thicker-walled arteries.

Endothelium and smooth muscle, the main components of vessel walls, play important roles in all types of vascular disease. A wealth of information is accumulating about the important properties and reactive capabilities of these seemingly simple cells. For example, in addition to maintaining a permeability barrier between blood and tissues, *the endothelium is recognized as the largest paracrine organ in the body.* It produces anticoagulants, procoagulants, fibrinolytic agents, prostanoids, connective tissue components, adenosine, and a host of other substances. Vascular homeostasis is maintained by complex interactions among endothelial cells, leukocytes, and platelets, each of which produce vasodilators and vasoconstrictors and can interact to inhibit or promote thrombosis.

Vascular **endothelial cells**, as well as forming the thromboresistant monolayer lining of the vascular system, mechanically insulate the circulating blood from the highly thrombogenic subendothelial materials. The thromboresistance of the endothelium is normally maintained by a balance between antithrombotic and thrombotic factors; stimulation of excessive prothrombosis can lead to clotting and thrombosis, whereas excessive antithrombosis can lead to ineffective hemostasis and bleeding. *Antithrombosis* is normally accomplished through:

- binding and inhibition of thrombin via activation by thrombomodulin of protein C and protein S, via accentuation of antithrombin activity by heparin-like molecules, and via the presence of alpha$_2$ macroglobulin;
- inhibition of platelet aggregation through elaboration of prostacyclin (PGI$_2$), a potent inhibitor of platelet aggregation and a vasodilator, and by ADPase-mediated conversion of pro-aggregating ADP to adenine nucleotide platelet inhibitors; and
- promotion of fibrinolysis by synthesis of tissue plasminogen activators.

Procoagulant activities include:

- the presence of minute amounts of tissue thromboplastin in endothelial cells, further production of which can be stimulated by endotoxin and by cytokines such as interleukin-1 and tumor necrosis factor;
- synthesis and secretion of von Willebrand factor, needed for adherence of platelets to subendothelial components, and secretion of platelet activating factor, an activator and aggregator of platelets; and
- inhibition of fibrinolysis through release of plasminogen activator inhibitors.

In addition to their role in hemostasis, endothelial cells participate in *modulation of blood flow and vascular reactivity* by production of endothelium-derived relaxing factor (nitric oxide); endothelin, a vasoconstrictor; angiotensin converting enzyme, which converts angiotensin I to angiotensin II; and prostacyclin. Endothelial cells modulate the actions of various vasoconstrictors (catecholamines, serotonin, arginine, vasopressin) and vasodilators (histamine, leukotrienes, adenine nucleotides), and in concert with the endothelium-derived factors can thereby allow blood vessels to rapidly adapt to changes in hemodynamic conditions.

Endothelial cells aid in the *regulation of inflammation and immunity* through production of interleukin-1 and various adhesion molecules, which moderate adhesion and hence emigration of leukocytes. The most important adhesion molecule pairs are the selectins, the immunoglobulins ICAM-1 and VCAM-1, and the β2 and β1 integrins. Endothelial cells also aid in *regulation of cell growth* by production of growth stimulators (platelet-derived growth factor, colony-stimulating factor, and fibroblast growth factor), and production of growth inhibitors (heparin, transforming growth factor-β). Endothelial cells produce basement membrane, and are capable of contraction. The endothelium is a semipermeable membrane that can also transport metabolites including proteins through the cytoplasm via pinocytotic vesicles. *Weibel-Palade bodies*, rod-shaped cytoplasmic organelles that store von Willebrand factor, serve as ultrastructural markers of endothelial cells.

Subendothelial connective tissues consist of various types of collagen, elastin, proteoglycans, fibronectin, laminin, and thrombospondin. Of these, fibrillar collagen is the most potent stimulus for platelet adhesion and activation; collagen also activates the intrinsic pathway of coagulation. *Fibronectin*, or "molecular glue," normally functions to stabilize cell-to-cell and cell-to-substrate attachments, but also becomes cross-linked to fibrin and helps to anchor hemostatic plugs.

Mild endothelial injury, as occurs in the course of inflammation, may be apparent histologically as hypertrophy of endothelial cells ("reactive" cells). As well, increased vascular permeability may lead to insudation of plasma proteins, including fibrinogen, into the subendothelial area, and hence contribute to vascular hyalinosis. Since the intima and inner media depend upon diffusion of metabolites from the vascular lumen for their nutrition, such subendothelial deposits may impair the viability of smooth muscle cells and contribute to degeneration, or *fibrinoid necrosis*, of vessel walls. Thus, the endothelial barrier must be preserved to maintain a normal environment for medial smooth muscle cells. Necrosis of endothelium, which may be caused by a wide variety of agents, leads to exposure of subendothelial collagen, a potent inducer of coagulation and platelet aggregation, and hence thrombosis. Endothelial cells at the edges of denuded areas will proliferate and migrate to repair the endothelial defect or to form an endothelial covering over (endothelialize) a mural thrombus. Severe microvascular damage, as occurs due to lipoperoxidation of endothelial membranes in vitamin E/selenium deficiency in pigs, can result in hemorrhage and organ failure.

Vascular smooth muscle cells are important both as effectors of vasoconstriction and dilation, and as cells capable of synthesizing basement membrane, collagen, elastin, and proteoglycans of the extracellular space. In response to growth factors derived from platelets and monocyte/macrophages, smooth muscle cells of the media migrate through fenestrae of the internal elastic lamina to the subendothelial area where, as "myointimal" cells, they proliferate and are responsible for organization or collagenization of deposits, including thrombi. Examples of endoarterial organization and arterio-occlusive disease are discussed later in this chapter. In common with other muscle cells, *medial smooth muscle cells respond to an increased workload by hypertrophy.* Medial hypertrophy is a prominent consequence, as well as a cause, of pulmonary hypertension. Sustained vasoconstriction, as is induced by ergot (*Claviceps purpurea*) or fescue grass (*Festuca arundinacea, Festuca eliator*) poisoning, can lead to gangrene of the extremities; fescue toxicosis in cattle (summer syndrome) is caused by infestation of the grass by the endophyte *Neotyphodium (Acremonium) coenophialum*, which produces the ergopeptide alkaloids, ergovaline, ergosine, and ergonine. Along with the other components of the vessel wall, smooth muscle cells may be involved in degenerative and inflammatory changes, as described below, and contribute to fibrinoid necrosis of the wall.

Normal aging changes of vessels include intimal thickening due to proliferation of myointimal cells and accumulation of their products, deterioration of medial elastic fibers, medial fibrosis and loss of medial smooth muscle, and accumulation of ground substance in the media. These slowly progressive changes are usually of little consequence given the large functional reserve of the vascular system, but do lead to loss of resilience of the vessel walls and may cause vessel wall thinning, stretching, and hence increased tortuosity.

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ARTERIES

Congenital anomalies

Minor variations occur among individuals of a species in the course and distribution of arteries, but these are of little significance except possibly in surgical techniques.

Arteriovenous fistula, the communication of an artery and vein that bypasses the capillary system, is a defect that *may arise developmentally or be acquired* subsequent to physical trauma, inflammatory necrosis involving adjacent vessels, neoplastic infiltration, or rupture of an arterial aneurysm into a vein. Fistulas occur in dogs, cats, horses, and cattle, but are uncommon. Clinically, fistulas most commonly occur in the extremities and appear as a pulsating, fluctuant swelling in which there is a palpable thrill and a machinery-like bruit on auscultation. In general, arteriovenous fistulas cause decreased peripheral resistance and increased venous return to the right heart. When 20–50% of the cardiac output is shunted through the fistula, high-output cardiac failure can occur. The veins involved are usually thick-walled ("arterialized"), may be dilated and extremely tortuous, and may have intimal sclerosis. *Hepatic arteriovenous fistula* (congenital hamartoma) occurs in dogs and cats, and can cause portal hypertension, portocaval shunts, and ascites.

Thebesian veins connect the cardiac chambers, between the trabeculae carneae, with the myocardial sinusoids and capillaries. They may also connect with coronary arterioles to form direct connections between the coronary arterioles and the cardiac chambers. Areas of myocardium in which the Thebesian vein system is richly persistent are randomly distributed and the true nature of the defect may be overlooked because of the tortuous aneurysmal dilation of the affected coronary artery. Injection studies are necessary to demonstrate the extent of the anastomosis.

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Degeneration of arteries

Arteriosclerosis

Arteriosclerosis literally means "hardening of the arteries," and is more fully defined as *chronic arterial change consisting of hardening, loss of elasticity and luminal narrowing resulting usually from proliferative and degenerative, rather than inflammatory, changes of the media and intima.* The term atherosclerosis is applied to lesions of arteriosclerosis in which degenerative fatty changes also occur. Atherosclerosis is the most common and important type of arteriosclerosis in humans, and the terms can thus be used interchangeably with little loss of meaning when discussing this species. *In domestic animals, arteriosclerosis is common, but of little importance, and atherosclerosis is rare.*

Arteriosclerosis develops slowly in animals, its extent or incidence being greater in older animals. The disease may become so widespread in individual animals that microscopically normal vessels may be difficult to find. The sclerotic vessels are usually not accompanied by significant disturbances of blood flow, although ischemic change, especially of brain and heart, may be seen. Well-developed lesions may be good sites for thrombus formation in animals when thrombogenic circumstances prevail. Arteriosclerosis uncomplicated by focal degeneration in the sclerotic plaques and with no or minimal lipid deposition is the lesion usually found in older horses, ruminants, and carnivores. There is a predilection for the abdominal aorta and points of arterial branching, but it is also seen in peripheral and pulmonary arteries and in the thoracic aorta. The extensiveness of the lesions is very variable, so that there is no clear differentiation between a permissible aging change and a pathologic process. The nature of the insult (or insults) to the vessel walls that result in sclerosis is not known, although there are numerous possibilities. Hemodynamic factors probably contribute, especially to development of plaques about the orifices of arterial branches.

Arteriosclerotic plaques produce a slight thickening and wrinkling of the intima, or more or less distinct white, oval or linear elevations, and they can be found microscopically in many animals in which they are not visible to the naked eye. Their presence in peripheral, including coronary and cerebral, vessels, can be correlated with arteriosclerosis of the abdominal aorta.

The initiating lesion(s) and the evolution of plaque formation are not fully elucidated. Microthrombi composed chiefly of platelets are deposited at natural sites of turbulence or of endothelial denudation, and may initiate the lesion. Platelets secrete platelet-derived growth factor (PDGF), epidermal growth factor, transforming growth factor-β, and other mitogens; PDGF stimulates migration and proliferation of smooth muscle cells. Alternatively, the initial change in the vessel wall may occur as the result of a nondenuding insult to the endothelium; endothelial cells themselves produce several mitogens, including PDGF. Histologically, the internal elastic membrane shows an early distinctive change, being irregular in outline, partially duplicated, and fragmented or discontinuous. Smooth muscle cells migrate into the intima from the media to function as do fibroblasts outside of arteries; these intimal smooth muscle cells produce most of the connective tissue matrix of the intimal plaque, including collagen, elastic tissue, and proteoglycans. In young plaques, or the deeper portions of older ones, the smooth muscle cells ("*myointimal cells*") are numerous and intimately mixed with collagen, elastic fibrils, basement membrane, and proteoglycans as the so-called *musculoelastic layer*. In large and older plaques, a more superficial elastic hyperplastic layer may be recognized by a more compact arrangement of collagen and elastica with relatively few cells. This lamination of plaques is not always clearly evident, and its development may depend on the age of the lesion as well as on the segment of vessel involved, whether chiefly muscular or chiefly elastic.

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Atherosclerosis

Atherosclerosis is of little practical importance in domestic animals, but is primarily of interest for the development of animal models of the human disease. The atherosclerotic susceptibility of animals varies; *rabbits, chickens, and pigs are atherosensitive, whereas dogs, cats, cattle, goats, and rats are atheroresistant*. Swine and nonhuman primates are usually considered to be the best animal models of human atherosclerosis.

In **humans**, *atherosclerosis and its complications of myocardial infarction, stroke, and peripheral vascular disease are major causes of morbidity and mortality in the western world*. Atherosclerosis affects the large elastic arteries (aorta, iliac) and the large and medium muscular arteries (carotid, coronary, femoral). The essential lesion is the *atheroma or fibrofatty plaque*, which is a focal, raised, intimal plaque with a core of lipid (cholesterol and its esters largely) covered by a fibrous cap. Complications of plaques include mineralization, ulceration, superimposed thrombosis, intraplaque hemorrhage, and aneurysmal dilation. *Fatty streaks*, another type of fatty intimal lesion, are common in the aorta and large arteries of domestic animals, especially ruminants and swine, and humans. The streaks, which are soft, smooth, nonelevated lesions ranging from pinpoint size up to several square centimeters, are rarely visible grossly unless the aorta is stained with a fat stain such as Sudan IV, which stains the lesions bright orange. Streaks and plaques may coexist in the same area, but fatty streaks also occur in areas in which plaques do not occur; thus, the role of fatty streaks in the genesis of atherosclerosis is unresolved.

Multiple pathogenetic influences can contribute to the development of plaques. The various events and theories of development of atherosclerosis have been unified in the "*response to injury*" *hypothesis*. Endothelial injury or dysfunction can occur as the result of hyperlipidemia, with the cholesterol present in low-density (LDL) and very-low-density lipoproteins (VLDL) seen as a primary culprit. Additional risk factors are hypertension, smoking, obesity, inactivity, and diabetes mellitus. Immunologic mechanisms, toxins, and viruses may also damage endothelium. Endothelial injury leads to platelet adhesion, monocyte adhesion and infiltration, and smooth muscle migration and proliferation, as discussed above under arteriosclerosis. The feature added in atherosclerosis is insudation of lipid, which is seen as extracellular lipid or as intracellular lipid in "foam cells." The lipid-laden foam cells that are characteristic of both fatty streaks and atheromatous plaques may be either altered macrophages or smooth muscle cells; macrophages predominate in streaks, whereas smooth muscle cells predominate in the fibrous cap of plaques. Endothelial cell dysfunction is emerging as an endocrinopathy that may contribute to the effects of atherosclerosis through impaired ability of endothelial cells to produce endothelial-derived relaxing factor and through increased production of endothelin.

Atherosclerosis develops commonly only in the **pig** amongst domestic species. Fatty streaks and atheromatous plaques develop in the aorta, extramural coronary arteries, cerebral and iliac arteries, as in man, and the plaques may cause considerable stenosis of vessels in swine 8–12 years old. The extent of lipid deposition can be influenced by feeding extraordinary diets containing much lipid, including cholesterol, but the atheromas rarely reach the degree of development seen in humans, and they do not lead to occlusive thrombus formation, which apparently requires softening and ulceration of the atheromatous plaque. The initial deposition of lipid occurs in the proliferated smooth muscle cells, which show signs of degeneration and which may become foam cells. Macrophages also appear, containing lipid, and fat may be demonstrable extracellularly, presumably released by degenerate cells. Deposits of cholesterol and mineral, not extensive, may be associated with softening of larger atheromatous plaques. However, except in pigs fed high-fat diets, lipid is usually a minor component of the predominantly fibromuscular plaques.

Of the domestic animals, the deposition of cholesterol and other lipids in the arteries in more than trace amounts occurs only in **dogs** (Fig. 1.54), and then almost always in association with *hypothyroidism or diabetes mellitus*. In dogs, hypercholesterolemia is chiefly related to hypothyroidism, but even in thyroidectomized, cholesterol-fed, hypercholesterolemic experimental dogs, only occasional dogs classed as "hyperresponders" developed atherosclerosis, perhaps as a result of an increased proportion of cholesterol-rich VLDL. A breed predisposition to atherosclerosis in miniature schnauzers may be the result of idiopathic hyperlipoproteinemia.

The deposition of lipids begins in the middle and outer layers of the media and occurs more extensively perhaps in the small muscular arteries than in the large elastic ones; if present, aortic lesions occur as intimal plaques. The veins are normal. The vascular changes can be severe enough to be seen on gross inspection. The vessels are enlarged, less pliable than normal, and their walls are thickened and ill defined because a hyperplastic adventitia merges irregularly with surrounding tissues. Projecting into the lumens of the vessels on cross-section are tiny yellow-brown nodules. In vessels large enough to be opened longitudinally, there are numerous small yellow flat elevations that may be confluent.

The media may be greatly increased in thickness by the accumulation of lipid, most of which is in foam cells, but some is present in identifiable muscle cells of the media or free in the interstitium as droplets or crystals (Fig. 1.55). The deposition of lipid in the internal layers of the media leads to disruption of the internal elastic lamina and involvement of the intima. The amount of lipid deposited can be, relatively, very great and lead to eccentric enlargement of the vessel. Associated with the lipidosis, and possibly as a response to it, there is progressive proliferation of fibrous connective tissue. Beginning with the formation of fine fibrils in and about the areas of lipid deposition, the fibrosis is progressive and may eventually completely transform the structure of the wall. The connective tissues become hyaline and relatively acellular and may be heavily impregnated with salts of calcium and iron, present as granules, large clumps, or crystalline plaques.

Atheromatosis of the degree described above in dogs is rare. Clinical consequences of severe atherosclerosis appear to be infrequent in dogs, even though the vessels most severely involved are

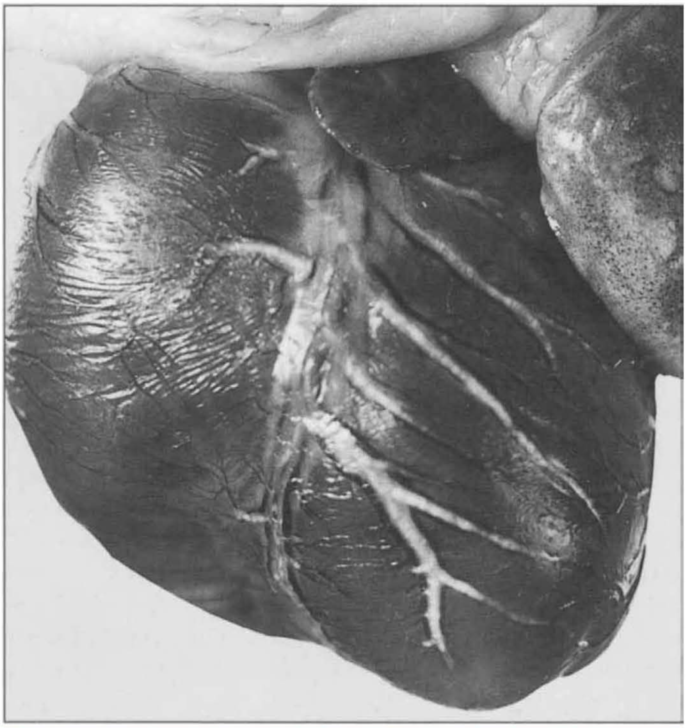

Figure 1.54 Irregular thickening of coronary arteries due to **atherosclerosis** in a dog.

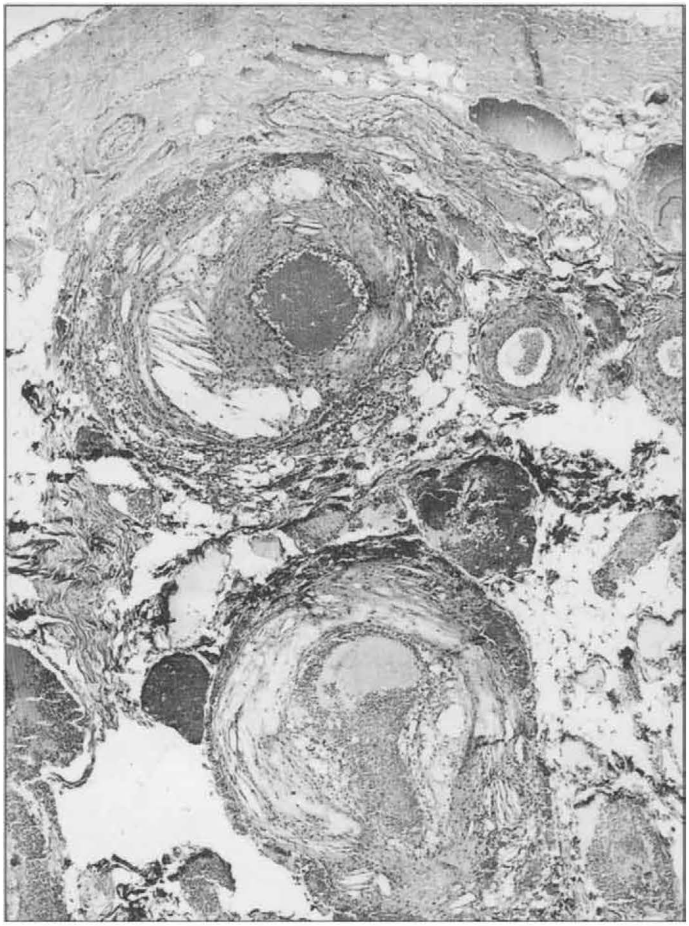

Figure 1.55 Atherosclerosis of extramural coronary arteries in a dog.

those of the heart, brain, and kidney. Occasionally, however, as in humans, rupture of the atheromatous area into the lumen with thrombosis or even widespread lipid embolism has been observed.

The morphology of atherosclerosis differs in dogs and in humans; *lipid is present in the intima but primarily in the media and adventitia of atherosclerotic canine arteries*, but is present primarily in the intima in man. The term "*xanthomatosis*" has been applied to the canine condition in the past, but its use is inadvisable as it may cause confusion with the human condition of primary xanthomatosis (type II hyperlipoproteinemia), in which there is premature atherosclerotic coronary artery disease, but also xanthomatous infiltration of the myocardium, dermis, and other organs with an associated granulomatous reaction. A similar condition in other animal species is referred to as xanthomatosis, and is characterized by the presence of lipid-laden foam cells in subcutaneous or intracutaneous accumulations known as *xanthomas*. A familial hyperlipoproteinemia occurs in lipoprotein lipase deficient cats, and results in the formation of xanthomata in a variety of organs; atherosclerosis is not a feature of this condition.

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Arteriolosclerosis

Arteriolosclerosis describes a heterogeneous group of arteriolar lesions that may be predominantly hyaline or predominantly hyperplastic. The major cause of these changes in vessels in humans is hypertension, but the pathogenesis of the lesions in domestic animals is often not understood. Endothelial damage is likely the primary event in these conditions. *Hyaline degeneration* – the microscopic appearance of amorphous, brightly eosinophilic material in vessel walls – can occur as the result of increased vascular permeability that allows insudation of various plasma components; it includes the amyloid deposits of renal glomeruli and elsewhere, and the fibrin and necrotic smooth muscle of "fibrinoid" necrosis. Fibrinoid necrosis may predate and initiate inflammatory cellular infiltration, after which the reaction would be termed arteritis. Thickening of the arteriolar wall may cause luminal narrowing and hence ischemia of the region supplied.

Systemic hypertension, or *persistently elevated systemic blood pressure*, is an important disease of humans, and occurs as *primary or essential hypertension* and as *secondary hypertension*. Both forms of hypertension are typically benign in their clinical course; *accelerated or malignant hypertension* develops in about 5% of affected individuals. Both primary and secondary hypertensions have been reported in dogs and cats; the primary form is rare and the secondary form is apparently uncommon, though hypertension may often go unrecognized. The diagnosis of hypertension could well become more frequent as noninvasive techniques for measurement of blood pressure, such as Doppler ultrasonic sphygmomanometry, come into common use. *Renal disease, likely the most common cause of hypertension in dogs and cats*, may be either a cause or an effect of hypertension, thus making difficult the definition of the occurrence of a hypertensive vascular lesion as a primary or a secondary event. Primary hypertensive lesions can lead to decreased renal perfusion, activation of the renin–angiotensin–aldosterone system, and hence more hypertension, but, on the other hand, primary chronic renal disease can lead to inadequate excretion of sodium and water, blood volume expansion, and hence secondary hypertension. In either case, *hypertension is self-perpetuating* as medial hypertrophy and hyalinization of renal arteries lead to more nephrosclerosis, more hypertension, and more pressure-induced vascular damage.

Hypertension, which would be classed as secondary, is said to occur in more than 60% of dogs with chronic renal disease, and may also occur in association with pheochromocytoma, hyperadrenocorticism, hypo- and hyperthyroidism, and diabetes mellitus. In cats, hypertension occurs in association with chronic renal disease, hyperthyroidism, and chronic anemia. Systemic and local hypertensions reportedly accompany acute and chronic laminitis in horses; hypertension is suggested to occur in acute and chronic laminitis in cattle. The hypertension of feline hyperthyroidism is reversible on resolution of the primary condition. *The presenting sign in canine and feline hypertension may be blindness due to hypertensive retinopathy*; ocular lesions include retinal vascular tortuosity, intraocular hemorrhage, and retinal detachment, the results of retinal arterial degeneration. In the face of hypertension, retinal and choroidal arterioles constrict; sustained vasoconstriction may lead to ischemic necrosis of the arteriolar smooth muscle, increased vascular permeability, hyalinization, edema, and hemorrhage. Left ventricular hypertrophy commonly occurs in hypertension in response to increased demand for cardiac output plus increased peripheral vascular resistance.

Pulmonary hypertension – *persistent elevation of pulmonary arterial blood pressure* – can occur both as the cause and as the result of pulmonary arterial disease (see Vol. 2, Respiratory system; and, in this chapter, Arterial hypertrophy, and Dirofilariasis (heartworm disease), p. 87).

Widespread arterial degeneration commonly occurs in dogs with **uremia**, and is most frequently noted in small muscular arteries and arterioles of the gastric submucosa, tongue, colon, gallbladder, urinary bladder, and kidneys. Less frequently affected are arteries of the small intestine, myocardium, and other organs. The arterial and arteriolar lesions, in concert with degenerative capillary lesions, produce ischemic injury, and contribute to grossly observed lesions of uremia such as uremic gastric infarction. The arterial lesions of acute renal insufficiency consist of subendothelial deposition of fibrin, disruption of the internal elastic lamina, necrosis of medial smooth muscle, mineralization, and sometimes a neutrophilic reaction. These lesions may be segmental or circumferential in the vessel wall. This arterial reaction is primarily degenerative rather than inflammatory, and is similar to **hyaline arteriolosclerosis** of benign nephrosclerosis (patchy ischemic atrophy) in humans. In chronic renal insufficiency, medial hypertrophy and adventitial fibrosis produce thickening and whorling of renal interlobular and intralobular arteries and afferent arterioles. These changes are similar to **hyperplastic arteriolosclerosis** of humans, which is characterized by concentric fibrosis and smooth muscle proliferation in the intima (*onion-skinning*); these hyperplastic changes, in combination with fibrinoid necrosis of the media of arterioles, are features of malignant hypertension.

Hyalinosis is commonly observed in the splenic arterioles of dogs and pigs, affecting most frequently those of the lymphatic nodules. This change is more severe in dogs with a variety of diseases including diabetes mellitus and chronic renal disease, and thus may be of hypertensive origin. A hypertrophic hyalinization is regularly observed in the uterine and ovarian arteries in pregnant and postpartum animals; the elastic tissue is increased in amount, the intima is markedly thickened and hyaline, and the muscularis may be atrophic.

Hypertrophic hyalinization, or hyalinosis, is observed in old dogs, affecting principally the **intramural coronary arteries** (Fig. 1.32) and small meningeal and cerebral vessels. The hyalinization is not clearly of clinical significance in the brain but *may cause multifocal intramural myocardial infarction* (MIMI) and, in concert with valvular endocardiosis, lead to congestive heart failure. The hyaline deposits consist predominantly of fibrin or glycosaminoglycans or, less commonly, amyloid. Other lesions encountered in the intramyocardial arteries include musculoelastic intimal thickenings, intimal cushions, and microthrombi; these lesions occur at sites of turbulent blood flow, namely bifurcations and branchings.

Sclerosis of intramural coronary arteries, predominantly of the left ventricle, occurs in dogs with congenital subaortic stenosis and occurs in the right ventricle of dogs with pulmonic stenosis. Intimal thickening due to fibroelastic and smooth muscle proliferation can result in myocardial infarction and scarring. The intimal proliferation may occur in response to decreased coronary perfusion pressure and increased intramural tension during systole.

Arteriolar hyaline degeneration is seen in swine as part of the pathological picture in meninges in organomercurial poisoning, in various organs in edema disease, and chiefly in the heart in hepatosis dietetica and mulberry heart disease. The progression of arterial lesions in **edema disease** (Fig. 1.56) is from mural edema, to hyaline degeneration, to medial necrosis and intramural and perivascular hemorrhage, at which stage acute severe hypertension develops, likely as a result of increased vascular resistance, and hypertension in turn exacerbates vascular damage; adventitial proliferation follows in survivors. Certain strains of *Escherichia coli* produce edema disease toxin (Shiga-like toxin-II variant, SLT-IIv), which preferentially targets vessels of the gastric and colonic submucosa and the cerebellar folia, perhaps because of concentration of a toxin receptor, such as globotetraosylceramide, at these sites. **Cerebrospinal angiopathy** in swine is characterized by lesions in arterioles, primarily in the brainstem, which include subendothelial hyaline deposits, degeneration or fibrinoid necrosis of the media, and perivascular eosinophilic droplets. The vascular lesions cause demyelination and malacia, and hence nervous signs, and are probably manifestations of subclinical edema disease (Fig. 1.57).

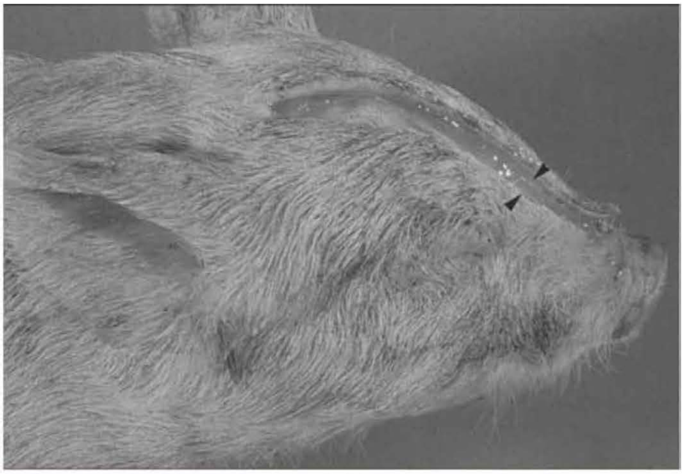

Figure 1.56 Marked subcutaneous edema (between arrowheads) over the forehead of a 4-week-old pig with **edema disease**. *Escherichia coli* O141:K85ab:K88ac was isolated from the spleen of the pig.

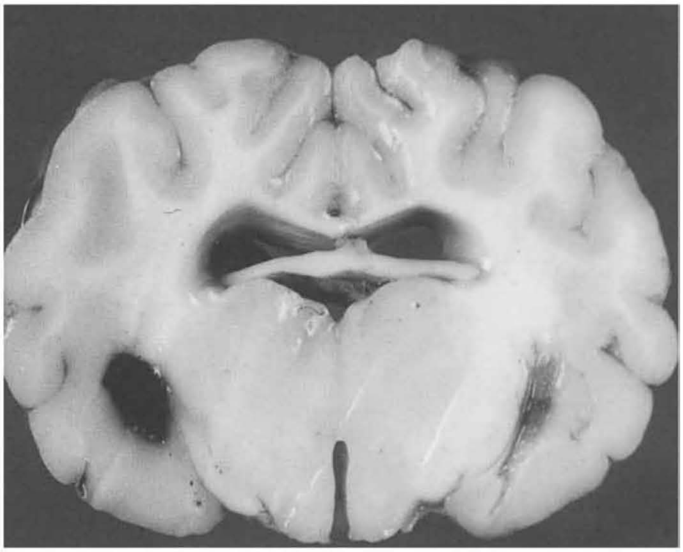

Figure 1.57 Hemorrhage and malacia in the brain of a pig, caused by **necrotizing cerebrospinal angiopathy.**

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Mineralization

Mineralization (calcification) occurs quite frequently in the arteries of animals, either as a dystrophic or metastatic process. **Dystrophic mineralization**, or *mineralization of dying or dead tissue*, occurs in areas of inflammation, degeneration, and thrombosis, and not necessarily in association with preexisting arteriosclerosis. **Metastatic mineralization** occurs as the *result of hypercalcemia and/or hyperphosphatemia*. The distinction between dystrophic and metastatic mineralization may be artificial; in vitamin D toxicosis for example, some maintain that degeneration occurs prior to mineralization as a result of direct vitamin D toxicity, and that hypercalcemia and mineralization are secondary events. Although the cardiovascular system is particularly susceptible to mineralization, mineralization of other soft tissues, such as the renal tubular and pulmonary alveolar basement membranes, endocardium and myocardium, and gastric mucosa, usually occurs concomitantly with vascular mineralization.

Vitamin D poisoning occurs in a variety of circumstances, and *leads to mineralization of vessels and other tissues in association with hypercalcemia and/or hyperphosphatemia*. The disease can be endemic in herbivores, cattle most often, that have consumed plants (such as *Solanum malacoxylon, S. torvum, Trisetum flavescens, Cestrum diurnum*) that contain 1,25-dihydroxycholecalciferol or a related compound. Horses and pigs are occasionally poisoned by inadvertent overdoses of vitamin D in feed. Accidental poisoning of dogs and cats with a *cholecalciferol (vitamin D_3)* rodenticide is common.

Mineralization occurs in dogs with hypercalcemia due to pseudohyperparathyroidism, in some dogs in association with chronic renal insufficiency, and in hypomagnesemic cattle. Mineralization also occurs spontaneously in animals of advanced age and, *in cattle especially, in a variety of chronic debilitating diseases such as Johne's disease*. In sporadic cases, the causes of mineralization are generally unknown and may be nonspecific.

Calcium and phosphorus are deposited in association with extracellular vesicles adjacent to elastic fibers and, in the smaller vessels, may produce a complete, concentric ring of medial mineralization; this is the domestic animal equivalent of *Monckeberg's arteriosclerosis (medial calcific sclerosis)* of humans. Mineralized lesions in the aorta and pulmonary artery are usually in the form of flat plaques and are often very striking grossly; complete encirclement is seldom seen. The button-like protuberances that are seen in the aorta about the orifices of smaller branches are not primarily aortic but, instead, concentric lesions in the small efferents that project when the opened vessel undergoes elastic recoil. The plaques in the large vessels are brittle and the smaller arteries are transformed into rigid tubes that can be fractured easily. Metaplastic ossification may occur (Fig. 1.58).

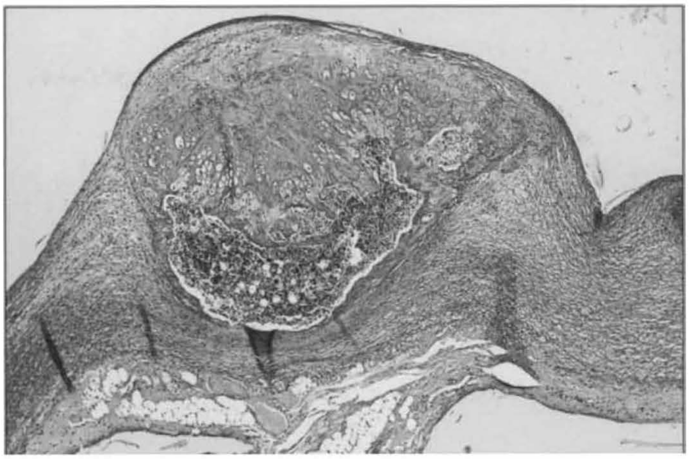

Figure 1.58 Cartilaginous and osseous metaplasia in arteriosclerosis of the aorta in a dog. Note hematopoietic tissue.

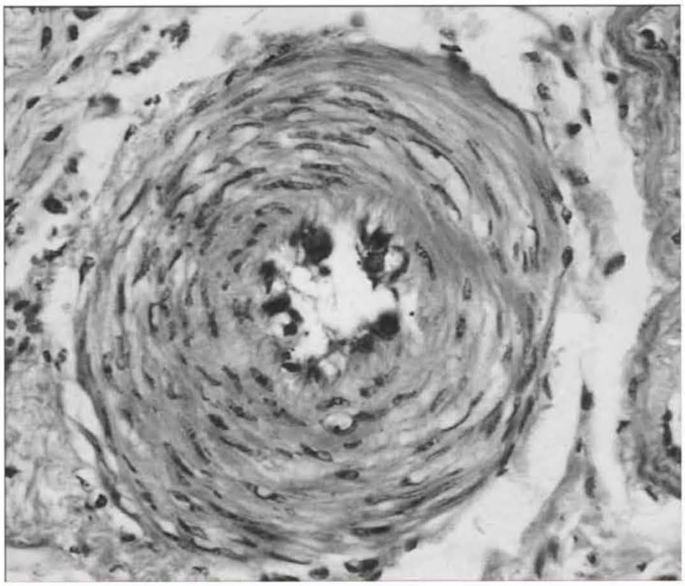

Figure 1.59 Intimal bodies in a submucosal artery of the intestine in a horse.

Intimal bodies *occur commonly in small arteries and arterioles of horses*, particularly in the submucosa of the intestines, but in other organs as well. Intimal bodies appear in section as multiple irregular mineralized masses covered by endothelium and usually protruding into the arterial lumen (Fig. 1.59). They occur in horses of all ages, are not associated with strongyle infection, and *have no apparent functional significance*. These bodies apparently arise from degeneration and mineralization of subendothelial smooth muscle cells and intercellular material.

The deposition of iron salts with calcium (**siderocalcinosis**) is observed commonly in horses, the incidence or severity of the change increasing with age. Vessels of all types are involved and sites of predilection include the globus pallidus and internal capsule in the brain. Mostly, the deposits are without notable consequence, but secondary malacia may occur. It is also possible that malacia of other cause may exacerbate the cerebrovascular siderosis.

The *mineralized nodules in the intima of the aorta in the horse* are probably all healed lesions of verminous arteritis (*Strongylus vulgaris* migration).

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Arterial rupture, aneurysms

Rupture of arteries as a result of physical trauma is common; spontaneous ruptures are not. The aortic lesions produced by *Spirocerca lupi* in dogs occasionally lead to rupture. *Rupture of a uterine artery* is a cause of fatal hemorrhage in aged mares at parturition and is associated with low serum copper levels. Mycotic ulceration of the internal carotid or maxillary artery in horses can lead to fatal guttural pouch hemorrhage.

Rupture of the aorta *is well known, but certainly uncommon, in the horse.* The ruptures occur during periods of excitement and activity, such as racing or in stallions while breeding, and are probably related to increased intra-aortic pressure. A predisposing aortic lesion has not been definitely identified, but fragmentation, degeneration, and mineralization of elastic fibers may contribute to weakening of the wall. "Cystic medial necrosis," the presence of pools of ground substance within the elastic media, has been suggested to be an underlying cause, but this change is present in the aortas of many normal horses. Intimal thickening and medial fibrosis of vasa vasora may predispose to aortic medial necrosis and rupture. Tears occur in the ascending aorta at any level from the aortic valvular ring to the level of the brachiocephalic trunk (Fig. 1.60), and range from 0.5 to 1 cm tears in the right coronary sinus to large transverse or three-cornered tears in the cranial wall of the aorta. Hemorrhage may occur into a number of different sites: *hemopericardium*, the most common sequel to aortic rupture, leads to sudden death; hemorrhage into, and disruption of, the atrioventricular node or bundle of His also leads to sudden death; dissection through the ventricular septum and into the right ventricle (*aortocardiac fistula*) causes progressive right heart failure; dissection of blood along the aortic wall may lead to formation of an *aneurysm*, which may in turn rupture.

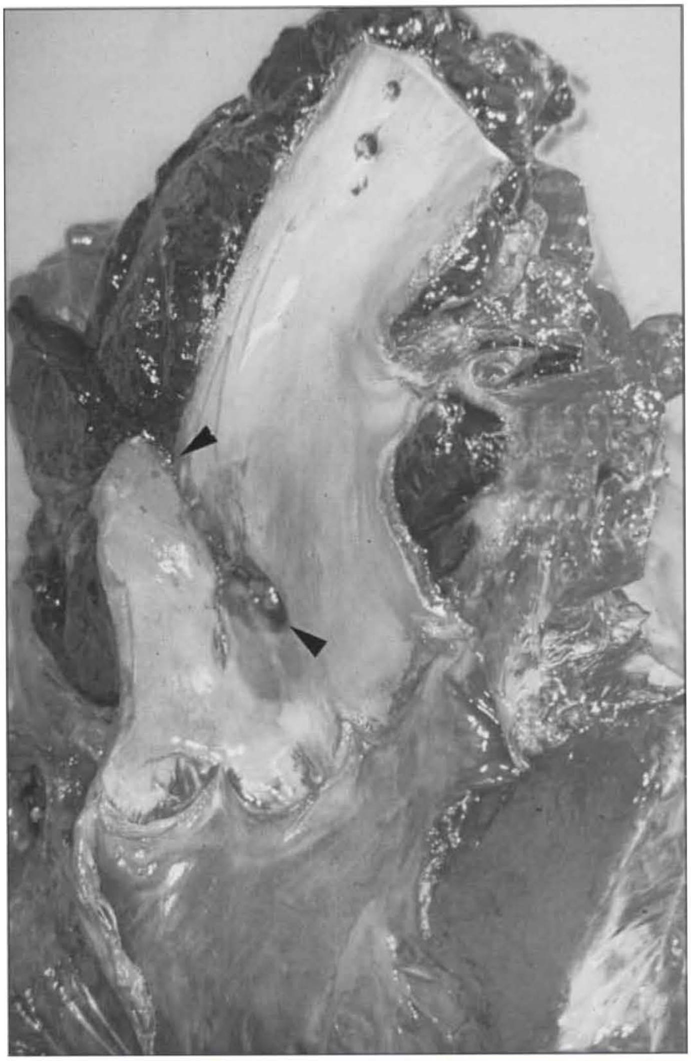

Figure 1.60 Rupture (between arrowheads) **of the ascending aorta** of a horse, which led to hemopericardium and fatal cardiac tamponade.

Spontaneous rupture of the *pulmonary artery* also occurs in horses, but is even less frequent than aortic rupture.

Rupture of the aorta, pulmonary artery, or coronary artery occurs experimentally, though not as a natural event, in *copper-deficient swine*, and has been extensively investigated because of similarities with *Marfan syndrome*, an inherited disorder in fibrillin metabolism in man and cattle in which dissecting aortic aneurysms occur. Degeneration of elastica appears to be the basis of the vascular lesions of copper deficiency, and is due to a deficiency of a copper-containing enzyme, lysyl oxidase, which is responsible for cross-linking of collagen and elastin. Similarly, feeding β-aminoproprionitrile to pigs, rats or turkeys causes dissecting aortic aneurysms and skeletal changes, an experimental model known as *lathyrism*, but is due to inhibition, rather than deficiency, of lysyl oxidase.

An **aneurysm** *is a localized abnormal dilation of any vessel.* Aneurysms are of most importance when they affect the aorta; they occur most commonly in humans as a complication of atherosclerosis, but are infrequent occurrences in domestic animals other than turkeys. On the basis of their gross appearance, aneurysms may be classified as *berry, saccular, fusiform, or dissecting.* In a **dissecting aneurysm**, blood enters the wall of an artery through an intimal tear, dissects between medial layers, and creates a cavity within the arterial wall. *Dissecting aortic aneurysm* (dissecting hematoma, or acute aortic dissection) is one manifestation of aortic rupture in horses, and may precede fatal aortic rupture. Dissecting aneurysms occur in the coronary and renal arteries of young, male, racing greyhounds, possibly as a result of arteriosclerosis and hemodynamic stresses, and can rupture fatally. Dissecting aneurysms may be of consequence by causing stenosis of the aortic lumen, as well as by rupturing. **True aneurysms** *are composed of all, or most, of the layers of the vessel wall.* Dilation of the cranial mesenteric artery may occur in the course of verminous arteritis in horses; the arterial wall is often greatly thickened, but the dilation could be termed an inflammatory rather than a true aneurysm. **False aneurysms** result from rupture of an artery or aneurysm and are essentially hematomas communicating with an arterial lumen; their walls are formed by the surrounding fibrous tissue.

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Arterial thrombosis and embolism

The three major predispositions to thrombosis are:

1. *injury to endothelium*, e.g., via infectious, toxic, or immunologic mechanisms;
2. *altered blood flow*, as occurs with stasis or turbulence; and
3. *hypercoagulability* of the blood, which may result from increased concentrations of activated procoagulants, increased numbers or stickiness of platelets, or decreased concentrations of inhibitors such as antithrombin (see nephrotic syndrome).

The relative contribution of each of the above three features of Virchow's triad may vary. Endothelial injury, by itself, is the major cause of thrombosis, especially in the heart and arteries. Stasis must usually be combined with endothelial damage and/or hypercoagulability to induce thrombosis.

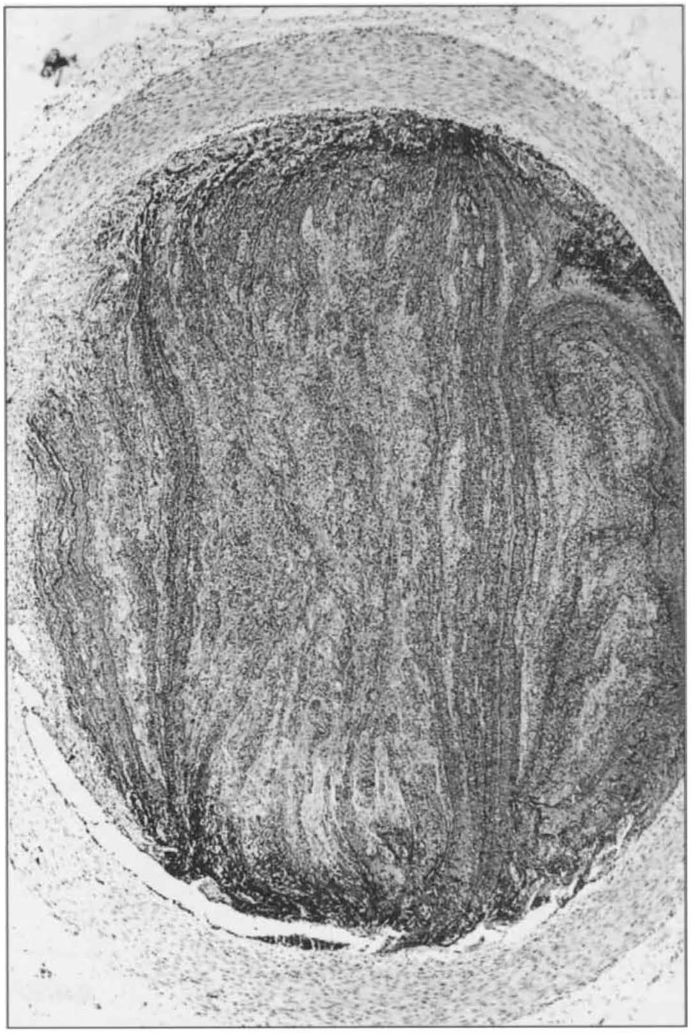

Figure 1.61 Laminations in an **arterial thrombus**, with organization from the intima and markedly reduced eccentric lumen, in aortic-iliac thrombosis in a calf.

Thrombi are of importance because they occlude the vessel at the site involved (Fig. 1.61), and because pieces of the thrombus break off giving rise to *emboli* that may occlude vessels distal to the site of thrombosis. Arterial occlusion is of significance in organs with an end-arterial blood supply, such as the kidney, because of the absence of collateral circulation and the development of infarction. Simultaneous occlusion of a large number of pulmonary arterioles or arteries by thrombi can lead to right heart failure (cor pulmonale) and death. Even in areas with extensive collateral circulation, the development of this circulation may be impaired by release of vasoactive substances such as serotonin and/or prostaglandins from the thrombus.

Specific causes and examples of thrombosis abound. As discussed below, thrombosis is a common complication of arteritis. Specific examples of thrombosis, namely aortic-iliac thrombosis in horses and disseminated intravascular coagulation, are discussed below.

Thromboembolism occurs in a variety of conditions. In verminous arteritis in horses, emboli arise from thrombi in the cranial mesenteric artery and cause intestinal ischemia, and possibly infarction. *Saddle thromboemboli of the aortic trifurcation in cats* arise from the dilated left atrium in cases of cardiomyopathy and cause posterior paresis of acute onset; *the clinical signs of aortic trifurcation obstruction, referred to as the five p's, are: pain, paresis, pulselessness, poikilothermy, and pallor.* Arterial thromboembolism occasionally occurs as a result of congenital cardiac defects and the formation of intracardiac thrombi. Emboli commonly arise from the cardiac valves in cases of vegetative bacterial endocarditis. Thrombi that develop in animals undergoing extended anesthesia can result in fatal pulmonary micro-thromboembolism, a common occurrence in humans, but a condition that is seldom recognized in domestic animals. *Pulmonary thromboembolism* may well complicate a variety of conditions, such as cardiac or renal disease, physical trauma, disseminated intravascular coagulation, hyperadrenocorticism, and neoplasia, but be underdiagnosed as a cause of morbidity.

Thrombosis is by far the most common antecedent to embolism, but *emboli other than thromboemboli occasionally occur.* Venous **air** embolism is a rare complication of pneumocystography, laparoscopy, or cryosurgery; an air trap develops in the right ventricle and may cause fatal blockage of circulation. **Foreign bodies**, such as pellets or bullets, may be embolic and occlude major vessels such as the aorta. Larval and adult **parasites**, such as strongyles and heartworms, may be embolic in the arterial system, especially if killed by therapy, and may cause arterial occlusion plus a granulomatous response. Emboli of **neoplastic** cells can result in distant metastases. **Fibrocartilaginous** embolism can result in necrotizing myelopathy in dogs, pigs, and humans. Fractures of bones, especially of those of the pelvis, and trauma of soft tissues can result in **fat** embolism; death may occur due to embolization of the central nervous system. Embolic fat may also originate from rupture of fat-laden hepatocytes.

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Aortic-iliac thrombosis in horses

Aortic-iliac thrombosis causes *exercise intolerance and hind-leg lameness* (intermittent claudication) in affected horses. The condition is seen most frequently in racing Thoroughbreds and Standardbreds, especially young males. The oldest lesions are located at the aortic quadrifurcation and in the distal portions of the femoral and internal iliac arteries (Fig. 1.62), and consist of partially or completely occlusive masses of well-organized and well-vascularized fibrous tissue, occasionally containing hemorrhagic or degenerate areas (Fig. 1.63). Proximal to these organized masses, large unorganized thrombi are often present. The tunica intima is obliterated by the occlusive masses, except for the internal elastic lamina, which usually remains intact. The tunica media is largely unaffected, except for ischemic necrosis of the media in greatly distended arteries or under thick plaques. The pathogenesis of the lesions is unknown. The lesions may result from organization of strongyle-related thromboemboli, or from progressive enlargement and organization of spontaneously developing fibrous intimal plaques.

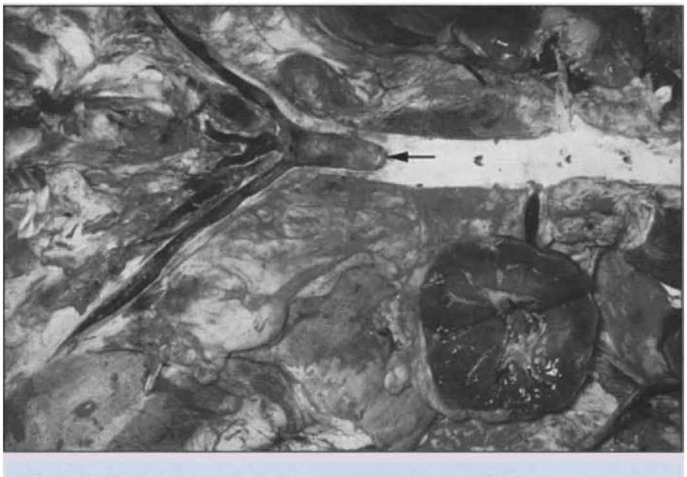

Figure 1.62 A large organized thrombus is present in the terminal aorta (arrow) and the internal and external iliac arteries in a horse with **aortic-iliac thrombosis**.

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Disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC) is a common and important intermediary mechanism of disease, but is not a disease in itself. It may be defined as a pathological activation of the coagulation system that leads to *generalized intravascular clotting* involving particularly arterioles and capillaries. The process may be *acute, subacute, or chronic*, and may be *localized or generalized*. Macrothrombosis does not usually develop in this disorder. The terms "consumption coagulopathy," "defibrination syndrome," and "consumptive thrombohemorrhagic disorder" are also used because of the massive consumption of coagulation factors which occurs and which may be sufficiently severe that a hemorrhagic diathesis results.

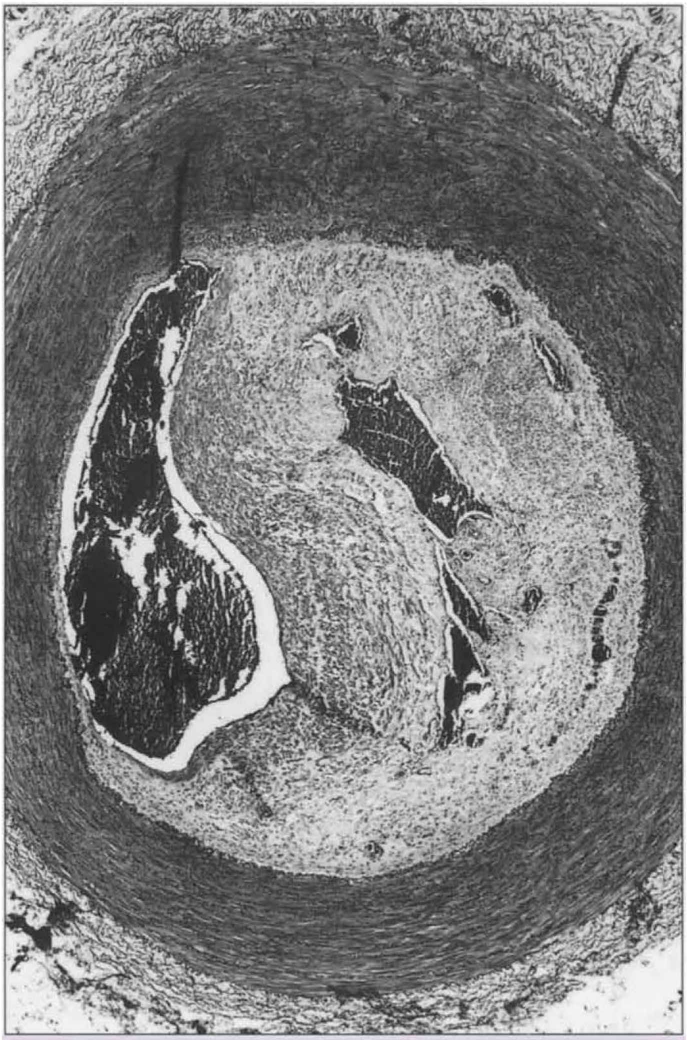

Figure 1.63 Aortic-iliac thrombosis in a horse. Lumen of artery is almost occluded by an organized, recanalized fibromuscular mass.

A wide array of agents and conditions will initiate coagulation (Table 1.7), basically either by causing *widespread endothelial damage* and thus exposing thrombogenic subendothelial collagen, or by directly *activating the coagulation cascade* via the intrinsic or extrinsic pathway. Exposure of monocytes, macrophages, and endothelial cells to disease agents or mediators will cause expression of tissue thromboplastin on the cell surfaces and activation of the extrinsic pathway of coagulation, and is likely a predominant pathway of DIC. As well as leading to expression of intravascular tissue factor, inflammation promotes coagulation by eliciting the expression of leukocyte adhesion molecules on intravascular cell surfaces, and by down-regulating the fibrinolytic and protein C anticoagulant pathways. Thrombin can in turn promote inflammatory responses, leading to further vascular injury. Stasis of blood flow and plasma hyperviscosity are other factors that may contribute to DIC.

Many of the agents listed in Table 1.7 may initiate DIC by more than one route. For example, gram-negative bacterial endotoxin can cause: endothelial damage (either directly or via mediators such as interleukin-1 and tumor necrosis factor), which exposes subendothelial collagen and leads to activation of platelets and Hageman factor; activation of the complement cascade and hence platelet activation; direct activation of Hageman factor and hence the intrinsic pathway; and leukocyte damage which results in thromboplastin

Table 1.7 Agents or conditions known to induce disseminated intravascular coagulation in animals

Bacteria	• Gram-negative (endotoxin) • Gram-positive
Helminths	• *Angiostrongylus vasorum* • *Dirofilaria immitis*
Protozoa	• *Babesia* spp. • *Sarcocystis* spp. • *Theileria* spp. • *Trypanosoma* spp.
Rickettsia	• *Rickettsia rickettsii* (Rocky Mountain spotted fever)
Viruses	• *African swine fever virus* • *Aleutian disease virus* of mink • *Bluetongue virus* • *Canine adenovirus 1* (infectious canine hepatitis) • *Classical swine fever virus* • *Epizootic hemorrhagic disease virus* of deer • *Feline infectious peritonitis virus*
Neoplasia	• Carcinoma • Hemangiosarcoma • Leukemia
Other	• Aflatoxicosis • Antigen–antibody complexes – incompatible blood transfusion • Gastric dilation-volvulus • Heat stroke • Hyperlipemia in ponies • Hyperosmolality • Immunologic endothelial injury • Ingestion of red maple leaves • Nephrotic syndrome • Proteolytic enzymes – pancreatitis, snakebite • Shock, vascular stasis, prolonged anesthesia, acidosis • Tissue necrosis – hepatic, pneumonia, postsurgery, burns

release. Viral infections are postulated to induce DIC by viral- or antiplatelet antibody-mediated platelet aggregation, by causing endothelial damage, and by causing Hageman factor activation by immune complexes. Also, in feline infectious peritonitis for example, hepatic necrosis impairs macrophage clearance of activated clotting factors and may potentiate the coagulation defect. Another important pathophysiologic event that accompanies activation of coagulation is activation of the kallikrein-kinin system; generation of kinins contributes to increased vascular permeability, hypotension, and shock.

When coagulation is initiated, activated Hageman factor simultaneously converts plasminogen to plasmin. Plasmin cleaves fibrinogen/fibrin to produce fibrinogen/fibrin degradation products that inhibit thrombin activity, fibrin polymerization, and platelet aggregation. A negative feedback system normally exists between thrombosis (coagulation) and fibrinolysis (anticoagulation) in which thrombin helps convert plasminogen to plasmin to cause fibrinolysis and hence limit thrombosis. In addition, a dynamic equilibrium exists between production of tissue plasminogen activator and production of plasminogen activator inhibitors by endothelial cells.

The clinical manifestations of DIC are protean and vary with the organ(s) in which thrombosis predominates and the severity of a hemorrhagic diathesis. Clinical presentations include shock, organ failure, hemorrhage, or hemolysis depending upon the relative activation of thrombin and plasmin and hence the dominance of thrombosis or hemorrhage. Hemorrhage is usually only seen in animals that survive acute episodes with thrombosis and shock, or in those reacting to a moderate procoagulatory stimulus. Perhaps the most common result of acute DIC is *shock*, which is due to reduced venous return to the right heart, plus systemic and pulmonary arterial hypertension due to thrombosis. Special examples of DIC include:

- the **generalized Shwartzman-like reaction** (GSR) (bilateral hemorrhagic renal cortical necrosis, induced experimentally by timed injections of endotoxin), which is seen as a cause of oliguric renal failure in septicemic or endotoxemic postpartum cows,
- **hemorrhagic adrenal necrosis** (Waterhouse–Friderichsen syndrome) seen in septicemic calves,
- **microangiopathic hemolytic anemia** (MHA) (an intravascular fragmentation anemia) seen in septicemic calves,
- **acrocyanosis and gangrene** seen in the extremities of calves recovering from septicemia,
- the **hemolytic-uremic syndrome** (HUS) in which coagulation is combined with microangiopathic hemolytic anemia and acute renal failure; a similar syndrome is seen in Greyhound dogs with idiopathic cutaneous and renal glomerular vasculopathy ("Alabama rot").

Animals that die with DIC may have petechial to ecchymotic hemorrhages on mucosae and serosae and in the skin, and may have variable thrombotic or hemorrhagic involvement of internal organs. Histologically, microthrombi may be associated with congestion, edema, hemorrhage, and necrosis. *Microthrombi are usually seen most easily in capillaries of brain, renal glomeruli, adrenals, lungs, and myocardium*, but may occur in any organ. They consist primarily of fibrin (*hyaline thrombi*), platelets (*granular thrombi*), or of fibrin degradation products (*hyaline globules or "shock bodies"*); pulmonary alveolar hyaline membranes are also said to be a form of hyaline microthrombi. Because fibrinolysis is an ongoing process that continues after death, *most microthrombi are lysed within three hours of death* and may not be found if postmortem examination is delayed. With routine hematoxylin and eosin staining, microthrombi appear as homogeneous pink masses in dilated small blood vessels. Identification of microthrombi is aided by the use of special stains such as Martius-scarlet-blue, which stains fibrin red, or phosphotungstic acid hematoxylin, which stains fibrin purple. Even with special stains, incompletely polymerized fibrin is difficult to demonstrate. Red cells that impinge on fibrin strands in the microcirculation may fragment; these red cell fragments (*schistocytes*) may be detected within blood vessels in histologic sections and may thus serve as indicators of DIC.

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Arterial hypertrophy

Hypertrophy of arteries may affect one or all components of the arterial wall. *High-altitude disease of cattle*, described below, is a result of hypoxia-induced pulmonary arterial constriction and hypertrophy that lead to pulmonary hypertension and eventual right heart failure (cor pulmonale). *Various cardiac anomalies* cause pulmonary arterial hypertension and hyperperfusion, which result in pulmonary arterial hypertrophy and additional pulmonary hypertension (see below). Arterial hypertrophy occurs in *collateral vessels* in response to the extra load they carry after occlusion of the artery that usually supplies an area. A specific form of hypertrophy occurs in the *pulmonary arteries of cats* and is described below. Systemic arterial involvement in the hypertension associated with chronic renal disease is described above.

"High-altitude disease" of cattle

This disease is caused by pulmonary hypertension that results in dilation and hypertrophy of the right ventricle with the ultimate development of cardiac decompensation and congestive cardiac failure. Edematous swelling of the venter, as is typical of congestive heart failure in cattle, is responsible for the synonym "*brisket disease*." The syndrome in cattle resembles in many respects the chronic mountain sickness of humans resident at high altitude, and represents failure of the cardiorespiratory system to adjust to the hypoxia of higher altitudes. There are species differences in the hypertensive response to hypoxia; sheep and dogs are hypo-responders, humans are intermediate, whereas cattle and pigs are hyper-responders and are prone to develop hypertensive heart failure at altitudes of about 2500 meters and above. There are also variations within species, and some cattle react dramatically to degrees of hypoxia that only mildly inconvenience others. Young cattle are more susceptible than adults, and the morbidity rate is highest in animals exposed to high altitudes for the first time. In animals imported from low altitudes to about 3500 meters, the incidence of severe pulmonary hypertension approaches 50%, whereas in herds maintained at such altitudes for many generations, high altitude disease may not affect more than 2%. The different morbidity rates are probably attributable to natural selection in the resident population.

Cattle exposed to critical altitudes, or experimentally to comparable degrees of hypoxic stimulation, develop a several-fold increase in pulmonary arterial pressure and pulmonary vascular resistance that may gradually be reduced to near normotensive levels when the animals are returned to low altitudes. A distinctive feature of the pulmonary vasculature in cattle is the inherently well-developed muscular media of the arteries and veins with diameters as small as 20 μm, which implies an unusual potential for vasomotor activity. *Hypoxia-induced vasoconstriction leads to work hypertrophy of the muscular media of pulmonary arteries and arterioles, and hence hypertension*, and may be compounded by reaction hypertrophy that occurs in response to increased arterial pressure. In cattle affected with high-altitude disease, there is uniformly prominent hypertrophy of the muscular media of pulmonary arteries and arterioles, and usually adventitial proliferation around pulmonary arteries of all sizes. The hypertension may also cause endothelial damage, thrombosis, intimal proliferation, and medial mineralization, especially in elastic pulmonary arteries.

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Cardiac anomalies and pulmonary arterial hypertension

Increased pulmonary arterial pressure (*hypertension*) and increased blood flow (*hyperperfusion*) occur with certain cardiac anomalies in which there is left-to-right shunting of blood, such as a large ventricular septal defect or patent ductus arteriosus. Pulmonary arterial hypertension, which may even lead to reversal of the shunt and cyanosis (*Eisenmenger's syndrome*), produces pulmonary arterial constriction and hypertrophy, increased pulmonary vascular resistance, and sustained pulmonary hypertension by means of a number of arterial responses. These responses range from persistence of the

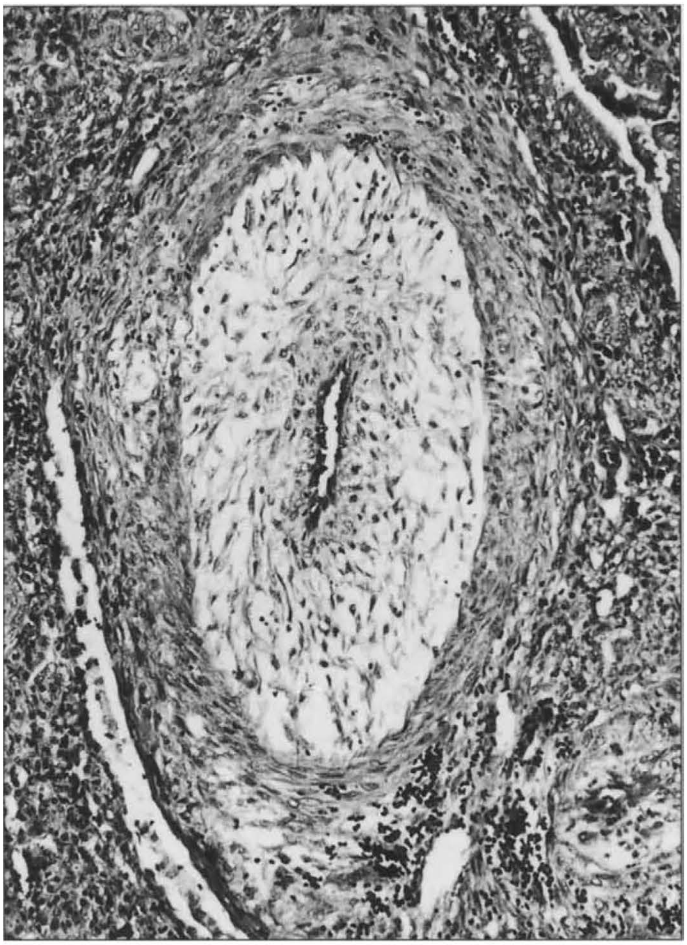

Figure 1.64 Idiopathic **intimal hyperplasia** in blood vessel in the lung of a kitten.

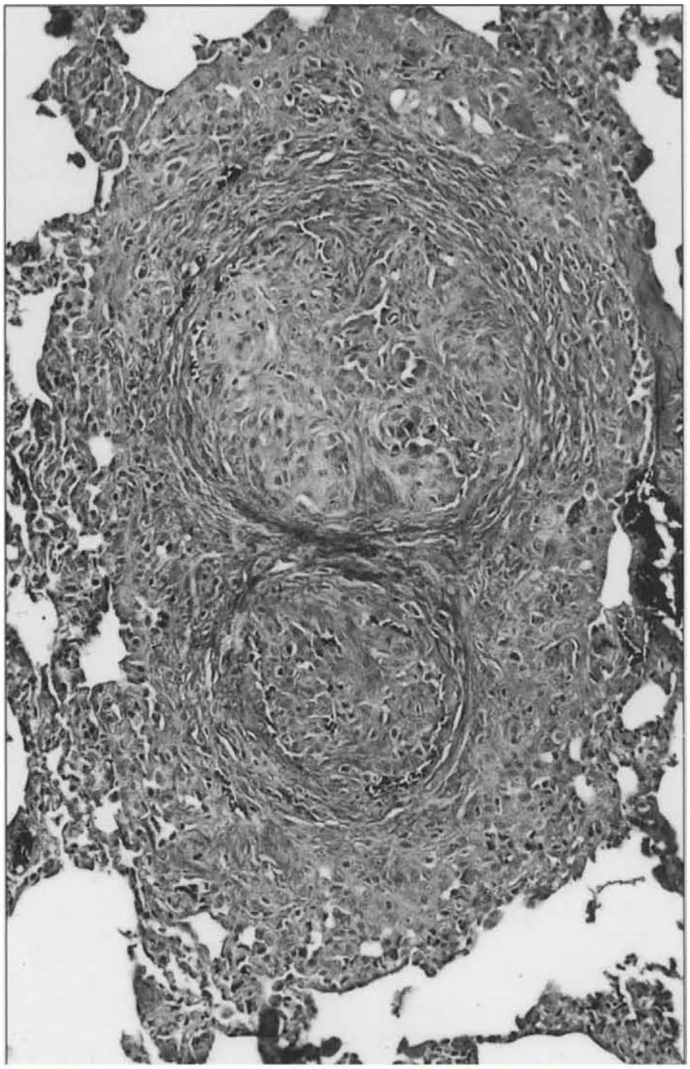

Figure 1.65 **Pulmonary arterial hypertension** in dog with a cardiac anomaly. Two pulmonary arteries with intimal fibromuscular hyperplasia, medial and adventitial hyperplasia. (Courtesy of JS Nimmo-Wilkie.)

normal thick muscular wall configuration of fetal life, to lesions that are the acquired response to hypertension (Fig. 1.64). *These acquired lesions include prominent medial hypertrophy, intimal proliferations that may be obliterative, disruption of elastic laminae, adventitial fibrosis, and plexiform lesions* (vascular occlusion by myointimal proliferations containing irregular vascular channels). These changes may be mediated by increased expression of various mediators, such as matrix metalloproteinases and endothelin-1. In the early, cellular phase of the lesion, migration of medial smooth muscle cells through the internal elastic lamina and into the intima, possibly in response to a chemical attractant such as endothelial cell-derived growth factor, is followed by their conversion to myofibroblasts and production of ground substance, collagen, and elastin, which produces marked intimal thickening (Fig. 1.65). Aged mature lesions become less cellular, myofibroblasts revert to smooth muscle cells, and thin elastotic septa separate wide vascular channels; these channels may be thrombosed. As the pulmonary vascular lesions progress, the arteries may dilate and their walls undergo fibrinoid degeneration. Together these lesions constitute "**plexogenic pulmonary arteriopathy**," which in its more severe manifestations is an end-stage irreversible arterial condition that is associated with a poor prognosis, even if the underlying cardiac anomaly can be corrected surgically.

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Medial hypertrophy of the pulmonary arteries of cats

Pulmonary medial hypertrophy and hyperplasia commonly occur in cats, but are of no clinical significance even when very severe and generalized. The change is seen with equal frequency in germ-free and conventional cats, displays no age, sex, or breed predilection, and appears to be *normal anatomic variation* in cats. The cat lungworm, *Aelurostrongylus abstrusus*, has been suggested as an etiologic agent, but while the parasite may produce similar lesions, it is not necessary for the development of the lesion. Pulmonary hypertension does not result from the vascular change, right ventricular pressure does not increase, nor does the right ventricle hypertrophy. The most severely affected vessels may be grossly visible on the cut surface of the lung and through the pleura when the lungs are collapsed, and are even palpable in some cases. The histologic spectrum of arterial changes ranges from mild sporadic or generalized hypertrophy of the tunica media, to marked medial hypertrophy and hyperplasia with concomitant intimal proliferation and severe luminal encroachment. Thrombosis has not been observed, but adhesion of endothelial surfaces across the lumen does occur providing sinuous channels and an appearance similar to that of an organized, recanalized thrombus. Frequently, the arterial hypertrophy is accompanied by fibromuscular hyperplasia in the pulmonary parenchyma and around alveolar ducts.

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Vasculitis

Vasculitis, or inflammation of a vessel, is characterized by the presence of inflammatory cells within and around the blood vessel wall with concomitant vessel wall damage as indicated by fibrin deposition, collagen degeneration, and necrosis of endothelial and smooth muscle cells. The fibrillary, homogeneous, or granular eosinophilic material observed by light microscopy and referred to as "**fibrinoid**" consists of fibrin, immunoglobulins, complement, and platelets; degenerate collagen and smooth muscle also contribute to increased eosinophilia of the vascular wall. Thrombosis may occur because of endothelial injury and initiation of coagulation. The above changes in the blood vessel wall distinguish vasculitis from perivascular infiltration with inflammatory cells. Neutrophils, lymphocytes, or macrophages may predominate in vasculitis, and the predominant cell type may be used to classify the vasculitis. Neutrophilic vasculitis may be further subdivided into **leukocytoclastic**, in which fragmented neutrophil nuclei or "nuclear dust" are present, and **nonleukocytoclastic**, in which there is little nuclear dust. The predominant cell type may vary over the course of the vasculitis, as the condition becomes chronic or resolves.

Vasculitis is an important component of a wide variety of inflammatory diseases, both **infectious** and **noninfectious** (Table 1.8), although its origin remains idiopathic in many cases. Vascular degeneration that lacks a significant inflammatory cell component occurs in a number of toxic and metabolic conditions, such as mercury poisoning and mulberry heart disease, and has been excluded from the classification. The terms *vasculitis* and *angiitis* are used interchangeably

Table 1.8 Causes of vasculitis in domestic animals

Infectious

- Viral
    - *African horse sickness virus*
    - *African swine fever virus*
    - *Bluetongue virus*
    - *Border disease virus*
    - *Bovine adenovirus*
    - *Bovine ephemeral fever virus*
    - *Bovine viral diarrhea virus*
    - *Classical swine fever virus* (hog cholera)
    - *Equid herpesvirus 1*
    - *Equine arteritis virus*
    - *Equine infectious anemia virus*
    - *Feline infectious peritonitis virus*
    - *Ovine herpesvirus 2* (sheep-associated malignant catarrhal fever)
    - *Porcine reproductive and respiratory syndrome virus*
    - *Visna/maedi virus* (ovine progressive pneumonia)
- Chlamydial
    - *Chlamydophila abortus* (*Chlamydia psittaci*)
    - *Chlamydophila pecorum* (sporadic bovine encephalomyelitis)
- Rickettsial
    - *Coxiella burnetii*
    - *Ehrlichia canis, E. equi*
    - *Ehrlichia (Cowdria) ruminantium* (heartwater)
    - *Rickettsia rickettsii* (Rocky Mountain spotted fever)
- Bacterial
    - *Actinobacillus pleuropneumoniae, A. suis*
    - *Corynebacterium pseudotuberculosis* (lymphangitis)
    - *Erysipelothrix rhusiopathiae*
    - *Haemophilus parasuis*
    - *Histophilus somni*
    - *Salmonella* spp.
    - *Streptococcus suis*
- Mycotic
    - *Aspergillus fumigatus, A. niger*
    - *Histoplasma farciminosum* (lymphangitis)
    - *Mucor* spp.
    - *Sporotrichum schenkii* (lymphangitis)
- Protozoal
    - *Besnoitia besnoiti*
    - *Encephalitozoon cuniculi*
    - *Hepatozoon americanum*
- Helminths
    - (medium to large vessels, usually endovasculitis)
    - *Aelurostrongylus abstrusus*
    - *Angiostrongylus vasorum*
    - *Brugia* spp. (lymphangitis)
    - *Dirofilaria immitis*
    - *Elaeophora* spp.
    - *Onchocerca* spp.
    - *Schistosoma* spp. (phlebitis)
    - *Spirocerca lupi*
    - *Strongylus vulgaris*

Noninfectious

- Immune-mediated
    - Anaphylactoid purpura
    - Food hypersensitivity
    - Foreign proteins (serum sickness)
    - Paraneoplastic, e.g., lymphosarcoma
    - Polyarteritis nodosa
    - Purpura hemorrhagica
    - Rheumatoid arthritis
    - Rabies vaccine reaction
    - Staphylococcal hypersensitivity
    - Systemic lupus erythematosus
- Nonimmune-mediated
    - Drug reaction, e.g., itraconazole, ivermectin, trimethoprim-sulfadiazine/sulfamethoxazole
    - Uremia

and may be used in preference to the more specific term *arteritis*, because arteries, capillaries, and veins may be involved simultaneously in some conditions. Also, the wall of the vessel may be obscured or obliterated by inflammatory changes, precluding definitive classification. The consequences of vasculitis depend upon the size, numbers, and types of vessels affected, and upon the degree of obstruction caused, as noted under thrombosis. We will discuss vasculitis in general and specific forms of arteritis in this section; *phlebitis* and *lymphangitis* are discussed with Veins and Lymphatics, respectively.

Vasculitides may be *classified* according to the size of the blood vessels involved (large, medium, small), the histologic characteristics of the lesion, or clinical effects. However, many cases overlap in their morphologic and clinical manifestations, and are simply termed "*systemic necrotizing vasculitis*." Improvement in the classification of necrotizing vasculitides will require further characterization of causes and of the spectrum of immune-mediated changes in vessels. Until such time, the use of accurate morphological descriptions of vascular lesions is preferable to placing vasculitides, especially those of known cause, in such ill-defined categories as "polyarteritis nodosa." The wide range of lesions observed probably reflects the variety of antigens or other agents involved, the intensity and chronicity of antigenic exposure, the relative contributions of immune-complex versus delayed-hypersensitivity reactions, the genetic variation among individuals, and the stage of development at which the lesion is observed.

Vasculitis may be of considerable significance in the pathogenesis of a condition, usually because of the occurrence of thrombosis, ischemia, and infarction (Fig. 1.66). As well as having local effects due to thrombosis and infarction, arteritis may affect distant organs, as occurs when obliterative pulmonary endoarteritis leads to congestive heart failure in dogs infected with *Dirofilaria immitis*. (The term *endo*arteritis is used in preference to *end*arteritis to avoid implying that end-arteries are primarily affected.) Arteritis of *hematogenous origin* occurs in the course of septicemias and bacterial endocarditis. The primary injury may be to the endothelium and intima or it may, when the organisms localize in the vasa vasorum, affect first the adventitia and outer lamellae. The arteritis in pigs with septicemic salmonellosis, erysipelas, or classical swine fever is conspicuously expressed as erythematous areas of purple discoloration on the ears, perineum, snout, and venter, which, if the animal survives, may become necrotic and slough. Lesions with this basis may also occur in other organs, and indeed are often seen as infarcts in the spleen in erysipelas and classical swine fever. In affected portions of vessels, the endothelium swells and proliferates, and vessel walls undergo acute fibrinoid necrosis. The formation of thrombi in these areas in turn gives rise to infarcts and necrosis. Vasculitis arising by *extension of inflammation and infection from adjacent tissues* may occur, especially if the original inflammatory process is suppurative or necrotizing. It is encountered most often in bacterial pneumonias, adjacent to abscesses, in purulent meningitis, in local lesions of aspergillosis and mucormycosis, and in acute metritis especially if complicated by necrobacillosis. Fungi of the family *Mucorales* have a distinct affinity for arteries and produce necrotizing, thrombotic arteritis.

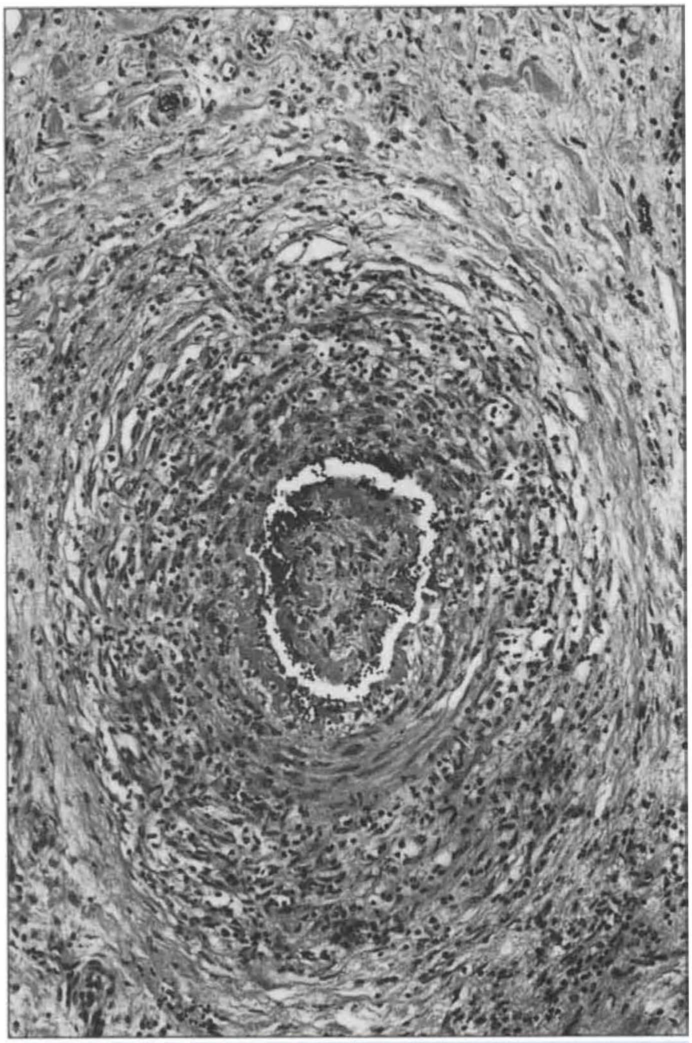

Figure 1.66 Panarteritis and thrombosis in the kidney of a pig.

Vasculitis may develop from within the vessel as a result of *endothelial injury* by infectious agents and/or immune reactions. Endothelial damage and exposure of subendothelial collagen leads to activation of Hageman factor, and hence activation of complement, kinin, and plasmin systems, and to increased vascular permeability and inflammation. Infectious agents may cause endothelial damage either directly or through the actions of various toxic products, such as endotoxins of gram-negative bacteria, or exotoxins of bacteria such as *Corynebacterium pseudotuberculosis*. *Mannheimia (Pasteurella) haemolytica* endotoxin (lipopolysaccharide, LPS) can directly damage endothelial cells; such damage may be reduced or increased by the activity of neutrophils, depending on the concentration of LPS present. The mechanism of LPS-mediated damage may involve prostaglandins or production of oxygen radicals by endothelial cells. In the absence of LPS, *M. haemolytica* leukotoxin may contribute to endothelial damage. In the case of *Actinobacillus pleuropneumoniae*, rather than endotoxin causing endothelial damage, cytotoxicity is apparently caused by a 104 kD hemolysin. *Histophilus somni* lipooligosaccharide has been shown to cause cytotoxicity and apoptosis of bovine endothelial cells through the generation of reactive oxygen and nitrogen intermediates. Many viruses are endotheliotropic, for example, the viruses of equine arteritis, infectious canine hepatitis, canine distemper, African swine fever, and classical swine fever (Table 1.8).

Immune reactions appear to be less important primary causes of vasculitis in domestic animals than in man, but they may contribute to vasculitis caused by an infectious agent, as occurs in malignant catarrhal fever. *Type III hypersensitivity reactions*, or Arthus reactions, cause necrotizing vasculitis by the deposition of immune complexes in vessel walls, usually in association with the endothelial basement membrane. The complexes fix complement, and complement fragments attract neutrophils that release lysosomal enzymes and oxygen radicals (superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorous acid) in the process of phagocytosing immune complexes, and hence cause necrosis. As the Arthus reaction matures, neutrophils diminish and mononuclear cells, including plasma cells, predominate. The morphology of immune-complex-mediated vasculitis can be modified by the relative concentrations of immune reactants or secondary mediators; increases in antibody or complement concentrations can change an Arthus-like reaction to a Shwartzman-like reaction. Immune complexes can be found in vessel walls in a variety of conditions, including systemic lupus erythematosus, Aleutian disease of mink, and feline infectious peritonitis. However, even though immunoglobulins or immune complexes are found in the walls of affected vessels, this is not definitive proof that the immune reaction caused the vasculitis. Conversely, the absence of immune complexes from a vessel wall lesion does not rule out immune-complex vasculitis, because immune reactants can disappear rapidly. The underlying lesion in *porcine dermatitis and nephropathy syndrome* (PDNS) is necrotizing systemic vasculitis, which has a predilection for skin and kidney, and is thought to have an immune-complex origin; *Porcine circovirus 2* antibody titers were excessively high in PDNS pigs in a case-control study.

Cell-mediated type IV, or delayed hypersensitivity, reactions may be involved in some types of vasculitis in which there is lymphocytic predominance, such as the arteritis of malignant catarrhal fever and of Border disease. In malignant catarrhal fever, the vasculitis is characterized by accumulations of principally lymphocytes and fewer macrophages in the adventitia and intima of affected small and medium arteries and veins of the alimentary tract, eye, kidney, liver, lung, and brain; neutrophils and plasma cells are rare. Lymphocytes may later invade the media, though adventitial infiltration continues to predominate, and there may be medial myocyte necrosis with variable amounts of fibrinoid degeneration and endothelial hyperplasia; thrombosis may be seen in advanced cases. There is a possibility of, but little direct evidence for, direct viral cytolysis in malignant catarrhal fever.

Vasculitis in humans has been associated with a variety of **drugs** (see *hypersensitivity vasculitis* on p. 72). Hypersensitivities to itraconazole, ivermectin, allergy immunotherapy (hyposensitization) injections, and ibuprofen have been reported in dogs.

Genetic predisposition to vasculitis occurs in a number of dog breeds, e.g., Beagle ("Beagle pain syndrome"), Greyhound ("Alabama rot"), Chinese Shar-Pei ("Shar-Pei fever"), German Shepherd, Bernese Mountain Dog. Vasculitis may be precipitated by vaccination or infection.

Arteritis may be found as an *incidental "background" lesion* in clinically normal animals. For example, mild idiopathic arteritis and intimal thickening occurs in extramural coronary arteries in 5–10% of young Beagle and mongrel dogs, which can complicate the interpretation of lesions in dogs used in arterial toxicity studies. A more severe, febrile syndrome, variously named *Beagle pain syndrome*, *canine juvenile polyarteritis syndrome*, or *idiopathic canine polyarteritis*, occurs in Beagles and other breeds. In this syndrome, necrotizing arteritis affects the coronary, mediastinal and cervical spinal arteries, resulting in thrombosis, infarction, and hemorrhage as well as progressive atrophy of temporal and cervical muscles; amyloidosis occurs in some chronically affected dogs. The syndrome appears to be immune mediated; some affected dogs have antineutrophil cytoplasmic antibodies (ANCA) and other immunologic irregularities similar to Kawasaki disease in humans.

An idiopathic vasculopathy occurs in kenneled and racing *Greyhound dogs*, involving skin and occasionally kidneys. Deep, slowly healing cutaneous ulcers occur as the result of fibrinoid arteritis, thrombosis, and hemorrhagic infarction. Renal lesions include acute necrosis of glomeruli and of afferent arterioles, glomerular capillary thrombosis, and acute tubular necrosis. The pathogenesis of the condition is thought to involve a genetic predisposition to an unidentified infectious agent.

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Polyarteritis nodosa (panarteritis, or periarteritis nodosa)

The term "polyarteritis nodosa" has been applied to a *heterogeneous group of arteritides*, which occur sporadically in all species of domestic animals, on the basis of similarities with *classic polyarteritis nodosa in humans, in which small and medium-sized arteries undergo severe necrotizing inflammation, often in a sharply segmental (nodose) pattern*, and with a predilection for branching points. As all layers of the arterial wall are involved, the lesion is also referred to as "*panarteritis*." Arterioles, capillaries, and venules are not involved, and glomerulonephritis is not present.

The only agent thus far associated with the condition in man is hepatitis B antigen, which is found in 25–40% of individuals with necrotizing vasculitis; high mortality occurred in the human disease before the advent of corticosteroid therapy. Polyarteritis nodosa has been reported in dogs having rheumatoid arthritis and systemic lupus erythematosus; separation of the vasculitis component from disease syndromes is of problematic value. Also, as noted above under *vasculitis*, a more appropriate term for idiopathic vasculitides is probably **systemic necrotizing vasculitis** rather than polyarteritis nodosa, which has tended to become a catchall term.

A wide variety of clinical and pathologic manifestations occur in polyarteritis nodosa as described in man and domestic animals. Although occasionally associated with the death of an animal, lesions are also noted in otherwise normal animals at slaughter. Renal, coronary, hepatic, and gastrointestinal vessels are perhaps most commonly involved (Fig. 1.67A, B). *Arterial lesions at all stages of development, namely acute, healing, and healed, may occur simultaneously in one individual and even within the same vessel.*

The acute lesions are often typical of immune-complex-induced arteritis, and inflammation and necrosis of the arterial wall may be segmental or circumferential. Thrombosis may occur and lead to infarction and hemorrhage. As the reaction progresses, neutrophils are replaced in the media by mononuclear cells, and fibroplasia may result in grossly visible fibrous thickening of the wall. The vascular lumen may be obliterated by organization of a thrombus plus intimal proliferation. The term "*periarteritis*" has been used in the past to describe a stage in the vascular reaction in which mononuclear cells surround the vessel, but the term is now little used.

Microscopic polyangiitis (*hypersensitivity vasculitis, microscopic polyarteritis, leukocytoclastic vasculitis*) affects smaller vessels in humans, namely *arterioles, capillaries, and venules* in skin, mucous membranes, lungs, brain, heart, gastrointestinal tract, kidneys, and muscle. Suspected causes include many common drugs, e.g., penicillin; microbes, e.g., streptococci; heterologous proteins; and tumor antigens. The reaction often occurs 7–10 days after exposure to the stimulus; remission usually follows removal of the agent. Lesions typical of leukocytoclastic vasculitis are seen. Since larger arteries are spared, macroscopic infarcts are uncommon. Hypersensitivity vasculitis appears to be the basis of some cases of *hemorrhagic purpura in horses*; other causes of subcutaneous edema and mucosal hemorrhages, such as equine viral arteritis, equine infectious anemia, and equine ehrlichiosis, must also be considered. Trimethoprim-sulfadiazine and trimethoprim-sulfamethoxazole have caused *hypersensitivity vasculitis in dogs*.

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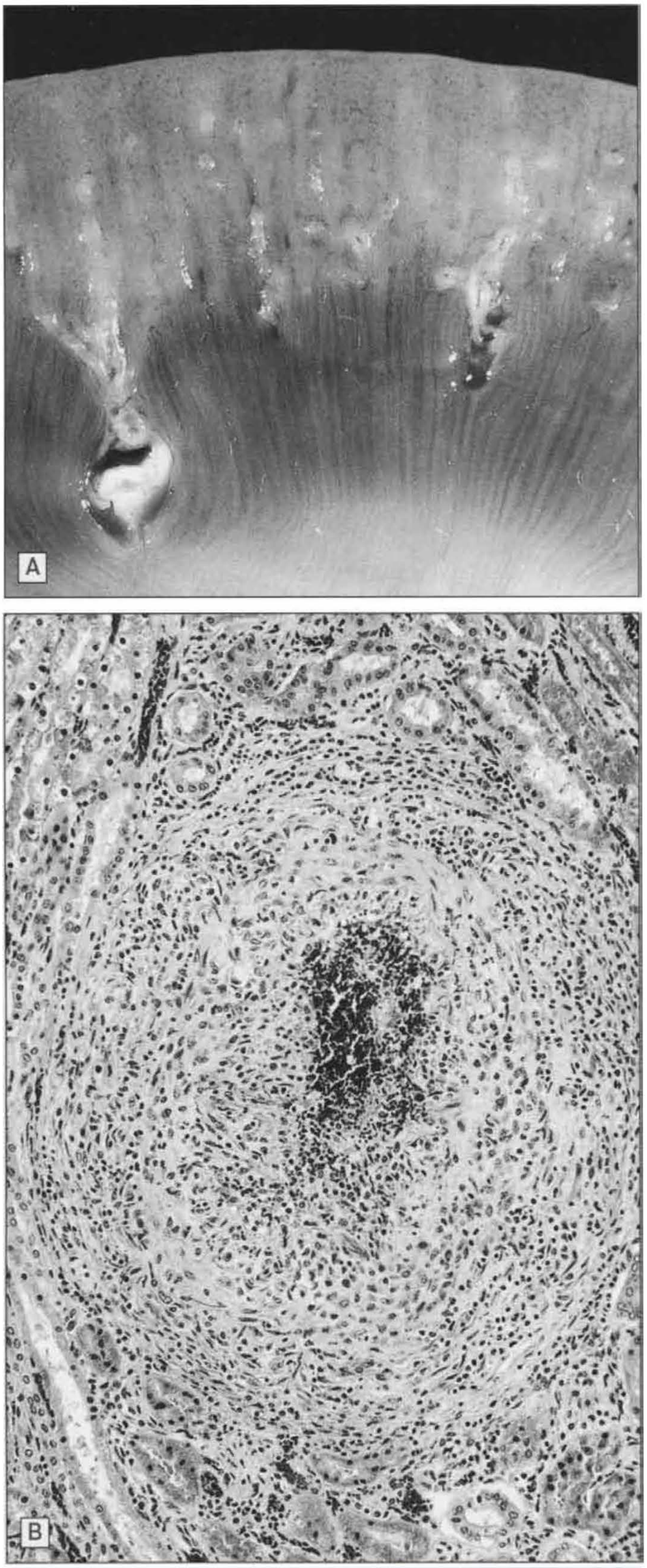

Figure 1.67 A. Systemic necrotizing vasculitis ("polyarteritis nodosa") in a sheep. **B. Panarteritis** in the kidney of a sheep with Border disease. (**B.** courtesy of J Orr.)

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Viral vasculitides

Equine viral arteritis

This disease is caused by ***Equine arteritis virus*** (EAV), an RNA virus of the family Arteriviridae, genus *Arterivirus*, which is pathogenic only for horses and is cytopathic in equine kidney culture. Although serologic surveys indicate that infection with EAV is common in North America and Europe, *clinical disease is uncommon*. Most strains of EAV are avirulent, and mortality is rare in natural outbreaks. Long-lasting immunity follows recovery. Transmission of virus occurs primarily by *respiratory and venereal routes* during the acute phase of infection; venereally infected mares can infect nonimmune mares by aerosol transmission. Long-term carrier stallions play an important role in perpetuation and dissemination of the virus; a carrier state has not been demonstrated in mares or foals.

The **clinical disease** is characterized by: fever; variable anorexia and depression; leukopenia; edema of the ventral body wall and limbs, especially the hindlimbs; skin rash, most commonly on the neck; serous, later mucopurulent, oculonasal discharge with rhinitis and conjunctivitis; and periorbital/supraorbital edema. Dyspnea, coughing, ataxia, and diarrhea are less frequent. Pregnant mares often *abort* during or shortly after the febrile period, probably due to myometritis and decreased progesterone production by a hypoxic placenta. Specific lesions are present occasionally in aborted fetuses, and include necrotizing vasculitis, especially of the placenta, and inflammatory foci in various organs. Newborn foals may succumb to interstitial pneumonia.

The virus is pathogenic to endothelial cells and causes ***panvasculitis****, that is, inflammation of veins, lymphatics, and arteries.* Following initial replication of the virus in macrophages, endothelial cells are invaded beginning three days after experimental aerosol infection. As inflammation progresses and neutrophils damage the internal elastic lamina, medial cells are invaded. The most severe edema occurs at days 6 and 7 when phlebitis, lymphangitis, and capillary damage are most pronounced. Arterial necrosis peaks at day 10, when edema has largely disappeared. The vascular lesions will resolve if the horse survives. Antibody appears to play little role in the pathogenesis of the disease, in contrast to the immune-complex component of some other viral vasculitides.

The **gross lesions** of the disease, in addition to the changes observed clinically, consist principally of hemorrhages and edema. Petechial hemorrhages are found on all serous membranes, in the substance of the lungs and the gastric mucosa. Larger hemorrhages may be present in the adrenals. There is excessive fluid which contains much protein and some strands of fibrin in all serous cavities. As much as 10 L may be present in the pleural and peritoneal cavities. The connective tissues and mesenteries of the body cavities are saturated with edema fluid and the wall of the gut may be thickened by edema. Enteritis, hemorrhagic or diphtheritic in character and usually more severe in the large than in the small intestine, is regularly present. The lungs are more-or-less severely edematous.

Characteristic **microscopic lesions** occur focally or segmentally in the media of small muscular arteries (Fig. 1.68). The muscle cells undergo necrosis and are replaced by hyaline or fibrinoid material. There is edema of the wall and adventitia and infiltration of leukocytes, chiefly lymphocytes, the nuclei of which undergo fragmentation with necrosis. The endothelium and intima may have been repaired so that thrombosis is unusual; thrombosis may however occur in the intestine and lungs. *The arterial lesions occur in many organs but are perhaps most consistently present in the gut and adrenals.* Infarction is most common in the intestines, particularly in the cecum and colon. Massive necrosis of lymph nodes also occurs. Extensive necrosis of the adrenals may result from direct viral injury and infarction.

Diagnosis is made through detection of seroconversion (serum neutralization, ELISA), or identification of the virus (virus isolation, polymerase chain reaction, immunohistochemistry) in the presence of compatible lesions. *Differential diagnoses* for EVA include diseases caused by other viruses (*Equid herpesvirus 1, Equine adenovirus, Influenza A virus, Equine infectious anemia virus, African horse sickness virus, Hendra virus, Getah virus*), plus purpura hemorrhagica, septic shock, and poisoning by the toxic plant hoary alyssum (*Berteroa incana*).

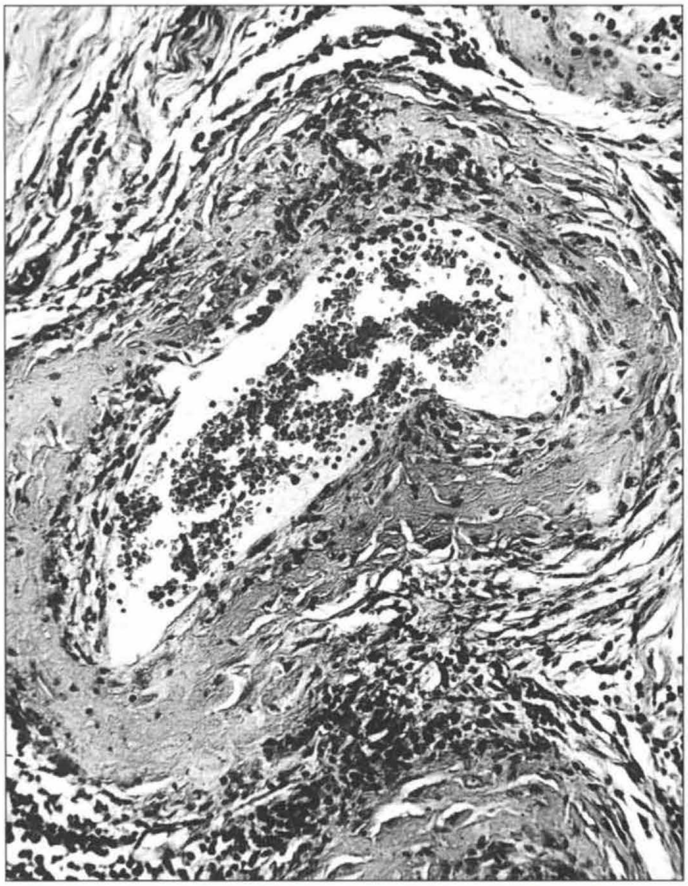

Figure 1.68 Equine viral arteritis in a horse. Artery of colonic submucosa with fibrinoid necrosis of muscularis and early leukocytic reaction. (Courtesy of JR Rooney.)

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African horse sickness

African horse sickness (AHS) is a vector-borne disease of solipeds, and occasionally of dogs and camels, caused by ***African horse sickness virus*** (AHSV), family Reoviridae, genus *Orbivirus*, that is closely related to bluetongue virus. The disease is endemic in sub-Saharan Africa, from where it spreads to southern Africa and occasionally northern Africa. It has also extended on occasion through the Middle East as far as India, and apparently with climate change, the vector has become established in southern Europe. *Its importance lies in the extreme lethal potential of the infection in susceptible horses and the very wide distribution of the competent insect vectors.*

The vectors of AHSV are primarily *Culicoides* biting midges, particularly *Culicoides imicola*. Transmission by mosquitoes or ticks is of minor importance. The AHSV requires an ambient temperature of ≥15°C to replicate and be transmitted by *Culicoides*. Historically, in enzootic areas south of the Sahara, outbreaks of disease occurred in seasons when nocturnal biting insects were active, outbreaks subsiding shortly after the first frosts. It is possible that, in climatically suitable areas, transmission might occur throughout the year. Annual recrudescence poses the question of reservoirs for over-winter persistence; the natural reservoir remains unknown, although the zebra is most likely. Even in districts where AHS occurs annually, the distribution tends to be limited to low-lying areas such as valleys, swamps, and areas of summer rain.

Horse sickness is primarily a disease of the horse and its close relatives, but a wide range of species is susceptible to the virus experimentally, including goats, guinea pigs, mice, ferrets, and rats. The horse is the most susceptible to infection and to illness; the mortality rate in susceptible populations of horses may be as high as 95%. The mortality rate in mules (about 80%) is less than that in horses but greater than that in the Egyptian donkey. The South African donkey and the zebra are resistant. The role of dogs, and other scavenging carnivores, in the disease cycle is not known. The disease is not contagious by contact, but dogs may acquire it from eating infected horseflesh. Infection detected by viral isolation or serologically can be common in dogs in some circumstances, but vector transmission from dogs is unlikely.

In many parts of Africa, the use of the horse, mule, and donkey is dependent on the control of AHS, but this control has been difficult to attain. The primary obstacle has been the multiplicity of antigenic types of AHSV. There are at present *nine major antigenic types*. These types have a basic antigenic relationship which may be demonstrated in the horse, but the relationship is not sufficiently close to provide solid immunity amongst types. Indeed, although all strains investigated can be fitted into one of the major types, the antigenic diversity of strains within a single type is such that very few strains are identical. This is certainly the situation in enzootic areas where a large number of different strains may be isolated during an outbreak. On the other hand, there has been close antigenic relationship between the viruses recovered during the epizootic outbreaks that occurred outside the reservoir-host range where the transmission was from horse to horse. This suggests that, although the AHSV is extremely mutable, mutations do not readily occur when the disease is transmitted from horse to horse. Recovered horses are immune to homologous challenge, and possess some immunity to heterologous challenge.

Four clinical forms of AHS are described: *pulmonary (peracute), mixed (acute), cardiac (subacute), and AHS fever (mild) forms*, from most to least severe. Most cases are of the mixed form, perhaps reflecting variations in the susceptibility of the host, the virulence of the virus, or the tropism of virus for different parts of the vascular system.

The **peracute or pulmonary form of the disease** – the most common form in epizootics – has an incubation period of 3–5 days. Fever occurs suddenly and is of short duration followed by the acute onset of respiratory distress. Death may occur in a few hours from *pulmonary edema*, and frothy fluid fills the respiratory tract and may flow from the nostrils.

The **subacute or cardiac form** is usually associated with infections that are not fully virulent or are encountered in animals in which infection by heterologous strains has stimulated some immunity. The incubation period is 7–14 days with clinical signs of fever lasting 3–6 days. As the fever subsides, edematous swellings appear in the temporal or supraorbital fossae and the eyelids. The swellings extend to the lips, cheeks, tongue, intermandibular space, and throat. Swelling from subcutaneous fluid may affect the upper neck and sometimes the chest and shoulders. Terminally, petechial hemorrhages usually occur on the conjunctiva and ventral surface of the tongue. The mortality rate may be as high as 50%, and is attributed to *cardiac failure and pulmonary edema*.

As observed above, many cases are **mixtures of the pulmonary and cardiac form**. Initial pulmonary signs of relatively mild degree may be followed by characteristic swelling of the head and death from cardiac failure, or the cardiac form may be followed by sudden onset of dyspnea and pulmonary edema.

The mild clinical form of the disease, **AHS fever**, may not be noticed in outbreaks. It is characterized by remittent fever lasting for a week or so. This is the form of the disease to be expected in animals with immunity to heterologous strains and in resistant species such as donkey and zebra. The mortality rate is about 70%.

The **pathogenesis** of the signs and lesions is related to sites of viral replication. Virus is found early and develops to highest titer in spleen, lymph nodes, lung, and the large intestine; the titer in myocardium is low. Viral infection causes degeneration and loss of endothelium in myocardial and pulmonary capillaries, with resulting increased vascular permeability, edema, hemorrhage, and microthrombosis. Vascular permeability of pulmonary capillaries may be increased through the action of inflammatory mediators released by activated intravascular macrophages. *Myocardial lesions* are seen microscopically in the cardiac form of the disease and are less severe in the pulmonary form. These consist of hemorrhage, edema, and focal myocardial necrosis (of hypoxic origin) with inflammatory infiltrates and slight proliferation of connective tissue.

The **gross lesions** observed at autopsy depend largely on the clinical form of disease. In the *pulmonary form*, the most characteristic changes are edema of the lungs and hydrothorax. In very acute cases, alveolar edema and mottled hyperemia of the lungs are seen, whereas in cases with a somewhat more protracted course extensive interstitial and subpleural edema are also present, but hyperemia is less evident. There may be diffuse alveolitis in some animals (Fig. 1.69A, B). Other lesions commonly observed are periaortic and peritracheal edematous infiltration, diffuse or patchy hyperemia of the glandular fundus of the stomach, hyperemia and petechial hemorrhages in the mucosa and serosa of the small and large intestines, subcapsular hemorrhages in the spleen, and congestion of the renal cortex. All the lymph nodes are enlarged and edematous, especially those in the thoracic and abdominal cavities. The pericardial sac may contain variable amounts of fluid, and numerous petechial hemorrhages occur in the pericardium. Cardiac lesions are usually not conspicuous, but epicardial and endocardial petechial hemorrhages may sometimes be evident.

In the *cardiac form*, the most characteristic lesions are the edematous infiltration of the subcutaneous, subfascial, subserous and intermuscular tissues. Only the head and neck may be involved, but in more severe cases the edema also involves lower parts of the neck, brisket, and shoulders. *Hydropericardium* is a constant finding, the pericardial sac often containing 2 L or more of fluid. The epicardium and endocardium show numerous, almost confluent ecchymotic hemorrhages. These are particularly conspicuous along the course of the coronary vessels, beneath the bicuspid and tricuspid valves, and in the papillary muscles adjacent to the insertions of the chordae tendineae. The lungs are usually normal or only slightly engorged and the thoracic cavity rarely contains an excess of fluid. The lesions in the gastrointestinal tract are generally similar to those found in the pulmonary form, except that submucosal edema tends to be far more pronounced. Ascites is unusual.

In the *mixed form*, the lesions are a combination of those observed in the pulmonary and cardiac forms. In fact, *most of the fatal cases of AHS can be considered to be of the mixed form*, with lesions typical of either the pulmonary or the cardiac form predominating.

The infective dose of AHS virus for **dogs** is large, and high infective titers are not known to be gained except by ingestion of infected horseflesh. The course of the disease in dogs is similar to the pulmonary form in horses with fever and severe respiratory distress. The mortality in dogs showing respiratory signs is very high. At autopsy, the lungs are edematous and may have some superimposed bronchopneumonia. There is much froth in the airways, copious hydrothorax in which the fluid gels on exposure, and saturation of mediastinal tissues. Histological changes are not helpful in diagnosis or understanding of pathogenesis.

The clinical signs of AHS are usually characteristic, although AHS might be confused with equine encephalosis, anthrax, equine infectious anemia, equine babesiosis, purpura hemorrhagica, equine viral arteritis, equine viral rhinopneumonitis, or trypanosomiasis.

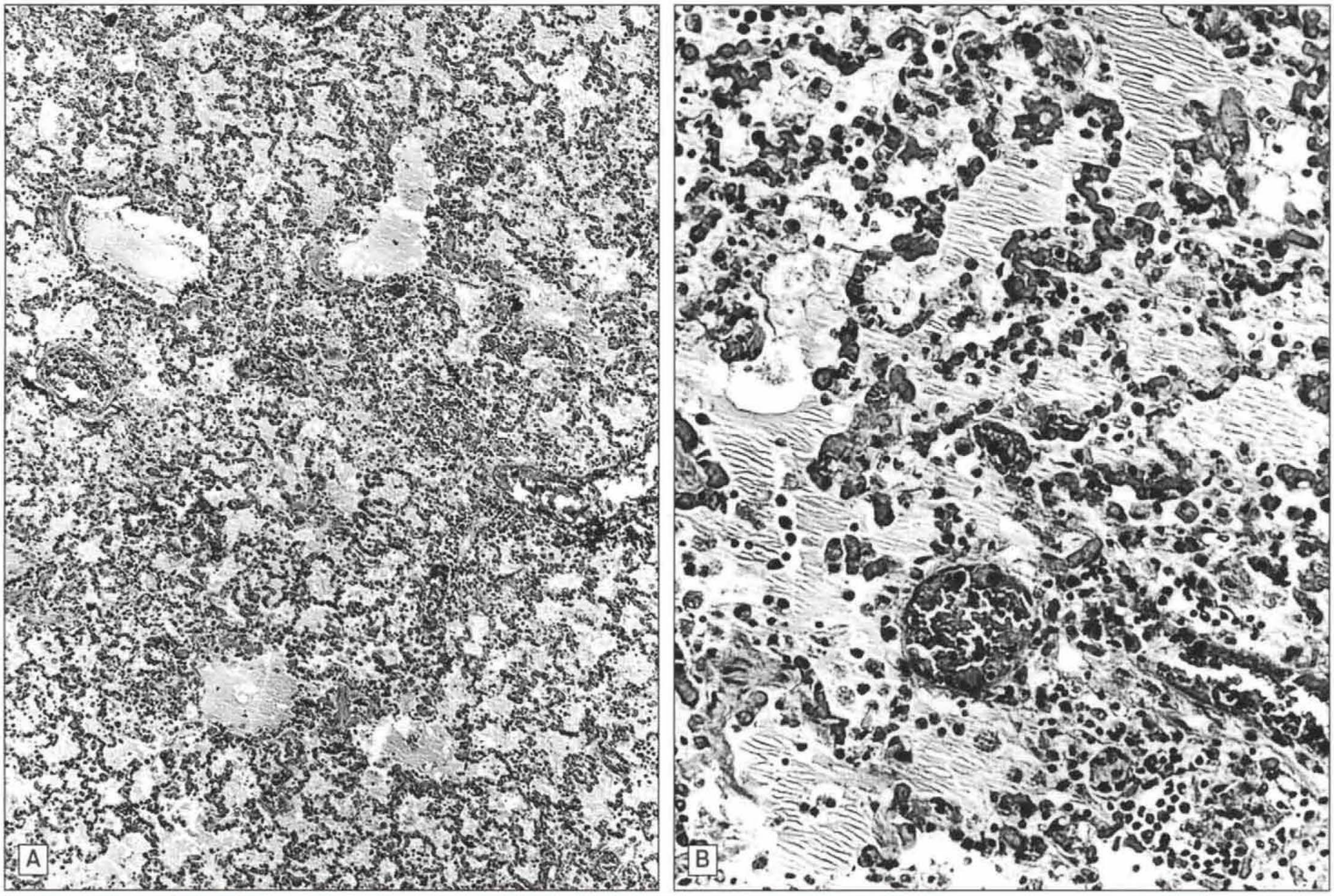

Figure 1.69 African horse sickness. A. Diffuse pulmonary edema with mononuclear cells in alveoli. **B.** Higher magnification of (**A**).

Diagnosis requires virus isolation, virus identification (ELISA, virus neutralization, PCR), or serology (ELISA, complement fixation, immunoblotting).

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African swine fever

African swine fever (ASF) is an acute-to-chronic, febrile, viral disease of swine, characterized by high fever, cutaneous hyperemia, abortions, edema, and hemorrhage in internal organs, particularly lymph nodes. ***African swine fever virus*** (ASFV) infects only members of the Suidae family and is a harmless companion of the warthog (*Phacochoerus aethiopicus*) and the bush pig (*Potamochoerus porcus*). However, the virus is a constant and major threat to domestic pigs. Originally limited to sub-Saharan Africa, the disease has appeared in southern and western Europe, the Caribbean, and Brazil, but remains endemic only in Sardinia outside Africa. *International spread of ASF is primarily through infected pork products in garbage fed to pigs.* Once a focus of infection is established, spread is most likely to occur by direct or indirect contact. In Africa, the transmission of the virus from wild Suidae to domestic pigs is primarily by the argasid tick *Ornithodoros moubata*, a true biological vector and a reservoir of the virus in nature. Additional arthropod vectors exist.

The causative agent, ASFV, is an enveloped DNA virus, family Asfarviridae, genus *Asfivirus*. It is a relatively resistant virus that may survive in the environment or in uncooked pork products for prolonged periods. There are several antigenically different strains of ASFV that also vary in their cultural characteristics and their ability to hemadsorb. Viral virulence is classified as high, moderate, and mild. In an area free of the disease, ASF is typically seen as a peracute or acute disease with high morbidity and mortality, but as virulence diminishes with time, subacute, chronic, and inapparent forms become increasingly evident. Survivors remain persistently infected.

The immunological response of swine to strains of ASFV is unusual and is incompletely understood. Susceptible animals infected with African field strains develop precipitating and complement-fixing, but rarely neutralizing or protective, antibodies. The animals remain viremic and usually die within 7–10 days. Pigs that recover are resistant to infection with the homologous virus. In the case of infection with highly virulent ASFV, even though 10 days may have elapsed since infection, pigs typically die before development of anti-ASF immunoglobulin, probably because of the demise of antigen-presenting cells in lymph nodes, spleen, and liver.

There is no cross-protection conferred by infections by *Classical swine fever virus* (CSFV) and ASFV. Failure of swine that are immune to CSFV to survive challenge with ASFV is the critical test for establishing the diagnosis of ASF in a suspected outbreak. The ability of most isolates of the virus to produce hemadsorption when grown in tissue culture contrasts with the CSFV, and this feature allows ready differentiation of most strains of the two viruses.

The ASFV replicates in and activates monocytes and macrophages of various lymphoid tissues and organs, resulting in release of cytokines such as interleukin-1 and tumor necrosis factor-α, and hence causes widespread cell death through apoptosis of T and B lymphocytes in lymphoid tissue and of endothelial cells in arterioles and capillaries, accounting for the lesions of the acute disease. Activation of pulmonary intravascular macrophages and release of cytokines incites pulmonary edema, neutrophil sequestration, and formation of microthrombi in septal capillaries. It also appears that the virus modulates signaling pathways in macrophages, hence interfering with the expression of host immunomodulatory genes; this evasion of host defences allows infections to be persistent.

The **peracute form** of ASF is *a severe acute viral hemorrhagic fever* characterized by a 1–3 day course of pyrexia, hyperpnea, and cutaneous hyperemia, with morbidity and mortality approaching 100%. The usual clinical course of ASF is acute. Following an incubation period of 4–10 days, there is a reduction in appetite, pyrexia, marked cutaneous reddening, dyspnea, and severe leukopenia. Pregnant sows may abort. The clinical signs in the less severe forms of the disease are variable. The signs may be a mild expression of those seen in the acute disease and may be confused easily with other diseases. There are recurring periods of pyrexia, dyspnea, growth retardation, and emaciation. Death may occur during one of the periods of pyrexia. *It is important to realize that the clinical signs of chronic ASF may be indistinguishable from those seen in classical swine fever (CSF).*

The ASFV tends to affect the same organs and tissues as does the CSFV. The anatomical differences tend to be quantitative and dependent on the more fulminating nature of ASF; *only splenomegaly and hematoma-like visceral lymph nodes are particularly characteristic of ASF* (Fig. 1.70). The vascular lesions that can develop rapidly, namely hemorrhage and edema, are apt to be more severe in ASF than in CSF, whereas the lesions that develop more slowly, such as infarction, tend to be absent.

The pig dead from **acute ASF** shows few or no signs of recent wasting. Gastrosplenic and renal lymph nodes are usually intensely hemorrhagic, and ecchymotic hemorrhages may be present on the serous membranes. The spleen is usually enlarged and may be markedly enlarged and friable; infarction cannot usually be recognized grossly. *Pulmonary edema*, not common in CSF, is present frequently in pigs with ASF. The pulmonary septa are thickened by a yellow gelatinous infiltrate. Pulmonary hemorrhage is also common. The gallbladder is often edematous, and the vessels of the wall are engorged and conspicuous. Both petechiae and ecchymoses are present on the serosal and mucosal surfaces. In some outbreaks of the disease, there are extensive pancreatic hemorrhages and necrosis. The renal changes are similar to those of CSF and consist of subcapsular petechiae (Fig. 1.71). The stomach often contains ingesta, but the mucosa is apt to be inflamed and eroded. Changes in the

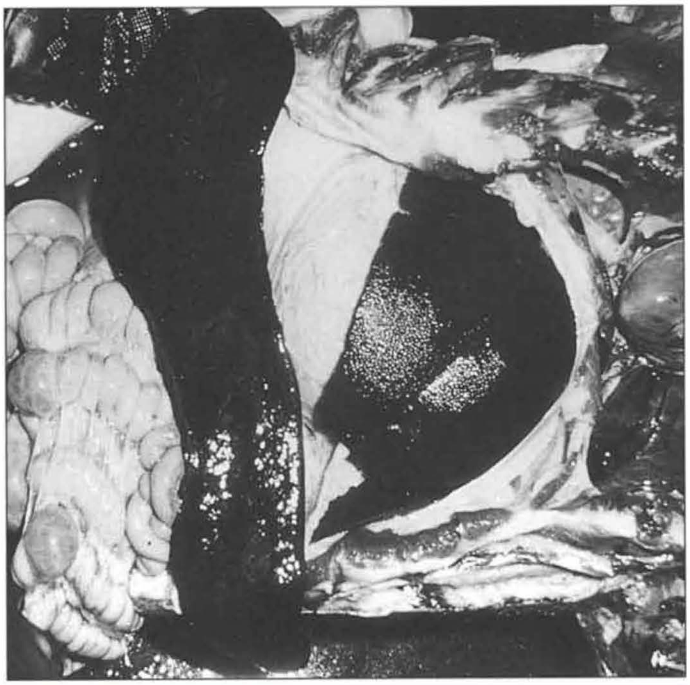

Figure 1.70 Splenomegaly in **African swine fever**. (Courtesy of C Brown.)

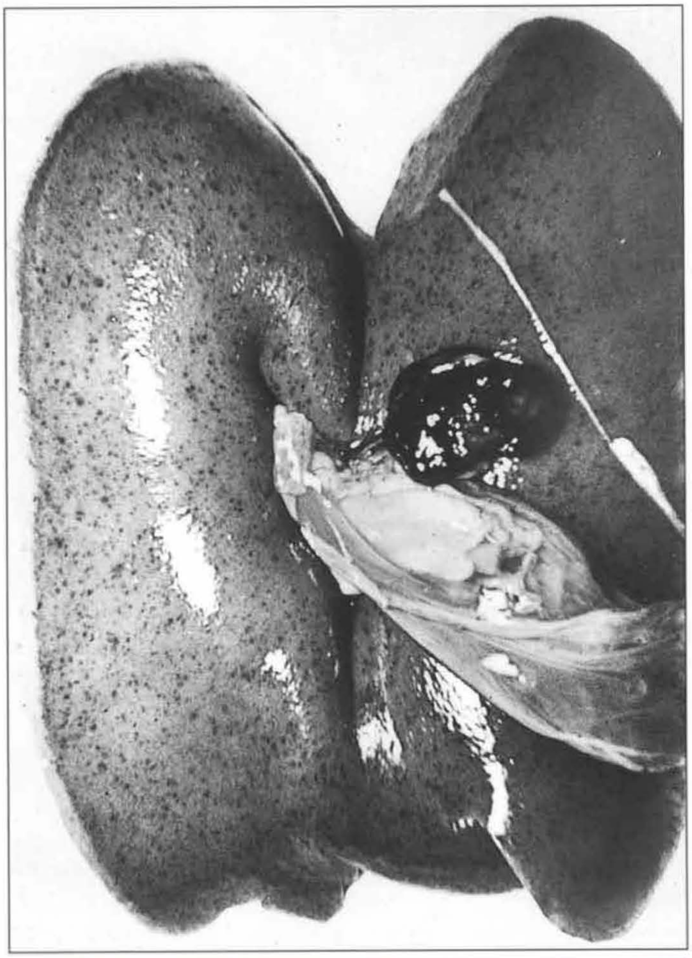

Figure 1.71 Renal cortical petechiae and hemorrhagic lymph node in **African swine fever**.

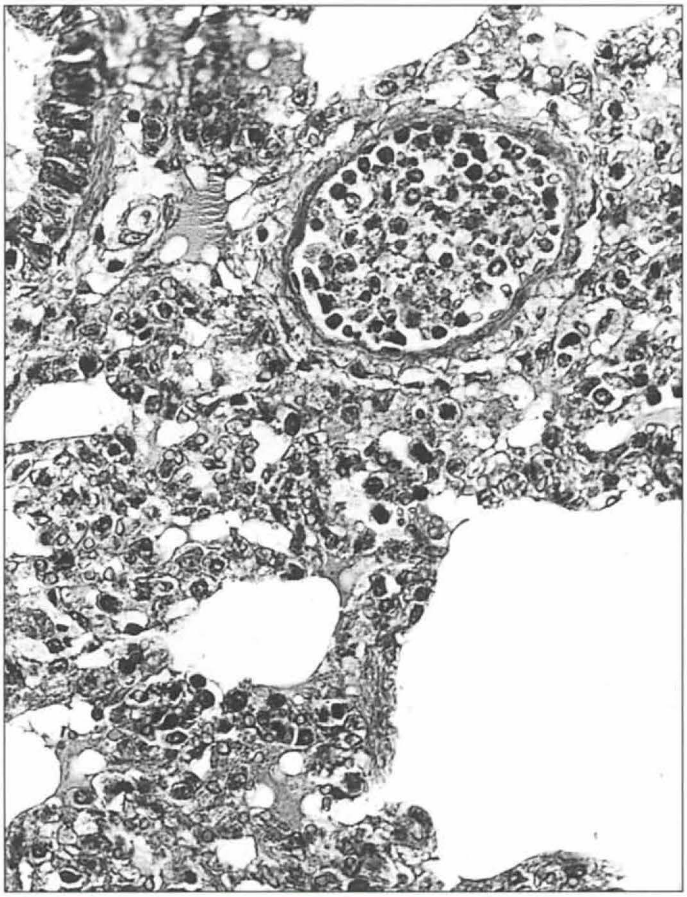

Figure 1.72 Cellular thrombus in pulmonary vessel, and alveolar thickening in **African swine fever.**

small intestine may be absent or consist of segmental areas of congestion and mucosal petechiation. More severe intestinal changes may be found in the large intestine; these may include large areas of hemorrhage, severe congestion, and ulceration. Lesions suggesting "button ulcer" are very rare in this disease, presumably because few animals survive long enough for their development.

The postmortem appearance of **subacute and chronic cases of ASF** varies considerably. In the subacute cases that die after 3–4 weeks of illness, there may be lymph node and renal hemorrhage, an enlarged, but not congested spleen, lobular consolidation of cranial lung lobes, and intestinal mucosal hemorrhage. Prominent features of the chronic form include fibrinous pericarditis and pleuritis; splenic and lymph node enlargement; lobular consolidation of the lungs, which may progress to necrosis and mineralization of an entire lobe; skin lesions ranging from raised hyperemic plaques to necrotic areas; swollen joints; and arthritis. The meningoencephalomyelitis and periportal hepatitis observed in the acute disease persist in the chronic disease. Lesions in *aborted fetuses* are inconsistent but include petechiation of placentas, skin, and myocardium, mottled lungs and liver, and anasarca.

Histologically, infection by a highly virulent strain of ASFV causes extensive necrosis of cells of the mononuclear phagocyte system, whereas infection with a moderately virulent strain causes little necrosis. *The histologic findings in acute ASF are very similar to those of CSF, but there are important differences.* A major difference is that ASFV does not infect epithelium. Necrosis with karyorrhexis in lymphoid tissue everywhere is often very obvious in ASF; frank necrosis is quite rare in CSF, although mature lymphocytes are apt to be absent in lymphoid tissue. Renal tubular degeneration with amorphous casts in the medulla is frequent in ASF, but rare in CSF. In ASF, necrosis of periportal hepatocytes and infiltrating lymphocytes is common, whereas microscopic hepatic lesions are usually absent in CSF. The vascular cuffs in the brain in ASF contain much more necrotic debris than do the lesions in CSF. *The degeneration of vascular endothelium and the fibrinoid arterial changes are identical.* Vascular endothelial damage in the lung may cause thrombosis (Fig. 1.72) of vessels and thickening of alveolar walls. Pigs dead of acute ASF may have glomerular capillary thrombosis; surviving pigs may develop focal segmental glomerulonephritis.

Since there is no treatment or effective vaccine against ASF, rapid diagnosis is critical to disease control. A **diagnosis** of ASF is confirmed by detection of ASFV antigen in tissues by direct immunofluorescence, detection of ASF antibody by indirect immunofluorescence or ELISA, detection of virus genome by PCR, and virus isolation. *Differential diagnoses* include classical swine fever (from which ASF cannot be differentiated by clinical or necropsy examination), erysipelas, salmonellosis, pasteurellosis, and other septicemias.

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Classical swine fever (hog cholera)

Classical swine fever (CSF) is a highly contagious viral disease of swine; it may occur as acute, subacute, chronic, or inapparent syndromes. Acute CSF is a disease of high morbidity and mortality caused by a virulent strain of virus; low virulence virus may cause inapparent disease. Classical swine fever is said to have developed de novo in Ohio, USA, in the early 1800s. It subsequently spread to almost all countries and is also known as *swine fever, Schweinepest, peste du porc, and peste svina.* The ***Classical swine fever virus*** (CSFV) produces disease only in swine. The disease has been eradicated from Australia, Canada, the UK, and the USA, but it remains a problem in Central and South America and several European countries.

Classical swine fever is caused by a *Pestivirus*, a member of the family Flaviviridae. Other members of the genus include *Bovine viral diarrhea virus* (BVDV) and *Border disease virus*. CSFV and BVDV are closely related antigenically, and although pestiviruses other than CSFV are thought to be harmless to pigs, *natural BVDV infection can cause clinical signs and necropsy lesions indistinguishable from those of chronic CSF*. Under appropriate circumstances, all three pestiviruses may produce transplacental infection, and hence persistent infections and congenital anomalies. The CSFV is an enveloped, single-stranded RNA virus that replicates intracytoplasmically. Most strains do not produce a cytopathic effect in porcine cell cultures. The enveloped nature of the virus makes it sensitive to lipid solvents and to desiccation. However, the virus may persist for prolonged periods in uncooked pork products.

Antigenic variation exists among strains of CSFV, and field strains vary widely in virulence. The interaction of a particular strain of virus and the host provides a range of disease syndromes. Strains of CSFV are usually classified as being of *high, moderate, or low virulence*, or as being *avirulent*. Highly virulent strains produce the features of the classical acute disease in pigs of all ages; the morbidity may reach 100%, with a mortality rate of up to 90%. Strains of moderate virulence induce subacute or chronic disease, and pigs may subsequently die or recover. In pigs infected postnatally, strains of low virulence produce few or no signs of disease and induce immunity, but may cause fetal abnormalities. Artificially attenuated strains used as vaccines are avirulent, even for fetuses. Virulence of CSFV may be unstable; virulence can increase by one passage through pigs.

Transmission of the disease is usually by direct contact of infected pigs or wild boar with susceptible pigs; CSF is endemic in wild boar in parts of Europe. The virus is present in urine, feces, and lacrimal and oronasal secretions of infected pigs, as well as semen of infected boars. With the less virulent strains, the virus may be excreted in the urine for periods up to 3 months. The major mechanism of spread of virus of low virulence occurs from continuous virus shedding by chronic or persistently infected asymptomatic pigs. Minor modes of transmission include fomites and arthropods.

Classical swine fever is characteristically an **acute disease** *of high morbidity and mortality*, most animals surviving only to 14 days after showing the first signs of illness, namely anorexia, depression, fever, and leukopenia. Persistent infection (that is, survival of greater than 30 days) is caused by CSFV strains of moderate or low virulence and may arbitrarily be divided into chronic and late-onset types. In **chronic CSF**, typical acute disease is followed by clinical improvement, the result of specific antibody formation, but later by immune exhaustion and chronic disease in which the pig is viremic and more susceptible to secondary bacterial infection. **Late-onset CSF** occurs in pigs that are persistently viremic and immunotolerant to CSFV as a result of fetal infection by CSFV of low virulence. The latest date for the development of a persistent infection appears to be about day 70 of gestation; by day 85 viral antigen may be detected in a few fetuses, but virus cannot be isolated. Signs that develop in viremic, but previously asymptomatic, pigs include anorexia, depression, leukopenia, conjunctivitis, dermatitis, diarrhea, runting, and posterior paresis. *The late-onset form of CSF is the porcine equivalent of mucosal disease in cattle*, in which BVDV infection of fetal calves results in persistently viremic, immunotolerant animals.

The **pathogenesis** of CSF is not fully understood, but involves the effects of the virus on the immune system (lymphoreticular cells and macrophages), the vascular endothelium, and epithelial cells. The effect of the monocytotropic CSFV on the immune system varies with the stage of differentiation; mature elements degenerate, whereas undifferentiated cells respond by proliferation. CSFV replicates in macrophages in lymph nodes and lymphoid tissues and can cause depletion of lymphocytes indirectly through expression of apoptotic cytokines, such as tumor necrosis factor-α. Similarly, intestinal epithelial necrosis appears be the product of release of chemical mediators from activated macrophages, rather than direct viral infection. Endothelial changes are primarily degenerative but some proliferative changes occur. *Damage to endothelial and other cells leads to thrombocytopenia, consumption coagulopathy, and in turn disseminated intravascular coagulation and hemorrhage.*

The classical or **acute** form of the disease, which affects pigs of all ages, commences by entry of the virus through the mucous membranes with initial replication in the tonsillar epithelium. This is followed by spread to the cervical lymph nodes with viremia within 16 hours of infection. The virus has a propensity to replicate in cells of the immune system, particularly in lymph nodes, bone marrow, and other lymphoid aggregates such as the spleen and Peyer's patches. After 3–4 days, the virus invades endothelial cells and epithelial cells including those of the pharyngeal mucosa, gastrointestinal tract, gallbladder, pancreas, salivary gland, uterus, adrenal, and thyroid. The clinical signs are not specific and do not provide good correlation with pathologic changes, although in the acute disease they may be sufficient for presumptive diagnosis where the disease is endemic.

Superficially, the eyelids are frequently adherent and sticky, and the carcass is dehydrated and soiled by terminal diarrhea. The irregular erythema that can be seen in the animal when alive is less obvious, but hemorrhages, particularly in unpigmented areas of skin, may be seen (Fig. 1.73). If present, they are most numerous on the abdomen and the inner aspects of the thighs.

Diagnostic lesions *may be sparse in CSF*, especially if peracute, and it may be impossible to establish the diagnosis on the basis of the gross lesions in a single animal. *The lesions most commonly present are hemorrhages in the periphery of the lymph nodes and renal petechiae.* The renal hemorrhages may be very few, and in all cases the kidney capsule should be removed and the surface examined in good light. Hemorrhages in the lymph nodes are more obvious; characteristically, only the periphery of the node is involved. In acute cases, this produces a distinctive bright red halo; if the case is more chronic, the halo may be the dirty brown of partially degraded hemoglobin. Less frequently, the hemorrhage in the node is diffuse. The nodal changes are generalized, but nodes most apt to show severe changes are the mandibular, colonic, hepatic, and iliac; changes in the renal lymph nodes are not useful in this context.

Splenic infarction is almost pathognomonic of acute CSF. Unfortunately, the tendency for infarcts to develop in the disease varies with the strain of the virus, with reports of incidence of 1–87%. When present, infarcts occur as single or multiple, dark red, pyramidal blebs 0.5–2 cm in diameter, usually along free edges but occasionally on the flat surface (Fig. 1.74). The spleen in CSF is not enlarged, as it is in septicemias, and is red-brown so that the infarcts contrast quite sharply with the meaty parenchyma. In addition to the hemorrhages of the kidney, petechiae are common in the urinary bladder

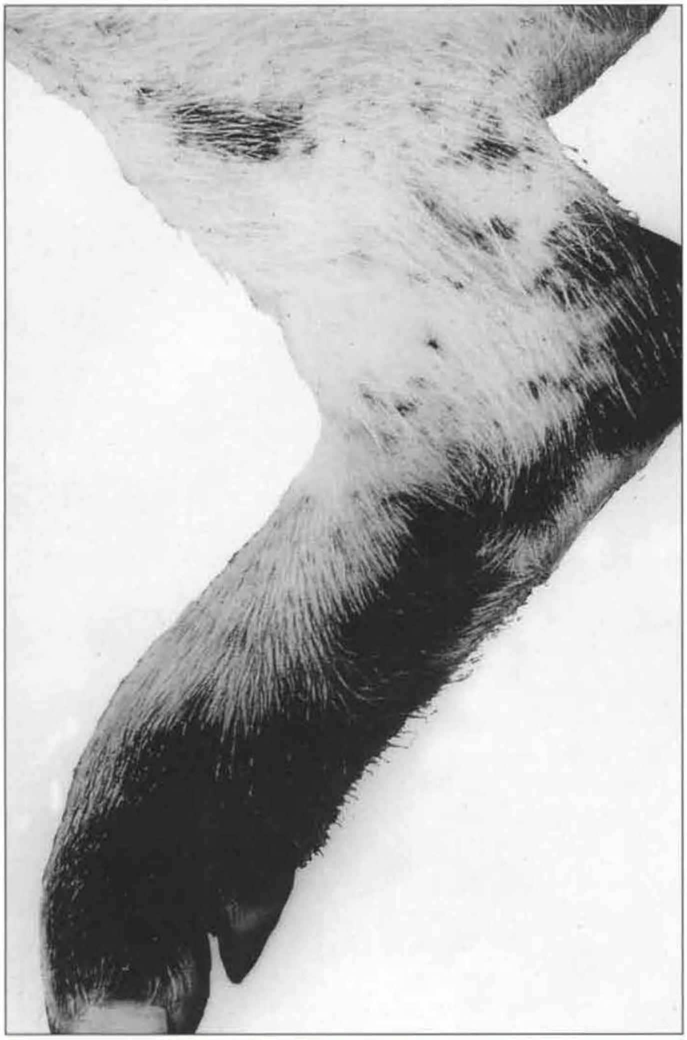

Figure 1.73 Diffuse and patchy subcutaneous hemorrhages in **acute classical swine fever**.

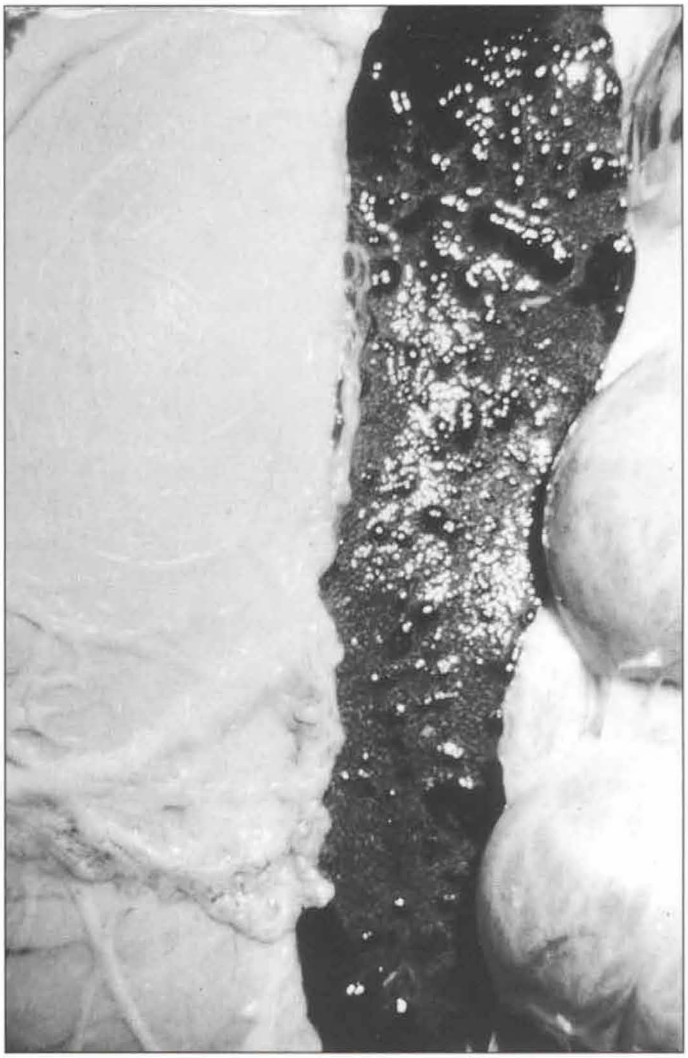

Figure 1.74 Multiple splenic infarcts in **classical swine fever**. (Courtesy of C Brown.)

(Fig. 1.75), the larynx (Fig. 1.76), the gastric mucosa, the lung, and the epicardium, in about that decreasing order of frequency. There is usually a small amount of straw-colored fluid in the pericardial sac. There may be hemorrhagic lobular pneumonia (Fig. 1.77).

Irregular, but sharply outlined areas of necrosis develop in the tonsils and posterior fauces (Fig. 1.78). The stomach of an animal dead of CSF is empty except for a small amount of watery mucus and ingesta. The fundic mucosa is congested, and mild or severe erosions may be present. Specific lesions do not occur in the small intestine, but the mesentery is usually congested. It is in the colon and cecum that the virus combines with the intestinal flora to produce craterous mucosal defects, "*button ulcers*," which are characteristic of the subacute or chronic stage of the disease. These lesions begin as sharply outlined circular areas of hemorrhage and necrosis (Fig. 1.79). The central area is yellow and dry. As the lesion ages, the rim, which is composed of necrotic epithelium, bacteria, and detritus, is raised above the surrounding mucous membrane and concentric rings form as if growth were cyclic. The center of lesions sinks giving a slight diskoid shape. If this necrotic tissue and debris is removed, a deep ulcer is revealed. Growth arrest lines may be seen in the ribs of chronically sick pigs. Gross lesions are not usually present in the brain, but the brain should be removed because it is there that the histological changes can be best appreciated.

Within the *brain*, all areas may be affected but the lesions are usually most conspicuous in the medulla, pons, mid-brain, and thalamus. The response is largely confined to the vessels and their supporting mesoderm. Many of the venules have eccentric cuffs, which are formed in part by transmural migration of monocytes. Mitotic figures and nuclear chromatin of necrotic cells are frequently present together in the cuff, as proliferation and necrosis are coexistent. Swelling and degeneration of the endothelial cells occur in the walls of the smaller vessels (Fig. 1.80). All changes from slight thickening to necrosis of the wall may be seen. The lumen is often compromised by these reactions. The response of the neural parenchyma appears to be entirely secondary to the vascular lesions; often it is nonexistent or confined to minimal neuronal degeneration, or it may consist of glial nodules formed about obstructed or destroyed capillaries. If severe cerebral edema develops, there may be widespread damage to the oligodendroglia. The vessels of the eyes, choroid plexuses, and the leptomeninges are similarly involved.

Vascular lesions usually are most severe in lymphoid tissue but may occur anywhere. The lesions vary from slight thickening of the capillary wall to

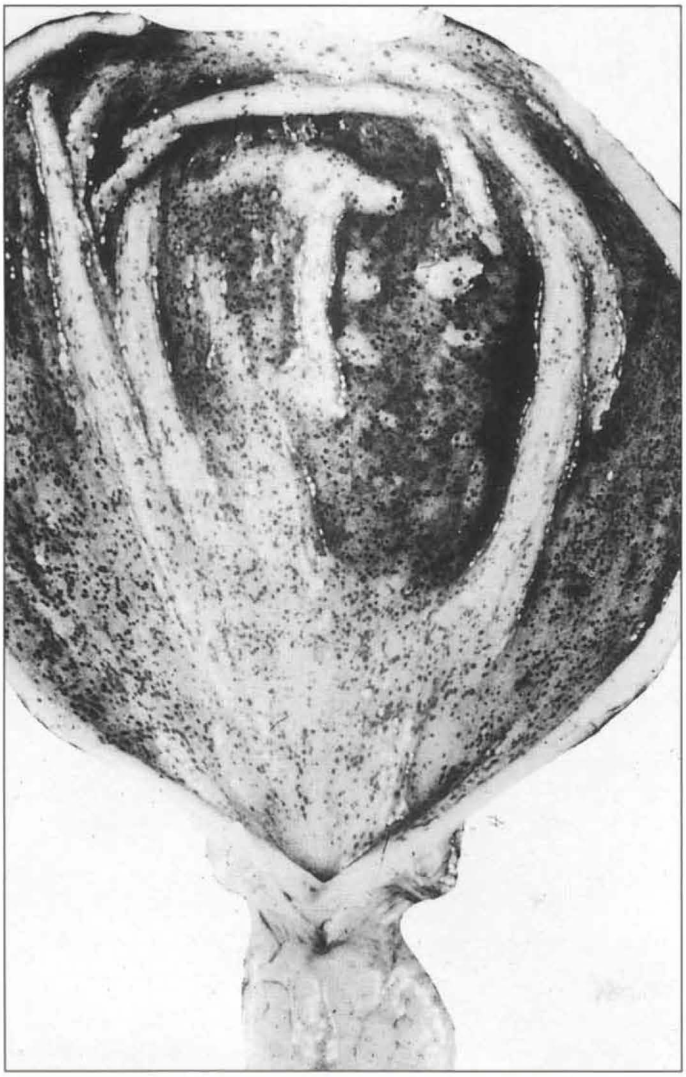

Figure 1.75 Hemorrhages in mucosa of urinary bladder in **acute classical swine fever**.

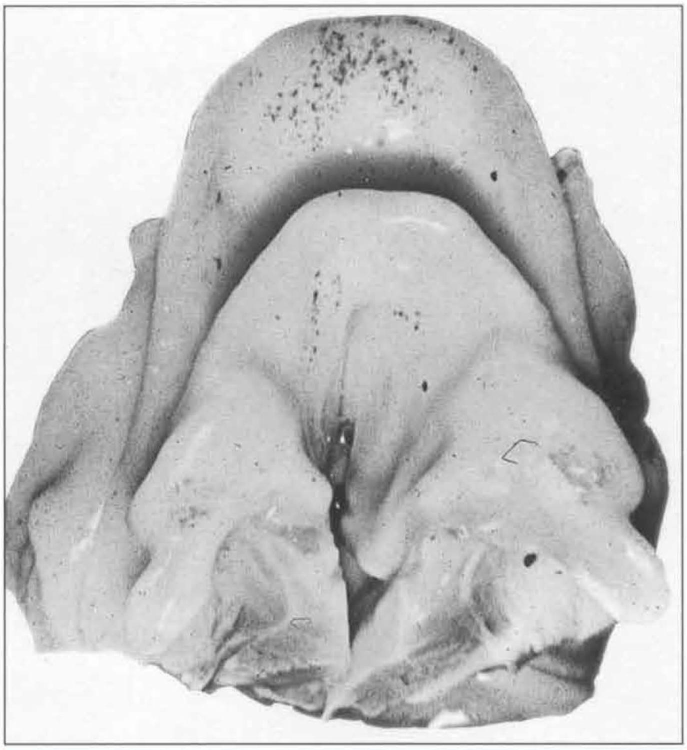

Figure 1.76 Laryngeal hemorrhages in **acute classical swine fever**.

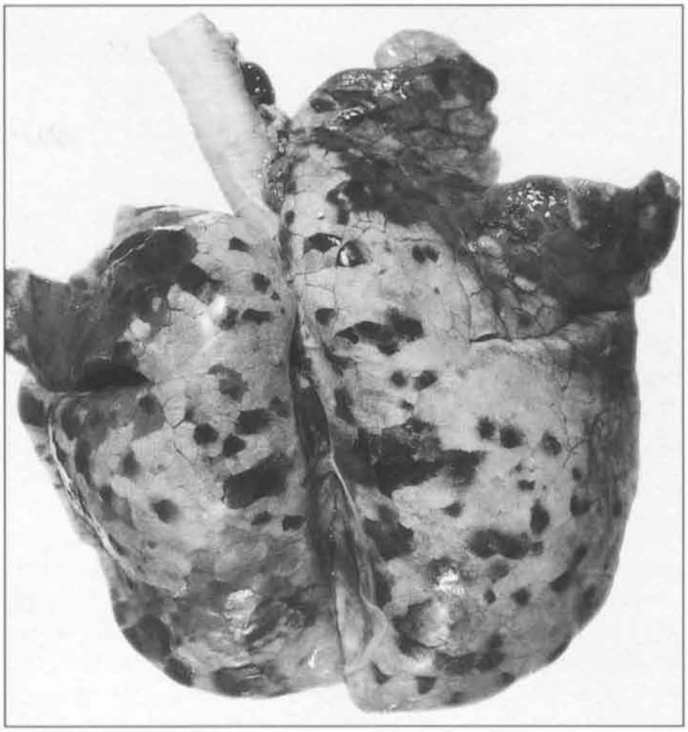

Figure 1.77 Hemorrhagic pneumonia with lobular distribution in **classical swine fever**. The cranial bronchopneumonia is probably not related.

fibrinoid necrosis of arterioles. As these changes develop, there is a tendency for extravasations to occur and for microthrombi to form.

Microscopic areas of *infarction* are common in the lymph nodes and skin, but only in the spleen, tonsil, gallbladder, and large intestine are these areas usually large enough to be grossly visible. In some cases, swelling and paleness of the capillary wall is obvious. The nuclei of these vessels are enlarged and the nuclear chromatin is dispersed as fine dust. Less often, the endothelial proliferation is so marked as to be the most conspicuous change. In general, in acute cases the degenerative changes are most prominent, whereas in chronic ones proliferative changes are more obvious.

In the periphery of *lymph nodes* (the equivalent of the medulla in other species), lesions vary from slight edema and proliferation of the reticuloendothelial elements to extensive hemorrhage. It is the intermediate stages that are the most common and in which the nature of the lesion is best visualized; in these, there is necrosis of the small vessels and secondary hemorrhages and parenchymal necrosis.

In the *spleen*, the most pronounced lesions are in the follicular arteries, particularly those at the apex of the pyramidal infarcts. The swelling and hyalinization of these vessels may be so severe as to occlude the lumen. Survival of tissue within the infarcted area will depend on how long the infarction preceded death. Even if the spleen is free of gross and microscopic infarction, vascular changes of the type seen elsewhere can usually be found. They are often

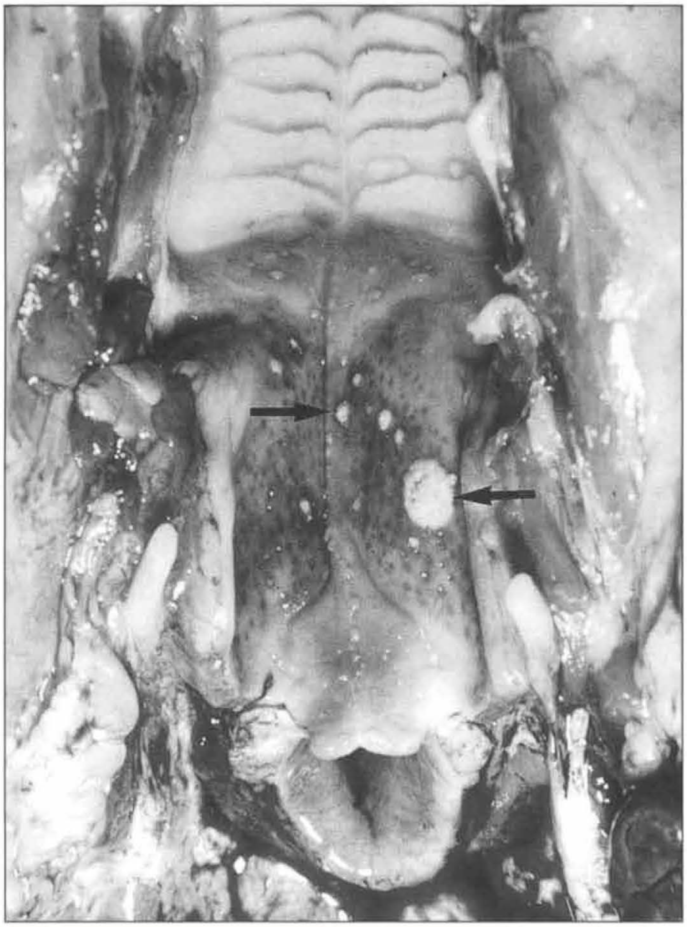

Figure 1.78 Necrosis of tonsils (arrows) in **classical swine fever**. (Courtesy of C. Brown.)

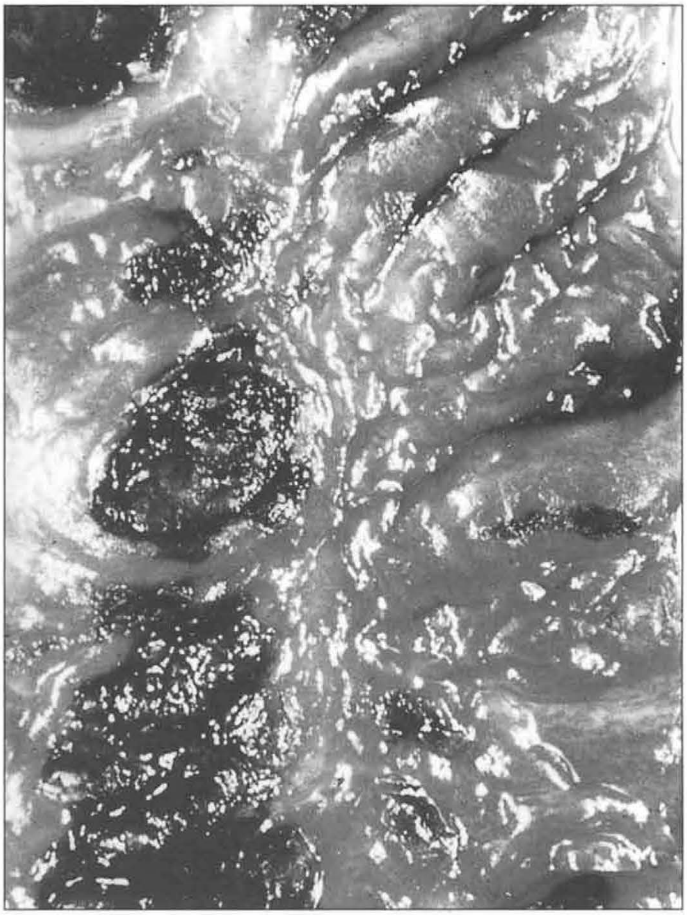

Figure 1.79 Hemorrhagic ulcers in the colon in **acute classical swine fever**.

associated with perivascular hemorrhage. In addition, there is reticuloendothelial hyperplasia and absence of mature lymphocytes.

The *kidney* tubules often show nonspecific degenerative changes. The subcapsular hemorrhages seen grossly are the result of increased vascular permeability and erythrodiapedesis. Immune-complex-mediated mesangioproliferative glomerulonephritis occurs, with deposition of IgM, IgG, and C1q in mesangial, subepithelial, and subendothelial sites, fusion of foot processes, infiltration of the mesangium by macrophages and later neutrophils, and the presence of viral particles in glomerular endothelial cells and podocytes.

In utero infections can result in a variety of effects, including abortion, mummified fetuses, and stillborn piglets. Fetal abnormalities such as ascites, petechiation of skin and other organs, hepatic nodularity, pulmonary hypoplasia, microcephalus, hydrocephalus, cerebellar hypoplasia, and hypomyelinogenesis may also be observed. Persistently infected pigs that survive may be runts and die 2–11 months after birth. These piglets have severe thymic atrophy and pale swollen lymph nodes; some may have focal colonic mucosal necrosis.

Serological **diagnosis** of CSF is difficult. Antibodies to CSFV and BVDV cross-react in many assays; they may be differentiated by specific virus neutralization tests. The prescribed tests in the OIE Manual are the neutralization peroxidase-linked assay, fluorescent antibody virus neutralization, and ELISA. CSFV antigen can be detected in live pigs by RT-PCR, and in formalin-fixed tissue by semi-nested RT-PCR or by in situ hybridization. *Differential diagnoses for CSF* include African swine fever (clinicopathologically indistinguishable), BVDV infection, porcine reproductive and respiratory syndrome (PRRS), postweaning multi-systemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), salmonellosis, erysipelas, acute pasteurellosis, other viral encephalomyelitides, streptococcosis, leptospirosis, and courmarin poisoning.

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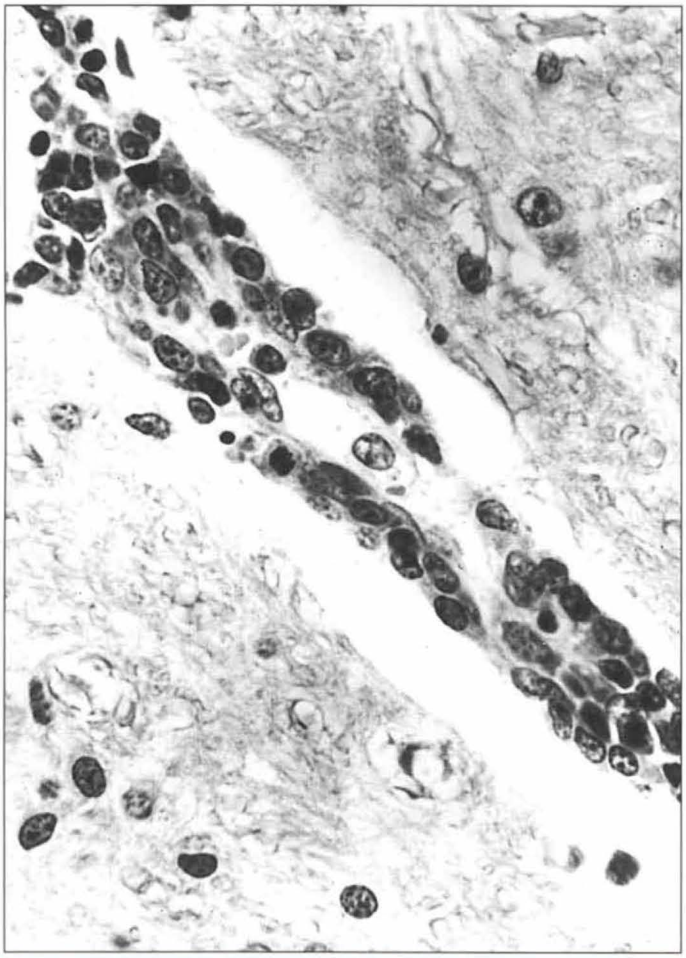

Figure 1.80 Cerebral venule cuffed primarily by multiplying mononuclear cells in **classical swine fever.**

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Bovine ephemeral fever

Bovine ephemeral fever (BEF), a non-contagious vector-borne viral disease of cattle and water buffalo, is characterized by sudden onset of fever, stiffness, and lameness, usually with high morbidity and low mortality, and with rapid spontaneous recovery within 3–4 days of the onset of clinical signs. The cause, ***Bovine ephemeral fever virus*** (BEFV), is in family Rhabdoviridae, genus *Ephemerovirus*. The disease is endemic in tropical regions of Africa and Asia and occurs as epidemics in subtropical and temperate regions of Africa, Asia, and Australia; it has not been reported from the Western Hemisphere, Europe, New Zealand, or New Guinea. In temperate climates, BEF is a disease of summer and autumn, and ceases abruptly at the first frost; the reservoir for overwintering or between epizootics is probably in wild ruminants. Infection usually confers lifelong immunity. The likely *vectors* are various species of *Culicoides* and mosquitoes.

Clinical disease ranges from almost imperceptible clinical signs to death, with considerable individual variation in an outbreak. Mild disease may be exacerbated by environmental stress, e.g., exposure causing dehydration. Disease is milder in young than in mature animals; in lean than in fat animals; in light steers and cows than in bulls; in dry cows than those in heavy lactation. The incubation period is usually 2–4 days. The fever may be biphasic, triphasic, or multiphasic; clinical signs are milder in early than in later phases of fever. Mildly affected animals may have fever, be lame or stiff for a day or two, and then recover completely. Neutrophilia with a left shift and concurrent lymphopenia, hyperfibrinogenemia, and hypocalcemia occur early in the course of disease. More severely affected animals are anorectic, have oculonasal discharge, may have muscle tremors, stiffness, lameness, and patchy edema of the face or jaw. Rumen motility ceases and the animal may be constipated. Animals in lateral recumbency are usually still able to rise. Lactation falls by an average of 50%, and does not usually recover to normal during that lactation. Cows in late pregnancy may abort. In very severe cases, there may be temporary or permanent paralysis of all limbs, inability to swallow, and drooling; in 1–4 days, loss of reflexes, coma, and death follow.

Whether the disease is mild or severe, recovery commences quite suddenly and is complete in 95–97% of uncomplicated cases, giving rise to the name "ephemeral fever." Although mortality is usually less than 1%, the most productive mature animals are often lost, plus there are losses of milk production, abortion losses, and temporary infertility in bulls. Other sequelae include fatal pulmonary emphysema, pneumonia, mastitis, and locomotor disturbances. The total economic loss in an outbreak can be severe.

The essential **gross feature** *of BEF is serofibrinous polyserositis*, which variously affects the synovial, pericardial, thoracic, and peritoneal cavities, and is the *result of increased vascular permeability*. The serosal membranes themselves are often edematous and may be hemorrhagic; the edema fluid in cavities often contains fibrin clots. Severely affected joints may be surrounded by brown or yellow gelatinous periarticular fluid that also extends along tendon sheaths and fascial planes. There may be patchy pulmonary edema, visceral and parietal pleuritis, epicarditis at the base of the heart, edematous lymph nodes in the febrile stages, and necrosis in some skeletal muscles; myonecrosis is likely the result of recumbency. Histologically, affected tissues are edematous and infiltrated by neutrophils, and may also be hyperemic and hemorrhagic. *Vascular changes* include endothelial swelling and hyperplasia, hyperplasia of pericytes, fibrinoid necrosis of arterioles, perivascular fibroplasia, and thrombosis of occasional vessels in muscles. Wallerian degeneration has been reported in the upper cervical spinal cord in cases with chronic paralysis, but may be secondary to trauma.

The **pathogenesis** of BEF is poorly understood, but is likely mediated through cytokines; the site of viral replication remains unknown. Neutrophils appear to play a central, but undefined, role; cattle in which neutrophils are experimentally depleted develop viremia but not clinical disease. Interferon α, interleukin 1, and tissue necrosis factor circulate in high titer during the acute phase of BEF.

The clinical signs observed in an epizootic are usually diagnostic. The **diagnosis** can be confirmed by virus isolation. Serological diagnosis may be complicated by cross-reaction with antibody to *Kimberley virus*, an arbovirus that induces production of low levels of serum neutralizing antibody to BEF virus but does not protect against BEF.

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Rickettsial vasculitides

Heartwater (cowdriosis)

Heartwater is a tick-borne rickettsiosis of ruminants that is endemic in sub-Saharan Africa and that has been identified in several Caribbean islands. The common name, heartwater, is derived from the *pericardial effusion* that is typically present in small ruminants but sometimes absent in cattle. Heartwater is a major vector-borne disease of ruminants in Africa, third in importance only to East Coast fever and trypanosomiasis, but has a wider distribution than either of these. Mortalities due to heartwater are estimated to be 3 times greater than those due to babesiosis and anaplasmosis. The causative agent is ***Ehrlichia* (*Cowdria*) *ruminantium***, order Rickettsiales. Isolates of *E. ruminantium* cross-react strongly with *Ehrlichia equi,* and to a lesser extent with *E. canis,* in indirect fluorescent antibody tests. The *vectors* are 3-host ticks of the *Amblyomma* genus, principally *A. variegatum*, *A. hebraeum*, and *A. pomposum*. *Amblyomma maculatum* and *A. cajennense* are suitable vectors present on the American mainland. Transstadial transmission occurs in vector ticks; transovarian transmission is infrequent. When a tick feeds on an infected host, organisms apparently first infect and develop within gut cells. Subsequent stages of the organism continue their development in hemolymph and salivary gland, and are transferred to the vertebrate host during tick feeding.

The various strains of *Ehrlichia ruminantium* are antigenically similar, but differ considerably in their virulence. The various domestic hosts also differ in their susceptibility. Imported breeds of cattle, sheep, and goats are as a rule much more susceptible than indigenous breeds. There is also an important age difference in susceptibility. Calves and lambs less than three weeks of age are highly resistant to heartwater. This is a true age resistance, and it is independent of maternal immunity. Ruminants are most susceptible at about the stage of early maturity, and in the absence of treatment *mortality is expected to be about 60% in cattle and close to 100% in European breeds of sheep*, although this will depend on the virulence of the infecting strain.

There also appear to be differences in susceptibility among the African antelopes. The blesbok and black wildebeest are known to be susceptible to infection without showing signs of illness. Wildlife may act as reservoirs of infection, though this is not necessary as the tick itself is a reservoir; infection survives in adult ticks for over 15 months. Domestic ruminants that have recovered from the disease seldom remain as carriers of the circulating organism for more than 3 weeks after recovery, but it is not clear whether the agent remains in a masked form in tissues. *Immunity of variable duration follows recovery, often as premunity*; sterile immunity may persist for a year or more after disappearance of the organism. Immunity in natural infections is expected to be reinforced by reinfections.

The early development of the parasite is not completely understood, but probably takes place in reticulum cells and macrophages of lymph nodes and spleen in which initial bodies are demonstrable at about the fourth day of infection. Organisms are then released into the blood and parasitize vascular endothelial cells. The organism is demonstrable in capillary endothelium of brain 1–3 days after it is demonstrable in lymph nodes. *Ehrlichia ruminantium* parasitizes endothelial cells but is also present in the blood; the infection is readily transmissible by inoculation of blood collected during the febrile stage or shortly thereafter. *The colonies (groups, clusters, morulae) of microorganisms in endothelial cells lie characteristically in membrane-bound vacuoles in the cytoplasm at the poles of the endothelial cell nuclei.* Colonies and organisms are pleomorphic; colonies are formed by binary fission of organisms. Organisms range in size from 0.2 to >2.5 μm and are termed small, medium, or large; each is surrounded by a double membrane, and based on their internal morphology they may be termed *elementary (electron-dense), intermediate, or reticulate bodies*; usually only organisms of the same form are found within a vacuole. They can be found in sections and are present in all organs and in vessels of all calibers but are best sought in the capillaries of the cerebral cortex in squash-smear preparations or in imprints from the endothelium of a large vessel such as the jugular vein. Giemsa is the preferred method of staining.

The natural incubation period of heartwater is 2–3 weeks but varies considerably. In the peracute form of the disease, death occurs rapidly in convulsions without warning. The usual **clinical syndrome** in susceptible animals is acute, with a course of up to 6 days. There is high fever and its attendant signs, and dyspnea. As the course advances, nervous signs develop. These are especially prominent in cattle and consist of chewing movements, protrusion of the tongue, twitching of eyelids and blinking, unsteadiness of gait, circling, and a terminal convulsive phase in which there is prominent cutaneous hyperesthesia. Diarrhea is common in cattle but less common in sheep. Mild progressive anemia may develop, as may leukopenia (neutropenia, eosinopenia, and lymphocytosis) and hypoalbuminemia. In the subacute syndrome, the course is prolonged and leads either to slow recovery or to collapse and death. Animals with high natural resistance or partial immunity may show transient fever only.

The **pathogenesis** of the increased capillary permeability seen in heartwater is unclear; the severity of edema is often thought to be out of proportion to the numbers of infecting organisms. Production of a toxin has been suggested, but not proven; *Ehrlichia* stains negatively with Gram's stain, has a cell wall similar to that of gram-negative bacteria, and has been suggested to produce endotoxin. Complement and

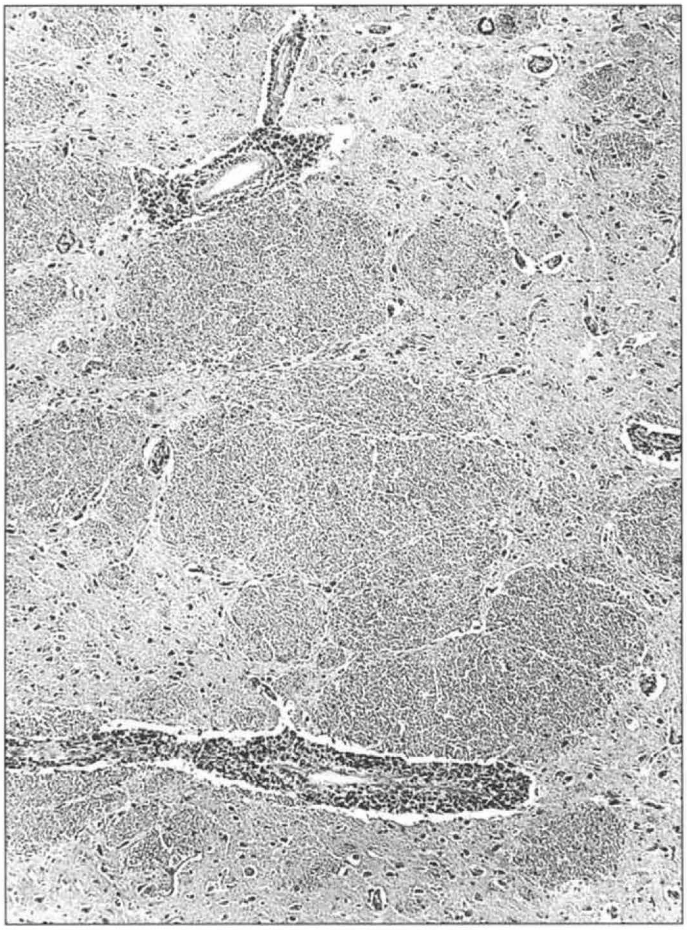

Figure 1.81 Perivascular cuffs around vessels in brain in **heartwater**.

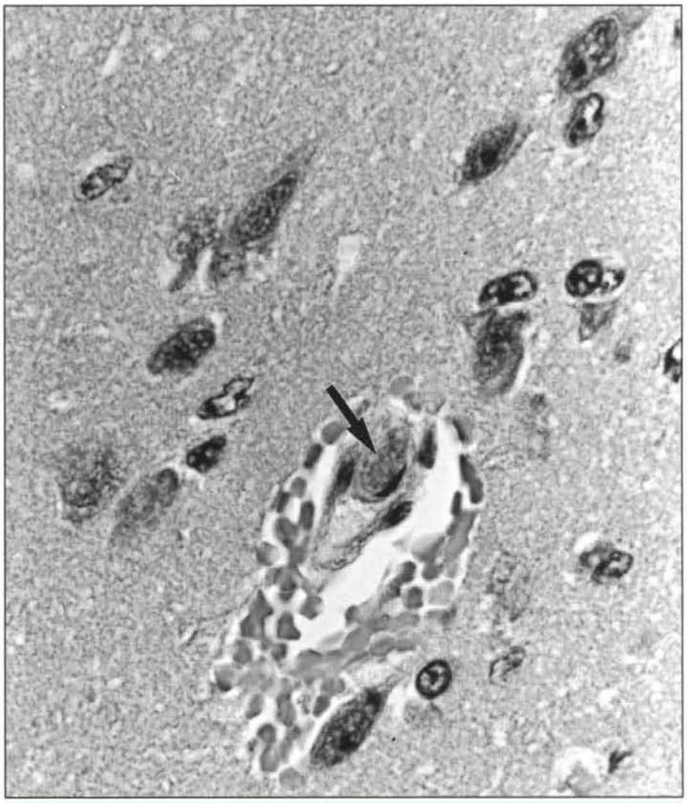

Figure 1.82 Groups of ***Ehrlichia*** *(Cowdria)* ***ruminantium*** organisms in an endothelial cell of a cerebral capillary (arrow) in heartwater, bovine. (Courtesy of TM Wilson.)

the products of arachidonic acid metabolism may play a role in the generation of vasoactive substances.

The **gross anatomical changes** in heartwater are the same in each ruminant species but generally are less prominent in cattle than in sheep or goats; lesions may be inconspicuous or absent in treated animals. The disease does not alter the general condition of the animal appreciably. Typical gross lesions include effusion in body cavities, hydropericardium, edema of lungs, brain, mediastinum, and associated lymph nodes, and splenomegaly, though all of the lesions rarely occur simultaneously. The name of the disease is derived from the hydropericardium, but this is sometimes absent. The effusion, which is serous with some fibrinous strands, is more constant and copious in sheep than in cattle. The other serous cavities also contain excess serous fluid; there may be several liters in the pleural and peritoneal cavities of the ox and up to half a liter in sheep and goats. When exudation is copious, the mediastinum and retroperitoneal tissues are also edematous. The membranes remain clear and glistening. The lungs are severely edematous, especially in the peracute form. The lymph nodes of the head, neck, and cranial mediastinum especially are swollen and edematous. The spleen is almost invariably much enlarged in sheep and goats, being sometimes up to 6 times its normal size, but splenomegaly in cattle is only moderate. There is hyperemia with submucosal edema in the lower alimentary tract but it is most pronounced in the abomasum. Petechial hemorrhages are present in the endocardium, mucosa of stomach and gut, tracheobronchial mucosa, and lymph nodes.

Lesions in the *brain* are common (Fig. 1.81) and account for the nervous signs that characterize the disease, especially in cattle, and for the failure of animals to recover with chemotherapy. Gross changes are inconstant on inspection of the surface of the brain. Petechial hemorrhages may be present over the cerebellum, and the choroid plexus may be dull and thickened. On slicing, petechial, ecchymotic, and larger hemorrhages may be numerous in all parts of the brain. The microscopic lesions in the brain are reflections of parasitism and injury to small vessels, especially capillaries and venules. Colonies of *E. ruminantium* are visible in endothelial cytoplasm of untreated cases (Fig. 1.82). There is acute inflammation of the choroid plexus with fibrinous exudate in the stroma, congestion, hemorrhage, and accumulation of inflammatory cells about the vessels. Within the brain, vascular injury leads to perivascular or larger hemorrhages and accumulation of protein droplets in the perivascular space. Focal or larger areas of malacia surround the vessels and are in various stages of development from edematous disruption of white matter with glial activation to microcavitation.

The **diagnosis** of heartwater depends largely on the demonstration of organisms in endothelial cells in brain either in smears or section; brain biopsy can be used to establish the diagnosis in the living animal. The pCS20 PCR assay is the most reliable and specific assay for the detection of *E. ruminantium* in ruminants and *Amblyomma* ticks. Various antibody detection assays are available, e.g., indirect fluorescent antibody, polyclonal competitive ELISA, major antigenic protein (MAP-1) ELISA, Western blot, but often lack specificity because of cross-reactivity with closely related ehrlichiae.

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Rocky Mountain spotted fever

Rocky Mountain spotted fever (RMSF), a febrile exanthema caused by ***Rickettsia rickettsii***, is an important rickettsiosis of dogs and humans; *R. rickettsii* or antigenically similar organisms infect a wide variety of mammals and birds. RMSF occurs in dogs throughout the Americas (and is known by various other names, e.g., Brazilian spotted fever), with most cases in North America occurring during the tick season (April to September). The *ticks* most commonly responsible for the transmission of RMSF are *Dermacentor variabilis* and *D. andersoni*; these ticks serve as vectors, reservoirs, and natural hosts for *R. rickettsii*. The rickettsiae are maintained in nature in a primary sylvan cycle, involving mostly small rodents. Dogs can develop a high and persistent rickettsemia, and are a potential public health danger; humans can be infected during tick removal by contact with hemolymph of engorged ticks or with tick excreta. Tetracycline therapy is effective, and dogs are immune after recovery.

Dogs are usually infected via the bite of infected ticks. *The rickettsiae invade and replicate within endothelial cells of small blood vessels*; endothelial damage is probably mediated by phospholipase A and a trypsin-like protease, of either rickettsial or host origin. There is little support for production of endotoxin or exotoxin by *R. rickettsii*. Endothelial damage leads to platelet activation and activation of coagulation and fibrinolytic systems; *thrombosis* in RMSF is thought to arise in reaction to endothelial damage. *Vasculitis* in RMSF is a product of endothelial swelling and necrosis with secondary contributions from the immune and phagocytic host responses mounted by lymphocytes and macrophages, but is not primarily caused by immune-complex formation or by cell-mediated mechanisms. The action of various mediators, such as those of the kallikrein-kinin system, in addition to direct endothelial necrosis, leads to increased vascular permeability and hence edema and multifocal hemorrhage.

Dogs less than two years old are predominantly affected. Purebred dogs, especially German Shepherds, appear to be more susceptible than are crossbreds. Following an incubation period of a few days, **clinical findings** in affected dogs include, in decreasing order of frequency, listlessness and depression, high fever, history or actual presence of ticks, anorexia, myalgia/arthralgia, lymphadenomegaly, vestibular deficits, dyspnea, conjunctivitis, paralumbar hyperesthesia, edema of face and extremities, petechiae or hemorrhagic diathesis, and vomiting and diarrhea. Vasculitis may be visible by means of ophthalmoscopic examination of retinal vessels.

Laboratory findings include anemia, leukopenia followed by leukocytosis, thrombocytopenia, prolongation of activated partial thromboplastin time, hyperfibrinogenemia, and increased concentrations of fibrin/fibrinogen degradation products. The thrombocytopenia is at least partly due to the development of anti-platelet antibodies. Serum biochemistry usually reveals hypoalbuminemia, increased alkaline phosphatase activity, and hypercholesterolemia. Increased concentrations of aldosterone and antidiuretic hormone may contribute to expansion of plasma and extracellular fluid volumes and hence edema. Edema of the medulla oblongata may contribute to cardiorespiratory depression. Death may occur in the acute stages of disease as the result of peripheral vascular collapse, hemorrhagic diathesis, and thrombosis, although fully developed DIC is an unusual event in RMSF in dogs. Necrosis of extremities may occur. Damage to the cardiovascular system, brain, or kidneys may cause death or permanent organ dysfunction; severely affected dogs may die with rapidly progressive meningoencephalitis.

Lesions seen at necropsy include edema of ears and muzzle; ulcerative glossitis; scrotal dermatitis; petechiation of mucous membranes, caudal abdominal skin, pleura, and gastric wall; hemorrhagic colitis; and hemorrhagic lymphadenopathy. *The prominent histologic lesion of RMSF is necrotizing vasculitis of small veins, capillaries, and arterioles*, with perivascular accumulations predominantly of lymphocytes and macrophages. This lesion is seen most commonly in skin, testes, alimentary tract, pancreas, kidneys, urinary bladder, myocardium, meninges, retina, and skeletal muscle. Acute meningoencephalitis may be present. There may also be acute splenitis, acute interstitial pneumonia, and multifocal necrosis of myocardium, adrenals, and liver. A moderate degree of glomerulonephritis may be present.

The most reliable *serologic test* for RMSF is the species-specific microimmunofluorescent method. High antibody titers develop to *R. rickettsii*, but usually decline after 3–5 months. Direct fluorescent antibody staining of a skin biopsy is positive in up to 80% of infected dogs; coccobacillary organisms are present in endothelial cells and vessel walls.

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Verminous arteritis

We are here concerned with those parasites whose natural habitat is the lumen or the wall of arteries. Parasites that, accidentally or as part of their life cycle, migrate in tissues may, depending on the route they take, cause vasculitis in various organs. The chief offenders in this regard are the larvae of the genera *Strongylus* and *Ascaris*; the vascular lesions of *Ascaris* infestations are described under diseases of the intestine (Vol. 2, Alimentary system). *Angiostrongylus vasorum*, which inhabits the pulmonary arteries of dogs, is described with the lungworms. *Spirocerca lupi*, which passes part of its history in the adventitia of the thoracic aorta, is described in its final habitat, the esophagus.

Dirofilariasis (heartworm disease)

The dog is the only mammal commonly infected by ***Dirofilaria immitis*** *(heartworm), and is the only significant reservoir of infection.* In areas in which heartworm infection is enzootic in dogs, a number of other mammals may become infected, including domestic cats and wild felids, wild canids (coyotes, foxes, wolves), ferrets, sea lions, muskrats, horses and, rarely, humans. If *D. immitis* develops to maturity in hosts other than the dog, microfilaremia is usually low or absent, and heart failure is rare. Pulmonary dirofilariasis is of considerable importance in humans because the spherical, subpleural granulomas produced may be mistaken radiographically for primary or metastatic lung tumors, leading to thoracotomy and excisional biopsy for diagnosis. *Dirofilaria immitis* has a tropical and subtropical distribution in southern Europe, Asia, Australia, and North and South America, although its range has extended into more northern temperate zones in North America and southern temperate zones in Australia.

Adults of *Dirofilaria repens* occur in subcutaneous connective tissues of dogs and other carnivores (and rarely humans). Similarly, *D. tenuis* is found in the subcutaneous connective tissue of the raccoon in the southern USA. None of these three filariids usually develops to maturity in humans, but they may be found in cysts in various parts of the body, especially in the lungs. *Dirofilaria striata*, a parasite of bobcats (*Lynx rufus*), has been reported in dogs in Florida. The "Florida" *Dirofilaria* differs slightly from *D. immitis* and *D. striata.*

Adult D. immitis worms live in the pulmonary arteries and right heart, but they can develop in various other arteries and veins, and have been reported from a variety of unusual sites, including the eye and brain. Adult males are 12–20 cm long by 0.7–0.9 mm in diameter; females are 25–31 cm by 1.0–1.3 mm. The adult female worms are viviparous, and release microfilariae into the bloodstream; mosquitoes obtain microfilariae in blood meals. The first, second, and early third larval stages of *D. immitis* are obligate parasites of mosquitoes, predominantly of the genera *Aedes, Culex,* or *Anopheles.* Larvae develop to the infective stage in about 13 days in the malpighian tubules. They then migrate to the cephalic spaces of the head or to the proboscis of the mosquito and escape into a new host when the mosquito feeds. The infective larvae are about 1.2 mm long when they enter the host, and are about 5 cm long when they reach the right ventricle 3–4 months later. In the meantime, the parasites have wandered and grown in the connective tissue, chiefly those of the subcutis and muscles, finally leaving these to migrate to the heart via the veins. They mature in a further 3 months. Thus the prepatent period is from 6–8 months. Adults may live for several years and microfilariae can survive in a dog for as long as 2.5 years. Adult heartworms may be found in cutaneous nodules occasionally in dogs, and rarely in cats.

The **clinical diagnosis** of dirofilariasis in dogs depends upon the detection of microfilariae in the blood, preferably by the microscopic examination of centrifuged sediment of lysed blood (*modified Knott's test*), or by a filter technique. Microfilariae of *D. immitis* (290–315 μm long, straight body and tail, tapered head) must be differentiated from those of ***Dipetalonema reconditum*** (270–290 μm, curved body, blunt head, most have a buttonhook tail), the adults of which are nonpathogenic and are commonly found in the subcutaneous connective tissue of dogs; these microfilariae can also be differentiated by PCR or histochemical means. The average number of circulating microfilariae per adult female *D. immitis* decreases as the number of adult worms increases. *Hence the numbers of circulating microfilariae indicate neither the numbers of adult worms present nor the severity of heartworm disease in an individual dog.* Although microfilariae are present in the blood more or less continuously, there is a tendency to periodicity, with maximum numbers occurring in the evening and in the summer, coincident with maximal activity of mosquitoes. Microfilariae are pooled in internal organs, especially the lungs, in the daytime. Fetal pups may be infected by microfilariae from the bitch and have microfilaremia, but the pups do not harbor adult worms. Cats infected with *D. immitis* are typically amicrofilaremic, given low numbers of adult female worms or male-only infections, hence antigen- or antibody-detection tests are required for diagnosis.

Occult dirofilariasis, that is, *heartworm infection without microfilaremia*, occurs in 10–67% of infected dogs, primarily as a result of destruction of microfilariae by immune mechanisms ("immune-mediated occult disease"), but also as the result of prepatent infections, single-sex infections, and drug-induced sterility of adult heartworms. Clinical diagnosis of occult dirofilariasis then rests on the findings of the indirect fluorescent antibody test, antigen detection tests, and cardiopulmonary imaging. Circulating antibodies are usually detectable only in dogs without microfilaremia.

The **clinical signs** of canine dirofilariasis are usually those of *cardiovascular dysfunction*, such as cough and tiring on exercise, which may progress to congestive heart failure. Heartworm disease is usually seen in dogs that are older than five years and have had continuous or multiple infections. There is a rough correlation between the numbers of worms present and the severity of the disturbance, but there are exceptional cases, and severe signs may appear in dogs with only a few worms. Clinically normal dogs may harbor up to about 30 worms, and clinically affected dogs 50 to more. Dirofilariasis in cats usually causes cough and dyspnea, but may as well cause vomiting, neurological signs, and malaise; sudden death is another possible outcome.

Adult heartworms are normally found in the pulmonary arteries and right ventricle (Fig. 1.83), but may be found in the right atrium and venae cavae in heavy infestations. Young adult worms usually develop first in small pulmonary arteries, and progressively involve more proximal arteries as they grow. Adult worms may be found in the right ventricle if more than about 25 are present, and in the right

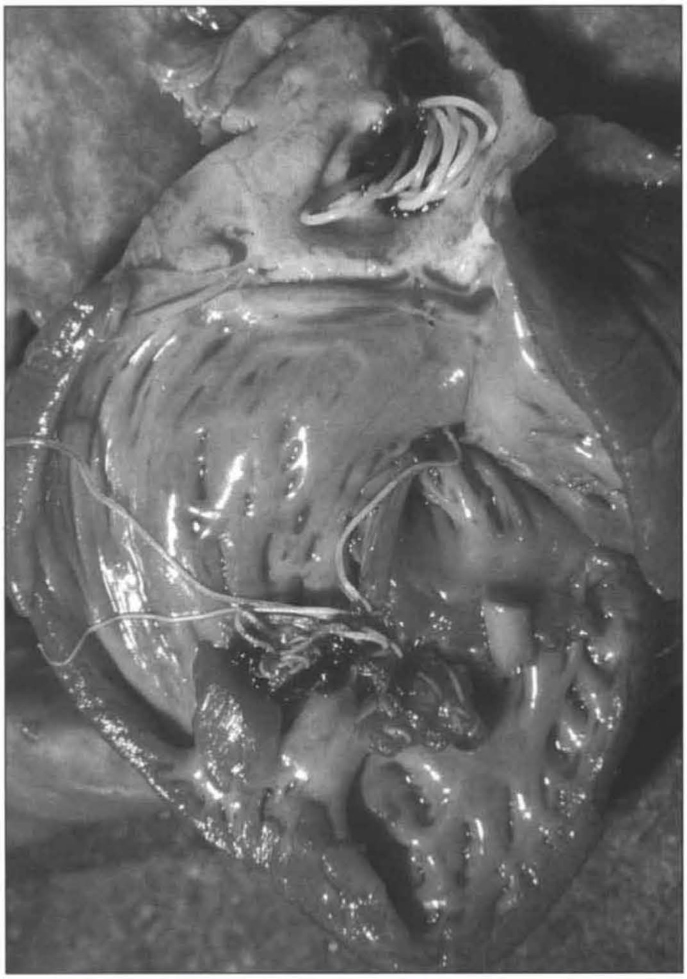

Figure 1.83 ***Dirofilaria immitis*** in right ventricle and pulmonary artery of a dog. (Courtesy of KG Thompson.)

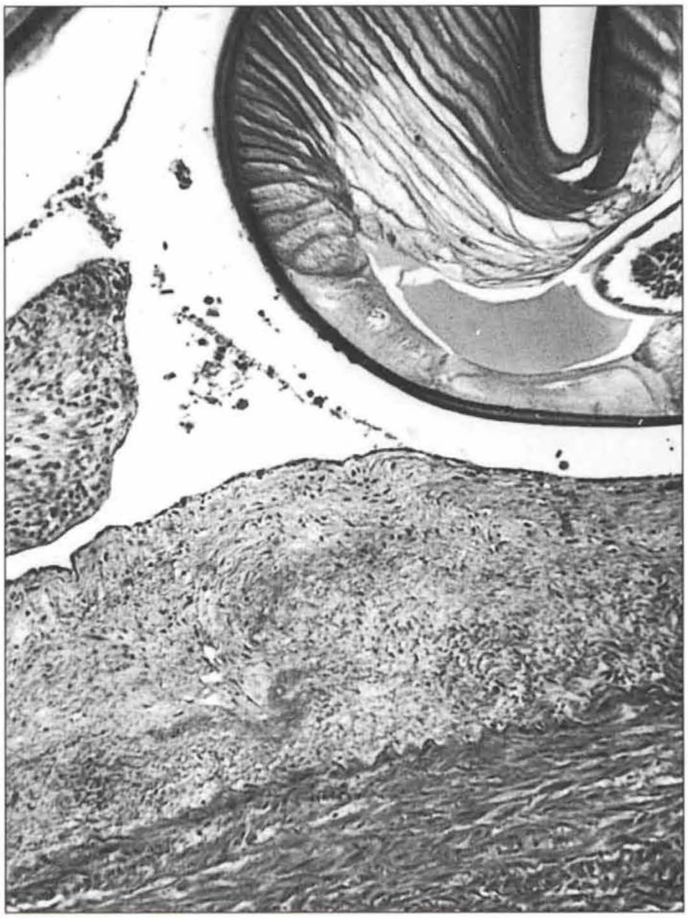

Figure 1.84 Sclerosis of pulmonary artery containing *Dirofilaria immitis* in a dog.

atrium and venae cavae if there are 50 or more worms. The caudal lobar pulmonary arteries are usually the most heavily infested vessels. Right heart failure develops as a consequence of *pulmonary hypertension*, which in turn is the result of pulmonary vascular sclerosis. Of secondary importance is mechanical interference of the worms with cardiac blood flow and valve function. Pulmonary sclerosis and hypertension slowly regress following removal of the adult worms.

Heartworm disease is primarily a pulmonary vascular disease, and is characterized by myointimal proliferations in small peripheral arteries initially, and later in the large lobar arteries, especially the right caudal lobar artery (Fig. 1.84). *The fibromuscular intimal proliferations in the larger vessels produce a grossly visible shaggy or roughened appearance, a change pathognomonic of heartworm disease.* The vascular reaction is to the immature and mature adult worms, and begins as an endoarteritis with infiltration of leukocytes, primarily eosinophils. Neutrophils are also present in the arterial wall, partly in response to the intracellular bacteria *Wolbachia* harbored by *D. immitis.* The leukocytic response subsides and is replaced by myointimal proliferation that produces irregular rugose to villus projections enmeshing the worms; the myointimal proliferation occurs at sites of direct contact with worms, is likely due to mechanical irritation and endothelial damage, and is possibly mediated by platelet-derived growth factor. *Thrombosis* may be associated with either live or dead worms, and *thromboembolism*, especially following adulticide therapy, may further exacerbate pulmonary hypertension; the presence of dead parasites initiates a granulomatous reaction in the vessel wall, which may extend into the pulmonary parenchyma. Thrombi may be organized and recanalized. Pulmonary infarction is an uncommon sequel to thromboembolism.

Pulmonary parenchymal changes that accompany the above vascular changes include periarterial granulomatous inflammation, hemosiderosis, diffuse interalveolar fibrosis, proliferation of alveolar epithelium, and fibrous pleural thickenings. Dead worms commonly incite *pulmonary granuloma formation.* Adulticide therapy causes embolization of worms and resulting thrombosis and granulomatous inflammation; resolution of these lesions is underway by 6 weeks post-treatment. Proliferative intimal lesions will progressively resolve after surgical removal of adult worms; resolution of advanced, fibrotic, obstructive arterial lesions is unlikely. Severe pulmonary arterial disease often results in chronic or low-grade DIC, and hence thrombocytopenia and hemoglobinuria. Microfilariae entrapped in the lung in a state of antibody excess are surrounded by neutrophils and eosinophils, producing *eosinophilic pneumonitis.* This allergic pneumonitis may be the precursor of *pulmonary eosinophilic granulomatosis*, which is characterized by 1–8 cm diameter nodules composed of eosinophils, lymphocytes, plasma cells, and macrophages, and devoid of microfilariae, plus peripheral blood eosinophilia.

Additional lesions of heartworm disease include those of *right heart failure*, such as chronic passive congestion of the liver, and occasionally ascites. *Membranoproliferative glomerulonephritis* occurs primarily due to glomerular deposition of immune complexes, either those formed in circulating blood in a state of antigen excess, or formed in situ in the glomeruli. The intensity of the immune-complex deposition varies directly with the intensity and duration of infection and the antibody response; the immune response may be to immature heartworms, microfilariae or adult heartworms. Physical damage to glomerular endothelium has been attributed to microfilariae. The result of glomerular disease in dirofilariasis is proteinuria, which is usually mild to moderate in severity. Renal failure and uremia are uncommon. Microfilariae are usually of little consequence to the dog, but when they die they may incite formation of microgranulomas in various organs, such as the lung, liver and kidney.

Aberrant migration of adult heartworms into the *systemic arterial circulation*, a rare event, results in thromboembolism and hence signs such as hindlimb lameness and interdigital ischemic necrosis.

The **vena caval syndrome** (venae cavae syndrome, liver failure syndrome) is a variant of heartworm disease seen usually in young dogs in which *large numbers of adult worms (100+) fill the right atrium and venae cavae*, likely as the result of retrograde migration from the pulmonary arteries. The syndrome is characterized by sudden onset of weakness, anorexia, bilirubinuria, hemoglobinuria, and anemia. Shock occurs because of obstruction and decreased venous return. The mass of worms may also interfere with valve function, causing tricuspid regurgitation, which together with pulmonary hypertension results in decreased left ventricular preload and congestive right ventricular failure. Hepatic congestion is very severe, hepatic lymphatics may be distended, and ascites may occur. Anemia develops due to right-atrial turbulence and shear-induced mechanical hemolysis, with perhaps microangiopathic hemolysis resulting from platelet activation and fibrin formation. Azotemia develops and death usually occurs in 1–3 days. Phlebosclerosis and thrombosis in the caudal vena cava and hepatic veins are similar to the reactions usually seen in the pulmonary arteries. Vena caval syndrome also occurs in cats, and untreated has a poor prognosis and high mortality rate in both dogs and cats; the treatment of choice is surgical removal of the heartworms.

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Strongylus vulgaris in horses

Horses are commonly infected with both large and small strongyles (see Vol. 2, Alimentary system), but *by far the most damaging to the host is the large strongyle* ***Strongylus vulgaris***. While the adults of *S. vulgaris* share their large intestinal habitat with many other strongylids, *S. vulgaris* is the only strongylid to undergo development in the horse's arterial system.

The *usual route of migration* of *S. vulgaris* is as follows. Infective third-stage larvae are ingested, exsheath in the small intestine, penetrate the mucosa and submucosa of the small intestine, cecum, and colon within three days of infection, and molt to fourth stage larvae by day 7. The larval penetration of the gut can cause the formation of large subserosal hemorrhages, called *hemomelasma ilei*. The fourth stage larvae, which are about 1 mm long, penetrate submucosal arterioles, migrate in or on the intima of arterioles and arteries, being constrained by the internal elastic lamina from penetrating to the media, and reach the cranial mesenteric artery, the predilection site for further development, between day 11 and 21. After 3–4 months, the larvae molt to the fifth stage (immature adults, 10–18 mm long), return to the wall of the cecum and colon via the intestinal arteries, and are encapsulated in subserosal nodules before returning to the gut lumen as adults when the nodules rupture. The adults require another 6–8 weeks to reach sexual maturity. The prepatent period of *S. vulgaris* is thus about 6–7 months.

Virtually all horses are infected with Strongylus vulgaris and have arterial lesions due to larval migration, although the prevalence of *S. vulgaris* infection has been markedly diminished by benzimidazole and avermectin anthelmintics. A degree of resistance to verminous arteritis is slowly acquired under natural conditions, but horses of all ages remain susceptible to infection. **Lesions** range from the very common, but insignificant, *tortuous intimal tracks*, to the less common, but more serious, *occlusive thrombotic lesions*. Lesions of verminous arteritis are often incorrectly called "verminous aneurysms," but these dilations are usually thick-walled due to inflammation and scarring, rather than thinned. Saccular or fusiform aneurysms may occasionally result from weakening of the arterial wall. *Lesions occur most frequently in the cranial mesenteric artery and its extension, the ileocecocolic artery*. This apparent larval predilection has been suggested to fit a random-walk model in which the larvae sense blood vessel curvature, rather than direction of blood flow, migrate longitudinally along arteries, and are essentially trapped in the cranial mesenteric artery because of its perpendicular connection with the aorta, a border which most of the larvae do not cross. Larvae that spill over into the aorta may be considered as aberrant and lost to their species, in that they are unlikely to return to the cranial mesenteric artery and hence to the gut.

Tortuous intimal tracks consist of fibrin, necrotic debris, and a mixture of inflammatory cells, including eosinophils, in the intima (Fig. 1.85). The tracks become covered by endothelium, the fibrin is removed, and the tracks are converted to fibromusculoelastic thickenings. More extensive larval migration causes more extensive thromboarteritis. Small mural thrombi are formed about the larvae attached to the intima, and there is a reactive leukocytic infiltration, edema, and resultant degeneration of the elastic and muscle fibers of the underlying media. The initial acute inflammation persists as long as the parasite remains but is soon accompanied by a productive connective-tissue response, initiated by smooth muscle cells from the media, with attempted organization of the overlying thrombus and the accumulation of large numbers of mononuclear leukocytes. The proliferating connective tissues of the intima and adventitia may ultimately replace the normal structure of the arterial wall. The affected walls become solid and very thick and the affected artery may be up to 20 cm in its greatest diameter. The luminal surfaces of active arterial lesions are always rough and covered with layered thrombi in which the parasite is found partially embedded. It is usual to find some worms, both fourth-stage larvae and immature adults, in the thrombi, but the number of worms in any location in any one case varies from a few to several hundred. Following migration of the fifth stage larvae back to the intestine, or destruction of the larvae by anthelmintics, the intimal lesions resolve as fibrous intimal thickenings, and the luminal diameter will hence increase. The arterial wall thickness also decreases.

The lesions and accompanying worms are most common in the cranial mesenteric artery (Fig. 1.86) although they are also found elsewhere, including the aorta, the renal arteries, the celiac artery and its branches, and the spermatic vessels. They may be in any branch of the cranial mesenteric artery but are concentrated largely in the right division and the colic artery, which is its continuation, and, to a slightly lesser extent, in the cranial branch and its continuation, the right colic artery. The worms are rarely found in the aorta caudal to the origin of the renal vessels; they are relatively common about the origin of the cranial mesenteric and celiac arteries, and less common again in the thoracic aorta. Although this statement on the prevalence of lesions in the thoracic aorta applies to actively thrombotic lesions containing worms, it is probable that older, warty, mineralized lesions of the type often seen in the intima of the aortic arch have this origin (see Fig. 1.30A); they occur frequently in sites where active lesions occur. The lesions in the aortic bulb are located immediately proximal to the aortic valves on the cranial wall of the bulb and in the adjacent cranial aortic and caudal right aortic sinuses, but rarely in the caudal wall and the left sinus. This localization has been explained on the basis of the curve of the aortic arch and the axis of systolic ejection which should, theoretically, provide a zone of turbulence and relatively low velocity of blood flow in the cranial segment of the aortic bulb.

The *sequelae of verminous arteritis* due to *S. vulgaris* are varied and depend upon the location of the arteritis. Involvement of the cranial mesenteric artery is almost constant in horses, but untoward sequelae are much less common, no doubt due to the extensive collateral arterial circulation to the equine intestine, which may mitigate against the effects of frequent thromboembolic episodes. *Colic*, which is commonly caused in horses by *S. vulgaris* if parasite control is poor, may occur for a number of reasons: thromboembolism and intestinal

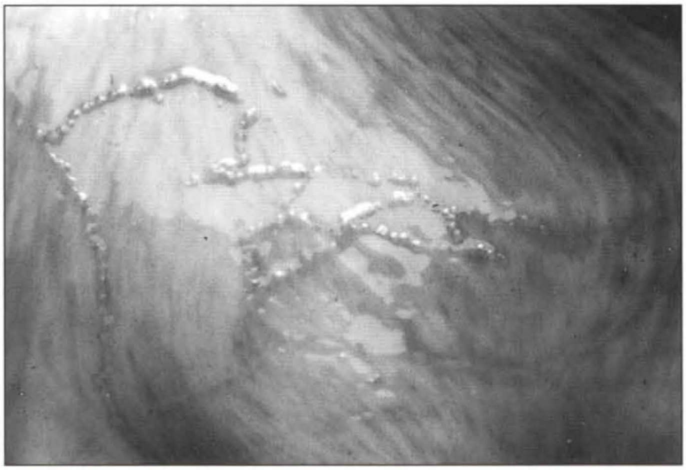
Figure 1.85 Tortuous intimal tracks due to *Strongylus vulgaris* migration in the aorta of a horse.

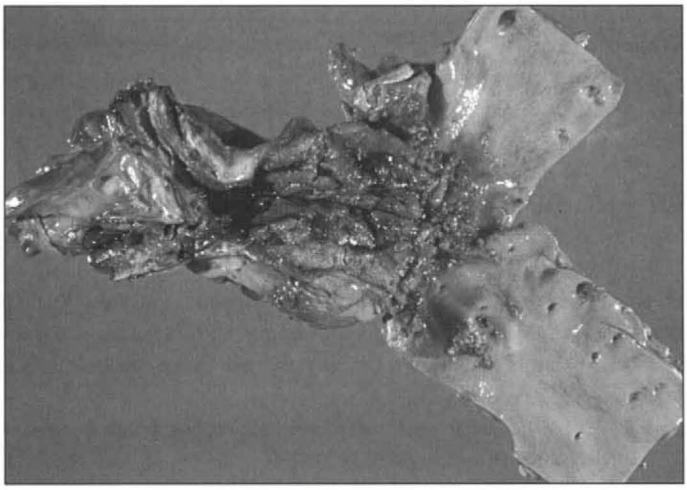
Figure 1.86 Severe **cranial mesenteric arteritis**, with dilation, thickening of arterial walls, and thrombosis of the artery (center) and of its divisions (left), caused by *Strongylus vulgaris* in a horse; opened aorta is to the right.

ischemia or infarction, interference with innervation of the gut due to pressure on abdominal autonomic plexuses, or release of toxic products from degenerating larvae. Fatal acute intestinal infarction occasionally ensues, with ischemia of large areas of the cecum or colon. Thromboembolism of the cecal and colic arteries may also lead to mucosal ulceration and diarrhea, and occasionally death. Hematochezia has been reported in a horse as a consequence of fistulous connections between a verminous cranial mesenteric arterial aneurysm and the cecum and ileum. As a result of successful suppression of *S. vulgaris* by anthelmintics, drug-resistant **cyathostomes** *(small strongyles)* have recently supplanted *S. vulgaris* as the most important cause of verminous colic. *Coronary arterial thrombosis and occlusion*, which occurs most commonly in the right coronary artery, can lead to myocardial infarction and death; similar consequences follow the development of verminous aortic valvular endocarditis (see Fig. 1.30A, B), a rare event in strongylosis. *Embolism of the brachiocephalic trunk* can occur and is especially significant when the emboli contain larvae that subsequently migrate in the brain causing encephalomalacia and either chronic incoordination or progressive fatal encephalitis. *Renal arterial verminous arteritis and thromboembolism* have resulted in renal infarction and, on rare occasions, passage of larvae in the urine. *Aortic-iliac thrombosis* may be a consequence of strongyle-related thromboembolism, but its pathogenesis remains uncertain. Thrombosis of the celiac artery and embolism of its branches is usually inconsequential.

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Onchocerciasis (parasitic aortitis)

Onchocerca armillata *is a parasite of the wall of the aorta of cattle, water buffaloes, goats, and camels*, in south Asia and equatorial Africa. Several other *Onchocerca* spp. are described with tendons and aponeuroses under diseases of muscle in Vol. 1, Muscle and tendon. The life cycle and vectors involved in the transmission of *O. armillata* are unknown; however, black flies (*Simulium*) and midges (*Culicoides*) are the intermediate hosts of other *Onchocerca* spp.

Although virtually all older cattle may be infested in enzootic areas, the lesions produced are apparently not of clinical significance. *The preferential site is the arch of the aorta.* With chronicity, lesions extend cranially to include the brachiocephalic trunk, costocervical arteries, and brachial arteries, and caudally to include the abdominal aorta to the level of the iliac bifurcation. The intima is corrugated, and *numerous sinuous tunnelings end in small nodules in the intima and media*; the nodules protrude into the vascular lumen or through the adventitia. The males, which are about 7 cm long, inhabit the nodules along with the anterior end of the female, which bears the vaginal opening, and the microfilariae, which escape into the bloodstream. The body of the female lies in the sinuous tracts, from which the complete specimen cannot be readily dissected, but its length is estimated as >100 cm. Parasitic tunnels in the inner media have a thin fibrous tissue lining without cellular infiltration. There may be acute transmural aortitis with a predominance of eosinophils, but *subacute or chronic focal granulomatous inflammation is more frequent and occurs around tunnels containing dead, degenerate, or mineralized worms*. The granulomas in the media and adventitia become encapsulated by fibrous tissue to form nodules of 0.5–2.5 cm diameter containing caseous material and intact and/or dead worms, and eventually dry mineralized debris. The granulomatous reaction will resolve with time, and, in very old cattle, the aortic wall becomes thinner, has a corrugated lining with irregular mineralized ridges, and may develop aneurysms up to 3.5 cm in diameter. The suggested causes of the granulomatous aortitis include hypersensitivity to the parasite, foreign body reaction to degenerate and dead onchocercal worms, and the release of toxic factors from the parasites.

Microfilariae can be found in the blood of infected animals at all times, but there is a definite tendency to nocturnal periodicity; infection can be detected by examination of skin snips for microfilariae. In some bulls with large numbers of circulating microfilariae, repetitive episodes of collapse and tetanic convulsion have been observed and attributed to the microfilariae. Some of the bulls showing convulsions eventually developed acute or recurrent ophthalmitis with ophthalmoscopic evidence of retinal pigmentary degeneration and other changes similar to those seen in ocular onchocerciasis in humans.

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Elaeophoriasis

The genus *Elaeophora* is another filarial parasite. Three species of interest are *E. poeli* and *E. schneideri*, which infect ruminants, and *E. bohmi*, a parasite of horses.

Elaeophora poeli occurs frequently in the *aorta of cattle and related species* in tropical areas of the Far East, but is of little clinical significance. Its life cycle is unknown. These are thread-like worms, the female of which may be up to 30 cm long. The lesions are found in the aorta and can be distinguished from those of *Onchocerca armillata* because the females of *E. poeli* are attached by the head with the body swinging free in the lumen of the vessel (Fig. 1.87). The males become encysted in intimal fibrous nodules that also contain the head of the female. In light infestations, the lesions are found chiefly on the dorsal wall of the aorta about the openings of the intercostal arteries. Heavy infestations are more diffuse and may provoke a prominent fibrous thickening of the vessel wall.

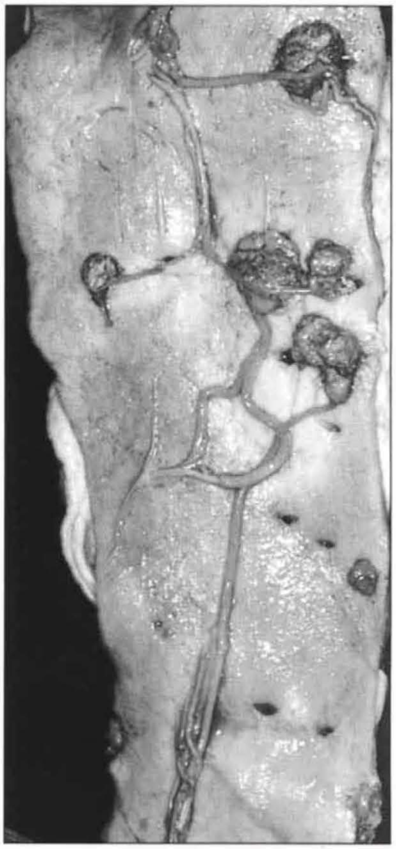

Figure 1.87 Intimal fibrous nodules in bovine aorta contain males and the heads of females of ***Elaeophora poeli***; the body of the female swings free in the aortic lumen. (Courtesy of PB Little and Can Vet J.)

Elaeophora schneideri is commonly found in the *arteries of mule deer and black-tailed deer*, as well as in white-tailed deer, domestic sheep and goats, elk (wapiti), moose, and bighorn sheep in mountainous areas of the western and southwestern USA and southwestern Canada, and in white-tailed deer in the southeastern USA. Mule deer and black-tailed deer are little affected by the parasite, and are considered the normal definitive hosts. Horseflies of the genera *Hybomitra* and *Tabanus* are the intermediate hosts. When the horsefly feeds, infective larvae invade the host and migrate to the leptomeningeal arteries, where they develop to immature adults that migrate against the blood flow to reside in the common carotid arteries. The parasites reach sexual maturity ~6 months later and begin producing microfilariae. Although adult worms normally inhabit the carotid arteries and their branches, they may be found in many other arteries through the body. Adult females are 6–12 cm long by 0.6–0.9 mm thick, and adult males are 5.5–8.5 cm long by 0.4–0.7 mm thick; adults live 3–4 years. Microfilariae are about 280 μm long by 18 μm thick, and are found in the capillaries of the forehead and face.

When larvae develop in the leptomeningeal arteries of elk, moose, domestic sheep and goats, sika deer, and possibly white-tailed deer, they can cause ischemic necrosis of brain tissue, resulting in "clear-eyed" blindness or sudden death. In elk, adult worms in the cephalic arteries cause mechanical irritation and hence endothelial damage, intimal proliferation and fibrosis, thrombosis, and embolism, which result in focal ischemia and infarction in the brain, eyes, optic nerves, ears, muzzle, nostrils, and other tissues of the head. Adult worms usually cause only mild intimal sclerosis in deer and sheep, and only rarely do they cause granulomatous verminous thrombosis. Microfilariae can cause multifocal myocardial infarction, myocarditis, and fibrosis in deer. Oral food impactions have been associated with *E. schneideri* in white-tailed deer, but a causal relationship has not been established.

Young domestic sheep and goats may develop nervous signs and die suddenly 3–5 weeks after infection as a result of thrombosis of cerebral and leptomeningeal arteries. In survivors, the most important consequence of elaeophorosis in sheep is *filarial dermatosis (sorehead)*, an often severe exudative dermatitis affecting the poll, face, abdomen, and lower parts of the legs, in up to 1% of sheep in enzootic areas. Stomatitis and rhinitis also occur. The lesions are due to microfilariae released by adult females located in arteries supplying the affected areas. Microfilariae can be demonstrated microscopically in the lesions and they are responsible for intense leukocytic infiltration, chiefly of eosinophils and mononuclear cells. There is hemorrhage, vesiculation, and ulceration of the epidermis with exudation of serum and leukocytes to form crusts. These lesions have the character of an allergic reaction and are presumably related to the alien nature of the sheep as a host, because they rarely occur in deer. The skin lesions are intensely pruritic and often lead to severe self-trauma. Lesions often persist for 3–4 years and will regress spontaneously following the death of adults and microfilariae.

Elaeophora bohmi was found in approximately 6% of a sample of Austrian horses, the vessels involved being arteries and veins of the metacarpus, metatarsus, and more distal extremities. The parasites rather selectively involve the media of the vessels avoiding the intima and adventitia, although the fibrous reaction that develops may cause stenosis of the lumen. The worms are coiled and entwined amongst the tissue layers, provoking *parasitic granulomas* with intense infiltration by eosinophils and macrophages. In cases of longer standing, diffuse or nodular fibrous thickenings are visible and palpable in the vessel walls. Microfilariae were found in the blood of 14 of 161 horses (9%) sampled in Iran. The infection appears to be of little clinical significance.

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VEINS

Venous disorders may be of clinical significance by predisposing to thrombosis and subsequent embolism, or by obstructing venous return and hence causing passive congestion of the affected areas. Obstruction of venous outflow may be followed by the development of collateral venous drainage.

Anomalies of veins occur uncommonly. Arteriovenous fistulas have been discussed above with arterial anomalies. A variety of congenital **portosystemic anastomoses** occur in dogs and cats and allow portal blood, with its absorbed toxic substances, such as ammonia, to bypass the liver and cause hepatic encephalopathy. The most common of these defects is *persistent ductus venosus*. Other portosystemic anastomoses occur extrahepatically between the portal vein and various branches of the caudal vena cava or the azygous vein. As well, portosystemic anastomoses may be acquired due to portal hypertension, which in turn is caused by impedance of blood flow through a diseased liver, an obstructed portal vein, or a kinked intrathoracic caudal vena cava. *Hepatic microvascular dysplasia* can occur in dogs and cats in the absence of macroscopic portosystemic shunts, and result in similar clinical signs. Portosystemic anastomoses and hepatic encephalopathy are discussed in more detail with Diseases of the liver.

Dilation of a vein, variously termed *phlebectasia, varicosity, or aneurysm*, occurs but is of little or no importance. It is common as the so-called "*varicocele*" *of the pampiniform plexus*. Dilation may occur because of congenital defects such as venous diaphragms, agenesis, hypoplasia, or ectopia, or may occur secondary to trauma, neoplasia, or surgical intervention. The initial dilation causes insufficiency of the venous valves, and the veins may become dilated, elongated and tortuous. Thrombosis or sclerosis may be sequelae. The acquired portosystemic anastomoses noted above usually result from dilation of preexisting microscopic venous anastomoses to produce collateral venous drainage.

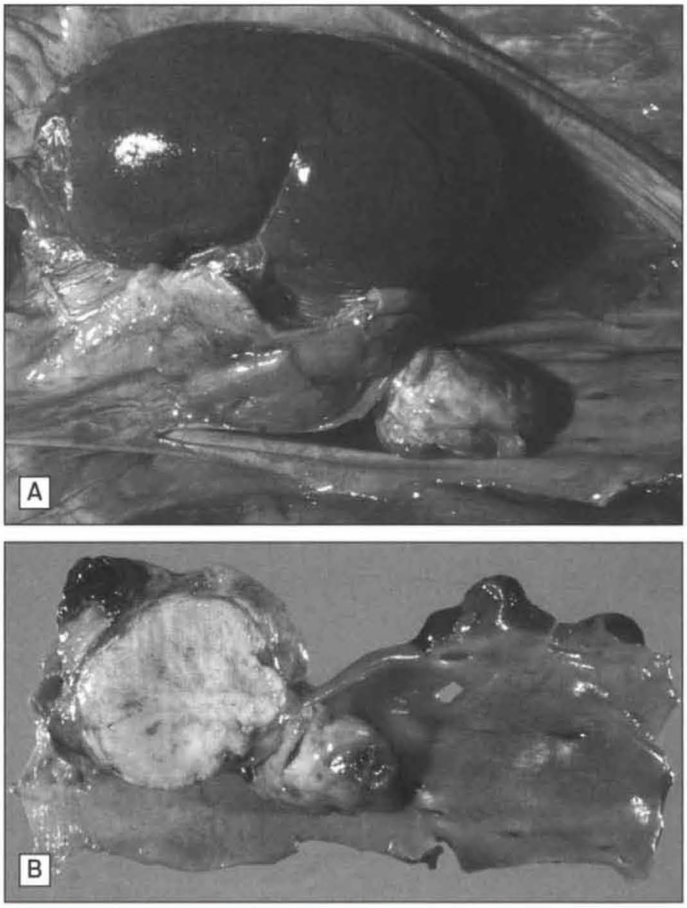

Figure 1.88 A. Pheochromocytoma of the right adrenal in an 8-year-old dog. **B.** Invasion of the caudal vena cava. The dog was found dead, but the pheochromocytoma was apparently functional as evidenced by acute catecholamine-induced myocardial necrosis.

Hepatic telangiectasis, peliosis hepatis, and hepatic "veno-occlusive disease" are described with diseases of the liver in Vol. 3, Liver and biliary system.

Rupture of a large vein, usually the result of physical trauma, may result in fatal hemorrhage. Spontaneous idiopathic rupture of the venae cavae or portal vein of horses occurs occasionally; rupture of the great coronary vein of the heart is a rare cause of unexpected death in horses.

Veins may be invaded by **neoplasms** (Fig. 1.88A, B), which may then metastasize, and may cause thrombosis or obstruction.

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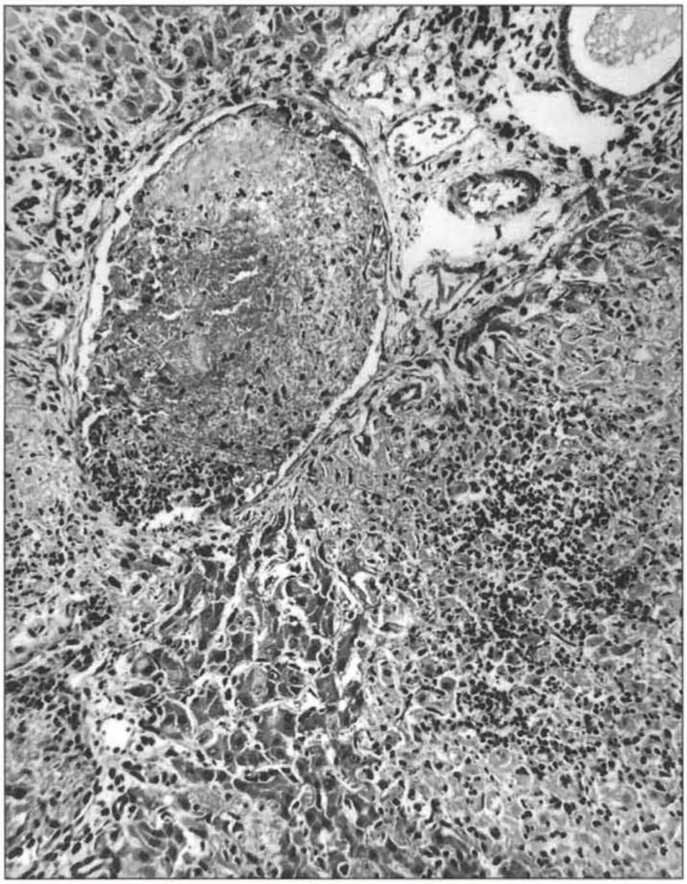

Figure 1.89 Intrahepatic **portal thrombosis** in a lamb with omphalophlebitis.

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Phlebothrombosis and thrombophlebitis

Venous thrombosis (**phlebothrombosis**) results from the usual pathogenetic factors and often leads to inflammation of the vein wall, and hence becomes **thrombophlebitis**. Conversely, *phlebitis inevitably leads to thrombosis*. Spontaneous thrombosis affects the venae cavae and portal veins occasionally, and the inciting factors are usually unknown. *Neoplastic invasion*, e.g., by pheochromocytoma, is one cause of thrombosis of the caudal vena cava. Thrombosis of the renal vein and vena cava occasionally occurs in dogs with the *nephrotic syndrome* as a result of urinary loss of antithrombin and resulting hypercoagulability of the blood. Stasis of venous blood results in thrombosis of the femoral tributaries of cattle recumbent for more than 4–6 hours ("*downer cows*"). Venous infarction commonly ensues, and necrosis of the medial muscle masses and sciatic nerves causes permanent posterior paralysis. The thickened, congested, red-black mucosa of the gastric fundus in swine with acute septicemia is a *venous infarct* resulting from thrombosis of veins of the mucosa and submucosa, probably as a sequel to endothelial damage. *Gastrointestinal infarction and ulceration* can occur in phenylbutazone-intoxicated horses as a result of degeneration of veins and phlebothrombosis. Thrombosis of the *jugular veins* follows inept or repeated venipuncture, injection of irritant solutions, and the use of indwelling catheters, especially if contaminated with bacteria. Important sequelae to jugular thrombosis are pulmonary embolism, and, if the emboli are septic, valvular endocarditis and pulmonary abscessation.

Endophlebitis may result from the implantation of bacteria carried in the blood stream, as in salmonellosis, and from the injection of irritant solutions. An important form of acute suppurative endophlebitis is the *omphalophlebitis of the newborn* resulting from infection of the uncicatrized umbilical cord. The resultant bacteremia may result in acute death or give rise to widespread suppurative lesions, often polyarthritis. Hepatic thrombophlebitis is especially common in "navel-ill" (Fig. 1.89).

In *feline infectious peritonitis*, phlebitis occurs as a result of antigen–antibody complex deposition in the walls of small veins and venules, which is followed by complement fixation and neutrophil chemotaxis. Neutrophils cause tissue necrosis, and hence thrombophlebitis, which is followed by the focal pyogranulomatous reaction typical of this disease (Fig. 1.90).

Phlebitis very commonly occurs by extension from areas of inflammation, and importantly provides for the hematogenous dissemination of infection. The entire thickness of the wall of the vein is inflamed, and thrombosis occurs rapidly. If the thrombus is infected, it is likely to soften, disintegrate, and produce emboli. An important example of this pathogenetic sequence is *thrombosis of the caudal vena cava in cattle*, which occurs as a sequel to rumenitis and hepatic abscessation (Fig. 1.91). Perivascular hepatic abscesses often involve the vena cava causing thrombophlebitis and a variety of consequences: pulmonary thromboembolism and arteritis, pulmonary abscessation, or pulmonary arterial aneurysms that may rupture and cause massive pulmonary hemorrhage (Fig. 1.92). Valvular endocarditis is an occasional sequel. As well, the abscess may erode the wall of the vena cava wall and rupture into the vena cava, causing massive septic embolization and sudden death.

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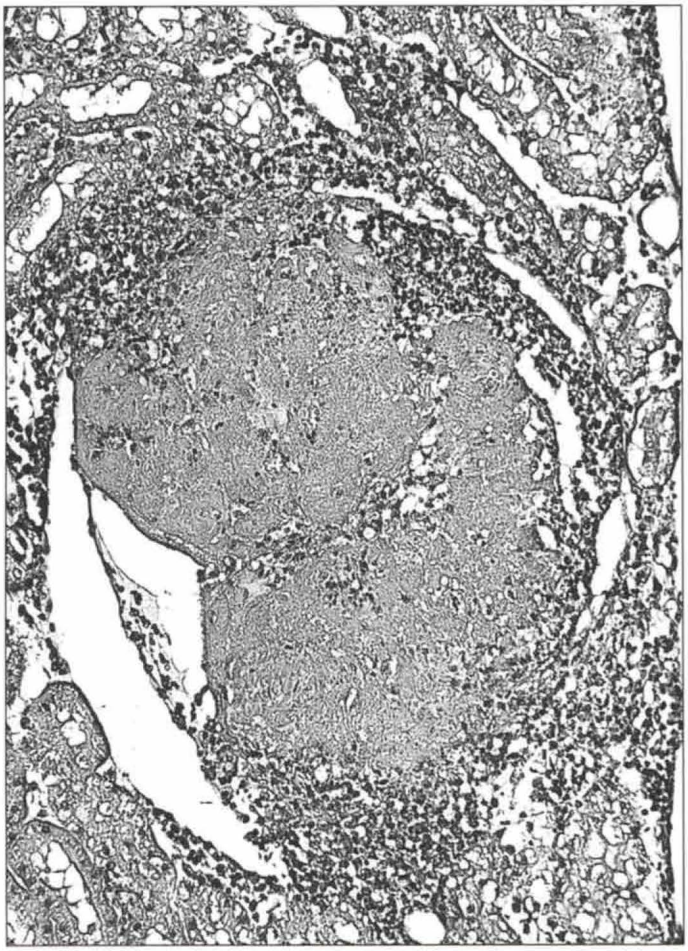

Figure 1.90 Thrombophlebitis of a renal stellate vein of a cat with feline infectious peritonitis.

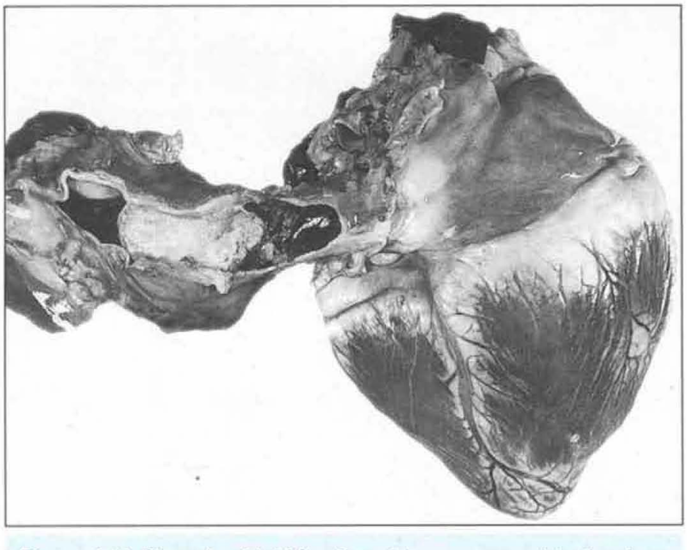

Figure 1.91 Thrombophlebitis of caudal vena cava originating from hepatic abscess, bovine.

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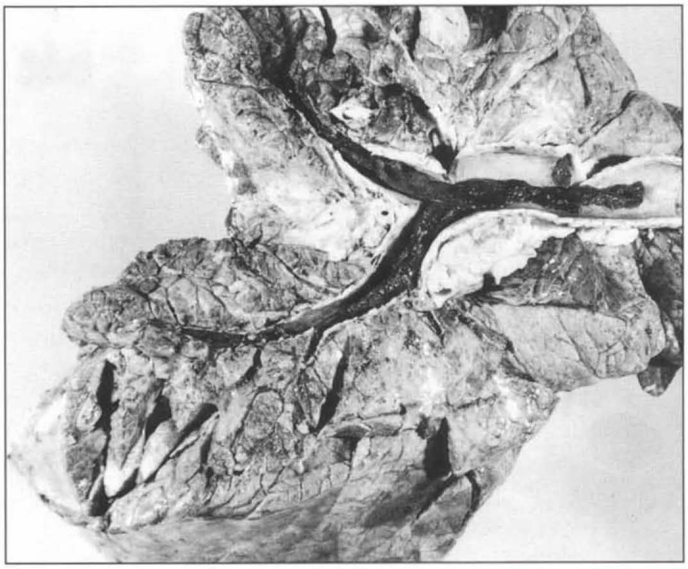

Figure 1.92 Hemorrhagic tracheal cast and acute pulmonary edema in a cow, as a sequel to rupture of hepatic abscess into the caudal vena cava.

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Parasitic thrombophlebitis

Schistosomes are important venous parasites in tropical Africa and Asia, and are discussed at length below. *Gurltia paralysans*, a metastrongyle, causes paralytic disease in domestic cats in South America. The adults lodge in the veins of the thigh and lumbar cord and cause thrombosis and ectasia.

Schistosomiasis (bilharziasis)

Schistosomiasis, a snail-borne fluke infection, is prevalent in domestic animals in Asia, Africa, and other tropical and subtropical areas, but does not usually occur as clinical disease; the importance of the disease may increase as irrigation projects enhance the habitat for the intermediate snail host. *Damage and clinical effects are the result of localization of permanently coupled adult male and female flukes in blood vessels of the liver, lungs, alimentary and urogenital tracts, and the nasal cavity.* Egg laying continues for many years, and schistosomiasis can be a disease of long chronicity.

Members of the family Schistosomatidae are the blood flukes of mammals and birds; species that parasitize mammals include those of the genera ***Schistosoma, Heterobilharzia***, and ***Orientobilharzia***. It is rather characteristic of the trematodes, including the blood flukes, that the intermediate stages are selective in their choice of hosts, mollusks, and that the infective and final phases do quite well in a variety of definitive hosts, although there is some host preference. The human schistosome, especially *S. japonicum*, may be discovered in domestic animals, and the mammalian schistosome may be discovered in man as patent or nonpatent infestations.

Based on egg morphology and intermediate snail hosts, the species that occur in domestic animals may be grouped as follows:

1. ***S. haematobium* group** – *S. bovis* in the portal and mesenteric veins of ruminants and occasionally horses, camels, and pigs, in southern Europe and tropical areas of Africa and Asia; *S. mattheei* in the portal and mesenteric veins, plus veins of the urogenital tract and stomach, of ruminants in central and southern Africa; *S. curassoni* in ruminants in west Africa; *S. margrebowiei* and *S. leiperi* in wild artiodactyls in central Africa.
2. ***S. mansoni* group** – *S. rodhaini* in dogs and other carnivores in central Africa.
3. ***S. indicum* group**, present in India and Southeast Asia – *S. spindale* in mesenteric veins of ruminants and, occasionally, of horses and dogs; *S. nasale* in nasal mucosal veins of cattle and, occasionally, of goats and horses; *S. indicum* in portal and mesenteric veins of herbivores; *S. incognitum* in swine and dogs.
4. ***S. japonicum* group** – *S. japonicum*, human by preference, in all domestic animals in the Far East; *S. mekongi* in dogs and humans in Southeast Asia.

Schistosoma bovis and *S. japonicum* are the most pathogenic of the schistosomes of cattle and sheep. Mixed infections with more than one species of schistosome occur; hybridization may occur in this circumstance.

Other members of the family Schistosomatidae of veterinary significance include: *Orientobilharzia turkestanicum* in mesenteric veins of herbivores and cats in the Near East, Russia, China, and Mongolia; *O. dattai* and *O. bomfordi* in artiodactyls in India; and *Heterobilharzia americana* in raccoons, bobcats, white-tailed deer, and occasionally dogs, in the southern USA.

The life cycles of the schistosomes are comparable to those of other flukes and follow the general pattern described elsewhere for the liver fluke, *Fasciola hepatica*. As a point of difference, the redia phase of *F. hepatica* in the intermediate host is replaced in the schistosome by a generation of daughter sporocysts.

The eggs of the schistosome are laid in the vessels that the adults inhabit. The eggs when laid are immature but contain a miracidium by the time they have worked their way through the overlying mucous membrane to escape to the exterior. The eggs hatch in water and the miracidia actively penetrate the soft tissues of aquatic snails to which they are physiologically adapted. The fork-tailed cercariae that emerge from the snail are the infective phase, and they enter the definitive host by burrowing through the skin; patent infestations can also develop if the cercariae are ingested with water. They will invade the skin of hosts that are quite alien to them, provoking allergic reactions and dermatitis (swimmer's itch, swamp itch).

Cutaneous lesions develop as a result of penetration of the skin by cercariae. They occur only on those portions of the skin that come in contact with infested water and are seldom observed in animals except under experimental conditions. The cutaneous lesions are tiny petechiated nodules in which there is a sharp leukocytic reaction to the parasite. Passage of a large number of parasites through the lungs will produce pneumonia. Ectopic localization in various organs, such as the kidney, produces small hemorrhages and inflammatory foci.

In the definitive host, the cercariae lose their tails and become *schistosomula*, which enter venules in the dermis and, thereafter, are conveyed passively in the blood, through the lungs, and into the systemic circulation. Once in the blood, there is probably no selective migration; those that are distributed to the vessels of their final habitat develop further and those that are distributed to other organs die. *Schistosoma nasale* develops in the veins of the nasal mucosa; all other species develop in veins of the abdominal cavity, principally in the portal vessels, until sexual maturity is attained, when they migrate out to the smaller veins of the mesentery (Fig. 1.93). In long-standing infections, *S. mattheei* may reside in veins of the urinary bladder as well as in portomesenteric veins. The prepatent period in the mammalian host ranges from 30 to 77 days.

In clinical cases of *schistosomiasis of portomesenteric distribution*, the animals are anemic, and may be severely emaciated in fatal cases. Diarrhea or dysentery occur at the time of patency, persist for a few weeks, and, in the absence of re-infection, may be followed by spontaneous recovery as the immune response of the host eliminates the parasites and suppresses the egg laying of survivors. The eggs can be found in the feces together with some mucus and blood. Blood loss leads to anemia and hypoalbuminemia, which may contribute to the presence of fluid in serous cavities. The wall of the intestine, chiefly the *large bowel*, is thickened by inflammatory and scar tissue, and may

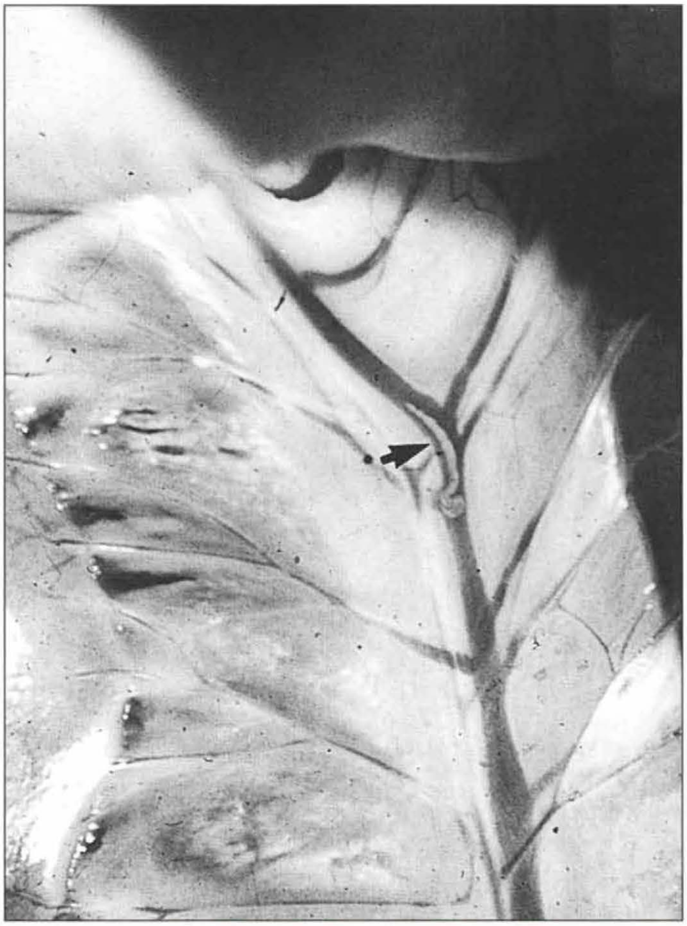

Figure 1.93 Adult **schistosome** (arrow) in a mesenteric vein in a lamb.

contain small granulomas and lymphoid nodules. Scarring begins in the mucosa and extends to involve all layers, including the serosa. The mucosa may bear polypoid and papillary proliferations. The mesentery is thickened and shortened by fibrous tissue, and the mesenteric nodes are enlarged and fibrotic. The adult parasites, which are about 1–3 cm long, can be found in the mesenteric and portal vessels. There is *fibrosis of the liver* of variable degree affecting particularly the portal vessels. The liver may be enlarged or small depending on the course and severity of the infestation. On cut surface, the periportal nature of the fibrosis is quite apparent but, with time and more severe infestations, the fibrosis becomes more diffuse and the surface of the liver may have a hobnailed appearance or be studded by numerous minute granulomas.

Proliferative granulomatous nasal lesions produced by *S. nasale* result in clinical signs of dyspnea and mouth breathing. Granulomatous lesions are frequently present in the *urinary bladder and ureters* of cattle with *S. mattheei* infection; lesions in the ureters, and occasionally renal pelvis, are linear, while those in the bladder become very extensive and polypoid.

The stage of oviposition and extrusion of eggs through the tissues and mucous membranes is the stage in which most damage is done. The mated schistosomes, in permanent copula, move out into the smallest venous radicles and the eggs are deposited first in the lumen of the vessel. They adhere to, and are eventually covered by, the endothelium. The eggs of some species are spinous and this probably facilitates passive migration into the tissue. The migration does appear, however, to be partly active by virtue of secretions elaborated by the developing miracidium, which diffuse through the shell of the egg into the tissues. These secretions may be also in part responsible for inciting the inflammatory reaction to the eggs. Initially most of the eggs are deposited in the lamina propria of the intestine, and break into the lumen with little more than mechanical injury. Rupture of congested mucosal venules accounts for the characteristic dysentery of schistosomiasis. Also contributing to diarrhea, loss of body condition, and failure to thrive are ganglionitis and degeneration of the enteric nervous system, which may result from a localized type I hypersensitivity reaction involving mast cell-derived chemical mediators.

As the disease progresses and the adults are excluded from smaller venules due to endophlebitis, eggs are laid in veins in deeper tissues where antigens released by the miracidium induce a delayed hypersensitivity response and formation of small granulomas ("*pseudotubercles*"). The granulomas are characterized initially by the infiltration of leukocytes, chiefly eosinophils, and later by the accumulation of mononuclear leukocytes, epithelioid and giant cells, and reactive fibrosis. Microabscesses occasionally form and rupture into the gut lumen. Eggs in submucosal granulomas degenerate and are removed, or may be mineralized and remain for a longer period of time. Some of the mature or disintegrating eggs may be coated with a bright eosinophilic deposit (*the Splendore–Hoeppli reaction*), the result of antigen–antibody complex formation. The granulomas gradually regress and are replaced by fibrous tissue. Host cell responses to eggs are primarily the result of host hypersensitivity to soluble egg antigens, and to a much lesser degree due to physical and chemical stimuli.

As would be expected, not all the eggs move in the proper direction. Some break into lymphatics and are conveyed to the regional nodes to produce granulomas but, more importantly, a great many of them pass to the liver producing portal phlebitis and granulomatous hepatitis, in concert with the adult flukes. In heavy infestations and possibly by means of venous shunts developed in the liver, many eggs may pass in venous blood to and beyond the lungs, producing granulomatous endoarteritis and adjacent granulomas wherever they lodge. The development of hepatic lesions follows the same course as for those that develop in the intestinal wall. In the liver, the eggs break out of the portal venules to become enclosed by granulomas. Sooner or later, they die and are mineralized, and by that time have provoked extensive surrounding fibrosis. The above course of events occurs similarly in urinary schistosomiasis caused by *S. mattheei*, wherein hematuria accompanies excretion of eggs in the urine, and *granulomatous cystitis and ureteritis* result from the host's immunological response to the schistosome eggs trapped in the tissues.

Adult schistosomes in mesenteric and portal veins elicit eosinophilic endophlebitis, often with irregular intimal proliferation, and may be found within thrombi. Adults may similarly cause reactions in veins of the pancreas and the mesenteric and portal lymph nodes, and in pulmonary arterioles. The dead parasites are removed by a granulomatous reaction that is often followed by the formation of a grossly visible lymphoid nodule. The lumen of the vein may be occluded, and the venous wall obliterated, by this lymphoid response. There may also be diffuse intimal proliferation and endophlebitis of intrahepatic portal veins, medial hypertrophy, and adventitial inflammation and fibrosis leading to prominent portal fibrosis, which may appear grossly as "clay pipe-stem" portal fibrosis. Irregular dilated vascular bypasses develop in the portal areas. The adults ingest erythrocytes and regurgitate hematin pigments, which are picked up by the monocyte-macrophage system, especially in the regional lymph nodes and liver; these black iron-porphyrin pigments may be responsible for the gray color of the liver and lungs in severe cases of schistosomiasis.

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LYMPHATICS

The richness of the lymphatic plexuses in almost all tissues and their important role as drainage channels for interstitial fluid makes for almost inevitable involvement of lymphatics by any inflammation and by the many neoplasms that metastasize via lymph channels. In most instances, the lymphatic lesions are so small as to have no significance, but in some cases the consequence of lymphatic involvement may be the major presenting clinical sign or lesion. Such, for example, is the case when attention is drawn to papillary carcinoma of the canine ovary by severe ascites; disrupted portions of the neoplasm permeate and obstruct the ventral diaphragmatic lymphatics, which are the main efferent channels of the peritoneal cavity.

Lymphedema *is defined as swelling of a part of the body by an increased quantity of lymph due to a lymphatic system disorder.* The term should not be used to describe edematous swellings due to venostasis, hypoproteinemia, heart failure, or cellulitis. Lymphedema may be classified as primary or secondary:

- *Primary lymphedema,* which is usually congenital, and may be hereditary, is due to anomalous development of the lymphatic system.
- *Secondary lymphedema* occurs because of obstruction of previously normal lymphatics, due to inflammation, neoplasia, surgery or trauma.

Lymphedema is of significance because it may predispose the affected area, usually a limb, to secondary bacterial infection and poor wound healing. *Prolonged lymphedema leads to fibrosis.*

A **lymphocele** is a lymph-filled space that does not have a distinct endothelial lining, and may be the result of disruption of lymphatics by trauma or surgery.

Congenital anomalies

There are many degrees of variation of the standard disposition of the large lymphatic vessels but these are of no importance. *Epicardial lymphatics in horses* are occasionally dilated and tortuous, and may be anomalous; they are of no apparent significance (Fig. 1.94).

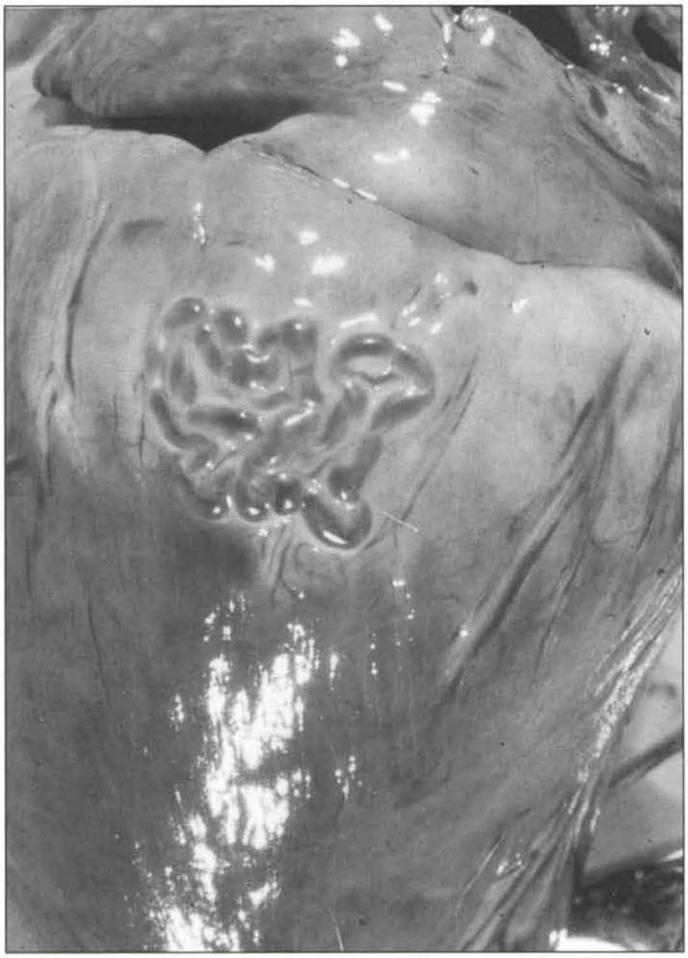

Figure 1.94 Dilated epicardial lymphatics in a foal.

Congenital hereditary lymphedema is a rare condition that has been reported in *calves, dogs, and pigs.* The lymphatic abnormality is inherited as an autosomal dominant trait in most cases examined. One of the regulators of lymphatic vasculature formation is T1α/podoplanin, a mucin-type transmembrane glycoprotein that is predominantly expressed by lymphatic endothelium and is regulated by the lymphatic-specific gene *Prox1.* T1α/podoplanin(–/–) mice die at birth and have defects in lymphatic vessel formation with consequent congenital lymphedema.

The central lymphatic system normally forms as outgrowths from the endothelium of primitive veins, and subsequent outgrowths form the peripheral lymphatic system. The primary lymph nodes develop from the primitive central lymphatic anlagen, the jugular and iliac lymph sacs. Secondary and tertiary lymph nodes later form along the peripheral lymphatic system and are the most severely affected nodes in congenital lymphedema. The earlier the stage at which lymphatic malformation occurs, that is, the more central the lesion, the more severe will be the clinical manifestations and the earlier will be their onset.

Grossly, the most severely affected individuals have generalized subcutaneous edema and fluid in serous cavities and are stillborn or must be delivered by cesarean section. The mortality rate of Ayrshire cows with lymphedematous fetuses has been high. Moderate degrees of lymphedema are seen as symmetrical swelling of the head, neck, limbs, and tail. The ears of lymphedematous calves often have edematous accessory lobes. Lesser degrees of lymphedema affect the distal parts of the limbs, especially the hindlimbs, and may not develop for several weeks postnatally. Mild regional lymphedema may be of little significance, although chronic subcutaneous edema will lead to fibrosis and may predispose to secondary bacterial infection. Mild lymphedema affecting only the hindlimbs will in some cases disappear, presumably due to postnatal development of the peripheral lymphatic system. The peripheral lymph nodes are usually smaller than normal or absent in affected animals. Although obstruction may be demonstrated by lymphangiography, nodal hypoplasia or absence does not necessarily lead to obstruction. Afferent and efferent nodal sinuses may be dilated and may be evident on gross examination. The thoracic duct may be dilated and tortuous in generalized lymphedema.

Histologically, a wide range of lymphatic abnormalities may be seen in the edematous, and possibly fibrotic, subcutis of affected areas. The lymphatics may be: *aplastic*, that is, no lymphatics are found; *hypoplastic*, in which case lymphatics are too few or too small; *or hyperplastic, or varicose*, in which case lymphatics are excessive in size and number, and dilation has resulted in valvular incompetence and lymphostasis. Ectatic lymph channels are commonly present around and within hypoplastic peripheral lymph nodes. Central nodes are similarly affected in animals with generalized lymphedema, and will be absent in cases of lymphvascular agenesis.

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Dilation and rupture of lymphatics

Dilation of normally developed lymphatic vessels (**lymphangiectasis**) *almost invariably results from some form of obstruction and leads to the accumulation of excess interstitial fluid in the drainage area.* Causes of such lymphatic obstruction include infiltrating neoplasms that may obstruct the lymphatic vessel or the drainage node, inflammatory thrombosis and sclerosis of the channels or nodal sinuses, and postsurgical scarring. The vessels become irregularly dilated and tortuous distal to the obstruction and the accompanying increase in interstitial fluid, if persistent, will cause an increase in interstitial and subcutaneous fibrous tissue.

Intestinal lymphangiectasis is the most common cause of *protein-losing enteropathy in dogs*, and contributes to intestinal protein loss in *cattle with Johne's disease and other enteritides*. Dilated lacteals in intestinal villi rupture or leak their contents into the intestinal lumen and severe hypoproteinemia can result. Leakage of protein from capillaries in the villi may also be an important contributor to protein loss. Chyle may leak from dilated subserosal lymphatics and contribute to the hypoproteinemia-induced ascites.

Chylothorax, the result of *leakage or rupture of the thoracic duct*, occurs infrequently in dogs and cats. The cause of chylothorax is usually not apparent; rupture due to thoracic trauma is often postulated but rarely proven. An injured thoracic duct has limited capacity for spontaneous healing. Reported causes of chylothorax include neoplasia, right-sided heart failure, dirofilariasis, thrombosis of the cranial vena cava, congenital anomaly of the thoracic duct, and fungal infections; many cases appear to develop spontaneously. Obstruction of thoracic duct drainage may lead to lymphangiectasis of intrathoracic lymphatics and leakage of chyle. **Chylopericardium** is an unusual accompaniment of chylothorax. The *diagnosis of chylothorax* is confirmed by analysis of the pleural fluid, which is opalescent to opaque, milky-white to yellow, and on standing forms a top layer of cream (chylomicron fat) that is ether-soluble. Microscopically, the fluid contains large numbers of lymphocytes, a few neutrophils, red cells, plasma cells, and fat globules. The site of the rupture is often difficult to locate surgically or at autopsy. Ligation of the thoracic duct leads to the development of alternative lymphaticovenous anastomoses, and cessation of the chylothorax.

Chylous ascites results from rupture of the cisterna chyli, and is a rare event.

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Lymphangitis

Lymphangitis with anatomic specificity is a prominent feature of a number of specific diseases. Most of the diseases are discussed elsewhere but warrant brief mention here also. Acute thrombotic lymphangitis, the thrombi containing large numbers of bacilli, with a marked tendency towards necrosis, is a feature of the restricted form of *anthrax* that is seen in pigs, dogs, and horses. Granulomatous lymphangitis occurs typically in *mycobacterial infections*; the mesenteric lymphatics stand out like threads beneath the serosa in Johne's disease, and are often beaded in intestinal tuberculosis; they bear the specific microscopic lesions of these infections (Fig. 1.95). The spread of *actinobacillosis* can often be discerned as long rows of small granulomas from the site of primary infection on the way to the regional nodes. The corded and nodular ulcerating lymphangitis of *cutaneous glanders* in horses must be distinguished from the specific forms of lymphangitis discussed below. Lymphangitis is often a prominent development in *cutaneous streptothricosis* in cattle, and can occur in various species with *cutaneous sporotrichosis*. Lipogranulomatous lymphangitis has been found in association with *intestinal lymphangiectasis* in dogs.

Bovine farcy is a chronic granulomatous disease of cattle in the tropics, and is characterized by nodular suppurative dermatitis, lymphangitis, and lymphadenitis. Most cases of bovine farcy are caused by *Mycobacterium farcinogenes* or *M. senegalense*.

Sporadic lymphangitis, *a nonspecific, nonulcerative lymphangitis affecting the hind legs of horses*, is relatively common. Synonyms include Monday morning disease, bigleg, and weed. The disease is ushered

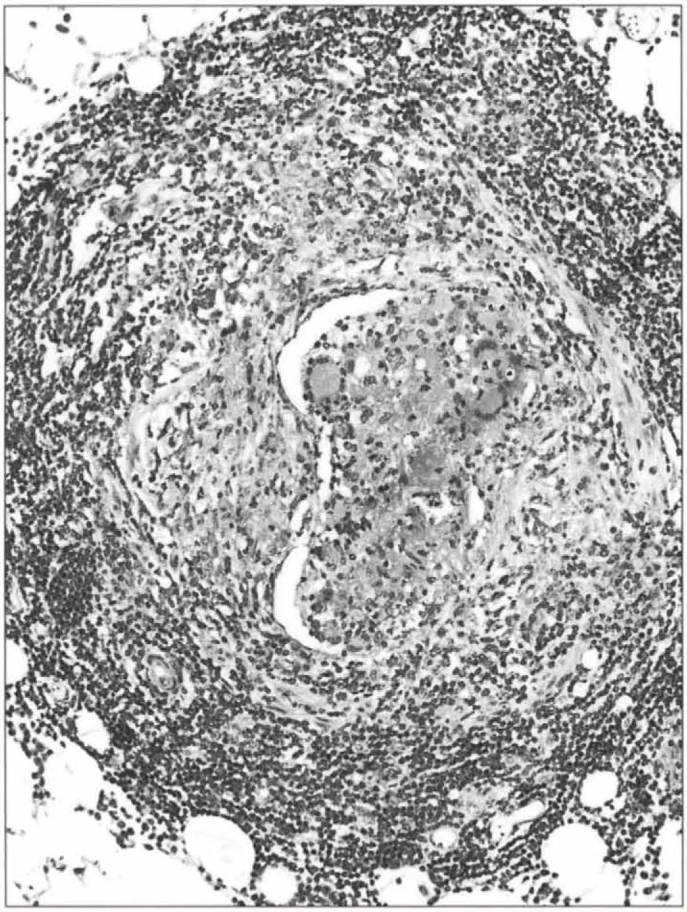

Figure 1.95 Lymphangitis in the ileal mesentery in Johne's disease. Note granulomatous inflammation with multinucleated giant cells.

in by acute lameness and severe cording of the lymphatics, although this is usually palpable only where the lymphatics lie near the saphenous vein on the inner aspect of the thigh. The limb swells rapidly, the swelling beginning about the pastern or metatarsus and reaching its zenith in 3 or 4 days, by which time it has usually extended beyond the stifle. There are transient general disturbances associated with fever. Recovery is usual although repeated attacks may occur and some cases are fatal, presumably as a result of septicemia. There is edema of the connective tissues of the distal portion of the limb with very rapid fibroplasia. Above the stifle, the edema is largely confined to the subcutis, this probably depending on the arrangement of fascia there. The lymphatics are dilated and the larger ones contain thick pus or a thinner mixture of pus and lymph. There is regional acute lymphadenitis.

The cause and pathogenesis of sporadic lymphangitis are unknown. Various pyogenic organisms have been recovered from the lesions, but it appears that, in the usual case, the initial acute infection is rapidly overcome. The occurrence of the disease after a period of inactivity, and the experimental production of a similar lesion by inoculation of bacteria into skin of the pasterns in undernourished horses, suggests that "stocking" of the hindlimbs (stagnant edema that is common in debilitated horses in periods of inactivity), predisposes to the progress of infection. However, most horses with stocking of the hindlimbs do not develop lymphangitis. The entry of infection is probably traumatic.

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Ulcerative lymphangitis

This is a chronic progressive inflammation of the subcutaneous lymphatics of horses. ***Corynebacterium pseudotuberculosis*** *(ovis)* is the cause of the classical condition, but similar lesions may be caused by other pyogenic organisms, including streptococci, staphylococci, *Rhodococcus equi*, and *Pseudomonas aeruginosa*. Two biotypes of *C. pseudotuberculosis* are recognized: a nitrate-negative type isolated from sheep and goats, and a nitrate-positive type isolated from horses and cattle. *Corynebacterium pseudotuberculosis* produces a potent phospholipase exotoxin that attacks the sphingomyelin of vascular endothelial cells, and may be important in aiding the spread of the bacteria by acting as a permeability factor. Ulcerative lymphangitis was an important cause of debility in the horse era, but is *now sporadic and rather rare*.

Some features of the biology of *C. pseudotuberculosis* are discussed with caseous lymphadenitis. In that disease, as in ulcerative lymphangitis, the infection begins in cutaneous wounds. *Ulcerative lymphangitis typically begins about the fetlocks of the hindlimbs.* As a result of lymphangitis, there is diffuse swelling in the leg soon followed by development of dermal nodules. These are abscesses that ulcerate and discharge thick creamy pus that may be bloodstained. Only a small area of skin may be sloughed so that, usually, the margins of the ulcer are undermined. The ulcers heal and leave small areas of depilated, depigmented skin. As the primary ulcers heal, new nodules form in adjacent skin, suppurate, ulcerate, and cicatrize, and in this way the disease progresses slowly. As the new nodules develop, the lymphatics between them become corded and as much as 1–2 cm thick, and fresh abscesses develop along the inflamed vessels. In uncontrolled infections, over a course of many months much of the skin of the body and neck as well as the limbs may be affected, and this leads to death. Typically, the regional lymph nodes, although moderately enlarged, do not become suppurative or fibrotic. In rare instances, bacteremia results in internal dissemination, especially to the kidneys.

Ulcerative lymphangitis also occurs in **cattle**, but as well as affecting the distal portions of the limbs, as in horses, the initial lesions may also develop within the dermis or subcutis of the neck, shoulder or flank. The initial abscesses are large and may be >5 cm in diameter. Extending away from them are thickened lymphatics along which new abscesses develop. The abscesses ulcerate on the skin and the ulcers persist, oozing a serum-like exudate. There is early lymphadenitis with swelling of the node and its fixation to the surrounding tissue. The inflammation spreads slowly and there is progressive suppurative lymphadenitis.

The equine biotype of *Corynebacterium pseudotuberculosis* causes *deep pectoral and ventral abdominal wall abscesses in horses* in the southwestern USA. The transmission of *C. pseudotuberculosis* in these cases is obscure, but may be by arthropod vectors. Ulcerative lymphangitis does not occur; dissemination is by bacteremia. Untoward sequelae include prolonged resolution of the abscesses, multiple and internal abscessation, and abortion.

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Epizootic lymphangitis

Epizootic lymphangitis, or *pseudofarcy*, is caused by ***Histoplasma capsulatum* var. *farciminosum*** (HCF) and clinically *resembles ulcerative lymphangitis and cutaneous glanders ("farcy")*. Epizootic lymphangitis occurs almost exclusively in *horses and mules*. "*Equine histoplasmosis*" is probably a more appropriate name, since HCF infection may be manifest as keratoconjunctivitis or pneumonia in the absence of past or concurrent lymphangitis.

Epizootic lymphangitis is enzootic in some Mediterranean countries, Africa, and the Near and Far East. The organism apparently exists as a soil saprophyte, and *enters the body through skin wounds of the lower limbs* to cause the classical epizootic lymphangitis, but may also invade castration wounds, ruptured abscessed lymph nodes after strangles, and gastric ulcers. *Histoplasma farciminosum* may cause sinusitis, and/or pneumonia via inhalation of contaminated dust, may cause rhinitis by contact with skin lesions, and may be transmitted by flies (*Musca* and *Stomoxys*) to cause keratoconjunctivitis.

The initial cutaneous lesion, or focus of infection in a wound, has a tendency to ulcerate, or it may undergo alternating periods of discharge and closure for some weeks before healing and scarifying. The infection may resolve, but often spreads centripetally in the adjacent tissues and along the lymphatics. In the adjacent tissues, which are swollen, small nodules develop (Fig. 1.96) which are about 1 cm in diameter and, in the course of a few days, these ulcerate to discharge at first a viscid gray exudate and, later, pus. The initial lesions are intradermal and are freely movable over the subcutaneous tissue. Spread of the infection is solely via the lymphatics and these convey the organisms into the subcutaneous and, occasionally, into the deeper tissues. The inflamed lymphatics are thickened and hard, and along their course new nodules form, ulcerate, discharge, and eventually heal with scarification, although there may be alternating periods of activity and quiescence with the growth of excessive granulation tissue. Sometimes the ulcers continue to enlarge and may coalesce. In this way, *the cutaneous infection spreads gradually but irregularly, but is always characterized by intradermal and subcutaneous nodules, ulcers, and irregularly swollen lymphatics*. In the early stages, the skin between the lesions remains normal and mobile, but in areas of extensive ulceration it becomes very thick, indurated, and firmly fused to the underlying tissues. When the thickened skin is incised, it presents the lardaceous appearance of granulation tissue in the horse and contains a number of small, yellow, purulent foci between which course the lymphatics, dilated and filled with pus and serous fluid. The regional lymph nodes are regularly involved and swollen. In the early stages, the swollen nodes contain many small foci of softening, but later the foci coalesce and are heavily encapsulated. The nodes may rupture. The condition is usually chronic, persisting for 3–12 months, and causes considerable debility, but low mortality.

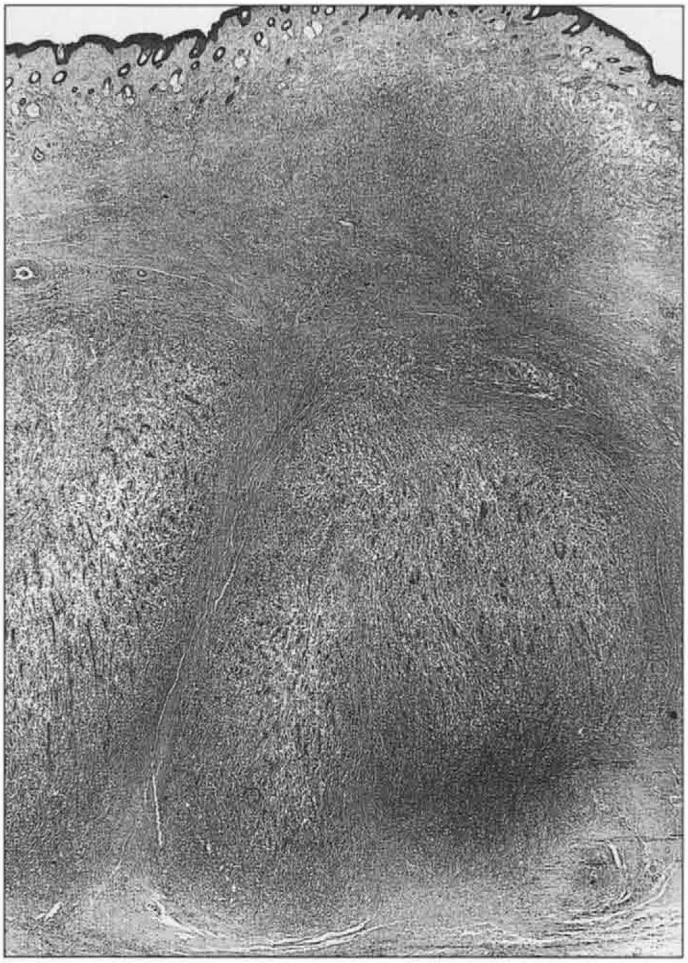

Figure 1.96 Intradermal nodes in **epizootic lymphangitis**. (Courtesy of DR Cordy.)

The disease may begin quite frequently on the *conjunctiva or nictitating membrane*, producing at first a small papule and a serous conjunctival discharge. The papules ulcerate to form flat, button-like growths of granulation tissue, the eyelids become severely swollen, and the inflammation extends to the tissues of the forehead.

Nasal infection is usually accompanied by a mucopurulent discharge containing large numbers of the fungus, and may be bloodstained. The lesions begin as yellow flat papules or nodules on the nasal mucosa and these soon break down to form craterous granulating ulcers that bleed easily. The nasal lesions are usually found near the external nares and they may extend from there on to the muzzle, or they may be found deep in the nasal cavity and in the pharynx. Similar lesions may occur in the nasal sinuses, the larynx, and the bronchi.

The *pulmonary lesions* may be solid granulomatous areas or they may be liquefied with pus-like contents. When ulcerative lesions are present on the nasal mucosa, there is suppurative regional lymphadenitis. The typical nodules of epizootic lymphangitis and the liquefied foci that form in deep tissues may also be found in the pleura, spleen, liver, testes, tunica vaginalis, and bone marrow.

The **diagnosis** of epizootic lymphangitis depends upon *demonstration of the fungus*, which is usually present in very large numbers in the contents of the nodules and fresh discharges from ulcers. The parasitic phase of the fungus is yeast-like, gram-positive, globose or oval in shape, and 2–3 μm in diameter. It occurs extracellularly and intracellularly in macrophages. The fluorescent antibody test is an effective means of diagnosis. In histologic sections, the organism is similar to *H. capsulatum*, consisting of a basophilic nuclear mass and thin capsule that can be demonstrated by stains for polysaccharides.

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Parasitic lymphangitis

Filariid worms of the genus **Brugia** (syn. *Wuchereria*) parasitize the lymphatic system of dogs and cats in tropical areas. *Brugia malayi* occurs in cats and primates in India and Malaysia; cats may be reservoir hosts for human infection. *Brugia patei* occurs in dogs and cats in Africa, *B. pahangi* in dogs and cats in Africa and the East, and *B. ceylonensis* in dogs in Sri Lanka. *Brugia* spp. are transmitted by mosquitoes, and their life cycles are similar to those of other filarial worms. Infective larvae enter peripheral lymphatics, migrate to the nearest lymph node and develop for two weeks before migrating down afferent lymphatics where they mature and *produce granulomatous lymphangitis, lymphangiectasis, and lymphadenitis*, but do not usually cause lymphedema and elephantiasis as occur in humans due to infection with *B. malayi* or the closely related *Wuchereria bancrofti*. The main importance of *Brugia* spp. lies in the differentiation of their microfilariae from those of *Dirofilaria immitis*, and in their role in human infections.

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VASCULAR NEOPLASMS

Tumors of endothelial origin are classified as benign or malignant, that is *angiomas* or *angiosarcomas*. Benign tumors consist of easily recognized vascular channels lined by a monolayer of relatively normal endothelial cells. Malignant tumors may not form well-organized vascular channels, are more cellular, and display cytologic anaplasia. Differentiation of vascular malformations or proliferations from hemangiomas may be difficult. Hemangiopericytoma is discussed with skin neoplasms.

There are rare reports of non-endothelial tumors, e.g., leiomyosarcoma, malignant melanoma, chondrosarcoma, arising from vessel walls, especially in the aorta or pulmonary artery.

The *nomenclature of benign vascular tumors or malformations* is confusing, and includes hemangioma, hemangioendothelioma, hamartoma, vascular malformation, telangiectasis, and angiokeratoma, and rare variants such as *angiolipoma, angiofibrolipoma*, and *angioleiomyoma*.

- **Hemangioma**, a benign tumor of endothelial cells, is supposedly a true neoplasm capable of independent growth. Some of the "growth" of a hemangioma may be due to congestion, hemorrhage, and thrombosis. Hemangiomas may be present at birth or soon after in children and foals, suggesting that they may in fact be vascular malformations.
- **Hemangioendothelioma** (HE) describes a benign vascular tumor with hypertrophic endothelial cells forming small hypercellular vascular channels, and is essentially a *very cellular capillary hemangioma*. In human pathology, HE is used to describe a vascular tumor that is histologically and clinically intermediate between hemangioma and angiosarcoma. Reports of rare variants of HE include *Kaposiform HE* in a dog, *epithelioid HE* in a dog, and *epithelioid/spindle cell HE* in a calf.
- **Hamartoma** is a general term that refers to *focal disordered overgrowth of mature tissue that is indigenous to the organ involved*. Since vascular tissue is ubiquitous, vascular hamartomas may occur in any site of the body; some authors refer to angiomas as vascular hamartomas. Most hamartomas are present at birth, and their growth is coordinated with that of the surrounding tissue.
- **Vascular malformation** is obviously a very broad term that may be included under the term hamartoma, and may include *arteriovenous fistula* in which thick-walled vessels occur in contrast to the thin-walled vessels of the other benign vascular proliferations.
- **Telangiectasis** refers to congenital or acquired *foci of abnormally dilated capillaries, sinusoids, arterioles, or venules*, usually seen as small masses in the skin and mucous membranes.
- **Angiokeratoma** is a rare benign tumor, usually of the nictitans and conjunctiva of dogs, in which *dilated vascular channels are associated with hyperplastic epithelium*.

Fortunately, these distinctions, though of pathogenetic interest, are of little practical importance since the lesions described are uniformly benign and excision, if possible, is curative.

Angioma

Hemangioma is a benign tumor of endothelial cells and may be classified as *capillary* or *cavernous* based on the size of the vascular channels formed. The tumor may be difficult to differentiate from vascular malformations and granulation tissue. Hemangiomas occur most commonly in older dogs, and are seen less frequently in other species. These tumors arise from vascular endothelium and hence may be found in any site in the body, but are *most common in the dermis and subcutis*, especially of the legs, flank, neck, face, and eyelid. Hemangiomas are usually single, ovoid, red-black masses of 0.5–3 cm in diameter, which ooze blood when cut. Histologically, blood-filled vascular spaces are lined by a single layer of well-differentiated endothelium, and may be thrombosed. The vascular spaces are separated by variable amounts of connective tissue stroma. Hemangiomas are not encapsulated, are not invasive, and do not recur after complete surgical excision. A rare form of cavernous hemangioma, analogous to the human **hemangioblastoma**, is reported in dogs, and is characterized by thin-walled capillaries separated by pleomorphic "stromal cells" of indeterminate origin.

Disseminated cavernous hemangioma is rare, but has been reported in calves, and a similar multifocal hemangioma has been reported in a pig. Multiple small tumors are present in skin, subcutis, gingiva, and internal organs. The condition has been termed *generalized congenital hemangiomatosis* and *juvenile bovine angiomatosis*, to include calves having either solitary, e.g., gingival or spinal canal, or multiple angiomatous lesions. Hemangiomas occur in the urinary bladder of cattle affected with enzootic hematuria, which is discussed in Vol. 2, Urinary system.

A number of hemangioma-like lesions occur in domestic animals. **Bovine cutaneous angiomatosis** of adult dairy cows is reported from Britain, France, and the USA. Nodular dermal vascular proliferations occur anywhere in the skin, but especially along the back, may have a considerable inflammatory component, and resemble "pyogenic granuloma" of humans. **Varicose tumor of the scrotum** of dogs is a benign vascular proliferation that resembles cavernous hemangioma when fully developed. Lesions resembling hemangioma occasionally develop in the skin of the scrotum or perineum of boars, or the vulva, mammary glands, or ovaries of sows. **Meningioangiomatosis**, a rare condition reported in dog, horse, and cow, is characterized by benign focal proliferation of blood vessels and meningothelial cells in the leptomeninges and underlying brain parenchyma.

Formerly thought to be due to neoplastic proliferation of endothelial cells and hence termed *malignant angioendotheliomatosis*, **intravascular lymphoma** is a rare large-cell lymphoma, seen in humans, dogs, and a cat, in which neoplastic lymphocytes proliferate within the lumens of blood vessels. **Lymphomatoid granulomatosis** is a rare pulmonary disorder of middle-aged dogs characterized by infiltration of lung and various other organs by neoplastic mononuclear cells admixed with eosinophils, lymphocytes, and plasma cells; originally thought to be angiocentric, this pulmonary neoplasm is now thought to be an atypical T-cell lymphoma that is angioinvasive.

Lymphangioma is a rare, benign tumor that consists of lymph channels forming capillary, cavernous, or cystic tumors. Lymphangiomas may occur as congenital malformations (hamartomas) or may develop spontaneously in adults. Cavernous and cystic lymphangiomas may progressively enlarge, dissect along fascial planes, and be difficult or impossible to remove surgically.

Glomangiomas (*glomus tumors*) of nonhuman primates, dogs, and cats, are rare benign neoplasms of the neuromyoarterial temperature receptors, primarily in the digits, characterized by branching vascular channels in a fibrous stroma that contains nests of specialized glomus cells, a type of smooth muscle cell. **Glomus jugulare and glomus pulmonale tumors** have been reported in dogs. The glomus jugulare and pulmonale are chemoreceptors, and tumors of these bodies resemble other chemodectomas, namely nests of monomorphic rounded cells with scant cytoplasm separated by thin septa. The tumor is encapsulated and grows expansively.

Angiosarcoma

Hemangiosarcoma (malignant hemangioendothelioma), a malignant tumor of endothelial cells, occurs most frequently in old dogs, but is less common than hemangioma. German Shepherd Dogs (Alsatians) are most commonly affected. Hemangiosarcoma occurs infrequently in cats, horses, cows, and sheep. The tumor may arise in any site of the body. *The most common primary sites in dogs are spleen, skin/subcutis, right atrium, and liver*; in cats, spleen, intestines, and subcutaneous tissue; in horses, ocular, cutaneous, and multicentric. *Hemangiosarcoma is the most common primary cardiac tumor of dogs*, and the tumor usually occurs subepicardially in the wall of the right atrium at the entrance to the auricle near the coronary groove (Fig. 1.97A) or in the auricular appendage. Hemangiosarcoma probably arises *de novo* and not from preexisting hemangiomas. The tumors have a gray to red-black hemorrhagic appearance, and may reach a diameter of 30 cm in the spleen as a result of hemorrhage within the tumor. Large hemorrhagic masses can often be found in the spleens of dogs; some are hematomas rather than either hemangiomas or hemangiosarcomas. The distinction is important because splenic hemangiosarcomas carry a very poor prognosis. However, the distinction can often be difficult because *splenic hemangiosarcomas often hemorrhage and if the resultant hematoma is not sampled carefully its neoplastic origin will be missed*. Following rupture, implants of splenic tissue or tumor may be found on the peritoneum. Hemangiosarcomas typically metastasize widely, especially to the lungs ("cannonball" metastases) (Fig. 1.97B). Histologically, these tumors consist of vascular spaces lined by elongated, plump, anaplastic endothelial cells. Vascular spaces must be identified in tumors with a highly cellular stroma to differentiate hemangiosarcoma from fibrosarcoma. Hemorrhage and necrosis commonly occur within these tumors, and death may occur due to hemorrhage into the peritoneal cavity, pericardial sac, or brain. When metastases are widespread, the primary tumor site may be difficult to determine, and multicentric origin is a possibility. Poorly differentiated hemangiosarcomas may usually be differentiated from spindle cell sarcomas or other nonendothelial neoplasms by immunohistochemical staining for factor VIII-related antigen, a marker of endothelial cells. *Ulex europaeus* lectin, a marker of neoplastic human endothelial cells, is unreliable as a marker of canine vascular tumors. Ultrastructurally, Weibel-Palade bodies are present in at least some of the typical neoplastic endothelial cells.

Lymphangiosarcoma (malignant lymphangioendothelioma) is an extremely rare tumor in domestic animals. The tumor is histologically similar to hemangiosarcoma, but the irregular vascular channels contain few red cells; it may be diffusely invasive and metastasize widely. Ultrastructurally, lymphangiosarcoma is distinguished from hemangiosarcoma, at least in cats and humans, by the general lack of a basal lamina, few micropinocytotic vesicles, few intercellular junctions, lack of pericytes, and a discontinuous endothelial cell layer in lymphangiosarcoma. Immunohistochemistry (factor VIII, vimentin, laminin) and lectinhistochemistry (RCA-I, PHA-E) may be other useful adjuncts in differentiating the two tumors.

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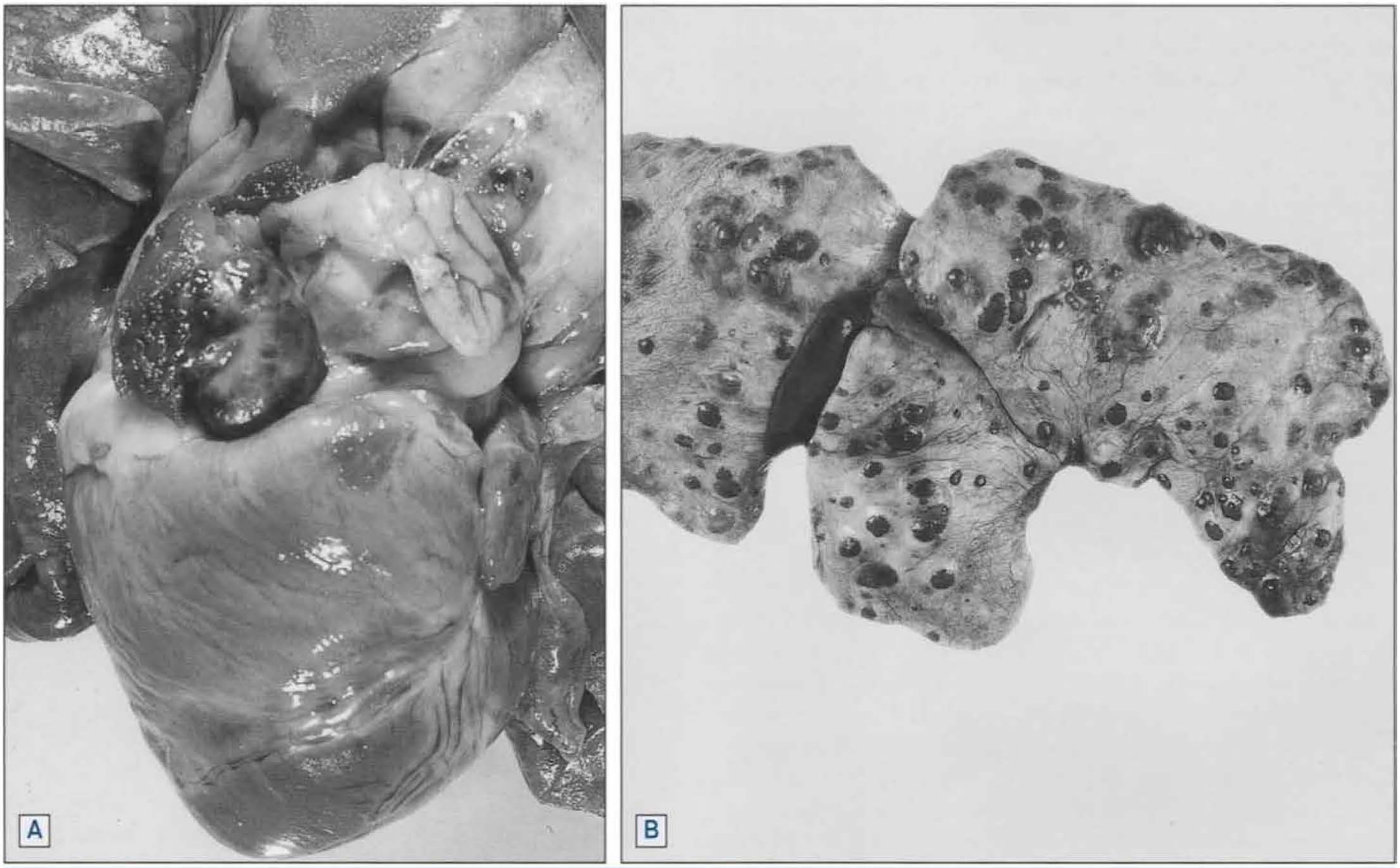

Figure 1.97 Hemangiosarcoma. **A.** In the right atrium of a dog. **B.** Multiple metastases of hemangiosarcoma to the lung in a dog.

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V. E. O. (Ted) Valli

With contribution from Patricia A. Gentry

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Stem cells

NORMAL HEMATOPOIESIS

The **hematopoietic system**, as used herein, refers to the system as a whole and *acknowledges the ultimate derivation of all fixed and circulating blood cells from a common pluripotential precursor that resides in the bone marrow of normal adults.*

The close relationship between bone and hematopoiesis is a constant finding in mammals and birds. While fetal and splenic hematopoiesis is largely erythroid, the full expression of all cell lines is largely confined to bone marrow. This intimate relationship appears to be dependent on the pattern of blood flow in bone and on a bone–bone marrow portal capillary system. The blood vessels of cortical bone that are derived from periosteal vessels arborize on the endosteal surface where they form a secondary capillary system that traverses the marrow cavity and drains into the central venous sinus. The radicles from the nutrient artery travel directly to the endosteum and penetrate the cortical bone to return via capillaries to the endosteal network. There is thus *anatomic evidence for a preferential microenvironment for hematopoiesis adjacent to endosteal bone* (Fig. 2.1), which may be generalized to explain the distribution of blood-forming activity in marrow and in ectopic or metaplastic bone.

Marrow capillaries form the conduit for delivery of blood cells to the systemic circulation via the vein which parallels the nutrient artery. All venous flow appears to be centripetal, with periosteal, osteal and marrow drainage all emptying into the central venous sinus. The marrow capillary endothelium is thin and the cells are without tight junctions. They are incompletely covered on the abluminal surface by a fenestrated basal lamina which is in turn covered to about 60% of area by cytoplasmic processes of adventitial reticular cells. These reticular cells are believed to be weakly phagocytic but are stromal in origin and distinct from the monocyte–macrophage system. They have no capability for hematopoietic differentiation but are **stromal stem cells** capable of forming adipocytes, osteoblasts, chondroblasts, and likely myoblasts. They form reticulin fibers that crosslink between vessels and form the structural support for bone marrow. These marrow stromal cells are hormonally active and essential for the maintenance, growth and differentiation of the hematopoietic stem cells by both cell–cell interactions and release of cytokines. Stromal cells secrete extracellular matrix proteins as well as producing cell surface transmembrane proteins that result in adhesion between the stromal cells and the developing hematopoietic cell lineages. A wide range of signaling molecules are produced, including M-CSF, G-CSF and IL-6,

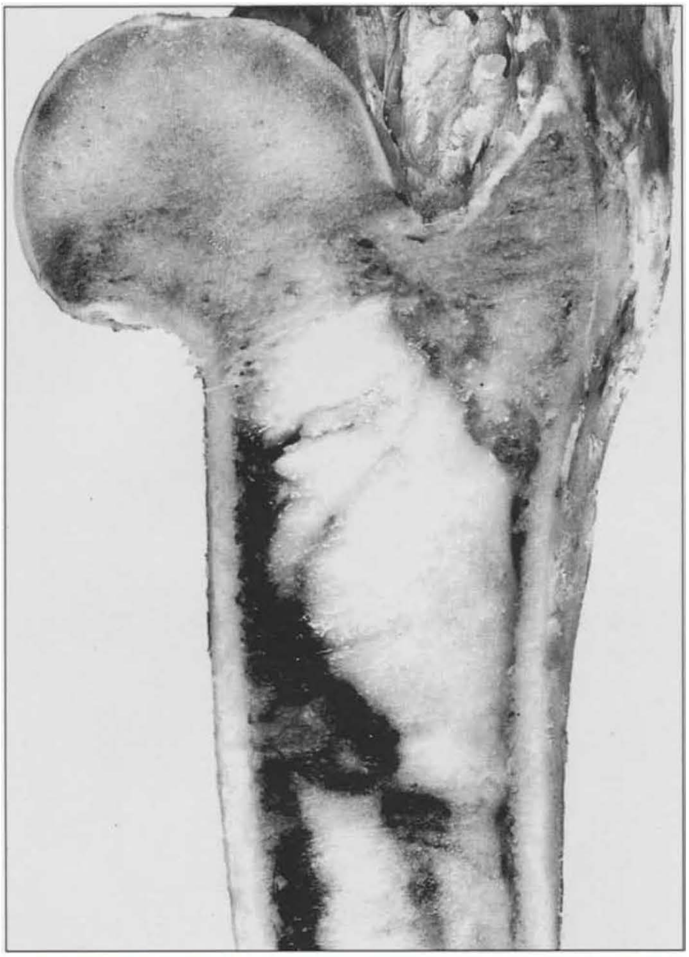

Figure 2.1 Expansion of erythropoietic marrow adjacent to **endosteum** in response to hemorrhagic anemia in a pig.

both in a constitutive and inducible manner, suggesting that the marrow stroma is able to adapt to body signals by altering the proportions of myeloid or lymphoid cell production.

The nonhematopoietic marrow includes nerves and fat cells. The adipocytes have a fatty acid content that differs from that in other sites. Marrow fat is more resistant to lipolysis in response to starvation. The nerves of bone marrow are both myelinated and unmyelinated and may innervate blood vessels.

In adults of all species, the hematopoietic marrow is concentrated in the spine, pelvis, sternum, ribs, calvarium, and proximal ends of the limb bones. When increased hematopoiesis is required, there is first lipolysis and myeloid expansion in those areas with residual red marrow. If requirements are further increased, there is hematopoietic conversion of fat in more distal areas. This expansion of activity, while focally irregular, follows a general preference for sites of higher blood flow which are found in close proximity to endosteal surfaces. Thus, *regenerative hematopoiesis is most prominent in areas of cancellous bone and in endosteal margins of metaphyses* (Fig. 2.1).

Coincident with the remodeling of fetal bone which provides a marrow cavity, there is colonization by hematopoietic stem cells which arise from the blood islands of the yolk sac. The cellular migration from the blood islands occurs in embryogenesis with seeding into the thymic anlage, the primordial lymph nodes, the liver and spleen. At birth, hematopoiesis is largely medullary with some persisting in liver and spleen and to a lesser extent in adrenals and kidney. In the adult, extramedullary hematopoiesis is largely limited to splenic erythropoiesis, as exemplified by the mouse.

The hematopoietic stem cell system *consists of a hierarchical lineage with the most primitive cells being capable of differentiation into all blood cell lines and almost unlimited self renewal* (Fig. 2.2). Growth and differentiation depends on the provision of a suitable inductive microenvironment dependent on an array of *growth factors* and *interleukins* (Figs 2.3, 2.4). Commitment to differentiation represents an interplay between inherent genetic capability and the selective hormonal pressure of the marrow microenvironment. More differentiated or committed stem cells can produce only one or two cell types and have limited proliferative capacity. The differentiation pathways result from the interaction of specific receptors for the various growth factors and the proportionate levels of the factors themselves. Thus, high levels of a particular factor may upregulate the production of its specific receptor and thereby recruit additional precursors to the production of a single cell lineage. Growth, differentiation, and maturation are forced by a higher proportion of receptors for the factors acting early in the proliferative cascade on primitive cells and vice versa (Fig. 2.2). These principles apply in leukemic as well as normal states and manipulation of this interplay is both a direct and supportive feature of antineoplastic chemotherapy. The cloning of growth factors and identification of their genetic origin to loci that are characteristically translocated in leukemias provides a molecular basis for understanding leukemogenesis. The process is most apparent when genes for growth factors translocate adjacent to potent promoters (oncogenes) thus providing the leukemic cells with the constitutive capability for stimulating their own proliferation.

Under normal circumstances, only a few of the primitive cells will be replicating at any time, the remainder being in resting phase and therefore relatively protected against insult. In the event of stem cell depletion by therapy or disease, the small number of active primitive cells is a limiting factor in the rate of recovery; and competition for the progeny most in demand (usually neutrophils) will decrease production of other cell lines (usually erythroid). *Primitive stem cells resemble medium lymphocytes and cannot be defined on a morphologic basis.* Maintenance of the stem-cell pool represents a significant proportion of the total marrow output.

Knowledge of relationships between precursor cells is provided by observations on stem cells in in vitro cultures. The precursors for the platelet and red cell series are related by genetic capability or by receptor status as are precursors for the neutrophil and monocyte series. These relationships provide an explanation for the clinical observation of concurrent increased production of red cells and platelets or of neutrophils and monocytes (Figs 2.3, 2.4). Hormonal factors influence the final defensive capability of leukocytes as well as their release from the marrow. Inflammation causes the release of a family of short- and long-acting mediators of which *tumor necrosis factor* stimulates growth factor production which increases marrow cell release. Failure of this final step in blood cell production results in *ineffective hematopoiesis* and death of the cells in the marrow. This syndrome is seen most frequently in the myelodysplasias of the cat and dog where there is the paradoxical concurrence of pancytopenia and hyperplastic marrow.

Knowledge of the cell kinetics and production times of blood cells and the proportions of their progenitors in normal marrow is essential to the

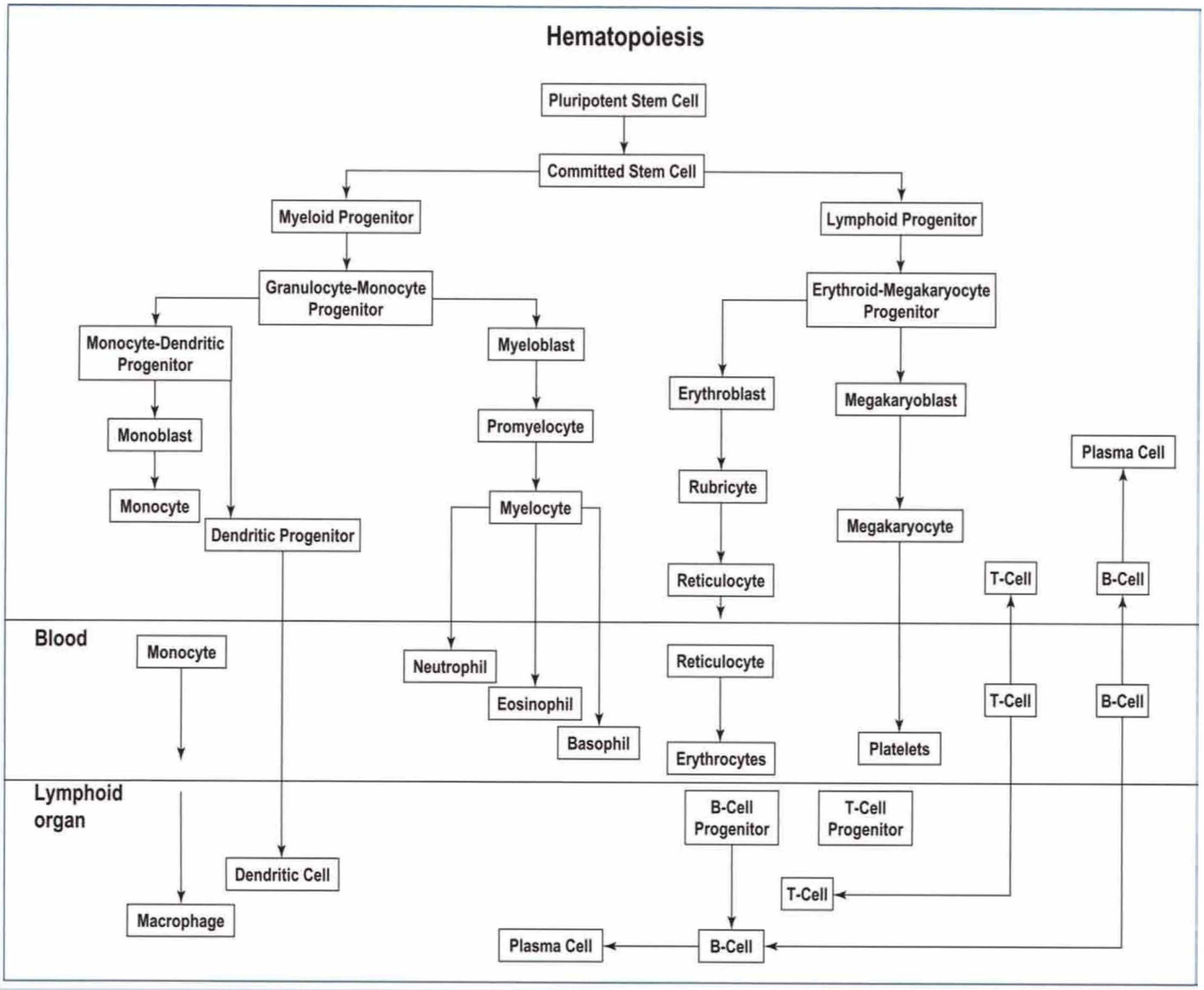
Hematopoiesis
Pluripotent Stem Cell
Committed Stem Cell
Myeloid Progenitor
Lymphoid Progenitor
Granulocyte-Monocyte Progenitor
Erythroid-Megakaryocyte Progenitor
Monocyte-Dendritic Progenitor
Myeloblast
Erythroblast
Megakaryoblast
Monoblast
Promyelocyte
Plasma Cell
Rubricyte
Monocyte
Myelocyte
Megakaryocyte
Dendritic Progenitor
Reticulocyte
T-Cell
B-Cell
Blood
Monocyte
Neutrophil
Reticulocyte
Eosinophil
T-Cell
B-Cell
Basophil
Erythrocytes
Platelets
Lymphoid organ
B-Cell Progenitor
T-Cell Progenitor
Dendritic Cell
T-Cell
Macrophage
Plasma Cell
B-Cell

Figure 2.2 Schematic representation of **hematopoietic stem cell system** illustrating the reduced capability for proliferation with increased pressure for differentiation. (Revised from Wintrobe's Clinical Hematology, 10th ed., and Schalm's Veterinary Hematology, 5th ed.)

interpretation of this tissue in disease. This information is summarized in Fig. 2.5.

The recognizable *blast cells* of the four lines (erythroid, neutrophilic, megakaryocytic, and monocytic) make up only about 1% of all marrow cells. Six morphological stages of myeloid and erythroid differentiation are recognized of which the first three are dividing cells and the last three are undergoing nuclear and cytoplasmic maturation. Very consistently in steady-state conditions, 25% of marrow cells are in the proliferative phase and 75% are in the maturation phase. An *increased proportion of proliferative phase* cells indicates active regeneration, leukemia, or ineffective hematopoiesis associated with nuclear maturation defects such as occurs in folate and vitamin B_{12}-related disorders. *Increased proportions of the maturation phase* cells with pancytopenia indicate ineffective hematopoiesis and myelodysplasia. Late asynchrony limited to the erythroid system concurrent with hypochromic anemia results from the *cytoplasmic maturation defect* of iron deficiency.

The proportions of proliferative phase cells indicate that *promyelocytes* and *prorubricytes* result from a simple doubling of the numbers of precursor blast cells. The proportions of myelocytes and basophilic rubricytes suggest that there is some proliferative activity in these stages, and nuclear labeling shows that metamyelocytes and polychromatic rubricytes can divide to a limited extent in conditions of increased demand. *Monocytes* have three morphological stages of differentiation of which the first two are dividing cells and the last is a maturation phase. The total production time of the monocyte appears to be only one quarter of that required to produce a neutrophil.

The *megakaryoblast* is about the size of a prorubricyte and can be identified by binucleation with interphase nuclei and intense cytoplasmic basophilia. Thrombopoietic cells can be divided into two broad groups. The proliferative phase cells are multinucleated and have minimal, very basophilic cytoplasm. Maturation phase cells have a multilobulated nucleus and abundant cytoplasm in which the

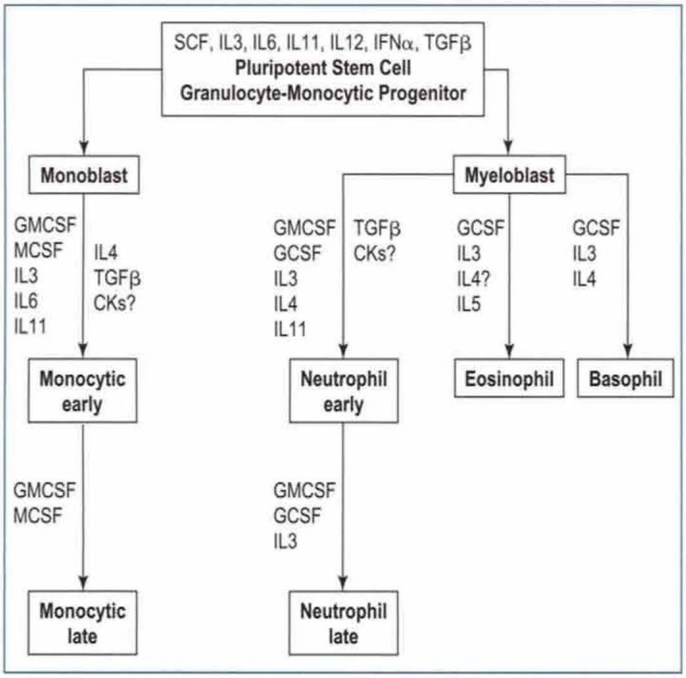
SCF, IL3, IL6, IL11, IL12, IFNα, TGFβ
Pluripotent Stem Cell
Granulocyte-Monocytic Progenitor
Monoblast
Myeloblast
GMCSF
MCSF
IL3
IL6
IL11
IL4
TGFβ
CKs?
GMCSF
GCSF
IL3
IL4
IL11
TGFβ
CKs?
GCSF
IL3
IL4?
IL5
GCSF
IL3
IL4
Monocytic early
Neutrophil early
Eosinophil
Basophil
GMCSF
MCSF
GMCSF
GCSF
IL3
Monocytic late
Neutrophil late

Figure 2.3 Cytokine regulation of granulocytic and monocytic production. (Revised from Wintrobe's Clinical Hematology, 10th ed., and Schalm's Veterinary Hematology, 5th ed.)

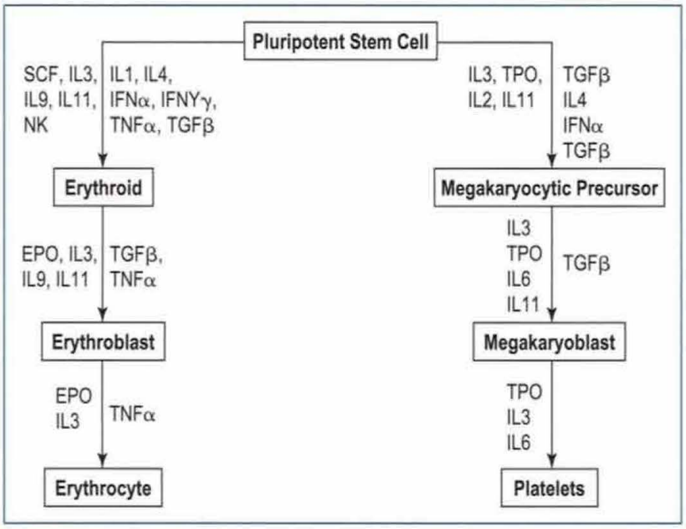
Pluripotent Stem Cell
SCF, IL3,
IL9, IL11,
NK
IL1, IL4,
IFNα, IFNYγ,
TNFα, TGFβ
IL3, TPO,
IL2, IL11
TGFβ
IL4
IFNα
TGFβ
Erythroid
Megakaryocytic Precursor
EPO, IL3,
IL9, IL11
TGFβ,
TNFα
IL3
TPO
IL6
IL11
TGFβ
Erythroblast
Megakaryoblast
EPO
IL3
TNFα
TPO
IL3
IL6
Erythrocyte
Platelets

Figure 2.4 Cytokine regulation of erythroid and thrombocytic production. (Revised from Wintrobe's Clinical Hematology, 10th ed., and Schalm's Veterinary Hematology, 5th ed.)

basophilia decreases as the granulation increases over the 4-day production period. Normal or increased nuclear ploidy with inadequate cytoplasmic volume is indicative of *ineffective thrombopoiesis*.

Lymphocytes are present in normal marrow at 3–5% of marrow cells. In inflammatory states, their number apparently increases by several percent and lymphocyte identification is complicated by an increased proportion of morphologically similar stem cells. Normally, *plasma cells* are present at very low levels in young animals and increase to 1–2% of marrow cells in adults. In chronic inflammatory disease, their numbers may rise to 25% or more of marrow cells. A large increase in plasma cells without cytologic atypia does not support a diagnosis of immunoblastic tumor. Germinal centers are not found in the marrow of normal animals at any age.

Cellularity of marrow *is used herein to indicate the proportions of hematopoietic to fatty marrow*, and **cell density** *to indicate the spatial relationship of hematopoietic cells to each other.* It is generally accepted that, excepting focal lesions such as tuberculosis, myeloma, or metastatic carcinoma, *red marrow obtained from any site will have the same cellularity and cell differential.* In other words, the marrow functions as an organ with proliferative and control mechanisms providing local and whole-body homogeneity. *Normal marrow functions at about 50% cellularity.* Persistently increased demands for cells may raise this proportion to 100%, in which event the marrow organ will be greatly expanded with conversion of fatty to red marrow in the endosteal marrow of the distal extremities. In normal animals, the hematopoietic cells are densely packed with their boundaries in close apposition. This close apposition is a function of cell–cell interaction in control mechanisms. In chronic disease with failing marrow, such as occurs in equine infectious anemia or bovine trypanosomiasis, the cell density is reduced, and in these circumstances the loss of marrow volume is partially compensated for by sinusoidal dilation. This latter change results in a marrow which appears "red" grossly but is cytologically hypofunctional.

Marrow iron is an important feature of myeloid evaluation. Stainable iron is rare in normal animals under a year of age and gradually increases with maturity. *Hemosiderin is intracellular in macrophages and in this state is only slowly mobilized for erythropoiesis.* Thus an animal with substantial marrow iron reserves will respond faster to acute hemorrhage if supplemental iron is provided. A coarse pattern of iron deposition in marrow indicates poor iron utilization, and is the characteristic pattern in the anemia of chronic disease. In hemolytic anemias, there is usually an increase in total marrow iron with abundant coarse and fine hemosiderin indicative of rapid turnover. A hypochromic microcytic anemia with late erythroid asynchrony and scant iron is found in iron deficiency anemia, while the same peripheral and marrow picture with increased hemosiderin strongly suggests copper deficiency.

In normal marrow, the **fat** is firm and opaque on gross examination. There is little change in volume on drying. The fat is completely removed in histologic processing leaving empty adipocytes. In contrast, in **serous atrophy** the marrow becomes grossly translucent and pink rather than clearly red or white, and the marrow shrinks rapidly on exposure of the medullary cavity to air indicating a higher than normal water content. Histologically, the fat cells in serous atrophy contain a thin, pink proteinaceous background. Serous atrophy of marrow fat is a late change in cachexia or starvation and is a significant lesion.

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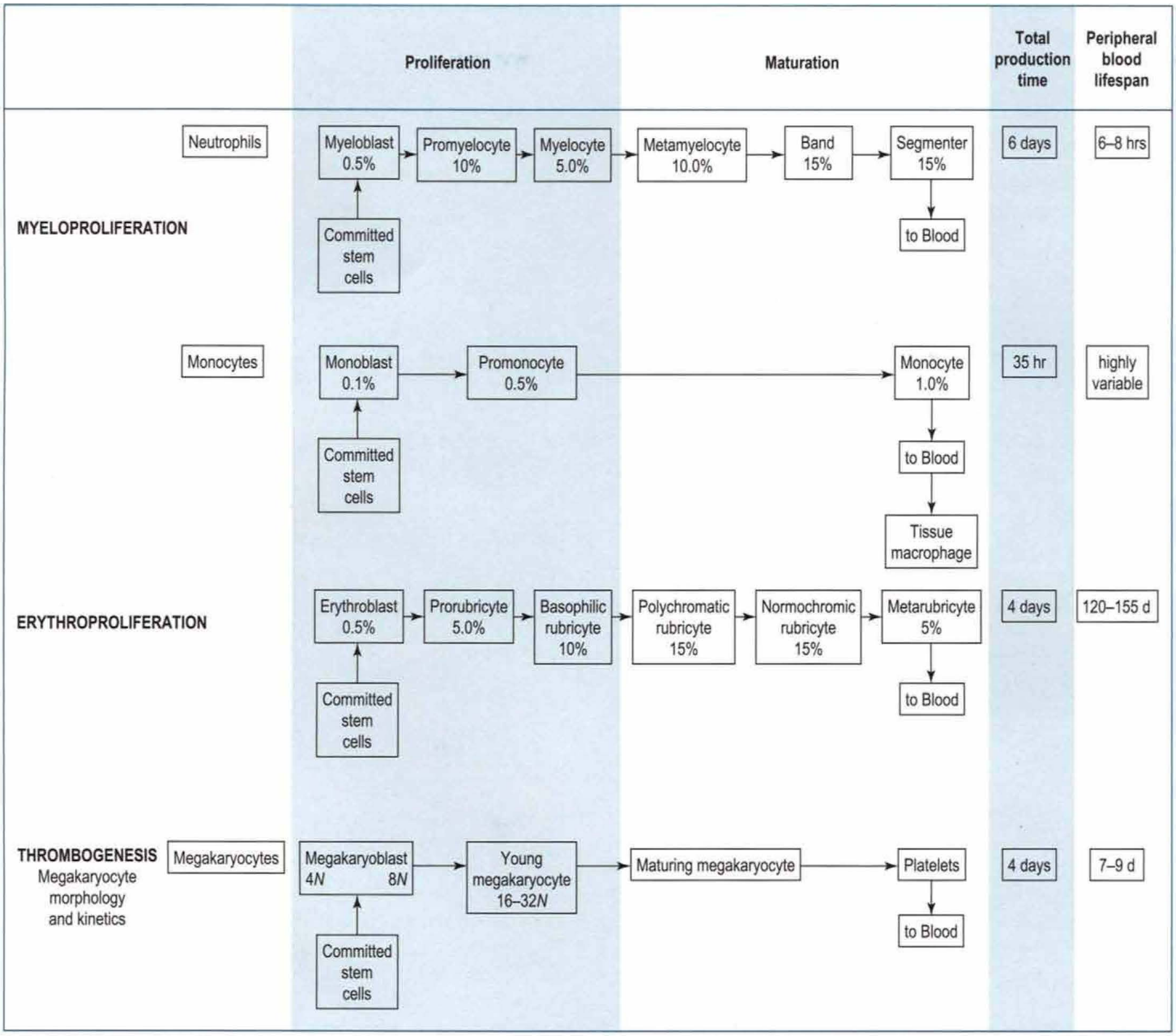
Proliferation
Maturation
Total production time
Peripheral blood lifespan
Neutrophils
Myeloblast 0.5%
Promyelocyte 10%
Myelocyte 5.0%
Metamyelocyte 10.0%
Band 15%
Segmenter 15%
6 days
6–8 hrs
MYELOPROLIFERATION
Committed stem cells
to Blood
Monocytes
Monoblast 0.1%
Promonocyte 0.5%
Monocyte 1.0%
35 hr
highly variable
Committed stem cells
to Blood
Tissue macrophage
ERYTHROPROLIFERATION
Erythroblast 0.5%
Prorubricyte 5.0%
Basophilic rubricyte 10%
Polychromatic rubricyte 15%
Normochromic rubricyte 15%
Metarubricyte 5%
4 days
120–155 d
Committed stem cells
to Blood
THROMBOGENESIS
Megakaryocyte morphology and kinetics
Megakaryocytes
Megakaryoblast 4N 8N
Young megakaryocyte 16–32N
Maturing megakaryocyte
Platelets
4 days
7–9 d
Committed stem cells
to Blood

Figure 2.5 Bone marrow and blood cell kinetics. Proportion of total marrow cells when myeloid:erythroid ratio is 1.0. Megakaryocytes at all stages account for 0.5–1.0% of marrow cells.

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DISORDERS OF STEM CELLS

Disorders of stem cell function include errors in the number and/or quality of cells produced. *Numerical disorders* consist of congenital cytopenias, acquired leukemias, toxic-induced neutropenia, idiopathic aplastic anemia, and pancytopenia. *Qualitative disorders* include congenital errors in cytoplasmic and nuclear development which result in defective protection against infection, oxygen transport, or clot formation.

Congenital abnormalities in blood cell numbers: cyclic hematopoiesis

Deficient cell production is seen in cyclic hematopoiesis of *humans* and *gray Collie dogs* and in the genetically anemic *W/W^v mouse* in which the error is in the restriction of the pluripotential stem cell pool. In contrast in the genetically anemic *Sl/Sl^d* mouse, the stem cell pool is adequate but marrow stroma is deficient resulting in an inadequate hematopoietic microenvironment. While not of clinical or diagnostic concern, these latter two diseases have contributed greatly to the understanding of hemopoietic dependence on a functional pluripotent stem cell in an appropriately fertile microenvironment. The diseases described in the next two sections are remarkable in that they combine a genetic origin with a dilution of melanin in pigmented hair and hematologic abnormalities.

Cyclic hematopoiesis *is a rare abnormality of pluripotent stem cells in gray Collies*, transmitted in an autosomal recessive manner. Affected puppies can be identified by their light silvery gray hair and by microphthalmia. The defect is lethal and death occurs before maturity. There is a trilineage cycling of blood cells with the deficits most apparent in neutrophils, which have the shortest peripheral lifespan estimated at 6–8 hours under steady-state conditions. In affected dogs, cycling occurs with a 12–14 day periodicity with severe neutropenia at each period of marrow hypoplasia followed by marked neutrophilia in rebound. The disease appears due to cycling levels of suppressor T lymphocytes which are in the ascendancy when hematopoiesis is failing and are reduced during recovery. The total leukocyte count of 2–20 × 10^9/L is treated by increasing the size of the stem cell pool by the administration of lithium carbonate, endotoxin, or prednisone. The latter acts presumably by reducing the level of the suppressive managerial T cells. The precursors for megakaryocytes and rubricytes go through similar cycles but, because of their longer peripheral lifespan, the deficits in these cells are less apparent. The dogs are immunologically competent, and lymphoid hyperplasia is an early compensatory mechanism for recurrent neutropenia. Amyloidosis is a common sequel in animals that survive 3 months or longer.

Dogs with cyclic hematopoiesis are difficult to maintain. *Disease is recurrent and related to sepsis.* Characteristically there are about 3 days of neutropenia followed by normal or increased neutrophil counts for 6–7 days. At periods of neutropenia, there is marked left shift with myelocytes present which have toxic vacuolation and granulation (Fig. 2.6) and Doehle bodies. Apparently cell supply is not the only error, and neutrophils have deficits in killing for a variety of bacteria. Animals may be mildly icteric and there is increased blood urea especially in those dogs which develop amyloidosis.

The disease tends to vary somewhat with the age of the animal, but *the same organ systems are affected at all ages*. The animal is usually in poor condition and there are often focal skin lesions with abscessation or cellulitis. Young dogs tend to have bronchopneumonia with purulent exudates, whereas older animals tend to have interstitial pneumonia with focal areas of scarring. There is frequently valvular endocarditis and catarrhal enteritis and frequently bleeding into the lower intestinal tract. The liver is usually pale and fatty and the spleen is uniformly enlarged. Lymph nodes are generally enlarged and may be focally abscessed. The bone marrow is uniformly reddened in mid-femoral shaft. Focal areas of sepsis, which may occur virtually anywhere in the monocyte/macrophage system, appear to impair bone remodeling. Dogs which die in the first few weeks of life tend to succumb to infections of the respiratory and enteric tract, whereas dogs which survive for several months frequently die of renal failure.

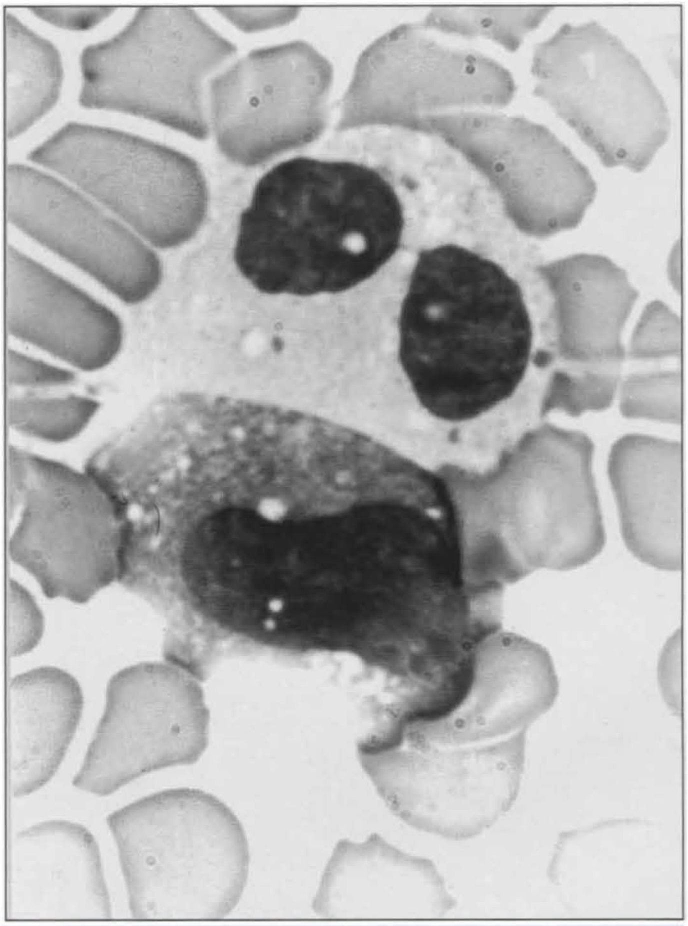

Figure 2.6 Cyclic hematopoiesis in a dog; blood smear. Immature monocyte, center. Bilobed neutrophil indicative of premature release from marrow contains toxic granules and vacuoles.

The lesions in the lung, kidney and gastrointestinal tract are those of chronic and recurrent inflammation usually due to common bacterial invaders. The principal changes occur in the hemic lymphatic system and consist of *widespread lymphoid atrophy* with reduced density of splenic periarteriolar sheaths (Fig. 2.7) and of germinal centers. In some dogs, the germinal centers may be markedly enlarged by both perifollicular and interfollicular deposition of amyloid which may appear as early as 3 months of age. The lymph nodes have normal numbers of germinal centers with poor cell density, and the paracortical areas have reduced area and cell density and there is characteristic sinus histiocytosis without medullary cord hyperplasia (Fig. 2.8). *The bone marrow varies widely depending on the stage of the cycle at the time of death.* Characteristic changes include a progressive increase in sinus and hemopoietic area with loss of fat and a generalized increase in connective tissue that becomes more prominent with survival. There is a characteristic

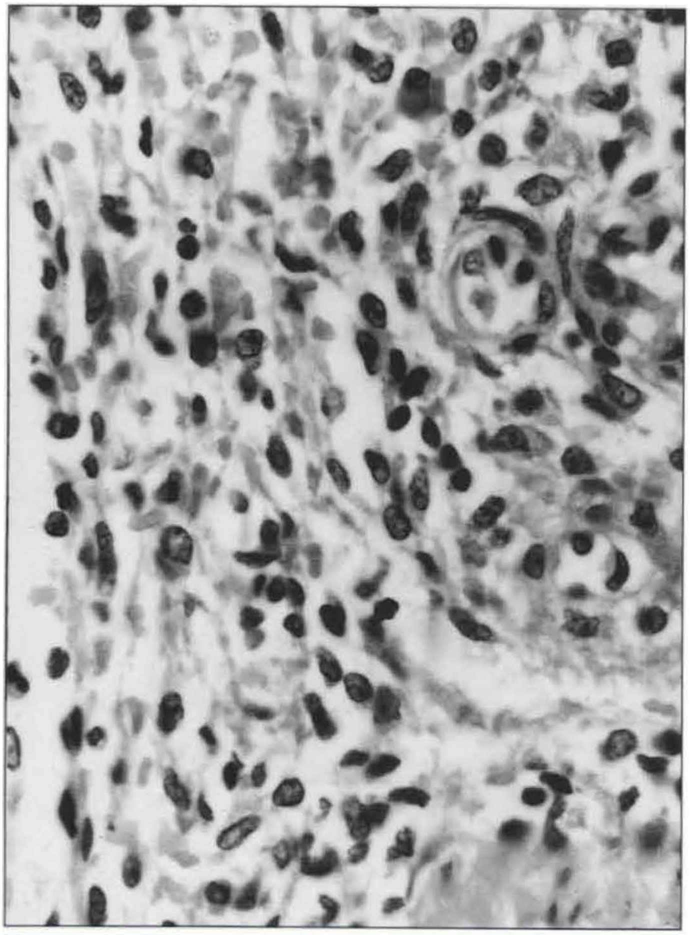

Figure 2.7 **Cyclic hematopoiesis** in a dog: spleen. Depletion of small lymphocytes in periarteriolar sheath.

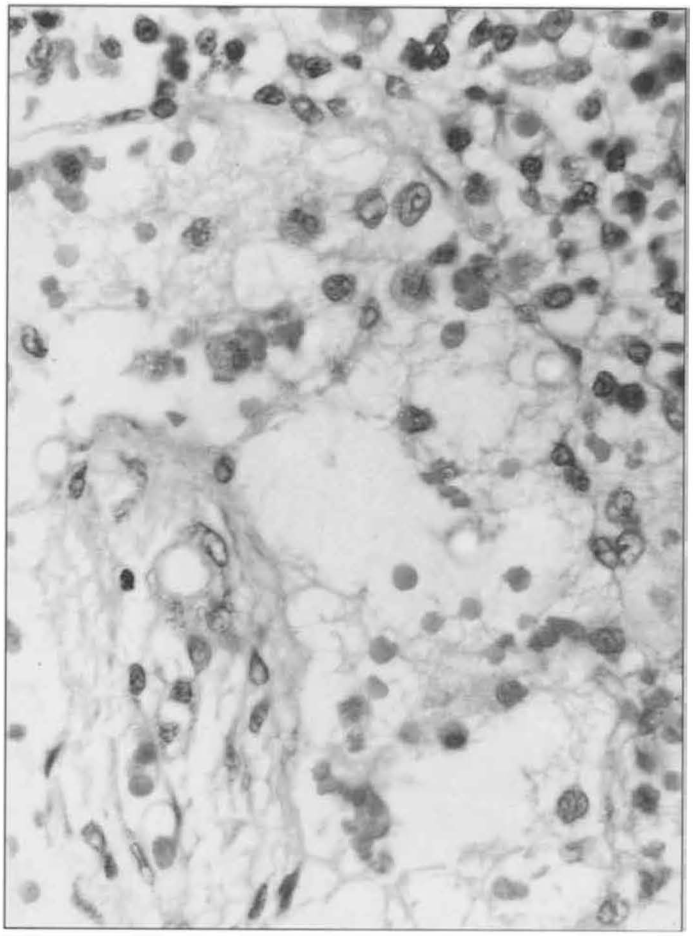

Figure 2.8 **Cyclic hematopoiesis** in a dog: lymph node. Cellular depletion of medullary cords and sinuses.

increase in marrow plasma cells and at the time of rebound, the marrow granulocyte reserves are increased, while at the time of aplasia there are few young cells present and the marrow is hypocellular. At the stage of rebound, there are many large, round cells with deeply stained cytoplasm which are likely myeloblasts and promyelocytes with a lesser number of erythroid precursors. There is thymic cortical atrophy.

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Congenital abnormalities in blood cell function

Chediak-Higashi syndrome

The Chediak-Higashi syndrome is a composite disorder of granule formation in cells and is of a simple recessive character in cattle of the Hereford, Brangus, and Japanese black breeds, cats, mink (Aleutian disease), mice, and humans. The condition may also occur in bison, killer whales, foxes, and white tigers. The disease is manifested clinically by *partial albinism or color dilution, high susceptibility to infections, and a hemorrhagic tendency.* It is a rare syndrome except in mink, in which all of the favored blue Aleutian strains are affected.

Enlarged cytoplasmic granules, which are the result of fusion of preexisting granules of normal size, are found in most types of cells which normally contain granules. The partial albinism is ascribed to *clumping of melanin granules* in such tissues as eye and skin and their fusion with lysosomes. There are fewer, larger granules than normal also in hepatocytes, renal epithelium, neurons, endothelial cells, and the blood leukocytes.

The prolonged bleeding time and hemorrhagic tendency is, at least in part, due to defects in platelets. The clumping response of platelets on exposure to collagen is deficient and is associated with reduced levels of platelet adenosine diphosphate.

Giant lysosomes are present in circulating granulocytes, lymphocytes, and monocytes. Phagocytosis appears to be normal but bacteriocidal activity is impaired. The impaired capacity of phagocytes to deal with ingested macromolecules or microorganisms appears to be due to *impaired fusion of lysosomes with phagosomes* and provides an explanation for the high incidence of recurrent infections.

Bovine leukocyte adhesion deficiency

Bovine leukocyte adhesion deficiency (BLAD) occurs in Holstein cattle as a heritable syndrome of chronic febrile disease characterized by poor growth and early death. *The disease is due to a congenital deficiency of leukocyte integrins essential for normal adhesion to endothelium and subsequent emigration to sites of inflammation.* These integrins have been classified as CD11/CD18 according to their alpha and beta subunits. Clinically, there is emaciation, dermatitis, persistent oral ulcers, enlarged lymph nodes, and slow healing of wounds and infections. There is anemia with marked persistent neutrophilic leukocytosis of up to 100×10^9/L and mild left shift. Bone marrow has marked myeloid shift with synchronous maturation of all cell lines, which appear normal by light and electron microscopy. There is polyclonal gammopathy with decreased albumin/globulin ratio. Morphologically, there is fibrosis of lymph nodes and wide spread infection of body surfaces but, significantly, neutrophils are present in large numbers in vessels but not in infected tissues. Neutrophils from these animals have reduced motility, phagocytosis, and intracellular killing. Since motility and phagocytosis are abnormal, this disease is distinct from "chronic granulomatous disease" of humans. The cases described all derived from a single sire, apparently as an autosomal recessive trait.

Canine leukocyte adhesion deficiency

Canine leukocyte adhesion deficiency (CLAD) occurs as the result of an *inherited autosomal recessive trait in young Irish Setter dogs* expressed as a syndrome of *recurrent severe bacterial infections*. The cause is a missense mutation in the beta-2 integrin subunit gene (ITGB2) that results in a defective beta-2 subunit of heterodimeric leukocyte adhesion proteins. The lack of the Cd11a,b,c and CD18 leukocyte adhesion proteins is similar to the granulocytopathy syndromes described in humans and in Holstein calves. Functionally the neutrophils from these dogs have severely diminished capacity for adherence, aggregation, chemotaxis, and reduced capability for phagocytosis. The neutrophils have normal oxidative burst activity and the animals have normal levels of complement and immunoglobulin. Morphologically the disease in dogs appears to be similar to BLAD described in affected Holstein calves with abundant neutrophils in small blood vessels in affected sites but not in the adjacent tissues and sites of bacterial colonization. Since the error lies in the mobilization and chemotaxis of the neutrophils but not the capacity for intracellular killing, CLAD is distinct from the pathogenesis of the chronic granulomatous disease syndrome of children wherein the defect is in the oxidative burst activity and intracellular killing of bacteria.

Pelger-Huet anomaly

The Pelger-Huet anomaly is a *benign dominant hereditary condition* characterized by *hyposegmentation of neutrophil nuclei* which have mature aggregation of chromatin despite their immature shape. The condition occurs in humans, dogs, and rabbits. The neutrophils respond normally in functional tests and heterozygotes survive normally. A homozygous form in rabbits is lethal. The main importance of the trait is to distinguish it from inflammatory states. Affected animals have a normal total leukocyte count; band neutrophils may be 30–70% of total cells. Metamyelocytes are present at much lower frequency, and myelocytes are not found in the blood of healthy dogs. An ephemeral form resembling this condition has been described in the dog possibly resulting from drug idiosyncracy.

Feline mucopolysaccharidosis

The type I or Hurler/Scheie and type VI or Maroteaux-Lamy forms of this disease occur in cats and are associated with *metachromatic, 0.5–1.0 μm diameter granules in the neutrophils*, which are not prominent with routine stains. Typical facial dysmorphia consisting of frontal bossing and depressed nasal bridge is most obvious in the type IV syndrome seen in the Siamese breed. Affected animals survive to reproductive age with normal mentation, small stature and cloudy corneas. They are not noted to be unusually susceptible to infection.

Sphingomyelinosis

A lysosomal storage disease due to an autosomal recessive trait resulting in a loss of sphingomyelinase occurs in humans and also in the cat, dog, and mouse. Storage in the hematopoietic system results in splenomegaly with psychomotor retardation as a result of accumulation in neurons. The enzyme is usually absent from leukocytes in the form seen in animals, and *lymphocytes and monocytes are characteristically vacuolated*. Nervous signs predominate.

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The leukon

LEUKOCYTE RESPONSES TO PERIPHERAL DISEASE

This section describes changes which occur in the different types of leukocytes. The following section deals with characteristic changes in leukocyte numbers in various species of domestic animals.

Neutrophils

Studies of granulokinetics using nuclear labels (tritiated thymidine) show remarkably similar results for humans and those domestic species studied. In the steady state, a total production time of 6 days is followed by a ripening period of 1 day prior to release from marrow. The peripheral circulation time is probably 6–8 hours. Neutrophils do not re-enter the circulation after tissue migration. Band neutrophils are released into the circulation in a bolus or discontinuous fashion.

The fine structure of neutrophils is quite similar for all domestic species. The **primary or azurophil granules,** most prominent in promyelocytes, contain myeloperoxidase, neutral and cationic proteases, are peroxidase-positive, and have microbicidal properties. The **secondary or specific granules** synthesized later in granulocytopoiesis contain lysozyme, collagenase and in most species, alkaline phosphatase, except in the dog, cat, mouse, rhesus monkey, and birds. The secondary granules are peroxidase-negative and form the background cytoplasmic density of the mature cells. The functional properties of neutrophils appear in a distinct order in granulopoiesis with Fc receptors first, followed by immune phagocytosis, complement receptors, oxygen-independent microbial killing, oxygen-dependent microbial killing, and finally chemotaxis. Phagocytosis is followed by activation of the NADPH-oxidase enzyme burst secretory system which is bactericidal for a wide variety of pathogens.

In *toxemic states*, the primary granules are visible in the cytoplasm of mature neutrophils as fine azurophilic or pink granules with Romanovsky stains. These primary granules are normally present in mature cells and can be demonstrated as artifactual change in aged or overstained neutrophils. Other indications of systemic toxemia include cytoplasmic vacuoles and Doehle body formation. In general, *toxic granulation represents the mildest change, followed by vacuolation and Doehle body formation.* In severe toxemias, such as those that occur in canine pyometra, bovine mastitis, and equine salmonellosis, all three changes occur together. Doehle bodies are composed of lamellae of rough endoplasmic reticulum persisting from the normal basophilia of immaturity. Since rough endoplasmic reticulum is part of the synthetic mechanism of the precursor cells, it is expected that as toxic changes increase, the specific or secondary granules are decreased. In contrast, animals with well-controlled or mild inflammatory disease usually have increased secondary granulation with minimal toxic changes. Presumably the functional capability of these cells varies directly with the level of granule-bound enzyme, as evidenced by cytoplasmic density in stained blood films.

There is considerable variation in enzyme content of granules in various species, with the dog and cat neutrophil lacking alkaline phosphatase. *Neutrophils function as secretory cells in the inflammatory reaction,* with granule exocytosis being mediated by a number of stimuli. The secreted material modulates and enhances the acute inflammatory reaction and appears to stimulate myelopoiesis. Such stimulation may partially explain why in diseases such as enteritis, mastitis, and dermatitis, in which neutrophils are lost from the body, blood neutrophils tend to be fewer than in tissue reactions where secretory granules are retained. The spilling of enzyme into tissues during phagocytosis may cause tissue injury in acute inflammation. The phagocytic vacuoles of neutrophils have a pH as low as 4.5, and adherence of activated cells to endothelium may cause vascular injury.

Leukocytes are usually quantitated on a numerical basis, however numbers and functional capability are not synonymous. Neutrophils may be hypofunctional and cytologically appear less dense in the same manner as red cells may be hypochromic and carry less oxygen. Neutrophils produced under conditions of severe toxemia have less specific granulation and are vacuolated; presumably their function is decreased in concert with their cytologic deterioration. In general, *the cytologic quality of neutrophil cytoplasm varies directly with leukocyte count and the clinical progress of the animal.* Thus animals in extremis tend to have very low leukocyte counts with severe toxic changes, whereas those that are responding well to their infection have high cell counts and the density of neutrophil cytoplasm is increased. Many factors may influence the quantitative and qualitative responses of the bone marrow in infectious disease.

Neutrophils complete their maturation under the influence of a number of factors including granulocyte-monocyte colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and interleukins-3, -4, -11 (Fig. 2.3). In *neonates*, free radical production in neutrophils and microbicidal capability but not phagocytosis is below adult levels, which may be a factor in their early susceptibility to infectious disease. In addition, severe *protein-calorie malnutrition* impairs neutrophil adherence and motility. Motility in neutrophils is accomplished by membrane flow in the direction of chemotaxis in a "tank-tread" manner. *Blood cells do not necessarily function independently,* for example, neutrophils and platelets act synergistically in both coagulation and inflammation. Thus selective cytopenias, such as neutropenia or thrombocytopenia, will impair the function of cells that are not numerically deficient. *Bacterial toxins* acting locally induce an inflammatory response, and result systemically in release of mature neutrophils and toxic changes in those in the process of maturation. Chronic infection with *Bovine viral diarrhea virus* decreases the functional reactivity of both neutrophils and lymphocytes. Phagocytosis is depressed in *hypercoagulable states*, likely due to the release of fibronectin products lysed by plasmin. Leukocyte function is impaired by *endogenous toxins* as occurs in uremia, and neutrophil enzyme is reduced in iron-deficient states. Leukocyte function may be significantly altered by *dietary factors*. For example, selenium deficiency causes reduced intracellular killing, and omega-3 fatty acids reduce leukotriene B4 and thus have anti-inflammatory effects. *Dexamethasone* depresses the functions of both neutrophils and lymphocytes, and this effect on neutrophils may be blocked by administration of ascorbic acid.

Antibiotics vary in their ability to enter neutrophils, but those that do, such as tetracycline and chloramphenicol, decrease chemotaxis;

tetracyclines and polymyxin B decrease both phagocytic activity and oxidative metabolism. Finally, the systemic effects of *cancer* include impairment of phagocyte function.

Eosinophils

The production kinetics of eosinophils are similar to those of neutrophils, but unlike the latter, they may survive many days in tissues. Eosinophils respond to different stimuli and their granulation is cell specific and, to some extent, species specific. They are capable of killing bacteria but are less efficient than neutrophils. Their armamentarium includes the major basic protein of their **specific granules**, a potent H_2O_2–peroxidase–halide system, and inactivators of the leukotrienes of anaphylaxis. They have *receptors* for complement 3b as well as for IgA, particularly secretory IgA, which may be why eosinophils tend to localize in mucosal surfaces as well as provide the means by which they bind to tissue parasites. Eosinophils express *leukocyte adhesion molecules*, but like lymphocytes are able to emigrate to sites of inflammation in animals with the leukocyte adhesion deficiency syndrome. In comparison to neutrophils, *eosinophils are primarily residents of tissues* and appear to be targeted to tissues on release from marrow such that while their level in the blood may not be elevated there may be heavy tissue accumulation. The association between parasitism and eosinophilia is less than is usually accepted. Eosinophils are late arrivals in the immune response to parasites, and their chemotaxis can be explained on the basis of specific lymphokines from prior sensitized T lymphocytes. The increase in mature eosinophils seen in mature sheep with hemonchosis is probably part of the syndrome of iron deficiency and not directly related to parasitism.

In blood films, eosinophils have more cytoplasmic basophilia than neutrophils. This is reflected in their wide spectral separation from other cells in fluorescent activated cell counters. In diseased animals, eosinophils frequently have more marked **toxic changes** than neutrophils. These changes consist of *reduced granulation, increased diffuse cytoplasmic basophilia, and large vacuoles.* The toxic changes are seen most often in diseases involving the gut, lung, and skin, and are not species specific. Eosinophils are most prominent in these three tissues, and this may provide an anatomic basis for the preference. Eosinophils can be considered to be concentrated on body surfaces, with greatest frequency in intestine and lesser numbers in dermis and hemiclymphatic tissue, lung, and uterus. Significantly, many tissues normally contain none at all. Cows in estrus usually have an eosinophilia of $0.5–1.5 \times 10^9/L$. Toxic changes are absent.

The **functions** of eosinophils are incompletely understood. They are known to interact with homocytotropic antibodies (IgE and IgG) and with mast cells and basophils. The antibody and T lymphocytes provide specificity to the reaction, and the IgE on mast cells attracts eosinophils which modulate the inflammatory reaction. The relative quantities of tissue IgE, extractable histamine, and eosinophils suggest that these components form a system which is most prominent on body surfaces, immunologically mediated, often parasite related, and frequently associated with eosinophilia. While eosinophils are able to phagocytose and kill bacteria, *their main role seems to be to react against large non-phagocytosable organisms, such as parasites, fungi and foreign bodies.*

When benign **eosinophilia** is marked and persistent, it can be assumed to be due to immune phenomena. Less clear is the distinction between benign hypereosinophilic syndrome and chronic myelogenous leukemia of the eosinophil type. While both conditions will cause tissue damage, the immaturity and atypia are more prominent in the malignant disease and are usually accompanied by some degree of basophilia. **Eosinopenia** is characteristic of acute infection. It appears that corticosteroid modulates the level of blood eosinophils, but their production and release may persist despite high levels of steroid if the peripheral stimulus is of sufficient intensity. Eosinophils appear to have a short circulation time in disease states, which may explain the apparent increased eosinopoiesis in marrow with focal tissue accumulation, without eosinophilia, such as occurs in eosinophilic myositis. The level of serum cationic protein derived from eosinophils may markedly increase in acute inflammation despite eosinopenia, suggesting that these cells are being produced and are degranulating rapidly but are not obvious in the blood because of rapid transit.

Basophils

Basophils arise from bone marrow precursors which can first be recognized at the promyelocyte stage. A number of cytokines are active in stimulation of basophil production including interleukins 11, 4, and 3, of which the last seems to be most important. Their maturation sequence and kinetics are similar to neutrophils with peak peripheral blood arrival of labeled cells 7 days after injection of tritiated thymidine. The circulation time is unknown but is probably short. Newly released cells are specifically directed; they do not have a random and age-dependent removal as do neutrophils. Basophils do not segment as fully as neutrophils, and in this respect resemble eosinophils. Basophils are about a third greater in diameter in blood films than neutrophils, and retain smooth nuclear boundaries unlike the irregular constrictions of mature neutrophils. These characteristics are of interest because *basophils are easily degranulated in slide preparation and may be mistaken for neutrophils.*

The relationship between basophils and mast cells appears to be functional, with a reciprocal variation in their relative proportions. *Basophils have segmented nuclei with moderate granulation, whereas mast cells are mononuclear and heavily granulated.* The granules of both cells contain *histamine and heparin*, but the histamine concentration in mast cells is some 20 times greater than in basophils. Basophil granules are like eosinophils, ultrastructurally distinct, contain major basic protein, and are peroxidase-positive. Mast cells contain hydrolytic enzymes and 5-hydroxytryptamine, which are not present in basophils.

It appears that basophils have a committed precursor at about the same level as neutrophils, and which is probably more primitive than the myeloblast. *Mast cells* are believed to be derived from an undifferentiated precursor in perivascular connective tissue that may in turn derive from monocytes. In this respect, mast cells differ from basophils and eosinophils which have their progenitors in marrow, whereas mast cells may arise in peripheral tissues as well. In cytologic preparations of mast cell tumors, it is common to find stromal type cells with bipolar and incomplete cytoplasmic boundaries which contain only one or two basophilic granules. These may be the stem cells for the bulk of the tumor mass.

Basophils are capable of phagocytosis and are weakly motile. Unlike mast cells, which degranulate by exocytosis and can then regenerate, *basophils largely degranulate by diffuse internal lysis and have less synthetic capability.* It is likely that basophils of most species have receptors for IgE and degranulate when attached antibody binds with specific antigen. There is, however, marked heterogeneity of response by basophils in different sites, as in mast cells, and activation and secretion is not an all-or-nothing phenomenon. The secretory activity of both basophils and mast cells is dependent upon the presence of calcium and magnesium ions. In addition to their specific involvement in hypersensitivity reactions, basophils release heparin during postprandial lipemia, which activates the enzyme lipoprotein lipase. Steroid hormones appear to cause a reduction in blood basophils, as well as eosinophils, and basophil counts are reduced in acute infections. Since basophils are rarely found in the blood of normal cats and dogs and are usually $<0.1 \times 10^9/L$ in the blood of other species, a reduction in basophils is seldom detected. **Basophilia** is seen in hypothyroidism, after protein injection (vaccination), estrogen injection, and accompanying eosinophilia. Basophilia may be seen in immune hemolytic anemia, after insect bites, severe cold, exposure to endotoxin, and in canine hyperadrenocorticism. Inhibition of basophil activation is induced by cyclosporine A and corticosteroids. Basophilia that accompanies a leukemoid reaction increases the likelihood of chronic myelogenous leukemia.

Monocytes

Blood monocytes derive from a marrow precursor in common with that for neutrophils and under cytokine pressure from GM-CSF, M-CSF, and interleukins 4, 11, and, most importantly, interleukin-3. There is some "switching" probably determined as late as the myeloblast stage. The peak of labeled blood monocytes occurs 2 days after injection of tritiated thymidine and, in contrast to neutrophils where the marrow and circulating pools of cells appear equal, *the monocyte pool is composed almost entirely of circulating elements*. Monocytes leave the peripheral blood with a half time of ~20 hours and are not believed to reenter the circulation.

Monocytes can be distinguished from lymphocytes on routine staining by their more irregular nuclear outline and uniform cytoplasmic density. Lymphocytes usually have a perinuclear halo of reduced cytoplasmic basophilia. Monocyte chromatin is consistently fine with or without a few large chromocenters, whereas lymphocyte chromatin varies widely with cellular activity. Cytochemically, monocytes have diffuse cytoplasmic staining for nonspecific esterase (α-naphthyl acetate esterase) activity which is absent in neutrophils of most domestic animals. Monocytic esterase is inhibited by sodium fluoride, which permits differentiation from early granulocytic precursors and from T lymphocytes. The specific granules of monocyte cytoplasm are not always resolvable by light microscopy. When present, they are similar in size and tinctorial properties to toxic granules of neutrophils. Newly released monocytes tend to have more cytoplasmic basophilia and lack vacuoles, while the opposite is true of older monocytes; vacuolation of monocytes is common in animals with inflammatory disease.

Monocytes leave the blood to replenish the entire spectrum of tissue macrophages, known specifically by location as macrophages of lung, spleen, marrow, peritoneum, and the lymph node sinuses as well as Kupffer, Langerhans, and giant cells. It is still undetermined whether the interdigitating reticular cells of germinal centers are derived from the monocyte–macrophage population. The terms reticulo- and reticulum should not be used with reference to the monocyte–macrophage system (formerly called the "reticuloendothelial system"). The metallophilic reticular cells of interstitial tissues produce reticulin or collagen and are weakly phagocytic. They are fibroblast-derived and distinct from macrophages, which do not produce fibers. Most of the tissue depots for macrophages are at the termination of the marrow–blood–tissue pipeline; however, in some sites, particularly lung and liver, there is local proliferation which may be accelerated in marrow failure.

The activities of the monocyte–macrophage system are almost as diverse as their tissue distribution and include phagocytosis, myeloproliferative control, antigen processing with expression of both MHC I and II antigens, and production of endogenous pyrogen. The ability of these cells to bind to antigens and lymphocytes is central to these functions. Macrophages have membrane receptors for the Fc portion of immunoglobulin, for C3b and C5a of the complement system, and for CD14 that binds to the LPS of gram-negative bacteria.

Phagocytosis is considered in three functional steps consisting of *particle attachment, internalization, and ingestion*. Attachment is a function of binding capabilities while the signal for ingestion may be either immunologically or nonspecifically activated. Ingestion is the energy-dependent step in phagocytosis. The energy is provided by anaerobic glycolysis, and macrophages characteristically function well in anoxic milieu such as occurs in abscesses and granulomas. In contrast, alveolar macrophages are critically dependent on a pO_2 of greater than 25 mmHg for phagocytosis. Once ingested, a foreign particle is subjected to an array of lysosomal enzymes after fusion of the primary vesicle with the membrane-bound enzyme to form the phagosomal digestion chamber.

Macrophages produce specific stimulatory molecules, called *colony-stimulating factor*, which are important in inducing myelopoietic differentiation. It is likely that other cells also produce this factor; however, the critical microenvironment in marrow probably requires a cell–cell interaction in which local macrophages are most effective. Marrow macrophages serve as nurse cells to erythropoietic islands and remove the nuclei (hematogones) from maturing rubricytes, recycling the digested nuclei at the nucleotide level into others at an earlier stage of development.

Monocytes and macrophages are involved in the immune system during *antigen recognition* and later as *effector cells during the cell mediated response*. Administered antigen first becomes associated with medullary macrophages and cortical dendritic cells of lymph nodes. This process, known as *antigen focusing*, appears to involve the phagocyte in antigenic degradation and adjuvanting with RNA, followed by transfer to competent B lymphocytes in intimate contact with the phagocyte membrane.

Temperature elevation is part of the host response to inflammation. *Leukocyte pyrogen* is produced de novo by all granulocytes but primarily by macrophages. Most inducers are themselves pyrogens, of which endotoxin is one of the most potent. Release of pyrogen, such as TNFα, accompanies phagocytosis, and like that process is energy dependent. The monocyte system also produces *interferon* and appropriately armed macrophages are able to kill tumor cells by mechanisms that are immunologically nonspecific.

In the dog, steroid injection causes prompt **monocytosis** which reaches $2–3 \times 10^9/L$ and persists for 8 hours. In the cat, horse, and cow, monocytes decrease in acute stress and return with marrow rebound. Monocytosis of $3–5 \times 10^9/L$ is characteristic of the dog with pyometra and, to a lesser degree, with immune hemolytic anemia. Monocytosis may accompany lymphoma, especially if there are large areas of tumor necrosis. Monocytosis may be seen in muscle bruising and with sterile hematomas.

Lymphocytes

Lymphoid precursors arise in the blood islands of the yolk sac and colonize *the primary lymphoid organs, the bone marrow and thymus,* in early embryonic life. Both of these sites remain active in lymphopoiesis with their progeny circulating to the *secondary lymphoid organs constituted by the lymph nodes, spleen, and gut- and lung-associated lymphoid tissue.* In general, the primary lymphoid tissues of marrow and thymus remain isolated from the activities which arise during cellular differentiation in the secondary or peripheral lymphoid tissues. Kinetically, the very great bulk of circulating lymphocytes are thymus-derived and long-lived (years), while a small number of cells with rapid turnover have a shorter lifespan (days). Lymphocytes more often terminate their sojourn in a particular compartment by division rather than death, and in this sense the concept of lifespan differs from that of granulocytes which are end-stage cells with a finite life.

Both B- and T-cell precursors are derived from the bone marrow from resident pluripotent stem cells. T-cell differentiation occurs primarily in the thymus but likely also to some extent in all body surfaces including the gut and lung. Naive T cells from the bone marrow (CD7+) mature in the thymus where, by contact with the epithelial cells of the thymic cortex, they are selected for histocompatibility (MHC) self-recognition and tolerance and undergo rearrangement of T-cell antigen receptor (TCR) genes. These 4 genes A, B, G, and D undergo recombination of their 3 major segments, comprising the variable, diversity, and joining sequences, which are themselves interwoven with nontemplate nucleotides by the enzyme TdT. This process produces T cells with a very large range of specificities within the two $\alpha\beta$ and $\gamma\delta$ lineages. Lymphocytes that do not pass the process of self-recognition undergo apoptosis with evidence of their passing seen as tingible body debris in the cytoplasm of thymic cortical macrophages. The pathway of naive lymphocytes through the thymus is thought to be by entry into the cortex and progression to exit near the corticomedullary junction.

The exiting TCRαβ+ T cells form two lineages. The TCRαβ+CD4+ become *helper/inducer cells* that are able and restricted to interact with antigen presented on the surface of MHC II positive antigen presenting cell (APC) macrophages. The other lineage comprising the TCRαβ+CD8+ cells *interact with antigen* presented on the surface of MHC I by any other nonhematopoietic cell in the body. Thus, *these two lineages become the managerial cells for adaptive immune responses by the combined B–T cell system.* Further combinations of T cells include the double positive CD4+CD8+αβ T cells and the double negative CD4-CD8-γδ T cells which make up the major circulating populations. The proportions of these 4 lineages vary both with age and species.

Naive B cells from the marrow migrate to the lymphoid areas of peripheral tissues and undergo a V, J, D Ig gene rearrangement process similar to that of T cells. Initial arrangements include the heavy Ig gene (V_H DJ) and later either the kappa or lambda light chain (V_L J). The initial maturation of B cells is antigen-independent and may occur in marrow with surface Ig of M or D types while the subsequent differentiation in the presence of antigen results in a class switch to Ig of G, A, or E types. The process of B-cell maturation is not associated with morphologic specificity. However, in the process of clonal proliferation of antigen-specific lineages, there is increase in nuclear size and cytoplasmic volume that usually includes the presence of nucleoli and variable nuclear indentations that may terminate in small mature lymphocytes or plasma cells. In this process, the B cells undergo an orderly succession of membrane markers that proceeds from CD79α and β in naive B cells to CD45, CD45R, CD19, CD20, CD21, and finally CD22. There are two major populations of B cells, with B-1 cells most prominent in the fetal/neonatal stages and expressing sIgM and CD5. The B-1 population is sparse in the adult but is the population of cells expanded in human B-CLL and in the benign lymphocytosis induced in cattle by BLV. Some lymphomas of humans and dogs may express CD5 but never CD3. The B-2 population of lymphocytes is continuously produced by the marrow throughout life and is the dominant B cell in the blood of most species.

In a kinetic sense, *the number of lymphocytes in the peripheral blood is the net result of recirculation from lymph to blood via the thoracic duct and from blood to tissue via the postcapillary venules of lymph node cortex.* Lymphopenia results in low transmural traffic across these venules while in lymphoid leukemia they may be dilated and tightly packed with tumor cells. Steroid therapy causes **lymphopenia**; this change is due partially to lympholysis and partially to redistribution of lymphocytes into tissues. Lymphopenia occurs in chronic renal disease, lymphangiectasia, chylothorax due to rupture of the thoracic duct, in canine parvoviral infection, distemper and viral hepatitis, in hyperadrenocorticism, and in chronic classical swine fever. In all species, persistent lymphopenia indicates a poor prognosis. **Lymphocytosis** occurs in ruminants responding well to chronic disease. Examples of the latter are found in tuberculosis, brucellosis, trypanosomiasis, and *Bovine leukemia virus* infection. Lymphocytosis occurs in cats as a fear reaction, and in dogs and cats with hypoadrenocorticism.

Platelets

Blood platelets are produced by **megakaryocytes** that arise from committed hematopoietic stem cells. There is a *common precursor for the erythroid and megakaryocytic systems* that differentiates at the same hierarchical level as the precursor for the neutrophil–monocyte system. Evidence for this relationship is found in in vitro culture systems where both megakaryocytes and rubricytes may develop under the inductive pressure of erythropoietin. In the fetus, megakaryocytes are found successively in the liver, spleen, and marrow and in adult mammals they are found in the marrow, lung, and spleen. Megakaryocytes develop from CD34+ stem cells, as do other hematopoietic lineages, but gain the thrombopoietin (TPO) receptor c-MPL. Their proliferation and maturation are induced by interleukins 3, 6, 11 and stem cell factor (SCF) Flt-3 ligand and TPO. As megakaryocytes mature, they gain specific markers including glycoprotein (GP)IIb-IIIa and GPIb.

The volume of marrow occupied by megakaryocytes bears a constant relationship to peripheral platelet consumption. Thus, with increased platelet utilization, there is an increase in megakaryocyte number and volume, giving rise to the concept of *megakaryocyte mass.* Normally there is also a *direct relationship between megakaryocytic ploidy and cytoplasmic volume.* Thus, increased output from the system is accomplished by both increased input from the stem cell pool and by increased output per cell as a result of increased nuclear reduplication. This duplication from 2 N to 16 to 32 N occurs by both endomitosis and mitosis, although the latter tends to be seen only in a stressed system. It has been estimated that about 50 platelets are produced per nuclear lobe or 1600 platelets from a 32 N cell. In most species, platelets can be produced in 4 days. They have a circulation time of 9 days, which is determined largely by age-dependent utilization and to a lesser extent by random removal at sites of endothelial injury. The cellular production from a hematopoietic system can be said to be effective when the peripheral arrival of new cells is consistent with the volume of marrow precursors. In immune thrombocytopenias, there is usually increased peripheral destruction with an appropriate increase in marrow production. Occasionally there is depression of the precursors, and the disease is then greatly complicated by decreased marrow production. A less obvious problem of ineffective thrombopoiesis occurs in humans in pernicious anemia and in calves with trypanosomiasis and probably in other conditions as well. *Ineffective thrombopoiesis* is characterized by increased megakaryocytic mass with increased nuclear volume and normal or decreased cytoplasmic volume in the face of a peripheral thrombocytopenia that is due primarily to decreased production. The rate of thrombopoiesis is probably controlled by regulatory T lymphocytes at the stem cell level and by the $\alpha 2$-globulin, thrombopoietin, at the level of differentiation and maturation.

Blood platelets are discoid cells generally one-third to one-half the diameter of homologous red cells. Platelets have mitochondria and are capable of glycolysis with pathways remarkably similar to those in skeletal muscle. The cell is sponge-like and perforated by a microtubular array that probably aids rapid release of granular contents during the secretory phenomenon known as the *platelet release reaction*. In stained blood films, platelet granules are pink and may be centralized into a granulomere leaving a basophilic agranular peripheral hyalomere. Platelets can be quantitated like red cells on the basis of hematocrit and mean volume. Platelets contain *fibrinogen* and the contractile protein, *thrombosthenin*, which provides the force for clot retraction. A variety of plasma proteins adhere to the platelet membrane and may function in establishing the electrical properties of platelets. Platelets undergo metabolic aging in the circulation undergoing progressive lipid peroxidation. This may be the mechanism of senescent removal. Young platelets tend to be larger with greater basophilia, whereas older platelets are less granular and may lack basophilia. Platelets larger than red cells and usually basophilic, are called *"shift" platelets*; they indicate increased production. Their detection in thrombocytopenias is thus prognostically significant in differentiating increased destruction from decreased production. Normally the blood platelets present a spectrum of size, basophilia and granulation which corresponds to cell age and indicates age-dependent removal. In thrombocytopenias with increased turnover, they may all appear young, or all appear old if utilization is normal and production has ceased.

The role of the platelet in **hemostasis** is discussed in a later section. Briefly, *the normal contractility of injured vessels is assisted by platelet adhesion, aggregation, release reaction, and contraction which is associated with a laminar layering of fibrin.* While blood will clot in the absence of platelets, the hemostatic plugs so formed are unstable due to deficient adhesion and contraction. *Platelets can be said to support endothelial integrity*, and thrombocytopenia results in red cell diapedesis through apparently intact endothelium. Clinically, endothelium appears to remain intact for some hours after thrombocytopenia develops, and similarly petechiation may persist for some hours after platelet production increases.

Platelets are not equal in their functional capability and may be deficient because of congenital or acquired defects. *Congenital thrombopathies* are recognized in dogs and cattle, and *acquired dysfunction* occurs in acute leukemias and with aspirin therapy. In routine hematopathology, the *clot retraction test* is a useful measure of the functional capability of the circulating platelets. This variability in function accounts for the variations in the platelet counts at which purpuric hemorrhage occurs. Thus, petechiae may not be seen at a level of 10×10^9/L if platelets are large and very functional, while purpura may be seen at 50×10^9/L or higher if function is poor or impaired.

Acquired thrombocytopenia was formerly termed idiopathic but is *now accepted to be immune in origin* unless shown to be otherwise. The spongy nature of platelets makes them susceptible to immune sensitization because of adsorbed drugs, toxins and virus. *Immune-mediated thrombocytopenia of unknown cause occurs in dogs, cats, cattle, and horses and probably in other species.* Thrombocytopenia is a constant finding in acute equine infectious anemia and in bovine trypanosomiasis. Low platelet counts due to decreased production occur in cyclic hematopoiesis, pancytopenia, and following estrogen toxicity or marrow irradiation. Concurrent hemolytic anemia and consumptive thrombocytopenia is seen in dogs, and a large spleen in any species may cause deficits in circulating cells. Thrombocytosis of small platelets is characteristic of iron and copper deficiency. Thrombocytosis is transient after splenic removal and occurs in polycythemia vera (of humans but not of dogs) and in megakaryocytic myelosis both of which are rare, but are seen most commonly in dogs.

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LEUKOPENIA-LEUKOCYTOSIS IN BENIGN DISEASE

The **hematologic profile** is a powerful tool in the assessment of response to disease. Serial blood profiles allow an assessment of an animal's response to a disease process and are of prognostic value. In general, *the three factors most important in predicting the magnitude of (neutrophilic) leukocytosis are* **animal species**, *and* **cause** *and* **localization** *of disease.* There is marked variation in hematologic reactivity among species of domestic animals, which is related to the normal ratio of neutrophils to lymphocytes in peripheral blood. Thus the dog, with a **neutrophil:lymphocyte ratio** of 2.4, is most reactive, whereas the cow, with a ratio of 0.5, is least reactive. The maximum leukocytosis likely to be encountered in each species can be predicted by multiplying the normal neutrophil:lymphocyte ratio times 50 as shown in Table 2.1.

In general, carnivores are more responsive than herbivores, and ruminants are the least reactive of the herbivores. Acute viral infections tend to involve large volumes of tissue and to injure body surfaces (gut, lung, skin) so that not only is the area of chemotaxis large but also leukocytes and their leukopoietic products may be lost from the body. Localized inflammatory processes are likely to be associated with leukocytosis regardless of species and type of agent. The differences between the cow and dog are largely in the rate of acceleration of cell production rather than in their potential for maximal response. The cow is able to mount a sustained neutrophilic leukocytosis like that of the dog with pyometra if given proper conditions of time and containment. These conditions are usually produced by abscessation of lung or liver which may be either miliary or single and massive. While sustained leukocyte counts in the cow greater than 50 × 10^9/L are rare, they do occur. The cow with a neutrophilic leukocytosis of 20–25 × 10^9/L that is sustained for more than a week can confidently be assumed to have abscessation.

Table 2.1 Leukocyte responses in benign disease

Species	Normal neutrophil: lymphocyte (N:L) ratio	Maximum expected leukocytosis (×10^9/L)
Dog	2.4	120
Cat	1.5	75
Foal	1.4	70
Horse	1.2	60
Pig	0.7	35
Goat	0.6	30
Sheep	0.5	25
Calf	1.0	50
Cow	0.5	25

In animals, *infection frequently causes leukopenia,* particularly in ruminants with mastitis and metritis and in carnivores with systemic viral infection such as infectious canine hepatitis and distemper in the dog and parvoviral infections in the dog and cat. Guidelines for leukocytic interpretation that are valid for all species, are that *the* **absolute increase** *in leukocytes indicates individual or species reactivity, whereas the* **proportional increase** *in mature and immature neutrophils and the level of toxic changes indicates the "effort of the response."* In all species, a low total leukocyte count with persistent neutrophil immaturity and toxemia is a *poor prognostic finding.* The return of the leukocyte count to normal from levels which were either increased or decreased, with reduction in immaturity and cytologic toxicity, is indicative of *recovery.* The return of eosinophils to the blood is usually prognostically favorable if accompanied by other signs of recovery.

MYELOID REACTIONS

Leukemoid reactions

Leukemoid reactions are abnormalities of peripheral blood which resemble leukemia but are due to other causes. They occur in all species but are most often seen in the *cat and dog.* The leukemoid reactions occur in a variety of hematologic perturbations including *infections, intoxications, severe hemorrhage or hemolysis, and in some malignant diseases.* In terms of cellular kinetics, a marked increase in blood leukocytes with immaturity is due to a combination of increased cell production and release and may be exacerbated by deficient cell removal and destruction. In the dog, leukemoid reactions are most often seen in association with *pyometra, peritonitis and hemolysis.* The cat is similar. The administration of tuberculin to previously sensitized rabbits can produce a leukemoid reaction with leukocyte counts in excess of 100 × 10^9/L that is primarily neutrophilic and with left shift.

Hematologically, there is *neutrophilic leukocytosis* which is usually in the range of 50–100 × 10^9/L. Mature neutrophils predominate in the peripheral blood, but there is immaturity to at least the promyelocyte stage. Blast cells are seen occasionally, but these are usually lymphoid and misinterpreted because of the myeloid immaturity. There is usually diffuse cytoplasmic basophilia of mature cells with reduced secondary granulation and variable cytoplasmic vacuolation. Toxic azurophilic granulation and Doehle bodies may be present. There is usually *anemia* of at least mild degree that is nonresponsive due to the intensity of the myeloid reaction. Mild rubricytosis of maturing cells is irregularly present. There is usually *monocytosis* of 2–4 × 10^9/L with mild immaturity and variable cytoplasmic vacuolation. Eosinophils are generally mature and less than 1 × 10^9/L and may have decreased secondary granulation with vacuolation and increased cytoplasmic basophilia. Basophils are absent.

Aspirated bone marrow is hypercellular, generally above 70% with synchronous myelopoiesis, a marked increase in mature reserves and less than 10% blast cells. There is erythroid atrophy, but not phthisis, and hemosiderin is usually coarse and increased.

Animals showing a leukemoid reaction often recover. Those that die show various lesions depending on the primary cause. Morphologically there is an increased volume of hematopoietic marrow. By definition, the leukemoid reaction must resemble leukemia. *The diagnosis of leukemoid reaction tends to be a working clinical designation used until biopsy, autopsy or recovery resolves the problem.*

Leukoerythroblastic reaction

The leukoerythroblastic reaction is characterized by leukemoid reaction with the presence in the peripheral blood of rubricytes as young as basophilic rubricytes. The leukoerythroblastic reaction is seen in all species and is *most common in the cat and dog*, and least common in the horse. Since this reaction is always part of other processes, there are no characteristic clinical signs. It is most apparent in *immune hemolytic anemia* of animals other than the horse. Immature cells of both systems are likely to appear in the peripheral blood when there is heightened marrow activity. The degree of immaturity is important and is less with reactive marrow than with malignant infiltration.

It is uncommon to have benign erythroid hyperplasia without concurrent myeloid hyperplasia. Thus, *responsive immune hemolytic anemia is usually accompanied by a sustained neutrophilia with mild left shift.* In contrast, myeloid hyperplasia is regularly accompanied by some degree of erythroid atrophy. Myeloid immaturity is thus expected in increased erythropoiesis, but rubricytes are not expected in myeloid hyperplasia unless there is a considerable level of stress. *The leukoerythroblastic reaction is therefore present at some point in all leukemias and is most common in the acute leukemias.*

Recognition of the leukoerythroblastic reaction does not constitute a diagnosis but rather a *condition of concurrent release of benign immature myeloid and erythroid cells.* It needs to be differentiated from malignancy in both the erythroid and myeloid systems and interpreted in the light of relative immaturity and the nature of the concurrent process.

MYELOID NEOPLASMS

Myeloproliferative disease *is a general term for diseases characterized by medullary and extramedullary proliferation of one or more marrow myeloid cell lines, with specific exclusion of the lymphomas and lymphoid leukemias.*

The **acute myeloid leukemias (AMLs)** are a biologically aggressive and morphologically diverse group of hematologic neoplasms characterized by *proliferation of poorly differentiated cells arising from the marrow myeloid progenitor cells.* On a morphologic basis, the acute myeloid leukemias are divided into four groups comprising the *granulocytic (neutrophil, eosinophil, basophil), monocytic, erythroid, and megakaryocytic lineages.* The presentation of AML is usually with proliferation of only one of these four cell lines, or less often with multiple lines involved in a panmyelopathy. The proliferation of the neoplastic clone causes phthisis of normal marrow elements with the result that animals with AML usually have some degree of marrow failure at the time of diagnosis including anemia and thrombocytopenia. The indications of AML in the blood may vary widely with very high leukocyte counts or severe leukopenia, but blast cells are always present. *The AMLs are classified here according to the revised WHO system and essentially on a morphologic basis* (Table 2.2). One of the major changes from the FAB (French–American–British) system of classification of the acute leukemias is that the WHO format reduces the level of blast cells required for a diagnosis of AML from 30 to 20% of nucleated cells in marrow or blood. A further proposal of the WHO classification is recognition that AMLs that arise in individuals that have had a prior diagnosis of myelodysplasia have a poorer prognosis than in the recognition of de novo acute leukemia, and therefore these cases should be given a separate category of classification. Further, if on the diagnosis of AML it is apparent that there are dysplastic changes in benign cell lines (multilineage dysplasia), then this finding should also require a separate category based on a poorer prognosis. In human pathology, lymphoid tumors can be classified largely on the basis of immunohistochemistry for routine diagnosis, whereas the AMLs require a battery of cytogenetic and molecular techniques for adequate characterization. With the sequencing of animal genomes now a reality, these more sophisticated techniques for the diagnosis of AML will be added to the morphologic identification of AML in animals. In the interim, there is every reason to rely on proven and available techniques such as the myeloperoxidase reaction and Sudan black B stain to differentiate the blast cells of the AMLs from those of the lymphoid system (Table 2.2).

Table 2.2 Cytochemistry for acute myeloid leukemias

Reaction	Normal cells manifesting reaction	Diagnostic utility
Myeloperoxidase	Neutrophil series Eosinophils (cyanide resistant) Basophils ± Monocytes ±	Myeloblastic leukemia without maturation – M1 Myeloblastic leukemia with maturation – M2
Sudan black B	Neutrophil series Monocytes ±	M1, M2
Chloroacetate esterase	Neutrophil series Monocytes ±	M1, M2
Nonspecific esterase (α-naphthyl acetate substrate, or α-naphthyl butyrate)	Monocytes (inhibited by sodium fluoride)	Monocytic leukemias Monocytic leukemia, differentiated – M5 Monocytic leukemia, poorly differentiated – M5 (acute monoblastic) Myelomonocytic leukemia – ??
Periodic acid-Schiff (PAS)		Erythroleukemia – M6 Acute lymphatic leukemias

With permission of R. D. Brunning, MD, Department of Pathology, University of Minnesota.

Leukemias result from the accumulation of chromosomal abnormalities that both permit and sustain neoplastic proliferation. Cytogenetic studies on human leukemias have established correlations between cytogenetic changes and clinical phenotypes for specific subtypes of leukemia. Because many of these chromosomal changes represent recurring types of leukemia, they have been molecularly cloned thus providing powerful tools for detection of specific types of genetic injury of both diagnostic and prognostic value. In addition, the presence or absence of these genetic changes provides clear and definitive distinction between benign and malignant disease. The structural basis for genetic change resulting in malignancy lies in the translocation of parts of chromosomes that both inactivates tumor suppressor genes and the normal caspase system of programmed cell death and permits the production of aberrant growth factors. Chromosomal translocations may alter gene function by *dysregulating gene expression*, as occurs in the lymphoid tumors due to breakages involving the immunoglobulin gene of B-cell tumors and the T-cell receptor genes of the T-cell tumors. These rearrangements cause inappropriate activation of an oncogene with the production or overproduction of a normal protein in a tissue where it is not normally found. The second mechanism by which translocations cause cancer is by the *joining of two genes normally on different chromosomes so that abnormal fusion proteins are produced.* Since the newly formed gene and its product are not found in normal tissues, the chimeric protein becomes tumor-specific and constitutes a tumor marker. These markers can be the basis for tumor diagnosis and for the detection of residual disease after therapy. In addition to activation of new proteins, chromosomal translocations or deletions may result in the loss of gene function such as occurs with tumor suppressor genes whose normal function is to limit cellular proliferation. Since there are two copies of each gene, both copies of the suppressor genes must be inactivated (loss of heterozygosity) in order for the implications of other translocations capable of causing cancer to become apparent.

In terms of pathogenetic mechanisms, the chronic leukemias have contributed greatly to understanding of the leukemic process since the description of the 9;22 (Philadelphia) chromosomal translocation characteristic of human chronic myelogenous leukemia.

Those acquired chromosomal alterations with high sensitivity and specificity for lineage specific malignancies are termed **primary changes**, as in the Philadelphia chromosome. Nonrandom acquired chromosomal changes which occur in the course of the accelerated phase of chronic myelogenous leukemia are termed **secondary changes** since they occur in other conditions and are not useful as tumor markers but are useful for staging and prognosis. Typically in late-stage human chronic myelogenous leukemia, there are further random changes in chromosomes which result from the basic instability of the malignant clone and lack lineage specificity but are associated with the level of tumor aggression and these are termed **tertiary chromosomal changes**. Tertiary changes are common in solid tumors and for years obscured the specificity of the less common primary and secondary changes. Clonal chromosomal abnormalities are found in the great majority of human leukemia-lymphoma and myelodysplasia suggesting that these changes have been inadequately looked for in spontaneous tumors of animals.

The major determination in the diagnosis of acute poorly differentiated or undifferentiated leukemia is the distinction between myeloid and lymphoid lineage. This distinction has traditionally been based on histochemistry (Table 2.2). However, the recognition and characterization of lineage-specific cell surface antigens has produced a large family of reagent antibodies directed against these antigens particularly for human and mouse cells. By international concordance these antigens are grouped by *cluster designation (CD)* in which CD4 defines T helper and CD8 defines T suppressor subsets, and CD13 defines poorly differentiated lymphoid clones whereas TdT defines poorly differentiated lymphoid clones.

The most useful reagents are those that are active in formalin-fixed, paraffin-embedded tissues. Many of the CD reagents are active only in flow cytometry, which limits their use to cases where the diagnosis has been largely determined by routine cytology and live cells can be prospectively prepared for in vitro staining and subsequent counting. Currently, *the most helpful CD antibodies for general use in histopathology are CD3 which is a pan-T-cell marker and CD79α as a pan-B-cell marker.* The CD3 antibody binds to the rearranged T-cell receptor and is thus able to mark all differentiated T cells. The CD79α peptide is Igα, and Cd79β is Igβ. They are both IgSF (superfamily) members and together form a heterodimer associated with surface IgM on B cells to form the B-cell receptor (BCR). These proteins appear on the surface of all differentiated B cells and the BCR is expressed prior to the appearance of the cytoplasmic μ chain. The anti-CD79α antibodies cross-react with endothelium in most domestic species, and with the cytoplasm of mature megakaryocytes and variably with hepatocytes. These are minor reactions and serve as an internal positive control. A less valuable feature of CD79α is that it may frequently mark the nuclei of T cells in a nonspecific reaction with only cytoplasmic marking indicating B-cell differentiation. *Both CD3 and CD79α mark the lymphocytes of most domestic species and rodents. These two reagents plus the histochemical reaction of Sudan black B are the major means of differentiating myeloid and lymphoid blasts.* Where time and additional unstained cytological preparations are available, the use of *myeloperoxidase* to identify granulocytic and monocytic precursors is an efficient means to prove that these are nonlymphoid lineages. Within the myeloid tumors, the *chloroacetate esterase stain* specifically identifies the granulocytic precursors whereas the *nonspecific or α-naphthyl acetate or α-naphthyl butyrate esterase stains* specifically mark the cytoplasm of monocytic precursors and macrophages.

As might be expected, the simplest techniques suffice in more differentiated malignancies, and in primitive tumors all modalities may be required to fully define cell lineage and thus management strategies. Collectively, these diagnostic techniques have added greatly to the understanding of leukemias particularly in the areas of apparent conflict. For example, in myeloid leukemia in blast crisis, in 25% of human cases the tumor cells carry lymphoid markers recapitulating the evolution of the clone from a totipotential stem cell capable of producing both myeloid and lymphoid progeny. Some 30% of human acute lymphoid leukemia in adults have the Philadelphia chromosome, and in some cases the response to treatment varies with the presence of specific cytogenetic alterations although the tumors may appear morphologically similar.

The myeloproliferative diseases occur mostly in dogs and cats; their recognition in other domestic and wild animals is relatively rare. In dogs, myeloproliferative disease constitutes about 5% of hematopoietic neoplasia. Cats have the greatest proportion and range of myeloproliferative diseases, which constitute 10–15% of hematopoietic neoplasms with a similar proportion of lymphoid leukemias and the rest lymphomas. Characteristically, the acute leukemias have a course of 1–2 months from diagnosis to termination, whereas the chronic leukemias have a natural course of 1–3 years. Acute leukemias of humans and animals, because of their high rate of proliferation, are most responsive to chemotherapy. In contrast, animals with chronic leukemia may derive little benefit from intensive cytoreductive chemotherapy based on cells in active proliferation because the mitotic rate of the tumor cells is similar to or less than that of residual normal marrow.

The descriptions provided are of the diseases unaltered by chemotherapy. Treatment of the acute leukemias causes lymphoid atrophy and reduced cellularity of bone marrow. Treatment of the chronic leukemias causes similar changes but with more generalized sclerosis.

Chemotherapy tends to reduce the size of the residual tumor cells and to reduce their lineage-specific differentiation. It is therefore highly desirable to base histologic and cytologic diagnoses on tissues derived prior to onset of therapy.

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Acute leukemias

M1, Myeloblastic leukemia without maturation (acute myeloblastic leukemia, AML)

M1 is defined as having *a predominance of blasts in blood and marrow with less than 10% having cytoplasmic granulation.* At least 5% of malignant blasts stain positively for Sudan black and myeloperoxidase. AML of the M1 and M2 type is largely a disease of young mature dogs and cats with a tendency for increased frequency in males. Swine are occasionally affected and other domestic animals very rarely. The M1 variant of AML is likely under-diagnosed since lymphoid tumors are much more common and, if the few cells with cytoplasmic granules are not recognized and cytochemistry is not carried out or is inconclusive, the tumor cells most resemble large malignant lymphocytes (Fig. 2.9A).

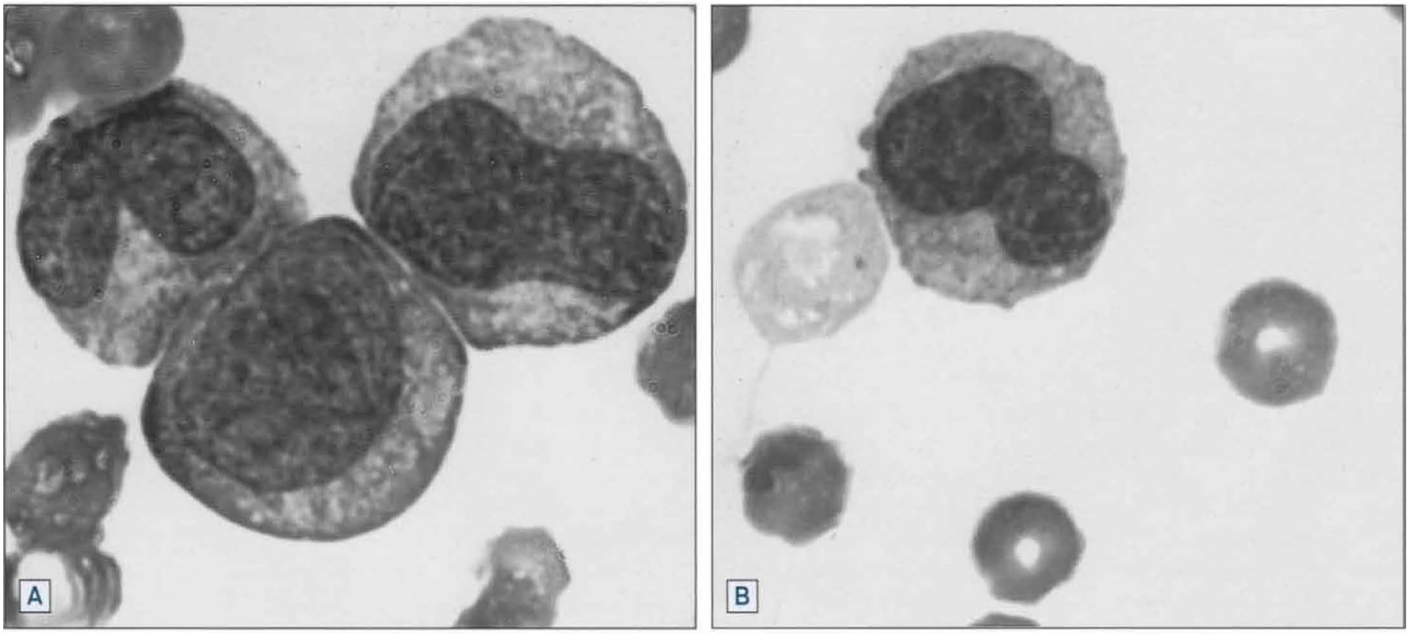

Figure 2.9 M2, Acute myeloblastic leukemia in a dog; blood smear. **A.** Poorly granulated myeloblasts. Myeloid origin can be deduced from the pattern of maturation that ends at the metamyelocyte stage. **B.** Bilobed progeny of malignant myeloid precursors. Cytoplasmic fragment from injured tumor cell that contains granules may be confused with a large platelet.

Histologically, M1 is indistinguishable cytologically from acute lymphocytic leukemia (ALL) or large cell lymphoma, thus *blood and marrow cytology is essential for the diagnosis of this disease.* Architectural differences between M1 and lymphoma are usually sufficient to identify the disorder as a primary leukemia but will not differentiate M1 from ALL.

M2, Myeloblastic leukemia with maturation (AML)

M2 is similar to M1 except that maturation proceeds to or past the promyelocyte stage in at least 10% of tumor cells (Fig. 2.9). Approximately half of the tumor cells stain positively with Sudan black and myeloperoxidase. Most animals are presented in good body condition because of epistaxis or melena of acute onset and have mucosal pallor often with thrombocytopenic petechiation. The liver and spleen are usually not palpably enlarged and the lymph nodes are characteristically of normal size. Acute myeloblastic leukemia usually has a course of 2–3 weeks from the time of diagnosis. Death is due to hemorrhage and sepsis, if not complicated by chemotherapeutic myelosuppression.

Anemia is moderate (80 g/L) and due to *myelophthisis*, or marked (20 g/L) when complicated by thrombocytopenic hemorrhage. Anisocytosis is mild and there are a few poikilocytes which are more numerous if platelets are deficient. Howell-Jolly bodies are usually present and indicative of reduced splenic function. Hypochromia will be present if the animal survives hemorrhage by a week or more. The erythroid response is mild or absent and usually less than the normal level of 60×10^9 reticulocytes per liter. Rubricytosis is often present to a mild degree, usually less than 1.0×10^9/L, and with cells as immature as prorubricytes and occasionally a rubriblast in the peripheral blood. These cells are benign and their release independent of age is indicative of leukemic myelophthisis (leukoerythroblastic reaction).

Thrombocytopenia is generally marked and in the $5–50 \times 10^9$/L range with immature platelets rare or absent. The platelets present tend to have uniform basophilia but reduced granulation and functional deficits accompany low numbers. Cytoplasm shed from the fragile tumor cells rounds up and with its sparse pink granulation may be mistaken for platelet material, especially large immature platelets.

The total leukocyte count is highly variable but may be in the normal range and is usually less than 25×10^9/L, but may rise to 50×10^9/L near termination. There is an absolute neutropenia and usually lymphopenia with myelocytes predominating followed by promyelocytes, metamyelocytes, and myeloblasts. *The tumor may be of the neutrophil, eosinophil, or basophil type, but the neutrophil type is most common.* Peripheral blood differentiation does not proceed past the metamyelocyte stage and if more mature cells are present they are likely to be the progeny of residual normal marrow. Hypersegmentation may be present in benign neutrophils due to hyposplenism and probably to folate deficiency. If neutropenia has persisted for a week or more, bacteremia is often present and the benign cells have toxic changes.

Cytologically (Fig. 2.9A), the leukemic cells in blood have round centrally placed nuclei 2.5 red cells in diameter with a fine uniform or cribriform chromatin pattern. One to three nucleoli which are round and from one-third to two-thirds the size of a red cell are visible in the leukemic myeloblasts and promyelocytes. The cytoplasm is moderate in volume and basophilia and has a smooth outline that is not usually indented by adjacent cells. There is usually sufficient fine azurophilic granulation, most visible in the Golgi clearing, to identify the cells as being of myeloid origin. In addition, the process of recognition by "the company they keep" is important; the mild reniform nuclear indentation in the most mature tumor cells suggests metamyelocyte differentiation (Fig. 2.9B). This indentation is much more shallow than the sharp folding characteristic of lymphocytes. Rarely in blood, benign promyelocytes and myelocytes will be recognized by their lower nuclear/cytoplasmic ratio, small nucleoli and increased granulation. They are part of the process of myelophthisis and the leukoerythroblastic reaction.

Aspirated bone marrow (Fig. 2.10A) has very high cellularity and cell density, with few fat vacuoles. There is *virtually complete phthisis of the normal elements* (Fig. 2.10B), resulting in loss of rubricytes, megakaryocytes, and mature granulocytes, and a coarse hemosiderin pattern. Characteristically the marrow cells are about one morphologic stage less mature than those in the peripheral blood. Early metamyelocytes are rare and myeloblasts and promyelocytes predominate. Mitoses are very frequent and granulation is usually recognized in metaphase cells when the obscuring basophilia is minimal. The animals are usually presented for examination when 50% or more of the marrow is occupied by tumor and marrow failure is imminent.

At *autopsy*, there is pallor of membranes and tissues and mild-to-moderate loss of condition. The lymph nodes are of normal size and often edematous. The liver is of normal size and pale. The spleen is usually mildly and uniformly enlarged and may be focally infarcted. Femoral marrow is uniformly vascular and a glistening pale tan rather than the hard white of normal fat. There are usually foci of hemorrhagic infarction if thrombocytopenia has been severe. The thymus is atrophic. Solid foci of the tumor in parenchymal organs are not found. Secondary lesions of marrow failure include hemorrhage, pneumonia and cellulitis. Because of the absence of neutrophils, even septic lesions may have minimal cell reaction.

Histologically the neoplastic cells are round with peripheralized chromatin, prominent pink nucleoli, and a moderate amount of amphophilic cytoplasm. There are frequent mitoses and some cells of metamyelocyte type are present. Mature cells of the normal series are rare and if the tumor is of the neutrophil type, most segmented cells will be residual, normal eosinophils. Megakaryocytes and rubricytes are rare or absent.

The spleen has lymphoid atrophy that is most prominent in thymic-dependent areas. The sinus areas contain few erythrocytes, are hypercellular due to tumor infiltration and have increased, coarse hemosiderin. Benign hematopoiesis, if present, is characteristically paratrabecular and largely limited to thrombopoiesis. There is usually some subendothelial colonization of the large muscular sinuses by tumor cells. The liver usually has periacinar ischemic degeneration. The tumor has a sinusoidal distribution and if the leukocyte count was low, tumor cells may not be obvious. Periportal colonization is minimal and absent from many triads; this is an important distinction from the lymphomas. Benign hematopoiesis is similarly inconspicuous, which suggests that there is exhaustion of benign stem cells. Lymph node histology is highly variable and may vary from minimal involvement occurring first in medullary cords (Fig. 2.11A) with moderate lymphoid atrophy to diffuse involvement with capsular colonization (Fig. 2.11B), usually without an increase in overall size. If transfusions have been given, there will be erythrophagocytosis in the medullary sinuses of the lymph nodes and sinuses of the spleen.

M2 myeloblastic leukemia is distinguished from acute lymphoblastic leukemia on the basis of cytoplasmic granulation and a positive

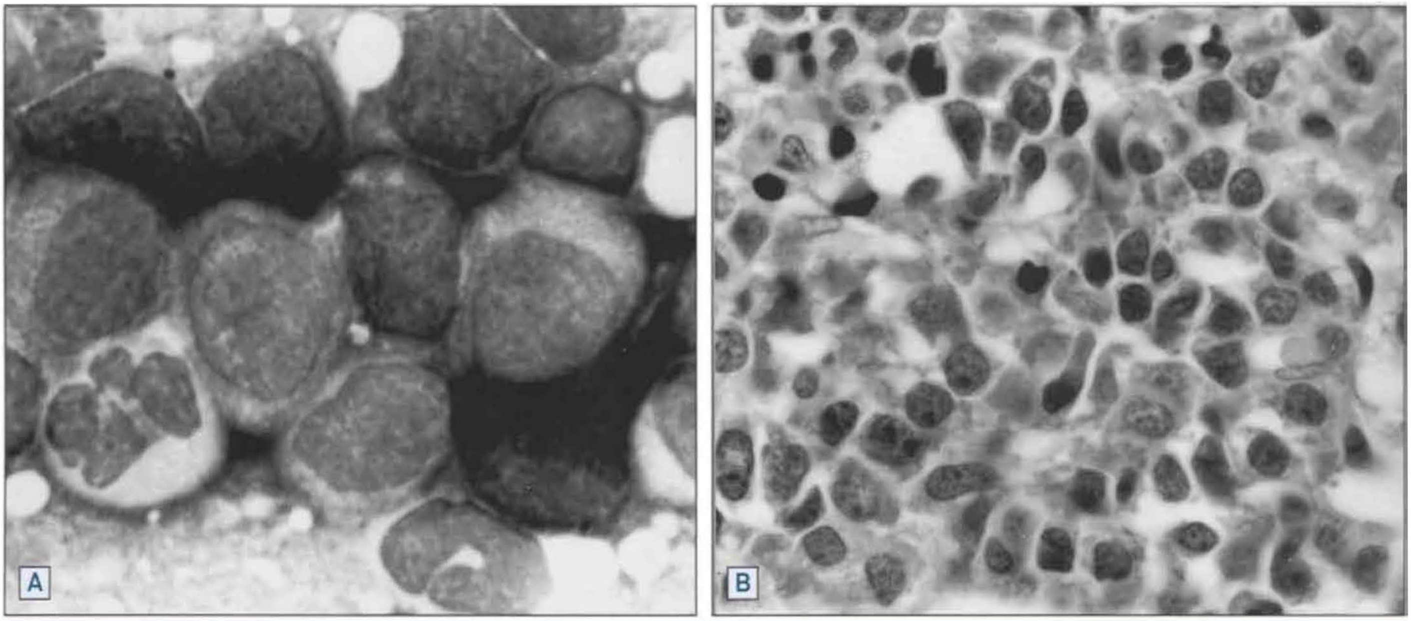

Figure 2.10 Acute myeloblastic leukemia. **A.** Bone marrow aspirate with early granulocytic asynchrony and phthisis of erythroid and megakaryocytic cells. **B.** Marrow histology. Phthisis of normal elements and reduction in mature granulocytes indicates the level of maturation of the malignant myeloid precursors.

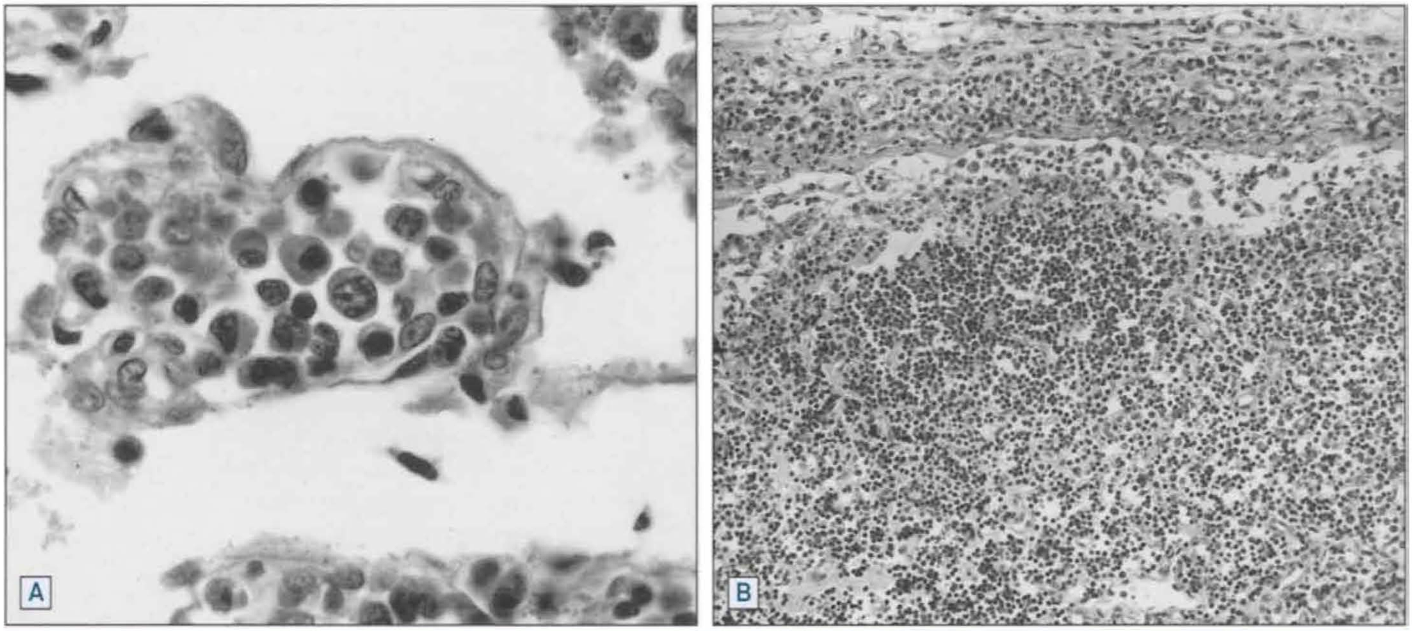

Figure 2.11 Acute myeloblastic leukemia. **A.** Medulla of node indicating pattern of tumor colonization in medullary cords. Empty sinuses indicate phthisis of marrow precursors of monocyte–macrophage system. **B.** Lymph node. Cortical and capsular colonization by tumor.

reaction of tumor cells to SBB and any progeny to the chloroacetate esterase stain.

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M3, Promyelocytic leukemia

Promyelocytic leukemia (Fig. 2.12) is a *rare disease of young mature animals*, most commonly recognized in *dogs and cats* but also in swine and likely occurs in species where it is looked for. Clinical signs are similar to M1/M2 myeloblastic leukemia but with a greater tendency for bleeding. The course of the disease is usually only 1–2 days from time of diagnosis. In humans, there are two forms of M3 with 80% of cases having very prominent cytoplasmic granulation in the malignant promyelocytes and less tendency for bleeding. The second type of human M3 has hypogranular promyelocytes with very fine azurophilic granulation and is associated with DIC.

Promyelocytic leukemia differs from myeloblastic leukemia by a predominance of promyelocytes in both blood and bone marrow. Most cases in animals are seen at necropsy and live presentation is rare. The M3 in animals associated with bleeding may resemble warfarin poisoning or other coagulopathy. The disease, while rarely occurring, may be even more rarely recognized because of the difficulty of making a specific diagnosis of acute myeloid leukemia solely on histologic examination. There is pancytopenia on presentation, and often severe and widespread hemorrhage and apparent bruising. The proportions of the heavy and lightly granulated forms of the disease in animals is unknown and, likely because of the association with coagulopathy and early death due to bleeding, the level of blast cells in the poorly granulated type never reaches the 20% level required for the diagnosis of other types of AML. Atypical promyelocytes are the predominant cell in both blood and marrow and further differentiation to myelocytes is minimal, and metamyelocytes arising from the malignant clone are rare. The malignant promyelocytes have nuclei the same size or larger than in AML of M0/M1 types but cytoplasm is much more abundant. The promyelocyte nuclei have shallow indentations but do not form typical myelocytes or metamyelocytes. The cytoplasmic granulation in both variants of this disease is intensely positive with Sudan black and myeloperoxidase stains. In M3 in humans, the cytoplasmic granules are procoagulant, and rapid cytoreductive therapy may result in disseminated coagulation. The disease in humans is associated with a specific primary 15;17 chromosomal translocation which results in fusion of the retinoic acid receptor-α gene on chromosome 17 with the zinc finger binding transcription factor (known as the promyelocytic leukemia or PML gene) on chromosome 15. This pathogenesis provides the basis for a very highly effective treatment of this disease with transretinoic acid.

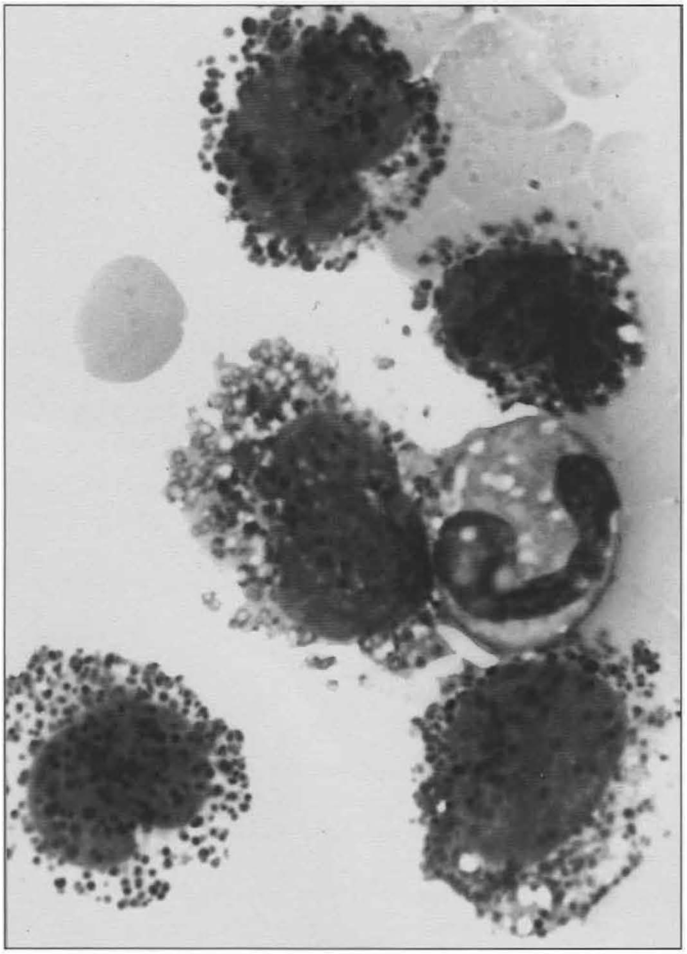

Figure 2.12 Promyelocytic leukemia, canine blood. Nuclear indentation indicates that this tumor is of basophil type rather than a mast cell leukemia.

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M4, Acute myelomonocytic leukemia

Myelomonocytic leukemia is a rare disease recognized in dog, cat, horse, and humans in which there is concurrent neoplasia of the neutrophil and monocytic cell systems. Most commonly the disease consists of concurrent clones of tumor derived from both the monocytic and neutrophil systems and as such the tumor cells mimic their normal counterparts in both morphology and histochemical reactions. As much as a third of human cases of M4 have a single tumor clone which combines the cytochemical characteristics of both cell lines and therefore represents an entity for which there is no normal counterpart. *AML of the M4 type have at least 20% of blast cells in blood or marrow and at least 20% of the marrow cells must be of monocytic lineage to distinguish M4 from M2.* In AML of M4 type in humans, there is often an increase in marrow eosinophils rather than neutrophils and these are atypical and have a mixture of eosinophil and basophilic granules. In older classifications, M4 is defined as having at least 20% of both tumor cell lines staining for the neutrophil series (chloroacetate esterase, Sudan black or myeloperoxidase activity) or for the monocytic series (fluoride-sensitive esterase). The requirement for at least 20% involvement by both tumor lines rules out confusion with hyperplasia. *The critical point in the diagnosis of this disease is that both types of lineage-specific staining must be found in cells which are independently determined to be malignant.*

Animals are presented in good body condition but with a history of weight loss of recent onset accompanied by malaise and anorexia. Clinically, there is pallor of mucous membranes, irregular enlargement of cervical nodes, mild splenomegaly and moderate fever. Anemia is moderate to marked and nonresponsive, with some fragmented cells, increased pallor and Howell-Jolly bodies. Thrombocytopenia is marked and poorly responsive. The leukocyte count is generally between 25 and 50 $\times 10^9$/L. Morphologically, the leukocytes resemble those of myeloblastic leukemia with some maturation, and acute monocytic leukemia, occurring in the same animal. Malignancy, as determined by nuclear atypia and immaturity in the peripheral blood is present in both systems. In general, the degree of esterase staining varies with the degree of cytoplasmic granulation, and both cell lines may be positive for peroxidase activity. The course is 4–6 weeks with termination due to sepsis and thrombocytopenic bleeding. Grossly there are widespread petechial hemorrhages, focal infarctions of liver, kidney, spleen and marrow and usually oral ulcerations.

Histologically there is widespread tumor infiltration in marrow, splenic sinuses, nodes, portal areas of the liver, and irregularly in other organs including lung. In untreated cases, benign extramedullary hematopoiesis may be found in adrenal glands and nodes. There is prominent phthisis of normal lines in marrow, particularly of megakaryocytes, and the diagnosis is dependent on cytology and cytochemistry. *M4 must be differentiated from monocytic leukemia (M5) and from myeloblastic leukemia M2, from which it is indistinguishable histologically.*

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M5, Acute monocytic leukemia

Acute leukemia of the monocytic lineage occurs as a *poorly differentiated* **acute monoblastic type M5a** *and in a more mature cell type* **acute monocytic M5b**. *The predominant cell in M5 is a monocytic precursor as defined by nonspecific (α-naphthyl-butyrate) esterase activity.* In the monoblastic M5a type, there are at least 20% of blast cells in marrow or blood and at least 80% of the cells are of monoblastic and promonocytic types. In the more mature M5b type, the same level of blast cells are present but promonocytes predominate in both marrow and blood with the total of both blastic and promonocytic forms equaling 80% of nucleated cells. In contrast to M4, less than 20% of cells mark with Sudan black or myeloperoxidase indicating granulocytic origin. The leukocyte count tends to be low or normal in the more acute form and elevated in the differentiated variant.

Monocytic leukemia occurs in young dogs and cats and in mature horses and cattle. The disease in the young tends to be of the M5a type whereas that in adults is M5b. This description is typical of the disease as occurs in the dog. M5a is an acute disease with clinical presentation similar to that of myeloblastic leukemia. Animals are presented because of general signs of lethargy, weight loss, vomition, diarrhea, and anorexia and more specifically recurrent epistaxis. Clinically there is pallor that appears worse than the PCV would dictate and is characteristic of this disease where there is sludging of tumor cells in the microvasculature. The course of the disease is generally 2–4 weeks.

Anemia is usually marked (<50 g/L) and nonresponsive with pallor in larger red cells. Thrombocytopenia is severe and nonresponsive. The leukocyte count is usually $<25 \times 10^9$/L at diagnosis but increases to between 50 and 100 $\times 10^9$/L terminally. There is often mild neutrophilia and left shift, which is likely due to growth factors produced by the tumor and a field effect of the autocrine pathogenesis driving the tumor cells themselves. *Cytologically, the tumor is relatively monomorphic, but both monoblasts and promonocytes are present, with the latter predominating in the blood* (Fig. 2.13). The monoblasts are large, 2.5 to 3 red cells in diameter with an irregularly indented nucleus whose boundaries are obscured by intense cytoplasmic basophilia. The promonocytes have slightly smaller nuclei, less than 3 red cells in diameter, with convoluted or cerebriform outline, a fine retiform chromatin pattern, and 1–2 nucleoli of moderate size. The cytoplasm is more basophilic than in young benign monocytes, and characteristically of uniform density from the nucleus to the cytoplasmic boundary. This is a useful distinction from the perinuclear clearing seen in lymphocytes. Very fine azurophilic granulation is apparent in the Golgi clearing, and clear vacuoles are frequent and often penetrate the nucleus. Rubricytes may be present, but the anemia is nonresponsive.

Aspirated marrow is highly cellular with relatively complete phthisis of erythroid and megakaryocytic cells. Myelopoiesis is synchronous to the neutrophil stage but relatively reduced in amount. Marrow cells are 80% undifferentiated blasts, monoblasts and promonocytes with nuclear hyperchromicity and prominent nucleoli and a moderate mitotic rate.

Lesions are similar to those of myeloblastic leukemia, often with focal hemorrhages, but less often with secondary infection. The tumor distribution (Fig. 2.14A, B) is like M2 with greater dissemination to the urinary system and adrenals. Histologically, the nuclear convolutions are less apparent than on cytologic examination and the tumor cells resemble cleaved lymphocytes with deeply amphophilic

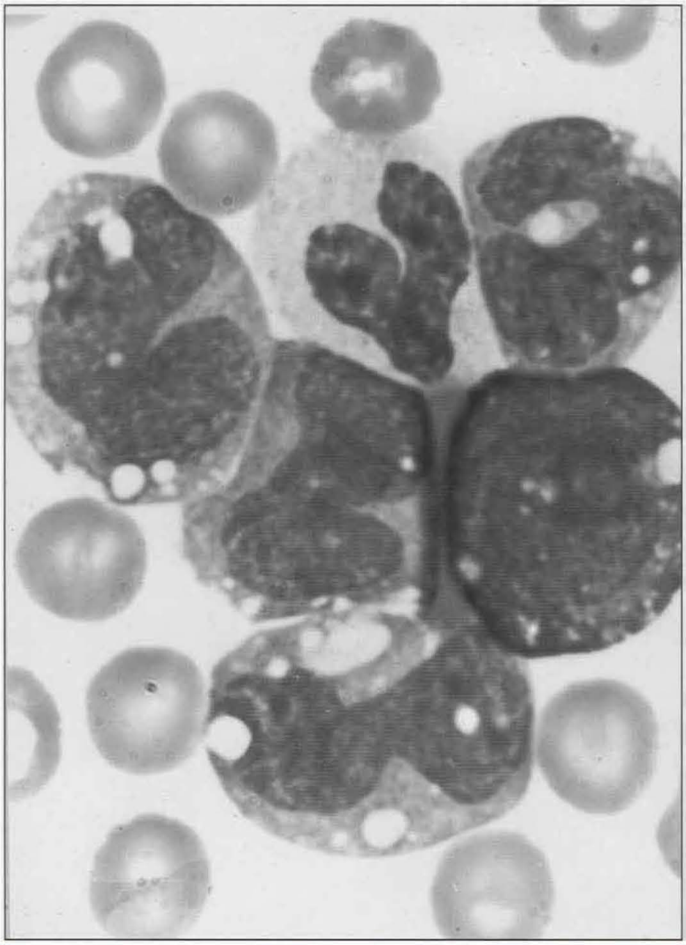

Figure 2.13 Monocytic leukemia, blood, dog. Irregularly convoluted and segmented nuclei with prominent cytoplasmic vacuoles indicate monocytic differentiation. Vacuolation characteristic of monocytes is present in the blastic cell at center right.

cytoplasm. The tumor cells are not regularly phagocytic and this criterion is not helpful in distinguishing this disease from acute lymphocytic leukemia (ALL) of the L2 type. *Definitive diagnosis requires cytochemical staining on blood and marrow aspirates or imprints.*

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M6, Acute erythroid leukemia

M6 occurs in two forms, of which **M6a (erythroleukemia)** (Fig. 2.15) *is a combined acute leukemia of both the erythroid and myeloid systems. In M6a leukemia, the erythroblasts are usually more than 50% of all marrow cells.* If this condition is present and erythroleukemia is suspected, then a second count of blast cells is made with the erythroid cells excluded. If in that count the level of myeloblasts equals or exceeds 20% of other marrow nucleated cells, then M6a or erythroleukemia is confirmed. In **M6b leukemia (erythremic myelosis)** (Figs 2.16–2.18), *the malignant erythroblasts are even more prominent and erythroblasts and prorubricytes constitute as much as 80% of all nucleated marrow cells.* Myeloblasts make up much less than 20% of the remaining marrow cells and some myeloid cells may have dysplastic changes. Erythroleukemia, or M6a, while rare in animals, is relatively less so in humans. On the other hand, erythremic myelosis or M6b, a disease associated with *Feline leukemia virus* infection in the cat, is much more common in animals than in humans where it is exceedingly rare. Hematopoietic proliferation in benign cells has *two loci of genetic control* to which the feedback system of growth factors provides the fine tuning. In malignancies, the system may become deregulated at either level resulting in either a poor- or well-differentiated tumor. In the erythroid system, autonomy at the early loci results in erythremic myelosis, whereas at the late loci the disease is polycythemia vera with an overproduction of normal appearing red cells. Within erythremic myelosis, there are variations in the general maturity of the tumor cells such that the more mature variant may initially be mistaken for a benign response to severe anemia while the immature variant resembles the hairy cell leukemia or "reticulotheliosis" described in cats. These variations are apparent histologically in the accumulation in the spleen and liver of rubricytes that are either relatively mature and well-saturated with hemoglobin or are primitive and resemble lymphocytes with round nuclei and an unusual level of anisokaryosis. The malignant erythroid cells mimic their normal counterparts in containing sufficient glycogen to stain positively with the periodic acid-Schiff (PAS) reaction in both cytologic and histologic preparations.

Erythremic myelosis is largely a disease of cats but we have seen it in a mature Holstein cow. It may appear from 1 year to old age and has a 2:1 predominance in males. The disease occurs rarely in other domestic animals.

Animals are presented with a history of depression lasting from a few days to 2 months. Anorexia develops and is occasionally accompanied by vomition and diarrhea. With recent onset, the animals are in good condition and later are thin with mild abdominal distension due to splenomegaly and mild hepatomegaly. The lymph nodes are of normal size or small and there is marked mucosal pallor, occasionally with mild icterus. The course of the disease is 1–3 months.

Anemia is severe with hemoglobin levels varying from 20–50 g/L. Hemotrophic mycoplasma infection (*Haemobartonella*) is often suspected and may occasionally be present but should be recognized as incidental because there is lack of polychromasia and blast cells are present in the blood. Anisocytosis is mild to moderate and in the cat the mean corpuscular volume is characteristically increased from a normal of 45 fL to the range of 60 fL. Changes in shape are slight and hemoglobin saturation is normal. Howell-Jolly bodies and spherocytes, and occasionally Heinz bodies, are present, but immature or polychromatic red cells average less than 1 per oil immersion field. The total nucleated cell count varies from 20 to 200 × 10^9/L and is

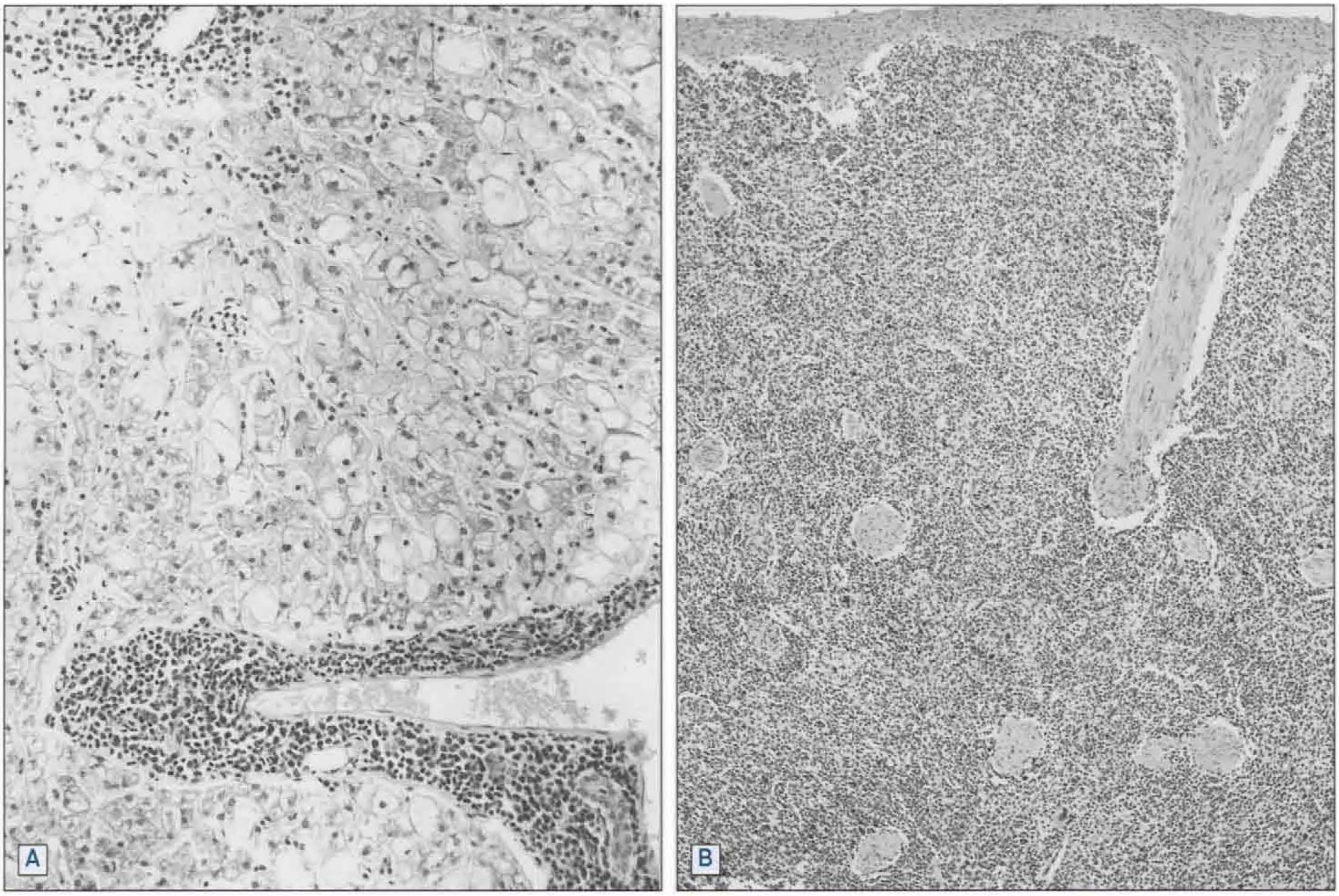

Figure 2.14 Monocytic leukemia in a dog. **A.** Colonization of periportal (below) and periacinar areas in the liver by malignant monocytes. **B.** Follicular atrophy and diffuse sinusoidal colonization in the spleen.

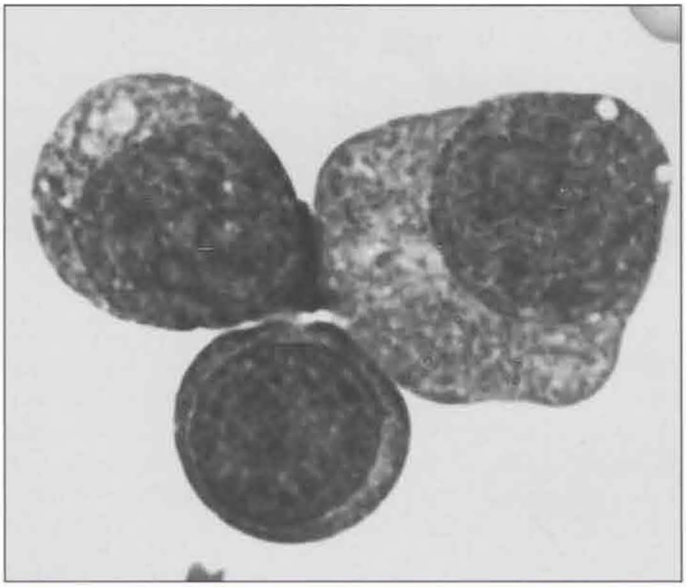

Figure 2.15 Erythroleukemia, canine blood. Concurrent neoplasia of granulocytic and erythrocytic systems. Malignant myeloblast (right) and malignant erythroid precursor (left).

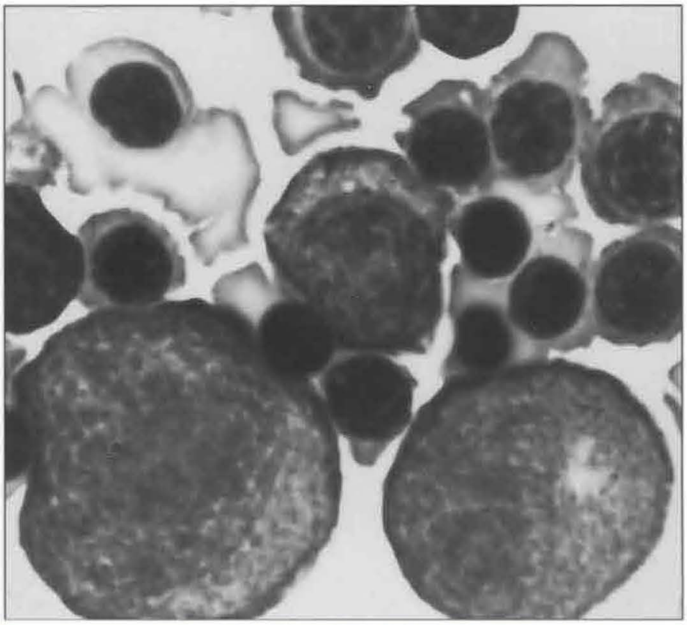

Figure 2.16 Erythremic myelosis in a cat; blood smear. Large malignant blast cells with a cluster of more mature progeny.

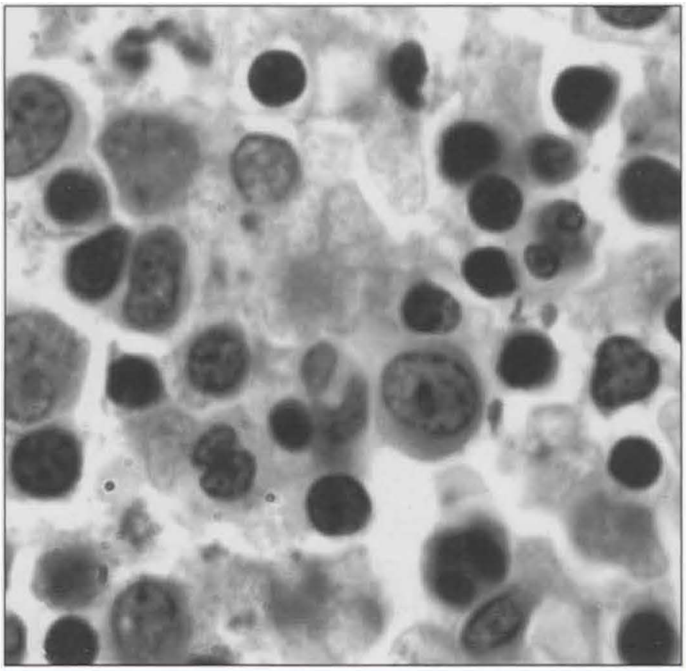

Figure 2.17 Erythremic myelosis in a cat. Histology of marrow. There is a predominance of maturation phase rubricytes.

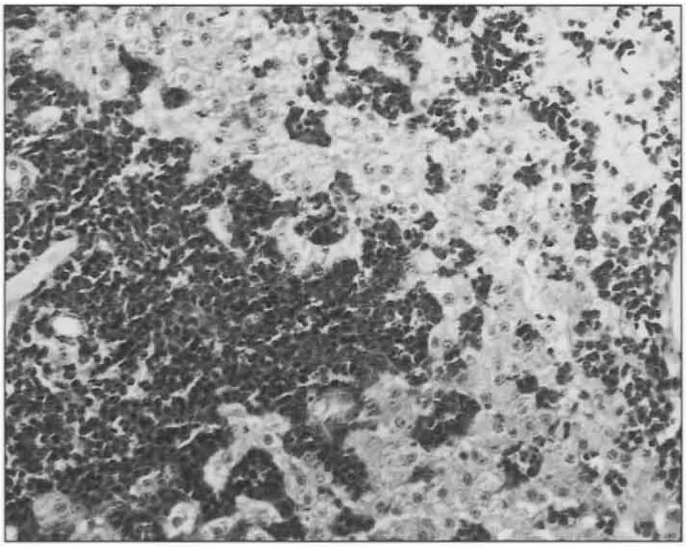

Figure 2.18 Erythremic myelosis in a cat. Liver with heavy periportal colonization (left) and multiple foci of tumor colonization in sinusoids and around central vein (right).

usually $>50 \times 10^9$/L. Neutrophils are at low normal or deficient levels and there may be a mild left shift. Lymphocytes may be $>10 \times 10^9$/L with higher counts probably reflecting the integrity of extramedullary lymphopoiesis. Rubricytes occupy 90% of the differential count and two-thirds of these are readily recognized polychromatic, normochromic and metarubricytes which have normal hemoglobin saturation but are larger than normal and have nuclei which appear inappropriately young for the degree of cytoplasmic maturation (early asynchrony). Presumably this larger size accounts for the persistent rise in MCV despite the lack of polychromasia, indicating that many red cells are the progeny of leukemic precursors. By association, the maturing rubricytes can be related to basophilic and prorubricytic precursors, but their significance may be missed if lymphocytosis is present and the numerous leukemic blasts are thought to be part of lymphoproliferation with benign rubricytosis. *Rubricytosis in the absence of polychromasia does not indicate a marrow response and this asynchronous production suggests neoplasia in the erythroid system itself.* The blasts (Figs 2.15–2.18) typically have round nuclei with dense cribriform chromatin and a single nucleolus which may be as large as a normal red cell and one third the nuclear diameter. The cytoplasm is moderate in volume, markedly eccentric, and basophilic. The malignant cells mimic benign rubriblasts in having a few fine azurophilic granules similar to those seen in myeloblasts. The granules differentiate these blasts from the lymphoid series, including the reticuloendotheliosis variant which these cells otherwise closely resemble. Platelets are generally $<75 \times 10^9$/L but immature platelets may be numerous. Many of the malignant rubricytes are injured in preparation of blood films and up to 25% of the differential count may be bare nuclei which in interpretation need to be included with their cohort.

Aspirated marrow is hypercellular and usually without fat or the usual coarse hemosiderin pattern of malignancy. There is an absence of mature granulocytes, and megakaryocytes are dysplastic but at near normal numbers, which may suggest that the leukemogenesis has occurred near the common precursor for the erythroid and platelet systems. There is marked early erythroid asynchrony with 70% of cells having large primitive nuclei which are described as "megaloblastic." Mild dyserythropoiesis is present with some bi- and trinucleation and small satellite nuclei. Mature rubricytes are rare considering their frequency in the peripheral blood.

On *gross examination*, all tissues are pale and there may be slight icterus. There is usually loss of condition and occasionally emaciation. The lymph nodes are of normal size or slightly enlarged. Lesions are largely limited to pallor, diffusely reddened femoral marrow, and moderate splenomegaly. The spleen is uniformly enlarged, and fleshy and dry on cut surface, without obvious lymphoid follicles.

Microscopically, the bone marrow has high cellularity with dense cellular packing (Fig. 2.16). Granulocyte reserves are absent. About 90% of the cells appear blastic with the rest much smaller and with apparent cytoplasmic hemoglobin. Megakaryocytes are present, usually in small clusters. The primitive cells have a fine granular chromatin pattern with thickened membranes and a single prominent central nucleolus. Nuclei are round or mildly irregular and the cytoplasm is moderate in volume, faceted against adjacent cells and deeply stained. The spleen has atrophy of thymic-dependent areas and increased sinus stroma with loosely packed, diffusely distributed tumor cells. Benign hematopoiesis is usually inapparent and hemosiderin is reduced. There is some subendothelial colonization of large muscular sinuses. Lymph nodes generally have cortical atrophy with sinus histiocytosis and occasionally contain focal neoplastic invasion. The liver usually has accentuation of zonation due to periacinar ischemia, and increased hemosiderin in Kupffer cells which suggests increased destruction of red cells. Tumor involvement is usually sinusoidal with dilation of periacinar sinusoids and mild focal investment of triads (Fig. 2.18).

Erythremic myelosis must be distinguished from hemolytic anemia. Cats with immune hemolysis may occasionally have marked rubricytosis of $50–90 \times 10^9$/L but these precursors are accompanied by $300–600 \times 10^9$ reticulocytes/L. Spherocytosis and rafting of red cells are present in immune hemolysis, the erythroid immaturity is less severe than in erythremic myelosis, and nuclear atypia is absent.

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M7, Acute megakaryoblastic leukemia

Acute megakaryoblastic leukemia is a rare disease mainly seen in dogs. This disease *may be underdiagnosed clinically* because of confusion with myeloblastic leukemia (M1), and acute lymphocytic leukemia (ALL) of the L2 type and morphologically with myeloid metaplasia or by new terminology acute panmyelosis with myelofibrosis. *M7 is characterized by 20% or more of blasts in the blood or marrow and at least 50% of the marrow cells must be of megakaryocytic lineage of some level of maturation.* The disease affects a wide age range in both humans and animals. Pathogenetically, the aberrant cytoplasm of the tumor cells is procoagulant resulting in fibrin deposition and repair by largely reticulin fibrosis. Platelet-derived growth factor (PDGF) is felt to be up-regulated and drives the *proliferation of benign stroma. The result is myelophthisis and very rapid marrow failure.* Clinical signs are typical of acute leukemia, often with fever, subcutaneous hematomas and gastrointestinal hemorrhage. The animals are usually in good condition indicative of rapid onset and typically are presented with hepatosplenomegaly. The course is usually 2–4 weeks.

There is pancytopenia with moderate to severe nonresponsive anemia and thrombocytopenia, and a total leukocyte count around $5 \times 10^9/L$ with neutropenia and peripheral blast cells. In blood, the megakaryoblastic nuclei are typically 1.5 to 3 red cells in diameter and round (Fig. 2.19), but irregular constrictions and binucleation may be seen. Chromatin is dense and cribriform and nucleoli are not prominent. Clear vacuoles may penetrate the tumor nuclei and cytoplasm and appear in platelets. Unlike malignant myeloblasts, the cytoplasm is not round and entire but scanty and may have one or two blunt pseudopodia. Basophilia is marked and obscures azurophilic granulation, which is variably present and is most easily seen in the cytoplasmic protrusions. Platelets vary widely in size and are basophilic but poorly granulated. If bleeding is prominent, there will be fragmented red cells.

Aspirated marrow may be highly cellular but the irregular sclerosis rapidly makes a core biopsy essential to evaluate marrow. There is early loss of erythropoiesis and granulopoiesis and loss of granulocyte reserves. Usually 70–90% of marrow cells are hyperchromatic blasts with irregularities in nuclear size, shape and number per cell. *The presence of nuclear fusion in some cells is highly significant and is characteristic of megakaryoblastic leukemia.* The tumor cells mimic their normal counterparts and about a third stain positively with the PAS reaction both cytologically and histologically depending on their content of cytoplasmic glycogen. Because cytoplasm is scanty in the mononuclear blasts, the PAS reaction is most prominent in the larger cells with lobulated nuclei and in these the reaction is highly variable in the same cell. Tumor cells stain negatively with Sudan black and myeloperoxidase and positive with α-naphthyl acetate esterase and for factor VIII antigen. Monocytic leukemia or malignant histiocytosis is ruled out by the absence of erythrophagocytosis and by lack of reaction to the α-naphthyl-butyrate esterase. *L2 is ruled out on the recognition of the multinucleated and lobulated nuclei on routine stains of aspirates and sections.*

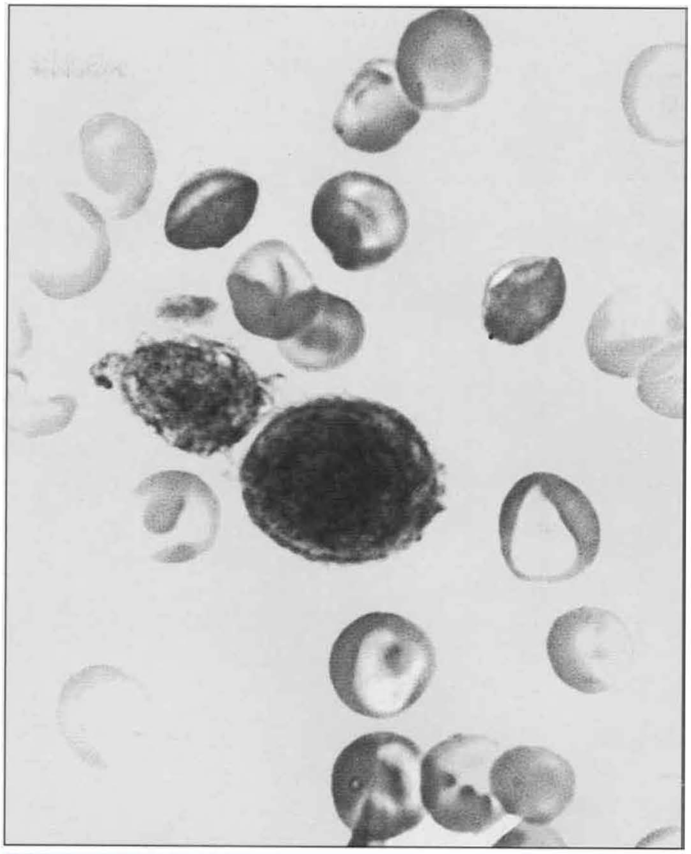

Figure 2.19 Acute megakaryoblastic leukemia, canine blood. Malignant megakaryoblasts with filiform and blunt cytoplasmic pseudopodia.

Grossly, there is pallor and irregularly hemorrhage that is more typical of factor deficiency than thrombocytopenia. The spleen is large and firm, and histologically there is lymphoid atrophy and marked sinus expansion with both blasts and irregular nests of megakaryocytic-like cells with marked atypia and occasionally very large nucleoli. Splenic stroma is increased and extramedullary hematopoiesis is absent or minimal. Tumor is present in the hepatic sinusoids and irregularly in nodes. There are remarkable tumor emboli in pulmonary arterioles with tumor cells apparently growing in syncytia (Fig. 2.20A). There is focal positivity for fibrin in these emboli indicating the propensity of this tumor to cause disseminated coagulation. In marrow, there is loss of fat and irregular phthisis of normal elements (Fig. 2.20B). The tumor cells proliferate in large foci that meld to diffuse areas that become fibrotic. In these foci, *the single and multilobular nucleated tumor cells greatly predominate and their apparent atypia rule out benign disease.* On specific staining, there is a marked and variable increase in reticulin but not of collagen.

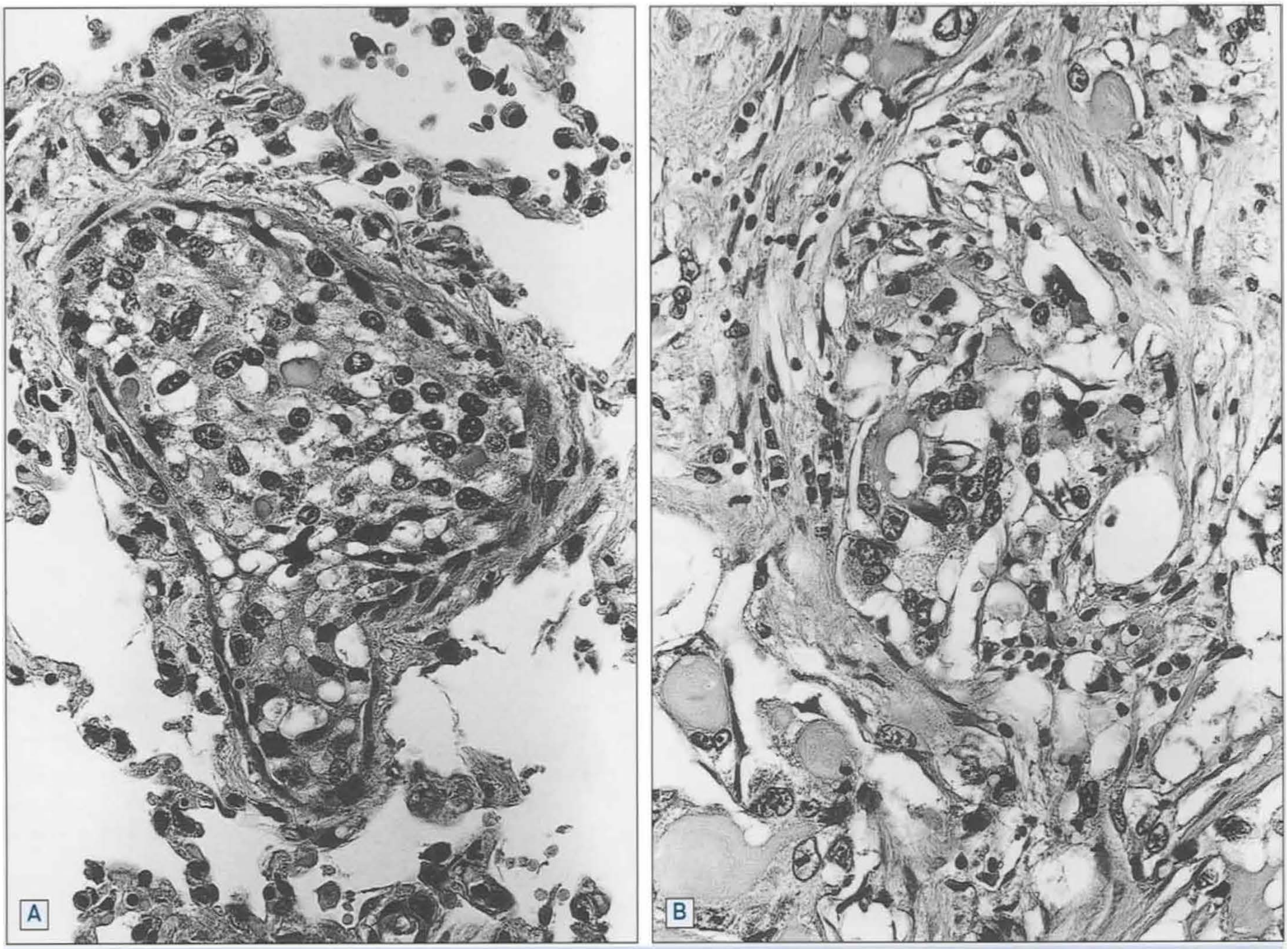

Figure 2.20 Acute megakaryoblastic leukemia in a dog. **A.** Lung, tumor emboli in arteriole. Small homogeneous areas are fibrin. **B.** Marrow with myelophthisis, myelofibrosis of benign stroma, and colonization by malignant megakaryoblasts.

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Chronic leukemias/myeloproliferative diseases

Fifty years ago, William Dameshek pointed out the similarities between the different types of chronic myeloid leukemia and noted that in many cases all lines of marrow production were involved as if the marrow was being driven to over-production by some abnormal stimulation. While one or more marrow lineages might be predominant in any particular case, the common presentation of slow insidious onset, indolent progression, with gradual development of hepatosplenomegaly, leukocytosis, thrombocytosis, and gradual onset of myelofibrosis and termination in acute leukemia suggested a single disease with a variety of early manifestations that were collectively termed the *chronic myeloproliferative diseases* (CMPDs). The individual disease entities in this grouping *included chronic myelogenous leukemia (CML), polycythemia vera (PV), chronic idiopathic myelofibrosis with myeloid metaplasia (CIMF), and essential thrombocythemia (ET) also known as megakaryocytic myelosis*. Subsequent work has shown that *a major feature linking the CMPDs is their clonal origin in a single marrow stem cell.* Thus even though a single cell lineage may have a proliferative advantage in a given case, multiple abnormal cell lines may be derived from the same abnormal stem cell.

The evidence for this relationship followed Dameshek's proposal by 10 years when the cytogenetic abnormality subsequently called the *Philadelphia chromosome* was found to be associated with most cases of CML, and not only in the neutrophil line but also in erythroid and megakaryocytic precursors and later in monocytes and some lymphocytes as well. This information *firmly established the CMPDs as clonal diseases with a variety of blood manifestations*. Each of the CMPDs is characterized by *early effective hematopoiesis with increased numbers of a neoplastic clone that reaches normal maturation with few morphologic or functional abnormalities.* Early signs of the CMPDs are related to the increased numbers of cells in the blood and to the accompanying increase in size of the spleen and liver. Finally, with progression of the disease, *each CMPD tends to progress to a point where hematopoiesis is ineffective and abnormalities develop in both maturation and function of the neoplastic clone with increasing immaturity to terminate in acute leukemia often with some features of myelofibrosis.* The proliferation of reticulin fibers and even collagen in the CMPDs was initially felt to occur because the marrow stroma itself was also part of the neoplastic clone. The advent of cytogenetic and molecular analyses has shown that the marrow stroma is not itself involved in the clonal abnormality but is responding appropriately to abnormal stimuli. Fibrosis is a common or constant finding in lesions characterized by megakaryocytic proliferation whether of the acute myeloid leukemia AML M7 type or CMPD of ET type or in the myelodysplastic diseases. Since there is some aspect of megakaryocytic proliferation in the CMPDs of other than the ET type, it has been assumed that platelet-derived growth factor, which stimulates proliferation of fibroblasts, and transforming growth factor-β (TGF-β), which regulates synthesis of extracellular matrix, are the operative stimuli. Subsequently it has been apparent that other myeloproliferative diseases like chronic myelomonocytic leukemia (CMML) and chronic monocytic leukemia also belong in the CMPD grouping. Newly recognized entities in human disease, including atypical CML (aCML) and juvenile myelomonocytic leukemia (jMML), are added to the CMPDs in the revised WHO classification of hematopoietic and lymphoid neoplasms.

CML has been the bellwether of things to come in our understanding of hematopoietic neoplasms. After the recognition of the micro-chromosome in CML, a great deal of work followed in the development of cytogenetics as a diagnostic tool. The advent of staining metaphase spreads of cells to identify specific areas in each chromosome or "banding" permitted each of the chromosomes to be specifically identified and numbered. Subsequently, Janet Rowley in 1973 recognized that the chromosomal abnormality in CML was not a deletion but rather a reciprocal translocation involving chromosomes 9 and 22. A decade later, work showed that the breakpoint cluster region (BCR) on chromosome 22 became linked to the ABL proto-oncogene from chromosome 9. The resulting fusion produced a new gene (BCR-ABL) that was not only capable of being transcribed to mRNA but was translated into a chimeric or fusion protein endowed with tyrosine kinase activity that is potentially oncogenic. There is current interest in work showing that the t[9;22] translocation and the formation of the BCR-ABL gene, while part of the process of clonal proliferation in CML, may not be the initial event but a secondary injury in a clone of stem cells that have already undergone a primary injury.

The ABL proteins have been shown to have a number of domains that allows for multimerization and also domains for DNA binding and for retention of the resulting protein in the nucleus. Variations in the resulting proteins permits elimination of the nuclear retention, and the subsequent cytoplasmic localization allows the hybrid protein with the tyrosine kinase activity to act on the RAS and RAF pathways which control cell proliferation. In human CML, the *BCR* breakpoint in chromosome 22 typically occurs in a region that spans the exons e12 to 16 and commonly known as b1 through b5 with the breakpoint usually at the b2 or b3 areas. In chromosome 9, the break in the ABL gene is usually at the beginning of the gene in an area known as b2 so that most of the gene is transferred to chromosome 22. Thus, the typical fusions are termed *b2a2* or *b3a2*. These two fusions are seen in virtually all cases of CML and in 20–50% of adult and 10% of pediatric cases of Ph+ acute lymphoblastic leukemia (ALL). Other breakpoint combinations occur and produce the fusion genes associated with the Ph+ ALL seen in the rest of the Ph + adult and pediatric cases. An *e19a2* fusion (also called *c3a2*) is felt to be the basis for a rare type of human CML in which there is very primarily neutrophil proliferation and known as chronic neutrophilic leukemia (CNL).

Typically, human CML progresses in 4–5 years to a terminal blast phase that is refractory to treatment. Since the blast phase of CML is like AML, the two may be confused if the chronic phase of CML was not recognized. *The blast phase may be focal or multifocal in marrow, and is best looked for by marrow core biopsy.* In addition, the blast phase may begin in an extramedullary site, making it important to examine any enlarging lymph node or other new proliferative lesion. Two thirds of the cases of human CML in blast phase are of the granulocytic lineage (M-BP) and resemble (AML M1-2) type or of granulocytic–monocytic (M4) type. Cytochemical reactions tend to be less intense than in the de novo AML and the blasts usually express CD13 and CD33. A rare type of myeloid blast phase is termed mixed-myeloid and is characterized by atypical appearing blasts of all three myeloid, erythroid, and megakaryocytic lineages. A third or less of human CML enters the blast phase with a lymphoid phenotype (L-BP). The L-BP blasts have very predominantly B-cell expression and mark with pan-B-cell reagents CD19 and CD20 and also co-express CD10. The blasts are TdT (terminal deoxynucleotidyl transferase)+ but do not express surface or cytoplasmic immunoglobulin.

While much has been learned about the CMPDs, *CML continues to be a fatally progressive disease.* The "designer" chemotherapeutic drug Cleevec has anti-tyrosine kinase activity and induces remission in human CML. Those cases failing remission after Cleevec therapy have developed a single amino acid substitution in the kinase fusion protein that prevents the drug from binding to it.

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Chronic myelogenous/granulocytic leukemia

Chronic myelogenous leukemia (CML) is recognized most frequently in mature dogs and cats and probably occurs in all species. Males cats are more often affected than females. The chronic form of myeloid leukemia CML is *less common than the acute form (AML).*

In contrast to the acute myeloid leukemias, there is a history of weight loss extending over several months with recurrent inappetence and diarrhea and often several treatments for slow-healing superficial lesions. Clinically there is mucosal pallor, dullness of hair, loss of condition, splenomegaly and increased heart rate. Lymph nodes are of normal size or slightly enlarged. The course is 1–4 years or longer. Late conversion to an acute leukemia is seen less often than in humans, probably because animals with this disease are usually destroyed when they enter the accelerated phase and prior to the development of a blast crisis.

Anemia is mild to moderate, nonresponsive, and less severe in dogs than in other species. A few relatively mature rubricytes are usually present plus a small number of fragmented and large red cells in a normochromic, normocytic or mild hypochromic background. The leukocyte count is usually $>50 \times 10^9$/L and often exceeds 200×10^9/L. Howell-Jolly bodies are usually present as a result of splenic overload, particularly if the leukocyte count is markedly elevated. By a similar mechanism, some hypersegmented neutrophils will be present and more prominent the higher the leukocyte count. *The major feature of chronic myeloid leukemia is that segmented neutrophils are always present in the peripheral blood and usually predominate.* Malignancy may occur in any of the three granulocytic lines, but *neutrophils are most often affected.* Leukemia involving the eosinophil line is seen most frequently in cats and basophil tumors in pigs. Secondary granulation is usually reduced, particularly as the disease progresses, and there may be toxic vacuolation (Fig. 2.21A). There is usually a reasonably synchronous distribution of myeloblasts to neutrophils, with the exception that myelocytes exceed the number of metamyelocytes. Myeloblasts are rare in peripheral blood and usually not found in a differential count of 100 cells. Cytoplasmic basophilia is usually mild and granulation is apparent in promyelocytes and myelocytes (Fig. 2.21A). There is usually a mild increase in normal appearing basophils. Platelet levels may be normal or increased but are usually around 100×10^9/L and with progression, deficiency may become limiting to life. Platelet granulation may be normal but is more often reduced, and there are occasional very large platelets.

Aspirated marrow is hypercellular during the chronic phase of CML with a myeloid to erythroid ratio of 50 or higher and a marked increase in myelocytes, metamyelocytes, and band neutrophils which, with a few segmented cells, occupy more than half of the marrow volume. Marrow basophilia and eosinophilia are commonly present. Myeloblasts are <5% of marrow cells and may total 10% combined with promyelocytes. Erythropoiesis is synchronous and never as deficient as in the acute leukemias. Hemosiderin is normal or reduced and megakaryocytes are at normal levels for long periods, but become deficient as the disease progresses and becomes more aggressive. Macrophages are noted to be atypical in a high proportion of human CML and may have blue cytoplasm due to the presence of birefringent crystals. The character of the cytoplasm may vary from faintly granular "tissue paper"-like to gray-green to clear pale blue and referred to as "sea-blue histiocytes." Similar cells have not been recognized in animal CML.

Grossly there is pallor and usually emaciation. There may be widespread focal hemorrhages, and enteric bleeding is common as are chronic oral or skin ulcers. The spleen is always enlarged, dry, and fleshy and may be many times normal size. The liver is enlarged to a variable degree, pale with an anemic lobular pattern, and may have focal hemorrhage, infarction, or infiltration. The nodes are irregularly enlarged, often due to septic drainage from secondary disease. There may be focal lesions in other organs but they are usually minimal. All marrow cavities are uniformly deeply reddened.

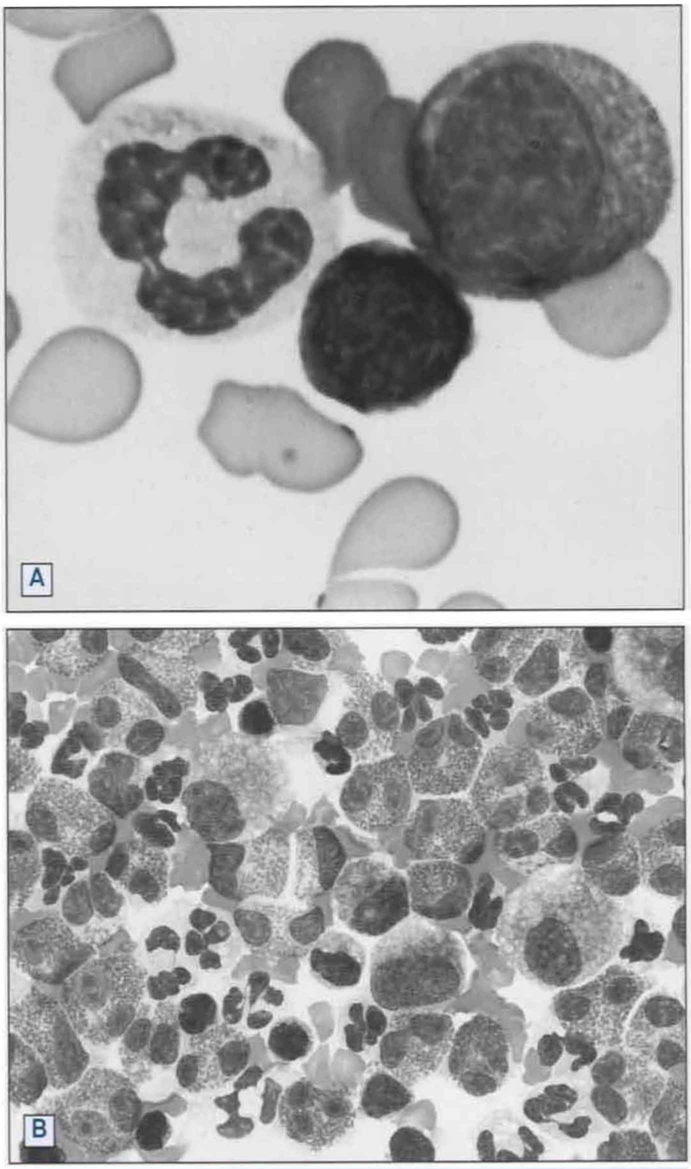

Figure 2.21 A. Chronic granulocytic leukemia, feline blood. Malignant promyelocyte (right) with a large poorly granulated neutrophil, probably the progeny of malignant precursors. There is nonresponsive anemia and mycoplasmas (hemobartonellae) are present on red cells (center). Benign basophilic rubricyte (center), in the absence of polychromasia, indicates myelophthisic erythroblastosis. **B. Hypereosinophilic syndrome** in a cat: bronchoalveolar lavage. A high proportion of mature eosinophils accompanied by neutrophils, large alveolar macrophages and a few small lymphocytes.

Histologically, the bone marrow is solidly cellular with dense cell packing and reduced sinus area. Myelocytes, metamyelocytes and bands predominate with numerous giant metamyelocytes. Erythropoiesis is relatively much reduced, but may be near normal on an absolute basis considering the greatly expanded marrow cavity. Megakaryocytes may be numerous and are hyperlobulated without the usual condensation to a single nuclear mass. They tend to have reduced cytoplasmic volume in proportion to nuclear size (ineffective thrombopoiesis). Primitive cells are numerous and tend to cluster in laminar fashion near bony trabeculae and around vessels in layers much thicker than the normal level of 1–3 cells to 5–10 cell layers. *An increase in depth of the primitive cell layer presages onset of the accelerated phase.* These proliferating cells have round or oval nuclei with coarsely granular chromatin and prominent single central pink nucleoli. Their cytoplasm is abundant and moderately amphophilic. Lymph nodes vary depending upon whether they are secondarily infected, but tend to have follicular hyperplasia with reduced cell density. Mature tumor cells may fill the peripheral sinus and permeate the capsule with cells in rows between the collagen layers. Primitive tumor cells will variably colonize the medullary cords while the mature cells predominate in sinuses. There is variable colonization of the medullary sinuses. This is most obvious if the tumor is of the eosinophil or basophil type, and if colonization is extensive it is often accompanied by medullary sclerosis. Benign extramedullary hematopoieses will also colonize the medullary cords and can be recognized by the presence of megakaryocytes. As the tumor progresses, the benign cells become displaced to the adjacent medullary sinuses.

The splenic capsule is thin and focally bridged. Follicles are present and small but sharply delineated with low cell density and reduced thymic cuffs. The sinuses are hypercellular containing focal clusters of primitive cells with prominent cytoplasmic basophilia, and also some mature cells but fewer than in the marrow. Tumor cells colonize the subendothelial areas of muscular veins and constitute a useful marker for the presence of leukemia/lymphoma. Hemosiderin is not increased and may be deficient if there has been recurrent hemorrhage.

The liver has both focal and sinusoidal involvement, the latter in transit and the former consisting of fixed leukemic infiltrates around both portal triads and the large veins. In perivenous areas, tumor cells replace hepatocytes within the endothelium of the hepatic cords forming a hematopoietic tissue and sinus relationship similar to that of marrow. Virtually all other tissues may be focally invaded, especially adrenal medulla, renal cortex around arterioles and, less regularly, the heart, reproductive, pulmonary and enteric systems. Lesions of cachexia are present in advanced cases.

Chronic myeloid leukemia must be differentiated from leukemoid reaction and from **hypereosinophilic syndrome** (Fig. 2.21B) *on the absence of blasts in the peripheral blood, an inflammatory target for increased myeloid production, and tissue destruction by the infiltrating tumor cells.*

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Chronic neutrophilic leukemia

Chronic neutrophilic leukemia (CNL) is rarely recognized in humans and has not been characterized in animals as a disease entity distinct from the CMPDs with "pure" neutrophil proliferation without accompanying changes in the other cell lineages. As such, it is more difficult to differentiate from the leukemoid reaction which tends to be an ephemeral response. The disease CNL in humans is recognized by sustained neutrophilia with granulocytic predominance in the marrow, and hepatosplenomegaly without presence of the Philadelphia chromosome in the tumor cell line.

Chronic eosinophilic leukemia/hypereosinophilic syndrome

Chronic eosinophilic leukemia (CEL) in humans is characterized by the *presence of the Philadelphia chromosome and the BCR-ABL fusion gene.* Sustained eosinophilic proliferation in the absence of the t[9;22] translocation is considered the criterion for diagnosis of the *hypereosinophilic syndrome* (HES). Chronic increase in eosinophil proliferation of either benign or malignant cause will result in tissue destruction in sites of eosinophil deposition due to release of the characteristic granules. CEL in animals is like CML of the neutrophil type with the exception that there will be marked collagenous fibrosis of peripheral tissues where eosinophils are concentrated. *Reactive eosinophilia* is usually of short duration with eosinophil counts in the 1.5×10^9/L range and an obvious target for the cellular production. Common causes of reactive eosinophilia include parasitism, asthma, eosinophilic enteritis and the eosinophilic granuloma complex, the latter seen primarily in cats. Both CEL and HES are primarily disease of cats. *HES and reactive eosinophilia can be distinguished from truly malignant CEL by the presence of blasts in the blood in CEL and by a greater shift to immaturity of eosinophils in the marrow and more severe anemia with a greater reduction in erythropoiesis* (Fig. 2.22). Marrow blasts are increased in CEL but seldom reach the 10% level for combined blasts plus promyelocytes that may be present in CML of the usual type. Oddly, although there may be quite marked fibrosis in liver, lung, nodes and spleen in CEL, there is only mild reticulin proliferation in marrow.

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Granulocytic sarcoma

Granulocytic sarcoma or *chloroma* is the *extramedullary growth of focal granulocytic neoplasms, which may be of neutrophil or eosinophil type*

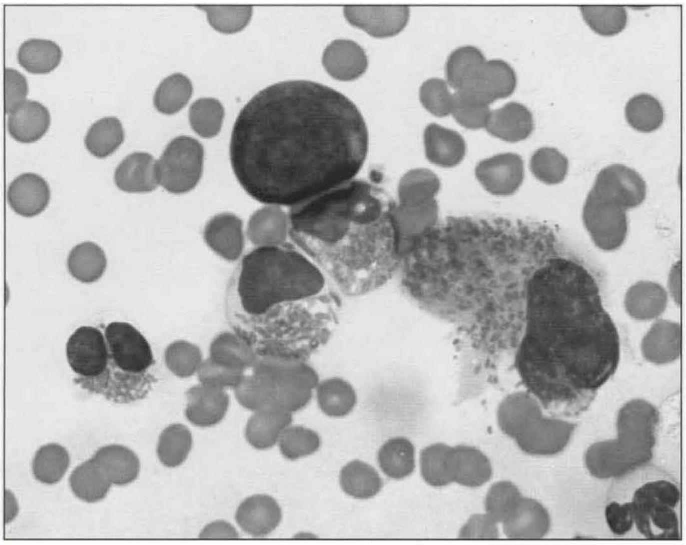

Figure 2.22 Chronic myelogenous leukemia, eosinophil type: feline bone marrow. Myeloblasts with injured eosinophil promyelocyte and maturing eosinophils.

(Fig. 2.23A–D). They are initially aleukemic and have variable differentiation, which, if minimal, usually leads to classification as lymphoma. They have typical granulocytic esterase (chloroacetate esterase, CAE) staining, and the ephemeral green hue on exposure to air is presumably due to a high level of myeloperoxidase. The disease in dogs tends to involve lung, gut and skin, but any tissue can be initially involved and skeletal muscle is characteristically affected in cattle. *There may be progression to acute myeloblastic leukemia* within months of biopsy. Most cases are diagnosed at autopsy. The detection of granulocytic sarcoma in an animal with a previous diagnosis of myeloid leukemia is indication of an accelerated phase with rapid progression and reversion to a less differentiated cell type. The major presentation of granulocytic sarcoma in humans follows 4 patterns of which the least common is as *an isolated mass without progression to acute leukemia, which would appear to be the most common presentation in animals*. The others are: as a localized lesion in patients who have AML, as a sign of impending conversion to AML in patients with MDS, and as a presenting lesion in the diagnosis of de novo myeloid leukemia.

Focal proliferation of eosinophils and precursors will result in *fibrosis* presumably as a result of tissue injury by lysosomal proteins. *Granulocytic sarcoma must be differentiated from benign hypereosinophilic syndrome and from eosinophil leukemia.* Cytochemical differentiation from lymphoma may be assisted by thin sections at 1–2 μm, in which the typical cytoplasmic granulation is most apparent, and by routine immunohistochemistry for B- and T-cell markers.

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Mast cell leukemia

Mast cells are widely distributed in connective tissues as a heterogeneous population. In the mouse and likely other species, the mast cell and the basophil share a common precursor. Neoplastic proliferation of mast cells to form *solitary or multiple tumors* is described in Vol. 1, Skin and appendages and in Vol. 2, Alimentary system. Diseases of the mast cell system in humans are relatively rare and most involve urticarial eruptions in the young. In contrast to animals, mast cell disease in humans involving the skin has a relatively much better outcome than systemic mast cell disease which almost always involves the bone marrow. *Systemic mastocytosis* involving primarily the hematopoietic organs in a pattern common to the leukemias is a rare disease seen primarily in humans and cats. Systemic mastocytosis occurs independently of cutaneous or alimentary mastocytoma. Mastocytemia may or may not be present but tends to be intermittent; *detection of mast cells in circulation is best accomplished on smears of buffy coat.* The hemogram may be normal or there may be eosinophilia and anemia.

The usual presenting signs of systemic mastocytosis in the cat are *vomiting and splenomegaly*. Gastric and duodenal ulceration is observed to accompany visceral and systemic mast-cell neoplasms, and is presumed to be caused by high levels of histamine produced by the neoplastic cells. The bone marrow may be involved, usually in a focal manner. The mast cells in marrow tend to be paratrabecular and are present both in mononuclear and spindle-shaped forms that resemble fibroblasts. There is usually a mixture of surrounding cells including eosinophils and lymphocytes. If frank leukemia is present, the marrow mast cells are more mononuclear and there is much more nuclear atypia. Nucleoli tend to be inconsistently present and their size and frequency do not appear to be of independent prognostic value. In the cat, splenomegaly which is often massive is consistently present. The spleen is firm and brown and there may be irregular white fibrous thickening of the capsule. Microscopically, the architecture of the splenic pulp is effaced by sheets of mast cells in diffuse distribution. The cytoplasm is bland and abundant and special stains are necessary to demonstrate the specific granules.

Hepatomegaly is frequently present. The infiltration of mast cells in the liver is diffuse and produces a fine stippled appearance when the triads are mainly infiltrated. Larger accumulations of the malignant cells in irregular foci may be visible as pale foci on the surface. The infiltrations in kidney, bone marrow and other organs tend to be more discrete than in the spleen and liver. *The major differentials to be considered in mast cell leukemia are AML of the M3 hypergranular type, manifestations of primary idiopathic myelofibrosis, and some cases of malignant fibrous histiocytoma that may have both eosinophilia and mastocytosis.* In animals, most cases of systemic mast cell disease have the diagnosis confirmed by the use of toluidine blue or other blood stains. Mast cells in animals, including malignant proliferations, generally stain with chloroacetate esterase stains in cytological preparations and with vimentin and lysozyme in paraffin-embedded tissues. In human diseases, the mast cells stain negatively for the integrins CD11a and b and for CD18, whereas basophils are positive for all three. Mast cells mark positively with CD117 [c-kit] and basophils are negative for this receptor, providing a means of distinguishing precursors of both cell lines. In both humans and animals, mast cell proliferations are heterogeneous in staining reactions in the same site in the same species which may in part be due to partial degranulation.

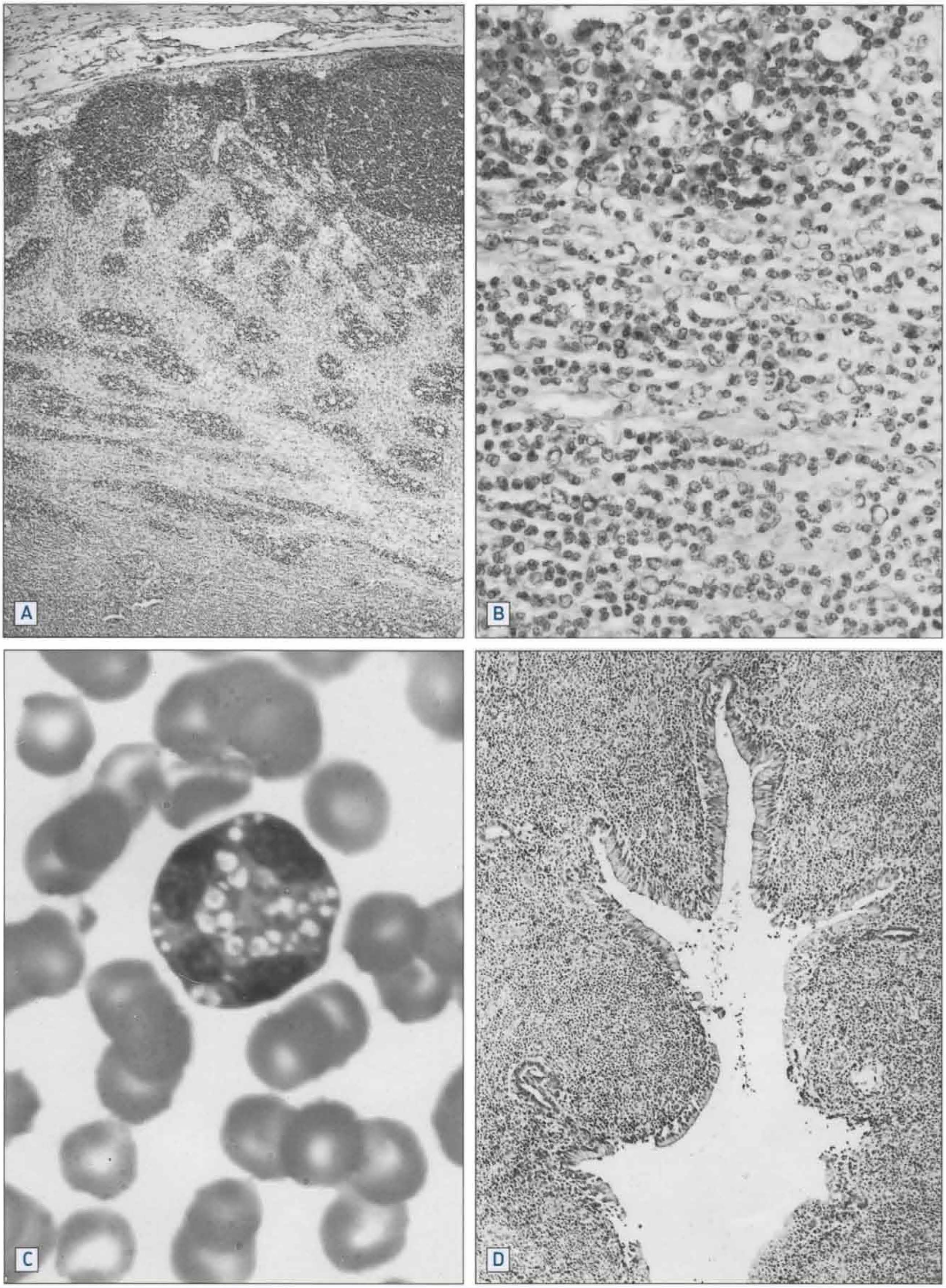

Figure 2.23 Granulocytic sarcoma in a dog. **A.** Lymph node. Follicular and paracortical atrophy. Sinus hypertrophy and colonization by granulocytic tumor. **B.** Detail of **A.** Medullary sclerosis and infiltration of eosinophils and precursors. **C.** Large atypical eosinophil in blood. **D.** Lung. Peribronchial infiltration with stenosis.

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Chronic idiopathic myelofibrosis/myeloid metaplasia with myelofibrosis

Chronic idiopathic myelofibrosis (CIMF) is the term that succeeds a variety of names for this same condition including *myeloid metaplasia with myelofibrosis* and *agnogenic myeloid metaplasia.* CIMF is the condition characterized by *clonal proliferation of bone marrow hematopoietic cells accompanied by the formation of connective tissue in the bone marrow as an event secondary to the hematopoietic proliferation.* Since by definition the disease is caused by excessive proliferation of hematopoietic cells with the presence of mobile stem cells that can move to other sites as the marrow becomes fibrotic, CIMF is usually accompanied by splenomegaly. Diagnosis is usually made when a hematopoietic dyscrasia evident in the peripheral blood prompts a bone marrow biopsy that results in a failed attempt to aspirate marrow – a "dry tap" – and is followed by a marrow core. Myelofibrosis in the marrow core may take the form of focal or diffuse reticulin sclerosis or focal or diffuse deposition of actual collagen. In well-advanced CIMF, there will be complete or almost complete phthisis of all marrow blood precursors, with the tissue consisting of a mixture of reticulin and collagen fibrosis and a few remaining fat cells. The only evidence of the original function of the tissue may be scattered hemosiderin-bearing macrophages that indicate a prior site of hematopoiesis.

In general, *CIMF is a disease characterized by inappropriate myeloid hyperplasia with synchronous maturation and without excess blast cells where the diagnosis of a leukemic process cannot be justified.*

CIMF occurs in dogs and cats. It is nearly always insidious in onset, and there is a history of reduced activity and weight loss. There is mucosal pallor and often minor oral ulcerations, and a history of recurrent and slow-healing skin lesions. Lameness associated with deep bone pain may be present as in myeloma, and the spleen is usually enlarged. The course is prolonged, generally 6 months to a year or more, usually with elective termination due to intractable anemia and recurrent infections.

Anemia is a constant finding even in the early "cellular phase" associated with high leukocyte counts, and is seen with variable degrees of hypochromia, anisocytosis and characteristic tear-drop-shaped red cells. Polychromasia is inappropriately mild, but relatively mature rubricytes are present as well as metamyelocytes as part of the *leukoerythroblastic reaction.* The leukocyte and platelet counts are increased in the early cellular phase and decreased later in the fibrotic phase of marrow failure. Hypersegmented neutrophils and toxemic changes are usually present in the later stages, but the early phase may have few of these and CML may be a differential consideration. Platelet size and shape is variable. Marrow aspirates are usually fibrillar and sparsely cellular with numerous bare stromal cell nuclei. The bone marrow is uniformly reddened and shells out of the femoral cavity in a cohesive cylinder.

Microscopically, the bone marrow loses its normal tendency for artefactual "cracking" during histological processing and is architecturally solid with few and irregular fatty areas. Cell density is reduced, as is sinusoidal area. The intercellular interstitium is pale, proteinaceous and not obviously fibrillar. In some areas, the specialized cells may be arranged in single file rows suggesting containment by reticulin sclerosis. Only a small proportion of cases survive until there is obvious collagen birefringence. With reticulin stains, the fiber proliferation is seen to be diffuse and spreading out from its normal perivascular location to invest individual marrow cells. Like the myelodysplastic syndromes, there is often venous ectasia with intravascular hematopoiesis. Stromal nuclei are increased and evident by their oval shape and indefinite cell boundaries. By the time collagen is demonstrable, hematopoiesis is absent and the extent of prior myeloid hypertrophy is indicated by hemosiderin-bearing macrophages surviving in poorly cellular areas of collagen matrix. The remaining hematopoiesis is largely granulocytic and megakaryocytic, with little erythropoiesis, and scattered foci of small lymphocytes (Fig. 2.24). Basophilic cells, which are prominent in early aspirates, are present but inconspicuous.

The spleen has follicular preservation and occasionally prominent hypertrophy. The sinus areas are solidly cellular and have obvious trilineage hematopoiesis. The liver has both periportal and sinusoidal colonies of hematopoietic cells, and similar foci with varying lineage can be found irregularly in almost all tissues except muscle. The lymph nodes are variable but usually enlarged with thickened capsules, medullary follicles, focal hematopoiesis and generalized sclerosis. This tendency for fibrogenesis to follow hematopoiesis is also evident

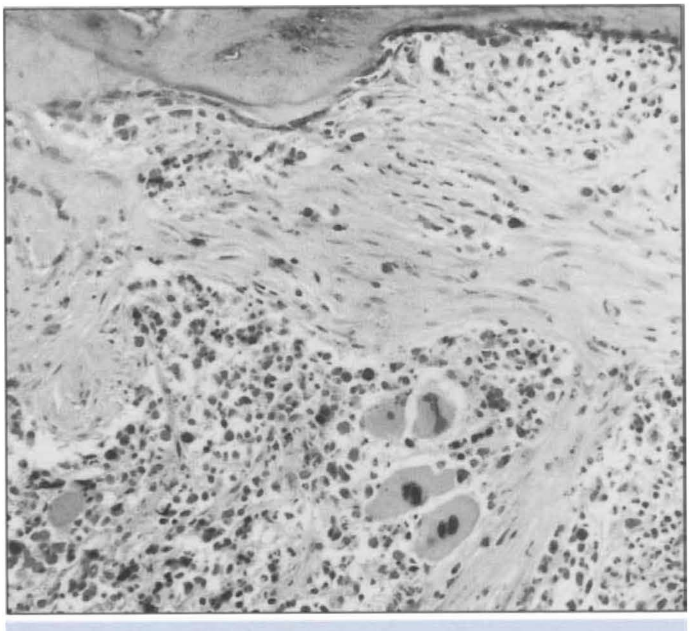

Figure 2.24 Myelofibrosis in dog marrow causing myelophthisic anemia.

in the spleen and liver, and suggests that the prolonged hyperplasia is attended by release of mediators that have been related to the megakaryocytic system and platelet-derived growth factor. *Unlike the myelodysplastic syndromes, CIMF regularly terminates in myelofibrosis not acute leukemia.* CIMF must be distinguished from reactive hyperplasia where there is a target for the increased cellular output, and from both myelodysplasia and CML where early asynchrony with increased blasts indicate a more aggressive diagnosis.

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Polycythemia vera

Polycythemia vera (PV) is a *rare disease seen mainly in dogs and cats* but occurs in cattle and can be induced in mice by the Friend leukemia virus. Mouse studies have shown that the "anemic" and "polycythemic" strains of this retrovirus interact with early and late acting genetic loci to produce either erythremic myelosis (M6) or polycythemia vera. In the latter disease, the erythroid stem cells will grow in culture without erythropoietin stimulation and were felt to be an autonomous tumor clone. Later work shows that other growth factors are involved in the erythroid hyperplasia, including IL-3, GM-CSF, and insulin-like growth factor (IGF-1) which appears to act cooperatively with erythropoietin (EPO). The endogenous erythroid colonies that grow in culture independent of EPO may be responding to minute amounts of insulin-like growth factor (IGF-1). The etiology of PV is unknown and, unlike the CMPDs, there are no consistent cytogenetic abnormalities.

The disease in humans is accompanied by leukocytosis and thrombocytosis and trilineage marrow hyperplasia in what is known as the "proliferative or erythrocytotic phase," which progresses to reticulin myelofibrosis and in most cases to a "spent phase," with marrow failure in most and acute leukemia in a minority, usually of the myeloid type. Leukemic transformation appears more common in patients treated with ^{32}P and chlorambucil than in those treated by phlebotomy. In humans, the disease is rare in patients under 60 years and most cases are diagnosed because of thrombotic sequelae. The standard criteria for making a diagnosis of PV in humans include the documentation of increased red cell mass to rule out plasma volume contraction or dehydration. Other criteria include normal arterial oxygen saturation and splenomegaly. If splenomegaly is absent, two additional abnormalities must be present, which might include leukocytosis, thrombocytosis, elevated neutrophil alkaline phosphatase activity, or elevated vitamin B12 levels.

Animals with polycythemia vera do not regularly develop thrombocytosis nor apparently myelofibrosis. This difference suggests that the myelofibrosis of the human disease may result from chronic megakaryocytic hyperplasia of the proliferative phase and attendant growth factor(s), or that animals with PV are more often terminated prior to the "spent" or marrow failure stage.

Affected animals are presented with reduced exercise tolerance and often polydipsia and signs of neuromuscular dysfunction, including head tremor and paraparesis. Clinically, there is mucosal congestion and cyanosis with labored respiration and signs of hemorrhage including epistaxis, hematemesis, hematochezia, and hematuria. Lymph nodes are normal, but occasionally there is splenomegaly. Thrombotic foci may be present and add to the hindlimb weakness. Males are affected more often than females, and the course is usually limited to months because of difficulties in management.

Hematologically, there is *marked erythrocytosis* with red cells in the $10–15 \times 10^{12}/L$ range, hemoglobin of 200–250 g/L and a hematocrit of 65–80%. The red cells are normochromic and normocytic unless there are thromboses, when poikilocytes will be present. Polychromasia or reticulocytes are usually not present, and the platelet count is low or normal. About one-third of cases have mature neutrophilic leukocytosis of up to $30 \times 10^9/L$. *Normal blood oxygen levels rule out secondary polycythemia.* The plasma volume is reduced, but the red cell mass is increased and may be doubled. Plasma iron turnover is greatly increased, and red cell life span is essentially normal.

Aspirated marrow has synchronous trilineage hyperplasia with cell proportions near a myeloid:erythroid ratio of 1.0. The cellularity is very high with little remaining fat.

Postmortem findings include generalized congestion and cyanosis with a tendency to arterial thrombosis. The spleen is enlarged in about 10% of cases, and bone marrow is diffusely reddened. Microscopically, there is dilation of hepatic sinusoids with hepatocellular atrophy and hyperpigmentation. The marrow has pancellular hyperplasia with high cellularity and reduced iron. There is splenic sinusoidal distention and congestion, compatible with increased blood volume.

Polycythemia vera must be distinguished from secondary polycythemia which is less rare and usually due to vascular anomalies causing anoxia. Rarely, primary or metastatic tumor in the kidney may also result in an erythropoietin-dependent polycythemia.

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Essential thrombocythemia/megakaryocytic myelosis

Essential thrombocythemia (ET) is the chronic counterpart of acute megakaryoblastic leukemia (M7). The disease in humans is regarded as a *clonal disorder*, but no consistent cytogenetic, morphologic or other laboratory marker has been associated with ET. In humans and animals, the disease is characterized by *sustained high-level thrombocytosis with marrow megakaryocytic hyperplasia.* ET is a rare disease of dogs and cats generally over five years of age with a clinical course of 1–3 years. *The disease must be distinguished from reactive thrombocytosis,* which is rarely persistent except in iron deficiency where the platelet counts are much below those of ET and the red cell changes are highly suggestive of the correct diagnosis. Other MPDs and the myelodysplastic syndromes need to be considered, but the blast cell count is low and the early asynchrony in marrow is largely limited to the megakaryocytic lineage.

Clinically, ET is characterized by bleeding and thrombosis of the tips of tail and ears and by massive thrombocytosis. Hematologically, there is *marked nonresponsive anemia* with mild anisocytosis and hypochromia and an occasional oval red cell. The leukocyte count is in the normal range, usually with reduced neutrophils and mild left shift, and mild to moderate basophilia, the latter being easily overlooked as the basophils are usually poorly granulated. The platelet count may be low or normal, but is most often dramatically increased to 2000–4000 × 10^9/L or higher which imparts a distinct blueness to the stained blood film. At any platelet level, there are marked morphologic changes with wide size variations, frequent vacuolation with pale basophilia, and irregular and reduced granulation (Fig. 2.25). The "shift" platelet reaction is marked, and functional tests of platelet activity, such as aggregation and clot retraction, are usually reduced. This thrombasthenia is made evident by serious hemorrhage resulting if even small amounts of aspirin are given to alleviate thrombosis in extremities.

Aspirated marrow is hypercellular with myeloid hyperplasia and early asynchrony involving the megakaryocytic and basophil lines, and erythroid phthisis with loss of mature granulocyte reserves. Hemosiderin has a coarse pattern and is increased in amount. With progression of the disease, aspiration of marrow is prevented by myelofibrosis and a core biopsy is required. Marrow aspirates are hypercoagulable, and nucleated cells are injured by fibrillar strands and obscured by masses of coherent platelets.

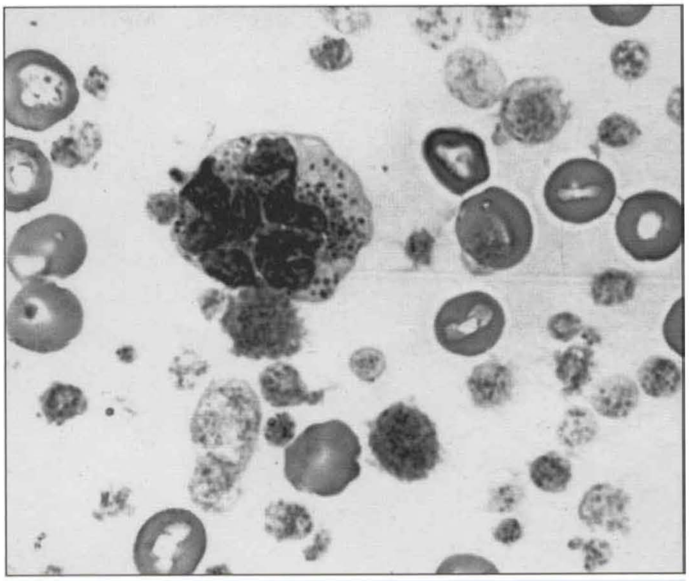

Figure 2.25 Essential thrombocythemia, canine blood. Marked anemia, largely normocytic and normochromic with severe thrombocytosis; platelets vary markedly in size, basophilia, and granulation. The cell in the center is an atypical basophil.

At postmortem, most tissues are pale, and the spleen is enlarged several times and dark, with a dry fleshy cut surface. The liver is symmetrically enlarged, dark to deep brown with rounded and notched borders. The kidneys are of normal shape and size but deep brown. The bone marrow is uniformly dark red and highly cohesive. Lymph nodes are of normal size.

Microscopically, the bone marrow is solidly cellular with generalized increase in reticulin fibers and focal reduction in cell packing where sclerosis is most advanced. There is massive megakaryocytic hyperplasia, often with normal cytoplasmic volume, and increased basophilia and hyperchromatic nuclei, but otherwise with little atypia. Isolated megakaryocytes are irregularly distributed in cortical areas of lymph nodes. Liver cells are atrophic with intrahepatic cholestasis, and sinusoids are 2–3 times normal width with few red cells and many megakaryocytes. Portal infiltration is mild and irregular. The spleen has lymphoid atrophy with hypercellular sinus areas which resemble bone marrow. Most other tissues are minimally affected.

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Myeloproliferative disease, unclassifiable (MPDU)

Animals may be presented with anemia and hyperplastic marrow and a spectrum of changes that are not typical of any of the diseases included in the CMPDs. If the cells are of myeloid origin and the blast cell level is below the 20% required for AML and less than 10% with some blasts in the peripheral blood, then a diagnosis of myeloproliferative disease, unclassifiable may be an appropriate working classification until the condition evolves into a more typical pattern. The diagnosis of MPDU may be thought of as a counterpart of acute myeloid leukemia not otherwise categorized for those myeloproliferative diseases with low blast cell counts and, dysplastic changes and uncertain lineage.

Myelodysplastic/myeloproliferative diseases

Chronic myelomonocytic leukemia (CMML)

Chronic myelomonocytic leukemia (preleukemia syndrome) is largely a disease of dogs over 5 years of age, and is likely under recognized in other species. The disease is characterized by persistent monocytosis with increased marrow monocytic precursors and variable prominence of trilineage dysplasia. There are circulating blasts that may be mistaken for atypical lymphocytes, and the marrow blasts plus promonocytes seldom exceed 10%.

There is a history of anorexia, lethargy, weight loss and reduced exercise tolerance. Affected dogs may have a poor hair coat and mucosal pallor, and often oral ulcerations. There is usually mild recurrent pyrexia with poor healing of minor injuries. The course is 6 months to 1–2 years. Some cases evolve into acute leukemia, but most are terminated because of recurrent infections and thrombocytopenic hemorrhage. Complete recovery probably does not occur. In CMML of humans, the criteria for diagnosis of this disease are an increase in marrow blasts in the 5–15% range but less than the 20% required for AML M5a or b.

Dogs with chronic myelomonocytic leukemia have mild-to-moderate nonresponsive anemia with mild anisochromia, anisocytosis, poikilocytosis and phthisic rubricytosis (leukoerythroblastic reaction). Most animals are thrombocytopenic with fewer than 100×10^9/L cells that have a relatively uniform basophilia and normal granulation. The leukocyte count is usually 4– 5×10^9/L, but may be as high as 30×10^9/L if animals are given steroid. Typically, there is neutropenia with about 1×10^9/L mature granulocytes, often with an equal number of cells as immature as myelocytes. Secondary granulation is reduced, and there is at least moderate toxemia and often Doehle bodies. Cases with low neutrophils and little immaturity tend to have some hypersegmentation. Lymphocytes are low normal. There are characteristically 1–4×10^9/L immature monocytes. Some are as young as promonocytes and have nucleoli (Fig. 2.26).

Aspirated marrow is always hypercellular and resembles refractory anemia with excess blast cells (Fig. 2.27A). There is early myeloid hyperplasia and loss or absence of granulocyte reserves, which paradoxically may be present concurrently with severe neutropenia. There is at least relative erythroid and megakaryocytic phthisis and qualitative changes are present, suggesting ineffective hematopoiesis (Fig. 2.27B). Marrow iron is coarse and increased.

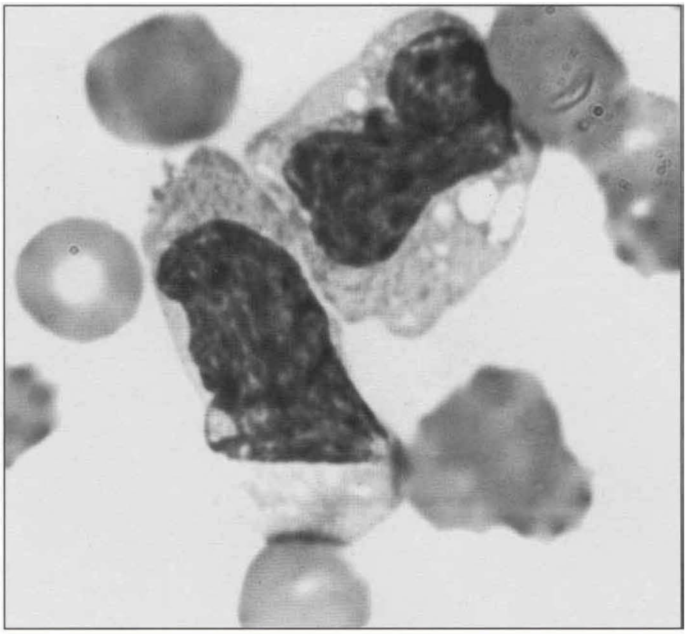

Figure 2.26 Chronic myelomonocytic leukemia, canine blood. Atypical "monocytoid" cells that on esterase staining appear to be of the neutrophil series.

At postmortem, there is anemia with normal lymph nodes and mild splenomegaly. Thrombocytopenic hemorrhage may be present. The microscopic findings are similar to those in refractory anemia with excess blast cells.

Esterase stains may be required to identify lineage if there is atypia and immaturity in both the neutrophil and monocytic systems (Table 2.2). *Chronic myelomonocytic leukemia can be differentiated from acute myelomonocytic leukemia on the basis of a longer course and lack of blast cells in the peripheral blood.*

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Atypical chronic myelogenous leukemia (aCML)

The name aCML has been applied to a specific human condition characterized by a disease that is in most respects like the majority of CML cases but lacks the BCR-ABL fusion gene. *The major differences between CML of the Ph+ type and aCML are that monocytes are more prominent in aCML and may suggest a diagnosis of CMML, and basophilia characteristic of both AML and CML is usually absent.* An

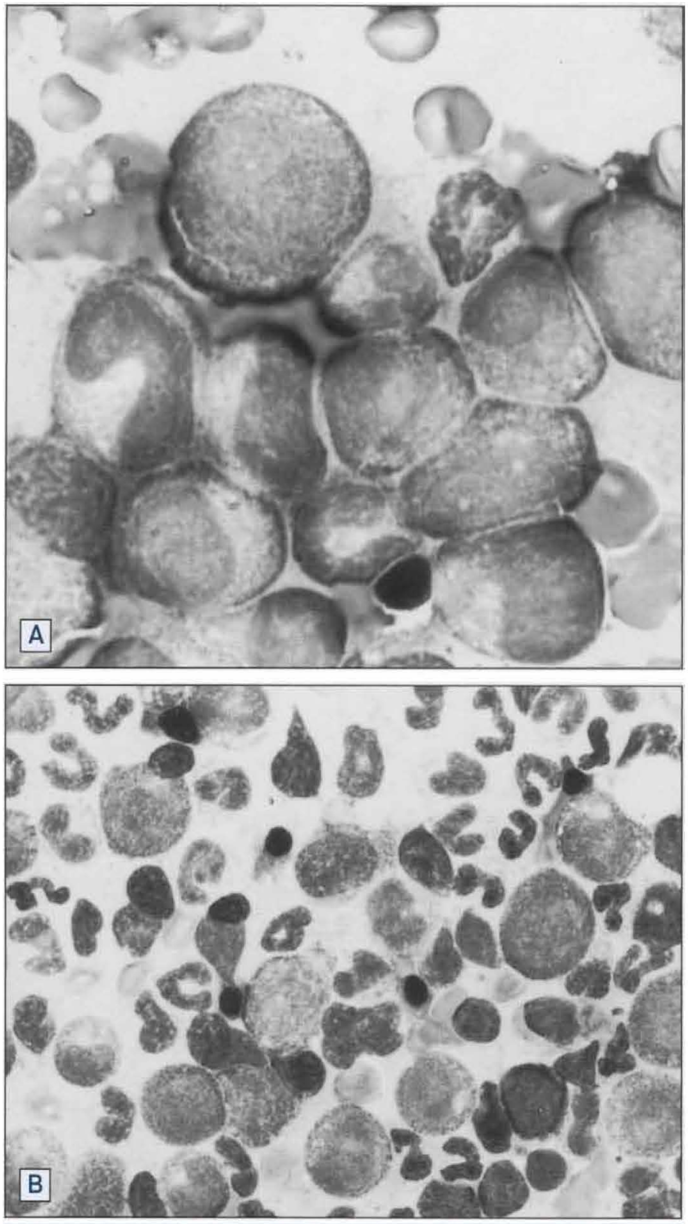

Figure 2.27 Chronic myelomonocytic leukemia in a dog, marrow aspirate. **A.** Area of early myeloid asynchrony with giant metamyelocytes. **B.** Paradoxical myeloid hyperplasia with increased mature granulocyte reserves and moderate erythroid phthisis.

unusual characteristic of aCML which may occur in animals is a type of dysplasia in the neutrophils series called "abnormal chromatin clumping." The change consists of very hyperchromatic chromatin arranged in prominent chromocenters each of about the same size of 0.5–7 μm and with added prominence because of surrounding parachromatin clearing. There are also pseudo Pelger-Huet nuclear changes and dysplastic changes in both the erythroid and megakaryocytic lineages. The peripheral blood red cells have frequent macro-ovalocytosis and there is usually thrombocytopenia.

Juvenile myelomonocytic leukemia (JMML)

Juvenile myelomonocytic leukemia is a disease of children generally less than 3 years of age. JMML has a type of genetic specificity in that it is Ph- and BCR-ABL negative and primarily involves the neutrophil and monocytic cell lines. The cells of this tumor also have a unique ability to grow in culture without added GM-CSF. This capability is lost by removing the monocytes and other adherent cells from the culture inoculum or by adding antibody to GM-CSF. The level of GM-CSF in spontaneously growing cultures is not above that of control levels, which suggests that there is hypersensitivity to GM-CSF as the basis for this syndrome. The patients are presented with fever and often pneumonia. Splenomegaly is always present, and half of cases have lymphadenopathy and a macropapular rash. There is leukocytosis of characteristically 10×10^9/L, of which at least 10% are monocytes accompanied by anemia and thrombocytopenia. The blast cell count including promonocytes is usually less than 5%. An unusual abnormality that constitutes a useful laboratory test is the presence of at least 10% of fetal hemoglobin. Treatment by chemotherapy is usually ineffective.

Myelodysplastic syndromes (MDS)

The myelodysplastic syndromes (MDS, dysmyelopoietic syndromes) are a *diverse group of clonal disorders of hematopoiesis characterized by refractory cytopenias accompanied by qualitative and quantitative dysplastic changes in marrow cells coincident with ineffective blood cell production.* About 20% (17–40%) of human cases terminate in acute myeloid leukemia; in animals most cases terminate or are terminated with marrow failure. These diseases were first felt to be maladies of old age, however it now appears that myeloproliferative disease in the young is preceded by a prodromal phase of myelodysplasia that has a high probability of evolving into acute myeloid leukemia. Thus, *myelodysplasia is a syndrome of clonal instability* that tends to evolve into acute myeloid leukemia with a high probability of progression in the young and a lower likelihood of progression in the aged. In humans, 80% of cases are characterized by cytogenetic abnormalities with determination of this diagnostic procedure considered an essential part of the evaluation of patients with MDS. In animals, cytogenetic changes are as yet undefined. Mechanistically, the stem cells in MDS are less responsive to GM-CSF than normal, whereas in acute myeloid leukemia they are hyperresponsive.

The term "myelodysplasia" replaces the previous label of "preleukemia," and in veterinary practice is a preferable diagnosis for an animal that might otherwise survive the disease but not the diagnosis. The FAB classification for myelodysplasia (1982) recognized *5 variants of ineffective myelopoiesis*, including refractory anemia with (RARS) and without (RA) ringed sideroblasts, refractory anemia with excess blasts (RAEB), and in transition to acute leukemia (RAEBt), and chronic myelomonocytic leukemia (CMML). The major utility of this classification was that patients with RA and RARS tended to have long survival while those with the other variants did not. The revised WHO Classification (2001) *proposes 6 variants of MDS* including RA, RARS, RAEB and 3 new categories: refractory cytopenia with multilineage dysplasia (RCMD), the 5q− syndrome, and MDS unclassifiable. The category CMML is placed in a new subdivision of myeloid dyscrasia that lies between frank acute myeloid leukemia (AML) and the myelodysplastic syndromes as they have been previously recognized. This new category is termed "myelodysplastic/myeloproliferative diseases" and includes 3 disease entities including CMML, plus atypical chronic myelogenous leukemia (aCML), and juvenile myelomonocytic leukemia

(JMML). With much additional experience since the introduction of the FAB classification, the WHO system categorized the MDS entities on a prognostic basis based on cytogenetic changes. Those MDSs involving only minor injury to chromosomes (5q− only or 20q− only) were placed in the "good" category, and "poor" with at least three complex chromosome abnormalities or with errors in chromosome 7, and in an "intermediate" risk category with other cytogenetic abnormalities. The application of this information led to the proposal for the myelodysplastic/myeloproliferative disease grouping to demonstrate the need for aggressive management of the 3 entities CMML, aCML, and JMML.

In general, the primary MDSs, that is those occurring without prior chemotherapy or other toxic exposure, are largely diseases of maturity in both humans (>50 years) and animals. The MDSs are much less common in children but those that do occur are aggressive lesions, primarily monosomy 7 and juvenile myelomonocytic leukemia JMML. This level of categorization in animals must await sequenced genomes and the development of diagnostic probes, since the greater number of chromosomes in animals makes cytogenetic analysis in these species much more difficult than in humans. In animals as in humans, most cases of MDS present with some aspect of marrow failure, usually recurrent infections due to deficits in neutrophils or nonresponsive anemia often associated with thrombocytopenic bleeding. Hepatosplenomegaly or lymphadenopathy are not usually present, but autoimmune disease involving marrow, skin or other areas may cloud recognition of underlying MDS. Fortunately, *there are in animals as in humans subtle but definable morphologic and quantitative changes in marrow and blood that enable the diagnosis of MDS to be made on currently available skill and technology*. The quantitative changes include early asynchrony or the inappropriate presence of increased numbers of myeloblasts that may be of type 1 or 2 (see AML, p. 125). Morphologic changes may involve one or all of the 3 leukocytic, erythrocytic or megakaryocytic lineages. Dysplastic changes in the neutrophil line include variations in cell size, decreased or abnormal granulation, and hypo- or hypersegmentation. In addition in most species of animals, giant metamyelocytes may be present in marrow and occasionally with the "donut" or rodent cell configuration. Erythroid dysplasia is manifested by asynchrony of nuclear and cytoplasmic development, megaloblastoid nuclear chromatin with finely distributed, less densely stained chromatin, and abnormalities of nuclear shape including lobulation, multinuclearity, pseudopodia and fragmentation. The resulting red cells will have increased variation in hemoglobin saturation and shape changes, including ovalocytes, dacryocytes (tear-drop shapes), and anisopoikilocytosis. Dysplasia in the megakaryocytic line includes marked variation in cell size with both very small and very large cells, both with nonlobulated nuclei: the larger may have very finely dispersed chromatin that lacks normal staining density like the megaloblastoid changes in the erythroid line. Dysplastic changes in megakaryocytic cytoplasm include asynchrony of nuclear and cytoplasmic maturation seen as pyknotic nuclei in small cells with little cytoplasm or in large cells with retention of largely agranular or hypogranular cytoplasm. In addition, there may be peripheralization or "rimming" of cytoplasmic basophilia and eccentric placement of cytoplasm, and even apparent phagocytosis of red cells.

The bone marrow in these syndromes is most often hypercellular (Fig. 2.28A), but hypocellular variants occur. Architectural abnormalities in marrow include topographic distortion with loss of the normal orientation of myelopoiesis near endosteum, and more centripetal location of erythroid islands, to a random distribution of lineages with loss of clustering of rubricytes. There is an irregular increase in stroma, which may be diffuse terminally accompanied by ectasia of sinuses with intravascular hematopoiesis. Plasmacytic cuffing of small vessels is increased, and there is increased remodeling of bone.

The usual clinical presentation is of a *thin lethargic animal with pale mucous membranes and a history of chronic recurrent infections often involving the respiratory tract*. While refractory anemias are relatively common in the cat and dog, *the types of myelodysplasia most commonly recognized in animals (as distinguished from most commonly occurring) are a form of RAEB in the cat and CMML in the dog.*

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Refractory anemia with excess blast cells (RAEB)

RAEB is a disease of humans and dogs and cats and likely other species where hematologic dyscrasias are given less attention. The disease in cats was felt to be a syndrome of FeLV infection, but since infection with that virus is now much less common but refractory anemia continues to occur, it must be assumed that feline RAEB results from a variety of causes. In all species, there is *nonresponsive pancytopenia and hyperplastic marrow with excess small (type 1) myeloblasts. The diagnostic criterion for RAEB is a level of marrow blasts increased to at least 5% and less than the 20% required for the diagnosis of AML.* The WHO classification divides the disease into two groups based on risk for progression to AML with those cases with 5–11% blasts in marrow termed *RAEB-1* and with 12–19% blasts *RAEB-2*. Further, in the human disease, focal areas of clustered blasts and promyelocytes may be present in marrow (*abnormal localization of immature precursors, ALIP*) and, if 3 or more are found, the disease has a higher risk of conversion to AML. The disease occurs in young mature animals without apparent sex predisposition. The course is 1–6 months, occasionally followed by recovery. Most animals are killed because of recurrent infections due to marrow failure.

The anemia is moderate to marked, mildly hypochromic and macrocytic with anisocytosis but no polychromasia. Both ovalocytes and nucleated red cells may be present in blood. The leukocyte count is generally below 5×10^9/L and there is neutropenia with moderate left shift. The neutrophils have reduced secondary granulation, diffuse basophilia, and toxic vacuolation. There is usually moderate thrombocytopenia with a lot of variation in size, mild uniform basophilia, and poor granulation.

Aspirated marrow is characteristically 60–80% cellular as determined by the proportion of cellular to fatty areas. There is early asynchronous maturation with as many as 5–10% blast cells, loss of granulocyte reserves, and an apparent "maturation arrest" at the myelocyte–metamyelocyte level. Most of the blasts are myeloid, but they are poorly granular and may be mistaken for lymphocytes, except that promyelocytes are increased and may be expected to be progeny of the atypical blasts (pattern recognition on the basis of "knowing them by the company they keep"). The erythroid nuclei have marked contrast between large chromocenters and clear areas; dyserythropoiesis with multinucleation is present to a variable degree. Since the early myeloid hyperplasia is not followed by mature reserves, the myeloid:erythroid ratio remains near 1.0. The megakaryocytes are normal or decreased but not increased. They have large "monocytoid" nuclei without normal lobulation. Cytoplasmic basophilia remains high in maturing cells and granulation is decreased. Marrow iron is coarse and increased.

At postmortem, there is pallor and emaciation with mild splenomegaly. Lymph nodes are normal or mildly enlarged. Secondary lesions of pleuritis, pneumonia and lymphadenitis are usually prominent. The bone marrow is uniformly pale, pink and opaque without serous atrophy, and is moderately cohesive.

Histologically, the bone marrow has high cellularity and focal absence of fat (Fig. 2.28A, B). Cell density may be variable due to reticulin sclerosis. Cytologically, 60% of the cells present appear primitive with round vesicular nuclei, prominent single nucleoli, and a thin rim of highly basophilic cytoplasm. Erythropoiesis is patchy with reduced cellular packing or density, and few mature rubricytes are present. Dyserythropoiesis is more prominent than on cytological preparations. Megakaryocytes appear immature due to irregular lobulation and low cytoplasmic volume. Marrow germinal centers may be present and numerous. The liver usually has some degree of periacinar ischemic degeneration, but hematopoiesis is minimal or

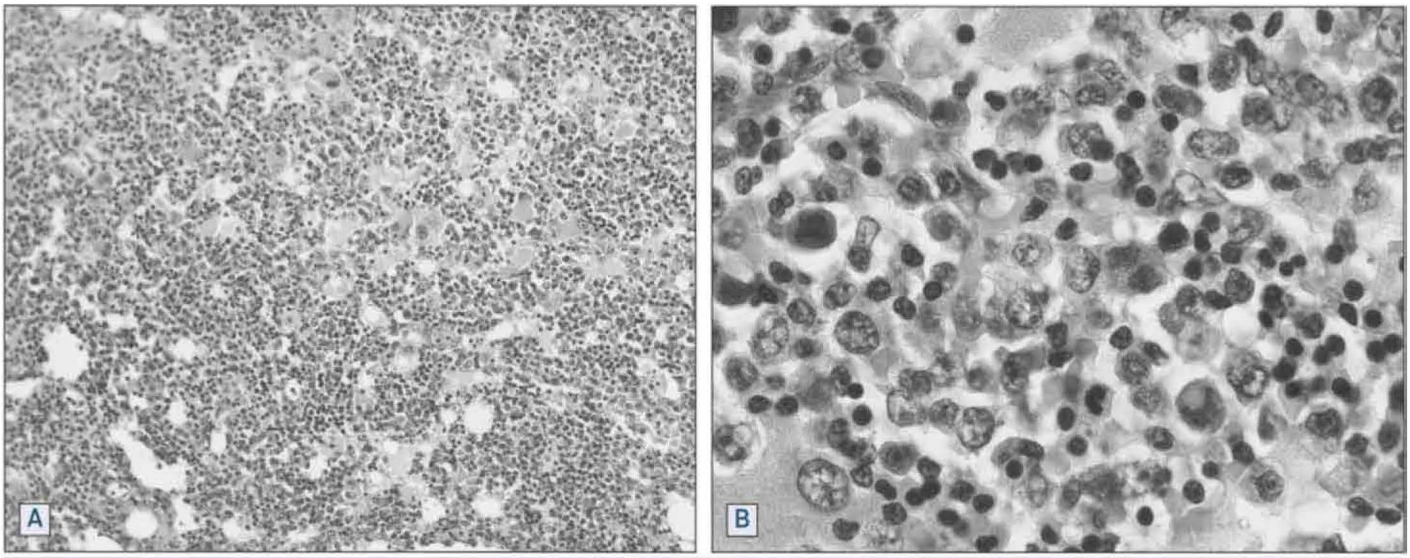

Figure 2.28 Myelodysplastic syndrome refractory anemia with excess blasts, feline bone marrow. **A.** There is almost complete loss of fat cells but with reduced density of remaining cells and dilation of marrow sinusoids. **B.** Detail of (**A**). There are increased numbers of large vesicular nuclei (early myeloid asynchrony) with loss of marrow granulocyte reserves. Most of the erythroid precursors are late stage with dyserythropoiesis characterized by abnormally lobulated erythroid nuclei.

absent. There is splenic follicular hyperplasia and increased sinus histiocytes. There are some paratrabecular megakaryocytes, but hematopoiesis is not prominent. The lymph nodes have follicular hyperplasia with medullary follicles and medullary cord hyperplasia. Neutrophils are not prominent either in sites of inflammation or their draining nodes because of the neutropenia.

Refractory anemia with ringed sideroblasts *occurs in animals on experimental diets deficient in vitamin B6.* Wintrobe and co-workers studied the function of this vitamin in swine, where its deficiency was found to be associated with a hypochromic microcytic anemia with iron overload. Biochemically, B6 is required for the formation of levulinic acid, in the early synthesis of the porphyrin rings of hemoglobin, and is the rate-limiting step in hemoglobin production when deficient. As a result of inability to form the protoporphyrin molecule, iron is not utilized but its absorption from the diet is increased. Iron accumulates in marrow macrophages but also in the mitochondria of developing rubricytes in stainable quantities seen with Prussian blue stain as "ringed sideroblasts." The MDS of the same name became recognized because of a syndrome of human refractory anemia with ringed sideroblasts that did not respond to supplemental vitamin B6. The MDS of this type is not currently recognized in animals. In humans with RARS, anemia is always present and of moderate degree with an unusual dimorphic pattern in red cells with some well saturated and a third or more very hypochromic and with the range and degree of poikiocytosis seen in iron-deficiency anemia. Occasionally stainable iron aggregates (Pappenheimer bodies) are present in peripheral red cells, visible as basophilic inclusions in routine blood stains and most likely to be seen if there is splenomegaly or asplenia. The platelets and neutrophils are normal in number and morphology, and there is marrow erythroid hyperplasia with >15% of rubricytes at all stages with stainable iron granules. The megakaryocytes and granulocytes appear normal morphologically and numerically. Some 50–10% of human RARS evolve to AML, and, if the anemic form is accompanied by trilineage dysplasia, the risk of malignant transformation is greater.

Myeloid metaplasia with myelofibrosis (MMM)/ chronic idiopathic myelofibrosis (CIMF)

Idiopathic myelofibrosis also known as *agnogenic myeloid metaplasia* is recognized as a clonal disorder of hematopoietic precursors and is *considered a type of chronic myeloproliferative disease (CMPD)* rather than a syndrome of myelodysplasia. See Chronic leukemias/myeloproliferative diseases, above.

Myelodysplastic syndrome, unclassified

The unclassified category of MDS includes those cases that are characterized by absence of blasts in the peripheral blood, minimal or no detectable increase in marrow blasts, with dysplasia in one or more marrow cell lineages, and refractory cytopenia of one or more cell lines in the peripheral blood. An appropriate working diagnosis would be MDS with refractory anemia, neutropenia, thrombocytopenia, or pancytopenia, as the case requires, to provide a mechanistic understanding of the principal abnormalities involved. The major criterion for use of this diagnosis is that *there must be evident and repeatable morphologic verification of dysplastic changes in one or more marrow cell lines.* A recurring syndrome where this diagnosis is appropriate is the cat or dog or other species that is presented with a moderate or severe nonresponsive anemia without thrombocytopenia or neutropenia but with marked marrow erythroid hyperplasia accompained by dyserythropoiesis but without an increase in marrow blasts. There may be dysplastic changes in the megakaryocytic, but not usually in the neutrophilic, line, and *the major differential diagnosis is the distinction from AML of the M6 type or acute erythroid leukemia.*

Since in animal medicine the cytogenetic differentiation between AML and MDS is not currently available, the rational management of these cases is to apply conservative therapy until it is apparent that it is not effective, and then more aggressive measures that would be appropriate for a diagnosis of frank leukemia.

HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS

Macrophages and dendritic cells are nonlymphoid mononuclear cells that are intimate participants in both the immune and nonimmune inflammatory reactions. They are involved to a minor or greater extent in the neoplastic processes of the various cells of both the myeloid and lymphoid neoplasms. In some diseases, these cells are themselves the principal cells involved in the hyperplastic or neoplastic process.

The **monocyte/macrophage/histiocyte cells** are marrow-derived from primitive myeloid precursor cells. The pattern of maturation is *from marrow precursor to monoblast, promonocyte, monocyte, and with further stimulus to histiocyte/macrophage,* with those latter two terms being used as synonyms. Macrophages express MHC class II DR receptors in most mammals and in chickens as well as a number of CDs (cluster designations) with the complement of CD: 45,11, 13,16 d 62 and cytoplasmic lysozyme varying in composition in various domestic animals. Cytochemically, macrophages stain positively for nonspecific esterase, acid phosphatase and lysozyme. They have receptors for a wide range of proteins including Ig's, complement, coagulation factors, some hormones and cytokines, including TNF, IL-1, INF-γ, and GM-CSF. Macrophages themselves are able with stimulation to produce a range of cytokines and growth factors and participate in nonimmune resistance to microorganisms. Macrophages become increasingly resistant to intracellular organisms by a process of activation under the influence of INF-γ and are able to recognize and clear apoptotic cells.

Dendritic cells have markedly different morphology (dendritic) to other mononuclear cells characterized by *long cytoplasmic tendrils* that in interdigitating cells are connected to others by desmosomes. The designation of dendritic, while indicating morphology, does not define histogenesis, and *includes Langerhans cells, indeterminate and veiled cells, interdigitating dendritic, follicular dendritic, and dermal dendrocytes.* Dendritic cells appear to arise from two lineages, myeloid and lymphoid. The **myeloid stream** evolves from CD34+ marrow precursor cells that differentiate under stimulation from GM-CSF and TNF-α to immature nonactivated Langerhans cells. In humans, the Langerhans cells are characterized by the presence of an ultrastructural organelle called the Birbeck granule which is a slender structure with a small bulb at one end that evoked the name "banjo" cell. These granules are present in the Langerhans cells of cattle but not in dogs and largely unknown in others. Langerhans cells express CD45 and CD1 and have intracytoplsmic MHCII products required for antigen-processing and antigen-presentation functions in interaction with B- and T-cell lymphocytes. In response to inflammatory cytokines or microbial antigens, Langerhans cells mature to dendritic cells with abundant surface MHCII complex. In this state, they migrate through the tissues as veiled cells to the paracortical areas of draining lymph nodes. In canine skin the Langerhans cells express CD1 and reside both within the epidermis and in the dermis located near postcapillary venules. In human skin, an indeterminate cell is identified that is morphologically and antigenically like the Langerhans cell but lacks the Birbeck granule; they are of indeterminate status and are thought to be either precursors or direct descendents of Langerhans cells. With activation by cytokines within the skin, the Langerhans cells in their "trained" state arrive in the node cortex having lost CD1 expression and complement receptors, but have surface MHC activity and as antigen-presenting cells (APC) they are able to produce an array of cytokines that interact with local paracortical T cells in the initiation of cellular immunity. An alternative pathway is proposed for the myeloid dendritic cells, which suggests that they differentiate under appropriate cytokine stimulation (TNF-α, IL-4) and become mature dendritic cells that circulate in the blood and home to sites of inflammation. A dermal dendrocyte/dermal dendrophage that is normally in the perivascular area of the papillary dermis is distinct from both the indeterminate and Langerhans cells and may have a role in humoral immunity by migration to become the follicular dendritic cell.

The second stream of dendritic cells of **lymphoid origin** are derived from committed marrow lymphoid precursors and migrate to the medulla of the thymus. In that area and under stimulation from IL-3, they differentiate to dendritic cells that then migrate to the paracortex of lymph nodes by traversing the postcapillary venules as plasmacytoid appearing T cells. Once in the node paracortex, the myeloid and lymphoid dendritic cells are not distinguishable but may have different functions with the lymphoid cohort responsible for immune tolerance. The dendritic cells of the germinal or follicular centers are believed to be of marrow origin and arrive in the nodes in afferent lymph where they are identified in the subcapsular sinuses by the KIM-4 marker. They subsequently migrate to the outer cortex and form a reticular network in the germinal centers being joined by thin branching cytoplasmic processes that link between cells by desmosomes. *The follicular dendritic cells are antigen-presenting cells* and retain immune complexes on their cell surfaces. They become multinucleated and are visible as the epitheliod appearing cells in germinal centers that have recently undergone severe lympholysis.

Macrophage/histiocytic neoplasms

Hemophagocytic histiocytic sarcoma (malignant histiocytosis)

Histiocytic sarcoma (HS) is the nonleukemic tissue phase tumor of the mononuclear phagocyte system. The older names are derived from the pattern of involvement where the disease involves lymph nodes. Thus, *malignant histiocytosis is the neoplastic counterpart of benign sinus histiocytosis when the sinus type of node involvement is apparent.* In contrast, the less-favored term "true histiocytic lymphoma" has been suggested as the designation for those neoplasms of malignant macrophages where effacement of node architecture is present, as in the aggressive large-cell lymphomas. *HS is a rapidly progressive disease characterized by proliferation of markedly atypical histiocytes which are usually avidly erythrophagocytic. The disease is rare and usually occurs in mature cats and dogs.* Clinically there is fever, depression, weight loss, irregular lymphadenopathy and most frequently hepatosplenomegaly.

Hematologically there is rapidly progressive anemia that is initially responsive and within a few weeks progresses to nonresponsive pancytopenia with marrow failure. The disease may be first noticed and diagnosed by an enlarged node that on aspiration yields very large cells with nuclei as large as hepatocytes and nucleoli as large as red cells. The cytoplasm is finely granular and stains positively with α-naphthyl acetate esterase and negatively for myeloperoxidase. Erythrophagocytosis is not present in all tumor cells, and hemosiderin in the absence of red cells indicates previous activity. In some cases, as many as 100 red cells may be identified within a single malignant histiocyte, which indicates that the anemia is initially due to intracellular hemolysis and later to both destruction and impaired production. A benign counterpart described in humans is termed "infection-associated hemophagocytic syndrome" and is thought to be induced by viral or other infectious agents, but has not been recognized in animals. Grossly, there is pallor, and hemorrhage may be present if thrombocytopenia has been severe. There is irregular firm enlargement of lymph nodes, which are dark on cut surface, and enlargement of spleen and liver, both of which may be focally infiltrated and infarcted. Bone marrow is irregularly reddened, and the disease usually advances so rapidly that fatty areas remain. Histologically, the tumor cells have distinct cytoplasmic borders, large round or oval often paired nuclei, prominent nucleoli, and abundant pale cytoplasm, often with ingested red cells and hemosiderin and less often platelets and leukocytes. The tumor cells may be discretely packaged and even mildly encapsulated in spleen and liver (Fig. 2.29A, B), or in more diffuse and infiltrative distribution as is more common in nodes and marrow. The diagnosis may need to be confirmed by histochemistry, but *the severe atypia and phagocytic tendencies suggest the correct interpretation.*

Most of the seminal papers on this subject were published before the advent of immunohistochemistry and detection of germ line genes for the B- and T-cell receptors. Where paraffin blocks have been available for review, many of the older papers describing HS are based on a mixture of diseases including B- and T-cell lymphoma, and benign infection-associated hemophagocytic syndrome. The human syndrome of "*histiocytic medullary reticulosis*" described a disease of malignant macrophages that was characterized by anemia, fever and weight loss with hepatosplenomegaly and widespread erythrophagocytosis, and seems to most closely fit the disease of HS as it is seen in dogs and cats.

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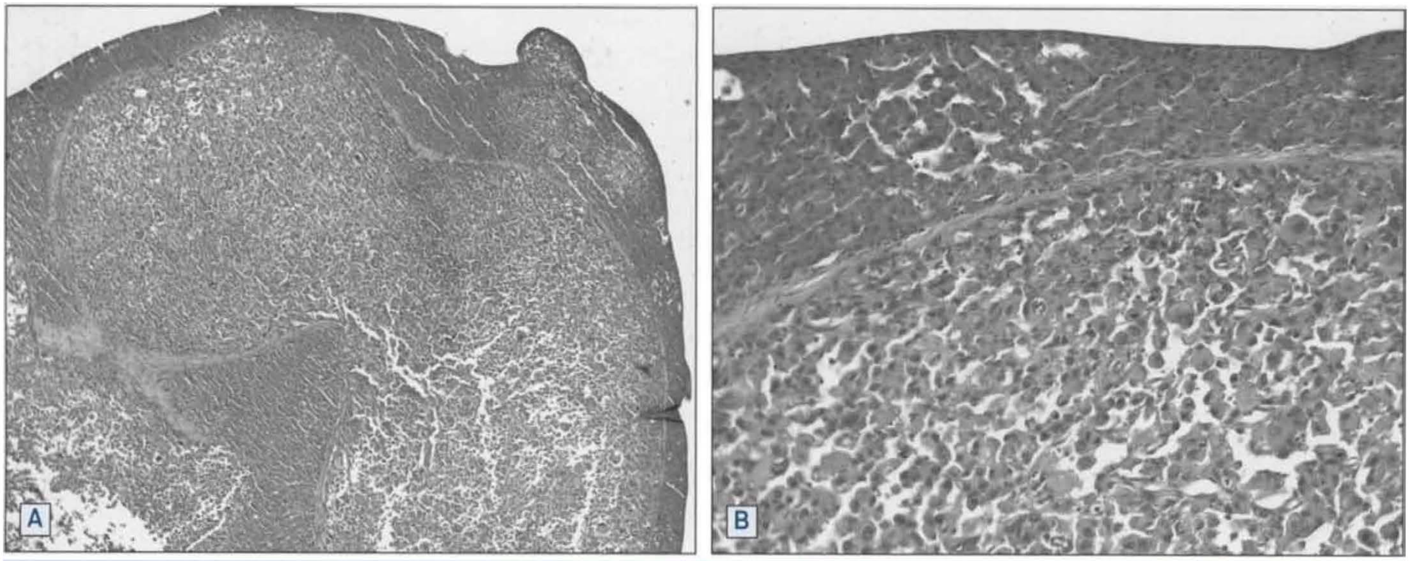

Figure 2.29 **Hemophagocytic histiocytic sarcoma**, liver, cat. **A.** Multifocal areas of tumor are displacing hepatic parenchyma with pseudoencapsulation of larger areas. **B.** The neoplastic cells are much larger than hepatocytes with marked anisokaryosis and abundant cytoplasm.

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Dendritic cell neoplasms

Langerhans cell histiocytosis (LCH)

The histiocytic diseases of animals arising from Langerhans cells are very largely diseases of *young dogs*. The dendritic cell neoplasms of both humans and animals are relatively recently defined lesions, and some migration of the terminology is to be expected. It is instructive that the current WHO classification system of hematopoietic and lymphoid neoplasms is the first major classification system to include a listing of dendritic cell neoplasms. Some interweaving of human and animal disease is required to apply the current WHO system to the dendritic cell diseases as seen in animals. There is however, a remarkable similarity of dendritic cell neoplasms in humans and animals.

Cutaneous histiocytoma is dealt with in the chapter on skin (See Vol. 1, Skin and appendages) but for clarity of organization all of the diseases arising from Langerhans cells are dealt with here. Histiocytoma is a frequently occurring, benign and usually solitary cutaneous neoplasm of dogs. *Histiocytomas are tumors of nonactivated epidermal dendritic or Langerhans cells* and as such express CD1, the defining antigen of dendritic antigen-presenting cells. They also express CD11c and MHC II, but not CD90 (thy1) or CD4, the latter being the marker for activated dendritic cells. Histiocytomas stain positively for vimentin but not lysozyme or myeloperoxidase. The histology of the lesions is described in Vol. 1, Skin and appendages. The human counterpart of histiocytoma (LCH) is primarily a pediatric disease with a median age of occurrence of less than 5 years. Unlike the disease in dogs with no gender predilection, in humans there is about a 2 to 1 male to female predominance. While the disease known as *Langerhans cell histiocytosis* or "solitary eosinophilic granuloma" in children may present with a single lesion like the dog, it is much more likely to be a deep-seated neoplasm involving bone, lymph node or lung, and more comparable to the disease in dogs known as systemic histiocytosis.

A much less common presentation of Langerhans cell disease in the dog is **cutaneous reactive histiocytosis**, wherein *lesions are multifocal and the result of proliferation of activated interstitial or dermal dendritic cells*. As such, they express CD1, CD11c, and MHC class II as in cutaneous histiocytoma but also CD90 (Thy1) and CD4. In cutaneous reactive histiocytosis, there is involvement of the deep dermis and subcutis with the superficial dermis inconsistently affected; epidermotropism is not a feature of the disease. The lesions of cutaneous reactive histiocytosis develop around the deep dermal vessels and are both angiocentric and occasionally angioinvasive with a mixture of lymphocytes and other inflammatory cells as well as the dendritic cells. Invasion of draining lymph nodes may occur. *This disease is not malignant but an immunoregulatory disorder*, and as such the lesions may respond to corticosteroids or other immunosuppressive therapy.

Systemic reactive histiocytosis

Systemic reactive histiocytosis is seen as a familial disease of Bernese Mountain Dogs and sporadically in other breeds. The disease is characterized by *multifocal involvement of skin by proliferating activated dermal or*

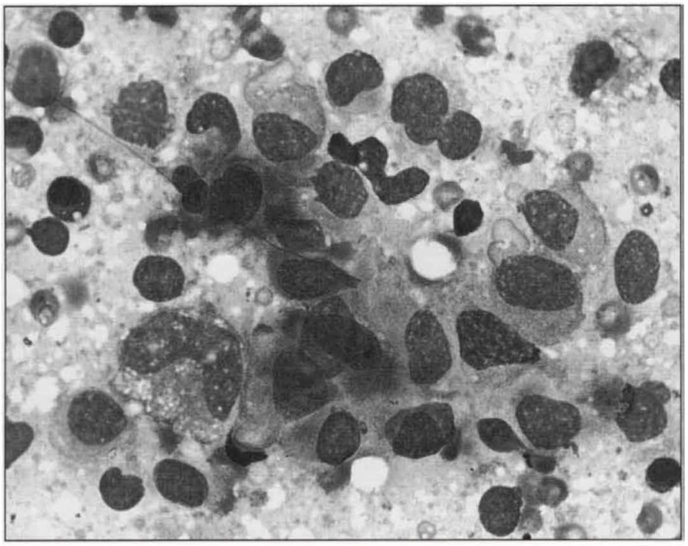

Figure 2.30 Systemic reactive histiocytosis, aspirate of a subcutaneous mass near the stifle joint of a Bernese Mountain Dog. The tumor nuclei are large and relatively bland without nucleoli and accompanied by abundant uniformly stained pale cytoplasm. (Courtesy of RM Jacobs.)

interstitial dendritic cells, and as such the invading cells express the identical constellation of antigens as seen in cutaneous histiocytosis. While the skin is consistently involved and in the same manner with angiocentric investment of deep dermal vessels with a polymorphous cellular infiltrate, there is also involvement of one or more of ocular and nasal mucosa, lymph nodes, lung, spleen, liver and bone marrow. Because of the involvement of vessels, *there may be focal infarction and the resulting mixed cellular reaction may suggest the presence of an infectious agent causing a granulomatous reaction*. The invading dendritic cells have bland chromatin with fine even distribution and normal staining density (Fig. 2.30). Nucleoli are small or unapparent, but the nuclear membranes are irregularly folded or creased giving characteristic linear nuclear clefts and grooving. Mitoses are relatively rare. The cytoplasm is abundant and of uniformly ground-glass density with indistinct cell boundaries. Like cutaneous histiocytosis, *the systemic disease is not malignant but an immunoregulatory disorder that may respond to aggressive immunosuppression.*

The multifocal but unisystem Langerhans cell histiocytosis (LCH) of humans is the disease formerly known as *Hand-Schuller-Christian syndrome* with the primary system of involvement usually bone. Finally, the multifocal and multisystem LCH disease of humans was known as *Letterer-Siwe syndrome* and is primarily a disease of infants and young children with strong male predominance. In general, the prognosis in these human conditions is most dependent on the extent of system involvement since the cells are cytologically similar in all variants of the dendritic LCH neoplasms. Patients who present with only one site involved seldom develop disseminated disease. Oddly, the absence of bone lesions is a poor prognostic sign and the presence of multiple osseous lesions is associated with a favorable outcome.

Histiocytic sarcoma

Histiocytic sarcoma has a breed disposition similar to that of systemic reactive histiocytosis and may occur as a solitary lesion or multiple lesions with rapid spread. *Histiocytic sarcomas result from the proliferation of dendritic antigen-presenting cells.* Tumors of this derivation have the immunological expression of CD1, CD11c, and MHC class II. They are variable in expression of CD90 and are negative for CD4 and are thus like cutaneous histiocytoma, but are negative for E-cadherin and have a much more aggressive cytomorphology. The primary sites for histiocytic sarcoma are on the limbs and typically overlying a joint such that curative excision is not possible. In disseminated disease, the more common sites include skin, lung, lymph node, bone marrow, spleen, and occasionally brain. Histologically, histiocytic sarcoma is characterized by marked cytologic variability accompanied by inflammatory cells and vascular invasion resulting in multifocal ischemic necrosis. Treatments are ineffective.

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Follicular dendritic cell sarcoma

This neoplasm is uncommon in humans, and may occur as histiocytic sarcoma of nodes in dogs. The median age of human patients is 40 years with equal sex distribution. Most cases present with an enlarged cervical or axillary lymph node, but extranodal occurrence may involve the oral cavity, tonsil, gastrointestinal system and breast. *A differential diagnosis that may be important in animals is the similarity of follicular dendritic cell sarcoma to inflammatory pseudotumor of the spleen and liver of dogs.* The biology of the follicular dendritic cell sarcoma is more like that of a stromal sarcoma than of a lymphoid tumor, with local recurrence more frequent than metastatic disease. These lesions can be large – usually 5 cm diameter in the periphery and as much as 20 cm in the abdomen. Histologically, the tumor has a spindle cell morphology often in a whorled or storiform pattern. Multinucleation is present and the nuclei are elongated with fine granular chromatin, and nucleoli are small and distinct. There may 5–10 mitoses per high-power field. The tumor cells closely resemble normal follicular dendritic cells in their complement of markers. They are consistently negative for CD1, desmin and keratin, and endothelial markers. They stain weakly with CD45 and consistently with vimentin, complement receptors, and the follicular dendritic marker R4/23.

LYMPHOID NEOPLASMS

Classification systems for lymphoid neoplasms

There has been a tremendous increase in understanding of benign and malignant lymphoid proliferation in the latter half of the twentieth century. Up until the time of World War II, some restricted the use of the term *lymphosarcoma* to well-differentiated lymphocytic proliferations, while others used the term, without regard to cytological differences, for all primary malignancies of lymph nodes. At the same time, others used the term *reticulum cell sarcoma* for large-cell lymphomas regardless of varying cytologic type. In 1942, **Gall and Mallory** reviewed 618 cases of human lymphomas and suggested a classification system including four entities: *Hodgkin disease, lymphosarcoma, reticulum-cell sarcoma, and giant follicular lymphoma.*

While this system received widespread use, it was apparent that the cellular heterogeneity of lymphomas and, the widely apparent inability of pathologists to fit all observed lesions into these four categories, provided the basis for further systems with more cytologic specificity.

Following a study of lymphomas in American servicemen recruited during World War II, Henry **Rappaport** pointed out the biological uniqueness of follicular lymphomas. Subsequently, in 1956, Rappaport proposed a classification for lymphomas that divided all lesions into "nodular" or "diffuse" categories at the architectural level, with the following categories based on cytologic type: *lymphocytic, well differentiated; lymphocytic, poorly differentiated; mixed lymphocytic–histiocytic; histiocytic; undifferentiated.*

Classification systems at this time were based on the concept that small, normal-appearing lymphocytes were end-stage cells without the ability to divide. Following this understanding, lymphoma of *lymphocytic, well-differentiated* type corresponded to our current understanding of the cells of chronic lymphocytic leukemia and small lymphocytic lymphoma, where the cells closely resemble normal lymphocytes of the thymic cortex. The *lymphocytic, poorly differentiated* category included cells now termed "intermediate" lymphocyte type. In this intermediate type, the nuclei were slightly larger and had greater internal nuclear detail than small mature lymphocytes, but lacked nucleoli. Neoplasms with this nuclear type with slightly increased amounts of eccentric cytoplasm were subsequently termed intermediate *lymphoplasmacytoid,* and those with greater cytoplasmic volume were called *lymphoplasmacytic.*

A major contribution of Rappaport was the recognition that within the group of lesions categorized as "lymphocytic, poorly differentiated," most were relatively indolent lymphomas with very slow progression, while one subtype had a very aggressive behavior with rapid progression. This aggressive cell-type was subsequently recognized as an entity and termed *lymphoblastic lymphoma.* Thus, establishing the understanding that *small-cell size is not in itself an indication of an end-stage cell without the ability to divide.*

It is at this point that the terminology of lymphomas in veterinary medicine diverged from human pathology, with the term "*lymphoblastic*" in animal reports being applied indiscriminately to large-cell lymphomas. The result of this divergence in the application of terms has meant that the *lymphoblastic lymphomas* of animals, as defined in the human context, have been very slow to come into general recognition. This has been particularly troublesome to veterinary oncologists because *lymphoblastic lymphomas* of animals, of B- and T-cell type, tend to respond very poorly to treatment, thus reducing the credibility of our accuracy in prognosis in advising owners of what can be expected as a result of undergoing treatment for lymphoma in any particular case.

The initial Rappaport classification was based on the assumption that any of the cytologic subtypes of human lymphoma could occur in either a nodular or diffuse form. As more experience with nodular lymphomas was achieved, it became apparent that only three subtypes of nodular lymphomas occurred with any frequency, and they subsequently became known as the small-cleaved cell, mixed small-cleaved, large-cell, and predominantly large-cell types. Consequently, the Rappaport classification was modified to separate lymphomas into the *nodular types* for which the names proposed at that time were *lymphocytic poorly differentiated, mixed lymphocytic–histiocytic, and histiocytic.* At the same time, the list of *diffuse lymphomas* was *extended to include lymphocytic of intermediate differentiation, a Burkitt type of undifferentiated lymphoma, as well as histiocytic and lymphoblastic.*

At this time, histochemistry was still in relative infancy and the specificity of esterase stains to differentiate monocyte–macrophage lineage from lymphoid lineage was not in use. As a consequence, there was a lot of confusion over the terminology of large-cell lymphomas with the terms of *reticulum-cell sarcoma* and *histiocytic lymphoma* all being used. Later, recognition of histiocytic sarcoma and malignant histiocytosis as specific diseases of the monocyte–macrophage lineage led to the use of the term "true-histiocytic lymphoma" as a designation for poorly differentiated solid tumors of the monocyte–macrophage system.

The modified Rappaport system of classification was widely used and brought much more confluence in the use of terminology by hematopathologists. Even today, more than 60% of lymphomas seen by human pathologists outside of medical centers are confined to the three entities of diffuse large-cell lymphoma, follicular lymphoma, or Hodgkin lymphoma. It is therefore understandable that, for most purposes, the modified Rappaport classification was widely favored. The need for further subdivision of lymphoid classification systems was driven by the dual recognition that small lymphocytes were not end-stage cells and that increased information on immune-cell function demonstrated that there were two principal classes of lymphocytes, thymic and bone marrow derived types, which are morphologically identical, but functionally heterogeneous. The development of two further systems of lymphoid classification, both appearing in 1974, were the *Kiel system* which was predominantly used in Europe and proposed by Karl Lennert, and in the United States, the *Lukes-Collins* classification system.

The **Kiel classification** divided lymphomas into *low-grade and high-grade malignancies* and introduced the term "*centrocytic*" for follicular neoplasms of small cells and "*centroblastic*" for large-cell follicular lymphomas. In this classification, Rappaport's follicular lymphoma of the mixed lymphocytic–histiocytic type became centroblastic–centrocytic and the term "lymphoplasmacytoid" was added to identify a subdivision of lymphocytic of intermediate differentiation, being a small-cell lymphoma of indolent type with a more abundant and slightly eccentric cytoplasm. The high-grade malignancies in the Lennert classification included centroblastic, lymphoblastic and Burkitt type, and introduced the new term "immunoblastic."

In the **Lukes-Collins classification**, the lymphomas were divided into *T-cell and B-cell types.* The *T-cell types* included small lymphocytic lymphoma and mycosis fungoides plus convoluted lymphocytic and immunoblastic sarcoma of T-cell type. The *B-cell types* included small-cell lymphocytic lymphoma, plasmacytoid lymphocytic lymphoma, as well as the follicular lymphomas and an immunoblastic sarcoma of B-cell type. The Lukes-Collins classification is based on the assertion that B- and T-cell type could be distinguished morphologically with oversight stains. One of the underlying assumptions behind this assertion was that lightly indented, or *cleaved-cell* lymphomas of small- and large-cell type, were likely to be B cells and of germinal center origin, while *convoluted* cells, which are those seen in mycosis fungoides and the Sezary syndrome, are likely to be of T-cell type. Again, with further experience, it became apparent that while the cells of follicular lymphoma could be assumed to be of B-cell type, *the cytologic features described for differentiating diffuse lymphomas into B- or T-cell phenotypes were unreliable.*

By 1980, there were *6 leading classification systems proposed for the diagnosis of lymphoma* and tremendous confusion among pathologists, both nationally and internationally, and particularly between pathologists and oncologists. As a consequence, the National Cancer Institute assembled an international cadre of hematopathologists who were asked to classify 1175 cases of human non-Hodgkin lymphoma that were staged and treated in a consistent manner with each pathologist grading each case by all six classification systems. This study showed that no one system was superior to the others in predicting survival, but *all systems were capable of dividing lymphomas into various degrees of progression as determined by survival rates.* The study resulted in the publication of the **National Cancer Institute Working Formulation (WF)** for clinical usage in 1982. The WF, as it came to be known, divided lymphomas into low, intermediate and high-grade types, and did not attempt to separate B- and T-cell types. The WF was proposed, not primarily as a system of lymphoid classification, but rather as a common language between the other classification systems in use. Since immunohistochemistry was still in its infancy, it was recognized that subdivisions of the working formulation would be made with the identification of phenotypic and genotypic subtypes.

In the latter part of the twentieth century, the WF became widely used for the classification of lymphomas in the United States while the Kiel classification was the system most widely used in Europe (plus a modified system in the UK). With the tremendous increase in the number of cluster designation (CD) antibodies identifying specific subtypes of lymphomas in flow cytometry, but particularly in paraffin-embedded tissues, the Kiel classification was updated in 1992 with lymphomas divided into major categories of B- and T-cell types, with each of these subdivided into low and high grade lymphomas. Subsequently, a US/British/European consortium, known as the *International Lymphoma Study Group*, proposed a **revised European/American lymphoma (REAL) classification** in 1994. The REAL system divides lymphomas into B- and T-cell types with the entities arranged from the more indolent to the more rapidly progressive lesions without specific subdivision into low- and high-grade types of tumors.

During this same era, a **French/American/British (FAB) working group** defined acute myeloid leukemias based on lineage, types M1–M7, and acute lymphoblastic leukemias based on morphology, L1–L3, with L1 and L2 being largely of T-cell type and L3 largely of B-cell type.

Finally, in 2001, a **working group of the World Health Organization** (WHO) completed a summary classification of tumors of the hematopoietic lymphoid tissues that includes all entities, including acute and chronic myeloproliferative diseases and separates lymphomas into B-cell, T-cell and NK-cell lymphomas as well as lymphoid leukemias. The WHO system includes updated classifications for myelodysplastic syndromes, Hodgkin lymphoma, a list of histiocytic and dendritic cell neoplasms, and a subdivision of systemic mastocytoses.

A further issue in the categorization of lymphoid tumors has been the *distinction between lymphomas and leukemias of the same cell type*. Some have long held that chronic lymphocytic leukemia and small-cell lymphocytic lymphoma were merely different manifestations of the same disease. The same can be said for acute lymphoblastic leukemia and lymphoblastic lymphoma. The updated Kiel classification included lymphoid leukemias of small cell B- and T-types, but does not include the continuity of these lesions with lymphomas of the same cell types. The Kiel system includes lymphoblastic lymphomas of B- and T-cell types, but does not include the corresponding acute lymphoblastic leukemias.

The REAL and updated WHO classifications recognize both leukemias and lymphomas of the B- and T-cell lymphoblastic (precursor cell) types, but only the REAL classification recognizes the continuity of both B- and T-cell chronic lymphocytic leukemias with their corresponding small cell lymphomas. In this context, *the REAL classification for lymphoid neoplasms is most applicable to the classification of lymphoid tumors of animals* since, unlike chronic lymphocytic leukemia of humans, the corresponding disease of dogs has been shown to be very predominantly of T-cell derived neoplasms.

In **summary**, *both the revised European/American (REAL) and the very similar WHO systems are appropriate for the classification of animal hematopoietic neoplasms.* The defining strategy of these new systems is that each entity is a fully characterized disease based on all information including cell type, tumor architecture, topography, age, gender, phenotype and in some cases genotype. Both systems recognize and separate lesions that may have similar morphology, but different phenotypes and rates of biological progression. Further, the REAL system can be applied very largely on the basis of routine histochemistry to define B- and T-cell lymphocyte derivation on paraffin-embedded tissues. The application of these systems to reviews of lymphomas in animals and their response to therapy should provide major impetus to the understanding of lymphomas in animals from the standpoint of research on spontaneous lymphoid neoplasms of outbred species, as well as treatment of lymphoid tumors in companion animals.

From an overall standpoint, *the 2001 revision of the WHO classification provides a comprehensive framework for the identification of both myeloid and lymphoid neoplasms as well as the histiocytoses and myelodysplastic syndromes.* It is likely that the current, much greater, understanding of myeloid neoplasms reflected in the WHO classification will permit and encourage more accurate definition of these neoplasms in animals.

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Lymphoproliferative disease

Lymphoproliferative disease may be defined as neoplastic proliferation of lymphocytes causing lymphoma or lymphoid leukemia. In general, the lymphoproliferative diseases form a spectrum with lymphoma and lymphoid leukemia at their extremes, and it is not always possible to determine whether the disease in a particular animal is primarily peripheral and a lymphoma, or of bone marrow origin and a leukemia. *The major distinction between leukemia and lymphoma is in the tissue area with the largest mass of tumor cells.* It is now understood that, in malignancies of the lymphoid system, there are always tumor cells in circulation whether or not they are recognized in the peripheral blood. The operative factors in the dissemination of tumors of the lymphoid system are the presence and type of *intercellular cytoplasmic adhesion molecules* (*ICAMs*) on the surface of the tumor cells that mediate adhesion to endothelium and the ability to emigrate to new tissue areas. In benign states, naive lymphocytes leaving the marrow "home" to the cortex of the thymus (*T cells*) and to the Peyer's patches of the intestine (*B cells*), and then to the peripheral tissues on the basis of these adhesion molecules. Malignant lymphocytes mimic this process, and those that do not home to tissue continue to circulate. All intermediate options exist, with no ability to adhere and undergo transmural endothelial passage, or with being tightly homed to the initial site of oncogenesis as occurs in neoplasms of the mucosal-associated lymphoid tissue (MALT) types. As a consequence of this understanding, *the leukemias and lymphomas of the same cell type, whether of acute or chronic cell maturity, are considered different manifestations of the same disease rather than separate entities.*

In lymphoproliferative disease, malignancy in an ontogenically primitive cell is likely to occur in the bone marrow, thus presenting as a leukemia in a young individual with the disease. In contrast, clonal autonomy in a mature lymphocyte is likely to occur in the peripheral tissues in a mature individual with presentation as a lymphoma. In general, the leukemias present with some degree of marrow failure characterized by anemia, thrombocytopenia, or neutropenia, which occurs when 50% or more of the bone marrow is involved by tumor. Under these circumstances, the blood and bone marrow are always diagnostic. In contrast, the lymphomas which involve peripheral tissues tend to leave the bone marrow relatively uninvolved and, at the time of diagnosis, these animals usually have normal hemoglobin, platelet and leukocyte levels.

In comparison to the complex molecular techniques required for identification of myeloid tumors, most of the identification of lymphoid neoplasms is done quickly and relatively cheaply with immunohistochemical reactions to identify B- and T-cell tumors and their various subsets. In general, *it appears that in both humans and animals the B-cell neoplasms have a better survival profile and response to therapy than the T-cell neoplasms.* Currently, it is possible to fingerprint lymphomas on the basis of their spectrum of gene activation. The mRNA is extracted from malignant lymphocytes with preparation of complementary DNA, which is then allowed to anneal with known transcripts on specifically prepared DNA microchips that detect tumor specific sequences in a screen that looks for many thousands of genes in a single analysis. These types of assays are able to detect subsets of tumor types within apparent single morphologic and phenotypic entities. This level of specificity in characterization of neoplasms holds the promise of better definition of the molecular pathogenesis of causation and for more specific and effective therapy.

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Precursor B-cell neoplasms

Precursor B-cell lymphoblastic leukemia/lymphoma (B-ALL/LBL)

Lymphoid neoplasms of the B-cell lymphoblastic type are rapidly progressive neoplasms that involve the bone marrow and peripheral tissues in various proportions with the same cell type in all areas. Acute B-cell lymphoblastic leukemia/lymphoma occurs in all species. There is a wide age range, and apparently some predisposition for intact males. The disease is seen most frequently in *calves and cats less than a year of age and in dogs under 5 years.* Prior to use of FeLV vaccines, it was assumed that most hematopoietic neoplasms in the cat were virus-associated, while in the calf under a year, malignant lymphoproliferation is not associated with *Bovine leukemia virus* infection. Most studies on phenotypic identification of B-ALL in dogs and cats have too few numbers for firm projections but it appears that about 60% of the ALL in cats and dogs are of the B-cell type. The phenotype of ALL cannot be predicted by routine stains, and is determined primarily by the use CD3 and CD79α reagents to identify neoplasms of T and B cells, respectively.

The *morphologic characteristics of the acute lymphocytic leukemias* according to the French/American/British (FAB) classification are based on nuclear/cytoplasmic ratio, nuclear size and shape, number and size of nucleoli, and cytoplasmic basophilia and vacuolation (Table 2.3). ALL is also *classified immunologically* in terms of degree of differentiation, and *cytogenetically*, as well as by *etiology and prognosis*. In human ALL, the disease in children is very predominantly (85%) of the L1 type, and a high proportion of these cases attain durable remissions on aggressive therapy. Immunologically, the disease in children is predominantly of early B type with rearrangement of the immunoglobulin gene. In contrast, ALL in adults is predominantly of the L2 type with chromosomal abnormalities occurring in two-thirds of cases, and the response to therapy is much poorer than that achieved in children. The L3 type is the leukemic equivalent of the Burkitt lymphoma cell and is more prone to abdominal and central nervous system spread. Prognosis tends to decline from L1 to L3. With the advent of universal phenotyping of ALL, the classifications based on morphology are less referred to and utilized. In human ALL, more than 80% of cases are of the B-cell type. In terms of the FAB classification, T-cell ALL may of either FAB L1 or L2 type, and the L3 types are predominantly of B-cell type as is the Burkitt lymphoma.

Despite their aggressive appearance, the proliferative fraction of these tumors is actually less than that of their normal counterparts,

Table 2.3 Cytologic correlates in lymphoblastc leukemia

FAB	Lymphoblastic L1	Lymphoblastic L2	Lymphoblastic L3
Incidence (%)			
Humans	80	15	5
Animals	10	60	30
Cytologic features			
Cell size	Small predominantly	Large, heterogeneous	Large, homogeneous
Chromatin	Homogeneous	Heterogeneous	Fine and homogeneous
Nucleoli	Absent or small	Absent or small	Prominent
Cytoplasmic volume	Low	Moderate	Moderate
Cytoplasmic basophilia	Moderate	Moderate	Strong
Cytoplasmic vacuolation	Absent	Absent	Prominent
Nuclear shape	Round, rarely cleft	Irregular clefting common	Regularly oval to round
Phenotype	Primarily T cell	Primarily T cell	Primarily B cell

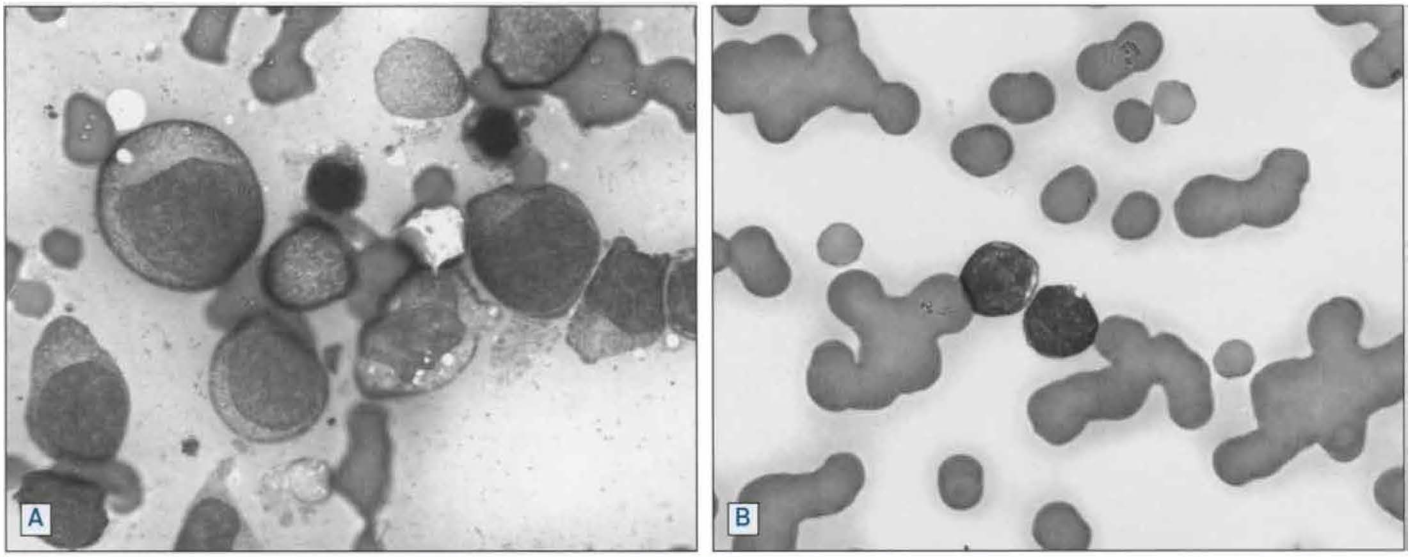

Figure 2.31 Lymphoblastic leukemia in a horse. **A.** Blood. Nuclei of neoplastic cells are approximately 1.5 red cells in diameter with deeply stained chromatin and with several small and inapparent nucleoli. The cytoplasmic vacuoles are the characteristic signature of the L3 leukemia. **B.** Bone marrow aspirate. Neoplastic cells are more variable in size and shape than in blood with homogeneous chromatin and 1 to 3 small nucleoli. Cytoplasmic vacuoles are characteristic of L3. and usually stain positively with Sudan black B.

and therefore cell-cycle-dependent cytoreductive therapy has the potential to injure the host more than the tumor. *In contrast to the acute myeloid leukemias, which compete directly for space with benign stem cells in subendosteal sites, the pattern of lymphoid colonization of marrow is random, and therefore the lymphoblastic leukemias tend not to result in marrow failure as rapidly as the acute or blastic tumors of the myeloid system.*

Since the phenotype of precursor cell leukemia in animals is largely undefined, the pathology of B- and T-cell ALL is also ill-defined. While there is little to suggest B-cell phenotype in the topography of precursor B-cell ALL, the T-cell ALL tends to begin in the thymus and rapidly spreads to the bone marrow.

With the exception of calves, animals are presented with minimal lymph node enlargement. There is some degree of mucosal pallor and splenomegaly, but hemorrhage is less common than with the acute myeloid leukemias. The course is one to two months.

Anemia at presentation is usually mild but tends to be more pronounced in cats. Red cells are normochromic, normocytic, without polychromasia, and there is an occasional poikilocyte. Dogs characteristically have Howell-Jolly bodies, indicating splenic involvement. The total leukocyte count is generally between 80 and 100 $\times 10^9/L$, but may be low or normal. The differential count is 80–100% lymphocytes, and leukoerythroblastosis is usually not present. Neutrophils are usually mature, and often hypersegmented with reduced secondary granulation. Metamyelocytes are occasionally seen, but more immature cells are unusual as are rubricytes.

Cytologically, B-cell ALL cells are intermediate in size with nuclei 2 red cells in diameter, round to oval, with finely distributed densely stained chromatin, indistinct or absent nucleoli, and minimal cytoplasm (Fig. 2.31A). Perinuclear clearing of basophilia with peripheral concentration is characteristic. Histologically, the nuclei of ALL cells are 1.5–2 red cells in diameter and may be small and uniform with inapparent nucleoli (L1), or larger with irregularly cleft nuclei (L2), or larger and of uniform size and shape with prominent nucleoli and highly basophilic cytoplasm often with vacuoles that stain positively for lipid (L3) (Fig. 2.31B). There may be a high level of apoptosis in histological preparations, with a resulting "starry-sky" appearance at low magnification due to a high number of macrophages containing tingible bodies.

A type of large granular lymphocyte tumor occurs in the Fischer rat and in dogs and cats and is considered with chronic lymphoid leukemia. The L1–L3 cell types are characteristic of the majority of the acute lymphoid leukemias seen in animals. A further type of lymphoid leukemia, formerly called "reticuloendotheliosis" and now "hairy cell leukemia," occurs in humans, and is related to infection with the HTLV-II. A counterpart of this disease has been described in cats, but appears to be very uncommon. Hairy cell leukemia has a very variable course in humans, but is generally considered with the chronic leukemias. Since in the cat survival is a matter of weeks after diagnosis, this entity is appropriately considered in the diagnosis of ALL. Morphologically, the tumor cells are characterized by thin cytoplasmic protrusions that are best seen in wet mounts and cytochemically by reaction for the tartrate-resistant acid phosphatase (TRAP) stain. Morphologically, the tumor has specificity in a broad rim of clear cytoplasm and by the manner in which it dilates, colonizes and lines hepatic and splenic sinusoids as well as the portal cuffing more typical of the lymphomas.

In general, in the acute lymphoid leukemias the platelets are usually less than 100×10^9/L and often severely deficient. Immature platelets are rare or absent but there may be confusion with agranular cytoplasm shed from injured lymphocytes.

Aspirated marrow is highly cellular with marked immaturity and trilineage phthisis. Bone marrow lymphocytes are characteristically slightly smaller and more uniform than those in the blood, possibly because of senescence in some of the latter. **Grossly**, there is usually only mild pallor and loss of condition, except in calves which may be cachectic and have marked symmetrical lymph node enlargement. Other species have only mild or irregularly enlarged nodes. The cervical and thoracic viscera are usually normal, except as noted for the calf. The liver is markedly enlarged in the calf with irregular pale areas and a lobular pattern on cut surface. In the cat and dog, the liver may be mildly enlarged. It is usually normal in other species. The spleen is moderately and symmetrically enlarged and is dry and fleshy on cut surface, occasionally with focal pale areas. The femoral bone marrow is uniformly reddened in cats, and may have foci of residual fat in dogs and horses. Calves have almost solid infiltration of all marrow cavities and large areas of infarction with yellow areas of necrosis surrounded by hyperemia. This degree of marrow involvement may be present in calves born with the tumor.

Microscopically, the bone marrow has 90–100% cellularity with a solid field of mononuclear cells. These are usually of medium size with a variable but minor proportion of large and small cells. Nuclei are open and vesicular with central nucleoli, and coarse granular chromatin with irregular deposition on the nuclear membrane. Cytoplasm is moderate in amount and deeply amphophilic. Only an occasional megakaryocyte and rubricyte can be found, and granulocyte reserves are absent. Lymph nodes have intact architecture with follicular atrophy. The cortical cell density is usually low, with tumor cells replacing the small paracortical cells. Postcapillary venules are tightly packed with malignant cells. The spleen has a thinned capsule, follicular atrophy, and solidly cellular sinus areas. There may be foci of necrosis. Benign hematopoiesis is minimal. Subendothelial colonization of splenic large muscular sinuses is well developed. The liver has diffuse sinusoidal and portal colonization. The tumor burden in the sinusoids roughly indicates the level of leukemia. There is irregular sinusoidal dilation, which contributes to the hepatomegaly, and usually some degree of periacinar ischemic degeneration. Foci of tumor may be widespread but are found in the kidney, testes, meninges, bowel, and pancreas.

Acute lymphocytic leukemia must be distinguished from acute myeloblastic leukemia on the basis of lack of cytoplasmic granulation and reaction to esterase stains. The differentiation of precursor cell leukemia from lymphoma of the same cell type is now considered of no diagnostic or prognostic value. Generally, *leukemias present with marrow failure and minimal lymphadenopathy*, whereas *lymphomas have a more normal neutrophil and platelet picture, and may not be anemic unless complicated by enteric hemorrhage. In lymphoma, there are usually localizing signs of peripheral involvement, including lymph node enlargement, central nervous system deficits, or melena.*

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Mature (peripheral) B-cell neoplasms

B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma

Chronic lymphocytic leukemia is an acquired clonal lymphoproliferative disorder, usually of B-cell origin, characterized by the accumulation of a relatively uniform population of immunologically incompetent but relatively mature lymphocytes. Cytologically, CLL is characterized by considerable variation in morphology from small cells with mature nuclei and minimal cytoplasm, to cells with nuclei nearly twice the diameter of a small, mature lymphocyte and with relatively abundant cytoplasm. The biological similarities in CLL of the various types of morphology are that they are characterized by a low proliferative rate and prolonged cellular survival. In contrast to many of the myeloproliferative diseases, *molecular and enzymatic studies indicate that the neoplastic clone in CLL involves only the cells apparent in the tumor without involvement of other cells of the myeloid or benign lymphoid systems.* While lymphoblastic leukemia is primarily a disease of the young in both humans and animals, CLL is a disease primarily of humans older than 80 years of age and *animals in late maturity*. In humans, but less so in animals, males appear to be affected more often than females, with an approximate 2:1 ratio. The risk factors for CLL in animals are unknown, but in humans, farmers who utilize an array of pesticides are at greater risk as are workers in the rubber and asbestos industry and those exposed to benzene. Unlike other types of leukemia, CLL in humans is not linked to prior exposure to radiation but genetic factors are involved as *CLL is the most common familial leukemia.* CLL occurs in younger humans and animals in which the clinical course is more variable, and the staging and prognostic criteria utilized for the older population are less useful and reliable. In humans, but not in animals, immunological changes have received a great deal of study in CLL. Characteristically, B-CLL cells have weak monotypic expression of surface immunoglobulin, usually of IgM but also of other types including IgD and even IgG. Human CLL has receptors for CD20, CD22 and CD11c, but CD79b and CD10 are not expressed. By this pattern of receptor development, *human CLL can be distinguished from other mature B-cell neoplasms such as mantle cell, prolymphocytic leukemia, follicular center cell lymphoma, and hairy cell leukemia.*

Cytogenetic changes in human CLL are difficult because of the rarity of mitoses available for analysis. More effective analyses, including fluorescence in situ hybridization (FISH), have assisted this process because the technique can be applied to interphase cells and mitotic activity is not required. These and molecular studies indicate that *karyotypic changes occur in only a minority of cases of human CLL*, with trisomy 12 being the most frequent abnormality found. More common genetic changes include 13q deletion or translocation, 14q+, 11q deletion or translocation, and a series of gene rearrangements involving the *BCL 1,2,3* genes.

The cell of origin of chronic lymphocytic leukemia is believed to be a lymphocyte normally present in peripheral blood at very low numbers and with an immunophenotypic pattern similar to CLL and in tissues present in the mantle cell areas of lymph nodes. The presentation of CLL in elderly humans is so characteristic that bone marrow studies may not be carried out. The minimal diagnostic criteria for human B-cell CLL include a peripheral blood lymphocyte count of at least 5–10 × 10^9/L with sustained lymphocytosis and a predominance of small mature-appearing lymphocytes, with less than 10% being prolymphocytes. Bone marrow cellularity is usually normal or increased, with lymphocytes constituting 20 to 50% of the bone marrow population. Several of the staging systems proposed for human CLL appear to be applicable to the disease in animals. The Binet system of classification includes three levels of presentation based on survival. The *three levels of tumor involvement* are:

1. No anemia or thrombocytopenia, with involvement of fewer than three lymphoid areas.
2. No anemia or thrombocytopenia, with involvement of three or more lymphoid areas.
3. Anemia with less than 100 g hemoglobin/L, or thrombocytopenia of less than 100 × 10^9/L.

Most cases of human and animal CLL maintain a stable cell type for long periods of time, but a small number of cases in both humans and animals evolve into ALL or an aggressive large cell lymphoma termed Richter's syndrome. The bone marrow involvement, which is a factor in staging, may be focal or nodular, interstitial or a combination of these two, or diffuse, with this sequence representing the order of progression and decreasing prognosis.

CLL in animals is primarily a disease of cats, cattle and dogs, usually eight to ten years of age or older. CLL is an insidiously progressive disease with few localizing signs and may be diagnosed without suspicion of disease in animals presented for routine clinical evaluation. The lymphocyte count on presentation in animals is highly variable and, while generally felt to be in excess of 50 × 10^9/L, is occasionally more than 500 × 10^9/L. This range in numbers is likely a function of CLL being diagnosed in animals relatively late in disease progression. It is likely that as routine blood examination in animals presented for annual or routine check-ups become more common, that CLL will become recognized as presenting with lymphocytosis at much lower levels and more characteristic of the situation in human CLL. Typically, anemia on presentation is moderate, normochromic and with minimal anisocytosis and poikilocytosis. Howell-Jolly bodies are occasionally seen, particularly if there is splenomegaly, and polychromasia and rubricytes are typically absent. Early in the course of CLL, neutrophil counts may be normal in absolute numbers and these may be maintained even while the lymphocyte count rises to very high levels and the neutrophils, as a percentage, are reduced to 5% or less of the peripheral blood cells. Thrombocytopenia is present in about 25% of dogs at diagnosis with CLL and is generally of mild degree. Major differences in phenotype are present in the dog as compared to humans. In one study of CLL in dogs, two-thirds of the population had T-cell leukemia and of that group almost half were of the LGL (large granular lymphocyte) type, one of which was likely an NK cell neoplasm. The canine B-cell CLL mark with CD79α and CD21, but unlike human B CLL, do not label with CD5. The phenotype of feline CLL is as yet less defined.

Cytologically, *the cells in the peripheral blood may be of several morphological types or combinations of types.* The most frequently described, but not the most frequently occurring, presentation is with a predominance of lymphocytes that are small with round nuclei that are the same size or slightly larger than the cells of the normal thymic cortex. The nuclei have a "blocky" or coarse chromatin pattern with large chromocenters whose density is characterized by irregular parachromatin clearing and an absence of nucleoli (Fig. 2.32A). The cytoplasm in this cell type is minimal and relatively lightly stained.

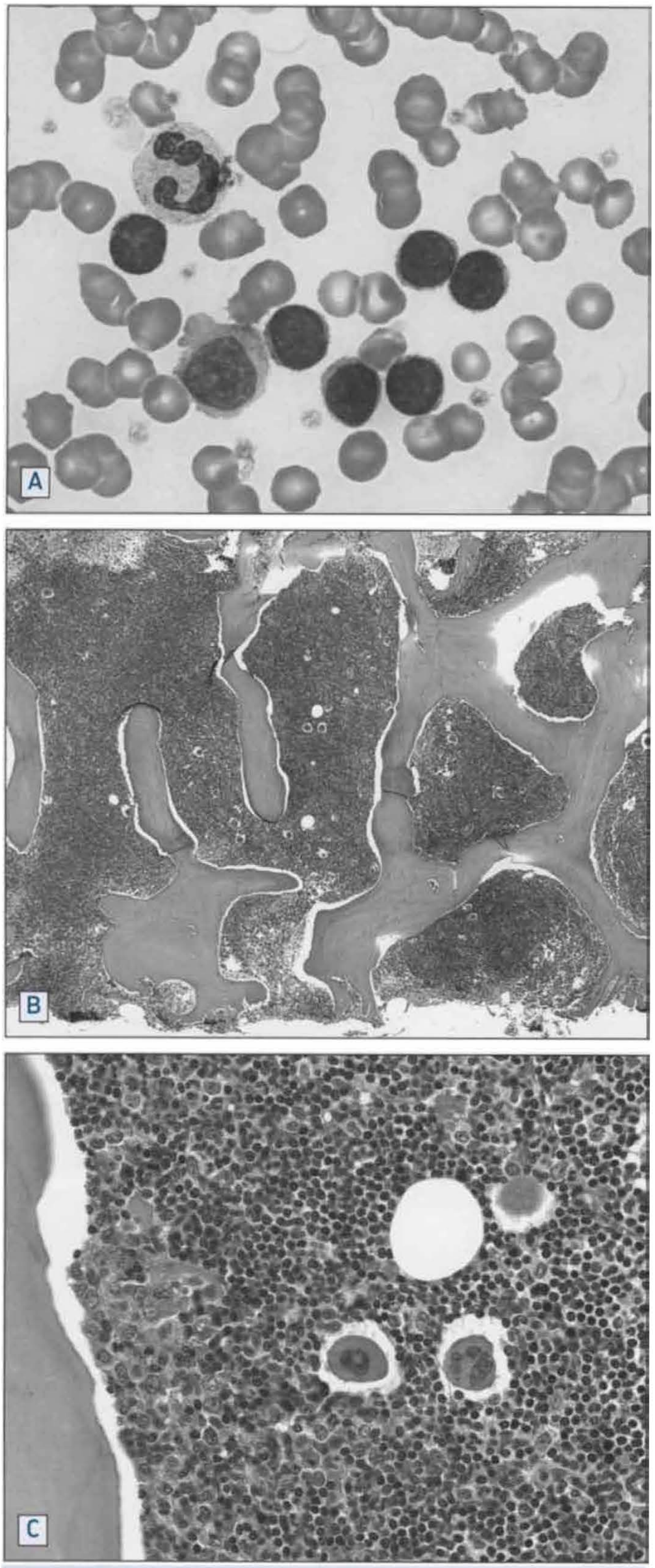

Figure 2.32 B-cell chronic lymphocytic leukemia in a dog. **A.** Blood. Small cell type with nuclei slightly larger than red cells and very little cytoplasm. The larger lymphocyte likely represents the proliferating population and has small nucleoli. **B.** Marrow. Relatively complete replacement of fatty areas by tumor cells represented by darker areas and residual hematopoiesis. **C.** Detail of (**B**). Interstitial pattern of marrow involvement with relatively solid areas of tumor surrounding the fatty vacuole and residual benign hematopoiesis adjacent to bone. (Courtesy of RM Jacobs.)

Cells of similar type are present in the bone marrow or in other sites of involvement. The more common presentation of CLL in animals is with a larger cell type in which the nuclei are round to oval and may be lightly cleft and are characteristically 2 red cells or slightly greater in diameter. The nuclei have densely stained chromatin, again with large chromocenters, with irregular lighter areas of parachromatin, and small nucleoli may occasionally be present. These cells characteristically have abundant cytoplasm in which the nuclei tend to be centrally placed with an irregular cellular shape resulting when the cells are seen in groups. The cytoplasm is lightly basophilic and agranular with perinuclear clearing. *CLL needs to be distinguished from prolymphocytic leukemia* in which the cells are a larger type, with the nuclei generally 2.5 red cells in diameter with accentuation of the large chromocenters and intervening parachromatin areas, one or two small nucleoli and relatively abundant cytoplasm.

Histologically, *the nuclei have the same characteristics as described in cytological preparation* except that the cells appear smaller in diameter and the variation in chromatin distribution is accentuated and more apparent. In both the small and larger cell types of CLL, deposition of chromatin in the nuclear membrane is not seen as in large-cell lymphomas, although chromocenters may impinge directly on the nuclear membranes. When the peripheral blood contains a mixture of cell types with either the small type or the large type predominating, it is likely that the disease began as a small-cell type CLL and is at a stage of progression to an accelerated phase with the larger cells representing prolymphocytes.

At the stage that most animals are diagnosed with CLL, the marrow aspirate is hypercellular with more than 90% of cells similar to lymphocytes in the peripheral blood (Fig. 2.32B, C). Animals with CLL usually are presented in relatively good body condition, often with abundant abdominal fat. *Splenomegaly is always marked in later stages of the disease* and the liver is at least mildly enlarged and palpable. There is generally not lymphadenopathy, and some nodes may be smaller than normal or there may be irregular focal nodal enlargement.

On gross postmortem examination, there is mild pallor with evidence of splenomegaly and the liver is mild to moderately enlarged, pale brown and with a lobular pattern that is more prominent as the anemia becomes more severe. On cut surfaces, the spleen is soft, fleshy, dry and deep red. Focal areas of tumor may appear as white nodules 5 mm or more in diameter in the spleen, liver and renal cortices. The nodes remain mobile and are never markedly enlarged and may be irregularly small or focally enlarged. The femoral bone marrow is uniformly pink in mid-shaft areas and is usually friable with heavy exfoliation on imprint preparation.

Microscopically, the bone marrow has greater than 90% cellularity and is usually seen as a solid field of small cells with occasional fat vacuoles and megakaryocytes (Fig. 2.32B, C). In later stages of the disease, it is not possible to determine whether the pattern of marrow involvement is focal, nodular or interstitial. The liver has tumor infiltration around virtually all triads, and there may be many lymphocytes in the sinusoids lining the hepatic cords. Unlike ALL, the degree of leukemia may not be reflected by the numbers of cells in hepatic cords, which is likely related to the pattern of L-selectins and integrins that determine the endothelial binding and homing pattern of the tumor cells. The spleen has atrophy of the thymic-dependent areas around small arterioles, and it may be apparent that the sinus infiltration is arising from cells around previous follicles or there may be

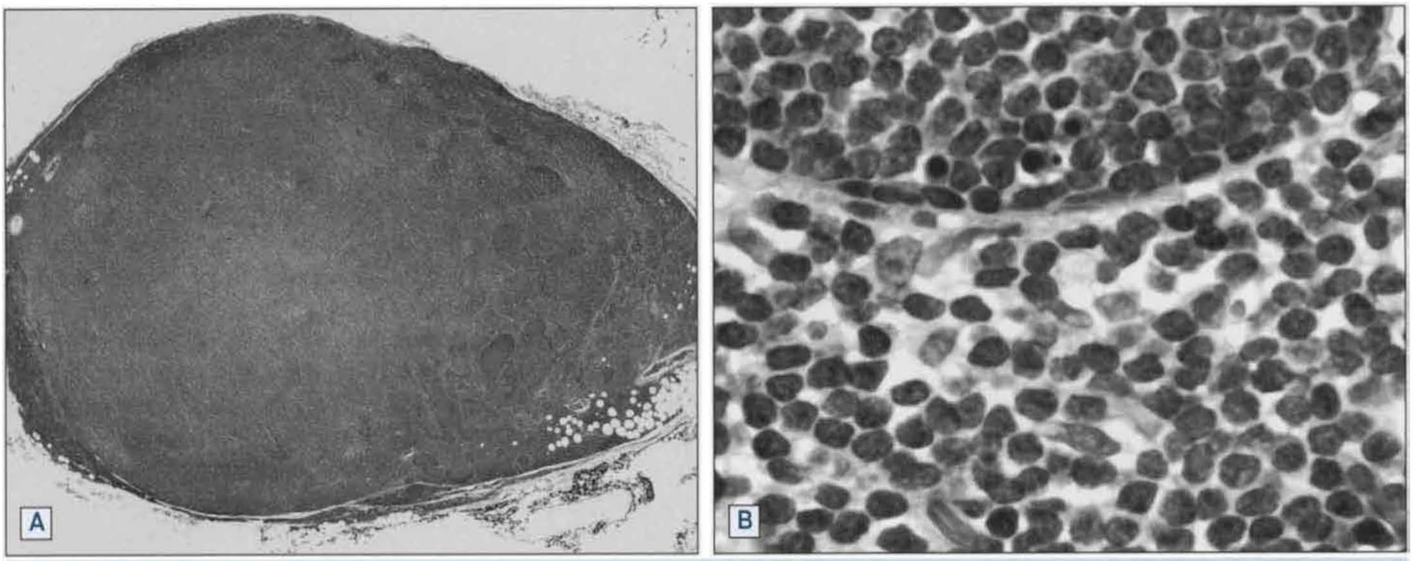

Figure 2.33 B-cell small lymphocytic lymphoma, lymph node, dog. **A.** There is effacement of normal architecture with compression of residual medullary cords and sinuses at the smaller end of the tissue. The irregular darker areas represent dilation of small veins with neoplastic cells, suggesting there is leukemia. The peripheral capsule is thinned and taut with irregular colonization of perinodal tissues. **B.** Detail of (**A**), area adjacent to dilated sinus. Nuclei are small with densely stained chromatin without nucleoli and minimal cytoplasm. Note apoptosis but absence of mitosis.

uniform sinus distention without a prior pattern of involvement being apparent. There is scattered hematopoiesis near the muscular trabeculae, most evident by a few remaining megakaryocytes. There is invariably subendothelial colonization of the large muscular veins. Nodal involvement in CLL is variable with some nodes atrophic with loss of germinal centers and sinus histiocytosis. Involved nodes tend to have diffuse cortical infiltration with dilated postcapillary venules, some of which are markedly dilated with tightly packed lymphoid cells. The tumor cells tend to occupy and expand the medullary cords and to compress and obliterate the sinuses. There is often small vessel dilation in the lung and kidney with many intraluminal lymphocytes, and focal tumor colonization may be found in most tissues including the brain and eye.

"Lymphoma of small lymphocytic cell type" (SLL) is virtually the same disease as chronic lymphocytic leukemia, and the diseases are now considered indistinguishable. If the disease presents primarily as a lymphoma, there are usually some nodes that are quite markedly enlarged and may even be affixed to surrounding tissues by extranodal tumor involvement. Both diseases are characterized by hepatosplenomegaly and progressive anemia, with thrombocytopenia and bleeding tendencies more common when there is a greater progression of tumor involvement in bone marrow than in the peripheral tissues. Histologically, the involved lymph nodes in SLL have a diffuse architecture with marked thinning of the peripheral capsule and focal colonization of the capsule and perinodal structure (Fig. 2.33A). There will be marked atrophy of lymphoid follicles, none of which may be present. Filling of the paracortex extends into the medullary region, with expansion of the medullary cords and compression but not destruction of the medullary sinuses. Focal lighter areas throughout the tumor are composed of slightly larger cells and are considered "*reaction centers*" consisting of prolymphocytes with a higher level of cellular proliferation. In the smaller cell types, the tumor cells resemble cortical thymocytes without nucleoli, and mitoses are rarely found outside of the reaction centers (Fig. 2.33B). In the CLL/SLL of the larger cell type, the cells histologically have greater internal nuclear detail, but again appear round with slightly greater cytoplasmic volume and greater internal nuclear detail. Mitoses again are rare.

Chronic lymphocytic leukemia and small lymphocytic lymphomas in both humans and animals are characterized by hypogammaglobulinemia and rarely monoclonal gammopathy of IgM type. The immunological implications of that abnormal globulin production may take the form of *autoimmune disease* of which immune hemolytic anemia is the most commonly observed type in humans and presumably in animals as well. Animals with hypogammaglobulinemia appear to benefit from globulin administration in terms of prevention of infectious disease. *CLL/SLL need to be differentiated from peripheralizing lymphoma of larger lymphocytes and chronic lymphocytic leukemia of the LGL type which are distinguished by their cytoplasmic granules to be of T-cell origin. The major differential diagnosis is with prolymphocytic leukemia (PLL).* CLL must have less than 10% prolymphocytes whereas disorders with 10 to 50% prolymphocytes are considered CLL/PLL, and PLL when greater than 50% of prolymphocytes are present in the peripheral blood.

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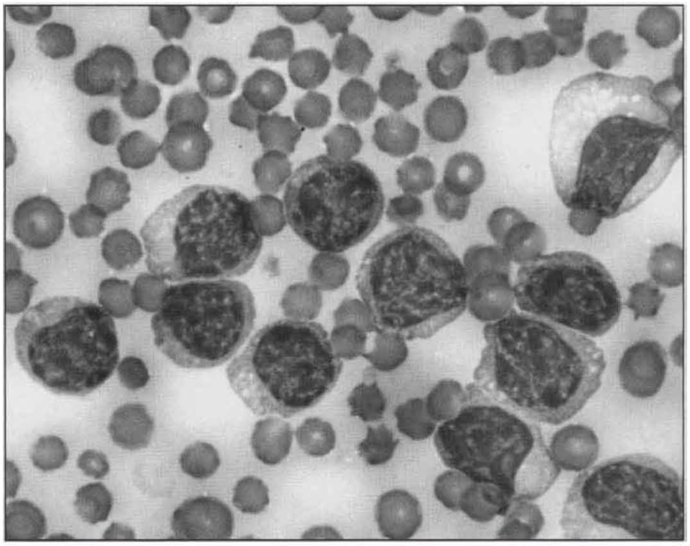

Figure 2.34 B-cell prolymphocytic leukemia, blood, dog. Nuclei are characteristically 2 red cells in diameter with large densely stained chromocenters separated by lighter areas of parachromatin clearing and joined by fine chromatin bands. Nucleoli are present and irregularly rimmed by chromatin. (Courtesy of RM Jacobs.)

B-cell prolymphocytic leukemia

B-cell prolymphocytic leukemia (PLL) has not been formally reported in animals. *It is certain, however, that the disease occurs and is unrecognized, because the illustrations of prolymphocytic leukemias in humans have a very characteristic cytomorphology which can confidently be said to occur in animals*, but has not been described as an entity as distinguished from chronic lymphocytic leukemia of smaller cell type. The disease was first described in humans in 1974, and while of much rarer occurrence than CLL, the disease has been well delineated. The characteristic presentation in humans is an elderly male who presents with marked splenomegaly and leukocytosis. Lymph node involvement at presentation is unusual, whereas bone marrow failure is much more advanced than is the usual case with CLL, in that *anemia, thrombocytopenia, and neutropenia are all common at initial diagnosis*. The cytomorphological characteristics of PLL may be found in both B- and T-cell leukemias, but it is *likely that 80% or more cases of human PLL are of the B-cell type.*

Both benign and malignant prolymphocytes have distinct morphology, with the neoplastic counterpart being an exaggerated form of the benign cells with more contrast between the chromocenters and parachromatin areas of the nucleus. In humans and animals, the cells in blood in PLL have abundant pale blue agranular cytoplasm that may be deeply indented by the surrounding cells. The chromatin in PLL is densely stained and has more definition of chromocenters than in the large-cell type of chronic lymphocytic leukemia, and much more chromatin aggregation than is present in lymphoblastic leukemia/lymphoma. The chromatin in PLL is distributed in 20 or more large dense chromocenters that are cross-linked with each other by chromatin bands bridging relatively lightly or unstained areas of parachromatin clearing. PLL cells are characterized by a single prominent nucleolus, usually relatively central, and by irregular "rimming" of chromatin that adds unusual definition to nucleolar size and shape (Fig. 2.34).

Animals with PLL usually are presented after some period of declining health, usually noticed by loss of weight, appetite and vigor. PLL in animals is seen most frequently in dogs and cattle and less frequently in cats, horses and other species. Anemia is present of moderate or greater degree with typically 50–80 g hemoglobin/L, and usually uncomplicated by recent enteric or thrombocytopenic hemorrhage. Anemia is normochromic with occasional poikilocytosis, and polychromatic red cells and rubricytes are typically absent. There is usually some degree of thrombocytopenia with the platelet count usually of 50–100 × 10^9/L, with leukocyte counts generally in the same range of magnitude. Unlike CLL, mitoses in peripheral blood leukocytes are not infrequent and are most profitably looked for at the feather tip of the blood smear. On aspiration, the bone marrow is hypercellular and usually greater than 80% occupied by tumor and residual hematopoietic cells with a few fat cells remaining. Erythropoiesis and thrombopoiesis are reduced and residual cells are without dysplastic changes. A coarse hemosiderin pattern may be present with increased marrow iron.

Morphologically, PLL in animals is like CLL/SLL with a more rapidly progressive pattern of growth. Tissues other than marrow that are usually involved include lymph nodes, spleen and liver. Unlike CLL, there may be extensive involvement of nodes that are not grossly enlarged. There is typically effacement of germinal centers and diffuse filling of the node cortex with abridgement of the peripheral sinus and some degree of involvement of perinodal structures. The nucleoli prominent on cytological preparations are also apparent histologically, with the tumor cells expanding in a concentric fashion around germinal centers providing an architectural picture similar to mantle cell lymphoma in tissues with early involvement. There is massive splenic involvement in PLL with a miliary pattern often visible grossly and, histologically, there is expansion of the white pulp and diffuse filling of the red pulp. Splenic involvement may be characteristic with the residual benign cells of germinal centers collapsing in small dense nodules within the areas of previous germinal centers surrounded by the larger tumor cells, giving an inverted pattern of germinal center formation. The liver has a sinusoidal pattern of involvement with less prominent portal cuffing than in CLL.

B-PLL must be differentiated from B-CLL by the greater proportion of prolymphocytes in the blood exceeding 50% of the total lymphocyte compliment. In animal pathology, the other differentials might include *acute lymphoid leukemia*, which can be ruled out because of the characteristic retention of large chromocenters in the nuclei of PLL cells.

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Lymphoplasmacytic lymphoma (LPL)

Lymphoplasmacytic lymphoma is a neoplasm of plasmacytoid lymphocytes with small B lymphocytes and some plasma cells that involves the bone marrow, lymph nodes and spleen. The typical cells of LPL have round or oval nuclei, slightly larger than those of small lymphocytic lymphoma (SLL), which lack nucleoli and have a moderate amount of densely stained, eccentrically placed cytoplasm. *In animals, LPL is one of the more common small cell lymphomas and is more common than SLL or small cell lymphomas of T-cell type.* Histologically, the LPL neoplasms have diffuse architecture and are characterized by cortical expansion of lymph nodes with effacement of normal architecture and involution of germinal centers. The disease is slowly progressive and mitoses are rare, with characteristically one present in 4 or 5 fields at ×400 magnification.

In animals, LPL tends to be a neoplasm with a relatively monotonous population of plasmacytoid small lymphocytes, whereas in human neoplasms of this type, there are numerous small lymphocytes and some more well-differentiated plasma cells. The neoplastic cells in human LPL have rearranged Ig heavy and light chain genes and express surface and cytoplasmic Ig, frequently IgM, but characteristically lack CD5 and CD10. Most human patients have a monoclonal paraprotein in the serum, commonly IgM, which corresponds to the syndrome known as *Waldenstrom's macroglobulinemia*. This paraprotein is seen in a significant portion of human patients with LPL, some of which have hyperviscosity syndrome or symptoms associated with cold agglutinins. While this aspect of animal pathology appears not to have been well studied, it is unlikely that LPL, as it is seen in animals, is very frequently associated with a paraproteinemia since this aspect of the disease is seldom one of the major presenting signs. In terms of the ontogeny of B-cell differentiation, *the LPL cells appear to be derived from germinal center B cells* in that they are characterized by having somatic mutations of their rearranged Ig genes. This change suggests antigenic selection and origin of the LPL cells from post-germinal center cells that did not complete differentiation to class-switching from IgM to one of the other immunoglobulin types. LPL is seen in some human patients with chronic *Hepatitis C virus* infection, many of whom have a mixed cryoglobulinemia. LPL developing in these patients has gene differentiation suggesting derivation from a highly selective and antigen-driven B-cell subset.

LPL has cytogenetic specificity with a t(9;14) (p13;q32) translocations as seen in approximately one-half of human cases. This change links a PAX 5 gene encoding a B-cell-specific transcription factor (BSAP) to the Ig heavy gene locus. This translocation leads to enhanced transcription of an unmutated PAX 5 gene and increased BSAP production. By definition, LPL does not have genetic alterations in the *BCL* area characteristic of other small B-cell lymphomas.

These genetic changes result in LPL cells having variable cytoplasmic staining for IgM and expressing the B-cell associated markers CD19, CD20, CD22 and CD79a. As noted earlier, they lack expression for CD5, CD10, CD23, and variable expression of CD43. CD25 or CD11c have faint expression in some cases. In summary, the typical morphology with lack of expression of CD5 and CD23 with strong reaction for surface immunoglobulin and CD20 confirms the diagnosis in human LPL. *In animals, the typical histo- and cytomorphology that is negative for CD3 and positive for CD79 constitutes confirmation of LPL.*

In animals, LPL is seen most frequently in *dogs and cats* but is also seen in cattle and occasionally in horses. LPL is a *disease of mature animals* and is characterized by *slow progression or enlargement of multiple peripheral lymph nodes* and may arise in skin, particularly in cats (Fig. 2.35A). Moderate splenic enlargement and colonization of the spleen is based on expansion of cells surrounding the germinal centers, presenting a miliary appearance on cut surface. In human LPL, particularly those associated with macroglobulinemia, it is characteristic to see cells with *Dutcher bodies* (intranuclear invagination of immunoglobulin containing cytoplasm found in both benign and malignant conditions) as well as some *Mott or flame cells* with multiple dilated vacuoles containing immunoglobulin. These are generally lacking in animal LPL and *the major differential diagnosis of this disease is from other small cell lymphomas, primarily mantle cell lymphoma, with less concern for differentiation from frank plasmacytoma.* LPL was referred to as lymphoplasmacytoid in the revised European–American lymphoma (REAL) classification System, but the category has been renamed lymphoplasmacytic in the WHO proposal. In previous classifications, both terms were used, with lymphoplasmacytoid being the term reserved for those small-cell lymphomas of intermediate nuclear type (slightly larger than benign cortical thymocytes) and with a narrow ellipse of eccentric cytoplasm. Lymphoplasmacytic was the term used for a tumor population with more variation in nuclear size and a greater volume of eccentric cytoplasm. In reviewing the literature on these lesions, it is apparent that the term lymphoplasmacytic as adopted by WHO better fits the majority of tumors of this type seen in human pathology than does lymphoplasmacytoid. In contrast, in animals, most of the indolent

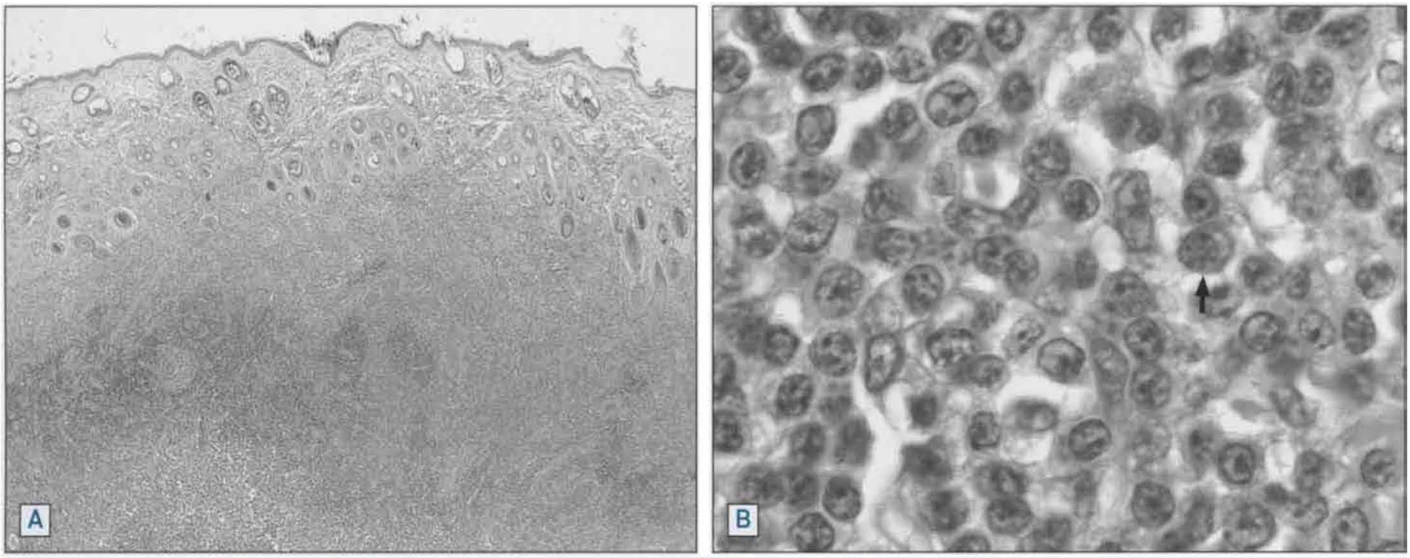

Figure 2.35 Lymphoplasmacytic lymphoma, skin, cat. **A.** There is relative sparing of the superficial dermis and adnexa with dense involvement of the panniculus. Small dark areas represent fading germinal centers, suggesting origin in benign lymphoid hyperplasia. **B.** Nuclei are slightly larger than red cells, vesiculated with peripheralized chromatin with nucleoli in larger cells. Cytoplasm is relatively abundant and deeply stained. Arrow indicates plasma cell differentiation.

small-cell lymphomas with plasmacytic differentiation have minimal cytoplasm and much better fit previous descriptions for lymphoplasmacytoid lymphoma (Fig. 2.35B).

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Splenic marginal zone B-cell lymphoma (+/− villous lymphocytes)

Cells of the splenic marginal zone were described as having architectural and immunological specificity in the mid-1980s. *Marginal zone lymphoma has since become a recognized entity in lymph nodes as well as in spleen and in extranodal sites where it is associated with mucosal-associated lymphoid tissue (MALT) types of neoplasms.* Marginal zone lymphomas occur in cats and dogs (Fig. 2.36A–E) and likely other species, but have only become recognized as entities in animal pathology during the last decade. In human neoplasms, cells of splenic marginal zone lymphoma express a variety of B-cell markers including surface immunoglobulin, usually IgM and *BCL2*, but do not express CD5, CD10, CD23, CD11c, or CD43. In animals, marginal zone lymphoma cells mark with CD79α and are negative as would be expected for the T-cell marker CD3. Marginal zone lymphoma in the human context is characterized by cells with nuclei of intermediate size, that is a small-cell lymphoma slightly larger than benign cortical thymocytes and with a broad circumference of relatively water-clear cytoplasm. Nuclei are generally round to oval and, while they are described as having a "monocytoid" appearance, the cells resemble monocytes in the volume of cytoplasm and not in terms of nuclear shape.

In animals, marginal zone lymphomas appear to follow a pattern of development over time that *begins with a widening zone of marginal cells encircling the normal mantle cell layer of germinal centers* and visible on low power examination because of the lighter staining characteristics due to the increased amounts of cytoplasm. In hyperplasias and early stages of marginal zone lymphoma, the nuclei are of intermediate-cell type with relatively abundant lightly stained cytoplasm, and cellular boundaries are generally indistinct. In normal benign tissues, cells of the same type can be found individually and in small clusters in the marginal zone region and also lining the inner surface of the subcapsular sinus of lymph nodes. At this stage, the cells generally do not have nucleoli, and nuclei largely resemble those of small benign lymphocytes with increased intranuclear detail because of contrast between densely stained large chromocenters and intervening areas of parachromatin clearing.

The *distinction between marginal zone hyperplasia in node or spleen and marginal zone lymphoma* is largely on the basis on whether or not the expanding areas outside of the germinal centers are neoplastic and becoming confluent, or are benign and remaining as distinct bands, usually two to three times as thick as the normal mantle cell layer. With time, these lesions apparently become more aggressive and the cell type gradually becomes that of a small immunoblastic-type lymphoma with nuclei approximately 1.5 red cells in diameter in histological section, with marked parachromatin clearing and chromatin deposition on the nuclear membrane and with a prominent single central nucleolus (Fig. 2.36E). The remarkable aspect of this lesion is that these cells have a very low mitotic rate and, despite their aggressive appearance, they remain solidly "homed" to the perifollicular regions for up to a year before progression proceeds to a diffuse architecture with obliteration of residual germinal centers.

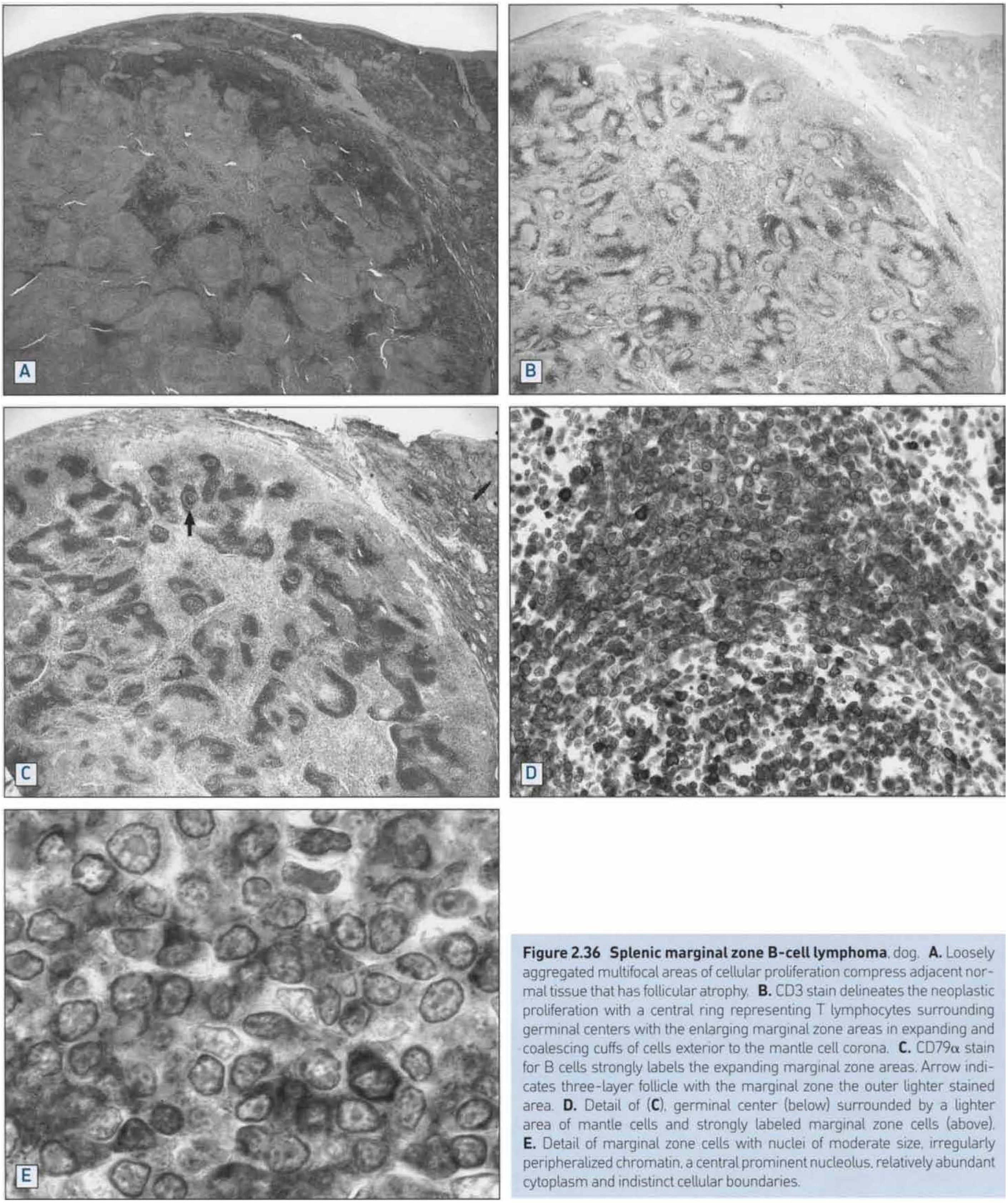

Figure 2.36 Splenic marginal zone B-cell lymphoma, dog. **A.** Loosely aggregated multifocal areas of cellular proliferation compress adjacent normal tissue that has follicular atrophy. **B.** CD3 stain delineates the neoplastic proliferation with a central ring representing T lymphocytes surrounding germinal centers with the enlarging marginal zone areas in expanding and coalescing cuffs of cells exterior to the mantle cell corona. **C.** CD79α stain for B cells strongly labels the expanding marginal zone areas. Arrow indicates three-layer follicle with the marginal zone the outer lighter stained area. **D.** Detail of (**C**), germinal center (below) surrounded by a lighter area of mantle cells and strongly labeled marginal zone cells (above). **E.** Detail of marginal zone cells with nuclei of moderate size, irregularly peripheralized chromatin, a central prominent nucleolus, relatively abundant cytoplasm and indistinct cellular boundaries.

Remarkably, the cells at the early stage of marginal cell lymphoma in spleen or node tend to mark much more lightly with CD79α than do more aggressive-appearing cells with the prominent single central nucleoli.

Marginal zone lymphoma is usually diagnosed in a peripheral node, but in those cases in which the disease appears to arise in the spleen, there is usually involvement of the liver and bone marrow and peripheral nodes at the time of diagnosis. Grossly, the spleen is dry on cut surface and shows miliary expansion of the follicular or white pulp areas. In the early stages of the disease in the spleen, there is a characteristic "bulls-eye" appearance to the splenic follicles due to hypocellularity of the germinal center, which is largely occupied by residual dendritic cells. At this stage, there is still a confluent cuff of small benign mantle cells surrounding the germinal center and inside the outer ring of lighter staining marginal zone cells. With progression, there is thinning and loss of the benign mantle cells with the rim of marginal zone cells remaining evenly centered on the involuting follicle. While it is difficult to determine in a particular case whether the lesion is splenic marginal zone hyperplasia or marginal zone lymphoma, some authors in the human literature confidently state that, if there are three zones in splenic follicles, the lesion is likely to be marginal zone lymphoma. On further progression of the lesion, particularly of lymph nodes, there is collapse of the residual mantle cells into the germinal center which then appears histologically as a focus of small dark cells at the center of an expanding circle of marginal zone cells that continue to be lighter staining because of their increased cytoplasmic volume.

In well-developed marginal zone lymphoma of spleen in the dog, the germinal centers are hypocellular and appear as epithelioid pale areas at the center of the expanding marginal zone. In the process of progressing from the three-ring "bulls-eye" appearance to the collapse of the germinal centers, the dendritic cell areas may become hyalinized or occupied by an amorphous proteinaceous insudation as evidence of progressing failure of the immune response and a kinetic absence of naive but competent B cells able to re-colonize the germinal follicles. The collapse and obliteration of the splenic germinal centers represents an important diagnostic finding that indicates the severity of impairment of the normal immune system and, thus, is less likely to be part of a hyperplastic marginal zone response than of marginal zone lymphoma. All of these changes may progress without marked enlargement of the spleen or impairment of sinus function, although there does appear to be some degree of increased phagocytic activity in splenic sinuses because of prominent hemosiderin-bearing macrophages in the red pulp or sinus areas and by progressive nonresponsive anemia.

In terms of **differential diagnosis**, marginal zone lymphoma of the spleen needs to be differentiated from *other B-cell lymphomas* that are centered on splenic white pulp. A major differential in human pathology is with villous lymphocytes or hairy cell leukemia that may not have a neoplastic counterpart in animals. Marginal zone lymphoma of the spleen is unlikely to be confused with lesions which, while causing lymphoid atrophy, are primarily centered on sinus areas such as the mast cell tumor of the spleen in cats.

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Hairy cell leukemia

Hairy cell leukemia (HCL) is currently not recognized in animals. The term "leukemic reticuloendotheliosis" was first used in human medicine in 1958 to describe the entity now called "hairy cell leukemia." In 1964, *Gilmore and Jones described a leukemic disease of cats they termed* "**reticuloendotheliosis**." The disease they described consisted of severe refractory anemia with splenomegaly and the presence of neoplastic cells in the blood, bone marrow, spleen and sinusoids of liver and lymph nodes. The disease occurred in cats 1 to 9 years of age with a strong male predominance. The animals were presented with anorexia, listlessness and weight loss, with most of the animals debilitated and often febrile. Moderate-to-severe enlargement of the spleen was a constant finding. Hematologic changes consisted of severe nonresponsive or poorly responsive anemia with leukocyte counts in ten cats varying from 6–50 × 10^9/L. The cells were considered inconsistent with any lineage definition and were felt to be precursor cells of varying myeloid or even lymphoid lineage. Nuclei were described as large and round, and in illustrations can be determined to be 1.5 to 2 red cells in diameter. The chromatin was described as having a fine woven pattern and nuclei were characterized by a large single nucleolus often eccentric to the nuclear center. The cytoplasm was moderate in amount and eccentrically placed, with a lighter stained area adjacent to the nucleus. The latter finding is suggestive of lymphoid origin and the cells were described as having a variable number of fine azurophilic granules. In illustrations, the cytoplasm is smooth and entire and does not resemble the fine stellate projections characteristic of hairy cell leukemia in humans. Significant pathological findings were limited to the bone marrow, spleen, liver, and lymph nodes, with the spleen dry on cut surface, dark red and with a fine granular appearance that was felt to be indistinct enlargement of splenic follicles. The bone marrow was hypercellular and primarily occupied by invading cells which were not considered clearly neoplastic. Involvement of the spleen was primarily in sinus areas with irregular dilation of sinusoids and relative follicular atrophy. A characteristic feature of hepatic involvement was irregular dilation of hepatic sinusoids which were filled with the invading "reticulum cells." Invading cells also were found in the sinusoids of lymph nodes, and in some cases, were found to have effaced normal nodal architecture.

It is unlikely that these authors were describing a disease comparable to hairy cell leukemia but, with the mention of fine azurophilic granules in the tumor cells, *it is possible that the description is comparable to chronic leukemia of large granular lymphocyte type, or the much less common* $\gamma\delta$ *hepatosplenic T-cell lymphoma of humans and animals.*

A description of hairy cell leukemia as it occurs in humans is presented here in the event that this is a lesion present but as yet undefined in animals. The disease in humans is of young to mature individuals with a 4:1 male predominance. The presenting signs include fatigue and infectious phenomena, with the latter of importance because of the concurrence of neutropenia and immunosuppression. Splenomegaly is almost always present, with hepatomegaly in 40% of cases. Peripheral lymphadenopathy is much less common. Pancytopenia is present in more than one-half of the patients at the time of presentation, along with anemia and thrombocytopenia, often accompanied by bleeding. HCL in humans is almost always accompanied by *monocytopenia* which is considered a useful diagnostic finding. While most patients are leukopenic, 10–20% present with a leukemic picture generally in excess of 10×10^9 lymphocytes/L, of which more than half are hairy cells. The hairy cells may be identified in routinely stained blood films but are most dramatically identified in *phase contrast preparations* of wet mounts of living cells. *In humans, the hairy cells in blood have nuclei slightly larger than red cells with a moderate amount of eccentrically placed cytoplasm that has a villous or shaggy cytoplasmic border.* Significantly, cytoplasmic granules are reported to be occasionally present. Bone marrow is considered essential to the diagnosis and, because the disease is associated with reticulin sclerosis of marrow, a core biopsy is usually required. Most patients have hypercellular marrow with phthisis of normal elements. In sections, the hairy cells appear to have abundant cytoplasm similar to marginal zone cells that cause regular separation of nuclei and a lighter appearance of areas with solid tumor infiltration in histological preparations. The nuclei are described as oval, indented, or bilobed and nucleoli are usually inconspicuous or absent as are mitoses. The cytochemical reaction of hairy cell leukemia is the presence of a *positive cytoplasmic reaction to the tartrate-resistant acid phosphatase (TRAP) test.* Characteristic immunophenotypic features of human hairy cells include features of a middle- to late-stage B cell with little somatic mutation of rearranged immunoglobulin genes, suggesting that the cells have not undergone antigenic selection. Human HCL cells react positively with CD45, the common leukocyte antigen, as well as pan-B-cell antigens including CD19, CD20, CD22, and CD79α. The cells do not express the immunoglobulin-associated membrane antigens CD79b, CD10, and CD21.

The characteristic pathology of HCL is present in the spleen where there is extensive involvement of the red pulp with white pulp atrophy. Histologically, splenic involvement in HCL consists of sheets of cells with bland-appearing nuclei and abundant cytoplasm. In the liver, there is portal and sinusoidal infiltration by the hairy cells that may be associated with reticulin sclerosis. In liver, there is irregular dilation of sinusoids that are lined with the tumor cells, and sinusoidal involvement does not correlate with the level of circulating hairy cells. These irregular dilations of the sinuses mimic peliosis hepatis and are believed to arise because of tumor cell adherence to and subsequent injury to sinusoidal membranes.

Most human cases terminate in septicemia and pneumonia, often due to gram-negative organisms.

The normal counterpart of the hairy cell is unknown, but has been suggested to be the *marginal zone cells surrounding lymphoid follicles* to which they are morphologically similar and like hairy cells are positive for FMC-7 but unlike hairy cells are negative for CD11c. Other considerations are the so-called *monocytoid B cells* found in the sinuses of lymph nodes which, like hairy cells, are CD11c positive but unlike hairy cells do not express CD25. *Marginal zone cells and monocytoid B cells constitute the differential diagnostic considerations in the diagnosis of hairy cell leukemia in histologic sections. In blood, the thin stellate cytoplasmic projections are the major distinguishing feature of HCL.*

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Plasma cell myeloma/plasmacytoma

Solitary plasmacytic tumors are largely diseases of the dog, and are rarely seen in the cat. In both species, they appear to be diseases of mature animals with most cases occurring in *animals over eight years of age. Plasmacytomas are considered monoclonal proliferations* in distinction to benign plasma cell hyperplasia, which may be characterized by gammopathy but of the polyclonal type. Benign plasma cell hyperplasias occur in diseases of chronic antigenemia and are seen in various tissues in equine infectious anemia and bovine trypanosomiasis. It is likely that the most marked example of benign plasma cell hyperplasia may occur in canine leishmaniasis, in which as much of 50% of bone marrow cells may be benign polyclonal plasma cells. Thus, *grading systems based on numbers and percentages of plasma cells are prone to error, and the distinction between monoclonal and polyclonal plasma cell proliferations is best based on a combination of cellular atypia and serum electrophoresis.*

Clonal plasmacytic tumors originate in soft tissues or bone and are referred to as **extramedullary plasmacytoma** *in soft tissues* and **solitary osseous plasmacytoma** *if occurring in bone.* The distinction is important, because *the osseous lesion may be expected to progress to a systemic multiple myeloma, whereas extramedullary plasmacytomas, particularly those of skin and the oral cavity, tend to be benign lesions that do not progress after simple excision.* In contrast, plasmacytomas of the esophagus, stomach, intestine and rectal area may be aggressive lesions that may become large locally and metastasize to regional nodes. Less commonly, plasmacytoma may occur in the spleen of the dog and the cat, with those in the dog tending to be focal or multifocal lesions that must be differentiated from nodular hyperplasia or lymphoma, while those in the cat may diffusely involve the sinus areas in a spleen of normal size and without surface irregularities (Fig. 2.37A).

The cytologic and histologic differentiation of benign and aggressive plasmacytoma and myeloma is not a clear-cut decision. In general, *plasmacytoma is characterized by a monotonous proliferation of cells that resemble plasma cells in any other location or site of aggregation* (Fig. 2.37B). Nuclei are round or oval, usually less than 1.5 red cells in diameter, and have densely stained chromatin with prominent chromocenters and lack nucleoli. Cytoplasm is usually abundant, eccentric and with an irregularly prominent Golgi zone. Binucleation of plasma cells occurs in benign plasmacytoma, but is relatively infrequent and mitoses are rare. In contrast, *in aggressive or anaplastic plasmacytoma there*

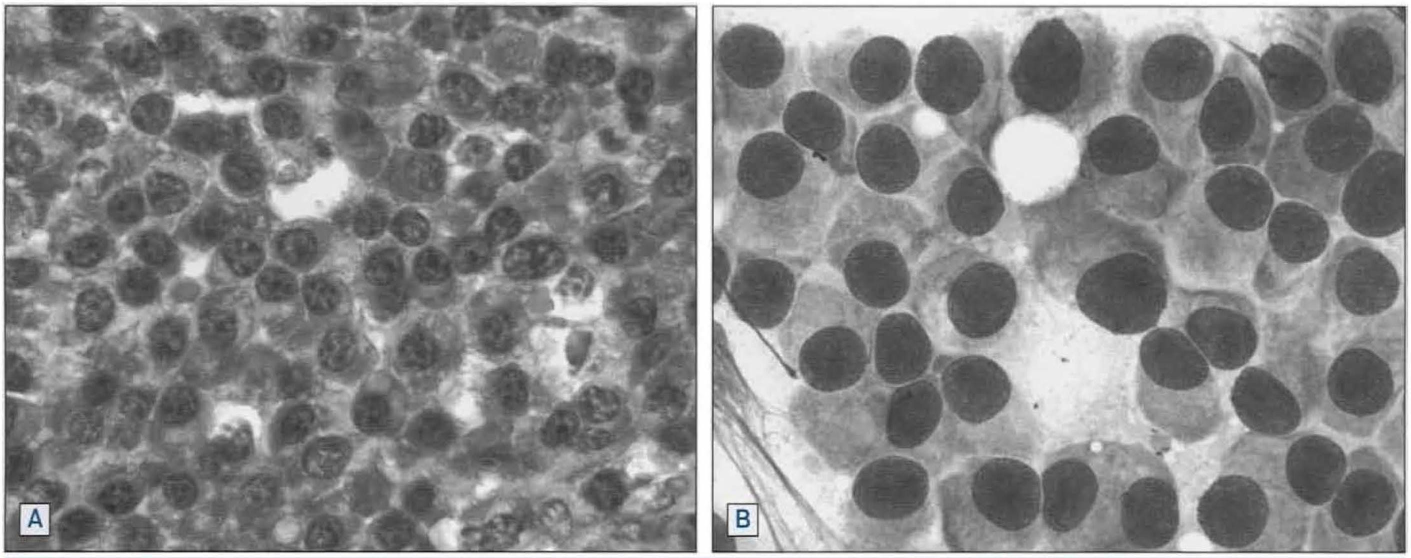

Figure 2.37 Plasmacytoma. A. Solid infiltrations of sinus areas with well-differentiated plasma cells in the spleen of a cat. **B.** Aspirate from subcutaneous mass near the elbow of a dog. Nuclei are round, regular with densely stained chromatin with cribriform pattern and small nucleoli. Cytoplasm is abundant, highly basophilic, and eccentrically placed. (Courtesy of RM Jacobs.)

is quite marked anisokaryosis, which in some cases, may be striking with scattered nuclei 4–5 red cells in diameter. Both aggressive plasmacytomas and myelomas differ from most other hematopoietic neoplasms and most carcinomas in that frankly malignant cells may maintain a lower than normal nuclear to cytoplasmic ratio than their benign counterparts. Thus, in aggressive plasmacytomas, cells with very large nuclei may have abundant cytoplasm. Besides marked anisokaryosis that may be expected to be present in aggressive plasmacytomas, the nuclei are hyperchromatic and characteristically have one or more prominent nucleoli. Binucleation is a frequent occurrence, and trinucleation or even multi-lobated nuclei may be present. In contrast to benign plasmacytoma, mitoses are of relatively frequent occurrence. In the dog, both the benign-appearing plasmacytomas of skin and the anaplastic-appearing lesions of the GI tract tend to be negative with CD79α, whereas plasma cell hyperplasias are strongly positive, a useful diagnostic distinction. All three lesions are strongly positive with vimentin.

Multiple myeloma is a disease of *mature and aged dogs* and is rare in the cat and other domestic species. *Myeloma is characterized by the production of monoclonal immunoglobulin, usually of the IgM type.* The immunoglobulin produced can represent a range of class of immunoglobulin or fragments of the molecule including both light and heavy chains of which the light chain is small enough to be passed through the glomerulus and represents a particular risk for renal failure (*myeloma kidney*). Small-cell lymphomas may also produce macroglobulinemia, known as Waldensdrom's macroglobulinemia in human pathology as noted in small-B-cell lymphomas. Thus, a variety of disorders may occur including dysproteinemia, paraproteinemia, and monoclonal gammopathy. *Plasma cell diseases have a risk of being followed by amyloidosis,* with deposition of a fibrillary protein that accumulates systemically and may cause fatal injury to the heart, liver and kidneys. Amyloidosis may be designated as *primary* when accompanying monoclonal plasma cell proliferation, and *secondary* when accompanying a chronic inflammatory or infectious process. Since monoclonal gammopathy may be present in the absence of localizing signs or focal lytic lesions of bone, the significance of monoclonal gammopathy may not always be clear. Human classifications of these conditions are given a working diagnosis of *monoclonal gammopathy of unknown significance* (MGUS).

The cause of myeloma is unknown. There is increased risk of developing this disease in humans with a background in cosmetology, farming, or employment in the petroleum, wood, leather or asbestos industries. This increased risk is related to exposure to agents such as *pesticides, petroleum products, asbestos, rubber, wood products, plastic, and high-dose gamma irradiation.* An association between myeloma and infection of *Human herpesvirus 8* has been suggested, with the pathogenesis being chronic infection of the marrow dendritic antigen-presenting cells which then over-produce IL-6 that drives plasma cell proliferation. *Long-standing or chronic infection with persistent antigenic stimulation has been considered a predisposing cause of myeloma in humans.* Most myeloma proteins are without specificity for a known antigen. Cases have been documented in which the M component in a canary breeder was related to canary droppings and to horse α-macroglobulin 30 years after passive therapy with equine anti-tetanus serum.

Dogs with myeloma are often presented with lameness, leading to detection of myeloma through aspiration of focal lytic lesions of bone identified radiographically (Fig. 2.38A). Hyperproteinemia in dogs may reach high levels of 80–140 g/L associated with high blood viscosity. These animals may also have a markedly enlarged spleen with focal areas of infarction and sepsis that makes these animals candidates for circulatory shock on even mild anesthesia for purposes of obtaining biopsy specimens. *Hyperviscosity syndrome* may be associated with bleeding tendencies due to inhibition of the large molecular weight clotting proteins by the M component produced by the myeloma cells.

The **diagnosis** *of myeloma is based on the identification of single or multiple foci of malignant cells associated with the production of the gammopathy* (Fig. 2.38B). In contrast to the aggressive plasmacytomas, *myelomas are usually characterized by homogeneity of cell type with the nuclei on histological examination characteristically 1.5 to 2 red cells in diameter.* The chromatin is densely stained with large chromatin aggregations and

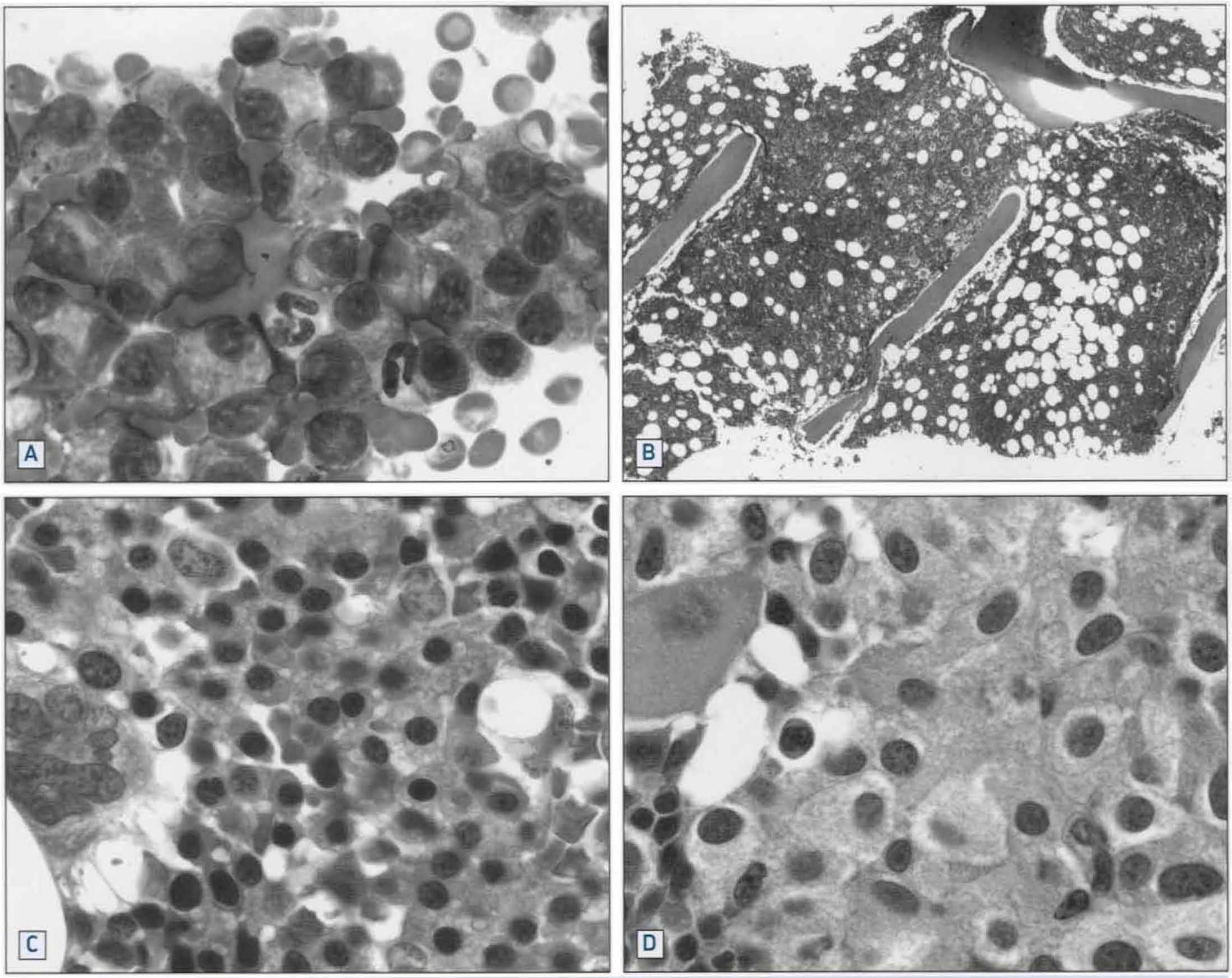

Figure 2.38 **Multiple myeloma**, bone marrow, dog. **A.** Aspirate. Nuclei are 1 to 1.5 red cells in diameter with mild anisokaryosis, densely stained nuclei with small inapparent nucleoli and abundant highly basophilic cytoplasm with prominent Golgi area of cytoplasmic clearing. (Courtesy of RM Jacobs.) **B.** The more dense areas with elimination of fat cells represent solid areas of interstitial and paratrabecular tumor infiltration. **C.** Residual megakaryocyte and densely stained myeloma cells with moderate amounts of densely stained cytoplasm. **D.** Different case illustrating type of tumor with much more abundant cytoplasmic volume. Note absence of mitoses in all illustrations.

irregular parachromatin clearing, with most cells having 1–3 relatively small nucleoli. The cytoplasm is usually abundant and stains uniformly pink with H&E (Fig. 2.38C, D); in cytologic preparations the cytoplasmic basophilia varies in intensity and in distribution (Fig 2.38A). Morphologically, foci of myeloma cells tend to grow in solid sheets displacing all normal bone marrow cells and causing lysis of bone that may be associated with *hypercalcemia*. Multiple foci are usually present. The most common sites for myeloma lesions are those areas that remain active in hematopoiesis in the adult animal, including spinal column, ribs, pelvis, calvaria and proximal long bones. Myeloma cells usually stain with strong cytoplasmic positivity for CD79α, and *the main differential is from benign plasmacytosis which usually has less cytoplasmic volume and absent nucleoli*. The mitotic rate of most myelomas is lower than that of normal bone marrow, and while mitoses may be found, they are not frequent. The low proliferative rate of myelomas needs to be considered in planning therapeutic management.

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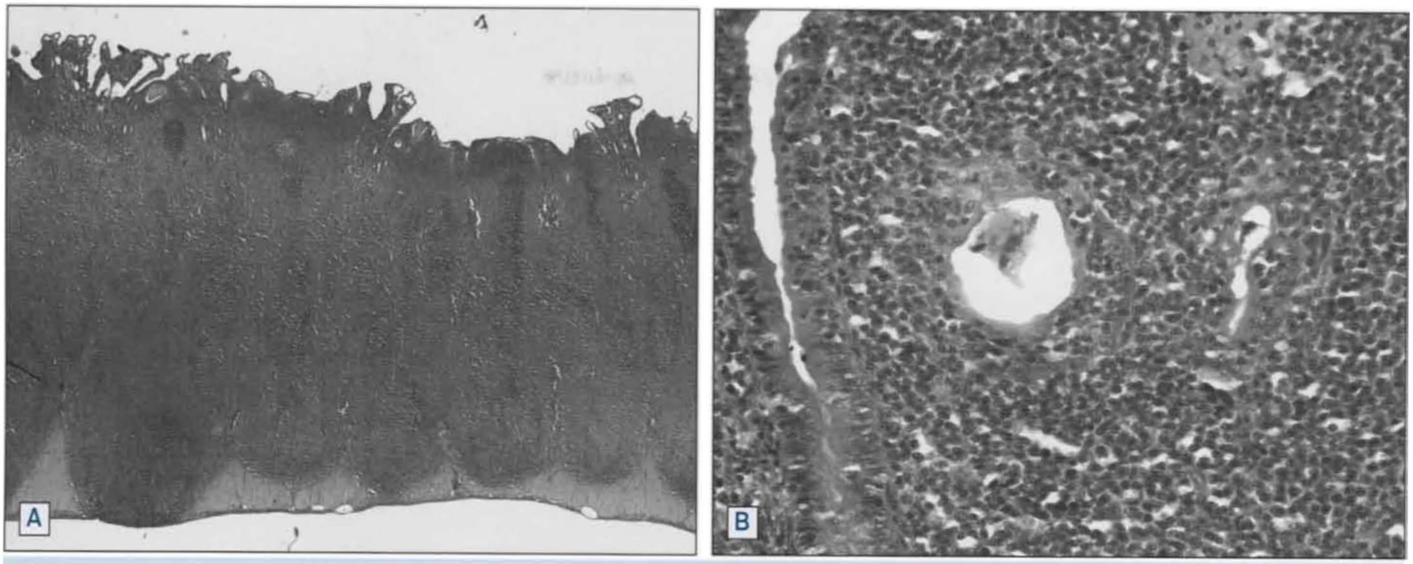

Figure 2.39 Extranodal B-cell lymphoma of MALT type, small intestine, cat. **A.** There is extensive filling and clubbing of villi, destruction of the muscularis mucosa, and marked and diffuse infiltration of the lamina propria. Dark areas of cellular accumulation represent fading areas of germinal center formation. **B.** Lympho-epithelial lesion characteristic of MALT lymphoma with extensive lymphocytic infiltration of two glands (center) and early invasion of glandular duct (left).

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Extranodal marginal zone B-cell lymphoma of MALT type

Extranodal lymphomas are common neoplasms in both humans and animals. Lymphomas of extranodal tissue may be of B- or T-cell type and vary in cell size, proliferative rates and aggressiveness. *Extranodal lymphomas of the mucosal-associated lymphoid tissue (MALT) type are a specific type of extranodal tumor characterized by B-cell phenotype, unique cytomorphology, a unique histological pattern of glandular invasion known as the "lympho-epithelial lesion," and indolent progression as well as being relatively curable by surgery.* Lymphomas involving mucosal surfaces likely occur in all species of animals but are most frequently recognized in *mature cats and dogs*. In the dog, the tissues most commonly involved are the lining of the stomach and the colon. In cats, mucosa in the area of ileocecal junction is a recurring site of MALT-type lymphoma. Besides these sites, virtually every mucosal surface, including salivary glands, oral cavity, thyroid gland, lung, mammary gland and reproductive tissues including uterus, ovary, testes and prostate, may be involved. In many instances, lymphomas in these regions follow prolonged benign lymphoid hyperplasias.

In humans, autoimmune disease of the salivary glands resulting in Sjogren's disease and in the thyroid gland Hashimoto's thyroiditis are examples of autoimmune disease of prolonged duration that may result in MALT lymphoma. Most MALT lymphomas are characterized by a bi-phenotypic or what may appear to be a mixed type of lymphoid reaction as a result of the residuum of benign polymorphous follicular hyperplasia de-differentiating into a focal area of apparent marginal zone-type cell proliferation. The morphology of the marginal zone cells, described under splenic marginal zone B-cell lymphoma, have the same morphology wherever they are prone to occur. In human pathology, the extranodal proliferation of marginal zone cells has been referred to "*monocytoid B-cell lymphoma*." There appear to be some differences between the marginal zone cells of spleen and node and those of mucosal surfaces involved in MALT lymphomas. It is certain that the lymphomas of mucosal surfaces have more heterogeneity of cell type than is observed in the enlarging marginal zone areas around germinal centers in the spleen and lymph node. Cytologically, as previously described, the nuclei of marginal zone lymphomas of nodal sites tend to have round-to-oval nuclei and may have prominent nucleoli. In contrast, monocytoid cells of mucosal surfaces have smaller nuclei, which are frequently indented or folded, and deeply stained chromatin with little internal nuclear detail. The monocytoid cells lack nucleoli and, like the marginal zone B-cell lymphomas, are characterized by a periphery of relatively abundant clear cytoplasm. Non-MALT lymphomas of mucosal surfaces tend to surround the deep glandular structures and, while glands may be isolated by proliferating cells, the neoplastic lymphocytes tend not to bridge the epithelial basement membrane. In contrast, *in MALT lymphomas, the lympho-epithelial lesion is characterized by heavy invasion of neoplastic lymphocytes into the epithelial layer with glandular destruction* (Fig. 2.39A, B).

In animals, as in humans, *it appears that the neoplastic cells in MALT lymphoma are rather tightly "homed" to the site of lymphoma.* Thus, while there may be multifocal sites of neoplastic transformation, particularly in lymphomas of the intestinal tract, the neoplastic cells tend to proliferate in situ and may become large and bulky in situ without spreading beyond the adjacent mesenteric lymph nodes. *This homing characteristic is extremely important to surgical management* since, if the lesion can be identified as a MALT-type lymphoma, there is logic in treating the condition by surgical removal of the affected tissue with an expectation of prolonged postoperative survival. Remarkably, this may occur even with the tumor persisting at the margins of a resected mass at the ileocecal junction in cats.

MALT lymphomas must be distinguished from other enteric lymphomas of large B-cell type, including T-cell-rich B-cell lymphomas and enteric-associated T-cell lymphoma (EATL), which is characterized by heavy infiltration of the surface epithelium with small mature T cells. Phenotypically, the majority of cells in MALT lymphomas will mark with CD79α and, in particular, those cells involved in the lympho-epithelial lesion.

An unusual lesion of mucosal surfaces seen in humans is the *NK/T-cell lymphomas of the sinonasal region*. The disease is common in the East, particularly in China, where many cases are associated with Epstein-Barr viral infection. The growth pattern of the tumor is frequently angiocentric, particularly in T-cell tumors of the γδ type which also occur in this region. Neoplasms with the angiocentric growth pattern are prone to have multifocal areas of ischemic necrosis. A neoplasm with similar growth type and immunophenotype has not been identified in animals.

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Nodal marginal zone B-cell lymphoma (+/– monocytoid B cells)

Marginal zone lymphoma (MZL) of lymph nodes is a slowly progressive proliferation of B cells with abundant relatively clear cytoplasm that accumulate in broad and uniform cuffs of cells outside the mantle cell layer of germinal centers. Marginal zone lymphoma is a relatively recently characterized entity in human lymphoma and has not yet been well described in animals. Marginal cell lymphoma in animals *is seen most frequently in mature dogs and cats* and likely occurs in other species. The benign counterpart of cells of marginal zone lymphoma were first described as immature sinus histiocytes which led to them being termed "monocytoid cells" and subsequently with phenotyping as "monocytoid B lymphocytes." In benign states in humans and animals, these cells can be found beneath the subcapsular sinus of nodes and lining the paratrabecular sinuses and occasionally in medullary sinuses in inflammatory states. They may proliferate in certain inflammatory processes in which the nodal architecture is relatively preserved with intact follicles and paracortical T-cell areas. Monocytoid B cells are found in relatively tight bands and clusters in human toxoplasmic lymphadenitis and in AIDS-associated lymphadenopathy. They are also prominent in lymph nodes in patients with infectious mononucleosis, cytomegaloviral lymphadenitis, as well as in nonspecific lymphoid hyperplasias and some granulomatous and purulent lymphadenopathies. Specific infectious disease association has not been defined in animals with a relatively pure proliferation of benign monocytoid B lymphocytes.

The morphology of nodal marginal zone lymphoma is similar to that described for splenic marginal zone lymphoma.

Phenotypically, the cells of human marginal zone lymphoma express B-cell markers with IgM more frequently than IgG. They are negative for IgD, CD5, CD23, CD10, and cyclin D-1, and they are positive for most B-cell markers. *Nodal marginal zone lymphoma is both an architectural and cytologic diagnosis with unique features on low power examination, with proliferation of cells in a continuous band surrounding the mantle cell layer of germinal centers.* Close association of marginal zone cells with reactive follicular hyperplasias suggests that they represent a stage of B-cell differentiation related to follicular proliferation. The follicular marginal zone is that area immediately external to the outside of the mantle cell corona of germinal centers. This area tends to be well developed and relatively easily identified in reactive B-cell areas whether spleen, Peyer's patches or lymph nodes. *In animals, the nodes most often reactive in benign states include those of the submandibular area and mesenteric sites as well as the popliteal lymph nodes of dogs.* In rats and mice, the splenic marginal zone is clearly delineated from the mantle cell corona by a marginal sinus. Within other species, the transition between mantle and marginal zone layers is less well defined.

In histologic section, the cells of *early* marginal cell lymphoma are small cells that resemble centrocytes, having round or oval nuclei or occasionally with shallow indentations and approximately 1.5 red cells in diameter. The chromatin pattern is like that of intermediate lymphomas of small cell type, with large chromocenters and irregular parachromatin clearing, and nucleoli are usually small or absent (Fig. 2.40A–E). Cells have abundant relatively water-clear cytoplasm with indistinct cellular boundaries. This volume of cytoplasm results in relatively even separation of the nuclei and a readily recognizable architectural region on low-power examination due to the lighter staining of the marginal zone cells. Marginal zone lymphomas in animals mark

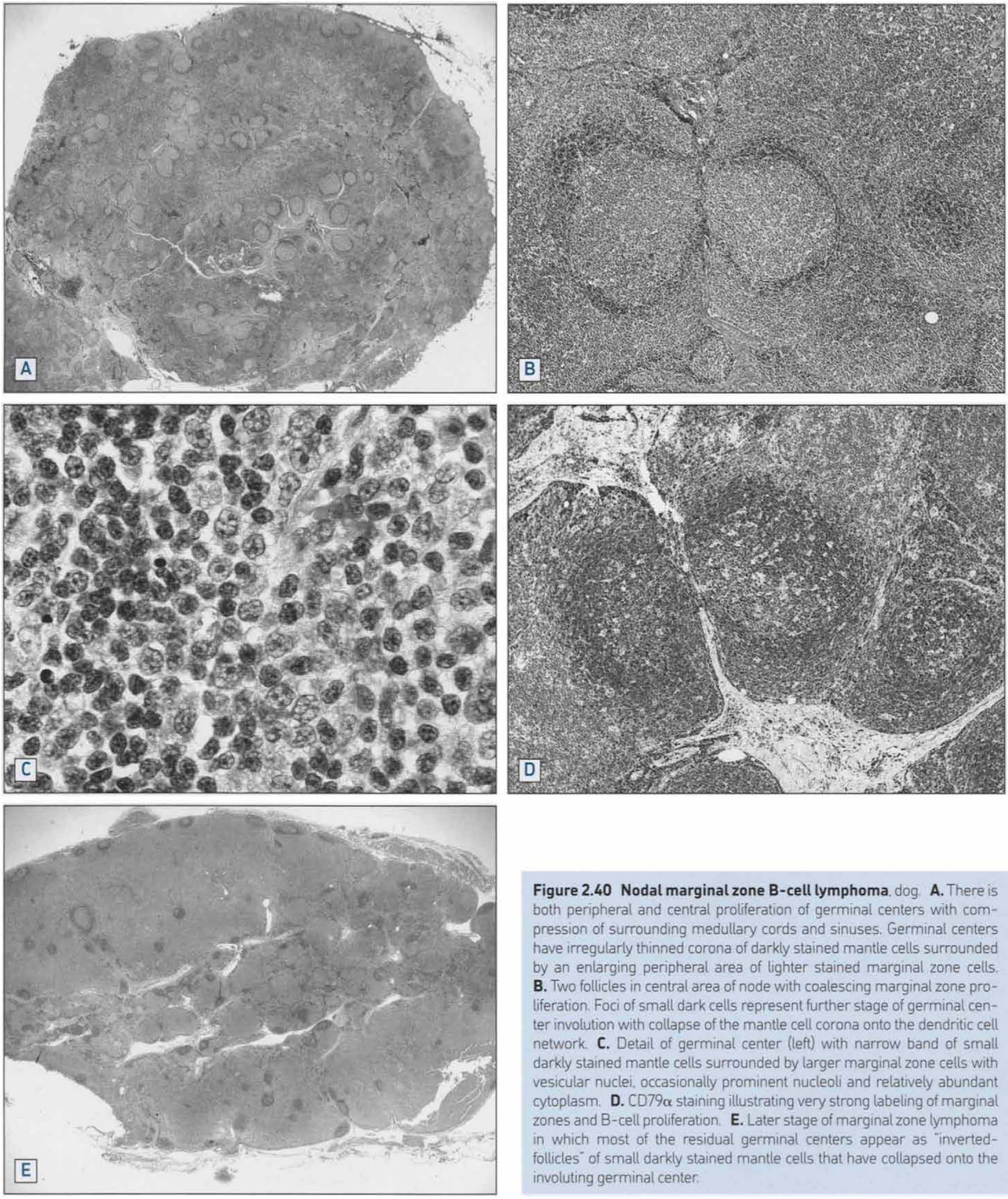

Figure 2.40 Nodal marginal zone B-cell lymphoma, dog. **A.** There is both peripheral and central proliferation of germinal centers with compression of surrounding medullary cords and sinuses. Germinal centers have irregularly thinned corona of darkly stained mantle cells surrounded by an enlarging peripheral area of lighter stained marginal zone cells. **B.** Two follicles in central area of node with coalescing marginal zone proliferation. Foci of small dark cells represent further stage of germinal center involution with collapse of the mantle cell corona onto the dendritic cell network. **C.** Detail of germinal center (left) with narrow band of small darkly stained mantle cells surrounded by larger marginal zone cells with vesicular nuclei, occasionally prominent nucleoli and relatively abundant cytoplasm. **D.** CD79α staining illustrating very strong labeling of marginal zones and B-cell proliferation. **E.** Later stage of marginal zone lymphoma in which most of the residual germinal centers appear as "inverted-follicles" of small darkly stained mantle cells that have collapsed onto the involuting germinal center.

strongly with CD79α and paradoxically, as the tumors become more aggressive, the cytoplasmic marking of the larger cell complement is more intense (Fig. 2.40D). *The differentiation between marginal zone hyperplasia and marginal zone lymphoma is not clear-cut.* In general, marginal zone hyperplasia is not associated with involution of the germinal centers nor diminution of cells of the mantle cell layer. Thus, marginal zone hyperplasia is generally seen with a florid follicular hyperplasia that may extend into the medullary areas of the node. At the level of the individual follicle in mantle cell hyperplasia, the germinal center is usually characterized by numerous tingible body macrophages, and the mantle cell layer is entire and of relatively uniform depth. Characteristically, the hyperplastic marginal zone layer will be 2–3 times the depth of the mantle cell layer, with irregularly sharp and intermingled definition with the surrounding paracortical areas. The postcapillary venules are always excluded from the marginal zone hyperplasias and marginal zone lymphoma. With progression, the marginal zones increase in depth and the mantle cell layers become thinned and discontinuous, whereas the germinal centers themselves show various stages of involution (Fig. 2.40B). With marginal zone lymphoma, the proliferating layers tend to become continuous between adjacent germinal centers and there is progressive involution of germinal centers in which the mantle cells have aggregated into the center of the follicle and appear as a cluster of small dark benign cells surrounded by the enlarging marginal zone lymphoma. This type of change is referred to as an "inverted follicle" (Fig. 2.40B, D). On the basis of very few cases, it appears to take about a year from the stage at which marginal zone lymphoma is apparent until there is a diffuse architecture with effacement of normal node structures and germinal centers are reduced to small clusters of residual mantle cells. Since most cases of nodal marginal zone lymphoma arise in a background of chronic follicular hyperplasia, *the transition from marginal zone hyperplasia to lymphoma is likely a year or more prior to evident neoplasia.* With further progression, there is increased presence of tingible body macrophages throughout the diffuse tumor area along with a shift in nuclear character to the vesicular nucleus that resembles small immunoblastic cells with peripheralized chromatin and a prominent single central nucleolus.

In dogs, marginal zone hyperplasia is characterized by a mixture of mantle cells and marginal zone cells of intermediate size with prominent central nucleoli. In early marginal zone lymphoma, marginal zone cells predominate and become more apparent due to peripheralized chromatin that makes the nucleoli appear more prominent. But in all cases, the mitotic rate of these cells is very low and, in early stages of the disease, marginal zone cells remain tightly homed to the periphery of the mantle cell cuffs. In marginal zone lymphoma in the dog, there is usually thickening of the capsule and the paratrabecular collagen bands, which suggests a prolonged period of reactive hyperplasia prior to the development of marginal zone lymphoma. The peripheral capsule remains intact until late in the involvement of the node when there is nearly complete effacement of normal node architecture, at which time there may be focal colonization of perinodal structures.

Marginal zone lymphoma needs to be differentiated from mantle cell lymphoma and follicular lymphoma.

- Marginal zone hyperplasia and early marginal zone lymphoma are characterized by germinal centers with 3 distinct cell layers including the germinal center, the mantle cell cuff, and the marginal zone layer.
- *Mantle cell lymphoma only has 2 cell layers,* with the germinal center and the mantle cell cuff, which is easily distinguishable from the surrounding heterogeneous cellular population of the paracortex.
- Mantle cell hyperplasia and lymphoma are characterized by proliferation of the mantle cell layer with gradual development of complete and diffuse encirclement of fading germinal centers.
- *Follicular lymphomas are characterized by closely facetted nodular proliferations surrounded by discontinuous residual mantle cell layers.*
- Marginal cell lymphoma in the dog may be confused with a type of *T-zone lymphoma* in which there is an architectural picture very similar to marginal zone lymphoma, but in which the perifollicular cells remain characteristically of small-cell type and mark strongly for CD3 and are CD79α negative.

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Follicular lymphoma

Follicular lymphoma (FL) is very predominantly a neoplasm of lymph nodes. Extranodal lymphoid tissue including the spleen and oropharynx (Waldeyer's ring) may be involved as well as bone marrow and liver and, less commonly, nonlymphoid organs such as skin, the ocular adnexa, gastrointestinal tract and female genital tract, the latter particularly in the cow. *Follicular lymphoma is defined as a lymphoma of follicular center B cells (centrocytes and centroblasts) that has at least a partially follicular pattern; FL is a neoplasm of germinal center B cells in which the centrocytes fail to undergo apoptosis.* This failure is due to a chromosomal rearrangement which in humans is shown to be t(14;18) that involves the anti-apoptosis gene *BCL2* that prevents the normal inactivation of this gene.

In order to understand the development and evolution of follicular lymphoma, it is useful to *review the temporal and cellular events in the formation of benign follicles.* Germinal centers tend to form several days after initial immunological stimulation of managerial T cells in the lymph node outer cortex. Typically, the node has a florid diffuse paracortical hyperplasia for a week following antigenic stimulation until follicular centers are formed. This fact has led to confusion in the biopsy of enlarged axillary nodes of children without knowledge of recent vaccination. The *timing of germinal center formation following vaccination* has been studied serially in nonhuman primates following immunization and in dogs given vaccinations into the foot pad with serial biopsy of the popliteal nodes. Similar timing is found in both dogs and primates, with *diffuse hyperplasia for at least a week followed by the development of follicular hyperplasia*. The *secondary response to antigen is much faster* and may result in new follicle formation in about 3–4 days or about one-half the time required for a primary immune response. Each new germinal center is believed to be formed by as few as 3–10 naive B cells to ultimately contain 10 000–15 000 B cells within the ten generations required to form a visible germinal center.

Initial reaction takes place between naive B cells on encountering T-cell-directed antigen in the paracortex, which then proliferate as IgM+ B blast cells. These blast cells migrate to the center of a primary follicle and fill the follicular dendritic cell network 3–7 days after antigen stimulation, forming an enlarging germinal center. The initial proliferation differentiates into Ig-negative large noncleaved follicular center cells, which appear as early as 4 days and accumulate at the dark or deep zone of the germinal center. These centroblastic large cells with vesicular nuclei have 1–3 prominent nucleoli that typically are peripheral and impinge on the nuclear membrane, a feature that is retained in follicular lymphomas. At this stage, the **centroblasts** have only a narrow rim of basophilic cytoplasm. These cells lack surface immunoglobulin and switch off the gene that encodes the BCL2, which permits these cells and their progeny to enter programmed cell death through apoptosis. At this time, the centroblasts express activation-associated antigens involved with recognition and interaction with T cells, including CD23, CD71, CD40 and CD86 as well as CD11a/18 and CD29, which permit their continued adhesion to the follicular dendritic cells. An early event in germinal center formation is the expression of the BCL6 protein with a nuclear zinc-fingered transcription factor essential for germinal center formation, and expressed by centroblasts and their progeny the centrocytes but not by naive or memory B cells, mantle cells or plasma cells. Normal inactive B lymphocytes express high levels of BCL6 messenger RNA but do not transcribe the protein. Subsequently, the mutations of the immunoglobulin and *BCL6* genes serve as markers of cells that have passed through the germinal center. At this early stage of germinal center development, the centroblasts begin a process of somatic mutation of the immunoglobulin gene variable regions, which permits the selection of cells with increasingly avid antibody for the inciting antigen. At the stage of variable chain mutation, the enzyme terminal deoxynucleotidyl transferase (TdT) adds a variable number of nucleotides at the heavy chain joining region thus adding a further area of diversity in the final antigen receptor gene. Examination of individual centroblasts picked from the dark zone of developing germinal centers suggests that there are initially many clones (5–10) of centroblasts with mutation of the variable region of the immunoglobulin gene, which subsequently diminishes to as few as 3 with progressive selection of cells producing the best antibody "fit" and antigen binding.

These early centroblasts and their progeny mature to nondividing smaller cells with irregular nuclei and inconspicuous nucleoli with scant cytoplasm then called **centrocytes**. Morphologically, these are first large and then small-cleaved follicular center cells that then accumulate at the opposite pole or light zone of the germinal center, which is typically oriented toward the subcapsular sinus of lymph nodes or towards the epithelium and source of antigen in Peyer's patches and tonsilar tissue. This peripheral and outer area of the germinal center also contains the highest concentration of follicular dendritic cells. The centrocytes now re-express surface immunoglobulin which has the same variable-diversity-joining (VDJ) gene rearrangement as the parent naive B cell and the centroblasts of the dark zone, but now has an altered antibody-combining site because of the somatic mutations in the immunoglobulin variable region, and in addition the cells may have undergone heavy-chain class switching. This process of somatic mutation uses a high level of intraclonal diversity of antibody-combining sites in a large population of cells derived from only a few precursors. Those centrocytes whose immunoglobulin gene mutations have resulted in decreased rather than increased affinity for antigen are rapidly eliminated by apoptosis. They are evident in the prominent tingible body macrophages of germinal centers. Those centrocytes with the most avid antibody binding are then able to present antigen to T cells in the light zone of the germinal center, and this interaction is thought to activate the T cells to express the CD40 ligand permitting them to bind to the CD40 antigen on the surface of the B cells. This ligation of the B-cell antigen receptor by antigen plus ligation of the CD40 receptor to T cells constitutes a functional "rescue" from death by apoptosis of B cells producing the most efficient antibody. Further interaction between the centrocytes and CD23 expressed on the follicular dendritic cells induces heavy-chain class selection and directs centrocyte differentiation into plasma cells. Alternatively, T cells in the germinal center light zone bearing the ligand for CD40 on the B cells are active in inducing maturation to memory B cells. The combined antigen-receptor ligation and CD40/T-cell ligation switch off BCL6 messenger RNA production and BCL6 protein expression in the B-cell progeny.

With development of polarity in the germinal center into a **dark and light zone**, the mantle cell layer becomes better defined and eccentric with a broader proportion throughout the light zone of the germinal center, usually nearest the subcapsular sinus. Those antigen-specific B cells produced in the germinal center migrate from the follicle to the periphery of the mantle cell layer to form the marginal zone cells. Marginal zone cells retain the slight irregularities of nuclei characteristic of the centrocytes but now develop more abundant pale-staining cytoplasm which gives this area the characteristic lighter appearance on low magnification of conditions of marginal zone hyperplasia or lymphoma. *Marginal zone B cells are thought to be memory B cells, and on exposure to the same antigen in a secondary immune response, the splenic marginal zone B cells migrate to the light zone of the germinal center and become immunoglobulin-positive blast cells and subsequently antigen-specific plasma cells*. There is apparently migration of marginal zone B cells in the spleen and Peyer's patches, indicating that they are not clonally related to an adjacent germinal center. In lymph nodes, in contrast to the spleen and Peyer's patches, the monocytoid B cells in reactive nodes do not appear to have mutated immunoglobulin gene variable regions, suggesting that they have not been through the antigen selection process of the germinal center.

In the current WHO Classification of Follicular Lymphomas, *the term has been defined more precisely to apply only to lymphomas of follicular center cells with a follicular pattern and not to lymphomas of other cellular origin with a nodular or follicular growth pattern*. The current understanding is that follicular lymphoma does not derive from reactive follicular hyperplasia but rather from an altered clone of B cells that mimic the activity of their benign counterparts in forming neoplastic follicles by the same manner as well-differentiated adenocarcinoma forming neoplastic glands. Neoplastic lymphoid follicles are composed of centrocytes and centroblasts in various combinations forming germinal centers that are relatively uniform in size, closely packed, lacking a mantle zone layer and are usually evenly distributed throughout the node obliterating medullary structures and extending outside of the capsule. Variations on this appearance include changes in the size, shape and distribution of follicles with the presence or absence of mantle cell cuffs, involvement of the intrafollicular areas, residual areas of diffuse paracortex and the presence of sclerosis. Within most follicular lymphomas of animals and humans, the follicles tend to be relatively uniform in size and shape and are closely facetted but may have markedly irregular shapes. *In most follicular lymphomas, the follicles themselves appear lighter on low magnification examination than the intrafollicular regions because they are composed of larger more pale-staining lymphocytes than the residual small cells of the intrafollicular region* (Fig. 2.41A–D). In most follicular lymphomas, the follicles are closely packed with obliteration of the paracortical T-zone areas. The intrafollicular area will contain the compressed residual high endothelial venules. There is loss of the cellular heterogeneity of the normal T-zone paracortex with the presence of neoplastic centrocytes and centroblasts similar to those cells within the neoplastic follicles. Chronic benign lymphoid hyperplasia appears to be a risk factor for lymphoma, and lymphomas of both follicular and diffuse types frequently are found in lymph nodes with collagenous thickening of the capsule and medullary raphe. In most cases, sclerosis in nodes is limited to broad collagen bands that are an exaggeration of normal node architecture, but occasionally there may be sclerosis of reticulin fibers and even fine collagen bands that encircle small nodules of lymphocytes. Vascular invasion occurs in both diffuse and nodular lymphomas and is most frequently seen in the larger peripheral veins with marked dilation of the vessel wall and gradual filling of subendothelial space with gradual compression and obliteration of the vascular lumen.

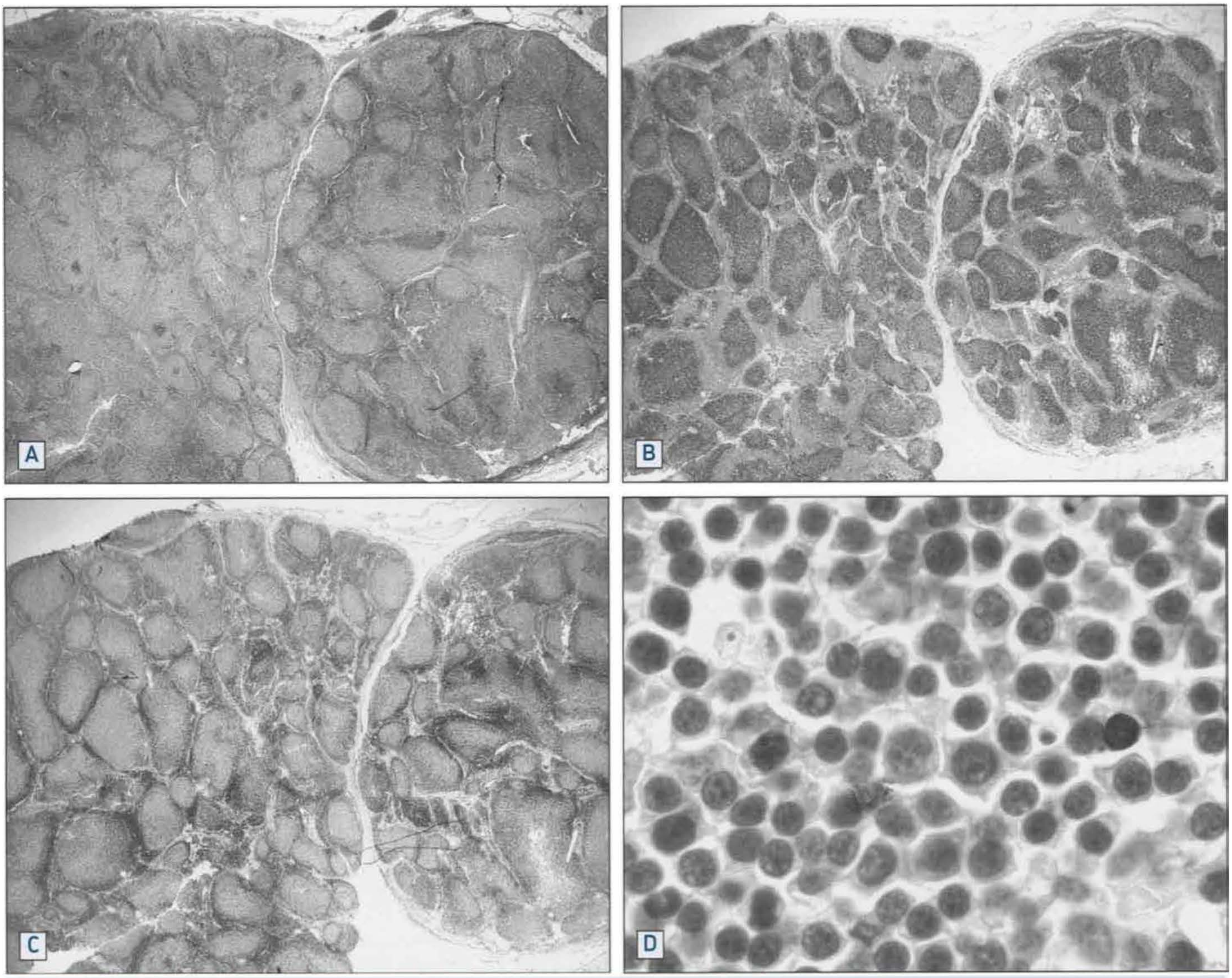

Figure 2.41 Follicular lymphoma, dog. **A.** The peripheral capsule is thin but intact, as is the peripheral sinus. The node is completely occupied by enlarging follicles of various sizes and shapes. Mantle cell coronas are absent from most follicles and of irregular thickness where present. **B.** CD79α stain distinctly marking the proliferative areas as of B-cell type. **C.** CD3 stain for T cells demonstrating marked involution of paracortex. **D.** CD3 stain of follicular center to demonstrate nuclear detail. Centroblasts (center) typically have 1 or more nucleoli impinging on nuclear membrane. Most cells present are centrocyte nuclei of intermediate small lymphocyte type with variation in depth of chromatin staining and absent nucleoli.

In terms of morphology, *the centrocytes and centroblasts of neoplastic follicles are similar or identical to those of benign germinal centers.* Unlike benign centrocytes, those of neoplastic follicles have a greater size variation and may be twice the size of the small-cleaved cells or centrocytes of benign follicles. The nuclei of centrocytes appear irregular or lobulated in histological sections and, while the term "cleaved" has been applied to these cells, the nuclear clefts are largely unseen. Chromatin of the centrocytes is less densely stained than that of benign small lymphocytes and relatively evenly dispersed, and 1 or 2 small nucleoli may be present. Centrocyte cytoplasm is scant, pale, and unobvious in H&E sections. Generally, the neoplastic centrocytes appear more monomorphous than those of normal follicles where there is a migration of cell types corresponding to the light and dark zones of a benign germinal center. The neoplastic centroblasts are much larger than small lymphocytes and may have nuclei 3–4 red cells in diameter. Centroblast nuclei are round or oval and may have shallow irregular indentations or obvious deep clefts. Nuclei are vesicular with peripheralization of chromatin and a clear center, often with some fine chromatin branching and 1–3 relatively prominent nucleoli usually near or impinging on the nuclear membrane (Fig. 2.41D). Centroblasts have a narrow rim of cytoplasm which is relatively inconspicuous on H&E preparations. In comparison to benign germinal centers, the mitotic activity is low within neoplastic nodules and a starry-sky pattern of tingible body macrophages normally seen within benign germinal centers is not present. In general, the number of mitoses in neoplastic follicles increases with the proportion of centroblasts to centrocytes.

In the classification of follicular lymphomas in the Working Formulation, *three types of follicular lymphomas were described based on cell type. These consisted of follicular lymphoma of small-cleaved cell type, follicular mixed small-cleaved and large-cell type, and follicular lymphoma of large-cell type.* The interpretation of survival data at that time was such that follicular small-cleaved and follicular mixed lymphomas were considered low-grade tumors, whereas follicular large cell lymphoma was included in the intermediate-grade neoplasms. Subsequent evaluations have indicated that follicular lymphomas have virtually the same survival pattern when given the same types of therapy. Thus, *follicular lymphomas are currently seen more as a continuum.* Variations in the proportions of centrocytes to centroblasts are their major distinguishing features. Currently, in the REAL and WHO classifications, follicular lymphoma is classed as Grade I if there are 6 or fewer centroblasts per neoplastic follicle, and Grade II if there are 6–15 centroblasts per follicle. Follicles with 15 or more centroblasts are classed as Grade III. These determinations are suggested to be made on the basis of counting centroblasts in 10 to 20 centers at ×400 magnification. Concerns expressed over the size of the field examined, whether using 10 × 18 mm diameter oculars or 10 × 22 mm diameter (wide-field) oculars, appear to require a level of precision in grading which is not borne out by subsequent survival data. *For purposes of diagnoses in animal lymphomas, it appears important to recognize follicular lymphoma as an entity with some indication of the proportions of centroblasts to centrocytes as an overall interpretation.*

Follicular lymphoma in humans express pan-B-cell antigens CD19, CD20, and CD22, as well as surface immunoglobulin with light-chain restriction. Follicular lymphomas in animals regularly mark with CD79α (Fig. 2.41B). CD3-positive T cells generally are restricted to residual cells in the mantle cell cuffs and focal areas of remaining paracortex (Fig. 2.41C). The use of CD79α and CD3 staining may be particularly useful in recognizing follicular lymphoma that is faintly nodular and largely composed of centrocytes or of centroblasts in the process of converting to a more aggressive, diffuse lymphoma. Follicular lymphomas in humans have rearranged immunoglobulin in heavy- and light-chain genes, demonstrable in southern blot analysis or polymerase chain reactions. In addition, like normal germinal center B cells, the follicular lymphomas have somatic mutations in the variable regions of immunoglobulin genes. The principle cytogenetic abnormalities in human follicular lymphoma are translocations involving the long arms of chromosomes 14 and 18, producing t(14;18) (q32;q21) in 75–90% of cases. This translocation between chromosomes 14 and 18 is not associated with a better or worse prognosis than those cases lacking this change. A study of the t(14;18) breakpoint revealed that a portion of DNA which is rearranged in most follicular lymphomas co-migrated with rearranged immunoglobulin genes in southern blots (of DNA). The gene encoded by that segment was given the name ***BCL2***. The protein product of the *BCL2* gene is expressed by resting B and T cells but not by normal germinal center cells, cortical thymocytes, or monocytoid B cells. Over-expression of the *BCL2* apparently provides a survival advantage to B cells by preventing apoptosis. Transgenic mice expressing the *BCL2* gene developed massive follicular lymphoid hyperplasia, with the persistence of a mature B-cell proliferation. Thus, follicular lymphomas are tumors of germinal center B cells in which the maturation from centroblasts to centrocytes fails to terminate in apoptosis because the t(14;18) translocation prevents inactivation of the anti-apoptosis gene *BCL2*.

Follicular lymphomas likely occur in all domestic species, but are most commonly recognized in the cat and cow and less commonly in the dog. Approximately 25–40% of human lymphomas are of follicular type, but fewer than 5% of animal lymphomas are considered follicular, assuming all of them are recognized. It is likely that these diseases are under recognized in animals because their lymphomas tend to be presented at an advanced stage when the architecture has become diffuse with melding of the follicles, and because immunophenotyping is not routinely done that tends to show the outline of the B-cell areas by residual T cells. Follicular lymphomas are indolent tumors that may reach quite large size in a single node in both the cat and dog. In about a third of human cases, follicular lymphoma will convert to a large-cell lymphoma of diffuse type with a more rapid subsequent clinical course that is usually refractory to treatment. Less information is available on the outcome of follicular lymphomas in animals, but it is likely that they are similar in becoming more aggressive with time. In humans, follicular lymphoma is a disease of adults generally older than 50 years. *In dogs, most cases of follicular lymphoma have the Grade III type*, with a high proportion of centroblasts, and the disease occurs in animals as young as 2 years but most occur in animals 10 years of age or older. In cats, the age distribution is much more variable with follicular lymphoma occurring in animals less than 2 years of age and as old as 22 years. Cats also appear to have more variability in grade, with the follicular mixed or Grade II lesion most common. In a large series of cat lymphomas derived from a number of contributing institutions, follicular lymphomas were less than 1% of the population, which suggests that follicular lymphoma was likely selected against, possibly because they were considered hyperplasia.

The main **differential diagnosis** *for follicular lymphoma is benign follicular hyperplasia.* Follicular hyperplasia usually has more variation in follicle size, with retention of the mantle cell cuff around each germinal center, and while the medullary structures may be compressed, there is not obliteration of the medullary cords and sinuses. *A major factor in differentiating follicular lymphoma from follicular hyperplasia is the presence of polarity in germinal centers of benign reactions and its absence in the neoplastic counterpart.* Polarity is best assessed near the peripheral capsule where orientation of the light and dark zones to the source of antigen can be more confidently assessed.

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Mantle cell lymphoma

Mantle cell lymphoma (MCL) is an aggressive small-cell lymphoma of B-cell type. The normal cell counterpart of mantle cell lymphoma appears to be naive B lymphocytes that home to and reside within primary lymphoid follicles and the mantle cell layers of secondary follicles. Cytologically, the cells of mantle cell lymphoma are slightly larger than their benign counterparts of the mantle cell cuff normally found around germinal centers. The nuclei tend to be round with shallow indentations, or more irregular and deeply indented. The nuclei are of "intermediate" type, slighter larger than 1.5 times the diameter of a red cell, with densely stained chromatin, incomplete parachromatin clearing, and inconspicuous or absent nucleoli. They have minimal cytoplasm, which is pale staining in H&E preparations, but of sufficient volume so that nuclei tend to be narrowly separated from each other. The early stage of mantle cell lymphoma is characterized by a reduction in size of the germinal centers, which have reduced cellular density and often display a background of bare follicular dendritic cells. The surrounding mantle cell cuffs are irregularly thickened and advancing in a discontinuous pattern into the surrounding paracortex. With progression of the disease, there is fusion of the proliferative areas between adjacent germinal centers, and on low-power examination, the cortex of the node is filled with small dark-staining cells in which the residual germinal centers appears as much lighter hypocellular areas. Because the tumor cells grow out of the mantle cell layers, there is never a three-layer appearance, as is the case with retention of the mantle cell layer around the germinal center in an expanding marginal zone lesion. Mantle cell lymphomas have been described as having a "blastoid" variant where the enlarging cuffs of cells have more of a vesicular nucleus with the irregular presence of small nucleoli. This variant has a tendency to become leukemic and appears to have a counterpart in animals, particularly dogs.

Mantle cell lymphoma may occur in the spleen, particularly in dogs, as focal or multifocal lesions within an expanding nodule of 1–2 cm in diameter (Fig. 2.42A–E). The mantle cell proliferations appear eccentric to a small arteriole, which suggests they may have arisen around an obsolescent germinal center (Fig. 2.42D). These proliferations appear as small, darkly stained masses on low-power magnification, which are separated from lighter stained areas that are more like the paracortical area of lymph nodes rather than splenic sinuses, and which are completely displaced to the periphery of the enlarging mass (Fig. 2.42A–C). Bone marrow is usually not involved, but involvement of the spleen usually coincides with involvement of the liver.

In animals, the MCL mark with variable intensity with CD79α (Fig. 2.42B) and strongly with BLA-36. They are routinely negative for CD3, but the use of that stain helps to delineate the areas of mantle cell proliferation in both the spleen and node (Fig. 2.42C,D). In humans, mantle cell lymphomas have a monoclonal B-cell phenotype, usually bearing surface IgM, with most cases expressing monoclonal lambda light chain. In early stages of mantle cell lymphoma, the cells within the remaining germinal centers are polyclonal and benign. Neoplastic mantle cells in humans stain with pan-B-cell markers including CD19, CD2, CD22 and CD24 and the histocompatibility antigen HLA-DR. There are negative for CD23, but typically stain for the T-cell antigen CD5 and express bcl-2 and cyclin D1. The characteristic cytogenetic abnormality in human MCL is a reciprocal translocation t(11;14) (q13;q32).

Mantle cell lymphoma is a relatively rare tumor of dogs and is apparently even more unusual in cats. It would be expected to occur in the spectrum of B-cell lymphomas in other animals. In dogs, mantle cell lymphoma appears to occur primarily in animals 10 or more years of age, and may be more common in males than females. In humans, the median age of mantle cell lymphoma is approximately 60 years, with a male predominance. If mantle cell lymphoma is a tumor of maturity onset in animals, then logically it would likely only be recognized in cats, dogs, and horses and possibly some exotic species.

Mantle cell lymphoma must be differentiated from other small cell lymphomas of similar cell type. The **diagnosis** of mantle cell lymphoma is *very predominantly on pattern recognition* on low-power examination of a lesion in the early stages of development characterized by irregular thickening of the mantle cell layers around involuting germinal centers. Mantle cell hyperplasia does not appear to occur as a component of follicular hyperplasia and, therefore, the recognition of proliferation within the mantle cell layer in the node or spleen with follicular involution is the key to recognition of MCL.

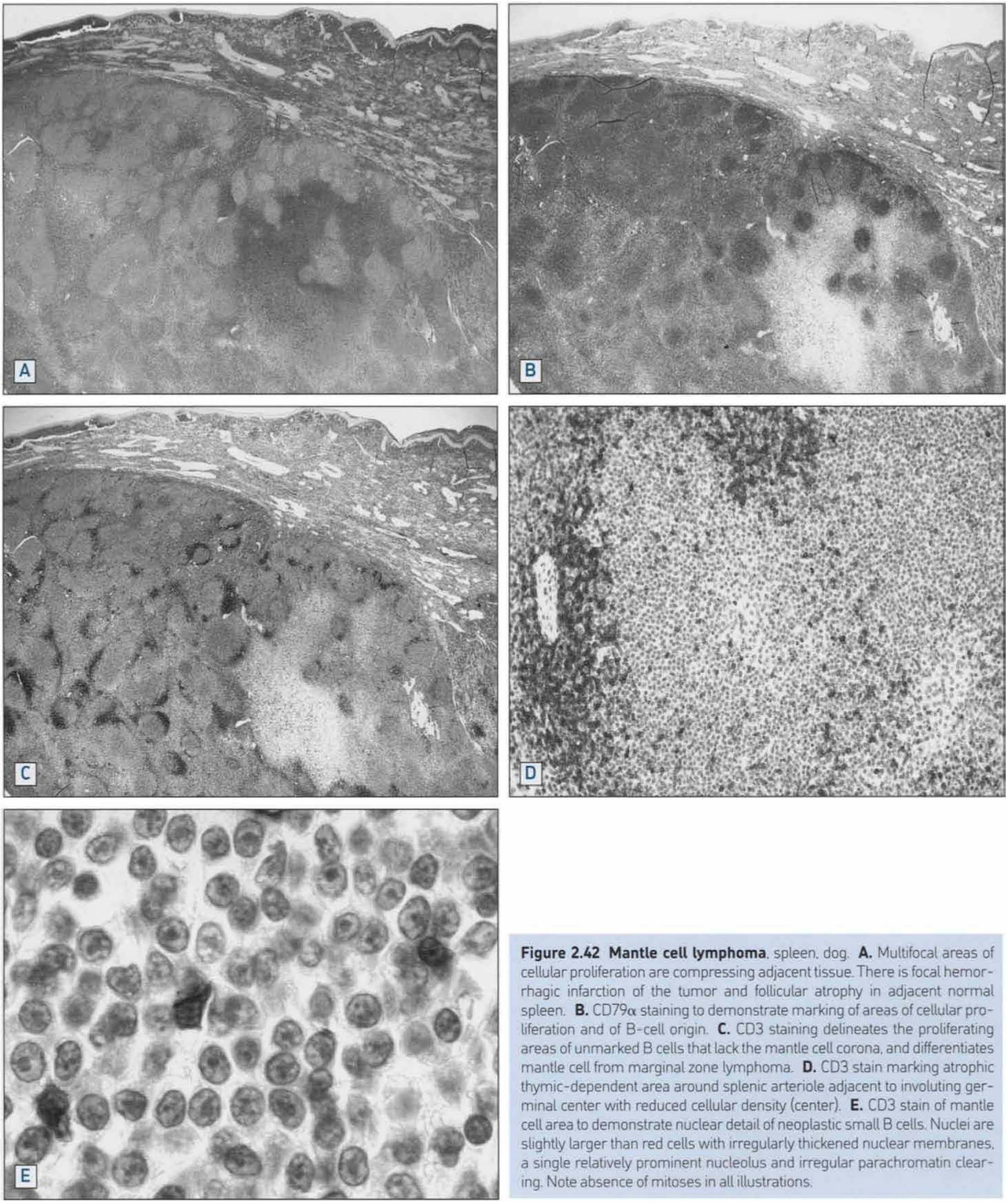

Figure 2.42 Mantle cell lymphoma, spleen, dog. **A.** Multifocal areas of cellular proliferation are compressing adjacent tissue. There is focal hemorrhagic infarction of the tumor and follicular atrophy in adjacent normal spleen. **B.** CD79α staining to demonstrate marking of areas of cellular proliferation and of B-cell origin. **C.** CD3 staining delineates the proliferating areas of unmarked B cells that lack the mantle cell corona, and differentiates mantle cell from marginal zone lymphoma. **D.** CD3 stain marking atrophic thymic-dependent area around splenic arteriole adjacent to involuting germinal center with reduced cellular density (center). **E.** CD3 stain of mantle cell area to demonstrate nuclear detail of neoplastic small B cells. Nuclei are slightly larger than red cells with irregularly thickened nuclear membranes, a single relatively prominent nucleolus and irregular parachromatin clearing. Note absence of mitoses in all illustrations.

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Diffuse large B-cell lymphoma

Lymphomas of diffuse architecture composed of large B cells constitute a very broad spectrum of lesions in both humans and animals. *Large-cell lymphomas that are presumptively of B-cell type are the most commonly occurring tumors in the cow, cat, horse and pig.* Two-thirds of the lymphomas in cattle are of the large B-cell type, about half of which are associated with *Bovine leukemia virus* infection and tend to have more variation in nuclear shape, most commonly sharp nuclear folds which has been referred to as the *diffuse large cleaved-cell type*. Twenty percent or more of the lymphomas of the dog are of the diffuse large B-cell type, while in the cat, approximately half of the lymphoid tumors are the large diffuse types including centroblastic, immunoblastic and large cleaved-cell types. The horse is unique in having a frequent association between a large B-cell lymphoma of the T-cell/histiocyte-rich type and multiple subcutaneous neoplasms. Finally, approximately two-thirds of the lymphomas of pigs are of the diffuse large-cell type, which are presumed to be of B-cell lineage.

In humans, the diffuse large B-cell lymphomas (DLBCL) represent as many as 40% of non-Hodgkin lymphoma cases, which are characterized by an aggressive natural history but usually have a good response to therapy with a complete remission rate in the 70–80% range and long-term disease-free survival of nearly 50%. Human large B-cell lymphomas usually occur in lymph nodes or in extranodal sites, of which those most frequently involved include the bone, skin, thyroid, gastrointestinal tract, and lung. *The DLBCLs are morphologically heterogeneous*, which has complicated classification for both humans and animals. Since the reproducibility rate of subtyping these lymphomas in human studies has been low, the European/American lymphoma (REAL) and revised WHO classifications have grouped the large B-cell lymphomas into a single category with description of a number of morphological variants and major subtypes.

A number of molecular genetic and immunophenotypic markers are employed in the characterization of human large B-cell lymphoma. In contrast to the enormous diversity in immunoglobulin gene rearrangements present in reactive B lymphocytes, *each neoplastic cell in a B-cell lymphoma has identically rearranged immunoglobulin genes, is derived from a single transformed B cell, and constitutes a monoclonal B-cell population.* Under normal circumstances, the naive B lymphocytes leave the bone marrow following successful immunoglobulin gene rearrangement, and in the peripheral B-cell pool, on encounter with antigen, their antibody may be modified by somatic hypermutation and class-switch recombination to provide an antibody with more avid binding to a particular antigen. This process of somatic hypermutation is apparently restricted to B-cell proliferation within the specific micro-environment of a lymphoid germinal center. Thus, the presence in a B-cell clone of somatically mutated immunoglobulin gene variable region constitutes the hallmark of germinal center B cells and their subsequent progeny, which may become either long-lived memory cells or plasma cells. Examination of the rearranged variable region genes of B-cell lymphomas by sequence analysis has become a useful tool by which to define the maturational stage at which neoplastic transformation has occurred and thus to assign the neoplastic clone to a cell type corresponding to their normal benign counterparts. The conclusions of these studies are that *the DLBCLs are, as a group, derived from antigen-experienced B cells that have participated in a germinal center reaction.*

Cytogenetic studies of B-cell lymphomas have identified a number of translocations including t(3;14) (q27;q32) as well as t(14;18) and t(8;14). The chromosomal translocation involving 3q27 involves a region of the immunoglobulin heavy chain gene. Cloning of the DNA from a novel gene of this region identified the *BCL6 (B-cell leukemia lymphoma-6) gene* which has a product apparently active in DNA binding and transcriptional regulation. High levels of expression of this protein have been found in a number of lymphoma subtypes. In gene knock-out mice with biallelically disrupted *BCL6* genes, the animals display normal B- and T-cell lymphocyte counts but completely fail to form germinal centers on exposure to T-cell dependent antigens. The animals suffer a high incidence of infectious diseases and inflammatory processes. It is concluded that *BCL6* is required for germinal center formation and normal antigen-dependent immune responses.

The t(14;18) (q32;q21) translocation characteristically found in follicular lymphomas places the *BCL2* gene into juxtaposition with a joining segment for the immunoglobulin heavy chain gene. Despite the mature phenotypes of the follicular lymphomas bearing this translocation, the change appears to occur at an early pre-B-cell stage of immune-differentiation. The t(14;18) translocation involving the *BCL-2* gene leads to over-expression of the BCL2 protein product by placing the *BCL2* proto-oncogene under the constitutive activation of the immunoglobulin gene IgH promoter. The resulting BCL2 fusion protein is located in the mitochondrial inner membranes and functions as an anti-apoptotic protein inhibiting cellular programmed death. This BCL2 protein over-expression occurs in up to 80% of DLBCLs with the differences between the large B-cell lymphomas and the follicular lymphomas suggesting that other changes must be responsible for their differentiation. The third translocation, t(8;14) (q24;q32), identified in diffuse large B-cell lymphomas involves the *MYC* oncogene located on chromosome 8 at band q 24, which is located to the IgH locus at 14q32. This defect has been reported in the great majority of Burkitt type lymphomas and is recognized in that all proliferating tissues express one member of the *MYC* family which is then downregulated when the cells reach terminal differentiation, which in the case of lymphocytes constitutes becoming long-lived memory cells in permanent G_0 resting phase. Transfection studies have shown that constitutive expression of *MYC* impedes inducible differentiation, whereas inactivation of *MYC* by antisense constructs permits the induction of cell differentiation. Thus, the expression of the *MYC* gene appears closely linked to the check-point decision between further proliferation and differentiation in lymphoid tissues.

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Morphologic variants of diffuse large B-cell lymphomas

Centroblastic variant

Diffuse large B-cell lymphomas of this type have morphologic features of the centroblast found in benign reactive germinal centers. These cells have medium-to-large, round-to-oval nuclei 1.5–2 red cells in diameter with vesicular nuclei, finely branched and peripheralized chromatin, and 2–4 nucleoli often impinging or bound to the nuclear membranes (Fig. 2.43). These cells are characterized by a very narrow boundary of moderately basophilic cytoplasm. Most of the cells in diffuse lymphomas of the centroblastic type have morphology as just described, but all have a certain proportion of cells with single central nucleolus known as *immunoblasts*. In addition, a third cell type may be present in which the nuclei are multi-lobated and polymorphic but retain the characteristic central nucleolus. Thus, as in the classification of large cell lymphomas by the Working Formulation, *the pathologist is faced with the decision of arbitrarily deciding which cell population is dominant among these various cell types.*

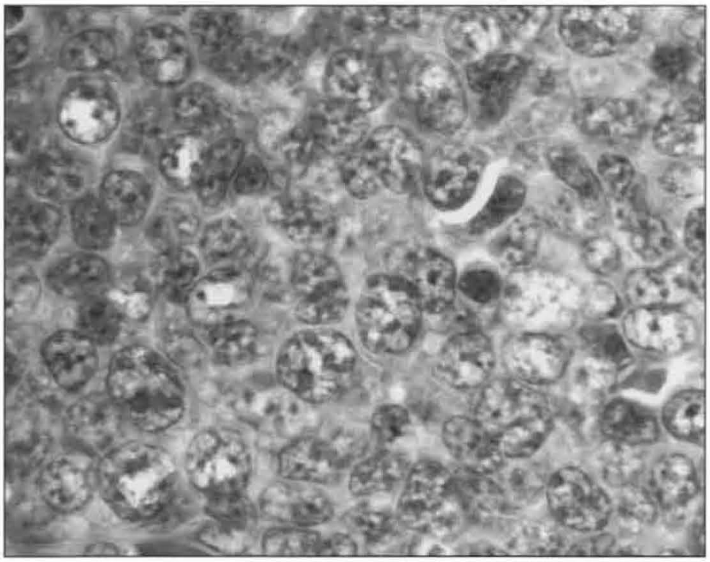

Figure 2.43 Diffuse large B-cell lymphoma, centroblastic variant, lymph node, dog. Nuclei are large (2 to 2.5 red cells in diameter) with small chromocenters, prominent parachromatin clearing and multiple small but prominent nucleoli usually adjacent to nuclear membranes. The neoplastic cells are caricatures of their counterpart in benign germinal centers.

Immunoblastic variant

By definition, more than 90% of the cells of this variant have a prominent single central nucleolus and, in contrast to the centroblastic large cell lymphomas, *the immunoblastic lymphomas may have more abundant cytoplasm with increased basophilia* (Fig. 2.44). Lymphomas of this type are felt to be relatively rare in human pathology but are *common in the dog and cat,* where a quarter to a third of lymphomas may be of this type, but are relatively rare in cattle, and constitute 5 to 10% of the lymphomas seen in the pig and horse. In animals, diffuse large B-cell lymphomas of the centroblastic and immunoblastic variants mark positively with CD79α and are negative for CD3. The human large B-cell lymphomas of this type mark with various pan-B-cell markers, including CD19, CD20, CD22, and CD79α, with surface immunoglobulins present in most cases with IgM followed by IgG and IgA in terms of frequency of immunoglobulin class types.

T-cell-rich/histiocyte-rich variant

One of the more intriguing variants of diffuse large B-cell lymphoma is the T-cell-rich variant in which *the malignant cells may constitute less than 25% of the tumor mass and may be as little as 5%.* Initial descriptions of the tumor credited the predominant T-cell component as a beneficial host response given the relatively indolent course of tumors of this type. The T-cell-rich/histiocyte-rich variant of B-cell lymphoma was known as the diffuse mixed cell lymphoma in the Working Formulation and was considered of intermediate grade in terms of its propensity for aggressive behavior. *It is currently felt that a*

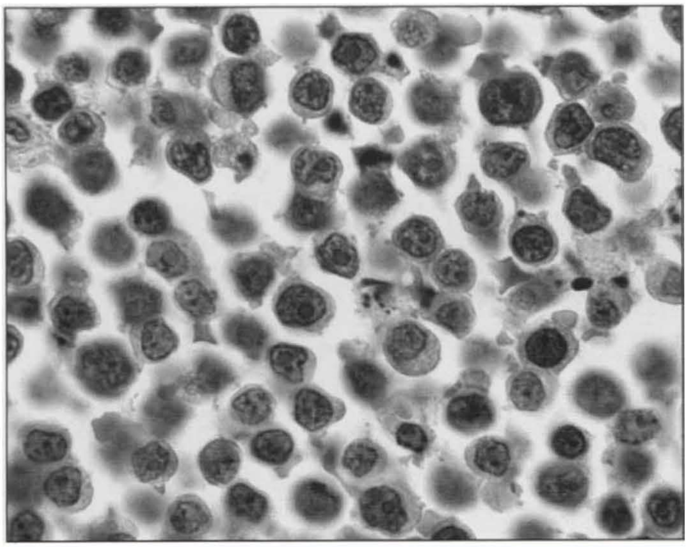

Figure 2.44 Diffuse large B-cell lymphoma, immunoblastic variant, lymph node, dog. The nuclei of immunoblastic lymphoma may be smaller or larger than those of the centroblastic variant and typically are round-to-oval with irregular thickening of nuclear membranes, irregular parachromatin clearing and prominent, usually single, central nucleoli. Cytoplasm is usually moderate-to-abundant and deeply stained.

number of entities may be present within the current definition of the T-cell-rich/histiocyte-rich variant of large B-cell lymphoma. The general criteria proposed for diagnosis of this lesion in humans include the following:

1. A diffuse growth pattern throughout the entire specimen on H&E-stained preparations.
2. Large neoplastic B cells with a highly variant morphology occurring singly constitute less than 10% of the total cell population and may occasionally show nodular grouping.
3. Other cells in the lesion are primarily benign T cells and histiocytes.
4. The clonality of the neoplastic B cells can be demonstrated by immunohistologic and molecular analyses.
5. While sharing features with lymphocyte-predominant Hodgkin lymphoma, the T-cell-rich B-cell lymphoma is usually associated with advanced clinical stage at presentation, and indolent behavior.

These criteria apply equally well to these neoplasms as they occur in animals, with the addition that in animals the lesions are characterized by fine reticulin sclerosis that is a highly consistent finding in all species. This characteristic of the T-cell-rich B-cell lymphomas is immediately apparent on low magnification examination of lymphomas of this type because they are completely cohesive and lack any architectural breaks in the tissue that are frequently seen in other lymphomas with a more friable architecture. Despite their apparent frequency, fibroblasts within these neoplasms are inconspicuous on histological examination, with the oval vesicular nuclei of moderate size relatively unapparent in the mixture of lymphocyte types. On specific staining with antibody against collagen type III or silver stain for reticulin, the background connective tissue is immediately apparent. Lymphomas of the T-cell-rich/histiocyte-rich large B-cell lymphoma are the characteristic type of subcutaneous lymphoma of the horse (Fig. 2.45A–D), which may be presented with literally of hundreds of small subcutaneous discoid masses 1–2 cm in diameter and up to 2 cm in depth. These proliferations are entirely subcutaneous and do not involve the skin or underlying musculature until they become very large, which may occasionally occur with single tumors weighing 4–6 kg. Tumors of this type are infrequently seen in the cow and dog, but constitute about 7% of lymphomas seen in the cat, and more than a third of cases in the horse.

In animals, T-cell-rich/histiocyte-rich B-cell lymphomas have a normal reaction for the benign T cells with CD3 and variable but consistent marking of the atypical large B cells with CD79α (Fig. 2.45D). The histiocyte population of these tumors has not been a matter of recognition in veterinary pathology, and specific staining to demonstrate their relative frequency has yet to be carried out. In human lesions, the T cells are predominantly CD4-positive while CD8-positive cells are present. The large atypical cells in human tissues mark for CD20 and CD45a as well as CD79α and other B-cell markers. An unusual lymphoma of cats of T-cell rich large B-cell type (Fig. 2.46A–E) has similarities to, and is described with, human Hodgkin lymphoma.

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Lymphomatoid granulomatosis variant

Lymphomatoid granulomatosis (LYG) is principally a disease of humans and dogs. A form of the disease may rarely occur in cats but has not been identified as part of this morphologic variant. *LYG was defined in humans as an angiocentric and angioinvasive lymphoproliferative disease that principally occurred in lung, but also occurred in other sites including skin, kidney, liver, and central nervous system.* The diagnosis of LYG in humans was based on recognition of *characteristic histologic findings of polymorphic lymphoid masses composed of small lymphocytes, plasma cells,*

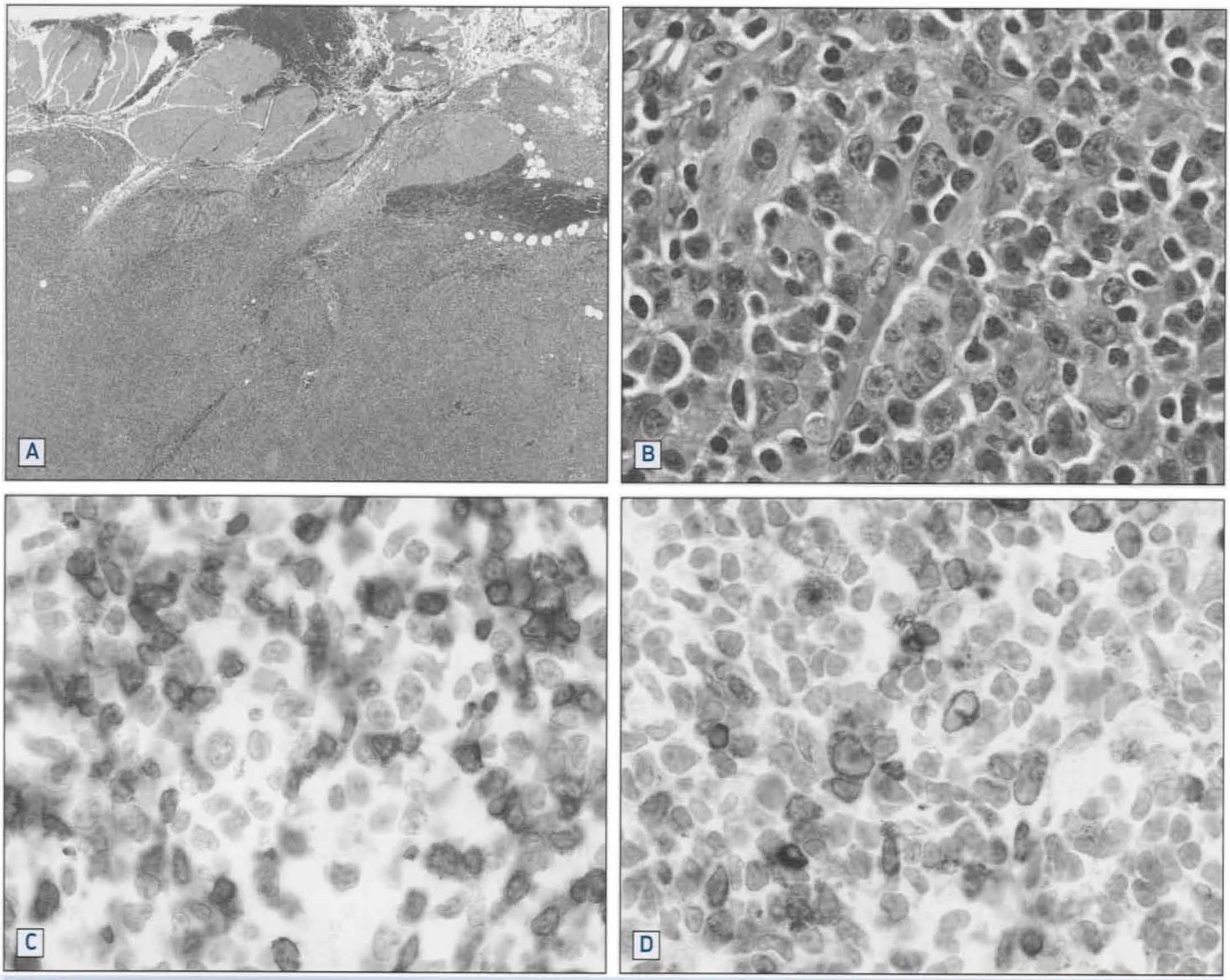

Figure 2.45 Diffuse large B-cell lymphoma, T-cell-rich/histiocyte-rich variant, subcutaneous mass, equine. **A.** The tumor has a solid appearance due to the presence of fine sclerosis and is invading subcutaneous muscle on an irregular broad front pattern. **B.** The lesion consists of a relatively even intermixing of a predominant population of benign small darkly stained T lymphocytes and large neoplastic B lymphocytes with atypical nuclei, multiple nucleoli and usually abundant cytoplasm. **C.** CD3 staining marks a high proportion of the small background lymphocytes with the larger atypical cells (center) negative. **D.** CD79α staining to demonstrate marking of the large atypical cells with most of the smaller background lymphocytes unmarked.

with scattered large atypical lymphocytes, and a lesion characterized by angiotropic transmural destruction of the walls of arteries and veins and the irregular occurrence of necrosis. It became apparent that the large atypical cells in human LYG were lymphoid cells with evidence of Epstein-Barr virus (EBV) infection of a phenotypic B cell. *The view has subsequently developed that most cases of human LYG are a form of T-cell-rich EBV-associated B-cell lymphoma.*

The disease in dogs is more variable and the lesion is less angiotropic and not angiodestructive, but focal ischemic necrosis does occur. In dogs, the predominant cells are small, apparently benign, T lymphocytes, with larger cells of irregular phenotype resembling centrocytes, and scattered large atypical cells with very large nuclei and nucleoli, plus the irregular aggregation of huge giant cells with many nuclei and abundant, often eccentrically placed, cytoplasm (Fig. 2.47A–D). LYG in the dog is characterized by *irregular fibrosis and multifocal areas of ischemic necrosis.* The cellular infiltrates surround and collapse the major airways and tend not to destroy bronchiolar walls or the epithelium. Phenotypically, LYG in the dog does not always make a convincing case for being a subtype of T-cell-rich/histiocyte-rich B-cell lymphoma. The small cells in the lesion regularly mark for CD3; whereas, the intermediate-sized cells have variable markings for both CD3 and CD79α and very large atypical cells, which appear to be the major or only neoplastic cells in the reaction, irregularly mark for CD79α (Fig. 2.47C, D). Macrophages are present but appear to be principally related to areas of necrosis. The giant cells are negative for lysozyme and a histochemical stain for myeloid and histiocyte cell peroxidases. One of the most typical characteristics of LYG in the dog is the topographical presentation with *the lesion characteristically forming a large mass in the upper caudal area of one lung lobe.* These lesions may become very large (10 cm or more in diameter) and ultimately involve mediastinal tissues.

In conclusion, the **diagnosis** of LYG in the dog is based on the location of the lesion in the mature animal, and is characterized by dense and irregular lymphoid cell infiltration with atypical large

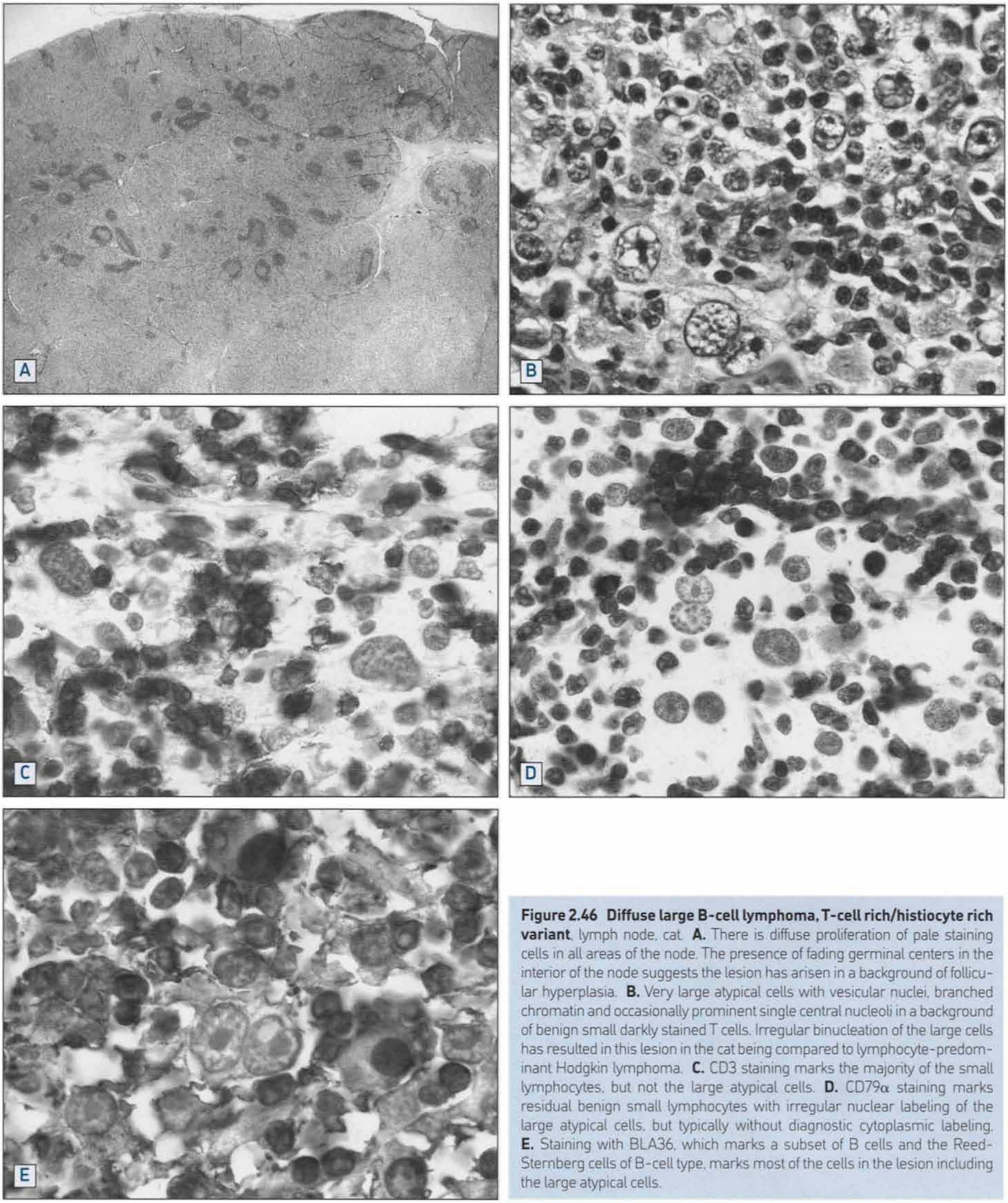

Figure 2.46 Diffuse large B-cell lymphoma, T-cell rich/histiocyte rich variant, lymph node, cat. **A.** There is diffuse proliferation of pale staining cells in all areas of the node. The presence of fading germinal centers in the interior of the node suggests the lesion has arisen in a background of follicular hyperplasia. **B.** Very large atypical cells with vesicular nuclei, branched chromatin and occasionally prominent single central nucleoli in a background of benign small darkly stained T cells. Irregular binucleation of the large cells has resulted in this lesion in the cat being compared to lymphocyte-predominant Hodgkin lymphoma. **C.** CD3 staining marks the majority of the small lymphocytes, but not the large atypical cells. **D.** CD79α staining marks residual benign small lymphocytes with irregular nuclear labeling of the large atypical cells, but typically without diagnostic cytoplasmic labeling. **E.** Staining with BLA36, which marks a subset of B cells and the Reed-Sternberg cells of B-cell type, marks most of the cells in the lesion including the large atypical cells.

cells and giant cells, multifocal areas of necrosis, and variable markings of the atypical cells for CD79α. Recent ability to stain with CD18 suggests that histiocytic sarcoma needs to be ruled out in the diagnosis of LYG.

Anaplastic large B-cell variant

Anaplastic large-cell lymphoma (ALCL) occurs as both a B- and T-cell lesion in humans and animals. In the early 1980s, an antibody was developed that marked Reed-Sternberg cells with the discovery of

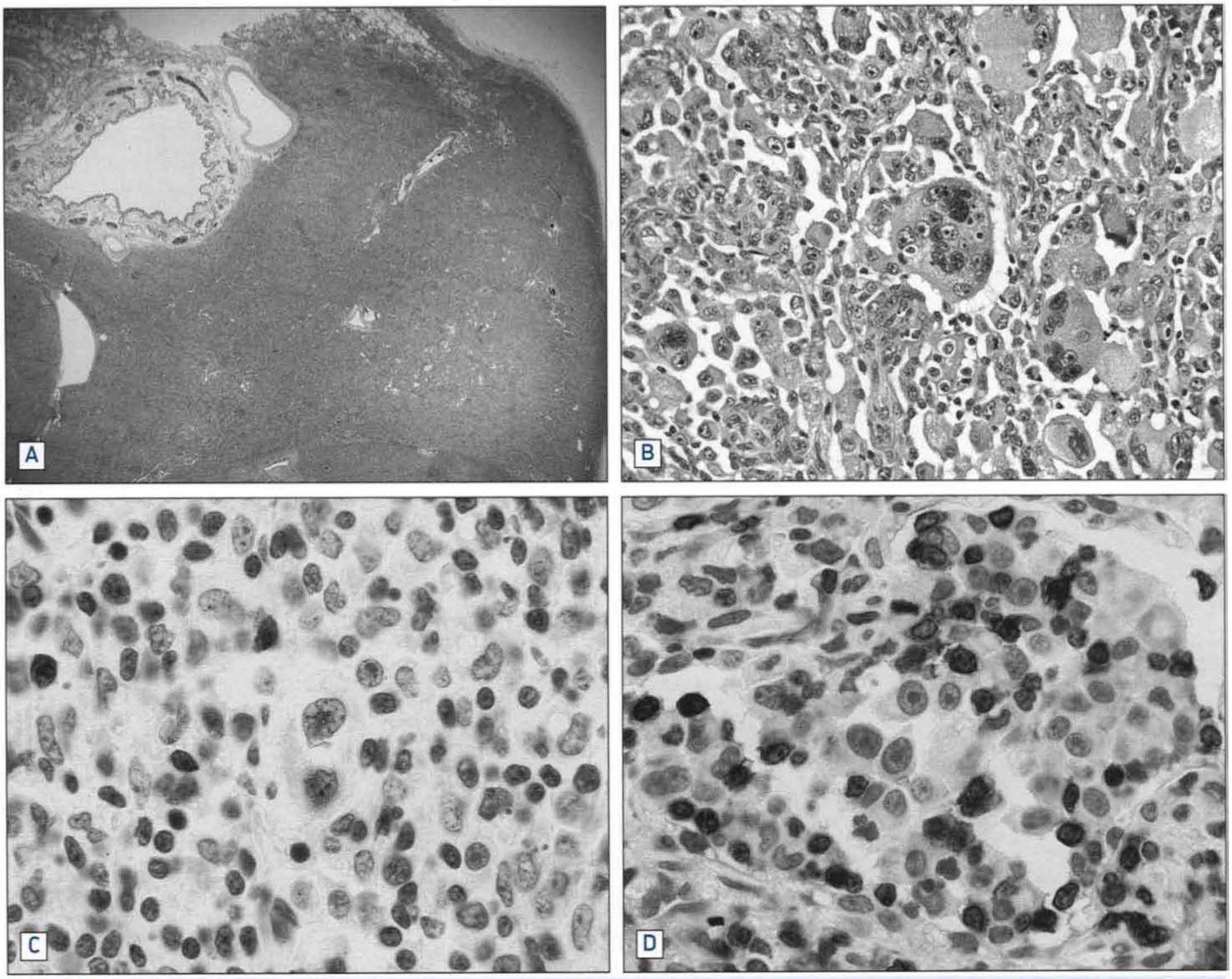

Figure 2.47 Diffuse large B-cell lymphoma, lymphomatoid granulomatosis variant, lung, dog. **A.** There is diffuse infiltration of pulmonary parenchyma with compression of vessels and smaller airways. **B.** Area with highly variable cell type including small and large lymphocytes and giant cells with marked anisokaryosis. Note giant cell undergoing necrosis. **C.** CD79α staining marks the nuclei but not the cytoplasm of the larger atypical cells and an occasional plasmacytic cell. **D.** Staining with CD3 marks many of the small lymphocytes as T cells but not the cytoplasm of the large atypical cells. Lymphomatoid granulomatosis in humans has been suggested to be a variant of the T-cell-rich B-cell lymphoma.

an antigen called "Ki-1" and subsequently clustered and recognized as CD30. Use of this antibody led to the identification of a heterogeneous group of human large cell neoplasms that marked with the CD30 reagent plus lymphoid markers, which were called "Ki-1 positive anaplastic large cell lymphoma." Recent classifications have recognized CD30-positive lymphomas of B-cell type and a T/null-cell phenotype usually bearing a chromosome 2 and 5 translocation. The disease is not well characterized in dogs and it appears that anaplastic large cell lymphoma tends to arise in body surfaces, i.e., skin, lung and gastrointestinal tract, with rapid spread to adjacent lymph nodes. Both B- and T-cell types of anaplastic large cell lymphoma have a similar morphology with nuclei that are 3–4 red cells in diameter and often indented or irregularly folded with peripheralized chromatin, marked parachromatin clearing, and 1 or 2 large central nucleoli (Fig. 2.48). Cytoplasm is moderately abundant and may be markedly basophilic. There is a high mitotic rate. The B-cell ALCLs tend to arise in the deep dermis and extend into the panniculus with multifocal areas of necrosis. The B-cell ALCL tend to have solid masses of atypical but clonal tumor, whereas the ALCL of the T-cell type tend to have a more pluricellular appearance with accompaniment of a mixture of small benign lymphocytes, mainly T cell.

Plasmablastic variant

The plasmablastic variant (PV) of diffuse large B-cell lymphoma appears to be a neoplasm that is intermediate in differentiation between clearly malignant B immunoblastic lymphoma and a plasmacytoma. This tumor in humans is found frequently but not exclusively in HIV-positive individuals and tends to arise in the oral cavity. *The disease is not described in dogs*, but may occur as a subset of those

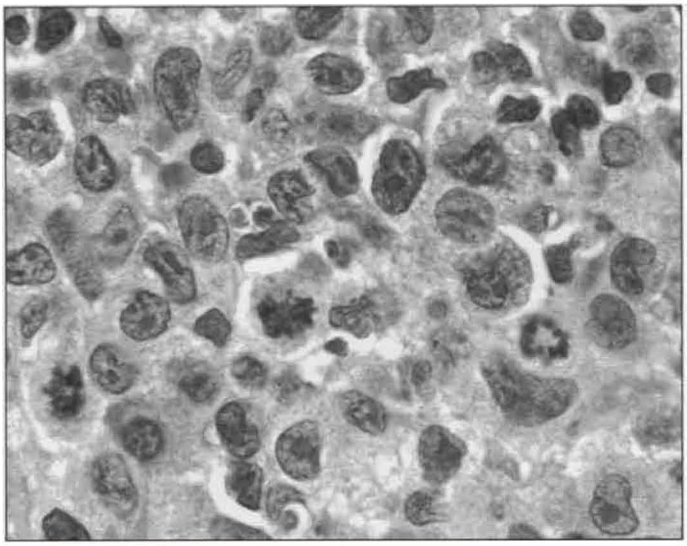

Figure 2.48 Diffuse large B-cell lymphoma, anaplastic large B-cell variant. lymph node, dog. Nuclei are generally large and irregularly folded and clefted with irregular thickening of nuclear membranes and 1 to 2 prominent nucleoli irregularly rimmed by small chromocenters. There are numerous mitoses and apoptotic bodies.

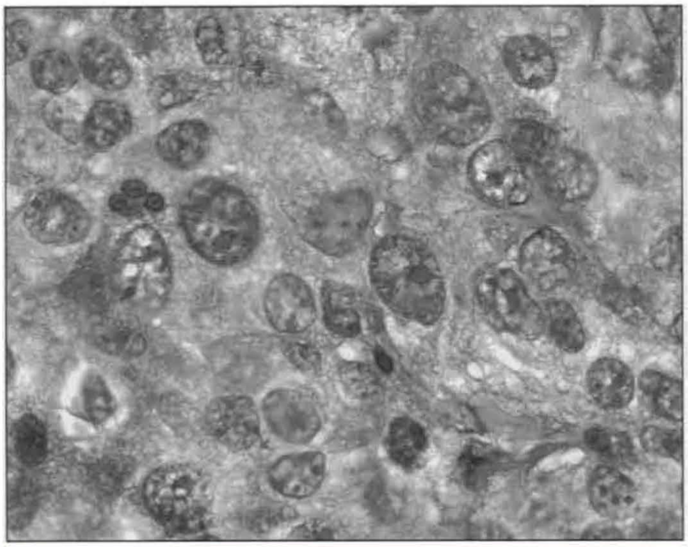

Figure 2.49 Diffuse large B-cell lymphoma, plasmablastic variant. lymph node, dog. Nuclei are 2 to 3 red cells in diameter with 1 to 2 prominent nucleoli and abundant moderately basophilic cytoplasm often eccentrically placed. There are frequent mitoses.

plasmacytomas found to be malignant (Fig. 2.49). The cytology of the disease in humans is of *relatively monomorphic plasmacytomas that have large and eccentric nuclei and single or several prominent nucleoli.* Cytoplasm is abundant and basophilic and there are numerous mitotic and apoptotic figures and frequent single cell necrosis. The phenotype of the human plasmablastic variant marks with strong expression of CD79α in half of the cases and strong consistent positivity with VS-38C. There are variable or weak reactions for CD45 and CD20. A similar disease in animals would likely be called an *anaplastic or atypical plasmacytoma.*

Topographic variants of diffuse large B-cell lymphoma

Mediastinal (thymic) large B-cell lymphoma

Primary mediastinal (thymic) large B-cell lymphoma in humans is an unusual neoplasm of young women that is characterized as a mediastinal mass of at least 5 cm in maximal dimension with no other lesion larger than that in the mediastinum. Characterization of the human lesion is demonstrated to be a lymphoma apparently arising within the thymus and of B-cell type. The human lesion is characterized by sclerosis with a predominance of large centroblast-like cells predominating. All cases have a B-cell phenotype and are positive for CD19, CD20, CD22, and CD79α, and negative for CD3.

In animals, the disease largely or primarily occurs in dogs and is only rarely encountered. These neoplasms in the dog *were called a multi-lobated B-cell lymphoma* and had a striking appearance in cytologic preparations derived from fine-needle aspiration and characterized by large and very large nuclei with multiple deep nuclear clefts and multiple prominent nucleoli (Fig. 2.50A, B). Older cases were found to be positive for IgM, and more recent cases mark strongly for CD79α. Histologically, the neoplastic cells have relatively abundant, lightly or clear-staining cytoplasm, and the sclerosis does not appear to be as prominent a factor in dogs as in the human disease. Very large cells with quite abundant cytoplasm are occasionally seen, which may suggest an epithelial cell arising in a thymoma, but these cells are negative for cytokeratin and weakly or strongly positive for CD79α. Mitotic rate is relatively low, but the lesions are aggressive and invade the lung and mediastinal tissues. The presentation is usually related to problems with cough, swallowing and exercise intolerance. *Mediastinal lymphomas in the cat are of a much more homogeneous cell type*, and usually composed of aggressive small B-cell lymphoma, more typical of the Burkitt-like lymphoma or small noncleaved cell of the Working Formulation.

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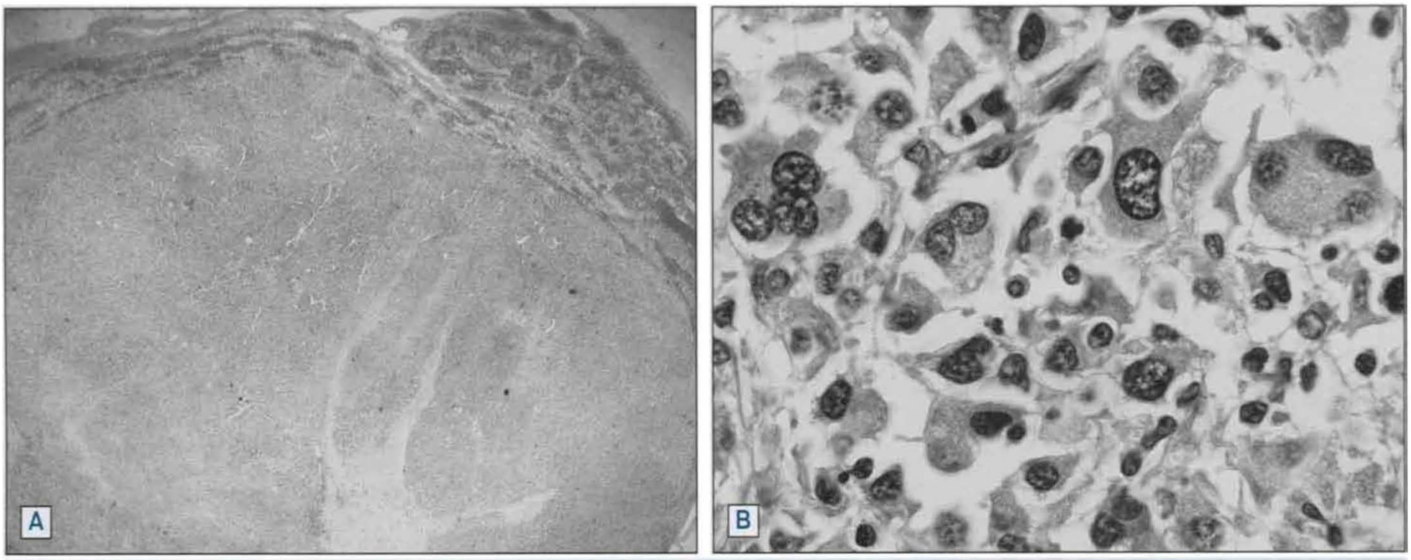

Figure 2.50 Diffuse large B-cell lymphoma subtype, mediastinal large B-cell lymphoma, dog. **A.** Enlarging diffuse proliferation is compressing atrophic medullary cords and sinuses of mediastinal node. **B.** Cytologically, the mass has marked variation in cell type with irregularly shaped large nuclei as well as multinucleation. Not all areas of tumor have this degree of pleomorphism.

Primary effusion lymphoma

Effusion lymphoma, also known as *body cavity lymphoma*, is a *rare neoplasm of large B cells* that in humans presents as a lymphomatous effusion in the absence of an adjacent lymphomatous mass. The disease is most frequently seen in immunodeficient patients with fairly consistent association with *Human herpesvirus 8* (HHV-8) infection. Effusion lymphoma apparently has a heterogeneous mutation profile of rearranged heavy chain genes that suggests that HHV-8 may transform B cells at various stages of maturation. Human effusion lymphoma lacks surface immunoglobulin and is positive for CD45, CD30, and CD38, but generally negative for CD19, CD20, and CD22.

Lymphoma is relatively frequently diagnosed in the cat and dog on the basis of the presence of neoplastic cells in body cavity fluids. The presence of neoplastic lymphocytes in aspirates of cerebrospinal fluid is the most common type of effusion lymphoma seen in animals. Neoplastic lymphocytes are generally more commonly found in thoracic than abdominal aspirates and, in both cases, the malignant cells in the effusion appear to be derived from exfoliation of lymphomatous masses that represent spread from a common type of large-cell lymphoma. No particular infectious agent is associated with lymphomatous effusions in animals, and the cytologic cell type is usually typical of immunoblastic or centroblastic lymphoma.

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Intravascular large B-cell lymphoma

Intravascular large B-cell lymphoma has gone through a series of name changes beginning as *angioendotheliomatosis* some 40 years ago on the presumption that the tumor arose from the endothelial cells. With recognition that the tumor was of lymphoid origin and B-cell type, the current terminology has been adopted. Intravascular lymphoma in humans marks with CD19, CD20, CD22, and CD79α. The predilection of the tumor for vascular channels is unexplained. Neoplasms of this type in humans lack the leukocyte adhesion molecule CD11a/CD18, which may explain the inability of these cells to extravasate to perivascular tissues. There appears to be no specific immunoglobulin gene rearrangements associated with intravascular lymphoma in humans, but infection with *Human herpesvirus 8* appears to be a condition for a diagnosis of this neoplasm in humans. The disease has a rapid clinical course in humans, and most cases are diagnosed at necropsy.

In animals, intravascular lymphoma of large B-cell type is a rare disease that is seen in the dog, and may rarely occur in other species. As in humans, most cases are diagnosed at necropsy after animals have been presented with sudden collapse that seems to involve failure of the cardiovascular or pulmonary systems. One case presented with paraplegia that rapidly progressed to cardiac failure. Necropsy demonstrated tumor emboli in the atria, myocardium and spinal cord (Fig. 2.51A). On necropsy of other animals, these large B-cell tumor emboli have been found in dilated vessels in the meninges (Fig. 2.51B) and in the choroid plexus of the central nervous system. Cytologically, the neoplastic cells are of immunoblastic and centroblastic type with multinucleation and a moderate amount of quite highly basophilic cytoplasm. *Involved vessels are almost always quite markedly dilated and the tumor cells appear to be coherent to each other but not particularly adherent to vascular endothelium.* Prior to CD immunochemistry, the neoplastic cells were found to mark with anti-canine IgM, and are strongly positive with CD79α. Cases of lymphoma of similar cytologic type and topographical distribution that are CD3-positive and that appear to begin in the nasal turbinates occur occasionally in animals.

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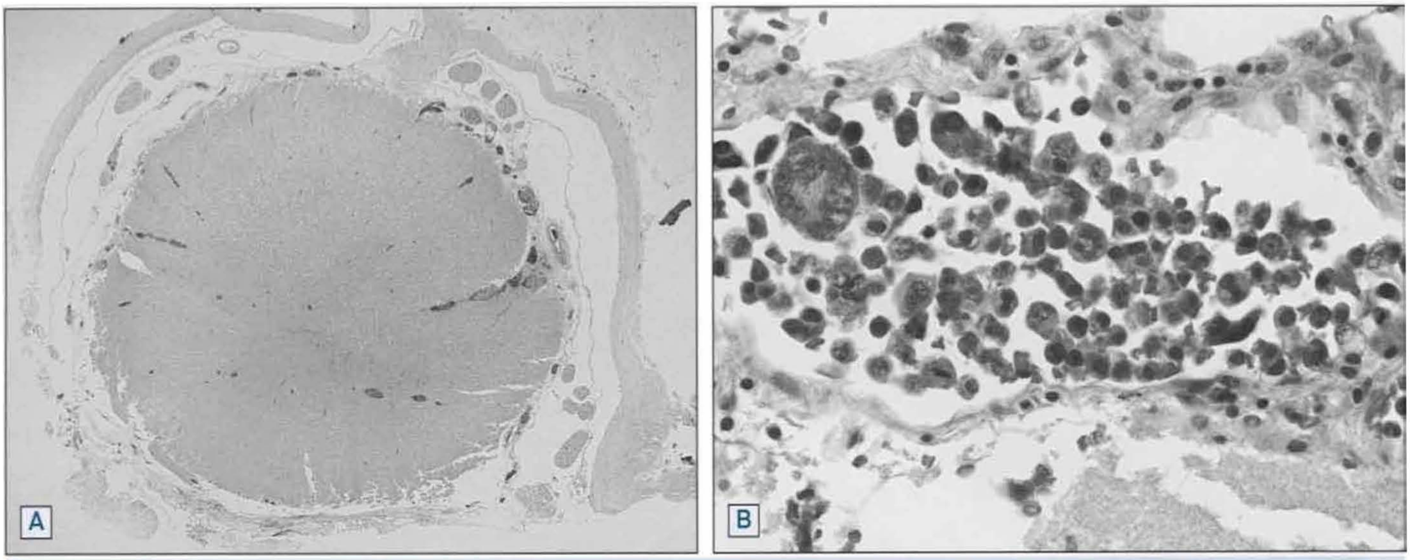

Figure 2.51 Diffuse large B-cell lymphoma, intravascular type, dog. **A.** Spinal cord. Prominent subdural and internal vessels represent areas of colonization by intravascular lymphoma. **B.** Brain, meningeal vessels. The cells have moderate anisokaryosis with occasional binucleation and multinucleation with relatively abundant deeply stained cytoplasm. (Courtesy of RM Jacobs.)

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Burkitt lymphoma/Burkitt cell leukemia

The revised WHO classification divides the category of Burkitt lymphoma into **morphological variants** and **subtypes**. The variants include *classical Burkitt lymphoma* characterized by a genotype of *MYC* but not *BCL2* rearrangements, with a B-cell phenotype and highly consistent uniformity of cell size, and features associated with a starry-sky pattern on low-power examination. The *Burkitt-like lymphoma* cannot be distinguished from the classical type on cytogenetic or phenotypic characteristics but only on morphology, with the Burkitt-like variant having moderate anisokaryosis and anisocytosis. It has been suggested that, in humans, the Burkitt-like variant is characteristically found in an older age group than the classical Burkitt lymphoma, and is more common in HIV-positive immunocompromised individuals.

In animal pathology, high-grade lymphomas with intermediate-size nuclei, vesicular chromatin pattern, and prominent nucleoli with a B-cell phenotype and starry-sky pattern were called *Burkitt-like lymphoma/leukemia*. In contrast, the category now termed Burkitt-like in the REAL and WHO classifications constitutes that category formerly known as *small-noncleaved cell lymphoma* in the Working Formulation. Further minor distinctions in identification of Burkitt lymphoma variants include cases with a plasmacytoid differentiation, usually associated with HIV-positive individuals, and subtypes based on geographic localization as endemic (African) and sporadic (European–American) cases.

The identification of Burkitt and Burkitt-like lymphomas in animals requires a careful consideration of their characteristics from a number of standpoints. In terms of *nuclear size*, tumors of Burkitt cell type are larger than those of the intermediate lymphomas of plasmacytic type and smaller than those of diffuse large-cell lymphoma, and are generally comparable in size to those of lymphoblastic lymphoma of both B- and T-cell type. In further consideration of nuclear size, the *Burkitt-type lymphoma can be identified in animals on the basis of the remarkable similarity and homogeneity of cell type, similar to disease in humans* (Fig. 2.52A, B). In contrast, the entity now known as Burkitt-like, and formerly known as small-noncleaved cell, characterized by greater variation in nuclear size, cannot be distinguished in morphometric studies as an entity separate from diffuse large-cell lymphoma. Thus, there has always been difficulty in assigning animal lymphomas with vesicular nuclei and multiple nucleoli to categories of either large-cell lymphoma or small-noncleaved cell lymphoma based largely on the subjective decision as to whether the nuclei were on the whole more appropriate in the large-cell or small-cell category. These difficulties have in no way been resolved by the newer tumor classification systems. Other minor distinctions between large-cell lymphoma and Burkitt-like lymphoma are that the large-cell type tends to have fewer and larger *nucleoli*, of which some characteristically impinge on the nuclear membrane as described for centroblastic neoplasms. Burkitt-like lymphomas may have 3–5 small nucleoli generally detached from the nuclear membranes, and as their previous name implied, the Burkitt-like lymphomas tend to have a round or oval nuclear shape without indentation or cleavage, whereas large-cell lymphomas may be of either type or mixed. Further, the Burkitt-like lymphomas tend to have a higher *mitotic rate* than large B-cell lymphomas and are more likely to have a starry-sky pattern on low-power magnification, but both of these distinctions are relatively arbitrary. Finally, the definition

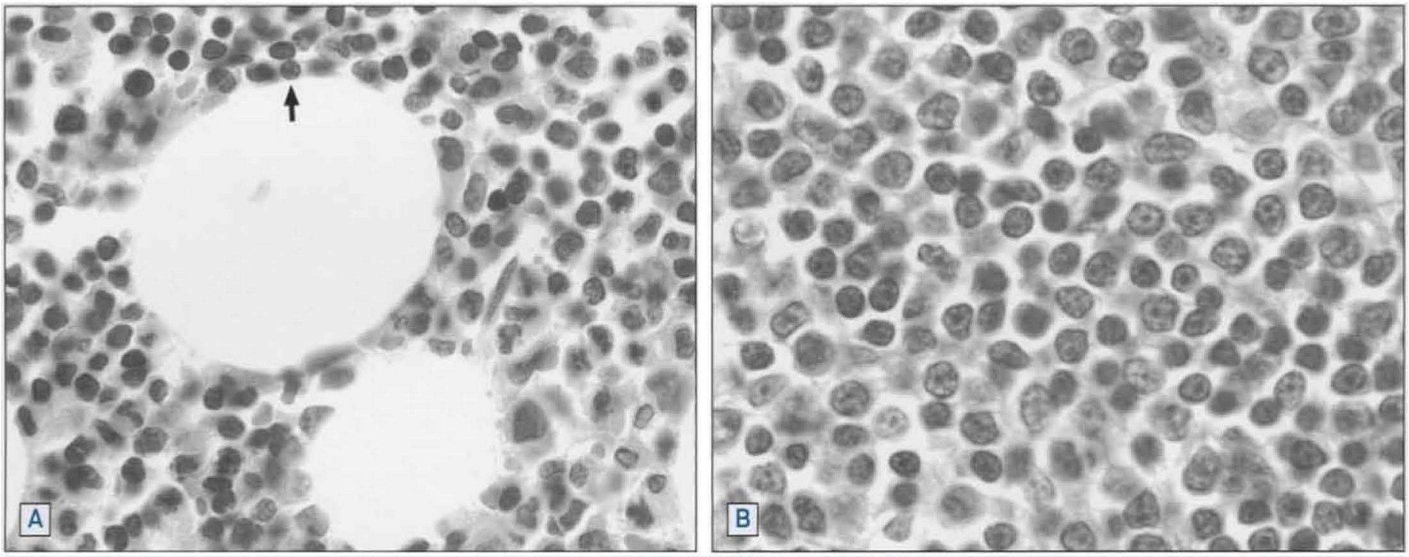

Figure 2.52 Burkitt-type lymphoma/leukemia, pig. **A.** Bone marrow. The small dark cells (arrow) represent focal interstitial colonization of bone marrow. **B.** Lymph node. Note homogeneity of cell size and nuclear type. Nuclei are generally densely stained with thickening of the nuclear membranes, irregular parachromatin clearing and occasional small prominent nucleolus. (Courtesy of T Dukes.)

of *chromatin distribution* in the Burkitt and Burkitt-like lymphomas is important to distinguish them from lymphoblastic lymphomas that have nuclei of the same or similar size. Lymphoblastic lymphomas are characterized by relatively dense and uniform chromatin distribution with absent or obscured nucleoli. In contrast, the nuclei of Burkitt and Burkitt-like lymphomas have a vesicular appearance due to peripheralization of chromatin, with thickening of the nuclear membranes and irregular well-defined parachromatin clearing that adds prominence to multiple and small nucleoli. Both lymphoblastic lymphomas and Burkitt-type lymphomas have high mitotic rates and may have a starry-sky appearance on low magnification examination. Further, subtle distinction exists between these two groups in that, like the internal nuclear detail that is poorly defined in lymphoblastic lymphomas, the metaphases in this category of neoplasm are also less densely stained and sharply defined than those of all other lymphoid neoplasms, and must be carefully looked for at a ×1000 magnification to be confident of an accurate assessment of their number.

It is suggested that eponyms such as Burkitt and Hodgkin diseases not be used directly to designate the counterpart neoplasms in animals, but rather that they be termed Burkitt or Hodgkin type. The Burkitt-like (formerly small-noncleaved cell) should have direct, comparable use in animals. *True Burkitt-type lymphomas are rare occurrences in animals*, occurring occasionally in the cow, dog, and horse. In contrast, *the Burkitt-like category constitutes approximately 20% of cases of lymphoma in the cow, 25% in the dog, 13–15% in the cat, 2% in the horse, 24% in the pig, and approximately 5% in humans*. In animals, as in humans, these two categories of lymphoma tend to reach complete remission with aggressive treatment that is more durable than that obtained with the T-cell counterparts.

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T-cell and NK-cell neoplasms

Precursor T-cell neoplasms

Precursor T-lymphoblastic lymphoma/leukemia

Precursor T-lymphoblastic lymphoma/leukemia (T-LBL/ALL) is a neoplasm of T-cell lineage composed of small to medium-sized blast cells, with scant cytoplasm, finely condensed chromatin, and indistinct nucleoli. There is variable involvement of bone marrow, as well as of thymus and lymph nodes. In humans, precursor T-cell neoplasms occur most frequently in late childhood to young adulthood with male predominance. These tumors comprise 15% of childhood and 25% of adult *acute lymphoblastic leukemias* with the prognosis typically worse than that for precursor B-cell neoplasms. Patients with T-LBL/ALL typically have elevated leukocyte counts at presentation, often over 50×10^9/L and are more likely to have irregular adenopathy and organomegaly. A cranial mediastinal mass is a highly specific predictor for T-cell phenotype in patients with ALL. In terms of staging, patients are defined as having *lymphoma* if there is a mediastinal or other mass and less than 25% blasts in the bone marrow, and as having *leukemia* if there are more than 25% bone marrow blasts with or without mediastinal or other masses.

Rearrangement of antigen-receptor genes is variable in human lymphoblastic neoplasms, and precursor T-cell neoplasms may have rearrangement of either or both TCR beta or gamma chain and

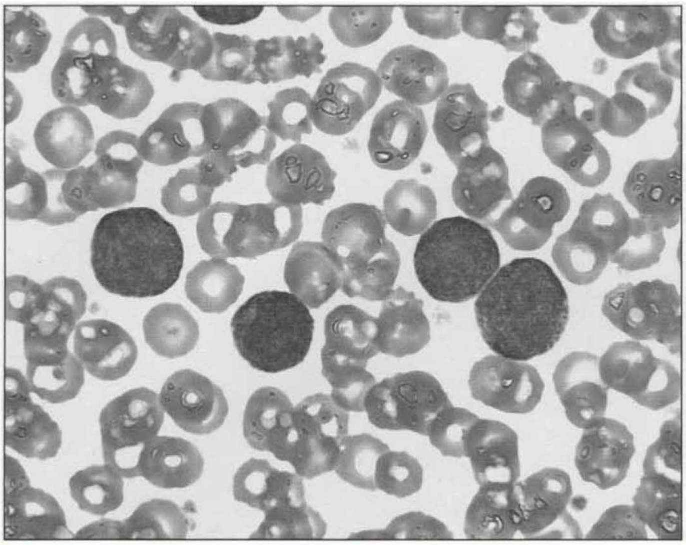

Figure 2.53 Precursor T-lymphoblastic lymphoma/leukemia, blood, dog. The blasts are usually of the L1 type. The blasts are typically small cells with nuclei 1.5 red cells in diameter and a very narrow rim of highly basophilic cytoplasm. The chromatin is densely stained with a coarsely cribriform pattern and nucleoli are present but relatively inconspicuous. (Courtesy of RM Jacobs.)

even the immunoglobulin heavy chain. Phenotypically, precursor T lymphoblasts are typically TdT positive and have variable expression for CD2, CD7, CD3, CD5, CD1a, CD4, and CD8, of which only CD3 is considered lineage specific.

Precursor T-cell neoplasms occur widely in animals but are seen most often in the cat, dog, calf and horse. The association with a thymic lesion probably occurs most often in the cat, although the relationship of this finding to T-cell phenotype is not generally considered. The small type of lymphoblast typical of the L1 FAB classification with a hyperchromatic cribriform chromatin pattern with inapparent nucleoli, nuclei about 1.5 red cells in diameter (10 μm) and scant cytoplasm is a relatively rare presentation in animals (Fig. 2.53). Approximately two-thirds of animal LBL/ALL are of the L2 type, with nuclei approximately twice the diameter of a red cell and nearly twice the diameter of a normal small lymphocyte. The chromatin pattern is fine granular to uniform with few or no large chromocenters and more obvious nucleoli. The cells have relatively abundant cytoplasm that is relatively pale staining with lighter perinuclear halo and increasing density toward the cellular margins. L1 and L2 types of neoplasms do not specify lineage and can be of B- or T-cell type, but most in animals appear to be T-type neoplasms. In contrast, the L3 type of ALL has relatively uniform round nuclei which are 1.5 times the diameter of L1, approximately the same size or slightly smaller than L2, and more uniform in size and shape. The chromatin is a more expanded version of L1, with hyperchromatic cribriform chromatin distribution with small, but relatively distinct, nucleoli and a moderate amount of highly basophilic cytoplasm that is frequently vacuolated. The L3 neoplasms tend to be characterized by numerous mitoses and apoptotic bodies and are the characteristic cytologic presentation of a Burkitt-type lymphoma and are usually of B-cell type. *T-type LBL/ALL in animals is characterized by rapid progression with anemia, thrombocytopenia with progressive marrow involvement, marrow failure and hepatosplenomegly.* T-cell LBL/ALL is not associated with a specific viral infection in animals. Cells of T-LBL/ALL regularly mark in high proportion in both cytoplasmic and histologic preparations with CD3.

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Mature (peripheral) T-cell neoplasms

T-cell prolymphocytic leukemia

T-cell prolymphocytic leukemia (T-PLL) is an aggressive T-cell neoplasm characterized by proliferation of medium-sized prolymphocytes with a mature T-cell phenotype. Prolymphocytic leukemia is a relatively infrequent disease in animals and probably occurs in all species. In terms of morphology, the leukemic T cells of T-PLL are larger than the blasts of L1 and approximately the same size of those of L3. Prolymphocytic leukemia cells are round and can be occasionally clefted with a narrow ellipse of moderately basophilic cytoplasm that is without vacuolation or granulation. *The characteristic feature of prolymphocytic leukemia is in the chromatin distribution, which characteristically consists of large densely stained chromocenters separated by branched areas of chromatin with parachromatin clearing providing a striking pattern of large chromocenters delineated by surrounding lightly stained zones.* T-cell PLL has prominent nucleoli and there may occasionally be broad-based cytoplasmic protrusions similar to the blasts of AML M7 (Fig. 2.54). There is usually extensive bone marrow involvement at presentation, and the peripheral lymphocyte count is typically high and in the 50–100 × 10^9/L range. The spleen and liver are involved, and lymph nodes tend to be involved in a paracortical T-zone manner with some sparing of follicles and medullary structures until late in the disease. T-PLL marks with CD3 in histological preparations, and while there is inadequate experience with immunophenotyping of cytological preparations, there is reason to expect that these would react positively to CD3 as well.

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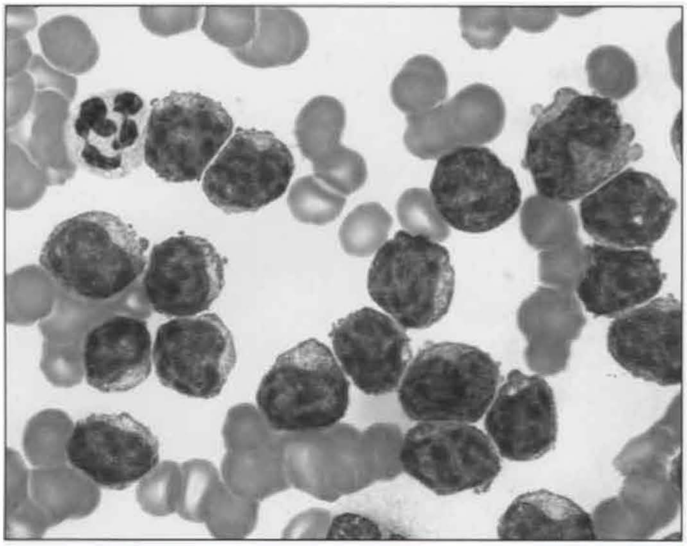

Figure 2.54 T-cell prolymphocytic leukemia, blood, dog. The characteristic feature of prolymphocytic leukemia is due to the retention of large chromocenters with irregular areas of parachromatin clearing. Nuclei may be round and occasionally cleft, unlike the more regular appearance of B-cell leukemias. Nucleoli are present but not prominent, and the cytoplasmic protrusions are characteristic of this neoplasm. (Courtesy of RM Jacobs.)

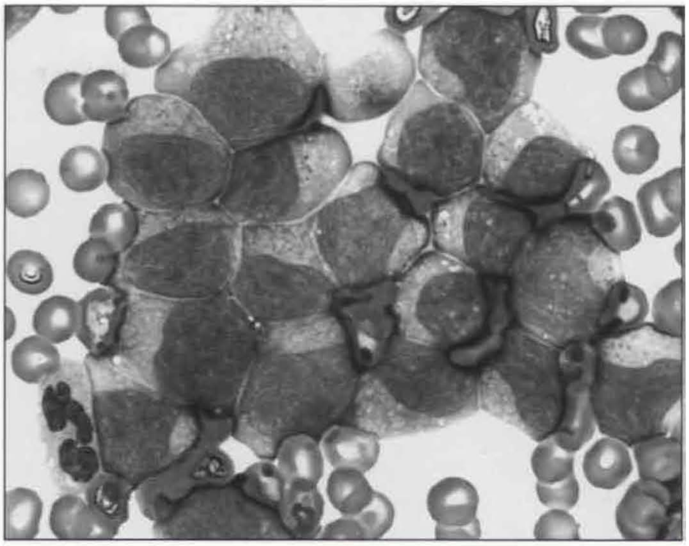

Figure 2.55 T-cell granular lymphocytic leukemia, blood, dog. The nuclei are 1.5 to 2 red cells in diameter and often irregularly indented. The chromatin pattern tends to be more homogeneous than that of prolymphocytic leukemia with retention of few large chromocenters and irregularly prominent nucleoli. The cytoplasm is abundant and irregularly basophilic, and small azurophilic granules are irregularly present.

T-cell granular lymphocytic leukemia

T-cell large granular lymphocyte leukemia (T-LGL) is defined as a neoplasm of mature CD8+ T lymphocytes characterized by cytoplasmic granulation with primary bone marrow involvement, and typically associated with neutropenia in humans but not apparently in animals. The human disease is characterized by adult onset with moderate lymphocytosis in the range of 5–20 × 10^9/L. Anemia and neutropenia are commonly found, usually with significant splenomegaly, but without major involvement of bone marrow, liver or lymph nodes. The course of the disease tends to be indolent, following a pattern of chronic lymphocytic leukemia. In the human disease, the T-LGL cells express CD2, CD3 and usually CD8 as well as the NK cell associated antigen CD16 and CD57, and genetically the T-cell receptor genes are clonally rearranged.

Neoplasms of LGL type occur in the dog, cat, horse, and rarely in the cow and have only been adequately described for the dog. Benign lymphocytosis of large granular lymphocytes may occur in most species, and is a typical response to inflammation in the cow. The disease in dogs is characterized by mature onset (>5 years of age) and there is persistent lymphocytosis with subgroups consisting of acute leukemia or chronic lymphocytic leukemia, with or without anemia. There is immunophenotypic variation between these groups in the dogs, but within a single individual, the neoplastic cells are of homogeneous immunophenotype. Dogs with T-LGL tend to be presented with high leukocyte counts in the 50–200 × 10^9/L range and two-thirds of the cases had splenomegaly on presentation. Most cases have T-cell receptor profiles of αβ type but TCR γδ was found in one-third of the cases. Both types of canine T-LGL mark with CD3 and those that have similar morphology with cytoplasmic granulation and are CD3-negative are considered likely NK-cell leukemias. The β2 integrin expression is distinctive in canine T-LGL, with CD11a consistently expressed, CD11B absent, and CD11c weakly expressed in two-thirds of the cases. The leukointegrin α subgroup D β2 is expressed in over 90% of cases.

Hematologically, T-LGL in the dog is characterized by peripheral blood cells that have nuclei 1.5 to 2 red cells in diameter and round-to-oval nuclei with a chromatin pattern irregularly similar to that of prolymphocytic leukemia, but with much more abundant and lightly basophilic cytoplasm with multiple (2–20) small azurophilic granules (Fig. 2.55). Like most cases of chronic lymphocytic leukemia, nucleoli are usually not present in animals with stable disease, and greater immaturity is present in the blood of animals that have rapidly progressive LGL more comparable to T-ALL. Histologically, there is relatively solid and uniform infiltration of sinus areas of the spleen with mildly atrophic, but not obliterated, follicles. While T-LGL in dogs is not associated with neutropenia as in the disease in humans, there does appear to be selective depression of erythropoiesis, which may be a characteristic of this neoplasm in the dog. A retroviral agent has been isolated from a clone of canine LGL leukemia in long-term culture.

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Aggressive NK-cell leukemia

Aggressive natural killer-cell leukemia (NKL) is defined in humans as a neoplasm of immature NK cells with peripheral blood lymphocytosis and a rapidly progressive clinical course. In humans, the cells in blood are described as larger than benign granular lymphocytes, and have irregularly indented and clefted hyperchromatic nuclei with distinct nucleoli. Lymph nodes and spleen have diffuse infiltration similar to other acute leukemias and appear to be involved prior to infiltration of the bone marrow. Phenotypically, in humans NKL express CD2 and CD56, but not CD3, and stain variably for CD11b. CD57 is usually not expressed and CD16 is positive in some cases. Genetically, the T-cell receptor genes are typically germline, and most human cases of NKL are associated with the clonal presence of Epstein-Barr virus (EBV) genomes. Clinical presentation is usually in the young adult with fever, hepatosplenomegaly, lymphadenopathy and leukemic lymphocytosis.

The most common example of aggressive NK-cell leukemia in animals is that found in Fischer 344 rats, in which 10–30% or more of animals over 18 months of age develop a spontaneous neoplasm arising in the spleen. The disease is rapidly progressive with peripheral leukocyte counts that may range from near normal to over 300 × 10^9/L. Leukemic rats consistently have immune-mediated hemolytic anemia, thrombocytopenia and coagulopathy suggestive of disseminated intravascular coagulation. Terminally, the animals are icteric with increased hepatocyte-specific enzymes. Peripheral blood is characterized by anemia with anisocytosis, spherocytosis, and moderately responsive anemia. The neoplastic cells are similar to those in other species with nuclei 2–2.5 red cells in diameter with densely stained large chromocenters and irregular parachromatin clear areas typical of prolymphocytic leukemia in other species (Fig. 2.56). Prominent nucleoli are present and often multiple with quite abundant, usually eccentric, cytoplasm with moderate basophilia and a small number of quite large azurophilic granules. Histologically, there is diffuse infiltration and expansion of the splenic red pulp by tumor, with depletion of the follicles and some degree of erythrophagocytosis believed to be by neoplastic cells. The liver has prominent sinusoidal infiltration, focal portal infiltration, and centrilobular ischemic necrosis. Mildly involved lymph nodes have tumor cells in the peripheral and medullary sinuses, which progresses to architectural obliteration by neoplastic cells. Bone marrow is involved in less than half of animals which develop the disease, and is characterized by myeloid hyperplasia and progressive osteosclerosis. The neoplastic LGL cells in the rat stain positively with beta-glucuronidase and acid phosphatase.

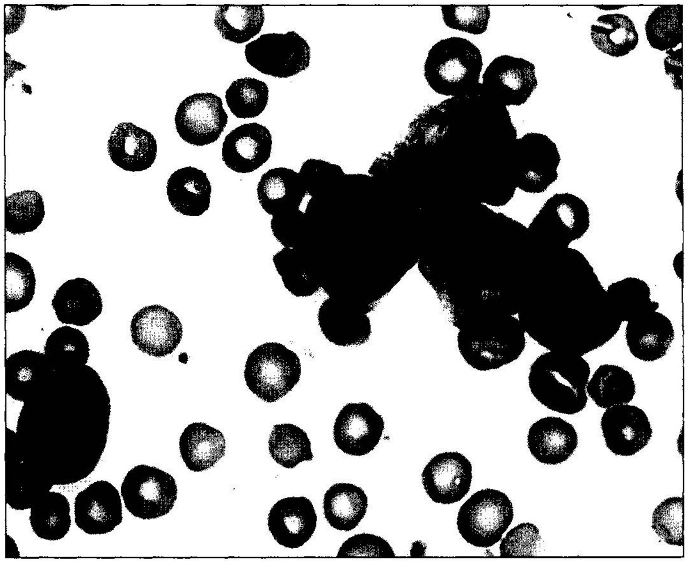

Figure 2.56 Aggressive NK-cell leukemia, blood, Fischer rat. Nuclei are generally 2 red cells in diameter with retention of some larger chromocenters in a finely woven chromatin pattern. Nucleoli are irregularly prominent. Cytoplasm is abundant, highly basophilic and contains numerous azurophilic granules. (Courtesy of RM Jacobs.)

It is likely that NK-cell leukemia occurs in both dogs and cats. The diseases are, as yet, undefined in those species. Cases of rapidly progressive leukemia with large granular lymphocytes in the dog and cat that are presented with splenomegaly and neoplastic cells in blood with the CD3-negative phenotype should be given more complete phenotypic and T-cell receptor analyses to further define this disease entity.

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Adult T-cell lymphoma/leukemia

Adult T-cell lymphoma/leukemia is a peripheral T-cell neoplasm in humans with a causal association with the human T-cell lymphotropic virus type-1 (HTLV-1). The FeLV-associated T-cell leukemia/lymphoma in the cat may be a counterpart for this disease in humans, and while *the lymphoma/leukemia associated with Bovine leukemia virus is of the B-cell type*, there may be pathogenetic as well as morphologic similarities in terms of induction by exogenous retrovirus. The human disease has a regional distribution based on endemic infection with the HTLV-1 virus, with a relatively high incidence of neoplastic transformation occurring in people in the south island of Japan and in the Caribbean basin. There are interesting morphologic similarities between the

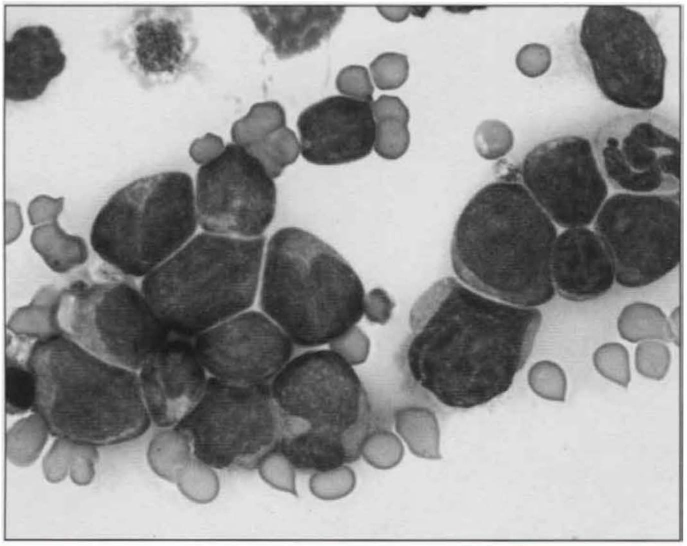

Figure 2.57 Adult T-cell lymphoma/leukemia, blood, cat, FeLV-positive. Nucleoli are present and irregularly apparent. Nuclei are 2 to 3 red cells in diameter and irregularly clefted, indented, or binucleated. Cytoplasm is moderate in volume and basophilia.

neoplastic cells in the human disease and those in animals with a retroviral-associated neoplasm. In humans, hyperlobated nuclei are frequently seen in cases of HTLV-1-associated lymphoma/leukemia known as the *floral variant, or flower cells*, and cells of this type are also seen in peripheral blood of BLV-associated lymphoma/leukemia in cattle and in FeLV-associated leukemia of cats (Fig. 2.57). In the human disease, there is always extensive lymph node enlargement, and bone marrow infiltrates tend to be patchy. Tumor cells express T-associated antigens CD2, CD3, and CD5, but usually lack CD7. Most cases are CD4-positive and CD8-negative; other combinations occur, but the disease is predominantly of helper-T-cell type. Genetic studies demonstrate clonally integrated HTLV-1 genes in all patients with this disease, and in all cases the T-cell receptor genes are clonally rearranged. Some human cases are lymphomatous and smoldering diseases with a relatively chronic progression, whereas the acute type, which is most common, presents with neoplastic cells in the blood, generalized lymphadenopathy with hepatosplenomegaly and often hypercalcemia.

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Extranodal NK/T-cell lymphoma, nasal type

The NK/T-cell lymphoma of nasal type was formerly called angiocentric lymphoma, and is a human disease having an NK-cell phenotype and expressing Epstein-Barr virus (EBV). There is a broad spectrum of cell types present, *with frequent necrosis and angioinvasion.* The presenting areas are most commonly in mid-facial region but also occur in other extranodal areas. The disease has been compared to lymphomatoid granulomatosis because of the angiocentric/angiodestructive features causing widespread necrosis, and very variable cellular population including neoplastic and reactive cells. The disease was *formerly known as lethal mid-line granuloma.* Polymorphic reticulosis and mid-line malignant reticulosis are the same or closely related lesions. Cells in the human lesion are CD2-positive but CD3-negative, and T-cell receptor arrangement is always absent. In paraffin sections, the neoplastic cells are usually positive for CD43 and CD56, but are negative for CD57 and CD11. The lesions are always positive for EBV genome.

It is not apparent that a counterpart to this disease occurs in animals, although lymphomas of the nasopharynx are a relatively frequent occurrence in both cats (Fig. 2.58A, B) and dogs. The disease in humans tends to present with mid-facial skin eruption that appears to be a progression of the lesion from the nasal sinuses. This form of presentation is unusual in animals, with lymphoid tumors of the nasopharynx tending to obstruct the airways and frontal sinuses with invasion of the cribriform plate. They may even involve tissues surrounding either eye, but tend not to be presented with a destructive lesion of the skin on the bridge of the nose.

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Enteropathy-type T-cell lymphoma

Enteropathy-type T-cell lymphoma in animals is *primarily a disease of dogs and cats* but likely occurs in other species. It is a disease of mature animals, which are presented with intermittent inappetence and diarrhea usually associated with some degree of weight loss. *The disease tends to involve the upper small intestine in both humans and animals and must be distinguished from inflammatory bowel disease of the lymphocytic–plasmacytic type, which it closely resembles.* In contrast to inflammatory bowel disease of the lymphocytic–plasmacytic type, there is usually much less lymphocytic proliferation in the submucosa, but there is prominent filling of the villi, and *the major distinction from inflammatory bowel disease of the lymphocytic–plasmacytic type is that there is a heavy but often segmental infiltration of the epithelium with small lymphocytes.* In histological sections, the neoplastic cells are found above the basal nuclei of the epithelial cells and apparently lie between adjacent epithelial cells and are characteristically surrounded by a clear

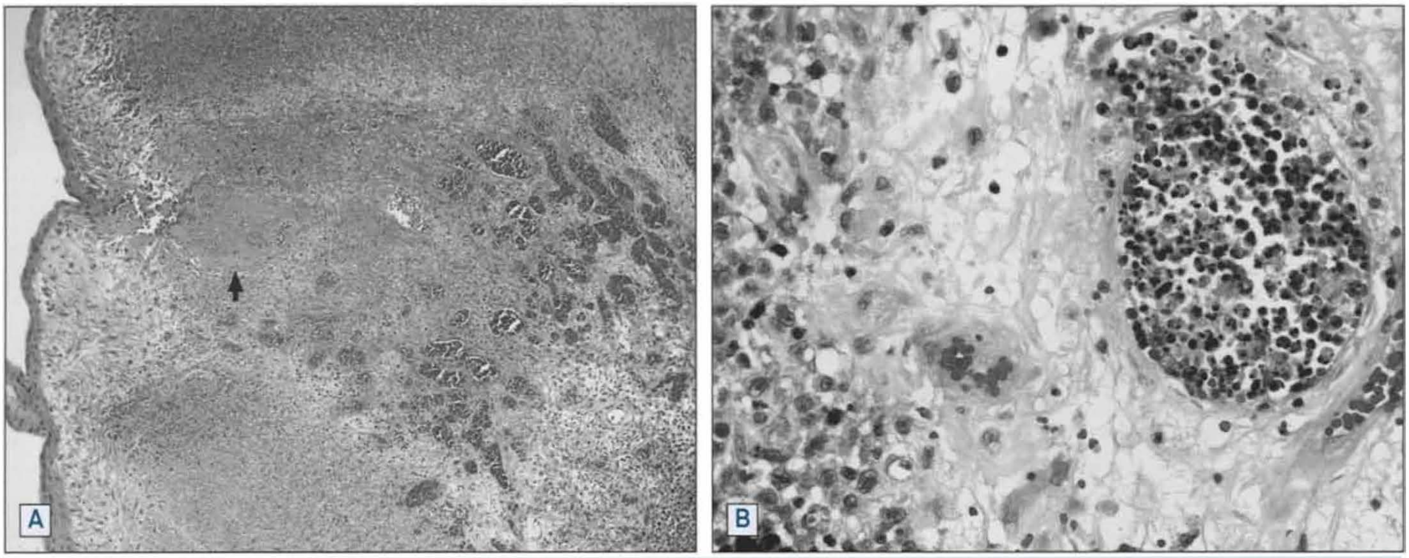

Figure 2.58 Extranodal lymphoma nasal type, nasal mucosa, cat. **A.** There is irregular dilation and thrombosis of mucosal vessels (arrow) and associated hemorrhage and infarction. **B.** Ischemic infarction of submucosa with extensive colonization and dilation of vein by tumor cells. The lesion was not phenotyped.

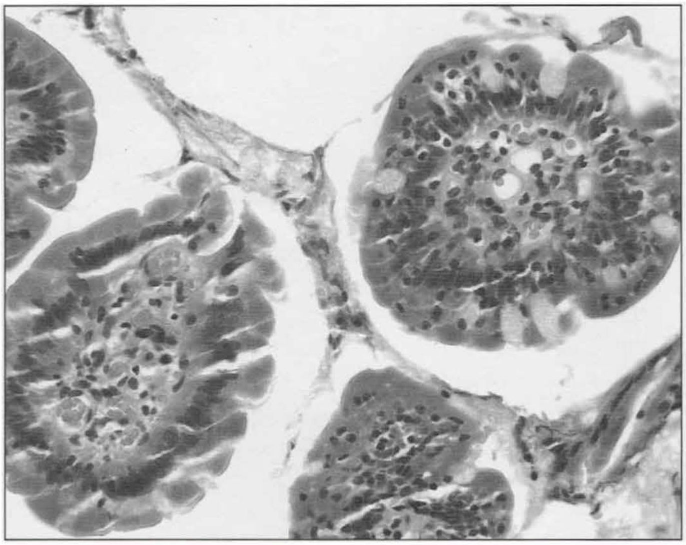

Figure 2.59 Enteropathy-type T-cell lymphoma, small intestine, cat. Early lesion characterized by irregular but extensive involvement of some villi with almost complete sparing of the epithelium of those immediately adjacent. Neoplastic nuclei are small, irregularly indented and deeply stained. Lamina propria and epithelial nuclei cells are CD3-positive. (Courtesy of FM Moore.)

halo, suggesting relatively abundant water-clear cytoplasm. In advanced cases, there is a blunting of villi and focal loss of epithelium, with much less heterogeneity of invading cells than is present in the lymphocytic–plasmacytic benign reactions. It has been suggested that *the key to early diagnosis of this disease is the presence of heavily involved villi with extensive epithelial invasion adjacent to others which have minimal or no involvement* (Fig. 2.59). This is in contrast to the benign lymphoid reactions of inflammatory bowel disease in which there tends to be relatively uniform involvement of all villi within the affected region of the small bowel.

In animals, the cells mark strongly with CD3, and in humans, the invading cells are CD3 and CD7 positive and usually CD8 positive and CD4 negative. Some cases of the human disease express cytotoxic T-cell-associated proteins granzym-B, TIA-1 and perforin.

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Hepatosplenic $\gamma\delta$ T-cell lymphoma

Hepatosplenic $\gamma\delta$ T-cell lymphoma is a neoplasm of mature, nonactivated, cytotoxic T cells, with prominent sinusoidal infiltrations of the spleen, liver, and bone marrow. The disease occurs rarely in humans and is *recently defined in animals*. It has been found that over one-third of the benign T cells in the splenic sinus areas are of the α_D β_2 positive $\gamma\delta$ T-cell type. *It has been suggested that the LGL lymphocytosis occurring in dogs may arise*

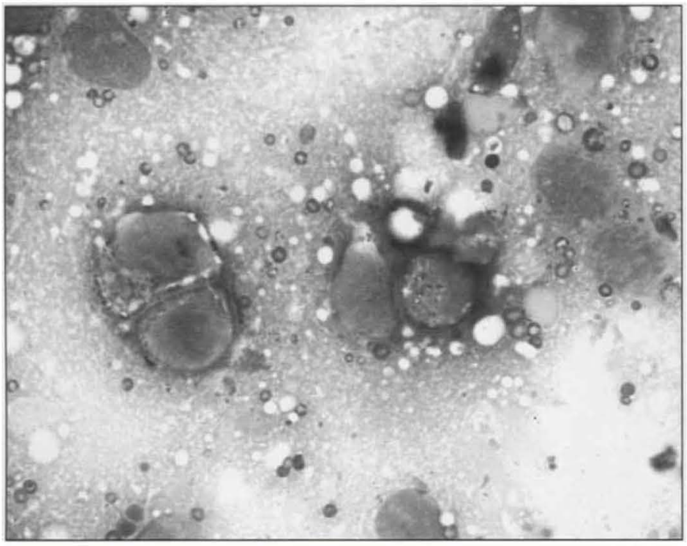

Figure 2.60 Hepatosplenic T-cell lymphoma, imprint of hepatic Tru-cut biopsy, cat. The nuclei are large and 2 to 3 red cells in diameter with relatively abundant cytoplasm and heavily granulated with large deeply azurophilic granules. The chromatin is coarsely granular with multiple small nucleoli. (Courtesy of RM Jacobs.)

from the large population of $\alpha_D\beta_2$ lymphocytes normally resident in the splenic red pulp.

In hepatosplenic γδ T-cell lymphoma of humans, the immunophenotype is generally positive for CD2, CD3, and CD16, negative for CD4, CD5, and CD8, with CD56 giving variable reactions. The neoplastic cells lack the αβ TCR protein and express the γδ complex. Typically, there is expression of the cytotoxic granule protein T1A-1, but granzyme-B and perforin are usually absent.

Clinically, the disease has only recently been characterized in humans and its occurrence in animals is still undefined. It is believed that previously cases occurring in humans were classified as T-cell CLL, with prolymphocytic leukemia or peripheral T-cell lymphoma of unspecified type. Most cases occur in young adult males who present with marked hepatosplenomegaly. There is usually minimal marrow involvement on presentation but neoplastic cells are present in the blood. The disease has been reported in patients receiving immunosuppressive drugs for solid organ transplants. Despite the relatively small size of the tumor cells, with relatively mature chromatin and low mitotic rate, the disease follows an aggressive pattern, often with prominent erythrophagocytosis by tumor cells in the spleen and liver. *A rare and highly aggressive hepatosplenic lymphoma occurs in cats and dogs in the absence of lymphadenopathy*; the disease in the cat is characterized by very large cytoplasmic granules (Fig. 2.60).

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Subcutaneous panniculitis-like T-cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma is defined in humans as a T-cell lymphoma that preferentially infiltrates subcutaneous tissue with variably sized neoplastic cells and characterized by prominent necrosis, karyorrhexis and accompanying benign inflammatory reaction. It is not clear that there is a well-defined counterpart of this tumor in animals. The characteristic non-epitheliotropic (i.e., not mycosis fungoides type) neoplasm in animals is a relatively "high-up" lesion that usually has a narrow Grenz zone or sparing of tissues in the superficial papillary dermis, with encirclement but not penetration of the adnexa and extension through the deep dermis into the subcutaneous tissues. These lymphomas are characterized by relatively small densely stained cells surrounded by a characteristically water-clear halo with relatively few mitotic figures. These lymphomas in animals mark strongly for CD3. They usually occur as single lesions, and appear to be the primary site of neoplastic transformation and not extensions from lymphoma in other sites. Alternatively, T-cell lymphoma may occur in animals, particularly in dogs, with multiple plaque-like areas of skin involvement and characterized by a very anaplastic neoplastic population described below as anaplastic large cell lymphoma T/null cell primary cutaneous type.

The panniculitis type of lesion in humans outwardly resembles a focus of fat necrosis and cases apparently may go through multiple biopsies before the diagnosis is changed from chronic panniculitis to T-cell lymphoma, assisted by increasing anaplasia in the neoplastic cells with disease progression. The neoplastic cells of human panniculitis like T-cell lymphoma express most T-cell antigens and they are usually CD8 positive but may be CD4 positive and express cytotoxic granule proteins TIA-1 and perforin. Most human cases express the αβ TCR rearrangement of occasional cells derived from γδ T cells. Remarkably, there is a high proportion of human cases of panniculitis that progress to a fatal hemophagocytic syndrome. The latter form of the disease progression does not appear to have a counterpart recognized in animals.

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Mycosis fungoides/Sézary syndrome

Mycosis fungoides occurs in humans and animals, where it is seen most frequently in dogs, but also occurs in cats and rarely in other domestic animals. *One of the more common presentations in animals is in the cutaneous type of T-cell lymphoma characteristic of 2-year-old cattle*

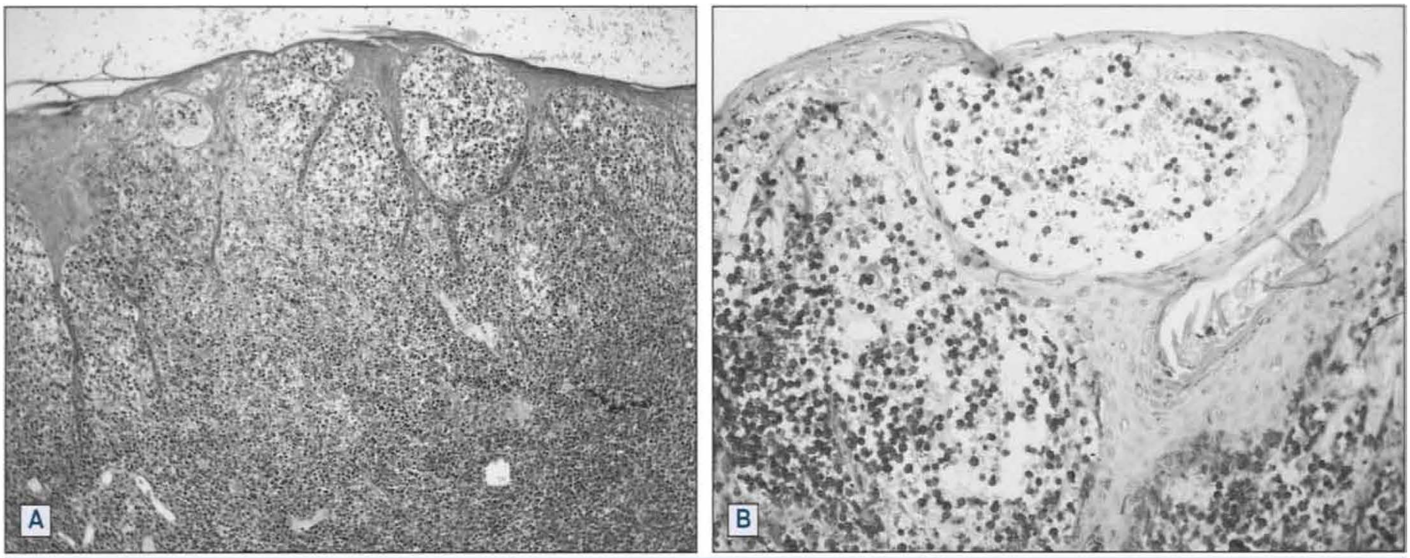

Figure 2.61 Mycosis fungoides/Sézary syndrome, skin, cat. **A.** There is marked cellular invasion of the reticular and superficial dermis with irregular cavitation of the epidermis and colonization of neoplastic cells. **B.** CD3 staining demonstrates T-cell phenotype of cells in superficial dermis and intraepithelial cysts (Pautrier's microabscess).

where the disease is not associated with Bovine leukemia virus. In the cow, the disease occurs as multiple plaque-like lesions with depilation and often ulceration, apparently due to irritation and rubbing on the part of the host. There may be systemic progression of the disease in dogs and cats, as is characteristic for cattle. These lesions wax and wane over a period of time and may become quite large, up to 10 cm or more in diameter, and because of the tendency of the lesions to be circular, they have been mistaken for ringworm infestations. The disease progresses relatively slowly, but over a period of a year, the lesions become systemic with involvement of the liver, spleen, lymph nodes, and bone marrow with the appearance of relatively characteristic **Sézary cells**, with convoluted nuclei in the peripheral blood.

Mycosis fungoides in other animals is a characteristic presentation of **epidermotropic lymphoma**, with proliferation of neoplastic cells immediately adjacent to the epidermal basement membrane and the formation of *tumor cell cysts (Pautrier's microabscesses)* in the epidermis, which is characteristically thickened over areas of tumor formation (Fig. 2.61A, B). There is generally some degree of sclerosis of the papillary dermis in areas of tumor formation and the disease regularly progresses to epidermal ulceration. From the deeper surface, the lesions progress through the reticular dermis, but tend not to invade deeply into the underlying structures. In both the dog and cat, the epidermotropic type of cutaneous T-cell lymphoma is a *disease of mature animals* with a mean age of onset in both species of about 10 years. The subtype of epidermotropic lymphoma in which the neoplastic cells primarily colonize the hyperplastic epidermis, known as *pagetoid reticulosis* or *Woringer-Kolopp disease*, is a very rare occurrence in the dog, and may occur in other species (Fig. 2.62A, B). The disease is indolent, but apparently has the potential to progress to systemic lymphoma.

The cytologic features of epidermotropic lymphomas in animals are similar to those in humans with tumor cells of intermediate to medium size and nuclei with moderate irregularity in nuclear shape. Ultrastructurally, it has been shown that the cells of mycosis fungoides in animals, and particularly the cow, have *cerebriform or deeply enfolded nuclear membranes*, like the counterpart in humans, and retain these characteristics on progression to the leukemic form, with Sézary-type cells in the peripheral blood. The chromatin has a granular or large chromocenter distribution without parachromatin clearing, and nucleoli are usually not evident in histological preparations. The neoplastic cells in animals regularly mark with CD3 and in humans the tumor cells are typically CD2, CD3, CD5 positive, with variable expression of CD7, and most cases express the β type of T-cell receptor. Most human cases are CD4 positive and it might be expected that the same situation prevails in animals, which would explain the tendency for animals to rub or injure the sites of tumor formation, however in dogs at least, most cases are of CD8 type.

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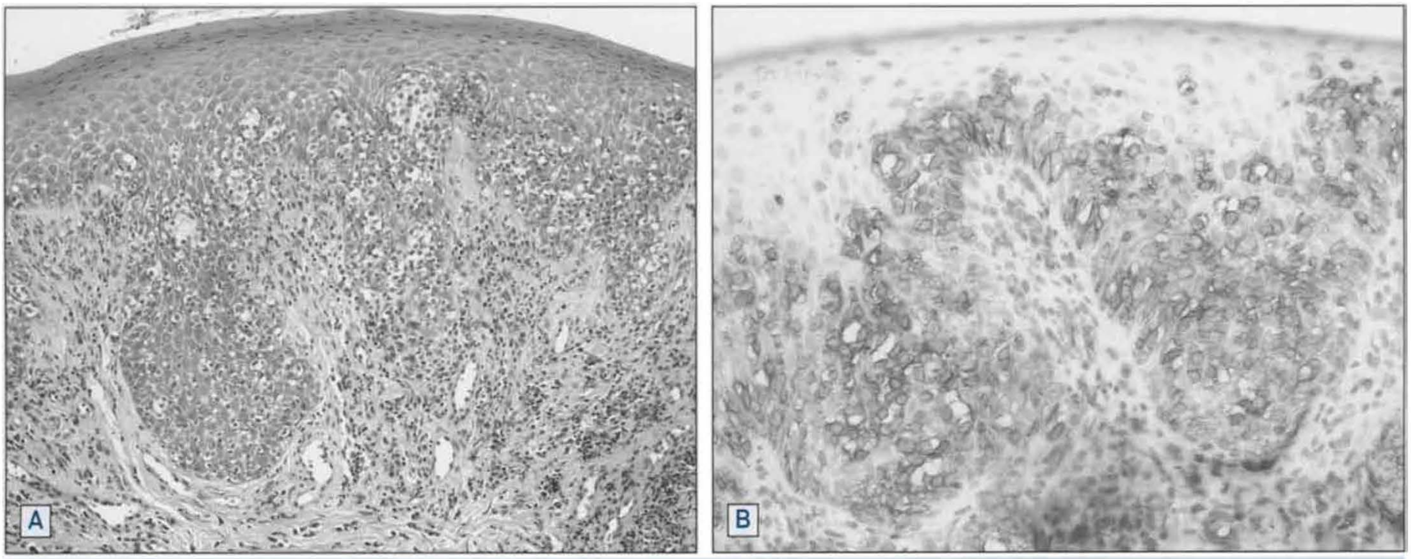

Figure 2.62 Pagetoid reticulosis/Worringer-Kolopp disease, skin, dog. **A.** There is epidermal thickening with infiltration of neoplastic cells into the epidermal basal cells forming small solidly cellular cysts of neoplastic cells. The cellular infiltration in the superficial dermis is of mixed B- and T-lymphocyte type, and benign. **B.** CD3 staining to demonstrate T-cell phenotype of the cells selectively colonizing the epidermis.

Anaplastic large-cell lymphoma, T/null cell, primary cutaneous type

Anaplastic large-cell lymphoma (ALCL) is defined as a neoplasm presenting in the skin without pre-existing lymphoproliferative disease and no current evidence of extracutaneous involvement at the time of diagnosis. ALCL, as it is described in humans, occurs in *mature dogs and cats*, and possibly in other species. In animals, ALCL appears to begin with involvement of the vessels of the papillary dermis and progresses to deep involvement of the dermis with irregular extension into subcutaneous structures. Cytologically, the tumors are characterized by remarkable irregularity of nuclear form with folded, indented and multinucleated shapes all present. Cytologically, the neoplastic cells have peripheralized chromatin, with irregular parachromatin clearing, and prominent but not large nucleoli (Fig. 2.63A–C). The neoplastic cells have relatively abundant cytoplasm, and cell boundaries are generally indistinct. ALCL of the primary cutaneous type in animals is accompanied by quite heavy infiltration of smaller benign cells of both B- and T-cell type. The tumor cells are highly variable on immunohistochemistry, and while they are negative for CD79α, they may also be negative for CD3, but in any particular lesion, some cells with highly atypical and presumed neoplastic nuclei are associated with positive CD3 cytoplasmic staining (Fig. 2.63C). Clinically, the disease may appear as a solitary plaque or as an erythematous nodule. The progression of the disease is not defined and, in some cases, extra patience may be curative.

In the human counterpart, the neoplastic cells mark for T-cell antigens and are usually CD4 and CD30 positive. In humans, the cutaneous form of ALCL is distinguished from the systemic form by the cells not expressing the ALK or epithelial membrane antigen.

Anaplastic large-cell lymphoma, T/null cell, primary systemic type

The primary systemic form of anaplastic large T- or null-cell lymphoma (ALCL) is defined as the neoplasm of large lymphoid cells with pleomorphic, often multi-lobated nuclei, abundant moderately basophilic cytoplasm, and a cohesive pattern of tumor expansion. The disease as it occurs in animals is rare, and is typically seen in a large breed dog of 1 year or less of age. The neoplastic cells in a particular case mark uniformly and strongly with CD3. The animals have a rapid onset of disease associated with edematous swelling of the limbs, with multiple areas of eryrthematous skin plaque oozing serum and lymphadenopathy. Cytologically, the tumor cells have the same anaplastic appearance as the primary cutaneous type with folded and multi-lobated neoplastic cells with peripheralized chromatin and prominent, often multiple, nucleoli with a moderate amount of cytoplasm. The lesions in the skin are characterized by deep involvement of the subcutaneous tissues and larger vessels, which are frequently invaded and obstructed by the neoplastic cells. There does not appear to be a particular angiotropism, but rather the tumor moving in a solid progression and tends to invade vessels, causing widespread necrosis and karyorrhexis. Lymph nodes have effacement of normal architecture with diffuse involvement of the glands and irregular bridging of the peripheral sinus with extranodal involvement (Fig. 2.64A–C). Animals are apparently not leukemic, but there is a tendency to involve the liver, spleen and gastrointestinal tract. In humans, the neoplastic cells are positive for CD30 and usually CD25 and ALK or epithelial membrane antigen. About 2/3 of human cases express CD3 and CD43. In the majority of human cases, the TCR genes are rearranged and 1/4 or more cases have no rearrangement of T-cell receptor or immunoglobulin genes. A t(2;5) translocation is present in up to 1/2 of human cases, which results in fusion of the nucleophosmin gene on chromosome 5 with a tyrosine kinase gene on chromosome 2 called *ALK* (anaplastic lymphoma kinase). In humans, the disease in both children and adults, while aggressive, seems to be potentially curable.

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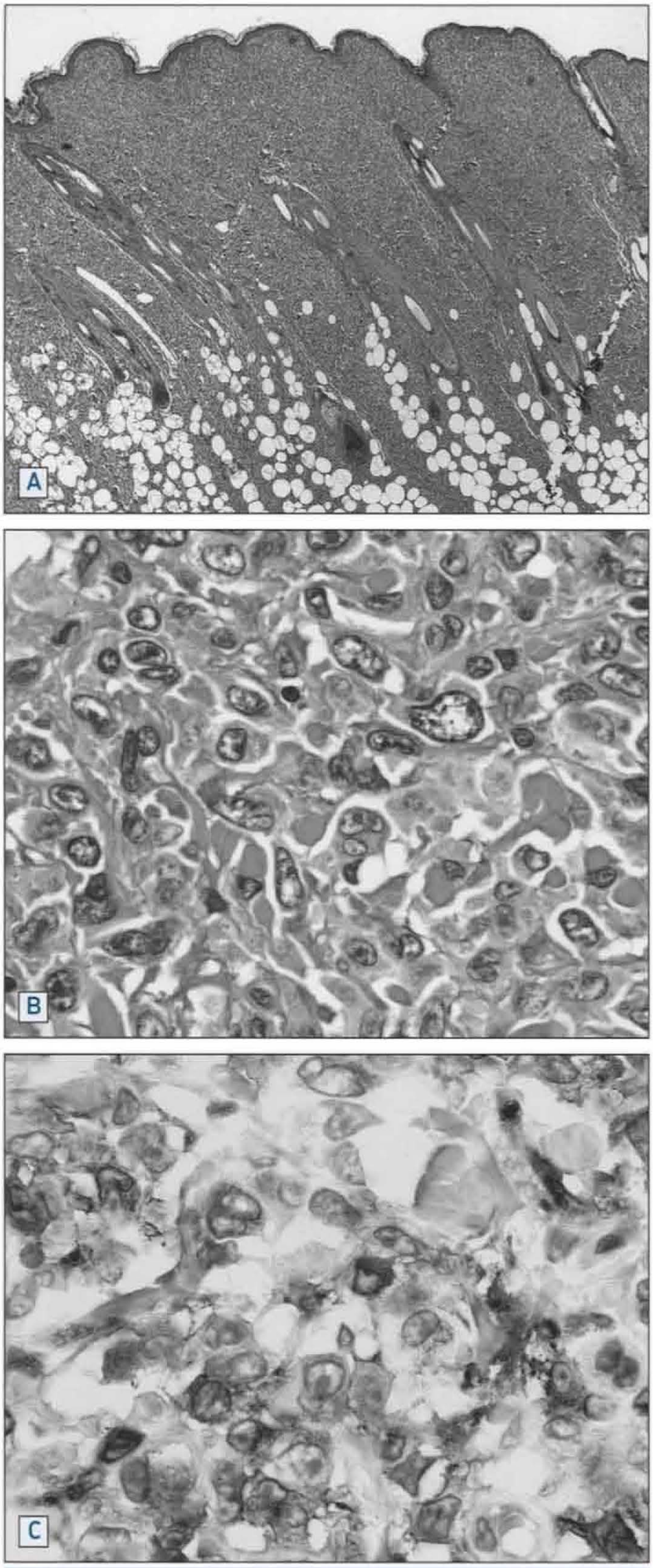

Figure 2.63 Anaplastic large-cell lymphoma, primary cutaneous type, skin, dog. **A.** There is solid and uniform infiltration into the superficial and reticular dermis with extension of the tumor through the adnexa and into subcutaneous fat. **B.** Nuclei are highly irregular in size and shape but have similar peripheralization of chromatin with deposition on the nuclear membrane, irregular parachromatin clearing and prominent, often multiple, nucleoli. The cytoplasm is relatively abundant and highly basophilic. **C.** CD3 staining to demonstrate T-cell phenotype of large atypical cells.

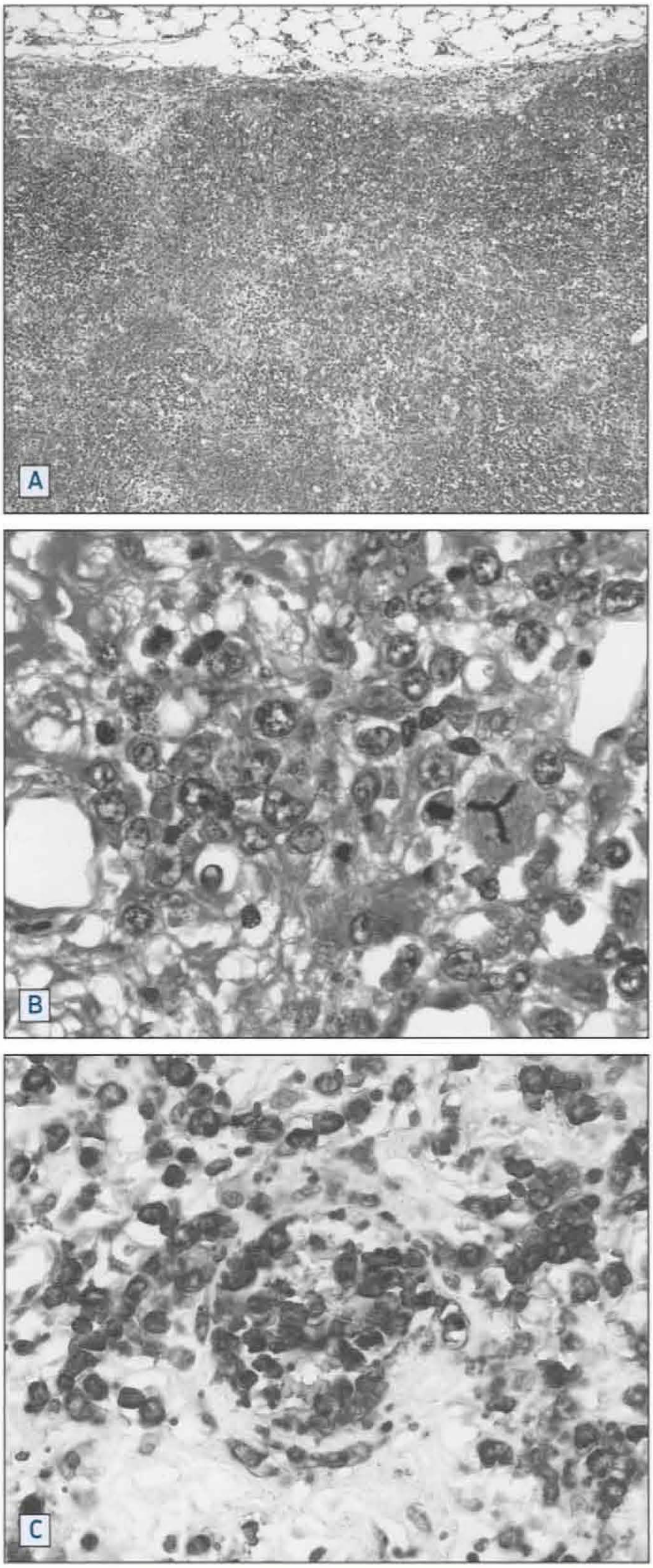

Figure 2.64 Anaplastic large-cell lymphoma, primary systemic type, lymph node, dog. **A.** There is diffuse involvement of the node, bridging of the peripheral sinus and involvement of perinodal tissues. Darker staining areas represent fading cortical germinal centers with diffuse involvement by tumor cells with effacement of architecture with focal ischemic and hemorrhagic infarction. **B.** The neoplastic lymphocytes have nuclei 1.5 to 2.5 red cells in diameter with irregular deposition of chromatin on nuclear membranes, parachromatin clearing and prominent nucleoli. Mitoses and single cell necrosis are frequent. **C.** CD3 staining of neoplastic cells dilating and invading small veins indicating T-phenotype.

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Peripheral T-cell lymphoma, not otherwise characterized

The category of peripheral T-cell lymphoma (PTCL) includes a spectrum of diseases in humans with the disease generally occurring in a lymph node or other lymphoid tissue, including spleen or Waldeyer's ring. *A variety of morphologic subtypes have been described with no consistent immunophenotypic, genetic or clinical features associated with them.* In humans, PTCL is typically composed of a mixture of small and large atypical cells which could be classified as diffuse, small-cleaved, mixed small and large, large cell or immunoblastic types, as used in the Working Formulation classification. The presence of an inflammatory background, including eosinophils and epithelioid macrophages, is common in some types of the tumors, with a subtype of these lesions characterized by focal clusters of histiocytes known as *lymphoepitheloid cells or Lennert's lymphoma.* Phenotypically, the neoplastic cells in humans are variable in antigen expression as in morphology, and mark variably for CD3, CD2, CD5, and CD7. CD4 is present more often than CD8, and the tumors may be double negative for CD4n and CD8. A more consistent finding is that B-cell associated antigens are always lacking. No specific cytogenetic or oncogene abnormality is identified with the variant subtypes of PTCL in humans.

PTCL in animals is equally lacking in definition, but several characteristic disease types occur. In the **dog**, the diagnosis of peripheral T-cell lymphoma is most often applied to a neoplasm of lymph nodes characterized by lesions with diffuse effacement of node architecture and the occasional occurrence of isolated, very large nuclei of 10 or more red cells in diameter (Fig. 2.65A–C). The lesions mark variably with CD3 and are negative for CD79. A much less common occurrence in the dogs is a subcutaneous lesion that resembles T-cell lymphoma of the panniculitis type, except that there is actual granuloma formation, and fat necrosis is a feature only at the periphery of these lesions. These neoplasms are remarkably pluricellular, with plasma cells, eosinophils, macrophages and fibroblasts. These lesions are usually subjected to multiple stains for identification of infectious agents or foreign bodies before neoplasm is suspected. The lesions are characterized by fine vessel proliferation and prominence with generalized sclerosis. The malignant T cells tend to cluster near the vessels and have relatively abundant cytoplasm. The nuclei are round, oval or indented with peripheralized chromatin, quite marked

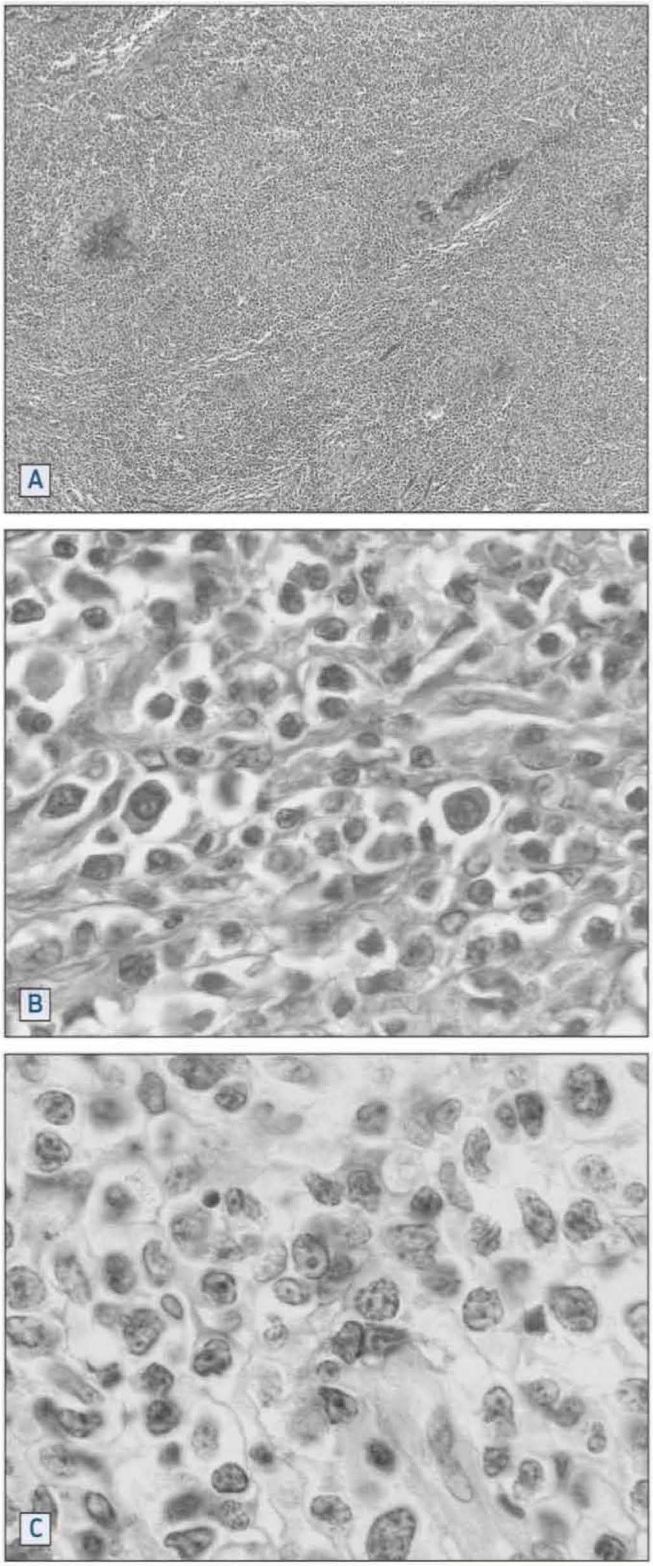

Figure 2.65 Peripheral T-cell lymphoma, not otherwise characterized, subcutaneous mass, abdomen, dog. **A.** The lesion has diffuse architecture with focal areas of necrosis surrounded by fibroblasts and large macrophages resembling an infectious granuloma. **B.** There is vibrant reticular sclerosis, mixed inflammatory cell type, including small, medium and large lymphocytes, eosinophils and plasma cells. Large cells with prominent nucleoli and relatively abundant basophilic cytoplasm occur singly, often near small vessels. **C.** CD3 staining demonstrating positive marking of a subset of large cells with atypical nuclei.

parachromatin clearing and a single prominent nucleolus that defines them as the only atypical-appearing cells in the lesion (Fig. 2.65B). Remarkably, these cells may constitute 5% or less of the lesion and appear to be a T-cell counterpart of the T-cell/histiocyte-rich B-cell lymphoma, where the neoplastic B cells are at the basis of the reaction but may be a small minority of the cells present. The neoplastic T cells mark irregularly with CD3, and the benign cells mark as would be expected for their lineage (Fig. 2.65C).

In the **cat**, peripheral T-cell lymphoma may take on a variety of histological subtypes, but characteristically is present in a cervical lymph node. These may become remarkably enlarged, and 4 cm or more in longest dimension, constituting discoid lesions lying on or beneath the jugular vein. Cases have been observed where the lesion may recur in the same site a year or more after the initial excisional biopsy, in a pattern of spread to contiguous lymphoid tissues not unlike that occurring in Hodgkin lymphoma in humans. The affected nodes have focal areas of benign follicular hyperplasia, but the major expansion is due to proliferation of medium-sized lymphocytes that resemble centroblasts with the scattered appearance of very large cells with nuclei 5 to 10 red cells in diameter and nucleoli as large as red cells. These atypical cells have abundant cytoplasm and are frequently undergoing single cell necrosis, with the cytoplasm becoming increasingly eosinophilic and retracting from surrounding cells. The nuclei are densely stained and pyknotic and progress to karyorrhexis but not apoptosis. There are scattered small lymphocytes and plasma cells throughout these lesions that mark strongly for CD79α, but the majority of the cells mark weakly for CD3 with the very large atypical cells often negative for both B- and T-cell reagents. These diseases in cats are remarkably indolent and tend to recur at the same site over a period of years. Neoplasms of this type in the cat have been called T-cell-rich/histiocyte-rich B-cell lymphoma, and it is likely that lesions of this type have promoted the feeling that a counterpart of Hodgkin lymphoma in humans may occur in the cat.

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Angioimmunoblastic T-cell lymphoma

An initial description of human angioimmunoblastic T-cell lymphoma was termed "*angioimmunoblastic lymphadenopathy*" because the disease was not felt to be uniformly a malignant neoplasm. The initial definition was a lymphoproliferative disorder associated with systemic signs, generalized lymphadenopathy, hepatosplenomegaly, immunologic dysfunction, and polyclonal hypergammaglobulinemia. A latter finding of dysproteinemia prompted the use of the acronym **AILD** indicating *angioimmunoblastic lymphadenopathy with dysproteinemia*. Subsequently, the term "*peripheral (now mature) T-cell lymphoma*" was suggested to include neoplasms with mature T-cell proliferations with broader presentation than that defined for AILD. The similarities between peripheral T-cell lymphoma (PTCL) and AILD included diffuse architectural effacement of nodes, a pleomorphic lymphoid proliferation, and prominent postcapillary venules. PTCL was felt to lack the inflammatory component of plasma cells and eosinophils seen in AILD; however, it became apparent that neither description was sufficiently specific and overlap between the two conditions occurred. For economy of terms of understanding, *it has been suggested that AILD is a particular form of peripheral or mature T-cell lymphoma*. AILD in humans is characterized by a number of unique features that suggest it needs to be considered a separate disease entity. These include frequent concurrence with immunodeficiency, polyclonal hypergammaglobulinemia and occasionally the coincident appearance of clonal B-cell proliferation verified by immunoglobulin gene rearrangements. Since some cases considered to be AILD lack both clonal T- and B-cell receptor gene rearrangements, these may constitute a *benign form of AILD* that might progress to clonal proliferations.

The characteristic histologic features in humans are effacement of nodal architecture with peripheral sinuses open and occasionally dilated, but with focal tumor involvement of perinodal tissues. Likely, the most characteristic feature of AILD that appears to occur in animals as well as humans is the *prominent proliferation of high endothelial venules*, many of which have thickened or hyalinized walls and appear more eosinophilic than normal with H&E staining. There are focal aggregates of dendritic cells surrounding the small vessel proliferation that may suggest the presence of an obsolescent follicle. The lymphoid complement is variable and consists of small-cleaved cells, others resembling the larger type of centrocytes and centroblasts, plus plasma cells, and finally large immunoblasts. The latter have a vesicular appearance with peripheralized chromatin, irregular thickening of the nuclear membranes and finely branched chromatin extending from a large central nucleolus to the nuclear membrane. Cytoplasm is usually abundant and pale staining.

AILD cells in humans mark with T-cell-associated antigens and usually CD4, but many CD8 positive cells can be present and may be benign. The follicular dendritic cells mark with CD21, and identification of the FDCs adjacent to fine vascular proliferation is an important diagnostic feature. Genetic changes include rearrangement of T-cell receptor genes in 3/4 of the patients, and an immunoglobulin heavy chain in 10% of cases, with Epstein-Barr virus (EBV) genomes apparently frequently detected and may be found in either T or B cells.

The clinical presentation in humans includes generalized lymphadenopathy, fever, weight loss, recurrent skin rashes, polyclonal hypergammaglobulinemia, and a history of recurrent infections. The course tends to be aggressive.

AILD in animals is most often seen as the disease of lymph nodes in dogs. The most characteristic changes are diffuse effacement of nodal architecture with atrophy of germinal centers and prominent arborization of small vessels with thickened endothelial walls (Fig. 2.66A–C). Numerous cells are marked with both CD79α and CD3 reagents, but the immunoblastic-appearing cells with the prominent single central nucleolus typically mark convincingly with CD3. Dogs with AILD tend to have a history of recent loss of weight and appetite, but the presence of dysproteinemia and unusual susceptibility to infections has not been recognized.

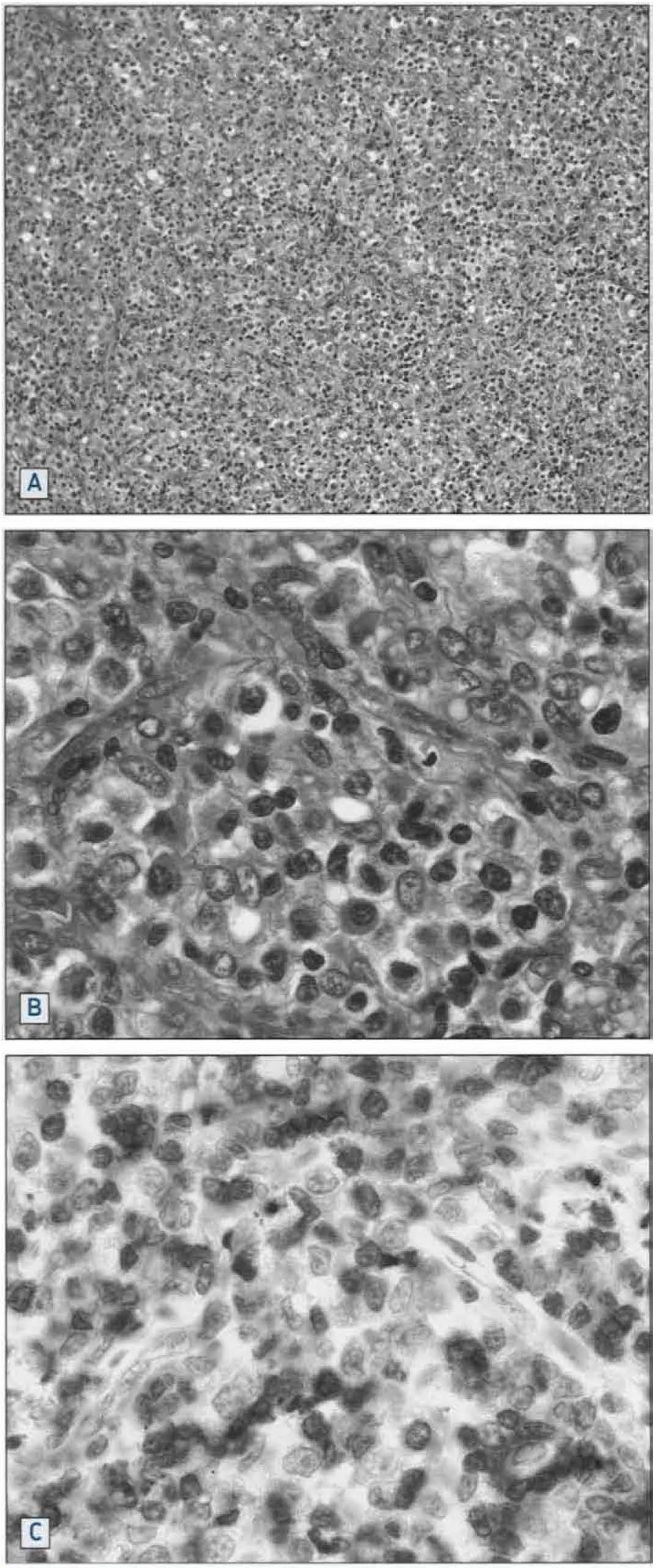

Figure 2.66 Angioimmunoblastic T-cell lymphoma, node, dog. **A.** There is obliteration of normal node architecture with a very prominent pattern of small vessel proliferation. **B.** The cellular infiltration is variable, including small, medium and large lymphocytes, plasma cells, fibroblasts and endothelial cells. **C.** CD3 stain demonstrating marking of small, medium and large cells as phenotypically T-cell derived. On staining with CD79α, only cells with plasmacytic appearance were marked.

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Hodgkin lymphoma

Recent technology permitting analysis of genes in single cells using polymerase chain reaction provided new information on the origin of **Reed-Sternberg (RS) cells** in Hodgkin lymphoma. Current classifications of Hodgkin lymphoma include the *three subtype categories – nodular sclerosis, mixed cellularity, and lymphocyte depletion* – in the single designation of *classic Hodgkin disease*. This grouping is to draw attention to the close histologic similarities among these subtypes of Hodgkin lymphoma and their distinction from the lymphocyte-predominant subtype. *There is ongoing concern with the definition of the lymphoid-predominant type of Hodgkin lymphoma because of the close similarity between this disease and some cases of T-cell-rich B-cell lymphoma.* It is in the possibility for confusion between these latter two diagnoses that consideration of Hodgkin lymphoma is of concern to animal pathology. The possibility for a variant form of Hodgkin lymphoma in animals is plausible, since *while Hodgkin lymphoma is generally not considered to occur in animals, the T-cell-rich B-cell lymphoma is recognized in most domestic species.*

An unusual lymphoma occurs in **cats** that has a unique presentation, morphology and biology. Animals 10 or more years of age are presented with an enlarged cervical node that may be >4 cm in length, discoid in shape and unilateral. They are encapsulated and relatively mobile, but excision may be followed in months or a year or more by regrowth apparently in another node in the same or contiguous area. Histologically, they resemble the T-cell/histiocyte-rich variant of large B-cell lymphoma in having a background of sclerosis with small and medium T cells, but scattered huge cells with nuclei 10 or more red cells in diameter and relatively abundant cytoplasm. These cells are variable in size and shape and may occur in binucleated forms with large nucleoli suggesting similarity to Reed-Sternberg cells of human Hodgkin lymphoma (Fig. 2.46B). The nuclei of these large cells mark with CD79α, but their cytoplasm does not or only rarely. They are positive with BLA36 and are frequently undergoing single-cell necrosis, like the RS cells of Hodgkin lymphoma. Like the situation in human pathology with the T-cell-rich/histiocyte-rich B-cell lymphoma, *this disease in cats is not well characterized.*

Single-cell studies from the classic grouping of Hodgkin lymphoma point to the *origin of the RS cell from a postgerminal center B-lymphocyte that has undergone immunoglobulin gene hypermutations.* In contrast, single-cell studies on RS cells from lymphocyte-predominant Hodgkin lymphoma have shown that these appear to be derived from B cells undergoing continued mutations within clonal immunoglobulin gene rearrangements. *This suggests that somatic hypermutation of immunoglobulin genes in RS cells appears to be an ongoing* *process in lymphocyte-predominant Hodgkin lymphoma.* Since the RS cells of classic Hodgkin disease are not characterized by ongoing mutations and appear to be derived from postgerminal center memory B-cells, there appears to be major differences in these genotypic subsets.

Others have suggested that the RS cells of Hodgkin lymphoma might be derived from follicular dendritic cells. This approach is prompted by the finding that both follicular dendritic cells and the RS cells of Hodgkin lymphoma mark with CD21 antibody. In one review, a subgroup of Hodgkin lymphoma had RS cells that expressed CD21 without any other B-cell marker. Further, in this subgroup of patients, none had RS cells with rearrangements of either the immunoglobulin heavy chain gene or the T-cell receptor genes. Other studies have suggested that the RS cells have clonal T-cell receptor gene rearrangements and expression of T-cell antigens. It has been further demonstrated that in some cases, RS cells have the presence of cytotoxic molecules including granzym-B, perforin and TIA-1 that are normally found in cytotoxic T lymphocytes and natural killer cells. Since these RS cells did not express other markers of natural killer cells, but did express T-cell antigens, *these RS cells appear to be derived from cytotoxic T lymphocytes.* It was concluded that the derivation of RS cells from T lymphocytes and in particular cytotoxic T-cell lymphocytes appears to occur in a small percentage of cases of classic Hodgkin lymphoma.

These findings demonstrate that *Hodgkin lymphoma represents a spectrum of diseases in which it is becoming clear that subtypes identified by immunophenotypic analysis have strong prognostic importance.* There is, therefore, an effort to redefine Hodgkin lymphoma with the incorporation of immunologic criteria in order to differentiate Hodgkin lymphoma and the RS cell from cases of non-Hodgkin lymphoma that are histologically similar and in some cases morphologically indistinguishable. The succinct definition of Hodgkin disease that unifies the different histologic subtypes is the *identification of diagnostic Reed-Sternberg cells in the appropriate cellular environment.* To assist this definition, the *immunological criteria for identifying RS cells in classic Hodgkin lymphoma include*: CD15-positive, CD30-positive, vimentin-positive, fascin-positive, CD40-positive, CD45-negative, CD20-plus/minus, CD3-negative, EMA-negative, and ALK-negative. The appropriate cellular environment is described as a surrounding population of T cell, predominantly CD4-positive and TIA-1-positive. *Veterinary pathologists will need to be aware of these criteria in proposing that lymphoproliferative diseases in animals may be a counterpart of human Hodgkin lymphoma* (Fig. 2.67A–C).

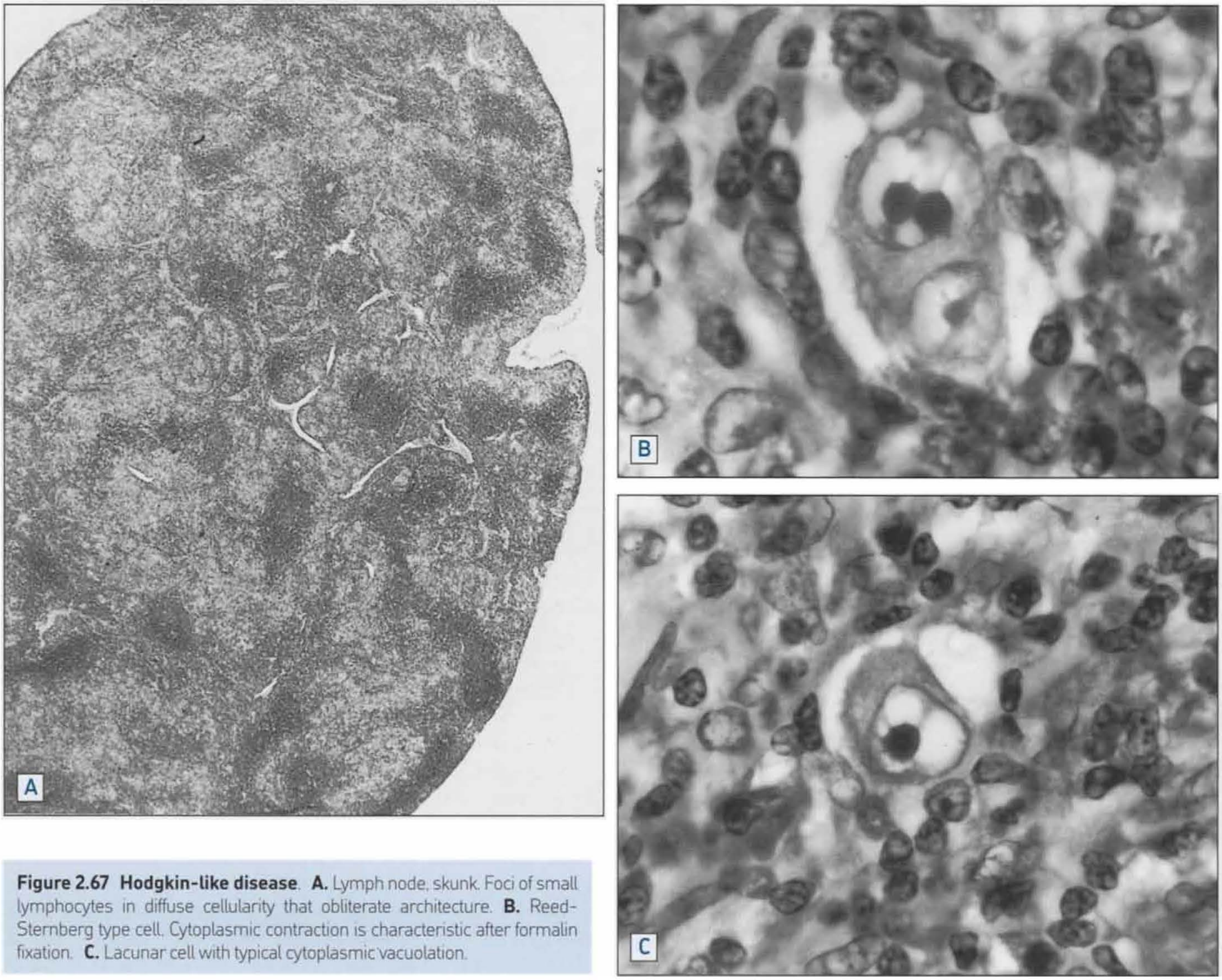

Figure 2.67 Hodgkin-like disease. A. Lymph node, skunk. Foci of small lymphocytes in diffuse cellularity that obliterate architecture. **B.** Reed-Sternberg type cell. Cytoplasmic contraction is characteristic after formalin fixation. **C.** Lacunar cell with typical cytoplasmic vacuolation.

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Lymphoma in animals

The general pathology and classification of lymphoma are discussed in the preceding sections. The following sections deal with the epidemiologic and topographical aspects of lymphoma in several domestic species.

Bovine lymphoma

Lymphoma in adult cattle is very largely the **enzootic bovine leukosis form** *associated with retroviral infection* that is transmitted horizontally by direct contact, including natural breeding and accidental transmission by contaminated needles, ear-tagging and dehorning equipment and by the use of whole-blood vaccines. Infection, once established, is lifelong and is characterized by the development of circulating antibody, which is also present throughout life. Antibody levels tend to increase with the number of viral antigens recognized, as the animal passes from an asymptomatic carrier, to lymphocytosis, and to tumorous state (should malignant disease develop). *Bovine leukemia virus (BLV)* is highly cell-associated, and viremia does not occur unless in the period immediately following infection and before antibody is formed. The virus survives in proviral state in bone marrow-derived lymphocytes and monocyte/macrophages, which may be induced to release the C type retrovirus on short-term in vitro culture in the absence of antibody.

The dynamics of infection in a particular animal and the spread of virus within a herd are highly dependent on genetic factors in both the virus and cattle. Progression of infection in an individual animal to persistent lymphocytosis is under control of the bovine major histocompatibility complex mapping adjacent to *BoLA* class II genes. Resistance factors in some animals control the rate of progression and result in reduced numbers of viral-infected cells and lower cell counts, both of which would tend to reduce the likelihood that resistant animals would transmit the infection. Transmembrane motifs are required for both infectivity and for viral propagation within an infected animal. Five of 10 animals' BLV-induced lymphomas were found to have mutations in the P-53 tumor suppressor gene. This information fits the observed phenomena that *only a very small percentage of BLV-infected cattle go on to develop lymphoma*, suggesting that dysregulation of both P-53 alleles must occur for malignant transformation of bovine B lymphocytes. The interaction of other agents in inactivation of the P-53 genes needs to be considered, including bovine papilloma and herpesviruses as well as pesticide exposure, which would correlate with the higher incidence of lymphoma seen in dairy cattle. The demonstration of concurrent infections with *Bovine immunodeficiency virus* and *Bovine syncytial virus* indicates further factors that may be involved in the progression of the BVDV infection in an individual animal.

The transformed phenotype of bovine lymphoma suggests that most cells are naive B cells with rearranged immunoglobulin genes, but have not undergone hypermutation or antigen-driven germinal center mutations. There appears to be some increase in transmission during the summer months, suggesting that biting insects might be vectors. It is, however, possible to maintain infected and noninfected herds on the same premises for prolonged periods with reasonable isolation procedures, which *suggests that direct contact is the primary mode of transmission*. Some herds tend to have a very high level of infection, whereas others have a low level of infection, and the proportions of infected animals tend to remain relatively stable over long periods. There is likely a genetic basis for this phenomenon.

Less than 3% of infected animals develop malignant disease, and then only if allowed to survive to the age of peak incidence at 6–8 years. Since viral transmission occurs primarily in closely confined dairy herds, and currently the rate of culling in dairy herds in North America is 20–30% per year, the number of cases of lymphoma appears to be dropping, while the infection rate remains relatively constant. In addition, the development of blood testing for BLV infection has permitted early identification of diseased animals, which are therefore more likely to be found at slaughter than in a clinical setting. Infected cattle which do not develop lymphoma appear to have few other specific problems related to retroviral infection, but may suffer a higher rate of culling for a variety of reasons that may be the result of subtle effects of the host–parasite relationship. *The sporadic forms of bovine lymphoma are not associated with BLV infection.*

Enzootic bovine lymphoma is therefore a disease of mature onset. Hematologically, the animals are normal or nearly so, without anemia or thrombocytopenia. There is a fluctuating lymphocytosis of moderate degree, which is the characteristic response of the cow to infection that is rather well controlled, and also occurs in trypanosomiasis and tuberculosis, and to a lesser extent in brucellosis. In herds in which the disease is endemic, infection is likely acquired in the first year of life. There may be little or no apparent effect of this exposure, but it does serve to perpetuate the virus in the herd. There may be a long period of occult transformation before clinical tumor develops. Cattle with the enzootic form of lymphoma react in lymphocyte stimulation tests against a membrane antigen derived from tumor lymphocytes, which constitutes a diagnostic test for occult tumor. This test remains positive until the later stages of the clinical disease when the animals become anergic. The same membrane antigen when given as a vaccine prevents lymphoma, but not BLV infection, in sheep subsequently challenged with live virus.

Clinically and morphologically, enzootic bovine lymphoma is subdivided on a topographic basis depending on the major organ systems involved. The most common presentation is an *enlarged lymph node* characteristically seen in high-producing dairy cows. Enlarged nodes may appear anywhere in the body but are common in the retrobulbar area where they cause unilateral or bilateral proptosis, and in the pharyngeal area where they result in dysphagia and stertorous respiration. The disease is frequently diagnosed on a pelvic examination on the basis of enlarged inguinal lymph nodes or involvement of the urogenital system.

The *enteric form of the disease* is, at least grossly, part of most cases and is characterized by diffuse thickening of the abomasal submucosa with eventual ulceration leading to hemorrhagic anemia. The latter tendency for gastric and also uterine homing suggests that, in many cases, the transformed lymphocytes are relatively mature and possess receptors allowing them to accumulate locally as a mucosal-associated lymphoid tumor. There is irregular involvement of the mesenteric nodes associated with the abomasal infiltration and these may, at times, be large enough to cause obstruction.

Hindlimb lameness, which progresses quickly to weakness and recumbency, reflects the spread of tumor from sublumbar nodes to the *spinal canal* where there is a relatively diffuse infiltration into the perineural fat and heavy involvement of the sheaths of the spinal nerves. Grossly, the perineural infiltration has only a slight fleshy pinkness to distinguish it from normal fat, although the distinction is readily made by an imprint of the affected tissues.

Animals occasionally are presented with signs of *central venous congestion* due to preferential involvement of the right atrium, colonization of the epicardial fat and irregular and often heavy infiltration into the myocardium that may be accompanied by tamponade.

Almost any organ can be involved, but *the liver is involved in most animals, as is the spleen*. Some cows die rapidly because of splenomegaly with rupture and abdominal hemorrhage. Rarely, the kidneys are primarily involved in a herd outbreak of lymphoma, again suggesting specific homing, in this case to renal tissue. Females may occasionally have primary and extensive involvement of the uterus and ovaries. On largely anecdotal evidence, it appears that cows that have been repeatedly superovulated for embryo transplant purposes may be more likely to develop lymphoma of the reproductive system. There is usually cytologic involvement of bone marrow in terminal cases which may become leukemic with very high cell counts, or rarely there is lymphopenia, but myelophthisis as is seen in the lymphocytic leukemia in the calf form is a rare occurrence. The hemolymph nodes are usually involved and occasionally they may be visible clinically as subcutaneous discoid masses 1–2 cm in diameter. The histologic type is consistent throughout the body and the duration of the disease is usually a matter of weeks once the disease has been recognized.

The **sporadic forms of bovine lymphoma** are not associated with BLV infection. They include the *calf type*, which is characterized by symmetrical lymphadenopathy and leukemia, the *juvenile type* with thymic lymphoma in yearlings, and the *skin type*, which occurs most often in 2-year-old cattle. These three sporadic types of bovine lymphoma do not react to membrane antigen from the enzootic form of bovine lymphoma in lymphocyte stimulation tests. As their name suggests, these diseases occur sporadically. It is not clear whether other viruses are involved in their genesis.

The **calf type** of lymphoma may be present at birth, but usually develops in the first 6 months of life and typically has symmetrical enlargement of lymph nodes. There is leukemia with leukocyte counts varying from 10 to 100×10^9/L, nonresponsive normochromic, normocytic anemia, and thrombocytopenia. Terminally, there is virtually complete myelophthisis and marrow infarction (Fig. 2.68) with neutropenia and thrombocytopenia but without hemorrhage. At autopsy, virtually all nodes are enlarged up to 10 times normal size, and the kidneys are usually diffusely involved but may not be markedly enlarged. The liver and spleen may be massively involved, or relatively spared. Calves may be born with lymphoma with the liver so enlarged at parturition as to cause dystocia. The cell type in the blood of the calf type of lymphoma resembles the L1 type of ALL, and histologically the nuclei are round and irregular in size and shape resembling Burkitt-type lymphoma. The phenotype of the calf-type tumors appears not to have been determined. The thymus is usually normal or atrophic, and skeletal muscle and even bone may be focally invaded by tumor.

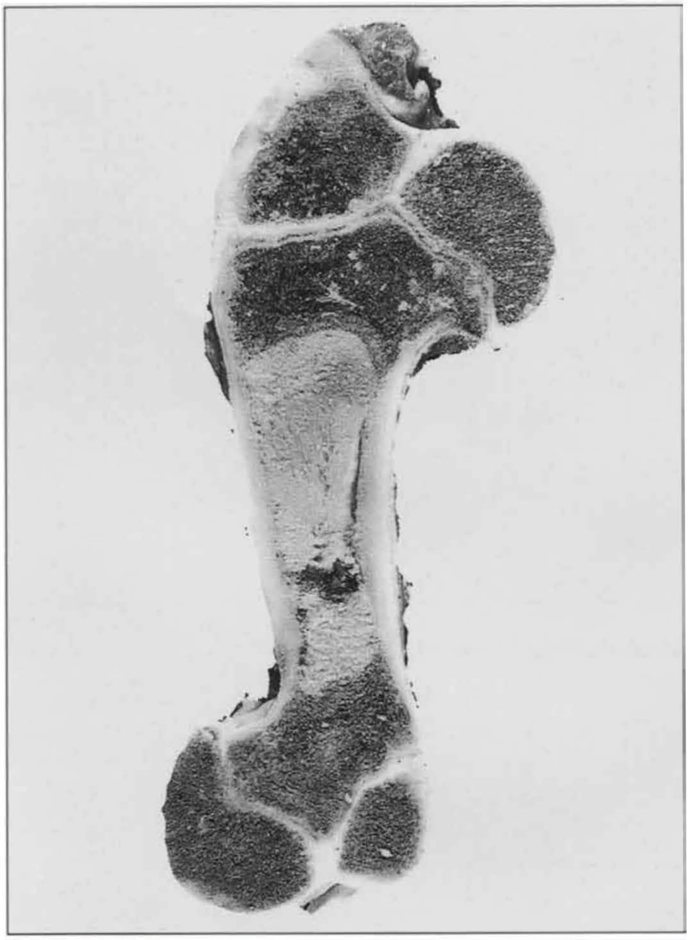

Figure 2.68 Lymphoma in a calf. Medullary infarction in humerus.

The **thymic form** of bovine lymphoma is described under diseases of the thymus (Thymic neoplasia, below). Most cases remain aleukemic.

The **skin form** of bovine lymphoma typically occurs in 2- to 3-year-old cattle, and is characterized by plaque-like, round, raised lesions which appear on the head, sides, and perineum, become depilated, and are frequently ulcerated. The lesions wax and wane over a period of months with some regressing entirely and new lesions appearing. The disease is remarkable in its indolent nature as compared to the other forms of lymphoma in cattle, and animals allowed to survive may do so for 12–18 months. Ultimately, there is deep organ involvement, and the visceral lesions are then indistinguishable from those of the enzootic type of bovine lymphoma. The skin lesions have architectural specificity and are dermotropic and characteristically result in dense infiltrations of tumor cells in the papillary dermis. There is focal invasion of the epidermis (Pautrier's microabscess) and, in the early stages, the disease resembles mycosis fungoides of humans but is more focally distributed, and in the advanced state with leukemia resembles Sézary syndrome. The tumor cells are of moderate size, with a very narrow rim of cytoplasm, and have cerebriform convolutions of the nuclear membranes. The skin form of bovine lymphoma is described under T-cell lymphoma of mycosis fungoides type.

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Ovine and caprine lymphoma

Ovine and caprine lymphomas are widely disseminated tumors similar to the calf or juvenile type of bovine lymphoma, but without leukemia and marrow involvement. A retrovirus indigenous to sheep shares major proteins with *Bovine leukemia virus* (BLV); however, most spontaneous lymphomas of sheep and goats, and virtually all of the those produced experimentally, are *caused by BLV*. Lymphoma can be produced in sheep in 30–60% of animals 2–3 years after infection with BLV. In sheep, as in cattle, infection with BLV once established is lifelong, and the virus persists in integrated provirus in the face of a strong and persistent antibody response. Unlike cattle, the host–parasite relationship between BLV and sheep does not produce persistent lymphocytosis but does produce tumor, and the earlier that lambs are infected, the greater will be the proportion of infected animals that develop lymphoma. Unlike cattle, BLV is able to induce lymphoma in sheep without prior mutations in the wild-type P53 tumor suppressor genes. It appears that, unlike cattle, some sheep after exposure to BLV will develop transient antibody and then are free of infection.

In sheep, BLV infection can be detected by the tests used for diagnosis in cattle. Sheep that develop lymphoma react in lymphocyte stimulation tests against membrane antigen derived from BLV-associated bovine lymphoma, providing a test for the presence of occult tumor.

Morphologically, there is a characteristic and consistent portal involvement of the liver, and there is often hepatomegaly. The spleen is symmetrically enlarged with focal and diffuse involvement and the heart and kidneys are often very heavily infiltrated. Histologically, the tumors are largely of the Burkitt-like (small-noncleaved cell type) with B phenotype and diffuse architecture. The disease has a very rapid course with most animals dying within 1–2 months of clinical diagnosis.

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Equine lymphoma

Lymphoproliferative disease in the horse, like that in the cow, has several characteristic topographically and cytologically distinct modes of presentation as *primarily leukemia or lymphoma.* In the **leukemic form**, which usually occurs in racing animals in training, there is primary marrow involvement with pancytopenia and severe thrombocytopenia, sudden onset of malaise, subcutaneous edema, and petechial hemorrhages. The bone marrow is diagnostic with myelophthisic destruction of normal cells by lymphocytes of the L2 or L3 type (see acute lymphoblastic leukemia; Fig. 2.31). The clinical course in this form of disease is less than a week. More commonly, the cells are more differentiated and typical of chronic lymphocytic leukemia, with leukemic lymphocytosis and the course is much longer. A disease like **mycosis fungoides** of humans occurs in horses, and progresses to a form of chronic lymphocytic leukemia with convoluted tumor cells like the human Sézary syndrome. The skin lesions may be minimal and confined to the mucous membranes, which are brick red and do not blanch on compression. Histologically, the tumor cells are dermotropic and of medium size, with cerebriform nuclear indentations, and mark positively with CD3.

The **solid forms** of lymphoid tumor in the horse can be characterized as *subcutaneous, alimentary, abdominal, splenic and thymic/multicentric.* **Subcutaneous** lymphoma is remarkable because of the indolent nature of the disease with some animals surviving for years, and because it occurs very predominantly in females. Regression of the disease has been observed during pregnancy and following removal of an ovarian granulose-theca cell tumor. A subsequent study of 29 cases of equine lymphoma for estrogen and progesterone receptor expression found that all cases were negative for estrogen receptors, but 16 of 29 were positive for progesterone receptors, of which 7 cases were of the T-cell-rich large B-cell type lymphoma. Affected animals may maintain body weight for many months, but are unsightly because of hundreds of subcutaneous masses 1–3 cm in diameter or larger. The lesions cluster on the lower half of the body, particularly on the chest, thorax, flanks and perineum, sparing the limbs and often involving the face and neck. One of the major reasons for biopsy of these animals is that an otherwise vigorous animal cannot be bridled or saddled without impinging on the masses. Histologically the tumors are truly subcutaneous, likely arise along lymphatics, and rarely involve the deep dermis which tends not to be fixed over them. Cytologically they are also diverse, but distinctive, and are *composed of a predominant population of benign small-cleaved T cells, and a minor population of large malignant B cells with prominent nucleoli.* Most of the small cell population marks positively with CD3 and the atypical large cells are positive with CD79α (see Diffuse large B-cell lymphoma). Characteristically, these subcutaneous neoplasms are interlaced at the cellular level with fine reticulin fibers, and on an architectural level with collagenous bands. Ultimately, there is involvement of regional lymph nodes, but deep organ involvement does not regularly occur in the time that most are allowed to survive. They remain hematologically normal, except for mild progressive anemia and hypoproteinemia. A second type of T-cell-rich B-cell lymphoma occurs in horses involving the dermis, and characteristically is seen as a protruding dorsolateral periorbital lesion 2–3 cm in length, with ulceration. Histologically, the lesions are sclerosing, but their most remarkable characteristic is a tendency to recur locally even after careful dissection. In all forms of the diffuse mixed tumor, the mitotic rate is very low and consistent with the indolent rate of progression.

The **alimentary form** of lymphoma occurs in adults that are characteristically thin, with reduced appetite and may be colicky, but usually do not have diarrhea. Anemia may be mild or quite severe, normocytic, and with hypoproteinemia. There is usually some degree of neutrophilia, with signs of toxemia due to ulceration of the intestinal mucosa, but leukemia is not present. There may be alpha heavy chain paraproteinemia as in Seligman's Mediterranean lymphoma of humans, but this aspect has not been frequently investigated. Some animals with this form of tumor will have hemolytic anemia that may dominate the clinical picture. Grossly, the peripheral and internally palpable lymph nodes are normal and the disease is difficult to diagnose short of an open biopsy. *The disease tends to be confined to the upper small bowel, pancreas and regional nodes, but there may also be involvement of the ileum* (Fig. 2.69A, B). There is laminar thickening of the intestinal wall with tight homing of the infiltrate to the muscularis mucosa and submucosa and relative sparing of the muscularis. The tumor cells are uniformly plasmacytoid, with round nuclei 1.5 red cells in diameter, and abundant highly basophilic and eccentric cytoplasm. There is mild nuclear atypia with nucleoli not generally present in mature-appearing plasma cells, hyperchromatic chromatin

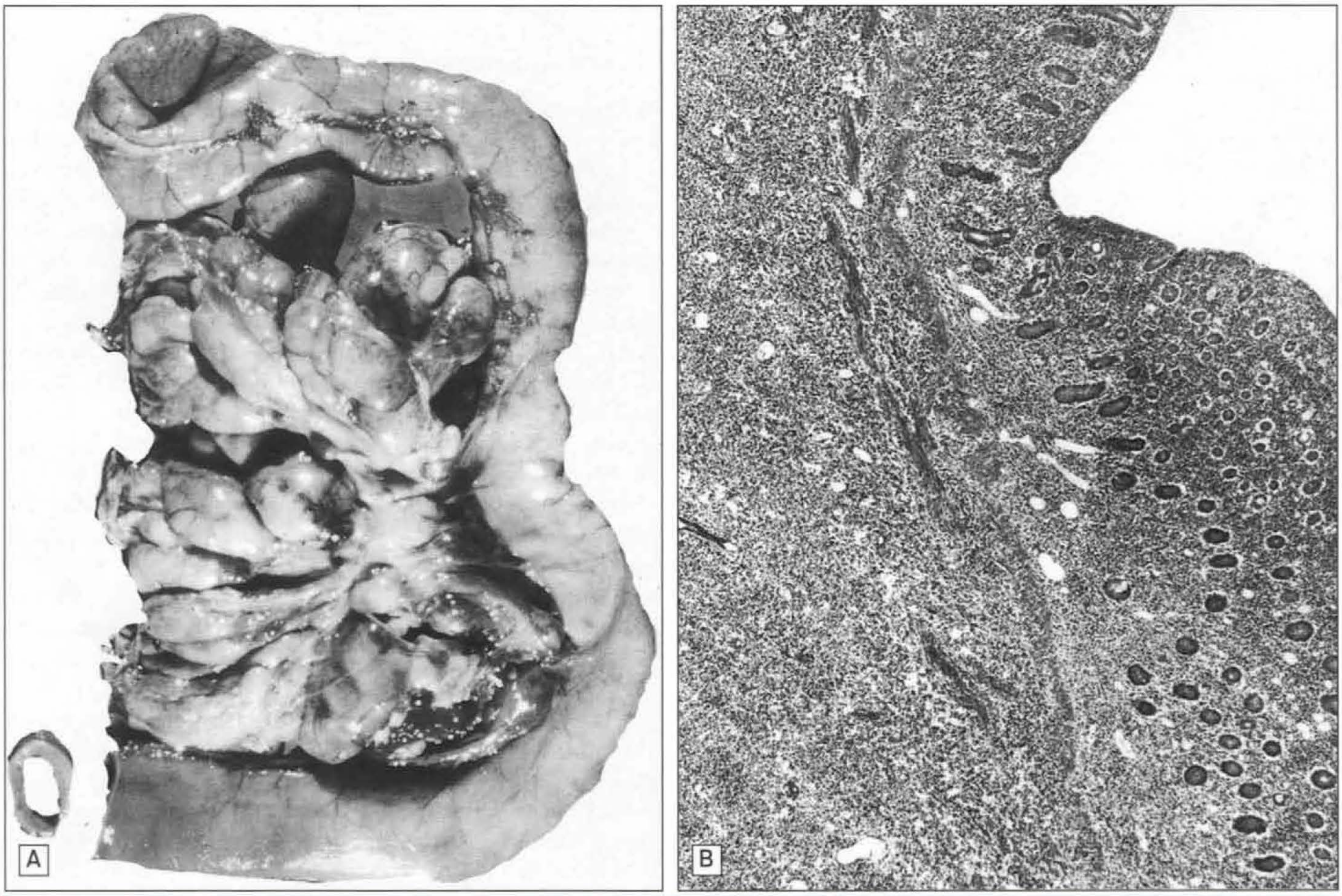

Figure 2.69 Lymphoma in a horse. **A.** Diffuse infiltration of duodenal wall and replacement of pancreas. **B.** Section of intestine of (**A**). The infiltrates may be confined to the mucosa but usually involve full thickness.

with an exaggerated heterochromatin clumping, and a low mitotic rate. Cytologically, in imprints, the tumor cells may be mistaken for plasma cell hyperplasia if the extent of the gross lesion has not suggested tumor. There is focal thinning and ulceration of the intestinal epithelium, and the tumor cells have the same morphology in the affected nodes, which tend to have thickened capsules and atrophy of normal cells. One is left with the impression that the disease begins as benign immune inflammation and progresses to lymphoma, albeit of well-differentiated type. The disease is an example of a well-ordered mucosal-associated lymphoid tumor with homing of the tumor cells to the submucosa until very late in the disease.

The **abdominal form** of equine lymphoma is not usually difficult to diagnose clinically because *tumor masses involve the large bowel and caudal abdominal nodes*. Clinically, the animals are thin and have poor appetite, dependent edema and recurrent diarrhea. The hematologic changes are typical only of chronic disease and inflammation. Grossly, there is widespread tumor in the abdominal cavity and there may be involvement of nodes in the thorax. There is splenomegaly, involvement of the hepatic hilar nodes, irregular thickening of the wall of the small and large intestine, and focal mucosal ulcers. There may be huge tumorous masses involving the intestinal serosa and mesenteric nodes. Histologically, the tumors are variable, but tend to be diffuse neoplasms of large B-cell type with quite a high mitotic rate. An undefined proportion of the abdominal form of equine lymphoma, particularly those with mediastinal involvement, appear to be neoplasms of the large granular lymphocyte type.

Splenic lymphoma is an isolated lesion that involves the lower part of the organ with a huge mass that may weigh 15 kg or more. This lesion is not usually palpable rectally and the superficial nodes are not enlarged. The animals are presented with responsive hemolytic anemia, and idiopathic immune or infectious equine anemia is usually suspected. There is an upward shift in the MCV and increased anisocytosis. Leukemia is not present, and the bone marrow is hyperplastic with an erythroid shift and synchronous maturation. The pathogenesis of the anemia appears to involve hypersplenism due to trapping of cells in the normal spleen adjacent to the tumor. Immune hemolysis may contribute as it does in other lymphoid tumors, but this has not been verified. Pathologically the disease usually is limited to the spleen, but occasionally adjacent organs are involved by direct extension. The tumors are of large B-cell type, typically with an immunoblastic nuclear configuration with a large single central nucleolus and a moderate amount of quite highly basophilic cytoplasm. The neoplastic cells mark with CD79α. Frequent use of ultrasound should permit the diagnosis of splenic lymphoma by directed needle aspiration. The major feature of this lesion is the difficulty in clinical diagnosis, and most cases are diagnosed at necropsy.

Multicentric lymphomas have a wide age range, but most occur in mature animals. Affected animals are usually in poor condition but continue to eat, and often have low protein levels and dependent edema. Many of these animals have a mediastinal mass as well as irregular enlargement of peripheral nodes, particularly those in the pharyngeal and mandibular area. Any of the superficial nodes may be involved, but the involvement is not symmetrical. Leukemia is occasionally present, but usually the blood is more typical of cachexia. Grossly, there is widespread tumor in the abdominal cavity, and often there is tumor in the mediastinum with involvement of the heart and even the lung. Cytologically, the tumors are not of consistent cell size, but most are large-cell lymphomas and may be of B- or T-cell type, and even the T-cell-rich B-cell lymphomas are found in the thorax.

Lymphoma in the horse, as in other species, may be very variable and primarily involve other tissues such as the central nervous system and urinary system.

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Canine leukemia/lymphoma

Hematopoietic tumors in urban dogs occur at an age-adjusted rate of some 30/100 000. It appears that the referral rates for leukemias and lymphomas at teaching institutions may not accurately reflect actual incidence of these neoplasms. Practitioners may be more willing to refer leukemias than lymphomas, but because of their perceived poor prognosis, leukemias appear less likely than lymphomas to be properly worked up for treatment.

Acute lymphoblastic leukemia in the dog has been inadequately studied from a phenotypic standpoint, but it appears that B-, T-, and null-cell types all occur, and there are indications that, in the dog, T-cell phenotype may predominate in ALL as it does in CLL. In the dog, 3/4 of a large series of well-characterized cases of CLL were found to be of T-cell type and more than 1/2 of all cases were CLL of large granular lymphocyte (LGL) type. The LGL cells of canine CLL are almost all CD8 positive and are predominantly of the αβ T-cell receptor type, with about a third having the γδ receptor configuration. Canine B-cell CLL is positive for CD21 and CD79α. Recent studies in human pathology indicate that the numbers of bone marrow blasts required for the diagnosis of leukemia have reduced markedly, and it appears that if 20% or more of marrow cells are blasts with a peripheral blood picture of ALL, a diagnosis of leukemia is confirmed. On the other hand, with CLL, the marrow is more likely to be occupied by a very high proportion of lymphocytes of which only a small proportion will be blasts, but the diagnosis is more easily reached because of very high lymphocyte counts in the peripheral blood (see ALL and CLL). Some progress has been made in genotypic characterization of canine lymphoma, with trisomy 13 found as a primary chromosomal abnormality in 25% of cases studied, and this finding was associated with a better prognosis. Unfortunately, the morphologic subtype of lymphoma associated

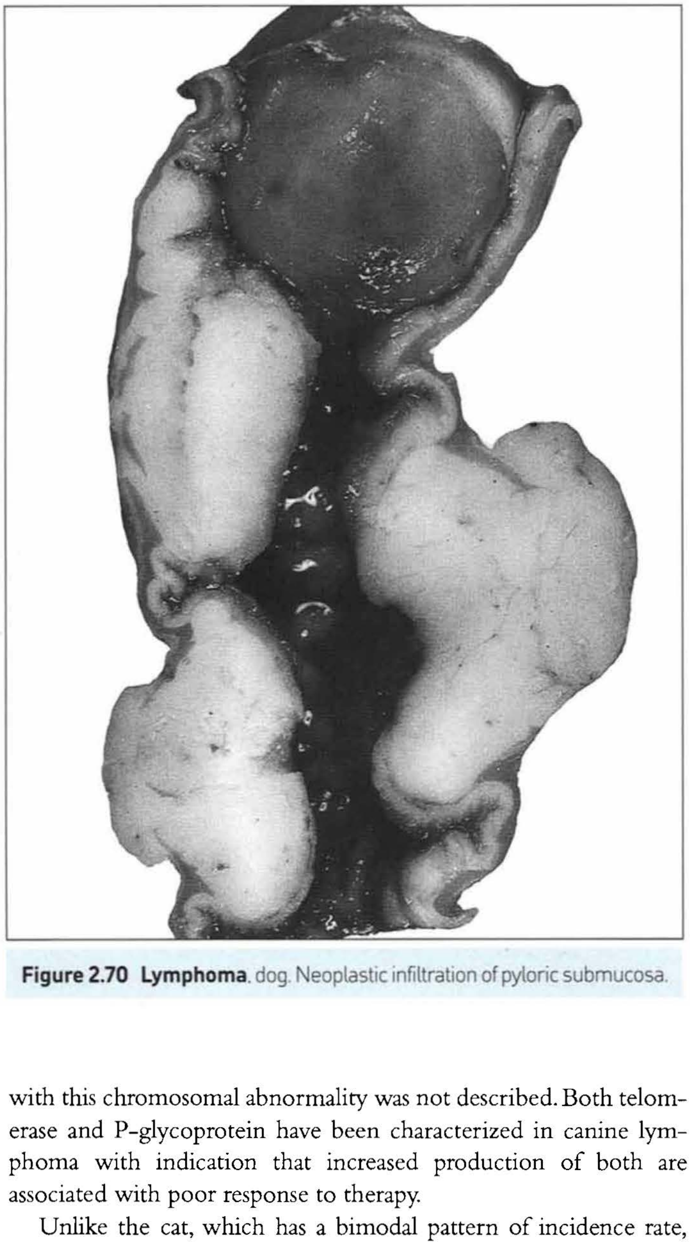

Figure 2.70 Lymphoma, dog. Neoplastic infiltration of pyloric submucosa.

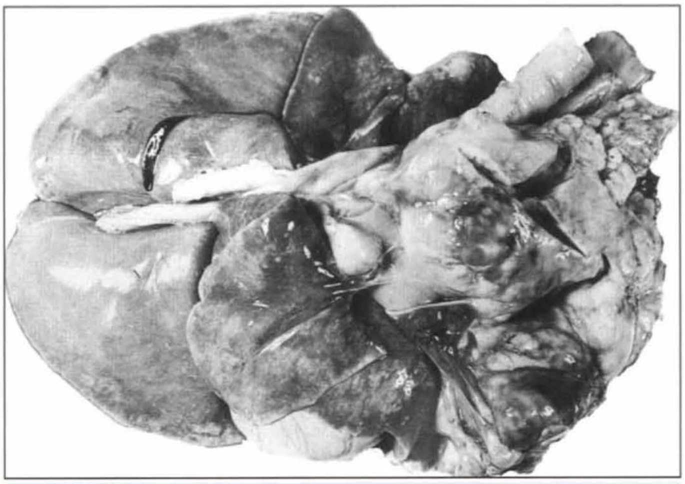

Figure 2.71 Thymic lymphoma of cranial mediastinum, dog.

with this chromosomal abnormality was not described. Both telomerase and P-glycoprotein have been characterized in canine lymphoma with indication that increased production of both are associated with poor response to therapy.

Unlike the cat, which has a bimodal pattern of incidence rate, hematologic malignancies in the dog occur with increased frequency throughout life. There is an equal sex incidence in intact animals, and a lowered risk for neutered males. *Most lymphomas in dogs are disseminated and of high-grade type*; however, there is some clustering of cases based on topographical distribution, with *multicentric most common, followed by enteric, cutaneous, and thymic types*. The presenting signs vary with the distribution of tumor and tendency, if any, for paraneoplastic changes. There is usually weight loss with some change in dietary habits. The **multicentric** type often has symmetrical lymphadenopathy and tends to be noticed when the animal is still in relatively good condition, as does the **cutaneous** form since the disease is usually focal and nodular. Dogs with hypercalcemia have polydipsia and polyuria and are usually in a state of collapse, while those with primary **enteric** lymphoma (Fig. 2.70) have some combination of vomiting and diarrhea, often bloody. There is mucosal ulceration in 50% of enteric cases, and these differ in having some degree of anemia accompanied by a moderate to marked neutrophilic leukocytosis. Dogs with **thymic** lymphoma (Fig. 2.71) have reduced exercise tolerance, often dysphagia and may have tamponade with severe impairment of circulation.

There are few correlations between cell type and topographical distribution of tumor; however, *there are more plasmacytoid tumors in the gut, skin and spleen than in other sites*, which suggests that for these areas malignant transformation is preceded by a period, perhaps prolonged, of benign immune hyperplasia. Cutaneous T-cell lymphoma in the dog may be of the epidermotropic or nonepidermotropic types, with the latter tending toward deeper involvement of the dermis and panniculus. Cutaneous B-cell lymphoma in the dog may be less common than the T-cell type and is more likely to be multifocal, presenting with a plaque or a nodular formation usually associated with erythema and variable hair loss. Canine cutaneous T-cell lymphoma (CTCL) of the nonepidermotropic type and canine cutaneous B-cell lymphoma (CBCL) are both characterized by a larger cell type than the epidermotropic T cells, but both may be very variable and have markedly atypical nuclei associated with anaplastic large-cell lymphoma subtypes (see Diffuse large B-cell lymphoma and Anaplastic large-cell lymphoma).

The enteric cases of canine lymphoma are also often preceded by the presence of lymphocytic-plasmacytic enteritis indicating prior long-standing benign inflammatory disease. The enteric counterpart of cutaneous epitheliotropic T-cell lymphoma occurs in the small intestine in both cats and dogs with heavy infiltration of small cells into surface and glandular epithelium. There is a clustering of the lymphoblastic type of lymphoma in the mediastinal cases, and T-cell phenotype and pseudohyperparathyroidism with hypercalcemia are also associated with mediastinal lymphoma.

The lymph nodes, liver and to a lesser extent the spleen (Fig. 2.72A), are involved in virtually all cases of canine lymphoma and, since there are usually lesions that are palpable or identifiable radiographically, most cases are diagnosed by fine needle aspiration biopsy. A canine counterpart of the hepatosplenic γδ T-cell lymphoma likely occurs in dogs and requires recognition and characterization. The lesions may be identified in almost any tissue including tonsils, which are often markedly enlarged (Fig. 2.72B), or in the eye. Involvement of lung (Fig. 2.73A) is uncommon, except as pleural implants from tumor in the cranial mediastinum. Secondary deposits in meninges and muscle tend to be diffuse, but those in myocardium tend to be more focal (Fig. 2.73B). About 50% of dogs have some degree of renal involvement and the proportion with impairment of

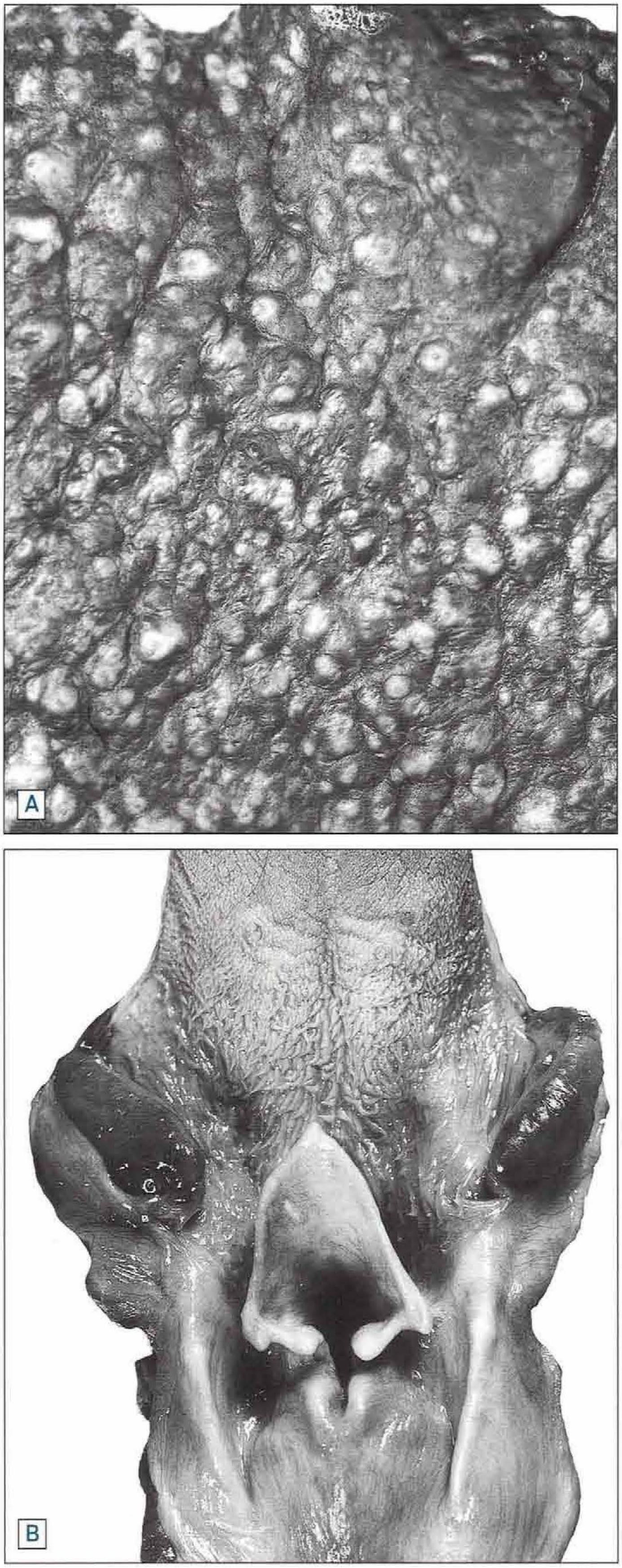

Figure 2.72 Lymphoma in a dog. **A.** Neoplastic enlargement of Malpighian bodies in the spleen. **B.** Bilateral tumor of tonsils.

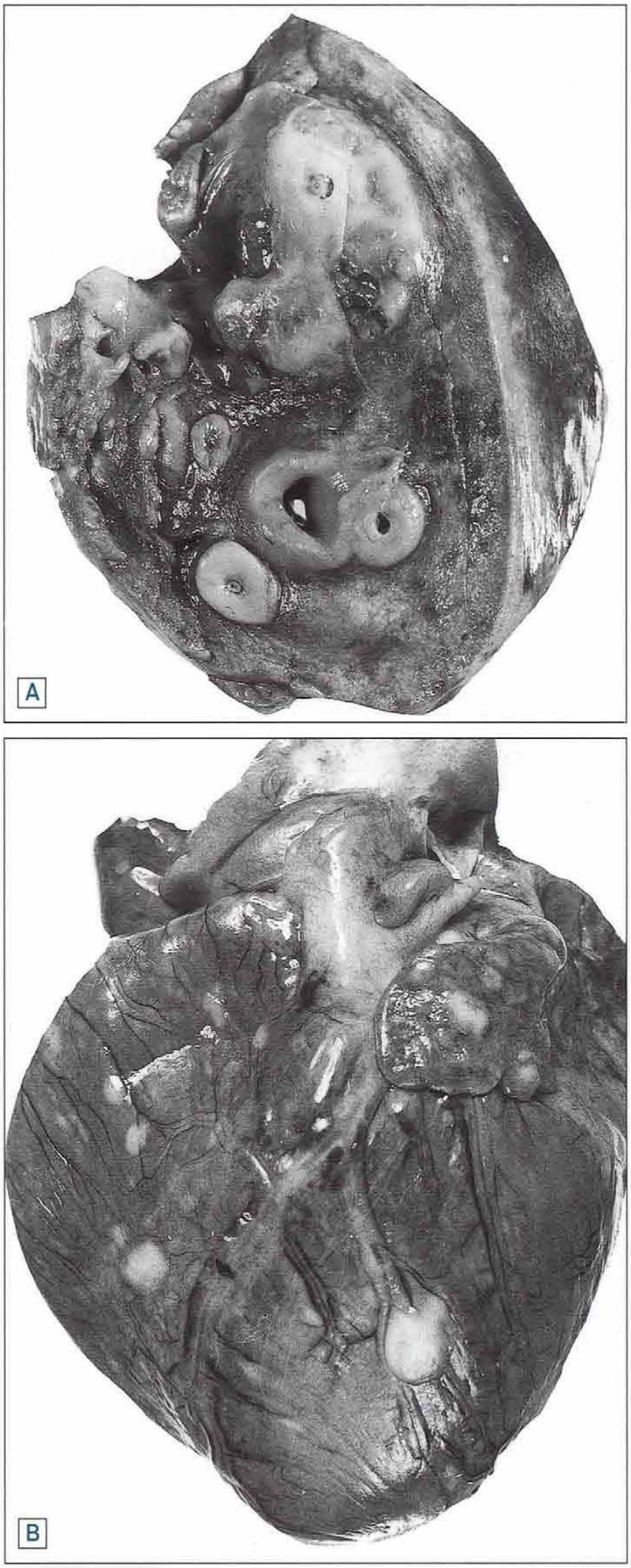

Figure 2.73 Lymphoma of Hodgkin type in a dog. **A.** Infiltrating perivascular and peribronchial lymphatics in lung. **B.** Multiple metastatic deposits in heart.

function, due to either or both tumor infiltration or hypercalcemia, is much higher. The bone marrow is involved to some degree in about one-third of cases and the histologic pattern of marrow involvement is a useful parameter in the evaluation of progression.

True follicular lymphomas in the dog appear to be relatively rare occurrences and constitute less than 5% of all cases. It is quite likely that the follicular variant of B-cell lymphoma is underdiagnosed because the lesions are either felt to be benign in their early stages of development and confused with follicular hyperplasia, or are dismissed as diffuse lymphomas in later stages of progression when the nodular follicular appearance is less apparent. The advent of routine use of immunophenotyping for B- and T-cell subtypes will likely significantly increase the rate of recognition of follicular lymphoma because these techniques serve to delineate follicular architecture even in advanced lesions. In the last decade, both mantle cell and marginal zone lymphomas have been recognized in the dog, and because both in the early stages can be seen as arising on germinal follicles are more likely to be recognized as having a unique and non-diffuse architecture. In the past, it has been felt that more than 80% of canine lymphoma was of high-grade or markedly aggressive histologic subtypes. With more accurate recognition of lymphoma subtypes in the dog, it is likely that a better approximation of prognostic categories would be 25% of cases as indolent, slowly progressing lesions over a period of 2 or more years, with another 25% as intermediately aggressive lesions that tend to progress to clinical attention over a year or more, and 50% of cases that are actually highly aggressive lymphomas, of which about 1/2 will enter complete remission with intensive combination chemotherapy.

Rarely, dogs develop a disease like human **myeloma** with focal lysis of bone, and are usually presented with lameness and nervous deficit. They have hyperproteinemia with monoclonal gammopathy that is in the 80–140 g/L range and may have heavy chain fragments in the urine. The spleen is often massively enlarged and may contain septic infarcts. The high level of high molecular weight plasma protein causes high blood viscosity, and anesthesia in these dogs for marrow or spleen aspiration may result in fatal hypotensive shock. The lesions may be widespread at the time of diagnosis, and qualify for the general term of "multiple myeloma," or only a single focus of tumor may be present. The tumor is always locally invasive and readily erodes bone, emphasizing the need for all destructive lesions of bone to be histologically evaluated so as to avoid inappropriate corrective measures such as excision arthroplasty of a femoral head that collapsed due to tumor lysis. Cytologically, the tumor is paradoxical in frequently having more rather than less cytoplasm than benign plasma cells. Nuclei are of moderate size, about 2 red cells in diameter, with more anisokaryosis than is seen in a plasmacytoma. They are round and hyperchromatic with irregular heterochromatin aggregation and small nucleoli. Binucleate cells are common. In the classic tumor, the cytoplasm is abundant, centered on the nucleus and pale staining so that the myeloma may resemble a clear cell tumor at low magnification. There may rarely be dedifferentiation of the myeloma to a more aggressive large type immunoblastic lymphoma in which the cells are smaller but the nuclei larger, with prominent central nucleoli. The latter type of lymphoma may on its own be secretory and associated with gammopathy.

Angiotropic large cell lymphoma is an unusual form of lymphoma that occurs in humans and dogs and likely in other species. In humans, there are a number of lymphomas characterized by angiocentric and angioinvasive characteristics including lymphomatoid granulomatosis and angioimmunoblastic lymphadenopathy. In these types of lymphoma, there is encirclement of small veins and muscular arteries with neoplastic cells that penetrate the wall of the vessel and colonize the lumen until it is completely obstructed with little overall distention of vessel diameter. These are characteristically T-cell neoplasms. In the intravascular lymphoma which is of B-cell type, there is colonization of the vascular lumen by neoplastic emboli which eventually obstruct the vessel (see Intravascular lymphoma; Fig. 2.51). *A third type of angiotropism occurs in animals in which the tumor cells selectively colonize and distend the space between the vascular endothelium and the vessel wall* (Fig. 2.74A, B). This lesion occurs in both veins and arteries and is characterized by marked distention of the wall with progressive compression of the lumen without penetration or colonization of the internal endothelial membranes by neoplastic cells. The form of angiotropic lymphoma seen in anaplastic large-cell lymphoma may occur in any tissue (Fig. 2.58), but is frequently seen in the skin and panniculus, whereas the variant involving subendothelial structures tends to occur in the heart and lung and lymph nodes in cases of small cell lymphoma. The intravascular variant is seen in the brain and spinal cord (Fig. 2.51) as well as other tissues. Each of these subtypes tends to be of large-cell type and the intravascular lymphomas mark strongly with CD79α, and those involving the subendothelium and characterized by angiocentric and angioinvasive properties mark strongly with CD3.

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Feline lymphoma

Acute lymphoblastic leukemia (ALL) in the cat, like the dog, has had inadequate definition of immunophenotype and it is apparent that, like the dog, B-, T- and null-cell types all occur. *There appear to more acute myeloid leukemias in the cat than in the dog* and more systematic work-up of acute leukemias in the cat may identify a higher proportion of myeloid tumors than are generally considered to occur. All three types of ALL, as defined by the French–American–British (FAB) classification system occur in the cat, but it appears that L1 and L3 occur most frequently. Like the dog, ALL is diagnosed in the cat when there are blastic cells in the peripheral blood and 20% or

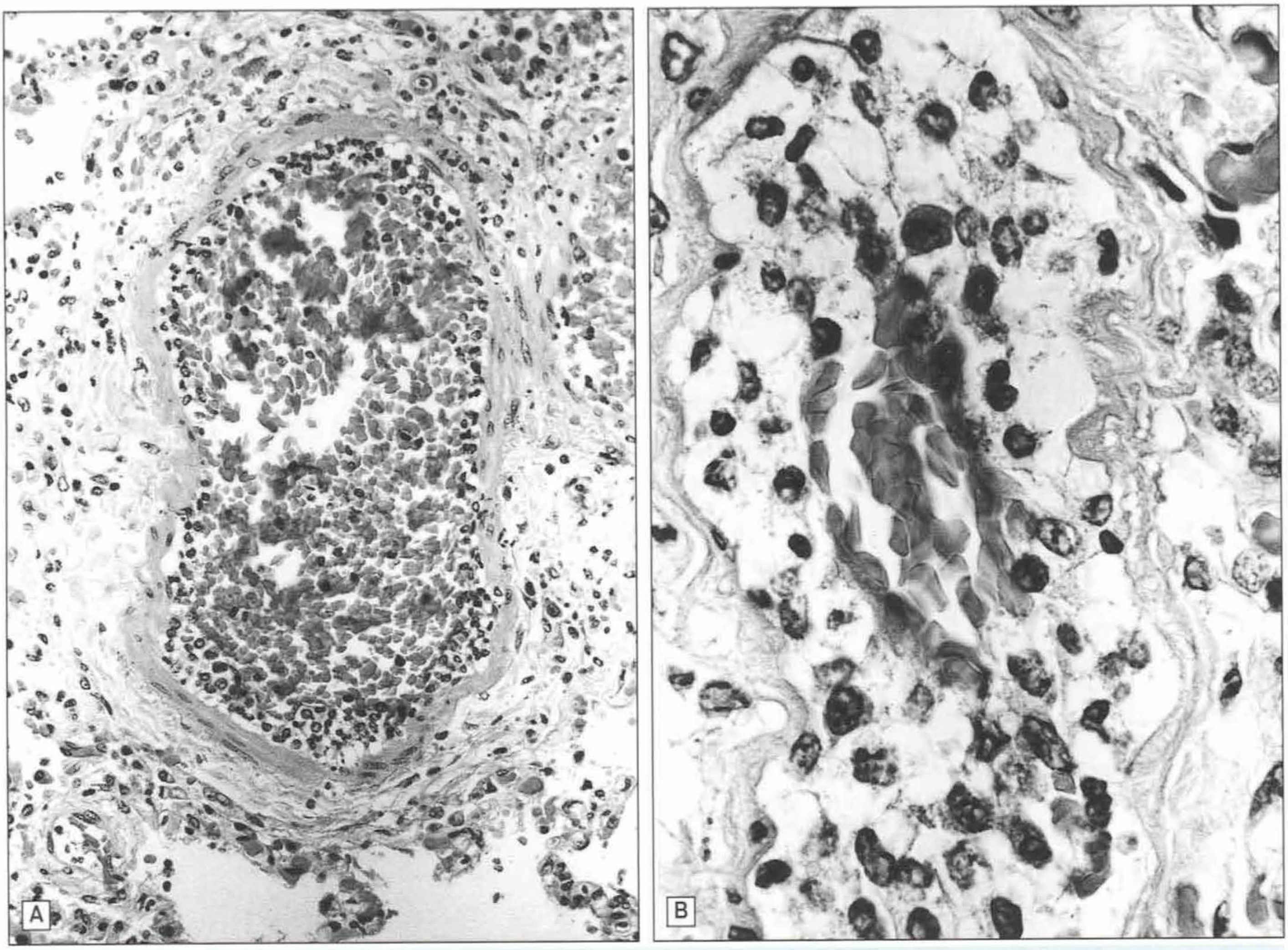

Figure 2.74 Angiotropic large cell lymphoma in the lung of a dog. **A.** Laminar subendothelial colonization of dilated artery. **B.** Detail of smaller arteriole. Marked luminal compression due to colonization of subendothelial area by lymphocytes with abundant clear cytoplasm.

more lymphoblasts in the bone marrow. *Chronic lymphocytic leukemia* (CLL) in the cat tends to be more of the small-cell type with very little cytoplasm and characterized by very high lymphocyte counts at presentation. Cats with CLL have greatly expanded areas of marrow hematopoiesis but solidly cellular areas of tumor in mid-femoral regions, suggesting that these lesions have been developing over a period of years.

Feline lymphoma is caused by the horizontally transmitted Feline leukemia virus (FeLV). The host–viral relationship in the cat, unlike the cow, includes persistent viremia which is associated with a high incidence of disease, but like the cow there may be latent infection with circulating antibody and persistence of provirus in target cells. The presence of a persistent viremic state in the cat likely explains the difference in rate of infection-associated disease in these two species. Like *Bovine leukemia virus* (BLV), the low molecular weight envelope proteins (p15E) are very highly immunosuppressive and, *in decreasing order of frequency, the disease syndromes associated with infection include leukemia/lymphoma, refractory anemia, feline infectious peritonitis, diseases of the oral cavity, and a variety miscellaneous problems.* Kittens are susceptible to infection with FeLV, and early infection with high rate of challenge and highly virulent virus favors the formation of myeloid rather than lymphoid tumor. It appears that feline leukemia is preceded by a period of myelodysplasia, which is likely true of leukemia in all species but is rarely observed outside of experimental infection.

The virus is worldwide in distribution with about 2% of cats infected. In a population of cats at veterinary clinics, as many as 90% of seropositive cats were ill with some form of disease. About 15% of cats infected with FeLV are also infected with *Feline immunodeficiency virus (FIV)*; however, the latter agent is not nearly as infectious as FeLV nor as likely to cause disease. The pattern of FeLV replication in cats begins first in lymphocytes and macrophages in the tonsils, and then is carried to the local lymph nodes of the head and neck. Viral replication is amplified in germinal centers. Within the first and second week of infection, the infected lymphocytes and macrophages carry the virus to the bone marrow, thymus, spleen, intestine and lymph nodes throughout the body. The most important of these tissues appears to be the intestinal lymphoid structures, with a critical balance or imbalance established between viral replication and the developing immune response. Those cats with an inadequate immune response develop persistent viremia that is life-long and increases the risk that the cat will die of FeLV-related disease within two to three years. In animals with a more effective immune response, the virus is controlled and may become latent, with a small number of cats testing negative by the FeLV, IFA, and even the ELISA, tests. Viral antigen may be detectable in body fluids and various tissues, particularly the spleen.

Commonly occurring syndromes of feline lymphoma include those with *thymic, alimentary, multicentric, and cutaneous presentations.* FeLV-related lymphomas have been identified as T-type in thymic lymphomas; however, some lymphomas of the multicentric type appear to arise from clones of very primitive cells which are of null cell type by phenotyping and have germ line organization of the T-cell receptor gene and the immunoglobulin heavy chain loci. Cytogenetic studies have been carried out on FeLV-infected cats with and without lymphoma, with chromosomal abnormalities identified in most cats with lymphoma with tetrasomy and trisomy observed but without correlation to histologic subtype or prognosis.

The means by which FeLV induces tumor is unknown, but the virus has been found to have multiple (up to 5) sites of integration into the feline genome with some of the sites consisting of incomplete virus. Molecular studies have shown that FeLV isolated from tumors contains a transduced cellular-myc gene with the viral-myc constituting the entire coding sequence of the cellular gene. Since FeLV is a slow-transforming retrovirus lacking an onc gene capable of direct leukemogenesis, the ability to acquire cellular oncogenes may be important in its ability to induce malignant transformation. In terms of detection of viral antigen or antiviral antibody, virus-positive cats are associated with the multicentric form of lymphoma whereas the thymic and alimentary forms of tumor predominate in cats negative for virus. However, in molecular studies, FeLV provirus was found in thymic lymphomas. Thus, viremia is associated with immunosuppression and secondary disease, while the latent form of infection (as in the cow) is not. Latent infection is, however, associated with tumor, suggesting that transactivation by integrated FeLV or action of its acquired v-myc oncogene is necessary for malignant transformation, but immunosuppression is not. A sterile vaccine based on a viral antigen-producing cell line appears able to produce protective antibody without inducing immunosuppression against FeLV or other feline vaccine viruses.

Since the distribution of lymphoid tumor in cats is so variable, the presenting signs are also variable, but *anemia and weight loss are the most consistent clinical signs.* Topographically, lymphoma in the cat is like that in the dog except for the more common presentation of the cat with advanced mediastinal lymphoma. In a histological review of 602 cases of feline lymphoma, 69 were of low-grade type, 210 of intermediate-grade type, and 323 of high-grade type, classified according to the NCI Working Formulation. The topographic distribution of tumor was 192 multicentric, 180 alimentary, 130 mediastinal, 36 renal, 35 peripheral node, 20 central nervous system, 12 cutaneous, 11 nasal, 8 eye/orbital, and 4 subcutaneous masses. In terms of cell type and topography, small-cell lymphomas were frequently found in cutaneous and alimentary tumors, and large-cell lymphomas were the most frequent types found in mediastinal, renal and multicentric cases. Tumor cells of the mediastinum were predominantly of the Burkitt-like or small-noncleaved cell type and, as would be expected, mitoses were more frequent in the high-grade lymphomas than in the intermediate- and low-grade types. Low mitotic rates were characteristic of lymphomas of the alimentary type, whereas high mitotic rates were characteristic of lymphomas of the mediastinum and renal tissues. In comparison with the dog, immunoblastic lymphoma is more common and constitutes a third of the cases in cats, but feline lymphoblastic lymphoma is much less common than in the dog. *Alimentary lymphoma in the cat* (Fig. 2.75) *deserves more attention* from a diagnostic sense because most reports have treated these diseases without regard to phenotype, and the enteric-associated T-cell lymphomas that are characterized by heavy epithelial colonization need to be distinguished from the B-cell lymphomas that tend to arise from lymphocytic–plasmacytic enteritis and may be of the diffuse B-cell type or of the mucosal-associated lymphoid tissue type (MALT) of which the latter may, in some cases, be surgically treatable diseases.

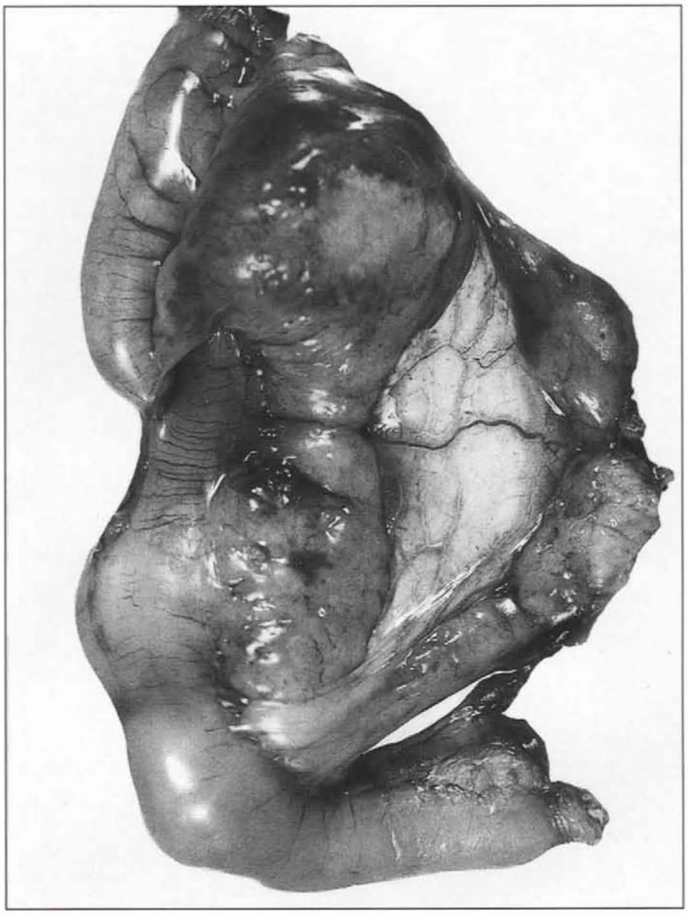

Figure 2.75 Lymphoma. Primary neoplasia in wall of jejunum in a cat.

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Porcine lymphoma

Lymphoma is the most common tumor in pigs, and in this species there is less diversity, both of topographical distribution and histologic type, compared to other domestic animals. B-lymphoma occurs in 25% of animals from herds of Large White pigs bearing an *autosomal recessive trait* for this condition. The disease may be detectable at 2–3 months of age and average survival is 4–6 months. It has not been possible to mate the homozygous affected animals because of their short survival. The disease appears to be similar in both the sporadic and familial occurrences, with generalized lymph node enlargement, splenomegaly, hepatomegaly and with particular involvement of the mesenteric nodes, stomach and intestines (Fig. 2.76). Discrete multifocal invasion of the renal cortex may be more common in the sporadic disease. There is gradual involvement of the marrow, and many cases die with leukemia, marrow failure, and widespread hemorrhages due to thrombocytopenia. Pigs with the inherited form of lymphoma have a heavy chain gammopathy with appearance of this fragment in the urine.

In lymphomas of outbred swine, most of which were diagnosed with tissues collected at slaughter, 60% were of diffuse large cell type, 24% Burkitt-like (small noncleaved cells), and 8% lymphoblastic. Acute lymphoblastic leukemia and lymphoma occur in outbred swine in animals as young as 3 weeks of age. An unusual form of lymphoma involves the lung, in which small arteries are ensheathed by a thick cuff of neoplastic T lymphocytes that have round nuclei, 2 red cells in diameter, and an immunoblastic appearance with a single central nucleolus, thickened nuclear membranes, and branched chromatin. The animals are presented with dyspnea and are suspected to have pneumonia. The neoplastic cells do not penetrate the walls of airways or vessels but cause dysfunction from compression of both and spread through the lung by encirclement of vascular channels. Neoplastic cells of similar type are found in the spleen.

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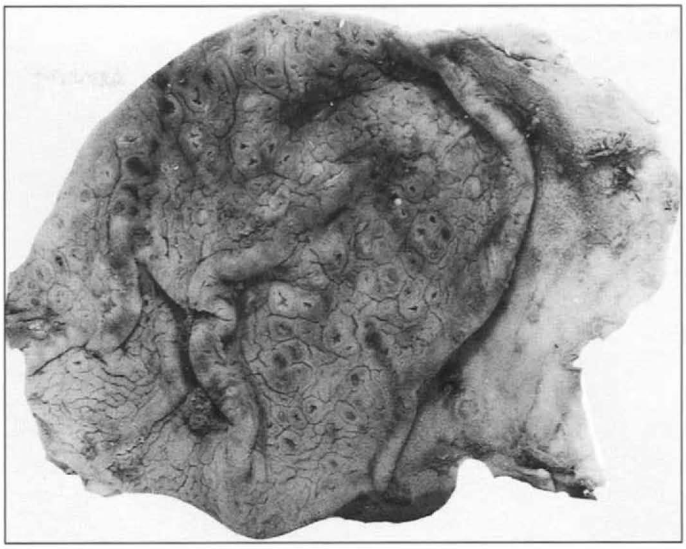

Figure 2.76 Lymphoma. Diffuse infiltration of stomach with pitting ulcers in a pig.

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Lymphomas in laboratory animals

Lymphomas are one of the most common tumors in rats and mice on 2-year or lifetime studies. There is much diversity of histologic type of tumor in various strains of animals, but the same types of tumors tend to cluster within a given strain. The murine lymphomas have been championed as models of human disease, but *unfortunately, rodent lymphomas have not been systematically reported as a function of animal genotype*, which is the information required by the pathologist dealing with the lesions in a specific strain of animal. A strain of *mice* infected with *Murine leukemia virus* (MuLV) developed a high incidence of hematopoietic tumors which are primarily B-cell lymphomas. Surprisingly, the tumors arising in these animals are more than 1/3 marginal zone lymphoma in histologic type as well as a much smaller proportion of follicular lymphomas and mantle cell lymphomas. Lymphomas occur as well in *ferrets* and in *hamsters* associated with a papovavirus. Lymphoma has been induced in *rabbits* by intravenous inoculation of Epstein-Barr virus (EBV) derived from cynomolgus leukocyte culture. One of the most studied and interesting spontaneous tumors is the large granular lymphocytic leukemia of the *Fischer rat* (F344; Fig. 2.56), which appears to be a tumor of natural-killer (NK) cells and has stimulated recognition of this tumor in many other species. Lymphomas have been increasingly found in *nonhuman primates*, particularly when infected with immunosuppressive lentivirus. They resemble their etiologic counterparts in humans, are very diverse, and have a tendency to involve the central nervous system.

Lymphomas may occur in *quail* (coturnix) utilized in laboratory studies, which may be associated with the avian reticuloendotheliosis virus. Breaks of lymphoma may occur in commercial *poultry* flocks as a result of infection with the same virus.

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Hodgkin-like lymphoma

Hodgkin-like lymphoma in animals that meets acceptable criteria as described for humans is a rare occurrence. In animals, Hodgkin-like lymphoma has been suggested to occur in the dog and more recently in the cat. *The diagnosis of Hodgkin disease rests on the demonstration of an acceptable Reed-Sternberg cell in an appropriate background of lymphoproliferation.* Few cases meet these criteria. The Reed-Sternberg cell (Fig. 2.67B) is apparently derived from a B cell in the classical subtypes of Hodgkin lymphoma, but is likely to be a dendritic cell in the lymphocyte-predominant Hodgkin lymphoma (see section on Hodgkin lymphoma). The lymphocyte-predominant type of Hodgkin lymphoma is of most interest in animal pathology because it is the subtype of this disease that most closely resembles the T-cell/histiocyte-rich B-cell lymphoma. There is confusion between the diagnosis of lymphocyte-predominant Hodgkin lymphoma and T-cell-rich B-cell lymphoma in humans and, therefore, it might be expected that lymphomas of the latter type in animals might be suggested to resemble Hodgkin lymphoma of humans. Both the classic type of Hodgkin lymphoma (includes subcategories of nodular sclerosis, mixed cellularity, and lymphocyte depletion), as well as the lymphocyte-predominant type, are characterized by artifactual contraction of the Reed-Sternberg cells from surrounding cells in formalin-fixed tissues. This artifact makes these cells more obvious on low-power examination and assists the identification of a rare Reed-Sternberg cell, or lacunal cell as they are called in early stages of development.

There is regional lymphadenopathy and the enlarged nodes tend to be very firm. The spread of the disease, particularly in cats, is by contiguity to adjacent lymphoid tissue, as in human Hodgkin lymphoma (see section on Hodgkin lymphoma). *Diagnosis is based on the presence of an unequivocal Reed-Sternberg cell in a background of homogeneous or heterogeneous lymphoid proliferation with mild or extensive sclerosis.* The disease tends to be indolent and animals may have large tumor masses and retain body condition.

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The erythron

The erythron may be defined as the circulating erythrocytes (red blood cells, RBCs), their nucleated precursors, and all elements involved in the control of erythroid proliferation. Diseases of the erythron are characterized by too many or too few red cells. *Polycythemia*, or too many red cells, is rare and usually due to dehydration and plasma volume contraction. *Anemia*, or too few red cells, is relatively common and is due to decreased red cell production or increased destruction or both.

ERYTHROCYTE PHYSIOLOGY

The erythrocyte is specialized to carry out gas transport, and in mammals is devoid of nuclei, mitochondria and ribosomes. Simplistically, the erythrocyte can be looked on as a cell membrane containing heme, globin and protective enzymes. The **red cell membrane** is a bilayer of lipid molecules oriented with the nonpolar groups directed inwards towards each other forming hydrophobic connections, while the hydrophilic overhead groups are directed outward, interacting with the plasma environment on the outer surface and cell contents on the inner surface. Studies of red cell membranes devoid of contents indicate the composition is 52% protein, 40% lipid and 8% carbohydrate by weight. Carbohydrate is largely made up of glycoproteins and a small amount of glycolipids. The *discoid shape of the red cell* ensures that all contents are close to a membrane surface, thus facilitating more efficient gas transport than would occur with a spherical shape. Another advantage of the discoid shape is its ability to undergo deformation in flow attaining an umbrella- or parachute-shaped configuration with the convex end oriented in the direction of flow, thus permitting red cells to pass through capillaries with the lumen only slightly more than 1/2 the diameter of the red cells. The indented area of the red cell is not fixed and can "flow" or move from one site to another as influenced by external forces. On the inner surface of the red cell membranes, fibrillar proteins form a supporting cytoskeleton. The lipid bilayer of the red cell is penetrated by a number of different transmembrane proteins, including glycophorin and ankyrin, both of which serve as anchor points for the fibrillar protein spectrin which, when linked by shorter chains of actin and tropomyosin, form an anchored "page-wire" network on the innersurface of the red cell membrane. *This fibrillar cytoskeleton provides and supports membrane shape, flexibility and durability.*

Cell volume is maintained by control of sodium and potassium levels through the action of ATP-dependent membrane enzymes. The membrane structural proteins are relatively fixed for the life of the cell, whereas the lipid, particularly cholesterol, is in dynamic interchange with that in plasma. This interchange is under enzymatic control and, in the presence of bile salts, cholesterol is gained by the cell causing increased membrane area and formation of the target cells associated with hepatic dysfunction.

The erythrocyte contents are largely proteins, of which some 95% is **hemoglobin** and the rest largely the enzyme systems required for glycolysis, protection of the heme and globin, and ATP production. The loss of the nucleus during maturation is accompanied by the loss of RNA and mitochondria. As a consequence, the mature erythrocyte has also lost the enzymes required for a functional tricarboxylic acid cycle, and the cytochromes essential for electron transport. The mature erythrocyte is, therefore, unable to derive phosphate-bond energy from the citric acid cycle and is unable to synthesize further heme. The remaining source of energy is through the metabolism of glucose to lactic acid to produce a functional level of ATP. This ATP functions to partition glucose from plasma, maintain homeostatic levels of intracellular sodium and potassium, maintain the biconcave shape, indirectly maintain iron in a ferrous state, and protect the integrity of the globin chains. As a consequence of glycolysis, there is production of the intermediate compound 2,3-diphosphoglycerate (2,3-DPG) which competitively binds to heme to control oxygen affinity and thus offloading. The balance between intracellular synthesis and utilization of glucose is such that glycogen is not formed and therefore erythrocytes are critically dependent on constant access to plasma glucose.

In circulation, red cells are constantly confronted with oxidant metabolites that are potentially injurious to heme, globin, enzymes and membranes. *The key antioxidant in the red cell system is reduced glutathione.* Reduced glutathione may be exhausted by oxidant attack from peroxides produced by macrophages or drugs. *This allows denaturation of the globin chains which form intraerythrocytic precipitates called* **Heinz bodies**. In animals, the major deficiency affecting glutathione regeneration is an (red cell) age-dependent deficiency of the glucose-6-phosphate dehydrogenase enzyme (G-6-PD). The G-6-PD enzyme is the rate-limiting factor in the hexose monophosphate shunt, and its deficiency in animals can be acquired by prolonged oxidant exposure as occurs in kale anemia or phenothiazine toxicity.

In order to function in oxygen transport, hemoglobin iron must be maintained in the reduced or ferrous state. Oxidant attack which reaches the heme iron forms ferric heme or *methemoglobin*, which is reduced by methemoglobin reductase or the diaphorase system. Some methemoglobin may be reduced by ascorbic acid and glutathione, but the diaphorase system is most important. Both the glutathione and the diaphorase reductase systems are critically dependent on a constant supply of glucose for energy. *Unlike the oxidation of globin, which results in hemolysis, the oxidation of heme causes cyanosis.* Deficiencies in methemoglobin reductase are inherited in humans and acquired in animals with nitrate poisoning.

Erythropoietin (EPO) is a constituent of plasma whose action is to increase the total number and volume of circulating red cells. The factor is class-specific, in that erythropoietin in one species of mammal is active in other mammals but not in birds or amphibians and vice versa. Erythropoietin is a glycoprotein synthesized by singly arranged cells within the renal cortex lined between the proximal convoluted tubules. Human erythropoietin contains 193 amino acids and consists of 39% carbohydrate with total molecular weight of 30 000 daltons. Erythropoietin must be glycosylated to be active, and is available as a recombinant pharmaceutical. Erythropoietin is able to pass the glomerular filtration process, and normally appears in urine at lower concentrations than in plasma. In anemic states, erythropoietin concentration in urine is greatly increased where its presence is indicative of a system responding normally to anemia and tissue anoxia.

Tissue anoxia is the specific stimulus for increased erythropoietin production and release. In this respect, arterial oxygen saturation is less basic to erythropoietin release than tissue saturation, since altered binding affinities may make blood oxygen unavailable to tissues. A DNA-binding protein in the area of the erythropoietin gene is tightly regulated by oxygen tension, and appears to be a physiological regulator of transcription from the erythropoietin gene.

Erythropoietin stimulates proliferation of primitive hematopoietic stem cells, which appear to be the target cells most sensitive to influence by erythropoietin and are identified by the highest density of erythropoietin receptors on their surface membranes. Hierarchically, these cells lie between the early committed stem cell and the proerythroblast, with the erythropoietin being absolutely essential for their survival, and, in the absence of adequate erythropoietin, these cells undergo programmed cell death. Continued presence of erythropoietin results in effective erythropoiesis and an increase in reticulocyte production. The continued administration of erythropoietin to humans with end-stage renal disease results in trilineage stimulation of bone marrow with an increase production of all cell types. Under increased hormonal pressure, accelerated hemoglobin synthesis occurs and, in sheep, a "switching" from synthesis of hemoglobin A to C, suggesting an action at the gene level above that of messenger RNA.

The net result of these changes is increased red cell production with an increase in mean cell volume and a greater proportion of maturation time spent in the peripheral blood. The presence of **"shift" polychromatic macrocytes** in the blood is erythropoietin-dependent, and they give a rough index of erythropoietin level and effect, indicating both increased levels of erythropoietin and a marrow capable and responding to it.

While in terms of evolutionary development, erythropoietin may be class-specific and human recombinant erythropoietin will stimulate increased erythropoiesis in domestic animals, the use of human recombinant erythropoietin in animals is not without risk. *Recombinant human erythropoietin (rhEPO) appears to be capable of causing the development of anti-rhEPO antibodies in both horses and dogs.* The human EPO genome appears to be 85% identical to that of cats and dogs and highly similar to the EPO genome of the horse. Since erythropoietin is a reasonably large polypeptide, it is not surprising that it could also act as an antigen in an unfamiliar host. Recombinant human EPO given to racing Standardbred horses, likely for non-medical reasons, later presented for medical examination and were found to have hematocrits of 16 and 24%. One horse had been given four injections of rhEPO and the second horse two administrations and both were presented for examination two months later when they were found to be anemic. Serum from these horses inhibited the growth of erythropoietin-dependent erythroid colony-forming units in culture assays, suggesting that their serum contained a factor that inactivated the rhEPO added to the culture median. Similar responses to rhEPO administration have occurred in the dog, whereas dogs given recombinant canine erythropoietin (rcEPO) responded normally with a temporary increase in reticulocytes and red cell counts, which was not followed by the development of anemia. *Androgenic steroids are able to increase endogenous EPO production and erythropoiesis,* which is accompanied by an increase in renal mass apparently associated with the proliferation of EPO-producing cells in the kidney.

Reticulocytes in the peripheral blood constitute a reliable index of new red cell production. Traditionally, reticulocytes have been enumerated using supravital stains in counting chambers which works well in the dog, cow and pig, but tends to be grossly inaccurate in the cat where large numbers of reticulocytes are produced with small amounts of residual RNA known as *punctate reticulocytes.* Cats also release reticulocytes with larger areas of RNA known as *aggregate reticulocytes,* which are more similar to those of other species. Reticulocyte counts in the cat based on aggregate reticulocytes provide an accurate assessment of erythropoiesis, but since the maturation from aggregate to punctate type of reticulocytes is a continuum, the differentiation between these two types and determination of their numbers is arbitrary and prone to error. In the *horse, which does not release reticulocytes into the peripheral blood,* the determination of increased erythropoiesis is largely dependent on changes in red cell numbers and the association of an increased mean cell volume with increased red cell production. The advent of electronic counting systems based on deflection of a laser beam rather than the Coulter system based on displacement of saline electrolyte has permitted the use of nucleic acid binding fluorochromes, which permits counting of reticulocytes in large numbers with more accurate results. It has also been possible to establish software gating programs that effectively differentiate between feline punctate and aggregate reticulocytes in electronic counting, permitting accurate determination of their numbers with very large samples counted. These results show that aggregate reticulocytes in the cat respond with up to 3% reticulocytes in response to phlebotomy, which is less than would be expected in the dog but adequate to provide a baseline for the species. The reporting of reticulocyte counts should always be made in *absolute numbers,* with percentage counts relatively meaningless without the added information of the total red cell count. Normal dogs with a total red cell count of 6×10^{12} red cells/L and 1% reticulocytes have 60×10^{9} new red cells/L to maintain homeostasis. This level of production is compatible with the red cell lifespan of 120 days at a turnover of approximately 1% of red cells per day. Evaluation of erythroid responses then needs to be based on a comparison of the absolute reticulocyte count in a canine patient in comparison to the normal level. Thus, a dog with 120×10^{9} reticulocytes/L in an absolute count has twice the normal output, and a dog with 300×10^{9} reticulocytes/L has five times the normal level and is at a level of production frequently reached by dogs with hemolytic anemia. Cats, in contrast, with anemia due to phlebotomy starting at a PCV of 20% and recovering to 27% over 12 days produced aggregate reticulocyte counts of 12×10^{9}/L prior to bleeding with gradual increases to 160×10^{9}/L on day 12. Over the same period, the punctate reticulocyte counts varied from 180×10^{9}/L to 1.7×10^{12}/L, without bearing any constant relationship to the number of aggregate reticulocytes.

Reticulocytes that are moderately larger (1.5 times the diameter) than a normal homologous erythrocyte lose their basophilia in about 1 day of circulation. *Macroreticulocytes or "shift" red cells* (2.0–2.5 times the diameter of normal erythrocyte) require 2 or more days to become normochromic, during which time they continue to synthesize hemoglobin. Remodeling of normochromic macrocytes continues for a week or more, during which time there is loss of membrane and hemoglobin until the cell is of characteristic diameter for the species. This distinction is important, since reticulocytosis is indicative of present production whereas macrocytosis indicates previous production and, in the absence of reticulocytosis in an anemic animal, signals recent cessation of red cell production.

Young red cells have unique characteristics that affect their behavior. Because of their larger size, lack of concavity and greater surface charge, they do not form rouleaux. Because of their reduced hemoglobin saturation, they have lower specific gravity and in the absence of rouleaux formation settle more slowly in sedimentation tubes giving a biphasic reaction. Since they contain mitochondria and are capable of aerobic glycolysis, they are more resistant to crenation and hypotonic lysis.

Prior to the understanding of the effects of EPO on the prevention of programmed cell death in erythroid precursors, it was frequently proposed that increased erythropoiesis was accomplished through an increase in numbers of erythropoietic cells and a decrease in mitotic intervals. Current understanding favors maintenance of normal kinetics of production with increased erythroid output, the result of a greater number of proliferative phase cells and the survival of their progeny through to the reticulocyte stage. These concepts are in agreement with earlier work on red cell production in calves where, in autoradiographic studies of bone marrow of calves given tritiated thymidine in vivo, the mean production time of labeled marrow metarubricytes in normal calves was 3.79 days, and in calves made leukopenic by endotoxin 3.83 days, and 3.76 days in calves bled to one-half their normal hematocrit level. Thus, it appears that *the production time of the red cell is very close to four days in most species* and the major difference between hematologic responses of the dog and the calf are in a much greater rapidity with which the dog can amplify the stem cell pool.

The **metabolism of iron** is central to the function of the erythron. Iron is tightly conserved in the body, and is lost in very small amounts in urine, sweat, and intestinal epithelial cells. The kinetics of iron absorption and its control are complex, but certain principles apply to all species. First, when storage iron is decreased, iron for absorption from the intestine is increased and vice versa. Second, iron absorption varies directly with the rate of erythropoiesis, being increased when erythropoiesis is increased and decreased in proportion to red cell production.

The key to iron absorption from the intestine resides in a mucosal absorptive cell that is able to absorb iron but is able to respond to reduced body iron stores by increasing the efficiency of iron absorption. The process is not understood, but an iron-binding protein called *mobilferrin* that may act as an iron messenger is built into the mucosal cell at the time of division in the intestinal crypts and programs for a given level of iron absorption. Transferrin, the plasma transport protein for iron, and its level of saturation are not the controlling factors in intestinal iron absorption since it has been found that subjects lacking transferrin have a normal level of iron absorption but abnormal transport and deposition in abnormal tissues. Iron is contained in a variety of compounds and compartments within the body. These are referred to as *iron pools,* and it is convenient to consider amount and proportions of iron present in a normal 70 kg human who has 4 g total body iron. In normal steady-state circumstances, 66% of those 4 g would be contained in hemoglobin, and thus there could not be sufficient reserves to replace the iron lost by significant hemorrhage. In young and growing animals, there are virtually no reserves and any iron loss is therefore more serious than in adults.

Free plasma iron, like other heavy metals, is toxic and a three-tiered system of protein-mediated transport and storage exists. *The most labile form of iron is contained in the beta globulin transport protein,* **transferrin**, which is produced in the liver and which is normally 40–50% saturated with iron. In the normal adult, transferrin contains 3 mg of iron and 0.07% of total body iron. In iron deficiency, transferrin levels may double, and levels decrease in protein starvation. The level of transferrin in serum is referred to as "*total iron-binding capacity*" while that portion carrying iron is called "*serum iron.*" Transferrin saturation levels of less than 16% are rate-limiting to erythropoiesis. This limiting level is easily reached in deficient states since, as serum iron drops, total iron-binding capacity increases. Each transferrin molecule is able to carry 2 molecules of iron, of which the first is more easily transferred than the second. The developing rubricytes have some 50 000 specific transferrin receptors that cleave the iron molecules into the erythroid cytoplasm. Although the erythroid marrow receives only 5% of cardiac output, 85% of the iron in transport is retained.

Iron must exist in the ferric state to bind to transferrin. **Ceruloplasmin**, *a copper-containing globulin, facilitates iron transport and converts ferrous to ferric iron in the presence of reducing metabolites.* Ceruloplasmin decreases in starvation and copper deficiency. It appears to act as an acute-phase protein and increases in late pregnancy, infections, leukemia-lymphoma, and in anemia of ineffective erythropoiesis of iron- or B12-deficient origin. Deficient ceruloplasmin results in impaired iron transport and hypochromic microcytic anemia indistinguishable from that caused by iron deficiency, except that marrow hemosiderin is increased rather than absent. Reticulocytosis following supplementation of deficient animals is delayed for several days while ceruloplasmin is synthesized.

Ferritin *forms the second level of iron transport and storage,* and acts as a buffer between transferrin, where iron turnover is very high, and hemosiderin, where iron turnover is low. Ferritin is an iron–protein complex occurring in most cells and plasma, and *consists of apoferritin protein subunits and iron.* Ferritin constitutes a labile source of storage iron and, in the normal human, 0.7–1.5 g of iron are bound to this protein. In inflammatory states, an acute-reacting system continuously scavenges iron from transferrin to ferritin where it is less available to both invading microorganisms and erythropoiesis. This system appears to be the basis for the paradoxical anemia of chronic disease, in which body iron stores are increased but serum iron is rate-limiting to erythropoiesis. *Serum ferritin levels, unlike serum iron levels, are an accurate measure of body iron stores.*

Hemosiderin *is the most stable and least available form of storage iron.* It is always intracellular, usually in macrophages, and is not found in plasma. Ferritin molecules have been observed in hemosiderin granules, and immunologically the proteins in both are identical. It appears that hemosiderin is a variable compound consisting of native and denatured ferritin with a total iron content of 25–30% by weight. The iron in hemosiderin is available only after what appears to be prolonged proteolysis. Thus, *hemosiderin constitutes the nonlabile iron storage system,* and the normal human adult has 1.2 to 1.5 g of total body iron bound in hemosiderin. Animals with abundant marrow hemosiderin do not respond maximally to blood-loss anemia unless supplemental iron is given. Iron from all sources, including that derived from hemolysis, hemosiderin, lysis and oral and parenteral supplementation, appears to be additive in achieving erythropoiesis.

Hemoglobin synthesis begins early in the sequence of erythroid differentiation. *The three essential components of hemoglobin are iron, protoporphyrin, and the globin chains.* The assembly of these components probably is closely coordinated so that the organic structures are not produced in excess if iron is limiting. Hemoglobin synthesis is under genetic control. The amino acid substitutions of the globin chain seen in human hemoglobinopathies are not found in animals. In animals, there is some switching of hemoglobin type as a result of alternate globin chains being produced under conditions of severe anemia and very high erythropoietin levels. This process may represent an acquired mutation that aids survival by substituting a chain with greater efficiency of oxygen delivery in anemic states. It also suggests that erythropoietin influences DNA transcription. Two-thirds of the erythrocytic hemoglobin is synthesized during the rubricyte stages, and one third is completed under the direction of messenger RNA left in the reticulocyte at denucleation.

The initial step in heme synthesis is the intramitochondrial union of glycine and succinyl-CoA to form *δ-aminolevulinic acid.* This step is dependent on the presence of pyridoxal phosphate of pyridoxine (vitamin B6) origin. The δ-aminolevulinic acid is then transported to the cytosol where two molecules are combined to form the ringed pyrrole *porphobilinogen.* Four of these pyrroles are then joined and closed to form the tetrapyrrole intermediates, *uroporphyrinogen* and *coproporphyrinogen,* which are then transported back into the mitochondria for the conversion to protoporphyrin and the addition of iron to form *heme.* In this sequence, *the porphyrinogens are the true intermediates, and the uroporphyrin and coproporphyin are byproducts of heme synthesis.*

The process of heme synthesis is accelerated by the presence of iron and is slowed by a build up of the end-product *hemin* (protoporphyrin–iron chloride) probably at the level of δ-aminolevulinic acid synthesis. *Lead poisoning* blocks the formation of protoporphyrin and causes δ-aminolevulinic acid to circulate in excess; its urinary excretion constitutes a test for lead toxicity. In addition, tetrapyrrols accumulate due to inhibition of iron insertion. These compounds are photodynamic and may be detected by fluorescence under ultraviolet examination.

The term **ineffective erythropoiesis** is used to indicate *hematopoietic production that does not result in functional release of new cells.* When erythropoiesis is ineffective, there is peripheral blood anisocytosis and poikilocytosis without appropriate reticulocytosis, and a tendency for increased bilirubin as a result of increased intramedullary erythrocyte catabolism.

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ANEMIA

Anemia is a sign of disease that results either from an *increased rate of destruction or loss of erythrocytes*, or from a *decreased rate of their production*. Anemia may be classified on the basis of the bone marrow response (*regenerative or nonregenerative*) or on the basis of red cell indices (*macrocytic/microcytic, normochromic/hypochromic*) which indirectly reflect the nature of the anemia itself. Anemia is classified here on an etiologic or pathogenetic basis with reference to various mechanisms for assessing bone marrow production. The descriptions which follow provide morphologic criteria to assist in the differentiation of the various types of anemias.

The function of hemoglobin is to supply oxygen to the tissues, and the signs and lesions of anemia are largely referable to hypoxia. Depending on the cause, however, other manifestations of the diseases of which anemia is a part are often foremost in appearance. Thus, in anemia of massive hemorrhage, the manifestations relate to hypoxia and sudden reduction in the volume of circulating blood; in hemolytic anemia, the prominent features may be icterus and hemoglobinuria, with fever whether or not the cause is infectious. The rate at which anemia develops will also influence the manifestations: when the development is rapid, signs of anoxia are likely to be predominant; when the development is slow, as in deficiency and aplastic anemia, the condition may be masked by physiologic compensations until some increased requirement, often exercise, places overwhelming demands on the organism.

The appearance of an anemic animal is characterized by *pallor*, perhaps obscured by icterus. Pallor is due to a reduction in total levels of hemoglobin, and, if the disease has developed slowly, to a dilution of the red cell mass by a proportionate increase in the plasma compartment in order to maintain normal blood volume. Grossly, the pallor is accompanied by edema of the *lung*, and tracheobronchial foam is typical. The *heart* is dilated and, if the anemia has been chronic, the myocardium tends to be flabby, friable, and pale as a result of fatty degeneration. The proportion of white to red blood clot is increased, and the *liver* and kidney are pale and may be fatty. In acute anemia, there is frequently the "nutmeg" appearance of periacinar hepatic necrosis. In acute hemolytic anemias, the *gallbladder* is distended and the feces are intensely stained with bile pigment. In chronic anemias of deficient hemoglobin production, the gallbladder is empty and the feces are pale. The character of the *spleen* varies with the cause and course of the disease. In the acute posthemorrhagic anemias, the spleen is contracted; in the acute hemolytic anemias, it is enlarged and pulpy; in the chronic hemolytic and deficiency anemias, it is enlarged and meaty. Since the spleen is a site of both production and destruction of red cells, the splenomegaly of hemolytic diseases may be the product of extramedullary hematopoiesis, or sinus hyperplasia of increased blood destruction, or both. In chronic anemia, the skeletal muscles tend to be pale in proportion to the loss of myohemoglobin. *Edema* is a frequent accompaniment of anemia, and serous fluid is found in the serosal sacs and in the dependent tissues of the limbs, ventral wall of the body, and throat. *Since the full characterization of an anemia may require the examination of both blood and tissues, it is important that diagnostic examinations begin with the live animal.* Cytologic preparations of bone marrow, like those of blood, can best be made prior to clotting and therefore both should be collected prior to death.

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Congenital abnormalities of red cell number and function: congenital aplastic/hypoplastic anemia

Anemias due to a congenital error in stem cell number (*reticular dysgenesis*) are rare or lethal defects that are infrequently identified in animals. In contrast, *those diseases characterized by normal or increased production of defective red cells with shortened life span are much more prevalent* and, because of their potential for dissemination through artificial breeding, they are of much more economic importance. Neonatal *llamas* suffer a severe anemia associated with slow growth, limb deformity, and hypothyroidism. The disease has not been adequately characterized, but may be a primary defect of red cells or a reflection of systemic errors in metabolism. A disease of *polled Hereford calves* characterized by anemia and cutaneous abnormalities is described in Vol. 1, Skin and appendages. A condition that strongly suggests congenital marrow aplasia has been described in a *Holstein calf*. A rare congenital pure red cell aplasia occurs in *humans* known as *Diamond-Blackfan anemia*, which has been treated with corticosteroids. It has been shown that interleukin-3 is able to support trilineage marrow production in these individuals. Animal counterparts of this condition have not been identified.

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Hereditary hemolytic disorders of red cell membrane or enzymes

Hereditary stomatocytosis

Red cells with a rectangular unstained area across their center are termed **stomatocytes** (Fig. 2.77). A hereditary anemia with these membrane changes occurs in *chondrodysplastic dwarf Alaskan malamute dogs* (see Vol. 1, Bones and joints). The anemia in these dogs is mild, and characterized by stomatocytosis, macrocytosis, reduced mean corpuscular hemoglobin, increased osmotic fragility, shortened red cell life span, reticulocytosis with marrow erythroid hyperplasia, and increased iron turnover. The glutathione levels are reduced. The anemia is thus hemolytic and partially compensated. Intracellular sodium and potassium are also abnormal in affected dogs. Changes are most severe in homozygotes in which both sodium and potassium are elevated. In heterozygotes, only sodium is elevated.

The hemolysis is low grade but intravascular, resulting in reduced serum haptoglobin, deposition of iron in renal epithelium, and hemosiderinuria. The defect is intrinsic to the red cells, since cells of affected donors have a shortened survival in normal dogs, whereas cells of normal donors survive normally in affected dogs. The reduced glutathione level does not render the affected cells more susceptible to oxidative denaturation of globin (Heinz bodies), because the enzyme present is unusually stable.

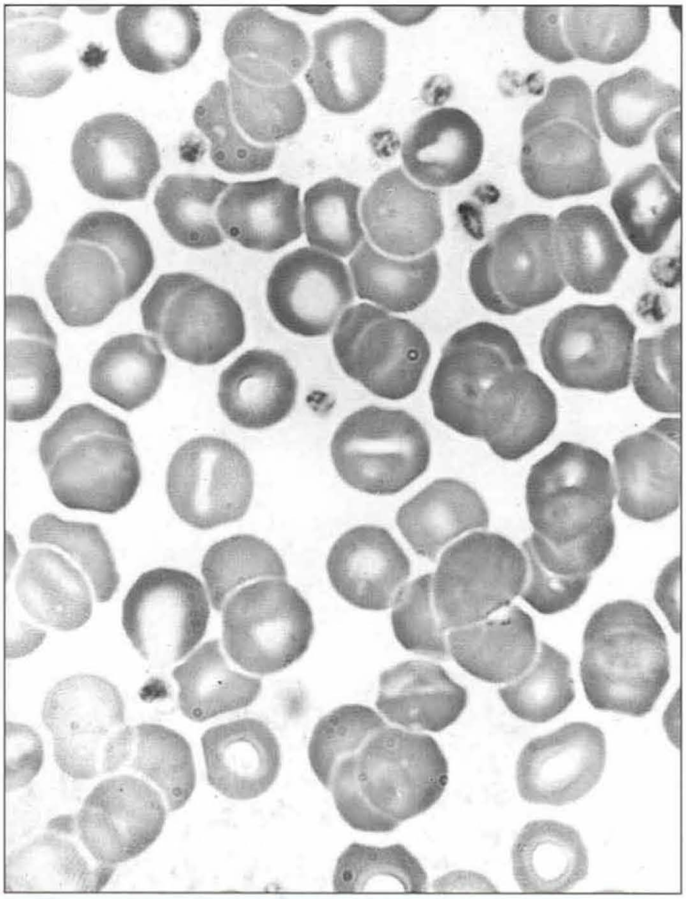

Figure 2.77 Hereditary stomatocytosis. dog. Central pallor of red cells with rectangular clear areas.

Familial anemia of *Abyssinian and Somal cats* has been described, in which there is prominent stomatocytosis and markedly increased somatic fragility.

Familial anemia in dogs

A familial hemolytic anemia found in Basenji and Beagle dogs is due to **pyruvate kinase deficiency**. The dogs are of normal appearance, size and weight. They are less active than normal, and on clinical examination have pale mucous membranes and often splenomegaly.

The anemia is moderately severe (60 g Hb/L), marocytic (MCV 86 fL), with large partially saturated cells (MCH 23 pg, MCHC 27%). There is a reduction in red cell count ($2–3 \times 10^{12}$/L) with a *remarkable reticulocytosis of 40%*. The young red cells with mitochondria are able to carry out aerobic metabolism and are protected until they mature. There is increased resistance to osmotic lysis for the same reason. There is a marked marrow erythroid hyperplasia with myeloid:erythroid ratios of 0.2–0.4. The apparent (^{51}Cr) red cell lifespan of normal Basenjis is about 20 days and 2.5–3.0 days in affected dogs. Plasma iron turnover rate is twice normal. Heterozygous carriers are asymptomatic but have higher than normal levels of red cell 2,3-diphosphoglycerate, as do the fully affected homozygous animals. The oxygen dissociation curve of affected animals is shifted to the right with reduced hemoglobin oxygen affinity due to the high intracellular levels of 2,3-diphosphoglycerate. Morphologically, Basenjis with pyruvate kinase deficiency develop *progressive myelofibrosis with osteosclerosis* as well as splenic and hepatic hemosiderosis.

Hereditary disorders of heme synthesis: the porphyrias

Porphyrias are diseases that result from abnormal metabolism of porphyrins which may be excreted to excess in urine and feces or deposited in various tissues. The pigments are fluorescent, unless iron is chelated in them. Fluorescent activation leads to the local production of free radicals in skin and exposed mucous membranes in the condition known as *photosensitization*.

Porphyrins are basic components of enzymes that contain heme, including hemoglobin, the cytochromes, catalase and many peroxidases. Porphyrias occur when toxic or inherited metabolic defects limit the activity of one of the enzymes in the synthetic chain.

Bovine protoporphyria affects *Limousin cattle* and is *caused by a deficiency in the activity of ferrochelatase*, the terminal enzyme which catalyzes the insertion of ferrous iron into protoporphyrin IX to form heme. The inheritance is recessive and heterozygotes are asymptomatic. Bovine protoporphyria differs from bovine congenital porphyria in that there is no excretion of porphyrins in the urine, no anemia and no discoloration of bone or teeth; *photodynamic dermatitis is the only consistent clinical manifestation.* Pigment is, however, deposited in the liver and heavy concentrations resembling lipofuscin are found in the cytoplasm of hepatocytes, Kupffer cells, endothelium, and portal stroma.

Congenital porphyria occurs in *humans, cattle, and cats* and results from the production of the *abnormal protoporphyrin isomer I.* It also occurs in swine, but the biochemical defect is not known. All cells in the body share this defect but, since the effects on the blood system are most apparent, the disease is usually called *erythropoietic porphyria*. Only isomer III can be synthesized into heme, although the enzymatic block is never complete and some normal heme is produced. The abnormal porphyrinogen intermediates accumulate in tissues and are readily oxidized to porphyrins capable of absorbing visible light and emitting visible fluorescence causing *photosensitization* (see Vol. 1, Skin and appendages). The disease is transmitted as a recessive trait (dominant in pigs) and heterozygotes are unaffected.

The enzyme uroporphyrinogen III cosynthetase is much reduced in affected animals and intermediate in heterozygotes. The disease results from either an absolute or relative deficiency of this enzyme because of overproduction of proximal cosynthetase enzymes. The type I isomer builds up in red cells and tissues and may be detected by fluorescence.

There is mild-to-moderate anemia (60–110 g Hb/L) exacerbated by exposure to light. The anemia is macrocytic normochromic in recovery. Polychromasia is mild and increases with light exposure. Marrow is hyperplastic with increase in late stage rubricytes. Red cells fluoresce and urine is brown. Red cell protoporphyrin is increased (350–450 mg/L of red cells, normal being 0.1–0.2 mg/L red cells). There is reduced ferrochelatase activity, but the level of immunoreactive protein is normal, suggesting a point mutation in the gene coding for the synthesis of this enzyme. Human patients with congenital porphyria develop hepatic hemosiderosis terminating in cirrhosis and suffer severe hemolytic anemia following hepatic orthopic transplantation. It is suggested that there was a consistent mild hemolytic anemia due to the hypersensitivity of the red cells to photo-oxidation such as might occur during a prolonged operative procedure.

Acquired porphyrias may follow exposure to *lindane* and related substances (see Vol. 1, Bones and joints).

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Anemia of deficient cell production

Anemias due to reduction in red cell production are characterized by hypoproliferation that primarily affects cell division as opposed to hemoglobin production. *The causes of inadequate cell production include immune phenomena, often drug-induced, toxins both exogenous and due to hepatorenal failure, myelophthisis, chronic inflammation, endocrine dysfunction and nutritional deficiency.*

Aplastic pancytopenia

Aplastic pancytopenia occurs in all species but is *most often seen in cats and dogs*. It may be congenital, but is *usually acquired and idiopathic*, and may be associated with specific infections or toxic agents. Marrow aplasia may be absolute and rapidly fatal or, more commonly, hypoplastic and chronic. Any of the three blood cell lines may become aplastic, but *characteristically the disease is either one of generalized aplasia resulting in pancytopenia, or of pure red cell aplasia* (Fig. 2.78) with a hyperplastic myeloid and megakaryocytic marrow. Rarely, diseases characterized by leukopenia are accompanied by marrow myeloid hyperplasia with apparent immune suppression of myeloid maturation. Thus, the apparent marrow hyperactivity is ineffective, and output is deficient. The accepted criteria for aplastic pancytopenia (aplastic anemia) are peripheral blood neutrophils less than 0.5×10^9/L with platelets less than 20×10^9/L, an

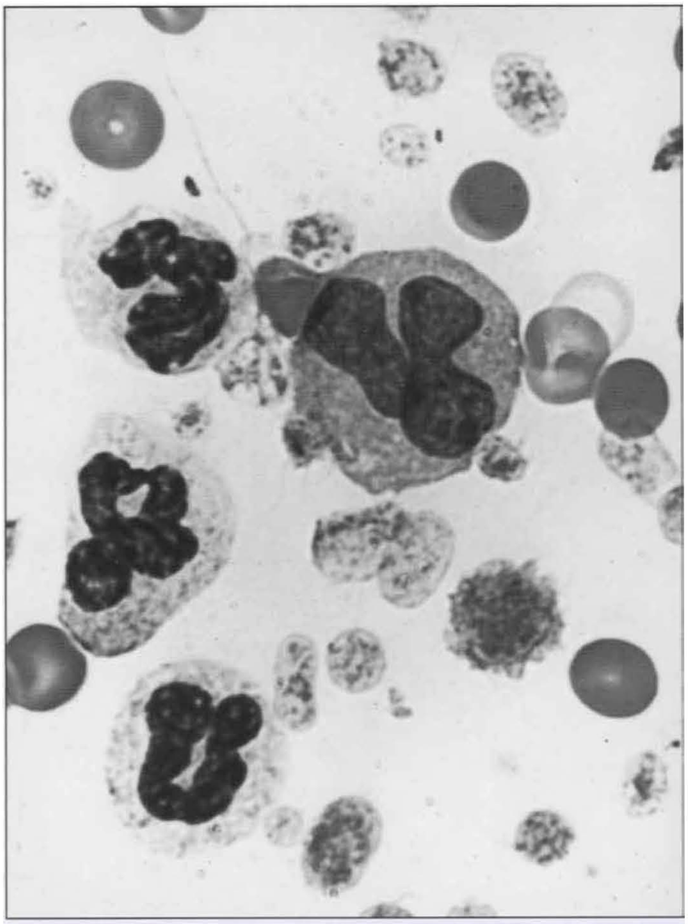

Figure 2.78 Pure red cell aplasia, peripheral blood from a dog. Nonresponsive anemia with excess neutrophils, monocytes, platelets.

absence of reticulocytes, and severe marrow hypocellularity in which fewer than 30% of residual cells are of hematopoietic type. *Suggested mechanisms for aplasia include decreased numbers of normal stem cells, inhibition of stem cell maturation, abnormal marrow microenvironment, lack of hematopoietic factors, and marrow depression associated with thymoma.*

Viruses may cause marrow depression. The most important of these in animals are the canine and feline parvoviruses and *Feline leukemia virus.* **Phenylbutazone** causes aplasia in humans but appears not to have this effect in animals unless there is prolonged exposure at high dosage. All forms of **estrogenic drugs** are potentially myelotoxic in dogs, and disease is characterized by an initial leukocytosis followed by thrombocytopenia, hemorrhage and aplasia which is usually irreversible. **Chloramphenicol** causes irreversible myeloid aplasia in humans as an apparent idiosyncratic reaction. It does not have this idiosyncratic effect in animals, in which marrow suppression is dose-related at above-normal rates of administration. **Furazolidone** at high dose rates is acutely neurotoxic in calves and pigs. When administered long-term to calves at dose rates used for prophylaxis of infection, furazolidine causes toxic depression of bone marrow manifested acutely as agranulocytopenia with widespread embolic infections, clinically best revealed as discrete necroses and ulcers of mucous membranes.

Animals with **aplastic pancytopenia** have epistaxis, bleeding into joints and dependent areas, and are often febrile due to sepsis. Animals with **pure red cell aplasia** are presented with mucosal pallor, lethargy and reduced activity, and are usually afebrile. Pancytopenia may be either moderate and chronic, or severe and acute with an absence of neutrophils. Those granulocytes present show toxic changes and little immaturity. Thrombocytopenia is severe and nonregenerative, with all platelets aged, small and with little basophilia. Anemia is moderate-to-severe and normochromic normocytic without polychromatic response. Differences in lifespan and rates of consumption of the cells of blood dictate that the aplastic syndromes are dominated by the purpura of thrombocytopenia and the sepsis of agranulocytosis. Morphologically, hemorrhage and sepsis will predominate in proportion to the relative deficits of platelets and/or neutrophils. If neutropenia is severe, there will be widespread bacterial emboli in lung, liver and kidney unaccompanied by the edema and cell reaction characteristically seen with a competent marrow.

Bracken fern poisoning

Bracken fern (*Pteridium aquilinum*) is abundant in many humid grassland areas of the world, and poisoning by it is often the factor that limits the utilization of pastures. There are other species in the bracken-fern genus, notably *P. esculentum*, the common bracken, which are toxic and the diseases produced are identical. *Cheilanthes sieberi*, the **mulga or rock fern**, contains much higher levels of thiaminase then does bracken, and produces the typical hematopoietic syndrome in cattle and polioencephalomalacia in sheep.

Bracken fern poisoning produces a variety of disease syndromes in grazing animals. The acute disease occurs in horses and pigs as a *neurologic and cardiac manifestation of thiamine deficiency*, in sheep as *polioencephalomalacia*, and in cattle as *aplastic pancytopenia*. The chronic syndromes occur in cattle and sheep as "*enzootic hematuria*" and "*bright blindness*," and in cattle there is an association with *epithelial tumors of the alimentary tract.* Some of these syndromes are appropriately discussed in other chapters.

Bracken fern appears to be most poisonous when it is green and growing actively. Hay made from the fern is also toxic, providing that the plants are well dried and retain some of the natural color. The fern is not particularly palatable and the ecological conditions which are conducive to the ingestion of toxic amounts of the plant are drought resulting in inadequate top growth of improved forage, or an excess of lush pasture in which animals seek out coarse growth in order to obtain sufficient roughage. The *poisoning is cumulative* and the disease may not be evident until after many weeks of continuous exposure, or for as long as 8 weeks after last exposure. *Cattle and horses are the most susceptible species*, sheep are relatively resistant as well as being fastidious eaters, and pigs, which eat the rhizomes, are occasionally poisoned. The manifestations of poisoning differ in the different species, but *depression of hematopoiesis is common to them all.* In cattle and sheep, the essential change is *irreversible trilineage marrow hypoplasia.* This results in **aplastic pancytopenia** and death due to thrombocytopenic hemorrhage (Fig. 2.79A, B) complicated by septicemia of neutropenia (Fig. 2.80A, B).

Horses and rats suffer myelosuppression but die of a nervous disease, which is curable by thiamine, before the effects of pancytopenia are fully developed. Horses whose diet contains 40% or more of bracken fern suffer inappetence, loss of weight, bradycardia, and nervous signs which progress to recumbency. The illness can be both prevented and treated with thiamine. The syndrome is comparable to that in horses poisoned by *Equisetum* spp., especially *E. arvense*, in which a thiaminase is present as it is in bracken fern and other pteridophytes. As expected in thiamine deficiency, there is, in horses and rats poisoned by bracken fern, a gradual rise in the pyruvate levels in blood. The hematological changes are not of prognostic significance in bracken poisoning in these species. The hematological changes are reversed by thiamine, but this effect appears to involve prevention of thiaminase toxicity rather than by correcting a thiamine deficiency, since a simple deficiency in rats does not cause anemia.

The disease in **cattle** apparently depends on a *direct inhibition of a pluripotent stem cell* and not a disturbance of the metabolism of thiamine. The principal hematological change in nonruminants is thrombocytopenia but there is also a tendency to neutropenia and anemia complicated by hemorrhage. The changes are consistent with what would be expected with inhibition of proliferation of blood cells in a "pipeline production" where the platelets have a 4 day production time and a life span of 9 days and the neutrophils a 6- to 7-day production time with a life span of only 6–8 hours. The anemia of hypoproliferation is less significant, unless complicated by bleeding, because of the long lifespan of the erythrocyte.

The morbidity from bracken poisoning in cattle is not high, but the mortality is, and recovery is rare once clinical signs are evident. This is logical since clinical signs are secondary to a complete loss of the "pipeline" of blood cell formation, and even a return of stem cell function would require survival through a week of thrombocytopenia and neutropenia before the results of renewed production become functional in the peripheral blood. In cattle, *thrombocytopenia occurs first* and is followed in hours by *leukopenia and neutropenia*, both likely complicated by increased consumption as hemorrhagic foci remove both from circulation. In contrast to the platelet and neutrophil precursors, some rubricytes persist in the

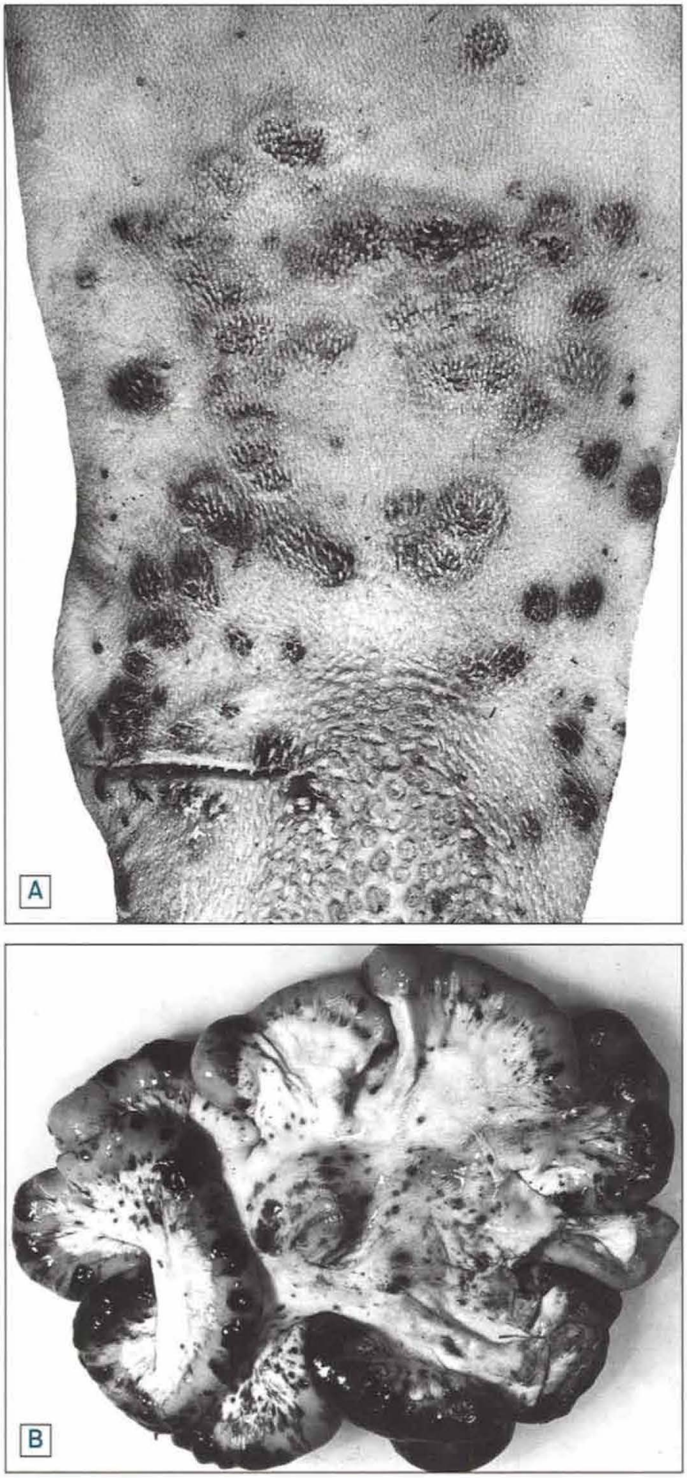

Figure 2.79 Bracken fern poisoning in an ox. **A.** Hemorrhagic infarcts in the tongue. **B.** Serosal hemorrhages of the intestine.

marrow, but the anemia is nevertheless largely nonresponsive. Because of the long (155 days) red cell lifespan in cattle, the anemia is, however, largely due to *hemorrhage* which is both enteric and generalized. As the disease develops, clot retraction is impaired out of proportion to the reduction in the platelet count, suggesting impaired function (thrombasthenia) as well as deficient numbers. Increased capillary fragility has also been proposed and is likely a consequence of unremitting thrombocytopenia.

The dominant features of the clinical disease are hemorrhages, bacteremia, and fever. The hemorrhages are due to thrombocytopenia, and the bacteremia due to neutropenia. Bacteremia and bacterial embolism contribute to the genesis of hemorrhage. The latent period of bracken poisoning in cattle is 2 months or more, and even longer in sheep, but the clinical episode develops suddenly and acutely, with high fever, hemorrhages on mucous membranes, and hemorrhages from natural orifices including the urethra. Calves frequently show excessive discharge of mucus, sometimes blood-stained, from the nose and mouth, edema of the throat, and focal, necrotic stomatitis and cheilitis (Fig. 2.79A). The course is usually less than 3 days. In the rare cases where recovery has been followed, the return of the leukocyte count to normal may require several months, which suggests continuing toxicity or injury to a very primitive stem cell pool of restricted numbers that requires weeks to replenish.

Grossly, *there is hemorrhage in almost all tissues but without any particular pattern* (Fig. 2.79B). They are particularly numerous, however, in the stomach and intestine, to the mucosal surface of which the blood tends to adhere in clots; in the large intestine, the hemorrhages are more diffuse, and copious free blood may be present in the lumen. *Hemorrhages beneath mucous membranes usually lead to overlying ulceration.* Hemorrhages in the liver may be sufficiently numerous to give it an odd variegated appearance, especially when it also contains many infarcts (Fig. 2.80A). The *hepatic infarcts* are spherical or wedge-shaped areas of white or yellow and similar to the lesions of black disease; they may be absent, few, or numerous. The hepatic infarcts, like those in other tissues, are caused by bacterial emboli which localize in small branches of the portal vein, the sinusoids, and the central veins. The bacteria are ordinary saprophytes in many cases and, if the postmortem interval is prolonged, the focal lesions expand about the colonies because of local postmortem putrefaction. The heart has extensive subserosal hemorrhage but, in addition, there are foci of *myocardial infarction* which are discrete yellow areas up to 1 cm long. There is usually little or no cellular reaction to these embolic bacteria or to the necrosis of tissue in the infarcts (Fig. 2.80B). *Infarcts in the kidney* are usually red and also caused by bacterial embolism. Extensive hemorrhages are rather consistently present in the walls of excretory passages of the urinary system. The red *bone marrow*, which in adults should be firm and fatty, is instead pink and soft, and it dehydrates and shrinks on exposure to air. Marrow cellularity is markedly diminished to the point where either only small islets of erythropoiesis remain, or there is complete aplasia with edematous stroma and dilated sinusoids, and only a few fat cells are present.

Pigs fed rhizomes show only *minor signs of toxicity* including, depression of growth rate, increases in blood pyruvate, and a reticulocytopenia, all of which can be corrected by administration of thiamine. Neurological signs such as occur in horses are not observed, but there is myocardial injury of the type observed in experimental thiamine deficiency in pigs. The rhizomes contain substantially more thiaminase than do the fronds.

The experimental production of bracken poisoning in **sheep** is very difficult. Sheep find the fern unpalatable and are slow to develop signs of poisoning. The clinical and pathologic manifestations of the disease are analogous to those in cattle, including the development of pancytopenia.

The discussion above relates to *two toxic factors in bracken fern*, one a *thiaminase* and the other a *substance that causes bone marrow depression*, especially in ruminants. A nosesquiterpene glucoside, *ptaquiloside*,

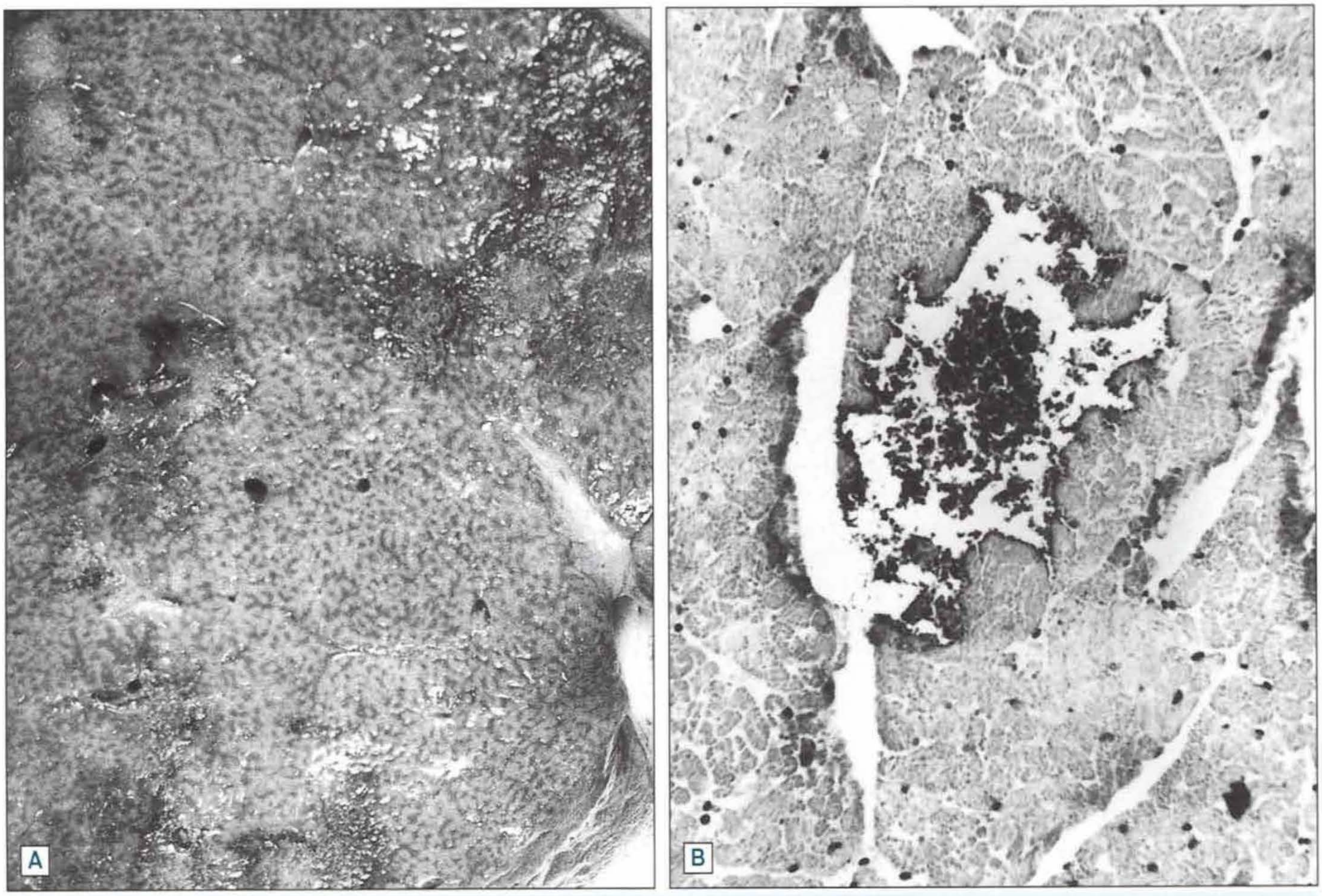

Figure 2.80 Bracken fern poisoning in an ox. **A.** Fatty liver with pale and red infarcts resulting from bacterial embolism. **B.** Bacterial embolism without inflammatory response.

extracted from bracken fern, has produced bone marrow depression in a calf but its significance to the naturally occurring disease is unclear. It appears to be the responsible agent in enzootic hematuria.

Disease in cattle, apparently identical to that produced by bracken fern, has resulted from feeding of *soya bean meal which had been extracted with trichlorethylene*, and in calves fed diets to which *nitrofurans* were added for bacteriostatic purposes.

Pure red cell aplasia

Pure red cell aplasia is seen in *humans and dogs*. Pathogenetically, there is *immune suppression of differentiated red cell precursors that is T-cell driven and mediated by interferon, and there may also be antibody against erythroid nuclei.* T-cell inhibition may be of several types, including that associated with the proliferation of benign clonal or malignant large granular lymphocytes, particularly those with the γδ T cells and some NK cells. In human disease associated with leukemias of large granular lymphocytes, there tends to be a more general suppression of bone marrow with neutropenia as the most important persisting deficit. In dogs, neutropenia does not appear to be a feature of neoplasms of large granular lymphocytes; however, nonresponsive anemia appears to be a consistent finding. Therapy, which is likely to be effective if vigorous, is indicative of the mechanism of aplasia and includes immunosuppression by drugs, antithymocyte globulin, and plasmapheresis.

Clinically, the disease is likely to occur in a mature dog and may result from exposure to a drug or virus, or be associated with a lymphoid tumor of the large granular lymphocyte type (see B-cell chronic lymphocytic leukemia/small lymphocytic leukemia). There is weight loss and exercise intolerance but the appetite is usually retained. There is pallor of membranes but no tendency for bleeding or infection. Hematologically, the anemia may be severe, with hemoglobin less than 50 g/L, and worse than the red cell numbers might suggest, since the red cells, while normochromic and normocytic, become small with age. While reticulocytes are consistently absent, there appears to be stimulation of those lineages able to respond and there is characteristically mature neutrophilia of 15–25 × 10^9/L and a thrombocytosis of 500–1000 × 10^9/L with marked variation in size, basophilia, and granulation, such that the larger platelets may interfere with the red cell count (Fig. 2.78). Monocytes are increased, likely as part of the stress reaction, to 2–3 × 10^9/L. The bone marrow has normal cellularity but the myeloid:erythroid ratio is greatly increased as are megakaryocytes, but rubricytes are rare. Grossly, *pallor is the dominant finding*, often with pulmonary edema and cardiac dilation. There is ischemic damage to periacinar hepatocytes and proximal renal epithelium. The spleen and nodes are of normal size without extramedullary erythropoiesis, reflecting the generalized suppression of the red cell system.

Feline panleukopenia

Feline panleukopenia (infectious feline enteritis), a *parvoviral infection* to which all Felidae are susceptible, is described in Vol. 2, Alimentary system. Clinically the disease may be of such sudden onset that poisoning is suspected. Signs begin 5–6 days after exposure of a susceptible animal and may be mild or fatal. Typically there is prostration with vomiting, diarrhea, and severe dehydration. Fever is variable and may not be present after onset of disease, likely because of the need for functional granulocytes for production of pyrogen. Pathogenetically, the disease is radiomimetic and the signs and lesions are referable to destruction of proliferating cells, principally in marrow, intestinal epithelium, spleen, lymph nodes and thymus. Hematologically the leukocyte counts decrease to less than one third of normal level on day 5 after experimental infection, remain low for 5 days, and then rise rapidly in cats that survive. In those that die, the leukocytes decline to less than $1 \times 10^9/L$ by the 7th and final day. This drop is almost entirely due to a *reduction in granulocytes*, and the timing of both the decline and recovery is related to the production time of the neutrophil, which can be deduced to be 5–6 days as in the calf and dog. Thus, there is complete cessation of all lines of hematopoiesis shortly after infection, and those cells in the postmitotic pool continue to mature and are delivered to the blood in pipeline fashion. After a short period of aplasia, there is recovery of the stem cell pool and an advancing front of early myeloid cells that appears as "maturation arrest" at days 7–8 when death due to dehydration usually occurs. The intestinal signs do not begin until granulocytes become deficient and, in the germ-free cat, there is a mild decline in total leukocytes but no loss of intestinal epithelium or diarrhea. In recovery, erythropoiesis is delayed until there is a peripheral blood neutrophilic leukocytosis (peak day 13) indicating a shunting of stem cell commitment to the lines essential for survival. The marrow in this disease is extremely labile and changes occur within hours, accounting for the variation seen with disease progression and recovery. Marrow cellularity drops to less than one-third of normal by the third day after inoculation, is less than 20% of normal between days 5–7, and returns to about half of normal on day 10. Platelet counts decrease to half the normal level on day 5, and in survivors return to original levels between days 7–10 postinfection. Animals that die have severe aplasia, with complete loss of the myeloid and erythroid proliferative phase cells. Survivors have the lowest levels of myeloblasts on day 3 followed by recovery on day 5, whereas erythroblasts reach their lowest level on day 5 and recovery is delayed until day 10.

Histologically, lymph nodes, spleen and Peyer's patches have *both thymus- and marrow-dependent hypocellularity*. Follicles are characteristically large and occupied by a background of pale dendritic reticular cells made apparent by lysis of the follicular center cells. Fragmented nuclei are usually absent, indicating that lympholysis occurred several days previously. Follicles are rimmed by a narrowed zone of small lymphocytes, and paracortical areas are also hypocellular. All tissues are lacking in neutrophils, and there is often extensive erythrophagocytosis in the medullary sinuses of mesenteric nodes. The latter is presumably the result of erythrocyte injury, possibly due to the adherence of luminal toxins during their passage through the capillaries of denuded villi. Striking changes occur in the thymus and consist of complete cortical collapse. The thymus becomes a thin band of facetted lobules composed of hypoplastic medullary areas with collapse of the fascial boundaries around the depopulated cortices. *Thymic atrophy* is the major lesion in experimental infection of germ-free cats where clinical disease is minimal.

Stachybotryotoxicosis

Stachybotryotoxicosis, as a *pancytopenic disease*, occurs in horses and ruminants in Eastern Europe and Russia. The responsible fungus *Stachybotrys alternans* (*atra*) grows on substrates rich in cellulose, and contaminates hay and cereal grain. *The nature of the marrow depressant toxin is not known*, but appears to be related to the trichothecenes produced by *Fusarium*. Although best documented as the cause of extensive outbreaks of disease in horses, episodes of comparable magnitude also occur in cattle, and sporadic episodes occur in sheep and swine. Other species including humans are susceptible. The syndrome occurs in *three stages*: the initial *local signs of irritation*; a period of *quiescence* in which blood dyscrasia occurs; and a final episode being the consequence of *severe pancytopenia*.

Following ingestion of contaminated food, there is drooling, hyperemia of buccal mucosa, and deep fissuring of the lips, leading to extensive inflammatory edema of the soft tissues of the buccal cavity. Local lymphadenopathy may persist for several weeks. There is early leukocytosis, followed by progressive depletion of formed elements of blood, especially platelets and leukocytes, with prolonged prothrombin and clot-retraction times. The terminal stage is fairly brief and manifested as *hemorrhagic diathesis and bacterial infection*.

The pathological picture in cattle closely resembles that observed in bracken-fern poisoning; in horses it is characterized by profuse hemorrhage and necrosis in many tissues. In sheep, the fleece may be shed at about the time when hemorrhages occur. Necrotic processes in the mucous membranes of the alimentary canal are characteristic, especially of lips, tonsillar fauces and large intestine. There is more or less severe depletion of hematopoietic and lymphopoietic tissues.

Myelophthisic anemia

Myelophthisic anemia of myelofibrosis is discussed with Myeloproliferative diseases, and the displacement of marrow in osteopetrosis and osteodystrophia fibrosa is discussed with Vol. 1, Bones and joints.

Myelophthisis of leukemia is seen most often in dogs, cats, and cattle. The anemia of leukemia has *4 mechanisms: stem cell displacement, panmyelosis, immune depression, and cachexia. Marrow failure with consequent loss of all normal cells represents the end stage of acute leukemia.* These cell deficits occur earlier and with greater diversity in acute myelogenous leukemia than in acute lymphocytic leukemia. The basic difference between these two diseases appears to depend on the relative distribution of the leukemic stem cells in the bone marrow. Normally the pluripotent stem cells are in highest concentration in the subendosteal marrow, apparently because of the tropism resulting from the bone-marrow portal capillary drainage. Leukemic lymphoid cells tend to be distributed randomly throughout the marrow cavity without predisposition for subendosteal hemopoietic areas, and, although the marrow may be solidly infiltrated by tumor, marrow failure is a late occurrence. In contrast, in acute myeloid leukemias, malignant stem cells compete directly with their benign counterparts driving them centripetally, in which location they are stimulated to enter a pattern of differentiation rather than of self-renewal, ultimately leading to depletion of the normal stem cell pool. The anemia in myelogenous leukemia may also be related to the fact that the erythroid cells are themselves the progeny of neoplastic precursors that do not respond to normal demands for proliferation.

In the lymphoid leukemias particularly, there are often immune phenomena associated with the appearance of the neoplastic clone. In chronic lymphocytic leukemia, therapy of the disease may result in the development of immune hemolytic anemia. Similar changes are observed in some lymphomas, and the effects may vary from a subtle immune depression of erythropoiesis to destruction of apparently normal peripheral blood erythrocytes.

All animals with leukemia that survive for more than a few weeks show some degree of the cachexia characteristic of cancer, due apparently to the systemic production of tumor necrosis factor by defensive cells. In addition to the wasting of cancer, there is the specific impairment of hemoglobin synthesis as a result of scavenging of serum transport iron, which is part of the biochemical response to chronic disease.

Anemia of uremia

Anemia of uremia occurs in all species but is most often recognized in the *dog and cat*, which are the domestic species that most commonly survive to old age. *Anemia is a characteristic feature of chronic renal failure*; urea levels of 35 mmol/L or higher are almost always associated with a hematocrit of 30% or less. There are *4 principal mechanisms* of anemia in renal failure. *These are excessive hemolysis of red cells due to retention of creatinine and guanidinosuccinic acid, toxic depression of erythropoiesis, loss of renal erythropoietin, and blood loss from the kidney*. All of these processes may be operative to some degree with variation in their relative importance from case to case, but the major limiting factor in the anemia of uremia in humans and animals appears to be *inadequate levels of erythropoietin*.

The anemia is normocytic, largely normochromic, and of mild-to-moderate severity. Polychromasia is rare or absent, and there is some poikilocytosis with an occasional hypochromic red cell. Toxic changes in neutrophils are usually prominent. The diagnosis is based on clinical signs and other biochemical evidence of uremia (see Vol. 2, Urinary system).

Anemia of endocrine dysfunction

Mild anemia of marrow hypoplasia is associated with loss of function of the pituitary, thyroid and adrenal glands, and to a lesser extent of the gonads. Anemia related to **pituitary dysfunction** appears to be due both to loss of thyroid stimulating hormone and adrenocorticotrophin, as both are required to maintain hemoglobin levels. **Hypothyroidism** is associated with normal red cell survival, but decreased red cell production, red cell mass and blood volume. Therapy may temporarily exacerbate the anemia, since the plasma volume recovers more rapidly than does the red cell mass. Mild normochromic normocytic anemia occurs in **adrenal insufficiency.** Its true extent is masked by a concomitant reduction in plasma volume. The anemia is corrected by steroid therapy, although the mechanism is unclear. There is often a mild increase in red cell numbers in hyperadrenocorticism, however, it is likely that the effect is erythropoietin mediated and not a direct effect of adrenal steroids on marrow. **Castration** lowers hemoglobin levels 10–20 g/L in male animals, to just above the normal female level. If females are spayed, their hemoglobin levels rise to that of castrated males.

Anemia is rarely of clinical significance in hormonal dysfunction, except in the need to distinguish it from anemia of other cause, and the reduction in hematocrit is usually an incidental finding. It is a mild normochromic normocytic anemia without toxemia or polychromatic response.

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Anemia of deficient hemoglobin production

The anemias of deficient hemoglobin may be subdivided on the basis of pathogenesis into *anemias of deficient heme and anemias of deficient globin*. In animals, *anemias due to insufficient heme are largely a result of iron or copper deficiency or chronic inflammatory disease*. In erythropoietic porphyria, there is production of an ineffective isomer of protoporphyrin and this disease is considered under congenital disorders of red cells. *Anemias due to deficient globin (e.g., thalassemia) are not of clinical importance in veterinary medicine*; however, true imbalances in the production of the α and β chains of globin have been identified in mouse models of this disease.

Iron-deficiency anemia

Iron is an essential component of hemoglobin, myoglobin, the cytochrome enzymes of mitochondria and hepatic microsomes, and the metalloflavoproteins (NADH), and is required as a cofactor for the function of other enzymes. Thus, *while anemia is the principal sign of iron deficiency, there are other diverse effects*, which include neutropenia, decreased capacity for killing ingested bacteria, impaired T-cell response to mitogens, and decreased capacity for interferon production. Iron deficiency results in alterations in several biogenic amines that are thought to be responsible for irritable behavior and cognitive defects observed in humans and animals. In addition, deficient states result in increased catecholamine levels leading to cardiac hypertrophy in rats and impaired temperature regulation and cold tolerance. A deficiency of body iron stores, as distinguished from iron deficiency anemia, may develop from deficiency of iron in the diet, impaired absorption, or excessive loss. In discussing the pathogenesis of iron deficiency, it is convenient to consider each of these possibilities.

The only natural **diet** of animals that can result in a **deficiency of iron** is milk, and therefore deficiency of this pathogenesis is seen only in sucklings. Supplementation of the diet of the dam does not influence the occurrence of iron deficiency in the sucklings. In terms of animal husbandry, the recognition of the need for supplemental iron for neonates has been a relatively recent development which followed serious losses when confinement rearing of swine came into vogue.

Impaired absorption of iron is rarely of significance in animals, but does occur in dogs with acquired malabsorption syndromes. Absorption takes place from the stomach and duodenum, and the degree of absorption within limits is geared to demand. About 5% of dietary iron is absorbed in the replete animal and this proportion may increase to nearly 20% in deficient states. Absorption varies with the form in which iron is ingested. Thus, heme iron is well absorbed, whereas inorganic iron is much less available but in adequate amounts is adequately absorbed. *Iron absorption appears to be regulated as a function of the level of saturation of the serum transport protein transferrin.* Transferrin has a high binding coefficient for iron and is normally about 50% saturated. In deficient states, saturation drops below the 16% level, at which point iron is not partitioned to the marrow rubricytes and iron supply becomes rate-limiting to red cell production. The developing enterocytes become imprinted with ferritin, the next level of iron transport, in proportion to body iron stores, and subsequently they absorb iron in proportion to the body's needs. Thus, absorption is partially blocked by high body stores and vice versa.

Free iron in serum in excess of the binding proteins is toxic and promotes bacterial invasion. In well-fed dams and sucklings, these binding proteins rapidly scavenge excess iron given parenterally, so that toxicity or infection does not occur. In debilitated animals, particularly the young, parenteral iron should be withheld until animals are gaining weight and serum proteins can be assumed to be replenished. Thus, teleologically, *the physiological anemia of the young is a defensive strategy to deny bacteria access to iron during the period of transition from passive to active immunity*. Since sucklings of all species are born with minimal stores, the increased requirements for iron concomitant with rapid growth and expansion of blood volume guarantees that they will develop iron deficiency if not given access to dietary iron or are otherwise supplemented.

Excessive loss of iron is the result of *chronic loss of blood* and is an important cause of iron deficiency; it is the only cause in adult animals, since normally only very small amounts of iron are excreted and lost.

The signs of iron deficiency are those of chronic anemia although they may be visibly complicated by the underlying pathologic process. Apart from the effects on blood and those changes which are the result of anemia, lesions specifically attributable to iron deficiency are limited to the recognition of the anemic state in conjunction with an absence of stainable iron in tissues. Body iron stores can be estimated as a function of plasma ferritin where 1 μg of ferritin can be equated with about 120 μg of iron/kg body weight. The *depletion of body iron* can be considered at *three levels of severity* based on the levels of saturation of transferrin, the level of hemoglobin, and of red cell protoporphyrin. At the *first stage of depletion*, the hemoglobin and transferrin saturation are normal, but ferritin is decreased and hemosiderin is absent. At the *second stage of depletion*, both ferritin and transferrin saturation are deficient and red cell production is slowed. Finally, in *iron-deficient anemia*, there is not only loss of all iron stores, but hemoglobin is decreased and red cell protoporphyrin is increased as a result of impaired heme synthesis. Rubricyte maturation is coordinated to produce heme and globin in proportional amounts. Lack of iron slows protoporphyrin and globin synthesis, and there is delayed rubricyte maturation which results in an increased proportion of mature rubricytes in the bone marrow (*mature asynchrony*). The marrow is hyperplastic with an erythroid shift but without peripheral blood reticulocytes. The marrow transit time of the rubricytes is extended beyond the normal 4 days, and erythropoiesis is thus termed *ineffective*.

Hematologically, *the anemia is classically hypochromic and microcytic in all species*, and accompanied by neutropenia with hypersegmentation and marked thrombocytosis of small platelets. Red cells are not only small and poorly saturated but also distorted in shape. The plasma is pale, as is the urine due to reduced formation of bilirubin. Serum iron is generally less than 9 μmol/L, and the total iron-binding capacity is increased, generally in the 70–110 μmol/L range. Apparently in a multiply transfused human, the typical anemia can develop in the face of adequate iron by the development of an antibody against the rubricyte transferrin receptor.

Anemia of iron deficiency is common in **piglets** and in **puppies** of the larger breeds; it is probably responsible also for the anemia which is often observed in *calves* on diets solely of milk, but it is seldom clinically important and probably never fatal. Unless special measures are taken to prevent the disease in piglets, a morbidity of 90% may occur with a mortality of approximately 10–50%. The higher figure for mortality includes those animals which die of secondary infections. As noted above, iron deficiency seriously impairs defensive mechanisms and predisposes piglets to a variety of infectious diseases, especially colibacillosis and swine dysentery, as well as spirochetal granulomas, omphalophlebitis, and streptococcal meningitis, pericarditis and polyarthritis, and other pyogenic processes.

The piglet is particularly predisposed because of its rapid growth rate. Mortality is higher in the winter than in other seasons, partly because of confinement and partly because of increased susceptibility to cold. The requirement of a young piglet for iron is set at about 7 mg per day; milk supplies only half of this amount.

The disease is varied in its manifestations and course, but it can be described in *three discrete syndromes*. The piglets are normal at birth. In the *classical syndrome*, they become progressively unthrifty after the first week of life and grossly there is pallor of all tissues, edema of muscles and connective tissues, edema of the lungs, and a thin-walled, flabby heart. In a *second syndrome*, the piglets often die suddenly in the third week. These piglets are well grown, pale and appear plump due as much to edema as to fat. At necropsy, all tissues are extremely pale, wet, and there is pericardial effusion with the heart dilated to more than twice its normal size. The *third syndrome is one of secondary infections*, and the age incidence is broadened to include pigs of 4–8 or 10 weeks of age and is seen where the piglets are not given parenteral iron and do not begin to feed from a creep feeder until they are 4–5 weeks old. In chronic cases, there will be dyskeratosis with abnormal ringing of horn and hooves or nails.

In all species with iron-deficiency anemia, the bone marrow has high cellularity and cell density with an erythroid shift and mature asynchrony with increased numbers of megakaryocytes and eosinophils (Fig. 2.81A, B). *The* **histologic diagnosis** *of iron-deficiency anemia is based on a hypercellular marrow with increased numbers of late stage rubricytes and an absence of hemosiderin.*

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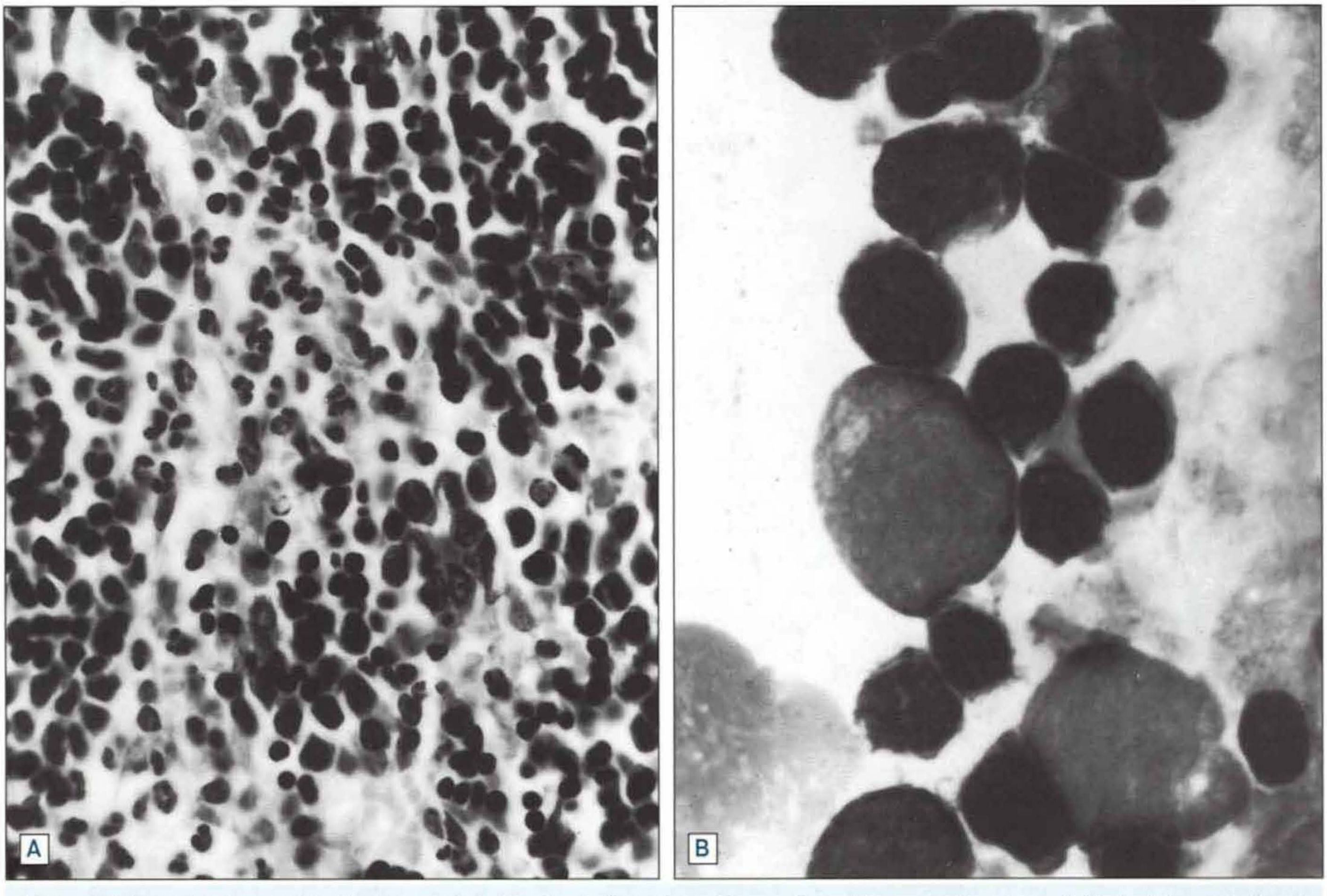

Figure 2.81 Iron deficiency. A. Erythroid hyperplasia. Density of cells indicates late asynchrony. Marrow section, sheep. **B.** Erythroid hyperplasia with increased rubricytes, late asynchrony. Marrow aspirate, calf.

Copper-deficiency anemia

Copper is a cofactor in a variety of oxidative enzymes of diverse function, and deficiency of the element may affect electron transport (cytochrome oxidase), the absorption of iron and its utilization in hematopoiesis (ceruloplasmin), protection from antioxidants (superoxide dismutase), tyrosine degradation and pigmentation (tyrosinase), neurotransmitter metabolism (dopamine hydroxylase), or cross-linkage of elastin and tropocollagen (lysyl oxidase). *Copper deficiency can therefore be manifested in a variety of ways and in several organ systems.* Cytochrome oxidase is a copper-dependent enzyme, and *diminished activity of cytochrome oxidase is a sensitive indicator of copper deficiency which apparently limits the synthesis of heme, the prosthetic group of the enzyme.* This association of copper with cytochrome oxidase is probably its fundamental physiological function, but its participation in other enzymological processes may account for the diverse manifestations of copper deficiency within and between species.

Disturbed hematopoiesis is not the sole or even the most important aspect of copper deficiency, but it is one aspect which is common to all species in which the deficiency is discussed in detail here; reference to neurological, vascular and cutaneous manifestations of the disease will be repeated briefly in the chapter on the appropriate system.

Uncomplicated copper deficiency is probably not of widespread occurrence in animals, but in many endemic areas there are chronic diseases with local names, due to a complicated or uncomplicated deficiency of copper. Factors which control the uptake of dietary copper and its excretion are discussed under chronic copper poisoning. There, reference is made to the ability of high dietary intakes of *molybdenum* in the presence of sufficient inorganic sulfate to limit hepatic retention of copper by sheep, either by lowering absorption or increasing excretion. There is some evidence for both. The experimental production of a conditioned copper deficiency by appropriate use of molybdenum and sulfate is sometimes successful, sometimes not, which, together with the observation that copper deficiency can occur in ruminants on diets normal with respect to copper, molybdenum and sulfate, indicates that there are additional unrecognized factors influencing the availability, absorption, excretion, storage and utilization of copper. There are differences between species, and between breeds of sheep, in the levels of activity of intestinal absorption mechanisms and, probably also, in hepatic excretory mechanisms.

Approximately 25% of ingested copper is absorbed, primarily from the stomach and upper small intestine. Absorption is like that of iron in that it is an energy-dependent, two-step process involving an intraepithelial metallothionein-like protein that may transfer the copper to the plasma carriers or be shed with the senescent enterocyte. It may be that absorption is more efficient in monogastrics, since deficiency can reliably be reproduced in swine whereas in sheep copper deficiency may be more a function of molybdenum toxicity. The absorption is influenced by the chemical form of the copper and by its tendency to form insoluble complexes in the gut. The absorbed copper is transported in blood loosely bound to serum albumin from which it is rapidly distributed to tissues and concentrated principally in liver, kidney, heart and brain. Copper in blood exists as copper–protein complexes in red cells, loosely bound to serum albumin, and as part of **ceruloplasmin**, a copper-containing α-2 globulin. Ceruloplasmin is produced in the liver and contains 7 copper atoms in each protein molecule, one atom being labile and the remainder structural. The copper of plasma is much more labile than that of red cells and is a better indicator of fluctuations in copper status than is the copper content of whole blood. Bile is the principal medium of copper excretion.

Copper is present in all cells with principal reserves contained in the liver, although significant amounts are in kidneys, bone marrow and central nervous system. The latter areas likely represent metabolically functional rather than storage forms. Considerably more copper is found in the liver of the fetus and newborn than in the liver of adults of the same species. An exception is in sheep; ewes, irrespective of their copper status, have much greater hepatic stores than their lambs. In adult ruminants, the levels of hepatic copper vary widely and reflect to some extent the animals' nutritional history with respect to this element. Much smaller, and rather stable, concentrations of copper are found in the liver of nonruminants. In general, determination of hepatic liver copper is a better indicator of the body's copper status than is determination of blood or serum copper concentrations.

Anemia is not invariably present in copper deficiency, but it is probable that anemia will occur if the deficiency is sufficiently severe and prolonged. Several mammalian species when made deficient in copper develop anemia, the severity of which depends on the duration of the deficiency as well as on host factors such as age and pregnancy. The morphological characters of the anemia vary with the species, that in rats, pigs, rabbits, and lambs is microcytic, hypochromic; in cattle and adult sheep, it is macrocytic, hypochromic; and in dogs, normocytic and normochromic.

Unlike iron, where the transport protein transferrin carries the metal in a high affinity bond with variable saturation, *copper circulates in the plasma in an integral bond with ceruloplasmin.* Since copper is required for the synthesis of ceruloplasmin, the ceruloplasmin level drops in copper deficiency. This is unlike iron deficiency in which the total iron-binding capacity rises as the serum iron drops. Ceruloplasmin acts as a specific oxidant for conversion of ferrous to ferric iron, in which state it is available for transferrin transport. The protein has been called *ferroxidase* to indicate this function.

The manner in which copper deficiency leads to anemia is not clear; however, the coincident development of marrow erythroid hyperplasia indicates that, as in iron deficiency, the stem cell system is not impaired but, because of reticulocytopenia, rubricyte maturation must be. *The disease is therefore one of* **ineffective hematopoiesis** *with hyperplastic marrow but deficient output.* The fact that the ineffective asynchrony is not uniformly of the late type in all species, as it is for iron deficiency, and that the red cells are not always hypochromic suggest that the mechanism of development is not solely one of iron inhibition. Rather it appears that the coordination of heme and globin synthesis, that is heme-limited in iron deficiency, is more generally affected in copper deficiency, and downregulation of rubricyte maturation may be mediated by deficits in both. In iron deficiency, marrow late-stage rubricyte mitoses are not prominent but probably do occur, and division without cytoplasmic production is the mechanism of microcytosis. In contrast, in the dog with copper deficiency the rubricytes are not predominantly late stage, but are generally younger. Mitoses are common and the anemia is normocytic, normochromic. Thus the net effect of copper deficient anemia in the dog appears to be defective maturation, increased intramedullary cell death and as in iron deficiency, deficient cell production. A shortened survival time of red cells occurs in copper-deficient, and also occurs in iron-deficient, pigs but kinetically the anemia in both is very largely due to reduced production.

Extensive hemosiderin deposits occur in sheep and cattle naturally deficient in copper, and in pigs made experimentally deficient, suggesting defective reutilization of endogenous iron. Copper-deficient swine have: impaired ability to transfer iron across the intestinal basement membrane; lowered plasma iron levels; an increase in plasma transferrin, and a lowered rate of turnover of iron from the liver. Thus, although the body is iron replete, there is impaired inter-organ transport, and in the pig the anemias of iron or copper deficiency look morphologically similar. There is abundant iron in the latter, however, and the thrombocytosis of iron deficiency is not seen in anemia of copper deficiency.

Anemia of suckling piglets associated with copper deficiency may account for some of the instances in which piglet anemia fails to respond to iron. In copper deficiency, the hemoglobin may fall to 10–20 g/L and the red cells to 2×10^{12}/L. The entire syndrome parallels that of iron deficiency except that it does not respond to iron; a good response follows the administration of copper but the reticulocytosis is delayed by the 3–8 days required for the synthesis of ceruloplasmin. In contrast, if plasma from a copper-replete animal is given, the response is immediate.

Anemia is an important sign of the enzootic maladies of copper deficiency, but is not invariably present, and in general, develops late in the course of the deficiency. The severity as well as the morphological features of the anemia are influenced by the severity of copper deficiency, the duration, whether complicated or not, and also by host characters such as age, growth rate, pregnancy, and parasitism. Rarely, anemia of copper deficiency may occur, where, for various reasons, there is dietary zinc excess with a competitive inhibition of enteric copper absorption.

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Cobalt-deficiency anemia

Cobalt deficiency is a disease of ruminants expressed as ill-thrift; if death occurs, it is due to inanition (see Vol. 2, Liver and biliary system). The disease is known in many parts of the world but the distribution, especially of marginal deficiencies, is not well plotted; occurrence is often seasonal and complicated by concurrent deficiencies of copper and phosphorus. The minimum concentration of cobalt in forage consistent with adequate nutrition of ruminants is one part per million on a dry weight basis, but the only effective test of cobalt deficiency is in the response to oral supplementation. Cobalt deficiency produces a *chronic wasting disease* to which sheep are slightly more susceptible than cattle and to which horses are immune; lambs and calves are even more susceptible than their respective adults. The disease is characterized by a gradual reduction of appetite for both food and drink, progressive emaciation, and death from starvation even though food may be plentiful. The European name, *licking disease*, places emphasis on the allotriophagia which accompanies inappetence in cattle. Abortion, temporary sterility and decreased production are common in cattle. Pallid mucous membranes are characteristic but, as mentioned above, *anemia may or may not be demonstrable by routine tests* and may be masked by blood volume contraction with loss of weight.

In recognized endemic areas, sickness is usually evident after the animals have been at pasture for 4–6 months. Calves and lambs may not survive more than a further 6 months, although adults introduced to deficient pastures may survive for 2 years or more. The postmortem picture is that of starvation.

Cobalt deficiency is a deficiency of vitamin B_{12}. Parenteral treatment of deficient animals with the vitamin will sustain them for long periods, suggesting that hepatic storage is efficient. Pair feeding studies show that the cachexia of cobalt deficiency is largely due to inanition although there is apparently some loss of absorptive or metabolic efficiency. Remarkably, the parenteral administration of cobalt as $CoCl_2$ to deficient sheep has no effect on the disease and much of the injected dose is lost in the urine. In contrast, the administration of cobalt orally, or vitamin B_{12} by any route, is curative if enough of either is given. Synthesis of the vitamin occurs in the rumen, and even if there is adequate storage of cobalt in the liver, the disease will progress unless there is enteric cobalt available for microbial metabolism. In horses, the concentration of vitamin B_{12} in the enteric contents increases from the stomach to the rectum. This may explain why *horses are refractory to cobalt deficiency*, and indicates that the supplementation of racing animals is unnecessary.

Energy metabolism in the ruminant depends on acetic, propionic and butyric acids, and the conversion of these to succinate. The enzyme, methylmalonyl-CoA mutase contains vitamin B_{12} and is responsible for the conversion of methylmalonyl CoA to succinyl-CoA. In cobalt deficiency, there is accumulation of methylmalonic acid in liver and blood, and high levels of propionate and acetate in blood; the latter may be responsible for inappetence. Vitamin B_{12} also participates via a methyltransferase in the conversion of homocysteine to methionine, an essential amino acid. It is possible that methionine deficiency is responsible for the fatty liver which occurs.

Inadequate ruminal cobalt is permissive of the chronic neurologic syndrome of *Phalaris* poisoning of sheep.

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Megaloblastic anemia

Anemia characterized by reticulocytopenia and marrow erythroid hyperplasia with early asynchrony of rubricytes occurs infrequently in cats and dogs. **Vitamin B_{12}** is stored in the liver in amounts sufficient to maintain animals for many months without further absorption. On the other hand, there is very little storage of **folic acid**, and signs of deficiency may appear after relatively short periods of disease in animals that are anorectic or receive chemotherapy. This may be due to acceleration of folic acid depletion with the inanition produced by rapidly growing tumors such as the high-grade lymphomas that cats and dogs are prone to develop. Vitamin B_{12} deficiency occurs rarely in the dog secondary to immune gastric atrophy. It is possible to produce the typical immune destruction of the gastric mucosa in dogs by immunization with homologous gastric juice or mucosal homogenate. A congenital anemia of *Giant Schnauzers* that is nonresponsive but not macrocytic is apparently due to an inability to absorb the vitamin since parenteral vitamin B_{12} reverses the syndrome. Dogs which survive *gastric torsion* with loss of most or all of the glandular portion of the stomach are at risk of developing vitamin B_{12} deficiency, but seem to have sufficient hepatic stores until they die of other causes. A variety of drugs may inhibit the absorption of either folic acid or vitamin B_{12}. Since analyses for these substances are rarely performed, the two deficiencies are not often specifically identified. They will be described together.

Hematologically, the first sign of disease due to deficiency of either folate or B_{12} is the presence of *large neutrophils with hypersegmented nuclei* in an animal with nonresponsive anemia, usually associated with acute leukemia or lymphoma. The marrow precursors of these neutrophils are *giant metamyelocytes* that have apparently become tetraploid but have not proceeded to division. In the early stages, the rubricytes appear of normal age distribution and the peripheral blood red cells are normochromic, normocytic. With progression, which appears most often in the dog, there is development of marrow erythroid hyperplasia with a predominance of young rubricytes, and macrocytosis in the blood with thrombocytopenia and increased variation in size of platelets. Pathogenetically, the disease is an example of an intact and functional stem cell system which is capable of producing a hyperplastic marrow that is ineffective in mature cell production, due to inhibition of DNA synthesis and subsequent cell division. Since divisions are skipped, the few resulting cells are larger than normal.

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Anemia of chronic disorders

In inflammatory states, acute-phase reacting proteins scavenge molecular iron from transferrin and tie it up in ferritin and later, if the reaction persists, in hemosiderin. Thus hypoferremia has been considered the primary mechanism in the development of the anemia of chronic disease. However, because the hypochromia is at most mild (MCHC less than 310 g/L) while the anemia may be moderate (Hb 70–100 g/L), *it appears that anemia is due primarily to reduced red cell production rather than inhibition of hemoglobin synthesis*. Further, while shortened red cell lifespan can be demonstrated in anemia of chronic disorders, the hemolysis is mild and the red cells survive normally in normal animals, demonstrating that the problem is environmental and not intrinsic to the red cell itself. Finally, it is recognized that in primarily hemolytic anemias, the bone marrow is highly productive and can increase output 6 or more times normal levels, yet in the anemia of chronic disorders the red cells are normocytic and there is reticulocytopenia out of proportion to the degree of the anemia. Cats with sterile abscessation and anemia respond partially to additional iron by continuous infusion and, more completely, to iron plus cobalt, the latter treatment to increase erythropoietin by tissue anoxia. Thus, pathogenetically, *the anemia of chronic disorders is primarily a problem of decreased and deficient production of red cell numbers* and, like the anemia of uremia, it may be compensated by increased levels of erythropoietin if iron is available. While the mechanisms operative in the syndrome of chronic disorders are defined, *the only practical therapy is reversal of the primary problem.*

Collectively, the *anemias associated with inflammatory, degenerative, immune and neoplastic disease form a large and important group in all species* (Fig. 2.82A, B). The common pathway by which erythropoiesis is selectively depressed in anemias of such diverse association is *related to tissue injury*, and mechanistically to the constant production of *tumor necrosis factor* or *"cachexin,"* a monokine produced by activated macrophages that has both toxic and cytostatic effects on the rubricyte system. Teleologically, this process appears to be a system to deny access to iron to invading microorganisms, with anemia as an acceptable exchange for survival. The anemias of chronic disorders are of moderate severity, largely normochromic, normocytic and poorly responsive, with associated toxic changes in leukocytes.

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Anemia of malnutrition

Anemia of malnutrition is most commonly a disease of the young and is seen most frequently in sheep and goats, and less often in kittens and puppies where poor nutrition may be complicated by ecto- and endoparasitism (Fig. 2.83A). Malnutrition in animals, as in humans, is sporadic and world wide in distribution. The disease is worthy of consideration because of the regular tendency for the anemia to worsen once corrective measures are taken. Hematologically, the anemia of malnutrition is largely normochromic, normocytic, but may be hypochromic if complicated by parasitism and blood loss. Kinetically, *the anemia is primarily due to reduced red cell production with minor hemolysis*, as in anemia of chronic disorders. Since malnutrition is so often associated with delayed healing and infection, the two syndromes are

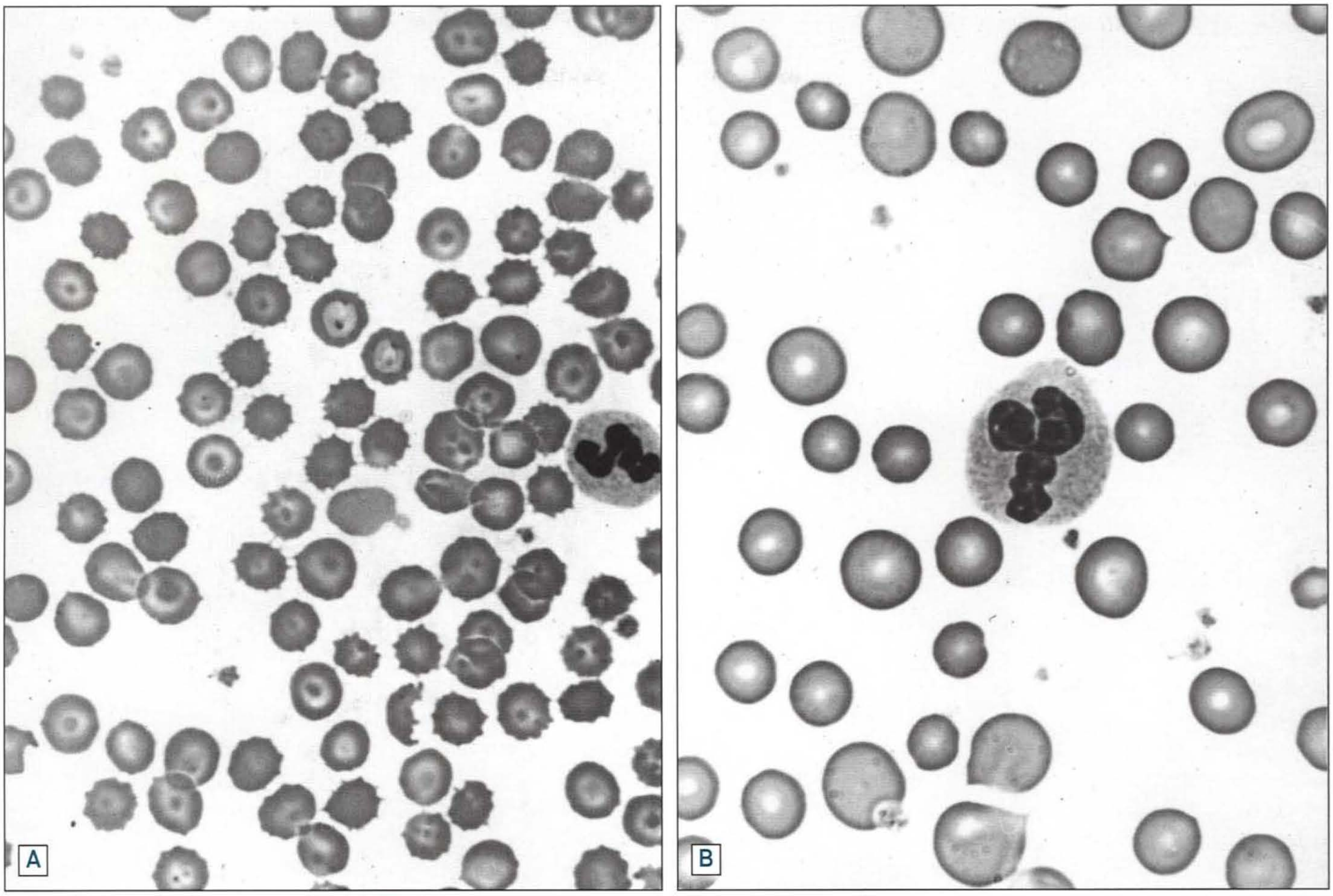

Figure 2.82 Anemia of chronic disease in a dog. **A.** Target cells resulting from altered serum cholesterol and lipoprotein in liver disease. Membrane pliability results in acanthocytosis and poikilocytosis. **B.** Microcytosis characteristic of portocaval shunting.

often coincident and may share a combined development. Malnutrition that is primarily due to **caloric deprivation** is called *marasmus*. In swine, the anemia is mild, and the response to phlebotomy is vigorous and curative, reflecting a functional stem cell system, adequate iron supply, and normal erythropoietin production.

Protein-calorie deprivation is more common, and when severe is called *kwashiorkor*. It is a syndrome of cachexia characterized by *hypoplastic marrow, mild anemia and reticulocytopenia*. Since serum proteins are also deficient, low serum iron is not rate-limiting to red cell production because transferrin is also low and the saturation level is adequate for iron delivery. The anemia in these syndromes is partially adaptive and due to reduced activity and partially to multifactor deficiencies. In the development of *starvation*, there is first a loss of muscle mass followed by parenchymal atrophy, anemia and lastly *a reduction in all serum proteins*. Since the latter are the major transport for many minerals, and the major source of amino acids, *their loss is most limiting to all aspects of hematopoiesis*. In repletion, these elements are replaced in reverse order and the recovery of all tissues is dependent on first increasing the levels of plasma albumin and globulins. As plasma protein levels rise, the plasma volume contraction that developed with loss of oncotic pressure is also reversed. Since this volume expansion is much more rapid than the expansion of the red cell mass, the anemia becomes more severe, and may be misinterpreted as therapeutic failure. The pattern of recovery is a rise in level of plasma proteins followed by blood volume expansion, reticulocytosis, a slow rise in hemoglobin, and finally a gain in body weight. The course of these changes takes 3–4 weeks, depending on the level of complicating factors such as specific vitamin deficiencies, parasitism and other infections. Grossly, starvation is characterized by atrophy of all organs but particularly the thymus, lymph nodes, spleen and liver. There is pancreatic acinar atrophy and serous atrophy of marrow fat (Fig. 2.83B).

Deficiencies of specific vitamins are capable of causing anemia in animals, but in general their importance is more in the prevention of related disease. Swine fed **vitamin E** deficient diets develop moderately severe nonresponsive anemia characterized by marrow erythroid hyperplasia with multinucleated dyserythropoiesis, increased turnover of serum iron, and increased unconjugated bilirubin. Red cell lipids are markedly reduced and red cells are highly susceptible to oxidant attack. The mechanism appears to be at least partially due to inhibition of pyrrole and thus heme synthesis. This mechanism is important in a practical sense since an abundant pool of protoporphyrin is an efficient sink for tying up iron in the synthesis of heme. Piglets that are vitamin E deficient are more susceptible to toxicity when given iron by any route. Premises where other vitamin E and selenium-related diseases have occurred are likely to have pale piglets that do not respond to repeated iron administration and have characteristic dyserythropoiesis.

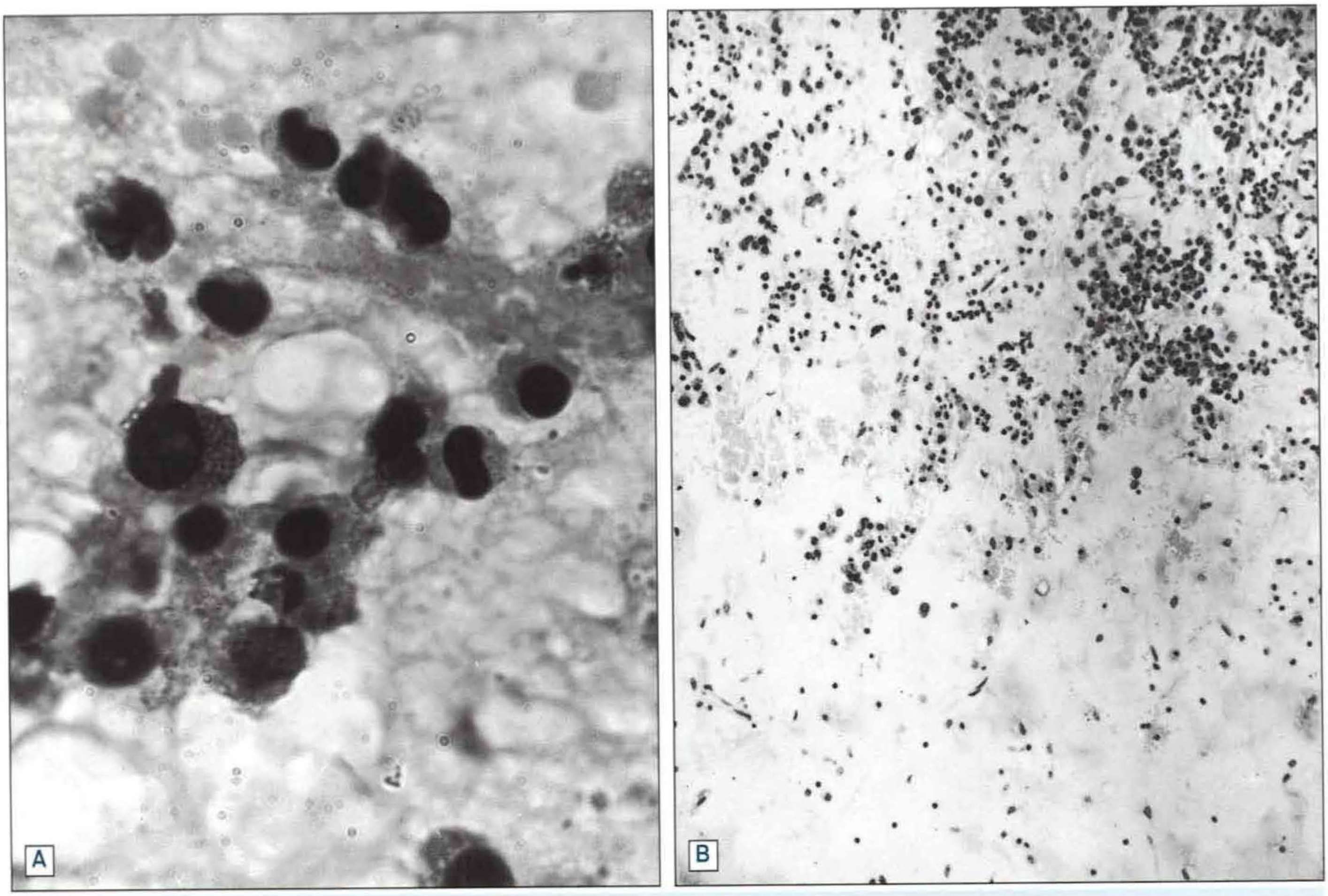

Figure 2.83 Anemia of starvation in a sheep. **A.** Late erythroid asynchrony and increased proportion of eosinophils. **B.** Serous atrophy of marrow fat with residual cuffs of subendosteal hematopoiesis.

Vitamin B_6 deficiency induced in swine results in a microcytic but not hypochromic anemia of moderate severity characterized by marrow erythroid hyperplasia and hyperferremia, and by hemosiderosis of marrow, spleen and liver, and hepatic centrilobular fatty degeneration. Vitamin B_6 is required for formation of the precursors of δ-aminolevulinic acid, which then forms the pyrrole ring and ultimately protoporphyrin. Blockage at this early stage of ring synthesis prevents iron utilization and leads to ineffective hematopoiesis. In the cat, in addition to anemia, vitamin B_6 deficiency causes stunting of growth, emaciation, convulsions and oxalate nephrosis.

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Hemolytic anemias

Anemia of hemolysis develops when the rate of destruction exceeds that of production. Since the products of hemoglobin catabolism are readily reutilized, *the anemias of hemolysis are characteristically and predominantly highly responsive with reticulocytosis and an upward shift in mean corpuscular volume (MCV).* It is stated that human marrow can increase output 6-fold in hemolytic anemia and the cat and dog have a similar capacity for regeneration while horses, pigs and ruminants are somewhat less responsive. The response to anemia is most efficiently measured by the **reticulocyte count**, which should be expressed in absolute numbers and not as a function of the number of leukocytes or peripheral blood rubricytes. Reticulocytes have a maturation time of about 1 day and *their level in the blood is always an indicator of current marrow output.* With very high levels of erythropoietin, some mitoses are skipped resulting in the release of even larger "shift" red cells which have a maturation time of 2–2.5 days. The presence of a few larger red cells that are not polychromatic in the absence of reticulocytosis indicates red cell shut-down within the last 1–2 days. Attempts to correct the reticulocyte count for the number of large shift red cells present is subjective, and introduces a misleading concept of precision to a process that is not reproducible. *The raw number of reticulocytes expressed in absolute terms is in itself the most accurate measure of marrow erythroid response*, just as the neutrophil count is an accurate measure of current myeloid output. In responsive anemias, there is a variable but much lower number of rubricytes accompanying the reticulocytes, while in stress erythropoiesis rubricytes may appear alone. *It is important that these nucleated precursors are not considered an indication of a responsive marrow.* If their absolute numbers, usually less than 10×10^9/L, are compared to expected levels of reticulocytes at $300–500 \times 10^9$/L, it can be seen that this level of contribution is minuscule and far below the normal level of production in a non-anemic animal. *Reticulocytes are not released to the blood in the horse*, but the upward shift in the MCV can be used as a relative measure of response. Blood loss in "hot blooded" (Thoroughbred and Standardbred) horses results in an upward shift of <6 fL, whereas in hemolytic anemia the shift will increase to >12 fL.

The iron economy may be rate-limiting in hemolytic anemia, with the contributions from recycling, enteric absorption and mobilization from stores being additive in their effect. This point is worth emphasizing, since *even animals that have abundant iron stores in hemosiderin will respond more strongly if given additional iron by any route.*

Intracellular destruction of red cells is the normal route of removal and occurs in the monocyte–macrophage system in marrow, spleen, liver and nodes. In hemolytic states, the spleen may become quantitatively more important than marrow. The actual process of lysis is rapid and, if erythrophagocytosis is observed, the level of destruction is likely also rapid. This catabolism releases iron from heme to transferrin or to storage as ferritin or hemosiderin, while the protoporphyrin ring is opened and released to the plasma as unconjugated bilirubin. Globin is reutilized via the amino acid pool.

Intravascular destruction of red cells occurs in normal circumstances at a very low rate as a result of high velocity trauma in small arteries. In some cases of accelerated hemolysis with complement fixation, red cells are lost by intravascular lysis at very rapid rates resulting in *hemoglobinuria*. Normally, *free plasma hemoglobin is rapidly scavenged from circulation by binding to first haptoglobin then hemopexin and finally albumin.* Free hemoglobin rapidly dissociates into dimers which are quickly bound with high affinity to haptoglobin and are then too large for renal excretion and are removed from circulation by the liver. If haptoglobin is saturated, iron unprotected by the normal cytosol reductase system is oxidized to the ferric state, causing breakage of the heme–globin bond. The free heme is then picked up by hemopexin which in the bound state is cleared with a half time of 7–8 hours by the liver. Even minor hemolysis will remove haptoglobin from circulation, but reduced levels of hemopexin constitute a sensitive test of intravascular hemolysis. Mild overloading of these systems allows the filtration of heme which is reabsorbed in the renal proximal tubules. The intracellular accumulation of hemosiderin constitutes a record of previous hemolysis that can be read by detecting the epithelial cells on urinalysis or on histological examination of the kidney. Severe intravascular hemolysis is a double hazard to the kidney because the toxic and vasoactive effects of the free heme are added to anemia, with resultant ischemic injury to the nephron (see Vol. 2, Urinary system).

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Immune-mediated hemolytic anemias

Adherence of antibody to the red cell membrane, with or without complement fixation, causes red cells to become spherical. Their lifespan is then reduced either by destruction by the monocyte–macrophage system or by intravascular hemolysis. Since the damage is usually to the most mature cells, *most hemolytic anemias are highly responsive.*

Immune anemias are classified on the basis of mechanism or association. Serologically there are three types: **warm-antibody** type with maximal activity at 37°C; **cold-antibody** type with maximal activity at 2–4°C; and those **without detectable antibody.** Classification by association is based on identification of an underlying cause and includes *primary (idiopathic) immune hemolytic anemia* and *secondary (symptomatic) immune hemolytic anemia.* These may be isoimmune, drug-induced, associated with infectious disease, associated with other immune disorders (systemic lupus erythematosus), or associated with lymphoid or other malignancy.

In **symptomatic immune anemias**, the cause is identified, and is usually adherence of drug or virus to red cells thereby sensitizing them to the immune system. In **idiopathic immune anemias**, which form the bulk of cases, it is not clear whether the error is due to an actual change in red cell antigenicity or to loss of self-recognition by the immune system.

The detection of antibody on the surface of red cells is performed by means of the **Coombs' (antiglobulin) test**. In this test, an antiglobulin for the intended species (e.g., dog) is prepared in another species (e.g., rabbit, sheep). Antiglobulins are usually prepared against both immunoglobulin and complement for broader reactivity. About 500 molecules of IgG must be bound to each red cell to give a positive Coombs' test, yet significant shortening of red cell lifespan can occur with as few as 24–34 molecules per red cell. Therefore, a negative Coombs' test does not rule out clinically significant hemolysis. The test is conducted at 37 and 4°C to detect warm- and cold-reacting antibodies. In general, the warm-reacting antibodies are IgG or IgA, and the cold-reacting are IgM. Cases of cold-reacting IgG have been described and these may be the cause of agglutination which appears due to complement alone.

The warm-reacting antibodies are termed "incomplete" and generally do not fix complement on the red cells. The anemias produced are characterized by a slower rate of intracellular hemolysis.

In contrast, the cold-reacting antibodies fix complement and may cause acute intravascular hemolysis with hemoglobinuria if extremities are cooled. The IgM antibodies tend to elute from red cells, but if complement is fixed it remains attached and may cause increased destruction in vivo and give a positive Coombs' test.

Whereas a negative Coombs' test does not rule out immune hemolytic anemia, a positive test may occur in the absence of anemia. Since macrophages do not pick up red cells bound by all classes of IgG, there may be cases that have a positive Coombs' test but are asymptomatic.

Five types of immune hemolytic disease occur:

1. erythrocytic type characterized by severe anemia with hyperplastic marrow (most common);
2. hemolytic anemia and immune thrombocytopenia with hyperplastic marrow;
3. pure red cell aplasia with selective immune depression of erythroid precursors;
4. pancytopenic type in which there is immune anemia, neutropenia and thrombocytopenia with hyperplastic marrow; and
5. the aplastic type which resembles pancytopenia but with marrow aplasia.

Isoimmune hemolytic anemia is recorded in kittens and pups but remains of most importance in foals, calves and piglets.

Isoimmune hemolytic anemia in the foal

Mares are isoimmunized naturally by focal placental hemorrhage which allows foal red cells to enter the maternal circulation. Clinical *isoerythrolysis* rarely occurs in primiparous mares and generally is not seen until the third or fourth foal. It is likely that a considerable amount of fetal blood is required for initial sensitization but restimulation in subsequent pregnancies may require much less blood of the same genotype, and therefore *repeat pregnancies by the same sire carry a greater risk of an affected foal.* Blood transfusions and tissue-origin vaccines may complicate the problem of maternal sensitization. Isoimmunization can only occur when the sire and foal possess a blood antigen absent in the mare, usually Aa or Qa. Stallions homozygous for an offending factor will always sire susceptible foals, while heterozygous sires will produce susceptible foals in about 50% of matings.

Foals are normal at birth but can absorb dangerous levels of colostral antibody for up to 48 hours after birth and likely longer. Foals that develop clinical disease and are removed from the dam may suffer recurrence of signs if put back on the dam a week or more later. Signs of the disease may occur as early as 8 hours or as late as 120 hours after birth. The severity of the anemia varies greatly depending on the amount and type of isoantibody that is absorbed. Hemolytic antibodies are most damaging and highest titers exist in the first milk. Thus, strong foals that nurse vigorously soon after birth may be most severely affected.

Earliest signs are lethargy and reluctance or inability to stand and infrequent suckling. Clinical signs include pallor of mucous membranes with icterus, dyspnea, pounding heart, and hemoglobinuria in severe cases. Mild disease may not be noticed.

There is severe normochromic anemia that becomes moderately macrocytic (Fig. 2.84A, B). Polychromasia is absent but an occasional rubricyte may be present. The marrow is hyperplastic with an erythroid shift. The hematocrit may drop below 15%, while the leukocyte count is elevated, usually to 25×10^9/L or more, and the platelet count is normal or elevated. The serum bilirubin may rise to 300 μmol/L or more, with much of it unconjugated and therefore fat soluble. Transaminase levels are elevated and a continuing rise suggests a poor prognosis. The urine is dark brown. The foal's cells are strongly Coombs-positive if the hemolysis is severe. Rouleaux formation, or rafting, of red cells is exaggerated (Fig. 2.84A). The **diagnosis** is confirmed by demonstration of antibody in maternal serum or milk that will agglutinate foal red cells and usually sire red cells. Foals with isoimmune anemia are usually treated with high levels of immunosuppressive steroids which, with high levels of iron released from lytic cells and inadequate passive transfer of antibodies in colostrum, *renders them extremely susceptible to bacterial infection.* In foals thus treated, the appearance of *toxic changes in blood neutrophils* with rising transaminases may be the first indication of infection, since fever and malaise are prevented by the steroids. Prophylactic antibiotics and/or very careful monitoring are indicated. Bovine colostrum has proven effective and may be helpful by stimulating development of the foal's complement system.

Foals that die of isoimmune anemia are usually *remarkably icteric*, and unless they are under a week of age there is usually some degree of *cachexia*. The lungs are pale, icteric and edematous, and there is increased pericardial fluid. The liver is turgid, friable, fatty and bile-stained. The spleen is enlarged with rounded borders and may have subcapsular hemorrhage. The splenic parenchyma is friable and bulging on cut surface but does not ooze blood on standing. The kidneys are pale with yellowed papillae, and the intestinal tract is usually empty of ingesta and moderately distended with gas. The femoral marrow is diffusely reddened, with translucent fat that rapidly retracts on drying.

The **histological lesions** vary with the treatment and period of survival. If repeated transfusions have been given, there will be *hemosiderosis* of the spleen, liver, marrow and lung despite the usual absence of storage iron in neonates. There is pulmonary alveolar flooding, often with foci of septic alveolitis. In animals that die acutely, there is ischemic hepatic periacinar degeneration, while those surviving 2 weeks or more and receiving steroid therapy have irregular ballooning of hepatocytes with canalicular cholestasis and hyperplasia of highly pigmented Kupffer cells. Extramedullary hematopoiesis is absent. The spleen has follicular and parafollicular lymphoid atrophy with germinal centers occupied by dendritic reticular cells with an epithelioid appearance. The sinus areas are densely cellular with increased fixed stromal cells and macrophages. Hemosiderin and erythrophagocytosis are prominent, but hematopoiesis is not. Kidneys have ischemic epithelial degeneration with flattened proximal epithelium that has heavy bile pigmentation and irregularly spaced nuclei. There are often granular and cellular casts in collecting tubules. The lymph nodes and thymus are hypocellular, and node sinuses are usually devoid of macrophages if steroid treatment has been prolonged. The marrow has a reduced amount of fat, and that present is undergoing serous atrophy and becoming eosinophilic. In acute cases, there is an erythroid shift and cellular packing is high. Later, the erythroid shift remains but the cell density is reduced and hemosiderin and erythrophagocytosis are prominent. Thrombopoiesis is normal and mature granulocytes are reduced.

Isoimmune hemolytic anemia in the calf

Spontaneous sensitization of cows to fetal red cell antigens is *very rare.* The calf derives its iron from maternal red cells deposited at the

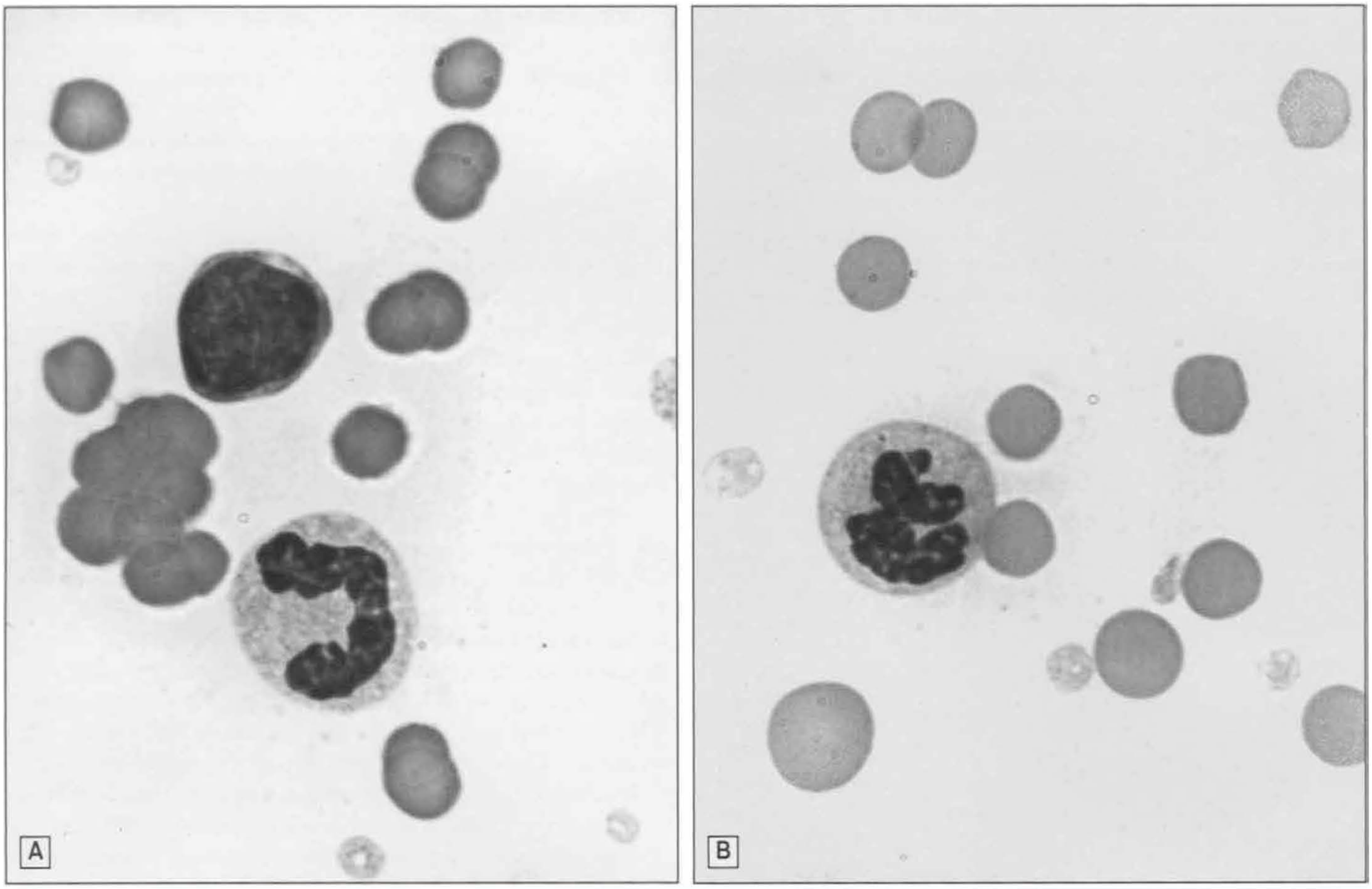

Figure 2.84 Isoimmune hemolytic anemia, in a foal. **A.** Rafting of red cells. **B.** Spherocytes and macrocytes indicative of defective erythroid response in this species.

trophoblastic junction, but the reverse cellular exchange does not appear to occur. Vaccines of blood origin for prevention of babesiosis and anaplasmosis may contain red cell antigens which immunize the dams. If the bull has the same red cell antigens as the vaccine donor, then the calves will share these antigens and may develop isoimmune hemolytic anemia when they receive colostrum. Gestation is normal.

The disease may be mild or peracute with signs occurring within hours of birth and severe and terminal dyspnea developing at 1–7 days of age. Severely affected animals are dull and have reduced activity. Jaundice develops in those that survive 1–2 days.

In the *peracute form* of the disease, there is hemoglobinuria, hypofibrinogenemia and fibrin degradation products in the blood, and death is rapid. At postmortem, the spleen is large and spongy and the kidneys are discolored by hemoglobin. There is excess blood-stained pleural fluid and the lungs are congested and edematous. Histologically, there are widespread fibrin thrombi, and *death is due to disseminated intravascular coagulation.*

In the *acute form,* the PCV drops to 0.06–0.07 L/L and there is often hemoglobinuria. Death is expected at ~5 days with anemia and icterus. The marrow is responsive but inadequate with only 1–2% reticulocytes, and rubricytosis that exceeds the level of leukocytes. The Coombs' test is positive and the dam's milk agglutinates the calf's red cells. Hemolysis occurs if complement is added. The A, B, C, F-V, and S-U antigen systems are most often the target of maternal antibody.

In the *mild form,* the hematocrit drops to 0.18 L/L at a week after birth and rises to 0.30 L/L at 3 weeks of age. The anemia is normochromic and macrocytic and recovery is slow.

Isoimmune hemolytic anemia in piglets

Isoimmune anemia of piglets was originally attributed to maternal sensitization by a crystal violet-inactivated classical swine fever vaccine of blood origin. However, *spontaneous transplacental sensitization occurs in swine and the disease is primarily an isoimmune thrombocytopenia with lesser effects on the red cell system.* The piglets are normal at birth and disease occurs after suckling. The isoantibodies are usually against the Ea, Ee, Gb, and Kb red cell antigens (see Platelet disorders).

Signs occur between 1–4 days after birth, and consist of pallor, inactivity, dyspnea and jaundice. These signs gradually subside. After 10–11 days, widespread petechiation develops, particularly in ventral areas, in association with *severe thrombocytopenia.* Most piglets die at this time. The first signs are due to red cell and platelet destruction in the peripheral blood. The marrow is at first responsive and the disease is self-correcting for the first week. As antibody continues to be absorbed, there is depression of marrow precursors and the terminal petechiae are associated with decreased platelet production because of marrow aplasia. The terminal anemia is due both to hemorrhage and hypoproliferation.

The erythrocyte count drops from a normal level of 4.5–5.3 × 10^{12}/L at birth to 1–3 × 10^{12}/L at 4 days of age. As the red cell count drops, anisocytosis and polychromasia develop, along with some circulating rubricytes. The mean corpuscular volume rises from a normal of 70 fL at birth to 100–120 fL at 7–14 days of age and returns to normal in survivors at four weeks of age. The neutrophils rise to 9–10 × 10^9/L at 4 days when the red cells are lowest and then decline to normal at 3–4 × 10^9/L. Platelets have a bimodal response and drop from 300 × 10^9/L at birth to 100 × 10^9/L at day one, then rise to normal at the first week, followed by severe thrombocytopenia after 10–14 days.

The bone marrow is hypoplastic in piglets which die, and megakaryocytes are absent. Red cells are Coombs-positive from day 1–7 and are negative by day 14.

Drug-induced hemolytic anemia

Many drugs have been associated with Coombs-positive hemolytic anemia and these same drugs may cause immune thrombocytopenia. *The most commonly implicated drugs are quinidine, quinine, para-aminosalicylic acid, phenacetin, penicillin, insecticides, sulfonamides, chlorpromazine, and dipyrone.*

There appear to be *two types* of immune hemolytic anemia associated with drugs. One is *caused by an antibody that reacts only with cells exposed to the drug*. In the other, the *antibody reacts with normal red cells in the absence of the drug*. The latter disease thus is indistinguishable from "auto"-immune hemolytic anemia except for the history of drug exposure and exacerbation with retreatment. Drug-related hemolysis is usually highly specific, but occasionally closely related drugs cause hemolysis in the same patient. Some drugs require only small doses to cause disease, while hemolytic anemia after penicillin therapy is limited to cases with prolonged high-level treatment. In most cases, the antibody eluted from sensitized red cells is drug-specific and does not react with normal red cells. Most cases caused by drugs other than penicillin have little IgG on red cells, but complement is usually present.

Since drugs and environmental contaminants are so ubiquitous, and since some drugs induce an "auto"-immune type hemolysis, *it is usually not possible to distinguish between spontaneous and drug-induced immune anemias.*

Primary or idiopathic immune hemolytic anemia

This type of anemia is *most common in dogs and cats*, less common in cows and horses, and probably occurs in all species.

Clinically, there is usually depression of sudden onset in an animal in good body condition. Occasionally the history may indicate previous attacks. Rarely, red urine is seen and the animal is examined for suspected urinary infection. There is mild-to-marked pallor and often mild icterus. When hemolysis and thrombocytopenia are present, there will be mucosal bleeding and often epistaxis, dark stools and hematuria. Heart rate and respiratory rate are increased in proportion to the degree of anemia, and a hemic murmur may be present. The spleen may be palpably enlarged.

Occasionally, immune hemolytic anemia in the dog may resemble systemic lupus erythematosus of humans. The canine form of **systemic lupus erythematosus** is characterized by Coombs-positive hemolytic anemia with thrombocytopenia, polyarthritis and glomerulonephritis. Rarely these lesions are accompanied by symmetrical facial dermatosis, serositis with accumulation of fluid in body cavities, and combinations of leukopenia, hepatosplenomegaly and lymphadenopathy. Antibodies to double-stranded DNA are relatively common, as are positive lupus erythematosus preparations. All breeds of dogs may be affected and the disease, as in humans, is most severe in young females. Canine systemic lupus erythematosus is considered also in Vol. 1, Bones and joints; Vol. 1, Skin and appendages; Vol. 2, Urinary system.

There is *moderate-to-severe anemia that is highly responsive*, with 10–20 polychromatic red cells per oil immersion field or 300–400 × 10^9 reticulocytes/L of blood. The anemia is macrocytic and slightly hypochromic with a normal mean corpuscular hemoglobin concentration. Spherocytosis, leptocytosis and marked anisocytosis are present (Fig. 2.85A–C), usually with rubricytosis of 1–20 × 10^9/L. Rouleaux formation is usually marked, and slide aggregation may be present. The direct Coombs' test is positive in the majority of cases, but may be negative; a negative result should not eliminate the diagnosis if the other findings are consistent with the disease. Large immature red cells occur in most microscopic fields.

There is *accompanying neutrophilic leukocytosis,* usually of 30 × 10^9/L in the dog and cat, which increases to 40 × 10^9/L with initial heavy steroid therapy. There is a mild left shift with eosinopenia, lymphopenia and characteristic monocytosis in dog and cow, and to a lesser extent in other species. Thrombocytosis is usually present with many large young platelets. The marrow is hypercellular with erythroid shift and normal maturation.

Biochemical icterus may be present and is usually less than 50 μmol/L except in the horse where 170 μmol/L or more may be present. In all species, free and conjugated bilirubin both tend to be markedly elevated. Icterus on presentation usually indicates previous attacks, or chronicity with ischemic hepatic damage. Icterus tends to increase with each blood transfusion, especially if the PCV is maintained for only 1–2 days. The erythrocyte sedimentation rate is very rapid.

Lesions are usually prominent. Those described here refer to the dog but the changes are similar in the cat, calf and horse. In the horse, immune hemolytic anemia is more often associated with (occult) lymphoma than in other species. Grossly, there is at least mild *icterus* and in some cases it is marked. The carcass is usually in good condition with some history of weight loss. In dogs, if steroid treatment has been prolonged, there may be abdominal enlargement and ventral hair loss. The lungs are edematous and may contain infarcts. The heart may be normal, or atonic, rounded and dilated if anemia has led to congestive failure. There is often a prominent yellow chicken-fat clot. The abdominal viscera are pale except the kidneys which have dark red-brown cortices if the disease has been prolonged. The liver is usually enlarged, fatty and friable with distended gallbladder and a nutmeg pattern visible through the capsule. The spleen is enlarged, sometimes up to 6–10 times normal weight. Lymph nodes are usually small if steroid treatment has been given, and red-brown medullary pigmentation due to hemosiderosis is present. The femoral marrow has subendosteal reddening if clinical signs have been present for a month, and diffuse reddening if the disease has persisted 3–4 months or longer. If the immune hemolysis is complicated by thrombocytopenia, *hemorrhagic lesions* may dominate the gross picture. In the combined immune destruction of erythrocytes and platelets, the lymph nodes are reddened, and may be enlarged, edematous, and cyanotic if draining an area with interstitial hemorrhage. Hemorrhages are most prominent on serosal surfaces including those of the enteric and respiratory systems, and on the heart and diaphragm.

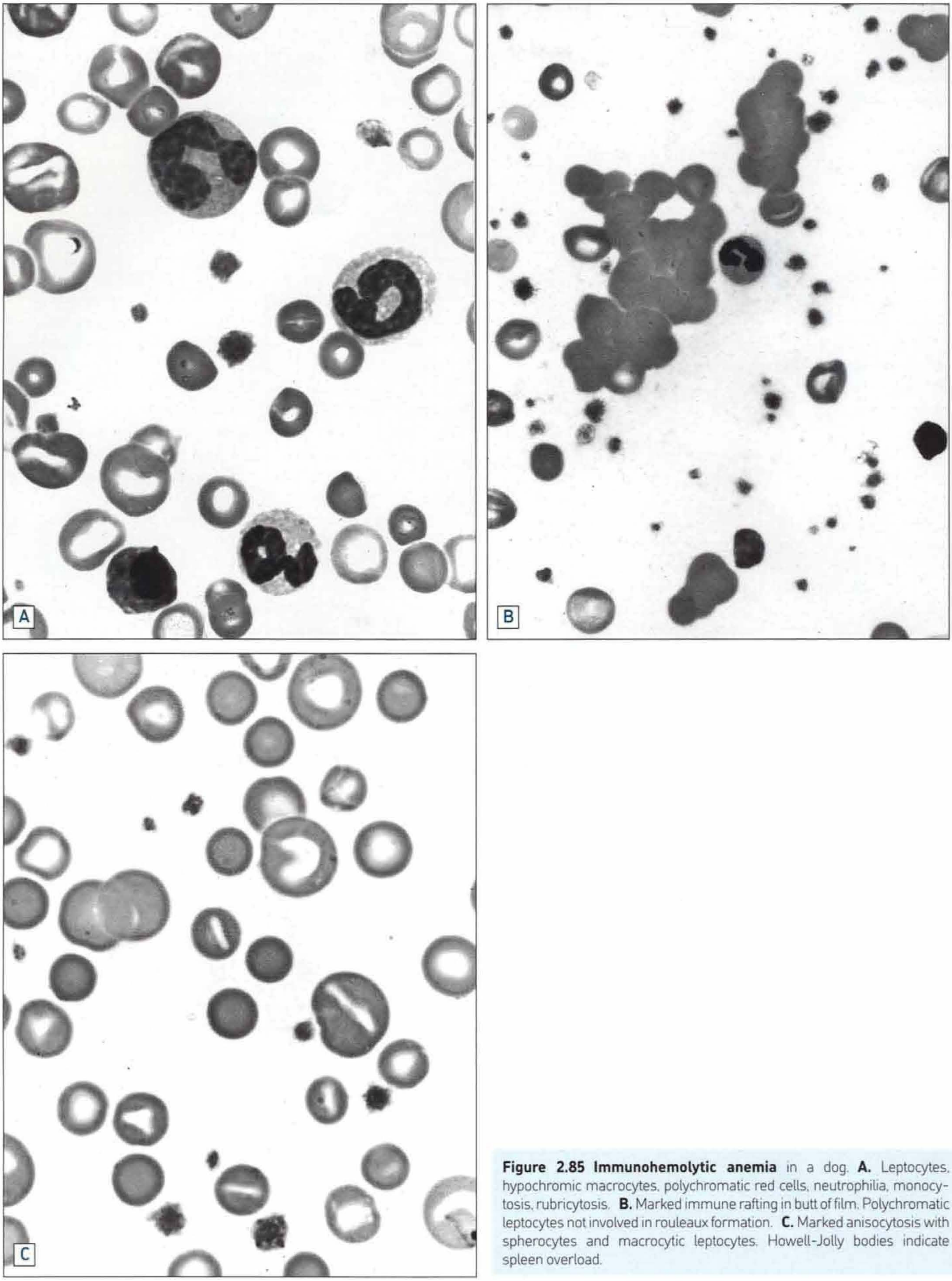

Figure 2.85 Immunohemolytic anemia in a dog. **A.** Leptocytes, hypochromic macrocytes, polychromatic red cells, neutrophilia, monocytosis, rubricytosis. **B.** Marked immune rafting in butt of film. Polychromatic leptocytes not involved in rouleaux formation. **C.** Marked anisocytosis with spherocytes and macrocytic leptocytes. Howell-Jolly bodies indicate spleen overload.

Histologically, the *thymus* is atrophic, though rarely there is a follicular reaction with plasma cell accumulation or a lymphoid thymoma. There is *pulmonary* alveolar flooding and, if congestive failure has developed, there are alveolar macrophages with hemosiderin. Some animals have pulmonary thrombosis with focal peripheral infarction. The *liver* has Kupffer cell hyperplasia of variable degree, and hemosiderin accumulation may be prominent. There is minimal periportal lymphoid reaction or hematopoiesis, and vacuolar degeneration is present in periacinar, and often in midzonal, hepatocytes. Inspissated bile plugs form in canaliculi, but the ducts themselves are empty. If changes of circulatory failure are added to the anemia, there is irregular necrosis of periacinar hepatocytes, swelling of sinusoidal endothelium and separation of hepatic cords. The *splenic* capsule is thin, and muscular trabeculae are narrow and sparse, reflecting splenomegaly and sinus hyperplasia. There is atrophy of follicles and periarteriolar sheaths. In addition, the normal small lymphocytes of the thymus-dependent cuffs are loosely rather than compactly arranged, and nuclear size is increased with vesiculation so that these areas are inconspicuous and identifiable by the vessel rather than the lymphoid cuff. Major changes occur in the sinus areas, which have diffuse extramedullary hematopoiesis with prominent erythropoiesis and thrombopoiesis. Hemosiderosis is often marked, with focal and diffuse clusters that may exceed 3–4 red cells in diameter. Erythrophagocytosis is present and often prominent. Plasma cells are increased and all free cells are enmeshed in a stromal network that is markedly hypertrophied and can be identified by the oval reticular nuclei and cellular cleavage planes created by their processes.

Lymph nodes have follicular hyperplasia with "bare" follicles due to paracortical atrophy. If steroid treatment has been intense and prolonged, there is generalized cortical atrophy and sinus expansion. There is always some degree of sinus histiocytosis, and variable accumulation of plasma cells in medullary cords. If hemorrhage has occurred, there is increased hemosiderin in sinus phagocytes, and erythrophagocytosis is often very marked. The *kidneys* have minimal to moderate thickening of glomerular basement membranes, and there are occasional hemosiderin granules in mesangial areas. The juxtaglomerular nuclei are prominent with large nucleoli, and the proximal epithelium is variably flattened and bile-pigmented. Occasionally, there are (incidental) loose lymphoid cuffs around corticomedullary arteries, and the kidney, like the lung, liver and spleen, may have focal thrombotic infarction. The *adrenal* zona glomerulosa is usually hypertrophic and the reticularis reduced.

Femoral bone marrow has 50–60% cellularity or higher in the normally fatty central medullary areas. Cellular packing (density) is high in highly responsive anemias, and reduced in animals which die with marrow depression. As in the spleen, hemosiderin is markedly increased and of both fine and coarse character, indicating chronic accumulation and continuing increased turnover. Erythrophagocytosis is often extensive and more easily identified than in the spleen. The marrow has an erythroid shift, and megakaryocytes are increased in numbers and multinuclearity. Animals with concurrent immune thrombocytopenia may have dysthrombopoiesis characterized by reduced megakaryocyte cytoplasmic volume and many pyknotic nuclei. Neutrophil migration through megakaryocytic cytoplasm is often prominent but is apparently without diagnostic value.

The *myeloid system* usually maintains a picture of early asynchrony due to steroid effects and early release of maturation phase cells. The relative reduction in myelopoiesis is apparently much more than compensated for by the expanded volume of hematopoietic marrow. Erythropoiesis is usually normal, but there may be mild early asynchrony if there is prominent peripheral blood rubricytosis with release of maturation phase cells. Occasionally there is mild dyserythropoiesis with satellite nuclei (incipient Howell-Jolly bodies), and mitoses may be observed in polychromatic rubricytes. Plasma cells are increased and may be found in cuffs around small arteries. *The overall picture is one of increased erythrocyte destruction followed by increased production and, in chronic cases, lymphoid atrophy, macrophage hyperplasia, hemosiderosis and secondary injury to parenchymal organs.*

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Infectious hemolytic anemias

Equine infectious anemia

Equine infectious anemia virus (EIAV) is a member of the Lentivirinae subfamily of retroviruses that infects horses, mules, and donkeys. The disease has a worldwide distribution and since the development of the agar gel immunodiffusion test, which detects anti-EIAV antibody, has been subject to control measures in many countries. The location of outbreaks in marshy areas led to the common name of "*swamp fever*," and their occurrence during the summer led to the demonstration that the virus is *arthropod-borne*. The likely vectors are the stable fly (*Stomoxys calcitrans*), horse flies (*Tabanus* spp.), and *Anopheles* mosquitoes. *Transmission is mechanical not biological*, and may also occur accidentally by contaminated needles, syringes and tattoo equipment or experimentally by parenteral administration of blood or virus. The virus is stable in the environment, but infection appears always to arise by close contact with an infected animal.

Infection is lifelong and viremia is cyclic, resulting in a clinical course varying from asymptomatic to acutely fatal and which resembles the idiopathic hemolytic anemias of dogs. *The virus infects cells of the monocyte–macrophage system* but, in acute viremic states, unintegrated viral DNA is present at levels two orders of magnitude higher in liver than in leukocytes and at lower levels in nodes, marrow, spleen and kidney. The relationship between the cyclic viremia, viral antigenic variation and the immune response is complex. The gp90, gp45 and p26 antigens appear to be most apparent to the immune system and, while these antigens are bound in early disease, neutralizing antibody is not detected before 2 months of infection. Subsequently, there is rapid variation in the glycoprotein envelope antigens that indicates that the preparation of an effective vaccine will be difficult as for the other

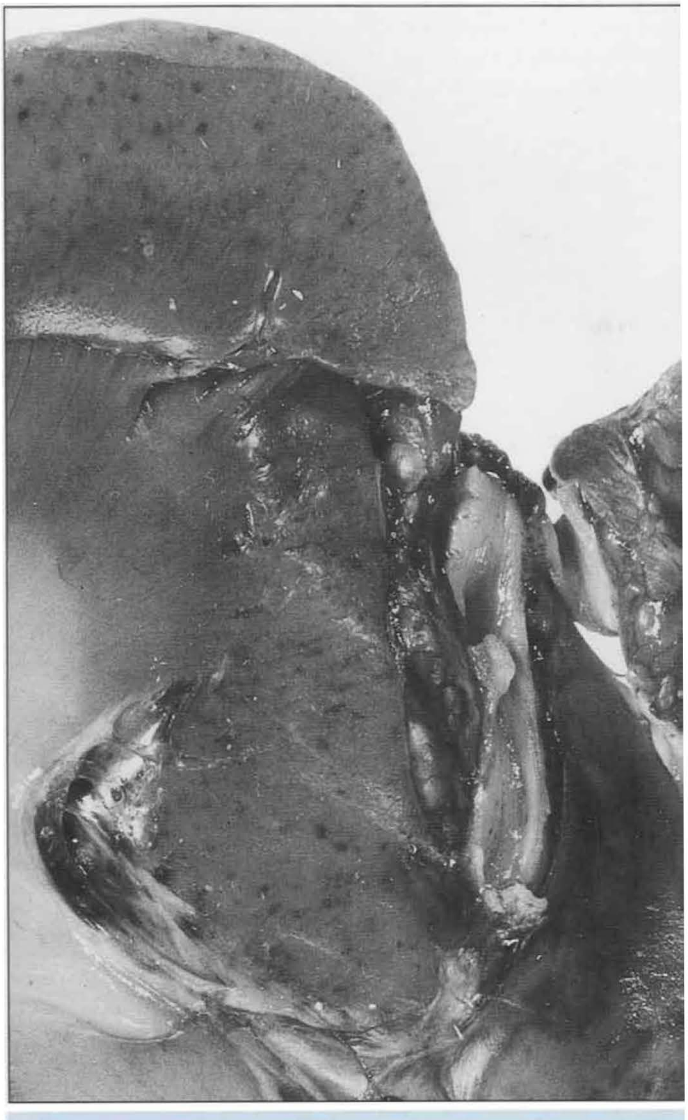

Figure 2.86 Equine infectious anemia. sagittal section of kidney. Prominent glomeruli with petechial hemorrhages.

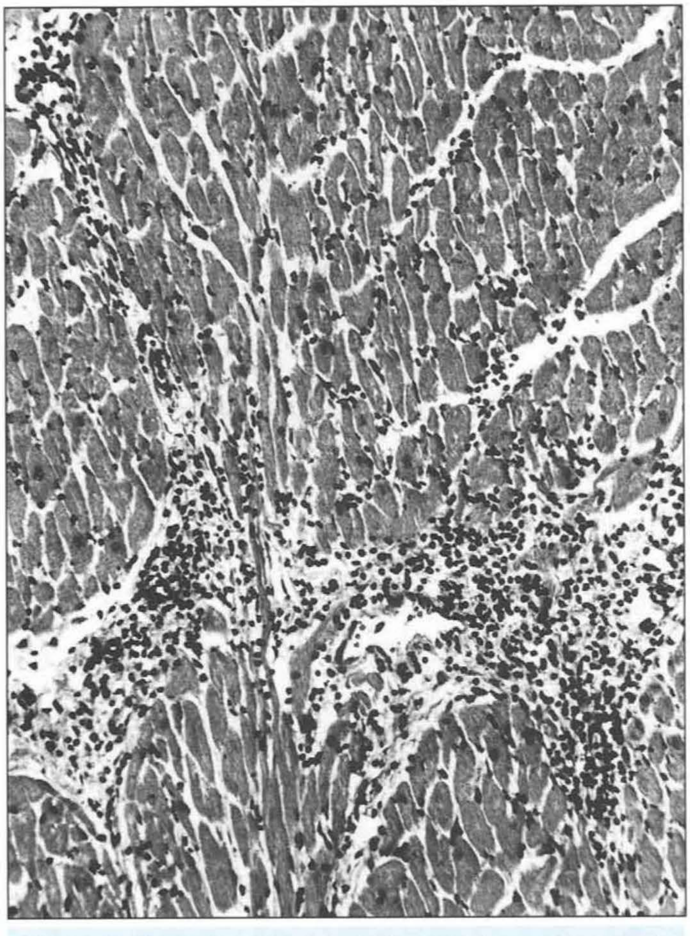

Figure 2.87 Equine infectious anemia. Lymphocytic interstitial myocarditis in acute disease.

retroviridae. The virus has both *cis*- and *trans*-acting response elements responding to viral regulatory proteins in controlling gene expression, and shares structural and sequence similarities with human immunodeficiency virus and simian immunodeficiency virus, and functional similarities with visna virus.

It has been suggested that the EIAV has little direct effect on tissues and that the pathogenesis of disease involves the indirect effects of the immune response. When foals with combined immunodeficiency (CID) and lacking functional B and T lymphocytes are infected with EIAV, the typical widespread inflammatory lesions of EIAV infection are not found, suggesting that the lesions in parenchymal organs are a function of the immune system. The foals develop anemia and, although they have a functional complement system, the characteristic fixation of C3 to red cells is not found. While CID foals are not able to clear virus from the blood, the level of viremia in these animals is no higher than that in infected conventional foals. Thus it appears that there may be NK cell or other factors operative in viral control besides antibody.

Following experimental infection of conventional horses, there is a variable incubation period of 5–7 days up to several weeks during which time there is asymptomatic viremia. All signs of disease correspond to the appearance of circulating antibody, which is complement-fixing but not initially virus-neutralizing. C3 appears on erythrocyte membranes, and this probably accounts for macrophage recognition and erythrophagocytosis. Antibodies of the IgG and IgM type are not found fixed to red cells, but it is believed that they have reacted specifically with virus adsorbed to erythrocytes, activated complement, and then been eluted off in the process of analysis. *Red cell destruction is thus of the "innocent bystander" type, with virus playing a similar role to compounds causing drug-induced immune hemolysis.* There is shortening of red cell lifespan to about 20–65% of normal. Hemolysis is largely intracellular, but in acute disease there is erythrocyte fragmentation due to microvascular injury, and decreased haptoglobin levels. The marrow is initially highly responsive with an upward shift in MCV and marked anisocytosis. Later the marrow becomes hypoproliferative. Evaluation of the erythroid response requires definition of the stage of the disease, and also the breed affected, since racing breeds tend to react differently than do ponies and heavy breeds.

The clinical disease is arbitrarily divided into *acute, subacute* and *chronic* forms, but death in the protracted cases results from acute exacerbation, so the signs are relatively constant. The acute disease is characterized by pyrexia and marked depression, with anorexia, weight loss and pitting, dependent edema. There are petechial hemorrhages most reliably seen on the ventral surface of the tongue but also present on ocular and vulvar mucosa. The tongue is most reliably examined by rotation in situ, since forced extraction tends to cause petechiation. Mild icterus develops after a short febrile period and is accompanied by pallor of mild-to-marked degree. The febrile periods may progress to death in less than a week, or

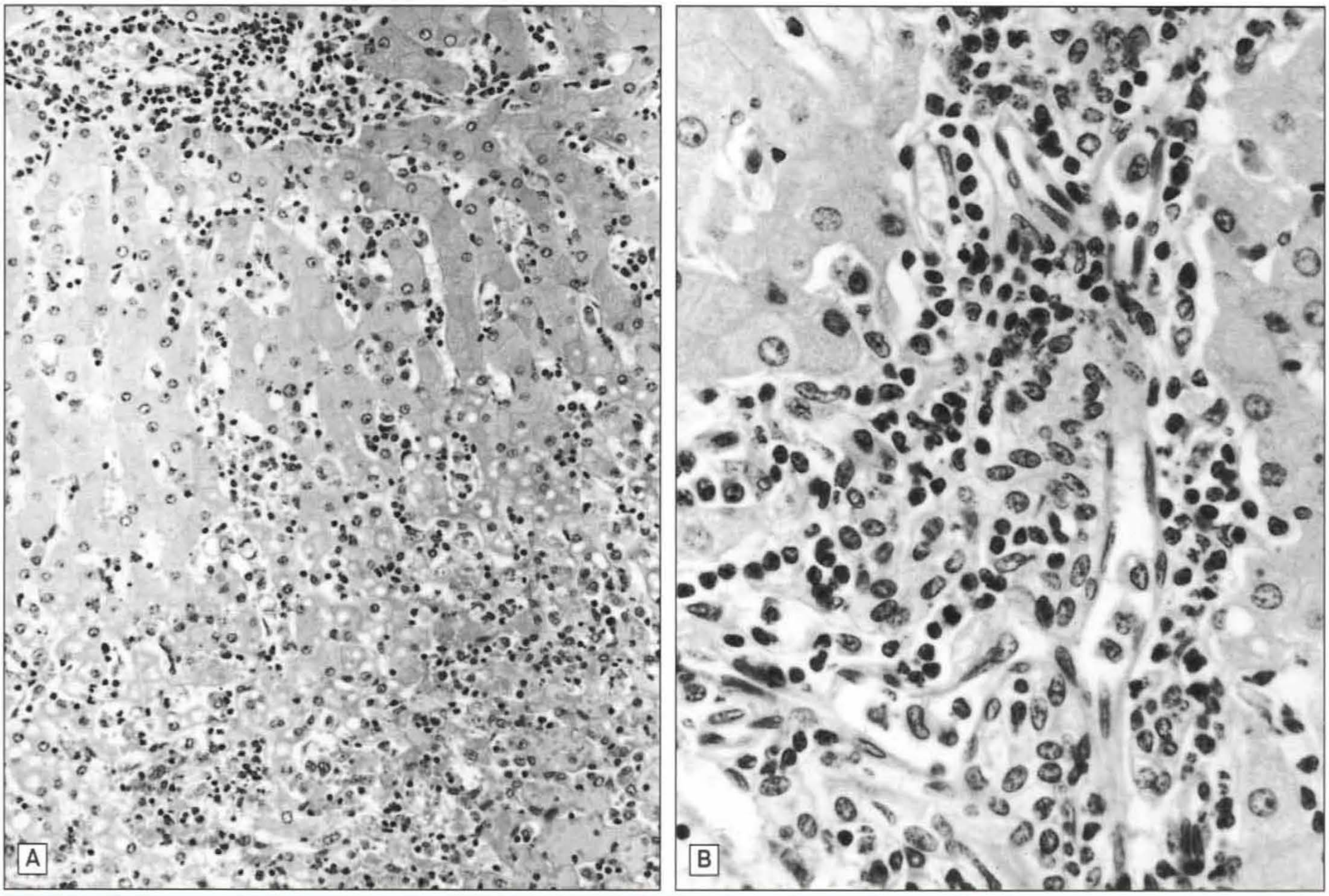

Figure 2.88 Equine infectious anemia. A. Hepatic portal and sinusoidal infiltrates of lymphocytes and plasma cells. Kupffer cell proliferation and periacinar necrosis in the chronic disease. **B.** Detail of portal area of (**A**).

regress and recur at irregular intervals. Rarely, an acute episode is followed by many years of an asymptomatic carrier state.

Anemia is severe and sufficient to cause death, with erythrocyte counts as low as 1×10^{12}/L, hemoglobin of 25–50 g/L, and hematocrits of 0.08–0.15 L/L. There is *consistent thrombocytopenia* during febrile periods with resulting petechiae. In the early stages of disease, there is anisocytosis, marked for the horse, with moderate poikilocytosis and, characteristically for the horse, no polychromatic red cells. Nucleated erythrocytes are not present in peripheral blood. In the early stage of an acute attack, there is macrocytosis, sometimes up to an MCV of 60 fL. Later the anemia is normochromic, normocytic and nonresponsive. There is accompanying leukopenia with neutropenia, minimal left shift, lymphopenia and at least a relative monocytosis. The monocytes often contain ingested red cells (*sideroleukocytes*), and stain positively for iron in buffy coat smears. Sideroleukocytes are specific not to equine infectious anemia but to intracellular hemolytic anemia but, prior to diagnosis by antibody, their presence at levels of 4/10 000 leukocytes or higher was considered a positive diagnosis. Sideroleukocytes are present in the blood within 2–3 days of a febrile episode and subside with the fever. The neutrophils have moderate toxic changes with vacuolation and azurophilic granulation, but not Doehle bodies. The mononuclear cells are large and basophilic and it is difficult to distinguish lymphocytes from young monocytes. The platelets are small and uniformly basophilic with a normal low level of granulation, and immature cells are rarely found. In the acute disease, the aspirated bone marrow is hypercellular with an erythroid shift, but cellularity and erythropoiesis are later reduced, and there is mild dyserythropoiesis and proliferation of macrophages and plasma cells.

Icterus is always present in febrile, anemic horses, and bilirubin is usually between 170 and 250 μmol/L; most is unconjugated. Lipemia is occasionally seen in the acute disease. With chronicity (30 days or more), there is a drop in albumin of around 10 g/L and a corresponding increase in gamma globulin so that the total protein is relatively unchanged but the albumin/globulin ratio is decreased. The serum iron rises in the acute disease and drops with chronicity, while the total iron-binding capacity remains normal or mildly elevated.

In horses dead of the acute disease, there is anemia, icterus and widespread foci of hemorrhage. In animals with protracted disease, there is anemia, emaciation and edema with serous atrophy of fat which is bilirubin-stained. In febrile disease, thrombocytopenia complicates minor trauma, and larger hemorrhages are found subcutaneously and around sites of parasitic migration. There is edema of the ventral abdominal wall and in the suspensory ligaments of the viscera. The spleen is enlarged, turgid and fleshy with capsular hemorrhages, a bulging but not oozing cut surface, and inapparent lymphoid follicles. The liver is enlarged, dark and turgid with a fine lobular pattern and focal capsular hemorrhages. Petechial hemorrhages are present on the renal capsules and in the perirenal tissues. On cut surface, there are multiple fine hemorrhages throughout the cortex and medulla (Fig. 2.86). The most significant gross lesions occur in bone marrow where the degree of reddening is in direct proportion to the duration of the disease. In acute cases, the conversion of fat to hematopoiesis in the femoral marrow occurs first in proximal cancellous and then subendosteal diaphyseal and distal cancellous areas. The red and yellow areas are initially firm and opaque, often with focal areas of hemorrhagic infarction. In chronic cases, the red conversion may include all of the medullary marrow and is alternately pink and translucent in areas of serous atrophy of fat, and cyanotic where congested sinuses have dilated as adjacent hematopoietic areas atrophy.

Microscopic lesions *occur in most tissues, but are most prominent in the heart, lungs, liver, spleen, kidney, marrow and lymph nodes.* Their severity varies with chronicity and they are described here in the fully developed state. The *myocardium* has fiber atrophy in chronic cases, and interstitial edema in acute cases, with perivascular lymphocytic aggregations that irregularly permeate the surrounding interstitium (Fig. 2.87). There is mild *pulmonary* alveolar thickening and an overall appearance of hypercellularity. Occasionally, hemosiderin-bearing macrophages are found in alveolar walls and are likely intravascular. The *liver* presents a spectrum of changes which varies from mild periportal lymphocytic infiltrates to atrophic cords with sinusoidal dilation, Kupffer cell hyperplasia, broad loosely arranged periportal lymphoid infiltrates, and increased interstitial connective tissue. As the disease progresses, hepatic hemosiderosis increases, largely in Kupffer cells. Generalized endothelial prominence, along with sinusoidal Kupffer cells and infiltrates, gives an overall picture of hypercellularity (Fig. 2.88A, B). In subacute cases there is periacinar fatty vacuolation, and in acute cases hemorrhage and necrosis. In animals in which the disease has been quiescent for some months, hepatocytes appear normal and lymphoplasmacytic infiltrates subside but sinusoidal hemosiderin-bearing macrophages remain as evidence of previous hemolysis.

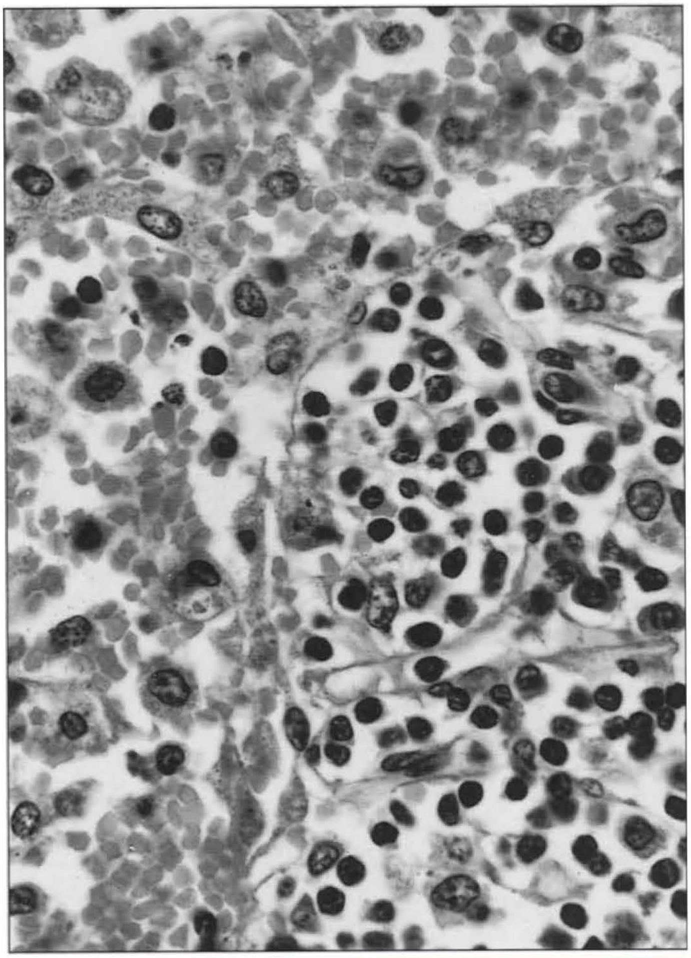

Figure 2.89 Equine infectious anemia, lymph node. Medullary cords contain few small lymphocytes and plasma cells. Active sinus histiocytes. Red cells in sinuses derived from thrombocytopenic bleeding.

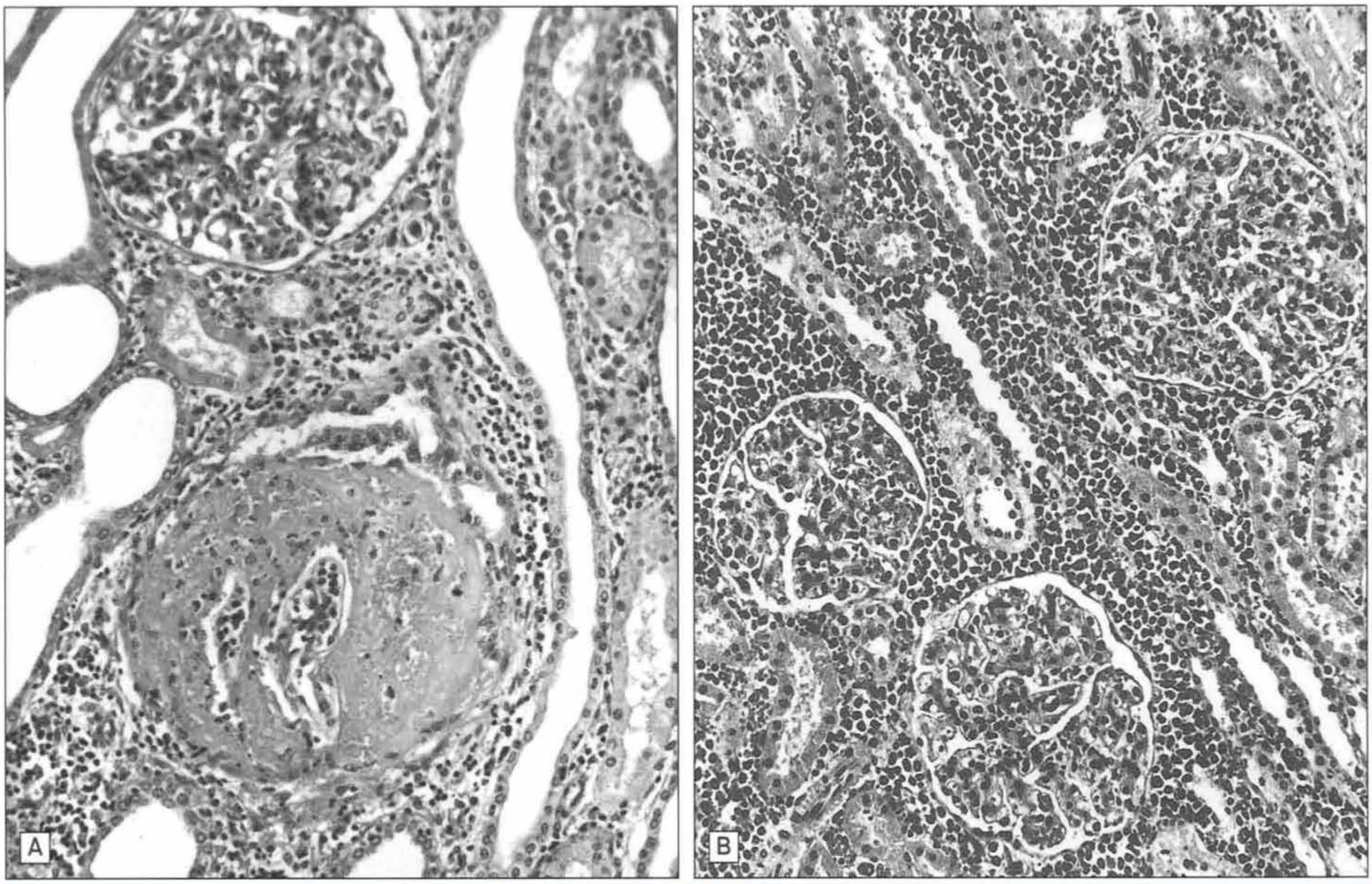

Figure 2.90 Equine infectious anemia, kidney. **A.** Periglomerular interstitial infiltrates of chronic disease, with glomerular hemorrhage from thrombocytopenia in acute exacerbation. **B.** Diffuse interstitial mononuclear cell infiltrate.

Splenic follicles are variably enlarged but hypocellular, often with a "bull's-eye" appearance due to a sharp distinction between the cells of the follicular center and the mantle and marginal layers, and a sharp transition to congestion in the surrounding sinusoids. In acute cases, the spleen is congested and, being largely composed of unsupported red cells, fractures on sectioning. Later there is sinus hyperplasia with stromal proliferation. The tissue is then cellular, fleshy and cohesive. Splenic hematopoiesis is never as prominent as in the dog with idiopathic immune hemolytic anemia, but hemosiderosis with macrophages and plasma cell proliferation is prominent. Lymph nodes are edematous and have medullary hemosiderosis with persistence of follicles and thin moth-eaten paracortical areas. In acute disease, the lymphoid tissue is highly reactive and there may be lymphocytic colonization of perinodal fat. Later there is sclerosis and lymphoid atrophy (Fig. 2.89).

Renal lesions in acute disease are largely hemorrhagic with some glomeruli obscured by erythrocytes and fibrin (Fig. 2.90A). Lymphocytic infiltrates may be intense and separate tubules in an irregular manner (Fig. 2.90B). There is some degree of epithelial atrophy and mild pigmentation that appears to be both bilirubin and hemosiderin.

The red *bone marrow* in acute disease has 40% or less of fatty areas and the hematopoietic cells are densely packed. There is an erythroid shift with synchronous maturation (Fig. 2.91), and a reduction in mature granulocytes, although eosinophils are prominent. Megakaryocytes are not increased, which may indicate that the thrombocytopenia may be at least partially due to ineffective production. With chronicity and repeated febrile periods, there is a progressive reduction of fat cells until the marrow is completely converted to hematopoiesis (Fig. 2.92A). At the same time, cellular packing decreases and sinusoidal expansion occurs so that marrow which appears red grossly can be seen histologically to be relatively hypoproliferative. Hemosiderin is prominent as is erythrophagocytosis (Fig. 2.92B). Large macrophages can be found which contain hemosiderin, red cells and rubricytes. This latter association does not appear to be a "nurse cell" relationship, but rather an extension of the peripheral disease to marrow with destruction of the precursors as well as the progeny. Dyserythropoiesis as evidenced by binucleated interphase rubricytes is present. Megakaryocyte cytoplasmic volume is normal or reduced but not increased, indicating that the response of this lineage is weakened and likely at least partially ineffective. Stromal cells are increased, endothelial nuclei are prominent, and there is a diffuse plasmacytosis. Hematopoiesis may regress from the central femoral cavity and leave fatty tissue with serous atrophy heavily laden with hemosiderin-bearing macrophages that indicate previous sites of production and hemolysis.

The disease is one of acute intracellular hemolysis followed by immunoproliferation and finally by hematopoietic and to a lesser extent immune exhaustion.

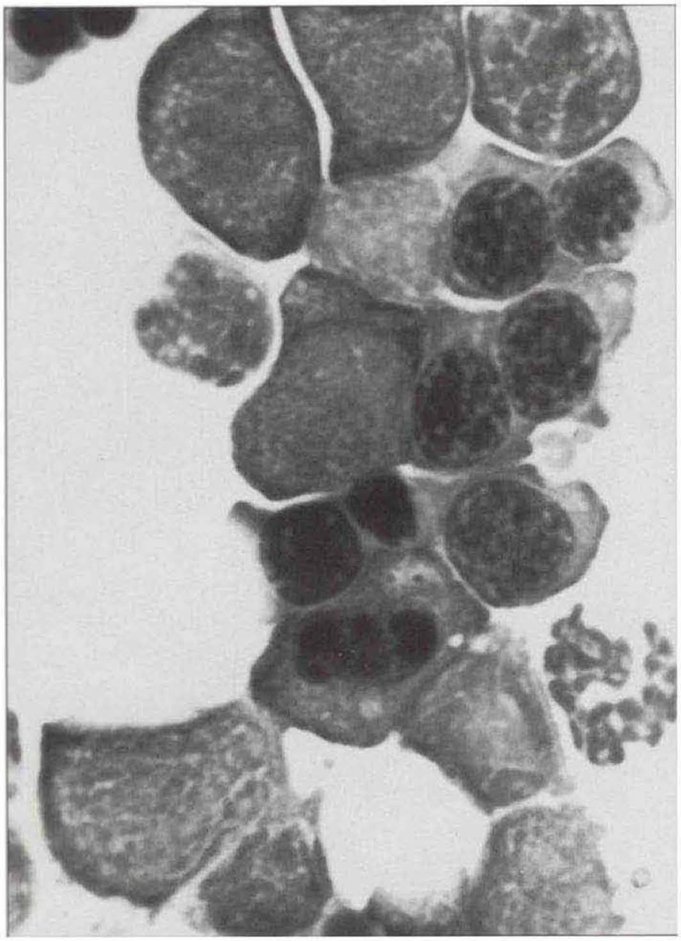

Figure 2.91 Equine infectious anemia. Marrow aspirate in acute disease. Productive synchronous erythroid hyperplasia.

Following the development of the agar gel immunodiffusion test, *steroid-responsive, idiopathic immune hemolytic anemia has been identified in the horse.* The lesions resemble those of immune hemolytic anemia of the dog, and lack the inflammatory reaction characteristic of equine infectious anemia. Horses with idiopathic immune hemolytic anemia are not usually thrombocytopenic and do not have cyclic fever.

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Anemia of blood cell parasitism

Babesiosis

The protozoan *Babesia parasitizes the erythrocytes of a wide range of mammals* including humans, and there is normally *quite strict host specificity. The agent is transmitted by ticks.* Natural transmission is largely *transovarial*, that is, the adult female becomes infected, and, depending on the babesial and tick species, the ensuing larval or nymphal generation then transmits the infection to a susceptible host. Transtadial transmission occurs in three-host ticks, infection being carried through a molt off the host, permitting transmission to the next host encountered. There is no evidence for transmission by biting insects or contaminated instruments (in contrast to the situation with *Anaplasma* infection), but infection is readily transmitted by experimental blood inoculation.

The severity of the disease produced in the mammalian host depends more on the strain and species of infecting *Babesia* than on the number of organisms inoculated. Pathogenic effects are, in most infections, related directly to *lysis of red cells by emerging parasites* but other mechanisms, including sludging of infected red cells, hemoglobinuric nephrosis, and release of vasoactive peptides, contribute to the signs and are responsible for death.

Different tick species are vectors in different countries for what are apparently the same species of *Babesia*. Nomenclature is not dealt with here, but rather the pathology and pathogenesis of babesiosis and, in particular, those naturally occurring infections that cause the most severe and economically important disease. *The severity of many of the anemias caused by parasites of blood cells is enhanced by splenectomy*, and this technique is used commonly in pathogenetic studies. Splenectomy is often performed in dogs following abdominal trauma or for treatment of neoplastic disease, and this may activate latent infections by organisms such as *Haemobartonella canis*. In human babesiosis, splenectomy appears to be a major risk factor for infection, primarily with *B. microti*.

Bovine babesiosis

Babesia bovis *(=B. argentina, B. berbera, B. colchica) is probably the most important cause of the "tick fevers" of cattle*, with local exceptions in some countries. The fact that some *Bos indicus* cattle breeds are relatively resistant to the effects of this parasite has led to the suggestion that the organism evolved in these breeds. Young cattle have pronounced resistance to severe infection. Immunity passively acquired from colostrum by very young calves may persist for up to 9 months of age. *Strong acquired immunity follows overt infection*, and may persist indefinitely in the absence of latent infection, although the latter is usual. Infection with a single strain of the parasite protects against subsequent infection for at least 4 years. For these reasons, the disease is

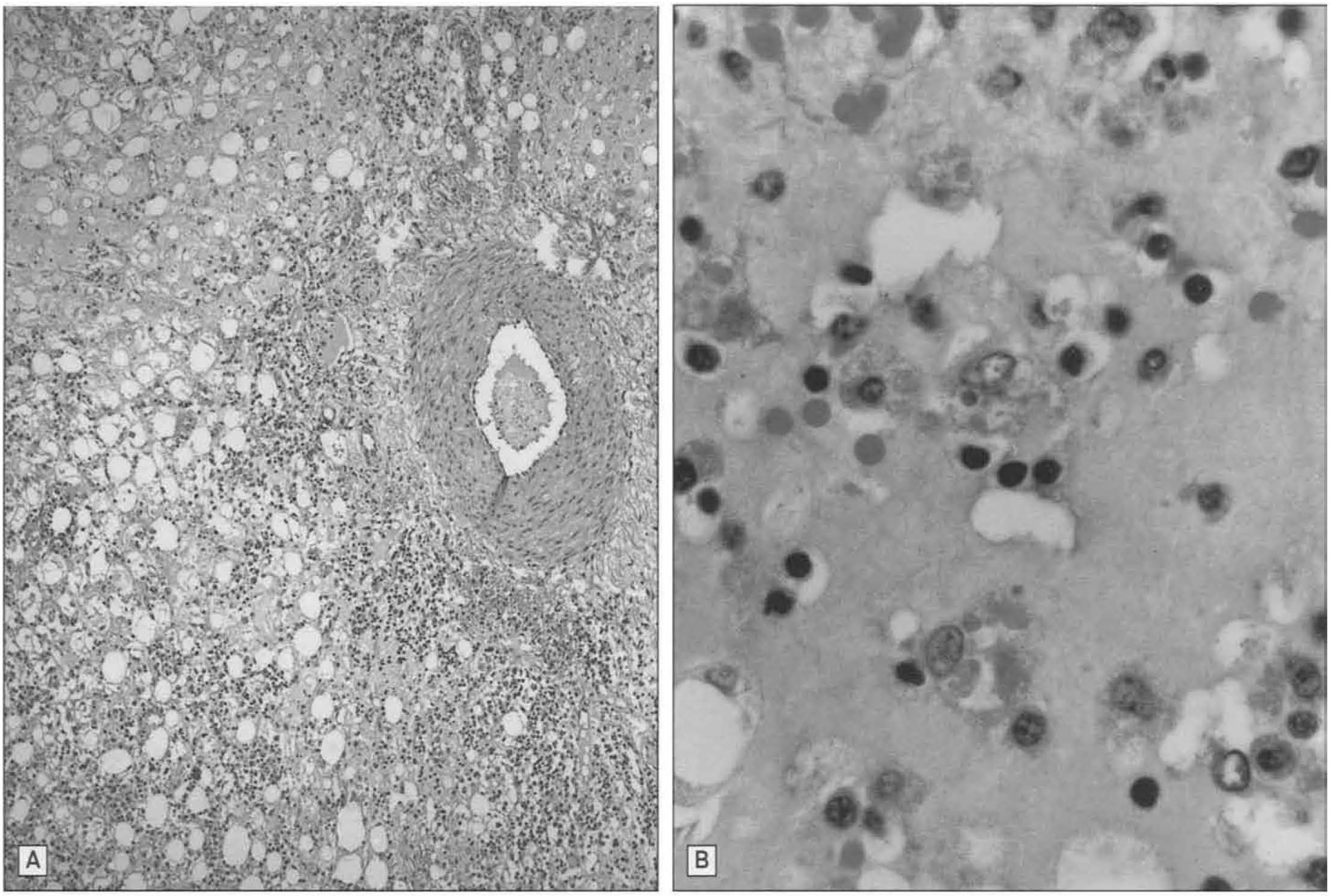

Figure 2.92 Equine infectious anemia. A. Central femoral marrow in chronic disease. Hypertrophy of marrow to occupy entire cavity followed by late reduced hematopoiesis and serous atrophy. **B.** Area of serous atrophy in marrow in chronic disease. Macrophages contain hemosiderin, indicating earlier hematopoiesis and erythrophagocytosis.

mostly seen in adult cattle recently introduced from tick-free areas, or in areas of greatly fluctuating tick populations.

Infection by *B. bovis* causes *severe febrile illness* which begins about 2 weeks after exposure to ticks. The animal may become extremely ill before severe anemia, parasitemia or hemoglobinuria are apparent, and sometimes the clinical picture is dominated by nervous derangement, likely associated with the tendency for parasitized red cells to sludge in cerebral capillaries as well as in the microvasculature of the adrenal and kidney of splenectomized animals. Usually, however, the clinical picture is characterized by *weakness, fever, hemoglobinuria, and anemia*; the latter is more severe in animals surviving more than a week. The parasite is never numerous in circulating blood, rarely being seen in more than 5% of circulating erythrocytes. Parasitized erythrocytes are much more likely to be found in smears of blood squeezed from cut skin capillaries than in routine blood samples from large veins. Recovery is usually fairly rapid in animals which survive the acute phase, although chronic ill-thrift has been described. Photosensitization may occur in the convalescent phase, probably due to overload of the phylloerythrin-conjugating sequence by bilirubin derived from hemolysis.

The most typical **postmortem findings** *are those to be expected of an acute intravascular hemolytic crisis.* There is anemia, variably severe jaundice and hemoglobinuria, and the kidneys are deep red-brown throughout as a result of hemoglobin staining and intense congestion of capillaries. There is capillary congestion of most organs, the spleen is always grossly swollen, soft and dark, and the liver is also acutely congested and may be heavily stained with bile pigment. The gallbladder is distended with thick, dark viscous bile.

In some acute cases, the lungs are congested and edematous and the larger airways contain stable foam. Recent hemorrhages are common in the thoracic serosal membranes. *The most characteristic macroscopic feature of B. bovis infections is striking, uniform congestion of the gray matter throughout the brain, imparting to it a dramatic, deep pink color (the cerebral flush)*, which contrasts strongly with the white matter; the latter is often stained a faint yellow by unconjugated bilirubin. In some peracute cases, there is minimal hemoglobinuria or jaundice, but the brain still shows the characteristic capillary congestion from which imprint preparations are usually diagnostic, with many intraerythrocytic organisms present.

Histologically, there is thickening and congestion of pulmonary capillaries with hemosiderin in intravascular macrophages. Kidneys and liver have the histological lesions to be expected of an acute hemolytic disease. There is variably severe hemoglobinuric nephrosis with severe congestion, focal hemorrhage and hemosiderin in tubular epithelium, and diffuse vacuolar degeneration with interstitial mixed mononuclear cell reaction. There is hepatic periacinar congestion

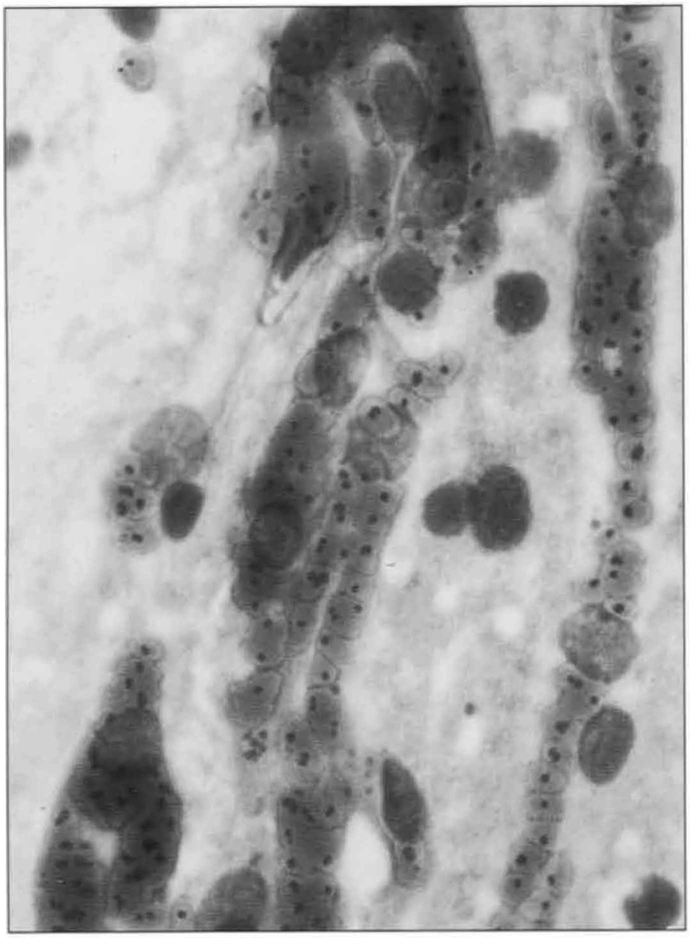

Figure 2.93 ***Babesia bovis***, ox. Giemsa stain. Sludging of parasitized red cells in cerebral capillaries. (Courtesy of HMD Hoyte.)

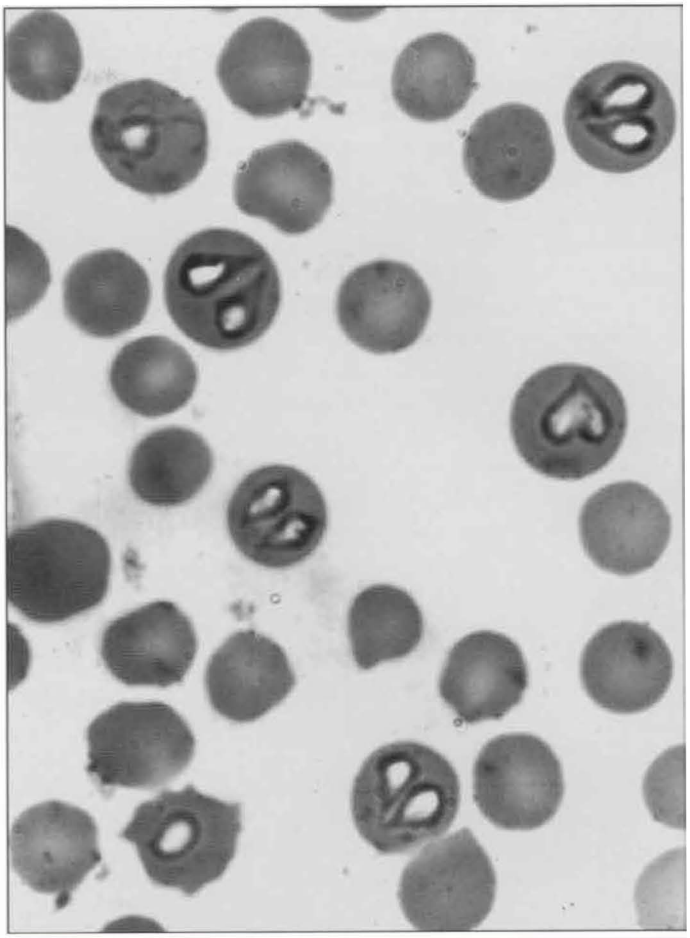

Figure 2.94 ***Babesia bigemina***, ox. Giemsa stain. Parasitized cells are large due to presence of parasites and selective parasitism of young cells.

with uninfected red cells, and vacuolar degeneration in midzone and periacinar hepatocytes, accompanied by canalicular cholestasis. There is hemosiderin accumulation in both hepatocytes and Kupffer cells, and the latter contain both infected and noninfected red cells. Lymphocytes and plasma cells accumulate in portal areas and around central veins. *Parasitized erythrocytes may be seen in vessels in all tissues*, but they are particularly common in interstitial capillaries in the kidney, in the gray matter of the brain, in the heart, and especially in skeletal muscle; in these locations, nearly every red cell packed into the distended capillaries appears to contain 1–2 parasites. *The organisms are faint blue in routine sections, and are best demonstrated in imprint preparations of fresh tissue.* In sections, Giemsa's stain demonstrates them as small paired or single spherical bodies with denser staining at one pole. In well-fixed fresh brain of acute cases, there may be edema of the neuropil around the clogged capillaries (Fig. 2.93).

Animals that die with peracute disease have necrosis of lymphocytes in germinal centers of node and spleen, while there may be some degree of recovery in animals surviving a week and general depletion of lymphocytes in those dying after protracted illness. Characteristic changes in the medullary region of nodes include sinus histiocytosis with extensive erythrophagocytosis. The histology of the spleen is often unhelpful, the organ being so suffused with intra- and extracorpuscular hemoglobin that evidence of erythrophagocytosis is obscured. There is little extramedullary hematopoiesis in liver or spleen. The bone marrow has an erythroid shift and, in animals that survive the acute stage, there is a strong reticulocytosis and slow recovery in red cell numbers. There is mild hemosiderin increase, suggesting that marrow is also a site of erythrolysis.

Babesiosis results in metabolic disease more complex than a simple syndrome of intravascular hemolysis. Severely affected animals may die before significant anemia has set in, and in these cattle the intense visceral congestion and pulmonary edema suggest that death may be at least partly due to circulatory shock. This has been supported by studies which have shown that the parasite is the source of proteases which activate plasma kallikrein which, as well as being a hypotensive agent, may activate bradykinin, another potent vasodilatory agent. Rather unexpectedly, metabolic alkalosis occurs in *B. bovis* infections, whereas acidosis is the rule in severe *B. canis* infections. *The result of these profound metabolic upsets is a syndrome of circulatory failure, likely due to extensive plugging of microvasculature by sequestered red cells.* The apparent anemia is therefore due in considerable measure to the vasodilatory effects of kallikrein and shift of red cells away from large veins. While plasmin activation occurs, the effects on the coagulation system are unlikely to be a significant factor in the pathogenesis since hemorrhage is not a major part of the morbid picture.

The actual mechanism of penetration of red cells by the merozoites of Babesia is as yet unclear, but complement is required and it appears that the organism causes activation by the alternative pathway, and by

C3b achieves adherence and later invasion. *The mechanism of hemolysis is also obscure.* It may be in large part spherocytic, and complicated by mechanical loss by fragmentation and exiting of parasites. Immune mechanisms do not appear to play a significant role in the early stages of the disease, but they may be a factor in delayed recovery. Osmotic fragility is markedly increased in nonparasitized cells, while the fragility of parasitized cells may actually decrease. Parasitized red cells form prominent stellate projections which appear to be the means by which they adhere to each other and to the endothelium. Fibrin may be involved in some of the sludging phenomena.

Babesia bigemina infection may be transmitted by the same tick as *B. bovis*, and both parasites may cause losses in the same geographic area. *Babesia bigemina*, however, usually causes a *much less severe syndrome*, notwithstanding the fact that parasitized erythrocytes circulate early (Fig. 2.94), sometimes before clinical signs are apparent, and in much greater numbers than they do in *B. bovis* infection. Like the latter parasite, *B. bigemina* causes *erythrocyte destruction*, but this appears to be directly proportional to the level of parasitemia, unlike *B. bovis* infection. There are few of the vasoactive and red cell adherence effects, so the disease is not attended by such profound shock-like signs. Thus the anemia in severe cases may be severe before the animal becomes ill and, as in any case of severe anemia, death may occur suddenly as a result of myocardial failure. Fever is not usually as severe as in *B. bovis* infection.

The pathology of *B. bigemina* and *B. bovis* infections is similar, except that in *B. bigemina* infection there is little capillary congestion of the viscera and none of the cerebral gray matter, *the presence or absence of the cerebral flush being the most reliable gross feature for differentiating the two infections.*

Pulmonary edema may be more often seen in fatal cases of *B. bigemina* infections, probably because the greater severity of anemia causes terminal left ventricular failure.

Babesia divergens and ***B. major*** are more or less restricted to northern and western Europe and Asiatic Russia and, for the most part, are of relatively minor importance to the cattle industries there, although significant outbreaks of disease and death may occur. Although *B. divergens* is small and resembles *B. bovis* in size, and *B. major* is as large as *B. bigemina*, the pathology and pathogenesis of the disease produced by these organisms is apparently very similar to that produced by *B. bigemina*. Thus there is intravascular hemolysis with little or no capillary agglutination of erythrocytes, and *death is due primarily to severe anemia*. Spontaneous splenic rupture has been reported in *B. major* infections, a complication rarely if ever reported in other bovine babesial infections.

Differential diagnosis of bovine babesial infections *in countries where more than one species of Babesia is present is important and at times difficult*, and may be complicated by dual infections and by the additional possibility of *Anaplasma* infection. *Babesia bovis* and *B. bigemina* may be distinguished from one another in *well-prepared blood smears* but erythrocytes parasitized by *B. bovis* are rare in jugular blood, or even in blood from deep skin punctures. They are best demonstrated in smears of blood expressed from a superficial skin scrape; this is most conveniently obtained from the *tailtip* in the live animal. *Babesia bigemina*-containing erythrocytes, on the other hand, are quite numerous in circulating blood while clinical signs are severe. The morphology of the parasites will usually be insufficiently preserved in postmortem material to allow distinction but the preference of *B. bovis* for capillaries of organs such as kidney, heart and brain will in most cases serve to distinguish the infections. It must be remembered that *Sarcocystis* spp. may be present in smears of myocardial blood. *Serological tests* are available for live animals and *direct fluorescent antibody techniques* may be used for differentiation in postmortem material. It is necessary to demonstrate a very heavy parasite burden in cerebral capillaries if brain smears are to be relied on as evidence of active *B. bovis* infection, as most clinically normal carriers of this organism can be shown to have some parasitized erythrocytes in this site.

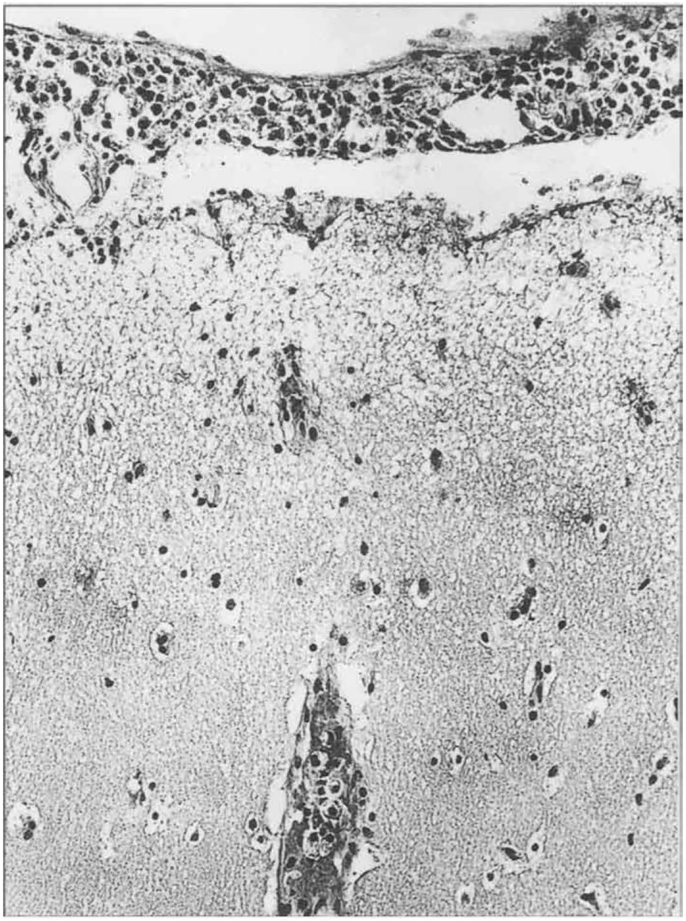
Figure 2.95 Sludging of red cells and meningeal infiltration in ***Babesia canis*** infection.

Blood smears should always be made from a peripheral site in a dead animal, particularly if fluorescent antibody techniques are available for diagnosis, because *such smears may be the only means of distinguishing an Anaplasma infection from one of the babesial infections.* Sterile immunity can be obtained with appropriately prepared antigens though these are not readily available.

Canine babesiosis

Babesia canis *is transmitted by different ticks in different countries*; it is not known if this is related to the apparent differences in virulence of the organism. The infection in dogs in Africa is severe and frequently causes death in all ages, whereas in Australia and in the United States the disease is relatively mild in mature animals. The disease is, however, *more severe in pups in all countries.* As distinct from bovine babesiosis, young dogs appear to be more susceptible than older animals.

Mildly affected animals develop *anemia and fever*, and are lethargic and have a poor appetite. Icterus and red urine are not seen and they recover after a clinical course of a few days. More severe cases show a wide variety of signs, including severe depression, drooling, vomiting, jaundice and hemoglobinuria, mucosal petechiae and congestion, ulcerative stomatitis, and angioneurotic edema of the head, legs and body. Anorexia and weight loss may be persistent. A few **dogs** may show nervous signs that are associated with erythrocyte sludging in cerebral capillaries and cellular infiltration of the meninges (Fig. 2.95). Acidosis is particularly important in this disease.

Grossly, there is staining of tissues both with bilirubin and hemoglobin. The kidneys are dark brown and there is copious thick bile in the gallbladder and splenomegaly. In addition, there is evidence of vascular injury in the form of hemorrhages and edema, which may be severe in the lung. Parasitized erythrocytes may be found plugging capillaries in smears and sections of cerebral cortex, reminiscent of *B. bovis* infection, but the phenomenon is never as severe as in that disease. *Disseminated intravascular coagulation* is a consistent occurrence in severe *B. canis* infection, and is presumably related to, and is likely to aggravate, the hemolysis and the vascular damage. Microthrombi can be demonstrated in many tissues.

Babesia canis and *B. bovis* infections clearly resemble one another in the severity of the syndromes and the involvement of pathogenetic mechanisms other than simple hemolysis.

Babesia gibsoni infection is apparently restricted to Asia, with the most severe losses in dogs imported into India. The pathogenesis of this infection seems to be analogous to that of *B. bigemina* in cattle, in that dogs may have heavy parasitemia and quite severe anemia without becoming very ill. The body temperature is not elevated as consistently as in the case of *B. canis* infection, and hemoglobinuria rarely if ever occurs. The pathogenesis of *B. gibsoni* infection appears to be related primarily to the intracellular destruction of erythrocytes, as the spleen becomes very large and there is no evidence of intravascular hemolysis. Death is due to anemia with PCVs dropping to less than 0.10 L/L.

Equine babesiosis

Babesiosis in horses is similar to that in dogs in that there are *two babesias of equids*, one larger than the other, but the larger (***B. caballi***) is usually considered less virulent than the smaller (***B. equi***). *Babesia caballi*, like *B. canis* and *B. bovis*, tends to accumulate in capillaries; thus these species have been referred to as "visceral," as distinct from *B. bigemina, B. equi*, and *B. gibsoni*, which are more evenly distributed throughout the circulation. There is, however, considerable overlap in virulence between all these species, all of which may cause death.

Babesia caballi infection is often mild and, if severe, the syndrome is characterized by fever, jaundice and anemia, but hemoglobinuria is unusual. *Babesia equi* infection produces severe anemia, often accompanied by hemoglobinuria, severe depression, lacrimation, and often ventral and periorbital edema and mucosal petechiae. Pelvic limb ataxia is not seen as frequently as in *B. caballi* infection. The lesions in both infections are very similar, and include generalized icterus, anemia and splenomegaly. More visceral edema and hemoglobinuric nephrosis is expected in *B. equi* infection. *Mixed infections are possible, and pathologic differentiation of equine babesiosis from equine infectious anemia may be difficult.*

Babesial infections of other species

These for the most part are mild or clinically inapparent, but severe reactions sometimes occur; the reasons for these are seldom obvious. The course of these diseases in general follows the pattern described for *B. bigemina* infection, in that there is quite severe parasitemia, anemia due to intravascular hemolysis, with hemoglobinuria in acute cases. At autopsy, there is splenomegaly and variably severe jaundice and, in some cases (notably *B. trautmanni* infection in pigs), edema and petechiae. *Babesia ovis* may cause cerebral congestion in sheep.

White-tailed deer may be infected by an organism resembling *B. divergens*, and mice may be infected with *B. rodhaini*, *B. hylomysci*, and *B. microti*. The latter infects other rodents as well. *Babesia hylomysci* causes severe hemoglobinuria and nephrosis.

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Cytauxzoonosis

An intraerythrocytic apicomplexan protozoal hemoparasite piroplasm classified in the family Theileriidae *infects wild ungulate species in Africa*, including the kudu, eland and giraffe, as well as *domestic and wild Felidae in North America*. Unlike *Babesia*, which only infects red cells of the mammalian host, *Cytauxzoon organisms have a schizogenous phase in intravascular macrophages as well as in red cells*. The natural mode of transmission is unknown, but *ixodid ticks are believed to be the most likely vectors*. The disease is not spread by cage contact but can be experimentally transmitted by fresh blood or by cryopreserved blood or tissue homogenates from infected animals.

The *clinical signs of cytauxzoonosis in domestic cats* include depression, anorexia, pyrexia, dehydration, pallor, icterus, dark urine and occasionally dyspnea. Affected cats are markedly febrile (40.9°C) in the early stages of the disease, and the hematologic picture is characterized by *pancytopenia with nonregenerative anemia*. In the early hemolytic stages of the disease, the urine is highly concentrated, acidic with high levels of protein and large amounts of bile and blood, including intact red cells and bilirubin crystals. Blood for detection of the intraerythrocytic parasites is best collected from peripheral tissues such as ear or tail. The intraerythrocytic organisms are similar in appearance to *Mycoplasma haemofelis* (*Haemobartonella*) and signet-ring forms are frequent. Rarely, multiple organisms may be present in the same red cell in a tetrad or Maltese cross configuration. Morphologically, *Cytauxzoon felis* (*C. felis*) cannot be reliably distinguished from *Babesia felis* by light microscopy. However, *C. felis* generates a strong and persistent antibody response with titers as high as 1:300 15 weeks after recovery, as measured by microfluorometric immunoassay. Effective immunity against apicomplexan hemoparasites requires a specific response by cytotoxic T cells. The disease is peracute with most animals dying within 1–2 days after onset of clinical signs. Treatment is largely supportive and recovery is slow, requiring several weeks for the hemoglobin to reach normal levels. In acute stages of the disease, there is prolongation of the clotting time as well as the prothombin time and partial thromboplastin time. These findings might be expected with rapid intravascular hemolysis releasing red cell stroma which is procoagulant.

While the disease is unusual, most reported cases have been fatal, with death occurring within a few days after the onset of clinical signs. The level of parasitemia is low and the piroplasms are commonly found in 1–4% of peripheral blood erythrocytes. While a much higher level of parasitemia may occur, in some reported cases no parasitized red cells were identified. *The sporadic appearance of the disease and fatal course suggest that domestic cats are an unusual host for the organism and that a reservoir host exists in the wildlife population.* The bobcat (*Lynx rufus rufus*) given blood from an infected cat develops persistent parasitemia without signs of clinical illness, suggesting that it may be the reservoir for the acute disease in domestic cats. Remarkably, there appear to be two strains of the organism which are indigenous to either the eastern or western bobcat. Cats are susceptible to both, and bobcats are susceptible to their non-indigenous strain. This likely explains why cats recovered from the bobcat transmission are still fully susceptible to other strains from domestic cats. **Diagnosis** *is dependent upon detection in red cells of the irregularly pear-shaped organisms.* They are often paired and are larger than *Mycoplasma haemofelis (Haemobartonella)* organisms. *There are apparently no pathognomonic gross or histological findings.*

Hepatozoonosis

Hepatozoonosis caused by the protozoan parasite ***Hepatozoon canis*** occurs worldwide in dogs and is seen in the southeastern and the southwestern United States. There are two species of *Hepatozoon* that affect dogs: *Hepatozoon canis* is spread by a variety of ticks, principally the brown dog tick, *Rhipicephalus sanguineus*; and ***Hepatozoon americanum*** is spread by the tick *Amblyomma maculatum*. Ticks ingest *gametocytes* in blood while feeding on an infected animal. Gametocytes sporulate in the tick, and *oocysts* containing 12 to 24 *sporozoites* are produced in the body cavities of the tick. When dogs ingest infected ticks, the sporozoites penetrate the intestinal epithelium and travel in blood to liver and other tissues where *intracellular cysts* are formed. *Merozoites* released from these cysts form *gamonts* in peripheral blood leukocytes and are spread to form *schizonts* in other areas of the body.

The prime presenting signs in the dog are *fever and depression*, characterized by mild anemia with extreme neutrophilic leukocytosis generally in the range of $100 \times 10^9/L$. The gamonts of *H. americanum* are larger than a red cell and egg-shaped, displacing the nucleus and relatively unstained in routine blood preparations. The level of parasitemia in leukocytes is highly variable, and *examination of buffy coat smears may be required to identify the pale "ice-blue" gamonts in the cytoplasm of monocytes or neutrophils.* The gamonts of *H. canis* are similar in appearance. Dogs with hepatozoonosis suffer *generalized myositis* with subsequent periostitis and generalized lymphadenopathy. The animals show signs of pain on palpation and movement and the schizonts may be identified in muscle; merozoites are present within histiocytes involved in areas of pyogranulomatous inflammation. The disease may become chronic with dogs serving as carriers of the infection. Commonly, the disease follows a chronic course without full recovery and death occurring in one to two years. There is currently no effective treatment.

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Anaplasmosis

There are three species of the genus ***Anaplasma***, order Rickettsiales: ***A. marginale*** and ***A. centrale*** are infectious for *cattle*; ***A. ovis*** infects *sheep and goats*. These parasites are now classified with the Rickettsiales notwithstanding that the disease produced, and its mode of transmission, have much in common with babesiosis. Division occurs by binary fission. The organisms are spherical or oval, from 0.3 to 1.0 μm in diameter, and situated in the erythrocytes (Fig. 2.96), usually near the margin (*A. marginale* and *A. ovis*).

Anaplasmosis in **cattle** is probably transmitted under field conditions by *Dermacentor* and other ticks, as well as by *Boophilus microplus*, and may be transmitted mechanically by blood-sucking flies. Mechanical transmission has resulted from the careless use of hypodermic needles during immunization procedures and bleeding, and by instruments used for dehorning, castration and ear tagging. The disease can be transmitted by blood from an infected animal, although in the chronic carrier state the level of parasitism is very low and organisms are difficult to find in the peripheral blood. Unlike *Babesia*, the intraerythrocytic antigens are relatively easily separated from red cells and form the basis of an *efficient diagnostic test based on complement fixation.*

Much of what has already been said of babesiosis is applicable to anaplasmosis. Young animals are susceptible to infection but relatively resistant to the disease; acquired infections in these are latent and endow the animals with resistance for the duration of the latent infection. *Carrier status and immunity are usual following recovery from infection.* This has epidemiological significance in that indigenous animals, wild ruminants as well as cattle, in enzootic areas acquire the infection early in life and, barring accidents, do not become diseased, but represent a source of infection for incoming animals and make eradication of the disease virtually impossible in many areas. Events that upset the balance between the organism and the host's immune system are not common, but include hemorrhage and especially other protozoan diseases that cause anemia. As in babesiosis, splenectomy is expected to lead to recrudescence. Acute disease can be regularly produced in splenectomized calves and infection appears to occur preferentially in older red cells.

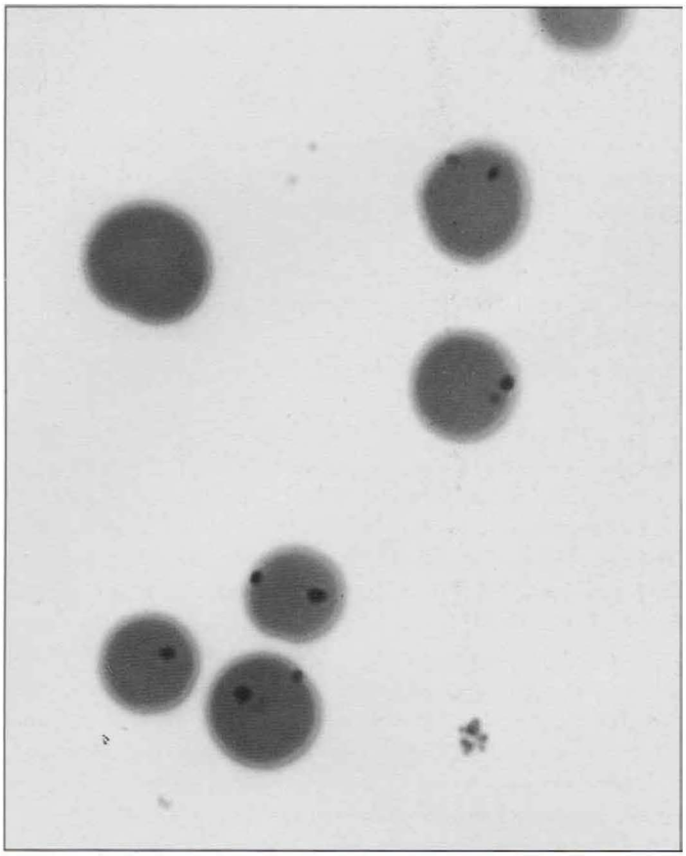

Figure 2.96 ***Anaplasma marginale*** in the peripheral blood of an ox.

The natural incubation period of anaplasmosis is 1–3 months or longer, and the disease which follows closely resembles babesiosis. One important distinction is that *hemoglobinuria does not occur in anaplasmosis* as the destruction of erythrocytes occurs intracellularly rather than intravascularly. While the mechanism of hemolysis is apparent, the means by which the phagocytic cells recognize injured or infected red cells is less clear. Serum factors sensitize erythrocytes to phagocytosis by the monocyte–macrophage system, and these opsonins increase in the circulation prior to the hemolytic crisis. Since the destruction of parasitized red cells seems to be selective, it is likely that the immune system identifies the erythrocytes containing parasites as abnormal and these are then removed by splenic and other macrophages. Spherocytosis may be prominent in the acute disease and is likely immune-mediated. Non-immune factors may be operative as well, and the decrease in plasma lipids with parasitemia may be related to the reduction in red cell numbers, and may contribute to that reduction by increasing membrane rigidity. Attempts to culture *Anaplasma* in vitro demonstrated that high magnesium levels were required to establish growth, and animals deficient in magnesium are refractory to clinical disease after experimental challenge. It appears that *Anaplasma* and other intracellular parasites require abundant ambient magnesium for their endogenous metabolism.

Animals with acute anaplasmosis have mucosal pallor, icterus, depression, and occasionally incoordination. The animals resist movement and have a rapid heart rate and increased respiratory rate. Urine may be deep brown but not red. *The clinical syndrome in cattle is usually subacute illness, with variable fever, anemia, weakness and ill-thrift.* The severity of the disease is related to the proportion of the erythrocyte mass destroyed, which may equal 70%.

At a variable period prior to the onset of clinical signs, usually 5–10 days, the *Anaplasma* organisms appear in red cells in the peripheral blood. Since the organisms continue to appear and increase in number, it is apparent that in the acute phase the parasitic multiplication proceeds more rapidly than removal of parasitized red cells in the monocyte–macrophage system. The erythrocyte count in acute disease drops to as low as 1×10^{12}/L and the hemoglobin to 30–40 g/L. The hematocrit tends to stabilize at 0.10–0.15 L/L, at which time there is rapid ongoing destruction with a competent marrow response resulting in a macrocytic, mildly hypochromic, anemia. At this time, as many as 50–60% of the red cells may be parasitized. *The strong erythroid response to anaplasmosis, which does not occur in babesiosis, is possibly due to the intracellular rather than intravascular destruction of erythrocytes*, thereby avoiding hemoglobinuria and allowing recycling of iron and proteins. Characteristically, anemia is accompanied by neutrophilic leukocytosis. Chronicity results in apparent recovery or chronic disease with debility and nonresponsive normochromic normocytic anemia in which the *Anaplasma* bodies are rarely observed in the peripheral blood. Biochemically, bilirubin, most of it unconjugated, is elevated in the acute disease.

There are no pathognomonic gross lesions of anaplasmosis. There is pallor of all tissues and mild icterus, with relatively good body condition in those animals dying acutely and cachexia in chronic cases. The lungs are pale and discolored and may have bullous emphysema if there has been severe terminal dyspnea. There are frequently ecchymotic hemorrhages on the epicardium, and the heart is flabby and dilated. The liver is anemic and icteric and the gallbladder is usually distended. The spleen is enlarged, turgid and congested in acute cases and firm, dark red and fleshy in chronic ones. The enteric tract is unremarkable and the bladder may contain deeply bilirubin-stained urine. The marrow cavity is variably expanded, depending on the stage of the disease, and may have serous atrophy with chronicity.

The tissue changes in anaplasmosis are similar to those in babesiosis, although hemosiderosis is much more marked in anaplasmosis.

Anaplasma centrale produces a natural infection of cattle, but is also employed, being a mild pathogen, as an immunizing agent against *A. marginale* in some areas of endemic infection. It usually produces a mild disease, although sometimes it can be severe, with fever and anemia but no icterus. *Anaplasma centrale*, too, causes a persistent infection.

Anaplasmosis in **sheep and goats** is caused by ***A. ovis***. Cattle are not susceptible to this parasite. On the other hand, sheep and goats develop a latent infection if inoculated with *A. marginale*. In the Mediterranean region, anaplasmosis occurs as a natural disease in sheep and goats, and is characterized by *progressive anemia and emaciation*, but with a relatively low mortality rate. In other regions in which the infection occurs, especially the United States and South Africa, the disease is milder unless the resistance of the animal is lowered by intercurrent disease, helminthiasis or inadequate nutrition. It is possible, however, that in endemic areas, ovine anaplasmosis of subclinical severity is responsible for unthriftiness of economic importance. Infected sheep develop complement-fixing antibodies.

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Mycoplasma (Eperythrozoon) suis

Parasites of the genus *Mycoplasma* (family Mycoplasmataceae, class Mollicutes) are *found on the surface of erythrocytes and occasionally free in the plasma*, rather than within the cells, and thus differ from *Anaplasma*. This location of the parasites is typical of the Mycoplasmataceae (Haemobartonellae), but *Mycoplasma* (*Eperythrozoon*) *suis* is distinguished by being predominantly coccoid with multiple organisms in a single red cell and irregularly arranged over the concavity, unlike the Mycoplasmataceae which tend to have coccoid and rod-shaped forms which typically appear in chains around the red cell periphery. This distinction is somewhat artificial since the round bodies of *Mycoplasma suis* when viewed on the periphery of the red cell membrane also appear rod-like. The ring-shaped structures frequently observed in Romanovsky-stained blood films are apparently artefactual alterations.

"*Eperythrozoonosis*" is, among domestic animals, *a disease of sheep, cattle, swine, and llamas, which is characterized by anemia and icterus*. Hemoglobinuria does not occur, and in **sheep** the red cells in clinical disease are Coombs-positive, which *suggests that the anemia is due to intracellular destruction of parasitized erythrocytes*. The disease in sheep has a seasonal incidence which suggests that infection may be associated with changes in the population of *Melophagus* and other blood-sucking insects. Infection can be transmitted artificially by inoculation of blood from a carrier animal, and clinical disease is most easily established in splenectomized recipients.

Mycoplasma (Eperythrozoon) ovis is parasitic in sheep in the form of ring-shaped, bacillary or coccobacillary forms, from 0.3 to 1.0 μm in diameter, attached to the surface of erythrocytes. Organisms are found free in the plasma, probably being dislodged from the erythrocytes during preparation of the smear. These parasites can cause disease in nonsplenectomized sheep, and relapses occur following splenectomy of carrier animals. The incubation period of the disease is approximately a week, after which parasitemia develops. The parasites multiply rapidly and in the course of a week far outnumber the erythrocytes, after which their relative numbers decline as anemia develops. In severe infections, icterus occurs. The mortality rate is insignificant, but repeated relapses can occur and can be induced by intercurrent disease. The erythrocyte count may drop to 1×10^{12}/L and there is concurrent monocytosis with lymphocyte counts up to 20×10^{9}/L.

Although *M. ovis* is widespread and ordinarily insignificant as a pathogen, a 10% mortality rate has been reported in naturally infected lambs that died with profound anemia. This suggests that the infection, either alone or as a complicating disease, may be responsible for more widespread anemia and unthriftiness than is usually recognized. Summer epizootics of the disease without mortality have been observed in which anemia was profound and affected animals developed edema of the head while grazing by day.

The pathological picture in clinical or fatal ovine eperythrozoonosis is principally that of anemia and serous effusions, in some cases with modest icterus. The anemia is hemolytic and, while hemoglobinuria is of unusual occurrence, bilirubin levels may be increased several-fold. The bone marrow has subendosteal reddening and shows evidence of regeneration. The kidneys may appear normal or be the blue color associated with hemoglobin, or more usually a rusty brown color that is due to large deposits of hemosiderin iron in the proximal tubules. The spleen is enlarged and soft and the enlargement may persist for some time after clinical recovery. There is lymphoid hyperplasia in the Malpighian corpuscles which are very prominent to the naked eye.

Mycoplasma (Eperythrozoon) wenyonii is the **bovine** parasite. It is of wide geographical distribution, and in most species resembles *M. ovis* but is host specific. It is not an important infection, and its potential for causing anemia and icterus is realized only during severe intercurrent disease and after splenectomy. The disease was believed to occur in outbreak form in first-calf dairy heifers following calving, and was characterized by edema of the teats and hind limbs believed due to vasculitis. *Eperythrozoon teganodes* is also a parasite of cattle. It is not pathogenic and is distinctive in that it is confined to plasma and not attached to red cells.

Mycoplasma suis and *E. parvum* are immunologically distinct, host-specific species in **swine**. The species *E. parvum* is usually coccoid with occasional ring forms less than 0.5 μm in diameter, and is benign. The natural disease in swine, which has been known as "icteroanemia," is caused by *M. suis*, an organism usually ring-shaped and about 0.8 μm in diameter, although in acute disease it may be up to 2.5 μm in diameter. Both parasites are enzootic in South Africa and the United States and probably elsewhere. The mode of natural transmission is not known, but *an arthropod is probably implicated*; the highest incidence of infection occurs during the summer months.

The morbidity in porcine haemotrophic *Mycoplasma* infection (eperythrozoonosis) is low, and pigs that acquire the infection remain as carriers. The disease is characterized by fever and anemia, which may be profound and in cold climates there may be necrosis of extremities. Icterus occurs only in the most severe cases. The usual manifestation of porcine hemotrophic *Mycoplasma* infection is the sudden death of a few pigs in a herd. Grossly, there is icterus, watery blood, serous effusions, and enlargement of the spleen. In the examination of smears of blood, precipitated stain should not be confused with the parasites. *The disease in all species is diagnosed by finding the organism in blood in conjunction with anemia, by serology, and by PCR techniques.*

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Hemotrophic *Mycoplasma* infection (hemobartonellosis)

The genus formerly known as *Haemobartonella* is closely related to the genus formerly called *Eperythrozoon* with nucleotide sequence data indicating that these organisms are part of the family Mycoplasmataceae, class Mollicutes, genus *Mycoplasma*. The organisms are attached to the surface of erythrocytes, principally as minute coccoid or bacillary forms that stain blue with Giemsa stain.

Hemotrophic mycoplasma are found in cattle, goats, cats and dogs, and in each species they are capable of causing hemolytic anemia. Geographically they are widespread, but they are unlikely to be seen unless looked for specifically. ***Mycoplasma haemocanis*** (*Haemobartonella canis*) is an opportunistic agent in **dogs** that is capable of causing hemolytic anemia in splenectomized animals, or those immunosuppressed by therapy or other disease. The sequence of the 16S ribosomal RNA gene of the dog appears to be almost completely similar to that of *M. haemofelis* permitting a PCR assay to be developed for detection of infection of hemotrophic *Mycoplasma* infection (hemobartonellosis) in both cats and dogs.

The type species is *candidatus M. haemomuris*, an ubiquitous parasite of the rat transmitted by lice. Rats harbor latent infections that can be activated by splenectomy, hemolytic poisons and intercurrent trypanosomiasis; anemia and parasitemia then develop rapidly and can be fatal. *M. haemocanis* of dogs has not been shown to have pathogenic significance, except that latent infections can be activated by splenectomy. Susceptible dogs develop acute hemolytic anemia that resembles idiopathic immune-mediated hemolytic anemia except for the presence of the organisms. ***Haemobartonella bovis*** of **cattle** is of no economic significance, but hemobartonellosis may complicate research on *Babesia* or *Anaplasma* where splenectomy has been carried out and dual infections occur. ***M. haemofelis*** is responsible for the natural disease "*feline infectious anemia*." The organism is ubiquitous, but the uncomplicated disease is unusual. Most often some other disease process has caused a reduction in resistance of the animal, and hemotrophic *Mycoplasma* is an opportunistic infection. One of the most common causes of reduced resistance in the cat is an occult lymphoma.

Hemotrophic *Mycoplasma* infection is presumably spread by fleas and other biting insects and is an important infection in **cats**. Males are at twice the risk of females of developing the clinical disease, and the risk of infection increases with age and in the spring. Direct contact is thought to be important in transmission. The anemia in cats may be Coombs-positive, and it is likely that either an antiparasitic antibody cross-reacts with normal red cell antigens, or parasitic antigen adsorbs to red cells and sensitizes them to the phagocytic system and selective removal by the spleen and marrow macrophages. The antibody in experimentally infected cats is of the cold-reacting type, which could be a factor in the development of clinical disease, since cats if permitted are regularly nocturnal. The red cell lifespan is halved in clinical disease.

The most common signs in cats are *lethargy and anorexia* for a short period of time. Often there is some other history of stress including transport or trauma resulting in sepsis. The *anemia is severe* and appears suddenly; the erythrocyte count may drop as low as 2×10^9/L with hemoglobin levels of 20–60 g/L and hematocrit of 0.06–0.23 L/L. Since the disease is characteristically secondary to other processes, the response to the anemia is highly variable, but in the rare uncomplicated case, the anemia is responsive and macrocytic and mildly hypochromic. **Diagnosis** *is dependent on identification of the rod- and ring-shaped organisms that are characteristically arranged in chains around the periphery of the erythrocyte membrane* (Fig. 2.97). The leukocyte count is

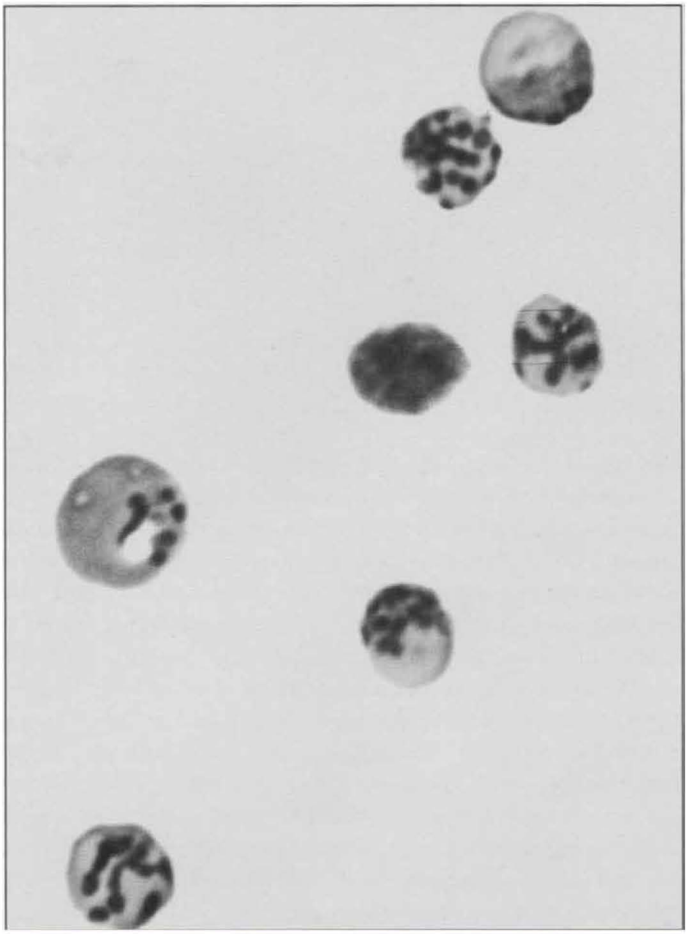

Figure 2.97 Hemotrophic *Mycoplasma haemofelis* (*Haemobartonella felis*) infection in the peripheral blood of a cat.

highly variable and there may be leukopenia due to marrow failure, or neutrophilic leukocytosis due to intercurrent sepsis, or leukocytosis of leukemia. Icterus is inconstant and generally mild. Since hemolysis is largely intracellular and not intravascular, the urine may be dark if the animal is icteric but hemoglobinuria is not a feature of the disease and the retention of iron and proteins resulting from hemolysis contributes to a degenerative response by the bone marrow in cats that are otherwise in good health.

The gross examination is dominated by pallor with or without mild icterus, and usually splenomegaly and mild generalized lymphoid hypertrophy. There is usually reddening of the femoral marrow. Histologically there is splenic sinus hyperplasia and moderate generalized follicular hyperplasia. In uncomplicated cases, the marrow is hypercellular with an erythroid shift. Not infrequently, the marrow and nodes are involved with a primary myeloproliferative or lymphoproliferative disorder that has reduced the efficiency of the monocyte–macrophage system in controlling the level of parasitism.

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Ehrlichiosis

Ehrlichieae of the family Rickettsiaeceae includes three species, ***Ehrlichia canis*, *E. bovis*** and ***E. ovina***, which produce disease in dogs, cattle and sheep respectively. A similar agent has been described (***E. equi***) which causes disease in the horse. The agent infecting the horse causes cytoplasmic morulae in granulocytes, whereas that infecting the dog causes inclusions in lymphocytes and monocytes. It appears that the equine agent occasionally also causes disease in the dog. A tick-borne anemia of dogs caused by *Rickettsia canis* has been known for many years. Subsequently a disease which occurred among introduced breeds in Southeast Asia became known as **canine tropical pancytopenia**. Unlike the disease in horses, which is self-limiting, the disease in dogs is characterized by fatal or protracted course in dogs of all ages. Although included here with the infectious hemolytic diseases, *canine ehrlichiosis when fully expressed is a symptomatic aplastic pancytopenia* with identification of the leukocyte inclusions giving specificity to the disease syndrome.

Ehrlichiosis in German Shepherd **Dogs** often produces a *severe hemorrhagic syndrome* that is less common in other breeds and in experimental infections. The disease is characterized by an incubation period of 10–20 days followed by a febrile phase with variable pyrexia, anorexia, weight loss, depression and weakness. Some animals die at this acute stage, but most enter a subclinical stage that is of variable duration but generally ends in about 90 days with either epistaxis and generalized hemorrhage or pancytopenia with progressive renal failure.

Typically, the most severe hematologic changes occur in platelets which are depleted by about the tenth day, with slow recovery over the next 30 days then fall progressively over 2 months or so as marrow aplasia develops. The erythrocyte numbers parallel proportionately with the changes in thrombocytes. The leukocyte count fluctuates until 80–90 days postinfection when there is *progressive and severe panleukopenia*. The anemia is characterized by moderate anisocytosis and polychromasia, with increasing poikilocytosis and loss of polychromasia as marrow failure progresses. There is marked variation of platelet size following the first attack of thrombocytopenia, but signs of production decrease terminally. The neutrophils and lymphocytes, though markedly decreased, retain relatively normal proportions and there is usually eosinopenia and some degree of monocytosis. **Diagnosis** *is based on hematological changes and the presence of the rickettsial inclusion bodies in the cytoplasm of lymphocytes and monocytes.* Typically the morulae of *E. canis* appear rounded and one-third to one-half the diameter of a red cell, with a granular internal structure consisting of basophilic elementary bodies each 0.5–1.0 μm in diameter. In the dog, the inclusions are typically in the cytoplasm of lymphocytes and monocytes, and a predominance of inclusions in the neutrophils associated with a milder disease syndrome suggests infection with *E. equi*. There is an increased erythrocyte sedimentation rate throughout the disease, progressive reduction in serum albumin with increased gamma globulin occasionally with polyclonal gammopathy and a much prolonged bleeding time. The late onset of marrow aplasia that becomes limiting as the globulin level peaks suggests that the marrow inhibition may have an immune rather than a direct infectious origin.

Grossly, there is *emaciation with subcutaneous and interstitial edema.* A consistent finding is epistaxis with petechial hemorrhages on gingiva and conjunctiva. Large subcutaneous hemorrhages are present and there are hemorrhages on the serosa and mucosa of the intestinal tract and on the bladder mucosa. There is mesenteric lymphadenopathy with irregular enlargement of other nodes and medullary discoloration due to hemorrhage. The liver is of normal size and consistency with pallor, focal areas of hemorrhage and an anemic periacinar pattern. The spleen is of normal size, or slightly enlarged and firm with congestion and obscured splenic corpuscles. Typically hemorrhages are found on the surface of the heart and in the myocardium, and the lungs are edematous and mottled, often with focal areas of hemorrhage associated with sepsis. *The* **postmortem diagnosis** *is best made by demonstration of morulae in the cytoplasm of pulmonary macrophages obtained from direct imprints of the cut surface of fresh lung.* There are focal subcapsular hemorrhages on the kidney with mild yellowing of the medulla.

Histologically, there is lymphoplasmacytic cuffing of veins in the central nervous system and around many vessels in other organs. The liver has panlobular atrophy of cords with sinusoidal dilation, focal plasmacytic cuffing in portal areas, and ischemic periacinar degeneration. There is mild alveolar septal thickening with hypercellularity and mononuclear cuffing around pulmonary vessels. In lymph nodes, the germinal centers are depleted, the paracortical areas are atrophic and there is marked medullary cord plasmacytosis and sinus histiocytosis, often with prominent erythrophagocytosis. Glomeruli are often ringed with aggregates of lymphocytes and plasma cells, and there are focal interstitial infiltrates of mononuclear cells. The bone marrow is hyperplastic early in the disease, with few fat cells and dense cellular packing, and aplastic terminally with dilated sinusoids and foci of hemorrhagic infarction. The disease in the dog has been called *radiomimetic* because of the prominent destruction of proliferating cell populations. The prominent plasmacytosis that persists in many organs should, however, alert the diagnostician that these lesions are more inflammatory in nature than those associated with idiopathic or drug-induced marrow aplasia.

Ehrlichiosis in **horses** is similar to that in dogs but less severe. In experimental disease, the incubation period varies from 1–9 days with the onset characterized by pyrexia, depression and complete anorexia. Edema of the ventral midline, limbs and prepuce persists for 1–2 weeks. There is leukopenia, thrombocytopenia, elevated plasma bilirubin and granular inclusion bodies, which are circular and 2–3 μm in diameter, in the cytoplasm of the neutrophils. The inclusions in granulocytes parallel the febrile period and are maximal on the third to fourth day and present for up to 15 days after infection. A rise in lymphocyte numbers heralds recovery, and a rise in platelets precedes the disappearance of ventral edema.

Gross lesions include petechial hemorrhage, ecchymoses and edema, particularly in subcutaneous tissues and on the ventral abdominal wall. There is often increased fluid in serous cavities as well as icterus, emaciation, and secondary bacterial infections, including bronchopneumonia, arthritis, lymphadenitis and cellulitis. Histologically there is vasculitis in the areas of edema and hemorrhage.

Potomac horse fever caused by *Ehrlichia risticii* is described in Vol. 1, Alimentary system.

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Anemias of vascular parasitism: trypanosomiasis

Trypanosomes are unicellular organisms, uniform in type but varying in morphologic details of taxonomic importance. Some species are morphologically stable while others are polymorphic, and the differentiation of species requires the familiarity of long acquaintance.

The important trypanosomes to human and animal health are *transmitted by arthropods* that carry the metacyclic stages in either the oral or anal tracts. Thus, ***Trypanosoma cruzi***, the cause of *Chagas' disease* in humans and animals in South America, is known as a *stercorarian parasite* because it is spread by passage in the feces of the reduviid insects (*Triatominae* spp.); after the insects bite (often around the mouth or eye), they defecate and the trypanosomes gain entry to the host through the bite wound, often assisted by the host scratching. The important African trypanosomiases of animals (***T. congolense***, ***T. vivax***, ***T. brucei brucei***) and of humans (*T. brucei rhodesiense* and *T. brucei gambiense*) are called *salivarian parasites* because of their transmission through the salivary glands of the tsetse fly. Other biting and sucking insects, such as tabanids, stable flies or fleas, and vampire bats may transmit trypanosomes mechanically. Infection of the definitive host, the tsetse fly, is probably lifelong. Some researchers feel that the disease resulting from fly transmission is more typical of field cases than that resulting from trypanosomes delivered by hypodermic needle.

Trypanosoma equiperdum, the cause of dourine, does not require a vector host but is transmissible by direct contact with mucous membranes. It is described in Vol. 3, Female genital system.

Not all species of *Trypanosoma* of mammals are pathogenic, and among the common nonpathogenic species of veterinary interest are ***T. melophagium***, ubiquitous in sheep and transmitted by the ked, *Melophagus ovinus*; ***T. theileri***, which is widespread in cattle and transmitted by several types of biting flies, including Tabanidae; ***T. theodori***, which occurs in goats in the Middle East, transmitted by the hippoboscid fly, *Lipoptena caprina*, and probably synonymous with *T. melophagium*; and ***T. lewisi*** of rats, which is of much interest as a convenient subject for research on the genus. ***Trypanosoma rangeli*** of South American cats, dogs and humans is also apparently nonpathogenic to the vertebrate hosts but differs from the above in that it can be transmitted by inoculation or contamination, the vector being the triatomine, *Rhodnius prolixus*. The species of trypanosomes listed above, although widespread in their hosts, are ordinarily sparse in blood and seldom observed unless specifically looked for in thick smears, after splenectomy, or by artificial culture. Occasionally, however, *T. melophagium* and *T. theileri* are found in very large numbers in the blood of cattle and sheep suffering from a primary and usually an immunosuppressive disease. It is not clear whether or not *T. theileri* may become pathogenic in the compromised host; however, its increase in number in association with other diseases suggests that it may be of secondary importance.

In endemic areas, the incidence of infection by pathogenic species varies depending on such factors as the availability of mammalian reservoirs, the density of vector tsetse flies, and the systems of husbandry adopted, but, *as a rule, domestic animals and trypanosomes cannot coexist.* Trypanosomiasis prohibits domestication of livestock in perhaps one-quarter of the total land surface of Africa. Knowledge of host–parasite relationships is not fully understood, either for the vector or the mammalian hosts. Within a species of the organism, local strains may be distinct in their basic and predominant antigens, but within the host in the course of chronic infections, a series of *genetically regulated antigenic variants* appears in succession so that the animal exhausts itself without clearing the infection. Successive waves of parasite and antibody dominance account for the characteristic recrudescences of the infection and for the known difficulty in producing an effective vaccine.

The pathogenic trypanosomes differ widely in virulence and are apparently nonpathogenic in mammalian reservoir hosts. Most of them are parasites of a variety of domestic and wild animals, in some of which they produce fulminating disease, in some chronic disease, and in others mild or unapparent infections. Strains within a species of the parasite also differ greatly in virulence, some strains producing rapidly fatal infections, some producing chronic infections with premunition, and some allowing complete recovery and acquired immunity. Much of the knowledge of trypanosomiasis has been gained by studies of *T. brucei* which conveniently infects dogs, rabbits, rats and mice and poses little problem of dissemination in developed countries where it has served as a research model. In terms of the overall problem, *T. brucei* is not nearly as important as *T. vivax* and *T. congolense* and the pathogenic trypanosomes of humans. Of the latter organisms, only *T. congolense* is studied with any frequency outside their natural habitats because the other trypanosomes are thought to constitute too great a hazard of dissemination even under conditions of laboratory restraint. Most of the research on *T. vivax* and *T. congolense* has been directed at defining the disease in British breeds of cattle. Efforts are now being made to understand the host–parasite relationship in indigenous species of animals, such as the Thomson's gazelle, wildebeest, cape buffalo and eland, which are heavily exposed and apparently carry the organisms, but rarely if ever have clinical disease.

The components of pathogenicity of trypanosomes are largely unknown. Their effects vary with their tissue tropism. The edema and interstitial reactions caused by *T. brucei* are due to the fact that this parasite resides in perivascular tissues, in contrast to the more serious pathogens of humans and animals which are obligate intravascular parasites. In many respects, bovine trypanosomiasis is similar clinically and pathogenetically to equine infectious anemia in that infection is followed by an asymptomatic period until antibody develops. There is then anemia and generalized compensatory hyperplasia of the mononuclear phagocyte system, which ultimately results in cachexia and hematopoietic and lymphoid exhaustion.

Bovine trypanosomiasis

In cattle, parasitemia regularly occurs a week after inoculation with 10^5 or more organisms that have been passed in rats and column-separated. By the second week of infection, there is a sharp drop in the red cell count and hemoglobin levels, accompanied by an erythroid shift and macrocytosis, *anemia being the predominant aspect of the disease.* There is *concurrent thrombocytopenia* of moderate degree and *hypocomplementemia*, with irregular appearance of immunoglobulin and complement on red cell membranes. Fibrinogen is reduced to about half of normal levels, and there is irregular appearance of fibrin split-products as animals go through febrile periods. Kinetically, the red cell lifespan is reduced to a half or less of normal, and iron utilization is initially rapidly increased, but slows down with cachexia and inhibition of the erythroid response due to inflammatory changes. The platelet lifespan is not shortened in infections with *Trypanosoma congolense*, although it may be with *T. vivax*. In the former, the truncation appears to be due to a proliferation of marrow megakaryocytes that are impaired in their maturation and produce fewer platelets that survive normally, thus constituting an ineffective response. The disease tends to stabilize at about 4–6 weeks in well-fed animals under laboratory conditions, and to proceed to death due to secondary causes in animals that are forced to forage for feed as well as maintain their immune defenses.

Red cells are destroyed in an "innocent bystander" fashion by adsorbing toxic and metabolic products of the trypanosomes, as well as suffering direct injury from a parasite-derived phospholipase. *There is competition between the stem cells for erythroid and myeloid proliferation in in vitro studies, which may explain the clinical finding that animals with severe anemia are more susceptible to secondary infection by bacteria and viruses.* A dilutional component to the anemia has been suggested, but no increase in blood volume has been detected. Calves infected during the first week of life have less severe hematologic disease than calves infected at 6 months or older. Some of these animals become cachectic and die, although it appears that infection at an early age results in a more tolerant host–parasite relationship that is less injurious to the host. *Trypanosoma vivax* and *T. congolense* differ in that *T. vivax* tends to circulate relatively freely and uniformly in the vascular system, and, in this respect, the level of parasitemia gives a reasonable estimate of the total parasitic burden. In contrast, *T. congolense* "homes" to the microvasculature of the brain and skeletal muscle, and the paucity of organisms in the peripheral blood makes diagnosis and estimates of total burden difficult. It appears that the tissue tropism of *T. congolense* is a response to developing immunity and is antibody-mediated. It is not known why brain and muscle are selectively parasitized, but the phenomenon may be related to trypanosomal energy requirements, since the endothelial cells in these areas tend to have more mitochondria than in other tissues.

Trypanosoma vivax is more virulent than T. congolense. Whereas both cause anemia and cachexia, infection with *T. congolense* regularly results in chronic disease, while in a high percentage of animals infected with *T. vivax* there is sudden death due to salmonellosis or other infections. Infected animals are moderately stunted in growth although this effect is less apparent on body weight than on dressed weight (the part of the carcass that is edible). This disparity is due to the fact that infected animals pool fluid in the intestinal tract and become pot-bellied while failing to deposit muscle and fat. When viewed from behind, infected animals have poorly covered bony prominences, poor "spring of rib," and a deep and pendulous abdomen. The skin is scurfy and rough, particularly about the head, ears and hind quarters. There is chronic low-grade pyrexia with intermittent temperature increases, and periodic watery diarrhea with passage of poorly digested ingesta of normal color. There is irregular oculonasal discharge, but no loss of appetite and the animals continue to eat. There is mild mucosal pallor without petechial hemorrhages, and the urine may be dark but never blood-tinged. The heart and respiratory rates are not remarkably altered under laboratory conditions but are increased in nonspecific response to anemia under field conditions, and animals may show open-mouth breathing on forced exercise. In the absence of secondary infection, there is little or no depression or abnormal behavior despite high levels of cerebral parasitism (Fig. 2.98A).

Anemia is of moderate degree, generally between 50 and 80 g/L, which is initially macrocytic and normochromic, and later becomes normochromic, normocytic and poorly responsive (Fig. 2.98B). In the well-developed disease, there are always some hypochromic red cells and at least mild poikilocytosis. In the early stages of infection, the radioiron uptake time is reduced from a normal of about 3 hours to about half that figure but in chronic disease is only mildly reduced. There is increased osmotic fragility of red cells without spherocytosis. The platelets are 100–200 × 10^9/L and are often lower during intercurrent infection. The leukocyte count is reduced to about half of normal (5–6 × 10^9/L) due to an absolute reduction in both neutrophils and lymphocytes. Changes are less severe in neonatally infected animals. Biochemical changes consist of a reduction in total protein, largely due to reduced albumin, but there are no consistent changes in transaminases or bilirubin. There is a consistent reduction in total serum lipids, cholesterol and triglyceride, and erythrocyte phospholipid levels are consistently elevated which may indicate that there is an acquired injury to red cell membranes which contributes to shortened lifespan. Aspirated marrow is hypercellular with an erythroid shift that drops the myeloid:erythroid ratio to 0.4 or lower in animals infected with *T. congolense*. There is a less marked erythroid response in *T. vivax* infection.

There are no pathognomonic **gross lesions** *of trypanosomiasis*. Chronically affected animals are cachectic and have a rough haircoat, and there is increased clear fluid in body cavities. There is generalized lymph node enlargement to 2–4 times normal, and the hemal nodes become prominent in subcutaneous areas and in association with the para-aortic and pelvic nodes where they may reach 1 cm in diameter or larger. The lungs are heavy and have increased density on palpation, and may show intercurrent cranioventral bronchopneumonia. The heart is generally flabby, with serous atrophy of pericardial fat, and there may be white foci 1–2 mm in diameter on the epicardium and on the cut surface. The liver and kidneys are symmetrically enlarged and constitute a greater than normal percentage of body weight. The liver is unusually firm and not easily penetrated by digital pressure, and there is a fine lobular pattern visible through the capsule and on cut surface. Renal medullary fat has serous atrophy. There are fine focal, raised, reddened areas 1–2 mm in diameter throughout both layers of the omentum. The enteric tract is unremarkable, except that the small intestine is atonic and contains an excessive amount of normal-appearing fluid content. *The most remarkable*

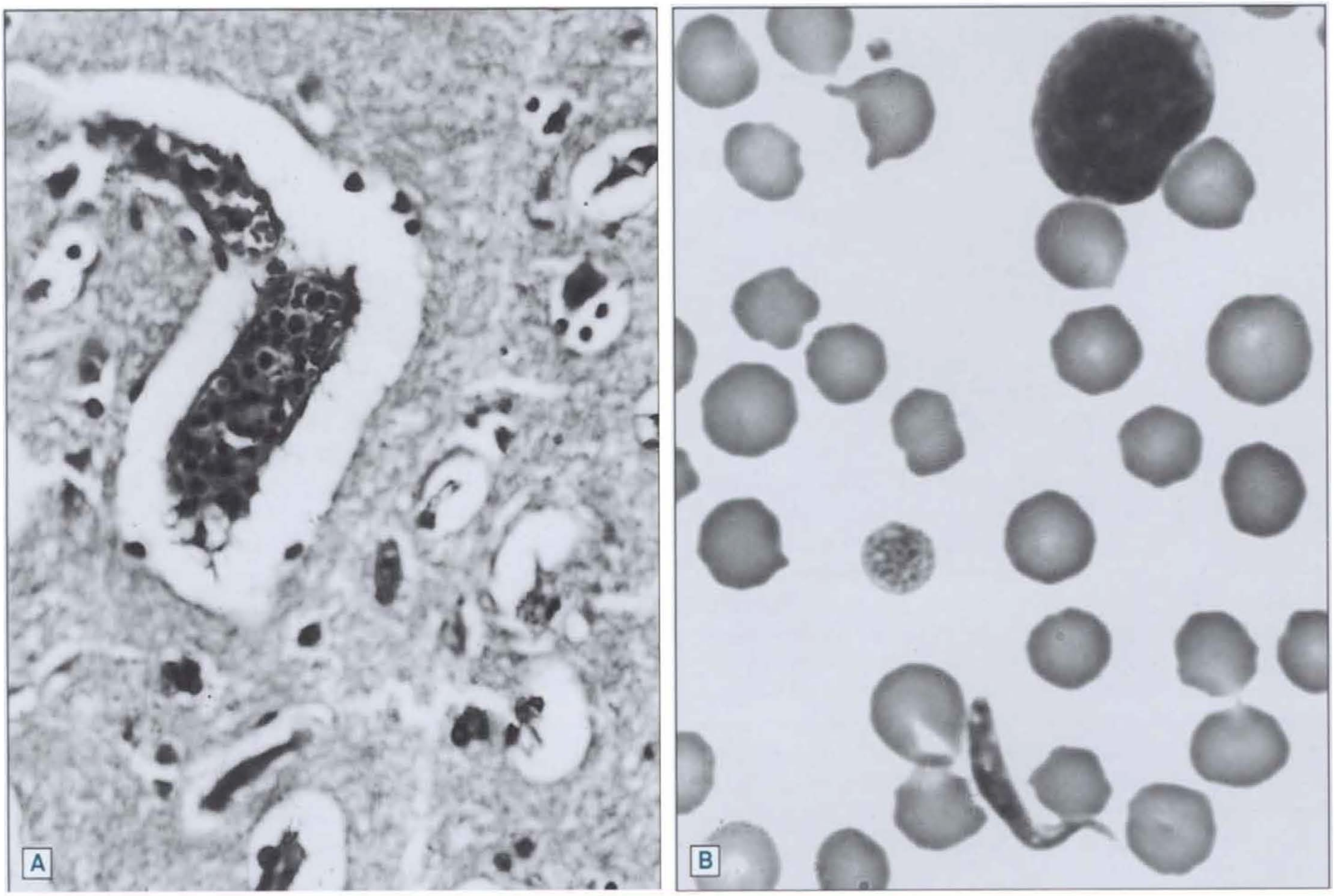

Figure 2.98 Trypanosomiasis in an ox. **A.** Microvascular thrombosis and early poliomalacia of the cerebrum. **B.** Moderate poorly responsive anemia, poikilocytosis and paucity of platelets, and a trypanosome, in peripheral blood.

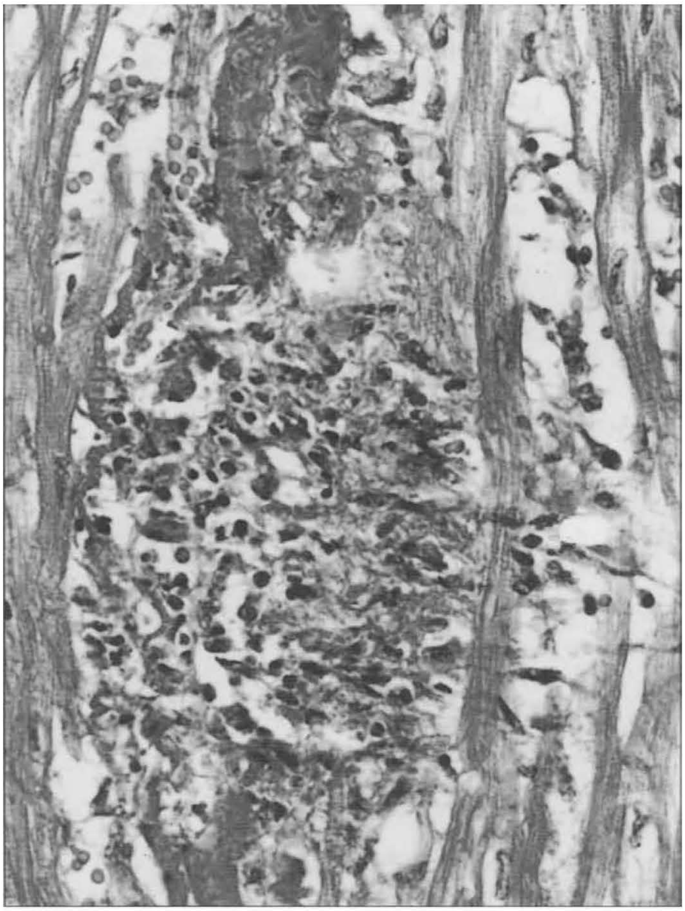

Figure 2.99 Trypanosomiasis in an ox. Lysis of myocardial fibers and myocarditis.

changes occur in lymph nodes, which have generalized medullary pigmentation, *and in bone marrow* where there is a regular increase in hematopoietic areas, which may occupy all of the cancellous and diaphyseal fatty areas in animals which have been infected 6 months or more. The spleen is uniformly enlarged and bulges, but does not ooze blood, on cut surface. The Malpighian corpuscles are generally visible grossly.

Microscopically, there is a generalized increase in septal width in the *lungs* and accumulation of intravascular hemosiderin-bearing macrophages. There are multifocal areas of fiber atrophy with sclerosis and lymphoplasmacytic proliferation in the *myocardium* (Fig. 2.99), most prominently in those animals which have succumbed to the disease. The *bone marrow* goes through a cycle of changes similar to those in equine infectious anemia. There is early conversion of fatty to hematopoietic marrow with high cell density, erythroid shift and plasmacytosis, followed by a reduction in cellular packing and dilation of sinusoids, and ultimately by reduction in hematopoiesis and serous atrophy of the remaining tissue. The rubricytes are increased in number and are relatively synchronous in maturation with numerous mature cells, suggesting late asynchrony associated with poor availability of iron. There is a variable degree of hemosiderosis, depending on the duration of the disease, and erythrophagocytosis may be observed in the marrow. Marrow granulocyte reserves are reduced, and, as a result, there is mild early asynchrony, although it is likely that with the expanded volume of hematopoeitic marrow there is a normal volume of granulopoietic tissue. Megakaryocytes are increased in number to about twice the normal level, and the nuclei are increased in diameter, but the cytoplasmic volume is reduced, indicating ineffective thrombopoiesis. There is a mild generalized increase in endothelial and true reticular cells in bone marrow, but myelofibrosis is not severe. Trypanosomes may be found free and phagocytosed in small vessels throughout the body, but are most commonly observed in the liver and cerebral cortex. *Hepatic changes* are constant, and consist of atrophy of hepatocellular cords with sinusoidal dilation and periportal lymphoplasmacytic proliferation. There is increased interstitial stroma, without change in lobular organization, and generalized Kupffer cell hyperplasia.

Changes in the lymphoid tissue are remarkable and constant in all areas of the body. There is first follicular hyperplasia (Fig. 2.100A), with a competent response and the formation of large and densely cellular germinal centers. These changes are accompanied by variable paracortical hyperplasia, and the paracortex has a moth-eaten appearance due to the many large lymphoblasts and tingible body macrophages. At this time, the lymph nodes are physically enlarged and there is thinning of the capsules with focal colonization of perinodal fat and a histologically compressed peripheral sinus. In chronic disease, the nodes remain enlarged but this is due, at least in part, to a marked increase in capsular and intranodal stroma with numerous collagenous septa extending from the medulla to the capsule. The medullary follicles remain, but their cellularity is reduced and there is marked atrophy of paracortical areas. Concurrently there is sinus histiocytosis and at least some degree of hemosiderosis without medullary cord hyperplasia. *The changes are thus those of continuing stimulation with an initial competent response followed by hyperplasia, then atrophy and sclerosis* (Fig. 2.100B).

Concurrent with the changes in lymph nodes, there is *remarkable atrophy of the thymus*, with the reduction most prominently affecting cortical regions. With the loss of cortical volume, there is a reduction in cell density and an increase in size of nuclei in cortical thymocytes. The *splenic lymphoid changes* reflect those in other areas of the body, with atrophy of periarteriolar cuffs and the formation of large hypocellular follicles, occasionally with "target" ringing of mantle cell and marginal zone layers. The number of follicles is not increased. The most marked splenic change is sinus hyperplasia characterized by a marked increase in fixed cells in the Billroth cord areas, which contain within their interstices many macrophages and plasma cells, with increased hemosiderin and an irregular appearance of extramedullary thrombopoiesis and erythropoiesis. *Renal changes* consist of a moderate generalized increase in interstitial connective tissue with mild epithelial atrophy and the appearance of large lymphoid cuffs around arterioles at the corticomedullary junction. Glomeruli are regularly increased in diameter and in cellularity and occasionally contain hemosiderin-laden macrophages. There are focal adhesions between the visceral and parietal layers of Bowman's capsule, and a mild epithelioid appearance to the central areas of the tuft. The nuclei of the juxtaglomerular cells are unusually prominent, and there is mild pigmentation by both iron and bilirubin in the proximal epithelium. The overall changes in glomeruli are compatible with *membranoproliferative glomerulonephritis*.

Subtle changes are consistently present in *skeletal musculature* and consist of an overall hypercellularity with a mild reduction in fiber diameter and increased perivascular lymphocytes. The

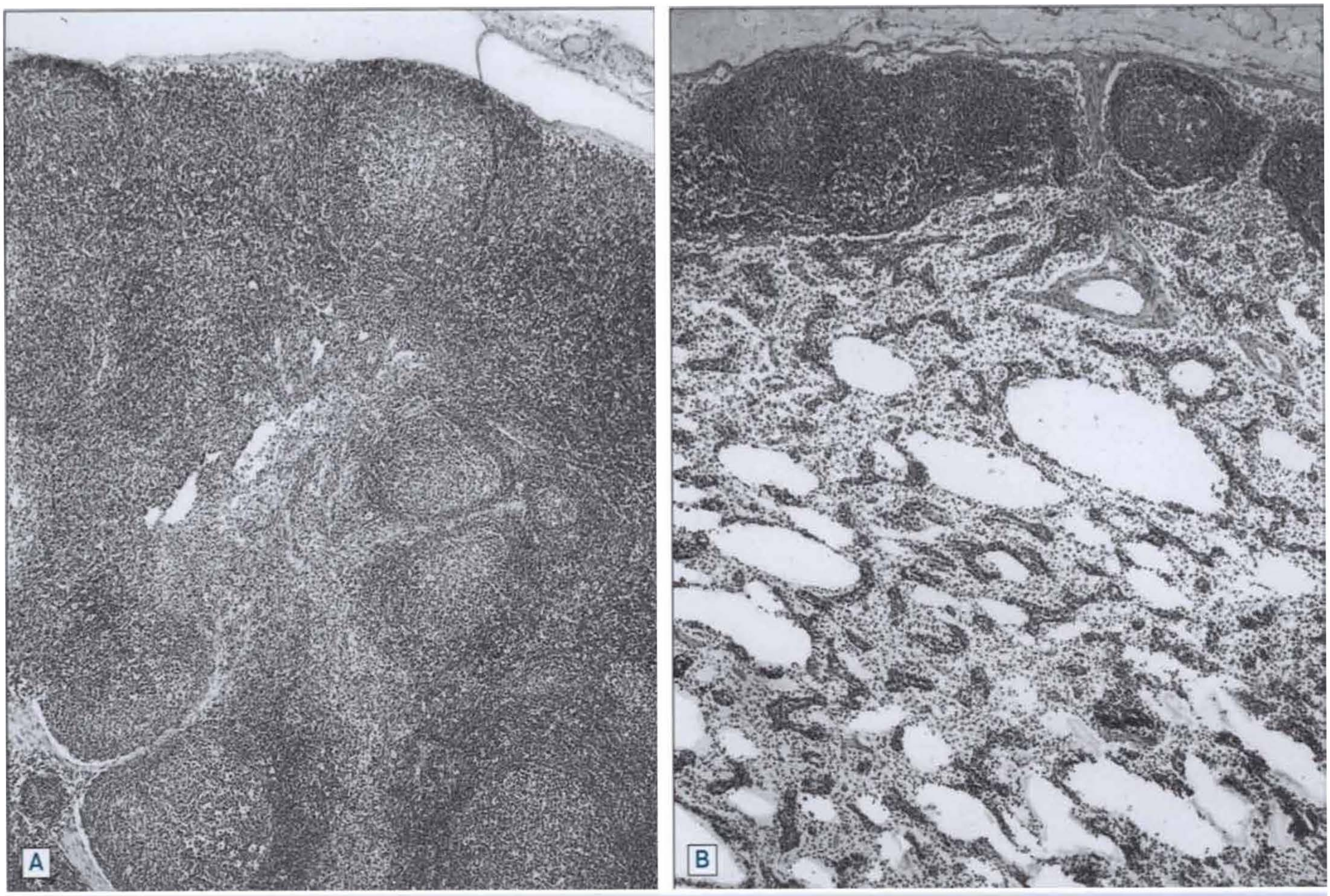

Figure 2.100 Trypanosomiasis in lymph nodes from cattle. **A.** Follicular hyperplasia. **B.** Paracortical atrophy, medullary sinus hyperplasia and histiocytosis, dilation of lymphatics in chronic disease.

reddened foci in the *omentum* consist of focal venous ectasia with mild interstitial sclerosis and perivascular lymphoplasmacytic reaction.

Trypanosomiasis in other species

Trypanosoma brucei rhodesiense and *T. b. gambiense* are human pathogens causing African sleeping sickness and are not important in domestic animals, although *T. gambiense* has produced meningoencephalitis in experimental goats and cats. These trypanosomes are morphologically indistinguishable from each other and from *T. brucei*, from which they are presumed to have evolved.

Trypanosoma brucei brucei is a cause of **nagana** in most domestic species in Africa, but in humans is refractory. Transmission is by *Glossina* spp. or mechanical. Equidae, small ruminants, camels and dogs are very susceptible, the disease is more chronic in cattle, and pigs may recover. Incoordination and spinal paralysis are reported in horses and dogs. Dogs may also develop parasitism and inflammation of the anterior segments of the eye. The neurological signs are undoubtedly due to invasion of cerebrospinal fluid and to inflammation of meninges, brain and cord. Diagnosis depends on demonstration of the organism in blood in acute or relapsing febrile cases, or otherwise in lymph nodes by direct smear, inoculation into the very susceptible laboratory mouse, or cultivation.

Trypanosoma evansi was originally shown to be the cause of **surra** in horses and camels, but it is pathogenic for most domestic species and distributed in North Africa, Asia, and Central and South America. Transmission is mechanical, chiefly by Tabanidae, but also by other blood-sucking flies, and apparently by vampire bats in the Americas. The disease in horses and dogs is severe, and probably uniformly fatal in the absence of adequate treatment. Cattle are mildly affected and act as reservoirs, although acute disease may occur in susceptible cattle introduced to endemic areas.

Trypanosoma equinum occurs in South America as the cause of **mal de caderas** of horses, a disease that resembles surra. The parasite is transmitted mechanically by tabanids. It is probably a stable variant of *T. evansi*.

Trypanosoma suis is a West African species transmitted by tsetse flies. It produces a nagana-like disease in pigs but is apparently nonpathogenic for other domestic animals.

Trypanosoma simiae was originally isolated from African monkeys. Although its pathogenicity for pigs is unpredictable, it is ordinarily highly virulent and causes death in a few days. Pathogenicity for other domestic species is insignificant. Transmission is cyclical in tsetse flies. Trypanosomes of the species *T. congolense*, *T. dimorphon*, *T. vivax*, and *T. uniforme* are causes of nagana in Africa. Most domestic species are susceptible to infection, although dogs are resistant to *T. vivax*. There is considerable variation in the pathogenicity of different strains, especially of *T. congolense*.

Trypanosoma cruzi is the cause of American trypanosomiasis (**Chagas' disease**), which is an uncommon illness of children, although infection of human adults and animals is apparently common. The reservoir hosts are chiefly wild species, but cats, dogs and pigs can be infected and act as reservoirs. Sheep and goats are susceptible to experimental infections. This trypanosome is of zoological interest because, both in the tissues of the vertebrate host and in the alimentary canal of the vector, it forms developmental phases resembling the other genera *Leptomonas, Crithidia*, and *Leishmania* of the Trypanosomidae.

Whereas some of the manifestations of *T. cruzi* infection may be attributable to parasitemia with the trypanosomal form, the disease is characterized by invasion of mesodermal tissues, and the growth therein of the leishmanial forms of the organism, which show a *preference for cardiac and skeletal muscle*. It has been suggested that, since the cysts are focal and the interstitial reaction may be widespread and diffuse, there may be an autoimmune component to the myocarditis associated with release of fiber proteins associated with parasitic injury.

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Hemolysis due to physical and chemical agents

Heinz-body hemolytic anemia

Hemolytic anemia due to denaturation of globin occurs in cattle, horses and dogs fed *onions*, and in cattle and sheep fed *rape or kale*. The same mechanism is operative in the anemia of horses with hemolysis after receiving *phenothiazine*, and in cats given *methylene blue* as a urine acidifier. Heinz-body anemia also occurs in horses that eat the leaves of *red (swamp) maple* (*Acer rubrum*). Heinz-body hemolytic anemia occurs as an *inherited deficiency of the glucose-6-phosphate dehydrogenase* (G-6-PD) enzyme in humans, and also rarely in sheep and dogs. In inherited deficiency, the level of G-6-PD may be 70–75% of normal, and low levels are associated with hemolysis on exposure to oxidants that may be dietary, such as *fava beans* or pharmaceuticals, such as the *primaquine* antimalarials. Cats appear particularly susceptible to Heinz-body formation, and Heinz bodies may be found in as many as 5% of apparently normal cats. The inclusions in cats were formerly called *erythrocyte refractile bodies*. Unstable hemoglobins resulting from inherited substitutions in the amino acids of the globin chains may have reduced avidity for the heme molecule, the loss of which is followed by globin denaturation and Heinz body formation. The latter pathogenesis is not of importance in domestic animals. Globin injury is involved to some degree in *postparturient hemoglobinuria in cattle* and in *chronic copper poisoning* in sheep. Heinz bodies or *intraerythrocytic globin precipitates*, when stained supravitally, appear as dense round structures 1–2 μm in diameter at the margin of the red cell.

Oxidizing agents which deplete red cell G-6-PD, the rate-limiting enzyme of the pentose shunt, diminish the protective biological antioxidant glutathione. Reduced glutathione is a hydrogen donor in the process catalyzed by glutathione peroxidase, a selenium-containing enzyme. *Selenium deficiency* alone may result in Heinz-body anemia. Failure to reduce oxidized glutathione results in formation of mixed disulfide linkages between glutathione and globin chains. These linked proteins, having lost the elasticity essential to the molecular shape change associated with reversible oxygenation, precipitate within the cell and aggregate to form Heinz bodies. *If the oxidizing attack is mild, the Heinz bodies are selectively removed by splenic phagocytes*, and the red cells return to the circulation with a slightly reduced volume. *If the reaction is severe, the Heinz bodies appear free in plasma* (Fig. 2.101), complicate platelet counts, and are removed by the spleen and liver. Hemoglobinemia, hemoglobinuria, and icterus occur in severe cases. Since young red cells have a higher G-6-PD enzyme level, *Heinz-body anemia is usually self-limiting and nonfatal*, as new red cells added to the circulation are more resistant to episodic toxic exposure. If the exposure to the toxic compound is dietary or continuous, the anemia may be progressive. The anemia may be moderate to severe, normochromic and moderately macrocytic, and responsive, with Heinz bodies free and projecting from the outer edge of red cells. Heinz bodies are unstained with Wright's stain, or stain similarly to hemoglobin. With supravital stains, such as new methylene blue, they are dark blue against red cells that are unstained. The level of red cell G-6-PD will be less than 25% of normal.

Copper poisoning

Copper poisoning may be acute or chronic, but the syndromes differ markedly, and the terms are misleading because *chronic copper poisoning is an acute and catastrophic syndrome*. The terms acute and chronic as applied to copper poisoning are not illogical since acute poisoning follows more or less immediately the acquisition of an overdose, whereas the chronic form represents cumulative poisoning which may, sooner or later, result in acute disease. *Chronic copper poisoning is a sporadic and enzootic disease of sheep, and a sporadic disease of cattle and swine.*

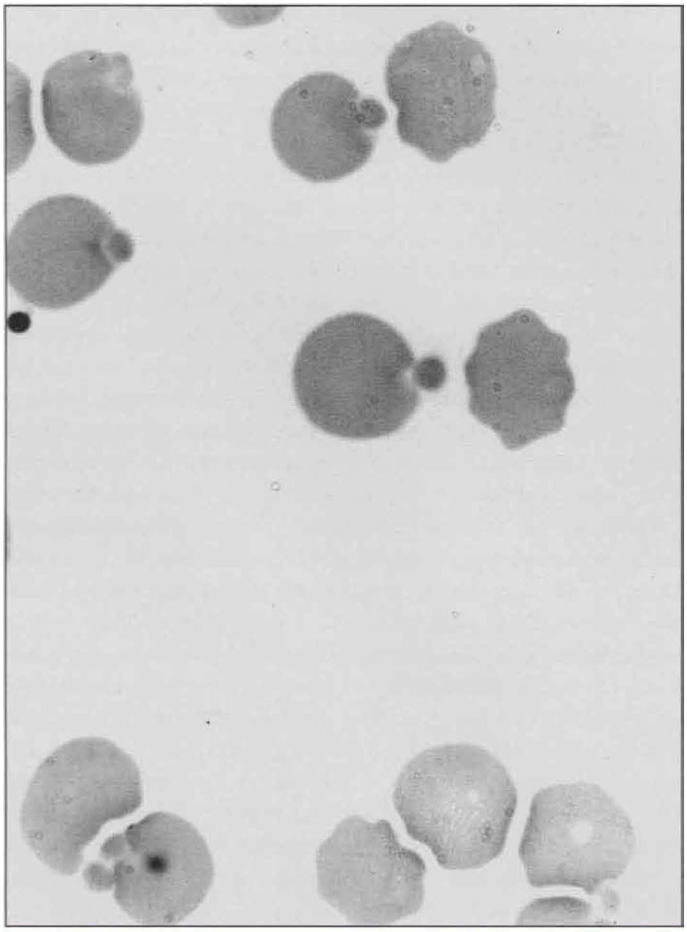

Figure 2.101 Phenothiazine toxicity. Heinz bodies in peripheral blood of a horse. Aggregates of denatured globin budding from red cell membranes.

Acute copper poisoning is characterized by *gastroenteritis* caused directly by the irritative effects of copper salts. The sole distinguishing feature is the passage of *bright blue-green fluid feces* accompanied by severe shock and rapid prostration. Grossly, there is *hemorrhagic and edematous abomasitis*. The copper salts are obtained by ingestion or by accidental therapeutic, chiefly anthelmintic, overdosage. The toxic dose of $CuSO_4$ for a sheep is ~20 mg/kg of live weight. Oral treatment at an appropriate level may precipitate the chronic syndrome if tissue stores of copper are high.

Chronic copper poisoning as defined here is an *acute episode of intravascular hemolysis caused by the release from hepatic storage into the blood of excessive amounts of copper*, until then stored by labile binding. The accumulation and release of copper may occur in a variety of ways, some being quite circuitous (see Vol. 2, Liver and biliary system).

Copper is an hepatotoxin, and during the period of copper accumulation there are progressive histologic, histochemical and ultrastructural changes in the liver, associated with an increase of hepatic enzymes in plasma. *The hemolytic crisis may be precipitated by stress; hepatocellular overload and lysosomal injury release toxic levels of copper into the blood.* As the capacity of carrier proteins is exceeded, excess copper rapidly enters the red cells, presumably exhausts the red cells' antioxidant system, and results in the denaturation of globin and the formation of Heinz bodies. Hemolysis lasts 2–6 days, with Heinz-body formation, hemoglobinemia and hemoglobinuria. If the animal does not die acutely, the new young red cells produced have higher enzyme levels and are more resistant to lysis than are older cells.

Coombs-positive red cells occur in sheep in the recovery stage of chronic copper poisoning. This is presumably the result of autosensitization by circulating red cell stroma and not a prior pathogenetic mechanism.

Hematologically, the hemolytic phase is characterized by a sharp increase in hematocrit to 0.60 L/L or higher in the hours preceding the onset of the hemolytic crisis, and is likely due to *dehydration*. The hemoglobin drops from a normal level of 100–110 g/L to 40–50 g/L in survivors. Since both free and cellular hemoglobin are measured by standard techniques, the massive hemoglobinemia will mask the actual amount of hemoglobin contained within intact erythrocytes. The erythrocyte count drops sharply to 2×10^{12}/L or less. There is neutrophilic leukocytosis during and following hemolysis. Morphologically, during hemolysis there is marked anisocytosis and poikilocytosis with moderate polychromasia and the appearance of Howell-Jolly bodies and rubricytes, with Heinz bodies budding from up to 15% of erythrocytes. *Reticulocytosis heralds the onset of recovery*. Serum bilirubin rises after the appearance of hemoglobinuria. Blood urea rises in parallel with bilirubin and then drops sharply in survivors, but does not return to normal. Levels of ceruloplasmin rise to 2–3 times normal during the hemolytic crisis, while red cell copper increases 15–20 times, plasma copper increases 3–6 times, and whole blood copper increases up to 10 times normal levels.

Cold hemoglobinuria

Hemoglobinuria after the ingestion of cold water occurs in calves and occasionally older cattle, principally as a disease of winter, and is seen in cattle which are housed in warm barns and given water at near-freezing temperature. It occurs also, but rarely, in calves which are maintained in hot barns in the summertime, and it can be readily reproduced by forcing them to drink cold water. The temperature of the water relative to the animals' environmental temperature is important since the disease rarely, if ever, occurs in cattle kept in cooler, open barns. *Hemolysis occurs most often in penned animals which are watered at irregular intervals and which therefore tend to drink large volumes when allowed access.* The disease is common although *rarely fatal.* The clinical signs consist of dyspnea with open-mouth breathing, followed by the passage of port-wine colored urine within 1–2 hours of drinking. Hemoglobinuria associated with the ingestion of cold water has also been observed in dogs and swine, but apparently is rare. The mechanism for the hemolysis in calves has been suggested to be osmotic lysis of red cells caused by the rapid ingestion of water, but this explanation is unconvincing since the osmotic effects of water should not be temperature dependent. The activity of cold agglutinins has been suggested as a cause of the hemolysis, but this seems even more unlikely since they are probably not widespread in cattle. The sporadic and ephemeral nature of the disease has not been conducive to study of the cause of the hemolysis.

Postparturient hemoglobinuria

The syndrome of postparturient hemoglobinuria occurs primarily in dairy cows and typically is seen within 2–6 weeks after parturition. The earlier the onset the more severe the disease. The name, however, is too restrictive because *the same disease, although slower in development, occurs before parturition, at other periods of the lactational cycle, and in males.* Most cases are, however, postparturient and severe. In North America, postparturient hemoglobinuria is a sporadic disease of multiparous high-producing dairy cattle, and is characterized by *intravascular hemolysis, hemoglobinuria, and anemia.* The disease is usually seen between the third and sixth lactation and tends to occur in the winter, particularly when preceded by a dry growing season. In New Zealand, the disease occurs in mid and late summer and in younger cows in first and second lactations. Like chronic copper poisoning of sheep, the disease may be precipitated by stress such as forced exercise.

The pathogenesis of erythrocyte destruction leading to intravascular hemolysis is not clear and may vary with geographic distribution. It is generally accepted, however, that *the disease is associated with severe hypophosphatemia*, and it has been produced when diets deficient in phosphorus are fed for long periods. Severe hypophosphatemia of the order observed with postparturient hemolysis is associated with depletion of red cell ATP and loss of membrane plasticity with formation of spherocytes and rigid, easily fragmented red cells. The latter syndrome is often recognized in humans on hyperalimentation or dialysis, and has been reproduced in dogs. In grazing cattle, an inverse relationship between the incidence of the disease and the fertility of the soil has been observed, and there is a direct relationship to use of beet pulp and cruciferous pastures as forage. In New Zealand, *copper deficiency* has been suggested as a possible factor in postparturient hemoglobinuria, possibly associated with the use of fertilizers containing molybdenum. Since copper is essential for the red cell superoxide dismutase antioxidant system, it is possible that cruciferous forage and copper deficiency work together to produce a Heinz-body type of hemolysis. While the dietary associations are varied, hypophosphatemia appears to be a significant part of the overall disease. The phosphorus levels in the blood, despite hemolysis, range from 0.45–0.59 mmol/L.

The signs of phosphorus deficiency which may accompany hemolytic anemia include *severe and shifting lameness, allotriophagia, decreased productivity, lordosis, and retention of long hair.* The animals may be emaciated. Usually there are no accompanying signs of phosphorus deficiency, however, and the disease occurs in well-fleshed animals at the peak of their productivity.

It is usual for hemoglobinuria to draw attention to the disease. Hemoglobinuria may not occur in all cases, and weakness with increased respiratory rates and mucosal pallor may be the initiating sign. The disease may progress to death in a day or in 4–5 days. The early mucosal pallor is, with time, replaced by jaundice, and milk production is sharply reduced and the secretion discolored with bile pigment.

Anemia is frequently severe, with erythrocyte counts dropping to 1×10^{12}/L or less. It is moderately responsive in animals which survive, and there is concurrent neutrophilic leukocytosis. Biochemical changes consist of hypophosphatemia and hyperbilirubinemia. The urine may contain high levels of hemoglobin that rapidly turns dark on conversion to methemoglobin with standing. There is concurrent albuminuria, characteristic of intravascular hemolysis and hemoglobinuric nephrosis, and the urine forms a stable froth on passage.

Grossly, *the principal changes are icterus, pallor, thin watery blood which is dark, and red-to-brown discoloration of the urine.* There is fluid accumulation in the serous cavities, and subcutaneous and interfascial edema. There are extensive ecchymotic hemorrhages on the cardiac surfaces, and the liver is slightly enlarged, turgid and pale with an ischemic nutmeg appearance caused by periacinar necrosis. The gallbladder is distended. The spleen is enlarged and congested in peracute cases but, in those that survive several days, it is of normal appearance. In spite of the frequent association of hemoglobinuria and death, it is unusual to find the blue-black pigmentation of kidneys characteristic of chronic copper poisoning in sheep. Icterus is often severe with canalicular cholestasis, which indicates extensive intracellular as well as intravascular hemolysis where the pigment is not lost in urine. Kidneys may be swollen, pale and soft, without hemoglobin pigmentation. The lungs are edematous and there is frequently interstitial and subcutaneous emphysema associated with terminal dyspnea.

Microscopic changes *are characteristic of intravascular and, to a lesser extent, intracellular hemolysis, and are not indicative of the cause.* There is panlobular hepatocellular swelling with ischemic periacinar coagulation necrosis. If animals survive for some hours with profound anemia, the margin of necrotic tissues within the functional lobules becomes progressively sharpened with a zone of fatty vacuolation separating viable and necrotic hepatocytes. Hypertrophy of Kupffer cells with hemosiderin accumulation becomes distinct in animals that survive for 3–4 days. There is congestion of splenic sinuses. Renal lesions are variable, usually with fatty degeneration of tubular epithelium, bile and hemosiderin pigmentation, and hyaline, granular and hemoglobin casts in the lower nephron. In more severely affected kidneys, there is patchy necrosis of the proximal tubular epithelium with desquamation, and interstitial edema.

Postparturient hemoglobinuria closely resembles the syndrome of hemolytic anemia produced in animals that are fed plants of *Brassica* spp. or *onions*. An unusual amino acid, S-methyl cysteine sulfoxide, is produced in these plants and is degraded in the rumen or colon to dimethyl disulfide. The sulfide causes the production of Heinz bodies in the red cells of all exposed species, but the resulting hemolytic anemia is most important in pregnant or parturient animals, especially those which are exposed to diets deficient in phosphorus. An intake by cattle of 10 g/100 kg body weight per day of the amino acid will produce low-grade anemia, and an intake of 15 g/100 kg body weight per day can produce severe fatal anemia.

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Hemolytic anemia due to mechanical damage to red cells

Microangiopathic or fragmentation hemolysis *occurs as a consequence of disseminated intravascular coagulation* and, like that syndrome, is secondary to primary disease involving microvascular injury. **Microangiopathic hemolytic anemia** (MHA) is characterized by severe poikilocytosis with intravascular hemolysis and hemoglobinuria (Fig. 2.102).

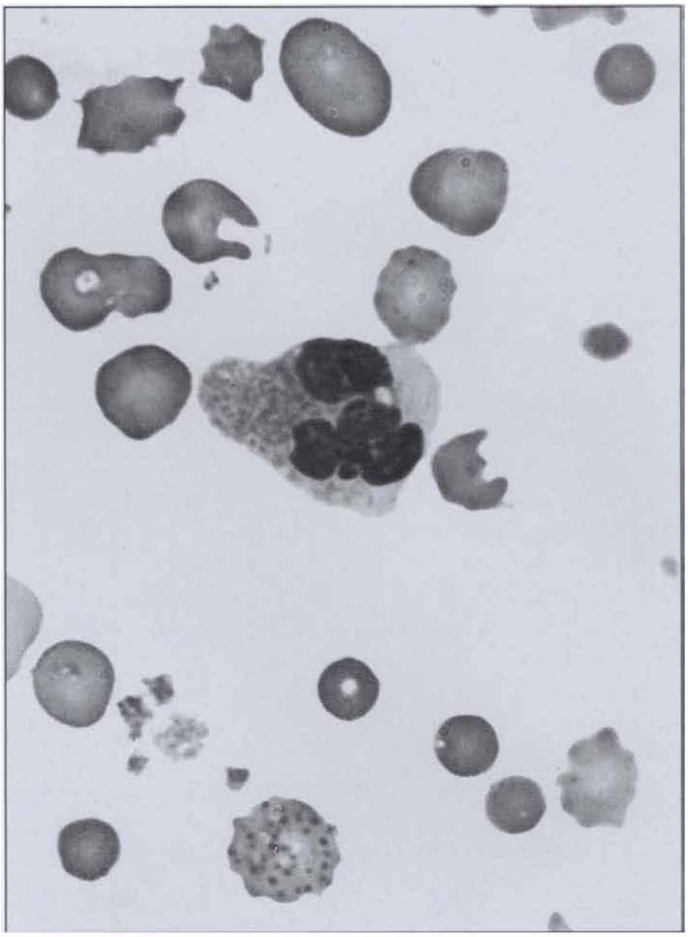

Figure 2.102 Microangiopathic hemolytic anemia, peripheral blood, in a calf with hemolytic-uremic syndrome. Nonresponsive anemia, microcytosis, poikilocytosis and basophilic stippling.

The basic mechanism underlying fragmentation hemolysis is the application of high shear forces to red cells moving at high velocity across a sharp drop in pressure. These conditions are most often met when *erythrocytes moving rapidly in small arterioles impinge on intraluminal fibrin strands and are fragmented*, usually with a characteristic "bite" out of the cell periphery. Thus, inflammatory lesions of capillaries and veins do not result in significant fragmentation, since the rate of flow in these vessels is too low. Fragmentation may occur as a result of arteriovenous fistulae when there is high turbulence and flow rates across a small orifice.

Fragmentation anemia occurs most often in association with infectious diseases which cause severe endothelial injury. Mild fragmentation occurs in animals with *salmonellosis* and with *fibrinous pneumonia*, but in these cases the degree of hemolysis is usually insignificant compared to the injury to the primary organ. Mild fragmentation also occurs in horses with colic in association with *verminous arteritis*. Since alterations in red cell shape in the horse are relatively rare, their presence in stained blood films is significant. Fragmentation anemia is seen in association with *somatic migration of parasites* and is prominent in animals affected by tissue phases of *Strongyloides stercoralis* and in dogs with *dirofilariasis*. Because of high renal blood flow and juxtaglomerular flow regulation, vascular lesions are prone to develop in the kidney, and fragmentation may occur there in association with a variety of renal diseases. Mild fragmentation occurs in association with *vegetative endocarditis*, particularly of the aortic and pulmonary valves, but is rarely seen in septal defects where the openings are usually large and the flow rate relatively low. In contrast, fragmentation occurs regularly in *patent ductus arteriosus* where the conduit is small and the pressure drop is high. Fragmentation of erythrocytes occurs during the development of *vitamin E/selenium deficiency of swine*, whereas marked poikilocytosis is seen in iron-deficiency anemia, and in lambs and, to a lesser extent, calves with nutritional myopathy. Presumably in the latter diseases, the hemolysis is largely due to intrinsic defects in erythrocyte membranes rather than to primary endothelial injury. Mild fragmentation occurs in *thrombocytopenia* in all species, presumably as a result of widespread endothelial deterioration, some of which involves small arteries. *Hemangiomas* cause focal coagulation and fragmentation in humans and animals, but the rate of destruction seldom results in anemia or red urine. Fragmentation of red cells may be significant in *widely metastatic carcinoma* and may be the presenting sign or may herald an accelerated phase of the disease in animals being treated. Hemoglobinuria occurs in event horses on *protracted exercise*, often in association with exertional myopathy and with a pathogenesis probably similar to that of "march hemoglobinuria" of humans. Markedly distorted red cells may be seen in hepatic failure in association with *severe anemia*. The major defect appears to be intrinsic to the red cell membrane as a result of low plasma lipid ("Zieve's syndrome"). The abnormal shapes and loss of membrane plasticity predispose these cells to fragmentation despite only minor vascular injury.

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Hemolytic anemia due to splenic hyperfunction

Increased erythrophagocytic activity results from any condition that causes enlargement of the spleen (**splenomegaly**). Splenomegaly may be the result of an immunoproliferative reaction due to infection, autoimmune disease, myeloid metaplasia, neoplasia or simply chronic congestion. Portal hypertension can cause congestive splenomegaly, but does so very rarely in animals. The stretching of the splenic reticulum in an enlarged spleen, regardless of the cause, is a specific stimulus to stromal hyperplasia. This increased sinus volume rapidly becomes populated with macrophages and, therefore, *splenic enlargement by definition leads to increased phagocytic function*. In the short term, conditions which cause splenomegaly with stretching, but not proliferation of the sinus stroma, are reversible. Blood pooled in an enlarged spleen is subjected to areas of slow flow and is biochemically characterized by low glucose, cholesterol and pH, which collectively cause premature aging and subsequent spherocytosis of erythrocytes, rendering them subject to phagocytic destruction. *The clinical disease resulting from hypersplenism may, therefore, be thrombocytopenic purpura, hemolytic anemia, neutropenia, or any combination of these*. If immune factors are added to the congestive pathogenesis, the disease is more severe.

Specific examples of conditions involving splenomegaly and hypersplenism include most if not all of the *infectious anemias*, but particularly those caused by protozoan agents such as *malaria, leishmaniasis and trypanosomiasis*. Generalized splenomegaly occurs in storage diseases in all species, but the effects in the nervous system and other organs are usually of more clinical importance than an enlarged and hyperfunctional spleen. Salers calves with β-mannosidosis have splenic involvement that is incidental to the impairment of the nervous system. Occasionally localized disease in the spleen will cause clinical anemia, as in *plasmacytoma of the spleen in the dog*. Occasionally, a focal area of splenic enlargement, such as might occur with *nodular hyperplasia*, or *marginal zone or mantle cell lymphoma*, will result in cellular deficits in the peripheral blood. Focal splenic lesions tend not to cause anemia but may cause thrombocytopenia due to slow and stagnant flow in irregularly dilated sinuses adjacent to a focal area of lymphoid proliferation. Clinical thrombocytopenia in the dog sufficient to result in petechial hemorrhages has resulted from a splenic lesion less than 2 cm in diameter that resolved on surgical removal. Histologically, there was apparent platelet sequestration in sinus "lakes" adjacent to the advancing margin of a *mantle cell lymphoma*. The latter conditions are not usually a diagnostic problem due to the efficiency of ultrasound in identifying focal lesions in body cavities of the dog and cat. In the horse, a type of *lymphoma* occurs as a localized splenic mass that may weigh as much as 15 kg. The animals are presented with a responsive hemolytic anemia that is usually suspected to be due to equine infectious anemia until proven serologically negative. Since the spleen is not palpable even with this size of mass, a clinical diagnosis depends on recognition of a nonimmunologic hemolytic process. It would be expected that lesions of this type would be evident on abdominal sonograms.

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Hemorrhagic anemia

Hemorrhage represents the simplest means of blood loss, although the pathogenesis may be most intricate. (The hemorrhagic diseases are given separate consideration later.) The causes of hemorrhage are very diverse with equally diverse results depending on the cause, the tissues directly involved, and on the rate of blood loss. Bleeding may be *acute* and the anemia of sudden onset, or *continuous* with loss of small amounts of blood over prolonged periods. The syndromes representing these two extremes are different, and therefore are discussed separately; however, between the extremes, the various signs form a continuous spectrum. The manifestations also vary depending on whether the hemorrhage is external with the blood lost from the

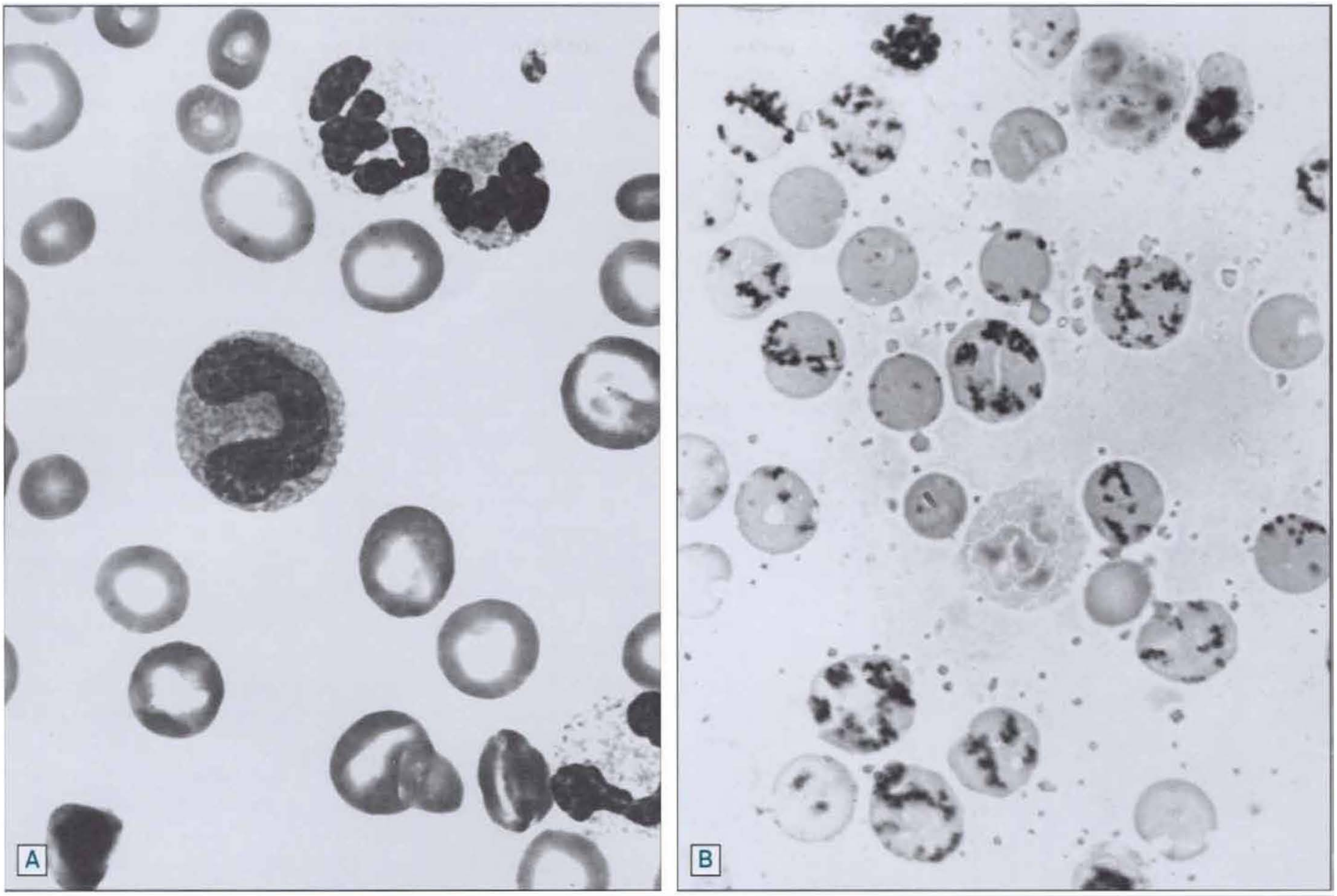

Figure 2.103 Hemorrhagic anemia of 1 week duration in an ox. **A.** Anisocytosis, hypochromia and poor response due to rate-limiting effects of iron utilization. **B.** Same case: reticulocyte preparation 2 days after parenteral iron administration.

body, or internal with the essential components of hemoglobin available for reutilization. These differences depend largely on the availability of iron and, to a lesser extent, on protein and essential amino acids (Fig. 2.103A, B).

Acute posthemorrhagic anemia with bleeding to the exterior has a number of possible causes, of which a common one is *trauma*. Rupture of blood vessels by *pathological processes* also occurs and is exemplified by massive hemorrhage from the nasal cavities, lungs and the female reproductive tract. *Esophagogastric ulceration of the porcine stomach* is a relatively common cause of acute anemia, as are *bleeding abomasal ulcers* in cattle. Hemorrhage into the stomach and intestines is accompanied by a moderate elevation of nonprotein nitrogen in blood. Parasitism, especially *hemonchosis, ancylostomiasis and coccidiosis*, may cause acute posthemorrhagic anemia. *Enzootic hematuria* of cattle is recognized as a cause of anemia in certain geographical areas.

In *acute hemorrhage*, immediate examination of the blood may reveal no change since all elements including plasma are proportionately reduced. Very shortly, however, there is an increase in the number of circulating platelets and of leukocytes, particularly neutrophils, which are largely mature. The leukocytosis is initially due to a redistribution of leukocytes from organs such as the spleen, splanchnic vessels and lung, in which there are normally sequestered a number equal in mass to the cohort normally in circulation. This neutrophilia and thrombocytosis persist as a reflection of increased marrow output directed principally to the production of red cells. As fluid is withdrawn from the tissues and alimentary tract to restore the plasma volume, the anemia becomes evident and it is, for the first 2 days or so, normocytic, after which the arrival of new red cells made macrocytic by the action of increased levels of erythropoietin add to the PCV, MCV and apparent anisocytosis. *Thus, the anemia of hemorrhage will be briefly macrocytic, and then scattered hypochromia will be seen, followed by a general reduction in red cell size and saturation as iron becomes limiting.* These changes appear to a greater or lesser degree regardless of the level of storage iron, since demand far outstrips mobilization, and the response will be increased incrementally by the addition of iron by both oral and parenteral means. The reticulocyte count, normally at 1% or 60 × 10^9/L in the dog and 0% in the cow will rise to 200–300 × 10^9/L depending on the iron supply and whether the blood was lost from the body. *In no case will the response equal that for hemolytic anemia.* As an example, a typical dog from a group of 36 with enteric bleeding from overdose of sulfa for coccidiosis had a PCV of 0.15 L/L and a reticulocyte count of 3.2 × 10^9/L, and a dog with factor VIII deficiency and an equivalent PCV had a similar level of response. Calves with blood loss anemia are even less responsive than dogs. Horses, which do not release reticulocytes into the circulation, will have an upward shift in MCV of 6 fL in response to removal of half the red cell mass, while in response to a similar loss due to Heinz-body hemolytic anemia the MCV rises 12 fL. Horses with roughly the same degree of anemia recovered their normal hemoglobin level in 42 days when made anemic by Heinz-body hemolysis, and in 84 days when made anemic by bleeding. Horses made anemic by bleeding increased their rate of hemoglobin production 1.5 to 2.5 times normal, while horses made anemic by hemolysis increased their hemoglobin production by 4.4 times normal. The red cells produced in response to hemolytic or hemorrhagic anemia had virtually normal lifespans. The response to anemia caused by a single episode of hemorrhage can be expected to restore a normal PCV in about 30 days, if not complicated by infection.

Following severe hemorrhage, rubricytes appear in the blood of most species except the horse. Their number, even when prominent by leukocyte standards, will only be in the $5–10 \times 10^9/L$ range and give no indication of response, as can be seen when their numbers are compared to the level of reticulocytes required to raise the PCV. *Reticulocytosis that persists longer than 1–2 weeks points to the probability that bleeding is continuing.* Significant red cell production can occur without apparent reticulocytosis, particularly in cattle, whose response is always subdued in comparison to dogs.

Marrow hyperplasia is evident grossly by an expansion of the hematopoietic areas along the endosteal surfaces of the long bones, and microscopically by an increase in proportion of hematopoietic to fat cells in red areas to nearly 100% from a normal proportion of about 50%. If iron deficiency is present, which it is likely to be in blood-loss anemia, there will be erythroid hyperplasia with late asynchrony or a predominance of late-stage rubricytes.

The immediate causes of chronic, posthemorrhagic anemia are, in general, likely to be enteric, occult and due to parasitism. Alternatively, the loss may be caused by external parasitism, as in *heavy flea infestation* of kittens and puppies. In the case of ulceration of the gastric squamous mucosa of swine, the loss may occur as recurrent acute episodes or continuous slight bleeding. By arbitrary but accepted definition, *chronic posthemorrhagic anemia is caused not only by loss of blood, but also by inadequate formation*, since continued bleeding depletes the body of hematopoietic substrates, principally iron. Under normal circumstances, 65–75% of the body iron is in the red cell mass, so severe hemorrhage represents a significant and irretrievable loss unless supplemental iron is given. Depending on the severity and duration of hemorrhage, the syndrome may be complicated by hypoproteinemia resulting from chronic loss of plasma proteins. *The anemia is hypochromic, microcytic, nonresponsive, and accompanied by thrombocytosis of small platelets.* Since, with deficiency of iron, hemoglobin synthesis lags behind the production of red cells, the hypochromia is more significant than the microcytosis. An indication of hypochromia can be obtained from a stained blood film in which the erythrocytes will be pale with increased central pallor in dogs, and show pallor in ruminants where it is not normally present. In addition, as part of the syndrome of iron-deficiency anemia, there will be poikilocytosis whose severity will vary directly with the degree of anemia. When the deficiencies are resolved, the response of the bone marrow is evident in prompt reticulocytosis. When anemia is excessively prolonged and severe, there may be some degree of extramedullary hematopoiesis by the liver and spleen. The spleen is enlarged and rather meaty, and microscopically there is increased sinus stroma.

Internal hemorrhage differs somewhat in its course from external hemorrhage. When acute, the main manifestations are the same but, in addition, there will be some increase in bilirubin even to the level of clinical icterus. Many cases, however, are too rapidly fatal for signs of degradation of the extravasated blood to become manifest. The principal causes of internal hemorrhage of a degree adequate to produce clinical anemia are *poisoning by sweet clover, Melilotus alba*, and *spontaneous or traumatic rupture of a viscus*, usually the liver or spleen as often occurs in dogs with hemangiosarcoma. Much of the blood lost into the peritoneal cavity is returned via the lymphatics to the circulation. In the latter disease in the dog, the previously extravasated red cells are apparent on the blood film as a second population of crenated cells in addition to the few acanthocytes characteristic of hemangiosarcoma. In any event of internal hemorrhage, most of the extravasated blood can be reutilized in hematopoiesis after its degradation by the monocyte–macrophage system. Hemosiderosis may therefore be a conspicuous feature in the liver, spleen, bone marrow and other organs in cases of internal destruction of blood but, as indicated earlier, hemosiderin iron is not readily available for reutilization.

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Disorders of hemoglobin

Congenital abnormalities of the synthesis of heme and of the molecular structure of hemoglobin are not known in animals, except for porphyria of cattle, pigs, and cats. The porphyrins are photodynamic, but otherwise appear to be inert (see Vol. 1, Skin and appendages). *Acquired disorders of hemoglobin are, however, rather common.* Increased protoporphyrins are associated with a number of diseases including lead poisoning, *iron deficiency*, and the porphyrias. Most hemoglobin disorders result in the formation of *methemoglobin*. *Carboxyhemoglobin* formation is rare unless poisoning is intentional.

Methemoglobinemia

Methemoglobin *is derived from hemoglobin by conversion of iron from the ferrous and functional state to the ferric form, where it is incapable of transporting oxygen.* A small amount of methemoglobin is normally present in blood, especially of herbivorous animals, and methemoglobin is probably formed at a steady rate but maintained at low concentration by an equilibrium with hemoglobin. Excessive concentrations of methemoglobin in the blood must then be due to increased production or to a defect in the mechanisms which reduce methemoglobin to hemoglobin. Methemoglobin reductase deficiencies have been described in the dog, but in the majority of cases the formation of methemoglobinemia in animals is due either to increased production of the pigment, under the influence of oxidizing poisons, or to irreversible oxidation of free hemoglobin in hemolytic diseases.

Many drugs and poisons are known to cause the oxidation of intracellular hemoglobin to methemoglobin, but the most important of these are the *nitrates, chlorates, phenacetin, and acetanilid.*

Nitrate poisoning and nitrite poisoning are often used synonymously, especially in relation to herbivores, in which reduction of nitrate to nitrite may occur in the alimentary tract. Reduction may also occur in forage before ingestion. Nitrate is, however, toxic as the nitrate radical and, although uncommon, poisoning has been observed in dogs and horses following ingestion of excessive amounts of *potassium nitrate.* Potassium nitrate is an irritant to the alimentary mucosa, and a diuretic, and signs of poisoning are referable to the alimentary and urinary systems but there are no specific lesions. Excessive dosage results in severe hemolytic anemia and icterus. The reduction of nitrate to nitrite is frequently insignificant in these circumstances and methemoglobinemia does not occur.

When *herbivores ingest nitrate,* it is reduced in the alimentary tract to nitrite and ultimately to ammonia. Since the amount of ammonia produced is rate-limiting, toxicosis by ammonia does not occur. Depending on a number of factors, including the initial concentration of nitrate, the flora of the rumen, and the diet of the animal, the conversion of nitrite to ammonia may be inadequate, in which case nitrite is absorbed and causes methemoglobinemia. The capacity of the rumen microflora to detoxify nitrate, or the nitrite derived from it, is energy-dependent and therefore progressively reduced under conditions of poor feeding. The conversion of nitrate to nitrite can and does occur externally as, for example, in hay stacked with a high moisture content.

The sources of nitrite for grazing animals are varied. *Agricultural fertilizers* which contain large amounts of nitrate or nitrite are frequently responsible for outbreaks of nitrate poisoning. In addition, a number of *plants* accumulate toxic levels of nitrate under natural conditions. The plants which are most frequently incriminated include the cereal crops, either green or as hay, the leaves of sugar beets and turnips and other Brassicaceae, *Sorghum* spp., and a number of *weeds*, especially variegated thistle (*Silybum marianum*), redroot pigweed (*Amaranthus retroflexus*), capeweed (*Cryptostemma calandulacium*), and *Tribulus* spp. The nitrate content of these and other plants is extremely variable and is influenced by the species of plant, the availability of the nitrogen of soil, the amount of shading, and the stage of growth of the plant. The application of herbicides may lead to an accumulation of nitrates in plants, especially sugar beet and wheat, and it is probable that a deficiency of other essential nutrients, such as sulfur, may result in metabolic disturbances in the plant and the accumulation of nitrate. Levels of nitrate, expressed as potassium nitrate, of less than 2% in plants are unlikely to result in toxicity. Outbreaks of nitrate poisoning are usually recorded on forage which contains 3–7% potassium nitrate.

Stagnant waters, especially *well water*, in areas of high fertility of soils tend to accumulate toxic levels of nitrate, and poisoning has been recorded in cattle and pigs which have ingested such water.

The pathogenesis of nitrate (nitrite) poisoning depends upon the oxidation of ferrous to ferric hemoglobin iron. It is likely that there are additional factors, including the oxidation of iron-containing respiratory enzymes and, since nitrates dilate blood vessels, a drop in blood pressure is to be anticipated. The blood vessels that are most sensitive to the dilatory effects of nitrite are those of the head, brain, meninges, and coronary vessels, with the visceral vessels being less sensitive. It is likely that the rapid collapse seen with nitrite poisoning is due to hemodynamic disturbances as well as to the inability of the oxidized hemoglobin to transport oxygen.

Cattle are more susceptible to poisoning than are sheep or horses. Clinically, nitrite poisoning is characterized by weakness, staggering gait, collapse, dyspnea, cyanosis, and rapid pulse. Death occurs in coma. Animals that are less severely affected urinate frequently. Grossly, there is little to see except for *cyanosis of mucous membranes and light brown discoloration of the blood and tissues.* An excess of blood-stained fluid is commonly present in the pericardial sac. Methemoglobin levels decrease rapidly after death.

Sodium chlorate is used as a herbicide and is occasionally responsible for death of cattle and sheep. It is a powerful but rather slow oxidizing agent, and its principal toxic effect is to cause methemoglobinemia. At high dosage, chlorate also causes intravascular hemolysis, so that hemolytic anemia and hemoglobinuria may accompany methemoglobinemia. When applied to plants, sodium chlorate is progressively converted to sodium chloride so that pastures are most hazardous immediately following spraying. The salt is palatable to livestock especially in areas of marginal deficiency of sodium chloride and this no doubt contributes to the likelihood of poisoning.

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Carboxyhemoglobinemia

Carbon monoxide *is formed by incomplete oxidation of combustible carbonaceous materials.* It is a common cause of poisoning in humans, *uncommon in most animals, even pets, but still occurs in housed pigs.* The usual sources of the carbon monoxide are illuminating gas and exhaust fumes from petroleum fuels that accumulate in tightly closed, heated barns. *Carbon monoxide has an affinity for hemoglobin that is 200–300 times the affinity of oxygen for hemoglobin, and the conjugated carboxyhemoglobin is incapable of carrying oxygen*; the conjugation is, for practical purposes, irreversible. Apart from the bright redness of the blood, which is directly due to carboxyhemoglobin, *the signs and lesions of carbon monoxide poisoning are those of anoxia.* The anoxia affects the brain most profoundly, and in acute poisoning the brain is congested and edematous and contains punctate hemorrhages. Degeneration of neurons may be widespread, and there is a tendency for foci of softening to develop. Recovery results in residual nervous defects in some cases.

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Lymphoreticular tissues

THYMUS

The thymus is a composite organ of epithelial and lymphoid tissues, with the epithelial part derived from the third pharyngeal pouch of the foregut endoderm. This epithelial anlagen is colonized by lymphocytes from the blood islands of the primordial yolk sac following migration of the epithelial component to the cranial mediastinal area. The epithelial components are derived bilaterally from the middle series of branchial pouches, which by epithelial outgrowths meet in the midline and are then displaced caudally in the neck.

There are *two streams of epithelial migration*, with that from the uppermost part of the pharyngeal pouch forming the *thymic duct epithelium* and later the *Hassall's corpuscles*, and that from the lower part of the pouch forming the *reticular epithelial component* of the cortex and medulla of the adult thymic lobule. Embryologically, the reticular epithelial component first invades the mediastinal interstitium with irregular, solid, rosette-like buds that enlarge to form the primordial lobules. This reticular epithelium forms loose cuffs around small vessels that persist in adult life and become obvious following lymphoid atrophy. The second component migrates as a system of branching ducts that ramify in the interlobular stroma and penetrate the lobules to form a central cord that broadly communicates between the lobules of a single lobe. In early development, this tubular system has a basement membrane and layered epithelium that becomes solid and cystic and loses the outer membrane in the mature medullary corpuscle. These relationships are pertinent to the histological interpretation of the thymus in pathologic states, as *in dysplasia, atrophy, regeneration, and hyperplasia, these components recapitulate their embryologic relationships*. Thus a thymoma with diffuse architecture and medullary predominance can be differentiated from hyperplasia by the presence of the reticular cuffs around the vessels of a thymoma. In terms of induction, it appears that these medullary ducts are essential to colonization of the anlagen by lymphocytes, and are the source of trophic thymic hormones. They are also the source of the cysts lined by ciliated epithelium found in the thymus and cranial mediastinum in many species and which, if large, may be of clinical significance as a cause of dyspnea. *A further cell type, which resembles striated muscle fibers*, is of indefinite origin, but has importance in the pathogenesis of myasthenia gravis.

In most species, the thymus reaches maximum development about the time of puberty. It can become hyperplastic, and in calves given repeated injections of endotoxin can extend from the rami of the mandibles to the base of the heart. Normally, *the thymus regresses in adult life*, and this regression may be accelerated during severe or chronic illness, but it never entirely disappears. Even a very small organ weighing less than 10 g can be immunologically potent in autoimmune dysfunction.

Thymic cortical lymphocytes are small cells with nuclei slightly larger than erythrocytes, little internal nuclear detail, and minimal cytoplasm. Despite their small size and lack of nucleoli, there is intense lymphopoiesis in the peripheral thymic cortex and continual migration of cells to the periphery from this area. Most (99%) of this proliferation is ineffective and balanced by a high rate of cell death, apparently in a process of immunologic selection. In normal states, cortical lymphocytes are closely packed so that the cortex appears uniformly dark histologically. In conditions of stress and atrophy, cortical epithelial cells and phagocytes become prominent and these larger cells with ingested nuclear debris give the cortex a moth-eaten appearance.

Thymic medullary lymphocytes are larger than their cortical precursors and have a more vesicular nucleus with small nucleoli and more cytoplasm. They appear less tightly packed than the cortical cells and, with the higher density of epithelial cells and the greater variability in nuclear size and shape, cause the medullary areas to appear lighter histologically, giving the thymus its *characteristic corticomedullary differentiation*. In many disease states where the animal is faring poorly, this corticomedullary boundary is indistinct and blurred by dilution of the cortical cells and an increase in macrophages in both regions. In normal states, the squamous ductal epithelium is obvious in central medullary areas, but the reticular epithelium of the cortex and medulla is not; the latter background of cells is only apparent following atrophy of the normal lymphocyte population.

Physiologically, *prothymocytes from bone marrow colonize the outer cortex of the thymus where they proliferate and are selected and trained by the cortical epithelium*. In this region, the epithelial cells form large membrane-lined cavities called *caveolae* in which the marrow-derived lymphocytes divide and undergo phenotypic development. On release from the marrow, prothymocytes have the ability to home selectively to the thymus but they lack helper, suppressor, or killer functions. In contrast, peripheral T lymphocytes are characterized phenotypically by CD4 (cluster derived, helper) or CD8 (suppressor) expression, functionally by the MHC determinants they recognize, and by their collective inability to react against normal self-antigens. The naive T cells arriving in the thymus from the bone marrow express CD34 and CD7 and are pluripotent in their ability to become types of T cells as well as dendritic or NK cells. They undergo a sequential pattern of antigen expression as a function of orderly gene rearrangements encoding the T-cell receptor (TCR). The earliest T-cell development occurs in the outer cortex with the expression of CD2 and CD44 and are termed "triple-negative" cells as they do not yet express CD3, CD4, or CD8. Most T cells develop a TCR heterodimer composed of α and β chains, a much smaller proportion develop the $\gamma\delta$ TCR configuration, and fewer still become intrathymic dendritic cells. Besides becoming CD3-positive with this level of maturation, the young thymocytes expressing the $\alpha\beta$ form of TCR also express CD4 and CD8 molecules and are known as double-positive cells during the time they express both molecules. Those cells that recognize antigen in association with class II MHC gain additional CD4 expression and lose CD8. Similarly, those cells that recognize antigen in association with class I MHC persist as CD8-positive cells. A much smaller population of $\gamma\delta$ T cells form a cytotoxic T-cell population as mature cells.

Prothymocyte development is believed to occur within the caveolae of the large epithelial "nurse cells." Nurse cells tend to express either MHC II, primarily in the cells of the outer cortex and dendritic cells of the inner cortex, or, in association with the more flattened reticular epithelium of the medulla, expressing both type I and II determinants. This close apposition of epithelium and young lymphocytes permits the transmission of inductive signals for proliferation and development, or for death of the great majority of the cells produced. The thymic nurse cells themselves produce interleukin-1 (IL-1), which drives lymphoproliferation, and in the course of maturation the T cells acquire receptor and production capability for IL-2, as well as other reactive lymphokines.

The progeny of this system are the recognition and regulation specialists of the adult immune system. They identify antigens presented to them by other cells as having either the MHC I or II determinants, and determine the presence or absence of foreign antigen, which is handled by killing or by stimulation of antibody development. T-cell development involves the rearrangement of the T-cell receptor genes analogous to the same process by which the B cells gain antibody diversity. *Whereas B cells recognize antigen on the basis of molecular shape, T-cell recognition is on the basis of peptides.* Since antigen is presented to the T cell by antigen processing phages and dendritic cells, denaturation of antigen is of no consequence since it is the amino acid sequence that is detected as foreign. In nonhematopoietic cells, internal antigen is displayed within a groove in the MHC-I molecule on cell membranes so that viral infection or tumor antigen are available for recognition by T lymphocytes. This process of cell recognition is accomplished by intracellular transporter pumps that display all of the proteins (self or foreign) produced within the cell on the cell surface. The diversity of T-cell recognition appears to be a function of receptor diversity and permits survival of cells with acceptable recognition of self to avoid "holes" in antigen detection. Thus, most NK (natural killer) cells recognize targets without MHC restriction, while the CD8+ cytotoxic cells with appropriate antibody mediate antibody-dependent cytotoxicity (ADCC). *Cytotoxic T cells and NK cells can induce apoptosis in adjacent cells by releasing cytotoxic granules called perforins or granzymes, both of which act in a Ca^{2+}*-dependent manner.

Cells exiting the thymus have not only managerial receptors but also *"homing" receptors* to guide them to appropriate areas in the peripheral nodes and Peyer's patches. Only 1–3% of thymocytes bear homing receptors, all of which are in the cortex and all of which are phenotypically mature. Thus, it appears that the homing direction is added last, with most of the cortical cells homed to nodes and likely medullary cells homed to Peyer's patches.

It appears that the thymic epithelium, besides making trophic hormone for the immune system, also makes the peptides *oxytocin and vasopressin* in common with the pituitary, thus indicating a mechanism for the interaction of the nervous, endocrine and immune systems.

In terms of ontogeny, lymphocytes first appear in the thymus of the fetal lamb at day 43, nodes at day 45, spleen at day 54, and Peyer's patches at day 65. These relationships are altered by fetal infection (see Vol. 3, Female genital system). In the dog, thymic epithelial enlagen are present by gestational age 23 days, lymphopoiesis begins by day 33 and corticomedullary differentiation with formation of Hassall's corpuscles is first evident at about 38 days.

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Developmental diseases of the thymus

Congenital immunodeficiency

Immunodeficiency is defined as an absence or defect in a class or subclass of the lymphocytes derived either from bone marrow or thymus. Problems not included in this discussion are congenital defects of phagocytic cells or of complement, and the failure to passively transfer immunoglobulin from dam to offspring. Immunodeficiency disease may be partial or complete. In individuals, the deficiency may affect bone marrow derived cells and be recognized by hypogammaglobulinemia, or involve thymus-dependent cells and be expressed as lymphopenia and deficient cell-mediated immunity. When the deficiencies involve both B- and T- derived classes of lymphocytes, they are called **combined immunodeficiency states**. Since deficiencies of the B and T lymphocytes tend to occur together, they are considered here under the thymus.

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Agammaglobulinemia

Immunoglobulin deficiencies occur in human males, foals and probably in other species. The disease in **foals** resembles X-linked agammaglobulinemia of human infants in both expression and progression. A selective deficiency of immunoglobulin M occurs in foals of Quarter Horse and Arabian breeding and both sexes are affected. Immunoglobulin levels vary from undetectable to 10% of normal, and the lymphocyte counts are normal, with normal proportions of B and T derived classes. These animals have neutrophilic leukocytosis without anemia and are presented for examination at 4–8 months of age with febrile disease involving the respiratory tract.

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Combined immunodeficiency (SCID)

Combined immunodeficiency occurs in humans, foals primarily of the Arabian breed, Jack Russell Terrier dogs, and in an inbred strain of the C.B-17 mouse. Both sexes are affected and the disease is due to an *autosomal recessive trait expressed as an absence of both B and T lymphocyte functions*. The syndrome is heterogeneous with a number of entities that differ in severity; however, since the disease is usually fatal in early life, *the acronym SCID is used to indicate* **severe combined immunodeficiency**. In mice, Arabian foals, and Jack Russell Terriers, SCID has been ascribed to defects in expression of DNA-PKcs (DNA-protein kinase catalytic subunit), which normally acts as a tumor suppressor. In the fully developed disease, there is extreme susceptibility to infectious agents, and in affected human infants, vaccination with smallpox and bacillus Calmette-Guerin is uniformly fatal. Affected foals survive about 2–5 months and infants 2 years, while SCID mice if maintained in microisolators will survive 40–80 weeks, or about three-quarters of a normal life span.

The disease occurs in some 2% of *Arabian bred foals* but the incidence of phenotypically normal heterozygotes will be much higher. Affected foals are normal at birth, but at about 10 days of age develop a range of diseases including pneumonia and diarrhea with which adenoviruses are peculiarly associated. In spite of intensive therapy, affected foals die at about 3 months of age. Hematologically, the animals are profoundly lymphopenic. There is hypoplasia of all lymphoid tissue including thymus, nodes and spleen, with histologic hypocellularity (Fig. 2.104A–D). Reduced lymphocyte levels in blood are usually more than compensated by neutrophilia, so that total leukocyte counts are usually in the normal range. *The mechanism of disease appears to be a defect in prolymphocytic stem cells at a hierarchical level that involves both bone marrow-derived and thymus-dependent systems.* Affected foals develop bilateral nasal discharge that becomes sufficiently profuse to impair suckling. There is progressive loss of weight and intermittent pyrexia with coughing, depression and rough haircoat. *Intractable respiratory disease is the most common clinical sign*, but diarrhea and swollen joints are also observed. There is severe lymphopenia, and *it is suggested that counts of less than* 1×10^9*/L are diagnostic for the disease.* Lymphopenia is present from birth and is constant throughout life. Since IgM is synthesized by the normal equine fetus, *absence of IgM from serum of foals that have not suckled supports a diagnosis of severe combined immunodeficiency disease*. In animals from 1 to 100 days of age, the serum IgM and IgA levels are low and remain between 0.2 and 0.6 g/L. The IgG, which is maternally derived, progressively drops from 8.0 g/L shortly after birth to 2.0 g/L in foals which approach 100 days of age. There is usually mild anemia that increases in severity with resistant and repeated infections, but is often masked by dehydration. Leukocyte counts are highly variable, being primarily dependent on the numbers of neutrophils.

The characteristic lesions associated with severe combined immunodeficiency in foals are *bilateral cranioventral bronchopneumonia in association with small spleen, lymph nodes and thymus.* The latter organ is identified only as a thin mediastinal raphe cranial to the heart. The bone marrow is unusually reactive for the age of the animal, and hematopoiesis persists in cancellous bone and partially occupies the central femoral cavity. Terminally, areas of marrow that appear red grossly may be hypoplastic with dilated sinusoids (Fig. 2.105A).

Histologically, *basophilic adenoviral inclusion bodies* are usually present in the epithelial nuclei of bronchi, bronchioles (Fig. 2.105B) and pancreatic ducts. The spleen is remarkable in that sinus areas are moderately cellular with macrophages and extramedullary hematopoiesis, while the small arterioles are devoid of lymphocytic cuffs. Architecturally, the nodes consist of a stromal framework that is essentially an unoccupied superstructure. Node capsules are delicate, with the peripheral sinus often heavily colonized by macrophages and granulocytes. Some nodes, particularly from the mediastinal area, may have cortical microabscesses. Follicles are absent, and the cortex consists of a reticular framework in which there are small foci of lymphocytes around small arterioles. Postcapillary venules have high vesicular endothelium and are usually well demonstrated because of the lack of perivascular and intramural lymphocytes. Medullary cords are collapsed, and medullary sinuses are dilated and contain neutrophils and macrophages. If thymus can be identified, it consists of small lobules of 1–2 mm in diameter surrounded by normal fat. The lobules have normal stromal capsulation and consist almost entirely of medullary structures with central Hassall's corpuscles and a vesicular reticular network with a very low lymphocyte population.

The presence of the **Hassall's corpuscles** is worthy of note since, besides identifying the tissue as thymus, their presence also indicates that there has been successful embryological migration of the epithelial duct system. In human infants, the corpuscles are present only in that variant with adenosine deaminase deficiency; however, this enzyme is not deficient in foals. In contrast, Hassall's corpuscles are not found in SCID mice, including the beige strain that also lacks NK cells. In the latter species, thymus can only be distinguished from mediastinal node by the presence of a peripheral sinus in the latter. Long-term survivors of the SCID mouse strain have a remarkable incidence of some 15% thymic lymphomas. Thymic hormone is not deficient in the SCID syndrome, and possibly the persistent high levels of interleukin-1 acting on a deficient target induce autonomous lymphoproliferation. The cellular defect is not well defined. There is failure of the lymphocytes to mature and, in the mouse, a defective recombinase system may prevent gene rearrangement both for immunoglobulin production and formation of the T-cell receptor. A variant of the SCID

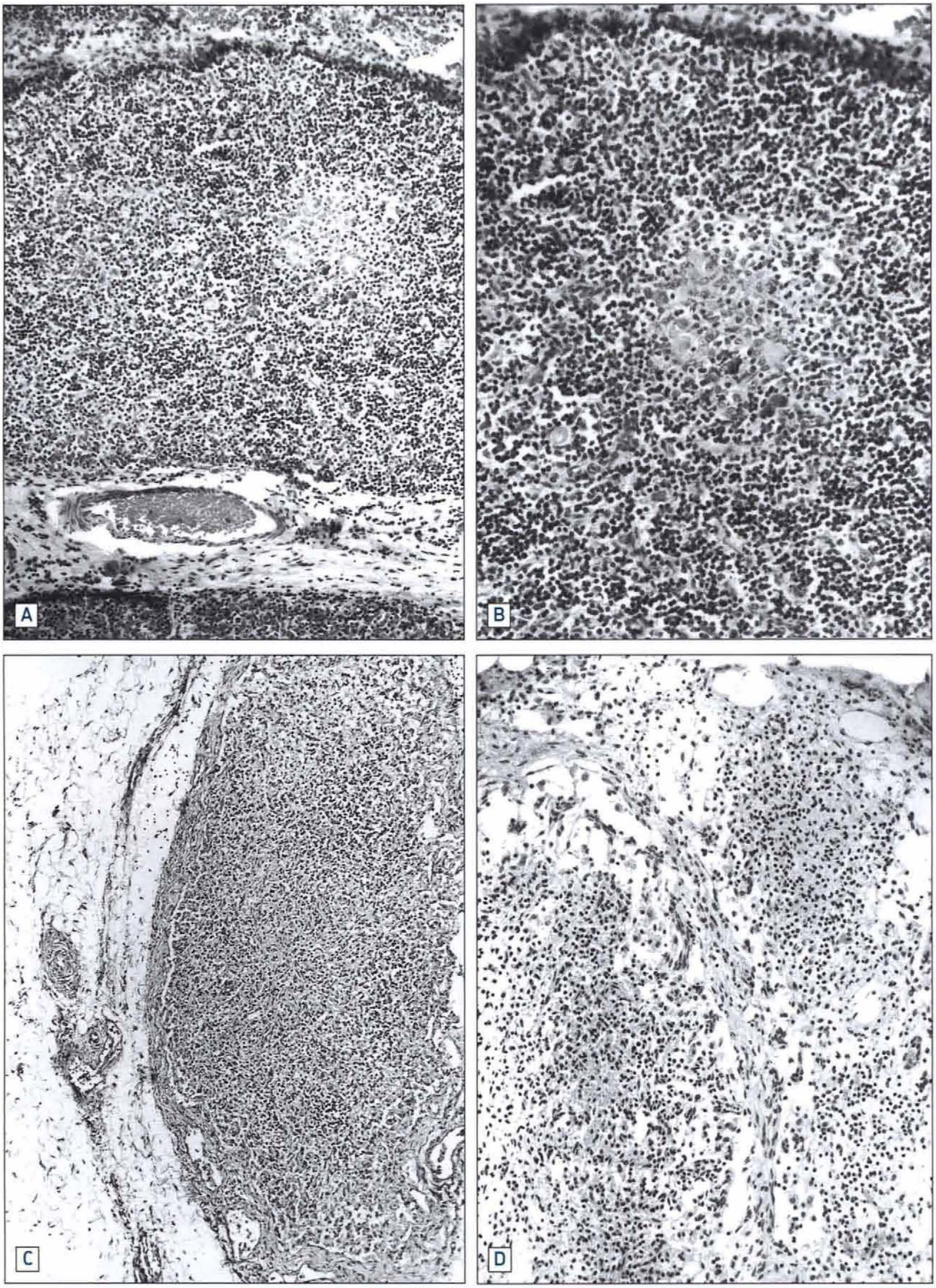

Figure 2.104 Congenital immunodeficiency in a foal. **A.** Thymic cortical hypoplasia. Cortical lobules small, reduced cellular density, lack of differentiation between cortex and medulla. **B.** Detail of hypoplastic thymus in (**A**). **C.** Hypoplasia of lymph node. **D.** Detail of (**C**) showing absence of follicles, and hypocellular paracortex.

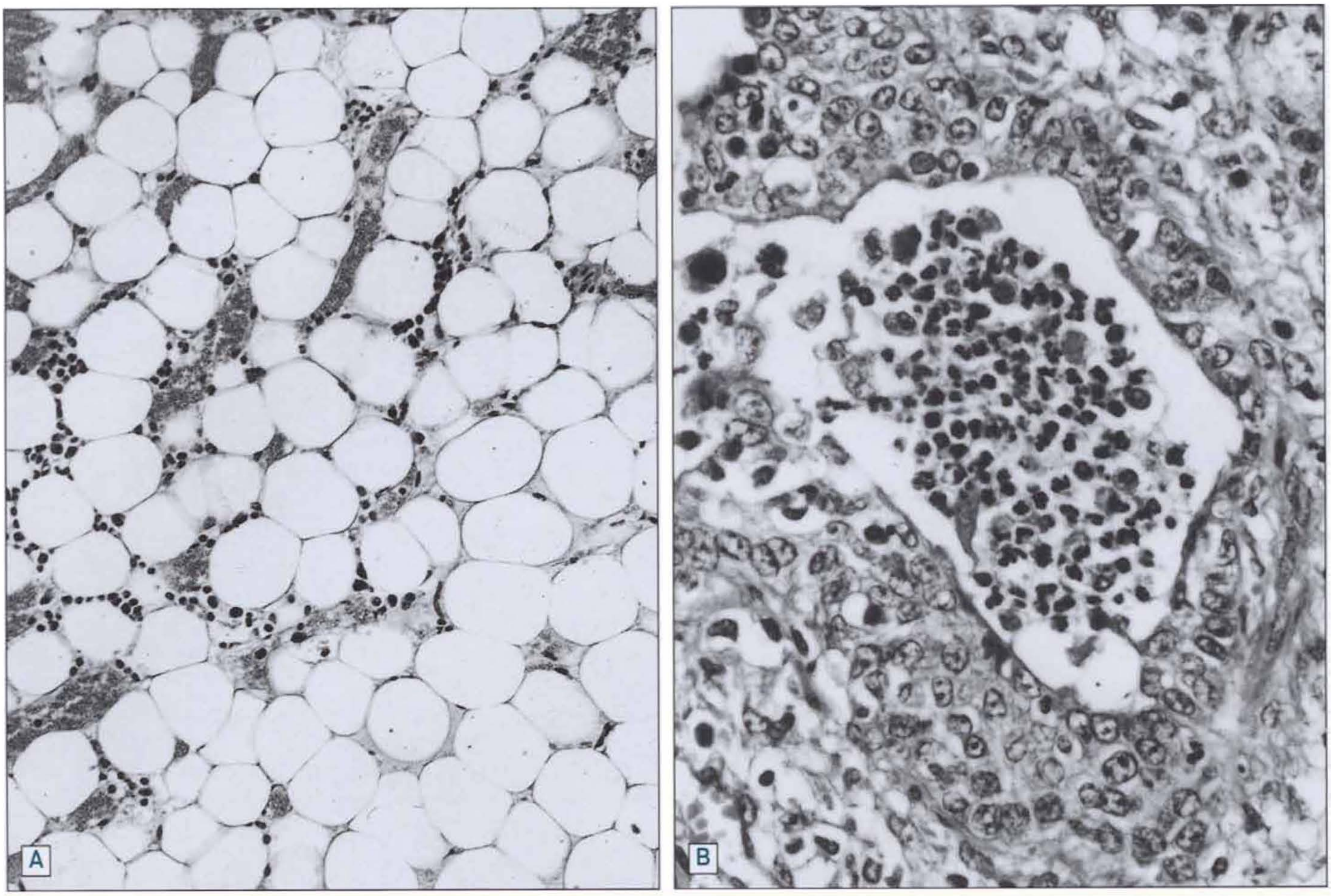

Figure 2.105 Congenital immunodeficiency in a foal. **A.** Hypoplasia of bone marrow. **B.** Necrotizing and hyperplastic bronchiolitis. Adenoviral inclusions are present in large sloughed epithelial cells.

syndrome in infants is termed the "bare lymphocyte syndrome," as the T cells lack HLA expression. It appears that some of the variability in these diseases may be due to the level of maternal cells in circulation that are functionally competent by standard tests. The SCID syndrome can be successfully treated in infants and foals by marrow transplant. Graft-versus-host disease follows transplantation of unmatched precursors.

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Acquired immune deficiency

Loss of normal immune reactivity occurs in a number of circumstances, most often as a result of therapeutic intervention, either specifically to combat autoimmune disease or indirectly as a result of antineoplastic chemotherapy. An unexpected cause of immune suppression is the *transfusion of otherwise compatible allogeneic blood*. The mechanism is apparently a blanking of the immune system by the massive introduction of antigen which, despite the beneficial hematologic effects to postsurgical patients, has a number of undesirable immune effects including a reduction in natural killer cell activity. *Normal pregnancy* induces a mild immune suppression which is usually only of significance in the face of epidemic viral disease, such as occurs with human influenza. The effect of pregnancy on the immune system is readily demonstrated experimentally in animals by alteration of the course of infection with viral, protozoal and helminthic agents. For example, in Uganda during an outbreak of lumpy skin disease, nonpregnant dairy cattle developed lymphadenopathy and high fevers of short duration followed by complete recovery, while pregnant cattle failed to develop severe lymphadenopathy and fever but subsequently developed skin lesions and protracted disease.

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Inflammatory diseases of the thymus

Immune inflammation of the thymus: myasthenia gravis

Myasthenia gravis is an autoimmune disorder of humans, dogs, and cats, characterized by muscle weakness and reduced exercise tolerance. All of the physiologic features of the disease can be accounted for by *reduction in acetylcholine receptors of neuromuscular junctions* (see Vol. 1, Muscle and tendon).

The disease arises as an immune-mediated phenomenon whereby a cellular and antibody reaction is developed against the thymic myoid cells which express acetylcholine receptors. The disease is mediated by the attachment of antibody to peripheral neuromuscular junctions. In dogs the syndrome occurs as an inherited disease of Smooth Fox Terriers, Springer Spaniels and Jack Russell Terriers, and spontaneously, often associated with thymoma. A possible role of bacteria in the pathogenesis of the spontaneous disease has been suggested because antibody against acetylcholine receptor cross-reacts with proteins found in *Escherichia*, *Klebsiella*, and *Proteus* spp. The thymus in myasthenia gravis of animals has not been well described, other than in the recognition of thymoma. In myasthenia gravis of humans, there are three characteristic types of non-neoplastic thymic histology, consisting of B-cell infiltration in a *follicular* or *diffuse* pattern, and *thymic atrophy*. The follicular type is most common and associated with the greatest density of thymic myoid cells with the diffuse lesion having an intermediate number and the atrophic type the fewest. The myoid cells tend to be associated with Hassall's corpuscles and the germinal centers, which resemble those of antigen-activated lymph nodes, and diffuse areas of B-cell proliferation are centered on the medullary areas with sparing of the cortex, which is often reduced in volume.

Myasthenia gravis in the dog is characterized by progressive weakness and is exacerbated by exercise, with only partial recovery on rest. Specific areas of involvement include the facial and extraocular muscles with the development of megaesophagus associated with difficult swallowing, regurgitation and aspiration pneumonia. Myasthenia gravis is characterized by sterile thymitis and the intrathymic production of acetylcholine-specific autoantibodies. It appears to arise completely within the thymus and appears to involve acetylcholine receptors on the myoid cells as a triggering antigen. The development of autoantibodies against acetylcholine and abnormal neurofilaments related to striated muscle, called *titin*, appear to result from aberrant T-cell selection and proliferation. It has been suggested that the acetylcholine receptor which is fully expressed in neonatal thymus abnormally persists and is amplified in cases of myasthenia gravis, and that this development may be the inciting antigen inducing the autoimmune reaction to peripheral acetylcholine receptors.

The association of myasthenia gravis with *thymoma* may not be related to the immunological role of the thymus. Some cases may be expressions of paraneoplastic autoimmunity associated with tumor-derived antigens not necessarily related to the acetylcholine receptors.

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Infectious inflammation of the thymus

Inflammation associated with some degree of atrophy of the thymus gland occurs frequently in infectious diseases. Thymic atrophy occurs in dogs with *canine distemper*, the changes being largely due to net emigration of lymphocytes rather than to lysis. Marked lymphocytolysis with involution occurs in foals aborted due to infection with *Equid herpesvirus 1* and in cats with *Feline panleukopenia virus*. In calves with *epizootic bovine abortion* and dogs with *salmon poisoning* (rickettsiosis), there is both lymphocytolysis and infiltration with other inflammatory cells. Chronic classical swine fever is characterized by progressive lymphoid depletion and death with complete thymic atrophy.

In foals aborted due to ***Equid herpesvirus 1*** infection, the thymus is grossly normal or of reduced size with edema. There is lack of distinction between cortical and medullary areas due to loss of cortical cells. Cytologically, there is extensive lymphocytolysis in the subcapsular areas, with pyknotic nuclear debris persisting in these areas (Fig. 2.106). The necrotic lesions tend to be patchy, but there is an overall reduction in cell density with edema of the lobules and separation of lymphocytes by clear fluid. Hassall's corpuscles may be surrounded by relatively clear acellular spaces, and viral inclusion bodies are often present in reticular epithelial cells. There is, in addition, a mild increase in monocytes and granulocytes in the interlobular connective tissue. The presence of nuclear debris in the outer cortices indicates acute lympholysis. Foals that survive 1–2

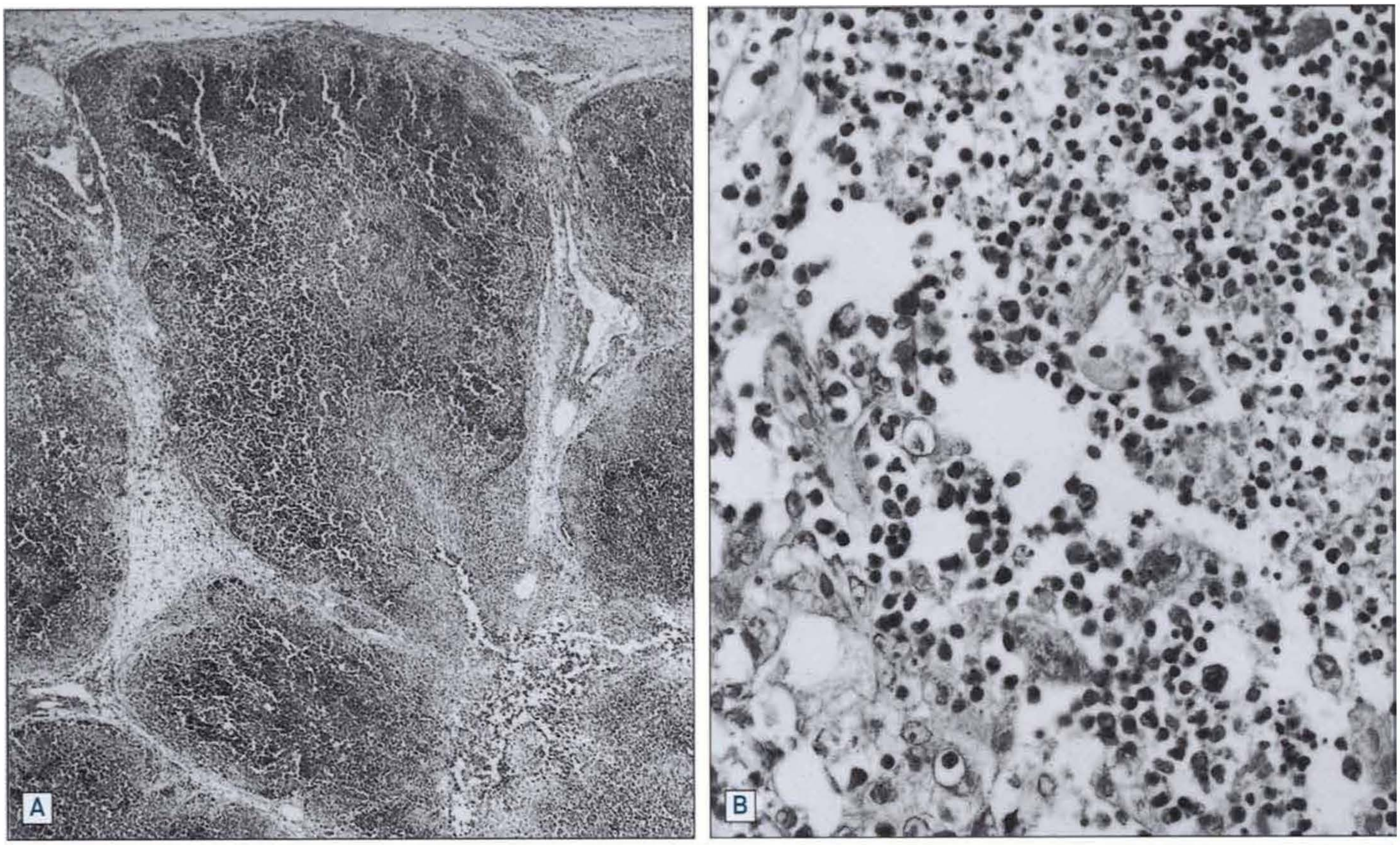

Figure 2.106 Thymitis caused by *Equid herpesvirus 1* in a fetal foal. **A.** Lobules are reduced in size and lack corticomedullary differentiation. The dark areas are viable lymphocytes. **B.** Detail of **A** to show extensive cell necrosis.

days may remove the cellular debris, and the cortices are then characterized by hypocellularity alone.

Cats which succumb to **feline parvoviral** infection (panleukopenia) have *marked thymic atrophy* (Fig. 2.107A–D) to the extent that the organ may not be identifiable grossly, and can only be examined histologically by systematically sampling the cranial mediastinal tissues. Microscopically, the thymus is characterized by collapsed lobules with angular facets to the lobes that become flattened and triangular. The changes are uniform in each lobule and consist of complete thymic atrophy (Fig. 2.108A, B), with only a very narrow rim of small lymphocytes 1–2 cells thick beneath the capsule. Hassall's corpuscles are prominent, and the lobules in effect consist of a condensation of medullary tissue and a prominent epithelial/stromal background that contains a few large lymphoblasts. Small lymphocytes typical of cortical cells are virtually absent from the medullary areas.

Cats infected with ***Feline leukemia virus*** (FeLV) suffer *thymic cortical atrophy of variable degree,* and a reduction in functional capability of neutrophils that may persist for a year after viremia is cleared. The p15E envelope protein appears to be the major factor in impaired T-cell function, apparently by inhibition of interleukin-2 production and responses. Gamma interferon production is inhibited and NK cell cytolytic function is impaired, but binding to target cells is unaffected. Circulating immune complexes contribute to the impairment of defenses and some improvement in condition can be achieved by their removal. There is a an age-related response to infection, with cats infected at under 8 weeks of age surviving about 3 months, and cats 4 months of age at infection surviving about a year. There is an early stage of lymphoid hyperplasia accompanied by weight loss, and followed by lymphoid depletion, lymphopenia, reduced lymphocyte response to lectins, hypogammaglobulinemia, ulcerative stomatitis, diarrhea with necrosis of crypt epithelium, and death. Thymic atrophy occurs in all cats that die, and terminally there is marrow hypoplasia.

The lesions associated with ***Feline immunodeficiency virus*** (FIV) appear, like the human disease, to be largely those of *secondary invaders*, and the most specific changes are largely in tests of immune cell number and function. Infected cats maintained in a clean environment survive indefinitely but have reduced numbers of CD4 lymphocytes and reversal of the CD4/CD8 ratio, suggesting specific infection of the helper cell population. Lymphocyte response to lectins is reduced and there is reduced interleukin-2 production by cells from infected cats. The disease process is likely accelerated when FeLV and FIV coexist, which occurs in about 1% of sick cats. Both viruses may be present in cats with lymphoma. It is remarkable how infrequently the thymus is examined in these diseases even when the focus of the investigation is on immunosuppression.

Bovine immunodeficiency virus (BIV) is an agent of wide distribution and low pathogenicity. In a proportion of infected cattle, there is a moderate lymphocytosis in the 10–15 × 10^9/L range, and mild generalized lymphadenopathy. The disease is very slowly progressive. Specific tests for this virus do not cross-react with *Bovine leukemia virus* or *Bovine syncytial virus*. The BIV agent will infect sheep and rabbits,

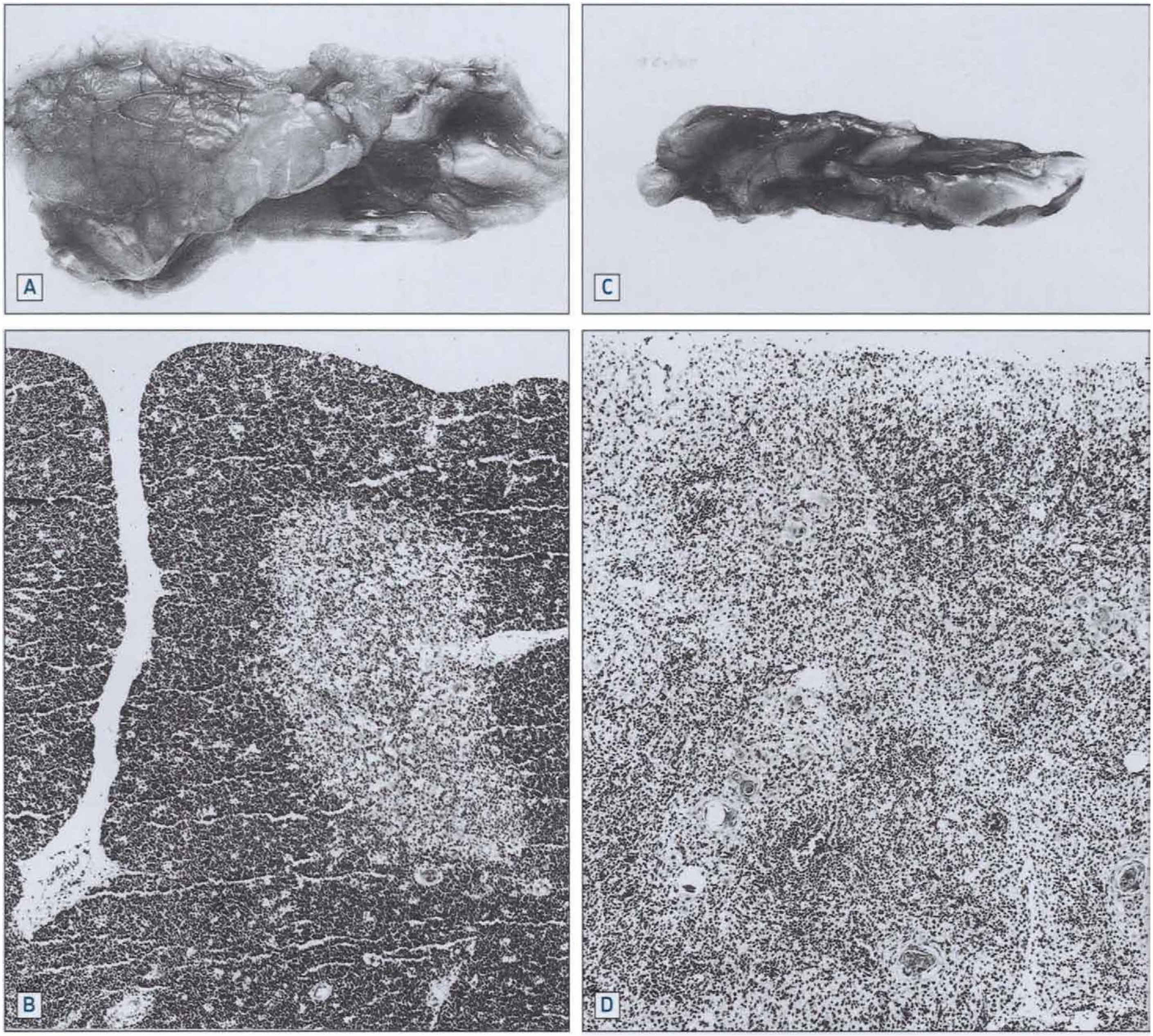

Figure 2.107 A, B. Normal thymus, gross and microscopic, of kitten at 8 weeks of age. **C, D. Degeneration of thymus** in panleukopenia in kitten at 8 weeks of age.

and they may be appropriate hosts for characterization of the virus and development of detection systems.

Infection with ***Bovine leukemia virus*** (BLV) is lifelong but, unlike infection with FeLV, there is no viremia after infection is established, the provirus persisting in the genome of B lymphocytes and macrophages. Chronic infection is characterized by the production of an ever-increasing array of antibodies against the various viral antigens, suggesting that the infection is not stable but continues to evolve with the development of lymphocytosis. An increased cull-rate has been shown for BLV-infected dairy cattle, which suggests that, while the cattle are outwardly normal, the anti-BLV immune response is at some cost to the animal.

A variety of **dietary constituents** can markedly affect thymic form and function, most often as a matter of deficiency. Animals that are *protein- and carbohydrate-deficient* during the adolescent phase have reduced thymic volume and fewer peripheral CD4-positive/CD8-positive T cells. Mice that were *protein- but not calorie-deficient* suffered thymic involution with elevated levels of endogenous steroid, which appeared to be the major mechanism in thymic involution. A number of trace elements including copper, zinc and selenium appear required for maintenance of an adequate immune system. Rats fed a *copper-deficient* diet developed hepatomegaly and increased heart weights with decreased thymus weights and reduction in production of immunoglobulin production and of cytotoxic NK cells.

Infection with *Human immunodeficiency virus* (HIV) causing AIDS is the major pandemic affecting this era. Infection with HIV induces thymic involution, as does infection with SIV in nonhuman primates. In the latter case where serial studies can be carried out,

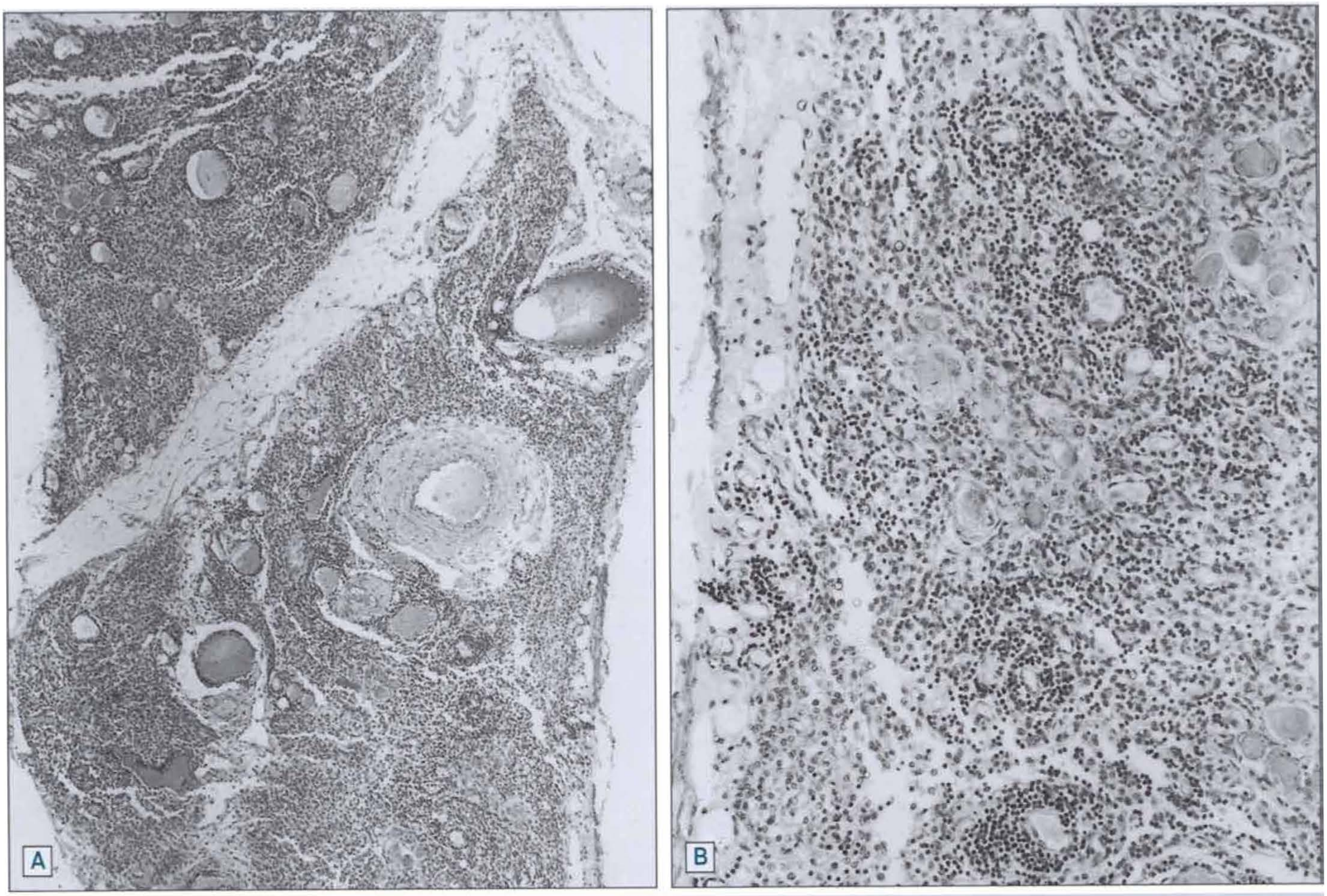

Figure 2.108 Thymic atrophy in feline panleukopenia. **A.** Lobules reduced in number and size. **B.** Proximity of Hassall's corpuscles to capsule indicates degree of cortical atrophy.

thymic involution associated with SIV infection was compared to the effects of normal aging and of stress and social deprivation (orphaned offspring) during the neonatal and adolescent periods. *Normal aging* was found to be associated with retention of the physical size of the thymic capsule with symmetrical atrophy of thymic cortex and medulla with the intervening space replaced by fatty tissue. The definition of the cortical and medullary regions was maintained, but cell division and apoptosis were decreased and there were no changes in general cell types. In contrast, the thymus in an orphaned monkey had severe thymic involution with loss of corticomedullary stratification. The overall thymus was reduced in size with collapse of the capsule and reduction in cellular density in residual corticomedullary areas. In contrast, in animals infected with SIV, there was general loss of thymic size, with condensation and folding of the capsule and apparent thickening of the interlobular septa. There was symmetrical reduction of thymic cortical and medullary areas, with irregular reduction in cellular density. Scattered plasma cells were found in the cortices of aged animals and an increased density in SIV-infected animals, but not in the atrophic thymus of the orphaned animal. The thymic involution associated with SIV infection is also associated with changes in the T-cell complement in the peripheral blood as well as the activity of effector T cells, with subsequent affects on antibody production. *These changes are replicated with considerable similarity due to leukemia virus and immunodeficiency virus infections in cats, mice and cattle.*

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Degenerative diseases of the thymus

Thymic involution *is part of the normal process of ageing and may be slowed by dietary restriction or accelerated by toxic insult.* It appears that a high caloric intake promotes early development of the endocrine and immune systems, and that the resulting high level of stimulation to end-organ systems results in an excess of tumor formation in secretory and reproductive organs, and early exhaustion of the immune system. This process may be augmented if a high proportion of the caloric intake is made up of animal fat. When a restricted diet of approximately two-thirds of free-choice intake is given to normal laboratory rats and mice, their lifespan is extended, and the level of spontaneous tumors is reduced. In addition, when animals with an inherited tendency for autoimmune disease are subjected to dietary restriction, a tripling of expected lifespan occurs. Thus, *unrestricted diet appears to increase the incidence of allergic and autoimmune phenomenon in the young and to contribute to early senescence.* It is suggested that these observations are explained by a slowing of the age-associated decline in DNA repair. The improved immune function in old animals maintained on restricted diets may have a similar basis. The recognition that a number of environmental and dietary contaminants may accelerate immune deterioration has resulted in the development of testing protocols specifically designed to assess adverse effects of this nature.

In a general sense, *thymic involution can be used as an index of disease severity and duration in young animals*, in which the organ would normally be much larger.

A further form of thymic involution occurs as a result of exposure to a variety of **environmental contaminants** with the most serious effects being those due to exposure to various forms of *dioxin* and various congeners of the *polychlorinated and polybrominated biphenyls (PCB, PBB)*. Much of the work that has been done in this area was based on a crude product known as Aroclor, which had been widely produced for use in the cooling and insulation of electrical transformers. These compounds are stable in the environment and tend to accumulate in the food chain and contaminate the fatty tissues of humans and free-living species. The various congeners within a mixture contained in Aroclor varied tremendously in their relative toxicity, most of which is directed at the immune system and evident in *marked thymic atrophy and degeneration*. In many ways, variation in toxicity of these various compounds had given conflicting information which permitted some studies to conclude that exposure to chlorinated biphenyls was relatively nontoxic, while others may be extremely toxic and several orders of magnitude more active in inducing target organ injury than other congeners with relatively similar atomic constituents but different molecular structure. In relative terms, the 126 isomer of PCB is approximately 3 orders of magnitude more toxic to target organs in the rat than the 128 isomer, with the 118, 156, 77, 153, 28, and 105 congeners intermediate in toxicity in descending order of effect.

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Hyperplastic and neoplastic diseases of the thymus

Thymic hyperplasia

Hyperplasia of the thymus occurs as an increase in volume of a histologically normal gland in the young, as the development of germinal centers in a gland of small, normal or increased size, or as a focal area of cortical hyperplasia in an older animal.

Diffuse hyperplasia may occur in any species, but is most likely to be recognized in calves, rabbits and birds that have been *repeatedly immunized*. Grossly, the glands may fill the cranial mediastinum and extend up the neck in calves. The capsules remain thin and delicate and the lobulation is distinct.

Follicular hyperplasia is the result of fully typical B-cell germinal centers forming, usually at the corticomedullary junction, in glands that may have a variety of other changes including reactive hyperplasia and atrophy. *Follicular hyperplasia is found not infrequently in thymoma*, suggesting some period of immune dysfunction preceding development of tumor. In humans, follicular hyperplasia is strongly associated with *autoimmune disease*, particularly myasthenia gravis, and 85% of patients with thymic lesions have this change. Follicular development occurs in dogs with immune hemolytic anemia and systemic lupus erythematosus but the frequency is not documented. Focal hyperplasia is an unusual and incidental finding in older animals, and is most often seen in rats and mice on lifetime studies where a strict protocol requires that the thymus is routinely examined histologically.

Focal cortical hyperplasia is an appropriate term for lesions characterized by proliferation of cortical lymphocytes in one or more lobules of the gland, usually with compression of surrounding, often atrophic, lobules *suggesting an attempt at regeneration* as is seen, for example, in the exocrine pancreas. The lesions are usually lightly encapsulated, often with some laminar infiltration of the perithymic tissues, and significantly with single epithelial cells scattered through a relatively diffuse area of lymphoid proliferation. Focal hyperplasia of the thymic cortex is visible on architectural or low-power examination by the presence of increased numbers of large tingible body macrophages imparting a "starry-sky" appearance similar to that seen in a reactive germinal center.

Focal medullary hyperplasia is seen most frequently in the thymus gland of rats from various studies and is characterized by a loosely defined focus of cells apparently arising at the corticomedullary junction that is recognizable on low-power examination by increased density due to a greater proportion of small cells than the surrounding thymic medulla. Epithelial cells are present throughout in single cell distribution, but Hassall's corpuscles appear not to be present within these nodules. *Their significance is unclear*, but they do not appear to proceed to thymic lymphoma. These masses do not produce clinical signs and are important primarily in their resemblance to early lymphoma.

Thymic neoplasia

Thymoma *is a tumor derived from the epithelial components of the thymus that is invested to a greater or lesser degree with benign lymphoid proliferation.* It is rare for thymomas to be purely epithelial, and the association of lymphocytes with epithelium, even in metastatic sites, suggests that the latter retains some lymphoid inductive capacity, notwithstanding the malignant state. *Thymomas tend to be slow growing, heavily encapsulated tumors that rarely metastasize.* They may be localized to one lobe, or more commonly a remnant of benign thymus may be found compressed to the periphery of the thymic capsule. Thymomas are classified on the basis of cellular composition and the type and degree of atypia of the epithelium, and thus are *predominantly lymphocytic, mixed, or predominantly epithelial.* The major diagnostic difficulty involves the recognition of epithelium in the lymphocytic type, which distinguishes it from the much more common mediastinal lymphoma. The epithelium may be very variable, but it is usually spindle-shaped in storiform or rosette pattern, vesicular in diffuse sheets, or squamous. The latter type is most likely to appear cytologically malignant and to metastasize.

In general, *lymphoid tumors of the cranial mediastinum* in the cat and bovine yearling are aggressive lymphomas, while in the goat, sheep, and occasionally in the dog, they are encapsulated masses that may cause clinical signs due to their size but rarely metastasize. In humans and likely in animals, there is rarely neoplasia of the myoid cells to form a *rhabdomyosarcomatous mass* which is malignant and metastatic.

Thymic lymphoma

Thymic lymphomas occur in *cats, young cattle and dogs*. In the **cat**, mediastinal lymphomas constituted 22% of 547 cases of lymphoma. Characteristically, the tumors reach a large size with dorsocaudal displacement of the lungs before they are presented for clinical examination. Thymic lymphomas are poorly encapsulated and tend to be traversed by irregular, fine, collagenous septa. It is possible that some of them arise in the cranial mediastinal lymph nodes, but at the time of diagnosis the thymus is obliterated and cannot be dissociated from the tumor. Histologically, two-thirds of feline thymic lymphomas are *high-grade tumors of Burkitt-like (small noncleaved cells) and immunoblastic type with a high mitotic rate.* They often have a "starry sky" appearance due to the presence of many tingible body macrophages associated with a high rate of apoptosis of the tumor cells. There is a wide age distribution of feline thymic lymphoma, with 90% occurring relatively evenly between the ages of 1 and 10 years. Less than 8% are of the *lymphoblastic type*, and true *small cell lymphomas of well-differentiated type* are even less common. Phenotypic studies of a series of feline thymic lymphomas are lacking but it appears that they are *usually T-cell type and CD-positive.* Involvement of the lung, even by direct extension, is unusual, although there may be infiltration into the atria and through the pericardium into the base of the heart and around the origins of the great vessels. Infiltration beneath the sternal pleura is consistently present. Metastases may be widespread, but they are rare and small compared to the mediastinal tumor, suggesting that these lesions spread by local expansion until late in the disease.

Thymic lymphoma in **cattle** characteristically occurs in yearlings of the beef breeds, is not associated with infection with *Bovine leukemia virus* (BLV), and does not share tumor-associated antigens with the BLV induced tumors. Typically, there is swelling, sometimes massive, at the base of the neck, and brisket edema. Bloating and dysphagia associated with esophageal compression are common. The animals are seldom leukemic, although in advanced cases there are usually neoplastic lymphocytes in the peripheral blood without changes in absolute numbers of cells, and tumor cells may be identified in sternal marrow aspirates. The tumors may extend from the rami of the mandibles to the base of the heart with a constriction at the entrance to the thoracic cavity, and may weigh 20 kg or more. They are lightly encapsulated and infiltrate the surrounding tissues, often with smooth, rounded, shiny implants appearing on the pulmonary and parietal pleura. There is compression of the lungs by tumor and excess pleural fluid. The lungs themselves may be focally invaded but are rarely extensively involved. Invasion of the pericardial sac produces pericardial effusion that contains neoplastic lymphocytes in large numbers. On cut surface, the tumors are gray with irregular yellow areas of infarction that have hyperemic borders, and they tend to have coarse collagenous septation. Histologically, thymic lymphomas in cattle have *diffuse architecture with a relatively minor stromal component*, and most of the septation is limited to rather heavy hyalinized bands that may be several centimeters apart. Cytologically, they are *high grade, largely of Burkitt-like (small noncleaved type), and with a disproportionately high number of lymphoblastic types.* All appear to be *T-cell type and CD3-positive.*

The thymic lymphomas of other species, including **dogs** and laboratory animals, do not differ markedly from those in the cat, but there is usually not extrathoracic tumor development.

Lymphoid thymoma

Lymphoid thymomas are seen most frequently in the dog, sheep and goat and rarely in the horse. In **dogs and horses**, the tumors are usually clinically apparent, with the animals presented for examination because of signs of respiratory and/or cardiovascular impairment. In **goats and sheep**, the lesions are usually found incidentally at autopsy. Lymphoid thymomas are irregularly shaped masses from 5 to 15 cm in diameter, with irregular rounded projections, and firm fibrous encapsulation. Grossly, the tumors appear as an exaggeration of normal thymic structure with heavy and irregular fibrous septation. Microscopic cysts are frequently observed, filled with pink proteinaceous material that resembles thyroid colloid. These cysts are branchial duct remnants and have a lining that varies from squamous to columnar and ciliated, and may change abruptly from one form to the other. The corticomedullary distinction is lost, and areas may more closely resemble cortex or medulla depending on the proportion of more pale-staining epithelial cells as compared to lymphocytes; however, the latter always predominate. Hassall's corpuscles are present and irregularly distributed throughout a background of benign but densely packed masses of lymphocytes of medium size. The predominant lymphocytes have nuclei only slightly larger than those of normal cortical thymocytes, and are uniformly interspersed with large lymphocytes with coarse retiform chromatin and prominent central nucleoli. *Lymphoid thymoma is distinguished from lymphoma on fine needle aspiration by the recognition of more regular intermixing of small and large lymphocytes and epithelial cells with pale nuclei and abundant cytoplasm throughout the material obtained.* If epithelial cells are not apparent in the aspirate, the irregular mixing of large and small lymphocytes helps to confirm the interpretation of thymoma. Alternatively, with current capability for immunocytochemistry, the epithelial cells are readily identified if of either round or strap-like configuration by staining for cytokeratin. In *lymphoma*, there is homogeneity of cell type, even if occasional epithelial cells are present. In contrast, in *thymic hyperplasia* the normal separation of cortex and medulla usually results in large and small lymphocytes being clustered separately, which suggests their origin from a tissue with normal corticomedullary distinction. There is *characteristic cuffing of small veins by reticular epithelial cells*, creating a clear space resembling a lymphatic, or the epithelium may invest the vessel with laminated stellate cells with plump vesicular nuclei in an onion-ring fashion. In dogs and cats, lymphoid thymomas may contain prominent *germinal centers*, and plasma cells may be present in their vicinity and along the fibrous septa. In these species, follicular activity in thymomas has a high association with autoimmune disease, particularly myasthenia gravis and less often polymyositis. In contrast, germinal centers are not found in thymomas of goats.

Lymphoepithelial thymoma

Tumors of this type are seen most frequently in the *goat and sheep* as space-occupying lesions. They have firm encapsulation with regular but coarse septation, but with minimal stroma in the pseudolobular areas. The proportion of lymphocytes and spindle cells varies from one area of the tumor to another, with focal predominance of either element. Cystic spaces are frequently present, and there may be large areas of hemorrhagic necrosis. *Hassall's corpuscles are present singly and in cornified clusters*, but tend to be less regularly present than in the lymphoid thymomas. Cytologically the lymphocytes are typical of the larger cells of the outer cortex of normal thymus, with compact chromatin and inapparent nucleoli. The epithelial cells are characteristically stellate or fusiform (Fig. 2.109A, B), with oval nuclei with sharply peripheralized chromatin and small central nucleoli. Alternatively, the epithelium occurs in relatively dense *"epithelioid" sheets* that appears syncytial without apparent cellular junctions, and with irregular appearance of small vacuoles containing small lymphocytes with a relatively large surrounding clear halo. Myoid cells may be absent or occur singly in association with lymphocytes rather than with epithelial areas, and may contain striated fibers. Perivascular epithelial cells are more variable than in the lymphoid thymomas, and may be present as focal clusters adjacent to vessels, arranged in rosettes rather than as perivascular cuffs. Germinal centers are not usually found.

Rarely **primary germ cell tumors**, outwardly resembling seminomas, occur in the thymus gland of male humans and animals in the absence of primary tumor in the testicle. Some of these have possibly been described as clear cell lymphomas if phenotypic identification was not carried out.

The revised WHO classification of hematopoietic and lymphoid tumors includes an entity called *mediastinal large B-cell lymphoma*, which is considered one of the variant types of large B-cell tumors. These lesions occur in the dog, and likely also in the cat, but apparently are distinct from the very large lymphomas that characteristically fill the cranial thorax in that species. The mediastinal B-cell lymphomas have a cytology like centroblastic lymphomas, with multiple relatively prominent nucleoli that tend to be located near the nuclear membrane. These lymphomas are also associated with some quite larger nuclei that are often irregularly lobated or indented, all of which mark convincingly with CD79α staining (see Topographic variants of diffuse large B-cell lymphoma, p. 182).

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LYMPH NODES

Structure and function of normal lymph nodes

In the dog, the thymus and spleen are formed at 27–28 days of gestation, and the primordial structures of the largest nodes are present

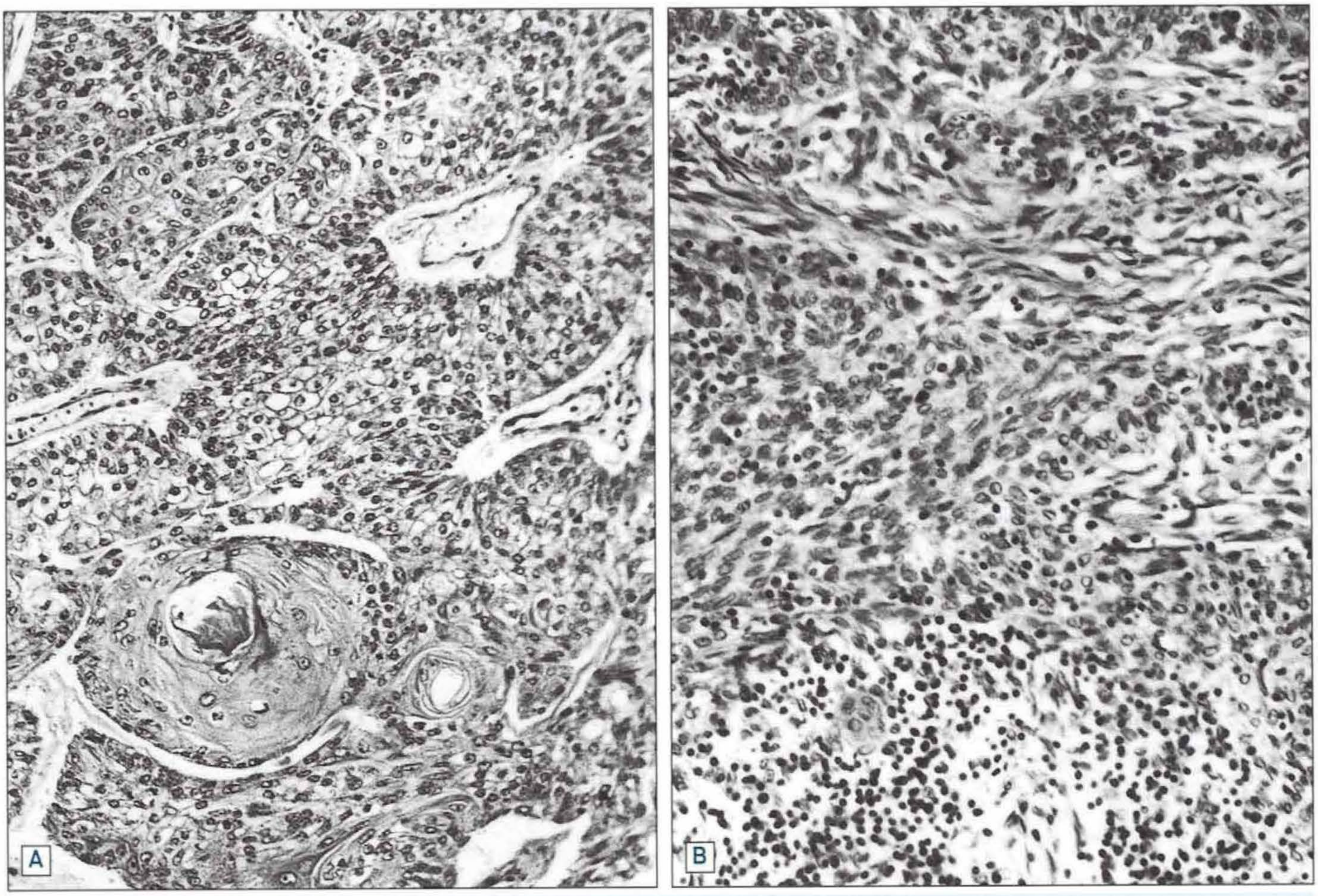

Figure 2.109 A. Thymoma of epithelial type in a dog. **B. Lymphoepithelioma of thymus** to show stromal character of epithelial component in an ox.

at 35–38 days. Lymphocytic colonization of the nodes and spleen is prominent at 52–53 days, and of the thymus at 62–65 days. The lymph node anlage appears to arise from periarteriolar mesenchyme. Lymphatic vessels develop prior to the nodes. At the confluence of lymphatics and arterioles, the lymphatics form cuffs enclosing the arterioles and outpouchings that form the capsule of the primordial lymph node, the hilar area being directed towards the arteriole of origin. As the tissue develops, proliferation of reticular cells within the lymphatic enclosure becomes eccentric to the hilar arteriole, entering the capsule in parallel with the draining efferent lymphatics. The periarteriolar reticular cells form a network that provides an appropriate environment for colonization by thymic and bone marrow-derived lymphocytes.

Lymph nodes, like the spleen, are variably involved in *myelopoiesis* in the fetal stage of development, and convert to *lymphopoiesis* shortly after birth. The actual level of lymph node development at birth varies widely with different species. Calves and foals have well-developed lymph nodes at birth, and may have germinal center formation if there has been intrauterine infection. In contrast, nestlings at birth have readily recognizable lymph nodes in the neck, mediastinal and mesenteric areas; the nodes have a loose reticular structure, very low lymphocyte density, and only limited organization into cortex and medulla.

The **microcirculation** of the mammalian lymph node is closely related to its function, and governs the architectural development of the node. *Afferent lymphatics* enter the capsule and bathe the cortex via the peripheral sinus. *Small lymphatic ducts* perforate the inner capsule of the peripheral sinus and form spherical microanastomotic networks that correspond in size to the germinal centers. On the medullary aspects of these spherical networks, the lymphatics anastomose into larger ducts that drain into the medullary sinuses and then into the major hilar *efferent lymphatics*. In contrast, *blood vessels* both enter (arteriole) and leave (vein) through the hilar region with small vessel arborization in the cortical area. In the outer cortex, small arteries form *meta-arterioles or precapillary arterioles* that perfuse the germinal centers and mantle cell corona. The transition to capillaries occurs at this site, and these vessels then enlarge to form the *postcapillary or high-endothelial venules* (HEV) of the paracortex, across which there is heavy traffic of lymphocytes from the blood to the lymph node parenchyma. These vessels then unite to form progressively larger veins which follow the medullary stroma to form the *efferent hilar vein*. In *pigs*, the cortex and medullary areas of lymph nodes are inverted, but the afferent lymph passes in lymphatics to the center of the node where the germinal centers are normally located. Like other mammals, recirculating lymphocytes enter lymph nodes through postcapillary high-endothelial venules and distribute to T and B areas, and emigrate directly into the peripheral venous system rather than the efferent lymphatics as in other mammals.

The various vascular structures of lymph nodes form characteristic associations with nodal tissue. The *lymphatics* are identifiable as major ducts entering the peripheral sinus but the intranodal lymphatics are not obvious, except for the large medullary sinuses and the efferent hilar vessels. The *arteries* can be identified entering the medullary or hilar area of the node, and they follow the fibrous trabeculae to the medullary sinuses. In this area, the arterioles form a regular and obvious network with the medullary cords, such that the arterioles are centrally placed in the sinuses and connected by a network of reticular stroma to the thin limiting membranes of the medullary cords. The smaller *arterioles* of the paracortex are irregularly visible, as are the terminal arteriolar vessels of the germinal centers and the mantle cell cuffs. In the adjacent paracortex, the *postcapillary venules* are readily identified. *These vascular structures of the blood and lymphatic systems through their interactions form three endothelium-lined compartments, intravascular, intralymphatic and interstitial,* which provide the functional architecture by which the lymph node filters lymph, processes antigen, and returns lymphocytes from blood to tissue.

Functionally, this microcirculatory arrangement directs antigens entering the cortical area in lymphatics to the germinal centers and the outer cortex. The **germinal center** *has a specific polarity directed at the source of antigen,* which in those germinal centers near the outer cortex can directly be seen to be oriented to lymph flow from the peripheral sinus. The germinal center is surrounded by an elliptical cuff of small B lymphocytes that is thicker at the superficial pole region near the peripheral sinus and thinner on the deep pole region adjacent to the deeper areas of paracortex. Within the germinal center itself, the cells in the superficial area have smaller nuclei with more abundant cytoplasm, which produces a lighter staining area on low-power examination. In contrast, the cells in the deep pole of the germinal center have larger nuclei with minimal cytoplasm, resulting in this area having a darker appearance. Within the central area of the germinal center, there are large macrophages that contain ingested apoptotic debris, representing lymphocytes that have undergone somatic hypermutation and have not been selected for further development. Depending on the plane in which the germinal center is sectioned, these large macrophages may form a complete field with a "starry-sky" effect across the circumference of the germinal center. *This polarity of germinal centers is a constant feature of lymphoid tissue in lymph nodes, tonsil, and Peyer's patch,* and is always most readily observed in relation to antigen in those follicles near the peripheral sinus. *Recognition of polarity is an important aspect of differentiating benign follicular hyperplasia from follicular lymphoma.*

Germinal centers that lie deep within the medulla in cases of follicular hyperplasia (Fig. 2.110A) do not have an obvious orientation to the capsule, but it can be assumed that the deep and superficial poles are oriented to the source of antigen and the fine network of lymphatics that drain the peripheral sinus. Paracortical tissue may appear as a solid band encompassing the germinal centers, as is typical of the young. With maturity, paracortical areas irregularly recede and regenerate in loosely defined, but densely aggregated, "*deep cortical units*" (Fig. 2.110B). These units are also defined by cellular composition and vascular supply as are the germinal centers, but, in contrast to the latter, *the deep cortical units are thymus-dependent areas populated by T cells.* There are *gaps* in the inner layer of the outer sinus by which the entering lymph can pass directly to the medullary sinuses. Presumably this route is taken by heavy but nonthreatening flow as occurs when frank blood enters the lymph node during biopsy (red cells appear in the medullary sinuses within minutes of the initiating surgical trauma).

The processing of antigen occurs at random in lymph node cortex but is always associated with a background of **dendritic reticular cells** *that function as antigen-presenting cells of the germinal center.* A portion, if not all, of the follicular dendritic cells arise from Langerhans cells that develop contact sensitization with antigen in epidermal areas drained by the particular lymph node. Langerhans cells in epithelial areas bind antigen relatively inefficiently, but within the germinal centers they undergo maturation in the process of forming tight junctions with other dendritic cells, thus forming a background network for the lymphoid follicles. Blood monocytes cultured in the presence of macrophage colony-stimulating factor (M-CSF) or granulocyte macrophage CSF and IL-4 differentiate without proliferation into CD14-positive macrophages or CD1A-positive dendritic cells. It is believed that this process is repeated in lymph nodes for the *maturation of Langerhans cells to dendritic cells of the germinal centers, and monocytes for differentiation into dendritic cells of the paracortical areas.* The process of B-cell maturation then involves the interaction of B lymphocytes that have undergone immunoglobulin chain rearrangement in preparation for antigen-driven selection by hypermutation. This process takes place with the assistance of T-helper cells and on the basis of antigen presentation in the context of MHC cellular recognition. B lymphocytes bound in this manner are selected for further division, finally to become plasma cells or memory B cells, while those that fail to be bound in the T-lymphocyte interaction undergo programmed cell death and are removed by follicular macrophages.

The dendritic cells of the germinal centers are long-lived and may retain antigen on their surface membranes for weeks or months in the process of antigen focusing, by which the specificity of a germinal center for a specific antigen is maintained. The dendritic cells of the paracortical nodules are also long-lived and, in superficial nodes of humans and cattle, they often contain Birbeck granules characteristic of the Langerhans cells of the skin, consistent with an origin and previous experience in that area. There is functional logic in this arrangement since the follicular macrophages and dendritic cells (B area) are efficient in the digestion and processing of large particulate antigens, whereas those in the paracortex (T area) are more efficient in trapping low molecular weight substances, such as agents producing contact sensitization in the skin, where the resident reticular cells likely had their initial training. *In general, the lymphocytes in the germinal centers are largely B cells while those in the paracortex are largely T cells.* The subsets of T cells are also segregated, but not as cleanly, with the *suppressor and NK cells confined almost entirely to the paracortex,* whereas *the helper cells are largely in the paracortex but also in the germinal centers and their small cell corona of mantle cells.*

The process by which lymphocytes exiting the marrow and thymus are directed to specific tissues is regulated by *homing receptors* on the lymphocytes, called *intercellular adhesion molecules (ICAM),* which bind to tissue specific *addressins* on high-endothelial or postcapillary venules in the peripheral lymphoid tissues. The characteristic development and binding of hematopoietic progenitor cells is mediated by adhesive functions of cell surfaces, called integrins and selectins, which are members of the immunoglobulin superfamily and identified by the CD44 reagent. There are more than 20 types of *integrins,* which are heterodimeric transmembrane proteins, and three major subtypes of *selectins* each with an affinity for various ligands of endothelial cells in different areas of the body. By these means, naive B and T cells leaving the bone marrow are guided to specific areas

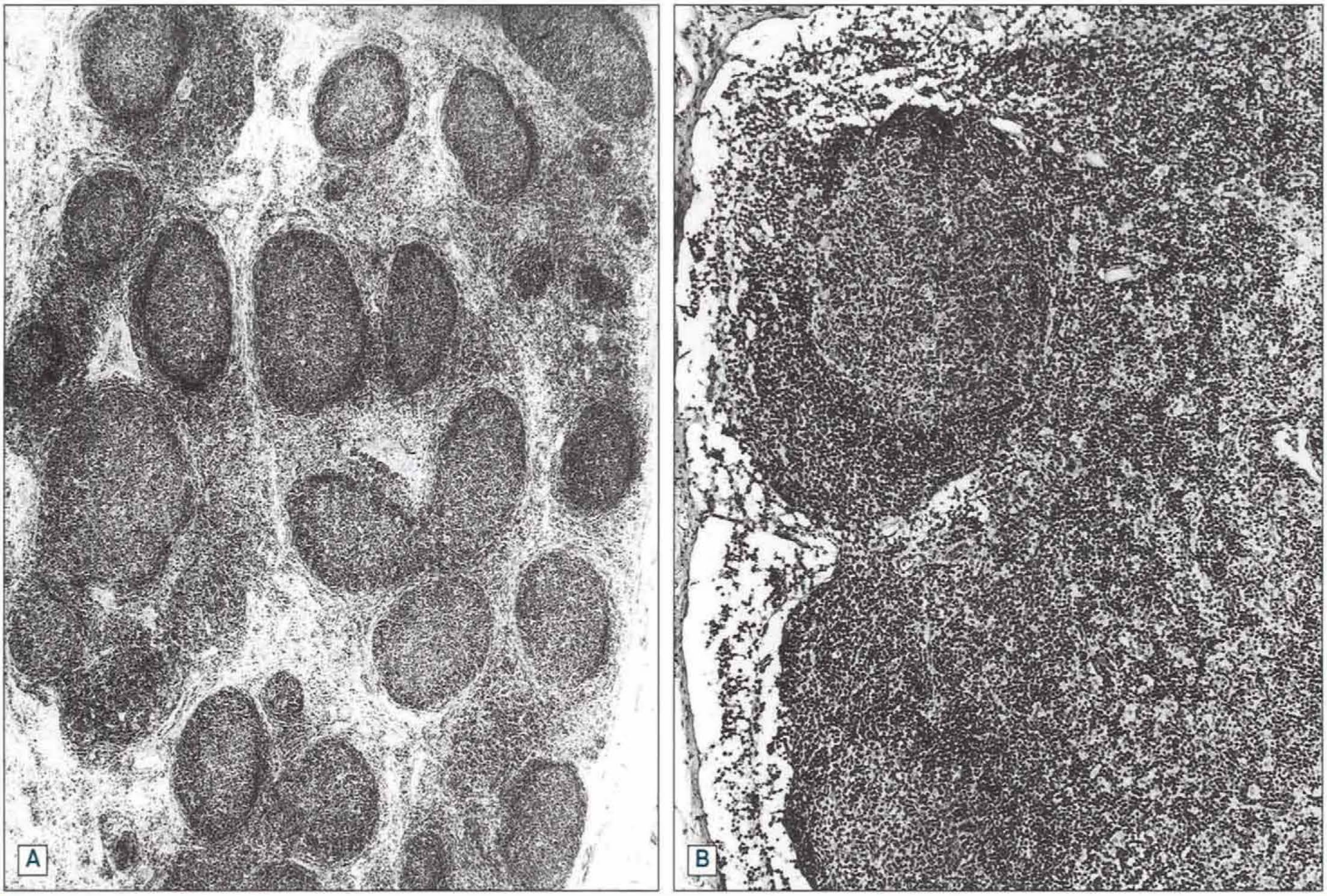

Figure 2.110 A. Follicular hyperplasia in a lymph node of a cat. **B. Paracortical hyperplasia** with typical "starry-sky" effect in stimulated lymph node of a dog.

of maturation in the thymus, intestine, skin and lymph nodes and memory cells are directed to the endothelium of specific tissues. It is worth mentioning in conclusion that *all of the hematopoietic-derived cells resident in lymph nodes are capable of forming malignant tumors including the interdigitating reticular cells.* This is in contrast to earlier concepts that the cells of lymphomas of B- and T-cell types remain relatively confined to the initial site of malignant transformation until late in tumor progression. It is now understood that *neoplastic lymphocytes from either bone marrow or peripheral lymphoid tissues may circulate at low levels early in the development of disease.* Their ability to disseminate is dependent upon the development of cell surface proteins, which mimic those of their benign counterparts, and permit them to bind to endothelium and extravasate to new tissue sites.

The general reactions of nodes to various stimuli are described in the introduction to the lymphomas, and that section is pertinent to the following consideration of non-neoplastic disease involving this tissue.

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Degenerative diseases of lymph nodes

The lesions associated with inherited diseases of the immune system are included in Developmental diseases of the thymus. In *severe combined immunodeficiency*, lymph nodes lack both germinal centers and paracortical lymphoid colonization. In *severe T-cell deficiency*, germinal centers are present, as is antibody, but paracortical areas are hypoplastic and the spleen lacks periarteriolar lymphoid sheaths. In *severe B-cell deficiency*, as occurs in agammaglobulinemic foals, germinal centers are not formed in node or spleen, and plasma cells are not found. The paracortical areas have normal cellularity, and cell-mediated immunity is intact.

A strain of black pied Danish cattle, and probably some other European breeds, carries an *autosomal recessive trait for thymic deficiency* (lethal trait A46). The calves appear normal at birth, but by 1–2 months of age they die of disseminated infection that originates in the skin. These calves are able to produce antibody, but *lack cell-mediated immune responses*. The defect appears to be an *inability to absorb zinc* and, if zinc is provided, they recover full immunologic capability and cutaneous signs of zinc deficiency disappear. *Lethal acrodermatitis*, a similar syndrome, occurs in Bull Terriers as an autosomal recessive trait; clinical signs are not relieved by treatment with zinc.

Thymic atrophy develops in an inbred strain of *Weimaraner dogs* that are able to produce antibody but in which cell-mediated responses are depressed.

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Developmental diseases of lymph nodes

Cachectic and senile atrophy lead to moderate reduction in the overall size of body nodes. Aging changes are largely limited to mild thickening of the capsule and medullary trabeculae, with reduced density of germinal centers.

- **Senile atrophy** is seldom of note in large domestic animals, and is only seen with frequency in *dogs, cats and primates*. Grossly, senile lymph nodes are small with edematous fascia, and characteristically have dark-brown pigmentation of medullary areas that may extend in decreasing concentration to the subcapsular sinus.
- **Cachectic atrophy** is frequently encountered in *old sheep and goats* with dental attrition. Nodal atrophy associated with cachexia is much more often encountered, and is characterized by retention of the overall architecture with marked reduction in cell density. Germinal centers are small with a hypocellular mantle cell corona, and paracortical areas are sparsely populated. In old animals with cachexia, there are numerous pigment-bearing macrophages in medullary sinuses, and thin proteinaceous fluid may be present in medullary cords and sinuses.

Hemorrhage originating within a node or in tissues drained by the node may be visible in the cortical or medullary sinuses. Red cells delivered to the node through the afferent lymphatics tend to pass through the cortex to the medullary sinuses where they are ingested by macrophages. The breakdown of erythrocytes within lymph nodes results in accumulation of *hemosiderin*, which is always most prominent in medullary areas. In cattle and sheep that die with acute respiratory distress, the cervical nodes are frequently red-black owing to the blood contained in them, much of which comes from the tracheal mucosa where extravasations begin in the lymphoid nodules.

Anthracosis is a regular finding in the pulmonary nodes of *dogs* that live in industrial areas, and is of lesser frequency and degree in cats. The carbon is retained solely in the phagocytes, principally those of the medullary cords, and therefore only the medulla is black. The pigment is inert and of no consequence. An unidentified black pigment, in appearance like melanin, is present in the hepatic nodes of *sheep and cattle* when they have, or have had, hepatic distomiasis. The same pigment occurs in bile ducts infested by *Fasciola hepatica*, and, in the case of *F. magna*, wherever the fluke has wandered.

In **emphysema** of lymph nodes, gas is confined to the sinuses. It affects the mesenteric nodes of swine in association with intestinal emphysema. Emphysema of the bronchial nodes commonly accompanies interstitial pulmonary emphysema of cattle. The lymph nodes are puffy and light. The sinuses are distended and their endothelium is lined by large macrophages and even giant cells, the mobilized cells occurring in small clusters of spotty distribution on the walls of the sinuses.

Lymphoid atrophy, with or without architectural alteration, occurs in a variety of circumstances. Lymphoid atrophy associated with marked dilation of medullary sinuses, termed *vascular sinus transformation*, results from blockage of the efferent lymphatics, and is exacerbated by the development of fibrosis if the venous drainage is also obstructed. These conditions may follow surgical intervention for lymphatic cannulation or occasionally develop in animals that have been recumbent for prolonged periods and suffered concurrent venous infarction of muscle.

Complete or focal infarction of node occurs with lymphoma, and may be the first sign of disease. Infarction is most likely to occur with large-cell lymphomas and, while vascular obstruction appears to be the cause, thrombosis is seldom observed. Infarction with lymphoma is most often seen in the calf form of sporadic bovine lymphoma.

Inflammatory diseases of lymph nodes

Lymphadenitis *results when an infectious agent is present in the lymph node*, as distinguished from *benign lymphoid hyperplasia*, in which the node is immunologically reactive but free of local invasion. Lymphadenitis may result from drainage to the node of the products of a distant inflammatory process that then progresses to involve the node directly. It may be possible to identify the infectious agent responsible for the lymphadenitis or to determine the type of invading agent from examination of the changes in the node (Fig. 2.111A, B).

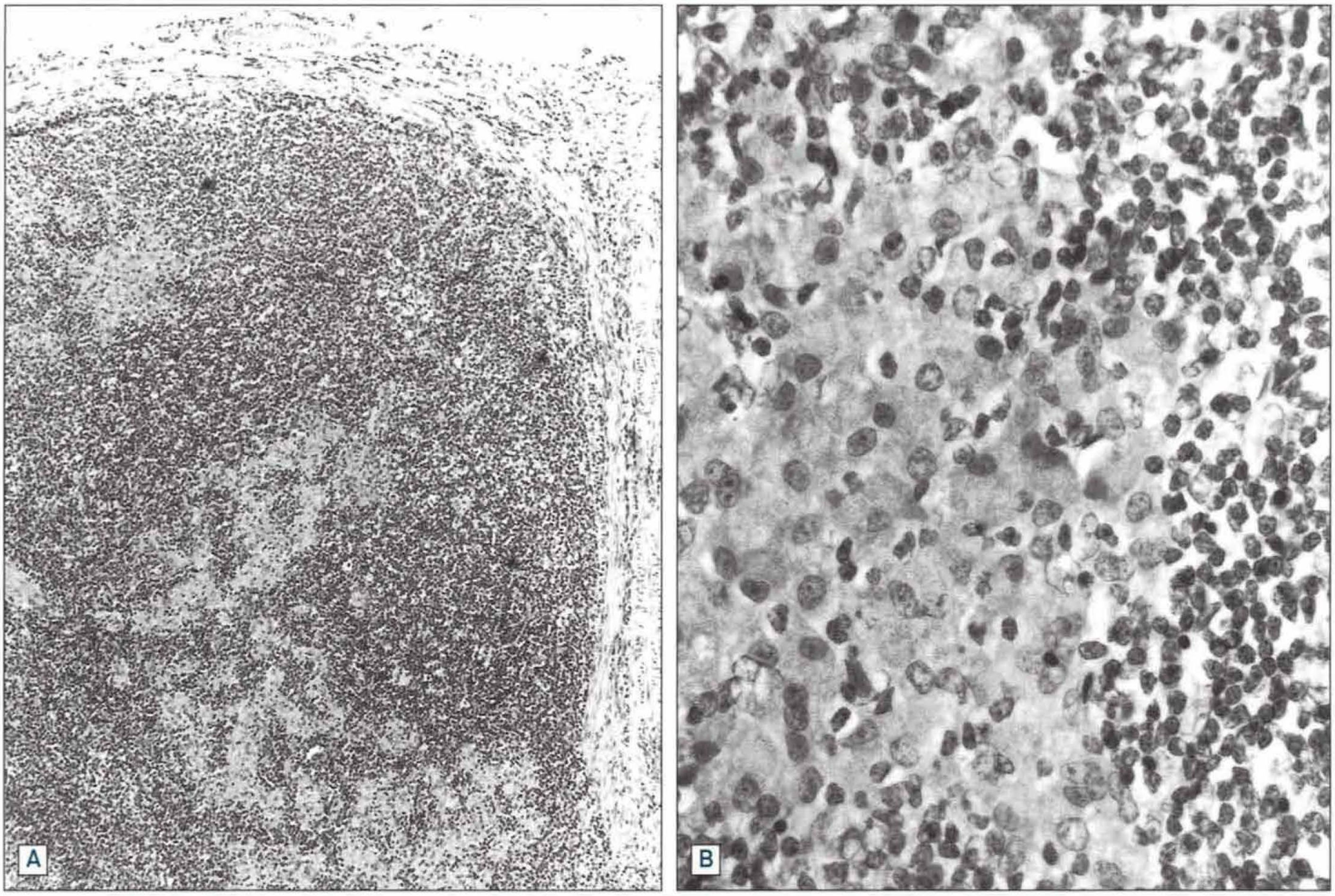

Figure 2.111 Johne's disease in a lymph node of a goat. **A.** Capsule thickened and colonized by inflammatory cells, peripheral sinus narrowed, paracortical hyperplasia. **B.** Epithelioid macrophages of sinuses of (**A**).

Lymphadenitis may be acute or chronic, suppurative, caseous, or granulomatous. These more or less specific types of lymphadenitis are discussed under specific diseases.

Cytologically, **acute lymphadenitis** is characterized by a mixture of small and large lymphocyte types accompanied by neutrophils and eosinophils that may constitute 25% or more of the cells present. Distribution of cell types present, including large macrophages, and the quality and character of the neutrophil nuclei are indicative of the cause of the process, which may be apparent if the phagocytic cells present have ingested bacteria. Characteristically, in lymph nodes involved with blastomycosis, there is a mixed cellular or granulomatous response with the infectious agents often obscured by tightly adherent neutrophils and macrophages. The chromatin pattern of lymphocytes in acute lymphadenitis differs from lymphoma in that large chromocenters persist in small lymphocytes, and larger lymphocytes, which are presumed to be the proliferative population, maintain larger chromocenters in benign conditions but tend to have a more uniform chromatin pattern characteristic of cells in cycle in lymphomas. Needle aspirates from lymph nodes may occasionally yield large numbers of necrotic cells, and it should be kept in mind that focal lymphadenopathy may be due to metastatic tumor.

Grossly in acute lymphadenitis, the lymph nodes are enlarged, soft, locally mobile and hyperemic to a variable degree. The capsule is taut and thinned due to the influx of cells, and on the cut surface the parenchyma bulges and exudes blood and lymph, but mainly the latter. Thus, if imprint preparations are made, it is necessary to repeatedly remove the exuding lymph from the cut surface in order to obtain enough adhesion for cellular exfoliation. There may be focal discoloration due to previous pigmentation or to infarction.

Histologically, acute lymphadenitis is characterized by marked hyperemia with unusual prominence of small vessels in cortical areas. Neutrophils are often present, both through drainage from the subcapsular sinus and by migration from the postcapillary venules. Some useful distinction can be made between granulocytes arriving from these two sources. In general, neutrophils in the peripheral and medullary sinuses indicate inflammation in the tissue area drained and are not necessarily indicative of local sepsis. On the other hand, foci of neutrophils in cortical areas are of hematogenous origin and almost always are reactions to bacterial colonization, as is likely to occur in the mesenteric node in bacterial enteritis. When the inflammation is due to one of the pyogenic organisms, abscessation is likely. Necrosis in lymph nodes is an attribute of some acute infections, such as salmonellosis and toxoplasmosis (Fig. 2.112).

In **chronic lymphadenitis**, hyperemia and edema are irregularly present, and the infected nodes are large and firm, and may be fixed to local tissues if there has been cellulitis. The capsule is thickened as

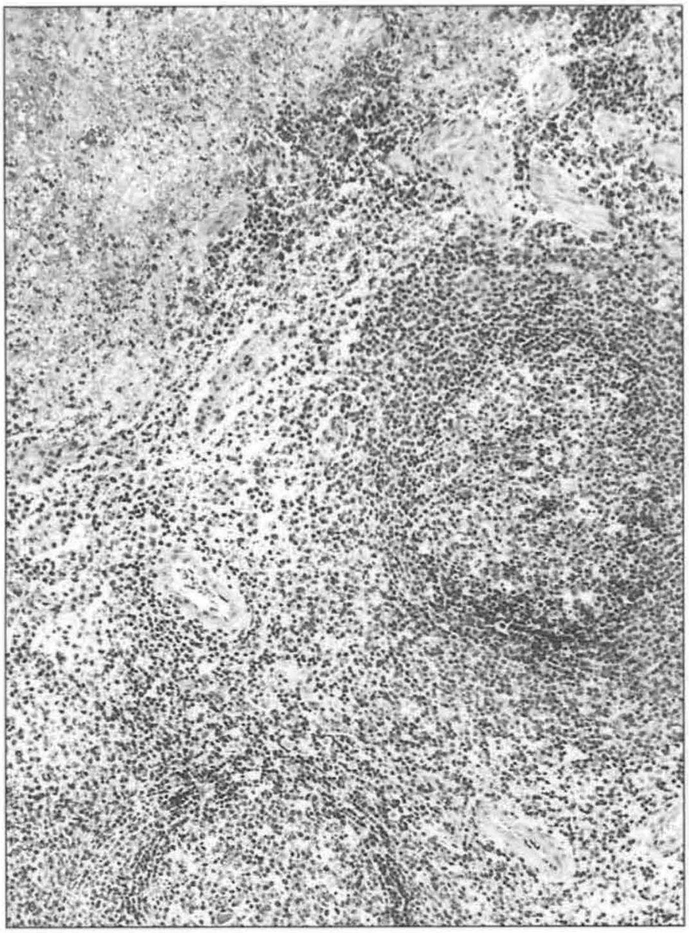

Figure 2.112 Acute lymphadenitis with subcapsular necrosis, in toxoplasmosis in the lymph node of a cat.

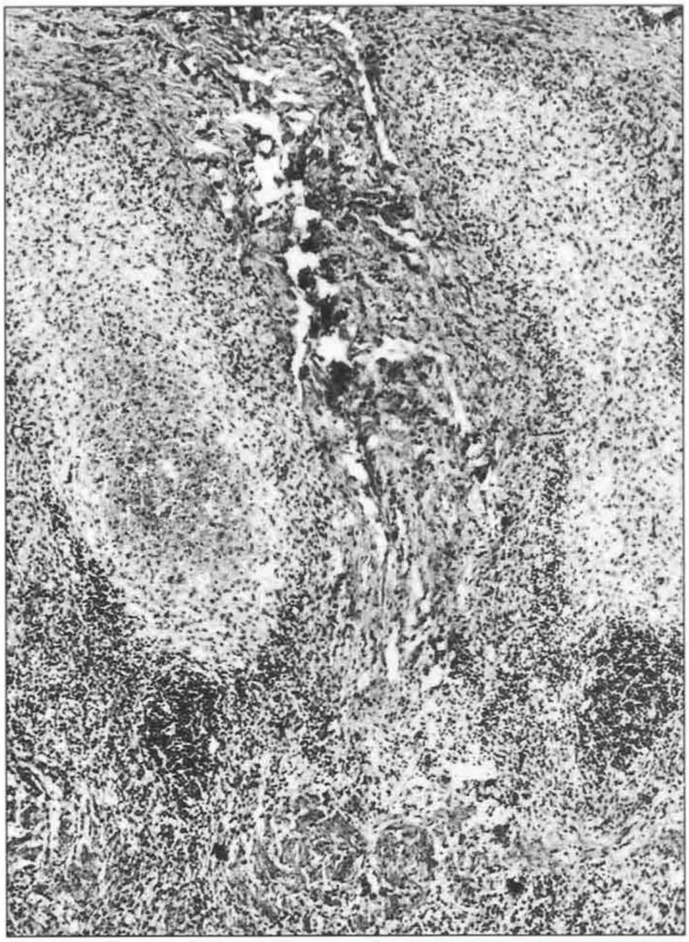

Figure 2.113 Lymphoid follicular necrosis in intestine, in parvoviral enteritis in a dog.

are the internal trabeculae, and with prolonged inflammation the node becomes dry and hard. Changes of this type are characteristically present in the supramammary lymph nodes of cows with brucellosis of long standing, and to a lesser extent in animals with chronic or recurrent bacterial mastitis. In the latter cases, the marked proliferation of the collagenous septa of the medulla may completely displace the medullary cords.

Lymphadenitis may be expressed by changes that are largely degenerative and affect the architecture of the tissue. Thus in some acute viral infections, there is rapid lysis of lymphocytes, as in *canine parvoviral infection* (Fig. 2.113) and in *rinderpest*. In *salmon poisoning* of dogs, the nodes may be much enlarged with follicular hyperplasia and accumulation of macrophages (Fig. 2.114A). In some chronic infections, particularly with the viruses causing immunodeficiency, the nodes are atrophic with wrinkled capsule and depletion of lymphocytes, and with sinus histiocytosis if the production of monocytes by the bone marrow remains competent (Fig. 2.114B).

Parasitic infestations of lymph nodes

The lymph nodes are not a final habitat for any nematode, but some species traverse them in migration and some lodge there accidentally. The larvae of *lungworms* travel by lymphatics from the intestine to the lungs but cause little injury in the course of these migrations. The first-stage larvae of those lungworms that reside in the alveolar spaces, *Muellerius* spp. and *Protostrongylus* spp. particularly, often find their way to the bronchial nodes and there produce granulomatous lymphadenitis. The nodes become enlarged, hard and irregular, and have many firm granulomas and encapsulated caseocalcareous nodules. The larvae of *Strongylus* spp. in horses occasionally produce hemorrhagic lymphadenitis of the abdominal lymph nodes that, when secondarily infected, may suppurate. *Larval trematodes*, probably *Fasciola hepatica*, are often found in the mesenteric nodes of ruminants, as are larvae of *Oesophagostomum columbianum* in sheep. Nodules develop in the cortex or medulla, as well as in capsular tissues. They are 2–5 mm in size and contain yellow-green pus, which in older lesions becomes caseous and calcified. The pus is largely the debris of eosinophils and it is surrounded by giant and epithelioid cells and a well-defined capsule. A very similar lesion is produced by the larvae of *Linguatula serrata*. The larvae of this parasite (see Vol. 2, Respiratory system) are, in some countries, very common in the mesenteric nodes of sheep and cattle. Viable larvae can be found in normal nodes, but usually the nodes show focal or diffuse hyperplasia and edema, with the larvae lying in cavities that contain a milky fluid resembling chyle. Apparently, the larvae die in the nodes and become encysted in abscesses, which may calcify. In active cutaneous infestations by *Demodex* spp. in dogs, the parasite is often found in the peripheral lymph nodes.

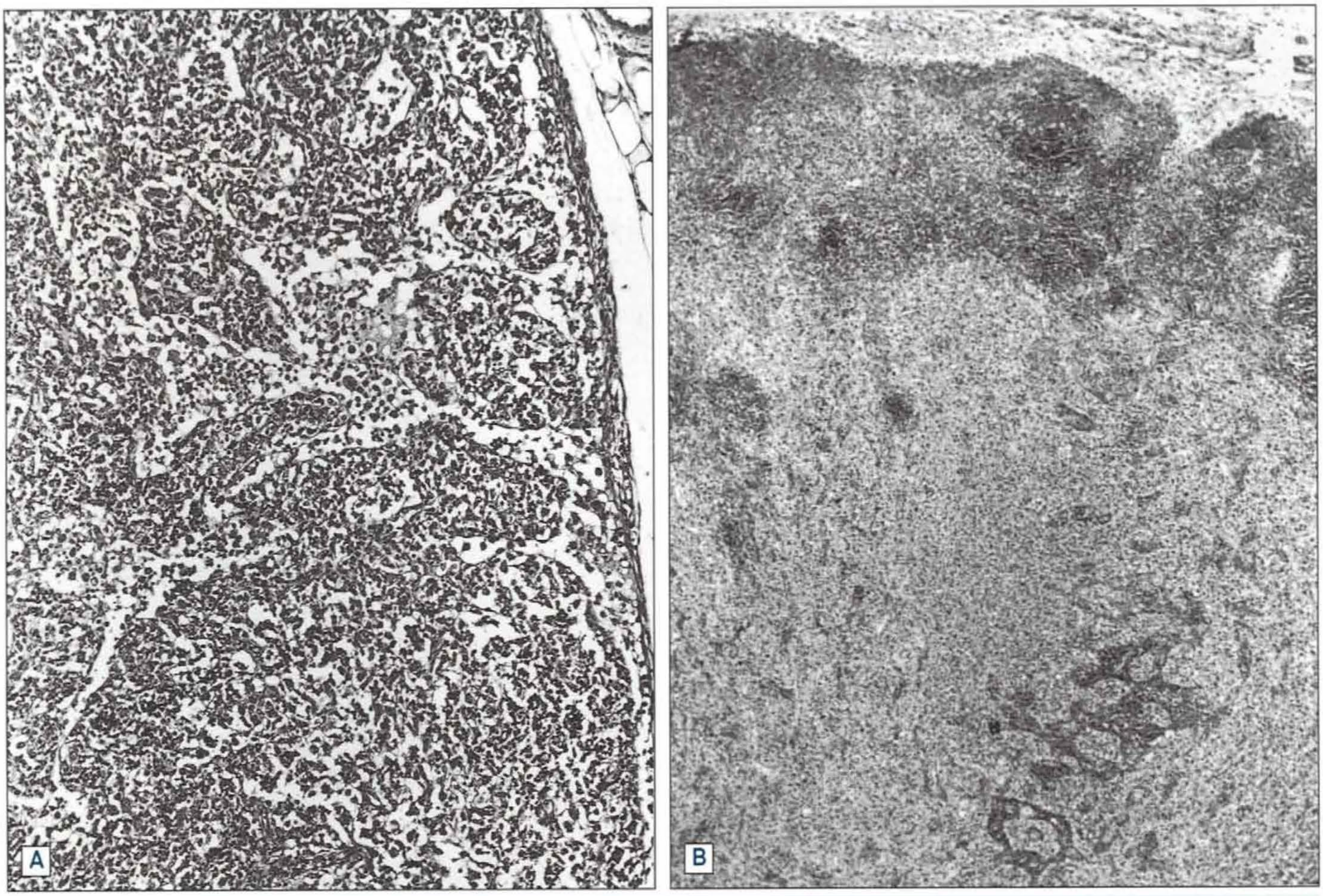

Figure 2.114 **A. Depletion of follicles and paracortex** and proliferation of sinus histiocytes in a lymph node, in salmon poisoning in a dog. **B. Medullary sinus hyperplasia** in a lymph node, in bovine viral diarrhea in an ox.

Hyperplasia of lymph nodes

Lymphadenopathy *is defined as a regional or generalized lymph node enlargement of unknown or unspecified cause.* **Lymphadenosis**, while not commonly used, more correctly defines generalized lymphoid hypertrophy, which the former term is used to indicate. *Local enlargement* usually reflects a pathologic process limited to the drainage area, particularly inflammatory or neoplastic disease. *Generalized enlargement* of lymph nodes is a more serious finding, since the possibility of primary tumor is much more likely. Infectious causes of generalized lymph node enlargement include tuberculosis, brucellosis, and protozoal infections. Generalized lymphadenopathy produced by an extraordinary degree of paracortical blastogenesis is typical of the early phases of malignant catarrhal fever and theileriosis in cattle. There may be mild generalized lymph node enlargement in adrenal atrophy, and lymphoid hyperplasia to some degree is transient in most young animals. Endocarditis or abscessation may cause generalized lymphadenopathy, as will lymphoma. In general, in acute septic inflammation the lymph nodes are normally mobile within the subcutaneous tissues, but may become fixed with chronic sepsis. Similarly, lymphadenopathy due to lymphoma usually does not cause fixation of the glands in the early stages, but there is usually perinodal colonization with loss of mobility in advanced stages.

The *cytologic picture* in benign lymphadenopathy is variable, with small and large lymphocytes, with cleaved and noncleaved nuclei, and macrophages, plasma cells and neutrophils. Unless the stimulation is less than 10 days old, this mixture of cells should be organized architecturally with evidence of follicular development. In contrast, in primary tumor there tends to be a monomorphic or bimorphic cell population and, in animals in which follicular lymphomas are uncommon, *finding a monomorphic cell population with diffuse architecture suggests lymphoma.* In primary tumor, macrophages may be present, and numerous if there is a high mitotic rate in the tumor and a high rate of cell death. In contrast, plasma cells and neutrophils tend to be less numerous than in lymphadenitis, although the presence of plasma cells in itself is no guarantee of a benign reaction. *The most important criteria for distinguishing lymphoma from hyperplasia are the integrity of normal architecture and the uniformity of cell and chromatin type.* Architecturally, the peripheral sinus is preserved, even in reactive states where there is perinodal colonization by benign lymphocytes. In contrast, the outer sinus is regularly encompassed and destroyed by even low-grade lymphoma of advanced stage.

In terms of the chromatin pattern, *benign and reactive lymphocytes have less internal nuclear detail than their malignant counterparts* and will have a variable amount of chromatin contained within large chromocenters irregularly distributed throughout the nucleus. In inflammatory disease, the chromatin tends to have a fine gradation from light to dark areas, which includes lightly stained but not clear areas. The nucleoli characteristically have a uniform rimming of aggregated chromatin, which makes them appear unusually prominent. In lymphoma, large chromocenters are fewer, and the chromatin pattern tends to be monotonously similar, most often with a retiform or branched pattern with heavy chromatin bands and irregular clear areas, as occurs in the small noncleaved and large cell tumors. Large-cell lymphomas of the centroblastic, and more particularly, the immunoblastic type, tend to have vesiculated nuclei that appear larger because of the internal parachromatin clearing, and prominent and often irregular peripheralization of chromatin causing apparent irregular thickening of the nuclear membrane. In neoplastic lymphocytes, the chromatin aggregations on nucleoli are irregular and discontinuous. Less commonly, tumor cells have cribriform or uniform chromatin patterns without chromatin aggregation, as occurs in lymphoblastic lymphoma. There is more variation in nucleolar size, shape and number in malignant nuclei than in benign nuclei.

One of the most difficult distinctions is between diffuse hyperplasia and lymphoma, and in these cases the decision must be based upon both the architectural changes and the character of the cells interpreted in terms of the history and clinical signs. In the early, marked, immune response, there will be diffuse parafollicular hyperplasia with effacement of pre-existing follicles. The paracortex has a moth-eaten appearance due to the mixture of cells present, which includes many large macrophages and pale dendritic reticular cells. Lymphomas of the T-cell-rich B-cell (diffuse mixed) type have in some proportion small-cleaved and large cells in every field, with the latter benign population predominating. *There are no lymphomas with three or more lymphocyte types regularly present*, and this fact tends to assist in correctly interpreting the early diffuse lymphoid proliferations. If a firm decision cannot be made, it is fully justifiable to request a repeat biopsy in 10 days, at which time a diffuse benign reaction will have developed a follicular pattern.

In **follicular hyperplasia**, even with extension of follicles to the medullary areas, there will be preservation of a narrow but intact mantle cell corona and at least a narrow band of paracortex between the follicles. In contrast, in **follicular lymphomas** the follicles tend to appear "bare" as a result of mantle cell and paracortical atrophy. Follicular lymphomas are composed of cells that are cytologically similar but of different density. In contrast, in follicular hyperplasia, there is marked variation in cell type across the diameter of the follicle as described earlier in regard to the *antigen-oriented polarity of the normal germinal center.*

Some of the more difficult distinctions between hyperplasia and primary malignancy of lymph nodes must be made in hyperimmune diseases. *Eosinophilic myositis* causes a remarkable alteration in lymph nodes with generalized sclerosis and thickening of the capsule, which is irregularly colonized by small lymphocytes and eosinophils in linear arrays, and marked thickening of the cortical and medullary trabeculae. There is diffuse follicular hyperplasia with paracortical atrophy and medullary cord hyperplasia with prominent plasma cell accumulation (Fig. 2.115A, B). Germinal centers vary markedly in size and shape and have accentuation of the large and small cell layers, and sinuses contain a dense matrix of epithelioid macrophages and eosinophils. Eosinophils are also prominent throughout the collagenous trabeculae. *Brucellosis* and *trypanosomiasis* present no problem in distinction from cancer, and result in a regular picture of follicular hyperplasia followed by paracortical atrophy, with generalized sclerosis and medullary cord hyperplasia.

An unusual type of follicular hyperplasia occurs in *old sheep and goats* that are in poor condition and may have been destroyed due to debility. There is regular capsular thickening and increased prominence of medullary trabeculae. The follicles may be very large, angular and facetted in an irregular fashion. Paracortical atrophy is marked, and there is medullary cord hyperplasia and empty medullary sinuses. The most marked changes affect the follicular vessels, which are large, tortuous arterioles with hyalinized media and often mineralized endothelium. The follicular centers include large epithelioid macrophages and often a very marked tingible body reaction. This reaction resembles *angiofollicular lymph node hyperplasia of the hyaline vascular type* as it is described in humans.

A *nodular reaction of the paracortical areas* occurs in lymph nodes draining malignant tumors as well as other conditions, and appears to represent an exaggerated thymus-dependent response. The nodules may be multiple and coexist with follicular atrophy, raising concern that they represent a multifocal diffuse lymphoma. *The nodules represent deep cortical units.* They are centered on networks of dendritic reticular cells and are densely cellular, but with the cytologic variation in cell type characteristic of paracortex. These paracortical nodules, unlike germinal centers, are irregularly defined from the surrounding paracortex by a lighter staining border of larger pale-staining dendritic cells and macrophages and fewer small densely stained lymphocytes.

A *distinctive peripheral node hyperplasia of young cats* is illustrative of the difficulty in separating strong reactive hyperplasia and neoplasia. It is usual in these cases, which range in age of onset from about 6 months to 2 years, for all peripheral nodes to be enlarged. The nodes are firm with smooth capsules and yellow-tan to white on cut surface. The architecture is severely distorted with loss of discernible trabeculae and of subcapsular, trabecular and medullary sinuses. Cortical lymphoid follicles may be many and large and in some areas they are encroached upon and partially obliterated by the expanded paracortex. The paracortex is comprised of a mixture of lymphocytes, blast cells, histiocytes and plasma cells with abundant mitoses and starry-sky appearance. The paracortical expansion may involve the capsule and adjacent adventitial tissue. High-endothelial venules are prominent in the paracortex. *The cause of this lymphadenopathy in cats is not known.* Recovery is expected after a course of variable duration. A similar lymphadenopathy can be caused by feline leukemia virus infection, as an unusual response to that virus. Argyrophilic intracellular bacteria are described in some of the spontaneous cases of lymphadenopathy but their nature and role remain to be determined; of interest is the morphologic similarity of these organisms to those described in humans affected by cat-scratch disease.

One of the more common indications for lymph node aspiration in dogs and cats are those associated with *chronic dermatitis or otitis.* Lymph nodes draining areas of pigmented skin that have been chronically inflamed frequently contain melanin-bearing macrophages in the outer cortex and these are frequently present in aspirates of draining nodes. Cytologically, *the melanin granules in melanocytes and macrophages from benign sources are of uniform size, shape and pigmentary density, and in the dog resemble tiny rice grains.* In contrast, melanin granules that are associated with pigmented melanomas are markedly

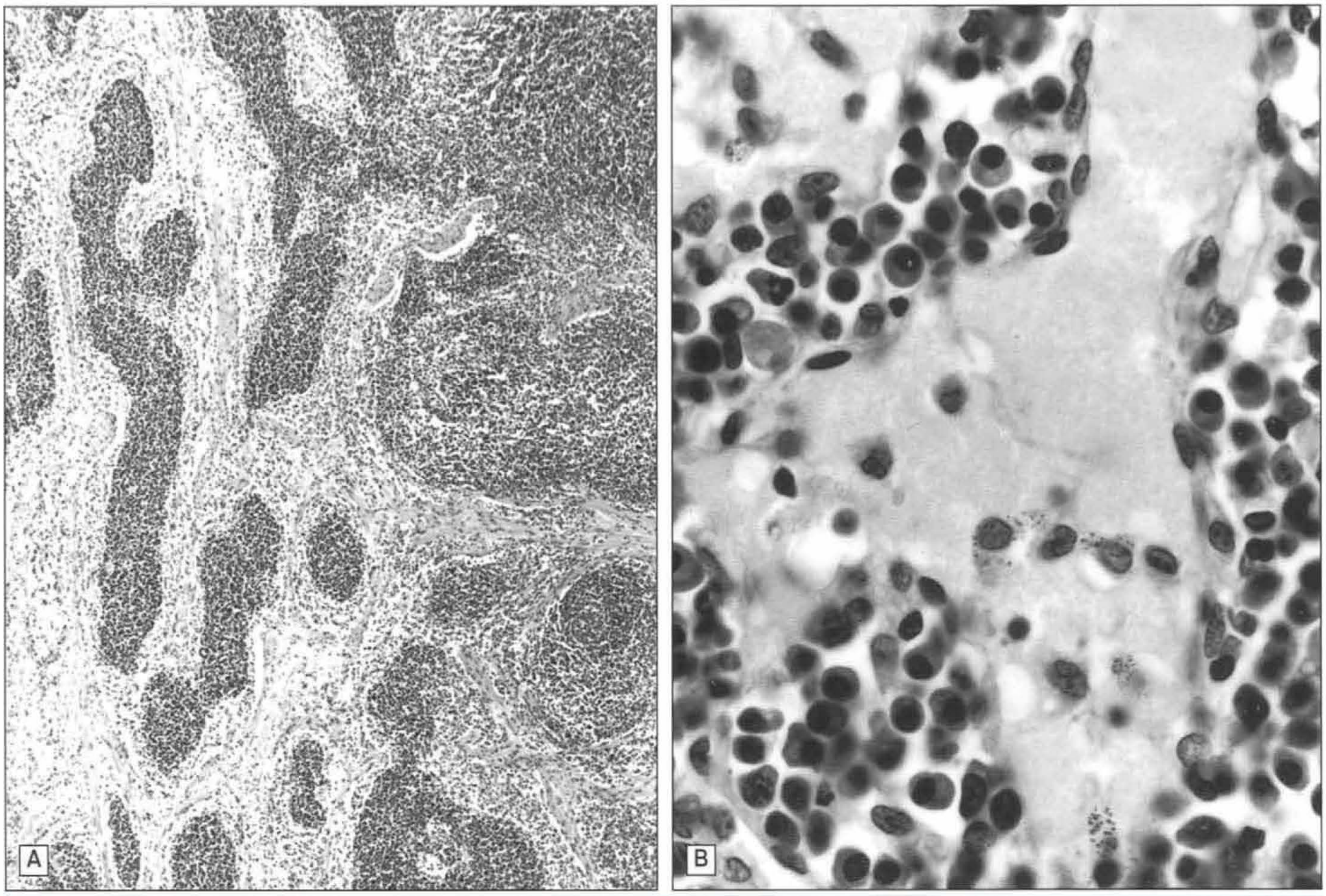

Figure 2.115 Medullary cord hyperplasia, lymph nodes. **A.** Characteristic of chronic immune response. Cords are sharply defined by high density of small lymphocytes and mature plasma cells. **B.** The sinus macrophages contain small granules of hemosiderin in a dog with leishmaniasis.

variable in size, shape and staining density and are more likely to be associated with a sclerotic reaction in the node.

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Neoplastic metastatic diseases of lymph nodes

The general reaction of lymph nodes to nonlymphoid cancer is atrophy of greater or lesser degree due to the systemic effects of cancer, apparently mediated by tumor necrosis factor. If the tumor causes inflammatory drainage to local nodes, these will have changes of *reactive hyperplasia and lymphadenitis*, as occurs in mesenteric nodes draining an area of ulceration due to carcinoma. It is significant that the specific reaction of local nodes to local tumor is usually little more substantive than that of nodes distant from the primary site of malignancy.

In general, *the response of local nodes to adjacent cancer is sinus histiocytosis*, and it has been felt that the strength of this reaction is as important to the prognosis in a given case as the characteristics of the tumor itself. *It is therefore part of the nature of the malignant process that the nodes are not more reactive and that the malignant clone was permitted to survive and spread.* Direct evidence of this process may be seen in dogs with mammary carcinoma where the inguinal and axillary nodes are usually buried in fat and hard to find unless enlarged due to growth of metastases. Normally, these nodes are thin, oval and discoid, and only 3–4 mm in thickness, and are identified by medullary pigmentation. Histologically, the capsule is lax and undulating and the peripheral sinus wide and poorly cellular. There is little follicular activity, paracortical atrophy with a few small deep cortical units that do not alter the depth of the node, and relatively empty medullary cords and sinuses. If metastases are present, they will consist of single cells and small clusters in the peripheral and medullary sinuses. The tumor cells appear to pass through the cortex to the medulla through the cortical gaps, and largely avoid processing through the cortical system of antigen surveillance. The sinus macrophages do not appear to be specifically attracted to the tumor cells, even when they invade the cortical parenchyma. Indirect evidence for the mechanism of this restrained lymphoid response has been found in that nodes draining an area containing melanoma (one of the more highly antigenic tumors) have an increased level of suppressor cell activity which presumably damps down the local defensive responses.

In human pathology, there is considerable interest in the concept of *sentinel node detection* in order to determine the lymph nodes most likely to be indicative of spread from an adjacent carcinoma. These studies are related to areas such as the axilla and mesentery where nodes may be small and multiple and embedded in fat and inobvious on surgical excision. Lymphatic mapping strategies have been devised using radio-labeled colloids to define drainage areas from a particular anatomic site. An immunohistochemical stain for cytokeratin on nodes draining an area of carcinoma easily and dramatically demonstrates single cell metastases as well as the often very subdued cellular reaction to them.

There are minor variations in this reaction with specific tumor systems. Highly secretory *adenocarcinomas* may have more reaction to their secretion than to the tumor cells themselves. *Metastatic mast cells* cause the same reaction in nodes as in primary sites, but tend not to incite a strong cellular and stromal reaction even when highly granulated. As noted earlier, the medullary cords of lymph nodes form a tissue–vascular boundary similar to marrow, and extramedullary hematopoiesis will colonize these sites.

Similarly, in *myeloid leukemias* the tumor cells colonize the medullary cords and, as in marrow, the benign cells are displaced, in this case to the adjacent medullary sinuses. In the acute myeloid leukemias, the primitive tumor cells have round nuclei and resemble the surrounding residual lymphocytes, making recognition of the metastatic disease difficult with oversight stains. Diagnosis is aided by the identification of megakaryocytes in either the cords or sinuses since, if conditions in the marrow are conducive to extramedullary colonization, it is likely that both malignant and benign stem cells are displaced and that the benign system will have trilineage progeny.

The most difficult metastatic tumor to recognize in a lymph node is a tumor of lymphoid origin. In situ lymphoma is rarely observed, but appears occasionally in nodes collected from abattoirs, suggesting that the animal affected was culled for poor performance even though the tumor was undetected. These cases provide a unique opportunity to observe the effects of lymphocyte homing in practical application. Lymphoid tumors represent clones of cells "frozen" in some state of maturation, either primitive for the high-grade tumors, or relatively mature for the low-grade lymphomas. The *lymphocyte-homing receptors* vary in their expression directly with the degree of lymphocyte maturity. There is, however, marked heterogeneity of homing receptor expression within a given histologic category of lymphoma. Thus those large-cell lymphomas that have well-developed homing receptors tend to be widespread at diagnosis because the tumor cells are able to bind to "*addressins*" on the *high-endothelial venules* of lymphoid tissue throughout the body and subsequently invade locally. In contrast, animals with large-cell lymphomas possessing poorly developed homing receptors tend to be presented with localized disease, since although the tumor cells may be in circulation they are unable to bind to endothelium in a site conducive to colonization. As would be expected, these interactions are complex and, while some primitive lymphomas, such as those in the mediastinum, may become very large locally without widespread dissemination, others such as the follicular lymphomas (Fig. 2.116) which tend to be clinically indolent have no, or low, levels of homing receptor but disseminate widely. The recognition of these receptors provides a logical mechanism for the observation that some lymphomas, such as the mucosal-oriented tumors (MALT, BALT), remain localized for long periods and may be best handled by surgical removal. In cattle, the effects of these homing tendencies are likely demonstrated by the plasmacytomas that convert whole nodes to medullary cords, and by the large-cell lymphomas where the tumor cells are recognizable by nuclear cleavage and are confined to the medullary sinuses, while the benign large cells occupy the cords.

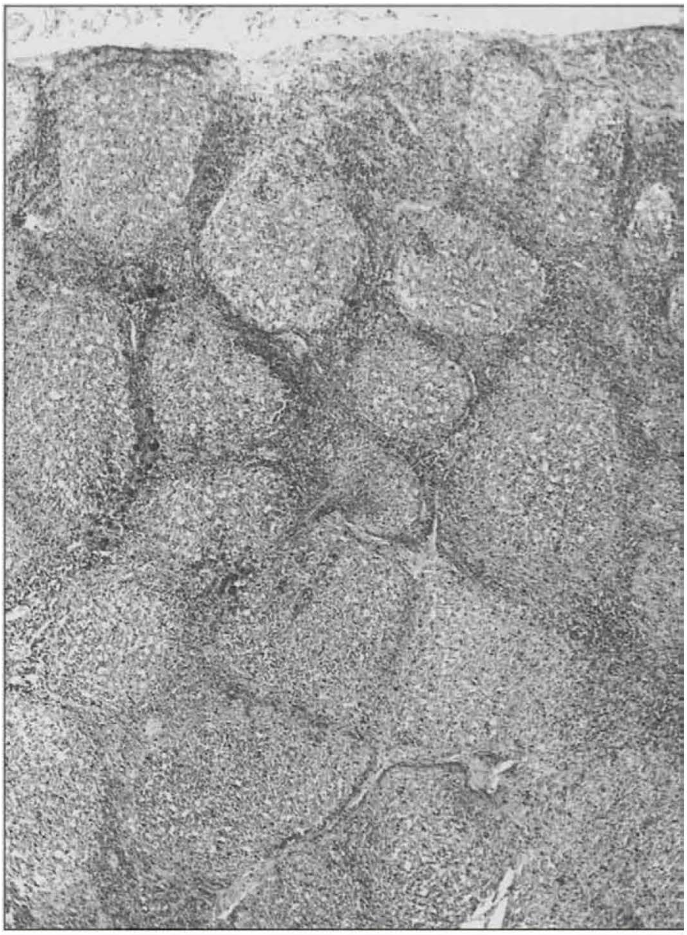

Figure 2.116 Follicular lymphoma in a lymph node of a dog.

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SPLEEN AND HEMOLYMPH NODES

The **spleen** *can be defined as a hematopoietic organ which filters blood through a sinusoidal system.* In contrast, **lymph nodes** *are part of the same organ system with capability for filtering lymph through sinusoids.* The vascular system of the spleen consists of arteries, veins and efferent, but no afferent, lymphatics. As a consequence, all antigen reaches the spleen through the blood, which determines that primary sensitization and antibody production take place in the spleen only if the first contact with antigen occurs by hematogenous sensitization. On subsequent challenge, the germinal centers of the spleen play an important role in the humoral anamnestic response, both by local production of antibody and by the provision of committed B memory cells to the peripheral lymphoid organ. The clearance of bacteria from the blood by the spleen predominates only if systemic opsonization is deficient. In other circumstances, the clearing appears to occur mainly in hepatic Kupffer cells. Thus, *splenectomy increases susceptibility to a number of blood-borne infections*, including septicemia and protozoal infections such as malaria, anaplasmosis, and hematotrophic mycoplasma infection (hemobartonellosis).

The **blood circulation of the spleen** is central to its function and consists of arborizing arterioles that terminate in the *penicilliary radicles*. As the arteriolar system of the spleen branches, the smaller muscular vessels become ensheathed with a cuff of T-cell lymphocytes forming the *periarteriolar lymphoid sheaths*. Irregularly along the length of these sheathed vessels, the lymphoid tissue widens eccentric to the arteriole and forms the B-cell derived germinal centers (Malpighian bodies). The germinal centers are supplied by a thin-walled branch of splenic sheathed arteriole providing both sustenance and antigen to the germinal centers. The germinal centers are surrounded by a *narrow cuff of small lymphocytes* analogous to the mantle cell layers of lymph nodes. Outside of the mantle cell cuff is an area of marginal zone cells that may vary from inobvious to a well-defined corona of larger cells with more abundant cytoplasm. The germinal centers are sheathed with definite boundaries by a narrow band of flattened connective tissue-like cells in rats and New World monkeys but not in other mammals. The sheathed arterioles at their termination form an arborization of penicilliary vessels that are ensheathed by a few plump reticular cells that form a potentially *contractile ellipsoid*. Under normal circumstances, the spleen functions largely as an "open" system with the blood from the penicilliary vessels entering more or less directly into large adjacent venous sinuses that drain into the splenic veins. Thus, normally almost all of the blood delivered to the spleen passes directly to the venous system, while as little as 3% perfuses the splenic cord areas where sorting and processing by the macrophages is carried out to remove effete or injured red cells. The flow rate through the spleen is relatively large and even with this low sorting fraction, *all of the blood passes through the splenic cord system once per day*. With increased arterial flow, or decreased direct drainage to veins, the spleen functions as a "closed" vascular system in which the penicilliary arterioles discharge largely into the Bilroth cords, and thus a much greater fraction of the arterial flow passes through the sinusoidal filter.

The structure of the **sinus walls** is central to the filtering function. They are composed of elongated endothelial cells that are irregularly supported by encircling reticular fibers, like the staves and hoops of a barrel. Adjacent endothelial cells are not joined by junctions, and they therefore form a lattice-work through which blood cells readily permeate to pass from the functional Bilroth cord, or filtering area, back into the venous or systemic circulation. The Bilroth cord/sinus areas or "*red pulp*" of the spleen consist of a system not unlike the medullary cords and sinuses of lymph nodes. The blood from the small penicilliary vessels appears able to enter directly into a sinus area or into a cord area that consists of a loose reticular stroma in which macrophages, lymphocytes and plasma cells are enmeshed. This system allows for slow flow for cells that enter a cord, with maximum opportunity for recognition of altered cells and their removal by splenic macrophages. Alternatively, those cells that in most circumstances are the majority enter a sinus area and rapidly regain the large veins and return to the systemic circulation essentially unfiltered. Under normal circumstances, there appears to be a constant relationship between splenic volume and body weight, as is the case for liver and kidney.

The marginal zone region receiving the blood exiting the germinal center is the area most densely populated with cells that have recently arrived from the general circulation. As a consequence, animals with marked neutrophilia will have many neutrophils in the area immediately surrounding the corona of the splenic follicle. Increased neutrophils in this area are more likely to be seen in the cat, dog, and horse that are characterized by response to infectious diseases such as salmonellosis with a strong neutrophilia, whereas in calves and mature cattle, bands and metamyelocytes may be found in this area. Since many of these animals die with neutropenia, neutrophil accumulation in this site is less frequently observed.

One of the most valuable aspects of phenotypic identification of B- and T-cell areas of the spleen is the opportunity to understand that the spleen is a highly ordered immune structure that varies in morphology as predictably as lymph nodes in response to various stimuli. In addition, the dissection of the phenotypic structure of the spleen clearly demonstrates the differences between hyperplastic and neoplastic lymphoid proliferations arising from one of the three layers of the splenic germinal center.

Hemal nodes (hemolymph nodes) occur in ruminants and a similar structure occurs in rats. They are enclosed by a fibromuscular capsule and have a general architecture similar to that of lymph nodes. *Hemal nodes have a blood vascular circulation and afferent and efferent lymphatics*, although the former are apparently much diminished in comparison to lymph nodes. The arteries entering through the hilar area arborize peripherally and are followed closely by veins that apparently communicate through a trabecular structure similar to that of the spleen. Hemal nodes have germinal centers, but the paracortical areas are much reduced in comparison to those of lymph nodes. Unlike lymph nodes, where the subcapsular sinus is poorly cellular, hemolymph nodes contain red cells in density similar to that of peripheral blood. Red cells are not present in the afferent lymphatics and very few are present in the efferent lymphatics; however, the larger sinuses of the hemonodes may be filled with blood and more closely resemble sinus areas of the spleen. Macrophages occupy the trabecular areas and phagocytosis occurs as in the spleen. Myelopoiesis does not occur in hemal nodes either under normal circumstances or under conditions of hematopoietic stress.

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Developmental diseases of the spleen

The spleen may be *congenitally absent* as occurs regularly in a strain of inbred mice, and rarely in humans and outbred animals when it usually occurs in conjunction with multiple anomalies. In nude mice and rats with thymic deficiency, there is loss of the periarteriolar lymphoid sheath areas of the spleen. The least rare anomaly is the presence of *accessory spleens*, one or more, usually present in the gastrosplenic omentum. *Acquired "accessory" spleens* are implants of splenic parenchyma following traumatic rupture of the main organ (see splenosis).

Duplication of the spleen is occasionally observed in normal swine and as one of multiple visceral defects in nonviable calves and lambs. *Ectopic pancreatic tissue*, either exocrine or endocrine, is occasionally observed in spleens that are otherwise developmentally normal.

Degenerative diseases of the spleen

Senile atrophy affects the spleen, as it does other lymphoid tissues, and is seen particularly in old dogs and old horses. Atrophy accompanies starvation and is seen in wasting disease. Microscopically, the capsule is contracted and thickened, and there is lymphoid atrophy, the severity of which is an indication of the duration and severity of the atrophic process. The sinus areas appear fibrous due to condensation of the sinusoids, and lack of blood and resident hematopoietic cells.

In *hyperimmune states*, there are characteristic changes in the germinal centers of animals that are severely stressed. Lympholysis occurs, and germinal centers appear epithelioid and hypocellular. If the cell loss is acute, there will be prominent tingible body macrophages, as in foals aborted due to *Equid herpesvirus 1* infection. If the reaction has persisted for more than a day, the cell debris is gone and only the dendritic cells remain. A variety of vascular changes occur, consisting of hyaline changes in small arterioles that may proceed to mineralization.

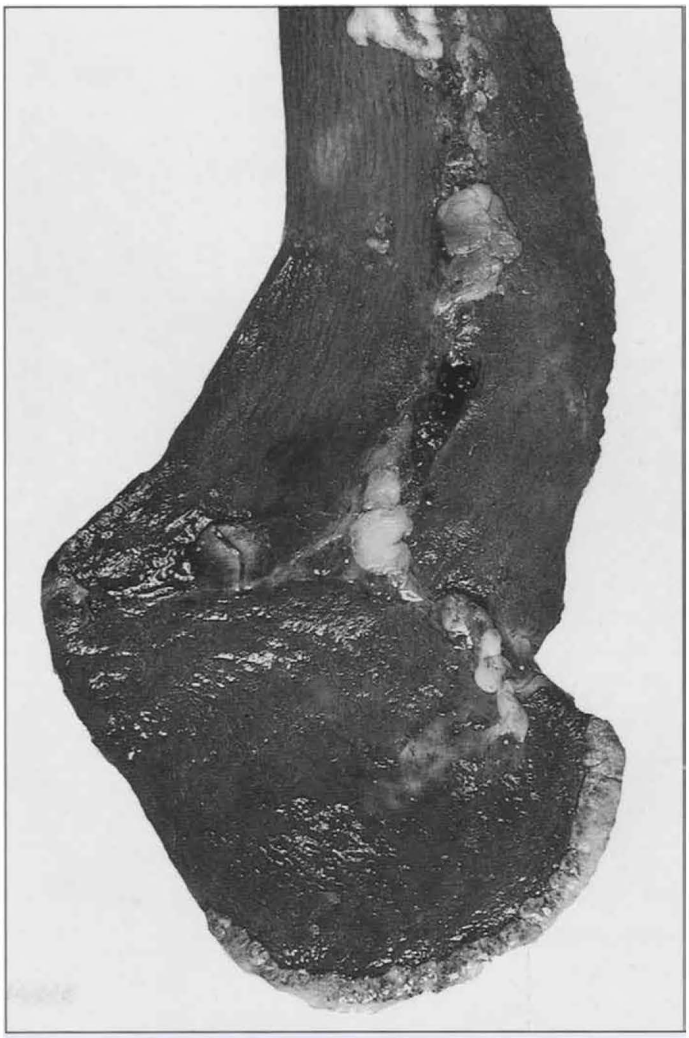

Figure 2.117 Siderotic encrustations of tip and capsule of spleen, dog.

Old dogs often have *yellow encrustations along the splenic margins*, or even covering most of the capsule (Fig. 2.117). Microscopically, there is condensation and thickening of the capsule, trabeculae and perivascular tissue. *The yellow or brown encrustations are known as* **siderotic nodules** *or Gamna-Gandy bodies, and represent deposits of iron and calcium in connective tissue, usually the trabeculae.* The salts become encrusted on connective tissue and elastic fibers, which are swollen, refractile, and stain with hematoxylin. The encrusted fibers occasionally are misinterpreted as fungal hyphae. In association with ceroid, these nodules likely represent the residual effects from areas of *hemorrhage*. Their deposition in the capsular margins and internally suggests that a dystrophic or storage phenomenon may be more likely.

Amyloidosis of the spleen (Fig. 2.118) occurs as part of generalized amyloidosis, but the deposits in the spleen may not be detectable grossly or histologically without appropriate stains. Amyloidosis of an obvious degree is not common, and when present it involves the germinal centers and may spare the sinus areas completely. This distribution of amyloid gives the organ the "sago-spleen" appearance in which the enlarged and waxy follicles protrude from the cut surface. The spleen is not enlarged. The deposition of amyloid occurs first in the small arteries of the lymphoid nodules, and minor depositions are common.

Histiocytes of spleen and lymph nodes are enlarged in most *storage diseases* (Fig. 2.119) (see Vol. 1, Nervous system).

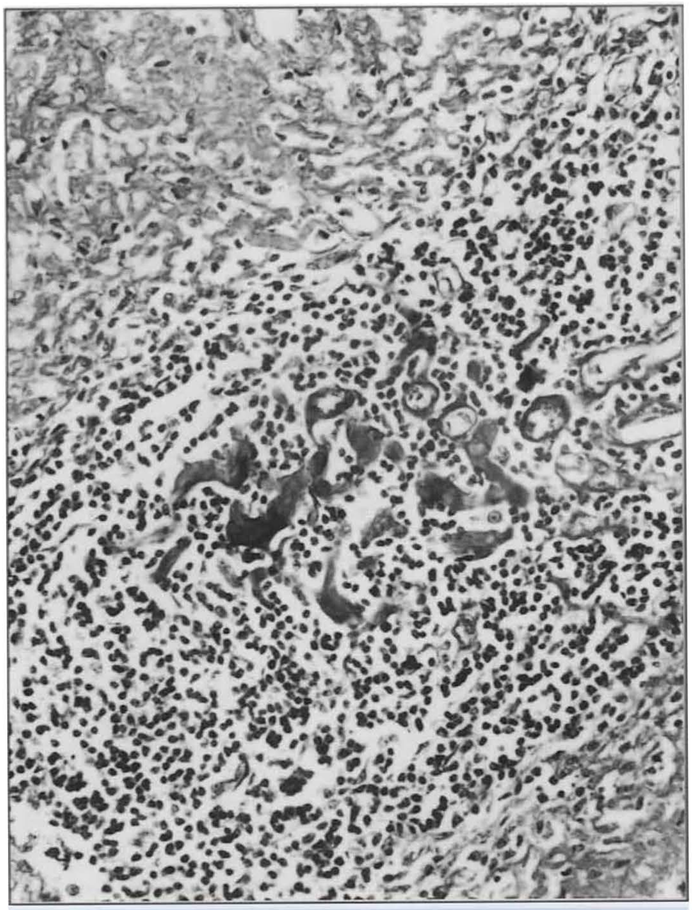

Figure 2.118 Amyloidosis in the spleen of a cat.

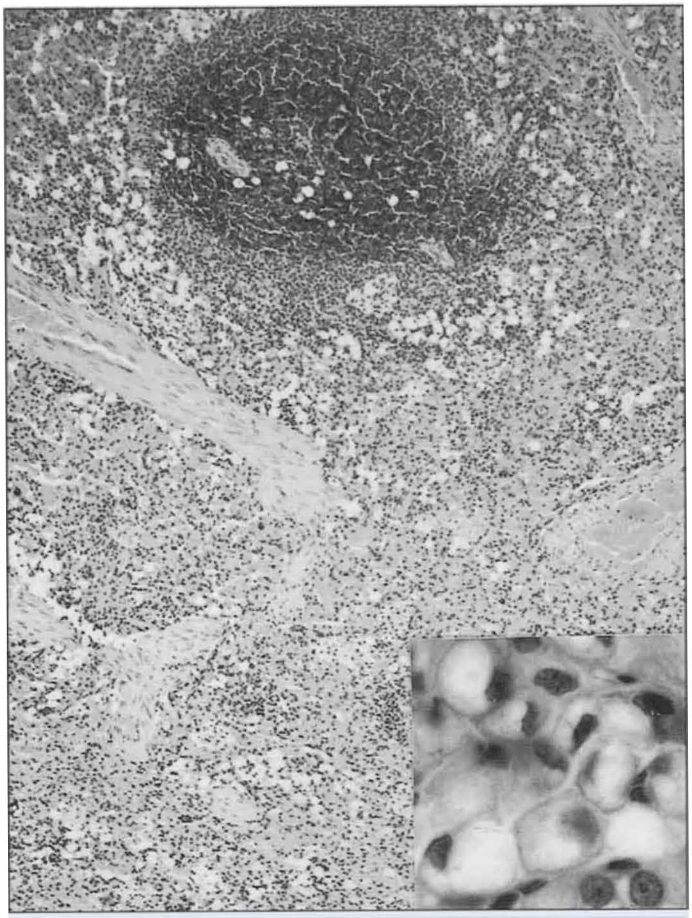

Figure 2.119 Sphingomyelin storage in the spleen of a dog. Clusters of large macrophages with clear cytoplasm in both follicles and pulp. Inset: storage cells.

Hemosiderin *is a storage form of iron and the only splenic pigment of importance.* The amount and form of storage iron varies greatly in normal animals with age and species. The pigment is usually present only in macrophages, but in long-standing iron accumulation it may be encrusted on fibers of connective tissue. The amount of hemosiderin is significant only when it is in excess to the point of causing tissue injury and fibrosis, and justifies the name hemosiderosis. Iron is absorbed in excess in any anemia where it is sufficiently available. Increased iron is one of the splenic changes in the hemolytic anemias. The patterns of iron storage in the spleen and marrow can yield information about the type of anemia. In nonresponsive anemias, the iron is very largely aggregated into large coarse granules within the phagocytes that themselves are unapparent. In responsive hemolytic anemias where there is rapid iron turnover, there is a spectrum of iron deposition from barely stainable, diffusely or focally distributed ferritin, to coarse hemosiderin. In the anemias of chronic disease, there is increased coarse splenic iron due to continued scavenging of the transferrin system by the acute phase reacting proteins.

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Rupture of the spleen

A normal spleen can be ruptured by the level of *trauma* that cats and dogs suffer regularly in automobile collisions. The result is more or less severe loss of blood into the abdomen.

There is usually a concurrent rise in transaminases, indicating *hepatic laceration* as an additional source of abdominal hemorrhage. The spleen may be fully divided into two or more parts, or merely ruptured and healed, the lines of healing producing notches and fissures. *Pathologic rupture* may occur in any species with only minor trauma if the spleen is enlarged and the capsule thinned. The latter pathogenesis may occur with splenomegaly of any cause including congestive, hemolytic, infectious, or neoplastic types (Fig. 2.120). Lymphoma is a common cause of splenic enlargement leading to fatal internal hemorrhage. Following rupture of the splenic capsule and spilling of the sinus cells into the abdominal cavity, there may be widespread seeding of splenic explants onto the serosal surfaces. This is called **splenosis**.

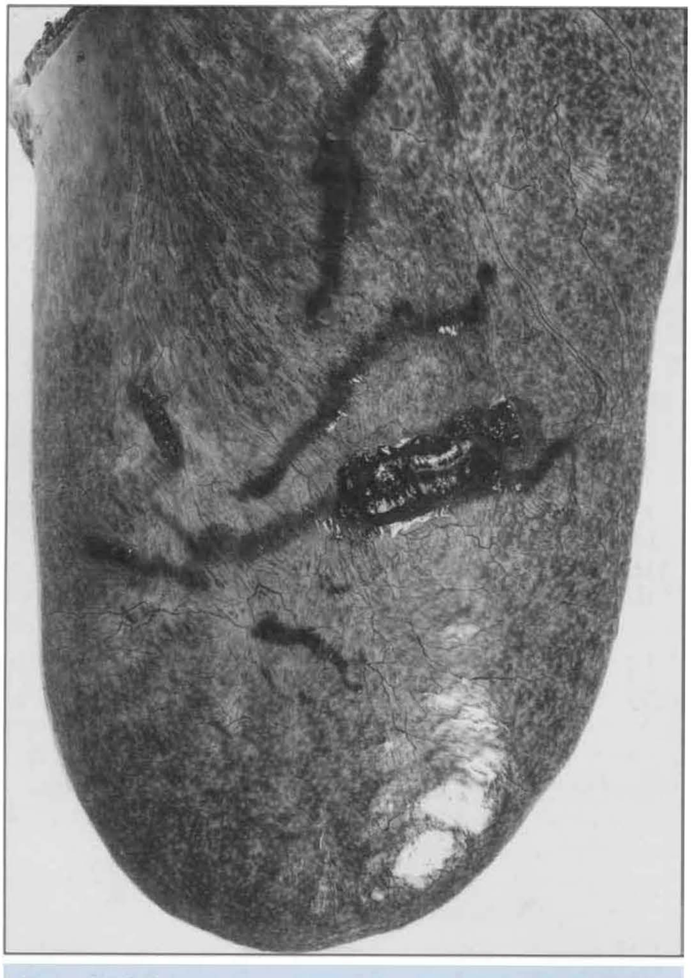

Figure 2.120 Spontaneous rupture of swollen spleen, lymphoma, ox.

These accessory spleens are functional and there may be hundreds on the omentum with a few on the peritoneum. They appear grossly like hemal nodes and histologically like normal spleen. Following splenectomy, any splenic implants present will increase in size and may give some protection against septicemic disease.

Torsion of the spleen

Torsion affecting only the spleen occurs in *pigs, dogs* and humans and rarely in horses, and *torsion of the spleen and stomach occurs in dogs.* When the whole spleen is twisted on its mesentery, there is severe congestion and *hemorrhagic infarction* due to occlusion of the vein and ultimately blockage of the artery. When the distal portion is twisted, only the vein tends to be occluded, causing hemorrhagic infarction and congestion. Splenic torsion in the **dog** occurs in large breeds and is characterized by enlargement of the upper abdomen with painful guarding on palpation, and the passage of dark-brown urine. Hematologically, there is a characteristic brown discoloration of plasma due to leaching of blood pigments through the capsule as it becomes necrotic. Associated signs consist of the postsplenectomy state with many distorted red cells, hypersegmented neutrophils and the presence of Howell-Jolly bodies and *red cell pits,* which are unusual in the peripheral blood of the dog with a normally functional spleen. The spleen, on surgical removal, may weigh as much as 5 kg and contain a large proportion of the original red cell mass. As is the case in humans who survive splenectomy, there is risk of infection by organisms normally controlled by the spleen. In the dog, hemotrophic mycoplasma infection and severe hemolytic anemia are the most likely adverse sequelae.

Splenic torsion in pigs usually occurs in **sows,** and often several are affected in the same herd over a period of a few days. Circumstantial evidence suggests that a change from once- to twice-daily feeding will prevent further losses, but other factors must be involved. Affected animals usually die acutely, and in white-skinned pigs cutaneous pallor suggests esophagogastric ulceration. Grossly the spleen is blue-black, massively and uniformly enlarged, and twisted 180° about its long axis.

Cysts

Splenic cysts are unusual. Occasionally, *Cysticercus tenuicollis* and hydatids are found there. *Pseudocysts* may result from cystic degeneration of hematomas. *Neoplastic cysts* are hemangiomas or hemangiosarcomas. The latter are relatively common in the dog and have a rather characteristic history of repeated bouts of malaise and weakness, with apparent recovery over 2–3 days. Severe anemia accounts for the weakness. Pallor may be noticed and results from rupture of the tumor with abdominal hemorrhage. The recovery over a few days is too rapid for new red cell production, and indicates autotransfusion of the extravasated blood. The hematologic picture is also typical with distorted red cells, even in the absence of abdominal hemorrhage, due to the effects of abnormal vascular walls and arteriovenous shunts. After hemorrhage, the red cell distortion is much worse and the formerly extravasated red cells appear tattered and effete, and the plasma is brown due to extensive hemolysis. There are usually Howell-Jolly bodies in the blood along with hypersegmented neutrophils. If abdominocentesis is performed, the presence of an occasional rubricyte is an indication of hemorrhage from the spleen rather than from another abdominal site.

Circulatory diseases of the spleen

Active hyperemia is common in acute systemic infections and also occurs in some acute bacterial intoxications, such as clostridial enterotoxemia of calves, erysipelas of swine, and *Streptococcus pneumoniae* infection in neonatal calves and goats. *Passive congestion* of the spleen may arise as a result of disturbances in the systemic and portal circulation, and is a feature of some of the acute hemolytic anemias. The most common cause of passive congestion is the use of barbiturate drugs. Central (cardiac or pulmonary) venous congestion does not usually cause congestion of the spleen in animals, partly because they usually do not live long enough, and partly because the splenic capsule is not elastic as in sheep, or is highly muscular as in the dog and horse and not easily distended. Portal obstruction, with chronic hepatic fibrosis and portal obstruction, causes significant splenic congestion in humans, but this sequence of events is rare in animals.

An acutely congested spleen is enlarged, moderately turgid, and cyanotic with the capsule blue-black. The normal architecture is not discernible on the cut surface, and the pulp is red-black and progressively exudes blood. Microscopically, the splenic sinuses are dilated with packed red cells, and the germinal centers are widely separated and trabeculae are thinned. The extent of acute congestion may make histologic examination difficult in the horse because the tissue, being largely clotted blood, fragments on sectioning. Splenic samples from horses destroyed with barbiturate should be taken from a thin marginal area where the parenchyma is supported on two sides by capsule. With chronic congestion, the organ becomes firm and the capsule and trabeculae thickened. There is lymphoid atrophy and sinus proliferation, with a marked increase in fixed cells in the red pulp and heavy sinus colonization with macrophages and hemosiderin.

Thrombosis of the splenic vein has been observed in association with traumatic reticulitis and portal thrombosis in cattle, and with splenic abscesses in horses. Thrombosis of both arteries and veins is seen in hypercoagulable states such as immune hemolytic anemias, purpura, and hemorrhagic pancreatitis. Spleens that are enlarged for any reason are prone to thrombosis and infarction, which is likely to occur in myeloma, lymphoma and in leukemia, due both to the thrombocytopenia and procoagulant content of hypogranular promyelocytic leukemia or more often the hyperviscosity of myeloma granulomas. In acute hog cholera, there is widespread endothelial swelling and proliferation with fibrinoid thrombosis of splenic arterioles in 50% of cases, resulting in raised dark infarcts 0.2–2.0 cm in diameter in the splenic capsule. The spleen may suffer septic infarction in the hyperviscosity syndrome associated with myeloma and gammopathy. The spleen is enlarged due to infiltration by tumor cells and flow is further compromised by the hyperviscosity of blood. The septic component is likely due to loss of normal antibody and displacement of phagocytes by the myeloma cells.

Embolism with infarction is not uncommon. The emboli are usually derived from valvular vegetations. The outcome has the usual dependence on whether the emboli are septic or bland.

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Inflammatory diseases of the spleen

Systemic inflammations cause a regular and fairly predictable pattern of response in the spleen. In septicemia and following injection of endotoxin or gram-negative bacterins, there is rapid accumulation of neutrophils in the splenic marginal zone and surrounding area of sinus. There is bacterial destruction and processing of antigen in this area followed by a predictable pattern of migration, the foreign material moving in specialized macrophages (*metallophils*) successively into the marginal zone and then to the small lymphocyte corona, reaching the germinal center in 10–12 hours. In contrast, immune complexes appear to move by flow and not by cellular transport. *Overwhelming infections* result in lympholysis of the follicular center cells, with the production of nuclear debris and the exposure of the underlying dendritic reticular cells, whose cytoplasm becomes more intensely stained in disease to attain the tinctorial properties and density of epithelioid macrophages. The nuclear fragments are rapidly removed so that the follicular center appears empty, with the nuclei of the dendritic cells relatively widely spaced and surrounded by their densely pink cytoplasm. These epithelioid germinal centers are seen in a variety of acute toxemic diseases in young animals. In older animals, the changes occurring in splenic germinal centers are often more severe and long-lasting, and consist of a depopulation of the follicular center cells and transudation of plasma proteins into the germinal centers to form a fairly persistent coagulum recognized as *intrafollicular hyalinosis*. These fibrinous exudates may be removed, with restitution of the follicular center cells or, if there is extensive vascular damage, they may become mineralized and result in involution of the germinal center.

Septicemia and bacteremia result in microbial deposition in the Billroth cord areas, where the abundance of phagocytic cells usually leads to their rapid destruction. Occasionally small foci of bacterial colonization develop in the cord and sinus areas of spleens, primarily in young animals with severe gram-negative bacterial infection of the enteric tract. In septicemic splenitis, there is splenic enlargement, with acute congestion and degeneration of the lymphoid follicles and hypercellularity of sinus areas. In very acute diseases, such as anthrax in cattle, the reaction is almost solely vascular. In the less severe septicemias such as erysipelas in swine, there is moderate reactive hyperplasia which may be more developed than that seen in acute fulminant gram-negative septicemias such as salmonellosis. Marked hyperplastic changes of the sinus areas are a feature of some diseases such as malaria, trypanosomiasis, equine infectious anemia and malignant catarrhal fever, although involution characterizes some infections.

Splenic abscesses may be miliary or large and focal, but both types are uncommon. Abscessation may be due to various organisms including *Arcanobacterium pyogenes*, but some commonly localize preferentially in the spleen (see Specific infections of the lymphoid tissues, this chapter). Purulent splenitis may develop by local extension in cattle from penetrating wounds of the reticulum and, in horses, by extension from the colon due to strongyle migration.

Hyperplastic diseases of the spleen

Nodular hyperplasia

Nodular hyperplasia is frequently observed in the spleens of *old dogs*, occasionally in old bulls, and rarely in other species. Most nodules are up to 2 cm in diameter and project hemispherically above the capsule, but may be 5 cm or more in diameter. On cut surface, the nodules vary from gray to pink to a variegated red and white, with yellow necrotic areas in the larger ones. The variegated pattern is due to persistent islands of sinus in a diffuse field of lymphocytic proliferation. Architecturally, the nodules are unencapsulated but compress adjacent trabeculae, and irregularly meld with surrounding sinus areas that have a much higher density of red cells. Lacking germinal centers, the nodules consist of monomorphic medium or large lymphocytes with moderate cytoplasmic volume and small nucleoli. The nuclei are round with thin nuclear boundaries, and are more open and less hyperchromatic than are lymphoma cells. There may be many mitoses, and *the lesions are judged to be benign more on a knowledge of behavior than cytology*. Architecturally benign focal lymphoid proliferation in the spleen tends to be arranged in large irregular islands that are loosely facetted in a relatively cohesive mass. In contrast, lymphomas of a mantle cell or marginal zone type can be seen to be related to the arteriolar structure of the spleen in multiple smaller foci, some of which contain remnants of germinal centers. Proliferation *immediately surrounding fading germinal centers* of small cell type are likely of mantle cell lymphoma. If the proliferation can be seen in some foci to surround *the outside of the mantle cell cuff* and is composed of intermediate-sized cells, then the lesion is likely marginal zone lymphoma. Focal lymphoid proliferations in the spleen, either hyperplastic or neoplastic, may be clinically apparent due to effects of the expanding mass on adjacent vascular sinuses resulting in vascular pooling and thrombocytopenia.

In contrast, **splenic plasmacytoma** is a rare condition of dogs with gross features indistinguishable from nodular hyperplasia. Cytologically the cells are uniform and of moderate size but with abundant, usually eccentric cytoplasm that stains deeply. Nucleoli are small but prominent and central and there is mild heterochromatin aggregation. Mitoses are less numerous than in the surrounding normal tissue and there may be some degree of gammopathy. Plasmacytoma may be indolent for long periods, but may progress to an immunoblastic type of lymphoma within a year of biopsy.

Hematopoietic alterations

Extramedullary hematopoiesis results when there is hormonal induction for increased cell production and pluripotential hematopoietic stem cells available. These cells normally circulate in very low numbers and, in preparation for extramedullary hematopoiesis, they return to embryonic sites of colonization, sparing the germinal centers and periarteriolar lymphoid sheaths. Extramedullary hematopoiesis is *characteristically trilineage* but one cell line may predominate, such as the myeloid system in canine pyometra or the erythroid system in hemolytic anemia (Fig. 2.121). Megakaryocytes are the most obvious hematopoietic precursor and characteristically lie adjacent to the smooth muscle trabeculae when stimulation is mild, but may become diffusely distributed in the sinus areas when stimuli are pronounced and prolonged. Splenic erythropoiesis is coincident with increased splenic red cell destruction in immune hemolytic anemias.

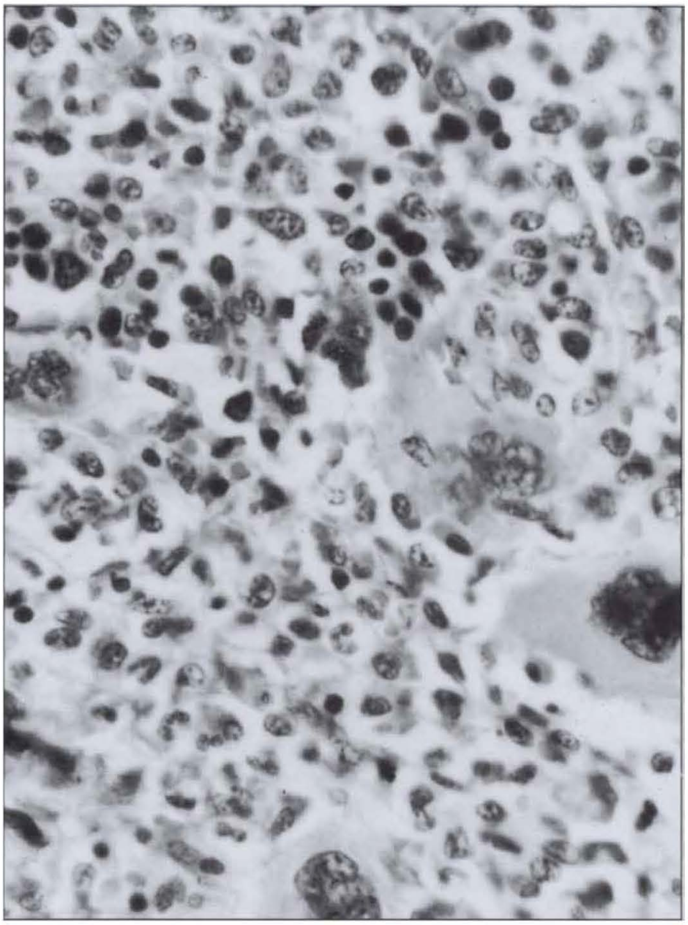

Figure 2.121 Extramedullary thrombopoiesis and erythropoiesis in pyometra in the spleen of a dog.

In contrast, **myeloid metaplasia** is a condition where there is marrow myeloid hyperplasia without an apparent target for the increased cell production (see Myelodysplastic syndromes (MDS), and Chronic idiopathic myelofibrosis/myeloid metaplasia with myelofibrosis). The disease tends to run a course of prolonged hyperplasia followed by myelofibrosis and displacement of the still functional stem cell system to embryonic sites of production. Grossly, the spleen is uniformly enlarged with turgid capsule and rounded borders. The parenchyma is dry, and the light pink of hematopoietic marrow rather than dark and cyanotic as with congestion. In myeloid metaplasia of the spleen, there is *myeloid predominance but all cell lines are present to some degree*. As part of the character of the disease, some dysplastic changes will be present in the extramedullary hepatopoietic cells in splenic sinusoids. **Dyspoiesis** may affect only one cell lineage, or more commonly all three, and is characterized by multinucleated rubricytes, incipient Howell-Jolly bodies, giant and donut forms of metamyelocytes, and hypofusion and segmentation of megakaryocyte nuclei. Myeloid metaplasia is accompanied by immune hyperplasia, hemosiderosis and splenic fibrosis. This is preceded by follicular hyperplasia of the spleen and nodes, and later hematopoietic colonization with gradual reduction in the lymphoid volume in the spleen, and eventual atrophy as the stem cell system becomes increasingly committed to myeloproliferation. Small infarcts develop and are likely the result of loss of plasticity, and fibrous impairment of vascular structures.

Rarely the spleen contains a **myelolipoma,** which stands out grossly as a focal pale area on the cut surface and histologically because the empty fat cells contrast sharply with the sinus parenchyma. Myelolipoma is composed of outwardly normal marrow with about 50% hematopoietic and the rest large typical fat cells.

Lymphoid hyperplasia of the spleen may be segmental and affect only the germinal centers, which may be enlarged and hypocellular as may be seen in immune hemolytic anemias followed by prolonged steroid treatment. Alternatively, there may be hyperplasia of the periarteriolar lymphoid sheath with prominent broad cords of lymphocytes surrounding the branches of the medium-sized arterioles. In contrast to both of these changes, there may be hyperplasias of the marginal zone cells which prominently surround all germinal centers and may fuse with adjacent germinal centers or actually surround adjacent periarteriolar lymphoid sheaths. The follicular structure of the spleen is usually not apparent on gross examination unless there is hyperplasia or neoplasia involving some or all of these lymphoid compartments. The germinal centers in hyperimmune states increase in both size and density, and the accentuation of the marginal zone, mantle cell corona, and follicular center cells is increased, often resulting in a "target" appearance. Since sinus and follicular hyperplasia of the spleen tend to occur together, their concurrence results in an overall increase in splenic weight.

Splenomegaly

The hematological effects of an enlarged spleen include pancytopenia or selective cytopenia, but almost always anemia and thrombocytopenia (see Hemolytic anemia due to splenic hyperfunction). **Splenomegaly** is an enlarged spleen, whereas **hypersplenism** describes a spleen that is overactive in cell destruction. Usually the two coincide but *whereas the enlarged spleen is always hyperactive, the hyperactive spleen may not always be enlarged.* Since the spleen is almost always involved to some degree in both red cell production and destruction, the decision on whether to remove the spleen in hemolytic anemia may require scintillation counting of labelled red cells to determine if splenectomy would be helpful. In concurrent hemolytic anemia and thrombocytopenia, as it occurs in dogs, immediate splenectomy is indicated to prevent fatal intracranial hemorrhage. The apparent decoupling of splenic size and function in cell destruction is mediated in large measure by the presence of opsonizing antibody which greatly reduces the chances of a given cell re-entering the vascular system after being shunted into the splenic cord system.

The degree to which splenic size departs from normal is difficult to assess, but weight is always a useful parameter, particularly in animals that die without exposure to barbiturate anesthetics. Splenomegaly is common, and the interpretation of splenic size at autopsy can be gained by reference to the whole carcass and to the cut surface of the organ. *The following is an arbitrary list of diseases to be considered when splenomegaly is present.* The absence of splenomegaly does not eliminate any of them, but makes them unlikely. Multiple focal lesions do not as a rule cause splenomegaly, although hemangiomas of the canine spleen and metastatic melanomas of the equine spleen are exceptions.

- Cattle and sheep
  - Anthrax
  - Salmonellosis
  - Lymphoma-leukemia
  - Babesiosis, trypanosomiasis, anaplasmosis
  - Congestive splenomegaly
  - Acute septic processes with bacteremia and toxemia

- Horses
  - Equine infectious anemia
  - Lymphoma-leukemia
  - Metastatic melanoma
  - Isoimmune hemolytic anemia
  - Salmonellosis
  - Anthrax

- Pigs
  - Torsion
  - Congestive splenomegaly
  - Salmonellosis
  - Erysipelas
  - Lymphoma-leukemia
  - Isoimmune hemolytic anemia
  - Protozoan diseases of blood
  - African swine fever

- Dogs and cats
  - Barbiturates
  - Lymphoma-leukemia
  - Acquired hemolytic anemias
  - Histoplasmosis
  - Amyloidosis
  - Leishmaniasis
  - Hemangiomas and hemangiosarcomas
  - Torsion (dogs)
  - Mast cell tumor (cats)

Splenic enlargement may occur in *storage diseases* of which there are many (see Vol. 1, Nervous system). Sphingomyelinosis of dogs is an appropriate example (Fig. 2.119).

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Neoplastic diseases of the spleen

The vascular tumors of the spleen (Fig. 2.122) are described in Vol. 3, Cardiovascular system, and leukemic diseases involving the spleen are described with the myeloproliferative and lymphoproliferative diseases (this chapter). *The spleen is expected to be involved in systemic malignancies of the lymphoid system, but this is not always the case or apparent.* In a review of lymphomas (and assuming that when lymphoma was suspected the spleen would always be examined), there was splenic involvement in only 41 of 72 cases (57%) in dogs, 35 of 81 cases (43%) in cats, and 27 of 90 cases (30%) in cattle. *Although the spleen may not be invaded in lymphoma, it is much more likely to be involved in frankly leukemic lymphoid and myeloid leukemia.* In all situations, but particularly in lymphoma, the size of the organ or the microscopic structure cannot be taken in itself as evidence for the presence or absence of tumor. Careful cytologic examination must be carried out to determine whether a suspect cell population is like that of unequivocal tumor elsewhere.

The evidence for well-defined tissue homing by lymphocytes, added to recognition of B-T subsets, facilitates the interpretation of lymphoid changes. Thus the loss of specific trophic factors, or the displacement of stem cells from a cellular compartment, explain the atrophy of lymphoid, particularly thymic-dependent, areas that are generally seen in cancer. More than 90% of peripheral lymphocytes are T cells and under normal circumstances they make up most of the cellularity of the sinus areas; consequently in lymphopenia the sinus areas of the spleen appear hypocellular and vice versa. In polyclonal B-cell hyperplasia, as occurs in cattle with trypanosomiasis and the persistent lymphocytosis stage of bovine leukemia virus infection, there is enlargement of the marginal zone areas around the splenic germinal centers.

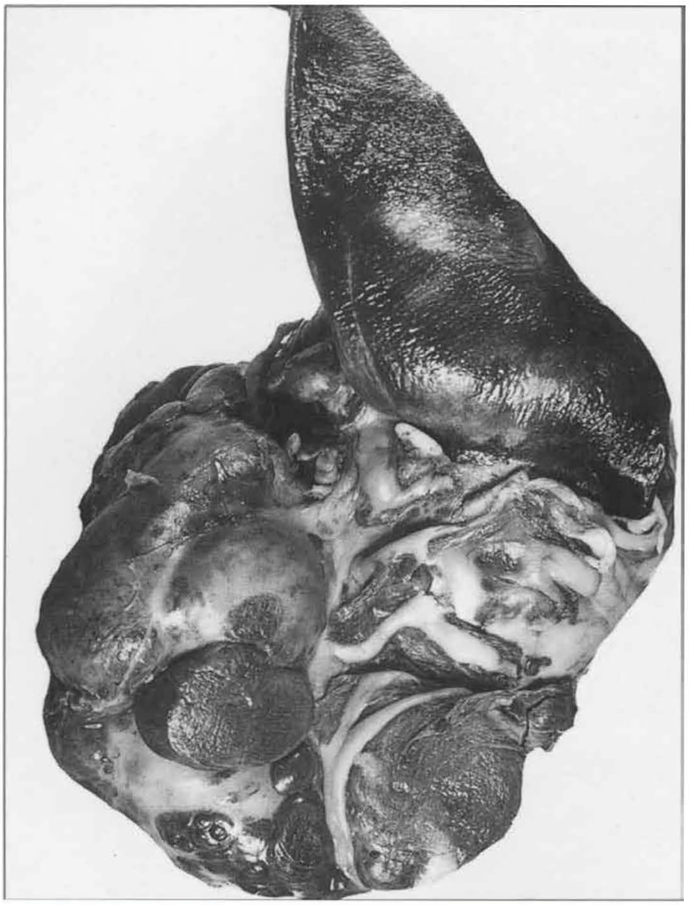

Figure 2.122 Hemangiosarcoma in the spleen of a dog.

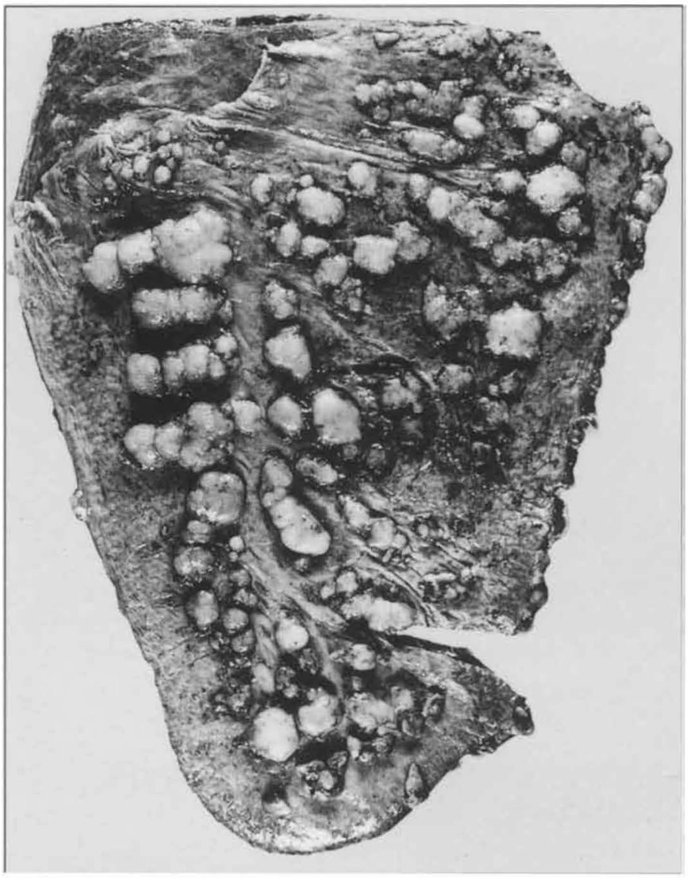

Figure 2.123 Implants of **squamous cell carcinoma** on splenic capsule, ox.

In cats and dogs with lymphoma, there is usually unequivocal involvement of lymph nodes, portal colonization of the liver, and atrophy of the periarteriolar lymphoid sheaths and germinal centers in the spleen. Splenic involvement is typically a late phenomenon in thymic lymphoma of all species. The identification of the hepatosplenic T-cell lymphomas of the cat and dog demonstrate that there is leukemia with heavy involvement of spleen but paradoxically not of bone marrow. Thus these are leukemic diseases that do not follow the usual pattern in which the marrow is the tissue most heavily involved.

When the spleen is symmetrically enlarged to a mild or greater degree, the histologic infiltration is usually diffuse, as occurs in leukemia, and occupies all of the sinus areas. There is initially, at least, mild atrophy of the germinal centers and periarteriolar lymphoid sheaths. A diagnosis of lymphoma in the spleen may be difficult when there is a prominent follicular pattern, and *the most reliable criterion of malignancy is subendothelial colonization of large muscular sinuses by lymphocytes*. Usually, when this change is found, lymphocytes of similar morphologic type will be identified in other areas, particularly the liver. In splenic follicular lymphoma of large-cell type (a rare disease), the tumor is multifocal and centered on germinal follicles, as can be demonstrated by collapse of the mantle cell corona and identification of residual benign small cells in the center of the enlarging tumor foci. In the horse and dog, lymphoma may be focal in the spleen, and in these cases there is more likely to be atrophy of lymphoid tissue in the benign area as the tumor increases in size. When the spleen is involved in adult cattle with diffuse (non-follicular) lymphoma, there is usually malignant colonization of the germinal centers, probably reflecting the bone marrow origin of the cells involved. A similar pattern of involvement occurs in cats and dogs, and there is usually a dense homogeneous cluster of lymphocytes 2–3 times the diameter of a normal follicle, with complete atrophy of the mantle cell corona and marginal zone cells. In contrast to follicular lymphoma, these are focal aggregates of diffuse tumor, and again their homing pattern can be demonstrated by identification of residual small benign cells in some of their centers as well as their constant relationship to the splenic arterioles.

Metastatic neoplastic disease of the spleen is uncommon. It is not likely that migration of tumor cells to the spleen is unusual, but rather that the functional efficiency of the cord and sinus macrophages usually prevents colonization. Metastatic carcinoma involving the spleen and liver usually arises from the pancreas or reproductive system. With neoplasia arising in the liver, metastases to the spleen are isolated and most are probably of arterial origin. Carcinomatous implants originating from other abdominal viscera can mimic peritoneal tuberculosis (Fig. 2.123).

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SPECIFIC INFECTIONS OF THE LYMPHOID TISSUES

The strategic location of the lymph nodes, and blood flow through the spleen, determine their role in resistance to infections and make their involvement, either locally or generally, directly or indirectly, inevitable in any infectious disease. For the most part, the lesions produced in the nodes and spleen are not specific for the cause. On the other hand, there are some infectious diseases in which these tissues are chiefly or consistently involved, and they are the subject of this section.

Caseous lymphadenitis

Caseous lymphadenitis (CLA) is a suppurative infection of the lymph nodes primarily of sheep and goats caused by ***Corynebacterium pseudotuberculosis*** *(ovis).* The disease occurs in sheep wherever they are raised, but horses, camels, deer, mules, and rarely cattle and humans may be affected. Cattle can develop a pathologic syndrome that resembles that in sheep, but they do so quite rarely, and the infection generally remains localized to 1–2 regional nodes draining an infected surface wound or a segment of intestine. *Coryne-bacterium pseudotuberculosis* is also the cause of *ulcerative lymphangitis* in cattle and horses, and of *pectoral abscesses* in horses.

There are two serotypes with *type I* in ovine, caprine and occasionally bovine isolates and *type II* in buffalo and most infections in cattle. An *exotoxin* that consists of a phospholipase D is an important aspect of virulence with effects including intravascular hemolysis, necrosis, pulmonary edema and shock.

C. pseudotuberculosis survives briefly in the environment and is able to spread indirectly, which is an important means of spread in sheep and goats that are routinely corralled on the same bed ground to avoid predation. Infection also occurs in shearing wounds in sheep and butting abrasions in males.

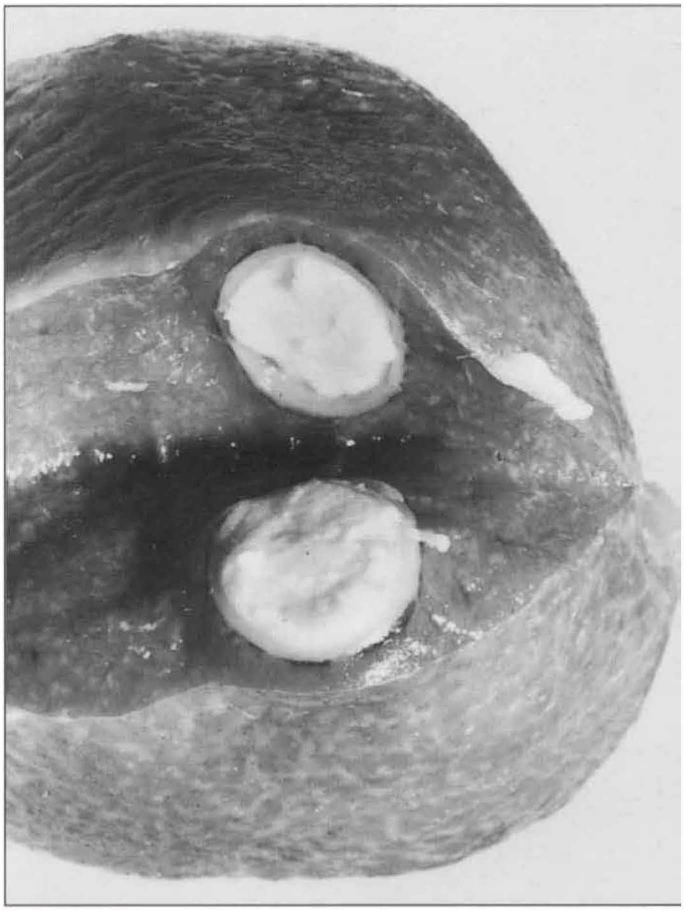

Figure 2.124 Abscess of melioidosis, spleen, goat. (Courtesy of WR Kelly.)

In **horses**, the disease occurs as ulcerative lymphagitis on the fetlocks, which is consistent with the concept that skin abrasions are important in spread of the organism. The seasonal incidence of abscesses in the pectoral and other regions of horses suggests that the organism may also be borne by arthropods.

The disease in **goats** can be more severe than in sheep, the most frequent lesions being in the *lymph nodes of the head and neck.* The lesions in goats closely resemble those of pseudoglanders or melioidosis (*Burkholderia (Pseudomonas) pseudomallei*), and it is important to distinguish these diseases (Fig. 2.124). Female goats and intact males have more extensive lesions than castrated males. In sheep and goats, there is slow spread of the disease with the prevalence of abscesses increasing as a function of age. *Caseous lymphadenitis is widespread in goats and the disease differs from sheep in the distribution of the abscesses.* In goats the mandibular lymph nodes, followed by the parotid nodes, are most often involved suggesting that the organism is acquired through the buccal mucosa, as well as through skin wounds. Superficial abscesses of the jaw and neck region are common and infection is probably acquired from feed stalls or collars carrying the bacterium. In only a small proportion of goats does infection spread to involve the mesenteric or mediastinal nodes grossly.

Caseous lymphadenitis in **sheep** almost always follows a *wound infection*, usually a shearing wound. The organism can penetrate the intact skin of freshly shorn sheep however, and may be transmitted by dipping fluids. Docking and castration wounds and the umbilicus are of minor importance. Occasionally, the infection may be acquired by ingestion, as indicated by confinement of the lesions to the nodes draining the buccal cavity. Less commonly the organism is inhaled, producing lung abscesses. Parasitic wounds of the lower alimentary tract are not a portal of infection.

While the phospholipase toxin is an important aspect of pathogenicity, the natural infection in sheep, even when generalized, gives scant clinical evidence of intoxication, though the exotoxin facilitates the spread of infection from the primary site. Vaccination with the exotoxin provides a degree of protective immunity. The leukotoxic surface lipid allows persistence within inactivated macrophages in which the organism is effectively a *facultative intracellular parasite*. The acquisition of host resistance may also be in part due to the selection of a population of macrophages resistant to and capable of destroying the toxic surface lipids of the bacteria.

The *sequence of events in progressive CLA* is infection of a superficial wound, spread of infection to the local lymph nodes which suppurate, and then lymphogenous and hematogenous extension to produce abscesses in internal organs. The progression is slow and may reach the bloodstream only in older animals, whereas in young animals the disease tends to be confined to the superficial lymph nodes, most commonly the precapsular and precrural.

Spontaneous recovery from the primary infection of cutaneous wounds, even when they suppurate, is more common than is progression of the infection to the lymph node, as judged by results in the experimental disease in which only about 20% of sheep develop abscesses in the nodes. Even when abscesses develop in the nodes, the primary cutaneous lesion heals, but secondary cutaneous lesions may develop as fistulae when the nodal abscess ruptures to the exterior. This occurs more often in goats than in sheep. *As a general rule, once the infection gains the nodes it is persistent*, although a few infections may be cleared when the node ruptures and discharges on to the skin.

The initial lesion in lymphoid tissues is diffuse lymphadenitis that is probably the result of the soluble exotoxin. When the organism reaches the nodes, multiple microscopic abscesses form in the cortex. Eosinophils are prominent in the reaction and cause the pus to be green. These foci rapidly coalesce and the central areas caseate to form a structureless mass which contains fragments of nuclear material and discrete clumps of bacteria. The abscesses are rapidly encapsulated, and when this occurs the acute reaction in the surrounding tissues subsides, but the abscesses continue to enlarge. With enlargement, there is progressive necrosis and re-formation of the capsule, which gives the lesion a very characteristic structure of concentric lamellations (Fig. 2.125); these are particularly prominent when calcareous granules are deposited in successive layers at the margin of the expanding lesion. In old lesions, the contents lose their green color, become inspissated and resemble putty. The lamellation is specific to the organism, not the organ involved. The nodal lesions often attain a diameter of 4–5 cm and exceptionally they may reach 15 cm. The larger lesions in superficial nodes cause pressure atrophy and depilation of the overlying skin; they frequently rupture to discharge chronically through a narrow fistula.

Caseous lymphadenitis is rarely fatal, and indeed it seldom even causes debilitation. Its economic importance is due to regulations concerning the trading of carcasses that show evidence of the disease. When fatalities do occur, they are caused principally by large pleuropulmonary abscesses. Rarely, *Corynebacterium pseudotuberculosis* causes small outbreaks of polyarthritis in lambs, but they recover spontaneously.

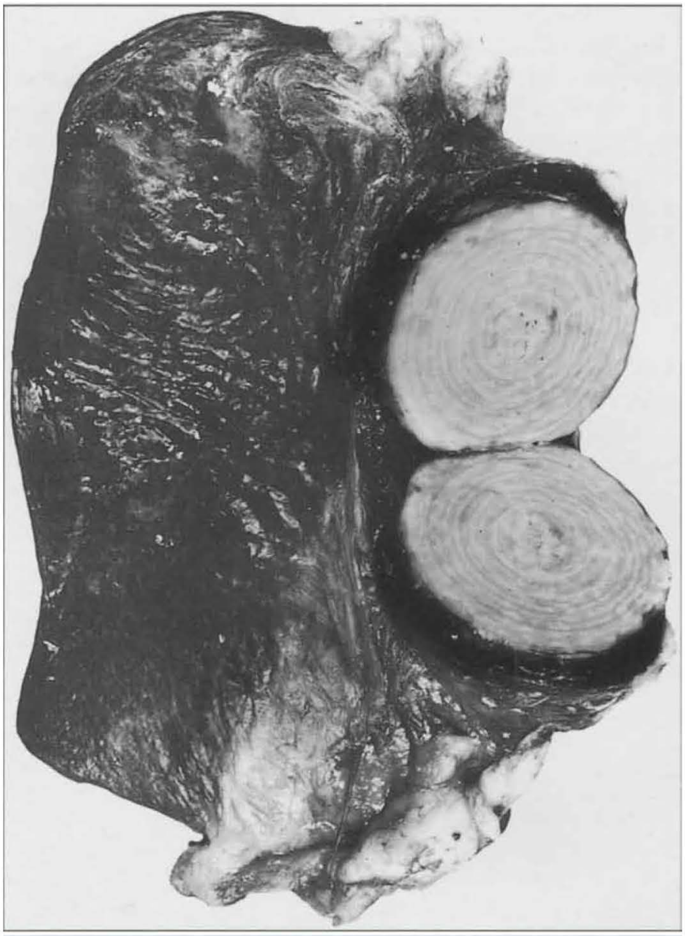

Figure 2.125 Laminated abscess of caseous lymphadenitis, spleen, sheep.

The mature lesion of CLA is an encapsulated abscess with pus of a distinct green hue and of caseous or caseopurulent consistency. The initial cutaneous lesion may not be evident, having resolved, but it is noted that occasional subcutaneous abscesses, principally of the face and belly, do occur without relation to known aggregations of lymphoid tissue. Mastitis, as occasionally observed in sheep and often observed in goats, probably represents an extension from a wound of the overlying skin; when the mastitis is early and acute it is diffuse and suppurative, and when chronic it is localized to encapsulated abscesses. The disease is easily diagnosed by fine-needle aspiration of the focal lesions, which allows both cytopathology and bacterial culture.

Spread from the lymph nodes produces lesions in the lungs, and these are rather common with advancing age. They also occur in young lambs, in which the progression is more rapid than in adults. The pulmonary lesions may consist of extensive bronchopneumonia, when abscesses rupture into bronchi in which there are soft caseopurulent foci, or there may be discrete nodules of various sizes and numbers. In cases of bronchopneumonia, and overlying those nodules which are immediately subserosal, there is pleuritis, often with adhesions. When the adhesions are few and localized adjacent to the nodules, the remaining pleural cavity may be normal. When the adhesions are more diffuse, there is a large amount of serous fluid in the cavities and a thin layer of fibrin on the pleura. The nodular lesions in the lung are similar to those in lymph nodes, and have a narrow zone of bronchopneumonia outside the capsule. With time, the pulmonary nodules become sharply circumscribed, encapsulated, subpleural abscesses. The pulmonary lesions are associated with characteristic lesions in *bronchial lymph nodes* which may be much enlarged. Dissemination of the infection from the lungs to other viscera is uncommon. Metastases are occasionally observed in the renal cortex as discrete abscesses or as descending pyelonephritis. Other viscera, chiefly the liver and spleen, may contain solitary abscesses of the typical form.

Antibody detection tests that may be useful in detect carriers include an enzyme-linked immunosorbent assay (ELISA) and a bovine gamma interferon ELISA. The agent itself can also be detected rapidly by PCR. Vaccines containing various combinations of whole cells and toxoids have had some success; DNA vaccines are under development. The organism is sensitive to a number of antimicrobials, but response to therapy is poor both because of the intracellular location of the organism and the characteristic formation of abscesses into which drug penetration is ineffective.

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Anthrax

Anthrax is caused by ***Bacillus anthracis***, a large, gram-positive, spore-forming organism that is *highly pathogenic for most herbivorous animals and humans*, whereas carnivorous birds and reptiles are resistant. Domestic animals are susceptible to *B. anthracis* in the decreasing order of goats, sheep, cattle, horses, pigs and dogs. Farmed mink are highly susceptible. *In ruminants, the disease is usually brief and septicemic; in horses, pigs, and dogs, it is frequently localized to the throat or intestine and may be fatal before invasion of the blood occurs.*

When the disease is septicemic, as it usually is in herbivores, the blood and tissues of the animal swarm with vegetative organisms which, when exposed to air or oxygen, form spores of most remarkable durability. *It is the combination of these two factors, the number of organisms and the resistance of spores, which is of paramount importance in the epidemiology of the disease.*

Bacillus anthracis probably has limited capacity for growth in the external environment, due in part to antagonistic soil bacteria. Growth may occur in alkaline soils with much decaying vegetable matter, and alternate periods of rain and drought and temperatures in excess of 15.5°C may also facilitate growth. The spores are known to remain viable in soil for at least 15 years, and probably much longer, since they have been noted to retain their vitality and virulence for 50 years in the laboratory. Natural survival of vegetative organisms is rather short, equivalent to the short duration of an infection; vegetative organisms do not survive in a carcass, but are rapidly killed by putrefactive bacteria. In the terminal stages of the disease, large numbers of bacilli are excreted in all natural excretions, as well as pathological exudates, and these organisms sporulate and perpetuate infections. As a general rule, *the spores are very resistant to methods of disinfection*, with the exception of chemical disinfectants which are oxidizing agents. Spores on skin have even survived tanning processes to become a hazard for humans.

Reliable details on how spontaneous infections are initiated in animals are not available, but the disease in wild herbivores often follows construction requiring excavation, such as fencing and excavation of dug-outs for watering livestock. It is accepted that dogs and pigs acquire the infection as a result of eating an animal that had anthrax, and deaths in humans have occurred after eating inadequately cooked meat from a goat dead of anthrax. Anthrax in pigs has been traced to the ingestion of bone meal which was not sufficiently sterilized. Vegetative bacilli are unlikely to cause the disease since they are rapidly destroyed in the acid medium of the stomach. Cattle and sheep are presumed to obtain the infection by ingestion of contaminated food and water, entry through mucous membranes possibly being aided by local trauma. Cutaneous infection is rare in cattle but has been reported in India, and pulmonary anthrax resulting from the inhalation of spore-laden dust can occur. Infection through the skin is occasionally seen in sheep, and may be assisted by grass seed infestation. Ingestion is an important mode of infection in horses and dogs, as indicated by the common occurrence of lesions in the throat. It is also thought that infection can be transmitted to horses by blood-sucking insects. Intestinal anthrax in pigs probably reflects infection by ingestion.

The **pathogenesis** *of anthrax is an initial lymphangitis and lymphadenitis* (Fig. 2.126), *which develops into septicemia.* This sequence is especially well illustrated in the pulmonary form of the experimental disease; spores that are inhaled are ingested by cells lining alveoli and transported in them to the tracheobronchial nodes, in which vegetation and true initiation of the infection occur. Spread to the blood is via lymphatics as well as by lymphovenous connections within lymph nodes, and numerous bacilli spread in the lymph from node to node as the filtering mechanism of each is successively swamped. Bacilli that enter the blood are taken up in other parts of the mononuclear phagocyte system, especially the spleen, to establish secondary centers of infection and proliferation.

There is notably little response on the part of a susceptible animal to the local establishment of anthrax infection. *Physiological disturbances, clinical signs and death depend on the development of a massive septicemia.* In immune animals, e.g., guinea pigs, infection is followed by a period of 2–4 hours in which the bacilli proliferate; the area then becomes infiltrated by leukocytes, and the bacilli fragment with little or no phagocytosis of intact organisms. The lysis of bacilli, of which the first sign is loss of capsule and staining properties followed by fragmentation, is apparently due to anthracidal substances in plasma or, more likely, liberated from leukocytes. Anthrax has always been regarded as dependent on invasiveness rather than toxigenicity, but it appears that antitoxic immunity does, in some manner, inhibit invasiveness.

Vegetative cells produce a small array of **toxins**. The organisms themselves and the capsular material are virtually nontoxic, although the capsular material may act as a spreading factor and inhibit the activity of leukocytes. The activity of toxins in septicemic anthrax is well illustrated by the demonstration that, once the degree of bacteremia passes a certain threshold, which is about 0.3% of the usual

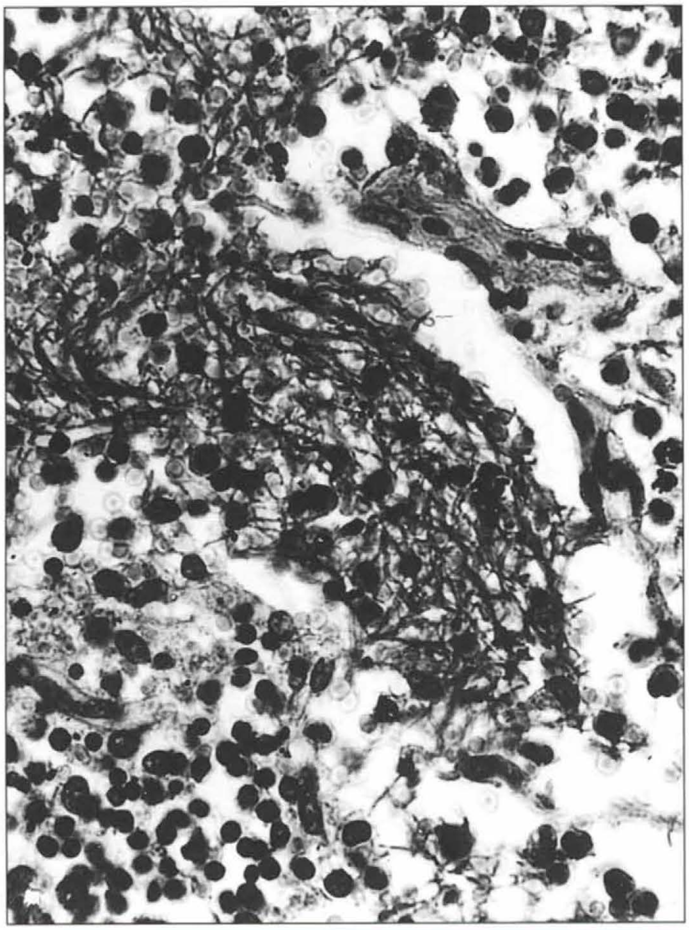

Figure 2.126 Anthrax bacilli in peripheral sinus, lymph node, ox.

maximum, death will occur even though all bacilli may have been destroyed by antibiotic therapy. *The toxin consists of three complementary components designated factors I, II, and III, or edema factor, protective antigen, and lethal factor*, respectively. *Edema factor* is an adenylate cyclase that increases cyclic AMP after activation by calmodulin. *Protective antigen* is likely a receptor-binding protein that appears to be essential for the biological effects of edema and lethal factors. *Lethal factor* is a CNS depressant, but its major effects may be elsewhere. The toxins have been assessed only in experimental laboratory animals, between species and strains of which there are considerable differences in sensitivity. The three toxins are serologically distinct and, since there is autostimulation, it is not surprising that they do not produce lesions when injected separately. *The combined effects of the three toxins are injury and inactivation of phagocytes, increased capillary permeability, anticomplementary activity, and impairment of coagulation.*

Immunity to anthrax appears to depend on the neutralization of toxin. Antibodies against the bacterial cells and capsules are useless, as is evident from the experience that completely avirulent or dead bacilli are not immunogenic; in fact, the most potent vaccines are of bacilli that proliferate and cause acute local inflammation but that do not invade the blood. The antigen that provokes antitoxic immunity is the protective antigen discussed above; it is presently regarded as being a nontoxic degradation product (toxoid) of the very labile toxin found in the blood of affected animals. The vaccine of Pasteur apparently resulted from the loss of a temperature-sensitive plasmid that encoded for the protective antigen.

When an animal is suspected of having died of anthrax, organisms should be detected in smears of blood or local exudate. All bacilli in internal organs are likely to be destroyed in 48 hours or less by putrefaction. Hence, *it is best to obtain blood for diagnostic purposes from close to the coronet or the tip of the tail*, places that are likely to be involved last by putrefactive processes that destroy the vegetative bacilli. *Bacillus anthracis* occurs in blood in pairs or in short chains of 3–4 cells. They are large truncate organisms that are easily observed. *They are differentiated from putrefactive bacteria by their distinct capsule, which stains pink with old methylene blue, and by having square ends when these are apposed.* The free ends of *B. anthracis* are often rounded as in *Clostridium* spp. The organism can be cultured readily from putrefied exudates if advantage is taken of its aerobic requirements and the heat resistance of the anthrax spores, to separate it from nonsporulating organisms and sporulating anaerobes. The organism can also be purified in guinea pigs by applying the inoculum to scarified skin. In nonsepticemic anthrax, such as occurs in swine and dogs, it is best to look for the organism in the local exudates and affected lymph nodes. For the diagnosis of anthrax, when other methods have failed or the tissues are old and dry, the Ascoli agar-gel precipitin test is useful but not completely specific.

Bovine anthrax is usually septicemic, and *sudden death* is usually the first indication of its presence in a herd. Even when cattle are observed closely, they may be dead within 1 hour of showing signs of illness, although some will show general signs of illness for about 24 hours before death. The signs of illness vary with the route of entry and when, as usually happens, entry is by inhalation or ingestion with no area of localization, the animals are depressed and listless. On examination there is high fever, increased heart and respiratory rates, and congested and terminally cyanotic mucosae that show evidence of bleeding. Animals that survive for a day may have dysentery, abortion, edematous swellings of the perineum, throat and abdominal wall, and blood-stained milk. Although infection may be virtually synonymous with disease and death in goats and sheep, it is not necessarily so in cattle, in which species *some infected animals probably recover*. This is suggested by the recovery of some animals that are experimentally inoculated and by the occurrence of transient febrile reactions attributable to no other causes, in herds in which fatal anthrax is occurring. For most animals, however, treatment to be effective must be administered early before there is marked septicemia and toxin production.

The carcass of an animal dead of this disease putrefies quickly, becomes very rapidly distended with putrefactive gases, and blood exudes from the natural orifices. These changes are, of course, not diagnostic, but when they are observed in an animal that has died suddenly in an area in which anthrax is endemic or has at any time occurred, the examination of smears of blood should always precede autopsy. Anthrax in the fulminating disease is very largely an intravascular infection with most of the organisms in the blood and the rest in the spleen. Septicemia in anthrax is a terminal event, and smears of blood may not be helpful when prepared more than a few hours before death.

The morbid picture of the disease in cattle is characterized by *splenomegaly*, multiple hemorrhages, and edematous effusions in connective tissues. *A very large soft spleen is the most significant lesion, and very rarely is it absent*. Splenomegaly occurs in other diseases of cattle, but rarely is it as large in association with sudden death. In anthrax the spleen is soft, sometimes it ruptures spontaneously, and when it is incised the pulp exudes very thick black-red blood which brightens in color on exposure to air. Smears and sections of the spleen reveal very large numbers of bacilli if the carcass is fresh but, when decomposition is advanced, they are destroyed by putrefactive changes. In some cases, splenomegaly is the only lesion. The histology of the spleen is not revealing. The sinus areas are distended with sludged red cells and the lymphoid follicles are widely separated and hypocellular, but numerous leukocytes and bacilli in chains are present. *It is typical of septicemic anthrax that the organisms are always intravascular.*

The blood is thick and dark, frequently described as tarry, and either it is not clotted or the clots are very soft and friable, compatible with the effects of the combined toxins in inhibiting the clotting system. There are likely nonspecific effects present, such as the disseminated coagulopathy and fibrinogenolysis that accompany hyperthermia. Small hemorrhages are common in the mucous and serous membranes and in the subcutaneous connective tissues. Loose connective tissues in any location may be infiltrated with gelatinous fluid, and accumulations of such fluid in serous cavities are stained with blood. There is congestion, swelling, and degeneration of parenchymatous organs. The myocardium is dull and flabby.

Cattle are moderately resistant to *B. anthracis* so that local lesions may occur at the site of entry. Local lesions are usually in the small intestine and take the form of *ulcerative hemorrhagic enteritis*, but acute inflammation in the abomasum and large intestine may also occur. The most severe lesions may be over the lymphoid tissues of the intestine, or extend for a considerable distance from these. The mucous membrane is intensely red, and at a greater distance is sprinkled with small hemorrhages. The contents of the intestine are then deeply stained with blood. Superficial necrosis and ulceration occur in some areas of most intense hyperemia. The corresponding mesentery, up to the regional nodes, is infiltrated with gelatinous fluid as a result of acute lymphangitis, and the fluid may be stained with blood. The regional nodes have the appearance of the spleen. They are enlarged, red-black, and on cut surface are moist and shiny. The vessels are intensely congested, and hemorrhage extends into the peripheral sinuses and cortex. Bacilli are numerous and leukocytes are present, but there is no necrosis.

In some cases in which the organisms gain entry through the oropharynx, there is hemorrhagic lymphadenitis of the nodes of the throat and edema of the connective tissues in these regions. The occasional case of *pulmonary anthrax* in cattle is characterized by acute congestion and consolidation of a portion of the lung with larger areas of interstitial edema, edema of the mediastinum, and regional hemorrhagic lymphadenitis. The pulmonary lesion is exudative, but lacks the full gamut of inflammatory change unless another cause of pneumonia is superimposed.

Sheep are more susceptible to *B. anthracis* than are cattle, and local lesions do not occur except in the unusual instances of percutaneous infection, in which the lesion may take the form of spreading edema from the outset or initially appear as hard circumscribed nodules. The disease in sheep takes the same course as that in cattle except that it is even more rapid. Splenomegaly is not as prominent in sheep as in cattle, likely because of the greater level of collagen in the splenic capsule of sheep. The parenchyma is, however, dark and soft. Edematous effusions do not occur in sheep.

Clinical signs of anthrax in **horses** may last for several days and are characterized by *colic* or by *large edematous swellings*. The swellings, which can be very extensive, occur on the ventral part of the abdomen and thorax, the legs, in the perineal region and about the external genitalia. Dysentery may accompany the acute colic. When ingestion is the route of infection in horses, the primary lesion may be in the throat or the intestine, and death may occur from the local reaction and without septicemia. Intestinal lesions are similar to those described above for cattle, and pharyngeal lesions are similar to those described below for swine. When septicemia occurs, the morbid changes are the same as in cattle, including very prominent enlargement and congestion of the spleen.

Pigs are relatively resistant to anthrax. They acquire the infection from eating infected flesh and the infection remains localized to the throat or intestine. Since septicemia is exceptional, splenomegaly is not a prominent part of the gross picture. *The characteristic sign is swelling of the pharyngeal region and neck.* Some pigs have diarrhea and dysentery, but it is unusual to have intestinal localization without pharyngeal localization. Anthrax without illness has been observed in swine; in such cases the disease is limited to isolated mesenteric or pharyngeal nodes.

The local lesion of anthrax is swine is a typical carbuncle at the point of entry, with acute regional lymphadenitis and lymphangitis. Some bacilli no doubt reach the blood, but they do not establish septicemia. Bacteria may localize in liver, spleen or kidney to produce a metastatic carbuncle but, usually, only individual nodes near the site of entry are involved. The lymphadenitis may be diffuse or focal but in both cases it is, in the initial stages, hemorrhagic. An intense leukocytic infiltration occurs, all cells within the affected portions of the node die, and the focus becomes encapsulated. With necrosis, the affected tissue changes from a brick-red to a gray friable mass that can be easily shelled-out when the gland is incised.

In *primary intestinal anthrax in pigs*, the initial lesion is focal or multifocal hemorrhagic enteritis, with a central zone of diphtheresis that eventually ulcerates. The adjacent serosa and mesentery are thickened with edema fluid and yellow, with foci and streaks of hemorrhage; they are the site of focal hemorrhagic necrosis due to acute necrotizing vasculitis and lymphangitis. These mesenteric lesions extend only as far as the regional nodes, which show the type of lymphadenitis characteristic of anthrax in swine.

Dogs are reputedly quite resistant to *B. anthracis*, but a number of outbreaks have been observed in kennels in which the dogs have inadvertently been fed meat from an animal that has died of the disease. Anthrax in dogs may pursue a *peracute course* to sudden death, it may be of the *pharyngeal type* in which extensive edema develops in the face, head and neck, or it may be of the *intestinal type* with signs of acute gastroenteritis. Anthrax may occur in **mink** with high mortality after feeding fresh meat from infected animals.

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Streptococcal adenitis in swine

Jowl abscess *is a cervical adenitis caused by* ***Streptococcus porcinus***, and like strangles of horses, the deep infection follows colonization of the oral cavity and likely the tonsils. The disease has become less common with the introduction of better hygiene, age-matched groups, and feeding equipment designed to avoid sharp projections. The organism has 8 serotypes, based on carbohydrate antigens, of which type 4 is most frequently isolated from cervical lymphadenitis of swine. Baby pigs have passive immunity, and transmission occurs through direct contact with infected animals that can shed organisms for months, and also from contaminated environment. Invasion of the nasopharynx is followed by mild fever and leukocytosis that resolve, and by lymph node enlargement in 2 weeks, which less often progresses to open drainage. The mandibular nodes are most often involved, followed by the retropharyngeal and parotid nodes. The disease is not often of clinical importance, and affected animals grow well, but losses result from condemnation at slaughter. Bacterins are less effective than live oral culture in prevention.

The abscesses are usually multiple and measure 1–10 cm in diameter. The pus is typically without odor, green, and creamy in consistency. *Streptococcus porcinus*, a beta-hemolytic *Streptococcus* that belongs to Lancefield's group E, can be isolated regularly from the pus.

Rhodococcus equi is often present in the submaxillary lymph nodes of swine and has been proposed as the cause of lesions typical of tuberculosis in these nodes. Evidence suggests, however, that *R. equi* is not responsible for the tubercle-like lesions, which are thought to be caused by *Mycobacterium* spp. of one or other variety. *Rhodococcus equi* has been isolated from lymphadenitis in cattle.

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Streptococcal adenitis in dogs

Streptococcal adenitis of dogs is similar to the disease in swine. It occurs in minor endemics in kennels and is characterized by pharyngitis, fever, conjunctival discharge and enlargement of the submaxillary nodes. The illness is transient in most cases, but in approximately 10% of dogs the course is prolonged for 2 weeks or more, and in these there is a tendency for the nodes to suppurate. The abscesses may be encapsulated and sterilized, or they may fistulate on to the skin and heal. Dysphagia, and occasionally asphyxia, occur in puppies. Typically, the *Streptococcus* belongs to Lancefield's group G and, coincident with the pharyngeal infection, bitches may have inflammation of the genital tract caused by the same organism.

Cervical adenitis caused by the group G organism may occur in **kittens**.

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Tularemia

Tularemia (deer fly fever) is caused by ***Francisella tularensis***. The disease was first described in Tulare county of California and is still more common in the western USA than elsewhere. The organism is a tiny, gram-negative, very pleomorphic coccobacillus that is a strict aerobe and shares many cultural and epidemiological features with *Yersinia pestis*, the cause of bubonic plague. *Francisella tularensis* is found worldwide except for Australia and Antarctica, but the biovar tularensis is found only in North America. *Francisella tularensis* infects a wide range of species, including most *domestic animals, humans, and wild rodents*, and it is in these last two that the disease is most often fatal.

The organism is abundant in nature as an infection of many species of rodents, and it is from these, either directly or by the mediation of insect vectors, that humans and domestic animals acquire the infection. The organism is able to penetrate intact skin and mucous membranes, but it is also infective by ingestion, inhalation, and inoculation by biting insects and ticks.

Tularemia in humans is a severe systemic disease, with various manifestations depending on dissemination or localization. *The disease in rabbits and rodents is recognized by the presence of miliary white foci 2 mm or more in diameter in the liver, spleen and lymph nodes.* They are indistinguishable grossly from the lesions caused by *Yersinia pseudotuberculosis*. Histologically, the lesions are characterized by *very focal but complete necrosis*. Neutrophils and pus may be present early and macrophages accumulate, but in slightly older and larger lesions there is total coagulative necrosis with a granularity that resembles caseation (Fig. 2.127). A very few fibroblasts and macrophages produce a sharp, narrow margin for the lesion. The bacteria can be demonstrated quite readily in lesions, in clumps in macrophages and especially as dead but still distinguishable ones in the center of the focus. The lesions in lymph nodes are often larger than those in the liver and may be readily visible grossly as wedge-shaped areas of cortical necrosis demarcated by a narrow zone of intense reactive hyperemia. The affected nodes are palpably enlarged in the living animal and may discharge a thin red pus on to the skin. The lymphadenitis may be generalized or restricted to the nodes draining the site of infection which, if visible, is an ulcerated papule.

Francisella tularensis is probably common as an infection in animals, but *the disease tularemia is not common*. It is probable, too, that

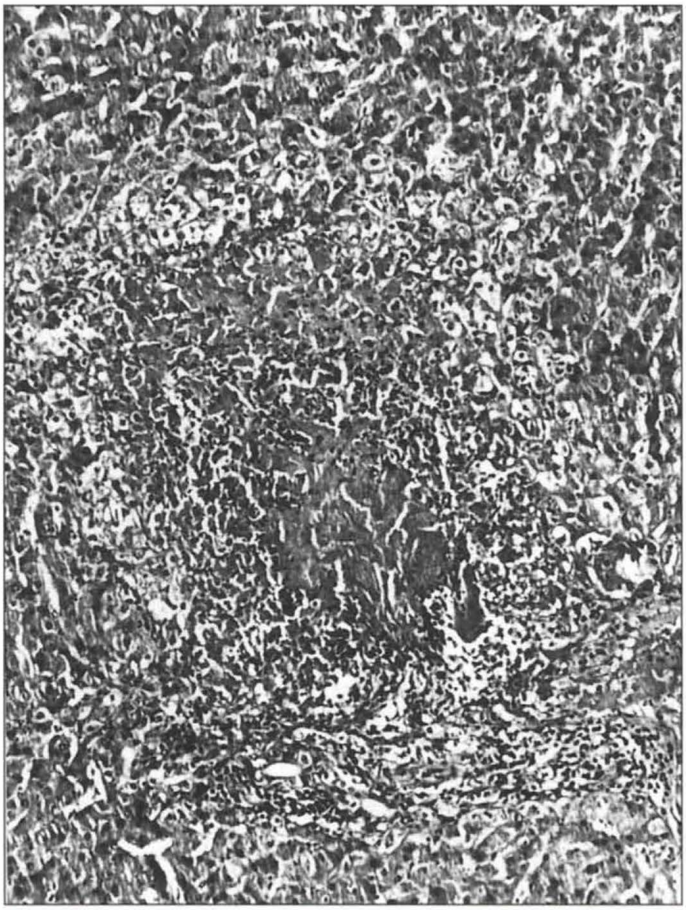

Figure 2.127 Pyogenic granuloma in tularemia in the liver of a sheep.

the infection may remain latent in domestic animals for long periods without causing ill health and with or without focal lesions, as has been observed in dogs. As a facultative intracellular parasite, it may persist for years as a latent infection, for which effective immunity is cell-mediated.

Naturally acquired, latent (subclinical) infections with *F. tularensis* have been observed in *dogs and cats* but, as a general rule, these species are resistant to the disease. Fatalities due to tularemia have been observed in *foals and sheep* in association with heavy infestations by ticks, and it is probable that the disease is endemic in sheep in areas where the reservoir rodents and ticks, especially *Dermacentor andersoni* and *Amblyomma americanum* abound. The disease in foals is characterized by a systemic and febrile illness with, at autopsy, enlargement of liver, spleen and kidneys, and the presence of the typical small necrotic foci of this disease. Tularemia in sheep is also associated with a heavy infestation of ticks; recovery can occur if the ticks are removed before the illness is far advanced. Affected sheep have high fever, stiffness of gait, depression, diarrhea and an increased respiratory rate. Lesions may be confined to the superficial lymph nodes or show the more classical localization. Pneumonia, probably of other cause, and anemia caused by the ticks are frequently present.

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Pseudotuberculosis

Yersinia pseudotuberculosis occurs worldwide in wild rodents and birds and is widespread in nature, recoveries having been made from soil, milk and feed. The organism regularly produces disease, often in epidemic proportions, only in *rodents and birds*. Sporadic infections with this organism and occasional outbreaks of disease occur in domestic species, laboratory colonies, mink and chinchilla colonies, and zoos. *Cats*, because of their contact with rodents and birds, are the domestic species most apt to be secondarily involved by outbreaks of the disease in its natural hosts. Losses of serious proportion, however, occur in *sheep* which are exposed to large numbers of organisms during outbreaks of the disease in rodents during cold weather. *The ovine disease is known as "pyemic hepatitis."*

The general pattern of this disease is the same in all species, the variation that occurs with respect to severity and duration being related to species susceptibility, and numbers and virulence of the organisms. *Yersinia pseudotuberculosis* is a *facultative intracellular parasite*, which explains the latent carrier state and the need for strong cell-mediated immunity for protection from infection. *The route of transmission is by ingestion* and, in susceptible animals, organisms enter the body through the intestine. Small necrotic foci develop in the Peyer's patches of the ileum and colon, and extend as lymphangitis to the regional nodes. The organism becomes septicemic and may kill susceptible rodents at this stage; more typically, and in all domestic species, *caseonecrotic foci form in the mesenteric nodes, spleen and liver* (Fig. 2.128A, B), often in association with fibrinohemorrhagic inflammation in the small intestine. The hepatic foci, which are the most obvious, are 1–10 mm in diameter, white and have no or scant tendency to encapsulation or softening. They are interspersed with irregular areas of parenchymal collapse that probably result from vasculitis and thrombosis (Fig. 2.129A). Microscopically, there is necrosis, with bacterial colonies and fragmented leukocytes surrounded by macrophages. Giant cells are absent, even from later contracting granulomas (Fig. 2.129B). The mesenteric nodes and spleen contain similar foci, and are enlarged by lymphoid and histiocytic hyperplasia. The mesenteric nodes in the cat may be 2–4 cm in diameter and can be grossly confused with intestinal toxoplasmosis or lymphomatosis.

Yersiniosis has emerged as a significant cause of disease in *farmed ruminants*, including deer. Affected deer may be found moribund or dead, but animals under observation are systemically ill with profuse diarrhea. The incidence of disease in younger age groups rivals that of salmonellosis. In addition to the *mesenteric lymphadenitis and hepatitis, enteritis* is consistently present and characteristic in its histologic expression. Numerous bacterial colonies are present in the lamina propria, associated with multiple suppurative foci or a diffuse suppurative enteritis.

In view of the extraordinarily wide host range of *Y. pseudotuberculosis* in natural infections, it is surprising that the infection is not recorded more often. This may be due in part to a preference of the organism for growth at room temperature rather than at 37°C.

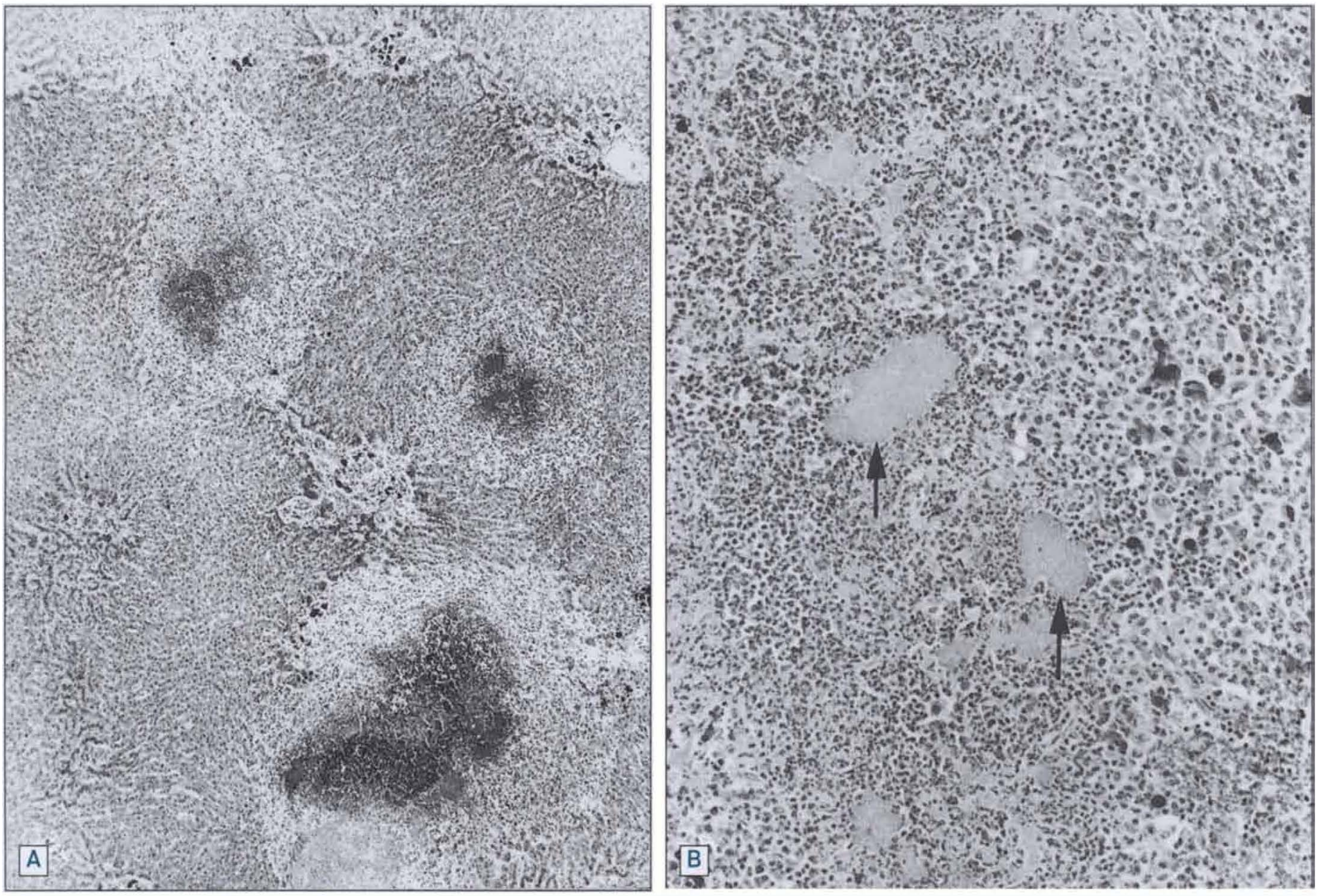

Figure 2.128 ***Yersinia pseudotuberculosis* infection** in a sheep. **A.** Multiple pyogenic granulomas in liver. **B.** Acute suppurative splenitis. Large bacterial colonies (arrows).

While susceptible laboratory species, such as guinea pigs and mice, are readily infected experimentally, there are few pathogenetic studies in domestic species. The frequency of natural infection in cats contrasts with the difficulty in establishing experimental infection in this species, which suggests the need for some predisposing alteration in the alimentary mucosa.

The related organism ***Yersinia enterocolitica*** causes *gastroenteritis and mesenteric adenitis* in a wide variety of species, including wild and domestic animals and is increasingly isolated from cattle. The organism has been isolated from deer, rabbits, dogs, pigs, horses, mink, various avian species, as well as sheep and goats. *Yersinia enterocolitica* shares an antigen with *Brucella abortus* that may result in false-positive agglutination reactions in serologic testing for brucellosis.

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Histoplasmosis

Histoplasmosis is caused by ***Histoplasma capsulatum***, a facultative intracellular parasite of macrophages, and is characterized by diffuse involvement of the mononuclear phagocyte system. *Histoplasma capsulatum* is a dimorphic ascomycete fungus. The *parasitic phase* is yeast-like, whereas the *nonparasitic phase* produces an abundant mycelium. The mycelium produces two types of spores: a small, smooth, globose *microconidium* 2–3 μm in diameter, and a large, thick-walled, globose *macroconidium or chlamydospore* that bears prominent surface projections. The chlamydospores are an identifying feature of the fungus and also the initiators of the parasitic phase.

Histoplasmosis has a worldwide distribution and is frequently diagnosed in the Mississippi, Ohio, and St. Lawrence river valley areas of North America. *It is a largely noncontagious disease of humans,*

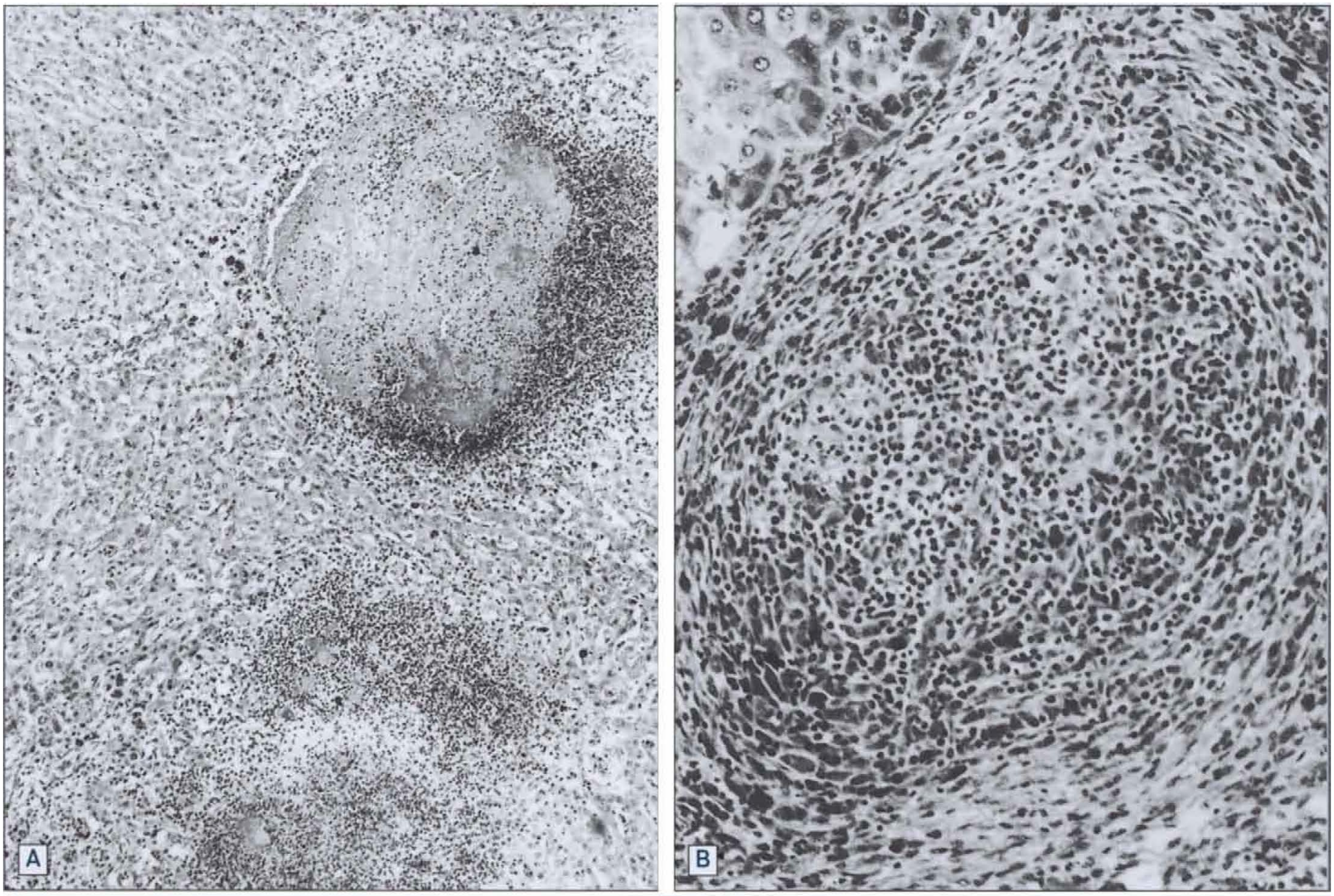

Figure 2.129 ***Yersinia pseudotuberculosis* infection** in the liver of a sheep. **A.** Septic phlebitis, sublobular vein. **B.** Residual granuloma of protracted infection.

dogs, cats, swine, cattle, horses, and wild animals, and it is suspected that the endemic distribution of the disease is related to factors that favor the persistence and growth of this environmental mold in the soil. The ubiquitous nature of the organism, its intracellular habitat, and its low infectivity suggests that cell-mediated immunity may be a major factor in the pathogenesis of disease.

It is generally agreed that *infection is obtained from soil*, either by ingestion or by inhalation of dust, and a distinct association has been repeatedly demonstrated between *Histoplasma capsulatum* and places where *pigeons* congregate, with abandoned *chicken* runs and houses, and with *bat* caves. Chickens themselves are not infected because their high body temperature is not conducive to growth of the organism.

The disease in animals is seen most frequently in *dogs* and less often in *cats*. Transmission from dog to dog has been established, but it is not known if dogs can transmit the disease to humans. When histoplasmosis becomes apparent clinically, the infection is disseminated, and the disease is then progressive and always fatal. Only a minority of infections become disseminated and cause disease, while *the majority occur without signs or lesions*. A high incidence of infection in endemic areas has been confirmed by cultural means, either by direct culture of organs or by first passing infected tissues through mice, which are highly susceptible to *H. capsulatum*. Bats, however, are resistant to infection with *H. capsulatum*, and frequently shed spores in their feces. Caves inhabited by bats are heavily contaminated with fungal spores, and it is suggested that this may be a further means of soil contamination particularly in the south and western United States. Positive cultures can be obtained from about 50% of unrelated cats and dogs.

Transmission is by inhalation of airborne conidia, followed by phagocytosis in the lung and then transport in phages to other areas of the body. This route accounts for the high incidence of infection in the bronchial and cervical lymph nodes. Since intestinal lesions are usually present, it is felt that ingestion might provide a direct route of infection. However, in disseminated disease, the intestine could be secondarily infected like many other tissues.

The course of *latent infections* in animals is not known, but the evidence suggests that such infections can persist for many months and possibly are permanent. Infection has been known to persist, without causing ill health, for 2 years in guinea pigs, 6 months in other laboratory rodents, and 4 months in kittens.

Animals with advanced disease due to *Histoplasma capsulatum* show emaciation, persistent diarrhea, pyrexia and enlargement of liver, spleen and lymph nodes. In a particular animal, the clinical signs are most commonly related to either the respiratory or the gastrointestinal tract.

Animals with histoplasmosis most consistently have mild nonresponsive, normochromic normocytic anemia. Leukocytic changes vary with the reserves of the animal and the stage of the disease. If the animal is in good body condition, the bone marrow is competent and there is neutrophilic leukocytosis with some degree of monocytosis. Later with debilitation, the leukocyte count drops to normal levels or lower with left shift and marked toxic changes, including the appearance of Doehle bodies. There is usually lymphopenia and eosinopenia. *Since the organisms are readily recognized on microscopic examination, fine-needle aspiration of liver, spleen, enlarged lymph nodes, marrow or skin provides adequate material for diagnosis in appropriate cases.* Occasionally the organism may be diagnosed in fine-needle aspirates of lung or bronchoalveolar lavage fluids, or lavage of the turbinates in animals with sinusitis. Cytologically, *the organisms are present in the cytoplasm of macrophages*, unless the cells have been injured, and are somewhat larger than when seen in histologic preparations.

Grossly, the *pulmonary lesions* of histoplasmosis may be in the form of gray, rounded nodules of 1–2 cm in diameter and with a distinct tendency to become confluent, or there may be a diffuse increase in the consistency of the lungs. When the *intestine* is involved, as it frequently is, the lesions are located chiefly in the lower part of the small intestine. The mucosa is the site of nodular thickenings or of corrugations similar to those seen in Johne's disease of cattle. The thickenings are due to infiltration of lymphocytes, plasma cells and macrophages in the lamina propria and submucosa, and they may extend also through the wall to the subserosa giving the gut a thickened pipestem appearance. When the thickening is extreme, ischemic ulcerations occur.

The *lymph nodes* are greatly enlarged, but are discrete and without adhesions. There may be no indication of normal architecture on the cut surface, with the uniformity resembling lymphoma, except that the nodes are firm and dry. Histologically, there are coalescing granulomas with histiocytosis, and cortical replacement by the reaction. The spleen is enlarged (Fig. 2.130), sometimes to several times its normal size, gray and firm. There is marked sinus expansion and filling by fixed cells of stromal origin and by colonization with macrophages, many of which contain the ingested organisms. There is lymphoid atrophy, varying in degree with the stage of debility of the animal. The liver is uniformly enlarged, firm and gray. The discoloration is diffuse and related to capsular thickening without focal lesions. The infiltrating cells collect in miliary foci in the portal triads and sinusoids, causing extensive displacement and atrophy of the parenchyma (Fig. 2.131A). The adrenal glands are often involved, the medulla, the cortex, or both, in histoplasmosis that is allowed to run its full course (Fig. 2.131B). Scant normal tissues may then remain in these organs.

Since *Histoplasma capsulatum* is an intracellular parasite of macrophages and causes diffuse involvement of the mononuclear phagocyte system, focal discrete lesions are not to be expected. *The enlargement of organs is due to extensive proliferation and infiltration with monocytes and epithelioid macrophages in whose cytoplasm many of the typical yeasts in small or large numbers are found* (Fig. 2.131B). The yeast bodies in sections and when stained with H&E appear as *basophilic dots with a clear halo*. The halo is part of the yeast's cell wall and can be demonstrated by stains for bound glycogen, the organism then appearing as a ring. Such stains are very helpful in distinguishing these organisms from cellular debris, but are not necessary when the yeasts are plentiful. Necrosis of tissues is not often present in histoplasmosis, although central caseation without mineralization may occur in large, dense accumulations of macrophages. Giant cells are seldom seen and there is only mild fibrosis.

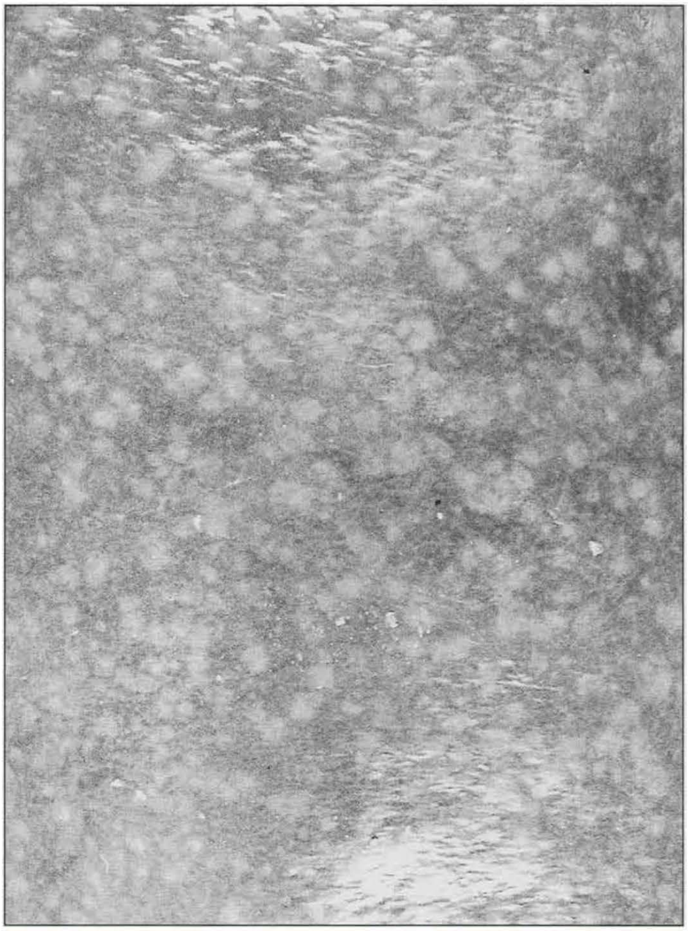

Figure 2.130 Histoplasmosis in a dog. Miliary epithelioid granulomas in the capsular surface of enlarged spleen.

The diagnosis of *occult histoplasmosis* can be made only by isolation of the organism from tissues. Responsibility of this organism for focal nonprogressive lesions can be accepted only when the fungus can be demonstrated histologically in the lesion, and this is rarely possible even in animals that are sensitive to histoplasmin.

The organs mentioned above are those most consistently and obviously involved in histoplasmosis. In the fatal disseminated disease, lesions of similar type may be found in other organs and tissues. *There is seldom any difficulty in demonstrating organisms in sections, although Grocott stain and PCR are more sensitive than H&E, and clinically they are readily cultured.* Culture should not be undertaken without precautions to prevent the inhalation of chlamydospores. Biopsy of enlarged lymph nodes and aspiration biopsy of bone marrow are useful for diagnostic purposes. In some cases, the organisms can be demonstrated in circulating monocytes, but they are never plentiful, so examination is best performed on buffy coat preparations.

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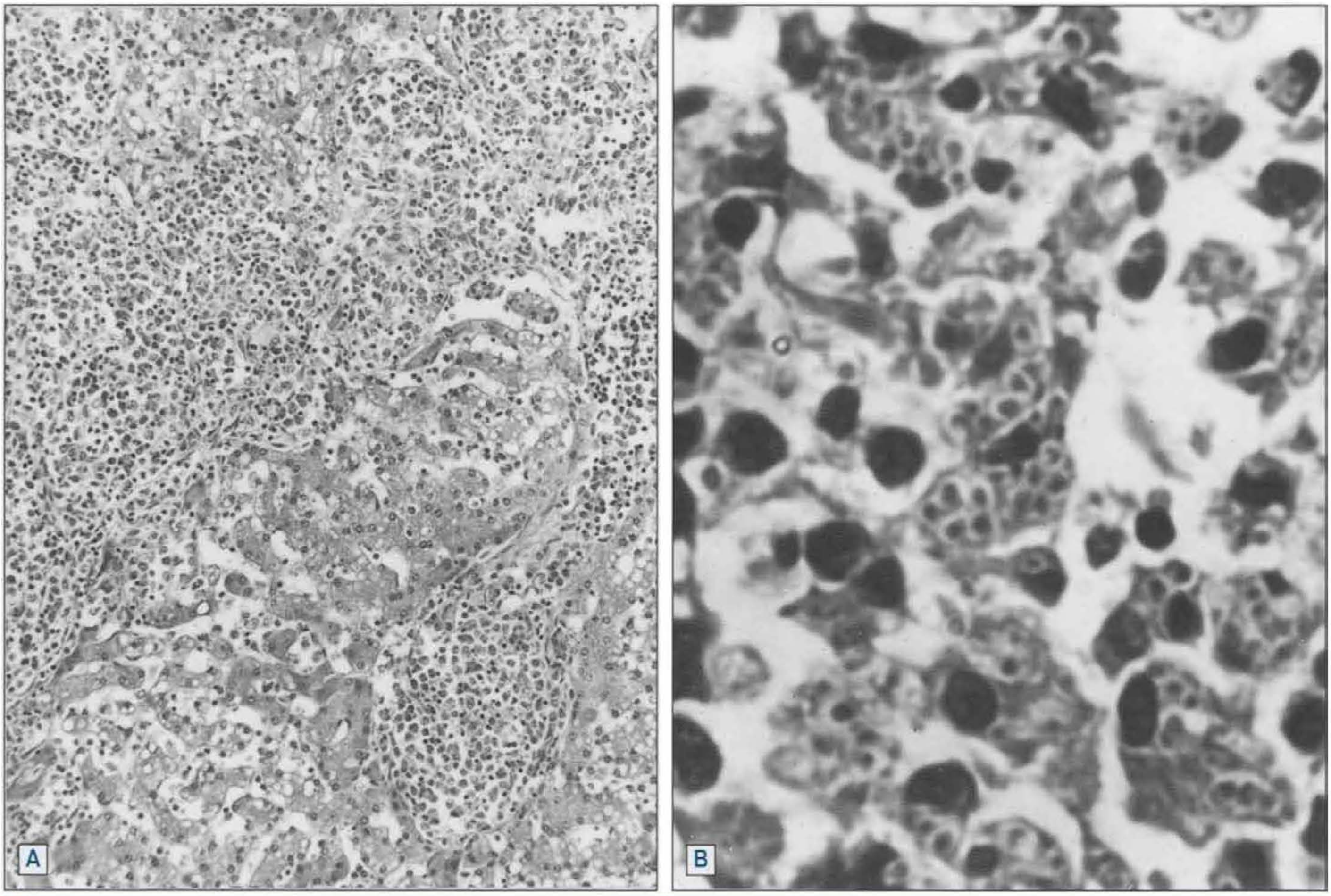

Figure 2.131 Histoplasmosis in a dog. **A.** Macrophage infiltration of liver **B.** Intracellular *Histoplasma capsulatum* in adrenal cortex.

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Leishmaniasis

The genus ***Leishmania*** is of protozoan parasites which are classified in the family Trypanosomatidae. Species of the genus are parasites of *humans, dogs, and other mammals*. Species of sandflies (*Lutzomia*) are the intermediate hosts and are essential for the maintenance of virulence, whereas other blood-sucking insects, including members of the genera *Phlebotomus* and *Stomoxys* and the tick *Rhipicephalus*, may act as mechanical vectors. Direct transmission and vertical transmission may occur in kenneled dogs.

The protozoa proliferate by binary fission in the midgut of the female sandfly and assume a *leptomonad form*, leaf-shaped with a single flagellum arising at the anterior pole. Infection of mammals occurs when the sandfly sucks blood, and in the mammalian host the protozoa assume the *leishmanial form* as rounded cells of ~2.0 μm in diameter with a vesicular nucleus and small kinetoplast but no flagellum. They multiply by binary fission and as leishmanial forms, the Leishman-Donovan bodies, are intracellular parasites of macrophages.

Leishmaniasis is important as a disease of humans, and wherever it occurs in humans it may also occur in dogs. Leishmaniasis actually includes three diseases:

- **cutaneous leishmaniasis** ("oriental sore") is caused by *L. tropica* and occurs in countries about the Mediterranean Sea;
- **mucocutaneous leishmaniasis** ("espundia") is caused by *L. braziliensis* and occurs in Central America;
- **visceral leishmaniasis** ("kala-azar") is caused by *L. donovani* and is endemic in parts of Europe, Africa, and Asia.

The species of *Leishmania* are not well distinguished; however, sequence data on kinetoplast DNA on several species permits their specific identification. Speciation depends on degrees of cross-immunity, different clinical manifestations, geographic location, and reservoir hosts. In some regions, such as India, the human population may be the reservoir but in other regions dogs, cats and other carnivores including rats probably serve as reservoir hosts.

Infections of animals with *Leishmania* spp. are probably quite common in endemic areas, but disease appears not to be. Latency of the infections, especially in the case of *L. donovani*, is also probable. *Both cutaneous and visceral forms of the disease have been described in dogs*, as well as cases of visceral disease in which the organisms are diffusely present in the dermis. Leishmaniasis is a disease of the monocyte–macrophage system, and the visceral disease mimics histoplasmosis. The protozoa

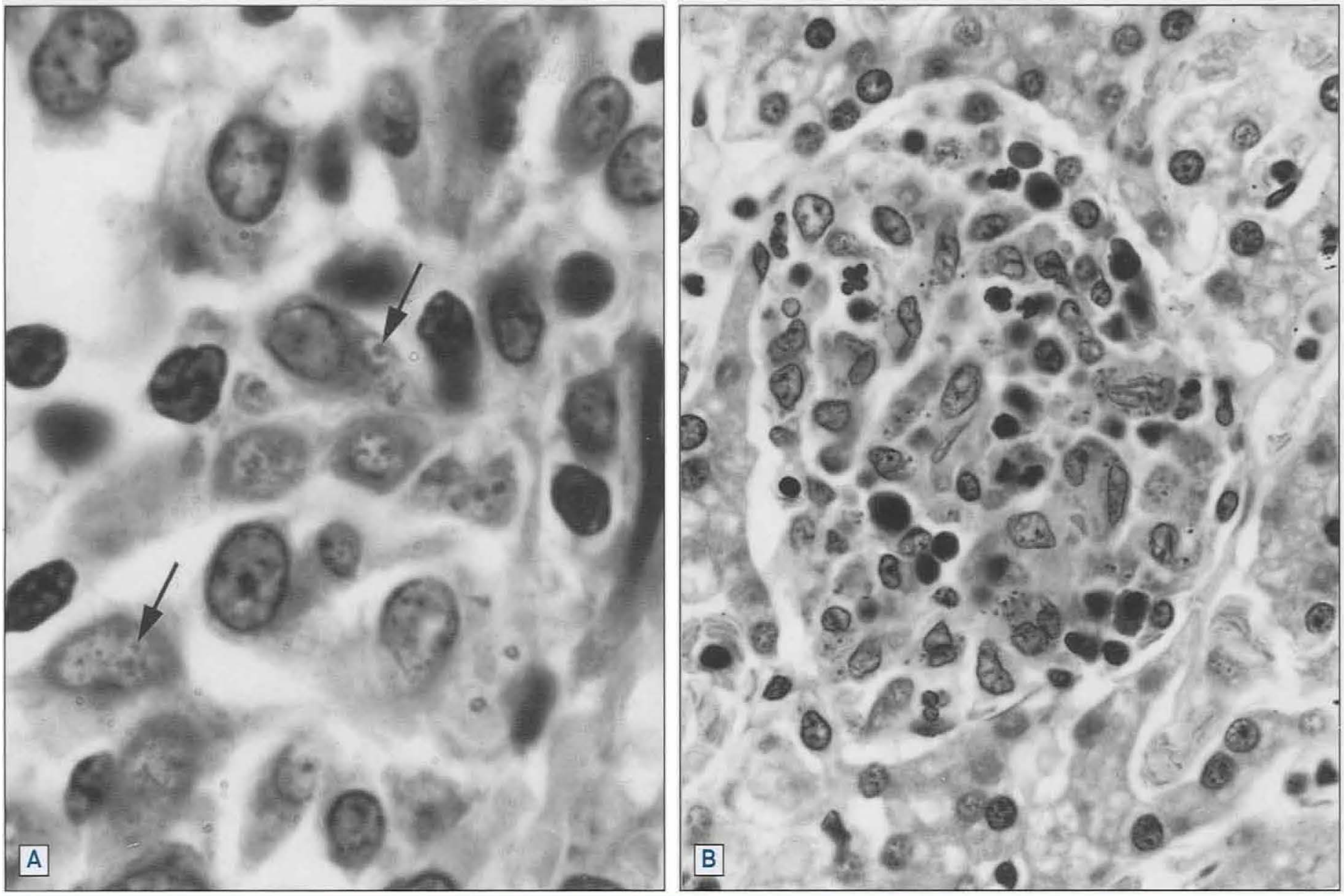

Figure 2.132 Leishmaniasis in a dog. **A.** Sinus area of spleen with macrophages, some containing *Leishmania* (arrows). **B.** Epithelioid granuloma in liver.

are not cytopathogenic in the usual sense, and destruction of host macrophages appears to be purely a mechanical consequence of proliferation of the protozoa in the cytoplasm.

Cutaneous lesions take the form of chronic ulcers that develop from inflammatory papules at the site of the insect bite. The organisms are inoculated by the biting insect and are soon ingested by histiocytes. Rapid proliferation of the protozoa disrupts the phagocytes, and the released organisms are ingested by further phagocytes to repeat the process. Lymphocytes and plasma cells surround the lesion and neutrophils are attracted to the debris. When the inflammation extends to the overlying epithelium, ulceration occurs. Numerous parasites are present within macrophages, and some are free in the tissue (Fig. 2.132A).

The *clinical signs of visceral leishmaniasis* in the dog are of chronic debility and often recurrent oculonasal discharge, with some crusting of the nose, and recurrent diarrhea. There may be mild enlargement of lymph nodes, and the spleen is always enlarged to some extent in the visceral form, but at any particular time may not attract clinical attention.

Grossly there are often focal scurfy skin lesions, but generally, in visceral leishmaniasis, the presentation is of a *mature dog in poor body condition with a rough hair coat.* The oral and cervical viscera are normal and the lungs generally have mild tan mottling but are otherwise normal, as is the heart. The liver contains numerous granulomas (Fig. 2.132B), and is symmetrically enlarged and dark brown. The spleen is 2–3 times or more normal size with symmetrical enlargement and is dark brown to black on capsular surface. There is mild irregular enlargement of lymph nodes with no other significant changes in the abdominal cavity except that the kidneys are darker than normal, and while of normal contour, there may be significant immune-complex glomerulonephritis and chronic renal failure. The bone marrow is uniformly reddened in midfemoral shaft in well-developed cases, but the fat is generally of normal character.

The lesions initially are of hemic-lymphatic hypertrophy with macrophage proliferation and focal granulomas (Fig. 2.132B). Splenic follicles are hyperplastic, often with follicular hyalinosis. In advanced cases, there is atrophy of nodes and spleen, and the sinus areas may be diffusely occupied by large macrophages heavily laden with intracytoplasmic organisms and numerous plasma cells (Fig. 2.133). The lesions in bone marrow may be focal, but consist of clusters of epithelioid macrophages with phagocytosed organisms (Fig. 2.134A, B). In the dog with a well-developed infection, the bone marrow will have remarkable plasma cell hyperplasia that may approach 50% of cells present and there is characteristically hypergammaglobulinemia. The protein is broadly polyclonal and the plasma cells lack atypia. Renal changes are variable and may consist of interstitial scarring with some parasitic involvement; however, the effects of hyperproteinemia and immune complexes appear to injure the kidney indirectly. Animals with leishmaniasis and amyloidosis have been described, and there may be concurrent changes due to both processes. *Diagnosis is based*

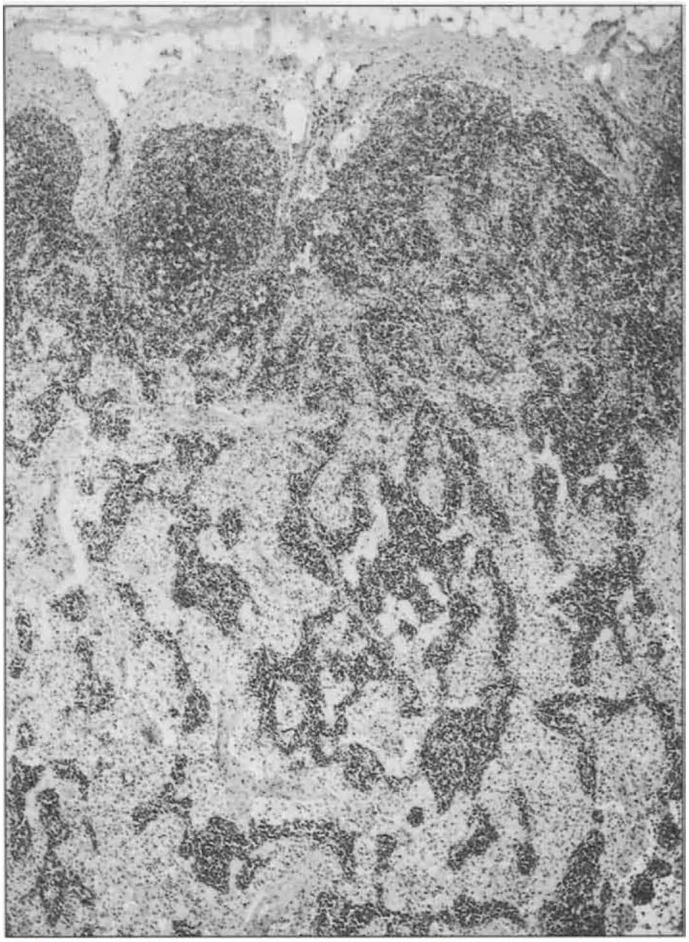

Figure 2.133 Leishmaniasis in a lymph node of a dog. Follicular and paracortical atrophy of chronic disease. Sinus histiocytosis and medullary cord hyperplasia.

on the demonstration of the organism in cytologic (Fig. 2.134A) *or in histologic preparation*, the former being easily achieved by aspiration of marrow, node, spleen, or liver.

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Theileriosis

Theileria are protozoan parasites of the order Piroplasmida, family Theileriidae. Speciation within the genus is not precise and the system in use depends on geographic distribution, vector, mode of pathogenesis, morphology, and frequency of schizonts in lymphocytes and of piroplasm stages in red cells, as well as on cross immunity and host specificity. Phylogenetic classification can be expected to evolve as more molecular-based investigations are conducted. The species presently recognized as pathogenic for cattle include:

- ***Theileria parva parva***, the cause of **East Coast fever** of east and central Africa;
- ***Theileria parva lawrencei***, the cause of **Corridor disease** (buffalo disease) of southern Africa;
- ***Theileria parva bovis***, the cause of **January disease** (Rhodesian theileriosis) of east and central Africa;
- ***Theileria annulata***, which causes **Mediterranean or tropical theileriosis** across north Africa and central Asia; and
- ***Theileria buffeli*** group (*T. buffeli, T. orientalis; T. sergenti* (invalid term)), which are widely distributed in East Asia and are relatively benign.

Theileria mutans causes benign theileriosis, a less important tick-borne disease of cattle in Africa, North and South America, Asia, Australia, and Europe. ***Theileria hirci*** and ***Theileria ovis*** are parasites of sheep in North Africa, the Middle East, and southern parts of Eurasia. *Theileria* spp. *reproduce by schizogony in lymphocytes (leukocytic or tissue phase) and are subsequently found in red cells (erythrocytic phase)*. A related genus is *Cytauxzoon*, which reproduces by schizogony primarily in macrophages and by fission in red cells.

The *Theileria* are spread by *biological tick vectors*, principally of the *Rhipicephalus* and *Hyalomma* genera. Larval and nymphal ticks ingest parasites in the erythrocytes of infected hosts. After engorgement, they detach from the host animal and molt through to the next instar. During this period, the parasite migrates to the acinar cells of the salivary gland of the tick. At this stage, the parasite in the salivary gland of the unfed tick is not infective for cattle. Once the infected tick has started to feed, the parasites multiply in the salivary gland, and maximum infective stages for cattle are excreted in the saliva between 3–5 days after commencement of blood feeding.

This description relates particularly to *T. parva* and the acute form of the disease, **East Coast fever**, which is encountered when susceptible animals are introduced to endemic areas and subjected to challenge by large numbers of infected ticks. The number of adult ticks provides an index of infection pressure, but the outcome of exposure is determined also by the susceptibility of the cattle and the virulence of the infecting strain of parasite. Thus, theilerial disease can

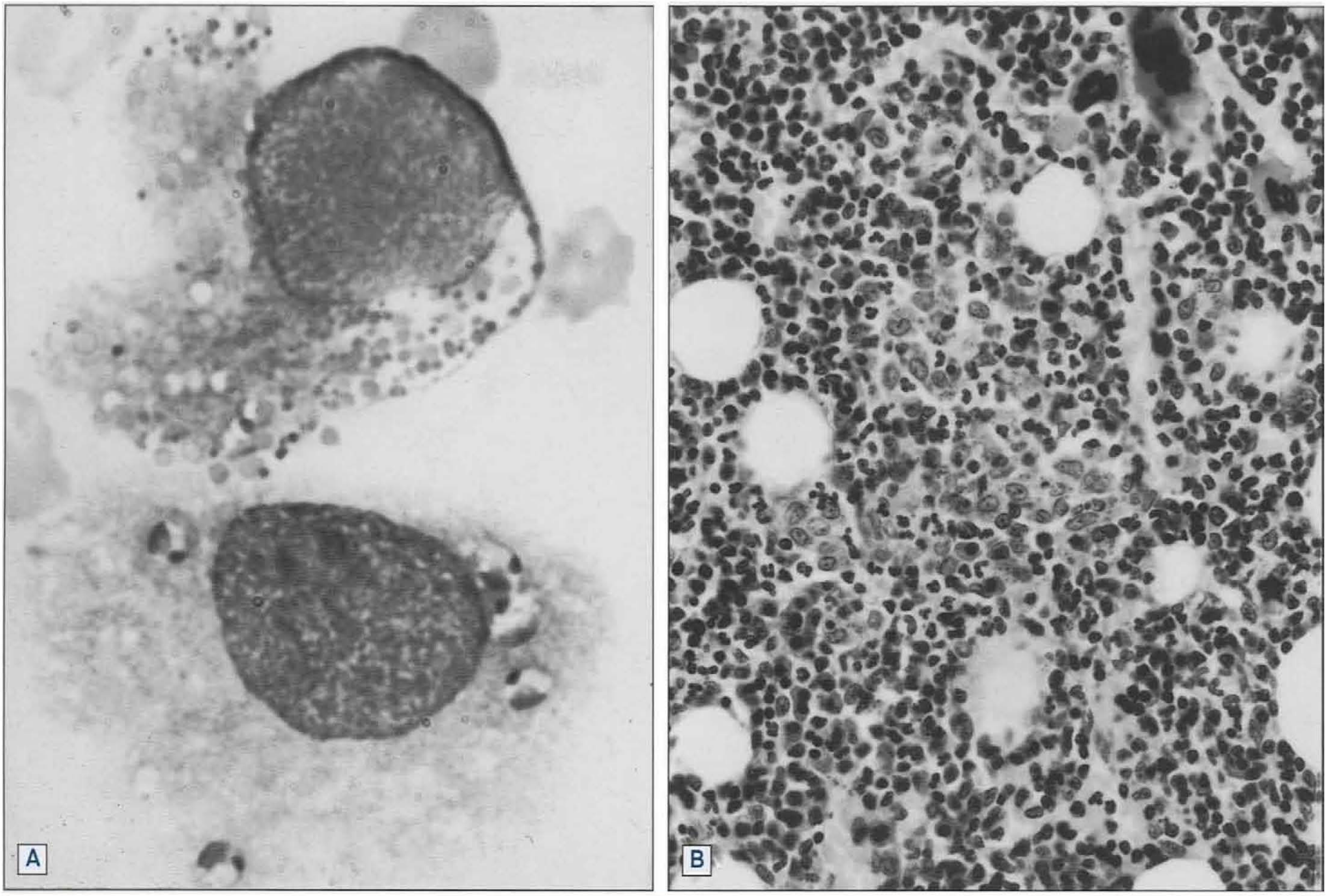

Figure 2.134 Leishmaniasis in a dog. **A.** Marrow aspirate. Epithelioid macrophage with intracytoplasmic *Leishmania*. Ruptured eosinophil at top. **B.** Marrow histology. Hyperplasia with dense cellularity, increased granulocytes and macrophages.

vary from a benign or nonclinical infection to one of acute course with high morbidity and mortality.

There is no evidence that different breeds of cattle vary in their initial susceptibility to *T. parva* or that, antigenic differences between strains notwithstanding, the parasites are capable of antigenic drift in the course of infection, although this might be expected. The components of virulence of *Theileria* organisms are incompletely understood. The sporozoites, of tick origin, invade and infect host lymphocytes, and this capability is one component of virulence and a result of receptor-mediated parasite-directed phagocytosis. Merozoite entry into red cells occurs by a similar process. *Infected lymphocytes are transformed in vitro into lymphoblastic cells, and this appears to parallel the in vivo event as the main indicator of pathogenicity.* Macroschizonts develop in the cytoplasm of the transformed cells and then divide synchronously with the host lymphocytes to infect their daughter cells. The rapid clonal expansion so induced causes the clinically recognizable **polylymphadenopathy.** While East Coast fever, and Corridor disease, are ultimately lymphocytolytic, and virulence is probably associated with macroschizogony, the pathogenesis of disease associated with some other species of the genus may be more intimately related to fission of the piroplasm forms in erythrocytes.

Most breeds of cattle are highly susceptible to *Theileria parva*, and with a high level of challenge mortality approaches 100%. The main vector is the tick *Rhipicephalus appendiculatus*. In enzootic areas, 40% of the calf crop may die of this disease if ticks are uncontrolled. The severity of the disease is directly proportional to the number of organisms inoculated into the animals by the ticks. From 4 to 20 days after infection by the transmission of **sporozoites** from an infected tick, **macroschizonts** are found in lymphoblasts of the lymph nodes draining the site of infection. These schizonts increase in number throughout the lymph nodes by an order of magnitude each 10 days, until virtually all of the lymphocytes are parasitized. Macroschizonts, 2-16 μm in diameter, may be identified in cytoplasm of infected lymphocytes using blood stains, and are known as **Koch's blue bodies** (Fig. 2.135), *which are considered diagnostic for the disease.* From the tenth day of lymph node infection, increasing numbers of macroschizonts enter the **microschizont** stage in which the host cells are destroyed and **merozoites** are released to invade erythrocytes. **Piroplasms**, which are the intraerythrocytic stage of the parasite, are infective for ticks and first appear on the 12th day after infection. They increase rapidly in number until death, at which point a high proportion of the erythrocytes are parasitized. They may be rod-shaped, round, oval, or anaplasma-like red cells, with light blue cytoplasm and red-purple nuclear staining with Geimsa stain.

Enlargement of lymph nodes is a typical sign of East Coast fever disease affecting all nodes, but the enlargement of other than the local nodes is not always apparent, and enlarged nodes shrink to about normal size terminally. The node of particular interest is that

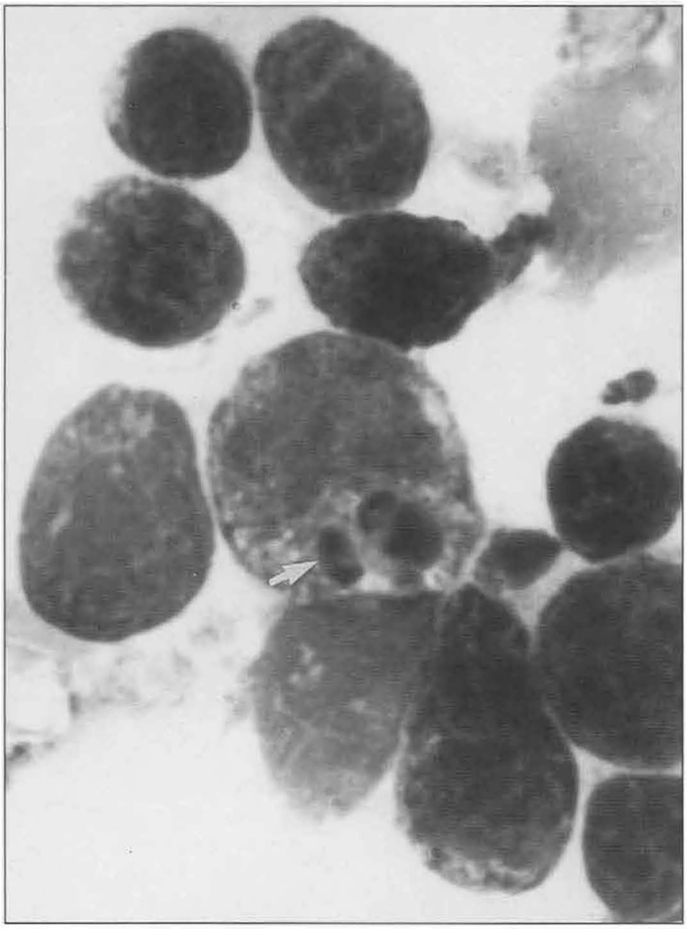

Figure 2.135 Theileriosis in an ox. Lymph node imprint, large lymphocytes with Koch's blue body (arrow).

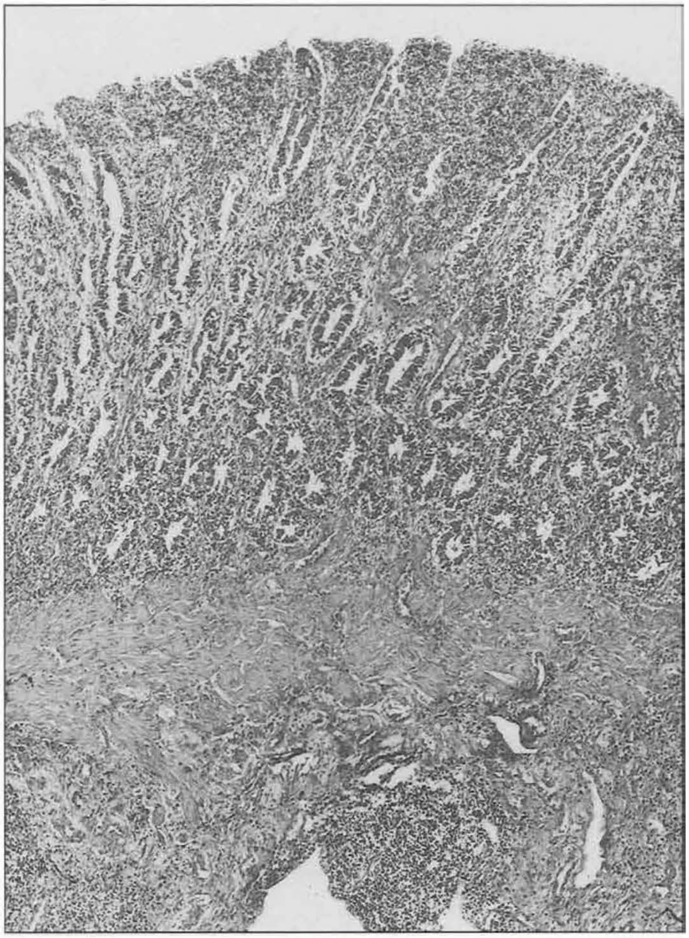

Figure 2.136 Theileriosis in an ox. Mucosal and submucosal lymphoid infiltration with mucosal erosion of the small intestine.

local to the site of infection which, with *Rhipicephalus appendiculatus*, is about the ears.

The end of the incubation period of ~2 weeks is heralded by *high fever*, followed in a couple of days by red cell parasitemia. Drooling, lacrimation, depression, diarrhea, and particularly progressive and prominent enlargement of the superficial lymph nodes give the disease a *close clinical resemblance to malignant catarrhal fever*. Severe pulmonary edema with dyspnea is common and terminal in many animals, while others become prostrate and comatose. The course of the disease is ~1 month and the mortality is ~95%. During the acute phase of the disease, more than 60% of lymphocytes may contain Koch's bodies. *The acute disease appears to be caused by massive lympholysis and progressive anemia.* An outstanding feature of East Coast fever is *leukopenia*, which is progressive from the onset of fever, and, terminally, very few leukocytes may be seen in blood smears. The total white count is seldom above 2×10^9/L and often only a fraction of this, with the cells remaining primarily neutrophils and lymphocytes. There is brief initial stimulation of myelopoiesis, but by the third day of disease there is neutropenia with immaturity and toxemia. The disease is progressive with loss of precursors, and ultimately there is a trilineage depression with accumulation of hemosiderin in macrophages. Terminally, the bone marrow is hypoplastic, and the remaining cells consist of large blastic parasitized lymphocytes and atypical erythroblasts.

Gross lesions *in the acute disease resemble those of malignant catarrhal fever*. Of principal interest is the *enlargement of lymphoid tissues, including Peyer's patches*. On cut surface, the lymph nodes are diffusely discolored with a red-brown cortex containing focal hemorrhages, and a dark red-brown medullary area. Serous effusion and gelatinous or hemorrhagic edema of connective tissues are common. The spleen is enlarged in the acute disease, but in cases with a prolonged course it may be shrunken and strap-like. There is ulcerative abomasitis, likely nonspecific. *The so-called "infarcts" of the liver and kidney are actually proliferative foci of perivascular lymphocytes.* These foci, which project slightly, produce a mottling of small gray-white patches visible on the surface of the liver and kidney. The lungs are congested and edematous with increased texture on palpation, and increased weight. Small hemorrhages associated with foci of hyaline degeneration occur in the muscles, and petechiae are commonly present under the tongue and in the vulva. Erosive or catarrhal enteritis overlies lymphocytic hyperplasia and infiltration of the gut mucosa (Fig. 2.136).

Histologically, in the early stages of infection, there is *diffuse lymphoid hyperplasia* (Fig. 2.137A), apparently at least partially due to constitutive production of IL-2 by infected lymphocytes. In animals that have died with East Coast fever, there is *widespread lympholysis* with hemorrhage and fibrinous exudate throughout the cortical areas of nodes. Lympholysis is prominent in germinal centers and there is a general loss of small lymphocytes, with those that remain

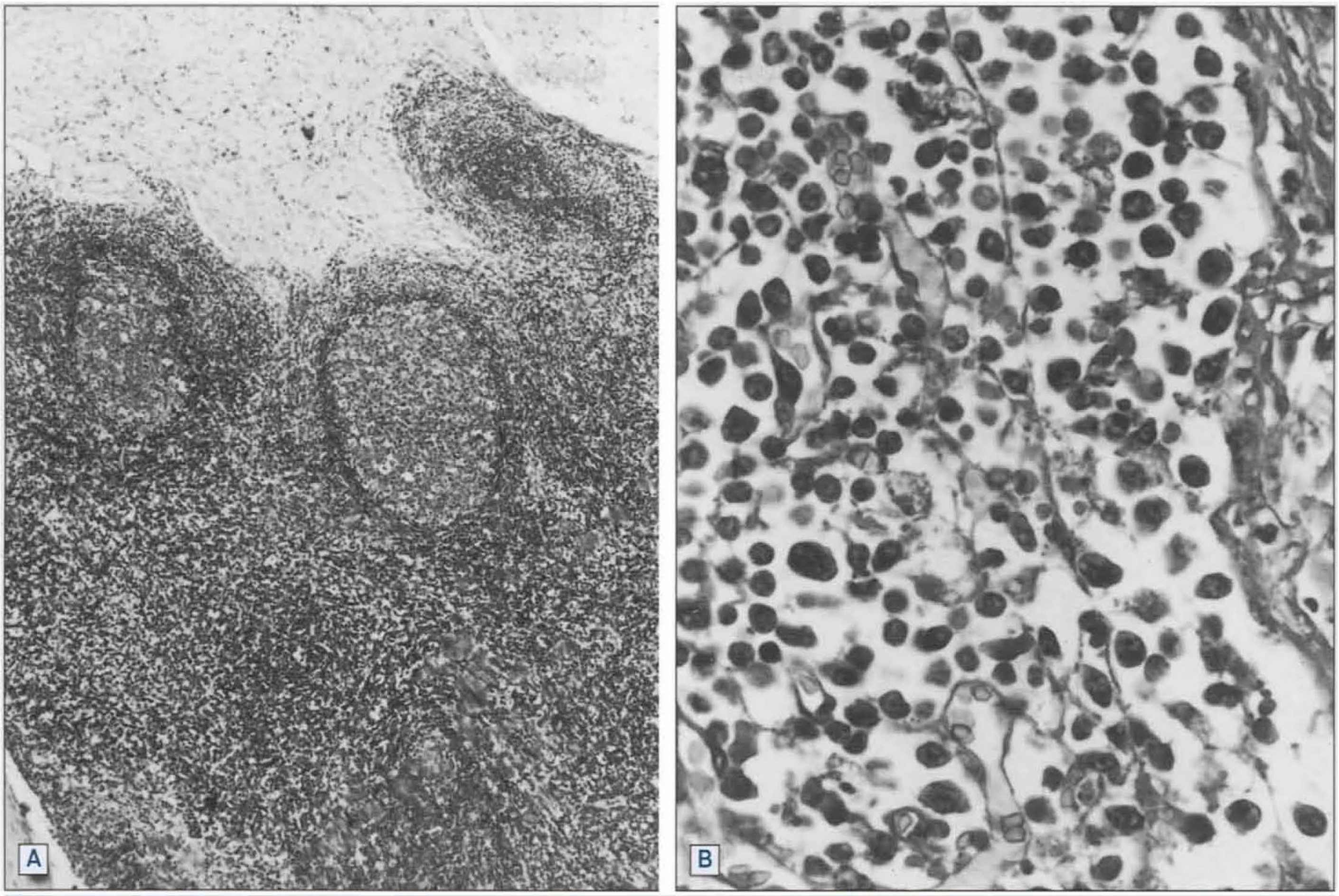

Figure 2.137 Theileriosis in an ox. **A.** Early follicular hyperplasia. Reduced paracortical density with moth-eaten appearance due to tingible body macrophages. **B.** Lymphoblastoid transformation in early disease.

appearing large and blastic (Fig. 2.137B). There is hepatic periacinar and, to a lesser extent, periportal lymphocytic infiltration and, in addition, there are focal infiltrations of the hepatic capsule that give rise to the raised foci seen grossly. In some cases, there is periacinar hepatocellular necrosis and irregular canalicular cholestasis with foci of inspissated bile. There is early splenic lymphoid hypertrophy that is later followed by lympholysis. Germinal centers remain prominent and are surrounded by areas of hemorrhage. The hypocellular follicular centers are usually occupied by fibrinous or hyaline exudate similar to that seen in lymph nodes. The kidneys are remarkably congested with focal hemorrhage, and there is interstitial infiltration with lymphocytes. The lymphocytic infiltration is prominent around vessels, and often around the parietal layer of Bowman's capsule. There is a variable level of parenchymal necrosis with the formation of hyaline casts and brown pigmentation of the remaining epithelium. The pulmonary changes are characteristic and consist of lymphocytic infiltration of the septa and interstitial tissues, resulting in widespread severe interstitial alveolitis (Fig. 2.138). The bone marrow is hypocellular with early asynchrony of the granulocytic system and a less severely affected erythroid system accompanied by proliferation of large lymphocytes similar to those infiltrating other tissues.

An aberrant expression of the disease is described as "**turning sickness**," although it is not clear whether the responsible organism is *T. parva*, *T. mutans*, or another. The condition occurs occasionally in partially resistant cattle re-exposed to heavy infestations of infective ticks. *Parasitized lymphocytes localize by embolism or sequestration in cerebrospinal vessels* (Fig. 2.139) *and produce hemorrhagic infarcts*. The infarcts are of usual character, but muddy discoloration of tissues and meninges with hemosiderin is suggestive of repetitive minor episodes of infarction. Infarcts of various ages may be found in other organs, especially kidney and spleen, but are small and easily overlooked. Koch's bodies may be rare or not demonstrable in peripheral blood or lymphoid organs, but are numerous in the clumped cells in vessels in the infarcts.

Theileria lawrencei is the cause of **Corridor disease** of calves, so named because it occurred in cattle moved into an area called "the Corridor" lying between two game reserves in South Africa. *Rhipicephalus* ticks collected in the area were able to infect cattle, but ticks feeding on infected cattle were not able to transmit the infection further to other cattle. Thus, cattle were not infective for ticks. While the disease in cattle closely resembled East Coast fever clinically and morphologically, there were differences in parasitic behavior. In cattle affected with Corridor disease, less than 5% of red cells in the blood and less than 5% of lymphocytes in smears of lymph nodes contained the parasite, whereas in East Coast fever the corresponding figures would be about 50 and 80%. Cross immunity, however, exists between *T. parva parva* and *T. parva lawrencei*. *Theileria lawrencei* is endemic in buffalo, which may be carriers, some strains are transmissible from cattle

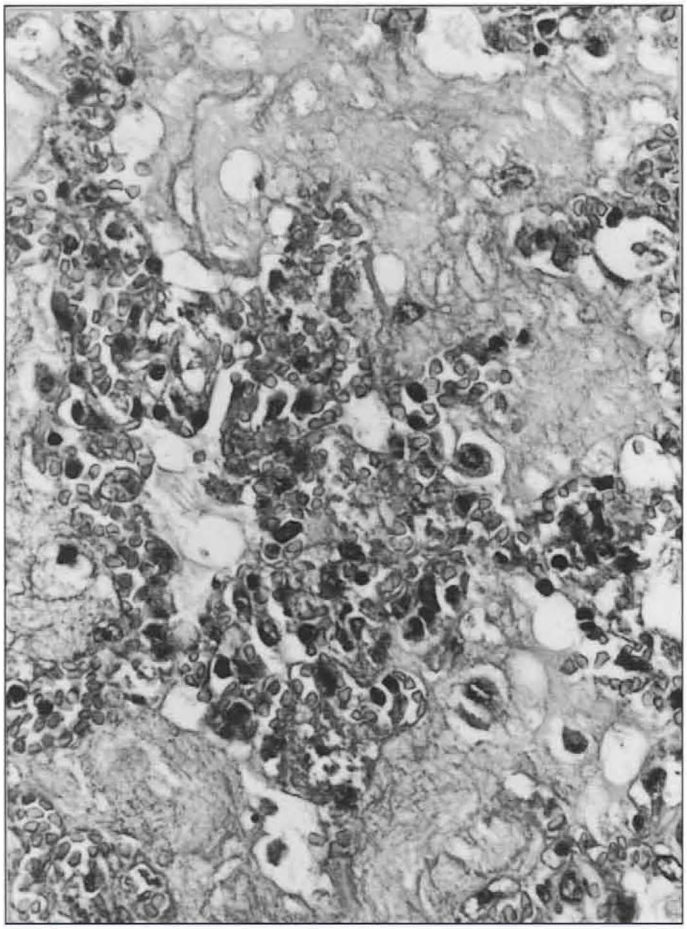

Figure 2.138 Theileriosis in an ox. Interstitial pneumonia with massive proteinaceous effusion.

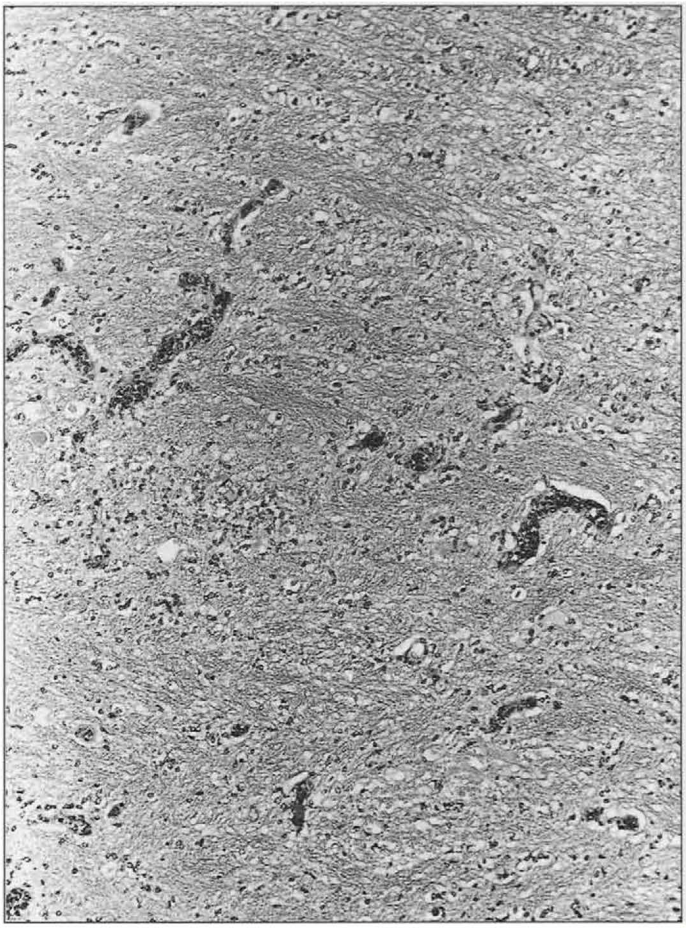

Figure 2.139 Theileriosis in an ox. Sequestration of parasitized cells in cerebral vessels in "turning sickness."

to cattle by ticks and, in these transmissions, *T. lawrencei* comes to behave like and to assume the morphological characters, particularly with respect to macroschizonts, of *T. parva*.

Theileria mutans is the cause of **Tzaneen disease** of cattle, usually a mild and inapparent infection. Sheep can be infected artificially, the infections remaining latent and persisting at least for some weeks; the organisms in sheep do not appear in smears of blood. *Theileria mutans* is widely distributed in tropical and subtropical countries and is transmitted naturally by ticks of the genera *Rhipicephalus*, *Amblyomma*, and *Haemaphysalis*, and possibly by ticks of other genera as well. The importance of the parasite lies in the need to differentiate it from *T. parva*. Cross-immunity trials are necessary for certainty, but it is useful that *T. mutans* can be readily transmitted by the inoculation of even small quantities of blood, and in this infection schizonts are rare or impossible to find. The phases in erythrocytes are rod-shaped and ring-shaped, in about equal numbers.

There are occasional reports of fatal *T. mutans* infections in adult cattle manifested as hemolytic anemia, icterus and hemoglobinuria. The lesions in lymphoid tissue are similar in type, but less severe than in East Coast fever. There is very heavy parasitism of erythrocytes, but schizonts are not observed. The pathogenesis of hemolysis is unclear, but there is some evidence that it is associated with the activities of macroschizonts in lymphocytes rather than with piroplasms in erythrocytes.

Theileria annulata is the cause of **tropical theileriosis** in North Africa, the Middle East, and central Asia. The vectors are ticks of the genus *Hyalomma*. Infection with *T. annulata* is persistent and latent, relatively benign, and often with no parasitemia detectable in smears of blood. It is, however, readily transmitted by inoculations of blood. Occasionally, acute cases do occur and these tend to be fatal, the disease closely resembling East Coast fever. The parasite, both within erythrocytes and forming Koch's bodies, becomes very numerous in the blood during acute attacks of the disease. The majority of the parasites in red blood cells are ring- or oval-shaped and only a few are bacilliform.

Theileria ovis is the ovine equivalent of *T. mutans*; it is benign. *Theileria hirci* is somewhat more virulent and is also pathogenic for goats, producing a disease closely resembling East Coast fever.

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Jembrana disease

A severe acute disease occurs in Bali cattle (*Bos javanicus*) in Indonesia, with a case fatality rate of 20%; mild or subclinical disease occurs in other cattle types and buffalo. The cause is **Jembrana disease virus** (JDV), a bovine lentivirus that is closely related to *Bovine immunodeficiency virus*. Virus titers are high in blood in the acute disease, and virus is present in saliva and milk; direct transmission may then occur to susceptible cattle via conjunctival, intranasal or oral routes. During the height of viremia, virus may also be transmitted mechanically by hematophagous arthropods. Recovered cattle may have persistent low-level viremia, but are probably an infrequent source of infection. Cattle of European breeds and sheep are resistant to the disease, although subclinical carrier status may persist for many months.

Clinically, following an incubation period of 5–10 days after infection, there is transient fever, dullness and anorexia. Mild oculonasal discharge, pallor of the mucosae, and diarrhea with blood-stained feces are common. Superficial lymph nodes are visibly enlarged, whereas examination of the blood during the febrile period usually reveals anemia, leukopenia, thrombocytopenia, and in some cases which may be fatal, an elevated blood urea level. Basophilic cytoplasmic inclusions are occasionally seen in circulating mononuclear cells.

At autopsy, lesions may not be remarkable, but several are constant and of diagnostic value. *Enlargement of lymph nodes with slight edema and blurred corticomedullary junctions are widespread but most marked when regional to other affected organs, e.g., lungs and liver.* During the acute phase there is *splenomegaly* and on incision the surface shows small gray-red foci. Scattered petechiae or ecchymoses occur irregularly in the serosae of the heart, gastrointestinal tract, kidneys and below the endocardium. The myocardium and liver contain gray foci 1–3 mm in size. Pulmonary lesions are subtle but can be detected in the cranial lobes, which are firmer than normal, blue-red, finely mottled and slightly edematous. Adjacent lobules show mild overinflation. Shallow erosions may occur in the gastrointestinal tract and caudal tongue, where an occasional ulcer is also possible. The kidneys are consistently pale and have small numbers of gray foci.

The general picture may be complicated by *concurrent infections*, which have included *Pasteurella* pneumonia and helminths of the liver, pancreas, or alimentary tract.

Histologically, *three phases* can be recognized in the acute disease process of about 6 weeks. During the first week, there is a general response by the lymphoreticular system, but in the second phase, up to the fifth week approximately, there is intense nonfollicular proliferation of reticular and large lymphoid cells. A similar infiltrative process occurs in the liver, kidneys, adrenal medulla and choroid plexus systems of the brain, although the central nervous tissue shows little change. In the cranial lung lobes, alveolar cells are enlarged, pleomorphic and proliferative alongside infiltrating mononuclear cells. Mid-sized pulmonary veins and arteries may be filled with large numbers of intravascular macrophages. In the third phase, the follicular lymphoid system is reactivated and plasma cells appear progressively. *The sequence is consistent with a transient immunosuppressive disease.* Indeed, the humoral immune response can be suppressed and delayed in JDV-infected cattle. Histologic lesions regress, but traces of change can be seen up to 60 days after infection.

Pleomorphic basophilic cytoplasmic inclusions are present in all affected tissues. By light microscopy, clumps of minute coccoid bodies about 0.5 μm, and larger, pleomorphic intravacuolar structures occur in reticular and large lymphoid cells, macrophages including Kupffer cells, pulmonary alveolar cells, and occasionally in vascular endothelium.

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Tick-borne fever

Tick-borne fever of sheep and goats and *pasture fever* of cattle is transmitted by the vector tick *Ixodes ricinus* and occurs in Great Britain, Ireland, The Netherlands, Norway, and Finland. The causative agent is ***Anaplasma phagocytophilum*** (formerly *Cytoecetes phagocytophila, Rickettsia phagocytophila, Cytoecetes bovis, Ehrlichia equi*), which is also the agent of human granulocytic ehrlichiosis in Europe and North America (where the vector tick is *Ixodes scapularis* or *I. pacificus*). The infection is transmitted stage to stage in ticks, but not hereditarily, and other blood-sucking arthropods may also transmit the agent. Similar diseases have been reported in sheep and cattle in North Africa. An agent in cattle identified as "*Ehrlichia bovis*" is thought to be transmitted by ticks of the genus *Hyalomma;* the infection in sheep is caused by "*Ehrlichia ovina*" transmitted by *Rhipicephalus* spp.

Tick-borne fever is a transient and mild illness that causes some loss of condition. The disease is, however, of some significance because it appears to predispose sheep to cerebrospinal inflammation in louping ill and to staphylococcal pyemia (tick pyemia), the virus of louping ill being conveyed by the ticks and *Staphylococcus aureus* being first established as an infection in the tick bites. Tick-borne fever also appears to predispose animals to pasteurellosis.

Clinically, adult ruminants are more susceptible to tick-borne fever than are the young. After incubation for 2–6 days, or a few days longer in cattle, there is sudden onset of moderate fever. The fever lasts 2–3 days if the agent is of relatively low virulence and subsides quickly. If more virulent organisms are involved, the febrile reaction may be prolonged for nearly 2 weeks, the decline to normal temperature being gradual. Ewes in late pregnancy may abort. In terms of laboratory diagnosis, *with the onset of fever, the organism is detectable in* *circulating granulocytes and large lymphocytes.* The most useful stains are Giemsa and polychrome methylene blue. As the fever declines, the organisms disappear from smears and there is progressive and quite severe neutropenia for a few days, after which the neutrophil count returns to normal. Organisms are no longer visible in smears unless there is a recrudescence with fever, but they do persist in the blood in some animals for a year or more. During fever, up to 95% of the neutrophils may contain parasites. The parasites are restricted to the cytoplasm and are of varied morphology, the variations probably representing developmental stages of the parasite. They may be round, rod-shaped or irregular masses measuring 0.75–3 μm in diameter present either singly or in clusters; ring-shaped bodies 2–3.5 μm in size breaking up into irregular fragments; round or oval morulae of 2.5–3.5 μm in diameter containing many small granules; or in occasional cells there are small masses of about 0.5 μm in diameter.

Persistence of infection after the febrile period is associated with a state of labile premunition, resistance to reinfection being demonstrable at ~5 weeks after the primary infection. Reactions to reinfection are usually much milder than those to the initial infection, but the situation is complicated by the immunologic differences and differences in virulence of strains of the organism. It is probable that in endemic areas sheep remain infected for life, reinfection occurring at each season of tick activity.

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Salmon poisoning in dogs

Salmon poisoning is a disease of dogs in the northwestern United States and western Canada, with reports from other areas usually being from dogs imported from those regions. Salmon poisoning is caused by ***Neorickettsia helminthoeca***. The disease is caused by dogs eating raw salmon or trout from areas where the disease is endemic. The dog appears to be the definitive host with the rickettsial agent infecting macrophages in its lymphoid tissues. *The agent is transmitted to dogs in cysts of the fluke Nanophyetus salmincola contained within the tissues of salmon or trout.* The presence of fluke eggs in dogs with signs of the disease are suggestive of the diagnosis. Eggs of the fluke are shed in dog feces which develop into a *miracidium*, and in the proper circumstances, these infect the freshwater snail *Oxytrema silicula*. The miracidium develops into a *redia stage* within the snail which then develops into a *cercaria*. Salmon are infected by ingestion of snails or by skin penetration by the cercaria. Some become encysted in various tissues and widely distributed in various tissues including the eyes, kidneys, liver, intestinal wall, musculature, and fins of salmonidae. The flukes apparently cause minimal damage or clinical signs to dogs, which shed the fluke eggs within about a week after ingestion of infected salmon and continue to shed for approximately 8 months. The rickettsial organism is present in all stages of the fluke life cycle and gains access to other tissues of the dog by an unknown process, but most likely through the attachment of the fluke to the intestinal wall.

Dogs are apparently susceptible to the infection at all ages, with the disease characterized by *lethargy and pyrexia with splenomegaly, generalized lymphadenopathy, vomiting, and diarrhea.* At the time the dogs are showing acute signs of salmon poisoning, there are usually large numbers of operculated fluke eggs in fecal smears. Mortality rate is very high, and approaches 100% in untreated animals. Organisms are present in virtually all enlarged lymph nodes, but usually the submandibular nodes are enlarged and easily aspirated, with the organisms present in the cytoplasm of macrophages. *The neorickettsial organisms are coccoid, less than 1 μm in length, and appear blue-purple with blood stains and blue with Macchiavello's stain.* Dogs succumbing to infection with salmon poisoning have marked enlargement of spleen and lymph nodes with focal hemorrhages in the thymus and pale often swollen liver with an exaggeration of the lobular pattern visible through the capsule. Histologically, the lesions of the nodes in dogs dying early in infection are those of massive lympholysis and loss of germinal centers and generalized cortical depletion of cells (see Fig. 2.114A). In animals that survive 10 to 12 days when there is prominent node enlargement, there is diffuse paracortical hyperplasia with a prominent "starry-sky" appearance but without the development of germinal centers. Splenic lesions are characterized by follicular lympholysis in the acute phases of the disease, as well as loss of cells in the periarteriolar lymphoid sheaths. There is marked congestion of sinus areas with numerous macrophages containing ingested debris as well as the neorickettsial organisms. Thymic lesions are similar with marked involution particularly of cortical areas in the acute phases of the disease with foci of hemorrhage and edematous separation of the lobules. In animals that survive a week or longer, lympholysis is less apparent but there is only minimal restoration of cells in periarteriolar lymphoid sheaths and in the thymic cortex. Hepatic lesions consist of isolated foci of necrosis with macrophage reaction.

Salmon poisoning needs to be distinguished from a related disease known as "**Elokomin fluke fever**," named after the river in Washington State where the disease was first recognized. There are antigenic differences between the agent of salmon poisoning and Elokomin fever, which were initially distinguished on the basis of the specificities of the antibody reaction in surviving animals applied in fluorescent antibody staining of the infectious agents. The lesions in both diseases are similar.

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Bovine petechial fever

Bovine petechial fever or **Ondiri disease** of Kenya and Tanzania is very similar to tick-borne fever in that the causative agent is rickettsial and parasitizes circulating neutrophils. The agent is most often identified as ***Ehrlichia (Cytoecetes) ondiri***. An arthropod vector is suspected, but unidentified. Several species of wild ruminants are susceptible to experimental infection, and the bushbuck and African buffalo may serve as amplifying and reservoir hosts.

Experimental transmission has been possible only by injecting blood, spleen or lymph node taken from affected animals, but transmission by these means is possible for only a limited number of passages. The disease occurs naturally only in restricted areas of Kenya at altitudes above 1500 m, and there appear to be some differences in breed susceptibility, with Borans susceptible but Ayrshires and Herefords resistant to experimental infection.

Clinically, the disease is often mild and, unless cattle are being closely observed, signs may not be noticed. After an incubation period of 5–14 days, the affected animals become febrile, and within 1–2 days *fine petechiae develop on the tongue, conjunctiva and the mucosa of the vulva.* The petechiae may persist for only 1–2 or up to 10 days. Occasionally the conjunctival lesions are severe, and the conjunctiva may become edematous and prolapse. Epistaxis and other hemorrhagic tendencies develop occasionally. Early in the course of the disease, affected animals have *leukopenia,* due to a drop in the numbers of circulating lymphocytes and monocytes, and *thrombocytopenia* of greater or lesser degree accounting for the variation in persistence of the petechiation. There follows in ~1 week a rebound leukocytosis produced by an increase in immature mononuclear cells. The organisms are found in neutrophils and lymphocytes, but are not common, and are not demonstrable in every case.

Grossly, the lymph nodes of affected animals are enlarged and congested. The petechial hemorrhages that are seen clinically are also found in the internal organs. The lungs are usually normal in experimental cases, but in severe field cases *death is often due to hemorrhage and edema of the lungs.* The folds of the abomasum are edematous and congested and the contents of the ileum and colon are tarry. The gallbladder is usually distended and the wall thickened by gelatinous edema. *The histologic appearance is nonspecific and the agent cannot be recognized in routine sections.*

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Hemorrhagic diatheses

Patricia A. Gentry

Hemostasis is a complex process involving multiple interdependent interactions among platelets, endothelial cells, white cells and fluid-phase proteins that exert both procoagulant and anticoagulant functions. Under normal circumstances, the **anticoagulant system**, which is designed to ensure that the coagulation mechanism is tightly regulated, predominates over the **procoagulant system**. In response to perturbations of vascular endothelium, procoagulant activators are released locally and blood platelets and plasma proteins become exposed to extravascular tissue and soluble activators. This initiates the process of **blood coagulation** and **platelet-mediated primary hemostasis** that initially stems the loss of blood at the site of the vascular lesion. **Thrombin** *is the most important enzyme of the coagulation system.* It has many biological functions in hemostasis, including activation of platelets, conversion of fibrinogen to the fibrin meshwork that is essential for thrombus formation, positive feedback amplification of coagulation, as well as activation of anticoagulants and the fibrinolytic system to regulate the extent of thrombus formation (Fig. 2.140). In addition to its function in hemostasis, thrombin also has important biological roles in cell proliferation and inflammation that are mediated through thrombin's ability to activate specific surface membrane receptors (protease-activated receptors, PARs) on target cells.

Thrombin formation

Initiation of thrombin formation

The primary event that initiates thrombin formation is the exposure of circulating blood to *tissue factor (TF),* which becomes exposed on the plasma membrane of activated endothelial cells or activated monocytes at sites of vascular injury or inflammation. This pathway of thrombin generation is termed the **tissue factor pathway** and is also known as the **extrinsic pathway.**

TF is a lipid-dependent transmembrane glycoprotein that is sequestered in quiescent endothelial cells and monocytes. Upon activation, the extracellular domain of TF is expressed on the cell surface in a configuration that facilitates the binding of one molecule of TF to one molecule of factor VII (FVII), or the active form of the enzyme, FVIIa. Although FVII is the predominant form of the protein in the circulation, a small fraction of the protein circulates as FVIIa. The binding of FVIIa to TF triggers coagulation by activating

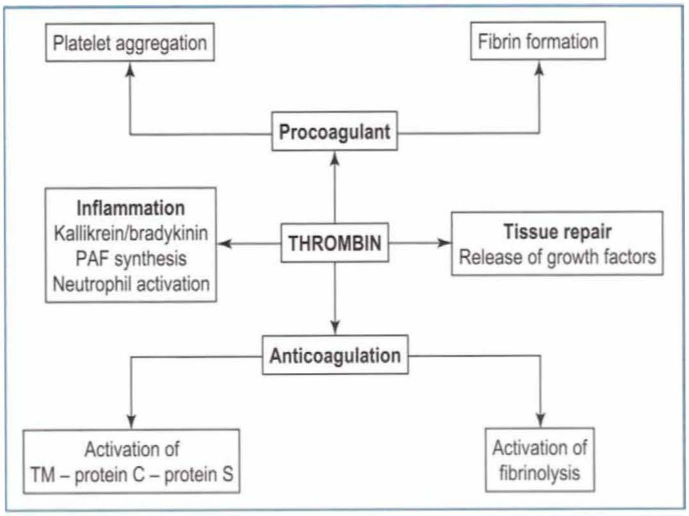

Figure 2.140 Selected multiple functions of **thrombin** that modulate coagulation, anticoagulation, inflammation and tissue repair. PAF = platelet activating factor. TM = thrombomodulin.

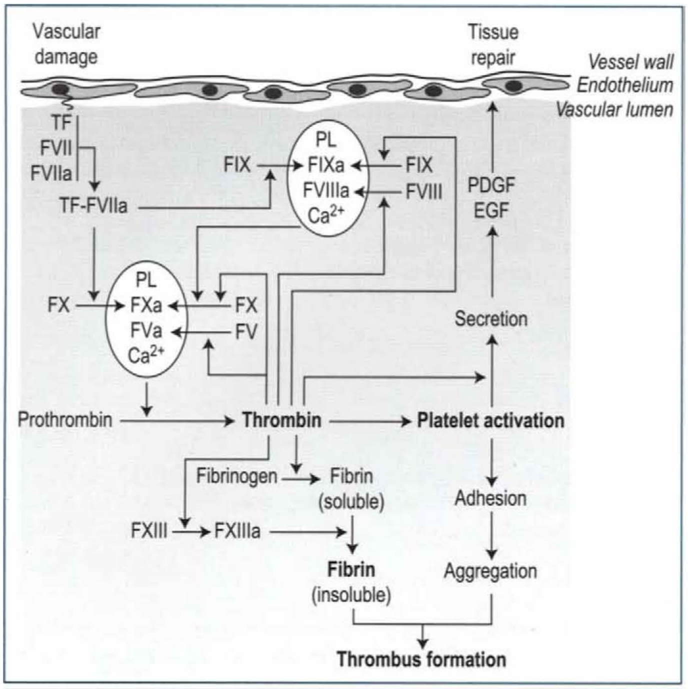

Figure 2.141 Schematic of the primary reactions of the **tissue factor pathway (extrinsic pathway)** leading to thrombin generation and platelet activation following vascular damage. EGF = epidermal growth factor. PDGF = platelet-derived growth factor. PL = phospholipid. TF = tissue factor.

additional TF-FVIIa molecules and by converting both factor IX (FIX) and factor X (FX) to their activated forms, FIXa and FXa, respectively (Fig. 2.141). Factors IXa and Xa can either remain associated with the TF-bearing cells or they can diffuse into the blood and bind to the surface of nearby activated platelets that have gathered around the site of vascular damage. Prothrombin is rapidly activated to thrombin by the "*prothrombinase complex*" that consists of FXa, phospholipid, calcium, and a protein cofactor, factor V (FV). During platelet activation, negatively charged phospholipids become exposed on the platelet surface. These phospholipids serve as binding sites for coagulation factors, protein cofactors and calcium to permit the assembly of the highly reactive enzyme–cofactor complexes that are essential for the rapid propagation of fibrin and thrombus formation. The platelet phospholipid contribution is also referred to as *platelet factor 3 (PF3).*

The first few molecules of thrombin generated by the "prothrombinase complex" initiate a positive feedback reaction that accelerates the rate of prothrombin activation and causes an increase in the amount of thrombin generated that, in turn, increases the rate of fibrin formation (Fig. 2.141). Thrombin directly increases the activity of the prothrombinase complex by activating FX to FXa and by proteolytic cleavage of FV to a more reactive form, FVa. Thrombin further facilitates thrombus formation at a site of vascular damage by promoting localized platelet activation, adhesion and secretory reactions that culminate in platelet aggregation (Fig. 2.141).

Maintenance of thrombin generation

The activation of prothrombin by the TF–FVIIa–FXa pathway initiates thrombin formation but it cannot sustain thrombin production long enough to ensure that sufficient fibrin can be formed to stem blood loss when a vessel wall is breached. This is due to the presence of a natural anticoagulant, *tissue factor pathway inhibitor (TFPI),* that circulates in blood bound to lipoproteins and platelets as well as being localized on endothelial cell surfaces in association with heparan sulfate. TFPI binds to FXa, and then forms an inactive quaternary complex, the FXa–TFPI–FVIIa complex, that effectively inhibits the TF pathway of thrombin generation. To circumvent the anticoagulant effect of TFPI, there are alternative pathways that result in activation of FX and hence thrombin production (Fig. 2.141).

Thrombin feedback not only activates FV but it also activates factor IX and converts FVIII to a more reactive form, FVIIIa (Fig. 2.141). Factor VIII normally circulates bound to *von Willebrand factor (vWF)*, an adhesive protein important for the generation of the initial platelet plug. After activation by thrombin, FVIIIa dissociates from vWF and forms a complex with FIXa, calcium and phospholipid molecules exposed on the surface of thrombin-activated platelets. The resulting highly reactive enzyme–cofactor complex, the "*tenase complex,*" proteolytically cleaves FX at the same reactive site as TF–VIIa and hence produces the same FXa product.

A reactive "tenase complex" leading to thrombin formation can also be generated by the **contact activation system**, also known as the **intrinsic pathway**. The contact activation system involves four components, *the proenzymes factor XII (FXII) and factor XI (FXI), prekallikrein (PK), and a protein cofactor, high molecular weight kininogen (HMWK)* (Fig. 2.142). This pathway is initiated when FXII interacts with a negatively charged surface, e.g., damaged endothelium or activated neutrophils, and undergoes a conformational change that results in its ability to cleave FXI to a proteolytically active form, FXIa, and to convert PK to kallikrein. This reaction is accelerated by the presence of HMWK. In plasma, FXI and PK each circulate separately as a complex with HMWK. FXIIa can convert HMWK to a modified form, HMWKa, that exhibits a high surface-binding affinity and thus brings large amounts of both FXI and PK to the charged surface in close proximity to the already adherent FXIIa. FXIa, in the presence of calcium, is a potent activator of circulating FIX and thus promotes thrombin formation. Direct activation of FXI by thrombin is another amplification loop, which, in turn, results in the generation of additional FIXa (Fig. 2.142).

The physiological role of the contact activation pathway is not fully understood because it does not appear to be important in trauma-mediated thrombin generation. However, kalllikrein not only functions as an activator of the fibrinolytic system, but it also stimulates both chemotaxis and degranulation of neutrophils attracted to an area of vascular perturbation (Fig. 2.142). Hence, *the contact activation pathway serves as a link between inflammation, thrombin formation and fibrinolysis.*

Fibrin formation

The final step in the coagulation pathway is the conversion of soluble fibrinogen to an insoluble fibrin meshwork in a multistep process mediated by thrombin and by activated factor XIII (FXIIIa) (Fig. 2.141). Fibrinogen is a large globular molecule composed of two sets of three identical protein chains ($A\alpha$, $B\beta$ and $\gamma\gamma$) forming a trinodular structure held together by disulfide bonds. Thrombin first interacts with fibrinogen by cleaving two small peptide chains, fibrinopeptides A and B, from the amino terminal end of the $A\alpha$ and $B\beta$ chains, respectively, thus altering the surface charge of the molecule. The

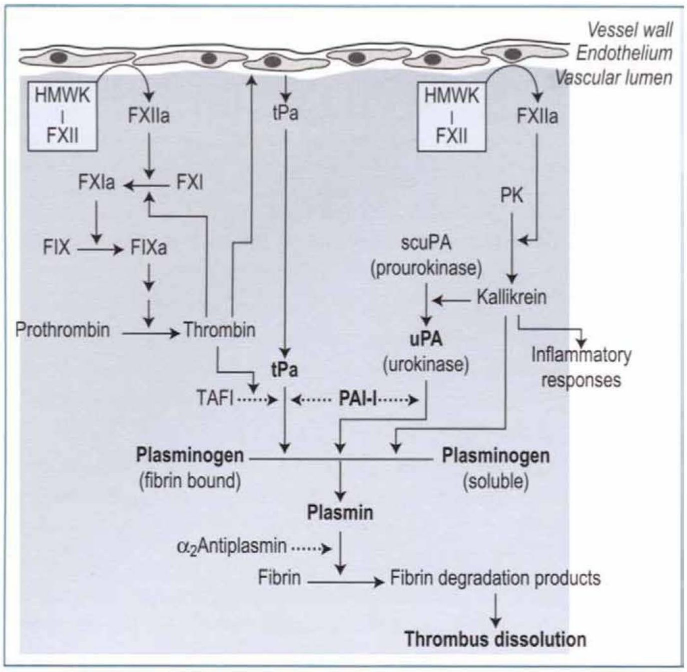

Figure 2.142 Schematic of interactions between the **contact activation pathway (intrinsic pathway)** system and activation of the fibrinolytic system that result in thrombus dissolution once tissue repair has occurred. Solid lines represent activation reactions; dashed lines represent inhibitory reactions. HMWK = high molecular weight kininogen; PAI-1 = plasminogen activator inhibitor-1, PK = prekallikrein, TAFI = thrombin-activatable fibrinolysis inhibitor, tPA = tissue-type plasminogen activator.

result is the formation of soluble fibrin monomers that can spontaneously polymerize by aggregating end-to-end and side-to-side to form a loosely knit mesh held together by covalent bonds. The transformation of this permeable structure to an insoluble fibrin mesh that forms the scaffolding for both thrombus formation and vascular repair requires the formation of stable peptide bonds between neighboring γ and α chains of the fibrin polymer. Factor XIIIa (FXIIIa), a transpeptidase enzyme activated by thrombin cleavage of the precursor, FXIII, catalyzes the formation of the internal peptide bonds between the fibrin chains in a calcium-dependent reaction.

Maximum thrombin generation occurs after the initial formation of fibrin strands. In addition to being important for the activation of FXIII, thrombin is important for activation of the protein known as *thrombin-activatable fibrinolysis inhibitor* (*TAFI*). This inhibitory protein not only prevents premature degradation of the fibrin clot but also participates in the activation of the *thrombomodulin-protein C-protein S (TM-protein C-protein S) anticoagulant system* that, in turn, prevents thrombin generation in the general circulation outside the fibrin clot (Figs 2.142, 2.143).

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Evaluation of thrombin and fibrin formation

Circulating levels of procoagulant activity

Although the quantitative amounts of various coagulant proteins in circulating blood vary between species, qualitatively the pattern is similar and is related to the specific role of each protein in hemostasis. For example, the amount of fibrinogen in plasma is generally 50 000 times higher than that of FVIII. This reflects the need for relatively large amounts of fibrinogen for the formation of the fibrin network, whereas *only a small amount of FVIII is required to catalyze the conversion of FIX to FIXa at a physiologically adequate rate.* Similar variations are observed for the vitamin-K dependent proteins, prothrombin, FVII, FIX and FX. Prothrombin is present in the circulation at higher levels than either FIX or FX which, in turn, exhibit higher levels than FVII. Thus, *proteins that interact in the initial stages of thrombin generation circulate at lower concentrations than proteins that act at the later stages*, which is consistent with the multiple reactions and amplification potential of the system (Fig. 2.141). The assembly of enzyme–cofactor complexes on negatively charged phospholipid surfaces not only increases the local concentration of the coagulation components but counteracts the inhibitory potential of circulating anticoagulants. Among the procoagulants, FVII has the shortest half-life, being in the order of hours rather than days as is observed for the other proteins. Fibrinogen and prothrombin exhibit the longest circulating half-lives.

The clinical manifestation of hemorrhagic problems is variable, depending on the nature and cause of the coagulopathy. In domestic animals, as in people, *hemorrhagic episodes are rarely encountered unless the biological activity of a single coagulation factor is severely compromised or the biological activity of multiple coagulation proteins is reduced below 30% of normal circulating levels.* Hemorrhage is typically into body cavities or tissues and from wounds. Subcutaneous hematomas and hemorrhage into urogenital and gastrointestinal tracts are also common.

Laboratory procedures

Appropriate blood sample collection and handling techniques are essential for reliable in vitro evaluation of hemostasis. While blood anticoagulated with EDTA is required for accurate platelet counts in mammals, *citrated plasma* is essential for the screening of the coagulation system and measuring individual components of the coagulation, fibrinolytic and inhibitory pathways.

Routine hemostatic screening involves determination of *platelet numbers*, the *one-stage prothrombin time* (OSPT or PT), the *activated partial thromboplastin time* (APTT), and the *thrombin time*, which can also be used to determine fibrinogen values. The PT assay measures the overall competency of the tissue factor pathway. The procedure involves the addition of a thromboplastin (TF)–phospholipid mixture to test plasma and the determination of the time for a fibrin clot to form after the sample has been recalcified. For the APTT assay, a FXII activating agent, such as celite or ellagic acid, mixed with phospholipid is added to test plasma prior to recalcification. Hence, this assay determines the competency of the contact activation system, the formation of the "tenase" complex as well as the formation of the "prothrombinase" complex in the test sample. A simplified, but less sensitive, version of the APTT assay is the *activated clotting time* (ACT). For this assay, the contact activator is added directly to whole blood and the time for clot formation to occur is recorded. The PT and APTT assays will detect a severe deficiency of a single procoagulant factor or a moderately reduced activity of several factors, but are not sufficiently sensitive to detect a mild to moderate reduction in a single factor. The assays will also be prolonged if circulating inhibitors are present in the patient's plasma.

Plasma fibrinogen concentrations can be quantitated by several methods, including physical and immunological procedures. A modified thrombin time assay, which quantifies the amount of fibrinogen in a diluted test sample on the basis of the rate of formation of fibrin, is currently commonly used in veterinary medicine. The biological activity of the specific coagulation factors, FXII, FXI, FIX, and FVIII are determined in an assay system based on a modification of the APTT assay, while the activity of FVII, FX, and FV require a modification of the PT assay. The ability of the test plasma sample to correct the clotting time of a plasma sample obtained from an individual with a proven hereditary defect of the specific factor relative to a reference standard is used to quantitate the activity of the specific factor. Prothrombin and FX activity can also be quantified by colorimetric methods based on the ability of enzymes isolated from snake venoms to selectively cleave the inactive zymogen into a reactive serine protease in phospholipid-independent reactions. An extract, Ecarin, from the venom of the saw-scaled viper (*Echis carinatus*) cleaves both carboxylated and noncarboxylated prothrombin, whereas Russell's viper venom selectively cleaves FX.

Specific laboratory assays that have been used to detect plasma proteins that function as natural anticoagulants or in the fibrinolytic system are described in the appropriate section below.

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Disorders of thrombin formation

Inherited disorders

Although FVIII and FIX are not involved in the initiation pathway of thrombin generation, inherited deficiencies in these factors are among the most commonly encountered in domestic animals and result in the most severe hemorrhagic episodes. The explanation for this apparent paradox has been obtained from studies with transgenic mice rendered deficient in a single coagulation protein. In mice, gene deletions for TF, FVII, FX, or prothrombin result in intrauterine death or fatal hemorrhage shortly after birth due, in part, to the role that thrombin plays in angiogenesis (Fig. 2.140). Murine gene deletions for any of FVIII, FIX or FXI result in viable offspring that exhibit various degrees of hemorrhage and mortality in the postnatal period. Both mice and people deficient in fibrinogen exhibit normal fetal development and moderate-to-severe bleeding incidents during later life.

Factor VIII deficiency (*hemophilia A*) is the most common of the naturally occurring inherited coagulopathies in veterinary medicine, being reported in many breeds of dogs, cats and occasionally in horses and cattle. It is an X-chromosomal recessively inherited disease that persists because *heterozygous females, carriers of the disease, are clinically asymptomatic.* In homozygous-deficient males, the clinical severity of the disorder can vary with the extent of reduction of circulating FVIII activity and with the size of the animal, with larger animals generally exhibiting more severe hemorrhagic problems. Unlike severely affected animals (<2% of normal FVIII activity), *mildly affected animals (5% of normal FVIII activity or above) seldom exhibit spontaneous bleeding.* Various types of stress, strenuous exercise, vaccination, surgery and trauma induce hemorrhagic episodes. In puppies, the first clinical signs may be cranial hematomas and gingival bleeding as permanent teeth erupt. As the dog matures, recurrent or shifting lameness due to intra-articular hemorrhage, particularly in the stifle joints, and subcutaneous and intramuscular hematomas are common clinical signs. Excessive bleeding resulting in large scrotal hematomas following castration is also typical.

Mildly affected male dogs can reach sexual maturity, and if they are mated with a heterozygous female, homozygous-deficient bitches can result. These animals may exhibit protracted estrus or postpartum hemorrhage.

In the laboratory, *FVIII deficiency is characterized by prolonged APTT, normal PT, and reduced plasma FVIII activity.*

Factor IX deficiency (*hemophilia B*) is another relatively common hemorrhagic disorder in dogs and cats. It has been described in several breeds of dogs and is the second most common inherited disorder in British shorthair cats. Like FVIII deficiency, it is inherited as an X-chromosomal recessive trait and has similar clinical characteristics, with both the circulating plasma levels of FIX and the size of the animal influencing the severity of hemorrhagic episodes. The reduced plasma FIX activity results in *prolonged APTT and normal PT laboratory results.*

A prolonged APTT and a normal PT is also the typical laboratory profile for animals with inherited defects involving proteins of the contact activation system, including, FXI, PK and FXII. The defect in each case is transmitted as an autosomal recessive trait.

- Among this group, **factor XI deficiency** is the only disorder in which affected animals can exhibit spontaneous bleeding episodes, most frequently associated with the urogenital tract. The disorder has been identified in English Springer Spaniels, Kerry Blue Terriers, and Great Pyrenees dogs, and in Holstein and Japanese Black cattle. Homozygous affected cattle have less than 5% of normal plasma FXI activity but the response to the trauma of surgery ranges from an uneventful cesarean delivery to catastrophic hemorrhage developing 12 to 48 hours after surgery.
- **Prekallikrein deficiency** is inherited as an autosomal recessive trait and is clinically asymptomatic. For example, PK deficiency was found fortuitously in a German Shorthaired Pointer who

subsequently underwent uneventful ovariohysterectomy surgery. Even in larger animals with PK deficiency, such as miniature and Belgian horses, hemorrhagic episodes are rare although bleeding has been reported in a horse following castration.

- **Factor XII deficiency** is inherited as an autosomal recessive trait, and has been observed in dogs and cats. No episodes of clinical bleeding occur in affected animals unless hemostasis is otherwise compromised. The absence of hemorrhage is consistent with observations that FXII has not been detected in plasma from birds, reptiles and amphibians.

Factor VII deficiency has only been reported in dogs and people. It is found in Beagles, Alaskan Malamutes, Boxers, Bulldogs, Miniature Schnauzers, and mixed-breed dogs, and is inherited as an autosomal trait. Dogs who survive to adulthood typically have plasma FVII activity of at least 5% of normal values, which is sufficient to initiate thrombin generation and induce adequate fibrin formation unless the animal is exposed to severe trauma. For example, FVII deficiency was diagnosed in a mixed-breed dog that exhibited abnormal bleeding following routine orchiectomy and failed to respond to vitamin K therapy. FVII deficiency is generally a mild disease, typified by easy bruising and a predisposition to demodicosis. Prolonged vaginal bleeding after whelping can occur in affected bitches. In the laboratory, *the PT is prolonged whereas the APTT is normal.*

As might be expected from its importance in the pathways of thrombin generation, severe **factor X deficiency** is lethal in severely affected dogs. It is inherited as an autosomal recessive trait, and dogs with >10% of normal plasma FX activity are usually stillborn or die as neonates from internal bleeding. The disorder has been reported in Jack Russell Terriers and in mixed-breed dogs. It has been eliminated from American Cocker Spaniels, the breed in which it was first identified. The *reduced plasma FX activity causes both the APTT and PT to be prolonged*. Both of these assays are also prolonged in canine **dysprothrombinemias**, which are characterized by reduced prothrombin coagulant activity and normal prothrombin antigen levels. This is a rare disorder that has been described in the Boxer, Otterhound, and English Cocker Spaniel breeds. Epistaxis and umbilical bleeding can occur in affected puppies, with mild mucosal bleeding being recorded in young adults.

Vitamin-K-dependent γ-glutamyl carboxylase deficiency has been identified in Devon Rex cats, Rambouillet sheep, and in a Labrador dog. In all species, the lack of adequate enzyme activity results in the synthesis of normal amounts of the vitamin-K-dependent coagulation proteins (FVII, FX, FIX, and prothrombin) but functional biological activity is depressed. In homozygous-deficient sheep, low levels of FIX (<16%), FX (<4%) and variably decreased prothrombin (11–36%) and FVII (20–33%) are typically found, whereas heterozygous carrier animals have normal vitamin-K-dependent coagulation factor activities. In affected sheep, lamb mortality is high and is associated with excessive blood loss at parturition through umbilical and subcutaneous tissue hemorrhages. Likewise, in affected kittens, bleeding episodes are severe or fatal and include hematomas and hemarthroses, as well as intrathoracic and intra-abdominal hemorrhages. In cats, administration of vitamin K_1 causes rapid recovery of circulating vitamin-K-dependent factor activity to near-normal levels. In an affected dog, circulating activity only rose to around 50–60% of normal canine values even after 3 months of vitamin K_1 therapy.

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Acquired disorders

Poisoning by vitamin K antagonists

In mammals, the only known function of vitamin K is as a cofactor for γ-glutamyl carboxylase, an endoplasmic enzyme involved in the post-translational carboxylation of glutamate (Gla) residues into γ-carboxyglutamate. Vitamin K hydroquinone (KH_2) forms the active coenzyme and its oxidation into vitamin K 2,3 epoxide (KO) provides the energy to drive the carboxylation reaction. Vitamin K is fat soluble, is widely available in green leafy plants and vegetables oils and, like γ-glutamyl carboxylase, is widely distributed throughout body tissues. Thus, impaired carboxylation of vitamin-K-dependent coagulation proteins is rarely due to a dietary insufficiency or impaired gastrointestinal absorption of Vitamin K, but rather is associated with the *ingestion of coumarin and its derivatives, or sulfaquinoxaline*, which has been used as a coccidiostat in livestock and poultry. These vitamin K antagonists inhibit the recycling of KO back to KH_2 through specific inhibition of vitamin K epoxide reductase. The progressive reduction in KH_2 effectively leads to a reduction in the bioavailability of the active form of vitamin K. Because the sole function of Gla residues in Factors VII, IX, X, and prothrombin is to facilitate their binding to the negatively charged phospholipids on the surface of activated endothelium and activated platelets, the rate of thrombin formation is reduced when the number of Gla residues per protein molecule is reduced. The nonfunctional forms of Factors VII, IX, X, and prothrombin are collectively referred to as PIVKAs (proteins induced by vitamin K antagonism). These proteins can be detected in plasma by a commercial Thrombotest assay.

The period between ingestion of a vitamin K antagonist and the appearance of clinical signs varies depending on the amount and type of toxicant consumed, the rate of its metabolism, and the amount and bioavailability of functional vitamin K in the patient prior to intoxication. Rodenticide consumption is the most common source of vitamin K antagonists in dogs but it has also been reported as causing hemorrhagic episodes in cats, horses and swine. The first-generation rodenticides, such as *warfarin* and *indone*, had relatively low toxicity and biological half-lives of under 24 hours, which rendered vitamin K therapy quite effective. The development of rodent drug-resistance led to the introduction of 4-hydroxycoumarins such as *bromadiolone* and *brodifacoum*, and indan-1,3-dione rodentidices, such as *diphacinone*, which are not only more potent but also have longer biological half-lives requiring more aggressive vitamin K therapy in affected animals. Further, the clinical severity of anticoagulant rodenticide poisoning can be exacerbated by underlying disease conditions, such as uremia, or by co-ingestion of pharmacological agents. For example, sulfonamides, corticosteroids and phenylbutazone can displace anticoagulant binding to serum proteins effectively increasing the bioavailability of the vitamin K antagonists.

Cattle, pigs, and rabbits are susceptible to an apparent avitaminosis K if hay or silage containing *moldy sweet clover (Melilotus alba)* is ingested. *Sweet clover contains coumarin, which is converted to dicumarol.* Young calves are more susceptible to dicumarol than are their dams, while sheep and horses appear resistant to this form of vitamin K antagonism.

Because FVII has the shortest circulating half-life (~4–6 hours), it is the first coagulation protein to show a decrease in circulating biological activity. Hence, in the initial stages of intoxication of vitamin K antagonists, the PT result may be prolonged while the APTT remains within the representative range. However, the APTT rapidly begins to also show prolongation because FIX and FX both have plasma half-lives of 14–18 hours whereas that of prothrombin is ~40 hours. Clinical signs are often broad and nonspecific and range from hemorrhage after physical trauma to apparently spontaneous external hemorrhaging in the form of epistaxis, melena or hematuria. Hemorrhagic episodes may not occur until several days after ingestion of the anticoagulant and may be acute in onset when the functional activity of several of the coagulation proteins is depressed. Bleeding into joint spaces can be associated with anemia, weakness and pallor, while dyspnea can be associated with hypovolemia and bleeding into the thoracic cavity.

Liver dysfunction

Although it is recognized that ~75% of human patients with liver disease develop hemostatic problems, *clinical expression of bleeding diatheses is reported relatively infrequently in domestic animals.* Part of the explanation may be due to the fact that chronic hepatic disease, in which cell mass is reduced by at least 70%, must be present before the hepatic synthesis of coagulant proteins is sufficiently reduced to prolong the PT and the APTT assays. Further, the hepatic synthesis of thrombin inhibitory proteins, such as protein C, and the fibrinolytic proteins, such as antiplasmin, will also be reduced, resulting in the maintenance of the hemostatic balance among thrombin/fibrin formation and plasmin/fibrin degradation. Additionally, in certain inflammatory diseases, such as canine hepatitis, the liver can increase the rate of synthesis of specific clotting factors such as fibrinogen and vWF, which together can promote the efficiency of platelet plug and hence thrombus formation.

In a retrospective study of *cats* with liver problems, 60% exhibited laboratory coagulation test abnormalities and only 23% of this group demonstrated bleeding episodes, which in each case was associated with invasive treatments such as insertion of intravenous catheters or venipuncture. The more severe the progression of the liver disease, the more likely the cat was to develop a clinical coagulopathy. A comparison of the PT, APTT, fibrinogen values and PIVKA results indicated that *a PIVKA prolonged more than 20% beyond control was a more reliable indicator of cats at hemostatic risk than were the other parameters.* This correlation was also observed for cats with small bowel disease and pancreatic exocrine insufficiency.

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Disorders of fibrin formation

Among domestic animals, hereditary **fibrinogen** defects have only been recognized in goats and dogs. **Afibrinogenemia** has been reported in one herd of Saanen goats, in which it was associated with severe umbilical bleeding, recurrent hemarthroses, and bleeding into subcutaneous tissues and from mucous membranes. **Hypofibrinogenemia** has been diagnosed in Bernese Mountain Dogs, Lhasa Apso, Vizsla, and Collie breeds. Like the **dysfibrinogenemia** that has been identified in an inbred Borzoi family and in a Border Leicester lamb, these fibrinogen abnormalities are expressed as a mild bleeding tendency that can be exacerbated by stress, trauma, or surgery.

Although fibrinogen responds as an acute-phase reactant in most domestic animals, with circulating levels rising several fold in response to inflammatory stimuli, thromboembolic problems are infrequently reported in veterinary medicine. Pulmonary thromboembolism occurs in dogs, usually as a secondary consequence of diseases such as *cardiac insufficiency, neoplasia, hyperadrenocorticism, or sepsis.* An elevation of plasma fibrinogen occurs in piglets in response to inoculation with *Actinobacillus pleuropneumoniae*, the causative agent of fibrinohemorrhagic pleuropneumonia in swine. Fibrinous pneumonia is a characteristic of shipping fever induced by *Mannheimia (Pasteurella)* in cattle. Hypercoagulation resulting in thrombosis of limb arteries has been reported in foals. In these animals, endotoxin-induced activation of thrombin generation was postulated to be a contributing factor along with elevated plasma fibrinogen. Similarly, elevated levels of fibrinogen, along with increases in circulating levels of prothrombin and FV, FVII, FIX, and FX, were observed in a study involving 56 dogs with Cushing syndrome. This increase in procoagulant activity was considered to be a contributing factor, along with a decrease in the circulating levels of the natural anticoagulant, antithrombin, in the development of a hypercoagulable state. In this group of Cushing syndrome dogs, a 4-fold increase in the incidence of pulmonary embolism and deep vein thrombosis and a higher mortality rate was observed.

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Platelet activation and thrombus formation

When vascular endothelial cells are injured or exposed to inflammatory mediators, the plasma membrane undergoes biochemical and conformational changes that convert this vascular layer from presenting an anticoagulant surface to flowing blood to one that presents a *procoagulant surface*. The availability of platelet activators, as well as surface-binding proteins, integrins, such as vonWillebrand factor (vWF), initiates platelet adhesion and congregation of platelets at the site of vascular perturbation at the same time as TF and surface bound phospholipids are becoming available to initiate fibrin formation. *The response of platelet accumulation is referred to as* "**primary hemostasis**."

Platelet activation and adhesion

The cytoplasm of megakaryocytes fragments into individual platelets as a result of shear forces of the circulating blood, primarily in the pulmonary circulation. Upon activation, platelets undergo a morphological transformation from discoid cells into spiny spherical cells with numerous filopodia. This "*shape change*" is caused by interaction of actin and myosin filaments in the cytoplasm and is accompanied by alterations in membrane conformation that activate platelet membrane glycoprotein, or *integrin*, receptors. These receptors mediate the adhesion of single platelets to subendothelial tissue that, in turn, triggers a cascade of reactions leading to recruitment and adhesion of additional platelets.

Both fibrinogen and vWF are required for the adherence of platelets to each other and to damaged endothelium. Glycoprotein Ib-V-IX is a constitutively active receptor that causes rapid platelet attachment to exposed perivascular vWF. This receptor is particularly important in areas of the circulation with high shear forces as it facilitates the stabilization of the platelet aggregates. The most abundant platelet integrin, glycoprotein IIa/IIIb, requires a conformational change during platelet activation to express receptor function for fibrinogen, and to a lesser extent vWF. Additional pools of this receptor are available in the surface-connected open canalicular system that is a characteristic feature of most mammalian platelets. Once fibrinogen is bound to glycoprotein IIa/IIIb receptors, it forms bridges between adjacent platelets that are essential for the development of stable platelet aggregates.

While thrombin can bind to the N-terminal domain of the glycoprotein Ib receptor enhancing its reactivity, *platelets, like endothelial cells, possess specific membrane thrombin receptors*. These receptors are members of the G-protein-coupled receptor family characterized by a single polypeptide with a seven-transmembrane spanning domain. Thrombin receptors are frequently referred to as *protease-activated receptors*, or PARs, because the receptors only acquire transmembrane signaling functions after thrombin has cleaved a peptide from the extracellular domain of its receptor. This cleavage unmasks a new amino acid terminus that binds intramolecularly with the body of the PAR receptor to form a reactive molecule. *Thrombin is recognized as the most potent agonist for platelets of all species.*

Platelet aggregation

The continued recruitment of circulating platelets and their incorporation into platelet aggregates around the site of already adherent platelets depends on the local accumulation of platelet agonists and the generation of procoagulant activity in the form of integrin and phospholipid exposure on the surface of activated platelets. The agonists include ADP, serotonin and epinephrine that are released from platelet dense granules, and newly synthesized reactive molecules, such as platelet activating factor (PAF) and thromboxane A_2 (TxA_2). Each of these compounds, like thrombin, interacts with its own G-coupled protein receptor to initiate a process of "inside-out" signaling. The activator–receptor interactions not only increase the avidity of the glycoprotein-integrin receptors for their ligands but also initiate intracellular downstream signaling via several pathways involving the activation of phospholipases, protein kinase K (PKC), and adenylate cyclase.

The hypo-adhesive and hypo-aggregable state of unactivated circulating platelets is maintained by low cytosolic levels of calcium and cyclic AMP (cAMP). Following platelet activation, phospholipase C-mediated hydrolysis of the membrane phospholipid, phosphatidylinositol-4,5-bisphosphate (PIP_2), results in the release of two secondary messengers, inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG), while phospholipase A_2 hydrolyses phosphatidylcholine to PAF and the TxA_2 precursor, arachidonic acid. Intracellular IP_3 directly induces calcium release from storage pools in dense granules, while DAG indirectly induces the same response through activation of PKC. Elevation of free cytosolic calcium, coupled with a reduction in intracellular cAMP, is a universal response among mammalian platelets and explains why drugs that function as calcium channel blockers, e.g., verapamil, universally depress the rate and extent of aggregation. In contrast, drugs, such as aspirin, which inhibit the reactivity of the constitutive form of the cyclooxygenase enzyme (COX-1), do not interfere with the aggregation response in platelets from those species, e.g., cow, rat, and some dogs, that are relatively insensitive to TxA_2 as an agonist.

Because of the multiplicity of the intracellular responses to receptor activation and because of the synergy among products of the enzyme activations, *the physiological response of circulating platelets to vascular damage is similar in all animals despite species and breed differences being observed when a single agonist is used to induce platelet aggregation in vitro.*

In all species, platelets also function as an essential component of the endothelial repair process. During aggregation, growth factors, such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), are released from their storage sites in α-granules (Fig. 2.141). Further, within the thrombus, the platelet cytoskeleton proteins, actin and myosin, in the presence of calcium, interact in a manner analogous to that of actin and myosin in muscle cells. *The activation of these contractile proteins results in the shrinkage of the platelet–fibrin meshwork, i.e., clot retraction.*

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Platelet disorders

Bleeding episodes associated with a lack of sufficient numbers of functional platelets are typified by petechial and ecchymotic hemorrhages from unsealed small endothelial lesions of mucosal membranes or of the microvasculature. *Epistaxis, gastrointestinal and urogenital bleeding and cutaneous bruising are common phenomena.* This type of bleeding is not observed in disorders involving thrombin generation because the platelet adhesion and aggregation responses are adequate to seal small lesions in areas of low blood pressure. For larger wounds and in areas of high pressure, hemostasis requires both fully functional platelet aggregation and fibrin formation so that a stable thrombus can form to stop blood loss and promote wound healing. *Thrombopathies* associated with prolonged bleeding times, normal platelet counts and normal coagulation profiles may be due to defects in platelet adhesion, aggregation or secretion.

Evaluation of platelet function

The *buccal mucosal bleeding time* (BMBT) is a measure of the time for bleeding to stop after a standardized cut is made with a spring-loaded disposable device in the mucosal surface of the upper lip. The BMBT is prolonged if there are insufficient numbers of functional platelets in the circulation, or if there are reduced levels of functional adhesive proteins, such as vWF. Platelet numbers can be estimated from a blood smear and/or a platelet count. The ability of platelets to aggregate in response to in vitro stimulation with known agonists can be determined in citrated blood or plasma, but because this requires specialized instrumentation, its usefulness is limited in many diagnostic situations.

Thrombocytopenia

Clinical signs of thrombocytopenia include petechiation and ecchymosis in tissues or mucous membranes, epistaxis, hematuria and prolonged bleeding after venipuncture. In the absence of any other underlying coagulopathy, these clinical signs are generally not observed in domestic animals unless the platelet count falls below 25–50 $\times 10^9$ platelets/L. In healthy animals, approximately 30–40% of the total platelet pool is sequestered in the spleen, and hence transient thrombocytopenia may be observed in healthy animals when physiological conditions, such as hypothermia, induce an increase in the splenic platelet pool.

Hereditary thrombocytopenia has only been reported in Cavalier King Charles Spaniels. In this breed the autosomal recessive trait is asymptomatic and is characterized by *macrothrombocytopenia.*

Acquired thrombocytopenia is one of the most commonly recognized hematological disorders. Thrombocytopenia associated with *decreased platelet production* may be secondary to megakaryocyte hypoplasia or neoplasias, such as lymphoma. However, thrombocytopenia can also occur because of a *decreased rate of platelet survival* within the peripheral circulation that can be either immune-mediated or drug-induced. At present there are no noninvasive tests that can differentiate between an increase and a decrease in platelet production.

Immune-mediated thrombocytopenia has been reported in many breeds of dogs and in both young and old animals. *Primary idiopathic, or autoimmune thrombocytopenia,* is caused by the development of autoantibodies without an apparent associated disease. Prolonged bleeding may be observed during estrus, at whelping, and following venipuncture or surgery. Neonatal *alloimmune thrombocytopenia* has been reported in pigs and horses. In pigs, the disorder usually occurs after two or more pregnancies. Piglets are frequently born healthy but develop thrombocytopenia at one week of age. Diagnosis of the disorder is one of exclusion, sepsis being the usual differential, because *infections are the most common cause of thrombocytopenia.*

Secondary immune-mediated thrombocytopenia can be caused by *anti-platelet antibodies* or may be the result of *antibody binding to non-self-antigens* that become absorbed onto platelet membranes. These antibodies may be produced in the animal in response to infectious agents, drug exposure, or neoplasia. For example, an increase in antibody titer to *Rickettsia rickettsii* corresponds to thrombocytopenia and the development of petechiae and ecchymoses in dogs with canine Rocky Mountain spotted fever. Thrombocytopenia associated with systemic lupus erythromatosus (SLE) has been reported in dogs, cats and horses. Likewise, thrombocytopenia frequently accompanies immune-mediated hemolytic anemia in dogs, and has also been observed in cats and horses.

All domestic species are susceptible to **drug-induced thrombocytopenia** because of the broad spectrum of drugs that can either suppress pro-platelet production in the bone marrow or increase the rate of platelet destruction as a consequence of drug-dependent antibodies that bind to complementary antigenic sites on platelet membranes. Mononuclear phagocytes in the liver and spleen then destroy the modified platelets. In horses, as in people, *heparin administration* can induce transient thrombocytopenia through its interaction with preformed antibodies that have developed against the specific platelet protein, platelet factor 4 (PF4). PF4 is one of the proteins secreted from the α-granules into the circulation after platelets are activated. It is a positively charged molecule that binds tightly to negatively charged heparin. Not only can the complex of antibody-PF4-heparin enhance the rate of clearance of platelets from the circulation, but it can also induce platelet activation. To avoid thrombotic complications, low-molecular-weight (LMW) heparin preparations are becoming more widely used in the treatment of disseminated intravascular coagulation (DIC) in people. Preliminary results indicate that LMW heparin will also prove useful in veterinary medicine, as trials in dogs indicate that it is effective as an antithrombotic agent and appears not to induce thrombocytopenic complications.

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von Willebrand disease

Although not an intrinsic platelet defect, *the primary cause of bleeding in von Willebrand disease (vWD) is failure of platelets to adhere and form aggregates around sites of vascular damage.* Although rare in cats, horses and cattle, *vWD is the most common canine inherited bleeding disorder.* In dogs, as in people, it represents a heterogeneous group of clinical disorders that are classified into *3 general types* on the basis of severity of bleeding, mode of inheritance, and biochemical abnormalities of the vWF protein. The vWF protein is primarily synthesized in endothelial cells as two subunits that subsequently undergo post-translational assembly into dimers and multimers ranging up to 100 vWF subunits that are stored in and subsequently released from endothelial cells. The high molecular weight subunits are most effective in promoting platelet-endothelial cell and intraplatelet bridges.

The release of vWF from cytoplasmic granules (Weibel-Palade bodies) occurs following endothelial cell stimulation with a variety of physiological compounds, such as thrombin, histamine, estrogen, thyroxine and the vasopressin analogue, 1-desamino-8-D-arginine vasopressin (DDAVP). Strenuous exercise also induces an increase in plasma vWF levels. In some species, such as people and cats, circulating platelets act as a secondary pool of circulating vWF because they contain megakaryocyte-derived vWF in their α-granules. However, canine platelets contain only very small amounts of vWF. Plasma vWF circulates in noncovalent complex with FVIII, which has the effect of prolonging the circulating half-life of the FVIII protein.

Based on breed studies in dogs, it appears that **type 1 vWD**, which is characterized by low circulating levels of vWF antigen and a proportional decrease in all multimeric forms, is transmitted in an incomplete dominant pattern. In contrast, the more clinically severe forms of vWD, **type 2 and type 3**, are recessive traits. In type 3, plasma vWF is absent whereas in type 2, high molecular weight multimers are absent and plasma vWF antigen levels are reduced (<50% of normal). In all forms of the disease, bleeding tendencies are exacerbated by concurrent thrombocytopenia or administration of drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), that impair platelet function.

Acquired vWD has been reported in a dog as a secondary consequence of lymphangioma. In ponies, administration of hydroxyethylene starch solutions as blood volume expanders can also induce a transient decrease in circulating vWF levels.

A prolonged bleeding time accompanied by a normal platelet count and a normal coagulation profile is suggestive of vWD. Diagnosis can only be confirmed by specific assay of plasma vWF antigen and the determination of vWF multimer distribution. Both of these analytes are determined by immunological procedures. *Because vWF is antigenically distinct among mammalian species, plasma samples must be analyzed with reagents appropriate for the patient.* A semi-quantitative assessment of functional vWF activity can be determined by evaluating vWF-dependent platelet agglutination. A molecular test for vWF based on variation in DNA sequence is available for some breeds of dogs.

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Inherited disorders of platelet function

Inherited thrombopathies are relatively rare in domestic animals compared to people, but they have been identified in several breeds of dogs and in cattle. *Four types of platelet dysfunction are recognized: disorders of platelet membrane glycoproteins, storage pool deficiencies, impairment of intracellular signaling, and a defect of platelet procoagulant activity.* Each of these disorders appears to be inherited as an autosomal trait. Clinical signs tend to be similar among the defects and involve mucosal bleeding that is more diffuse than the "pinpoint" hemorrhages observed with thrombocytopenia. Gingival hemorrhage can occur during shedding of deciduous teeth in puppies. Epistaxis, cutaneous ecchymoses, hematuria and prolonged or excessive bleeding at sites of surgery or trauma are common in older dogs. In cattle, subcutaneous hematomas and prolonged hemorrhage from puncture wounds, such as vaccination, ear tagging and insect bites, are typically encountered.

Thrombocytopenic thrombopathia, a variant of type I Glanzmann's thrombasthenia, has been documented in the Otterhound and Great Pyrenees breeds, and in a Boxer dog. The disorder is characterized by a decrease in the number of the platelet glycoprotein IIb/IIIa membrane receptors that serve as binding sites for vWF and fibrinogen. Thus, platelets from homozygous-deficient animals undergo a normal shape-change response following stimulation with agonists such as ADP, collagen, thrombin or PAF, but fail to form stable aggregates. Clot retraction is also impaired. Because platelet morphology is normal, a definitive diagnosis requires the analysis of platelet membranes by electrophoretic or flow cytometric techniques using antibodies specific for α- and β-subunits of the glycoprotein receptor.

The bleeding diathesis in **Chediak-Higashi syndrome** *(CHS) is due to a dense-granule storage pool deficiency.* The disease has been identified in Hereford, Brangus, and Japanese Black cattle, in Aleutian mink, Persian cats, and blue and silver foxes. The dense granules, the amine storage organelles, are either absent or ultrastructurally indistinct in platelets from affected animals. The in vitro aggregation response to ADP or collagen is reduced as a result of a decrease in the secretion of ATP, ADP and serotonin, which may or may not be accompanied by a decrease in intracellular calcium mobilization. Similar biochemical changes have been observed in several American Cocker Spaniels with normal platelet morphology.

Defective intracellular signaling pathways have been identified as the cause of platelet dysfunction in Basset Hounds, and Spitz dogs and in both purebred and crossbred Simmental cattle. In Basset Hounds, impaired cyclic AMP metabolism and abnormal phosphodiesterase activity result in elevations in basal platelet cAMP, which interferes with intracellular signaling following platelet activation. Platelet shape change is normal, but platelet adhesion and aggregation in response to most agonists is depressed. A similar response is observed in platelets from affected Spitz dogs, although the molecular basis for the defect in this breed has not been fully elucidated. In Simmental cattle, the platelet defect is characterized by a failure of platelets to form aggregates in vitro in response to ADP, collagen, PAF or other agonists that induce intracellular calcium mobilization. It has been proposed that the molecular basis for the dysfunction may be due to abnormal cytoskeleton interactions that prevent the assembly of the cytoplasmic domain of the GPIIb/IIIa receptors into the functional complexes necessary to support intracellular signaling.

A **defect in platelet procoagulant activity** has been identified in both male and female members of an inbred German Shepherd colony. As in the comparable human disorder known as Scott syndrome, platelet morphology is normal, as is platelet function as assessed by bleeding time, clot retraction, and aggregation. Total platelet phospholipid levels are normal but there is a reduction in the amount of phosphatidylserine that becomes exposed on the platelet surface during the activation process. Because of this, the platelets cannot provide an adequate phospholipid platform for the formation of the "tenase" and "prothrombinase" complexes, and consequently the rate of thrombin generation and fibrin formation is impaired. This delay in clot formation is clinically manifest by epistaxis, hyphema, intramuscular hematoma, and prolonged bleeding with cutaneous bruising after surgery.

Thrombopathies due to a combination of signaling and storage pool defects have been reported in a gray Collie and are associated with CHS in Japanese Black cattle.

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Acquired platelet disorders

Considering the broad range of pharmacological agents that impair various biochemical aspect of platelet function, *it is probable that transient decrements in platelet hemostatic responses are relatively common in veterinary medicine but are rarely documented.* This is likely due to the relatively short half-lives of circulating platelets so that new, fully functional platelets are replenishing the circulating pool, and to the fact that a drug may selectively inhibit one intracellular signaling pathway thus reducing, but not completely blocking, platelet adhesion and aggregation. Further, in the absence of a preexisting coagulopathy, such as vWD or thrombocytopenia, a partial suppression of platelet reactivity is not, by itself, sufficient to induce clinical hemorrhage in the absence of trauma. However, hemorrhagic episodes are commonly observed when both thrombocytopenia and platelet dysfunction occur. For example, in calves infected with **type 2 *Bovine viral diarrhea virus***, spontaneous hemorrhages occur because of a decline in both circulating platelet numbers and their aggregation response. Dogs infected with *Ehrlichia canis* also develop thrombocytopenia and exhibit a reduction in both platelet adhesiveness and aggregation. The tendency to bleed and the alterations in platelet function in dogs with **canine ehrlichiosis** may be due to the appearance of anti-platelet antibodies.

Because of the subtle quantitative differences in the relative contributions of the various activation and intracellular signaling pathways among platelets from different species, it is difficult to make generalized statements about the inhibitory actions of anti-inflammatory and antibacterial agents. For example, nonsteroidal anti-inflammatory drugs, such as aspirin, that inhibit the endogenous platelet cyclooxygenase enzyme (COX-1), suppress platelet aggregation in most species. Bovine platelets are unresponsive to the inhibitory effects of aspirin and likewise are unresponsive to the β-lactam antibiotic, penicillin. In other species, this group of antibiotics binds to platelet membranes and inhibits glycoprotein receptor mediated adhesion and aggregation. The platelet inhibitory effects of barbiturates and cardiovascular drugs, such as diltiazem and verapamil, which act as calcium-channel blockers, also vary among species. Buccal mucosal bleeding time and platelet adhesion are prolonged in uremic dogs, but the molecular mechanisms involved have not been identified.

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Regulation of coagulation: endogenous anticoagulants

It is essential for the maintenance of blood flow to tissues downstream from a site of vascular damage that thrombus formation be confined to the site of vascular damage, and that any procoagulant enzymes, such as FXa and thrombin, that escape from the thrombus into the circulation be neutralized by circulating inhibitors.

Antithrombin (AT, formerly called antithrombin III, or heparin cofactor) *is the major inhibitor of both thrombin and thrombin generation in plasma*. It is a member of the superfamily of "*serpins*" (serine protease inhibitors) that also includes α_1-antitrypsin, α_2-antiplasmin, and α_2-macroglobulin. Plasma AT is an α_2-globulin derived from both hepatocytes and endothelial cells. It preferentially inhibits the free forms of thrombin, FIXa and FXa, and thus has limited inhibitory activity against the "prothrombinase" and "tenase" complexes. The inhibitory action of AT is derived from the interaction of its reactive arginine residue with a serine residue at the reactive site of thrombin, or other serine proteases. The resulting thrombin–AT (TAT) complex prevents thrombin cleavage of fibrinogen to fibrin (Fig. 2.143). The inhibitory efficiency of AT is enhanced by heparan sulfate complexes exposed on the surface of activated endothelial cells. Either intravenously administered heparin, or heparin released from mast cells during an inflammatory reaction, also increases the rate of formation of TAT complexes. This mechanism forms the molecular basis for the therapeutic use of heparin as an anticoagulant. The TAT complexes are removed from the circulation either through clearance by the mononuclear phagocyte system or by becoming bound to the endothelial cell integrin, *vitronectin*, which results in their endocytosis and lysosomal degradation.

The **protein C anticoagulant system** regulates coagulation by modulating the activity of FV and FVIII. It represents a unique "on-demand" inhibitory system that is activated by thrombin generation. Protein C is synthesized in the liver as a vitamin-K-dependent zymogen of an anticoagulant protease. It is converted to an *activated form, APC*, on the surface of intact endothelial cells by thrombin that has become bound to the membrane protein, **thrombomodulin** (TM) in the T-TM complex (Fig. 2.143). Thrombomodulin is present on endothelial cells in arteries, capillaries, veins, and lymphatics. It is present in highest amounts in capillaries where blood flow is slower and thrombin concentrations highest. APC can cleave the phospholipid-membrane-bound cofactors to form the inhibitory products FVai and FVIIIai that, in turn, cause the suppression of both "prothrombinase" and "tenase" activity. The cleavage of FVa and FVIIIa within the phospholipid–enzyme complexes is facilitated by the vitamin-K-dependent cofactor, **protein S**, which forms a membrane-bound complex with APC. Only the free circulating form of protein S can complex with APC. This form represents about 30% of the total plasma concentration, as the major portion of protein S circulates bound to the complement regulatory protein C4b-binding protein.

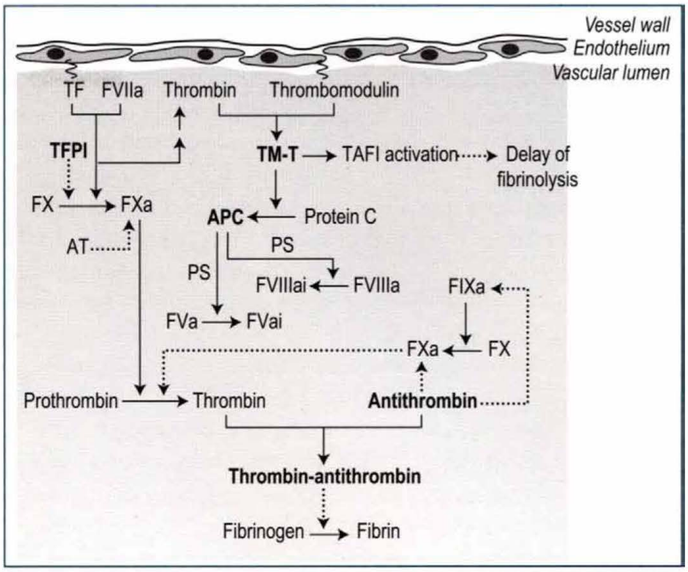

Figure 2.143 Schematic of the **major anticoagulant pathways**. Solid lines represent activation reactions and dashed lines represent inhibitory reactions. APC = activated protein C, AT = antithrombin, FVai = inhibitory form of FV, FVIIIai = inhibitory form of FVIII, PS = protein S, TAFI = thrombin-activatable fibrinolysis inhibitor, TFPI = tissue factor pathway inhibitor, TM-T = thrombomodulin–thrombin complex.

In vivo, APC does not cleave FVIII circulating bound to vWF, but it can cleave circulating FV and FVa, in part because both FV and FVa can bind to phospholipids in proximity to the APC-protein S membrane-bound complex. The cleaved FV can function in synergy with protein S as an APC cofactor in the degradation of FVIIIa. Thus, the anticoagulant potential of FV may be important in the regulation of the "tenase" complex by APC and protein S. Although few studies have been conducted with the APC-protein S system in domestic animals, its physiological importance is demonstrated by the severe thromboembolic disease that is associated with homozygous deficiency of protein C in both people and mice.

Disorders of endogenous anticoagulants

Although several types of inherited **antithrombin deficiency** have been characterized in people, this disorder has not been identified in domestic animals. Both functional and immunological laboratory assays have been developed to quantify AT levels in mammalian plasma. This has led to the recognition that, in many species, sepsis and DIC produce acquired deficiencies in circulating AT activity that are frequently accompanied by the appearance of circulating TAT complexes. One of the common hemostatic abnormalities observed in horses with colic is a decline in plasma AT activity. An acquired deficiency in AT activity has also been reported in dogs as a secondary consequence of protein-losing nephropathies or enteropathies. In dogs, the hypercoagulable state associated with these disorders, and the thrombosis that is an uncommon complication of hyperadrenocorticism, diabetes mellitus and cardiovascular disease, is attributed to reduced plasma AT activity. Reduced plasma AT was also one of the major hemostatic abnormalities found in a foal with thrombosis of the brachial artery. In contrast, increases in AT activity have been reported in cats associated with various inflammatory conditions. This is likely related to the fact that AT responds as a positive acute-phase reactant in cats.

Both immunological and functional laboratory techniques are available to quantify the protein C component of the thrombin–thrombomodulin–protein C–protein S system in animal plasma. As in human infants, foals exhibit lower functional protein C activity than do adult horses. Protein C activity is reduced in cases of vitamin K deficiency or antagonists, with protein C antigen levels being unaffected. DIC-induced consumption of protein C has been reported in dogs and primates. Lupus-like anticoagulants are phospholipids that promote thrombosis through inhibition of the protein C–protein S complex.

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Fibrinolysis

The fibrinolytic system is an essential component of the hemostatic process. It is required for the dissolution of the thrombus through the proteolytic degradation of fibrin after tissue repair has occurred. The central enzyme in lysis of fibrin is **plasmin**, which is formed from its precursor, *plasminogen*, by one of two endogenous activators, tissue-type plasminogen activator (tPA) and urokinase (uPA) (Fig. 2.142). Plasminogen is synthesized by hepatocytes as a single chain glycoprotein and is the fibrinolytic component present in the highest concentration in the circulating blood. *Plasmin is a trypsin-like enzyme with broad substrate specificity*. In addition to degrading fibrin strands, it can degrade fibrinogen, FV and FVIII, various extracellular matrix components, and can activate other proteases, including matrix-degrading metalloproteinases. Like thrombin, both plasmin formation and its activity are highly regulated. For example, both tPA and uPA have short circulating half-lives relative to plasminogen, in part because of their rapid clearance via LDL-receptor related proteins (LRP) on cell surfaces. Further, *in healthy animals, the balance between circulating inhibitors and activators of plasminogen favors inhibition of plasmin formation.* The major inhibitor of plasminogen activation is *plasminogen activator inhibitor-1 (PAI-1)*, which can block the action of both tPA and uPA (Fig. 2.142). In normal plasma, PAI-1 is in five-fold excess over tPA, so most plasma tPA circulates as an inactive tPA-PAI-1 complex. This balance can be changed by the increased release of tPA from endothelial cells in response to exercise, vascular occlusion, or in response to locally generated thrombin at a site of vascular damage. In plasma, the primary inhibitor of plasmin activity is α_2-antiplasmin, with α_2-macroglobulin and α_1-antitrypsin capable of acting as "back-up" inhibitors.

Surface-mediated fibrinolysis

In the initial stages of thrombus formation, the activation of plasminogen is suppressed. Although thrombin is one of the potent promoters of the synthesis and secretion of tPA by endothelial cells, this profibrinolytic action is effectively opposed by the localized activation of TAFI (Figs 2.142 and 2.143). High concentrations of thrombin generated both by the positive feedback activation of FXI to FXIa and activation of the "tenase" complex, combined with the formation of thrombin–thrombomodulin complexes, enhance the activation of TAFI, which in turn inhibits tPA activation of plasminogen. Platelets represent the main circulating pool of PAI-1, which is locally released from α-granules during platelet activation. This platelet contribution of PAI-1 accounts, at least in part, for the premature fibrinolysis that has been observed in platelet-poor thrombi compared to platelet-rich thrombi.

Once an organized thrombus has been formed, plasmin formation and fibrinolysis is promoted. Most of the proteins of the fibrinolytic system appear in thrombi at final concentrations representing 10–30% of their concentration in plasma. This is consistent with simple trapping of plasma proteins in the thrombus. Fibrin potentiates fibrinolysis by bringing together plasminogen and tPA on its surface, which not only facilitates enzyme–substrate interactions but also protects tPA from inhibition by TAFI and PAI-1. Further plasmin, formed on a fibrin surface, interacts with fibrin strands at both its active site and at specific lysine-binding sites. This linkage has the effect of decreasing the ability of α2-antiplasmin to interact with and inhibit plasmin generated within the fibrin meshwork (Fig. 2.142).

Fluid-phase fibrinolysis

In contrast to tPA, uPA readily activates plasminogen in the fluid compartment of the circulation. At present, it is unclear how uPA participates in hemostasis, but it is evident from transgenic and knockout mouse studies that the tPA and uPA activators can compensate for each other. Fibrin is rapidly deposited after wounding, not only to prevent blood loss but also to provide a scaffold for the invasion of cells during the subsequent healing process. *Plasmin and uPA are known to be important in the slow dissolution of fibrin that occurs once tissue repair is complete.*

Single-chain urokinase-type plasminogen activator (scuPA, prourokinase) is synthesized in renal tissue and in macrophages as a zymogen. The molecule contains an EGF domain that is responsible for the binding of scuPA to specific receptors that are present on the surface of a variety of cells. *Kallikrein*, either generated at the site of vascular trauma or as part of an inflammatory response, is one of the important physiological mediators that selectively cleaves scu-PA to a proteolytically active two-chain form (uPA) (Fig. 2.142). Unlike tPA, uPA has no specific affinity for fibrin and thus can activate circulating and fibrin-bound plasminogen indiscriminately. Initially, plasmin generated outside a fibrin clot can be rapidly neutralized by α_2-antiplasmin. However, if the formation of plasmin exceeds the availability of this inhibitor, then plasmin can begin to degrade circulating plasma proteins such as fibrinogen, FV and FVIII. Different bacterial strains produce efficient plasminogen activators that can act as exogenous activators, frequently inducing inappropriate levels of plasmin formation. One of the best characterized compounds is *streptokinase*, which is produced by those strains of streptococci that use plasmin to their advantage in increasing the efficiency of their invasive capacity. In many mammalian species, streptokinase acts by forming a 1:1 stoichiometric complex with either plasminogen or plasmin. In this form, the general proteolytic action of streptokinase is decreased, but its plasminogen-activating activity is increased several fold.

Primary disorders of the fibrinolytic system have not been characterized in domestic animals. Unlike the situation in people, there appear to be no significant postoperative changes in the fibrinolytic system in dogs. However, alterations of the fibrinolytic system associated with inflammatory conditions are frequently recognized as a prelude to DIC.

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Inflammation and neoplasia

Inflammatory mediators, released either from an infectious agent and/or by the host, can manipulate procoagulant/anticoagulant equilibrium and cause secondary hemorrhagic or thrombotic complications. *The role of the contact activation system as a link between hemostasis and inflammation has long been recognized.* In addition to initiating thrombin formation through the FXI-tenase pathway (Fig. 2.142), activation of FXII causes the conversion of PK to kallikrein that, in turn, converts HMWK to the inflammatory mediator *bradykinin*. A close connection between the tissue factor pathway and inflammation has more recently been elucidated.

An increase in TF expression on the plasma membrane of peripheral blood cells and endothelial cells occurs in response to products from both gram-negative and gram-positive organisms. Activated FVII, FX and thrombin, generated following exposure to TF, trigger intracellular signaling cascades in leukocytes by binding to PAR-type receptors that currently have no known role in hemostasis. *Among the inflammatory responses induced by these interactions are leukocyte recruitment, release of additional cytokines, and production of reactive oxygen species.* Systemic activation of thrombin generation can result in the cleavage of fibrinogen and the deposition of fibrin in the microvasculature of various organs. The formation of fibrinous exudates can benefit the host by encapsulating microorganisms. On the other hand, because the exudates can incorporate a large number of bacteria that are protected from phagocytosis once they are sequestered, bacterial proliferation can occur.

Blood platelets are also recognized as inflammatory mediators, either through release of their cytoplasmic granules, e.g., histamine and serotonin, or through the formation of cell membrane-derived neutrophil and monocyte agonists, e.g., PAF and leukotrienes. After activation, mononuclear phagocytes can produce and release a wide range of inflammatory mediators including: IL-6 that can stimulate increased hepatocyte fibrinogen synthesis, PAF and prostaglandins that can induce further platelet activation, and tumor necrosis factor (TNF), which can initiate fibrinolysis through the release of plasminogen activators. Thus, *in the initial phase of inflammation, a balance between procoagulant activity, fibrin formation, and the lysis of formed fibrin can be maintained.* However, within several hours of the initiation of an inflammatory response, the fibrinolytic system is suppressed due, in part, to the increased synthesis and release of PAI-1. The fact that thrombosis is not a consistent clinical observation during inflammation can be explained by the thrombin- and/or cytokine-induced activation of the TM–protein C–protein S anticoagulant system (Fig. 2.143). The suppression of both thrombin formation and plasmin generation maintains a hemostatic equilibrium.

Dysequilibrium among procoagulant, anticoagulant and fibrinolytic activity, similar in clinical manifestation to the situation that occurs in sepsis, is a common occurrence in people with various types of cancer. The association between hemostatic abnormalities and **neoplasia** is less well understood in domestic animals, although abnormal bleeding can be a clinical sign in animals with malignancies such as hemangiosarcoma, multiple myeloma and mast cell tumors. *Abnormal laboratory hemostatic profiles are frequently associated with untreated neoplasia in dogs and cats.* For example, in a study of 100 dogs, 83 exhibited at least one abnormal parameter with thrombocytopenia (36 dogs), prolonged APTT (32 dogs), and either elevated or reduced fibrinogen values (50 dogs) being the most common findings. *Anti-neoplastic drugs can also impair the hemostatic process.* For example, vincristine has been shown to inhibit canine platelet function. L-asparaginase, which inhibits protein synthesis, and actinomycin D, which directly antagonizes vitamin K, can produce transient reductions in the circulating activity of multiple hemostatic proteins. Functional inhibition of clotting factor activity by antibodies produced by neoplastic cells can also contribute to hemorrhagic problems. These pathological inhibitors, or circulating anticoagulants, may either be immunoglobulins directed against the functional epitopes of hemostatic proteins or antiphospholipid antibodies that interfere with the normal rate of formation of the "tenase" and "prothrombinase" complexes (Fig. 2.141). The presence of acquired plasma inhibitors is suspected when deficits in single or multiple coagulation factors cannot explain abnormal laboratory hemostasis profiles.

Disseminated intravascular coagulation (DIC) is an acquired syndrome that has been defined as "*the activation of intravascular* *coagulation up to intravascular fibrin formation, which may or may not be accompanied by secondary fibrinolysis or inhibited fibrinolysis.*" Due to the pathological activation of coagulation and inappropriate hypercoagulation with or without fibrinolysis, thrombosis and/or hemorrhage may occur at multiple sites, which in turn cause organ dysfunction or failure. It is a potential complication of several conditions, including sepsis and neoplasia. In horses, DIC has been reported as a complication of acute gastrointestinal tract disease. It is estimated that approximately 50% of dogs with *immune-mediated hemolytic anemia* exhibit evidence of a hypercoagulable state at the time of clinical presentation. Initially, an increased rate of thrombin generation can be compensated for by the circulating anticoagulant system, i.e., TFPI, APC and antithrombin (Fig. 2.143), resulting in a condition referred to as "*compensated DIC*." Variable laboratory results are obtained at this stage. Because of the increased amount of thrombin formation, PT and APTT results may be shortened and variable alterations observed for platelet counts and fibrinogen levels. Definitive laboratory diagnosis requires the evaluation of molecular markers such as TAT complexes (Fig. 2.143) and peptide fragments, such as prothrombin fragment 1 + 2, that are released during activation of procoagulant factors.

If the triggers to thrombin generation persist, the patient may progress to the *second phase of DIC*. When the reserve of circulating inhibitors is consumed, thrombin generation and fibrin formation are unrestrained. This results in the depletion of circulating platelets and the consumption of both procoagulant proteins and fibrinogen, hence the use of the terms "*consumptive coagulopathy*" *or* "*decompensated DIC*" to characterize this stage of DIC. The depletion of both circulating platelets and hemostatic proteins generally results in the development of either spontaneous hemorrhaging or hemorrhages in response to minor trauma. The abnormalities in the laboratory profile include *decreased platelet count, prolonged PT and APTT results, and decreased levels of fibrinogen, antithrombin, and protein C*. In dogs experimentally infected with *Angiostrongylus vasorum*, the consumptive coagulopathy that develops when the larvae migrate into the pulmonary circulation is marked by a decrease in plasma FV and FVIII activity along with an increased APTT.

Frequently, at some stage during DIC development, the fibrinolytic system is also activated resulting in increased amounts of intravascular plasmin being generated. Concurrent fibrin polymerization and fibrinolysis exacerbates the tendency for hemorrhage to occur. During plasmin proteolysis of fibrinogen and fibrin, peptide fragments, known as *D-dimers* and *fibrin degradation products (FDPs)*, respectively, are released into the circulation. Hence, *the evaluation of circulating levels of FDPs and D-dimers can be used as a diagnostic indicator of uncompensated DIC*. Excessive generation of plasmin and production of FDPs both contribute to *organ failure*, which is often the ultimate cause of death in animals with untreated DIC. For example, plasmin can induce vascular and cellular damage through activation of matrix metalloproteinases that degrade a wide range of cytoskeletal filaments. FDPs can enhance local inflammatory reactions by triggering the release of IL-1 and IL-6 from monocytes and macrophages.

There are a number of hemorrhagic conditions in which endothelial injury induced by infectious agents may be the underlying problem. One of the more severe and more commonly encountered disorders in this category is **purpura hemorrhagica**, a vasculitis syndrome, associated with *Streptococcus equi* infection in horses. In dogs, hemorrhage associated with *heartworm disease, Rocky Mountain spotted fever*, and *leishmaniasis* has been attributed, at least in part, to vascular damage. *Exercise-induced pulmonary hemorrhage* in horses is also likely due to an alteration in capillary permeability, because platelet function was found to be identical in horses that exhibited clinical bleeding and those that did not.

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3

Endocrine glands

Charles C. Capen

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GENERAL CONSIDERATIONS

Endocrine glands are collections of specialized cells that synthesize, store, and release their secretions directly into the bloodstream. They are sensing and signaling devices located in the extracellular fluid compartment and are capable of responding to changes in the internal and external environments to coordinate a multiplicity of activities that maintain homeostasis.

Secretions of endocrine glands are peptide, steroid, catecholamine, or iodothyronine hormones that are transported by the blood to influence the functional activity of target cells elsewhere in the body. Other populations of cells are concerned with degradation of hormone after it has exerted its physiologic function. This is accomplished by peptidases on the cell surface, uptake by cells and degradation by lysosomal enzymes, or conjugation with glucuronide or sulfate and excretion in the bile or urine.

Endocrine glands are small compared with other organs, widely distributed in the body, and connected with one another by the bloodstream. They are richly supplied with blood, and there is a close anatomic relationship between endocrine cells and the capillary network. To facilitate rapid transport of raw materials and secretory products between the blood and endocrine cells, the peripheral cytoplasmic extensions of capillary endothelial cells have *fenestrae* covered by a single membrane.

Types of hormones

Polypeptide hormones

The primary site of action for polypeptide hormones is the plasma membrane of target cells. Receptor proteins for the hormone are present on the outer surface of the plasma membrane. These hormones are water soluble, have a *short half-life in blood* (usually measured in minutes), and lack specific plasma-binding proteins.

The receptors for polypeptide hormones on the plasma membrane of target cells perform two key functions. *First, they recognize the active hormone* from among many other proteins to which the cell is exposed. The concentration of hormone in extracellular fluids often is much lower than that of other proteins. The hormone binds to the receptor site and forms a reversible hormone-receptor complex. *Second, the receptor conveys the message of the bound hormone from the outside to the inside of the target cell.* The magnitude of this transmembrane signal depends on the concentration of hormone to which the target cell is exposed, the affinity of the receptor for the hormone, and the concentration of receptors on the target cell.

There appears to be a *single common intracellular pathway* for many different polypeptide hormones. It begins with the activation of an enzyme, *adenylate cyclase*, in the plasma membrane of target cells. Cyclic adenosine monophosphate (cAMP) is formed intracellularly from adenosine triphosphate (ATP) and activates cAMP-dependent protein kinases. These protein kinases activate or inactivate a variety of enzymes by phosphorylating them, using adenosine triphosphate as a source of phosphate. The intracellular pathway for each polypeptide hormone subsequently branches into a multiplicity of pathways leading to a variety of effects on any given target cell.

Endocrine cells that produce polypeptide hormones have a well-developed endoplasmic reticulum with many attached ribosomes where the hormone is assembled, and a prominent Golgi apparatus for packaging hormone into granules for intracellular storage and transport. *Secretory granules* are unique to polypeptide hormone- and catecholamine-secreting endocrine cells and provide a mechanism for intracellular storage of substantial amounts of preformed active hormone. These membrane-limited granules are macromolecular aggregations of active hormone, often associated with a specific binding protein. When the cell receives a signal for hormone secretion, secretory granules are directed by microtubules to the periphery of the endocrine cell, probably by the contraction of microfilaments. The limiting membrane of the granule then fuses with the plasma membrane of the cell. The hormone-containing granule core is extruded through a hiatus into the extracellular perivascular space by emiocytosis or exocytosis. The granule core is fragmented subsequently and hormone is rapidly transported through capillary fenestrae into the circulation. Hormone synthesized in excess of requirement is degraded by fusion of the hormone-containing granules with lysosomes, a process termed *crinophagy*.

Steroid hormones

Steroid hormone-secreting endocrine cells are characterized by large lipid bodies in the cytoplasm that contain cholesterol and other precursor molecules. The lipid bodies are in close proximity to an extensive tubular network of smooth endoplasmic reticulum and large mitochondria that contain the hydroxylase and dehydrogenase enzyme systems. These enzymes function to attach various side chains to the basic steroid nucleus. *Steroid-producing cells lack secretory granules and do not store significant amounts of preformed hormone.* They are dependent on continued biosynthesis to maintain the normal secretory rate for a particular hormone.

Steroid hormones having a basic nucleus of three cyclohexane rings and one pentane ring account for approximately 15% of mammalian hormones. *The primary site of their action is the nucleus of target cells.* Receptors are proteins that bind the hormone primarily in the nucleus resulting in *genomic activation of target cells*. The interaction of steroid hormones with the genetic information results in increased transcription of messenger ribonucleic acid (mRNA), which directs new protein synthesis by specific target cells. The steroid hormones are lipid-soluble, which facilitates their transport through the cell membrane. They have a *long half-life in blood* (typically measured in hours) and reversibly bind to high-affinity, specific binding proteins for transport in plasma.

Catecholamine and iodothyronine hormones

This chemical group of hormones are *tyrosine derivatives*. They account for approximately 5% of mammalian hormones and include the *catecholamines (epinephrine, norepinephrine)* secreted by the adrenal medulla, and *iodothyronines (thyroxine (T_4), triiodothyronine (T_3))* produced by follicular cells of the thyroid gland. Catecholamines share similar mechanisms of action with polypeptide hormones, whereas iodothyronines more closely resemble the steroid hormones.

Proliferative lesions in endocrine glands

Characteristics

Endocrinologically active (functional) neoplasms derived from polypeptide hormone-secreting endocrine cells consist of one predominant cell type and usually are associated clinically with the autonomous secretion of one polypeptide hormone. However, there is evidence from immunocytochemical and electron microscopic investigations that some endocrine tumors are composed of more than one type of neoplastic cell and are capable of synthesizing multiple hormones.

Neoplasms derived from steroid hormone-secreting endocrine cells are characterized by having large lipid bodies in their cytoplasm that contain cholesterol and other precursor molecules for hormone synthesis. The lipid bodies are in close proximity to an extensive tubular network of smooth endoplasmic reticulum and large mitochondria. Steroid hormone-producing cells lack secretory granules and are unable to store significant amounts of preformed hormone.

The histologic separation between nodular hyperplasia, adenoma and carcinoma often is more difficult in endocrine glands than in most other organs of the body. For many endocrine glands (especially thyroid C cells, secretory cells of the adrenal medulla, and specific trophic hormone-secreting cells of the adenohypophysis) there is a continuous spectrum of proliferative lesions between diffuse or focal hyperplasia and adenomas derived from a specific population of cells.

It is a common feature of endocrine glands that prolonged stimulation of a population of secretory cells predisposes to the subsequent development of a higher than expected incidence of tumors. Long-term stimulation leads to the development of clones of cells within the hyperplastic endocrine gland that grow more rapidly than the rest and are more susceptible to neoplastic transformation when exposed to the right combination of promoting carcinogens.

Nodular hyperplasia usually appears as multiple small foci, in one or both (for paired) endocrine gland(s), that are well demarcated but not encapsulated from adjacent normal cells. Cells comprising an area of nodular hyperplasia closely resemble the cell of origin; however, the cytoplasmic area may be slightly enlarged and the nucleus more hyperchromatic than in the normal endocrine cell.

Adenomas are solitary nodules, in one (or occasionally both for paired) endocrine gland(s), that are larger than the multiple foci of nodular hyperplasia. They are *sharply demarcated from the adjacent normal glandular parenchyma,* often by a thin, partial to complete, fibrous capsule. The adjacent parenchyma is compressed to varying degrees depending upon the size of the adenoma. Cells comprising an adenoma closely resemble the cell of origin morphologically and in their architectural pattern of arrangement; however, there often are histologic differences such as multiple layers of cells lining follicles or vascular trabeculae and solid clusters of secretory cells subdivided into packets by a fine fibrovascular stroma.

Carcinomas usually are larger than adenomas and produce enlargement in one (or occasionally both, for paired) endocrine gland(s). The separation between adenoma and carcinoma of an

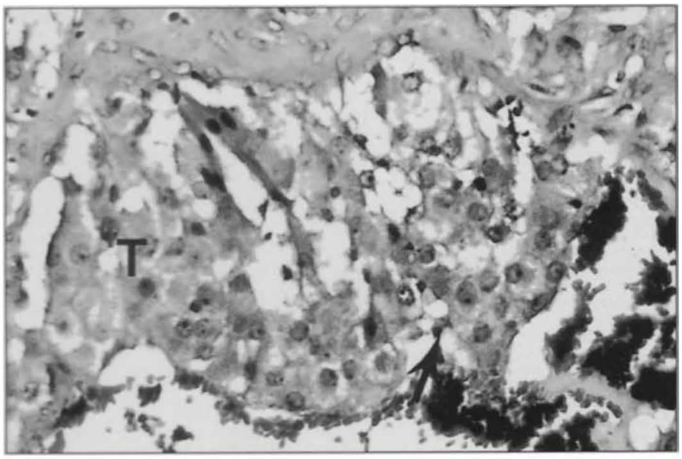

Figure 3.1 Subendothelial growth of tumor cells (T) in a **pheochromocytoma**, rat. The blood-filled space is lined by endothelial cells (arrow).

endocrine gland often is more difficult than in other tissues. *Histopathologic features that are suggestive of malignancy in an endocrine tumor include intraglandular invasion, invasion into and through the capsule of the gland with establishment of secondary foci of growth in the periglandular fibrous and adipose connective tissues, formation of tumor cell thrombi within vessels (especially muscular-walled), and particularly the establishment of metastases at distant sites.* The growth of neoplastic cells in a subendothelial location in highly vascular benign endocrine tumors should not be mistaken for vascular invasion and clusters of neoplastic cells may be artefactually forced into the lumen of thin-walled vessels and be misinterpreted as tumor cell emboli (Fig. 3.1). Malignant endocrine cells often are more pleomorphic (including oval or spindle-shaped) than normal, but nuclear pleomorphism is not a consistent criterion to distinguish adenomas and carcinomas. Mitotic figures may be frequent in malignant endocrine cells, but the significance of this criterion can vary considerably with the degree of background stimulation of the endocrine gland.

Functional aspects of endocrine tumors

Many neoplasms derived from endocrine glands are endocrinologically active (functional), secrete an excessive amount of hormone either continuously or episodically, and result in clinical syndromes of hormone excess. Many examples occur in different animal species including the hypoglycemia of beta cell neoplasms of the pancreatic islets in dogs, hyperthyroidism associated with adenomas and carcinomas derived from thyroid follicular cells in cats and dogs, hypercalcitoninism in bulls and other animal species with thyroid C-cell tumors, hyperadrenocorticism either associated with adrenocorticotropin (ACTH)-secreting pituitary corticotroph adenomas or neoplasms derived from the adrenal cortex (zona fasciculata) in dogs, among many others.

Measurement of hormone levels in serum or plasma in the basal, suppressed, or stimulated state and, in some instances, hormonal metabolites in the urine over a 24-hour period of excretion is essential to confirm that an endocrine tumor is releasing hormone at an abnormally elevated rate. An endocrine tumor also can be interpreted as being endocrinologically active if either the rim of normal tissue around the tumor or the opposite of paired endocrine glands undergoes atrophy due to negative feedback inhibition by the elevated hormone levels or an altered blood constituent. In response to the autonomous secretion of hormone by the tumor, these non-neoplastic secretory cells become smaller than normal, especially in the cytoplasmic area, and eventually the number of cells is decreased. Functional pituitary neoplasms secreting an excess of a particularly trophic hormone (e.g., adrenocorticotropin) will be associated with hypertrophy and hyperplasia of target cells in the adrenal cortex (zonae fasciculata and reticularis).

Mechanisms of endocrine disease

Primary hyperfunction of an endocrine gland

In primary hyperfunction of an endocrine gland, cells (often neoplastic and derived from the gland) synthesize and secrete hormone at an autonomous rate in excess of the body's ability to utilize and subsequently degrade the hormone, thereby resulting in *functional disturbances of hormone excess*. These include hyperfunction of parathyroid chief cells, thyroid C (parafollicular) cells, beta cells of the pancreatic islets, secretory cells of the adrenal medulla, follicular cells of the thyroid, among many others.

The *autonomous secretion of parathyroid hormone* results in progressive and generalized demineralization of the skeleton, leading to hypercalcemia, which results in soft tissue mineralization and the development of renal calculi. The accelerated osteoclastic resorption of bone results in marked thinning and osteoclastic tunneling of cortical bone, and predisposes bone to the development of pathologic fractures.

The *autonomous hypersecretion of thyroxine and triiodothyronine* is a common endocrinopathy in older cats. This hypersecretion is associated with a spectrum of proliferative lesions of thyroid follicular cells. Although the majority of the functional thyroid lesions are adenomas derived from follicular cells, other distinctly multifocal lesions in one or both thyroids are more consistent with multinodular hyperplasia. Functional thyroid lesions in cats should be considered potentially malignant because a few are adenocarcinomas that may metastasize to regional lymph nodes. The functional disturbances of hyperactivity, weight loss despite increased appetite, hyperthermia, and tachycardia reflect long-term stimulation of multiple populations of target cells by the abnormally elevated blood levels of thyroid hormones.

Secondary hyperfunction of an endocrine gland

In secondary hyperfunction of an endocrine gland, a lesion in one organ (e.g., adenohypophysis) secretes an excess of a trophic hormone that leads to long-term stimulation and hypersecretion of hormone by a target organ. An example of this pathogenic mechanism in animals is the adrenocorticotropic hormone (ACTH)-secreting tumor derived from pituitary corticotrophs in the pars distalis and intermedia of dogs (see Fig. 3.11).

The clinical signs and lesions in the animal primarily are the result of the elevated blood cortisol levels associated with the ACTH-stimulated hypertrophy and hyperplasia of the zonae fasciculata and reticularis of the adrenal cortex. The syndrome of cortisol excess in dogs is characterized by progressive alopecia, hyperpigmentation, and muscle wasting caused by the protein catabolic effects of glucocorticoids. Some dogs, particularly Poodles, have a similar marked adrenocortical enlargement and clinical evidence of cortisol excess, but without a tumor in the pituitary gland. These dogs may have a change in their set point to the negative feedback signal (e.g., blood cortisol). This can be caused by an abnormal accumulation of certain neurotransmitter substances (e.g., serotonin) near neurosecretory neurons in the hypothalamus that secrete corticotrophic hormone-releasing factor. The end result is corticotroph hyperplasia, elevated ACTH levels in the blood and long-term stimulation of the adrenal cortex, which results in hyperplasia of the zona fasciculata and zona reticularis and the clinical syndrome of cortisol excess (see Fig. 3.10).

Primary hypofunction of an endocrine gland

In primary hypofunction of an endocrine gland, *hormone secretion is subnormal* because of extensive destruction of secretory cells by a disease process, the failure of an endocrine gland to develop properly, or the result of a specific biochemical defect in the synthetic pathway of a hormone.

- *Immune-mediated injury* causes hypofunction of several endocrine glands in animals, including the parathyroid gland, adrenal cortex, and thyroid gland. Thyroiditis caused by this mechanism is characterized by marked infiltration of lymphocytes and plasmacytes, and deposition of electron-dense immune complexes along the follicular basement membranes with progressive destruction of secretory parenchyma.
- *Failure of development* also results in primary hypofunction of an endocrine gland. An example of this mechanism in animals is the failure of oropharyngeal ectoderm to differentiate into trophic hormone-secreting cells of the adenohypophysis in dogs, resulting in pituitary dwarfism and a failure to attain somatic maturation (see Fig. 3.9). A large, multicompartmented cyst is present on the ventral aspect of the brain in the pituitary region of these dogs. The cyst compresses the normally developed neurohypophysis and results in disturbances of water metabolism.
- Another form of primary hypofunction is *failure of hormone synthesis caused by a genetically determined defect in a biosynthetic pathway or lack of a specific enzyme.* One of the best-documented examples of this condition in animals is vitamin D-dependent rickets in pigs. It is caused by a lack of 25-hydroxycholecalciferol-1-α-hydroxylase in the proximal convoluted tubules of the kidney. This enzyme is needed to synthesize the hormonal form of vitamin D. Blood calcium and phosphorus levels progressively decrease due to the subnormal ability of the pig to convert 25-hydroxycholecalciferol to the biologically active, hormonal form (1,25-dihydroxycholecalciferol) in the kidney. The lowered blood concentrations of calcium and phosphorus lead to a failure of mineralization of osteoid and persistence of cartilage in the physes, leading to severe deformities in the skeleton (see Vol. 1, Bones and joints).

Congenital dyshormonogenetic goiter in sheep, goats, and cattle is another example of primary hypofunction caused by failure of hormone synthesis. The low blood thyroxine and triiodothyronine levels and clinical evidence of severe hypothyroidism in these animals are due to an inability of follicular cells to synthesize thyroglobulin. The molecular defect has been shown to be a defective processing of the primary transcripts for thyroglobulin messenger ribonucleic acid (mRNA) and aberrant transport of mRNA from the nucleus to ribosomes. This results in subnormal amounts of thyroglobulin mRNA in follicular cells, particularly mRNA that is attached to membranes of the endoplasmic reticulum in the cytoplasm.

Thyroglobulin is one of the major components of colloid in the lumen of thyroid follicles. This high molecular weight glycoprotein is synthesized on ribosomes of the rough endoplasmic reticulum in follicular cells. Thyroglobulin is packaged into apical granules that are secreted into the follicular lumen to serve as an extracellular matrix for the stepwise iodination of the tyrosyl residues incorporated into its structure, resulting in the formation of thyroxine and triiodothyronine. These animals do not have a defect in the ability of the thyroid to concentrate ^{131}I; however, there is a greatly reduced ability to iodinate tyrosyl residues and form thyroid hormones. The subnormal blood levels of thyroxine (T_4) and triiodothyronine (T_3) are detected by the hypothalamus and adenohypophysis, resulting in an increased secretion of thyroid stimulating hormone (TSH) or thyrotropin (TTH) and intense hyperplasia of follicular cells (see Fig. 3.66B).

Secondary hypofunction of an endocrine gland

In secondary hypofunction of an endocrine gland, *a destructive lesion in one organ, such as the pituitary, interferes with the secretion of trophic hormone*. This results in hypofunction of the target endocrine glands. Large, endocrinologically inactive, pituitary neoplasms in adult dogs, cats, and other animal species may interfere with the secretion of the multiple pituitary trophic hormones and result in clinically detectable hypofunction of the adrenal cortex, follicular cells of the thyroid, and gonads (see Fig. 3.15).

Target endocrine organs respond dramatically to a lack of normal production of pituitary trophic hormones. The adrenal glands of animals with large nonfunctional pituitary adenomas are small and often difficult to find at necropsy. The adrenals consist primarily of medullary tissue surrounded by a narrow zone of atrophic cortex. The adrenal cortex appears as a thin yellow-brown rim composed of a moderately thickened capsule and secretory cells of the outer (aldosterone-producing) layer, zona multiformis (glomerulosa), which is not under ACTH control. The zonae fasciculata and reticularis are severely atrophied compared with those in normal adrenal glands, secrete subnormal amounts of glucocorticoid hormones, and have a blunted increase in cortisol or corticosterone following the administration of exogenous ACTH. By comparison, thyroid glands in animals with large pituitary adenomas disrupting normal trophic hormone (e.g., TSH) production either are near normal size or only slightly reduced in size to a much lesser degree than is the adrenal cortex. The majority of thyroid follicles are large, lined by flattened (atrophic) cuboidal follicular cells, and distended with densely stained colloid with little evidence of endocytotic activity because of a lack of TSH. Calcitonin-secreting thyroid C cells function normally since they are not controlled by pituitary trophic hormones. Seminiferous tubules in the testis are small with little evidence of active spermatogenesis and the ovarian cortex is devoid of functional graafian follicles.

Secondary hypofunction of an endocrine gland also occurs when *insufficient iodide ion* is available for thyroid follicular cells to synthesize adequate amounts of thyroid hormones to meet the animal's daily requirements. The thyroid gland attempts to compensate by increasing the efficiency of iodide trapping and by preferentially synthesizing more T_3 than T_4, thereby saving a molecule of iodide for every molecule of thyroid hormone produced. *Dietary iodine deficiency resulting in diffuse hyperplastic goiter* (see Fig. 3.61) was common in many areas of the world before the widespread addition of iodized salt to animal diets and still occurs worldwide in domestic animals and human beings living in iodine-deficient areas. Marginally iodine-deficient diets that contain goitrogenic compounds may cause severe thyroid follicular cell hyperplasia and goiter. Goitrogenic substances include thiouracil, sulfonamides, anions of the Hofmeister series, and a number of plants of the family Brassicaceae. Offspring of females fed iodine-deficient diets are likely to develop severe thyroidal follicular cell hyperplasia and have clinical signs of hypothyroidism. The affected lobes are firm and dark red because an extensive interfollicular capillary network develops under the influence of long-term TSH stimulation. Follicles are irregular in size and shape in hyperplastic goiter because they contain various amounts of lightly eosinophilic and vacuolated colloid. Some follicles collapse because of the lack of colloid. Their lining epithelial cells are columnar and have deeply eosinophilic cytoplasm and small hyperchromatic nuclei that often are situated in the basilar portion of the cell. The follicles are lined by single or multiple layers of hyperplastic follicular cells that form papillary projections into the lumens of some follicles.

Endocrine hyperactivity secondary to diseases of other organs

A common example of endocrine hyperactivity secondary to diseases of other organs in animals is the **hyperparathyroidism** *that develops secondary to chronic renal failure or nutritional imbalances.* In the **renal form**, the retention of phosphorus early and subsequent progressive destruction of cells in the proximal convoluted tubules interferes with the metabolic activation of vitamin D_3 by the 1-α-hydroxylase in the kidney. This is the rate-limiting step in the metabolic activation of vitamin D_3 and is tightly controlled by parathyroid hormone (PTH) and several other factors. The impaired intestinal absorption of calcium results in the development of progressive hypocalcemia. Parathyroid stimulation in animals with chronic renal disease can be directly attributed to the hypocalcemia that develops. As the phosphorus concentration increases, blood calcium decreases reciprocally due largely to the suppressive effects of the hyperphosphatemia on the renal 1-α-hydroxylase resulting in an interference in production of the active form of vitamin D_3 ($1,25\text{-}(OH)_2D_3$), thereby reducing intestinal absorption of calcium. All 4 parathyroid glands are enlarged due initially to organellar hypertrophy and later to chief cell hyperplasia that results in an increased PTH synthesis and secretion in response to the hypocalcemia. Although skeletal involvement is generalized with hyperparathyroidism, it does not affect all parts uniformly. Bone lesions include resorption of alveolar socket bone and loss of *lamina dura dentes* that occur early in the course of the disease. This results in loose teeth that may be dislodged easily and interfere with mastication. Due to the accelerated rate of resorption, cancellous bones of the maxilla and mandible become weakened, are readily pliable ("rubber jaw"), and the jaws fail to close properly.

Nutritional hyperparathyroidism develops in animals fed abnormal diets that are low in calcium, high in phosphorus or oxalate, or deficient in cholecalciferol for certain non-human primates. Unsupplemented all-meat diets fed to carnivores fail to supply the daily requirements for calcium, leading to progressive hypocalcemia that stimulates the parathyroid gland to increased activity. The normal kidneys in these animals respond to the increased parathyroid hormone secretion by increasing phosphorus excretion and lowering blood phosphorus levels. After a carnivore is fed this imbalanced diet for several weeks to months, its skeleton becomes severely demineralized and predisposed to fractures. The cortices of long bones are thin and the medullary cavity widened due to intense osteoclastic resorption of bone that is stimulated indirectly by the increased secretion of parathyroid hormone (see Vol. 1, Bones and joints).

Hyperfunction of an endocrine organ also can be the result of hormonal imbalances induced by **xenobiotics**. For example, hyperactivity of the pituitary gland in rodents during chronic toxicity testing often results in increased development of tumors in the gonads or mammary glands. Excess production of luteinizing hormone (LH), usually due to disruption of negative feedback control by estrogen or testosterone, increases the incidence of tubulostromal adenomas and granulosa cell tumors in the ovary of mice, and Leydig (interstitial) adenomas of the testes in rats. The tubulostromal adenomas are the most important of the ovarian tumors in mice and they are the tumors whose incidence often is increased by various endocrine perturbations associated with chronic exposure to xenobiotics, senescence or inherited genic deletion. Tubulostromal adenomas are unique lesions that develop frequently in the mouse ovary, accounting for approximately 25% of naturally occurring ovarian tumors in this species. They are uncommon in rats, rare in other animal species, and are not recognized in the ovaries of women.

Factors that destroy or greatly diminish the numbers of ovarian follicles, such as senescence, genetic deletion of follicles, X-radiation, drugs and xenobiotics, and early thymectomy with the development of autoantibodies to oocytes all diminish sex steroid hormone secretion by the ovary. This results in elevated circulating levels of gonadotrophins, especially LH, due to decreased negative feedback on the hypothalamic–pituitary axis by estrogens and possibly other humoral factors produced by graafian follicles. The long-term stimulation of stromal (interstitial) cells, which have receptors for LH, and, indirectly, the ovarian surface epithelium appears to place the mouse ovary at increased risk for developing the unique tubular or tubulostromal adenomas.

Leydig (interstitial) cells of the testis frequently undergo proliferative changes with advancing age and following chronic exposure to large doses of xenobiotics that result in hormonal imbalances in rodents. Pathogenic mechanisms reported to be important in the development of proliferative lesions of Leydig cells include hormonal imbalances, irradiation, species and strain differences, and exposure to certain chemicals. Hormonal imbalances are important factors in the development of focal proliferative lesions of Leydig cells, including increased estrogenic steroids in mice and hamsters and elevated pituitary gonadotrophins in rats resulting from the chronic administration of xenobiotics. Many xenobiotics when administered chronically to rats disrupt the hypothalamic–pituitary–testis axis at one of several possible sites (e.g., androgen receptor antagonists, 5-α-reductase inhibitors, testosterone biosynthesis inhibitors, GnRH agonists, and aromatase inhibitors) interfering with negative feedback control, resulting in hyperfunctioning of the pituitary gland

and over-production of LH that leads to the proliferative changes (hyperplasia, adenoma) in Leydig cells.

Hypersecretion of hormones by nonendocrine tumors

Certain neoplasms of nonendocrine tissues in both animals and humans either secrete hormones or humoral substances that share chemical and/or biologic characteristics with the "native" hormones secreted by an endocrine gland. *Most of the recently discovered humoral substances secreted by nonendocrine tumors are peptides* rather than steroids, iodothyronines or catecholamines, which require more complex biosynthetic pathways. For example, **humoral hypercalcemia of malignancy (HHM, "pseudohyperparathyroidism")** is a clinical syndrome produced primarily by the autonomous hypersecretion of a new hormone, **parathyroid hormone-related peptide (PTH-rP)**, by cancer cells. PTH-rP is able to interact with the parathyroid hormone receptor in target cells (e.g., bone and kidney) and result in persistent, often life-threatening, hypercalcemia.

Humoral hypercalcemia of malignancy is associated with diverse malignant neoplasms in animal and human patients. Characteristic findings in patients with HHM include hypercalcemia, hypophosphatemia, hypercalciuria (often with decreased fractional calcium excretion), increased fractional excretion of phosphorus, increased nephrogenous cAMP, and increased osteoclastic bone resorption. *The direct effects of PTH-rP on bone and kidney, and indirect effects on the intestine produce hypercalcemia.* Increased osteoclastic bone resorption is a consistent finding in HHM with increased calcium release from bone. The kidney plays a critical role in the pathogenesis of hypercalcemia and hypophosphatemia, since renal calcium reabsorption is stimulated by PTH-rP and phosphorus reabsorption is inhibited due to binding to and activation of the renal PTH/PTH-rP receptors. PTH-rP binds to the N-terminal PTH/PTH-rP receptor in bone and kidney, but does not cross-react immunologically with native PTH. PTH-rP stimulates adenylate cyclase and increases intracellular calcium ion in bone and kidney cells by binding to and activating the cell membrane PTH/PTH-rP receptors. This results in stimulation of osteoclastic bone resorption, increased renal tubular calcium reabsorption, and decreased renal tubular phosphate reabsorption. In some forms of HHM, there are increased serum 1,25-dihydroxyvitamin D_3 levels that may increase calcium absorption from the intestine. Although excessive secretion of biologically active PTH-rP plays a central role in the pathogenesis of hypercalcemia in most forms of HHM cytokines such as interleukin-1, tumor necrosis factor-α, transforming growth factors, and 1,25-dihydroxyvitamin D_3 may have synergistic or cooperative actions with PTH-rP.

A well-characterized example of this disease mechanism in animals is the *adenocarcinoma derived from the apocrine glands of the anal sac in dogs.* Serum PTH levels are lower in dogs with apocrine carcinomas and hypercalcemia than in controls and PTH levels are undetectable in tumor tissue. The parathyroid glands are small and difficult to locate or not visible macroscopically in dogs with persistent cancer-associated hypercalcemia. Atrophic parathyroid glands are characterized by narrow cords of inactive chief cells with abundant fibrous connective tissue stroma and widened perivascular spaces. The inactive chief cells have markedly reduced cytoplasmic area, prominent hyperchromatic nuclei, relatively straight cell membranes with uncomplicated interdigitations, and are closely packed together. These morphologic findings indicate that the malignant neoplasms are not producing a substance that stimulates parathyroid hormone secretion by chief cells, but rather that the parathyroid glands are responding to persistent hypercalcemia by undergoing trophic atrophy. Thyroid parafollicular cells (C cells) often respond to the persistent elevation in blood calcium by undergoing diffuse or nodular hyperplasia.

Endocrine dysfunction due to failure of target cell response

Endocrine dysfunction due to a failure of target cell response has been recognized coincident with a more complete understanding of how hormones convey their biologic message. Steroid and iodothyronine hormones penetrate the cell membrane and are transported to the nucleus where they interact with the genetic information in the cell to increase new protein synthesis. Polypeptide and catecholamine hormones bind to receptors on the surface of target cells and activate a membrane-bound enzyme that generates one of several possible intracellular messengers (e.g., cAMP) which elicits the physiologic response. *Failure of target cells to respond to hormone may be due to a lack of adenylate cyclase in the cell membrane or to an alteration in hormone receptors on the cell surface.* Hormone is secreted in normal or increased amounts by cells of the endocrine gland.

Insulin resistance associated with obesity in both animals and humans can result from a decrease or "downregulation" of receptors on the surface of target cells. This develops in response to the chronic increased insulin secretion stimulated by the hyperglycemia that results from excessive food intake. Large pituitary neoplasms that extend dorsally out of the sella turcica destroy critical hypothalamic nuclei that regulate the intake of food. These animals often develop insulin-resistant hyperglycemia and glycosuria as a result of a downregulation of insulin receptors on target cells induced by the chronic excessive intake of food and hyperinsulinemia (Fig. 3.2). Secretory cells in the corresponding endocrine gland (i.e., pancreatic islets) undergo

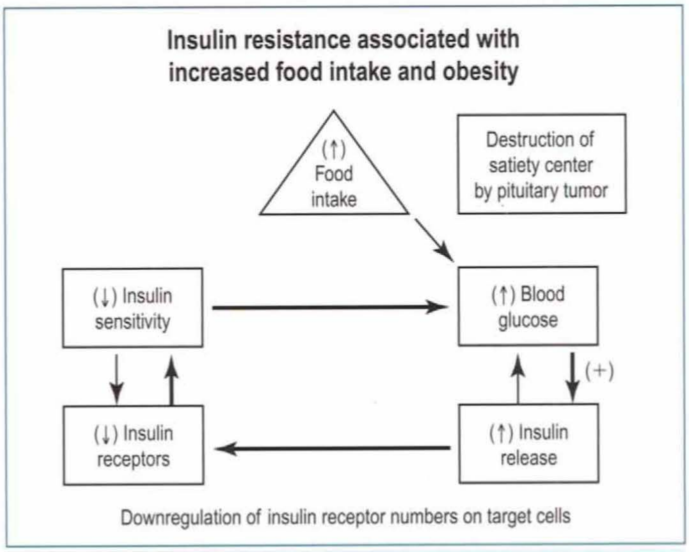
Insulin resistance associated with increased food intake and obesity
(↑) Food intake
Destruction of satiety center by pituitary tumor
(↓) Insulin sensitivity
(↑) Blood glucose
(+)
(↓) Insulin receptors
(↑) Insulin release
Downregulation of insulin receptor numbers on target cells

Figure 3.2 Downregulation of insulin receptors on target cells in response to chronic increased food intake resulting in decreased insulin sensitivity (resistance) and hyperglycemia. (From Capen CC. Toxicol Pathol 2001;29:8–33.)

compensatory hypertrophy and hyperplasia in an attempt to secrete additional hormone.

An interesting form of hypoparathyroidism has been reported in human patients in which the inability of target cells to respond is due to a defect in the cAMP-mediated signal transduction resulting from a lack of specific nucleotide regulatory protein in the cell membrane. Patients with "pseudohypoparathyroidism" develop hypocalcemia and hyperphosphatemia in spite of hyperplastic parathyroids and elevated blood levels of PTH. Similarly, animals and humans with nephrogenic diabetes have defects in the vasopressin receptor on cells of the distal nephron and collecting ducts in the kidney resulting in a lack of adenylate cyclase stimulation and generation of cyclic AMP after vasopressin binding to its receptor on target cells.

Failure of fetal endocrine function

Subnormal function of the fetal endocrine system, especially in ruminants, may disrupt normal fetal development and result in prolongation of the gestation period. In Guernsey and Jersey cattle, a genetically determined failure of development (aplasia) of the adenohypophysis results in a lack of fetal pituitary trophic hormone secretion during the last trimester and hypoplastic development of target endocrine organs (e.g., adrenal cortex, thyroid follicular cells, and gonads). Fetal development is normal up to approximately seven months gestation but subsequently fetal growth ceases irrespective of how long the viable fetus is retained in utero.

Prolongation of gestation also occurs in ewes that ingest the plant *Veratrum californicum* early in gestation. Toxins in the plant cause extensive malformations of the central nervous system (CNS) and hypothalamus in lambs. Although the adenohypophysis is present, it is unable to secrete normal amounts of trophic hormones (e.g., adrenocorticotropin (ACTH)) because it lacks the necessary fine control derived from the releasing hormones of the hypothalamus. Target endocrine organs in the fetus are hypoplastic and the adrenal cortex does not differentiate completely into the three distinctive zones that secrete corticosteroid hormones. The plant contains potent steroidal alkaloids that inhibit neural tube development when ingested by the ewe between days 9 and 14 of gestation. Cyclopia and extensive CNS malformations are found in lambs. Arrhinencephaly and lack of development of nasal bones accompany the formation of a proboscis-like structure. The lambs retained in the uterus continue to grow beyond the normal gestation period.

The concepts that have emerged from the study of these naturally occurring diseases are: *first*, fetal hormones are necessary for final growth and development in utero in certain animals; and *second*, normal parturition at term requires an intact fetal hypothalamic–adenohypophyseal–adrenal cortical axis in these species working in concert with trophoblasts of the placenta. Although the presence or absence of functional adenohypophyseal tissue determines whether the fetus continues to grow in utero, the pathogenesis of prolongation of the gestational interval is similar in these two examples. The subnormal development of the fetal adrenal cortex results in inadequate secretion of cortisol and failure to induce the 17-α-hydroxylase in the placenta that converts precursor molecules (e.g., progesterone) to estrogen. The fetal adrenal gland is small and there is incomplete development of the three distinct zones of the cortex (Fig. 3.3). The dam's circulating progesterone is maintained near mid-gestational concentrations, and there is a lack of the marked increase in estrogens that normally occurs near term and results in parturition by stimulating the local synthesis of prostaglandins in the uterus. This normally results in the smooth muscle contractions and biochemical changes in collagen along the birth canal that permit delivery of the fetus.

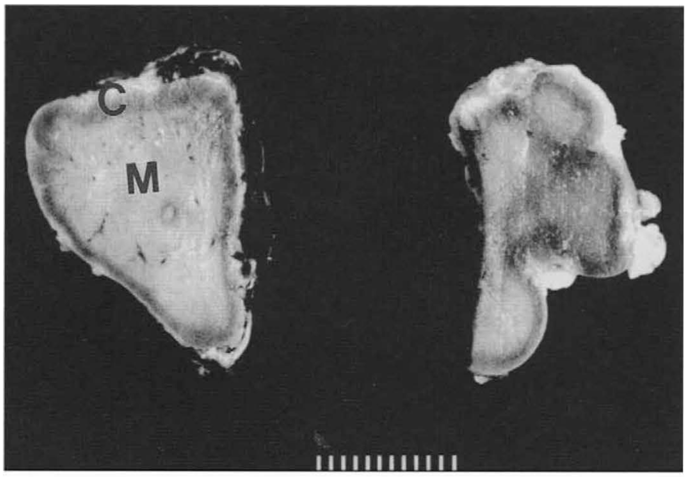

Figure 3.3 Failure of fetal endocrine function. **Adrenal cortical hypoplasia** in a calf with prolongation of the gestation period. The adrenal cortex (C) is markedly reduced in thickness and the adrenal medulla (M) is relatively more prominent. Scale in mm. (From Capen CC. Toxicol Pathol 2001;29:8–33.)

Endocrine dysfunction resulting from abnormal degradation of hormone

Increased degradation of hormone. The long-term administration of *various xenobiotics* (e.g., phenobarbital and others) results in the induction of liver enzymes (e.g., T_4–uridine diphosphate (UDP)-glucuronyl transferase) that increases the degradation of thyroxine, especially in laboratory rodents. Endocrine gland and target cell function are normal. The chronic disruption of the thyroid–pituitary axis and augmented TSH secretion in rodents, especially male rats, often increases the development of thyroid follicular cell tumors in chronic toxicity and oncogenicity studies with certain drugs and chemicals. Hepatic microsomal enzymes play an important role in thyroid hormone economy because glucuronidation is the rate-limiting step in the biliary excretion of T_4 and sulfation, primarily by phenol sulfotransferase for the excretion of T_3. Long-term exposure of rats to a wide variety of different chemicals induces these enzyme pathways and results in chronic stimulation of the thyroid by disrupting the hypothalamic–pituitary–thyroid axis. The resulting chronic stimulation of the thyroid by increased circulating levels of TSH in rats often results in a greater risk of developing tumors derived from follicular cells in chronic toxicity/carcinogenicity studies with these compounds (Fig. 3.4). The chronic administration of phenylbutazone, a nonhormonal antipyretic compound used in the medical management of inflammatory conditions of the musculoskeletal system, especially in horses, which is a hepatic microsomal enzyme inducer, often results in moderate thyroid enlargement by a similar mechanism described above.

Decreased degradation of hormone. The rate of secretion of hormone by an endocrine gland may be normal with this mechanism,

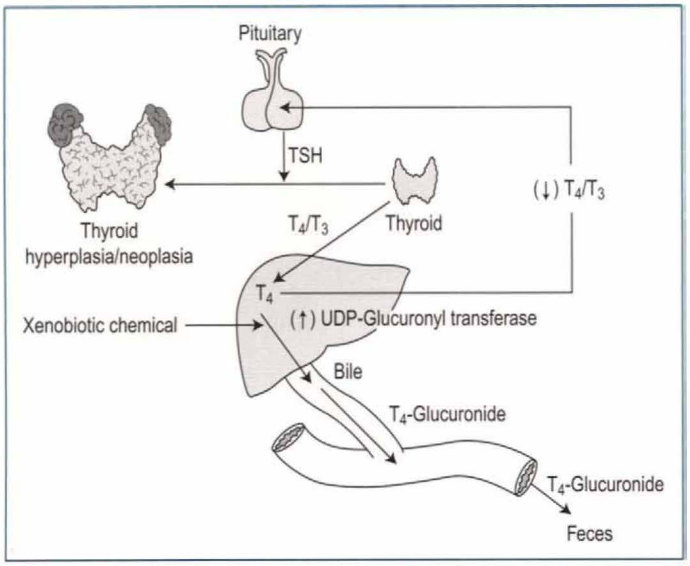

Figure 3.4 Hepatic microsomal enzyme induction by the chronic administration of xenobiotics in rodents leading to thyroid follicular cell hyperplasia and neoplasia in chronic studies with large doses of compound. (From Capen CC. Toxicol Pathol 2001;29:8–33.)

but blood hormone levels are persistently elevated, thereby simulating a syndrome of hypersecretion, due to a decreased rate of hormone degradation. The classic example of this pathogenic mechanism is the syndrome of feminization due to hyperestrogenism associated with cirrhosis and decreased hepatic degradation of estrogens. Chronic renal disease in dogs occasionally is associated with persistent hypercalcemia due, in part, to decreased degradation of PTH (along with decreased urinary excretion of calcium) by the diseased kidney.

Syndromes of iatrogenic hormone excess

Direct effects. The administration of hormone, either directly or indirectly, influences the activity of target cells with this mechanism and results in important functional disturbances. It is well recognized that the administration of potent preparations of adrenal cortical steroids at inappropriately high daily doses for prolonged intervals in the symptomatic treatment of various diseases can produce most of the functional disturbances associated with endogenous hypersecretion of cortisol. Similarly, the administration of excessively large doses of insulin can result in hypoglycemia, and an excess of exogenous T_4 or T_3 may result in hyperthyroidism, especially in certain species (e.g., cats) that have limited capacity to conjugate T_4 with glucuronic acid and enhance biliary excretion.

Indirect effects. *The administration of progestins to dogs indirectly results in a syndrome of growth hormone excess.* The injection of medroxyprogesterone acetate for the prevention of estrus in dogs appears to stimulate expression of the growth hormone gene in the mammary gland leading to an elevation in circulating growth hormone levels, and results in the classic clinical manifestations and lesions of acromegaly (e.g., excessive skin folds, respiratory stridor due to increased soft tissue in oropharyngeal region, expansion of interdental spaces, and hyperglycemia) (see Fig. 3.20).

It was assumed initially that the reversibly elevated circulating levels of growth hormone (GH) in response to the administration of synthetic progestins in dogs were from direct stimulation of somatotrophs in the pituitary gland; however, somatotroph hyperplasia or adenomas have not been found in the adenohypophysis of progestin-treated dogs. The elevated GH levels were associated with progressively increasing circulating concentrations of insulin-like growth factor (IGF) and insulin. The progestin-induced excessive GH secretion in dogs was characterized by disappearance of the pulsatile secretion pattern and insensitivity to stimulation of GH secretion by both growth hormone-releasing hormone and clonidine, as well as inhibition of GH release with a somatostatin analog. The stimulation of growth hormone release in dogs by progestins differs from the situation in humans in whom high prolactin levels result from treatment with these drugs.

Transphenoidal hypophysectomy 4–6 weeks after the last progestin injection in dogs with markedly elevated plasma GH concentrations did not change the circulating levels of GH. Subsequent removal of all mammary tissue in the progestin-treated dogs resulted in a rapid decline of plasma GH concentrations to values in the reference range. Measurement of GH immunoreactivity in tissue homogenates from progestin-administered dogs revealed the highest GH content was present in normal and neoplastic mammary tissue. Immunohistochemical evaluation further confirmed the mammary gland as the source of the excessive GH production. In the dogs treated with progestins, GH immunoreactivity was present in the cytoplasm of both normal active ductal epithelial cells and benign neoplasms derived from mammary ductal epithelium. There was no GH immunoreactivity in the stroma or in mammary tissue from control dogs. Interestingly, progestins therapy has been used successfully to treat dogs with juvenile-onset panhypopituitarism and dwarfism due to failure of development of the adenohypophysis and severe GH deficiency.

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PITUITARY GLAND

Although the pituitary gland is derived from the oropharyngeal ectoderm, it is completely separated from the oral cavity in adult animals. It is situated in the sella turcica, a concavity of the sphenoid bone, and enveloped by an extension of dura mater. The pituitary gland (hypophysis) is subdivided anatomically into the **adenohypophysis** and **neurohypophysis**.

Development, structure, and function

Embryologic development of the pituitary gland

The pituitary gland develops embryologically from a dorsal evagination of oropharyngeal ectoderm (Rathke's pouch) (Fig. 3.5) and a ventral downgrowth of diencephalic neuroectoderm, as shown schematically in the diagram. The point of fusion of the two primordia develops into the pars intermedia. The pars distalis undergoes extensive proliferation to form the major part of the adenohypophysis and is responsible for the secretion of the multiple trophic hormones.

The pituitary gland has two preformed cavities, the residual lumen of Rathke's pouch (cleft) and the infundibular recess of the third ventricle (central cavity). Separation of the developing adenohypophysis from the oropharynx is completed by formation of the sphenoid bone.

Structure of the adenohypophysis

The adenohypophysis consists of three portions, the *pars distalis, pars tuberalis, and pars intermedia*. In many species, the adenohypophysis

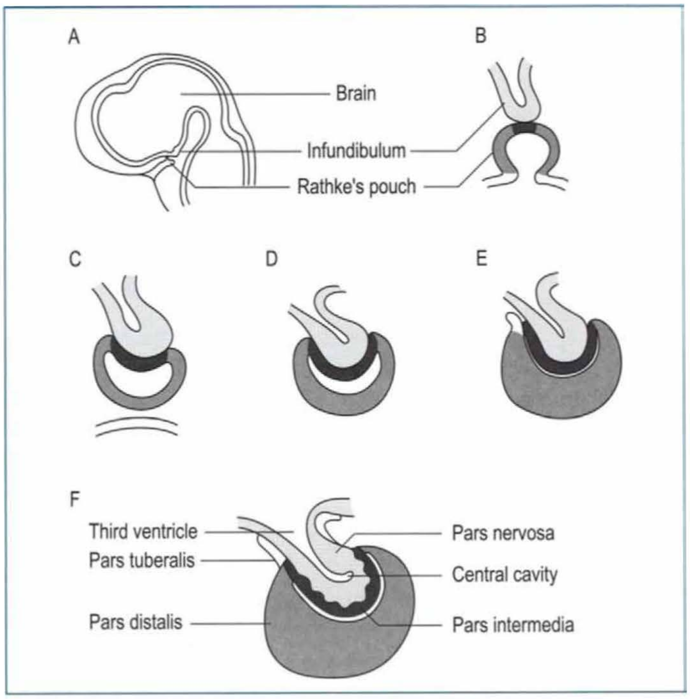

Figure 3.5 Embryologic development of the **pituitary gland**. (From Turner CD, Bagnara JT. In: General Endocrinology, 1971.)

completely surrounds the **pars nervosa** of the neurohypophyseal system.

- The **pars distalis** is the largest of the three parts of the adenohypophysis and contains the multiple populations of endocrine cells that secrete the different pituitary trophic hormones. The secretory cells are supplied with abundant capillaries that have fenestrae in their peripheral cytoplasmic extensions and are supported by the cytoplasmic processes of stellate (follicular or sustentacular) cells.
- The **pars tuberalis** consists of dorsal projections of cells along the infundibular stalk. It functions primarily as a scaffold for the capillary network of the hypophyseal portal system during its course from the median eminence to the pars distalis.
- The **pars intermedia** forms the junction between the pars distalis and pars nervosa. It lines the residual lumen of Rathke's pouch and contains two populations of cells. In the dog, one of these cell types synthesizes and secretes adrenocorticotropic hormone (ACTH), similar to corticotrophs in the pars distalis.

Functional cytology of the adenohypophysis

A specific population of endocrine cells is present in the pars distalis (and in the pars intermedia of dogs for ACTH) that synthesize and secrete each of the pituitary trophic hormones. Secretory cells in the adenohypophysis are subdivided into *acidophils, basophils, and chromophobes* based on the staining of their secretory granules with pH-dependent histochemical stains.

- **Acidophils** are further subdivided functionally into *somatotrophs* that secrete growth hormone (GH, somatotropin) and and *luteotrophs* that secrete uteotrophic hormone (LTH, prolactin). Their granules contain hormones that are simple proteins that stain with orange G, azocarmine or erythrosin.
- **Basophils** include both *gonadotrophs* that secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH), and *thyrotrophs* that secrete thyrotrophic hormone (TSH). Secretory granules of basophils contain glycoproteins that react with the periodic acid-Schiff reagent.
- **Chromophobes** are pituitary cells that do not have obvious cytoplasmic secretory granules on light microscopic evaluation. They include the pituitary cells concerned with the synthesis of ACTH and melanocyte-stimulating hormone (MSH), nonsecretory follicular (stellate) cells, degranulated chromophils (acidophils and basophils) in the actively synthesizing phase of the secretory cycle, and undifferentiated stem embryonic cells of the adenohypophysis.

Specific immunocytochemical staining of the adenohypophysis in dogs has demonstrated that ACTH- and MSH-staining cells are polyhedral to round, sparsely granulated, and most numerous in the ventrocentral and rostral portions of the pars distalis. They are less frequent in the dorsal and caudal regions of the pars distalis and throughout the pars tuberalis. In the pars intermedia of dogs, most cells demonstrate immunoreactivity to either ACTH, or α-MSH.

Thyrotrophs are large polyhedral cells situated singly or in small groups ventrocentrally in the paramedian plane in the pars distalis of dogs. Gonadotrophs (cells reacting with antisera to human FSH and/or bovine LH) are oval to polyhedral and distributed singly in the pars distalis, particularly in the dorsocranial region and in the caudal extensions along the pars intermedia.

Immunoreactive prolactin cells occur in small groups of large polygonal cells with prominent granules in the ventrocentral and cranial portions of the canine pars distalis. A diffuse increase in this population of cells occurs in females near parturition. Growth hormone-secreting cells are present singly along capillaries in the dorsal region of the pars distalis near the pars intermedia. They are small, round to oval, and have numerous cytoplasmic granules. Somatotrophs frequently undergo diffuse hyperplasia and hypertrophy in old dogs, especially females with mammary dysplasia or neoplasia.

Hypothalamic control of the adenohypophysis

Each population of endocrine cells in the pars distalis is under the control of a corresponding releasing hormone from the hypothalamus (Fig. 3.6). These **releasing hormones** are small peptides synthesized via

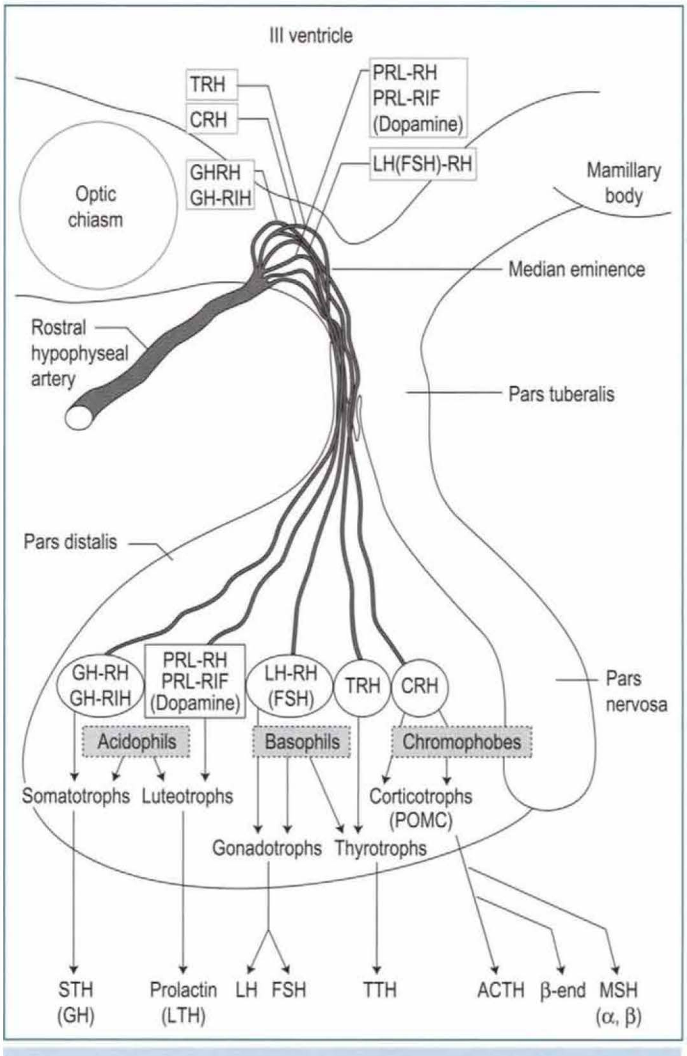

Figure 3.6 Separate **hypothalamic releasing hormones or factors** control the secretion of each trophic hormone by the adenohypophysis. Trophic hormones that do not act on a target endocrine organ to release a final endocrine product (e.g., somatotrophin and prolactin) also are regulated by a hypothalamic release inhibiting hormone (RIH) or factor (e.g., dopamine for prolactin).

neurosecretion by neurons in the hypothalamus. Axonal processes transport them to the median eminence where they are released into capillaries and conveyed by the hypophyseal portal system to specific endocrine cells in the adenohypophysis, where each stimulates the rapid release of secretory granules containing a specific preformed trophic hormone.

There are separate hypothalamic releasing hormones that regulate the rate of secretion of each trophic hormone secreted by the adenohypophysis. *For most pituitary trophic hormones,* **negative feedback control** *is accomplished by the blood concentration of the hormone produced by the target endocrine gland,* e.g., thyroid gland, adrenal cortex, ovary, or testis. The hormone produced by the endocrine glands exerts negative feedback control either on the neurosecretory neurons in the hypothalamus that synthesize the corresponding releasing hormone or on trophic hormone-secreting cells in the adenohypophysis or at both sites. However, GH, LTH, and MSH do not act on target endocrine organs to stimulate secretion of a hormone. *Negative feedback control of these three pituitary hormones is effected by production of a corresponding* **release-inhibiting hormone** *("factor") by neurons in the hypothalamus.* The relative local concentrations of the specific releasing hormone and release-inhibiting hormone appear to govern the rate of release of GH, LTH and MSH from the adenohypophysis. Growth hormone-release inhibiting hormone ("*somatostatin*"), which controls the release of somatotropin, also is produced by endocrine cells in the pancreatic islets. Dopamine is the principal release-inhibiting hormone for prolactin.

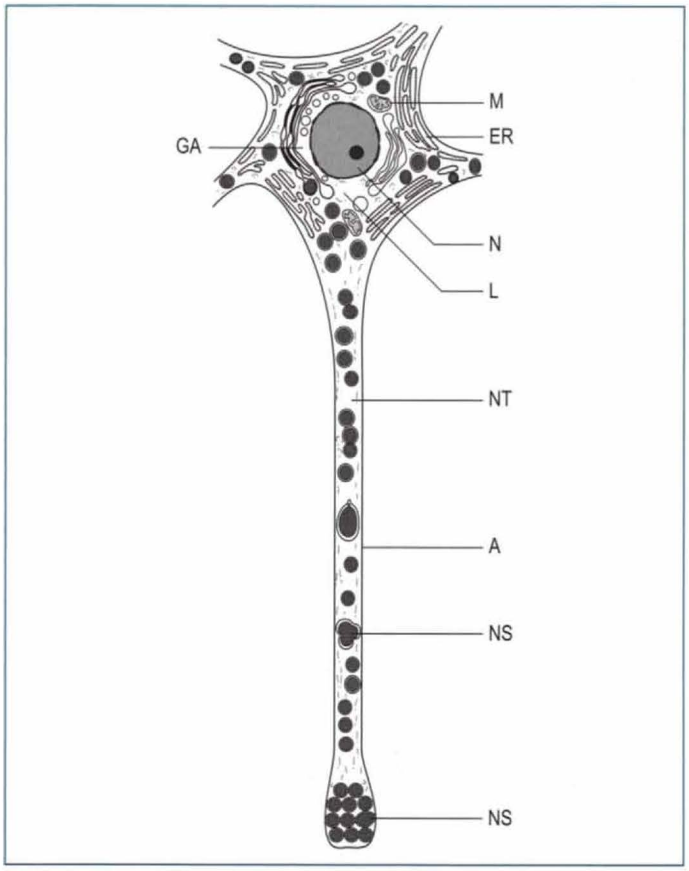

Figure 3.7 Structural characteristics of a **neurosecretory neuron**. Nerve cell body (N, nucleus) has dendritic and axonal (A) processes, arrays of rough endoplasmic reticulum (ER), a prominent Golgi apparatus (GA), and large mitochondria (M). Hormone-containing, membrane-limited neurosecretory granules (NS) are transported along axon to site of release in the pars nervosa. NT = neural tubules, L = lysosomes.

Structure and function of the neurohypophysis

The **neurohypophysis** has three anatomic subdivisions. The **pars nervosa** represents the distal component of the neurohypophyseal system. It is composed of numerous capillaries that are supported by modified glial cells (*pituicytes*). The capillaries in the pars nervosa are termination sites for the nonmyelinated axonal processes of neurosecretory neurons in the hypothalamus. Secretion granules that contain the neurohypophyseal hormones, *oxytocin* and *antidiuretic hormone*, are synthesized in **hypothalamic neurons** but are released into the bloodstream in the pars nervosa. The **infundibular stalk** joins the pars nervosa to the overlying hypothalamus and is composed of nonmyelinated axonal processes from neurosecretory neurons.

Neurosecretory neurons in the hypothalamus receive neural input from higher centers and translate this into endocrine output in the form of hormonal secretion. In addition to the usual structural features of neurons, they contain prominent lamellar arrays of rough endoplasmic reticulum, large Golgi apparatuses, and numerous membrane-limited neurosecretory granules in the cell body and axonal process (Fig. 3.7).

The neurosecretory neurons concerned with hormone synthesis are segregated into anatomically defined regions, termed *nuclei*, in the hypothalamus. The **supraoptic nucleus** is concerned primarily with the synthesis of antidiuretic hormone, whereas oxytocin is produced predominantly by neurons in the **paraventricular nucleus**.

Antidiuretic hormone and oxytocin are nonapeptides synthesized by neurons situated in either the supraoptic or the paraventricular nucleus. Antidiuretic hormone and its corresponding neurophysin appear to be synthesized as part of a common larger biosynthetic precursor molecule, termed propressophysin. The hormones are packaged into membrane-limited neurosecretory granules with a corresponding transport protein (*neurophysin*) and transported to the pars nervosa by axonal processes of the neurosecretory neurons. These axons terminate on fenestrated capillaries in the pars nervosa and release ADH or oxytocin into the circulation.

Antidiuretic hormone is transported by the bloodstream to its site of action in the kidney, where it binds to specific receptors on epithelial cells in the distal part of the nephron and collecting ducts. The overall effect of ADH on the kidney is to increase the active renal tubular reabsorption of water from the glomerular filtrate. The hormone (ADH)–receptor complex activates the membrane-bound enzyme, adenylate cyclase, resulting in the intracellular formation of cyclic adenosine monophosphate (cAMP) from ATP. The intracellular accumulation of cAMP activates protein kinases involved in the phosphorylation of proteins in the luminal membrane of distal tubular cells that increase their permeability to water.

Blood supply to the pituitary gland

The neurohypophysis in most animals is supplied directly by the caudal hypophyseal arteries that branch from the internal carotid arteries. Branches of the rostral hypophyseal arteries originate from the internal carotid arteries and form the caudal communicating arteries of the circle of Willis. Arteriolar branches penetrate the pars tuberalis, lose their muscular coat, and form a capillary plexus near the median eminence. These vessels subsequently drain into **hypophyseal portal veins** that supply the pars distalis and transport the hypothalamic releasing hormones to the pituitary. A small artery that arises from the caudal hypophyseal artery may provide a minor blood supply to the adenohypophysis.

Diseases of the pituitary gland

Hypophyseal changes associated with alterations in target organs

In response to surgical removal or disease processes in a target endocrine organ of the pituitary, there is a progressive decline in the circulating concentration of hormone produced by the target organ. This reduction in circulating hormone level is detected by the hypothalamic–adenohypophyseal system and results in structural changes in the specific trophic hormone-secreting cell population in the pars distalis.

The initial reaction is rapid release of storage granules containing preformed trophic hormone from one population of endocrine cells in the pars distalis. For example, only thyrotrophic basophils degranulate following thyroidectomy or thyroid disease, corticotrophic chromophobes degranulate after adrenalectomy, and gonadotrophic basophils release their secretory granules in response to gonadectomy. After an interval of several days following surgical ablation or extensive destruction of a target endocrine organ, the corresponding degranulated trophic hormone-secreting cell in the pars distalis undergoes hypertrophy with expansion of the cytoplasmic area in response to the sustained increased demand for the particular trophic hormone. The abundant cytoplasm contains extensive organelles concerned with hormonal synthesis (rough endoplasmic reticulum) and packaging into secretory granules (Golgi apparatus) plus large mitochondria.

If the demand for trophic hormone secretion is sustained for days or weeks, one specific population of endocrine cells in the pars distalis undergoes hyperplasia. Groups of hyperplastic trophic hormone-secreting cells are present, scattered as small nests throughout an otherwise normal pars distalis. In response to long-term (weeks to months) stimulation, the hypertrophied cytoplasm of the cells becomes vacuolated due to the extensive distention of profiles of rough endoplasmic reticulum with finely granular, moderately electron-dense material. The multiple small cytoplasmic vacuoles coalesce subsequently to form a large vacuole that may displace the nucleus eccentrically, characteristic of cells in the pituitary gland that degranulate following thyroidectomy, adrenalectomy, or gonadectomy.

Pituitary cysts

Cysts derived from distal ("sellar") end of the craniopharyngeal duct

Cysts may develop from remnants of the distal craniopharyngeal duct, which normally disappears by birth in most animal species. The cysts are lined by cuboidal to columnar, often ciliated, epithelium and contain mucin. In dogs, especially of the brachycephalic breeds, *cysts from these remnants are often found at the periphery of the pars tuberalis and pars distalis*. Cystic remnants of the craniopharyngeal duct in one survey were found in 53% of dogs of several breeds.

Craniopharyngeal duct cysts occasionally become large enough to exert pressure on the infundibular stalk and hypophyseal portal system, median eminence, or pars distalis. Structures adjacent to the cysts atrophy to varying degrees owing to compression and interference with the blood supply. Disruption of a large cyst with escape of the proteinaceous contents into adjacent tissues may incite intense, local inflammation with subsequent fibrosis that may interfere with pituitary function. Clinical signs can include visual difficulties due to pressure on the optic chiasm, diabetes insipidus, obesity, and hypofunction of the adenohypophysis leading to gonadal atrophy, decreased basal metabolic rate, and hypoglycemia.

Cysts derived from proximal end of craniopharyngeal duct (pharyngeal hypophysis)

The proximal portion of the adenohypophyseal anlage may persist in the dorsal aspect of the oral cavity in adults as undifferentiated remnants of cells along the craniopharyngeal canal or as differentiated cells similar to those of the definitive adenohypophysis. These remnants, called the **pharyngeal hypophysis**, have been described in dogs, cats, other animal species, and humans. The pharyngeal hypophysis is physically separated from the sellar adenohypophysis in dogs, but in cats these structures may be continuous due to persistence of the craniopharyngeal canal.

The pharyngeal hypophysis is seen most often in brachycephalic breeds of dogs. It is a tubular structure lined by ciliated columnar epithelium, located on the midline of the nasopharynx, and is frequently continuous with a multilocular cyst that is lined by ciliated cuboidal or columnar epithelium. The cyst contains colloid material and cellular debris. A mass of differentiated acidophilic, basophilic, and chromophobic cells, similar to those of the sellar adenohypophysis, usually extends from the cyst wall.

Cysts (up to several centimeters in diameter) may be derived from the oropharyngeal end of the craniopharyngeal duct and project as a space-occupying mass into the nasopharynx in dogs. The predominant clinical sign may be related to respiratory distress due to ventral displacement of the soft palate and occlusion of the caudal nares. The cyst wall may be hard on palpation due to the presence of partially mineralized woven bone. The contents of the cyst are often yellow-gray and caseous due to the accumulation of keratin and desquamated epithelial cells from the cyst lining. The squamous epithelial lining of the cyst is derived from metaplasia of the remnants of the primitive oropharyngeal epithelium.

Cysts resulting from failure of differentiation of oropharyngeal ectoderm of Rathke's pouch

Pituitary dwarfism in dogs usually is associated with a failure of the oropharyngeal ectoderm of Rathke's pouch to differentiate into trophic hormone-secreting cells of the pars distalis. This results in a progressively enlarging, multiloculated cyst in the sella turcica and absence of an adenohypophysis. The cyst is lined by pseudostratified, often ciliated, columnar epithelium with interspersed mucin-secreting goblet cells. The mucin-filled cysts eventually occupy the entire pituitary area in the sella turcica and severely compress the pars nervosa and infundibular stalk (Fig. 3.8A, B). Few differentiated, trophic hormone-secreting chromophils are present in the pituitary region that immunocytochemically stain for the

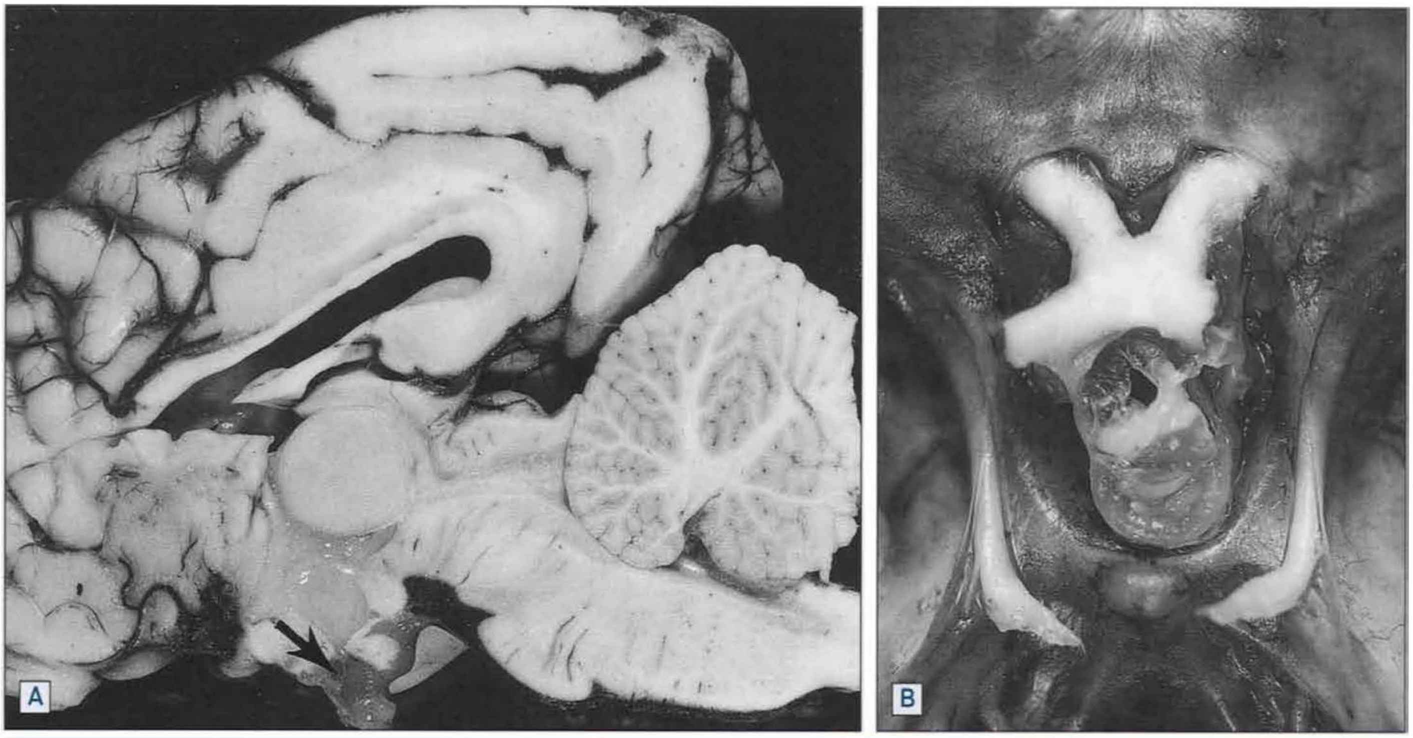

Figure 3.8 Failure of oropharyngeal ectoderm to differentiate into trophic hormone-secreting cells of the adenohypophysis in a German Shepherd Dog with **dwarfism**. **A.** Sagittal section. Pars nervosa compressed by mucin-filled cyst (arrow). **B.** Dorsal view of cyst.

specific trophic hormones. An occasional small nest or rosette of poorly differentiated epithelial cells is interspersed between the multiloculated cysts, but the cytoplasm of these cells is usually devoid of hormone-containing secretory granules.

Juvenile panhypopituitarism occurs most frequently in German Shepherd Dogs, but it has also been reported in other breeds such as the Spitz, Toy Pinscher, and Carelian Bear Dogs from Denmark. The dwarf pups appear normal or are indistinguishable from littermates at birth and until ~2 months of age. Subsequently the slower growth rate than the littermates, retention of puppy hair, and lack of primary guard hairs gradually become evident in dwarf pups. German Shepherd Dogs with pituitary dwarfism appear coyote- or fox-like due to their small size and soft woolly coat (Fig. 3.9). Bilaterally symmetrical alopecia develops gradually and often progresses to complete alopecia except for the head and tufts of hair on the legs. There is progressive hyperpigmentation of the skin until it is uniformly brown-black over most of the body. Adult German Shepherd Dogs with panhypopituitarism vary in size from as small as 2 kg up to nearly half normal size, depending upon whether the failure of formation of the adenohypophysis is nearly complete or only partial.

Permanent dentition is delayed or completely absent. Closure of epiphyses is delayed as long as 4 years, depending on the severity of hormonal insufficiency. There are few trabeculae in the primary and secondary spongiosa of the metaphysis of long bones, and osteoblasts are decreased in dwarf pups when compared with normal littermates. The external genitalia usually remain infantile. The testes and penis are small, mineralization of the os penis is delayed or incomplete, and the penile sheath is flaccid. In females the ovarian cortex is hypoplastic and estrus irregular or absent.

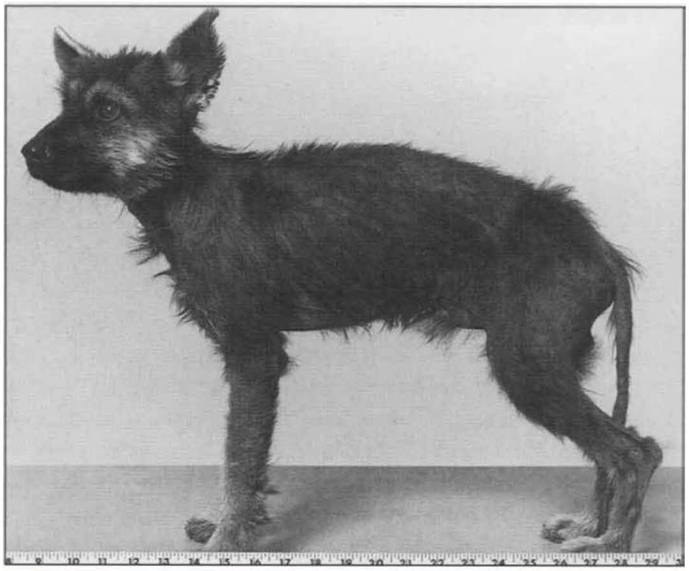

Figure 3.9 Panhypopituitarism ("pituitary dwarfism") in a 1-year-old German Shepherd Dog.

The shortened lifespan in dogs with pituitary dwarfism results not only from the panhypopituitarism but also from the resulting secondary endocrine dysfunction, such as hypothyroidism and hypoadrenocorticism. The increase in blood thyroxine and cortisol levels in response to challenge by exogenous TSH and ACTH are subnormal, owing to hypoplasia of thyroid follicular cells and the zonae fasciculata and reticularis of the adrenal cortex. The variation in severity and onset of the lesions in pituitary dwarfism appears to be related to the degree of penetrance of the defect. The degree to which the oropharyngeal epithelium fails to differentiate is variable as is the rapidity with which the mucin-filled cysts enlarge and exert pressure on adjacent structures.

Cutaneous lesions include hyperkeratosis, follicular keratosis, hyperpigmentation, adnexal atrophy and loss of elastin fibers, and the loose network of collagen fibers in the dermis. Hair shafts are absent and hair follicles are primarily in the telogen (resting) phase of the growth cycle.

Panhypopituitarism in German Shepherd Dogs often occurs in littermates and related litters, *suggesting a simple autosomal recessive mode of inheritance.* The activity of **somatomedin** (IGF-1) (a cartilage growth-promoting peptide whose production in the liver and plasma activity is controlled by somatotropin) is low in dwarf dogs. Intermediate somatomedin activity is present in phenotypically normal ancestors suspected to be heterozygous carriers. Useful diagnostic aids for pituitary dwarfs include comparison of height with littermates, radiographs of open epiphyseal lines, thyroid function tests, and skin biopsy.

Assays for somatomedin (a non-species-specific, somatotropin-dependent peptide) provide an indirect measurement of circulating growth hormone activity in dogs suspected to be heterozygous carriers. Basal levels of circulating canine growth hormone are detectable but low (normal range: 1.75 ± 0.17 μg/L) in pituitary dwarfs and fail to increase following a provocative test for growth hormone secretion provided by clonidine injection as in normal dogs. Insulin hypersensitivity has been demonstrated in pituitary dwarf dogs, most likely due to a change in insulin receptor numbers or affinity of binding in response to the low growth hormone levels. Dwarf dogs develop more profound hypoglycemia following an insulin injection than do normal dogs. Their response is similar to that in experimentally hypophysectomized dogs.

Cysts associated with pituitary dwarfism morphologically are distinct from the cysts that develop following the abnormal accumulation of colloid in the residual lumen of Rathke's pouch. The normally developed pars distalis and pars nervosa are compressed to various degrees by the abnormal accumulation of colloid in a normal cavity of the pituitary.

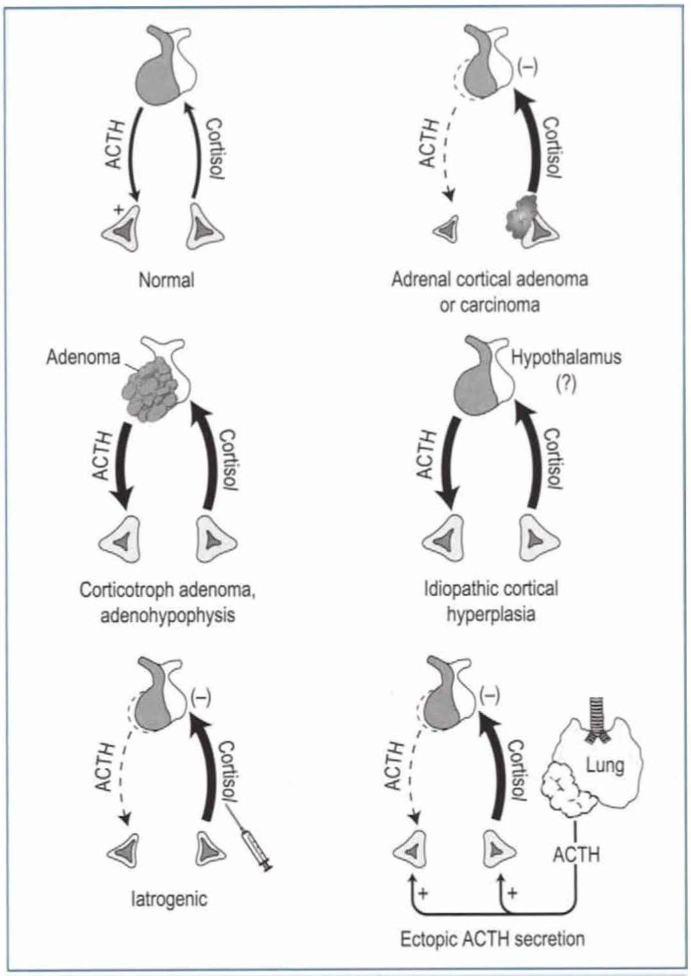

Figure 3.10 Multiple pathogenic mechanisms of **cortisol-excess** in dogs.

Neoplastic diseases of the pituitary

Corticotroph (ACTH-secreting) adenoma of the adenohypophysis

Functional tumors arising in the pituitary gland may be derived from corticotroph (ACTH-secreting) cells in either the pars distalis or the pars intermedia of dogs. *They are the most common cause the clinical syndrome of cortisol excess* **(Cushing's disease)** in dogs (Fig. 3.10). These neoplasms are encountered most frequently in dogs and infrequently in other animal species. Corticotroph adenomas develop in adult-to-aged dogs and have been reported in a number of breeds. Boxers, Boston Terriers, and Dachshunds appear to have a higher incidence of ACTH-secreting pituitary tumors than other breeds. *The spectrum of clinical manifestations and lesions that develop is primarily the result of long-term overproduction of cortisol by hyperplastic adrenal cortices.* These changes are the result of the combined gluconeogenic, lipolytic, protein catabolic, and anti-inflammatory actions of glucocorticoid hormones on many organ systems of the body. In horses pituitary tumors arise almost exclusively from the pars intermedia and are associated with dramatic clinical signs due primarily to space-occupying effects on the hypothalamus and other parts of the pituitary gland.

The pituitary gland is consistently enlarged in dogs with corticotroph adenomas (Fig. 3.11). Neither the occurrence nor the severity of functional disturbances appears to be directly related to the size of the neoplasm. Small corticotroph adenomas are as likely to be endocrinologically active as are larger neoplasms. The larger adenomas are often firmly attached to the base of the sella turcica but without evidence of erosion of the sphenoid bone. In the dog, the diaphragma sellae is incomplete. Therefore, the line of least resistance favors dorsal expansion of the gradually enlarging pituitary mass and invagination into the infundibular cavity, dilation of the infundibular recess and the third ventricle, with eventual compression or replacement of the hypothalamus, and possible extension of the tumor into the thalamus.

Dorsal expansion of larger corticotroph adenomas results in either a broadbased indentation and compression of the overlying hypothalamus, or extension into and replacement of the parenchyma of the hypothalamus and occasionally the thalamus. In the larger neoplasms, there are often focal areas of hemorrhage, necrosis, mineralization, and liquefaction. Growth of the pituitary tumor along the basilar aspects of the brain may result in incorporation of the

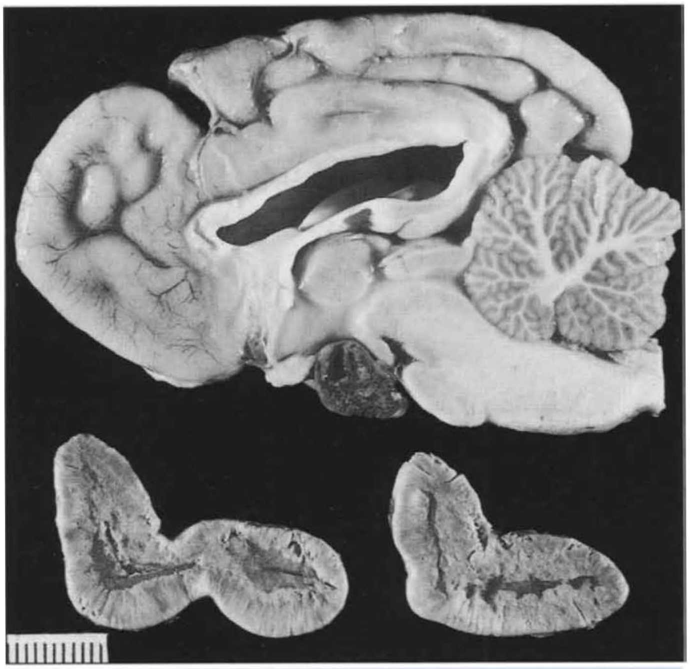

Figure 3.11 Corticotroph (ACTH-secreting) chromophobe adenoma of the pituitary with bilateral adrenal cortical hyperplasia, dog. Scale in mm.

second, third, and fourth cranial nerves, leading to disturbances of their function.

Bilateral enlargement of the adrenal glands occurs in dogs with functional corticotroph adenomas (Fig. 3.11). This enlargement often is striking and is due to increased cortical parenchyma, primarily in the zonae fasciculata and reticularis. Nodules of yellow-orange cortical tissue often are found outside the capsule in the periadrenal fat, as well as extending down into the adrenal medulla. The corticomedullary junction is irregular and the medulla is compressed.

Pituitary corticotroph adenomas are composed of well-differentiated, large or small, chromophobic cells supported by fine connective tissue septa. They can be divided into *sinusoidal* and *diffuse types* on the basis of the predominant pattern of cellular architecture. The cytoplasm of the tumor cells usually is devoid of secretory granules detectable by routine histochemical procedures used for pituitary cytology. However, pituitary corticotroph adenomas arising in both the pars distalis and the pars intermedia associated with the syndrome of cortisol excess in dogs are composed of polyhedral cells that immunocytochemically stain selectively for ACTH and MSH. *Nodules of focal hyperplasia* and microadenomas, composed of similar ACTH/MSH cells, often are present in the adenohypophysis of older dogs.

Pituitary adenomas arising in both the pars distalis and pars intermedia of dogs have positive immunocytochemical staining for ACTH, β-lipotrophin, and β-endorphin. Immunocytochemical studies have demonstrated that ACTH- and MSH-staining cells are polyhedral to round, sparsely granulated, and most numerous in the ventrocentral and cranial portions of the pars distalis in normal dogs. Corticotrophs are less numerous in the dorsal and caudal regions of the pars distalis and throughout the pars tuberalis.

Although remnants of the pars distalis can be identified near the periphery of corticotroph adenomas, the demarcation between the neoplasm and pars distalis often is not distinct. The pars distalis either is partly replaced by the neoplasm or severely compressed. The pars nervosa and infundibular stalk either are infiltrated and disrupted by tumor cells or completely incorporated within the larger neoplasms.

Cells constituting functional corticotroph adenomas have definite ultrastructural evidence of synthetic and secretory activity. Organelles concerned with protein synthesis (endoplasmic reticulum) and packaging of secretory products (Golgi apparatus) are well developed in tumor cells. *Hormone-containing secretory granules can be demonstrated by electron microscopy in functional corticotroph adenomas of dogs.* This is in contrast to the absence of demonstrable secretory granules within neoplastic cells observed with light microscopy. The granules vary in number from cell to cell, are spherical, and are surrounded by a delicate limiting membrane. They are small (mean diameter, 170 nm), electron-dense, often situated peripherally in the cell, and may have a prominent submembranous space.

A number of distinctive clinical and functional alterations develop in dogs with corticotroph (ACTH-secreting) adenomas, resulting in the **syndrome of hyperadrenocorticism** (*hypercortisolism*). Centripetal redistribution of adipose tissue leads to prominent fat pads on the dorsal midline of the neck, giving the neck and shoulders a thick appearance. Appetite and intake of food often are increased, either as a direct result of the hypercortisolism or involvement of hypothalamic appetite control centers by a large pituitary tumor. The muscles of the extremities and abdomen are weakened and atrophied. The loss of abdominal muscles and muscles of the appendicular skeleton results in gradual abdominal enlargement ("pot belly"), lordosis, muscle trembling, and a straight-legged skeletal-braced posture in order to support the body's weight. Profound atrophy of the temporal muscles may result in concave indentations and readily palpable prominences of underlying skull bones. Hepatomegaly due to increased fat and glycogen deposition plus vacuolation of hepatocytes produces a distended, often pendulous, abdomen. Functional alterations of the skin, muscle, lung and other tissues are the direct effects of elevated blood cortisol levels. They also are similar to those resulting from a primary cortisol-secreting adrenal cortical tumor and will be described elsewhere in this chapter.

Adenoma of the pars intermedia

Adenomas derived from cells of the pars intermedia are *the most common type of pituitary tumor in horses*, the second most common type in dogs, and rare in other species. They develop frequently in older horses, with females affected more frequently than males. Nonbrachycephalic breeds of dogs develop adenomas in the pars intermedia more often than brachycephalic breeds.

Adenomas of the pars intermedia in dogs result in only moderate enlargement of the pituitary gland. The pars distalis is readily identifiable and sharply demarcated from the rostral margin of the neoplasm. The tumor may extend across the residual hypophyseal lumen and result in compression atrophy, but usually does not invade the parenchyma of the pars distalis (Fig. 3.12A). The neurohypophysis is incorporated within the tumor, but the infundibular stalk is intact. Degenerative changes within the neoplasm are minimal.

Adenomas of the pars intermedia in dogs appear to arise from the lining epithelium of the residual hypophyseal lumen covering the pars nervosa. They often are relatively small and more strictly localized than corticotroph adenomas arising in the pars distalis of dogs.

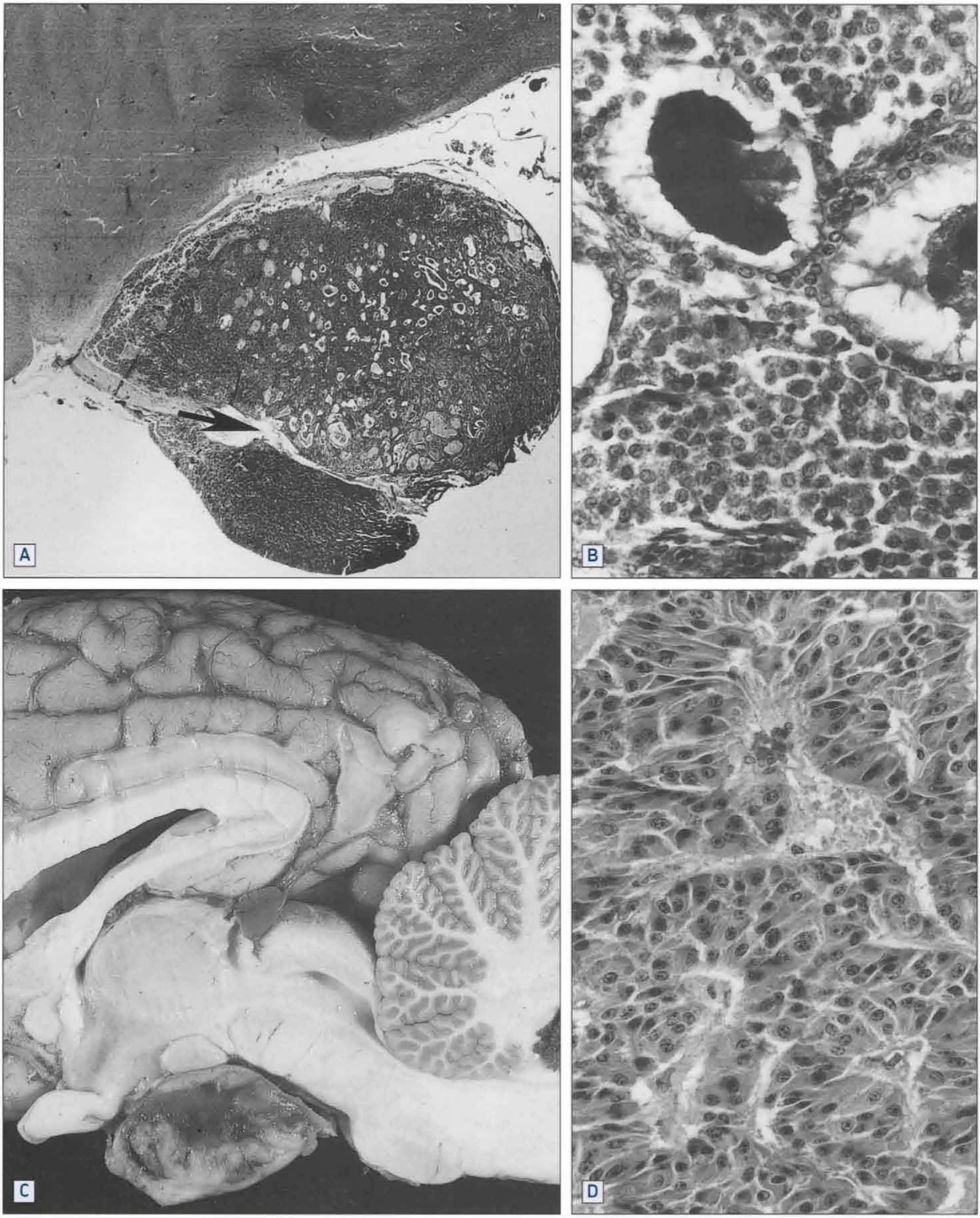

Figure 3.12 Corticotroph adenoma of pars intermedia in a dog. **A.** Neoplasm sharply demarcated from pars distalis. Arrow indicates the residual hypophyseal lumen. **B.** Nests of chromophobic cells interspersed between colloid-filled follicles. **Adenoma of pars intermedia** in a horse. **C.** Dorsal extension of tumor compressing hypothalamus and optic nerves with invasion of pars nervosa. **D.** Spindle-shaped tumor cells arranged in cords.

Adenomas of the pars intermedia are sharply demarcated from the pars distalis, usually by an incomplete layer of condensed reticulum and focal accumulations of lymphocytes, but are not encapsulated. The histologic appearance is different from corticotroph adenomas of the pars distalis, in that *there are numerous colloid-filled follicles interspersed between nests of large chromophobic cells* (Fig. 3.12B). The follicles are lined by simple columnar epithelium, which is partly ciliated, and contain interspersed mucin-secreting goblet cells. The follicular colloid is densely eosinophilic and periodic acid-Schiff (PAS) positive. The nests of cells between the follicles are primarily chromophobic, but occasional cells contain secretory granules of simple protein (acidophilic) or mucoprotein (basophilic). Endocrinologically active (ACTH-secreting) adenomas of the pars intermedia in dogs have prominent groups of large corticotrophs with abundant eosinophilic cytoplasm interspersed with variable numbers of smaller, more basophilic, cells and widely scattered follicles. Dense bands of fibrous connective tissue are occasionally interspersed between the follicles and nests of chromophobic cells, particularly in the endocrinologically inactive adenomas of the pars intermedia. Mitotic figures are observed infrequently. The neoplastic cells compress and often invade the pars nervosa and infundibular stalk.

Adenomas of the pars intermedia in dogs either are endocrinologically inactive and associated with various degrees of hypopituitarism and diabetes insipidus, or endocrinologically active and secrete excessive ACTH, leading to bilateral adrenocortical hyperplasia and a syndrome of cortisol excess. The clinical signs in the dogs with functional adenomas are similar to those described for corticotroph adenomas of the pars distalis.

Two cell populations have been identified in the pars intermedia of normal dogs by immunocytochemistry. The predominant cell type (**A cell**) stains strongly for α-MSH as in the pars intermedia of other species. A second cell type (**B cell**) in the canine pars intermedia stains intensely for ACTH but not for α-MSH. This second cell population accounts for the high bioactive ACTH concentration found in the pars intermedia of dogs, and appears to be the cell of origin of corticotroph adenomas of the pars intermedia in dogs with cortisol excess.

Adenomas of the pars intermedia in horses can be large tumors that extend out of the sella turcica and severely compress the overlying hypothalamus (Fig. 3.12C). The adenomas are yellow to white, multinodular, and incorporate the pars nervosa. On sectioning of the pituitary mass, the pars distalis usually can be identified as a compressed subcapsular rim of tissue on the rostral margin. A sharp line of demarcation remains between the neoplasm, which is partly encapsulated, and the compressed and atrophic pars distalis. The tumors are subdivided into nodules or compartments by fine septa of connective tissue that contain numerous capillaries and rare inflammatory cells. Tumor cells are large cylindrical, spindle-shaped or polyhedral, with an oval hyperchromatic nucleus (Fig. 3.12D). The histologic pattern is often reminiscent of the prominent pars intermedia of normal horses. Occasionally, cuboidal cells form follicular structures that contain dense eosinophilic colloid. In other areas, the spindle-shaped cells may assume a more sarcomatous pattern and palisade around vessels. The cytoplasm is lightly eosinophilic and granular.

Electron microscopy of the large cells comprising adenomas of the pars intermedia in horses reveals that the rough endoplasmic reticulum and Golgi apparatus are particularly well developed suggesting they are synthesizing and packaging considerable amounts of protein (pro-opiomelanocortin for secretion). The neoplastic cells contain numerous membrane limited secretory granules in their cytoplasm with a mean diameter of ~300 nm that are surrounded by a closely applied limiting membrane.

The *clinical syndrome* associated with tumors of the pars intermedia in horses is characterized by polyuria, polydipsia, increased appetite, muscle weakness, somnolence, intermittent hyperpyrexia, and generalized hyperhidrosis. The affected horses often develop striking *hypertrichosis* (*hirsutism*) because of failure of the seasonal shedding of hair (Fig. 3.13). The hair over most of the trunk and extremities is long (up to 9–10 cm), abnormally thick, wavy, and often matted together. Biopsy of skin reveals normal hair follicles uniformly in the anagen phase, normal epidermis and dermal collagen, and absence of the characteristic skin lesions observed in dogs with Cushing's disease due to cortisol excess.

Horses with larger tumors may have hyperglycemia (insulin-resistant) and glycosuria, most likely the result of downregulation of insulin receptors on target cells induced by the chronic excessive intake of food and hyperinsulinemia (see Fig. 3.2). *The disturbances in carbohydrate metabolism, ravenous appetite, hypertrichosis, and hyperhidrosis are considered to be primarily a reflection of deranged hypothalamic function caused by the compressive and destructive effects of the large pituitary tumors.* Adenomas of the pars intermedia in horses often extend out of the sella turcica and expand dorsally because of the incomplete diaphragma sellae, severely compress the overlying hypothalamus, and result in localized necrosis of nuclei in this important regulatory center. The hypothalamus is the primary autonomic center for homeostatic regulation of body temperature, appetite, cyclic shedding of hair, among many other functions.

Plasma cortisol and immunoreactive adrenocorticotropin (ACTH) levels are either normal or only modestly elevated in horses with adenomas of the pars intermedia. The cortisol levels often lack the

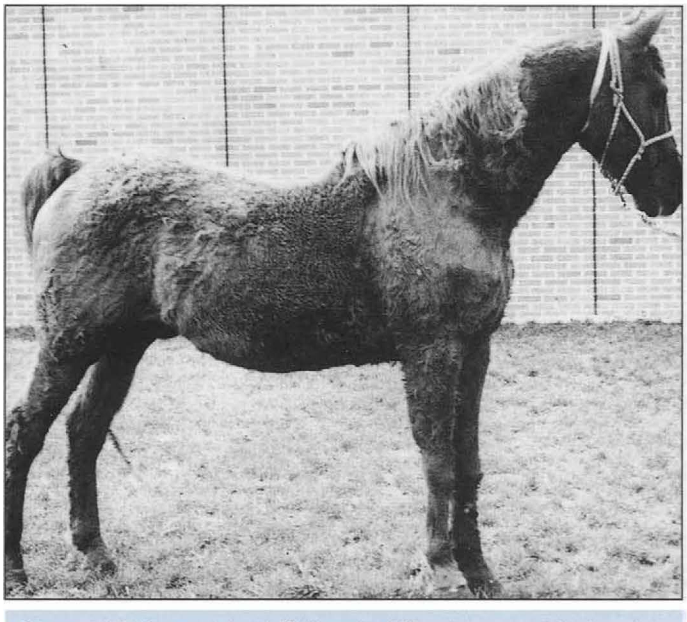

Figure 3.13 Hypertrichosis ("hirsutism") in a horse, resulting from failure of the cyclic shedding of hair associated with an adenoma of the pars intermedia. (From Capen CC. In: Special Veterinary Pathology, 3rd ed., 2001.)

normal diurnal rhythm and are not suppressed by either high or low doses of dexamethasone.

Tumor tissue and plasma from horses with adenomas of the pars intermedia contain high concentrations of **immunoreactive peptides** (*corticotropin-like intermediate lobe peptide (CLIP), α- and β-melanocyte-stimulating hormones (α- and β-MSH),* and *β-endorphin (β-END)*) derived from *pro-opiomelanocortin (pro-OMC)* and processed in the pars intermedia (Fig. 3.14). This biosynthetic precursor is a high molecular weight (31 000–37 000 daltons) glycoprotein that undergoes different post-translational processing in the pars distalis and pars intermedia. In the normal pars distalis, pro-OMC is processed to ACTH (4500 daltons), β-lipotropin (β-LPH) and γ-LPH, whereas in the normal pars intermedia the same precursor molecule is cleaved into α-MSH, CLIP (that contains amino acids 18–39 of the ACTH molecule), β-MSH, and β-END. Plasma cortisol strongly inhibits ACTH secretion by the pars distalis but has much less effect on peptides secreted by the pars intermedia, which are under tonic dopaminergic inhibitory control (Fig. 3.14).

The modest elevation of plasma immunoreactive adrenocorticotrophin appears to be due to the different processing of pro-OMC in tumors derived from cells of the pars intermedia. This may explain the normal or slightly elevated blood cortisol levels and normal or mildly hyperplastic adrenal cortices observed in some horses with adenomas of the pars intermedia. The plasma and tumor levels of pars intermedia-derived peptides (CLIP, α- and β-MSH, β-END) are disproportionately elevated (40 times or more) compared to those of ACTH, apparently as the result of selective post-translational processing of pro-OMC in a similar manner as the normal pars intermedia. Extracts of adenomas derived from pars intermedia also contain immunoreactive peptides of larger molecular weight than those present in the normal pituitary tissue. The smallest (38 500 daltons) of these peptides could represent pre-pro-OMC with an attached signal or leader sequence of ~26 amino acid residues. The larger peptides (47 000 and 63 000 daltons) may be derived from improper intranuclear processing of pro-OMC mRNA with retention of additional coding nucleotide sequences.

Horses with pituitary adenomas derived from pars intermedia develop a clinical syndrome that is associated, in part, with the autonomous production of excess pro-OMC-derived peptides. Immunocytochemical evaluation of adenomas of the pars intermedia reveals a diffuse moderate-to-strong staining for pro-OMC, α-MSH, and β-END. Although many of the functional disturbances in horses with pituitary adenomas (e.g., diabetes insipidus, polyphagia, hyperpyrexia, hyperhidrosis, and hypertrichosis (Fig. 3.13)) appear to be the result of hypothalamic or neurohypophyseal dysfunction, other signs (e.g., docility and diminished responsiveness to painful stimuli) may be related to the elevated plasma and cerebrospinal levels of β-END. Although ACTH is demonstrable in adenomas of the pars intermedia, the staining intensity is patchy and considerably weaker than that of pro-OMC, α-MSH, and β-END. These findings are in accord with biochemical studies that report markedly elevated concentrations of immunoreactive pro-OMC and pro-OMC-derived peptides including α- and β-MSH, corticotrophin-like intermediate lobe peptide, and β-END in adenomas and plasma of affected horses relative to ACTH. The overall processing of peptides in adenomas in the pars intermedia appears to be similar to that in the normal equine pars intermedia. *The clinical syndrome in horses with pituitary tumors leading to hypothalamic and neurohypophyseal derangement as well as autonomous production of excess amounts of pro-OMC-derived peptides is distinctly different from Cushing's disease that occurs in dogs, cats, and human patients.*

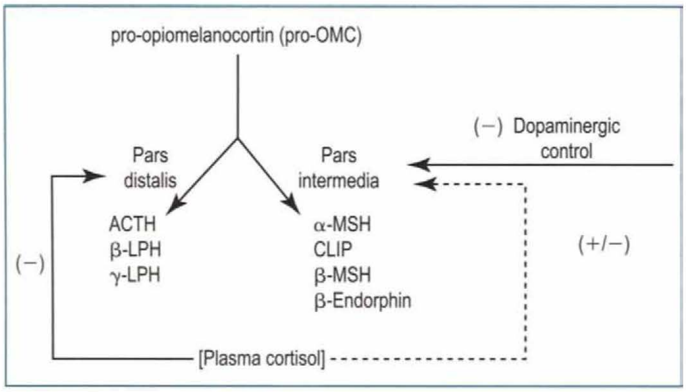

Figure 3.14 Post-translational **processing of pro-opiomelanocortin** (pro-OMC) produced by corticotrophs in pars distalis and pars intermedia. The plasma concentration of cortisol regulates processing in the pars distalis, whereas the pars intermedia is primarily under dopamine control.

Corticotrophs in the pars distalis of horse have strong immunostaining for ACTH, whereas only a few cells stain for α-MSH. These immunocytochemical findings illustrate the differences between adenomas of the pars intermedia in horses and corticotroph adenomas of the pars distalis (also pars intermedia in dogs) that result in the classical Cushing's disease with cortisol excess in humans and dogs. Corticotroph adenomas associated with Cushing's disease are characterized by strong immunostaining for ACTH and weak-to-moderate immunostaining for α-MSH.

Hormonally inactive chromophobe adenoma of the pars distalis

Nonfunctional pituitary tumors occur in dogs, cats, laboratory rodents, and parakeets, but are uncommon in other species. *Although chromophobe adenomas appear to be hormonally inactive, they may cause significant functional disturbances and clinical signs by compression of adjacent portions of the pituitary gland and dorsal extension into the overlying brain.*

Nonfunctional pituitary adenomas result in clinical disturbances either by interfering with secretion of pituitary trophic hormones and diminishing target organ function or dysfunction of the central nervous system. Affected animals often are depressed, have incoordination and other disturbances of balance, are weak and may collapse with exercise. In long-standing cases, there may be evidence of blindness with dilated and fixed pupils due to compression and disruption of optic nerves by dorsal extension of the pituitary tumor (Fig. 3.15).

Animals with hormonally inactive pituitary adenomas often have progressive loss of weight (*pituitary cachexia*), with muscle atrophy due to a lack of the protein anabolic effects of growth hormone. Compression of the cells that secrete gonadotrophic hormones or the corresponding hypothalamic releasing hormone(s) results in atrophy of the gonads, resulting in decreased libido or anestrus. Affected animals appear dehydrated, as evidenced by a lusterless dry haircoat, and they consume increased amounts of water.

Hormonally inactive pituitary adenomas often attain considerable size before they cause obvious signs or kill the animal. The proliferating tumor cells incorporate the remaining structures of the

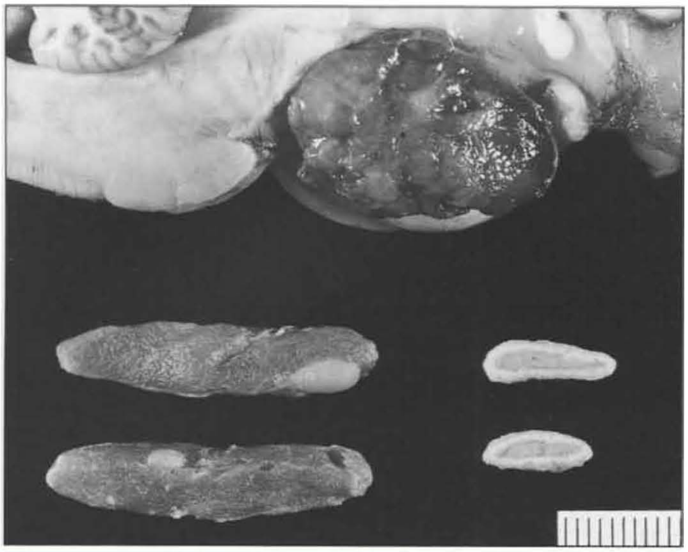

Figure 3.15 Destruction of pituitary by **nonfunctional adenoma** with trophic atrophy of adrenal cortex and thyroid follicular cells. Accumulation of colloid in involuted follicles prevents marked reduction in thyroid gland size. Scale in mm.

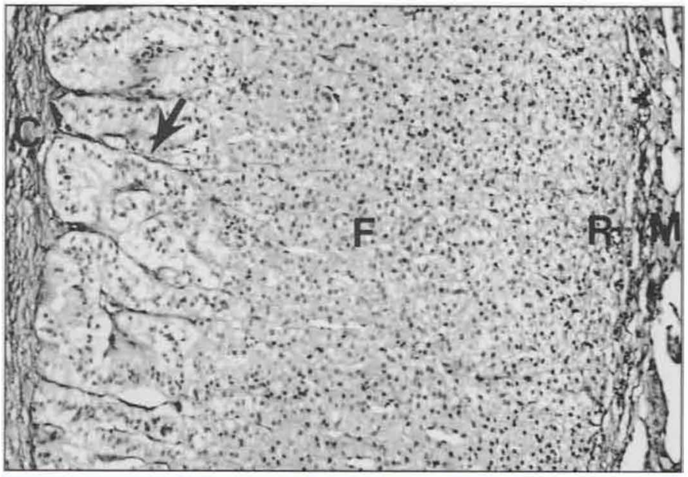

Figure 3.16 Adrenal cortex from a dog with a large **nonfunctional pituitary adenoma**. The inner zonae fasciculata (F) and reticularis (R) are atrophic due to subnormal production of ACTH. The outer zona glomerulosa (arrow) is normal but relatively more prominent. C = adrenal capsule, M = medulla.

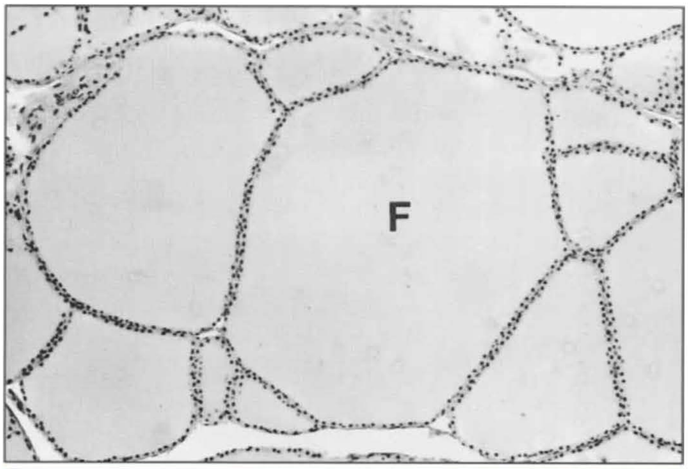

Figure 3.17 Colloid involution of thyroid follicles due to subnormal production of TSH by the adenohypophysis in a dog with a large **nonfunctional pituitary adenoma**. The follicular cells have undergone trophic atrophy and are low cuboidal because of a lack of TSH, and the follicles (F) are greatly distended by the accumulation of colloid. (From Capen CC. Toxicol Pathol 2001;29:8–33.)

adenohypophysis and infundibular stalk. The neoplasms attach firmly to the base of the sella turcica, but cause no erosion of the sphenoid bone. The incomplete diaphragma sellae permits dorsal growth of the adenoma along lines of least resistance. The entire hypothalamus may become compressed and replaced by the tumor.

The adrenal glands in animals with large nonfunctional pituitary adenomas are small and consist primarily of medullary tissue surrounded by a narrow zone of cortex. The adrenal cortex appears as a thin yellow-brown rim composed of a moderately thickened capsule and secretory cells of the outer zona glomerulosa (Figs 3.15, 3.16), which are not predominantly under the control of ACTH. The zonae fasciculata and reticularis are severely atrophied. Thyroid glands in animals with large pituitary adenomas are either normal size or smaller than normal, though less atrophied than the adrenal cortex (Fig. 3.15). Most of the atrophic thyroid follicles are large, lined by flattened cuboidal epithelium, and have few endocytotic vacuoles near the interface between the colloid and luminal aspect of the follicular cells (Fig. 3.17). The thyroid lesion is due to lack of TSH-induced endocytosis of colloid. Seminiferous tubules of the testes are small and show little evidence of active spermatogenesis.

The cells comprising nonfunctional pituitary adenomas are cuboidal to polyhedral and either arranged in diffuse sheets or subdivided into small packets by fine connective tissue septa. Special histochemical techniques for pituitary cytology fail to demonstrate specific secretory granules within the cytoplasm of tumor cells. The histogenesis of nonfunctional chromophobe adenomas often is difficult to define precisely, but they appear to be derived from less differentiated pituitary cells that neither store nor secrete a specific hypophyseal trophic hormone.

The daily administration of calcitonin to Sprague-Dawley, and to a lesser degree Fischer 344, rats for 1 year has been reported to increase the incidence of nonfunctional pituitary adenomas. Immunohistochemical analysis revealed that the tumor cells were negative for all of the major pituitary hormones, and serum levels of growth hormone, prolactin, ACTH, LH, and FSH were unchanged in calcitonin-treated rats. Immunohistochemistry and in situ hybridization analysis demonstrated that the nonfunctional pituitary tumors expressed the glycoprotein hormone α-subunit and serum levels of α-subunit were markedly increased (approximately 20-fold).

Acidophil adenoma of the pars distalis

Although **growth hormone-secreting acidophils (somatotrophs)** are one of the major cell types in the adenohypophysis, *development of functional adenomas from this population of acidophils is infrequent in animals.* Neoplasms derived from granulated acidophils are uncommon in all domestic animal species, but have been reported in the cat, dog and sheep; however, they are common in many strains of old laboratory rats.

Acidophil adenomas enlarge the pituitary gland and indent the overlying hypothalamus or extend into the overlying brain to various degrees. The enlarged hypophysis is composed of irregular columns of acidophils interspersed between numerous large blood-filled sinusoids. The fibrous stroma is sparse. Although the degree of cytoplasmic granulation of acidophils varies from cell to cell, the predominating type of neoplastic acidophil usually contains many secretary granules. The nuclei of the densely granulated acidophils are small, oval, and hyperchromatic. Sparsely granulated ("chromophobic") cells are interspersed between the densely granulated acidophils. Their cytoplasm is more abundant and lightly eosinophilic but contains only an occasional secretory granule. Secretory granules of acidophils are bright red when stained with acid fuschin-aniline blue and with Crossman's modification of Mallory's trichrome. Orange-G stains the granules an intense yellow-orange, but they are PAS-negative.

Colloid-containing follicles lined by follicular cells are found occasionally within **acidophil adenomas in dogs**. The colloid is intensely PAS-positive. Numerous sinusoids are distended with erythrocytes, detached neoplastic cells, and large masses of fibrin. The pars nervosa and infundibular stalk are infiltrated at the periphery by neoplastic cells, compressed, and partly replaced by fibrous astrocytes.

Electron microscopic evaluation reveals that *two types of acidophils* occur within pituitary acidophil adenomas. The *predominant type of acidophil* is smaller and contains many secretory granules. The granules are spherical to oval, uniformly electron-dense, finely granular, and surrounded by a delicate limiting membrane. The submembranous space of the granule is narrow. The mean diameter of mature secretory granules in the neoplastic acidophils is 420 nm (range 320–600 nm). The plasma membranes of adjacent cells are relatively straight with uncomplicated interdigitations and are connected by an occasional desmosome. The Golgi apparatus is comparatively small and associated with few prosecretory granules. The rough endoplasmic reticulum is composed of small, flattened membranous sacs with attached ribosomes. A few mitochondria are randomly distributed throughout the cytoplasm. Acidophils of this type are interpreted to be in the "storage phase" of their secretory cycle.

The *less common type of neoplastic acidophil* has a greater cytoplasmic and nuclear area but contains numerous organelles and few mature secretory granules. The rough endoplasmic reticulum is extensive and consists of aggregates of lamellar arrays of granular membranes. The Golgi apparatus is prominent and associated with numerous small prosecretory granules. Mitochondria are observed more often in the cytoplasm of this type of acidophil. These hypertrophied acidophils are considered to be secretorily active cells. Cells with various amounts of organellar development and numbers of mature secretory granules are present between the extremes of storage and actively synthesizing acidophils.

In the pars distalis of normal dogs immunoreactive prolactin cells occur as small groups of large polygonal cells with prominent granules in the ventrocentral and rostral portion. A diffuse increase in this population of cells occurs in female dogs near parturition. In comparison, growth hormone-secreting acidophils are present singly along capillaries in the dorsal region of the pars distalis near the pars intermedia. They are small, round to oval, and have fine cytoplasmic granules. Somatotrophs frequently undergo hypertrophy and diffuse hyperplasia in old dogs, especially females with mammary dysplasia or neoplasia.

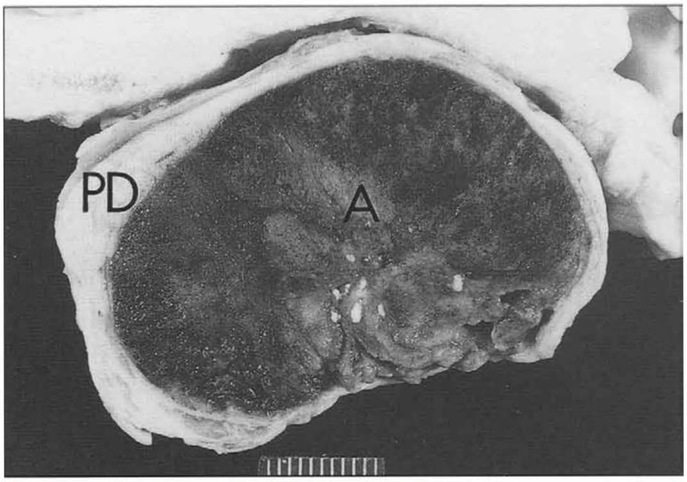

Figure 3.18 Acidophil adenoma (A) from a ewe with severe compression of the pars distalis (PD) and overlying brain. Scale in mm. (From Capen CC. In: Tumors of Domestic Animals, 4th ed., 2002.)

In none of the reported acidophil tumors of dogs has conclusive evidence been presented that there were functional disturbances resulting from hypersecretion of somatotropin or prolactin. **Acromegaly** is a disease characterized by an overgrowth of connective tissue, increased appositional growth of bone, coarsening of facial features, and enlargement of viscera due to chronic excessive secretion of growth hormone (somatotropin). However, acidophil adenomas in dogs have been associated with thickened cranial bones and metahypophyseal diabetes with fewer than normal pancreatic islets.

Acidophil adenomas in cats have been associated with clinical signs of diabetes mellitus with degranulation of pancreatic islets and vacuolar changes in beta cells. The persistent elevation of serum somatotropin in cats also is associated with proliferation of joint cartilage, leading to degenerative arthropathy, and with kidney disease due to periglomerular fibrosis and mesangial proliferation.

Functional acidophil adenomas in cats secrete excess growth hormone, which results in downregulation of insulin receptors and resistance to the action of insulin at the target cell level. Several reports have described acidophil adenomas in cats associated with **insulin-resistant diabetes mellitus** and acromegalic features with immunocytochemical localization of growth hormone in the cytoplasm of tumor cells. The circulating growth hormone levels were approximately 100 times normal and were nonsuppressible by an exogenous glucose load. Clinical evidence of acromegaly included an enlarged abdomen and prognathia inferior.

Acidophil adenomas in **sheep** may attain considerable size (Fig. 3.18) and remain confined to the sella turcica because sheep have a complete diaphragma sellae separating the pituitary fossa from the brain. The remaining adeno- and neurohypophysis are compressed severely (Fig. 3.18), and the sella turcica is enlarged and deepened due to pressure-induced osteolysis (Fig. 3.19).

Changes in plasma growth hormone, prolactin, cortisol, and progesterone levels in beagles following intramuscular injection of 75 mg/kg medroxyprogesterone acetate (6 α-methyl-17-acetoxyprogesterone) every 3 months for 17 months have been correlated with the development of acromegalic features and mammary nodules. Medroxyprogesterone acetate increased mean growth hormone levels, incidence of acromegaly-like changes (Fig. 3.20),

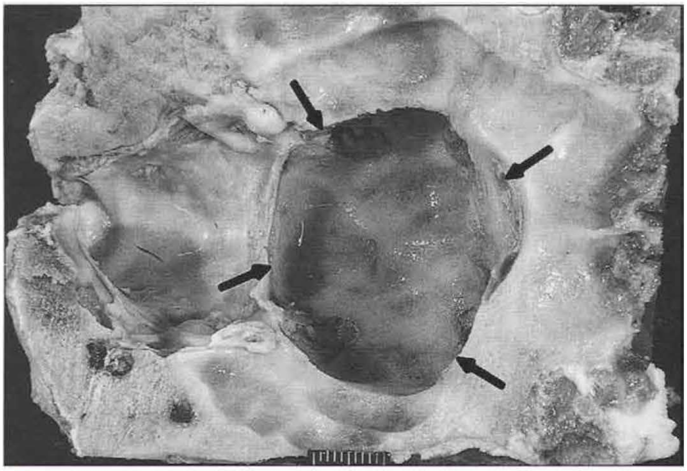

Figure 3.19 Enlargement and deepening of sella turcica (arrows) associated with a large **acidophil adenoma** (prolactin-secreting cells) of the pituitary gland that remained confined to this region due to the complete diaphragma sellae. Scale in mm. (From Capen CC. In: Tumors of Domestic Animals. 4th ed., 2002.)

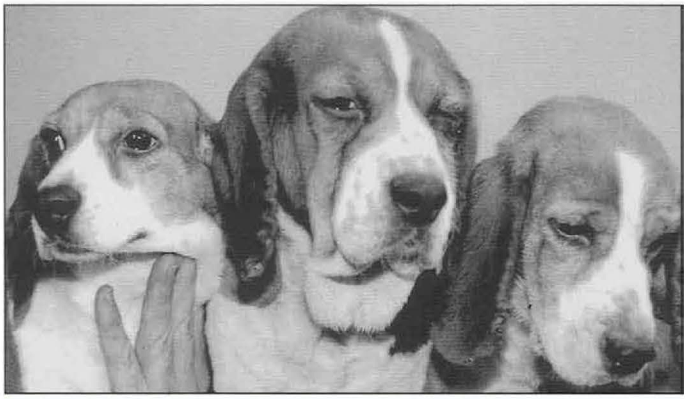

Figure 3.20 **Iatrogenic acromegaly** in a beagle (center) compared with unaffected littermates (left and right). Note the coarseness of facial features and marked thickening and folding of the skin of the face. These characteristic changes are the result of the protein anabolic effects of somatotropin, produced in excess by hyperplastic mammary ductular epithelial cells that have been stimulated by the exogenous administration of medroxyprogesterone acetate. (Courtesy of P Concannon.)

and frequency of palpable mammary nodules in beagles under controlled conditions. The growth hormone increase is the result of increased expression of the growth hormone gene in ductal cells of the mammary gland and not by stimulation of somatotrophs in the pituitary gland.

Growth hormone levels were elevated (2.5 μg/L and above) in all female beagles with acromegalic features compared with placebo controls and dogs receiving crystalline progesterone implants. All dogs with elevated growth hormone levels had multiple mammary nodules. The elevation in growth hormone levels and development of acromegalic features was greater in older than in younger dogs. Initial elevations of growth hormone occurred after 8 months of medroxyprogesterone acetate treatment. Serum prolactin levels were not changed by either medroxyprogesterone acetate or crystalline progesterone implants, but serum cortisol levels were suppressed significantly compared with those in controls. The latter most likely was the result of medroxyprogesterone-induced suppression of pituitary ACTH secretion and corresponding decrease in cortisol synthesis by the adrenal cortex. Elevated growth hormone levels also are reported in dogs with spontaneous mammary tumors.

Craniopharyngioma (intracranial germ cell tumor)

Craniopharyngioma *is a benign tumor that is derived from epithelial remnants of the oropharyngeal ectoderm of the craniopharyngeal duct (Rathke's pouch)*. It occurs in animals younger than those with other types of pituitary neoplasm and is present in either a suprasellar or infrasellar location. It is one cause of *panhypopituitarism and dwarfism* in young dogs due to subnormal secretion of somatotropin and other trophic hormones beginning at an early age, prior to closure of the growth plates.

Pleomorphic tumors in the suprasellar region often are large and grow along the ventral aspect of the brain where they can incorporate several cranial nerves. In addition, they extend dorsally into the hypothalamus and thalamus. The *clinical signs* resulting from this type of rapidly growing tumor in the pituitary region often are a combination of

1. lack of secretion of pituitary trophic hormones resulting in trophic atrophy and subnormal function of the adrenal cortex and thyroid, gonadal atrophy, and failure to attain somatic maturation due to a lack of growth hormone secretion;
2. disturbances in water metabolism (polyuria, polydipsia, low urine specific gravity and osmolality) from an interference with the release and synthesis of antidiuretic hormone by the large tumor;
3. deficits in cranial nerve function; and
4. central nervous system dysfunction due to extension into the overlying brain.

Histologically, the tumors have alternating solid and cystic areas. The solid areas are composed of nests of epithelial cells (cuboidal, columnar, or squamous) with focal areas of mineralization. The solid areas are composed of nests of epithelial cells (cuboidal, columnar, or squamous cells) with prominent focal areas of keratinization and occasional mineralization that compress the overlying hypothalamus. The areas of keratinization are densely eosinophilic and frequently are associated with fragments of nuclear chromatin. The neoplastic epithelial cells are large and pleomorphic with vesicular nuclei and prominent nucleoli, and frequently have discrete vacuoles in their cytoplasm. There frequently is an admixture with smaller cuboidal to polyhedral cells that have acidophilic secretory granules that stain positive for either prolactin or growth hormone by immunocytochemistry. The cystic spaces are lined by either columnar or squamous cells and contain keratin debris and colloid. Colloid-containing follicles may be formed that are lined either by cuboidal or by columnar cells and contain variable amounts of eosinophilic colloid.

It has been proposed that pleomorphic neoplasms in the suprasellar region of younger dogs be classified as **germ cell tumors** *rather than craniopharyngiomas*. The diagnosis of germ cell tumors was based upon three criteria:

1. midline suprasellar location,
2. presence within the tumor of several distinct cell types (one population resembling a seminoma or dysgerminoma, and

others suggesting teratomatous differentiation into secretory glandular and squamous elements), and

3. positive staining for α-fetoprotein.

This interpretation suggests that these suprasellar pleomorphic neoplasms in dogs are of germ cell origin and that the secretory (glandular) and squamous elements represent teratomatous differentiation. Additional cases need to be studied utilizing a spectrum of immunocytochemical stains for the protein and glycoprotein pituitary hormones, alpha subunit, chorionic gonadotrophin, α-fetoprotein, and placental alkaline phosphatase to determine which of the pleomorphic neoplasms in the suprasellar region are derived from remnants of the oropharyngeal epithelium of the craniopharyngeal duct and which have their origin from multipotential germ cells.

Basophil adenoma of the pars distalis

Tumors composed of granulated basophils are one of the least common pituitary tumors in all animal species. Cushing's disease in human beings was initially attributed to a hypersecretion of ACTH by small basophilic adenomas in the pars distalis. Current evidence suggests they are a possible cause for a small percentage of patients with Cushing's disease. Several of the early reports on ACTH-secreting pituitary tumors in dogs with hyperadrenocorticism reflected this concept and interpreted them to be basophil adenomas. Corticotroph (chromophobic) adenomas of the pars distalis and pars intermedia are responsible for the great majority of cases of Cushing's-like disease in dogs.

Basophil adenomas in people also may secrete thyrotropin (TTH) resulting in bilateral enlargement of both thyroid lobes ("goiter"). Serum thyroxine, triiodothyronine, and TTH are elevated and may be responsive to TRH from the hypothalamus. The neoplastic cells contain small secretory granules (diameter less than 150 nm) with prominent rough endoplasmic reticulum and Golgi apparatus, characteristic of pituitary thyrotrophs.

Pituitary chromophobe carcinoma

Pituitary chromophobe carcinomas are uncommon compared with pituitary adenomas, but have been seen in older dogs and cows. They are usually endocrinologically inactive but may result in significant functional disturbances by destruction of the pars distalis and neurohypophyseal system, leading to panhypopituitarism and diabetes insipidus.

Pituitary carcinomas are large and extensively invade the overlying brain, along the ventral aspect of skull, and into the sphenoid bone of the sella turcica inducing resorption of the bone. Metastases may occur to regional lymph nodes or to distant sites, such as the spleen or liver.

Malignant tumors of pituitary chromophobes are highly cellular and often have large areas of hemorrhage and necrosis. Giant cells, nuclear pleomorphism, and mitotic figures are encountered more frequently than in chromophobe adenomas.

Tumors metastatic to the pituitary gland

The pituitary gland occasionally is either partially or completely destroyed by metastatic tumors from distant sites. Examples include malignant lymphoma in cattle and dogs; malignant melanoma in horses and dogs, transmissible venereal tumor and adenocarcinoma of the mammary gland in dogs. In addition, the pituitary may be compressed or destroyed by local infiltration from osteosarcomas of the sphenoid bone, ependymomas arising in the infundibular recess of the third ventricle, by abscesses (Fig. 3.21A), meningiomas (Fig. 3.21B), and gliomas ("infundibuloma") of the infundibular stalk.

Granular cell tumors of pituitary

Granular cell tumors (GCT) *are uncommon in most animal species*, but have been reported in dogs, horses, and cats. They are encountered most commonly in the oral cavity; occasionally in the heart, lymph nodes, brain, and skin; and rarely involving the pituitary gland in a parasellar location. A recent report describes involvement of the pituitary, optic chiasm, and ventral pyriform lobes by a GCT resulting in blindness, seizures, ataxia, weakness, and behavioral changes.

The neoplastic cells in GCT have abundant eosinophilic granular cytoplasm with round-to-oval eccentrically placed nuclei (Fig. 3.22A). The cytoplasm stains diffusely positive with the PAS reaction, but immunohistochemical staining for pancytokeratin, vimentin, S-100 protein, glial fibrillary acidic protein, neuron-specific enolase, and synaptophysin is uniformly negative. Ultrastructurally, the neoplastic cells contain numerous membrane-bound lysosomal bodies that displace the nucleus to an eccentric location and obscure other cytoplasmic organelles (Fig. 3.22B). A similar entity in this region of human patients is referred to as parasellar or pituitary symptomatic GCT.

Mechanisms of pituitary tumor induction

Pituitary tumors can be induced readily by sustained uncompensated hormonal derangements leading to increased synthesis and secretion of pituitary hormones. The absence of negative feedback inhibition of pituitary cells leads to unrestrained proliferation (hyperplasia initially, neoplasia later). This effect can be potentiated by the concurrent administration of *ionizing radiation* or *chemical carcinogens*. The administration of *estrogens* is a reproducible method for inducing pituitary tumors in certain experimental animals. The effect of exogenous estrogen on the rat pituitary includes stimulation of prolactin secretion and the induction of prolactin-secreting tumors. The administration of estrogens in susceptible strains results in elevated serum prolactin levels, increased numbers of prolactin cells within the pituitary, enhanced incorporation of tritiated thymidine within the gland, and increased mitotic activity. Other agents, including caffeine, have been implicated in the development of pituitary adenomas in rats.

The *high frequency of spontaneous pituitary adenomas in laboratory rats* is a well-recognized phenomenon that must be considered in any long-term toxicological study. The incidence of pituitary tumors is determined by many factors including strain, age, sex, reproductive status, and diet. Both hereditary factors and the levels of circulating sex steroids have been suggested as important etiological mechanisms. The hypothalamus has also been incriminated in the development of these tumors. Age-related hypothalamic changes may result in diminished activity of dopamine, the major prolactin-inhibitory factor. Numerous studies have demonstrated the striking degree of strain variation in the incidence of pituitary tumors in rats, which has been reported to range from 10% to more than 90%. Rapid body growth rates and high levels of conversion of feed to body mass in early life, or high protein intake in early

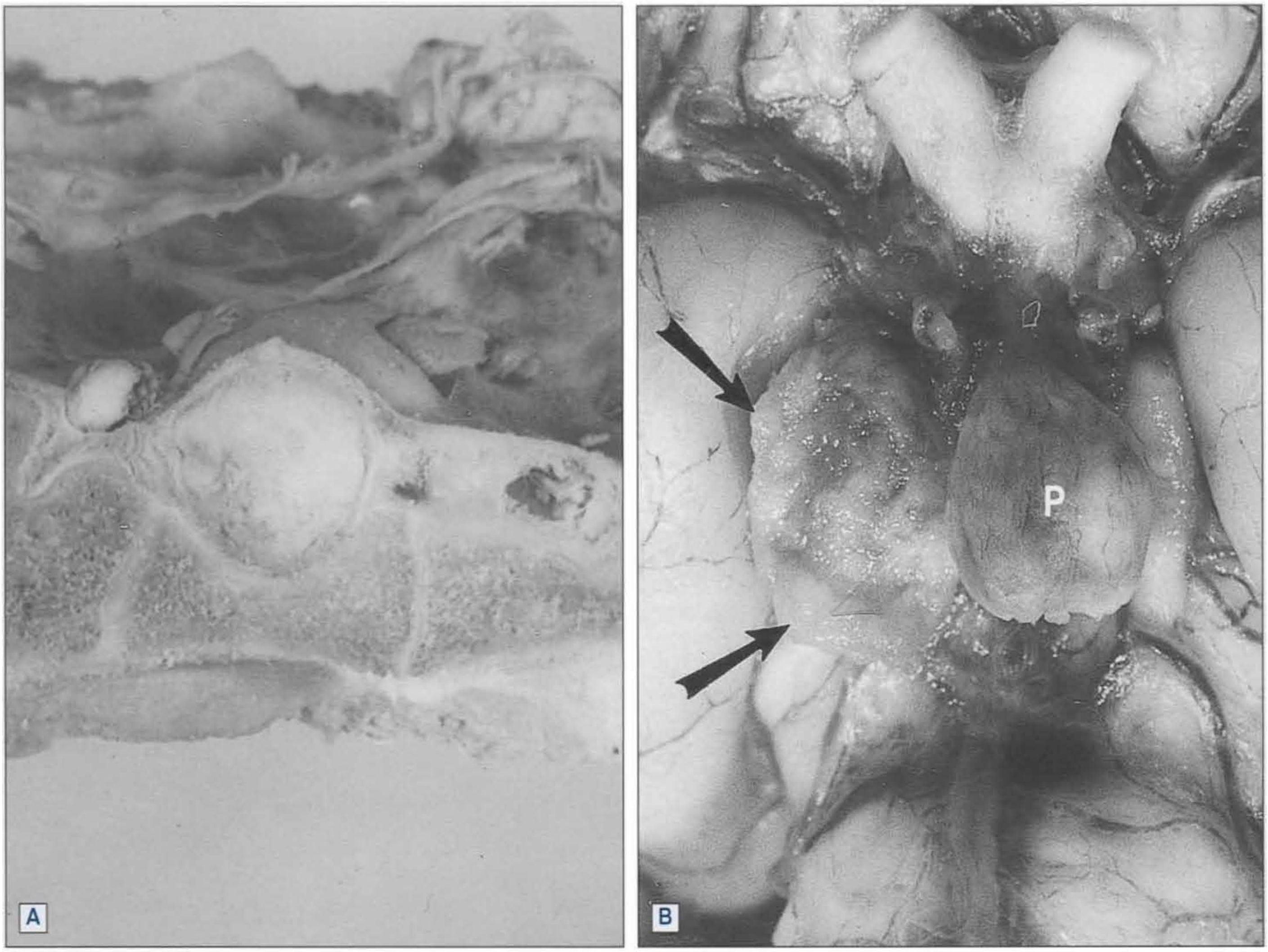

Figure 3.21 A. Pituitary abscess in a calf, mid-sagittal section. **B. Meningioma** (arrows) on ventral aspect of brain exerted pressure on the hypothalamic-hypophyseal portal system in a dog. P = pituitary gland.

adult life, predispose any strain of rat to the development of pituitary adenomas.

Diseases of neurohypophysis

Diabetes insipidus is a disorder in which inadequate antidiuretic hormone (ADH) is produced (*hypophyseal form*), or target cells in the kidney are unable to respond to the secretion of normal or elevated circulating levels of hormone (*nephrogenic form*).

The **hypophyseal form of diabetes insipidus** develops as a result of compression and destruction of the pars nervosa, infundibular stalk, or supraoptic nucleus in the hypothalamus. The lesions responsible for the disruption of ADH synthesis or secretion include large pituitary neoplasms (endocrinologically active or inactive), dorsally expanding cysts or inflammatory granulomas, and traumatic injury to the skull with hemorrhage and glial proliferation in the neurohypophyseal system. The neurohypophysis, infundibular stalk, and hypothalamus are compressed or disrupted by neoplastic cells. This interrupts the nonmyelinated axons that transport ADH from its site of production, primarily in the supraoptic nucleus of the hypothalamus, to the site of release in the capillary plexus of the pars nervosa. Compression of neurosecretory neurons in the supraoptic nucleus of the hypothalamus by the dorsally expanding neoplasm may also result in decreased ADH synthesis. Axons in the compressed pars nervosa with hypophyseal diabetes insipidus associated with pituitary neoplasms are depleted of ADH-containing, dense neurosecretory granules, unlike those in normal animals (Fig. 3.23A, B). Sporadic cases of hypophyseal diabetes insipidus may be the result of an *inherited biochemical defect in the synthesis of ADH* and its corresponding neurophysin I, as has been described in the Brattleboro strain of rat.

In the **nephrogenic form of diabetes insipidus**, blood levels of ADH are normal or elevated, but target cells in the distal nephron and collecting ducts are unable to respond due to a lack of adenylate cyclase in the plasma membrane.

Animals with diabetes insipidus excrete large volumes of hypotonic urine, which in turn obliges them to take in equally large amounts of water to prevent hyperosmolality of body fluids and dehydration. Urine osmolality is decreased below normal plasma osmolality (~300 mmol/L) in both hypophyseal and nephrogenic

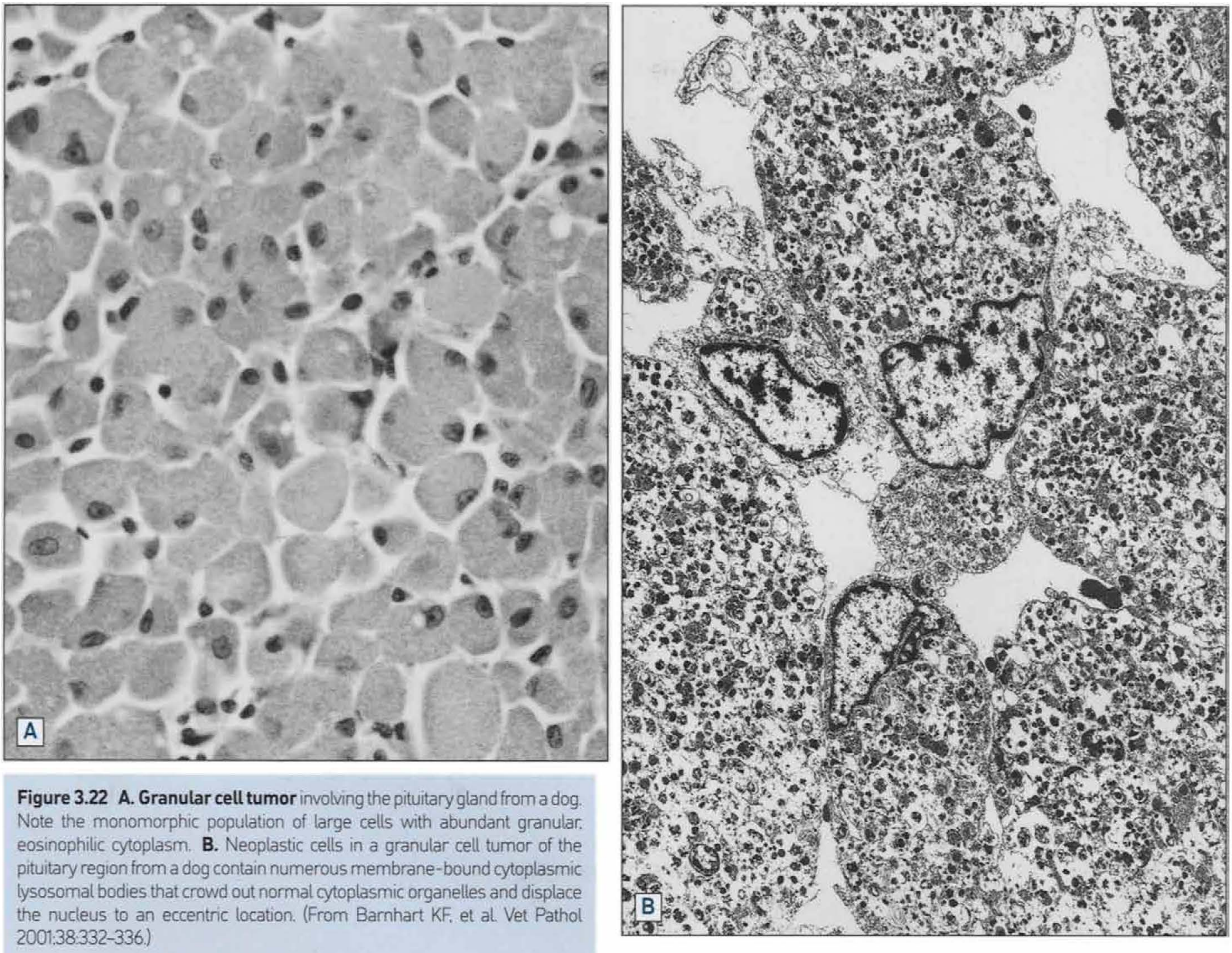

Figure 3.22 **A. Granular cell tumor** involving the pituitary gland from a dog. Note the monomorphic population of large cells with abundant granular, eosinophilic cytoplasm. **B.** Neoplastic cells in a granular cell tumor of the pituitary region from a dog contain numerous membrane-bound cytoplasmic lysosomal bodies that crowd out normal cytoplasmic organelles and displace the nucleus to an eccentric location. (From Barnhart KF, et al. Vet Pathol 2001;38:332–336.)

forms of diabetes insipidus. In response to water deprivation, urine osmolality remains below that of plasma in both forms in contrast to what is observed in normal animals. *The elevation of urine osmolality above that of plasma in response to exogenous ADH in the hypophyseal form, but not in nephrogenic diabetes insipidus, allows separation of these two forms of the disease.*

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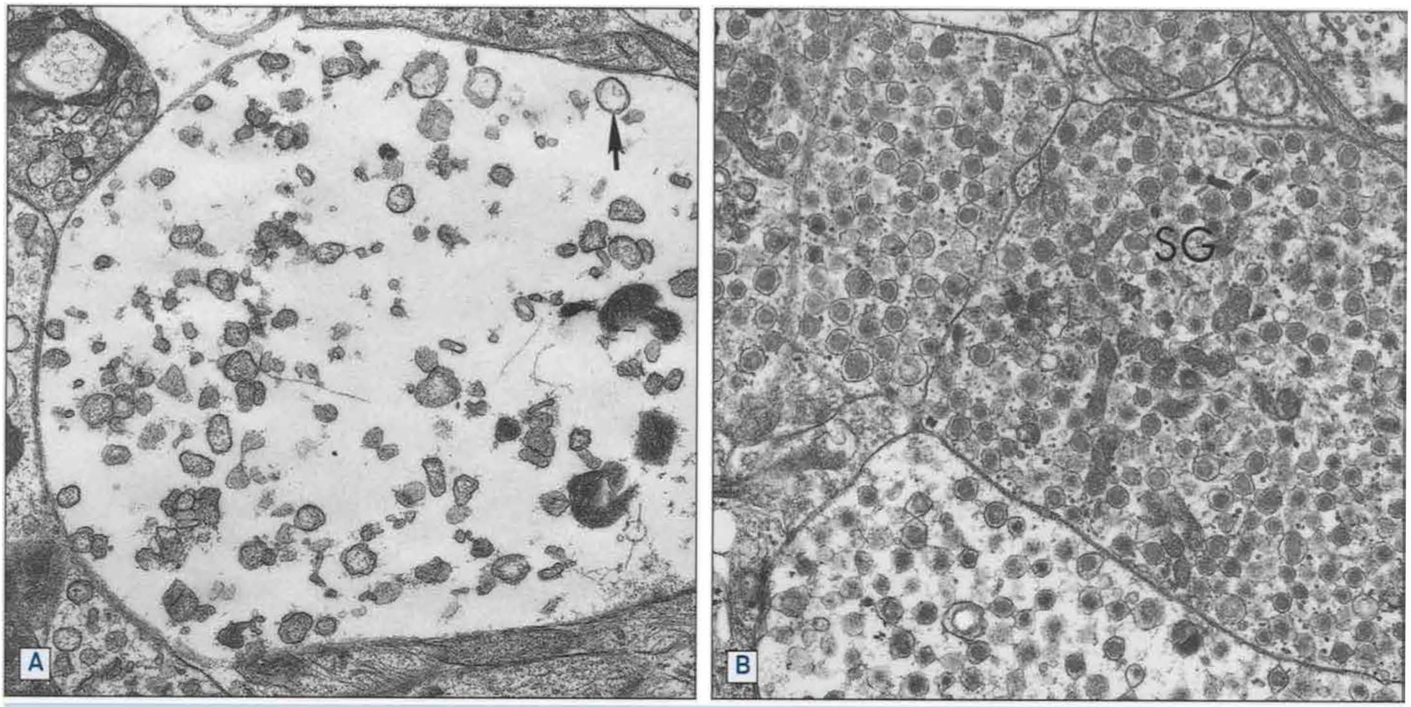

Figure 3.23 A. Cross section of axonal process in pars nervosa of dog with **hypophyseal diabetes insipidus** associated with a pituitary adenoma. Axonal swelling contains few neurosecretory granules with dense cores, but occasional irregularly shaped, empty vesicles (arrow). **B.** Cross section of axonal process in pars nervosa of **normal dog** illustrating numerous membrane-bound, antidiuretic hormone-containing, neurosecretory granules (SG).

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CALCIUM-REGULATING HORMONES

Calcium ion plays a key role in many fundamental biologic processes including muscle contraction, blood coagulation, enzyme activity, neural excitability, hormone release, and membrane permeability, in addition to being an essential structural component of the skeleton. To maintain a constant concentration of calcium, despite variations in intake and excretion, endocrine control mechanisms have evolved that primarily consist of the interactions of three major hormones (Fig. 3.24). Although the direct roles of *parathyroid hormone, calcitonin, and vitamin D* frequently are emphasized in the control of blood calcium, *other hormones such as adrenal corticosteroids, estrogen, thyroxine, somatotropin, and glucagon, also contribute to the maintenance of calcium homeostasis under certain conditions.*

Calcium (Ca) serves two primary functions in the body: *(1) structural integrity of bones and teeth, and (2) as a messenger or regulatory*

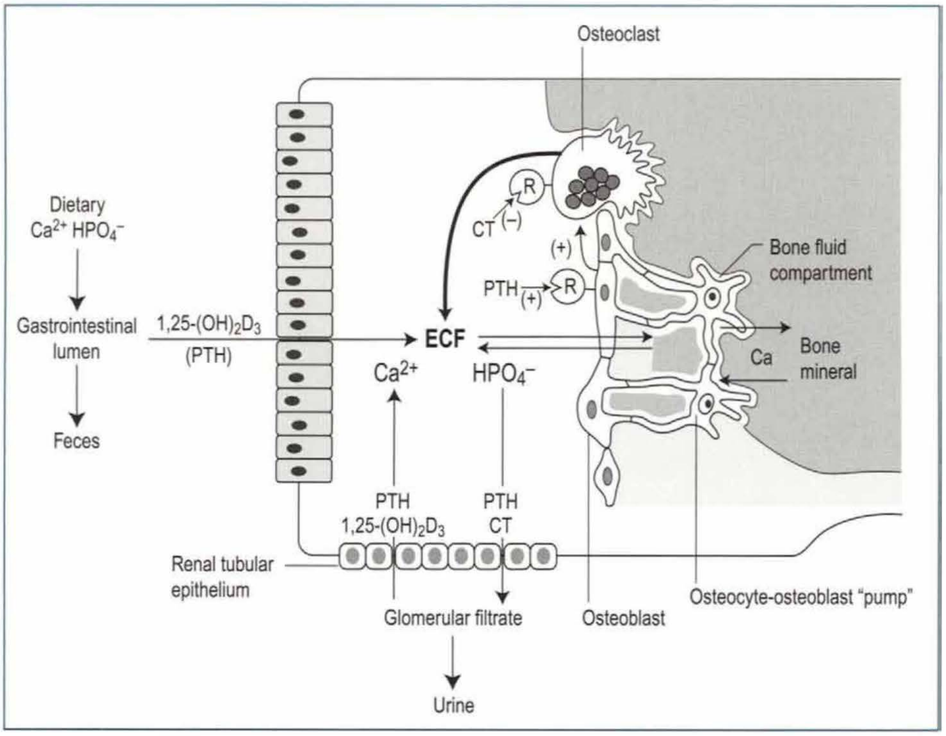

Figure 3.24 Interrelations of parathyroid hormone (PTH), calcitonin (CT), and 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$ D_3) in the **hormonal regulation of calcium and phosphorus** in extracellular fluids (ECF). (From Rosol TJ, Capen CC. In: Clinical Biochemistry of Domestic Animals, 5th ed., 1997.)

ion. The 10 000-fold concentration gradient of calcium ion (Ca^{2+}) between the extracellular fluid (1.2 mmol/L) and the cytoplasm (100 nmol/L) permits Ca^{2+} to function as a signaling ion to activate intracellular processes. The lipid bilayer of the cell membrane has a low permeability to Ca^{2+}; therefore, influx of Ca^{2+} into the cytoplasm is controlled by a heterogeneous group of *Ca channels* regulated by membrane potential, cell membrane receptors or intracellular secondary messengers. Influx of Ca^{2+} into cells can: (1) regulate cellular function by interactions with Ca-binding proteins (e.g., calmodulin) and Ca-sensitive protein kinases, and (2) stimulate biologic responses such as neurotransmitter release, contraction, and secretion. *Ionized calcium (Ca^{2+}) also plays an important role in cell adhesion and blood coagulation.* In addition, Ca^{2+} may regulate cellular function by binding to a G-protein-linked Ca^{2+}-sensing receptor in the cell membrane, such as in parathyroid chief cells or renal epithelial cells.

It is critical for cells to maintain the normal low level of intracellular Ca^{2+}. If cellular calcium homeostasis fails due to anoxia, an energy-deprived state or perturbed membrane integrity, cell viability is threatened due to uncontrolled entry of Ca^{2+} through the plasma membrane or from intracellular stores.

There are two sources of calcium in bone that can enter the circulation: (1) readily mobilizeable Ca salts in the extracellular fluid, and (2) hydroxyapatite crystals that require digestion by osteoclasts before Ca^{2+} can be released from bone. The nature and regulation of the readily mobilizable Ca in bone is poorly understood; however, it is present in small amounts and likely plays a role in the fine regulation of serum Ca concentration. If there is a significant need for Ca from bone it must come from osteoclastic resorption of hydroxyapatite crystals. In adult animals, there is a stable balance between Ca deposition associated with bone formation by osteoblasts and Ca release associated with osteoclastic bone resorption. In young animals, bone has a positive Ca balance due to the relative excess of bone formation. Conditions that result in excessive bone resorption (e.g., humoral hypercalcemia of malignancy, osteolytic bone metastases, or primary hyperparathyroidism), can release large amounts of Ca from bone and contribute to the development of life-threatening hypercalcemia.

Parathyroid glands and parathyroid hormone

Development and structure

Parathyroids are of entodermal origin and derived from the third and fourth pharyngeal pouches in close association with the primordia of the thymus (Fig. 3.25). Anatomic location of parathyroid glands in relation to thyroids and related structures in several species of animals is shown in Fig. 3.26.

The parathyroid glands contain a single basic type of secretory cell, the chief cell, which is concerned with the elaboration of a single hormone. The chief cells are in different stages of secretory activity, and in certain species are in transition to oxyphil cells. Chief cells interpreted to be in an inactive (resting or involuted) stage of their secretory cycle predominate in the parathyroid glands under normal conditions. Inactive chief cells are cuboidal and have uncomplicated interdigitations between contiguous cells. The relatively electron-transparent cytoplasm contains poorly developed organelles and secretory granules are sparse. The cytoplasm often has either numerous lipid bodies and lipofuscin granules or aggregations of glycogen. Chief cells in the active stage of the secretory cycle are in the minority in the parathyroid glands of most species. The cytoplasm of active chief cells has increased electron density due to the close proximity of organelles and secretory granules, increased density of the cytoplasmic matrix, and loss of glycogen particles and lipid bodies.

The second cell type in the parathyroid glands of certain species, including humans, is the **oxyphil cell**. These cells are absent in rats,

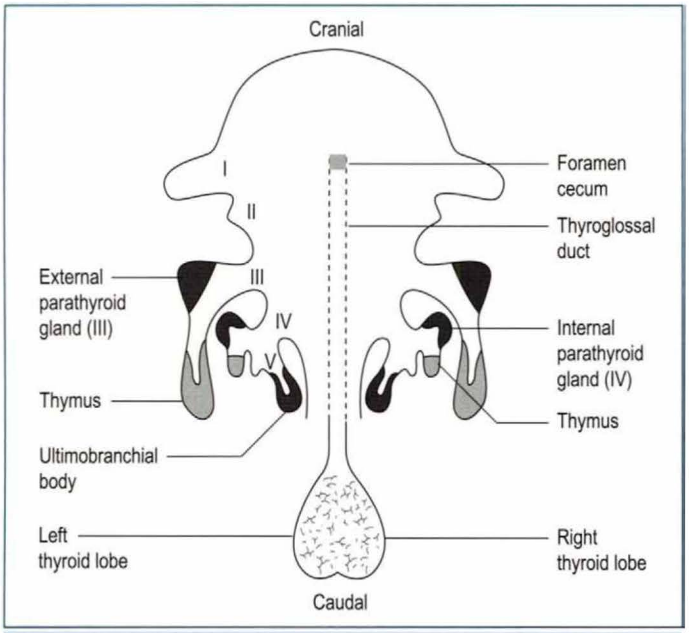

Figure 3.25 Embryologic development of thyroid and parathyroid glands.

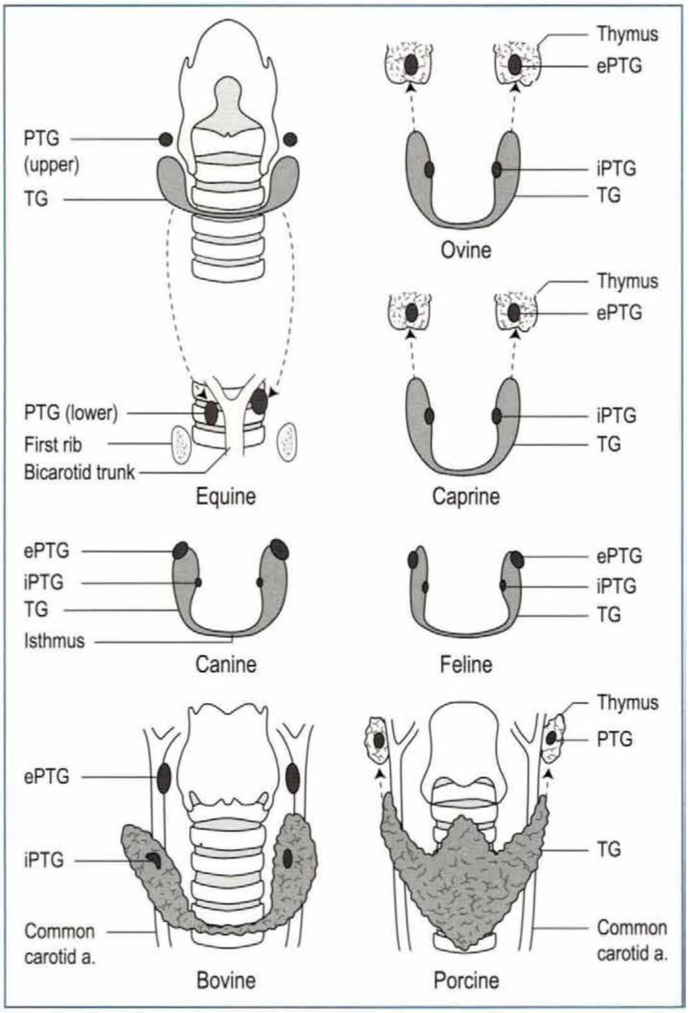

Figure 3.26 Anatomic location of parathyroids and thyroids in various species. A = artery. ePTG = external parathyroid gland. iPTG = internal parathyroid gland. TG = thyroid gland. (Modified from Grau H. Dellmann HD. Z Mikrosk Anat Forsch 1958;64:192–214.)

chickens, and many species of lower animals. Oxyphil cells occur either singly or in small groups interspersed between chief cells. They are larger than chief cells and their abundant cytoplasm is filled with numerous large, often bizarre-shaped, mitochondria. Glycogen particles and free ribosomes are interspersed between the mitochondria. Granular endoplasmic reticulum, Golgi apparatuses, and secretory granules are poorly developed in oxyphil cells of normal parathyroid glands, *suggesting that oxyphil cells do not have an active role in the biosynthesis of parathyroid hormone.* Oxyphil cells have a higher oxidative and hydrolytic enzyme activity than chief cells, associated with the marked increase in numbers of mitochondria.

Cells are observed with cytoplasmic characteristics intermediate between those of chief and oxyphil cells. These **transitional oxyphil cells** have numerous mitochondria but other organelles are present, including rough endoplasmic reticulum, Golgi apparatuses, and secretory granules.The significance of oxyphil cells in the pathophysiology of the parathyroid glands is not completely understood. They are not altered in response to either short-term hypocalcemia or hypercalcemia in animals, but both oxyphil cells and transitional forms may be increased in response to long-term stimulation of human parathyroid glands.Therefore, oxyphil cells do not appear to be degenerate chief cells, but rather are derived from chief cells as the result of aging or some other metabolic derangement.

Biosynthesis and secretion of parathyroid hormone

The production of parathyroid hormone involves the biosynthesis of a large precursor on ribosomes of the rough endoplasmic reticulum in chief cells. This initial translation product is **pre-proparathyroid hormone** (pre-proPTH). It is composed of 115 amino acids and contains a hydrophobic signal sequence of 25 amino acid residues that facilitates the penetration and subsequent vectorial discharge of the nascent peptide into the cisternal space of the rough endoplasmic reticulum. Pre-proPTH is rapidly converted (within 1 min or less) to **proparathyroid hormone** (proPTH) by the proteolytic cleavage of 25 amino acids from the NH_2-terminal end of the molecule. The intermediate precursor, proPTH, is composed of 90 amino acids and moves within the membranous channels of the rough endoplasmic reticulum to the Golgi apparatus. Enzymes within membranes of the Golgi apparatus cleave a hexapeptide from the NH_2-terminal (biologically active) end of the molecule forming active parathyroid hormone. Active PTH is packaged into membrane-limited, macromolecular aggregates **(secretion granules)** in the Golgi apparatus for subsequent storage in chief cells. Under certain conditions of increased demand, PTH may be released directly from chief cells without being packaged into secretion granules.

Biologically active parathyroid hormone secreted by chief cells is a straight chain polypeptide consisting of 84 amino acid residues with a molecular weight of 9500. Although the principal form of active parathyroid hormone secreted from chief cells is a straight chain peptide of 84 amino acids, the molecule is rapidly cleaved into amino-and-carboxy-terminal fragments in the peripheral circulation and especially in the liver. The purpose of this fragmentation is uncertain since the biologically active amino-terminal fragment is no more active than the entire (1-84) PTH molecule. The plasma half-life of the N-terminal fragment is considerably shorter than that of the biologically inactive carboxy-terminal fragment of parathyroid hormone. The C-terminal and other portions of the PTH molecule are degraded primarily in the kidney and tend to accumulate in the circulation with chronic renal disease.

Chief cells synthesize and secrete another major protein termed **"parathyroid secretory protein (I)"** or **chromogranin A**. It has a higher molecular weight than PTH being composed of 430–448 amino acids, and is *co-stored and secreted with parathyroid hormone.* A similar molecule has been found in secretory granules of a wide variety of peptide–hormone-secreting cells (e.g., thyroid C cells, pancreatic islets, adrenal medulla, endocrine cells of the gastrointestinal tract, among others) and in neurotransmitter secretory vesicles of sympathetic nerves. An internal region of the parathyroid secretory protein or chromogranin A molecule is identical in sequence to **pancreastatin**, a C-terminal amidated peptide that inhibits glucose-stimulated insulin secretion. This 49 amino acid proteolytic cleavage product (240–280 amino acids) of parathyroid secretory protein *inhibits low calcium-stimulated secretion of parathyroid hormone and chromogranin A from parathyroid cells.* These findings suggest that one function of chromogranin A-derived peptides is to act locally in an autocrine manner to inhibit the secretion of active hormone by endocrine cells, such as those of the parathyroid gland.

Chromogranin A (CGA) shares considerable homology between species. Immunologic cross reactivity to mammalian proteins has been observed in reptiles, amphibians, fish, and *Drosophila* tissues. Chromogranin A is synthesized as a pre-protein and is directed to the internal cavity of the rough endoplasmic reticulum (RER) by the N-terminal pre-region of the peptide. Once inside the RER, the pre-region is cleaved by a signal peptidase. *Although the functions of CGA are still under investigation, several roles have been postulated.* Chromogranin A is suspected to play an important role in the maturation of secretory granules. Inside the Golgi apparatus, CGA is involved in the packaging of contents into newly formed vesicles. Chromogranin A precipitates as it diffuses into the trans-Golgi network. Other secretory products such as parathyroid hormone become entrapped in the growing CGA conglomerate and subsequently are packaged into a secretory granule. Chromogranin A has a large calcium-binding capacity that may enhance vesicle stability. As secretory granules mature, they accumulate large amounts of calcium (up to 40 mmol/L), which also may serve as a route of Ca^{2+} secretion. However, free (ionized) calcium concentrations remain in the micromolar range since most Ca^{2+} is bound to CGA. Chromogranin A-calcium complexes are important in maintaining the integrity of the secretory granule, since the absence of calcium will cause dissociation of protein complexes and result in osmotic lysis of the vesicle. Therefore, *the intragranular functions of CGA include hormone packaging, stabilization of the granule against osmotic gradients, and excretion of intracellular calcium.*

During the process of secretion, the contents of secretory granules are extruded into the pericapillary spaces. The pH and calcium concentration of the extracellular fluid promote dissociation of CGA complexes and solubilization of its bound calcium and other contents of the granule. Once solubilized, extracellular peptidases cleave CGA into biologically active peptides that act as paracrine or autocrine regulators of secretion. Most of the CGA-derived peptides have been reported to decrease hormone secretion.

Secretory cells in the parathyroids of most animals store relatively small amounts of preformed hormone but are capable of responding to minor fluctuations in calcium ion concentration by rapidly altering the rate of hormonal secretion, and more slowly by altering the rate of hormonal synthesis. In contrast to most endocrine organs that are under complex controls, *the parathyroids have a unique feedback control system based primarily on the concentration of calcium (and to a lesser extent of magnesium) ion in blood.* If the blood calcium is elevated, then circulating levels of parathyroid hormone rapidly and markedly decrease. Conversely, if the blood calcium is lowered, parathyroid hormone levels increase. The concentration of blood phosphorus has no direct regulatory influence on the synthesis and secretion of the hormone; however, certain disease conditions with hyperphosphatemia are associated clinically with secondary hyperparathyroidism. An elevated blood phosphorus level may lead indirectly to parathyroid stimulation by virtue of its ability to lower blood calcium according to the mass-law equation when the serum is saturated with these two ions. Hyperphosphatemia also suppresses the rate of formation of the biologically active, hormonal form of vitamin D_3 (1,25-dihydroxycholecalciferol) in the kidney, which further contributes to the development of hypocalcemia and parathyroid stimulation.

Magnesium ion has an effect on parathyroid secretory rate similar to that of calcium, but its effect is not equipotent to that of calcium. The more potent effects of calcium ion in the control of parathyroid hormone secretion, together with its preponderance over magnesium in the extracellular fluid, suggest a *secondary role* for magnesium in parathyroid control.

Calcium ion controls not only the rate of biosynthesis and secretion of parathyroid hormone, but also other metabolic and intracellular degradative processes within chief cells. Increased calcium ion in extracellular fluids rapidly inhibits the uptake of amino acids by chief cells, synthesis of proparathyroid hormone and conversion to active parathyroid hormone, and secretion of the stored hormone. The shifting of the percentage of flow of proparathyroid hormone from the degradative pathways to the synthetic route represents a key adaptive response of the parathyroid gland to a low-calcium diet. During periods of long-term calcium restriction, the enhanced synthesis and secretion of parathyroid hormone is accomplished by an increased capacity of the entire pathway in individual chief cells and subsequently through hyperplasia of active chief cells. Recently synthesized and processed active parathyroid hormone may be released directly in response to increased demand and bypass the chief cell's storage pool of mature secretory granules in the cytoplasm. *Bypass secretion of parathyroid hormone* can be stimulated only by a low circulating concentration of calcium ion and not by other secretagogues such as cyclic adenosine monophosphate and beta agonists (epinephrine, norepinephrine and isoproterenol) for parathyroid hormone. Degradation of "mature parathyroid hormone" by lysosomal enzymes occurs after prolonged exposure of chief cells to a high calcium environment.

Serum calcium ion (Ca^{2+}) binds to a Ca receptor on the chief cell that permits the serum Ca^{2+} to regulate chief cell function. The receptor is present on the plasma membranes of parathyroid chief cells, renal epithelial cells, and other cells that respond to extracellular Ca^{2+}. Mutations in one or both of the Ca^{2+}-sensing receptor genes in humans result in familial hypocalciuric hypercalcemia or neonatal severe hypercalcemia, respectively. Interaction of serum Ca^{2+} with its receptor on chief cells results in the formation of an inverse sigmoidal relationship between serum Ca^{2+} and PTH concentrations. The serum $[Ca^{2+}]$ that results in half maximal PTH secretion is defined as the serum calcium "set-point" and is stable for an individual animal. The sigmoidal relationship between serum [Ca^{2+}] and PTH secretion permits the chief cells to respond rapidly to a reduction in serum Ca^{2+}. *The major inhibitors of PTH synthesis and secretion are increased serum [Ca^{2+}] and 1,25-dihydroxyvitamin D_3.* Inhibition of PTH synthesis by 1,25-dihydroxyvitamin D_3 completes an important endocrine feedback loop between the parathyroid chief cells and the renal epithelial cells, since PTH stimulates renal production of 1,25-dihydroxyvitamin D_3.

Biologic action of parathyroid hormone

Parathyroid hormone is the principal hormone involved in the minute-to-minute, fine regulation of blood calcium. It exerts its biologic actions by directly influencing the function of target cells primarily in bone and kidney, and indirectly in the intestine.

The action of PTH on bone is to mobilize calcium from skeletal reserves into extracellular fluids. The administration of parathyroid hormone causes an initial decline followed by a sustained increase in circulating levels of calcium. The transitory decrease in blood calcium probably results from a sequestration of calcium phosphate in bone and soft tissues. The subsequent increase in blood calcium results from an interaction of parathyroid hormone with osteocytes and (indirectly) osteoclasts in bone. The initial binding of parathyroid hormone to osteoblasts lining bone surfaces causes the cells to contract, thereby exposing the underlying osteoid-covered bone matrix. Osteoblasts secrete latent collagenase and neutral proteases that result in degradation of the osteoid and expose the underlying bone mineral, which attracts osteoclasts.

The response of bone to parathyroid hormone is *biphasic*. The immediate effects are the result of increasing the activity of existing osteoclasts and osteocytes present in bone. This results in an increased flow of calcium from deep in bone-to-bone surfaces through the coordinated action of osteocytes and endosteal lining cells (inactive osteoblasts). Osteoclasts are primarily responsible for the long-term action of parathyroid hormone on increasing bone resorption and overall bone remodeling. If the increase in parathyroid hormone is sustained, the active osteoclast pool in bone is increased and the mineral and organic components (e.g., hydroxyproline) released from bone are released into the extracellular fluid compartment. A long-term increase in parathyroid hormone secretion, such as occurs with certain disease conditions, may also result in the formation of greater numbers of osteoblasts with a resultant increase in bone formation as well as resorption. However, bone resorption usually is greater than bone formation, leading to a net negative skeletal balance.

Osteoclasts are primarily responsible for the catabolic action of PTH on bone by increasing resorption. PTH not only stimulates increased activity of preformed osteoclasts, but increases their differentiation from precursor cells of the osteoclast lineage. However, receptors for PTH are present on osteoblasts, not on osteoclasts. Isolated osteoclasts respond to PTH only in the presence of osteoblasts. The mechanism by which binding of PTH to osteoblasts results in stimulation of osteoclasts appears to include direct effects on the osteoblast as well as the local release of factors by osteoblasts that are capable of stimulating osteoclastic bone resorption (Fig. 3.27).

Bone resorption is a complex, multistep, process that involves the activation of multiple genes and the action of multiple hormones. Most hormones and cytokines that are involved in the regulation of bone resorption (e.g., parathyroid hormone, calcitriol, interleukin-1β, tumor necrosis factor (TNF), interleukin-6, prostaglandin E_2) act via receptors on osteoblasts. Three new family members of the TNF ligand and receptor signaling system that have been identified and cloned recently play a critical role in the regulation of bone resorption. In the presence of permissive concentrations of macrophage colony-stimulating factor (M-CSF), these three new TNF superfamily molecules constitute the common pathway for regulation of osteoclast formation and function by cells of the osteoblast lineage, thereby mediating the biologic effects of many upstream hormones and cytokines.

The **receptor ligand (RANK ligand** or **RANKL)** is a membrane-bound protein on osteoblasts that serves as a common mediator for osteoclastic bone resorption. This ligand (initially termed **osteoclast differentiation factor (ODF)** and **osteoprotegrin ligand (OPGL)**) is produced by osteoblast lineage cells and *stimulates differentiation of cells of the osteoclast lineage, enhances the functional activity of mature osteoclasts, and prolongs osteoclast life by inhibiting apoptosis.* Targeted deletion of the receptor ligand in mice leads to osteopetrosis, shortened bones, impaired tooth eruption, and immunologic abnormalities. Expression of RANKL on RNA in osteoblasts/stromal cells is up-regulated by osteotrophic factors such as calcitriol, PTH, and interleukin-11. This molecule is identical to TNF-related activation-induced cytokine (TRANCE) or receptor activator of NF-kappa B ligand (RANKL) reported to stimulate T-cell growth and dendritic cell function in the immunology literature.

The **membrane receptor (RANK)** for the ligand (RANKL) on cells of the osteoclast lineage is identical with a receptor of similar name identified previously on immune cells. The receptor also has been referred to in the literature as **osteoclast differentiation and activation receptor (ODAR)**. *Binding of the ligand to this receptor activates signaling pathways in osteoclasts that lead to increased functional activity.* Osteoclast precursors express RANK, recognize RANKL through cell-to-cell interaction with osteoblasts/stromal cells, and differentiate into prefusion osteoclasts in the presence of M-CSF. Mice made deficient in RANK by targeted deletion of the gene develop severe osteopetrosis due to decreased osteoclast function and fail to develop peripheral lymph nodes.

Osteoprotegrin (OPG) ("decoy receptor") is a novel member of the TNF receptor superfamily produced by cells of the osteoblast lineage/stromal cells and a negative regulator of bone resorption. *When this soluble decoy receptor binds to the ligand (RANKL), it prevents the ligand from binding to its biological membrane receptor (RANK) thereby resulting in its inactivation.* Overexpression of this decoy receptor in transgenic mice leads to osteopetrosis associated with decreased osteoclast formation and function. In contrast, targeted gene ablation in mice results in severe osteoporosis and arterial mineralization.

These findings clearly indicate that *the microenvironment in bone marrow provided by osteoblast lineage/stromal cells regulates osteoclast differentiation and function.* Osteoclast formation appears to be determined by the ratio of RANKL:OPG, and alterations in this ratio may be a major cause of bone loss in certain metabolic disorders such as estrogen deficiency and glucocorticoid excess.

Binding of PTH to specific receptors on bone cells results in the activation of adenylate cyclase in the plasma membrane. Adenylate cyclase catalyzes the conversion of ATP to cAMP in target cells. Accumulation of cAMP in target cells functions as an intracellular

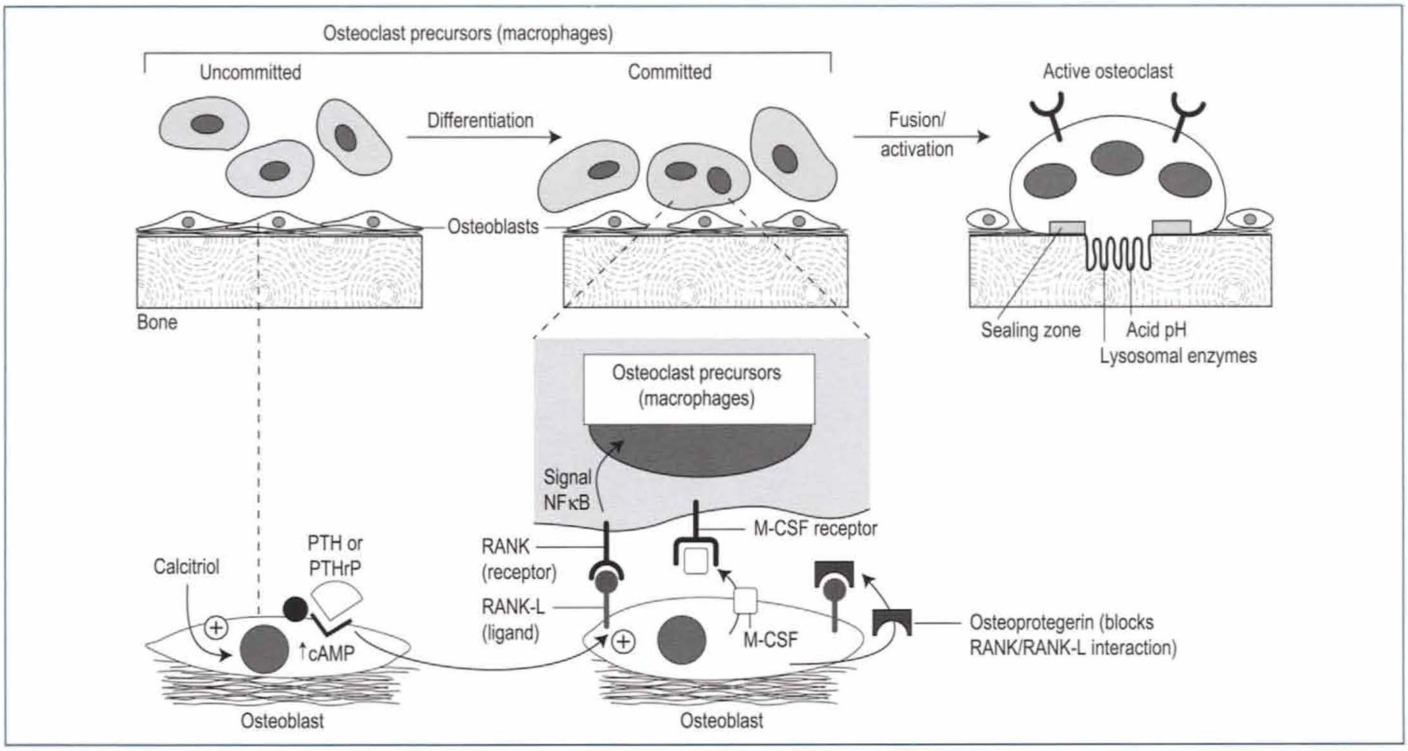

Figure 3.27 Cellular control of bone resorption. Specific receptors for parathyroid hormone (PTH) are present on osteoblasts but not on osteoclasts. RANK-L, or osteoclast differentiation factor, is produced by cells of the osteoblast lineage, serves as a common mediator for osteoclastic bone resorption, and its expression is up-regulated by osteotrophic factors such as PTH, PTHrP and calcitriol. M-CSF = macrophage colony stimulating factor; R = receptor for either PTH or PTHrP; RANK-L = receptor activator NF-kappa B ligand.

messenger of PTH action in osteoblasts. Parathyroid hormone also induces an increase in cytoplasmic calcium and stimulates phosphatidylinositol turnover in osteoblasts. The increase in cytosolic calcium is partially dependent on cAMP accumulation. Calcium concentration in the osteoblast may also be increased by the activation of protein kinase C, resulting in the production of inositol triphosphate and subsequent release of calcium from the endoplasmic reticulum. PTH also appears to use a second intracellular messenger in target cells by stimulating inositol triphosphate and diacylglycerol production.

Parathyroid hormone has a rapid (5–10 min) and direct effect on renal tubular function leading to decreased reabsorption of phosphorus and phosphaturia. The site where PTH blocks tubular reabsorption of phosphorus has been localized by micropuncture methods to the proximal tubule of the nephron. PTH binds to a receptor on the basolateral aspect of renal epithelial cells. The hormone stimulates adenylate cyclase, increases intracellular cAMP, and inhibits phosphorus reabsorption across the brush border through the actions of protein kinases. The parathyroid hormone receptor has been cloned and sequenced from cDNA isolated from kidney cells. The PTH receptor has a striking degree of sequence homology (~56%) with the calcitonin receptor, but lacks similarity with other G protein-linked receptors. The receptors for these calcium-regulating hormones belong to a family of G protein-linked receptors with seven transmembrane spanning domains that activate adenylate cyclase.

Although the effects of PTH on the tubular reabsorption of phosphorus have been considered to be of major importance, there is substantial evidence that the ability of PTH to enhance the reabsorption of calcium contributes in a significant manner to the maintenance of calcium homeostasis. *This effect of PTH upon tubular reabsorption of calcium appears to be due to a direct action on the distal convoluted tubule.* The biochemical mechanism by which PTH enhances calcium reabsorption also is coupled to increases in intracellular cAMP.

The other important effect of parathyroid hormone is the regulation of the conversion of inactive 25-hydroxycholecalciferol to biologically active (1,25-dihydroxycholecalciferol) and other metabolites of vitamin D_3. Under normal conditions parathyroid hormone is secreted continuously from chief cells. In the liver, kidney, peripheral circulation, and at target cells, PTH is cleaved into a biologically active (N-terminal) and a biologically inactive C-terminal portion.

Age-related changes in the parathyroid gland

Parathyroid structure has been evaluated by light and electron microscopy in neonatal (2–6 days of age), growing (2–5 months), and adult (2–3 years) dogs. **Dark (active) chief cells** with an electron-dense cytoplasm, prominent rough endoplasmic reticulum and Golgi apparatuses, numerous membrane-limited secretory granules, and frequent interdigitations of the plasma membrane predominated in the parathyroid glands of growing and adult dogs. Parathyroid glands of neonatal dogs were different, with more numerous **light (inactive) chief cells** that were interpreted to be cells in transitional stages with slightly distended cisternae of rough endoplasmic reticulum, inconspicuous Golgi apparatuses, and few secretory granules. Ultrastructural morphometric analysis has established that the volume of the predominant parathyroid cell in neonatal dogs is significantly less than that of chief cells of growing dogs, but the cell surface was not significantly different between groups. The volume of chief cells occupied by mitochondria, rough endoplasmic reticulum, and lysosomes in growing dogs is greater than in neonatal dogs, but the surface of the Golgi apparatus and rough endoplasmic reticulum did not differ between groups. These findings suggest that the secretory activity is greater in parathyroid glands of neonatal dogs than in those of growing dogs. *The storage capacity for PTH is low in the canine parathyroid gland (similar to other species), and the biosynthesis of PTH by chief cells can be stimulated or inhibited within minutes by changes in the circulating concentration of calcium ion.*

Thyroid C cells and calcitonin

Development and structure of thyroid C cells

A second calcium-regulating hormone, **calcitonin**, secreted by the **thyroid C cells** in response to hypercalcemia, lowers plasma calcium. C cells are distinct from follicular cells, which secrete thyroxine and triiodothyronine. They are situated either within the follicular wall between follicular cells or as small groups between follicles. They do not border the follicular colloid directly, and their secretory polarity is oriented toward the interfollicular capillaries.

The **ultimobranchial body** (last, usually fifth pharyngeal pouch), which delivers the neural crest-derived C cells to the postnatal thyroid gland, fuses with each thyroid lobe at the hilus and distributes C cells throughout each lobe to various degrees in different species. In the dog, nodular aggregates of C cells frequently persist along the course of the major vessels to the thyroid. There are occasional ultimobranchial-derived, colloid-containing follicles within the focal accumulations of C cells along the course of vessels within the thyroid lobe or in the connective tissues of the thyroid hilus in dogs. Islands of lightly stained cells, termed **C-cell complexes**, are present in the thyroid glands (especially prominent in dogs) often near the internal parathyroid gland. They are remnants of the ultimobranchial body that can give rise to thyroid follicles and C cells, and should not be misinterpreted as areas of C-cell hyperplasia. *C-cell complexes in the thyroid gland contain four cell types*:

1. *follicular cells* that stain positive for thyroglobulin,
2. *C cells* staining positive for calcitonin, calcitonin gene-related peptide, and neuron-specific enolase,
3. *stellate and cuboidal cells* forming follicle-like structures that stain positive for somatostatin, and
4. *undifferentiated cells* staining negative for all antigens.

The C-cell complex may be the origin of the unique **mixed thyroid carcinoma** (differentiated thyroid carcinoma, intermediate type) in human patients or **ultimobranchial thyroid neoplasms** in bulls that are composed of cells that have a positive immunoreactivity for both thyroglobulin and calcitonin.

Cysts derived from remnants of the ultimobranchial body are observed frequently in the postnatal thyroid of rats lined by squamous epithelium and containing keratin debris. Occasional follicles derived from ultimobranchial primordia contain heterogeneous material in the lumen and scattered ciliated epithelial cells in the follicular wall. In rats, CT-immunoreactive cells have been demonstrated in the wall of the ultimobranchial tubule as well as thyroglobulin-positive staining in adjacent follicles and solid clusters. These findings, plus the presence of numerous mitoses and PAS-positive microfollicles, support the hypothesis that the ultimobranchial body contributes to the formation of follicles in certain areas of the thyroid lobes.

In submammalian species, C cells and calcitonin activity remain segregated in the **ultimobranchial gland**, which is anatomically distinct from both the thyroid and the parathyroid glands. In the avian ultimobranchial gland, a network of stellate cells with long cytoplasmic processes supports the C cells.

The concentration of calcium ion in plasma and extracellular fluids is the principal physiologic stimulus for the secretion of calcitonin by C cells. Calcitonin is secreted continuously under conditions of normocalcemia, but the rate of secretion is increased greatly in response to elevations in blood calcium. Magnesium ion has an effect on calcitonin secretion similar to that of calcium, but this effect is observed only under experimental conditions with nonphysiologic levels.

Biosynthesis and secretion of calcitonin

The complete amino acid sequence of porcine, bovine, ovine, canine, salmon, and other animal species, as well as human calcitonin has been determined. Synthetic human calcitonin has been prepared and shown to be biologically active. The structure of calcitonin differs considerably between species. The molecules in 8 selected species share only 9 of the 32 amino acid residues. However, the amino terminal of the calcitonin molecule is similar in all species. It consists of a seven-membered ring enclosed by an intrachain disulfide bridge. The complete sequence of 32 amino acids and the disulfide bond are essential for full biologic activity. Salmon calcitonin is more potent in lowering blood calcium on a weight basis than any of the other calcitonins when administered to mammals, including humans. The reason for the greater biologic potency of salmon calcitonin in mammals is uncertain but probably is related to an increased resistance to metabolic degradation or a greater affinity for receptor sites in bone and other target tissues.

Hyperplasia of C cells occurs in response to long-term hypercalcemia. When the blood calcium is lowered, the stimulus for calcitonin secretion is diminished and numerous secretory granules accumulate in the cytoplasm of C cells. The storage of large amounts of preformed hormone in C cells and rapid release in response to moderate elevations in blood calcium probably reflect the *physiologic role of calcitonin as an "emergency" hormone to protect against the development of hypercalcemia.*

Calcitonin secretion is increased in response to a high-calcium meal, often before a significant rise in plasma calcium can be detected. The cause of this increase in calcitonin secretion could be either a small undetectable rise in plasma ionized calcium or direct stimulation by the oral calcium load of certain gastrointestinal hormones, which in turn act as secretagogues for calcitonin release from the thyroid gland. Gastrin, pancreozymin, and glucagon all stimulate calcitonin release under experimental conditions. Thus **gastrointestinal hormones** may be important in triggering the early release of calcitonin to prevent the development of hypercalcemia following ingestion of a high-calcium meal.

Biologic action of calcitonin

Calcitonin exerts its function by interacting with target cells primarily in bone and kidney. *The actions of parathyroid hormone and calcitonin are antagonistic on bone resorption but synergistic on decreasing the renal* *tubular reabsorption of phosphorus*. The hypocalcemic effects of calcitonin are primarily the result of decreased entry of calcium from the skeleton into plasma due to transient inhibition of parathyroid hormone-stimulated bone resorption. The hypophosphatemia develops from a direct action of calcitonin increasing the rate of movement of phosphate out of plasma into soft tissue and bone, as well as from the inhibition of bone resorption. The action of calcitonin is not dependent on vitamin D, since it acts both in vitamin D-deficient animals and following the administration of large doses of vitamin D.

The action of calcitonin on inhibiting bone resorption stimulated by parathyroid hormone and other factors results from blockage of osteoclastic bone resorption. Calcitonin produces specific structural alterations osteoclasts, which have specific receptors for the hormone on their surfaces. Osteoclasts withdraw from resorptive surfaces and the brush border and transitional zone become atrophic. In addition, there are fewer osteoclasts in bone. Although calcitonin can block bone resorption completely, the inhibition is a transient effect. Eventually the effects of parathyroid hormone on increasing bone resorption become manifest in the presence of calcitonin.

Both calcitonin and parathyroid hormone decrease renal tubular reabsorption of phosphate, leading to phosphaturia; however, the adenylate cyclase-linked receptors for calcitonin are found in the ascending limb of the loop of Henle and the distal convoluted tubule. In addition, calcitonin results in diuresis of sodium, chloride, and calcium, whereas parathyroid hormone causes renal retention of calcium and hydrogen ions.

Serum calcitonin is best measured by radioimmunoassay. Due to the low degree of homology of calcitonin between species, there is poor cross-reactivity of radioimmunoassays (RIAs) for calcitonin. In veterinary medicine, the need to measure circulating levels of calcitonin is associated with calcitonin-secreting thyroid neoplasms and other clinical disorders resulting from abnormal blood levels of calcitonin. In humans, calcitonin RIA is useful to identify functional C-cell (medullary) tumors of the thyroid gland. RIAs for calcitonin are necessary also to experimentally investigate regulation of C cells in vivo or in vitro. Since the sequencing of canine calcitonin, a canine-specific RIA has been developed and has been used to measure serum calcitonin in dogs.

Calcitonin and parathyroid hormone, acting in concert, provide a dual negative feedback control mechanism to maintain the concentration of calcium in extracellular fluids within narrow limits. Parathyroid hormone is the major factor involved in the minute-to-minute regulation of blood calcium under normal conditions. In most higher mammals that live in a relatively low-calcium high-phosphorus environment, protection against the development of hypocalcemia by parathyroid hormone is a life-sustaining function. *Calcitonin appears to function more as an "emergency" hormone* to prevent the development of hypercalcemia during the rapid postprandial absorption of calcium and to protect against excessive loss of calcium and phosphorus from the maternal skeleton during pregnancy.

Cholecalciferol (vitamin D_3)

Biosynthesis of vitamin D_3 and metabolites

The third major hormone involved in the regulation of calcium metabolism and skeletal remodeling is **cholecalciferol** or **vitamin D_3**. *Although this compound has been designated a vitamin for a long time, it can equally be considered a hormone.* Cholecalciferol is ingested in small amounts in the diet and can be synthesized in the epidermis from precursor molecules (e.g., 7-dehydrocholesterol) through a previtamin D_3 intermediate form. This reaction is catalyzed by ultraviolet irradiation (wave length 2900–3200 Å) from the sun. A high-affinity vitamin D-binding protein transports cholecalciferol in the blood.

Vitamin D must be metabolically activated before it can produce its known physiologic functions. Vitamin D_3 from dietary sources is absorbed by facilitated diffusion and bound to an α-2 globulin in the blood for transport. Endogenous cholecalciferol synthesized in the skin from 7-dehydrocholesterol also is protein-bound for transport to the liver. In the liver, cholecalciferol is converted to 25-hydroxycholecalciferol ($25(OH)D_3$) by a hepatic microsomal enzyme, **calciferol-25-hydroxylase**, which is associated with the endoplasmic reticulum in hepatocytes.

This first (inactive) metabolite, 25-hydroxycholecalciferol ($25(OH)D_3$), is transported to the kidney and undergoes further transformation to a more polar active metabolite, 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$). Other metabolites are formed, such as 24,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$). The formation of $1,25(OH)_2D_3$ is catalyzed by **25-hydroxycholecalciferol-1-α-hydroxylase** (1-α-hydroxylase) in mitochondria, primarily in cells of the proximal convoluted tubule. *The conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ is the rate-limiting step in vitamin D metabolism* and is the primary reason for the delay between vitamin D administration and expression of its biologic effects.

The control of this final step in the metabolic activation is complex and is regulated in part by the plasma calcium concentration and its influence on the rate of secretion of parathyroid hormone. Parathyroid hormone and conditions that stimulate its secretion increase the transformation of $25(OH)D_3$ to $1,25(OH)_2D_3$. Low blood phosphorus increases the formation of $1,25(OH)_2D_3$, whereas high blood phosphorus suppresses the activity of the 1-α-hydroxylase (Fig. 3.28).

The rates of synthesis of $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$ appear to be reciprocally related and controlled by similar factors. When $1,25(OH)_2D_3$ synthesis increases, the synthesis of $24,25(OH)_2D_3$ declines, and vice versa. $24,25(OH)_2D_3$ may play a role in bone formation and egg hatchability in certain animals, and with $1,25(OH)_2D_3$ may exert negative feedback control on the parathyroid gland.

Other hormones also may increase the activity of renal 1-α-hydroxylase and the formation of $1,25(OH)_2D_3$ under certain conditions. Prolactin, estradiol, placental lactogen, and somatotropin all enhance 1-α-hydroxylase activity. Increased secretion of these hormones, either alone or in combination, appears to be important in the efficient adaptation to major calcium demands, such as pregnancy, lactation, and growth.

Biologic action of hormonal form of vitamin D

The active metabolites of vitamin D increase the absorption of calcium and phosphorus from the intestine. From a functional point of view, *vitamin D can be thought to bring about the retention of sufficient mineral ions to ensure mineralization of bone matrix*, whereas *parathyroid hormone maintains the proper ratio of calcium to phosphate in extracellular fluids*.

The major target tissue for $1,25(OH)_2D_3$ is the mucosa of the small intestine, where it increases the active transcellular transport

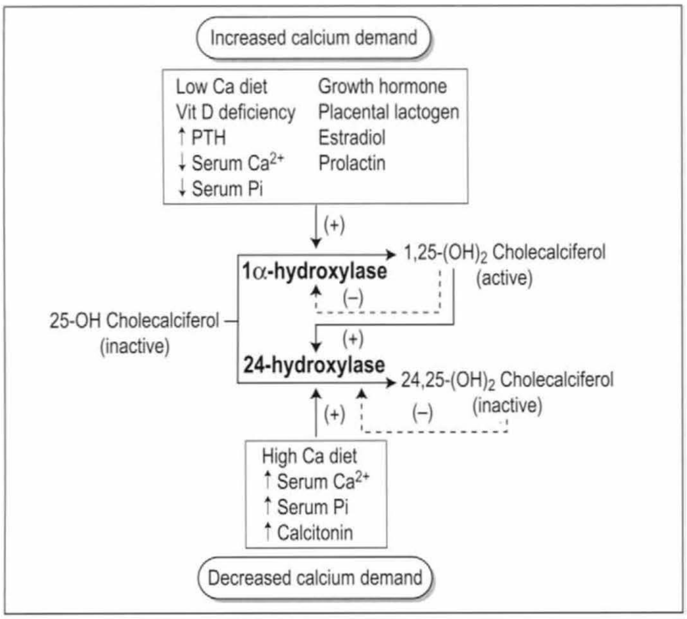

Figure 3.28 Multifactorial control of the final step of metabolic activation of vitamin D in the kidney. Conditions associated with increased calcium demand result in the stimulation of the production of 1,25-$(OH)_2$ cholecalciferol from 25-OH cholecalciferol by increasing the activity of 1α-hydroxylase in the mitochondria of renal tubular cells. Under conditions of decreased calcium demand, the production of 1,25-$(OH)_2$-cholecalciferol is diminished and 24,25-$(OH)_2$-cholecalciferol (an inactive metabolite) is formed by the enzymatic action of a 24-hydroxylase.

of calcium (proximal part) and phosphorus (distal part). Following synthesis in the kidney, 1,25$(OH)_2D_3$ is transported in a protein-bound form to specific target cells in the intestine and bone. Free 1,25$(OH)_2D_3$ penetrates the plasma membrane of target cells and initially may bind to a cytoplasmic receptor in cells of the intestine. However, the hormone–receptor complex is rapidly transferred to the nucleus, and 1,25$(OH)_2D_3$ binds to specific receptors in the nuclear chromatin. In the nucleus, it stimulates gene expression with increased messenger RNA formation, which directs the synthesis of vitamin D-dependent proteins.

Intestinal absorptive cells are concerned with the transport of calcium from the lumen to the blood stream and in response to 1,25$(OH)_2D_3$ they synthesize a specific **calcium-binding protein** (CaBP, calbindin). The highest concentration of CaBP is at the luminal aspect of intestinal absorptive cells, where it complexes with calcium ions for transport to the basolateral aspect of the cell. Calcium-binding protein has also been isolated from kidney, parathyroid gland, and the shell gland of laying hens, and a vitamin D-dependent calcium binding protein is present in bone, particularly in the spongiosa and cartilaginous growth plate.

The absorptive capacity of the intestine for calcium is a direct function of the amount of CaBP present. Vitamin D or low-calcium diets stimulate the synthesis of CaBP. The physiologic function of CaBP may be to protect absorptive cells against high cytosolic concentrations of calcium ion during the transcellular transport of calcium from the luminal border to the laterobasilar border of intestinal cells. At the basilar aspect of intestinal absorptive cells, calcium is exchanged for sodium and enters the extracellular fluids.

The active metabolites of cholecalciferol also act on bone. Rickets and osteomalacia develop in young and mature animals respectively, when it is deficient (see Vol. 1, Bones and joints). Vitamin D also is necessary for osteoclastic resorption and calcium mobilization from bone in adults. Small amounts of active vitamin D metabolite are necessary to permit osteolytic cells to respond to parathyroid hormone ("permissive effect") under physiologic conditions. Both 25$(OH)D_3$ and 1,25$(OH)_2D_3$, and vitamin D_3 in pharmacologic doses, stimulate osteoclastic proliferation and the resorption of bone.

Less is known regarding the action of cholecalciferol and its active metabolite on the kidney. *The active metabolite(s) of vitamin D appear to stimulate the retention of calcium by increasing renal tubular reabsorption, probably in the distal part of the nephron.* The active metabolite may have a direct effect on the parathyroid gland, in addition to its well-characterized action on intestine and bone.

Most assays for vitamin D and its metabolites used clinically employ competitive protein or radioreceptor techniques using vitamin-D-binding proteins isolated from tissues and tritium-labeled tracers; however, newer ^{125}I-based radioimmunoassays have been developed. 25$(OH)D_3$ and 1,25$(OH)_2D_3$ are the most frequently measured metabolites in clinical veterinary medicine. Because the structures of the common vitamin D metabolites do not vary between species, the same assays can be used to measure serum metabolites in animals and humans. *1,25$(OH)_2D_3$ is the most potent metabolite in regulating calcium balance; therefore, its measurement is often necessary to understand abnormal calcium regulation.* The circulating concentration of 1,25$(OH)_2D_3$ is approximately 25 pg/mL (65 pmol/L) in adult animals and is greater in younger animals. Measurement of serum 25$(OH)D_3$ is useful to evaluate the potential of dietary vitamin D deficiency or excess since serum levels are much greater and less tightly controlled compared to 1,25$(OH)_2D_3$. Normal serum concentrations of 25$(OH)D_3$ are 10–50 ng/mL (25–125 nmol/L). Animals that have ingested excess vitamin D may have serum 25$(OH)D_3$ concentrations 4- to 20-fold greater than normal.

Disorders of vitamin D metabolism

Deficiency of vitamin D results not only from simple dietary lack or inadequate exposure to sunlight, but also from a deficiency of the hydroxylase enzymes essential for metabolic activation of precursor molecules. **Vitamin D-dependent rickets, type I,** *in both pigs and humans, is a familial disease inherited as an autosomal recessive.* Newborn pigs appear healthy and have normal blood calcium and phosphorus concentrations. At 4–6 weeks of age, blood Ca and P decrease, serum alkaline phosphatase activity increases, and clinically detectable rickets develops during the following 3–4 weeks. The pigs develop deformities of bone in the axial and appendicular skeleton, severe pain, and classic lesions of rickets. Serum 25$(OH)D_3$ levels are markedly increased compared to those of control pigs whereas serum 1,25$(OH)_2D_3$ levels are depressed in rachitic pigs compared with normal pigs. Homozygous, rachitic animals have no renal 25$(OH)D_3$-1-α-hydroxylase in kidney homogenates or mitochondrial preparations, which is responsible for the insufficient renal production of 1,25$(OH)_2D_3$.

Vitamin D-dependent rickets, type II, *is a group of disorders characterized by target organ resistance to 1,25$(OH)_2D_3$.* The disease is characterized by increased serum 1,25$(OH)_2D_3$ levels, hypocalcemia, secondary hyperparathyroidism, and clinically by rickets or osteomalacia. New World primates have a relative end-organ resistance to $1,25(OH)_2D_3$ and require high levels of vitamin D_3 in their diet. The common marmoset has been shown to represent a model of vitamin D-dependent rickets, type II. Marmosets have increased serum $1,25(OH)_2D_3$ concentrations compared to rhesus monkeys, and some marmosets developed osteomalacia even when on high vitamin D_3 diets. The resistance to $1,25(OH)_2D_3$ is likely due to low affinity binding of $1,25(OH)_2D_3$ to its nuclear receptor (see vitamin D-dependent rickets in Vol. 1, Bones and joints).

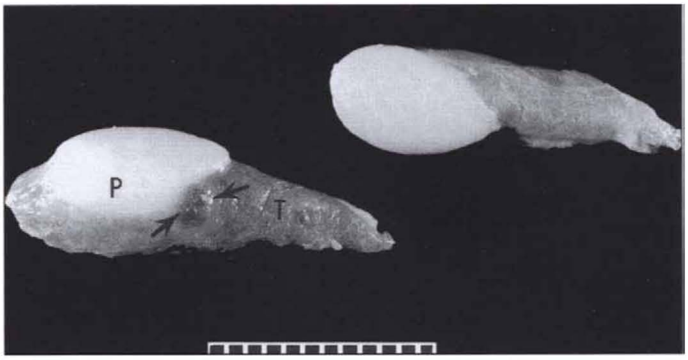

Figure 3.29 Parathyroid cyst adjacent to a hyperplastic parathyroid gland (P), from a dog with chronic renal failure. The cyst (arrows) was formed from the persistence and distention of the embryonic duct that connects parathyroid-thymic primordia in the III and IV pharyngeal pouches (Kürsteiner's cyst). T = thyroid gland. Scale in mm. (From Capen CC. In: Special Veterinary Pathology, 3rd ed., 2001.)

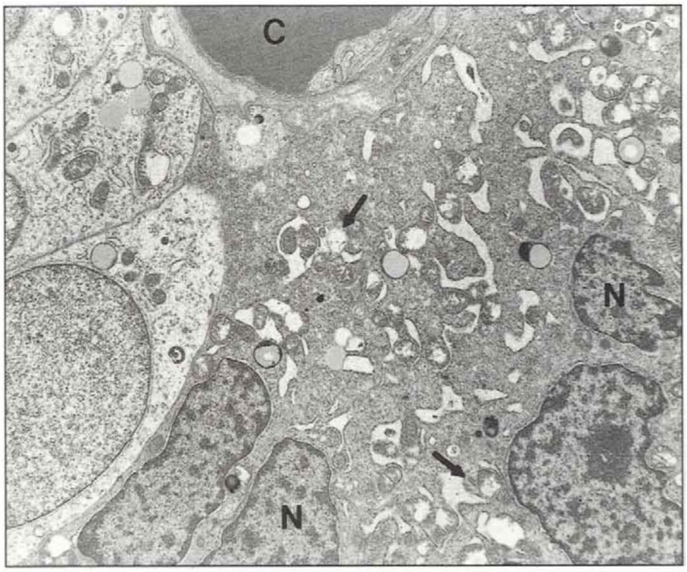

Figure 3.30 Syncytial ("giant") cell (right) and adjacent inactive chief cells (left) in canine parathyroid gland. The large cytoplasmic area of the syncytial cell is formed by the fusion of adjacent chief cells following the disruption of plasma membranes. The multiple nuclei (N) are smaller and more electron-dense than in normal chief cells (left), and they have condensed chromatin. The mitochondria are swollen with disruption of cristae in the syncytial cells, and profiles of endoplasmic reticulum are distended (arrows). C = capillary. (From Capen CC. In: Pathobiology of the Aging Dog, 2001.)

Diseases of the parathyroid gland

Parathyroid and related cysts

Parathyroid (Kürsteiner's) cysts are observed within the parenchyma of the parathyroid or in the immediate vicinity of the gland, frequently in dogs and occasionally in other animal species. Parathyroid cysts usually are small, multiloculated, lined by cuboidal to columnar (often ciliated) epithelium, and contain densely eosinophilic proteinaceous material. Chief cells adjacent to larger cysts may be moderately compressed. *Parathyroid cysts appear to be dilated remnants of the duct of pharyngeal pouches III and IV* that connects the parathyroid and thymic primordia during embryonic development (Figs. 3.25, 3.29). Similar cysts may be present in the cranial mediastinum when remnants of the embryonic duct are displaced with the caudal migration of the thymus. They are lined by pseudostratified columnar epithelium and contain proteinaceous material.

Other cystic structures may occur in the thyroid–parathyroid area:

- Midline cysts derived from remnants of the **thyroglossal duct** are lined by multi-layered thyroidogenic epithelium, which often has colloid-containing follicles, and usually are located on or near the midline, from the base of the tongue caudally into the mediastinum.
- **Ultimobranchial duct cysts** often are present in the parenchyma of the thyroid (near the thyroid hilus in ruminants) and have a keratinizing squamous epithelial lining. Ultimobranchial duct cysts are common in thyroids from laboratory rats. They are derived from remnants of the ultimobranchial body (last, usually fifth, pharyngeal pouch) that fuses with the lateral thyroid lobes during embryonic development, and distributes calcitonin-secreting C cells (derived from neural crest) into each thyroid lobe.
- **Branchial cysts** are located lateral to the parathyroid–thyroid area, often near the base of the ear and angle of mandible, attached deeply to cervical structures, and clinically are referred to as "*lateral neck cysts*." They are lined by pseudostratified columnar or focally squamous, partially ciliated, epithelium that is derived from remnants of the second pharyngeal pouch.
- **Salivary mucoceles** may also be present in the parathyroid–thyroid region. These cysts are lined only with granulation tissue that develops in response to the escape of saliva into the interstitium following disruption of a salivary duct.

Degenerative changes of the parathyroid gland

Parathyroid glands of dogs and rats may develop **multinucleated syncytial giant cells.** They often are more numerous near the periphery of the parathyroid gland, but this variable and considerable numbers can be present in the more central portions of the gland. The number of syncytial cells varies considerably between parathyroid glands in the same animal but may account for up to half of the parenchyma of the gland. They appear to form by the fusion of the cytoplasmic area of adjacent chief cells. The cytoplasm of syncytial cells is densely eosinophilic and homogeneous, and the plasma membranes between adjacent cells are often indistinct (Fig. 3.30). The nuclei are smaller, more hyperchromatic and oval, than those in adjacent chief cells.

The mechanism by which syncytial cells form is uncertain, but it does not appear to be related to improper fixation of the parathyroid, as they have been observed in glands perfused for electron microscopy. They usually do not occur in sufficient numbers to interfere significantly with parathyroid function.

Chemical-induced toxic injury of parathyroids

Ozone. Inhalation of a single dose of ozone for 4–8 hours has been reported to produce light and electron microscopic changes in parathyroid glands. In response to longer exposure to ozone, many chief cells undergo compensatory hypertrophy and hyperplasia with areas of capillary endothelial cell proliferation, interstitial edema, degeneration of vascular endothelium, formation of platelet thrombi, leukocyte infiltration of the walls of larger vessels in the gland, and disruption of basement membranes. Chief cells had prominent Golgi complexes and endoplasmic reticulum, aggregations of free ribosomes, and swollen mitochondria. Inactive chief cells with few secretory granules predominate in the parathyroids in the later stages of exposure to ozone. Plasma membranes of adjacent chief cells were disrupted resulting in coalescence of the cytoplasmic area. *The parathyroid lesions in ozone-exposed animals shared certain features with isoimmune parathyroiditis.* Antibody against parathyroid tissue was localized near the periphery of chief cells by indirect immunofluorescence.

Aluminum. Evidence for a direct effect of aluminum on the parathyroid was suggested from studies of patients with chronic renal failure treated by hemodialysis with aluminum-containing fluids or orally administered drugs containing aluminum. These patients often had normal or minimal elevations of immunoreactive parathyroid hormone (iPTH), little histological evidence of osteitis fibrosa in bone, and a depressed response by the parathyroid gland to acute hypocalcemia. An increase in aluminum concentration in vitro over a range of 0.5–2.0 mM in a low-calcium medium (0.5 mM) progressively inhibits the secretion of PTH. PTH secretion was inhibited by 68% at 2.0 mM aluminum, while high calcium-containing medium (2.0 mM) without aluminum maximally inhibited PTH secretion only 39%. The inhibition of PTH secretion by aluminum does not appear to be related to an irreversible toxic effect, because normal secretion was restored when parathyroid cells were returned to 0.5 mM calcium medium without aluminum. *Aluminum appears to decrease diglyceride synthesis*, which is reflected in a corresponding decrease in synthesis of phosphatidylcholine; however, phosphatidylinositol synthesis was not affected by aluminum.

L-asparaginase. Rabbits administered L-asparaginase develop severe hypocalcemia and tetany characterized by muscle tremors, opisthotonos, carpopedal spasms, paralysis, and coma. This drug was of interest in cancer chemotherapy because of the beneficial effects of guinea pig serum against lymphosarcoma in mice. *Parathyroid chief cells appeared to be selectively destroyed by L-asparaginase.* Chief cells were predominantly inactive and degranulated, with large autophagic vacuoles present in the cytoplasm of degenerating cells. Cytoplasmic organelles concerned with synthesis and packaging of secretory products were poorly developed in chief cells. Rabbits developed hyperphosphatemia, hypomagnesemia, hyperkalemia, and azotemia in addition to acute hypocalcemia. Rabbits with clinical hypocalcemic tetany did not recover spontaneously; however, administration of parathyroid extract prior to or during treatment with L-asparaginase decreased the incidence of hypocalcemic tetany.

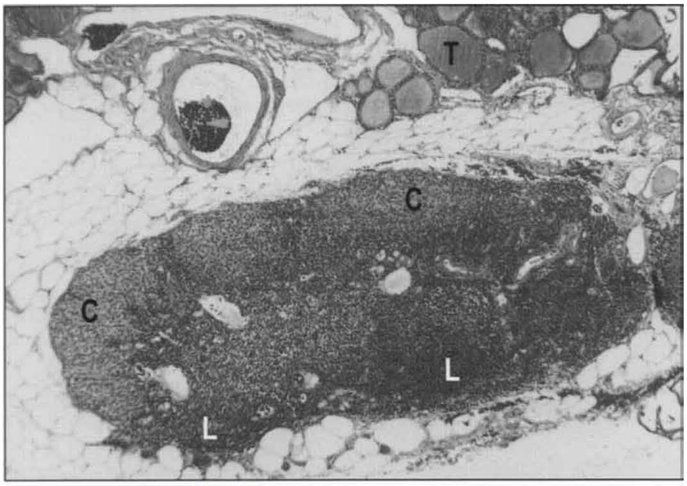

Figure 3.31 Lymphocytic parathyroiditis in a dog with hypocalcemia. There are multifocal areas of lymphocytic (L) infiltration and parenchymal destruction. In this early stage of the disease, there are multiple areas of nodular regenerative hyperplasia of chief cells (C). T = thyroid follicle.

Lymphocytic parathyroiditis and hypoparathyroidism

In hypoparathyroidism, either subnormal amounts of parathyroid hormone are secreted by pathologic parathyroid glands, or the hormone secreted is unable to interact normally with target cells. Hypoparathyroidism has been recognized in dogs, particularly in smaller breeds such as Schnauzers and Terriers, but infrequently in other animal species. *The functional disturbances of hypoparathyroidism primarily are the result of increased neuromuscular excitability and tetany.* Bone resorption is decreased because of a lack of PTH and blood calcium levels diminish progressively.

Several pathogenic mechanisms can result in inadequate secretion of parathyroid hormone. *Idiopathic hypoparathyroidism in adult dogs* is associated usually with diffuse lymphocytic parathyroiditis, resulting in extensive degeneration of chief cells and replacement by fibrous connective tissue. In the early stages, there is infiltration of the gland with lymphocytes and plasma cells, and nodular regenerative hyperplasia of remaining chief cells (Fig. 3.31). Later, the parathyroid gland is completely replaced by lymphocytes, fibroblasts, and neocapillaries, with only an occasional viable chief cell.

Lymphocytic parathyroiditis most likely is an immune-mediated disease. Similar lesions are produced in dogs by injection of parathyroid tissue emulsions and by repeated injections of parathyroid emulsions with Freund's adjuvant.

Other possible causes of hypoparathyroidism include invasion and destruction of parathyroids by primary or metastatic *neoplasms* in the cranial cervical region and trophic atrophy of parathyroids associated with long-term *hypercalcemia*. The parathyroid glands may be damaged or inadvertently removed during the course of *surgery* to remove thyroid tumors or multifocal hyperplasia ("goiter"). If the parathyroid glands or their vascular supply is intact but damaged, there is often regeneration of adequate functional parenchyma and subsequent disappearance of clinical signs. The presence of numerous *canine distemper virus* particles in chief cells of the parathyroid gland may contribute to the low blood calcium in certain dogs with this disease. *Agenesis* of both pairs of parathyroids is a rare cause of congenital hypoparathyroidism in pups.

Parathyroid stimulation associated with acute hypocalcemia near parturition

Parturient paresis *is a complex metabolic disease characterized by the development of severe hypocalcemia and hypophosphatemia near parturition and the initiation of lactation in dairy cattle.* The serum calcium (total and ionized) concentration falls to less than 50% of normal in spite of increased secretion of parathyroid hormone.

The parathyroid glands are capable of responding to the acute hypocalcemia by increased development of cellular organelles for hormonal synthesis and by the secretion of parathyroid hormone. Chief cells in the active stage of synthesis and secretion predominate in the parathyroid glands (Fig. 3.32A); however, decreased numbers of storage granules in chief cells suggest that the increased rate of hormone secretion exceeds the rate of synthesis by the gland. There is a significant increase in plasma parathyroid hormone levels in cows with parturient hypocalcemia as compared to normal parturient cows.

The composition of the prepartum diet is a significant factor in the pathogenesis of parturient hypocalcemia. High-calcium diets increase the incidence of the disease and low-calcium diets, or prepartum diets supplemented with pharmacologic doses of vitamin D, reduce the incidence of the disease.

Cows fed a high-calcium diet have higher blood calcium levels prepartum, but are less able to maintain serum calcium near the critical time of parturition. Plasma parathyroid hormone levels are lower prepartum than in cows fed a balanced diet, and decline further at 48 hours postpartum. Chief cells in the inactive stage of the secretory cycle predominate in cows fed high calcium diets (Fig. 3.32B), whereas actively synthesizing chief cells are most numerous in parathyroids of cows fed balanced prepartal diets. In response to the elevated blood calcium in cows fed high-calcium prepartum diets, thyroid stores of calcitonin are diminished, and C cells are partially degranulated and appear to be actively synthesizing more calcitonin. This stimulation of C cells is accompanied by a decrease in bone turnover near parturition. Trabecular bone surfaces are inactive and few osteoclasts are resorbing bone.

Calcium homeostasis in pregnant cows fed a high-calcium diet appears to be maintained principally by intestinal calcium absorption. This greater reliance on intestinal absorption than on parathyroid hormone stimulated bone resorption probably is a significant factor in the more frequent development of profound hypocalcemia near parturition. Cows fed a high-calcium diet are more susceptible to the decreased calcium available for absorption as a result of the anorexia often associated with the high blood estrogen levels near the time of parturition.

Calcium homeostasis in cows fed balanced or relatively low-calcium diets prepartum appears to be more under the fine control of parathyroid hormone secretion with the approach of parturition. The higher levels of parathyroid hormone secreted during the prepartum period by an expanded population of actively synthesizing chief cells results in a larger pool of active bone-resorbing cells to fulfill the increased needs for calcium mobilization at the critical time near parturition and the initiation of lactation. Cows fed a low-calcium diet are less susceptible to the influence of decreased intestinal calcium absorption and flow into the extracellular calcium pool resulting from the anorexia and intestinal stasis associated with parturition.

Less is known about the development of hypocalcemic syndromes in animal species other than in the cow. In dogs, **puerperal tetany** (eclampsia) is most frequently encountered in the small, hyperexcitable breeds. The clinical course is rapid and the bitch may proceed from premonitory signs of restlessness, panting, and nervousness to ataxia, trembling, muscular tetany, and convulsive seizures in 8–12 hours. There is no evidence to suggest that puerperal tetany in heavily lactating bitches is the result of interference in parathyroid hormone secretion. Severe hypocalcemia and often

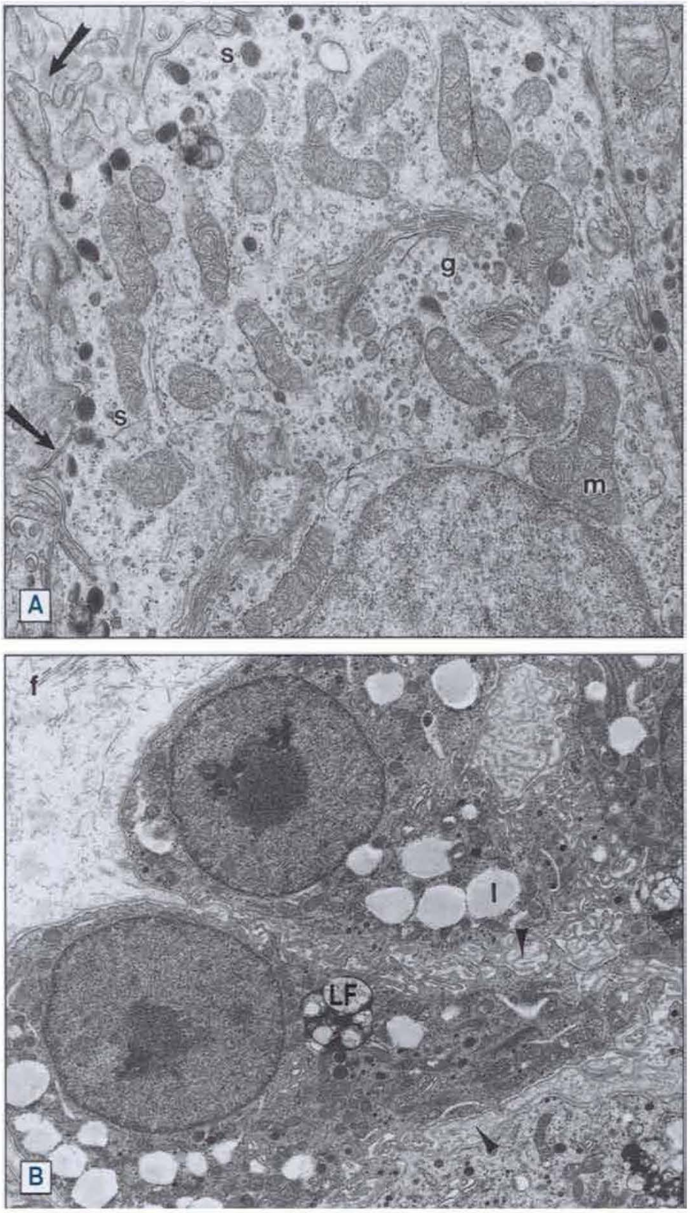

Figure 3.32 A. Acute parathyroid stimulation in response to severe hypocalcemia associated with parturition and initiation of the lactational drain for calcium. Parathyroid hormone-containing secretory granules (s) migrate peripherally and fuse with plasma membrane of actively secreting chief cells (arrow). Golgi apparatus (g) is hypertrophied. Many large mitochondria (m) are present. **B. Chronic parathyroid suppression** in a cow fed a high-calcium diet. Irregularly shaped atrophic chief cells are separated by cytoplasmic processes (arrowheads) in widened intercellular spaces. The electron-dense cytoplasm contains many lipid bodies (l), large lipofuscin bodies (LF), and occasional secretory granules. Cytoplasmic organelles other than mitochondria are poorly developed. Widened perivascular space contains many collagen fibers (f).

hypophosphatemia develop near the time of peak lactation (approximately 1–3 week postpartum), most likely the result of a temporary failure in calcium homeostasis resulting in an imbalance between the rates of inflow to and outflow from the extracellular fluid calcium pool. The administration of intravenous calcium to stop the tetany, combined with temporarily decreasing the lactational drain of calcium by removing the pups, corrects the disruption of calcium homeostasis in the majority of bitches. Supplemental dietary calcium and vitamin D have proven useful in preventing relapses in certain bitches with puerperal tetany.

Functional disturbances associated with hypocalcemia in cows are related to paresis, whereas in the bitch they are primarily the result of neuromuscular tetany. *The occurrence of either tetany or paresis in response to hypocalcemia appears to be the result of basic physiologic differences in the function of the neuromuscular junction of the cow and the bitch.* The release of acetylcholine and transmission of nerve impulses across neuromuscular junctions are blocked by hypocalcemia in cows (but not in the bitch) leading to muscle paresis. The dog appears to have a higher margin of safety in neuromuscular transmission in that the degree to which the endplate potential exceeds the firing threshold is greater than in the cow. Excitation–secretion coupling is maintained at the neuromuscular junction in the bitch with hypocalcemia, and tetany occurs as a result of spontaneous repetitive firing of motor nerve fibers. Due to the loss of stabilizing membrane-bound calcium, nerve membranes become more permeable to ions and require a stimulus of lesser magnitude to depolarize.

Parathyroid hyperplasia

Focal (nodular) hyperplasia. Chief cell hyperplasia may affect the parathyroids in a distinctly focal or multifocal distribution. In focal parathyroid hyperplasia there are single or multiple nodules in one or both glands in which there is an increased number of closely packed chief cells often with an expanded cytoplasmic area. The foci of chief cell hyperplasia are not encapsulated and are poorly demarcated from adjacent parenchyma. Chief cells within the nodules have a relatively uniform composition with a high cytoplasm:nucleus ratio and a slightly more hyperchromatic nucleus than adjacent normal chief cells. There may be slight compression of adjacent chief cells around larger areas of focal hyperplasia. Focal chief cell hyperplasia often is difficult to separate from a chief cell adenoma using only morphologic criteria. *The presence of multiple nodules of various sizes and uniform cellularity in one or both parathyroids with minimal compression and no fibrous encapsulation is more compatible with focal hyperplasia than chief cell adenoma.*

Diffuse hyperplasia. Parathyroid hyperplasia, such as seen with chronic renal failure and long-term dietary imbalances, results in uniform enlargement of all parathyroid glands due to both hypertrophy and hyperplasia of chief cells. There is not a peripheral rim of compressed atrophic parathyroid parenchyma as occurs around a functional adenoma, but rather *a uniform population of hyperplastic chief cells extending to the capsule of the gland.* The chief cells often are packed together with indistinct cell boundaries. The expanded cytoplasmic area of chronically stimulated chief cells is lightly eosinophilic with occasional distinct vacuoles. A more prominent fibrovascular stroma in some diffusely hyperplastic parathyroids may result in a lobulated appearance, and in others chief cells form distinct acinus-like structures.

Hyperparathyroidism secondary to renal disease

Secondary hyperparathyroidism as a complication of chronic renal failure is characterized by excessive production of parathyroid hormone in response to chronic hypocalcemia. When the renal disease reaches the point at which there is a significant reduction in the glomerular filtration rate, phosphorus is retained and progressive hyperphosphatemia develops, which contributes to parathyroid stimulation by virtue of its ability to lower blood calcium.

Impaired intestinal absorption of calcium due to an acquired defect in vitamin D metabolism probably also plays a significant role in the development of hypocalcemia in chronic renal insufficiency and uremia. Chronic renal disease impairs the production of $1,25(OH)_2D_3$ by the kidney and thereby diminishes intestinal calcium transport and increases mobilization of calcium from the skeleton (Fig. 3.33).

Chronic renal insufficiency occurs frequently as a result of several acquired or congenital kidney lesions. All four parathyroid glands undergo marked chief cell hyperplasia (see Fig. 3.37D) and the bones have varying degrees of generalized osteodystrophia fibrosa (see Vol. 1, Bones and joints). Chief cells in the parathyroid glands of dogs with chronic renal disease are primarily in the actively synthesizing stage of the secretory cycle.

The pathogenesis of secondary renal hyperparathyroidism is complex, but it has been recently recognized that $1,25(OH)_2D_3$ (calcitriol) plays an important role in this disease. *Animals with advanced chronic renal failure (CRF) have decreased circulating concentrations of $1,25(OH)_2D_3$* caused by decreased renal synthesis. Initially, the elevated blood phosphorus depresses the activity of the 1-α-hydroxylase in the kidney. In the later stage of chronic renal disease, there are decreased numbers of tubular epithelial cells with 1-α-hydroxylase activity to form the active form of vitamin D. Early stages of CRF are often associated with normal concentrations of circulating $1,25(OH)_2D_3$ (even with the loss of nephrons) due to the correcting effects of increased PTH on renal 1-α-hydroxylase that enhance renal tubular synthesis of $1,25(OH)_2D_3$.

$1,25(OH)_2D_3$ also is an important regulator of parathyroid chief cell function and acts to decrease PTH mRNA expression, increase expression of the vitamin D receptor, and control the set-point of chief cell responsiveness to negative feedback by the serum calcium concentration. The function of $1,25(OH)_2D_3$ serves to complete the endocrine feedback loop with the parathyroid gland to control serum calcium concentration. *Decreased circulating levels of $1,25(OH)_2D_3$ in animals with CRF results in chief cell hyperplasia and increased secretion of intact PTH.*

All four parathyroid glands are enlarged due to organellar hypertrophy initially and cellular hyperplasia later as compensatory mechanisms to increase hormonal synthesis and secretion in response to the hypocalcemic stimulus. Since the parathyroid gland stores comparatively little preformed hormone, the rate of peptide synthesis appears to be rate-limiting for hormonal secretion. Although the parathyroids are not autonomous, the concentration of PTH (especially –COOH terminal fragment) in the peripheral blood in patients with chronic renal failure may exceed that of primary hyperparathyroidism. Therefore, greatly increased numbers of chief cells are required to sustain the increased rates of hormonal secretion in patients with long-standing renal disease.

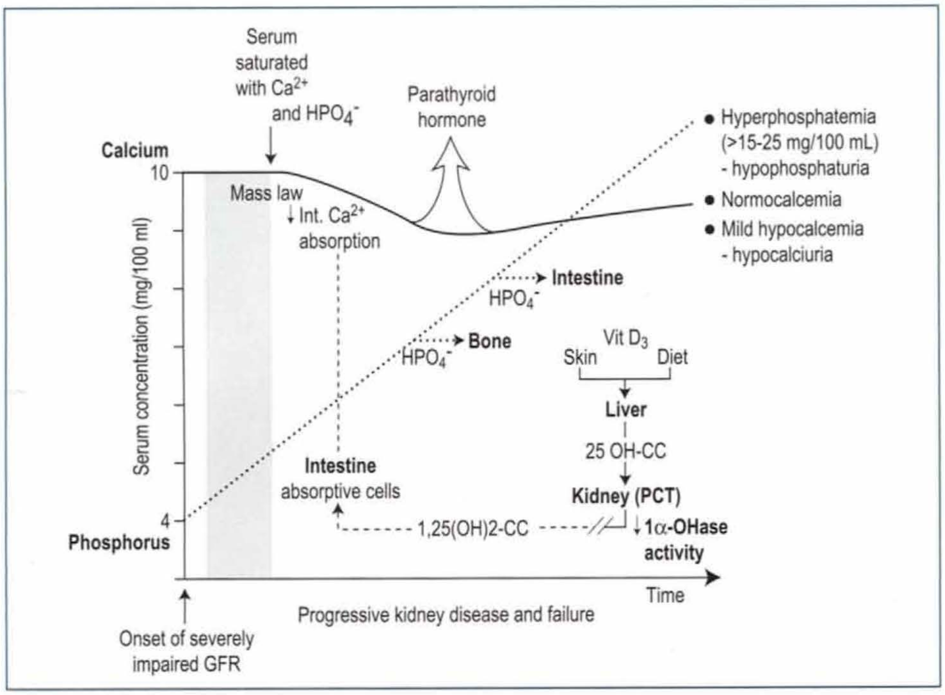
Serum saturated with Ca^{2+} and HPO_4^-
Parathyroid hormone
Calcium
10
Mass law
↓ Int. Ca^{2+} absorption
• Hyperphosphatemia (>15-25 mg/100 mL)
- hypophosphaturia
• Normocalcemia
• Mild hypocalcemia
- hypocalciuria
Intestine
HPO_4^-
Bone
HPO_4^-
Vit D_3
Skin
Diet
Liver
25 OH-CC
Kidney (PCT)
Intestine absorptive cells
1,25(OH)2-CC
↓ 1α-OHase activity
Serum concentration (mg/100 ml)
4
Phosphorus
Time
Progressive kidney disease and failure
Onset of severely impaired GFR

Figure 3.33 Sequential alterations in serum calcium and phosphorus during the pathogenesis of **secondary hyperparathyroidism** associated with progressive renal failure. (From Rosol TJ, Capen CC. In: Clinical Biochemistry of Domestic Animals, 5th ed., 1997.)

Hyperparathyroidism secondary to nutritional imbalances

Nutritional secondary hyperparathyroidism occurs commonly in cats, dogs, certain non-human primates, horses, domestic and captive birds, and reptiles. The increased secretion of parathyroid hormone is a compensatory mechanism directed against a disturbance in mineral homeostasis *induced by diets with either low calcium or excessive oxalate content, or high phosphorus with normal or low calcium content.* Inadequate vitamin D_3, besides causing rickets, also causes lesions of secondary hyperparathyroidism in some species. The significant result of these imbalances is hypocalcemia, which results in parathyroid stimulation. In response to the diet-induced hypocalcemia, chief cells undergo hypertrophy and eventually hyperplasia (Fig. 3.34A). The expanded cytoplasmic area is lightly eosinophilic and vacuolated compared with chief cells in normal animals (Fig. 3.34B). Perivascular spaces are narrow and there are few fat cells in the interstitium. The skeletal lesions of nutritional hyperparathyroidism are discussed with Vol. 1, Bones and joints.

Primary parathyroid hyperplasia

Primary parathyroid hyperplasia has been described in *German Shepherd pups* associated with hypercalcemia, hypophosphatemia, increased immunoreactive parathyroid hormone, and increased fractional clearance of inorganic phosphate in the urine. Clinical signs included stunted growth, muscular weakness, polyuria, polydipsia, and diffuse reduction in bone density. Intravenous infusion of calcium failed to suppress the autonomous secretion of parathyroid hormone by the diffuse hyperplasia of chief cells in all parathyroids. Lesions included nodular hyperplasia of thyroid C cells and widespread mineralization of lungs, kidney, and gastric mucosa. The disease is inherited as an autosomal recessive trait.

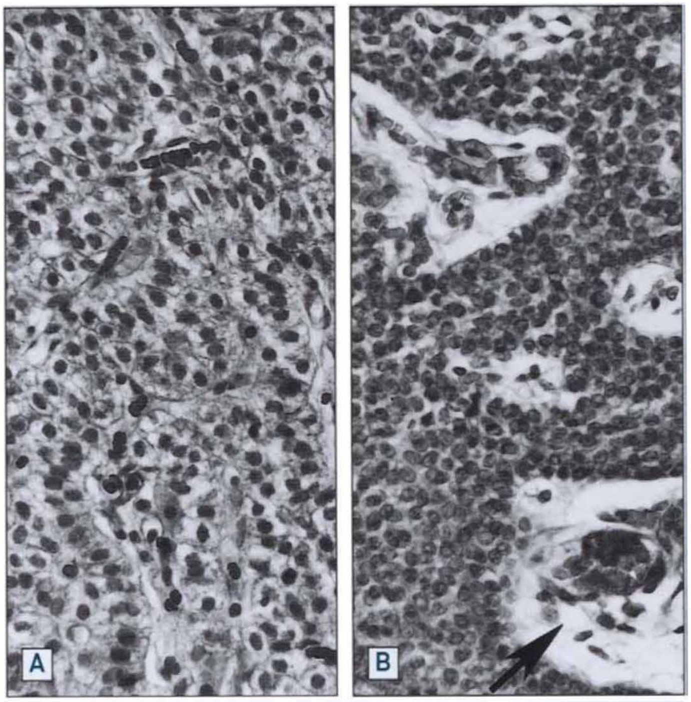

Figure 3.34 **A. Parathyroid chief cell hypertrophy and hyperplasia** in a kitten fed a low-calcium (all-meat) diet. The enlarged chief cells are closely packed, and perivascular spaces are narrow. **B.** Chief cells in parathyroid gland of kitten fed a diet that supplied daily requirements for calcium and phosphorus. Note the more prominent perivascular spaces (arrow).

Parathyroid suppression associated with vitamin D intoxication from calcinogenic plants and rodenticides

Livestock grazing on various calcinogenic plants develop a progressive debilitating disease with widespread mineralization of soft tissues. Cestrum diurnum

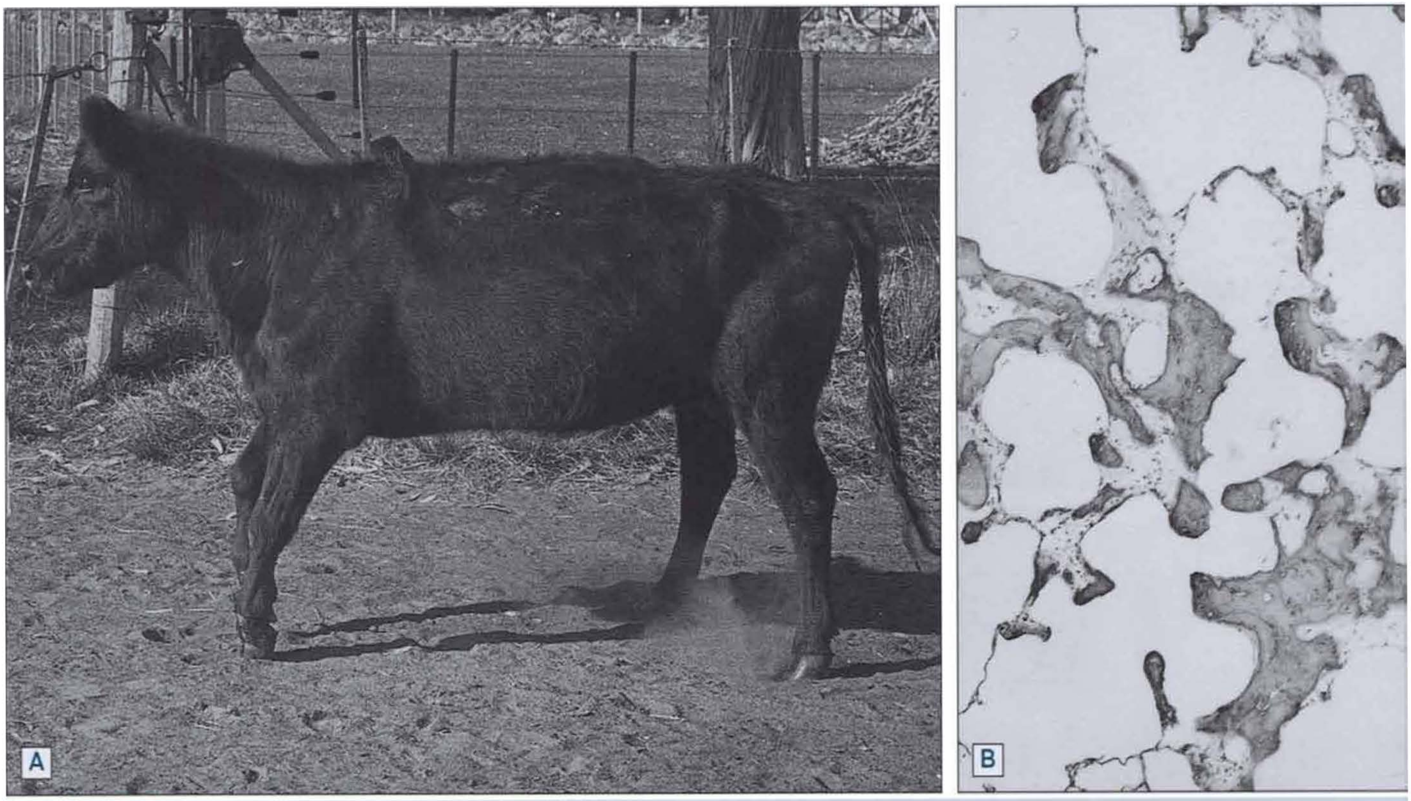

Figure 3.35 ***Enteque seco.*** **A.** Contraction of tendons and kyphosis in affected heifer. **B.** Pulmonary ossification.

(day-blooming jessamine) in Florida and elsewhere in the southern United States and *Trisetum flavescens* in the Bavarian and Austrian Alps cause calcinosis in horses, cattle, and sheep. *Solanum malacoxylon* in Argentina and Brazil produces the disease *enteque seco* or *espichamento* in cattle, which is characterized by the development of hypercalcemia, hyperphosphatemia, and severe soft tissue mineralization. Morphologically similar diseases occur in Jamaica (Manchester wasting disease) and Hawaii (*Naalehu* disease). Other plants that contain vitamin D-like activity are *Solanum torvum*, *S. verbascifolium*, *Dactylis glomerata*, and *Medicago sativa*. Sporadic cases of extensive mineralization occur in cattle throughout the world.

The leaves of these calcinogenic plants contain a substance(s) possessing vitamin D-like biologic activity. The dried leaves of *Solanum malacoxylon* contain a steroid–glycoside conjugate in which the steroidal component is identical to $1,25(OH)_2D_3$. The leaves are not palatable when green, but are eaten readily when dry. The calcinogenic plants appear to produce disease only in herbivores after ingestion of leaves. Intoxication produces rapid wasting and marked elevations of the calcium and phosphorus levels in the blood. *The patterns of these elevations of blood electrolytes as well as the soft tissue mineralization are comparable to those produced by intoxication by vitamin D.* The leaves of *S. malacoxylon* are remarkably potent with as little as 2 g being capable of producing elevations of calcium and phosphorus in the blood of adult cattle. The typical wasting syndrome is seen clinically when animals are forced to eat large quantities of the leaves. The animals develop kyphosis with contraction of the tendons and ligaments resulting in an inability to completely straighten their front limbs (Fig. 3.35A). Contraction of the abdominal aponeurosis gives a unique tucked-up line to the abdomen resulting in affected animals having a racehorse-like appearance. Many animals develop severe vascular and pulmonary disease as a result of irregular or occasional exposure to the plant due to mineralization and ossification of interalveolar septa in the lung (Fig. 3.35B).

These diseases associated with the ingestion of calcinogenic plants are not restricted to cattle but also occur also in goats, sheep and horses, although with lesser frequency and severity. Mature animals are most commonly affected, usually producing progressive debility. The earliest signs are those of stiffness and wasting. There is a subtle progression to permanent lameness due to contraction of the tendons. At this stage, affected animals often are excitable, tire easily if made to exercise, and may show signs of acute cardiac insufficiency. If they are removed from affected areas, the stiffness and deformity are not lessened but the wasting is stopped, and the animals gain weight if good pasture is available.

Necropsy reveals widespread mineralization of the tissues, particularly the aorta, heart, and lungs (Fig. 3.36A, B) *often with areas of ossification.* Small mineralized blood vessels are encountered in the subcutis, and mineralized plaques are present on the parietal and visceral pleura. Portions of the lungs fail to collapse and these represent areas of mineralization in which the parenchyma is disrupted and emphysematous. The pulmonary lesions primarily involve the caudal lobes and consist of irregular thickening of alveolar septa due to mineralization and collagen deposition. The opened heart often reveals mineralization of the right atrium but not of the right ventricle. The endocardial mineralization is most severe in the atrium and ventricle (chiefly the basal portion of the latter) also involving the semilunar cusps, the bicuspid valve, and chordae tendineae, and extending into the aorta. The aortic mineralization in early cases is restricted to the zones about the orifices of large branches, but in

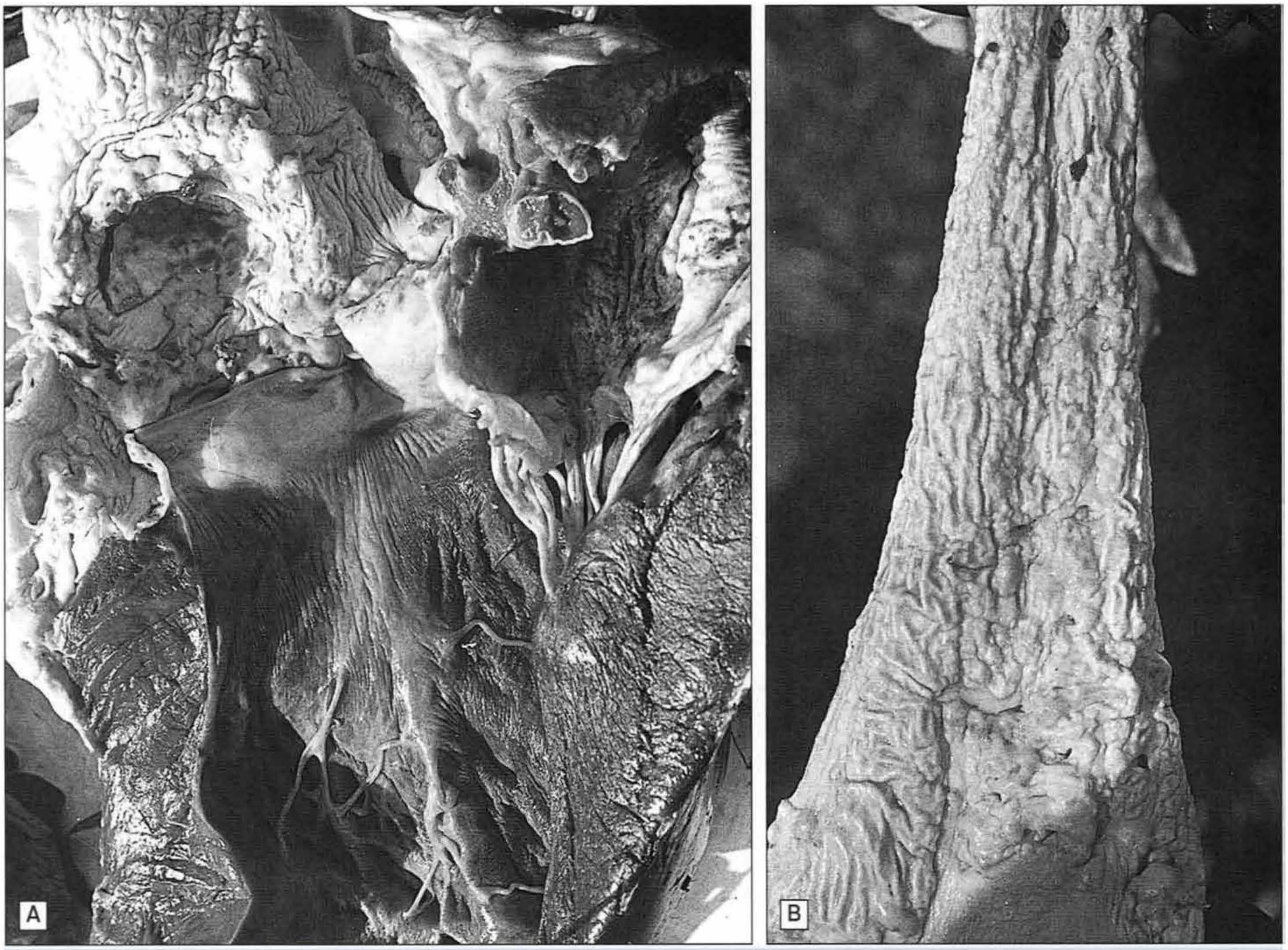

Figure 3.36 Mineralization in poisoning of a cow by *Solanum malacoxylon*. **A.** Heart. **B.** Aorta.

advanced cases it is more diffuse. In advanced cases of intoxification, many small arteries, especially the coronary and mesenteric arteries, are mineralized severely but irregularly. The arterial mineralization begins in the intima and, with continuous accretion, extends to the media disrupting the elastic laminae of the vessels. Areas of mineralization are present microscopically in the mucosa of the abomasal fundus as well as in other fibroelastic tissues. Mineralization of ligaments and of the fibrocartilagenous portions of tendons is responsible for the stiffness along with the degenerative arthropathy that usually is present in affected animals.

In chronic cases, the bones become extremely dense as new trabeculae are formed in the marrow spaces. These trabeculae appear to develop in an atypical basophilic matrix similar to that deposited in vitamin D poisoning (see Vol. 1, Bones and joints,). Parathyroid chief cells appear initially to accumulate secretory granules after feeding *S. malacoxylon* and later to undergo involution and atrophy.

The incidence of hypercalcemia due to vitamin D intoxication in **small animals** *has increased due to use of vitamin D (cholecalciferol) as a pesticide and rodenticide.* The ingested vitamin D is converted to 25-hydroxy vitamin D in the liver and is the major toxic metabolite. 25-hydroxy vitamin D has a much reduced binding affinity for the vitamin D receptor (500- to 1000-fold) than $1,25(OH)_2D_3$ (active form of vitamin D), but is present at such high concentrations that it stimulates hypercalcemia by increasing intestinal absorption of calcium. Only small amounts of the 25-hydroxy vitamin D are converted to the active form of vitamin D, $1,25(OH)_2D_3$, which often is present in the circulation at normal to subnormal levels. This is due to inhibition of renal 1-α-hydroxylase by low serum PTH levels, hypercalcemia, hyperphosphatemia, and negative feedback from $25(OH)D_3$ that binds to the vitamin D receptor. The hypercalcemia of hypervitaminosis D is usually accompanied by hyperphosphatemia (since vitamin D also increases intestinal absorption of phosphorus in addition to calcium) and normal serum alkaline phosphatase activity. Skeletal disease is not a consistent feature, since the increased concentrations of blood calcium and phosphorus are derived principally from augmented intestinal absorption; however, widespread mineralization of soft tissue occurs similar to animals ingesting calcinogenic plants.

Neoplasms of the parathyroid glands

Functional adenomas and carcinomas of *parathyroid glands secrete parathyroid hormone in excess of normal, causing a syndrome of* **primary hyperparathyroidism** (Fig. 3.37). The normal control mechanism

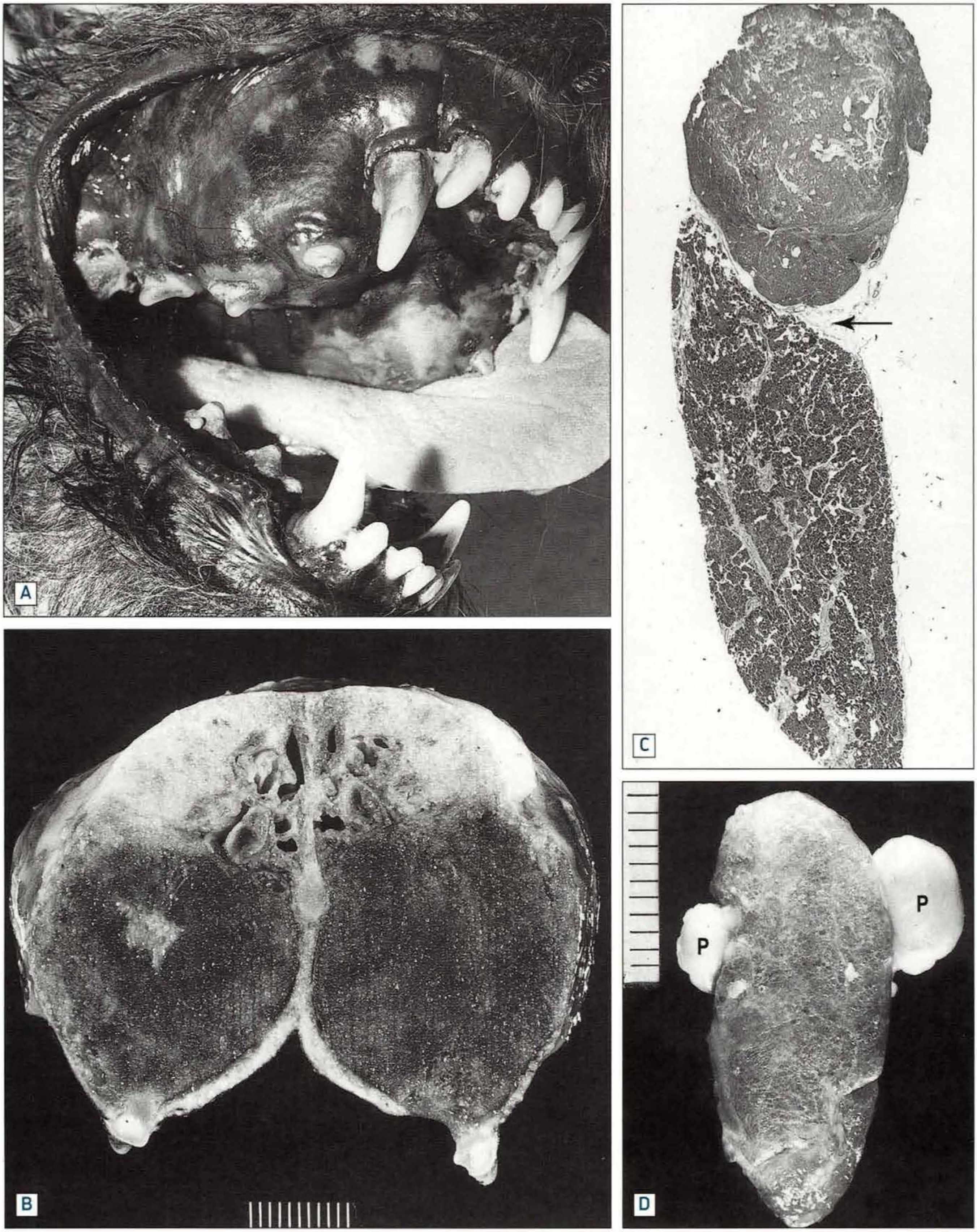

Figure 3.37 Hyperostotic fibrous osteodystrophy in the maxilla of a dog with primary hyperparathyroidism. **A.** Bony enlargement of maxilla. **B.** Cross section showing encroachment on the nasal cavity by extensive proliferation of woven bone. **C. Chief cell adenoma** (top) sharply demarcated from and compressing the adjacent thyroid (arrow) in a dog with primary hyperparathyroidism. **D. Chief cell hyperplasia** resulting in enlargement of internal and external parathyroids (P) in a dog with chronic renal failure.

for parathyroid hormone is lost and hormone secretion is excessive in spite of an increased level of blood calcium. Cells of the renal tubules are sensitive to alterations in the amount of circulating parathyroid hormone. The excretion of phosphorus and retention of calcium initially are enhanced. A prolonged increased secretion of parathyroid hormone accelerates osteoclastic bone resorption and results in generalized fibrous osteodystrophy.

Adenomas of parathyroid glands are encountered in older animals, particularly dogs and occasionally cats. The number of cases studied in dogs is inadequate to determine any breed or sex predisposition. Tumors of parathyroid chief cells in animals do not appear to be sequelae of long-standing secondary hyperparathyroidism of renal or nutritional origin.

Chief cell adenomas usually result in considerable enlargement of a single parathyroid gland. They are light brown to red and are located either in the cervical region by the thyroids or infrequently within the thoracic cavity near the base of the heart. Parathyroid neoplasms in the precardiac mediastinum are derived from ectopic parathyroid tissue, displaced into the thorax with the expanding thymus during embryonic development. Parathyroid adenomas are sharply demarcated and encapsulated from the adjacent thyroid gland (Fig. 3.37C). Multiple white foci may be seen in the thyroids of dogs with functional parathyroid tumors. These represent areas of C-cell hyperplasia in response to the long-term hypercalcemia (Fig. 3.38). In secondary hyperparathyroidism, all four parathyroids are enlarged 2–5 times normal size (Fig. 3.37D). In comparison, a parathyroid adenoma will enlarge a single gland to a much greater degree; however, the remaining parathyroids will be atrophic. *Histologic demonstration of a compressed rim of parathyroid parenchyma and a partial-to-complete fibrous capsule in an enlarged gland points to the diagnosis of adenoma rather than chief cell hyperplasia* (Fig. 3.39).

Parathyroid adenomas are composed of closely packed chief cells subdivided into small groups by fine connective tissue septa with many capillaries. The chief cells are cuboidal or polyhedral, and the cytoplasm stains lightly eosinophilic. Occasional oxyphil cells, water-clear cells, and transitional forms may be distributed throughout the adenoma. The transitional oxyphil cells contain organelles concerned with hormonal synthesis and packaging, as well as occasional secretory granules. This is in contrast to the oxyphil cell of normal parathyroid glands, which has a cytoplasm filled with tightly packed mitochondria, but a poorly developed endoplasmic reticulum and Golgi apparatus. Fat cells and mast cells often are present in the stroma of adenomas.

Adenomas are surrounded by a fine, partial to complete, connective tissue capsule, and may compress the adjacent thyroid gland. A rim of compressed and atrophic parathyroid parenchyma usually is present outside the capsule of a small adenoma (Fig. 3.39). The parathyroid tissue in the rim, and in other parathyroids, of an animal with a functional parathyroid adenoma is chronically suppressed by the elevated serum calcium (Fig. 3.40). The suppressed chief cells have numerous droplets of lipofuscin and lipid. These atrophic chief cells are small, irregular in shape, and have a small amount of densely eosinophilic cytoplasm and densely basophilic nuclei.

Parathyroid adenomas usually are slow growing and compress the adjacent thyroid to various degrees. They are well encapsulated and can be surgically excised without difficulty. Successful removal of a functional parathyroid adenoma results in a rapid decrease in circulating parathyroid hormone levels, since the half-life of parathyroid hormone in plasma is short (~5 min). *Plasma calcium levels in animals with overt bone disease may decrease rapidly following surgical removal of the parathyroid adenoma* due to mineralization of osteoid matrix and reach subnormal levels within 12–24 hours, resulting in life-threatening hypocalcemic tetany (Fig. 3.41). Postoperative hypocalcemia is the result of depressed secretory activity in the remaining parathyroid tissue due to long-term suppression by chronic hypercalcemia, decreased bone resorption, and accelerated mineralization or organic matrix formed by the hyperplastic osteoblasts along bone surfaces. Infusion of calcium gluconate, feeding a high-calcium diet, and supplemental vitamin D therapy will correct this postoperative complication.

Chief cells comprising functional parathyroid adenomas are in the actively synthesizing stage of the secretory cycle by electron microscopy. Multiple large lamellar arrays of rough endoplasmic reticulum and clusters of free ribosomes are present in the cytoplasm. However, few mature secretory ("storage") granules are present in the cytoplasm suggesting that parathyroid hormone is secreted at a faster rate than synthesis and storage in autonomous chief cells. Large mitochondria and prominent Golgi apparatuses are present in neoplastic chief cells. The annulate lamellae that occur frequently in parathyroid adenomas from human patients have not been reported in either normal or neoplastic chief cells of animals. Parathyroid adenomas may contain secretory granules in chief cells as well as in transitional oxyphil cells with well-developed organelles concerned with hormonal synthesis and packaging. Occasional parathyroid adenomas in both dogs and human beings may be composed predominantly of large eosinophilic oxyphil cells.

Parathyroid carcinomas *are larger than adenomas, invade the capsule and adjacent structures (e.g., thyroid glands and cervical muscles), and may metastasize to regional lymph nodes and lung.* They are uncommon in animals but have been observed in dogs and cats. Parathyroid carcinomas are composed of chief cells with a highly variable development of cytoplasmic organelles. Alterations of the nuclear morphology have been described in malignant chief cells. There usually is a greater degree of cellular pleomorphism than in chief cell adenomas with more frequent mitotic figures and microscopic evidence of invasion into the adjacent parathyroid, thyroid, or periglandular connective tissues.

Primary hyperparathyroidism. The clinical disturbances observed with functional parathyroid tumors primarily are the result of the persistent hypercalcemia and a weakening of bones by excessive resorption. Hypercalcemia results in anorexia, vomiting, constipation, depression, polyuria, polydipsia, and generalized muscular weakness due to decreased neuromuscular excitability. Cortical bone in longstanding cases is thinned due to increased resorption by osteoclasts stimulated by the autonomous secretion of parathyroid hormone. Lameness due to fractures of long bones may occur after relatively minor physical trauma. Compression fractures of vertebral bodies can exert pressure on the spinal cord and nerves, resulting in motor or sensory dysfunction, or both. Facial hyperostosis due to extensive osteoblastic proliferation and deposition of poorly mineralized osteoid, and loosening or loss of teeth from alveolar sockets, has been observed in dogs with primary hyperparathyroidism (Fig. 3.37A, B). Primary hyperparathyroidism occurs in older animals. They usually have a history of multiple fractures associated with severe generalized skeletal demineralization and normal renal function. Radiographic evaluation reveals

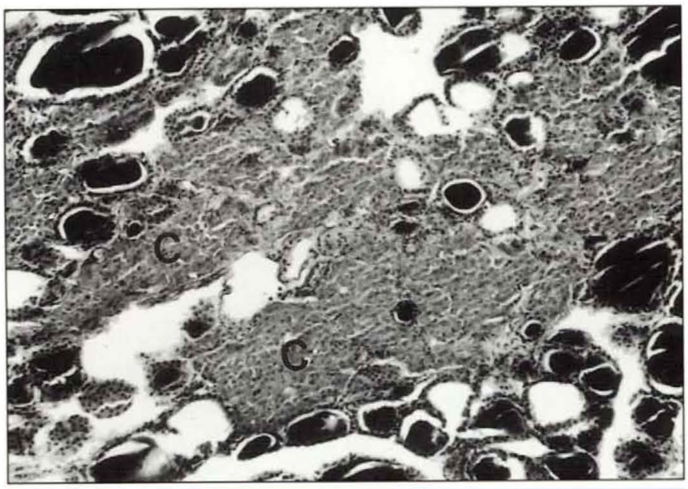

Figure 3.38 Multifocal C-cell hyperplasia (C) in a dog with primary hyperparathyroidism and persistent hypercalcemia. (From Capen CC. Toxicol Pathol 2001:29:8–33.)

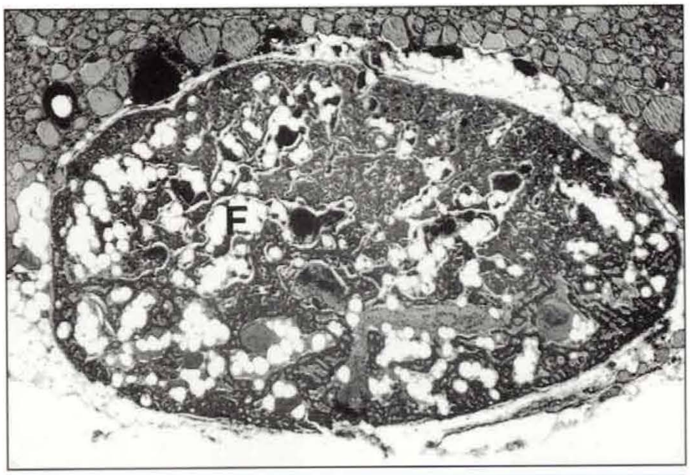

Figure 3.40 Diffuse trophic atrophy of nonneoplastic parathyroid gland in a dog with primary hyperparathyroidism and persistent hypercalcemia. There are prominent fat cells (F) in the interstitium between the groups of atrophic chief cells. (From Capen CC. Toxicol Pathol 2001:29:8–33.)

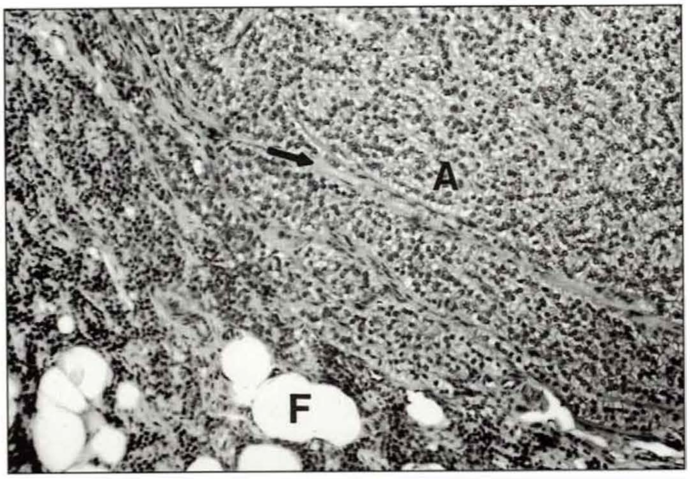

Figure 3.39 Functional chief cell adenoma (A) in the parathyroid gland sharply demarcated and partially encapsulated (arrow) from the rim of adjacent nonneoplastic parathyroid tissue that has undergone severe atrophy in response to persistent hypercalcemia. Prominent fat cells (F) are present in the interstitium between groups of atrophic chief cells in the suppressed parathyroid gland. (From Capen CC. Toxicol Pathol 2001;29:8–33.)

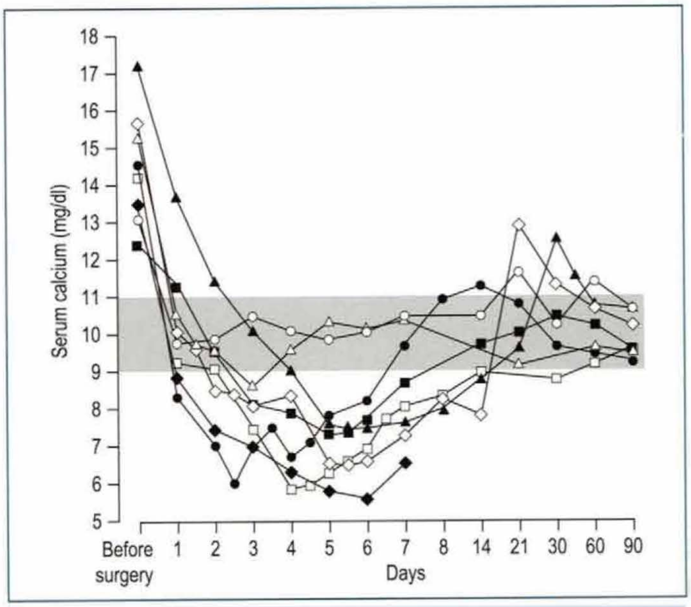

Figure 3.41 Postoperative hypocalcemia in dogs following surgical removal of functional parathyroid adenomas. The remaining atrophic parathyroid glands (as in Fig. 3.40) were unable to maintain the serum calcium because of a subnormal rate of PTH secretion. Supplemental vitamin D and calcium carbonate slowly returned the blood calcium to the reference range (shaded area). (From Berger B. Feldman EC. J Am Vet Med Assoc 1987:191:350–356.)

areas of subperiosteal cortical resorption, loss of lamina dura around the teeth, soft tissue mineralization, bone cysts, and a generalized decrease in bone density with multiple fractures in advanced cases.

The laboratory tests most useful in establishing the diagnosis of primary hyperfunction of the parathyroid glands is quantitation of total blood calcium and phosphorus, and circulating levels of parathyroid hormone (N-terminal or immunoradiometric assay (IRMA)). Although other laboratory findings may be variable, *persistent hypercalcemia (>12 mg/dL, >3.0 mmol/L) is a consistent finding.* Dogs evaluated with primary hyperparathyroidism often have had a greatly elevated (13–20 mg/dL, 3.25–5.0 mmol/L, or higher) blood calcium level. The blood phosphorus level is low or in the low-to-normal range (<4 mg/dL, <1.3 mmol/L) because of inhibition of renal tubular resorption of phosphorus by excess parathyroid hormone. Serum alkaline phosphatase activity may be increased due to increased osteoblastic activity as a response to mechanical stress of bones weakened by excessive resorption or due to direct (receptor-mediated) stimulation of osteoblasts by the elevated PTH level. The urinary excretion of calcium and phosphorus is increased and may predispose to the development of nephrocalcinosis and urolithiasis. Accelerated bone matrix catabolism is reflected by increased excretion of hydroxyproline in the urine. Repeated palpation of parathyroid gland tumors may result in infarction due to vascular thrombosis with extensive ischemic necrosis of tumor cells and the rapid development of hypocalcemia similar to that seen following surgical removal.

Hypercalcemia associated with neoplasms of nonparathyroid origin

Hypercalcemia is a common disorder that affects animals and has many causes. *The most common cause of hypercalcemia in animals and human beings is cancer-associated hypercalcemia.* There are *three mechanisms* of increased serum calcium induced by neoplasms:

1. humoral hypercalcemia of malignancy (HHM), or pseudohyperparathyroidism,
2. hypercalcemia induced by metastases of solid tumors to bone, and
3. hematologic malignancies.

Hypercalcemia results from an imbalance of calcium released from bones, calcium excretion by the kidney, and/or calcium absorption from the intestinal tract. The functional disturbances of hypercalcemia are similar regardless of underlying cause and depend on the rapidity of onset and magnitude of the increased serum ionized calcium levels. *Animals with serum calcium values in excess of 16.0 mg/dL (4.0 mmol/L) generally have the most severe clinical signs.* Exceptions to this rule occur, and occasionally animals with severe hypercalcemia have mild clinical signs especially if the calcium is primarily protein-bound. Horses and rabbits have normal total serum calcium concentrations greater than other domestic animals, which should be considered before hypercalcemia is diagnosed in these species.

Metabolic acidosis will enhance the severity of clinical signs since it will result in an increase in the ionized fraction of serum calcium. Increased serum ionized calcium will induce clinical signs related to the gastrointestinal, neuromuscular, cardiovascular, and renal systems. Decreased contractility of the gastrointestinal smooth muscle may be associated with anorexia, vomiting or constipation. There may be generalized motor weakness due to decreased neuromuscular excitability. Polyuria and polydipsia are commonly encountered in animals with hypercalcemia. Initially, polyuria and polydipsia are due to impaired renal concentrating ability resulting from an inhibition by the hypercalcemia of the antidiuretic hormone-dependent resorption of NaCl in the diluting segment of the nephron by decreasing adenylate cyclase activity. Hypercalcemia also has a toxic effect on renal tubules either directly or from ischemia induced by vasoconstriction. Tubular epithelial cells undergo degeneration with the collecting system most severely affected.

Humoral hypercalcemia of malignancy (HHM) is one form of cancer-associated hypercalcemia that is induced by the secretion of humoral factors that have effects distant to the site of the neoplasms. Some neoplasms that cause HHM may metastasize to bone (Fig. 3.42A), but the primary mechanism of hypercalcemia involves the distant effects of humoral factors and not the localized effects of bone metastases. *Multiple humoral factors have been associated with HHM including parathyroid hormone, parathyroid hormone-like proteins, cytokines, steroids such as $1,25(OH)_2D_3$, and prostaglandins.* Many of the humoral factors produced by tumor cells that alter calcium metabolism and bone resorption can also be produced by osteogenic cells, such as osteoblasts.

The clinical syndrome of HHM in animals mimics primary hyperparathyroidism, and a term used previously to describe HHM was "*pseudohyperparathyroidism*." However, HHM does not mimic hyperparathyroidism in all aspects, as there are distinct differences in bone remodeling and vitamin D metabolism between patients with HHM and with primary hyperparathyroidism. *The most consistent feature of HHM in animals is increased osteoclastic bone resorption distant to the site of the neoplasm.* Other features include hypercalciuria, decreased fractional excretion of calcium, increased nephrogenous 3′,5′-cyclic adenosine monophosphate (cAMP), hypophosphatemia, and hyperphosphaturia.

The similarities of these clinicopathologic alterations to hyperparathyroidism initially led to the hypothesis that the neoplasms secrete parathyroid hormone (PTH) or PTH-like substances; however, *native PTH is not produced by the vast majority of neoplasms associated with HHM.* Immunoreactive PTH levels are normal or low in patients with HHM, and the tumors do not produce PTH mRNA. There are well-validated reports of ectopic production of PTH by some tumors, but these occurrences are uncommon.

Purification of the PTH-like biologic activity from neoplasms associated with HHM resulted in the discovery of a hormone named **parathyroid hormone-related protein (PTHrP)** (previously referred to as *parathyroid hormone-like peptide* or *hypercalcemia of malignancy factor*). PTHrP plays a central role in the pathogenesis of HHM since it is a consistent feature of HHM and shares most or all of the biologic activities of PTH. PTHrP acts alone in some forms of HHM but may act synergistically or additively with other humoral factors in the pathogenesis of hypercalcemia (Fig. 3.42B).

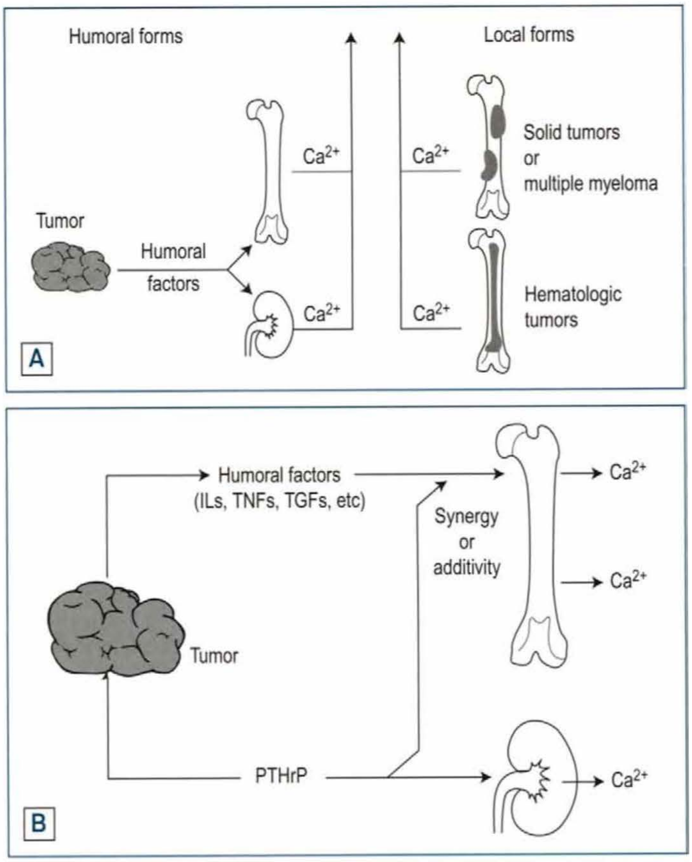

Figure 3.42 A. Humoral and local forms of **cancer-associated hypercalcemia** increase circulating concentrations of calcium by stimulating osteoclastic bone resorption and increasing renal tubular reabsorption of calcium. **B.** Multiple humoral factors can act additively or synergistically to induce hypercalcemia associated with humoral hypercalcemia of malignancy (HHM). (IL = interleukin, TNF = tumor necrosis factor, TGFs = transforming growth factors, PTHrP = parathyroid hormone-related protein. (From Rosol TJ, Capen CC. Lab Invest 1992;67:680–702.)

It is possible that some examples of HHM are not associated with PTHrP production by the neoplasms, but rather are due to recognized or unrecognized humoral factors, cytokines or hormones that are capable of stimulating osteoclastic bone resorption and result in hypercalcemia.

Humoral hypercalcemia of malignancy occurs as a spontaneous disease in domestic and laboratory animals. Malignant neoplasms that are commonly associated with HHM in animals include the *adenocarcinoma derived from apocrine glands of the anal sac in dogs, some T-cell lymphomas of dogs, myelomas, and miscellaneous carcinomas that sporadically induce HHM in various species, such as cats and horses (gastric squamous cell carcinoma)*. The dog is most often affected. HHM occurs in 10–40% of dogs with lymphoma (usually thymic or multicentric forms), and in the majority of dogs with adenocarcinomas derived from apocrine glands of the anal sac. Lymphoma is a relatively common tumor in older, large-breed dogs, whereas the apocrine adenocarcinoma is less common and occurs most often in older female dogs. HHM also occurs sporadically in dogs, usually in association with other carcinomas.

There are three primary mechanisms by which humoral factors can induce hypercalcemia:

1. stimulation of osteoclastic bone resorption,
2. increase in calcium reabsorption from the kidney (Fig. 3.43), and
3. increase in calcium absorption from the intestine.

Increased osteoclastic bone resorption is a fundamental component of the pathogenesis of hypercalcemia and is a consistent feature of HHM. The kidney also plays a central role in the induction of hypercalcemia. Increased osteoclastic bone resorption would increase the flux of calcium into the circulation, but this could be readily excreted by the kidney under normal circumstances. In HHM, fractional excretion of calcium often is reduced, which results in decreased excretion and increased tubular reabsorption of calcium. Therefore, bone and renal mechanisms both appear to be responsible for hypercalcemia in HHM. The role of intestinal absorption of calcium in the pathogenesis of hypercalcemia has not been well evaluated in domestic animals.

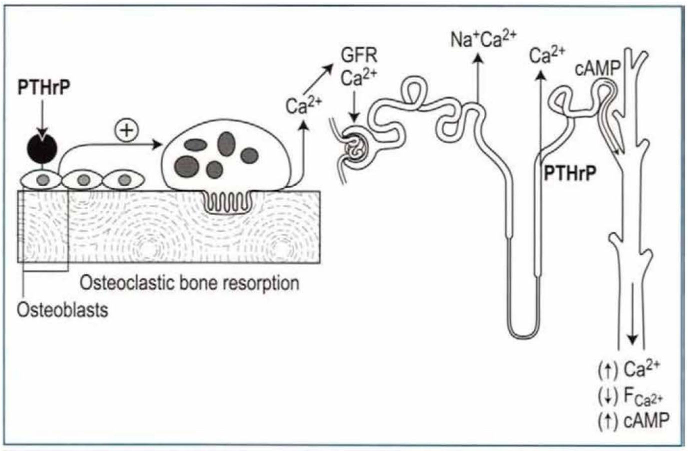

Figure 3.43 Humoral hypercalcemia of malignancy (HHM) develops in animal and human patients when neoplasms constitutively secrete parathyroid hormone-related protein (PTHrP). PTHrP binds to receptors on osteoblasts, which subsequently signal osteoclasts to increase bone resorption, and renal epithelial cells in the distal nephron to enhance calcium reabsorption (decreasing fractional (F) calcium (Ca^{++}) excretion), resulting in persistent hypercalcemia. cAMP = cyclic adenosine monophosphate. (From Capen CC. Toxicol Pathol 2001;29:8–33.)

Most of the humoral factors implicated in the pathogenesis of HHM are capable of stimulating osteoclastic bone resorption in vivo and in vitro. The humoral factors must be secreted by the neoplasms in large enough quantities to increase circulating concentrations and stimulate bone resorption alone or by cooperative interactions with other humoral factors. None of the humoral factors identified stimulates osteoclast formation or activation directly. Most factors, including PTHrP and cytokines, bind to receptors on osteoblasts, which are responsible for coordinating the stimulation of osteoclastic bone resorption.

Parathyroid hormone-related protein (PTHrP) was initially purified from human tumors associated with HHM; subsequently, the cDNA and gene for PTHrP were isolated, sequenced, and characterized. The PTHrP gene is complicated and contains at least 6 exons, two 5′-untranslated regions each with its own promoter, a preprohormone coding region, a main coding region, and 2 additional 3′ regions that have coding and noncoding regions. There is alternate splicing of PTHrP mRNA so that 3 mature peptides can be formed composed of 139, 141 and 173 amino acid residues. The PTHrP gene is located on the short arm of chromosome 12 in humans and is thought to have evolved from the PTH gene on the short arm of chromosome 11 through chromosome duplication. The PTH gene is simpler and has only three exons (5′-untranslated region, preprohormone coding region, and main coding region with a 3′-noncoding region) and encodes for a protein of 84 amino acids.

Parathyroid hormone-related protein binds to the well-defined PTH receptors in bone and kidney with similar affinity to PTH. The first 34 N-terminal amino acids of PTHrP contain the PTH-like biologic activity. This is similar to PTH in which the first 34 N-terminal amino acids (of a total of 84) contain most of the biologic activities. There is 70% sequence homology of the first 13 N-terminal amino acids of PTHrP and PTH, but little homology after amino acid 13. The N-terminus is important in the activation of the PTH receptor. Sequence homology in this region is important for the ability of PTHrP to stimulate PTH receptors. The biologic actions of PTHrP in bone and kidney are similar to PTH. Both PTHrP and PTH induce their cellular actions in osteoblasts and renal cells by stimulating adenylate cyclase, phospholipase C, and pathways leading to increased intracellular calcium concentration. The efficiency of signal transduction by PTHrP in different cells or membranes may be affected by differences in species specificity for PTHrP. N-terminal PTHrP stimulates osteoclastic bone resorption in vivo and in vitro with similar potency to PTH.

Parathyroid hormone-related protein plays a central role in the pathogenesis of HHM. It is produced by most tumors and is present in the circulation at increased concentrations in animals and human patients with HHM. The administration of anti-PTHrP antibodies to animal models of HHM decreases serum calcium levels and bone resorption. Increased circulating concentrations of PTHrP also have been detected in dogs with spontaneous HHM as well as in experimental animal models of HHM. Dogs with adenocarcinomas derived from apocrine glands of the anal sac have markedly elevated concentrations of PTHrP (10–100 pM), suggesting that PTHrP plays a primary role in the pathogenesis of the hypercalcemia associated with these tumors. In contrast, dogs with HHM and lymphoma have PTHrP concentrations which range from undetectable to 17 pM. PTHrP appears not to be the sole humoral factor responsible for the induction of hypercalcemia in dogs with lymphoma, but may interact cooperatively with other humoral factors.

The syndrome of humoral hypercalcemia of malignancy in elderly female dogs most frequently is associated with *perirectal adenocarcinomas derived from apocrine glands of the anal sac.* The dogs have persistent hypercalcemia and hypophosphatemia that return to normal following surgical excision of the neoplasm in its entirety. The hypercalcemia persists following removal of the parathyroid glands. All tumors have histologic features of malignancy and most metastasize to iliac and sublumbar lymph nodes. Apocrine carcinomas are poorly demarcated from adjacent normal tissues. They frequently extend projections of tumor cells along connective tissue planes and early in the course of the disease invade thin-walled lymphatics and veins to form tumor cell emboli (Fig. 3.44). Functional disturbances in dogs with HHM include generalized muscular weakness, anorexia, vomiting, bradycardia, depression, polyuria, and polydipsia. These clinical signs primarily are the result of severe hypercalcemia, and complicate the problems associated with the malignant neoplasm.

Apocrine adenocarcinomas develop as firm masses in the perirectal area, ventral-lateral to the anus, in close association with the anal sac, but are not attached to the overlying skin. The tumor arises in the wall of the anal sac and projects as a mass of variable size into its lumen (Fig. 3.45A). *It forms glandular acini with projections of apical cytoplasm extending into a lumen* (Fig. 3.45B) *and is histologically distinct from the more common perianal (circumanal) gland tumor in this region of dogs.* Most neoplasms are histologically bimorphic with glandular and solid areas (Fig. 3.45C). The solid pattern of arrangement of neoplastic cells is characterized by sheets, microlobules, and packets separated by thin fibrovascular stroma. Pseudorosettes are common in solid areas adjacent to small blood vessels.

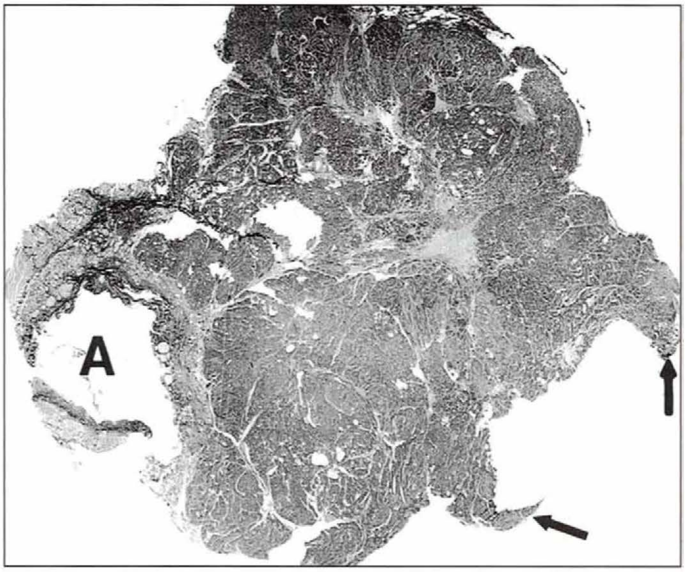

Figure 3.44 Infiltrative carcinoma arising from apocrine glands in the wall of the anal sac (A). extending numerous projections (arrows) into the surrounding perineal connective tissues and into lymphatics in this region. Dog had persistent hypercalcemia and unilateral enlargements of sublumbar lymph nodes due to tumor metastasis. (From Capen CC. In: Tumors of Domestic Animals. 4th ed., 2002.)

The tumor cells in adenocarcinomas derived from apocrine glands of the anal sac contain well-developed rough endoplasmic reticulum, clusters of free ribosomes, large mitochondria, and prominent Golgi apparatuses. Prominent blebs of apical cytoplasm project into the lumens of glandular acini. Small, membrane-limited, secretory granules often are present in the apical cytoplasm of neoplastic cells. These granules are similar in size and electron density to PTH-containing storage granules in chief cells of normal parathyroid glands; however, it has not been determined whether they contain hormonal activity. It appears that the neoplastic cells secrete PTHrP in a constitutive rather than a regulated manner as most tumor cells have few, if any, secretory granules in their cytoplasm.

Skeletal demineralization in dogs with HHM is mild in comparison with other causes of hypercalcemia and usually undetectable by conventional radiographic methods. Neoplastic cells from the perirectal adenocarcinomas rarely metastasize to bone and cause osteolysis. Variable numbers of osteoclasts are present on bone surfaces in dogs with marked hypercalcemia (Fig. 3.45D), possibly reflecting different states in the course of the disease and phases of bone remodeling activity. Osteocytic osteolysis is not detected microscopically and the cement lines are smooth and linear.

Renal mineralization is detected histologically in ~90% of dogs with pseudohyperparathyroidism associated with apocrine adenocarcinomas of the anal sac, particularly when the Ca × P product expressed as mg/100 mL is 50 or greater. Tubular mineralization is most pronounced near the corticomedullary junction but also is present in cortical and deep medullary tubules, Bowman's capsules, and glomerular tufts. Mineralization is present less frequently in the fundic mucosa of the stomach and endocardium.

The parathyroid glands are small and difficult to locate or not visible macroscopically. Microscopically, they are characterized by narrow cords of inactive chief cells with abundant fibrous connective tissue stroma and widened perivascular spaces (Fig. 3.46). This suggests that apocrine adenocarcinomas do not stimulate parathyroid hormone secretion, but rather the parathyroids respond to the persistent hypercalcemia by undergoing trophic atrophy. Thyroid C cells respond to the persistent elevation in blood calcium by undergoing diffuse or nodular hyperplasia. There is an inappropriate serum concentration of 1,25-dihydroxycholecalciferol for the degree of hypercalcemia in tumor-bearing dogs, and the rapid decrease following excision of the tumor suggests that the tumor may secrete a substance(s) that alters the activity of the 1-α-hydroxylase in the kidney.

Physiologic role for parathyroid hormone-related protein. Parathyroid hormone-related protein (PTHrP) is a 139–173 amino acid peptide, originally isolated from human and animal tumors associated with humoral hypercalcemia of malignancy. However, PTHrP is not strictly a calcium-regulating hormone as it appears to have an important physiologic role in may normal tissues. It have been determined that PRTHrP is widely produced in the body and acts as a paracrine factor in the tissues in which it is produced.

In contrast to parathyroid hormone, which is produced only by the parathyroid gland, PTHrP is produced by many normal tissues in adult animals, including stratified squamous epithelium, endocrine glands (adrenal cortex and medulla, fetal and adult parathyroid glands, adenohypophysis, thyroid), skeletal, cardiac and smooth muscle, kidney, bone, lactating mammary gland, lymphocytes, lung, skin, bone, brain, pancreas, testis, prostate, ovary, myometrium, placenta,

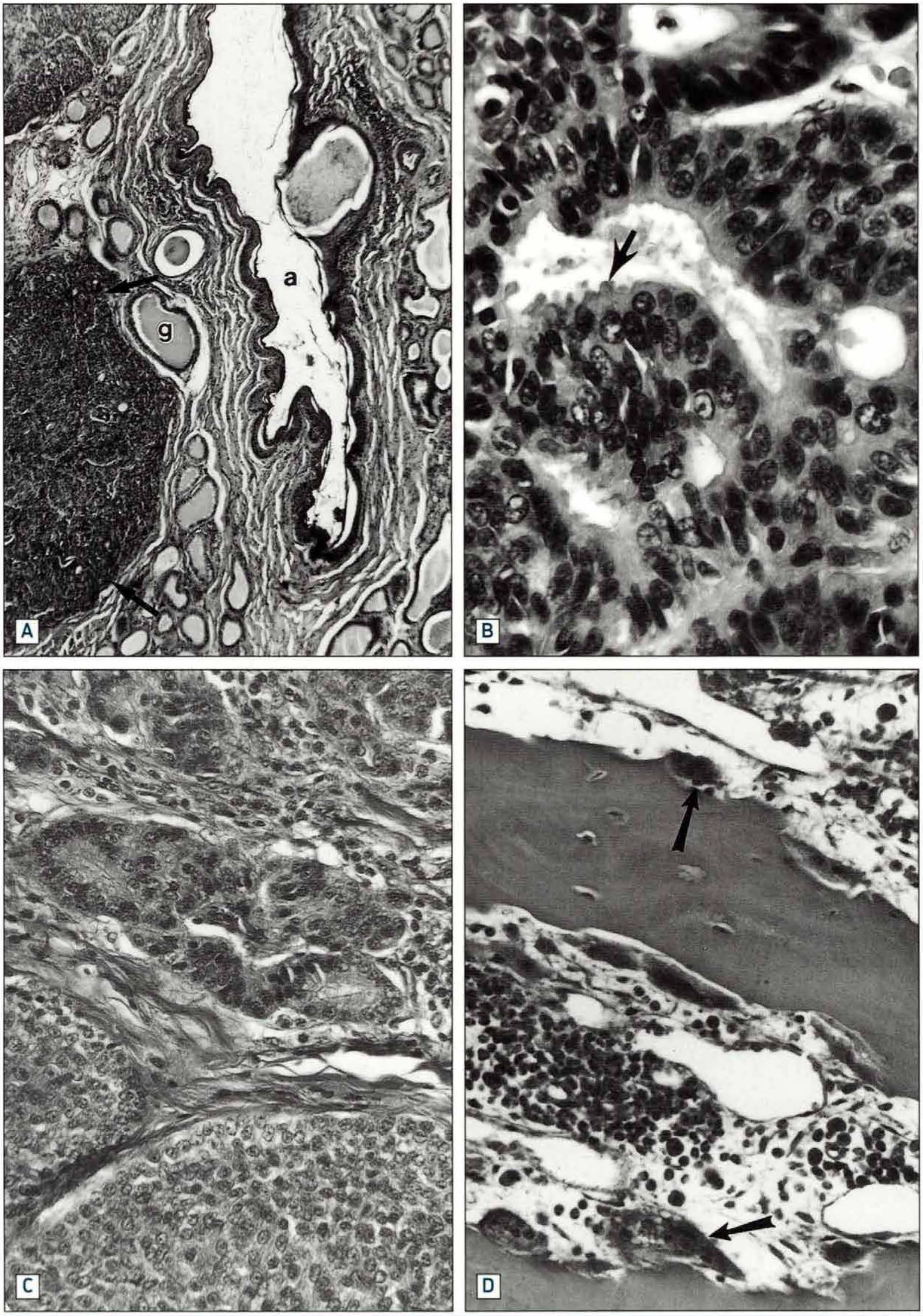

Figure 3.45 Apocrine adenocarcinoma of anal sac in a dog. **A.** Adenocarcinoma (arrow), normal glands (g) and anal sac (a). **B.** Glandular acini lined by neoplastic cells with apical cytoplasmic projections (arrow). **C.** Bimorphic growth pattern of apocrine adenocarcinoma of anal sac in a dog. **D.** Osteoclastic bone resorption. There are numerous osteoclasts with excavations (arrows) aligned on trabecular bone surfaces. (From: Meuten DJ, et al. Vet Pathol 1981;18:454–471.)

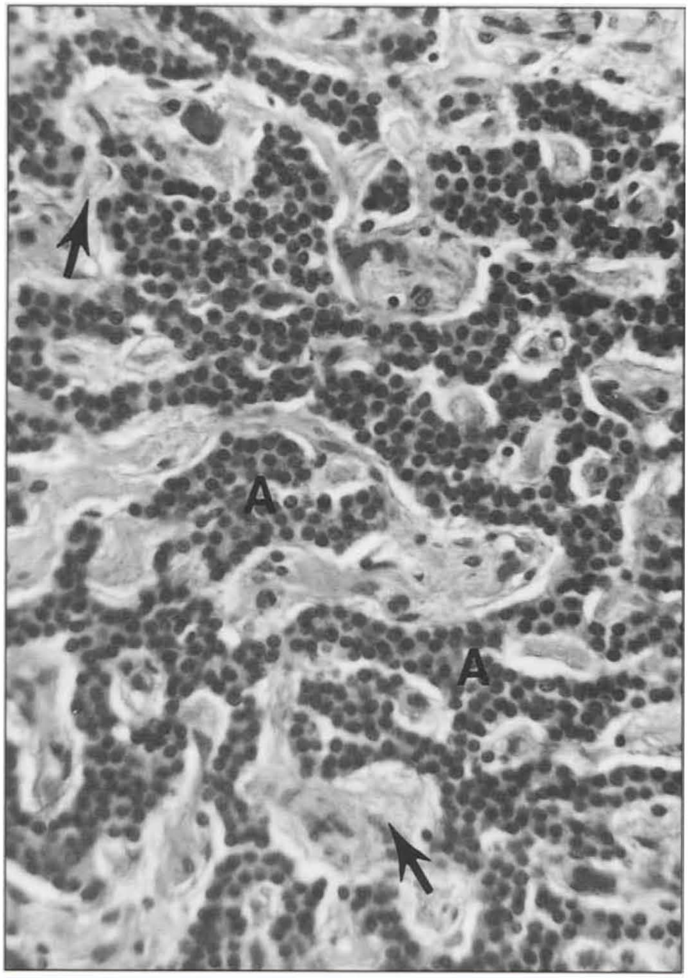

Figure 3.46 Atrophic parathyroid gland from a dog with cancer-associated hypercalcemia. Narrow cords of atrophic chief cells (A) are separated by interstitial spaces with increased connective tissue (arrows).

and avian oviduct. *Present evidence suggests that PTHrP acts as an autocrine or paracrine regulator in these normal tissues,* although its exact function in most normal adult tissues is largely unknown. Circulating concentrations of PTHrP in normal animals and humans are low (<1 pM) and the PTH/PTHrP receptor is often expressed on the same or adjacent cells in tissues that synthesize PTHrP. Epidermal keratinocytes produce PTHrP, which plays a role in their proliferation or differentiation.

The fetus maintains higher concentrations of serum calcium compared to the dam. Because the fetal parathyroid glands produce low levels of PTH, the mechanism of maintaining increased serum concentrations of calcium was unknown until the finding that *PTHrP maintains calcium balance in the fetus and is the major hormone secreted by the chief cells of the fetal parathyroid glands.* The PTHrP produced by the placenta also stimulates the uptake of calcium by the fetus. Parathyroid hormone-related peptide plays a role in the differentiation of many tissues during gestation and is especially important in the growth and development of bone. Growth of cartilage at the epiphyseal plate is regulated by PTHrP, which stimulates chondrocyte proliferation, inhibits apoptosis, and inhibits the maturation of chondrocytes from the proliferative zone to the hypertrophic zone.

The greatest concentration of PTHrP is found in milk (10–100 nmol/L) and is 10 000- to 100 000-fold greater than in the serum. The function of PTHrP in the mammary gland and in milk is poorly understood at present. However, recent studies have reported that over-expression of PTHrP in the mammary gland during glandular development prior to lactation results in glandular hypoplasia due to a reduction in the morphogenesis and branching of the mammary ducts. Biologically active PTHrP produced by alveolar epithelial cells during lactation results in the high concentration of PTHrP in milk and this PTHrP may play a role in stimulating the transport of calcium by alveolar epithelial cells from serum to milk. Synthesis of PTHrP by the mammary gland abruptly ceases when suckling stops and the gland undergoes involution. The PTHrP peptide is enzymatically cleaved in milk, but the N-terminal PTHrP fragment retains biologic activity. Smooth muscle including blood vessels, uterus, urinary bladder, gastrointestinal tract, and the oviduct of the hen produce PTHrP. In general, PTH-rP expression is increased when smooth muscle is stretched and PTHrP induces relaxation of smooth muscle and attenuation of contraction. With progressive distension of the uterus during pregnancy or during descent of the ovum in the hen's oviduct, *PTHrP likely functions as a paracrine regulator of vascular tone causing vasodilation and modulating vasoconstriction by other vasoactive compounds.*

Lymphosarcoma is the most common neoplasm associated with hypercalcemia in dogs and cats. Estimates of the prevalence of hypercalcemia in lymphoma dogs vary from 10 to 40%. Peripheral lymph node enlargement may or may not be detected, but there usually is evidence of cranial mediastinal or visceral involvement. *Most lymphomas associated with HHM in dogs are of the T-cell subset,* whereas lymphomas associated with normal blood calcium are usually of B-cell origin. Most dogs with lymphoma and hypercalcemia have increased circulating concentrations of PTHrP but the concentrations are not as high as those in dogs with HHM and apocrine adenocarcinomas, and PTHrP does not correlate with serum calcium concentration. It is not completely resolved whether the hypercalcemia develops from the production of humoral substances by neoplastic cells or from physical disruption of trabecular bone due to frequent marrow involvement, or from both mechanisms. Dogs with lymphosarcoma cells in bone marrow often have increased numbers of osteoclasts on trabecular bone surfaces opposite a surface lined by osteoid and large columnar osteoblasts (Fig. 3.47A, B).

Hypercalcemia with tumors metastatic to bone. *Solid tumors that metastasize widely to bone can produce hypercalcemia by the induction of local bone resorption associated with tumor growth. This is not common in animals,* but is an important cause of cancer-associated hypercalcemia in human beings. Tumors that often metastasize to bone and induce hypercalcemia in human patients include breast and lung carcinomas. The pathogenesis of enhanced bone resorption is not completely understood, but the *two primary mechanisms* include: (1) secretion of cytokines or factors that stimulate local bone resorption, and (2) indirect stimulation of bone resorption by tumor-induced cytokine secretion from local immune or bone cells. Cytokines or factors that may be secreted by tumor cells and stimulate local bone resorption include PTHrP, transforming growth factor-α and -β, and prostaglandins (especially PGE_2). In some cases, bone-resorbing activity can be inhibited by indomethacin, which suggests that prostaglandins are either directly or indirectly associated with the stimulation of bone resorption. The cytokines most often

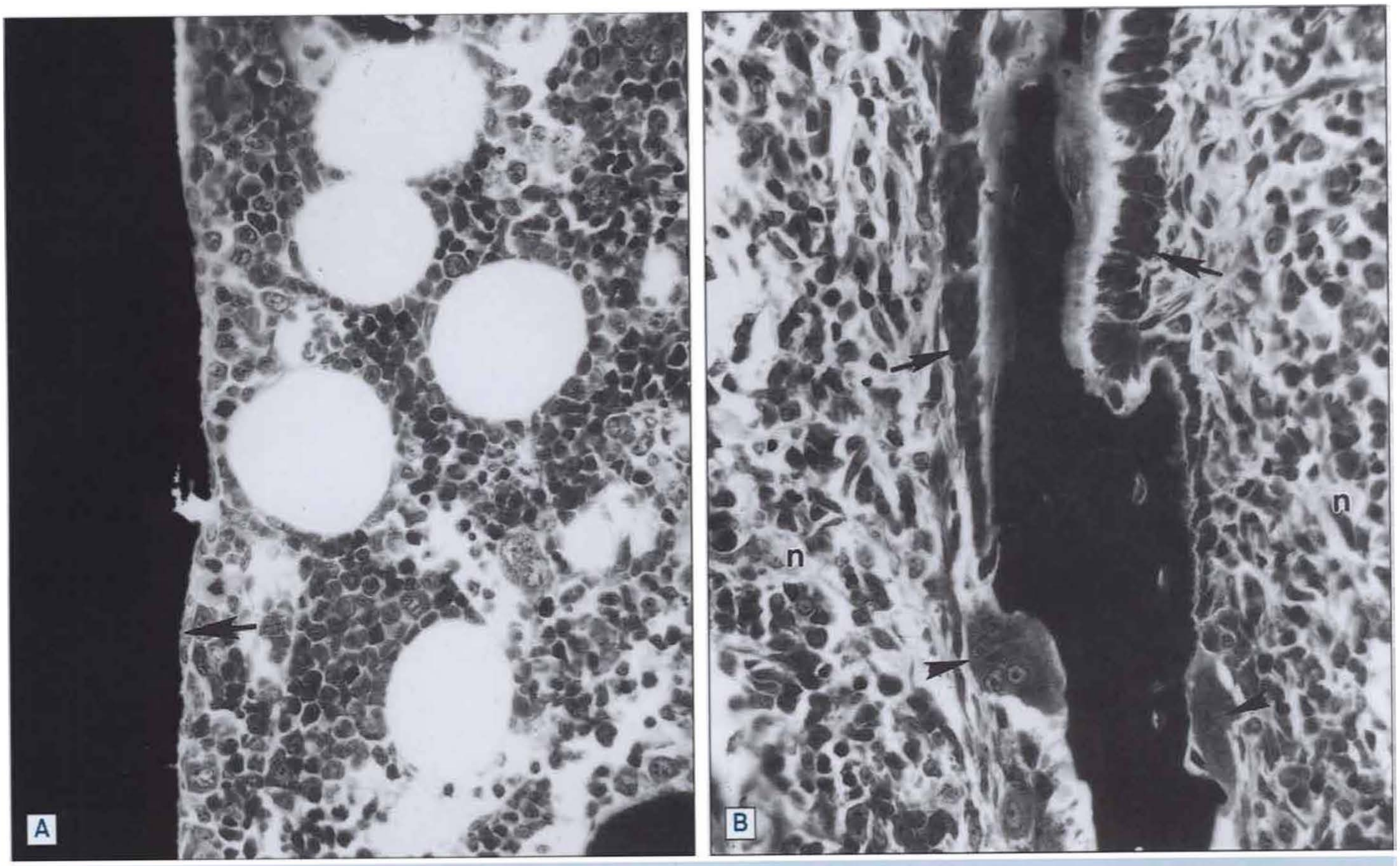

Figure 3.47 Lumbar vertebrae from a dog, von Kossa tetrachrome. **A.** Normocalcemic dog with smooth bone surface and flattened osteoblasts (arrow). **B. Hypercalcemic dog with lymphosarcoma of marrow** (neoplastic cells, n). Several osteoclasts (arrowheads) are present in lacunae. Hyperplastic osteoblasts (arrows) and prominent osteoid seams are present adjacent to resorptive surfaces. (From Meuten DJ, et al. Lab Invest 1983:49:553–562.)

implicated in indirect stimulation of bone resorption by local immune cells include interleukin-1 and tumor necrosis factor.

Differential diagnosis of humoral hypercalcemia of malignancy. *Lymphoma is the most common neoplasm associated with hypercalcemia in dogs and cats.* Peripheral lymph node enlargement may or may not be detected, but evidence usually exists for cranial mediastinal or visceral involvement. Serum immunoreactive PTH levels have been found to be subnormal in hypercalcemic dogs with lymphoma, and plasma immunoreactive PGE_2 levels did not differ from levels in control dogs. Culture media from normal lymphoid tissue and control media had no effect on release of ^{45}Ca from prelabeled fetal mouse forelimb bones; however, media from tumor tissue increased ^{45}Ca release. These findings suggested that the local production of bone-resorbing factors was important in stimulating calcium release from bone in certain dogs with lymphoma and hypercalcemia.

Evaluation of parathyroid function

Parathyroid function may be altered by a wide variety of diseases or by exposure to chemicals that either elevate or lower the blood concentration of calcium (particularly calcium ion). In response to hypocalcemia, chief cells undergo hypertrophy and eventually hyperplasia. On formalin- or Bouin's-fixed sections, the expanded cytoplasmic area is lightly eosinophilic and vacuolated. Perivascular spaces are narrow in a hyperplastic parathyroid and there are few fat cells in the interstitium. In response to hypercalcemia, the cytoplasmic area of chief cells is decreased and more densely eosinophilic often with a widening of intercellular and pericapillary spaces. If the hypercalcemia is prolonged, there is an overall reduction of glandular parenchyma with increased fibrous or adipose connective tissue in the interstitium. Subtle differences between affected and normal animals can be best evaluated by morphometric evaluation. The area ratios of parenchyma:interstitium of the glands and cytoplasm:nucleus of chief cells should be assessed.

Ultrastructural evaluation of chief cells is a sensitive means of morphologically evaluating the parathyroid gland. Perfusion of the thyroid–parathyroid area with glutaraldehyde-based fixatives followed by post-fixation with osmium tetroxide results in the best retention of structural detail in parathyroids of animals. Morphometric studies can be used to quantitate total cytoplasmic area and area occupied by a particular organelle (e.g., secretory granule).

In response to an acute lowering of blood calcium, a larger percentage of chief cells ultrastructurally will be in the active stage of synthesis and secretion than under steady-state conditions. This is indicated by a peripheral migration of secretory granules and alignment along the plasma membrane, aggregation of the endoplasmic reticulum into lamellar arrays, and enlargement of the Golgi apparatus associated with many small dense granules in the process of formation. Conversely, chief cells in response to hypercalcemia are predominantly in the inactive stage of the secretory cycle as evaluated by electron microscopy with dispersed profiles of endoplasmic reticulum, small Golgi complexes with few granules, and often accumulations of either glycogen or lipid (depending upon species) in the cytoplasm. Secretory granules accumulate initially in response to elevated blood calcium, but subsequently decrease due to degradation by lysosomal enzymes by the process of crinophagia. Atrophic chief cells develop in response to sustained and/or more severe hypercalcemia. Their cytoplasm is more electron-dense and irregularly shrunken with widened intercellular spaces. Cytoplasmic organelles are poorly developed and may have early degenerative changes suggested by mitochondrial vacuolation with disruption of cristae and distention of endoplasmic reticulum with loss of ribosomes.

The metabolism of PTH and the formation of multiple circulating forms of PTH have made development of clinically useful immunoassays challenging. The principal circulating forms of PTH include intact PTH (1-84) and C-terminal peptides (PTH 35-84). Parathyroid hormone has two functional domains. N-terminal PTH (1-34) is the region of major biological activity in relation to calcium regulation. N-terminal PTH does not exist in the circulation in a biologically relevant concentration, but administration of exogenous PTH (1-34) will induce the typical actions of PTH on bone and kidney cells. The function of C-terminal PTH-derived peptides is less well understood.

PTH is secreted from chief cells in two forms, *viz.* intact PTH (1-84) and C-terminal peptides. PTH (1-84) is the circulating form of biologically active PTH and is rapidly removed from the circulation by endopeptidases on Kupffer cells or glomerular filtration. Its half-life is less than 5 minutes. Some of the C-terminal peptides are released from the Kupffer cells back into the circulation and are excreted by glomerular filtration. Therefore, C-terminal PTH has a longer serum half-life than PTH (1-84) and is present in the serum at greater concentrations (50–90% of total PTH) than PTH (1-84), especially in states of hyperparathyroidism. End organs (bone and kidney) also are responsible for degradation of a small fraction of serum PTH. This involves internalization of PTH–receptor complexes and intracellular degradation by lysosomal enzymes. Rapid removal of PTH from the circulation allows the in vivo actions of PTH to be controlled by the rate of PTH secretion from the parathyroid chief cells.

Immunoassays for PTH initially were single-site radioimmunoassays (RIA) for C-terminal peptides. These assays were suboptimal, since the biologically active form of PTH was not measured; however, they were clinically useful in states of hyperparathyroidism in patients with normal renal function. Midregion and C-terminal RIAs measure both intact PTH and C-terminal PTH, which make them less clinically relevant. In addition, conditions that reduce glomerular filtration rate, such as renal failure, will result in a large increase in the serum concentration of C-terminal PTH. *Intact serum PTH concentrations are best measured by two-site immunoradiometric assay (IRMA) or N-terminal RIA.* Serum intact PTH can be measured in dogs and cats with assays developed for human PTH due to the cross-reactivity of the antisera used in the assays. Intact PTH has been successfully measured in dogs or cats with either a human N-terminal RIA or two-site IRMA with good correlation between the two assays.

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THYROID GLAND

Development, structure, and function

Embryologic development of the thyroid gland

The thyroid gland originates as a thickened plate of epithelium in the floor of the pharynx. It is intimately related to the aortic sac in its development and this association leads to the frequent finding of *accessory thyroid parenchyma* in mediastinal structures, especially in dogs. This accessory thyroid tissue may undergo neoplastic transformation. Branched cell-cords develop from the pharyngeal plate and migrate dorsolaterally but remain attached to the pharyngeal area by the narrow *thyroglossal duct.* The entodermal bud that forms the thyroid gland arises on the midline at the level of the first pharyngeal pouch (see Fig. 3.25). This gives rise to the thyroglossal duct, which migrates caudally. The proliferation of cell cords at the distal end of the thyroglossal duct forms the follicles of each thyroid lobe. The area at the base of the tongue marking the origin on the thyroid gland is referred to as the *foramen cecum linguae* in postnatal life. Calcitonin-secreting C cells of neural crest origin reach the postnatal thyroid gland by migrating into the *ultimobranchial body.* This last pharyngeal pouch moves caudally in mammals to fuse with the primordia of the thyroid gland and distribute C cells into each thyroid lobe.

Folliculogenesis is a complex process in the thyroid that begins by the proliferation of irregularly arranged cell cords derived from the entoderm of the thyroglossal duct on the midline of the developing pharyngeal gut. The solid cell clusters extend caudally into the primitive mesenchyme and develop complex interdigitations of plasma membranes of adjacent cells. Primitive follicles develop either by invagination of the plasma membrane of individual follicular cells to form intracytoplasmic colloid-containing microfollicles that subsequently coalesce to form larger follicles, or by the secretion of colloid in the narrow intercellular spaces limited by zonulae adherens and desmosomes. In rats with a normal 21-day gestation period, thyroid follicles begin to form between days 15 and 18 of gestation. Thyroid stimulating hormone (TSH, thyrotropin) may not be required for the formation of the initial primitive follicles, but follicles formed on day 17 and subsequent days of gestation are responsive to TSH for their final development in rats.

A portion of the thyroglossal duct may persist postnatally and form a cyst. **Thyroglossal duct cysts** are present in the ventral aspect of the cranial cervical region in dogs. Their lining epithelium may undergo neoplastic transformation (see discussion of cervical cysts in parathyroid–thyroid area, above).

Accessory thyroid tissue *is common in the dog,* and may be located anywhere from the larynx to the diaphragm. About 50% of adult dogs have accessory thyroid tissue embedded in the fat on the intrapericardial aorta. These nodules are usually 2–5 mm in dimension and may be multiple (Fig. 3.48). They lack C (parafollicular) cells, which secrete calcitonin, but their follicular structure and function (ability to concentrate iodine and iodinate tyrosyl residues) are the same as that of the main thyroid lobes. Attempts to induce hypothyroidism in the dog by surgical thyroidectomy are not consistently successful because the follicular cells in the accessory thyroids readily respond to the prompt increase in endogenous TSH and undergo hyperplasia sufficient to sustain adequate hormone production.

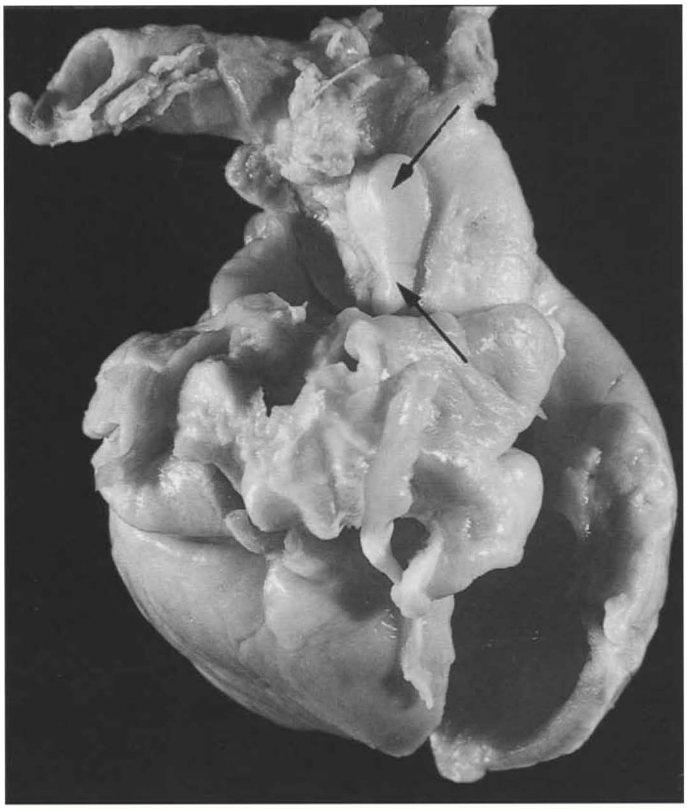


Figure 3.48 Ectopic thyroid tissue (arrows) at the base of the heart from a dog.

Structure of the thyroid gland

The two thyroid lobes in most species are located on the lateral surfaces of the trachea. In pigs, the main lobe of the thyroid is in the midline in the caudal cervical region with dorsolateral projections from each side.

The principal blood supply to each lobe in the dog is from the cranial thyroid artery (a branch of common carotid), and the major venous drainage is via the caudal thyroid vein that enters the internal jugular vein. Lymph drainage from the cranial pole of the thyroid lobes in dogs is to the retropharyngeal lymph nodes. Lymph flow from the caudal aspect of each thyroid lobe is variable, but it often bypasses any lymph nodes before entering the brachiocephalic trunk. Efferent lymphatics usually enter directly into the cervical lymphatic trunk or internal jugular vein. *This explains the frequent occurrence of pulmonary metastases from thyroid carcinoma in dogs prior to development of secondary foci in regional lymph nodes.* Small efferent lymphatics may pass through the caudal cervical lymph nodes along the ventral surface of the trachea before entering the cranial mediastinum. These vascular arrangements are significant for the spread of thyroid carcinomas.

The thyroid gland is the largest of the endocrine organs that functions exclusively as an endocrine gland. The basic histologic structure of the thyroid is unique for endocrine glands, consisting of follicles of various sizes (20–250 μm) that contain colloid produced by the **follicular cells (thyrocytes)**. The follicular cells are cuboidal to low columnar (under conditions of normal iodine intake), and their secretory polarity is directed toward the lumen of the follicles. An extensive network of interfollicular capillaries provides the follicular cells with an abundant blood supply. Follicular cells have extensive

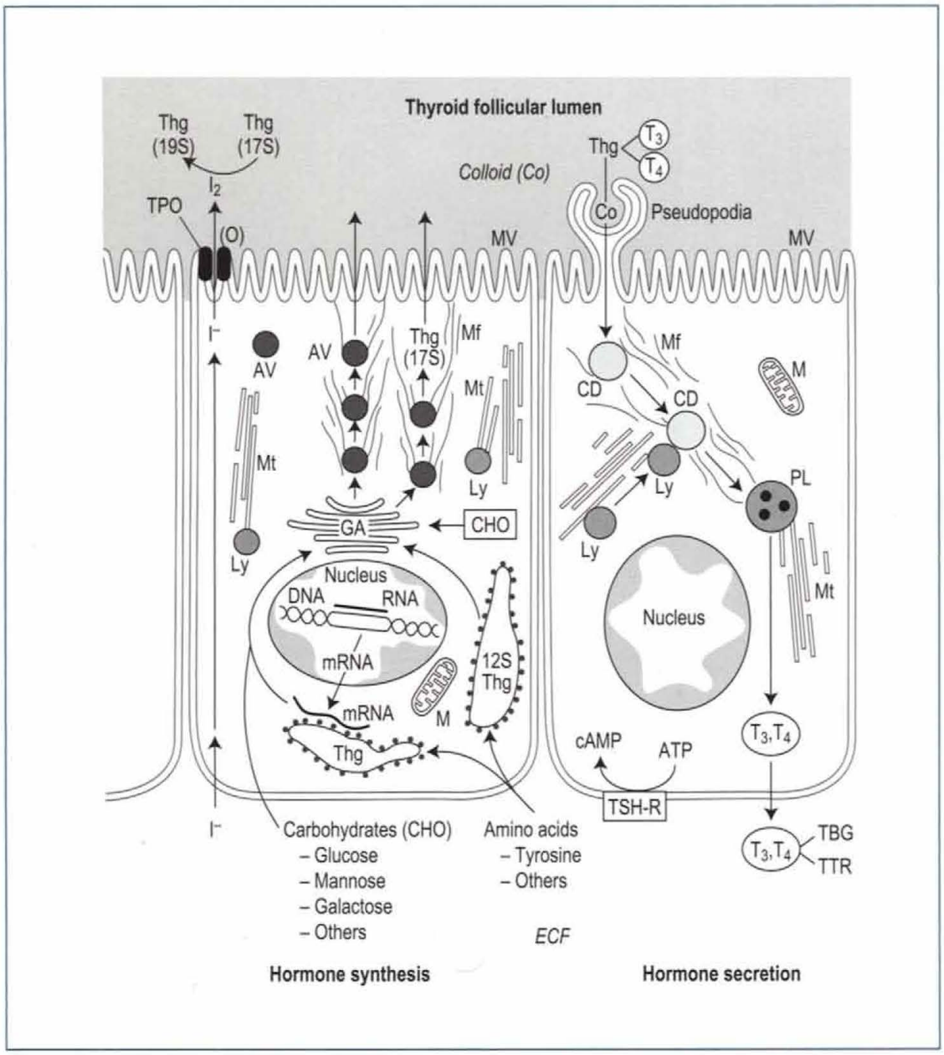


Figure 3.49 Thyroid follicular cells illustrating two-way traffic of materials from capillaries to follicular lumen. Raw materials, such as iodide ion (I^-), are concentrated and rapidly transported into follicular lumen (left side of drawing). Amino acids (e.g., tyrosine) and sugars are assembled by follicular cells into thyroglobulin (Thg), packaged into apical vesicles (AV), and released into lumen. Iodination of tyrosyl residues occurs within the thyroglobulin molecule to form thyroid hormones (iodothyronines) in the follicular lumen. Elongation of microvilli (MV) and endocytosis of colloid by follicular cells occurs in response to TSH stimulation (right side of drawing). Intracellular colloid droplets (CD) fuse with lysosomal bodies (Ly) and active thyroid hormones (T_4 and T_3) are enzymatically cleaved from thyroglobulin and free T_4 and T_3 are released into circulation. (Modified and redrawn from Bastenie PA, et al. In: Molecular Pathology. 1975.)

profiles of rough endoplasmic reticulum and a large Golgi apparatus in their cytoplasm for synthesis and packaging substantial amounts of protein (e.g., thyroglobulin) that is then transported into the follicular lumen. The interface between the luminal side of follicular cells and the colloid is modified by numerous microvillus projections.

Various numbers of lysosomes, histochemically stainable for enzymes such as acid phosphatase, are found in the apical portion of follicular cells. Soon after stimulation of follicular cells by TSH, intracellular droplets of ingested colloid (phagosomes), corresponding to those demonstrated light microscopically by the PAS reaction, are more numerous than in the resting state. Some of these form phagolysosomes in follicular cells by fusion with lysosomes.

Biosynthesis of thyroid hormones

The synthesis of thyroid hormones is unique among endocrine glands because the final assembly of hormone occurs extracellularly within the follicular lumen. Essential raw materials, such as **iodide ion (I^-)**, are trapped by follicular cells from plasma, transported rapidly against a concentration gradient to the lumen of the follicle, and oxidized by a peroxidase enzyme in the microvillus membranes to **iodine (I_2)**.

The assembly of thyroid hormones within the follicular lumen is made possible by a unique protein (**thyroglobulin**) synthesized by follicular cells (Fig. 3.49). Thyroglobulin is a high molecular weight glycoprotein synthesized in successive subunits on the ribosomes of the endoplasmic reticulum in follicular cells. The constituent amino acids (tyrosine and others) and carbohydrates (mannose, fructose, galactose) are derived from the circulation. Recently synthesized thyroglobulin leaving the Golgi apparatus is packaged into apical vesicles that are extruded into the follicular lumen.

The amino acid **tyrosine**, an essential component of thyroid hormones, is incorporated within the molecular structure of thyroglobulin. Iodine is bound to the tyrosyl residues in thyroglobulin at the apical surface of follicular cells to form successively **monoiodotyrosine (MIT)** and **diiodotyrosine (DIT)**. These biologically inactive iodothyronines subsequently are coupled together under the influence of thyroperoxidase to form the two **biologically active iodothyronines** (**thyroxine (T4), triiodothyronine (T3)**) secreted by the thyroid gland.

The functionally important **thyroperoxidase** enzyme in the thyroid hormone synthetic pathway is present in the apical plasma membrane and microvilli as well as in other membranous structures of the follicular cells. Thyroperoxidase is a membrane-bound, heme-containing glycoprotein composed of 933 amino acids with a transmembrane domain. This important enzyme oxidizes (in the presence of hydrogen peroxidase) iodide ion (I^-) taken up by follicular cells

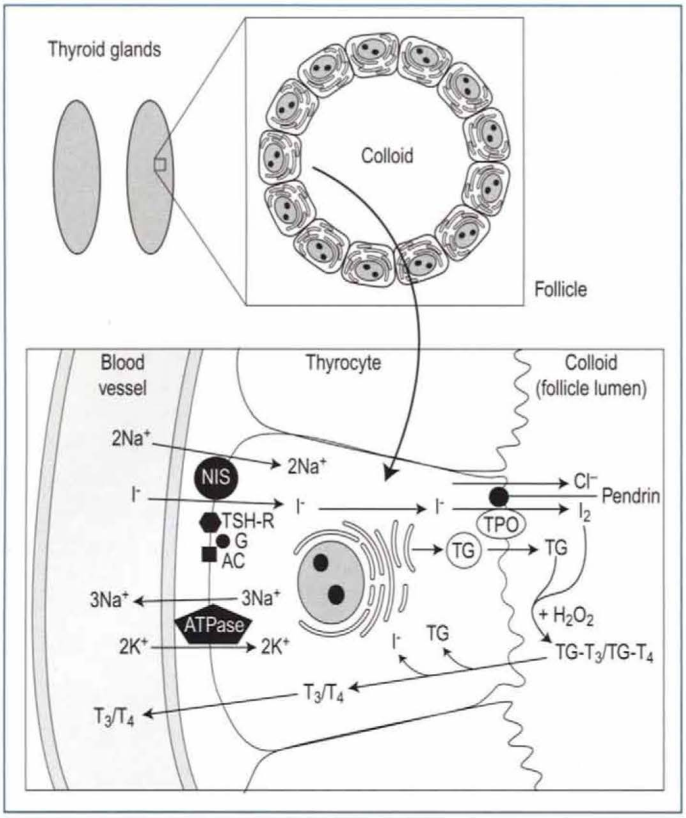


Figure 3.50 Sodium iodide symporter (NIS) and thyroid hormone biosynthesis. The NIS located in the basolateral membrane of thyroid follicular cells is responsible for the rapid uptake and concentration of iodide ion (I^-) from the circulation. The enzyme thyroperoxidase (TPO) in microvilli on the lumenal surface oxidizes I^- in the presence of H_2O_2 to reactive iodine (I_2) that subsequently binds to tyrosyl residues in the thyroglobulin (TG) molecule of the colloid. AC = adenylate cyclase. G = G-protein. T_3 = triiodothyronine. T_4 = thyroxine. TSH-R = thyroid stimulating hormone receptor. (Courtesy of La Perle K. Encyclopedia of Hormones. 2002.)

through the function of the sodium iodide symporter into reactive iodine (I_2), which binds to the tyrosine residues in thyroglobulin. Iodine is incorporated not only into newly synthesized thyroglobulin recently delivered to the follicular lumen, but also into molecules already stored in the lumen. Thyroperoxidase also functions as a coupling enzyme to combine MIT and DIT to form triiodothyronine (T_3), or two DITs to form thyroxine (T_4).

The mechanism of active transport of iodide has been shown to be associated with a **sodium iodide (Na^+–I^-) symporter (NIS)** present in the basolateral membrane of thyroid follicular cells (Fig. 3.50). Transport of iodide ion across the thyroid cell membrane is linked to the transport of Na^+. The ion gradient generated by the Na^+–K^+ ATPase appears to be the driving force for the active co-transport of iodide. The transporter protein is present in the basolateral membrane of thyroid follicular cells and is a large protein containing 643 amino acids with 13 transmembrane domains.

Other tissues, such as salivary gland, gastric mucosa, choroid plexus, ciliary body of the eye, and lactating mammary gland, also have the capacity to actively transport iodide, albeit at a much lower level than the thyroid. In the salivary glands, NIS protein has been detected in ductal cells but not in acinar cells. Only the thyroid follicular cells accumulate iodide in a TSH-dependent manner. The NIS gene is complex (15 exons, 14 introns) and its expression in the thyroid is up-regulated by TSH. *The functionally active iodine transport system in the thyroid gland has important clinical applications in the evaluation, diagnosis, and treatment of several thyroid disorders, including cancer.* The NIS and active transport of iodide can be selectively inhibited by competitive anion inhibitors (e.g., perchlorate, thiocyanate); thereby, effectively blocking the ability of the gland to iodinate tyrosine residues in thyroglobulin and synthesize thyroid hormones.

The ability of NIS to concentrate iodide holds clinical relevance, in addition to its physiological importance, by providing the basis for using radioactive iodine in the treatment and management of various thyroid diseases. Radioactive iodine is commonly used to destroy overactive thyrocytes in patients with thyrotoxicosis, to ablate normal and malignant thyroid tissues in patients who have undergone total thyroidectomy for thyroid carcinoma, and to perform whole-body scans for the detection of recurrent and metastatic thyroid cancer.

Once within the thyrocyte, iodide ion is passively transported via **pendrin** into the follicular lumen. Pendrin, an ~86 kDa chloride/iodide transport protein, is located at the apical membrane of thyrocytes, and is responsible for the transport of iodide ion into the follicular lumen. It is the product of the gene responsible for *Pendred syndrome*, a genetic disorder in human patients characterized clinically by sensorineural hearing loss and goiter. Pendrin is characterized by 11 or 12 putative transmembrane domains and is closely related to a family of sulfate transport proteins; however, pendrin does not transport sulfate and unlike NIS does not require sodium for its transport of iodide.

Thyroid hormone secretion

The secretion of thyroid hormones from stores within luminal colloid is initiated by elongation of microvilli on follicular cells and formation of pseudopodia. These elongated cytoplasmic projections are increased by pituitary TSH, extend into the follicular lumen, and indiscriminately phagocytize a portion of adjacent colloid. Colloid droplets within follicular cells subsequently fuse with numerous lysosomal bodies that contain proteolytic enzymes. Triiodothyronine (T_3) and thyroxine (T_4) are released from the thyroglobulin molecule and, because of their hydrophobic nature, diffuse out of follicular cells into the adjacent capillaries. The biologically inactive iodotyrosines (MIT, DIT) simultaneously released from the colloid droplets are deiodinated enzymatically, and the iodide generated is either recycled to the follicular lumen to iodinate new tyrosyl residues or released into the circulation under normal conditions. Thyroxine is rapidly bound in plasma to albumin and several globulin fractions (especially high-affinity **thyroxine-binding globulin (TBG)**), and triiodothyronine is bound to albumin (**transthyretin**) and one globulin fraction in dogs.

Negative feedback control of thyroid hormone secretion is accomplished by the coordinated response of the adenohypophysis and specific hypothalamic nuclei to circulating levels of thyroid hormones (especially T_3). A decrease in thyroid hormone concentration in plasma is sensed by groups of neurosecretory neurones in the hypothalamus that synthesize and secrete a small peptide (3 amino acids), **thyrotropin-releasing hormone (TRH)**, into the hypophyseal portal circulation. Thyroid stimulating hormone (TSH) or thyrotropin (TTH) is conveyed to thyroid follicular cells where it binds to the basilar aspect of the cell, activates adenyl cyclase, and increases the

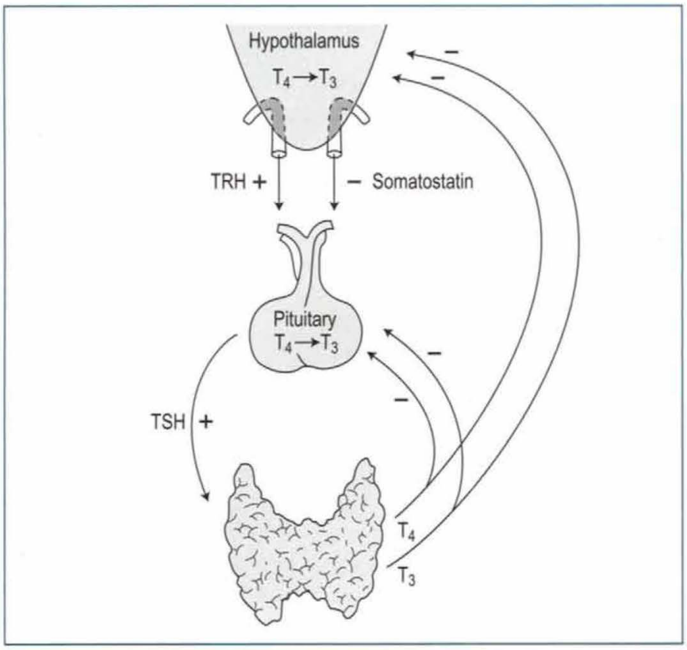


Figure 3.51 Hypothalamic-pituitary-thyroid axis. Thyroid stimulating hormone (TSH) from the pituitary stimulates the secretion of both tetraiodothyronine (T_4) and triiodothyronine (T_3). These act at the pituitary level to control secretion of TSH by a negative feedback mechanism. In addition, T_4 is converted to T_3 within the pituitary by a monodeiodinase. Secretion of TSH is stimulated by thyrotropin-releasing hormone (TRH) from the hypothalamus and inhibited by somatostatin and to a lesser extent by dopamine. Thyroid hormones act at the hypothalamus to stimulate the secretion of somatostatin. T_4 also is deiodinated to T_3 within the hypothalamus and this conversion which plays a role in feedback regulation. (Redrawn from Reichlin S. In: Williams Textbook of Endocrinology, 9th ed., 1998.)

rate of biochemical reactions concerned with the synthesis and secretion of thyroid hormones (Fig. 3.51).

One of the initial responses by follicular cells to TSH is the formation of numerous cytoplasmic pseudopodia, resulting in increased endocytosis of colloid and release of preformed thyroid hormones stored within the follicular lumen. If the secretion of TSH is sustained (hours or days), thyroid follicular cells become more columnar, and follicular lumina become smaller due to increased endocytosis of colloid. Numerous PAS-positive **colloid droplets** are present in the luminal aspect of the hypertrophied follicular cells. The converse of what has been just described occurs in response to an increase in circulating thyroid hormones and a corresponding decrease in circulating pituitary TSH. Thyroid follicles become enlarged and distended with colloid due to decreased TSH-mediated endocytosis of colloid. Follicular cells lining the involuted follicles are cuboidal and there are few endocytotic vacuoles at the interface between the colloid and follicular cells.

There are differences in thyroid morphology and function between canine breeds of European origin and the Basenji, which originated in Africa. At the same level of iodine intake, thyroidal turnover of iodine is 2–3 times faster in the Basenji than in European breeds. The corresponding differences in thyroid morphology in the Basenji include smaller follicles, more widespread and uniform vacuolation of colloid, more columnar follicular epithelium, and ultrastructural features of follicular cells that more closely resemble those of the TSH-stimulated thyroid in European breeds, such as the Beagle.

Action of thyroid hormones

Once released into the circulation, **thyroxine (T_4)** and **triiodothyronine (T_3)** act on many different target cells in the body. The overall functions of the hormones are similar, though much of the biologic activity is the result of monodeiodination by **5′-deiodinase** to 3,5,3′-triiodothyronine (active T_3) prior to interacting with target cells. Under certain conditions (protein starvation, neonatal animals, liver and kidney disease, febrile illness, etc.) or exposure to xenobiotics, thyroxine is preferentially monodeiodinated by 5′-deiodinase to 3,3′,5′-triiodothyronine (**"reverse T3"**) (see Fig. 3.68). Since this form of T_3 is biologically inactive, monodeiodination to form reverse T3 provides a mechanism to attenuate the metabolic effects of thyroid hormones in peripheral tissues.

The subcellular mechanism of action of thyroid hormones resembles that for steroid hormones, in that free hormone enters target cells and binds loosely to cytosol-binding proteins. Free triiodothyronine initially binds to receptors on the inner mitochondrial membrane to activate mitochondrial energy metabolism, and subsequently binds to nuclear receptors and increases transcription of the genetic message (mRNA) to facilitate new protein synthesis (e.g., structural, enzymatic, and binding proteins).

Thyroid hormone functions in target cells are mediated by three nuclear receptors: $T_3R\alpha_1$, $T_3R\beta_1$, and $T_3R\beta_2$ that are encoded by two genes, viz. $T_3R\alpha$ and $T_3R\beta$. Thyroid hormones are essential for numerous postnatal developmental processes including growth and neurogenesis. The $T_3R\alpha$ gene is widely expressed from early development, while the $T_3R\beta$ gene is highly restricted and expressed later in development. Mice with targeted disruption of the $T_3R\alpha$ gene have markedly reduced production of both T_4 and T_3, associated with growth arrest and delayed development of bones and small intestine. By comparison, mice with targeted disruption of the $T_3R\beta$ gene have overproduction of thyroid hormones and impairment of auditory function but no developmental defects.

The *overall physiologic effects of thyroid hormones* are to: increase the basal metabolic rate; make more glucose available to meet the elevated metabolic demands by increasing glycolysis, gluconeogenesis, and glucose absorption from the intestine; stimulate new protein synthesis; increase lipid metabolism and conversion of cholesterol into bile acids and other substances; activate lipoprotein lipase and increase sensitivity of adipose tissue to lipolysis by other hormones; stimulate heart rate, cardiac output, and blood flow; increase neural transmission, cerebration, and neuronal development in young animals.

Diseases of the thyroid gland

Developmental disturbances of the thyroid gland

Aplasia and **hypoplasia** are uncommon and may be bilateral, in species with paired thyroids, or unilateral. Bilateral aplasia or hypoplasia of the thyroid in a young animal may result in dwarfism and other manifestations of early-onset hypothyroidism (i.e., subnormal central nervous system development, failure to grow at a normal rate, etc.).

Congenital hypothyroidism has been reported with primary thyroid hypoplasia in Scottish Deerhound pups. Thyroid glands were smaller than normal and composed of small follicles with little or no colloid (Fig. 3.52A) compared to age-matched normal

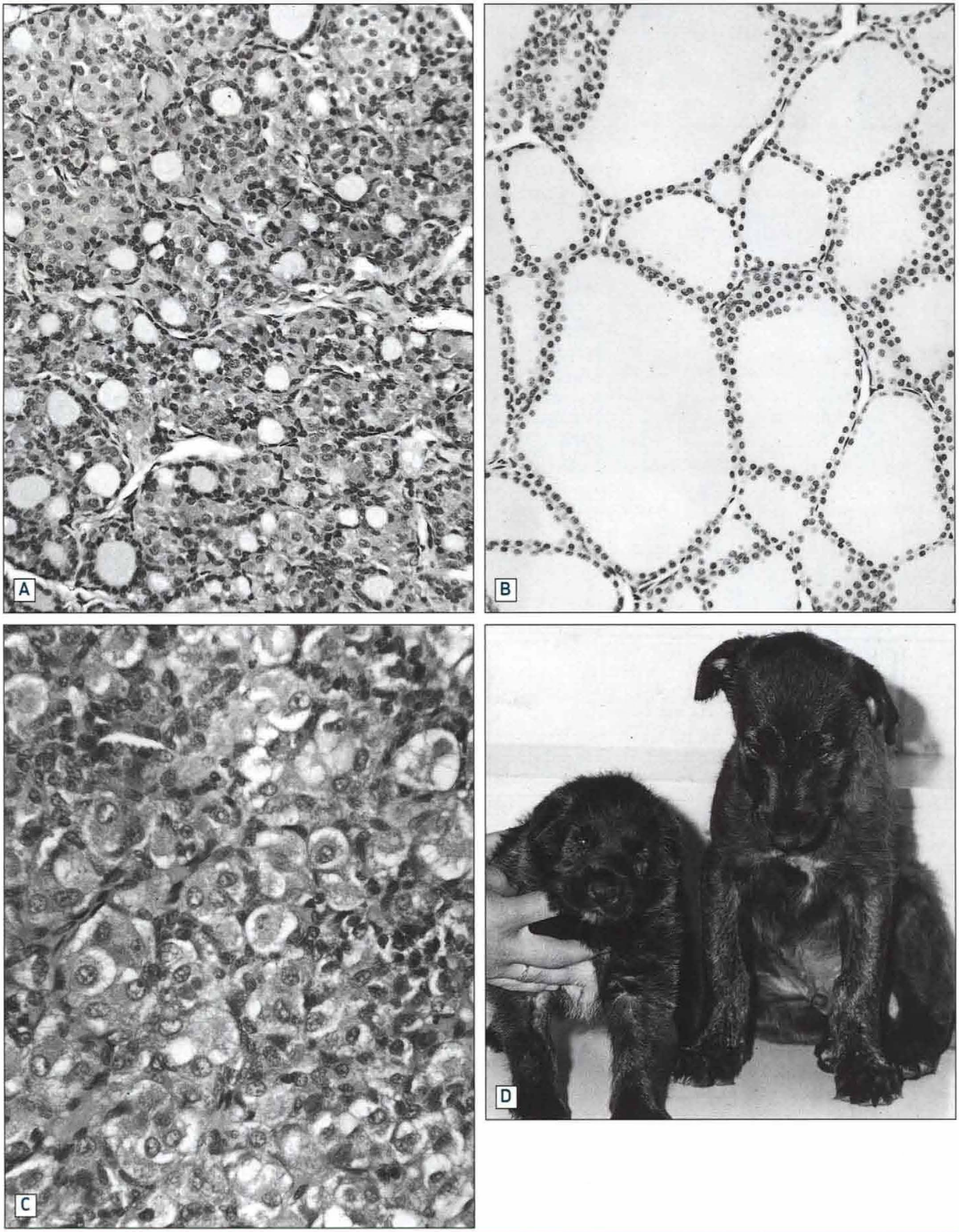


Figure 3.52 Primary thyroid hypoplasia. Scottish Deerhound with nongoitrous congenital hypothyroidism. **A.** Small thyroid follicles with little stored colloid. **B.** Normal thyroid gland from 8-week-old Scottish Deerhound pup with large colloid-filled follicles lined by low cuboidal follicular cells. **C.** Adenohypophysis from pup with thyroid hypoplasia. Numerous vacuolated trophic hormone (TSH)-producing cells are admixed with a few granulated acidophils. **D.** The hypothyroid pup (left) is smaller, has a shortened domed head, and a fine haircoat. (From Robinson WF, et al. Aus Vet J 1988;65:386–389.)

pups (Fig. 3.52B). The pituitary gland was normal macroscopically, but contained few granulated acidophils with a high percentage of markedly vacuolated cells with few cytoplasmic granules, similar to thyroidectomy cells (Fig. 3.52C). Serum thyroxine levels were markedly decreased and did not increase in response to exogenous TSH. The affected puppies had retarded growth, fine hair coat, weakness, difficulty in walking, mental depression and somnolence. Pups with thyroid hypoplasia were smaller than their littermates and had short limbs and a short broad head (Fig. 3.52D). There was an absence of ossification of epiphyses in the axial and appendicular skeleton (see Vol. 1, Bones and joints).

Thyroglossal duct cysts develop most frequently in dogs and pigs and occasionally other animals by persistence of portions of the midline embryologic primordium of the thyroid that migrates caudally from the floor of the primitive pharynx to form the postnatal thyroid lobes. Thyroglossal duct cysts are present in the ventral midline of the cervical region in dogs as fluctuant masses that may rupture and form a fistulous tract to the exterior. Their lining epithelium consists of multiple layers of follicular cells with occasional colloid-containing follicles that may undergo neoplastic transformation and give rise to papillary carcinomas (see Neoplasms of the thyroid gland).

Other cysts that develop in the thyroid region that must be differentiated from thyroglossal duct cysts include parathyroid cysts, branchial ("lateral neck") cysts, ultimobranchial duct cysts, follicular ("colloid") cysts, and salivary mucoceles. These are discussed under Parathyroid and related cysts.

Degenerative changes of the thyroid gland

The formation of densely basophilic spherules or granules in the colloid of thyroid follicles occurs frequently in adult to aged dogs and occasionally in other species. These **corpora amylacea-like bodies** appear to represent the precipitation of mineral on aggregated colloid (possibly with abnormal chemical structure). **Colloid mineralization** develops in animals with normal blood calcium and phosphorus levels, and does not appear to be consistently associated with other diseases. It is distinctly different from the mineralization of interfollicular capillaries associated with chronic renal failure. The mineralization of colloid usually is not associated with any interference with thyroid function, even though it affects many follicles.

The accumulation of **lipofuscin** in follicular cells occurs in many animals and accounts for the progressive darkening (brown-red) of the thyroid with advancing age. The numerous PAS-positive lipofuscin granules accumulate particularly in the luminal aspect of follicular cells, but do not appear significantly to interfere with thyroid function.

The deposition of **amyloid** in the interstitium between thyroid follicles has been observed in dogs, cats (especially certain breeds such as Abyssinian), cattle, and occasionally in other animals. Affected thyroids may be slightly enlarged, pale, and firmer than normal. The interstitial accumulation of amyloid may be extensive and compress adjacent follicles, but usually not to a degree that interferes with thyroid function clinically. Amyloid in the thyroid of dogs and cattle almost always is part of generalized amyloidosis associated with a chronic suppurative process elsewhere in the body.

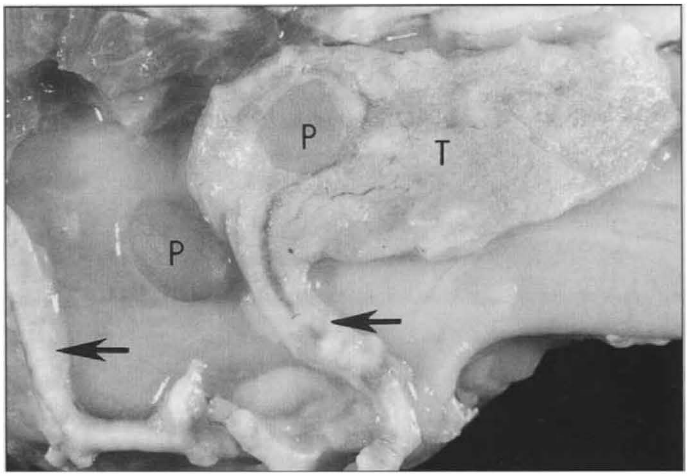


Figure 3.53 Idiopathic follicular atrophy. Thyroid (T) is reduced in size compared to adjacent parathyroids (P) and lighter in color due to increased adipose connective tissue. Thyroid arteries (arrows) are thickened and prominent due to atherosclerosis.

Hypofunction of the thyroid gland

Hypothyroidism of adult onset is a well-recognized clinical disease in dogs, but is encountered only occasionally in other animals. (Congenital hypothyroidism is discussed under Developmental diseases of the thyroid gland.) Although hypothyroidism may occur in many adult purebred and mixed breed dogs, certain breeds (Golden Retrievers, Doberman Pinschers, Dachshunds, Shetland Sheepdogs, Irish Setters, Miniature Schnauzers, Cocker Spaniels, and Airedales) have been reported to be more commonly affected. *Hypothyroidism in dogs usually is the result of* **primary** *lesions in the thyroid gland, particularly lymphocytic thyroiditis and idiopathic follicular collapse.* Less common causes of hypothyroidism in dogs are *bilateral nonfunctional thyroid tumors* or *severe iodine-deficient hyperplastic goiter.* Hypothyroidism associated with long-standing pituitary or hypothalamic lesions that prevent the release of either TSH (**secondary**) or thyrotropin-releasing hormone (TRH) (**tertiary**) is much less common in dogs. The thyroid gland in secondary hypothyroidism is moderately reduced in size and composed of colloid-distended "involuted" follicles lined by flattened follicular cells (see Fig. 3.17).

Idiopathic follicular atrophy ("collapse")

In follicular atrophy, *there is progressive loss of follicular epithelium and replacement by adipose tissue with a minimal inflammatory response.* The gland usually is smaller and lighter in color than normal (Fig. 3.53). The early lesion of follicular atrophy that is seen in dogs with mild clinical signs of hypothyroidism appears to be confined to one part of the thyroid. The affected part of the thyroid gland contains small follicles lined by tall columnar follicular cells, often with little colloid (Fig. 3.54A). Immediately adjacent thyroid follicles are normal. Individual or small groups of degenerate follicular cells with eosinophilic cytoplasm and pyknotic nuclei are present in the follicular wall, colloid, and interstitium (Fig. 3.54B).

A more advanced form of follicular atrophy is seen in dogs with clinical hypothyroidism and low circulating concentrations of thyroid hormones. The thyroid gland is composed predominantly of

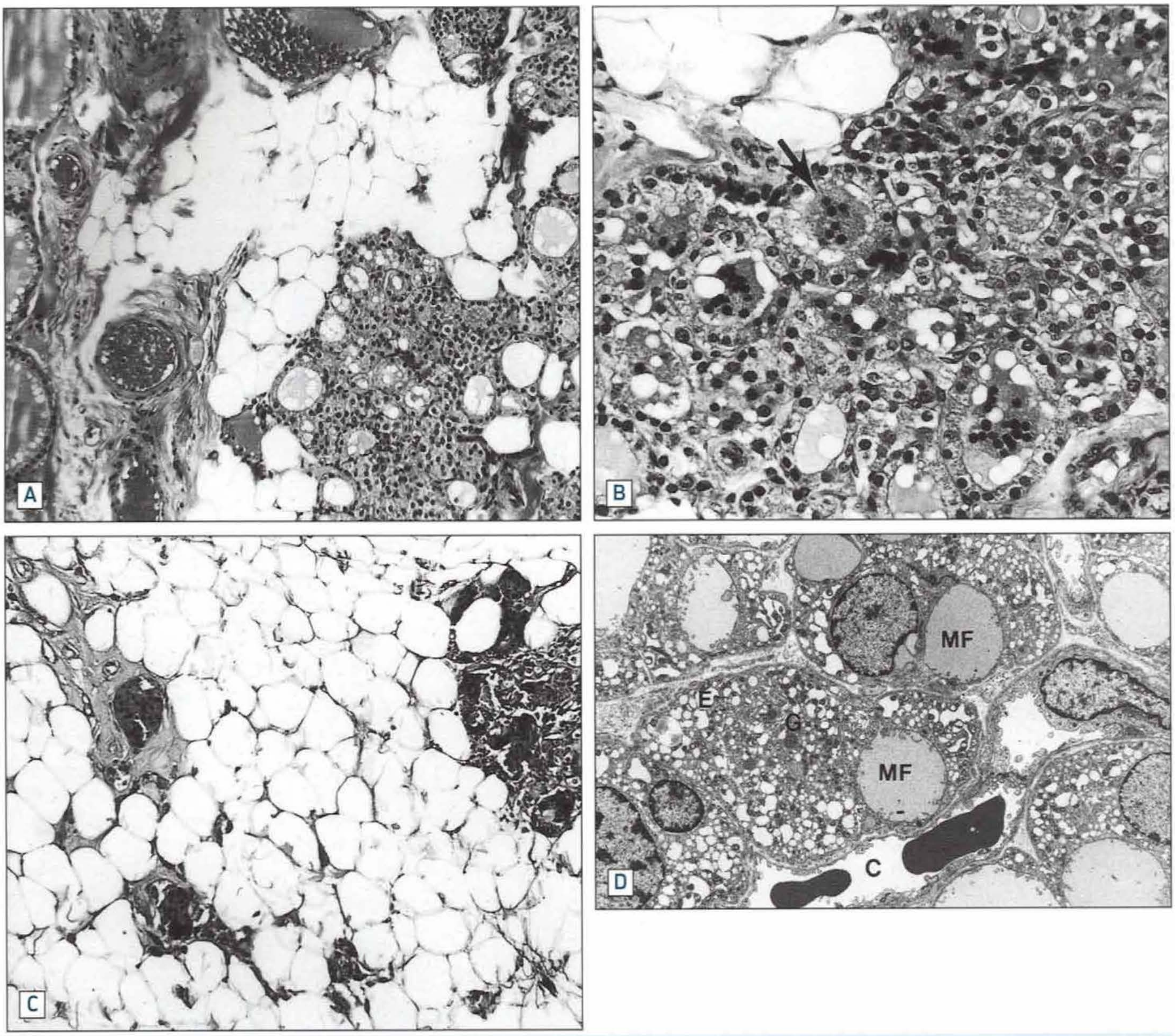


Figure 3.54 Idiopathic follicular atrophy in a dog. **A.** Early stage. **B.** Degenerate follicular cells in follicular wall and colloid (arrow). **C.** Late stage. Thyroid consists mainly of adipose tissue, scattered small follicles and nests of C cells. **D.** Note the lack of normal thyroid follicles and the presence of microfollicles (MF) in the cytoplasm of individual follicular cells. The remaining follicular cells are hypertrophied with dilated endoplasmic reticulum (E) and well-developed Golgi apparatuses (G). C = capillary. (From Gosselin SJ, et al. Vet Pathol 1981;18:299–309.)

adipose tissue with only occasional clusters of small follicles containing vacuolated colloid (Fig. 3.54C). When the thyroid is reduced markedly in size, it consists primarily of small follicles and individual follicular cells with PAS-positive colloid in microfollicles in the cytoplasm (Fig. 3.54D). Small foci of lymphocytes and plasma cells may be associated with disruption of thyroid follicles and release of colloid. The mild increase of connective tissue in the interstitium appears to be the result of condensation of the normal stroma. Focal aggregates of C cells appear relatively more prominent due to progressive loss of follicular cells. Occasionally, thyroid glands with idiopathic follicular atrophy have either an encapsulated microadenoma or a nonencapsulated area of nodular hyperplasia of follicular cells.

The earliest lesion of idiopathic follicular atrophy observed by electron microscopy is degeneration of individual follicular cells lining thyroid follicles. The advanced stage of follicular atrophy is characterized ultrastructurally by a lack of normal thyroid follicles and the presence of microfollicles in the cytoplasm of individual follicular cells. The remaining hypertrophic follicular cells form small nests and are arranged closely along capillaries. The microfollicles appear to form by invagination of the apical surface of the follicular cells. Long microvilli extend into the colloid and membrane-limited colloid droplets are present in the cytoplasm near the microfollicle, suggesting increased endocytotic activity. Degenerate follicular cells with poorly defined plasma membranes, an irregularly shrunken nucleus, markedly dilated profiles of rough endoplasmic reticulum, and swollen mitochondria with disrupted cristae often are scattered in the interstitium. Macrophages with membrane-limited vacuoles containing myelin bodies, other membranous debris, and lysosomes are present near the degenerate follicular cells. The basement membrane may be thickened focally around remaining follicular cells. Small groups of thyroid C cells remain in thyroids with idiopathic follicular atrophy. They appear normal ultrastructurally and have a normal complement of membrane-limited secretory granules in their cytoplasm.

Idiopathic follicular atrophy ("collapse") appears to be a primary degenerative disease of the thyroid and differs distinctly from the trophic atrophy of follicular cells secondary to diminished secretion of TSH. Under the latter conditions, thyroid follicles undergo involution, are lined by a low cuboidal epithelium, and distended by uniformly dense PAS-positive colloid with little evidence of endocytosis. Hypertrophy and hyperplasia of thyrotrophic basophils occurs in the pars distalis of dogs with idiopathic follicular collapse and hypothyroidism.

Lymphocytic thyroiditis

Lymphocytic thyroiditis in dogs, an obese strain of chickens, nonhuman primates, and Buffalo rats closely resembles Hashimoto's disease in humans. Though the exact mechanism in the dog is not well established, evidence suggests a polygenic pattern of inheritance similar to that observed in the human disease. *The immunologic basis for the development of chronic lymphocytic thyroiditis in both humans and dogs appears to be through production of autoantibodies directed against thyroglobulin, thyroperoxidase (a microsomal antigen), or the TSH receptor.* Thyroglobulin autoantibodies have been found in a high percentage of dogs with hypothyroidism, and may be related to the development of the thyroiditis. Laboratory Beagles with spontaneous lymphocytic thyroiditis also have circulating thyroid autoantibodies, but the focal thyroiditis usually is not associated with clinical hypothyroidism. Thyroiditis in laboratory Beagles is similar serologically to human thyroiditis in that antibodies are present against thyroglobulin and thyroperoxidase; however, there is not a positive correlation between the occurrence or height of the thyroglobulin antibody titers and the occurrence or severity of thyroiditis.

Thyroid glands of dogs with lymphocytic thyroiditis may be slightly enlarged and tan-white, normal, or reduced in size. Histologic alterations include *multifocal to diffuse infiltrates of lymphocytes, plasma cells and macrophages.* Lymphoid nodules are present occasionally between follicles. The remaining follicles are small and lined by columnar epithelial cells. Lymphocytes, macrophages, and degenerate follicular cells often are present in the vacuolated colloid (Fig. 3.55A, B). Thyroid C (parafollicular) cells are seen in small nests or nodules between follicles and often are relatively more prominent than in normal dogs.

Lymphocytic thyroiditis is characterized ultrastructurally by lymphocytes, plasma cells, and macrophages in the interstitium, migrating through the follicular wall, and into the lumen where they are mixed with colloid and degenerate follicular cells (Fig. 3.55B). Either the basement membrane around thyroid follicles is thick, or discrete electron-dense deposits are present between the follicular cells and the basement membrane. These electron-dense deposits are similar to those reported to represent immune complexes in humans with Hashimoto's thyroiditis.

The thyroid lesion of lymphocytic thyroiditis progresses to replacement of the thyroid gland by mature fibrous connective tissue with only a few remaining scattered foci of inflammatory cells (Fig. 3.55C). Thyroid follicles are small and widely separated in the "end-stage" of thyroiditis and contain only a small amount of vacuolated colloid. There is a marked increase in collagen fibers surrounding small follicles or groups of follicular cells.

Many of the follicular lesions with lymphocytic thyroiditis are the result of lymphocytes and plasma cells migrating between thyroid follicular cells. This migration causes separation of adjacent follicular cells from the basement membrane, exfoliation of follicular cells, migration of lymphoid cells into the lumen, and eventual destruction of the follicle. To compensate for the progressive destruction of thyroid follicles by inflammatory cells and the decreased production of thyroid hormones, follicular cells in the remaining follicles often undergo hypertrophy, presumably in response to increased secretion of TSH.

Oncocytes with abundant eosinophilic cytoplasm often are seen in thyroids of dogs with long-standing lymphocytic thyroiditis. They contain abundant oxidative enzymes but sparse rough endoplasmic reticulum, suggesting an imbalance between the respiratory and synthesizing activities of the cell. Thyroid hormone synthesis appears to be severely compromised in these modified follicular cells. The occurrence of oncocytes in thyroid glands may be related to ageing or overstimulation.

Extrathyroidal lesions and functional disturbances in hypothyroidism

Many functional disturbances associated with hypothyroidism are due to a reduction in basal metabolic rate. A *gain in body weight* without an associated change in appetite occurs in some hypothyroid dogs. The weight gain varies from slight to striking, and hypothyroid dogs usually are less active than normal.

Lesions in skin and haircoat occur in the majority of hypothyroid dogs. Thyroxine stimulates the anagen or active phase of hair growth, whereas reduced blood levels of thyroid hormones favor the telogen or resting phase. Telogen hairs are more easily dislodged from the hair follicles, resulting in thinning of the haircoat and often a bilaterally symmetrical alopecia. Areas affected initially by hair loss are those receiving frictional wear such as the tail and cervical area. The tail may become almost completely bare in dogs with long-standing hypothyroidism.

Hyperkeratosis is a consistent finding in hypothyroidism and results in increased scaliness of the skin. It may become severe and occur in circular scaling patches resulting in skin lesions suggestive of seborrhea. Microscopic examination consistently reveals marked hyperkeratosis which includes the external root sheath. The excessive keratin formation and accumulation within hair follicles often results in *follicular keratosis* that may cause a grossly observable distention of follicles.

Hyperpigmentation occurs in many dogs with hypothyroidism, especially in localized areas of alopecia such as the dorsal aspect of the nose and distal portion of the tail. Increased numbers of melanocytes are present in the basal layer of the epidermis. Changes in the thickness of the epidermis are variable in dogs with hypothyroidism. Epidermal atrophy is reported in ~50% of hypothyroid dogs, normal thickness in ~20%, and mild-to-moderate epidermal thickening due to a prominent stratum granulosum with an atrophic stratum spinosum in the remaining 30% of dogs.

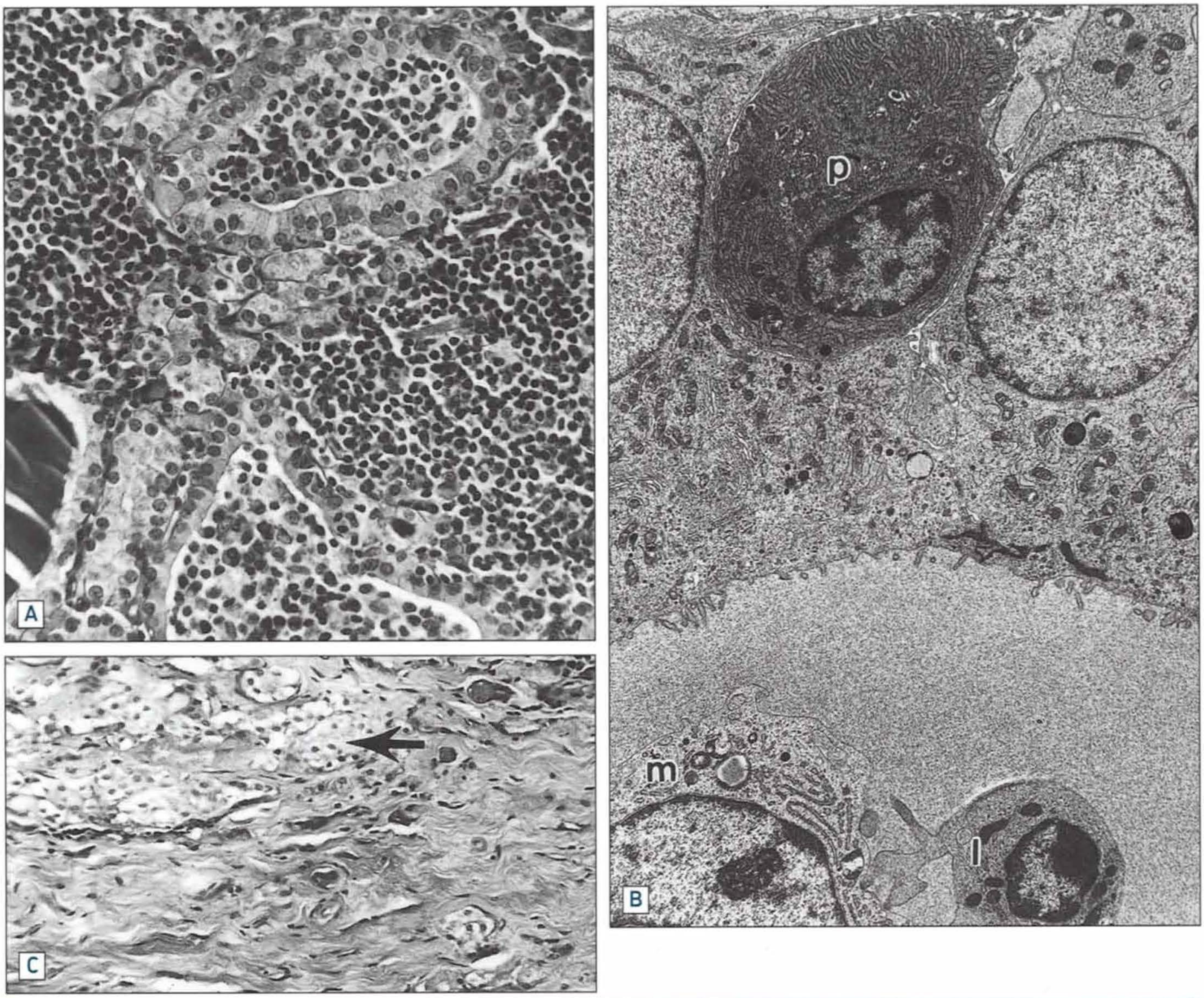


Figure 3.55 Lymphocytic thyroiditis in a dog. **A.** Infiltration of interstitium and follicular lumens with lymphocytes and plasma cells. **B.** Lymphocyte (l) and macrophage (m) are present in colloid of follicle and a plasma cell (p) is infiltrating between follicular cells. (From Gosselin SJ, et al. Am J Pathol 1977;90:285–289.) **C.** End-stage thyroiditis with fibrosis, few follicles, and nests of C cells (arrow).

Myxedema may develop and produce a characteristic clinical appearance in long-standing or severe hypothyroidism. *Mucin (neutral and acid glycosaminoglycans combined with protein) accumulates in the dermis and subcutis.* This material binds considerable amounts of water and produces marked thickening of the skin. This is most obvious around the face and head where accentuation of the normal skin folds causes a "tragic" appearance (Fig. 3.56). The eyelids appear thick and drooping, thus contributing to the sad facial expression. The skin feels thick and doughy but the characteristic pitting observed with other types of edema does not occur with myxedema. Histologically, mucin appears as a blue-purple, granular or fibrillar material that disrupts the normal collagen and elastin fibers on skin sections. Mucin is not well visualized on skin sections stained with hematoxylin and eosin, but disruption of the normal pattern of collagen and elastin fibers is evident in the dermis.

Abnormalities in reproduction are common in hypothyroidism. Lack of libido and reduction in sperm count may occur in males, whereas abnormal or absent estrous cycles with reduced conception rates may result in females. The spermatogenic epithelium in the testis often is markedly atrophic in long-standing cases of hypothyroidism. Impaired joint function with evidence of pain and joint effusion also can result from severe or prolonged hypothyroidism.

Hypothyroidism in dogs is accompanied by reduced circulating concentrations of thyroid hormones, low protein-bound iodine (PBI) levels, and decreased ^{131}I uptake by the thyroid gland. The occurrence of mild, nonresponsive, normocytic, normochromic anemia is consistent with a diagnosis of hypothyroidism.

The marked **hypercholesterolemia** seen with long-standing and severe hypothyroidism results in a variety of *secondary extrathyroidal lesions, including atherosclerosis, hepatomegaly, and renal glomerular and corneal lipidosis.* Hypercholesterolemia results from the decreased rate of lipid metabolism with diminished intestinal excretion of cholesterol and conversion of lipids into bile acids and other compounds.

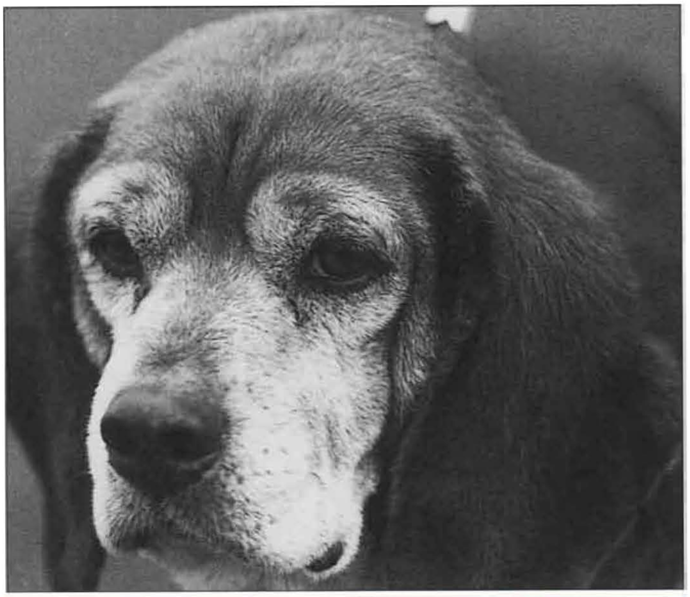


Figure 3.56 Hypothyroidism in a dog. Myxedema with thickened facial skin folds and "tragic" facial expression.

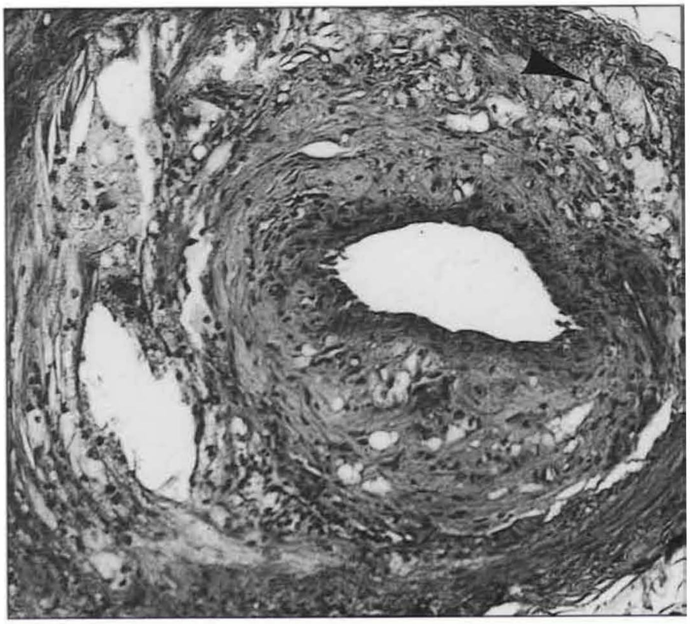


Figure 3.57 Hypothyroidism in a dog. Coronary atherosclerosis with lipid-filled macrophages (arrowhead).

- Atherosclerosis of coronary, cerebral and other vessels may develop in dogs with severe hypothyroidism and long-standing hyperlipidemia (Figs 3.53, 3.57). This occasionally results in hemorrhage and ischemic necrosis of the myocardium due to impingement on the vessel lumina by numerous lipid-laden macrophages in the tunica media and adventitia.
- In dogs with markedly elevated plasma lipids, renal glomeruli may become plugged with lipid, resulting in progressive renal failure. The lipid-filled glomeruli can be seen macroscopically as small yellow-white foci throughout the kidney cortex (Fig. 3.58).

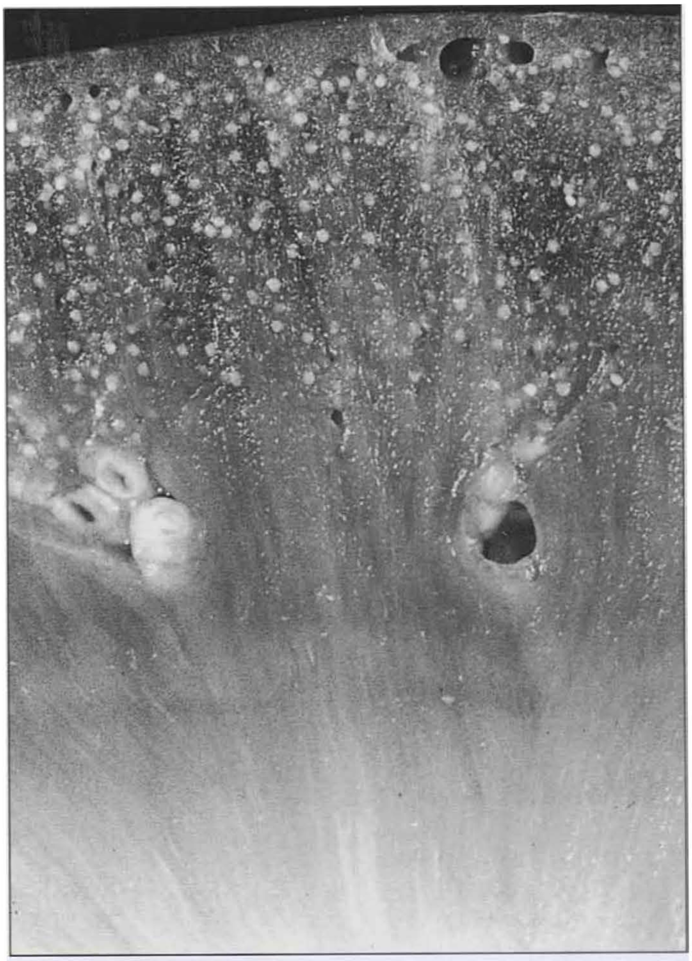


Figure 3.58 Glomerular lipidosis in a dog with severe and long-standing hypothyroidism with marked hypercholesterolemia.

- Accumulation of excessive lipid in the liver often results in various degrees of hepatomegaly with abdominal distention and hepatic failure.
- Corneal lipidosis is observed occasionally in hypothyroid dogs with hypercholesterolemia. This lesion often is unilateral because the lipid is deposited in corneas that have been previously injured and have a network of "ghost" vessels from which the lipid diffuses into the connective tissue stroma.

Evaluation of thyroid function

Serum cholesterol concentration is an indirect and valuable index of the peripheral action of thyroid hormone. Two-thirds of dogs with spontaneous hypothyroidism have fasting serum cholesterol concentration >300 mg/100 mL (>7.75 mmol/L). Hypercholesterolemia is as dependent, however, upon the composition of the dog's diet as upon the severity and duration of hypothyroidism. Cholesterol values tend to be higher in the general population of pet dogs fed table scraps or home diets than in dogs maintained exclusively on commercially manufactured dry dog foods (e.g., under laboratory conditions). Hypercholesterolemia also occurs in some dogs with cortisol excess, a disease that must be considered in the differential diagnosis of hypothyroidism. Therefore, *the measurement of serum cholesterol is not a specific or dependable test of thyroid function, but fasting cholesterol values*

>600 mg/100 mL (>15.5 mmol/L), which are often observed in hypothyroidism, infrequently occur in any other disease of dogs.

Moderate normocytic normochromic anemia often is associated with subnormal function of the thyroid in dogs. This anemia also has been observed in humans and in experimental animals and is of a nonregenerative type. The stained blood smear characteristically has little or no evidence of active erythrogenesis such as anisocytosis, polychromasia, or nucleated red cells. Leptocytosis may be especially prominent. The hemogram is characteristic of anemia associated with a variety of chronic diseases including neoplasia and chronic infection.

The most sensitive and accurate method for evaluation of thyroid function is *measurement of total blood thyroxine (T_4) and triiodothyronine (T_3) levels by radioimmunoassay*. The normal blood level of total T_4 in the dog is 1.5–3.6 μg/dL (13–51 nmol/L) (mean: 2.48 μg/dL, 32 nmol/L) and for total T_3 48–154 ng/dL (0.74–2.37 nmol/L)(mean: 95 ng/dL, 1.46 nmol/L). In dogs with hypothyroidism, the total T_4 level usually is <1.0 μg/dL (<13 nmol/L) and T_3 is <50 ng/dL (<0.77 nmol/L). When levels are borderline, clearer separation of dogs with hypothyroidism from euthyroid dogs can be made by injecting TSH. In euthyroid dogs, the T_4 level will at least double 8 hours after IV or IM administration of TSH. In dogs with hypothyroidism, T_4 and T_3 levels do not change significantly after injection of TSH. Plasma T_4 levels are no more than 0.2 μg/dL (2.6 nmol/L) above control values at 8 hours post-TSH, and plasma T_3 is increased by no more than 10 ng/dL (0.15 nmol/L) after TSH. The increase in serum T_3 after TSH is more variable in normal dogs than for T_4, but in dogs with primary hypothyroidism there usually is little (10 ng/dL, 0.15 nmol/L; or less) change in serum T_3 at 8 hours after TSH administration.

Free thyroxine can be measured by equilibrium dialysis with tracer. Other methods of measuring free T_4 are technically less difficult but are much less accurate. The serum free T_4 in normal dogs ranges from 0.5–2.7 ng/dL (6.7–34.7 pmol/L). The *advantages of measuring free T_4 over total T_4* include: (1) higher correlation with thyroid secretory function, (2) less overlap of hypothyroid and euthyroid levels, and (3) slower decrease with nonthyroidal illness.

Histologic examination of a thyroid biopsy is a useful and reliable aid in the diagnosis of thyroid disease when either the results of serum assays for T_4 and T_3 are equivocal or a nodule is palpated in the thyroid area. The removal of the caudal one-fourth of either lobe of the thyroid for histologic examination is a relatively simple surgical procedure without significant risk, even when the dog is hypothyroid. In most cases of hypothyroidism, there is either marked loss of thyroid follicles with replacement by adipose connective tissue or multifocal lymphocytic thyroiditis and interstitial fibrosis that develops on an immunologic basis.

In the evaluation of clinically important thyroid disease problems and potential thyroid toxicity of various xenobiotics, an accurate quantitation of circulating levels of thyroid stimulating hormone (TSH or thyrotropin) is important in the critical assessment of thyroid hormone economy. Thyrotropin is the major regulator of the synthetic and secretory processes of thyroid follicular cells. However, the immunoassay for TSH is highly species-specific, with considerable interanimal and interassay variations. Thyrotropin is a glycoprotein synthesized by thyrotrophic basophils in the adenohypophysis and is composed of α and β subunits. The α subunit is similar to that of several pituitary hormones of the same family, but the β subunit is specific for TSH. The hormone circulates primarily in the free (unbound) form and has a short (approximately 15 min) plasma half-life. Serum plasma samples for TSH assay should be collected in the morning before noon to minimize the effects of diurnal variation on hormone levels. A serum TSH procedure has been recently introduced to measure canine TSH and is available either as an immunoradiometric, luminescence assay, or ELISA. The β-subunit of canine TSH has an 89% homology with the human subunit, and circulating TSH levels in dogs are 10- to 20-fold lower than those in humans. Although the first generation canine TSH assay is considerably less sensitive than the rat and human TSH assays, an increased TSH level has been demonstrated in dogs with primary hypothyroidism that returned to normal 24 hours after once daily T_4 therapy. A peak increase in serum TSH occurred at 30 min postinjection of thyrotropin-releasing hormone (TRH) that subsequently rapidly returned to baseline values.

Hyperplasia of the thyroid gland

Non-neoplastic and noninflammatory clinical enlargements (**"goiter"**) *of the thyroid* develop in all domestic mammals, birds, and submammalian vertebrates. The major pathogenetic mechanisms responsible for the development of thyroid hyperplasia include *iodine-deficient diets, goitrogenic compounds that interfere with thyroid hormone synthesis, dietary iodide excess, and genetic enzyme defects in the biosynthesis of thyroid hormones* (Fig. 3.59). All of these factors result in inadequate thyroid hormone synthesis and decreased blood levels of T_4 and T_3. The low blood levels are detected by the hypothalamus and pituitary to increase the secretion of TSH, which results in hypertrophy and hyperplasia of follicular cells.

Diffuse hyperplastic and colloid goiter

Iodine deficiency that resulted in diffuse thyroid hyperplasia was common in many goitrogenic areas throughout the world before the widespread addition of iodized salt to animal diets. Although iodine-deficient goiter still occurs in large areas of the world in domestic animals, the outbreaks are sporadic and fewer animals are affected. Marginal iodine-deficient diets containing certain *goitrogenic compounds* may result in severe thyroid hyperplasia and clinical evidence of goiter. These substances include thiouracil, sulfonamides, anions of the Hofmeister series, and a number of plants from the family Brassicaceae (see Thyrotoxic effects of drugs and chemicals, below). Young animals born to females on iodine-deficient diets are more likely to develop severe thyroid hyperplasia and have clinical signs of hypothyroidism (Fig. 3.60).

Although seemingly paradoxical, *excess iodide in the diet also can result in thyroid hyperplasia in animal and human beings*. Foals of mares fed dry seaweed containing excessive iodide may develop thyroid hyperplasia and clinically evident goiter. The thyroid glands of the young are exposed to higher blood iodide levels than the dam because of concentration of iodide, first by the placenta, and second by the mammary gland. High blood iodide interferes with one or more steps of thyroid hormone synthesis and secretion, leading to lowered blood T_4 and T_3 levels, and a compensatory increase in pituitary TSH secretion. Excess iodine appears to primarily block the release of thyroid hormones by interfering with the fusion of colloid droplets and lysosomal bodies thereby disrupting the proteolysis of colloid and release of T_4 and T_3.

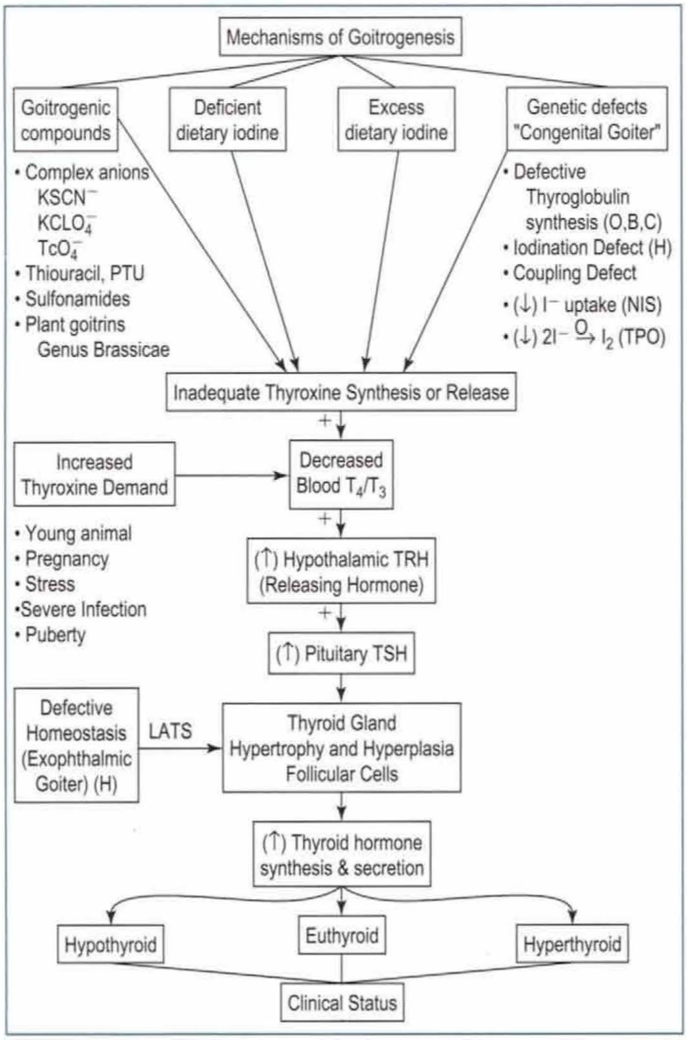

Mechanisms of Goitrogenesis
Goitrogenic compounds
Deficient dietary iodine
Excess dietary iodine
Genetic defects "Congenital Goiter"
• Complex anions
$KSCN^-$
$KCLO_4^-$
TcO_4^-
• Thiouracil, PTU
• Sulfonamides
• Plant goitrins
Genus Brassicae
• Defective Thyroglobulin synthesis (O,B,C)
• Iodination Defect (H)
• Coupling Defect
• (↓) I^- uptake (NIS)
• (↓) $2I^- \xrightarrow{O} I_2$ (TPO)
Inadequate Thyroxine Synthesis or Release
+
Increased Thyroxine Demand
Decreased Blood T_4/T_3
• Young animal
• Pregnancy
• Stress
•Severe Infection
• Puberty
+
(↑) Hypothalamic TRH (Releasing Hormone)
+
(↑) Pituitary TSH
Defective Homeostasis (Exophthalmic Goiter) (H)
LATS
Thyroid Gland Hypertrophy and Hyperplasia Follicular Cells
(↑) Thyroid hormone synthesis & secretion
Hypothyroid
Euthyroid
Hyperthyroid
Clinical Status

Figure 3.59 Mechanisms of goitrogenesis. Multiple pathogenic factors (goitrogenic compounds, deficient and excessive dietary iodine intake, and genetic defects) result in inadequate thyroxine (T_4)/triiodothyronine (T_3) synthesis and lead to long-term stimulation of thyroid follicular cells (hypertrophy and hyperplasia) by increased secretion of pituitary thyroid stimulating hormone (TSH). B = bovine, C = caprine, O = ovine, H = human, $KCLO_4$ = potassium perchlorate, KSCN = potassium thiocyanate, LATS = long acting thyroid stimulator, an autoantibody that binds to the TSH receptor and stimulates the synthetic and secretory activity of follicular cells. NIS = sodium iodide symporter, PTU = propylthiouracil, TcO_4^- = pertechnetate, TPO = thyroperoxidase, TRH = thyrotropin releasing hormone. (From Capen CC. In: Tumors of Domestic Animals, 4th ed., 2002.)

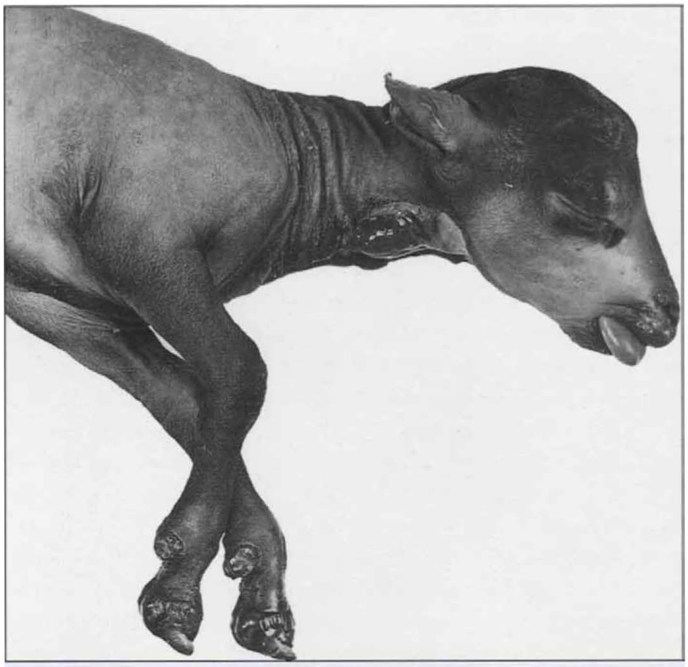


Figure 3.60 Congenital goiter due to iodine deficiency in a lamb. Note large thyroids, poor development of wool, and swollen tongue.

Iodine deficiency may be conditioned by other *antithyroid compounds* present in animal feeds and be responsible, in particular situations, for a high incidence of goiter. Prolonged low-level exposure to thiocyanates, which are produced by ruminal degradation of cyanogenetic glucosides from plants such as white clover (*Trifolium*), couch grass (*Cynoden*) and linseed meal, and by degradation of glucosinolates of the *Brassica* crops, is associated with congenital goiter in ruminants. *Goitrin* (5-vinyl-oxazolidine-2-thione) derived from the glucosinolates of *Brassica* spp. inhibits organification of iodine. *Leucaena leucocephala* and other legumes of the genus are native or cultivated in many subtrophical areas and contain the toxic aminoacid, *mimosine*. The effects of mimosine are discussed with Vol. 1, Skin and appendages. It is not directly goitrogenic but is converted in the rumen to 3-hydroxy-4(1*H*)-pyridone which prevents organic binding of iodine by the thyroid.

Goiter in adult animals usually is of little clinical significance and, except for occasional local pressure influences, the general health is not impaired. However, it does continue to be of significance as a disease of newborn animals, although the previous serious losses in endemic areas are now controlled by the prophylactic use of iodized salt.

Congenital hypothyroidism in domestic animals usually is associated with hyperplastic goiter, even though the dam may show no evidence of thyroid dysfunction. Gestation often is prolonged, the larger goiters may cause dystocia, and there is a tendency to retain the fetal placenta. Affected foals show extreme weakness and die within a few days after birth. The thyroids may be only slightly enlarged. Calves are somewhat more resistant to the effects of hypothyroidism. Although up to 70–80% may have large goiters in endemic areas, the majority survive and thrive. A few calves are partially or completely hairless, but these are either born dead or die soon after birth. Newborn goitrous pigs, goats, and lambs (Fig. 3.60) frequently have myxedema and alopecia. The mortality rate is high, with the majority being born dead or dying within a few hours of birth. Enlarged thyroid glands are readily palpable or visible in kids and lambs, but are not readily apparent in piglets because of the combination of a short neck and the development of myxedema. The tongue is swollen and there is edematous swelling of the fauces and larynx, which probably are contributory factors to death of the animals. Asphyxiation may result also from pressure by the enlarged thyroid gland on the trachea and adjacent structures. Young, goitrous animals that are treated and survive usually do not show permanent ill effects.

Congenital hyperplastic goiter with hypothyroidism is not a prominent feature of thyroid disease in *domestic carnivores*. However, degrees of follicular cell hyperplasia will be found that parallel changes in the maternal gland. In puppies, the thyroid enlargement

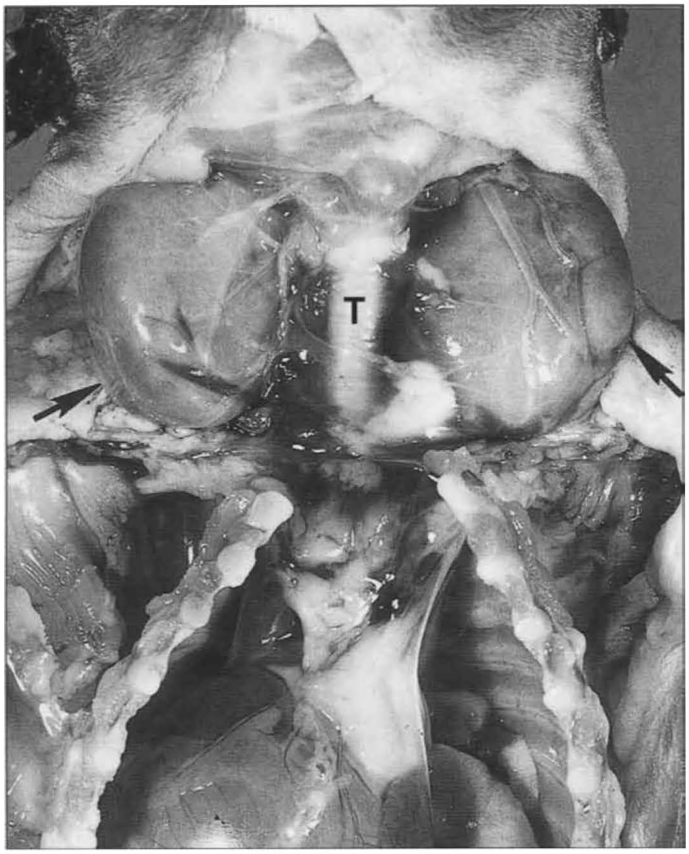


Figure 3.61 Symmetrical hyperplastic goiter (arrows) in a pup. T = trachea.

may be sufficient to cause dystocia or asphyxiation (Fig. 3.61). In the most severe form, death occurs shortly after birth and those affected have poorly developed coarse hair, anemia, and defective bone mineralization. Less severe forms are more common and affected pups often recover spontaneously. They are characterized by dry, coarse and sparse hair coat, narrow palpebral fissures, broad skulls and legs, which are relatively thicker and heavier than normal.

Both lobes of the thyroid are uniformly enlarged in young animals with diffuse hyperplastic goiter (Fig. 3.61) *due to extensive follicular cell hypertrophy and hyperplasia with enhanced endocytosis of colloid and collapse of follicles* (Fig. 3.62). The enlargements may be extensive and result in palpable swellings in the cranial cervical area. The affected lobes are firm and dark red because an extensive interfollicular capillary network develops under the influence of long-term TSH stimulation.

Colloid goiter *represents the involutionary phase of diffuse hyperplastic goiter and is usually seen in young adult and adult animals.* The markedly hyperplastic follicular cells continue to produce colloid, but endocytosis of colloid is decreased due to diminished pituitary TSH levels in response to the return of blood thyroxine and triiodothyronine to normal. Both thyroid lobes are diffusely enlarged but are more translucent and lighter in color than with hyperplastic goiter. The differences in macroscopic appearance are the result of reduced vascularity in colloid goiter and development of macrofollicles distended with colloid (Fig. 3.63A, B).

The changes in diffuse hyperplastic and colloid goiters are consistent throughout the diffusely enlarged thyroid lobes. The follicles are irregular in size and shape in hyperplastic goiter because of variable amounts of

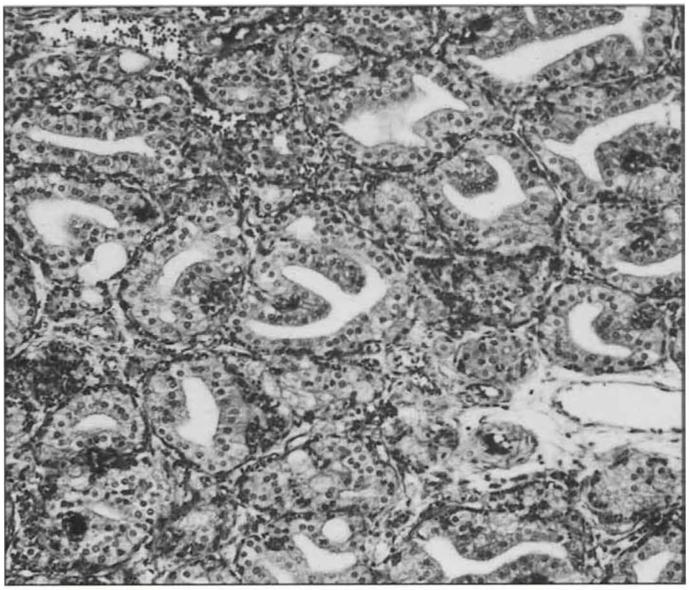


Figure 3.62 Hypertrophy and hyperplasia of thyroid follicular cells in **congenital hyperplastic goiter** with collapse of many follicles.

lightly eosinophilic and vacuolated colloid in the lumen. Some follicles are collapsed due to lack of colloid (Fig. 3.62). The lining epithelial cells are columnar with a deeply eosinophilic cytoplasm and small hyperchromatic nuclei that often are situated in the basilar part of the cell. The follicles are lined by single or multiple layers of hyperplastic follicular cells, which in some follicles may form papillary projections into the lumen. Similar proliferative changes are present in ectopic thyroid parenchyma in the neck or mediastinum.

Colloid goiter may develop either after sufficient amounts of iodide have been added to the diet of animals with iodine-deficient hyperplastic goiter or after the requirements for thyroid hormones have diminished in an older animal. Blood thyroid hormone levels return toward normal and the secretion of TSH by the pituitary gland is correspondingly decreased. Follicles are progressively distended with densely eosinophilic colloid due to diminished TSH-induced endocytosis. The follicular cells lining the macrofollicles are flattened and atrophic. The interface between the colloid and luminal surface of follicular cells is smooth and lacks the characteristic endocytotic vacuoles of actively secreting follicular cells (Fig. 3.63B). Some involuted follicles in colloid goiter have remnants of the papillary projections of follicular cells extending into their lumen. Interfollicular capillaries are less well developed than those with diffuse hyperplastic goiter as a result of the lower TSH levels.

Nodular hyperplasia

Nodular hyperplasia (goiter) in thyroid glands of old horses, cats, and dogs appears as multiple white to tan nodules of variable size (Fig. 3.64). The affected lobes are moderately enlarged and irregular in contour. *Nodular goiter in most animals is endocrinologically inactive and encountered as an incidental lesion at necropsy*; however, functional thyroid adenomas often develop in glands with multinodular follicular cell hyperplasia in old cats.

Nodular goiter consists of multiple foci of hyperplastic follicular cells that are sharply demarcated, but not encapsulated, and result in minimal compression of adjacent thyroid parenchyma. The microscopic appearance

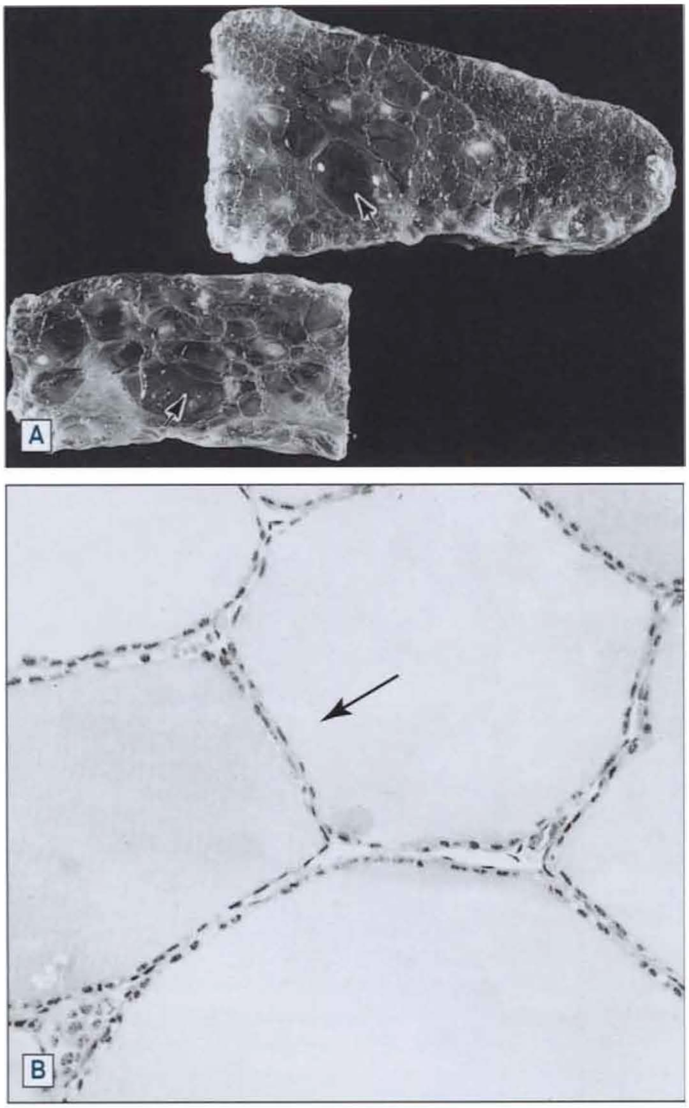


Figure 3.63 A. Colloid goiter in a lamb with large follicles (arrows) throughout sections of each lobe. **B.** Colloid goiter with distended follicles lined by flattened inactive cells (arrow).

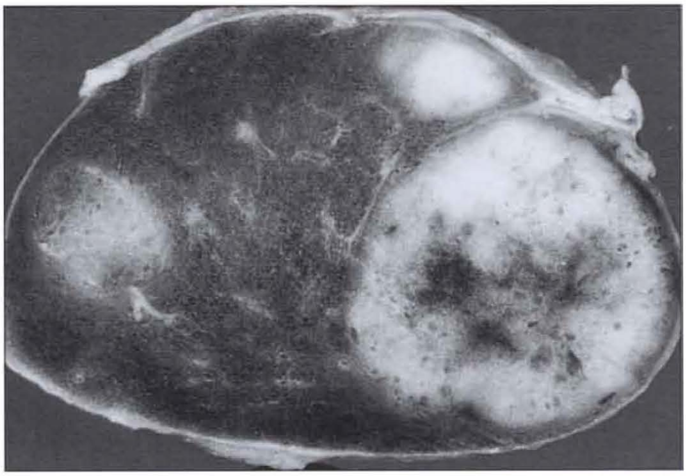


Figure 3.64 Nodular goiter with **multiple hyperplastic nodules** in a horse.

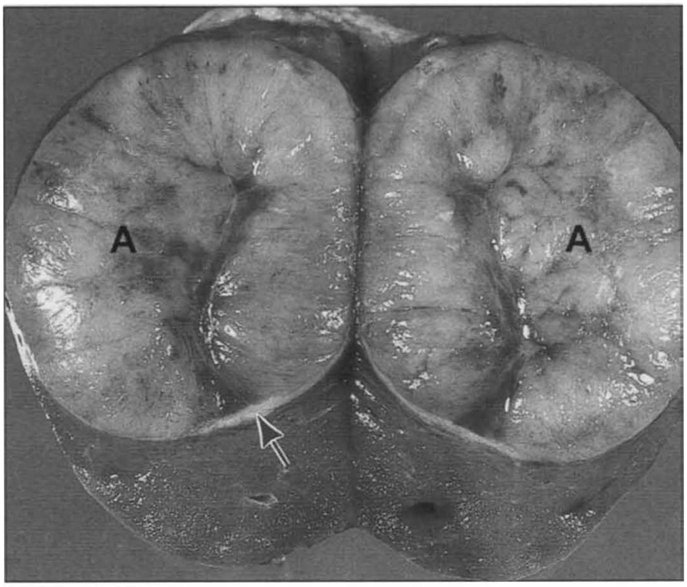


Figure 3.65 Solitary **thyroid follicular cell adenoma** (A) with prominent fibrous capsule (arrow) in a horse.

within a nodule is variable. Some hyperplastic cells form small follicles with little or no colloid. Other nodules are formed by larger irregularly shaped follicles lined by one or more layers of columnar cells that form papillary projections into the lumen. Some of the follicles are involuted and filled with densely eosinophilic colloid. These changes appear to be the result of alternating periods of hyperplasia and colloid involution in the thyroid glands of old animals. The areas of nodular hyperplasia may be microscopic, as in old cats, or grossly visible causing enlargement of the thyroid, as in old horses (Fig. 3.64).

There are no absolute morphologic criteria for distinguishing hyperplastic nodules and adenomas derived from thyroid follicular cells. As a general rule, hyperplastic nodules are multiple, poorly or not at all encapsulated, variable in their histologic structure, and do not cause compression of adjacent thyroid parenchyma. Adenomas on the other hand tend to be solitary, well encapsulated, fairly uniform in histologic structure, and cause compression of the surrounding parenchyma owing to progressive expansile growth (Fig. 3.65). Endocrinologically active thyroid adenomas result in colloid involution of follicles in the rim of surrounding thyroid due to inhibition of TSH secretion.

Inherited dyshormonogenetic goiter

An inability to synthesize and secrete adequate amounts of thyroid hormones beginning before or at birth has been documented in human infants and in several animal species. Congenital dyshormonogenetic goiter is inherited as an *autosomal recessive trait* in Corriedale, Dorset Horn, Merino, and Romney sheep; Afrikander cattle; and Saanen dwarf goats. The subnormal growth rate, absence of normal wool development or a rough sparse hair coat, myxedematous swellings of the subcutis, weakness, and sluggish behavior suggest that the affected young are *clinically hypothyroid.* Most lambs with congenital goiter either die shortly after birth or are highly sensitive to the effects of adverse environmental conditions.

Thyroid glands are symmetrically enlarged at birth due to intense diffuse hyperplasia of follicular cells (Fig. 3.66A). Thyroid follicles are lined by tall

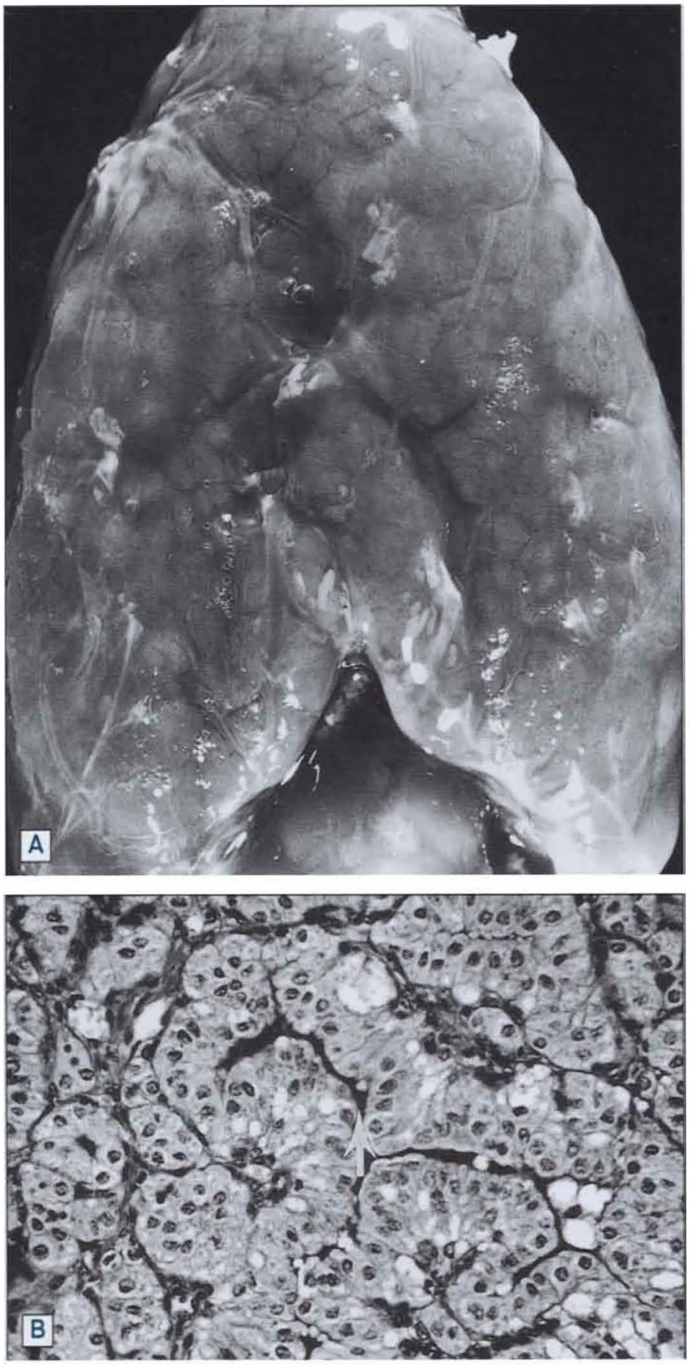


Figure 3.66 A. Congenital dyshormonogenetic goiter in a Corriedale lamb. **B.** Congenital goiter in a lamb with severe diffuse TSH-mediated follicular cell hypertrophy and hyperplasia. Thyroid follicles have collapsed and have slit-like lumens (arrow, PAS+ colloid) due to a lack of colloid resulting from the inability to synthesize thyroglobulin. (From Capen CC. Toxicol Pathol 2001;29:8–33.)

columnar cells, but often are collapsed because of lack of colloid resulting from the markedly increased endocytotic activity and diminished ability to synthesize thyroglobulin (Fig. 3.66B). The tall columnar follicular cells lining thyroid follicles have extensively dilated profiles of rough endoplasmic reticulum and large mitochondria, but there are relatively few dense granules associated with the Golgi apparatus and few apical vesicles near the luminal plasma membrane. Numerous long microvilli extend into the follicular lumen.

Although thyroidal uptake and turnover of ^{131}I are greatly increased compared with euthyroid controls, *levels of circulating* T_4 *and* T_3 *are consistently low*. The absence of a defect in the iodide transport mechanism and organification or dehalogenation, together with an absence of normal 19-S thyroglobulin in goitrous thyroids *suggests impairment in thyroglobulin biosynthesis* in animals with inherited congenital goiter. A closely related or similar defect appears operational in the examples of congenital goiter in sheep, cattle, and goats. The protein-bound iodine levels in sheep, cattle, and goats with inherited congenital goiter are markedly elevated. This appears to be the result of iodination of albumin and other plasma proteins by the thyroid gland under long-term TSH stimulation, since hormonal iodide levels (T_4 and T_3) are significantly lower than in controls. Hypothyroid goats with congenital goiter can be returned to a state of euthyroidism by the addition of iodide (1.0 mg/day) to the diet. Although the goats remain unable to synthesize thyroglobulin, supplementation with excess iodide results in sufficient formation of T_3 and T_4 in the abnormal iodoproteins to make the animals euthyroid.

The presence of messenger ribonucleic acid (mRNA) coding for thyroglobulin has been investigated to elucidate further the molecular basis for the impairment of thyroid hormone biosynthesis in congenital goiter. Although thyroglobulin-mRNA sequences are present in the goitrous tissue, their concentration is reduced to one-tenth to one-fortieth of normal, and the intracellular distribution is abnormal (nuclear: 42% of normal; cytoplasmic: 7%; membrane fraction: 1–2%). *The lack of thyroglobulin in these examples of congenital goiter in animals appears to be due to a defect in thyroglobulin-mRNA*, leading to aberrant processing of primary transcripts or transport of the thyroglobulin mRNA from the nucleus to the ribosomes of the endoplasmic reticulum.

Thyrotoxic effects of drugs and chemicals

Many of the significant physiologic and pathologic data in the literature on thyroid function and structure have come from studies in animals. Although the basic hypothalamic–pituitary–thyroid axis functions in a similar manner in animals and humans, *there are differences between species that are important when extrapolating animal data from toxicity and carcinogenicity studies for human risk assessment.*

Long-term perturbations of the pituitary–thyroid axis by various xenobiotics or physiologic alterations (e.g., iodine deficiency, partial thyroidectomy) are more likely to predispose laboratory animals to a higher incidence of proliferative lesions (e.g., hyperplasia and adenomas of follicular cells) than is the case in the human thyroid gland. This appears to be particularly true in male rats, in which there usually are higher circulating levels of TSH than in females. The greater sensitivity of the animal thyroid to derangement by drugs, chemicals and physiologic perturbations also is related to the shorter plasma half-life of T_4 (12–24 hours) than in humans (5–9 days) due, in part, to the considerable differences between species in the transport proteins for T_4.

There also are marked species differences in the sensitivity of the functionally important thyroperoxidase enzyme to inhibition by xenobiotics. *Thioamides* (e.g., sulfonamides) and other chemicals can selectively inhibit thyroperoxidase and significantly interfere with the iodination of tyrosyl residues incorporated in the thyroglobulin molecule, thereby disrupting the orderly synthesis of T_4 and T_3. Long-term administration of sulfonamides results in the development of thyroid "nodules" (e.g., focal hyperplasias and adenomas) frequently in the sensitive species (such as the rat, dog, and mice) but not in species resistant (e.g., monkey, guinea pig, chicken, and humans) to the inhibition of peroxidase in follicular cells.

The *goitrogenic effects of sulfonamides* have been known for more than 50 years, since the reports of the action of sulfaguanidine on the rat thyroid. Sulfamethoxazole and trimethroprim exert a potent goitrogenic effect in rats, resulting in marked decreases in circulating T_3 and T_4, a substantial compensatory increase in TSH, and increased thyroid weights due to follicular cell hypertrophy and hyperplasia. The dog also is a species sensitive to the effects of sulfonamides, resulting in markedly decreased serum T_4 and T_3 levels, hyperplasia of thyrotrophic basophils in the pituitary gland, and increased thyroid weights. By comparison, the thyroids of monkeys and human beings are resistant to the development of changes that sulfonamides produce in rodents (rats and mice) and the dog. Rhesus monkeys treated for 52 weeks with sulfamethoxazole with and without trimethroprim had no changes in thyroid weights and the thyroid histology was normal.

Studies comparing the effects of propylthiouracil (PTU) and a goitrogenic sulfonamide (sulfamonomethoxine) on the activity of thyroperoxidase in the rat and monkey (using the guaiacol peroxidation assay) demonstrated the concentration required for 50% inhibition of the peroxidase enzyme (inhibition constant$_{50}$ [IC_{50}]) was 50 times greater in the monkey than in the rat. Sulfamonomethoxine was almost as potent as PTU in inhibiting the thyroperoxidase in rats; however, it required 500 times the concentration to inhibit the enzyme in the monkey compared to the rat. Studies such as these with sulfonamides demonstrate *distinct species differences between rodents and primates in the response of the thyroid to chemical inhibition of hormone synthesis*; therefore, it is not surprising that the sensitive species (e.g., rats, mice, and dogs) are much more likely to develop follicular cell hyperplasia and thyroid tumors after long-term exposure to sulfonamides than the resistant species (e.g., subhuman primates, human beings, guinea pigs, and chickens).

The plasma thyroxine half-life in rats is considerably shorter (12–24 hours) than in humans (5–9 days). This is related in part to considerable differences between species in the transport proteins for T_4 and T_3. In humans and the monkey, circulating T_4 is bound primarily to thyroxine-binding globulin (TBG); however, this high affinity binding protein is not present in rodents, birds, amphibians or fish. Triiodothyronine (T_3) is transported bound to TBG and albumin in human beings, monkey, and dog, but only to albumin in mouse, rat, and chicken. In general, T_3 is bound less avidly to transport proteins than T_4, resulting in a faster turnover and shorter plasma half-life in most species.

In the evaluation of potential thyroid toxicity of various xenobiotics in animals, accurate quantitation of circulating levels of TSH is essential in order to determine whether proliferative lesions of follicular cells are mediated by a chronic hypersecretion of TSH. *The immunoassay for TSH is highly species-specific with considerable interanimal and interassay variations*. Xenobiotics that disrupt thyroid hormone synthesis, secretion, or peripheral metabolism often result in prompt increases in circulating TSH levels. Thyroid stimulating hormone levels are higher in male than female rats, and castration decreases both the baseline serum TSH and response to thyrotropin-releasing hormone (TRH) injection. Follicular cell height often is greater in male than female rats in response to the greater circulating TSH levels. The administration of exogenous testosterone to castrated male rats restores the TSH level to that of intact rats.

Chemical disruption of thyroid hormone synthesis and secretion in animals may occur at a number of different steps in thyroxinogenesis. These include blockage of iodine uptake, organification defects, blockage of hormone release, drug-induced thyroid pigmentation, inhibition of 5′-deiodinase, and induction of hepatic microsomal enzymes (Fig. 3.67).

Blockage of iodine uptake

The initial step in the biosynthesis of thyroid hormones is the uptake of iodide from the circulation, and transport against a gradient across follicular cells to the lumen of the follicle. A number of anions act as competitive inhibitors of iodide transport in the thyroid, including perchlorate (ClO_4^-), thiocyanate (SCN^-), and pertechnetate. Thiocyanate is a potent inhibitor of iodide transport and is a competitive substrate for thyroperoxidase, but it does not appear to be concentrated in the thyroid. Blockage of the iodide-trapping mechanism has a disruptive effect on the thyroid–pituitary axis similar to iodine deficiency. The blood levels of T_4 and T_3 decrease, resulting in a compensatory increase in the secretion of TSH by the pituitary gland (Fig. 3.67). The hypertrophy and hyperplasia of follicular cells that follows sustained exposure results in increased thyroid weight and the development of thyroid enlargement or goiter.

Organification defect

A wide variety of chemicals, drugs, and other xenobiotics affect the second step in thyroid hormone biosynthesis. The step-wise binding of iodide to the tyrosyl residues in thyroglobulin requires oxidation of inorganic iodide (I^-) to molecular (reactive) iodine (I_2) by thyroperoxidase present in the luminal aspect (microvillar membranes and apical cytoplasm) of follicular cells and adjacent colloid. Classes of chemicals that inhibit the organification of thyroglobulin include:

1. *thionamides* (such as thiourea, thiouracil, propylthiouracil, methimazole, carbimazole, and goitrin);
2. *aniline derivatives and related compounds* (e.g., sulfonamides, *para*-aminobenzoic acid, *para*-aminosalicylic acid, and amphenone);
3. *substituted phenols* (such as resorcinol, phloroglucinol, and 2,4-dihydroxybenzoic acid), and
4. *miscellaneous inhibitors* (e.g., aminotriazole, tricyanoaminopropene, antipyrine and its iodinated derivative (iodopyrine)).

These chemicals exert their action by inhibiting thyroperoxidase, which results in a disruption both of the iodination of tyrosyl residues in thyroglobulin but also the coupling reaction of inactive iodotyrosines (e.g., MIT and DIT) to form active iodothyronines (T_3 and T_4) (Fig. 3.67). Propylthiouracil (PTU) has been shown in rats to affect each step in thyroid hormone synthesis beyond iodide transport.

Blockage of thyroid hormone release

Relatively few chemicals selectively inhibit the secretion of thyroid hormone from the thyroid gland. An *excess of iodine* inhibits secretion of thyroid hormone and occasionally can result in goiter and hypothyroidism in animals and human patients. Several mechanisms have been suggested for this effect of high iodide levels on

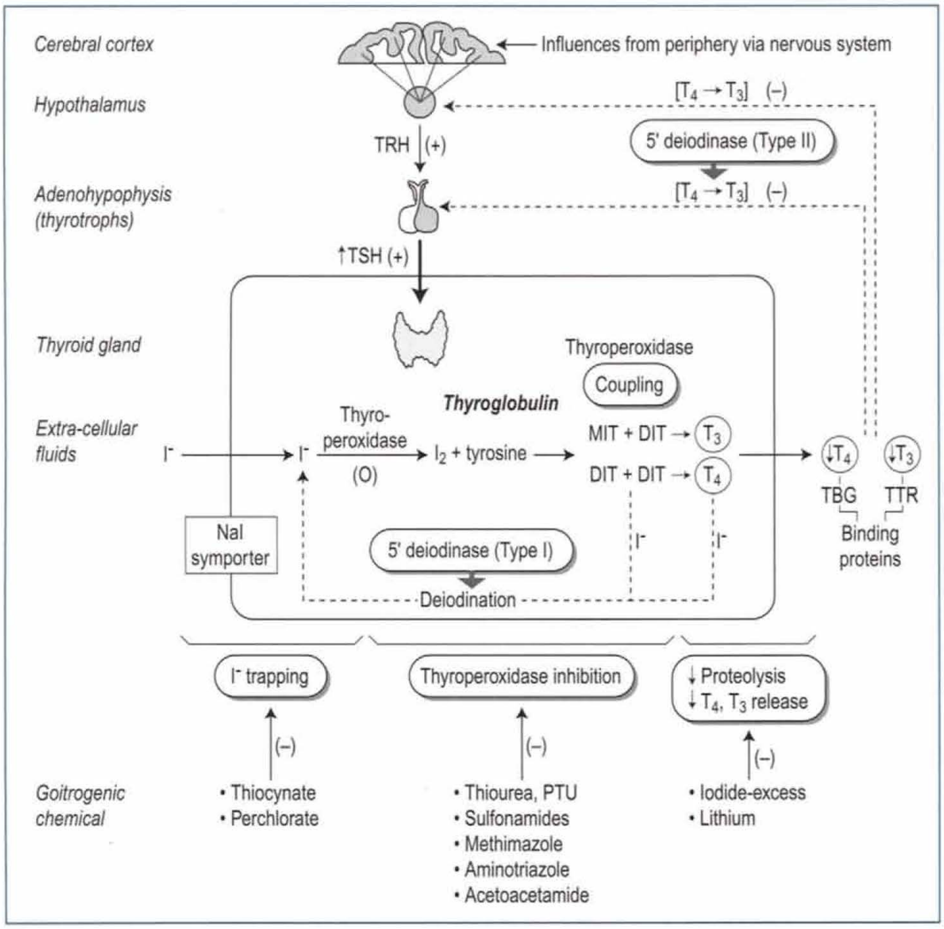

Cerebral cortex
Influences from periphery via nervous system
Hypothalamus
[T4 → T3] (–)
TRH (+)
5' deiodinase (Type II)
Adenohypophysis (thyrotrophs)
[T4 → T3] (–)
↑TSH (+)
Thyroid gland
Thyroperoxidase
Coupling
Thyroglobulin
Extra-cellular fluids
I⁻
I⁻
Thyro-peroxidase
(O)
I2 + tyrosine
MIT + DIT → T3
DIT + DIT → T4
↓T4
↓T3
TBG
TTR
Binding proteins
NaI symporter
5' deiodinase (Type I)
I⁻
I⁻
Deiodination
I⁻ trapping
Thyroperoxidase inhibition
↓ Proteolysis
↓ T4, T3 release
(–)
(–)
(–)
Goitrogenic chemical
• Thiocynate
• Perchlorate
• Thiourea, PTU
• Sulfonamides
• Methimazole
• Aminotriazole
• Acetoacetamide
• Iodide-excess
• Lithium

Figure 3.67 Mechanism of action of goitrogenic chemicals on thyroid hormone synthesis and secretion. Chemicals can interfere with thyroid function by: (1) blocking iodide ion trapping, (2) inhibiting organic binding-coupling, or (3) disrupting proteolysis and release of T_4 and T_3 from colloid.

the thyroid hormone secretion, including a decrease in lysosomal protease activity (human glands), inhibition of colloid droplet formation (mice and rats), and inhibition of TSH-mediated increase in cAMP (dog thyroid slices). Rats fed an iodide-excess diet have hypertrophy of the cytoplasmic area of follicular cells with an accumulation of numerous colloid droplets and lysosomal bodies. There is limited evidence ultrastructurally of fusion of the membranes of these organelles and degradation of the colloid necessary for the release of T_4 and T_3 from the thyroglobulin (Fig. 3.67).

Lithium also has a striking inhibitory effect on thyroid hormone release. The widespread use of lithium carbonate in the treatment of manic states occasionally results in the development of thyroid enlargement with either euthyroidism or occasionally hypothyroidism in human patients.

Drug-induced thyroid pigmentation

The antibiotic *minocycline* produces a striking black discoloration of the thyroid lobes in laboratory animals and humans with the formation of brown pigment granules within follicular cells. The pigment granules stain similarly to melanin and are best visualized on thyroid sections stained with the Fontana-Masson procedure. Electron-dense material first accumulates in lysosome-like granules and in the rough endoplasmic reticulum. The pigment appears to be a metabolic derivative of minocycline, and administration of the antibiotic at high dose to rats for extended periods may result in disruption of thyroid function and development of goiter. The release of T_4 from perfused thyroids of minocycline-treated rats is significantly decreased, but follicular cells retain the ability to phagocytize colloid in response to TSH and have numerous colloid droplets in their cytoplasm.

Other chemicals (or their metabolites) selectively localize in the thyroid colloid resulting in abnormal clumping and increased basophilia to the colloid. Brown-to-black pigment granules may be present in follicular cells, colloid, and macrophages in the interthyroidal tissues resulting in macroscopic darkening of both thyroid lobes. The physicochemically altered colloid in the lumina of thyroid follicles appears to be less able than normal colloid either of reacting with organic iodine in a step-wise manner to result in the orderly synthesis of iodothyronines, or of being phagocytized by follicular cells and enzymatically processed to release active thyroid hormones into the circulation. Serum T_4 and T_3 are decreased, serum TSH levels are increased by an expanded population of pituitary thyrotrophs, and thyroid follicular cells undergo hypertrophy and hyperplasia and eventually develop tumors.

Inhibition of 5′-deiodinase

Erythrosine (FD&C red no. 3) is one the best characterized chemicals that acts as a 5′-deiodinase inhibitor and results in perturbations of thyroid function. It is a tetraiodinated derivative of fluorescein, with iodine accounting for ~58% of the molecular weight of the color. Erythrosine is a red dye used widely as a color additive in foods, cosmetics, and pharmaceuticals. *Amiodarone* is an organic

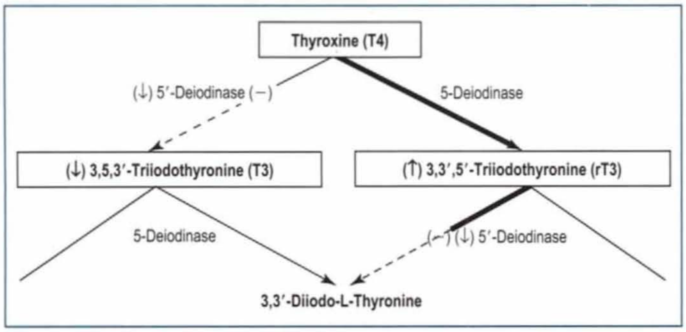


Figure 3.68 Effects of food color, erythrosine (FD&C red no. 3), on peripheral metabolism of thyroxine. Inhibition of 5′-deiodinase by erythrosine results in lowered serum T_3 and a compensatory increased secretion of TSH by the pituitary gland, leading to follicular cell stimulation. Preferential monodeiodination of thyroxine by 5-deiodinase results in markedly elevated blood levels of inactive reverse T_3 (rT3). (From Capen CC, Martin SL. Toxicol Pathol 1989;17:266–293.)

iodinated antiarrhythmic compound that also disrupts thyroid hormone economy by inhibiting 5′-deiodinase. *Iopanoic acid* and *flavonoids* also inhibit the enzyme in hepatocytes.

Erythrosine is an example of a xenobiotic that causes changes in circulating levels of thyroid hormones and morphologic evidence of follicular cell stimulation by producing alterations in the peripheral metabolism of T_4. *Inhibition of 5′-deiodinase in the liver and kidney by erythrosine explains the lower circulating T_3 levels.* The monodeiodination of T_4 by another enzyme (5-deiodinase) to reverse-T_3 and inhibition of 5′-deiodinase (which is necessary to further degrade this inactive iodothyronine to 3,3′-diiodothyronine (T_2)) results in the striking accumulation of serum reverse-T_3 (Fig. 3.68). The pituitary, sensing the lowered circulating levels of T_3, compensates by increasing the secretion of TSH, which results in the morphologic evidence of follicular cell stimulation (Fig. 3.68). Erythrosine does not appear to be a direct-acting thyroid oncogen. Rather, in massive doses (4% of diet over lifetime beginning in utero), it acts through a secondary mechanism to promote the development of benign thyroid tumors in laboratory animals.

Induction of hepatic microsomal enzymes

Hepatic microsomal enzymes play an important role in thyroid hormone economy since *glucuronidation is the rate-limiting step* in the biliary excretion of T_4, and sulfation, for the excretion of T_3 by phenol sulfotransferase. Long-term exposure to many chemicals may induce these enzyme pathways and result in chronic stimulation of the thyroid by disrupting the hypothalamic–pituitary–thyroid axis (Fig. 3.69). The resulting chronic stimulation of the thyroid by increased circulating levels of TSH often increases the risk of developing tumors derived from follicular cells in chronic studies with these compounds in certain species. **Xenobiotics** that induce liver microsomal enzymes and disrupt thyroid function include central nervous system drugs (e.g., phenobarbital, benzodiazepines); calcium channel blockers (e.g., nicardipine, bepridil); steroids (spironolactone); retinoids; chlorinated hydrocarbons (chlordane, DDT, TCDD), polyhalogenated biphenyls (PCB, PBB). Most of the hepatic microsomal enzyme inducers have no apparent intrinsic carcinogenic activity and produce little or no mutagenicity or DNA damage.

Phenobarbital has been studied extensively as the prototype for hepatic microsomal inducers that increase a similar spectrum of cytochrome P450 isoenzymes. *Polychlorinated biphenyls (PCBs)* also induce hepatic microsomal enzymes and disrupt thyroid function. PCBs are commonly used industrial compounds that are widespread environmental contaminants. Their disease-producing capability includes alterations in reproduction, growth, and development. PCBs cause a significant reduction in serum levels of thyroid hormones due to alterations in thyroid structure, in addition to the induction of hepatic UDP-glucuronyl transferase and increased secretion of thyroxine-glucuronide in the bile.

In contrast to the previous categories of indirect acting thyrotoxic compounds, *certain chemicals and irradiation appear to have a direct effect on the thyroid gland resulting in genetic damage that leads to cell transformation and tumor formation in animals.* Examples of thyroid initiators include 2-acetylaminofluorine (2-AAF), N-methyl-N-nitrosourea (MNU), N-bis(2-hydroxypropyl) nitrosamine (DHPN), methylcholanthrene, dichlorobenzidine, and polycyclic hydrocarbons. Chemicals in this group often increase the incidence of both benign and malignant thyroid tumors. Iodine deficiency is a strong promoter of MNU-initiated thyroid tumors in rats.

Understanding the mechanism of action of xenobiotics on the thyroid gland provides a rational basis for extrapolation of findings from long-term rodent studies to the assessment of a particular compound's safety for humans. Many chemicals and drugs disrupt one or more steps in the synthesis and secretion of thyroid hormones, resulting in subnormal levels of T_4 and T_3, associated with a compensatory increased secretion of pituitary TSH (Fig. 3.69). When tested in highly sensitive species, such as rats and mice, early on these compounds resulted in follicular cell hypertrophy/hyperplasia and increased thyroid weights, and in long-term studies they produced an increased incidence of thyroid tumors by a secondary (indirect) mechanism associated with hormonal inbalances. In the secondary mechanism of thyroid oncogenesis in rodents, the specific xenobiotic chemical or physiological perturbation evokes another stimulus (e.g., chronic hypersecretion of TSH) that promotes the development of nodular proliferative lesions (initially hypertrophy, followed by hyperplasia, subsequently adenomas, infrequently carcinomas) derived from follicular cells. Compounds acting by this indirect (secondary) mechanism with hormonal imbalances usually show little or no evidence for mutagenicity or for producing DNA damage. The literature suggests that prolonged stimulation of the human thyroid by TSH will induce neoplasia only in exceptional circumstances, possibly by acting together with some other metabolic or immunologic abnormality.

Neoplasms of the thyroid gland

Most thyroid tumors are of follicular origin. They are common in dogs and cats, moderately so in horses, and distinctly rare in cattle, sheep, and swine. These tumors usually develop in animals that often are permitted to live to an advanced age such as dogs, cats and horses.

Follicular cell adenoma

Adenomas are usually white to tan, relatively small, solid nodules that are well demarcated from the adjacent thyroid parenchyma. The affected thyroid lobe is only moderately enlarged and distorted. A distinct

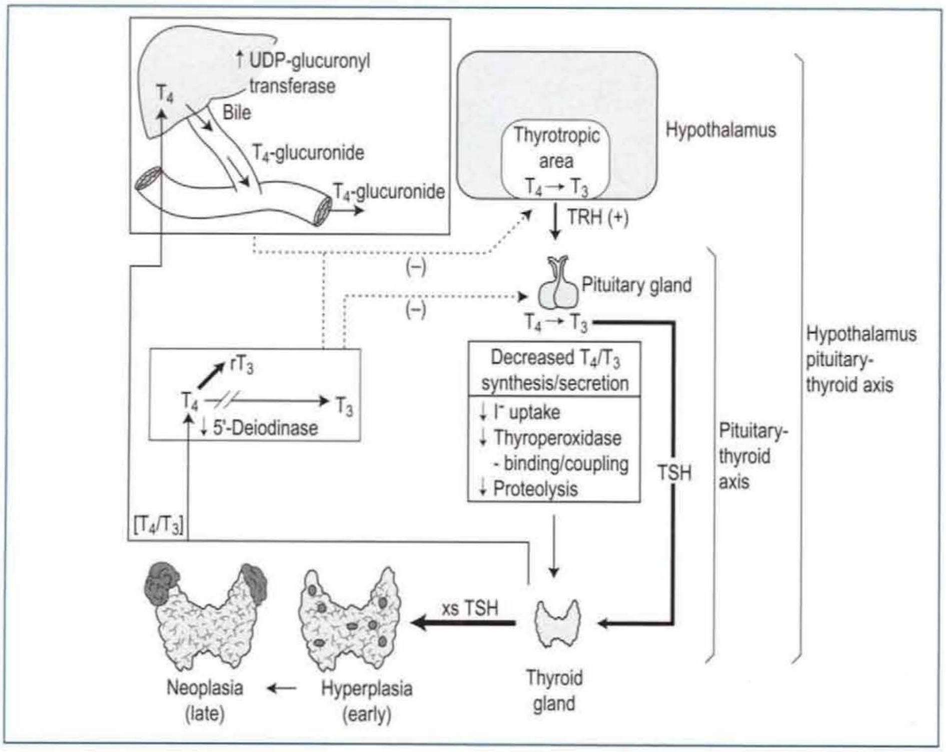


Figure 3.69 Multiple sites of disruption of the hypothalamic-pituitary-thyroid axis by xenobiotics. Chemicals can exert direct effects by disrupting thyroid hormone synthesis or secretion and indirectly influence the thyroid through inhibition of 5′-deiodinase or by inducing hepatic microsomal enzymes (e.g., T_4-UDP-glucuronyl transferase). All of these mechanisms can lower circulating levels of thyroid hormones (T_4 and T_3), resulting in a release from negative feedback inhibition and increased secretion of TSH by the pituitary gland. The chronic hypersecretion of TSH predisposes the sensitive rodent thyroid to develop an increased incidence of focal hyperplastic and neoplastic (predominantly adenomas) lesions by a secondary (epigenetic) mechanism due to hormonal imbalances. (From Capen CC. Toxicol Pathol 1997;25:39–48.)

white fibrous capsule of variable thickness separates the adenoma from the compressed parenchyma. Only a single adenoma usually is present in a thyroid lobe (see Fig. 3.65). Other thyroid adenomas are composed of fluctant thin-walled cysts filled with a yellow to red fluid. The external surface of **cystadenomas** is smooth and covered by an extensive network of blood vessels. Small masses of neoplastic tissue remain in the wall and form rugose projections into the cyst lumen. The thyroid parenchyma of the affected lobe may be completely obliterated.

Adenomas are classified into *follicular and papillary types.* They are sharply demarcated and encapsulated from the adjacent compressed thyroid parenchyma by a partial or complete fibrous capsule of variable thickness. *Adenomas derived from follicular cells that retain the ability to form follicles, according to one of several patterns, are considerably more common than papillary adenomas in animals.* Each **follicular adenoma** tends to have a consistent growth pattern within itself.

There are several different patterns of growth for follicles, similar to those of the normal thyroid. *Microfollicular adenomas* consist of tumor cells arranged in miniature follicles with small amounts of colloid or an absence of colloid. *Macrofollicular adenomas* are comprised of irregularly shaped large follicles that are greatly distended with colloid and lined by flattened follicular cells. There often is extensive hemorrhage and desquamation of follicular cells into the lumina of the distended follicles.

Cystic adenomas consist of one or two large cavities filled with proteinaceous fluid, necrotic debris, and erythrocytes. Focal accumulations of tumor cells, forming either follicles or solid nests, are present in the capsule of dense fibrous connective tissue. These adenomas may develop by progressive cystic degeneration of one of the several types of follicular adenomas. **Trabecular adenomas** are the most poorly differentiated of the follicular type. The tumor cells are small and are arranged in narrow columns separated by an edematous fibrous stroma. There is little evidence of follicle formation.

Oxyphilic adenomas are composed (predominantly or entirely) of large cells with densely eosinophilic granular cytoplasm arranged in indistinct follicles with little or no colloid formation. Oxyphilic (Hürthle) cells appear to be metabolically altered follicular cells that accumulate abnormally large numbers of mitochondria in their cytoplasm.

Papillary adenomas of thyroid origin are recognized infrequently in most animal species. Columnar or cuboidal follicular cells are arranged in a single layer about a thin vascular connective tissue stalk. *These papillary projections extend into the lumina of cystic spaces of various sizes.* The cysts contain desquamated tumor cells, colloid, erythrocytes, and occasionally laminated foci of mineralization resembling psammoma bodies.

Hyperthyroidism associated with thyroid tumors

Multinodular hyperplasia and follicular cell adenomas are common lesions in thyroid glands of adult to aged cats that develop a clinical syndrome of hyperthyroidism. Follicular cell adenomas, often developing in a thyroid with multinodular hyperplasia, are encountered more commonly than thyroid carcinomas. Adenomas and carcinomas are most likely to be encountered in aged cats, whereas nodular hyperplasia can occur at any age. The mean age of cats with benign tumors has been reported to be 12.4 years, and thyroid carcinomas as 15.8 years.

There has been a dramatic increase in the incidence of thyroid neoplasms and other focal proliferative lesions in cats resulting in hyperthyroidism since the late 1970s and at present it is one of the two most common endocrine diseases in adult cats (diabetes mellitus being the other). Prior to 1980, clinical hyperthyroidism was diagnosed infrequently in cats. The reason for the apparent increased incidence is uncertain, but appears to be related, in part, to: (1) a larger population of older cats seeking veterinary medical care since 1980, (2) improved assays for thyroid hormones, and (3) detailed characterization of the clinical syndrome and increased awareness of its common occurrence in adult-to-aged cats by veterinary clinicians. In addition, there does appear to be a "real" increase in the incidence of feline hyperthyroidism over the last 30 years. *Potential risk factors* have been reported to include a predominantly indoor environment, regular treatment with flea powders, exposures to herbicides and fertilizers, a diet primarily of canned food, and non-Siamese breeds (10 times greater occurrence). It has been suggested that wide variations (excessive to inadequate) in dietary iodine intake over prolonged periods may play a role in the pathogenesis of thyroid disorders in cats.

The disease in cats is mechanistically different from the clinically important *Grave's disease in humans*, as hyperthyroid cats do not have elevated circulating levels of thyroid-stimulating immunoglobulins comparable to long-acting thyroid stimulator (LATS) (an autoantibody that binds to the TSH receptor and activates follicular cells). Purified immunoglobulin G (IgG) preparations from hyperthyroid cats significantly increased ^{3}H-thymidine incorporation into DNA and stimulated cell proliferation 15-fold but did not stimulate intracellular cAMP. The former could be inhibited completely by a specific TSH receptor-blocking antibody. These data suggest that elevated titers of thyroid growth IgG's are present in cats with hyperthyroidism and most likely act by the TSH receptor. This important thyroid disease in cats most closely *resembles toxic nodular goiter in humans*. Hyperplastic and neoplastic thyroid tissue from cats is transplantable into athymic (nude) mice and continues to overproduce T4 and T3 in a subcuticular location.

Studies utilizing primary cultures of enzymatically dissociated follicles from thyroid proliferative lesions from cats with hyperthyroidism have reported that organification and ^{3}H-thymidine labeling continue in the absence of TSH, in contrast to follicles from normal cat thyroids. *These findings suggest that an intrinsic alteration in follicular cell function occurs in thyroids of cats with multinodular goiter leading to autonomy of cell growth and persistent overproduction of thyroid hormones.* A recent study reported an over-expression of the c-ras oncogene in areas of nodular hyperplasia and adenomas derived from follicular cells in cats with hyperthyroidism, suggesting that *mutations in this oncogene may play a role in the pathogenesis of these proliferative lesions.*

Point mutations in the thyrotropin receptor (TSHR) gene cause two forms of thyrotoxicosis in humans, namely, autonomously functioning toxic follicular adenomas and hereditary (autosomal dominant) toxic thyroid hyperplasia. The normal feline TSHR sequence between codons 480 and 640 is highly homologous to that of other mammalian TSHRs, with 95, 92, and 90% amino acid identity between the feline, canine, human, and bovine TSHRs, respectively. Analysis of single-stranded conformational polymorphisms in thyroid DNA from sporadic cases of feline thyrotoxicosis and leukocyte DNA from two cases of familial hyperthyroidism in cats failed to identify mutations between codons 480 and 640 of the TSHR gene. These interesting findings suggest that TSHR gene mutations are not a common cause of the focal proliferative lesions of thyroid follicular cells that result in feline thyrotoxicosis.

The syndrome of hyperthyroidism in aged cats can be associated with adenomas, multinodular hyperplasia, or adenocarcinomas derived from follicular cells. The most common clinical sign is *weight loss*, in spite of normal or increased appetite (Fig. 3.70). Polydipsia and polyuria, increased frequency of defecation, increased volume of stools, and increased activity occur. A common functional disturbance is tachycardia accompanied by premature beats and/or a systolic murmur. Cardiomegaly due to left ventricular hypertrophy may be evident on radiographs or at necropsy.

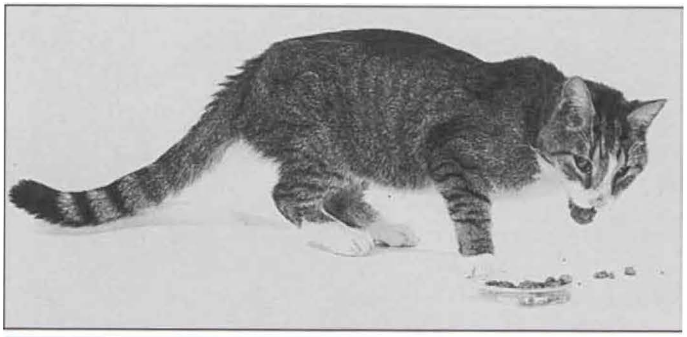


Figure 3.70 Hyperthyroidism in a cat with a functional adenoma derived from thyroid follicular cells. The cat progressively lost weight in spite of increased food consumption.

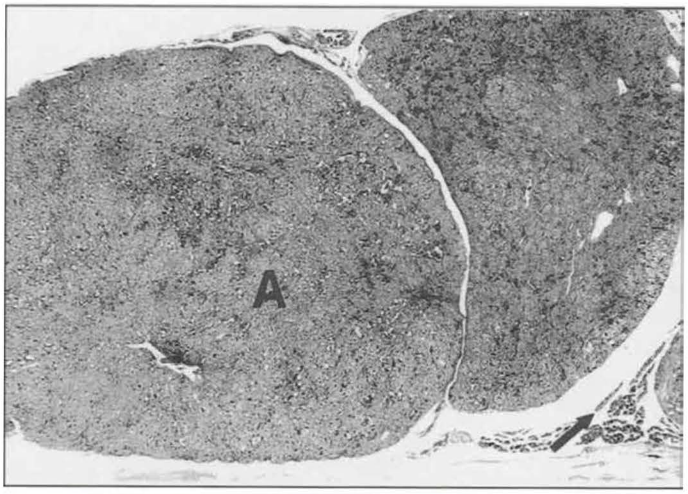


Figure 3.71 Follicular cell adenoma (A) in a cat with hyperthyroidism. The adenoma is lobulated, reflecting its likely development from coalescence of multifocal areas of adenomatous hyperplasia. The tumor is sharply demarcated from a rim of normal thyroid (arrow). (From Capen CC. Toxicol Pathol 2001;29:8–33.)

Thyroid adenomas in cats usually appear as solitary, soft, often lobulated nodules that enlarge and distort the contour of the affected lobe (Fig. 3.71). A thin, partial fibrous capsule separates the adenoma from the adjacent, often compressed, thyroid parenchyma (Fig. 3.72). Follicles in the surrounding rim of suppressed thyroid are markedly enlarged and distended by colloid, a lesion referred to as "*colloid involution*." The follicular cells are low cuboidal and atrophied with little evidence of endocytotic activity in response to the elevated levels of thyroid hormones produced by the adenoma resulting in suppressed levels of TSH (Fig. 3.72). Focal areas of necrosis, mineralization, and cystic degeneration are present in larger adenomas. Functional follicular adenomas often develop in thyroids that have multinodular hyperplasia of follicular cells in both lobes.

Functional thyroid adenomas are composed of cuboidal to columnar follicular cells with occasional papillary infoldings that form follicles containing variable amounts of colloid. The follicles usually are partially collapsed and contain little colloid due to the intense endocytotic activity of neoplastic follicular cells. Long

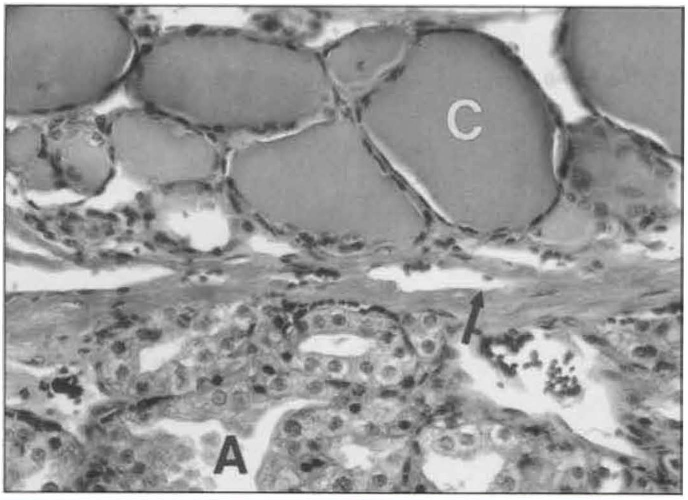


Figure 3.72 Follicular cell adenoma (A) composed of follicles with little stored colloid as a result of autonomous and excessive secretion of thyroid hormones. Follicles in the rim of normal thyroid have undergone colloid involution (C) and are distended with colloid as a morphologic response to suppressed TSH levels. A thin fibrous capsule (arrow) separates the adenoma from the peripheral rim of normal thyroid. (From Capen CC. Toxicol Pathol 2001;29:8–33.)

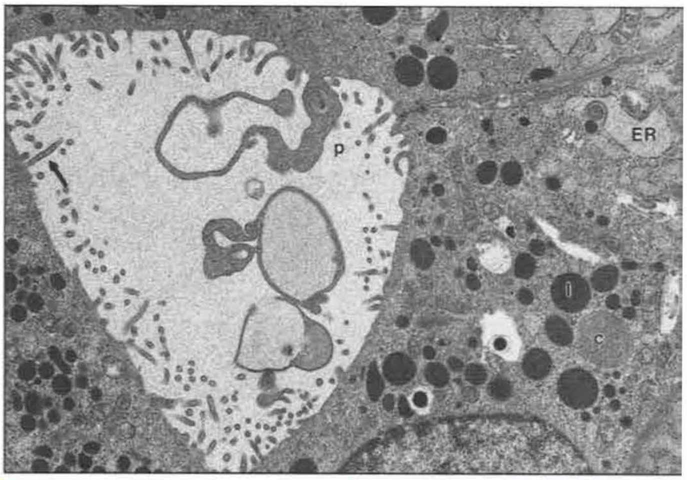


Figure 3.73 Functional follicular cell adenoma in a cat. Numerous cytoplasmic processes (p) extend from the luminal surface of neoplastic follicular cells to engulf colloid by endocytosis. Note lysosomal (l) bodies associated with colloid droplets (c) and long microvilli (arrow) on surfaces bordering colloid. Profiles of rough endoplasmic reticulum (ER) are dilated by finely granular material.

cytoplasmic projections extend from the follicular cells into the lumen to endocytose colloid (Fig. 3.73). As a result of the marked endocytotic activity, numerous colloid droplets are present in the apical cytoplasm of follicular cells in close proximity to the many lysosomal bodies.

Hyperthyroidism in cats also occurs in association with bilateral multinodular ("adenomatous") hyperplasia. These multiple areas of thyroid hyperplasia usually do not appreciably enlarge the affected lobe(s) (Fig. 3.74). The hyperplastic nodules are composed of irregularly shaped, colloid-filled follicles lined by cuboidal follicular cells. The multiple nodules of follicular cell hyperplasia eventually may coalesce to form macroscopically observable thyroid adenomas.

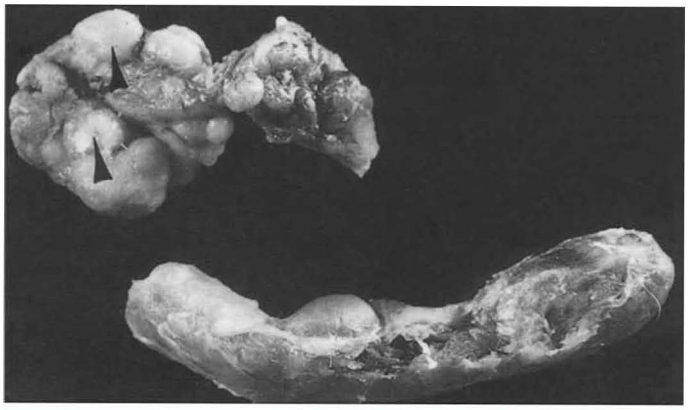


Figure 3.74 Multinodular follicular cell hyperplasia (arrowheads) in both thyroid lobes from a cat with hyperthyroidism.

Cats with hyperthyroidism usually have markedly elevated serum thyroxine and triiodothyronine levels. Normal feline serum levels of T_4 measured by radioimmunoassay are ~1.5–5.0 μg/dL (19–64 nmol/L) and serum T_3 levels are 60–200 ng/dL (0.9–3.1 nmol/L). The serum T_4 levels in cats with hyperthyroidism range from 5.0 to >50 μg/dL (64–640 nmol/L) and serum T_3 levels range from 100 to 1000 ng/dL (1.5–150 nmol/L). Moderately increased liver-derived serum enzyme levels, including AST (SGOT), ALT (SGPT), and especially alkaline phosphatase, occur in hyperthyroid cats.

The likelihood of developing clinical hyperthyroidism associated with thyroid neoplasms in animals depends upon: (1) the capability of tumor cells to synthesize T_4 and T_3 (e.g., well-differentiated thyroid tumors that form follicles and produce colloid are more likely to synthesize thyroid hormones than poorly differentiated solid neoplasms), and (2) the degree of elevation of circulating levels of T_4 and T_3, which depends upon a balance between the rate of secretion of thyroid hormones by the tumor and the rate of degradation of thyroid hormones. For example, *dogs have a much more efficient enterohepatic excretory mechanism for thyroid hormones than cats and infrequently have clinical signs associated with functional thyroid tumors.* Cats are very sensitive to phenol and phenol-derivatives, and have a poor ability to conjugate phenolic compounds (such as T_4) with glucuronic acid and excrete the T_4-glucuronide into the bile. The capacity of conjugation of T_3 with sulfate is limited and easily overloaded. Therefore, *cats with relatively small functional proliferative lesions of thyroid follicular cells often have marked elevations in circulating levels of T_4 and T_3 with clinical signs of hyperthyroidism,* whereas dogs with functional thyroid tumors have only slight elevations of serum T_4 and T_3 and less frequently develop clinical hyperthyroidism.

When first evaluated, a small percentage of hyperthyroid cats have T_4 concentrations within the upper limits of the reference range. This most likely is due to early detection of the disease attributable to clinician awareness and readily available screening tests. Hyperthyroidism usually can be confirmed in these cats by repeating the thyroid hormone assays or performing a T_3 suppression test. In addition, a wide variety of nonthyroidal illnesses in hyperthyroid cats also may suppress the serum T_4 concentration into the normal range. Hyperthyroid cats with increased circulating T_4 and T_3 levels do not respond to exogenous TSH stimulation by increasing hormone levels two or three fold as in normal cats. The neoplastic follicular cells are unable to respond to the exogenous TSH, and follicular cells in the adjacent normal rim of thyroid have undergone trophic atrophy with colloid involution of follicles and are unable to increase blood T_4 and T_3 levels after only short-term stimulation by TSH.

Hyperthyroid cats often have disturbances of calcium homeostasis and diffuse chief cell hyperplasia in the parathyroid glands. Compared to reference intervals, blood ionized (not total) calcium and plasma creatinine concentrations often are significantly decreased and plasma phosphate and intact parathyroid hormone levels are increased. Hyperparathyroidism occurred in 77% of hyperthyroid cats, with parathyroid hormone levels elevated up to 19 times the upper limit of the reference range. Hyperphosphatemia was present in approximately 40% of hyperthyroid cats. The mechanisms for the development of hyperphosphatemia in feline hyperthyroidism are uncertain but may be related, in part, to polyphagia with increased intestinal phosphate absorption, increased catabolism of muscle proteins and release of phosphate due to the gluconeogenic effects of the elevated thyroid hormone levels, and increased bone resorption with release of phosphate into the blood. The hyperparathyroidism and chief cell hyperplasia appear to be related to the reciprocal decline in circulating levels of ionized calcium in response to the hyperphosphatemia. An elevated blood phosphate also could inhibit the renal 1 α-hydroxylase and decrease the production of the active form of vitamin D thereby reducing intestinal calcium absorption; however, circulating levels of $1,25(OH)_2D_3$ were not decreased in the limited number of hyperthyroid cats evaluated.

Serum levels of the bone isoenzyme of alkaline phosphatase are elevated consistently in cats with hyperthyroidism; however, there is no correlation between the magnitude of increase of alkaline phosphatase, osteocalcin, and serum T_4 concentrations. Although the total calcium usually is within the reference interval, the serum ionized calcium is reduced in approximately 50% of cases. *These findings suggest that hyperthyroid cats have altered bone metabolism*; however, the bone disease usually is not clinically significant in adult-to-aged cats with hyperthyroidism.

Surgical excision of affected thyroid lobe(s), medical management by thyroid blocking drugs (e.g., methimazole, propylthiouracil), and radioactive iodine are effective treatments available for cats with hyperthyroidism. Bilateral thyroidectomy is necessary if both glands are abnormal in appearance at surgery. At least one parathyroid gland should be left with an intact blood supply if bilateral thyroidectomy is performed. A thyroid-blocking agent, such as methimazole or propylthiouracil, can be used before surgery to alleviate some of the severe clinical effects (e.g., tachycardia and cardiomegaly) of hyperthyroidism.

Follicular cell carcinoma

Thyroid carcinomas are palpable masses in the ventral cervical region and they may be unilateral or bilateral. Most thyroid tumors are palpated near the larynx, but larger ones may extend toward the thoracic inlet. The tumor may be firm or soft. Respiratory distress occurs if the tumor encroaches upon the larynx or trachea. Carcinomas become fixed in position by extensive local invasion of adjacent structures, whereas adenomas are freely movable under the skin.

In dogs, thyroid carcinomas occur more often than adenomas, but in cats adenomas occur more frequently than carcinomas. No sex prevalence has been observed in dogs. It has been reported that Boxers develop thyroid carcinomas more frequently than any other breed of dog. There also are data indicating that Beagles, Boxers, and Golden Retrievers have a significantly greater risk for thyroid carcinoma than all other breeds of dogs combined.

Adenocarcinomas of the thyroid are larger than adenomas, are coarsely multinodular, and often have large central areas of hemorrhage and necrosis. Unilateral involvement is about twice as frequent in dogs as is involvement of both thyroid lobes. Carcinomas are poorly encapsulated and invade locally into the wall of the trachea, cervical muscles, esophagus, larynx, nerves, and vessels. Early invasion into branches of the cranial and caudal thyroid veins with the formation of *tumor cell thrombi* (Fig 3.75A, C) leads to multiple *pulmonary metastases* (Fig. 3.75D, E), often before involvement of the retropharyngeal and caudal cervical lymph nodes. Focal white gritty areas of mineralization or bone formation are scattered throughout some tumors. Although adenomas and carcinomas derived from follicular cells usually arise in the neck from the thyroid lobes, they may develop from *ectopic thyroid parenchyma in the mediastinum* and must be included in the differential diagnosis of "*heart-base tumors*" in dogs.

Malignant tumors of thyroid follicular cells generally are more highly cellular and have a greater degree of cellular pleomorphism than adenomas. On the basis of the predominant histologic pattern of growth, *"differentiated" thyroid adenocarcinomas are subdivided into follicular, papillary, and compact cellular (solid) types.* In dogs, where thyroid carcinomas are seen most frequently, they often have both a follicular and compact cellular growth pattern (Fig. 3.75B), whereas papillary carcinomas are uncommon.

In **follicular adenocarcinomas**, *the majority of tumor cells are arranged in a recognizable follicular pattern.* It is possible to subdivide follicular carcinomas further on the basis of size of follicles, as described for follicular adenomas, but this often is difficult because of the admixture of growth patterns present in any one tumor. Such subdivisions appear to be of little prognostic value in animals with thyroid cancer. The tumor cells are tall cuboidal to columnar, and form follicles of variable size, shape, and colloid content. Mitotic activity in the tumor cells is minimal. The colloid in follicular lumina occasionally is clumped and extensively mineralized.

In **compact cellular carcinoma**, tumor cells form aggregations or solid sheets of cells often separated by fibrous stroma with little or no attempt at follicle formation and colloid secretion. The polyhedral cells are closely apposed and have eosinophilic cytoplasm that is finely granulated or vacuolated. Immunocytochemical and ultrastructural studies have demonstrated that compact cellular ("solid") carcinomas in dogs are derived from follicular cells and not from C cells.

Follicular-compact cellular carcinoma *is the most common type of malignant thyroid tumor in dogs*, having approximately equal follicular and compact cellular growth patterns (Fig. 3.75B). The follicles formed often are smaller and contain less colloid than do pure follicular carcinomas. The tumor cells arranged in compact or solid areas appear to be morphologically and functionally less differentiated than those that form follicles and secrete colloid.

Papillary carcinomas in which tumor cells form papillae extending into cystic spaces are *uncommon in animals*. Single or multiple layers of cuboidal cells surround fibrovascular stalks that project into cystic spaces. Their nuclei are vesicular and pleomorphic with prominent nucleoli. The nuclear vacuoles seen by light

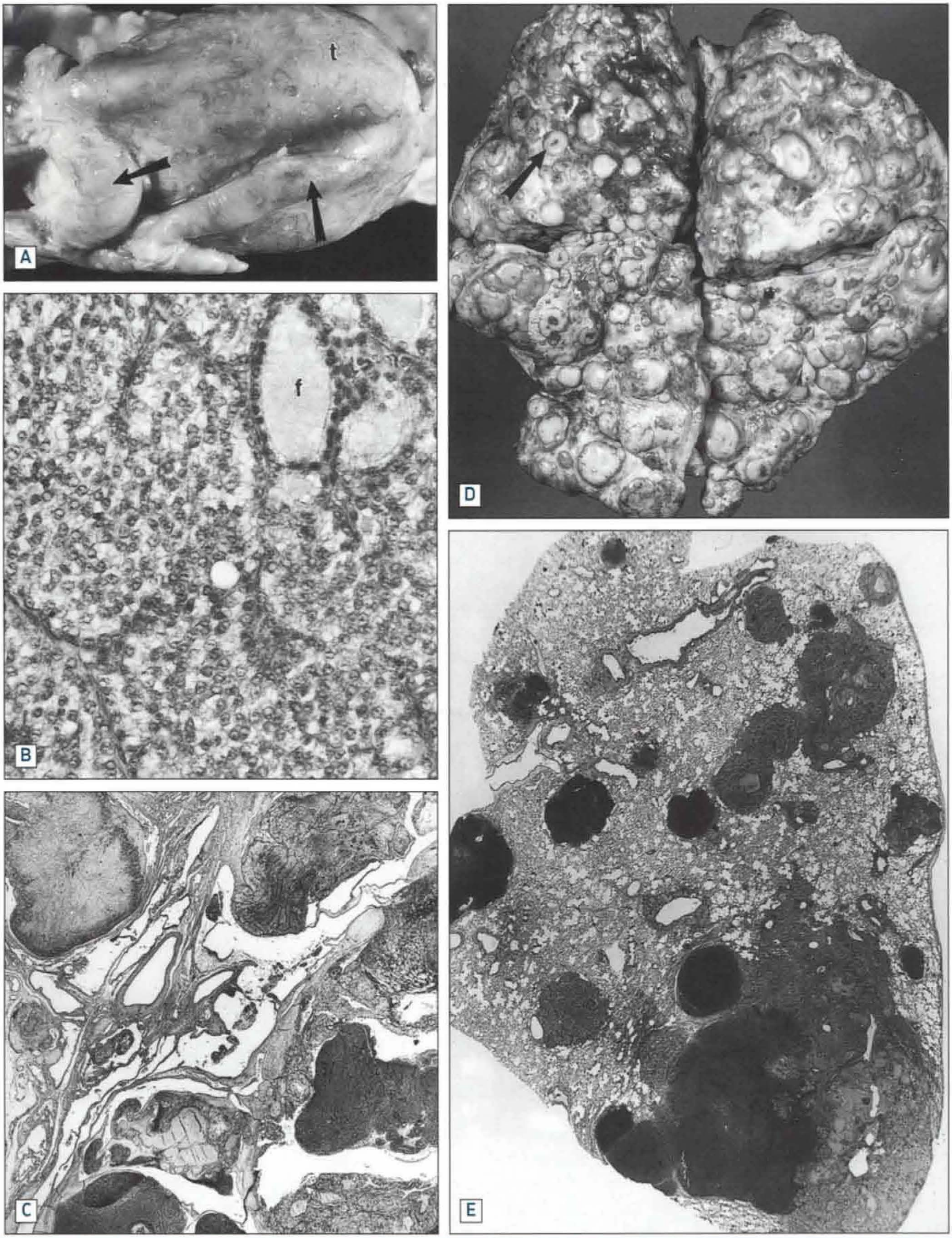


Figure 3.75 Adenocarcinoma of thyroid follicular cells, dog. **A.** Prominent tumor thrombi distending thyroid veins (arrows) adjacent to the enlarged thyroid (t). **B.** Follicular-compact cellular pattern. Colloid-containing follicle (f). **C.** Vascular invasion by tumor cell thrombi. **D.** Extensive pulmonary metastases; many nodules have an indented surface (arrow). **E.** Multiple metastases in lung.

microscopy have been shown by electron microscopy to represent cytoplasmic evaginations into the nucleus. Infiltration of tumor cells through the fibrous connective tissue capsule and into adjacent tissues is frequent in thyroid adenocarcinomas.

Undifferentiated thyroid carcinomas *lack a characteristic architectural pattern of arrangement of tumor cells.* They are an uncommon form of thyroid carcinoma in animals. **Small-cell carcinoma** is one type of undifferentiated thyroid carcinoma. They are composed of highly malignant follicular cells with either a diffuse or compact pattern of growth. The small tumor cells are uniform in appearance and are closely packed within clusters separated by fibrous stroma. The scant cytoplasm is eosinophilic, and the oval nucleus is densely hyperchromatic. Mitotic figures are frequent.

Giant cell carcinoma is a highly malignant thyroid tumor derived from poorly differentiated follicular cells. *The anaplastic tumor cells are large, pleomorphic, and often spindle-shaped, making differentiation from a fibrosarcoma difficult.* The demonstration of identifiable epithelial structures may require multiple sections from several areas of the tumor. Follicular remnants of transitional forms suggest that giant cell carcinomas are derived from thyroid follicular cells.

Malignant mixed thyroid tumors have been reported in the dog. The tumors contain both malignant thyroid follicular cells and mesenchymal elements, usually osteogenic or cartilaginous.

Thyroid carcinomas often grow rapidly, invading adjacent structures such as the trachea, esophagus, and larynx, and usually are fixed in position. *The earliest and most frequent site of metastasis is the lung* (Fig. 3.75D, E), because thyroid carcinomas tend to invade branches of the thyroid vein. Tumor cords may be palpated in the thyroid or jugular veins in some animals with thyroid carcinoma. The retropharyngeal and caudal cervical lymph nodes are less frequent sites of metastasis.

Some thyroid tumors in the dog secrete sufficient thyroid hormone to produce mild clinical signs of *hyperthyroidism*. It is surprising that hyperthyroidism occurs even with functional tumors since experimental induction of hyperthyroidism in the dog requires daily administration of ~25 times the normal replacement dose of desiccated thyroid or *l*-thyroxine. The clinical signs of hyperthyroidism in dogs with functional thyroid tumors include weight loss, polyphagia, polyuria, polydipsia, weakness and fatigue with exercise, intolerance to heat, and nervousness.

Thyroid carcinomas occur much less frequently in **cats** than either adenomas or multinodular hyperplasia. They often result in considerable enlargement of one or both thyroid lobes and may invade adjacent structures. Thyroid carcinomas are characterized by neoplastic invasion of vessels and connective tissue capsule. Metastases to regional lymph nodes (retropharyngeal, mandibular, deep cervical) and distant sites have been reported infrequently in thyroid carcinomas of cats. The well-differentiated thyroid adenocarcinomas are composed of a uniform pattern of small follicles containing variable amounts of colloid. Strands of dense connective tissue with an abundant capillary network and foci of lymphocytes subdivide the neoplastic cells into small lobules.

Thyroid carcinogenesis and radiation. Epidemiologic investigations in humans and animal studies have indicated that the risk of developing thyroid cancer is increased following exposure to external (localized) X-rays (especially to the cervical region) or to internal ^{131}I irradiation. For example, results of a large study in female Long-Evans rats revealed that the proportion of rats with thyroid carcinomas was similar for ^{131}I and X-irradiation within the dose range of 0–1000 rads. There was a significant dose-dependent increase in follicular cell carcinomas in rats administered ^{131}I or exposed to external X-radiation.

Neoplasms of thyroglossal duct remnants

Tumors arising in cystic remnants of the thyroglossal duct are *rare in animals* but have been reported in dogs. They appear as well-circumscribed, fluctuant, movable enlargements (~2–4 cm diameter) on the ventral midline in the cranial cervical region. The clinical history usually indicates a slowly progressive expansion of the cervical mass. On cross-section, multilocular cystic areas containing translucent proteinaceous fluid alternate with white solid areas. The thyroid glands appear to be normal in the few cases studied in dogs. These tumors appear to develop from the epithelium of the thyroglossal duct and are not a cystic metastasis from a primary carcinoma in the thyroid gland.

Neoplasms of thyroglossal duct remnants are well-differentiated papillary carcinomas. Multiple papillary outgrowths covered by multiple layers of tall cuboidal to columnar epithelial cells extend from the cyst wall into the lumen (Fig. 3.76). The cyst wall is composed of dense fibrous connective tissue with focal areas of hemorrhage and cholesterol clefts. Aggregations of thyroidogenic epithelium in the form of small follicles and cell cords are present within the fibrous capsule and in surrounding connective tissue. These follicles are lined by low cuboidal epithelium and contain variable amounts of colloid. Carcinomas of thyroglossal duct remnants appear to be well-differentiated and slow growing with limited evidence of local tissue invasion.

Thyroid C (parafollicular) cell tumors

Tumors derived from C (parafollicular) cells of the thyroid gland are most frequently encountered in adult-to-aged bulls (ultimobranchial body origin), certain strains of laboratory rats, adult-to-aged horses, but infrequently in other species. A high percentage of aged bulls have been reported to develop C-cell tumors (30%) or hyperplasia of C cells and ultimobranchial derivatives (15–20%) when they were fed diets with a high calcium content similar to that fed to cows. *These frequently occurring hyperplastic and neoplastic lesions of C cells have been observed only in bulls and not in cows.* The incidence

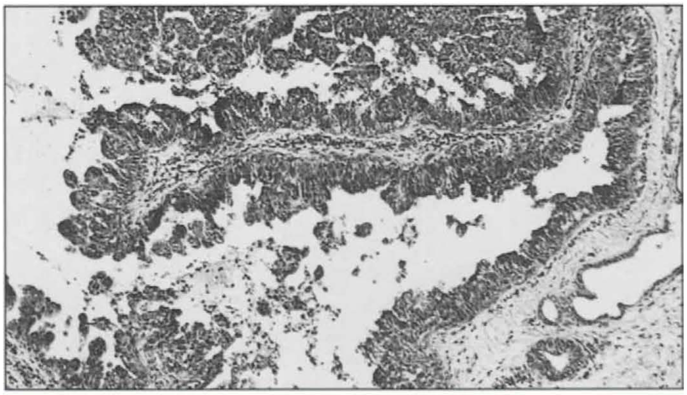


Figure 3.76 Papillary cystadenocarcinoma derived from thyroglossal duct remnants in a dog.

of C-cell tumors increases with advancing age in bulls, and may be associated with the development of vertebral osteophytes.

The syndrome of C-cell tumors in bulls shares many similarities with medullary thyroid carcinoma in humans. *Multiple endocrine tumors*, especially bilateral pheochromocytomas and occasionally pituitary adenomas, are detected coincidentally in bulls and human patients with C-cell tumors. This may represent a simultaneous neoplastic transformation of multiple endocrine cell populations of neural crest origin in the same individual. A high frequency of thyroid C-cell tumors and pheochromocytomas has been reported in a family of Guernsey bulls, suggesting an autosomal dominant pattern of inheritance. Diffuse or nodular hyperplasia of secretory cells in the adrenal medulla appears to precede the development of pheochromocytoma.

The calcitonin-producing C cells were described initially in dogs as light or gray cells and are particularly prominent in this species. Nodular aggregations of C cells are especially prominent in dogs, either near the thyroid hilus in the perithyroidal connective tissues, or within the thyroid lobes along the course of the major branches of the thyroid artery. The ultimobranchial body (last, usually fifth pharyngeal pouch) that delivers the neural crest-derived C cells to the postnatal thyroid gland fuses with each thyroid lobe at the hilus and distributes C cells throughout each lobe to various degrees in different species. In the dog, nodular aggregations of C cells frequently persist along the course of the major vessels to the thyroid; therefore, *C-cell hyperplasia in dogs should be diagnosed only when there is a definite increase in C-cell numbers throughout each thyroid lobe compared to age-matched controls.* Both thyroid lobes should be sectioned longitudinally in a consistent manner for microscopic evaluation. This will minimize the prominent regional differences of C cells in the thyroid glands of normal dogs that can result in the over-interpretation of these focal aggregations of C cells as a significant lesion. The C cells in the focal aggregations have abundant, lightly eosinophilic, finely granular, cytoplasm and a spherical-oval nucleus. There are occasional ultimobranchial-derived, colloid-containing follicles lined by more basophilic cells within the focal accumulations of C cells along the course of vessels within the thyroid lobe or in the connective tissues of the thyroid hilus in dogs.

Focal (nodular) hyperplasia of C cells often precedes the development of C-cell neoplasms in animal and humans. *The histological distinction between focal hyperplasia and adenoma of C cells often is difficult and somewhat arbitrary.* The diagnosis of C-cell hyperplasia refers to a focal or diffuse increase of C cells between thyroid follicles and/or within the follicular basement membrane. The C cells appear normal, with abundant, lightly eosinophilic, granular cytoplasm and a round-to-oval nucleus with finely stippled chromatin (Fig. 3.77). Cell boundaries often are indistinct. Focal hyperplasia of C cells forms a nodule with an approximate diameter equal to or less than 5 average colloid-containing thyroid follicles with minimal evidence of compression of adjacent follicles (guidelines for rat thyroids). Hyperplastic C cells within the basement membrane may compress individual thyroid follicles. Calcitonin immunoreactivity is localized to the cytoplasm of hyperplastic C cells.

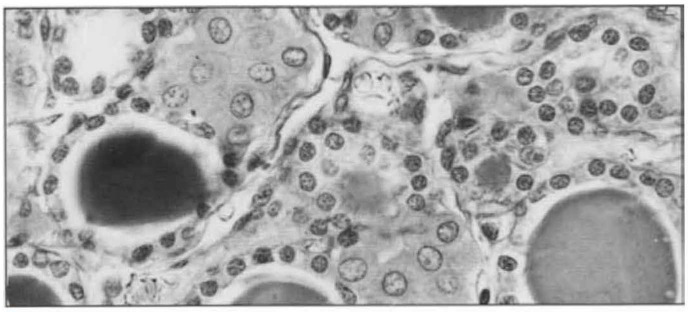
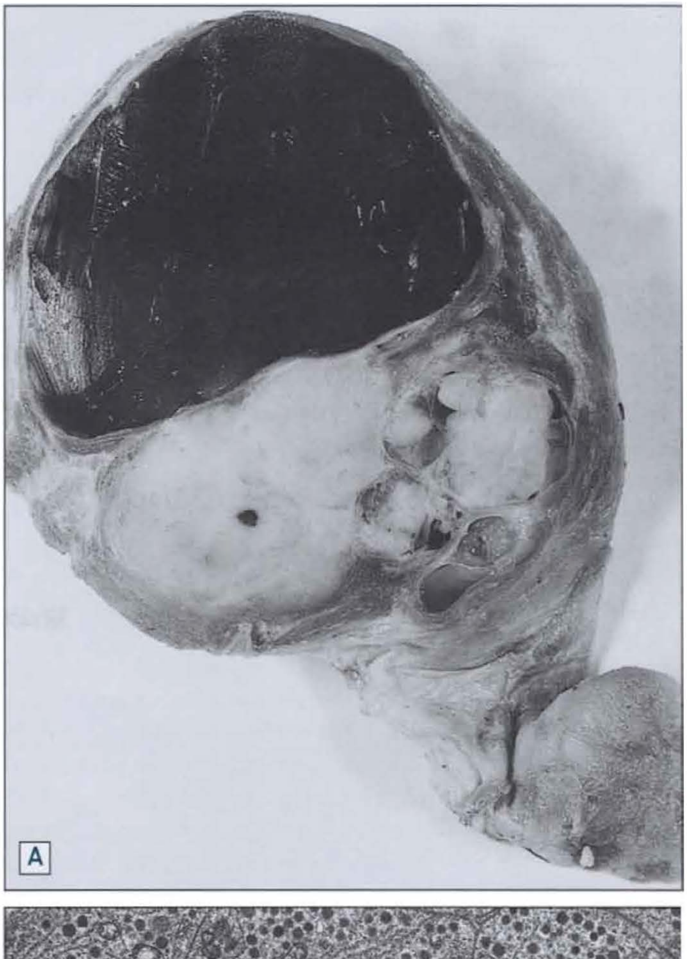


Figure 3.77 Hyperplasia of thyroid C cells in a dog.

C-cell adenomas appear as discrete, single or multiple, gray to tan nodules in one or both thyroid lobes (Fig. 3.78A). Adenomas are smaller (~1–3 cm diameter) than carcinomas, and are separated from the thyroid parenchyma by a thin, partial to complete, fibrous



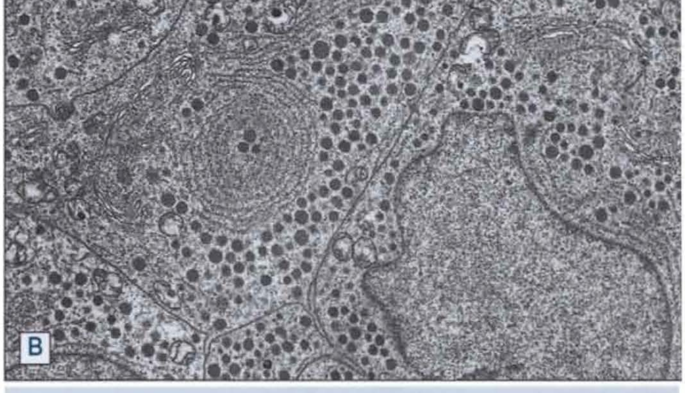


Figure 3.78 A. C-cell adenoma with cyst formation in a bull. **B.** C-cell adenoma in a horse, composed of well-differentiated cells that contain numerous membrane-limited secretory granules, circular profiles of rough endoplasmic reticulum, and prominent Golgi apparatuses.

connective tissue capsule. The adjacent thyroid is compressed but not invaded by neoplastic C cells. In horses, C-cell adenomas may result in a palpable enlargement in the cranial cervical region. Larger C-cell adenomas incorporate most of the thyroid lobe, but a rim of dark brown-red thyroid often is present on one side.

Thyroid C-cell adenomas microscopically are discrete, expansive masses of cells greater in diameter than 5 average colloid-containing thyroid follicles. They are well circumscribed or partially encapsulated from adjacent follicles that are compressed to various degrees. C-cell adenomas may be sub-divided into packets of cells by fine or coarse connective tissue septa and capillaries. The neoplastic C cells are well differentiated and have abundant cytoplasm that is lightly eosinophilic or clear. Areas of subendothelial growth of neoplastic C cells should not be over-interpreted as vascular invasion and evidence of malignancy. The well-differentiated cells comprising C-cell adenomas have numerous membrane-limited secretory granules, circular and lamellar profiles of rough endoplasmic reticulum, and prominent Golgi complexes (Fig. 3.78B). Some C-cell adenomas are composed of larger cells with amphophilic cytoplasm, large nuclei with coarsely clumped chromatin, and prominent nucleoli. These cells bear a histological resemblance to ganglion cells and the potential of primitive neuroectodermal cells that give rise to the adrenal medulla to differentiate into either sympathetic ganglion cells or catecholamine-secreting endocrine cells. Occasional amyloid deposits may be found both in nodular hyperplasia and in adenomas.

Thyroid **C-cell carcinomas** result in more extensive multinodular enlargements of one or both thyroid lobes. The proliferating neoplastic tissue may incorporate the entire thyroid gland. Multiple metastases in cranial cervical lymph nodes may be large and have areas of necrosis and hemorrhage. Pulmonary metastases are present infrequently and appear as discrete tan nodules throughout all lobes of the lung. C-cell carcinomas are more highly cellular and the tumor cells are more pleomorphic than are C-cell adenomas. They often have evidence of intrathyroidal and/or extracapsular invasion, occasionally with metastasis to distant sites. The neoplastic cells are polyhedral to spindle-shaped with lightly eosinophilic, finely granular, indistinct cytoplasm (Fig. 3.79). The vesicular nuclei are oval or elongate and have more frequent mitotic figures than do adenomas. Malignant C cells often are subdivided into small groups by fine connective tissue septa that contain small capillaries.

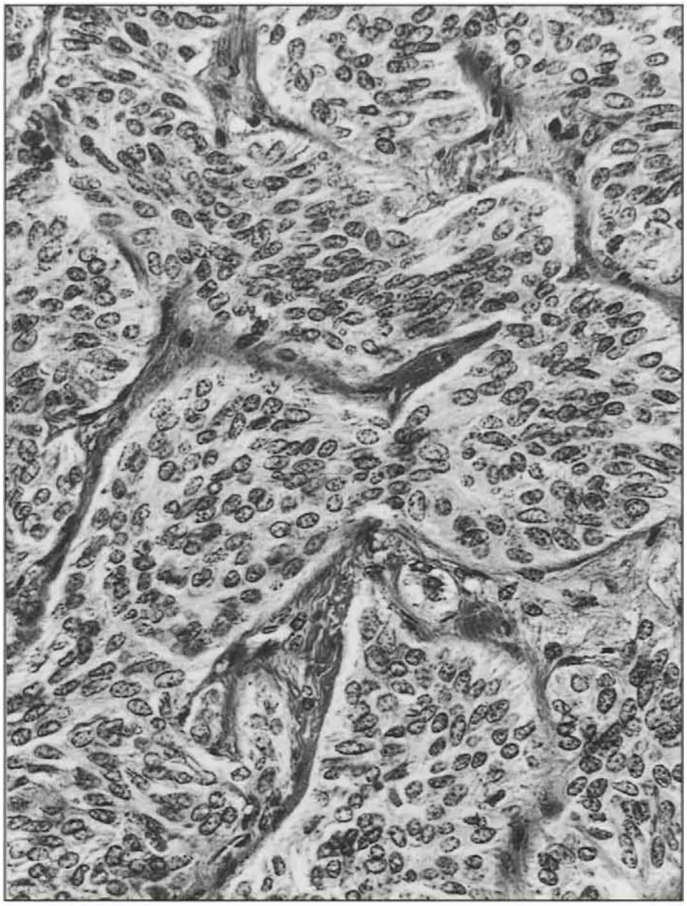


Figure 3.79 C-cell carcinoma illustrating neuroendocrine pattern in a bull.

Thyroid C-cell tumors in bulls, other animals and humans often are firm and in some areas the stroma consists of dense bands of fibrous connective tissue. In both adenomas and carcinomas, there may be deposits of homogeneous eosinophilic material that stains positively for *amyloid*. Ultrastructurally, large aggregations of fine amyloid fibrils are observed between the bundles of collagen fibers. The etiology of the localized amyloid deposition in thyroid C-cell neoplasms is uncertain but it is not associated with chronic suppurative lesions in other organs. Amyloid production is consistently associated with medullary thyroid carcinoma in humans and also has been reported in certain other endocrine tumors. Chemical differences exist between amyloid fibrils of immunoglobulin origin and those produced by endocrine tumors.

Bioassay of C-cell adenomas and carcinomas from bulls has demonstrated the presence of *calcitonin activity in tumor tissue* and calcitonin has been detected at higher than normal levels in plasma of bulls by immunoassay. Calcium infusion to raise serum calcium increases plasma calcitonin-like activity. Mean serum calcium levels in bulls with calcitonin-secreting thyroid tumors are only slightly lower than in adult control bulls. The near normal or mild decrease in serum electrolyte values in animals with chronic hypersecretion of calcitonin probably is a result of the low turnover rate of bone and compensatory mechanisms of other endocrine organs such as the parathyroid glands.

The etiology of C-cells neoplasms is unknown, but a possible relationship has been suggested between the long-term dietary intake of excessive calcium and the high incidence of these tumors in bulls. Depending upon the feeding regimen, adult bulls may ingest 3.5–6.0 times the amount of calcium normally recommended for maintenance. *The chronic stimulation of C cells by high levels of calcium absorbed from the digestive tract may be related to the pathogenesis of C-cell neoplasms.* A significant decline in the incidence of C-cell tumors has been reported when bulls are switched from a high-calcium intake to a reduced calcium intake. Cows do not develop proliferative lesions of C cells under similar dietary conditions, possibly because of the high physiologic requirements for calcium imposed by pregnancy and lactation.

Ultimobranchial tumors in the thyroid glands of bulls often have a more complex histologic structure than the typical C-cell (medullary) neoplasms in human patients, horses, dogs, and certain strains of laboratory rats. Focal areas in the tumor are composed of more differentiated C cells that have abundant lightly eosinophilic cytoplasm and are located either in the wall of thyroid and ultimobranchial follicles or as larger nodules with a solid histologic structure. This often is accompanied by multifocal hyperplasia of

C cells in other parts of the thyroid lobes and hilus. The neoplastic C cells often are embedded in an increased amount of hyalinized stroma that may contain amyloid. Other areas of this unique thyroid neoplasm in bulls appear to be derived from less differentiated ultimobranchial remnants and consist of follicle-like structures, cysts, and tubules composed of immature small basophilic cells. They closely resemble undifferentiated or stem cells of the normal ultimobranchial body in bulls and other species that have the potential to differentiate into both C and follicular cells. Thyroid follicles and cribriform structures with colloid-like material formed by cells resembling differentiated follicular cells often are present in the neoplasms in close association with these more primitive ultimobranchial-derived structures. The heterogeneous histologic structure of ultimobranchial neoplasms in bulls resembles an unusual type of thyroid carcinoma of human patients. This variant, designated as an **intermediate type of differentiated thyroid carcinoma**, has structural and immunocytochemical characteristics of both medullary (C-cell) and follicular carcinomas.

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ADRENAL CORTEX

Development, structure, and function

Embryonic development of adrenal gland

The adrenal glands of mammals consist of two distinct parts that differ not only in morphology and function but also in origin. Because of their close structural relationships, the outer cortex and inner medulla of the adrenal gland usually have been considered parts of one organ (Fig. 3.80). The **adrenal cortex** develops from cells of the celomic epithelium that are of mesodermal origin. The chromaffin tissue and sympathetic ganglion cells of the **adrenal medulla** are derived from ectoderm of the neural crest. It is not until relatively late in fetal development that a definitive relationship between the two primordia occurs.

Structure of adrenal gland

The adrenal glands are richly vascularized, receiving arterial branches either directly from the aorta or from the phrenic, renal and lumbar arteries. The arteries form a vascular plexus in the capsule that eventually supplies the entire adrenal gland through separate channels to the capsule, cortex, and medulla. A sinusoidal network is formed about the cell columns of the adrenal cortex that empties into the venous tree at the periphery of the medulla. The larger branches of the venous tree empty into the adrenal vein.

The adrenal cortex classically is subdivided into three layers or zones, although the demarcation between zones often is not distinct.

- The **zona glomerulosa** (multiformis, arcuata) is composed of columns of cells that have a sigmoid arrangement next to the capsule. It represents ~15% of the cortex and is responsible for the secretion of *mineralocorticoid hormones*.

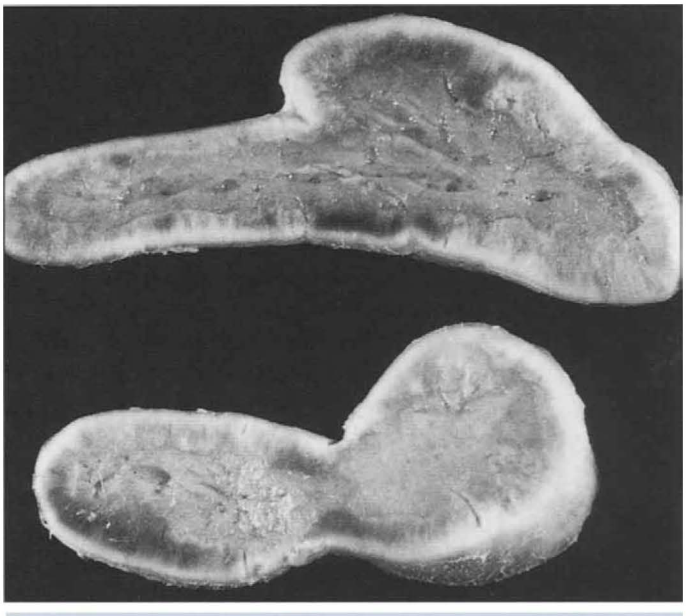

Figure 3.80 Normal adrenal glands (top = right adrenal; bottom = left adrenal) from a dog. Cortical:medullary ratio is approximately 2:1.

- The secretory cells of the **zona fasciculata** are arranged in long anastomosing cords separated by numerous small capillaries. This middle zone, which comprises ~70% of the cortex, is composed of cells that contain abundant cytoplasmic lipid and are responsible for the secretion of the *glucocorticoid hormones*.
- The **zona reticularis** accounts for the remaining 15% of the cortex. The secretory cells are arranged in small groups surrounded by capillaries. This inner layer is responsible for the secretion of *sex steroids* by the adrenal gland.

Cortical cells may project for a short distance into the medulla.

Biosynthesis and action of adrenal cortical hormones

Mineralocorticoids *are adrenal steroids that have their principal effects on ion transport by epithelial cells, resulting in a loss of potassium and conservation of sodium.* The most potent and important naturally occurring mineralocorticoid is *aldosterone*. The enzymatically controlled electrolyte "pumps" in epithelial cells of the renal tubule and sweat glands respond to mineralocorticoids by conserving sodium and chloride and by excreting potassium. In the distal convoluted tubule of the mammalian nephron, a cation exchange mechanism exists for the resorption of sodium from the glomerular filtrate and secretion of potassium into the lumen. These reactions are accelerated by mineralocorticoids but proceed at a much slower rate in their absence. A lack of secretion of mineralocorticoids (such as in idiopathic adrenal atrophy of dogs) may result in a lethal retention of potassium and loss of sodium.

Glucocorticoid hormones secreted by the adrenal cortex are concerned with the intermediary metabolism of glucose. *Cortisol* and lesser amounts of *corticosterone* are the most important naturally occurring glucocorticoids secreted by the adrenal gland in domestic animals. *In general, the actions of glucocortocoids on carbohydrate, protein, and lipid metabolism result in sparing of glucose and a tendency to hyperglycemia and increased glucose production.* The acute effects of glucocorticoids are observed within 15–30 min prior to when the compensatory effects of insulin become prominent. There is a decrease in glucose uptake in adipose tissue, skin, fibroblasts, and lymphoid tissue followed shortly by increased catabolism in these tissues and muscle. This provides the amino acids for gluconeogenesis, which is increased mainly in the liver. In addition, glucocorticoids decrease lipogenesis and increase lipolysis in adipose tissue, which results in release of glycerol and free fatty acids.

Glucocorticoids also function to *suppress inflammatory and immunologic responses* and thereby attenuate the associated tissue destruction and fibroplasia. However, under the influence of high levels of glucocorticoids there is an enhancement of the spread of infections and reduced resistance to a number of bacterial, viral, and fungal diseases. Glucocorticoids may impair the immunologic response at any stage from the initial interaction and processing of antigens by cells of the monocyte–macrophage system through the induction and proliferation of immunocompetent lymphocytes and subsequent antibody production. Inhibition of a number of lymphoid cell functions by glucocorticoids forms part of the basis for suppression of the immunologic response.

Glucocorticoids also *decrease the initial inflammatory reaction* and its classic manifestations of heat, swelling, and pain. The degree of hyperemia, extravasation, cellular migration, and infiltration at the site of injury is decreased. Especially important are the effects of glucocorticoids on the usual vascular responses of increased permeability, diapedesis, and extravasation. Capillary blood flow is decreased and there is less endothelial swelling. In addition, a number of *phagocytic mechanisms are inhibited* by glucocorticoids and clearance of particulate substances from the blood and lymph is impaired. The accumulation of engulfed antigens in macrophages probably is related in part to the *enhanced stability of lysosomal membranes* caused by glucocorticoids. There is a diminished capacity of lysosomes to interact with phagocytized material and to release hydrolytic enzymes involved in intracellular digestion.

Glucocorticoids exert a *profound negative effect on wound healing*. Dogs receiving high therapeutic levels of adrenal corticosteroids or animal patients with hypercortisolism may have wound dehiscence following surgery. The basis mechanism involved is inhibition of fibroblast proliferation and collagen synthesis leading to a decrease in scar tissue formation.

Secretion of **adrenal sex hormones** by cells of the zona reticularis occurs under normal conditions but in minute amounts that probably are of minor physiologic significance. Secretory cells of the inner zone of the cortex synthesize progesterone, estrogens, and androgens. Under pathologic conditions, excessive secretion of adrenal sex steroids infrequently may occur associated with a neoplasm arising in the zona reticularis. The clinical manifestations of virilism, precocious sexual development or feminization depend upon which steroid is secreted in excess, sex of the patient, and the age of onset.

The **renin–angiotensin system** is the major regulator of *aldosterone* production by the zona glomerulosa of the adrenal cortex. Renin is an enzyme secreted into the circulation by cells of the juxtaglomerular apparatus in the kidney. It acts to cleave the plasma globulin, angiotensinogen, to form angiotensin I. This decapeptide is further hydrolyzed to angiotensin II by a converting enzyme. Angiotensin II is both a potent vasoconstrictor and a trophic hormone for the zona glomerulosa of the adrenal cortex, resulting in the synthesis and secretion of aldosterone. It is a very labile peptide that is quickly inactivated in plasma and tissues by angiotensinases.

A number of factors are concerned with the regulation of renin secretion by the kidney. The "short loop" of negative feedback control is the direct inhibition exerted by circulating levels of angiotensin II. The "long loop" of this servo-mechanism is exerted by an indirect feedback inhibition by aldosterone on renin secretion. Renin release and eventually aldosterone secretion are increased by conditions that compromise blood flow and pressure to the kidney, severe dehydration that results in decreased intravascular blood volume, and sodium depletion.

Adrenocorticotropin (ACTH) secreted by the adenohypophysis is the principal regulator of adrenal cortical growth and secretory activity, particularly of cells in the zonae fasciculata and reticularis. The adrenal cortex secretes physiologic quantities of cortisol only in response to ACTH stimulation. ACTH is one of many polypeptide hormones known to exert its action on target cells through receptor-mediated activation of adenylate cyclase and generation of the intracellular mediator 3′,5′-adenosine monophosphate (cyclic AMP). Cyclic AMP serves as the intracellular mediator of ACTH action by stimulating certain key enzymes (e.g., protein kinases) to initiate the biochemical events leading to the biosynthesis of corticosteroid hormones.

Control of the secretion of ACTH by the adenohypophysis is governed by the hypothalamus largely through the secretion of **corticotropin-releasing factor (CRH)**. This peptide is secreted by neurons of the hypothalamus into capillaries that form the *hypothalamic–pituitary portal system* and convey CRH to corticotrophs of the pituitary. Corticotropin-releasing factor acts by stimulating cyclic AMP formation within ACTH-secreting cells resulting in the rapid release of preformed secretory granules containing ACTH.

Negative feedback control of ACTH secretion is exerted primarily by the circulating level of cortisol acting on secretory cells in the hypothalamus or adenohypophysis gland or both. When plasma cortisol levels are elevated beyond the normal physiologic range (as occurs following the exogenous administration or with cortisol-producing adrenal tumor), ACTH secretion is suppressed, secretory cells in the zonae fasciculata and reticularis decrease the rate of synthesis and release of corticosteroid hormones, and the adrenal cortex undergoes trophic atrophy. Conversely, when cortisol levels are subnormal, there is increased release of ACTH from the pituitary gland in an attempt to increase cortisol secretion and return blood levels towards normal.

Diseases of the adrenal cortex

Developmental disturbances of the adrenal cortex

The adrenal glands are composites of steroid hormone-secreting cells of mesodermal origin and catecholamine hormone-secreting cells of neural crest origin that become associated anatomically to varying degrees in different animals. The development of one component does not depend on the development of the other, nor does it depend on the presence or absence of the kidney.

Unilateral agenesis of an adrenal gland occurs occasionally in dogs, most frequently on the left side. **Total agenesis** of the adrenal cortex is fatal in all species due to the inability to produce mineralocorticoids, but the medulla is not essential for life. Developmental anomalies or diseases that decrease or prevent the production or release of ACTH from the fetal pituitary result in hypoplasia or atrophy of the zonae fasciculata and reticularis but not the zona glomerulosa. **Accessory adrenal cortical** tissue is frequent in many species. It may be found in the capsule of adrenal, in the periadrenal or perirenal adipose tissue, and in the mesorchium. Accessory adrenocortical tissue is seen frequently in the vicinity of the equine testis.

Hypoplasia of the adrenal cortex is associated with maldevelopment of the hypophysis associated with anencephaly, in some cases of cyclopia, and in hypophyseal aplasia. The adrenal cortices are small and histologically have nests of cortical cells without distinct zonal development, similar to the arrangement of the fetal adrenal cortex (see Fig. 3.3). The adrenal medulla is normal.

Foci of hematopoietic cells are found incidentally in the adrenal glands and usually are not associated with anemia or other evidence of bone marrow depression or extramedullary myelopoiesis. They are observed most commonly in cattle as round white foci (up to 3–4 mm in diameter) and histologically are composed predominantly of eosinophils. Foci of lymphopoiesis are seen in the cortex of both sheep and cattle and also may be present in the medulla.

Congenital enzyme defects of adrenal cortical steroidogenesis

Several genetically determined enzyme defects in the adrenal cortex of humans interfere with the synthesis of certain cortical steroids and lead to various degrees of hypoadrenocorticism. For example, a lack of 3-hydroxycorticosteroid dehydrogenase blocks the synthesis of cortisol, aldosterone, and adrenal sex steroids. It is uncertain whether any of these congenital enzyme defects occurs in domestic animals.

Rabbits (III VD/J strain) with a congenital (autosomal recessive gene) deficiency of cholesterol-20α -hydroxylase in the adrenal cortex and testis have marked adrenal cortical hyperplasia and feminization at birth. This is a lethal trait and results in death soon after birth. The blockage at an early stage of steroidogenesis in the adrenal cortex interferes with the ability to synthesize cortisol (probably other corticosteroids as well) and leads to a marked accumulation of substrate (e.g., cholesterol) in cortical cells. The marked hyperplasia of the zonae fasciculata and reticularis in the adrenal cortex is a response to the elevated ACTH levels stimulated by the low blood cortisol. The hyperplastic cortical cells are vacuolated and lipid-laden due to the accumulation of cholesterol. The lack of cholesterol-20α -hydroxylase in the testis interferes with the synthesis of androgenic steroids. Inadequate androgen levels during development result in failure of virilization of structures derived from the urogenital sinus and account for the finding of female external genitalia in ~80% of rabbits with the hyperplasia (ah/ah) genotype.

Degenerative changes of the adrenal cortex

Mineralization of the adrenal glands with extensive deposits of calcium salts occurs frequently in adult cats. A 30% incidence has been reported for cats compared with <6% for dogs. Mineralization also is common in adrenal glands of monkeys. *The cause is unknown.* The mineral deposits, although often bilateral and extensive, usually are not associated with clinical signs attributable to hypoadrenocorticism. The mineralized adrenals may be detected at necropsy or during routine radiographic evaluation of the abdomen.

Extensively mineralized adrenal glands are coarsely nodular, firm and mottled with multiple yellow-white foci throughout the cortex and extending into the medulla. They are gritty and difficult to cut. Histologically, there are large areas of necrosis with mineral deposition adjacent to areas of nodular regenerative hyperplasia of remaining viable cortical cells. These hypertrophic cortical cells have abundant lipid in their expanded cytoplasmic area and appear to be able to maintain near normal blood cortisol levels in response to an apparent increased secretion of ACTH.

Capsular sclerosis affects the adrenal glands of old cows often with ovarian follicular cysts. The connective tissue changes often proceed to collagenous and osseous metaplasia in old bulls. There may be an associated moderate reduction in the width of the cortex.

Amyloid deposition in the adrenal glands usually involves the cortex but not the medulla. It occurs in all species and is regularly part of the syndrome of generalized amyloidosis in cattle. Affected adrenal cortices often are widened and the amyloid deposits may be grossly visible as translucent areas. Amyloid deposition begins around the sinusoids in the inner portions of the zona fasciculata and often is largely confined to this zone. Signs of adrenal cortical insufficiency usually do not develop.

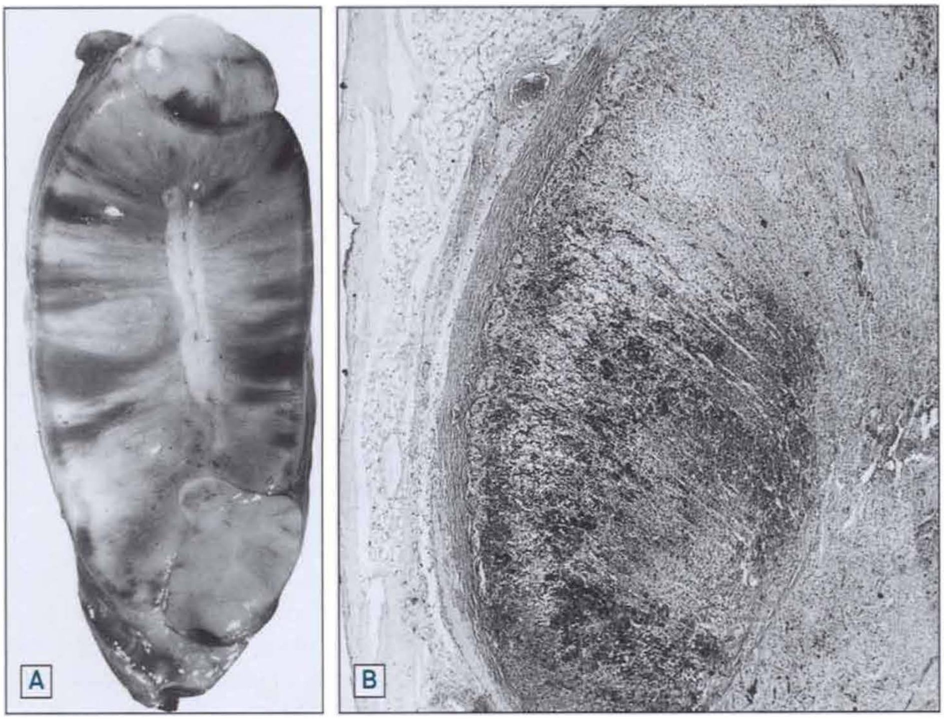

Figure 3.81 A. Multiple hemorrhages in the adrenal cortex in a severely stressed horse. **B.** Necrosis with hemorrhage in adrenal cortex of a lamb with coliform septicemia.

Hemorrhages in the adrenals occur in the newborn of any species. They are presumed to be due to *birth trauma*. Widespread hemorrhage and early degeneration occurs in the adrenal cortex as part of the exhaustion phase of the "*stress response*." It is seen in wild animals that die suddenly during restraint and horses that die from *overexertion* (e.g., struggling to free themselves from being trapped in a fence). *Toxemia* (e.g., intestinal torsion in horses) and *septicemia* also may injure the endothelial lining of adrenal sinusoids and result in extensive cortical hemorrhage (Fig. 3.81A) or the formation of hematomas.

Telangiectasis of the adrenal cortex occurs in middle-aged or older animals as single or multiple dark foci near the corticomedullary junction. These areas typically are depressed on cut surfaces. Areas of telangiectasis appear to develop subsequent to degeneration and loss of cortical cells and ectasia of adrenal sinusoids in the area. Persisting parenchymal cells in these foci either may be hypertrophied and contain much lipid or be small and atrophic.

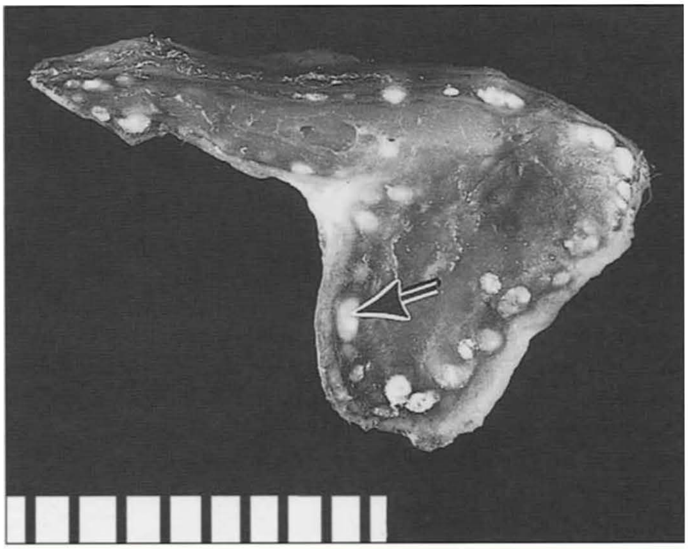

Figure 3.82 Multifocal granulomatous adrenalitis in a dog. The cortices are thin with multiple granulomas (arrow). Scale in mm.

Inflammation of the adrenal cortex (adrenalitis)

Infectious and parasitic agents frequently localize in the adrenal gland and elicit various degrees of inflammation and necrosis. *There is good experimental evidence that the high local concentration of anti-inflammatory steroids in the adrenal cortex suppresses local cell-mediated immunity and permits the preferential progressive growth of certain fungi, protozoa, and bacteria in the adrenal gland.* Focal inflammations usually are suppurative arising in the course of bacterial septicemia. The adrenal capsule provides an effective barrier against direct invasion by inflammatory processes in adjacent tissue. Gram-negative bacteria, especially coliforms, produce suppurative inflammation with necrosis as part of septicemia in many species (Fig. 3.81B). Emboli often lodge in the adrenal sinusoids to produce focal necrosis and suppurative infection. Tuberculosis of the adrenal is encountered mainly in cattle and humans. *Toxoplasma gondii* localizes in the adrenal cortex of many animals and produces focal areas of necrosis with minimal inflammation. Granulomatous inflammation due to *Histoplasma capsulatum*, *Coccidioides immitis*, or *Cryptococcus neoformans* occasionally occurs in dogs and cats in areas where these fungi are endemic. Multiple distinct granulomas with central areas of necrosis and mineralization may nearly destroy the entire cortex (Fig. 3.82). Small nodules of viable hypertrophic cortical cells are present between the granulomas. The reaction to *Cryptococcus* in the adrenal may vary from no reaction to extensive infiltration by macrophages.

Several *viruses* affect the adrenals and focal lesions are expected in herpesviral infections such as pseudorabies. The adrenal lesions are characterized by intranuclear viral inclusion bodies, necrosis, and hemorrhage in the cortex and medulla. Lymphocytes and macrophages infiltrate the necrotic and hemorrhagic foci in the later stages of the infection.

Inflammatory edema of the adrenal glands occurs in older animals with acute infectious diseases. These conditions would be more likely to cause cortical hemorrhages in young animals. The glands are enlarged due to swelling of the cortex from hyperemia and edema. The cortical cells are swollen, granular, depleted of lipid, and dissociated by the inflammatory edema.

Mechanisms of toxicity of the adrenal cortex

Adrenal cortical cells contain large stores of lipid used as substrate for steroidogenesis. Many compounds that are toxic for the adrenal cortex are lipophilic and accumulate in these lipid-rich cells. **Mechanisms of toxicity** include:

- **Impairment of steroidogenesis**, which is an important mechanism of toxicity in the adrenal cortex that can occur by inhibition of cholesterol biosynthesis or metabolism, and by disruption of cytochrome P450 enzymes. Both of these mechanisms will lead to the accumulation of increased cytoplasmic lipid in the form of discrete droplets.
- **Toxin activation by mitochondrial cytochrome P450 (CYP450) enzymes** in the cortical cells. Activation of toxins can result in the generation of reactive oxygen metabolites, result in membrane damage, and produce phospholipidosis in the cells.
- **Exogenous steroids**, which can disrupt normal function and structure of the adrenal cortex. Agonists will induce negative feedback inhibition of ACTH secretion by the pituitary and will result in atrophy of the zonae fasciculata and reticularis. Some steroids, such as the sex steroids, can induce proliferative lesions in the adrenal cortex. Antagonists will block steroid hormone action, lead to increased ACTH secretion, and diffuse hyperplasia of the cortex.
- **DNA damage,** by agents such as carcinogens or radiation, may induce neoplasia of the adrenal cortex.

There is considerable *species variation* in the response of the adrenal cortex to exogenous chemicals. This is due to both inherent differences in the sensitivity to certain drugs and differences in the metabolic pathways of steroidogenesis. An interesting example is o,p′-DDD (mitotane) which was originally developed to treat metastatic adrenal cortical cancer in humans. However, humans are relatively insensitive to the effects of o,p′-DDD and the drug was not effective in the treatment of adrenal cancer. In contrast, dogs are considerably more sensitive to the effects of o,p′-DDD and it has been used effectively in a dose-dependent manner in the medical management of pituitary-dependent hyperadrenocorticism due to autonomous secretion of ACTH by pituitary (corticotroph) tumors. o,p′-DDD is a selective toxin for the zona fasciculata and reticularis; thereby, sparing the important life-sustaining functions of the zona glomerulosa and production of aldosterone.

Classes of chemicals known to be toxic for the adrenal cortex include short chain (3 or 4 carbon) aliphatic compounds, lipidosis inducers, and amphiphilic compounds. A variety of other compounds also may affect the medulla. The most potent aliphatic compounds are of 3-carbon length with electronegative groups at both ends. These compounds frequently produce necrosis, particularly in the zonae fasciculata and reticularis. Examples include acrylonitrile, 3-aminopropionitrile, 3-bromopropionitrile, 1-butanethiol, and 1,4-butanedithiol. By comparison, lipidosis inducers can cause the accumulations, often coalescing, of neutral fats that may be of sufficient quantity to cause reduction or loss of organellar function and eventual cell destruction. Cholesterol is the precursor substrate required to synthesize steroid hormones. Steroidogenic cells obtain cholesterol exogenously from serum lipoproteins and endogenously from de novo synthesis via the acetyl coenzyme A pathway (Fig. 3.83A). The adrenal cortical cells and ovarian interstitial (OI) cells in the rat preferentially utilize serum high-density lipoproteins (HDLs) for their primary source of cholesterol and resort to de novo synthesis if HDL does not meet the demand of steroidogenesis. This is in contrast to Leydig cells of the testis, which preferentially utilize de novo synthesis of cholesterol and use an exogenous source only when intracellular synthesis does not meet the demand and the cholesterol pool has been depleted.

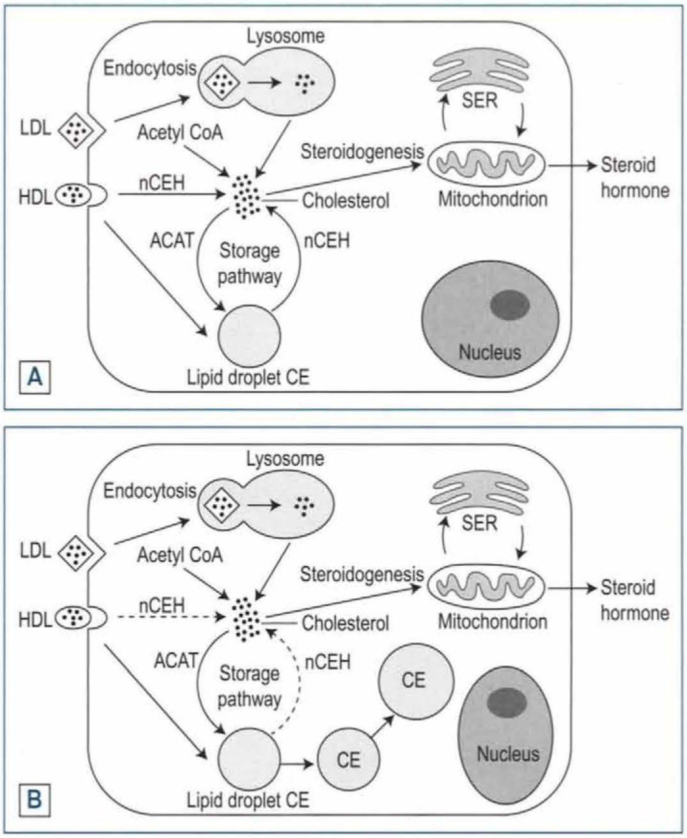

Figure 3.83 A. Cholesterol metabolism and steroid hormone biosynthesis in adrenocortical cells. Cholesterol is the substrate for steroid hormone biosynthesis. Conversion of cholesterol to pregnenolone occurs in the mitochondria, and oxidative reactions catalyzed by P450 enzymes occur in the smooth endoplasmic reticulum (SER) and mitochrondia. Sources of cholesterol include lipoprotein (LDL and HDL) uptake from serum, de novo synthesis from acetate via the acetyl coenzyme A pathway, and hydrolysis of cholesteryl ester (CE) by neutral CE hydrolase (nCEH). The storage pool in the form of lipid droplets is derived principally from the conversion of free cholesterol to CE catalyzed by acyl coenzyme A:cholesterol acyltransferase (ACAT). **B. Pathogenesis of cholesteryl lipidosis in adrenocortical cells.** Inhibition of nCEH by a xenobiotic can result in the accumulation of CE in the form of lipid droplets in the cytoplasm. The levels of ACAT that catalyze the formation of CE from cholesterol activity remain near normal. (From Latendresse JR, et al. Toxicol Appl Pharmacol 1993;122:281–289.)

The zonae reticularis and fasciculata appear to be the principal targets of xenobiotics in the adrenal cortex. Examples of the compounds causing lipidosis include aminoglutethimide, amphenone, and anilines. Tricresyl phosphate (TCP) and other triaryl phosphates cause a defect in cholesterol metabolism by blocking both the uptake from serum and storage pathways. An inhibition of cytosolic neutral cholesteryl ester hydroxylase (nCEH) by triaryl phosphate results in the progressive accumulation of cholesteryl ester in the form of lipid droplets in the cytoplasm of adrenal cortical and OI cells (Fig. 3.83B) but not in testicular Leydig cells of rats. Acyl coenzyme A: cholesterol acyl transferase (ACAT), an enzyme that esterifies cholesterol to make cholesteryl ester, was depressed to a lesser extent but contributed to the elevated intracellular storage of cholesterol in the form of lipid droplets by the triaryl phosphate (Fig. 3.83B).

Biologically active cationic amphiphilic compounds produce generalized phospholipidosis that involves primarily the zonae reticularis and fasciculata and results in the development of microscopic phospholipid-rich inclusions. These compounds affect the functional integrity of lysosomes, which appear ultrastructurally to be enlarged and filled with membranous lamellae or myelin figures. Examples of compounds known to induce phospholipidosis include chloroquine, triparanol, and chlorphentermine.

Adrenal lesions associated with hypoadrenocorticism

Idiopathic adrenocortical atrophy. *The most frequently observed lesion in dogs with* **hypoadrenocorticism** *is bilateral idiopathic adrenocortical atrophy in which all layers of the cortex are markedly reduced in thickness* (Fig. 3.84A, B). There usually is deficient production of all classes of corticosteroids (mineralocorticoids, glucocorticoids, and adrenal sex steroids). The adrenal cortex is reduced to one-tenth or less its normal thickness and consists primarily of the adrenal capsule. The adrenal medulla is relatively more prominent and with the capsule makes up the bulk of the remaining adrenal glands. The pathogenesis of idiopathic adrenocortical atrophy is unknown, but it has been *proposed that the lesion is immune-mediated.* Multiple foci of lymphocytes and plasma cells often are seen interspersed between adrenal sinusoids and groups of fibroblasts in the earlier stages of the disease. The entire three zones of the cortex are nearly absent in dogs that die from untreated hypoadrenocorticism. The capsule appears thickened due to collapse of the adrenal cortex plus fibroblastic proliferation.

No obvious pituitary lesions other than compensatory hyperplasia of corticotrophs have been observed in dogs with idiopathic adrenal cortical atrophy. All zones of the adrenal cortex are involved, including the zona glomerulosa, which is not under ACTH control. In comparison, trophic atrophy of the adrenal cortex secondary to a destructive pituitary lesion that decreases ACTH secretion is characterized by severe atrophy only of the inner two cortical zones. These animals do not have the characteristic electrolyte abnormalities present in hypoadrenocorticism since the secretion of aldosterone remains within normal limits.

Causes of adrenocortical insufficiency in the dog include granulomatous inflammation caused by such diseases as histoplasmosis, blastomycosis or tuberculosis that destroy the cortex; thrombosis of adrenal vessels with infarction of the cortex associated with disseminated intravascular coagulopathies; invasion and destruction of adrenal cortex by metastatic neoplasms; hemorrhage and necrosis

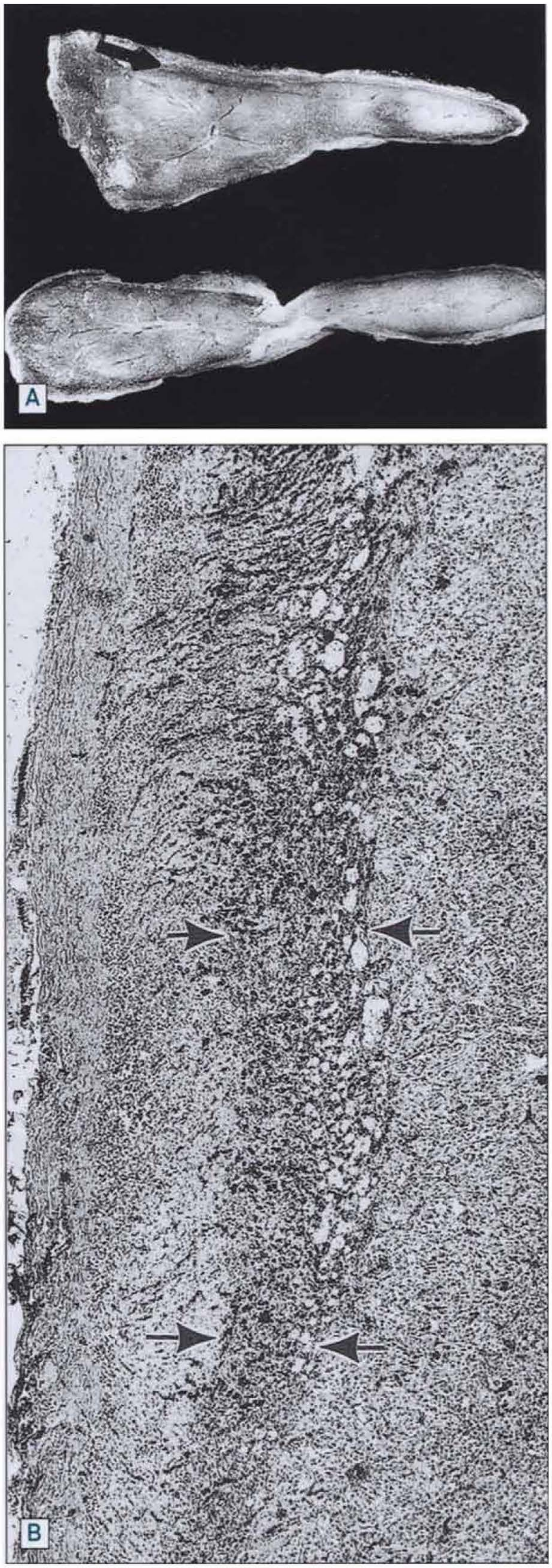

Figure 3.84 A. Idiopathic adrenal cortical atrophy in a dog. All three layers of the adrenal cortex from both glands are markedly reduced in thickness (arrow) due to atrophy as a sequela of immune-mediated adrenalitis and destruction of parenchymal cells. **B.** Severe adrenal cortical atrophy in a dog with hypoadrenocorticism. Medulla lies between arrows.

with subsequent replacement by fibrous connective tissue; and extensive amyloid deposition along adrenal sinusoids.

Many of the *functional disturbances* of hypoadrenocorticism associated with chronic adrenal insufficiency are not specific and include recurrent episodes of gastroenteritis, slowly progressive loss of body condition, and failure to respond appropriately in stressful situations such as minor illnesses or surgery. Less commonly, the dog is presented in a shock-like coma with no history of previous illness. Although hypoadrenocorticism occurs in dogs of any breed or sex and at any age, idiopathic adrenocortical insufficiency occurs more frequently in *young adult dogs*. This may be related to its likely immune-mediated pathogenesis with the generation of autoantibody against cortical cells, progressive degeneration of parenchymal cells, and lymphoplasmacytic adrenalitis.

Reduction in the synthesis and secretion of mineralocorticoids, primarily aldosterone, results in marked alterations of serum potassium, sodium, and chloride levels. Less potassium is excreted by the kidney, resulting in a progressive rise in serum potassium levels and *severe hyperkalemia* (5.5–9.0 mmol/L, 5.5–9.0 meq/L). Less sodium and chloride are reabsorbed from renal tubules, leading to various degrees of hypernatriuria and hyperchloruria and a corresponding decline in blood levels. The severe hyperkalemia frequently produces marked cardiovascular disturbances that are reflected by changes in the electrocardiogram.

Although the development of clinical signs often is insidious and not readily apparent, the dog frequently is presented with *acute circulatory collapse* and evidence of *renal failure*. Progressive decrease in blood volume contributes to hypotension, weakness, and microcardia. Peripheral circulatory collapse may result from progressive hemoconcentration. Increased excretion of water by the kidney, due to hyponatremia and hypochloremia, results in progressive dehydration and hemoconcentration. *Emesis, diarrhea, and anorexia* are common in dogs with hypoadrenocorticism and they contribute to the animal's deterioration. *Weight loss* is frequently severe.

Decreased production of glucocorticoids results in several characteristic functional disturbances of hypoadrenocorticism. Failure of gluconeogenesis and increased sensitivity to insulin contribute to development of *moderate hypoglycemia*. *Hyperpigmentation* of the skin occurs in some dogs with long-standing adrenocortical insufficiency. This change results from lack of negative feedback on the pituitary gland, increased release of ACTH, and "specificity spillover" with binding to melanocyte-stimulating hormone (MSH) receptors on melanocytes in the skin.

Peripheral lymph nodes may be moderately increased in size due to *lymphoid hyperplasia*. Histologically, prominent germinal centers are observed in lymph nodes, and substantial numbers of eosinophils may infiltrate the peripheral lymph sinus. There often are increased numbers of eosinophils and lymphocytes in the circulation as a result of the diminished cortisol levels.

Hematologic alterations that occur in hypoadrenocorticism include *hemoconcentration* due to dehydration and loss of intravascular fluid volume. In some dogs, the numbers of eosinophils and lymphocytes in the blood are not increased above normal, but the differential count is not consistent with the animal's severely "stressed" appearance. Blood urea and creatinine levels begin to rise as renal perfusion and urine output decrease. The serum glucose level ranges from low normal to moderately subnormal in affected dogs, but this usually is not responsible for functional disturbances.

Hypercalcemia (up to 3.75 mmol/L, 15 mg/dL) occurs in approximately 30% of dogs with hypoadrenocorticism. The elevated serum calcium concentration returns to normal when the adrenal cortical disease is treated with adequate corticosteroid replacement therapy. The mechanism of hypercalcemia is not understood completely, but the ionized fraction of serum calcium remains normal whereas the nonionized fraction increases and there is inappropriate hypocalciuria for the degree of hypercalcemia. Since the ionized serum calcium concentration is not increased, the hypercalcemia often is not deleterious to the animal; however, some of the clinical signs of hypoadrenocorticism (vomiting, anorexia, polyuria, and polydipsia) are similar to those of hypercalcemia.

The *plasma cortisol levels* in dogs with hypoadrenocorticism range from 28 to 41 nmol/L (0.1–1.5 μg/dL). Due to the severe atrophy or destruction of the adrenal cortex, there is little or no increase in blood cortisol levels following administration of exogenous ACTH.

Carbadox (Mecadox) is a synthetic antibacterial agent and growth promotant belonging to the family of quinoxaline-di-*N*-oxide compounds. It has been used in young pigs as a growth promotant at 50 ppm in feed and as a preventive or therapeutic agent for diarrhea up to 100–150 ppm. Exposure to 150–200 ppm of carbadox in the feed for 2–3 weeks or 100 ppm for 4–5 weeks can produce a disease in pigs that is characterized by growth retardation and dehydration associated with *lesions in the adrenal zona glomerulosa* and in the kidney. Prolonged exposure to even 50 ppm can produce disease, the only safe level being 25 ppm or less. The related compounds olaquindox and cyadox have similar effects to carbadox but the latter drug is somewhat less toxic.

The adrenal changes consist of disorganization of the zona glomerulosa with loss of distinction from the zona fasciculata. The cells of the glomerulosa develop hydropic degeneration and eventually there is atrophy, mild diffuse fibrosis and mononuclear cell infiltrates in the zone and fibrosis of the capsule, which consistently contains cells with PAS-positive cytoplasmic granules. Depending on duration and severity of exposure, adrenal lesions may persist following removal of the drug. In the kidney, there is degeneration and desquamation of epithelium in the collecting tubules of the medulla and in the pelvic epithelium. Plasma aldosterone and sodium levels are reduced significantly after 2 weeks exposure to toxic levels of carbadox, and potassium is increased.

Hyperplasia of the adrenal cortex

Accessory cortical nodules are common in the adrenal glands of adult to aged animals and are found in the capsule, cortex, and medulla (Fig. 3.85A). Many accessory cortical nodules either arise as evaginations of outer cortex into the capsule and surrounding periadrenal fat or invaginations of the cortex into the medulla.

Nodular hyperplasia (measuring from <1.0 to 2.0 cm) also is common in the adrenal as well-defined spherical nodules in the cortex or attached to the capsule. Hyperplastic nodules are usually multiple and bilateral, yellow, and may involve any of the three zones of the cortex. Histologically, the nodules near the capsule *resemble zona glomerulosa*, sometimes with areas like zona fasciculata. The cells either are about the same size as those of the adrenal cortex or hypertrophied. The lipid content in these hyperplastic nodules is not depleted in circumstances that reduce the amount of lipid in the rest of the

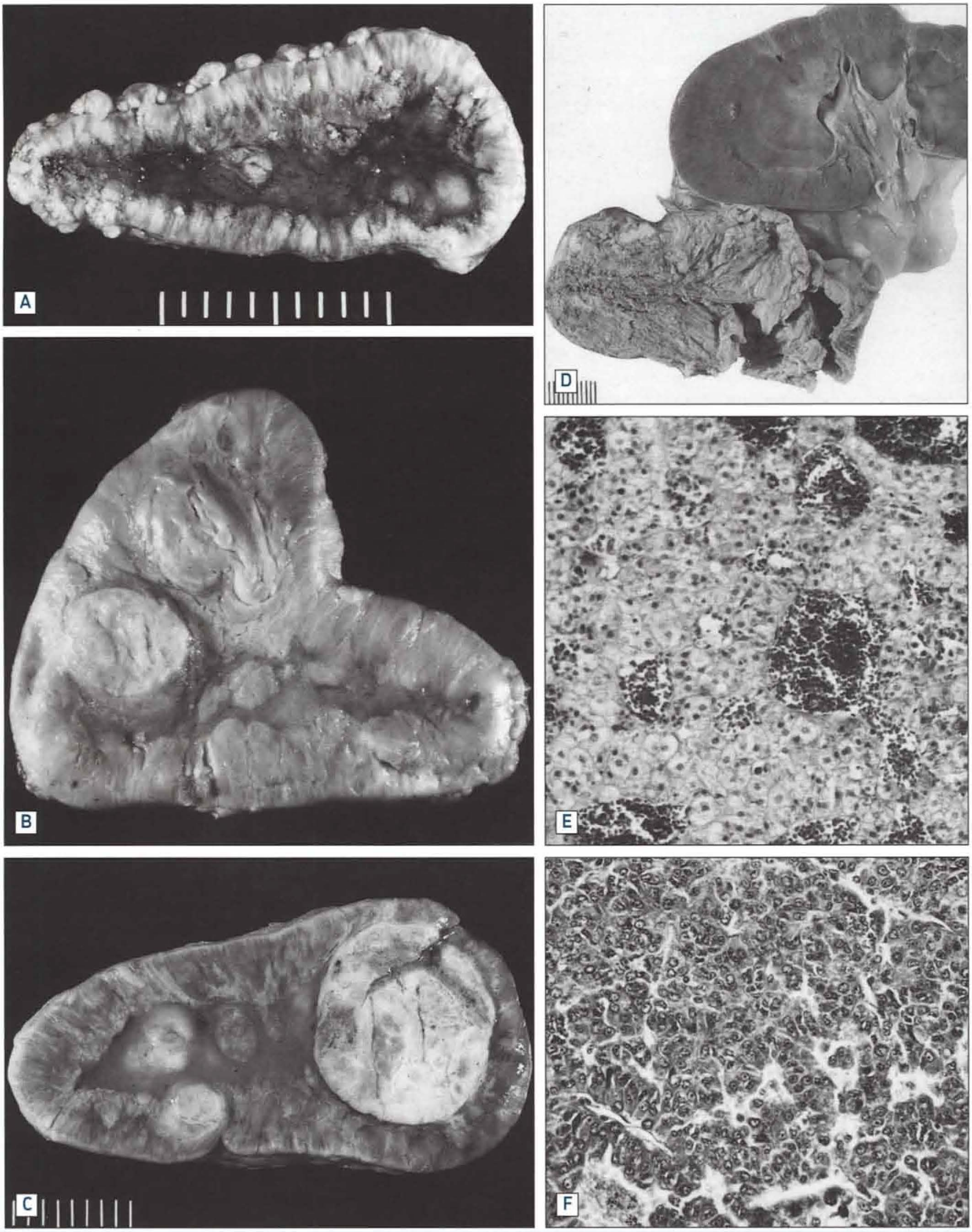

Figure 3.85 A. Discrete **accessory adrenal cortical nodules** in capsule and medulla in a dog. **B.** Discrete area of **nodular hyperplasia** in a diffusely hyperplastic adrenal cortex. Dog with pituitary corticotroph adenoma and hypercortisolism. **C. Cortical adenoma** (right) in an adrenal with multinodular hyperplasia (left) from a dog. **D.** Functional **adrenal cortical carcinoma** in a dog with the syndrome of cortisol-excess. The adrenal gland was completely incorporated within the mass at the cranial pole of the kidney (top). **E. Adrenal cortical adenoma** with hematopoiesis. **F. Adrenal cortical carcinoma** from a dog. Scale in mm in (**A**), (**C**), (**D**).

cortex. Hyperplastic cortical nodules are most common in *older horses, dogs, and cats*.

Nodular hyperplasia of the zona reticularis may appear as discrete foci of cortical parenchyma extending into the medulla and result in an irregular corticomedullary junction. This adrenal lesion has been seen in animals with functional disturbances suggesting androgen excess (e.g., greater muscle mass, well-developed crest, hypertrophy of clitoris, and involution of mammary glands).

Diffuse cortical hyperplasia results in uniform, usually bilateral, enlargement of the adrenal cortex. There is a marked hypertrophy and hyperplasia of cells in the zonae fasciculata and reticularis in response to autonomous hypersecretion of ACTH by a corticotroph adenoma of the pituitary. The hyperplastic cells in the zona fasciculata are vacuolated (lipid-rich) and arranged in straight columns separated by sinusoids. Cells in the outer zona glomerulosa often are compressed by the expanded inner two zones. The corticomedullary junction is irregular in diffuse hyperplasia, and projections of cortical cells often extend into the medulla. Areas of discrete nodular hyperplasia may be present in a diffusely hyperplastic cortex (Fig. 3.85B).

Hyperplasia of zona glomerulosa occurs as part of the compensatory mechanism of the body to a variety of factors that stimulate long-term release of renin from the juxtaglomerular apparatus of the kidney. Chronic sodium depletion or potassium excess, decreased blood volume to the kidney, or a sustained reduction in systemic blood pressure all will increase aldosterone production by stimulating renin release. Renin results in the formation of angiotensin II, a trophic hormone for the zona glomerulosa of the adrenal cortex.

Neoplasms of the adrenal cortex

Myelolipoma

This is a *benign lesion* encountered in the adrenals of cattle, nonhuman primates, and infrequently in other animals. It is composed of accumulations of well-differentiated adipose connective tissue cells and hematopoietic tissue, including both myeloid and lymphoid elements. Focal areas of mineralization or bone formation may occur in the tumor. Although the origin of these nodular aggregations of fat, bone and myeloid cells is uncertain, they appear to develop by *metaplastic transformation* of cells in the adrenal cortex.

Cortical adenoma

Adenomas of the adrenal cortex are seen most frequently in *old dogs* (8 years and older) and sporadically in horses, cattle, and sheep. They usually are encountered as *incidental findings* at autopsy. Castrated male goats are reported to have a higher incidence of cortical adenomas than intact males.

Cortical adenomas are well-demarcated, usually single, nodules in one adrenal gland, but may be bilateral. Larger cortical adenomas are yellow to red, distort the external contour of the affected gland, and are partially or completely encapsulated. Adjacent cortical parenchyma is compressed and the tumor may extend into the medulla.

Discrete cortical adenomas often develop in an adrenal with multiple areas of nodular hyperplasia (Fig. 3.85C). Smaller cortical adenomas are more yellow and similar in color to the normal adrenal cortex because of the high lipid content. They are surrounded on all sides by mildly compressed cortex with attempts at encapsulation, and may be difficult to distinguish from nodular cortical hyperplasia in old dogs. However, nodular hyperplasia consists of multiple small foci usually in both adrenals with no evidence of encapsulation and often is associated with extracapsular nodules of hyperplastic cortical tissue.

Cortical adenomas are composed of well-differentiated cells that resemble secretory cells of the normal zonae fasciculata or reticularis. Tumor cells are arranged in broad trabeculae or nests separated by small vascular spaces. The abundant cytoplasmic area of tumor cells is lightly eosinophilic, often vacuolated, and filled with many lipid droplets. Adenomas are partially or completely surrounded by a fibrous connective tissue capsule of variable thickness and a rim of compressed cortical parenchyma. Focal areas of mineralization, hematopoiesis (Fig. 3.85D), and accumulations of fat cells may be found in cortical adenomas.

Cortical carcinoma

Adrenal cortical carcinomas occur less often than adenomas and have been reported most often in *cattle* and *older dogs*, but infrequently in other species. Carcinomas develop in adult to older individuals, and there is no apparent breed or sex predisposition.

Adrenal carcinomas are larger than adenomas and more likely to be bilateral. In dogs, they are composed of variegated, yellow-red, friable tissue that incorporates the affected adrenal gland (Fig. 3.85E). They are often fixed in location because of extensive invasion of surrounding tissues with *extension into the caudal vena cava*, forming large tumor-cell thrombi. Carcinomas may attain considerable size in cattle (up to 10 cm or more in diameter) and have multiple areas of mineralization or ossification.

Carcinomas are composed of more highly pleomorphic secretory cells than adenomas that are subdivided into small groups by fibrovascular stroma of variable thickness. The architecture of the affected adrenal usually is completely obliterated by the carcinoma. The pattern of growth varies between individual tumors and within the same carcinoma. Tumor cells usually are large and polyhedral with prominent nucleoi and densely eosinophilic or vacuolated cytoplasm (Fig. 3.85F). Areas of hemorrhage within the tumors are common because of rupture of thin-walled sinusoids.

Carcinomas and adenomas of the adrenal cortex in dogs occasionally are functional and secrete excessive amounts of hormone, usually cortisol. The clinical picture of adrenal cortical carcinoma may be complicated by compression of adjacent organs by the large tumor, invasion into the aorta or caudal vena cava leading to intra-abdominal hemorrhage, and metastases to distant sites.

Functional cortical adenomas and carcinomas are associated with atrophy of the contralateral cortex because of negative feedback inhibition of pituitary ACTH secretion by the elevated blood cortisol levels. The atrophic cortex consists primarily of the adrenal capsule and zona glomerulosa. Few secretory cells remain in the zonae fasciculata and reticularis. A similar atrophy may be present in the remnants of compressed adrenal cortex around functional adenomas. The adrenal medulla in the contralateral gland appears expanded and relatively more conspicuous because of the lack of cortical parenchyma.

Hypercortisolism

The manifestations and lesions associated with hyperadenocorticism, mainly in dogs, result from long-term overproduction of cortisol by hyperactive adrenal cortices. As a result of the cortisol excess,

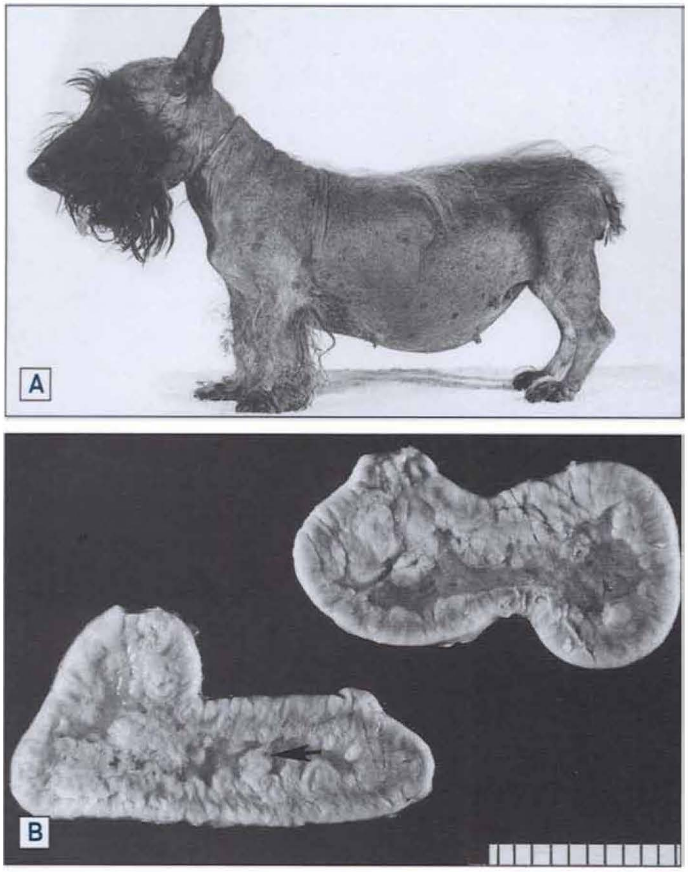

Figure 3.86 Hypercortisolism in a dog. **A.** Hypercortisolism with alopecia extending over most of the body. The skin is thin, wrinkled, and hyperpigmented. Muscle asthenia is evident from the pendulous abdomen and swayed back. **B.** Idiopathic adrenocortical hyperplasia with bilateral cortical thickening and irregularity of corticomedullary junction (arrow). Scale in mm.

affected dogs develop a spectrum of functional disturbances and lesions from the combined gluconeogenic, lipolytic, protein catabolic, and anti-inflammatory effects of the glucocorticoid hormones on many organs. The course of the disease is insidious and slowly progressive. *Cortisol excess is one of the most frequent endocrinopathies in adult to aged dogs*, occurs occasionally in cats, but is encountered infrequently in other species of domestic animals.

The elevation in circulating cortisol levels in dogs with hyperadrenocorticism may result from one of several different pathogenetic mechanisms. The most common mechanism is a *functional corticotroph (ACTH-secreting) adenoma of the pituitary gland* (pars distalis or pars intermedia in dogs) that causes bilateral adrenal cortical hypertrophy and hyperplasia (see Fig. 3.10). Hypercortisolism associated with *idiopathic adrenal cortical hyperplasia* occurs most frequently in poodles and occasionally in other breeds (Figs 3.86A, 3.87). The cortex of both adrenal glands is thickened considerably owing to diffuse or nodular hyperplasia of secretory cells, primarily in the zona fasciculata, and the corticomedullary junction is irregular (Figs. 3.86B, 3.87E).

Functional adrenal neoplasms are infrequent (10–15% of cases) causes of the syndrome of cortisol excess in the dog. Many of the clinical signs and lesions of naturally occurring hyperadrenocorticism can be induced by the long-term, daily administration of large doses of corticosteroids for the treatment of other diseases.

The appetite and intake of food often are increased as a direct result of either the hypercortisolism or the destruction of hypothalamic centers that control appetite by a dorsally expanding pituitary tumor. The muscles of the extremities and abdomen are weakened and atrophied. The loss in tone of the abdominal muscles and muscles of the appendicular skeleton results in gradual abdominal enlargement, lordosis, muscle trembling, and a straight-legged, skeletal-braced posture (Fig. 3.86A). Profound atrophy of the temporal muscles may result in obvious concave indentations and readily palpable prominences of underlying skull bones. Hepatomegaly due to increased fat and glycogen deposition may contribute to the development of the distended, often pendulous, abdomen. The muscular asthenia and wasting is the result of increased catabolism of structural proteins, combined with diminished protein synthesis, under the influence of long-term cortisol excess.

Skin lesions occur in a high percentage of dogs with hypercortisolism. The initial changes in the skin often are observed over points of wear such as neck, flanks, behind the ears, and over bony prominences. These initial skin changes spread in a bilaterally symmetrical pattern to involve a significant percentage of the body surface. The basic skin lesion of cortisol excess is atrophy of the epidermis and pilosebaceous apparatus, combined with loss of collagen and elastin fibers in the dermis and subcutis (Fig. 3.87D). The skin is of fine texture and often paper-thin. Most of the hair follicles are inactive and in the telogen phase. Accumulation of keratin in the atrophic hair follicles disrupts the base of attachment of the hair shaft, thereby permitting easy dislodgement from the follicle. The epidermis usually is markedly thinned and consists of only one or two cell layers derived from the basilar stratum germinativum. However, the stratum corneum is considerably thickened owing to the accumulation of multiple layers of keratin on the surface (Fig. 3.88).

Cutaneous mineralization is a characteristic lesion in dogs with hypercortisolism, but occurs only in long-standing cases. Although mineral deposition may occur in any region of the body, the dorsal midline, ventral abdomen, and inguinal region are frequently affected sites. Numerous mineral crystals are deposited along collagen and elastin fibers in the dermis and may protrude through the atrophic and thinned epidermis (Fig. 3.87B). The epidermis remains intact in less severe cases and appears irregularly elevated by the opaque white deposits of mineral. A narrow rim of hyperemia and foreign-body granulomatous inflammation often surrounds the areas of mineralization. The mineral deposits occur in dogs with normal blood calcium and phosphorus levels. The pathogenesis of this manifestation of hypercortisolism most likely is related to the gluconeogenic and protein catabolic action of cortisol, resulting in the rearrangement of the molecular structure of proteins such as collagen and elastin, and the formation of an organic matrix that attracts and binds calcium.

Severe mineralization also occurs in several other tissues. The lungs are most frequently affected (>90%) (Fig. 3.87A) but active skeletal muscles (Fig. 3.87C) and the wall of the stomach also may have extensive areas of mineralization. Large, basophilic mineral crystals are deposited in interalveolar and bronchiolar walls with little accompanying inflammation. Pulmonary function may be compromised when mineralization is extensive.

The syndrome of long-term cortisol excess often is complicated by *increased susceptibility to bacterial infections of the skin, urinary tract, conjunctiva, and lung.* Multifocal areas of suppurative folliculitis and

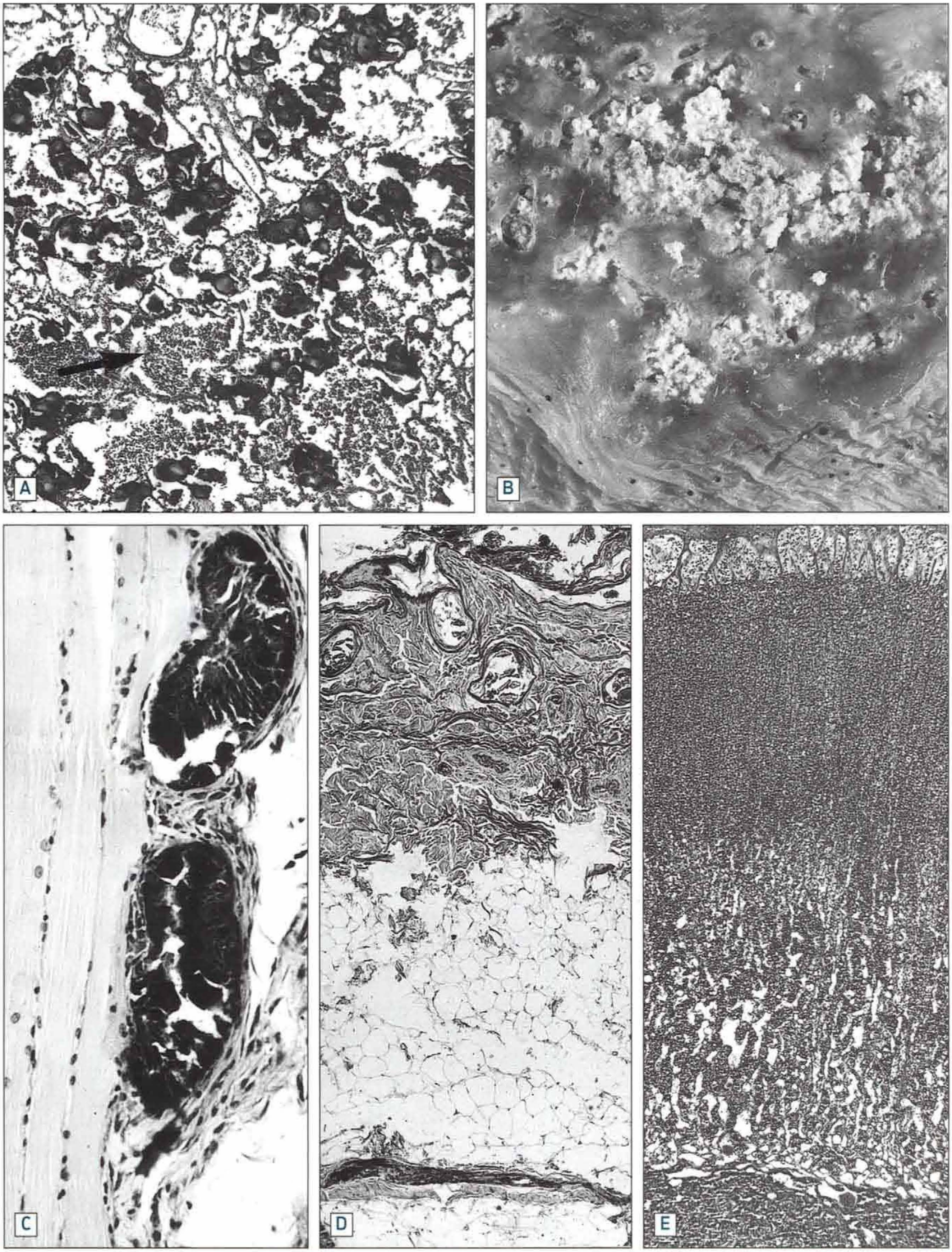

Figure 3.87 Hypercortisolism in a dog. **A.** Pulmonary mineralization and suppurative bronchopneumonia (accumulation of leukocytes, arrow). **B.** Severe mineralization of the skin. Note empty hair follicles and mineral crystals protruding through atrophic epidermis. **C.** Mineralization of connective tissue between muscle bundles. **D.** Atrophy of epidermis with accumulation of layers of keratin and follicular keratosis, and loss of collagen and elastin fibers in subcutis. **E.** Hyperplasia of zonae fasciculata and reticularis in adrenal cortex of a dog with a corticotroph adenoma of the pituitary. Outer zona glomerulosa (top) is approximately normal thickness or slightly compressed.

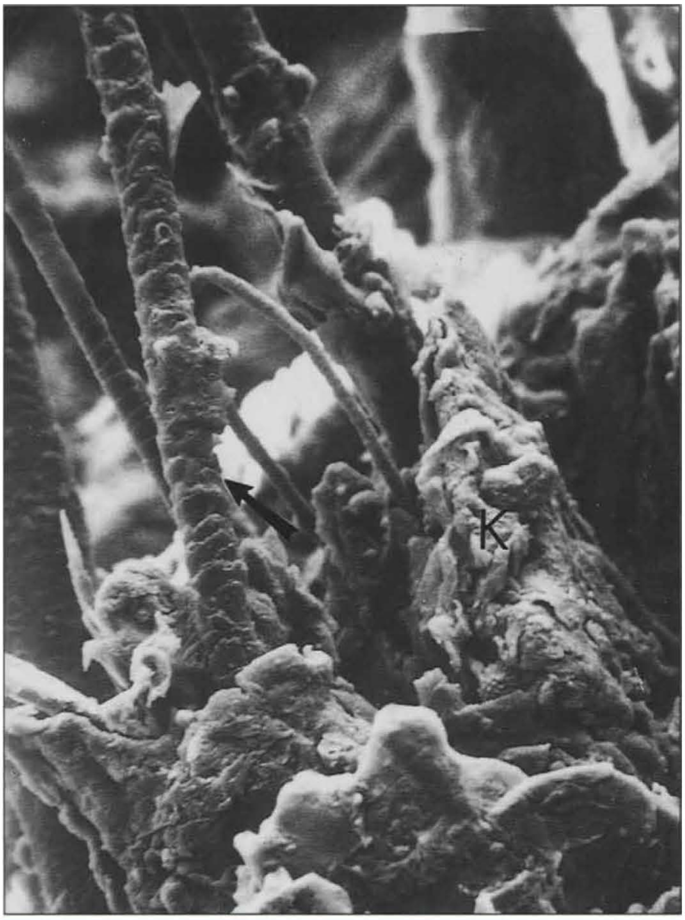

Figure 3.88 Skin of a dog with **hypercortisolism**. The skin surface is rough and scaly due to the accumulation of interlapping plates of keratin (K) in the stratum corneum. A few hair shafts (arrow) are emerging from the hair follicles. Scanning electron micrograph.

dermatitis develop near the lip folds and footpads and elsewhere in the skin. Suppurative bronchopneumonia frequently is a serious, life-threatening, complication since it develops in lungs whose function is already partially impaired by extensive mineralization of alveolar walls and terminal bronchioles.

The consistent effects that elevated blood levels of corticosteroids have on the hematopoietic system may help establish a diagnosis of adrenal cortical disease. Involution of lymphatic tissue results in significant *lymphopenia*. There is intravascular destruction of eosinophils or sequestration of circulating eosinophils in the lung and spleen, which leads to *eosinopenia*. An excess of corticosteroids can cause neutrophilia and hence leukocytosis by stimulating the formation of neutrophils and decreasing the migration of these cells into areas of infection. The nuclei of neutrophils in dogs and cats with naturally occurring or iatrogenic hyperadrenocorticism may become hypersegmented as a reflection of their prolonged lifespan in the circulation.

Laboratory confirmation *of the syndrome of cortisol excess is accomplished by a combination of suppression and stimulation tests of the hypothalamic–pituitary–adrenocortical axis.* A single blood cortisol determination usually is not diagnostic due to the wide range of normal in "stressed" dogs in an abnormal environment (such as a veterinary hospital). Low doses of dexamethasone (15 μg/kg) suppress ACTH production and subsequently plasma cortisol levels in normal dogs, but do not suppress cortisol levels in dogs with pituitary-dependent hyperadrenocorticism or functional adrenal cortical neoplasms. High doses of dexamethasone (1.0 mg/kg) usually suppress plasma cortisol levels in dogs with pituitary-dependent hyperadrenocorticism but do not significantly influence levels in dogs with cortisol-producing adrenal cortical neoplasms. Radioimmunoassays developed to measure plasma ACTH in the dog have demonstrated a mean concentration of 10 pmol/L, range 3.7–21.6 pmol/L (45.8 pg/mL, range 17–98 pg/mL). Assays for plasma ACTH are useful in differentiating pituitary-dependent (elevated) and adrenal-dependent (suppressed) causes of the syndrome of cortisol excess. Dogs with functional adrenal cortical neoplasms have plasma ACTH concentrations two standard deviations or more below the mean value for normal dogs.

Dogs with the syndrome of cortisol excess caused by diffuse cortical hyperplasia (corticotroph adenoma of pituitary or idiopathic adrenal hyperplasia) can be managed medically by the oral administration of the drug *ortho,-para*′-2,2-bis (2-chlorophenyl-4-chlorophenyl)-1,1-dichlorethane (**o,p′-DDD**, mitotane). The drug is an isomer of the insecticide DDD and is selectively cytotoxic, at the recommended dosage, for the zonae fasciculata and reticularis of the canine adrenal cortex. In dogs, the cells of these zones are highly susceptible to the cytotoxic effects of o,p′-DDD. After 5–10 days of daily treatment (50 mg/kg), the secretory cells of the inner two zones are swollen, vacuolated, and undergo necrosis. Mitochondria are vacuolated, profiles of smooth endoplasmic reticulum are disrupted, and lipid accumulates in secretory cells of the zona fasciculata after o,p′-DDD administration. Necrosis is followed progressively by collapse of the two inner zones, reduction in cortical thickness, and dilation of vascular sinusoids. After several months of treatment with o,p′-DDD, the cortex is markedly thinned and consists of an intact zona glomerulosa surrounded by a fibrous capsule. The levels of plasma cortisol decline progressively in dogs with hyperadrenocorticism treated with o,p′-DDD. The exaggerated increase in plasma corticosteroids in response to ACTH stimulation in dogs with adrenal cortical hyperplasia is eliminated after treatment with o,p′-DDD. The weekly doses of o,p′-DDD must be continued for the life of the dog in order to prevent recurrence of the functional disturbances and lesions of hypercortisolism. If the intermittent doses of o,p′-DDD are discontinued, small nests of viable cortical cells which remain in the zonae fasciculata and reticularis will regenerate the pretreatment diffuse cortical hyperplasia, with the return of the functional disturbances and the biochemical alterations. Dose levels of o,p′-DDD that are effectively cytotoxic to hyperplastic adrenal cortices usually will not destroy functional adrenal cortical adenomas and carcinomas in the dog unless high doses of drug are used. Higher dose levels of the drug can be toxic to several parenchymal organs.

Adrenal tumors in ferrets

Adrenal tumors are being recognized with greater frequency in ferrets as they are being kept as household pets and living to a more advanced age. The adrenal enlargements are either bilateral (approximately 45%) due to diffuse (most frequent) or nodular hyperplasia, or unilateral (approximately 55%) due to adrenal cortical carcinoma (more common) or cortical adenoma. Clinical signs in ferrets with adrenal cortical tumors include *vulvar enlargement*, *bilaterally symmetrical alopecia* especially on the ventral abdomen and medial aspects of the rear legs, *polyuria*, *polydipsia*, and the presence of a *palpable mass at the cranial pole of the kidney* (left side has a greater frequency than the right).

Adrenal tumors develop in adult-aged ferrets (mean age 5 years) with females more frequently affected than males (sex ratio of 2:1 or greater). The history frequently indicates the ferrets were gonadectomized at an early age (5 or 6 weeks). Other functional disturbances including anemia, thrombocytopenia, pyometra, and endometrial hyperplasia are consistent with *overproduction of estrogenic steroids by the adrenal tumors*. Some of the functional disturbances resemble intact females with persistent or prolonged estrus; ferrets being seasonally polyestrous and induced ovulators. About one third of ferrets with adrenal cortical tumors also have neoplasms derived from the insulin-producing beta cells of the pancreatic islets that can be associated with hypoglycemia and elevated levels of serum insulin, resulting in seizures, episodic lethargy, ptyalism, ataxia, and hind leg weakness.

The most consistent endocrinologic change in ferrets with adrenal tumors is *elevation of plasma levels of estradiol-17β*. It is presumed that the estradiol-17β is produced by the tumor directly, but an alternative possibility would be that the adrenal tumors secrete androgenic steroids that are aromatized in the skin and possibly elsewhere to estrogenic steroids. There is no increase in circulating estradiol-17β levels in response to exogenous ACTH, but plasma levels decrease following adrenalectomy. Plasma cortisol and corticosterone levels in ferrets with adrenal tumors either are in the range of normal or below, and do not show an exaggerated increase in response to exogenous ACTH. There is not a decrease in plasma cortisol following unilateral adrenalectomy, and the contralateral adrenal cortex is not atrophic as would be expected if the adrenal tumor were secreting excess cortisol. However, the urinary cortisol:creatinine ratio has been reported to be elevated in ferrets with adrenal cortical tumors. The clinical signs in ferrets with adrenal cortical tumors can be effectively reversed by adrenalectomy (especially of the left side if there is no macroscopic enlargement) but not by chemotherapy with o,p′-DDD. Complete regrowth of hair usually occurs by 2–3 months postadrenalectomy.

ADRENAL MEDULLA

Development, structure, and function

Embryonic development and structure

The adrenal medulla has a different embryonic derivation than the cortical portion of the adrenal gland. It is derived from neuroectoderm of the neural crest. Secretory cells in the adrenal medulla are involved in biosynthesis of the *catecholamine group of hormones*.

The medulla is completely surrounded by the adrenal cortex in mammals, and venous drainage from the cortex bathes the medullary cells with blood containing the highest concentration of corticosteroids of any fluid in the body. This close anatomic association between the adrenal cortex and medulla in mammals is not fortuitous, because the N-methylating enzyme (phenylethanolamine-*N*-methyl transferase), which converts norepinephrine to epinephrine, is corticosteroid hormone-dependent.

A variety of techniques may be used for the demonstration of catecholamines in tissue sections. The chromaffin reaction is the oxidation of catecholamines by potassium dichromate solutions and results in the formation of a brown-to-yellow pigment in medullary tissue. The chromaffin reaction as traditionally performed has a low level of sensitivity and should not be used for the definitive demonstration of the presence of catecholamines in tissues. Similarly, both the argentaffin and argyrophil reactions, which have been used extensively in the past for the demonstration of chromaffin cells, have low sensitivity and specificity. *Fluorescence techniques using formaldehyde or glyoxylic acid represent the methods of choice for the demonstration of catecholamines at the cellular level.* These aldehydes form highly fluorescent derivatives with catecholamines, which can be visualized by ultraviolet microscopy. *Immunohistochemistry* provides an alternative approach for the localization of catecholamines in chromaffin cells and other cell types. Antibodies are available that permit epinephrine- and norepinephrine-containing cells to be distinguished in routinely fixed and embedded tissue samples. Several of the important enzymes involved in the biosynthesis of catecholamine hormones also may be demonstrated by immunohistochemical procedures. Antibodies to chromogranin-A can be used for the demonstration of this unique protein in chromaffin cells.

Biosynthesis of catecholamine hormones

The main biosynthetic pathway for catecholamines in mammals starts with *tyrosine* derived either from the diet or formed through hydroxylation of phenylalanine in the liver. Tyrosine is first converted to 1-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase, and DOPA is then decarboxylated to 1-dihydroxyphenylethylamine (DOPAmine) by l-amino acid decarboxylase, which subsequently undergoes β-hydroxylation (by DOPAmine β-hydroxylase) to *norepinephrine* (Fig. 3.89).

Though the adrenal medulla does not appear to be absolutely essential for life, there are a number of physiologic conditions (e.g., sudden hypoglycemia and acute stress) in which release of epinephrine from the medulla is beneficial. Stimulation by preganglionic sympathetic fibers that synapse on secretory cells of the adrenal medulla also increases the output of catecholamines, primarily by increasing tyrosine hydroxylase and DOPAmine beta-oxidase activity.

Catecholamines are degraded in peripheral tissues by catechol-*O*-methyl transferase and monamine oxidase to several metabolites, such as vanillylmandelic acid, metanephrine, and normetanephrine that are excreted in the urine. Measurement of the urinary excretion of these metabolites plus the relatively small amount of free catecholamines excreted in the urine are useful in the diagnosis of certain disorders characterized by overproduction of adrenal medullary hormones. This is best done on urine samples collected over a 24-hour period since catecholamine overproduction often is episodic rather than continuous.

Neoplasms of adrenal medullary secretory cells

Pheochromocytoma and malignant pheochromocytoma

Pheochromocytomas are the most common neoplasms arising in the adrenal medulla of animals. They develop most often in cattle and dogs, and infrequently in other domestic animals. In bulls and humans, pheochromocytomas may develop concurrently with

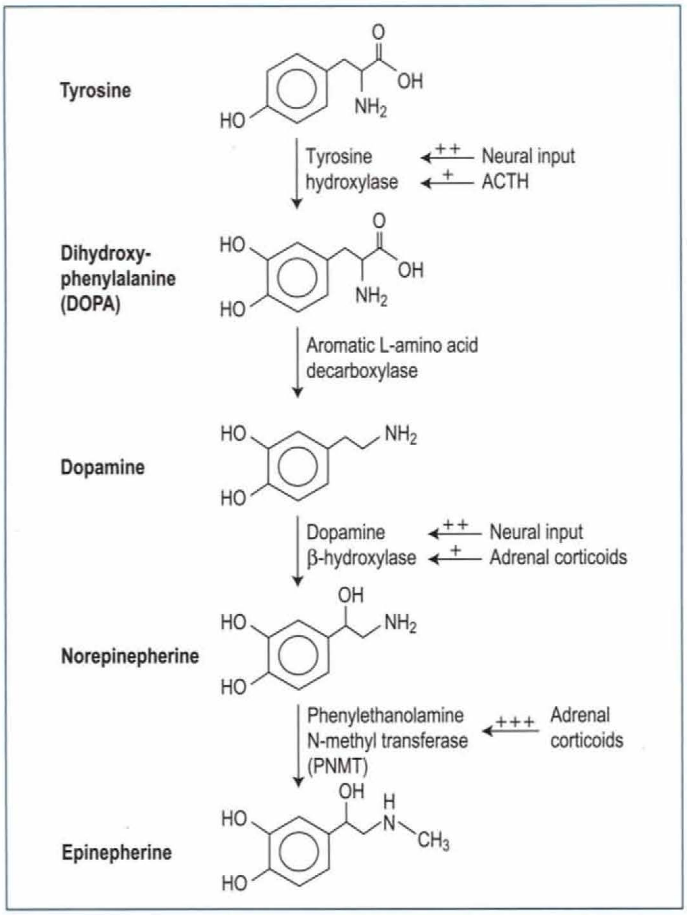

Figure 3.89 Synthesis of norepinephrine and epinephrine in chromaffin cells of the adrenal medulla. Tyrosine hydroxylase is the rate-limiting enzyme. Enzymatic conversion of tyrosine to DOPA and dopamine occurs in the cytoplasm. Synthesis of norepinephrine occurs in the secretory granules, which is the site of dopamine β-hydroxylase. Norepinephrine leaves the secretory granules and is converted to epinephrine in the cytoplasm by phenylethanolamine-N-methyltransferase (PNMT) and then re-enters the secretory granules. (From Rosol TJ, et al. Toxicol Pathol 2001;29:41–48.)

calcitonin-secreting C-cell tumors of the thyroid gland. In some animals, this appears to represent neoplastic transformation of multiple types of endocrine cells of neuroectodermal origin in the same individual. *Malignant pheochromocytoma* often is used to designate medullary tumors that invade through the adrenal capsule and into adjacent structures or that metastasize to distant sites.

Pheochromocytomas are tumors of chromaffin cells and are almost always located in the adrenal gland of animals. They can be either unilateral or bilateral. Although size varies considerably they often are large (10 cm or more in diameter) and incorporate the majority of the affected adrenal gland. A small remnant of the adrenal gland often can be found at one pole (Fig. 3.90A). Smaller tumors are completely surrounded by a thin compressed rim of adrenal cortex. Large pheochromocytomas are multilobular and variegated light brown to yellow-red due to areas of hemorrhage and necrosis. A useful aid in macroscopic diagnosis of pheochromocytoma is the *Henle chromaffin reaction* with either potassium dichromate or iodate. Application of Zenker's solution to the flat-cut surface of a freshly resected tumor results in oxidation of catecholamines, forming a dark-brown pigment within 20 min or less (Fig. 3.90B).

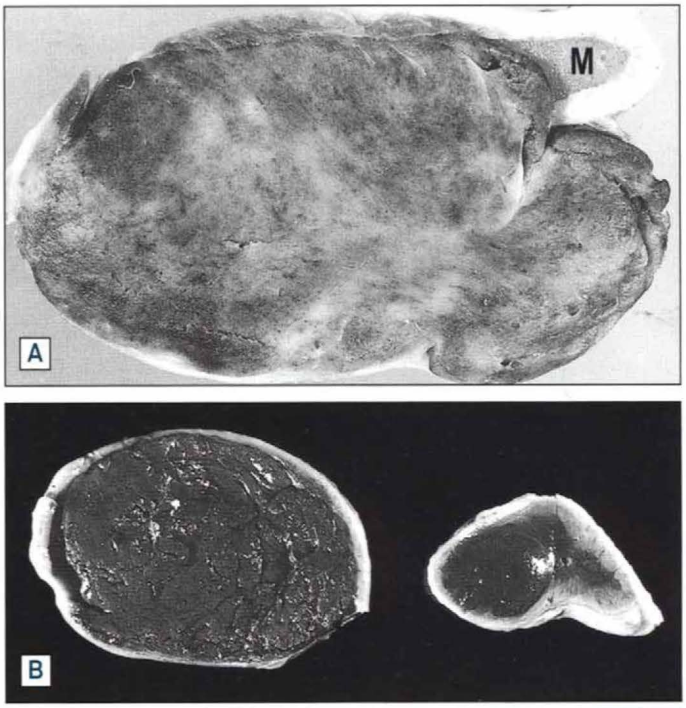

Figure 3.90 A. Pheochromocytoma in a dog with remnant of adrenal medulla (M) at top right. **B.** Chromaffin-positive reaction in bilateral pheochromocytomas, bovine.

Tumor cells in pheochromocytomas vary from small cuboidal or polyhedral cells to large pleomorphic cells with multiple hyperchromatic nuclei. The cytoplasmic area is lightly eosinophilic, finely granular, and plasma membranes often are indistinct because of early onset of autolysis in adrenal medullary tissue. Tumor cells are characteristically subdivided into small lobules by fine connective tissue septa and capillaries (Fig. 3.91A).

Pheochromocytomas are composed of epinephrine-secreting cells, norepinephrine-secreting cells, or both. The principal distinguishing morphologic feature of these two populations of cells is in the fine structure of their secretory granules. Pheochromocytomas from which norepinephrine is the principal catecholamine extracted are composed of cells with secretory granules that have an eccentrically situated electron-dense core surrounded by a wide submembranous space (Fig. 3.91B). When epinephrine is the principal catecholamine the secretion granules in tumor cells have a coarsely granular internal core of lower density and a narrow submembranous space (Fig. 3.91C).

Small pheochromocytomas are well encapsulated and remain confined to the affected adrenal gland. Larger malignant pheochromocytomas may exert pressure on and invade adjacent tissues, particularly the caudal vena cava and aorta. Tumor cells often invade the capsule and penetrate through the wall of the caudal vena cava forming a large thrombus that partially occludes the venous return from the caudal extremities. Metastases occur in ~50% of malignant pheochromocytomas to the liver, regional lymph nodes, spleen, and lungs in dogs. Growth of tumor cells in a subendothelial location along vascular sinusoids and artefactual displacement of clusters of tumor cells into the lumen of thin-walled veins during sectioning and forced through the capsule should not be misinterpreted as evidence of malignancy in adrenal medullary neoplasms (Fig. 3.92).

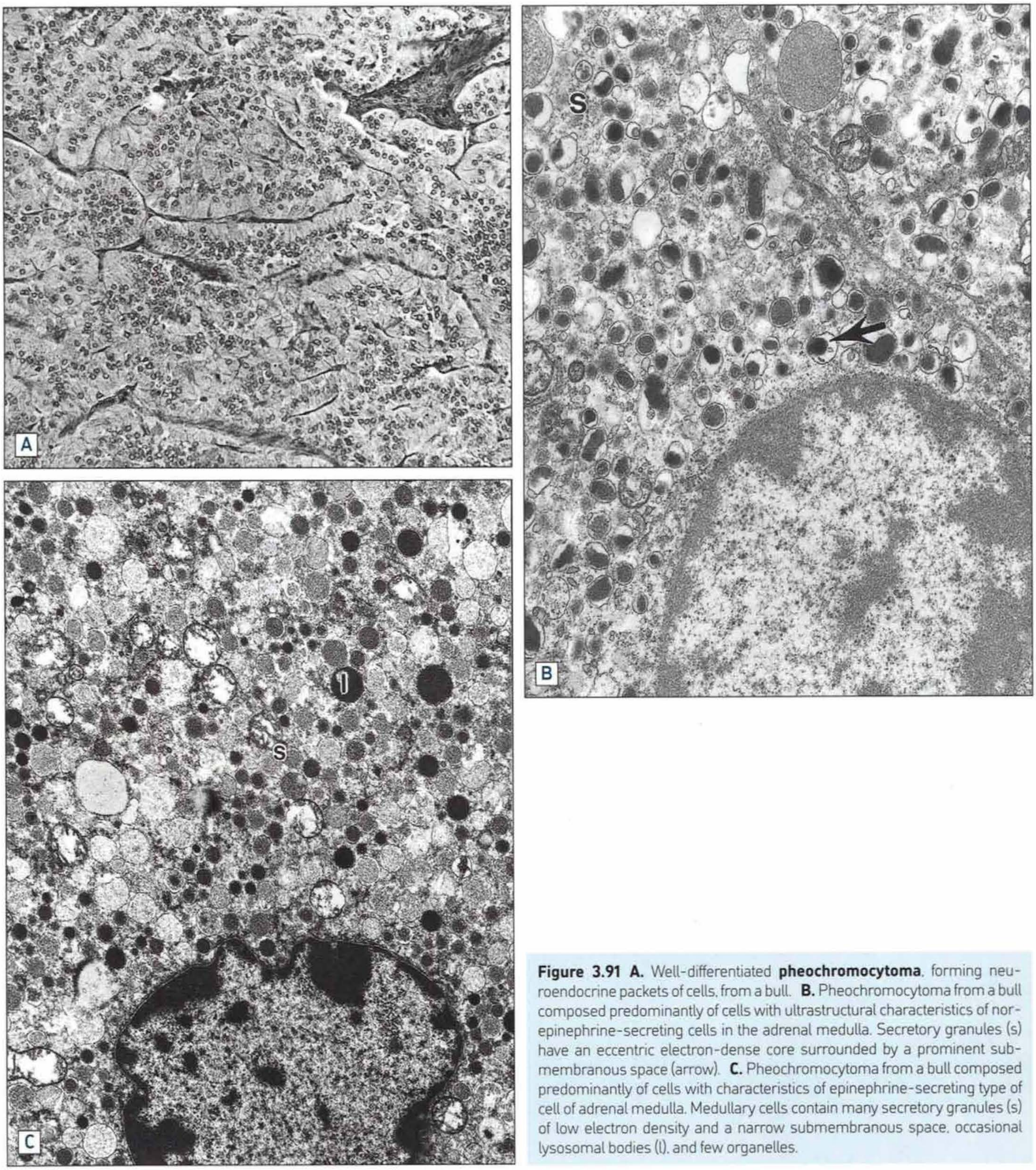

Figure 3.91 A. Well-differentiated **pheochromocytoma**, forming neuroendocrine packets of cells, from a bull. **B.** Pheochromocytoma from a bull composed predominantly of cells with ultrastructural characteristics of norepinephrine-secreting cells in the adrenal medulla. Secretory granules (s) have an eccentric electron-dense core surrounded by a prominent submembranous space (arrow). **C.** Pheochromocytoma from a bull composed predominantly of cells with characteristics of epinephrine-secreting type of cell of adrenal medulla. Medullary cells contain many secretory granules (s) of low electron density and a narrow submembranous space, occasional lysosomal bodies (l), and few organelles.

Functional pheochromocytomas with clinical signs related to catecholamine overproduction occur infrequently in animals. Tachycardia, edema, and cardiac hypertrophy observed in dogs and horses with pheochromocytomas have been attributed to excessive catecholamine secretion. Arteriolar sclerosis and widespread medial hyperplasia of arterioles have been reported in dogs with pheochromocytomas that were associated with clinical signs suggestive of paroxysmal hypertension. The catecholamine content in pheochromocytomas from bulls with concurrent C-cell tumors of the thyroid gland is higher than in the normal bovine adrenal medulla. Urinary excretion of vanillylmandelic acid and free unconjugated catecholamines are elevated in bulls with pheochromocytomas.

Mechanisms of adrenal medullary tumor development

There are *multiple factors* involved in the pathogenesis of pheochromocytomas. This has been most completely studied in laboratory rats. *The factors involved include genetic background, chronic high levels of growth hormone or prolactin associated with pituitary tumors, dietary factors, and stimulation of the autonomic nervous system.* Chronic administration of reserpine to rats results in decreased blood pressure, neural stimulation of the adrenal medulla, and development of hyperplasia and pheochromocytoma. In aged F344 rats, the incidence of pheochromocytoma has been reported to be greater in animals with more severe chronic progressive glomerulopathy. Nutritional factors have an important modulating effect on the spontaneous incidence of adrenal medullary proliferative lesions. Several sugars and sugar alcohols have produced adrenal medullary tumors at high dosages, including xylitol, sorbitol, lacitol, and lactose at concentrations of 10 to 20% in the diet. Although the mechanism involved is not completely understood, a role for calcium has been postulated. High doses of slowly absorbed sugars and starches increase the absorption and urinary excretion of calcium. In addition, vitamin D_3 induces proliferative lesions (hyperplastic nodules and pheochromocytomas) in the rat. Hypercalcemia is known to increase catecholamine synthesis in response to stress, and low-calcium diets will reduce the incidence of adrenal medullary tumors in xylitol-treated rats. Other compounds that might act via altered calcium homeostasis include the retinoids (which can produce hypercalcemia) and conditions such as progressive nephrocalcinosis in aging male rats treated with nonsteroidal anti-inflammatory agents.

Calcium ions as well as cyclic nucleotides and prostaglandins may act as mediators capable of stimulating both hormonal secretion and cellular proliferation. The likelihood of medullary lesions also appears greater in rats with metastatic calcification and nephrocalcinosis. *Vitamin D is the most potent in vivo stimulus yet identified for chromaffin cell proliferation in the adrenal medulla.* Vitamin D_3 (5000; 10 000; or 20 000 IU/kg/day in corn oil) resulted in a 4- to 5-fold increase in bromodeoxyuridine (BrdU) labeling at week 4 that diminished to a 2-fold increase by week 26. An initial preponderance of epinephrine-labeled cells (phenylethanolamine-N-methyl transferase positive (PNMT) cells) subsequently gave way to more norepinephrine-labeled cells. Eighty-nine percent of rats receiving the two highest doses of vitamin D_3 had focal medullary proliferative lesions (BrdU-labeled focal hyperplasias) and pheochromocytomas by week 26 in contrast to absence of medullary lesions in controls. The proliferative lesions usually were multicentric, bilateral, peripheral in location in the medulla, nearly all were PNMT-negative, and appeared to represent morphologic continuum rather than separate entities. Earlier studies had demonstrated that the vitamin D_3-induced increase in chromaffin cell proliferation was observed as early as 1 week but had declined by 4 weeks. These findings support the hypothesis that altered calcium homeostasis is indirectly involved in the pathogenesis of pheochromocytomas in rodents, most likely via effects on increasing chromaffin cell proliferation. Vitamin D_3, lactose, and xylitol all failed to stimulate directly the proliferation of chromaffin cells in vitro.

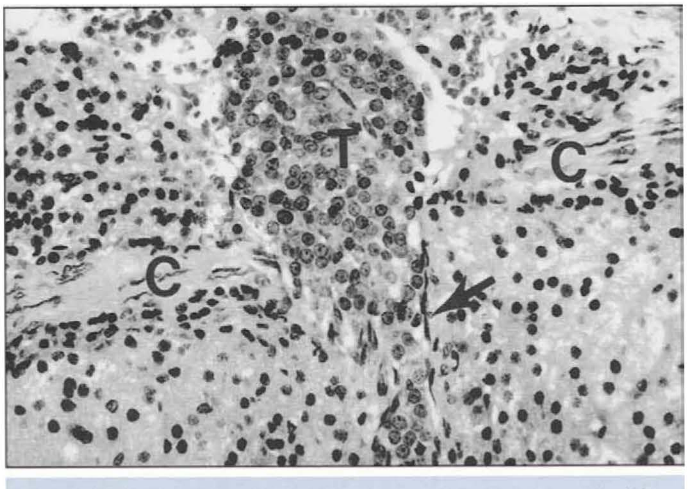

Figure 3.92 Pheochromocytoma from a rat. Tumor cell thrombus (T) in endothelial cell-lined adrenal vein passing through adrenal capsule C. The thrombus likely was artefactually forced through the capsule (arrow) during sectioning of the enlarged adrenal gland.

Adrenal medullary hyperplasia

Diffuse or nodular adrenal medullary hyperplasia appears to precede the development of pheochromocytoma in bulls, humans with C-cell tumors of the thyroid gland, and laboratory rats. Diffuse proliferation of chromaffin cells is non-encapsulated but compresses the surrounding adrenal cortex (Fig. 3.93). The hyperplastic cells are columnar to cuboidal and have a lightly basophilic cytoplasm. Bulls with prominent diffuse medullary hyperplasia often have a few small foci of intense nodular (focal) proliferation of medullary cells. Medullary hyperplasia is detected by increased total adrenal weight, a decrease in corticomedullary ratio due to an increase in the size and number of medullary cells, and the presence of frequent mitotic figures in the adrenal medulla.

Neoplasms of cells of the sympathetic nervous system in adrenal medulla

Neuroblastoma

Neuroblastomas arise from primitive neuroectodermal cells, often in younger animals, and form a large intraabdominal mass. They are differentiated from pheochromocytomas by being composed of small tumor cells with hyperchromatic nuclei and scant amounts of cytoplasm that often resemble lymphocytes and tend to form pseudorosettes (Fig. 3.94). Neurofibrils or unmyelinated nerve fibers can be demonstrated in neuroblastomas.

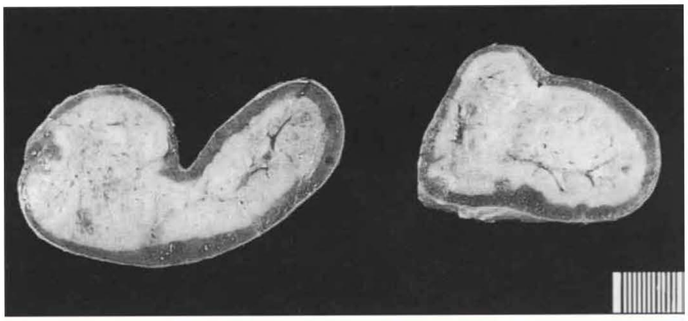

Figure 3.93 Bilateral diffuse hyperplasia of adrenal medulla from a bull. Scale in mm. (From Yarrington JT, Capen CC. Vet Pathol 1981;18:316–325.)

Ganglioneuroma

Ganglioneuromas are benign tumors in the medulla composed of multipolar ganglion cells and neurofibrils with a prominent fibrous connective tissue stroma (Fig. 3.95A–C). They usually are well-differentiated small tumors that in cattle often contain melanin pigment. The surrounding adrenal cortex is compressed to various degrees depending upon the size of the tumors. Neoplastic cells in medullary tumors occasionally may differentiate in two directions resulting in adjacent pheochromocytomas and ganglioneuromas in the same adrenal gland.

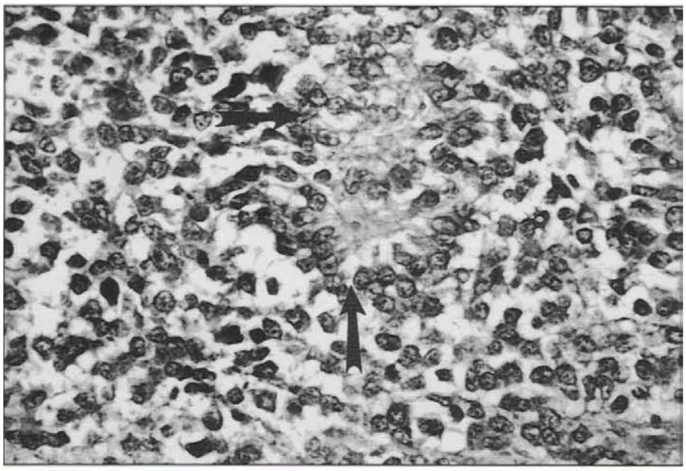

Figure 3.94 Neuroblastoma in a canine adrenal gland, with pseudorosette formation (arrow). The tumor cells have small hyperchromatic nuclei and poorly defined cytoplasm. (From Capen CC. In: Tumors of Domestic Animals, 4th ed., 2002.)

Metastatic tumors of the adrenal medulla

Secondary foci of neoplastic growth in the adrenal glands are common in cases of disseminated neoplasia and originate primarily as tumor cell emboli. The metastases usually are bilateral and the medulla is an early site of tumor growth due to the low-pressure venous blood supply. Direct invasion of the adrenal glands may occur from primary or secondary tumors of contiguous structures; however, direct invasion is uncommon due to the adrenal capsule.

Involvement of the adrenal glands by lymphosarcoma and mammary carcinoma may be extensive, and both adrenals are likely to be affected. Tumor emboli lodge in sinusoids of the adrenal medulla and may grow to an extent that they compress the surrounding adrenal cortex.

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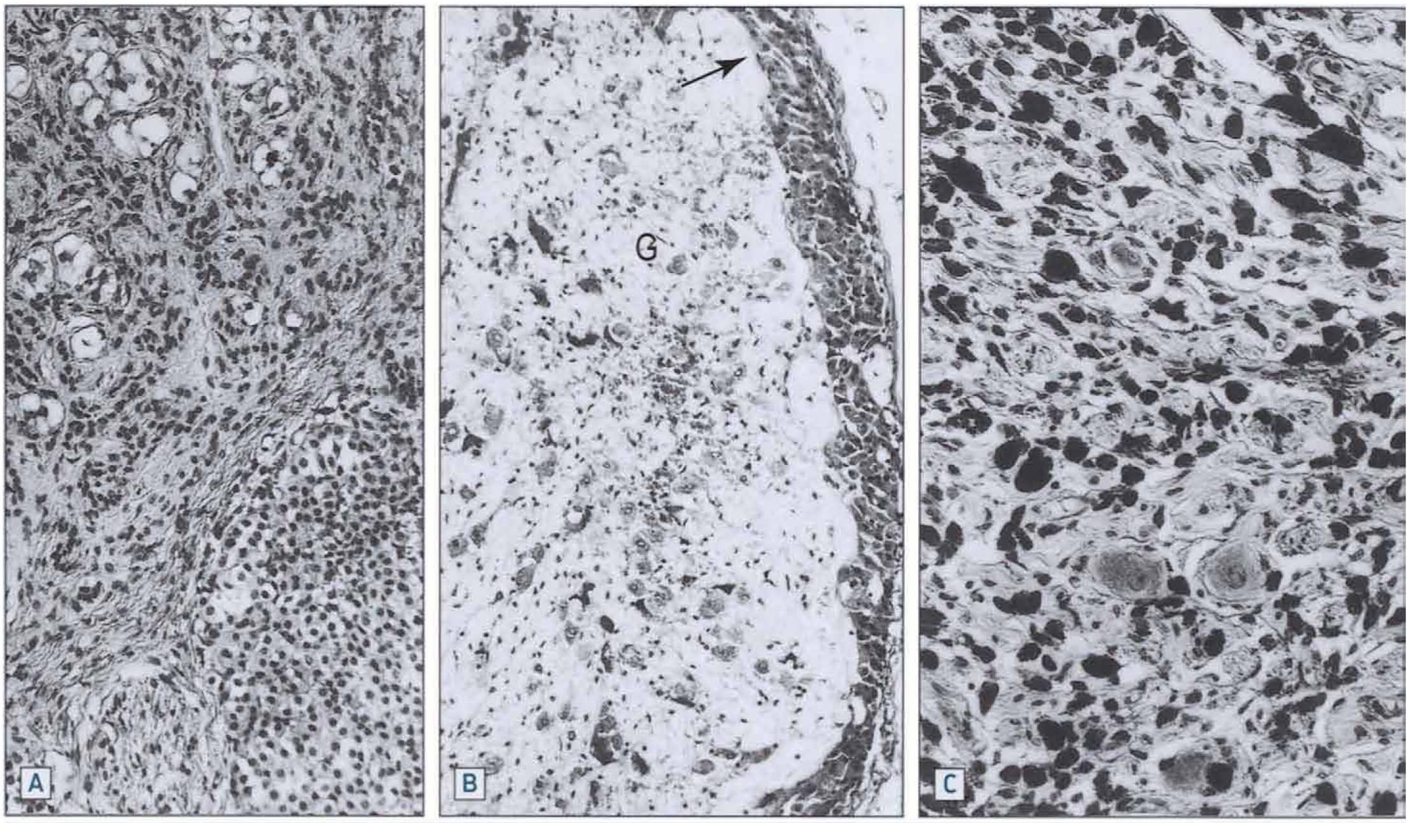

Figure 3.95 A. Ganglioneuroma in a bovine adrenal medulla. **B.** Ganglioneuroma (G) in a rat adrenal medulla, composed of multipolar neurons compressing surrounding cortex (arrow). **C.** Pigmented ganglioneuroma in a bovine adrenal medulla.

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CHEMORECEPTOR ORGANS

Development, structure, and function

The chemoreceptor organs (*nonchromaffin extra-adrenal paraganglia*) are sensitive to changes in the blood carbon dioxide content, pH, and oxygen tension, thereby aiding in the regulation of respiration and circulation. **Carotid and aortic bodies** can initiate an increase in the depth, minute volume, and rate of respiration by way of parasympathetic nerves and result in an increased heart rate and elevated arterial blood pressure by way of the sympathetic nervous system. *They are composed normally of parenchymal (chemoreceptor, glomus) cells and stellate (sustentacular) cells.* Nerve endings with synaptic vesicles as well as nerve fibers are seen in close association with the chemoreceptor cells.

Chemoreceptor tissue is present at several *sites* in the body including the carotid body, aortic bodies, nodose ganglion of the vagus nerve, ciliary ganglion in the orbit, pancreas, bodies on the internal jugular vein below the middle ear, and glomus jugulare along the recurrent branch of the glossopharyngeal nerve. Aortic bodies of dogs normally consist of clusters of cells embedded in adventitia at multiple sites including the innominate artery immediately below the origin of the right subclavian artery, on the cranial surface of the aortic arch, beneath the arch between the aorta and pulmonary artery, between the ascending aorta and pulmonary artery near the left coronary artery, and scattered in the wall of the pulmonary artery. Although chemoreceptor tissue appears to be widely distributed in the body, *tumors develop principally in the aortic and carotid bodies of animals.*

Aortic body adenoma and carcinoma

Aortic body neoplasms are encountered more frequently than neoplasms of the carotid body in animals, but the reverse is true for humans. These tumors develop primarily in dogs, and infrequently in cats and cattle. Brachycephalic breeds of dogs such as the Boxer and Boston Terrier are highly predisposed to develop tumors of the aortic and carotid bodies.

Aortic body tumors appear most frequently either as single masses or as multiple nodules within the pericardial sac near the base of the heart (Fig. 3.96). They vary considerably in size (0.5–12.5 cm) with carcinomas, in general, being larger than adenomas. Solitary small **aortic body adenomas** either are attached to the adventitia of the

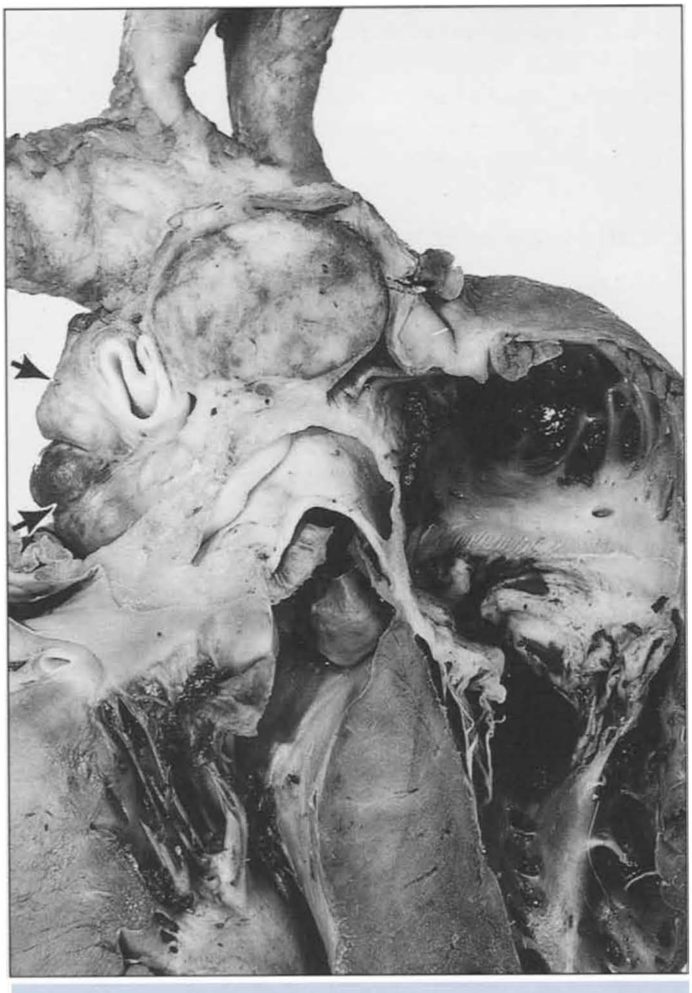

Figure 3.96 Aortic body tumors (arrows) at base of heart of a dog.

pulmonary artery and ascending aorta or are embedded in the adipose connective tissue between these major vascular trunks. They have a smooth external surface and on cross-section are white, mottled with red-to-brown areas. Larger adenomas may indent the atria or displace the trachea, are multilobular, and partially surround the major arterial trunks at the base of the heart. Although the vessels may be completely surrounded by neoplastic tissue, there usually is little evidence of vascular constriction.

Aortic body carcinomas occur less frequently in dogs than adenomas. Carcinomas may infiltrate the wall of the pulmonary artery to form papillary projections into the lumen or invade through the wall into the lumen of the atria. Although tumor cells often invade blood vessels, metastases to the lung and liver occur infrequently.

Aortic body tumors in animals tend to be more benign than tumors of the carotid body. They grow slowly by expansion and exert pressure on the vena cava and atria. Aortic body carcinomas may invade locally into the atria, pericardium, and adjacent large thin-walled vessels. When they metastasize, secondary foci of growth are found most frequently in the lung and liver.

Tumors of the aortic bodies in animals are not functional (i.e., they do not secrete excess hormone into the circulation), but may result in a variety of functional disturbances as *space-occupying lesions*. These include manifestations of cardiac decompensation due to pressure on the atria, vena cava or both associated with larger aortic body adenomas and carcinomas. There may be dyspnea, coughing, vomiting, cyanosis, hydrothorax, hydropericardium, ascites, edema of the subcutaneous tissue (involving head, neck, and forelimbs), and passive congestion of the liver. The accumulation of serous, often blood-tinged, fluid in the pericardial sac results from the invasion of tumor cells into lymphatics at the base of the heart or the compression of small pericardial veins.

Carotid body adenoma and carcinoma

Carotid body neoplasms arise near the bifurcation of the common carotid artery in the cranial cervical area. They usually appear as a unilateral slow-growing mass and only rarely develop on both sides in the same animal.

Carotid body adenomas are well encapsulated, have a smooth external surface, and vary from 1 to 4 cm in diameter. The bifurcation of the carotid artery is incorporated in the mass, and tumor cells adhere firmly to the tunica adventitia. A branch of the glossopharyngeal nerve may be traced into the capsule of the tumor by careful dissection. Adenomas are firm, white with scattered areas of hemorrhage, and extremely vascular. Complete surgical excision or biopsy often is difficult due to the high degree of vascularity and intimate relationship with major arterial trunks in the neck.

Carotid body carcinomas are larger (up to 12 cm in diameter) and more coarsely multinodular than adenomas. Although malignant carotid body tumors appear to be encapsulated, tumor cells invade the capsule and penetrate into the walls of adjacent vessels and lymphatics. The external jugular vein and several cranial nerves (in addition to the carotid bifurcation) may be incorporated by the neoplasm. Larger tumors can result in extensive dorsolateral deviation of the trachea. Metastases of carotid body tumors occur in about one third of cases, and have been found in the lung, bronchial and mediastinal lymph nodes, liver, pancreas, and kidney. *Multicentric neoplastic transformation of chemoreceptor tissue occurs frequently in brachycephalic breeds of dogs.* About two thirds of the reported cases of carotid body tumors also had aortic body tumors.

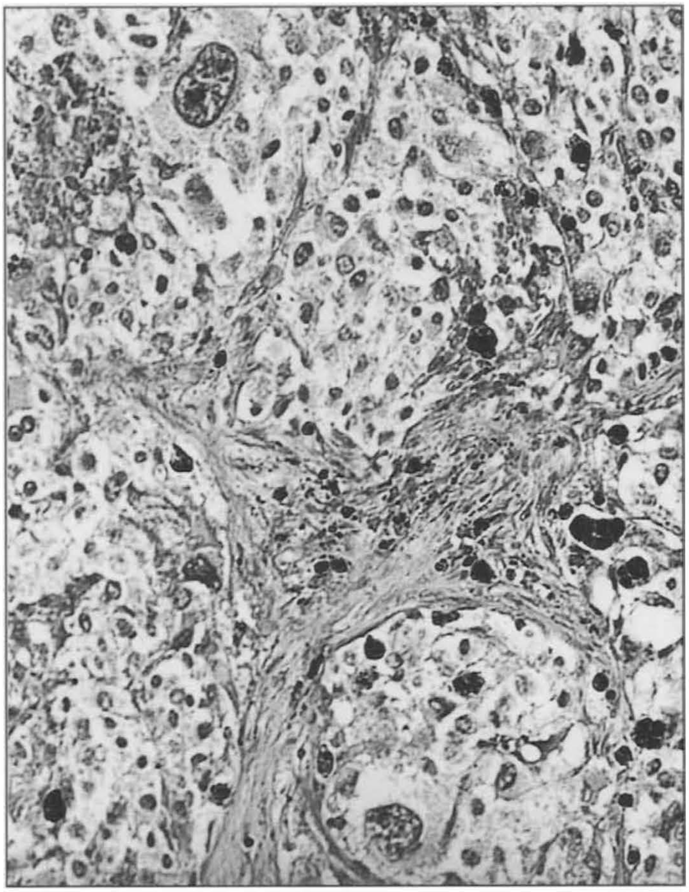
Figure 3.97 Cellular pleomorphism in a **carotid body tumor** from a dog.

The histologic characteristics of chemoreceptor tumors ("chemodectomas") are essentially similar whether they are derived from the carotid or aortic body. The neoplastic chemoreceptor cells are subdivided into lobules by prominent branching trabeculae of connective tissue that originate from the fibrous capsule (Fig. 3.97). They are further subdivided into small compartments by fine septa that contain collagen and reticulin fibers plus small capillaries. Tumor cells are commonly aligned along and around small capillaries.

The tumor cells of chemodectomas are discrete, cuboidal to polyhedral, and closely packed together. The cytoplasm is lightly eosinophilic, finely granular, and often vacuolated. Cells comprising chemodectomas rapidly undergo autolysis. Cell boundaries become indistinct and the cytoplasm appears clear if the postmortem interval is prolonged. *Chromaffin granules cannot be demonstrated in the cytoplasm of cells comprising chemodectomas*, in contrast to pheochromocytomas of the adrenal medulla.

Cells of aortic and carotid body tumors in the dog resemble those in normal chemoreceptor tissue, but lack the normal relationship to sustentacular, neural, and vascular elements. The large polyhedral chemoreceptor or parenchymal cells are arranged in small clusters. The cytoplasmic density of the neoplastic cell varies from light to dark depending on the development of secretory organelles and numbers of storage granules. Small spherical mitochondria, parallel arrays of rough endoplasmic reticulum, and Golgi apparatuses with prosecretory granules are scattered in the cytoplasm.

Variable numbers of small, electron-dense, membrane-limited secretory granules are present in the tumor cells and are important in establishing the diagnosis of an aortic or carotid body tumor. The electron density of secretory granules in chemoreceptor cells varies considerably with the fixative used, but the granules are well preserved with glutaraldehyde. In general, secretory granules are more numerous in cells of adenomas than in those of carcinomas of the aortic and carotid bodies. Stellate cells often extend long cytoplasmic processes around and between the tumor cells in carotid body adenomas from dogs. These processes appear to terminate near perivascular spaces. Similar sustentacular cells have been described in the carotid body of normal animals and are considered to have a supportive rather than a secretory function. *Ultrastructural studies are helpful in differentiating between heart-base tumors derived from chemoreceptor cells of the aortic body and ectopic thyroid follicular cells in the dog.* The neoplastic follicular cells are arranged predominantly in a compact cellular (solid) pattern with only an occasional colloid-containing follicle, and they may closely resemble an aortic body tumor. Although thyroid follicular cells contain large lysosomal (dense) bodies, they lack the small membrane-limited secretory granules characteristic of chemoreceptor cells in the aortic and carotid bodies.

Carotid body tumors are very vascular and have numerous muscular arterioles, large thin-walled veins, and an abundant network of capillaries in the connective tissue septa. There are focal areas of hemorrhage from disruption of thin-walled vessels and areas of coagulation necrosis. Several layers of tumor cells may radiate along fine connective tissue septa from the thin-walled vessels. Tumor cells frequently invade blood vessels and lymphatics with the formation of emboli. Carcinomas have evidence of tumor cell invasion through the capsule and into the walls of large muscular arteries and adjacent structures.

Dogs with carotid body tumors usually are presented with a palpable, slowly enlarging mass in the craniolateral cervical region near the angle of the mandible. Larger neoplasms interfere with swallowing due to pressure on the esophagus and result in circulatory disturbances from compression of the larger veins in the neck. Other functional disturbances may be related to the presence of an aortic body tumor in the same animal.

Carotid body tumors tend to be more malignant than aortic body tumors, and metastases are present in about one third of reported cases. The presence of tumor cell emboli in vascular or lymphatic spaces in a biopsy of a primary chemodectoma does not necessarily indicate that metastases are present in distant organs.

The etiology of carotid and aortic body tumors is unknown, but it has been suggested that a *genetic predisposition aggravated by chronic hypoxia* may account for the higher risk of certain brachycephalic breeds such as the Boxer and Boston Terrier. Carotid bodies of several mammalian species, including dogs, undergo hyperplasia when subjected to chronic hypoxia by living in a high-altitude environment. People living at high altitudes have 10 times the frequency of chemodectomas of those living at sea level.

Heart-base tumors derived from ectopic thyroid

Adenomas and carcinomas derived from ectopic thyroid tissue account for ~5–10% of "heart-base" tumors in dogs. They often compress or invade structures in the cranial mediastinum near the base of the heart. Areas of ectopic thyroid tumors with a compact cellular (solid) pattern of arrangement are difficult to distinguish histologically from aortic body tumors. *Cells comprising ectopic thyroid tumors generally are smaller than in aortic body tumors, with more hyperchromatic nuclei and eosinophilic cytoplasm.*

Neoplastic thyroid follicular cells are not consistently subdivided into small packets by fine strands of connective tissue. Tumor giant cells are infrequent in ectopic thyroid tumors and the stroma is less prominent. Multiple sections usually reveal the formation of *primitive follicular structures or colloid-containing follicles* by neoplastic follicular cells in ectopic thyroid tumors but not in aortic body tumors. Ectopic thyroid carcinomas arising at the base of the heart in dogs either remain localized and enlarge in the cranial mediastinum or occasionally metastasize to extrathoracic sites.

Orbital (extra-adrenal) paragangliomas (neuroendocrine tumors)

A unique extra-adrenal paraganglioma occurs in *horses*, resulting primarily in clinical signs related to *exophthalmos*. The lesion appears to be derived from cells of neural crest origin, most likely nonchromaffin paraganglia near the ciliary ganglion. The neoplastic cells usually invade locally in the retrobulbar space but may infiltrate more aggressively through the optic canal into the cranial vault resulting in lysis of bone and extension into the nasal cavity.

The tumors are densely cellular with nests or packets of neoplastic cells subdivided by a fine fibrovascular (reticulin-positive) stroma characteristic of neuroectodermal-derived tumors. The cytoplasmic area is abundant, lightly eosinophilic, and finely granular, often with indistinct individual cell boundaries. The neoplastic cells have a positive argyrophilic reaction (either by Pascual's silver inpregnation method or the Grimelius technique), stain positive by immunohistochemical methods for chromogranin A and neuron-specific enolase, but are negative for S-100 protein and glial fibrillary acidic protein. Ultrastructurally, neoplastic cells of orbital paragangliomas in horses are similar to those in canine aortic and carotid body tumors, with typical membrane-bound secretory granules and prominent arrays of rough endoplasmic reticulum.

Surgical removal of the tumors by transpalpebral orbital exenteration has been performed with variable success depending upon the degree of local tissue invasion of the tumor beyond the orbit. Manipulation of the tumors during surgery may result in hypotension.

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4

Female genital system

Donald H. Schlafer and Richard B. Miller

Pathology of the genital system of the nongravid female

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Pathology of the genital system of the nongravid female

GENERAL INTRODUCTION

Unique challenges associated with pathology of the female reproductive system

In no other system does one commonly encounter so great a variety of gross and microscopic changes in "normal" tissues as those that are routinely encountered in reproductive organs in the normal cycling and gravid female. The complex physiologic events that are associated within the estrus cycle, in pregnancy, or during the postpartum periods are accompanied by dramatic tissue changes in the gonads, tubular, and external genitalia, such as marked hyperplasia, atrophy, necrosis, hemorrhage, and tissue invasion. These are changes that pathologists usually associate with pathological processes. Consequently, when presented with reproductive tissues, an examining pathologist must start with a firm understanding of the normal embryology, anatomy, and reproductive physiology associated with the species under study.

Remarkable species variations exist, which limits the usefulness of broad generalizations and makes casual extrapolations fraught with error. A good clinical history that includes stage of cycle, prior reproductive status, and supporting endocrinology is especially important; if not provided, it should be aggressively sought!

It must also be appreciated that, to a significant extent, the commercial value of individual animals can depend on the pathologist's report (for example, endometrial biopsy from a broodmare). Not only do the descriptive and diagnostic sections of the report have to be accurate and precise, clinicians and owners will likely place great weight on the pathologist's interpretation and assessment of clinical significance. This part of the report must be carefully formulated.

Pathology of the reproductive system is unquestionably both fascinating and challenging.

SEXUAL DEVELOPMENT AND ANOMALIES ASSOCIATED WITH SEXUAL DIFFERENTIATION

Abnormalities of sexual development are relatively common and affect all domestic animal species. Although the majority of these do not affect reproductive performance and are considered incidental findings, their presence can add confusion when confronted during clinical examinations (for example, detection of cysts in or near the ovary during ultrasound examination), and during routine surgery or postmortem examinations. Basic knowledge of the normal process of sexual differentiation, including a full appreciation of the anatomical changes that occur, is essential for the pathologist doing routine diagnostic pathology. The first section of this chapter provides a brief overview of normal sexual development. This is followed by a discussion of frequently encountered lesions associated with anomalous development of reproductive organs.

Normal sexual differentiation

Knowledge of the essential elements of the developmental anatomy of the reproductive organs is important for appreciation of the anatomy and histology of the gonads, tubular and external genitalia of the female, and is essential for comprehension and interpretation of congenital anomalies, the histogenesis of various neoplasms, or the pathogenesis of many other ovarian lesions (e.g., cysts, abnormalities of placentation).

Stage of sexual development

There are **three essential sequential series of events** that occur during **normal sexual development**:

1. The initial **establishment of genetic sex** (at fertilization).
2. The subsequent modeling of embryonic gonadal tissue as ovarian tissue, thereby **establishing gonadal sex**.
3. Controlled, programmed regression and growth of different parts of the indifferent tubular and external genital tissues of the early embryo to form those definitive structures that constitute the normal female reproductive pattern (the female phenotype). This is referred to as the **establishment of phenotypic sex**.

Establishment of genetic sex

The determination of genetic sex is fixed at the time of fertilization when egg and sperm unite. The genetic sex, or chromosomal sex, as it is sometime referred to, is then imposed on the undifferentiated indifferent gonad and thus the sequence of stages in sexual development progresses.

Each embryo will have either XX (in females) or XY (in males) sex chromosomes. These chromosomes carry many different genes required for sexual development, and recent research has shown that these genes interact as cascades, with certain genes acting as controlling, or master genes. Aberrations in genes associated with sexual differentiation, therefore, can lead to a broad variety of developmental anomalies. Knowledge gained through study of spontaneous cases of abnormal sexual differentiation in animals and humans has been greatly expanded through work using engineered mutations in mice. However, the precise mechanisms through which genes control each aspect of sexual differentiation are complex and only superficially understood.

It has been known for some time that the Y chromosome contains the genes that regulate differentiation leading to the male phenotype. Those genes responsible were postulated to be the **testis-determining factor(s)** of the Y chromosome, with the inference that it would be shown to be a single gene. This gene has been called the *SRY* gene (sex-determining region of the Y chromosome). The list of genes that when mutated involve anomalies in sex differentiation is continuously expanding. These include *WT1, Sf-1, SOX9, DAX1, SRY, MIS,* and *AR*. The genetics of gonadal development is complex and beyond the scope of this chapter (see MacLaughlin and Donahoe, in the Bibliography below).

Development of gonadal sex

Growth and development of the gonads require a complex series of steps that are only slowly becoming evident. The ovary arises from paired gonadal primordia called the *genital ridges* located between the mesonephros and attachment of the mesentery. Primordial germ cells migrate to the developing indifferent gonad through the wall of the yolk sac and the wall of the hindgut to become associated with primitive sex cords. *The sex cords go on later to organize as follicles containing the germ cells.*

In a normal XX female embryo, the outer cortical layer of the indifferent gonad becomes the ovary, while the medullary region of the indifferent gonad develops as the testis in normal male (XY) embryos. It is necessary to appreciate this to understand the range of abnormalities in gonadal developments in intersex animals. *Gonadal hormones (limited to products from testicular tissue) control subsequent differentiation of the rest of the reproductive organs.* Only hormones produced by testicular tissue act to control further modeling of the tubular and external genitalia. Female sexual differentiation does not require products from the fetal ovary. A male fetus castrated in utero early in development, although a genetic male, will develop female internal and external organs and appear as a phenotypic female.

The two critical products originating from the fetal testis are both hormones: **Müllerian inhibitory substance (MIS)** and **testosterone**.

Phenotypic sexual development

Female tubular and external genital development progresses in genetic female embryos only in the absence of hormones, whereas in the male, *MIS and testosterone function to force tissues of the indifferent, early embryo towards differentiation into male structures.* These hormones influence the regression of female embryonic tubular structures and support the formation of male tubular organs (epididymis, vas, accessory sex glands) and stimulate the progressive development of the genital tubercle to become the penis, the genital folds to close as the prepuce, and the genital sinus to differentiate into the pelvic structures.

Modeling of the tubular genitalia involves growth, fusion, and extension of the embryonic paramesonephric ductal system to form the internal female nongonadal organs (uterus, cervix, and cranial vagina); and changes in the urogenital recess, genital tubercle, and genital folds to form the vulva, clitoris, vestibule, and caudal vagina. The presence of androgens, either endogenous (from testicular tissue, for example in intersex individuals with ovotestes) or from exogenous sources (androgens administered during pregnancy) can influence this development, leading to partially masculinized females (*ambiguous genitalia*).

Development of the tubular genitalia

Even as the indifferent gonads are developing from the genital ridge, in embryos destined to become females, cylindrical (ductal and tubular) elements are forming in the abdomen that will further differentiate into the tubular genitalia.

During the early stages, male embryos and female embryos contain the same tissues and cannot be differentiated based on morphology. Paired **mesonephric (Wolffian) ducts** arise from primitive renal tissues and paired **paramesonephric (Müllerian) ducts** form de novo from longitudinal folds along the lateral inner abdominal wall (Fig. 4.1). Tubules branching from the cranial segment of the mesonephric ducts contribute to development of the rete and sex cords.

The mesonephric and paramesonephric ducts run parallel to one another. Both ductal systems are symmetrically paired and extend caudally toward the urogenital sinus. The cranial ends of the developing paramesonephric ducts do not close and open directly into the peritoneal cavity, corresponding to the tissue that will become

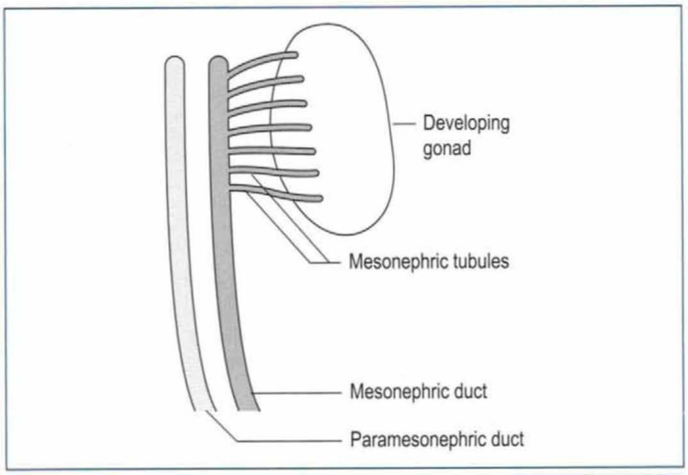

Figure 4.1 Embryonic development of the female genital system. Initial development of the indifferent ducts, tubules, and external genitalia is the same in male and female embryos. Anatomically, the internal organs in the indifferent stage consist of two bilaterally symmetrical ductal systems, the mesonephric (Wolffian; male) and paramesonephric (Müllerian; female) ducts, and the developing, but uncommitted, gonad. Additionally, the mesonephric duct develops tubular extensions at its cranial end that extend into the hilar area of the gonad. In the male, these will become the rete and the efferent tubules of the epididymis. Although the uterine tubes, uterine horns, body, cervix, and cranial vagina develop from the paramesonephric duct, the mesonephric tubes become the rete and contribute to the sex cords of the ovary. Normally, the ducts of the opposite sex regress; however, their embryonic remnants are frequently encountered in otherwise normal adult animals, and are also present as cystic structures in or near the ovary, uterus, cervix, or vagina. (Illustration prepared by Mr. Michael Simmon.)

the fimbrial part of the uterine tube. In domestic animals, the paramesonephric ducts fuse at their caudal ends to form the body of the uterus, the cervix, and the cranial vagina, each with a single lumen.

Female differentiation occurs in the absence of a functional male gonad. Sexual development in all individuals would be feminine if not prevented by the influence of two hormones produced by the embryonic testis. An important feature of gonadal differentiation is the chronological differences that exist between the sexes. In all mammalian species studied, *males differentiate earlier than females.*

Masculine differentiation is imposed on the system at an early stage by testosterone produced by the Leydig cells of the developing testis. The second hormone critical for normal male differentiation, MIS, is produced by sustentacular (Sertoli) cells of the developing testis. MIS causes the paramesonephric (Müllerian) ducts to regress, leaving only the mesonephric ducts and tubules to grow and differentiate. The presence of an embryonic testis producing testosterone supports further differentiation of mesonephric duct growth to become the vasa deferentia, seminal vesicles, and the epididymides. Testosterone also plays a central role in the development of the external genitalia. Testosterone is converted to dihydrotestosterone by 5α-reductase, and this hormone induces development of the penis and scrotum from the bipotential external genital primordia. In a female, in the absence of testosterone or MIS, the mesonephric ducts atrophy, the paramesonephric ducts become the uterine tubes, horns and body, cervix, and cranial vagina, and the indifferent external genitalia become the vulva, clitoris, vestibule, and caudal vagina.

Abnormalities of sexual development

Disorders of genital development occur in all domestic species, but are common in none. They are caused by abnormalities of genetic or chromosomal origin or inappropriate hormone exposure. The mechanisms involved in the production of many abnormalities are not known and a condition cannot often be classified precisely. *Hermaphroditism is a broad grouping.* **Hermaphrodites** have ambiguous genitalia, with part or all of the genital organs of both sexes present. The intersex is subclassified into true and pseudo, the distinction being based on the *presence of both types of gonadal tissue in the true hermaphrodite.* The **pseudohermaphrodite** has only a single type of gonadal tissue and the individual is classified as either a male or female pseudohermaphrodite on the basis of the gonadal tissue present. These terms serve only limited goals of description. The application of sophisticated techniques to the study of abnormal sexual development has elucidated, in some cases, the mechanism by which the anomalies developed, and as well has clarified some of the intricate hormonal and genetic mechanisms by which normal sexual development occurs.

Intersex conditions

Abnormal sexual differentiation commonly results in abnormal-appearing external genitalia. Some of the more dramatic examples of this occur in intersex animals that commonly have ambiguous external genitalia. As demonstrated in Figure 4.2, a photo of an intersex mare with bilateral ovotestes, the external genitalia have features that are partially female and partially male. Testosterone produced by testicular tissues (interstitial cells) of her ovotestes masculinized her external genitalia. The vulva is located more ventrally than in a normal female, the vulvar lips are variably fused, and there is clitoromegaly. In true hermaphrodite bitches, owners frequently observe an abnormally enlarged clitoris that may protrude from the vulva. Less subtle cases may be presented in fertility work-ups. The spectrum of changes ranges from nearly normal-appearing females, with grossly normal ovaries and uteri, to males with small genitalia, perhaps hypospadias, and gonads contained in scrotal sacs that look like testes with epididymides, but histologically containing ovarian tissue.

It is not uncommon to find normal-appearing testes attached to complete and well-formed uterine horns in intersex bitches (Fig. 4.3).

Ovotestes will contain both ovarian and testicular tissues (Fig. 4.4). It is not uncommon to find secondary or antral follicles, and occasionally follicles can ovulate, and there may be corpora lutea in gonads that also contain male (seminiferous and interstitial) tissue.

Ovotestes will vary greatly in both their gross and microscopic appearance, depending on the relative amounts of ovarian and testicular tissue present (Fig. 4.5). Ovotestes composed predominantly of ovarian tissues are usually contained in a bursa. There may be identifiable epididymal tissues at the caudal pole, near the attachment of the proper ligament of the ovary. Ovotestes composed of mostly testicular tissue will look like hypoplastic testes, have large epididymides, and may be near or in the inguinal canal or scrotal sac. They will, however, be attached to uterine horns.

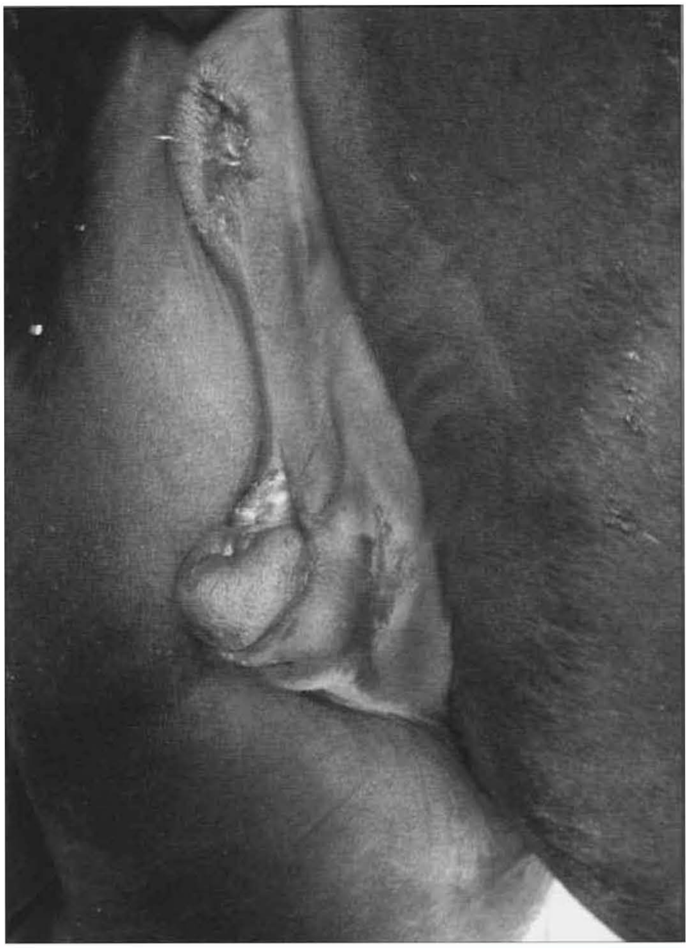

Figure 4.2 The **external genitalia of an intersex horse** with clitoromegaly and partial fusion of the vulvar lips. The external female genitalia were partially masculinized from exposure to testosterone released from testicular tissue (interstitial cells) from her ovotestes during embryonic development.

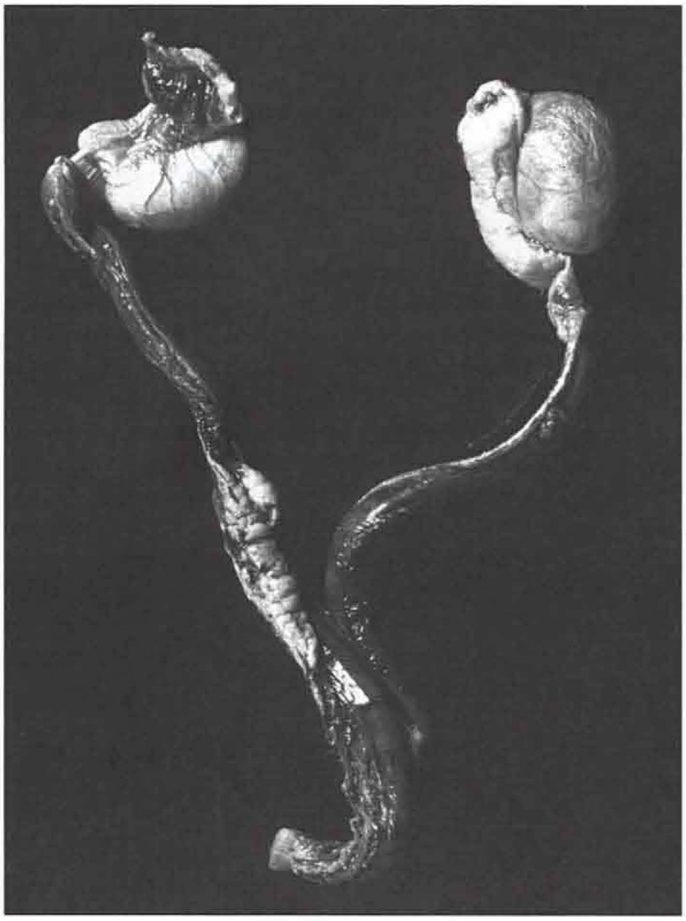

Figure 4.3 Internal reproductive organs from an **intersex bitch** revealing a near normally formed, but hypoplastic, uterus (paramesonephric duct derived) with bilateral ovotestes that are predominantly testicular with attached epididymis (derived from mesonephric tubules).

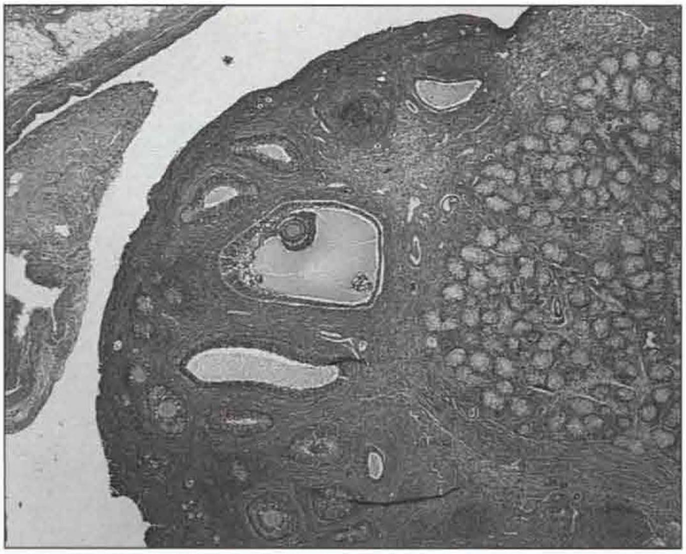

Figure 4.4 Section of **ovotestis from an intersex bitch**. The gonad is contained within a bursa (visible on the left) and has both ovarian tissue (with primary and antral follicles on the left) and lobules of hypoplastic testicular tissue in the medullary area (on the right). Even relatively small areas of testicular tissue in ovotestes produce sufficient amounts of two hormones, testosterone from the interstitial cells and Müllerian inhibitory substance from Sertoli cells, that act during early gestation to masculinize the developing mesonephric and paramesonephric ducts and tubules and the external genitalia.

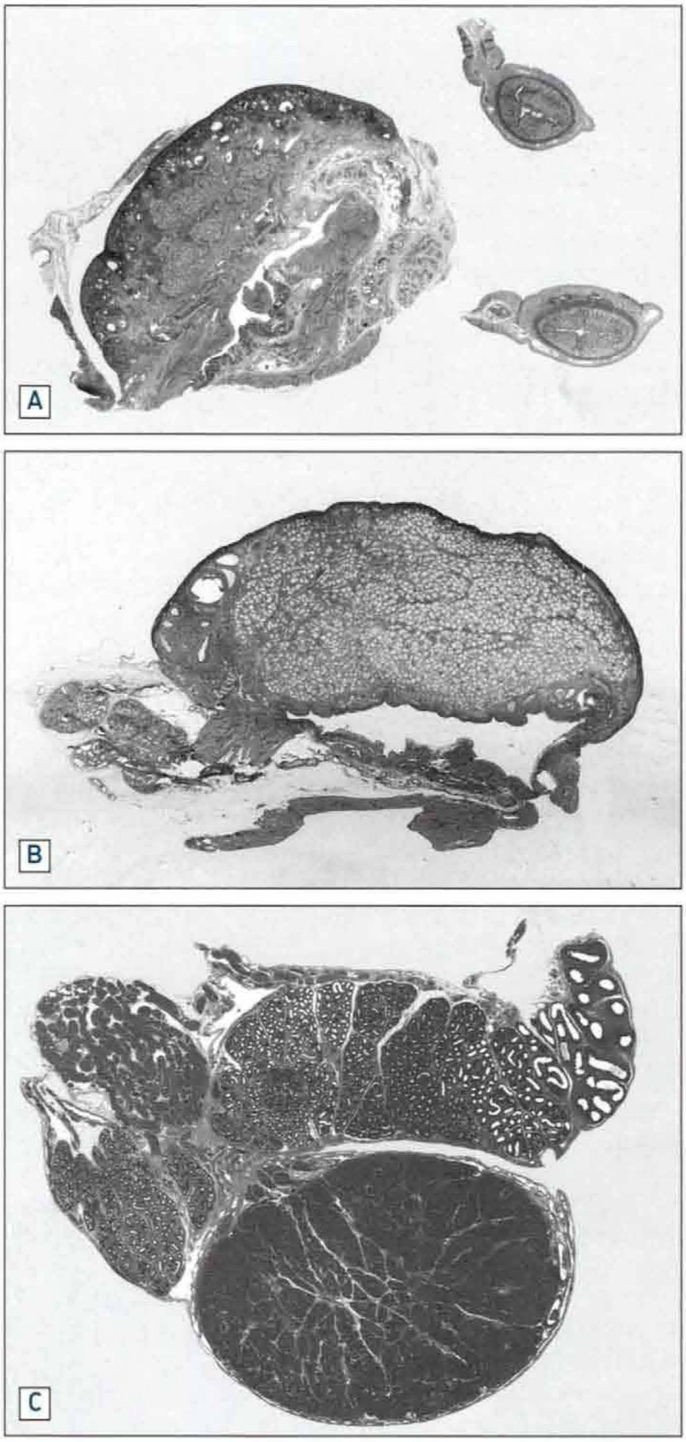

Figure 4.5 Ovotestes from intersex bitches. These three subgross photographs of gonadal sections demonstrate the variability in amounts of ovarian and testicular tissue found in ovotestes. An important pattern to recognize is that ovarian tissues will be found in the cortex and testicular tissues in the medulla. **A.** The ovotestis is mostly ovarian with only a few lobules of hypoplastic seminiferous tubules in the medulla. **B.** The ovotestis is mostly testicular tissue (seminiferous tubules forming a lobular pattern), but also note the presence of antral follicles in the ovarian tissue at the left pole. **C.** The ovotestis is nearly entirely testicular and has a large epididymis attached.

In the canine species, ovotestes are commonly contained within a bursa, and careful inspection reveals the presence of both uterine tubes and epididymal structures (Fig. 4.6).

Examination of the tubular structures from intersex animals commonly reveals the presence of both paramesonephric and mesonephric ducts (Fig. 4.7). In phenotypically female intersex animals, persistent mesonephric duct segments are most commonly found adjacent to, or opposed to, the lateral wall of the cranial segments of the uterus (paramesonephric ductal structure).

Freemartinism

Freemartinism occurs most commonly in cattle. The bovine freemartin is a genetic female born co-twin with a male (twinning occurs in 1–2% of pregnancies in cattle). The twinning is dizygotic in most cases; anastomoses develop between the placental vascular systems of the two fetuses (Fig. 4.8). Normal development of the gonads, tubular and external genitalia of the female embryo is dramatically altered by hormones and cells she receives through the common

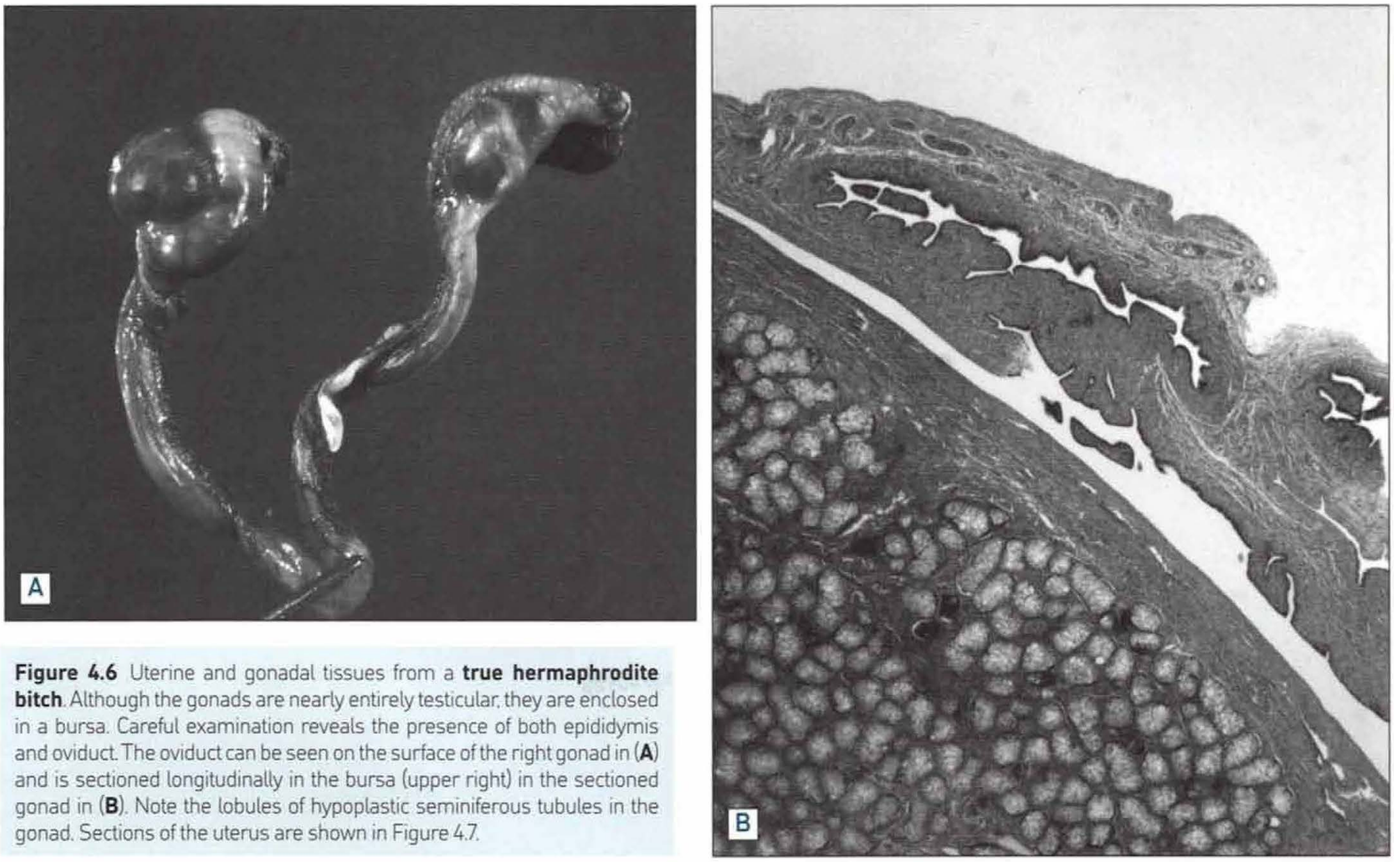

Figure 4.6 Uterine and gonadal tissues from a **true hermaphrodite bitch**. Although the gonads are nearly entirely testicular, they are enclosed in a bursa. Careful examination reveals the presence of both epididymis and oviduct. The oviduct can be seen on the surface of the right gonad in (**A**) and is sectioned longitudinally in the bursa (upper right) in the sectioned gonad in (**B**). Note the lobules of hypoplastic seminiferous tubules in the gonad. Sections of the uterus are shown in Figure 4.7.

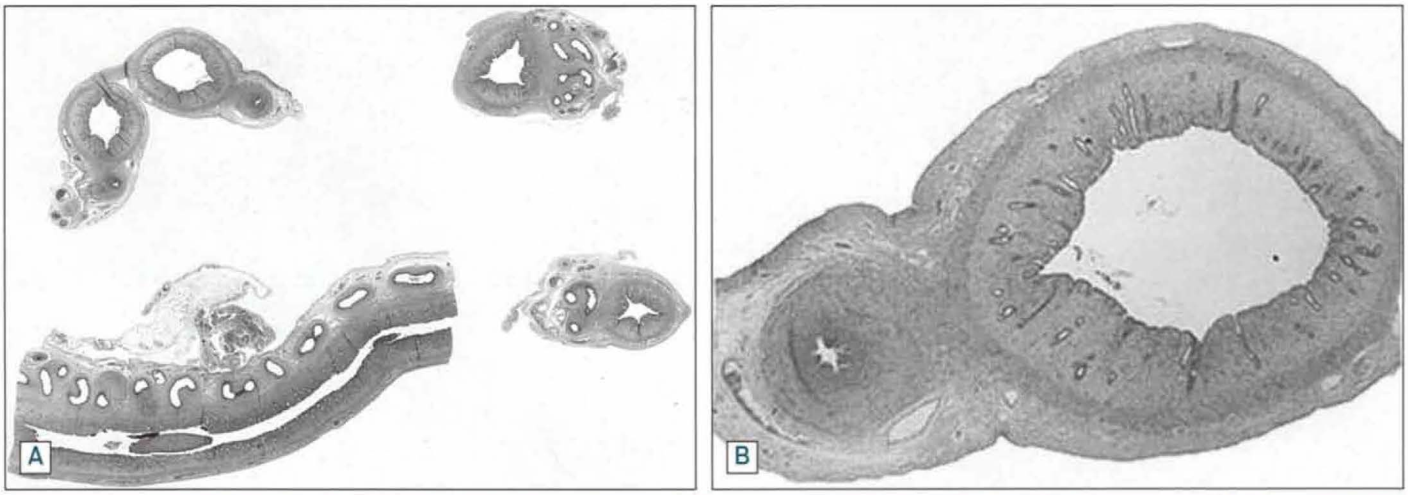

Figure 4.7 A, B. Subgross pictures of sections of the uterine horns from the **intersex bitch** in Figure 4.6. Note the presence of derivatives of both the **paramesonephric and mesonephric ductal systems** (uterine horn and vas deferens, respectively). The hypoplastic vas is present in both the cross-sections as smaller round structures at the point of attachment of the broad ligament (mesometrium) and in the longitudinal section (lower right), where it has been cut multiples times as it runs and bends along the uterine horn. Panel (**B**) is a high magnification of one of the uterine horns showing the small well-developed vas with its smooth-muscle wall and epithelial lining (smaller tubular structure on the left).

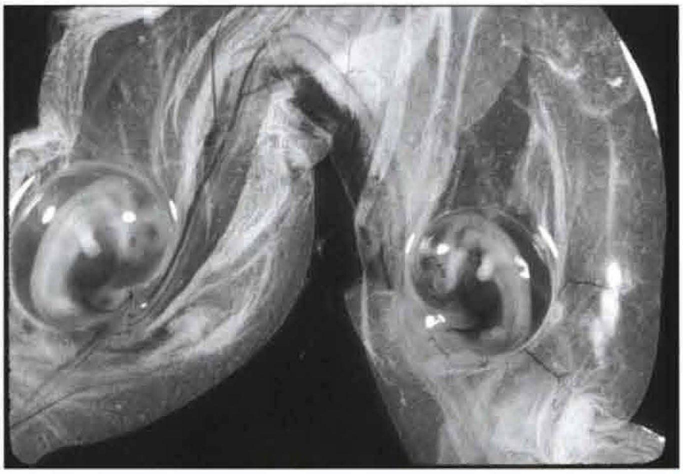

Figure 4.8 Twin bovine embryos contained within their amnions with fusion of branches of their umbilical arteries. Each embryo becomes a chimera and, if the twins are a male and a female, development of the female's reproductive organs will be masculinized; the constellation of changes that develop in her reproductive organs is known as the "freemartin condition."

circulation with her male twin. If anastomoses fail to develop, the female is not damaged, but *nearly all male–female twinning in cattle results in the female becoming a freemartin.*

The effect of the association on the male is always minimal. In the female, the gonads are stunted and frequently contain hypoplastic seminiferous tubules (ovotestes). The uterine horns are hypoplastic, or may be nearly completely absent or lack communication with the vagina (Fig. 4.9). Some parts of the mesonephric ductal system are commonly present. *A consistent feature (and therefore a key diagnostic feature) is the presence of poorly developed vesicular glands that are found attached to the lower fused segment of the paramesonephric ducts* (Fig. 4.10A). The vagina is short and nonpatent. The external genitalia commonly have enlargement of the clitoris, a small vulva, and a prominent tuft of hair (Fig. 4.10B).

Because of the clear association between freemartinism and the presence of interplacental anastomoses, and because the male gonads develop earlier than those of the female, it was presumed that male sex hormones were carried from the male fetus to the female, sterilizing and modifying the latter. The inadequacy of this explanation is demonstrated by the fact that androgens can masculinize a female fetus but they cannot inhibit the ovaries or the paramesonephric ducts. A critical observation was that *embryonic blood of the twins is*

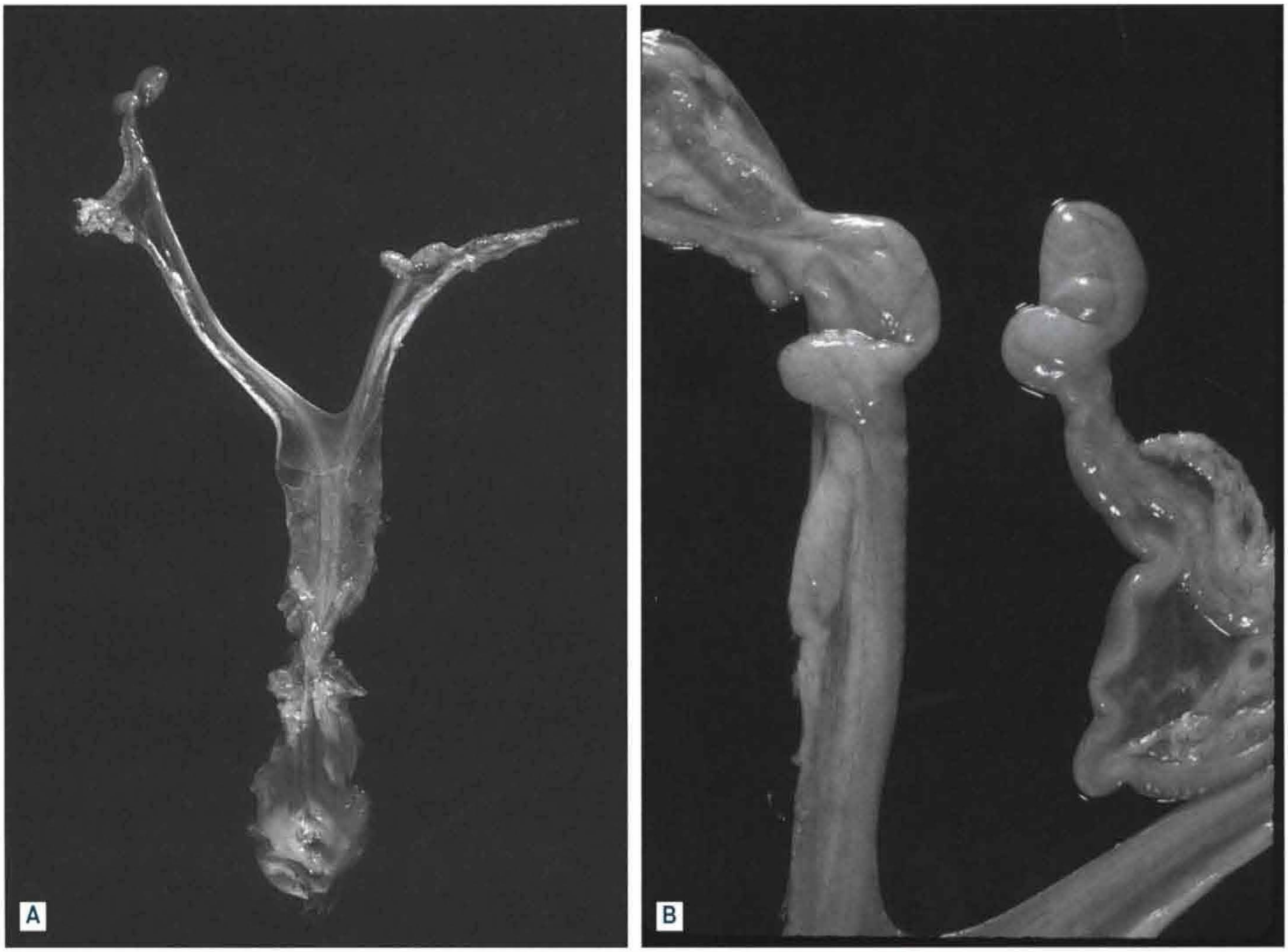

Figure 4.9 A. Reproductive organs of a **newborn bovine freemartin calf**. Note the markedly hypoplastic uterine horns, failure of the paramesonephric ducts to fuse to form a single uterine body, presence of paired seminal vesicular glands, and abnormal gonads. **B.** The gonads are ovotestes and are partially surrounded by epididymal tissues. A uterine tube is seen extending vertically below ovotestis on the right. (Therefore, derivatives of both the paramesonephric (uterine tube) and mesonephric (epididymis) ductal systems are present.)

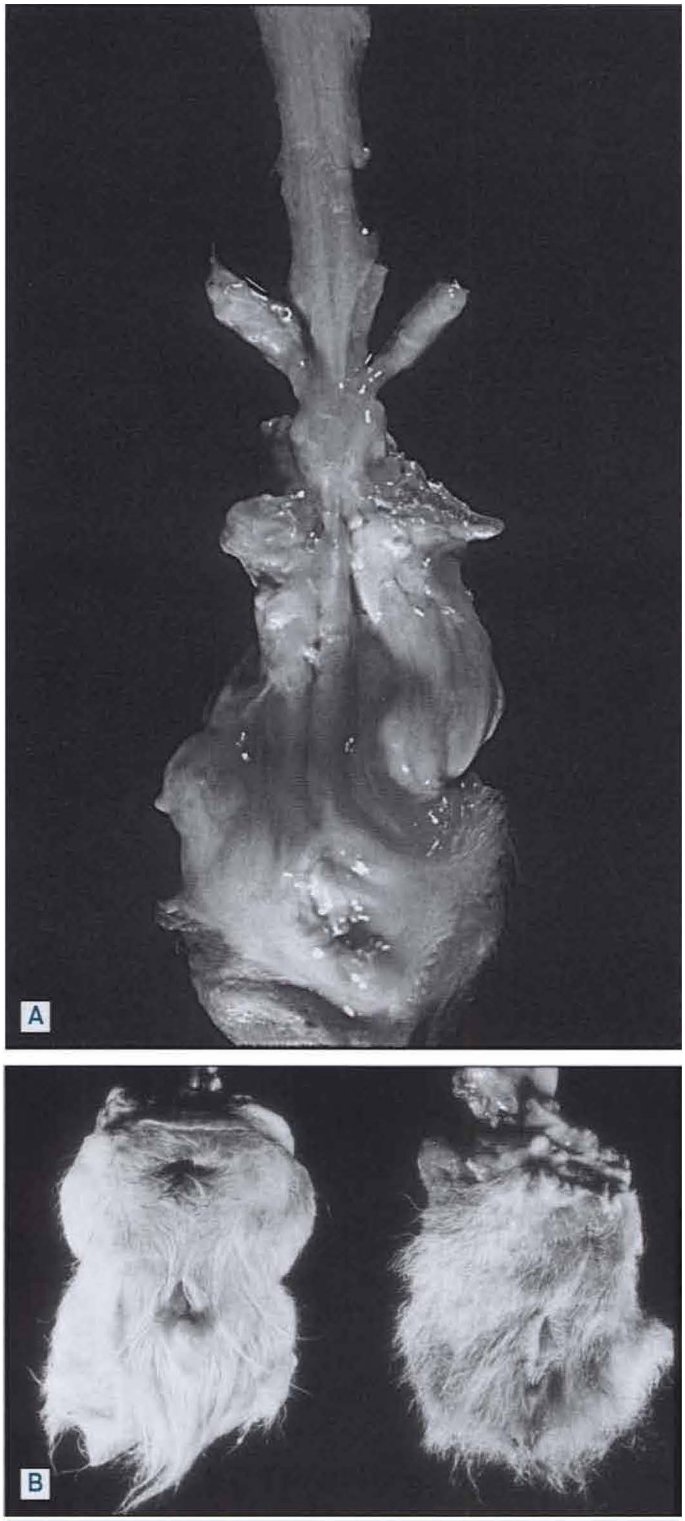

Figure 4.10 The caudal aspect of the tubular genitalia and vulva from the **freemartin heifer calf** in Figure 4.9. **A.** *A key diagnostic feature is the paired hypoplastic seminal vesicular glands* that consistently develop near the caudal segments of the paramesonephric ducts (which may or may not have fused). **B.** Comparison of the external genitalia of a normal newborn freemartin calf on the right and the prominent clitoris and a long tuft of hair attached ventrally to the freemartin calf's vulva on the left.

exchanged and leads to permanent colonization by hematopoietic cells, with the result that each twin develops an acquired tolerance for the blood cells of the other. This condition, known as **chimerism**, appears to be confined to hematopoietic cells. Although unproven, exchanges of nonhematopoietic cells do not appear to take place in freemartinism. *It is now believed that the male fetus sterilizes the female by the partial expression of testis-determining factor of the Y chromosome carried by the bloodstream via the placental anastomoses to the female gonad where it brings about ovarian inhibition.* This inhibition is manifest first functionally; estrogen production by the freemartin ovary is reduced and androstenedione is elevated as early as 40 days of gestation. This is well before morphologic evidence of damage can be recognized.

The gonad of a freemartin is usually a cord-like thickening in the cranial border of the ovarian ligament. Various degrees of modification of the gonad may occur. The rete is well developed, but other ovarian structures may be absent. Alternatively, greatly reduced ovarian tissue can be present but it lacks the normal germ cell complement. The morphologic variation shown in gonadal development is causally reflected in the variation of the tubular genitalia.

The epididymis is absent and the spermatic cord is poorly developed in cases with the lowest degree of male transformation. With increasing degrees of differentiation towards a testis, the spermatic cord and epididymis become better developed. The tubular genitalia, of paramesonephric duct origin, may vary from cord-like structures, without any lumina, to well-developed uterine horns with lumina and endometrial glands. *Communication with the vagina is always absent, no matter how well developed the uterus may be.*

Vestigial seminal vesicles are always present, the vagina is hypoplastic or nonpatent with a complete hymen, the vulva and vestibule are hypoplastic, and the clitoris is enlarged. Many freemartins have a prominent fold of skin on the median plane of the body, extending from a position ventral to the vulva to an area near the umbilicus. The mammary gland fails to undergo normal development.

The allantoic fusion in bovine twin pregnancies occurs at about the 10 mm stage of development. Approximately 90% of females born co-twin with males are freemartins and sterile. Freemartins occur in bovine triplets, quadruplets, and quintuplets as well as twins.

In *sheep*, dizygotic twinning is common and freemartinism does occur, but it is much less common; only 1% of heterosexual twins are freemartins in this species. The reason for this is the infrequency with which large-caliber interplacental anastomoses develop. Freemartinism does occur in *goats*, complete with blood chimerism, but is rare because, as in sheep, large anastomoses between fetuses are rare. Large anastomoses have been demonstrated between chorionic sacs in *swine* and freemartinism occurs in that species. However, because of the large litter size, it is difficult to identify the neighbor of intersex piglets, and the condition has not often been proven. Twin ovulation is relatively common in *horses*. Most twins do not survive to term, but vascular anastomoses with erythrocyte chimerism are frequent in equine twins, although freemartinism has not been convincingly demonstrated.

XX sex reversal

As described earlier, the female role in sexual differentiation is largely passive: the undifferentiated gonad will develop into an ovary unless the testis-determining factor is present, and the undifferentiated tubular genital tract will develop into normal female genitalia unless male hormones are present.

Testis induction during development is controlled by a master gene, the Y-linked *SRY* gene. There are, however, several syndromes of sex reversals in genetic females with XX karyotypes (referred to as XXSR). Much of what we know about these conditions we have learned from the condition in mice in which an autosomal dominant gene, *Sxr*, acts like a Y chromosome. Thus XX mice that carry the *Sxr* gene develop testes and male tubular genitalia. A somewhat similar condition has been observed in dogs, pigs, and goats, although in these species the autosome-associated gene with the Y action functions as a recessive gene.

Sex reversal caused by male differentiating genes on the X chromosomes of females has been reported in over 15 breeds of *dogs*. For some time, this condition has been recognized in the American Cocker Spaniel breed, in which XX phenotypic males and XX true hermaphrodites have been described. Some of the true hermaphrodites have functional ovarian tissue and have given birth to pups, but testicular tissue present in the gonads of either the XX males or the XX true hermaphrodites has invariably lacked germ cells. The condition is inherited as an autosomal recessive. Recent work has shown that XXSR dogs are *SRY*-negative, and has implicated other genes, possible *SOX9*, as being responsible for initiating male differentiation in XX female canine embryos.

The condition has also been studied in *goats* in which the autosomal gene with the Y effect is on the chromosome that controls polling or is closely linked to it. The polled gene is a dominant autosome, a single copy of which produces hornlessness without affecting sex differentiation. In the homozygous condition, however, XX goats are rendered hermaphrodites. They have testes or ovotestes. It has been presumed that the translocation of a subcritical portion of the testis-determining gene to the autosome that contains the gene for polling has produced the recessive mode of sex determination. There is a considerable range of masculinization. The affected individuals may have largely female appearance with only an enlarged clitoris and abdominal testes. Other animals have the appearance of near-normal, although sterile, males.

Sex reversal is common in *pigs*. The mode of inheritance is not well understood, and some cases may have been confused with freemartinism. The masculinizing effect is incomplete. The gonads in

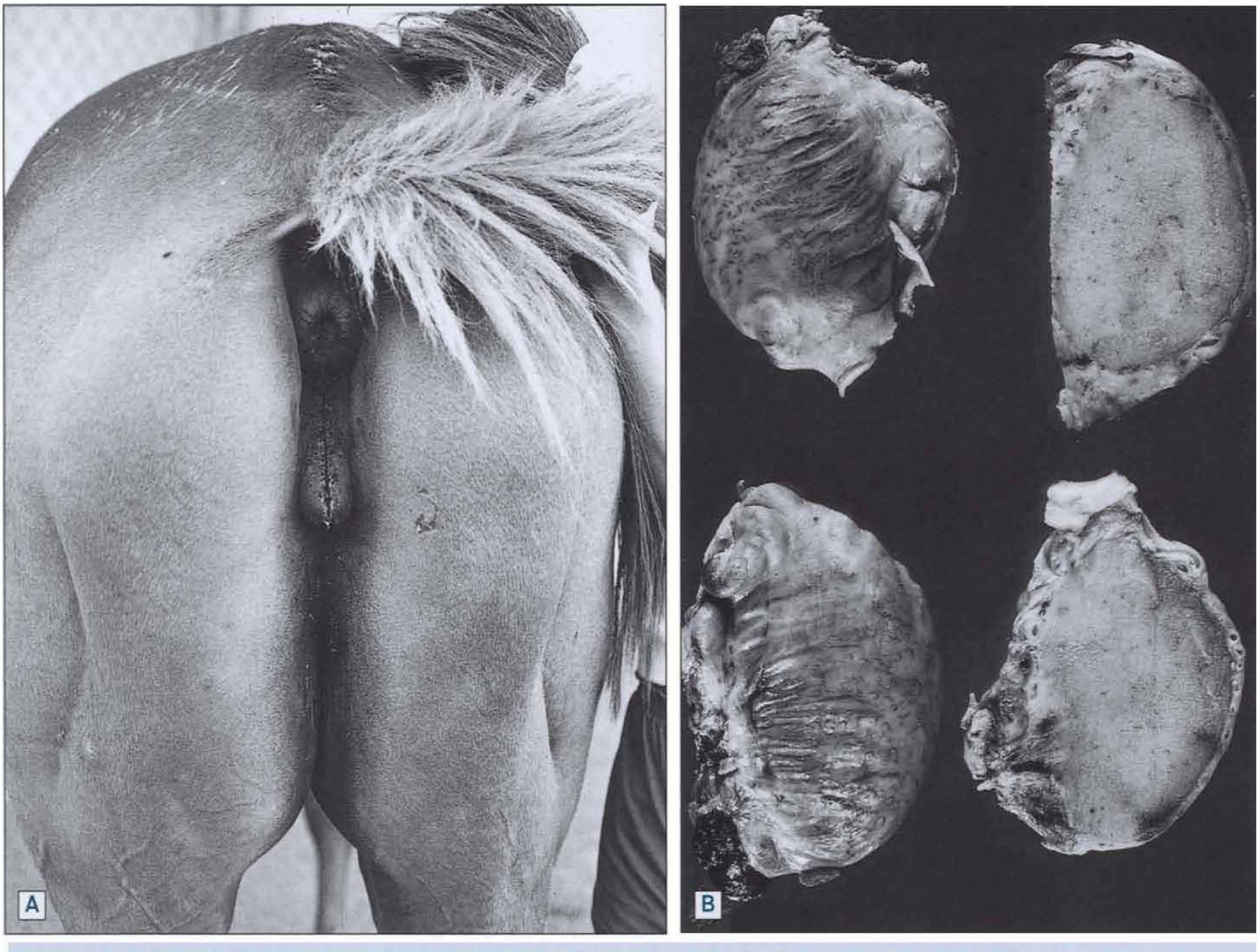

Figure 4.11 A. Androgen insensitivity (testicular feminization) in a mare. The external genitalia appear as a normal mare, but the genetic sex of this animal is male and the gonads are testicular (intra-abdominal, hypoplastic), as shown in panel **B.** The tissues of the external genitalia lack androgen receptors. Even though the genetic and gonadal sex is male and the fetal gonads produce testosterone and the appropriate enzyme systems for conversion of testosterone to dihydrotestosterone, the absence of receptors for dihydrotestosterone in the developing external genital tissues leads to failure of masculinization.

affected individuals are often ovotestes and, although the testicular tissue lacks germ cells, ovulation and pregnancy have been reported. This condition can be distinguished from freemartinism by demonstrating that blood chimerism does not exist in affected individuals.

XY sex reversal in the mare

Individual examples of domestic animals that had XY karyotypes but were phenotypically female have been reported. In the horse, an inherited XY sex-reversal syndrome has been identified. Pedigree analysis suggests that the trait is inherited as the result of an autosomal sex-limited dominant gene or as a Y chromosomal mutation with variable expression. There is a considerable range of phenotypic expression in this syndrome. Affected individuals vary from phenotypically normal but sterile mares with inactive ovaries and normal tubular genitalia to individuals with streak gonads or ovotestes and severely hypoplastic or aplastic tubular genitalia.

Androgen insensitivity

A syndrome of androgen insensitivity has been recognized in humans, mice, rats, cats, cattle, and horses. This inherited syndrome is most often called *testicular feminization* and the *controlling mutation identified as Tfm*. Affected individuals are of XY genotype and have testosterone-producing testes. The external genitalia are female in type. The *Tfm* mutation produces a deficiency of intracellular androgen receptors, rendering all cells insensitive to androgens. MIS is still produced by the testes of affected animals, and therefore paramesonephric duct derivatives are absent because of their sensitivity to this hormone.

Equine male pseudohermaphrodites of this syndrome have normal-appearing female external genitalia (Fig. 4.11A). The vagina ends in a blind sac, with no cervix, uterus, or fallopian tubes being present. The gonads are small and clearly testicular, but present in the normal ovarian position (Fig. 4.11B).

The seminiferous tubules are small and lined by inactive Sertoli cells (Fig. 4.12A). Rare spermatogonia can be found, but spermatogenesis does not occur. Well-differentiated Leydig cells are closely applied to the seminiferous tubules (Fig. 4.12B).

Horses with the syndrome have no detectable male accessory sex glands. The cattle that have been described have rudimentary seminal vesicles and ampullae. Both cattle and horses that lack androgen receptors have normally formed female mammary glands. Horses are unique among the species in which the syndrome has been

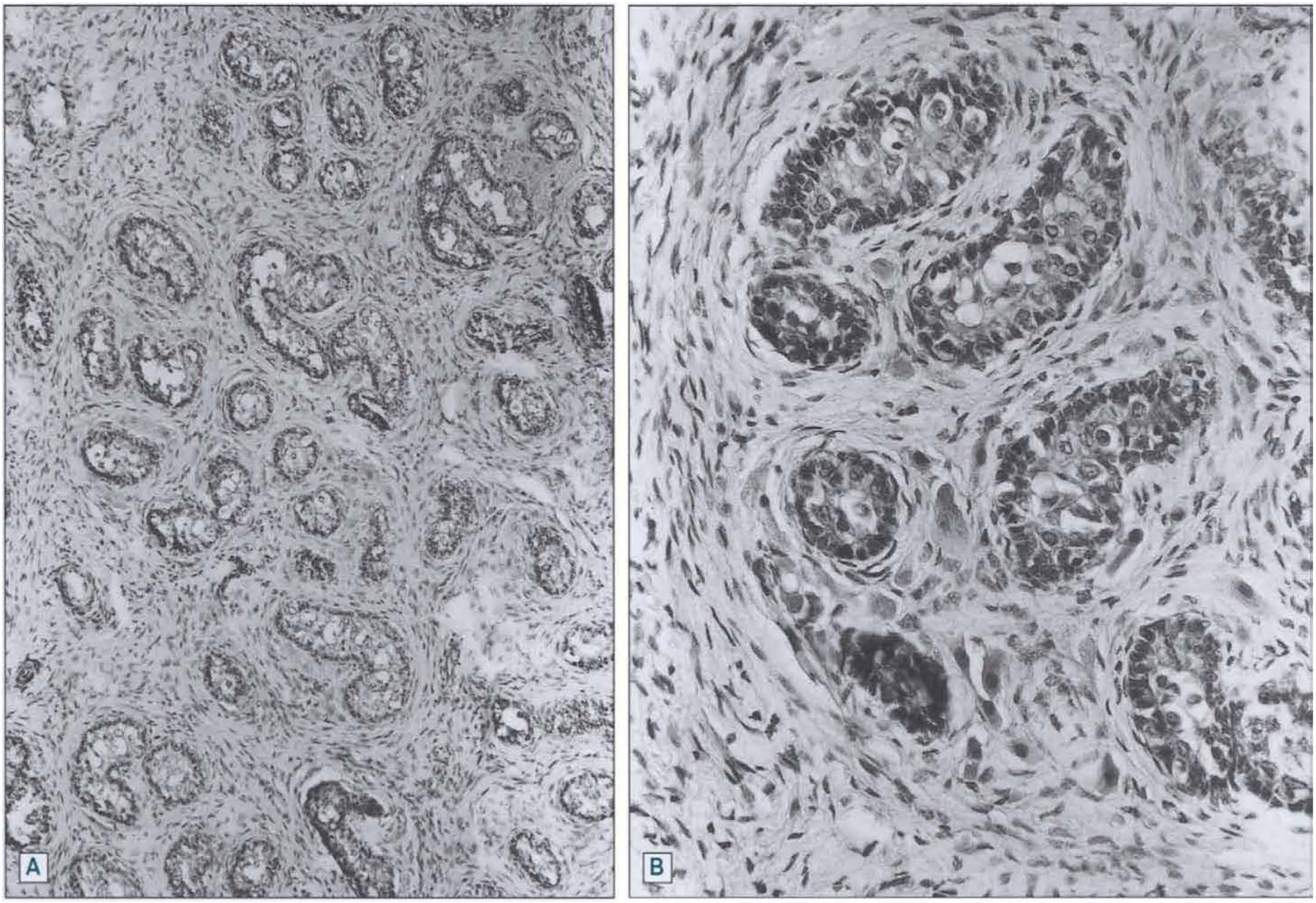

Figure 4.12 A. Photomicrograph of tissues from the **intra-abdominal testicle** from the mare in Figure 4.11 demonstrating the presence of many interstitial cells in the stroma separating hypoplastic seminiferous tubules. The interstitial cells produce testosterone that is converted to dihydrotestosterone, which causes the phenotypic masculinization in those tissues containing the appropriate receptors. **B.** Higher magnification of hypoplastic seminiferous tubules.

reported in that affected individuals show male behavior patterns. This syndrome of male pseudohermaphroditism can be distinguished from others by assaying the cytosol receptors of the androgen target cells. This can be done most conveniently by culturing fibroblasts from the genital skin. It is important that labial tissue be used, because receptor activity is sharply reduced in nongenital skin.

Ovary – developmental anomalies

Agenesis of one or both ovaries is rare, but has been observed in ruminants, swine, and dogs. In bilateral agenesis, the tubular genitalia may be absent as part of the defect or, if present, are infantile or underdeveloped.

Duplication of ovarian tissues is very rare. Although there are no controlled studies, duplication may in reality be splitting of ovarian gonadal tissue. Ovaries from a young calf with ovarian duplication are shown in Figure 4.13.

Ovarian remnants: interest in the occurrence of anomalous ovarian duplication commonly arises when either cats or dogs, supposedly surgically neutered, exhibit signs of estrus. This is referred to as the *ovarian remnant syndrome.* In the bitch, the effects of circulating estrogen can be relatively easily detected by doing serial vaginal cytology. Ultrasonographic and exploratory examinations are done when the animal is in estrus and has large follicles, or during the luteal phase of the cycle, when corpora lutea would also make ovarian tissue easily identified. Although occasionally proposed, there is little information concerning whether small pieces of ovarian tissue inadvertently released during canine or feline surgery might be able to implant on the peritoneal surface in the abdomen.

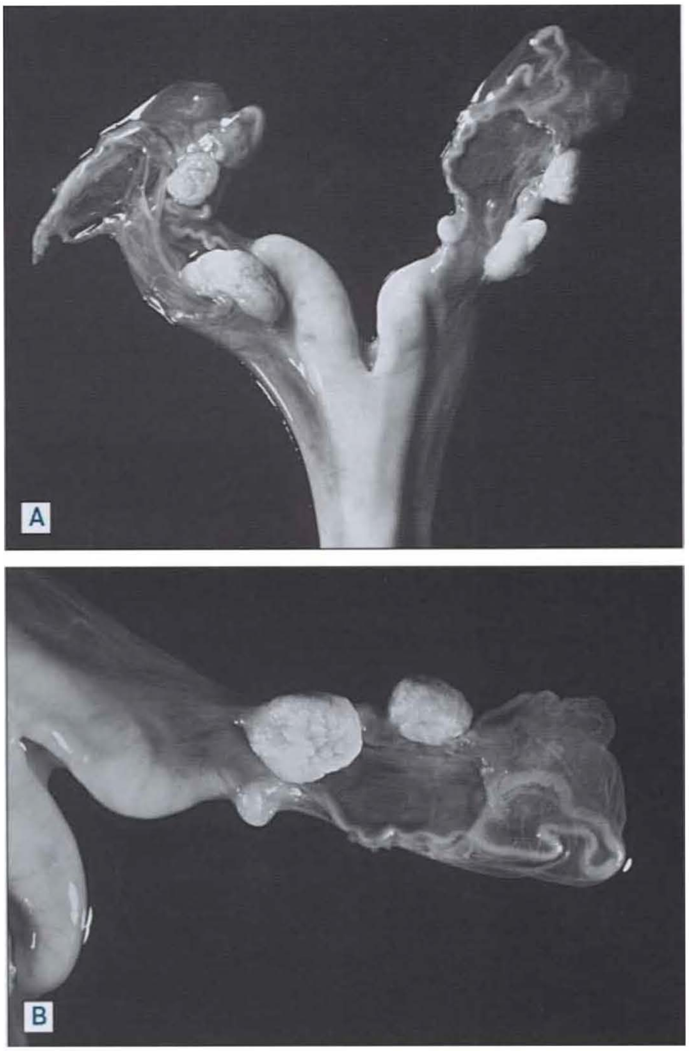

Figure 4.13 A. Duplication of the ovaries occurs very rarely. The gonads in this newborn calf were each divided, giving the impression that two separate gonads had formed. An isthmus attaches the pair of ovaries. **B.** The two pieces of the left gonad are more clearly shown.

Vascular hamartomas of the ovary have been described in swine and cattle. Since they are often confused with neoplasms, they are described in the section on ovarian tumors (see p. 456).

Hypoplasia of the ovaries has been studied primarily in cattle, but occurs in other species. *It is usually bilateral but varies considerably in its severity and symmetry,* so that "severe hypoplasia" or "partial hypoplasia" may be applicable to one or both ovaries. In severe hypoplasia, the defective gonad varies in size from a cord-like thickening in the anterior border of the mesovarium to a flat, smooth, firm, bean-shaped structure in the normal position. There are neither follicles nor luteal scars and, microscopically, the ovary is largely composed of medullary connective tissues and blood vessels.

Ectopic adrenal tissue is usually found incidentally during histopathology. These small nodules are composed of adrenal cortex and are most commonly found on the surface of the ovary of mares or adjacent to the ovary of queens (Fig. 4.14).

Cysts arising from remnants of developmental structures

Cystic lesions in and near the ovary and uterine tubes are common, especially in the mare. Figure 4.15 demonstrates the embryonic origin of the most common of these cysts.

Cysts arising from either the cranial or caudal segments of the mesonephric tubules are found as thin-walled, movable cysts at either the cranial or caudal pole of the ovary and are called **cystic epoophoron or paroophoron**, respectively. Cystic epoophorons are more common and can become quite large, especially in the mare (Fig. 4.16), and are discussed below. **Cystic rete** are common

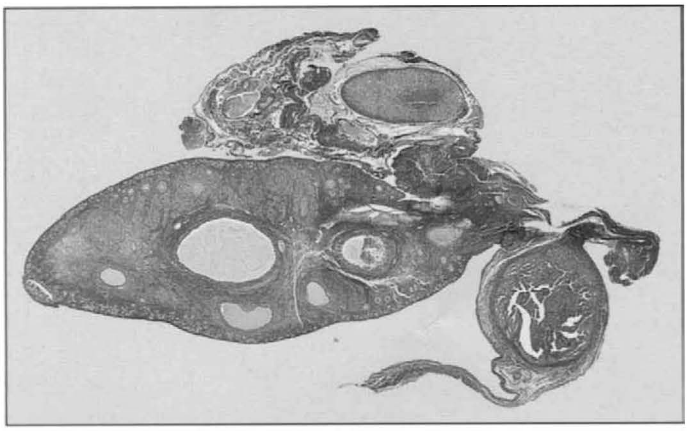

Figure 4.14 Ectopic adrenal tissue is most commonly found adjacent to, or attached to, the tunic of the ovary of the queen and mare. A nodule of ectopic adrenal tissue (upper right) is evident adjacent to the ovary and uterine tube in this subgross photograph of tissues from a queen.

in the cat and dog, and arise from the rete ovarii, which are also derived from the mesonephric tubules. These arise in the hilar area of the ovary. All derivatives of the mesonephric tubules commonly have variably prominent smooth muscle in their walls. The epithelial lining is cuboidal in small cysts, tends to become squamous in slightly larger cysts, and is usually absent due to pressure atrophy in larger cysts. Only the cystic rete commonly progress to compress ovarian tissues to such a degree as to compromise ovarian function. Adenomatous hyperplasia of the rete epithelium is common, and cystic adenomas occur, most commonly in the bitch.

By far the most common type of cyst arising from remnants of the embryonic ducts or tubules is found in fillies and mares. These are *cystic apical segments of the paramesonephric duct*. These are also called **fimbrial cysts** and appear as one or two thin-walled cysts, up to 1 cm in diameter, that are attached to the fimbria of the uterine tube (Fig. 4.17). Careful examination of the fimbria during necropsy will reveal that these are very common. During clinical examinations, they may be detected by palpation or by ultrasonography, but do not reduce fertility. The differential diagnosis is a cystic remnant of the mesonephric duct, but these are found adjacent to the uterine tube or along the lateral border of the uterine tube, uterine body, or in the cervix or vagina.

Mesonephric duct remnants are frequently found as small cysts lying along the lateral side of the uterine tubes, uterine horns, uterine body, cervix, or cranial vagina. They are in or near the attachment of the mesosalpinx or mesometrium of the uterine tube and uterine horns, but those found in the uterine body and cervix tend to be found within the myometrium, and those in the vagina are commonly multiple or linear cystic structures in the submucosa. Intersex animals with a predominantly female phenotype often have remnants of persisting segments of mesonephric ducts that are located in the attachment of the broad ligament and run parallel with the uterine horns (Fig. 4.18). These cystic remnants of the mesonephric duct have the microscopic appearance of the vas deferens and have

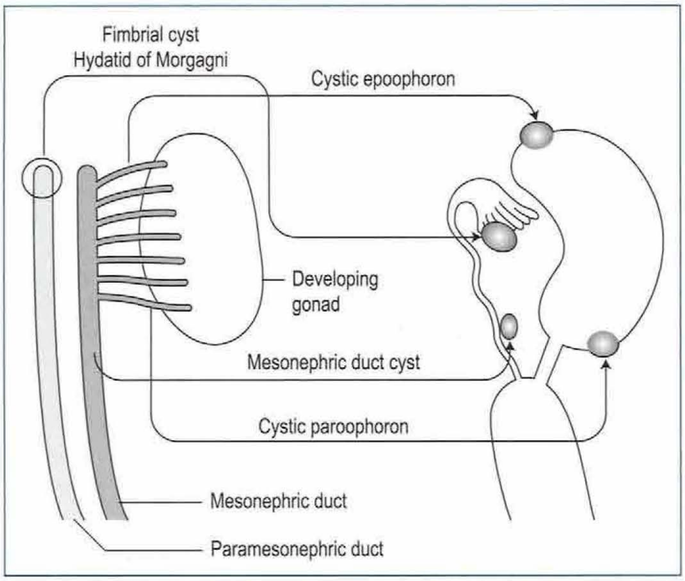

Figure 4.15 Cysts in or near the ovary and along the uterus are common, but usually incidental findings. The embryonic origin of several common cysts is shown in this drawing. Details are provided in the text and examples of some of these cysts as they occur in different species are shown in the following figures. (Illustration by Michael Simmon.)

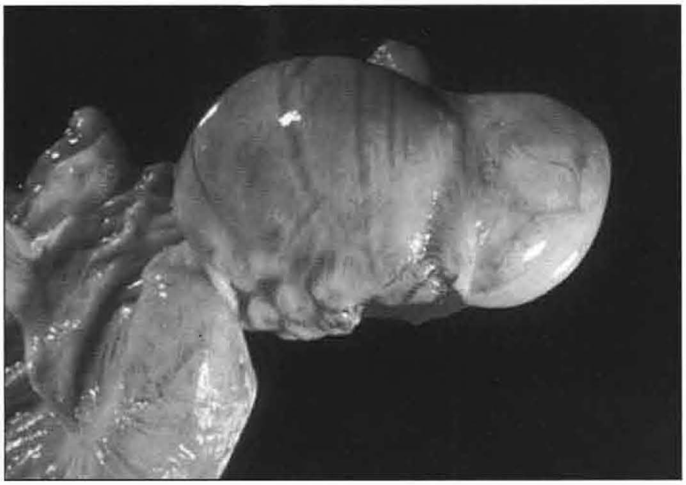

Figure 4.16 A large **cystic epoophoron** extends from the proximal pole of the ovary of a mare. These cysts arise from remnants of the cranial mesonephric tubules, and although they can become large, do not reduce fertility. They are palpable per rectum and can be seen during ovarian ultrasonography.

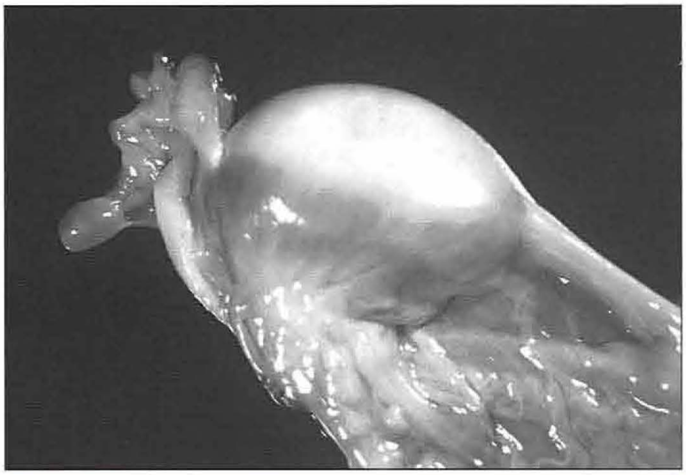

Figure 4.17 Paramesonephric duct cyst (fimbrial cyst) extending from the fimbria of the uterine tube of a young filly.

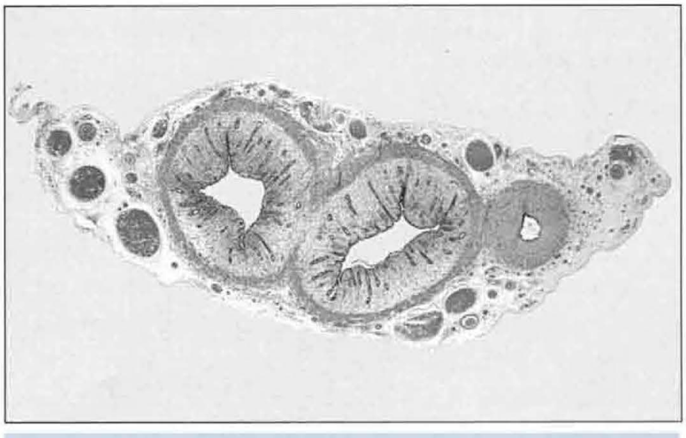

Figure 4.18 Cross-section of two uterine horns and **unilaterally persistent mesonephric duct** (right side) from an intersex bitch. These remnants occur as either cysts or short segments of hypoplastic vas deferens.

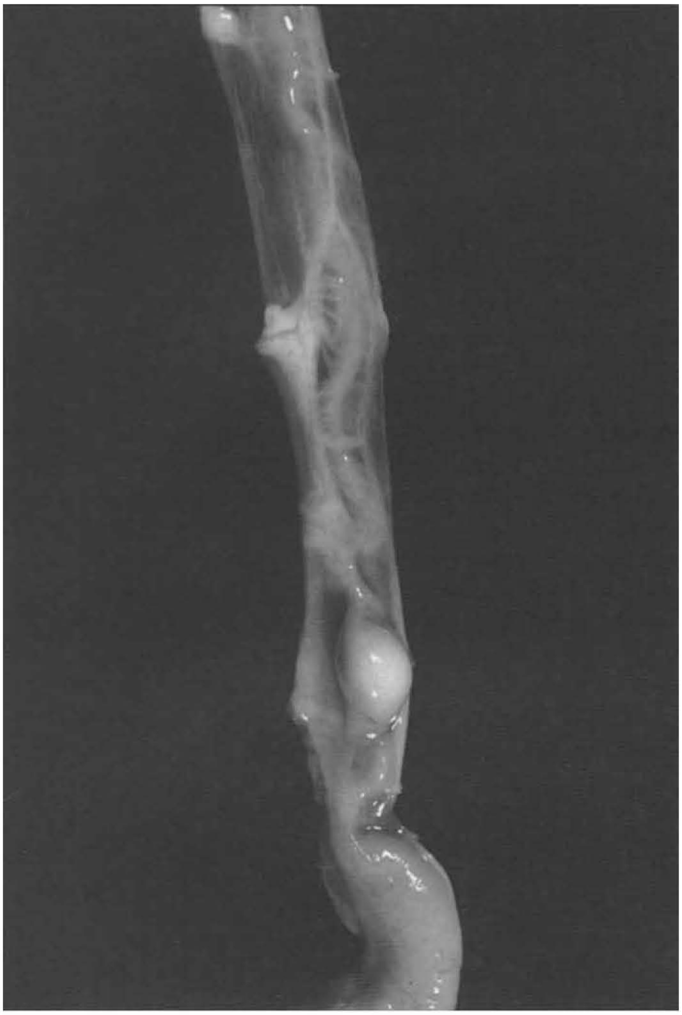

Figure 4.19 Cystic remnant of a mesonephric duct adjacent to the uterine tube from a cow.

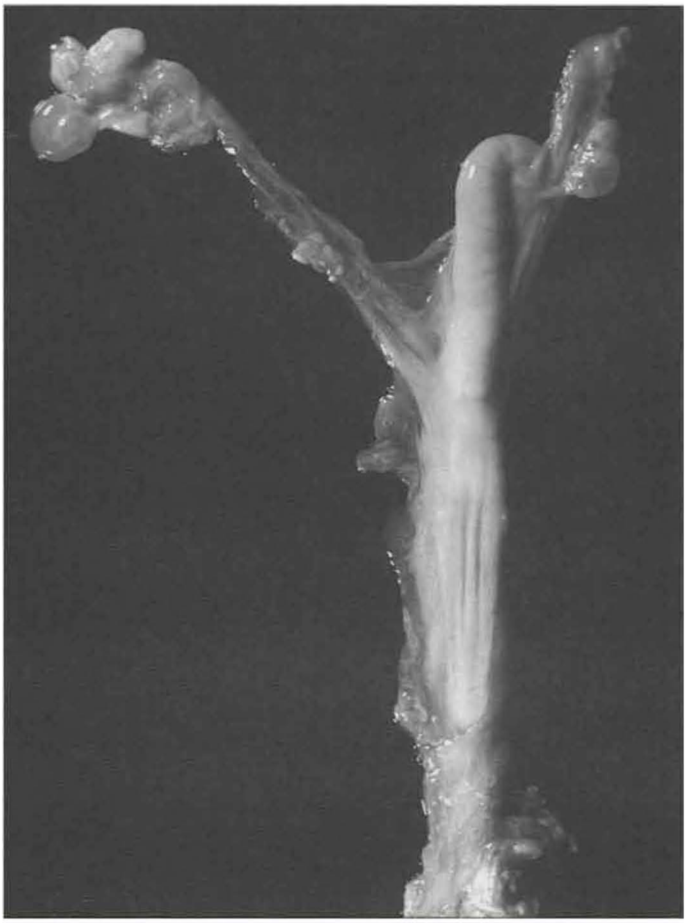

Figure 4.20 Segmental aplasia of the left uterine horn of a heifer caused by failure of a segment of the paramesonephric duct of this side to develop normally. Also note the hydrosalpinx.

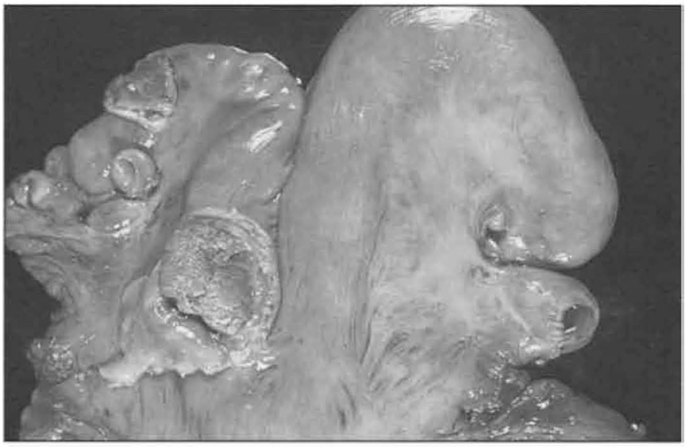

Figure 4.21 Segmental aplasia of the left uterine horn of an adult cow opened to demonstrate soft tan **concretions** formed from accumulated uterine secretions and sloughed cells.

a lumen lined by epithelial cells and a relatively prominent small muscle wall.

Cystic mesonephric duct remnants are common in the cow. The fluid they contain is usually clear, but may be opaque (Fig. 4.19).

Arrests in the development of the paramesonephric ductal system

There are three patterns of defect:

1. failure of segments of the paramesonephric ducts to develop,
2. failure of the caudal parts of the two paramesonephric ducts to fuse appropriately and develop a single lumen,
3. failure of the caudal ends to the fused paramesonephric ducts to fuse with the invaginated urogenital sinus, leading to failure of the lumen of the fused ducts to become continuous with the caudal vagina.

Segmental aplasia of the paramesonephric duct system is found as either failure of short or long segments of the uterine horn to develop (Fig. 4.20). This was commonly found in white Shorthorn cattle and gave rise to the name "white heifer disease" for the syndrome, but no breed is exempt from the condition. The arrested development is thought to be autosomal recessive. Complete absence of an entire horn is referred to as **uterus unicornis**.

Within the isolated segments of uterus, secretions and sloughed epithelial cells become inspissated and often form soft tan concretions within the distended isolated lumen of the uterine horn proximal to the area of segmental aplasia (Fig. 4.21).

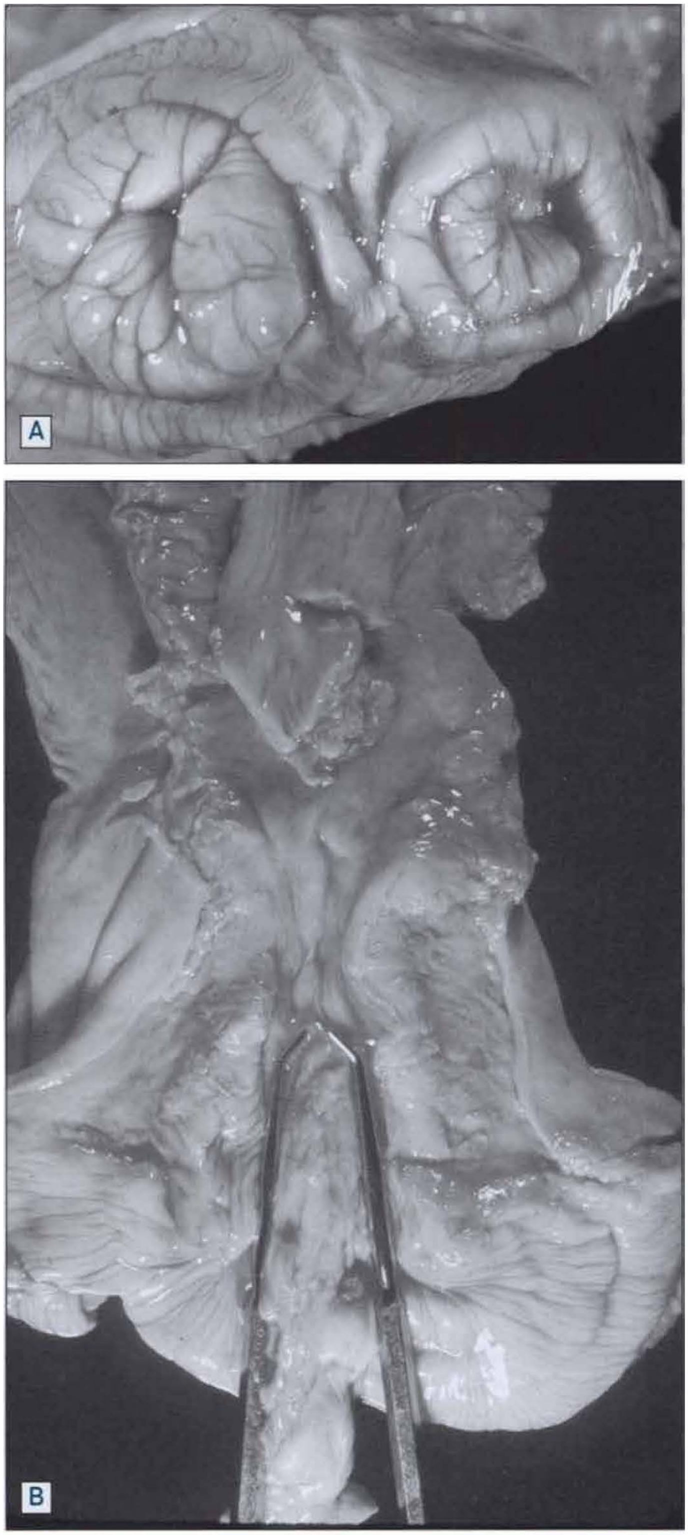

Figure 4.22 **A. Double cervical os** (as viewed from the vagina) caused by failure of the mesonephric ducts that normally join and fuse during embryonic development to do so. **B.** The defect as demonstrated in the opened uterus and cervix in (**B**) is common. A single lumen in the uterine body (normal: formed by fusion of the two paramesonephric ducts) and two separate cervical lumens (demonstrated by the probes placed in the two cervical lumens) are seen in the most common form of this defect in cattle. Complete division of both the uterine body and cervix is less common and is called *uterus didelphus*.

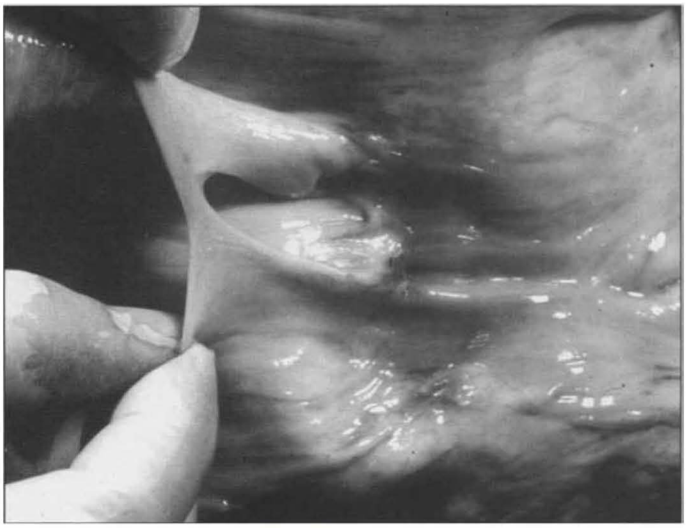

Figure 4.23 **Incomplete hymen** in a young heifer. The hymen is a persistent membrane of tissue that was formed by the inward expansion of the urogenital sinus on one side (forming the vagina) and the caudal development of the joined paramesonephric ducts on the other. Complete or partial hymen is commonly found in young calves, and occasionally in young females of other species. They are usually torn during breeding or parturition, usually presenting no clinical problems.

Failure of the paramesonephric ducts to fuse is most commonly found in the cow and usually involves the cervix, and less commonly the uterine body. Partial failure of the paramesonephric ducts to fuse with loss of the joining wall to form a single lumen is more common than complete failure to fuse. An example of the more common appearance is shown in the tissues in Figure 4.22. The uterine body and cranial cervix have developed normally, but there is a median septum in the cervix, leaving two cervical canals and the appearance of two cervices. The apparent septum is the medial walls of the two paramesonephric ducts that failed to regress.

Less commonly, there may be failure of the caudal aspects of the paramesonephric ducts to fuse. This occurs on occasion in the bitch and results in the presence of a longitudinal median band of tissue extending from the mid dorsal to the mid ventral walls of the vagina cranial to the urethral orifice.

Failure of fusion of the paramesonephric ducts with the urogenital sinus results in persistence of a tissue band running across the vagina just cranial to the opening of the urethra. This tissue forms an **imperforate hymen** that can either be complete, or more commonly is only a small piece of the wall (Fig. 4.23). In association with imperforate hymen, the remainder of the genital tract may be normal, but if the hymen is complete, in time and with the accumulation of secretions (there may be 10 liters or more), the vagina, cervix, and uterus become distended and atonic. If the hymen is perforated and the distension relieved early, the genital tract may function normally, but prolonged and severe dilation leads to atrophy and permanent loss of tone of the walls. Bacteria may be fortuitously implanted in the uterine contents to produce pyometra.

Moderate **hypoplasia of the cervix**, in which some rugae are absent and the canal widely patent, may militate against functional closure of the canal and predispose to persistent invasion of the uterus by microbes, and chronic endometritis. Occasionally, the cervical canal is irregular in its course or even tortuous. Although

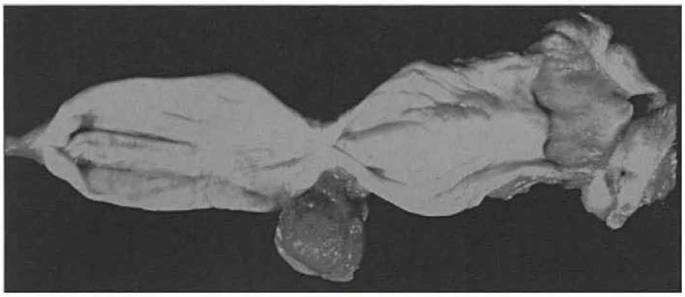

Figure 4.24 Segmental stenosis of the tubular genitalia is uncommon in dogs or cats. The middle region of the vagina of this bitch was completely occluded and the lumen of the cranial vagina was distended with tan fluids.

this may not interfere with conception following natural breeding, insertion of an inseminating catheter may be difficult or impossible. Repeated attempts to pass a pipette can lead to cervical trauma, occasionally complicated by formation of traumatic inclusion cysts or abscesses.

Dilations and diverticula of the cervix have been observed as a cause of infertility in heifers. The malformations occur at the level of the third and fourth rugae, and the cervical canal is usually constricted caudal to the defect. The dilated areas are usually spherical and located near the internal os. Diverticula are dorsal or dorsolateral and eventually become filled with tenacious mucus. The cause of the condition has not been established, but it is assumed to be developmental.

Vaginal anomalies

Vaginal stenosis is uncommon and may be either congenital or acquired. The stenosis in Figure 4.24 was complete and had caused dilation of the proximal vagina.

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PATHOLOGY OF THE OVARY (NONDEVELOPMENTAL LESIONS)

Reproductive organs of sexually mature animals normally undergo dramatic changes during the estrous cycle. These morphological changes associated with follicular development, ovulation, luteinization, and regression of the corpus luteum are referred to as the **ovarian cycle**. Species variations exist that can potentially be very confusing, both during gross and microscopic examination. Albeit beyond the scope of this chapter, the importance of acquiring basic information from the relevant literature, including basic texts on animal reproduction and theriogenology, cannot be overemphasized.

Miscellaneous lesions

Intrafollicular hemorrhage occurs commonly in calves and is also found in follicular cysts in the bitch, and occasionally in atretic follicles of cows. Hemorrhage occurs during ovulation in all species of domestic animals, but is usually minimal and largely confined to the cavity of the collapsed follicle. However, it can be severe and occasionally even lethal in the mare. In the mare in the autumn at the beginning of the period of seasonal anestrus, hemorrhage occurs into anovulatory follicles ("autumn follicles").

Focal areas of serositis develop adjacent to corpora lutea, indicating that they occur following ovulation. The recently formed strands of tissue, which are referred to as **ovulation tags**, are composed of fibrin, proliferating capillaries, and leukocytes. As the lesion regresses, neutrophils are replaced by lymphocytes, macrophages, and plasma cells, and mesothelial cells cover the tags. Fine bursal adhesions may result, but they are too delicate to interfere with ovulation or the passage of ova into the oviduct. Ovulation tags occur in all species, but most frequently in the cow and mare. Scars that form on the surface of the ovary are small and of no consequence.

A significant form of **ovarian hemorrhage** is that which follows the older clinical practice of manual enucleation of corpora lutea in cattle. The blood loss may vary from 0.5 liters to several liters and cause death. Hemorrhage is more profuse in pregnant cows and those with pyometra than in normal cycling cattle. In cases of pyometra and salpingitis, the release of inflammatory detritus into the bursa often results in the formation of extensive adhesions between the oviduct and ovary. The expressed mass of luteal tissue persists indefinitely as a roughly spherical, flattened soft mass. The mass develops a fibrous capsule enclosing the necrotic luteal cells and can be differentiated from a lipoma.

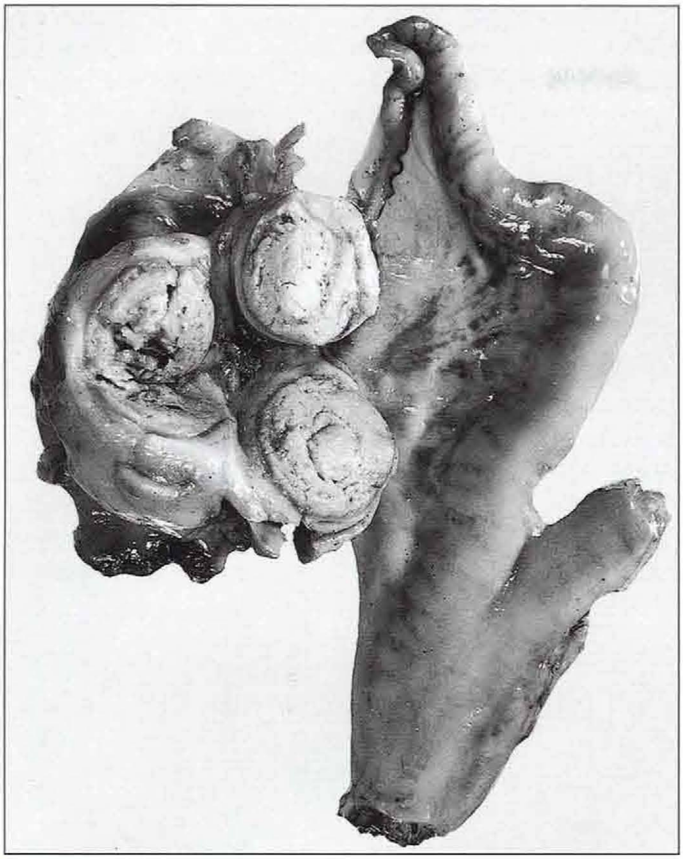

Figure 4.25 Caseous oophoritis caused by *Corynebacterium pseudotuberculosis* in a ewe.

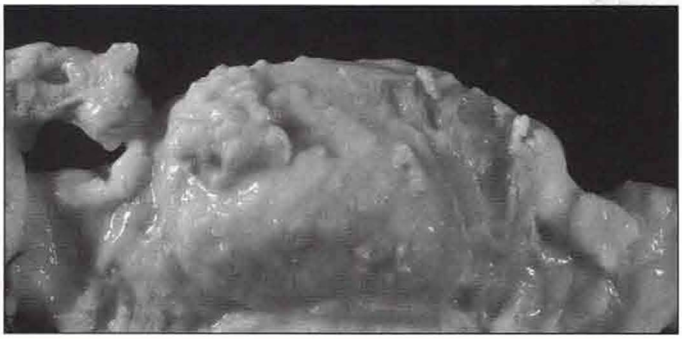

Figure 4.26 Purulent oophoritis nearly completely destroying the ovary of a cow. The cow also had suppurative salpingitis and metritis.

Postparturient vascular lesions occur frequently in the bovine ovary. The changes consist of intimal proliferation of mucoid tissue in ovarian arteries and hyalinization of the walls of arteries and arterioles. Some of the more severely affected vessels occasionally undergo thrombosis. Degeneration of arterioles also occurs in rapidly regressing corpora lutea associated with abnormally short estrous cycles.

Oophoritis is relatively rare and, when it occurs, it is usually pyogenic (Fig. 4.25).

Abscessation of the ovary may follow enucleation of the corpus luteum in cows with pyometra or from oocyte retrieval using needle aspiration (Fig. 4.26). Serosal granulomas on the ovary occur in bovine peritoneal tuberculosis and in brucellosis, visible macroscopically as small red nodules or tags. Similar lesions are usually visible on adjacent serosal surfaces of the genital organs and adnexa. These infective granulomas remain localized to the surface of the ovary and do not penetrate its substance.

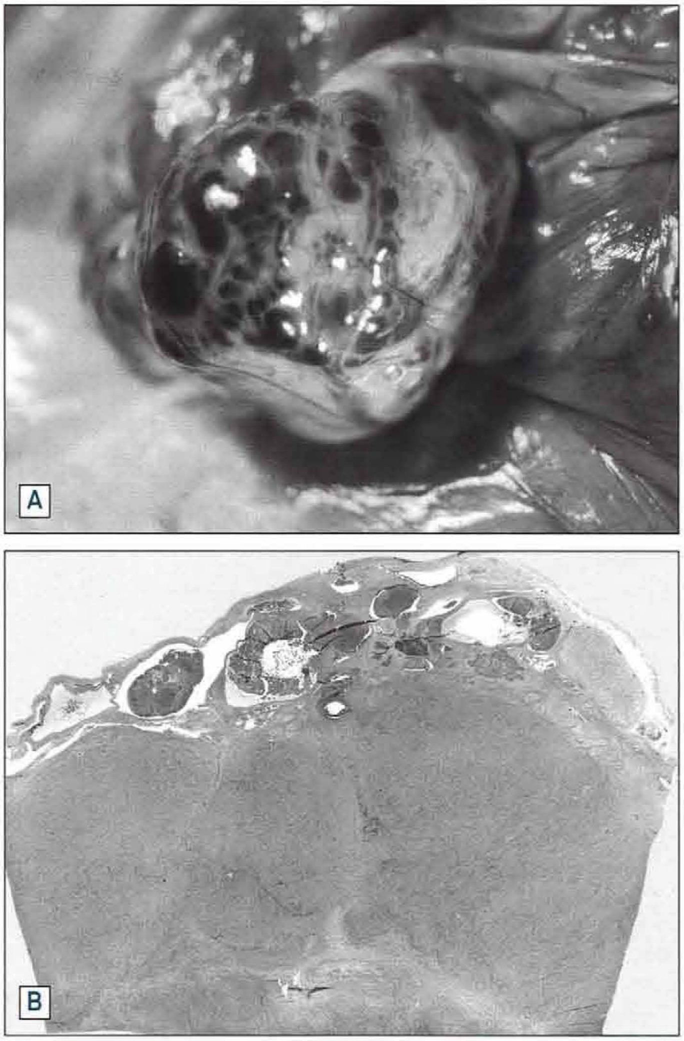

Figure 4.27 A. Varicose veins on the surface of the ovary of an older mare. **B.** Subgross photograph showing distended veins on the surface of this ovary.

Age-related degenerative changes

Equine ovarian varicosities

The surface of the equine ovary is richly endowed with veins that, with age, commonly become varicose. Thrombi may form, and if extensive can lead to infarction, or ischemic damage to the ovary. Ovarian varicosities do not usually occur in other species. They appear as dark, nearly black serpentine fluid-filled tissue on the surface of the ovary (Fig. 4.27).

Ovarian cysts

Cysts arise either within, or adjacent to the ovary. Several cysts that develop from embryonic structures, the mesonephric duct, mesonephric tubules, and paramesonephric duct, were discussed above where an illustration (see Fig. 4.15) was used to summarize the embryonic structures from which some common cysts arise. These cysts will be briefly discussed in this section.

There are three types of **cysts that develop from mesonephric tubules**: cystic rete, cystic epoophoron, and cystic paroophoron. **Cystic epoophoron** (see Fig 4.16) develops from the cranial mesonephric tubules, whereas **cystic paroophoron** occurs less commonly and is found on the caudal surface of the ovary. Because these cysts are found near the ovary, they are casually referred to as *paraovarian cysts*. More precise identification can be achieved either through closer examination (the location relative to the ovary is very helpful), or by histopathology. Cysts of the epoophoron and paroophoron are movable cysts on the cranial surface of the ovary and have a thin wall of connective tissue and muscle fibers, are lined by low columnar epithelium with clear cytoplasm, and have a basement membrane. Follicles may protrude from the surface of the ovary, but are not movable.

Cystic rete (Fig. 4.28) develop from mesonephric tubules that form the rete ovarii within the ovary. Less commonly, cystic extraovarian rete are found. **Cystic rete tubules** have been observed in the bitch, queen, and cow. Those in the cow seldom attain significant size, but those of the bitch and queen become large enough to be confused with cystic follicles. Cystadenomatous tumors of the ovary may arise focally in these cysts.

Cysts of paramesonephric duct origin are very common in the mare. They are located on the fimbria of the oviduct and are called *fimbrial cysts, hydatids of Morgagni, or accessory oviducts.*

Cystic subsurface epithelial structures (cystic SES) of the bitch. The modified peritoneal cells covering the surface of the ovary of the bitch normally extend into the ovary a short distance where they are arranged as small single epithelial-lined cavities called "subsurface epithelial structures" or SES. Their importance is twofold. They frequently give rise to single, or more commonly, multiple cysts extending along the ovarian surface, and SES occasionally undergo neoplastic transformation. Neoplasms of the SES are usually adenomas, but carcinomas do occur. These tumors tend to form cysts and have a folded, papillary pattern.

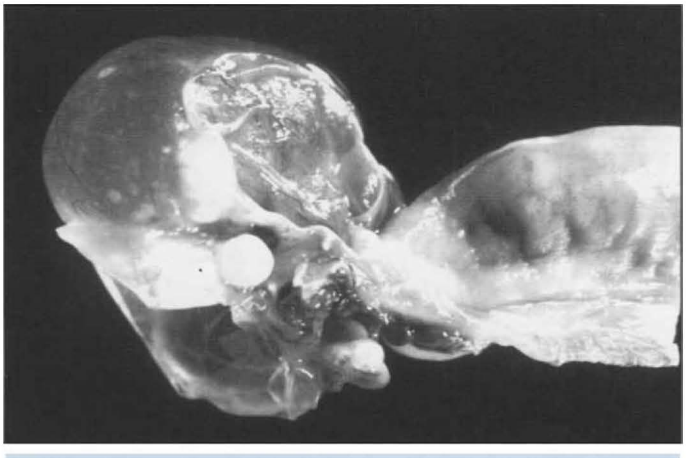

Figure 4.28 Cystic rete ovarii have replaced and compressed the remaining ovarian tissue in this cat. Cyst rete are common in queens and frequently occur in bitches. They develop in the hilar area of the ovary, a useful feature in identifying them both grossly and microscopically.

The incidence of cysts of the SES increases with age. They are small, seldom larger than 5 mm in diameter, and can be located anywhere within the ovary, but their relationship to the surface is usually obvious. These cysts are lined by cuboidal epithelium. They do not appear to have any effect on fertility but, since the SES are hormonally sensitive, cyst formation may be associated with hormonal dysfunction. The lining of the cysts, like the surface epithelium and the SES, expresses cytokeratin and this allows differentiation from atretic follicular cysts.

Germinal inclusion cysts of the mare

Entrapment of small segments of peritoneum is associated with ovulation, and in the mare leads to the development of inclusion cysts that are found near the ovulation fossa (Fig. 4.29). They are also referred to as *fossa cysts*. Although rather commonly encountered as one or a few small cysts lined by epithelial cells (small follicles are lined by granulosa cells) near the ovulation fossa, they are rarely numerous

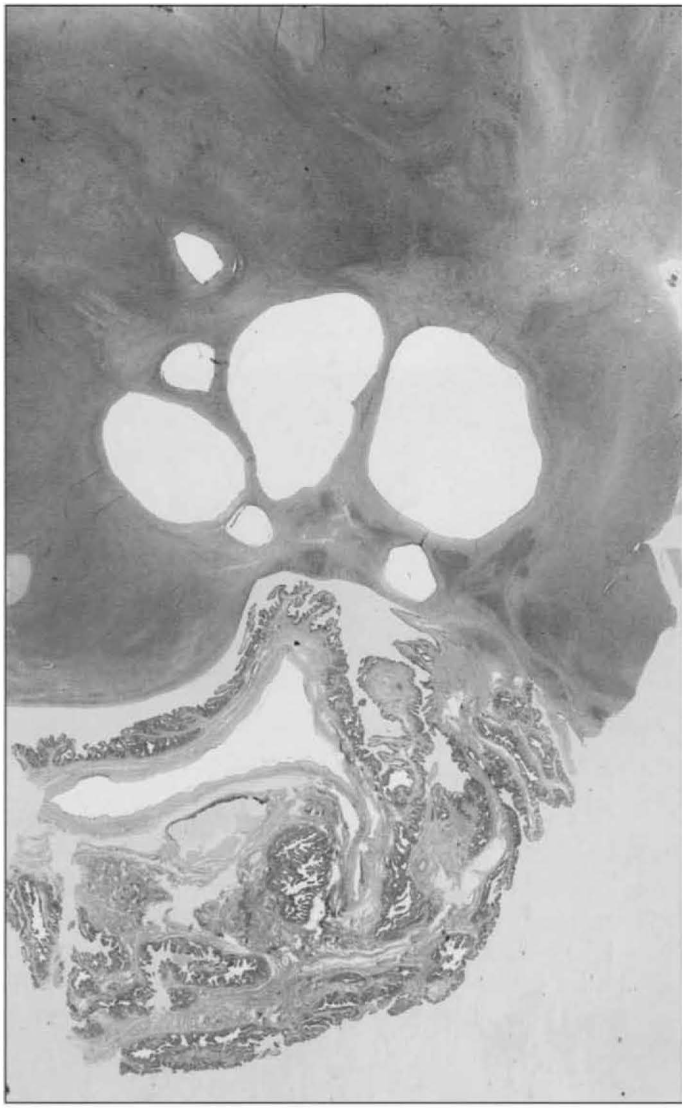

Figure 4.29 Germinal inclusion cysts adjacent to the ovulation fossa in a mare. The cysts form from small pieces of surface epithelium (modified peritoneum) that becomes entrapped after the surface is disrupted at ovulation.

or large enough to interfere with ovulation. Occasionally, their expansion can lead to atrophy of compressed ovarian tissue with loss of function (Fig. 4.30).

Cystic follicular disease

Anovulatory cystic ovarian disease occurs in most, if not all, species. Its importance as a disease entity varies greatly among species; in domestic species, *it is only a serious problem in cows and sows.* Cystic ovarian disease occurs infrequently in the bitch and queen and rarely in the ewe, doe, and mare.

Diagnosis depends in part on differentiating anovulatory cystic follicles from normal antral or tertiary follicles. An important criterion for this differentiation is size. Follicles larger than they should normally be at ovulation, persistence, and possible associated signs of hyperestrogenism are features of cystic follicular disease.

Tertiary follicles vary greatly in size between species, ranging from about 3 mm in the queen up to 7 cm in the mare! Normal follicular diameters near ovulation are approximately 2 cm in the cow, 1 cm in the sheep and pig, and 0.75 cm in the dog. Knowledge of the stage of the cycle is also helpful, but disappointingly is provided inconsistently in the histories provided with clinical case submissions.

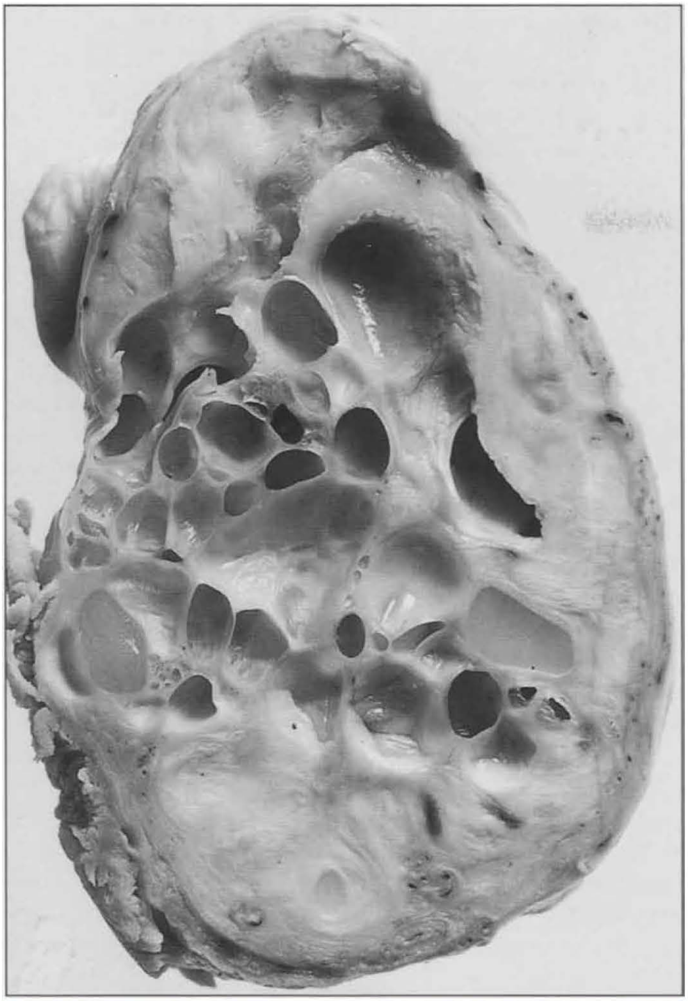

Figure 4.30 Multiple germinal inclusion cysts from a mare (hemisectioned ovary). (Courtesy of V Osburne.)

The examining pathologist should persist and insist on receiving as complete a reproductive history as possible.

It is only in cattle that the condition has received detailed study. Accordingly, the major consideration here is of the bovine disease.

Cystic ovarian disease in cows

A well-known feature of anovulatory follicular cysts is their relation to nymphomania, but *most ovarian cysts are not associated with signs of persistent estrus.* The behavior of cows with cystic ovaries is variable. The majority of cows with ovarian cysts are anestrous. The disease arises from the failure of mature follicles to ovulate. It occurs most often before the first postpartum ovulation. Approximately 45–60% of animals that develop anovulatory follicular cysts will re-establish normal ovarian cycles spontaneously. Cystic follicles may also develop after postpartum ovarian cycles have been established, and these cysts are more likely to persist if effective treatment is not instituted.

The *cause* of cystic ovarian disease is not understood in any species. The disease in cattle occurs more frequently after parturient or postparturient disease, and there is evidence that *intrauterine infections* play a role in the pathogenesis of the disease. There is clearly a *genetic predisposition* to the disease in certain families. The daughters of cows that have had cystic ovaries have a substantially increased risk of developing the disease as compared to the general population. The disease tends to involve primarily dairy cows, but it can occur in cattle of any breed if they are withheld from breeding for a prolonged period of time. One of the factors that has made the understanding of the disease difficult is the definition of the disease itself. *Follicular cysts in cattle are usually defined as follicles greater than 2.5 cm in diameter that fail to ovulate and may persist.* By the time a follicle can meet the criteria to be defined as cystic, the conditions that led to its formation have passed and are not available for study. To offset this difficulty, anovulatory ovarian disease has been produced by a variety of experimental techniques. Unfortunately it is not known whether any, or all, of the experimental manipulations induces anovulatory cystic disease by the mechanisms that operate in the natural disease.

The most widely held theory of the *origin of cystic ovarian disease* has involved aberration of the preovulatory surge of luteinizing hormone, either the absence of the surge or the mistiming of the surge. This hypothesis, or some modification of it, is still the most attractive. Cows that develop cystic ovaries as the result of estrogen and progesterone treatment have an increased mean basal concentration of luteinizing hormone secretion with increase in the frequency and amplitude of pulses, but the characteristic preovulatory luteinizing hormone surge is deficient. This increase in luteinizing hormone secretion is thought to be due to aberrant hypothalamic function altered experimentally by steroids or naturally by ovarian secretion.

The cysts may be single (Fig. 4.31) or multiple on one or both ovaries. These cysts may persist, but during the course of the disease additional cysts may be recruited and some cysts undergo atresia. Patches of luteal tissue can be seen grossly in the wall of some of the cysts and can be recognized histologically in about a quarter of them. The walls of the cysts show the same type of degeneration that occurs in normal atresia. Degeneration of the granulosa cells takes place first, the cells undergoing pyknosis and karyorrhexis and

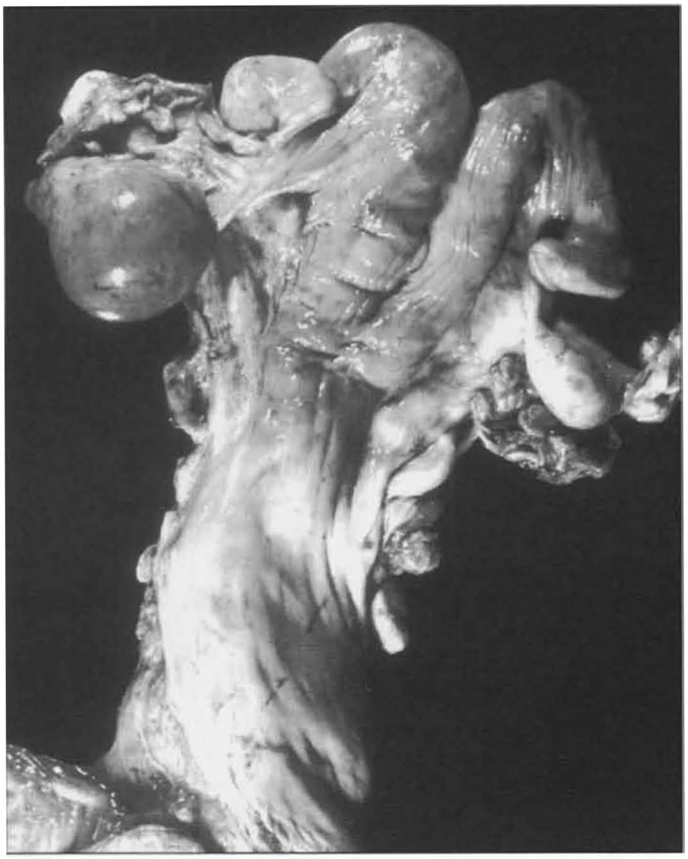

Figure 4.31 Cystic follicle in the ovary of a cow. This condition is thought to be due to inadequate luteinizing hormone release with persistence of the anovulatory follicle.

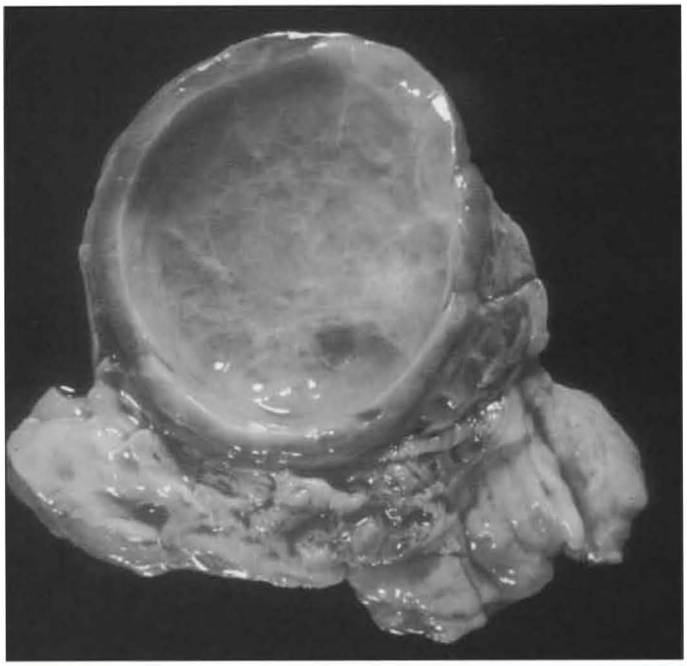

Figure 4.32 Luteinized follicular cyst from a cow. Note the uniform layer of luteinized granulosa cells, which both grossly and microscopically appear as luteal tissue, lining the inner wall of this anovulatory follicle. There is no ovulation papilla.

sloughing into the cyst lumen. The oocyte also undergoes degeneration. The changes of the theca interna are variable. The theca is partially luteinized in some cases, whereas in others it degenerates and is infiltrated by fibrous tissue. The luteal tissue may occur in patches or form a crescent of variable thickness.

Luteinized cyst

This type of cyst develops when ovulation fails to occur and the theca undergoes luteinization. There is no ovulation papilla and the luteal mass is smooth and rounded (Fig. 4.32). The cavity of the cyst is spherical and lined by a layer of fibrous tissue adjacent to the zone of luteinized cells. Luteinized cysts occur more frequently in *cattle and swine* than in other species of domestic animals. Partial luteinization of the wall of follicular cysts is a common feature in the bitch. In cattle, they usually occur as single cysts. Single luteinized cysts may occur in pregnant sows, but multiple cysts are associated with infertility.

Anovulatory luteinized cysts should not be confused with cystic corpora lutea. A cystic corpus luteum is a corpus luteum that has formed after ovulation and in which a central cavity has persisted in a mass of developing luteal tissue. Cystic corpora lutea are not evidence of ovarian malfunction. They form after ovulation, and they do not affect the length of the estrous cycle. If the cow has been successfully bred, the cavity will slowly become obliterated. Large central cysts may occasionally persist for 30–40 days after conception. *Cystic corpora lutea can be distinguished from luteinized cysts by the ovulation papilla that distorts the outline of the cyst at the point of ovulation.*

Ovarian epidermoid cysts have been recognized in cattle only recently. These are reported to be multiple and small and have a squamous epithelial lining that forms keratin.

Extraovarian lesions associated with cystic ovarian degeneration in cows

Changes in other organs develop only if the ovarian cysts persist. Since most cases are responsive to treatment, this is now the exception. It has been shown that in cystic ovarian disease the hormone limits are within normal levels; however, the toxicity of ovarian hormones depends not on the absolute levels, but in the loss of cyclicity and on persistence.

In established cases of cystic ovarian disease, *the uterus is usually altered*, the differences in degree being related to the duration of the condition. In association with functionally active follicular cysts, the uterus is enlarged and the wall is edematous (Fig. 4.33).

The *cervix* is enlarged, the external os patent, and the plicae edematous. The endometrium may appear grossly normal, being smooth, semitransparent, gray-pink, and moist. There may be some degree of *cystic endometrial hyperplasia* detectable by the naked eye as tiny gray-white elevated blisters in the surface of the endometrium, which is overlain by a thin layer of clear viscous mucus. The accumulation of mucus (*mucometra*) and the development of cystic endometrial hyperplasia progress with time and are most striking in those animals that have been affected long enough for atrophy of the ovarian cysts to have occurred and the animal to have become anestrous. By then, the endometrium has become very cystic and the volume of mucus accumulated to 100–1000 mL or more.

The cervix may be hypertrophic or atrophic, as described above. It usually contains thick, viscid, gray-white, and cellular mucus.

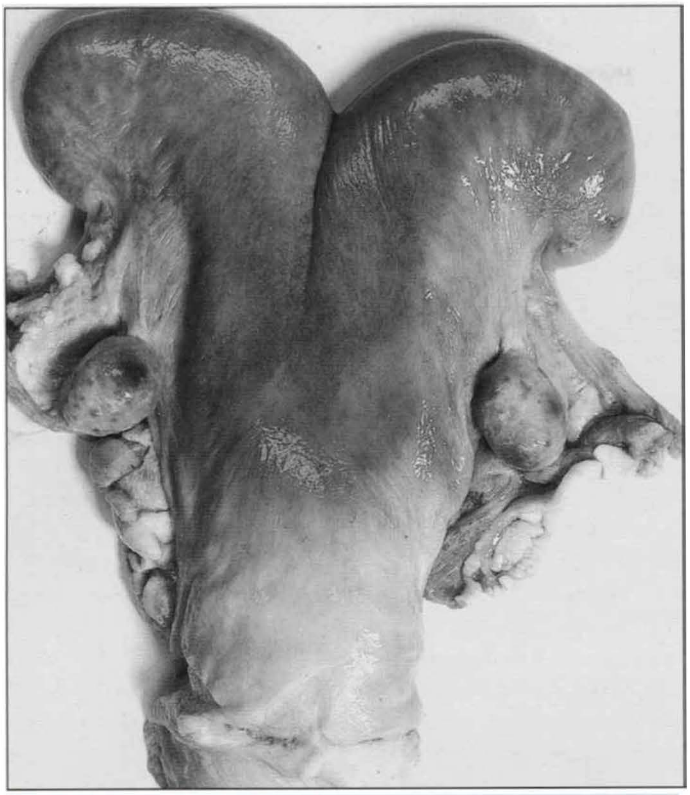

Figure 4.33 Mucometra in the uterus of a cow with a follicular cyst. The endometrium undergoes hyperplasia caused by the long-term release of estrogens from follicular cysts.

The epithelium undergoes squamous nonkeratinizing metaplasia of mild degree. The vagina is edematous when the cysts are active, but otherwise the most striking change is the formation of *cysts in Gartner's ducts*. These mesonephric duct vestiges that lie in the wall of the vagina, one on each side of its floor, are normally visible only microscopically as continuous or discontinuous ducts lined by a simple pavement or cuboidal epithelium. When chronically stimulated by estrogen, the epithelium becomes squamous and the ducts cystic and visible, or palpable, as a series of blebs or as tubules up to 1.0 cm in diameter on the floor of the vagina.

Cystic Gartner's ducts are usually accompanied by *cystic Bartholin's glands*. Either may become abscessed. Bartholin's glands, one on each side of the floor of the vulva, undergo cyclic secretory changes during the estrous cycle and a squamous epithelium on the ducts is normal in estrus. Exaggeration of this change following long-term exposure to estrogens causes occlusion of the ducts and the formation of retention cysts. The vulva may be edematous when the cysts are active, and the clitoris may be enlarged in long-standing cases.

Cystic ovarian degeneration in other species

In **swine**, *cystic ovaries are common and are important as a cause of infertility*. Failure of a single follicle to ovulate need not interfere with pregnancy and single large cysts, 2–3 cm in diameter, may be found in pregnant animals. They are presumed to represent mature follicles that did not ovulate, but single anovulatory cysts may also be associated with irregularity of the estrous cycle. The association is more clearly manifested with multiple cysts. The cysts vary in type from follicular, which are lined by normal granulosa cells, to luteinized cysts. Multiple luteinized cysts with some cysts up to 5 cm in diameter are a distinctive feature of cystic ovarian disease in swine. The luteal tissue may form a complete rim or be present in patches. The endometrium in cases of multiple luteal cysts shows hyperplasia of progestational type, and in long-standing cases the clitoris is enlarged.

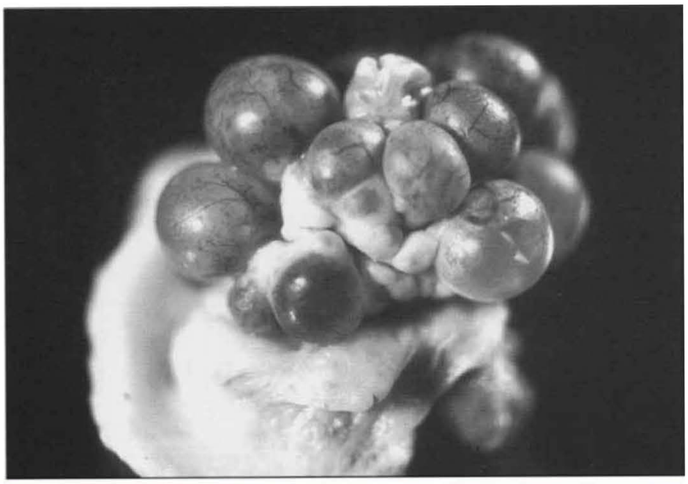

Figure 4.34 Multiple follicular cysts in a bitch. Many of the nodular structures have the appearance of corpora lutea. Some appear as more typical follicles containing clear fluid, though larger than normal. These are cystic follicles in which the inner walls of the anovulatory follicles have undergone partial luteinization.

Cystic degeneration of the Graafian follicle with the associated loss of cyclicity and infertility comparable to that of the cow and the sow does not appear to occur in the **mare**, but the matter is not clear because anovulatory follicular cysts do develop during the winter anestrous period, and some of these mares will show irregular signs of estrus. The number and size of these anovulatory cysts vary considerably. There is no evidence that development of these cysts, regardless of their size, is indicative of reproductive disease.

In the **bitch**, anovulatory cysts of both follicular and luteinized type tend to occur in older age groups. Grossly they tend to be multiple and commonly have partial luteinization of cyst walls (Fig. 4.34). The incidence of cystic ovarian disease is low in the bitch, but there is some confusion on this point because cystic rete tubules, cysts of SES, paraovarian cysts, and cystadenomas have been confused with follicular cysts.

Polycystic ovarian disease in the bitch is rare, but, because it can cause hyperestrogenism, it carries a special risk in the dog because of the species' sensitivity to either endogenous or exogenous estrogen, which can induce lethal bone marrow suppression. The condition can involve either one or both ovaries, the affected ovary being greatly enlarged by multiple, thin-walled cysts that vary from 1 to 12 mm in diameter. The disease develops in mature animals that may be either nulliparous or multiparous. If clinical signs occur, they are apt to be associated with *hyperestrogenism* and include persistent estrus with cornification of the vagina and swelling of the mammary glands or the toxic effects of hyperestrogenism on the bone marrow with resultant pancytopenia. This pancytopenia can result in anemia, thrombocytopenia with bleeding, most often occurring as epistaxis, or granulocytopenia with increased susceptibility to infection. The mechanism of the inhibitory action of estrogen on the hematopoietic tissues in estrogen-sensitive species is not known. Estrogen appears to inhibit the differentiation of pluripotent stem cells while stimulating the differentiation and maturation of committed stem cells.

Most of the cysts are obviously of follicular origin but in various stages of atresia. Degenerating oocytes can be identified in some of the cysts, but the disease is not just one of anovulation because in some cases hundreds of follicles have been recruited. The granulosa and theca lining of the cysts is attenuated in the larger ones. Patchy areas of luteinization form in the walls of some of the cysts. This luteinization may involve individual cells or sheets of cells and either the theca or the granulosa.

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NEOPLASTIC DISEASES OF THE OVARY

General considerations

Enlargement of the ovary or localized tissue proliferation within the ovary can result from a number of conditions that include: normal cyclical changes (tertiary follicles, developing corpora lutea), atretic follicles (autumn follicles in the mare), hematoma, oophoritis, or cysts of ovarian or paraovarian structures. These conditions must be differentiated from primary ovarian neoplasms that occur with variable frequency in all species of domestic animals.

Ovarian neoplasms tend to occur in mature or older animals and may be associated with behavioral changes or changes in target tissues if the tumor is functional (e.g., stallion-like behavior in mares with granulosa cell tumors that are producing androgens). Although rare, metastases from neoplasms in other sites may also be found in ovaries.

Tumors arising from tissues that are specifically ovarian can be divided into three broad categories: tumors of the **surface celomic epithelium**, tumors of the **gonadal stroma**, and tumors of **germ cells**. Tumors developing from nongonadal supporting tissues of the ovary can be of the usual variety of fibroblastic, smooth-muscle, and vascular tumors, but are, in fact, uncommon in domestic animals.

Tumors of the surface epithelium and its tubular invaginations, the SES, give rise to *papillary and cystic adenomas* and, less frequently, *papillary adenocarcinomas*. These tumors are common only in the bitch among domestic animals.

Gonadal stromal tumors are tumors of granulosa and theca cells and their luteinized counterparts. They are also referred to as *sex cord-stromal tumors* to reflect the concern that the granulosa cells may arise from the sex cords of the surface celomic epithelium or the mesonephric tubules. In spite of the histogenetic uncertainty, these tumors must be dealt with as a group because *granulosa cells and theca cells often coexist in the same tumor*. This tendency to coexistence is dealt with by hyphenation, referring to the tumors as *granulosa-theca cell tumors*. Included in this group are tumors that resemble testicular tumors of the Sertoli cell and Leydig cell types. Whether these are, in fact, Sertoli cells and Leydig cells or whether the ovarian stromal cells are merely growing in patterns of their testicular homologs is difficult to determine. The question is made more difficult by the fact that *this group of tumors frequently produces hormones*. The hormones are sometimes appropriate products for the apparent cells of origin, that is, estrogens and progesterone, but many of the ovarian stromal tumors produce androgens. This is particularly true of the ovarian tumors of the mare. It is not useful to attempt to classify ovarian tumors on the basis of hormone production, because, in addition to variations in hormone production from a single class of ovarian tumors, variation in hormone production from a single tumor can occur over time.

The **germ cell tumors** of the ovary, like their testicular counterparts, arise from germ cells, and are usually divided into two main categories in domestic animals: the **dysgerminoma** and the **teratoma**. *The dysgerminoma is composed of cells that show morphologic similarity to primordial germ cells and resemble their testicular homolog, the seminoma. In teratomas, totipotential germ cells have undergone somatic differentiation giving rise to two or more germinal layers with a variety of tissues present in the tumors.* Germ cell tumors are rare in domestic species, except for the testicular seminoma of dogs, and further subdivision of these two categories is probably unwise at this time; however, some germ cell tumors do not fit well into this simple two-category classification. When more extensive series are available for study and analyzed by sophisticated methods, it is likely that the germ cell tumors of animals will show the wide variability that human germ cell tumors display. In addition to the germ cell tumors that show no somatic differentiation (the dysgerminomas and the seminomas) and tumors that have extensive differentiation into tissues of multiple germinal layers (the teratomas), human germ cell tumors have shown various stages of differentiation, i.e., endodermal sinus tumors (yolk sac tumors), choriocarcinomas, and embryonal carcinomas, as well as tumors from single germ layers. This is the variability that is to be expected in tumors arising from pluripotential germ cells.

Ovarian tumors occur most frequently in the bitch, mare, and cow. In the bitch, they are often bilateral, especially those arising from the surface epithelium and the SES. In the cow, ewe, and mare, they are usually unilateral and of the gonadal-stromal type. In cows, there is a tendency for the tumor to occur in the daughters of affected dams and, in these animals, removal of the affected ovary may be followed by the development of a tumor in the other ovary. Very few ovarian neoplasms have been reported in the feline and porcine species. The tumors of the cat are similar to those of the bitch.

Sex cord-stromal tumors

These tumors arise from sex cords or the ovarian stroma and include granulosa cell tumors and tumors of the theca or, rarely, luteal tissue.

The **granulosa-theca cell tumor** is the most common tumor in this group. They are *generally unilateral, usually nonmalignant tumors* in any species. Although they may be observed in young animals, the incidence increases with age. Part of their interest lies in the production of steroids by these tumors.

In *mares*, in which species the tumors are common and have been studied most carefully, three behavioral patterns have been recognized: *anestrous, continuous or intermittent estrus, and male behavior*. Surprisingly, testosterone levels in the peripheral plasma are elevated in most cases. Male behavior is usually only seen in those cases in which the testosterone levels are above 100 pg/mL plasma. Some mares with granulosa-theca cell tumors also have elevated estrogen levels, but these elevations are less clearly related to behavioral patterns. Even in cases in which hormone production associated with the tumor is low, *atrophy of the opposite ovary usually occurs*. The cause of this atrophy has not been determined. It was thought to be the result of testosterone production by the tumor, but convincing association between testosterone levels and ovarian atrophy has not been established, and androgen production by tumors other than the granulosa-theca group has not caused atrophy of the contralateral ovary. Granulosa cells produce the peptide hormone, *inhibin*, which inhibits release of follicle-stimulating hormone. This hormone has been demonstrated to be elevated in granulosa cell tumors, and it may be that the production of inhibin produces the characteristic ovarian atrophy seen in this disease. Normal function of the atrophic ovary usually returns within a year of removal of the tumor. In bitches with sex cord-stromal tumors, *cystic endometrial hyperplasia and pyometra* are common. Vaginal changes can be used to monitor estrogenic influences.

The surface of the granulosa-theca cell tumor is smooth (Fig. 4.35) and the cut surface may be solid or cystic (Figs 4.35, 4.36). The solid portions of the tumor are white or yellow, depending on the lipid content. The cells in these tumors resemble their counterparts in normal follicles, but their histologic arrangement is quite varied. These tumors commonly contain areas with characteristic gland-like or rosette patterns of abortive follicles, some of which may contain a secretory globule resembling an ovum and called a *Call–Exner body* (Fig. 4.37A).

This type of differentiation is frequently observed in the early stages of development of bovine neoplasms, but is less common in other species and in large tumors. Instead, the arrangement of cells is usually diffuse with, in some tumors or parts of tumors, pseudoalveolar pattern, depending on the disposition of the stroma. There is a tendency for some granulosa-theca cell tumors to develop a tubular pattern similar to that of the Sertoli-cell tumor of the testis (Fig. 4.37B). The stroma consists of broad irregular bands of dense collagen. Cyst formation and hemorrhage are common. The more thecomatous portions of such tumors may resemble normal theca cells and, at the other extreme, be distinguishable from plump fibroblasts only by the demonstration of sudanophilic droplets in the cytoplasm or by histochemical techniques for steroids.

The cysts that often make up the bulk of the tumor are lined by granulosa cells that are surrounded by a variable population of thecal cells (Fig. 4.38). In some equine tumors, particularly those associated with high testosterone levels, embedded in the thecal cell layer are large polyhedral eosinophilic cells resembling the testosterone-producing Leydig cells. Ancillary diagnostic testing includes endocrinology. Testosterone and estradiol concentrations tend to be variable, but α-inhibin concentrations have been shown to correlate to tumor mass and to be more consistently elevated.

Tumors composed only of theca cells, **thecoma** or **luteoma**, are much less common in all domestic animals than those arising from granulosa cells or containing a mixture of theca and granulosa cells. The few tumors reported have been firm, white-to-orange, and composed of streaming oval or spindle cells that resembled the cells of the theca interna. The demonstration of lipid in the cytoplasm allows differentiation from fibromas.

Tumors of the surface celomic epithelium

Neoplasms arising from the epithelium covering the surface of the ovary are very important tumors in women, but in nonhuman mammals, they occur commonly only in the bitch. *Papillary cystadenoma and cystadenocarcinoma* have been reported primarily in the bitch. As with the granulosa theca-cell tumor, many of the affected bitches have *cystic hyperplasia of the endometrium*. It should be emphasized that hyperplasia of the endometrium has been observed in bitches with a variety of ovarian neoplasms. It appears that the tumor, irrespective of type, may occasionally stimulate the ovarian stroma with concomitant production of steroid hormones. Papillary cystadenocarcinomas should be considered in the differential diagnosis of *ascites* in adult entire bitches. The ascites develops from obstruction of the diaphragmatic lymphatics by permeating tumor fragments, with perhaps a contribution by secretion by the tumor epithelium. The tumors are frequently multicentric in origin and bilateral. They vary in size from small neoplasms that are not detectable on gross examination to irregular-shaped masses 10 cm or more in diameter. Confinement of the tumor within the rather complete ovarian bursa of the bitch, with consequent compression of the papillae, gives them a typical cauliflower-like appearance (Fig. 4.39A). Once free of the bursa, the papillary nature becomes obvious (Fig. 4.39B) and peritoneal implantation occurs readily. The presence of such papillae is indicative of malignancy. Cysts of various sizes are usually scattered throughout the neoplastic mass (Fig. 4.40).

The epithelium varies from low cuboidal to columnar, with stratification in some areas, and mitotic figures are scant, even in the malignant form. The tumor appears to develop from the surface and from tubular structures (cortical tubes, SES) of the ovarian cortex. Many of the tubules are continuous with the surface epithelium and responsive to estrogenic stimulation. Papillary tumors have been induced in the bitch by prolonged administration of diethylstilbestrol. The experimentally induced tumors regress following withdrawal of hormone treatment. *Tumors of the surface epithelium can occur in association with sex cord-stromal tumors*, possibly by hormonal induction by the granulosa-theca tumors. *The separate identification of the two tumors can be difficult, particularly in areas where the tumors join, but is aided by immunohistochemical staining.* Cytokeratin intermediate filaments are expressed in the surface epithelium and the SES. On the basis of limited investigation, it appears that this specificity has been conserved during tumor development, and affords easy differentiation of the above from cytokeratin-negative tumors of the sex cord-stromal group.

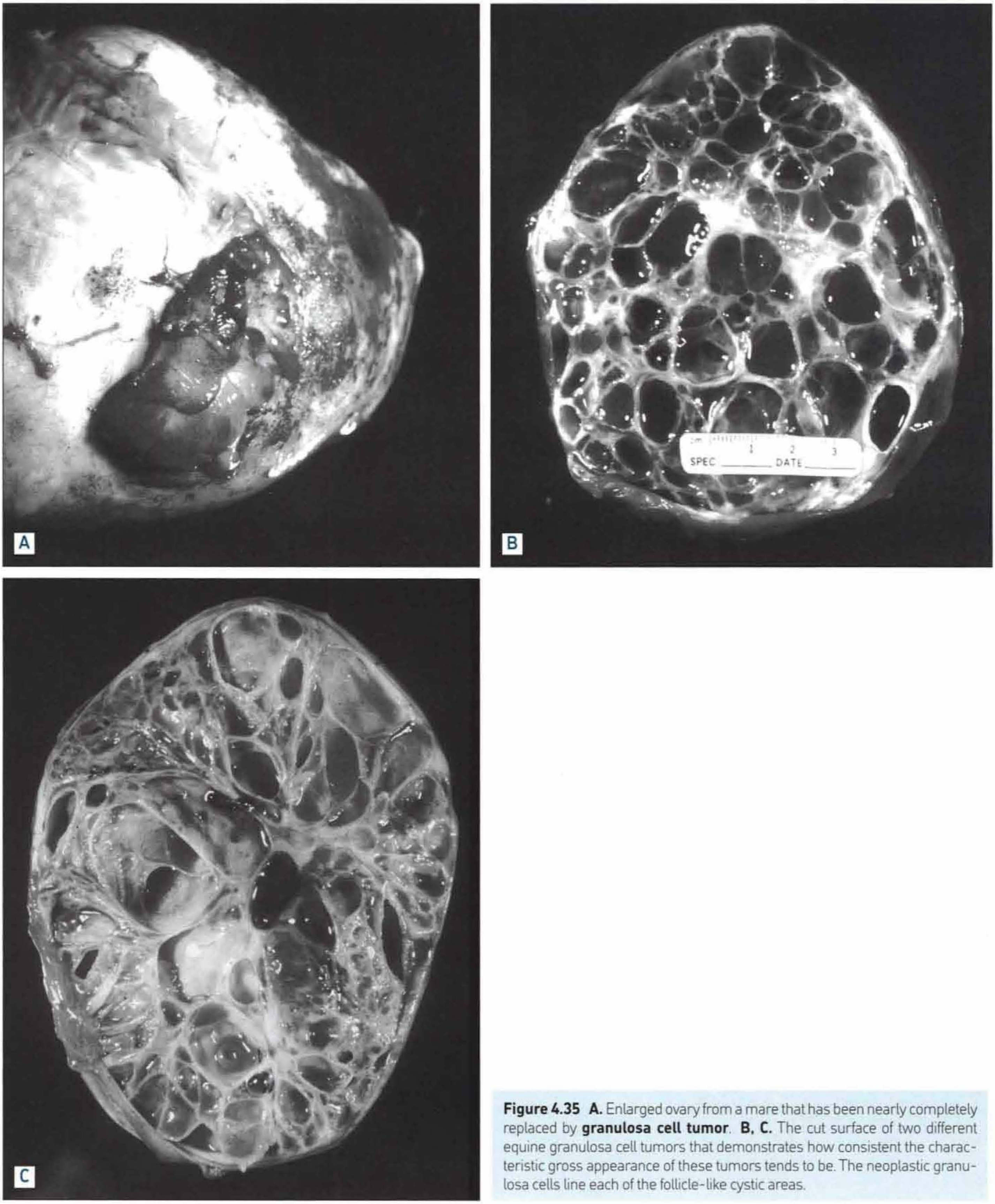

Figure 4.35 **A.** Enlarged ovary from a mare that has been nearly completely replaced by **granulosa cell tumor**. **B, C.** The cut surface of two different equine granulosa cell tumors that demonstrates how consistent the characteristic gross appearance of these tumors tends to be. The neoplastic granulosa cells line each of the follicle-like cystic areas.

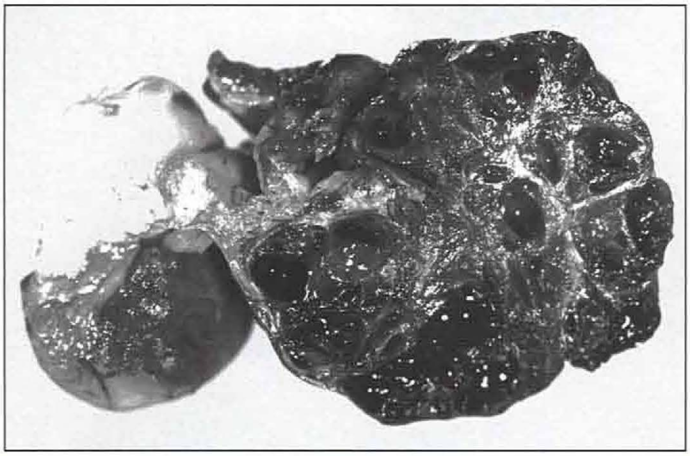

Figure 4.36 **Granulosa cell tumor** extending from the surface of the ovary from a bitch. Note the similar cystic pattern as that seen in the equine granulosa cell tumors in Figure 4.35. Following surgical removal, the fluid within the cystic spaces is commonly hemorrhagic.

Tumors of germ cells

The **dysgerminoma** is a *rare tumor* that is comparable to the more common seminoma of the testicle in gross and microscopic features. *It is usually unilateral and has been observed in the bitch, cow, mare, and sow.* The tumor is smooth, or nearly so, and relatively soft. The cut surface is gray and may have areas of hemorrhage or yellow patches of necrosis. It is composed of a uniform population of large rounded cells with large central chromatic nuclei. Mitotic figures and giant cells are frequent. The pattern of growth is diffuse; the stroma is always scanty. Just as in the canine testicular seminoma, accumulations of lymphocytes are present within the tumors.

As the tumors are uncommon, it is difficult to generalize as to age of occurrence and clinical behavior in various species. In the bitch, in which most cases have been reported, the tumor is one of advanced age, and metastases have occurred in 10–20% of the cases. They have a more aggressive biological behavior in horses.

Ovarian teratomas *are rarely observed in domestic animals, but have been reported in the bitch, sow, mare, and cow.* Most are well differentiated and benign. They usually have solid and cystic areas containing

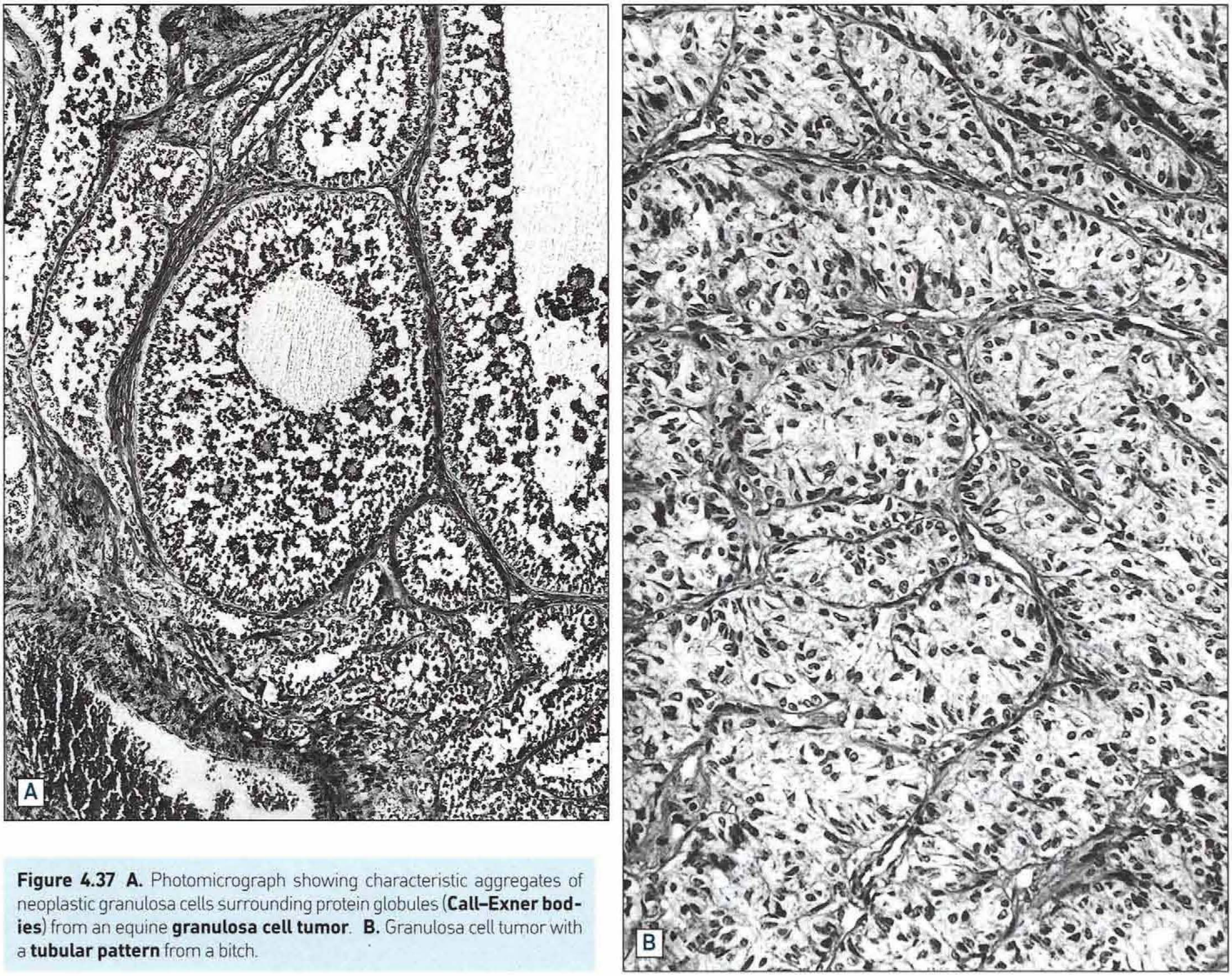

Figure 4.37 A. Photomicrograph showing characteristic aggregates of neoplastic granulosa cells surrounding protein globules (**Call-Exner bodies**) from an equine **granulosa cell tumor**. **B.** Granulosa cell tumor with a **tubular pattern** from a bitch.

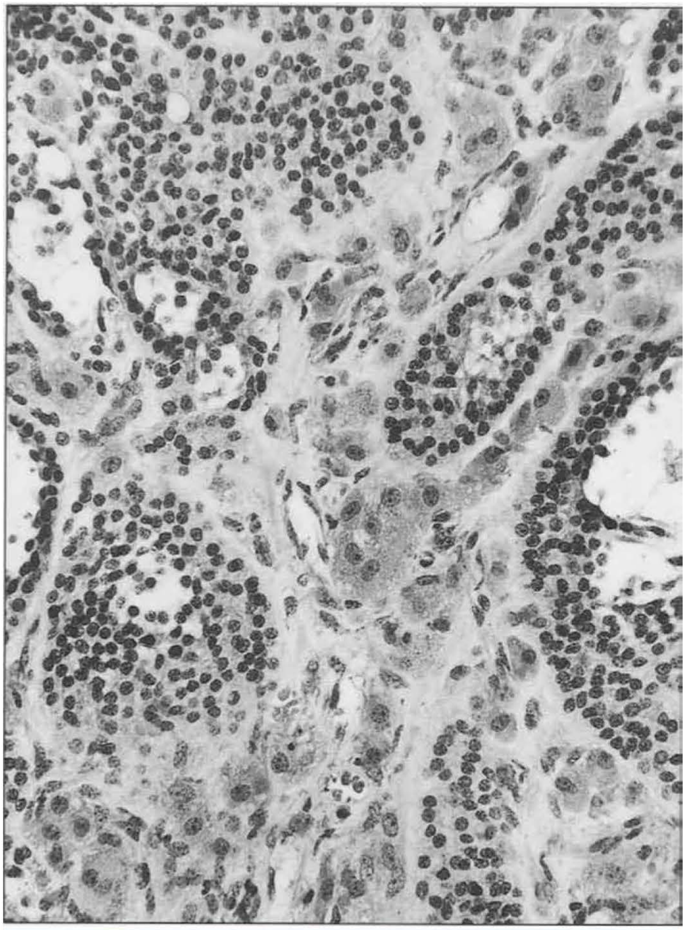

Figure 4.38 Photomicrograph of a **granulosa-thecal cell tumor** from a mare demonstrating presence of both neoplastic granulosa cells and the presence of many interstitial cells.

sebaceous material and hair (Fig. 4.41). A wide variety of other tissues is often present, including neural tissue, adipose tissue, bone, teeth, and respiratory epithelium (Fig. 4.42).

There were several theories on the histogenesis of teratomas, but it is now believed that the benign cystic ovarian teratomas are *parthenogenic tumors* that develop from a single germ cell that has completed its first meiotic division, but not its second. The evidence for this has come from a series of elegant cytogenetic and biochemical studies that have shown that tissues from ovarian teratomas are unique in that they are XX diploid cells, but are homozygous at chromosomal loci for which the host is heterozygous. This lack of heterozygosity is most reasonably explained as being the result of meiotic division.

Tumors of nongonadal tissues

Ovarian hemangioma is rare in all domestic animals except the pig, in which species it is the most common ovarian tumor in mature and aged sows. Ovarian hemangiomas are globular, well circumscribed, and tan to red-brown. Their surface is smooth and glistening and contains prominent vessels. The tumors arise in the ovarian cortex, and they are occasionally bilateral. The tumor is composed of well-differentiated endothelial cells that line vascular clefts and spaces. The tumors are often subdivided by bands of connective tissue. Malignant vascular tumors of the ovary have not been reported.

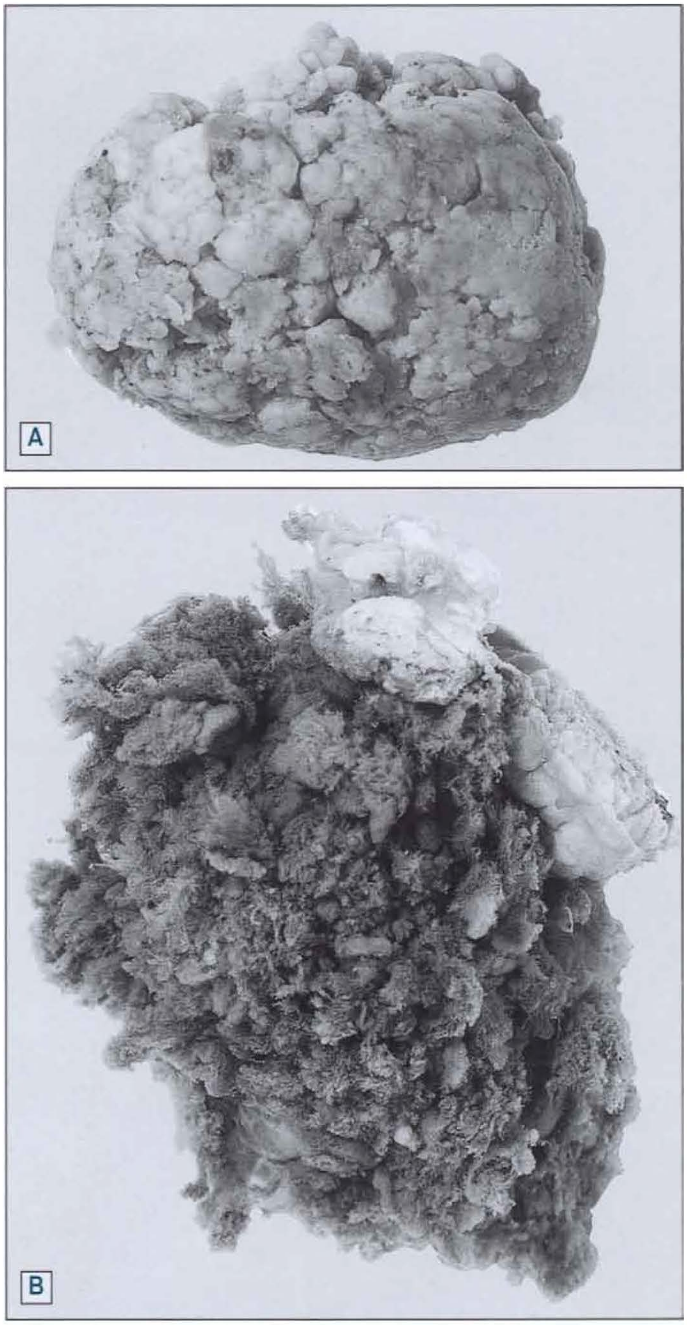

Figure 4.39 Papillary cystadenocarcinoma of the ovary, bitch. **A.** The ovary is contained within the bursa and its surface is irregular and cauliflower-like. **B.** When growing free of the bursa, the villous nature of the tumor is more apparent.

Ovarian tumors of other supporting tissues are very infrequent. **Leiomyomas** developing from the smooth muscle in the mesovarium have been reported in the bitch, queen, and sow, and mesovarial leiomyomas have been induced in rats treated with β-adrenergic receptor stimulants.

Lymphoma can affect the ovary of cattle. Neoplastic foci tend to develop within corpora lutea (Fig. 4.43).

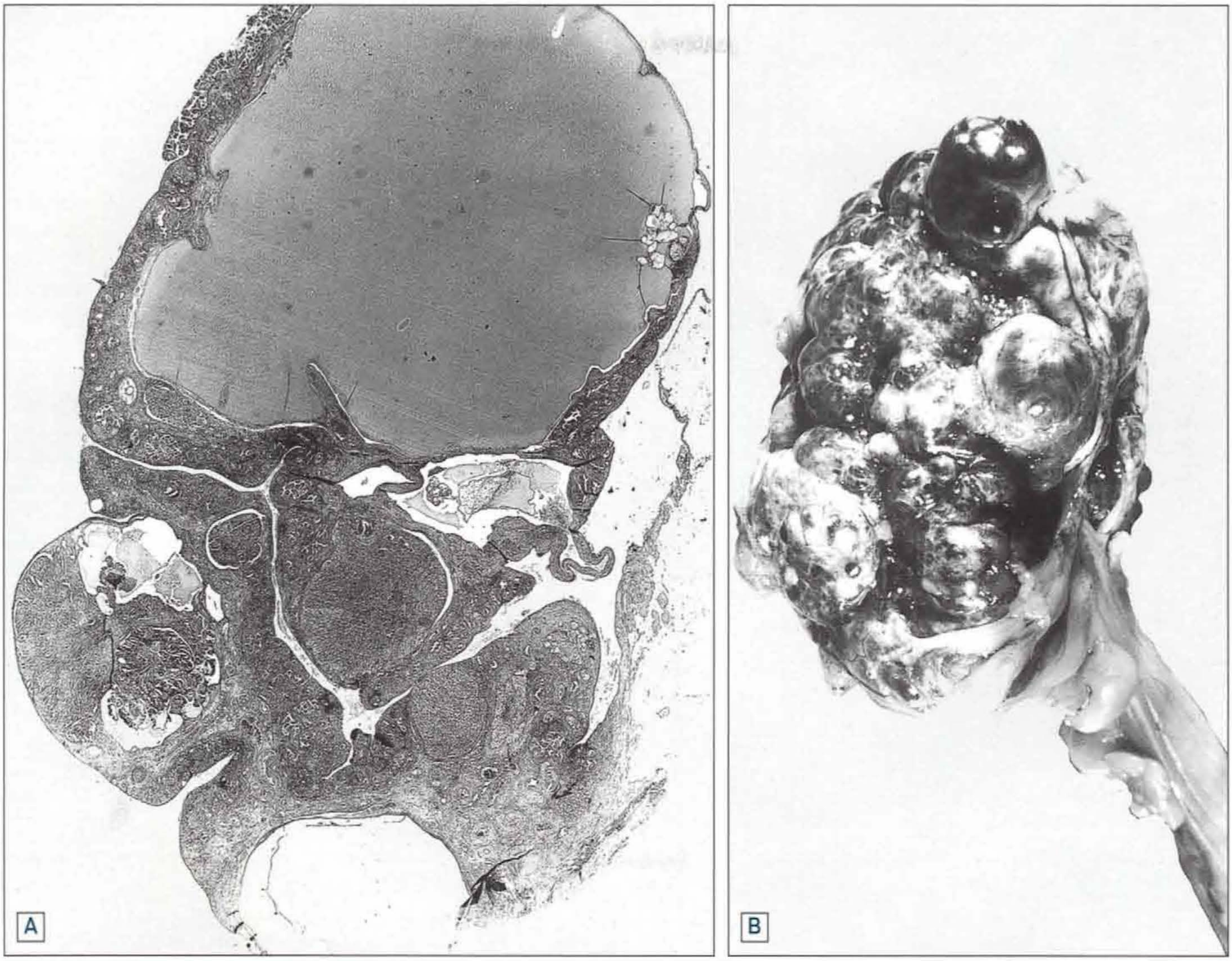

Figure 4.40 A. The cut surface of canine ovary with **papillary cystadenocarcinoma**. This neoplasm develops from the subsurface epithelial structures and is a common ovarian tumor in older animals. The neoplasm usually has a pattern of cystic structures with prominent papillary ingrowth into their cavities. **B.** The bursa has been removed, revealing the very irregular neoplastic growth pattern.

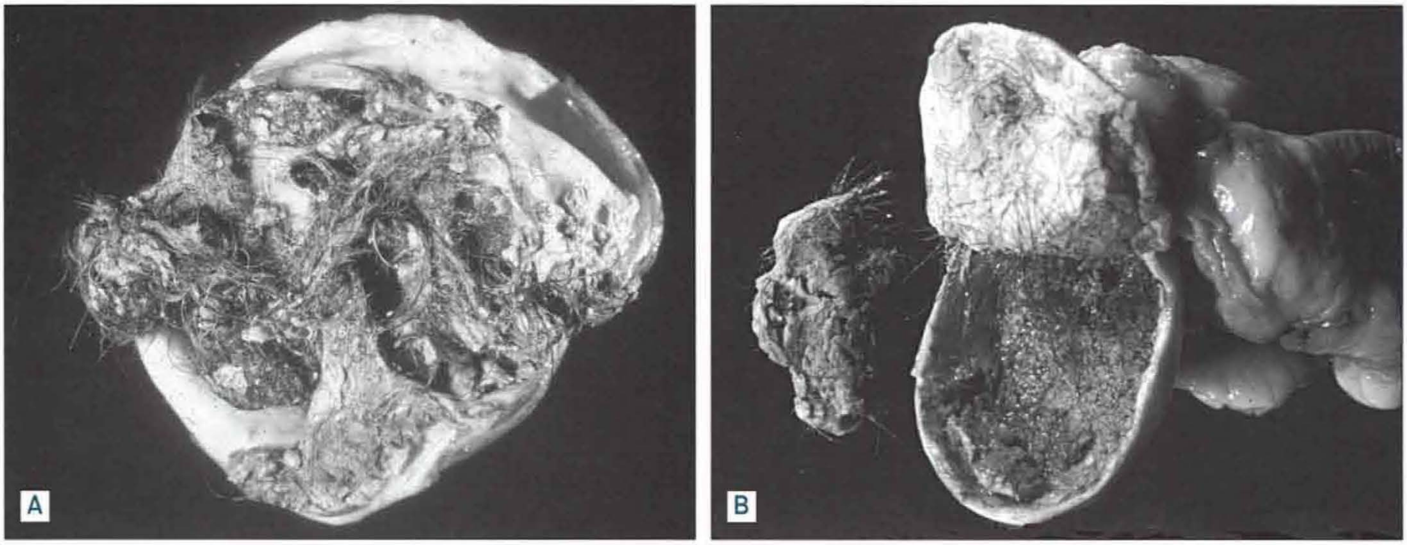

Figure 4.41 A. Equine ovarian teratoma. The normal ovarian parenchyma has been completely effaced by a large cystic mass containing mats of hair. A variety of tissues can often be identified in these tumors, including bone, teeth, and muscle. **B.** Opened **granulosa cell tumor** from a **bitch**. The wall contained firm areas and the central cavity was lined by dermis with hair.

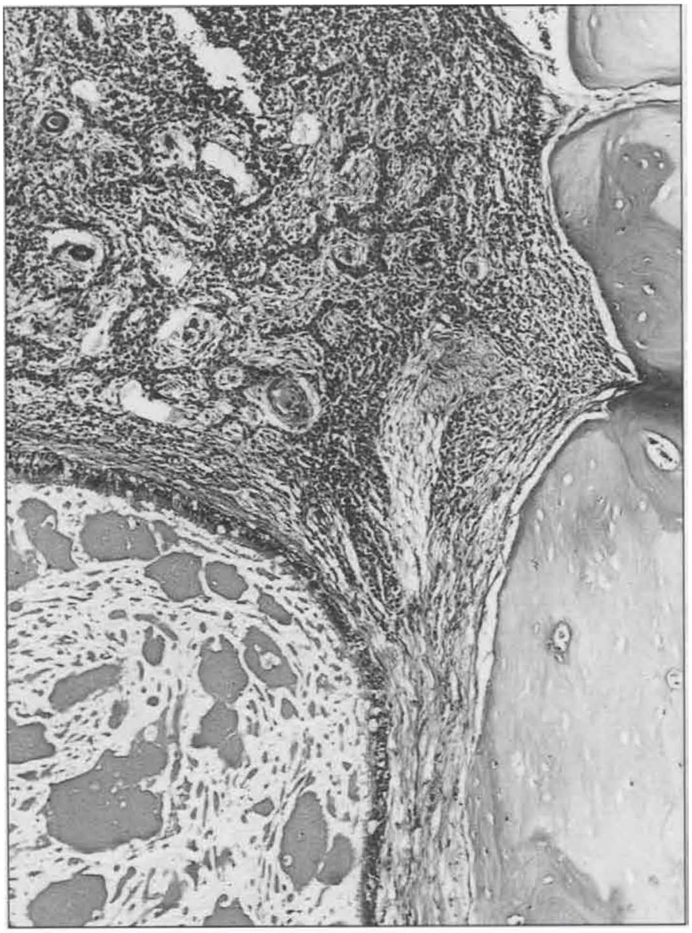

Figure 4.42 Photomicrograph of a **typical teratoma** showing the presence of multiple tissue types.

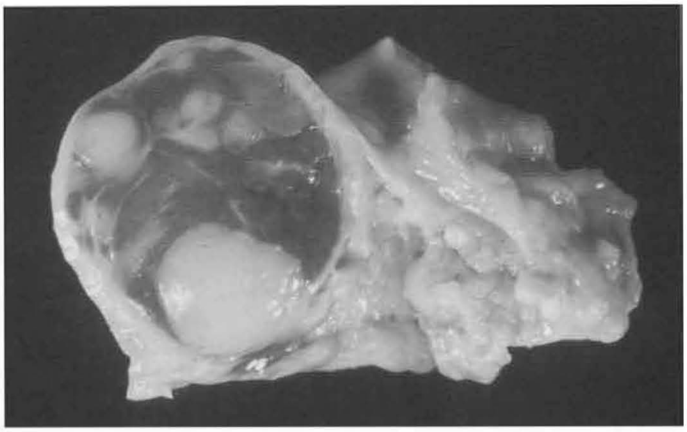

Figure 4.43 Bovine ovarian lymphosarcoma. Several soft pale masses of neoplastic lymphocytes are present within a corpus luteum. Although the uterus is commonly involved, corpora lutea seem to be the tissue within ovaries that are most commonly involved.

Metastatic tumors

Secondary tumors of the ovary are probably not less rare than primary ones, the relatively high incidence largely being attributable to secondary deposits of lymphomas. Mammary carcinomas in the bitch and intestinal carcinomas in the cow may metastasize to the ovary and apparently have an affinity for corpora lutea.

Vascular hamartomas

Vascular hamartomas of the ovary are included with tumors because they may be confused with neoplasms, and because granulosa theca-cell tumors occasionally occur in the same gonad. *Hamartomas are tumor-like malformations that are present at birth* and cease to grow after the animal reaches maturity unless the mass is subjected to trauma, infection, or vascular embarrassment. They have been observed in the cow and sow. The mass of malformed vessels may vary in size from those barely visible on gross examination to those weighing several kilograms. The smaller hamartomas are composed principally of mature-appearing, tortuous arteries and veins with relatively little intervascular connective tissue. In the mature individual, the increase in size of the hamartoma is due to thrombosis, with subsequent edema, hemorrhage, and necrosis, followed by proliferation of fibrous connective tissue. Small hamartomas are clearly demarcated from the adjacent ovarian tissue, but the ovary is gradually replaced as the mass enlarges. *The distinction between hemangiomas and vascular hamartomas is a difficult one*, and it may be that some of the reported ovarian hemangiomas are in fact vascular hamartomas that have grown as the result of vascular accidents.

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PATHOLOGY OF THE UTERINE (FALLOPIAN) TUBES

Primary lesions in the uterine tubes are uncommon. *Hydrosalpinx, pyosalpinx, and salpingitis* are the only ones of importance, and these are usually secondary to disease of the uterus or to manual manipulation of the ovary. As regards incidence of salpingeal lesions, the only agreement is that they are much more common than is their diagnosis. They are recognized to be important in the cow and sow, but not in other species.

Hydrosalpinx

Hydrosalpinx is so called because the uterine tube is distended, uniformly or irregularly, up to 1.5 cm or so with clear watery mucus that fluctuates. The tube is also increased in length and tortuosity and is thin-walled. Histologically, there may be extensive multilocular cyst formation in the mucosa with obliteration of the lumen, and, in some chronically inflamed uterine tubes, there is extensive interstitial fibrosis.

Distension of the uterine tube by fluid follows loss of patency of the lumen (Fig. 4.44). *The obstruction may have a congenital or inflammatory basis.* Congenital anomalies involving the tubes are very rare, except in freemartins, but secondary hydrosalpinx can be associated with obstructions and segmental aplasias of the uterine horns. In the latter instances, the apex of the horn is usually distended with fluid also. Acute septic inflammations are more likely to produce *pyosalpinx*; chronic infective inflammations tend to produce loculations and stenosis of the lumen with *secondary hydrosalpinx*. As almost all infective inflammations are of ascending type from coexisting uterine inflammation, the most severe changes and the obstruction in such cases are usually at or near the uterine end of the tube.

A common form of inflammation that results in hydrosalpinx in cattle is at least in part *traumatic in origin, following manual manipulation of the ovary*. Uterine irrigations are somewhat less important, but, in either event, it is the adhesion between the tube and adnexa (usually the ovary with partial or complete obliteration of the bursal cavity) that causes obstruction of the abdominal ostium and secondary hydrosalpinx. Squashing of the normal diestrual corpus luteum (there is at best only partial expression of the body) is not difficult but, because of its vascularity, there is always some hemorrhage, varying in extent from very slight to fatal. Small clots, which are usually retained in the bursa, can be completely resorbed. Larger clots must be organized, and this results in the formation of adhesions within the bursa and the fimbriated portion of the tube. With more extensive hemorrhage, adhesions may form to adjacent abdominal viscera and genital adnexa. Expression of pathologically persistent corpora lutea may result in little hemorrhage but a lot of tissue damage, and a more or less proportional degree of organization by fibrosis will result. The important types of persistent corpora lutea occur in association with pathological distension of the uterus. If this latter is pyometra, an additional complication is involved in that, as well as the manual trauma, there is liability to spillage of inflammatory products and extension of infection to traumatically devitalized tissue.

Therapeutic uterine irrigation in cattle, especially if performed in the early postpartum period, has also caused the type of adhesion referred to and follows one of two complications: the irrigation fluid may leak through the tube into the bursa and provoke an inflammatory reaction, the usual types of irrigating fluid being irritative, or the infusion of an excessive volume of fluid causes rupture of the uterus and perimetritis. Uteri vary in the amount of fluid they may retain, but any amount in excess of 120 mL is liable to cause uterine rupture, which occurs on the lesser curvature at the mesometrial attachment with permeation of fluid through or beneath the serosa. Inflammatory thickening is the usual result, and the development of serosal adhesions produces tubal obstruction and dilation.

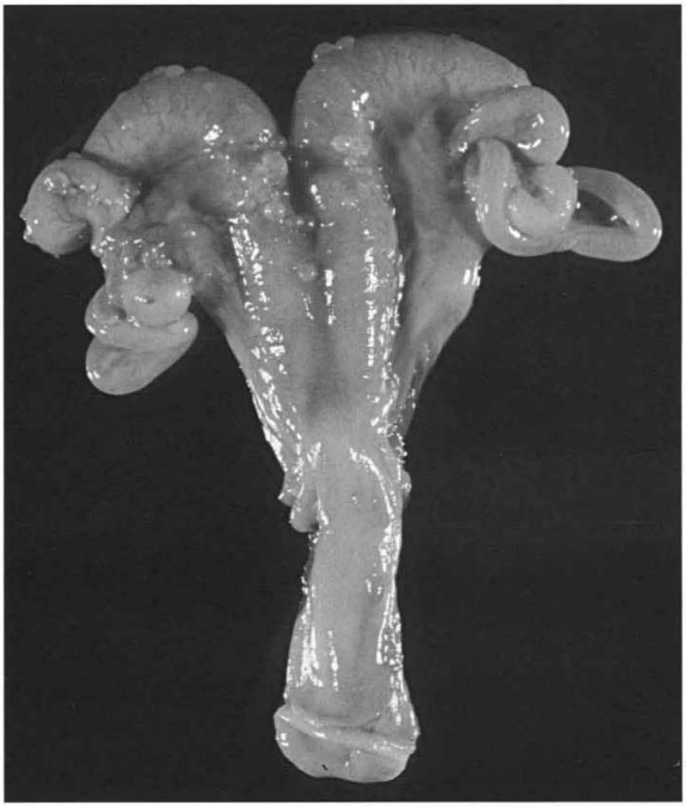

Figure 4.44 Bilateral hydrosalpinx in a sheep. There are also many serosal inclusion cysts on the surface of the uterine horns.

Salpingitis

The uterine tube is a rather simple structure histologically, but even minor inflammatory changes, evidenced by slight congestion or the presence of a few plasma cells, appear to be important because of the readiness with which the epithelial cells desquamate or lose their cilia. The proper function of the living epithelium is necessary for the propulsion of the ovum, for the dissolution of the cumulus oophorus prior to fertilization, and for the maintenance of a luminal environment suitable to survival of the ovum. The salpingeal mucosa has much less capacity for restitution than does the endometrium.

Inflammation of the uterine (fallopian) tubes without significant enlargement is the most common and most important tubal lesion. It is usually bilateral, is usually not detectable macroscopically, and may show serous, catarrhal, or fibrinous inflammation. In the mildest forms of salpingitis, the mucosa alone is affected and changes of functional significance may be slight enough to be overlooked histologically.

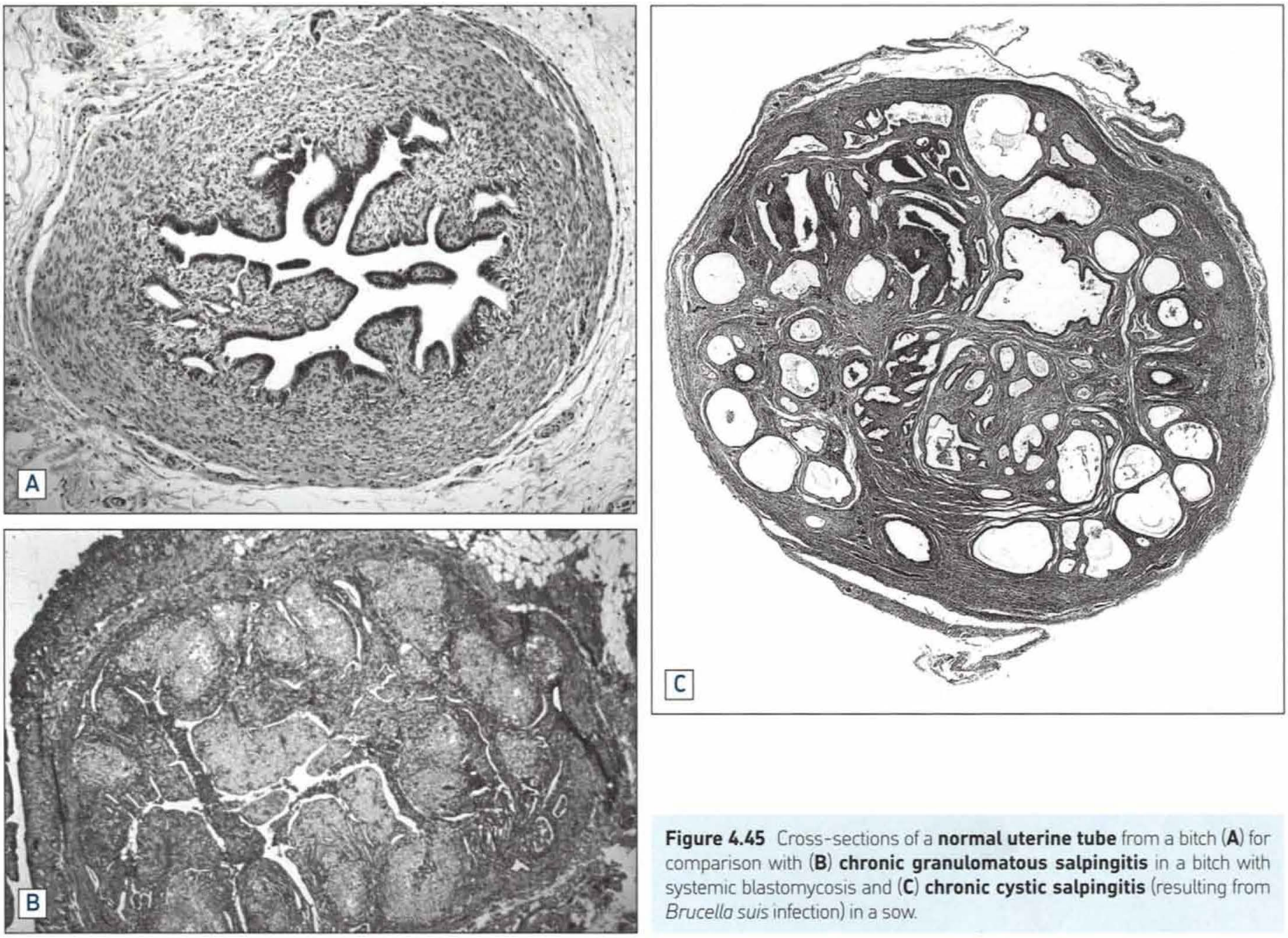

Figure 4.45 Cross-sections of a **normal uterine tube** from a bitch (**A**) for comparison with (**B**) **chronic granulomatous salpingitis** in a bitch with systemic blastomycosis and (**C**) **chronic cystic salpingitis** (resulting from *Brucella suis* infection) in a sow.

Congestion of the mucosal vessels, mononuclear-cell infiltration, loss of epithelial cilia, and some desquamation of epithelium may be the only changes detectable. With more severe infections, catarrhal exudate collects in the lumen, the mucosal folds are thickened by cellular infiltration and congestion, and the epithelium is in large part destroyed. Loss of epithelium occurs first in the free edges of the mucosal folds and these denuded areas tend to fuse and adhere to produce intramucosal cysts. Alternatively, in chronic catarrhal salpingitis, the mucosa is virtually destroyed and replaced by proliferated connective tissue and cellular infiltrations with more or less occlusion of the lumen (Fig. 4.45).

Salpingitis is a common lesion in animals, with both *Mycoplasma* and *Ureaplasma* infections. Nonspecific infections causing salpingitis almost invariably do so following spread from the uterus. *There is probably 70–75% association between uterine and salpingeal inflammation* when diagnosis of the latter is based on histological evidence. In some cases, there will be perimetritis with adhesions, pyosalpinx, or bursal abscess. *Adhesions* of the infundibulum of the mare are very common. The cause of these adhesions is unknown. Some are associated with perimetritis, but most are not. It has been suggested that they may develop as a result of ovulatory hemorrhage. However, this explanation does not easily account for the substantial predominance of adhesions of the right infundibulum.

Granulomatous salpingitis is uncommon. When it does occur, the uterine tube will be firm and distended (Fig. 4.45B).

Pyosalpinx

This is less common than hydrosalpinx, and typically follows metritis in the same manner as do other forms of salpingitis. *The significant anatomic difference is the accumulation of pus in the tube following obstruction of the lumen.* The obstruction may be produced by inspissated exudate, inflammatory thickening, and fusion of the mucosal folds, or chronic granulation tissue. The length of the tube is usually not uniformly involved by the inflammatory process; rather, there are segments in which the reaction is more acute or more advanced so that the obstruction tends to involve irregular segments with the intervening portions distended with exudate.

The entire thickness of the wall of the duct is infiltrated with neutrophils, lymphocytes, and plasma cells, and the same cells collect in the lumen and in the mucosal cysts formed by adhesions between the denuded epithelial folds. Surviving epithelium may be

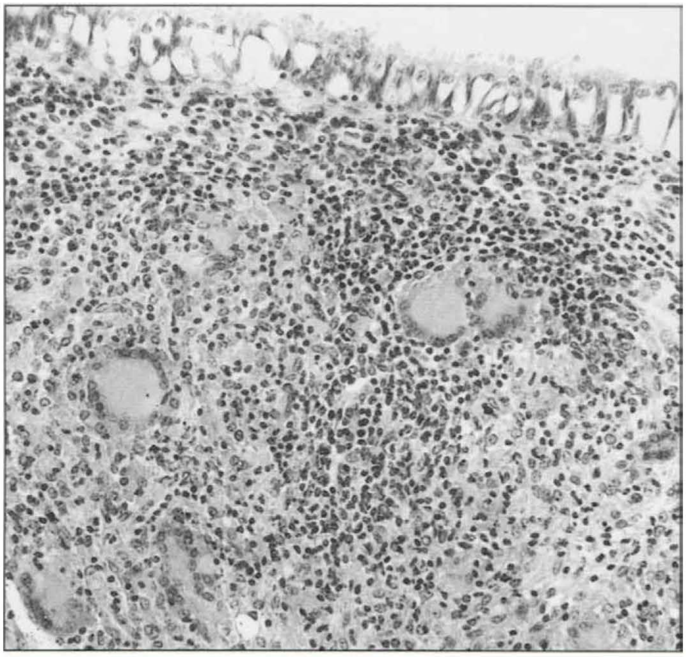

Figure 4.46 Granulomatous salpingitis from tuberculosis in a cow.

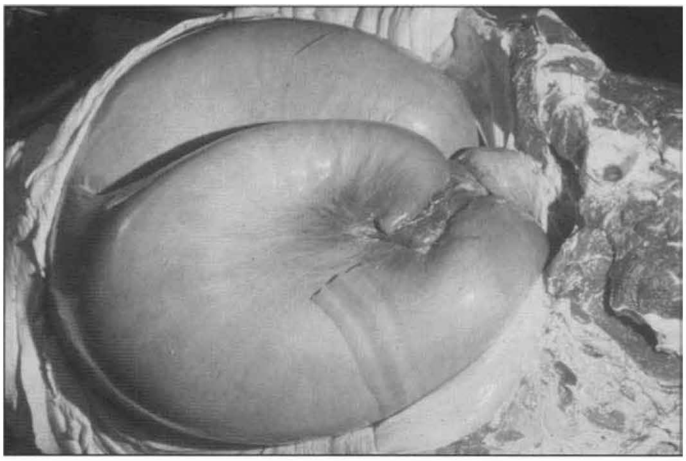

Figure 4.47 Uterine torsion in a cow.

partly squamous. Eventually, the bacteria will be destroyed and the exudate converted to a watery fluid (hydrosalpinx). Frequently accompanying pyosalpinx are the *bursal adhesions* and *local peritonitis* described earlier.

Among the organisms that may be found in inflammatory diseases of the uterine tube are streptococci, staphylococci, *Escherichia coli*, and *Arcanobacterium pyogenes*, with the latter as the most common and important. *Brucella suis* in swine and *Mycobacterium tuberculosis* in cattle are responsible for specific forms of salpingitis; the lesions are as described for these infections in the uterus (Figs 4.45C, 4.46).

PATHOLOGY OF THE UTERUS

Abnormalities of position or location

Torsion *of the uterus is uncommon except in the cow and mare.* In almost all cases, such twisted uteri are pregnant (Fig. 4.47), but torsion may also occur with pyometra and hydrometra. *The torsion is of the same nature as an intestinal volvulus and occurs about the transverse axis of the organ with the mesovarium as one fixed point.* In uniparous species (cow) in which a well-developed intercornual ligament does not permit much independent movement of the horns, the entire organ is involved in the torsion, which is about the mesovarium and vagina or cervix as fixed points. In multiparous species (bitch, cat) with long horns and no intercornual ligament, the torsion will involve part of one horn or the entire horn, the fixed points in the latter instance being the mesovarium and the site of attachment of the horn to the uterine body. There seem to be no rules governing the direction of the twist. Minor degrees of torsion (up to 90°) are fairly common in cows and apparently resolve themselves. *The condition becomes of importance only when the torsion is 180° or more and results in dystocia.* Any twist in excess of 180° may also result in local circulatory embarrassment. The thinner-walled veins are obstructed before the arteries, and the uterus becomes congested and edematous, with edema of the placenta and death of the fetus. The devitalized uterine wall becomes friable and susceptible to rupture or, if cesarean section is performed, the friability of the wall makes suturing difficult. Death of the fetus may be followed by mummification if the cervix remains closed; if air and infection enter the uterus, the fetus putrefies. In the bitch and cat, transverse rupture of the twisted segment near parturition can release the dead fetuses into the peritoneal cavity (Fig. 4.48).

The fetuses that escape into the peritoneal cavity undergo mummification, attach to the omentum, liver, or intestine, and become covered by a rather thin membrane. Occasionally, a fetus becomes dismembered and fetal bones may be scattered throughout the omentum. In some cases, the mummified fetuses remain in the peritoneal cavity for months or years without causing clinical signs of disease. If the horn does not rupture, the fetus may die, become mummified, and the uterine tube will shrink in size (Fig. 4.49).

Prolapse *of the uterus occurs fairly commonly in ruminants*, exceptionally in other species. Predisposing causes in the cow are essentially those that cause, or are associated with, uterine hypotony and probably also with dysrhythmia of involutionary contractions. Amongst the most common associations in the cow are *prolonged dystocia relieved by forced traction, retained placenta, and postparturient hypocalcemia.* Probably the same sort of influences operate in ewes and, in addition, uterine prolapse after parturition is a common complication of the *hyperestrogenism* which results from the ingestion of legumes with a high content of estrogens.

In any species, usually only the previously gravid horn prolapses. In the cow and ewe, the nongravid horn, and sometimes intestine and bladder also, may be present within the everted horn. The pathologic sequelae of prolapse are comparable to those of intestinal intussusception with the added factor of trauma. Congestion and edema are followed by hemorrhage, necrosis, and sepsis. Gangrene may supervene.

Rupture of the uterus may occur spontaneously, but is usually a result of *obstetrical manipulations.* Most ruptures occur in the fundus adjacent to the pelvic brim as irregular tears that may involve

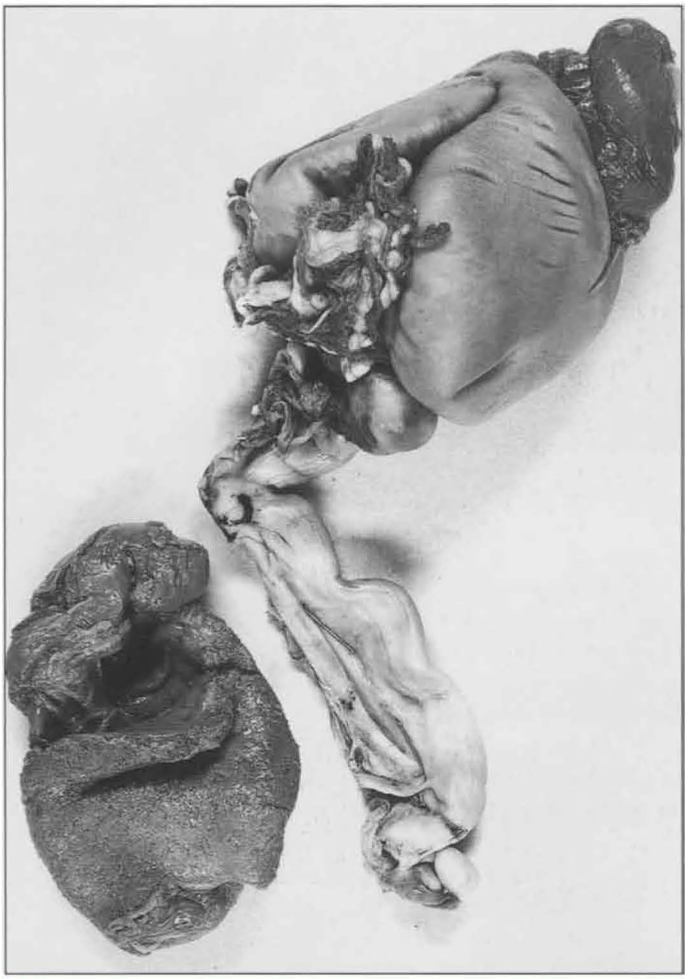

Figure 4.48 Uterine torsion and rupture in a bitch with release of a fetus and placenta into the abdominal cavity.

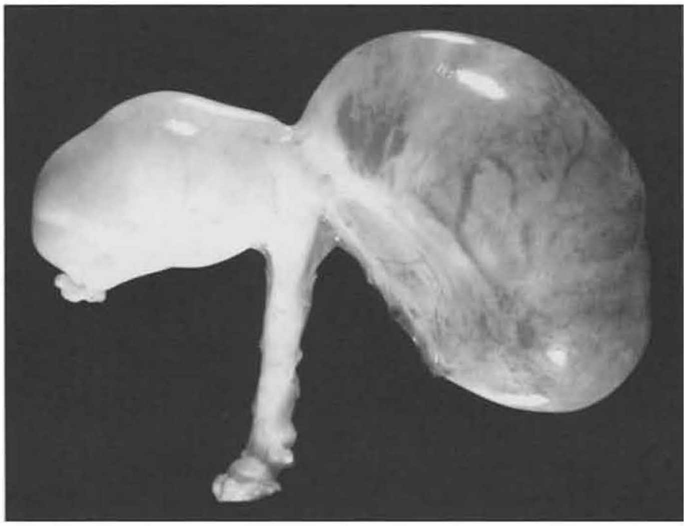

Figure 4.49 Uterine torsion of a gravid uterine horn in a cat.

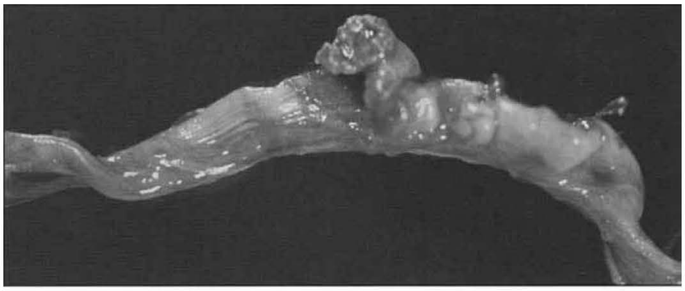

Figure 4.50 Uterine artery rupture in a mare. The resulting hematoma can dissect along fascial planes in the broad ligament, or extend into the abdominal cavity where they continue to bleed and may lead to exsanguination.

the full width of the wall or only the mucosa. Mucosal ruptures are of little consequence. Complete ruptures are often fatal either by virtue of hemorrhage, or spread of uterine inflammation to the peritoneum, or displacement of retained membranes into the abdominal cavity. The majority of ruptures occur in uteri that are devitalized as a result of torsion or prolonged dystocia.

Rupture may also follow acute distension of the uterus produced by *infusion fluids*. This is not an uncommon accident. The rupture occurs on the lesser curvature along the line of attachment of the mesometrium, and the irrigating fluids spread into the ligament.

Circulatory disturbances

Endometrial hyperemia and edema occur normally at *estrus* and reach the greatest relative development in the bitch in proestrus. The resulting diapedesis and endometrial exfoliation account for the uterine hemorrhage at proestrus in this species. A small amount of mucosal hemorrhage is common in heifers, less common in older cows, and occurs immediately after estrus. The source of the hemorrhage is the endometrial capillary bed immediately cranial to the cervix. It is probably an estrogen-withdrawal effect and the nearest thing to menstruation in domestic animals. Punctate hemorrhages occur in the uterine serosa in heifers in estrus.

Hemorrhage of pathologic import follows *torsion and inversion*, by obvious mechanisms. Perhaps the most common association of abnormal bleeding is *endometrial hyperplasia* in the bitch, especially if there is superimposed infection. A less common cause in this species is the *uterine fibroleiomyoma* (fibroid). Vaginal hemorrhage from uterine leiomyosarcomas was a fairly common clinical feature in Saanen goats.

Rupture of the uterine artery with hemorrhage into the broad ligament, with or without extension into the abdominal cavity, occurs in the mare. The latter situation commonly results in the death of the mare from exsanguination. The uterine artery near the rupture commonly contains chronic degenerative changes (Fig. 4.50).

PATHOLOGY OF THE ENDOMETRIUM

Irregularities of endometrial growth

Atrophy

Atrophy of the endometrium results from loss of trophic ovarian function. Senile atrophy is not important in large domestic animals. Atrophy is common after ovariectomy, but may reflect hypopituitarism of chronic inanition or wasting disease, or a primary hypophyseal lesion. The more superficial portions of the endometrium are the more atrophic and, in advanced atrophy, the lining mucosa covers a thin layer of condensed stroma in the depths of which are the inactive glandular remnants that are sometimes cystic.

The endometrium of those species that have a seasonal period of anestrus undergoes a normal atrophic change. In mares, in which these changes have been studied by endometrial biopsies, the luminal and glandular epithelium may become cuboidal and the glands are straight during the winter anestrous period, but there is considerable individual variation in the degree of atrophy mares develop.

Hyperplasia

General considerations

Endometrial hyperplasia in the bitch is common and usually involves cystic distension of endometrial glands. The condition is called **cystic endometrial hyperplasia** and, if extensive, results in accumulation of endometrial secretions in the uterine lumen. In turn, infection of the uterus may follow. This association has led to the sequence of endometrial hyperplasia and subsequent infection being known as the *cystic endometrial hyperplasia–pyometra syndrome.*

Experimental studies have shown that a variety of *sterile substances*, such as sterile silk or autogenous tissue, when placed in the lumen of the uterus of a bitch in the luteal phase of the estrous cycle, will result in dramatic endometrial proliferation and remodeling. These studies demonstrated how sensitive and responsive the canine endometrium is during the period of the estrous cycle when it would be an intimate part of placental structures if the bitch were pregnant. *Bacteria* might also provide appropriate stimulation that could drive the endometrium to undergo hyperplasia and hypertrophy. An alternative plausible pathogenesis for cystic endometrial hyperplasia–pyometra is that an initial event is the establishment of a *low-grade, subclinical infection* that during the luteal phase (also referred to as the secretory or progestational phase) causes the endometrium to proliferate. Changes in the uterine environment, accumulation of secretions, and other progestational effects could then lead to massive proliferation of bacteria and inflammatory cellular infiltration into the endometrium with accumulation of purulent exudate in the uterine lumen.

Cystic endometrial hyperplasia can involve either a single or a few glands, or endometrial glands extending along segments of endometrium (Fig. 4.51). Sometimes, the entire endometrial surface is involved. Individual endometrial glandular cysts can become quite large, up to 1 cm. More diffuse involvement can leave the endometrium thickened and, on cross-section, effaced by variably dilated glands (Fig. 4.51C, D).

Endometrial hyperplasia can also be attributed in some species to *excessive and prolonged estrogenic stimulation*. Sources of estrogen can be endogenous, for example from granulosa cell tumor, or exogenous, as in ingestion of certain plants. Secretions from a markedly chronically stimulated endometrium can lead to gross accumulation of mucoid fluid in the uterus, a condition called *mucometra* (Fig. 4.52).

Progesterone has been shown to play the major role in the induction of endometrial hyperplasia in the dog and cat, but even here the endometrial response to progesterone depends upon *estrogen priming*. Estrogens act by binding to the estrogen receptors that are

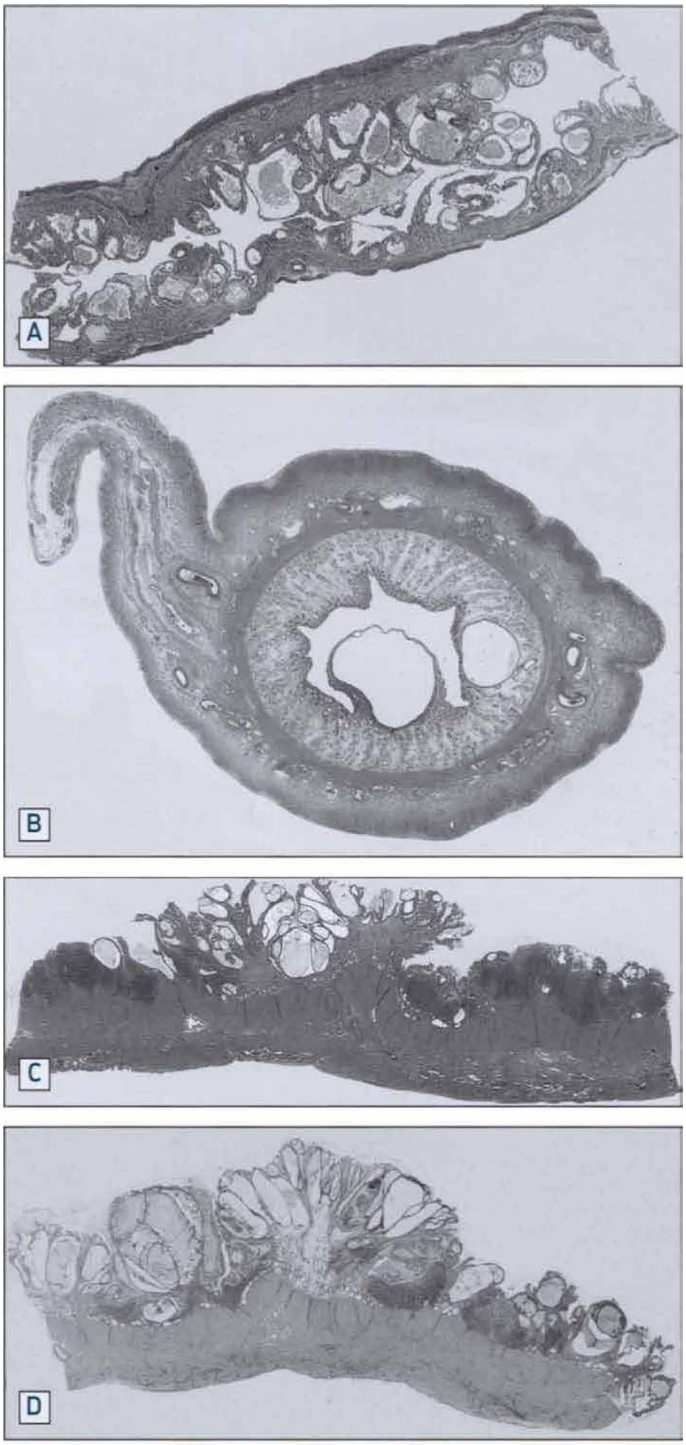

Figure 4.51 A–D Cystic endometrial hyperplasia in uterine sections from four bitches. The hyperplasia can involve one or a few glandular units (upper panel), or have a much more diffuse, locally extensive involvement. Occasionally, the entire endometrium is involved. The hyperplastic endometrium in the latter cases tends to secrete large amounts of fluid. The darker areas in the endometrium in the lower photographs are mononuclear cellular infiltrates that become neutrophilic in response to secondary bacterial proliferation. *The neutrophilic exudate and endometrial hyperplasia are characteristic features of pyometra.*

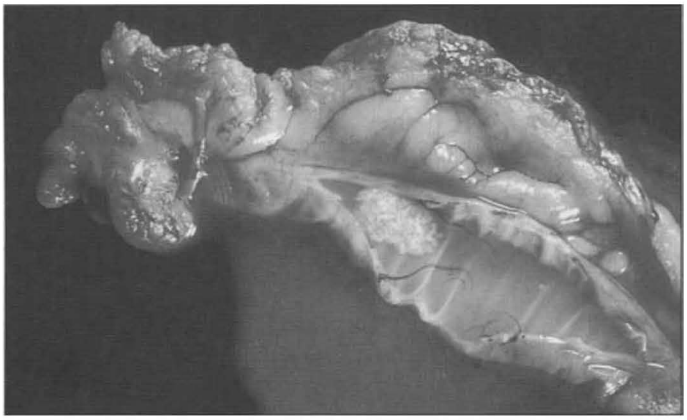

Figure 4.52 Mucometra distending the uterus of a bitch. The physical pressure placed on the endometrium can lead to endometrial atrophy over time.

present in the endometrial cells and act on these susceptible cells to induce the synthesis of intracellular receptors for progesterone. The progestational effect of conversion of the endometrium to its secretory mode depends on this estrogenic priming, and it is likely that in some cases the disturbances of endometrial growth giving rise to endometrial hyperplasia are to be found in the disturbances of the timing and duration of the priming, although cystic endometrial hyperplasia can be produced in the bitch in the normal early-diestral uterus by mild trauma (see also section on Pyometra, in the bitch and queen, below). Many cases of cystic hyperplasia of the endometrium have developed following the use of long-acting progestational compounds to delay the onset of estrus in bitches. Cystic endometrial hyperplasia in the *cow* is associated with ovarian follicular cysts or granulosa-cell tumors, both of which can produce prolonged hyperestrogenism. Cystic endometrial hyperplasia is very uncommon in the *mare*. It has not been associated with granulosa-theca cell tumors in this species.

Ovarian tumors, especially granulosa theca-cell and papillary cystadenocarcinomas, are present in some cases of endometrial hyperplasia of the bitch, but in the majority of clinically recognizable cases, the ovaries contain apparently normal corpora lutea. The hyperplasia develops during the long luteal phase that is normal in the bitch. Often paraovarian cysts are present in cases of endometrial hyperplasia.

Noncystic endometrial hyperplasia is not recognizable macroscopically except as an equivocal thickening of the endometrium. The thickening is due to increased size and number of glands, which are irregular in their distribution and course, the normal parallel alignment being lost. The stroma is not hyperplastic but is edematous. The glands may show both proliferative and secretory activity. In the bitch, the glands are tortuous and secretory; the mucosal epithelial cells are typically progestational, being hypertrophied with clear cytoplasm; the glands of the basal endometrium are also active; and there is little evidence of the normal partitioning of the endometrium into layers. There is usually some degree of adenomyosis. Cystic hyperplasia, the so-called "Swiss-cheese" endometrium, is the histologic extreme of the condition and is irreversible. *It is probable that endometrial hyperplasia is but an exaggeration of the normal proliferative activity of the endometrium in response to ovarian hormones.*

Endometrial hyperplasia is a significant precancerous lesion in women. In domestic animals, this is not so. Endometrial hyperplasia is very common in the bitch but uterine cancer is extremely rare.

Canine segmental hyperplasia

The endometrium of the bitch occasionally undergoes an unusual, but very characteristic, endometrial segmental hyperplasia consisting of localized proliferation of the endometrial glands and endometrial surface that *closely resembles pregnancy implantation sites* (Fig. 4.53). The unopened uterus may have one or more ovoid distended areas that may resemble pregnancy sites. Prior to opening the uterus, other differential diagnoses would include endometrial polyp, chronic pyometra, leiomyoma, or fetal mummification.

Segmental endometrial hyperplasia can occur in virgin bitches, but they may also represent persistence of endometrial remodeling at sites where an embryo has been lost. Similar segmental areas of hyperplasia have been experimentally produced by placing various sterile materials in the lumen of bitches that are in the luteal phase of their cycles. It has been suggested that these are florid decidual reactions, and have been referred to as *deciduomas*, but use of this term is discouraged.

The microscopic features are characteristic (Fig. 4.54). The hyperplastic endometrium may appear as a broad-based polypoid mass extending into the uterine lumen (Fig. 4.53A), or may form a continuous band of protruding tissue (Fig. 4.54A). The endometrium is organized in three discrete layers, corresponding to the deep glandular zone, condensed connective tissue band, and folded luminal epithelial junctional zones. The luminal surface is always necrotic, and no fetal placental tissues are found.

Estrogenic plants

Pasture legumes as sources of estrogenic activity have claimed attention as a cause of a spectacular syndrome of *infertility in* **sheep**, and as a cause of less obvious depression of fertility in sheep. Although many estrogenic substances are known to occur in plants, pasture plants that have been found to produce estrogenic effects are mainly some varieties of *Trifolium subterraneum, T. pratense, Medicago sativa,* and *M. truncatula*. The estrogens of the clover species are usually *isoflavones*; alfalfa and the barrel medic contain *coumestans*.

Extensive metabolism of phytoestrogens occurs in the rumen. During such metabolism, compounds of very different estrogenic potency from those in the plant can be produced. Among the isoflavones, formononetin, a compound with little estrogenic activity in itself, is the main compound producing histologic effects in sheep after its conversion in the rumen to the potent estrogenic metabolite, equol.

There is variation in estrogenic potency between strains of clover and also with the season or stage of growth, activity being greatest in winter and early spring. Potency is diminished if the clover is allowed to wilt and dry, or if it is made into hay, but the activity is retained if the clover is artificially dried.

Estrogenic pastures grazed during the breeding season may temporarily impair fertility, and ewes so affected may have a very low lambing rate and a high incidence of dystocia and uterine prolapse. Wethers on such pastures develop enlargement of the bulbourethral glands (see Vol. 3, Male genital system). The fertility of affected ewes

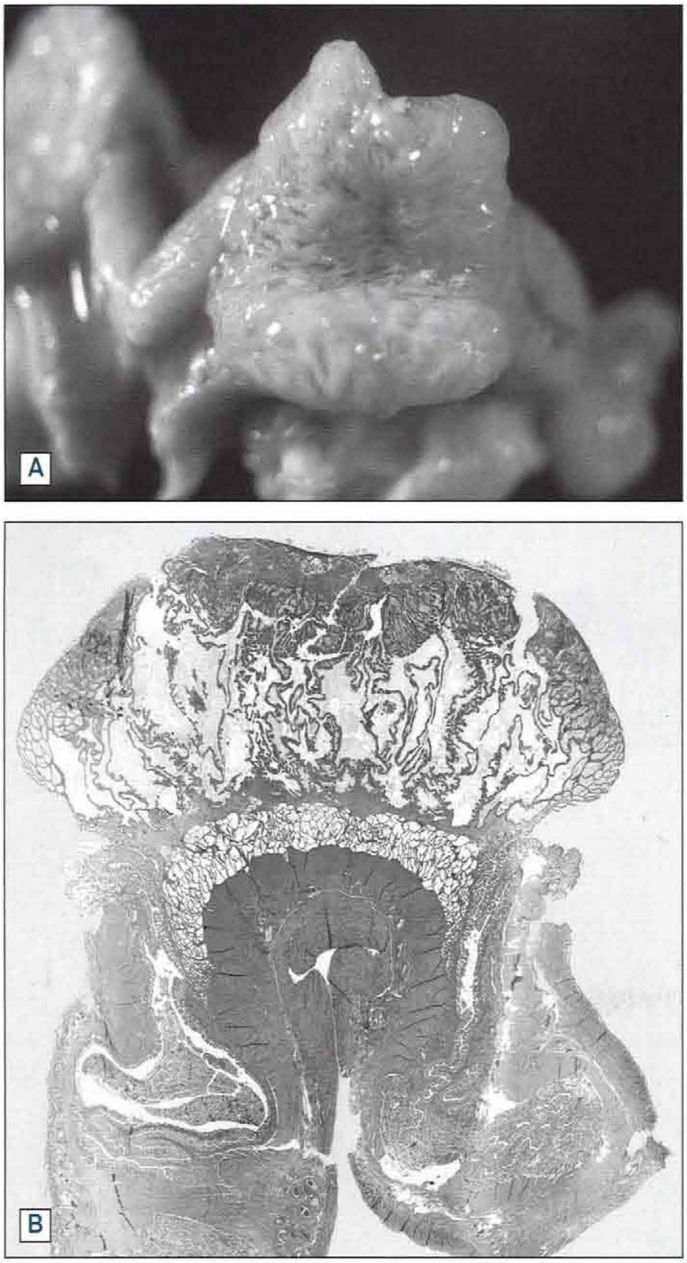

Figure 4.53 A, B. Segmental endometrial hyperplasia in a bitch. A less common form of endometrial hyperplasia occurs during the luteal phase in some bitches. The endometrium along one or more discrete sections of the uterine horn undergoes a highly organized hyperplasia that is very similar to the endometrial tissue remodeling that occurs at placentation sites during normal pregnancy. The uterine horn is distended and, when cut open, the proliferative band of endometrial tissue protrudes from the endometrial surface.

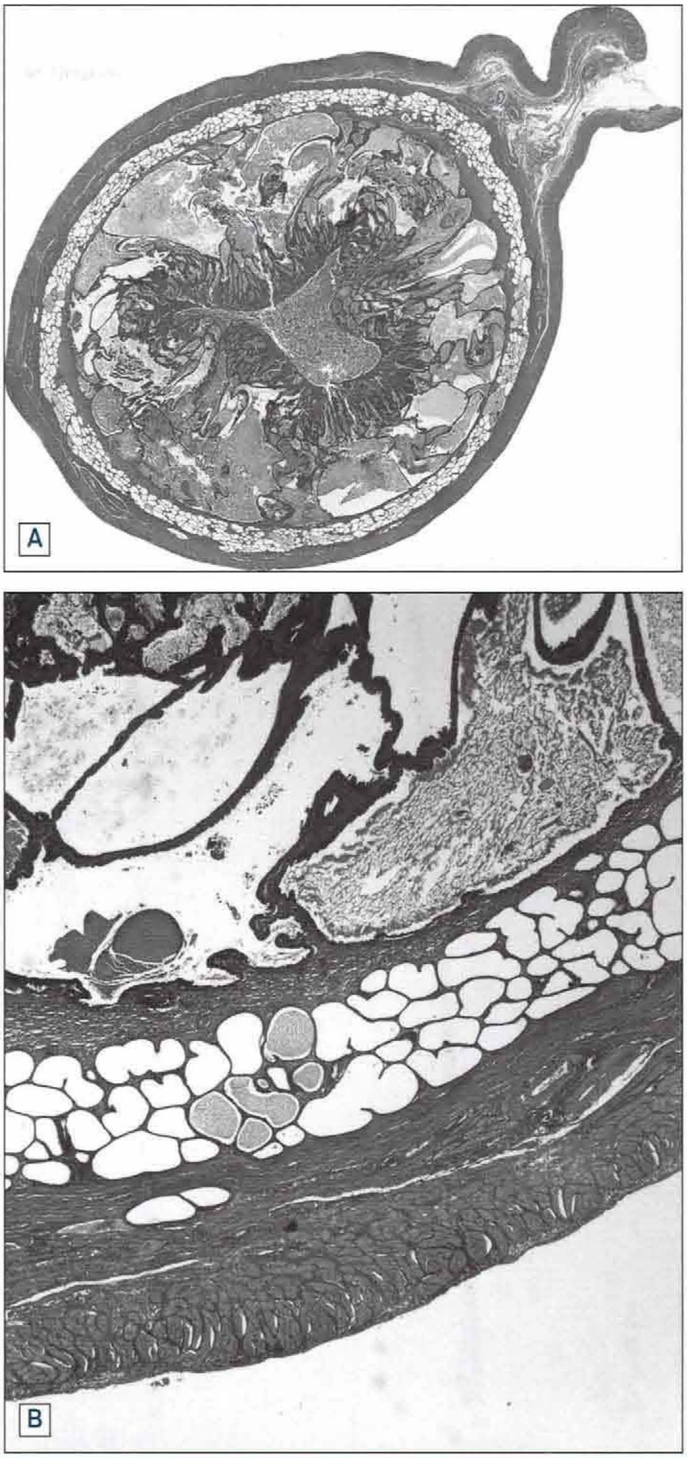

Figure 4.54 Subgross photograph of **segmental endometrial hyperplasia** in the bitch. Note the uniform distension of the deeper areas of endometrial glands. The luminal epithelium has many thin folds, lined by secretory epithelial cells. The luminal aspects of these folds are usually necrotic and the necrotic debris is admixed with mucoid endometrial secretions. The subgross photograph in (**A**) is a section of uterine horn cut through the center of one of these areas. Panel (**B**) is a higher magnification of the deep glandular area with a thin band of tissue separating the highly proliferative villous folds that resemble the junctional zone that develops in normal canine placentation beneath the area of zonary attachment.

is returned after removal from the offending pastures. The infertility is in large part attributable to failure of transport of sperm through the cervix due to changes in the cervical mucus. Estral cycles are normal, and normal ovulation apparently occurs. The dystocia that is common is attributed to reduced myometrial tone at parturition; it results in a high rate of maternal and neonatal death. Uterine prolapse occurs in maiden and nonpregnant ewes as well as postpartum ewes; mammary development and lactation occur in nonpregnant ewes.

The principal morphologic changes in the cervices of affected ewes consist of greater glandular development, reduced numbers of goblet cells, and a reduced amount of stratified epithelium. These changes are associated with an increased incidence of cervicitis. The endometrium is hyperplastic, and macroscopic cysts are often present. Hydrometra and pyometra are occasionally seen. These are the signs and lesions that were described in the original reports of infertility in ewes caused by estrogenic plants, commonly known as *clover disease.*

The development of varieties of clover low in formononetin has made it possible through changes in management and agronomic measures to control the most severe forms of the disease. But, as the original syndrome has been brought under control, *a much more insidious form of estrogenic infertility in ewes has emerged as the result of chronic or repeated ingestion of low levels of phytoestrogens.* This syndrome causes less severe reproductive losses, but the infertility tends to be permanent. Its anatomic basis consists primarily of subtle cervical changes that also impair sperm transport. The cervical lesions are those of blunting of the cervical folds with a reduction of the number of folds and crypts associated with an increase in the stroma of the lamina propria. There is an increase in coiled tubular glands of the endometrial type in the cervical lamina propria. This change is most severe at the cranial end of the cervix. This glandular induction is the reason the disease has been referred to by the awkward name of *estrogen-induced transdifferentiation.* The persistent effects of estrogen also bring about modification of sexual behavior; some ewes develop a more male behavior, clitoromegaly, and fusion of the ventral labia. These changes are mild and the disease can be best identified by careful cervical histologic examination, determining the number of folds and quantifying the area of the lamina propria. Since this is unknown territory for most pathologists, adequate control material is essential.

The effects of estrogenic pastures or of the various isoflavones on **cows** have not been conclusively examined, but there is little doubt from information accrued in Israel and Tasmania that the effects are important; alfalfa (lucerne) and the clovers listed above have been blamed. The principal features of the syndromes reported are similar to those produced by cystic follicles. The infertility rate is high and is associated with persistent cystic ovaries, aberrations of the estrous cycle with abnormally long estrus, swelling of the vulva independent of estrus, and sometimes enlargement of the clitoris, increase in size of the uterus and cervix with cystic endometrial hyperplasia, and enlargement and function of the virgin mammary gland.

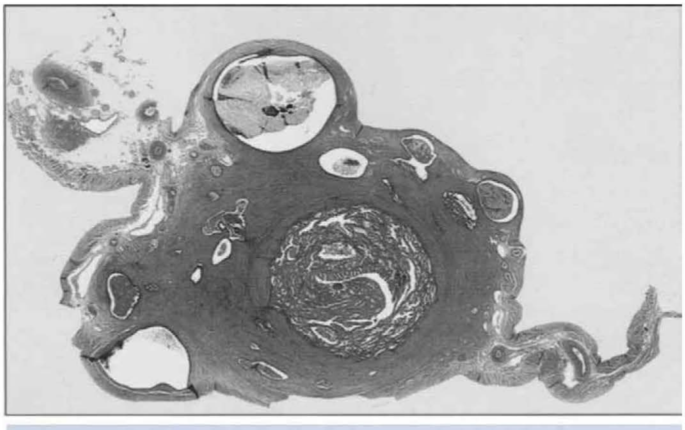

Figure 4.55 Canine adenomyosis. Adenomyosis can appear as single small gland profiles within the myometrium, or become marked, as demonstrated in this subgross section. Variably sized cystic endometrial glands are scattered throughout the myometrium and protrude from the serosal surface of this cross-section of uterine body. The epithelial lining of these ectopic glands ranges from normal-appearing (see Fig. 4.56) to attenuated in the larger cysts.

Adenomyosis

This term applies to the presence of endometrial glands and stroma between the muscle bundles of myometrium (Figs 4.55, 4.56).

In some cases it is a malformation and in others it arises by hyperplastic overgrowth of the endometrium. It is not a common lesion in any domestic species, but is seen in the bitch with cystic endometrial hyperplasia. Adenomyosis is occasionally observed in cows as part of the local disarray of segmental aplasia. It may also be present as a malformation of the tips of the uterine horns in cows.

Adenomyosis as seen in domestic animals shares features with *endometriosis* of menstruating primates. However, there are important

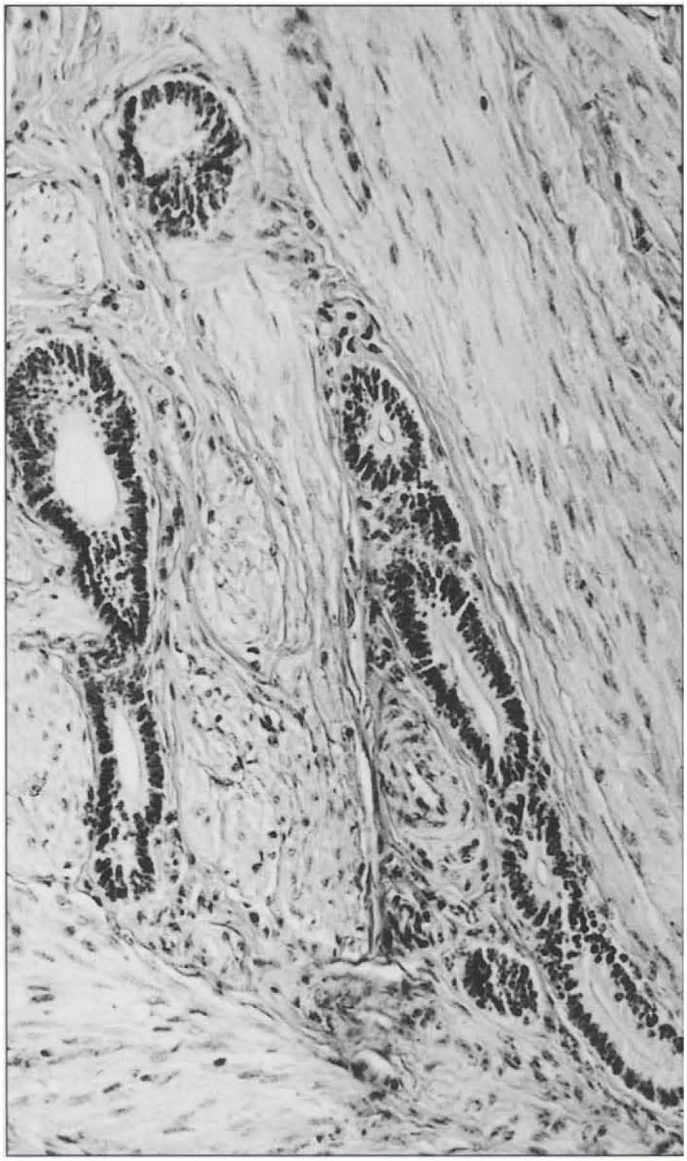

Figure 4.56 Photomicrograph of **canine adenomyosis**. These slightly irregular endometrial glands are present within the myometrium.

distinctions. Endometriosis is a condition in which actively growing endometrial tissues are explanted to aberrant sites within and outside the uterus. One of the aberrant sites can be the myometrium; in this site the two conditions are histologically similar.

Endometrial polyp

This lesion is seen most commonly in the bitch and queen and is striking when the polyps are large (Fig. 4.57). Segmental distension of the uterine horn(s) can be confused with tumors of the myometrium (leiomyomas), chronic pyometra, pregnancy or sites of fetal resorption, or segmental cystic endometrial hyperplasia. In contrast to the polypoid form of endometrial hyperplasia, *the true polyp contains substantial connective tissue stroma in addition to dilated glands, and is pedunculated* (Fig. 4.58). Polyps may be multiple or isolated, and their shape is molded to the uterine lumen.

Polyps may provide sufficient mass that uterine contractions can mechanically force prolapse of the affected horn. Endometrial polyps may be observed protruding through the cervix into, and sometimes even from, the vagina.

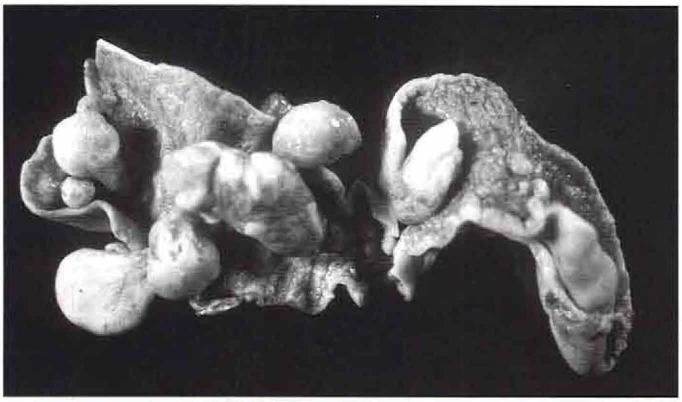

Figure 4.57 Multiple **endometrial polyps** in a queen. This queen had several firm segmental enlargements involving both uterine horns. Endometrial polyps can be single or multiple and occur most commonly in older queens and bitches.

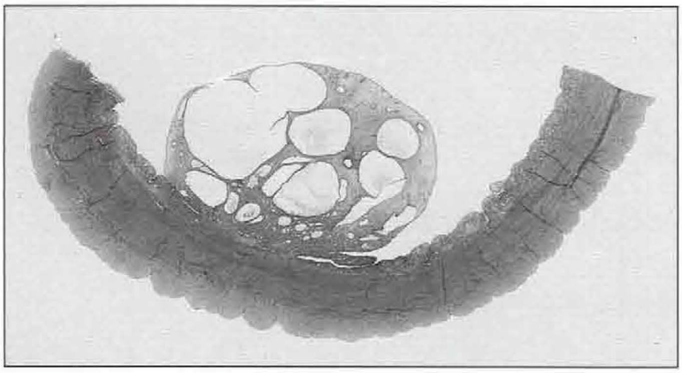

Figure 4.58 Canine **endometrial polyp** extending into the uterine lumen. Note the cystic spaces that are dilated endometrial glands. These polyps contain fibrous connective tissue bands, and progress from locally extensive areas of cystic hyperplasia that become fibrotic, compromising release of secretions from the glands. With uterine contractions and limitation by the lumen, these become pedunculated. Larger polyps assume the shape of the lumen and can become fusiform.

Uterine accumulation of secretory or inflammatory exudates

Hydrometra and mucometra

The two conditions are considered together as the difference is probably only in physical properties and depends on the degree of hydration of the mucin, which in turn may be related to the relative activity of estrogenic hormone. *The accumulation of thin or viscid fluid in the uterus is concurrent with the development of endometrial hyperplasia or is proximal to an obstruction of the lumen of the uterus, cervix, or vagina.* Grossly, there is uniform distension of the uterus (Fig. 4.59). If hydrometra or mucometra persists for a long time, the endometrium becomes markedly attenuated and will atrophy (Fig. 4.60).

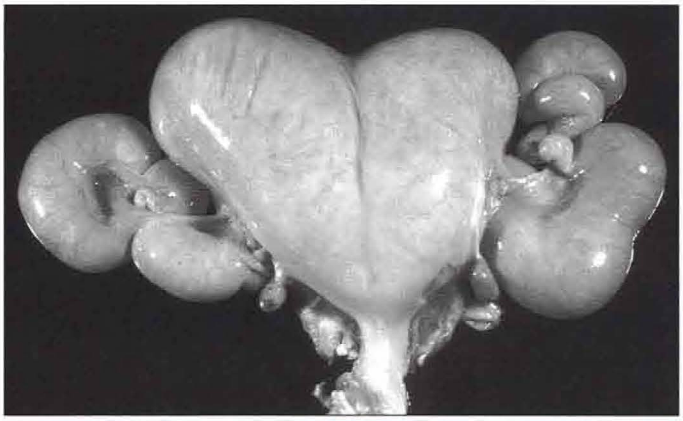

Figure 4.59 **Hydrometra** in a goat. The uterine wall becomes thinner as fluids accumulate. Accumulation of viscous fluid within the uterus is called *mucometra*.

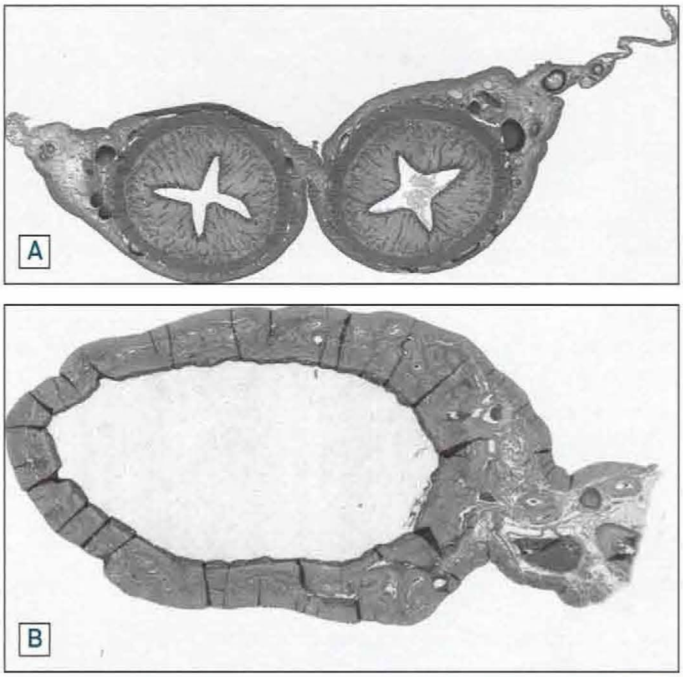

Figure 4.60 **A.** Subgross photograph of a cross-section of normal uterine horns from a bitch for comparison to the distended uterine lumen from a bitch with **hydrometra** in (**B**). Long-standing hydrometra or mucometra can lead to atrophy of the endometrium.

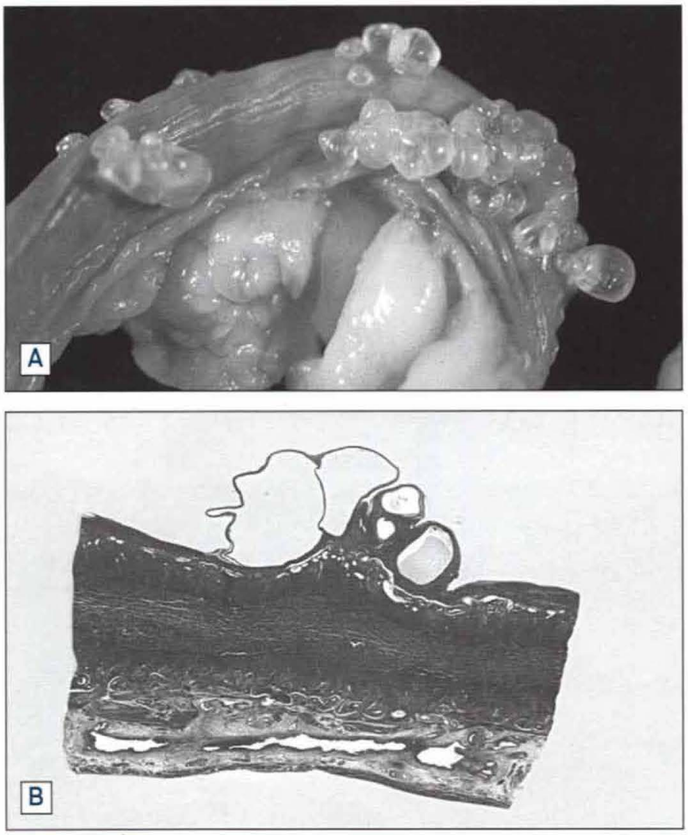

Figure 4.61 A. Serosal inclusion cysts on the surface of a uterine horn from a bitch. These cysts develop following adhesion of the serosa to itself in areas of linear folds and, most likely, in association with mild or minimal perimetritis. No inflammation is present by the time the cysts are detected. **B.** Cysts are most commonly multiple, thin-walled, and contain clear fluid, as shown in this subgross photograph taken from tissues from a different bitch. They can be differentiated from mesonephric duct cysts that are only found at the attachment of the mesometrium (broad ligament) and are usually single or tubular. They also occur in other species, but are most commonly found in older multiparous bitches and small ruminants.

In cases of congenital or acquired obstruction of the lumen of the tubular genitalia, the volume of fluid that accumulates proximal to the obstruction depends on the site of the obstruction. In uterus unicornis, there may be 500 mL; in imperforate hymen, there may be 10 L or more. The fluid is slightly cloudy and watery but, in some cases of segmental aplasia where the volume of retained secretion is not great, it may be very viscid, ocher-colored, and sometimes inspissated to rubbery masses of mucin and cellular detritus (see Fig. 4.21). In these cows, the ovaries are normal. If affected uteri become infected, an intractable pyometra results. An abnormally long and tortuous cervix may result in a form of mucometra caused by the retention of uterine secretions.

Serosal inclusion cysts

Uterine serosal inclusion cysts are thought to arise by pinching off of surface epithelial indentations. They are occasionally observed in the aged pluriparous bitch and less commonly in ruminants, and appear as thin-walled cysts containing clear watery fluid and vary in size from a few millimeters to several centimeters. Serosal inclusion cysts form from small folds of peritoneum that adhere and form entrapped pieces of serosal epithelium that slowly accumulate secreted fluids. They tend to occur either during uterine involution or in association with perimetritis; however, inflammatory cellular infiltrates are not usually present in or near these cysts. The gross appearance of these cysts can be striking. Most lie on the surface of the uterus (see Figs 4.44, 4.61). They contain clear fluid and have thin translucent walls. They may vary from a few millimeters to 2 cm or more in diameter. Serosal inclusion cysts can be distinguished from paraovarian cysts by their location. Similarly, although a differential diagnosis would also include mesonephric duct cyst, the latter are located along the broad ligamentous attachment, tend not to be multiple, are not as superficially located, and microscopically would have smooth muscle in their wall.

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INFLAMMATORY DISEASES OF THE UTERUS

General considerations

Inflammation limited in extent to the endometrium is termed **endometritis**; involvement of the entire thickness of the wall is **metritis**; of the serosa, **perimetritis**; and of the suspensory ligaments, **parametritis**. The classification is to some extent a useful index of the severity of reaction and of the pathogenesis. The great majority of inflammatory conditions of the uterus begin in the endometrium and are in some manner associated with the reproductive process. The predisposing factors are to be sought then at either end of the gestation period.

The normal nonpregnant uterus is endowed with a high degree of resistance to infection, and even in the case of the specific genital diseases, brucellosis, trichomoniasis, and *Campylobacter* infection, is incapable of supporting bacterial growth or even the persistence of bacteria for any extended period. Something is known of factors that render the uterus susceptible, at least temporarily, to infection, but little is known of the mechanisms of its resistance. The self-limiting nature of most infections, other than those associated with pyometra, has long been recognized and formed the basis for the recommendation of a period of sexual rest for animals with uterine infections. Even the specific genital infections are self-limited in duration; active infection of a uterus with *Campylobacter fetus* or *Tritrichomonas foetus,* in the absence of pregnancy, survives only for two to three estrual cycles. *Brucella abortus* causes infection of the pregnant uterus but does not persist well in the nonpregnant uterus, although it is apparently capable of persisting in the mammary gland and lymph nodes of infected cows. *Uterine resistance varies during the estrous cycle, susceptibility being greatest during the luteal phase of the cycle.* Several factors may be involved. Uterine motility is increased during estrus and this aids in the physical clearance of microorganisms. Uterine synthesis and secretion of immunoglobulin G (IgG) and IgA vary somewhat during the cycle, but the variations are not great and the timing varies between species. The functional activity of the polymorphonuclear leukocytes migrating into the uterus may also be affected by the stage of the cycle, being more active during estrus than diestrus, but much of the reduced uterine resistance during diestrus and pregnancy is related to secretion into the uterine lumen of progesterone-induced immunosuppressants that are capable of inhibiting lymphocyte proliferation. It has been well demonstrated that *the uterus that is under the influence of progesterone (which includes the pregnant uterus) is very susceptible to many nonspecific bacteria*, and that a uterus not under the influence of progesterone is remarkably resistant to the same organism, even when its expulsion is prevented by ligation of the cervix. This comparison can be carried a step further to show that even greater susceptibility to infection is present at the implantation sites in the pregnant uterus. The uterus of the castrate is resistant to infection but does not clear infections as promptly as does the uterus under the influence of estrogens. These factors of susceptibility and resistance provide some insight into the pathogenesis of postcoital uterine infections and pyometra in the dog and cat.

A different set of influences operates on the puerperium. *It is well recognized that uterine infections are likely to follow any abnormal parturition, such as an abortion, retained placenta, twin births, dystocia, and traumatic lacerations of the genital canal.* Of the specific abortive agents in the cow, *C. fetus* and *T. foetus* typically cause early death of the conceptus that is not complicated by placental retention or metritis. But with other causes, *B. abortus* especially, abortion may occur later in pregnancy, at which time the well-developed and manifold interdigitations of the cotyledons favor retention of the placenta and the development of acute metritis. But in these instances much of the uterine disease is the result of secondary invasion by bacteria that gain access to the uterus as the result of an abnormal delivery of a dead or diseased fetus.

Some cases of postpartum metritis are a continuation and exaggeration of a gestational uterine infection, but *most puerperal infections of the uterus can be viewed as analogous to wound infections, with the organisms entering via the cervix.* Probably all mares have uterine infections by streptococci within 1–3 days of parturition, but these do not persist for more than 2–3 days. In the cow, too, it has been observed that 25–30% are infected in the normal puerperium but that most recover spontaneously from these infections with *Arcanobacterium pyogenes, Escherichia coli,* and other bacterial mixtures. Clearly, the outcome will be determined as much by the number and virulence of invading organisms as by the environment within the uterus. The recognized predisposing causes outlined above will all be associated with retarded uterine involution, as will most cases of metritis. With the more virulent anaerobic bacteria, such predisposition is not essential.

The period necessary for normal involution varies with the species and is determined by the nature of placentation (depth and nature of invasion – deciduate versus adeciduate) and the rate of endometrial repair. It is well advanced in the mare within 9 days, as judged by the capacity for fertile mating at that time, in contrast to the involutionary process in the bitch, which involves deposition of material and extensive, slowly progressive remodeling. In the latter case, abnormalities of involution are common and termed *subinvolution of placental sites*. Most of these are probably never recognized clinically and spontaneously regress.

Endometritis

In endometritis, *the endometrium or uterine mucosa is mainly involved.* Almost all uterine infections begin as endometritis, and many such cases progress to involve the myometrium, becoming metritis. The mildest forms are seen postcoitus. In the mare, semen is ejaculated directly into the uterus and induces a transient postcoital endometritis. Infectious agents tropic for the uterus include *T. foetus* and *C. fetus* or pyogenic cocci and coliforms of low pathogenicity.

Uterine infection frequently occurs in **mares** both following foaling and after coitus by α-hemolytic streptococci, *Klebsiella pneumoniae, Escherichia coli* and *Taylorella equigenitalis,* the contagious equine metritis organism. The endometritis is usually mild, but the impact on fertility can be substantial.

Mild endometritis may not be overtly evident clinically. A slight opacity of the normally crystal-clear estrual mucus may be all that is seen. Histologically, the changes are not striking and consist for the most part of a diffuse but light infiltration of inflammatory cells with slight desquamation of the superficial epithelium and no significant vascular changes. Involvement of the glands is minimal. The significance of a few leukocytes in the stroma is always equivocal in cattle; they follow within 2–3 days of parturition and are present during estrus. However, the presence of neutrophils in the stroma of the endometrium is evidence of inflammation in the mare. *The best indication of mild endometritis in all species consists of accumulations of plasma cells and foci of lymphocytes in the stroma.* Resolution of this type of endometritis may occur with no more residue than a few cystic glands with periglandular fibrosis (Fig. 4.62), although during its course it may be responsible for a series of aborted embryos. If the endometritis is more severe or persists for a longer time, the cumulative damage to the endometrium may render the mare sterile.

Contagious equine metritis is a *venereal disease of mares* caused by ***Taylorella equigenitalis***, a gram-negative, microaerophilic coccobacillus. The disease produced by this organism does not appear to be significantly different from other common infecting organisms of the mare's genitalia. The interest in this disease stems from its apparently abrupt appearance in horse-breeding establishments in England in 1977 and its rapid spread to equine studs around the world. Strict control measures seem to have limited its spread and the clinical disease is now rare.

The disease causes temporary infertility in mares and a mucopurulent discharge that lasts 2–3 weeks. The organism can persist in infected mares for several months, and recovered mares represent an important reservoir of infection. Stallions transmit the organism by genital contact but do not develop clinical disease.

The temporary infertility that is the clinical hallmark of the disease is the result of mild to moderate inflammation of the endometrium and adjacent structures. After experimental introduction of the organism into the uterus, and presumably after natural infection, the bacteria can be regularly demonstrated in the uterine tubes, endometrium, cervix, and vagina for a 2–3-week period, during which time the organism elicits a mild to moderate inflammatory response. The endometrial folds are turgid and swollen and covered by a small amount of cloudy viscid exudate. At this stage the endometrium is edematous and the inflammatory infiltrate consists mainly of neutrophils. As the reaction subsides, the edema disappears and plasma cells predominate. Changes in the cervix parallel those of the endometrium. Salpingitis develops in some cases but is a less regular feature of the infection. Infection produces no gross changes in the vagina or vaginal vestibule, and histologic changes are mild. No lesions develop in the clitoral fossa or sinus, but the organism can be recovered from these sites after it has disappeared elsewhere.

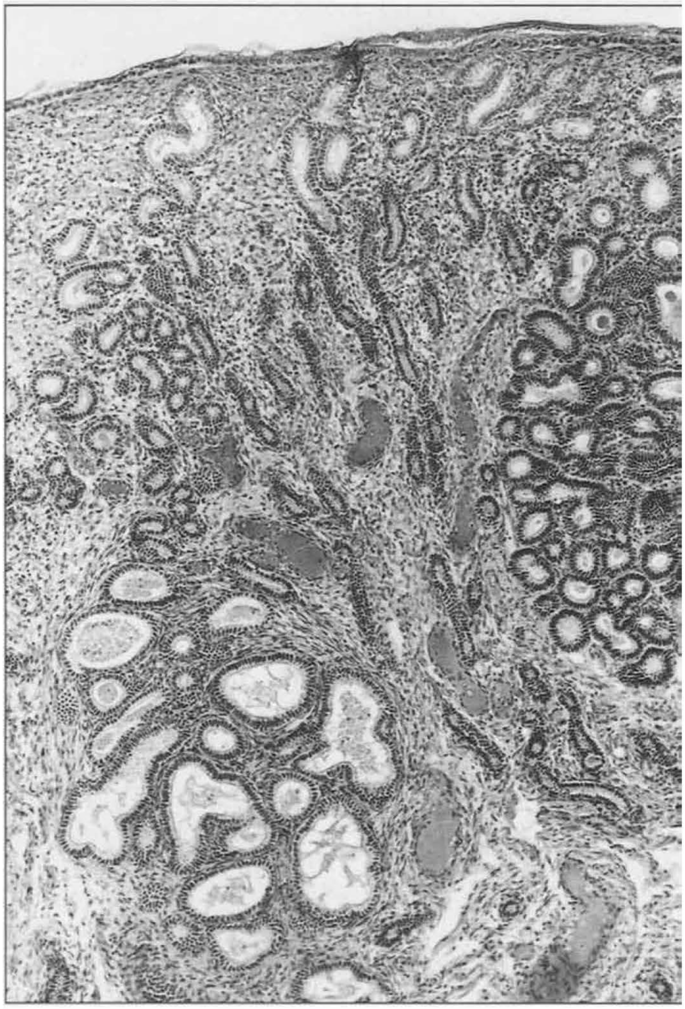

Figure 4.62 Endometrial tissue from a mare showing **glandular nesting**. Endometrial biopsy is routinely done in mares, tissues are evaluated, and a category (score) is assigned based on the presence and severity of endometrial fibrosis, inflammation, lymphatic dilation, and loss of glands. The clinical history is also considered. The most common system used, the Kenney–Doig, uses four categories (I, IIa, IIb, and III).

More severe grades of endometritis are common to the puerperium in **cattle**. Nothing of significance may be visible on the serosal surface, but the organ is enlarged and flabby, and collapsed rather than firm and contracted. The lumen contains chocolate-colored lochia that is slightly tenacious and often without foul odor. With the admixture of inflammatory exudate and placental detritus, the uterine content becomes progressively dirty gray-yellow. The endometrium is congested and swollen, and the intercotyledonary areas are ragged and tattered with shreds of mucosa free in the lumen. Small hemorrhages are common in the congested mucosa, and there is a prominent leukocytic infiltration massing at the surface involving all mucosal elements, including the glands. Where suppuration and superficial necrosis produce the tattered mucosa, the surface is comparable to a pyogenic membrane. The remainder of the genital canal may show nothing more than the traumatic lesions incident to parturition. If the uterus is paretic, there may be no discharge in the vagina.

Sporadic outbreaks of suppurative endometritis associated with *Bovine herpesvirus 4* have been reported. The histopathology is typical for herpesvirus infections, with focal necrosis, ulceration, and common secondary bacterial infections. Intranuclear viral inclusion bodies are found in endometrial epithelial and endothelial cells.

Metritis

The distinction drawn here between endometritis and metritis for purposes of description is that, *in metritis, all layers of the uterine wall show evidence of acute inflammation.* The uterus is paretic and there may be little or no vaginal discharge. The wall of the uterus is thickened with suffused blood and edema fluid and is very friable. The serosa is dull and finely granular with "paintbrush" hemorrhages and a thin deposition of fibrin, or the subserosal vessels may be darkly congested. Other than traumatic rupture, perforation with secondary peritonitis is not common except in anaerobic infections; death in untreated cases usually occurs first from toxemia or septicemia.

The secretion may be scant or abundant, is fetid, and is dirty yellow to red-black. The microscopic picture is that of purulent inflammation. Subserosal connective tissues are edematous and infiltrated with leukocytes, and the same process surrounds the blood vessels of the myometrium and permeates bundles of, and individual, muscle fibers, which themselves undergo granular degeneration. In metritis, as in acute endometritis, leukocytes mass on the mucosal surface and are associated with extensive hemorrhage, necrosis, and sloughing. Invasion of blood vessels, both arteriolar and venous, aggravates the lesion. Thrombosis may extend to the vessels of mesometrium with the usual sequelae of hemorrhage and infarction.

Chronic endometritis

Recovery from the acute phase of the infection often results in chronic endometrial involvement. With greater-or-lesser degrees of endometrial destruction and replacement by granulation scar tissue, the uterus takes on the nature of a fistulous tract. The changes depend on the duration and severity of the inflammation, but *consist essentially of productive fibrosis and leukocytosis in which lymphocytes and plasma cells predominate.* Thickening of the endometrium is by inflammatory tissue; the glands are depleted; those that survive are atrophic, flattened, and attenuated, or cystic due to periglandular fibrosis. The lining mucosa may be intact, denuded in places, or show foci of polypoid hyperplasia or squamous metaplasia, as do any chronically irritated mucous membranes. The exudate in the lumen is not copious and may be serous, catarrhal, or frankly purulent. Much of the labile endometrial stroma, especially that of caruncles, may be replaced by useless scar tissue, and dystrophic mineralization of necrotic portions of the endometrium may sometimes be extensive enough that the lining of the uterus feels gravelly.

It is estimated that 20% of cows with generalized **tuberculosis** and 4% of all tuberculous cows have involvement of the endometrium. There are three routes of infection, namely, hematogenous, via the uterine (fallopian) tubes from the peritoneum, and coital; of these, the last is exceptional. As in tuberculous lesions generally in cattle, *there are two anatomical forms of the lesion, miliary tuberculosis and diffuse caseating tuberculosis,* although transitional forms do exist. It is generally accepted that the disseminated miliary lesion is of hematogenous origin during the phase of early dissemination.

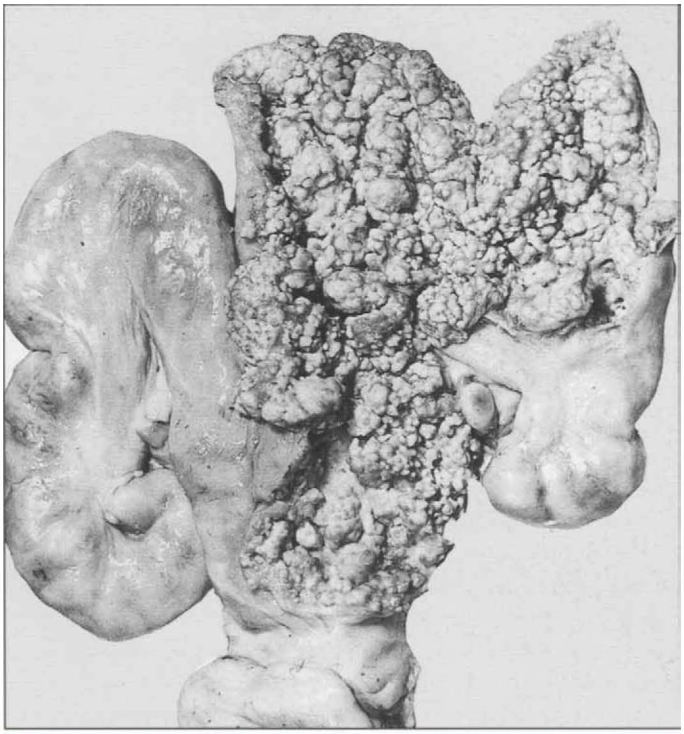

Figure 4.63 Granulomatous metritis in bovine tuberculosis.

In *miliary tuberculosis,* the uterus may appear normal externally. In the early stages, there may be no exudate in the lumen, but later, as the granulomas enlarge and ulcerate, the uterus will contain yellow purulent exudate. The granulomas are visible as few or many nodules in the mucosa, usually the more superficial portions, and microscopically are of typical tuberculoid structure. A common site is near the bifurcation of the uterus or in the caruncles of the pregnant uterus.

Caseous tuberculosis causes thickening and rigidity of the horns with serofibrinous or purulent fluid in the lumen. The endometrium is thickened, dry, and extensively caseous (Fig. 4.63). There may be intense leukocytic infiltration and marked exudation. The caseated area is usually demarcated by a zone of epithelioid cells from a margin of connective tissue.

In association with the uterine lesion in tuberculosis, there is often involvement of other portions of the genital tract. There may be multiple red granulations on the surface of the ovary and its ligaments (Fig. 4.64), or there may be enlargement of the ovaries with parenchymal tubercles. Tuberculous salpingitis or pyosalpinx is probably the rule when the uterine tubes are the portal of uterine infection. Similar lesions may be found infrequently in the lower

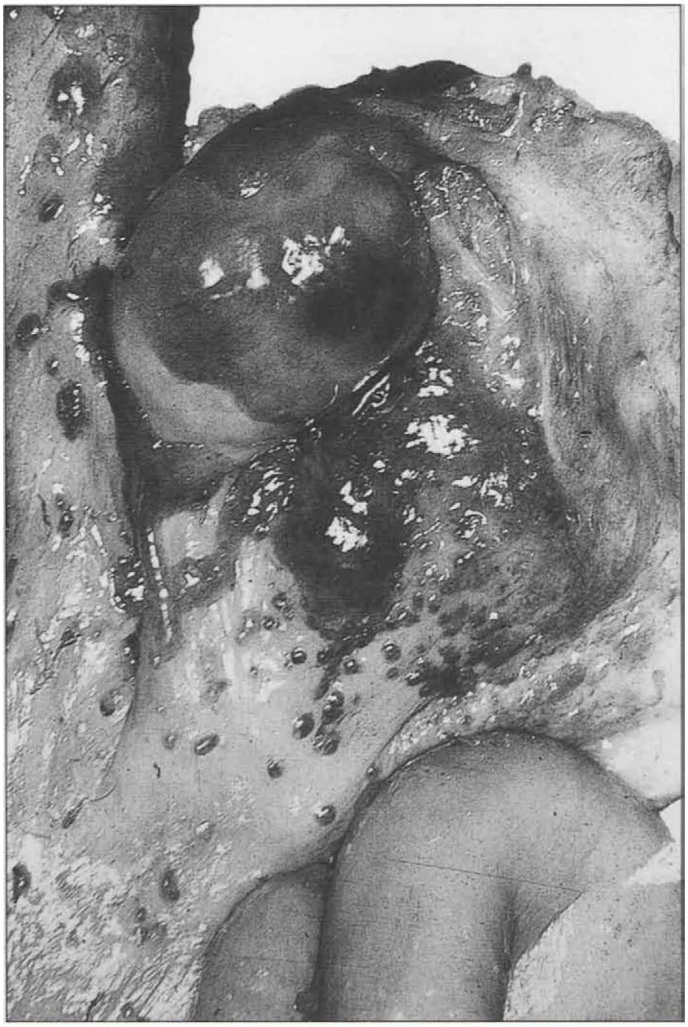

Figure 4.64 Serosal surface of mesovarium and ovary with granulomatous inflammation in **bovine tuberculosis**.

genital tract. Infection of cows with the avian strain of the organism also produces tuberculous metritis and placentitis with multiple small endometrial granulomas.

Uterine abscess

This is not a common observation. The localization of an infection to one part of the uterine wall is thought to follow severe metritis or localized traumatic injury to the infected endometrium. Such an abscess may reach 15 cm in diameter and is usually well encapsulated, although there may be some perimetrial adhesion and, in few instances, rupture into the peritoneal cavity or an adjacent hollow viscus.

Uterine abscesses are observed more frequently in cattle than in other species. There appears to be a relationship between the frequency of abscesses and uterine manipulations involving the use of instruments. *In cattle, most large abscesses are located in the dorsal wall of the uterine body.* This is the area most subject to trauma during the passage of insemination pipettes and uterine catheters. Abscesses that develop following severe metritis are usually small (1–3 cm) and do not have preferential sites.

Parametritis and perimetritis

Chronic adhesive peritonitis involving the genital tract does not usually result from septic metritis because the uterine serosa offers an efficient barrier to the spread of infection, and spontaneous rupture of an infected uterus is not common. Few virulent infections spread to the supporting ligaments. Excluding an origin from an extragenital focus, *perimetritis and parametritis in cattle usually follow manual manipulation of the ovary, pyosalpinx, obstetrical operation, removal of retained placenta, and uterine irrigations.* In each of the latter three circumstances, there may be accidental perforation or rupture of the uterus. The extent of the adhesion may vary from a few fibrous bands to dense connective tissue that obscures the contour of the organs and fixes them to adjacent viscera (Fig. 4.65). Abscesses may form in the adhesions of the ovarian bursa and the rectovaginal pouch.

Salpingitis

See the section on Pathology of the uterine (fallopian) tubes, above.

Pyometra

Pyometra is acute or chronic suppurative infection of the uterus with accumulation of pus in the uterine lumen. The escape of the pus is usually prevented by a functionally closed cervix. But the discharge may be prevented by an acquired or congenital cervical stenosis, and in mares the gravitational pull of the flaccid, distended uterus over the brim of the pelvis may limit the discharge of pus. Pyometra may occur as a sequel to uterine infections of the types described in the previous sections, but as it is a pathologic entity with a number of factors entering into its pathogenesis, it is considered separately here. Pyometra is an uncommon condition in the ewe and the sow. *It is relatively common in the bitch, queen, cow, and mare,* but the circumstances under which the disease develops in these species vary.

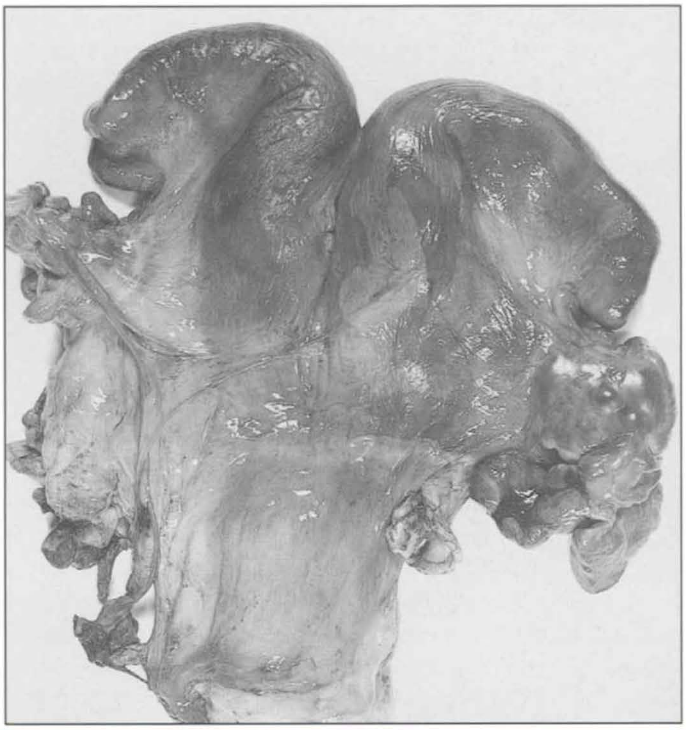

Figure 4.65 Pyometra with secondary adhesions in the uterus of a cow.

Pyometra in the bitch and queen

Pyometra in the **bitch** is a disease that characteristically affects older animals, especially those that are not bred. *The condition most often develops a few weeks after estrus.* Affected animals are depressed and anorexic, frequently vomit, and have polyuria and polydipsia, usually accompanied by a vaginal discharge. The pathologic findings vary with the stage of the disease. In less advanced cases, the uterus may be only slightly enlarged with mild endometrial hyperplasia and inflammation. In the more advanced stages, there is a remarkable distension of the uterine horns, which may come to occupy most of the peritoneal cavity (Figs 4.66, 4.67). (In contrast, pyometra in cattle most commonly develops in the postpartum period and is frequently associated with retention of fetal membranes (Fig. 4.68). If the purulent exudate does not drain from the uterus, it can become dehydrated with time and the inspissated material is found as soft concretions in the uterine lumen (Fig. 4.69).)

Distension of the cornua may be symmetrical or asymmetrical, uniform or ampulla-like dilatations, as in the uterus of mid-pregnancy. The cervix is completely or almost completely closed as a functional response to luteal hormones. The serosal surface of the uterus is dark and the vessels are congested and prominent. The wall is friable and either rupture or perforation with secondary peritonitis is not uncommon. There may be obvious inflammation of the peritoneal serosa and suspensory ligaments, but this is unusual. The nature of the uterine content is variable. In the more severe cases, usually those infected with *Escherichia coli* and *Proteus* spp., the exudate is thick, viscid, tenacious, opaque, red-brown, and with a characteristic fetid odor. In other cases, usually those infected with streptococci and staphylococci, the exudate is more typically purulent. The mucosa is irregular in thickness, necrotic, and ulcerated in portions with irregular superficial hemorrhages,

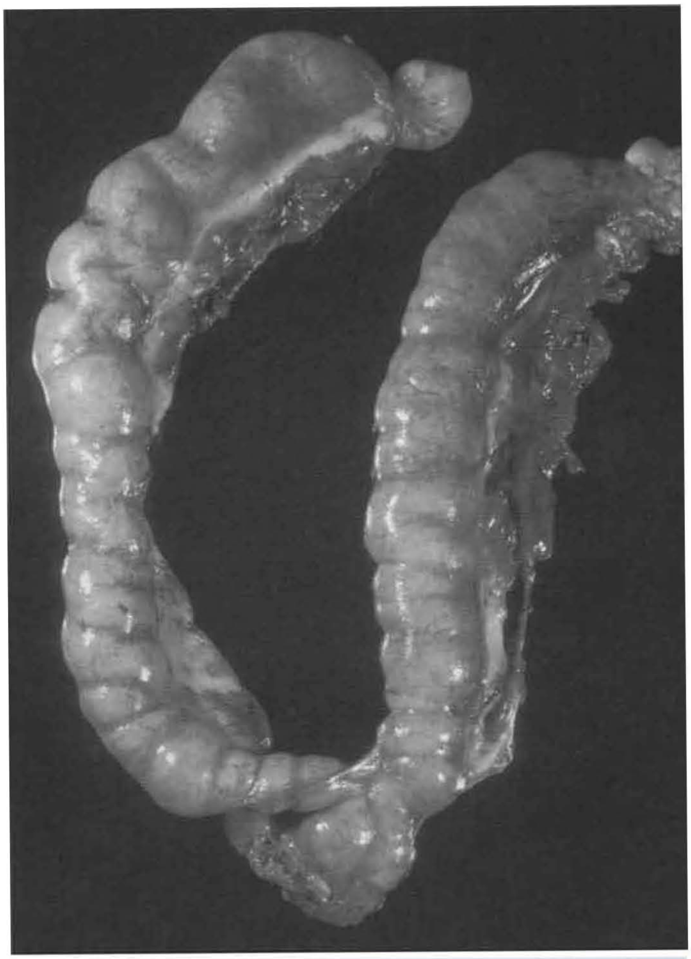

Figure 4.66 Pyometra in a bitch. Note the spiraling of the uterine horns, a change that occurs when the canine uterus is under the influence of progesterone. Both horns are mildly distended by purulent exudate. Cystic endometrial hyperplasia is commonly present (see Fig. 4.71).

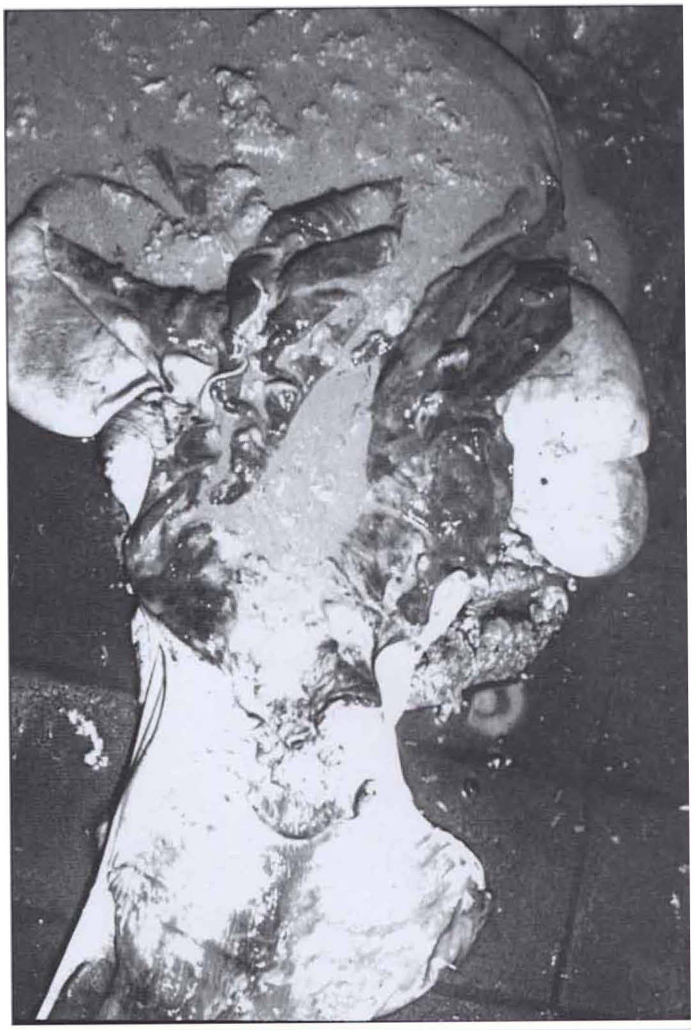

Figure 4.68 Pyometra in a cow.

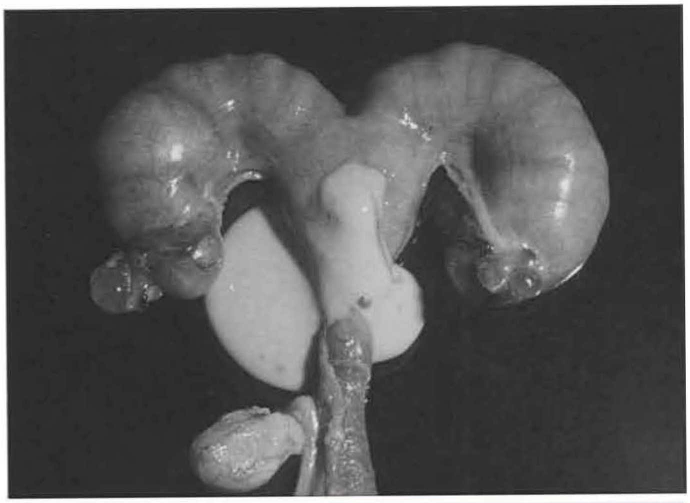

Figure 4.67 Pyometra in a queen.

and in other portions obviously hyperplastic, dull-white, and dry in appearance with small cysts visible in these hyperplastic areas.

Microscopically, the most significant feature is the remarkable endometrial hyperplasia and progestational proliferation in almost all cases (Figs 4.70, 4.71). The cells of such progestational epithelium are enlarged, columnar, vacuolated, and have small pyknotic nuclei. In some cases, the normal single layer of cells piles up to produce pseudostratification or localized papillary proliferations. Whatever remains of the endometrial lining may show this development, or it may be patchy and alternating with normal epithelium.

These changes are produced by the luteal hormones that inevitably pave the way for the development of this disease. *The histological changes due to infection vary with the bacterial cause and time.* Masses of neutrophils collect in the uterine lumen and in the glands, although there is relative sparing of the glands unless they are cystic. The migrating leukocytes collect near the surface and then penetrate the epithelium. In milder cases, there may be few neutrophils in the endometrial stroma, but they are not many when compared with the numerous plasma cells and lymphocytes. There may not be much vascular reaction over and above that of hormonal origin, although perivascular reaction and leukocytosis of lymphatic vessels are almost constant in the myometrium. Sometimes the reaction is much more severe than that just described, and in the endometrial stroma there are all the exudative phenomena of acute inflammation accompanied by the early reparative response of granulation tissue. The blood vessels are very congested and some show thrombosis, and about others there is diapedesis or larger hemorrhage.

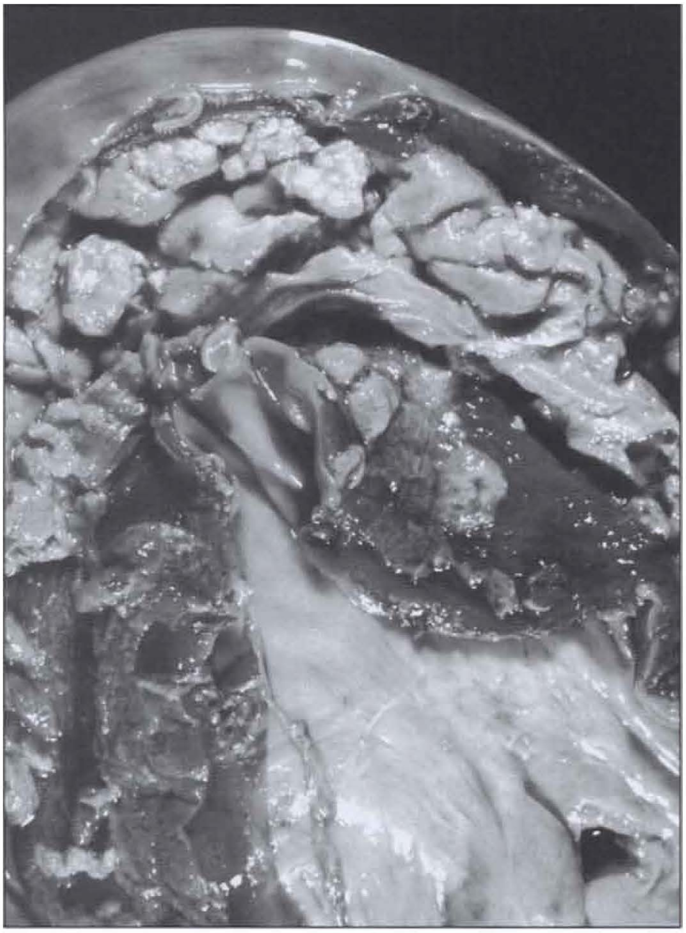

Figure 4.69 Chronic pyometra in a cow. Note the inspissated purulent exudate.

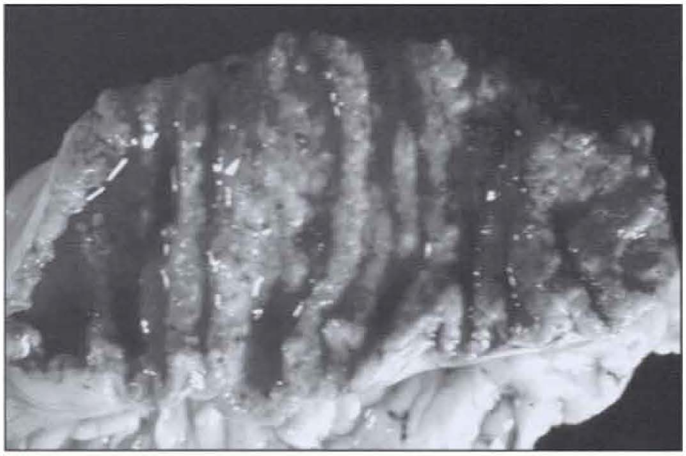

Figure 4.70 Cystic endometrial hyperplasia in a bitch. This uterine horn is opened, exposing the endometrial surface. The endometrium is thickened and there is uniform cystic distension of endometrial gland lumens. The bitch had purulent exudate in the lumen (pyometra).

The stroma is edematous and bullae often lift off the overlying epithelium. Numerous neutrophils infiltrate the stroma and collect in the lumen. There is little formation of fibrin, and rarely are there microabscesses in the mucosa.

It is clear that most cases of pyometra in the bitch are associated with endometrial hyperplasia. It is usually a disease of the diestral (metestrus) period, a time at which corpora lutea are present and progesterone levels are high and, since the condition has been produced both experimentally and accidentally by the administration of progestins, it is clear that *progesterone is an essential feature in the pathogenesis.*

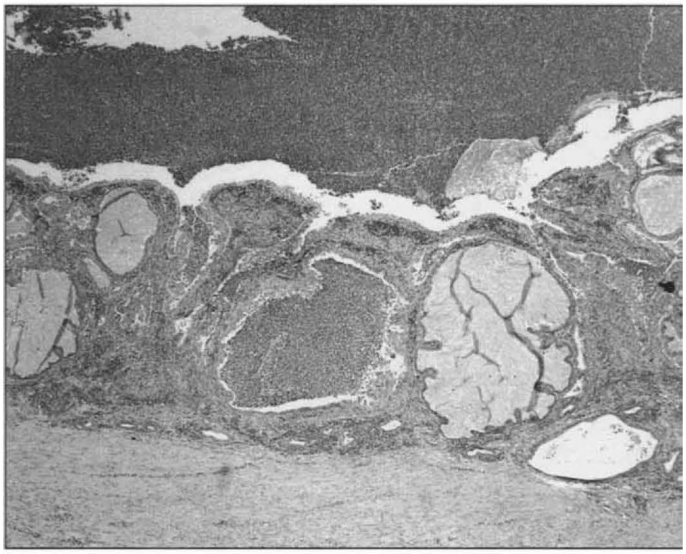

Figure 4.71 Canine pyometra with cystic endometrial hyperplasia (subgross photograph). During the luteal phase of the cycle, the canine endometrium is very sensitive to stimuli, responding by undergoing glandular and luminal epithelial hyperplasia with accumulation of secretions. This is followed by lymphocytic inflammation that becomes purulent when secondary bacteria invade the uterus.

Since cystic endometrial hyperplasia had been produced in dogs by administration of high levels of progesterone for extended periods of time, and since cystic endometrial hyperplasia was a hallmark of canine pyometra, it was presumed that cystic endometrial hyperplasia induced by abnormal progesterone secretion, or response, preceded and paved the way for the bacterial component. Some doubts concerning the validity of this view were generated when hormonal abnormalities could not be demonstrated in cases of pyometra. Now it has been shown that cystic endometrial hyperplasia can be produced in the normal, early-diestral uterus by mild trauma to the endometrium. The investigators scratched the endometrium with a thin wire, but endometrial biopsy has caused the same lesion. More directly to the point, inoculation of appropriate strains of *E. coli* into the uterine lumen, if given 1–2 weeks after estrus, causes a pyometra that has all the features of the natural disease, including cystic endometrial hyperplasia.

It appears that an appropriate stimulus applied to the progesterone-primed endometrium produces cystic endometrial hyperplasia and that one such stimulus can be a bacterial infection. Accordingly, rather than pyometra in the bitch occurring as the result of bacterial infection being superimposed on an abnormal endometrium, *it appears that the bacterial infection of the progesterone-primed endometrium produces both the pyometra and the cystic endometrial hyperplasia.*

The bacterium most commonly present in pyometra in the bitch is *E. coli*, and uterine *E. coli* isolates are typically identical or very similar to isolates from feces of the same bitch. Most of the strains involved are urinary tract pathogens and possess *uropathogenic virulence factors*, including P fimbriae, α-haemolysin, and cytotoxic necrotizing factor 1, that probably enhance the pathogenicity of the strain in the canine genital tract and may facilitate the colonization of the progesterone-primed endometrium. Urinary tract infections are common in bitches with pyometra, and the strains of bacteria infecting both are often identical. It seems probable that the urinary infection predisposes to pyometra, and that the uterus is infected at the stage that suitable receptors are developed in the endometrium in response to hormone stimulation. This explanation of the bacterial infection can be only a partial one as not all bitches with pyometra have urinary tract infection, and not all infecting organisms are urinary pathogens. In some cases of pyometra, bacteria cannot be recovered.

The clinical signs and extragenital lesions associated with pyometra in the bitch are due to severe intoxication and probably also intermittent bacteremia. Renal signs and lesions are common. Profound hypotension may reduce renal perfusion and lead to prerenal uremia. This is worsened by membranoproliferative glomerulonephritis that often develops as a result of immune-complex deposition. Polyuria, which is such a common feature of the disease, is due to impaired tubular ability to concentrate urine. The mechanisms involved in this are not completely understood, but they are thought to be brought about by *E. coli* antigen and have an immunologic basis. Most animals have profound leukocytosis, reflected in the bone marrow as an increased myeloid-to-erythroid ratio. There is myeloid hyperplasia in bone marrow and extramedullary myelopoiesis in liver, spleen, lymph nodes, and adrenals.

Pyometra in the **queen** is roughly comparable to the disease in the bitch, but there are differences, some of which are related to the differences in the estrous cycle of the two species. Cats are induced ovulators, and usually if not bred have repeated estrous periods. However, *pyometra develops most often 2–5 weeks after estrus*, and usually after spontaneous ovulation and presumably corpora lutea formation. Unlike the bitch, many queens with pyometra do not have corpora lutea present in their ovaries at death or surgery, but approximately half do, and *there is an obvious correlation between the presence of corpora lutea and the development of pyometra in queens.* The correlation with endometrial hyperplasia is less clear. Most queens with pyometra do have some degree of cystic endometrial hyperplasia, but this change is very common in the age group affected.

Pyometra in the cow

Pyometra in the cow is also associated with corpus luteum activity in the ovary. However, in contrast to the situation in the bitch in which the high progesterone levels of early diestrum predisposes to uterine infection, *in the cow, uterine disease causes the corpus luteum to persist and maintain a high progesterone level.* The retention of the corpus luteum appears to be due to a reduction in or inhibition of the synthesis and release of the luteolytic factor, prostaglandin-$F_{2\alpha}$, by the diseased endometrium.

Broadly, there are two periods in which a uterine infection can lead to retention of a corpus luteum with the accompanying hormonal effects that *convert an endometritis to a pyometra.* They are during the *early postpartum period*, following dystocia, retained placenta, and metritis, and at *various times after breeding*, as a result of venereal infections with early embryonic death. Insemination during the luteal phase of the cycle or similar operations that can introduce contamination into the uterus during the luteal phase can mimic venereal infection.

The role of retained luteal tissue in the pathogenesis of pyometra appears clear. The secreted progesterone endows the uterus with a high degree of susceptibility to infection, maintains functional closure of the cervix, and inhibits myometrial contractility.

The amount of pus retained in the uterus of a cow with pyometra varies from a few milliliters to more than several liters and is thick, rather mucinous, and cream or gray-green colored. The cervix has no seal of mucus so, although contracted, there is usually escape of a small amount of pus into the anterior vagina. The wall of the uterus is thick, doughy, and paretic but, in long-standing cases, especially as complications of mucometra, the walls are thin or fibrosed. The histological changes do not differ significantly from those of endometritis of comparable duration and severity. The organisms involved are hemolytic streptococci, staphylococci, coliforms, *Arcanobacterium pyogenes*, and *Pseudomonas*. The venereally transmitted protozoan, *Tritrichomonas foetus*, can be the cause of pyometra after breeding.

Rarely, the anomalous developments of the uterus that were described earlier can lead to pyometra. In the segmental aplasias and with imperforate hymen, the lumen proximal to the site of obstruction becomes distended with mucus and cellular detritus. These closed cavities provide a satisfactory environment for bacterial growth, but this is not a frequent complication. When it does occur, the source of infecting organisms is probably hematogenous. Pyometras seldom spontaneously resolve and, although the condition is not usually life-threatening as it is in bitches, unless cyclic activity can be reinstituted, the condition will persist.

Pyometra in the mare

Pyometra in the mare differs from the disease in the bitch and the cow in several particulars. While some cases develop following difficult parturitions with infections, as they do in cattle, many do not. Remarkably, most mares continue to cycle during the disease, and the hormonal influences that are so marked in the bitch, queen, and cow are much less important in the mare.

In some mares, cervical adhesions and closure may lead to pyometra, but in most instances the purulent material collects without demonstrable cervical closure, and in some cases the cervix is fully dilated. Copious amounts of pus can be discharged under such circumstances, particularly during estrus. *Pyometra in the mare seldom leads to evidence of systemic disease*, although some mares develop mild anemia.

The length of the estrous cycle appears to be related to the severity of the endometrial damage. In rare cases, where the endometrial damage is severe, the cycles are prolonged with long luteal phases due to delayed or inadequate prostaglandin $F_{2\alpha}$ release. Mares with less severe endometritis have normal or shortened cycles.

A variety of organisms may be present in pyometra in the mare: *Streptococcus zooepidemicus* is the most common, but *E. coli*, *Actinomyces* spp., *Pasteurella* spp., and *Pseudomonas* are often present.

Endometrial biopsy

Endometrial biopsy is most firmly established in *equine theriogenology*. The evaluation system is based on identification and scoring of four microscopic lesions plus consideration of the mare's reproductive history. *The microscopic features evaluated include inflammation, dilation of lymphatics, loss of glands, and presence of fibrosis.*

Only a small amount of information has been published on endometrial biopsy in other species. Pathologists are asked to evaluate endometrial biopsies for cattle and from bitches, and as more information is gathered and evaluated, recommendations for scoring will be followed by validation of their prognostic value.

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Pathology of the gravid uterus, placenta, and fetus

General considerations

It is difficult to generalize on the subject because of the remarkable variation in the reproductive affairs of the various species. Because the mechanisms by which the gestation period is initiated, the pregnancy maintained, and the delivery triggered have such profound effects on how diseases of the conceptus will be manifest, some review of the special features of reproductive patterns in the various domestic species is given here.

For a pregnancy to proceed, cattle, sheep, goats, swine, and horses require that the normal cyclic lysis of the corpus luteum be prevented. This is achieved in **cattle** by secretion by the blastocyst of interferon-γ, which inhibits synthesis and release of prostaglandin $F_{2\alpha}$ from the endometrium. This spares the corpus luteum and allows it to serve as the corpus luteum of pregnancy, and it then provides part, or all, of the hormonal support of the pregnancy. In cattle, up to 40% of total embryonic loss probably occurs between days 8 and 17 of pregnancy. Early embryonic death will permit release of prostaglandin $F_{2\alpha}$ and lysis of the corpus luteum and subsequent reinstitution of the estral cycles in these species. The delay between death of the conceptus and the return of the dam to estrus is usually sufficient to allow for complete or near-complete autolysis and dissolution of the fragile embryo. As a result, *the early diseased products of conception are rarely available for study.* Venereal infections cause some of the early losses, but on the basis of the few studies that have been done, and on the experience in other species, *it is presumed that chromosomal abnormalities account for most of these early losses.* In monotocous species, embryos that die undergo prompt dissolution, the products are expelled, and the disease is recognized only as infertility with slightly prolonged estral cycles. The death of a single embryo, or even several, does not interrupt the pregnancy in swine and dead embryos are resorbed or mummified. Rarely, the death of the conceptus may be caused by organisms that convert the pregnancy to a pyometra, the active infection preventing the lysis of the corpus luteum. Trichomoniasis of cattle is an example of a venereal infection that can lead to pyometra.

In the **mare**, gonadotropic hormone (equine chorionic gonadotropin) is produced in ulcer-like structures in the endometrium, *the endometrial cups* (Fig. 4.72). These structures form early in pregnancy and were originally thought to be maternal in origin and part of the mare's adaptation to pregnancy, but it has been shown that the cells that make up the cups are fetal in origin and develop from a band of elongated trophoblast cells of the chorionic girdle. These cells become detached from the fetal chorion on about day 35 of gestation and burrow into the stroma of the endometrium. There the cells enlarge and take on the appearance of decidual cells.

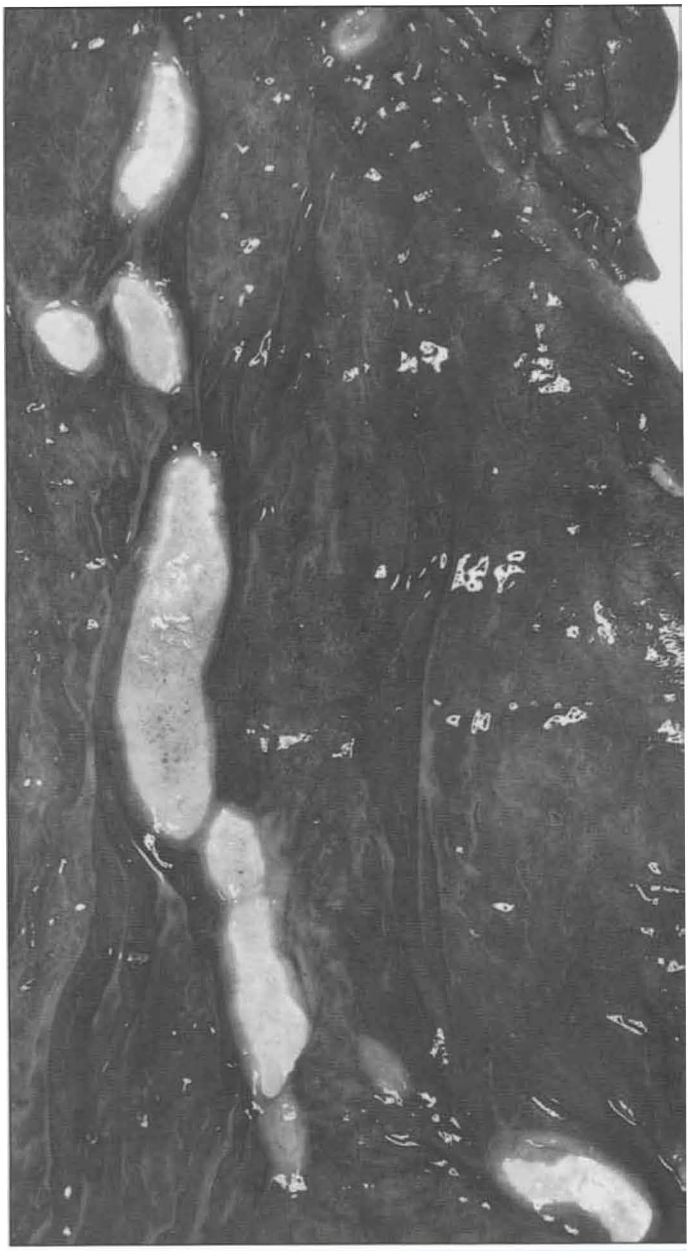

Figure 4.72 Endometrial cups in the uterus of a mare; these are the sites of production of gonadotropic hormones.

The cup tissue becomes visible at about day 40 of gestation as a horseshoe or ring-shaped band in the endometrium of the pregnant horn. The cups increase in size until day 60 of gestation, at which time their hormone production is at a maximum. Hormone levels fall after day 60, and the cups become pale and slough between days 75 and 100 of gestation. The degeneration of the cups resembles graft rejection. The cells of the fetal chorion are surrounded by maternal lymphocytes and undergo prompt degeneration. The necrotic slough is detached and lies free between the endometrium and allantochorion.

This potent chorionic gonadotropin in mares stimulates ovarian corpora lutea to develop, and these maintain the pregnancy until approximately 126–140 days, when the fetal chorion takes over the production of progesterone. If the conceptus dies after day 35 of gestation, at which time the fetal cells have invaded the endometrium, the cups form and secrete chorionic gonadotropin, accessory corpora lutea are produced, and the mare fails to return to estrus and continues for a variable period in a state of pseudopregnancy.

Domestic carnivores do not have estral cycles that require interruption in order to begin a pregnancy. Once formed, the corpora lutea usually persist during most of the gestation period. They develop after ovulation, spontaneously in the case of the bitch and induced by coitus in the cat. Once formed, their lifespan appears to be inherent and the hormone production by them to be independent of a pregnancy until the last stages of gestation. As a result, death of the embryos, or death or sickness of the fetuses, shortens the length of the gestation period only slightly. Fetal death before the terminal stage does not usually lead to premature delivery; rather, the process of autolysis and resorption or mummification begins, and the products are expelled at or near term. *As a consequence, it is very rare to obtain diseased fetuses from these species in a satisfactory state for study.* Most studies of fetal diseases in carnivores have been based on experimental investigation.

The corpus luteum is essential for the maintenance of pregnancy during the entire gestation period in swine and goats, as it is in dogs and cats, before term. The corpus luteum is only essential during the first half of pregnancy in the cow, mare, and ewe. During this time, the effects of fetal death are unpredictable. In some cases the corpus luteum may undergo luteolysis and the dead fetus or fetuses are expelled, usually severely autolysed, but in some cases the corpus luteum will not be lysed and will persist, and the fetus will be resorbed or more likely expelled. If the fetus is older, and particularly in cattle, it may be mummified. During the last third of gestation in these species, the pregnancy requires hormonal support from the fetus, and fetal death at this stage leads to expulsion within a few days. Even a few days allow time for autolysis to be evident; the fetal tissues are pale, the red cells lysed, and pleural and peritoneal cavities are filled with hemoglobin-stained fluid.

The **mechanisms of parturition** are similar in most mammalian species. Labor requires the coordinated contractions of the smooth muscles of the uterus. These are supplemented during the expulsive phase of labor by contraction of the abdominal muscles. Preceding the muscular phase, there must be changes in the connective tissues in the cervix, uterus, and vagina, allowing these tissues to become compliant.

The *timing of parturition* is species-specific and related to appropriate fetal maturity. How the fetal maturity is sensed and how it is translated into maternal myometrial contraction appears to vary between species and is unknown for most. Knowledge of fetal control of parturition has been gained largely in sheep, a species in which fetal control of parturition is nearly absolute. In other species, the fetus plays a lesser role or none at all.

Parturition in the **ewe** is initiated by a sharp rise in cortisol secretion by the adrenal cortex of the fetal lamb. The increased secretion is partly due to increased responsiveness to corticotropin and partly to increased fetal adrenocorticotropic hormone production. It has been shown experimentally that either hypothalamic or pituitary destruction or bilateral adrenalectomy in the lamb fetus will prevent its initiation of parturition and indefinitely prolong gestation. Fetal cortisol induces enzyme synthesis in the placenta. These enzymes synthesize estrogen from progesterone, increasing placental estrogen production and decreasing progesterone. The change in ratio of estrogen to progesterone stimulates release of prostaglandin $PGF_{2\alpha}$ from the maternal endometrium. $PGF_{2\alpha}$ induces compliance in the cervix by changing the physical properties of the collagen fibers. These alterations are based in part on loss of collagen but more to an increase in glycosaminoglycans and water that loosen the matrix in which the collagen fibers are imbedded. The $PGF_{2\alpha}$ also enhances the myometrial response to oxytocin and stimulates uterine contractions. The mechanism by which parturition is triggered in cattle appears to be similar to those in sheep. Parturition in the corpus luteum-dependent species is initiated by regression of the corpus luteum and a corresponding fall in circulating progesterone. The details are only known in the goat, in which species the fetal adrenal cortex produces a cortisol surge that produces $PGF_{2\alpha}$ release and luteolysis. It is unlikely that the fetal control of parturition in other corpus luteum-dependent species is as strong as it is in goats.

The mechanisms by which dead fetuses are expelled in cattle and sheep are not known, but it is probable that they share features with normal parturition. They must involve $PGF_{2\alpha}$ release, cervical compliance, and coordinated myometrial contraction. Chronic fetal disease in cattle and sheep results in premature delivery of live but diseased fetuses. Since fetal stress activates the same hypothalamic–pituitary–adrenal endocrine chain used to initiate normal parturition, it is probable that chronic fetal disease operates through the same chain to cause abortions of live fetuses. Equine herpesviral infection regularly produces abortion of live, diseased, equine fetuses, but very little is known of the mechanisms involved in either these abortions or normal parturition in this species.

Embryonic death

There is little that can be said with profit of the pathology of the ovum, zygote, or early embryo, but a brief consideration is included here for purposes of orientation.

When fertilized ova die, they undergo progressive cytolysis within the zona pellucida, which often remains intact after the cytoplasm and nucleus are dead. The whole structure then disintegrates and is resorbed or discharged at the next estrus. It is rare to recover abnormal ova from the bovine uterine tube during the first 72 hours postestrus. Presumably, in those cases in which estrus occurs after a normal cycle, the ova die most commonly between days 4 and 10 of the cycle. Most embryonic deaths in cows, however, are associated with prolongation of the interestrual period. In the embryonic stage, the embryonic tissue proper disappears first and the trophoblast remains for a while before degenerating.

The incidence of zygotic and embryonic mortality is remarkably high in the species that have been studied, and probably is in all species. Humans and the domestic species studied suffer a zygotic loss of 15–30%. The causes are presumably diverse and may vary somewhat from species to species. However, the demonstration of abnormal karyotypes in early porcine zygotes and in a high percentage of unselected spontaneous human abortions *suggest that chromosomal abnormalities are an important cause of this mortality*. The frequency with which chromosomal abnormalities are found varies with the stage of pregnancy; they are most common in the early stages of pregnancy. This is the pattern one would anticipate since some of the most severe anomalies are not compatible with attachment or implantation. Most of these chromosomal abnormalities represent numerical changes such as monosomy, polysomy, polyploidy, and mixoploidy. Only a small percentage is structural. The monosomies and polysomies are the result of nondysjunction during meiosis or early cleavage stages. Triploidy can be caused either by two sperm fertilizing one egg, or by the suppression of the second polar body and one sperm fertilizing a diploid egg; both possibilities increase with increased age of the gametes. Tetraploidy occurs as a result of suppression of the first cleavage division of the zygote. Mixoploids develop as a consequence of mitotic error during cleavage division of the zygote. Structural anomalies are caused by spontaneous breakage and reunion. Viruses, drugs, and radiation are known to cause this type of chromosome damage.

Fetal death

A degenerate zygote or early embryo may be resorbed or expelled from the uterus. At a later stage in development, *the dead fetus may be mummified, macerated, or aborted; an* ***abortion*** *is defined as the expulsion of a fetus prior to the time of expected viability*. A dead fetus delivered within the period of expected viability is arbitrarily referred to as **stillborn**. It should be appreciated that these different terms might be applied to fetuses suffering from the same basic disease.

In uniparous domestic animals, death of the fetus in late pregnancy is usually followed by abortion. In multiparous species, if most of the fetuses die at the same time, all are likely to be aborted, but it is more usual for one or several dead fetuses to be retained with the remaining viable ones and delivered at parturition. It is often apparent that the deaths have occurred at different ages, the dead fetuses being of different sizes and degrees of mummification or maceration (Fig. 4.73).

Mummification of fetus

Mummification of a dead fetus is seen occasionally in any, but usually multiparous, species and most commonly in the sow. In the mare, it is typically one of twin fetuses that is mummified. Mummification may also occur in mares receiving progesterone in the early stages of gestation in order to prevent abortion associated with failure of endometrial cup development and subsequent failure of accessory corpora lutea formation and thereby progesterone production. The injected progesterone inhibits contractions of the uterus and, if the fetus dies, prevents abortion, so the fetus mummifies. *A prerequisite for mummification is that bacterial infection is not present*. The fluids are resorbed and the membranes become closely applied to the desiccated fetus. The whole mass becomes brown or black and rather leathery, moist on the surface with sticky mucus, but without odor or exudate. The time required for complete mummification will depend to some extent on the size of the fetus. Dehydration is advanced by 7 days in sheep fetuses; however complete mummification probably requires as long as 6–8 months in the case of a 6-month bovine fetus. All stages may be observed from the earliest of beginning separation of the placenta with hemoglobin staining of the tissues, to the latest, when all that remains is a firm and shrunken remnant consisting almost wholly of dried skin and bones. Until the time of expulsion, which on occasion occurs spontaneously, or parturition in multipara that still carry some viable fetuses, the cervix remains closed and sealed. In uniparous animals, the mummified fetus may be retained indefinitely. Animals that have had and delivered a mummified fetus usually breed normally on subsequent occasions so there cannot be any serious uterine lesion accompanying the fetal death. Genetic diseases (both inherited and chromosomal abnormalities), viral and protozoan

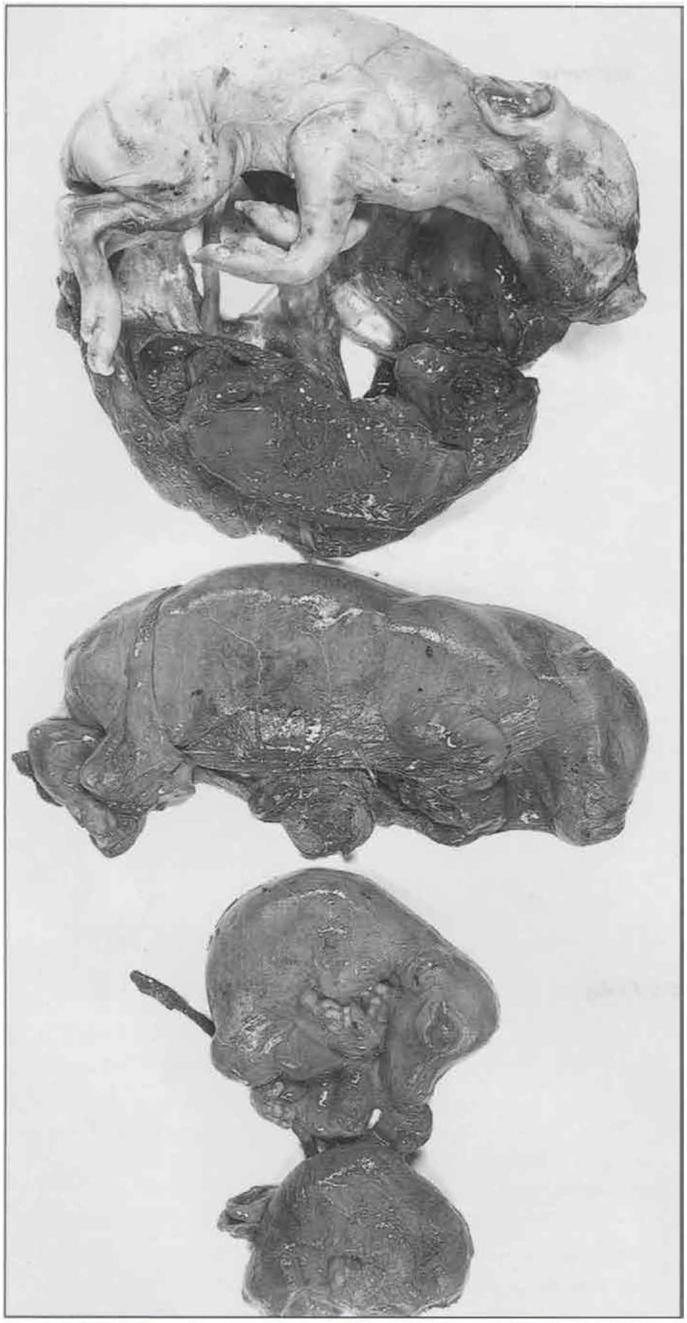

Figure 4.73 Porcine littermates that have died at differing stages of gestation and are undergoing **mummification**.

infections, and placental insufficiencies have been proposed as the causes of fetal death leading to mummification, but the cause is rarely firmly established in any particular case. Very likely all the proposed causes and others can produce fetal death at an appropriate time, and mummification can result. *The critical features for mummification appear to be the retention of the dead fetus within the uterus through the action of a functional corpus luteum, and fetal skin mature enough to resist autolysis.*

Fetal maceration and emphysema

Maceration and emphysema of the fetus require the presence of an infection in the uterus. If the early embryo succumbs to uterine or embryonic infection, maceration is usually followed by resorption from the uterus or expulsion along with a small amount of purulent exudate. This is the usual course of events in venereal infections by *Campylobacter fetus* and *Tritrichomonas foetus* in cows, but is also to be expected with any sort of nonspecific endometrial infection. The causes of infectious death of the conceptus during the period of the fetus are the same as above, but the consequences, pyometra or acute endometritis, are usually more severe because of the presence of decomposing fetal remnants. The differences between pyometra and endometritis are as discussed earlier; in pyometra, the corpus luteum may persist, the cervix remains sealed, and the uterus withholds its contents. If the corpus luteum and cervical seal break down, there is a purulent discharge of the contents, and except for the presence of fetal remnants, this endometritis does not differ much from that of the puerperium. After about the third month of pregnancy in the cow, complete fetal maceration does not occur. The bones, either more or less complete or only the ossification centers, resist maceration (Fig. 4.74). They may be discharged or retained in the pus of pyometra indefinitely, often near the cervix. The uterine pus is usually thick and intensely fetid, but this depends on the nature of the infection; in trichomoniasis, the exudate is watery and odorless.

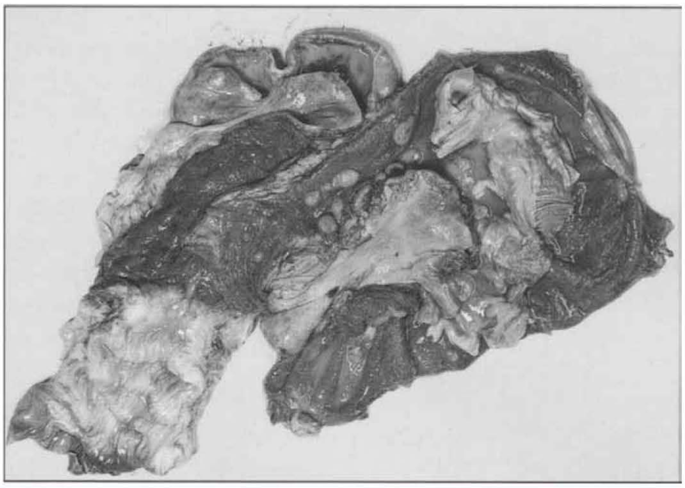

Figure 4.74 Macerating bovine fetus.

The usual infectious causes of fetal death and endometritis are not potent gas-formers; the development of fetal emphysema almost invariably depends on patency of the cervix and on invasion of the uterus and dead fetus by putrefactive organisms from the vagina. There are two common antecedents to emphysema: dystocia at or near term, and incomplete abortion. In incomplete abortion, the cervix is open but not completely dilated and the fetus may be delivered into the cervix or anterior vagina or, because of malpresentation, uterine inertia, or inadequate dilation of the cervix, it may be retained in the uterus. The fetus putrefies, becomes distended with foul gas and crepitates. If the fetus is small, the dam may survive the initial acute episode of fetal emphysema, after which maceration occurs in a chronic, foul purulent metritis. The cervix is not sealed, but because of uterine paresis, the pus is retained in the flaccid, dependent organ.

Advanced uterine lesions accompany the macerated fetus. The uterine wall is thickened and the reaction within it varies from the acute exudative inflammation of pyometra to more or less complete sclerosis and replacement by granulation tissue in long-standing cases. In these, the uterus closes firmly about the bones, and the bones may cause perforation.

Fetal emphysema, at or near term, complicating dystocia is fatal unless treated, and maceration is not an expected sequel. In some cases of uterine torsion, however, the twisted cervix and vagina produce an adequate seal against bacterial invasion of the dead fetus, and mummification, rather than maceration or emphysema, results.

A special form of fetal emphysema occurs in ewes, caused by *Clostridium chauvoei*, the organism responsible for blackleg. It affects fetuses near full term, causing acute tympanitic distension of the uterus, typical hemorrhagic and necrotizing lesions of the fetus, and the accumulation of dark thin discharge in small amounts in the uterus. The pathogenesis of this special infection is not entirely clear, but there is usually a relation to rough handling such as at shearing.

Embryonic death with persistence of membranes (cystic placental mole)

This is an uncommon development that can follow embryonic death. In the period of the embryo, the fetal membranes are in volume and mass the greater part of the products of conception, and *it is possible for the embryo to die and to be resorbed while the membranes persist and continue to grow. The remaining empty cyst has been referred to as a* **cystic placental mole**. Most empty placental cysts correspond in size to fetal membranes at 3–4 months of gestation. There is usually no patent lumen to the cyst. It is filled with a mass of clear-gelled fluid together with placental stroma. In those cases with placentary development, the allantoic and amniotic epithelia may be present. The condition may become infected and converted to a pyometra, or undergo necrosis and be discharged. Infections that result in rapid death of the fetus (e.g., bovine herpesvirus, *Arcanobacterium pyogenes*, *Histophilus somni*) usually result in a severely autolysed fetus, as time is required for the corpus luteum to lyse, the cervix to dilate, and for prostaglandin to contract the uterus and expel the fetus. In infections that are chronic and take time to kill the fetus (e.g., *B. abortus*, most mycotic abortions, *Yersinia pseudotuberculosis*), the preparations for abortion may be complete by the time the fetus dies and therefore the fetus will be aborted in a relatively well-preserved condition. In infections that are largely confined to the amnion, such as *Ureaplasma diversum*, the probable mechanism involves macrophage activation resulting in cytokine-induced production of prostaglandin by the endometrium, lysis of the corpus luteum, and premature delivery of a well-preserved or slightly autolysed fetus, depending on how quickly death follows the infection.

Adventitial placentation (semiplacenta diffusa)

The development of intercotyledonary placentation in cattle is a mechanism of compensation for inadequate development of placentomes. The inadequate number of placentomes is primarily endometrial, and may be congenital or acquired. There are normally 75–120 caruncles in the cow, and 40–125 in the ewe and goat. Not all of them are fully utilized in a normal single pregnancy. Occasionally, the numbers are much less as a congenital disorder of endometrial organization; more commonly, the reduction in number is acquired by inflammatory destruction of portions of the endometrium. Compensation consists of a great increase in the size of remaining caruncles during pregnancy, many of which may fuse, and by the development of a more primitive villus placentation between the placentomes (Figs 4.75, 4.76). *The adventitious placenta develops first adjacent to the placentomes*; the process may remain localized or involve virtually the entire intercotyledonary placenta, chiefly along the floor of the uterus. In the latter instances, pregnancy is insecure and may not proceed beyond mid-term. Hydrallantois is a complication. Adventitial placentation has not been observed in the sheep or goat placenta.

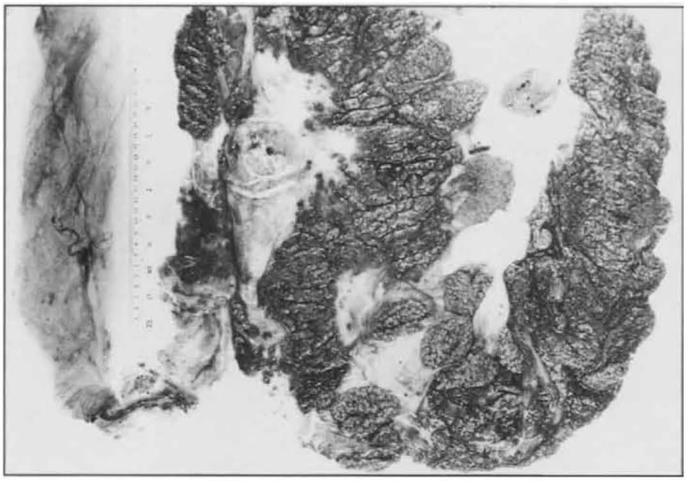

Figure 4.75 Adventitial placentation in the placenta of a cow.

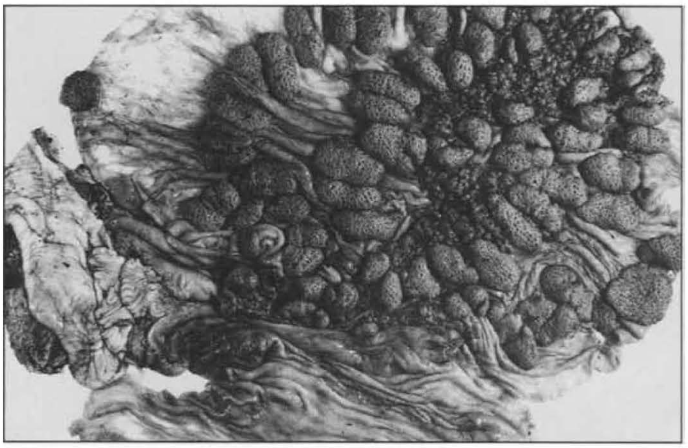

Figure 4.76 Uterus from a cow with **adventitial placentation**.

Hydramnios and hydrallantois

Excessive accumulations of fluid in the amniotic and allantoic sacs are infrequent diseases of pregnancy. They occur most often in cattle, and are rare in other species. Excess fluid may accumulate in both sacs, but this is the exception. The source, nature, and control of the fluid in the two sacs are different, and the conditions that give rise to excess fluid in each sac tend to be different. The total amount of fetal fluid increases progressively throughout pregnancy, and the volume at term in cattle is between 15 and 20 liters. This represents a fivefold increase during the last 4 months of gestation. The relative quantity of fluid in each sac varies during the gestation period. During the first and third trimesters, the amount of allantoic fluid is greater.

The nature of the fluid in each sac varies during gestation. At term, the amniotic fluid is glary, slightly viscous, and consists largely of fetal salivary secretion, the volume being largely controlled by fetal swallowing. The allantoic fluid is thin and watery and is derived from the fetal kidney via the urachus.

Hydramnios, or hydrops of the amnion, is usually associated with malformation of the fetus. The malformations may be either inherited or acquired. A variety of inherited diseases can cause hydramnios. There is a high incidence of the condition: in bison-cattle hybrids, in association with chondrodystrophic and muscle contracture malformations of bovine and ovine fetuses, as well as in other types of fetal malformation, particularly those involving gross facial anomalies.

Hydrallantois in cattle is most often associated with uterine disease with inadequate numbers of caruncles and the development of adventitial placentation. It also occurs with increased frequency in cows bearing twins. The quantity of excess allantoic fluid may be as much as 170 liters, but it is not notably different in quality from normal. The fetal membranes are only slightly thickened, but may be tough and rupture with difficulty. Dystocia with uterine paresis, retention of placenta, and metritis are sequelae of those few cases that do not abort earlier in gestation. The fetuses are usually dead when aborted or delivered at term and small for their age, but may have anasarca and ascites.

The disease occurs but rarely in *mares*. The conditions that predispose in this species are not known.

Amniotic plaques, placental mineralization, and avascular chorion

These are normal and their inclusion here is warranted only to avoid confusion to anyone seeing them for the first time. *Amniotic plaques are foci of squamous epithelium on the internal surface of the amnion.* They may or may not be keratinized. They are about 2–4 mm in diameter, flat, and resemble lesions of the poxes. They are especially concentrated on the umbilical stump where they are taller, cylindrical, or papilliform. They seem to be constantly present on the bovine amnion during the middle trimester, and they do also occur in other species but have not received much attention.

The *deposition of mineral*, visible about the small blood vessels of the placenta as white streaks and spots, occurs in many species from about the end of the first to the middle of the second trimester. The degree of mineral deposition is quite variable, and is more extensive in the allantois than in the amnion (Fig. 4.77). It can be distinguished from dystrophic mineralization in that it is not gritty but resembles milk in appearance and texture. There is no indication of why the mineral is deposited nor what, if any, place it has in placental economy.

Metaplastic ossification complete with large multinucleated osteoclast-like cells occurs commonly in the placenta of sows giving birth to normal litters but is rarely seen in other species.

The *avascular chorion,* commonly called the "necrotic tips," is a normal region, which is avascular and avillus, and occurs at the extremities of the chorioallantoic membrane in porcine, ovine, and bovine placentas. It is almost invariably present from the period of elongation to term. Sometimes the region may extend for several centimeters along the chorion. In appearance, it is clearly demarcated from the vascular chorion, varies from white to brown, and is usually wrinkled.

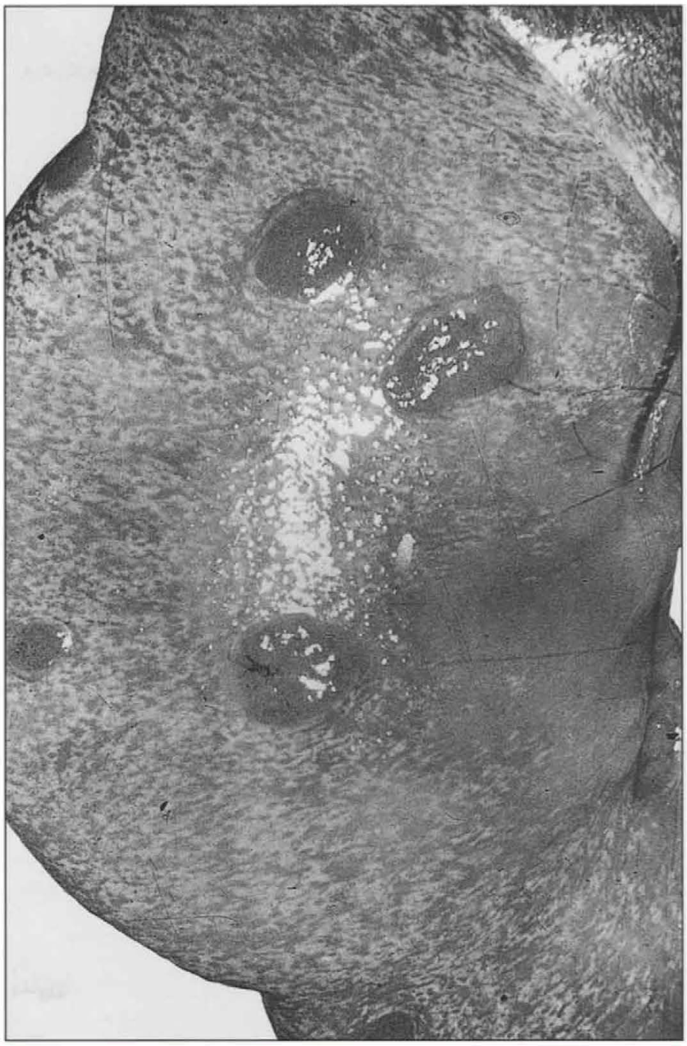

Figure 4.77 Normal mineralization in the placenta of a bovine fetus.

Prolonged gestation

Syndromes characterized by abnormally long gestation periods occur in cattle and sheep. Some of these syndromes have been only partially defined, but in all cases adequately documented, *evidence of fetal anomaly, either anatomic or functional, has been present.* Two of the syndromes in **cattle** have a genetic basis, the trait being governed by autosomal recessive genes, the defective fetus being homozygous.

- The first of these, which is most common in the Holstein and Ayrshire breeds, produces a nonviable large calf after a gestation period which is approximately 2 months longer than normal. The parturition is abnormal; the maternal preparations of relaxation of the pelvic ligaments and filling of the udder are minimal, and assistance is usually necessary. The calves are large, but of nearly normal proportions. They suffer from severe respiratory distress. Death is due to either these respiratory difficulties or uncontrollable hypoglycemia. The *adrenals are hypoplastic* and this is thought to be the lethal defect in the syndrome.
- In the second type of inherited prolonged gestation, which is seen in Guernsey, Jersey, and Swedish Red and White breeds, *fetal monsters* are common. Many of these animals have severe head

anomalies of the cyclopian type, but in some the defect is discrete and consists only of the absence of the pituitary. *Adenohypophyseal aplasia* is common to all of them. These fetuses fail to develop after approximately the seventh month of gestation, and the length of gestation appears to be determined by the viability of the fetus. Some gestation periods last 17 months; at this time, presumably, the placenta can no longer adequately provide for the fetus and the dead fetus, still developmentally immature, is expelled.

Not all syndromes of prolonged gestation in cattle fit this pattern either genetically or anatomically, and sporadic examples of fetuses unable to terminate their gestation occur. These are usually grossly deformed giants with severe brain anomalies; these animals regularly die in utero.

Prolonged gestation occurs in **sheep** as a result of chemically induced teratogenesis. If ewes ingest *Veratrum californicum* on or about day 14 of pregnancy, they may fail to begin labor at term. Pregnancy then continues for weeks beyond term, leading ultimately to rupture of pelvic ligaments and maternal death. The fetuses damaged by the weed have cyclopian deformities and attain giant proportions. Originally, the pituitaries were thought to be absent. It appears that in most cases the gland is displaced in the malformed head.

The functional defects that prevent the various types of fetuses from making their timely contribution to their delivery can now be interpreted in the light of experimental studies. These indicate that, for a fetal lamb to initiate parturition, the fetal hypothalamus, its connection to the pituitary, the pituitary, and the adrenals must be functionally competent. If any of these links is defective, prolonged gestation results. Only hypophyseal absence, however, leads to fetal dwarfism.

An additional syndrome of prolonged gestation has been recognized in ewes that are fed the African shrub *Salsola tuberculata*. The mechanisms by which this plant poisoning interferes with parturition are unknown. They appear to be different from previously reported ones, in that the effects of the plant are not teratogenic, and the intoxication prevents parturition when fed during the last 50 days of gestation. The plant may inhibit fetal hypothalamic-releasing factors.

S. tuberculata is drought-resistant, and the disease occurs when other feed is lacking. The Karakul breed of sheep is the breed raised where the plant is common and is the breed most often affected, but other breeds are susceptible to the toxic effects of the plant. The affected ewes fail to develop normal preparturient udder enlargement, but show no other signs of intoxication.

The fetuses continue to grow and usually initiate parturition 10–20 days past term. At delivery, the affected fetuses are large, lethargic, and suffer a high mortality rate. The pelts are overgrown and worthless, the hooves long, and the teeth erupted. The adrenals of fetuses are hypoplastic and the pituitaries are small and normal granulation of the cells of the adenohypophysis is lacking.

ABORTION AND STILLBIRTH

It will be apparent from the foregoing discussion that diseases of the conceptus may result in death with resorption, fetal mummification, abortion, or stillbirth depending on the age of the conceptus and the species involved, but not all fetal infections result in fetal death. Some viral infections appear to cause little harm to fetuses, at least during certain stages of gestation, and in some the effects are subtle. Many fetal diseases, such as brucellosis of cattle and sheep, epizootic bovine abortion, *U. diversum*, and mycotic fetal infections in cattle are chronic and may lead to the premature delivery of small-for-gestational-age and diseased fetuses.

The list of bacterial and mycotic infections known to produce sporadic abortion is so long as to be nearly valueless because *any bacteremia or systemic fungal infection occurring during pregnancy carries with it a great risk of bacterial or mycotic colonization of fetal chorion.* The entire basis of this susceptibility is not known; however, factors within the conceptus, such as isolation from the maternal immune system, decreased oxygen concentration, elevated temperature, sluggish inflammatory and immune responses, and the provision of preferred nutrients for many organisms, must be important. The organisms involved in fetal infection are the same irrespective of the period of gestation. The effects vary greatly and overall, fetal resistance increases with age. There is some evidence suggesting that bacterial colonization of the fetal membranes does not occur as readily before embryo attachment.

Bacterial and mycotic fetal infections are most common in cattle and horses. There are, however, *important differences in the pathogenesis of the infections in the two species.* Except for the venereal infections early in gestation, bacterial fetal infections in cattle are probably hematogenous in origin. *Leptospira* and *Ureaplasma* infections of cattle, however, may reside in the uterine tube and move to the placenta during pregnancy and perhaps during subsequent infections. Bacterial infections of the equine placenta may arise hematogenously (probably route for nocardioform and some *Streptococcus equi* infections), but are more likely to infect the fetal membranes via the less protective cervix. The route of the infection can usually be determined by examination of the placenta. Transcervical infection involves the chorion adjacent to the internal os of the cervix.

The fetal effects of viral infections depend on the virus and the age of the fetus. Where the effects are known in detail, the pattern is that infections early in gestation are more likely to kill or produce serious teratologic effects; later in gestation, the effects are less severe. There are important exceptions, and the details are not known for most viral infections. Frequent mention is made throughout these volumes to abortion in relation to specific viral diseases. The fact that no mention is made of fetal disease should not be implied to mean that the virus in question is incapable of reaching the fetus or harming it. And, alternatively, the fact that fetal disease is associated with a specific viral disease should not be construed to mean that fetal disease is produced at all stages of gestation, or that, if it is, the resultant fetal lesions are the same throughout the gestation period.

Toxic, nutritional, genetic, and physical diseases are also important causes of reproductive failure and some mention is made of them elsewhere, but often the details are not available. Selected here for description are those infections that characteristically result in abortion as a major manifestation.

Diagnosing the infectious causes of abortion

The diagnostic rate on the causes of abortion, based on examination of samples submitted to veterinary laboratories throughout the world, varies from 5 to 50%. The proportion of abortions that are caused by infection is not known; however, approximately 90% of those abortions in which the cause is determined are assigned to infection. The purpose of this discussion is to assist in maximizing the diagnostic rate on the infectious causes.

Not all of the infectious causes can be recognized by the examination of fetoplacental submissions. The effects of infectious and noninfectious causes may be manifest indirectly through the dam or directly through the placenta and fetus. *Conditions in the dam affecting the fetus and placenta and which may result in abortion include hemoconcentration, circulatory failure, anemia, fever, endotoxemia, and respiratory disease.* With these conditions, no lesion of diagnostic value will be found in the fetus or placenta.

Pregnant animals may contact infectious agents by many routes: through the respiratory tract, vagina, mouth, or, if insect-derived, through the circulation. Some agents may be carried into the reproductive tract with the semen or embryo transfer fluids. In cattle, *U. diversum*, *Bovine herpesvirus 1* (BoHV-1), and *Bovine viral diarrhea virus* (BVDV) may contaminate either of these, and being preserved by freezing and resistant to the antibiotics commonly used, they survive there. In addition, it appears that some agents, such as *Leptospira hardjo,* may be maintained in the uterus or uterine tube over the open period and infect the conceptus as it develops. The role of the changing hormonal influence in the reproductive tract on the multiplication of organisms may be substantial. *Campylobacter fetus* moves from the vagina to the uterus as pregnancy advances and the hormonal influence is altered. Some organisms may multiply in the conceptus for a very long period before abortion. *U. diversum*, for example, may remain in amniotic fluid for up to 117 days before abortion occurs. Similarly, *Aspergillus fumigatus* may multiply in the placenta for at least 25 days before producing abortion, and it is suspected that BoHV-1 remains in the placenta for a prolonged period before invading the fetus.

Cooperation among all parties involved (owner, clinician, pathologist, bacteriologist, virologist) is often necessary to reach a diagnosis. Eventually, other specialists, including nutritionists, toxicologists, botanists, and epidemiologists, may be consulted.

Because the act of abortion rarely points to a definite cause, it is important to collect all information at the first contact. While the history seldom points directly to the cause of abortion, clues may be found that will indicate what needs to be done to determine the diagnosis. Abortion may occur some time after the initiating illness, and therefore health, performance, and travel records become important in suggesting the diagnosis.

As the dam, placenta, and fetus may equally be involved, each should be examined and sampled. In addition, and perhaps more importantly, a representative portion of the remaining animals that have not aborted should be examined.

- Samples should include *serum* from the dam and herd (flock, or kennel), swabs, or samples from the uterus (the caruncle in cattle may be useful), placenta, and fetus.
- If the *placenta and fetus* cannot be quickly and suitably transported in their entirety, samples may be removed and one portion placed in fixative and another in plastic bags on ice for culture.
- Tissues may, in addition, be *frozen* for future culture or testing for agents using one of the many immunologically based techniques presently available.
- Samples from the conceptus for *culture* should include placenta, stomach content, lung, and kidney.
- Tissues essential for *histology* are a sample of the endometrium (caruncle), placenta, brain, eyelid (with conjunctiva), thymus, lung, heart, liver, spleen, kidney, adrenal, and intestine. To be most useful, tissues for histology must be properly fixed or chilled (not frozen) if transport is to exceed 3 hours.

As with the history, it is important to maximize sample collection initially, thereby permitting the use of all the necessary diagnostic aids as the cause is gradually revealed. Samples not required are easily discarded later. The results of serology are more easily interpreted on a herd basis than on an individual animal, and may be particularly useful when samples are collected before pregnancy or at the time of pregnancy diagnosis and again at the time of abortion. This technique has proven very useful in the diagnosis of leptospirosis. A representative portion to establish a baseline titer need only be sampled. Two samples from a dam which has just aborted may contribute little useful information as many agents that subsequently cause abortion have initiated production of a high antibody titer long before abortion occurs. Serum antibody titers are often within the "normal" range at the time of abortion. Interpretation of the significance of antibody titers may be further complicated in that many animals may not respond to the agent with the production of antibody and yet the animal may be carrying the organism and abort because of it. Up to 22% of animals aborting because of *Leptospira hardjo* may not have a significant titer (microscopic agglutination test) at the time of abortion.

It is important not to eliminate a disease from the list of possibilities because of a history of vaccination. Many diseases may occur and result in abortion in spite of previous vaccination, e.g., BVDV and BoHV-1, and of course, vaccination with certain live vaccines during pregnancy may cause abortion. Vaccination titers may be difficult to distinguish from titers due to infection, particularly if exposure has followed inoculation of even a single dose of vaccine. Baseline values need to be established.

Frequently, lesions pointing to the diagnosis may only be present in the placenta. As the placenta is a very large organ, and as much of it may be normal, *the more of the placenta that can be examined, the more accurate assessment of it is likely to be.* It rates in importance for examination equal to both the alimentary and respiratory tracts of an animal after birth. Unless the entire placenta is expelled, at least part of the portion retained internally should be examined, as it often has the most severe lesion and is less contaminated by bacteria from the environment. If the placenta cannot be found, endometrial samples become more crucial and should be requested. Samples may be divided in three and utilized for bacterial culture and histology, and a portion frozen for virology. **The optimal submission includes the fetus and placenta plus serum from the dam**. Serum is best collected at pregnancy diagnosis and then resampled at time of abortion. This method has been found of particular use in the diagnosis of leptospirosis in dairy cows on drylot where the fetus is often not discovered until trampled. Examination of a "fresh" fetus is 53 times more likely to contribute a diagnosis than an autolysed one. Submissions including the entire fetus, placenta, and serum are 4.28 times more likely to result in a diagnosis than where pieces only of the fetus are submitted. Titers of fetal antibody may also be useful in diagnosis of BVDV, *Neospora*, or leptospiral infections.

Some lesions and normal findings that may be confusing will be described briefly. To examine the **caruncle** it should be cut sagittally and the cut surface examined for infarction or suppuration. Infarction is most commonly recognized in mycotic infections but may be caused by a variety of bacteria, including *Brucella* and *Salmonella*. Infarction frequently results in retention of caruncular material by the cotyledon. A variety of bacteria, including *A. pyogenes*, may cause suppurative placentitis. Occasionally, fibrosis of caruncles is seen and may represent normal caruncular maturation or be the result of a chronic infection caused by organisms such as *Yersinia pseudotuberculosis.*

To examine the **chorioallantoic placenta,** it should be completely spread out and inspected carefully for lesions on both surfaces. Lesions in the bovine placenta are commonly observed towards the tip of the pregnant horn and should not be confused with the normal avascular chorion in this site. On histologic examination of hematoxylin and eosin-stained sections of the chorionic villus, a blue finely granular to amorphous material may be observed in the interstitium. This may be glycosaminoglycans, mineral, bacteria, or DNA, and special stains and even close examination will differentiate it. Glycosaminoglycans are considered normal, as is mineral in the early stages of gestation. In the later stages, however, mineral is commonly observed in areas of necrosis and feels gritty on gross palpation.

The trophoblast cells should be thoroughly examined on high power as a variety of bacteria may be phagocytosed, or at least contained, by them. The erythrophagocytic trophoblast has been shown to phagocytose *Brucella,* and the organism has been seen by electron microscopy in the rough endoplasmic reticulum. *Coxiella burnetii* is also found in the trophoblast and can usually be distinguished from bacteria in that the cytoplasm of the cell appears foamy compared to the finely granular appearance of bacteria.

Lesions of variable intensity are usually seen in vessels of the placenta. Discrete necrotizing vasculitis of the endothelial cells in small vessels of the villi regularly occurs with infectious bovine rhinotracheitis infection. Mycotic infections cause very severe vasculitis, resulting in thrombosis and infarction of the caruncle and cotyledon. *Histophilus somni,* while perhaps rare as a cause of abortion, may cause a similar lesion. *Chlamydophila* infections of the placenta may also cause severe vasculitis, but usually without thrombosis. The bacteria-packed vessels in *Salmonella* placentitis are so near the surface that they are easily mistaken for phagocytosed bacteria in trophoblasts.

Lesions of hemorrhage, necrosis, mineralization, and fibrosis may also be observed on the chorionic and allantoic surfaces and are commonly seen with a variety of fungi and bacteria, including *U. diversum.*

The inner surface of the **amnion** may be severely stained with meconium, particularly in chronic progressive infections of the chorion. It is frequently the site of lesions associated with *U. diversum* infection, and is there characterized by multifocal to confluent areas of necrosis, mineralization, and fibrosis, with marked thickening and mild vasculitis being most common. Mycotic and *Mycoplasma* infections may penetrate the chorion and allantois to the amnion and result in a similar lesion.

Discrimination must be used in interpreting the results of cultures derived from samples of fetus, placenta, or even the stomach contents, as organisms may move, or be carried, from the vagina to the conceptus when the cervix dilates during an impending abortion due to another cause. These organisms may rapidly contaminate the placenta, grow in the fetal fluids, and may even be swallowed by a viable fetus, thereby appearing in the stomach contents. *To avoid confusion, vaginal samples should be taken as soon as a vulvar discharge is seen or impending abortion is suspected.* Vaginal discharges from animals with impending abortions are much less likely to be contaminated by extraneous organisms than are samples from an animal that has aborted several days previously.

Observing the relative state of preservation of the fetus may contribute information about the cause. Acute infections, such as those caused by BoHV-1, usually result in the discharge of a severely autolysed fetus, as the fetus is killed rapidly. Chronic infections, (such as *U. diversum, B. abortus, Yersinia pseudotuberculosis,* or *Aspergillus fumigatus*), or other persistent stresses, however, allow the fetus to prepare itself for delivery and hence it is discharged in a fresh state, perhaps small-for-gestational-age, and sometimes alive. *Evidence of the significance of an isolate may only be obtained by histologic examination of certain tissues.*

A few of the less commonly examined tissues which may be useful in making the overall decision about the cause of abortion will be briefly considered. Microscopic examination of the **eyelid** (surface and palpebral conjunctiva) will reveal lesions in about two-thirds of the fetuses aborted due to infectious causes. Lesions are most frequently observed on the conjunctival surface, and vary from a slight infiltrate of mononuclear cells to a severe necrotizing reaction. As in other areas, the cellular infiltrate may be determined by the duration of the infection before death of the fetus. In cattle, *Yersinia* sp., *Ureaplasma,* and mycotic infections are commonly associated with a heavy focal mononuclear cell infiltrate just under the conjunctiva, whereas *A. pyogenes* infection may result in complete loss of conjunctival epithelium, and the accumulation of large numbers of bacteria, but few inflammatory cells, on the surface. Many bacteria, including *A. pyogenes* and *Listeria,* continue to multiply after the death of the animal. BVDV infection may initiate perivascular mononuclear cell infiltration and cause injury to hair follicles. The pattern of follicular hair growth when compared to the age of the fetus may determine the time of infection. Mycotic infections cause folliculitis; however, the lesion is very variable, not only as to its presence but also its severity, and while intracorneal pustules may be seen, frequently either no lesion is observed or traces of inflammation and fungi are only found in the outer sloughing keratinized debris. This material may be lost unless care is taken in sectioning.

Examination of the **brain,** particularly with the discovery of the importance of protozoan infections as a cause of abortion in ruminants, is an essential part of any necropsy examination. Many viral infections manifest their effects in the brain. While the brain often appears soft and unworthy of fixation, and some are literally poured into the fixative, interesting and informative lesions are often found only in this site.

The **lung** *ranks among the most common organs in which to see lesions.* Aspiration of meconium into airways, with or without evidence of an agent, is seen in the terminal phases of many bacterial, and less commonly in mycotic, infections, and is probably related to the rapidity of the development of the lesion and the degree of impairment of placental function. Frequently there will be no local reaction in the lung to the agent and in these the exudate present probably represents aspiration of the agent and inflammatory cells from a placentitis. Many of the inflammatory cells may be maternal in origin. In *U. diversum* and *Bovine parainfluenza virus 3* infections in bovine fetuses, airways may remain relatively clear and alveolar ducts become thickened and the lumina pavemented with many macrophages and a few neutrophils. Severe necrotizing bronchitis may be seen with *Equid herpesvirus 1* infection in horses, whereas BoHV-1 in cattle usually produces only multifocal necrosis and no visible pneumonia.

Examination of the **intestine** contributes much information in some specimens. Sections should be removed from the small and large intestine, with care taken to preserve the content intact. Bacteria may be seen colonizing the luminal content and are used as an indicator of whether or not the bacterial isolate is significant. Terminal swallowing of an agent may transport it to the stomach; however, finding the agent in the intestinal lumen is evidence that it has been in the animal for at least several hours. The fetus swallows amniotic fluid beginning at a very early age, and bacteria and fungi can be easily seen in meconium. In one survey, lesions were observed in association with a variety of agents in 32% of 50 bovine fetuses examined. Lesions varied from mild with *A. fumigatus* to focal areas of necrosis in the mucosa with BoHV-1, diffuse necrotizing colitis with *Listeria*, cryptal necrosis and Peyer's patch lymphocyte hyperplasia with *Bacillus* sp., loss of crypts, and heavy mononuclear and plasma cell infiltrates with *Y. pseudotuberculosis*.

Isolation of different organisms concurrently from the products of abortion makes interpretation difficult. BVDV is commonly detected along with other organisms also considered to be causes of abortion, and in one study was found in 3 of 16 fetuses with *Bacillus* sp., 6 of 30 with *A. pyogenes*, and 17 of 45 with fungal infection. In these animals, immunosuppression associated with BVDV infection may permit the organism to circulate in the dam and localize in the placenta or it may be that, as just stated, the animal was aborting due to BVDV infection and the other organisms were contaminants. In contrast, abortion may occasionally be classified as infectious because of histologic lesions observed, but where no organism is identified. Failure to grow the organism may be due to improper handling of the samples or destruction of the organism in severely autolysed fetuses.

Portions of *chilled kidney* are useful for the fluorescent antibody detection of BoHV-1, BVDV, and *Leptospira*. *Stomach content* is useful for bacterial or mycological culture and direct examination for bacteria, fungi, and rickettsia (*Coxiella*). In some diagnostic laboratories, all samples of stomach content and impression smears of placenta are examined using a modified acid-fast stain. Using this stain and others, an experienced microbiologist can distinguish a variety of organisms. Certain organisms are extremely fragile, and care in handling samples becomes very important. Samples to be cultured for *U. diversum*, for example, should be collected aseptically, chilled to 4°C and delivered to the laboratory immediately. Leptospires may be equally fragile.

Fetal serum or thoracic fluid samples for antibody detection may be collected but the antibody titers, as in the dam, must be interpreted with caution. The fetus is capable of responding to a variety of agents with antibody production relatively early in gestation, and the presence of specific antibody in fetal fluids may be considered presumptive evidence of fetal infection. It has been shown, however, at least in sheep, that placental vascular damage may allow antibodies to cross the placenta from the dam to the fetus, thereby making interpretation difficult. When antibody passage occurs, concentrations in the fetus will presumably be lower than in the dam. Fetal antibody detection has been useful in diagnosing fetal BVDV, and leptospiral infections in third-trimester fetuses.

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Bacterial causes of abortion

Brucellosis

Bacteria of the genus *Brucella* are small, gram-negative bacilli or coccobacilli that are strictly parasitic, prefer the intracellular habitat, and produce in animals chronic infections with persistent or recurrent bacteremias typically manifested by abortion.

Three classic species of *Brucella* were described and defined originally largely on the basis of host of origin – ***B. melitensis***, goats, ***B. abortus***, cattle, and ***B. suis***, swine – but now by biochemical and serological reactions. The differences between the species are slight and quantitative rather than qualitative, and the number of biotypes within each species is large. This has led to the recommendation that all should be considered a single species, *B. melitensis*, and that present species and variants with fixed properties be regarded as biotypes of the one species. This suggestion has much to recommend it. The current nomenclature is confusing to the nonspecialist. However, most of the old truths still apply; the disease in cattle is caused by strains of *B. abortus*, the disease in sheep and goats by *B. melitensis*, and *B. suis* is found chiefly in swine. Cross-infections do occur and almost all domestic species are susceptible. But changes in the nomenclature will not solve the fundamental problem posed by the tendency of organisms of the genus to acquire new characteristics and infect new hosts. The animals in which the new varieties of organisms have been discovered have usually been hosts not previously included in the reservoir system of the genus. Examples of this phenomenon are the discovery of organisms now known as *B. suis* type 2 in hares, *B. neotomae* in wood rats, *B. ovis* in rams, and *B. canis* in dogs. The generation of new features is presumably the result of evolutionary forces; why the changes are occurring so rapidly is unknown. It seems likely that what has been done in the laboratory to change speciation has been done in nature, and that rapid evolutionary changes will continue to be the norm of the genus. And this in turn will continue to pose identification problems and disrupt control and eradication programs.

The biological similarities among the various species and biotypes of *Brucella* are in keeping with the remarkable similarities in the diseases produced in the different hosts by the various strains of the organism. Infections by any of these organisms of the genus *Brucella* are, initially at least, systemic, and relapsing bacteremic phases are well-established events in the persisting infections. Localization and persistence of infection may occur in many organs, perhaps to a greater extent with *B. suis* than with the other species. Some organs, however, notably the genitalia and placenta, are noted for the regularity with which they develop intense persistent foci of infection.

Bovine brucellosis caused by *Brucella abortus*

Brucellosis occurs in cattle in most parts of the world. In some countries the incidence of the disease is low, either because of measures taken to prevent its entry or to eradicate it but, where the disease is endemic and uncontrolled, the incidence may approach 20–30%.

The usual source of infection for cattle is an aborted fetus or placenta, or contaminated uterine discharges, and the usual route of infection is alimentary. Infection can also occur per vaginam, via the conjunctiva, and through the broken or unbroken skin. The relative importance of these latter routes is not known. Coital infection can occur but is uncommon, especially if genital infection in the male is longstanding, possibly because fewer organisms are excreted in semen from chronic lesions than from early lesions in the genitalia of bulls. Infection can be transmitted at artificial insemination if semen from infected bulls is used. Irrespective of the route of infection, the development and establishment of infection are probably comparable and will depend on the age and reproductive status of the animal, its inherent resistance, and on the dose and virulence of the infecting strain of the organism. Young cattle are relatively resistant up to about the age of puberty. They can be infected by the usual routes and means, including the ingestion of milk in which the organisms are intermittently excreted. Calves normally throw off the infection in the course of a few months.

Once infection is established in sexually mature animals, females especially, it tends to persist indefinitely. Some, perhaps many, will recover completely, but which ones will, and when they will, cannot be predicted. The organisms extend quickly to the lymph nodes regional to the point of entry, and there they provoke acute lymphadenitis. The inflamed nodes are enlarged, often much so, hyperplastic with no clear corticomedullary distinction, and frequently bear small or large medullary hemorrhages. The sinuses are infiltrated with neutrophils and eosinophils; germinal centers and proliferative activity become obvious; and there is slow but remarkable accumulation of plasma cells in the medullary sinuses. The changes in the regional nodes take some weeks to develop fully, and they persist for a prolonged period. There is no fibrosis or necrosis in the nodes.

The infection may be overcome in the regional nodes, but once established, it is expected to spread during the phase of acute regional lymphadenitis. Spread is chiefly hematogenous and bacteremia may persist for several months, the duration of persistence apparently depending on the susceptibility or resistance of the host. As the infection becomes chronic, bacteremia becomes intermittent, ceases in some animals, and recurs irregularly for at least 2 years in 5–10% of animals. Also, it tends to recur at parturition. It might be expected that the bacteremic episodes would result in localization and persistence of the organism in many tissues but, curiously, localization is largely restricted to the spleen, mammary glands, mammary lymph nodes, and pregnant uterus of the female, and to the lymphoid tissues, testis, and accessory glands in the male, and such localizations occur in the early bacteremic phases. The organism has little or no predilection for the kidney (although microscopic interstitial nephritis may be present), ovary, bone marrow, or mesenteric lymph nodes, and appears not to be excreted in the urine or feces. Localization does occasionally occur in synovial structures to produce purulent tendovaginitis, arthritis, or bursitis, but whether localization and persistence occur in synovium which is healthy is not clear; some pre-existing inflammatory changes may be necessary.

Infected animals, almost without exception, excrete *B. abortus* in the colostrum. Thereafter, excretion of the organism in milk may cease, but frequently it continues, although intermittently, throughout the period of lactation. Organisms excreted in milk are an important source of infection for children; and also for calves, but they recover without significant effect. It is still not clear whether and to what extent *B. abortus* causes anatomical changes in the mammary glands. This must be expected, but they must also be mild and difficult to distinguish from the focal inflammatory reactions commonly present in mammary glands. Dense infiltrations of the interstitial tissue by plasma cells, lymphocytes, and histiocytes, and exudation of neutrophils in acini probably constitute the usual changes. The mastitis is focal and not attended by gross changes. The cellular content of milk is increased.

B. abortus has special affinity for the pregnant endometrium and fetal placenta to which it spreads hematogenously during the initial or later bacteremia. Experiments using *B. abortus* in goats have shown that the organism is carried by the bloodstream to the periphery of the caruncle, where at the extremities of the maternal villi capillaries leak into the narrow space (hemophagous organ) adjacent to the fetal erythrophagocytic trophoblast of the chorion. Either cells of the erythrophagocytic chorionic trophoblast phagocytose the *Brucella* organisms, or they invade; regardless, they multiply and spread via the uterine lumen and endocytosis, or by cell-to-cell transfer, to the rough endoplastic reticulum of the adjacent chorionic trophoblast cells. They multiply here and spread to the fetus following ulceration of the trophoblast and invasion of the fetal chorionic villi. The process may be similar in the bovine caruncle where hematomas appear at the ends of maternal septa late in gestation.

Gross lesions *in the placenta are characteristic but not pathognomonic;* similar lesions of less or equal severity may be caused by other bacterial infections, and fungi produce similar lesions, usually of greater severity. There is considerable variation in the severity of the placental lesions, and this is reflected to some extent in the course of the local infection. If the lesion is severe, abortion or premature birth is the likely outcome, and if the lesion is of minor severity, the calf may be delivered normally at term and be viable or nonviable. The intrauterine lesions apparently progress very slowly, because an interval of many months may elapse between infection and abortion or normal birth. Abortion occurs most often in the seventh and eighth months of gestation. Once the infection localizes in a pregnant uterus, it almost certainly remains there and remains active until the fetus and placenta are delivered and for some time thereafter. The nonpregnant uterus is not particularly susceptible to *B. abortus*, and following abortion or parturition, the organism is cleared from the uterus in a few weeks, or longer in some cases.

The external appearance of an infected pregnant uterus is normal. Sometimes the placenta is normal. Typically, *between the endometrium and chorion in the intercotyledonary area, there is more-or-less abundant exudate* that is odorless, dirty yellow, slightly viscid and slimy, and which contains gray-yellow, pulpy floccules of detritus. The fetal membranes and the umbilical cord are saturated with clear edema fluid, and the membranes may be 1.0 cm or more thick. The fetal fluids are usually normal, although occasionally fluid in the amnion is viscid and stained with meconium as the lesion extends.

The placental lesions are not uniform; some cotyledons may appear more-or-less normal and others will be extensively necrotic, while still others are diseased to intermediate degrees; similarly, the intercotyledonary placenta varies in the extent to which it is changed, lesions being most prominent adjacent to the cotyledons. Affected areas of intercotyledonary placenta are thickened with yellow gelatinous fluid, opaque and tough, and the normal smooth glistening surface takes on an appearance resembling yellow to gray Morocco leather with, on the surface, a patchy coagulum of inflammatory exudate and desquamated, degenerate epithelial cells. Affected cotyledons or portions of them are necrotic, soft, yellow-gray, and may be covered with the sticky, odorless, brown exudate that is usually referred to as resembling soft caramel candy.

Histologically, edematous placental stroma contains increased numbers of leukocytes, largely mononuclear but with some neutrophils. The chorionic epithelial cells are stuffed with bacteria (Fig. 4.78), and many of them, with their inhabitants, desquamate into the uterochorionic space. The organisms in intact epithelial cells are coccoid, but free in the exudates; they assume a more elongate form even while still contained within the ghosts of dead epithelial cells. Over the placentomes, the same sort of placentitis is present, but the parasitism is not so extensive in the epithelial cells covering the cotyledonary villi, except at their base, or in the epithelium lining the caruncular crypts, although many of the syncytial trophoblastic cells may be necrotic. The intervillus portions of the placenta, the so-called placental arcades, are quite severely affected, and exudate accumulates between the arcades and the expanded outer extremities of the maternal septa. There is normally some placental exudate and minor hemorrhage in these spaces, but this is greatly exaggerated in placentitis and contains infiltrated leukocytes, epithelial debris, and bacteria. The maternal portions of the placentome are not much involved except for the expanded ends of the maternal septa where these are bathed in the exudate of the placental arcades, and in consequence, become denuded and superficially necrotic. Beneath the necrotic tips of the maternal septa, there is more-or-less dense infiltration of leukocytes and prominent productive inflammation, the

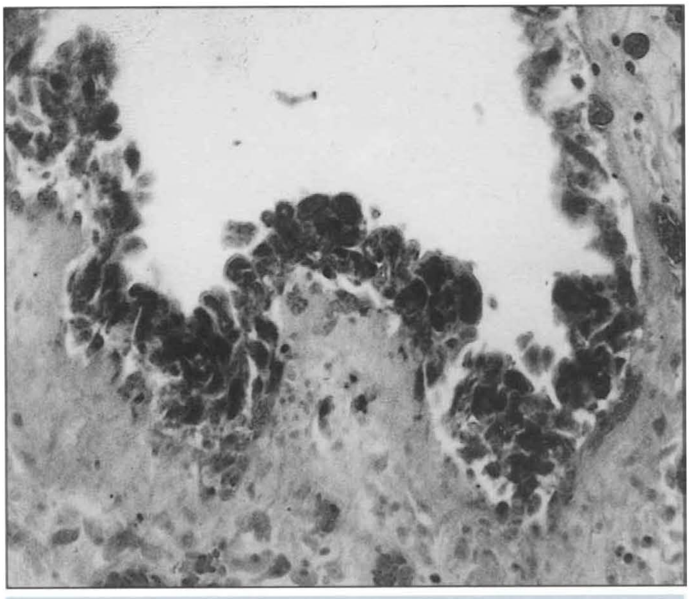

Figure 4.78 Colonies of ***Brucella abortus*** in cells of chorionic epithelium.

fibrosis extending some distance along the sides of the septa. Inflammatory enlargement of the terminal portions of the maternal septa produces an increased degree of placental interlocking, and probably contributes to retention of the placenta. Adhesions, in the usual sense of connective-tissue fusion, between placenta and uterus do not occur. The endometrium is relatively unscathed in the early infections. The zona basalis shows some increase in lymphocytes and plasma cells, and there may be scattered microscopic granulomas of epithelioid cells. Even the intercaruncular epithelium may not be overly disturbed. Later, there is severe endometritis.

(For a discussion of lesions in bulls caused by *B. abortus*, see Vol. 3, Male genital system.)

The **fetus** is usually somewhat edematous with blood-tinged subcutaneous fluid. The same fluid is present in excess in the body cavities and the dorsal retroperitoneum. The normal abomasal content of a fetus is clear, translucent, thick, and viscid; in brucellosis, it often becomes very turbid, of a lemon-yellow color, and flaky. *The important fetal lesion in brucellosis is pneumonia that is present to some degree in most fetuses aborted in the last half of pregnancy.* The lungs may appear grossly normal, but histologic examination reveals scattered microscopic foci of bronchitis and bronchopneumonia (Fig. 4.79). When severely affected, the lungs are enlarged and shaped to the thoracic contour, firm on palpation, reddened on the pleural surface or hemorrhagic, and with fine yellow-white strands of fibrin on the pleura. Microscopically, there may be any stage from the minor changes mentioned through a well-developed catarrhal bronchopneumonia to the fibrinous variety. The predominant inflammatory cells are mononuclear, although many immature and mature neutrophils may be present in some areas. The septa may be edematous and the perivascular lymphatics infiltrated with leukocytes. *By the time the placentitis has advanced to an extent where abortion is inevitable, acute diffuse endometritis, without histologic specificity, has developed.* Variable lesions in the fetus include necrotizing arteritis, especially of pulmonary vessels, focal areas of necrosis, and granulomas with giant cell formation in lymph nodes, liver, spleen, and kidney. The organism may be identified by culture, immunofluorescence, or immunohistochemistry.

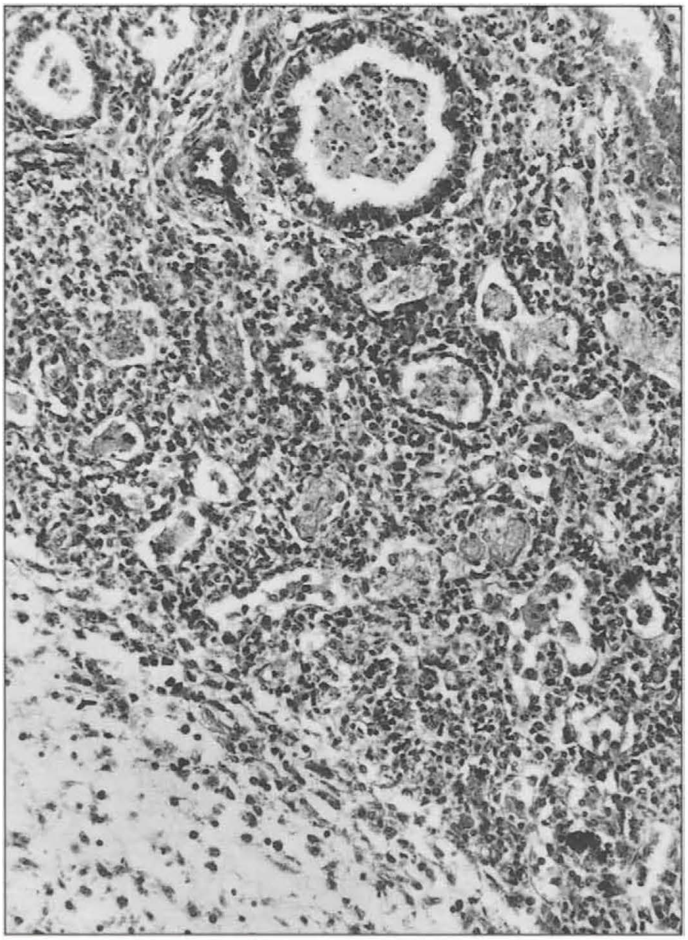

Figure 4.79 Pneumonia due to brucellosis in a bovine fetus.

Brucellosis in swine, caused by *Brucella suis*

Pigs are susceptible to *B. melitensis,* and slightly susceptible to *B. abortus*. The disease is usually caused by *B. suis*, and this organism presents important problems in many countries. *There are some differences in the diseases produced by B. suis and B. abortus, and these depend largely on the frequency with which B. suis in swine produces focal granulomatous lesions with coagulative necrosis, the affinity of this organism for the skeleton and joints, and its tendency to remain in granulomatous foci in the nonpregnant endometrium.*

The early stages of the pathogenesis of the infection are comparable with the early stages of bovine brucellosis. Infection can occur by the same variety of routes, but, *in swine, brucellosis is chiefly transmitted by coitus.* Boars are as readily infected as sows, and most infected boars develop lesions in the testes or accessory genitalia, from which the organisms are shed in the semen, often for life. They may also be shed in the urine from a focus of infection in the bladder. Infected females may discharge the organism from the uterus for up to 2.5 years. Suckling piglets can be infected, although they are less susceptible than weaners or adults, and although most infected piglets cast off the infection, a few carry the infection into adulthood.

With the development of lymphadenitis regional to the point of entry, the infection becomes bacteremic. *The bacteremia may be transient or persist for many months or even for some years.* Localization may occur in many organs, but especially in the male and female genitalia, the skeleton, including the vertebral column, synovial structures, mammary glands, lymph nodes, spleen, liver, kidney, bladder, and even in the brain.

B. suis can grow and multiply in phagocytes, and the typical granulomatous lesion begins with the accumulation of histiocytes and epithelioid cells. Perhaps as a response to developing hypersensitivity to the organisms, as the lesion enlarges, caseous necrosis occurs centrally and fibrous tissue forms a capsule. The granulomas enlarge progressively, and the necrotic tissue attracts neutrophils. Giant cells are absent or scarce. Calcium salts may be deposited in the necrotic foci.

Articular lesions caused by B. suis are quite common. They begin as a synovitis and chiefly affect the compound and large joints of the limbs. The reaction is purulent or fibrinopurulent. *Osteomyelitis in this disease is typically vertebral* (or usually observed there). As with other causes of osteomyelitis, localization is typically in the vertebral epiphyses of the lumbar region. There is, however, an unusual tendency to involve and destroy the intervertebral cartilages. The smaller bony lesions are typically granulomatous with dry caseation necrosis, but the necrotic cores of larger lesions may liquefy and the suppurative reaction extends to the meninges or fistulae to produce paravertebral abscesses.

In the uterus and uterine tubes, there are often conspicuous and characteristic lesions, which are not dependent on an association with pregnancy. They have been referred to as *miliary uterine brucellosis* and, as the name infers, there are few or very many pale yellow nodules with an average diameter of 2–3 mm seeded in the mucosa. When the nodules are numerous, they may coalesce to form irregular plaques and then are associated with thickening of the uterine wall and stricture of the lumen. The same lesion is usually also present in the uterine tubes, where obstruction results in pyosalpinx. Incised, a small quantity of caseous exudate can be expressed from the nodules. Small, red, flat, and irregular granulomas are often scattered over the surface of the supporting ligaments; grossly, they resemble fetal fat and are easily overlooked. There is some stromal fibrosis and diffuse cellularity in the tubes and endometrium due to infiltrated plasma and lymphoid cells. In addition, well-developed and multiple hyperplastic lymphocytic nodules are evident. There are few neutrophils in the stroma, but with the mononuclear cells, they can be seen pushing their way through the epithelium to collect in abundance in the lumen of the uterus and tube and in the more superficial glands. The glands are dilated and the leukocytes are enmeshed in strands of mucin and mixed with amorphous globs of mucus. The deeper glands are cystically dilated with attenuated epithelium, serous or thin mucinous content, and few, if any inflammatory cells. The epithelium lining the lumen and superficial glands is in part retained, in part desquamated, and in part shows the development of a remarkable degree of squamous metaplasia, even to the development of rete pegs and intercellular bridges. The organism is not visible in the lumen.

Abortion usually occurs between the second and third month of pregnancy, but the incidence of abortion is relatively less than in the bovine disease; *there is also a high incidence of stillborn and weak piglets born at term,* and probably also a high incidence of early undetected embryonic deaths. The placenta may be retained. The specific uterine lesions of porcine brucellosis are described above. Apparently, the miliary lesions may develop during pregnancy to a superimposed diffuse catarrhal type of endometritis with patchy congestion, hemorrhage, and edema and a small amount of creamy-pink catarrhal exudate that contains large numbers of organisms. The fetal placenta may not show conspicuous changes, but as a rule, it is congested with small hemorrhages and patchy edema. Usually too, there is a thin layer of exudate that is slimy, gray-yellow, or gray-brown, like mucopus. It is more copious than the secretion normally present in the interplacental space, and smears from it show numerous free organisms and many epithelioid cells, presumably chorionic, which contain clumps of bacteria. The fetus shows subcutaneous edema, which is especially prominent about the umbilicus, and effusions into the body cavities. The edema fluid is often blood-stained. The stomach contents may appear normal or be slimy, turbid, or yellowish and may contain small flakes like curd.

Brucellosis in sheep, caused by *Brucella ovis*

A *Brucella* mutant, *B. ovis*, which was identified less than 35 years ago, causes a specific form of epididymitis in rams in most parts of the world in which sheep are raised. The organism also causes placentitis in pregnant ewes, but *epididymitis is the more common and important manifestation of the infection*, and the main discussion of the disease is included in Vol. 3, Male genital system.

There are probably as many modes of transmission as there are for the *Brucella* in general, but in this disease, *the coital route is important; infected rams excrete large numbers of the organism in the semen.* The infection passes through a stage of generalization, but the disease is never generalized. The organism is a humble one and not very virulent. It grows abundantly but harmlessly in the incubated egg, and survives for a long time in the rat testis without provoking anything. Even the epididymitis of rams is largely an indirect effect. The epididymal lesion produced by the organism, possibly immune-mediated, is a modest one, but it does predispose to sperm stasis and extravasation, which produce the *characteristic chronic granulomatous epididymitis*, by which the disease is recognized. In contrast to the organism's nonpathogenic nature in the nonpregnant ewe, *the organism readily parasitizes the placenta and produces abortion*; this is not common, although it may be important in individual flocks. While the manifestations of infection in both ewes and rams are solely genital, the organism can be cultured in large numbers from other organs in which it does not, however, produce lesions.

The *placenta* is grossly edematous, being thickened to 2–5 cm by gelatinous fluid. *Periarteritis and arteritis are distinctive features*, as in most forms of placentitis. The amnion is adherent to the chorioallantois and thickened in patches. The intercotyledonary placenta has plaque-like thickenings, which may coalesce to resemble yellow-white chamois leather (Figs 4.80, 4.81). Diseased cotyledons may become partially detached; they are firmer than normal, enlarged, and pale yellow. There is extensive necrosis of the epithelial elements of the cotyledons with edema and cellular infiltration of the stroma. Organisms in abundance inhabit the epithelial cells of the chorion.

Most lambs are alive at the commencement of parturition, but mummification or progressive autolysis is also common. As usual, the fetus tends to be moderately edematous, the fluid blood-stained, and that in the serous cavities may contain flecks or strands of fibrin. When present, calcified plaques on the walls, soles, or both, of the hooves and accessory digits are characteristic. *The fetus shows little histological evidence of systemic infection*, even though the gastric contents may be heavily infected. Mild pneumonic alterations and lymphadenitis, especially in anatomical association with the lungs, may be present together with the development of germinal centers and plasma cells in nodes and spleen. Acute interstitial nephritis, particularly about the corticomedullary junction, and inflammatory infiltrates in portal triads are common.

The nonpregnant uterus is insusceptible to the infection. Infection of the fetus may be produced experimentally at any stage, but following systemic exposure, as per conjunctiva, the period of greatest susceptibility to intrauterine localization is from about 21 to 90 days of pregnancy.

Following artificial exposure, there is a lag of 2–3 weeks before bacteremia develops, and the bacteremic phase, in rams and probably in ewes, lasts about 2 weeks. Breeding ewes to rams with open epididymal infection is more likely to result in infertility than abortion. The routes of natural exposure of pregnant ewes are not known. Placental localization is followed by slow development of the intrauterine infection, and the pregnancy may survive for 2–3 months after first being infected.

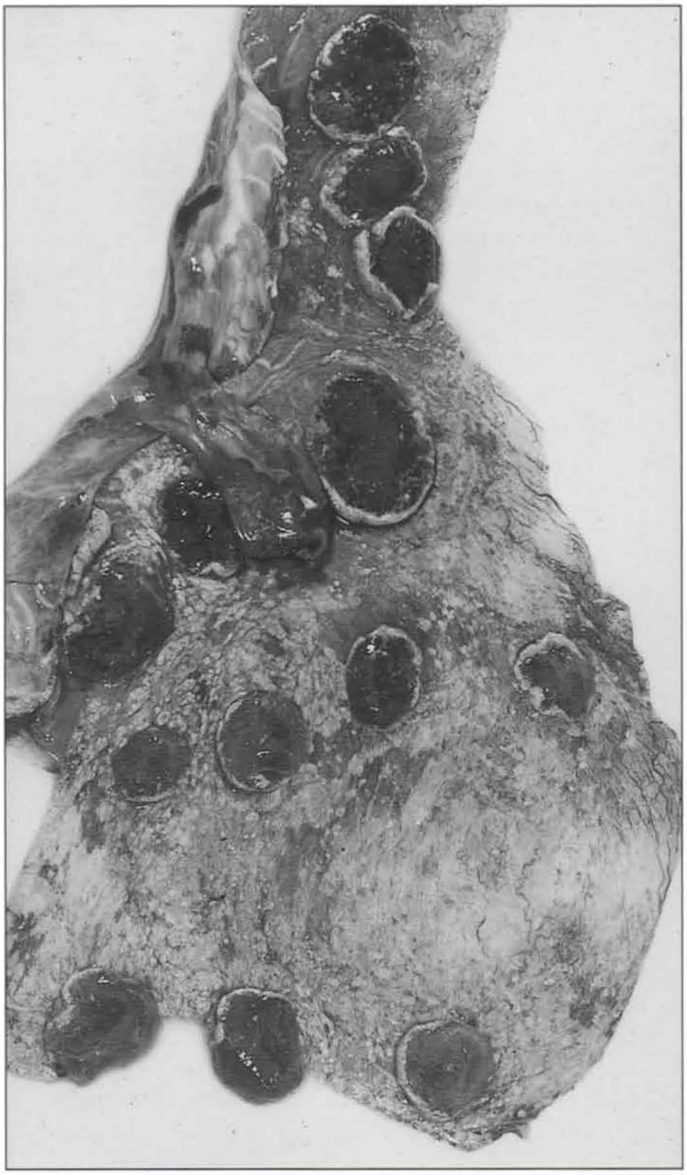

Figure 4.80 Placentitis caused by *Brucella ovis* in a sheep.

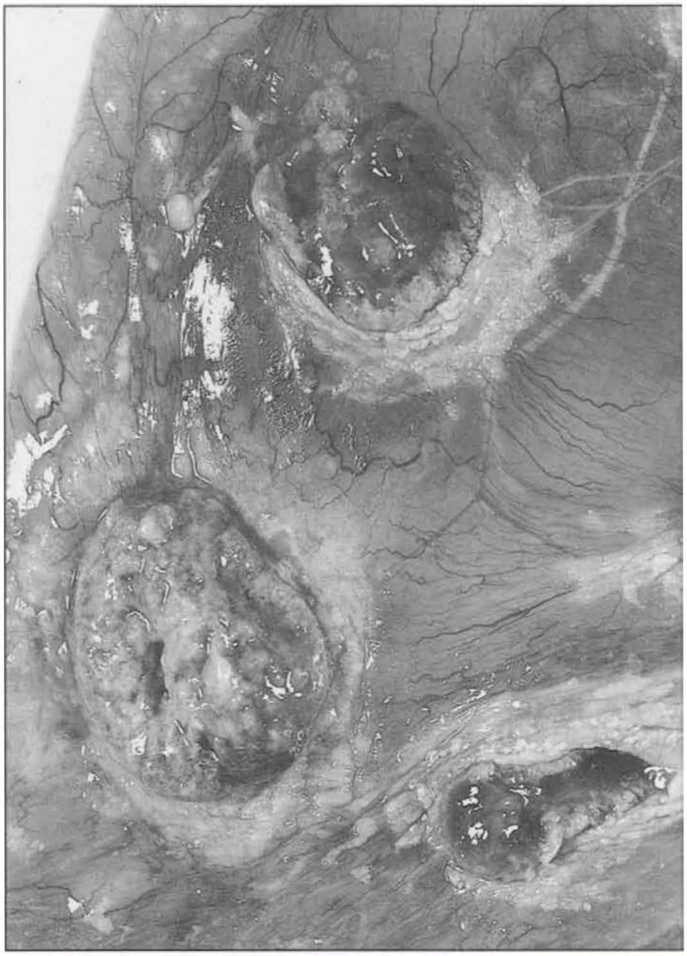

Figure 4.81 Details of **pericotyledonary placentitis** caused by *Brucella ovis* in a sheep.

Brucellosis in sheep and goats, caused by *Brucella melitensis*

B. melitensis is the principal cause of brucellosis in sheep and goats, although natural infections with *B. abortus* occur occasionally. Brucellosis in goats and sheep is prevalent in countries bordering the Mediterranean, in the Near East, and in South America.

In most respects, *the caprine disease resembles bovine brucellosis*, but the disease in sheep is usually less protracted and spontaneous recovery is fairly common in a few weeks or months. In the early bacteremic phases, goats may suffer severe illness and even die, but many infections are asymptomatic. The early signs of the disease may be referable to acute mastitis with palpable nodules in the gland and a secretion that is clotted and watery. The organism is excreted in the milk, usually only for a few weeks in sheep, but often for several months or even some years in goats.

As is usual for ruminants, *abortion may be the only sign observed and it tends to occur late in pregnancy*. The uterine and vaginal discharges after pregnancy or parturition contain large numbers of organisms. Abortion or stillbirth may terminate successive pregnancies.

Brucellosis in dogs, caused by *Brucella canis*

B. canis was first isolated and recognized as a cause of abortion and epididymitis in dogs in 1966. The disease was identified in Beagle colonies in North America at that time. Since then, it has been found in many parts of the world and in a variety of breeds. However, accurate incidence rates are not available; in the USA, where the infection is widespread, the reported incidence rates range from 0.5% to 5%.

The disease is caused by a mucoid strain of *Brucella* that shares biochemical features with *B. suis*. Because of the mucoid nature of the organism, it does not possess the smooth (0) antigens of *B. abortus* or *B. melitensis*. As a consequence, the conventional brucellosis test antigens that are used for diagnosis of the disease in other domestic species are ineffective in the diagnosis of *B. canis* infections.

Transmission of the disease can be *by ingestion* of infected vaginal discharge after abortion or *venereally* by infected seminal fluids. The seminal fluids may remain infected for months. Pregnant bitches may abort after 30 days of gestation, but *most abortions occur after 50 days. Testicular degeneration and epididymitis are the usual manifestations of the* *disease in male dogs*. In response to the discomfiture of the epididymitis, the dogs frequently lick the scrotal skin, producing severe ulcerations. Persistent bacteremias are common in many dogs that show no signs of disease. Bacteria have been recovered from the blood for periods as long as a year, even though high agglutinating titers were maintained throughout the bacteremic period. The nongenital lesions consist initially of nonspecific enlargement of the retropharyngeal and mandibular lymph nodes. Later the lymph nodes throughout the body may be enlarged due to diffuse hyperplasia of lymphocytes and macrophages. The aborted fetuses may be dead or alive when expelled, but live fetuses usually die within a few hours or days. Pneumonia, endocarditis, and hepatitis are observed in the infected fetuses.

Focal necrosis is prominent in the chorionic villi and many of the trophoblastic epithelial cells are laden with bacteria. In the bitch, serosanguineous to gray-green vaginal discharge may be evident for 1–6 weeks following abortion, and provides a source of infection for other dogs.

Brucellosis in other domestic species

Brucella abortus or *B. suis* is regularly found in the bursal lesions described elsewhere as "**poll evil**" and "**fistulous withers**" (see Vol. 1, Bones and joints). There are other isolated reports of suppurative skeletal or synovial lesions in horses caused by these organisms.

Whereas **cats** are resistant to natural infection with *Brucella* spp., **dogs** are relatively resistant to the classical strains of *Brucella*, but natural infections caused by all three species of the organism do occur. The majority of such infections are asymptomatic and detected serologically. Orchitis and epididymitis have been observed. Other lesions described in dogs with brucellosis, chiefly minute granulomas in liver, kidney, and lymph nodes, are neither specific nor necessarily related to the infection.

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Campylobacter infections of the genital tract in sheep and cattle

Infections caused by *Campylobacter* species are common and widespread in humans, cattle, sheep, swine, and chickens and also occur occasionally in dogs. In sheep and cattle, diseases caused by these organisms were previously grouped under the title of "vibriosis," given their classification in the genus *Vibrio* at that time. The principal patterns of disease are *genital*, characterized by infertility or abortion, or *intestinal*, characterized by enteritis and diarrhea.

- *C. fetus* subsp. *venerealis* (previously *V. fetus* var. *venerealis*) causes a true genital infection and is an important cause of infertility in cattle and can cause abortion in cattle and sheep.
- *C. fetus* subsp. *fetus* (previously *V. fetus* var. *intestinalis*) is a common resident of the ovine and bovine intestine and can cause abortion in both species but does so more commonly in sheep.
- *C. jejuni* (previously *V. jejuni*) is a common inhabitant of the intestinal tract in sheep and cattle and may cause enteritis in humans and occasionally animals. It frequently causes abortion in sheep and less often in cattle, although heavy losses in individual cattle herds do occur.

Genital infection of cattle caused by Campylobacter fetus subsp. venerealis

The disease in cattle due to *C. fetus* subsp. *venerealis* is a specific venereal disease, transmitted by coitus. Infected bulls may carry the organism indefinitely in the preputial cavity, although some recover spontaneously. Bulls do not become permanent carriers until they are at least 4 years of age, and most do not become readily infected until they are 5–6 years old. Epithelial crypts develop in the penile mucosa with advancing age and provide a favorable habitat for the bacteria. This also appears to be the natural habitat for these organisms, and once the infection is established in these older animals, it tends to be retained permanently as a locally innocuous surface contaminant together with the nonpathogenic *C. sputorum* subsp. *bubulus*. The organism may also survive for long periods without producing lesions on the surface of the bovine vagina, but not in the uterus. Infected cows develop immunity and control the infection. *The outstanding feature seen with the infection is not abortion but temporary sterility or repeat-breeding with prolongation of the interestrual periods.* It is probable that the delayed return to estrus is the consequence of early embryonic death following fertilization and embryo attachment. Detectable abortions occur at any time but chiefly about the fourth to sixth month of gestation. The placenta is not usually retained.

The endometrial lesions in cows that are repeat-breeders are mild, and consist of lymphocytic infiltrations and nodules and scattered cystic glands. Aborted placentas are often autolysed, indicating that fetal death occurs some time before expulsion. *Placental lesions resemble those in brucellosis but are less severe.* The intercotyledonary placenta is edematous, opaque, and may be leathery; there is a diffuse infiltration of cells, which are mainly histiocytes. Diseased cotyledons are yellow and pulpy; many have yellow necrotic villi at the margins and in others these are scattered throughout. There may be dense accumulations of neutrophils among the denuded villi and in the stroma. The degree of placentitis is quite variable and may be inconspicuous. The parasitism of the chorionic epithelium that is characteristic of *Brucella* occurs but is less evident in placentitis caused by *C. fetus* subsp. *venerealis*. *Lesions in the fetus are nonspecific.* There are commonly blood-stained effusions in the subcutis and body cavities with loose deposits of fibrin on the serous membranes. The normal colorless thick viscid mucus of the stomach content becomes yellow, very turbid, and flaky.

Genital infection of sheep and cattle caused by Campylobacter jejuni or Campylobacter fetus subsp. fetus

The chief manifestations of these Campylobacter infections in sheep are late abortion, premature birth, and the birth of weak lambs. There is not usually retention of the placenta or sterility. Occasional maternal deaths occur as a result of metritis. *Infection is not synonymous with abortion*, as infected ewes may deliver infected but clinically normal lambs.

Transmission of *C. jejuni* most often occurs by the oral route, and fecal contamination of water supplies is relatively frequent. The organism is carried in the intestinal tract of poultry, and it is a commensal of cattle, sheep, and swine. The organism is also a frequent inhabitant of the intestine in dogs and cats and many rodents.

When strains of *C. fetus* subsp. *fetus* isolated from feces or bile are given orally to susceptible sheep, there is transient bacteremia followed by localization in the gut and bile, and the infection can spread by contact between sheep. In pregnant, nonimmune ewes, localization also occurs in the uterus. Surface (S)-layer proteins appear to be essential for colonization and/or translocation of *C. fetus* subsp. *fetus* to the placenta. With both of these *Campylobacter* species, the genital infection is primarily an intestinal infection; transmission is by ingestion, and *uterine localization is an accidental outcome of a brief bacteremic phase in a nonimmune sheep.* The incidence of abortion will depend on the number of ewes pregnant more than 1 month and on their experience with the infecting strain. Immunity following infection or abortion usually lasts for 2 years; however, if other strains are introduced, they may cause abortion, as cross-protection between strains is incomplete. This also applies to vaccination strains.

The abortion rate in natural outbreaks varies between 5% and 70% but is usually about 25%. *Aborted fetuses may have only nonspecific edematous changes; however, some have rather specific hepatic lesions.* Affected livers have few or many light tan areas, from 1–2 mm up to 1–2 cm in diameter, and randomly distributed (Fig. 4.82). Frequently they have a "target pattern" with a more or less distinct, slighty raised white outer rim and a depressed tan inner zone. The abdomen is frequently distended with fluid, fibrin, and the enlarged liver. If the lamb has lived a while before succumbing, these lesions must be differentiated from those of putrefactive origin and from the focal necrobacillary hepatitis of umbilical origin. Histologically, *the lesion is multifocal necrotizing hepatitis*, the necrotic region represented by the tan area and the outer rim by cellular infiltrate and necrosis (Fig. 4.83). There is frequently bronchopneumonia with large numbers of neutrophils in major airways and alveolar ducts. Small renal cortical hemorrhages may also be present.

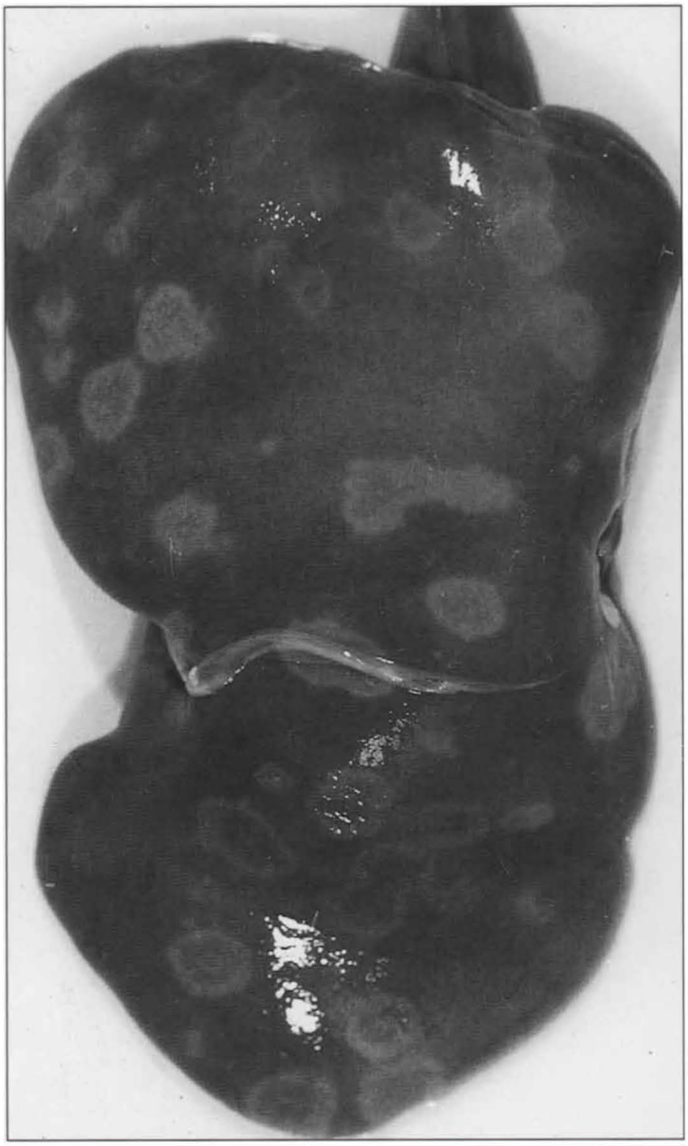

Figure 4.82 Focal hepatitis caused by *Campylobacter fetus* in a fetal lamb.

The cotyledons are enlarged, pale yellow to tan, dull and pulpy, and covered with brown exudate. The chorionic stroma is edematous and infiltrated with leukocytes, which are mainly histiocytes. There is frequently vasculitis. There is mild or acute endometritis with rather more prominent inflammatory exudation in the caruncular septa. The placental lesions are more severe over the placentomes than in the intercotyledonary areas, and are qualitatively similar to the lesions described in the placenta in bovine brucellosis.

Diagnosis *is based on the typical hepatic lesions and demonstration of the organism.* Distinctive organisms may be observed in smears of abomasal content or of affected cotyledons and show up well with special stains. Dark-field or phase-contrast microscopy may be used

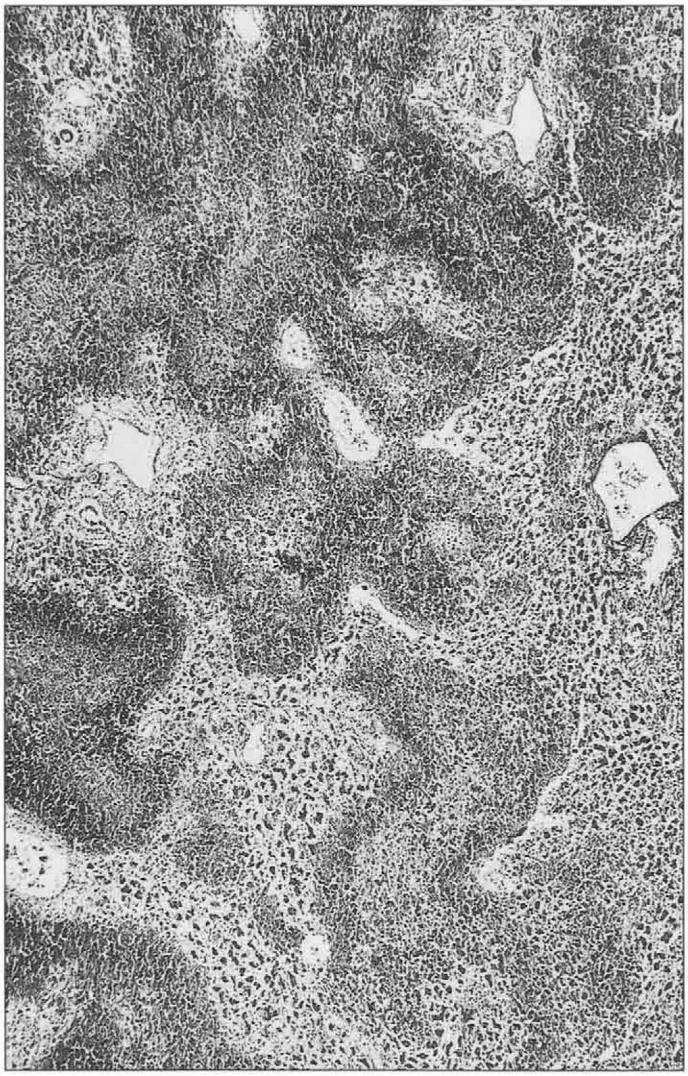

Figure 4.83 *Campylobacter* sp. **necrotizing hepatitis** in an ovine fetus.

to see the characteristic darting motility. Discrimination of the species may be made by culture or application of specific fluorescent antibody techniques.

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Flexispira rappini infections

Flexispira rappini is a highly motile anaerobic rod-shaped bacterium with multiple flagella at both of its tapered ends. Although *F. rappini* is the generally accepted name, it has not been firmly classified. The organism is closely related to organisms in the genus *Helicobacter* but is distinguished from them by its unusual spiral grooves visible on electron microscopic examination. Comparable organisms are frequently found in feces of dogs and pigs, and in the stomachs and intestines of a variety of other species.

Flexispira rappini infections in pregnant ewes result in fetal mummification, abortion, and the birth of infected lambs, born weak. It is not considered to be the cause of epizootics of abortion and, as the lesions closely resemble those occurring in abortion due to *Campylobacter* sp. (which does cause epizootics), it is important that it be differentiated.

Naturally or experimentally infected ewes usually abort in the last trimester of pregnancy. The fetus is usually well preserved; however, if it is retained in the uterus, it decomposes rapidly, and the liver may be liquefied. An infected lamb may be born alive, and occasionally only one lamb of a twin may be affected.

Gross and microscopic lesions may be completely absent but, when present, are in the placenta and liver. In the placenta, they consist of a light covering of brown-to-gray exudates on the chorionic surface. In the abdomen, there may be a fibrinous peritonitis, with a thin, loosely attached layer covering the liver. The liver may be congested and enlarged or tan and soft. Multifocal to coalescing pale, brown-to-gray, clearly demarcated, irregularly shaped areas (0.5 cem to several centimetres across) are often visible on the surface and extend into the parenchyma. In both the placenta and liver, lesions not visible grossly may be obvious on microscopic examination. In the placenta, foci of necrosis with accumulations of neutrophils and mononuclear cells may be present on chorionic epithelium. There is heavy infiltration of neutrophils and macrophages in the villus stroma, and severe neutrophil-rich vasculitis may be present in the placental vessels. Capillaries in the villus stroma may be distended with organisms. In the liver, there are multiple, sometimes connecting, foci of necrotic hepatocytes infiltrated by large numbers of degenerating neutrophils, macrophages, and lymphocytes. Hepatocytes in surrounding areas are dissociated.

The condition should be suspected when these lesions are present and *Campylobacter* cannot be isolated. The organism can be seen on dark-field or phase-microscopic examination of a drop of stomach content. It stains weakly gram-negative and is more easily visualized using Giemsa or crystal violet. Special conditions are needed for culture. The organism also produces abortion in guinea pigs.

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Listeriosis

Infections by *Listeria monocytogenes* occur worldwide and in a variety of animals, including humans. In ruminants, infections are traditionally associated with cerebral localization and *encephalitis* (for general discussion of the diseases associated with this organism, see Vol. 1, Nervous system). However, *localization also occurs in the pregnant uterus and abortion or stillbirth is then the common sign.* The syndromes of encephalitis and abortion may occur together in a herd or flock, but, rather surprisingly, this is the exception. More commonly one or the other syndrome occurs exclusively. *L. monocytogenes* varies greatly antigenically – serovars 1 and 4b are commonly isolated from cattle, and serovars 4b and 5 from sheep. Serovar 5 (*L. ivanovii*) seems particularly pathogenic for sheep. *L. ivanovii* as a cause of abortion in sheep and cattle occurs less frequently than *L. monocytogenes* and rarely causes other conditions.

L. monocytogenes is shed in the feces of normal carriers and clinically affected animals alike, and has been reported to survive in some soils for as long as 2 years. It is most often *spread by ingestion* of food or water contaminated by feces, urine, placenta, or vaginal discharges from aborting animals. Infection by the oral route, and others, has been shown experimentally to produce abortion. *Silage* is often the source of the organism in severe outbreaks of the infection. The aerobic environment just beneath the surface of a silage bale provides conditions for productive growth of the bacteria. *L. monocytogenes* can multiply in poorly preserved silage with a high pH (6–7.8) but dies at a pH lower than 5.5.

The gravid uterus is highly susceptible to infection, and abortion is experimentally induced by the intravenous injection of pregnant ewes rather than by ingestion. Fetal infection is considered to be by hematogenous spread from the placenta with an incubation period of about 5–12 days. Abortions in both cattle and sheep due to *Listeria* occur during the last trimester of pregnancy. The percentage of pregnant animals that abort within any group is variable; usually the abortions produced are sporadic, but sometimes 50% of pregnant animals may abort. If uterine infection develops during the early part of the last trimester, the organism quickly invades the placenta, and the fetus dies as a result of septicemia. The dead fetus is expelled in approximately 5 days; by this time autolytic changes mask minor gross lesions produced by the organism and all organs teem with bacteria, which continue to multiply after death of the fetus. Abortions at this stage are not usually accompanied by severe systemic disease in the dam. The placenta is typically retained as a result of a mild metritis, but the organisms and associated inflammation are quickly cleared from the nonpregnant uterus.

If the fetus is near term when the infection develops, abnormal parturition is instituted in which dystocia is the rule, severe metritis and septicemia being the most common complications. Lesions in the fetuses and placentas in this group are less likely to be obscured by autolytic changes. Gross lesions consist of *tiny pinpoint yellow foci in the liver* (Fig. 4.84). Similar foci, but usually only visible microscopically, are present in the lung, myocardium, kidney, adrenal, spleen, and brain. These foci have a central area of necrosis in which the organism can be visualized along with small numbers of degenerating neutrophils and mononuclear cells (Fig. 4.85). If the

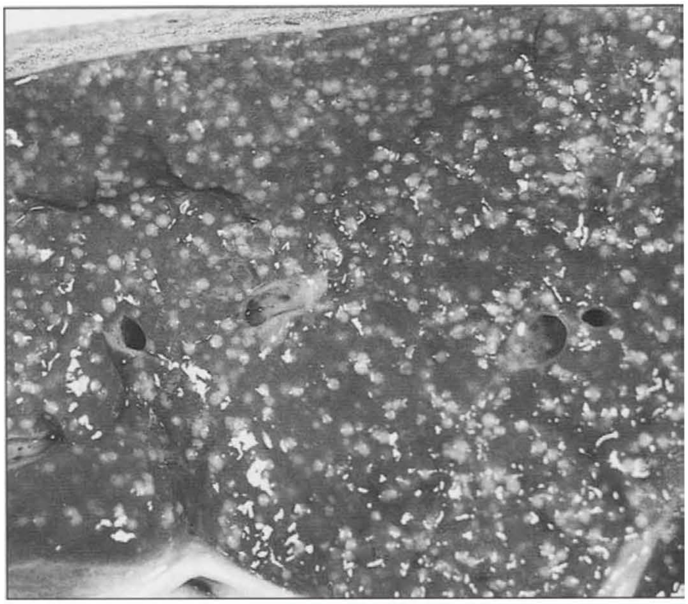

Figure 4.84 Multifocal hepatitis in a fetal calf, caused by *Listeria monocytogenes*.

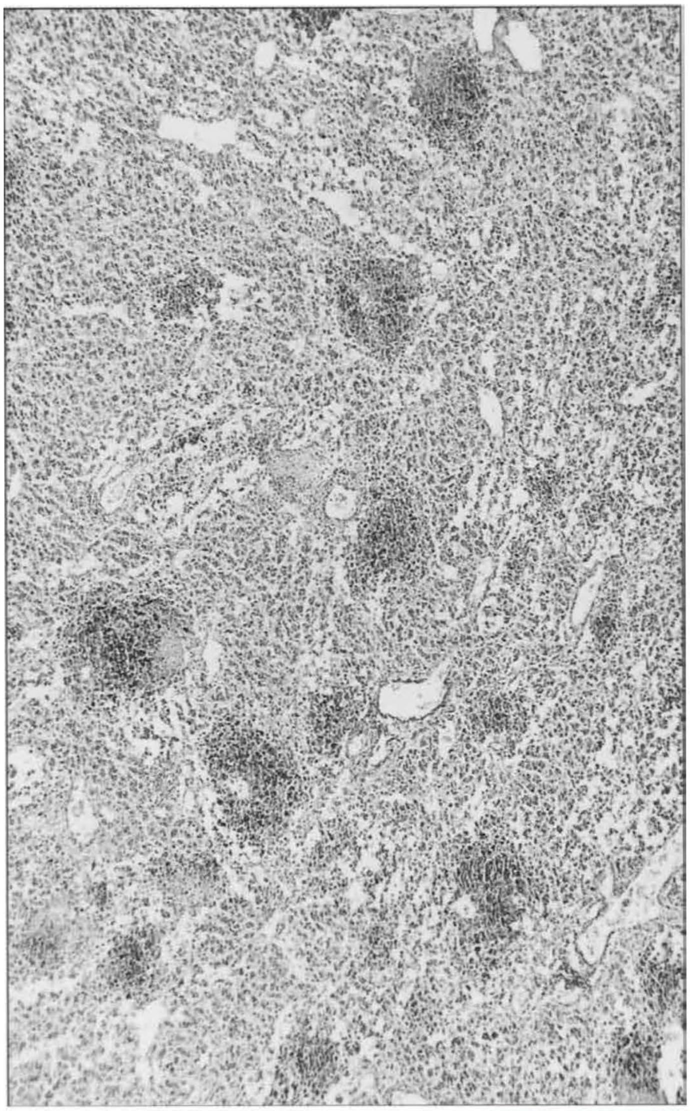

Figure 4.85 Multifocal hepatitis caused by *Listeria monocytogenes* in a bovine fetus.

fetus is near term, there may be severe diffuse nonsuppurative cerebrospinal meningitis in which the organism can also be seen. In the bovine fetus, in spite of marked autolysis, the most dramatic and distinguishing lesion may be in the mucosa of the colon, where *severe necrotizing enteritis* with clusters of colonizing gram-positive bacteria can be seen (Fig. 4.86). The jejunum is autolysed but otherwise normal. Grossly and microscopically, the placental lesion is severe. The necrotic tips of the cotyledonary villi are covered by purulent exudate in which many bacteria are present. There is also focal-to-diffuse red-to-brown *exudative intercotyledonary placentitis*.

Recovery of the organism is sometimes difficult and is probably associated with the advanced autolysis or contamination of some samples. These difficulties can frequently be overcome by refrigerating the specimen (4°C) and reculturing after a period of storage using enriched or selective media.

Clinically affected animals respond well to antibiotic therapy; however, pregnant animals may still abort following recovery.

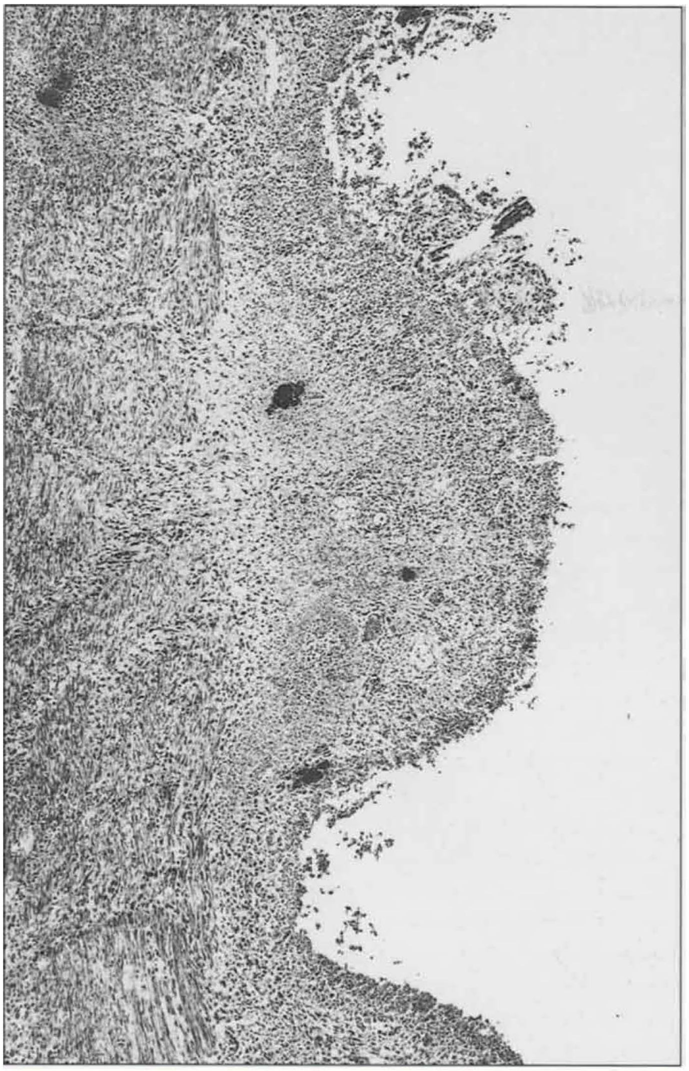

Figure 4.86 Necrotizing colitis caused by *Listeria monocytogenes* in a bovine fetus. Note the dark-staining colonies of bacteria.

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Leptospira infections causing abortion in cattle

Leptospirosis is a zoonotic disease, widespread throughout the world, and is an important cause of abortion and infertility in cattle. Most often, leptospirosis in cattle is caused by serotypes of the species *interrogans*, specifically, ***Leptospira interrogans*, serovars *pomona* and *hardjo***. *L. interrogans* serovar *hardjo* is maintained by cattle and is found worldwide. Serovar *hardjo* occurs in two strains, *hardjobovis* and *hardjoprajitno*. Strain *hardjobovis* is the only type isolated in North America. This organism is more or less widely accepted to be *L. borgpetersenii* serovar *hardjo* type *hardjobovis*. *Hardjoprajitno* is a highly virulent type of leptospira and has not been isolated in North America but has mainly been associated with cattle in Europe. Both serotypes *hardjobovis* and *hardjoprajitno* are spread by urine, postabortion discharges, infected placenta, and sexual contact, or by in utero infection to the fetus. Serovar *pomona* is maintained by swine and some other animals and is a common cause of incidental leptospirosis in cattle. Vaccines used in the USA to protect against *L. hardjo* type *hardjobovis* infection only contain *L. hardjo hardjoprajitno*.

Exposure of nonvaccinated dairy cows to *L. hardjo hardjobovis* has been shown to be associated with increased breedings required per conception. Diagnosis may be confirmed using immunologically based tests to reveal and identify the organism and routine testing of fetal cavity fluids for antibody. It has become a relatively common diagnosis (0–40% of bovine fetuses submitted), depending on the geographic location. Infection of cattle by either *L. hardjo* or *L. pomona* results in bacteremia followed by damage in various parenchymal organs (especially in the young) and eventually excretion of organisms in milk and urine. Antibody titers develop by 4–6 weeks and may stay up for a year or more. Leptospires may then localize in the proximal renal tubules. Persistence and intensity vary with the organism and the host. *L. hardjo* excretion in urine is usually high for 4–6 weeks but may persist at a lower intensity for 6–12 months or life. Cessation is usually associated with a marked increase in urinary antileptospiral IgG and IgA antibodies. *L. hardjo* may localize in the uterine tube and descend into the uterus and placenta during pregnancy. Systemic infection may produce a febrile response and "flaccid agalactia" with the udder secretion becoming scant, yellow, and thick. The organism may also localize in the placenta and, in some animals, produces fetal disease and abortion.

Most of the abortions due to Leptospira occur in the last trimester of pregnancy and often follow acute infection of the dam by 1–6 weeks (*pomona*) or 4–12 weeks (*hardjo*). *L. autumnalis* usually causes abortion in the second trimester. *L. hardjo* may cause early embryonic death. Fetal infection is not invariably fatal, and weak calves may be born with a substantial antibody titer. *Fetuses that die in utero because of Leptospira infection are usually expelled in an autolysed state.* Abortion rates with *L. pomona* may be as high as 50%, whereas with *L. hardjo* they are generally around 3–10%, and infertility is a common observation in infected herds.

Gross and histologic lesions in the fetus and placenta are nonspecific; however, *the presence of Leptospira-like organisms in trophoblast cells, nonsuppurative meningitis, and nephritis warrants further testing.*

The placenta may be edematous, but inflammatory changes are modest in spite of the fact that there may be clumps of leptospires in the chorionic epithelium. The fetus is often autolysed, and calves in which leptospiral antigen is detected in the placenta weigh 6–10 kg less than calves with no antigen in the placenta. Histologically, multifocal necrotizing tubular necrosis or interstitial inflammatory infiltrates, usually with plasma cells, can be found in the kidneys of some of the fetuses. Occasionally, the fetus will have nonsuppurative meningitis (Fig. 4.87).

The organism may be demonstrated in smears of homogenates of fetal kidney, lung, liver, and placenta, or in fetal aqueous humor by the indirect fluorescent antibody test, or by culture. The organism can also be demonstrated in sections of fixed fetal kidney by immunohistochemistry, or in frozen sections using the fluorescent antibody technique. In stillborn calves, the adrenal glands, lung, and placenta are the most useful organs to examine for leptospiral antigen. Cultures of fetuses can often be misleading. Calves infected with *Leptospira* have been found to be more likely to be infected by *Arcanobacterium pyogenes* or *Bacillus* spp.; the latter is probably an incidental finding, but more easily demonstrated than the *Leptospira*. Cultures of specimens can be productive but are time-consuming and cumbersome with a long delay before results. Fetuses which die when the infection is generalized may not have formed antibody, but antibody is frequently present in cavity fluids by the time the organism has localized in the fetal kidneys. Cows aborting fetuses infected with *L. hardjo* may have high microscopic agglutination titers, but up to 40% are reported as not having a significant titer, and over half of those may have no detectable titer. Prospective serology on a herd basis has been reported to be of considerable value in sorting out titers resulting from vaccination, and either previous or present infections.

Leptospira infections of the pregnant uterus in swine

Abortion is often the only evidence of leptospiral infection in a swine herd. There may be no relationship between return to estrus and leptospirosis. Several serovars of *Leptospira interrogans* regularly infect swine: *canicola, grippotyphosa, icterohaemorrhagiae, sejroe, pomona,* and *tarassovi*. The latter two serotypes, which are adapted to spread in swine, are most commonly associated with fetal disease and abortion. Serological testing has indicated that infection by serovar *bratislava*, or a closely related serovar, is also widespread; and abortions, stillborn, and weak liveborn piglets may have the organism and antibodies to it. Serovar *bratislava* has also been associated with infertility without abortions. In one report, *Leptospira bratislava* was detected in samples of the uterine tube by means of immunofluorescence. Some of the serum samples from these animals were positive for antibodies, whereas others were negative. In one study, where many animals had a positive titer to *Leptospira*, failure to identify *L. bratislava* by immunofluorescence was believed to be associated with transient infections early in life.

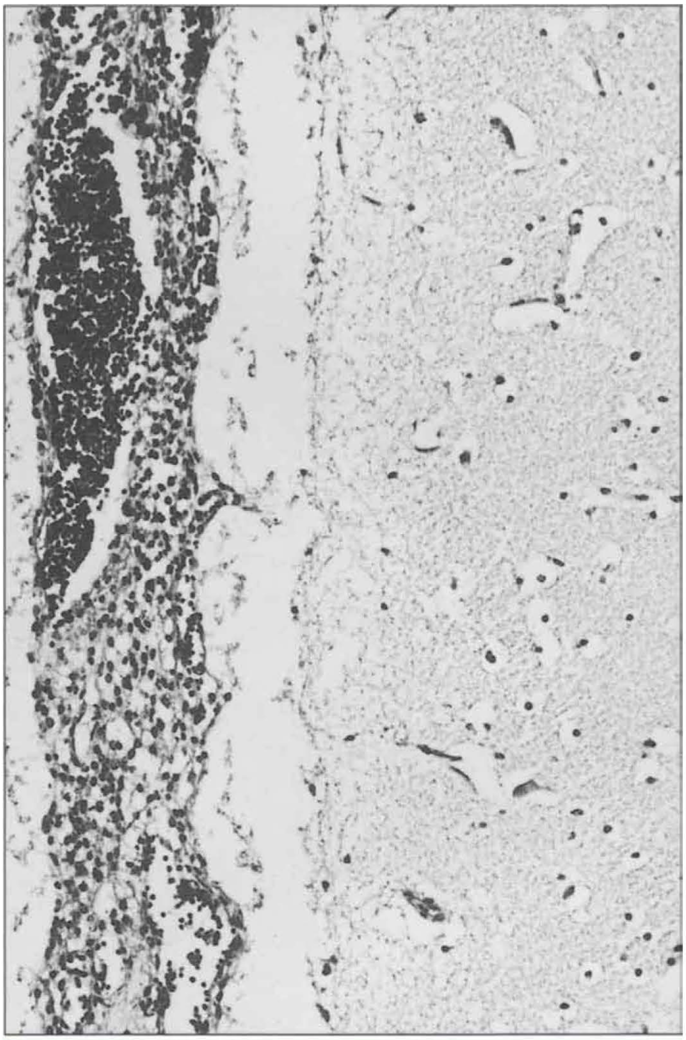

Figure 4.87 Nonsuppurative meningitis caused by *Leptospira* sp. in a bovine fetus.

Leptospires are passed in the urine, and the organisms can enter through mucous membranes or breaks in the skin. Poor management of effluent from piggeries may result in contamination of drinking water, resulting in transmission of the organism to swine and other animals inducing leptospirosis in cattle, sheep, horses, and dogs. Infected swine develop bacteremia before the leptospires localize in the kidneys and uterus, where they may persist and can be shed for months. Systemic signs, if they occur at all, develop during the stage of leptospiremia. It is also during this stage that the organism invades the placenta and infects the fetus. *Most of the abortions due to leptospirosis occur late in gestation, but stillbirths, sometimes with a few partially mummified fetuses, and births of live but weak pigs are also part of the pattern.* Seropositive dams may have a greater risk of having weak newborn piglets and also of having more weak newborn piglets per litter. Infected fetuses often die in utero and undergo autolysis, which obscures any lesions. Some aborted fetuses have elevated immunoglobulin levels.

The placentas of infected pigs may be edematous, but the most severe lesions are seen in those piglets delivered alive, but sick, at or near term. Some of these will be icteric, have patchy hepatic necrosis, fluid, and fibrin in the peritoneal cavity, and inflammatory changes in multiple organs. Focal aggregations of lymphocytes in the renal cortex, medulla and pelvis are characteristic but inconstant. Leptospires can be demonstrated in the kidneys or lungs on section using a silver stain. Special stains are much more useful on tissue sections taken from swine than cattle; however, the fluorescent antibody test for the organism on kidney, lung, and placenta homogenates gives a more definitive answer and allows visualization of the lesion with the antigen. In addition, leptospires may frequently be demonstrated by dark-field microscopy or specific fluorescent antibody test in the urine of the dam up to 2 weeks after abortion and occasionally in fetal cavity fluids from fresh or autolysed fetuses. Leptospires are rarely recognized in fluids from fetuses, which have been frozen, or in fetuses in any condition when aborted because of serovar *bratislava*. Fetal cavity fluids and maternal serum may contain specific antibodies and, along with fluorescent antibody testing of fetal tissues for antigen, makes a practical diagnostic procedure. As in cattle, maternal antibodies may only be useful in diagnosis if examined on a herd basis.

Leptospira infections causing abortion or stillbirth in horses

Until recently, leptospires have not generally been considered to be a major cause of reproductive failure in horses. In Ireland, where tissues from 22 aborted equine fetuses were cultured and examined for leptospires, 9 fetuses were found to contain organisms representing four serogroups: *Australis, Hebdomadis, Icterohemorrhagiae* and *Pomona*. Serovars *bratislava, kennewicki*, and *pomona* are considered to be of major importance in horses, and in one large group of horses tested in Ireland, 89% were found to carry antibody to *bratislava*. The horse is considered to be the maintenance host for *bratislava*.

Foals are usually aborted in the last trimester of pregnancy or stillborn, and gross and microscopic lesions are seldom present. Gross lesions occasionally observed in aborted foals include icterus, yellow mottled liver, mild hepatomegaly, and petechial hemorrhages on mucous membranes. Histopathology may reveal dissociation of hepatocytes, disruption of hepatic cords, multifocal hepatic necrosis, large multinucleated hepatocytes, and renal tubular necrosis. Cerebral perivascular hemorrhage, thrombosis, and severe meningitis with heavy neutrophil and macrophage infiltrates have been observed. In a recent study, leptospires were identified as the cause of abortion in four aborted foals with giant cell hepatitis. For diagnosis, high titers of fetal antibody have been detected in fetal fluid by the microscopic agglutination test and the organism has been identified in impression smears or histologic sections of kidney and placenta by immunofluorescence.

Leptospira infections causing abortion in sheep

A variety of serovars, including *bratislava, ballum*, and *pomona*, will cause abortion and stillbirths in sheep; however, *the major serovar is hardjo*, and with this serovar there seems to be a sheep reservoir that does not include cattle. Sheep seem somewhat resistant to leptospirosis but have a vulnerable period for 2 weeks before lambing and 1 week after. Available evidence suggests that, with different serovars, sheep are usually infected from an environment contaminated by other animals. Feeding newborn lambs with colostrum from *Leptospira*-infected cows is believed to cause death in the lamb. Research has also led to the speculation that leptospirosis is generally less common in sheep than in horses, cattle, and pigs; however, *under conditions of intensive management, leptospirosis can cause severe losses in the last 2 weeks of gestation and early postpartum period.* Losses include abortion, stillbirth, and birth of weak lambs. The grazing lands for sheep are usually arid and therefore do not support leptospires, and the drinking habits of sheep are more fastidious in that they do not normally drink out of puddles.

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Ureaplasma diversum infections

Ureaplasma (formerly T-*Mycoplasma*) *is an important cause of reproductive failure in cattle* and ranks second only to *Bovine viral diarrhea virus* as a cause of abortion identified by some laboratories. The true prevalence of abortion due to this organism is obscure, however, as few laboratories have the capability to grow the organism. *U. diversum* is a bacterium in the family Mycoplasmataceae. The organism is frequently present on the mucous membranes of the nasal passages, vulva, and vagina of the cow, the sheath of bulls, and in semen and embryo transfer fluids. It remains in these sites in the animal for long periods, and can be found in urine, and vaginal and nasal discharges. It may be transmitted to cows (or bulls) during breeding, and as the organism is preserved by freezing, it remains viable in diluted frozen semen.

Virulent strains of *U. diversum* may produce *vulvitis, embryonic death, abortion, or the birth of dead or weak calves at term*. Epidemics of abortion are rare in chronically infected herds, but may occur where large groups of naive animals are congregated. Abortion usually occurs in the last trimester of gestation, or infection may manifest as premature delivery, with the birth of weak or dead calves near term. The fetal membranes may be retained. On gross examination, *the amnion is frequently the most severely affected portion of the placenta*. It has patchy thickening with fibrosis and, in addition, may have foci of necrosis, hemorrhage, fibrin exudation, mineralization, and areas that are stained with meconium. The chorioallantois may

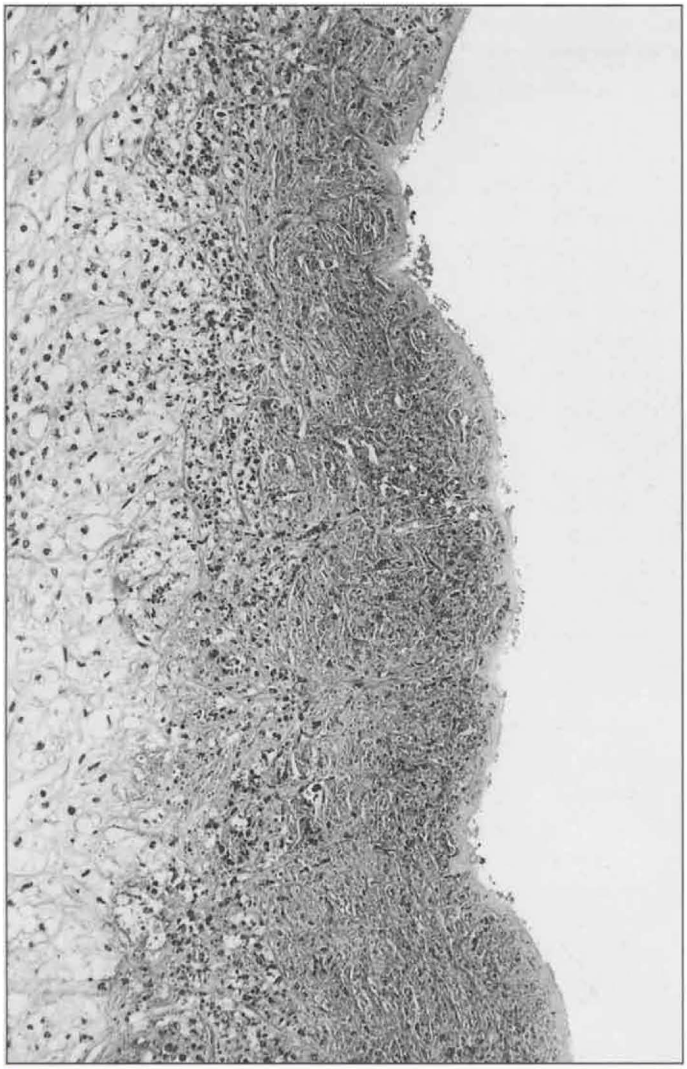

Figure 4.88 Chronic amnionitis due to *Ureaplasma diversum* infection of a bovine fetus.

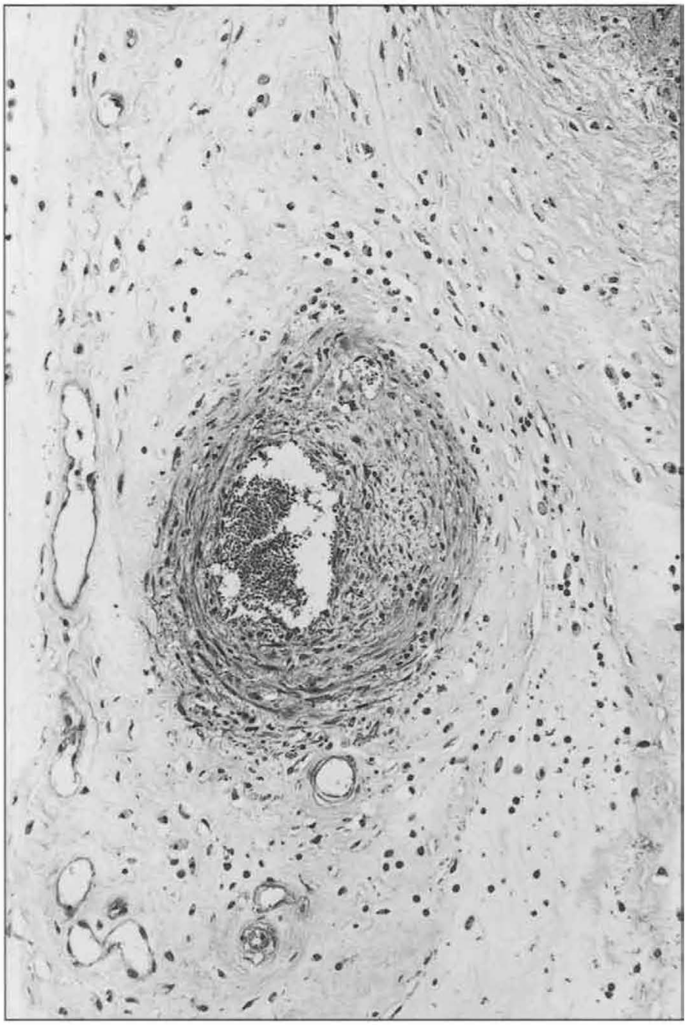

Figure 4.89 Placental vasculitis caused by *Ureaplasma diversum* infection.

be similarly affected, and if so, the lesion is often more severe on the allantoic surface. On histologic examination of the placenta, fibrosis and ongoing interstitial necrosis are extensive. The inflammatory cell component is mainly mononuclear, with macrophages and plasma cells predominating (Fig. 4.88). Mild arteritis is also present (Fig. 4.89).

The fetus is usually well preserved and may be stained with meconium. The lungs are firm and, even if the calf is born alive, are poorly aerated. There is an erosive conjunctivitis with prominent goblet-cell formation of the palpebral conjunctiva. Foci of lymphocytes, including plasma cells, are present in the lamina propria (Fig. 4.90). The lungs have nonsuppurative alveolitis, and periairway lymphocytic mononuclear cell infiltrates may be prominent (Fig. 4.91). *The lesions in the amnion, chorioallantois, and lung are characteristic but not pathognomonic, and require validation by positive culture from placenta, stomach contents, or lung.*

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Arcanobacterium pyogenes as a cause of abortion in cattle and sheep

Arcanobacterium (Actinomyces, Corynebacterium) pyogenes is widespread throughout the world as a common cause of pyogenic infection in a variety of domestic animals. The organism is commonly isolated from aborted fetuses and is fairly consistently diagnosed in 1–15% of bovine fetuses submitted. It also causes abortion in sheep, but this is considered less frequent. *It is believed to be a primary pathogen in the cause of abortion.*

The organism is a common contaminant on the mucous membranes, and is found in the tonsils and vagina of healthy cattle. As to the pathogenesis of abortion, it is suspected the organism may penetrate the bloodstream from a mucosal surface, resulting in transient bacteremia, which, in a pregnant animal, results in placental localization and abortion. *Abortion is usually a single event in a herd or flock but is*

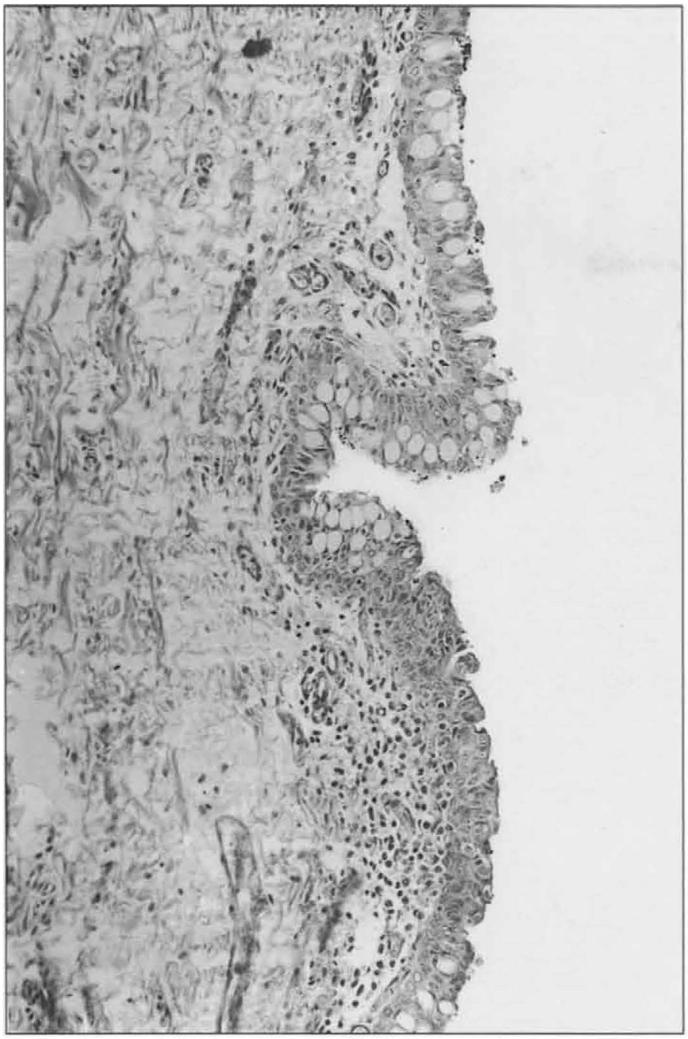

Figure 4.90 Conjunctivitis, with goblet-cell metaplasia, caused by *Ureaplasma diversum* infection of a bovine fetus.

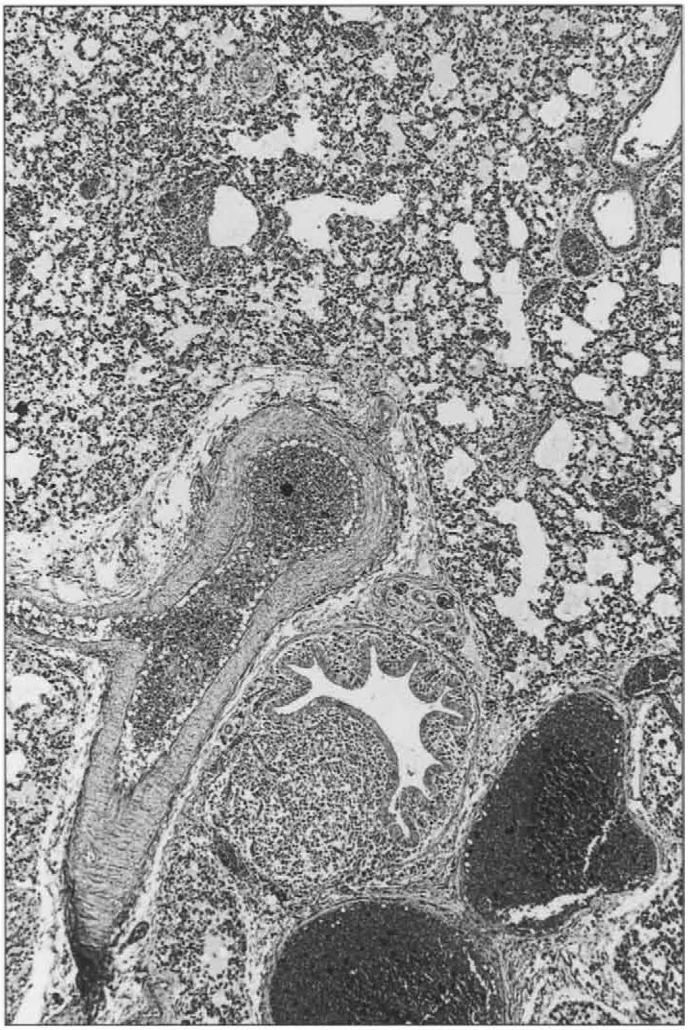

Figure 4.91 Nonsuppurative **alveolitis** and periairway lymphocyte accumulation in reaction to *Ureaplasma diversum* infection of a bovine fetus.

occasionally multiple. Abortion may occur at any stage of gestation but is usually in the last trimester. Dams may be ill following abortion, and some die with suppurative endometritis, arthritis, or mastitis.

The fetus may be severely autolysed or well preserved, and *gross lesions are visible in the placenta, trachea, and lung.* The placenta is often retained and when submitted may be heavily contaminated. Gross examination of portions submitted reveal marked autolysis and suppurative placentitis with yellow-to-brown exudate over swollen edematous cotyledons (Fig. 4.92). In some fetuses, a hemorrhagic cast is present in the trachea (Fig. 4.93). On gross examination of the lung, it is dark red and swollen with minute yellow foci visible on the surface.

On microscopic examination of the placenta and fetus, lesions may be modest (with large numbers of bacteria but no inflammatory cells in placenta and lung) or excessive (with a severe necrotizing, suppurative process in the placenta and acute fibrinous bronchopneumonia in the lung). In addition, bacteria may colonize in vessels, on the skin surface, and on the conjunctiva with destruction of the epithelium.

The **diagnosis** *may be made on seeing the typical lesions and isolating the organism, preferably from the placenta, lung, and stomach contents.* In the absence of lesions, the diagnosis can be made with less confidence by culture only. It has been suggested that the young fetus may be unable to respond to the organism.

Salmonella as a cause of abortion in sheep and cattle

The bacterium *Salmonella* is in the family Enterobacteriaceae. It is a facultative anaerobe, a gram-negative rod, and usually motile. There are over 1300 serotypes in the genus *Salmonella.* Some of these are auxotrophic and have an affinity for just one host, while others are ubiquitous and found in many animals. *S. typhimurium* is ubiquitous, whereas *S. abortus ovis* is most prevalent in sheep and therefore considered auxotrophic. Many of the serotypes causing abortion in livestock are ubiquitous. Salmonellosis is of worldwide concern as a human disease and causes major losses in calves. It is also an important cause of abortion in both cattle and sheep and, in some situations, in mares.

Clinically normal hosts may carry *Salmonella.* It is carried by many mammals and birds, including cattle, sheep, pigs, horses, mice, rats, various birds, including seagulls, and is excreted in the saliva, milk, feces, and urine and in the fluids discharged when animals abort. Animals may become infected through their feed, water, bedding, or the equipment used in feeding, handling, and cleaning. *Abortions are often precipitated by a stressful event such as transport, change in diet, or feeding spoiled feed.* It is not completely defined how these

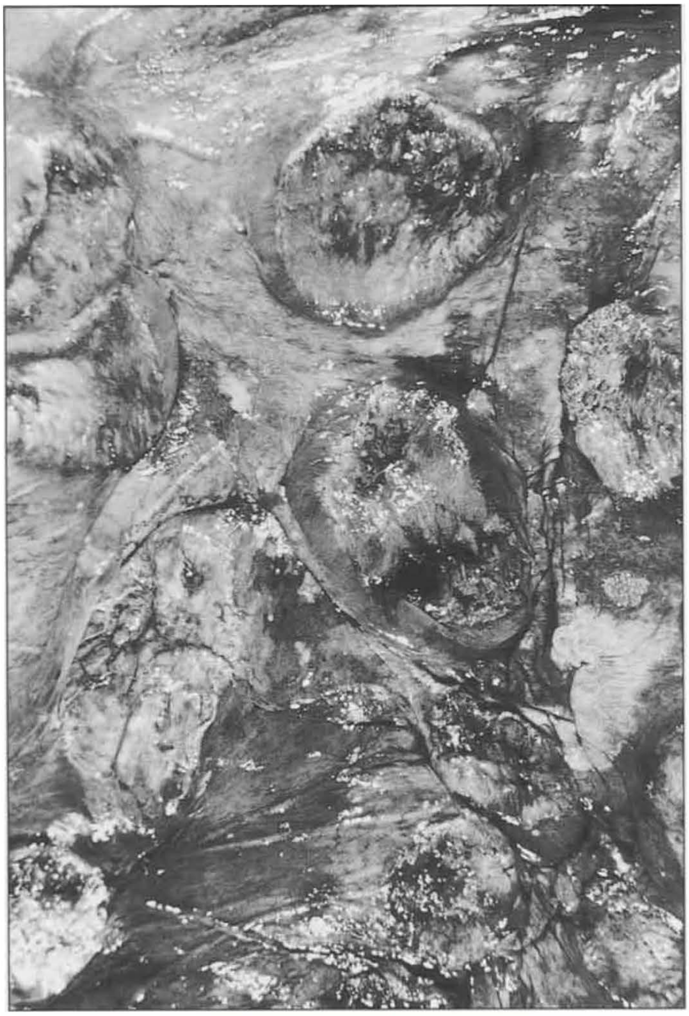

Figure 4.92 Suppurative placentitis caused by *Arcanobacterium pyogenes* infection of a bovine fetus.

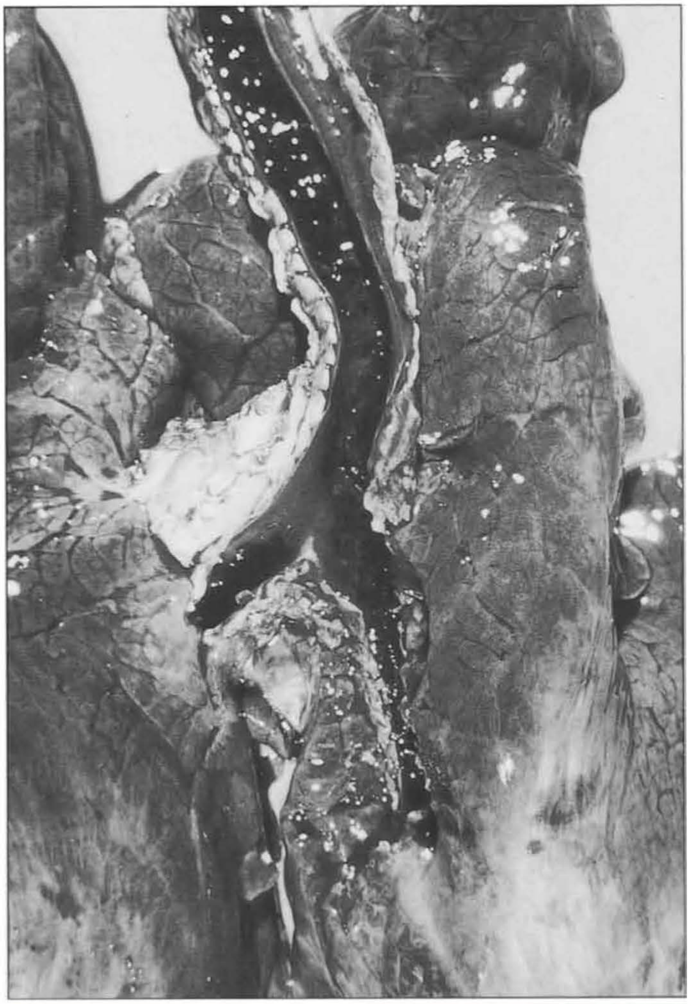

Figure 4.93 Hemorrhagic tracheal cast in *Arcanobacterium pyogenes* abortion of a bovine fetus.

events precipitate increased multiplication of the organism, but it may partly be due to disturbance of the normal intestinal flora. While specific nutrients in the placenta which enhance the growth of *Salmonella* have not been demonstrated, in vivo experiments have shown marked growth enhancement of *Salmonella* following inoculation of whole placental extracts into mice.

The organism may stay in a herd for several years, and *abortions may be sporadic or epizootic, usually occurring in late gestation.* Abortion can occur without illness in the dam or may follow within a week of clinical signs. Cattle may suffer with a variety of clinical manifestations, and in one report, the main manifestations exhibited by *S. dublin* infection varied from pneumonia, to abortion and enteritis, to polyarthritis, in successive years. Signs of enteritis and abortion appear to be commonly associated. Sheep may have a mild upset with fever and anorexia shortly before aborting.

The following *pathogenesis* of placental infection has been proposed. The organism first localizes in the intestine of the dam and this is followed by a brief bacteremia with localization in the lymph nodes, spleen, and lung. After a further period of growth, a second bacteremia occurs in which the placentome becomes infected. Excessive growth of the organism leads to almost complete destruction of the fetal villi, which is followed by abortion and may occur without invasion of the fetus. If *S. dublin* infection occurs within a month of term, the calf may be born alive, carrying the organism.

Following abortion, the placenta is often retained and the fetus is usually autolysed. On gross examination, the chorioallantois is found to be thickened with amber fibrin-containing fluid, the chorionic surface is diffusely gray to red, and there is yellow exudate on the variably tan and red cotyledons. Portions of the caruncle may remain adherent to the cotyledon. Histologically, there is mineralization of individual trophoblast cells, interstitium of the villi, and chorioallantoic arcade. When the chorion is severely affected, mineralization may also be extensive on the allantoic surface. Accompanying the mineral, exuberant bacterial growth expands the villi (Fig. 4.94). In some villi, a heavy infiltrate of neutrophils is present and, if the caruncle remains attached to the cotyledon, there is a clear line of demarcation between the affected villus within the maternal crypt and the near-normal maternal septum. Dilated capillaries, immediately under the sloughed trophoblast cells in the arcade zone, are stuffed with bacteria and in cross-section resemble large rounded trophoblast cells filled with bacteria (Fig. 4.95). In spite of the severe placentitis, there

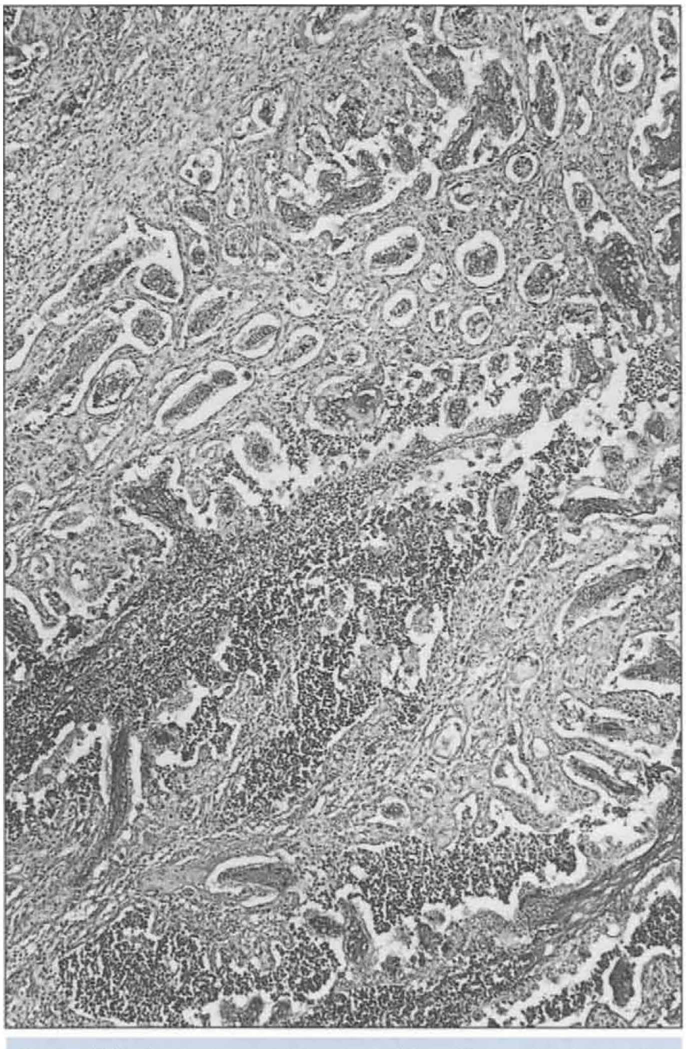

Figure 4.94 Acute suppurative placentitis with complete destruction of villi in the crypt region of a placentome in *Salmonella* abortion in a cow; adjacent caruncle is near normal.

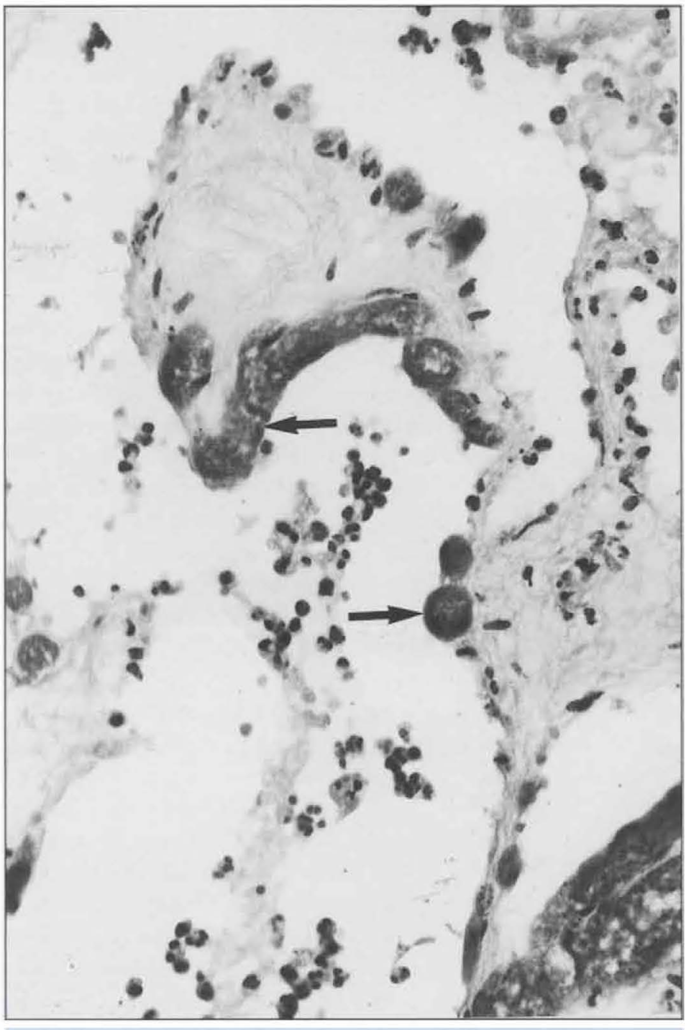

Figure 4.95 Chorionic arcade region, with absence of trophoblast cells in ***Salmonella* abortion** in a cow. Bacteria fill vessels (arrows).

may be no lesions in the lung; however, when present, lesions consist of a moderate to light accumulation of neutrophils in bronchi and bacteria may be seen colonizing airway epithelium. In the liver, there can be multifocal suppurative hepatitis.

Diagnosis is based on recovering the organism from placenta and stomach content. Tissues in the fetus are sometimes negative on culture in spite of marked placentitis. Nearly pure cultures may be obtained from samples of vaginal discharge taken just before abortion. Animals have a remarkable increase in titer following abortion, but this is not a commonly used test needed for diagnosis.

Yersinia pseudotuberculosis as a cause of abortion in cattle, sheep, and goats

Yersinia pseudotuberculosis is a gram-negative coccobacillus in the family Enterobacteriaceae. The DNA of *Y. pestis*, the cause of human plague, and *Y. pseudotuberculosis* are at least 90% interrelated. Six serogroups of *Y. pseudotuberculosis* are recognized based on immunoreactivity of the O antigens. *Y. pseudotuberculosis* causes ileitis, lymphadenitis, and abscessation in humans, and infects a wide variety of domestic, laboratory, and native animals and birds throughout the world. The organism is frequently isolated from the feces of normal cattle, and from abortions in sheep, goats, and cattle. In sheep the organism also causes abdominal abscessation and inflammation in the testis and epididymis.

The *pathogenesis* of abortion probably follows invasion of the intestinal epithelium, transient bacteremia, and localization in the maternal caruncle, followed by passage to the chorioallantois and fetus. It is revealing that in the description of the experimental production of disease in sheep that follows, uterine, and specifically caruncular, disease was present as in naturally occurring abortion. It is possible that nutrients in the caruncle favor localization and growth of *Yersinia* that is followed by villus infarction and invasion of the chorion.

Lesions reported are based on limited observations that are generally similar in aborted fetuses and stillborns from sheep, goats, and cattle. The aborted conceptus is usually well preserved and contains a variety of lesions. Placentitis is largely confined to the cotyledons. Cotyledons are red or tan, thickened and relatively devoid of exudate on the surface. Portions of the caruncle may remain attached. The intercotyledonary region is usually translucent but a frosting of fibrin and some fibrosis may closely surround the affected cotyledons (Fig. 4.96). The thoracic and abdominal cavities contain excess amber fluid with some fibrin. In the fetal liver, there may be pale tan focal areas of necrosis ranging in size from 0.1 to 1 cm. No other gross lesions are observed.

Histologically in the placenta, there is necrosis of villi and moderate infiltration of granulocytes, macrophages, and mononuclear cells in the interstitium of the chorioallantoic arcade. In placental vessels, there is fibrinoid necrosis of the media accompanied by mononuclear cell and neutrophil infiltrates. Lesions in the attached portions of caruncle consist of thrombosis of septal vessels, hemorrhage, and necrosis, with a heavy diffuse infiltration of neutrophils and mononuclear cells. In the lamina propria of the conjunctiva, there are focal infiltrations of mononuclear cells, plasma cells, and a few neutrophils (Fig. 4.97). In the lung, there are a few mononuclear cells in airways. Hepatic lesions consist of focal areas of necrosis infiltrated by granulocytes and mononuclear cells. Similar foci are visible on microscopic examination of myocardium and lymph nodes. Peyer's patches are precociously cellular. The lamina propria and submucosa of the colon are heavily populated by plasma cells and mononuclear cells, and within the lumen, colonies of the organism and scattered inflammatory cells can be seen in the meconium (Fig. 4.98).

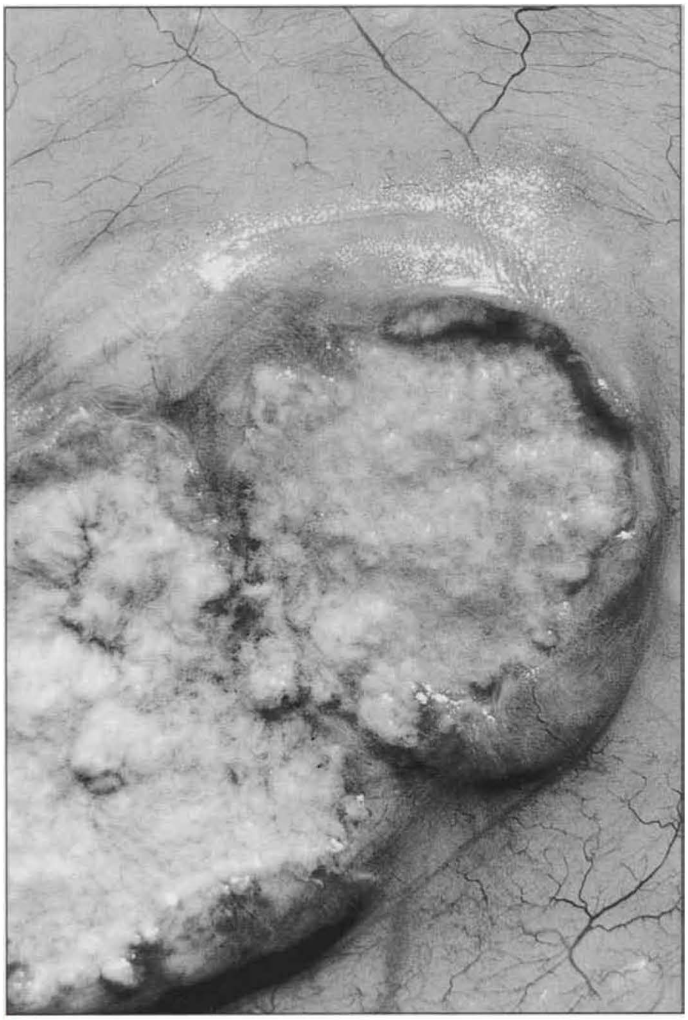

Figure 4.96 Chronic suppurative cotyledonary placentitis and pericotyledonary fibrin in *Yersinia pseudotuberculosis* abortion in a cow.

Extensive lesions in the uterus follow the experimental production of abortion in sheep. They consist of thrombosis of septal vessels, necrosis of septa, and heavy infiltrations of granulocytes in caruncles and intercaruncular regions.

Diagnosis *is based on recovery of the organism from placenta, stomach content, or lung, and the lesions described.*

Histophilus somni infections of the pregnant bovine uterus

Histophilus somni (formerly *Haemophilus somnus*) is the only species of the genus *Histophilus*, family Pasteurellaceae, and encompasses bacteria isolated from cattle and previously described as *Haemophilus somnus*, as well as ovine isolates formerly referred to as *Histophilus ovis* and *Haemophilus agni*. *H. somni* is a fastidious gram-negative coccobacillus that is considered normal flora of the male and female bovine genital tract. There are marked differences in the ability of different isolates

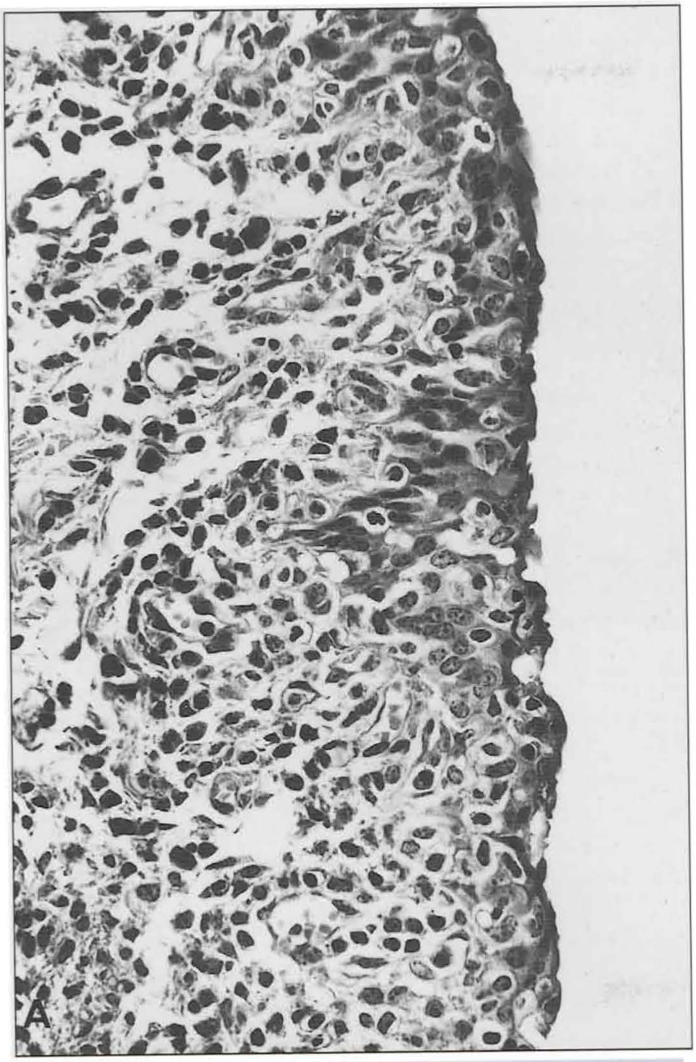

Figure 4.97 Nonsuppurative conjunctivitis in aborted calf with *Yersinia pseudotuberculosis* infection.

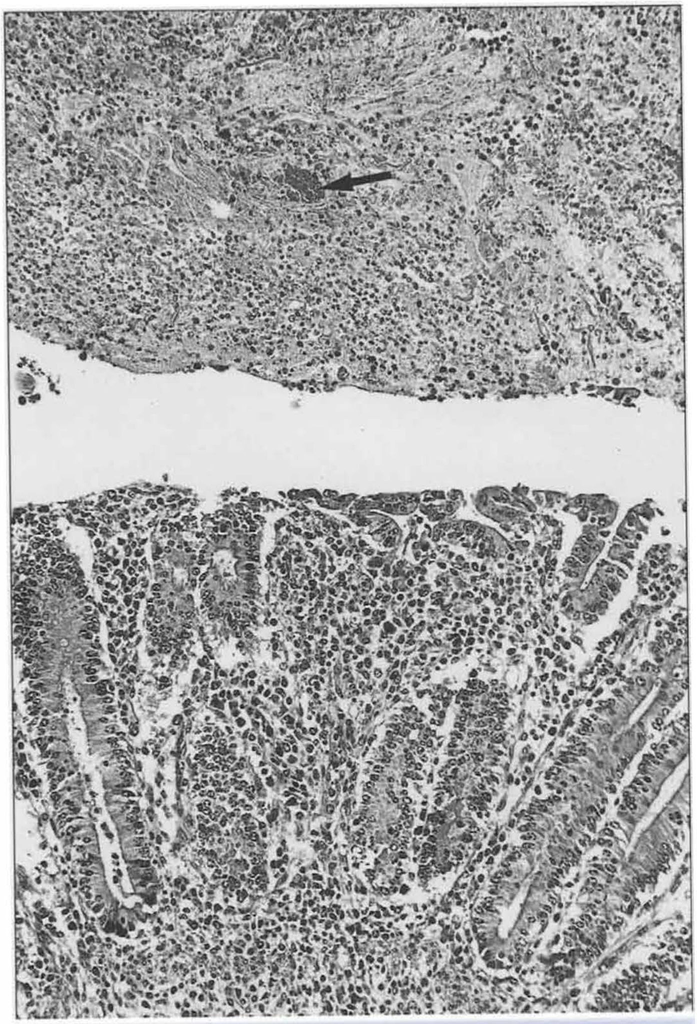

Figure 4.98 Lymphoplasmacytic colitis, with colonies of *Yersinia pseudotuberculosis* in meconium (arrow).

from the reproductive tract to produce central nervous system disease. Preliminary experiments have not shown the reverse to be true.

H. somni is best known for its association with central nervous system disease and pneumonia in cattle, and these are discussed under those sections. *The organism is often associated with vaginitis, infertility, abortion, and occasionally with fatal endometritis.* Usually abortions induced by *H. somni* involve solitary animals; however, occasionally an entire herd will develop reproductive disease, including vaginitis and infertility attributed to this organism. When this occurs, predisposing factors, in the form of malnutrition, concurrent virus infection, or stress, should be considered.

The organism is commonly resident in the vagina of cows (3–76% of animals sampled), and in the prepuce of bulls (more than 75% positive). Natural breeding therefore easily spreads it. Most strains of the organism are sensitive to the antibiotics used in the preparation of frozen semen. Sampling of a naturally infected herd revealed over 50% of calves infected by 7 months of age. The presence of the organism in the vagina does not imply clinical disease.

The *pathogenesis* of abortion has not been clarified. The organism shows some ability to penetrate the cervical area to infect the placenta; however, lesions in the placenta are usually in cotyledons, which suggests a hematogenous route. Bacteremia following a vaginal or respiratory infection is likely. *Death of the fetus after infection is usually rapid, resulting in the abortion of a severely autolysed fetus.*

Lesions in the placenta are primarily confined to the cotyledon and consist of acute nonsuppurative placentitis, which does not appear to penetrate to either the amnion or allantoic surface. On histologic examination, the most distinctive lesion in the placenta consists of severe fibrinoid necrosis and thrombosis of large and small arteries (Fig. 4.99). The cellular infiltrate in vessel walls and adjacent villi is mainly macrophages with some neutrophils. There is sparing of veins and capillaries. Colonies of bacteria are visible next to the trophoblast cells. Lesions in the fetus are usually sparse; however, acute fibrinous bronchopneumonia is occasionally observed. On histology, airways are seen to contain degenerating mononuclear cells, macrophages, but few neutrophils. Bacteria are frequently observed adjacent to the epithelium of alveolar ducts, and swirling (oat-type) macrophages are numerous in these areas. Fibrin often fills lymphatics, distending interlobular septa.

Diagnosis *is based on observing the characteristic lesions in the placenta and lung, and isolation of the organism from the stomach content or placenta.*

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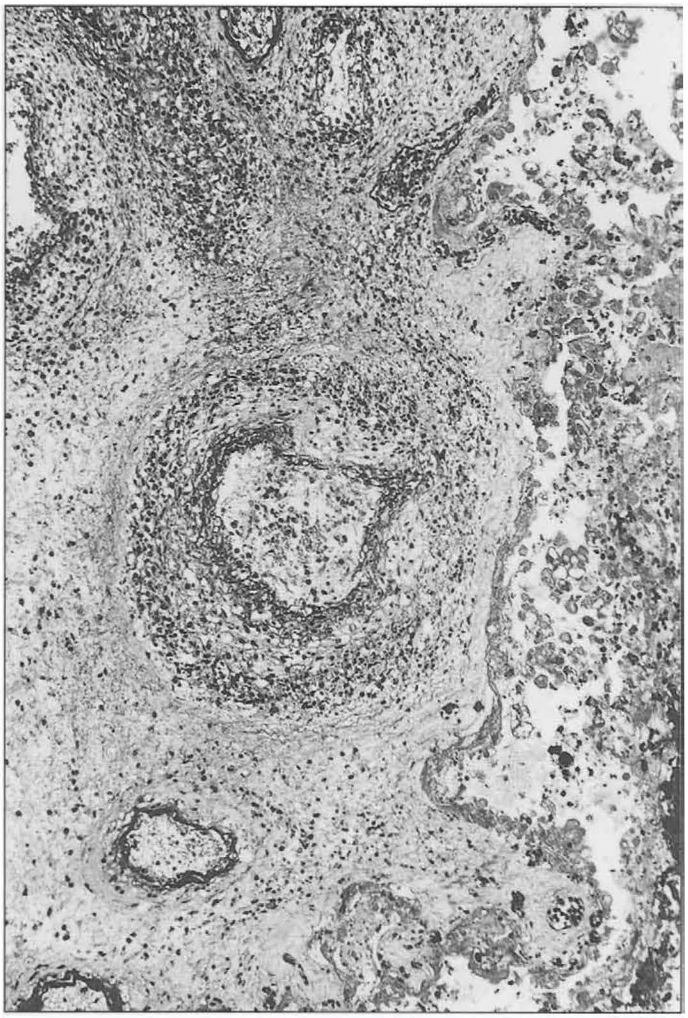

Figure 4.99 Acute nonsuppurative **vasculitis** and cotyledonary **placentitis** in the chorion, due to *Histophilus somni* infection in a cow.

Chlamydophila (Chlamydia) infections causing abortions

Chlamydophilae (chlamydiae) are obligate intracellular parasites that multiply in membrane-bound vacuoles in a variety of cells. They have two distinct forms, the reticulate body, which is not infectious, and the elementary body, which is infectious and released from the cytoplasm by a mechanism not fully understood but which involves disruption of the cell. The inclusions, which can be seen on light microscopy, consist of hundreds of gram-negative *Chlamydophila* cells bound by a membrane in a cytoplasmic vacuole. Based on DNA sequence analysis, the family Chlamydiaceae is divided into two genera: *Chlamydophila* and *Chlamydia*. The species of *Chlamydophila* and *Chlamydia* of veterinary interest include the re-designated forms of *Chlamydia psittaci*, namely *Chlamydophila abortus* (former ovine serovar (immunotype) 1), *C. psittaci* (former avian serovar), *C. felis* (feline serovar), *C. caviae* (guinea pig serovar); plus *C. pecorum* (mammals), and *Chlamydia suis* (former porcine serovar of *C. trachomatis*). *C. suis*, *C. pecorum*, and *C. abortus* have all been associated with reproductive

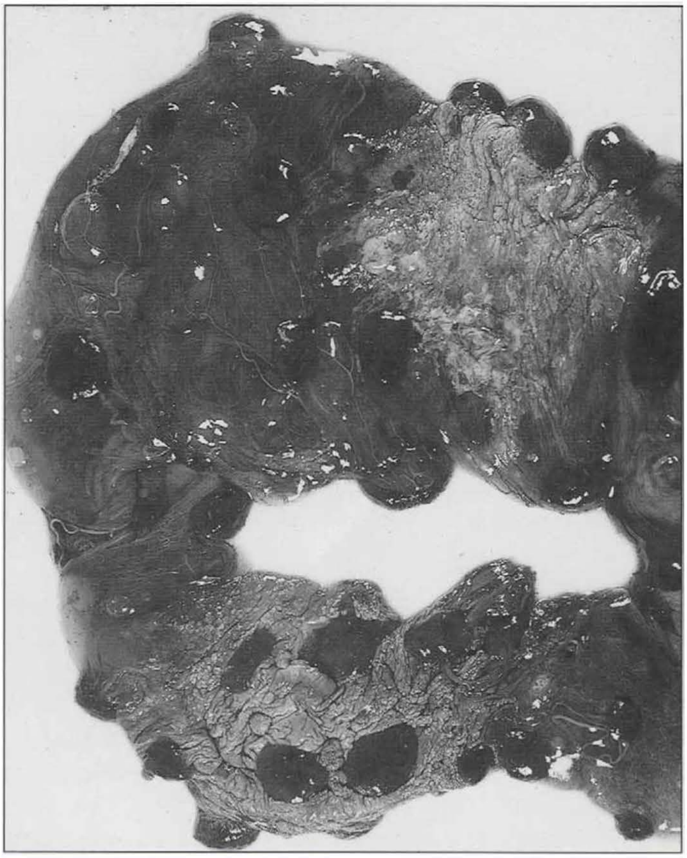

Figure 4.100 **Chlamydial placentitis** in a sheep.

failure and abortion in swine. Chlamydiae have also been associated with abortion in horses. Of significance as human pathogens are *C. psittaci*, *C. pneumoniae*, and *C. trachomatis*.

Chlamydophila abortion in sheep, goats, and cattle

Chlamydophila abortus (C. psittaci) is an important cause of in utero infections in sheep and goats, resulting in abortion, stillbirth, and the birth of weak offspring, and can cause abortion in women in close contact with these aborting ruminants. The disease in sheep has been known as "ovine enzootic abortion," and "enzootic abortion of ewes," but will be referred to here as *Chlamydophila* abortion.

Contamination of foodstuffs with feces or the products of abortion containing the organism transmit the organism orally. On entry into sheep and goats, *C. abortus* may infect the intestine permanently, and from here invade the bloodstream as a chlamydophilemia, and infect the placenta and frequently the fetus in pregnant animals. Naive animals, including newly introduced sheep and females pregnant for the first time, are most vulnerable. *The infection is chronic and abortion usually occurs in the final trimester of pregnancy, or infection may manifest as stillbirths or weak lambs.* Extensive losses (30%) may occur on introduction of the organism to a flock, and yearly rates of 5% are common when it exists in the enzootic form.

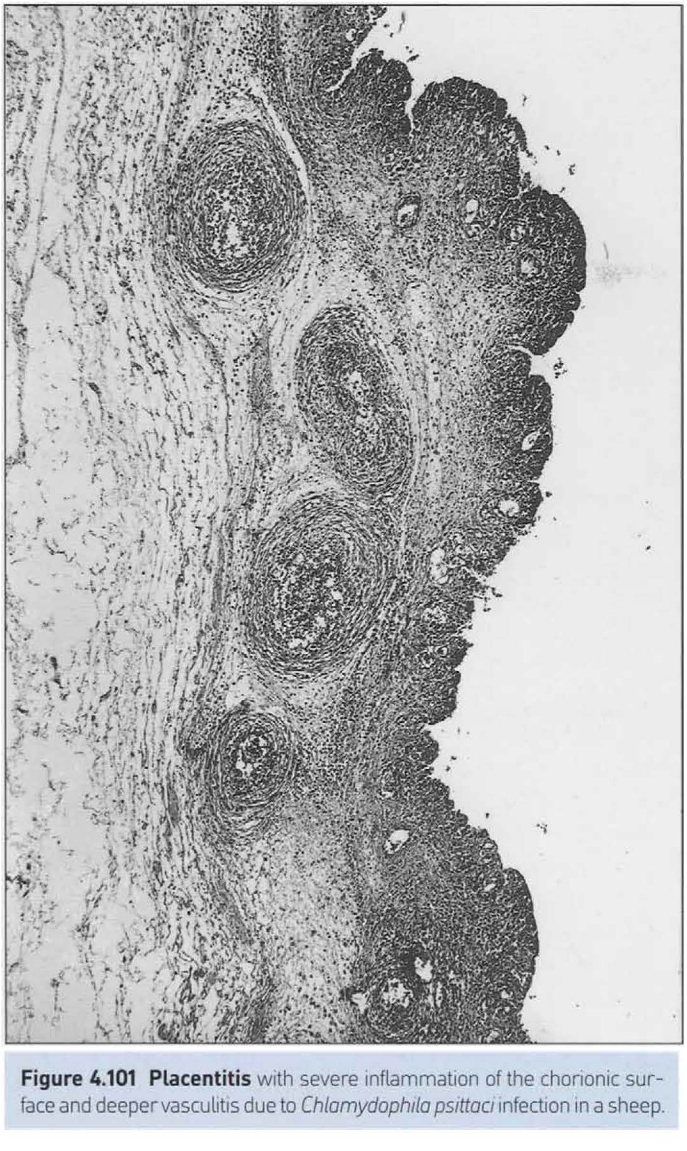

Figure 4.101 Placentitis with severe inflammation of the chorionic surface and deeper vasculitis due to *Chlamydophila psittaci* infection in a sheep.

Retention of fetal membranes may occur in some animals, and aborting females may occasionally be ill. *The placenta resembles that seen in bovine brucellosis* and there is patchy, almost equal involvement of cotyledons and intercotyledonary regions. Affected cotyledons are a dull-clay or dark-red color, firm, and matted with a dirty-red exudate. The intercotyledonary region is red to brown and has irregular patches of edema amidst a dry, leathery thickening of the chorioallantois (Fig. 4.100). These thickenings are often concentrated in ridges, which on histology are seen to consist of medium to large vessels prominent because of *marked vasculitis*. The vasculitis is characterized by fibrinoid necrosis of the media with a moderate to heavy infiltrate of neutrophils and some mononuclear cells (Figs 4.101, 4.102). In the chorion, there is superficial necrosis, and characteristic inclusions can be seen in the cytoplasm of trophoblast cells. Abortion is likely the result of several factors, including tissue destruction by *C. abortus*, vasculitis, and thrombosis, and a fetal inflammatory response. Production of tumor necrosis factor-α by fetal macrophages that express major histocompatibility complex II molecules may be significant in the pathogenesis of abortion.

The fetus is usually well preserved but may have few gross lesions. These consist of scattered hemorrhages in the subcutis, thymus, lymph

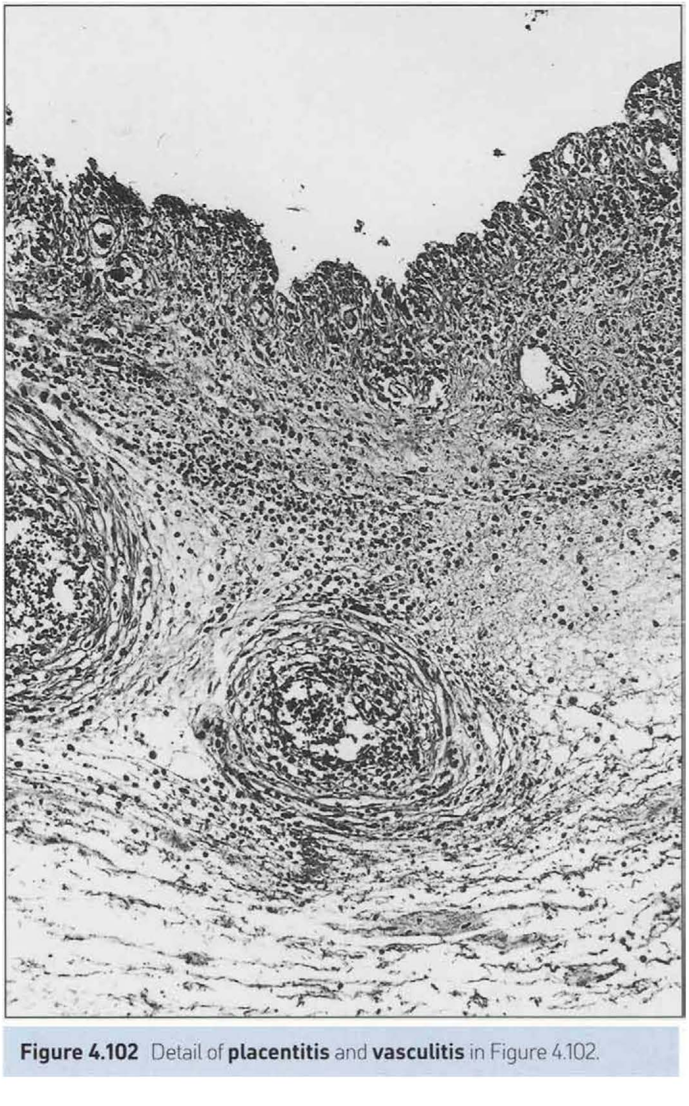

Figure 4.102 Detail of **placentitis** and **vasculitis** in Figure 4.102.

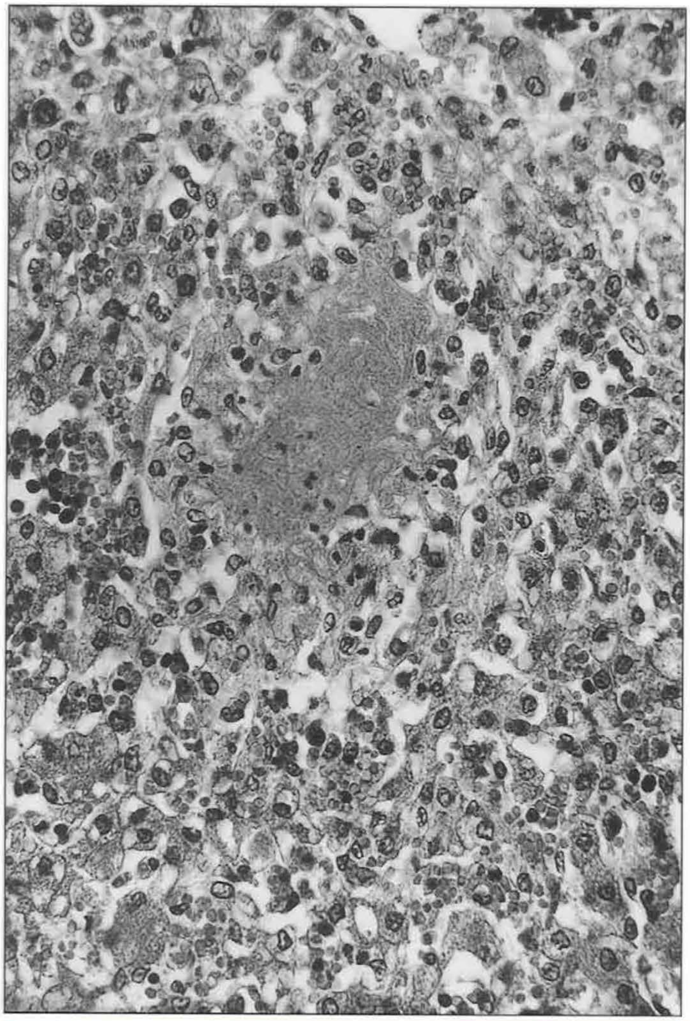

Figure 4.103 Focal coagulative necrosis in liver in *Chlamydophila psittaci* abortion.

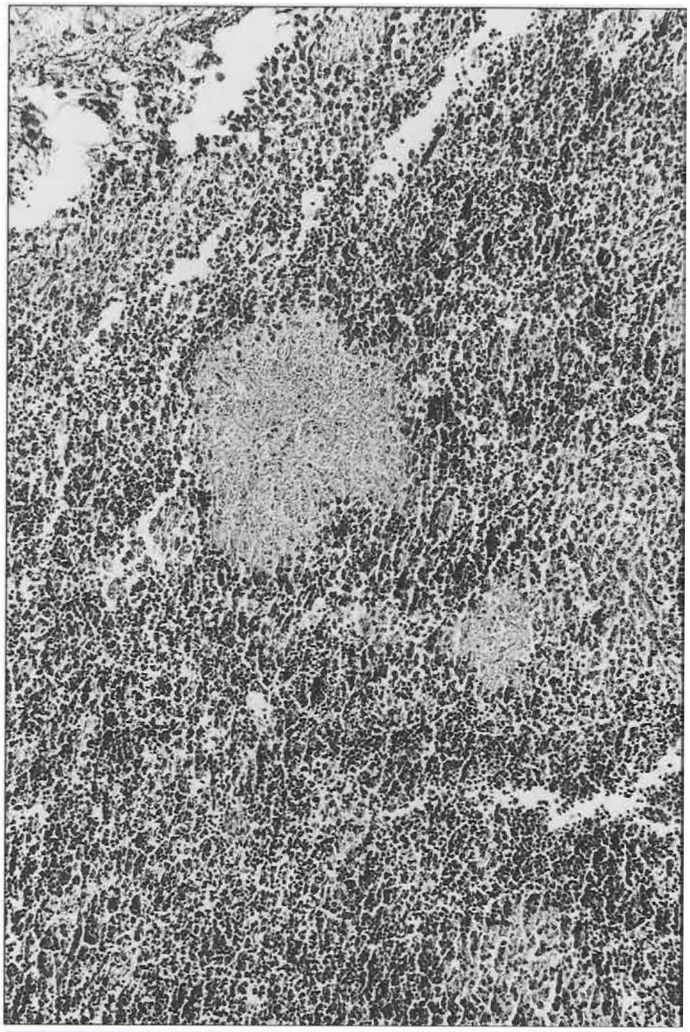

Figure 4.104 Focal coagulative necrosis in spleen of a sheep.

nodes, and muscles. The liver is sometimes swollen and has pinpoint yellow foci on the surface. Histologic lesions in the fetus are modest but characteristic. *There are focal areas of coagulative necrosis in the liver and spleen.* These foci consist of as few as five cells, stain pale pink, and are variably surrounded by a scant infiltrate of mononuclear cells (Figs 4.103, 4.104). Throughout the liver and concentrated in portal areas there is an increase in mononuclear cells. In the lung, alveolar septa are thickened by mononuclear cell infiltrates. Mild meningoencephalitis with vasculitis and hemorrhage has also been reported.

Cattle do carry *Chlamydophila* in the intestine, as do sheep, and the organism in the intestine can produce abortion. Abortions in cattle are usually sporadic, occurring throughout the year with occasional outbreaks in isolated herds. Fetal lesions are similar to those in lambs. *Waddlia chondrophila*, another member of the order *Chlamydiales*, is also responsible for abortion in cattle.

In order to establish *Chlamydophila* as the cause of an abortion in cattle or sheep, the organism should be demonstrated in a smear (preferably of placenta) stained by a modified Ziehl–Neelsen, Gimenez, or Giemsa method. The organism can also be demonstrated in sections from the diseased placenta using a specific fluorescence antibody test or immunohistochemistry. The presence of specific antibody in the fetus is confirmative, but antibody is not always present. Demonstration of antibody in the dam is of little use as a large proportion of cattle and sheep have been exposed and test positive. Specific monoclonals are becoming available to detect antigens of known pathogenic strains of *Chlamydophila*. Eluates, prepared from specimens containing the organism, can be grown on appropriate cell lines on coverslips, fixed, stained, and examined within 3 days. This method is very effective in detecting *Chlamydophila*.

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Abortion in sheep, goats, and cattle caused by *Coxiella burnetii*

Coxiella burnetii is in the family Rickettsiaceae and is the only member of the genus *Coxiella*. The organism is well known as the cause of Q (query) fever in humans. It is an obligate intracytoplasmic parasite. *Dairy cows, goats, and sheep are the common reservoirs of the organism,* and some forms of wildlife may also carry it. They are probably not the source of the organism but acquire it from domestic animals. Human and domestic animal infection occurs when contaminated dust is inhaled; however, massive contamination of a paddock or pasture facilitates oral transmission. Infection apparently persists indefinitely in sheep and cattle, and organisms are shed at parturition and in the milk and feces. Abortion usually follows initial exposure and may be unusual in endemically infected flocks.

Abortion tends to occur late in the gestation period, and weak lambs and kids may be born during an outbreak. The aborted fetus may be well preserved or autolysed. *Gross lesions are confined to the placenta, which is thickened and leathery*, with multifocal to confluent areas of mineralization. The exudate is copious, off-white, and most obvious in the intercotyledonary region (Fig. 4.105). If the cotyledon is involved, the early lesion appears as a white outer ring with flecks of white scattered in the central region. Histologically, there is acute diffuse suppurative placentitis with heavy neutrophil infiltration and extensive necrosis of cotyledonary villi and intercotyledonary epithelium (Fig. 4.106A). In contrast, the cellular infiltrate in the interstitium of the chorioallantoic arcade is largely mononuclear, with a predominance of plasma cells. The lesion, which on gross section appears to be largely confined to the intercotyledonary region, is frequently found on histology to be also extensive in the cotyledons, especially at the periphery. The vasculitis that develops with *Chlamydophila* infections is not usually a feature of placentitis due to *Coxiella*. *Smears of the placental exudate contain large numbers of organisms*, which can be stained by the modified Ziehl–Neelsen or Macchiavello's stain. Invasion of the cells of the chorion by the organism produces microcolonies, which distend the cytoplasm of the infected cells (Fig. 4.106B). These microcolonies can be seen in conventionally stained sections, but must be distinguished from similar colonies that *Chlamydophila* organisms form. With hematoxylin and eosin stains, *Chlamydophila* inclusions tend to stain poorly and appear more homogeneous, whereas with *Coxiella*, cells containing the organism frequently have a characteristic foamy appearance with multiple unstained vacuoles within a pale blue cytoplasm. The nucleus, if visible, is usually pushed against the cell wall and assumes a crescent shape.

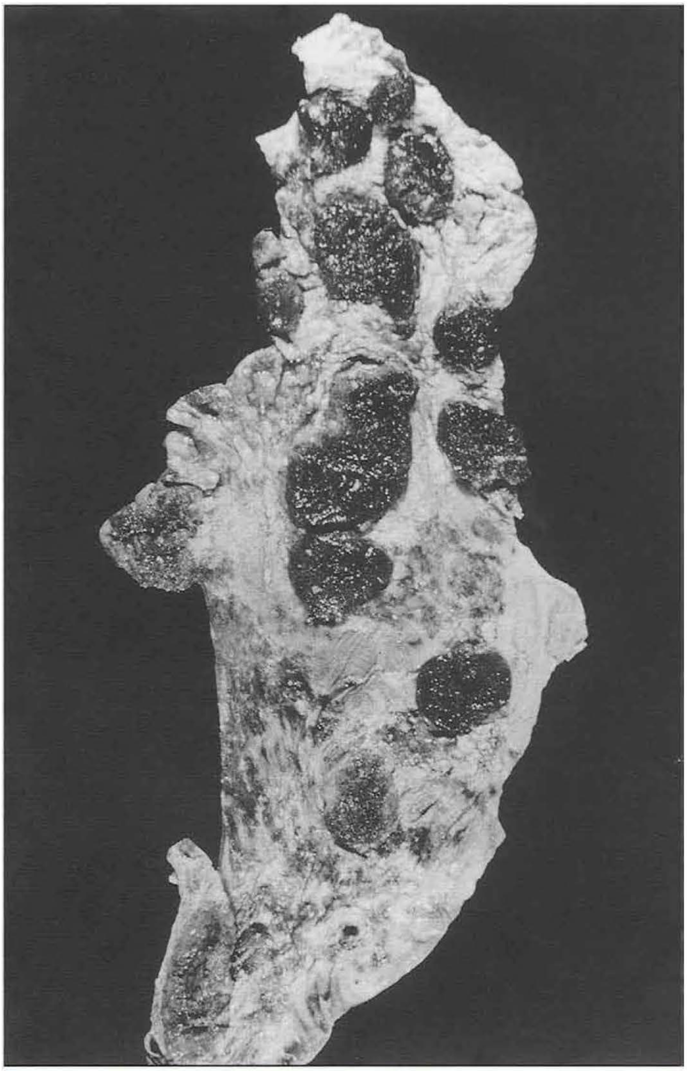

Figure 4.105 Suppurative intercotyledonitis in *Coxiella burnetii* abortion in a goat.

The histologic lesions in affected fetuses are usually modest. Granulomatous hepatitis and nonsuppurative pneumonia with occasional focal lymphoid accumulations around bronchioles have been described. A few lymphocytes and macrophages may infiltrate the renal medulla and surround the portal vessels of the liver.

Bovine fetuses may also be aborted due to *Coxiella* infections. The lesion in the placenta is characteristic and similar to that observed in sheep and goats. Care must be taken in attributing the simple presence of *Coxiella* as sufficient evidence for the cause of abortion. Animals may carry the organism for a prolonged period and, although they appear to abort only once, there may be large numbers of organisms in the placenta in subsequent pregnancies. Assessment of the extent of the placental lesion may be helpful.

Diagnosis is routinely based on the characteristic placental lesions and the appearance of the organism in impression smears stained by a modified acid-fast technique. More definitive methods utilizing direct or indirect fluorescent antibody techniques are available.

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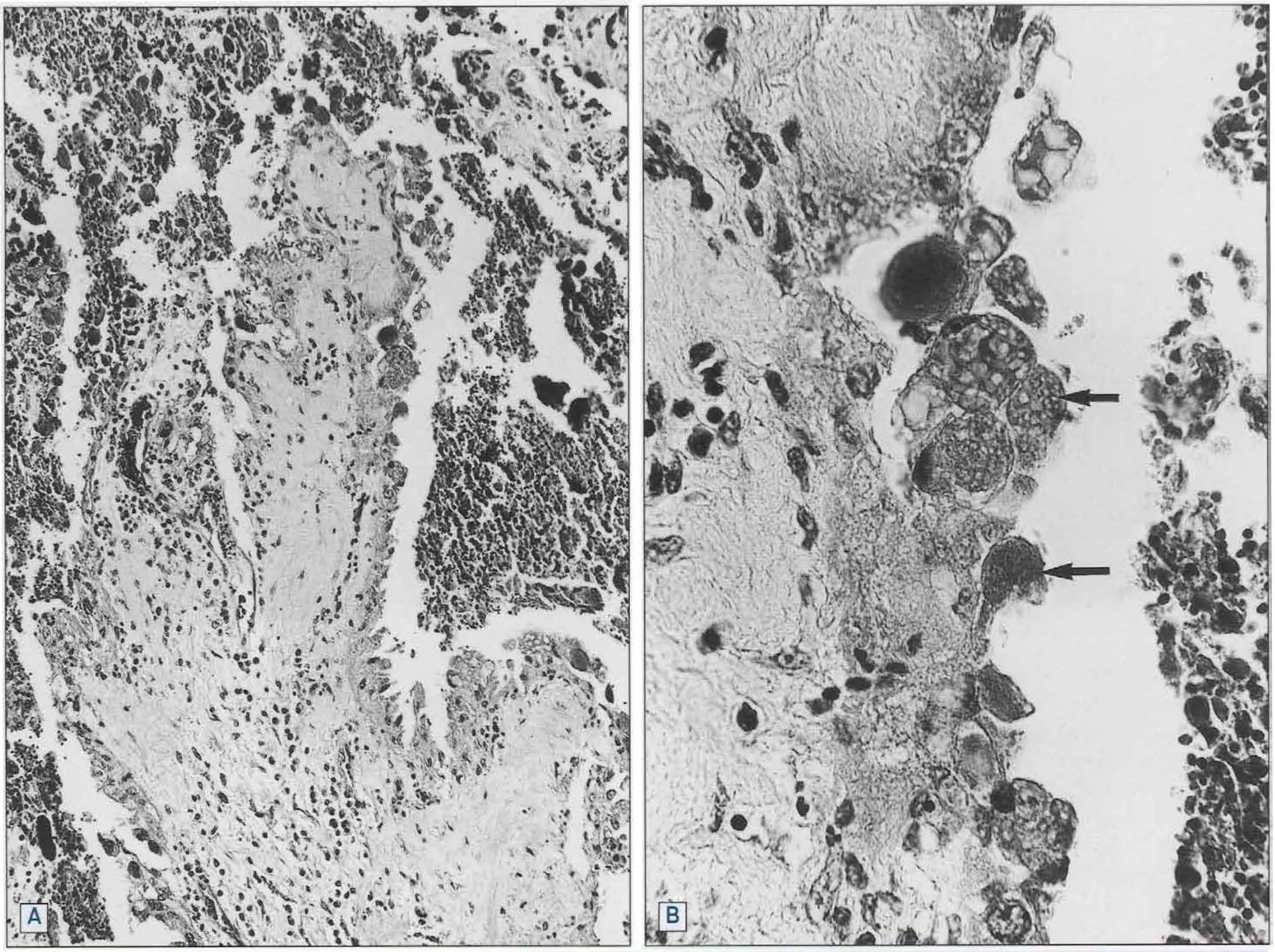

Figure 4.106 A. Suppuration and necrosis of placental villi; plasma cells in the interstitium in *Coxiella burnetii* abortion in a sheep. **B. *Coxiella burnetii*** within trophoblast cells (arrows) in a sheep placenta.

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Mare reproductive loss syndrome and late-term abortions

A syndrome of abortion reached epidemic proportions in the spring of 2001 and to a lesser extent in 2002. It is of major concern to the equine industry in various states but especially in *Kentucky*. Several hundred late-term fetuses were examined. Most were aborted or stillborn at term or several weeks before term, well preserved, and enclosed within the placenta. There were few or no premonitory signs of illness in the mares. Lesions observed in the fetus included hyphema and little or no inflation in the lungs. Hemorrhages were frequently observed on the chorion, amnion, and amniotic segment of the umbilical cord, pleura, and heart. The surface of the amniotic segment of the cord was dull gray to yellow, roughened, and thickened by stromal edema. On microscopic examination, the amniotic cord had bacteria on the surface and where there was loss of epithelium there were light to heavy infiltrations of neutrophils and macrophages. Similar light infiltrates were seen in the stroma of the allantochorion.

Cultures of the fetus and placenta yielded non-β-hemolytic *Streptococcus* spp. and/or *Actinobacillus* spp. in 50% and 20% of the specimens cultured. Similar bacteria were also cultured from lung, stomach content, and placental membranes. Microscopic lesions included funisitis, amnionitis, pneumonia, fetal bacteremia, and sometimes chorionitis. Further investigations supported unusually warm weather and rate of change from cool to warm as major factors, which correlated with increased bacterial growth and *eastern tent caterpillar (ETC)* development. In a controlled study, investigations involving the feeding of ETC to pigs caused abortion. A similar study in mares revealed that abortion occurred only on feeding of the insect exoskeleton, and that hair remnants resembling ETC setae were embedded in the submucosa of the digestive tract of aborting gilts and of an aborting mare. *A factor in or on the ETC cuticle may be responsible for equine abortions.*

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Nocardioform placentitis in mares

This disease, caused by actinomycete bacteria, is primarily found in *central Kentucky,* with a few similar cases reported elsewhere. All ages and breeds of mares are affected. The mare shows no outward signs of illness, except, as with other near-term abortions, premature development of the mammary gland and lactation may occur. Infection is cleared early and rebreeding has not been accompanied by problems. The bacteria involved are *gram-positive branching filamentous organisms,* and include at least three different genera of bacteria: *Crossiella equi, Streptomycin,* and three species of *Amycolatopsis.* No risk factors have been identified, nor has the condition been reproduced.

The lesion in the placenta is somewhat unique in that infection does not seem to start at, nor communicate with, the cervical star. The chorionic surface of the most cranial portion of the body and entrance to the uterine horns is covered with thick brown exudate containing sloughed chorionic cells, neutrophils, and bacteria. The bacteria invade the chorionic epithelium but have not been identified in fetal fluids and organs.

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Miscellaneous infections causing abortion in mares

That group of *bacterial infection* in foals producing the syndromes variously known as joint ill, navel ill, foal septicemia, and pyosepticemia neonatorum can also produce abortions and stillbirths. The implications of this are that these diseases of the newborn can be continuations of intrauterine disease. The organisms involved, in approximately the order of frequency, are: β-hemolytic streptococci, *Escherichia coli, Pseudomonas, Staphylococcus aureus, Klebsiella pneumoniae,* and *Actinobacillus equuli.* These organisms may occasionally reach the chorion by way of the bloodstream of the dam, but most ascend through a patent cervix (Fig. 4.107). This produces inflammatory thickening of the placenta adjacent to the internal os of the cervix.

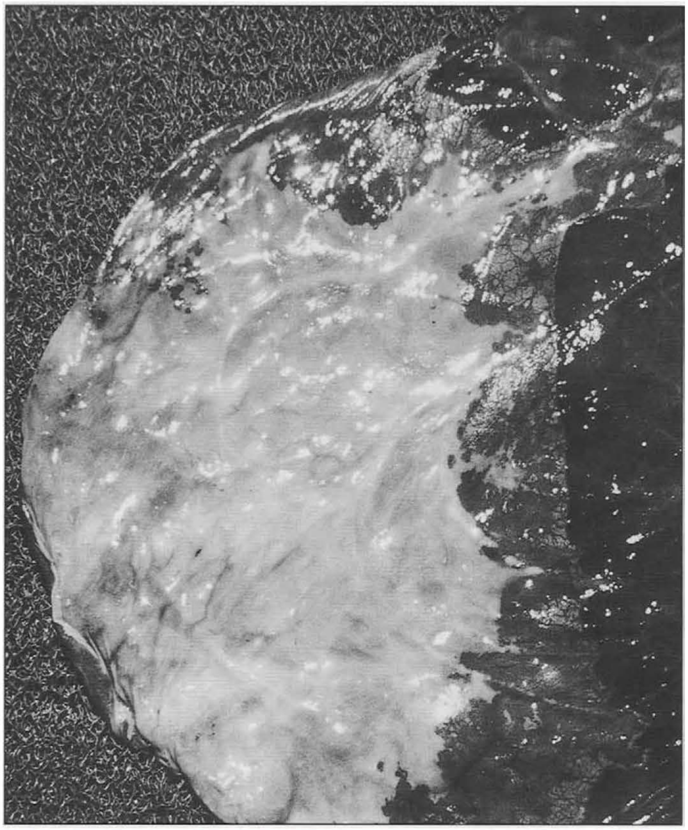

Figure 4.107 Placental thickening following transcervical infection with *Streptococcus* sp. in a mare.

Fungal infections frequently have the same pathogenesis and produce a similar placental lesion with a sticky brown exudate covering the surface of the affected areas of the chorion (Fig. 4.108). *Aspergillus fumigatus* is the most commonly isolated fungus, but *Absidia corymbifera* and other *Aspergillus* species have been recovered.

Actinobacillus equuli is an important cause of neonatal death of foals in most countries. Foals may become infected in utero and be aborted, as discussed above, or they may die of septicemia in the early neonatal period, or they may survive for several days and develop foci of localization in many tissues. It is probable that some of these infections are acquired during or after parturition. Aborted foals and those dying acutely of septicemia do not have distinctive lesions. After a course of 3 or 4 days or more, localization of the infection with miliary microabscessations and polyarthritis develops. The microabscesses, which are embolic in origin, may be found in many organs, and they are readily visible to the naked eye in the renal cortices. The renal abscesses are small, being only 1–3 mm in diameter, and numerous. Histologically, bacterial colonies are obvious in the glomeruli and intertubular capillaries and are enclosed in foci of intense suppuration. The arthritis is fibrinopurulent.

There is little information on the epidemiology and pathogenesis of actinobacillosis. Mares, which are caused to abort by this infection, are not ill, and the organism does not persist for long in the uterus after abortion. However, the same mare may abort successive pregnancies, a fact which suggests that the uterus can be reinfected from some endogenous asymptomatic focus. *A. equuli* can occasionally be found in the intestine and tissues of healthy animals, and also as an

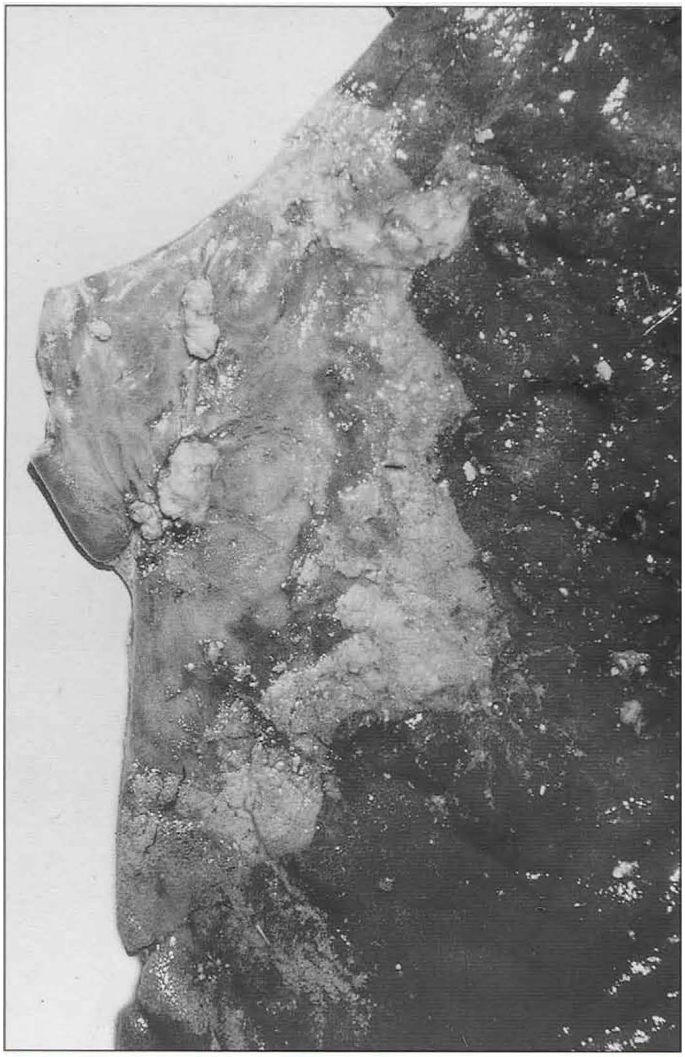

Figure 4.108 Mycotic placentitis following transcervical infection in a mare.

opportunist in pathological tissues such as verminous thromboses. It is, however, seldom of significance in adult animals, although septicemic infections have been observed.

Rhodococcus equi is an important cause of pneumonia and colitis in foals less than 6 months of age, and a rare cause of placentitis and abortion. The fetus may be expelled within the membranes. The cervical star and surrounding region may be red; the lungs are pale and fail to float. *R. equi* may be isolated in pure culture from the placenta, lungs, liver, and stomach content. The lesion in the placenta consists of a perivascular macrophage infiltrate with a few lymphocytes and neutrophils. The macrophages contain numerous gram-positive coccobacilli. Inflammation is most extensive on the allantoic surface. On microscopic examination, there is multifocal hypertrophy and hyperplasia of allantoic epithelial cells, and small numbers of neutrophils and large macrophages are loosely scattered within the amnion. Pulmonary alveoli contain a few granulocytes and many macrophages, some of which are multinucleated.

Encephalitozoon cuniculi is a microsporidial organism that is a rare cause of placentitis and abortion in a wide range of animals, including horses. A Quarter Horse mare aborted a fetus and placenta 6 weeks before term; viscous yellow exudate was scattered over the chorionic surface of the placental body and both horns. In addition, the joints of the foal were swollen and contained tan to red gelatinous material. On microscopic examination, there was necrotizing placentitis and trophoblasts contained large numbers of intracytoplasmic vacuoles that were filled with elongated gram-positive organisms. These were positively identified by polymerase chain reaction and electron microscopy as *E. cuniculi*. No organisms were identified in the joints.

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Mycotic abortion in cattle

Sporadic cases of bovine abortion occur due to infection with a variety of fungal species, with *Aspergillus fumigatus* the most frequent isolate. Zygomycetes (*Absidia, Mortierella, Rhizomucor, Rhizopus*) are also commonly responsible. Mixed fungal infections are relatively common, in which case both septate and nonseptate hyphae may be observed in the placenta. A small number of abortions result from *Pseudallescheria boydii* and yeast (*Candida, Torulopsis*) infections. The portal of entry is not known, but the initial development of lesions in placentomes indicates *hematogenous arrival*. Extension from either respiratory or rumen infections is possible. With the exception of *Rhizopus*, these organisms are well-known secondary pathogens. Abortion occurs late in gestation, between the sixth and eighth month, and the placenta is often firmly retained.

The fetus may appear normal, but often there are characteristic cutaneous lesions in the form of irregular elevated plaques resembling ichthyosis or extensive ringworm. These lesions are seen most commonly about the periorbit, occiput, shoulders, back, and sides (Fig. 4.109). Affected

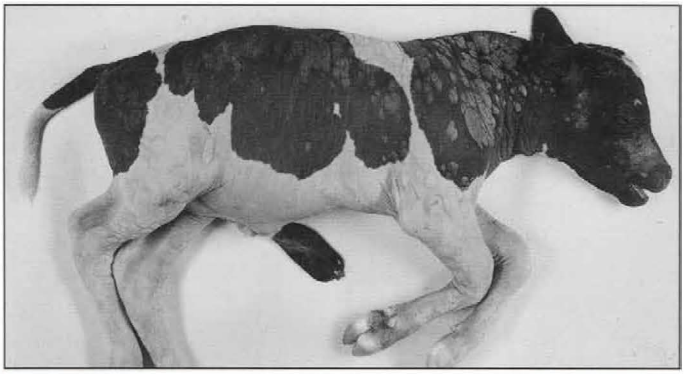

Figure 4.109 Mycotic dermatitis in a fetal calf in mycotic abortion from a cow.

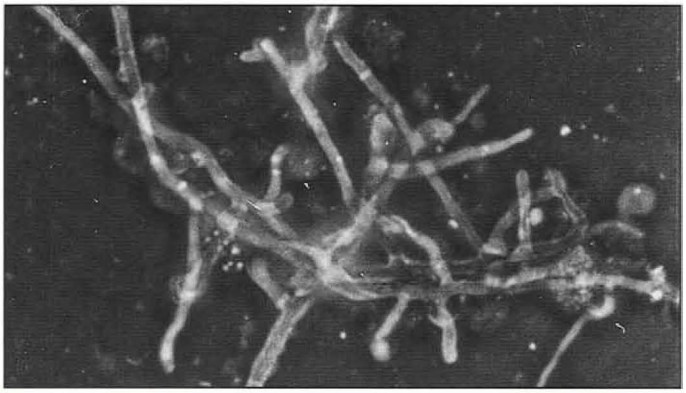

Figure 4.110 *Aspergillus* sp. in smear from fetal stomach (phase-contrast microscopy) in mycotic abortion from a cow.

areas are slightly elevated, gray, and irregular in outline and tend to coalesce. Histologically, the infection is seen to be superficial, involving the epidermis mainly, with parakeratosis, and edema and inflammatory cell infiltration of the underlying dermis. Sometimes the fungus invades the hair follicles and dermis but, with the exception of an occasional case of bronchopneumonia, there are no lesions in the internal organs. The fungus can be isolated from the stomach contents (Fig. 4.110). *The placental lesions are remarkable.* The gross appearance resembles that seen in brucellosis but is often much more severe. The allantois-chorion is then leathery with extensive superficial necrosis. The placentomes are greatly enlarged and necrotic with swollen margins, and are firmly incarcerated (Fig. 4.111). *The infecting organisms are readily demonstrated in the necrotic tissue*, and typically extend along the blood vessels to produce necrotizing vasculitis.

Lesser degrees of placentitis are probably quite common, with the lesions restricted to placentomes as areas of hemorrhage, softening, and necrosis (Fig. 4.112). The infection appears to begin in the placentomes, with later spread to the interplacental space between the cotyledons, where a rich growth of hyphae occurs. With placentitis of lesser degree, there are usually no cutaneous lesions in the fetus, although the fungi can be found in the gastric contents. The endometrial lesions are less severe than those in the placenta. Secondary infections may follow retention of the placenta. The majority of cows recover sufficiently after abortion to allow subsequent pregnancies to be carried to term, but in some, endometrial destruction is severe.

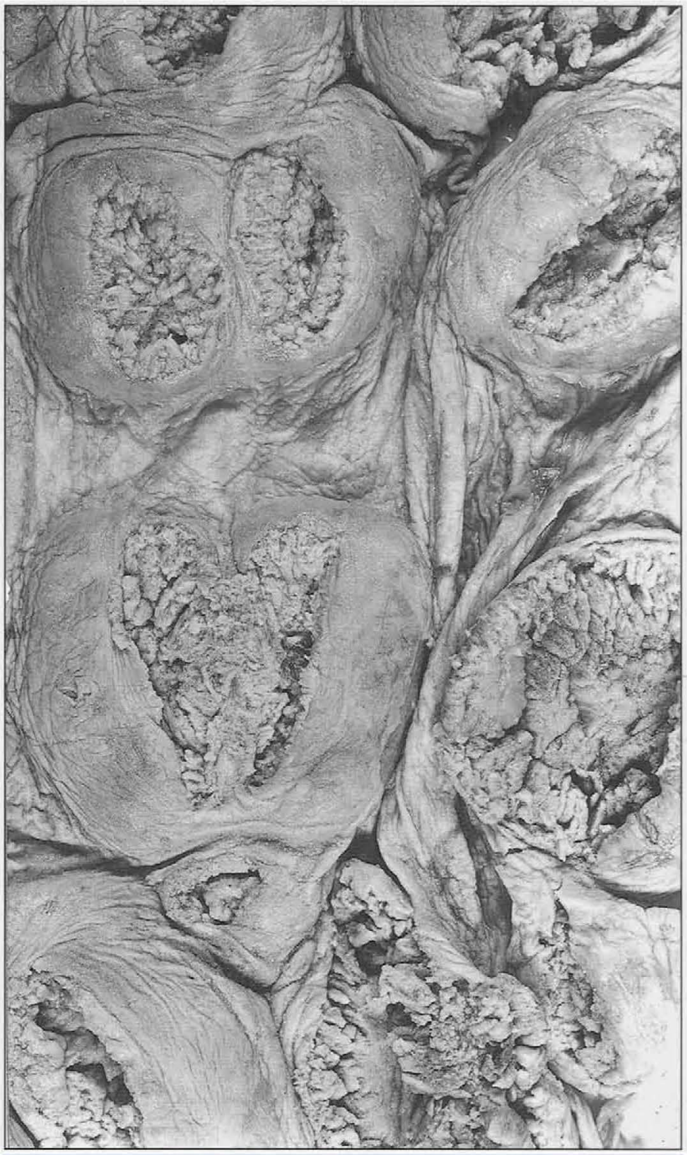

Figure 4.111 Mycotic placentitis, bovine.

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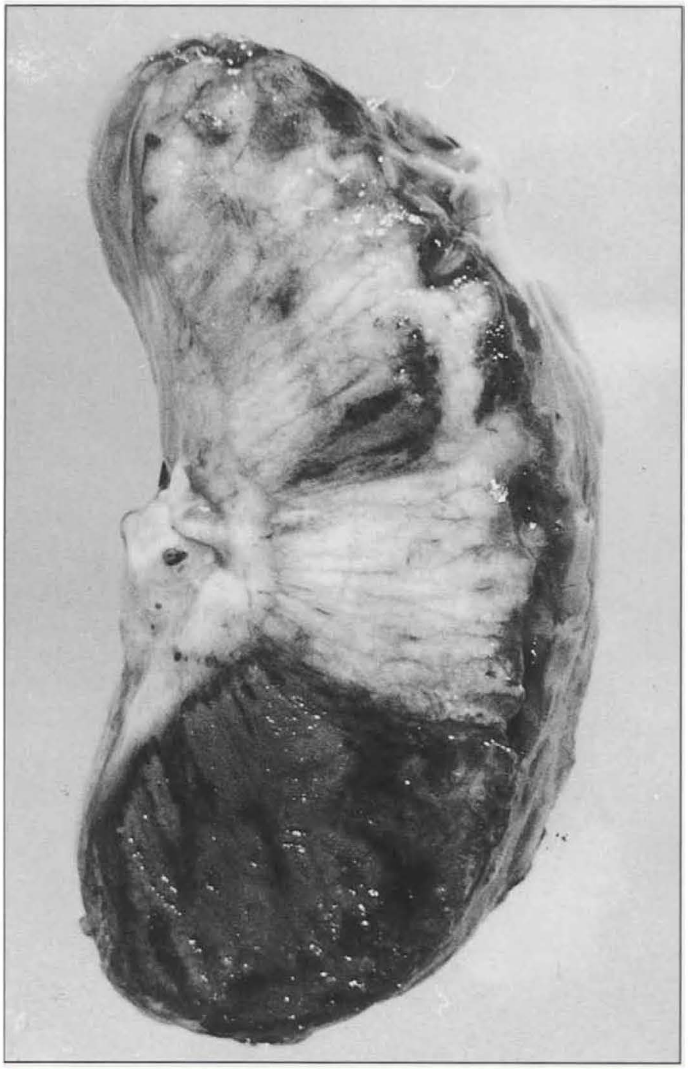

Figure 4.112 Infarction of a placentome in mycotic abortion from a cow.

Epizootic bovine abortion

This disease is a tick-borne infection of cattle that produces chronic fetal disease and abortion. The name is a misnomer, as epizootic bovine abortion (EBA) is actually provincial and endemic. Its distribution is apparently limited by that of the vector, the argasid tick *Ornithodoros coriaceus*. The precise geographic range of the tick is not known. It is known to inhabit brushy foothills of California and adjacent areas of Nevada, Oregon, and northern Mexico. The most common hosts of the tick are cattle and deer. The tick is presumed to transmit an agent from the deer to cattle, as the infection remains endemic in ranges on which no cattle have been grazed. *The cause of EBA is unknown; the primary suspect is the spirochete Borrelia coriaceae.* Although this spirochete has been detected in salivary glands and other tissues, in a high percentage of adult *O. coriaceus* ticks in areas endemic for EBA, isolation attempts have been unsuccessful. The search in the infected fetus has narrowed on the thymus primarily.

Cattle exposed to the ticks for the first time are primarily at risk. *The infection produces no recognizable clinical signs in cattle of any age, but if the animals are pregnant, especially at 2–6 months, the infection is passed to their fetuses, where a chronic disease develops.* The fetus at this stage of gestation has not fully developed a functional immune system. This, coupled with a cow that has not been previously exposed to the agent, allows the agent to cross the placenta to the fetus and initiate chronic infection. There is a 3-month or longer incubation period between the exposure of the dam to ticks, development of the entire spectrum of lesions, and death and abortion of the fetus. Since the lesions gain their specificity only during the latter part of this prolonged period, the disease can only be confidently diagnosed in those animals exposed before the last trimester.

Not all diseased fetuses are aborted. The fetus is likely to have developed a functional immune system by 7–8 months of gestation, and may not be aborted if infected by the agent in that time period. Instead, the calf may not die from the infection but be born weak. *Weak calves* are often seen associated with outbreaks of abortion in this disease. Cows that abort due to the disease are not usually ill and the placenta is shed without difficulty. Cattle develop immunity and do not abort even if they are re-exposed to the ticks and the agent they carry. If there is little movement of animals, abortions may be rare in endemic areas. The disease becomes a major cause of abortion in a herd only when pregnant nonimmune animals are moved into an area where the infection is endemic.

The fetuses affected by the disease are most often aborted during the last trimester. Most trigger their own deliveries and die either during delivery or shortly thereafter. Only rarely do they die in utero and undergo autolysis. Many, but not all, affected fetuses have characteristic abdominal distension due to ascites, which is a striking, but inconstant, lesion. Lymph nodes throughout the carcass are enlarged, usually impressively so. The normal superficial cervical lymph node of a term bovine fetus weighs between 3.5 and 7.0 g. Fetuses aborted in this disease have cervical lymph nodes that weigh 16 g or more. The prescapular lymph node is especially easy to see beneath the skin immediately in front of the shoulder blade. The spleens of affected fetuses are similarly enlarged; the thymus, in contrast, is reduced in size.

Petechial hemorrhages are regularly seen in the conjunctival and oral mucosa as well as in the mucosa of the trachea. The thymus is often embedded in massive hemorrhages and edema. These hemorrhages are, at least in part, traumatic, developing during parturition, the portion of the thymus within the thoracic cavity being spared. The *enlarged, coarsely nodular liver* is, when present, an impressive gross change in fetuses affected by EBA (Fig. 4.113). However, the lesion is not present in all diseased fetuses, and similar hepatic congestion with ascites occurs in fetuses with cardiac anomalies. Many aborted fetuses breathe, and the lungs of these are partially aerated. Small gray foci of inflammation can be seen in a variety of tissues, but are most conspicuous in organs such as kidney and heart that provide a dark background.

The changes that develop in the lymphoid organs are the most specific and diagnostic. *The enlargement of lymph nodes and spleen consists of remarkable lymphoid and mononuclear cell hyperplasia.* Well-defined secondary follicles develop in the cortical and paracortical regions of the lymph nodes. The sinuses are stuffed with macrophages that form sheets in the medullary areas (Fig. 4.114). The spleen shows a similar hyperplastic response involving lymphocytes of the follicles and periarterial sheaths; there is also widespread infiltration of macrophages. Foci of acute necrosis are often superimposed on the chronic proliferative change (Fig. 4.115).

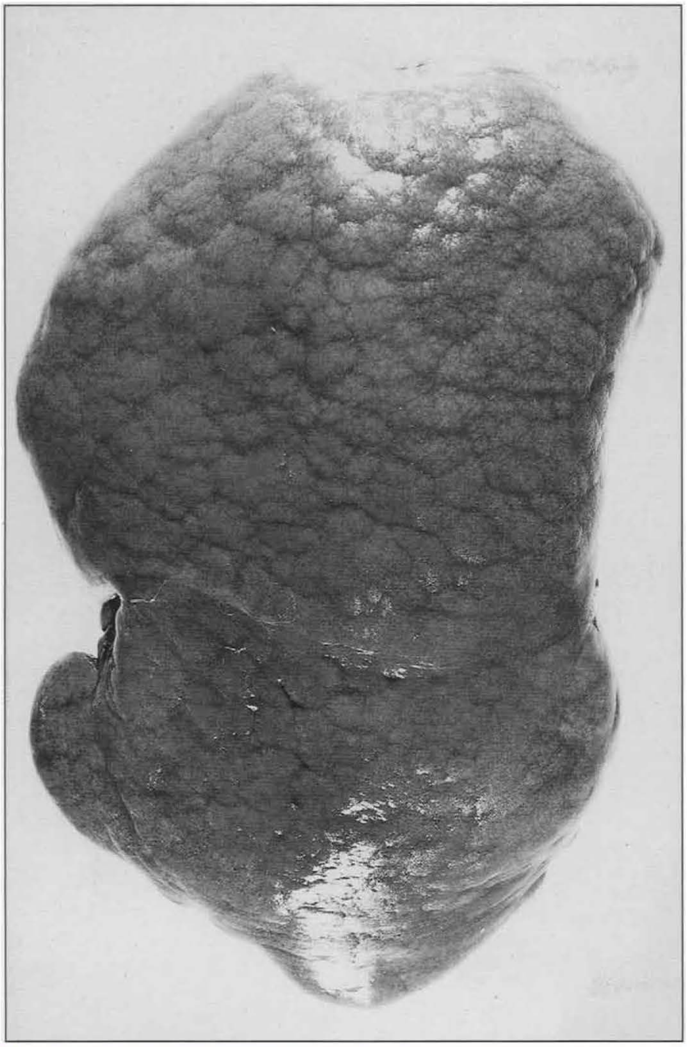

Figure 4.113 Congestive nodularity of liver in an aborted fetus, in epizootic bovine abortion.

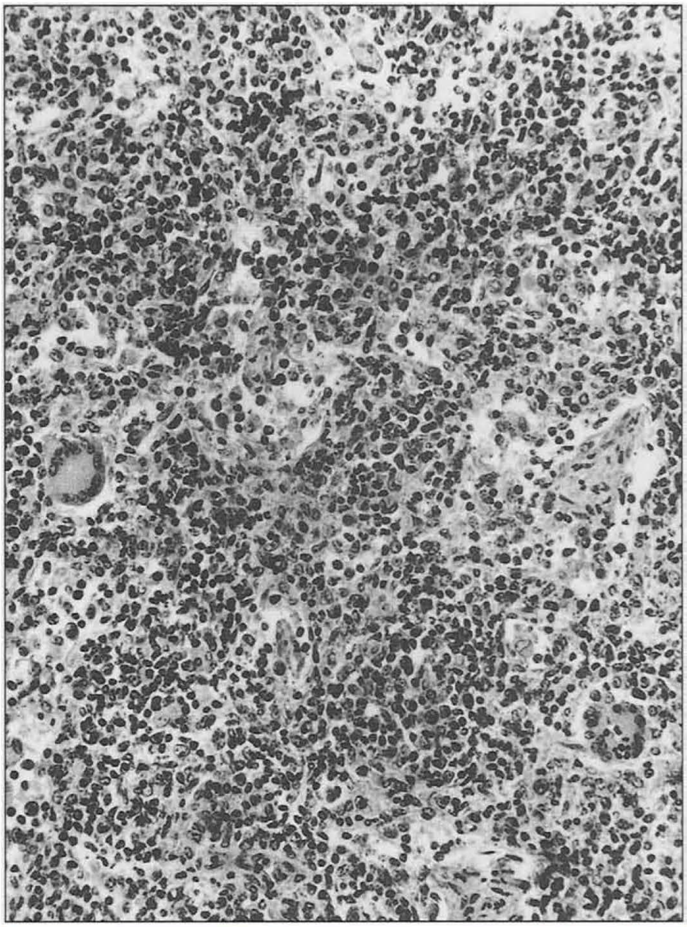

Figure 4.114 Proliferative lymphadenitis with giant cell formation in an aborted calf, in epizootic bovine abortion.

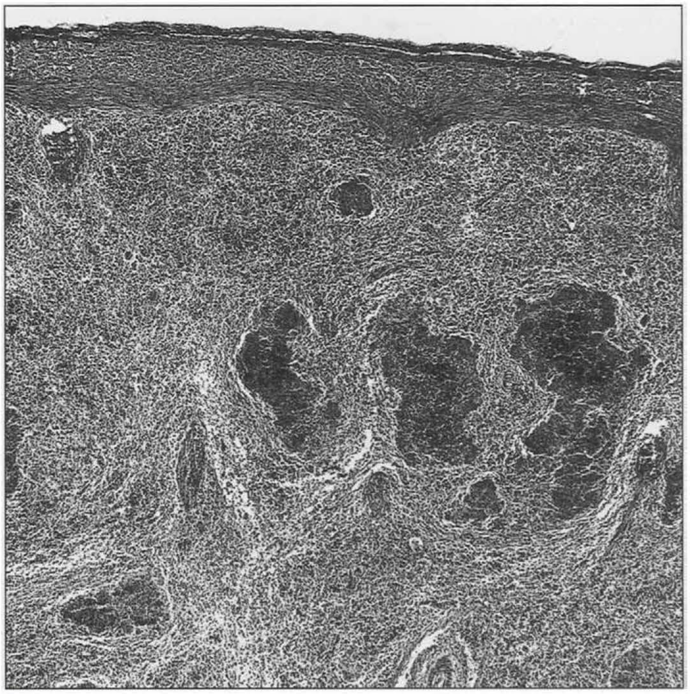

Figure 4.115 Focal areas of **acute necrotizing splenitis** in a fetus, in epizootic bovine abortion.

The most distinctive fetal lesion is a *remarkable thymic inflammatory change*. The cortical mantle of thymocytes is greatly reduced, and macrophages diffusely infiltrate both the medulla and the septa of the gland (Fig. 4.116). The central veins of the liver are distended and the liver plates thinned. Mononuclear cell infiltration is present around portal vessels. Foci may be 100 μm in diameter and are often granulomatous in appearance.

Affected fetuses may have vasculitis involving small- and large-caliber vessels in any, or all, organs (Fig. 4.117). Lesions that are particularly useful in establishing the diagnosis are regularly present in the lung and brain. The alveolar walls of the lung are thickened, and granulomatous inflammatory foci are present in the septa. In the brain, foci of vasculitis are scattered throughout and the meninges are thickened by granulomatous inflammation (Figs 4.118, 4.119).

Lesions do develop in the placenta but are usually mild and involve the loose connective tissue. They contrast with the acute severe inflammatory reaction of the chorionic surface seen in bacterial and *Chlamydophila* infections.

Diagnosis of EBA is based on a combination of gross and histologic findings in an aborted fetus in the geographical area endemic

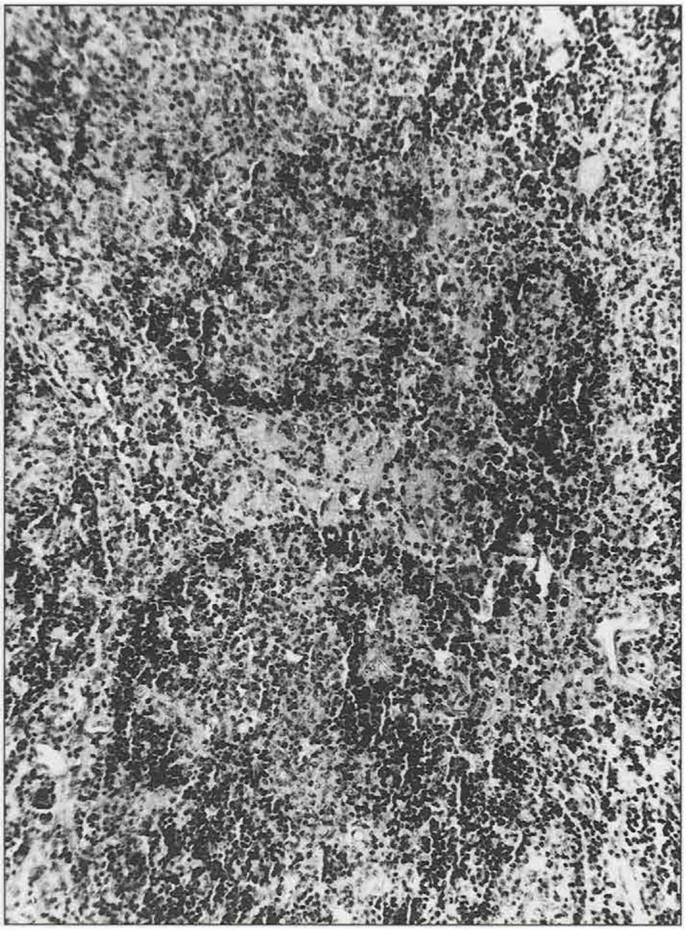

Figure 4.116 Thymitis with distortion of thymic architecture by infiltrating macrophages in epizootic bovine abortion in a fetus.

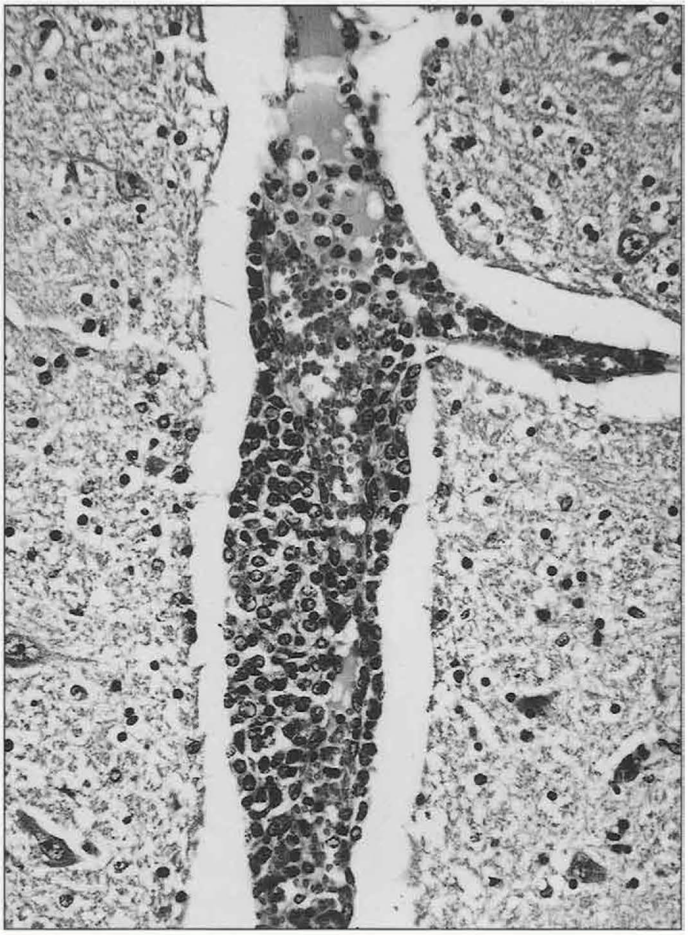

Figure 4.118 Cerebral vasculitis in a fetus, in epizootic bovine abortion.

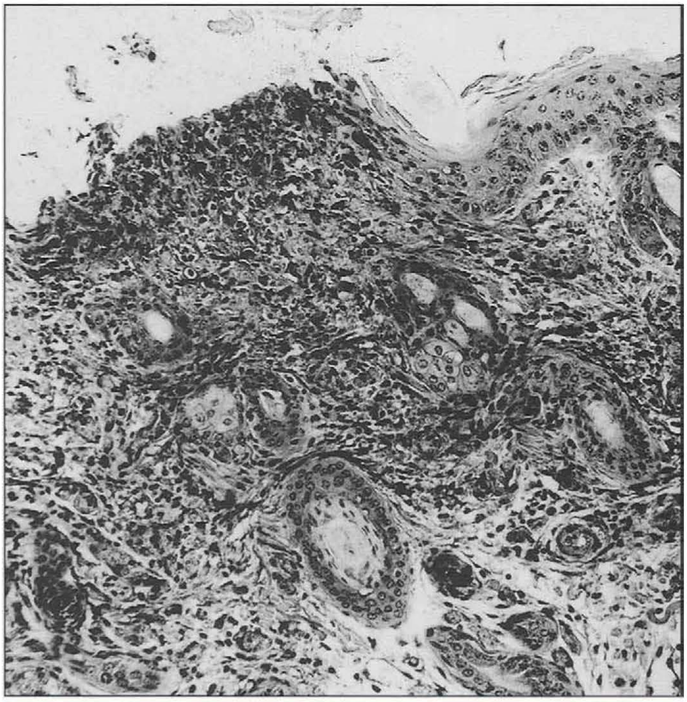

Figure 4.117 Focal necrotizing dermatitis, in epizootic bovine abortion.

for *O. coriaceus* ticks. Antibody titers to borrelia must be interpreted carefully, as cross-reactivity exists among antibodies to *Borrelia theileri*, *B. burgdorferi*, and *B. coriaceae*.

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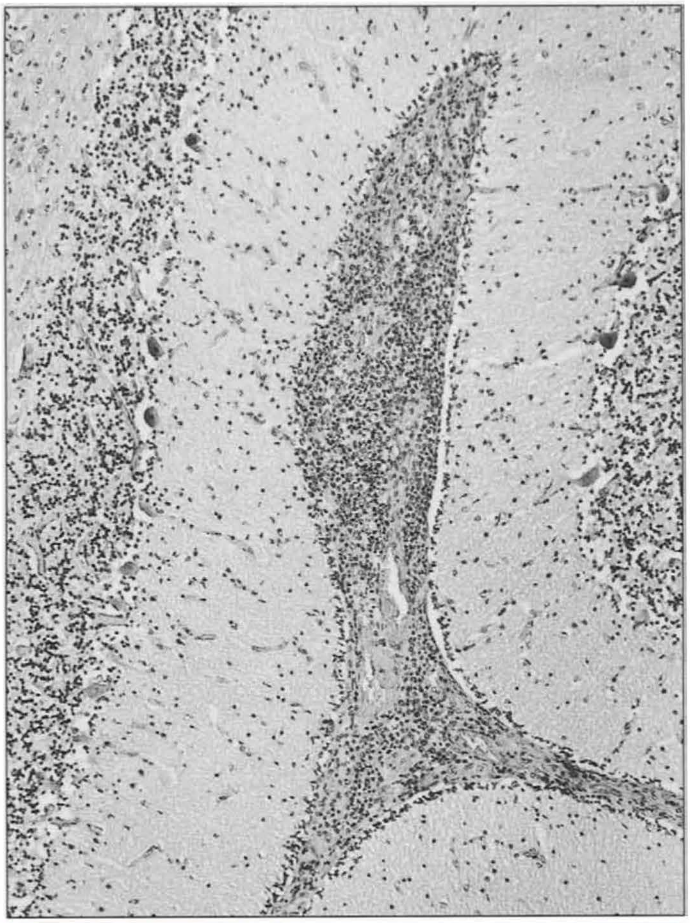

Figure 4.119 Granulomatous meningitis, in epizootic bovine abortion.

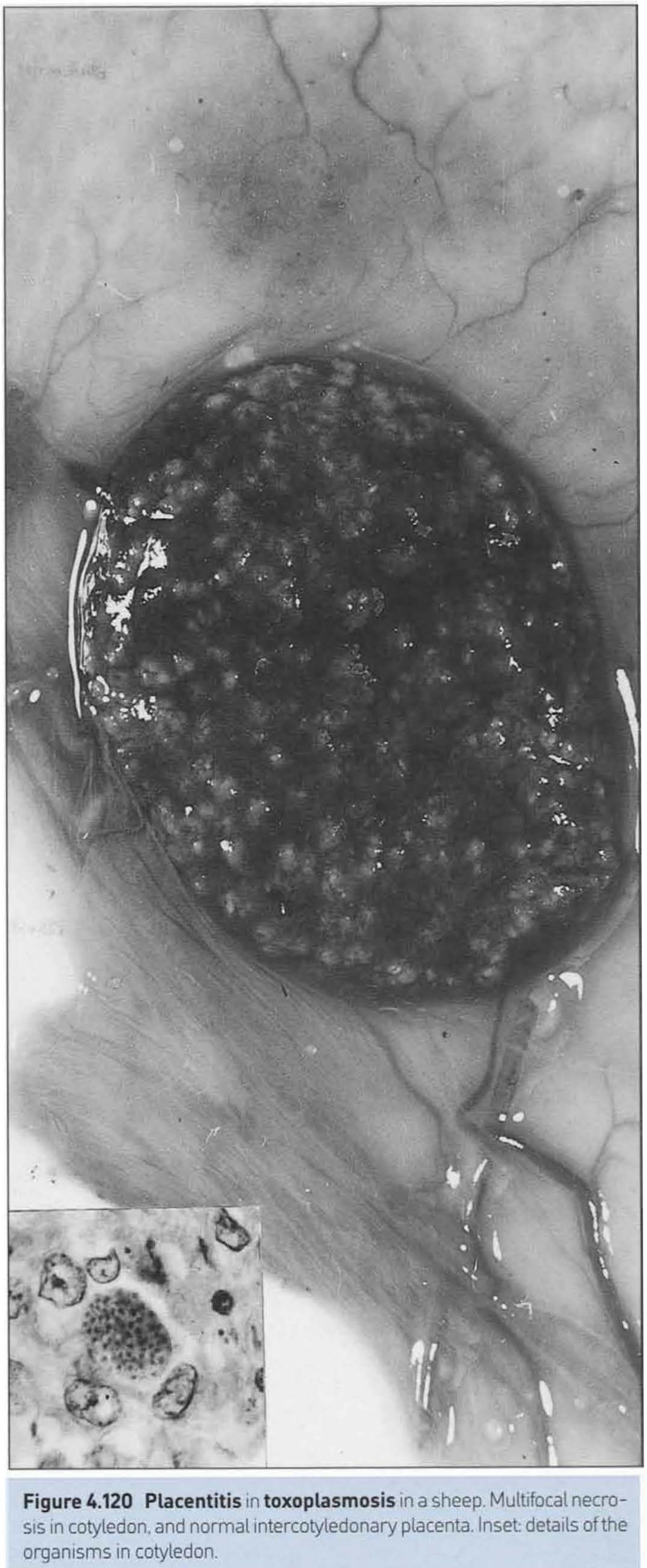

Figure 4.120 Placentitis in **toxoplasmosis** in a sheep. Multifocal necrosis in cotyledon, and normal intercotyledonary placenta. Inset: details of the organisms in cotyledon.

Protozoan infections causing abortions

Toxoplasmosis

Infection with ***Toxoplasma gondii*** appears to be very common in many species, but the disease toxoplasmosis is much less common and its diagnosis is still somewhat of an event. The present discussion is limited to genital manifestations. A more complete discussion of the disease is given with coccidial disease of the intestine (see Vol. 2, Alimentary system).

Toxoplasma is second only to *Chlamydophila* as a cause of fetal death and resorption, abortion, or stillbirth in sheep, and is also an important abortifacient in goats; both pathogens may occur simultaneously in the same abortion outbreak. Animals that are particularly at risk of *Toxoplasma* abortion are those moved late in pregnancy to areas heavily contaminated with cat feces. This occurs most often when ewes are housed in barns. Under such circumstances abortion rates can be high. Although *Toxoplasma* can cause abortion, congenital transmission from ewe to lamb also occurs in a high percentage of normal healthy lambings.

The ewes and does show no signs of infection. The aborted lambs and kids show no significant gross lesions; in only a few are lesions and parasites demonstrable histologically in myocardium, lung, and brain. Irregular small or large foci of leukomalacia are common in the hemispheral white matter. *The fetal placenta bears what are probably characteristic lesions*. The cotyledons are bright to dark red as compared with a normal deep purple color; scattered amongst the fetal villi are *numerous white flecks or small soft white nodules* 1–3 mm in diameter (Fig. 4.120). The villi are edematous, and there is focal necrosis and desquamation of trophoblastic epithelium. In some there may be caseous and calcified cotyledonary nodules. The organism is identified either free or in pseudocysts. The intercotyledonary placenta shows edema only. Encysted organisms can frequently be found in the endometrium. It may be assumed that placental infection can occur at any time the infection is active and the organism is in the proliferative phase.

Cattle rarely, if ever, abort due to toxoplasmosis. Although the organism may be present in cattle, it does not appear to cause disease. *Toxoplasma* has been reported to cause abortion in *swine*, with fetal lesions, but this appears to be an uncommon event.

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Neospora infections causing reproductive failure in cattle, sheep, dogs, and cats

Neospora is a newly recognized protozoan parasite belonging to the class Apicomplexa. *N. caninum* was first isolated from a litter of puppies, and domestic dogs are the only recognized definitive hosts of the parasite. *It is a major cause of abortion in both dairy and beef cattle throughout the world.* Although observed in tissues from aborted bovine fetuses for many years, the organism was first associated, and in 1987 identified, as the cause of an abortion storm in cattle in New Mexico. It differs morphologically and antigenically from *Toxoplasma* and *Sarcocystis*.

The organism can cause severe neuromuscular paralytic disease in dogs and cats. Experimentally induced infections in pregnant dogs and cats lead to in utero death and birth of infected offspring, some of which die while others remain normal. Orally induced infection in nonpregnant cats subsequently bred results in clinically normal young, born infected with the parasite.

In **cattle**, the natural route of infection is probably by consumption of oocysts excreted in the feces of dogs and other canids. Eating infected carcasses or placentas from infected fetuses may infect canids. Most reports of abortion involve dairy cattle and particularly cows on drylot. This is believed to be associated with the density of population and methods of feed storage providing increased opportunity for contamination, transmission, and spread of the organism. Beef cows however also abort. Conception does not seem to be affected; however, animals have been reported to abort with *Neospora* in three sequential pregnancies. Abortion storms, where fresh or autolysed fetuses are aborted, may be followed by the birth of mummified fetuses for several months. Animals aborting in one year may or may not abort in the next gestation. Herd investigations have

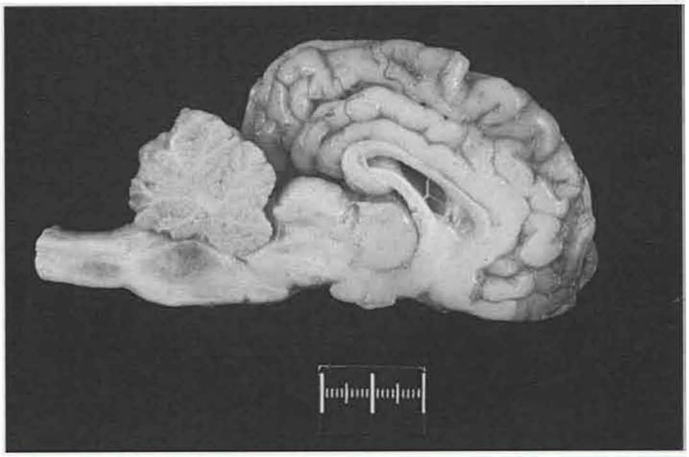

Figure 4.121 Focal malacic lesions in a fetal bovine brain, due to *Neospora* infection.

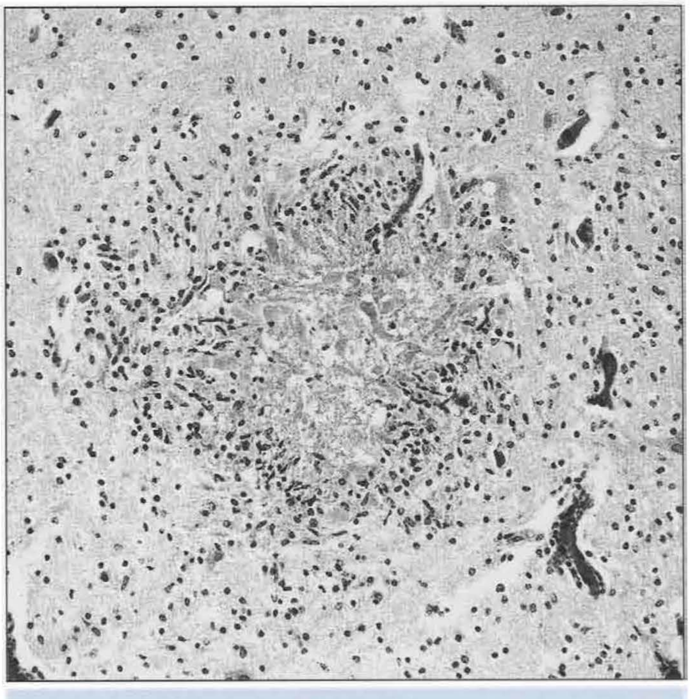

Figure 4.122 Focal necrosis surrounded by narrow zone of glial cells in fetal bovine brain, due to *Neospora* infection. (Courtesy of Barr BC and Vet Pathol 1990;27:354–361.)

shown that 23% of pregnant cows and heifers abort during the first year of infection. Although protective immunity develops in infected cows, this immunity does not prevent transplacental infection, and seropositive cows are more likely to abort than are seronegative cows. Seronegative cows probably always produce seronegative calves. Most *Neospora*-induced abortions occur at 5–6 months of gestation; this mid-gestation abortion frequency is distinctive from other kinds of abortion in dairy cattle. Pregnancy-induced immunosuppression is a possible, but unproven, cause of reactivation of latent *Neospora* infections. The economic impact of *N. caninum* infection in dairy cattle extends beyond that from abortion to include premature culling due to the diminished milk production that occurs in seropositive cows. Cows infected with *Neospora* are usually not clinically ill, nor do they have an increased risk of dying compared to seronegative cows.

Congenitally infected full-term calves may not show signs of disease, but still have a high titer to the organism. *Neospora*-infected calves may be born small-for-gestational-age, unable to rise, ataxic, or have no clinical signs. Fore- and hindlimbs may be flexed or hyperextended; there may be loss of conscious proprioception.

On necropsy, *there are often no useful identifying gross lesions*. Bovine fetuses are often expelled in the second or third trimester and may be fresh, autolysed, or in early stages of mummification. In the placenta, the cotyledons are necrotic and the intercotyledonary region is normal. Rarely, there are scattered tan-to-gray soft foci visible in the brain, brainstem (Fig. 4.121), skeletal muscle, and heart. These consist of *necrosis, mononuclear cell infiltrates, perivascular cuffing, and sometimes mineralization* (Fig. 4.122). Cysts may be difficult to find or conspicuous, and are probably more common in spinal cord than brain. *N. caninum* invades many different cell types and tissues in the fetus (Fig. 4.123). The tachyzoites proliferate intracellularly by endodyogeny; massive proliferation leads to host cell lysis, and newly formed parasites are released to invade neighboring cells.

Neospora tachyzoites may be identified in focal brain lesions; however, *free protozoa are difficult to recognize and are most easily identified using immunohistochemistry*. Intracellular zoites may be seen in myocytes and Purkinje fibers in myocardium. Less consistent lesions include multifocal hepatic necrosis (Fig. 4.124), focal nonsuppurative interstitial nephritis, interstitial pneumonia, and adrenal adenitis. Large numbers of *N. caninum*-like zoites are rarely observed in poorly defined cysts within trophoblasts, and more often no significant lesions are seen in the placenta. Mononuclear cell infiltrates may be associated with areas of focal necrosis in the cotyledons. *Neospora*, although rarely identified in placenta by immunohistochemistry, may be positive on polymerase chain reaction.

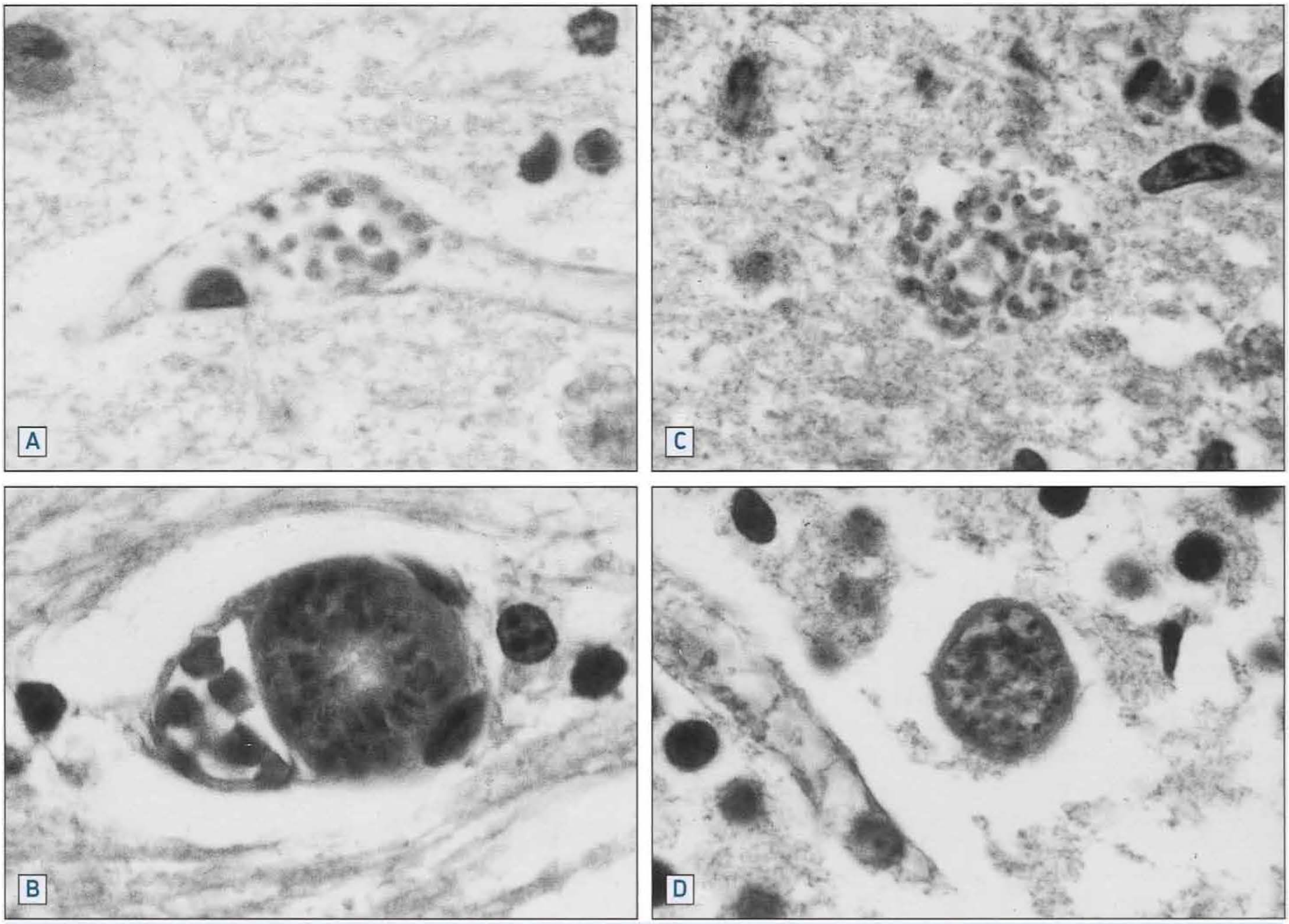

Figure 4.123 **A.** Cluster of ***Neospora* zoites** in fetal brain capillary. **B.** Cluster of *Neospora* zoites within an endothelial cell. **C.** Cluster of *Neospora* zoites lying free in brain parenchyma. **D.** Cluster of *Neospora* zoites surrounded by cyst-like wall in brain parenchyma. (Courtesy of Barr BC and Vet Pathol 1990;27:354–361.)

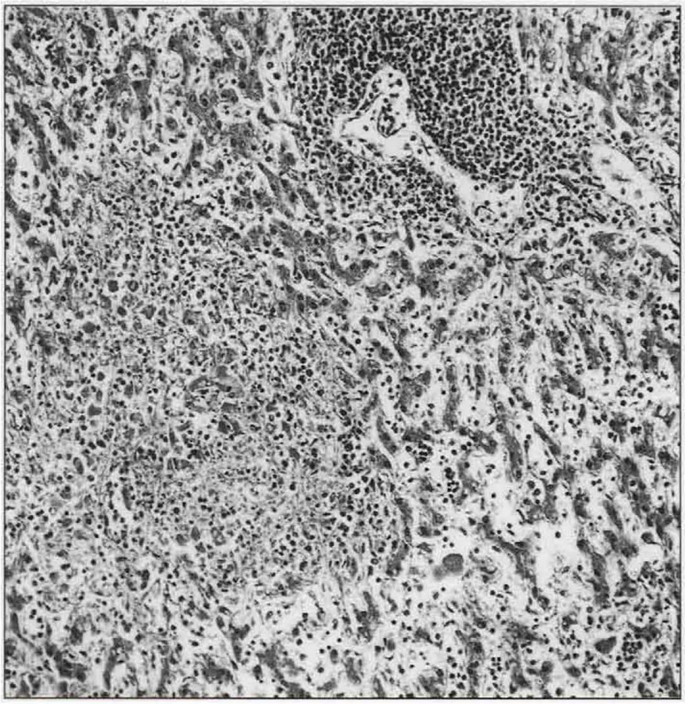

Figure 4.124 Focus of **hepatic necrosis** (below, left) and mononuclear cell infiltrate in portal area in a bovine fetus, aborted due to *Neospora*. (Courtesy of Barr BC and Vet Pathol 1990:27:354–361.)

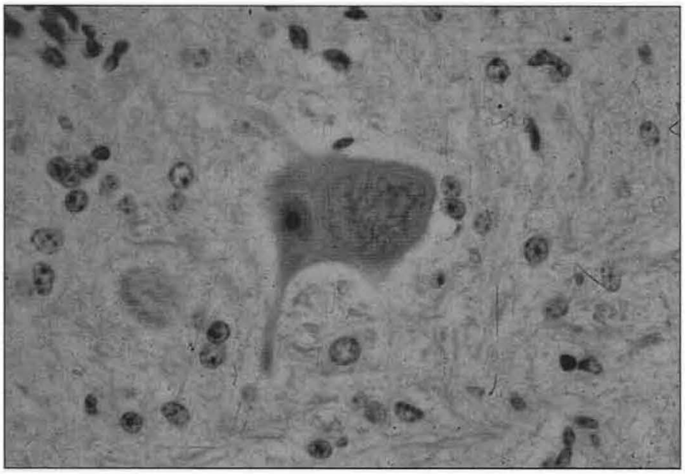

Figure 4.125 Immunohistochemically stained section of fetal bovine brain demonstrating ***Neospora*** organisms in a neuron. (Courtesy of Debbie Haines.)

On light microscopy, *N. caninum* can be distinguished from cysts of *Toxoplasma* and *Sarcocystis*, as the cyst wall is slightly thicker. *All stages can be differentiated from other protozoans using immunohistochemistry*, which is successfully used to demonstrate protozoa in brain (Fig. 4.125), spinal cord, lung, kidney, heart, and skeletal muscle of fresh or autolysed fetuses and also, but with more difficulty, in mummified fetuses. Isolation of *Neospora* from autolysed tissues in cell cultures is rarely successful because of the effects of autolysis. Tissue cysts may be rare in in utero-infected fetuses; however, serology on fetuses aborted at greater than 5 months' gestation has proved useful in detecting antibodies to *Neospora*. Maternal antibodies to *Neospora* may also be detected but decline within 2–5 months of abortion.

N. caninum has also been identified as a cause of abortion in **sheep** and possibly in **goats**. Lesions appear to be similar in bovine and ovine aborted fetuses, and clinically affected bovine, ovine, canine, and feline neonates. The role of *Neospora* (*N. caninum* or *N. hughesi*) in **equine** abortions is under investigation.

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Sarcocystis species causing abortion in cattle, goats, sheep, and pigs

Sarcocystis species cause abortion, stillbirth, and neonatal deaths in cattle, goats, sheep, and pigs. In contrast to some of the other protozoa, they are usually specific for their intermediate host and may be named using the intermediate and definitive host in the species name; for example, *Sarcocystis bovicanis (*syn. *S. cruzi)*, *Sarcocystis capracanis (*syn. *S. tenella)*. Transmission, pathogenesis, lesions, and diagnosis are similar in all intermediates, and only lesions observed in cattle will be described. Ingesting food or water contaminated by feces containing oocysts, which are immediately infective to the intermediate host, infects the animal. Definitive hosts include the dog, coyote, wolf, red fox, and raccoon for the pathogenic organism *S. bovicanis*.

The pathogenesis of *Sarcocystis*-induced abortion is uncertain, but may involve anemia in the dam leading to fetal hypoxia and death,

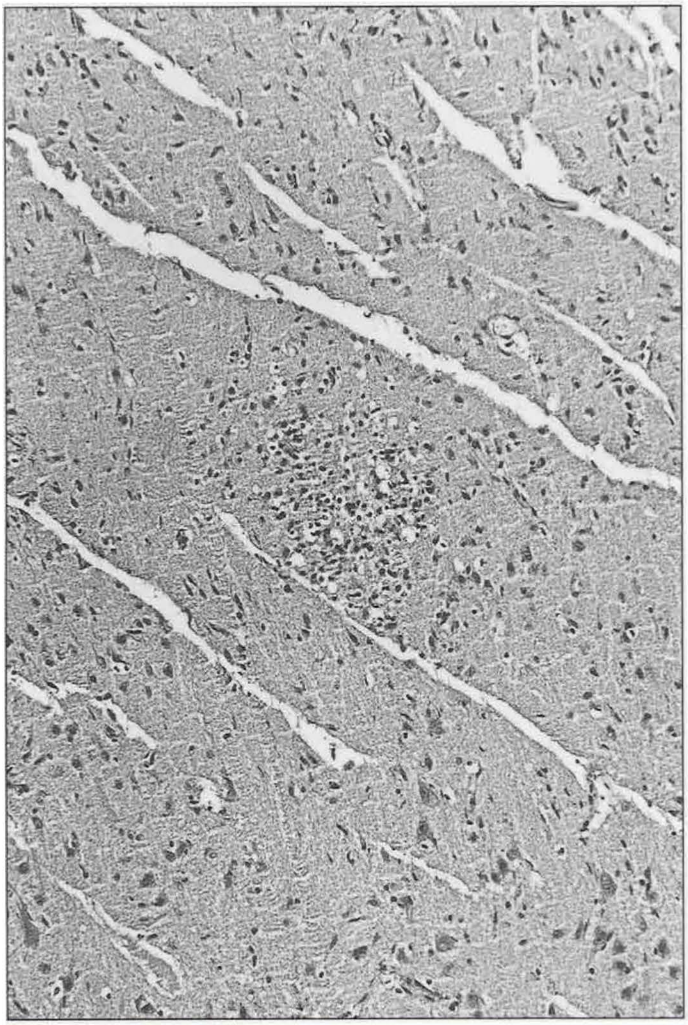

Figure 4.126 Nonsuppurative encephalitis in *Sarcocystis* abortion of a bovine fetus.

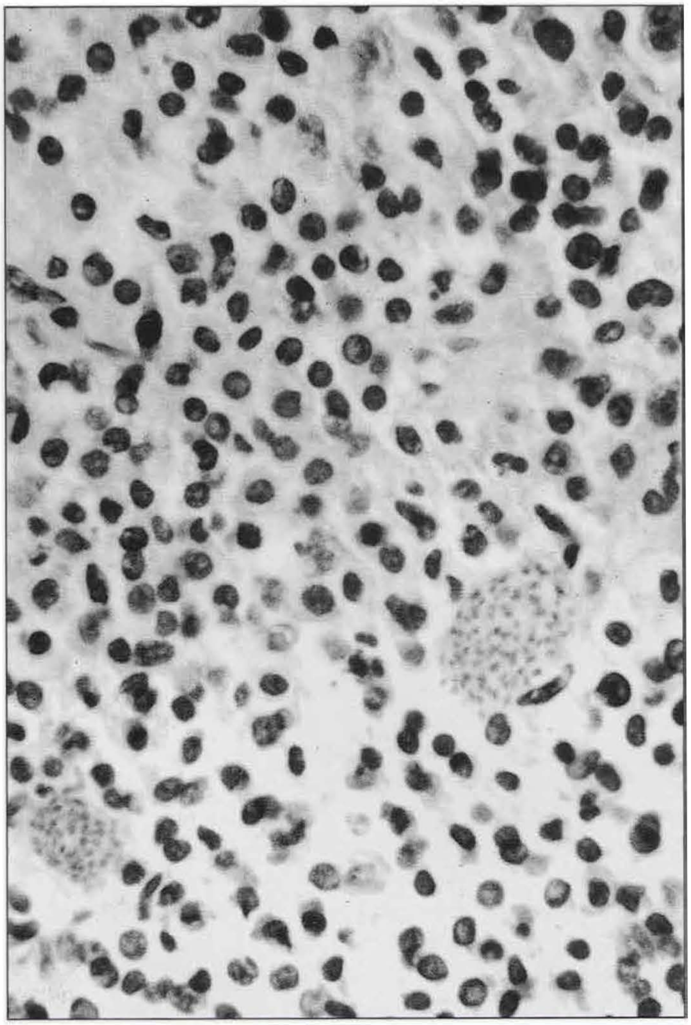

Figure 4.127 Sarcocyst in endothelium of small vessel in Peyer's patch of a bovine fetus.

premature induction of parturition through release of prostaglandin $F_{2\alpha}$ from damaged endothelial cells, fever, or direct infection of the fetus. Infection in the dam may result in acute necrotizing endometritis followed by multiple foci of necrosis in the soft tissues of the fetus. Why the organism is sometimes visible only in the dam but at other times also in the fetus is not known. In the dam, the zoite-containing cysts may be observed in the endometrium or caruncle following abortion, but if it passes to the fetus, in spite of autolysis and near absence of gross lesions, extensive lesions are evident microscopically. In the brain and meninges, there are multifocal areas of necrosis surrounded by undifferentiated mononuclear cells and lymphocytes (Fig. 4.126). Cardiac muscle, kidney, liver, lung, and chorioallantoic membranes may have similar lesions, some of which are mineralized and undergoing fibrosis. Endothelial cells in several soft tissues may contain thin-walled cysts that bulge into the lumen (Fig. 4.127) of the frequently thrombosed vessel. The cysts, packed with elongate zoites, though often difficult to find, are most easily distinguished in nervous tissue, maternal caruncle, and endometrium away from the site of inflammation.

Diagnosis is based on finding typical lesions of nonsuppurative meningoencephalitis, multifocal necrosis in soft tissues, and the presence of cysts containing zoites with a tropism for endothelium. If the organism cannot be found on routine sectioning, it can readily be located using specific fluorescent antibody, immunogold, or avidin–biotin complex techniques.

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Genital tritrichomoniasis

This is a specific contagious venereal disease of cattle caused by the flagellated protozoan *Tritrichomonas foetus* and transmitted at coitus or artificially via contaminated semen.

Infection in the bull remains in the preputial cavity and must be considered, in the absence of effective treatment, as permanent. In early infections, there is a balanoposthitis of moderate severity with preputial swelling and a slight purulent discharge. As the infection becomes chronic, the inflammatory reaction disappears and the organisms become fewer in number. There is a tendency for them to concentrate on the glans penis and adjacent areas of the prepuce, but they are quite difficult to find in preputial washings and may be detected only by test-mating suspected bulls to susceptible heifers.

Females are not readily infected, if at all, except by service or experimentally by placing a culture of the organism in the vagina. A few days after infection, acute vaginitis with swelling of the vulva develops and there is a moderate amount of mucoid floccular discharge in the vagina. The protozoa may be easy or impossible to find in this exudate. The vaginitis resolves shortly, and the infection localizes in the uterus and cervix. Immediately prior to the estrus, which follows the infective service, large numbers of organisms can usually be found in exudate aspirated from the cervix, but as estrus advances the number of protozoa is greatly reduced.

The manifestations of established trichomoniasis in females are cervicitis and endometritis that result in repeat breeding, abortion, or pyometra. The inflammatory changes in the endometrium and cervix are relatively mild and nonspecific, although the mucopurulent exudate may be rather copious. Discharge of exudate into the vagina may be more or less continuous or intermittent, and the numbers and activity of the trichomonads in the discharge vary considerably over short periods. The discharge may not be apparent at the vulva.

The pattern of repeat breeding in tritrichomoniasis is the same as that in venereal *Campylobacter* infections. Return to service occurs at irregular intervals, a fact that indicates that fertilization and implantation are followed by embryonic death. When the embryo or fetus dies, it may be resorbed, aborted, or retained with the development of pyometra. Trichomonad abortions may occur at any time, but mainly in the first half of pregnancy. *There are no specific fetal lesions, but large numbers of protozoa may be found in the fetal fluids and stomach.* The placenta is not severely altered as in brucellosis; it may be covered by white or yellow flocculent exudate in small amounts, thickened and slightly tough; hemorrhage without much necrosis may be evident on the cotyledons. Pyometra is one of the remarkable changes of trichomoniasis, but it is a relatively uncommon complication. Its pathogenesis follows the scheme outlined earlier, and its character is remarkable only for the copiousness of the exudate; any volume up to 4 liters or more may be present. The exudate may be watery with floccules, colostrum-like, or brown and sticky. It is without odor and swarms with trichomonads.

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Viral infections of the pregnant uterus

Porcine reproductive and respiratory syndrome (PRRS)

The syndrome has gone under various names such as "blue ear," because of ear discoloration in the pneumonic form, and "mystery swine disease" when the cause was yet to be identified. The syndrome is caused by *Porcine reproductive and respiratory syndrome virus* (PRRSV), family Arteriviridae, genus *Arterivirus*, a positive-strand RNA virus. All ages of nonimmune pigs are affected. Death rates can be high in piglets born with pneumonia or infected before or after weaning. Deaths in piglets are also associated with secondary bacterial infections, including *Salmonella choleraesuis*, *Streptococcus suis*, *Actinobacillus pleuropneumoniae*, and *Haemophilus parasuis*. These secondary infections often account for major reproductive losses. Viremia and circulating antibodies may coexist. Strain differences may account for some of the reinfections. Nonimmune animals vaccinated with modified-live PRRSV vaccines may experience increased abortions, stillbirths, and high mortality in newborns.

The effects of PRRSV on reproductive performance are variable between strains, and even though a gilt develops antibodies to one strain she may be susceptible to another. Similar differences in pathogenicity occur in respiratory disease. Effects also vary greatly depending on the stage of gestation and may result in early farrowing of stillbirths and mummified fetuses.

Initial clinical findings in infected pregnant gilts are anorexia, lethargy, depression, and mild fever; cyanosis of the snout, ears, tails, vulva, and abdomen occurred commonly in European outbreaks but are rarely observed in North America. This is commonly followed by a sudden increase in *early farrowing, late-term abortions, stillbirths, mummified fetuses, infertility,* and sometimes death. Live piglets with respiratory signs, muscle tremors, and splayleg also occur. Transplacental passage may result in fetal antibody production. After an outbreak of reproductive failure, previous levels of reproductive performance may not be achieved. Variable degrees of endometritis and myometritis may be present.

Gross and microscopic lesions in piglets are variable to absent and often obscured by autolysis. "Paintbrush" hemorrhages have been observed on the placenta and petechial hemorrhages, and necrosis occurs on umbilical vessels, skin, and renal cortex. Microscopic lesions include lymphoplasmacytic and histiocytic infiltrates within and around small capillaries at the periphery of the tunica adventitia of umbilical arteries. Mild to moderate, multifocal to diffuse, histiocytic and proliferative interstitial pneumonia characterized by mononuclear cell septal infiltration is commonly observed. Hypertrophy and hyperplasia of type 2 pneumocytes is occasionally present. Mild segmental necrotizing and lymphohistiocytic arteritis has been seen in lungs. Mild segmental necrotizing and lymphohistiocytic, suppurative and, less often, eosinophilic hepatitis may be present. Mild multifocal perivascular lymphohistiocytic myocarditis and, rarely, multifocal myocardial hemorrhages are present.

The **diagnosis** *is established by isolating the PRRSV, polymerase chain reaction (PCR) assay, or by in situ hybridization or immunohistochemical (IHC) detection of the virus in formalin-fixed tissues.* Immunogold has been found to be superior to immunoperoxidase as a marker. The virus is rapidly inactivated in aborted, stillborn, or mummified fetuses and isolation is therefore inconsistent. However, reverse transcriptase PCR testing modalities appear to be impacted the least by the adverse effects of autolysis. PCR becomes valuable in diagnosis as there are no consistent gross or microscopic lesions of this syndrome in fetuses and in utero autolysis rapidly inactivates the virus and interferes with isolation from aborted transplacentally infected fetuses.

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Classical swine fever virus infections of the pregnant uterus in sows

Classical swine fever virus (CSFV, syn. Hog cholera virus), a pestivirus, family Flaviviridae (single-stranded, positive-sense RNA genome), is a serious and contagious viral disease of swine; fortunately it has been eradicated from many countries. *Pestiviruses include CSFV, Border disease virus (BDV) of sheep, and Bovine viral diarrhea virus (BVDV). Infection by any of these three viruses can have similar results,* namely early embryonic losses due to in utero transmission to the fetus, severe congenital abnormalities and, particularly with BVDV, lifelong persistent infections.

The virulence of CSFV varies from avirulent to highly virulent. Pigs infected with highly virulent CSFV may have a high fever, depression, incoordination, reddening of the skin, diarrhea, respiratory distress, and death associated with widespread hemorrhage due to *severe necrotizing vasculitis*. Moderately virulent and virulent strains of CSFV cause subacute and chronic disease, with similar but milder signs. *During an inapparent viremic phase in a pregnant sow, the virus may be carried to the fetus and result in embryonic death, malformations, mummification, or stillbirth, or birth of live clinically normal pigs or pigs with tremors. Persistently infected piglets* may be born at term and appear clinically normal or weak but, in either circumstance, eventually succumb to the effects of the virus.

The virus can be transmitted from carrier or clinically affected pigs through their secretions and excretions, or through the ingestion of contaminated wastes. Birds, parasites, and fomites have also been identified as the source of virus in outbreaks. At one time, the use of vaccines containing CSFV of low virulence was the cause of severe losses due to in utero infections in pigs.

After exposure of a pregnant sow, the virus multiplies in the tonsils and spreads to other lymphoid organs, eventually reaching the fetus between 13 and 18 days. During the viremic stage, the virus crosses the uteroplacental interface infecting, all or only some fetuses. In the latter situation, the virus may continue to pass from fetus to fetus in utero, eventually infecting many.

Lesions produced in a fetus following in utero infection are very variable and depend on the virulence of the infecting virus, the stage of gestation when the infection occurs, and an apparent variation in the development time of immune competence to CSFV in fetal piglets. Generally, and with many exceptions, early infections result in embryonic or fetal death or persistent infections. Early to midgestation infections result in *malformations*, including pulmonary hypoplasia, pulmonary artery malformation, microagnathia, arthrogryposis, and central nervous system malformations (including cerebellar hypoplasia, microcephaly, and defective myelination in brain and spinal cord). Infections during the last trimester may produce no abnormalities, or may result in mummification or stillbirths.

Persistent infections in piglets most commonly follow exposure to the virus from 22 to 70 days of gestation and, rarely, up to 100 days. These fetuses may or may not die in utero and often show retarded development and runting. Many show no signs until some time after birth and persistently infected pigs living to 11 months have been reported. The latter commonly have marked depletion of lymphocytes (especially in the thymic cortex), and B-cell-dependent regions of lymph nodes and spleen. Lesions are also observed in heart (degeneration of endothelium and valvular fibrosis), liver (portal fibrosis), intestine (villus atrophy and ulceration), lungs (interstitial pneumonia), skin (necrosis and degeneration of epithelial cells), and central nervous system (neuronal degeneration and hydrocephalus). Many persistently infected piglets are born alive with a congenital tremor associated with central nervous system dysgenesis, cerebellar hypoplasia, and hypomyelination. Piglets with tremor, which survive for long periods, have reduced clinical signs but continue to excrete CSFV. The similarity of this to the syndrome in border disease virus-infected "shaker lambs" suggests a similar pathogenesis.

Many fetal piglets show some ability to respond immunologically to CSFV between 70 and 90 days of gestation with an immunoglobulin of low specificity and avidity. In accordance with this, fetuses collected from sows infected during this period may show general lymphoid hyperplasia and development of primary follicles. Kidney and brain may have mild lesions with scattered focal mononuclear cell infiltrates, and spleen and kidney may have foci of necrosis and hemorrhage.

For **diagnosis**, CSFV can be isolated from a variety of fetal tissues, including the placenta, and from the serum of persistently infected swine. Fetal antibody may be present in late-gestation fetuses, but carrier sows may or may not have antibody. In addition, the virus may be identified by the fluorescent antibody technique in tissue sections. In some laboratories, monoclonal antibodies to CSFV are routinely being used to differentiate CSFV from BVDV infections and, in some instances, CSFV vaccine strains from field strains. This may be very important in large eradication and control programs.

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Bovine viral diarrhea virus infections of the pregnant uterus

Bovine viral diarrhea virus (BVDV) is classified as genus *Pestivirus* in the family Flaviviridae. It is closely related to *Border disease virus* of sheep and less closely to *Classical swine fever virus*. For further discussion, see the section on *Border disease virus* infection in sheep and goats, below. *These three viruses have been shown to cross-infect naturally between species.* The various strains of BVDV, while similar, differ in virulence and can be distinguished antigenically. Two *biotypes* exist, one that is cytopathic for cell cultures (cp-BVDV = CB, cytopathic biotype), and one that is not cytopathic (ncp-BVDV = NCB, noncytopathic biotype). Both cause disease in cattle. In addition, there is *type 2 BVDV* that can infect fetuses, and exists in cytopathic and noncytopathic forms as well.

BVDV and *Akabane virus* are probably the most important viral pathogens of the bovine fetus. BVDV is distributed worldwide, and persistently infected cattle are reported to make up about 1% of the general population. They do not develop antibody to the homologous strain to which they are infected, but may respond to heterologous strains used in vaccines. Cattle persistently infected with ncp-BVDV or temporarily infected with cp-BVDV may excrete virus in feces, semen, urine, milk, nasal, ocular, and uterine secretions. Susceptible animals can acquire the virus by inhalation, ingestion, or when bred with contaminated semen. Cows may also become infected from contaminated embryo transfer fluids where serum from carrier animals is used. Cytopathic BVDV is derived from mutations of ncp-BVDV. *The ncp biotype may persistently infect the immunologically incapable fetus.* There is no report of a cytopathic biotype being isolated from an infected animal without the noncytopathic biotype also being present; the cp form has no means of maintaining itself in the population of animals without the ncp form.

Reproductive disease in cattle induced by BVDV includes oophoritis, fertilization failure, embryonic death, absorption or abortion, mummification, stillbirth, birth of calves small-for-gestational-age or with congenital defects, and weak calves or near-normal-looking calves born persistently infected (PI) with ncp-BVDV. Abortion storms can occur, and occasionally severe losses due to BVDV may follow superovulation and embryo transfer. This may be related to the creation of a population of fetuses of the same gestational age (and therefore equally susceptible to the infection), or contamination of transfer fluids by BVDV. Present evidence suggests that ncp-BVDV infects the bovine fetus following oronasal exposure of the dam, and only causes embryo injury when it mutates to the cp form of fetal infection.

Fertilization failure may occur when the virus is introduced in utero to seronegative cows. Conception and pregnancy rates in animals viremic at or shortly after insemination were approximately half of noninfected controls at gestation day 77. Surviving embryos were not infected. These data strongly support BVDV infection as being detrimental to early embryo development. Acutely infected bulls shed the virus in their semen for at least 14 days, and may have abnormal semen for several months. Spread of the virus is more likely to occur with natural service than with artificial insemination. Carrier and newly infected cows infect the fetus through the placenta. Bovine embryos or fetuses exposed to ncp-BVDV following viremia in the dam between 40 and 120 days of pregnancy may die and be absorbed, aborted, or mummified, supposedly following mutation to the cytopathic form. Fetuses dying from the infection may remain in the uterus for up to 50 days after death; recovery of the virus may be difficult.

In the period from 45 to 125 days' gestation, the ncp biotype will probably not cause fetal loss, but if it changes to the cp form, it may result in intrauterine growth retardation. Only the ncp biotype can cause persistent infection. If born alive, these PI animals constitute the main source of virus for other animals. *Central nervous system malformations* following infections with BVDV may continue to occur somewhat later in gestation than do infections resulting in persistent viremia. This is probably associated with the stepwise development of immune capability in the fetus along with the corresponding gradual development of its nervous system. Abnormalities attributed to BVDV include microencephaly, cerebellar hypoplasia, hydranencephaly, hydrocephalus, hypomyelinogenesis, myelination defects, microphthalmia, cataracts, retinal degeneration, optic neuritis, brachygnathism, thymic aplasia, hypotrichosis, alopecia, pulmonary hypoplasia, and growth retardation with growth-arrest lines in long bones. Porencephaly, hydranencephaly, and hydrocephalus have also been described, but the association with BVDV is less clearly documented. It is believed by some that only the cp biotype causes abnormalities. Cerebellar hypoplasia may be inapparent or severe. Normal and severely affected folia may be adjacent. Early lesions consist mainly of edema, hemorrhage, and necrosis of external germinal cells. Remnants of severely affected folia consist of fluid-filled cavities lined by flattened astrocytes surrounded by a thin layer of neuropil containing many hemosiderin-laden macrophages. Some folia have only reduced myelin content. Purkinje cells may be reduced in number and with a disorderly or absent external germinal cell layer. Perivascular accumulations of mononuclear cells and a few segmented cells may be present in neural parenchyma and meninges in cerebrum and cerebellum. Spongiform alterations of brainstem white matter have been recorded, along with spongiform alteration and liquefactive necrosis of ventricular funicular white matter in the spinal cord. Focal malacia of the ventral horns occurs following degeneration of ventral horn neurons along with glial satellitosis. Cataracts and retinal atrophy may be present in the eye, and the latter is presumed to follow focal-to-diffuse necrotizing, nonsuppurative retinitis and usually accompanies the cerebellar lesions. Microphthalmia and optic neuritis with subsequent atrophy also occur.

Other lesions associated with BVDV infections in the fetus include thymic cortical atrophy (with only epithelial stroma and macrophages persisting in the cortex), multifocal myocarditis, peribronchiolar lymphoid hyperplasia, pulmonary hypoplasia, multifocal perivascular dermatitis, and hypotrichosis. Cellular accumulations are usually mononuclear, lymphocytic, and plasma cell-rich. Plasmablasts, plasma cells, and lymphoblasts may be prominent in lymphoid tissues.

Antibody in fetal fluids and culture of virus from fluid and tissues are sensitive methods to determine if fetal infection has occurred. The presence of specific antibody in an aborted fetus only indicates the fetus was infected in utero. Fluorescent antibody and immunohistochemical detection of virus are frequently used to indicate the association of the virus with a lesion.

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Border disease virus infection in sheep and goats

Border disease virus (BDV), *Classical swine fever virus* (CSFV), and *Bovine viral diarrhea virus* (BVDV) are in the family Flaviviridae, genus *Pestivirus*. All are related antigenically and cause significant disease in animals. BDV and BVDV are closely related, while CSFV is distinct. Some strains of BDV appear to be more closely related to certain strains of BVDV than to other strains of BDV, while others cannot be differentiated from each other. BDV, BVDV, and CSFV have been shown to cross-infect between species and this may occur naturally. Border disease is the result of in utero infection of pregnant sheep or goats by BDV; prevalence of BDV is variable in sheep and rare in goats. *Infection can result in embryonic or fetal death, abortion, mummification, dysmorphogenesis, early postnatal death, and birth of weak or clinically normal young.* Clinically normal kids or lambs can be persistently infected (PI). Infection in the nonpregnant adult animal may result in fever and leukopenia, but clinical signs are usually absent.

Transmission of BDV is believed to occur mainly between sheep; however, natural and experimental infections of BVDV from cattle to sheep have been reported and can cause BDV outbreaks. BDV can be transmitted by oral, conjunctival, intranasal, and genital routes. *As congenitally infected sheep can have lifelong viremia, they are probably the main reservoir for the virus.* PI ewes can transmit the virus to their fetus through the placenta (over several pregnancies), and in rams, the virus has been observed in the seminiferous tubules of the testes and can be transmitted in the semen. BDV has been demonstrated in the germinal cells of the ovaries of sheep; the significance of this site in the transmission of the virus is not known.

A wide variety of fetal tissues are affected by BDV infection, and the name "*hairy shaker*" was coined because of the frequently observed hairy appearance of the wool and the tremor associated with hypomyelinogenesis. Abnormalities induced in the fetus, as in cattle, depend mainly on the stage of gestation, and reflect the stepwise development of fetal immunocompetence to the virus. Strains of the virus also vary in virulence, as does the susceptibility of breeds of sheep to the effects of the agent. As the ovine and caprine fetus becomes immunocompetent to BDV by about 90 days, most injury follows infections occurring before this time.

Central nervous system lesions include hypoplasia and dysplasia of the cerebellum, microcephaly, porencephaly, hydranencephaly, leukoencephalomalacia, and reduced weight and length of the spinal cord. The most consistent lesion is *dysmyelination or hypomyelination*, and usually occurs without signs of inflammation.

The mechanisms responsible for hypomyelination have not been completely defined. It has been suggested that BDV has a specific affinity for oligodendrocytes; however, evidence supporting this is lacking. There is strong evidence that the hypomyelination seen in the brain and spinal cord in BD may be due to lowered activity of oligodendrocytes associated with subnormal thyroxine production. In the tested lambs with BDV, there were significantly decreased concentrations of serum triiodothyronine and thyroxine, and many of the thyroid follicle cells contained BDV. As in many other tissues occupied by BDV in persistently infected animals, little cell death or associated inflammation was present. Further support for the hormonal theory of hypomyelinogenesis came on finding significantly lower concentrations of the thyroid hormone-dependent enzyme 2',3'-cyclic nucleotide-3'-phospho-diesterase (CNP) in oligodendrocytes from affected animals. CNP is normally found in oligodendrocytes and its activity, which is dependent on the presence of both thyroxine and growth hormone, increases with myelination. Work in congenitally hypothyroid mice and thyroidectomized rats has shown that growth hormone and thyroxine are necessary for normal myelination of nerve fibers.

Abnormalities in the fleece are commonly seen in fetuses following in utero infections with BDV. In smooth-coated ewes and nannies infected between 50 and 85 days of gestation, the fleece of the fetus may become hair-like and curly. The hairiness is associated with enlargement of primary follicles, increased size of fiber, increased proportion of fibers with a central medulla, and increased diameter of the medulla. Around 90 days of gestation, animals become immunocompetent to the virus, and infection at that time may result in only a diminished number of secondary follicles. The reduction has been suggested to be the result of reduced fetal nutrition because of placentitis. In pigmented breeds, there may be large patches of abnormally pigmented black or brown fleece, especially along the neck. Exactly why these changes are induced has not been determined. However, using immunofluorescence, BDV antigen has been demonstrated in skin follicles of fetuses infected at 45 days of gestation, starting at 40 days postinoculation and continuing in lambs up to 5 months of age.

Intrauterine growth retardation commonly occurs with BDV infection in goats and sheep and was reported to be of similar degree to that seen in fetal starvation, cardiac anomalies, and placentitis, except for lung and brain, which were more severely affected. Growth-arrest lines are commonly observed in the long bones of BDV-infected fetuses and lambs. Arthrogryposis and kyphoscoliosis occur infrequently in conjunction with hydranencephaly, cerebellar dysmorphogenesis, and hypoplasia in BDV-infected fetuses.

BDV infection in sheep and goats causes *placentitis* that is particularly evident when infection occurs during the first trimester of gestation. Early in the infection, multiple white 1–2 mm foci of necrosis appear in the caruncles at the base of the crypts. These may fuse into a continuous band of necrosis, which may become mineralized and infiltrated with connective tissue and a few macrophages, or the entire base of the caruncle may be infarcted, necrotic, and infiltrated with neutrophils. The lesion is believed to be associated with endothelial swelling and thrombosis of capillaries in the endometrium and adjacent caruncle. The trophoblast does not usually undergo necrosis but may atrophy.

When several kids or lambs show typical signs of BDV infection, definitive **diagnostic tests** can be selected with confidence. However, diagnosis may be difficult when BDV occurs only as reproductive failure with embryo or fetal death followed by absorption, abortion, or mummification. Frequently in goats, abortion may be the principal sign as the placentitis is severe and proceeds quickly. In aborted fetuses with congenital abnormalities, diagnosis requires the detection of the virus, virus-specific antigens, or fetal antibodies against the virus in cavity fluids. The cotyledons, or preferably the uterine caruncles, are the best source of virus. It is variably recovered from aborted fetuses and almost never from those that are mummified. While isolates are usually noncytopathic, viral antigen can be demonstrated by direct or indirect immunofluorescence, immunoperoxidase, or avidin-biotin techniques in cryostat sections of selected tissues from affected lambs or the caruncle following abortion.

Sheep have been reported to retain BVDV in the uterus from one estrus to the next and to produce persistently infected offspring when naturally bred or artificially inseminated by semen collected from PI rams.

Naturally occurring BVDV infections in pregnant sows have been reported to pass to the fetus, causing stillbirth and neonatal death similar to chronic swine fever. Piglets infected in utero can either carry the virus (without antibody), develop antibody later, or become clinically affected and develop intestinal lesions depending on the stage of gestation when infection occurs. On necropsy, infected piglets may also have nonsuppurative meningitis and choroiditis. Swine fetuses dying early in gestation from in utero infections with BVDV may also be absorbed.

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Equine arteritis virus infections of the pregnant uterus in mares

Equine viral arteritis (EVA) is a disease of horses caused by *Equine arteritis virus* (EAV), family Arteriviridae, genus *Arterivirus*. Genetic diversity of the virus is generated in the course of persistent infection of the carrier stallion and the genetic changes that maintain EVA in the stallion's reproductive tract likely affect phenotypic properties of the virus, such as virulence. Strains of the virus vary greatly in virulence. Consequences of infection range from subclinical to mild fever; ocular and nasal discharges; edema around the eyes, legs, and scrotum; skin rash; and, in severe forms, diarrhea, respiratory distress, and rarely death. Embryonic death and resorption rates are permanently increased, and 40–80% of pregnant animals abort or deliver a stillborn foal. Abortion usually occurs 1–4 weeks after the onset of fever, and is probably the most grievous manifestation of the disease, although in some outbreaks severe interstitial pneumonia and necrotizing enteritis may occur in foals.

The virus is spread by aerosolization of respiratory secretions among closely associated animals. *Stallions are probably the most important means of spread*, as they shed the organism in semen for a short or very long period, even for life, and transmit the disease sexually. There are minimal consequences of sexually acquired infection with little effects on conception rate, unless a mare is infected in the late stages of gestation, in which case she may abort. Stallions that are shedding the organism should not be used for breeding unless they are bred to seropositive mares that have received the organism from either vaccination or natural infection. In contrast, while mares may spread the virus during their illness or shortly after, they generally do not remain carriers and after recovery neither mares nor stallions shed the virus in nasal passage secretions, urine, or blood. Most horses develop a long-term immunity to EAV after natural infection of the virus.

Aborted fetuses are usually well preserved, but gross and microscopic lesions are rarely observed as the virus seldom produces active infection in the fetus. In foals surviving for more than 24 hours, fever, leukopenia, and/or thrombocytopenia are sometimes observed. Widespread inflammatory lesions in sections of the brain, liver, and spleen have been recorded. On microscopic examination, findings include interstitial pneumonia, lymphocytic periarteritis with fibrinoid necrosis of the tunica media, and renal tubular necrosis. Moderate arteritis with necrosis and mononuclear cell infiltrate has been reported in the myocardium of a few fetuses. In the uterus of the mare, however, there may be acute multifocal necrotizing myometritis associated with replication of the virus in smooth-muscle cells. Uterine infection might be the critical process contributing to abortion.

In the live mare and stallion during the early stages of disease, the presence of EAV may be determined by culture or polymerase chain reaction assay of swabs taken from nasopharynx and conjunctiva, from white blood cells, or from semen. The virus may be recovered from semen or revealed by breeding unexposed mares, which may then develop a fever or become clinically ill. Serological tests are available to detect previously infected animals. Samples for culture from the conceptus should include placenta, lung, and spleen. Diagnosis can also be obtained by immunohistochemistry combined with virus isolation.

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Wesselsbron virus infections of the pregnant uterus in sheep and cattle

Wesselsbron virus (WESSV) is in the family Flaviviridae, genus *Flavivirus*, in the yellow-fever virus group. It is spread primarily by mosquitoes. Infection is often fatal in the ovine and bovine fetus, in newborn lambs and pregnant sheep. It may also produce congenital anomalies in bovine and ovine fetuses. Human illness is not fatal and is characterized by fever, chills, muscle pain, hyperesthesia, and a skin rash. Information is largely incomplete with regard to the pathogenesis and pathology of this infection in pregnant animals.

The disease is widespread in the southern and western parts of Africa in sheep and cattle; however, in spite of a high prevalence and high annual infection rate, mortality is low. In a naive flock of sheep, however, mortality in newborn lambs and pregnant ewes may be high. Pregnant ewes may die before or after abortion, whereas nonpregnant ewes, wethers, and yearlings usually show no signs of illness. *Changes in the liver due to WESSV infection may precipitate acute copper toxicity*, thereby inflating the perceived mortality directly due to this virus.

Pregnant sheep infected with WESSV develop a fever associated with initial viremia, and often abort at this stage due to the systemic effects of the virus on the dam. Under these circumstances, neither virus nor antibody is detected in the fetus. If the ewe does not abort during the primary viremia, the virus can invade the placenta and fetus in association with a second growth phase and result in death of the fetus. The fetus may be absorbed, mummified, aborted, or carried to term and be delivered dead. The death rate for lambs infected in utero approaches 100%, and even those born alive die soon after. Both wild and attenuated strains of WESSV have been shown to cause nonsuppurative menigoencephalitis, hydranencephaly, arthrogryposis, and hydrops amnii in fetal sheep; however, this is probably infrequent under field conditions.

In pregnant cattle, WESSV probably only crosses the placenta and infects the fetus in a low percentage of animals, even in the face of prolonged maternal viremia. When the virus does infect the fetus, abortion may occur or the calf may be born weak and die shortly after. These calves have typical liver lesions and precolostral antibody to WESSV. However, virus may not be recovered. Many pregnant cows, even after prolonged viremia, give birth to normal live calves that show no serological or virological evidence of infection. In cattle, as described in sheep, certain strains of the virus occasionally produce nonsuppurative meningoencephalomyelitis, porencephaly, and cerebellar hypoplasia in the fetus.

Gross and microscopic descriptions of findings in aborted fetuses, or stillborn lambs and calves, are limited. Experimentally infected newborn lambs have a slight to moderately enlarged, yellow to orange, variably congested liver. On microscopic examination, hepatocyte necrosis is slight to extensive, but is usually single-cell and generalized. *Eosinophilic, intranuclear inclusion bodies of various shapes are frequently present*. Separated, individual hepatocytes may be round, shrunken, and have intensely pink-staining cytoplasm and nuclear lysis (Councilman bodies). Kupffer cell hyperplasia is prominent, particularly in the central vein region, and Kupffer cells filled with yellow or pink granular material often bulge into sinusoids. The sinusoids also contain large numbers of mononuclear cells and degenerating neutrophils. Bile ducts and canaliculi are frequently distended with bile. In portal areas, there is bile duct hyperplasia, moderate numbers of macrophages, and some neutrophils and plasma cells. There are petechial and ecchymotic hemorrhages on the mucosal surface of the abomasum and the content is dark brown. Other nonspecific lesions, consistent with a viremia, are also described.

The **diagnosis** *of WESSV infection in pregnant animals is based on clinical findings, histology, and culture from liver and brain*. The lesions produced by Rift Valley fever virus infection may easily be confused with Wesselsbron disease, and, although the liver lesions tend to be different, complications due to precipitation of copper toxicity may make the differentiation difficult.

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Porcine parvovirus infections of the pregnant uterus

Porcine parvovirus (PPV) is in the family Parvoviridae, subfamily Parvovirinae, genus *Parvovirus*. *PPV is an important and common cause of reproductive failure in pigs, and manifests as embryonic death with reabsorption, mummification, stillbirths, and reduced litter size.* Farrow-to-finish farms are affected nearly twice as often as feeder pig farms, probably due to the maintenance of a continual reservoir of PPV in the finishing pigs. The virus is most commonly spread by the ingestion of contaminated feces, and will adhere to the zona pellucida of embryos. Viremia develops in susceptible pigs, and in pregnant pigs the virus enters the placenta and passes to the embryo or fetus 23–32 days following oral inoculation. Up to a fetal age of 70 days, the virus may cause death of the embryo (with absorption) or fetus (with mummification); however, the porcine fetus becomes increasingly resistant to the effects of the virus after 36 days and is immunocompetent by about 70 days. Death of the fetus may be due to the total effect of the virus on rapidly dividing cells, including neurons and capillary endothelium of the cortical laminae in cerebellum and cerebrum, and cells in the lung, liver, pancreas, and chorioallantois. Movement of virus from one fetus to another may be affected by development of maternal antibody, increasing resistance of fetuses with advancing age, and the variability of the circulation and contact between the placental membranes of adjacent fetuses.

When PPV is introduced by the uterine route, it causes inflammation in the ovaries and degeneration of uterine epithelium, both of which may contribute to reproductive failure but do not otherwise cause clinical signs. Uterine changes may include marked variation in surface epithelial cell height, epithelial cell basophilia, degeneration, and ulceration. Light infiltration of neutrophils, eosinophils, and lymphocytes may be present throughout the endometrium. Corpora lutea contain foci of mononuclear cells, many of which are lymphoplasmacytic. *In the fetus, lesions are most commonly observed in older fetuses and may be present in kidney, liver, brain, and fetal placenta.* In the cerebrum, which is the most dependable site, perivenous accumulations of adventitial cells, plasma cells, and other mononuclear cells occur in white and gray matter and in meninges. Similar accumulations are observed in the renal interstitium, hepatic portal regions, and in the chorioallantois.

For **diagnosis**, viral antigen is demonstrable by the fluorescent antibody test in affected tissues of embryos, mummified and stillborn fetuses, and a highly sensitive DNA probe, specific for PPV-infected cells, is available. *Identification of specific antibody, particularly IgM, in fetal fluids indicates fetal infection, and with other appropriate findings is supportive evidence for the cause of reproductive failure.* When the only manifestations are small litters or pigs that recycle, the causal relationship of these signs with PPV is difficult to establish, and knowledge of titers of antibody against PPV in the gilt or sow before and after the event may be the only incriminating evidence. Various tests, including an enzyme-linked immunosorbent assay, have been developed for this purpose.

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Bovine parvovirus infections of the pregnant uterus in cattle

Bovine parvovirus (BPV) is in the family Parvoviridae, and is the type species for BPV-like viruses; it is widely distributed in cattle populations throughout the world. *It is reported to cause enteritis and diarrhea in young calves, and abortion and birth of weak calves when infections occur in naive pregnant cows. The organism is not considered a major cause of abortion*, but this may be because it is not looked for consistently in submissions. Anywhere from 10 to 100% of the cattle in a population will have antibody to parvovirus and work in the USA suggests that, when reproductive problems, including repeat breeding, embryonic mortality, and abortion are considered, animals with antibody to bovine parvovirus had about three times as many of these problems as compared to control seronegative animals.

Bovine parvovirus is excreted in the feces of affected and normal animals, and the oral route may be important in transmission. Inoculation of parvovirus into pregnant cows can cause in utero fetal death and abortion in the early stages of gestation. Some calves may be born with cerebellar hypoplasia due to lysis associated with the multiplication of BPV in cells of the external granular layer. Cerebellar hypoplasia is believed to follow infection occurring in the period of rapid cell multiplication (107–150 days' gestation) in the cerebellum. *Cowdry type A inclusions* are seen in external granular cells, hepatocytes, adrenal cortical cells, and intestinal crypt cells associated with areas of necrosis. Bovine fetuses are capable of forming IgM antibody to the virus by 93 days' gestation; infections in the third trimester usually only result in antibody formation with no abortion.

For **diagnosis**, BPV may be recovered in a variety of tissues, including placenta, amniotic fluid, adrenal glands, and liver. When infection occurs in mid-gestation, detection of specific antibody in fetal serum may be used as putative evidence of the cause of abortion. The presence of antibody may interfere with recovery of the virus but probably will not completely prevent isolation, as the virus is frequently cell-associated.

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Canine minute virus

Canine minute virus (CMV) is a species distinct from *Canine parvovirus 2* and *Feline panleukopenia virus* in the genus *Parvovirus*, and antibody to it is widespread among dogs. It may occasionally cause mild diarrhea in pups, but its significance is poorly defined. Experimental oral–nasal infection is clinically inapparent, but it may produce inclusion bodies in the cells of the duodenal crypts. Transient lymphocytolysis in the cortex of lymph nodes, thymus, and in gut-associated lymphoid tissue has been reported.

The virus seems capable of transplacental transmission to the fetus, and exposure of pregnant dogs has been associated with fetal resorption, or birth of dead or weak pups, depending on gestational stage at exposure. Anasarca and myocarditis are reported in dead or weak puppies. Spontaneous reproductive failure associated with MVC is not reported.

The *pathogenesis* of MVC and its clinical significance are not yet known, but preliminary findings suggest it may be responsible for a portion of deaths in pups less than 4 weeks of age and it may cause reproductive failure. Virus isolation is difficult, possibly because of antibodies in infected dams at the time they resorb their fetuses; however, histological examination of tissues from dead pups may reveal viral inclusion bodies in small intestinal villus epithelial cells or bronchial epithelial cells. Much more information is necessary to ascertain the role of this virus in canine disease. *Reports of deaths and abortions suggest it is more common than currently recognized.*

Diagnosis can be confirmed by immunofluorescence or immunohistochemistry.

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Bluetongue virus infections of pregnant sheep and cattle

Bluetongue virus (BTV) is a nearly worldwide viral infection of sheep and other ruminants. In sheep and, with much less severity and frequency, in cattle, the disease is characterized by fever, erosive to ulcerative lesions of the upper gastrointestinal mucosa, and focal hemorrhage and necrosis of smooth, skeletal, and cardiac muscles. BTV infections are rarely fatal; and in cattle, most infections are subclinical. *Infections occurring during pregnancy, however, can cause heavy losses due to early in utero death and absorption, abortion, mummification, or stillbirth with severe fetal growth retardation and multiple congenital anomalies.*

BTV is a member of the family Reoviridae and is the prototype virus for the genus *Orbivirus*. There are several serotypes of BTV, and they vary greatly in virulence. The virus is mainly spread by, and can multiply in, various species of biting gnats in the genus *Culicoides*. With many serotypes of BTV, viremia exists for a longer period in cattle than in sheep, and in both, antibody and virus may coexist for several days. The prolonged viremia seen in cattle has led some investigators to designate them as the reservoir host for this virus. The virus has been found in the semen of a few bulls; however, it is usually transient and therefore of less importance than arthropods in transmission.

When an animal becomes infected, primary virus replication occurs in the lymph nodes draining the site of inoculation. This is followed by extension through the lymphatics and bloodstream to the spleen and other lymph nodes, where the virus propagates and is carried via the blood to a variety of tissues. The organism appears to be closely associated with erythrocytes during viremia. Species-specific differences in the production and activities of endothelial cell-derived inflammatory and vasoactive mediators appear to contribute to the greater sensitivity of sheep than cattle to BTV-induced microvascular injury.

The consequences of infection during pregnancy depend on the stage of gestation when infection occurs and on the serotype of virus. Infections in sheep up to approximately 50 days of pregnancy may result in death of the conceptus and absorption or abortion. Between 50 and 80 days of gestation, *necrotizing encephalopathy* occurs; and in the last third of gestation, as immunocompetence increases, there may be no obvious disease. On the other hand, infections of near-term and newborn lambs can result in a sluggish immune response, possibly associated with the rise in fetal cortisol at parturition and the ingestion of colostrum, both of which may suppress the active immune response.

Lesions in the brain of the *ovine fetus* infected during the 50–70-day period consist of diffuse infiltration of mononuclear cells in the meninges and necrosis of the cerebral cortex. The meninges and areas of necrosis gradually become infiltrated with macrophages and, unless the fetus is expelled early, appear as variably sized fluid-filled cavities (porencephaly and hydranencephaly), frequently accompanied by some degree of hydrocephalus in the new or stillborn lamb. Cerebellar dysgenesis, retarded development, and skeletal growth-retardation lines have also been described in fetuses inoculated with BTV. The lungs in fetuses inoculated with virus at 80–100 days become infiltrated with large mononuclear cells within the interstitium of small arterioles. These accumulations eventually form continuous overlapping nodules of large mononuclear cells that have abundant cytoplasm and indented nuclei. Similar lesions are occasionally seen in the renal medulla and hepatic portal areas. Cortical regions of lymph nodes are enlarged by large numbers of primary and secondary lymphoid follicles that contain numerous plasma cells. In the spleen, small white nodules are visible on the cut surface. These consist of greatly expanded periarteriolar lymphoid tissue.

Lesions in the *bovine fetus* develop similarly, and up to 70 days of gestation, BTV infection may result in in utero death and absorption or abortion of the conceptus. Between 70 and 130 days of gestation, serotypes 10, 11, 13, and 17 can cause hydranencephaly and death of the fetus. Two distinct processes may be involved in the development of central nervous system lesions due to BTV infections in bovine fetuses: *infarction*, associated with viral-induced vascular injury, is probably responsible for the large areas of necrosis and cavitation occurring in the rostral regions of the cerebrum; whereas *viral destruction* of clusters of individual cells, including glial cell precursors, results in foci of necrosis and accounts for microcavitation. Collapse of these cavities may facilitate dilation of the lateral ventricles and subsequent hydrocephalus. Death of calves with hydranencephaly pre-empts them spreading the virus by an arthropod vector, even if they are viremic. The earliest evidence of antibody activity against BTV group antigen in bovine fetuses occurs at around 145 days of gestation; by 200 days, there is significant neutralizing antibody activity, therefore no virus is detectable at birth.

Diagnosis of in utero infections in cattle and sheep is based on finding the typical lesions and specific fetal antibody to BTV in cavity fluid or blood. Virus isolation after fetal immunocompetence to BTV is unlikely but not invariable. Maternal precipitating antibody (agar gel immunodiffusion test) may be absent at term, while neutralizing antibody may be more persistent. The c-enzyme-linked immunosorbent assay for specific neutralizing antibody is more specific and sensitive. To culture the virus from blood, animal inoculation or washed erythrocytes may need to be used because the organism is closely associated with red cells. BTV and EHDV can be detected and differentiated by multiplex RT-PCR.

Epizootic hemorrhagic disease virus infections of the pregnant uterus in cattle, sheep, and white-tailed deer

Epizootic hemorrhagic disease virus (EHDV) is an arthropod-borne virus in the family Reoviridae, genus *Orbivirus*. It exists as at least two serotypes, 1 and 2, and is present in Africa, Australia, North America, and Japan. *Ibaraki disease virus*, first isolated in Japan, is a serotype-2 strain. EHDV is spread by *Culicoides* spp., and *causes fatal hemorrhagic disease of white-tailed deer.* In cattle, the Ibaraki strain can cause severe ulceration of the mucosa of the mouth, tongue and esophagus with necrosis of esophageal musculature and marked pain on swallowing. Many animals die. In many respects the disease is similar to BTV infection in sheep, but, in contrast to BTV, *EHDV has very low pathogenicity for sheep.* Although EHDV cross-reacts with BTV in some serological tests, it is considered to represent a separate population of viruses. Experimental inoculation of BTV has been reported to cause abortion and mummification in white-tailed deer. Because of its similarity to BTV, EHDV is also believed to be a cause of abortion and congenital anomalies in cattle, but this has not been proven.

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Chuzan disease of cattle

Chuzan virus (CHUV, syn. *Palyam virus*) has been isolated from cattle and the midge *Culicoides oxystoma*. The virus is in the family Reoviridae, genus *Orbivirus*, Palyam serogroup, and is most closely related to *African horsesickness virus* within the orbiviruses. CHUV has been incriminated as the cause of an epizootic in the Kyushu district of Japan, wherein 2463 calves, mostly beef, were affected. A high percentage of newborn calves were born with *hydranencephaly and cerebellar hypoplasia.* A subsequent seroepidemiological investigation revealed evidence to suggest the organism had been spread from the most southerly island of Japan to the mainland in the north. Cattle in the region did not have antibody to the virus previous to this epizootic.

Calves involved in the epizootic were born alive and were normal in size and weight. Clinical signs and congenital abnormalities included corneal opacity, blindness, nystagmus, cerebellar ataxia, tremor, and opisthotonos. Necropsy lesions included hydranencephaly, hydrocephalus, microcephaly, and cerebellar hypoplasia. No lesions were seen in other organs and no virus was isolated from any of the calves; however, 44 of 49 precolostral serum samples checked for neutralizing antibody to CHUV were positive. Experimental infection of 15 cows at 89–150 days' gestation resulted in 14 normal calves and one calf with severe hydranencephaly and cerebellar hypoplasia.

For **diagnosis**, CHUV can be recovered from blood for up to 56 days after inoculation. It is closely associated with red blood cells, even in the presence of antibody. Demonstration of specific antibody in the serum of precolostral calves will be incriminating.

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Canid herpesvirus infection in pups and pregnant bitches

Canine herpesvirus (*Canid herpesvirus 1*, CaHV-1), family Herpesviridae, subfamily Alphaherpesvirinae, is in the genus *Varicellovirus*. In adults or weaned puppies, CaHV-1 may produce mild upper respiratory infection, and inapparent infections are common. Infection of the neonate is possible by various routes. It may be transmitted from maternal lesions of genital herpes to the pup as it traverses the birth canal, from initial maternal infection, or by recrudescence of existing latent virus. Viral recrudescence can be induced in recovered animals by corticosteroids. There is considerable homology between endonuclease fragments of CaHV-1 and *Felid herpesvirus 1*.

Canine herpesvirus infection is regularly fatal for newborn puppies. Resistance to the disease is sharply age-related; pups exposed after 2 weeks of age will not develop severe illness. The disease has an incubation period of 3–7 days, after which the affected puppies rapidly sicken and die, usually within 2 days. The clinical signs lack specificity. Affected animals are not usually febrile; most vomit and refuse food. Their breathing becomes shallow and rapid, and shortly before death, the pups give evidence of abdominal pain.

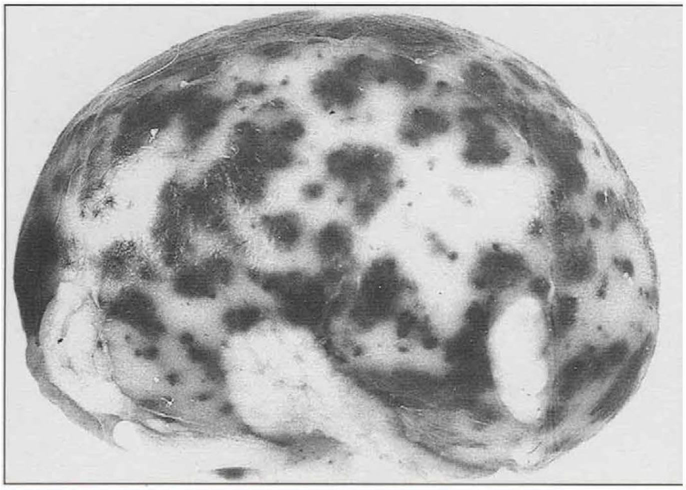

Figure 4.128 Renal hemorrhages in newborn pup due to *Canine herpesvirus* infection.

At autopsy, there is usually pleural and peritoneal effusion that may be blood-tinged. *Petechial and ecchymotic hemorrhages* are scattered throughout the subserosal tissue, usually representing the most impressive gross feature of the disease. Large hemorrhages are regularly seen in the kidney (Fig. 4.128). The lungs are wet, and frothy fluid fills the bronchi and bronchioles. Lymph nodes are enlarged and reddened, and splenomegaly is present. Tiny red foci, determined microscopically to be foci of necrosis, are occasionally seen in the liver. *Foci of necrosis are characteristic of the disease and may occur in any organ* (Fig. 4.129). Lung, heart, kidney, intestine, pancreas, adrenal, and spleen are common sites. The inflammatory response associated with these disseminated focal areas of necrosis is slight or absent. Viral inclusion bodies are not numerous, but can best be seen adjacent to the areas of necrosis in the liver or lung, or in the renal glomeruli. The inclusion bodies are intranuclear; most are basophilic, but some are faintly acidophilic.

Discrete microscopic lesions are present in the brains of the pup as early as 72 hours after experimental infection. These increase in severity as the disease progresses. They consist of nonsuppurative meningoencephalomyelitis, characterized by destruction of gray

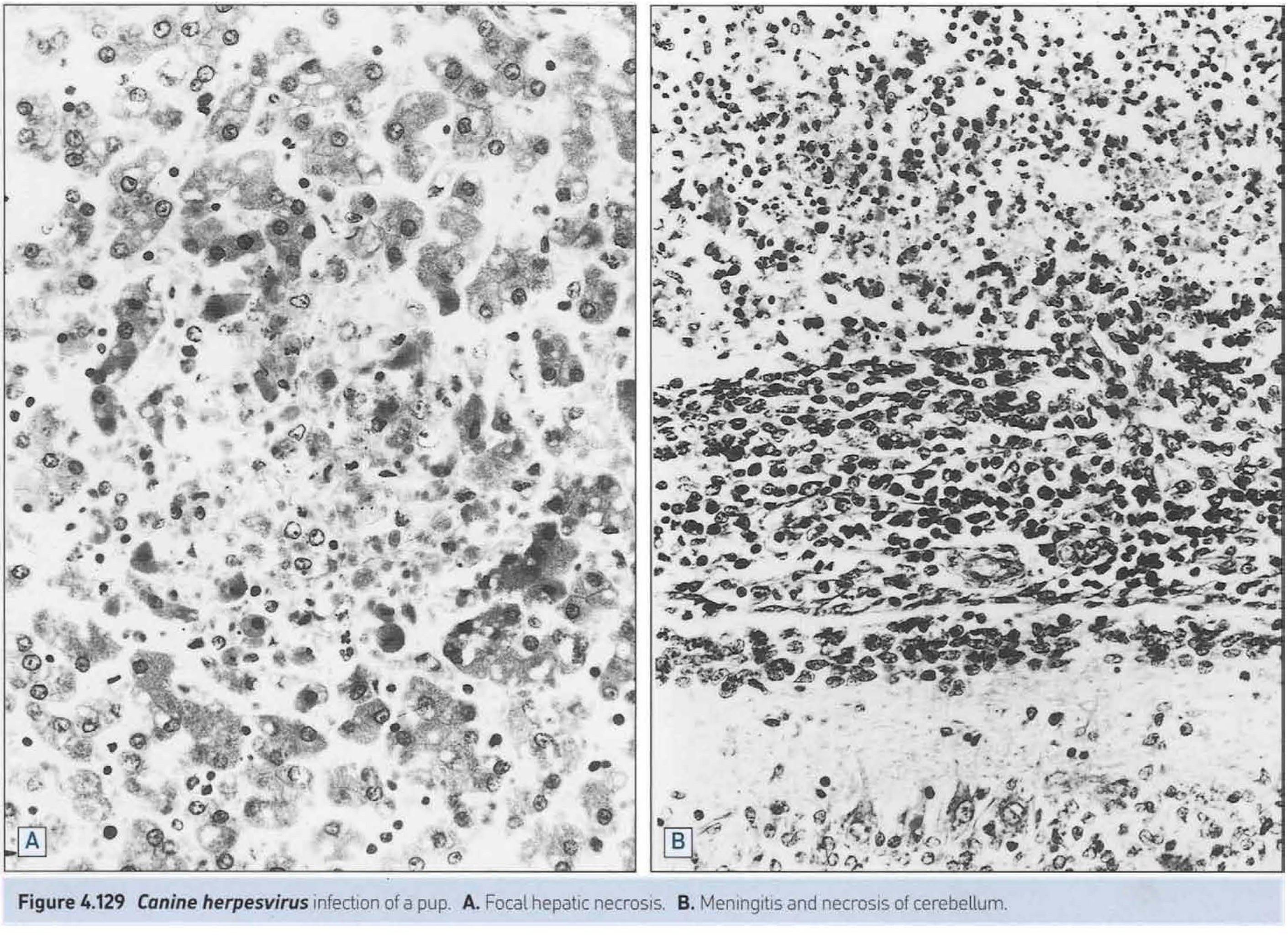

Figure 4.129 ***Canine herpesvirus*** infection of a pup. **A.** Focal hepatic necrosis. **B.** Meningitis and necrosis of cerebellum.

and white matter; the gray is somewhat more severely involved. There are focal and segmental areas of destruction of the cerebral cortices with concurrent microgliosis. Vascular changes vary from endothelial swelling to mononuclear cuffing. Diffuse pial meningitis with infiltrates of mononuclear cells and neutrophils is frequent, as is ganglioneuritis of the Gasserian ganglia.

Canine herpesvirus has been associated with abortion, stillbirth, and infertility. Depending on the time of gestation, inoculation of bitches with CaHV-1 has been reported to result in death and expulsion of fetuses, mummification, or premature delivery of live puppies. Focal areas of necrosis with intranuclear inclusions were variably observed in liver, spleen, kidney, placental labyrinth, and heart. Endothelium and trophoblast were commonly involved. Virus was recovered from many of the fetuses.

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Suid herpesvirus 1 infections causing abortion in swine

Suid herpesvirus 1 (SuHV-1, Pseudorabies virus, Aujeszky's disease virus) is in subfamily Alphaherpesvirinae, genus *Varicellovirus. SuHV-1 is capable of causing reduced fertility in boars and sows, fetal resorption, mummification, abortion, and stillbirth in sows, and fatal meningoencephalitis in young pigs, sheep, cattle, and dogs.* Strains of SuHV-1 vary in their ability to invade the placenta and fetus; however, abortion without invasion of the conceptus may occur secondary to fever and nervous disease in the sow. When the virus does invade the placenta and fetus, characteristic gross and microscopic lesions are produced. There is a strong correlation between the ability of strains to cause syncytial formation in cell culture and their ability to produce disease in pigs and cattle.

The disease has a worldwide distribution, and the carrier sow is the most important source of the virus. Recrudescence and shedding through nasal mucus may occur following stress, and there is some evidence for long-distance airborne transmission, although this would appear not to be a major threat. Boars may carry SuHV-1, spreading it through nasal mucus and semen. The virus can survive in a pen for up to 2 weeks.

The virus enters the nasal passages of the susceptible pig and passes via the pharyngeal and olfactory nerves to the brain, where it rapidly spreads to the rest of the brain, causing nonsuppurative meningoencephalitis. It has affinity for the respiratory tract, and may induce severe rhinitis, tonsillitis, and pneumonia.

Abortion usually occurs about 10 days after the onset of clinical illness in the sow. If the placenta and fetus are invaded, lesions consist of multifocal coagulative necrosis of chorionic villi and focal coagulative necrosis in many organs, including liver, adrenal, and spleen. *Intranuclear inclusions* have been observed in trophoblast and interstitial cells and hepatocytes around areas of necrosis.

Virus may be demonstrated in nasal or vaginal swabs and fetal tissues by isolation and fluorescent antibody or enzyme-linked immunosorbent assays; polymerase chain reaction assays are also available.

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Suid herpesvirus 2 (cytomegalovirus) infections of the pregnant uterus of the pig

Suid herpesvirus 2 (SuHV-2, Swine cytomegalovirus) is in the family Herpesviridae, unassigned to a subfamily or genus. Infection by this virus is normally confined to pigs, and was first described in England in 1955 as *inclusion body rhinitis* and, subsequently, as a transplacental infection of fetal pigs, in 1961 in Australia.

In neonates, SuHV-2 causes *mild to severe nonsuppurative necrotizing rhinitis* characterized by the presence of *large basophilic intranuclear inclusions* in the epithelium of the mucous glands and ducts in the nasal mucosa. The virus usually affects young pigs (less than 4 weeks old) but in naive herds may cause death in pigs of 4–12 weeks. The virus occasionally causes severe systemic disease in mature pigs. *When infection occurs in pregnant sows, they may deliver small litters, mummified fetuses, or stillborn or weak piglets either before or on the due date.* Pigs infected in utero may be born alive, small-for-gestational-age, and may develop systemic signs and die. Subsequent reproductive performance in infected sows may be reduced and manifested as decreased conception rates and decreased litter size.

Surveys of pig herds for serum antibody to SuHV-2 indicate infection to be widespread throughout the USA, Canada, Europe, Australia, and New Zealand. Transmission occurs when newly infected pigs excrete the virus in nasal and ocular secretions and urine. Boars may also excrete it in semen, and sows in cervical discharges. The virus has the capacity to remain latent in macrophages and other cells of the monocyte–macrophage system. Animals with latent virus may also excrete it, even in the face of serum antibody, following administration of corticosteroid and probably following periods of stress. Heavy losses are infrequent in commercial herds; however, in gnotobiotic pigs or minimal-disease herds in which pigs have had no previous experience with the virus, the introduction of actively or latently infected animals may prove disastrous.

When a sow is infected with SuHV-2, multiplication occurs first in the nasal mucosa. This is followed by viremia and dissemination of virus to many body organs, including the placenta in pregnant sows. The virus seems to pass through the placenta without inducing injury; experimentally the virus has not been isolated from the placenta, even when the fetus that has just died is positive. Neither has antibody been detected in piglets that become infected in utero. Fetuses die 4–6 weeks after inoculation of the sow, and the time is independent of the stage of gestation. Casualties of infection are scattered randomly throughout the uterus in various stages of decomposition and mummification, much as with porcine parvovirus infection. Following experimental infection, no gross lesions were seen on necropsy of the autolysed fetuses. However, on microscopic examination there was cytomegaly, and intranuclear inclusions were observed in liver and lung. In a natural outbreak of SuHV-2 infection, pigs born alive with a congenital SuHV-2 infection usually died within a week. Gross lesions in these consisted of pulmonary edema and congestion, hydrothorax and hydropericardium, and multiple firm, gray foci in the most ventral portions of the lungs. Mediastinal lymph nodes were enlarged and petechial hemorrhages were visible on the heart, lungs, intestine, and kidneys. Histologically, there was pulmonary edema and hemorrhage; alveolar septa were thickened, mainly by macrophages, but lymphocytes and neutrophils were also present. Large basophilic intranuclear inclusions were present in capillary endothelial cells and macrophages. Similar inclusions were present in capillaries of the renal medulla and tubular epithelium. Two types of inclusion have been observed: the usual large basophilic type found in pulmonary macrophages and glands and ducts of the nasal mucosa and renal tubular epithelium, and a small herpesvirus-like inclusion found mainly in monocyte–macrophage cells. Nonsuppurative meningoencephalitis has been reported in some pigs and was characterized by perivascular lymphocytic infiltrates and gliosis in the choroid plexus and vessels outside the cerebral cortex.

Experimental infections of piglets in utero or newborns less than 2 weeks of age have shown them to be very susceptible and to develop severe debilitating disease, with death a common sequel. This severe infection has been related to viral infection in the cells of the monocyte–macrophage system. This results in disseminated hemorrhage and edema in the lung, kidney, adrenal, liver, and lymph nodes, all of which is associated with viral destruction of macrophages and capillary endothelial cells. In the less severe and nonfatal forms of the disease, there is invasion of the mucus-producing cells of the nasal mucosa, the cells of the renal tubules and, less frequently, the epithelium of the salivary glands, Harderian and lacrimal glands, seminiferous epithelium, epithelium of ductus epididymis, mucous glands of the esophagus, duodenum, jejunum, and hepatocytes. Invasion of macrophages and endothelium or epithelium is not completely exclusive and both occur often.

The cytomegalovirus inclusions seen on histology when correlated with appropriate signs are **diagnostic** *of SuHV-2 infection in pigs.* Infection in a herd may be revealed by detecting antibody in the maternal serum using an indirect immunofluorescent (IIF) antibody test on infected lung macrophages. To detect virus, samples of nasal mucosa, lung, and kidney can be cultured directly on lung macrophages. Viral antigen may be detected in frozen sections of affected lung or kidney using the IIF test. As stated previously, the organism is not found or seen in the placenta, and antibody is not present in the fetus but may be present in piglets infected after birth.

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Bovine herpesvirus 1 infections of the pregnant uterus in cattle, goats, sheep, and pigs

Bovine herpesviruses cause a wide variety of diseases in several species, including cattle, sheep, goats, wildebeest, and other wild ruminants. They are divided into six different groups, designated BoHV-1 to BoHV-6. The infections of the pregnant uterus caused by bovine herpesviruses that will be discussed in this section include BoHV-1 (infectious bovine rhinotracheitis virus, IBRV; infectious pustular vulvovaginitis virus, IPVV), and BoHV-4 (Movar virus, a cytomegalovirus).

Bovine herpesvirus 1 is in the family Herpesviridae, subfamily Alphaherpesvirinae, genus *Varicellovirus*. Based on restriction endonuclease analysis and cross-hybridization, BoHV-1 is subdivided into three antigenic variants or subgroups: subgroup 1 – IBRV; subgroup 2 – IPVV; and subgroup 3 – neuropathogenic. *IBRV and IPVV both cause abortion. IBRV is considered to be more virulent than IPVV* and, in addition to abortion, causes severe upper respiratory disease, endometritis, oophoritis, mastitis, dermatitis, and fatal diarrhea of young calves. IPVV primarily causes inflammation of the penis and sheath in bulls, inflammation in the vagina and vulva in cows, and abortion in cows.

BoHV-1 is distributed worldwide. Cattle-to-cattle transmission is the principal method of spread, and virus is easily dispatched from naturally infected or vaccinated cattle to susceptible animals through respiratory, ocular, or vaginal discharges. *All BoHV-1 strains*, including thermosensitive and thymidine kinase-negative strains, *are capable of becoming latent* and therefore undergoing recrudescence and shedding from mucous membranes. Virus is more abundant in excretions from primary than from recrudescent infections; however, transmission may occur from either. Live vaccine viruses may become latent and none of the vaccines can prevent latency by a superinfecting challenge virus, but, if the antibody titer is high in the vaccinate, the incidence of latent superinfection and amount of virus excreted may be lowered. Recrudescence may be induced by superinfections, corticosteroids, transport, parturition, and other stressful situations. Semen may also be an important source of the virus and embryo transfer fluids less so.

Sheep, goats, and pigs often have antibody titers to BoHV-1 and may occasionally show clinical signs of disease. Infection may manifest as an upper respiratory disease, which usually goes unnoticed; however, sheep may develop pneumonia. Abortions, from which BoHV-1 has been recovered, have been described in sheep and pigs. Sheep, goats, pigs, and wild ruminants are rarely the source of BoHV-1 virus for other animals; it is more likely that cattle transmit the virus to them, and disease is an infrequent event.

When the virus enters the mucosa of the respiratory or genital tract in pregnant animals, it multiplies at that site and is carried to the rest of the body in infected leukocytes and blood to the uterus. It has been postulated that, on reaching the caruncle, the virus passes

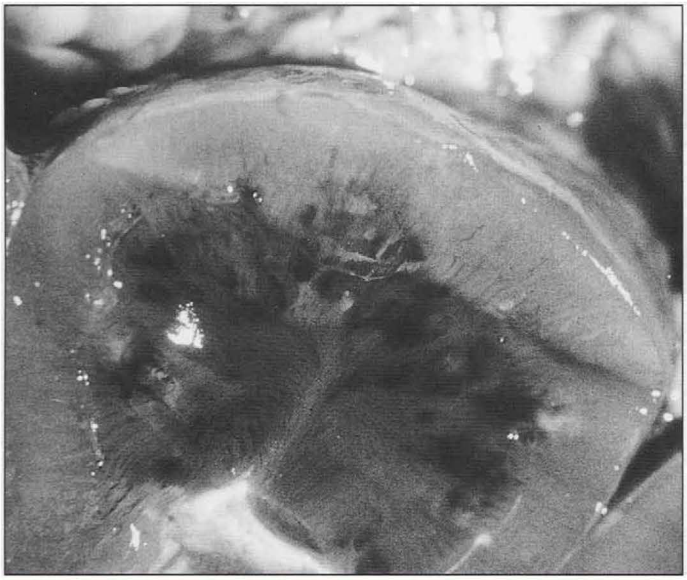

Figure 4.130 Focal renal hemorrhage and necrosis due to *Bovine herpesvirus 1* infection in a day-old calf.

though the trophoblast to the interstitium of the villus, therein infecting endothelium, mesenchyme, and then trophoblast. Death is due to tissue destruction in the fetus and the placenta. Early hatched embryos are also susceptible to IBRV infection.

Abortion rates in a herd of cattle may reach 25%; however, the prevalence of abortion in a region declines with a reduction in the naive population. Most cows infected with BoHV-1 do not abort until 3–6 weeks following the initial infection, and a significantly greater number of cows abort between 5 and 8 months of gestation than earlier. It is speculated the virus can stay in the placenta for a prolonged period before invading the fetus. As the virus invades, the fetus dies quickly with no preparation for delivery. *Expulsion occurs 3–5 days following fetal death, and the carcass is in a state of advanced autolysis.* Rarely, fetal death coincides with the time of normal delivery and the fetus is discharged well preserved.

Gross lesions in the fetus are usually absent or masked by autolysis, but when visible include white to tan 1–3 mm diameter foci of necrosis under the liver capsule and, more rarely, on the surface of the lung. Perivascular renal hemorrhage may be present in addition to focal hemorrhages at the corticomedullary junction in the kidney (Fig. 4.130).

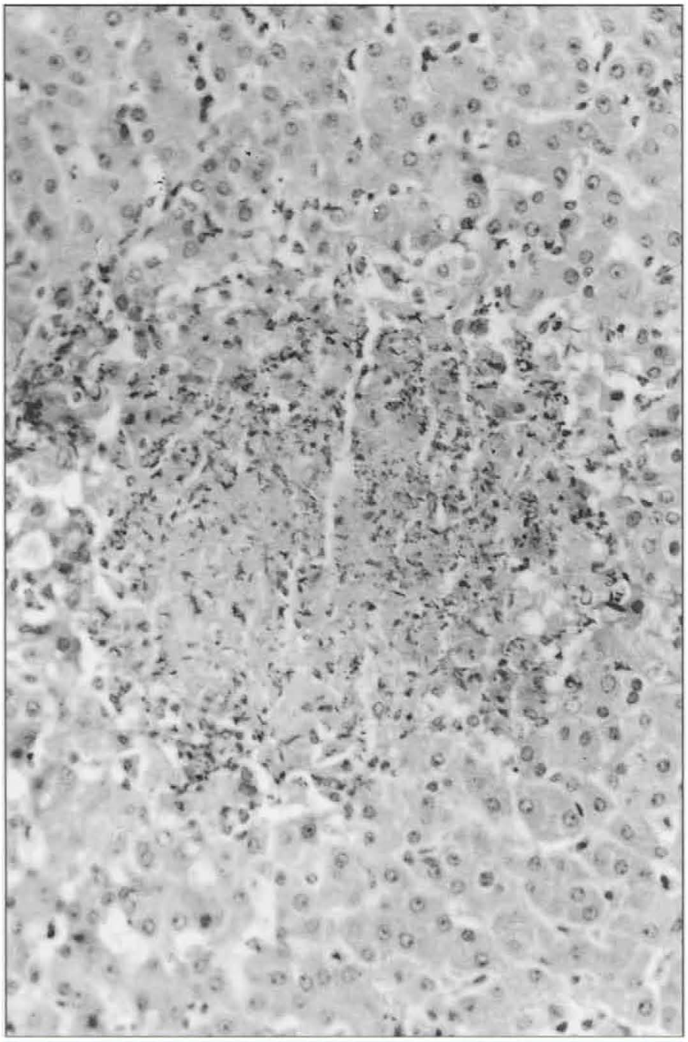

Figure 4.131 Focal hepatic necrosis due to *Bovine herpesvirus 1* infection.

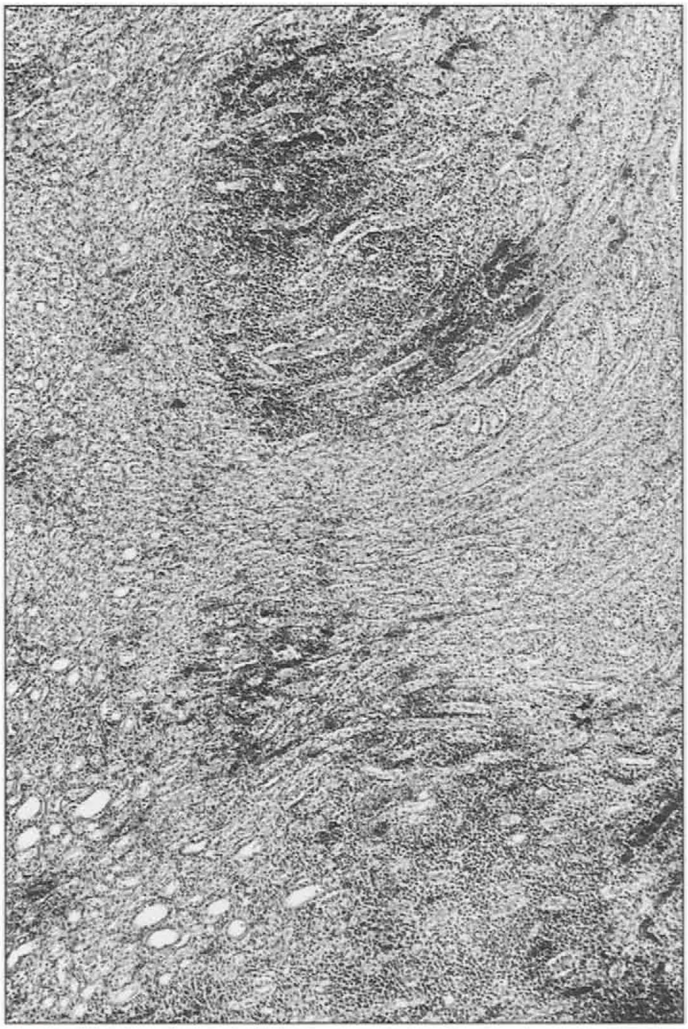

Figure 4.132 Renal necrosis due to *Bovine herpesvirus 1* infection in a day-old calf.

On microscopic examination, foci of necrosis with minimal cellular infiltrate can be seen in many tissues, including liver, adrenal, kidney, intestine, lymph node, lung, and spleen (Figs 4.131, 4.132). As autolysis advances, *inclusion bodies* are difficult to distinguish but are most profitably sought in the adrenal within the more normal cells surrounding a focus of necrosis. Necrotizing vasculitis is consistently present in the small vessels of placental villi (Fig. 4.133).

To confirm the **diagnosis**, the immunoperoxidase test using monoclonal antibodies for BoHV-1 antigen is specific, highly sensitive, can be done on fixed tissue sections, and requires no tissue cultures. Results are also available quickly. Fluorescent antibody techniques on frozen tissue sections of kidney, liver, or placenta, and tissue cultures of these samples can also be used for detection of virus. To identify antibodies to BoHV-1, the enzyme-linked immunosorbent assay has a higher validity in differentiating a negative from a positive sample than the serum neutralization test. With BoHV-1, cows abort such a long time after initial infection that most animals have very low titers to the virus at that time. On an individual basis, therefore, antibody titers are of little value in diagnosis. Also, as the fetus dies quickly, there is usually no evidence of fetal antibody production.

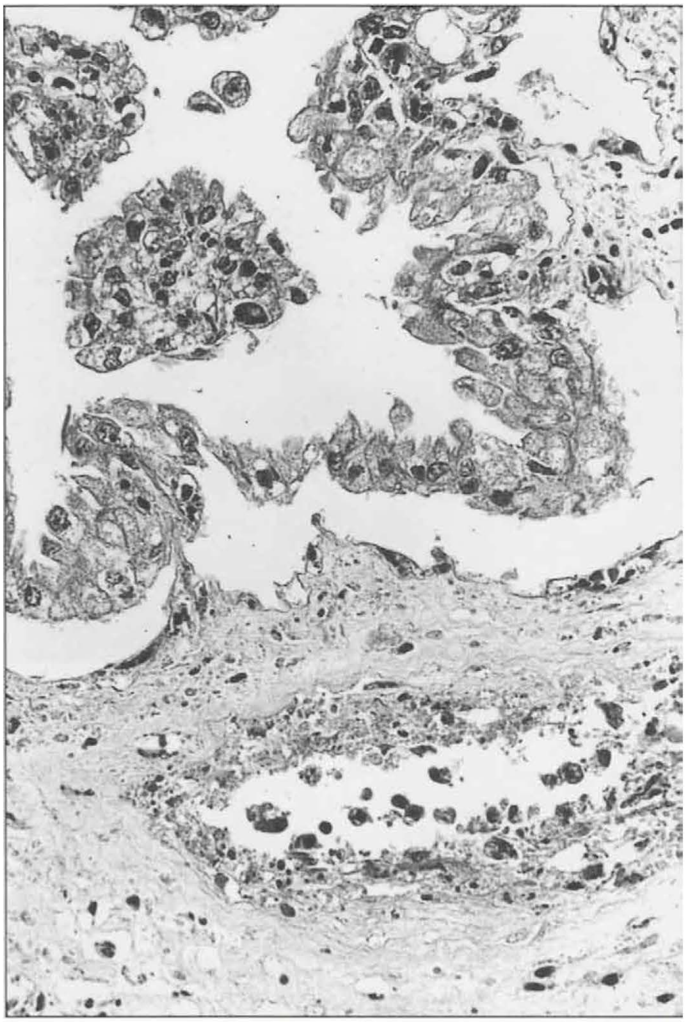

Figure 4.133 Necrotizing vasculitis in the placenta of an aborted bovine fetus, due to *Bovine herpesvirus 1* infection.

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Bovine herpesvirus 4 (cytomegalovirus) infections of the pregnant uterus in cattle

Bovine herpesvirus 4 (BoHV-4, Bovine cytomegalovirus, Movar virus) is in the family Herpesviridae, subfamily Gammaherpesvirinae (cell-associated herpesviruses), genus *Rhadinovirus*. It has worldwide distribution in cattle and has been associated with a wide variety of clinical conditions, including pneumonia, enteritis, metritis, mammillitis, and the disease syndrome "epivag." Epivag is characterized by vaginitis, salpingitis, and oophoritis, or epididymitis.

BoHV-4 is considered an important cause of abortion in cattle; however, experimental evidence confirming a causal relationship is incomplete. The virus is less virulent than BoHV-1, and concurrent infections of BoHV-4 with other pathogens are frequent (up to 75%). It has been suggested that the virus is immunosuppressive and thereby intensifies the effects of other agents. BoHV-4 has been isolated along with bovine viral diarrhea virus and border disease virus from abortions, and with *Mycobacterium paratuberculosis* from animals with Johne's disease.

As the virus is carried within mononuclear blood cells, grows on mucosal surfaces, and is also considered a poor immunogen, it elicits a low level of neutralizing antibody that is also of low avidity. It is transmitted from cow to cow in oral and nasal secretions and, during viremia, is carried to the placenta, where it multiplies and invades the fetus. The virus may remain latent in mononuclear blood cells and in the trigeminal nerve ganglion. Recrudescence can be induced with dexamethasone. Herds tested for BoHV-4 antibodies vary greatly in the proportion of cattle with antibody to the virus.

Mummification and birth of weak pigs have also been reported in association with BoHV-4 infections in sows. Fetal pigs are capable of developing antibody to the virus by 74 days' gestation. Recrudescence induced by dexamethasone has been reported in sheep and pigs, with spread of virus to other animals.

Descriptions of the lesions seen in the aborted bovine fetus are limited to one report. No gross lesions were observed in the fetus, but on histological examination there was thickening of alveolar septa in the lungs, where many large intranuclear inclusions were observed in large alveolar cells. Similar inclusions were also seen in cells of bile duct epithelium, myocardium, spleen, and epithelium of renal tubules. The virus was not isolated from this animal, but the inclusions seen on light and electron microscopy were typical of cytomegaloviruses.

To confirm the **diagnosis**, fetal lung, liver, and kidney should be cultured for the virus, and fluorescent antibody techniques may also be used to demonstrate its presence. Neutralization tests are usually unsatisfactory for the detection of antibody as the virus elicits a low antibody response. The enzyme-linked immunosorbent assay and an indirect immunofluorescent test have been found to be more sensitive and may have value on a herd basis. Antibodies may also be detected in cavity fluid of aborted fetuses and are evidence at least of fetal infection. A nested duplex polymerase chain reaction assay has been used to detect BoHV-1 and BoHV-4 in lymph nodes and peripheral blood leukocytes.

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Equid herpesvirus 1 abortion in horses

Equid herpesvirus 1 (EHV-1, Equine abortion virus) is in the family Herpesviridae, subfamily Alphaherpesvirinae, genus *Varicellovirus*. It is widespread throughout the world and causes respiratory, neurologic, and generalized neonatal disease, as well as abortion. The virus can be separated into two distinct subtypes (1 and 2) on the basis of restriction endonuclease cleavage of the viral DNA. Both subtypes cause respiratory disease, abortion, and neonatal disease. Subtype 1, however, causes a more severe (but less frequently encountered) respiratory disease than subtype 2, and is the more frequent isolate from aborted foals and neonatal disease. Also, it is probably the only type causing neurologic disease. Subtype 2 is commonly isolated from mild upper respiratory disease in horses and less so from abortion. Respiratory disease due to EHV-1 is common in some areas, whereas abortion is rare; this may be explained to some extent by the presence of a subtype less likely to produce abortion but capable of producing respiratory disease.

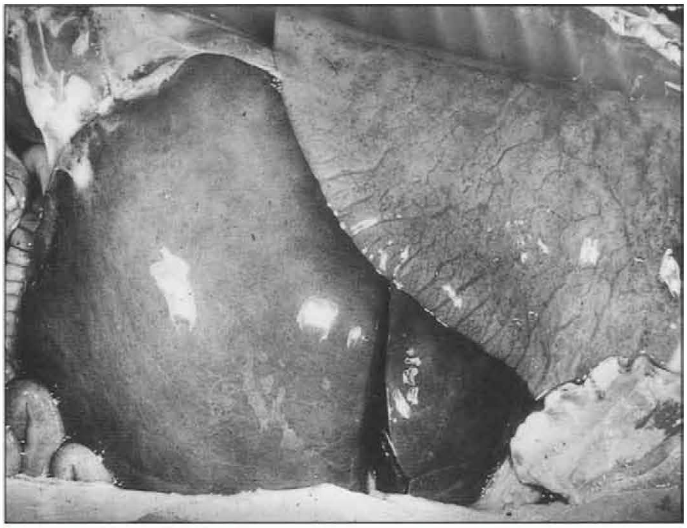

Figure 4.134 Pulmonary edema in an aborted equine fetus, due to *Equid herpesvirus 1* infection.

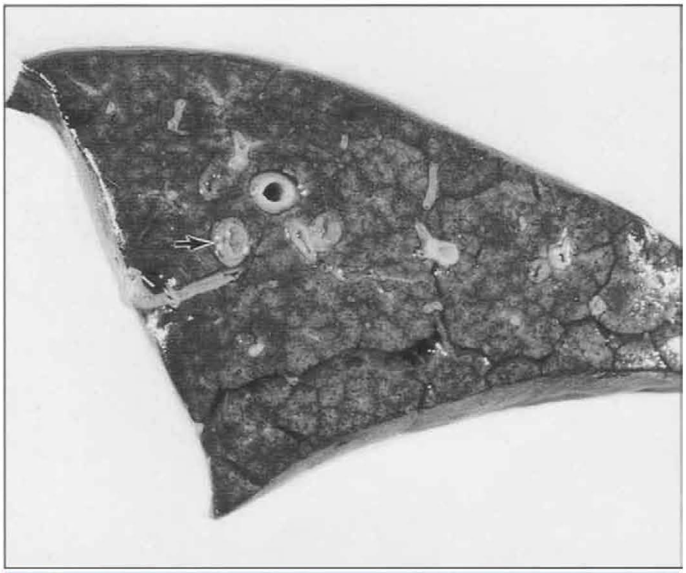

Figure 4.135 Fibrin casts in fetal bronchi, due to *Equid herpesvirus 1*.

EHV-1 is very widespread, and first exposure usually occurs before the foal is 1 year old. Mild-to-severe upper respiratory disease is produced, and secondary bacterial infection, frequently with *Streptococcus zooepidemicus* or other streptococci, is common. As the virus is so widespread and contact so likely at an early age, abortion usually occurs in an animal that has had previous experience with the virus. As with herpesviruses generally, the level of immunity is low, and the virus is transported in leukocytes through the bloodstream to the placenta and hence the fetus. Death of the fetus does not occur until the onset of the usually prompt and uncomplicated abortion. The dam shows no premonitory signs and the fetus is aborted in a fresh state. The time from exposure – whether by recrudescence of latent virus or reinfection – until abortion varies from 9 days to several months. Respiratory disease in the mare is usually not observed following the infection that results in abortion. It is reported that 95% of the abortions due to EHV-1 occur in the last 3 months of pregnancy, and naturally acquired infection has not been observed to produce abortion before 5 months of gestation. Exactly where the virus is, and in what state, during the protracted incubation period has not been determined.

The aborted fetuses may show *characteristic and diagnostic lesions* that are variable in their development and may be modest. Edema of the subcutis and fascia and accumulated amber fluid in the body cavities are common in aborted fetuses. There may be slight general icteric discoloration and meconium staining of the footpads and amnion. The most consistent gross lesion is *severe pulmonary edema*. The lungs are heavy and rubbery, show the impressions of the ribs, and exhibit a pitting response to pressure (Fig. 4.134). There is also edema of the interlobular septa. Their color may be darker or lighter than normal, and tan-to-white foci of necrosis (2–4 mm in diameter) and petechial hemorrhages may be visible on the surface. Casts

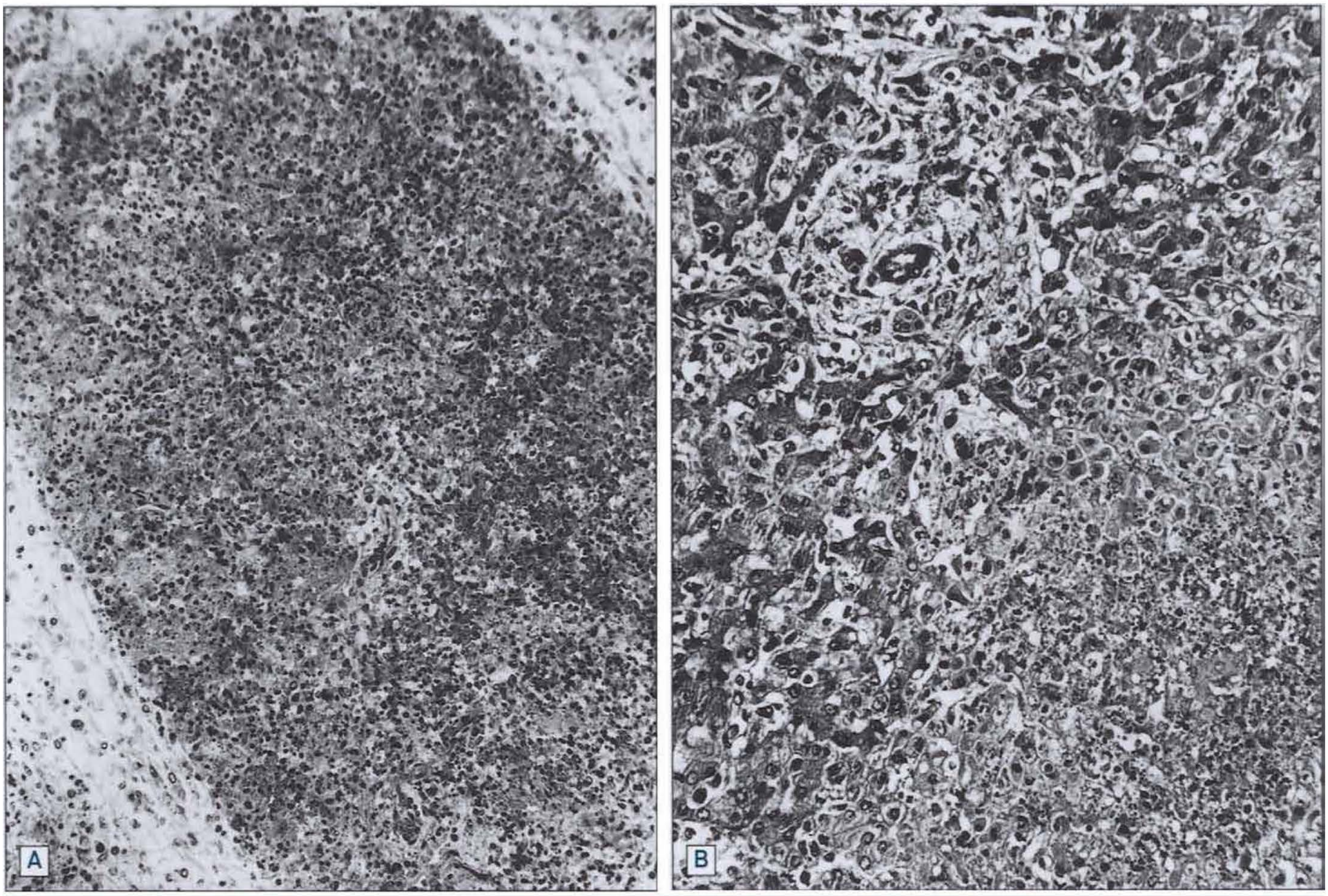

Figure 4.136 *Equid herpesvirus 1* abortion of an equine fetus. **A.** Acute necrotizing **pneumonia** in aborted equine fetus. **B.** Acute **hepatitis** with focal necrosis.

of fibrin are occasionally present in the bronchi (Fig. 4.135) and, rarely, in the trachea. Beneath the capsule of the liver there are, in about 50% of aborted fetuses, gray-to-white foci of necrosis varying in size from minute up to 5.0 mm in diameter. Such foci may be few or numerous. The spleen is usually enlarged, with petechial hemorrhages on the capsule and unusual prominence of the follicles on cut surface. Petechial or ecchymotic hemorrhages may occur anywhere, but chiefly in the upper respiratory mucosae. Occasionally, there is hemorrhagic necrosis of the renal cortices.

Histologically, the pulmonary interlobular septa are edematous and infiltrated with mononuclear inflammatory cells. The edema and spotty necrosis and hemorrhage involve the whole organ uniformly; there is fibrinous alveolar exudation and necrosis of bronchial and alveolar epithelial cells (Fig. 4.136A). *The acidophilic inclusion bodies found in the nuclei of the bronchial and alveolar epithelium are specific.* The foci of hepatic necrosis are not so common as the changes in the lungs: they are often minute and may be missed in a section. Acidophilic inclusion bodies also form in the nuclei of hepatic parenchymal cells, but they are not constant and are never numerous. If present, they can usually be found around the areas of focal necrosis. There is edema of the liver, and leukocytic infiltration in the necrotic foci and portal triads is common (Fig. 4.136B). Rarely, there is a diffuse hepatitis without focal necrosis. Necrosis of germinal centers occurs in the enlarged splenic follicles and other lymphocytic tissues, including thymus. Intranuclear inclusion bodies may be found in the primitive reticular cells in such foci. There are hemorrhages in the splenic pulp and about the malpighian corpuscles. The placenta is normal.

Foals infected with this virus in utero may be born alive at, or near, term. Whether any survive or not is not known. Many of them die in the first few days with severe interstitial pneumonia and secondary bacterial septicemia. Focal hepatic necroses are, as a rule, not present in these animals; however, focal necrosis of crypt epithelium with hemorrhage in the intestine is sometimes observed (Fig. 4.137).

The **diagnosis** of abortion can be made on observing typical microscopic lesions, including the presence of inclusions. The demonstration of EHV-1 on cell cultures from samples of lung, liver, spleen, or thymus or by the immunoperoxidase technique on placenta is definitive. Determination of the specific subtype may be accomplished using monoclonal antibodies. Serological examination for antibody is of little use in the diagnosis of abortion as most animals are exposed to the virus at several intervals in their life and abortion may occur too long after the last exposure, making interpretation impossible, even on the basis of paired samples.

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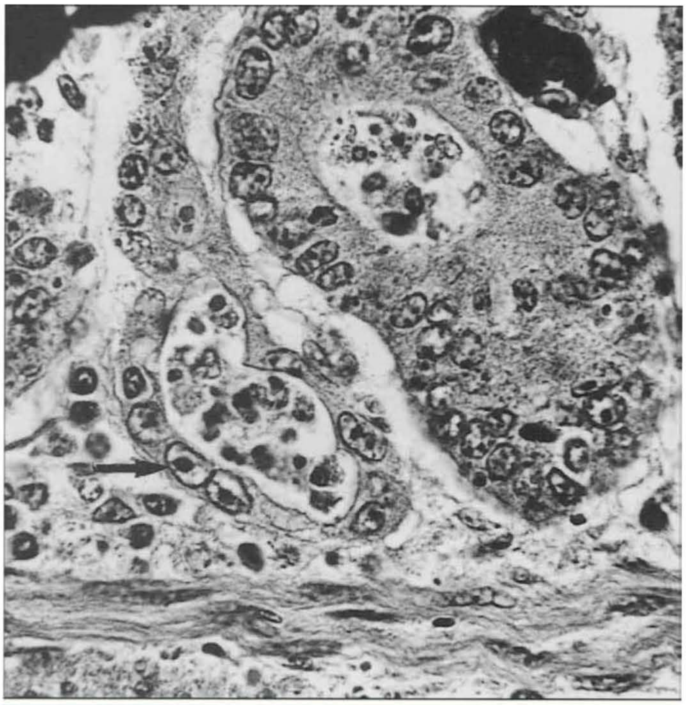

Figure 4.137 Intranuclear inclusions (arrow) and necrosis in intestinal crypts in a 2-day-old foal, due to *Equid herpesvirus 1* infection.

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Akabane virus infections of the pregnant uterus

Akabane virus (AKAV) is in the family Bunyaviridae, genus *Orthobunyavirus*, Simbu serogroup. In Australia, the Simbu serogroup includes Akabane, Tinaroo, Peaton, Aino, Douglas, Thimiri, and Facey's Paddock viruses; Akabane and Tinaroo viruses are naturally occurring reassortants. *Akabane disease*, commonly known as *enzootic bovine arthrogryposis and hydranencephaly* (ag/he), occurs in Australia, Japan, Korea, Israel, and Kenya. Evidence of disease in postnatal cattle, sheep, and goats is minimal; only slight leukopenia in goats and mild keratoconjunctivitis in cattle have been reported, plus one report of encephalomyelitis in cows. In contrast, infection of nonimmune pregnant sheep, goats, and cows has resulted in extensive losses in Japan, Israel, and Australia.

AKAV is transmitted by various insects, including the hematophagous midge *Culicoides brevitarsis* in Australia, and various species of mosquitoes, including *Aedes vexans, Culex tritaeniorhynchus,* and *C. pipiens*. Losses are seasonal and confined geographically by the range of the insect. Animals beyond this range constitute a naive population, and if for some reason infected insects invade this area, extensive losses may occur.

Infections by AKAV before 30 days in gestation can produce multiple congenital abnormalities and can result in normal or malformed fetuses that may be aborted, mummified, or delivered alive or stillborn, early, on due date, or late. Many are delivered with difficulty because of the congenital malformations. Anomalies observed include microcephaly, porencephaly, hydranencephaly, hydrocephalus, hydromyelia, arthrogryposis, kyphosis, lordosis, scoliosis, brachygnathia, and muscle atrophy. Additional abnormalities reported in lambs include spina bifida, cyclops, Arnold–Chiari syndrome, and anal atresia. When the fetus is infected between 41 and 50 days' gestation, thick perivascular cuffing of macrophages, lymphocytes, and plasma cells is observed, coupled with very prominent glial nodules. The large variety of central nervous system malformations caused by AKAV is attributed to differences in the fetal age at infection, the vulnerability of neurons to direct injury, and the nature of tissue response to such injury. Affected lambs are reported to weigh significantly less than normal lambs, even though gestation length overall is slightly extended. Infection of the fetus after 90 days' gestation is less severe; however, there are inflammatory lesions without malformation in the brainstem.

AKAV is carried by a midge or mosquito from a viremic to a susceptible animal, and at no time does the dam show clinical signs of infection. Viremia lasts for about 4 days, during which time the virus invades the placenta, replicates in trophoblastic cells, and finally crosses the placenta and infects the fetus. If AKAV is inoculated immediately after the integration of the placental cotyledon with the maternal caruncle, viral invasion of the chorioallantoic membrane occurs rapidly. Lesions in the brain and possibly in muscle are probably due to the direct cytopathic effect of the virus on developing neurons. A regular sequence of events follows the primary insult of polioencephalitis; focal necrosis in the brain may progress to malacia and, depending on the extent of the injury and time, to porencephaly and hydranencephaly. Neuronal death in brain and ventral spinal cord motor neurons is followed by neurogenic atrophy of muscle and arthrogryposis.

Necropsy findings in affected species are similar. *In the brain, gross lesions have a broad range, including microcephaly, hydrocephalus, porencephaly, and hydranencephaly.* Cystic cavities vary from being visible only microscopically to having almost complete loss of parenchyma. The cerebellum may be normal to markedly atrophic. Microscopically, there is encephalitis with macrophages, plasma cells, and lymphocytes infiltrating areas of necrosis and accumulating around vessels. Lesions are sometimes most prominent in the brainstem and spinal cord. The atrophic cerebral cortex may have multifocal areas of necrosis with accumulations of glial cells and perivascular mononuclear cells. In the cerebellum, there may be loss and malpositioning of Purkinje cells and reduced size of molecular and granular layers.

Lesions induced in the *brain* early in gestation, because of extensive destruction of cells, may proceed to hydranencephaly with subsequent arthrogryposis. Lesions produced late in the first trimester usually consist of multifocal encephalitis and proceed only to porencephaly. Infections late in the second trimester may produce lesions consisting of multifocal necrosis and gliosis with less cyst development.

Lesions in the *spinal cord* can be bilateral or unilateral and consist of mineralization of meninges, atrophy of the cord, and hydromyelia. Necrosis and depletion of ventral horn motor neurons may proceed to cavitation. Light or heavy accumulations of perivascular lymphocytes may be present throughout the cord with decreased numbers of axons and hypomyelination in the lateral and central tracts.

Joints of the front legs are more commonly affected than those of the hind, and both may be fixed in either flexion or extension. There is slight-to-marked atrophy of skeletal muscle groups in affected limbs. The muscles are edematous, pale, and flabby to firm. On microscopic examination of muscle, sarcoplasmic nuclei are either swollen or pyknotic. Loss of striations, granular degeneration, and empty sarcolemmic cylinders may be present. Replacement of muscle by connective tissue and fat occurs finally. Well-preserved fibers may be present adjacent to areas of complete loss of muscle mass. Polymyositis, from which the virus can be isolated, has been reported in some infected fetuses.

The **diagnosis** of AKAV infection may be made by demonstrating virus or antibody in affected animals. Virus may be recovered from various fetal tissues and fluids, including brain, spinal cord, cerebral fluid, skeletal muscle, chorioallantois, and amnion. It may be grown on hamster lung cells and can cause reduced litter size, transplacental infection of the fetus, and birth of live, weak young. AKAV can also grow in the brain of intracranially inoculated suckling mice. As the fetus is capable of producing antibody from an early stage of gestation, inferred evidence of the cause of malformation and abortion may be determined by finding specific antibody to AKAV in fetal cavity fluids, including fluids from stored tissues or precolostral blood samples from affected newborn animals.

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Aino virus infections of the pregnant uterus in cattle

Aino virus (AINOV, syn. *Shuni virus*) is closely related to *Akabane virus*, and is similarly in the family Bunyaviridae, genus *Orthobunyavirus*, Simbu serogroup. As with AKAV, AINOV is mainly transmitted by the midge *Culicoides brevitarsis* – but has also been isolated from mosquitoes, including *Culex tritaeniorhynchus* and a mixture of *C. pipiens* and *C. pseudovishnui*. The range of the organism, as determined by serum antibody titers, extends along the northern and eastern coasts of Australia (from just within Western Australia to central New South Wales) and is well within the range of *C. brevitarsis*. The virus is also present in Japan. Using antibody titers as evidence of previous infection, fewer species overall and fewer animals within affected species appear to be infected with AINOV compared to AKAV.

Cattle that are infected do not have clinical signs; however, insofar as precolostral antibodies to the organism are found in deformed newborns, *the organism is capable of crossing the bovine placenta and causing abortion*. Lesions consist of placental edema and necrosis, renal tubular necrosis with interstitial lymphoplasmacytic infiltrates, nonsuppurative meningitis, and interstitial pneumonia with mononuclear cells and some neutrophils. Experiments conducted with sheep indicate that, while the virus can infect the fetus after direct inoculation, it does not cross the placenta after inoculation into the pregnant ewe.

Lesions induced in young calves following intracerebral inoculation of AINOV and AKAV are reported to be different. With AINOV, nonsuppurative encephalomyelitis developed first in the cerebrum adjacent to the ventricles and spread to the medulla and the brainstem, but remained mild in the spinal cord. Using animals inoculated with AKAV as controls, nonsuppurative encephalomyelitis was produced in the brainstem and spinal cord only. Intravenous inoculation of virus into susceptible pregnant cows at various stages of gestation may give a more valuable comparison.

Diagnosis is based on immunohistochemistry or fluorescent antibody testing on frozen sections or smears of homogenates of fetal kidney, lung, and liver. Prospective serology on cows at pregnancy diagnosis and at abortion may be useful. Aino and Akabane bunyaviruses can be differentiated by nested polymerase chain reaction.

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Cache Valley virus infection of the pregnant uterus in sheep

Cache Valley virus (CVV, syn. *Bunyamwera virus*) is in the family Bunyaviridae, is a serotype of *Bunyamwera virus*, and is widely distributed in North America. Serological data indicate it infects pigs, horses, raccoons, foxes, and domesticated and wild ruminants, including cattle, sheep, caribou, and deer, as well as humans. It is believed to be spread by insects, and has been isolated from a variety of mosquitoes and midges, including *Culiseta inornata, Psorophora columbiae, Aedes sollicitans, Ae. taeniorhynchus, Anopheles grabhamii, An. crucians, An. uadrimaculatus*, and *Culicoides* spp.

There is considerable evidence supporting CVV as the cause of a severe epizootic in a flock of sheep in Texas where, of 360 lambs delivered, 92 were mummified, stillborn, or born with congenital anomalies. Sixty-nine lambs had *arthrogryposis and hydranencephaly*. All of the ewes bearing affected lambs had neutralizing antibody to CVV and affected lambs had precolostral antibody to the virus. In the months before pregnancy, 5% of ewes had antibody to the virus and after pregnancy, 63.4% were positive. Following the outbreak, a sentinel sheep was placed in the area and CVV was eventually recovered from a blood sample.

Examination of 15 lambs stillborn at term during the epizootic revealed that central nervous system, and muscle and joint lesions were frequently present in the same animal, and lesions between lambs mainly differed in severity. Gross lesions in limb joints consisted of articular rigidity with articulations held in the flexed position. The limbs appeared long for the size of the body. In addition, kyphosis, scoliosis, and torticollis were frequently observed. Muscles associated with the affected limbs and spine were described as shrunken, pale, and firm. Lesions in the brain consisted of *hydrocephalus, porencephaly, and hydranencephaly*. The cerebrum, cerebellum, and spinal cord were often markedly reduced in size and the cerebellum was occasionally mildly lissencephalic.

Microscopic lesions were observed in skeletal muscles, brain, and two placentas. In affected muscle, fibers were small, lacked striations, and nuclei were sparse. Fibers were separated by loose connective tissue and adipocytes; rarely, a few neutrophils and mononuclear cells were present. Histologic examination of the brain confirmed gross findings, and cavities not visible grossly were occasionally observed. In regions of marked hydranencephaly, the cerebral cortex was reduced to a few layers of neurons within the meninges. Entire lobes of the cerebellum were often absent but adjacent lobes were often near-normal, with only minor loss of cells in the molecular layer. The density of axons in the spinal cord was markedly reduced, especially in dorsal tracts, and, whereas ventral horn neurons were only slightly reduced in the cranial cord, hardly any remained in caudal portions. Inflammatory cells were scant in lesions, except in two of the five placentas examined wherein a mild perivascular neutrophil infiltrate was evident.

Inoculation of 36 ovine fetuses in utero with CVV has added further credibility to the association of CVV as the cause of the epizootic. Following in utero inoculation of the virus, several of the fetuses developed arthrogryposis and hydranencephaly; some were mummified, others died and were absorbed, and a few had oligohydramnios. Lambs infected at 27–35 days of gestation had the greatest mortality rate, while lambs infected between days 36 and 45 of gestation had more congenital anomalies. Virus was recovered from fetuses before 69 days but not after 76, regardless of when they were infected, and this was coincident with the development of neutralizing antibody in the fetus. The virus was recovered from allantoic fluid in 11 of 17 fetuses euthanized at less than 76 days' gestation.

The **diagnosis** of CVV infections resulting in fetal loss and congenital anomalies in lambs is most easily accomplished by examining precolostral serum or cavity fluids of lambs with arthrogryposis and hydranencephaly for antibodies to CVV. Attempts to demonstrate virus in tissues of lambs born with congenital abnormalities may be futile, as by the time of birth the virus is rarely present. Differential diagnoses should include infection by *Akabane virus*, as the lesions are remarkably similar.

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Rift Valley fever virus infections of the pregnant uterus in cattle and sheep

Rift Valley fever virus (RVFV) infects many domestic, wild, and laboratory animals and humans. It occurs in at least 18 countries over a 6700-km north–south range in Africa, plus Saudi Arabia. *Infection by RVFV causes a disease characterized by rapid onset and high mortality in lambs, low mortality in adult sheep and calves, and even lower or no mortality in cattle. There is frequently a high rate of abortion, approaching 100% in sheep and cattle.* People may also become infected and develop influenza-like illness; mortality is generally low, but morbidity is high and the degree of debility often extreme. *Hepatic necrosis is the prominent lesion, and occurs in all affected species and age groups, including the fetus.* Host resistance to fatal hepatic disease has been shown in laboratory animals to be inherited as a simple Mendelian dominant gene. Susceptibility is age- and sex-related; the young are more susceptible, and males less resistant than castrates and females. Although two-thirds of sheep are genetically resistant to fulminant hepatic disease, *sheep are regarded as the most susceptible domestic animal.*

The virus is in the family Bunyaviridae, and is the type species of genus *Phlebovirus* (Zinga virus is a strain of RVFV.) *It is transmitted primarily by mosquitoes*, and *Culex pipiens* is a major vector in parts of Africa. Generalized infection in the mosquito is necessary before transmission occurs effectively, and movement of virus from the insect gut is facilitated by the presence of microfilariae in ingested blood. *Aedes* spp. are capable of transovarial transmission. *Aedes albopictus*, a strain of mosquito in the USA, can transmit the virus and movement of animals either incubating infection or in viremia raises the possibility of international spread of the virus. Biting midges and ticks are also implicated in transmission. The virus probably does not spread from animal to animal in the absence of insects; however, experimentally the virus can be transmitted by just about every route, including aerosols or application into nasal passages, to scarified skin or on the conjunctiva. *Aborted fetuses and membranes have a very high titer of virus.* Epizootics often follow a period of heavy rain, particularly after drought, and outbreaks may be separated by periods of 10–15 years without a case.

In mature sheep or cattle, the disease is manifest in *peracute, acute, subacute, chronic, and convalescent forms.* In sheep, the subacute form, in which the animal is febrile for 24–96 hours, is common, and up to 100% of pregnant ewes can abort during this phase or when convalescing. In cattle, the peracute form is rare and abortions occur during the acute or convalescent period and vary from 10 to 85% of pregnancies. Deaths in cows are uncommon. Infertility occurs in both sheep and cattle and may follow retention of fetal membranes and endometritis. Signs are unpredictable in goats. They tend to be less susceptible than sheep, but if infected can abort.

During viremia, the organism is carried to the placenta and fetus, where *the liver appears to be the primary site of multiplication.* Hepatocyte destruction is probably related to the repeated cycles of virus growth and release of virus from infected cells.

Lesions are much the same in cattle or sheep fetuses and, except for one report where experimental inoculation of pregnant ewes with an attenuated RVFV resulted in hydranencephaly and arthrogryposis, *infection by RVFV usually results in death of the fetus* in utero. This occurs at any gestational age and, as it occurs rapidly, the fetus is aborted in an autolysed state. On gross examination, the liver is swollen and discolored orange to brown and occasionally to dark red. Microscopically, the most consistent lesion seen in almost 100% of fetuses examined is *multifocal to massive hepatic necrosis.* A few degenerating neutrophils may be present in these lesions. *Acidophilic intranuclear inclusions* of various shapes (fusiform, round, oval) are frequently present in hepatic cells of ovine fetuses and are helpful in the diagnosis. These are seen less often in bovine fetuses and may be difficult to find when autolysis is advanced. Mineralization of individual or clusters of hepatocytes is also common. Cholestasis may be evident in canaliculi. Other lesions reported include degeneration of lymphocytes in spleen and lymph nodes, and degenerative lesions in heart muscle and renal tubules.

The hepatic lesion is considered diagnostic; however, in areas where the condition is not endemic, definitive **diagnosis** depends on identification of the virus. It can be detected early in infection in blood or serum by reverse transcriptase-polymerase chain reaction. It is abundant in fetal tissues and placenta and may be cultured or detected using the indirect fluorescent antibody technique on frozen sections of liver, or by immunohistochemistry. Differential diagnoses in sheep include infections by *bluetongue virus* and *Wesselsbron virus.*

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Circovirus postweaning multisystemic wasting syndrome

Porcine circovirus type 2 (PCV2), family Circoviridae, genus *Circovirus*, is associated with the postweaning multisystemic wasting syndrome (PMWS), and appears to act with other cofactors that cause stimulation of the pig's immune system. Antibody to the agent has been demonstrated for several years and it is suspected that a new highly virulent PCV2 virus has recently emerged in several countries. Clinical PMWS has not been reported in Sweden; however, antibodies to PCV2 are present in many Swedish pigs tested. PMWS was first reported in western Canada in 1991 and has now been found in the USA and in many European and Asian countries. *The disease primarily affects pigs 5–18 weeks of age and is characterized by failure to grow and eventual emaciation.* Affected pigs may have stomach ulcers in addition to a wide variety of lesions, including pneumonia and diarrhea.

A *reproductive failure syndrome* has been observed in several herds and is characterized by late-term abortions of mummified, macerated, or autolysed piglets, and piglets born weak or stillborn. Lesions in these piglets included enlargement of the liver, congestive heart failure, with excess fluid in the thorax and abdominal cavities. The heart may be enlarged and irregular in shape and on histological examination extensive areas of myocardial degeneration, edema, and necrosis with mild fibrosis and diffuse moderate infiltrations of lymphocytes and macrophages may be observed. The virus can be demonstrated by immunohistochemistry in heart muscle cells. No other viruses were detected in this investigation. Stress factors and co-infections with other porcine viruses were not detected; however, environmental, husbandry, and genetic factors may be important in the progression of clinical PMWS.

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MISCELLANEOUS LESIONS OF THE POSTPARTUM UTERUS

Endometrial cysts, *adjacent to caruncles, develop in some cows and ewes during the process of uterine involution* (Fig. 4.138). Adhesion of the caruncular stalk to adjacent glandular tissue causes blockage of the underlying glands. The retention cysts enlarge progressively with age, and care must be taken to differentiate them from estrogen-linked cystic hyperplasia.

Subinvolution of placental sites in the bitch is manifested clinically by a prolonged, blood-tinged vaginal discharge. In the normal bitch, overt uterine bleeding usually ceases within 7–10 days following whelping, but the affected bitch may bleed for several weeks or months. In some cases, the blood loss causes severe anemia and occasionally death. *Ellipsoidal enlargements*, located in the areas of previous placental attachment, are evident in the uterine cornua (Fig. 4.139). The endometrium in the affected areas is hemorrhagic, irregularly thickened, and gray to brown. The endometrium between the enlargements is normal in appearance. The surface epithelium is often detached, but when present, it has heavily vacuolated cytoplasm, indicating progestational stimulation. Corpora lutea are invariably present, but progesterone levels have been low in the few cases in which the determination was made. The uterine mass adjacent to the uterine lumen is composed of an admixture of amorphous eosinophilic debris, thrombotic material, degenerating placental site tissue, and regenerating endometrium (Fig. 4.140). The deeper tissue is permeated with numerous irregular-shaped cells with large nuclei

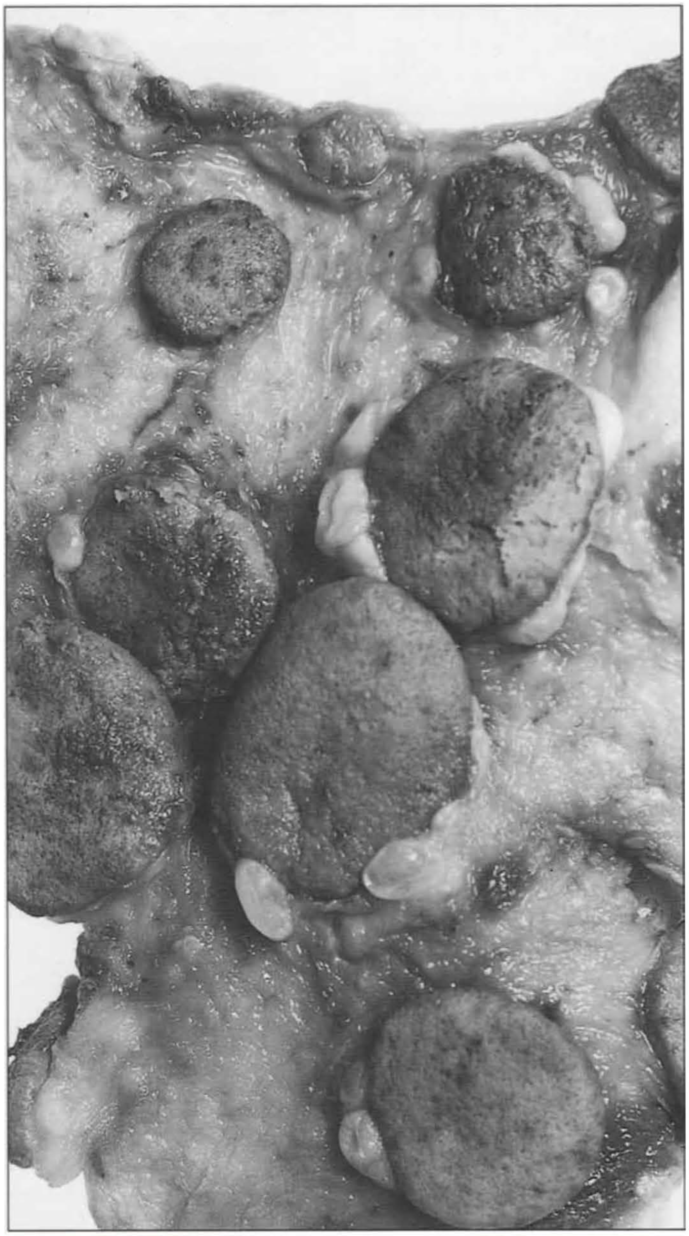

Figure 4.138 Endometrial cysts in a cow.

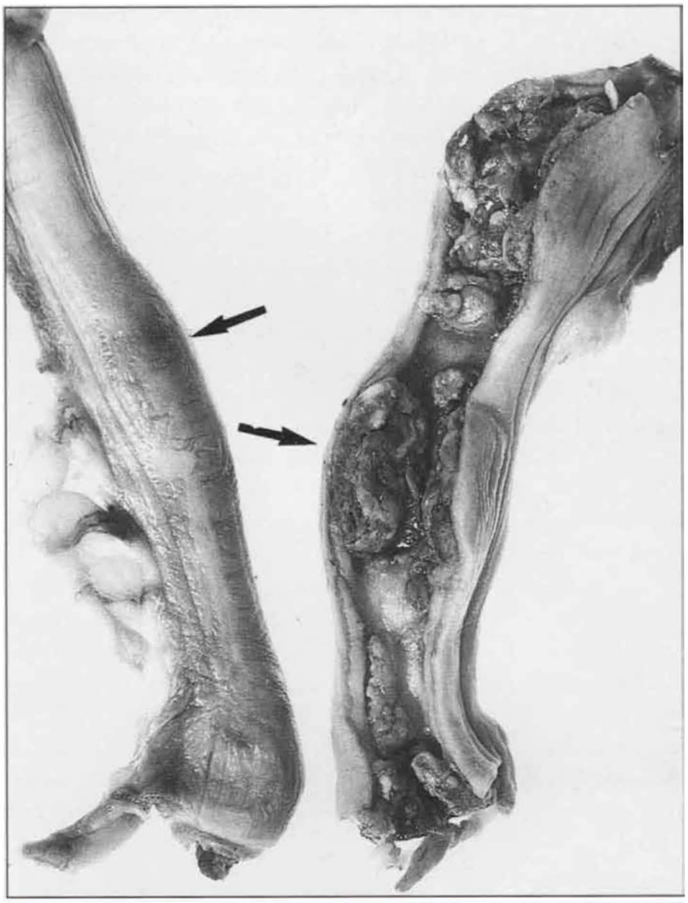

Figure 4.139 Subinvolution of placental sites in a bitch. Note fusiform enlargement of uterine horns at sites of failure of involution (arrows).

and abundant vacuolated cytoplasm (Fig. 4.141). They are interpreted as syncytial trophoblasts or alternatively as decidual cells, and are far more numerous than in the normal gravid uterus. Some degree of invasion of the myometrium by these cells is not unusual, and in some cases there is perforation of the serosa, allowing uterine contents to escape into the peritoneal cavity. The condition appears to be more prevalent in young bitches, but *the cause has not been established.*

Pathology of the cervix, vagina, and vulva

PATHOLOGY OF THE CERVIX

Cysts of the cervix occur in cows, and probably all are *retention cysts* formed by fusion of the rugae. Loss of the original epithelium of the rugae is first necessary and may be incidental to the lacerations of parturition, artificial insemination, or inflammation. The cysts are usually small and not significant. Larger ones may cause partial occlusion of the cervical canal, but this is seldom of importance.

Stenosis of the cervix is extraordinary. It is *acquired* rather than congenital, and consists of fusion across epithelial surfaces and scarification. It may follow severe laceration or long-standing inflammation.

Cervicitis is not in itself an entity but, instead, is an *extension of endometritis or vaginitis*. The mucus-secreting epithelium provides good defense against bacterial invasion and, if the epithelium breaks down, it exposes densely arranged connective and muscular tissues, which are not especially sensitive to the actions of bacteria. In the cow, the epithelium lining the external os and the adjacent few rugae is of the simple vaginal type, and this is more susceptible to irritation than the mucus-secreting surface cranial to it. Most inflammations then are superficial, but there are some exceptions, such as necrobacillosis. The circumstances in which cervicitis occurs have been mentioned in the section on pathology of the uterus or will be mentioned

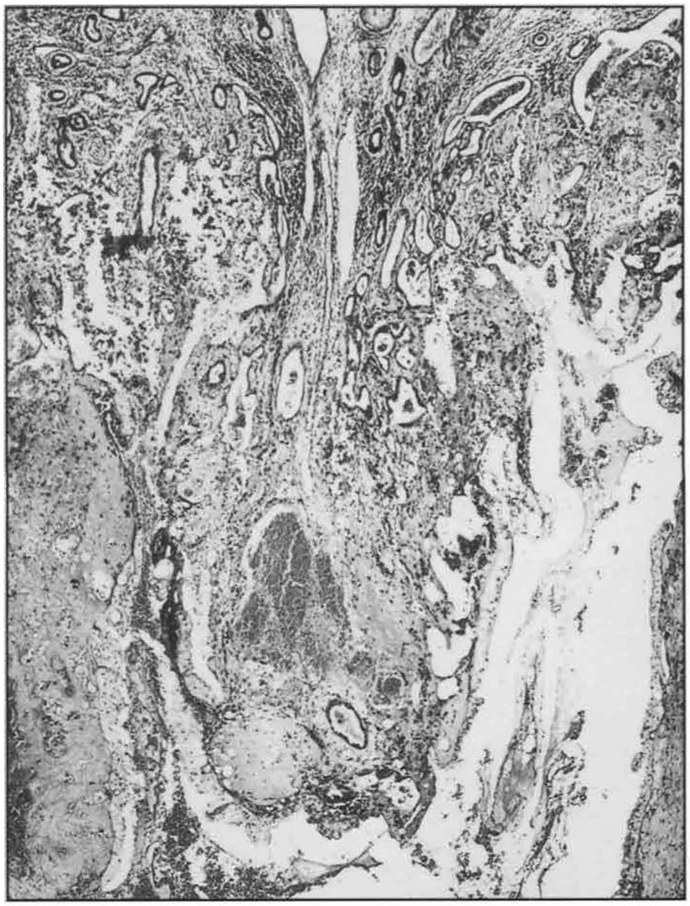

Figure 4.140 Subinvolution of placental sites in a bitch. Note hemorrhage and necrotic debris in pedunculated endometrium.

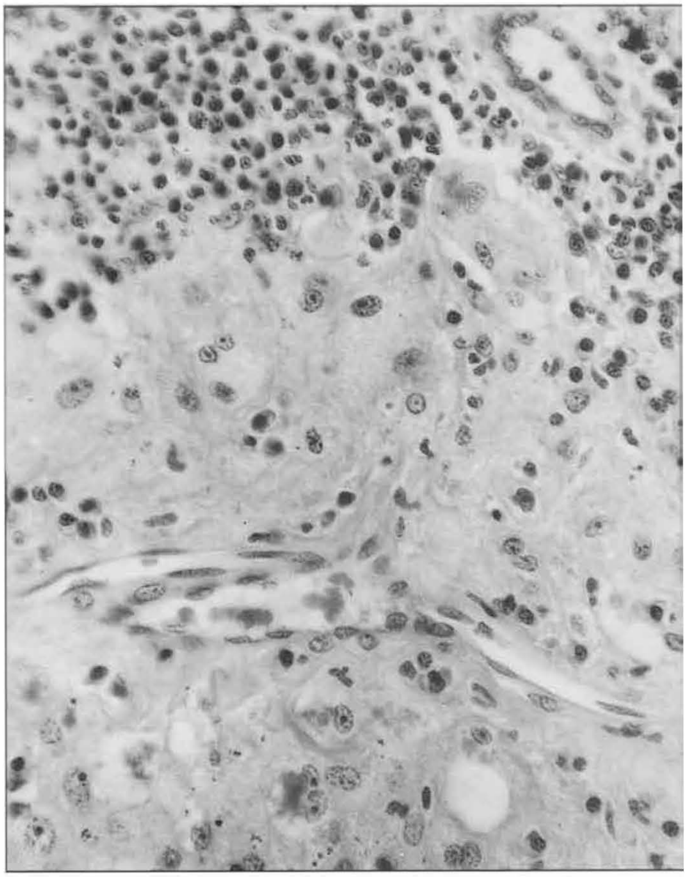

Figure 4.141 Decidual cells and surrounding inflammatory cells deep in the placental site, in subinvolution of placental sites.

under pathology of the vagina and vulva, below. The usual form of bovine *simple cervicitis* is seen as a swelling of the caudal annular rugae, which are edematous and hyperemic. They soon protrude through the external os into the vagina, and thin mucopurulent exudate accumulates between the folds and collects in the vagina. Histologically, there is epithelial degeneration and desquamation and infiltration by inflammatory cells that are predominantly mononuclear. Neutrophils penetrate the epithelium and become mixed with mucus, or in more acute cases there may be frank suppuration. Cervicitis is not usually more severe than this, although in older cows the prolapse and tumefaction of the rings may be grossly obvious. Although degrees of prolapse result from inflammation, they may also predispose to it. Slight but progressive degrees of eversion of the cervical rings occur with succeeding pregnancies to expose portions of the cervical mucosa to the contaminated environment of the vagina. *Chronic cervicitis* may lead in time to enlargement and induration of the cervix with some stenosis, but enlargement is not a criterion of inflammatory sclerosis. Discrete *cervical abscesses* or *suppurative fistulous tracts* occasionally result from accidental injury acquired during uterine irrigation or artificial insemination.

PATHOLOGY OF THE VAGINA AND VULVA

Cysts in the vagina or vulva are not important in themselves, but they do have some diagnostic significance. They occur as *cystic dilations in Gartner's ducts and Bartholin's glands* in cattle. Gartner's ducts are remnants of the embryonic mesonephric (Wolffian) ducts, which lie, one on each side of the floor of the vagina, beneath the epithelium. They are invariably present to some degree in cattle, although, when normal, they are only detectable microscopically as discontinuous ducts lined by simple epithelium and found in the cranial vagina and disappearing caudally. They become cystic in cows poisoned with highly chlorinated naphthalenes, in cows with ovarian follicular cysts, and occasionally following acute vaginitis. With lesser degrees of dilation, the cysts are more readily palpable than visible, but in well-developed instances they are clearly visible through the elevated and thinned vaginal wall (Fig. 4.142). The cysts may be isolated or they may have a string-of-beads disposition, or the whole duct may be present, dilated to 1–2 cm and tortuous. *Cysts of Bartholin's glands* occur under the same stimuli, but chiefly as a consequence of inflammation. The glands lie one on each side of the floor of the vestibule and normally are about 3 × 1.5 cm in dimension. They become visible when enlarged, especially if the vestibular mucosa is slightly everted. These glands are very sensitive to estrogens, responding with the elaboration of thin mucus and hyperplasia of the ductal epithelium. Gross exaggeration of responses in hyperestrogenism accounts for the cystic development. Large retention cysts follow inflammatory stricture of the short excretory ducts. Abscessation may follow localization of infection in the cysts.

Ruptures of the vagina and vulva are quite frequently acquired as *parturient injuries*. The mucosa alone may be ruptured, or the

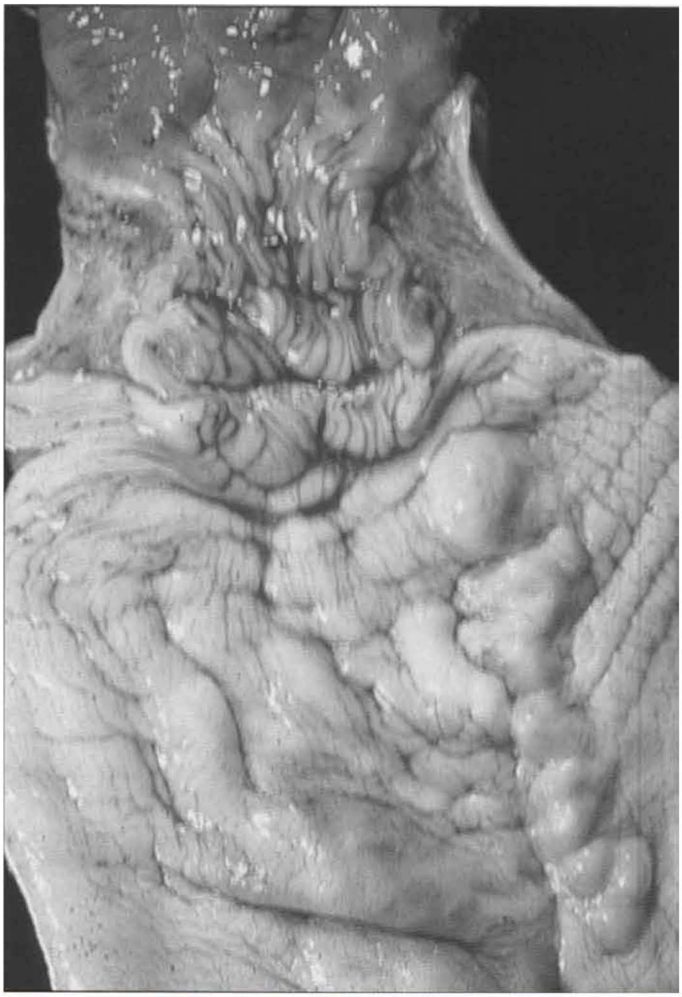

Figure 4.142 Cystic segment of a mesonephric duct in the vagina of a cow (cystic Gartner's duct). These cysts are common in cattle and are found in the ventrolateral area of the floor of the cranial vagina.

entire thickness of the vaginal wall. Hemorrhages occur into the vagina or the perivaginal wall from fetal pressure or vascular disruption. *Pelvic fat may herniate into the vagina*; it is often mistaken for a neoplasm. Defects that extend deeper than the mucosa heal with cicatrization, which may, in turn, result in partial stricture. Otherwise, the outcome depends on whether the lacerations become infected and, if so, with what. Occasionally, diffuse phlegmon, abscess, gangrene or peritonitis are sequelae.

Tumefaction of the vulva

Tumefaction of the vulva is a physiological response to estrogens. Vulvar enlargement may be marked during the normal estrous period or may be persistent when resulting from exposure to endogenous or exogenous estrogens. Abnormal patterns and concentrations of endogenous estrogens may occur from *cystic follicular disease, estrogen-secreting tumors (granulosa cell tumors), or from exogenous sources such as mycotoxins.* Vulvar tumefaction is most common in the sow and bitch, and must be differentiated from vulvitis (bite wounds), physical trauma, or dystocia.

Vulval tumefaction in **swine** may be due to exposure to mycotoxins. A distinctive syndrome characterized by vulval hyperemia and edema occurs in swine associated with the feeding of moldy grains. The mycotoxin that causes this estrogenic effect is *zearalenone or F-2*, which is produced by at least four different *Fusarium* species, *F. graminearum* being the most common.

Young gilts are chiefly affected, older animals being more resistant. There is remarkable edematous swelling of the vulva and vagina, which may be severe enough to lead to eversion and prolapse of the mucosa. Prolapse of the vagina may occur in up to 30% of cases, and secondary rectal prolapse occurs in a lesser percentage. The uteri of affected animals may be enlarged by endometrial gland hyperplasia and edema. The ovaries become inactive and atrophic. Gilts may also show mammary gland enlargement with squamous metaplasia of the ductal epithelium. Decreased libido, testicular atrophy, and balanoposthitis have been reported in young boars.

Strains of *Fusarium* vary in toxigenic potency, in regard not only to zearalenone but also to trichothecene toxins, which, if produced in quantity, may broaden the clinical picture to include anorexia and vomition.

Inflammatory diseases of the vagina and vulva

The mucous membrane of the vagina and vulva shares, with mucous membranes in general, a sensitivity to irritants. Although the vagina is of paramesonephric duct origin, the original epithelium is replaced by stratified squamous epithelium from the urogenital sinus. This epithelium proliferates and matures under the influence of estrogen and is then more resistant to infection. This enhanced resistance may be due to mechanical factors in the thickened keratinized epithelium and to local production of lactic acid from the glycogen that is deposited in the epithelium under the influence of estrogens.

Granular venereal disease

Papular eruptions of the vulval mucosa are common in most domestic species but are best known in the *bovine* species by the above name or as "*nodular venereal disease*" or "*granular vaginitis.*" The term vaginitis is a misnomer, as the papules are strictly limited to the vulval mucosa and are not found in the vagina, although in acute cases there may be an associated nonspecific vaginitis. Vulval granules may be found in any herd, affecting animals of any age, but are usually more prominent in heifers bred naturally. They are much less common in pregnancy and are almost never present about the time of parturition. In severe cases, the papules may be found on all aspects of the vulval mucosa, but they are usually clustered in the ventral commissure about the clitoris as pale or pink elevations a few millimeters in diameter and covered by a normal intact vulval mucosa. When numerous, they are likely also to be more active, larger, often coalescent, congested, and red, with concurrent catarrhal vulvitis and vulval swelling. The overlying epithelium is then easily injured and bleeding occurs freely from the papules. The resting papules are composed of respectably organized lymphoid follicles (Fig. 4.143).

When the vulval mucosa is irritated, these become congested, with small intrafollicular hemorrhages and edema, and hyperplastic, the mitotic frequency being quite high. The same lesions occur on the penis and prepuce of the bull and often persist for many months, as do those of the vulva. These papules have been produced experimentally with ureaplasmas and occur commonly in herds with natural *Ureaplasma* infections but are *not specific for this organism.* The development of subepithelial lymphocytic foci is a characteristic response of mucous membranes to mild persistent or recurrent

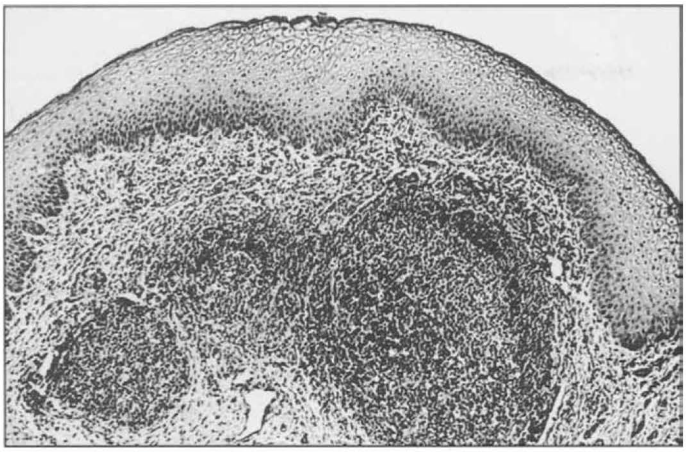

Figure 4.143 Bovine granular venereal disease, with submucosal lymphoid hyperplasia.

irritation and the simplest explanation of granular venereal disease is that *it is the result of mild inflammation of the vulval mucosa.*

Infectious bovine cervicovaginitis and epididymitis ("epivag")

This is a specific infectious disease that has been an important cause of infertility in east and southern Africa. A slow-growing *cytomegalovirus of the Bovine herpesvirus 4 group* has been recovered from infected animals. In limited studies, these isolates have not reproduced the full spectrum of clinical signs, so there remains some uncertainty about their role in the disease. The slow-growing herpesviral strains are not serologically related to the *Bovine herpesvirus 1* of infectious bovine rhinotracheitis.

Natural transmission is solely by coitus. Experimental transmission is easy if infective discharges are placed in the vagina or prepuce. After incubating for a few days, diffuse purple inflammatory streaks or patches develop in the cranial vagina, to be followed by the development of copious tenacious creamy discharge in which there are large numbers of neutrophils but few or no organisms. The infection spreads readily from the vagina to the cervix and uterus, also with the production there of the same copious pus. About 25% of infected females are sterile because of the development of *chronic salpingitis with hydrosalpinx and bursal adhesions.* In the bull, there is early but slight palpable enlargement of the spermatic cord and epididymis. The disease usually commences in one epididymis but later involves both organs and spreads from the tail of the epididymis to the head of it and ultimately to the testicle. The lesion is apparently an *interstitial epididymitis* with the production of excessive peritubular fibrosis and tubular obstruction. Testicular changes are probably secondary to those in the epididymis and to obliterative adhesions in the cavity of the tunica vaginalis. Similar productive inflammatory lesions occur in the ampullae and the seminal vesicles.

Infectious pustular vulvovaginitis of cattle

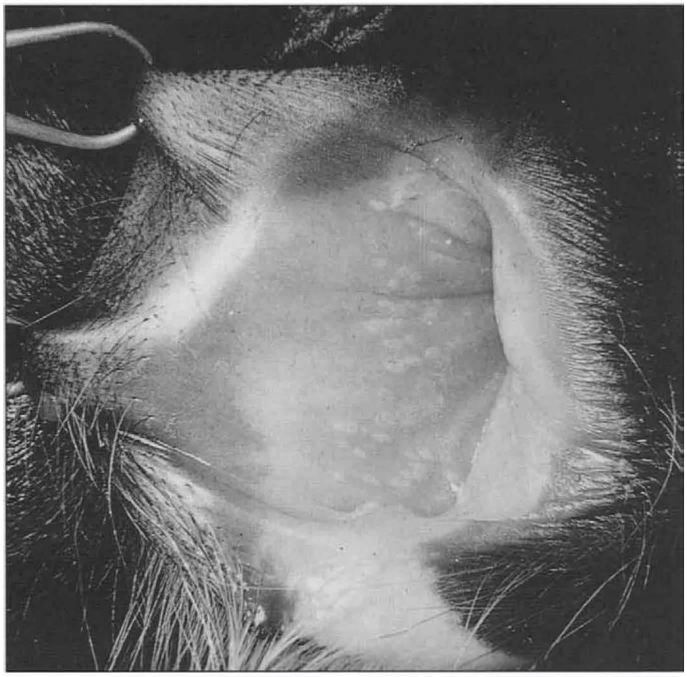

Figure 4.144 Multifocal necrotizing vulvitis caused by *Bovine herpesvirus 1.* (Courtesy of Baker JA et al. and Cornell Vet 1960;50:156–170.)

We are assuming for this description of infectious pustular vulvovaginitis that it is the disease that for many decades has been variously termed vesicular venereal disease, vesicular vaginitis, coital exanthema, coital vesicular exanthema, and Blaschenausschlag. The assumption is probably valid, but proof is lacking and may no longer be obtainable. Infectious pustular vulvovaginitis is caused by the same herpesvirus (*Bovine herpesvirus 1*) that causes infectious bovine rhinotracheitis (see Vol. 2, Respiratory system), or at least is caused by a subtype of that virus that is serologically indistinguishable from it. As a rule, nasal and vaginal infections behave epidemiologically as distinct diseases, although occasionally the syndromes occur together in individual animals. The infection can be transmitted to sheep and goats, producing vaginitis.

Infectious pustular vulvovaginitis is *highly contagious.* It is frequently transmitted by coitus, but it can also be transmitted by other mechanical means and is contagious by close contact. It may involve individual or a few animals in a herd, but frequently spreads rapidly to involve all exposed females in a few days. The disease subsides in about 10 days, leaving immunity that is fragile and transient. Reinfection can occur, but early reinfection produces only mild disease.

The incubation period is 1–3 days but may be as brief as 12 hours. The lesions are restricted to the genital tract, but a viremic phase probably occurs because there is early fever and leukopenia. Initially, the vaginal and vulval mucosa is hyperemic with focal hemorrhages in the lymphocytic follicles of the submucosa. *The severity of the vulvovaginitis increases rapidly, and edema of the vulva and mucopurulent vaginal discharge develop.* The focal lesions replace the hemorrhages over the lymphoid follicles and consist of small (2–3 mm) pock-like foci, slightly elevated, pale, soft, and friable (Fig. 4.144).

The focal lesions, being related to the lymphoid follicles, may be in short linear arrangements. The epithelium in the focal lesions erodes or ulcerates so that in a few days the foci are flat, gray, semitransparent plaques the size of the original lesions.

The virus is epitheliotropic, the initial and most severe alterations occurring in the epithelium of the vagina and vulva. There is ballooning degeneration of the epithelial cells, and, at about 24 hours, *intranuclear inclusions* can be found in the epithelium (Fig. 4.145A).

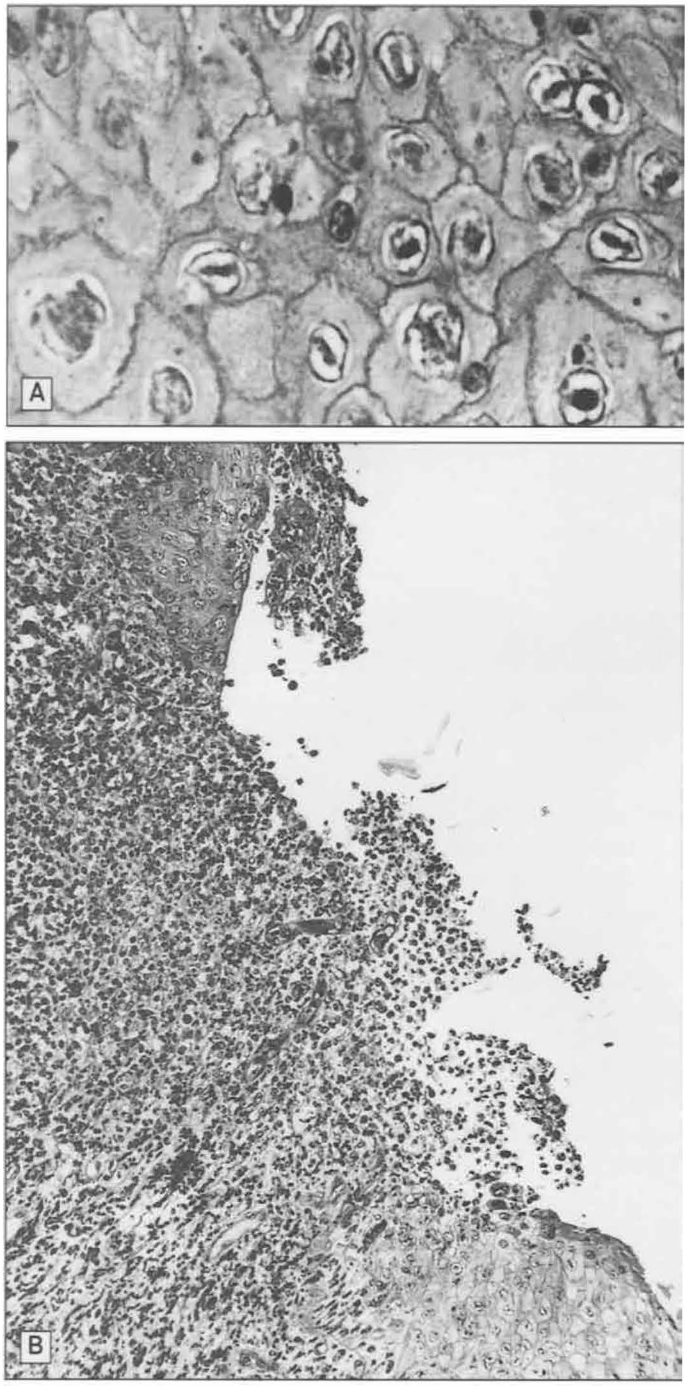

Figure 4.145 A. Intranuclear inclusion bodies in vaginal epithelial cells in *Bovine herpesvirus 1* infection. **B.** Histologic detail of **(A)**. Ballooning degeneration and ulceration of epithelium.

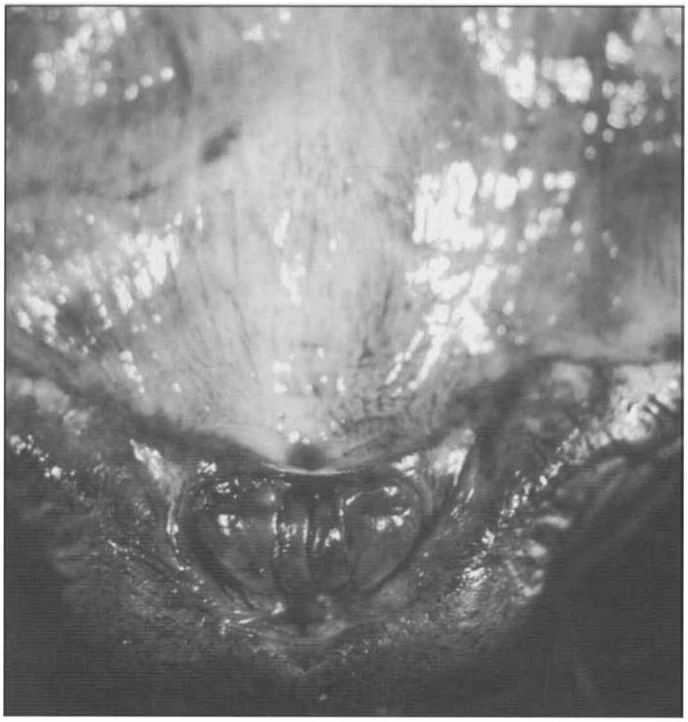

Figure 4.146 Contagious equine metritis. The clitoris and clitoral fossa of a mare infected with *Taylorella equigenitalis* appear grossly normal but are the sites of chronic infection in the mare. Mats of bacteria can be found on the mucosal surface of the sinuses and crypts of the clitoris.

The inclusions are lightly acidophilic or amphophilic and large; they can be found for 3–4 days, by which time the lesion has reached its zenith and is beginning to resolve. The infected cells undergo necrosis, and epithelial disruption and ulceration occur, accompanied by an intense infiltration by neutrophils (Fig. 4.145B). Vesicles and true pustules do not form. Acute inflammation occurs in the lamina propria, with hyperemia and edema and the exudation of numerous plasma cells and lymphocytes. Many of the small vessels are occluded by adventitial and endothelial swelling. The lymphocytic follicles are remarkably hyperplastic and edematous, and their outlines are obscured by the infiltrating cells in the lamina propria. Resolution occurs in about 8 days, with hyperplastic lymphoid follicles and slight epithelial thickening as residues.

Although most cows that are served naturally by infected bulls do not appear to experience infertility, susceptible heifers, which are inseminated with semen containing virus, fail to conceive. Intranuclear inclusions may be found in the epithelial cells of the zona compacta within 48 hours but are absent by 72 hours after exposure. The surface epithelium and the underlying connective tissue become necrotic and infiltrated with neutrophils. There is pronounced edema of the lamina propria and infiltration of mononuclear cells, especially lymphocytes. The oviducts are involved, but to a less severe degree.

The virus can produce similar lesions on the mucous membrane of the penis of infected bulls. Since recrudescence with viral shedding is a feature of this, as well as other, herpetic diseases, animals with inapparent infections can also transmit the disease.

A herpesvirus identified as *Equid herpesvirus 3* produces a comparable genital disease in horses, *equine coital exanthema.* It is discussed with diseases of the penis, in Vol. 3, Male genital system.

Contagious equine metritis

Contagious equine metritis, caused by *Taylorella equigenitalis,* is a sexually transmitted disease of horses. As discussed in the section of this chapter on endometritis, above, acute infection of mares causes purulent endometritis and cervicitis. In mares that have become carriers the *Taylorella* organisms reside within the crypts and fossa of the around the clitoris. Although the mare's clitoral area, shown

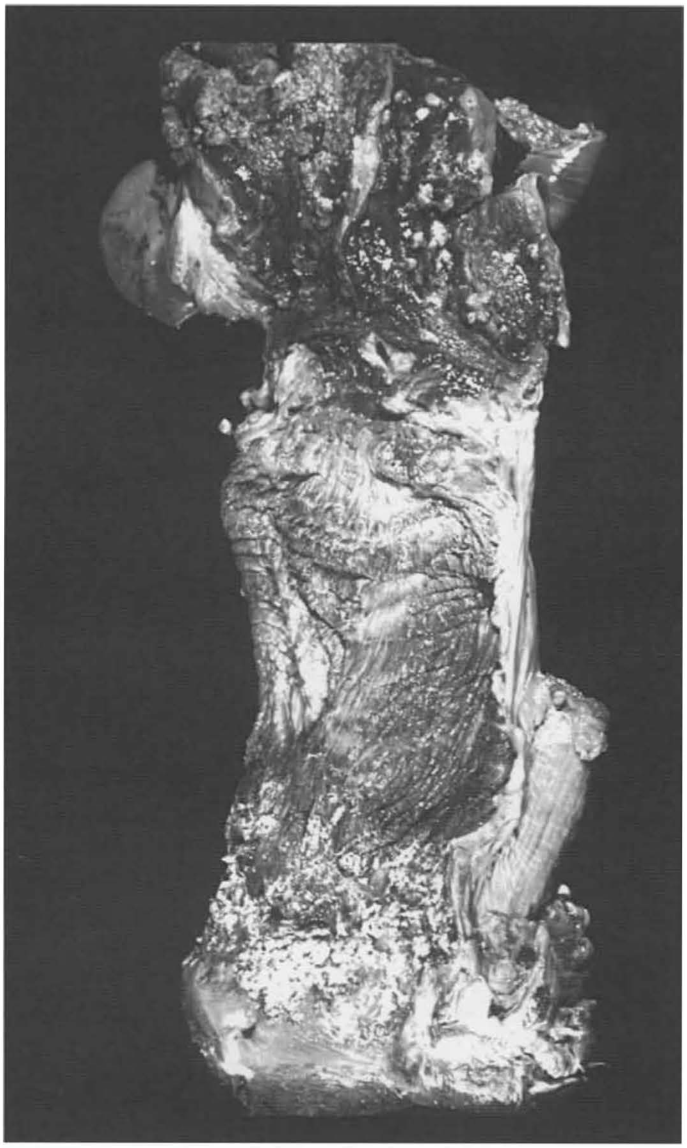

Figure 4.147 Necrotizing vaginitis in a cow, associated with dystocia and associated pressure necrosis.

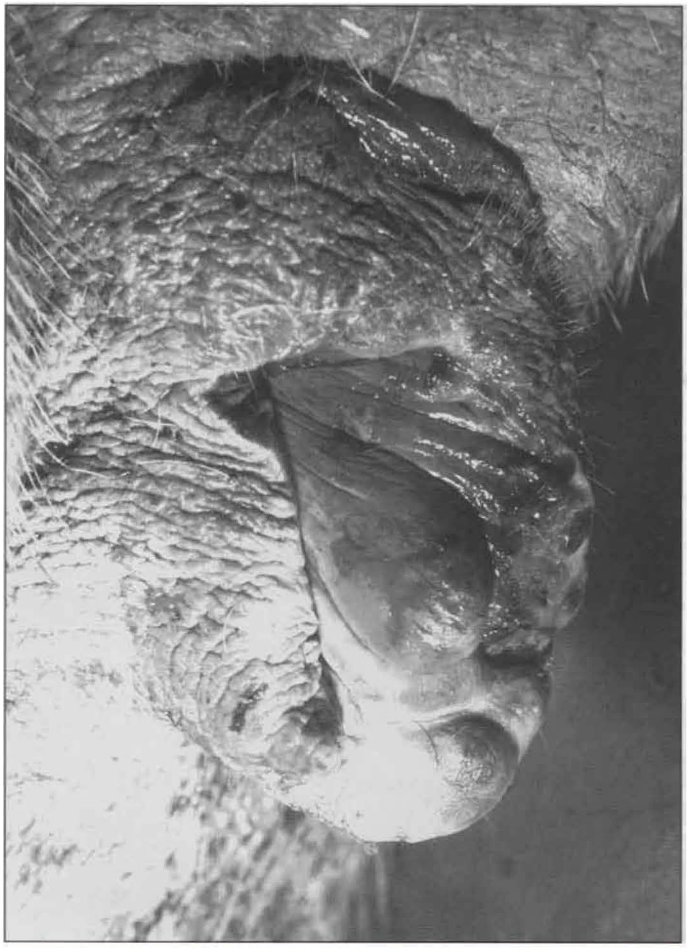

Figure 4.148 Disfigured vulva of a sow, resulting from trauma during delivery.

in Figure 4.146, is grossly normal, she had large numbers of *Taylorella* coccobacilli within the clitoral fossa that was associated with only a mild lymphocytic reaction. An atypical *Taylorella* sp. also capable of causing transient endometritis and endometritis has been isolated from *donkeys* in the USA.

Necrotic vaginitis and vulvitis

Necrotic vaginitis is a deep diphtheritic inflammation of the vaginal mucosa; it occurs in two fairly distinct syndromes, either vulvovaginitis or cervicovaginitis. *Necrotic vulvovaginitis* is uncommon, but may involve a number of cows in a herd. It is primarily due to trauma with contamination, often the result of bite wounds by pigs or dogs. *Necrotic cervicovaginitis* (Fig. 4.147) is a complication of parturition and is chiefly observed in ewes and cows as a consequence of dystocia. A number of influences, mainly prolonged pressure necrosis, laceration, and abrasion, usually acting in combination, is responsible for the lesion. Severe necrotic cervicovaginitis is frequently fatal, either in association with direct extension of the inflammation to the peritoneum or in association with the uterine complications of prolonged dystocia and fetal emphysema.

Lacerations associated with dystocia commonly occur in mares, sometimes extending to involve the rectum and perineum. *Scarring* of the vulva from previous trauma associated with delivery is a common finding in large domestic animals (Fig. 4.148). *Primary skin tumors* that occur in the perineal area and frequently involve the vulva include squamous cell carcinomas and melanomas in mares (Fig. 4.149).

Dourine

Dourine of horses and their relatives is caused by ***Trypanosoma equiperdum*** and is *primarily a venereal disease*. The other trypanosomiases of domestic animals are discussed in Vol. 3, Hematopoietic system, being essentially hemic infections with arthropod vectors. Dourine differs from other trypanosomiases in that the organisms are in the blood only intermittently or in very virulent infections, and transmission is effected not by blood-sucking insects but by contact of infected mucous membranes. *Natural transmission is by coitus*, so the disease is almost exclusively one of stallions and breeding mares. Rare cases occur in unbred animals and in young foals. The disease prevails

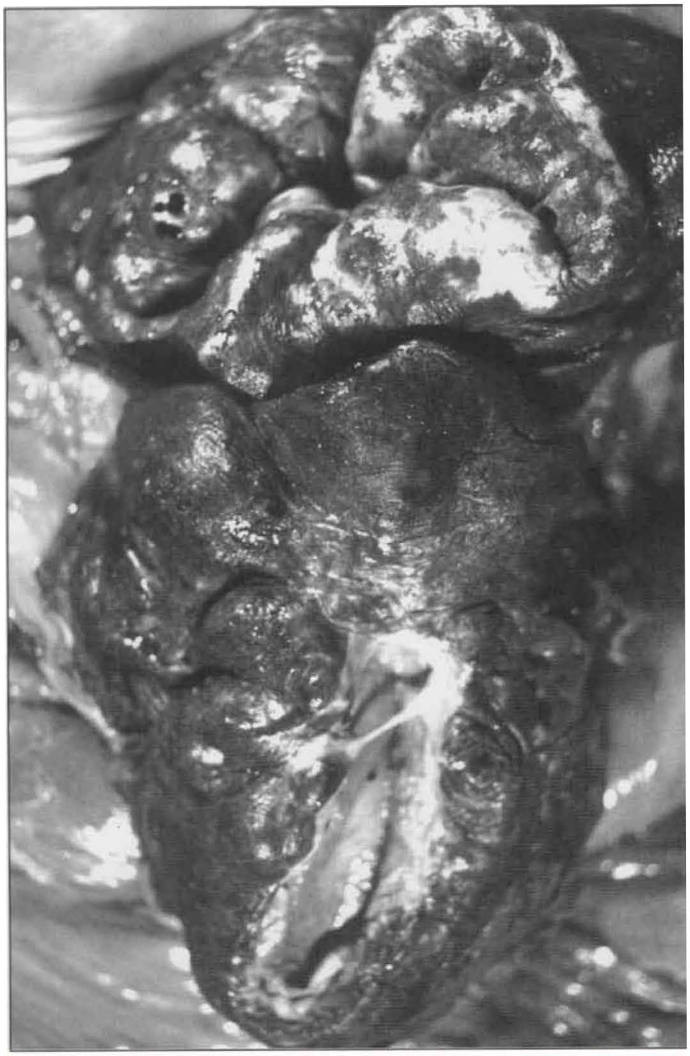

Figure 4.149 Multifocal nodular pigmented masses involving the vulva and perineum of a mare. This **malignant melanoma** had also metastasized to regional lymph nodes. These are common neoplasms that involve the vulva and perineum of the mare. Squamous cell carcinomas also occur in this area.

in the Balkans, much of Africa, Asia, and South America. It has been eradicated from most of Europe and North America.

Strains of *T. equiperdum* vary considerably in their virulence, although not notably in their capacity to infect. Irrespective of the route of experimental infection in horses, the trypanosomes demonstrate some predilection for genital mucosae. Following natural infection, the organisms are frequently numerous in vaginal discharges and the male urethra. There are periods, however, sometimes of several weeks or months, in which the organisms are not present in these sites, so infected animals are not always infective for others at breeding. The organisms penetrate intact mucosa at the site of implantation and proliferate in the submucosal lymph spaces. The incubation period may be several weeks or months during which time trypanosomes are present in the genital discharges, so there is a possibility that they may proliferate in the lumen of the genital tract for long periods before invading the tissues. From the initial lesions, the organisms are disseminated in the blood to other parts of the body, and edematous swellings occur where they localize. Infection of the blood may be intermittent or continuous, and there may be few organisms or many, depending on the virulence of the strain and the susceptibility of the host. Strains of low virulence may not be demonstrated in the blood by direct smears, but may be demonstrated by centrifuging plasma or directly by transfusing blood in large volumes. In these mild infections, it may also be possible to demonstrate the organisms in genital washes or discharges, but diagnostic problems are simplified by an efficient complement-fixation test.

The signs and course of the infections vary considerably depending on the virulence of the organism and on the susceptibility of the host. The resistance of the host can be greatly modified by climatic conditions, physical condition, intercurrent disease, nutritional status, and so forth, and the South African varieties of the disease can be asymptomatic in animals that are well husbanded. Animals affected with this insidious type of the disease do act as carriers and reservoirs of infection. When the infection is attended by clinical signs, the course is variable; it may be severe and fatal in a few weeks, fatal after a chronic or intermittent course of from several months to 2 years, or clinical recovery may occur.

The signs of dourine can be divided into genital, cutaneous, nervous, and general manifestations, which occur separately or concurrently. The initial signs are usually genital, but may be nervous or cutaneous. The incubation period of the genital signs varies from several days to several months, during which the organisms are present in genital discharges or washings. The external genitalia are swollen and doughy, but characteristically the swellings are neither hot nor painful. The degree of swelling varies considerably from case to case and periodically in an established case, with a tendency to be permanent due to induration in chronic infections. The swelling, when severe, extends to the perineum and ventral abdominal wall. The lymphocytic follicles of the mucosa of the female genitalia become hyperplastic and ulcerate, and at this stage there may be copious fluid discharge. In males, the swelling involves the glans penis as well as the prepuce, and may cause prolapse of the urethra and penis. In virulent infections, flat circular ulcers develop in the glans penis. *Healed ulcers in both males and females often remain as depigmented scars*, and pigmentary atrophy of the skin and genital mucosa may also occur in the absence of ulceration, the loss of pigment being, when present, a very characteristic sign of this disease.

Cutaneous lesions may never occur in the mild disease. In virulent infections, edematous urticaria-like plaques, usually circular but sometimes linear or in rings, occur in the skin, especially on the sides of the body and croup. The swelling may be up to 15 cm in diameter and painless and free of itch. They disappear in a few days and new ones form. There is usually no residuum, but sometimes there are local disturbances of sweating and pigmentation.

Nervous manifestations develop late in the course and usually lead to death. There is acute hyperesthesia initially, which may be generalized or localized to the distribution of particular nerves. Later, there is diminished sensitivity or even anesthesia, and this is accompanied by paresis or paralysis of individual motor nerves. The pareses are either unilateral in distribution or asymmetrical in severity and most commonly involve the facial nerves and the motor nerves of the hindlimbs. Decubitus follows.

The *general manifestations* are chiefly of continued or remittent fever, emaciation, and severe anemia. Noninflammatory synovial effusions, superficial lymphadenopathy, uveitis, and optic atrophy are described.

Apart from changes in the peripheral nerves that are responsible for the paralytic phenomena, pathological changes additional to those that are clinically observable are not reported. Degenerative changes occur chiefly in the lumbar and fifth and seventh cranial nerves, being most severe in the roots and also involving the ganglia. The neuropathy is probably preceded by edematous infiltrations, and these may still be present at death. The large nerve trunks are transformed into fibrous cords that are fused with the surrounding muscular fascia. Microscopically, there is edema, mononuclear infiltration and fibrosis of the perineurium. There is slight endoneural reactivity in fascicles in which there is extensive fiber degeneration. Sclerosing changes are also present in the ganglia.

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NEOPLASTIC DISEASES OF THE TUBULAR GENITALIA

Isolated reports have ascribed parentage of both benign and malignant tumors to all histologic components of the tubular genitalia, sometimes with doubtful credentials. Some of the least uncommon tumors warrant brief discussion.

Tumors of the vulva could be discussed with tumors of the skin. *Squamous cell carcinoma* is well known in the mare and cow, and, indeed, a high incidence in cows is reported from Kenya. These bovine cases are analogous to the orbital tumors of cattle, thought to be initiated in a solar dermatosis, their incidence negatively correlated with the degree of local epithelial pigmentation.

Leiomyoma

Leiomyomas are the most common tumors of the tubular genitalia of the bitch. Leiomyomas are less common in other species but, regardless of species, leiomyomas have characteristic gross features. Their origin is from the smooth muscle in the wall of the uterus, cervix, or vagina, and, although they may be solitary, they are usually multiple. The tumor in the bitch is neither malignant nor wholly autonomous, being, in some unknown manner, *endocrine-dependent*. It rarely occurs in the bitch earlier than middle age and is frequently associated with ovarian follicular cysts or estrogen-secreting tumors, and often also with endometrial hyperplasia, mammary hyperplasia, and mammary neoplasia. Bitches castrated early in life are exempt, and established tumors regress following castration. This tumor has been provoked in guinea pigs by continuous low-level doses of estrogen but, for spontaneous leiomyomas as well as endometrial and mammary neoplasms, there is as yet no precise knowledge of the role of estrogen.

The genital leiomyoma may grow to be as large as 10–12 cm in diameter but is not invasive. The smaller tumors are fleshy but, as they enlarge, become firm or hard (hence the clinical term "*fibroid*") due to the connective-tissue stroma. On cut surface, they have a watered-silk appearance and the color, whether more fleshy or white, depends on relative amounts of muscle and connective tissue. Figure 4.150 shows the gross features of a large leiomyoma in the uterus of a cow. The tumor is not encapsulated but is well demarcated and easily shelled out. In almost all cases, the tumors project as globose or elliptical masses or as bulbous polyps into the lumen of the vagina, uterus, or cervix, but some project outward and a few are found in the mesometrium. Figure 4.151 is a photograph of two leiomyomas in the uterus of a bitch. Occasionally large numbers of small leiomyomas are found in the vagina (Fig. 4.152).

Degenerative changes occur in the larger tumors and are of two types: either progressive replacement by fibrous tissue of the stroma, or edema and liquefaction with cyst formation. In the first type, the fibrosis may progress through hyalinization to mineralization. The second type of degenerative change afflicts vaginal leiomyomas especially, probably because these more often become pedunculated and susceptible to circulatory embarrassment.

Histologically, the tumor is composed of whorling bundles of smooth-muscle cells with abundant stroma but scant intercellular connective tissue. The organization does not depart much from normal, and often the presence of neoplasm is best appreciated by the naked-eye appearance of the tissue.

Leiomyosarcoma

Cytologic features, such as pleomorphism, rate of mitoses, and invasiveness, help differentiate leiomyomas from leiomyosarcomas.

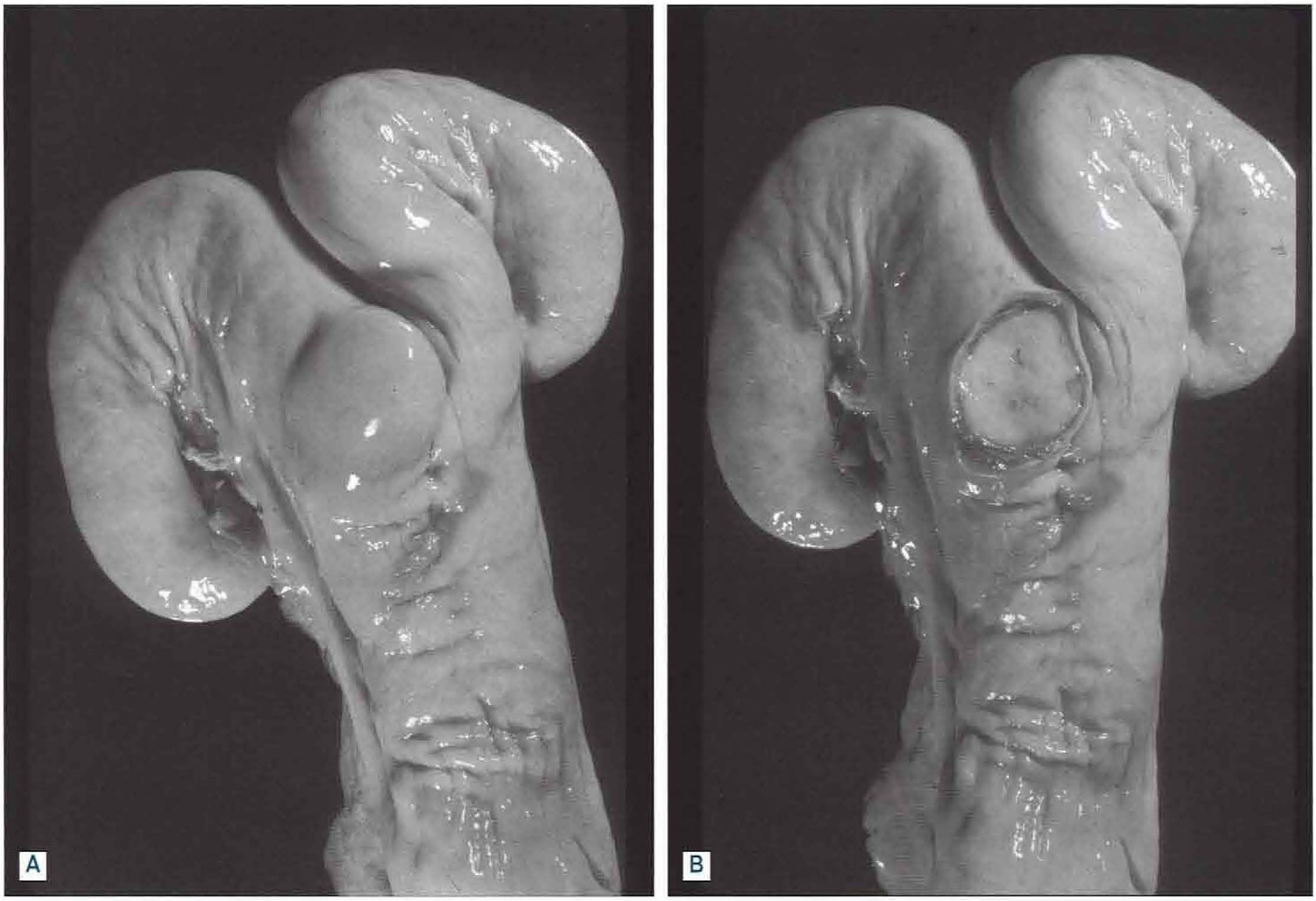

Figure 4.150 **A. Leiomyoma** seen as a single mass in the dorsal wall of the uterus of a cow. **B.** The serosa and myometrium over the mass and outer half of the mass have been removed to reveal the typical firm white appearance of leiomyomas.

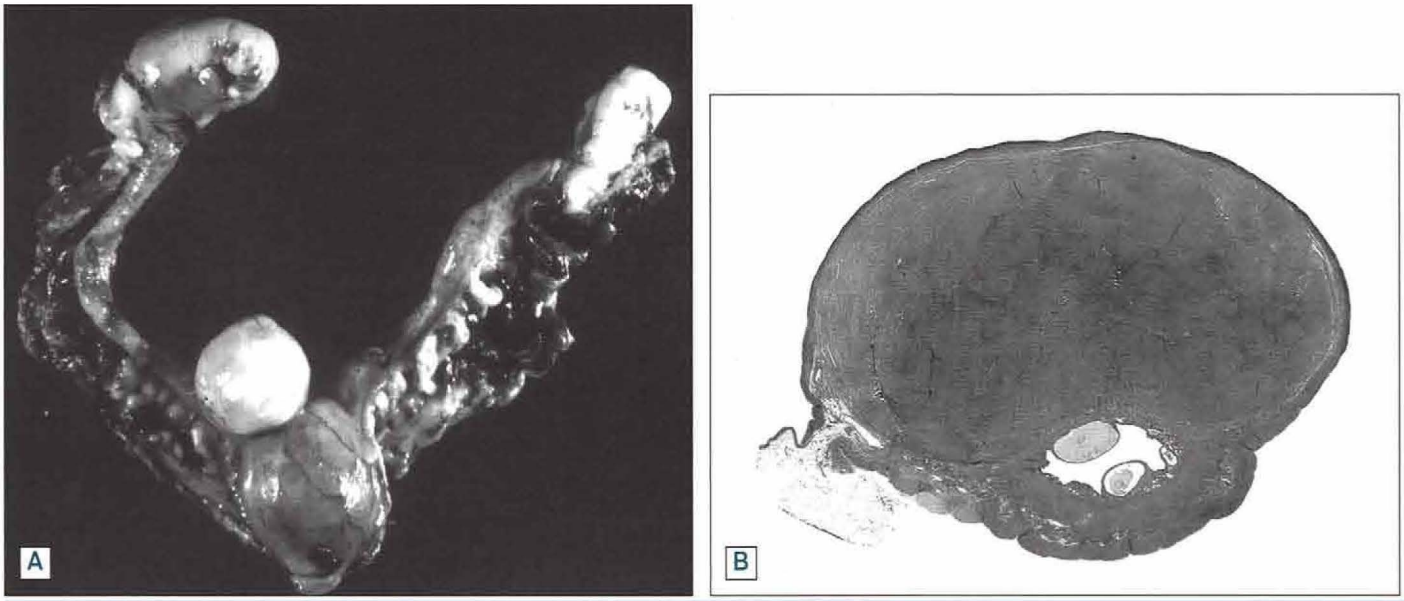

Figure 4.151 **Leiomyomas** can appear as masses within the uterine wall or extend inward or outward. **A.** Two leiomyomas are present in the body of this bitch's uterus, one extending outward from the surface of the base of the uterine horn, and the other filling the lumen of the uterine body. **B.** Subgross section of a uterine leiomyoma from tissues of a different bitch. The tumor is within the wall and has distorted the shape of the uterus. Note the uniform dense nature of the mass. Two endometrial cysts are also present.

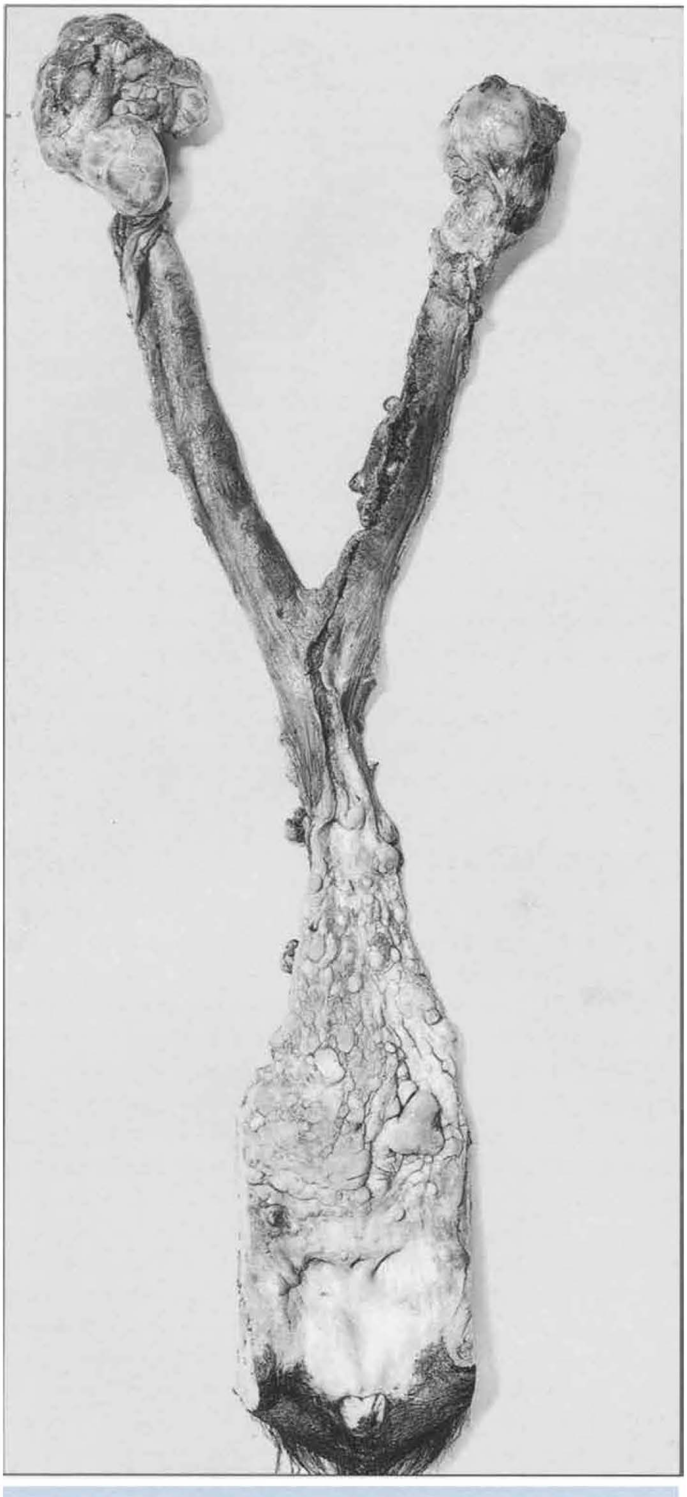

Figure 4.152 Miliary **leiomyosarcoma** involving the vagina of a bitch.

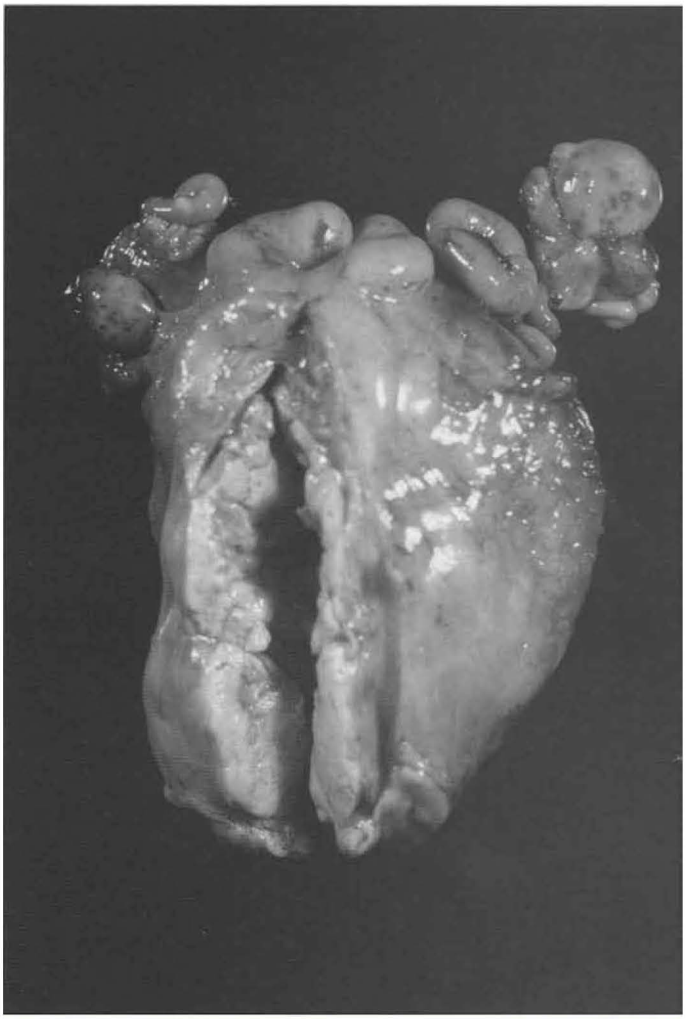

Figure 4.153 Leiomyosarcoma effacing the uterine body, cervix, and cranial vagina in a Saanen goat, which has a predilection for this tumor.

Leiomyosarcomas are *uncommon*, but have been found in older Saanen goats (Fig. 4.153), with occurrence reported in siblings, suggesting that in this breed there may be a genetic defect.

Transmissible venereal tumor of dogs

This tumor is a contagious neoplasm, transmitted most commonly by coitus, and occurs on the external genitalia of either sex. It is also known as Sticker's sarcoma. The noncommittal name of the tumor, transmissible venereal tumor, is preferred to such terms as lymphosarcoma, histiocytoma, and sarcoma, which, suitably prefaced to indicate its transmissibility, have been used. Because the host's tissues do not contribute to the growth, the histogenesis is obscure and likely to remain so. This tumor differs from other infectious tumors in that *the infecting cells are transplanted and grow like a graft.* This was the first neoplasm in the history of pathology to be transmitted experimentally; this was accomplished by the Russian veterinarian Novinsky in 1863.

The tumor has been reported from Europe and Asia as well as from the Americas. There are marked fluctuations in its prevalence in endemic areas over the course of a few years. The tumor tends to be common where dogs are allowed to run free and is rare where dogs are controlled. The tumor is not confined to the genitalia, but in some few cases is found in other cutaneous locations. *Metastasis sometimes occurs to regional lymph nodes, but spontaneous regression usually occurs in less than 6 months.*

It seems unlikely that a tumor of such character would originate in multiple geographic centers; more probably its wide distribution, especially in tropical and subtropical areas, is due to a manner of spread akin to that of infectious disease. This is an assumption

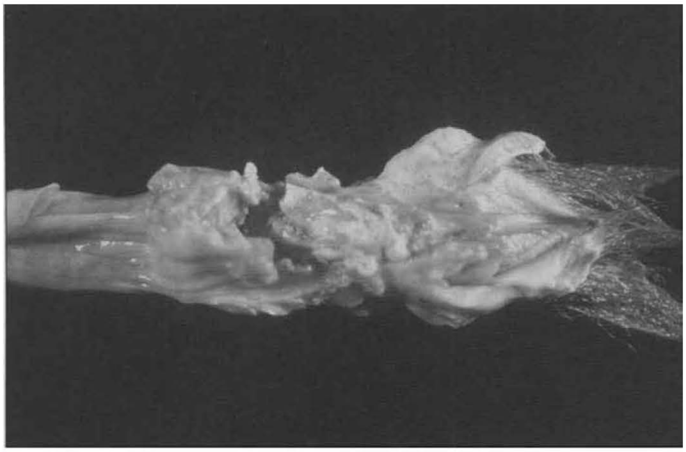

Figure 4.154 Transmissible venereal tumor in the vagina of a bitch. These tumors are transmitted from dog to dog by transplantation of neoplastic cells.

impossible to prove, but it is supported by the fairly constant karyotypes of the tumor cells to the extent that these have been examined. The diploid number of chromosomes in dogs is 78, and, normally, they are acrocentric except for X and Y. The chromosome complement of tumor cells originating in separated areas of Japan and North America is closely similar, most tumor cells containing 59 chromosomes; the minor variations in chromosome numbers in some cells and in chromosome morphology are within the variability accepted for stem-lines of transplantable tumors and long-term cell cultures.

In the vagina, the tumor takes origin in the submucosa, usually of the dorsal wall, as one or more papular or papillary proliferations (Fig. 4.154). Expansive growth also occurs mainly in the submucosa, and the overlying epithelium becomes stretched and attenuated. With rupture or penetration of the epithelium, the tumor projects into the vagina as an irregular, ulcerated, and friable mass that may protrude from the vulva. Histologically, the tumor varies depending on the stage of growth or regression. During the early stage of growth, the tumor is composed of round, oval, or polyhedral cells with indistinct boundaries and poorly stained or clear cytoplasm. The nuclei are large in proportion to cell size with a single, well-defined nucleolus and many chromatin granules. Variability in the size of the cells is rather characteristic and mitoses are frequent. Electron microscopic studies have shown that most cells in growing tumors are round cells with microvilli, whereas regressing tumors have spindle-shaped cells suggestive of tumor cell differentiation toward fibroblastic cells. The number of infiltrating lymphocytes increases as growth of the tumor slows and the tumor begins to regress. Most of the infiltrating lymphocytes are T cells.

In the skin also, the tumor seems to arise in the subcutis, and the epidermis is not usually penetrated. It is suggested that natural cutaneous implantation occurs in bite or other wounds. In any locale, the tumor shows a marked tendency to break down after a few months of rapid progression, and on incision the necrotic tissue resembles pus.

It is not known how this tumor can be transplanted with living cells across major histocompatibility barriers and survive for a substantial time before regressing. Infected dogs do develop antibodies, but in dogs with growing tumors some of these antibodies are blocked by a tumor-associated antigen. This suggests that the expression of this tumor-associated antigen may play a role in blocking systemic immune responses and facilitate tumor growth. Unlike tumor-associated antigen, which is expressed in progressive tumors, class I and class II major histocompatibility antigens are only expressed when regression begins. Dogs are resistant to challenge after natural regression of the tumor. Secondary tumor challenges begin to regress on day 9 and disappear after about 2 weeks. The secondary tumors are rapidly infiltrated by lymphocytes, most of which are T cells, and undergo degeneration. The tumor is transmissible not only between dogs but also to the fox, coyote, and jackal.

The life history of the tumors as outlined above and especially the limited metastatic capability are not constant, and greater contagiousness and more virulent expressions may be expected in immunosuppressed individuals and in a canine population of suboptimal physiologic status. Such appears to be the case, for example, in the promiscuous, scavenging, malnourished, feral populations. There, metastases are common in both sexes as sequelae to the primary lesions. In the female, extension occurs to the uterus, cervix, and uterine tubes. In both sexes, there is early spread to inguinal nodes, which become large and firm. Cutaneous involvement is also common with few or many lesions, some up to 6 cm in diameter, often ulcerated and hemorrhagic. Buccal lesions and large periorbital tumors are also described. Ocular lesions are common in this disease, but, apart from the sites mentioned, conventional metastatic patterns in internal viscera do not occur.

Fibropapilloma of the vulva

This is probably the most common tumor of the bovine vulva and affects young animals primarily. It is, briefly, a wart growing on a mucous membrane, the nature of the host tissue apparently determining the reaction to the virus of bovine verrucae (*Bovine papillomavirus*). This virus infecting the keratinized bovine skin provokes the typical papilloma of relatively scant connective-tissue core and abundant epidermal overgrowth. Transplanted instead to the penile or vulval mucosa, the contribution of the two moieties is reversed, the bulk of the tumor being connective tissue with just enough epithelium to cover it. Histologically, *the bulk of the tumor consists of interlacing bundles of fibrocytes*. In the younger tumors, there may be many mitotic figures, and such cases have often been misdiagnosed as fibrosarcomas. Many of the plump spindle cells have large nuclei with bizarre nucleoli and sometimes pale, eosinophilic inclusion-like intranuclear structures. Collagen formation from the stroma is progressive with duration. Surface ulceration is followed by superficial inflammatory infiltration.

The vulval tumors are usually sessile, rounded growths when young, but become progressively more cauliflower-like. Those attached to the penis are often pedunculated. The natural history of the genital fibropapilloma is the same as that of the common cutaneous papilloma; *spontaneous regression occurs in 1–6 months or so*. Within this period, surgical excision may be followed by recurrence.

Transmissible genital papilloma of the pig

Ordinary cutaneous warts are not described in pigs. A transmissible papilloma occurs in the *preputial diverticulum of the boar*. It is transmissible to the lightly scarified vulval mucosa with an experimental incubation period of about 8 weeks. The lesions are 1–3 cm in size and

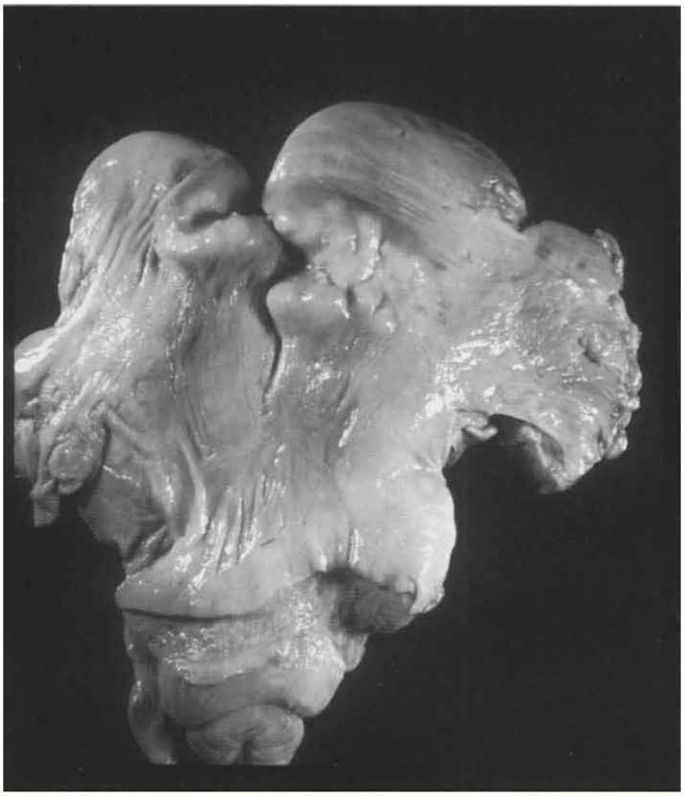

Figure 4.155 Uterine adenocarcinoma in the wall of a uterine horn in a cow. These neoplasms stimulate a marked desmoplastic response, which results in the typical firm, depressed, and contracted appearance of the invading tumor – features that are used to diagnose this tumor grossly.

papular, some of the larger lesions being papillary. The clinical course is not many weeks, after which the lesions begin to regress and are eventually sloughed. Recovered animals are immune to reinfection.

The lesion histologically is typically papillomatous with extensive uneven acanthosis and epithelial overgrowth, but with none of the abundant mesodermal reaction that occurs in the bovine disease outlined above. The broadened malpighian zone contains scattered cells with *intracytoplasmic inclusions* that are large, spherical, homogeneous with acidophilic stains, and often surrounded by a halo. An inflammatory response with mononuclear cell infiltration occurs in the underlying dermis.

Carcinoma of the endometrium and cervix

These are *rare neoplasms* in domestic animals and therein lies their interest. The rarity is real and not, as has been implied, apparent because of inadequate postmortem examination. The most popular current opinion on the pathogenesis of the endometrial carcinomas in women relates them to prolonged estrogenism, with cystic endometrial hyperplasia of the same cause as the precancerous lesion. In domestic animals, on the other hand, cystic endometrial hyperplasia is common but carcinoma is rare and, when it does occur, does not seem to require prior hormonal conditioning of the endometrium.

Carcinoma of the endometrium appears to occur more frequently in *cattle* than in other domestic animals. The tumors may be single or multiple, hard, nodular masses of variable size in the uterine wall. Desmoplastic reaction is commonly a prominent feature, which leads to umbilication of the serosal surface (Fig. 4.155). The regional lymph nodes and the lungs are the usual sites of metastatic lesions.

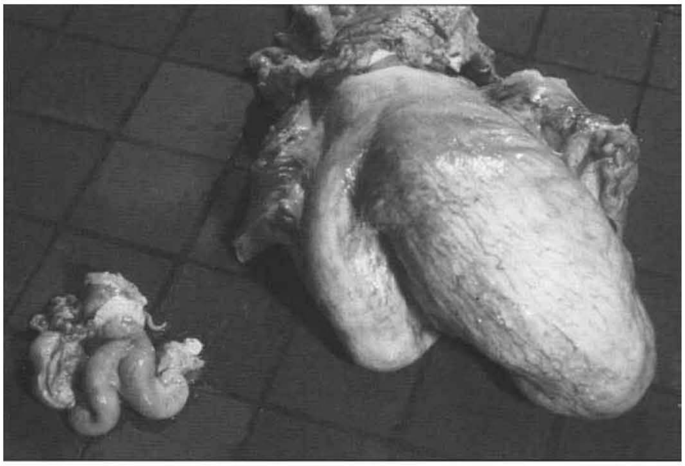

Figure 4.156 Lymphosarcoma diffusely involving the uterus on the right has massively enlarged the uterus. The uterus on the left is from a normal cow.

As noted earlier, focal proliferative lesions of the endometrium and normal or prolonged endometrial repair postdelivery may be confused as being neoplastic changes in the endometrium. This is especially true in the bitch and queen, and caution should be exercised before making a diagnosis of endometrial carcinoma in those species.

Lymphosarcoma

Involvement of the uterus is not uncommon in the multicentric form of *bovine* lymphosarcoma but is distinctly so in other species. As elsewhere, there are two anatomic forms of uterine involvement, diffuse and nodular. In either event, the initial deposition seems to occur in the endometrium. The *diffuse lesion* involves both horns, body, and occasionally the cervix in a more or less uniform thickening, leaving a uterus that can be very large, but retaining its typical shape (Fig. 4.156). The thickened wall loses its elasticity and on cut surface is rather structure-less with an appearance like firm fat (Fig. 4.157). Gentle pressure may expel small quantities of cloudy, highly cellular fluid. There is patchy ulceration of the endometrial mucosa. There is nothing peculiar to the *nodular form* of the disease. These nodules, few or many, may attain large size and cause corresponding deformity. Central liquefactive necrosis is common in them. Microscopically, there is, with either form of the disease, gradual replacement of the normal uterine structures by infiltrating tumor cells. Lesions of corresponding type occur in the vagina, but do so less commonly.

Metastatic tumors

With the exception of lymphosarcoma, secondary neoplastic diseases of the tubular genitalia are rather *rare*. Serosal implantations occur in peritoneal carcinomatosis. There are a few reports of results of immunohistochemical staining of tumors involving either the uterus or ovary in cattle.

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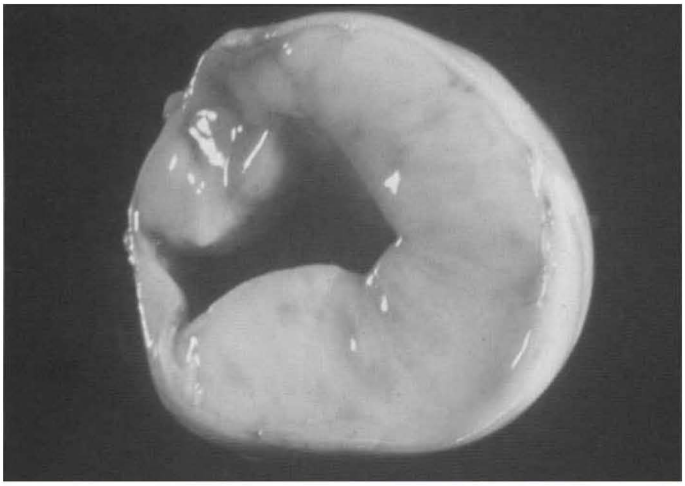

Figure 4.157 Cross-section of a **uterine horn** from a cow with **lymphosarcoma**. The wall is irregularly thickened by the invading neoplasm and has a characteristic tan, slightly soft appearance.

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Pathology of the mammary glands

The mammary glands are modified cutaneous glands with the important roles of providing nourishment to immature neonates and providing to the newborn of many species passive immunologic protection against pathogenic microbes. The mammary glands may, however, provide a source of infection by microorganisms such as *Mycobacterium paratuberculosis* and the Brucellae, which arrive in the gland hematogenously and provoke minimal pathologic change, or *Pasteurella* spp. and *Streptococcus* spp. during the course of galactogenic infection and inflammation. A number of helminth parasites have adapted their life cycles in ways that ensure their transfer to neonates in milk.

Diseases affecting the skin of the mammary glands and neoplastic diseases, of especial prevalence and importance in dogs and cats are discussed in Vol. 1, Skin and appendages.

DEVELOPMENTAL CONSIDERATIONS

The weight of evidence supports the derivation of mammary glands as specializations from sweat glands. The myoepithelial cells are thought to be of epithelial origin and the relationship to sweat glands possibly accounts for the structural and behavioral similarities, and the participation of myoepithelial cells, of mammary and sweat-gland carcinomas in the bitch.

The glands form on the *mammary line or ridge* detectable in the ventrolateral ectoderm of the embryo. The ectodermal cells of the ridge, which are destined to develop into the mammary glands, congregate in specific areas, of number and location appropriate to the species, to form the *mammary buds*. From the buds, primary sprouts push into the mesenchyme, the number of sprouts from each bud determining the number of openings that will eventually develop in each teat. Only one *primary sprout* develops from each bud in cattle, and is destined to form the *streak canal* and *teat cistern* and, proximally, the *gland cistern*. Secondary sprouts develop from the primary sprout to form the early *milk ducts*. In species such as cattle, sheep, and goats with one teat orifice, the gland is a single large secretory structure. There are usually two streak canals in horses and pigs, 3–7 in the cat, and 8–14 in the dog. In species with multiple teat orifices, a mammary gland is in reality a composite of glands, the number corresponding to the number of streak canals, and each is autonomous and separate from its neighbors.

Supernumerary teats or nipples are common, especially in cattle in which the incidence may reach 30%, and are probably subject to a genetic control mechanism. They occur in males as well as females (except in male horses, which do not develop nipples), and are located in relation to the embryonic mammary ridge, although some may be displaced laterally. In many cases the supernumerary teat will have functional mammary tissue associated with it.

Mammary gland development in *males* is generally similar to that in females in the embryonic and fetal stages. In those species such as rats, mice, and horses, the males of which do not have teats or nipples, the primary sprouts separate from the surface epithelium of the mammary bud under the influence of androgenic hormone and regress. The male mammary gland is susceptible to hormonal stimulation but is not as sensitive as the female. Alveolar structures are not present and the enlargement that occurs under the influence of estrogen is due to cystic ductal hyperplasia. This is most often seen in dogs with estrogen-secreting tumors of the testes.

INFLAMMATORY DISEASE OF THE MAMMARY GLANDS: GENERAL CONSIDERATIONS

Mastitis is inflammation of the mammary gland, and this inflammatory response is nearly always a response to invasion of the gland by one of a number of microbes. The route of invasion may be hematogenous, as

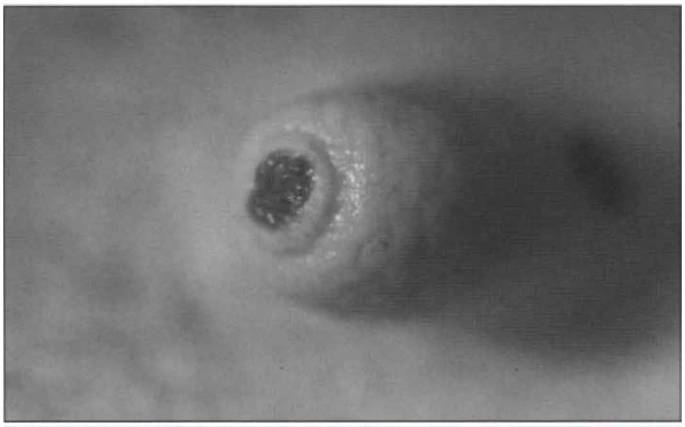

Figure 4.158 Mechanical damage to the teat end from improper milking equipment. The area of the orifice is ulcerated and scabbed, interfering with the barrier function of the streak canal and predisposing to ascending infection. This is a common iatrogenic lesion with important health and economic implications.

in tuberculosis and brucellosis, it may be percutaneous as a complication of local dermatological conditions of the skin or teat, but *the usual route of invasion is through the teat canal.* The relative roles of innate and acquired resistance, damage to the teat sphincter, and factors associated with mechanical milking are complex. The pathogenesis and characteristics of the disease, which are intensively studied only in cows, are dependent on factors in microbial ecology and the nature of the pathogen as well as on host factors.

A primary and critical barrier to infection is the teat canal. The establishment of a bacterial population in the teat cistern is usually followed by inflammation, but the application of even large numbers of bacteria to the teat orifice of an otherwise healthy teat results in infection in only a few cases. The length of the teat canal seems not to be important. Successful defense systems, both innate and acquired, control entry, proliferation, and tissue invasion and involve a spectrum of tissue, cellular, and cytokine responses.

Mechanical injury

Machine milking of dairy cattle is prone to induce significant damage to the teat ends when milking procedures and the milking equipment are not optimal (Fig. 4.158). This damage associated with high vacuum, poor liners, ineffective pulsations, and overmilking leads to partial eversion of the canal, ulceration, and fibrosis with associated bacterial growth (Fig. 4.159). Teat sinus injury from *overmilking* is associated with reduction of the *keratin plug* that helps protect from ascending infections.

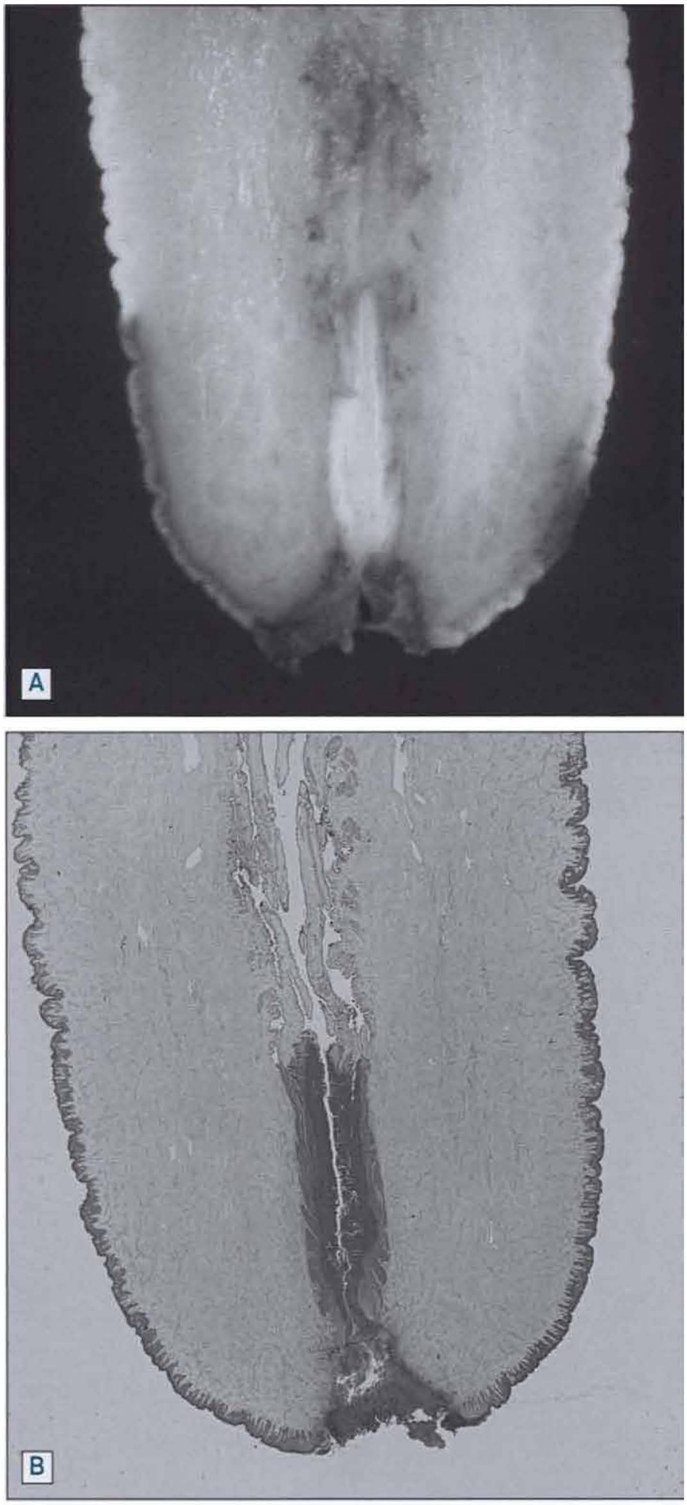

Figure 4.159 A. Sectioned fixed teat shown in Figure 4.158. **B.** Hematoxylin and eosin-stained section showing the teat cistern and keratin-lined teat canal with **focal ulceration** caused by trauma during milking.

INNATE AND ACQUIRED RESISTANCE OF MAMMARY GLANDS

Resistance to mastitis caused by *Streptococcus agalactiae* is rather strong in some cows, so it is possible to compare cows that are highly susceptible with those that are highly resistant. The difference between resistant and susceptible animals is only apparent if the infecting organism is applied to the teat orifice or within the teat canal; disposition of the organism, even in small numbers, within the sinus rather regularly results in infection – apparently the inherited mechanisms of resistance have been bypassed. Resistance is not an all-or-none phenomenon. It declines progressively with age and is, no doubt, subject to the general influence of body health, but at any single exposure to the teat orifice or canal it is all or none. In a susceptible animal, exposure of the canal to even small numbers of the organism is followed by their multiplication and entry into the cistern, whereas, in resistant animals, the inoculated organisms multiply briefly, if at all, in the teat canal, and disappear in the course of 2–3 milkings. *But resistance to this sort of exposure, which most nearly parallels natural exposure, can be removed by removing the smegma of the teat canal.* The teat canal is lined by stratified squamous epithelium that is surfaced by a layer of keratin-like material and then an innermost layer of smegma – a waxy material probably composed of epithelial debris and milk solids. Although the lining of the teat canal is an invagination from the normal surface epithelium, its chemical composition, as judged by staining reactions, is quite different. It is possible that there is in the smegma of the teat orifice of resistant cows some substance that is actually inhibitory to the growth of *S. agalactiae*. The phenomenon of resistance can be demonstrated also to operate in infections by *Staphylococcus aureus*, but with two differences. There is no increasing-with-age susceptibility to staphylococcal infections, and cows that are resistant to *Streptococcus agalactiae* are not necessarily resistant to *Staphylococcus aureus* and vice versa; apparently, the ingredients of resistance to the two infections are not the same.

There are humoral and cellular factors in normal milk that are inhibitory to bacterial growth and that may be increased in inflammation.

Lactoferrin is an iron-binding protein present in secretions and in neutrophils, which is inhibitory to the multiplication of bacteria with high iron requirements. The high concentration of citrate in normal milk prevents the action of lactoferrin, but it is effective in the secretion of the nonlactating gland.

Lysozyme is locally synthesized in the gland and, although the concentration in milk is low, there is some correlation between milk titer and susceptibility to infection. The titer may be increased during inflammation.

Lactoperoxidase is synthesized by mammary epithelium and is potentially inhibitory of *S. aureus* and most streptococci. It is a source of hydrogen peroxide available as a byproduct of bacterial fermentation of milk carbohydrates.

There are differences between species in the nature of **immunoglobulins** in colostrum and milk. In the cow, the immunoglobulins are predominantly of the *IgG* class, and are selectively transferred from serum to milk. The levels are low in normal glands but permeability changes in inflammation allow the titer to be much increased. The same class of antibody is identified with the concentration of plasma cells in the distal teat cistern and proximal streak canal, and may have an effect against organisms resident in the streak canal. *The major role of these antibodies is to promote opsonization of microorganisms. IgA* is also present in milk and may have a defensive effect on bacterial adherence to epithelial surfaces. Gram-negative bacteria can be killed by the *complement-antibody system*. The activity of this system in milk is normally low but can be greatly increased in inflammation as a result of permeability changes in mammary vasculature. There is variation among animals in the level of bacteriocidal activity in serum, but this appears to be the main mechanism limiting the duration of acute coliform infections.

Phagocytosis is the most important component of defense against gram-positive bacteria that have gained entry to the gland. Neutrophils enter the secretion in large numbers during mastitis, but the efficiency of phagocytosis and killing of engulfed bacteria is less in milk than in serum. The reduced efficiency may be due to depletion of energy substrates or lysosomal exhaustion following ingestion of lipid droplets or other solid materials in milk. Nonetheless, leukocytes do have a very significant role in controlling infection and in preventing superimposed infections in inflamed glands.

BOVINE MASTITIS

Bovine mastitis is typically a response to ascending infection of the gland by way of the teat canal. In spite of diligent efforts to limit infective opportunities and to treat established infections, the incidence of the disease and of reinfection is, overall, not much changed. The problems appear to reside in limited defense against colonization by ascending infection and in clearing mechanisms of limited effectiveness.

Potential mammary pathogens are ubiquitous. Modern techniques of microbial classification have identified more than 100 species, subspecies, and serovars isolated from the mammary gland. *Streptococcus agalactiae and perhaps some types of Staphylococcus aureus are obligate parasites of the gland and inevitable pathogens, but the great majority of infections are opportunistic.* It is perhaps useful to view the mammary gland and its microbiota in the same way as the bacterial colonization of other epithelial surfaces is viewed, whereby the local epithelial microenvironment is available for colonization by competent microbes. In this view, the consistent presence of *Corynebacterium bovis* and coagulase-negative staphylococci as well as other ill-characterized microaerophilic and anaerobic organisms reflects the *normal ecology* on which from time to time populations of other microorganisms may be superimposed.

Mastitis, excepting that caused by obligate pathogens, might best be regarded as an *inflammatory disease rather than an infectious disease*, and is one expressed by tissue injury when the microbial balance is upset and becomes dominated by organisms that are injurious by virtue of tissue invasiveness or toxigenicity. The pathologic process may range from transient to persistent and from mild or subclinical to peracute and fatal.

Notwithstanding the large number of microbial species that may be isolated from the diseased mammary gland, *the epidemiological picture of bovine mastitis is dominated by the streptococci, staphylococci, and coliforms.* Some infections, such as by *Cryptococcus* and the atypical mycobacteria, are usually iatrogenic. Some infections, such as by *Pseudomonas* and *Prototheca*, reflect heavy environmental contamination. Once the inflammatory process is established, additional microbial species, particularly the anaerobes, may participate by permission of a changed intramammary environment, allowing growth of resident organisms or altered competence of the teat canal, allowing entry of additional species.

Streptococcal mastitis

Bacteria of the family Streptococcaceae are important causes of bovine mastitis. *Streptococcus agalactiae, S. dysgalactiae,* and *S. uberis* are the most frequently implicated. Sporadic disease has been associated with *S. equi* and its subspecies *zooepidemicus, S. bovis, S, pyogenes, S. pneumoniae, Enterococcus faecalis, E. faecium,* and *E. durans.*

Bovine mastitis due to *Streptococcus agalactiae* was by far the most common and important of the forms of bovine mastitis until it was subdued by therapy. *The causative agent has the mammary gland of the cow and goat as its natural and sole habitat*; resistance to the extramammary environment is low, although the organism may survive for a month or more on fomites, but environmental factors are important in the transfer of infection from cow to cow.

The only significant portal of entry of *S. agalactiae* into the mammary gland is through the teat canal. Mastitis is always a possible complication of traumatic teat injuries, but infection in such cases is more often due to environmental organisms than to *S. agalactiae.*

Streptococcal mastitis is usually permanent. The organism is capable of maintaining its numbers in the cisterns in opposition to inflammatory products and the irrigative force of milking. How it remains in equilibrium with its host in the infection phase is not clear. At a variable period following the establishment of infection, the bacterial population may suddenly increase and the tissue becomes invaded for a brief period producing a clinical crisis. This proliferation of bacteria within the cisterns precedes tissue penetration and is associated with altered virulence of the organism, as measured by mouse-pathogenicity tests. Whether there is also some change in the host system is not known, but change ought perhaps to be expected, because the detrimental effects of improper machine milking, in terms of height of vacuum, rate of pulsation, and period of application, are expressions, largely, of flare-ups of established quiescent infections. Repeated invasions usher in a series of inflammatory and reparative reactions, which, if unchecked therapeutically, culminate in fibrosis and involution of the affected quarter.

There are various degrees of severity of the clinical reaction of streptococcal mastitis, the more acute cases being associated with systemic disturbances and the less acute ones not. The anatomic basis lies not in the acuteness of the inflammation, but in the amount of mammary tissue involved. A greater or lesser amount of normal uninflamed tissue is always present, the inflammatory reaction being confined to those areas in which the organisms manage to penetrate the duct epithelium. It appears that the streptococci are unable to penetrate the interstitial tissues to a significant degree, although sometimes they are found in leukocytes in the lymphatics and supramammary lymph node, and that the inflammatory reaction is due to diffusing toxic products of the organisms. This period of epithelial penetration is brief, being of only a few hours' duration, and the organism in tissue is rapidly destroyed by leukocytes. This destruction of the organism may at times sterilize the udder or so diminish numbers that bacterial cultures of milk taken during a crisis may be negative, a finding that also applies to other acute mammary infections.

The first response to the penetration of streptococci is remarkable interstitial edema and extensive migration of neutrophils into the interlobular tissue and secretory acini. Stromal lymphatics are widely dilated and contain numerous leukocytes that are delivered to the regional lymph node. The acinar epithelium becomes vacuolated and desquamated, or in places heaped up and ragged, over accumulations of macrophages and fibroblasts that make their appearance very early in the course of the reaction. Streptococci are very numerous at this stage, both within the ducts and acini and in and under the epithelium. *The acute exudative reaction gives way to two processes that proceed together – pathologic fibrosis and involution.* A few organisms still persist in the larger ducts and the neutrophil reaction is reduced, but macrophages and fibroblasts continue to increase in number (Fig. 4.160A) and eventually obliterate many of the acini. Lymphocytic

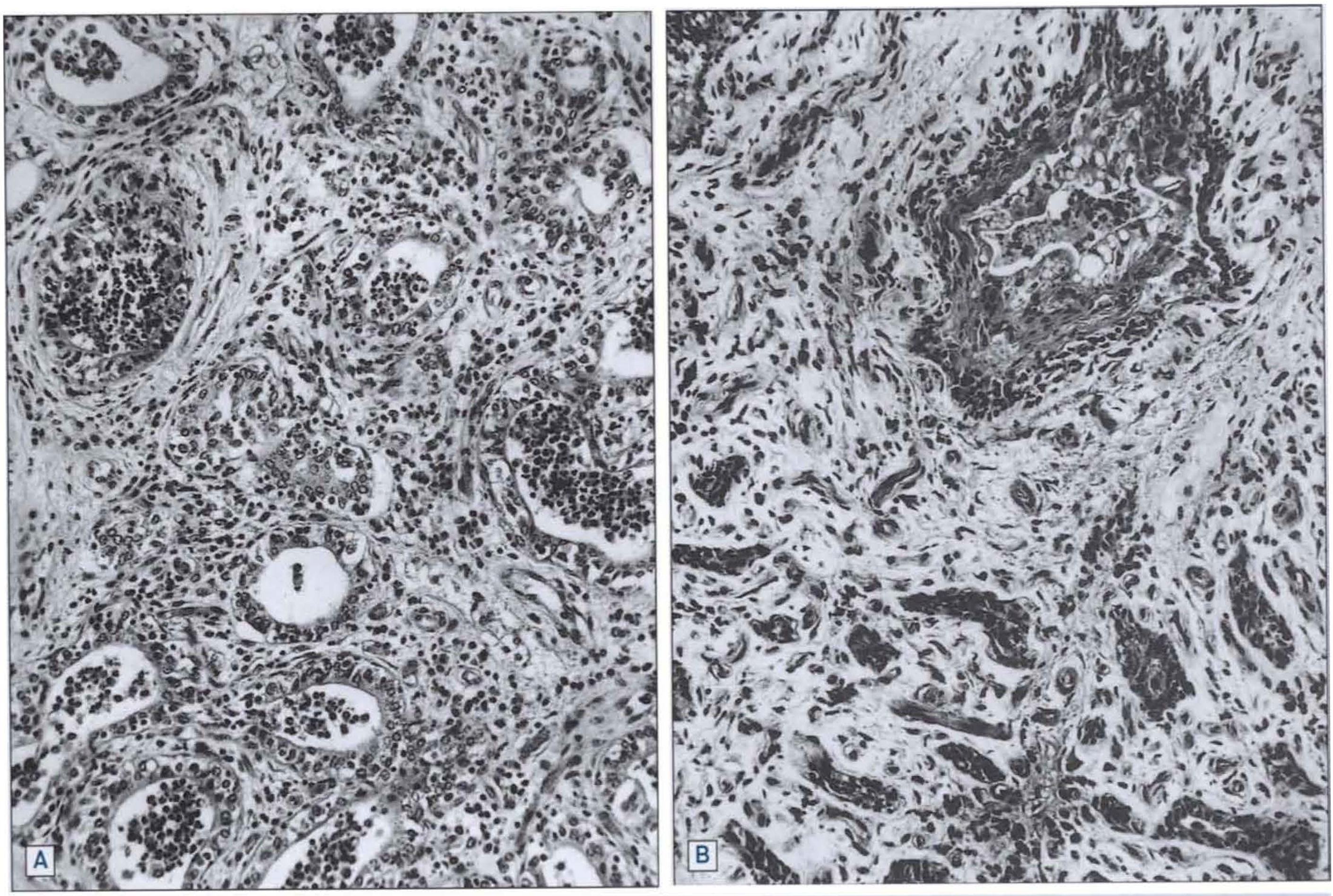

Figure 4.160 A. Acute streptococcal mastitis in a cow. **B. Chronic streptococcal mastitis** is characterized by fibrosis, atrophy of the glandular secretory epithelium, and squamous metaplasia of the ducts.

foci begin to develop in the interstitial tissue. Other acini become dilated and rounded and contain a stringy coagulum with intact cells and cellular debris, which constitutes the first indications of the involution that follows interruption of secretion and acinar stagnation.

The involution affects lobules directly involved in the exudative inflammation as well as those surrounded by interacinar tissue to which the fibroplasia has extended. Stagnation of secretion also occurs in many of the smaller ducts. It is pertinent to note that this is the stage that on palpation is swollen, firm, and painful and often referred to as "fibrosed." But there is as yet little fibrosis, *"induration" being due instead to retained secretion.* These nodular or diffuse indurations are firm and liver-like in consistency but of normal color and are easily cut, in contrast to the normal slack elasticity of mammary tissue that makes it difficult to cut. The processes of fibrosis and involution continue until the end-stages, when some lobules show normally involuted tissue, some are obliterated by fibrosis (Fig. 4.160B), and others show a variable balance between both processes. Such a gland is atrophic, dry, and, because of the fibrosis, poorly elastic. Some lobules escape for a time, often a long time, only to be involved ultimately in a flare-up or by extension of the disease process. It is evident then that the many lobules involved are often out of phase with each other, but that in each the succession of events proceeds rapidly.

Adjacent to the spots where the streptococci invade the epithelium of the smaller ducts, *granulation tissue develops rapidly and exuberantly and protrudes into the duct lumen*, which is dilated and contains stagnant milk, exudate, and numerous organisms. These polypoid proliferations may completely obstruct the duct or, by fusing with the opposite surface, produce loculations resembling abscess cavities that are clearly visible macroscopically. There is concomitant proliferation of periductal fibrous tissue spreading centrifugally to involve and obliterate large amounts of lobular tissue. Such granulation tissue in time cicatrizes and the duct epithelium is restored. Similar changes occur in the larger ducts and teat cisterns at points of bacterial penetration, but they are less exuberant and the epithelium is shortly transformed to a squamous, sometimes keratinizing, type that is later desquamated and reconstituted as the acute phase subsides to the chronic one of involution and as fibrosis supervenes. *Involution has been regarded as a defense mechanism, but this misconstrues its purely passive development as a consequence of stagnation.* Such involuted lobules are capable of function at subsequent lactations. Whether there is any replacement of destroyed lobules by regeneration from persistent ducts has not been established, but it cannot amount to much.

The *gross appearances* of streptococcal mastitis vary according to the stage of the disease. Usually more than one quarter is involved, but not uniformly, most of the change taking place in the distal portion of the gland about the cisterns and large ducts. The cisterns and ducts are filled with secretion in the early stage of the disease, the secretion being serous and floccular, or distinctly purulent. The mucous membrane of the cistern may not be appreciably altered or it may be hyperemic and granular. The glandular tissue is swollen and turgid. It cuts easily with a knife, and on the cut surface the lobulations are distinct because the swollen lobules protrude. It is not possible to recognize the interstitial edema. The affected lobular tissue is gray and can be distinguished from the milky whiteness of normal lactating tissue, but not readily from involuted parenchyma.

In the later stages of the disease, the most obvious changes occur in the cisterns and large ducts as moderate thickening of the epithelium and small rounded polypoid proliferations into the lumen of the cistern and large ducts. Fibrosis is recognizable in these cases surrounding the ducts, surrounding lobules and obliterating some, and diminishing in severity towards the base of the gland.

Infection with ***Streptococcus uberis***, an environmental pathogen, may produce transient inflammation, often accompanied by systemic signs of illness, and infection may become persistent. Infection tends to be sporadic, but occasionally there are severe outbreaks in individual herds. Mammary gland infection with *S. uberis* is recognized as one of the least well-controlled causes of mastitis in dairy cattle. Any of *S. agalactiae, S. dysgalactiae,* and *S. uberis* may be present with *Arcanobacterium pyogenes* in the severe summer mastitis of dry cows and heifers.

Mastitis caused by ***S. pyogenes*** is of some importance because the organism is a human pathogen; the secretion from infected quarters may contain enormous numbers of these organisms, but they proliferate barely, if at all, in drawn samples of milk. The mammary infections may arise from close association with infected humans, and outbreaks of upper-airway infection have occurred in people consuming milk from infected cows.

Staphylococcal mastitis

Staphylococcal mastitis is predominantly an infection of the younger age groups, and there is no increase in susceptibility with age.

Pathogenic strains of staphylococci are always of human or animal origin and persist as permanent inhabitants of the skin and mucous membranes, although they are capable of more than average resistance to the general environment. It is generally accepted that the infection of the udder is *contagious*, and it seems probable that entry through the teat canal is, in general, subject to the same sort of local controls as is the entry of *S. agalactiae*.

Pathogenic staphylococci can be broadly grouped as to whether they are catalase-positive or catalase-negative. The ability of a strain to produce catalase and hemolysis is the best single criterion of pathogenicity for animals. The coagulase-positive staphylococci include the important ***Staphylococcus aureus***, as well as *S. intermedius* and *S. hyicus*. The coagulase-negative staphylococci comprise a large number of species, of which *S. epidermidis* is the species most frequently isolated from milk. These species are of low pathogenicity and may perhaps be best regarded as part of the normal flora. They are commonly recovered from normal milk samples and from cases of subclinical mastitis expressed as elevated somatic cell counts in drawn samples.

Mastitis paralleling the natural disease can be produced by infusing the udder with the α toxin; the β toxin is nonirritant. Strains of the organism, not separable by other criteria, differ in their toxigenicity, but this is not necessarily related to pathogenicity; one strain may produce gangrenous mastitis on some occasions and only a mild disease on other occasions.

Clinically, staphylococcal mastitis may be peracute and fulminating or milder and more chronic; the latter is the more common. The acute forms of the disease, typically, occur shortly after parturition and tend to produce gangrene of affected quarters and high mortality. The affected quarters are swollen and tense, hot and firm, and very painful. There is almost complete stagnation of secretion and only a few milliliters of brown, blood-stained, or straw-colored watery fluid can be expressed from the teat. Uninfected quarters of the same udder are also swollen and tense and the secretion is reduced but otherwise normal – an effect probably due to the diffusion of toxins through the vascular bed of the gland.

Gangrene usually affects first the teat and adjacent portions of the udder and may not be more extensive, or it may extend even to involve the whole quarter. The tissues become blue and eventually black and are softer, insensitive, and cold. There is pitting edema of the inguinal area, flank, and venter, and in a day or so the necrotic skin begins to exude serum and to slough, and crepitating gas bubbles develop beneath it. The changes are those of ordinary moist gangrene remarkable only for the abundance of fluid exudation, and attributable to the direct action of toxin on the acinar tissue and to venous thrombosis. The amount of tissue involved in the gangrenous process is quite variable and groups of necrotic lobules adjoin others that are near normal. Natural separation of the gangrenous areas begins about a week after the onset but proceeds slowly with the development of a suppurative surface and fistulae.

The acute, nongangrenous, and mild forms of the disease progress more closely along the lines already described for streptococcal mastitis, but with some important differences that will be mentioned later. It appears that early events are comparable to those of streptococcal infection and that the bacterial population, resident in the ducts and cisterns during the infection phase, multiplies rapidly (Fig. 4.161A) and then penetrates the duct wall to the interacinar tissue. If the penetration is massive and the organisms are highly toxigenic, the acute and gangrenous forms of the disease occur. But the invasion, even if massive, is only massive in patches and intervening areas remain normal for some time (Fig. 4.161B).

The differences in the manner of progression of streptococcal and the milder staphylococcal mastitis depend on different toxigenicity of the genera and the ability of the staphylococci to invade more deeply into the interacinar tissue and to establish themselves there as persistent foci of infection to provoke the granulomatous reaction known as "*botryomycosis*" (Fig. 4.162).

The initial reaction is necrotizing and these necrotic foci are soon surrounded by an intense leukocytic response, and fibroplasia develops rapidly to wall off the irritant foci, thus obliterating large portions of the normal mammary structure. Each granulomatous focus may be no more than 1–2 cm in diameter, but they may be numerous and involve a large proportion of the gland with, between them, a residuum of involuted lobules that is surrounded by septa greatly thickened by confluence of fibrosis. The thickened septa and the granulomas are readily visible to the naked eye and small amounts of pus can be expressed from them. Microscopically, the granulomas closely resemble those of actinomycosis and have been so called, but the coccal organisms are easily visible in the microabscesses. Walled off by connective tissue, the bacteria are not exposed to the action of antibiotics. The infection is always a localized one, and even in peracute cases bacteremia does not occur.

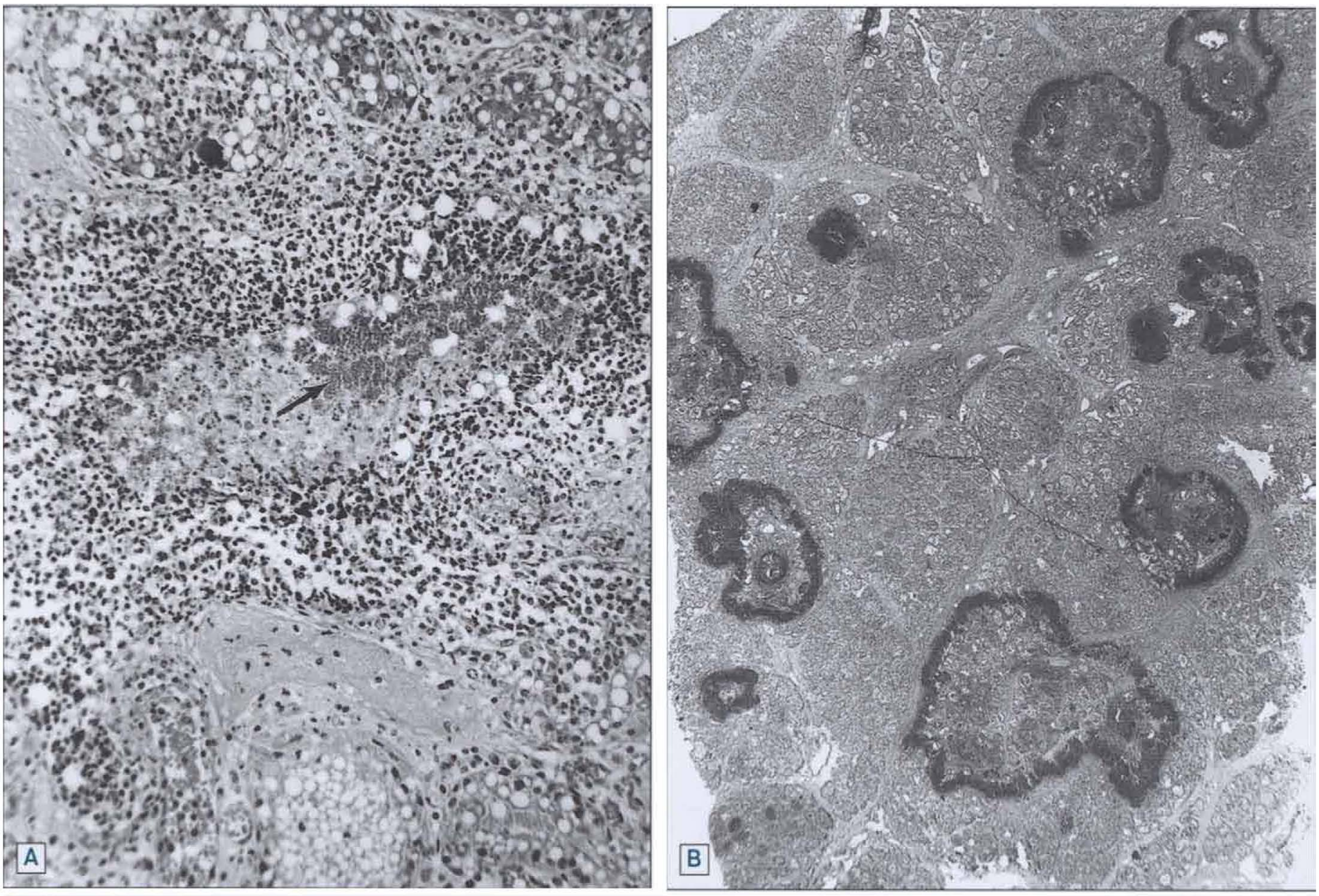

Figure 4.161 A. Acute staphylococcal mastitis in a cow, with bacterial colonies present. **B.** Multifocal areas of locally extensive **acute necrotizing mastitis** in staphylococcal mastitis.

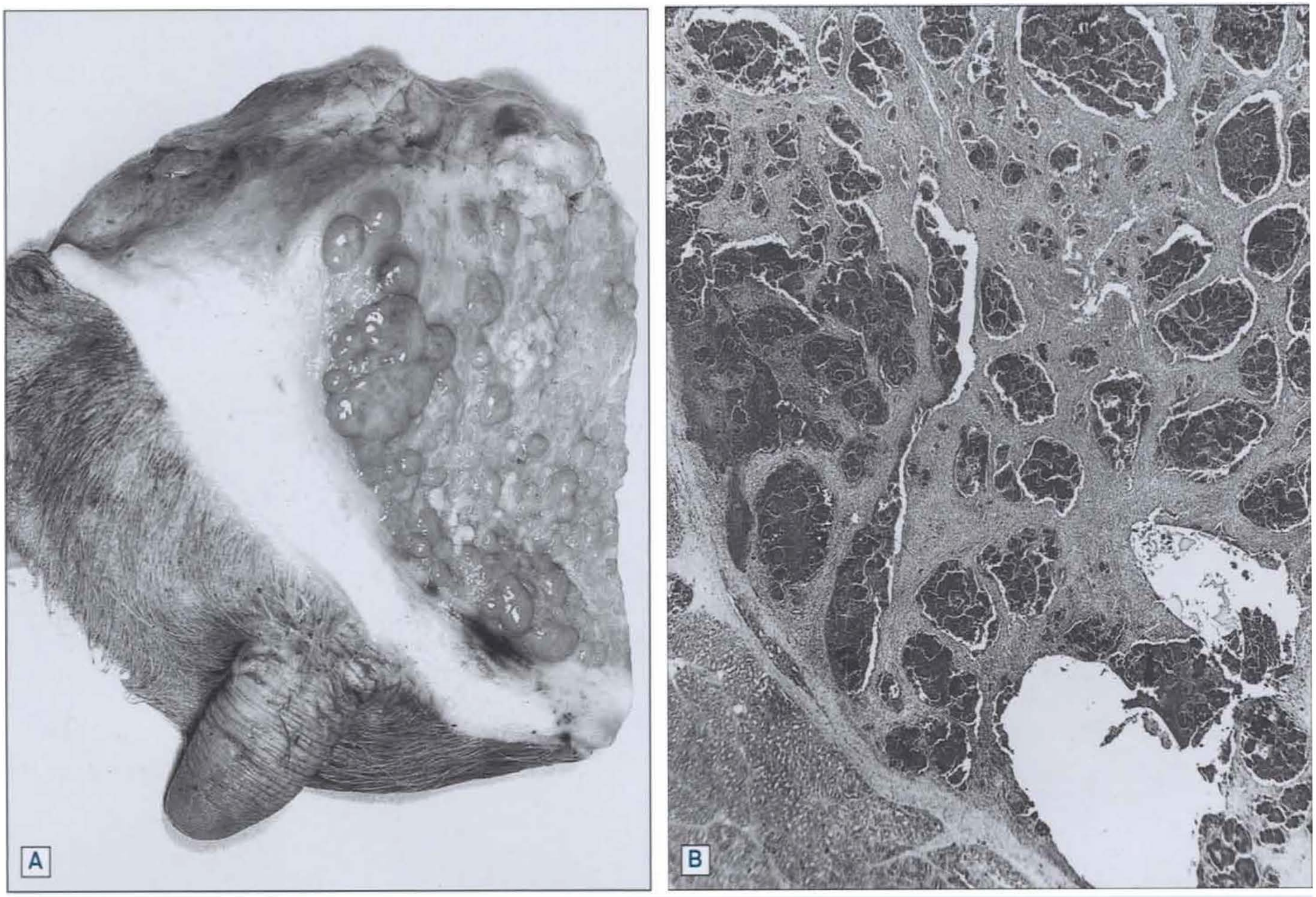

Figure 4.162 A. Multifocal granulomas with associated fibrosis in **chronic staphylococcal mastitis** in a cow; referred to as a **botryomycotic type**. **B.** Photomicrograph of the lesion in **(A)**. Note the thick bands of fibrous connective tissue.

The organ changes in glands shedding nonhemolytic, coagulase-negative staphylococci of low pathogenicity without clinical signs of infection are mild progressive fibrosis and lobular atrophy. Hemolytic coagulase-positive staphylococci produced lobular lesions of greater extent and rate of progression. The different stages of the inflammatory reaction in different lobules is quite like that in the streptococcal disease, and the patchy progression corresponds to the irregular distribution of cultivable organisms in affected glands. It is proper to emphasize the importance in this mild disease of partial or complete occlusion of small collecting ducts or intralobular ducts by exudate or granulation tissue as mechanisms inducing acinar atrophy. Occluded ducts also produce a locus for bacterial persistence and growth leading to small abscesses and obliterative granulomas.

Staphylococcus epidermidis is an intramammary pathogen, but not a serious one. Infections tend to be eliminated spontaneously.

Coliform mastitis

Coliform bacteria are more common and important than their treatment as a miscellany suggests, but very little is known of the pathogenesis and lesions. The bacteria usually grouped in this category include ***Escherichia coli*** and species of the genera ***Enterobacter, Klebsiella, Citrobacter, Serratia,*** and ***Proteus***. *These organisms are part of the* **environmental** *flora, and they do not have a particular predilection for the mammary gland; mammary infection may be correlated with the level of environmental exposure.* Each usually produces a clinically acute form of the disease with systemic reaction and, especially in the case of *Escherichia coli, septicemia*. Acute tendovaginitis may also occur. Both *E. coli* and *Enterobacter aerogenes* may be associated with clinical flare-ups of the milder progressive type of disease with a history closely resembling that caused by *Streptococcus agalactiae*. With these infections also, negative cultures may be obtained during the acute phase.

Coliform mastitis is a serious problems in herds in which the more common mammary infections are suppressed or eliminated. *Coliform mastitis can be viewed as a disease of a normal lactating gland,* the microbe being ineffectual in initiating infection in glands that, as a result of prior irritation, produce large numbers of neutrophils in the secretion. Coliform bacteria are ubiquitous in the dairy environment and microbial density may be enormously increased by well-intentioned husbandry procedures.

In accord with convention, *the mammary changes produced by coliform organisms are attributed to the action of endotoxins.* Cellular susceptibility to the actions of endotoxins are of course important, but the tissue changes that dominate the response are the result of endotoxic injury to the microvasculature of the alveolar walls and

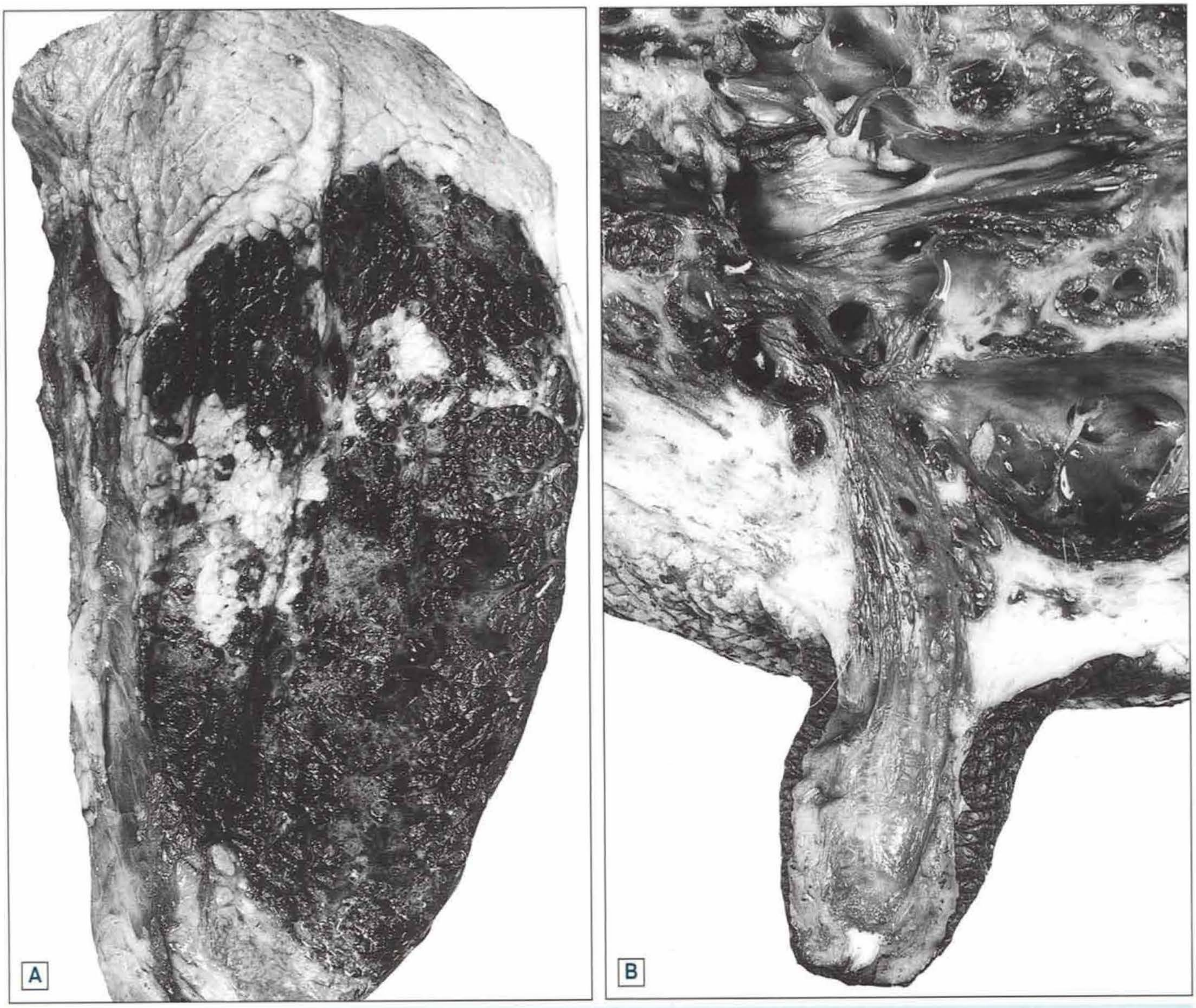

Figure 4.163 Coliform mastitis. **A.** Acute locally extensive hemorrhagic necrotizing mastitis. **B.** Hemorrhagic purulent **galactophoritis**.

mammary interstitium. Acute infections that provoke massive neutrophil release may be self-cleansing. Chronic infections do however occur, characterized by irregular exacerbations, but the mechanisms of persistent infection are unclear and may be related to the adequacy of host responses and the sensitivity of the bacteria to the lytic effects of the antibody/complement mechanism.

The pathology of the coliform infections has not been studied in detail. Indeed, there is even some debate on the pathogenesis of those infections produced by the enterobacteria, a hematogenous route of infection frequently being postulated because mastitis often coexists with puerperal infection of the uterus. *It appears much more likely that infection is galactogenic*, because the cisterns and ducts bear the brunt of the injury and contain numerous bacteria whereas neither bacteria nor significant leukocytosis can be demonstrated in the interstitial tissue in the peracute infections, which are the fatal ones.

The infection is often limited to one quarter and, being of short course, the inflammation may be predominantly serous with much edema, or it may be severely hemorrhagic (Fig. 4.163A). The secretion in the cistern is scanty and may be watery, or cloudy and blood-stained; it contains floccules of fibrin and coagulated casein. Similar floccules can often be seen as plugs in the milk ducts (Fig. 4.163B). There is usually severe edema of the subcutis.

Microscopically, the inflammatory reaction is seen to be centered on the ducts. The lining of the larger ducts is destroyed and replaced by fibrinocellular exudate. The lining of the smaller, intralobular ducts is also destroyed and in these there are plugs of necrotic detritus (Fig. 4.164).

The acini are filled with serous fluid in which there are vacuolated desquamated epithelial cells, but leukocytes are few or absent, either in the alveoli or in the septa. The septal tissues, especially the interlobular septa, are widened by edema fluid, and the greatly dilated lymphatics contain many plugs of fibrin. In areas of hemorrhagic inflammation, the septal vessels are suffused with blood and tortuous and there is extensive hemorrhage into the stroma and into the acini.

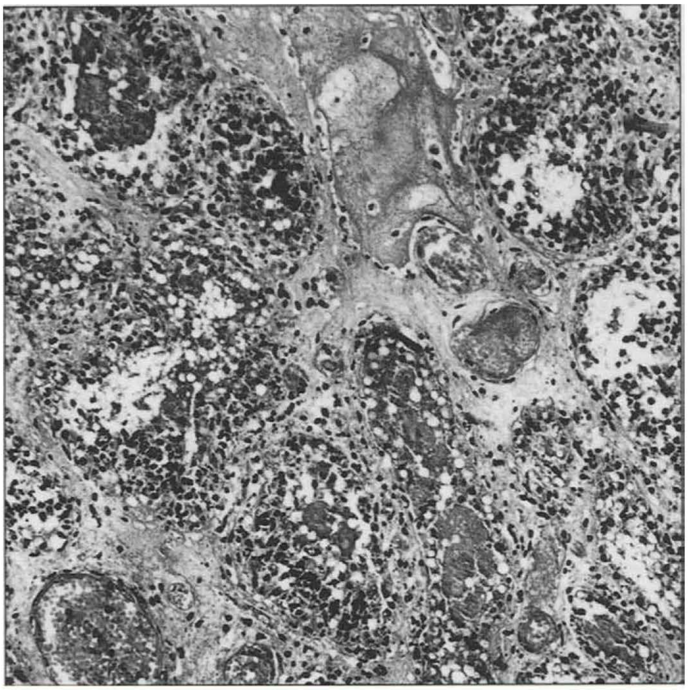

Figure 4.164 Acute fibrinous necrotizing mastitis, in bovine coliform mastitis.

If the course of the peracute inflammation is prolonged beyond 1–2 days, *extensive necrosis of tissue may occur*. Gangrene is not produced by coliform bacilli alone. The necrosis is evident by the altered stainability of the tissues; the histoarchitecture of the tissue is retained for some time. If the animal survives, the necrotic tissue, usually most of one quarter, is sequestrated.

Summer mastitis

Arcanobacterium (formerly *Actinomyces, Corynebacterium*) ***pyogenes*** causes sporadic cases of acute mastitis as a result of penetrating injury and is frequently associated with a form of mammary disease known in the UK as summer mastitis and in continental Europe as Holstein udder plague. *This is usually a mixed bacterial infection affecting immature and nonlactating glands of animals at pasture.* While *Arcanobacterium pyogenes* can cause sporadic cases of suppurative mastitis and can be experimentally transmitted via the teat canal, it cannot always be recovered from the exudates of summer mastitis. It is more usual to recover a mixture of organisms that may include *A. pyogenes, Streptococcus dysgalactiae, Peptostreptococcus indolicus, Bacteroides melaninogenicus, Fusobacterium necrophorum,* microaerophilic, and unidentified obligate anaerobes. Infection is acquired via the teat canal, probably as a result of contamination by flies attracted to pre-existing teat lesions such as impetigo, papular stomatitis, trauma, and photosensitization.

The sequence of events in actinomycotic mastitis has not been studied, but the lesion is clearly a *necrotizing suppurative galactophoritis* with slight primary involvement of the acinar tissue. Grossly visible abscesses form where the exudate remains stagnant in the ducts. The smaller abscesses occur in the intralobular ducts, and these are associated with desquamative changes and the exudation of a few leukocytes into the acini, and with rapid fibroplasia and infiltration of leukocytes, including many plasma cells, in the septa. The smaller intralobular abscesses may heal with obliteration of the ducts and scarification of the lobule. The large abscesses are centered on the larger ducts, the walls of which are remodeled by exuberant granulation tissue, although still lined in part by hyperplastic and squamous epithelium. Bacteria are numerous in the abscesses and in the secretions in other parts of the duct system. The walls of the teat canal and cistern are thickened by granulation tissue and the mucosa is ragged. The cistern is narrowed by fibrosis, and the teat canal may be stenotic or atretic.

Mycoplasma mastitis

The mycoplasmas are difficult organisms to handle, and much sorting out of them remains to be done in terms of their classification, ecology, and pathogenic capabilities. They are prominent causes of bovine mastitis. In herds where sincere attempts are made to control mammary infections, their presence is suggested by high attack rates, rapid spread to other quarters and within the herd, unrewarded efforts to culture bacteria, and very poor response to therapy. Shedding of the organisms from infected quarters is inconsistent, adding to the challenge of clinical diagnosis and control.

The diseases produced by *Mycoplasma* spp. and *Acholeplasma* are not clearly separable pathologically or clinically. The following discussion relates in particular to ***Mycoplasma bovis*** which, being the most frequent of these infections, is used here as the type infection, although infections by *M. canadense, M. bovigenitalium,* and *M. californicum* are also common.

The disease is characterized by *sudden onset of agalactia*. The mammary gland is initially swollen, firm, and painless. Ordinarily there are no systemic signs, but arthritis may complicate some outbreaks. When clinical signs are more extravagant, coexisting mammary infections should be suspected. The disease spreads rapidly in a herd, and animals in full lactation tend to be affected simultaneously in all glands. The milk as drawn appears normal but rapidly forms a floccular deposit and clear supernatant on standing. The presence of pus or blood probably indicates coexistent bacterial infection, although the mycoplasmas do cause massive emigration of neutrophils.

Affected quarters are, in the active stages, swollen and firm but later become slack as rapid involution occurs. The altered secretion and glandular enlargement may persist for several weeks. Clinical recovery may occur without return to full normal function, and such animals may continue to excrete the organism intermittently for more than a year. It is not clear whether clinical relapses occur or whether over this long period there is cumulative mammary injury.

The route of infection of the mammary gland is not clear. The disease can be produced experimentally, usually in an acute form, by applying the organisms on the teat or within the canal, as well as by intravenous injection. The mode of spread suggests a galactopoietic infection, but the occurrence of arthritis suggests that systemic dissemination occurs in some cases.

The pathological changes in the gland appear fairly distinguishable from other causative types of mastitis, and the available descriptions of tissue changes have much in common. *The distinguishing features appear to be the abundant exudation of granulocytes in the early stages, and the hyperplasia of alveolar as well as ductal epithelium in later stages.*

The exudation of granulocytes begins within a few hours of experimental infection as perivascular accumulations in walls of the cistern, in the lobular interstitium, which is edematous, and mixed

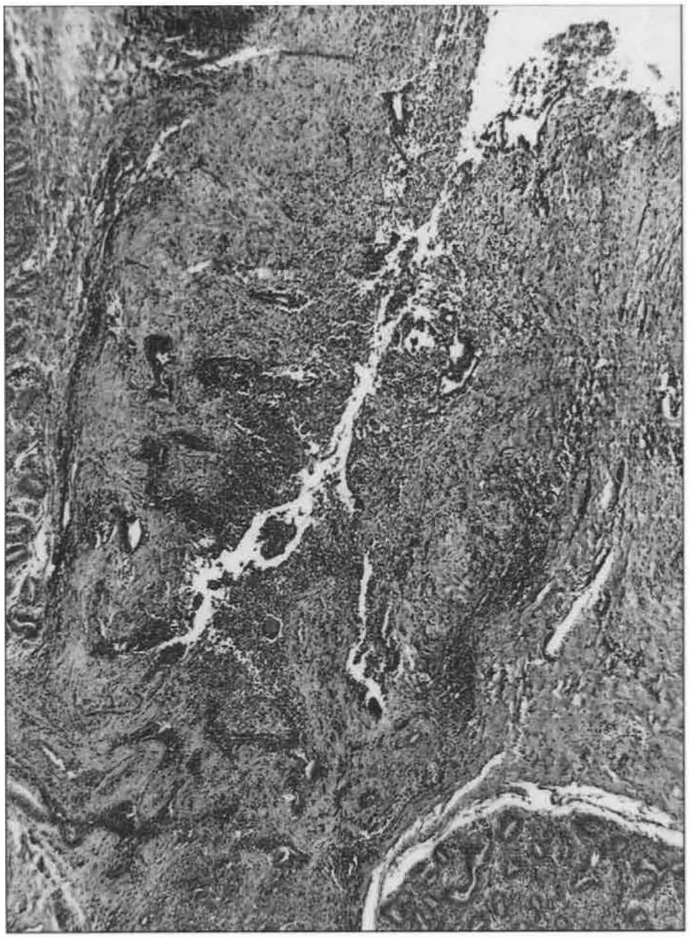

Figure 4.165 Chronic granulomatous galactophoritis, in bovine tuberculosis.

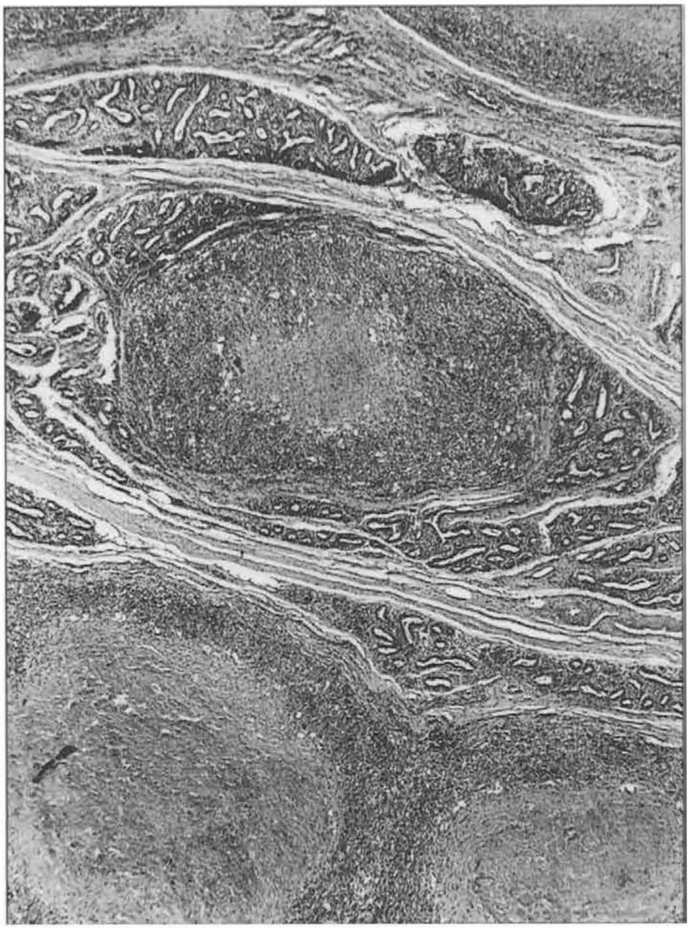

Figure 4.166 Multifocal (miliary) **tuberculous mastitis** in a cow.

with the alveolar secretion. Initially patchy, the reaction extends quickly to involve most of the parenchyma, and, after several days, the reaction begins to subside; mononuclear cells, including plasma cells and macrophages, appear in the exudates; and dense focal lymphocytic accumulations develop in intralobular and periductal connective tissue. The lymphocytic infiltrations persist for many months. The epithelial cells of alveoli and ducts develop large fat vacuoles that are discharged into the lumen. The alveolar epithelium shortly becomes hyperplastic, the rather undifferentiated cells being present in several layers. In the course of some weeks, fibrosis of intralobular septa is accompanied by alveolar atrophy. Hyperplastic and metaplastic alterations also occur in the ductal epithelium, around which is progressive fibroplasia and lymphocytic infiltration. There is extensive but discontinuous erosion of the epithelium, and similar changes occur in the epithelium of the milk cistern. Exuberant granulation tissue forms, especially at the sites of epithelial erosion, producing polypous protrusions into the ducts and cistern. Retention of exudate occurs in small ducts. The exudate tends to mineralize and serve as a nidus for discrete granulomas.

Bovine tuberculous mastitis

Various figures have been quoted for the incidence of mastitis in bovine tuberculosis, but no satisfactory estimation has so far been made. The most significant generalization is that involvement of the mammary glands is second only to that of the lungs. Its public health importance, however, depends on the observation that, in all cases, regardless of age or activity of the lesion, the ducts are involved and, therefore, every case of mammary tuberculosis is an open case.

There are three major anatomical forms of mammary tuberculosis; *disseminated miliary tuberculosis, chronic organ tuberculosis,* and *caseous tuberculous mastitis*. In the great majority of cases, the infection is blood-borne, with subsequent spread along the ducts (Fig. 4.165). The anatomic form of the lesions is deductively explained on the basis of degrees of resistance and hypersensitivity, but these are essentially imprecise (see Vol. 2, Respiratory system). The disease may occur with outstanding duct involvement following infusion with material contaminated with ***Mycobacterium bovis***.

Disseminated miliary tuberculosis develops as part of an early generalization and is not common, even in a highly infected community. The lesions are rather like those of miliary tuberculosis elsewhere, occurring as nodules up to 1 cm or so in diameter which project above the cut surface. The *tubercles* are not evenly distributed, but rather tend to occur in groups in the deeper tissues. *Some are caseous, others are caseous and mineralized, and all are of typical tubercle structure, often with very heavy capsules*. The foci begin to develop in the interacinar connective tissue and remain localized to lobules that are completely destroyed (Fig. 4.166), but there is no direct progression between adjacent lobules or encroachment on the interlobular connective tissue. The reaction is typically tuberculous, and often there is more than one tubercle in each lobule affected. The interlobular ducts are also extensively involved and the dilated lumen is filled with cellular exudate. The wall itself is greatly increased in thickness by tuberculous granulation tissue and epithelial hyperplasia. The *supramammary lymph nodes* in such cases usually contain many typical tubercles.

Chronic organ tuberculosis *is the most common form, representing 80–90% of cases.* It is rather different from the ordinary tuberculous lesion, and the *regional lymph nodes are not usually involved,* although some may show numerous microscopic foci of infection with few organisms. The mammary tissue is very firm and cuts readily. The lobulated structure is exaggerated and, when cut, projects above the surface of the organ, giving a smoothly bumpy appearance surrounded by the indentation of the interlobular tissue. The lobules that are affected – and most of them tend to be – vary from gray-red to white, and the surface is dry. The lesions begin as one or more foci of granulation tissue within the lobules and they expand and coalesce to involve the entire lobule. A few of them break down and caseate. Histologically, the lobular outlines are retained and the interlobular septa are not involved. The process begins in the intralobular septa, and the diffuse tuberculous granulation tissue overruns and obliterates the acinar tissue. *Typical tubercules do not form,* although the types of cells involved in the reaction are as usual. The intra- and interlobular ducts are always involved and the walls are greatly thickened by granulation tissue. The surface tends to caseate, although some epithelium, altered to a squamous type, may persist. Caseous exudate collects in the sinuses. In this form of the disease, *intramammary spread is entirely by way of the ducts,* and the reaction is typical of any chronic organ tuberculosis and of exogenous or endogenous reinfection in an animal highly resistant by virtue of previous exposure.

Caseous tuberculous mastitis causes great enlargement of affected glands, and they are not nodular. The caseous areas are large and irregular, with the dry yellow caseous appearance and hyperemic margin suggestive of ischemic infarction. Other portions of the gland may show areas of chronic organ tuberculosis, often with commencing caseation and transformation to the diffuse type. Confluence is the rule and there is no limitation of spread by interlobular boundaries. The histological change is characterized by inflammatory exudation of fibrin and numerous leukocytes. The caseated areas are surrounded by a zone of hyperemic granulation tissue of the sort that occurs in chronic organ tuberculosis, and hemorrhage in it occurs frequently. This form of the disease appears to develop from chronic organ tuberculosis, or possibly as part of an early generalization of the disease, in animals whose resistance is lowered by nonspecific factors or in which a high degree of sensitivity to the organism has developed.

There is, in addition, a form of mammary tuberculosis in which the process centers on the interlobular ducts with subsequent spread to the larger ducts. In all cases of tuberculous mastitis there is duct involvement, but in the types already described duct involvement is by spread from initial foci in the intralobular tissues. In this last form, so-called **tuberculous galactophoritis**, there may be no discoverable acinar lesion, or, if the lesions are present, they are more recent than, and obviously derived from, the duct infection. The glandular tissue in such cases retains its lobular character. The ducts are dilated and filled with an exudate of mixed inflammatory cells. The duct epithelium undergoes patchy squamous or keratinizing metaplasia and the walls are greatly thickened by granulation tissue and infiltrated mononuclear cells but have few or no tubercles. *The infectious process apparently begins in the walls of the larger interlobular ducts and extends up and down the duct system.* Proximal progression leads to involvement of the smaller interlobular ducts and their obstruction, and this is followed by involution of the associated lobules. Tubercles occur in the supramammary lymph gland in this form of the disease.

Mammary tuberculosis usually develops insidiously without signs of acute inflammation, except in a few cases of the diffuse caseous type. The gland progressively increases in size and firmness. Rather importantly, *the milk may be physically normal for a long period after the onset of the disease, even though it contains the bacilli.* In the later stages of the disease there is a reduction in the amount of the secretion, and it is converted to a thin whey-like fluid that contains floccules of caseous exudate and very large numbers of bacilli. Because of the predominance of duct involvement, all cases are "open" and bacilli may be passed in the milk before gross lesions of tuberculosis are evident.

Miscellaneous infections

Included within this grouping are bacteria (*Nocardia asteroides, Mycobacterium* spp.), fungi (*Cryptococcus neoformans*), yeasts (*Candida* spp.), and algae (*Prototheca zopfii*). With the exception of *N. asteroides*, these infections develop after infusion of the udder with compounds, usually penicillin, suspended in oil and tend, therefore, to involve a number of animals in individual herds.

Mastitis caused by ***Nocardia asteroides*** (primarily a pathogen of humans and dog) is sporadic, although occasionally it reaches significant proportions in a herd. The source of infection is predominantly the soil, and under unknown circumstances entry is gained to the udder via the teat canal. Although perhaps best regarded as a sporadic infection of individual quarters, it is possible the outbreaks in herds could have resulted from repeated mammary infusions for the control of mastitis.

N. asteroides may be present in mammary secretions for many months without causing clinical signs of mastitis. In other cases, the response to infection is a mild chronic involvement of the mammary gland, while in yet other cases, the reaction is of acute onset and severe clinical course. Severe reactions seem particularly likely to occur in infections acquired near the time of parturition. The severe reactions are characterized by fever that may last for several weeks with other signs of systemic illness, and spread of infection to supramammary nodes and lungs has been demonstrated in some of these cases. Affected quarters enlarge rapidly and in one week may be very firm from fibrosis. In some cases, there is a palpable nodularity, the nodules being 2–5 cm in diameter. These may be small sinus tracts discharging on to the skin, or larger defects that qualify as ruptures. The acute infections may be purulent in small foci, but larger abscesses suggest a mixed infection with *Pseudomonas aeruginosa, Arcanobacterium pyogenes*, or other organisms.

The mastitis is essentially lobular, with the ducts bearing the brunt of the injury. The lining of the ducts and sinuses is thick and corrugated. The drawn milk may be near normal or grossly altered, and in the early stages of infection may contain small white granules up to 1 mm in size, which are tangled masses of bacteria. Similar colonies may be found in the discharge of sinus tracts. The glandular lesions remain fairly restricted to lobules without much coalescence across interlobular septa, except in virulent infections or those with much suppuration. The cut surface of the tissues shows extensive fibrosis

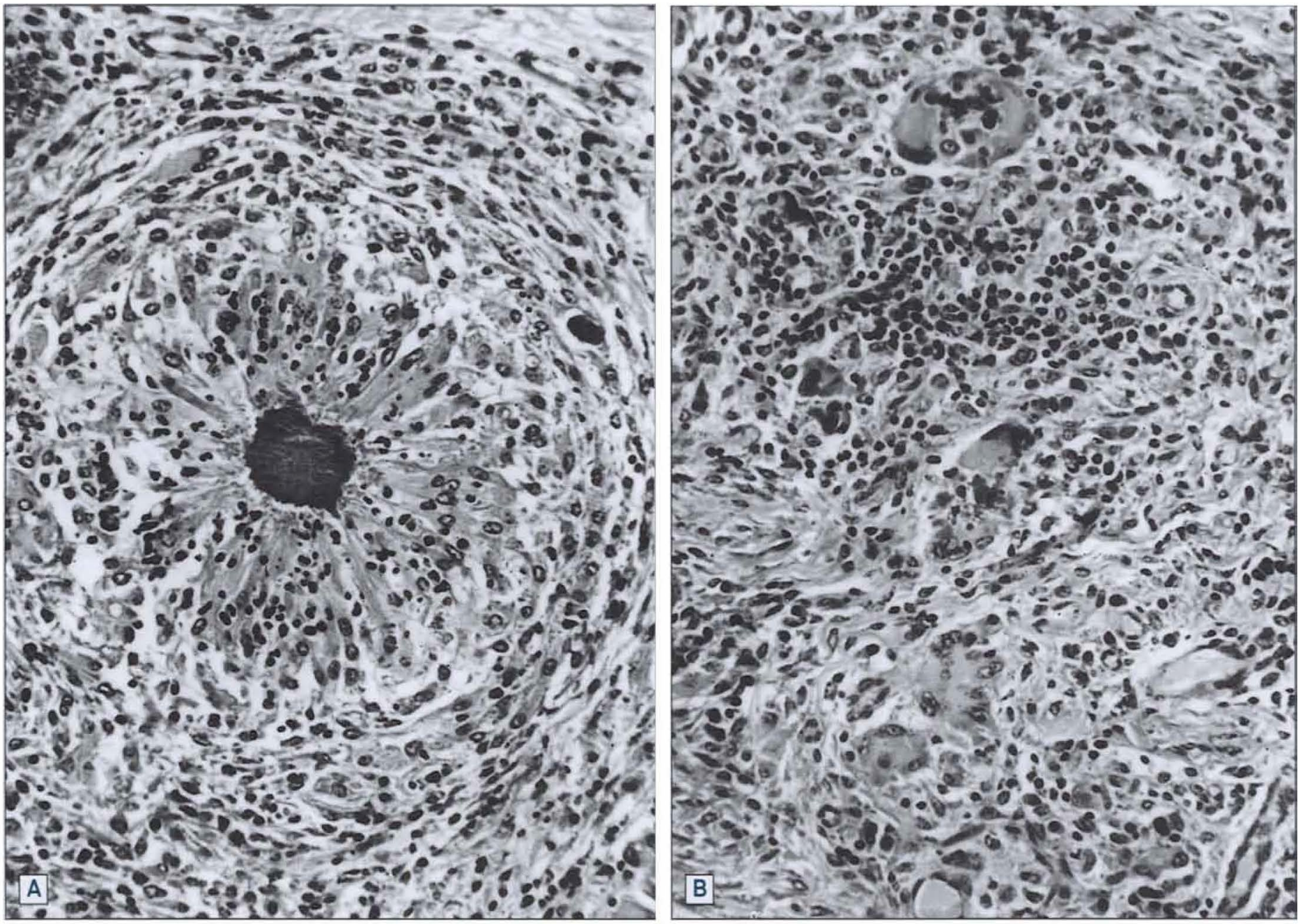

Figure 4.167 Severe **granulomatous mastitis** in bovine mammary tissue, due to *Nocardia*. **A.** Note the **bacterial colony** in the center of the granuloma. **B. Giant cells** predominate in the inflammatory reaction in this field.

and a lumpy lobular appearance that may be beset with small abscesses. The process from there is typically granulomatous without club formation (Fig. 4.167).

Cryptococcal mastitis caused by *Cryptococcus neoformans* has, in most reported instances, followed repeated udder infusion, usually with aqueous or oily penicillin. There is usually involvement of more than one quarter with, in the acute stages, rather severe swelling and increased firmness of the gland, and subcutaneous edema that when chronic produces tight adhesion of the skin to the gland. Changes in the milk occur after 2–3 weeks, when it becomes watery and flaky, although large numbers of organisms are excreted during the acute phase. The diseased tissue is abnormally fleshy, firm, and gray-white with spotty hemorrhages. Lobulation is exaggerated in the acute stages and the cut surface is very slimy with viscid gray secretion that makes the gland hard to handle. Later the tissues become involuted and gray and contain numerous small granulomas.

The histological picture is variable. *In severely affected areas there tends to be complete liquefaction affecting epithelial cells*, and the acini therefore become distended and confined only by the skeleton of connective tissue (Fig. 4.168). The organism is present in very large numbers, and sometimes even the connective tissue is lysed to produce multilocular cystic acini. Thus far the inflammatory reaction is minor, but there develops a massive histiocytic response. Large vacuolated histiocytes infiltrate the acini and ductules and crowd beneath remaining epithelium, lifting it from its basement membrane. These cells engulf large numbers of yeasts and the histolytic effect of the organisms is no longer so obvious. *More chronically, there develops extensive inter- and intralobular fibrosis and a variable number of more typical granulomas.* The corresponding lymph node is greatly enlarged and the lesions in it parallel those in the udder (Fig. 4.169). The infection may metastasize to the lungs.

Mastitis caused by ***Mycobacterium*** spp. other than *M. bovis* also follows mammary infusion measures. These infections too cause very marked enlargement of the gland, but there is no systemic reaction, nodularity, or subcutaneous edema. *The basic tissue reaction is granulomatous and well restricted to lobules.* Giant cells are common, but the main infiltrating cells are histiocytes and lymphocytes organized in granulomatous formations that are often centered on oil droplets. Many of the lobules are completely destroyed by granulation tissue and there is considerable thickening and lymphocytic infiltration in the interlobular connective tissue. In the early stages, a ring of neutrophils may surround the oil droplets that contain large numbers of bacilli. Neither the organisms alone nor the oil alone is capable of provoking a granulomatous mastitis.

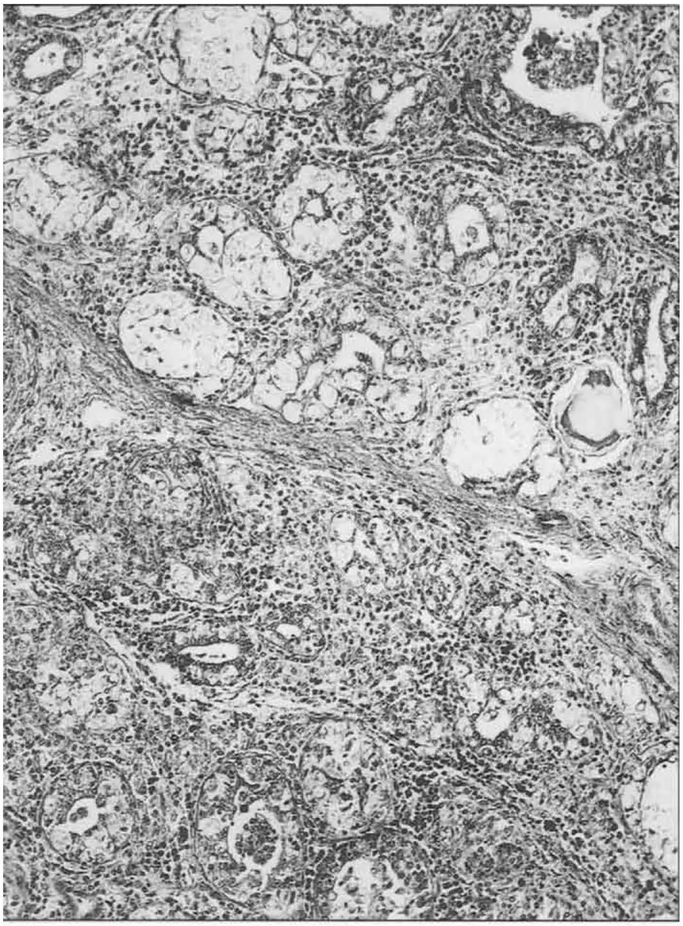

Figure 4.168 Chronic glandular atrophy with diffuse interstitial fibrosis in bovine **cryptococcal mastitis**.

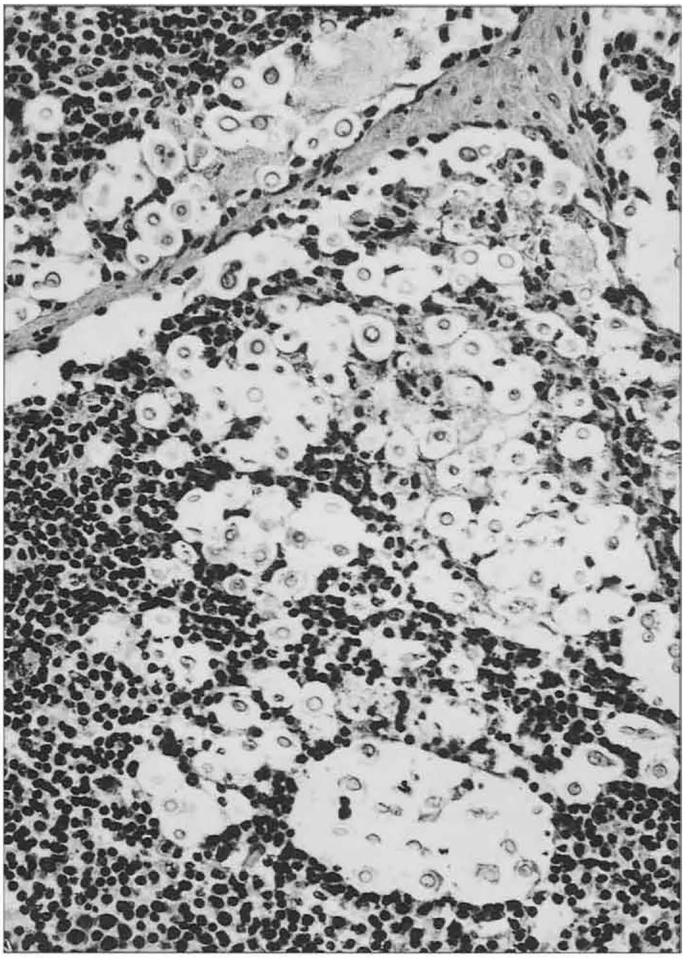

Figure 4.169 Supramammary lymph node from a cow with **cryptococcal mastitis**, showing characteristic "soap-bubble" appearance.

Candida spp. infection has usually occurred under the same conditions of infusion. Mastitis caused by ***Trichosporon*** species is also reported. The tissue changes were not examined in either of these infections, but the infection produced by *Candida* spp. is often acute and transient, the infecting organism disappearing in about 3 weeks. Candidiasis is more likely to occur in animals being fed brewers' grains.

Infection with ***Prototheca zopfii***, an achlorophyllous alga, can cause sporadic or epidemic mastitis that is refractory to treatment. Environmental wetness, muddiness, and the presence of organic material are conducive to the growth of *Prototheca* spp. Improper milking procedures and unsanitary infusion of intramammary antibiotics contribute to mammary gland infection. The mastitis caused may be mild clinical or chronic subclinical, and may cause a dramatic drop in milk production. Histological lesions consist of interstitial infiltrates of macrophages, plasma cells, and lymphocytes, with neutrophils in acinar lumens. The reaction becomes granulomatous with time. Algae are present in macrophages and free in the alveolar lumen and interstitium, and are more easily identified if stained with periodic acid–Schiff.

MASTITIS IN SHEEP AND GOATS

Mastitis in the ewe and goat is usually caused by *Staphylococcus aureus* or *Mannheimia haemolytica*. *Corynebacterium pseudotuberculosis* and *A. pyogenes* cause "cold" abscesses and probably gain entrance through skin wounds. Mastitis in the ewe and doe is important where milk is used for human consumption either directly or in products such as cheese, and because the mortality rate can be as high as 50%. Except for infection by *Mycoplasma agalactiae*, clinical mastitis usually affects only one gland. The range of organisms causing bacterial disease includes the species pathogenic in the cow.

S. aureus causes a galactogenic infection occurring very early in lactation, with a course and consequences paralleling the disease in the cow. The morbidity, however, is higher and may approach 25% of a flock. *Mannheimia haemolytica* causes mastitis in sheep on summer range at or near the end of lactation. It too is probably galactogenic and affects ~5% of a flock. There is an early acute phase with systemic reaction, but in the course of 48 hours the systemic reaction has subsided. The affected gland, usually one side, is greatly enlarged and tense, and the secretion becomes watery and contains flakes. Later there may be developing bluish discoloration and widespread necrosis of tissue. Animals that do not die develop abscesses in the course of a week or so with rupture and fistulation, and eventually most of the affected gland sloughs. Pneumonia in lambs has been reported to accompany this form of mastitis.

Contagious agalactia is primarily a disease of goats, sheep being slightly less susceptible, and is caused by *Mycoplasma agalactiae*. The disease is endemic in countries bordering on the Mediterranean sea. Diagnostic aids include improved serological testing and use of the polymerase chain reaction.

The name, contagious agalactia, merely emphasizes one, although perhaps the most consistent, feature of the disease, namely mastitis. *The disease is initially septicemic, and often fatal in this phase.* If the infected animal survives the acute stage, there may develop signs of localization in the eyes and periarticular tissues and, in the case of a lactating female, in the mammary gland. These are the sites in which localization is obvious, but it might be anticipated that *M. agalactiae* shares the affinity of this class of microorganisms for connective tissues and lymphatics and that it localizes in other less obvious places. The organisms are eliminated in the secretions and discharges. Infection is probably by ingestion, but it can be galactogenic and per conjunctivam.

Lactating females and kids are particularly susceptible to the infection and likely to succumb in the septicemic phase of the disease. Pregnant females may abort or deliver live but infected fetuses. There are no specific lesions in the septicemic phase, but the organism can be cultured from all tissues and from the blood. In infections of lesser severity or following recovery from the peracute disease, the febrile reaction subsides, and lameness, keratoconjunctivitis, and alterations of the mammary secretion occur. This complete triad is not always present, and even when there are signs of localization, the animal may continue to decline and die in 2 weeks or so.

The mastitis apparently is an inflammation primarily of the interstitial tissue with secondary changes in the acini. Mild inflammations, although they cause cessation of lactation, may be followed by full functional recovery, but the more severe reactions may lead to progressive fibrosis with parenchymal atrophy. The organisms are eliminated in the milk and in other discharges for many months in animals that survive the initial stages of the disease. Lameness is indicative of *arthritis and periarthritis* and this is more common at the carpal and tarsal joints than at others. Inflammatory edema thickens the periarticular tissues. The synovial membranes may be normal or they may be hyperemic or ulcerated with an increased volume of turbid fluid that may be blood-stained. The ocular lesions, which are present in ~50% of cases, consist of mucopurulent conjunctivitis and keratitis that may be complicated by ulceration and staphyloma.

Mastitis affecting both glands of ewes is a common manifestation of infection by the lentivirus of **maedi-visna** (ovine progressive pneumonia; see Vol. 2, Respiratory system). The mammary disease can be of much earlier onset and of much higher incidence than neurologic or pulmonary disease, although the expression of the various syndromes may be strongly influenced by breed and genotype of the hosts. The presenting indications of lentivirus mastitis are reduced milk yield, poor preweaning growth of lambs, and progressive atrophy and induration of both glands. The virus is present in the mammary gland shortly after exposure. The severity of the lesions increases with the duration of infection. The virus replicates in circulating monocytes and more freely in tissue macrophages. The presence of macrophages in the mammary interstitium encourages a local inflammatory response consisting largely of lymphocytes and plasma cells. *The lymphocytic-plasmacytic infiltrations are diffuse in the interstitial and periductal connective tissue and lymphatic nodules are formed.* Fibrosis is not a feature of the response. Degeneration and loss of acinar and ductular epithelium that is present in advanced lesions may be a consequence of the infiltration and not a direct virus effect.

The lentivirus of **caprine arthritis-encephalitis** is closely related to that of maedi-visna, and the pathogeneses of the infections in the separate species have very much in common. Mastitis does occur in the caprine disease and has similar morphological features to that in the ewe but is not a prominent component of the syndrome.

The toxic principle of the **avocado**, *Persea americana*, is *persin*; only some varieties of the plant are toxic. Mammary injury is reported in lactating goats. The gland becomes swollen, firm, and congested, especially in dorsal parts, and there may be subcutaneous edema affecting the gland and adjacent body wall. Histologically, there is *widespread degeneration and necrosis of secretory epithelium*, more severe at the centers of the lobules, and patchy loss of duct epithelium. Cellular inflammatory response is minimal. Restitution of function may be incomplete.

MASTITIS IN DOGS AND CATS

In the dog and cat, inflammations of the breast are uncommon and nonspecific. The most important is *nonspecific acute mastitis*, which is usually confined to early lactating or pseudopregnant periods. The exciting agents are staphylococci and streptococci, the former being the more common, and both are believed to gain entrance via fissures in the nipples and adjacent skin and to spread by way of both the ducts and the lymphatics. The staphylococci may cause gangrene, but they tend to localize in the course of a few days and form abscesses, while the streptococci tend to produce a more diffuse and spreading purulent inflammation. The acutely affected glands are large, firm, and edematous, and the overlying skin becomes taut and shiny. Only a small amount of gray secretion can be expressed; it may be blood-tinged or contain pus. The *chronic cases of mastitis* in these species cannot be satisfactorily distinguished from neoplasms until examined cytologically. Indeed, mastitis is often superimposed on cystic mammary hyperplasia and on mammary neoplasia, especially those tumors that involve the ducts.

MASTITIS IN SWINE

Chronic granulomatous mastitis, affecting one or more glands, is occasionally observed in old sows. The lesions are of *botryomycotic* type, being suppurative granulomas, and most of them are produced by *Staphylococcus aureus*. *Actinomyces bovis* was an important cause of this form of mastitis some decades ago, but presently appears to be of very low incidence. It is true for sows as for cows that the designation "actinomycosis of the udder" refers more usually to staphylococci than to actinomycotic infection. In either event, it appears that the infections are percutaneous, entry being gained through wounds of the skin.

Mastitis caused by coliform bacteria occurs as a very acute infection shortly after parturition. It usually affects more than one gland and produces lesions that are similar to those of cows with coliform mastitis. Hemorrhagic mastitis is very rare in sows, and the usual gross appearance is of slight to moderate reddening of the glands with severe edema. Affected mammae are firmer than normal. The severe systemic reaction and the occasional presence of clinical synovitis indicate that septicemia may occur. The pathogenesis is unknown, but ascending infections from environmental contamination may be important.

The important syndrome in sows is known as the **mastitis–metritis–agalactia syndrome**. It occurs in sows 12–48 hours after parturition and is seen as lethargy, fever, swelling, and firmness of the mammary glands, and agalactia. There is some overlying hyperemia, and edema may be extensive. *There are probably several causes for the presence of agalactia in postpartum sows*, and acute infectious mastitis such as that due to coliforms is one. The mastitis–metritis–agalactia syndrome is most common in intensive units, and hormonal dysfunction stimulated by sudden changes in management may be precipitating. Metritis is seldom a significant part of the syndrome.

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5

Male genital system

Robert A. Foster and Philip W. Ladds

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GENERAL CONSIDERATIONS

Mastering pathology of the male reproductive system is more than knowing how to recognize the diseases that affect each part of the tract. Understanding mechanisms and identifying clinical significance are very important. Arriving at an accurate diagnosis is facilitated by similarities among the various species, such that familiarity with a disease in one species generally permits the pathologist to make the same diagnosis in another species. There is a short list of common diseases, which we will identify, for each of the anatomical locations in the male reproductive tract.

Understanding the basic mechanisms of disease, especially in companion animals, is much more problematic since there has been little interest in or funding for detailed research. Prospective studies are very difficult to manage for the same reasons. Diseases of production animals frequently are handled pragmatically, i.e., cull the affected animal. Hence, there are few studies of the mechanisms of disease of the male genital tract in domestic animals. Information gleaned from a variety of human, laboratory animal, and/or toxicological studies has been used to draw possible parallels with diseases in domesticated species.

The testes are often considered the center of the "male reproductive universe," but not every disease of the scrotal contents involves the testes. "Orchitis" is a common clinical diagnosis even though the inflammation and/or infection usually involve the tunica vaginalis, epididymis, or spermatic cord. The testes only infrequently are involved directly in inflammatory reactions. Similarly, the ability of spermatozoa to stimulate a florid inflammatory reaction is overlooked, and bacterial involvement and abscesses are high on the list when inflammatory conditions are suspected. *We will highlight common misconceptions in the diagnosis of male reproductive disease.*

From a functional and pathogenetic point of view, the male reproductive system, focused on successful delivery of viable spermatozoa to the female, is composed of a *production unit* (the testis), a *maturation* *and maintenance unit* (epididymis), and a *transportation unit* (various parts of the ducts and accessory genital glands). Each will be dealt with in turn. The male still comprises 50% of the reproductive unit.

Spermatogenesis

Successful spermatogenesis is a complex process that now is being unraveled, because of interest in the effects of toxicants and drugs on fertility. Early studies examined the effects of temperature on spermatogenesis, and individual instances of failure were investigated morphologically. However, now we have the ability to investigate the role of gene expression, cellular interactions, accelerated apoptosis, various cytokines, and hormonal interactions on the function of the germinal compartment.

Spermatogenesis requires a complex interaction among germ cells, Sertoli cells, and interstitial endocrine (Leydig) cells.

Sertoli cells are very complex in their structure and function. The majority of Sertoli cells are present at birth or soon after. They extend from the basement membrane to the luminal surface, and have a large surface area. Attachment to adjacent Sertoli cells and to germinal cells is by a variety of junctional complexes, and this forms the blood–testis barrier. Sertoli cells function to maintain the integrity of the seminiferous epithelium, compartmentalize the seminiferous epithelium into basal and luminal compartments, secrete fluid, form the tubular lumen, phagocytose degenerating and necrotic cells, deliver nutrients to germ cells, metabolize steroids, translocate germinal cells, secrete proteins, regulate the spermatogenic cycle, and mediate hormonal effects on the germ cells. These many functions are potential targets for abnormalities.

Spermatogenesis begins with stem cells that proliferate by mitosis, undergo a meiotic phase, and finally differentiate. These stages correspond to *spermatogonia, spermatocytes, and spermatids*, respectively. The spermatogonia are subdivided into proliferative and differentiating types of stem cell. They divide by mitosis to produce most of the cells that go on to form spermatozoa. Meiotic division has a prolonged prophase, but subsequent phases proceed rapidly. The spermatids are formed as a final, differentiating step. *Spermiation* is the active release of spermatozoa by Sertoli cells to the lumen, for subsequent transport through the duct system.

Spermatogonia are exposed to interstitial tissue fluids. Occluding junctions of Sertoli cells mostly form the blood–testis barrier. Spermatocytes and spermatids within the tubule are "external" to the blood–testis barrier. Apoptosis of germ cells at each stage of development occurs as a natural phenomenon (see below), and Sertoli cells phagocytoze degenerative and necrotic cells.

Control of spermatogenesis involves a complex interaction of central and local factors. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are the major pituitary hormones regulating spermatogenesis. Interstitial endocrine cells have LH receptors that, when coupled with LH, induce testosterone production. Testosterone is essential for spermatogenesis; Sertoli cells, and perhaps germinal cells, have testosterone receptors. Testosterone is known to inhibit apoptosis of germ cells. FSH directly stimulates the Sertoli cells and perhaps the germinal cells. FSH and testosterone probably act synergistically.

Local control of spermatogenesis is also highly dependent on local autocrine and paracrine factors; the number of factors recognized is increasing exponentially. Sertoli cells produce inhibin, which inhibits FSH production. Germinal cells, Sertoli cells, peritubular cells, and interstitial endocrine cells secrete many cytokines/growth factors. These cells are influenced, in turn, by a large number of similar signaling molecules. Germ cells express Fas (APO-1, CD95), a transmembrane receptor molecule that transmits an apoptotic signal in the cell when it is bound by a Fas ligand. Sertoli cells express Fas ligand, and regulators of apoptosis are found in the testis, including the Bcl-2 family of proteins and the p53 protein. Apoptosis is a normal phenomenon regulating cell populations, but induction of apoptosis or increased apoptosis also occur with exposure to toxic compounds, depletion of growth factors, alteration of hormonal support, exposure to heat, radiation, transient ischemia, or treatment with chemotherapeutics. Testes with impaired spermatogenesis, such as hypospermatogenesis or maturation arrest, may have increased apoptosis.

There are well-recognized changes in spermatogenesis with **age**. The size of the testis decreases progressively from puberty into adult life. Initially, the seminiferous tubules increase in length and diameter, and the testicular volume increases. However, from early adulthood onward, there is a gradual reduction in tubular diameter. The absolute number of Sertoli cells probably declines from puberty on. A decline in the number of spermatocytes and an increase in apoptosis both contribute to reduced testicular mass to various degrees in different species. A concurrent increase in the mass of the testicular interstitial tissue typically occurs with age, and the basal lamina of the seminiferous tubule increases in thickness.

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Immunity to spermatozoa

Any condition that causes leakage of spermatozoa or spermatozoal antigens into the extratubular compartment is potentially complicated by inflammatory and immunologic responses. The response to spermatozoa is variable, but innate or nonspecific immunity and humoral and cellular mechanisms are involved. Spermatozoa have a cell wall of lipids and phospholipids; they incite a foreign body-type reaction, and the large amount and keratinoid composition means that degradation is slow. In addition, immune responses are florid and result in the accumulation of many immunoglobulin-producing cells as well as CD4 and CD8 lymphocytes. Up-regulation of major histocompatibility complex (MHC) I in epithelial cells also occurs. The reaction leads to chronic inflammation and fibrosis, and continued obstruction and disruption of tubules. Spermiostasis, spermatocele, and/or further sperm granulomas are the consequence.

Antispermatozoal antibodies are found in serum, genital secretions, or both. The presence of these immunoglobulins in plasma is associated with infertility in several species including humans. There is a lack of information on their presence in genital fluids, where they have the greatest impact. Spermatozoal antigens gain entry to the interstitium due to trauma or rupture of a duct or tubule following inflammation or obstruction. Specific conditions that result in sperm granulomas include blind-ended efferent ductules (sperm granuloma of the epididymal head or efferent ductules), segmental aplasia of the mesonephric ducts, adenomyosis, and epididymitis. Infection of dogs with *Brucella canis* can cause the formation of antispermatozoal antibody, and it is anticipated to occur in vasectomy and other causes of obstruction of ducts.

Serum immunoglobulin may gain access to the spermatozoa through any part of the reproductive tract, but the efferent ductules are probably the most accessible site, because the barrier between the internal and external compartments is less tight. Local immunoglobulin production can occur at any location in the tract, but the accessory genital glands are the most common site (see below).

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Immunity in the reproductive tract

Innate, humoral, and cellular immunity are all expressed in the male genital tract. The innate mechanisms are, arguably, the most important. The male genital tract has little ability to compensate for direct injury. The testes are bounded by the tough and relatively inelastic tunica albuginea, and any sudden increase in internal pressure or swelling, be it caused by hemorrhage or edema, results in testicular necrosis. The epididymis and deferent duct (ductus deferens) form a single small-diameter tube that is easily obstructed and damaged, and it is free from the usual systemic immune surveillance. There is always the specter of inflammation and acquired immunity to spermatozoal or secretory products.

Normally very little immunoglobulin enters the seminal fluids, and with a few exceptions, lymphocytes are not found in the interstitium of reproductive structures in normal male animals, although they are occasionally seen in the interstitium of the epididymis of the dog. Their presence in the interstitium of the testis, epididymis, and deferent duct is presumed to be acquired. Macrophages are present in the normal interstitial tissues of all species. Epithelium can express MHC II molecules if inflammation is present. The various ducts of the male reproductive tract contain intraepithelial leukocytes, especially CD8 lymphocytes. The accessory genital glands are believed to be the main location for plasma cells, which presumably add immunoglobulins to the seminal fluid to help prevent access of ascending agents to the deferent duct and epididymis. Local humoral immunity develops in the accessory genital glands, and is predominantly IgA-based in those species that have been examined.

One of the unfortunate side effects of having spermatozoa not recognized as "self" is the necessity of maintaining these cells completely separate from immune surveillance. This provides microbial organisms a location within the lumen of ducts and tubules where they can grow and cause damage. Once the body recognizes the injury and recruits inflammatory cells, there often is too much damage to permit repair or regeneration. In addition, the very processes of inflammation, including the release of free radicals by leukocytes, damage the tract further. As in many other sensitive tissues, *the damage done by inflammation may be a major cause of dysfunction.*

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Anomalies of development

Production of a normal ejaculate depends on a combination of factors. These include normal development of the reproductive tract, and normal spermatogenesis, spermatozoa maturation and transport, normal accessory genital gland function, and nervous, musculoskeletal, and psychological factors. While the exact mechanism of development of the male genital tract has not been determined, research in several species, especially rodents, suggests the following.

Reproductive differentiation requires appropriate sequential development and regression of the gonadal mesenchyme, paramesonephric (Müllerian) and mesonephric (Wolffian) tubules and ducts, and the tissues of the gonadal ridge. When all steps occur normally, genotypic, gonadal, and phenotypic sex is matched. Male domesticated mammals normally have an XY genotype, although XX males can occur if a sex-determining factor is present. In XY males, this is the *testis-determining factor* (TDF), the production of which is governed by the sex-determining region (SRY) of the Y chromosome.

During early embryonic development, germ cells migrate from the yolk sac to the genital ridge. The constituents of the primitive gonad are the germ cells, mesenchymal cells, coelomic epithelial cells, and mesonephric epithelial cells. These form the four major cell types in the gonad – the germ cells, supporting cells, steroid-producing

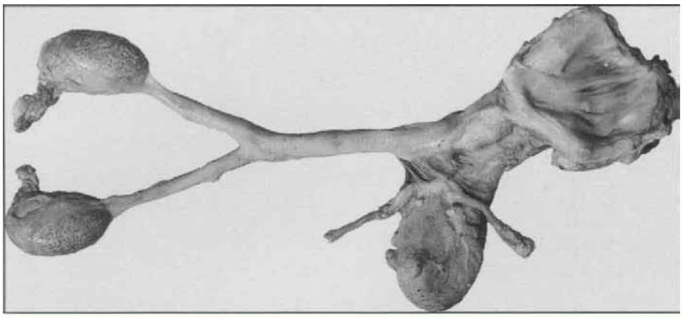

Figure 5.1 **A male pseudohermaphrodite bovid** with tubular genitalia that is predominantly female, but both gonads are testes.

cells, and unspecialized mesenchyme. The TDF causes the germ cells to go into mitotic arrest, the supporting cells become the Sertoli cells, the steroid-producing cells become the interstitial endocrine cells (of Leydig), and the mesenchyme develops the testicular pattern. The ducts are derived from the mesonephric ducts. The genital sinus and external genital tubercle are bipotent. Male differentiation occurs before female differentiation and is dependent on the production of hormones such as testosterone.

The male phenotype depends on the production by the Sertoli cells of *Müllerian duct inhibitory substance* (MIS), which causes the paramesonephric (Müllerian) duct to regress. Interstitial endocrine cells produce testosterone that inhibits further female differentiation, prevents the mesonephric ducts from regressing, and induces development of the penis and scrotum. The mesonephric ducts give rise to the epididymides, deferent ducts, and vesicular glands.

Developmental abnormalities of the male genital system include XXY (Klinefelter's syndrome), XX sex reversal, persistent Müllerian syndrome, translocations, and androgen insensitivity (testicular feminization). These are manifested clinically as *ambiguity in phenotype* and/or *infertility*.

- Cats, bulls, dogs, pigs, horses, and sheep that have an **XXY genotype** or a mosaicism with XXY chromosomes may have hypoplastic internal genitalia. The most well known of these are male *tortoiseshell or calico cats*, which are almost always infertile, and have testicular hypoplasia.
- **XX sex reversal** occurs in goats, dogs, and pigs. In goats this is called the *polled/intersex syndrome* (PIS) because it is common in polled animals, where polled XX homozygotes are hermaphrodites. They range from sterile males to females with testes. In dogs, affected American Cocker Spaniels can vary from XX males to XX true hermaphrodites. In pigs, it is a common anomaly that may be confused with freemartinism.
- Animals with **androgen insensitivity (testicular feminization)** are male pseudohermaphrodites – they have testes, no tubular genitalia, but are phenotypically female (Fig. 5.1). Cats, horses, and cattle are reported with this condition.
- Animals with the **persistent Müllerian duct syndrome** are phenotypically male and may be fertile. Affected Miniature Schnauzer dogs may develop hydrometra and pyometra of the remnants. Many are also cryptorchids. The defect appears to be insensitive to MIS rather than a lack of production of MIS.

Gynecomastia is occasionally seen as a solitary lesion in males. There are reports of bucks from high milk production lines that develop mammary glands.

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SCROTUM

The scrotum is an outpouching of perineal skin and is lined by evaginations of the peritoneum. The theory that the development of the scrotum is directed solely to providing a superficial cool environment for the testis is too limited. The storage receptacles for mature spermatozoa in most mammals also need to be located in sites in which the temperature is less than core temperature. Furthermore, since maturation of spermatozoa is a slow process, a fact reflected in the extraordinary length of the epididymal tubule, the scrotum may have evolved to accommodate the epididymis. Finally, the testicular artery, which is quite separate from the blood supply to the epididymis, has, by virtue of its length, physiological consequences that are assisted by the development of an abdominal evagination.

The structure of the scrotum is adapted to its function of maintaining the testis at a temperature less than core. The scrotal skin is thinner than skin elsewhere and is well covered with hair only in the cat, although in all species it is well supplied with apocrine sweat glands; there is little or no subcutaneous fat or connective tissue. The contractility of the scrotum is a property of the *dermal dartos* that consists of fibroelastic tissue and smooth muscle. Thermoregulatory function is shared by the *cremaster muscle*, which regulates the proximity of the testis to the abdominal wall, and the *pampiniform plexus* of veins, which ensures maximum contact of cooled venous circulation with the warmer arterial blood. If the testes of the common domestic animals are maintained at or above normal body temperature, degeneration or arrest of development of the seminiferous epithelium occurs, although impairment of the interstitial endocrine cells is minimal or absent. Experimental "shortening" of the scrotum of ram lambs results ultimately in mature rams that have small testes and epididymides, low spermatozoa production, and in most cases no epididymal spermatozoa reserves.

The formation of the scrotum from the paired genital swellings of the embryo, and its full development at puberty, requires gonadotrophin-induced androgens, and is dependent therefore on the development of testes. **Anomalies of scrotal development** are simple in their expression in spite of the complicated steps in the development of the paired primordia. *Apparent absence of the scrotum* has been observed in cryptorchidism. Various degrees of *bifurcation of the scrotum*, and *scrotal clefts*, represent failures of fusion

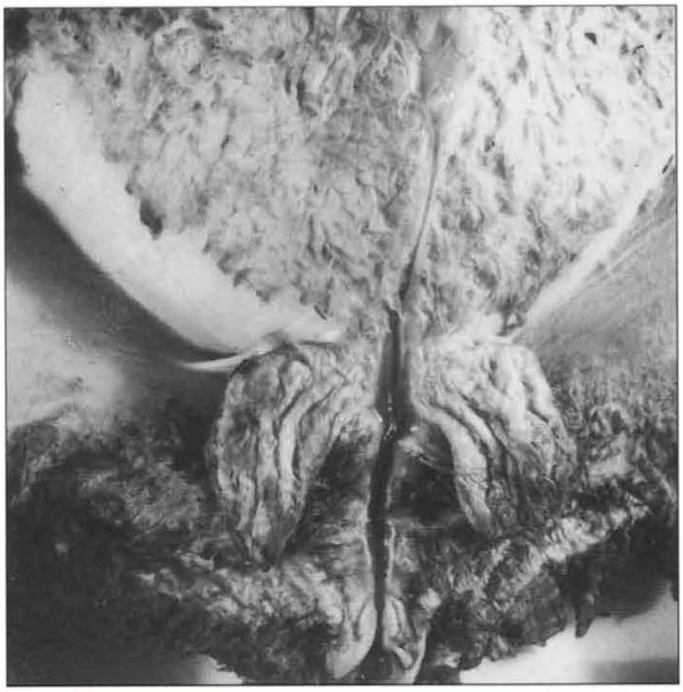

Figure 5.2 A ram lamb with complete **hypospadias**, including bifurcation of the scrotum.

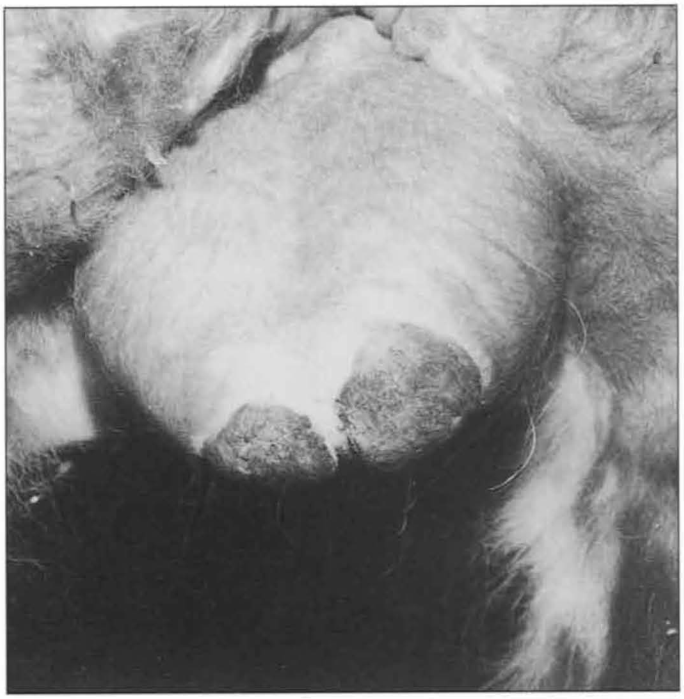

Figure 5.3 **Scrotal frostbite** in a bull. (Courtesy of WF Cates.)

(Fig. 5.2). Such defects may be local and accompanied by hypospadias and other defects such as atresia ani, or reflect gonadal dysgenesis in intersex states.

Range bulls exposed to extreme cold may develop **scrotal frostbite**. A higher incidence of lesions in old bulls is attributed to their more pendulous scrotums. Lesions consist essentially of necrosis of skin on the ventral aspect of the scrotum and vary from mild (about 3 cm diameter) to severe, in which more than 75% of the scrotum is affected (Fig. 5.3). Associated changes include scrotal swelling, tunic adhesions, and decreased semen quality.

Enlargement of the scrotum is mostly due to edema, which has local or systemic causation (see under Tunica vaginalis). Skin changes resulting from a dietary deficiency of zinc may involve the scrotum. In dogs, white-yellow crusts may occur, and parakeratosis is seen histologically. In sheep, there is cessation of wool growth on the scrotum. Pemphigus erythematosus and drug eruption, such as in dogs in response to the antifungal flucytosine, may also be manifested by changes in scrotal skin.

Because of its delicacy, scrotal skin is especially vulnerable to inflammation. The expressions of inflammation are influenced by the abundant arteriovenous anastomoses that are present, and the effects include impairment of the function of the tunica dartos. **Scrotal dermatitis** frequently is caused by nonspecific environmental irritants. Important specific causes in ruminants include *Dermatophilus congolensis*, *Besnoitia besnoiti*, fungi, and various ectoparasites, especially *Chorioptes bovis*. Poxvirus infection of the scrotum of bulls may occur as diffuse or focal lesions. In dogs, the scrotum may also be a preferred site for localization of *Prototheca wickerhamii*, causing diffuse pyogranulomatous dermatitis with ulceration. These infections are discussed in Vol. 1, Skin and appendages.

Although the scrotal skin of bulls seems especially susceptible to infection by *Dermatophilus congolensis*, possibly because of the role of ticks in transmission, testicular degeneration does not necessarily ensue. However, severe, prolonged infection may result in thickening of the skin to 1 cm, with advanced testicular degeneration and sterility. Infection of scrotal skin of bulls and goats by *Besnoitia besnoiti* also may lead to severe testicular degeneration. This infection is not limited to skin, and the small nodules containing white gritty material may be observed grossly in the subcutis of the scrotum as well as in the tunics, the parenchyma of the testis, and in the epididymis. Vascular damage is considered important in the development of lesions, and large numbers of *Besnoitia* cysts may be present in the intima of vessels in the pampiniform plexus. A feature of the testicular lesions is extensive intratubular mineralization.

Infestation by *Chorioptes bovis* is common on the scrotum of rams. Indeed, the scrotum appears to be a preferred site for the mite. The lesions can be mild but are frequently sufficiently severe to influence the size and function of the testis. In such cases there is marked thickening of skin and matting of overlying hair and wool by secretions and exudate. *Linognathus pedalis*, the foot louse of sheep, and *Haematopinus eurysternus* in cattle also can be important causes of scrotal dermatitis. Sarcoptic mange, with typical crusted lesions, may especially involve the scrotum in goats and cats.

The flea *Tunga penetrans* also may cause scrotal dermatitis in the boar by burrowing into the skin. Scrotal myiasis in most species is caused occasionally by screwworm, including both *Chrysomya bezziana* and *Cochliomyia hominivorax*, and other fly larvae. Prior ulceration or laceration predisposes to myiasis.

Any of the **neoplastic diseases** of the skin may occasionally arise on the scrotum. *Melanotic tumors* and *mast-cell tumors* are not uncommon in the scrotal skin of the dog. Varicose tumors of blood vessels in the scrotal skin, referred to as **hemangiomas**, occur in dogs and boars. In dogs, the lesions commence as focal pigmented areas and progress to a small plaque and then to capillary, then cavernous, hemangioma. Microscopically, small arterioles are seen between cavernous spaces that may extend quite deeply into underlying tissue.

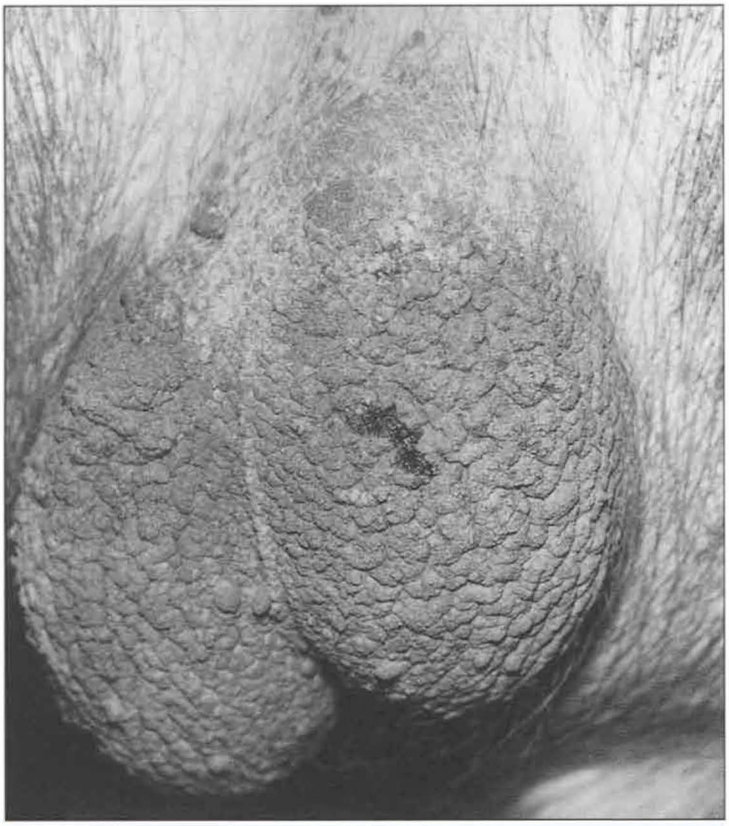

Figure 5.4 Multiple **scrotal hemangiomas** in a boar.

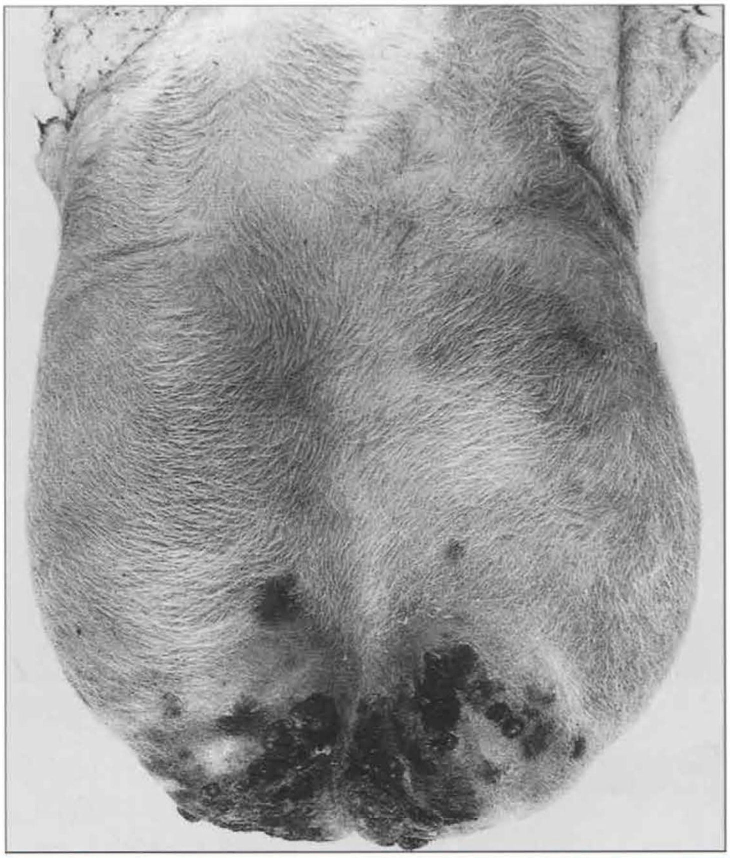

Figure 5.5 **Varicose dilation** of scrotal veins in a bull.

The lesions may become ulcerated and severe hemorrhage may occur. In boars, the multiple "warty" lesions are unsightly and may occasionally bleed, but do not appear to affect fertility (Fig. 5.4). Such lesions are benign and, as in dogs, they may not be true neoplasms. Age, altered hemodynamic forces in the capillary bed of the scrotal dermis, and genetic predisposition are factors that might be involved in the development of these abnormalities.

Varicose dilations of scrotal veins occur in older bulls. They appear as flattened and irregular thickenings of the scrotal skin. The overlying epithelium usually is normal unless it ulcerates, when oozing of blood occurs (Fig. 5.5). The affected venous channels are irregular in shape, widely dilated, and have thickened walls. Thrombosis occurs when the lesion is traumatized.

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TUNICA VAGINALIS

The tunica vaginalis is an extremely thin mesothelial layer continuous with and structurally similar to the peritoneum. The scrotum and testis, which are covered by the parietal and visceral layers, are separated by the cavity of the tunica vaginalis, and must be considered in any condition involving the latter.

The cavity of the tunica vaginalis communicates with the peritoneal cavity and is susceptible to the accumulation of fluid, **hydrocele**, in conditions leading to ascites, anasarca, or local lymphedema. Hydrocele may lead to quite severe testicular degeneration on the involved side and to lesser damage on the opposite side, mostly in response to impaired thermoregulation. Scrotal hydrocele, with diminished sperm quality, has been identified as a herd problem in bulls. Clinically, the fluctuant nature of the enlarged scrotum, with a scrotal circumference up to 60 cm, is indicative of edema, and if affected bulls are examined at necropsy there is obvious ascites. If hydrocele persists, the tunics become increasingly fibrotic. Ostertagiasis and eperythrozoonosis have been incriminated as causes of such hydrocele.

Lymphatic drainage from the cavity of the tunica vaginalis is through scrotal lymphatics beneath the parietal tunic, and this

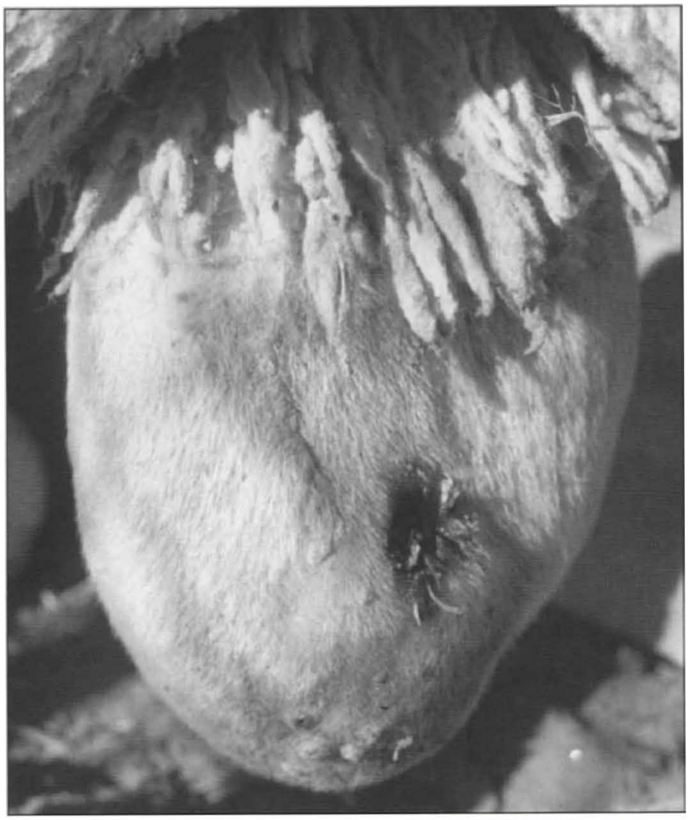

Figure 5.6 Fistulous tract through the scrotum of a ram with severe epididymitis and periorchitis.

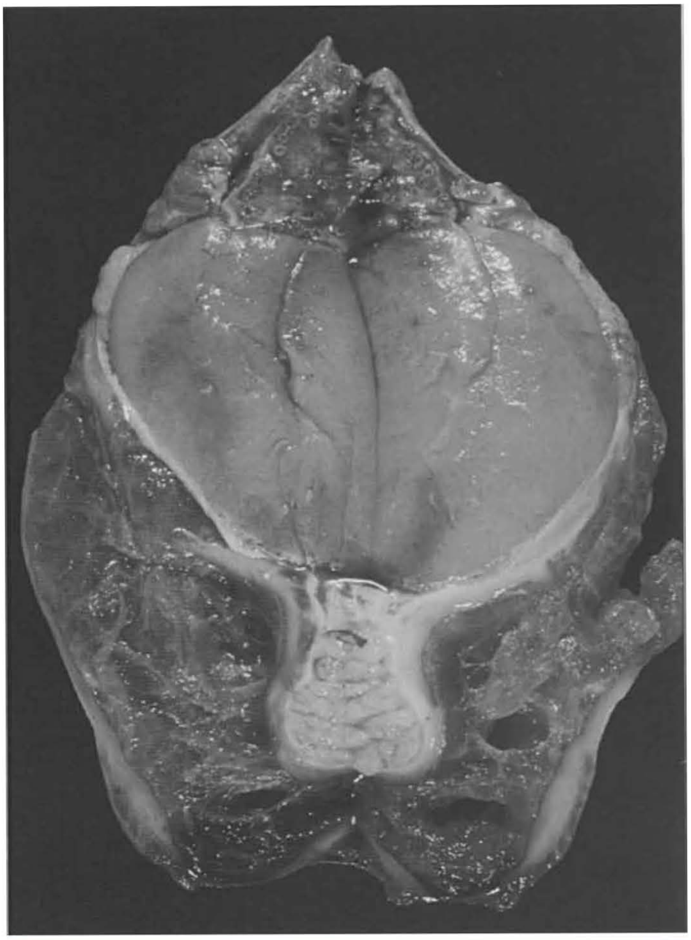

Figure 5.7 Periorchitis secondary to epididymitis in a ram. Fibrin and edema fluid are present within the cavity of the tunica vaginalis.

accounts for the spread of inflammatory processes from the cavity of the tunica to the scrotum. In the stallion, scrotal swelling, with edema of the prepuce, is seen in many conditions, including peritonitis, equine viral arteritis and other vasculitides including purpura hemorrhagica, and surra, caused by *Trypanosoma evansi* infection.

Hematocele, the accumulation of much blood in the vaginal cavity, is mostly the result of trauma. It is not uncommon in boars housed together.

The visceral tunic is an integral part of the testicular capsule, the other two layers being the *tunica albuginea* and *tunica vasculosa*. The tunica albuginea is composed of inelastic collagen and fibrous tissue, and smooth muscle through which the tunica vasculosa is normally visible. In spite of the presence of smooth muscle, the capsule is not significantly contractile; it probably contributes to the flow of semen by maintaining intratesticular pressure. The tunic is thickened and opaque in testicular hypoplasia and atrophy.

Inflammatory changes in the tunica vaginalis may be part of disseminated infection, with lesions typical of the infection, such as feline infectious peritonitis, tuberculosis, caseous lymphadenitis, and the various diseases causing polyserositis in pigs. Suppurative inflammation also occurs as an extension of scrotal injury or from epididymitis (Fig. 5.6).

Severe periorchitis is a complication of epididymitis irrespective of cause, but is particularly found in animals with *Trypanosoma brucei* infection, and in rams infected with *Brucella ovis* or *Actinobacillus seminis* (Fig. 5.7). Ectopic parasites, such as equine strongyles, can be found on the tunics. *Setaria labiatopapillosa* causes granulomatous periorchitis in bulls, and lesions occur particularly after worms are killed by anthelmintic treatment. A high occurrence of eosinophilic, then granulomatous, periorchitis in buffaloes is also attributed to *Setaria* sp. Cysts of taenid larvae, mostly *Cysticercus tenuicollis*, may be found in the scrotal cavity of rams, unassociated with inflammation. In dogs, rare peritoneal cestodiasis caused by larvae of *Mesocestoides* sp. may involve the vaginal tunics, with saccular dilatations within the tunics, thickening of the spermatic cord, and pyogranulomatous inflammation involving the tunica albuginea and extending into the testes.

Adhesions between the visceral and parietal layers of the tunica vaginalis are common, especially in older animals. Adhesions are at first fibrinous, reflecting acute periorchitis, but later become fibrous. Fine, threadlike, fibrous adhesions located at the epididymal tail in bulls are normal. Although adhesions may result from infectious periorchitis and may be associated with epididymitis, it seems probable that most are the result of trauma. Most adhesions are focal and solitary and are not accompanied by lesions in the testis or epididymis; they probably are of little consequence.

Cystic lesions of the tunica vaginalis may be loculations within adhesions. Very small cysts, probably of paramesonephric duct origin, are common adjacent to the head of the epididymis in the horse, and are referred to as "appendix testis" or Morgagni's hydatid.

Neoplastic disease *involving the tunica vaginalis is rare*, even as secondary extension from the testis or scrotum. *Mesotheliomas* with primary involvement of the tunica vaginalis occur in the dog and

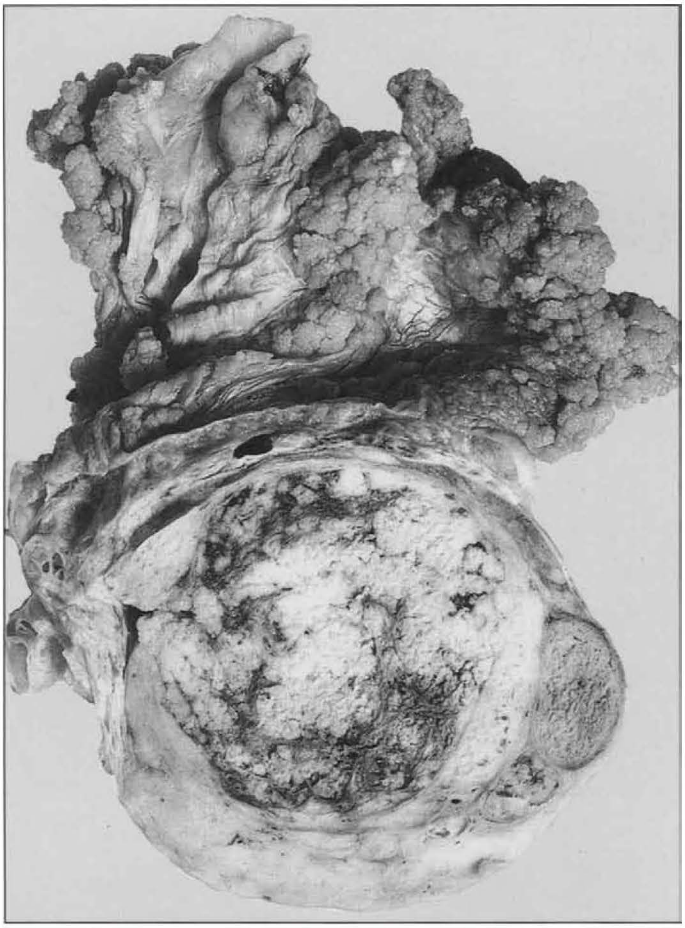

Figure 5.8 Papillary mesothelioma of the tunica vaginalis that covers the scrotal contents of a dog.

bull (Fig. 5.8). Extension of the tumor into the peritoneal cavity has been observed in the dog. Ultrastructural or immunohistologic study may be necessary to differentiate such mesotheliomas from adenocarcinoma. Origin of intrascrotal mesothelioma from the paramesonephric duct remnant has been suggested. There is a report of a lipoma occupying the right scrotal cavity in a ram, causing compression of, but apparently not directly involving, the testis.

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TESTIS AND EPIDIDYMIS

Spermatozoa develop in the *seminiferous tubules* and pass through the *straight tubules* into the *intratesticular rete* within the mediastinum testis. They then leave the testis via the *rete testis* and enter the *efferent ductules*. The efferent ductules number between 13 and 20, depending on the species. Most join the single duct of the *epididymis*, but it is common in most species for one or more to end blindly. The epididymis is long and tortuous, and divided into many distinct areas. For this discussion, three main areas will be referred to: the *head, body, and tail*. The tail terminates in the *deferent duct*. Disease of one area has effects on other regions, and some diseases affect all areas.

Pathologists often examine individual testes or small biopsies rather than whole groups of animals. Gross and histological assessment of reproductive tissues is a valuable tool if used appropriately. Assessment of a problem often requires collaboration of the cytogeneticist, endocrinologist, theriogenologist and pathologist, who, alone, may not be able to determine the complete pathogenesis. For the pathologist to function optimally, appropriate samples must be collected and processed. For many of the diseases and the tissues, routine fixation with 10% formalin is adequate, but for a detailed assessment of spermatogenesis, or the histological appearance of seminiferous tubules, rapid fixation after death or collection (within minutes in many species) in Bouin's fluid or Zenker's fluid, with paraffin embedding, is necessary. Plastic embedding is the method of choice for a detailed assessment of the spermatogenic cycle. Inadequate fixation or suboptimal processing of testicular tissues will cause artifacts that cannot be distinguished from degeneration.

Normal spermatogenesis requires a normal scrotal testis in domestic mammals. The testis varies in its gross and microscopic appearance among the species, mostly in the extent to which the testis is divided into regions by fibrous tissue septa. This division is marked in the stallion, less so in the dog and boar, and mostly absent in ruminants. The basic microscopic appearance is similar – the testis is divided into the *interstitial or intertubular compartment* and the *seminiferous tubular compartment*. The interstitial endocrine cells are the major resident of the intertubular compartment; macrophages are another important cell type.

The interstitial compartment is a unique immunological environment. Nonspecific immunity is normal, but humoral and cell-mediated immunity is altered such that allogeneic and xenogeneic tissue transplants are maintained for a prolonged period. This state is dependent on the presence of testosterone, and is related in part to Fas ligand expression by Sertoli cells that induces apoptosis of Fas-expressing T cells. This environment is thought to provide protection to the germinal cells, and prevent autoimmune reactions or recognition of germinal cells by the immune system.

The peritubular cells and the basement membrane of the seminiferous tubule delineate the tubular compartment. Sertoli cells and germinal cells make up the intratubular compartment. The normal tubular diameter is 146 μm in the stallion, 180 μm in the dog, and 236 μm in the boar.

The normal testis is pale pink or even almost white. This is presumably because of the large quantity of chromatin material. Abnormalities that result in a lowering of spermatogenesis result in the dominance of other pigments – especially lipochrome pigments. The testes of boars become progressively brown with age, and are brown if degenerate.

A frequent misdiagnosis is made in equine fetal and neonatal gonads. Fetal testes undergo hypertrophy because of hyperplasia of the interstitial endocrine cells. These cells contain lipochrome pigments that are especially noticeable during the atrophic stage that

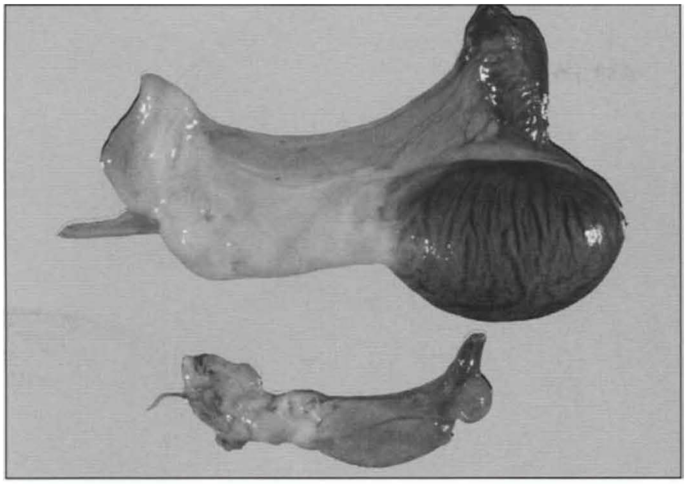

Figure 5.9 Equine **fetal** testis and epididymis (upper) with **physiological gonadal hypertrophy**; testis is dark brown. Testis undergoes atrophy to become the size of the **normal neonatal testis** (lower) before it passes through the inguinal canal.

occurs prior to birth. Many a practitioner and pathologist have been fooled by the apparent brown discoloration of the testicular parenchyma of the neonatal testis (Fig. 5.9).

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Anomalies of development

Cryptorchidism

Incomplete descent of the testes and associated structures (cryptorchidism) is one of the most common abnormalities of the male reproductive system, and is the most common genital abnormality of the male cat and horse. Descent of the testes, epididymides, and spermatic cord (including the testicular artery and vein and the deferent duct) is a complex series of events that requires hormonal, constitutive, and nervous control. Individual cases of cryptorchidism may be due to genotypic or environmental causes. Complete testicular descent usually occurs prior to birth in most species, with the exception of the dog.

Maldescent creates several problems. Most cases are presumed to be inherited, and, as such, breeding from affected individuals is not recommended. *Retained testes lack spermatogenesis*, and *fertility may be compromised. Increased rates of testicular neoplasia* are associated with cryptorchidism in several species, most notably the dog. Finally, *testicular torsion* is found almost exclusively in maldescended testes, although the stallion may be an exception.

Testicular (and therefore epididymal) descent occurs in three main stages: *the relative transabdominal migration phase, the intra-inguinal phase, and extra-inguinal migration*. The first phase may be partially controlled by Müllerian inhibitory substance, the second phase requires increased intra-abdominal pressure, and the third involves interaction of androgen, calcitonin gene-related protein, the genitofemoral nerve, and perhaps other factors. The exact mechanisms are yet to be elucidated in domestic mammals, but they no doubt are complex and involve an interrelationship of systemic and local factors. The effects of endocrine disruptors on male development are increasingly important, as multiple anomalies such as reduced anal–genital distance, delayed preputial separation, hypospadias, and ectopic and maldescended testes can all be induced by antiandrogenic and estrogenic chemicals.

In early fetal life, a cranial gonadal suspensory ligament supports the testis. It is retained when there is a lack of exposure to androgen, in which case subsequent development of the *gubernaculum testis* also is impaired. The gubernaculum testis is a gelatinous cord of tissue that extends from the caudal pole of the testis to the inguinal area and it exerts traction on the testis. A mutation in the *GREAT* gene, which is highly expressed in the gubernaculum and may mediate response to hormones, has been associated with intra-abdominal cryptorchidism in humans and, experimentally, in mice.

Diagnostic pathologists are frequently asked to ensure that the tissue removed during cryptorchidectomy was testis. Where the tissue resembles normal testis, there is little challenge, but, all too often, the retained testis has degenerated or is twisted and only a small remnant remains. The most difficult cases are those where necrosis of the testis presumably has occurred and all that remains is scar tissue and hemosiderin- and lipofuscin-containing macrophages. *Anorchia* is very rare and, where possible, should be separated from degeneration or death of a retained testis.

The structural anomalies associated with the failure of the testis to migrate to the scrotum include splenogonadal fusion, retention of the cranial gonadal suspensory ligament, and abnormalities of the gubernaculum and vaginal process.

A *hereditary basis* for cryptorchidism has been established or suggested in all species. Chromosomal abnormalities are implicated in many species including rams, bulls, and stallions. Chromosomal anomalies were detected in humans in 4% of a group of 160. However, a hereditary basis is unlikely in outbreaks of cryptorchidism, and *hormonal and environmental factors* are more likely.

Retained testes in pubescent animals are smaller than their normal counterparts (Fig. 5.10). This is because of the deleterious effect of body temperature on spermatogenesis. The seminiferous tubules are smaller than normal and often present with the "Sertoli cell only" pattern. Germ cells may be recognized in young animals, but in older animals, degenerative changes such as interstitial fibrosis and thickening of basement membranes superimpose on the hypoplastic appearance. In extreme forms, the outlines of distorted basement membranes are seen in a sclerotic tissue. Hyperplastic foci of Sertoli cells occur in some retained testes and these may be sites of development of Sertoli cell tumors. In unilateral testicular maldescent, which is typical, the contralateral testis is hypertrophied.

Cryptorchidism is the most common disease of the reproductive tract in male **cats**, and Persians are over-represented. The failure of testicular descent in cats tends to be unilateral without a bias to one side or the other. The testis can be anywhere along the migration path, although inguinal cryptorchidism predominated in one study.

In **dogs**, cryptorchidism may be an inherited disease (autosomal recessive) in some breeds, although there is some doubt that this is the main cause. Although it is a frequent clinical finding, little is known of the mechanism and features of the condition. There are

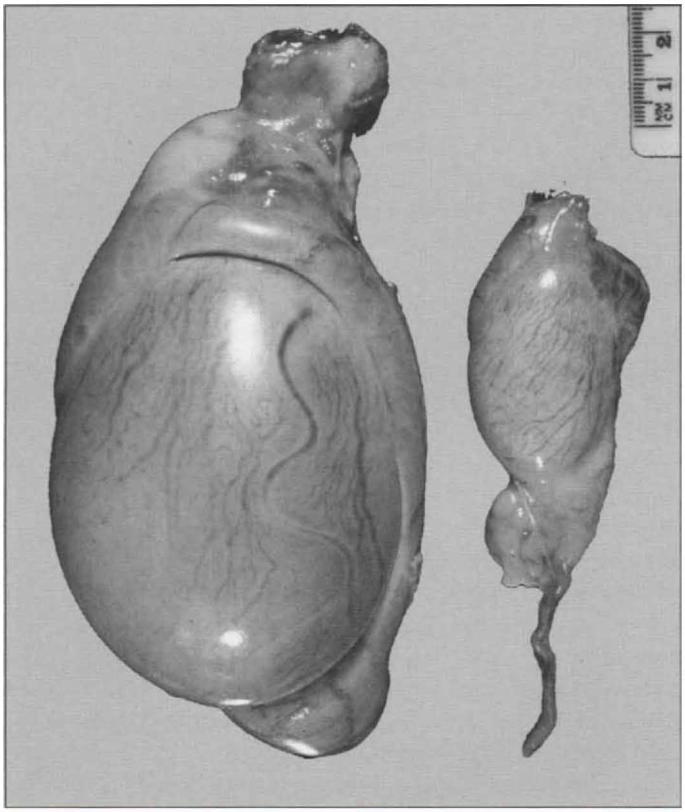

Figure 5.10 Unilateral **abdominally retained testis** (right) from a ram. The contralateral testis (left) was within the scrotum and is more bulbous than normal because of compensatory hypertrophy.

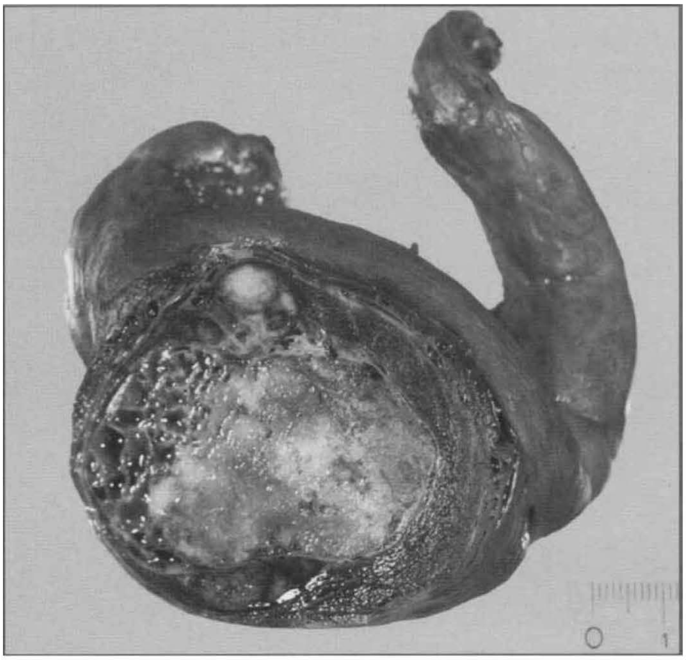

Figure 5.11 **Intra-abdominal testis** of a dog that developed a Sertoli cell tumor. The testis subsequently underwent torsion and venous infarction.

associations of cryptorchidism with several other diseases in dogs. *The association between testicular neoplasia and testicular maldescent is well recognized.* Sertoli cell tumors are the most common, especially in abdominally retained testes. Seminomas are the second most common neoplasm, and they are mostly in inguinally retained testes. Persistent Müllerian duct syndrome in Miniature Schnauzers is associated with unilateral or bilateral testicular maldescent. Testicular torsion usually involves cryptorchid testes – especially if there is a concomitant testicular neoplasm (Fig. 5.11). Other diseases associated with ectopic testes are patellar subluxation, hip dysplasia, penile and preputial defects, and umbilical and inguinal hernias.

Dogs are more likely to have retention of the right testis – perhaps because of a longer pathway for descent of the testis, or a less well-defined gubernaculum on the right. Inguinal testes predominate over intra-abdominal. Testicular neoplasms – Sertoli cell tumor and seminoma – are more prevalent in the right side also. Descent is usually complete by 3 months of age in the dog. Hormonal studies suggest that LH is lower in cryptorchid dogs, and the cryptorchid testis mediates the inhibition of LH secretion.

Cryptorchidism is common in **boars**. There is a hereditary basis, with the trait being recessive, and in the Duroc breed there may be involvement of recessive genes at more than one locus. Abnormal development of the gubernaculum including underdevelopment, excessive growth, and an abnormal location is the main cause of cryptorchidism in pigs.

Cryptorchidism is infrequently reported in **bulls**, but this does not necessarily indicate a low prevalence – because it is easily recognized by laypersons who do not report its occurrence. Retention of the testis and epididymis is mostly in the inguinal region, and occurs about twice as often on the left as compared to the right side. Polled Hereford and Shorthorn cattle are more at risk than other breeds. Little is known of the pathogenesis of cryptorchidism in bulls, although it is believed to be hereditary. The appearance of the cryptorchid testis is similar to that of hypoplastic testes and epididymides. Neoplasia is very rare, with an interstitial cell tumor being reported in a cryptorchid calf.

In **rams**, cryptorchidism is most likely the result of an autosomal recessive mode of inheritance, but it may also be due to a dominant gene with incomplete penetrance. Unilateral maldescent is more common than bilateral involvement, and the right testis is most often retained. Castration of ram lambs in many parts of the world is done by laypersons, and hemicastration is likely in unilateral cryptorchid animals. Some castrated animals have only their scrotum removed, leaving the testes in an "induced cryptorchid" state. Cryptorchidism in sheep has an increased prevalence in polled animals.

Cryptorchidism occurs sporadically in **goats**, mainly involving the right testis. It is a regular feature of the goat polled/intersex syndrome.

There are many reports of cryptorchidism in **stallions**, and the condition is particularly noted because of the aggressive tendencies of stallions as compared to geldings. Testicular retention is usually unilateral, with about equal frequency as to side affected. Abdominal retention of left testes is more common than inguinal; the reverse is the case on the right side. Neoplasia of the retained testis is occasionally seen, with teratomas, seminomas, and Sertoli cell tumors being reported.

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Other testicular anomalies

Monorchia is the presence of only one testis. As a general term, monorchism is the result of cryptorchidism, severe unilateral testicular degeneration or agenesis. *True agenesis* occurs when there is a failure of one testis to develop. The differentiation between true agenesis and a cryptorchid with total testicular degeneration is impossible, but true agenesis is considered to be very rare. The presence of *supernumerary testes* or *fusion of abdominal testes* is also very rare.

The presence of testicular tissue outside the testis is reported in pigs, where nodules of tissue are found throughout the peritoneal cavity. In the cat, heterotopic interstitial endocrine cells are reported in the epididymis, and neoplasia of this tissue is reported.

Fusion of the testes to abdominal organs such as the spleen is reported, and is a cause of cryptorchidism.

Accessory or ectopic adrenal tissue is occasionally found in horses and rarely in other species. Most are located in the region of the head of the epididymis and distal spermatic cord (Fig. 5.12).

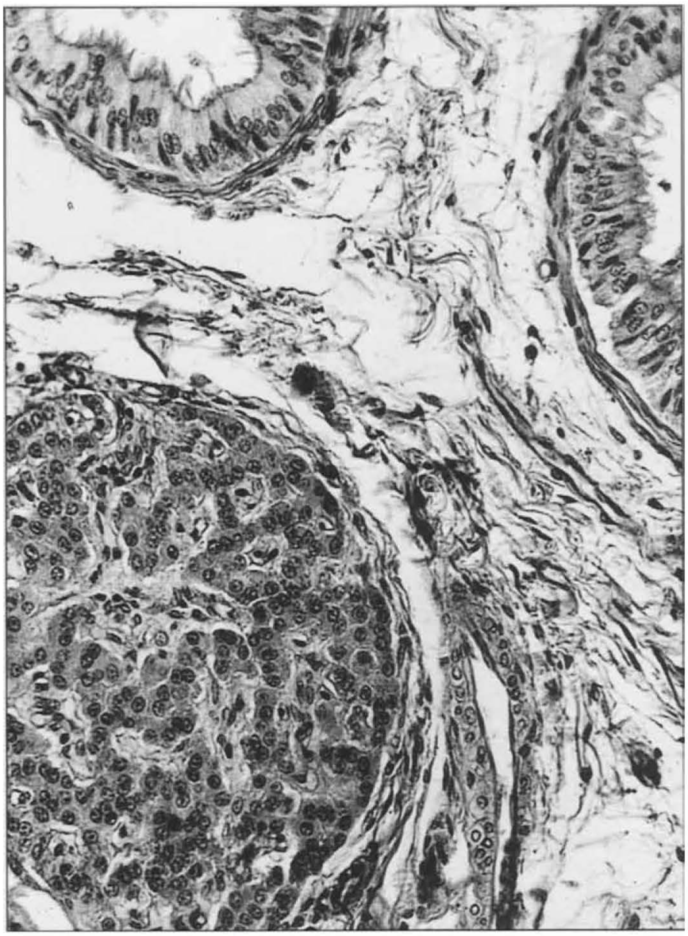

Figure 5.12 Microscopic **ectopic nodule of adrenal cortex** in the epididymis of a ram.

Variations in the shape of testes are described. "Hour-glass" shaped, round, and horizontally placed testes are all reported.

A variety of cystic structures have been described in and around the testis and epididymis. Some arise from the rete testis and others are remnants of mesonephric ducts or ductules, or paramesonephric ducts.

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Ductular anomalies

The ducts that transport spermatozoa and fluid from the testis join the intratesticular rete testis to the pelvic urethra. Maturation and storage of spermatozoa occurs in this conduction system. There is a single duct from the efferent ductules to the urethra, and any interruption has potentially catastrophic effects to the fertility of that side. The eventual effect of continuous spermatozoal production and disruption of one of the efferent ductules has a similar outcome.

Many ductular anomalies have minor significance. Melanosis of the epididymis occurs in rams and bulls. Adrenal ectopia, various cystic remnants of the mesonephric and paramesonephric ducts

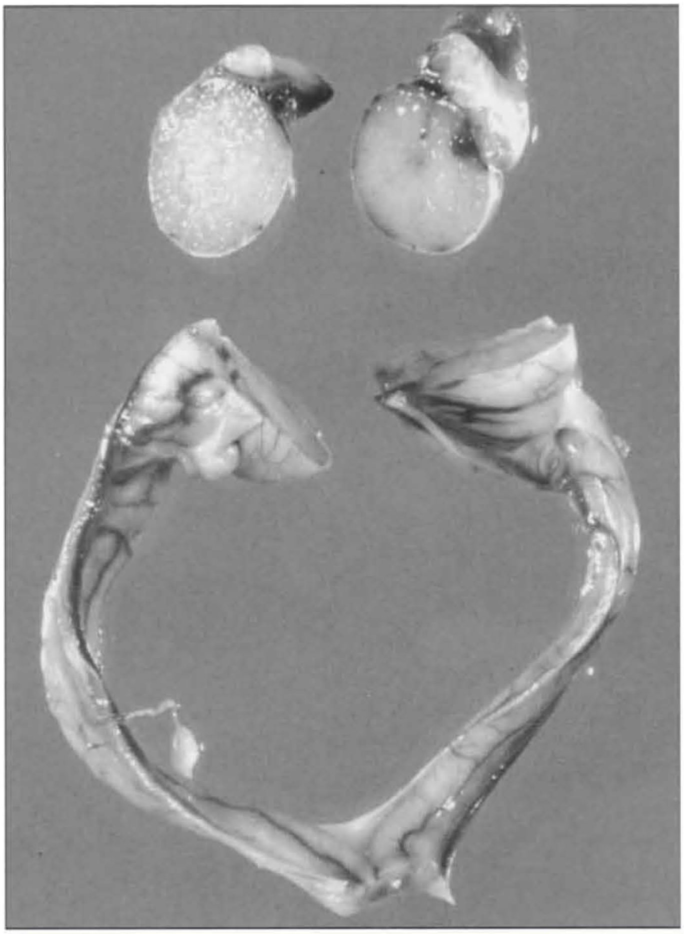

Figure 5.13 Miniature Schnauzer dog with **persistent Müllerian duct syndrome**. This dog is phenotypically male with retained testes (sectioned) and well-developed uterine horns and body.

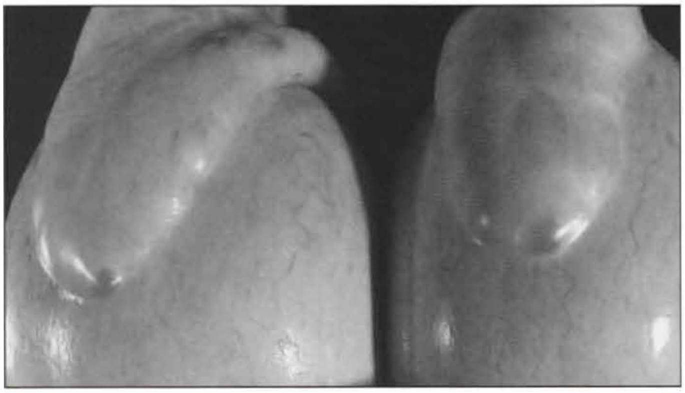

Figure 5.14 Bilateral congenital **retention cysts** at the junction of the head of the epididymis and testis of a ram.

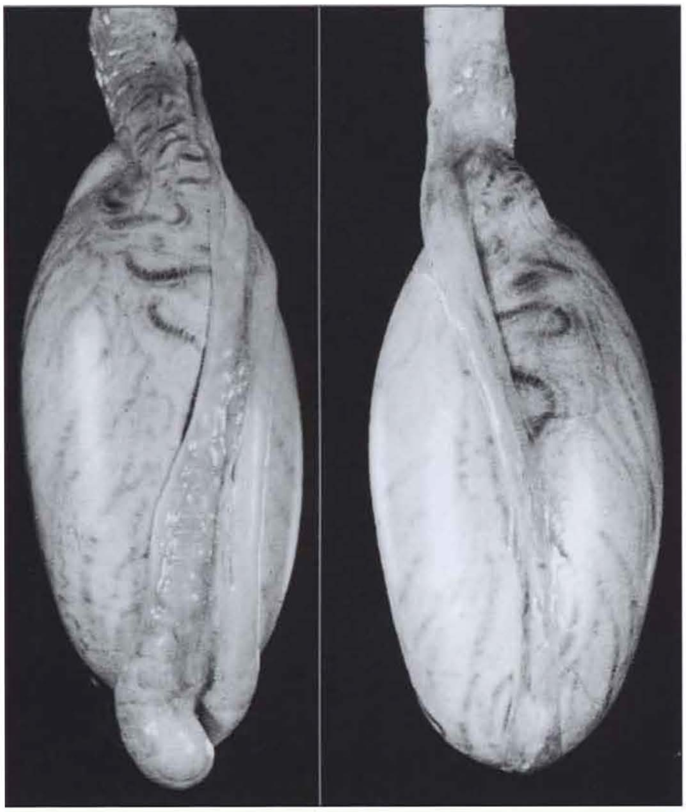

Figure 5.15 **Unilateral aplasia** of the tail of the epididymis (right) in a bull.

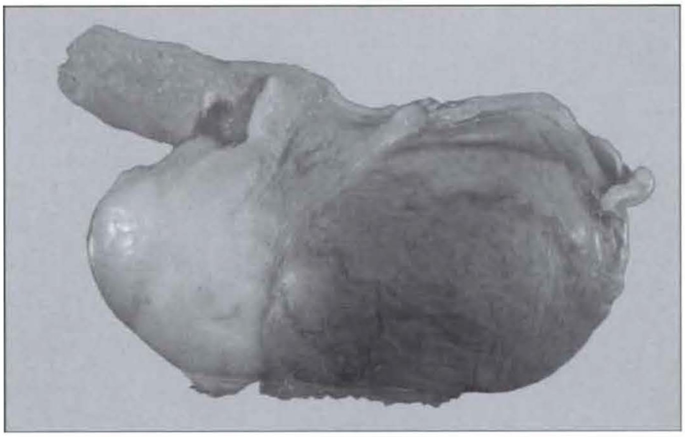

Figure 5.16 **Sperm granuloma** of the **epididymal head** in a dog.

(Figs 5.13, 5.14), and inclusion cysts also are reported in the epididymis. Most are incidental findings and are important as differential diagnoses for epididymitis.

Segmental aplasia of the mesonephric duct is an important condition because it has a profound effect on fertility of the affected side (Fig. 5.15). Segmental aplasia is rare, but further examination of species (such as the cat) in which it has not been reported would no doubt complete the list of species in which it is known to occur. Unilateral aplasia may be an inherited condition. The lack of one part of the epididymis or deferent duct is most likely to be identified if the tail of the epididymis is missing, so it is not surprising that this is the most frequently recognized anomaly. Apart from an absence of the structure, continuous production of spermatozoa results in the complications of spermiostasis, tubular dilation, formation of sperm granulomas, dilation of the mediastinum testis, and testicular atrophy. Examination of the accessory genital glands will usually reveal a smaller vesicular gland on the affected side – presumably because of a lack of trophic stimuli. Hypertrophy of the contralateral testis is also likely.

Sperm granuloma of the epididymal head (Fig. 5.16) is often mistaken for infectious epididymitis. Failure of one or more of the 12–25

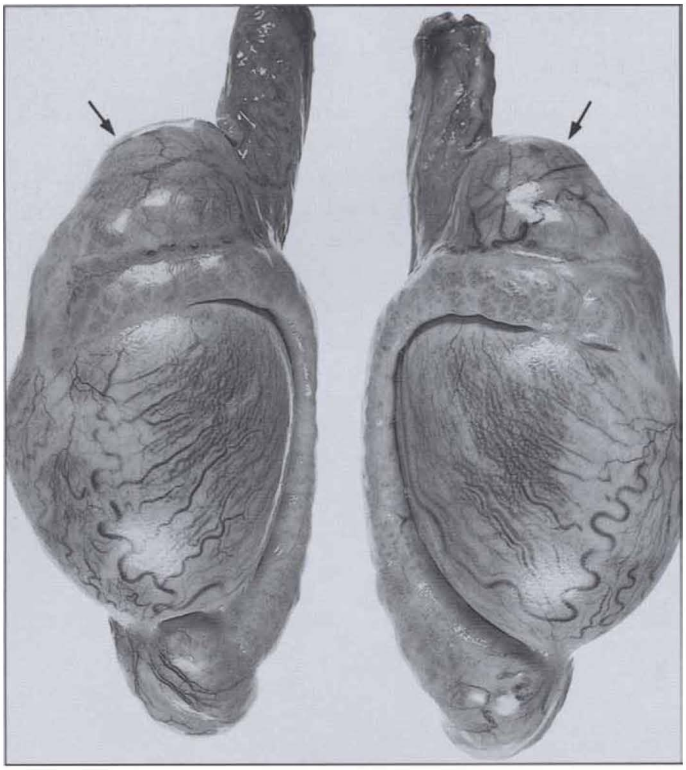

Figure 5.17 Bilateral **sperm granulomas** of the **epididymal head** (arrows) in a goat. Each efferent ductular region, and the head of the epididymis are large and contain sperm granulomas.

efferent ductules to join with the epididymal duct is believed to be the underlying defect. After puberty, the blind-ended ductule(s) fill with spermatozoa and, with spermiostasis, may rupture and/or form a sperm granuloma. The associated inflammation and fibrosis will eventually cause obstruction of other efferent ductules or the epididymal duct. The result will be complete obstruction to flow of spermatozoal-rich fluid and progressive formation of more sperm granulomas. The distal epididymal tissues are reduced in size, the mediastinum testis dilates, and the testis undergoes pressure atrophy. This condition is often misdiagnosed as epididymitis and the potential congenital or hereditary basis for the problem is missed. Sperm granuloma of the epididymal head is a common finding in polled Saanen goats, where they may be bilateral (Fig. 5.17).

Ectopic interstitial endocrine cells and *ectopic interstitial cell tumors* have been reported in the tunica albuginea, mediastinum testis, or spermatic cord of cats.

Many *cystic lesions* have been reported in the tunica albuginea, epididymis, spermatic cord, and adjacent to the accessory genital glands. These are presumed to be remnants or duplications of the paramesonephric and mesonephric duct systems. Remnants of the mesonephric duct include the paradidymis (interna and externa), epididymal cysts, and appendix epididymis. So-called *retention cysts* are very common in the region of the head of the epididymis, especially in rams (see Fig. 5.14). They are usually up to 3 mm in diameter, contain clear fluid, and are lined by epithelium similar to that of the epididymis. The paradidymis is seen proximal to the epididymis in the spermatic cord as one or several small cysts, particularly in neonates. Other cysts, seen particularly in the epididymis, can be microscopic or up to 4 cm in diameter.

Remnants of the paramesonephric ducts include the appendix testis, the uterus masculinus near the accessory genital glands, and, in some cases a completely retained duct that runs beside the testis. Histologically these may have the appearance of a juvenile uterus with prominent musculature and uterine glands.

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Variation in testicular and epididymal size

The daily spermatozoa output of the testes is directly and highly correlated to testicular volume, most commonly evaluated using scrotal circumference, for which reference ranges have been established by species or breed. Testes above this range are hypertrophied; those below the range are too small because of atrophy or hypoplasia. *Even experienced pathologists have difficulty in differentiating hypoplastic from atrophic testes in adults.* This is because there are variations in the degree of hypoplasia and, over time, hypoplastic testes may undergo degeneration. The dogma is that in atrophic testes, the ratio of testicular to epididymal weight is reduced since the reduction in size of the testis is greater than the reduction in size of the epididymis. The basement membrane of appropriately fixed atrophic testes is buckled or has a "wavy" outline. In hypoplastic testes, the ratio of the weight of the testis to epididymis is similar to that of normal testes. The size of both is smaller that normal and there are histological features of spermatogenic arrest in tubules that are smaller than age- and breed-matched controls. The best method to be sure that degeneration has occurred is to demonstrate a decrease from normal testicular weight or size over time. In contrast, hypoplastic testes are smaller than normal and remain so. Since pathologists usually must make an assessment based on a single observation, a diagnosis of "small testes" is not necessarily an indication of ignorance; rather, it is an indication of appropriate caution.

Testicular hypertrophy

Enlargement of an otherwise normal testis is rarely a primary disorder. *Secondary or compensatory hypertrophy* is a unilateral condition that indicates an underlying disease such as orchitis or neoplasia in the contralateral testis. It is a well-recognized phenomenon in hemicastrates, and in hypoplasia, cryptorchidism, or atrophy of the contralateral testis (see Fig. 5.10). Hemicastration of prepubertal boars, especially in those younger than 3–4 months, results in dramatic hypertrophy of the contralateral testis. In rams and bulls, hypertrophy appears to occur mostly when the unilateral condition is present during the peripubertal period and is associated with increased FSH production. The increase in size can be up to 100%,

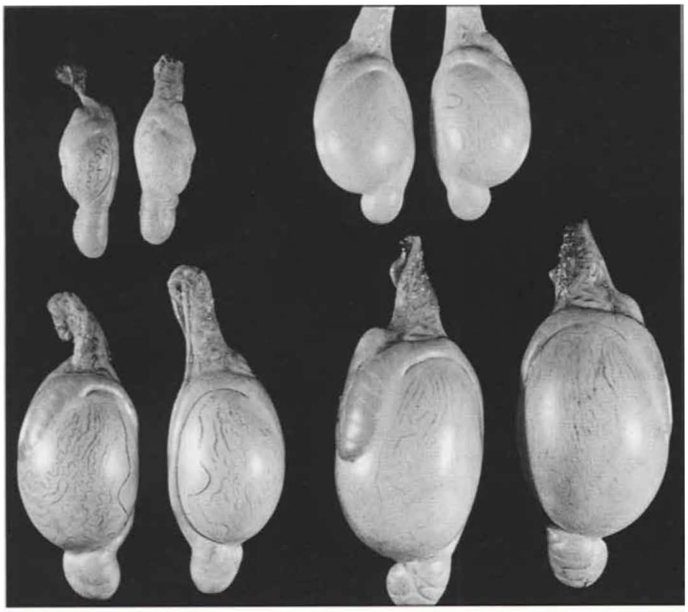

Figure 5.18 Testes and epididymides of four rams of the same age. There is a gradation of **testicular hypoplasia** from severe (upper left) to normal (lower right).

and is due to an increase in the size of Sertoli cells and in the number of germinal cells per Sertoli cell. In some instances there may be an increase in the number of Sertoli cells and spermatogonia, and an increase in diameter and length of seminiferous tubules.

The presence of *dilated cystic remnants and pseudocysts* can also cause, or be confused with, enlargement of a testis. Occlusion of the efferent ductules can lead to dilation of the rete and mediastinum testis and appear as testicular enlargement, although the thickness and fibrous nature of the tunica albuginea makes sudden enlargement of the testis less likely. Adhesions and thickenings of the tunica vaginalis can also appear clinically as testicular enlargements. To the unsuspecting, lesions of the scrotum or its contents are frequently mistaken as testicular enlargement; hydrocele, epididymitis, or the presence of sperm granulomas are examples.

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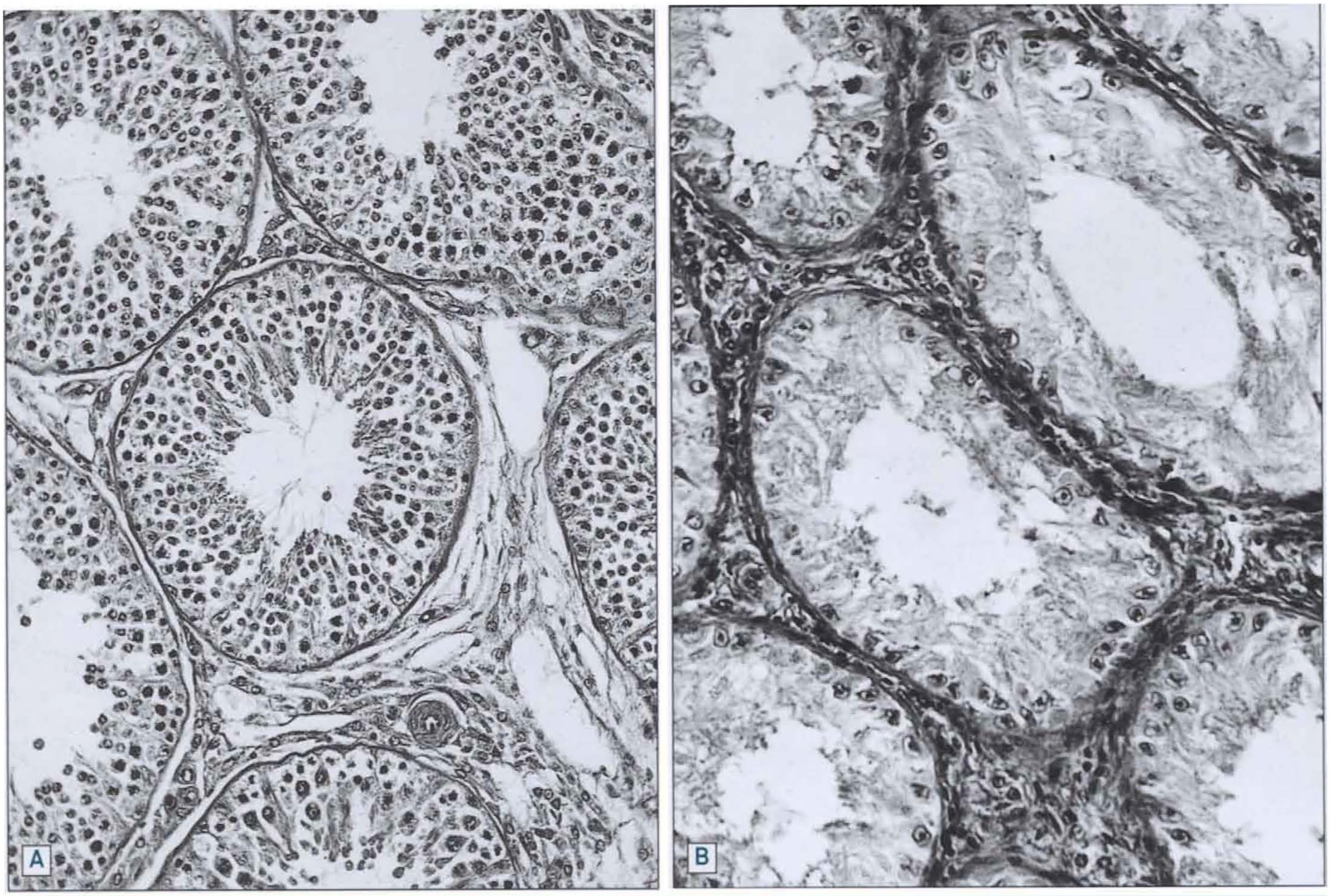

Figure 5.19 A. Normal seminiferous tubules of an adult bull. **B.** Severe **testicular hypoplasia** in a bull, with the Sertoli cell only pattern and a complete lack of germ cells.

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Testicular hypoplasia

Hypoplastic testes have failed to grow to a normal size. *Testicular hypoplasia occurs in cryptorchidism, some intersex conditions, and as an "uncomplicated" disease.* It can be unilateral or bilateral, and the small size is because of a reduction in the amount of the seminiferous epithelium. An affected testis is grossly similar to a normal testis in almost every way, except size (Fig. 5.18).

Hypoplasia is the potential outcome of a large number of different abnormalities that may operate at a systemic or local level, as perhaps occurs in the case of unilateral disease. The small size is theoretically because of an abnormally small number, length, or diameter of tubules, or one or more combinations of these (Figs 5.19A, B and 5.20). Germ cells may be absent, or be present but fail to produce enough spermatozoa. Germ cells may have failed to migrate to the genital ridge in utero, failed to migrate in sufficient numbers, failed to survive, or they may have arrested development, undergo excessive apoptosis, or undergo degeneration at some stage of spermatogenesis (Fig. 5.21).

Studies of hypoplasia have been mostly observational, at the clinical, gross, or microscopic level. Microscopic abnormalities include a total lack of germ cells, arrested spermatogenesis in all tubules, or arrested spermatogenesis that varies from tubule to tubule (low germ cell resistance). Hypoplastic testes with a total lack of germ cells are very small and fail to enlarge from their size at birth. Those testes with arrested development of all tubules fail to progress beyond a certain stage of spermatogenesis.

A deficiency of gonadotrophins is associated with hypoplasia (hypogonadotrophic hypogonadism) in men and mice, but studies in domesticated mammals indicate a normal or raised serum concentration of FSH and LH. Testosterone concentration was lower than normal in some bulls with hypoplasia; in sheep, testosterone and inhibin concentration may be normal.

At the chromosomal level, testicular hypoplasia in animals with a condition resembling Klinefelter's syndrome is recognized. Affected cats, bulls, dogs, pigs, horses, and sheep have an XXY genotype or a mosaicism with XXY chromosomes. The best known of these is the male tortoiseshell or calico cat. In goats of the Saanen breed with the gene for polledness, testicular hypoplasia is seen in genetically female pseudohermaphrodites. A variety of other chromosomal abnormalities have been identified as being associated with hypoplasia.

At the genetic level, hypoplasia is known or suspected to be *hereditary* in the bull, ram, and buck. The exact genetic abnormality

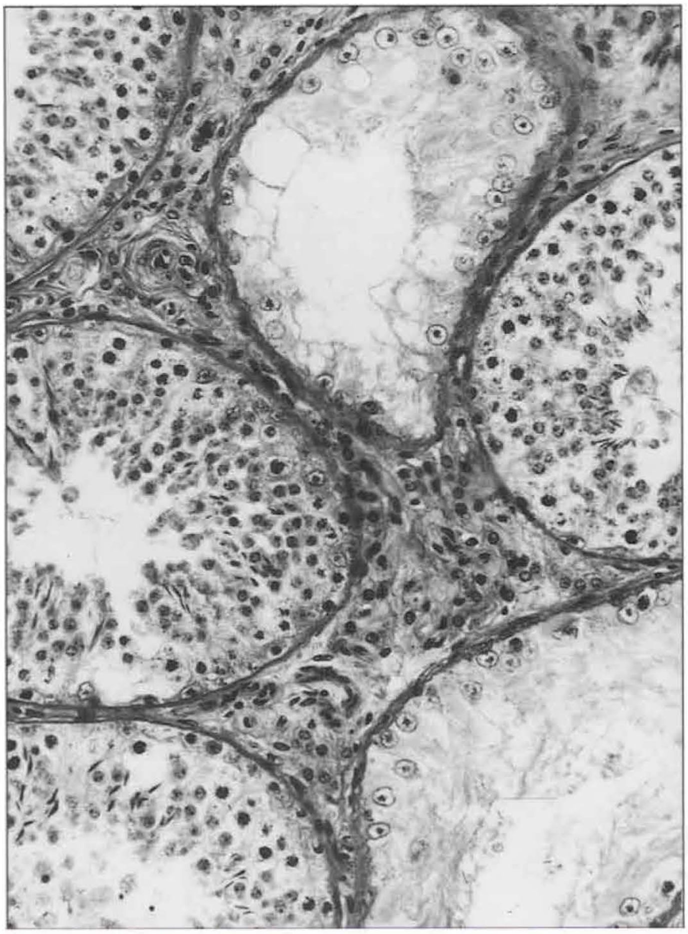

Figure 5.20 Hypoplastic seminiferous tubules adjacent to normal tubules of a bull.

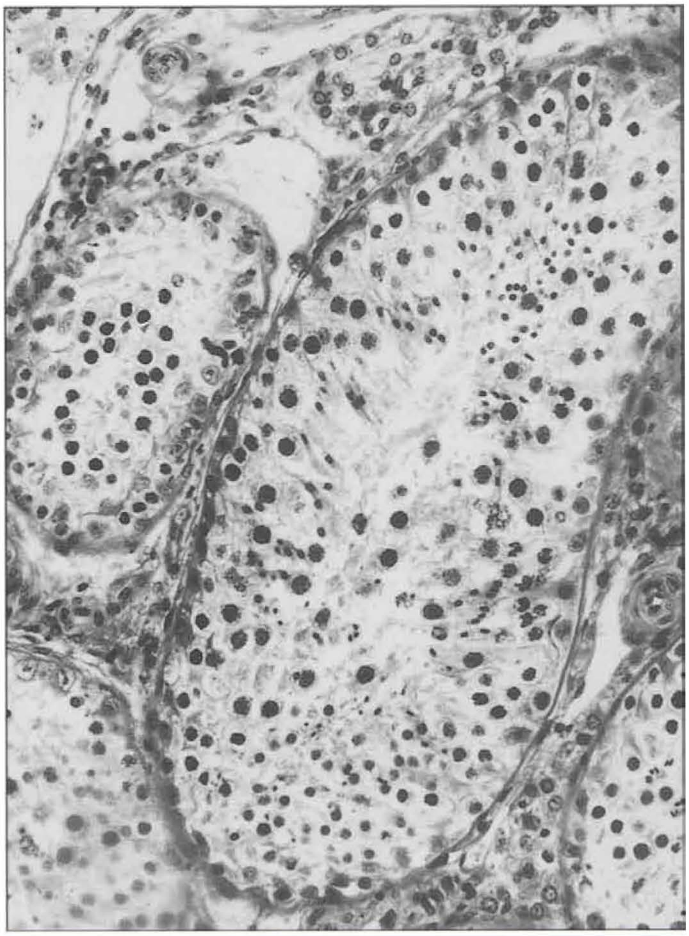

Figure 5.21 Seminiferous tubules of a bull with **testicular hypoplasia**. Tubules have arrested spermatogenesis.

is often not established. There are few studies of hypoplasia in domesticated animals, but there are some special features of the disease in selected species discussed below.

Hypoplasia in bucks

There are several types of hypoplasia recorded in bucks. It is a common abnormality in the polled/intersex (XX sex reversal) syndrome of polled Saanen goats, where the animals are genotypically female but phenotypically male. The testes of affected animals have rudimentary seminiferous tubules. Other sporadic forms of hypoplasia occur, and the cause is not known.

Hypoplasia in bulls

As with other species, testicular hypoplasia in bulls probably is a multifactorial condition. Studies suggest it is hereditary, particularly in the Swedish highland breed, where it has a recessive inheritance with incomplete penetrance. Animals with white body and ears are particularly likely to have hypoplasia. The majority of unilateral hypoplastic testes are on the left side.

The defect in hypoplasia varies from case to case. Some are regarded as a "germ cell weakness," whereby germ cells are present in most seminiferous tubules but there is marked pyknosis of primary spermatocytes. Some are described as having "sticky chromosomes" with the presence of multipolar spindles in primary spermatocytes, or having germinal epithelium that does not progress to spermatogenesis. Other cases of hypoplasia have a Sertoli cell-only pattern or they have some normal and abnormal tubules intermixed, often with affected tubules being in the more dorsal parts of the testis. Despite the various abnormalities observed, there generally appears to be a cessation of spermatogenesis at some stage, with either degeneration or excessive apoptosis of the germinal epithelium.

Hypoplasia in cats

Testicular hypoplasia is a sporadic disease of cats. It is most frequently seen on cryptorchid animals and in cats with a tricolor or calico coloration. Males with tricolor or calico coloration often have an XXY genotype. Affected cats may have no germ cells and markedly hypoplastic testes with small diameter tubules. Chimeric animals may be fertile.

Hypoplasia in dogs

A diagnosis of testicular hypoplasia in dogs is usually made only after azoospermia is found during an investigation of infertility. Hypoplasia cannot be implicated as a cause of azoospermia if the dog has been previously fertile, and can only be considered if acquired azoospermia can be ruled out with certainty. Confirmation of congenital azoospermia because of testicular hypoplasia requires historical information or testicular biopsy after the expected date of puberty.

Congenital azoospermia occurs in some lines of dogs. This suggests a familial or hereditary basis, implicating inbreeding.

Hypoplasia is commonly seen in animals with failure of testicular descent, and in chromosomal abnormalities such as XX sex reversal and XXY syndrome. Offspring of bitches treated with diethylstilbestrol may have hypoplastic testes.

Hypoplasia in rams

Testicular hypoplasia of rams usually is a sporadic disease that may be either bilateral or unilateral. The disease is not well studied. A condition resembling Klinefelter's syndrome, with an XXY genotype is reported. Zinc deficiency has been implicated in some cases.

Various degrees of hypoplasia are recognized, and affected testes may have tubules that, at the extreme are about 90 μm in diameter (normal 160 μm) and are lined by mostly fetal Sertoli cells. An outbreak of gonadal hypoplasia that affected rams born within a short time frame is reported. The affected testes were uniformly small and had a "Sertoli cell-only" pattern histologically. An environmental cause was suspected.

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Table 5.1 Causes of testicular degeneration

- Advancing age
- Chemicals
  1. Chemotherapy
  2. Chlorinated naphthalenes
  3. Halogenated compounds, including hexachlorophene
  4. Nitrogen-containing compounds, including benzimidazoles, nitrofurans
  5. Metal compound toxicity
- Epididymitis
- Heat
- Hormones
  1. Dexamethasone
  2. Estrogen
  3. Testosterone
  4. Zearalenone
- Neoplasia
  1. Pituitary tumors
- Nutritional disorders
  1. Negative energy balance
  2. Fatty acid deficiency
  3. Hypovitaminosis A
  4. Hypervitaminosis A
  5. Hypovitaminosis B
  6. Hypovitaminosis C
  7. Hypovitaminosis E
  8. Protein and amino acid deficiency
  9. Zinc deficiency
- Plants
  1. Locoweed (*Astragalus*)
  2. Lysine seeds (gossypol)
- Radiation
- Stress/corticosteroid therapy
- Trauma
- Ultrasound
- Viral infection
  1. *Porcine reproductive and respiratory syndrome virus*
  2. *Canine distemper virus*

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Testicular degeneration

Testicular degeneration is manifested clinically and grossly as atrophy, mineralization, and fibrosis. It usually occurs because of influences external to the testis (see Testicular hypoplasia). There is a seemingly endless list of potential causes, as outlined in Table 5.1. The close interrelationship of the Sertoli cells, interstitial endocrine cells, and germ cells means that insults to any one or several of them eventually affect them all. Even though the insult is external to the testis, the manifestation in the testis is not always bilateral or uniform. The testes of aged bulls frequently degenerate from the ventrum, and those from rams appear to degenerate from the dorsum.

Testes undergoing degeneration are usually reduced in size. In early or rapidly progressing degeneration, the testis is soft and flabby, lacks turgor, and the cut surface does not bulge. Wrinkling of the tunica albuginea may be apparent. With ongoing loss of fluid and reduction in the germinal epithelium relative to the stroma, the result is a small testis of firm consistency. The substance of the testis takes on a darker color that is often brown. Since the epididymis usually is less affected than the testis, it ultimately will appear to be disproportionately large (Fig. 5.22A, B). With continued degeneration and fibrosis, the testis becomes increasingly hard and variable mineralization may occur (Fig. 5.23).

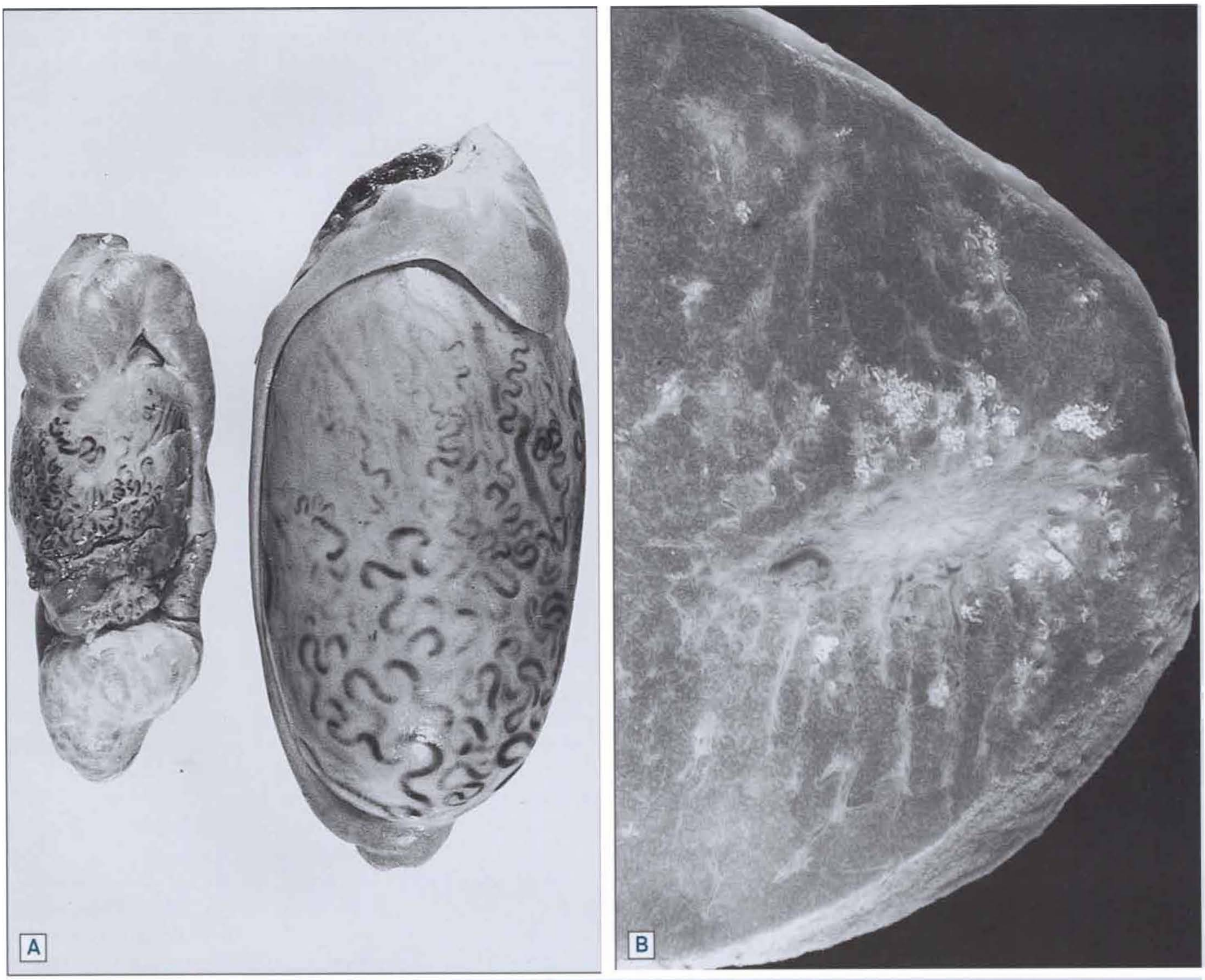

Figure 5.22 **A.** Unilateral **testicular degeneration** in a bull. The epididymis of the atrophic testis appears disproportionately large. **B.** Discoloration (brown) of the testis and mineralization of seminiferous tubules in a bull with testicular degeneration/atrophy.

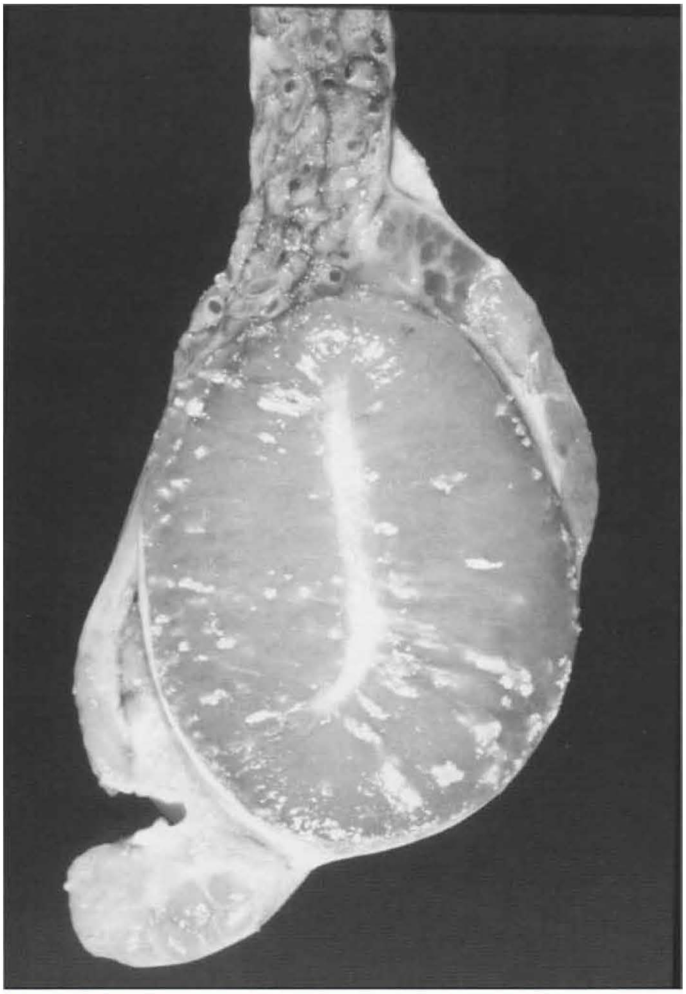

Figure 5.23 Testicular atrophy and resultant darker testicular color, and multifocal mineralization in a buck.

Testicular degeneration may be uni- or bilateral, and *it is assumed that unilateral degeneration is the result of a local event, while bilateral degeneration usually is the result of a systemic problem.*

Histological changes in degeneration (Fig. 5.24A, B) vary in degree, but are relatively stereotypic. In the early stages, Sertoli cells develop either a fine basal vacuolation or a more dramatic vacuolation of the apical cytoplasm. There also is disorganization and exfoliation of germ cells. In some cases there may be failure of release of germ cells from Sertoli cells (spermiation) and spermatozoa are phagocytized by Sertoli cells.

Early changes in the germ cells include failure of maturation of spermatozoa and degeneration of spermatids; many spermatids are necrotic and others produce characteristic spermatidic multinuclear giant cells. When the degeneration is more advanced, the affected areas are more extensive and degenerative changes appear in the precursors of spermatids. Depending on the insult, there may be cytoplasmic vacuolation and nuclear pyknosis, or apoptotic bodies are seen. Progression of the changes results in loss of germinal cells, and eventually, loss of even the resistant Sertoli cells. Basement membrane thickening is a frequent finding, and as a result of shrinkage and collapse of tubules, it becomes wavy and buckled. The PAS stain clearly accentuates the basement membrane change. In ultrastructural studies of the testes of normal bulls and bulls with atrophy or hypoplasia, mean thicknesses of the actual basal lamina were approximately 0.7, 1.5, and 1.0 μm, respectively.

Once-only scrotal heating can cause the appearance of giant cells in degenerate testes and perhaps in the semen. Two types of giant cells, mononuclear giant cells probably derived from pachytene spermatocytes that fail to differentiate further, and multinucleate cells considered to be derived from coalescence of identical spermatids, are observed histologically. Even extremely brief heating, of several minutes only, induces giant cell formation. Such giant cells are seen as early as 6 hours and as late as 7 weeks post-heating, but they seem to be most prevalent at about 1 week. The fate of these cells is unclear. They may disintegrate or pass out of the testis. In addition to giant cell formation, minor increases in testicular temperature in sheep produce a marked accumulation of B-type spermatogonia; histologically this can be demonstrated by the presence of many cells in which mitosis is incomplete.

Granulomas can form in degenerate testes (Fig. 5.25A, B). This and the presence of lymphocytes and plasma cells indicates an immune response to spermatozoa, but complicates the differentiation of simple degeneration from interstitial orchitis. *Osseous metaplasia* sometimes occurs in tubules affected by spermiostasis for a long time.

Mineralization is a common sign of degeneration, and it may involve all or part of one seminiferous tubule, or whole regions (Fig. 5.26). This latter pattern of change is only significant if the testes are to be biopsied, wherein the section may not be representative of the whole.

The basic mechanism of degeneration of the cells that make up the germinal epithelium is presumed to be via *apoptosis.*

The testis is very susceptible to the effects of increased scrotal temperature. Both testicular and epididymal function are altered when intrascrotal temperatures increase. In the epididymis, an increase in temperature reduces mRNA expression of a spermatozoal membrane glycoprotein (CD52) in a testosterone-independent manner. An inability to maintain the testes at a temperature lower than normal body temperatures can occur with pyrexia, increased environmental temperatures, scrotal thickening and hair and wool cover, and by the presence of intrascrotal inflammation or scrotal dermatitis. Varicocele is also implicated in the failure to maintain testicular thermoregulation. The progressive degeneration of maldescended testes is believed to be the result of abnormally high testicular temperature.

Degeneration of the testes with advancing age is a recognized phenomenon. The cause is not fully known but it may be secondary to degenerative vascular lesions within the testis. Old bulls and rams have a diffuse increase in intertubular stroma and a decreased proportion of tubular mass. Other changes include increased thickness of tunics and tubular basement membrane, increased proportion of degenerate tubules, and an apparent increased number of interstitial endocrine cells, which contain increased lipofuscin.

Gonadotrophins are essential for the normal development and function of the reproductive tract. The pathology of *disruptors of endocrine function* is a major focus because of the effects of antiandrogenic and estrogenic substances on male reproduction. Substances that reduce androgen concentrations, such as lead and ethanol, adversely affect spermatogenesis and cause degeneration. Sometimes the degeneration is subtle – such as reducing the number of

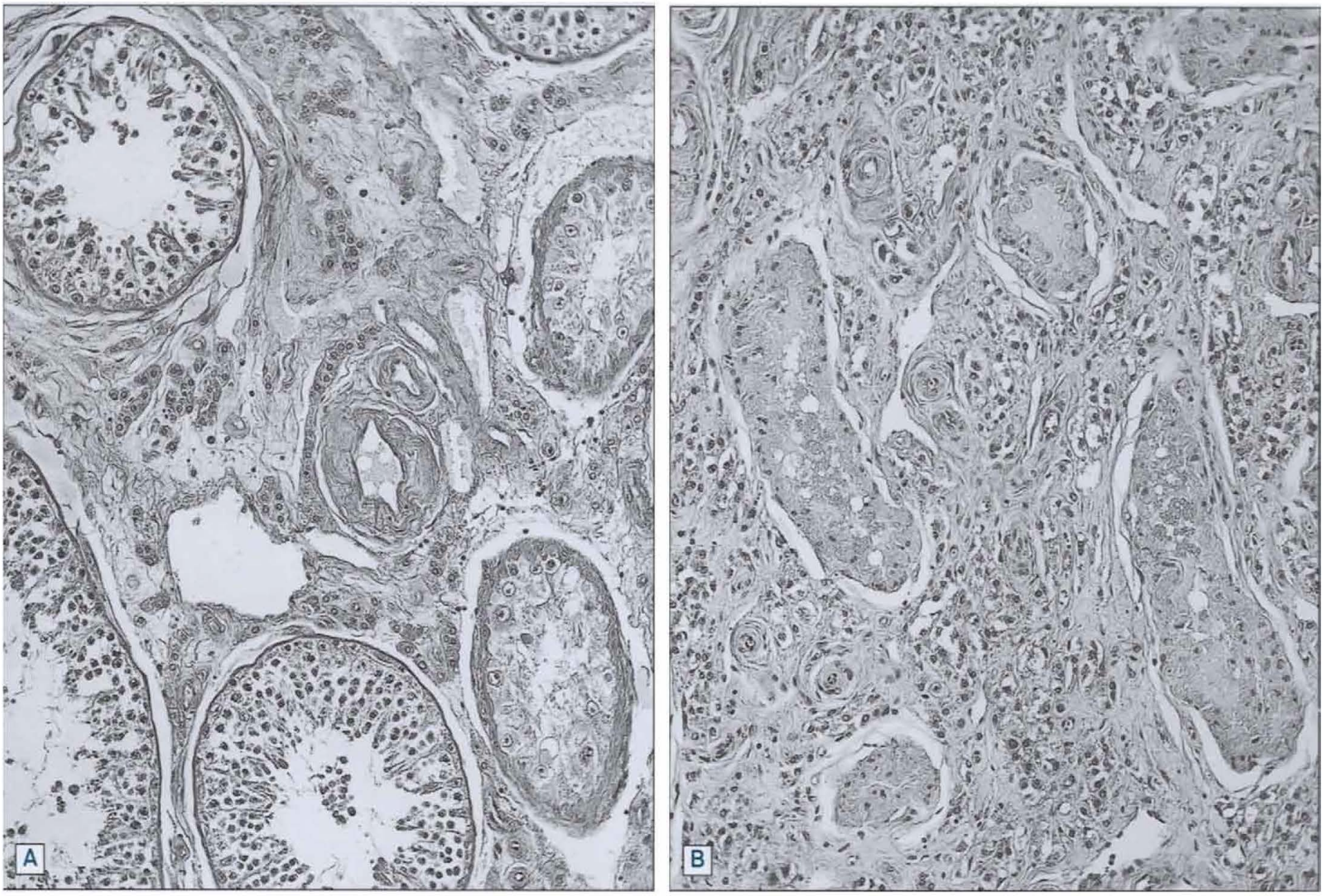

Figure 5.24 Testicular degeneration in a bull. **A.** Tubules are at various stages with changes that include a Sertoli cell only pattern, reduced spermatogenesis, and buckling of basement membranes. Hyalinized blood vessels are in the interstitium. **B.** More advanced degeneration with hyalinized tubules and depletion of both germinal and Sertoli cells.

spermatids. Drugs and chemicals (phenobarbital, DDT) that stimulate the hydroxylases that reduce testosterone activity also can create antiandrogenic effects.

Some *directly acting toxicants* are known in domesticated mammals. These may be derived from plants, or from other environmental or therapeutic sources. Biotransformation of such compounds can occur in the testis, as those formed elsewhere may not attain significant concentrations in the testis. The toxicant may affect any one or several of the cell types, including the Leydig cells, Sertoli cells, spermatogonia, spermatocytes, spermatids, spermatozoa, or the epididymal tissues.

Interstitial endocrine cell toxicants, as expected, affect testosterone production. Ketoconazole, ethanol, acetaldehyde, and cannabinoids are examples. Their effects include alteration of Sertoli cell function and maturation of germ cells, especially pachytene spermatocytes and spermatids.

Direct Sertoli cell toxicants will have detrimental effects on the blood–testis barrier, orientation and translocation of germ cells, hormonal and nutritional support of germ cells, and phagocytosis of residual bodies. The targets for toxicants include the actin filaments, intermediate filaments, and microtubules. Cytochalasin disrupts actin filaments, acrylamide disrupts intermediate filaments, and colchicine, vinblastine, and vincristine affect microtubules. Fortunately, many of these drugs, when used at therapeutic doses, have a temporary effect that is reversed when therapy ceases.

Effects seen microscopically include vacuolation or swelling and germ cell changes. Failure of spermiation can occur.

Toxicants that affect the germ cells are those that are used to prevent rapid mitotic division, and the spermatogonia are the major targets. Adriamycin and cyclophosphamide are examples. Damage by these compounds to the genetic composition of stem cells will also have effects on the later stages of development, up to spermatozoa. Toxicants of the other germ cells are little recognized in domestic species, although in rodents, ethyl glycol alkyl ethers affect spermatocytes, nitroimidazoles affect spermatids, and compounds that impinge on energy metabolism affect spermatozoa. Damage to various stages causes apoptosis, with a rapid uptake of the detritus by Sertoli cells. Within 48 hours, there may no longer be evidence of apoptosis – just the appearance of "maturation arrest."

Toxicants can also affect the efferent ductules and epididymis and/or the spermatozoa in transit. The difficulty with investigating efferent ductular toxicosis is separating the effect due to alteration in testosterone concentration, and direct toxic effects. Substances that affect the efferent ductules include cyclophosphamide, methyl chloride, and reserpine. Such toxicants decrease spermatozoal concentration and cause spermiostasis, sperm granuloma formation,

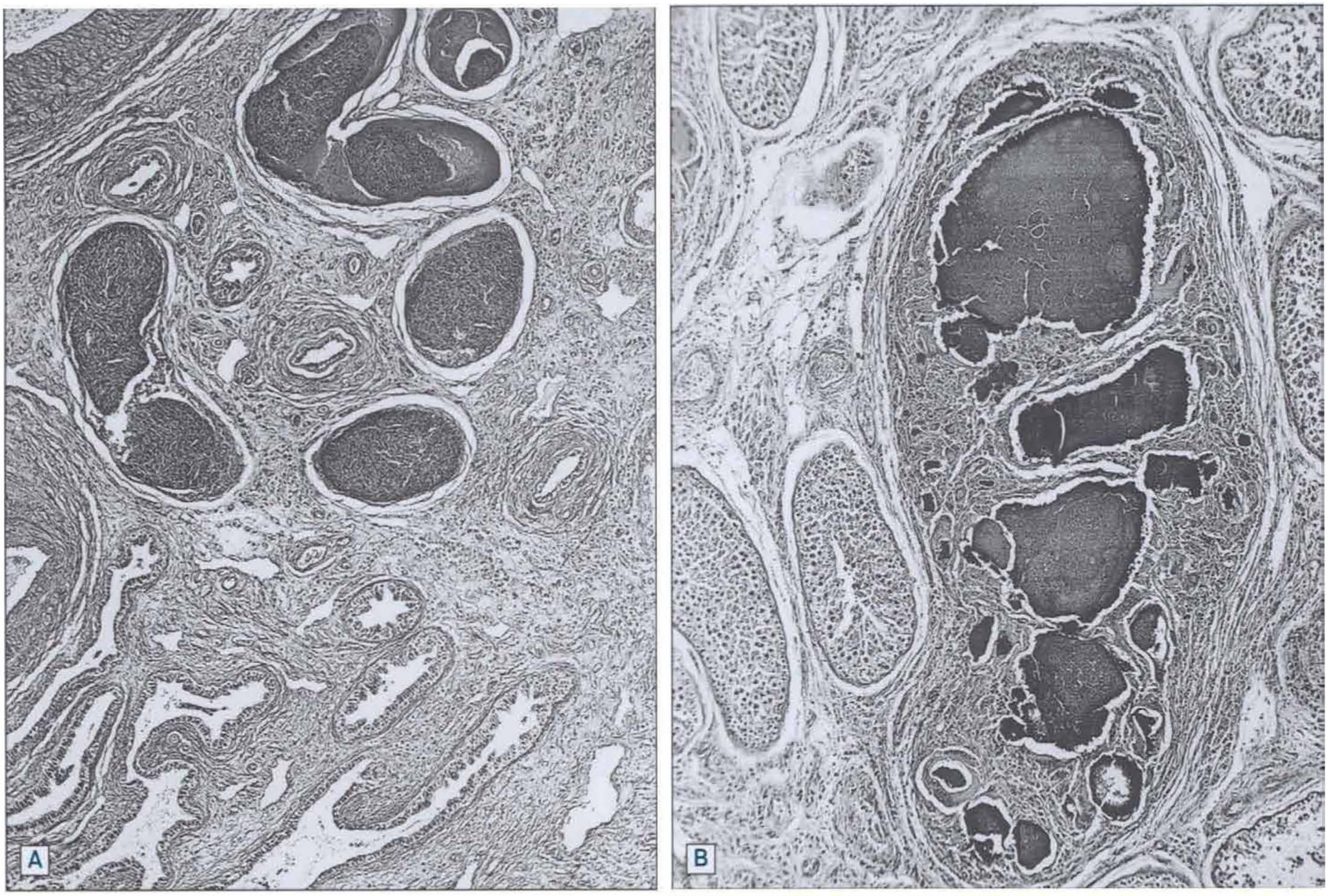

Figure 5.25 **A. Spermatozoal stasis** in remnants of seminiferous or straight tubules near the mediastinum testis. **B.** Sperm granuloma in a focus of spermatozoal stasis in the testis of a bull.

and necrotic and degenerative changes to the epithelium. Several have direct effects on spermatozoa in the ducts.

There are few reports of *direct viral infection* and their effects on spermatogenesis. However, *Porcine reproductive and respiratory syndrome virus* replicates in germ cells, alters spermatogenesis, and induces apoptosis. *Canine distemper virus* infection of dogs can cause testicular degeneration.

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Inflammation of the testis and epididymis

Orchitis

Apart from bulls in areas endemic for *Brucella abortus* or tuberculosis, orchitis is a rare and sporadic disease in domesticated animals. *The vast majority of cases diagnosed clinically as orchitis are actually epididymitis* (see below). Focal accumulations of lymphocytes are occasionally seen in the testes of most species as incidental findings (Fig. 5.27). Lymphocytic (or nonsuppurative) inflammation is seen in some infertile animals; an immunological pathogenesis is invoked after it was demonstrated that immunization of guinea pigs and bulls with spermatozoa induced inflammation of the rete testes. Efferent ductules are also involved experimentally.

Orchitis as the primary and severe disease has historically been attributed to brucellosis or tuberculosis. Tuberculous orchitis is a multifocal granulomatous disease that is much less common now because of eradication in many countries. Brucellosis is similarly reduced in

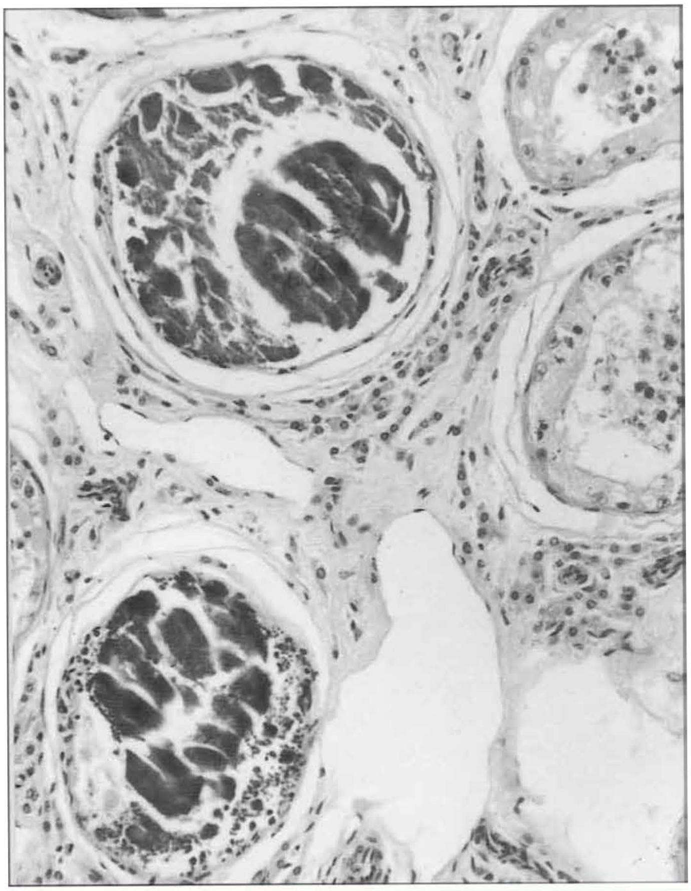

Figure 5.26 Mineralization of seminiferous tubules in the testis of a bull with testicular degeneration.

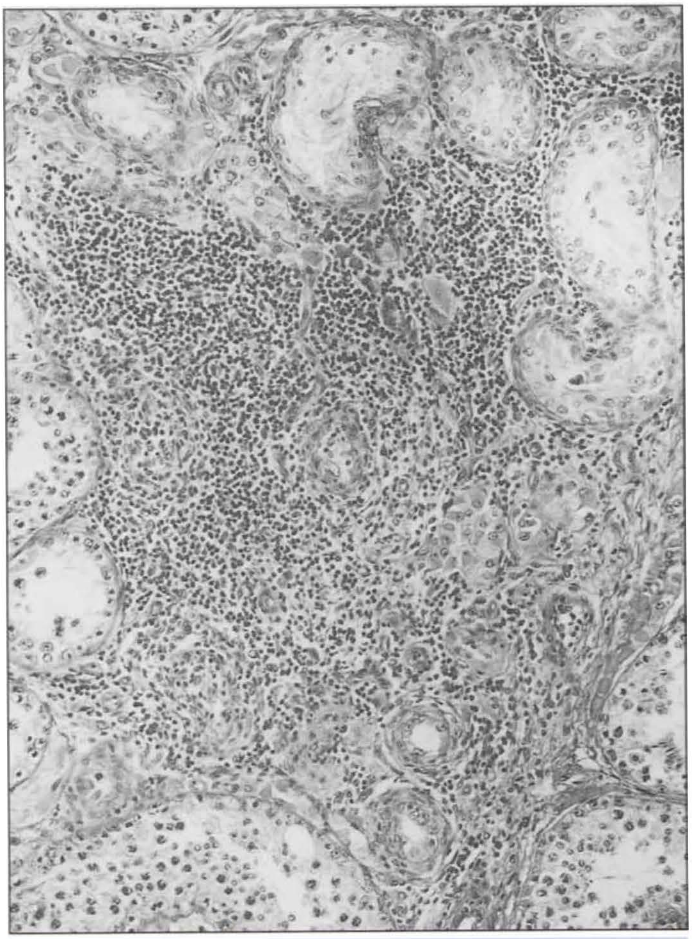

Figure 5.27 Focal interstitial accumulation of lymphocytes with seminiferous tubular degeneration in a stallion.

prevalence. *Brucella abortus* (bulls), *Brucella suis* (pigs), *Brucella canis* (dogs), and *Brucella melitensis* (goats) can cause orchitis as a dominant change. However, epididymitis is often the primary manifestation.

Orchitis occurs sporadically in cats with feline infectious peritonitis, in rams and bucks with *Corynebacterium pseudotuberculosis*, in pigs with *Burkholderia pseudomallei,* and in stallions with migrating larvae of *Strongylus* spp. nematodes. Sporadic infection with other bacteria will no doubt occur from time to time. The relative isolation of the testis suggests that infection with the various agents is mostly hematogenously derived, or occurs by direct penetration.

Orchitis has been divided into three major categories: interstitial orchitis, intratubular or granulomatous orchitis, and necrotizing orchitis.

Interstitial orchitis

Interstitial orchitis may not be recognized macroscopically, but histologically it is characterized by *lymphocytic infiltration of intertubular stroma, with concurrent or subsequent fibrosis* (Fig. 5.28). In bulls, small mononuclear infiltrates are frequently observed adjacent to seminiferous or rete tubules or efferent ductules of otherwise normal testes. Such foci may be of infectious or immune origin, the latter being in response to antigen leaking from a damaged tubule. In stallions, interstitial lymphocytic foci, often perivascular, are particularly common and occur in areas of tubule degeneration and vasculitis. Foci of lymphocytes in cats are considered an age-associated change.

Intratubular orchitis

Intratubular orchitis probably results from *ascending infection.* Macroscopically, solitary or multiple white–yellow foci of up to ~1 cm in diameter are seen. Histologically, the tubule outline is retained in the affected area, but the seminiferous epithelium is obliterated and replaced by numerous macrophages and multinucleated giant cells that surround neutrophils and debris (Fig. 5.29A, B). The pathogenesis of this granulomatous orchitis is comparable to sperm granuloma formation in the epididymis. Sertoli cell hyperplasia and mineralization may accompany these changes.

Necrotic orchitis

Necrotizing orchitis is characteristic of *brucellosis* but may result from other infections, or conditions causing severe trauma or ischemia of the testis. Severe periorchitis may reduce blood supply to the testis so that it dies and becomes a necrotic mass encased within the markedly thickened tunics. Necrotic areas are dry, yellow, often laminated, and slightly mineralized. The histological picture is ultimately one of coagulative necrosis bordered by fibrosis and inflammatory cells. Abscessation and fistulation through the scrotum may accompany necrotizing or other forms of orchitis.

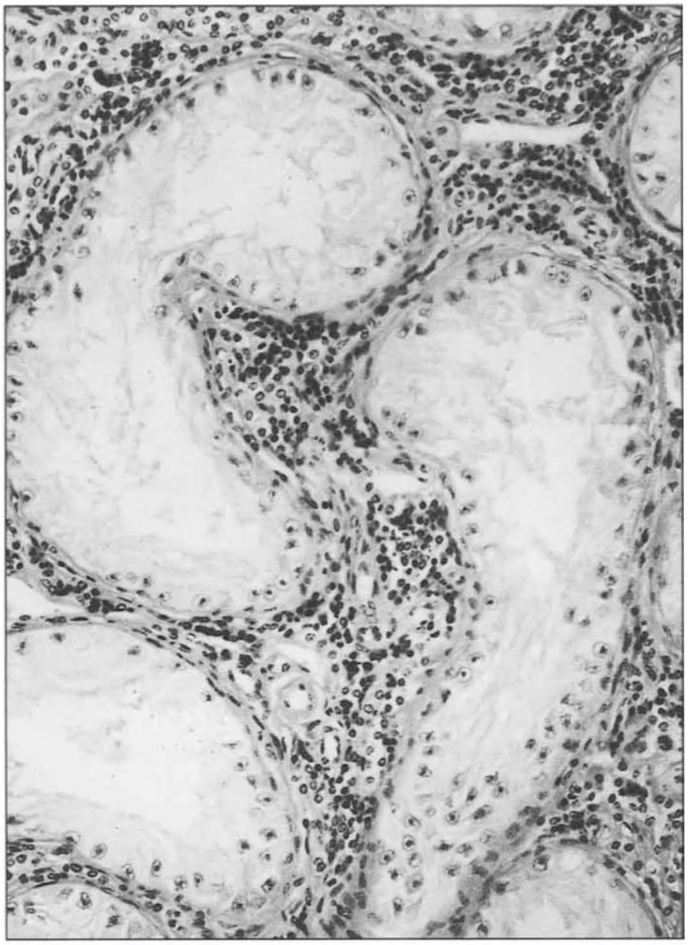

Figure 5.28 Lymphocytic infiltrates in the interstitium of a bull with **interstitial orchitis** and degeneration of seminiferous tubules.

Many infectious agents have been isolated from testes or semen of animals with orchitis, but the significance of many and perhaps most isolates is unclear.

Orchitis in bulls

In bulls, viruses have been isolated from testes or semen, but changes in the testes are observed in association with only some of them. Persistent infection with *Bovine viral diarrhea virus* causes spermatozoal defects, but no distinct histologic lesions in the testis. Severe interstitial orchitis and testicular degeneration associated with inflammation of spermatic arteries occurs in malignant catarrhal fever. In experimental bluetongue virus infection in bulls, interstitial orchitis is also associated with arteritis. Clinical orchitis and aspermatogenesis have been observed in association with enteroviral infection, but lesions are not described. A focal nodular orchitis may be observed in lumpy skin disease of bulls.

Orchitis caused by *Brucella abortus* occurs in regions of endemic **bovine brucellosis**. The live vaccine strain (strain 19) is also capable of producing the lesion. In most instances, the orchitis is acute and the lesion is irreversible. It may be unilateral but affected animals are sterile. The scrotum swells and is hot and doughy due largely to inflammatory changes in the tunics and to a lesser extent in the epididymis (Fig. 5.30). Swelling of the testis is limited by the inability of the tunica albuginea to stretch, and any swelling constricts venous and then arterial flow causing infarction. The cavity of the tunica vaginalis is distended with fibrinopurulent exudate. Scattered yellow foci of necrosis coalesce to produce total testicular necrosis. Sequestration by inflammation and thickening of the tunica soon follows. Sometimes the necrotic parenchyma liquefies and the organ then is a pus-filled cavity surrounded by a thick connective-tissue capsule. Rupture may occur but is unusual. Occasionally, the necrotic foci may not expand and coalesce but rather remain as dry areas of necrosis surrounded by a thick layer of fibrous tissue.

Microscopically, the inflammatory involvement of the tunics is similar to any serous membrane with organization resulting in adhesions between the parietal and visceral layers. These adhesions may be extremely dense. Within the testes, the infection appears to progress along the lumen of the seminiferous tubules. The seminal epithelium becomes necrotic and desquamates. Large numbers of the organisms are visible in the lumen, especially when stained by the modified Ziehl–Neelsen method. At the early stage, a variety of leukocytes invade the interstitial tissues and form cuffs about the tubules. The tubules and the interstitial tissues then become necrotic. There is often focal necrotizing epididymitis complicated by the development of sperm granulomas.

Tuberculous orchitis in bulls is an uncommon lesion, even in areas of endemic infection. The testis is less frequently involved than the epididymis or tunics. The granulomatous response to the tubercle bacilli is similar to the granulomas that occur to spermatozoa. Involvement of the testis may be either miliary or regional. In the miliary form, small or large caseous and mineralized foci are irregularly scattered throughout the testes but may spare the epididymis entirely. Broad bands of caseous necrosis radiate out from the rete testis in the more regional or diffuse form, and the epididymis is usually involved. This distribution is due to the extension of the chronic tuberculous process within the seminiferous tubules. The path of infection in such cases is probably intratubular from a primary epididymal lesion.

Other bacteria causing orchitis in bulls, sometimes in association with overt abscessation, include streptococci, staphylococci, *Arcanobacterium pyogenes*, *Escherichia coli*, *Histophilus* spp., and *Salmonella* spp. *Actinomyces bovis*, *Actinobacillus* sp., and *Nocardia farcinica* may also cause bovine orchitis. In nocardiosis, the lesions are at first nodular but ultimately transform the whole testis into an abscess, the capsule of which is the tunica vaginalis.

Infection of bulls with *Chlamydophila* spp. causes orchitis, and in field cases focal granulomatous lesions have been observed. The spontaneous occurrence of orchitis and epididymitis has been described in a bull with *Mycoplasma* sp. infection.

Orchitis in boars

Pigs experimentally infected with *Porcine rubulavirus*, a paramyxovirus that causes "blue eye," may develop orchitis and epididymitis. The virus targets the head of the epididymis where it causes interstitial inflammation and sperm granulomas. Interstitial fibrosis is seen in animals that recover. Viral antigen is seen in the epithelial cells. Seminiferous tubular degeneration and interstitial orchitis occur in some animals. *Suid herpesvirus 1* infection (pseudorabies, Aujeszky's disease) may cause edema of the scrotal region, but

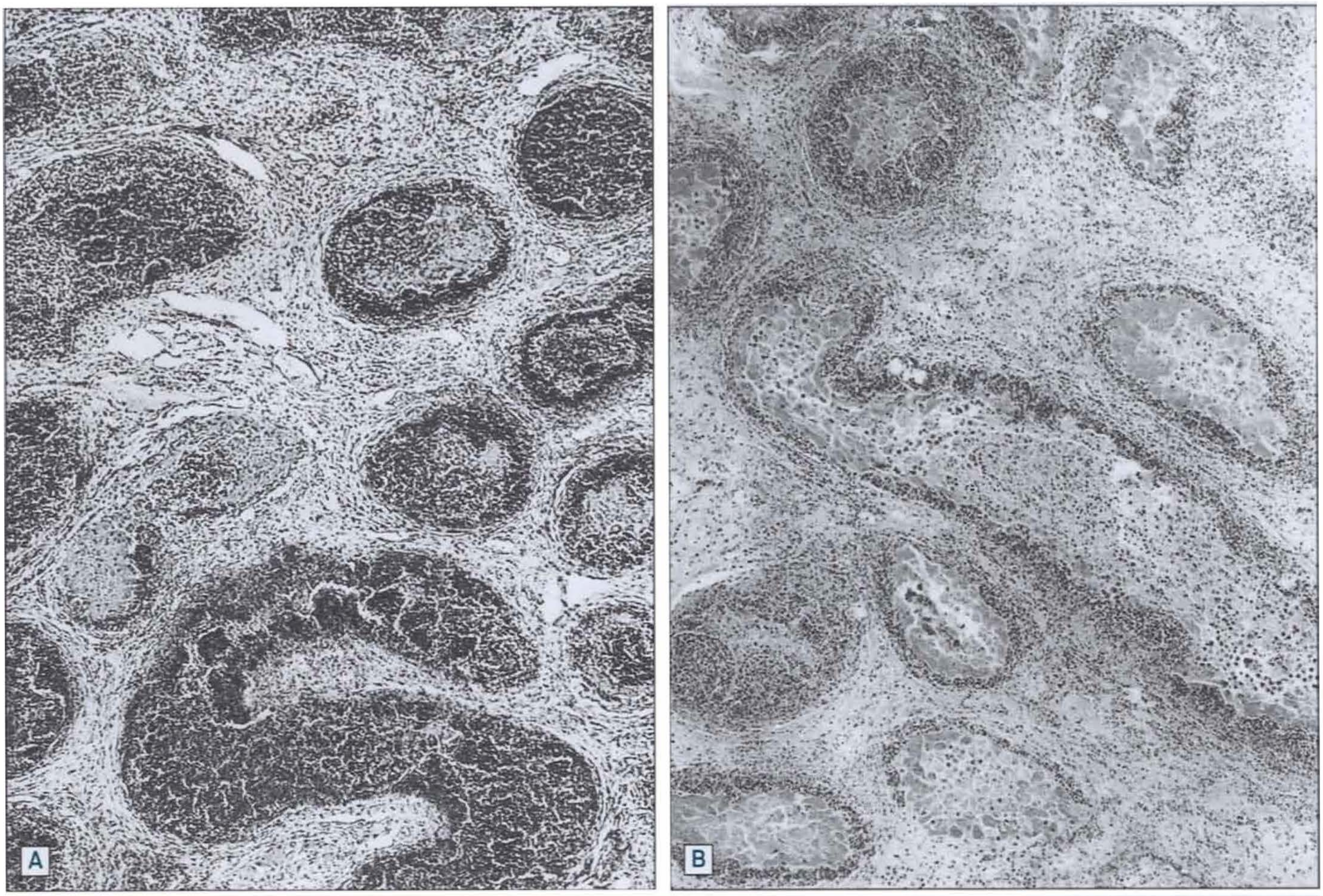

Figure 5.29 Orchitis in a bull, caused by *Brucella abortus*. **A.** The inflammatory reaction is predominantly intratubular. **B.** Necrosis of tubules and inflammatory infiltrates of leukocytes, fibrin, and edema.

results of experimental infection have varied. Viral replication occurs in the serosa after intratesticular inoculation; exudative periorchitis results. Intratesticular inoculation of boars with porcine parvovirus caused testicular degeneration. Enteroviruses and parvovirus have been isolated from semen.

Orchitis caused by *Brucella suis* results in multiple abscesses rather than confluent necrosis. Some cases have fibrinopurulent and hemorrhagic periorchitis. Abscessation develops in the epididymis as well as in the testis; there is central caseation surrounded by a zone of epithelioid macrophages, and these in turn by a broad connective-tissue capsule infiltrated by leukocytes.

In some tropical countries, orchitis caused by infection with *Burkholderia pseudomallei* occurs in boars and other small domestic animals. Lesions may also be found in the vesicular glands (seminal vesicles), prostate, and other organs. Extreme enlargement of the testis due to accumulation of purulent exudate may occur. The main histological lesion is multifocal caseous necrosis with marked mononuclear cell infiltration and encapsulation by much fibrous connective tissue. Severe testicular degeneration accompanies orchitis. Other organisms isolated from boars with orchitis include *Arcanobacterium pyogenes, Streptococcus zooepidemicus*, and *Streptococcus equisimilis*.

Orchitis in stallions

In stallions, mild interstitial orchitis is common. Similar lesions may be part of generalized vascular involvement in equine viral arteritis. Infarcts may also occur in equine infectious anemia. Orchitis can occur as part of systemic disease, and is reported in glanders (*Burkholderia mallei*), and as an acute suppurative, sometimes abscess-forming orchitis in infection with *Salmonella abortus-equi, Streptococcus equi*, and *Streptococcus zooepidemicus*. Focal lesions due to the larvae of *Strongylus edentatus* may be seen in the testis, tunics, and epididymis, especially of young horses. Cryptorchid testes are more commonly affected. Hemorrhagic 2 mm wide tracts containing the migrating larvae are seen. The histological lesions are initially hemorrhagic and then eosinophils infiltrate. *Halicephalobus gingivalis* can cause granulomatous orchitis as a consequence of systemic spread. A pseudocyst, or fluid-filled cavities that have no epithelial lining, is reported in a horse as a sequel to fibrinonecrotic orchitis. The lesion was associated with trauma and secondary infection with *Streptococcus zooepidemicus* occurred.

Periorchitis in horses occurs as part of generalized septic disease, and as a complication of trauma, penetrating injury, or surgery.

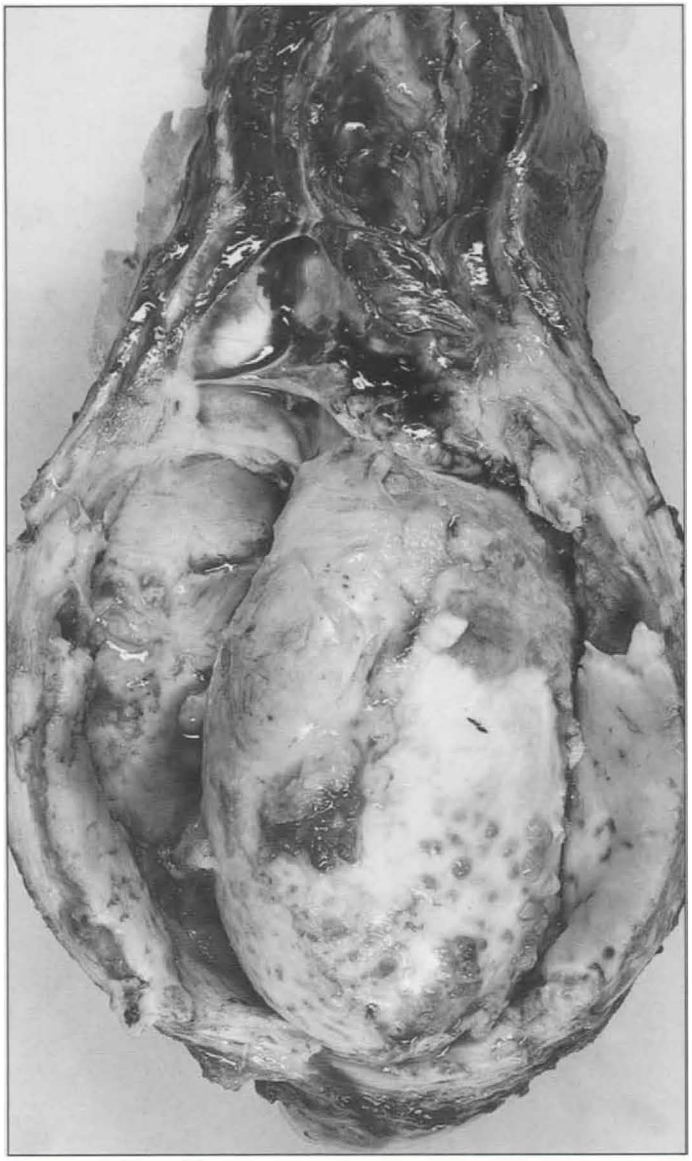

Figure 5.30 Orchitis and periorchitis in a bull, caused by *Brucella abortus*. Fibrin is adherent to tunica vaginalis, epididymis, and testis.

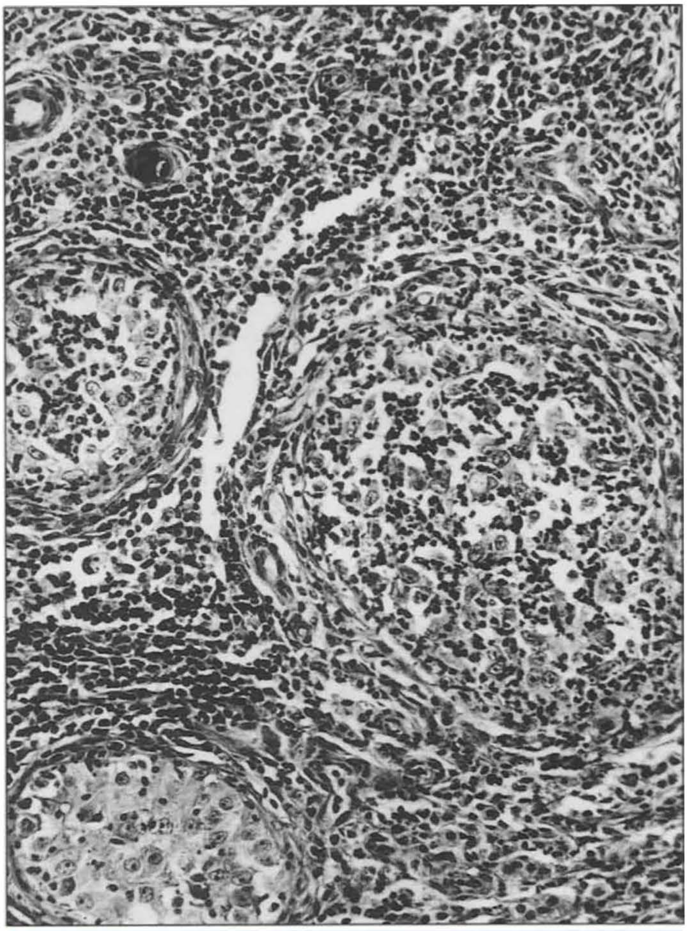

Figure 5.31 Orchitis in a dog, caused by *Escherichia coli*. There are interstitial and intratubular infiltrates of macrophages, neutrophils, and lymphocytes.

Orchitis in small ruminants

Nodular orchitis occurs in sheep pox, and chronic interstitial orchitis, but not epididymitis, has been observed in rams infected with the *Visna/maedi virus*. Sporadic testicular abscesses result from infection with *Arcanobacterium pyogenes* and *Corynebacterium pseudotuberculosis*. In bucks, orchitis, which in many respects resembles brucellosis in bulls, may result from infection with *Brucella melitensis*. Orchitis also occurs in breeding goats as a component of besnoitiosis. Orchitis in rams occurs mostly in association with epididymitis.

Orchitis in dogs and cats

In dogs, orchitis is usually accompanied by epididymitis. Intranuclear and cytoplasmic inclusions are found in the Sertoli cells in mature dogs with distemper. The majority of seminiferous tubules degenerate, and inflammation occurs in a few tubules. Penetrating wounds of the scrotum may occasionally be implicated in the pathogenesis of epididymoorchitis in the dog, but the commonest route is by reflux along the deferent duct from the bladder, urethra, or prostate of infection, chiefly by *Escherichia coli*, *Proteus vulgaris*, and other miscellaneous organisms. An acute inflammatory response in either the epididymis or testis is usually suppurative, with the formation of one or more abscesses (Fig. 5.31). The tunica vaginalis may be involved by extension of the inflammatory process, and fistulation through the scrotal skin to the exterior may occur. Alternatively, dogs will self-traumatize the scrotum and cause a fistula to develop. Acute inflammation usually is centered on the ducts, with degeneration and desquamation of the epithelium, and edema and mononuclear cell infiltrates in the surrounding stroma. Healing occurs with dense scarring, which in the epididymis will cause some tubular obstruction with spermatocele formation. Subacute and chronic inflammatory lesions present no special features. The affected testis is usually firm, small, and irregular, although the epididymis may be enlarged and woody. Lymphocytes and plasma cells now predominate and fibrosis is well developed. Neutrophils predominate in the lumen of the epididymal tubules.

Other bacterial causes of orchitis in dogs are *Brucella canis* and *Burkholderia pseudomallei*, both of which are associated with epididymitis (see below). A familial occurrence of interstitial, lymphocytic orchitis, associated with testicular atrophy and reduced fertility, has been observed in inbred Beagle dogs with lymphocytic thyroiditis; immune factors have been implicated.

Orchitis in **cats** is very rare and may be a manifestation of feline infectious peritonitis. Periorchitis is also a presenting complaint in feline infectious peritonitis and in traumatic injury to the scrotum; orchitis can be a sequel to these conditions.

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Epididymitis

Inflammation of the epididymis is one of the more common inflammatory diseases of the genitalia, but frequently it is diagnosed clinically as "orchitis." Regardless of cause, damage to the epididymal duct is complicated by the formation of sperm granulomas and subsequent development of epididymitis. The exception is in prepubertal animals where there are no spermatozoa; in these cases, true abscesses occur. Epididymitis in the absence of sperm granulomas is possible in adults, but it usually is a subclinical or early event.

Epididymitis is often *infectious*, and infectious disease frequently causes a spectrum of lesions, including inflammation of the accessory genital glands. The effects of epididymitis are so much more dramatic than prostatitis, vesicular adenitis, or ampullitis that these latter manifestations are often overlooked. *Sterile epididymitis* does occur, and is caused by congenital ductal anomalies, adenomyosis, trauma, and the reflux of urine.

Bacteria cause most infections of the epididymis, although viruses such as *Equine arteritis virus* and others are reported to induce epididymitis.

Primary infection with *Brucella* spp. in each species results in epididymitis. *Brucella ovis*, *B. canis*, *B. melitensis*, and *B. suis* are especially virulent for the epididymis. It is assumed that the infection is systemic and the bacterium localizes in the epididymis.

Direct infection of the epididymis by penetrating injury is a rare event. Secondary infection from periorchitis, or peritonitis is an occasional possibility.

Almost all species develop infection of the epididymis by the ascending route. This has been studied in the ram where *Actinobacillus seminis* and *Histophilus somni* (*Haemophilus somnus/Histophilus ovis*) are common isolates. While the exact mechanism is not known, it seems that preputial organisms migrate to the accessory genital glands and infect the epididymis by retrograde movement. The privileged environment of the lumen of the epididymal duct allows the organisms to infect the organ and incite damage. The formation of sperm granulomas means that the reproductive potential of the affected side is lost. Complete return to normal is rare. In the dog, the sequel of self-trauma of the scrotum, and systemic affects of infection with endotoxin-producing bacteria such as *Escherichia coli* further complicate epididymitis by causing systemic illness.

Local immunity to prevent epididymitis is not likely to be achieved in the short term. The epididymis has no natural local immune system of antigen receptors, nor known recirculation of immunocytes, and no local plasma cell population. Some aggregates of lymphocytes are occasionally seen in otherwise normal animals. After infection, the epididymis must develop a local immune system, but unfortunately, the damage is usually so extensive, and the sequel so severe, that the response is too late. Even so, the epithelial cells do have the ability to express MHC I and II, and lymphocytes and plasma cells can be recruited after challenge.

Once initiated, the course of epididymitis is variable. The acute stage with edematous enlargement may be followed by abscess and sperm granuloma formation, sometimes with perforation, periorchitis and peritonitis, and increasing fibrosis.

Macroscopically, an increased epididymal size and dissymmetry in shape is apparent especially in unilateral cases in comparison with the contralateral side. Fibrinous and then fibrous adhesions may be present between affected epididymis and adjacent tunics. Consistency will depend on duration of inflammation and the development of sperm granulomas. In chronic epididymitis, the marked increase in fibrous tissue both within the epididymis and between the epididymis and tunics results in atrophy of epithelial elements and the recognizable epididymis becomes hard and nodular, perhaps enclosing dilated remnants of epididymal duct (Fig. 5.32). Distinct hard "sperm stones," the end products of spermiostasis, may be present within such ducts. Concurrent testicular atrophy will also result in the epididymis appearing disproportionately large in relation to the testis.

Histologically, affected epididymal ducts contain fibrin, neutrophils, and spermatozoa in various stages of disintegration, damaged epithelium, macrophages, and multinucleate giant cells, many of which contain spermatozoa. Other features of sperm granuloma, with prominent interstitial accumulation of lymphocytes and plasma cells, become apparent (Fig. 5.33). Epithelial hyperplasia, with the development of intra-epithelial lumina has been observed in most domestic species in association with epididymitis, but such lumina also occur in noninflammatory lesions. In chronic epididymitis, squamous metaplasia of epithelium may also occur in affected ducts in association with progressive fibrosis, and there may be hypertrophy of smooth muscle surrounding ducts.

Epididymitis may also be caused by sperm granulomas resulting from congenital ductal anomalies, adenomyosis, trauma, and infections.

Epididymitis in bulls

Infectious and immunologic causes of epididymitis in bulls are largely the same as those for orchitis, but bulls develop a specific infectious epididymitis, so-called epididymitis-vaginitis ("epivag"), first described in Kenya and later in South Africa. Lesions in the bull consist of initial soft swelling of the epididymis with subsequent enlargement and fibrosis. Associated lesions are "abscess" formation, tunic adhesions, ampullitis and vesicular adenitis, and testicular degeneration. Sometimes, however, the vesicular glands only are

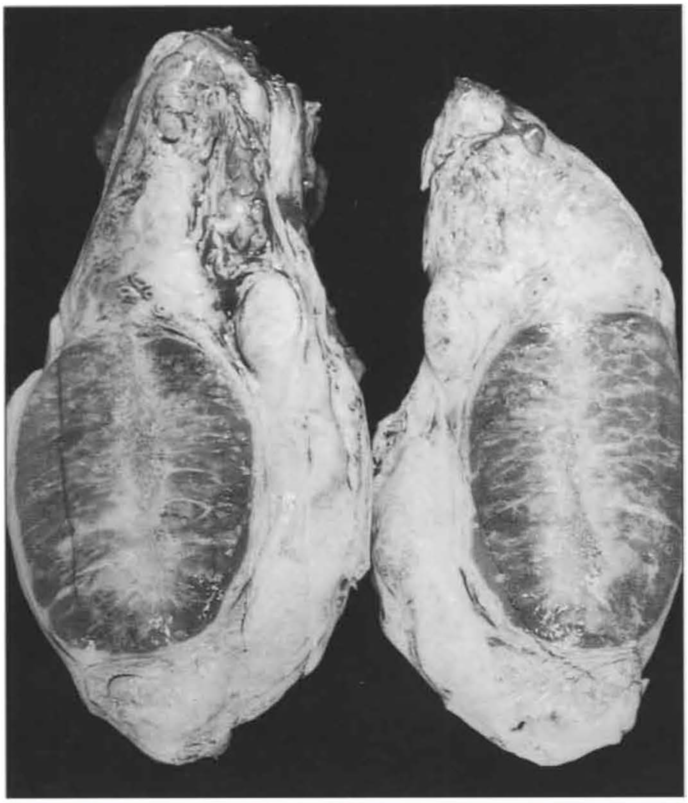

Figure 5.32 Chronic epididymitis in a bull, with marked epididymal fibrosis, adhesion of the tunica vaginalis, and marked testicular atrophy.

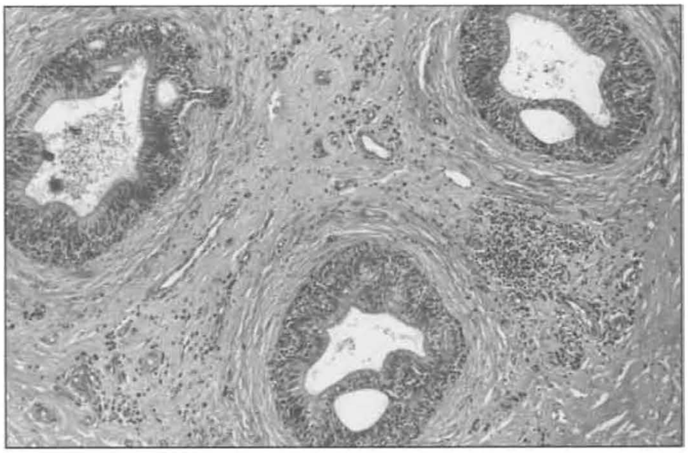

Figure 5.33 Chronic epididymitis in a ram, with interstitial fibrosis and epididymal epithelial hyperplasia and intraepithelial lumina formation.

affected. The possible role of a herpesvirus in the pathogenesis of "epivag" is discussed in Vol. 3, Female genital system.

Brucella abortus rarely causes epididymitis in the absence of orchitis. *Actinobacillus seminis*, a frequent cause of epididymitis in rams, has been isolated from the semen of a bull with bilateral epididymitis. Epididymitis has been observed in bulls with vesicular adenitis induced by inoculation of *Mycoplasma bovigenitalium*, but the roles of mycoplasmas and chlamydias in causing epididymitis await clarification. Epididymitis may accompany orchitis, periorchitis, and testicular degeneration in cattle, sheep, goats, horses, and dogs infected with *Trypanosoma brucei*.

Epididymitis in small ruminants

Epididymitis is of particular importance in rams, in which it is a frequent and serious cause of reduced fertility. Although one of two organisms *Brucella ovis* or *Actinobacillus seminis* are usually associated with the condition, many other bacteria, such as *Histophilus somni*, *Mannheimia haemolytica*, *Escherichia coli*, and *Arcanobacterium pyogenes* have been isolated from cases of epididymitis. Whereas *Brucella ovis* is a cause of epididymitis in mature rams, other gram-negative pleomorphic organisms are found more commonly in epididymitis in virgin rams, suggesting the existence of two separate disease entities, dependent upon sexual experience of the animal.

Ovine epididymitis caused by *Brucella ovis* is an important cause of reduced fertility in many countries and has been extensively studied. Progression of the disease is very slow, from a local infection persisting in the exposed mucous surface for 1 month, to a regional one involving adjacent lymph nodes, leading to bacteremia. The bacteremic stage appears to subside after about 2 months, but organisms localize in the genital tract, spleen, kidney, and liver where they persist for an indefinite period. Following experimental infection, neither gross nor microscopic lesions are seen in organs other than genitalia.

In approximately 90% of epididymides with lesions caused by *Brucella ovis*, the epididymal tail is involved, and lesions in this location probably occur in all epididymides infected with this organism. Initial localization of the bacteria produces edema, and lymphocytic and macrophage infiltration. Later, neutrophils are added when spermatozoa enter the interstitium. Early epithelial changes include hyperplasia and hydropic degeneration, with the formation of intraepithelial lumina (Fig. 5.33). At the same time there is increasing fibrosis in interstitial areas. The combination of fibrosis and epithelial hyperplasia obstructs the lumen and causes spermiostasis. These changes develop over many months, and large numbers of organisms are excreted in the ejaculate. Subsequent events depend on the extravasation of spermatozoa and formation of sperm granulomas. The tail of the epididymis in these cases may be enlarged 4–5 times (Fig. 5.34), and the lesion is often bilateral. If the extravasated spermatozoa enter the cavity of the tunica vaginalis, adhesions will result and testicular degeneration increases. Unlike brucellosis in the bull, there is no primary orchitis. Lesions in the deferent duct similar to those in the epididymis may occur, but are not associated with sperm stasis or leakage. There is pronounced epithelial hyperplasia, with thickening and folding of the wall, and the lamina propria is densely infiltrated with lymphocytes, plasma cells, and histiocytes. Unfortunately for control purposes, many rams do not develop detectable gross lesions or they develop them only late in the course of the disease. Identification of *B. ovis* in histological sections is difficult, but can be aided by immunohistochemistry.

Actinobacillus seminis and related strains of the so-called gram-negative pleomorphic organisms also induce epididymitis (Fig. 5.35). These bacteria consist principally of *Histophilus somni* (*Histophilus ovis*) and *Actinobacillus seminis*. Both *H. somni* and *A. seminis* are temporarily resident in the prepuce and become opportunistic pathogens by ascending infection, under appropriate conditions, such as those resulting at puberty from elevated levels of LH and FSH releasing hormones. The pathogenesis of *E. coli* epididymitis in rams probably is similar. Typically, these are acute infections that occur mostly in young rams, and there may be severe and diffuse periorchitis. In epididymitis caused by *Actinobacillus seminis*, there is abscessation of one

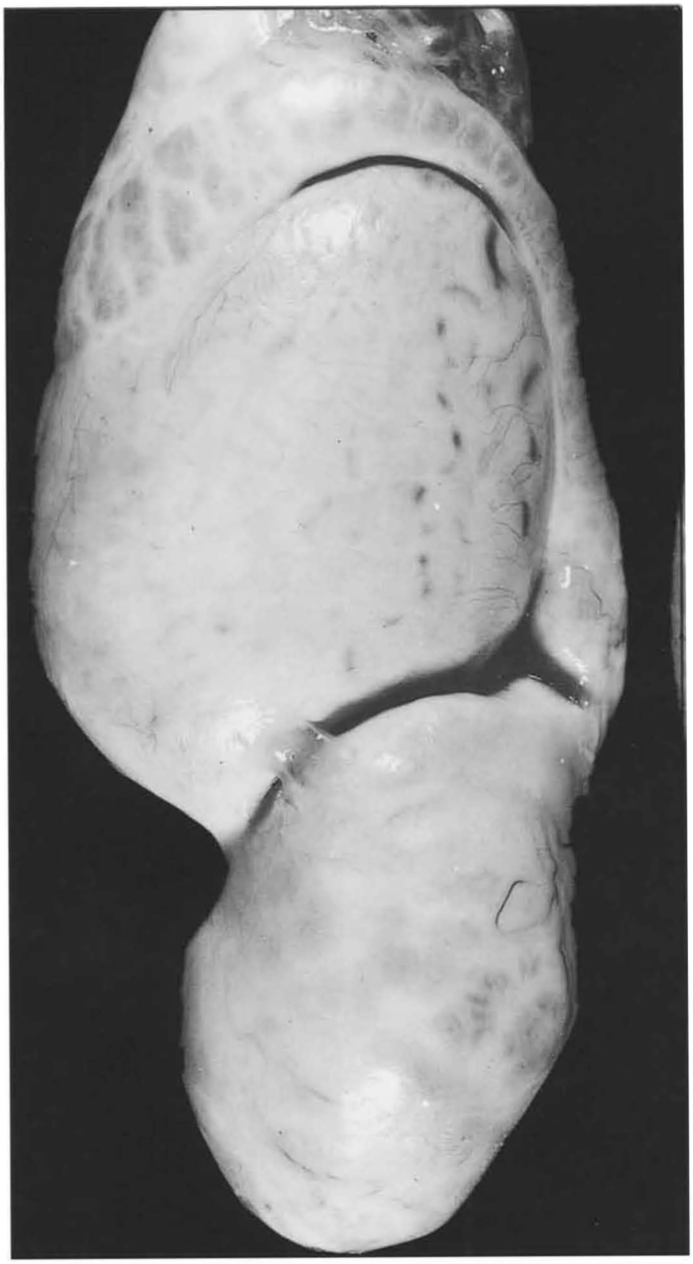

Figure 5.34 Chronic epididymitis in a ram, caused by *Brucella ovis*. The epididymal tail is enlarged with fibrosis and sperm granulomas (not visible). The testis is also atrophic.

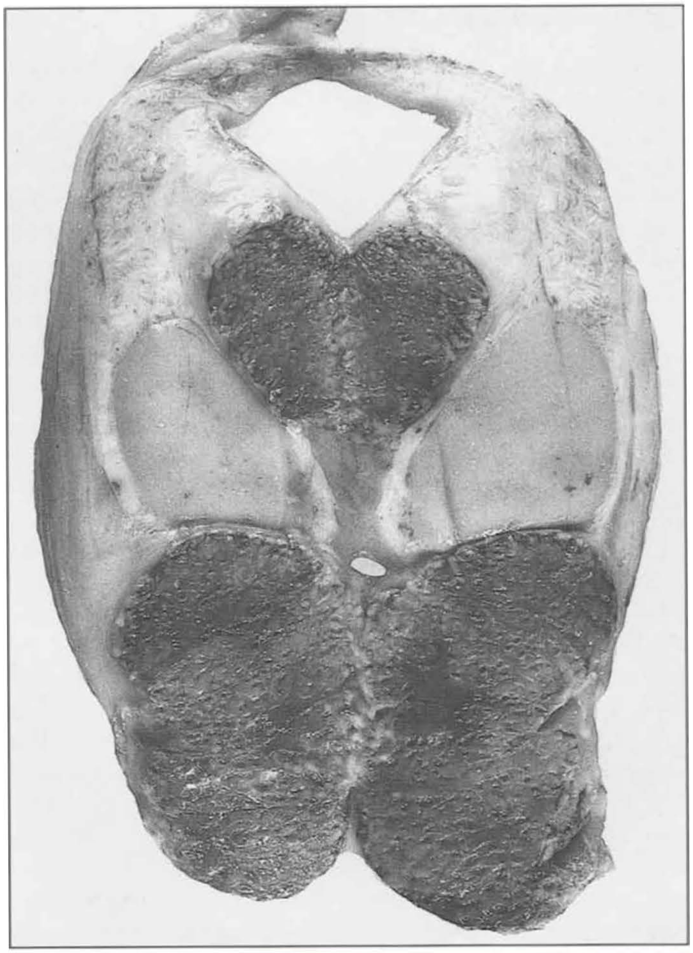

Figure 5.35 Acute epididymitis caused by *Actinobacillus seminis* in a ram. (Courtesy of KL Hughes.)

or both epididymides, and these may fistulate through the scrotal wall. Histologically, the initial epididymal lesion is similar to that of *Brucella ovis*, being characterized by intraepithelial lumina. In the chronic form of the disease seen in older rams, the epididymides are enlarged and fibrotic, and the testes are atrophic. The vesicular glands or prostate also may be affected. Experimental inoculation of rams by various routes with *Actinobacillus seminis* has shown that part of or the entire genital tract may become infected, but that the epididymis is most constantly involved.

Lesions in the epididymis caused by *Histophilus somni* are similar. The disease occurs sporadically, is sometimes accompanied by a febrile response, and the most frequent lesion is a large multilobulated abscess affecting one epididymis. The abscess cavities contain much fluid and green-yellow flocculent material, often with lumps of necrotic material. Fibrous adhesions of the tunics also occur. In early cases with epididymal abscessation, the deferent duct is usually patent and contains pus and spermatic fluid but in older cases that part of the epididymis adjacent to the deferent duct is usually empty and has grossly fibrosed walls. Microscopically, the early response is severe suppurative inflammation involving the walls of epididymal tubule. In appropriately stained sections, numerous bacteria can be demonstrated in intraluminal necrotic debris. Subsequently, infection may extend to the stroma, so that in chronic cases there is extensive interstitial epididymitis with much fibrosis. As with brucellosis, the testis is only involved secondarily.

Epididymitis is less studied in **bucks** than in rams. *Brucella melitensis*, *Actinobacillus seminis, Staphylococcus aureus*, *Escherichia coli*, or *Pseudomonas* spp. may be isolated from lesions, and there is a report of possible *Brucella ovis* epididymitis in an angora goat.

Epididymitis in boars

In boars, the macroscopic lesions caused by *Brucella suis* infection are quite variable. Single or multiple "abscesses" are frequent in the epididymis, but less so in the testes, which may be enlarged or atrophic. Enlargement of the vesicular glands due to localization of *B. suis* may also occur, and abscessation, perhaps seen only microscopically, occurs in the vesicular glands and prostate and bulbourethral glands.

Epididymitis in dogs and cats

In male dogs infected with *Brucella canis,* there is epididymitis, prostatitis, scrotal dermatitis, and testicular atrophy. These changes can be unilateral. Infected animals are bacteremic and the organism can persist in certain tissues, particularly the prostate, for many months, but recovery can eventually occur and recovered dogs are immune to reinfection. Venereal transmission to females by infected males can occur, although the organism is not consistently isolated from semen. Scrotal swelling is apparent 1–2 weeks after experimental intravenous inoculation, or 3–5 weeks after oral infection. Such swelling is caused by accumulation of fibrinopurulent exudate in the cavity of the tunica vaginalis. Scrotal ulceration is the result of persistent licking of the scrotum caused by the pain of epididymitis. Testicular abscesses are seldom if ever observed but testicular necrosis (as observed in the bull) may occur rarely, and is accompanied by marked fibrous thickening of tunics.

Microscopically in such cases, there is coagulative necrosis with a predominantly mononuclear, reparative response in peripheral areas where necrotizing vasculitis and associated thrombosis may also occur. More frequently, however, histological findings are those of interstitial epididymitis and prostatitis, and testicular atrophy. Lymphocytic infiltration of epididymal stroma is variable. Fibrosis may be extensive but, in contrast to brucellosis in other species, obliteration or stricture of ducts is unusual. Lymphocytes, neutrophils, and macrophages are present in the epididymal duct. In chronic cases, there is marked enlargement of the epididymis, especially the tail, with possible spread of inflammation to the deferent duct. As well as containing inflammatory cells and an increased percentage of abnormal spermatozoa, the ejaculate of male dogs with chronic brucellosis contains spermatozoa agglutinins, and the resulting infertility may, in part, be mediated by isoimmune reactions resulting from the heightened nonspecific phagocytic activity of inflammatory cells attracted to the sites of bacterial growth in the epididymis. *Brucella suis* also may cause spontaneous granulomatous epididymitis and prostatitis in the dog.

Escherichia coli or other gram-negative bacteria cause most sporadic cases of canine epididymitis (Fig. 5.36). The epididymitis caused by these agents is often seen as an acute disease and the dogs may be systemically ill with endotoxemia. Scrotal swelling and mutilation is relatively common. Following infection of dogs with *Burkholderia pseudomallei*, epididymitis, orchitis, and scrotal edema may occur in association with pyrexia, depression, swelling of one or more limbs, and lameness. Macroscopically, the epididymides are enlarged to perhaps 3–4 times normal size, are firm and hemorrhagic, and may contain small abscesses or necrotic foci. The testis and deferent duct may also be involved.

Mycoplasma canis can cause urinary tract infection in dogs, with subsequent purulent epididymitis and prostatitis.

Mycotic epididymitis caused by *Rhodotorula glutinis* or *Blastomyces dermatitidis* produces granulomatous epididymitis with a reaction pattern similar to disease in other tissues. The complication of sperm granulomas adds to the granulomatous nature of the disease.

Epididymitis occurs in sexually mature dogs with *canine distemper.* The histological appearance is similar to that seen in epididymitis of other causes, but, in addition, cytoplasmic and intranuclear inclusions are present in the epithelial cells. Some care is necessary to differentiate the specific inclusions from the eosinophilic cytoplasmic bodies normally present in the head of the epididymis and from the intranuclear bodies that are present in normal dogs; immunohistochemistry will differentiate inclusions in question.

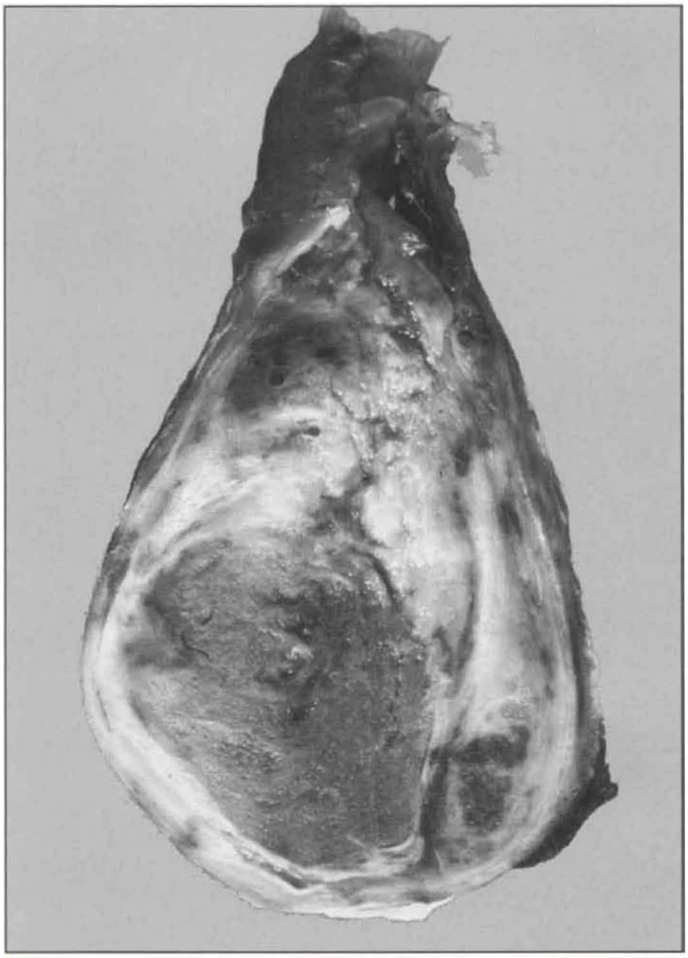

Figure 5.36 Severe **epididymitis and periorchitis** in a dog. The tail of the epididymis is large and edematous, and there is fibrin and exudate in the cavity of the tunica vaginalis.

Epididymitis is exceedingly rare in **cats**. It has been seen as fibrinonecrotic vasculitis and epididymitis in association with genital involvement in feline infectious peritonitis. Ascending epididymitis also occurs.

Epididymitis in stallions

In the stallion, migrating strongyle larvae are considered a likely cause of epididymitis and sperm granuloma of the epididymis; adenomyosis has been shown to be a further cause. Rare sporadic cases of bacterial epididymitis are also reported. *Streptococcus zooepidemicus* has been isolated from such cases.

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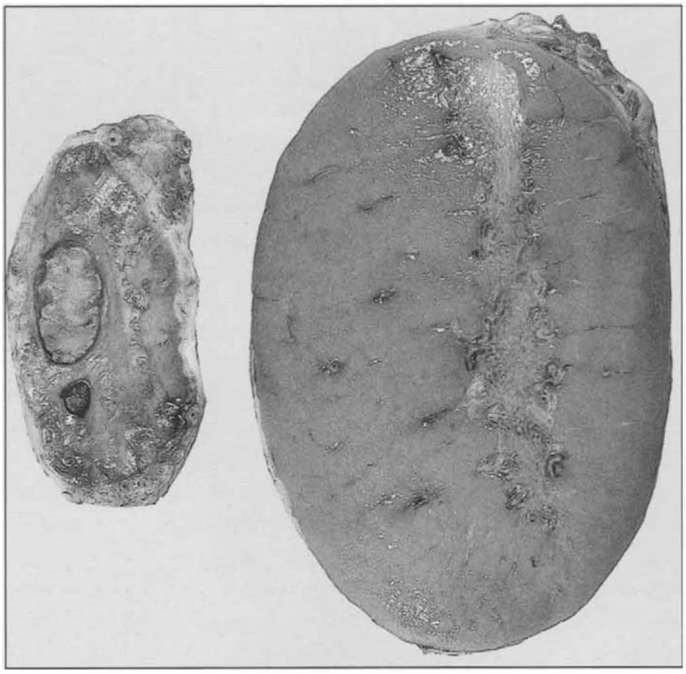

Figure 5.37 Atrophy and infarction of the left testis of a bull due to compression of the spermatic cord while removing tissue for biopsy. The right testis is normal.

Circulatory disturbances

The spermatic artery is very long because of the coiled portions proximal to the testis. The degree of coiling varies between species, being quite pronounced in ungulates, in which the spermatic artery is several meters long. Testicular blood flow is low in relation to metabolic needs, and hypoxia develops quickly if increasing testicular temperature increases metabolic demand or if blood flow is impaired. By the time the artery penetrates the tunica albuginea, pulsatile flow is almost eliminated and the structure of the vessel changes. The diameter enlarges, the wall becomes thinner, and the elastic fibers are reduced. Increased intratesticular pressure will have dramatic effects on circulation.

The interstitial tissue of the testis is richly supplied with *lymphatics*. In addition to the usual role in fluid exchange, they probably assist in transportation of hormones between interstitial endocrine cells and the tubules. Edema of the testis occurs after trauma and is a frequent early change in orchitis. Although the tunica albuginea is relatively indistensible, some enlargement can occur and serous fluid drips from the cut surface. There is distinct separation and dilation of tubules, diffuse vacuolation of germinal epithelium, and dilation of lymphatics.

Hyaline degeneration of the walls of arterioles is associated with wedge-shaped areas of fibrosis in the testes of old bulls, but the precise cause of this lesion is not clear. In old dogs, hyaline degeneration of both arterioles and arteries accompanies testicular degeneration. Initial lesions occur in the tunic and parenchyma, but larger vessels in the spermatic cord are subsequently involved. Focal areas of infarction may occur as a result of severe vascular lesions. Atheromatous lesions are rare; in dogs they may indicate hypothyroidism or diabetes mellitus.

Thrombosis of testicular arteries, usually of unknown cause, is seen occasionally in bulls. Such thrombi are present in both the parenchyma and tunics and may be partially mineralized and sometimes occluding. The mild degenerative changes in germinal epithelium in association with thrombi emphasize the good collateral circulation. In experimental *Trypanosoma vivax* infection in sheep, thrombosis of testicular vessels probably contributes to testicular degeneration. Subsequent infarction and necrosis may sometimes occur. Occluding venous thrombosis has been observed in the testes of rams. Some had a concurrent varicocele; testicular degeneration associated with such thrombi was mild.

Inflammation of the testicular artery occurs frequently in the horse. The known causes are migrating strongyle larvae and *Equine arteritis virus*, but the cause is not established in many cases. The usual morphologic manifestations in testicular tissue include focal infiltration of lymphocytes and degeneration of seminiferous tubules adjacent to the inflamed arteries and arterioles. The inflammatory reaction is rarely of such a severe degree as to cause thrombosis and infarction. Horses infected with *Equine arteritis virus* may have an acute lesion with a necrotic vasculitis, edema, and hemorrhage. Chronic cases will have lymphocytic vasculitis and perivasculitis.

A striking *vasculitis* with marked interstitial epididymitis, orchitis, and testicular degeneration occurs in malignant catarrhal fever in buffaloes and bulls in association with generalized vasculitis. Dogs may develop vasculitis as part of a localized or generalized polyarteritis, and it may occur in systemic necrotizing vasculitis ("Beagle pain syndrome").

Occlusion of the testicular artery with resulting ischemia of the testis may result from torsion, contusion of the spermatic cord, inappropriate placement and/or hypotension during surgery, or the use of an emasculatome for castrating young lambs and calves (Fig. 5.37). Experimentally, destruction of germinal epithelium can be demonstrated after ischemia of more than 1 hour. Necrosis of testicular parenchyma follows after 4–6 hours, although spermatozoa are relatively resistant to lysis and may retain their staining characteristics for weeks or months. Following ligation of the spermatic artery in prepubertal rams, however, revascularization of the tunica albuginea occurs by penetration of capillaries from the epididymal arteries. Islets of seminiferous tubules associated with the tunic (Moskoff's islets) may thus survive, and considerable regeneration occurs during the ensuing months. As the regenerated tubules lack a normal drainage system, there is spermiostasis and ultimately degeneration.

Torsion *of the testis is rare unless there is incomplete descent.* The stallion appears to be the exception, as spontaneous torsion is a recognized cause of "colic." In dogs especially, testicular neoplasia is often also present, to provide sufficient weight to maintain the torsion. The usual clinical presentation is acute abdominal pain, and the testis is blackened due to venous infarction, and is sometimes unidentifiable as testis. Spontaneous torsion of the cryptorchid testis of boars is seen commonly at slaughter. Torsion with complete ischemia and infarction will cause complete loss of spermatogenesis of the affected testis. Correction of torsion in laboratory animals and humans can save the testis, but some continue to be aspermatogenic even if infarction does not occur. Interstitial endocrine cells and Sertoli cells retain their ability to function, but reactive oxygen species may induce germ cell apoptosis and loss.

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Neoplasms of the testis and epididymis

Testicular neoplasms are most commonly found in the dog. There is no satisfactory explanation for this, but intact dogs are allowed to live as long as they are able, and they tend to be watched closely. Male cats tend to be castrated early and fewer of those that are intact live to old age. Neoplasms arise in all species sporadically, and with the exception of the dog, it is possible to predict the histologic type of neoplasm based on species and age. Seminoma is the common tumor of the aged horse, and teratoma is more common in the young. Cats, boars, rams, and bucks rarely get testicular neoplasms, and both seminoma and Sertoli cell tumor are reported. Bulls are more likely to have interstitial cell tumors, but Sertoli cell tumors are also reported.

The three main testicular neoplasms of dogs are the Sertoli cell tumor, the interstitial (endocrine) cell tumor, and the seminoma. The fourth most common, but still rare, is a mixed germ cell and stromal cell neoplasm. Multiple types of neoplasia may be found in one testis. *Most primary testicular neoplasms in dogs are benign.* Exceptions are rare, but malignant Sertoli cell tumors and seminomas are reported. Identification of metastasis in lymphatics, spermatic cord, lymph node, or distant sites is the only way to determine that the neoplasm is malignant; there are no good cytological or histologic markers of malignancy.

Most neoplasms cause enlargement of the testis. In general, seminomas are white, soft, and usually bulge on cut section. Sertoli cell tumors induce fibrous tissue so they are white and are tough. The interstitial cell tumor is yellow, often contains areas of hemorrhage, and is soft. *Sertoli cell tumors, and rarely interstitial cell tumors, may produce a hyperestrogenism syndrome and feminization.* In dogs, this is usually manifested by attractiveness to other male dogs, gynecomastia, and alopecia. Prostatomegaly and squamous metaplasia of the prostate also occur. Affected animals return to normal after removal of the neoplasm. The signs are not always associated with estrogen production and not all dogs will have a raised serum estrogen concentration. In these instances, inhibin secretion by the neoplastic Sertoli cells inhibits the secretion of FSH and LH by the pituitary, which, in turn, inhibits testosterone production and presumably alters the ratio of estrogen to testosterone. Some animals will develop bone marrow suppression, and poorly responsive pancytopenia. Feminization is much more common when the neoplasm is larger, and therefore is more common in cryptorchid dogs. It is also in these dogs that the unfortunate sequel of testicular torsion can occur.

Testicular tumors are seen mostly in mature and old animals; the occurrence of interstitial cell tumors in dogs is especially age associated. Canine testicular tumors are found more frequently in the right than in the left testis, and this is also true for the cryptorchid testis.

Other neoplasms of the testis include the *mixed germ cell–sex cord–stromal neoplasms* that have been reported in the horse and dog, *rete adenoma and adenocarcinoma*, and *embryonal carcinoma* in the horse.

Metastasis of neoplasms to the testis is rare. Lymphoma in the horse, dog, and bull, and hemangiosarcoma in the boar and dog are reported. The list will no doubt increase in length if pathologists routinely examine the testes at all necropsies.

Epididymal neoplasms are exceedingly rare. Ectopic interstitial cell tumors have been described in the cat. Lymphoma or any other systemically metastatic neoplasm may affect the testis and epididymis.

Gonadal stromal tumors

Interstitial (Leydig) cell tumor

Interstitial cell tumors are derived from the endocrine cells of the testicular interstitium. They are grouped with Sertoli cell tumors in being derived from tissue of the sex cords or stroma. Lesions that are less than 1 cm in diameter may be hyperplasia, although this distinction may have little biologic significance.

In the dog, interstitial cell tumors are found chiefly in older animals. It also occurs in the bovine testis, is found in the older age groups, and mainly in Guernseys. In horses, the tumor develops almost exclusively in cryptorchid testis. There are very few reports of testicular neoplasia in boars, but interstitial endocrine cell hyperplasia and tumor are more common than the others.

Normal interstitial endocrine cells produce androgens, and some interstitial cell tumors of dogs have been shown to produce excess androgen, but *most tumors do not cause signs of hyperandrogenism.* Signs of hyperestrogenism have been observed in a few dogs with interstitial cell tumors and estradiol concentration in testicular venous blood of affected dogs may be elevated. The condition has been corrected by removal of the neoplasm. However, in dogs,

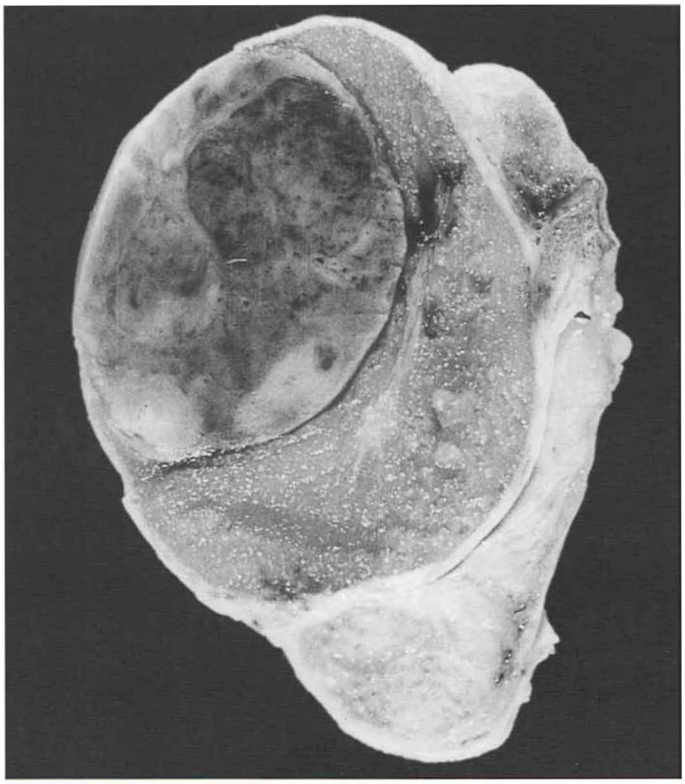

Figure 5.38 Intratesticular **interstitial cell tumor** in a dog. These are soft and tan, with regions of hemorrhage.

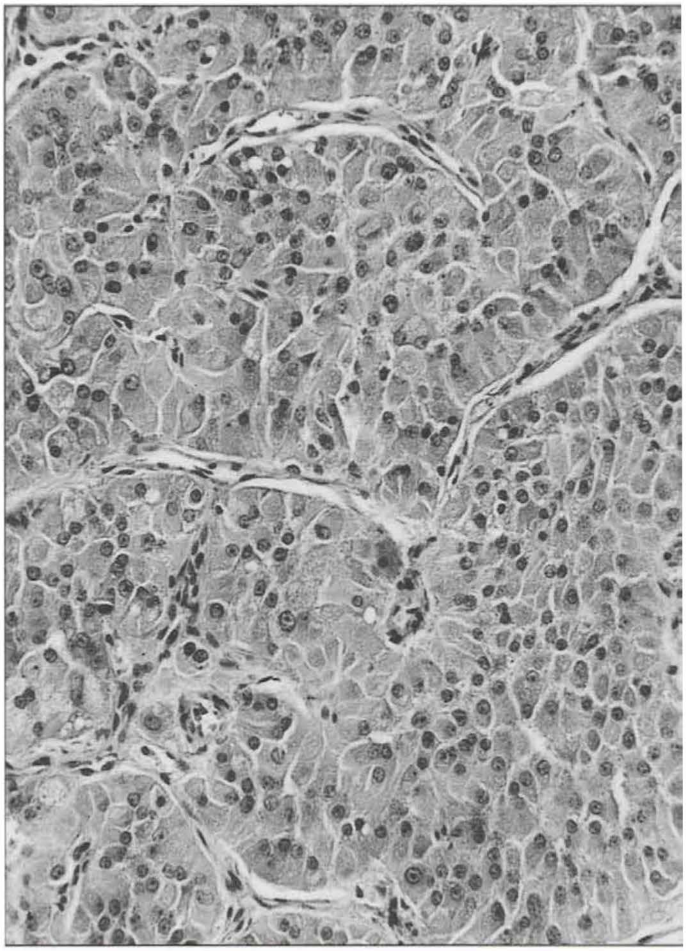

Figure 5.39 Endocrine pattern, with large polygonal cells packed into groups by a fine fibrous stroma, of an **interstitial cell tumor** in a dog.

a common association is with perianal gland neoplasia, tail gland hyperplasia, and prostatic enlargement, which suggests that secretion of excess androgens also occurs.

Interstitial cell tumors in stallions have been seen most often in undescended testes. They contain two cell types; the first is essentially a hypertrophic interstitial endocrine cell but the other is a pleomorphic fusiform cell with fibrillar, vacuolated cytoplasm and indistinct borders. Hormone determinations have not been reported on horses with these tumors, but viciousness, which was corrected by castration, has been observed.

A high incidence of telangiectasis of the liver, thyroid C (parafollicular) cell tumors, and infertility has been observed in Guernsey bulls with interstitial cell tumors, but it is not known whether the lesions are in any way interdependent or possess a common denominator.

Nodular hyperplasia is probably a preneoplastic change in some instances of interstitial cell tumor of the dog. The hyperplastic nodules occur principally in testes that have undergone senile atrophy and, although they may be macroscopically visible, the nodules are small, nonencapsulated, and consist of an increased number of interstitial endocrine cells in the intertubular stroma. The apparent diffuse hyperplasia of interstitial endocrine cells in cryptorchid and hypoplastic testes does not predispose to the development of tumors. In both nodular and diffuse hyperplasia, the hyperplastic cells are regular in form and size with increased acidophilia of the cytoplasm and without mitoses.

Interstitial cell tumors in the dog are often multiple, but may be solitary and unilateral or bilateral (Fig. 5.38). Commonly they are from 1 mm to 2 cm or more; only exceptionally are they large enough to increase the size of the organ, but they may lend the organ an irregularity in contour, the rounded bulge of the tumor being visible in an otherwise small, soft, atrophic testis. On cut surface, the tumors are well demarcated but only lightly encapsulated, spheroidal, and yellow. The tumor is predisposed to hemorrhage which causes dark discoloration, and to cyst formation in areas. Even a large tumor may be obviously composed of confluent nodules indicating multicentricity of origin, and this is readily apparent in the form of discrete tumors when they are small. The consistency is soft, there being little stroma, and the cut surface is slightly greasy.

Interstitial cell tumors grow slowly and expansively with surrounding compression atrophy, and often with a thin condensed capsule. They are not notably invasive. In bulls with interstitial cell tumors, semen production and fertility may be reduced.

Typically, the component cells in the dog are rather respectable interstitial endocrine cells, being round or polyhedral, with abundant cytoplasm that may be granular or vacuolar and that often contains yellow lipochrome pigment (Fig. 5.39). The neoplastic cells in the bull are not vacuolated and contain very little lipid. Sometimes and in some tumors, the cells have a more mesenchymal appearance, being spindle-shaped with indistinct cytoplasmic outline and a streaming arrangement. It is in such tumors especially that necrosis and cyst formation occur. The nuclei are regular in size and staining affinity, and mitoses are rare. The stroma is scant, supporting a capillary network, but occasionally suffices to give the tumor an endocrine appearance.

Intranuclear cytoplasmic invaginations or inclusions have been observed consistently in up to 15% of neoplastic cells in canine interstitial cell tumors. The inclusions, which are strongly PAS-positive and which are composed of smooth and rough endoplasmic reticulum, vesicles and lipid vacuoles, myelin figures, and disrupted membranous profiles, have not been demonstrated in other testicular tumors. Nuclei containing these invaginations are enlarged. Except where invaginations are present, the ultrastructural appearances of neoplastic and normal interstitial endocrine cells in the dog are similar.

Sertoli cell tumor

The Sertoli cell tumor is rare in domestic species other than the dog, but has been observed in the bull, horse, ram, and cat. These tumors often cause enlargement of the affected testis, and there is, sometimes, the development of a *feminization syndrome* in the host. The feminizing effect of these tumors is due to either their high content of estrogen or to secretion of inhibin. The degree of differentiation of the neoplastic cells will influence the content of hormone but, usually, it is larger tumors that are responsible for feminizing syndromes, which suggests that the mass of the tumor is related to the quantity of hormone elaborated. The attractiveness of affected dogs to other male dogs is well known, but a hyperestrogenism syndrome also is manifest as reduction of libido, female distribution of body fat, cutaneous and pilosebaceous atrophy leading to symmetrical alopecia, atrophy of testes and penis, an estrogenic form of mammary development, swelling of the prepuce, and hyperplasia or squamous metaplasia of the prostate, which may be accompanied by perineal hernia. Prostatic metaplasia can progress to the stage where quite large keratin accumulations fill glandular lumina. Enlargement of the seminal colliculus with partial obstruction of the urethral lumen may also accompany prostatic changes in these dogs.

As well as feminization, there may be *depression of bone marrow activity* with resultant clinical signs of hemorrhage caused by thrombocytopenia, anemia caused by blood loss and/or diminished erythrocyte production, and infection and fever associated with granulocytopenia. Recovery may follow castration and supportive therapy.

The gross appearance of a Sertoli cell tumor can be quite distinctive. The larger tumors are somewhat irregularly ovoid, lobulated and enclosed in a tense tunica albuginea (Fig. 5.40). The cut surface bulges and usually is white and quite firm or even hard, although sometimes it may be discolored or cystic. *The firmness of the tumor is due to the abundance of its stroma,* something the other two common types of tumor have in small amounts only.

Although metastasis of Sertoli cell tumor to the regional lymph node is unusual, local extension of neoplastic tissue into the testicular vein and associated lymphatics may result in hydrocele with massive swelling of the scrotum.

Histologically, Sertoli cell tumors may be of two types, either intratubular (with or without invasion as determined by penetration of the basement membrane) or diffuse. *There seems to be little correlation, however, between histologic type and metastasis.* Stroma is always plentiful, and it may be hyalinized. The stromal tissues are commonly arranged to provide a tubular pattern in which the neoplastic cells tend to palisade. Such arrangements to some degree can be found in most Sertoli cell tumors (Fig. 5.41A, B). In the early and well-differentiated tumors, the cells resemble normal Sertoli cells, being rather elongate with foamy acidophilic cytoplasm and small, basally situated, dark-staining nuclei. In less differentiated varieties, the cells are still elongate and possess eosinophilic cytoplasm, but the nuclei are elongate and pleomorphic and no longer basally located against the trabecular pole (Fig. 5.41A, B). In the less common form of the tumor, the cells show little or no tendency to palisade but are discrete and spherical with well-defined eosinophilic cytoplasm and some nuclear irregularity. Mitoses are sparse. Lipids are demonstrable in the neoplastic cells as large droplets and globules in more differentiated tumors and as fine droplets in the least differentiated tumors.

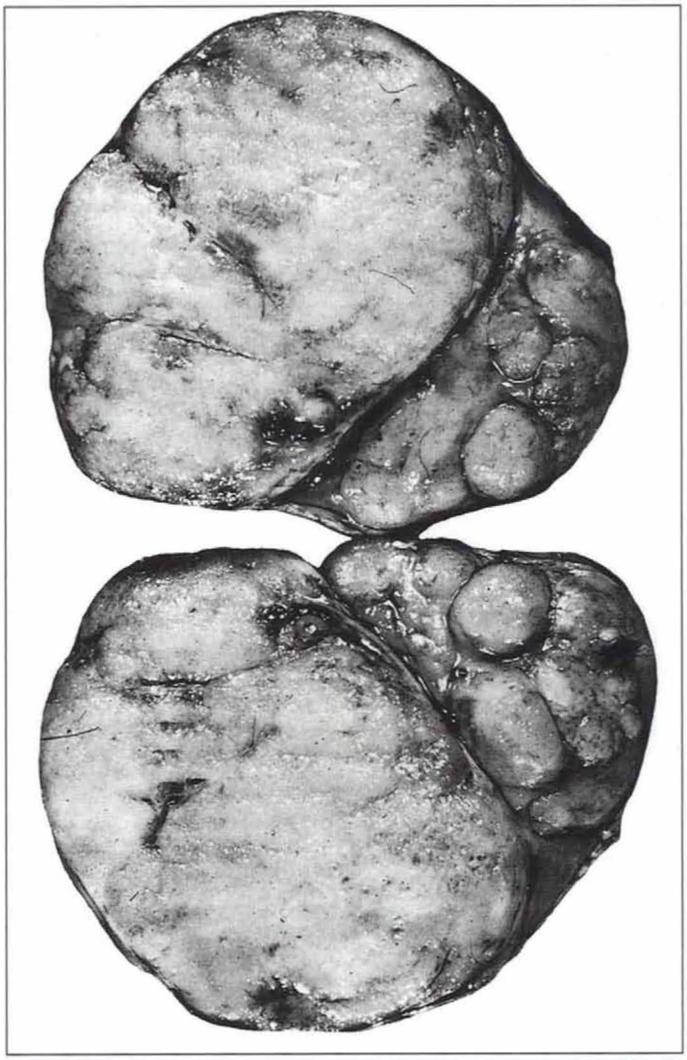

Figure 5.40 Malignant **Sertoli cell tumor** in a dog. Presence of metastasis is the only criterion to differentiate malignant from benign neoplasia.

Sertoli cells are reported to be the only cells of the testis that stain immunohistochemically with antibody to neuron-specific enolase, wherein the stain is intracytoplasmic. They stain diffusely intracytoplasmically with vimentin (as do interstitial endocrine cells). Cytokeratin stains are negative. There are varied reports of staining with other antibodies, including S100, melan A, and inhibin. Because of the conflicting results from different laboratories, it is not possible to generalize about their staining characteristics.

Ultrastructural examination of canine Sertoli cell tumors reveals that the characteristic specific intercellular junctions and crystals of

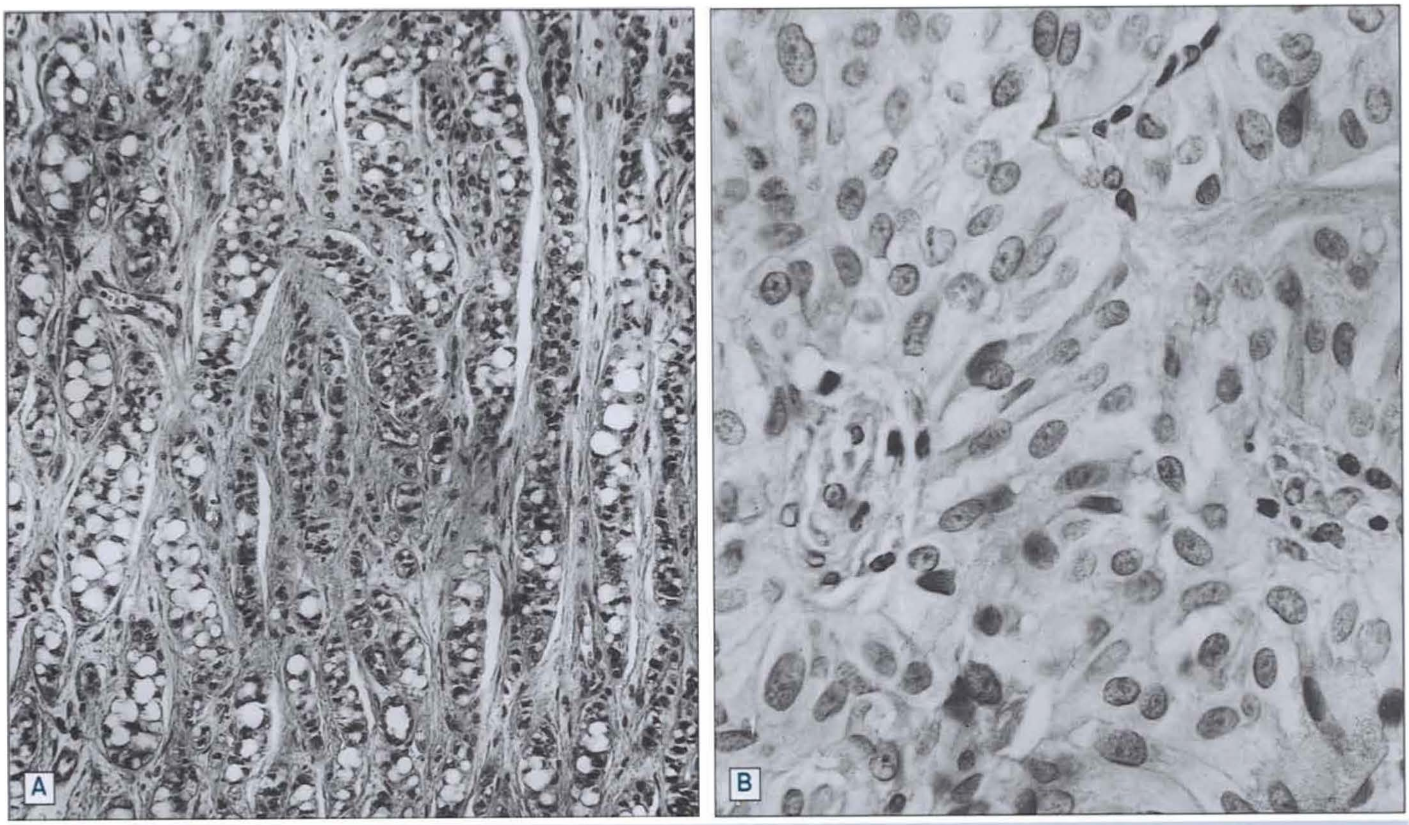

Figure 5.41 A. Histological appearance of **Sertoli cell tumor** in a dog showing tubular structures separated by abundant fibrous tissue. **B.** On higher magnification, the cells tend to bridge from one side of the tubular structure to the other.

Charcot–Bottcher do not occur in neoplastic cells. Nevertheless, a number of common features do persist so that these and the abundant intracytoplasmic organelles permit differentiation from interstitial cell tumors and seminomas. Prominent intercellular gap junctions have been observed in a Sertoli cell tumor in the dog and it is possible that these were induced by the hormonal activity of the tumor.

Early age of slaughter of most food animals precludes studies on true tumor occurrence with increasing age. However, in one study, in which **bulls** and buffaloes were kept until 9–14 years for draft purposes, 20 of 161 testes examined contained neoplasms; all but one were Sertoli cell tumors. Seven tumors were in undescended testes. In contrast to Sertoli cell tumors in the dog, those in cattle have been observed in newborn or young calves with sufficient frequency to suggest that impaired embryogenesis, possibly of genetic origin, might have some role in causation. In support of this suggestion is the presence, in some bovine Sertoli cell tumors, of laminated intratubular concretions resembling those seen in bovine testicular hypoplasia and cryptorchidism. Moreover the simultaneous occurrence of Sertoli cell tumor and epididymal aplasia has been observed. One described bovine Sertoli cell tumor was in a testis of an animal in which castration by the burdizzo method had been attempted 5 years previously. In general, the gross and microscopic appearance of Sertoli cell tumors in the bull resembles those in the dog (Fig. 5.42A, B). Metastasis of Sertoli cell tumor in the bull has not been observed, and there is no clear evidence of hyperestrogenism.

In **rams**, occasional Sertoli cell tumors, comparable to those in the bull, have been observed. Hyperplastic Sertoli cells occur in cryptorchid rams (Fig. 5.43), but whether these could potentially develop into neoplasms seems unlikely, given the rarity of the tumors.

One case of Sertoli cell tumor in a **stallion** involved the single descended testis; a ductal pattern of neoplastic Sertoli cells, which contained clusters of distinct hyaline bodies, was evident.

Germ cell tumors

Seminoma

Seminomas are common in canine testes and have also been observed in the stallion (and mule), ram, buck, and bull. They occur in *older animals* and are disproportionately common in cryptorchid testes. They arise from cells of the spermatogenic series, presumably from basal spermatogonia, and they are usually multifocal of origin in the affected testes. These tumors do not produce hormones. They are not often malignant, but are probably more so than either of the other two types. However, *they tend to be locally invasive* and there are no known factors to predict the metastatic potential of seminomas.

Seminomas often attain a size of 6 cm or more before removal, and by then the stretched tunica albuginea encloses neoplastic tissues only. Sudden enlargement of the testis, and pain caused by hemorrhage and necrosis in the tumor, are often the presenting signs in dogs. In all species, the sectional surface is coarsely lobulated by a few fine trabeculae, the color is usually white or gray-white, and the texture is soft to moderately firm (Fig. 5.44). If lightly squeezed, a milky fluid may exude from the cut surface. The texture and color closely resemble that of neoplastic lymphoid tissue.

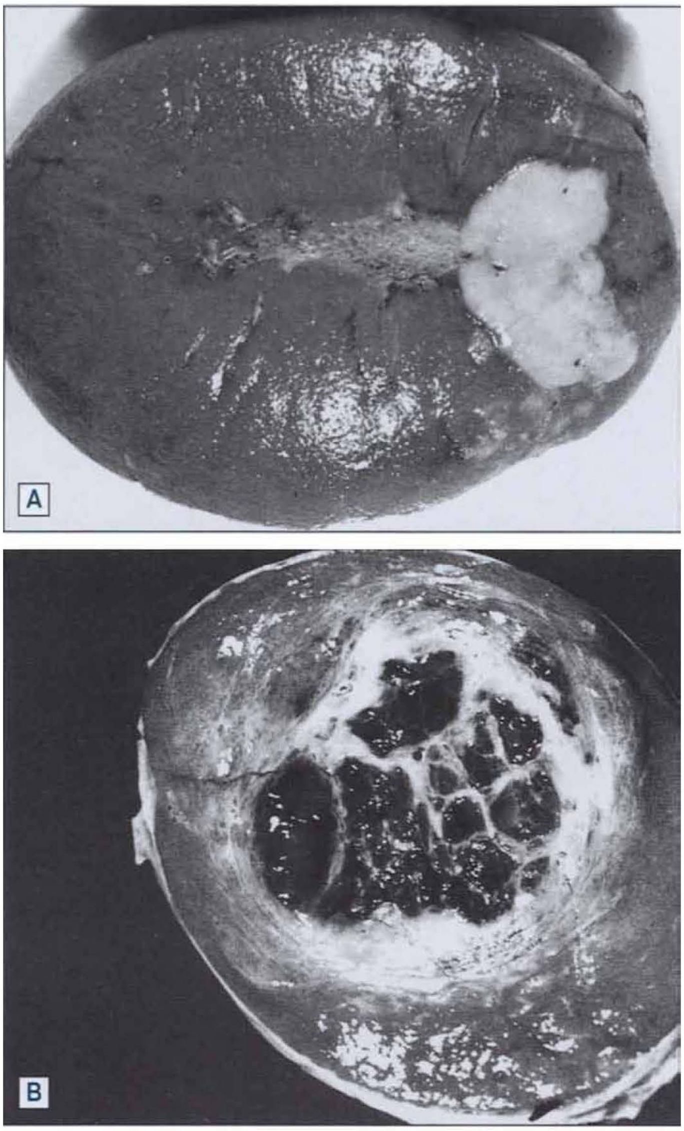

Figure 5.42 Sertoli cell tumor in a bull. Macroscopic appearance varies from (**A**) a solid to (**B**) a cystic type. (Both courtesy of J Comp Pathol.)

Microscopically, intratubular, and diffuse types are recognized. The earliest development of the tumor is intratubular and even in some large specimens intratubular growth is still evident and undoubtedly comprises one method of spread (Fig. 5.45A). Rupture of the tubules soon occurs and the growth becomes confluent, forming broad sheets of closely packed cells with scant supporting stroma. There is only slight cytological variation from tumor to tumor. The cells are large and polyhedral and fairly discrete, with a rim of visible cytoplasm that may be basophilic or eosinophilic (Fig. 5.45B). The nuclei are large and rounded and usually strongly chromatic with one or more large acidophilic nucleoli. In some tumors, the nuclei are larger and vesicular but still regular in shape, and the cells are closely packed with scant or invisible cytoplasm. In many seminomas, it is possible to find scattered mono- or multinucleate giant cells with abundant granular acidophilic cytoplasm. *Focal or diffuse accumulations of CD8+ lymphocytes occur in most seminomas and are a useful distinguishing feature.* Whereas atrophy of tubules at the edge of the tumor is normally evident, occasional foci of marked intratubular spermatogonial proliferation and early seminoma formation in this location are sometimes seen.

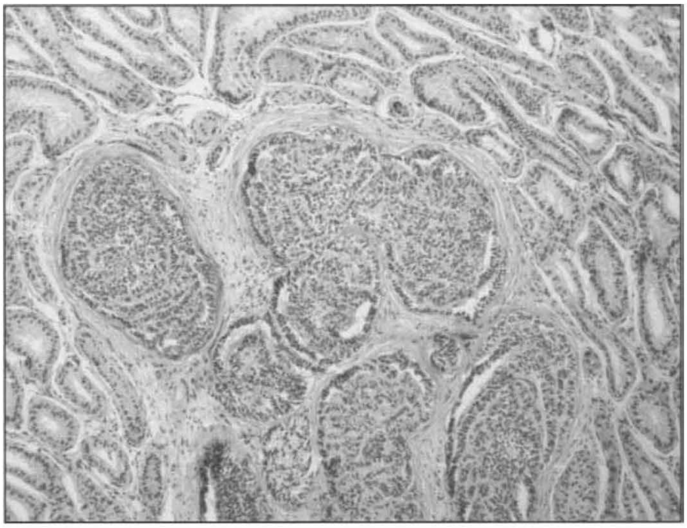

Figure 5.43 Focal **Sertoli cell hyperplasia** in the cryptorchid testis of a ram.

Germ cells stain by immunohistochemistry with *vimentin* in a perinuclear pattern, but they are negative for neuron-specific enolase and cytokeratin.

When examined by electron microscopy, tumor cells in canine seminoma resemble normal germinal epithelium and are characterized by a relative scarcity of cytoplasmic organelles, oval nuclei, straight cell borders, and distinct Golgi complex. Intercellular bridges, as seen in normal germinal cells, are present in seminomas. In all cases, the cells are markedly distinct from cells of interstitial or Sertoli cell tumors.

Presumably because abdominal location of seminomas in retained testes precludes their early clinical recognition, they may become quite large and metastasize widely. In the stallion, seminomas up to 9 kg have been found, and metastasis to most parts of the abdominal cavity and also the thoracic cavity, has been observed.

Intratubular seminomas have been reported in mature and aged rams with testicular degeneration. The proliferating spermatogonia are confined to the seminiferous tubules and do not attain sufficient size to be recognized on gross examination. It appears that this may be a dysplastic rather than a neoplastic process. A similar dysplastic process in otherwise degenerate testes is also observed in dogs. Several such foci occur in the organ, the affected tubules being slightly dilated and the abnormal cells distinctively large, rounded or polyhedral, usually multinucleate, and with pigments in the cytoplasm. Rarely, a highly malignant form of seminoma occurs in the ram, producing overall enlargement of the testis, and hemorrhage, necrosis, and massive neoplastic infiltration of the entire testis.

Seminomas are exceedingly rare in boars and cats.

Teratoma

Teratoma of the testis is a neoplasm that is virtually unknown in domesticated mammals other than the horse, in which it is the most frequently reported testicular tumor in the young animal. Equine teratoma is a benign tumor found in the scrotal, or more often, cryptorchid testis of young animals. It is probable that its presence in a fetal testis would prevent normal descent. There is a report of testicular teratoma that caused partial obstruction of the colon in a neonatal foal.

Macroscopically, teratomas are single or multiple and quite varied in color and texture. They are usually less than 10 cm in diameter

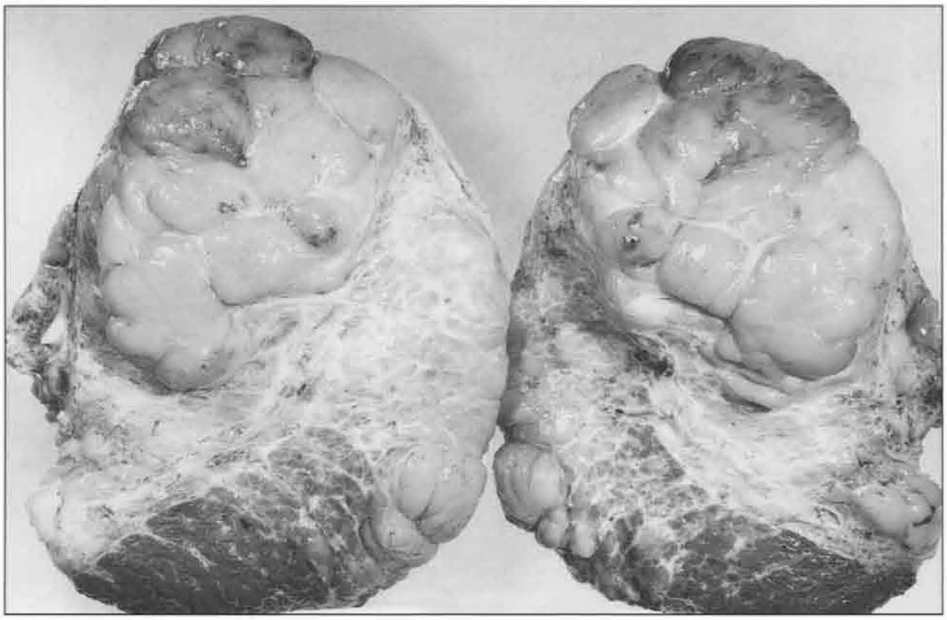

Figure 5.44 Multinodular appearance of a **seminoma** in a horse. The neoplastic tissue varies from white to tan, and can be firm. The adjacent testis is atrophic.

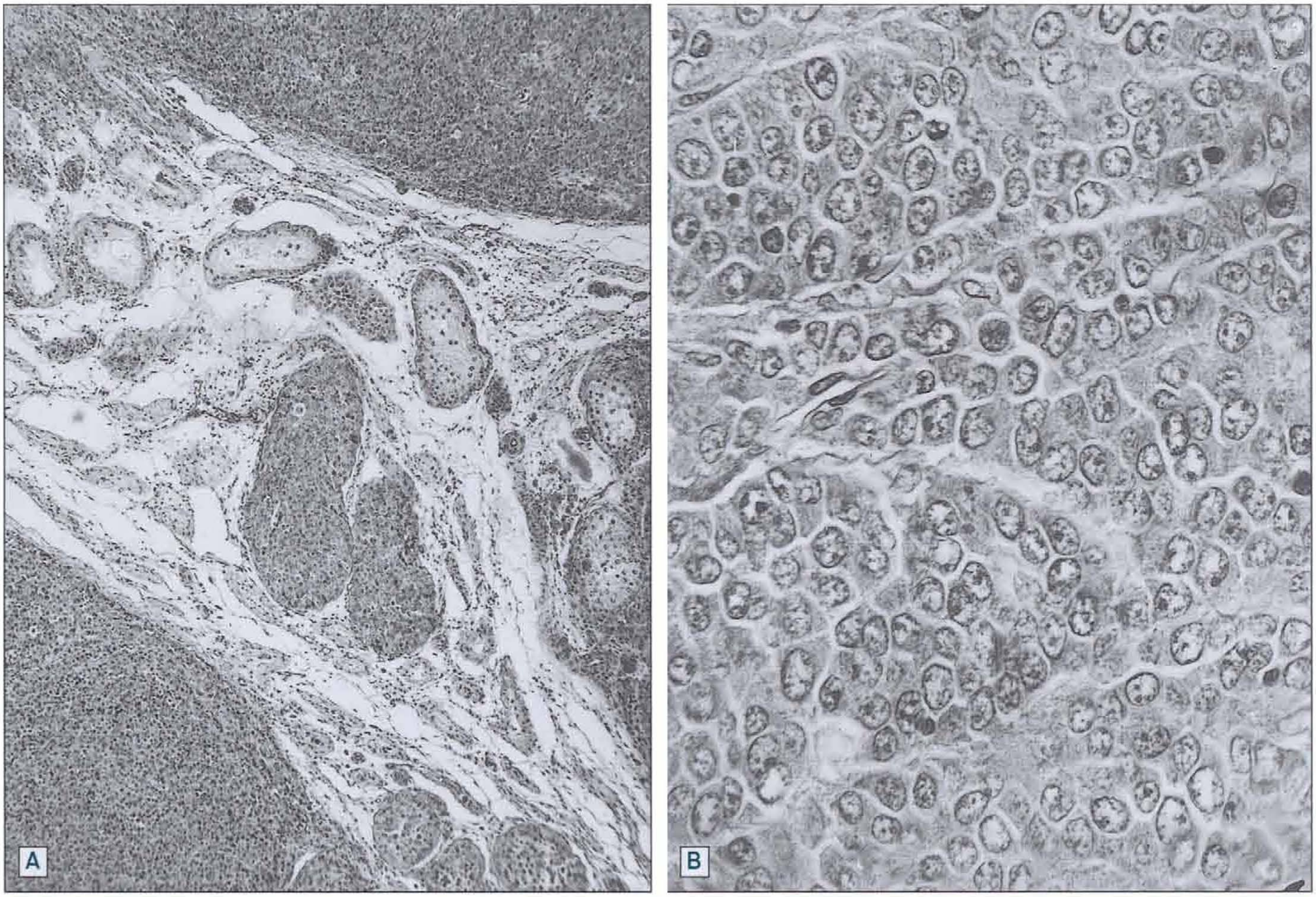

Figure 5.45 A. Seminomas usually have a combination of diffuse and intratubular growth. The adjacent testis may be atrophic as was the case in this horse. **B.** Histologically, the cells are large round cells with scant cytoplasm and large nuclei with prominent nucleoli. Mitoses are frequent.

but may be greater than 25 cm. A cystic and/or multilocular structure is common and, on section, hair and mucoid or sebaceous-like secretions are often seen, hence frequent use of the term "dermoid cyst." Yellow-white solid masses with fibrous, adipose, cartilaginous, and bony tissue are also frequent.

Histologically, *structures derived from all embryonic germ layers may be present*, including ectodermal (dermoid cysts, hair, teeth), neuroectodermal (nervous tissue, melanoblasts), entodermal (salivary gland, respiratory), or mesodermal (fibrous or adipose tissue, bone, muscle). Nervous tissue is almost always present, and adipose tissue is also very common. Testis adjacent to a teratoma may show reduced spermatogenesis, with various degrees of tubular atrophy. The histogenesis of gonadal teratoma is discussed in Vol. 3, Female genital system.

Embryonal carcinoma

Embryonal carcinoma is rare in animals but is of significant frequency among the germinal tumors of humans in which it is highly malignant. It is probably best regarded as belonging, histogenetically, with the teratomas but not displaying the tissue differentiations that identify the teratoma. The cells are of indifferent embryonic types. Trophoblastic differentiation and demonstrable α-fetoprotein in the epithelial cells supports the diagnosis.

Teratocarcinoma, a neoplasm with features of teratoma and embryonal carcinoma, is reported in the horse.

Mixed germ cell–sex cord stromal tumor

These neoplasms are a combination of germ cells and cells derived from either the primitive sex cords or the stroma of the gonad. *Gonadoblastoma* is another name for this neoplasm. There is a suggestion that these have been missed and, hence, underreported. The lesions grossly are firm, white to tan, and expansile. Larger neoplasms may be hemorrhagic. *The histological features are a combination of seminoma and Sertoli-like cells with tubular structures intimately associated with germ cells.* Immunohistochemical staining with neuron-specific enolase for Sertoli cells and with vimentin for germ cells may confirm the dual nature of this neoplasm.

Other primary tumors

Adenomas and adenocarcinomas presumably originating from the rete testis are described in dogs and horses. These are tubulopapillary structures with scant supporting stroma. The papillae and anastomosing tubules are lined by small closely packed cells with scant cytoplasm either in single or multiple layers. Evidence of transformation from normal to neoplastic rete epithelium is a useful criterion for diagnosis, and it is desirable to exclude other teratomatous structures or distant primary tumor elsewhere. Immunohistochemically, cytokeratin is stainable in epithelium of the rete testis.

Reports of *mesenchymal tumors* of the testis are mostly of individual cases. They include leiomyoma and leiomyosarcoma, hemangioma and hemangiosarcoma, mast cell tumor, peripheral nerve sheath tumor, and histiocytic sarcoma, to name a few.

Neoplasia of the epididymis is a very rare event. Any tissue may transform, and metastatic neoplasia or involvement of systemic neoplastic conditions such as lymphoma may occur. Interstitial cell tumor of the epididymis of the cat is a recognized disease and arises from ectopic interstitial endocrine cells.

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Miscellaneous diseases of the testis and epididymis

Sperm granulomas occur at any location that spermatozoa may be found. The most recognized syndrome is *sperm granuloma of the epididymal head*, a condition believed to be associated with blind-ending efferent ductules (see Ductular anomalies). It has been seen in virtually every species, except perhaps the cat.

Adenomyosis of the mesonephric ducts is a histological finding where *diverticula of the epithelium of the efferent ductule, epididymis, or deferent duct protrude through the muscular wall.* Spermatozoa may become entrapped and ultimately incite an inflammatory reaction. Hyperestrogenism may be an inciting cause. Adenomyosis is the suspected underlying defect when a sperm granuloma is found in a location apart from where blind-ending efferent ductules would be expected. Virtually every species has been reported to develop adenomyosis of the mesonephric duct.

The **rete testis** is a series of interconnected channels that join the seminiferous tubules to the efferent ductules. It has intratesticular and extratesticular segments. Spermatophagia occurs in this area, and immune reactions to spermatozoa may be manifested by inflammation in this region. There are two main primary diseases of the rete: *neoplasia and cyst formation.* The epithelial cells are the origin of adenomas and adenocarcinomas in horses, dogs, and other species. Cysts of this region are reported in the stallion, cats, and

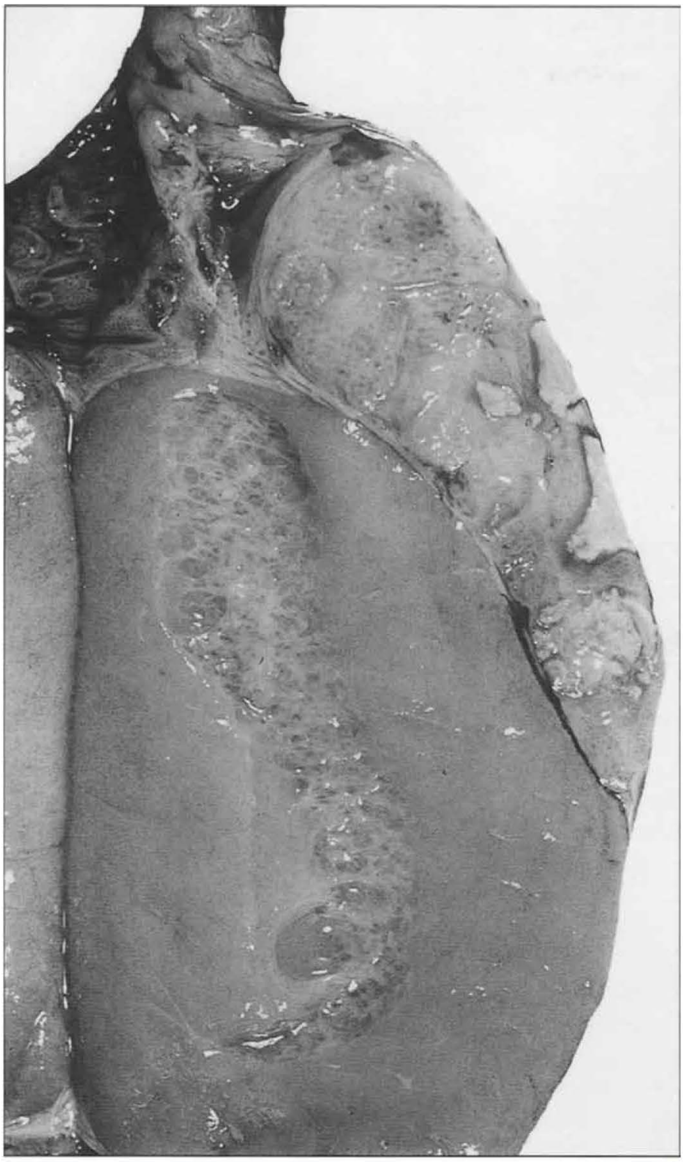

Figure 5.46 Dilation of rete tubules and the mediastinum testis secondary to obstruction and increased pressure caused by sperm granuloma of the epididymal head in a ram.

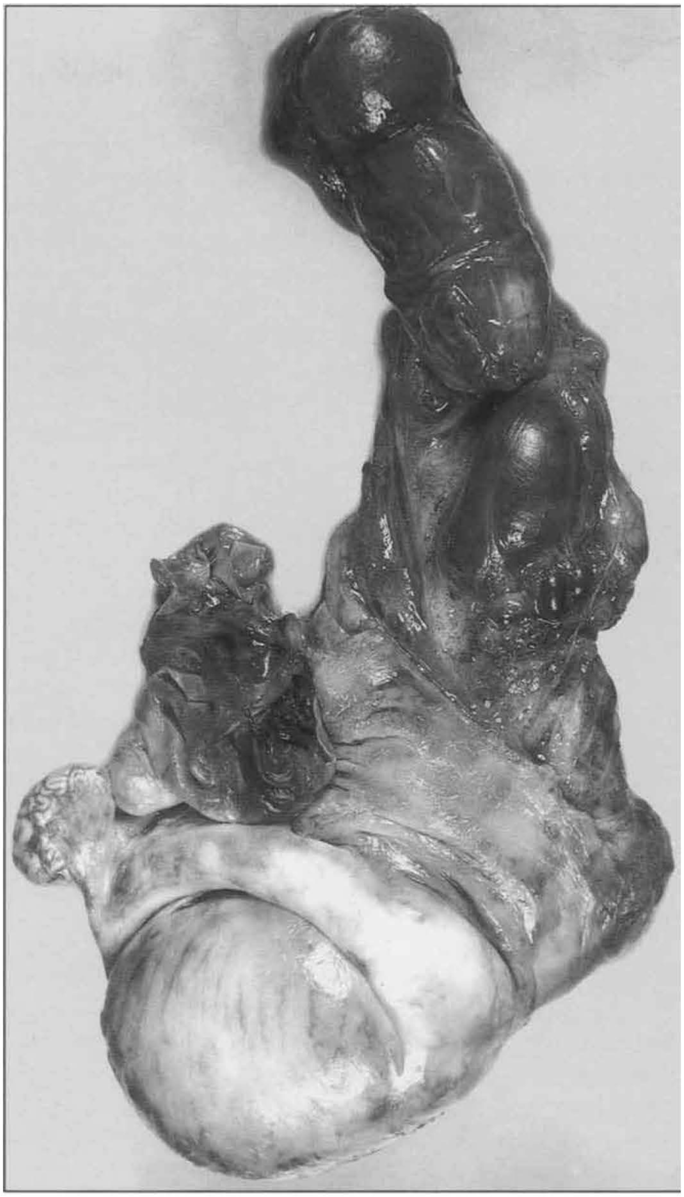

Figure 5.47 Varicocele with thrombosis in a stallion. The grossly dilated and thrombosed pampiniform plexus causes a large multilobular mass above the testis in the spermatic cord.

dogs. Secondary dilation of the rete testis occurs with obstruction of the efferent ductules or epididymis (Fig. 5.46).

SPERMATIC CORD

The spermatic cord is made up of the testicular artery, vein, and nerves, and the deferent duct. They are critical structures for the function of the testis and epididymis. Scrotal hernia and varices of the pampiniform plexus are probably the most commonly recognized disease of the spermatic cord. Most of the other diseases become differential diagnoses for these.

Varicocele

A varicocele is a dilation and tortuosity of the veins of the pampiniform plexus and the cremasteric veins. Varices of the spermatic veins are most commonly seen in old rams. They occur sporadically in other species, such as the stallion (Fig. 5.47), as an incidental finding or secondarily to conditions where there is constriction of the veins of the spermatic cord. Varices are seldom recognized unless they are thrombosed, and inexperienced persons may miss them. There is no concrete evidence that small varicoceles are detrimental, but the very large ones are, by virtue of their size and the reduced ability of the animal to raise the testis to maintain thermoregulation. The pathogenesis of varices in animals is not well understood. In humans, the left unilateral location is regarded as the result of altered hemodynamics from insertion of the testicular vein to the renal vein rather than directly to the vena cava. Evidence in humans with varicose veins of the legs suggests that the elastic properties of the walls of the veins and arterial flow are major factors and varicoceles in rams may be similar. Many rams and bucks have intimal sclerosis of the spermatic vessels but no varicocele.

Varicoceles appear as dark red nodules, 1–3 cm or more in diameter, enclosed in fascia of the spermatic cord proximal to the testis. Dissection of varicoceles may reveal large organizing laminated thrombi. *Differential diagnoses* of varicocele include neoplasia (mesothelioma), scrotal hernia, scrotal lymphadenitis, sperm granuloma of the deferent duct, and retained structures and cysts.

Varicocele in the ram is bilateral, or unilateral with no apparent predisposition as to side. Large varicoceles are always thrombosed and are associated with overt testicular degeneration, occasional thrombosis of testicular vessels, and reduced testis:body weight ratios, the latter possibly resulting from compromised thermoregulation in the testis. Detailed histological studies have revealed an apparently independent sclerosis involving almost 50% of both arteries and veins in the spermatic cord of rams. The arterial changes, which tend to be bilateral, consist of fibroplasia of the tunica intima. A variable degree of intimal and or medial mineralization may also be seen in branches of the testicular artery in the spermatic cords of rams and bucks. Apparent hypoplasia of veins of the spermatic cord has been observed in bulls associated with thickening and degeneration of the testicular artery.

Although studies in the bull have not demonstrated a direct connection between venous and arterial blood vessels of the pampiniform plexus, functional *arteriovenous anastomoses* through small vessels do occur in normal bulls, boars, and rams, and may represent a further means of thermoregulation of the testis. A large anastomosis of the spermatic artery and vein has been observed in the stallion associated with an abdominal testis.

Scrotal hernia

Scrotal or inguinal hernia is a rare condition in all species except the horse. It is reported in dogs, rams, and stallions. In rams, it tends to involve the left side. It is believed to be a genetic and recessive disorder.

In **stallions**, the disorder is either congenital and often resolves by 3–6 months of age, or it is acquired and is potentially life threatening because of intestinal strangulation. In the *congenital form*, a portion of the jejunum enters the vaginal sac; rupture of the vaginal tunics at birth may result in the loop of intestine lying in a subcutaneous location. In the *acquired form* in entire animals, there may be an association with exercise or some activity such as serving a mare. Left- and right-sided hernias occur with equal frequency. Herniation of the intestine usually obstructs vascular supply to the testis, necessitating castration at the time of surgical repair. The acquired form is very rare in geldings.

Miscellaneous diseases of the spermatic cord

Other lesions of the spermatic cord may involve any of the structures present, including the deferent duct, blood vessels, nerves, cremaster muscle, or tunics. Some of these diseases, particularly congenital anomalies, are described elsewhere in more detail.

Inflammation of the spermatic cord is **funiculitis**; it follows open castration. It may be acute and necrotizing, as is often seen in the pig, in those species in which there is ample opportunity for contamination, or it may be chronic, as in the typical "*scirrhous cord*" of horses and cattle. The nature of the infecting organisms determines the nature of the reaction. In the pig, it is often a necrotizing purulent response; there may be very little granulation tissue, and the cutaneous incision may not heal. In this species too, ascending infection provoking diffuse peritonitis, and tetanus, are common complications. The classic scirrhous cord of the gelding is a pyogenic infection, usually by staphylococci, which produces typical "botryomycosis," an exuberant granulation tissue enclosing multiple small abscesses and containing many fistulae.

A *pseudocyst* of the spermatic cord of a gelding is reported.

Neoplasms arising in these structures, or metastasizing from other locations may be found. Any of the structures can be involved, including fibrous tissue, smooth muscle, endothelium, or mesothelium.

Verminous granulomas caused by wandering larvae of *Strongylus* spp. are occasionally observed in the spermatic cord and testes of horses.

Sperm granuloma of the deferent duct is seen mostly as a sequel to vasectomy, but is occasionally observed as spontaneous disease.

Ectopic adrenal tissue is occasionally found in the spermatic cord.

Disease of the *scrotal lymph node* can be confused with disease of the spermatic cord. Caseous lymphadenitis in sheep and lymphoma in dogs are two relatively common diseases that may cause enlargement of the node and be mistaken for varicocele or some other abnormality. Drainage of lymph and therefore inflammatory infiltrates, spermatozoa, and metastases from intrascrotal neoplasia can be to the superficial inguinal, sub-iliac, or lateral and medial iliac nodes.

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ACCESSORY GENITAL GLANDS

Vesicular glands (seminal vesicles) and ampullae

Anomalies of development

The chief congenital anomaly affecting the vesicular glands (seminal vesicles) and ampullae of the deferent duct is **segmental aplasia of the mesonephric duct**. This condition appears to occur most frequently, and has been most studied in the bull, in which it seems to be heritable. Aplasia or hypoplasia of this duct may affect any or all of the genital organs derived from it so that the deferent duct and epididymis may be simultaneously affected. Aplasia is mostly unilateral and the ampulla on the affected side is completely absent or rudimentary and the vesicular gland is markedly hypoplastic and sometimes cystic. Vesicular gland hypoplasia of variable severity may occur independently of aplasia of the ampullae or deferent duct. Other anomalies of the vesicular glands and ampulla of the bull include fusion of the vesicular glands (Fig. 5.48A) and

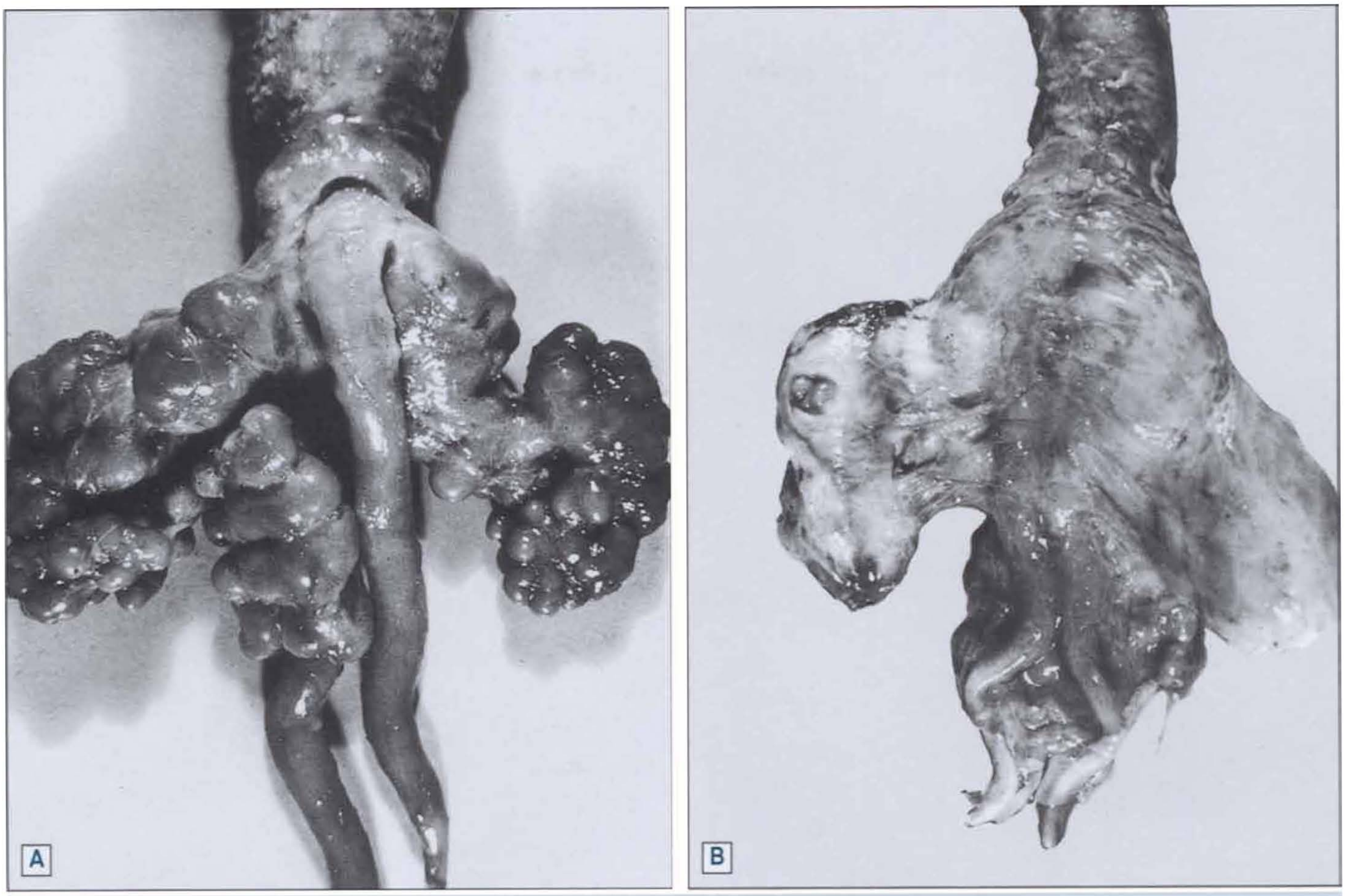

Figure 5.48 **A.** The **vesicular glands are fused** and there is a ventrally displaced ampulla in this bull. **B.** Chronic **vesicular adenitis** in this bull caused fibrosis and shrinkage of the glands.

appendages of the ampullae. There is variation in the relationship of ampullae to the vesicular glands in normal bulls; the ampullae are entirely dorsal to the vesicular glands in 40% of bulls, are entirely ventral to the vesicular glands in 40% of bulls, and in the remaining 20% of animals are intermediate. Realization of these anomalies and anatomical variations is important in rectal examination of bulls. Although the anomalies per se seem incidental, and unrelated to diminished fertility, evidence suggests that anomalous glands are predisposed to inflammation.

Cystic dilation of the lumina of occasional lobules, or a general dilation of duct lumina in one, and sometimes both, vesicular glands, occurs in bulls, horses, and swine.

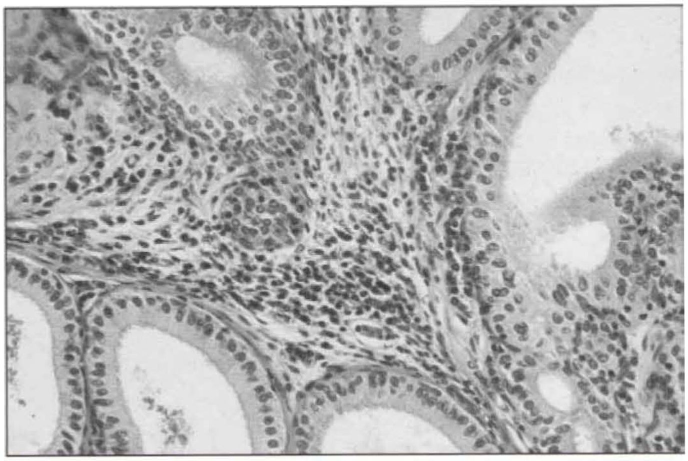

Figure 5.49 Microscopically visible **chronic vesicular adenitis** in a ram with *Brucella ovis* infection. Large numbers of lymphocytes and plasma cells are in the interstitium.

Inflammation of the vesicular glands

Vesicular adenitis, which is frequently accompanied by similar reactions in ampullae, is a common lesion in the bull, and is seen rarely in the stallion and boar (Fig. 5.48B). Macroscopically apparent vesicular adenitis is quite rare in the ram but histological lesions accompany the rather frequent localization of *Brucella ovis*, *Actinobacillus seminis*, and *Histophilus somni* (*Histophilus ovis*) in the vesicular glands and ampulla, in association with epididymitis caused by these organisms (Fig. 5.49). Typically, vesicular adenitis is characterized clinically by palpable changes in the vesicular glands on rectal examination, and by changes in the semen, particularly the presence of pus. Young bulls, less than 2 years of age, are frequently affected.

Two forms of vesicular adenitis are recognized in the **bull**, a *chronic interstitial form* characterized by a considerable increase in size, excessive fibrosis, firm consistency, and loss of lobulation, and a

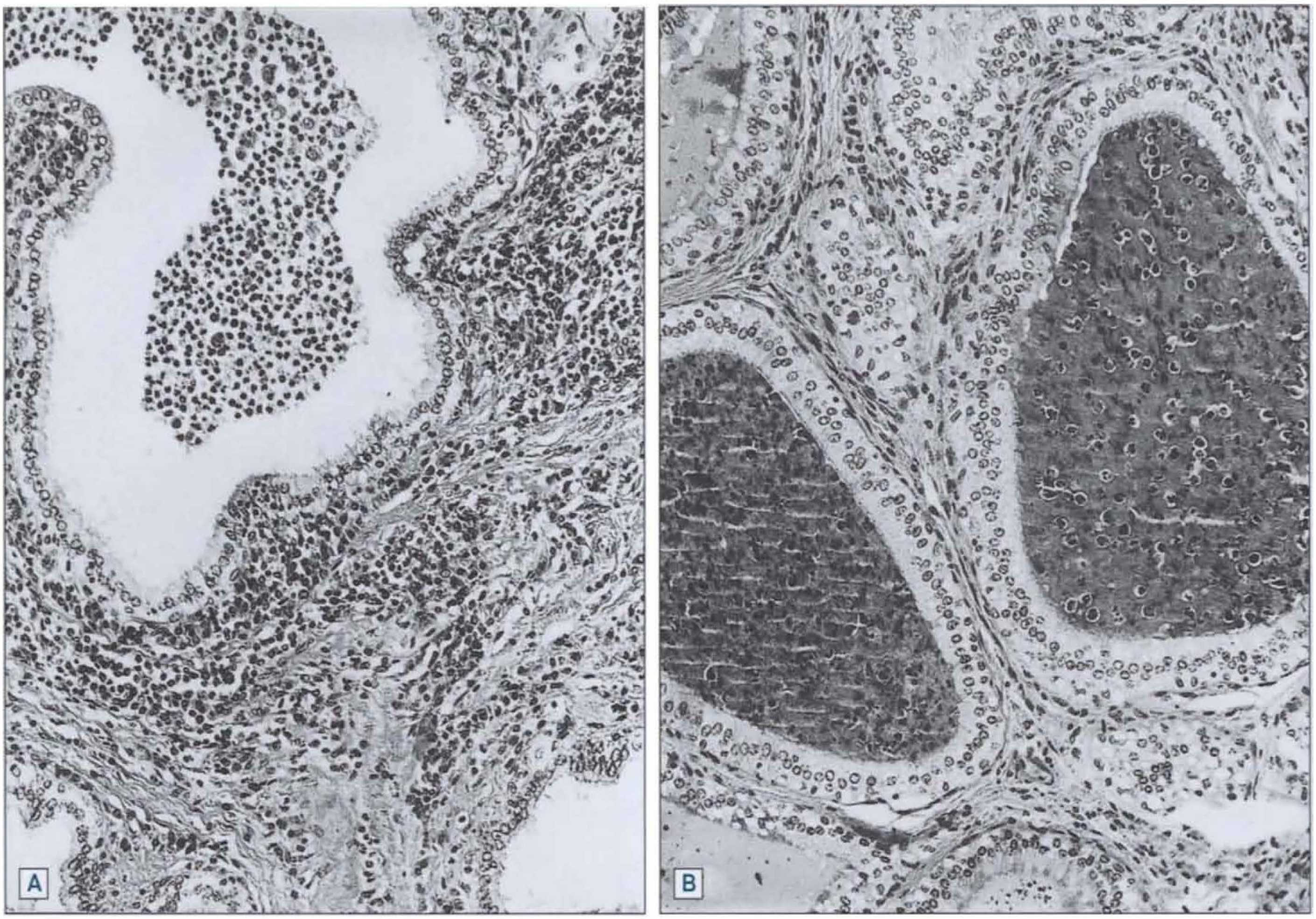

Figure 5.50 Histological lesions of **vesicular adenitis** in the bull. **A.** Chronic interstitial vesicular adenitis with lymphocytes, plasma cells and fibrous tissue in the interstitium. Glandular lumina contain neutrophils. **B.** Acini are filled with basophilic debris.

predominantly *degenerative form* characterized by at most a slight increase in size and consistency.

Microscopically, the chronic interstitial form is characterized by fibrosis and cellular infiltration of stroma with lymphocytes, plasma cells, histiocytes, neutrophils, and occasional eosinophils (Fig. 5.50A). The epithelium usually is normal but may be metaplastic in some areas. Acini contain a small amount of finely granular eosinophilic material, occasional neutrophils and desquamated epithelium. In the predominantly degenerative form of vesicular adenitis, only a slight increase in stroma is observed, but leukocytic infiltration is often present. In addition, neutrophils, sloughed epithelium, and intensely basophilic material are present in the acini in clumps or strands (Fig. 5.50B).

Numerous *infectious agents* may be isolated from bovine vesicular adenitis, but the precise roles of most have yet to be determined. In chronic vesicular adenitis, bacteriological investigations are often negative. *Arcanobacterium pyogenes* appears to be the most common isolate, and lesions in general are those of chronic interstitial vesicular adenitis. Large abscesses may be present also. These may involve adjacent tissue, and adhesions and fistulas form with the rectum or bladder. Staphylococci and streptococci generally do not form large abscesses, but more often small focal or diffuse suppurative inflammation. Vesicular adenitis caused by *Brucella abortus* is fibrinopurulent, progressing to necrosis, suppuration, and dystrophic mineralization; granuloma formation is rarely observed. Tuberculous vesicular adenitis causes a great increase in the size of vesicular glands with characteristic nodulation and caseation.

Several viruses, including the virus of infectious bovine rhinotracheitis–infectious pustular vulvovaginitis (*Bovine herpesvirus 1*), may infect the accessory genital glands of the bull. Clinical signs of vesicular adenitis in such cases are reported to be mild and transitory. The vesicular glands and prostate are the most productive sites for viral replication in *Bovine viral diarrhea virus* infection in bulls, but neither gross nor microscopic lesions are observed. *Mycoplasma bovigenitalium* and ureaplasmas have been isolated from bulls with vesicular adenitis, and unequivocal lesions have resulted from experimental infection with these organisms and *Mycoplasma bovis*. Apart from a slight to pronounced infiltration of eosinophils into these lesions, they are nonspecific, and it is unclear to what extent mycoplasmas cause spontaneous vesicular adenitis. *Mycoplasma bovigenitalium* can be isolated from the vesicular gland secretions from a large proportion of normal pubescent bulls. As with the mycoplasmas, *Chlamydophila* spp. have been isolated from the semen and epididymides of bulls, and its possible role in vesicular adenitis has yet to be clarified. The vesicular glands may also be an important site for localization of *Leptospira* spp. *Leptospira interrogans* serovar *hardjo* has been isolated with equal frequency from vesicular glands and kidneys of naturally infected bulls, thereby suggesting the possibility of venereal transmission.

The *pathogenesis* of bovine vesicular adenitis is as yet unresolved. Hematogenous infection of the glands from a primary point of infection elsewhere seems probable in most cases but ascending infection from the urethra and infection via peritoneal fluid, perhaps following navel infections during calfhood, are also possible. Establishment of infection may be aided by stress, reflux of urine or semen into the vesicular glands, and by congenital defects in the duct system of the pelvic organs.

In **sheep**, **goats**, and **pigs**, vesicular adenitis is of lesser importance than in the bull, but it occurs in brucellosis caused by *Brucella ovis, Brucella melitensis*, and *Brucella suis*, respectively. The characteristic lesion is chronic, and resembles that seen in the bull (Fig. 5.49). In boars, abscessation of the vesicular glands and prostate may accompany orchitis caused by *Burkholderia pseudomallei*. Abscesses in the vesicular glands of barrows may be the result of infection at the time of castration.

In the **stallion**, purulent, abscess-forming vesicular adenitis has been described, but appears to be uncommon. Following experimental infection with *Equine arteritis virus*, virus is shed in semen of experimentally infected stallions for months, and the ampullae and bulbourethral glands seem to be predilection sites. It is unclear whether there are lesions in infected tissues, or associated spermatozoal abnormalities. Bacterial vesicular adenitis and ampullitis occur sporadically and may be a cause of "colic."

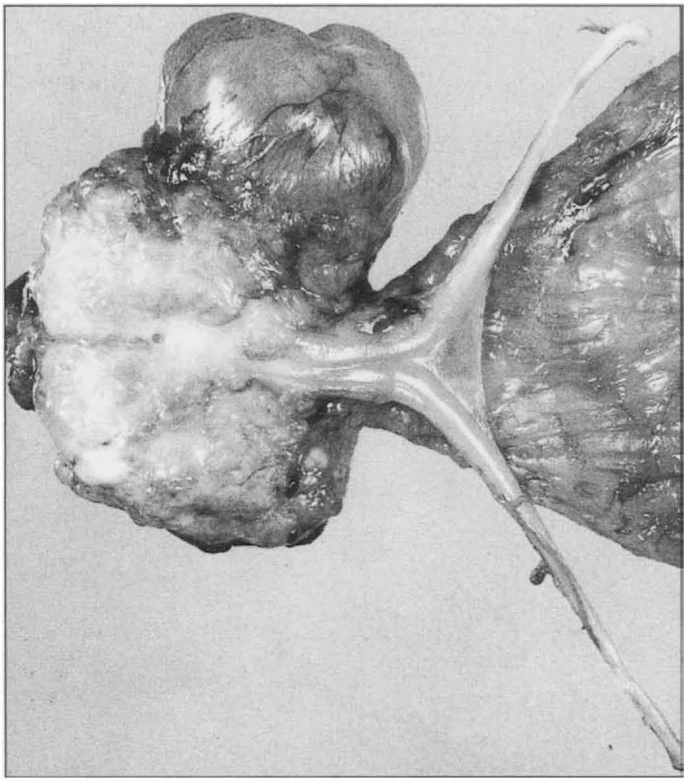

Figure 5.51 Paraprostatic cyst distorting the contour of the prostate in a dog.

Prostate and bulbourethral glands

These accessory genital glands are both derived from urogenital epithelium and their responses to some adverse influences are similar. Studies on the accessory genital glands of normal bulls and rams have demonstrated that a protective local secretory immune system is present and especially involves the bulbourethral glands and prostate, which both have a predominance of IgA-containing cells in subepithelial locations.

Anomalies of development

Anomalies of the bulbourethral gland include *congenital retention cysts* in bulls, rams, and cats, and *aplasia, hypoplasia, and fusion* in bulls. *Melanosis* of the bulbourethral glands has been observed in the bull and in swine. Malformations of the prostate are infrequent and are usually seen together with anomalies of the remaining genitalia – especially in intersex states and in cryptorchidism. In some dogs, the dorsal median groove of the prostate may be indistinct so that the bilobed nature of the gland is not clearly evident. Other prostatic anomalies include the *prostatic appendage in the bull* with polyp-like protrusion of prostatic tissue into the urethral lumen, and congenital retention cysts in the bull and cryptorchid boar.

Prostatic cysts in the dog may be congenital or be secondary to hyperplasia, neoplasia, or inflammation (see below). Classification of prostatic cysts in the dog into four types – *multiple cysts associated with prostatic hyperplasia, retention cysts, paraprostatic cysts, and cysts associated with squamous metaplasia* – has been proposed. Although their precise origin is still unclear, paraprostatic cysts probably result from anomalous development. Most cysts appear to be attached to the prostate at a small localized area but without a definite stalk (Fig. 5.51). The smaller cysts are up to ~7 cm in diameter and have a wall 2–5 mm thick, whereas larger cysts may be up to 24 cm long and 14 cm in diameter and contain much collagen and even bone in their walls. Both types of cysts may be lined by epithelium that appears to be secretory. Accumulations of fibrin on the inner aspect of the larger cysts, as well as cauliflower-like bony lesions extending into the lumen, are common. Cysts may rarely become infected and rupture but their content is almost always sterile and is devoid of pus, urine, and spermatozoa. It has been suggested that such cysts arise from vestiges of the paramesonephric duct, the uterus masculinus, but most reported cysts appear to have arisen from one or the other prostatic lobe. Enlarged remnants of ducts that form prostatic or paraprostatic cysts may be expected to arise in the dorsal aspect of the prostate and have their origin in the midline. Where such cysts are bilobed and lined by simple columnar to pseudostratified columnar epithelium that resembles endometrium, their designation as *cystic uterus masculinus* is justified. Cystic enlargement, which occurs mostly in old dogs, appears to be stimulated by estrogenism, as in response to a Sertoli cell tumor, and may cause constipation, dysuria, or anuria.

A *uterus masculinus* is also frequently observed in males of other species (Fig. 5.52). As in the dog, it may be cystic or communicate with the urethra.

Inflammation of the prostate and bulbourethral gland

Inflammation of the bulbourethral gland in the bull often accompanies vesicular adenitis, which it resembles both in causation and tissue response. Concretions may result from chronic inflammation.

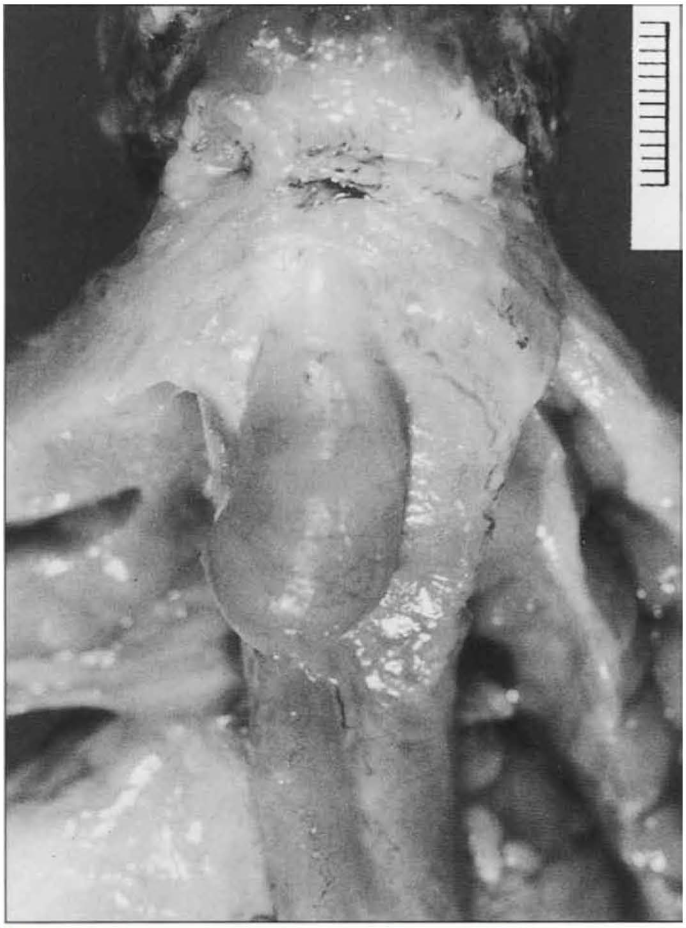

Figure 5.52 Cystic uterus masculinus (center) overlies the ampullae of this bull.

Prostatitis is common in the dog. It is often a disease of older dogs in which hyperplastic prostatic changes are present. The inflammatory changes contribute to the enlargement in a significant proportion of the cases, but the disease may occur in younger dogs with normal prostates. The infecting agents are urinary pathogens, *Escherichia coli*, *Proteus vulgaris*, streptococci, and staphylococci, which invade via the prostatic urethra.

Prostatitis is often acute, with systemic signs of illness, and about two-thirds of affected dogs have a history of urinary tract signs that include gross blood and or pus in the urine, urethral discharge, incontinence, or dysuria. Except for dramatic elevations in neutrophil counts with prostatic abscesses, hematological data are mostly equivocal, so that cytological evaluation and culture of prostatic fluid collected by prostatic massage or ejaculation is needed to assist in the differential diagnosis of prostatic disease in the dog.

Acute prostatitis is a diffuse or focal suppurative process with a tendency to abscess formation. The abscesses may be minute and multiple, or large with confluent areas of necrosis. The larger abscesses fluctuate on capsular palpation. Such abscesses may lead to metastatic sepsis, septicemia, peritonitis, and death, especially if there is sudden rupture.

In *diffuse prostatitis*, the gland is often asymmetrically enlarged, congested, and edematous (Fig. 5.53) with a soggy consistency, and pressure causes welling of pus over the cut surface. Depending on the duration and severity of the inflammation, the reaction may be centered on the acini with mild exudation and only slight stromal involvement, or a neutrophilic infiltrate may pervade all structures (Fig. 5.54A). Localization in and destruction of acini proceeds to abscess formation and these may become confluent, converting the whole gland into a multiloculate abscess cavity, but this is unusual. Acute inflammation may resolve and leave only extensive scarring, or it may become chronic, especially if ducts are obstructed, and the infection then tends to persist in small walled-off foci.

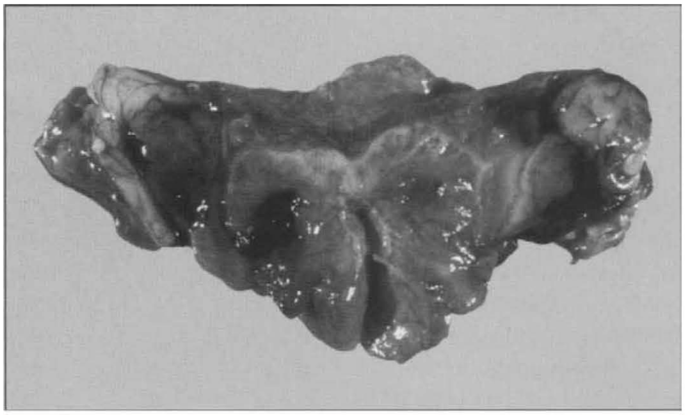

Figure 5.53 Transverse section of a canine prostate with **acute prostatitis**. Hemorrhage and edema involve the prostate and the periprostatic tissues.

Chronic prostatitis in the dog is also a common lesion (Fig. 5.54B). The prostatic epithelium is atrophic and its cytoplasm loses its characteristic eosinophilic staining quality. The lumina of the gland contain a variable number of neutrophils and macrophages, and debris. The inflammation is apt to involve the gland segmentally and may spare large portions. Aggregates of lymphocytes in the fibromuscular stroma, especially about the ducts, are very common.

Prostatitis is a constant feature of *Brucella canis* infection. Typically, involvement is extensive but lobular in distribution, and consists of a generalized lymphocytic infiltration with destruction of adjacent epithelium and associated fibrosis. Prostatitis may become emphysematous in the presence of gas-forming organisms. Systemic fungal infections, such as that caused by *Blastomyces dermatitidis,* may involve the prostate.

Metaplasia and hyperplasia of the prostate and bulbourethral gland

Reversible enlargement of the bulbourethral glands with squamous metaplasia, hyperplasia, and cystic dilation occurs in wethers ingesting strains of *clover possessing high estrogenic potency*, e.g., *Trifolium pratense, T. repens, T. subterraneum*. In wethers, but not in entire male sheep, a few weeks grazing on potent green clover pastures provides adequate estrogenic stimulus for the induction of profound and extensive metaplastic changes in the accessory sex organs, particularly the bulbourethral glands. The bulbourethral glands become enlarged and firmer than normal and are marbled on cut surface. They may also contain cysts, and in a small percentage these cysts may become enormous and cause fluctuating swelling of the perineal region (Fig. 5.55). The large cavity fills with urine and cellular detritus and communicates with the lumen of the urethra through the dilated pores of bulbourethral glands in the dorsal wall of the pelvic urethra. The epithelial hyperplasia, together with the formation

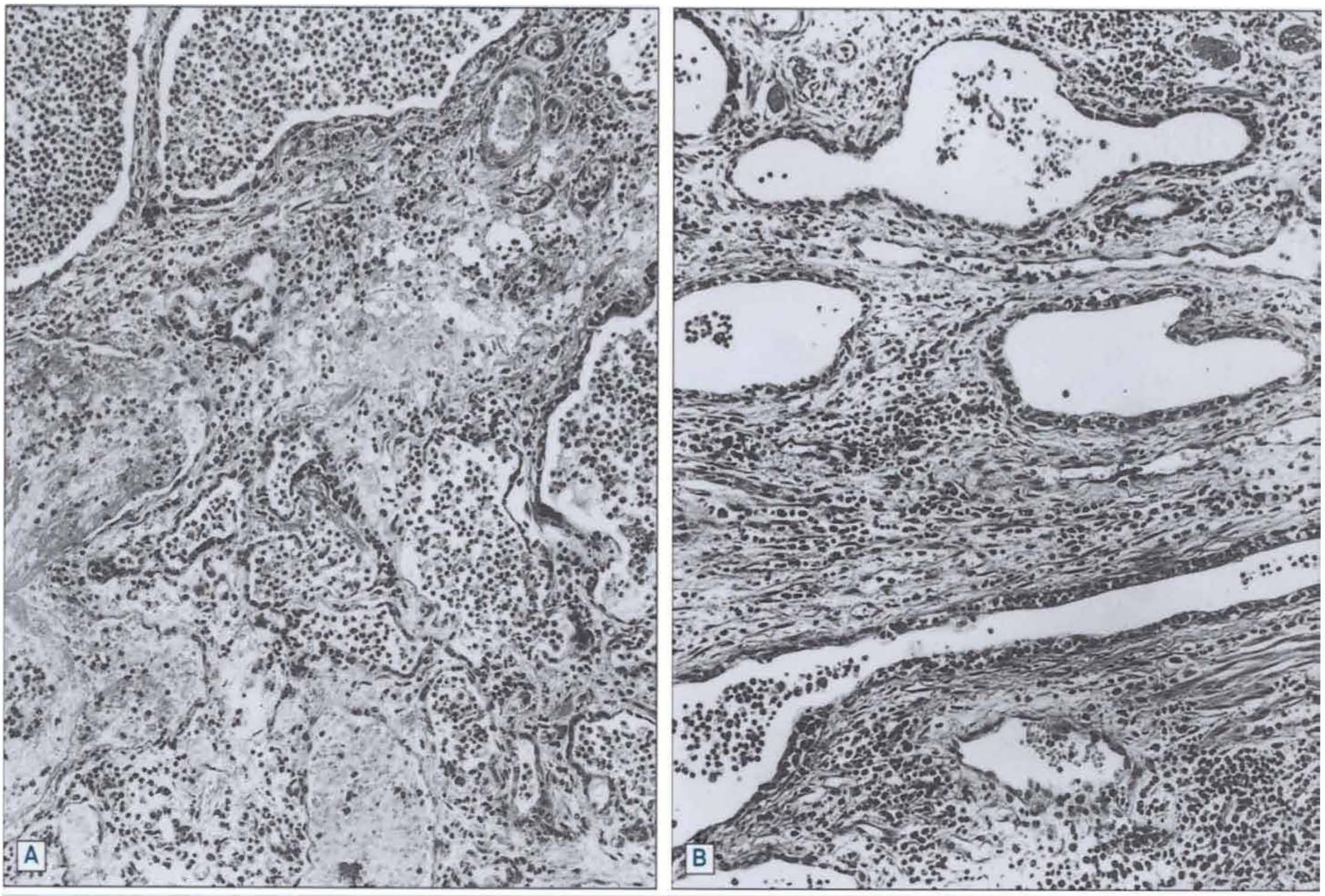

Figure 5.54 A. Histological appearance of **acute prostatitis** with abundant neutrophils within glandular lumina, and interstitial edema and leukocytes. **B. Chronic interstitial prostatitis** with lymphocytes and plasma cells with abundant fibrous tissue in the interstitium of the prostate of a dog.

of cellular casts, may produce urethral obstruction with death. Prolapse of the rectum is not uncommon. Cysts of the uterus masculinus occur and castrated males show mammary development (for effect on females, see Vol. 3, Female genital system). The *prostatic changes* consist of squamous metaplasia with conversion of the acini to keratinaceous cysts. There is also increased stroma in the prostate, and the changes are grossly visible as chalky or yellow streaks and flecks. In addition, the prostatic urethra may show metaplastic changes.

After *stilbestrol* administration, comparable changes have been observed in the bulbourethral glands, prostate, and vesicular glands of feedlot lambs and bull calves. In bulls, histology and quantitation of cytokeratin by immunohistochemistry may be used to detect illegal use of estrogenic compounds as growth promotants.

Marked squamous metaplasia of glandular genital organs, but particularly the vesicular glands of bulls, occurs following ingestion of *chlorinated naphthalenes*. Nodular hyperplasia of the bulbourethral glands of rams occurs occasionally.

Hyperplasia–hypertrophy of the prostate is observed in the bull, but it commonly occurs only in the dog; the organ is more-or-less diffusely involved, although in some instances normal or atrophic lobules may be interspersed in small numbers among hyperplastic ones. Some degree of hyperplasia is often evident in dogs 4–5 years of age, and the prevalence and degree increase with advancing years, such that 80% or more of mature or old dogs may have enlarged prostates. Squamous metaplasia of the epithelium of the prostate gland in the male dog may occur spontaneously in association with *neoplasia of the testes*, particularly Sertoli cell tumors, or following the administration of *estrogens*. The metaplastic change may involve acini in all parts of the gland as well as the prostatic urethra, uterus masculinus, and ducts. Affected epithelium is converted to stratified squamous, from the surface of which squames are shed into the lumen (Fig. 5.56). Neutrophils and macrophages are often numerous in lumina containing squames that have become fused into amorphous masses. Squamous metaplasia is associated with increased proliferation of basal cells. Although squamous metaplasia of the canine prostate predisposes it to inflammation, there is no evidence that this is a preneoplastic change. Squamous metaplasia of the prostate, comparable to that in the dog, has also been observed in swine. In cats, following estrogen administration, prostatic enlargement with epithelial hyperplasia and cystic dilation of glands occurs, but metaplastic cornification is restricted to urethral epithelium.

Enlargement of the prostate is frequently associated with *constipation*, presumably caused by pressure on the rectum. Less common, but more important, is *interference with urination*. It is not agreed just why urinary retention occurs. Compression with stenosis of the prostatic urethra has been advanced as an explanation, but is unsatisfactory because, at least postmortem, it is impossible to demonstrate any compression of the urethra, and because in the living animal incontinence

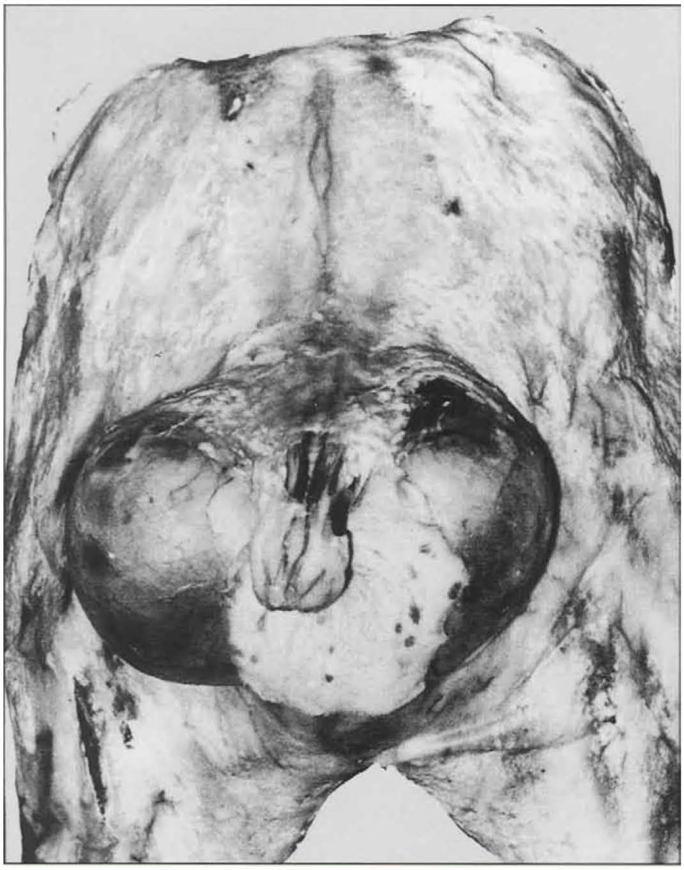

Figure 5.55 Phytoestrogenism causing massive enlargement of the bulbourethral gland in a wether.

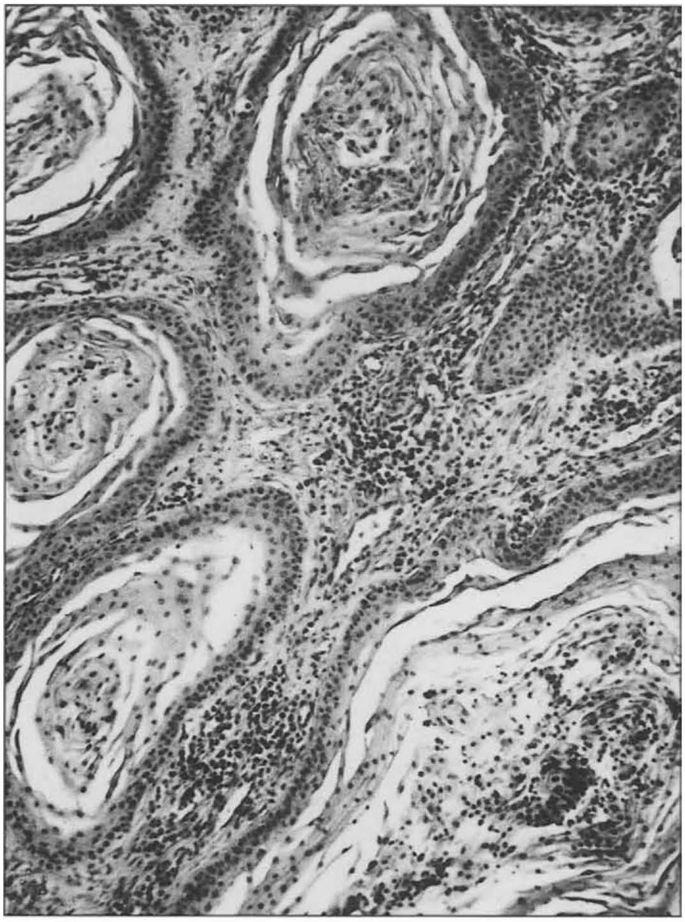

Figure 5.56 Squamous metaplasia of the glandular acini in the prostate of a dog. There are also interstitial infiltrates of lymphocytes.

is common and slight manual pressure on the bladder will serve to eliminate urine. Nor is the urethra completely surrounded by the prostate gland. It is possible that pressure of the enlarged gland on the sacral parasympathetic outflow causes paresis of the bladder. It is also possible that attenuation of the urethral lumen by longitudinal stretching may contribute in those cases in which the prostatic enlargement is sufficient to cause its displacement forward out of the pelvic cavity. Acute infections of the urinary tract and hydronephrosis often complicate the urinary obstruction.

There is little doubt that hormonal imbalances are important causes of prostatic hyperplasia. Hyperplasia does not occur in castrated dogs and castration is a useful therapeutic measure, although there are rare exceptions in which an adrenocortical tumor in a castrated dog has been associated with hyperplasia. *An altered androgen:estrogen ratio seems to underlie prostatic hyperplasia in dogs.* Two phases in the development of lesions are suggested. In the first phase in young dogs, actual epithelial hyperplasia is associated with normal testicular interstitial endocrine cells and normal androgen production, but with the secretion in the testes of an as-yet-unidentified estrogen-like molecule. In the second, cystic, phase there is decreased androgen production and decreased activity of testicular interstitial endocrine cells but continued production of the estrogenic substance. Importantly, however, although levels of circulating testosterone may decrease in old dogs, actual concentrations of dihydrotestosterone in hypertrophied or hyperplastic tissue are increased. Also it has been demonstrated that in such tissue in aged Beagles, nuclear androgen receptors are elevated. Therefore, estrogens appear to act synergistically with androgens to potentiate hyperplasia and act directly on the prostate. It is unclear in spontaneous prostatic hyperplasia, however, whether and to what extent estrogen induces accompanying stromal changes.

The hyperplastic gland is almost invariably enlarged, sometimes up to four times its normal size, and the surface is irregularly nodular (Fig. 5.57A), in some cases obscuring the normal bilobed appearance. Palpably fluctuating cysts and venous and lymphatic ectasias may be present beneath the capsule. The appearance of the cut surface varies depending on the degree of acinar and stromal hyperplasia and on the presence and size of cysts. The lobules vary much in size and may be poorly defined, or well defined if there is a prominent increase in size of the interlobular trabeculae.

The glandular elements are often spongy, and close examination reveals numerous small cysts containing milky fluid. The larger readily visible cysts are irregularly distributed, but the largest ones tend to be located beneath the capsule. The prostatic urethra may be somewhat distorted with small yellow elevations of the mucosa corresponding to the papillae of the excretory ducts. Microscopically, the structure is very diverse and consists of adenomatoid hyperplasia, stromal hyperplasia, and condensation, and cystic formations variously interspersed (Fig. 5.57B). The adenomatoid or acinar hyperplasia consists of epithelial hyperplasia leading, as in the thyroid gland, to papillary proliferations into the lumen, but always in a single layer on an intact basement membrane. Within the same lobule, some acini may be cystic, and these are of irregular size, rounded, and lined by

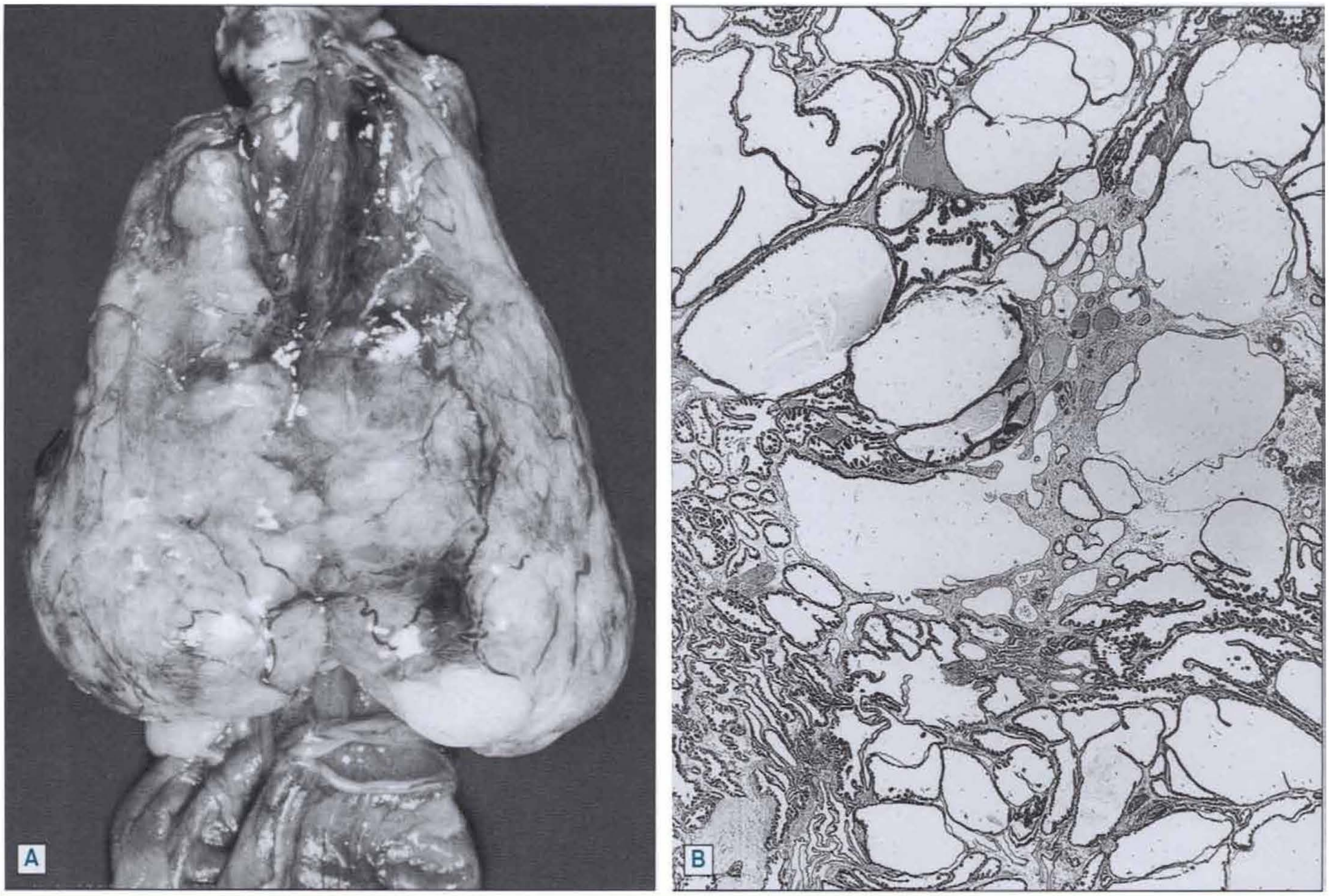

Figure 5.57 A. Prostatic hyperplasia in a dog typically causes almost symmetrical enlargement of the prostate. **B.** Microscopically, the glands are both cystic and hyperplastic.

epithelium that has become pressed and flattened. There may be secretion in the lumen but usually not. The interlobular connective tissue is frequently and irregularly increased in amount and condensation, and may exist as broad sheets with some extension into the intralobular stroma. In most instances, this interstitial tissue contains accumulations of mononuclear inflammatory cells, but the infiltrates do not involve the epithelium or acinar lumen.

There is no clear distinction between a normal prostate and one that is in the early stages of hyperplasia, although arbitrary decisions may be made on the weight or size of the gland relative to body weight. The relative weight corrected for age is fairly consistent, except in Scottish Terriers in which the relative weight of the prostate is about four times that of any other breed. Although estrogens in some cases are therapeutically effective in causing temporary regression of an enlarged prostate, these hormones in suitable dosage cause rapid enlargement of the gland.

Hypertrophy induced by estrogen per se is distinguishable by virtue of squamous metaplasia in the gland, and frequently in the uterus masculinus also.

Neoplasms of the prostate and bulbourethral gland

Neoplasia of the accessory genital glands is rare in all domestic animals. Hemangiosarcoma has been observed in the bulbourethral gland of the goat.

Major interest centers on **prostatic carcinoma in dogs**. There are different opinions about the origin and classification of neoplasia of the canine prostate. While the origins may be from acinar or ductular epithelium, neoplasia may also arise from the pelvic urethra and this seems especially plausible in dogs castrated prior to puberty (Fig. 5.58). Studies indicate that the glandular types of carcinoma are correlated with the lifetime exposure of testicular hormones. Immunohistochemistry has been used to separate transitional cell (urothelial) carcinoma arising in the urethra from prostatic glandular carcinoma (adenocarcinoma), but many studies do not appear to make the distinction. There is also the problem of dealing with mixed neoplasia wherein there are glandular and urothelial elements. Attempts to differentiate neoplasms are made difficult because they have a similar cell of origin. *It seems better to group the neoplasia as carcinoma of the prostate, and to acknowledge that glandular, urothelial, squamoid, or mixed types occur.*

Carcinoma of the prostate is uncommon. Neoplasia of the canine prostate is considered not to result from hyperplasia. *The cause of prostatic carcinoma is unknown* but, as with hyperplasia, it seems clear that the neoplasm may develop in an environment of hormonal imbalance, probably with other causative factors also being involved. Prostatic neoplasia is not causally related to testicular neoplasia. Castration has been shown to increase the risk of development of prostatic carcinoma.

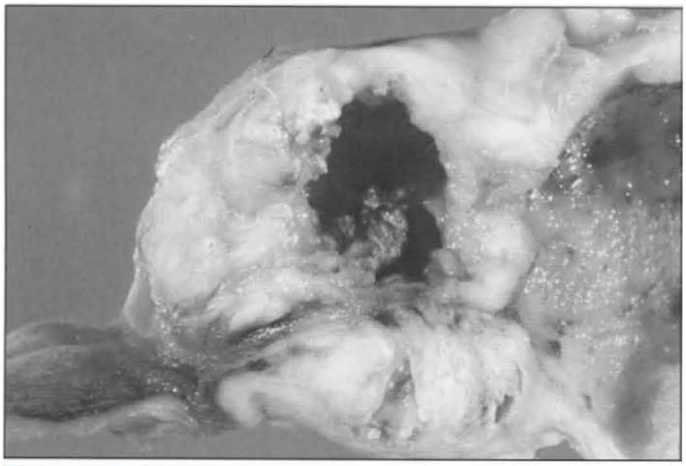

Figure 5.58 . Carcinoma of the prostate in an older dog castrated before puberty. The phenotype was a **transitional cell carcinoma** that had a small primary with central cavity of necrosis. This neoplasm was widely metastatic.

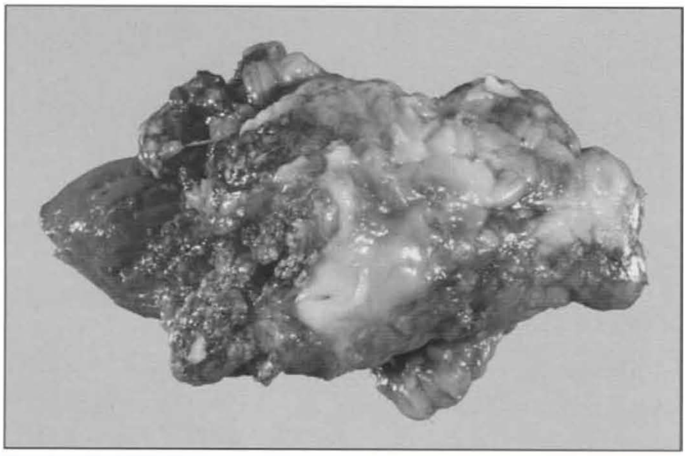

Figure 5.59 Carcinoma of the prostate in a dog. The phenotype of this carcinoma was an **adenocarcinoma**. Extensive invasion of the neoplasm into the surrounding tissues with filling of the pelvis is evident. No distant metastases were found.

Prostatic carcinoma is seen mostly in old dogs, especially those older than 10 years. Clinical signs are those of prostatic disease generally, but dogs with carcinoma seem more likely to exhibit emaciation and hindlimb locomotory disturbances, apparently due to metastasis of the tumor to the lower lumbar vertebrae, bones of the pelvis, and perhaps long bones of the rear limbs. Metastasis to bone is common, and is accompanied and probably preceded by visceral metastasis. In addition to the pelvis and lumbosacral spine, metastasis may involve the ribs, scapula, or digits. Bone metastasis may result from spread of neoplastic cells in the vertebral venous plexus, systemic circulation, or direct extension. Macroscopically, enlargement of the prostate with carcinoma is more likely to be asymmetric and irregular than is the case in hyperplasia (Fig 5.59).

A severe fibrosing reaction to invading tumor cells may be evident, and the neoplasm usually is quite hard, sometimes containing foci of ossification. The capsule of the prostate and adjacent organs often is invaded by the neoplasm, which may be adherent to the pubis or pelvis probably more commonly than in prostatic hyperplasia. Cyst formation in association with the neoplasm is common. Neoplastic tissue may extend into the sublumbar area, and metastasis to iliac, lumbar and pelvic lymph nodes, bone, kidneys, bladder, lungs, liver, heart, mesentery, and omentum occurs. Neoplastic cells may also be observed in the urine and occasionally in blood smears. Prostatic carcinoma metastatic to the regional lymph nodes and urinary bladder, but not the lungs, has been associated with hypertrophic osteopathy. Histological confirmation of the prostatic origin of metastases in bone and other tissues may be aided by immunohistochemistry. However, whereas both normal and hyperplastic canine prostatic epithelium label strongly with antibodies to both canine and human prostatic antigen, most reported canine prostatic carcinomas did not.

Microscopically, *it is important to note that most prostates with carcinoma will also contain areas of hyperplasia*, so on preliminary examination features of hyperplasia, and especially the tendency or otherwise for cells to be arranged on a basement membrane, need to be assessed. Also, as with malignant neoplasia generally, careful attention needs to directed to the extent of any associated necrosis and inflammation and the likely contribution of tumor growth in causing this. Heterogeneity is a feature of prostatic carcinoma. About one-third are adenocarcinomas with glandular differentiation including typical variation in acinar size, shape, and spacing, acini lined by cuboidal or columnar cells with enlarged nuclei and prominent nucleoli, sometimes a signet-ring pattern of tumor cells, and mucin often present within lumina of acini. A greater proportion of prostatic carcinomas, however (~50%), show mixed morphology, so that even within one tissue section, two or more patterns of differentiation – such as urothelial, squamoid, or sarcomatoid, as well as glandular – may be present. In the urothelial type, cells with round nuclei are present in dense nests. In squamoid tumors, closely packed non-keratinizing epithelial cells with abundant cytoplasm have prominent intercellular bridges. Sarcomatoid change, which is characterized by spindle-shaped nuclei in tumor cells, seems rarely, if ever, to predominate.

Prostatic carcinoma is described in the **cat**, in which the prostate is normally placed more caudally than in the dog and covers the urethra dorsally and laterally. As in the dog there is a history of dysuria and hematuria. Affected prostates measure 2 cm or greater, are white to cream, and microscopically have acinar or ribbon arrangement of pleomorphic epithelial cells. Mitoses are frequent. Pulmonary metastases may occur.

Other primary tumors of the prostate are leiomyoma, fibroma, and sarcomas such as fibrosarcoma, leiomyosarcoma, and hemangiosarcoma. Canine prostatic leiomyomas and leiomyosarcomas have been associated with stranguria, asymmetrical enlargement of the prostate, and metastasis to various organs. Microscopically, multinucleate and anaplastic smooth muscle cells were apparent but immunohistochemistry was used to confirm the diagnosis.

Secondary tumors, especially tumors of the lymphoreticular system, are not too uncommon although uncommonly sought. It is sometimes difficult to differentiate lymphomatous infiltration from the common senile infiltration of lymphocytes. Carcinomas of the neck of the bladder may invade the prostate.

Miscellaneous conditions of accessory genital glands

Atrophy of the accessory genital glands occurs following castration, in advanced age, and sometimes with chronic inflammation. The glands become small, dense, and of tough consistency.

In the dog, the atrophic prostate may be reduced to one-half or one-fourth its normal size. The response to androgen deprivation caused by castration is sudden; ultrastructural studies have revealed that within 3 days many prostatic acinar cells were atrophic and contained large lipid droplets. There was, however, concurrent proliferation of basal cells. Microscopically, after castration, acinar and ductal epithelial cells decrease in size, become less differentiated and more basophilic, and within 3 months epithelium is flat, and acinar lumina small or nonexistent. The stroma concurrently becomes more conspicuous, and smooth muscle of the capsule and trabeculae disappears and is replaced by dense fibrous tissue. Senile atrophy occurs normally in dogs over 11 years of age. Atrophy may also result from distemper, diabetes mellitus, and other systemic diseases.

Concretions in the vesicular glands, bulbourethral glands, or prostate are attributed to precipitation of retained secretions or to chronic inflammation. *Corpora amylacea* may be found in the accessory genital glands of domestic animals. In bulls, vesicular concretions are up to 1.5 cm or more in diameter, irregular, friable, rough externally, and distinctly laminated on section. Histologically, the concretions consist of amorphous eosinophilic debris with occasional clumps of enmeshed nuclear material. They are usually found in inflamed vesicular glands and the lining of the concretion cavity varies from normal to pseudostratified. Concretions are composed of organic components, phosphates, carbonates, and spermatozoa – so-called "*semen stones*." Microconcretions are common in the vesicular glands of rams, unassociated with inflammation.

In dogs, the occurrence of prostatic concretions or calculi is extremely rare and such calculi may be confused with urinary calculi. One report of a large (~30 × 20 × 10 mm), laminated prostatic calculus composed of calcium carbonate and struvite associated it causally with reflux of urine. Calculi are normally 1–5 mm in diameter, hard, white, and spherical, and consist of phosphates and carbonates of calcium as well as urates and oxalates. These form around a crystallization point of organic material – mostly desquamated gland epithelium. The presence of calculi seldom appears to elicit clinical signs. Prostatic calculi have also been observed in sheep.

Metaplastic ossification of prostatic stroma occurs in the dog in association with neoplasia and other chronic conditions. Foci of mineralized bone could, on radiographic examination, be mistaken for calculi.

Intraductal foreign (plant) material has been observed in the bulbourethral gland of the bull associated with chronic adenitis.

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PENIS AND PREPUCE

It is convenient and logical for the pathologist to consider these together. There are a number of developmental penile and preputial abnormalities that are of surgical importance but which do not require the contributions of the pathologist; those which do, tend to involve both the penis and prepuce.

Congenital lymphedema in Hereford calves, involving the prepuce, limbs, and tail, is associated with aplasia of lymphatic vessels and nodes. *Penile and preputial hypoplasias* occur mainly as a result of early castration or in intersex states associated with other defects of the urogenital system. In the male cat, the presence of *penile spines* is an indicator of the presence of testicular tissue. A case of *diphallia* or duplication of the glans penis in the cat is described, but as in the ram it was associated with other abnormalities indicative of an intersex state. *Partial or complete lack of the sigmoid flexure of the penis* has been observed in rams and bulls. In the bull, a form of hypoplasia described as *congenital short penis*, possibly associated with shortening of the retractor penis muscle, has been described. Clinical studies on adult bulls with short penis, which would appear to relate also to gross necropsy findings, revealed penile protrusion (penile tip to preputial orifice) of 10–22 cm as opposed to 25–42 cm in normal matched controls. *Hypoplasia of the glans penis* only has been reported. Other rare anomalies in the bull include abnormal insertion of the retractor penis muscle, with stretching of the skin cranial to the testis during erection, partial or complete duplication of the penis, supernumerary ectopic penis, and detached urethral process in which the free end of the penis has a bifid appearance resembling diphallia. Congenital dilation of the penile urethra has been described in the goat. Directional deviations of the erect penis may be attributed to congenital curvature of the os penis in the dog, to asymmetrical development of the corpus cavernosum penis in the horse and donkey, and to *persistence of the penile frenulum* in the bull, buck, boar, dog, and cat (Fig. 5.60), or malfunction of the apical ligament in the ram and bull. Caution is needed in differentiating true persistent frenulum from the balanopreputial fold in normal immature males. Although studies in the boar have indicated some breed susceptibility for persistent

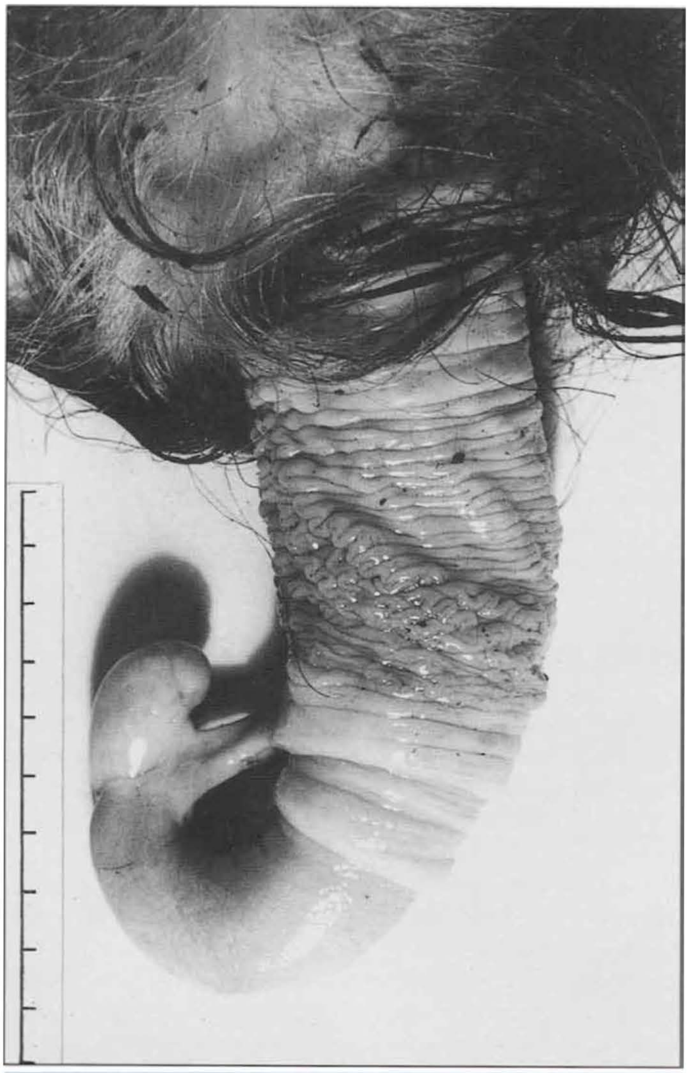

Figure 5.60 Persistent penile frenulum causing ventral deviation of the penis in a bull.

frenulum, there appears to be no definite evidence for inheritance of this trait in any domestic species.

Erection of the penis requires participation of the local circulatory apparatus. Contraction of the ischiocavernosus muscles allows arterial input to the corpus cavernosum and spongiosum, but occludes venous drainage. Abnormalities in vascular function may lead to hemorrhage from the corpus spongiosum to the urethra. Forced deviation of an erect penis may exaggerate the vascular pressures and the structural tensions of the penis. Several important vascular defects of the penis that cause failure of erection, and impotence have been studied extensively in the bull and boar, but their precise diagnosis is not possible at necropsy unless prior study of abnormal angioarchitecture of the penis has been achieved by radiography or injection of plastic into vessels to form casts. These defects in the bull include *rupture of the corpus cavernosum penis* with subsequent hematoma formation, mostly at the distal bend of the sigmoid flexure (Fig. 5.61) but sometimes proximal to it, and *vascular shunts* from the corpus cavernosum to the corpus spongiosum of the penis or to peripenile vasculature which prevent effective erection. *Penile hematoma* resulting from forced deviation of the penis is seen in rams and bulls. Rupture of the corpus cavernosum has also been observed in the stallion, but in this species hemorrhage from vessels outside an intact tunica albuginea is considered more frequent; trauma is the likely cause. Whereas in the bull these defects seem mostly to have an acquired origin, shunts in the boar from the corpus cavernosum penis to neighboring veins appear to be developmental in origin and may be inherited. Immunohistochemical studies of impotence in the boar have provided some evidence that a contributing defect may be defective enervation with depletion of vasointestinal polypeptide (VIP) reactivity in penile nerves. Stallions may develop *varices* of the penile periurethral veins.

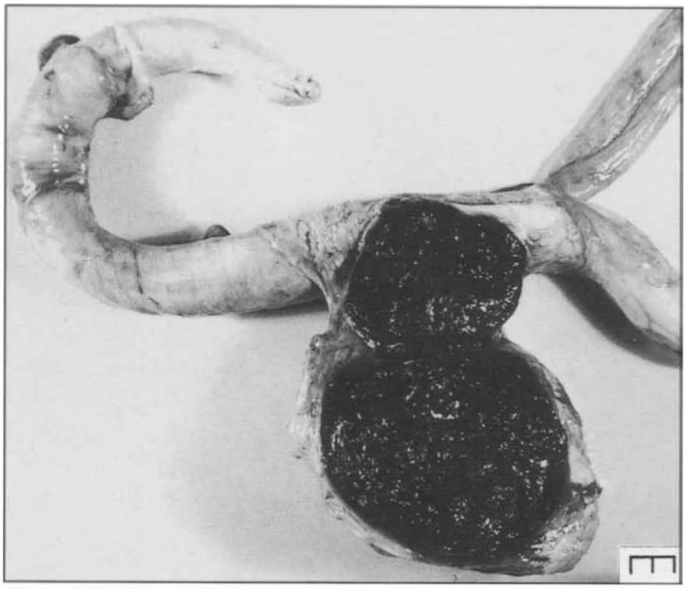

Figure 5.61 Forced deviation of the penis resulted in rupture of tunica albuginea and **hematoma** at the point of attachment of retractor penis muscle at the sigmoid flexure in a bull.

Surgical conditions of the prepuce include *varicosities* of preputial veins in stallions that may lead to thrombosis, edema, and inflammation. In dogs, *trauma to the corpus cavernosum or root of the penis* may occur during attempted copulation and is associated with hindquarter pain and/or lameness, dysuria, and perirenal edema but no obvious hematomas. Extensive *thrombosis* of the corpus cavernosum penis was noted in cats and stallions with a history of priapism of 1–3 weeks' duration, and it was unclear whether the thrombi were the cause or result of pain and persistent erection; in separate stallions, concurrent nematodiasis and generalized malignant melanoma, perhaps with metastases exerting pressure on the pudendal nerve, were, however, considered the primary cause. *Eversion of the preputial mucosa* as a temporary event is common in bulls of the *Bos indicus* species and does occur in polled breeds of *Bos taurus*. The eversion is permitted by inadequate muscular arrangements in the prepuce, and its importance lies in the injuries to which the everted epithelium is exposed. Trauma and desiccation lead to edema and inflammatory change (Fig. 5.62). A *preputial diverticulum* is anatomically normal in the boar but abnormal in the bull and buffalo. Deflection of the penis into the diverticulum results in accumulation of debris, urine, and semen that predispose to local infection and inflammation.

Tearing of preputial mucosa, typically at the ventral preputial fornix, is a common injury that occurs in bulls used for artificial insemination. The precise cause is unknown.

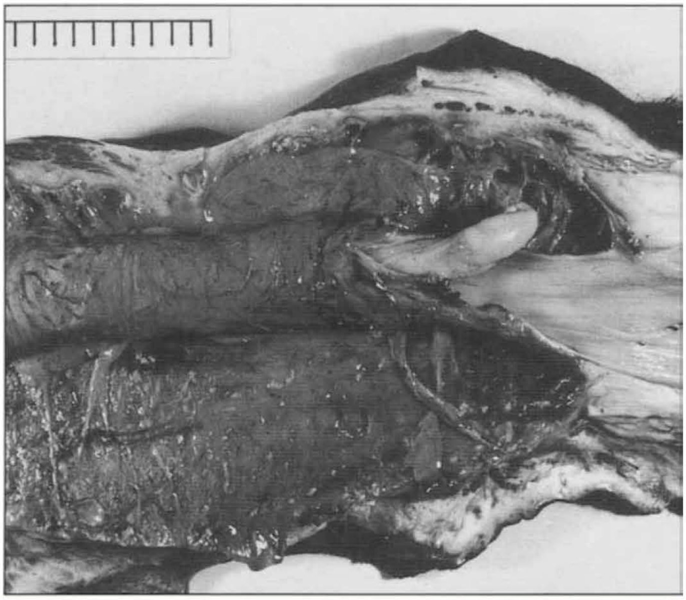

Figure 5.62 Gangrene of the prepuce and peripenile tissues in a bull with preputial prolapse.

Paraphimosis, or inability to retract the penis, is a particular problem in the stallion. Trauma is the most common cause, but inflammation of other causes, neoplasia, or primary penile paralysis may be involved. *Penile paralysis* occurs in severely debilitated stallions, after administration of certain phenothiazine derivative tranquilizers, or as a result of local neurologic disorders.

In the dog, *fracture of the os penis* with resultant callus formation may lead to obstruction of the urethra. Mineralization and ossification of the penis caudal to the os penis is a frequent (usually radiographic) finding and its prevalence increases with age. Urethral obstruction with hydronephrosis and bladder rupture due to aberrant localization of *Ancylostoma caninum* in the caudal os penis has been described.

Inflammation of the penis and prepuce (balanoposthitis)

Inflammation of the glans penis (*balanitis*) is frequently accompanied by inflammation of the prepuce (*posthitis*). Fundamental to an understanding of the causes and pathogenesis of balanoposthitis is an appreciation of the large variety and number of organisms resident in the preputial cavity, and of the existence of local immunity in this location. Viruses such as *Bovine parainfluenza virus 3* in bulls and herpesviruses in many species, nonpathogenic and potentially pathogenic bacteria such as *Corynebacterium renale* and *Histophilus somni*, fungi, mycoplasmas and ureaplasmas, chlamydia, and protozoa are readily isolated from preputial cavities that are normal on clinical and pathological examination. Studies on *Mycoplasma* species and *Ureaplasma diversum* in bulls suggest that the prepuce and distal urethra are areas these organisms mostly colonize.

Reports on whether these organisms cause lesions in the prepuce are conflicting. Lymphoplasmacytic infiltrations of the preputial mucosa, and sometimes distinct lymphoid follicles, probably are responses to the presence of these organisms, but these histologic changes are sufficiently frequent to be considered normal. Immunoglobulins are present in preputial washings, and it seems logical that these are derived from plasma cells of the prepuce. In the bull, the prevalence of these plasma cells tends to increase with age. Possibly, small (T) lymphocytes, frequently seen in preputial and penile epithelium, participate in cell-mediated immune reactions at the epithelial surface. Mucous glands are absent from the epithelium of the penis and prepuce, thus limiting the analogies that can be drawn with inflammatory processes on mucous membranes.

Balanoposthitis in the bull can be caused by ***Bovine herpesvirus 1***. This virus causes both respiratory disease (infectious bovine rhinotracheitis, IBR) and genital disease (infectious pustular vulvovaginitis, IPV). The genital disease in the bull is characterized clinically by thin purulent preputial discharge. Simultaneous occurrence of the respiratory and genital forms of the disease is rare. In the acute stage of balanoposthitis, 2–3 days postinfection, numerous small gray-white opaque foci of necrosis are present (Fig. 5.63A, B). These areas of necrosis may form confluent and flat efflorescences. In severe cases, edematous swelling of the penis and prepuce may occur at this time.

The foci of necrotic mucosa, which exist for 1–2 days only, subsequently become indistinct, the surface sloughs and sharp ulcers or erosions remain, especially in the area of the glans. A distinct zone of hyperemia surrounds many ulcers. Healing commences after 6–8 days and in uncomplicated cases is complete in 2 weeks. For a variable period thereafter, however, lymphoid follicles are present as pale to dark red nodules, arranged separately or in clusters, and seromucoid exudate is present.

The microscopic lesion is one of epithelial necrosis with neutrophilic accumulation, lymphocytic infiltration of surrounding stroma, and the transient appearance of intranuclear inclusion bodies in degenerating epithelial cells. The residual changes are nonspecific. As with other herpesvirus infections, latency occurs and viral production can be reactivated by natural mechanisms or in response to corticosteroids. Although orchitis has been observed in bulls in association with this herpetic infection, it is exceptional.

Herpesviral infection of male goats may also cause similar lesions that may, however, progress to extensive suppurative and necrotizing balanoposthitis involving the glans, fornix, and entire urethral process.

The lesions and pathogenesis of **equine coital exanthema**, caused by *Equid herpesvirus 3*, are in many respects comparable to those of infectious bovine rhinotracheitis in cattle, although the penile and preputial pustules and subsequent ulcers are somewhat larger, being up to about 15 mm in diameter (Fig. 5.64). Lesions in the stallion tend to involve the body of the penis more frequently than the glans. They first appear 2–5 days postinfection as "watery blisters" but rapidly progress to circumscribed yellow pustules with raised borders and depressed centers. Resolution usually occurs within a few weeks leaving depigmented spots, but it is unclear whether the virus remains in the penis and prepuce in a latent form.

In dogs, the genital lesions of ***Canid herpesvirus 1*** infection consist of hyperemia, petechial hemorrhages, and the development of lymphoid nodules, especially over the base of the penis and the preputial reflection. There is an associated serous discharge from the preputial orifice. Lesions appear about 3 days after experimental infection, but are self-limiting, and regress 4–5 days subsequently, with no apparent sequelae. Simultaneous conjunctivitis may sometimes occur. Recurrence of lesions, an important feature of the disease in bitches, seems to be less common in the male. Pustule formation and ulceration do not appear to be a feature of genital herpesvirus infection in

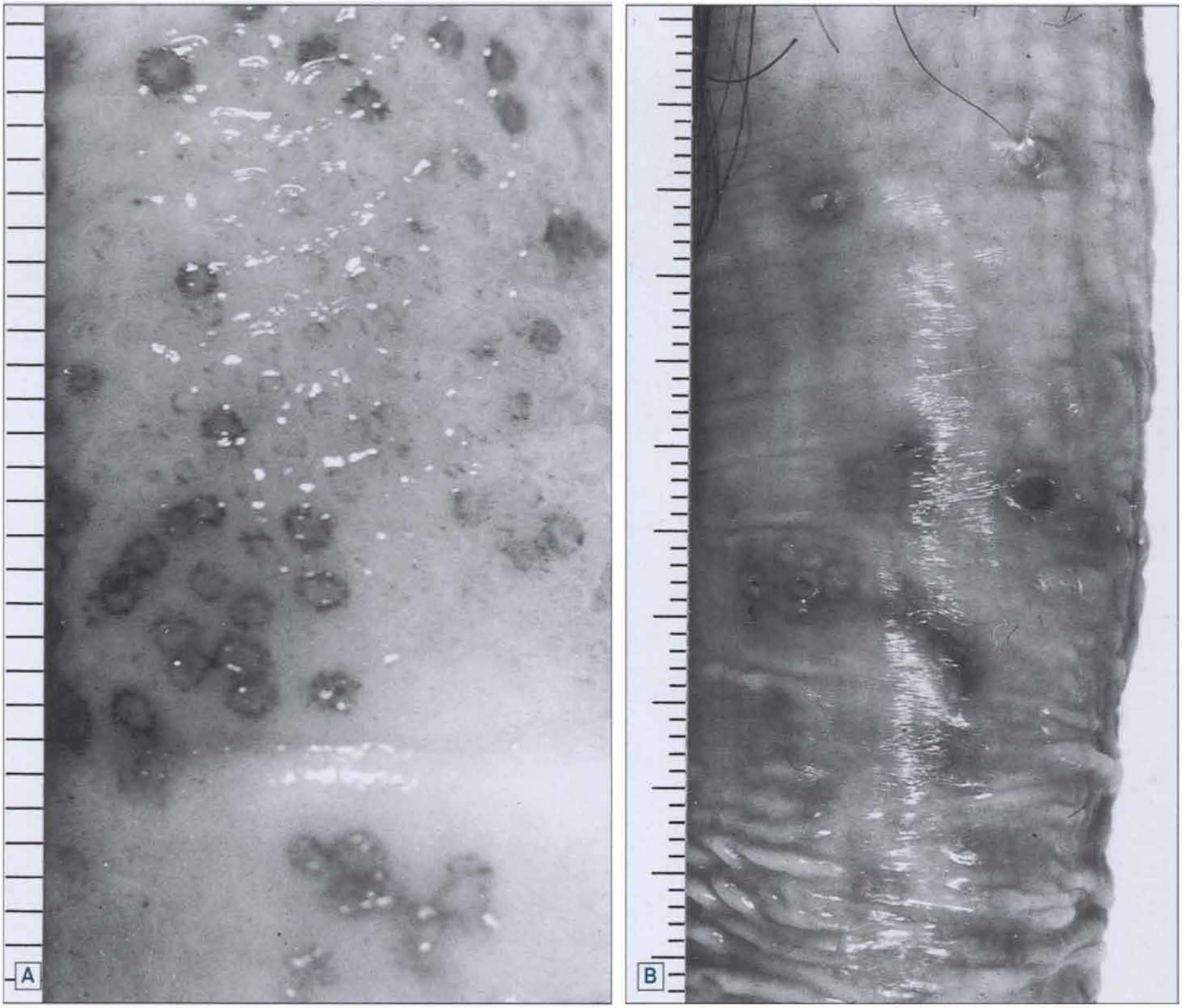

Figure 5.63 *Bovine herpesvirus 1* (BoHV, IBRV) infection producing **multifocal ulcers** on the penis. **A.** Earlier stages have distinct zones of hyperemia. **B.** Later on, ulceration of epithelium occurs.

the male dog even though virus can be isolated from the penis of infected dogs following immunosuppression with prednisolone.

A mild but *chronic balanoposthitis*, not associated with herpesvirus infection and presumed to be bacterial in origin, is common in dogs. Depending upon duration, there is intense hyperemia of the epithelium, mucopurulent exudates, and sometimes ulceration. Lymphoid follicles become enlarged and prominent. Hemolytic strains of *Escherichia coli* are most frequently isolated from such cases, other common isolates being *Proteus vulgaris* and hemolytic streptococci.

Multiple pale dome-shaped papules up to ~3 mm in diameter on hairless parts of the equine prepuce, and on the muzzle, may result from infection with the *Molluscum contagiosum virus* (for details see Vol. 1, Skin and appendages). Histologically, discrete epithelial proliferations with characteristic "molluscum bodies" are present.

In sheep, **ulcerative posthitis of wethers** particularly, but also of rams, is common and important. Vulvitis may occur in ewes in affected flocks. Differences in susceptibility between wethers and rams are probably attributable to incomplete development of the prepuce and penis in the former, and the tendency of the wether to urinate within its sheath.

Development of the primary lesion depends on the occurrence of a transmissible, urea-hydrolyzing bacterium, considered to be *Corynebacterium renale*, and on the excretion of urine rich in urea. In one outbreak, *Rhodococcus equi* and *Corynebacterium hofmanni*, both of which also produce urease, were isolated from lesions. Other factors in urine, possibly hormonal in origin, may also be involved. High protein and especially leguminous diets predispose to the disease and the incidence is lowest during the summer. It is presumed that wethers are infected by the transmission of material on contaminated bedding, herbage, or by flies. Venereal transmission from rams to ewes also occurs.

The lesion in wethers begins as a small yellow area of epidermal necrosis, usually on the dorsal part of the bare tip of the prepuce. This area ulcerates, and slow expansion of the lesion may result in

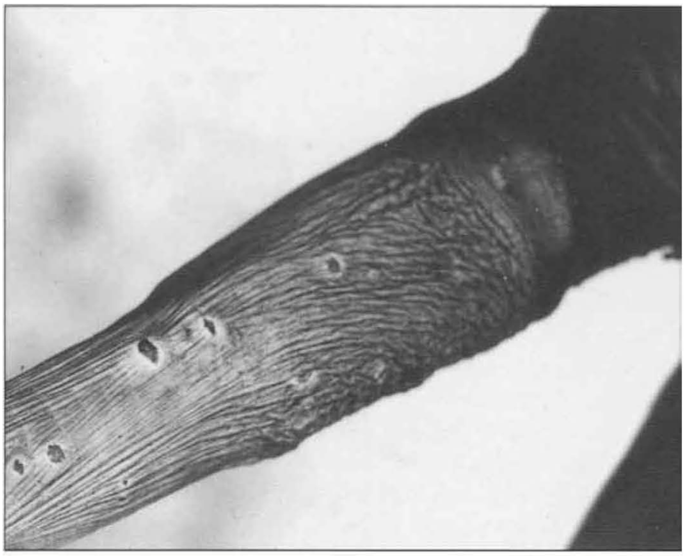

Figure 5.64 Healing ulcers of penis due to *Equid herpesvirus 3* (**coital exanthema**).

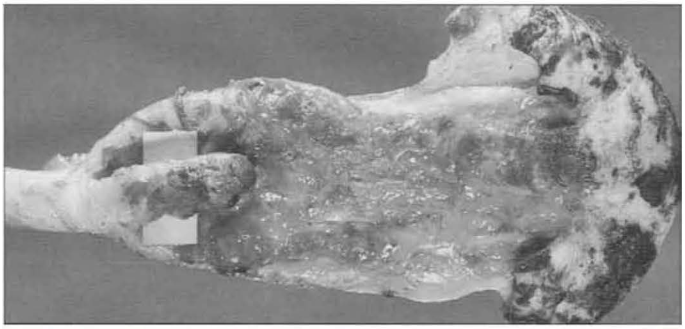

Figure 5.65 Ulcerative **posthitis and balanitis** in a wether. (Courtesy of WJ Hartley.)

occlusion of the preputial orifice. Secondary lesions occur when the prepuce becomes swollen due to the accumulation of urine or pus. At this stage, there is more or less extensive internal ulceration of the prepuce that may slough, destruction of the urethral process, and ulceration of the glans penis (Fig. 5.65). The common name, "*sheath rot,*" describes the condition as this stage. Secondary lesions are uncommon in rams.

A severe **ulcerative balanitis**, distinct from the above condition, occurs in border Leicester rams, in association with vulvovaginitis in ewes mated to them. Morbidity rate can be quite high. Acute deep ulcerations up to several centimeters in diameter occur on the ventral surface of the penis. Excessive granulation tissue and varying hemorrhage become apparent in ulcerated areas and the lesion may extend well above the surface of the penis. Much necrotic and purulent material covers the glans and urethral process, which is markedly thickened. Adhesions of the penis and prepuce may result. Ewes in contact with affected rams have shallow ulcers on the ventral commissure of the labia and posterior vagina. The causative agent has not been identified and it is not clear why border Leicester rams are predisposed.

Traumatic injury by the cocklebur (*Xanthium strumarium*) may cause outbreaks of severe "acroposthitis" in rams with subsequent suppurative vaginitis in ewes mated to them.

Outbreaks of **ulcerative posthitis in bulls** occur occasionally, but these are less severe than in sheep and it is unclear how closely the cause and pathogenesis of this condition parallels ovine posthitis. *Corynebacterium renale*, an inhabitant of the prepuce of apparently healthy bulls, frequently can be isolated from bulls with ulcerative posthitis, and as animals on a high plane of nutrition are predisposed, a parallel of this condition with ovine posthitis is suggested. However, it is not clear why steers are infrequently affected. Mild to severe erosions and ulcerations with associated edema are confined to the preputial orifice and anterior ventral area of the prepuce. Lesions begin as small raised areas of necrosis that are easily dislodged to leave shallow erosions. The ulcers that subsequently develop in these locations are irregular in shape, may be several centimeters in diameter, are sharply defined and frequently bleed. The histological appearance of lesions is nonspecific, with superficial necrosis, reactive epithelial hyperplasia, underlying granulation tissue, and a mixed cellular infiltration. Causal organisms cannot be demonstrated histologically in early lesions, preceding ulceration. In more severe forms of this condition there is increasing edema, abscessation, and myiasis. Deformity of the preputial orifice and phimosis may occur following healing of ulcers.

In bulls, the larvae of *Strongyloides papillosus* may cause severe balanoposthitis. Traumatic injury with subsequent infection by such organisms as *Arcanobacterium pyogenes*, *Escherichia coli*, streptococci, and staphylococci is also common in bulls. Tuberculous balanoposthitis, with characteristic granulomas involving the penis or prepuce, is seen occasionally in bulls from infected herds, sometimes as the only lesion. Genital transmission is possible. The presence of *Campylobacter fetus* subsp. *venerealis* in the preputial sac is not associated with gross or histological changes, although the recognized higher susceptibility of older bulls has been associated with an increase in size and number of crypts in the epithelium of the penis. It seems unlikely that *Campylobacter* spp. can colonize the preputial cavity of male goats; three species (*C. fetus subsp. venerealis, C. fetus subsp. fetus, and C. jejuni*) did not persist following experimental intrapreputial inoculation.

Specific lesions do not accompany preputial infection in bulls by *Tritrichomonas foetus*. Microscopic examination of infected penile and preputial epithelium may reveal cells and debris in crypts, and a slight but mixed cellular infiltrate. Rarely can trichomonads be demonstrated in histological sections. Recognition of the carrier state in bulls in the absence of lesions in both campylobacteriosis (see above) and trichomoniasis emphasizes the need for diagnosis by culture or molecular techniques such as PCR.

Proliferative lesions of the penis and prepuce occur in dogs with **leishmaniasis**, and may be misdiagnosed as transmissible venereal tumor. The lesions, which develop over a period of several months, are apparent as multiple ulcerated nodules up to about 1 cm in diameter located on the penile tip and glabrous preputial epithelium, as well as other organs. Histologically, there is a predominance of macrophages that contain amastigotes of *Leishmania infantum*. Immunohistochemistry is helpful in confirming the diagnosis.

Balanoposthitis is an uncommon lesion in the **stallion** but occurs in dourine, caused by *Trypanosoma equiperdum* (see also Vol. 3, Female genital system) and in cutaneous habronemiasis. Nodular lesions in the preputial lamina of the stallion may also be caused by adults, larvae, and ova of *Halicephalobus* (*Micronema*) sp., or by mycotic infection, particularly pythiosis. Primary infection in **dourine** occurs in the genitalia, following transmission by coitus. There is marked edema of the prepuce and adjacent areas, and phimosis may result. In many cases, yellow-red nodules up to ~8 mm in diameter may subsequently appear on the penis especially near the urethral orifice. These later transform to shallow ulcers, which after healing may leave distinct nonpigmented foci on the skin of the penis, prepuce, scrotum, and perineum. In **cutaneous habronemiasis** (for detailed discussion see Vol. 1, Skin and appendages) also known as "genital bursatti" and "summer sores," the verminous lesions may also be present on other parts of the body. The lesions may affect the penis only, may be around the urethral meatus, or may at the same time involve the preputial mucosa at about the point of its contact with the end of the retracted penis. The lesions are elevated, ulcerated, fungating, and very liable to hemorrhage; they consist of exuberant fibrous tissue enclosing few or many larvae of *Habronema* spp. and may be sufficiently large to cause paraphimosis. There is very dense infiltration by eosinophils, and these accumulate as small eosinophilic abscesses about immobilized and dead larvae. They may be discernible with the unaided eye as small yellow foci on the cut surface. The lesion develops during the summer months. *These larvae very often infect squamous cell carcinomas*, and all "summer sore" lesions should be examined with this in mind. *Geldings* develop a nonspecific posthitis with accumulation of smegma and detritus, presumably because of reduced penile extrusion.

Marked and apparently selective infestation of muscles of the penile urethra in **pigs** may occur several months after infection with *Sarcocystis miescheriana* from dogs. Microscopically, there is hyaline degeneration of muscle fibers and marked infiltration of eosinophils with fewer lymphocytes and macrophages.

Preputial diverticulitis occurs in swine, and the characteristic plaques and ulcers occur in the preputial diverticula of 25–40% or more of castrated and entire male pigs. The lesions commence as foci of hyperkeratosis and progress to distinct white-gray plaques 2–4 mm in diameter with obvious para- and dyskeratosis. As a result of central necrosis, sloughing of epithelium, and neutrophilic infiltration, they form discrete yellow-brown ulcers up to ~10 mm in diameter with raised borders (Fig. 5.66A, B). Such lesions may become confluent, and hemorrhage from them is common. Ulceration is seen most often in older pigs. Calculi may be present in the diverticulum. The precise cause of preputial diverticulitis in pigs is unclear, but accumulation and decomposition of urine are considered of primary importance. *Eubacterium suis*, a cause of pyelonephritis and cystitis in sows, may

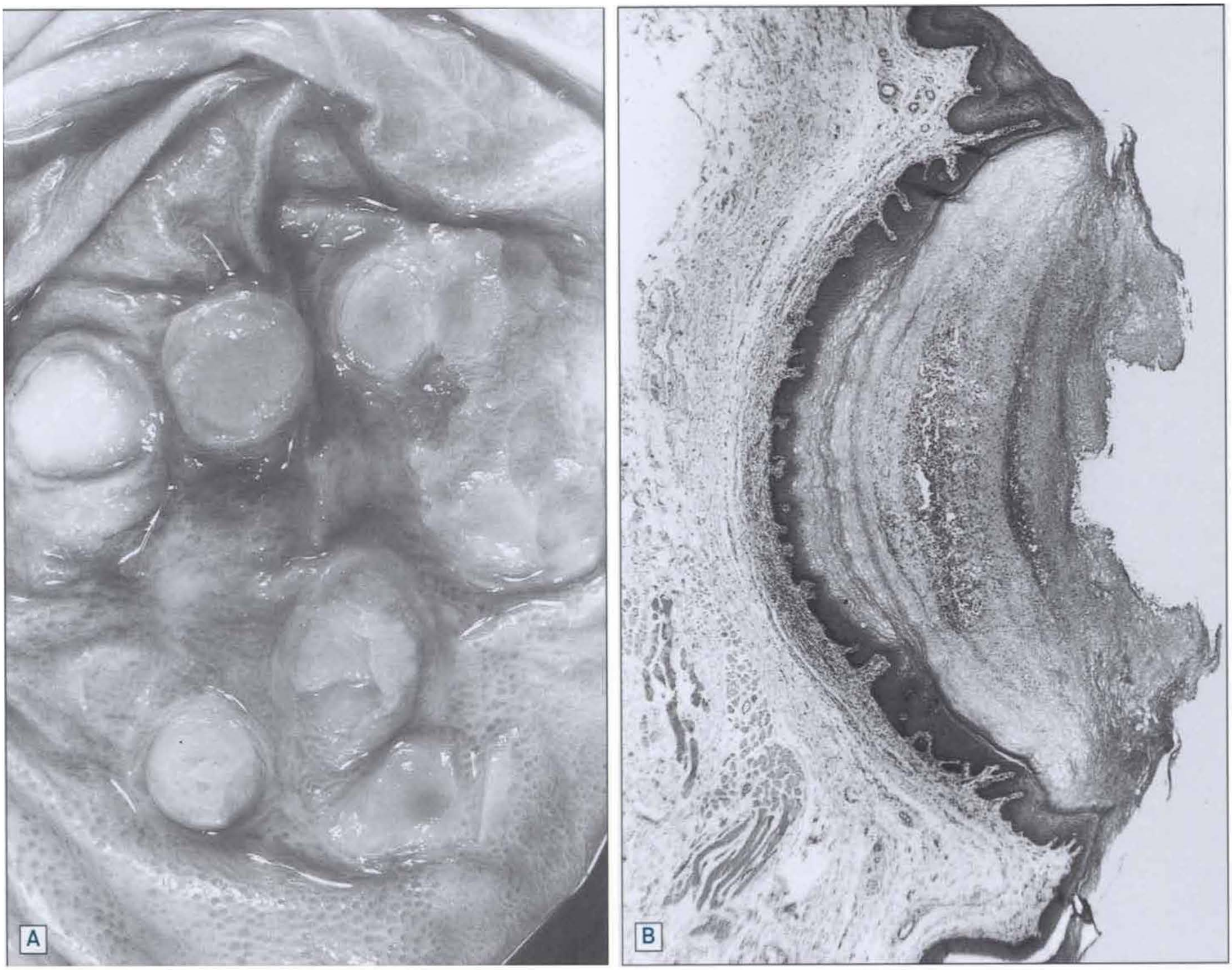

Figure 5.66 A. Plaque and ulcer of the preputial diverticulum in a boar. **B.** Histologic appearance of a plaque in the preputial diverticulum.

be isolated from the prepuce of boars with diverticulitis and healthy boars; the likelihood of venereal transfer exists. Other bacteria frequently present in the diverticulum include *Proteus* spp. and staphylococci. There appears to be no correlation between types of bacteria present and boar performance. An age-related change in bacterial flora of the diverticulum has been demonstrated, *Eubacterium suis* being generally more common in pigs over 4 months of age and in adult boars than in younger animals. Spirochetes also colonize the preputial diverticulum and may contribute to inflammation.

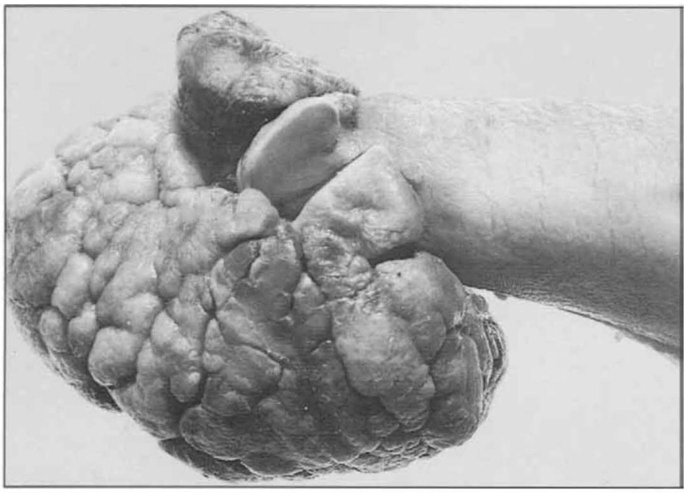

Figure 5.67 Transmissible fibropapilloma in a bull. The exophytic proliferation may be confused with a squamous cell carcinoma.

Miscellaneous conditions causing variable inflammation of the penis and prepuce include *intrapreputial foreign bodies*, and "*hair ring*" in the bull, buck, and cat, in which in-drawn preputial hairs encircle and may constrict the penis.

Neoplasms of the penis and prepuce

The important primary tumors are transmissible fibropapilloma in the bull, squamous papilloma and squamous cell carcinoma in the horse, and transmissible venereal tumor of dogs. Additional details on bovine fibropapillomatosis and canine venereal tumor are given in the Female Genital System.

Fibropapillomas in bulls caused by *Bovine papillomavirus 2* occur on the glans penis. They are most common in young bulls, 1–2 years of age, and may not be noticed until after service, when hemorrhage occurs. They are usually multiple and up to several centimeters in diameter (Fig. 5.67). Tumors are pink or gray-white on section and are composed mainly of fibrous tissue with an epithelial covering of variable thickness (Fig. 5.68A, B). Histologically, there

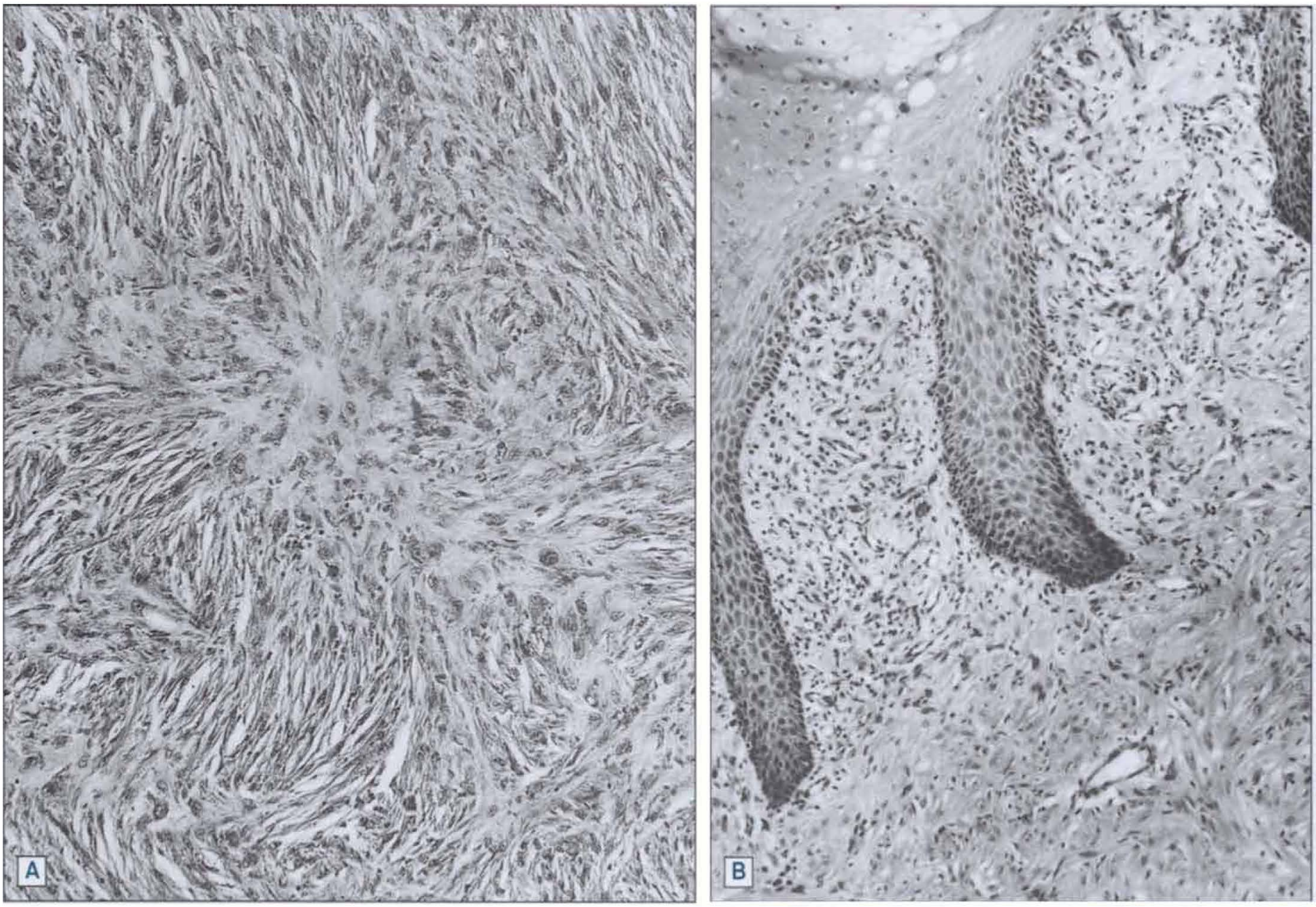

Figure 5.68 Histological details of **transmissible fibropapilloma** in a bull. **A.** Marked fibrous proliferation. **B.** Interface of the connective tissue with the characteristic long projections of epithelium.

are differences between tumors in young and old bulls, those in young bulls being more cellular with frequent mitoses.

Although benign, large fibropapillomas in bulls may interfere with free movement of the penis through the preputial orifice and retraction of the glans may be prevented. In such cases, preputial hairs may engage the prolapsed penis and cause strangulation. Alternatively, continued growth of the mass within the preputial cavity may prevent penile protrusion. The urethra may then be so compressed that rupture occurs and extensive cellulitis results from infiltration of urine in the peripenile tissues.

A *transmissible genital papilloma* has been reported in **swine**. Both the natural and experimental neoplasms regress with time. Papilloma virus antigen is present in the tumor, but the virus has not been recovered. The tumors are small, round, and project 3 cm from the mucosal surface. Histologically, they are characterized by excessive thickening of the stratum spinosum with exaggeration of the rete pegs. There is little proliferation of the underlying connective tissue. A thin layer of keratinizing cells covers the hyperplastic epithelium.

Squamous papilloma in all species is a benign, keratinizing epithelial papilliform tumor with little fibrous stroma; it is usually small and is frequently associated with lymphoplasmacytic infiltration. Squamous papilloma is most common in the *horse*, however, and is the benign counterpart of squamous cell carcinoma.

Squamous cell carcinoma (SCC) of the penis and prepuce has been described in the horse, dog, and bull, but it is possible the bovine cases were fibropapillomas. In the **horse**, SCCs occur with about equal frequency in stallions and geldings and the average age is greater than 12 years. Because SCCs in the equine penis and prepuce have occurred at sites of prior removal of squamous papilloma, any papilloma in these locations should be considered as premalignant. As with SCC elsewhere, over-expression of p53 seems to be involved in development of this tumor in the equine penis. Tumors arise mostly from the glans. Superficial ulceration and necrosis of large tumors are common (Fig. 5.69). Histologically, the penile tumor is usually well differentiated and keratinization is almost always present. Infiltration of inflammatory cells, especially eosinophils, around the tumor is common, and foci of necrosis and mineralization are frequent. Extension of the tumor occurs either by infiltration into the corpus cavernosum or by metastasis, especially to the inguinal lymph nodes and less frequently to other organs such as lung or liver. In the **dog**, SCC of the penis and prepuce has been associated with papillomavirus. It is of similar appearance to that in the horse but may be non-keratinizing. Approximately 25% of reported cases had metastasized, chiefly to the inguinal lymph nodes.

Transmissible venereal tumors in the male dog mostly arise on the penis or within the prepuce. They may be single or multiple, sessile or pedunculated, nodular or papillary, soft to firm consistency, and up to ~15 cm in diameter (Fig. 5.70). Histologically, the usual pattern is one of solid sheets of uniform round, ovoid, or polyhedral cells with large round vesicular nuclei. Mitoses are common. Metastasis occurs in up to 5% of cases and may involve the superficial inguinal lymph nodes and various organs. Most cutaneous metastases are considered to result from trauma and mechanical implantation. Spontaneous regression of tumors sometimes occurs, and usually they are sensitive to chemotherapy with vincristine. Immunohistochemical staining characteristics suggest they are of histiocytic origin, as they are positive when stained with

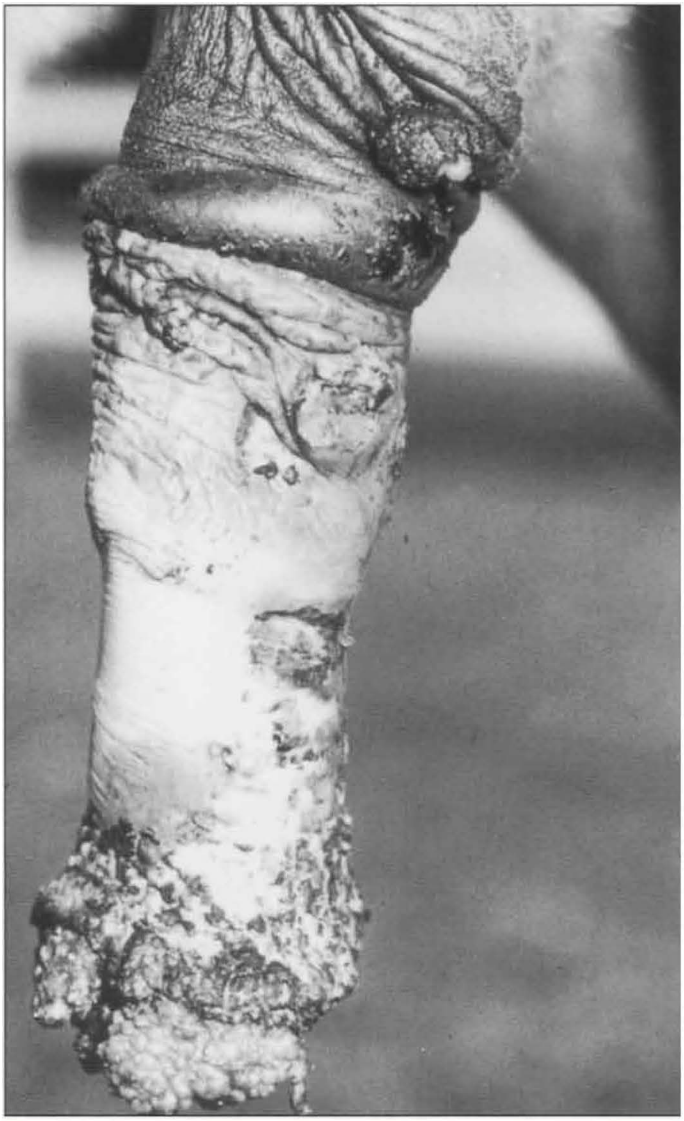

Figure 5.69 Squamous cell carcinoma of the penis of a horse. Squamous cell carcinomas often cause enlargement of the penis, which becomes hard and tough from the desmoplastic response to the neoplastic cells. (Courtesy of KG Johnston.)

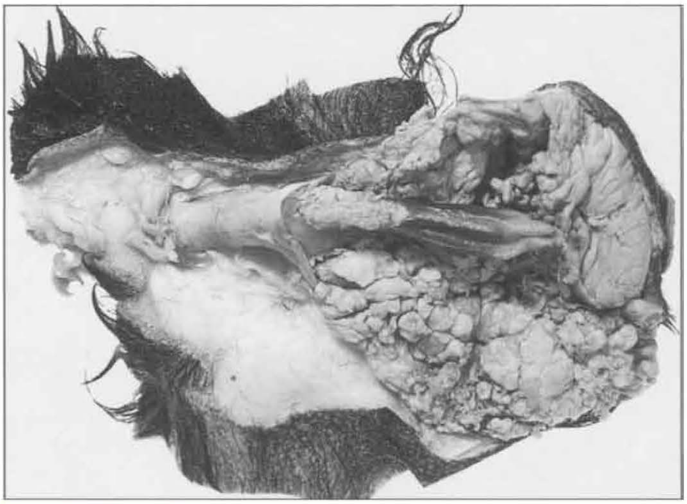

Figure 5.70 Transmissible venereal tumor on the penis and within the prepuce in a dog.

vimentin, often positive with α1-antitrypsin and lysozyme, and negative for CD3, immunoglobulins, and cytokeratin (for more detail see Female genital system).

Less common tumors of the penis or prepuce include apocrine sweat gland adenocarcinoma of the equine prepuce, mast cell tumor, malignant melanoma, hemangiosarcoma, lymphosarcoma in which cell infiltration may be either diffuse or nodular, osteosarcoma, chondrosarcoma, and ossifying fibroma of the os penis in a dog.

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